CLINICAL STUDY PROTOCOL

Protocol No. BCX1812-311

IND No. 76,350

A PHASE 3 MULTICENTER, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRAMUSCULAR PERAMIVIR IN SUBJECTS WITH UNCOMPLICATED ACUTE INFLUENZA

THE **IMPROVE 1** STUDY

(**I**ntra**M**uscular **P**eramivir for the **R**elief **O**f symptoms and **V**irologic **E**fficacy)

Short title: Intramuscular Peramivir for the Treatment of Uncomplicated Influenza

Protocol Date: Version1.0: 24 August-2007

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1 TITLE PAGE

Protocol Number:	BCX1812-311
Study Title:	A phase 3 m ulticenter, random ized, double-blind, placebo-controlled s tudy to evaluate th e effic acy and safety of intram uscular peram ivir in subjec ts with uncomplicated acute influenza
IND Number:	76, 350
Investigational Product:	Peramivir (BCX1812)
Indication Studied:	Uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive Birmingham, AL 35233
Development Phase:	3
Sponsor Medical Officer:	W. James Alexander, M.D., M.P.H. Senior Vice President, Clinical and Regulatory Operations Chief Medical Officer Phone: +1 919 859 1302 Fax: +1 919 851 1416 Email Address: jalexander@biocryst.com
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Final Protocol Date:	Version 1.0: 24-August-2007
Amendment(s) Date(s):	None

1.1 Protocol Approval Signature Page

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

BioCryst Pharmaceuticals, Inc.
Reviewed and Approved by:

W James Alexander 24 August 2007

W. James Alexander, M.D., M.P.H. Date
Senior Vice President, Clinical and Regulatory
Operations and Chief Medical Officer

1.2 Clinical Study Protocol Agreement

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practices, and all applicable regulatory requirements.

Investigator's Signature Date

Name (Print)

2 SYNOPSIS

Protocol No.	BCX1812-311
Protocol Title:	A Phase 3, Multicenter, Random ized, Double-Blind, Placebo-Controlled Stu dy to Evaluate the Efficacy an d Safety of Intram uscular Peram ivir in Subjects with Uncomplicated Acute Influenza
Sponsor:	BioCryst Pharmaceuticals, Inc.
Investigators/Study Sites:	Multinational
Development Phase:	3
Objectives:	
Primary:	To evaluate the efficacy of peram ivir adm inistered intramuscularly com pared to p lacebo on th e tim e to alleviation of clinica l sym ptoms in adult sub jects with uncomplicated acute influenza.
Secondary:	1. To evalua te the saf ety and tolera bility of pe ramivir administered intramuscularly; 2. To evaluate seconda ry clinical outcom es in response to treatment; 3. To evaluate changes in in fluenza virus titer (viral shedding) in response to treatment; 4. To assess p harmacoeconomic m easures in resp onse to treatment 5. To assess c hanges in inf luenza vir al suscep tibility to neuraminidase inhibitors following treatment
Number of Subjects:	Total enrollm ent: up t o 800 subjects random ized (160 subjects in the placebo treatment group, 320 subjects in the peramivir 150m g treatm ent group and 320 subjects in the peramivir 300m g treatm ent group). The study will close after enrollment of at least 500 subjects who have either a positive Influenza A or B antigen te st (Rapid Antigen Test – RAT) at screening, or who ha ve a negative RAT at screening but a positive influenza A or B PCR a ssay result from a baseline nasopharyngeal swab.
Study Design:	This is a multinational, random ized, double-blind study comparing the efficacy and safety of two single dos e regimens of peram ivir administered intramuscularly versus placebo in adults with uncom plicated acute influenza. Each subjec t's ass ignment to trea tment will be stra tified according to the Rapid Antigen Test (RAT) result at screening and current sm oking be havior, with 80% of subjects ce ntrally rand omized to one of the two activ e

	single dose regimens of peramivir (2:2:1 randomization) Treatment Group 1: Peramivir 150mg Treatment Group 2: Peramivir 300mg Treatment Group 3: Placebo Enrollment of subjects into the RAT negative stratum will be permitted at individual sites that have identified 3 RAT positive subjects at screening within a 10 day period, indicating activity of influenza in the site's vicinity. After identification of 3 RAT positive subjects within 10 days, a site may enroll 1 RAT negative subject that fulfills the inclusion/ exclusion criteria. One additional RAT negative subject may be enrolled thereafter for each preceding RAT positive subject that is identified. Study drug will be administered as one 2mL intramuscular injection in each gluteal muscle (total of 4mL injected in divided doses). Subjects eligible for screening will have an anterior nasal or pharyngeal swab collected for testing by RAT for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated with a different commercially available RAT kit. Subjects meeting the inclusion/ exclusion criteria may be enrolled into the study. All enrolled subjects will record the following in a Study Diary: • Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol), aspirin, ibuprofen or other NSAID. • Assessment of severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14 • Assessment of subject's ability to perform usual activities, (rated as 0–10 on a visual analog scale) once daily through Day 14 • Assessment of subject's time lost from work or usual activities and productivity compared to normal (rated as

	0-10 on a visual analog scale) once daily through Day 14 • Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14 Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. In a subset of a minimum of 200 subjects, an additional virology sample will be collected on day 2. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result). All virologic assessments will be performed by a central laboratory. At the study day 3 visit, when all subjects are evaluated for safety and a blood draw is completed for clinical laboratory investigations, a single pharmacokinetic (PK) sample will also be drawn. This single PK sample will be analyzed for plasma concentrations of peramivir (ng/mL) and evaluated in an exposure response analysis. At selected sites a separate sub-study will be conducted to collect limited PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.
Study Population:	Male and female subjects, 18 years of age and older, with symptoms consistent with a diagnosis of uncomplicated acute influenza infection may be screened for enrollment. Subject eligibility will be dependent on the presence of two or more symptoms consistent with acute influenza as well as the results obtained from a rapid antigen test (RAT) for influenza A and B at screening.
Inclusion Criteria:	1. Male and non-pregnant female subjects age ≥18 years 2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate specimen collected from an anterior nasal or pharyngeal swab, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated with a different commercially available RAT kit. A limited number of RAT negative

	subjects may be enrolled in accordance with a defined screening algorithm. 3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. For subjects with a positive RAT at the time of screening, a subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at time of screening. For subjects with no positive RAT at screening, fever as defined above must be documented at time of screening 4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of any severity (mild, moderate, or severe) 5. Presence of at least one constitutional symptom (myalgia [muscle aches], headache, feverishness, or fatigue) of any severity (mild, moderate, or severe) 6. Onset of symptoms no more than 48 hours before presentation for screening 7. Written informed consent
Exclusion Criteria:	1. Women who are pregnant or breast-feeding 2. Presence of clinically significant signs of acute respiratory distress 3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma 4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class IV functional status within the past 12 months 5. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min) 6. Current clinical evidence of active bacterial infection at any body site that requires therapy with oral or systemic antibiotics 7. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy 8. Current treatment for active viral hepatitis C 9. Presence of known HIV infection with a CD4 count <350 cell/mm^3 10. Current therapy with oral warfarin or other systemic anticoagulant 11. Receipt of any doses of rimantadine, amantadine,

	zanamivir, or oseltamivir in the 7 days prior to screening 12. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days 13. History of alcohol abuse or drug addiction within 1 year prior to admission in the study 14. Participation in a previous study of peramivir as treatment for acute influenza or previous participation in this study 15. Participation in a study of any investigational drug within the last 30 days 16. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.
Study Endpoints:	
Primary Endpoint:	*Clinical:* Time to alleviation of clinical symptoms of influenza
Secondary Endpoint(s):	*Safety* Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests *Clinical:* Time to resumption of subject's ability to perform usual activities Time to resolution of fever Incidence of influenza related complications *Virologic:* Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$) and/or by quantitative polymerase chain reaction (PCR) assay *Pharmacoeconomic*: Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.
Exploratory Endpoint:	*Viral Susceptibility:* Change in influenza virus susceptibility to neuraminidase inhibitors
Investigational Product, Dose, and Mode of Administration:	Peramivir (BCX-1812), 75mg/mL or placebo (buffered diluent), 2mL per injection, administered intramuscularly in the gluteal muscle, bilaterally.
Duration of Treatment:	Following treatment on day 1, study duration for individual

	subjects is expected to be up to 14 days (including all visits).
Statistical Methods:	Descriptive statistical methods will be used to summarize the data from this study, with statistical testing utilized for the primary and secondary efficacy endpoints. Unless otherwise noted, all statistical testing will be two-sided, and will be performed using a significance (alpha) level of 0.05. For assessment of the primary efficacy endpoint, the overall significance level will be maintained by a Bonferroni adjustment for the planned comparisons between the two active treatment groups and placebo. Detailed statistical procedures will be provided in a full statistical analysis plan (SAP) completed prior to database lock and study unblinding.
Sample Size:	From previous studies of neuraminidase treatment of uncomplicated influenza it is expected that the median time to alleviation of symptoms will be 103.3 hours for subjects receiving placebo. Additionally, it is expected that the median time to alleviation for the low dose peramivir arm will be 69.9 hours, yielding a hazard ratio of 0.68. Using these assumptions, a sample size of 200 influenza-infected subjects per active treatment group and 100 infected subjects in the placebo group (a total of 500 influenza-infected subjects) is sufficient to provide at least 80% power to detect a hazard ratio of 0.68 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months). Up to 800 subjects will be enrolled to achieve the target number of at least 500 subjects with diagnostic evidence (RAT or PCR) of an acute influenza infection.
Efficacy:	The intent-to-treat infected population will include all subjects who are randomized, received study drug, and have proven influenza by any one of the following: primary viral culture, PCR, or paired serology showing $\geq$ 4-fold increase in antibody to influenza A or B. The primary efficacy variable is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when each of seven symptoms of influenza are either absent or are present at no more than mild severity level and remain at no worse than this severity status for a 24 hour period. Descriptive statistics for the primary efficacy variable will

	be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment difference will be assessed using a Cox Regression model with effects for RAT result at screening, current smoking behavior, treatment group, and influenza season at randomization. Pairwise comparisons between each active group and placebo will be constructed from the Cox Regression model. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing assessment. Time to resumption of usual activities and time to resolution of fever will be analyzed in a similar manner. Changes in viral titers will be compared using the van Elteren statistic controlling for RAT result at screening, current smoking behavior and influenza season at randomization. Analyses of other continuous endpoints will be analyzed in a similar manner.
Safety:	Safety analyses will be presented for all subjects in the safety population, defined as all randomized subjects who receive at least one dose of study drug. Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. The occurrence of treatment-emergent AEs will be summarized using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs that are related to study medication will be generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Descriptive summaries of vital signs and quantitative clinical laboratory changes will be presented by study visit. Frequency and percentages of subjects with abnormal laboratory test results will be summarized by toxicity grade. Concomitant medications will be mapped to a WHO preferred term and drug classification. The number and percent of subjects taking concomitant medications will be summarized using preferred terms and drug classifications. The number and percent of subjects experiencing each

	abnormal physical examination finding will be presented. The number and percent of subjects discontinuing study as well as the reasons for discontinuation will be summarized by treatment group.
Protocol Date	Version 1.0: 24-August-2007

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3.1 List of Figures

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0–72}$	area under the curve from time 0 to 72 hours
$AUC_{0–\infty}$	area under the curve extrapolated from time 0 to infinity
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
C_{max}	maximum plasma concentration
CK	creatine kinase
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRF	Case Report Form
CV	coefficient of variation
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRC	influenza related complications
ITT	intent-to-treat
ITTI	intent-to-treat infected
IUD	intrauterine device
IVRS	interactive voice response system
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRU	medical resource utilization
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction

RAT	Rapid Antigen Test
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
$t_{1/2}$	elimination half-life
$t_{1/2}\lambda z$	terminal half-life
$TCID_{50}$	time weighted change from baseline in log 10 tissue-culture infective $dose_{50}$
TEAEs	treatment-emergent adverse events
T_{max}	time to attain maximum plasma concentration
UPEP	urine protein electrophoresis
WBC	white blood cell
WHO	World Health Organization

5 INTRODUCTION

5.1 Background

Influenza virus is a member of the *orthomyxovirus* family and causes an acute viral disease of the respiratory tract. Typical influenza illness is characterized by abrupt onset of fever, headache, myalgia, sore throat, and nonproductive cough.[1] The illness is usually self-limiting, with relief of symptoms occurring within 5 to 7 days. Nevertheless, it is an important disease for several reasons, including ease of communicability, short incubation time, rapid rate of viral mutation, morbidity with resultant loss of productivity, risk of complicating conditions, and increased risk of death, particularly in the elderly. During 19 of the 23 influenza seasons between 1972/1973 and 1994/1995, estimated influenza-associated deaths in the United States ranged from approximately 25 to more than 150 per 100,000 persons above 65 years of age, accounting for more than 90% of the deaths attributed to pneumonia and influenza.[2]

Presently, only a few measures are available that can reduce the impact of influenza: active immunoprophylaxis with an inactivated or live attenuated vaccine and chemoprophylaxis or therapy with an influenza-specific antiviral drug. Neuraminidase inhibitors are the current mainstay of antiviral treatment for influenza. Marketed neuraminidase inhibitors include zanamivir (Relenza®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche-Gilead), an oral prodrug of the active agent, oseltamivir carboxylate. Influenza neuraminidase is a surface glycoprotein that cleaves sialic acid residues from glycoproteins and glycolipids. The enzyme is responsible for the release of new viral particles from infected cells and may also assist in the spreading of virus through the mucus within the respiratory tract. The neuraminidase inhibitors represent an important advance in the treatment of influenza with respect to activity against influenza A and B viruses, with proven therapeutic value in reducing influenza lower respiratory complications,[3] and lower rates of antiviral drug resistance[4].

The use of currently available neuraminidase inhibitors has been limited by concerns including, the degree of effectiveness, the requirement for an inhaler device (zanamivir), and the emergence of resistant influenza virus variants in some treated populations.[5] In addition, there are risks of bronchospasm with zanamivir; and gastrointestinal side effects, with oseltamivir.

Peramivir is a neuraminidase inhibitor that represents a potentially promising addition to the armamentarium of drugs for the treatment of influenza infections due to its potential for parenteral administration and lower frequency of dosing.

5.2 Rationale for Study

An oral formulation of peramivir has previously been evaluated in a full range of safety, tolerability, pharmacokinetic, and efficacy studies. In a multinational phase 3 clinical trial conducted in 1999-2001, oral peramivir demonstrated antiviral activity against influenza A and B infections, and improvement in the relief of clinical symptoms. Because of the limited bioavailability of peramivir following oral administration (<5%), it was determined that the parenteral route of administration is more appropriate for the

delivery of peramivir. Subsequent phase 1 studies of intravenous and intramuscular formulations of peramivir have confirmed that parenteral routes of administration result in plasma levels of drug that are as much as 100 times those achieved via the oral route. Further details of these studies are provided below and in the Investigator Brochure.

A phase 2 randomized, double blind, placebo controlled study of two single dose regimens (150mg or 300mg) of intramuscular peramivir in subjects with uncomplicated acute influenza infections (study BCX1812-211) was initiated in early 2007 in North America and the UK. The study has been extended to sites in Hong Kong, Australia, New Zealand and South Africa and is expected to complete enrollment in mid-2007. At a scheduled meeting on 21 May 2007, an independent data monitoring committee for this study completed a blinded review of all reported adverse events and grade 3 and 4 clinical laboratory evaluations from the first 135 subjects randomized. Following this blinded safety review the DMC provided a written recommendation that the study continue as planned.

Because of the previous demonstration of significant antiviral activity, the strong suggestion of clinical efficacy of oral peramivir previously demonstrated in acute influenza, and the encouraging pharmacokinetic and preliminary safety profile of the intramuscular formulation of peramivir demonstrated to date, this phase 3 study will be conducted to evaluate the efficacy and safety profile of intramuscular peramivir and to determine the optimal single dose regimen.

5.3 Non-Clinical Experience with Peramivir

5.3.1 In vitro Assays

Peramivir is a selective inhibitor of viral neuraminidase, with 50% inhibitory concentrations (IC_{50}) for bacterial and mammalian enzymes of >300µM.[6] In an *in vitro* study, 42 influenza A and 23 influenza B isolates were collected from untreated subjects during the 1999–2000 influenza season in Canada.[7] These isolates were tested for their susceptibility to the neuraminidase inhibitors zanamivir, oseltamivir carboxylate, and peramivir using a chemiluminescent neuraminidase assay. Inhibition of Type A influenza neuraminidase by peramivir was approximately an order of magnitude greater than inhibition of neuraminidase from Type B viruses. IC_{50} values for the Type A enzymes ranged from <0.1 to 1.4nM, whereas the Type B enzymes ranged from <0.1 to 11nM, with three out of four values in the 5- to 11nM range. Peramivir was the most potent drug against influenza A (H3N2) viruses with a mean IC_{50} of 0.60nM as well as most potent against influenza B with a mean IC_{50} of 0.87nM.

In another *in vitro* comparison of peramivir, oseltamivir, and zanamivir, using a neuraminidase inhibition assay with influenza A viruses, the median IC_{50} of peramivir (approximately 0.34nM) was comparable to that of oseltamivir (0.45nM) and significantly lower than zanamivir (0.95nM). For influenza B virus clinical isolates, the IC_{50} of peramivir (1.36nM) was comparable to that of zanamivir (2.7nM) and lower than that of oseltamivir (8.5nM).[8]

The potency of peramivir was evaluated against five zanamivir-resistant and six oseltamivir-resistant influenza viruses.[9] Peramivir remained a potent inhibitor against all

oseltamivir-resistant viruses including the mutations H274Y, R292K, E119V, and D198N, with IC_{50} values ≤40nM. Peramivir also potently inhibited (IC_{50} ≤ 26nM) the neuraminidase activity of zanamivir-resistant strains, which had the following mutations: R292K, E119G, E119A, and E119D. However, one zanamivir-resistant influenza B virus, B/Mem/96, with a mutation R152K isolated from cell culture, was relatively resistant to all neuraminidase inhibitors, including peramivir (IC_{50} = 400nM).

5.3.2 Animal Models

In a mouse model of influenza infection, a single intramuscular injection of peramivir (10mg/kg) given 4 hours prior to inoculation with an A/NWS/33 (H1N1) influenza strain resulted in 100% survival in contrast to 100% mortality in a control group injected with saline.[6] In the same mouse model, treatment of mice up to 72 hours after influenza infection using peramivir (20mg/kg) resulted in 100% survival, compared to 100% mortality in the control group injected with vehicle.[10]

Peramivir has also demonstrated activity in animal models utilizing a clinical H5N1 isolate as the infecting virus strain. In a mouse model, a single intramuscular dose of peramivir (30mg/kg) injected 1 hour after inoculation with the highly pathogenic (H5N1) A/Vietnam/1203/04 strain, resulted in a 70% survival rate that was similar to that seen in mice treated with oseltamivir given orally at 10mg/kg/day for 5 days [11]. In similar experiments, mice inoculated with the same strain of H5N1 virus that were then treated for up to 8 days with intramuscular peramivir exhibited 100% survival [12]. This longer duration of peramivir treatment also prevented viral replication in the lungs, brain and spleen at days 3, 6 and 9 post inoculation.

5.4 Previous Phase 3 Clinical Experience with Oral Peramivir

An oral formulation of peramivir has previously demonstrated antiviral activity and preliminary clinical efficacy in challenge studies in human volunteers, as well as in treatment studies in patients with uncomplicated acute influenza infections during the influenza seasons of 1999-2001. A Phase 3 multinational study (BC-01-03) of oral peramivir was conducted. Two dose regimens of oral peramivir, 800mg QD for 5 days, or 800mg QD on Day 1, followed by 400mg QD for 4 days, were compared to a matched placebo treatment group. A total of 1246 subjects were randomized to treatment at sites in the USA, Western and Eastern Europe, South America, Australia and New Zealand. As presented in the table below, the primary end-point of time to relief of influenza symptoms was not found to be significantly different (p=0.17) between the three treatment groups.[13] A sub-group analysis of the time to relief of symptoms by country or region demonstrated marked differences in the primary endpoint.. In the subset of influenza-infected subjects enrolled at sites in the US, clinically meaningful differences in time to relief of influenza symptoms between the placebo and the two peramivir arms were observed, that just missed statistical significance (p=0.07). However, a number of secondary end-points in this phase 3 study, such as time to overall well-being, time to normal activity, incidence of influenza related complications and quantity of viral shedding, reached or approached statistically significant differenced between the peramivir and placebo treatment groups (p=0.03-0.06).

Results of Study BC-01-03

	Median Time to Relief of Influenza Symptoms (Hours)	
Dose and Regimen	**Overall Results (n=1246)**	**US Sites (n=206)**
Peramivir 800mg po x 5d	89.0	70.8
Peramivir 800mg po x 1d and 400mg po x 4d	91.7	88.8
Placebo x 5 days	104.4	106.8
p value	0.17	0.07

5.5 Previous Phase 1 Experience with Intramuscular Peramivir

Two phase one studies evaluating the safety and pharmacokinetics of an intramuscular formulation of peramivir have been conducted in a total of 45 healthy volunteers receiving peramivir.

Study Peramivir-Him-06-111 evaluated the single dose pharmacokinetics and tolerability of 75mg, 150mg and 300mg doses of peramivir administered as intramuscular (i.m.) and intravenous (i.v.) injections in a crossover design (9 subjects per group). Peak plasma levels of i.m. peramivir generally occurred within 30 minutes following injection. Plasma pharmacokinetic parameters for i.m. peramivir are summarized below for the three intramuscular single dose regimens evaluated:

Dose (mg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (hr·ng/mL)	$t_{½}$[a] (hr)
75 i.m.	4296±812	11659±1123	19.8±7.9
150 i.m.	7612±884	23952±3804	24.3±4.1
300 i.m.	15150±2367	49649±5619	22.8±2.5
[a]terminal half life			

In a second phase 1 study, Peramivir-Him-06-112, the same dose levels of peramivir were administered as single i.m. injections on two consecutive days (6 subjects per group). This double-blind study also included a placebo arm. The pharmacokinetic parameters of i.m. peramivir following the second day of dosing were consistent with those seen following single doses of the drug.

The observations of safety and tolerability of i.m. peramivir in each of the 2 phase 1 studies were unremarkable. No serious adverse events were reported. The most commonly observed adverse events or laboratory abnormalities were headache, several reports of signs and symptoms of vasovagal reactions following injections, and transient increases in creatine kinase. No consistent differences in frequency of adverse events were observed between the active and placebo treatment groups, with the exception that CK elevations appeared to be dose related in the peramivir treatment groups. The vasovagal reactions were attributed to the receipt of relatively large volumes of i.m. injection (2 injections each of 2mL) in the fasted state.

An ongoing phase 2 study, BCX1812-211, is a randomized, double-blind placebo-controlled study to evaluate the efficacy and safety of two single dose regimens of peramivir. Up to 100 subjects per arm will receive either 150mg or 300mg of peramivir or placebo. The primary endpoint of the study is the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza. An independent data monitoring committee reviewed grouped blinded safety data on the first 135 subjects randomized. The study remains blinded. The frequency of vasovagal reactions reported as adverse events in this ongoing study is 2/135 (to date) which is lower than that seen in the phase 1 volunteer studies.

5.6 Dose Rationale

Oseltamivir is approved for the treatment of uncomplicated acute influenza at a dosage of 75mg twice daily in adults[14]. Oseltamivir was shown to be clinically effective in a Phase III study of oral oseltamivir versus placebo in naturally occurring seasonal influenza, and these data were sufficient for regulatory approval for marketing of oseltamivir. At least 75% of an oral dose of oseltamivir reaches the systemic circulation as oseltamivir carboxylate. When oseltamivir is administered orally at a dose of 75mg twice daily, the serum C_{max} of oseltamivir carboxylate is approximately 348ng/mL and the AUC_{0-48} is 10,876 h·ng/mL. The clinical data indicate that this level of exposure to oseltamivir was sufficient to provide clinical improvement in uncomplicated acute influenza.

The serum pharmacokinetic data (C_{max} and $AUC_{0-\infty}$, respectively) following intramuscular doses of peramivir are approximately 7600ng/mL and 24,000 h·ng/mL for the 150mg dose and are approximately 15,000ng/mL and 49,000 h·ng/mL for the 300mg dose. Previous studies have assessed the concentrations of the neuraminidase inhibitor zanamivir in nasal and pharyngeal secretions after parenteral administration of this drug. . Within several hours after administration, the concentrations in secretions were approximately 100-fold lower than in serum or plasma. In theory, relatively high levels of a neuraminidase inhibitor in respiratory secretions are desirable in order to rapidly inactivate influenza virus and to delay or prevent the development of resistance in infecting virus strains. Intramuscular doses of peramivir, including doses of 150mg and 300mg have been shown to be well tolerated in previous Phase 1 studies and since no identified safety signal has been noted by an independent data monitoring committee in the ongoing Phase 2 study, it is appropriate for these two dose regimens to undergo further evaluation in this Phase 3 study.

6 STUDY OBJECTIVES

6.1 Objectives

6.1.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated influenza.

6.1.2 Secondary Objective(s)

The secondary objectives of this study are:

1. To evaluate the safety and tolerability of peramivir administered intramuscularly;
2. To evaluate secondary clinical outcomes in response to treatment;
3. To evaluate changes in influenza virus titer (viral shedding) in response to treatment;
4. To assess pharmacoeconomic measures in response to treatment;
5. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment;

6.2 Study Endpoints

6.2.1 Primary Endpoint

Time to alleviation of clinical symptoms of influenza

6.2.2 Secondary Endpoint(s)

Secondary efficacy endpoints will include evaluations in each subject of:

1. Safety parameters, including: treatment- emergent adverse events (TEAEs) and treatment-emergent changes in clinical laboratory tests;
2. Time to resolution of fever;
3. Time to resumption of subject's ability to perform usual activities;
4. Incidence of influenza related complications
5. Quantitative change in influenza virus shedding measured by viral titer assay ($TCID_{50}$) and/or by quantitative polymerase chain reaction (PCR) assay;
6. Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.

6.2.3 Exploratory Endpoint

An exploratory endpoint will evaluate change in influenza virus susceptibility to neuraminidase inhibitors in viral isolates recovered from subjects pre and post treatment.

7 STUDY DESIGN

7.1 Overall Study Design and Plan

This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. Up to 800 subjects will be enrolled into the study. Each subject's assignment to treatment will be stratified according to a Rapid Antigen Test (RAT) result and current smoking behavior, with 80% of subjects centrally randomized via an Interactive Voice Response (IVR) system to one of the two active single dose regimens of peramivir (2:2:1 randomization):

Treatment Group 1:	Peramivir 150mg	maximum n=320
Treatment Group 2:	Peramivir 300mg	maximum n=320
Treatment Group 3:	Placebo	maximum n=160

Previous studies evaluating the efficacy of neuraminidase inhibitors enrolled subjects with influenza-like illness on the basis of presence of specific clinical signs and symptoms. These studies were conducted before the widespread availability of rapid influenza antigen diagnostic test kits (Rapid Antigen Tests-RAT), and typically between 50-70% of subjects enrolled were subsequently confirmed by viral culture to have an active influenza infection. The sensitivity of commercially available RAT kits ranges from 62% to 83%, depending upon the virus sub-type. [15] In the phase 2 study of intramuscular peramivir (study BCX1812-211) a positive RAT test was required for study entry. It is likely that a number of subjects with an influenza infection were excluded from this study due the sensitivity of this assay.

In this phase 3 study a limited number of RAT negative subjects will be enrolled in accordance with the following algorithm to minimize false negative test results: once an individual site has identified 3 RAT positive subjects at screening within a 10 day period, indicating influenza activity is present in the site's vicinity, a RAT negative subject that meets the inclusion / exclusion criteria may be enrolled. One additional RAT negative subject may be enrolled there after for each preceding RAT positive subject that is identified. The RAT result for each subject screened will be recorded on the study Interactive Voice Response System (IVRS) to manage this enrollment algorithm.

A baseline nasopharyngeal swab specimen will be sent to the central virology laboratory for each RAT negative subject that has been randomized into the study. These specimens will be tested for influenza A and B infection using a PCR assay. The PCR results for these specimens will be reported to BioCryst in real time. The study will close after enrollment of at least 500 subjects who have either a positive Influenza A or B RAT at screening, or who have a negative RAT at screening but a positive influenza A or B PCR assay result from a baseline nasopharyngeal swab.

Study drug will be administered as one 2mL intramuscular injection in each gluteal muscle (total of 4mL injected in divided doses).

At screening, subjects will have an anterior nasal or pharyngeal swab collected for testing with a commercially available RAT kit for influenza A and B in accordance with the RAT manufacturer's instructions. If this test is positive and the subject is enrolled, additional specimens will be obtained for isolation and culture of influenza virus and quantitative PCR assay. If the initial RAT is negative, the test should be repeated with a different commercially available RAT kit. A limited number of RAT negative subjects may be enrolled in accordance with the screening algorithm defined above.

Enrolled subjects will record the following in a Study Diary:

- Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol- provided), aspirin, ibuprofen or other NSAID.
- Assessment of seven symptoms of influenza on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe), twice daily (AM, PM) through Day 9, then once daily (AM) through Day 14
- Assessment of subject's ability to perform usual activities, (0–10 on a visual analog scale) once daily through Day 14
- Assessment of a subject's time lost from work or usual activities and productivity compared to normal (0-10 on a visual analogue scale) once daily through Day 14
- Doses of antipyretic (e. g. acetaminophen/ paracetamol), expectorant, and/or throat lozenges administered each day through Day 14.

Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for influenza viral culture and quantitative PCR assay. In a subset of a minimum of 200 subjects an additional viral culture/ PCR sample will be collected on Day 2. Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Days 1, 3, 5, and 9 for all subjects, and on Day 2 in the subset of 200 subjects, to assess for potential changes in influenza viral susceptibility to neuraminidase inhibitors in response to treatment.

At selected sites a separate sub-study will be conducted to collect limited PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate sub-study protocol, BCX1812-311PK.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1.1 Inclusion Criteria

Subjects must meet all of the following criteria for inclusion in this study:

1. Male and female subjects age ≥18 years
2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate specimen collected from an

anterior nasal or pharyngeal swab, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated with a second commercially available RAT kit. A limited number of RAT negative subjects may be enrolled in accordance with a defined screening algorithm..

3. Presence of fever at time of screening of ≥38.0 C (≥100.4 ºF) taken orally, or ≥38.5ºC (≥101.2 F) taken rectally. For subjects who test RAT positive at the time of screening, a subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify in the absence of documented fever at time of screening. For subjects who test RAT negative at screening, fever as described above must be documented at time of screening.
4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of any severity (mild, moderate, or severe)
5. Presence of at least one constitutional symptom (myalgia [muscle aches], headache, feverishness, or fatigue) of any severity (mild, moderate, or severe)
6. Onset of illness no more than 48 hours before presentation
7. Females of child-bearing potential must have a negative urine pregnancy test (Beta HCG) at screening/baseline AND report one of the following:
    - Be surgically sterile or practice monogamy with a partner who is surgically sterile
    - Have been sexually abstinent 4 weeks prior to date of screening evaluation and be willing to remain abstinent through 4 weeks after study drug administration
    - Use oral contraceptives or other form of hormonal birth control including hormonal vaginal rings or transdermal patches and have been using these for 3 months prior through 4 weeks after study drug administration
    - Use an intra-uterine device (IUD), or double barrier contraception (such as condom or diaphragm with spermicidal gel or foam) as birth control 4 weeks prior to date of screening through 4 weeks after study drug administration
8. Provide written informed consent

8.1.2 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

1. Women who are pregnant or breast-feeding
2. Presence of clinically significant signs of acute respiratory distress
3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma
4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class IV functional status within the past 12 months (section 15.1).

5. History of chronic renal impairment requiring hemodialysis or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min)
6. Clinical evidence of active bacterial infection at any body site requiring therapy with oral or systemic antibiotics
7. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy.
8. Current treatment of active viral hepatitis C
9. Presence of known HIV infection, with a CD4 count <350 cell/mm^3
10. Current therapy with oral warfarin or other systemic anticoagulant
11. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening
12. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days
13. History of alcohol abuse or drug addiction within 1 year prior to admission in the study
14. Participation in a previous study of peramivir as treatment for acute influenza or previous participation in this study
15. Participation in a study of any investigational drug within the last 30 days
16. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.

8.1.3 Removal of Subjects from Therapy or Assessment

All subjects are permitted to withdraw from participation in this study at any time and for any reason, specified or unspecified, and without prejudice. The Investigator or sponsor may terminate the subject's participation in the study at any time for reasons including the following:

1. Adverse event;
2. Intercurrent illness;
3. Non-compliance with study procedures;
4. Subject's decision;
5. Administrative reasons;
6. Lack of efficacy;
7. Investigator's opinion to protect the subject's best interest.

Any subject who withdraws because of an adverse event will be followed until the sign(s) or symptom(s) that constituted the adverse event has/have resolved or is determined to represent a stable medical condition.

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary, or if it is the desire of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject and determine the

subject's medical condition. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data.

9 TREATMENTS

9.1 Treatments Administered

Peramivir is an investigational drug. Peramivir for intramuscular injection is a small-volume parenteral and will be supplied as a 75 mg/mL solution in sodium citrate/ citric acid buffer. The pH is approximately 3.0.

A matched placebo solution of sodium citrate/ citric acid buffer with 1.2% sodium chloride at a pH of approximately 3.0 will be supplied.

9.2 Identity of Investigational Product(s)

Peramivir and placebo peramivir will be supplied in clear 2mL vials. An individual study drug kit will contain 2 vials of blinded study drug (peramivir and/or placebo, depending upon the treatment group), 2 syringes and 2 needles in which to draw up the solution for intramuscular injection. All materials will be packaged in a labeled box container. All study drug kits must be stored at 2-8 ^{o}C. Each individual study drug kit will be labeled with some or all of the following information as required by local regulations:

- Sponsor name and contact information, study protocol number, kit number, description of the contents of the container, instructions for the preparation of the syringe and administration of the study drug, conditions for storage, statement regarding the investigational (clinical trial) use of the study drug and date for retest or expiry date.

Each vial of study drug will be labeled with some or all of the following information as required by local regulations:

- Sponsor name, study protocol number, description of the contents of the vial, instructions for the preparation of the syringe, statement regarding the investigational (clinical trial) use of the study drug and lot number.

9.3 Method of Assigning Subjects to Treatment Groups

Subjects will be centrally randomized in a ratio of 2:2:1 to one of three treatment groups: single dose peramivir 150mg, single dose peramivir 300mg or placebo, in accordance with a computer-generated randomization schedule prepared by a non-study statistician. Subjects will be stratified according to the result of a RAT test and current smoking behavior. Once a subject is eligible for randomization, he/she will be assigned a study drug kit number that will be obtained by study staff from the study interactive voice response system (IVRS). Once a study drug kit number has been assigned to a subject, it cannot be reassigned to any other subject.

9.4 Study Medication Accountability

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the sponsor, issued to the subject or directly administered to the subject (including date and time), and any drug accidentally destroyed. The sponsor will supply a specific drug-accountability form. At the end of the study, information describing study drug supplies (e.g., lot numbers) and disposition of supplies for each subject must be provided, signed by the Investigator or designee, and collected by the study monitor. If any errors or irregularities in any shipment of study medication to the site are discovered at any time, the Project Manager must be contacted immediately.

At the end of the study, all medication not dispensed or administered and packaging materials will be collected with supervision of the monitor and returned to the sponsor or destroyed on site as dictated by the appropriate Standard Operating Procedure at the participating institution.

9.5 Blinding/Unblinding of Treatments

This is a double-blind study. The treatment group assignment will not be known by the study subjects, the investigator, the clinical staff, the CRO or Sponsor staff during the conduct of the study.

Section 11.2.4provides information regarding the process for unblinding the treatment assignment, if necessary, in the event of an SAE.

9.6 Prior and Concomitant Therapies

All medications, by any route of administration, used during this study must be documented on the Case Report Form (CRF). Prescription as well as non-prescription medications should be recorded. Medication used for the treatment of influenza-related symptoms will be captured by the subject in the diary card provided by BioCryst.

9.7 Overdose and Toxicity Management

To date there is no experience with overdose of intramuscular or intravenous peramivir. If overdose occurs, subjects should receive indicated supportive therapy and evaluation of hematologic and clinical chemistry laboratory tests should be conducted. The effect of hemodialysis on elimination of peramivir is unknown.

9.8 Dose Interruption

As this is a study of a single injection of peramivir or placebo, guidelines for treatment interruption for drug related SAEs or toxicities are not applicable.

10 STUDY CONDUCT

A study schedule of evaluations is presented in Figure 1. A detailed list of the evaluations is also provided in the following sections.

10.1 Evaluations

All subjects enrolled in this study will undergo the following evaluations:

10.1.1 Medical History

Medical history, influenza vaccination status within the previous 12 months and demographic data (including smoking behavior) will be recorded at Screening/Baseline.

10.1.2 Rapid Antigen Test for Influenza

At Screening/Baseline, a commercially available, rapid antigen test (RAT) for influenza A and B will be performed on an adequate specimen collected by swabbing the anterior nose or pharynx, in accordance with the RAT manufacturer' instructions. A negative initial RAT should be repeated with a different commercially available RAT kit. Refer to the Study Manual for instructions regarding the use of the RAT kits provided for this study. Sites may use the kits provided by the Sponsor or any other commercially approved RAT available at their site to document a confirmed influenza infection.

A limited number of RAT negative subjects will be enrolled in accordance with the following algorithm: Once an individual site has identified 3 RAT positive subjects at screening within a 10 day period, indicating that influenza activity is present in the site's vicinity, a RAT negative subject that meets the inclusion/exclusion criteria may be enrolled. One additional RAT negative subject may be enrolled thereafter for each preceding RAT positive subject that is identified. The RAT result for each subject screened will be recorded on the study Interactive Voice response (IVR) system to manage this enrollment algorithm.

10.1.3 Physical Examination and Influenza-related Complications Assessments

The Investigator will perform a physical examination at Screening/Baseline. Subject's weight will be recorded at Screening/Baseline.

Study personnel will be provided with an influenza-related complications (IRC) checklist in the CRF to evaluate the subject for the presence of clinical signs and /or symptoms of the following influenza-related complications: sinusitis, otitis, bronchitis and pneumonia. At each follow up assessment a targeted physical examination will be conducted to record the presence of influenza related complications. If the investigator determines that the subject experiences (or is presumed to experience) an IRC as noted above, he/she will record that assessment on the IRC CRF page and any medication used to treat the condition will be recorded on the concomitant medication page. Such information describing IRC signs and/or symptoms should not be reported as adverse events. Any injection site reactions noted will be recorded in the CRFs as adverse events.

10.1.4 Vital Signs

Vital signs evaluations will include blood pressure, pulse rate, and respiration rate. The investigator will record oral body temperature at baseline. Thereafter the subject will record oral temperature twice daily in the study diary card.

Vital signs will be measured at Screening/Baseline, pre-dose, and at 15 minutes following the study drug injection on Day 1, then once daily on Days 2 (for those subjects who are

seen on day 2), 3, 5, 9, and 14.

10.1.5 Electrocardiogram Measurements

A 12-lead elec trocardiogram (ECG) will b e obtained at Screen ing/ Baselin e. I f this baseline ECG is interpreted by the conductin g physician as m eeting the exclusionary criteria listed in secti on 8.1.2 the subject will no t be enrolled in this study. If the EC G is interpreted as being abnorm al and does not m eet the exclusionary criteria (e.g. acute ischemia, medica lly sig nificant dys rhythmia) th en this subject m ay be enrolled, taking into con sideration th e subjec t's m edical history. Appropriate follow-up evaluations, including but not lim ited to repeat E CGs, should be com pleted at s ubsequent study visits as directed by the investigator.

10.1.6 Clinical Chemistry Profiles

Clinical ch emistry pro files will include a C hemistry 20 panel (includes sod ium, potassium, chloride, total CO_2 [bicarbonate], creatinine, gluc ose, urea nitrogen, albu m in, total calcium , total m agnesium, phosphor us, alkaline phosphatase, alanine aminotransferase (ALT), aspartate am inotransferase (AST), total bilirubin, d irect bilirubin, lactate dehydrogenase [LDH], total protein, total creatine kinase, and uric acid)

Blood samples for clinical chem istry profiles will be collected at Screen ing/Baseline, and on Days 3, 5 and 14.

10.1.7 Hematology Profiles

Hematology will include complete blood count (CBC) with differential.

Blood sa mples for he matology prof iles will be collected at Screening/Baseline, and on Days 3, Days 5 and 14.

10.1.8 Serology for Influenza

Paired blood samples for determ ination of antibody to influenza A and B (serology) will be obtained with the clinical laboratory tests at Screenin g/Enrollment and at Day 14. These specimens will be stored at the central laboratory and will be analyzed if needed to confirm the diagnosis of influenza.

10.1.9 Urinalysis and Evaluation of Protein in Urine

Urinalysis will in clude dipstick tes ts for protein, glucose, ketones, b lood, urobilin ogen, nitrite, pH, and spec ific gr avity a nd m icroscopic evaluation for R BCs and WBCs. Samples for urinalysis will be collected at Screening/Baseline, and on Days 3, 5, and 14.

10.1.10 Urine Pregnancy Test

Females of childbearing potential will be ev aluated for pregnancy at Screening/Bas eline and Day 14 using a urine pregnancy test.

10.1.11 Samples for Virologic Laboratory Assessments

An adequate spec imen will b e co llected by sw abbing the anter ior nos e (bila teral) and posterior pharynx for virologic laboratory a ssessments including culture for the isolation of influenza virus and/or quantitative PCR assa y at Screenin g/Baseline, and at Days 3, 5, and 9. In a subset of a m inimum of 200 subjects an additio nal sam ple will be taken at

Day 2. Refer to the Laboratory Manual for instructions regarding the processing and shipment of these specimens.

10.1.12 Subject Self Assessments

Subject self assessments will be performed beginning pre-dose on Day 1 and recorded in the subject's Study Diary including the following:

- Oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol, provided) aspirin, ibuprofen or other NSAID. The times of each temperature determination will be recorded in the Study Diary. The baseline temperature will be recorded at the screening/Day 1 visit prior to dosing, regardless of whether the subject had recently taken an anti-pyretic; the time of anti-pyretic use pre-treatment will be recorded in the CRF, if applicable.
- Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [muscle aches], headache, feverishness, and fatigue) on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily, beginning pre-dose on Day 1 and through Day 9, then once daily through Day 14.
- Assessment of the subject's ability to perform usual activities using a 0–10 visual analogue scale once daily through Day 14.
- Assessment of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 visual analogue scale once daily through Day 14.

The subject's diary card will be reviewed by study staff at each visit for completion of the record of all required items, with particular emphasis on alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms. Study staff will not attempt to ask subjects to retrospectively complete missing diary card data for any scheduled assessments that have not been completed prior to the clinic visit. Study staff should, however, remind the subject to complete the diary card at all scheduled times.

10.1.13 Concomitant Medications

All concomitant medications used during this study, with the exception of those medications taken for symptomatic relief of influenza symptoms, which will be recorded by the subject in their diary card, must be documented on the Case Report Form (CRF).

10.1.14 Adverse Events

AEs will be assessed from the time of administration of study medication through the final study visit.

10.1.15 Single Pharmacokinetic Exposure Sample

On study day 3 a single PK sample will be drawn in concert with the day 3 safety clinical laboratory blood draw. This sample will be sent for subsequent analysis of the concentration of peramivir at this time point. Data from this single PK sample will be combined with data from the PK sub-study (BCX1812-311PK) to perform an exposure-response analysis. This analysis will be described as part of the sub-study analysis plan..

10.2 Screening

10.2.1 Informed Consent

The nature and purpose of the study and the expectations of a participating subject will be described to potential study subjects, their questions will be answered, and the subjects will then be asked to sign an informed consent document. Study subjects will then undergo the screening evaluation as noted in Section 10.2.2

10.2.2 Screening/Baseline Evaluation and Enrollment

Screening/baseline evaluation may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subjects may be enrolled and receive treatment with study drug prior to receiving results of the laboratory assessments (with the exception of urine pregnancy test result, which must be known).

Eligible subjects will be enrolled and randomized to blinded study treatment. The randomization will include stratification by RAT status and current smoking behavior. The Investigator will prepare a request for blinded study drug assignment which includes the subject's screening number. The Investigator or designee at the clinical study center will contact the central randomization Interactive Voice System (IVRS call center). The IVRS call center will advise the study center of the investigational study drug kit number that is assigned to that subject at enrollment.

Subjects that are determined to be ineligible will be advised accordingly, and the reason for ineligibility will be discussed. If desired by the subject the reason for ineligibility may be provided/discussed with their health-care provider by the Investigator or designee.

Ineligible subjects who have been screened for the study will also be entered on the IVRS. For such subjects, the screening number assigned, subject's date of birth and a reason for ineligibility will be entered on to the IVRS. **All <u>ineligible</u> subjects must be entered onto the IVRS within 24 hours of screening,** to assist with surveillance analysis during the course of the study.

10.3 Treatment Period—Study Day 1

Day 1 represents the only day of study drug dosing. Study drug administration should

occur as soon as possible following inform ed consent, screening and random ization. Therefore, it is expec ted that the da te of Screening/ Baseline and Day 1 will usua lly be the same date.

10.3.1 Pre-dose Evaluations

Following an explanation of the Subject Self Assessment measures (Section 10.1.11), the subject shall complete the record of these assessments in their Study Diary prior to dosing. The subject will be counseled regarding the expectations for recording these assessments through Day 14.

Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) and a 12 lead ECG will be obtained prior to dosing. At Hour 0, the blinded study drug will be administered intramuscularly (one injection in the left gluteal muscle, and one injection in the right gluteal muscle within a period of ≤ 10 minutes.). The calendar date and 24-hour clock time of the first and second injections will be recorded.

The following evaluations will be performed post-dose on Study Day 1:

- Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) at 15 minutes after the study drug administration
- Record any concomitant medications
- Record any AEs

10.4 Post-Treatment Assessment Period

10.4.1 Days 2, 3, 5, 9 and 14

Study evaluations will be perform ed on Days 2 [subset], 3, 5, 9 and 14 in accordance with the schedule of evaluations (Figure 1).

Visits may be conducted in the investigator's o ffice or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

The Day 2 assessm ent will be con ducted in pe rson only f or the subse t of subjects who will p rovide additional Day 2 viro logy samples. For all o ther study subjects, study staff will attem pt to contact the subjects on Day 2 by telephon e to confirm their com pliance with com pletion of the Subject Self Assessm ents, to note any concom itant m edications and adverse events. Any adverse events repo rted by the subject during this telephone contact will be recorded on the adverse event form and verified during the visit on day 3.

At each v isit it is im portant that the sub ject's Study Diar y record be rev iewed for completion of daily Subject Self Assessm ents. The subjects should be counseled as necessary regarding self assessments and Study Diary record requirements. The subject's diary card w ill be reviewed by study staff for alleviation of symptom s as well as relapse of sym ptoms. Relapse is defined as the r ecurrence of at least one resp iratory sym ptom and one con stitutional symptom (both greater th an mild in s everity) for 24 hours an d the presence of fever (unless influenced by antipyr etic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms.

Day 3:

The results of the CK assay at Day 3 will not be made available to investigators, to BioCryst or to any study personnel unless the CK result at Day 3 is ≥ 2000 IU/mL. A single PK sample will be drawn as part of the safety clinical laboratory study on Day 3.

Day 14:

If a subject does not have resolution of all influenza-related symptoms (defined as a patient-recorded symptom score of moderate [2] or severe [3]), the investigator will schedule a follow-up assessment with the patient at Day 21 and, if required, Day 28. These follow-up assessments may be conducted in person or by telephone at the investigator's discretion. The subjects will not be required to continue to record daily symptom scores, but will report these scores to the investigator on Day 21 and, if required, on Day 28. No protocol-mandated follow up will occur after Day 28. Further management of the subject will be in accordance with the investigator's standard practice.

Figure 1 Study Measurements and Visit Schedule

	Screening [1] (Baseline)	Treatment Period Day 1[1]	Assessment Day Day 2[2]	Day 3	Day 5 (±1 day)	Day 9 (±3 day)	End of Study Early Withdrawal Day 14 (±3 day) [8]
Informed Consent	X						
Rapid Antigen test for Influenza A & B	X						
Medical History/Physical Exam[3]	X						
Influenza-related complications checklist[3]	X		X	X	X	X	X
Inclusion/Exclusion	X						
Clinical Chemistries[4]	X			X	X		X
Hematology[4]	X			X	X		X
Exposure Pharmacokinetic Sample				X[4]			
Serology (serum) Sample	X						X
Urinalysis[4]	X			X	X		X
Urine Pregnancy Test	X						X
Vital Signs[5]	X	X	X	X	X	X	X
ECG[6]	X						
Sample (nasopharyngeal swab) for Influenza Virus Culture/ PCR assay and for resistance studies		X	X	X	X	X	
Study Drug Administration		X					
Subject Diary Review[7]		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

[1] It is expected that the date of Screening and Day 1 (date of administration of study drug) will be the same. Visits at Screening and on subsequent study days may occur in subject's home by the investigator (all visits) or appropriately trained study center staff (Day 2, 3, 5, 9 visits).

[2] Day 2 visit required only for a subset of subjects for whom additional Day 2 virology sample is required. For all other subjects, Day 2 will be a telephone contact with the subject to ensure compliance with diary card completion, concomitant medication and adverse event review.

[3] Medical history and physical exam at screening to include weight, and smoking behavior. Targeted physical examinations will be performed to complete the influenza-related complications checklist by the appropriate medical personnel at appropriate visits.

[4] Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subject may be enrolled and begin treatment with study drug prior to receiving results. On Day 3 an extra tube will be included with the safety blood sample for evaluation of peramivir concentrations.

[5] Vital sign measures will include blood pressure, pulse rate and respiration rate. Vital signs will be recorded at Screening, pre-dose and at 15 min following the study drug administration on Day 1, then once on remaining days as stipulated. The investigator will record oral temperature at baseline. Thereafter the subject will report oral temperature measurements twice daily in the Study Diary

[6] If the baseline ECG is interpreted by the conducting physician as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal and does not meet the exclusionary criteria (e.g. acute ischemia, medically significant dysrhythmia) then this subject may be enrolled, taking into consideration the subject's medical history. Appropriate follow-up evaluations, including but not limited to repeat ECGs, should be completed at subsequent study visits as directed by the investigator.

[7] Subjects record symptom assessment in Study Diary, twice daily, beginning pre-dose on Day 1 through Day 9, then once daily through Day 14; subjects record ability to perform usual activities once daily, beginning pre-dose on Day 1 through Day 14. Subjects record oral temperature twice daily throughout as well as all concomitant medication and adverse events

[8] For any subject with unresolved influenza symptoms(moderate or severe) or unresolved adverse events, a follow up assessment (in person or by phone) will be scheduled at Day 21 and Day 28 if required

11 ADVERSE EVENT MANAGEMENT

11.1 Definitions

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

AEs may be designated as "nonserious" or "serious" (see Section 11.1.2).

Surgical procedures are not AEs but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [muscle aches], headache, feverishness, and fatigue) will be documented in a subject's study diary and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as AEs unless the symptom(s) worsen to the extent that the outcome fulfils the definition of an SAE, which then must be recorded as such (see Section 11.1.2). Likewise, a RAT for influenza is required at screening in order to determine eligibility for the study, and therefore a positive RAT is not considered an AE.

11.1.2 Serious Adverse Event

A SAE is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

11.2 Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of AEs are to be collected from the time of study drug administration through the follow-up period ending on Day 28. The Investigator or designee must completely and promptly record each AE on the appropriate CRF. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

The Investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or for 30 days after the subject's last study visit, whichever comes first.

11.2.1 Definition of Severity

All AEs will be assessed (graded) for severity and classified into one of four clearly defined categories as follows:

- **Mild:** (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
- **Moderate:** (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- **Severe:** (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- **Life-threatening:** (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization.

11.2.2 Definition of Relationship to Study Drug

The blinded Principal Investigator must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the

subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication, is confirmed by improvement upon stopping the medication (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

11.2.3 Reporting Serious Adverse Events

Any SAE must be reported to BioCryst or its designee within 24 hours of the Investigator's recognition of the SAE by first notifying the Medical Monitor at the number listed below:

Telephone: **Europe: +44 1628 548000; North America: 1-888-724-4908**

Facsimile: **Europe: +44 1628 540028; North America: 1-888-887-8097 or 1-609-734-9208**

The site is required to fax a completed SAE Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the SAE must be reported and sent by facsimile to BioCryst or its designee as soon as they are available.

The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (e.g., revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and for retaining a copy in their files.

If the Investigator becomes aware of any SAE occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify BioCryst or its designee.

Any SAEs considered possibly related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch reporting system in

accordance with FDA and other applicable regulations. However, the Investigator is not obligated to actively seek reports of AEs in former study participants.

While pregnancy is not considered an AE, all cases of fetal drug exposure via parent as study participant (see Section 4.4) are to be reported immediately to BioCryst or its designee. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee.

11.2.4 Emergency Procedures

In the event of an SAE, the Principal Investigator may request the unblinding of the treatment assignment for the subject affected. If time allows (i.e., if appropriate treatment for the SAE is not impeded), the Principal Investigator will first consult with the Medical Monitor regarding the need to unblind the treatment assignment for the subject. At all times, the clinical well-being of any subject outweighs the need to consult with the Medical Monitor.

The Principal Investigator may contact the IVRS central randomization center and request the unblinding of the treatment assignment that corresponds to the affected subject. The IVRS center will record the name of the Investigator making the request, the date and time of the request, the reason for the request, the subject number and study drug kit number, and whether the Medical Monitor was consulted prior to the request being made. The Sponsor will be informed within 24 hours if unblinding occurred.

12 STATISTICAL METHODS

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and other selected efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. The term "treatment group" refers to randomized treatment assignment: peramivir 150 mg, peramivir 300 mg, or placebo. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date within each subject number.

Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted with the SAS® System, version 9.1.3 or higher.

12.1 Data Collection Methods

The data will be recorded on the CRF approved by BioCryst. The Investigator must submit a completed CRF for each subject who signs an informed consent form (ICF), regardless of duration. All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF.

Documents (including laboratory reports, hospital records subsequent to SAEs, etc.) transmitted to BioCryst should not carry the subject's name. This will help to ensure

subject confidentiality.

The Investigator must consult the "CRF Completion Manual" for comprehensive instruction for completion of the CRF.

12.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be created prior to the review of any data. This document will provide a more technical and detailed description of the proposed data analysis methods and procedures.

12.3 Sample Size Estimates

From published results, it is expected that the median time to alleviation of symptoms will be between 103.3-116 hours for subjects receiving placebo.[16,17] For sample size calculations the best placebo response (103.3 hours) will provide the most conservative estimate of an observed hazard ratio. Additionally, it is expected that the median time to alleviation for the lowest dose peramivir arm will be 69.9 hours, yielding a hazard ratio of 0.68. Using these assumptions, a sample size of 200 infected subjects per active treatment group and 100 infected subjects in the placebo group is sufficient to provide at least 80% power to detect a hazard ratio of 0.68 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months). Up to 800 subjects will be enrolled to achieve the target number of at least 500 subjects with diagnostic evidence (RAT or PCR) of an acute influenza infection (200 per active treatment; 100 receiving placebo) as described in section 12.4.2

12.4 Analysis Populations

The populations for analysis will include the intent-to-treat (ITT), intent-to-treat infected (ITTI), and safety populations.

12.4.1 Intent-To-Treat Population

The ITT population will include all subjects who are randomized. Subjects will be analyzed in the treatment group to which they were randomized. The ITT population will be used for analyses of accountability and demographics.

12.4.2 Intent-To-Treat Infected Population

The ITTI population will include all subjects who are randomized, received study drug, and have proven influenza by any one of the following: culture, PCR, or paired serology showing $\geq$ 4-fold increase in antibody to influenza A or B, and received study drug. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI population will be used for primary analyses of efficacy.

12.4.3 Safety Population

The safety population will include all subjects who received study drug. Subjects will be analyzed according to the treatment received. This population will be used for all safety analyses.

12.5 Interim and End of Study Analyses

Interim Analysis

An independent DMC will review safety data on an ongoing basis. Safety analyses will be presented in a manner consistent with the presentations intended for the final analysis.

End of Study Analysis

A final analysis is planned after the last subject completes or discontinues the study, and the resulting clinical database has been cleaned, quality checked, and locked.

12.6 Efficacy Analyses

12.6.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when a subject has Alleviation of Symptoms. A subject has Alleviation of Symptoms if all of the seven symptoms of influenza (nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, feeling feverish) assessed on his/her subject diary are either absent or are present at no more than mild severity level and at this status for at least 21.5 hours (24 hours - 10%).

Descriptive statistics for the primary efficacy endpoint will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized overall and for individual symptoms for each treatment group. Overall treatment differences will be assessed using a Cox Regression model with effects for RAT result at screening, current smoking behavior, treatment group, and influenza season at randomization (if necessary). Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing post-baseline assessment. Pairwise differences in time to alleviation of symptoms among the treatment groups will be evaluated using contrast statements from the final Cox model. In order to maintain the overall type I error in the presence of the planned comparisons between the two peramivir treatments and placebo, a Bonferroni correction will be applied to the primary efficacy endpoint analysis. P-values for the planned comparisons of each peramivir arm to placebo will be adjusted via a Bonferroni correction (i.e., if the unadjusted p-value for an active comparison versus placebo, p, is less than 0.05, then p^a=p*number of planned comparisons=p*2; otherwise, p^a=p). Superiority of peramivir to placebo will be established if the adjusted p-value is less than or equal to 0.05.

12.6.2 Secondary Efficacy Endpoints

All secondary endpoints will be summarized using descriptive statistics by treatment group and study day/time, if appropriate. Statistical comparisons for each endpoint will be constructed without adjustment for multiple endpoints.

The reduction in viral shedding will be assessed as the change in viral titers defined as the time-weighted change from baseline in $\log_{10}$ tissue culture infective dose$_{50}$ ($TCID_{50}$/mL) and will be summarized for each treatment group. The time-weighted average change from baseline will be calculated on a by-subject basis through Day 9 using the trapezoidal rule with all available post-baseline on-treatment data (data after initiation of study treatment) minus the baseline value. Specifically, the time-weighted area under the curve for time a (t_a) to time b (t_b) is given by the formula

$$TWAUC = \frac{AUC(t_a - t_b)}{(t_a - t_b)},$$

where $AUC(t_a - t_b) = \sum_{i=a}^{b-1} \frac{(y_i + y_{i+1})(t_{i-1} - t_i)}{2}$ and t_i represents the date of the i^{th} viral titer assessment and y_i represents the $\log_{10}$ value of the i^{th} viral titer assessment. If there is a baseline value and only one follow-up value, y_i then the time-weighted change from baseline is defined as the difference between y_i and baseline. If there is a baseline value and no follow-up value, the subject is excluded from analysis. The differences between each of the peramivir treatment groups and placebo will be evaluated using a van Elteren Test adjusting for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Analyses of the PCR results will be analyzed in a similar manner.

Subject's ability to perform usual activities as determined from the visual analog scale will be summarized by study visit day and treatment group. Differences between the treatment groups will be assessed using the van Elteren Test adjusting for smoking behavior and influenza season at randomization (if necessary). The time (days) to resumption of a subject's ability to perform usual activities (i.e., subject scores ability to perform usual activities as 10) will be estimated using the method of Kaplan-Meier. Differences between each of the peramivir treatment groups and placebo will be assessed using the log rank statistic adjusting for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Subjects who do not return to the pre-study level of performance of usual activities will be censored at the time of their last non-missing post-baseline visual analog scale value.

Subject's oral temperature will be summarized by study visit and treatment group. Differences between the treatment groups will be assessed using the Wilcoxon Rank Sum Test controlling for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). A subject has Resolution of Fever if he/she has a temperature < 37.2°C (99.0°F) and no antipyretic medications have been taken for at least 12 hours. The time to resolution of fever will be estimated using the method of Kaplan-Meier using temperature and symptom relief medication information obtained from the subject diary data. Difference between the treatment groups will be assessed using the

log rank statistic controlling for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Subjects who do not have resolution of fever will be censored at the time of their last non-missing post-baseline temperature assessment.

The MRU, MRU-related direct costs, and indirect costs attributable to days missed of work and work productivity and/or performance losses will be summarized by treatment group. Differences between each of the peramivir treatment groups and placebo group will be evaluated using both parametric and/or non-parametric tests, as appropriate. If necessary, bootstrapping techniques will be used to calculate confidence intervals around the incremental differences in costs.

12.6.3 Exploratory Endpoint

Genotypic (including Hemagglutinin and Neuraminidase), phenotypic, viral culture and PCR data will be listed for each subject. These listings will be constructed in a manner consistent with the FDA June 2006 Guidance Document: *"Guidance for Submitting Influenza Resistance Data"*.[18] Additionally, the number and percentage of genotypic changes from wild-type amino acid will be summarized separately for treatment group, protein type, and study visit.

12.7 Safety Analyses

AEs will be mapped to a MedDRA-preferred term and system organ classification. The occurrence of TEAEs will be summarized by treatment group using MedDRA-preferred terms, system organ classifications, and severity. If a subject experiences multiple events that map to a single preferred term, the greatest severity and strongest Investigator assessment of relation to study drug will be assigned to the preferred term for the appropriate summaries. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to study drug will be generated.

Descriptive summaries of vital signs and clinical laboratory results will be presented by study visit. Laboratory abnormalities will be graded according to the DAIDS Table for Grading Adverse Events for Adults and Pediatrics (Publish Date: December 2004). The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized by treatment group. Laboratory toxicity shifts from baseline to Day 3, Day 5, and Day 14 will be summarized by treatment group.

Abnormal physical examination findings will be presented by treatment group. The number and percent of subjects experiencing each abnormal physical examination finding will be included.

Concomitant medications will be coded using the WHO dictionary. These data will be summarized by treatment group.

Subject disposition will be presented for all subjects. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

12.8 Sub-Study and Pharmacokinetic Analysis

The sub-study to exam ine exposure respons e, along with the corresponding single PK samples collected on all subjects on study day 3 will be co mpleted as part of the sub-study. All s tatistical m ethods will be outlin ed as part of the sub-stu dy protocol and exposure-response analysis plan. All sub-study analyses will be reported in a separate sub-study report.

12.9 General Issues for Statistical Analysis

12.9.1 Multiple Comparisons and Multiplicity

In order to m aintain the overall typ e I e rror in the presen ce of the plann ed com parisons between the two peram ivir treatm ents and pl acebo, a Bonferroni correction will be applied to the prim ary efficacy end point an alysis. No oth er ad justments for m ultiple comparisons are planned.

12.9.2 Covariates

Primary and secondary efficacy analyses will be adju sted for RAT result at screen ing, current smoking behavior and influenza season at randomization (if necessary).

12.9.3 Planned Sub-Groups

The prim ary efficacy endpoint will be summ arized separately by stratification group (current sm oking behavior [sm oker or non-smoker] and RAT result at screening [negative or positive]) and by viral subtype using descriptive statistics by treatment group and study day, if appropriate. No formal statistical testing will be utilized.

Additional analyses m ay be perform ed by count ry, if necessary, for subm ission to local regulatory authorities.

12.9.4 Missing Data

Every effort will be m ade to obtain required data at each s cheduled ev aluation from all subjects who have been random ized. No attemp t will be m ade retrospectively to obtain missing subject reported data (including in fluenza symptom severity assessm ents, temperature, ability to perf orm usual activi ties, m issed days of work and im pact of influenza on subject's work perform ance and/or productivity) that has not been completed by the subject at the time of return of the subject diary to the investigative site. In situa tions where it is not possible to obt ain all da ta, it m ay be necessary to im pute missing data.

In assessing the p rimary efficacy endpoint, fo r subjects w ho withdraw or who d o no t experience allev iation of sym ptoms, m issing data will be censored using the date of subject's last non-m issing assessm ent of infl uenza sym ptoms. Missing assessm ents of influenza sy mptoms conservatively will be im puted as h aving severity above absen t or mild (as f ailures). For the subjec t diary data, the f ollowing data con ventions will be utilized. Missing diary com pletion will be im puted as 11:59 for diary entries desig nated

as morning and 23:59 for evening and daily reported values. Entries with values exceeding the 24-hour clock will be set to 23:59 of the day recorded. Select exploratory sensitivity analyses may be conducted to ascertain the effect, if any, of these methods. These sensitivity analyses are further described in the SAP. Secondary efficacy endpoints with time to event data will be censored using the date of subject's last non-missing assessment of the given endpoint.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1.1 Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; GCPs, including International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator should submit written reports of clinical study status to their Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) annually or more frequently if requested by the IRB/ IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the IRB/ IEC should be maintained in the study documents file. Copies of clinical study status reports (including termination) should be provided to BioCryst.

13.1.2 Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent form, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the investigational medicinal product is released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The Investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the Investigator will provide the IRB/IEC with a report of the outcome of the study.

13.1.3 Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any

study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by BioCryst and by the reviewing IRB/ IEC. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and BioCryst policy.

The Investigator must explain to potential subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry in to the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the ICF. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1.4 Payment to Subjects

Reasonable compensation to study subjects may be provided if approved by the IRB/IEC responsible for the study at the Investigator's site.

13.1.5 Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his /her IRB/IEC as required.

13.2 Study Monitoring

During trial conduct, BioCryst or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow BioCryst monitors or its designees and appropriate regulatory authorities direct access to source documents to perform this verification.

13.3 Quality Assurance

The trial site may be subject to review by the IRB/IEC, and/or to quality assurance audits performed by BioCryst, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to

the process.

13.4 Study Termination and Site Closure

BioCryst reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by BioCryst, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5 Records Retention

To enable evaluations and/or audits from regulatory authorities or BioCryst, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to BioCryst. The Investigator must obtain BioCryst's written permission before disposing of any records.

13.6 Study Organization

13.6.1 Data Monitoring Committee

BioCryst will assemble an independent Data Monitoring Committee (DMC) to assess safety parameters of the trial on a periodic, ongoing basis while the trial is in progress. The committee will include a statistician and three physicians, two of whom will be Infectious Disease specialists. Full details of the composition of the DMC and how the DMC is to operate will be described in a separate DMC charter.

13.7 Confidentiality of Information

BioCryst affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by BioCryst. However, in compliance with federal regulations, BioCryst requires the investigator to permit BioCryst's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study.

BioCryst will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health

information of subj ects participating in BioCryst-sponsored Clinical Trials. "Authorization" is required from each research subject, i.e., specified permission granted by an individual to a covered entity for the us e or disclosure of an indi vidual's protected health information. A valid au thorization must meet the implem entation specifications under the HIPAA Pri vacy Rule. Authorizat ion m ay be com bined in the Inform ed Consent docum ent (approved by the IRB/IE C) or it may be a separate docum ent, (approved by the IRB/IEC) or provided by the Investigator or Sponsor (without IRB/IEC approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appr opriate ind ividual. HIPAA authoriz ations are required for U.S. sites only.

13.8 Study Publication

All data generated from this study are the propert y of BioCryst and shall be held in strict confidence along with all inform ation furnis hed by BioCryst. Independent analysis and/or publication of these data by the Investig ator or any member of his/her staff are not permitted without prior written consent of BioCryst. W ritten perm ission to the Investigator will be contingen t on the review by BioCryst of the statistical ana lysis and manuscript and will provide fo r nondisclosure of BioCryst confidential or proprietary information. In all case s, the partie s agree to s ubmit all m anuscripts o r abstracts to all other par ties 30 days prior to su bmission. This will e nable all parties to pr otect proprietary information and to provide comme nts based on infor mation that m ay not yet be available to other parties.

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15 APPENDICES

15.1 NYHA Functional Classification Criteria: Heart Failure and Angina

NYHA Functional Classification of Heart Failure

Class I
No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea.
Class II
Symptoms with ordinary physical activity. Walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold weather, in wind, or when under emotional stress causes undue fatigue or dyspnea.
Class III
Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea.
Class IV
Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea.

NYHA Functional Classification of Angina

Class I
Angina only with unusually strenuous activity.
Class II
Angina with slightly more prolonged or slightly more vigorous activity than usual.
Class III
Angina with usual daily activity.
Class IV
Angina at rest.

CLINICAL STUDY PROTOCOL

Protocol No. BCX1812-311

IND No. 76,350

A PHASE 3 MULTICENTER, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRAMUSCULAR PERAMIVIR IN SUBJECTS WITH UNCOMPLICATED ACUTE INFLUENZA

THE **IMPROVE 1** STUDY

(**I**ntra**M**uscular **P**eramivir for the **R**elief **O**f symptoms and **V**irologic **E**fficacy)

Short title: Intramuscular Peramivir for the Treatment of Uncomplicated Influenza

Protocol Date: Version1.0: 04 September 2007

BioCryst Pharmaceuticals, Inc.
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1 TITLE PAGE

Protocol Number:	BCX1812-311
Study Title:	A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza
IND Number:	76, 350
Investigational Product:	Peramivir (BCX1812)
Indication Studied:	Uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive Birmingham, AL 35233
Development Phase:	3
Sponsor Medical Officer:	W. James Alexander, M.D., M.P.H. Senior Vice President, Clinical Development Chief Medical Officer Phone: +1 919 859 1302 Fax: +1 919 851 1416 Email Address: jalexander@biocryst.com
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Final Protocol Date:	Version 1.0: 04 September 2007
Amendment(s) Date(s):	None

1.1 Protocol Approval Signature Page

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

BioCryst Pharmaceuticals, Inc.
Reviewed and Approved by:

W James Alexander 4 SEP 2007

W. James Alexander, M.D., M.P.H. Date
Senior Vice President, Clinical Development
Chief Medical Officer

1.2 Clinical Study Protocol Agreement

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practices, and all applicable regulatory requirements.

Investigator's Signature Date

Name (Print)

2 SYNOPSIS

Protocol No.	BCX1812-311
Protocol Title:	A Phase 3, Multicenter, Random ized, Double-Blind, Placebo-Controlled Stu dy to Evaluate the Efficacy an d Safety of Intram uscular Peram ivir in Subjects with Uncomplicated Acute Influenza
Sponsor:	BioCryst Pharmaceuticals, Inc.
Investigators/Study Sites:	Multinational
Development Phase:	3
Objectives:	
Primary:	To evaluate the efficacy of peram ivir adm inistered intramuscularly com pared to p lacebo on th e tim e to alleviation of clinica l sym ptoms in adult sub jects with uncomplicated acute influenza.
Secondary:	1. To evalua te the saf ety and tolera bility of pe ramivir administered intramuscularly; 2. To evaluate seconda ry clinical outcom es in response to treatment; 3. To evaluate changes in in fluenza virus titer (viral shedding) in response to treatment; 4. To assess p harmacoeconomic m easures in resp onse to treatment 5. To assess c hanges in inf luenza vir al suscep tibility to neuraminidase inhibitors following treatment
Number of Subjects:	Total enrollm ent: up t o 800 subjects random ized (160 subjects in the placebo treatment group, 320 subjects in the peramivir 150m g treatm ent group and 320 subjects in the peramivir 300m g treatm ent group). The study will close after enrollment of at least 500 subjects who have either a positive Influenza A or B antigen te st (Rapid Antigen Te st – RAT) at screening, or who ha ve a negative RAT at screening but a positive influenza A or B PCR a ssay result from a baseline nasopharyngeal swab.
Study Design:	This is a multinational, random ized, double-blind study comparing the efficacy and safety of two single dos e regimens of peram ivir administered intramuscularly versus placebo in adults with uncom plicated acute influenza. Each subjec t's ass ignment to trea tment will be stra tified according to the Rapid Antigen Test (RAT) result at screening and current sm oking be havior, with 80% of subjects ce ntrally rand omized to one of the two activ e

	single dose regimens of peramivir (2:2:1 randomization) Treatment Group 1: Peramivir 150mg Treatment Group 2: Peramivir 300mg Treatment Group 3: Placebo Enrollment of subjects into the RAT negative stratum will be permitted at individual sites that have identified 3 RAT positive subjects at screening within a 10 day period, indicating activity of influenza in the site's vicinity. After identification of 3 RAT positive subjects within 10 days, a site may enroll 1 RAT negative subject that fulfills the inclusion/ exclusion criteria. One additional RAT negative subject may be enrolled thereafter for each preceding RAT positive subject that is identified. Study drug will be administered as one 2mL intramuscular injection in each gluteal muscle (total of 4mL injected in divided doses). Subjects eligible for screening will have an anterior nasal or pharyngeal swab collected for testing by RAT for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated with a different commercially available RAT kit. Subjects meeting the inclusion/ exclusion criteria may be enrolled into the study. All enrolled subjects will record the following in a Study Diary: • Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol), aspirin, ibuprofen or other NSAID. • Assessment of severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14 • Assessment of subject's ability to perform usual activities, (rated as 0–10 on a visual analog scale) once daily through Day 14 • Assessment of subject's time lost from work or usual activities and productivity compared to normal (rated as

	0-10 on a visual analog scale) once daily through Day 14 • Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14 Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. In a subset of a minimum of 200 subjects, an additional virology sample will be collected on day 2. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result). All virologic assessments will be performed by a central laboratory. At the study day 3 visit, when all subjects are evaluated for safety and a blood draw is completed for clinical laboratory investigations, a single pharmacokinetic (PK) sample will also be drawn. This single PK sample will be analyzed for plasma concentrations of peramivir (ng/mL) and evaluated in an exposure response analysis. At selected sites a separate sub-study will be conducted to collect limited PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.
Study Population:	Male and female subjects, 18 years of age and older, with symptoms consistent with a diagnosis of uncomplicated acute influenza infection may be screened for enrollment. Subject eligibility will be dependent on the presence of two or more symptoms consistent with acute influenza as well as the results obtained from a rapid antigen test (RAT) for influenza A and B at screening.
Inclusion Criteria:	1. Male and non-pregnant female subjects age ≥18 years 2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate specimen collected from an anterior nasal or pharyngeal swab, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated with a different commercially available RAT kit. A limited number of RAT negative

	subjects may be enrolled in accordance with a defined screening algorithm. 3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. For subjects with a positive RAT at the time of screening, a subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at time of screening. For subjects with no positive RAT at screening, fever as defined above must be documented at time of screening 4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of any severity (mild, moderate, or severe) 5. Presence of at least one constitutional symptom (myalgia [muscle aches], headache, feverishness, or fatigue) of any severity (mild, moderate, or severe) 6. Onset of symptoms no more than 48 hours before presentation for screening 7. Written informed consent
Exclusion Criteria:	1. Women who are pregnant or breast-feeding 2. Presence of clinically significant signs of acute respiratory distress 3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma 4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class IV functional status within the past 12 months 5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia. 6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min) 7. Current clinical evidence of active bacterial infection at any body site that requires therapy with oral or systemic antibiotics 8. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy 9. Current treatment for active viral hepatitis C 10. Presence of known HIV infection with a CD4 count <350 cell/mm^3 11. Current therapy with oral warfarin or other systemic

	anticoagulant 12. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening 13. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days 14. History of alcohol abuse or drug addiction within 1 year prior to admission in the study 15. Participation in a previous study of peramivir as treatment for acute influenza or previous participation in this study 16. Participation in a study of any investigational drug within the last 30 days
Study Endpoints:	
Primary Endpoint:	*Clinical:* Time to alleviation of clinical symptoms of influenza
Secondary Endpoint(s):	*Safety* Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests *Clinical:* Time to resumption of subject's ability to perform usual activities Time to resolution of fever Incidence of influenza related complications *Virologic:* Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$) and/or by quantitative polymerase chain reaction (PCR) assay *Pharmacoeconomic*: Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.
Exploratory Endpoint:	*Viral Susceptibility:* Change in influenza virus susceptibility to neuraminidase inhibitors
Investigational Product, Dose, and Mode of Administration:	Peramivir (BCX-1812), 75mg/mL or placebo (buffered diluent), 2mL per injection, administered intramuscularly in the gluteal muscle, bilaterally.
Duration of Treatment:	Following treatment on day 1, study duration for individual

	subjects is expected to be up to 14 days (including all visits).
Statistical Methods:	Descriptive statistical methods will be used to summarize the data from this study, with statistical testing utilized for the primary and secondary efficacy endpoints. Unless otherwise noted, all statistical testing will be two-sided, and will be performed using a significance (alpha) level of 0.05. For assessment of the primary efficacy endpoint, the overall significance level will be maintained by a Bonferroni adjustment for the planned comparisons between the two active treatment groups and placebo. Detailed statistical procedures will be provided in a full statistical analysis plan (SAP) completed prior to database lock and study unblinding.
Sample Size:	From previous studies of neuraminidase treatment of uncomplicated influenza it is expected that the median time to alleviation of symptoms will be 103.3 hours for subjects receiving placebo. Additionally, it is expected that the median time to alleviation for the low dose peramivir arm will be 69.9 hours, yielding a hazard ratio of 0.68. Using these assumptions, a sample size of 200 influenza-infected subjects per active treatment group and 100 infected subjects in the placebo group (a total of 500 influenza-infected subjects) is sufficient to provide at least 80% power to detect a hazard ratio of 0.68 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months). Up to 800 subjects will be enrolled to achieve the target number of at least 500 subjects with diagnostic evidence (RAT or PCR) of an acute influenza infection.
Efficacy:	The intent-to-treat infected population will include all subjects who are randomized, received study drug, and have proven influenza by any one of the following: primary viral culture, PCR, or paired serology showing $\geq$ 4-fold increase in antibody to influenza A or B. The primary efficacy variable is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when each of seven symptoms of influenza are either absent or are present at no more than mild severity level and remain at no worse than this severity status for a 24 hour period. Descriptive statistics for the primary efficacy variable will

	be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment difference will be assessed using a Cox Regression model with effects for RAT result at screening, current smoking behavior, treatment group, and influenza season at randomization. Pairwise comparisons between each active group and placebo will be constructed from the Cox Regression model. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing assessment. Time to resumption of usual activities and time to resolution of fever will be analyzed in a similar manner. Changes in viral titers will be compared using the van Elteren statistic controlling for RAT result at screening, current smoking behavior and influenza season at randomization. Analyses of other continuous endpoints will be analyzed in a similar manner.
Safety:	Safety analyses will be presented for all subjects in the safety population, defined as all randomized subjects who receive at least one dose of study drug. Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. The occurrence of treatment-emergent AEs will be summarized using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs that are related to study medication will be generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Descriptive summaries of vital signs and quantitative clinical laboratory changes will be presented by study visit. Frequency and percentages of subjects with abnormal laboratory test results will be summarized by toxicity grade. Concomitant medications will be mapped to a WHO preferred term and drug classification. The number and percent of subjects taking concomitant medications will be summarized using preferred terms and drug classifications. The number and percent of subjects experiencing each

	abnormal physical examination finding will be presented. The number and percent of subjects discontinuing study as well as the reasons for discontinuation will be summarized by treatment group.
Protocol Date	Version 1.0: 04 September 2007

3 TABLE OF CONTENTS

3.1 List of Figures

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0–72}$	area under the curve from time 0 to 72 hours
$AUC_{0–\infty}$	area under the curve extrapolated from time 0 to infinity
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
C_{max}	maximum plasma concentration
CK	creatine kinase
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRF	Case Report Form
CV	coefficient of variation
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRC	influenza related complications
ITT	intent-to-treat
ITTI	intent-to-treat infected
IUD	intrauterine device
IVRS	interactive voice response system
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRU	medical resource utilization
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction

RAT	Rapid Antigen Test
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
$t_{1/2}$	elimination half-life
$t_{1/2}\lambda z$	terminal half-life
$TCID_{50}$	time weighted change from baseline in log 10 tissue -culture infective $dose_{50}$
TEAEs	treatment-emergent adverse events
T_{max}	time to attain maximum plasma concentration
UPEP	urine protein electrophoresis
WBC	white blood cell
WHO	World Health Organization

5 INTRODUCTION

5.1 Background

Influenza virus is a member of the *orthomyxovirus* family and causes an acute viral disease of the respiratory tract. Typical influenza illness is characterized by abrupt onset of fever, headache, myalgia, sore throat, and nonproductive cough.[1] The illness is usually self-limiting, with relief of symptoms occurring within 5 to 7 days. Nevertheless, it is an important disease for several reasons, including ease of communicability, short incubation time, rapid rate of viral mutation, morbidity with resultant loss of productivity, risk of complicating conditions, and increased risk of death, particularly in the elderly. During 19 of the 23 influenza seasons between 1972/1973 and 1994/1995, estimated influenza-associated deaths in the United States ranged from approximately 25 to more than 150 per 100,000 persons above 65 years of age, accounting for more than 90% of the deaths attributed to pneumonia and influenza.[2]

Presently, only a few measures are available that can reduce the impact of influenza: active immunoprophylaxis with an inactivated or live attenuated vaccine and chemoprophylaxis or therapy with an influenza-specific antiviral drug. Neuraminidase inhibitors are the current mainstay of antiviral treatment for influenza. Marketed neuraminidase inhibitors include zanamivir (Relenza ®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche-Gilead), an oral prodrug of the active agent, oseltamivir carboxylate. Influenza neuraminidase is a surface glycoprotein that cleaves sialic acid residues from glycoproteins and glycolipids. The enzyme is responsible for the release of new viral particles from infected cells and may also assist in the spreading of virus through the mucus within the respiratory tract. The neuraminidase inhibitors represent an important advance in the treatment of influenza with respect to activity against influenza A and B viruses, with proven therapeutic value in reducing influenza lower respiratory complications,[3] and lower rates of antiviral drug resistance[4].

The use of currently available neuraminidase inhibitors has been limited by concerns including, the degree of effectiveness, the requirement for an inhaler device (zanamivir), and the emergence of resistant influenza virus variants in some treated populations.[5] In addition, there are risks of bronchospasm with zanamivir; and gastrointestinal side effects, with oseltamivir.

Peramivir is a neuraminidase inhibitor that represents a potentially promising addition to the armamentarium of drugs for the treatment of influenza infections due to its potential for parenteral administration and lower frequency of dosing.

5.2 Rationale for Study

An oral formulation of peramivir has previously been evaluated in a full range of safety, tolerability, pharmacokinetic, and efficacy studies. In a multinational phase 3 clinical trial conducted in 1999-2001, oral peramivir demonstrated antiviral activity against influenza A and B infections, and improvement in the relief of clinical symptoms. Because of the limited bioavailability of peramivir following oral administration (<5%), it was determined that the parenteral route of administration is more appropriate for the

delivery of peramivir. Subsequent phase 1 studies of intravenous and intramuscular formulations of peramivir have confirmed that parenteral routes of administration result in plasma levels of drug that are as much as 100 times those achieved via the oral route. Further details of these studies are provided below and in the Investigator Brochure.

A phase 2 randomized, double blind, placebo controlled study of two single dose regimens (150mg or 300mg) of intramuscular peramivir in subjects with uncomplicated acute influenza infections (study BCX1812-211) was initiated in early 2007 in North America and the UK. The study has been extended to sites in Hong Kong, Australia, New Zealand and South Africa and is expected to complete enrollment in mid-2007. At a scheduled meeting on 21 May 2007, an independent data monitoring committee for this study completed a blinded review of all reported adverse events and grade 3 and 4 clinical laboratory evaluations from the first 135 subjects randomized. Following this blinded safety review the DMC provided a written recommendation that the study continue as planned.

Because of the previous demonstration of significant antiviral activity, the strong suggestion of clinical efficacy of oral peramivir previously demonstrated in acute influenza, and the encouraging pharmacokinetic and preliminary safety profile of the intramuscular formulation of peramivir demonstrated to date, this phase 3 study will be conducted to evaluate the efficacy and safety profile of intramuscular peramivir and to determine the optimal single dose regimen.

5.3 Non-Clinical Experience with Peramivir

5.3.1 In vitro Assays

Peramivir is a selective inhibitor of viral neuraminidase, with 50% inhibitory concentrations (IC_{50}) for bacterial and mammalian enzymes of >300µM.[6] In an *in vitro* study, 42 influenza A and 23 influenza B isolates were collected from untreated subjects during the 1999–2000 influenza season in Canada.[7] These isolates were tested for their susceptibility to the neuraminidase inhibitors zanamivir, oseltamivir carboxylate, and peramivir using a chemiluminescent neuraminidase assay. Inhibition of Type A influenza neuraminidase by peramivir was approximately an order of magnitude greater than inhibition of neuraminidase from Type B viruses. IC_{50} values for the Type A enzymes ranged from <0.1 to 1.4nM, whereas the Type B enzymes ranged from <0.1 to 11nM, with three out of four values in the 5- to 11nM range. Peramivir was the most potent drug against influenza A (H3N2) viruses with a mean IC_{50} of 0.60nM as well as most potent against influenza B with a mean IC_{50} of 0.87nM.

In another *in vitro* comparison of peramivir, oseltamivir, and zanamivir, using a neuraminidase inhibition assay with influenza A viruses, the median IC_{50} of peramivir (approximately 0.34nM) was comparable to that of oseltamivir (0.45nM) and significantly lower than zanamivir (0.95nM). For influenza B virus clinical isolates, the IC_{50} of peramivir (1.36nM) was comparable to that of zanamivir (2.7nM) and lower than that of oseltamivir (8.5nM).[8]

The potency of peramivir was evaluated against five zanamivir-resistant and six oseltamivir-resistant influenza viruses.[9] Peramivir remained a potent inhibitor against all

oseltamivir-resistant viruses including the mutations H274Y, R292K, E119V, and D198N, with IC_{50} values $\leq$40nM. Peramivir also potently inhibited ($IC_{50} \leq 26$nM) the neuraminidase activity of zanamivir-resistant strains, which had the following mutations: R292K, E119G, E119A, and E119D. However, one zanamivir-resistant influenza B virus, B/Mem/96, with a mutation R152K isolated from cell culture, was relatively resistant to all neuraminidase inhibitors, including peramivir (IC_{50} = 400nM).

5.3.2 Animal Models

In a mouse model of influenza infection, a single intramuscular injection of peramivir (10mg/kg) given 4 hours prior to inoculation with an A/NWS/33 (H1N1) influenza strain resulted in 100% survival in contrast to 100% mortality in a control group injected with saline.[6] In the same mouse model, treatment of mice up to 72 hours after influenza infection using peramivir (20mg/kg) resulted in 100% survival, compared to 100% mortality in the control group injected with vehicle.[10]

Peramivir has also demonstrated activity in animal models utilizing a clinical H5N1 isolate as the infecting virus strain. In a mouse model, a single intramuscular dose of peramivir (30mg/kg) injected 1 hour after inoculation with the highly pathogenic (H5N1) A/Vietnam/1203/04 strain, resulted in a 70% survival rate that was similar to that seen in mice treated with oseltamivir given orally at 10mg/kg/day for 5 days [11]. In similar experiments, mice inoculated with the same strain of H5N1 virus that were then treated for up to 8 days with intramuscular peramivir exhibited 100% survival [12]. This longer duration of peramivir treatment also prevented viral replication in the lungs, brain and spleen at days 3, 6 and 9 post inoculation.

5.4 Previous Phase 3 Clinical Experience with Oral Peramivir

An oral formulation of peramivir has previously demonstrated antiviral activity and preliminary clinical efficacy in challenge studies in human volunteers, as well as in treatment studies in patients with uncomplicated acute influenza infections during the influenza seasons of 1999-2001. A Phase 3 multinational study (BC-01-03) of oral peramivir was conducted. Two dose regimens of oral peramivir, 800mg QD for 5 days, or 800mg QD on Day 1, followed by 400mg QD for 4 days, were compared to a matched placebo treatment group. A total of 1246 subjects were randomized to treatment at sites in the USA, Western and Eastern Europe, South America, Australia and New Zealand. As presented in the table below, the primary end-point of time to relief of influenza symptoms was not found to be significantly different (p=0.17) between the three treatment groups.[13] A sub-group analysis of the time to relief of symptoms by country or region demonstrated marked differences in the primary endpoint.. In the subset of influenza-infected subjects enrolled at sites in the US, clinically meaningful differences in time to relief of influenza symptoms between the placebo and the two peramivir arms were observed, that just missed statistical significance (p=0.07). However, a number of secondary end-points in this phase 3 study, such as time to overall well-being, time to normal activity, incidence of influenza related complications and quantity of viral shedding, reached or approached statistically significant differenced between the peramivir and placebo treatment groups (p=0.03-0.06).

Results of Study BC-01-03

	Median Time to Relief of Influenza Symptoms (Hours)	
Dose and Regimen	**Overall Results (n=1246)**	**US Sites (n=206)**
Peramivir 800mg po x 5d	89.0	70.8
Peramivir 800mg po x 1d and 400mg po x 4d	91.7	88.8
Placebo x 5 days	104.4	106.8
p value	0.17	0.07

5.5 Previous Phase 1 Experience with Intramuscular Peramivir

Two phase one studies evaluating the safety and pharmacokinetics of an intramuscular formulation of peramivir have been conducted in a total of 45 healthy volunteers receiving peramivir.

Study Peramivir-Him-06-111 evaluated the single dose pharmacokinetics and tolerability of 75mg, 150mg and 300mg doses of peramivir administered as intramuscular (i.m.) and intravenous (i.v.) injections in a crossover design (9 subjects per group). Peak plasma levels of i.m. peramivir generally occurred within 30 minutes following injection. Plasma pharmacokinetic parameters for i.m. peramivir are summarized below for the three intramuscular single dose regimens evaluated:

Dose (mg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (hr·ng/mL)	$t_{½}$[a] (hr)
75 i.m.	4296±812	11659±1123	19.8±7.9
150 i.m.	7612±884	23952±3804	24.3±4.1
300 i.m.	15150±2367	49649±5619	22.8±2.5
[a]terminal half life			

In a second phase 1 study, Peramivir-Him-06-112, the same dose levels of peramivir were administered as single i.m. injections on two consecutive days (6 subjects per group). This double-blind study also included a placebo arm. The pharmacokinetic parameters of i.m. peramivir following the second day of dosing were consistent with those seen following single doses of the drug.

The observations of safety and tolerability of i.m. peramivir in each of the 2 phase 1 studies were unremarkable. No serious adverse events were reported. The most commonly observed adverse events or laboratory abnormalities were headache, several reports of signs and symptoms of vasovagal reactions following injections, and transient increases in creatine kinase. No consistent differences in frequency of adverse events were observed between the active and placebo treatment groups, with the exception that CK elevations appeared to be dose related in the peramivir treatment groups. The vasovagal reactions were attributed to the receipt of relatively large volumes of i.m. injection (2 injections each of 2mL) in the fasted state.

An ongoing phase 2 study, BCX1812-211, is a randomized, double-blind placebo-controlled study to evaluate the efficacy and safety of two single dose regimens of peramivir. Up to 100 subjects per arm will receive either 150mg or 300mg of peramivir or placebo. The primary endpoint of the study is the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza. An independent data monitoring committee reviewed grouped blinded safety data on the first 135 subjects randomized. The study remains blinded. The frequency of vasovagal reactions reported as adverse events in this ongoing study is 2/135 (to date) which is lower than that seen in the phase 1 volunteer studies.

5.6 Dose Rationale

Oseltamivir is approved for the treatment of uncomplicated acute influenza at a dosage of 75mg twice daily in adults[14]. Oseltamivir was shown to be clinically effective in a Phase III study of oral oseltamivir versus placebo in naturally occurring seasonal influenza, and these data were sufficient for regulatory approval for marketing of oseltamivir. At least 75% of an oral dose of oseltamivir reaches the systemic circulation as oseltamivir carboxylate. When oseltamivir is administered orally at a dose of 75mg twice daily, the serum C_{max} of oseltamivir carboxylate is approximately 348ng/mL and the AUC_{0-48} is 10,876 h·ng/mL. The clinical data indicate that this level of exposure to oseltamivir was sufficient to provide clinical improvement in uncomplicated acute influenza.

The serum pharmacokinetic data (C_{max} and $AUC_{0-\infty}$, respectively) following intramuscular doses of peramivir are approximately 7600ng/mL and 24,000 h·ng/mL for the 150mg dose and are approximately 15,000ng/mL and 49,000 h·ng/mL for the 300mg dose. Previous studies have assessed the concentrations of the neuraminidase inhibitor zanamivir in nasal and pharyngeal secretions after parenteral administration of this drug. . Within several hours after administration, the concentrations in secretions were approximately 100-fold lower than in serum or plasma. In theory, relatively high levels of a neuraminidase inhibitor in respiratory secretions are desirable in order to rapidly inactivate influenza virus and to delay or prevent the development of resistance in infecting virus strains. Intramuscular doses of peramivir, including doses of 150mg and 300mg have been shown to be well tolerated in previous Phase 1 studies and since no identified safety signal has been noted by an independent data monitoring committee in the ongoing Phase 2 study, it is appropriate for these two dose regimens to undergo further evaluation in this Phase 3 study.

6 STUDY OBJECTIVES

6.1 Objectives

6.1.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated influenza.

6.1.2 Secondary Objective(s)

The secondary objectives of this study are:

1. To evaluate the safety and tolerability of peramivir administered intramuscularly;
2. To evaluate secondary clinical outcomes in response to treatment;
3. To evaluate changes in influenza virus titer (viral shedding) in response to treatment;
4. To assess pharmacoeconomic measures in response to treatment;
5. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment;

6.2 Study Endpoints

6.2.1 Primary Endpoint

Time to alleviation of clinical symptoms of influenza

6.2.2 Secondary Endpoint(s)

Secondary efficacy endpoints will include evaluations in each subject of:

1. Safety parameters, including: treatment- emergent adverse events (TEAEs) and treatment-emergent changes in clinical laboratory tests;
2. Time to resolution of fever;
3. Time to resumption of subject's ability to perform usual activities;
4. Incidence of influenza related complications
5. Quantitative change in influenza virus shedding measured by viral titer assay ($TCID_{50}$) and/or by quantitative polymerase chain reaction (PCR) assay;
6. Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.

6.2.3 Exploratory Endpoint

An exploratory endpoint will evaluate change in influenza virus susceptibility to neuraminidase inhibitors in viral isolates recovered from subjects pre and post treatment.

7 STUDY DESIGN

7.1 Overall Study Design and Plan

This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. Up to 800 subjects will be enrolled into the study. Each subject's assignment to treatment will be stratified according to a Rapid Antigen Test (RAT) result and current smoking behavior, with 80% of subjects centrally randomized via an Interactive Voice Response (IVR) system to one of the two active single dose regimens of peramivir (2:2:1 randomization):

Treatment Group 1:	Peramivir 150mg	maximum n=320
Treatment Group 2:	Peramivir 300mg	maximum n=320
Treatment Group 3:	Placebo	maximum n=160

Previous studies evaluating the efficacy of neuraminidase inhibitors enrolled subjects with influenza-like illness on the basis of presence of specific clinical signs and symptoms. These studies were conducted before the widespread availability of rapid influenza antigen diagnostic test kits (Rapid Antigen Tests-RAT), and typically between 50-70% of subjects enrolled were subsequently confirmed by viral culture to have an active influenza infection. The sensitivity of commercially available RAT kits ranges from 62% to 83%, depending upon the virus sub-type. [15] In the phase 2 study of intramuscular peramivir (study BCX1812-211) a positive RAT test was required for study entry. It is likely that a number of subjects with an influenza infection were excluded from this study due the sensitivity of this assay.

In this phase 3 study a limited number of RAT negative subjects will be enrolled in accordance with the following algorithm to minimize false negative test results: once an individual site has identified 3 RAT positive subjects at screening within a 10 day period, indicating influenza activity is present in the site's vicinity, a RAT negative subject that meets the inclusion / exclusion criteria may be enrolled. One additional RAT negative subject may be enrolled there after for each preceding RAT positive subject that is identified. The RAT result for each subject screened will be recorded on the study Interactive Voice Response System (IVRS) to manage this enrollment algorithm.

A baseline nasopharyngeal swab specimen will be sent to the central virology laboratory for each RAT negative subject that has been randomized into the study. These specimens will be tested for influenza A and B infection using a PCR assay. The PCR results for these specimens will be reported to BioCryst in real time. The study will close after enrollment of at least 500 subjects who have either a positive Influenza A or B RAT at screening, or who have a negative RAT at screening but a positive influenza A or B PCR assay result from a baseline nasopharyngeal swab.

Study drug will be administered as one 2mL intramuscular injection in each gluteal muscle (total of 4mL injected in divided doses).

At screening, subjects will have an anterior nasal or pharyngeal swab collected for testing with a commercially available RAT kit for influenza A and B in accordance with the RAT manufacturer's instructions. If this test is positive and the subject is enrolled, additional specimens will be obtained for isolation and culture of influenza virus and quantitative PCR assay. If the initial RAT is negative, the test should be repeated with a different commercially available RAT kit. A limited number of RAT negative subjects may be enrolled in accordance with the screening algorithm defined above.

Enrolled subjects will record the following in a Study Diary:

- Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol- provided), aspirin, ibuprofen or other NSAID.
- Assessment of seven symptoms of influenza on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe), twice daily (AM, PM) through Day 9, then once daily (AM) through Day 14
- Assessment of subject's ability to perform usual activities, (0–10 on a visual analog scale) once daily through Day 14
- Assessment of a subject's time lost from work or usual activities and productivity compared to normal (0-10 on a visual analogue scale) once daily through Day 14
- Doses of antipyretic (e. g. acetaminophen/ paracetamol), expectorant, and/or throat lozenges administered each day through Day 14.

Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for influenza viral culture and quantitative PCR assay. In a subset of a minimum of 200 subjects an additional viral culture/ PCR sample will be collected on Day 2. Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Days 1, 3, 5, and 9 for all subjects, and on Day 2 in the subset of 200 subjects, to assess for potential changes in influenza viral susceptibility to neuraminidase inhibitors in response to treatment.

At selected sites a separate sub-study will be conducted to collect limited PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate sub-study protocol, BCX1812-311PK.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1.1 Inclusion Criteria

Subjects must meet all of the following criteria for inclusion in this study:

1. Male and female subjects age ≥18 years
2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate specimen collected from an

anterior nasal or pharyngeal swab, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated with a second commercially available RAT kit. A limited number of RAT negative subjects may be enrolled in accordance with a defined screening algorithm..

3. Presence of fever at time of screening of ≥38.0 C (≥100.4 ºF) taken orally, or ≥38.5ºC (≥101.2 F) taken rectally. For subjects who test RAT positive at the time of screening, a subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify in the absence of documented fever at time of screening. For subjects who test RAT negative at screening, fever as described above must be documented at time of screening.
4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of any severity (mild, moderate, or severe)
5. Presence of at least one constitutional symptom (myalgia [muscle aches], headache, feverishness, or fatigue) of any severity (mild, moderate, or severe)
6. Onset of illness no more than 48 hours before presentation
7. Females of child-bearing potential must have a negative urine pregnancy test (Beta HCG) at screening/baseline AND report one of the following:
    - Be surgically sterile or practice monogamy with a partner who is surgically sterile
    - Have been sexually abstinent 4 weeks prior to date of screening evaluation and be willing to remain abstinent through 4 weeks after study drug administration
    - Use oral contraceptives or other form of hormonal birth control including hormonal vaginal rings or transdermal patches and have been using these for 3 months prior through 4 weeks after study drug administration
    - Use an intra-uterine device (IUD), or double barrier contraception (such as condom or diaphragm with spermicidal gel or foam) as birth control 4 weeks prior to date of screening through 4 weeks after study drug administration
8. Provide written informed consent

8.1.2 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

1. Women who are pregnant or breast-feeding
2. Presence of clinically significant signs of acute respiratory distress
3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma
4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class IV functional status within the past 12 months (section 15.1).

5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.
6. History of chronic renal impairment requiring hemodialysis or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min)
7. Clinical evidence of active bacterial infection at any body site requiring therapy with oral or systemic antibiotics
8. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy.
9. Current treatment of active viral hepatitis C
10. Presence of known HIV infection, with a CD4 count <350 cell/mm^3
11. Current therapy with oral warfarin or other systemic anticoagulant
12. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening
13. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days
14. History of alcohol abuse or drug addiction within 1 year prior to admission in the study
15. Participation in a previous study of peramivir as treatment for acute influenza or previous participation in this study
16. Participation in a study of any investigational drug within the last 30 days

8.1.3 Removal of Subjects from Therapy or Assessment

All subjects are permitted to withdraw from participation in this study at any time and for any reason, specified or unspecified, and without prejudice. The Investigator or sponsor may terminate the subject's participation in the study at any time for reasons including the following:

1. Adverse event;
2. Intercurrent illness;
3. Non-compliance with study procedures;
4. Subject's decision;
5. Administrative reasons;
6. Lack of efficacy;
7. Investigator's opinion to protect the subject's best interest.

Any subject who withdraws because of an adverse event will be followed until the sign(s) or symptom(s) that constituted the adverse event has/have resolved or is determined to represent a stable medical condition.

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary, or if it is the desire of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject and determine the

subject's medical condition. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data.

9 TREATMENTS

9.1 Treatments Administered

Peramivir is an investigational drug. Peramivir for intramuscular injection is a small-volume parenteral and will be supplied as a 75 mg/mL solution in sodium citrate/ citric acid buffer. The pH is approximately 3.0.

A matched placebo solution of sodium citrate/ citric acid buffer with 1.2% sodium chloride at a pH of approximately 3.0 will be supplied.

9.2 Identity of Investigational Product(s)

Peramivir and placebo peramivir will be supplied in clear 2mL vials. An individual study drug kit will contain 2 vials of blinded study drug (peramivir and/or placebo, depending upon the treatment group), 2 syringes and 2 needles in which to draw up the solution for intramuscular injection. All materials will be packaged in a labeled box container. All study drug kits must be stored at 2-8 ^{o}C. Each individual study drug kit will be labeled with some or all of the following information as required by local regulations:

- Sponsor name and contact information, study protocol number, kit number, description of the contents of the container, instructions for the preparation of the syringe and administration of the study drug, conditions for storage, statement regarding the investigational (clinical trial) use of the study drug and date for retest or expiry date.

Each vial of study drug will be labeled with some or all of the following information as required by local regulations:

- Sponsor name, study protocol number, description of the contents of the vial, instructions for the preparation of the syringe, statement regarding the investigational (clinical trial) use of the study drug and lot number.

9.3 Method of Assigning Subjects to Treatment Groups

Subjects will be centrally randomized in a ratio of 2:2:1 to one of three treatment groups: single dose peramivir 150mg, single dose peramivir 300mg or placebo, in accordance with a computer-generated randomization schedule prepared by a non-study statistician. Subjects will be stratified according to the result of a RAT test and current smoking behavior. Once a subject is eligible for randomization, he/she will be assigned a study drug kit number that will be obtained by study staff from the study interactive voice response system (IVRS). Once a study drug kit number has been assigned to a subject, it cannot be reassigned to any other subject.

9.4 Study Medication Accountability

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the sponsor, issued to th e subject or directly adm inistered to the subject (including date and tim e), and any drug accidentally destroyed. The sponsor will supply a specific dru g-accountability form . At the en d of the study, information describing s tudy drug s upplies (e.g. , lot num bers) and disp osition of s upplies for each subject m ust be provided, signed by the Invest igator or designee, and collected by the study m onitor. If any errors or irregularities in any shipm ent of study m edication to the site are discovered at any time, the Project Manager must be contacted immediately.

At the end of the study, all medication not dispensed or administered and packaging materials will be collected with supervision of the m onitor and returned to the sponsor or destroyed on site as dictated by the appropr iate Standard Operating P rocedure at the participating institution.

9.5 Blinding/Unblinding of Treatments

This is a double-blind study. The treatm ent group assignm ent will not be known by the study subjects, the investigator , the clinical staff, the CRO or Sponsor staff during the conduct of the study.

Section 11.2.4provides inform ation regarding the process for unblinding the treatment assignment, if necessary, in the event of an SAE.

9.6 Prior and Concomitant Therapies

All m edications, by any route of adm inistration, used during this study m ust be documented on the Case Report Form (CRF). Prescription as well as non-prescription medications should be recorded. Medication us ed for the treatm ent of influenza-related symptoms will be captured by the subject in the diary card provided by BioCryst.

9.7 Overdose and Toxicity Management

To date there is no experience with overdose of in tramuscular or intrav enous peram ivir. If overdose occurs, subjects should receive indicated supportive therapy and evaluation of hematologic and clinical chem istry laboratory tests should be conducted. The effec t of hemodialysis on elimination of peramivir is unknown.

9.8 Dose Interruption

As this is a study of a single injection of peramivir or placebo, guidelines for treatm ent interruption for drug related SAEs or toxicities are not applicable.

10 STUDY CONDUCT

A study schedule of evaluations is presented in Figure 1 . A detailed lis t of the evaluations is also provided in the following sections.

10.1 Evaluations

All subjects enrolled in this study will undergo the following evaluations:

10.1.1 Medical History

Medical history, influenza va ccination s tatus within th e previous 12 m onths and demographic data (including smoking behavior) will be recorded at Screening/Baseline.

10.1.2 Rapid Antigen Test for Influenza

At Screening/Baseline, a comm ercially availa ble, rapid antigen tes t (RAT) for influenza A and B will be performed on an adequate specimen collected by swabbing the anterior nose or pharynx, in accordance with the RAT manufacturer' instru ctions. A negative initial RAT should be repeated with a different commercially available RAT kit. Refer to the Study Manual f or instru ctions regarding the use of the RAT kits provided for this study. Sites m ay use the kits provided by the Sponsor or any other commercially approved RAT available at their site to document a confirmed influenza infection.

A lim ited num ber of RAT negativ e subje cts will be enr olled in a ccordance with the following algorithm: Once an ind ividual site has identif ied 3 RAT positive sub jects at screening within a 10 day period, indicating that influenza activity is present in the site's vicinity, a RAT negative subject that m eets the inclus ion/ exclus ion criteria m ay be enrolled. O ne additional RAT negative subj ect m ay be enrolled th ereafter for each preceding RAT positive subject that is iden tified. The RAT result for each subject screened will be record ed on the s tudy Inte ractive Voice response (IVR) system to manage this enrollment algorithm.

10.1.3 Physical Examination and Influenza-related Complications Assessments

The Investigator will perform a physical ex amination at S creening/Baseline. Subject's weight will be recorded at Screening/Baseline.

Study personnel will be provide d with an influenza-related complications (IRC) checklist in the CRF to evalua te the subjec t for the pr esence of clinical signs and /or symptoms o f the following influenza-related complications: sinusitis, otitis, bronchitis and pneumonia. At each follow up assessm ent a t argeted physical exam ination will be conducted to record the presence of influenza related com plications. If the investigator determines that the subject experiences (or is presumed to experience) an IRC as noted above, he/she will record that assessm ent on the IR C CRF pa ge and any m edication used to treat the condition will be reco rded on the concom itant m edication page. The investigato r will promptly provide appropriate treatm ent fo r any suspected or proven IRC, as such treatment will not affect the efficacy assessm ents. Such information describing IRC signs and/or sym ptoms should not be reported as adverse events. Any inj ection site reactions noted will be recorded in the CRFs as adverse events.

10.1.4 Vital Signs

Vital signs evaluations will include blood pressure, pulse rate, and respiration rate. The investigator will record oral body temperature at baseline. Thereafter the subject will record oral temperature twice daily in the study diary card.

Vital signs will be measured at Screening/Baseline, pre-dose, and at 15 minutes following the study drug injection on Day 1, then once daily on Days 2 (for those subjects who are seen on day 2), 3, 5, 9, and 14.

10.1.5 Electrocardiogram Measurements

A 12-lead electrocardiogram (ECG) will be obtained at Screening/ Baseline. The principal investigator will be responsible for interpretation of the Screening ECG. This interpretation may be performed by the investigator or he/she may delegate this action to another physician and the investigator will acknowledge the interpretation. If this baseline ECG is interpreted as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal but does not meet the exclusionary criteria, the subject may be enrolled unless other exclusion criteria apply. The principal investigator is responsible to ensure that such an enrolled subject be informed of the nature of the abnormal ECG and that any medically indicated repeat ECG examinations and/or referral of the subject for further evaluation is made either during subject's participation in the study or immediately after the subject's discharge from the study.

10.1.6 Clinical Chemistry Profiles

Clinical chemistry profiles will include a Chemistry 20 panel (includes sodium, potassium, chloride, total CO_2 [bicarbonate], creatinine, glucose, urea nitrogen, albumin, total calcium, total magnesium, phosphorus, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, lactate dehydrogenase [LDH], total protein, total creatine kinase, and uric acid)

Blood samples for clinical chemistry profiles will be collected at Screening/Baseline, and on Days 3, 5 and 14.

10.1.7 Hematology Profiles

Hematology will include complete blood count (CBC) with differential.

Blood samples for hematology profiles will be collected at Screening/Baseline, and on Days 3, Days 5 and 14.

10.1.8 Serology for Influenza

Paired blood samples for determination of antibody to influenza A and B (serology) will be obtained with the clinical laboratory tests at Screening/Enrollment and at Day 14. These specimens will be stored at the central laboratory and will be analyzed if needed to confirm the diagnosis of influenza.

10.1.9 Urinalysis and Evaluation of Protein in Urine

Urinalysis will include dipstick tests for protein, glucose, ketones, blood, urobilinogen, nitrite, pH, and specific gravity and microscopic evaluation for RBCs and WBCs.

Samples for urinalysis will be collected at Screening/Baseline, and on Days 3, 5, and 14.

10.1.10Urine Pregnancy Test

Females of childbearing potential will be evaluated for pregnancy at Screening/Baseline and Day 14 using a urine pregnancy test.

10.1.11 Samples for Virologic Laboratory Assessments

An adequate specimen will be collected by swabbing the anterior nose (bilateral) and posterior pharynx for virologic laboratory assessments including culture for the isolation of influenza virus and/or quantitative PCR assay at Screening/Baseline, and at Days 3, 5, and 9. In a subset of a minimum of 200 subjects an additional sample will be taken at Day 2. Refer to the Laboratory Manual for instructions regarding the processing and shipment of these specimens.

10.1.12 Subject Self Assessments

Subject self assessments will be performed beginning pre-dose on Day 1 and recorded in the subject's Study Diary including the following:

- Oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol, provided) aspirin, ibuprofen or other NSAID. The times of each temperature determination will be recorded in the Study Diary. The baseline temperature will be recorded at the screening/Day 1 visit prior to dosing, regardless of whether the subject had recently taken an anti-pyretic; the time of anti-pyretic use pre-treatment will be recorded in the CRF, if applicable.
- Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [muscle aches], headache, feverishness, and fatigue) on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily, beginning pre-dose on Day 1 and through Day 9, then once daily through Day 14.
- Assessment of the subject's ability to perform usual activities using a 0–10 visual analogue scale once daily through Day 14.
- Assessment of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 visual analogue scale once daily through Day 14.

The subject's diary card will be reviewed by study staff at each visit for completion of the record of all required items, with particular emphasis on alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms. Study staff will not attempt to ask subjects to retrospectively complete missing diary card data for any scheduled assessments that have not been completed prior to the clinic visit. Study staff should, however, remind the subject to complete the diary card at all scheduled times.

10.1.13 Concomitant Medications

All concomitant medications used during this study, with the exception of those medications taken for symptomatic relief of influenza symptoms, which will be recorded by the subject in their diary card, must be documented on the Case Report Form (CRF).

10.1.14 Adverse Events

AEs will be assessed from the time of administration of study medication through the final study visit.

10.1.15 Single Pharmacokinetic Exposure Sample

On study day 3 a single PK sample will be drawn in concert with the day 3 safety clinical laboratory blood draw. This sample will be sent for subsequent analysis of the concentration of peramivir at this time point. Data from this single PK sample will be combined with data from the PK sub-study (BCX1812-311PK) to perform an exposure-response analysis. This analysis will be described as part of the sub-study analysis plan..

10.2 Screening

10.2.1 Informed Consent

The nature and purpose of the study and the expectations of a participating subject will be described to potential study subjects, their questions will be answered, and the subjects will then be asked to sign an informed consent document. Study subjects will then undergo the screening evaluation as noted in Section 10.2.2

10.2.2 Screening/Baseline Evaluation and Enrollment

Screening/baseline evaluation may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subjects may be enrolled and receive treatment with study drug prior to receiving results of the laboratory assessments (with the exception of urine pregnancy test result, which must be known).

Eligible subjects will be enrolled and randomized to blinded study treatment. The randomization will include stratification by RAT status and current smoking behavior. The Investigator will prepare a request for blinded study drug assignment which includes the subject's screening number. The Investigator or designee at the clinical study center will contact the central randomization Interactive Voice System (IVRS call center). The IVRS call center will advise the study center of the investigational study drug kit number that is assigned to that subject at enrollment.

Subjects that are determined to be ineligible will be advised accordingly, and the reason for ineligibility will be discussed. If desired by the subject the reason for ineligibility may be provided/discussed with their health-care provider by the Investigator or designee.

Ineligible subjects who have been screened for the study will also be entered on the

IVRS. For such subjects, the screening number assigned, subject's date of birth and a reason for ineligibility will be entered on to the IVRS. **All ineligible subjects must be entered onto the IVRS within 24 hours of screening,** to assist with surveillance analysis during the course of the study.

10.3 Treatment Period—Study Day 1

Day 1 represents the only day of study drug dosing. Study drug administration should occur as soon as possible following informed consent, screening and randomization. Therefore, it is expected that the date of Screening/ Baseline and Day 1 will usually be the same date.

10.3.1 Pre-dose Evaluations

Following an explanation of the Subject Self Assessment measures (Section 10.1.11), the subject shall complete the record of these assessments in their Study Diary prior to dosing. The subject will be counseled regarding the expectations for recording these assessments through Day 14.

Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) and a 12 lead ECG will be obtained prior to dosing. At Hour 0, the blinded study drug will be administered intramuscularly (one injection in the left gluteal muscle, and one injection in the right gluteal muscle within a period of ≤ 10 minutes.). The calendar date and 24-hour clock time of the first and second injections will be recorded.

The following evaluations will be performed post-dose on Study Day 1:

- Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) at 15 minutes after the study drug administration
- Record any concomitant medications
- Record any AEs

10.4 Post-Treatment Assessment Period

10.4.1 Days 2, 3, 5, 9 and 14

Study evaluations will be performed on Days 2 [subset of subjects only], 3, 5, 9 and 14 in accordance with the schedule of evaluations (Figure 1). Subjects with persistent moderate or severe influenza symptoms at day 14 will also complete a Day 21 visit.

Visits may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

The Day 2 assessment will be conducted in clinic or by study staff only for the subset of subjects who will provide additional Day 2 virology samples. For all other study subjects, study staff will attempt to contact the subjects on Day 2 by telephone to confirm their compliance with completion of the Subject Self Assessments, to note any concomitant medications and adverse events. Any adverse events reported by the subject during this telephone contact will be recorded on the adverse event form and verified during the visit

on day 3.

At each visit it is important that the subject's Study Diary record be reviewed for completion of daily Subject Self Assessments. The subjects should be counseled as necessary regarding self assessments and Study Diary record requirements. The subject's diary card will be reviewed by study staff for alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms.

Day 3:

The results of the serum CK result obtained at Day 3 will not be made available to investigators, to BioCryst or to any study personnel, unless the CK result at Day 3 is ≥ 2000 IU/mL. A single PK sample will be obtained on Day 3 at the same time as the clinical laboratory blood specimen is obtained.

Day 14:

If a subject has one or more persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit then the subject must be evaluated in further follow-up visits, at day 21 (± 3 days), and if required at day 28 (± 3 days) If a subject reports moderate or severe influenza symptoms then the investigator will record the intensity of each of the influenza symptoms on a visit specific CRF page at the day 21 and day 28 visits. After day 14 the subject will not record symptoms in a diary.

Day 21 (if applicable):

The day 21 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 14. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at the Day 21 visit and such action(s) will be recorded on the Day 21 CRF page. The investigator will recall the subject for a further study visit at day 28 (± 3 days) if moderate or severe symptom(s) of influenza persist at Day 21.

Day 28 (if applicable):

The day 28 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 21. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at this visit and such action(s) will be recorded on the Day 28 CRF page. No further follow-up visits beyond day 28 are to be formally scheduled unless in the clinical judgment of the investigator further follow-up is required. The investigator will use his/her clinical judgment to manage the subject, referring the subject, if appropriate, for further care.

10.4.2 Adverse Events Reported at Post-treatment Visits

In this study, symptoms of influenza will be considered separately from adverse events reported during the post-treatment period. Accordingly, adverse events that have onset in the post-treatment period will be assessed and followed as specified in 11.2. Specifically,

the investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

Figure 1 Study Measurements and Visit Schedule

	Screening [1] (Baseline)	Treatment Period Day 1[1]	Assessment Day Day 2[2]	Day 3	Day 5 (±1 day)	Day 9 (±3 day)	End of Study Early Withdrawal Day 14 (±3 day) [8]
Informed Consent	X						
Rapid Antigen test for Influenza A & B	X						
Medical History/Physical Exam[3]	X						
Influenza-related complications checklist[3]	X		X	X	X	X	X
Inclusion/Exclusion	X						
Clinical Chemistries[4]	X			X	X		X
Hematology[4]	X			X	X		X
Exposure Pharmacokinetic Sample				X[4]			
Serology (serum) Sample	X						X
Urinalysis[4]	X			X	X		X
Urine Pregnancy Test	X						X
Vital Signs[5]	X	X	X	X	X	X	X
ECG[6]	X						
Sample (nasopharyngeal swab) for Influenza Virus Culture/ PCR assay and for resistance studies		X	X	X	X	X	
Study Drug Administration		X					
Subject Diary Review[7]		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

[1] It is expected that the date of Screening and Day 1 (date of administration of study drug) will be the same. Visits at Screening and on subsequent study days may occur in subject's home by the investigator (all visits) or appropriately trained study center staff (Day 2, 3, 5, 9 visits).

[2] Day 2 visit required only for a subset of subjects for whom additional Day 2 virology sample is required. For all other subjects, Day 2 will be a telephone contact with the subject to ensure compliance with diary card completion, concomitant medication and adverse event review.

[3] Medical history and physical exam at screening to include weight, and smoking behavior. Targeted physical examinations will be performed to complete the influenza-related complications checklist by the appropriate medical personnel at appropriate visits.

[4] Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subject may be enrolled and begin treatment with study drug prior to receiving results. On Day 3 an extra tube will be included with the safety blood sample for evaluation of peramivir concentrations.

[5] Vital sign measures will include blood pressure, pulse rate and respiration rate. Vital signs will be recorded at Screening, pre-dose and at 15 min following the study drug administration on Day 1, then once on remaining days as stipulated. The investigator will record oral temperature at baseline. Thereafter the subject will report oral temperature measurements twice daily in the Study Diary

[6] If the baseline ECG is interpreted by the conducting physician as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal and does not meet the exclusionary criteria (e.g. acute ischemia, medically significant dysrhythmia) then this subject may be enrolled If the conditions highlighted in section 10.1.5 are met for the subject.

[7] Subjects record symptom assessment in Study Diary, twice daily, beginning pre-dose on Day 1 through Day 9, then once daily through Day 14; subjects record ability to perform usual activities once daily, beginning pre-dose on Day 1 through Day 14. Subjects record oral temperature twice daily throughout as well as all concomitant medication and adverse events

[8] For any subject with unresolved moderate or severe intensity influenza symptoms a follow up assessment will be scheduled at Day 21 (± 3 days) and Day 28 (± 3 days) if required (See Section 10.4.1).

11 ADVERSE EVENT MANAGEMENT

11.1 Definitions

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

AEs may be designated as "nonserious" or "serious" (see Section 11.1.2).

Surgical procedures are not AEs but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [muscle aches], headache, feverishness, and fatigue) will be documented in a subject's study diary and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as AEs unless the symptom(s) worsen to the extent that the outcome fulfils the definition of an SAE, which then must be recorded as such (see Section 11.1.2). Likewise, a RAT for influenza is required at screening in order to determine eligibility for the study, and therefore a positive RAT is not considered an AE.

11.1.2 Serious Adverse Event

A SAE is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

11.2 Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of AEs are to be collected from the time of study drug administration through the follow-up period ending on Day 14. The Investigator or designee must completely and promptly record each AE on the appropriate CRF. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

The Investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

11.2.1 Definition of Severity

All AEs will be assessed (graded) for severity and classified into one of four clearly defined categories as follows:

- **Mild:** (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
- **Moderate:** (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- **Severe:** (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- **Life-threatening:** (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization.

11.2.2 Definition of Relationship to Study Drug

The blinded Principal Investigator must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the

subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication, is confirmed by improvement upon stopping the medication (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

11.2.3 Reporting Serious Adverse Events

Any SAE must be reported to BioCryst or its designee within 24 hours of the Investigator's recognition of the SAE by first notifying the Medical Monitor at the number listed below:

Telephone: **Europe: +44 1628 548000; North America: 1-888-724-4908**

Facsimile: **Europe: +44 1628 540028; North America: 1-888-887-8097 or 1-609-734-9208**

The site is required to fax a completed SAE Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the SAE must be reported and sent by facsimile to BioCryst or its designee as soon as they are available.

The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (e.g., revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and for retaining a copy in their files.

If the Investigator becomes aware of any SAE occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify BioCryst or its designee.

Any SAEs considered possibly related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch reporting system in

accordance with FDA and other applicable regulations. However, the Investigator is not obligated to actively seek reports of AEs in former study participants.

While pregnancy is not considered an AE, all cases of fetal drug exposure via parent as study participant (see Section 4.4) are to be reported immediately to BioCryst or its designee. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee.

11.2.4 Emergency Procedures

In the event of an SAE, the Principal Investigator may request the unblinding of the treatment assignment for the subject affected. If time allows (i.e., if appropriate treatment for the SAE is not impeded), the Principal Investigator will first consult with the Medical Monitor regarding the need to unblind the treatment assignment for the subject. At all times, the clinical well-being of any subject outweighs the need to consult with the Medical Monitor.

The Principal Investigator may contact the IVRS central randomization center and request the unblinding of the treatment assignment that corresponds to the affected subject. The IVRS center will record the name of the Investigator making the request, the date and time of the request, the reason for the request, the subject number and study drug kit number, and whether the Medical Monitor was consulted prior to the request being made. The Sponsor will be informed within 24 hours if unblinding occurred.

12 STATISTICAL METHODS

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and other selected efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. The term "treatment group" refers to randomized treatment assignment: peramivir 150 mg, peramivir 300 mg, or placebo. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date within each subject number.

Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted with the SAS® System, version 9.1.3 or higher.

12.1 Data Collection Methods

The data will be recorded on the CRF approved by BioCryst. The Investigator must submit a completed CRF for each subject who signs an informed consent form (ICF), regardless of duration. All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF.

Documents (including laboratory reports, hospital records subsequent to SAEs, etc.) transmitted to BioCryst should not carry the subject's name. This will help to ensure

subject confidentiality.

12.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be created prior to the review of any data. This document will provide a more technical and detailed description of the proposed data analysis methods and procedures.

12.3 Sample Size Estimates

From published results, it is expected that the median time to alleviation of symptoms will be between 103.3-116 hours for subjects receiving placebo.[16,17] For sample size calculations the best placebo response (103.3 hours) will provide the most conservative estimate of an observed hazard ratio. Additionally, it is expected that the median time to alleviation for the lowest dose peramivir arm will be 69.9 hours, yielding a hazard ratio of 0.68. Using these assumptions, a sample size of 200 infected subjects per active treatment group and 100 infected subjects in the placebo group is sufficient to provide at least 80% power to detect a hazard ratio of 0.68 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months). Up to 800 subjects will be enrolled to achieve the target number of at least 500 subjects with diagnostic evidence (RAT or PCR) of an acute influenza infection (200 per active treatment; 100 receiving placebo) as described in section 12.4.2

12.4 Analysis Populations

The populations for analysis will include the intent-to-treat (ITT), intent-to-treat infected (ITTI), and safety populations.

12.4.1 Intent-To-Treat Population

The ITT population will include all subjects who are randomized. Subjects will be analyzed in the treatment group to which they were randomized. The ITT population will be used for analyses of accountability and demographics.

12.4.2 Intent-To-Treat Infected Population

The ITTI population will include all subjects who are randomized, received study drug, and have proven influenza by any one of the following: culture, PCR, or paired serology showing $\geq$ 4-fold increase in antibody to influenza A or B, and received study drug. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI population will be used for primary analyses of efficacy.

12.4.3 Safety Population

The safety population will include all subjects who received study drug. Subjects will be analyzed according to the treatment received. This population will be used for all safety analyses.

12.5 Interim and End of Study Analyses

Interim Analysis

An independent DMC will review safety data on an ongoing basis. Safety analyses will be presented in a manner consistent with the presentations intended for the final analysis.

End of Study Analysis

A final analysis is planned after the last subject completes or discontinues the study, and the resulting clinical database has been cleaned, quality checked, and locked.

12.6 Efficacy Analyses

12.6.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when a subject has Alleviation of Symptoms. A subject has Alleviation of Symptoms if all of the seven symptoms of influenza (nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, feeling feverish) assessed on his/her subject diary are either absent or are present at no more than mild severity level and at this status for at least 21.5 hours (24 hours - 10%).

Descriptive statistics for the primary efficacy endpoint will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized overall and for individual symptoms for each treatment group. Overall treatment differences will be assessed using a Cox Regression model with effects for RAT result at screening, current smoking behavior, treatment group, and influenza season at randomization (if necessary). Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing post-baseline assessment. Pairwise differences in time to alleviation of symptoms among the treatment groups will be evaluated using contrast statements from the final Cox model. In order to maintain the overall type I error in the presence of the planned comparisons between the two peramivir treatments and placebo, a Bonferroni correction will be applied to the primary efficacy endpoint analysis. P-values for the planned comparisons of each peramivir arm to placebo will be adjusted via a Bonferroni correction (i.e., if the unadjusted p-value for an active comparison versus placebo, p, is less than 0.05, then p^a=p*number of planned comparisons=p*2; otherwise, p^a=p). Superiority of peramivir to placebo will be established if the adjusted p-value is less than or equal to 0.05.

12.6.2 Secondary Efficacy Endpoints

All secondary endpoints will be summarized using descriptive statistics by treatment group and study day/time, if appropriate. Statistical comparisons for each endpoint will be constructed without adjustment for multiple endpoints.

The reduction in viral shedding will be assessed as the change in viral titers defined as the time-weighted change from baseline in $\log_{10}$ tissue culture infective dose$_{50}$ (TCID$_{50}$/mL) and will be summarized for each treatment group. The time-weighted average change from baseline will be calculated on a by-subject basis through Day 9 using the trapezoidal rule with all available post-baseline on-treatment data (data after initiation of study treatment) minus the baseline value. Specifically, the time-weighted area under the curve for time a (t_a) to time b (t_b) is given by the formula

$$TWAUC = \frac{AUC(t_a - t_b)}{(t_a - t_b)},$$

where $AUC(t_a - t_b) = \sum_{i=a}^{b-1} \frac{(y_i + y_{i+1})(t_{i-1} - t_i)}{2}$ and t_i represents the date of the i^{th} viral titer assessment and y_i represents the $\log_{10}$ value of the i^{th} viral titer assessment. If there is a baseline value and only one follow-up value, y_i then the time-weighted change from baseline is defined as the difference between y_i and baseline. If there is a baseline value and no follow-up value, the subject is excluded from analysis. The differences between each of the peramivir treatment groups and placebo will be evaluated using a van Elteren Test adjusting for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Analyses of the PCR results will be analyzed in a similar manner.

Subject's ability to perform usual activities as determined from the visual analog scale will be summarized by study visit day and treatment group. Differences between the treatment groups will be assessed using the van Elteren Test adjusting for smoking behavior and influenza season at randomization (if necessary). The time (days) to resumption of a subject's ability to perform usual activities (i.e., subject scores ability to perform usual activities as 10) will be estimated using the method of Kaplan-Meier. Differences between each of the peramivir treatment groups and placebo will be assessed using the log rank statistic adjusting for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Subjects who do not return to the pre-study level of performance of usual activities will be censored at the time of their last non-missing post-baseline visual analog scale value.

Subject's oral temperature will be summarized by study visit and treatment group. Differences between the treatment groups will be assessed using the Wilcoxon Rank Sum Test controlling for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). A subject has Resolution of Fever if he/she has a temperature < 37.2°C (99.0°F) and no antipyretic medications have been taken for at least 12 hours. The time to resolution of fever will be estimated using the method of Kaplan-Meier using temperature and symptom relief medication information obtained from the subject diary data. Difference between the treatment groups will be assessed using the

log rank statistic controlling for RAT result at screening, current smoking behavior and influenza season at randomization (if necessary). Subjects who do not have resolution of fever will be censored at the time of their last non-missing post-baseline temperature assessment.

The MRU, MRU-related direct costs, and indirect costs attributable to days missed of work and work productivity and/or performance losses will be summarized by treatment group. Differences between each of the peramivir treatment groups and placebo group will be evaluated using both parametric and/or non-parametric tests, as appropriate. If necessary, bootstrapping techniques will be used to calculate confidence intervals around the incremental differences in costs.

12.6.3 Exploratory Endpoint

Genotypic (including Hemagglutinin and Neuraminidase), phenotypic, viral culture and PCR data will be listed for each subject. These listings will be constructed in a manner consistent with the FDA June 2006 Guidance Document: *"Guidance for Submitting Influenza Resistance Data"*.[18] Additionally, the number and percentage of genotypic changes from wild-type amino acid will be summarized separately for treatment group, protein type, and study visit.

12.7 Safety Analyses

AEs will be mapped to a MedDRA-preferred term and system organ classification. The occurrence of TEAEs will be summarized by treatment group using MedDRA-preferred terms, system organ classifications, and severity. If a subject experiences multiple events that map to a single preferred term, the greatest severity and strongest Investigator assessment of relation to study drug will be assigned to the preferred term for the appropriate summaries. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to study drug will be generated.

Descriptive summaries of vital signs and clinical laboratory results will be presented by study visit. Laboratory abnormalities will be graded according to the DAIDS Table for Grading Adverse Events for Adults and Pediatrics (Publish Date: December 2004). The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized by treatment group. Laboratory toxicity shifts from baseline to Day 3, Day 5, and Day 14 will be summarized by treatment group.

Abnormal physical examination findings will be presented by treatment group. The number and percent of subjects experiencing each abnormal physical examination finding will be included.

Concomitant medications will be coded using the WHO dictionary. These data will be summarized by treatment group.

Subject disposition will be presented for all subjects. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

12.8 Sub-Study and Pharmacokinetic Analysis

The sub-study to exam ine exposure respons e, along with the corresponding single PK samples collected on all subjects on study day 3 will be co mpleted as part of the sub-study. All s tatistical m ethods will be outlin ed as part of the sub-stu dy protocol and exposure-response analysis plan. All sub-study analyses will be reported in a separate sub-study report.

12.9 General Issues for Statistical Analysis

12.9.1 Multiple Comparisons and Multiplicity

In order to m aintain the overall typ e I e rror in the presen ce of the plann ed com parisons between the two peram ivir treatm ents and pl acebo, a Bonferroni correction will be applied to the prim ary efficacy end point an alysis. No oth er ad justments for m ultiple comparisons are planned.

12.9.2 Covariates

Primary and secondary efficacy analyses will be adju sted for RAT result at screen ing, current smoking behavior and influenza season at randomization (if necessary).

12.9.3 Planned Sub-Groups

The prim ary efficacy endpoint will be summ arized separately by stratification group (current sm oking behavior [sm oker or non-smoker] and RAT result at screening [negative or positive]) and by viral subtype using descriptive statistics by treatment group and study day, if appropriate. No formal statistical testing will be utilized.

Additional analyses m ay be perform ed by count ry, if necessary, for subm ission to local regulatory authorities.

12.9.4 Missing Data

Every effort will be m ade to obtain required data at each s cheduled ev aluation from all subjects who have been random ized. No attemp t will be m ade retrospectively to obtain missing subject reported data (including in fluenza symptom severity assessm ents, temperature, ability to perf orm usual activi ties, m issed days of work and im pact of influenza on subject's work perform ance and/or productivity) that has not been completed by the subject at the time of return of the subject diary to the investigative site. In situa tions where it is not possible to obt ain all da ta, it m ay be necessary to im pute missing data.

In assessing the p rimary efficacy endpoint, fo r subjects w ho withdraw or who d o no t experience allev iation of sym ptoms, m issing data will be censored using the date of subject's last non-m issing assessm ent of infl uenza sym ptoms. Missing assessm ents of influenza sy mptoms conservatively will be im puted as h aving severity above absen t or mild (as f ailures). For the subjec t diary data, the f ollowing data con ventions will be utilized. Missing diary com pletion will be im puted as 11:59 for diary entries desig nated

as morning and 23:59 for evening and daily reported values. Entries with values exceeding the 24-hour clock will be set to 23:59 of the day recorded. Select exploratory sensitivity analyses may be conducted to ascertain the effect, if any, of these methods. These sensitivity analyses are further described in the SAP. Secondary efficacy endpoints with time to event data will be censored using the date of subject's last non-missing assessment of the given endpoint.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1.1 Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; GCPs, including International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator should submit written reports of clinical study status to their Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) annually or more frequently if requested by the IRB/ IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the IRB/ IEC should be maintained in the study documents file. Copies of clinical study status reports (including termination) should be provided to BioCryst.

13.1.2 Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent form, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the investigational medicinal product is released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The Investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the Investigator will provide the IRB/IEC with a report of the outcome of the study.

13.1.3 Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any

study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by BioCryst and by the reviewing IRB/ IEC. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and BioCryst policy.

The Investigator must explain to potential subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry in to the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the ICF. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1.4 Payment to Subjects

Reasonable compensation to study subjects may be provided if approved by the IRB/IEC responsible for the study at the Investigator's site.

13.1.5 Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his /her IRB/IEC as required.

13.2 Study Monitoring

During trial conduct, BioCryst or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow BioCryst monitors or its designees and appropriate regulatory authorities direct access to source documents to perform this verification.

13.3 Quality Assurance

The trial site may be subject to review by the IRB/IEC, and/or to quality assurance audits performed by BioCryst, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to

the process.

13.4 Study Termination and Site Closure

BioCryst reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by BioCryst, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5 Records Retention

To enable evaluations and/or audits from regulatory authorities or BioCryst, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to BioCryst. The Investigator must obtain BioCryst's written permission before disposing of any records.

13.6 Study Organization

13.6.1 Data Monitoring Committee

BioCryst will assemble an independent Data Monitoring Committee (DMC) to assess safety parameters of the trial on a periodic, ongoing basis while the trial is in progress. The committee will include a statistician and three physicians, two of whom will be Infectious Disease specialists. Full details of the composition of the DMC and how the DMC is to operate will be described in a separate DMC charter.

13.7 Confidentiality of Information

BioCryst affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by BioCryst. However, in compliance with federal regulations, BioCryst requires the investigator to permit BioCryst's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study.

BioCryst will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health

information of subj ects participating in BioCryst-sponsored Clinical Trials. "Authorization" is required from each research subject, i.e., specified permission granted by an individual to a covered entity for the us e or disclosure of an indi vidual's protected health information. A valid au thorization must meet the implem entation specifications under the HIPAA Pri vacy Rule. Authorizat ion m ay be com bined in the Inform ed Consent docum ent (approved by the IRB/IE C) or it may be a separate docum ent, (approved by the IRB/IEC) or provided by the Investigator or Sponsor (without IRB/IEC approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appr opriate ind ividual. HIPAA authoriz ations are required for U.S. sites only.

13.8 Study Publication

All data generated from this study are the propert y of BioCryst and shall be held in strict confidence along with all inform ation furnis hed by BioCryst. Independent analysis and/or publication of these data by the Investig ator or any member of his/her staff are not permitted without prior written consent of BioCryst. W ritten perm ission to the Investigator will be contingen t on the review by BioCryst of the statistical ana lysis and manuscript and will provide fo r nondisclosure of BioCryst confidential or proprietary information. In all case s, the partie s agree to s ubmit all m anuscripts o r abstracts to all other par ties 30 days prior to su bmission. This will e nable all parties to pr otect proprietary information and to provide comme nts based on infor mation that m ay not yet be available to other parties.

14 REFERENCES

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15 APPENDICES

15.1 NYHA Functional Classification Criteria: Heart Failure and Angina

NYHA Functional Classification of Heart Failure

Class I
No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea.
Class II
Symptoms with ordinary physical activity. Walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold weather, in wind, or when under emotional stress causes undue fatigue or dyspnea.
Class III
Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea.
Class IV
Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea.

NYHA Functional Classification of Angina

Class I
Angina only with unusually strenuous activity.
Class II
Angina with slightly more prolonged or slightly more vigorous activity than usual.
Class III
Angina with usual daily activity.
Class IV
Angina at rest.

CLINICAL STUDY PROTOCOL

Protocol No. BCX1812-311

IND No. 76,350

A PHASE 3 MULTICENTER, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRAMUSCULAR PERAMIVIR IN SUBJECTS WITH UNCOMPLICATED ACUTE INFLUENZA

THE **IMPROVE I** STUDY

(**I**ntra**M**uscular **P**eramivir for the **R**elief **O**f symptoms and **V**irologic **E**fficacy)

Short title: Intramuscular Peramivir for the Treatment of Uncomplicated Influenza

Protocol Date(s):
Version 1.0: 04 September 2007
Version 2.0: 05 October 2007

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1 TITLE PAGE

Protocol Number:	BCX1812-311
Study Title:	A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza
IND Number:	76, 350
Investigational Product:	Peramivir (BCX1812)
Indication Studied:	Uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive Birmingham, AL 35233
Development Phase:	3
Sponsor Medical Officer:	W. James Alexander, M.D., M.P.H. Senior Vice President, Clinical Development Chief Medical Officer Phone: +1 919 859 1302 Fax: +1 919 851 1416 Email Address: jalexander@biocryst.com
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Final Protocol Date:	Version 1.0: 04 September 2007
Amendment(s) Date(s):	Version 2.0: 05 October 2007

1.1 Protocol Approval Signature Page

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

BioCryst Pharmaceuticals, Inc.

Reviewed and Approved by:

W James Alexander MD 05 October 2007

W. James Alexander, M.D., M.P.H. Date
Senior Vice President, Clinical Development
Chief Medical Officer

1.2 Clinical Study Protocol Agreement

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, random ized, double-blind, placebo-controlled study to evaluate the effic acy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practices, and all applicable regulatory requirements.

Investigator's Signature Date

Name (Print)

2 SYNOPSIS

Protocol No.	BCX1812-311
Protocol Title:	A phase 3, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc.
Investigators/Study Sites:	Multinational
Development Phase:	3
Objectives:	
Primary:	To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza.
Secondary:	1. To evaluate the safety and tolerability of peramivir administered intramuscularly 2. To evaluate secondary clinical outcomes in response to treatment 3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment
Exploratory:	1. To assess pharmacoeconomic measures as response to treatment 2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment
Number of Subjects:	Total enrollment: a total of 750 evaluable subjects will be randomized to treatment (150 subjects in the placebo treatment group, 300 subjects in the peramivir 150mg treatment group and 300 subjects in the peramivir 300mg treatment group). An evaluable subject is one who is randomized, receives study drug, and has confirmed acute influenza by primary viral culture or PCR. A positive Rapid Antigen Test (RAT) at screening will be required for enrollment. Because results of clinic-based RAT tests may not precisely indicate presence of influenza infection, it is expected that at least 850 subjects will be randomized to treatment to ensure that 750 evaluable subjects are treated.
Study Design:	This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza.

	Each subject's assignment to treatment will be stratified according to body mass index (BMI). Two BMI strata are planned: Normal-Overweight (≤ 29.9 kg/m^2), and Obese (≥ 30.0 kg/m^2). The number of subjects enrolled who have a BMI ≤ 29.9 kg/m^2 (Normal-Overweight) will be at least 75% of the total enrollment. The number of subjects enrolled who have a BMI ≥ 30.0 kg/m^2 (Obese) will be ≤ 25% of total enrollment. All subjects will be centrally randomized to one of three treatment groups according to BMI strata in a ratio of 2:2:1 such that 80% of subjects are randomized to one of the two single dose regimens of peramivir. Treatment Group 1: Peramivir 150mg Treatment Group 2: Peramivir 300mg Treatment Group 3: Placebo Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in equally divided doses). Procedures for gluteal intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual. Subjects eligible for screening will have an anterior nasal swab collected for testing by rapid antigen testing (RAT) for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/exclusion criteria may be enrolled into the study. All enrolled subjects will record the following information in a Study Diary: • Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14 • Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol) or other anti-pyretic medications. • Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14 • Doses of antipyretic, expectorant, and/or throat lozenges

	taken for symptomatic relief each day through Day 14 Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result on culture) as well as other virologic assessments (e.g. PCR, genotypic testing) All virologic assessments will be performed by a central laboratory. Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis. At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.
Study Population:	Male and female subjects, 18 years of age and older, with symptoms consistent with a diagnosis of uncomplicated acute influenza infection may be screened for enrollment. Subject eligibility will require the presence of two or more symptoms consistent with acute influenza as well as positive results obtained from a rapid antigen test (RAT) for influenza A or B at screening.
Inclusion Criteria:	1. Male and non-pregnant female subjects age ≥18 years. 2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one-hour. 3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening. 4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity.

	5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity. 6. Onset of symptoms no more than 48 hours before presentation for screening. 7. Written informed consent.
Exclusion Criteria:	1. Women who are pregnant or breast-feeding. 2. Presence of clinically significant signs of acute respiratory distress. 3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma. 4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the past 12 months. 5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia. 6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min). 7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza. 8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics. 9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening. 10. Currently receiving treatment for viral hepatitis B or viral hepatitis C. 11. Presence of known HIV infection with a CD4 count <350 cell/mm^3. 12. Current therapy with oral warfarin or other systemic anticoagulant. 13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening. 14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days. 15. Immunized against influenza with inactivated virus vaccine within the previous 14 days. 16. Receipt of any intramuscular injection within the previous

	14 days. 17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study. 18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study 19. Participation in a study of any investigational drug or device within the last 30 days.
Study Endpoints:	
Primary Endpoint:	*Clinical:* Time to alleviation of clinical symptoms of influenza.
Secondary Endpoint(s):	*Safety:* Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests. *Clinical:* Time to resolution of fever. Incidence of influenza related complications. *Virologic:* Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).
Exploratory Endpoint(s):	*Pharmacoeconomic*: Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity. *Virologic:* Quantitative change in influenza virus shedding, measured by PCR. Change in influenza virus susceptibility to neuraminidase inhibitors.
Investigational Product, Dose, and Mode of Administration:	
Peramivir (BCX-1812), 75mg/mL, 2mL per injection, administered as bilateral injections.	
Reference Therapy, Dose, and Mode of Administration:	
Matching Placebo (buffered diluent), 2mL per injection administered as bilateral injections.	
Duration of Treatment:	Following treatment on day 1, study duration for all subjects is expected to be up to 14 days (including all visits). Presence of unresolved adverse events and/or treatment-emergent laboratory findings at the Day 14 visit, or persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit, will require additional follow up.
Statistical Methods:	
Study Hypothesis:	The null hypothesis (H_0) for this study is that the time to

<table>
<tr><td></td><td>alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir 150mg (H_{01}) or peramivir 300mg (H_{02}).

The alternative hypothesis (H_1) is that subjects treated with peramivir 150mg (H_{11}) or peramivir 300mg (H_{12}) have an improvement in time to alleviation of influenza symptoms over those treated with placebo.</td></tr>
<tr><td>Sample Size:</td><td>From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation for the 150 mg dose peramivir arm will be reduced by 30% compared to placebo (see table below) yielding a hazard ratio of 0.70.

<table>
<tr><td colspan="3">Median Time To Alleviation of Symptoms (Hours)</td></tr>
<tr><td>Placebo</td><td>Peramivir 150mg</td><td>Difference (hours)</td></tr>
<tr><td>145.0</td><td>101.5</td><td>43.5</td></tr>
<tr><td>140.0</td><td>98.0</td><td>42.0</td></tr>
<tr><td>135.0</td><td>94.5</td><td>40.5</td></tr>
<tr><td>130.0</td><td>91.0</td><td>39.0</td></tr>
<tr><td>125.0</td><td>87.5</td><td>37.5</td></tr>
<tr><td>120.0</td><td>84.0</td><td>36.0</td></tr>
<tr><td>115.0</td><td>80.5</td><td>34.5</td></tr>
<tr><td>110.0</td><td>77.0</td><td>33.0</td></tr>
<tr><td>105.0</td><td>73.5</td><td>31.5</td></tr>
<tr><td>100.0</td><td>70.0</td><td>30.0</td></tr>
</table>

Using these assumptions, a sample size of 300 evaluable subjects per active treatment group and 150 evaluable subjects in the placebo group (a total of 750 evaluable subjects) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).</td></tr>
<tr><td>Efficacy:</td><td>The intent-to-treat infected population (ITTI) will include all subjects who are randomized, received study drug, and have confirmed influenza by primary viral culture or PCR. The primary efficacy variable is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when each of seven symptoms of influenza are either absent or are present at no more than mild severity level and remain at no worse than this severity status for a 21.5 hour (24 hours – 10%) period.</td></tr>
</table>

	Descriptive statistics for the primary efficacy variable will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment difference will be assessed using a Cox Regression model with effects for BMI at screening, influenza type by PCR at screening, treatment group, and, if necessary, influenza season at randomization. Pairwise comparisons between each active group and placebo will be constructed from the Cox Regression model. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing assessment. Time to resolution of fever will be analyzed in a similar manner. Efficacy analyses will be repeated for a Per-Protocol Infected population (PPI). This population will include those subjects in the ITTI population who received an adequate intramuscular injection. Details of this population will be described in the statistical analysis plan. The PPI population analysis will be used as supportive to the primary analysis with the ITTI. Changes in influenza virus $TCID_{50}$ (viral titers from nasopharyngeal specimens) will be compared using the van Elteren statistic controlling for BMI at screening, influenza type by PCR, and, if necessary, for influenza season at randomization. Analyses of other continuous endpoints will be analyzed in a similar manner. The number and percentage of subjects experiencing influenza related complications (IRC) will be summarized by complication preferred term and treatment group. The difference between the treatment groups will be assessed using a logistic regression model with factors for treatment group, BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary). Pairwise differences between the treatment groups will be evaluated using contrasts from the final logistic regression model.
Safety:	Safety analyses will be presented for all subjects in the safety population, defined as all randomized subjects who receive at least one dose of study drug. Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. The occurrence of treatment-emergent AEs will be summarized using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs that are related to study medication will be

	generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Descriptive summaries of vital signs and quantitative clinical laboratory changes will be presented by study visit. Frequency and percentages of subjects with abnormal laboratory test results will be summarized by toxicity grade. Concomitant medications will be mapped to a WHO preferred term and drug classification. The number and percent of subjects taking concomitant medications will be summarized using preferred terms and drug classifications. The number and percent of subjects experiencing each abnormal physical examination finding will be presented. The number and percent of subjects discontinuing study as well as the reasons for discontinuation will be summarized by treatment group.
Date of Protocol:	Version 1.0: 04-September-2007
Amendment (Dates):	Version 2.0: 05-October-2007

3 TABLE OF CONTENTS

3.1 List of Figures

3.2 List of Tables

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0–72}$	area under the curve from time 0 to 72 hours
$AUC_{0–\infty}$	area under the curve extrapolated from time 0 to infinity
BMI	Body Mass Index in kg/m^2
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CIOMS	Council for International Organizations of Medical sciences
C_{max}	maximum plasma concentration
CK	creatine kinase
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRF	Case Report Form
CV	coefficient of variation
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRC	influenza related complications
ITT	intent-to-treat
ITTI	intent-to-treat infected
IUD	intrauterine device
IVRS	interactive voice response system
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRU	medical resource utilization
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction
RAT	Rapid Antigen Test
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan

SD	standard deviation
SUSAR	Suspected Unexpected Serious Adverse Event
$t_{1/2}$	elimination half-life
$t_{1/2}\,\lambda z$	terminal half-life
$TCID_{50}$	tissue-culture infective $dose_{50}$
TEAEs	treatment-emergent adverse events
T_{max}	time to attain maximum plasma concentration
WBC	white blood cell
WHO	World Health Organization

5 INTRODUCTION

5.1 Background

Influenza virus is a member of the *orthomyxovirus* family and causes an acute viral disease of the respiratory tract. Typical influenza illness is characterized by abrupt onset of fever, headache, myalgia, sore throat, and nonproductive cough.[1] The illness is usually self-limiting, with relief of symptoms occurring within 5 to 7 days. Nevertheless, it is an important disease for several reasons, including ease of communicability, short incubation time, rapid rate of viral mutation, morbidity with resultant loss of productivity, risk of complicating conditions, and increased risk of death, particularly in the elderly. During 19 of the 23 influenza seasons between 1972/1973 and 1994/1995, estimated influenza-associated deaths in the United States ranged from approximately 25 to more than 150 per 100,000 persons above 65 years of age, accounting for more than 90% of the deaths attributed to pneumonia and influenza.[2]

Presently, only a few measures are available that can reduce the impact of influenza: active immunoprophylaxis with an inactivated or live attenuated vaccine and chemoprophylaxis or therapy with an influenza-specific antiviral drug. Neuraminidase inhibitors are the current mainstay of antiviral treatment for influenza. Marketed neuraminidase inhibitors include zanamivir (Relenza®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche-Gilead), an oral prodrug of the active agent, oseltamivir carboxylate. Influenza neuraminidase is a surface glycoprotein that cleaves sialic acid residues from glycoproteins and glycolipids. The enzyme is responsible for the release of new viral particles from infected cells and may also assist in the spreading of virus through the mucus within the respiratory tract. The neuraminidase inhibitors represent an important advance in the treatment of influenza with respect to activity against influenza A and B viruses, with proven therapeutic value in reducing influenza lower respiratory complications,[3] and lower rates of antiviral drug resistance[4].

The use of currently available neuraminidase inhibitors has been limited by concerns including, the degree of effectiveness, the requirement for an inhaler device (zanamivir), and the emergence of resistant influenza virus variants in some treated populations.[5] In addition, there are risks of bronchospasm with zanamivir; and gastrointestinal side effects, with oseltamivir.

Peramivir is a neuraminidase inhibitor that represents a potentially promising addition to the armamentarium of drugs for the treatment of influenza infections due to its potential for parenteral administration and lower frequency of dosing.

5.2 Rationale for Study

An oral formulation of peramivir has previously been evaluated in a full range of safety, tolerability, pharmacokinetic, and efficacy studies. In a multinational phase 3 clinical trial conducted in 1999-2001, oral peramivir demonstrated antiviral activity against influenza A and B infections, and improvement in the relief of clinical symptoms. Because of the limited bioavailability of peramivir following oral administration (<5%), it was determined that the parenteral route of administration is more appropriate for the delivery of peramivir. Subsequent phase 1 studies of intravenous and intramuscular formulations of peramivir have confirmed that parenteral routes of administration result in plasma levels of drug that are as much as 100 times those achieved via the oral route. Further details of these studies are provided below and in the Investigator Brochure.

Because of the previous demonstration of significant antiviral activity, the strong suggestion of clinical efficacy of oral peramivir previously demonstrated in acute influenza, and the encouraging pharmacokinetic and preliminary safety profile of the intramuscular formulation of peramivir demonstrated to date, this phase 3 study will be conducted to evaluate the efficacy and safety profile of intramuscular peramivir and to determine the optimal single dose regimen.

5.3 Non-Clinical Experience with Peramivir

5.3.1 In vitro Assays

Peramivir is a selective inhibitor of viral neuraminidase, with 50% inhibitory concentrations (IC_{50}) for bacterial and mammalian enzymes of >300μM.[6] In an *in vitro* study, 42 influenza A and 23 influenza B isolates were collected from untreated subjects during the 1999–2000 influenza season in Canada.[7] These isolates were tested for their susceptibility to the neuraminidase inhibitors zanamivir, oseltamivir carboxylate, and peramivir using a chemiluminescent neuraminidase assay. Inhibition of Type A influenza neuraminidase by peramivir was approximately an order of magnitude greater than inhibition of neuraminidase from Type B viruses. IC_{50} values for the Type A enzymes ranged from <0.1 to 1.4nM, whereas the Type B enzymes ranged from <0.1 to 11nM, with three out of four values in the 5- to 11nM range. Peramivir was the most potent drug against influenza A (H3N2) viruses with a mean IC_{50} of 0.60nM as well as most potent against influenza B with a mean IC_{50} of 0.87nM.

In another *in vitro* comparison of peramivir, oseltamivir, and zanamivir, using a neuraminidase inhibition assay with influenza A viruses, the median IC_{50} of peramivir (approximately 0.34nM) was comparable to that of oseltamivir (0.45nM) and significantly lower than zanamivir (0.95nM). For influenza B virus clinical isolates, the median IC_{50} of peramivir (1.36nM) was comparable to that of zanamivir (2.7nM) and lower than that of oseltamivir (8.5nM).[8]

The potency of peramivir was evaluated against five zanamivir-resistant and six oseltamivir-resistant influenza viruses.[9] Peramivir remained a potent inhibitor against all oseltamivir-resistant viruses including the mutations H274Y, R292K, E119V, and D198N, with IC_{50} values ≤40nM. Peramivir also potently inhibited ($IC_{50} \leq 26$nM) the neuraminidase activity of zanamivir-resistant strains, which had the following mutations: R292K, E119G, E119A, and E119D. However, one zanamivir-resistant influenza B virus, B/Mem/96, with a mutation R152K isolated from cell culture, was relatively resistant to all neuraminidase inhibitors, including peramivir (IC_{50} = 400nM).

5.3.2 Animal Models

In a mouse model of influenza infection, a single intramuscular injection of peramivir (10mg/kg) given 4 hours prior to inoculation with an A/NWS/33 (H1N1) influenza strain resulted in 100% survival in contrast to 100% mortality in a control group injected with saline.[6] In the same mouse model, treatment of mice up to 72 hours after influenza infection using peramivir (20mg/kg) resulted in 100% survival, compared to 100% mortality in the control group injected with vehicle.[10]

Peramivir has also demonstrated activity in animal models utilizing a clinical H5N1 isolate as the infecting virus strain. In a mouse model, a single intramuscular dose of peramivir (30mg/kg) injected 1 hour after inoculation with the highly pathogenic (H5N1) A/Vietnam/1203/04 strain, resulted in a 70% survival rate that was similar to that seen in mice treated with oseltamivir given

orally at 10mg/kg/day for 5 days[11]. In similar experiments, mice inoculated with the same strain of H5N1 virus that were then treated for up to 8 days with intramuscular peramivir exhibited 100% survival[12]. This longer duration of peramivir treatment also prevented viral replication in the lungs, brain and spleen at days 3, 6 and 9 post inoculation.

5.4 Previous Phase 3 Clinical Experience with Oral Peramivir

An oral formulation of peramivir has previously demonstrated antiviral activity and preliminary clinical efficacy in challenge studies in human volunteers, as well as in treatment studies in patients with uncomplicated acute influenza infections during the influenza seasons of 1999-2001. A Phase 3 multinational study (BC-01-03) of oral peramivir was conducted. Two dose regimens of oral peramivir, 800mg QD for 5 days, or 800mg QD on Day 1, followed by 400mg QD for 4 days, were compared to a matched placebo treatment group. A total of 1246 subjects were randomized to treatment at sites in the USA, Western and Eastern Europe, South America, Australia and New Zealand. As presented in the Table 1 below, the primary end-point of time to relief of influenza symptoms in 694 subjects with confirmed influenza was not found to be significantly different (p=0.17) between the three treatment groups.[13] A sub-group analysis of the time to relief of symptoms by country or region demonstrated marked differences in the primary endpoint.. In the subset of influenza-infected subjects enrolled at sites in the US, clinically meaningful differences in time to relief of influenza symptoms between the placebo and the two peramivir arms were observed, however statistical significance (p=0.07) was not achieved. However, a number of secondary endpoints in this phase 3 study, such as time to overall well-being, time to normal activity, incidence of influenza related complications and quantity of viral shedding, achieved or approached statistically significant differences between the peramivir and placebo treatment groups (p=0.03-0.06).

Table 1 Results of study BC-01-03

Dose and Regimen	Median Time to Relief of Influenza Symptoms (Hours)	
	Overall Results (n=694)	US Sites (n=198)
Peramivir 800mg po x 5d	89.0	70.8
Peramivir 800mg po x 1d and 400mg po x 4d	91.7	88.8
Placebo x 5 days	104.4	106.8
p value	0.17	0.07

5.5 Previous Phase 1 Experience with Intramuscular Peramivir

Two phase 1studies evaluating the safety and pharmacokinetics of an intramuscular formulation of peramivir have been conducted in a total of 45 healthy volunteers receiving peramivir.

Study Peramivir-Him-06-111 evaluated the single dose pharmacokinetics and tolerability of 75mg, 150mg and 300mg doses of peramivir administered as intramuscular (i.m.) and intravenous (i.v.) injections in a crossover design (9 subjects per group). Peak plasma levels of

i.m. peramivir generally occurred within 30 minutes following injection. Plasma pharmacokinetic parameters for i.m. peramivir are summarized in Table 2 below for the three intramuscular single dose regimens evaluated.

Table 2 Pharmacokinetic parameters from study Him-06-111.

Dose (mg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (hr·ng/mL)	$t_{1/2}$[a] (hr)
75 i.m.	4296 ± 812	11659 ± 1123	19.8 ± 7.9
150 i.m.	7612 ± 884	23952 ± 3804	24.3 ± 4.1
300 i.m.	15150 ± 2367	49649 ± 5619	22.8 ± 2.5
[a]terminal half life			

In a second phase 1 study, Peramivir-Him-06-112, the same dose levels of peramivir were administered as single i.m. injections on two consecutive days (6 subjects per group). This double-blind study also included a placebo arm. The pharmacokinetic parameters of i.m. peramivir following the second day of dosing were consistent with those seen following single doses of the drug.

The observations of safety and tolerability of i.m. peramivir in each of the 2 phase 1 studies were unremarkable. No serious adverse events were reported. The most commonly observed adverse events or laboratory abnormalities were headache, several reports of signs and symptoms of vasovagal reactions following injections, and transient increases in creatine kinase. No consistent differences in frequency of adverse events were observed between the active and placebo treatment groups, with the exception that CK elevations appeared to be dose related in the peramivir treatment groups. The vasovagal reactions were attributed to the receipt of relatively large volumes of i.m. injection (2 injections each of 2mL) in the fasted state.

5.6 Phase 2 Experience with Intramuscular Peramivir

A phase 2 study BCX1812-211 was completed in 2007. This study was a randomized, double-blind placebo- controlled study to evaluate the efficacy and safety of two single dose regimens of peramivir. A total of 344 subjects were enrolled into this study with 115 subjects randomized to Placebo; 114 subjects randomized to peramivir 150 mg; and 114 subjects randomized to peramivir 300 mg. The primary endpoint of the study was the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza. Based on preliminary data, the primary endpoint of time to alleviation of clinical symptoms in BCX1812-211 did not achieve statistical significance in the pre-planned ITTI study population (Table 3). Based on pre-planned and post hoc analyses, it appeared that a majority of subjects within this phase 2 study did not receive an adequate intramuscular injection.

In phase 1 studies (Him-06-111 and Him-06-112) of i.m. peramivir, significant increases in creatine kinase (CK) were observed at Day 3 compared with Baseline (Day 1) in all subjects who received active study drug or placebo. CK is a well established marker of muscle damage, and it was hypothesized that CK increase may act as a surrogate marker of an adequate i.m. injection.

Within the phase 2 study, an increase in CK between Baseline (Day 1) and Day 3 was not observed in a majority of subjects. In the phase 1 studies study drug was administered with a 1½

inch needle. In the phase 2 study a shorter needle (1 inch) was supplied with the study drug, with guidance that a longer needle (1½ inch) should be used for larger subjects. Based on the observed lack of CK increases at Day 3 compared to baseline, the Sponsor hypothesized that the needle used for injection failed to penetrate muscle and deliver intramuscular study medication in many subjects.

A sub group of subjects was identified in which a Day 3 CK increase of at least 50U/L was observed over baseline. Within this adequate intramuscular injection sub-group, notable improvements in the time to alleviation of symptoms were observed for both peramivir dose groups: 44.6 hours for peramivir 150 mg treatment and 64.8 hours for the peramivir 300 mg treatment (Table 3). These efficacy data support the further development of peramivir as a single dose, intramuscular treatment for acute influenza.

Table 3 Summary of Efficacy from BCX1812-211.

	Placebo	Peramivir 150mg	Peramivir 300mg
Intent-to-Treat Infected Population[1] (n=313) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=107 137.0 115.9-163.8	n=104 114.1 95.2-145.5 22.9	n=102 115.9 77.8-136.6 21.1
Adequate Injection Population[2] (n=101) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=40 152.2 103.8-183.9	n=32 107.6 76.8-175.1 44.6	n=29 87.4 40.8-163.8 64.8
[1]: Intent-to-Treat Infected Population: PCR+ for either Influenza A and/or Influenza B at baseline/screening visit. [2]: Adequate Injection Population: ITTI subjects in who study drug reached target muscle tissue, as evidenced by an increase in serum CK levels of ≥ 50 U/L over baseline at the Day 3 study visit.			

An independent data monitoring committee reviewed grouped blinded safety data throughout study BCX1812-211. In the overall safety population (n=342), doses of peramivir 150 mg and 300 mg were both found to be well tolerated and no safety concerns were identified by the DMC. The three treatment groups were similar with respect to the frequency and severity of adverse events. Two serious adverse events were reported in the study, and neither was considered by the investigator to be related to treatment. One SAE (pyelonephritis) occurred 5-days after study treatment in a subject who received placebo, and one SAE (meningitis, resulting in death) occurred 10-days after study treatment in a subject who received 300 mg of peramivir. There were no meaningful differences among the three treatment groups with respect to the frequency or severity of graded laboratory toxicities. A summary of the adverse events and graded toxicities, together with a list of the most frequently reported adverse events, is presented in Table 4.

Table 4 Summary of Safety from BCX1812-211.

Safety Parameters	Placebo (N=114)	Peramivir 150 mg (N=113)	Peramivir 300 mg (N=115)
Any Clinical Adverse Event	49 (43%)	43 (38%)	44 (38%)
Any Graded Laboratory Toxicity	99 (87%)	93 (82%)	92 (80%)
Any Serious Adverse Event	1 (<1%)	0	1 (<1%)
Most Frequent Adverse Events Assessed as Study Drug-Related			
Diarrhea	5 (4%)	5 (4%)	6 (5%)
Nausea	7 (6%)	7 (6%)	9 (8%)
Vasovagal Reaction	4 (4%)	2 (2%)	0

5.7 Dose Rationale

Oseltamivir is approved for the treatment of uncomplicated acute influenza at a dosage of 75mg twice daily in adults[14]. Oseltamivir was shown to be clinically effective in a phase 3 study of oral oseltamivir versus placebo in naturally occurring seasonal influenza, and these data were sufficient for regulatory approval for marketing of oseltamivir. At least 75% of an oral dose of oseltamivir reaches the systemic circulation as oseltamivir carboxylate. When oseltamivir is administered orally at a dose of 75mg twice daily, the serum C_{max} of oseltamivir carboxylate is approximately 348ng/mL and the AUC_{0-48} is 10,876 h·ng/mL. The clinical data indicate that this level of exposure to oseltamivir was sufficient to provide clinical improvement in uncomplicated acute influenza.

The serum pharmacokinetic data (C_{max} and $AUC_{0-\infty,}$ respectively) following intramuscular doses of peramivir are approximately 7600ng/mL and 24,000 h·ng/mL for the 150mg dose and are approximately 15,000ng/mL and 49,000 h·ng/mL for the 300mg dose. Previous studies have assessed the concentrations of the neuraminidase inhibitor zanamivir in nasal and pharyngeal secretions after parenteral administration of this drug. . Within several hours after administration, the concentrations in secretions were approximately 100-fold lower than in serum or plasma. In theory, relatively high levels of a neuraminidase inhibitor in respiratory secretions are desirable in order to rapidly inactivate influenza virus and to delay or prevent the development of resistance in infecting virus strains. Intramuscular doses of peramivir, including doses of 150mg and 300mg have been shown to be well tolerated in previous Phase 1 studies. In the completed Phase 2 study, both doses of peramivir (150 mg and 300 mg) were well tolerated and no safety concerns were apparent. Therefore, it is appropriate for these two dose regimens to undergo further evaluation in this Phase 3 study.

6 STUDY OBJECTIVES

6.1 Objectives

6.1.1 Primary Objective

To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza.

6.1.2 Secondary Objective(s)

The secondary objectives of this study are:

1. To evaluate the safety and tolerability of peramivir administered intramuscularly,
2. To evaluate secondary clinical outcomes in response to treatment,
3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment.

6.1.3 Exploratory Objective(s)

The following exploratory objectives have been identified for this study.

1. To assess pharmacoeconomic measures as response to treatment.
2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment.

6.2 Study Endpoints

6.2.1 Primary Endpoint

The primary clinical endpoint is the time to alleviation of clinical symptoms of influenza for each subject.

6.2.2 Secondary Endpoint(s)

Secondary safety, clinical, and virologic endpoints will include evaluations in each subject of:

Safety: Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests.

Clinical: Time to resolution of fever; Incidence of influenza related complications.

Virologic: Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).

6.2.3 Exploratory Endpoints

Pharmacoeconomic and virologic evaluations in each subject for exploratory endpoints will also be assessed and include:

Pharmacoeconomic:	Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.
Virologic:	Quantitative change in influenza virus shedding, measured by PCR; Change in influenza virus susceptibility to neuraminidase inhibitors.

7 STUDY DESIGN

7.1 Overall Study Design and Plan

This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza.

Each subject's assignment to treatment will be stratified according to body mass index (BMI). Two BMI strata are planned: Normal-Overweight (≤ 29.9 kg/m^2), and Obese (≥ 30.0 kg/m^2). The number of subjects enrolled who have a BMI ≤ 29.9 kg/m^2 (Normal-Overweight) will be at least 75% of the total enrollment. The number of subjects enrolled who have a BMI ≥ 30.0 kg/m^2 (Obese) will be $\leq 25\%$ of total enrollment. All subjects will be centrally randomized to one of three treatment groups in a ratio of 2:2:1 such that 80% of subjects are randomized to one of the two single dose regimens of peramivir.

Treatment Group 1: Peramivir 150mg
Treatment Group 2: Peramivir 300mg
Treatment Group 3: Placebo

Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in equally divided doses). Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

Subjects eligible for screening will have an anterior nasal swab collected for testing by RAT for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/ exclusion criteria may be enrolled into the study.

All enrolled subjects will record the following information in a Study Diary.

- Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14.

- Oral temperature measurements will be taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol) or other antipyretic medication.

- Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14

- Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14

Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result) as well as other virologic assessments (e.g. PCR, genotypic testing). All virologic assessments will be performed by a central laboratory.

Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis.

At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK. Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in divided doses). Procedures for intramuscular injection, with a recommended needle length appropriate to the size and weight of the subject, are provided in the study drug administration manual.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1.1 Inclusion Criteria

Subjects must meet all of the following criteria for inclusion in this study:

1. Male and non-pregnant female subjects age ≥18 years.
2. A positive Influenza A or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one hour.
3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening.
4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity.

5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity.
6. Onset of symptoms no more than 48 hours before presentation for screening.
7. Written informed consent.

8.1.2 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

1. Women who are pregnant or breast-feeding.
2. Presence of clinically significant signs of acute respiratory distress
3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma. (See Section 15.2).
4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the past 12 months. (See Section 15.1).
5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.
6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min).
7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza.
8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics.
9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening.
10. Currently receiving treatment for viral hepatitis B or viral hepatitis C.
11. Presence of known HIV infection with a CD4 count <350 cell/mm^3.
12. Current therapy with oral warfarin or other systemic anticoagulant.
13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening.
14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days.
15. Immunized against influenza with inactivated virus vaccine within the previous 14 days.
16. Receipt of any intramuscular injection within the previous 14 days.
17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study.
18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study.
19. Participation in a study of any investigational drug or device within the last 30 days.

8.1.3 Removal of Subjects from Therapy or Assessment

All subjects are permitted to withdraw from participation in this study at any time and for any reason, specified or unspecified, and without prejudice. The Investigator or sponsor may

terminate the subject's participation in the study at any time for reasons including the following:

1. Adverse event;
2. Intercurrent illness;
3. Non-compliance with study procedures;
4. Subject's decision;
5. Administrative reasons;
6. Lack of efficacy;
7. Investigator's opinion to protect the subject's best interest.

Any subject who withdraws because of an adverse event will be followed until the sign(s) or symptom(s) that constituted the adverse event has/have resolved or is determined to represent a stable medical condition.

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary, or if it is the desire of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject and determine the subject's medical condition. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data.

9 TREATMENTS

9.1 Treatments Administered

Peramivir is an investigational drug. Peramivir for intramuscular injection is a small-volume parenteral and will be supplied as a 75mg/mL solution in sodium citrate/ citric acid buffer. The pH is approximately 3.0.

A matched placebo solution of sodium citrate/ citric acid buffer with 1.2% sodium chloride at a pH of approximately 3.0 will be supplied.

The gluteal site of injection and the syringe needle length are to be recorded in the subjects CRF. Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

9.2 Identity of Investigational Product(s)

Peramivir and placebo peramivir will be supplied in clear 2mL vials. An individual study drug kit will contain 2 vials of blinded study drug (peramivir and/or placebo, depending upon the treatment group), 2 syringes and 2 needles in which to draw up the solution for intramuscular injection. All materials will be packaged in a labeled box container. All study drug kits must be stored at 2-8^{o}C.

Each individual study drug kit will be labeled with some or all of the following information as required by local regulations:

- Sponsor name and contact information, study protocol number, kit number, description of the contents of the container, instructions for the preparation of the syringe and administration of the study drug, conditions for storage, statement regarding the investigational (clinical trial) use of the study drug and date for retest or expiry date.

Each vial of study drug will be labeled with some or all of the following information as required by local regulations:

- Sponsor name, study protocol number, description of the contents of the vial, instructions for the preparation of the syringe, statement regarding the investigational (clinical trial) use of the study drug and lot number.

9.3 Method of Assigning Subjects to Treatment Groups

Subjects will be centrally randomized according to BMI strata in a ratio of 2:2:1 to one of three treatment groups: single dose peramivir 150mg, single dose peramivir 300mg or placebo, in accordance with a computer-generated randomization schedule prepared by a non-study statistician. Eighty percent (80%) of subjects will be randomized to treatment with one of the two single dose regimens of peramivir, 20% will be randomized to treatment with placebo.

Each subject's assignment to treatment will be stratified according to body mass index (BMI). Two BMI strata are planned: Normal-Overweight (≤ 29.9 kg/m^2), and Obese (≥ 30.0 kg/m^2). The number of subjects enrolled who have a BMI ≤ 29.9 kg/m^2 (Normal-Overweight) will be at least 75% of the total enrollment. The number of subjects enrolled who have a BMI ≥ 30.0 kg/m^2 (Obese) will be $\leq 25\%$ of total enrollment.

Once a subject is eligible for randomization, he/she will be assigned a study drug kit number that will be obtained by study staff from the study interactive voice response system (IVRS). Once a study drug kit number has been assigned to a subject, it cannot be reassigned to any other subject.

9.4 Study Medication Accountability

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the sponsor, issued to the subject or directly administered to the subject (including date and time), and any drug accidentally destroyed. The sponsor will supply a specific drug-accountability form. At the end of the study, information describing study drug supplies (e.g., lot numbers) and disposition of supplies for each subject must be provided, signed by the Investigator or designee, and collected by the study monitor. If any errors or irregularities in any shipment of study medication to the site are discovered at any time, the Project Manager must be contacted immediately.

At the end of the study, all medication not dispensed or administered and packaging materials will be collected with supervision of the monitor and returned to the sponsor or destroyed on site as dictated by the appropriate Standard Operating Procedure at the participating institution.

9.5 Blinding/Unblinding of Treatments

This is a double-blind study. The treatment group assignment will not be known by the study subjects, the investigator, the clinical staff, the CRO or Sponsor staff during the conduct of the

study.

Section 11.24 provides information regarding the process for unblinding the treatment assignment, if necessary, in the event of an SAE.

9.6 Prior and Concomitant Therapies

All medications, by any route of administration, used during this study must be documented on the Case Report Form (CRF). Prescription as well as non-prescription medications should be recorded. Medication used for the treatment of influenza-related symptoms will be captured by the subject in the diary card provided by BioCryst.

9.7 Overdose and Toxicity Management

To date there is no experience with overdose of intramuscular or intravenous peramivir. If overdose occurs, subjects should receive indicated supportive therapy and evaluation of hematologic and clinical chemistry laboratory tests should be conducted. The effect of hemodialysis on elimination of peramivir is unknown.

9.8 Dose Interruption

As this is a study of a single injection of peramivir or placebo, guidelines for treatment interruption for drug related SAEs or toxicities are not applicable.

10 STUDY CONDUCT

A study schedule of evaluations is presented in Figure 1. A detailed list of the evaluations an visits is also provided in the following sections.

10.1 Evaluations

All subjects enrolled in this study will undergo the following evaluations:

10.1.1 Medical History

Medical history, influenza vaccination status within the previous 12 months and demographic data (including smoking behavior) will be recorded at Screening/Baseline.

10.1.2 Rapid Antigen Test for Influenza

At Screening/Baseline, a commercially available, rapid antigen test (RAT) for influenza A and B will be performed on an adequate specimen collected by swabbing the anterior nose in accordance with the RAT manufacturer' instructions. A negative initial RAT should be repeated within one hour. Refer to the Study Manual for instructions regarding the use of the RAT kits provided for this study. Sites may use the kits provided by the Sponsor or any other commercially approved RAT available at their site to document a confirmed influenza infection.

10.1.3 Physical Examination and Influenza-related Complications Assessments

The Investigator will perform a physical examination at Screening/Baseline. Subject's height and weight, and BMI will be recorded at Screening/Baseline in the subjects CRF.

Study personnel will be provided with an influenza-related complications (IRC) checklist in the CRF to evaluate the subject for the presence of clinical signs and/or symptoms of the following influenza-related complications: sinusitis, otitis, bronchitis and pneumonia. Note that subjects with clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis, and/or pneumonia at screening are not eligible for enrollment in this study (See Section 8.1.2 exclusion criteria number 8).

A targeted physical examination will be conducted at each visit to record the presence/absence of influenza related complications (IRC). If the investigator determines that the subject experiences (or is presumed to experience) an IRC as noted above, he/she will record that assessment on the IRC CRF page and any medication used to treat the condition will be recorded on the concomitant medication page. The investigator will promptly provide appropriate treatment for any suspected or proven IRC. Such information describing IRC signs and/or symptoms should not be reported as adverse events. Any injection site reactions noted will be recorded in the CRFs as adverse events.

10.1.4 Vital Signs

Vital signs evaluations will include blood pressure, pulse rate, and respiration rate. The investigator will record oral body temperature at baseline. Thereafter the subject will record oral temperature twice daily in the study diary card.

Vital signs will be measured at Screening/Baseline, pre-dose, and at 15 minutes following the study drug injection on Day 1, then once daily on Days 3, 5, 9, and 14.

10.1.5 Electrocardiogram Measurements

A 12-lead electrocardiogram (ECG) will be obtained at Screening/Baseline. The principal investigator will be responsible for interpretation of the Screening ECG. This interpretation may be performed by the investigator or he/she may delegate this action to another physician and the investigator will acknowledge the interpretation. If this baseline ECG is interpreted as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal but does not meet the exclusionary criteria, the subject may be enrolled unless other exclusion criteria apply. The principal investigator is responsible to ensure that such an enrolled subject be informed of the nature of the abnormal ECG and that any medically indicated repeat ECG examinations and/or referral of the subject for further evaluation is made either during subject's participation in the study or immediately after the subject's discharge from the study.

10.1.6 Clinical Laboratories

Clinical chemistry profiles will include a Chemistry 20 panel (includes sodium, potassium, chloride, total CO_2 [bicarbonate], creatinine, glucose, urea nitrogen, albumin, total calcium, total magnesium, phosphorus, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, lactate dehydrogenase [LDH], total protein, total creatine kinase, and uric acid).

Hematology will include complete blood count (CBC) with differential.

Urinalysis will include dipstick tests for protein, glucose, ketones, blood, urobilinogen, nitrite, pH, and specific gravity and microscopic evaluation for RBCs and WBCs.

Clinical laboratory studies (clinical chemistries, hematology, and urinalysis) will be completed at Screening/Baseline, and on Days 3, 5 and 14.

10.1.7 Urine Pregnancy Test

Females of childbearing potential will be evaluated for pregnancy at Screening/Baseline and Day 14 using a urine pregnancy test.

10.1.8 Serology for Influenza

Paired blood samples for determination of antibody to influenza A and B (serology) will be obtained with the clinical laboratory tests at Screening/Enrollment and at Day 14. These specimens will be stored at the central laboratory and will be analyzed if needed to confirm the diagnosis of influenza.

10.1.9 Samples for Virologic Laboratory Assessments

An adequate specimen will be collected by swabbing the anterior nose (bilateral) and posterior pharynx for virologic laboratory assessments including culture for the isolation of influenza virus and/or quantitative PCR assay at Screening/Baseline, and at Days 3, 5, and 9. Refer to the Laboratory Manual for instructions regarding the processing and shipment of these specimens.

10.1.10 Subject Self Assessments

Subject self assessments will be performed beginning pre-dose on Day 1 and recorded in the subject's Study Diary including the following:

- Oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol, provided) or other anti-pyretic medications. The times of each temperature determination will be recorded in the Study Diary. The baseline temperature will be recorded at the screening/Day 1 visit prior to dosing, regardless of whether the subject had recently taken an anti-pyretic; the time of anti-pyretic use pre-treatment will be recorded in the CRF, if applicable.

- Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily, beginning pre-dose on Day 1 and through Day 9, then once daily through Day 14.

- Assessment of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 visual analogue scale once daily through Day 14.

The subject's diary card will be reviewed by study staff at each visit for completion of the record of all required items, with particular emphasis on alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever

(unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms. Study staff will not attempt to ask subjects to retrospectively complete missing diary card data for any scheduled assessments that have not been completed prior to the clinic visit. Study staff should, however, remind the subject to complete the diary card at all scheduled times.

10.1.11 Concomitant Medications

All concomitant medications used during this study, with the exception of those medications taken for symptomatic relief of influenza symptoms, which will be recorded by the subject in their diary card, must be documented on the Case Report Form (CRF).

10.1.12 Adverse Events

AEs will be assessed from the time of administration of study medication through the final study visit.

10.1.13 Pharmacokinetic Exposure Samples

All subjects will have two pharmacokinetic (PK) samples drawn to assess peramivir drug levels. The first PK sample will be drawn on day 1 between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be drawn at the day 3 visit in all subjects. The sample will be drawn at the same time as the blood draw is completed for clinical laboratory investigations. The 30-60 minute sample (treatment day 1) and the day 3 PK sample will be analyzed for plasma concentrations of peramivir (ng/mL) and evaluated in a population exposure response analysis.

At selected sites a separate sub-study will also be conducted to collect additional PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK. Data from these two PK samples in all subjects will be combined with data from the PK sub-study (BCX1812-311PK) to perform a population based exposure-response analysis. This analysis will be described as part of the sub-study analysis plan.

All PK samples will be processed at a central bioanalytical laboratory. Refer to the instructions provided regarding the processing and shipment of these PK samples.

10.2 Screening Period

10.2.1 Informed Consent

The nature and purpose of the study and the expectations of a participating subject will be described to potential study subjects, their questions will be answered, and the subjects will then be asked to sign an informed consent document. Study subjects will then undergo the screening evaluation as noted in Section 10.22

10.2.2 Screening/Baseline Evaluation and Enrollment

Screening/baseline evaluation may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Clinical laboratory assessments performed at Screening are for the purpose of establishing a

baseline. Subjects may be enrolled and receive treatment with study drug prior to receiving results of the laboratory assessments (with the exception of urine pregnancy test result, which must be known).

Eligible subjects will be enrolled and randomized to blinded study treatment. The randomization will be stratified by BMI: Normal-Overweight (BMI ≤ 29.9 kg/m^2) and Obese (BMI ≥ 30.0 kg/m^2). The Investigator will prepare a request for blinded study drug assignment which includes the subject's screening number. The Investigator or designee at the clinical study center will contact the central randomization Interactive Voice System (IVRS call center). The IVRS call center will advise the study center of the investigational study drug kit number that is assigned to that subject at enrollment.

Subjects that are determined to be ineligible will be advised accordingly, and the reason for ineligibility will be discussed. If desired by the subject the reason for ineligibility may be provided and/or discussed with their health-care provider by the Investigator or designee.

Ineligible subjects who have been screened for the study will also be entered on the IVRS. For such subjects, the screening number assigned, subject's date of birth and a reason for ineligibility will be entered on to the IVRS. **All <u>ineligible</u> subjects must be entered onto the IVRS within 24 hours of screening,** to assist with surveillance analysis during the course of the study.

10.3 Treatment Period—Study Day 1

Day 1 represents the only day of study drug dosing. Study drug administration should occur as soon as possible following informed consent, screening and randomization. Therefore, it is expected that the date of Screening/ Baseline and Day 1 will usually be the same date.

10.3.1 Pre-dose Evaluations-Study Day 1

Following an explanation of the Subject Self Assessment measures (Section 10.1.11), the subject shall complete the record of these assessments in their Study Diary prior to dosing. The subject will be counseled regarding the expectations for recording these assessments through Day 14.

Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) and a 12 lead ECG will be obtained prior to dosing.

10.3.2 Post-dose Evaluations-Study Day 1

The blinded study drug will be administered (hour 0) as bilateral intramuscular injections within a period of ≤ 10 minutes. The calendar date and 24-hour clock time of the first and second injections will be recorded. The gluteal site of injection and the syringe needle length are also to be recorded in the subjects CRF. Sites are instructed to follow procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, provided in the study drug administration manual.

The following evaluations will be performed post-dose on Study Day 1:

- Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) 15 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the vital sign measurements in the subjects CRF.

- Draw a PK sample between 30 and 60 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the blood draw.

- Record any concomitant medications.

- Record any AEs.

10.4 Post-Treatment Assessment Period

10.4.1 Days 2, 3, 5, 9 and 14

Study evaluations will be performed on Days 2, 3, 5, 9 and 14 in accordance with the schedule of evaluations (Figure 1). Subjects with persistent moderate or severe influenza symptoms at day 14 will also complete a Day 21 visit, and if required a Day 28 visit.

Visits may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Study staff will attempt to contact the subjects on Day 2 by telephone to confirm their compliance with completion of the Subject Self Assessments, to note any concomitant medications and adverse events. Any adverse events reported by the subject during this telephone contact will be recorded on the adverse event form and verified during the visit on day 3.

At each visit it is important that the subject's Study Diary record be reviewed for completion of daily Subject Self Assessments. The subjects should be counseled as necessary regarding self assessments and Study Diary record requirements. The subject's diary card will be reviewed by study staff for alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms.

Day 3:

The second PK sample for all subjects will be obtained on Day 3 at the same time as the clinical laboratory blood specimen is obtained. The exact 24-hour clock time of the blood draw will be recorded in the subjects CRF.

Day 14:

If a subject has one or more persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit then the subject must be evaluated in further follow-up visits, at day 21 (± 3 days), and if required at day 28 (± 3 days) If a subject reports moderate or severe influenza symptoms then the investigator will record the intensity of each of the influenza symptoms on a visit specific CRF page at the day 21 and day 28 visits. After day 14 the subject will not record symptoms in a diary.

Day 21 (if applicable):

The day 21 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 14. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at the Day 21 visit and such action(s) will be recorded

on the Day 21 CRF page. The investigator will recall the subject for a further study visit at day 28 (± 3 days) if moderate or severe symptom(s) of influenza persist at Day 21.

Day 28 (if applicable):

The day 28 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 21. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at this visit and such action(s) will be recorded on the Day 28 CRF page. No further follow-up visits beyond day 28 are to be formally scheduled unless in the clinical judgment of the investigator further follow-up is required. The investigator will use his/her clinical judgment to manage the subject, referring the subject, if appropriate, for further medical care.

10.4.2 Adverse Events Reported at Post-treatment Visits

In this study, symptoms of influenza will be considered separately from adverse events reported during the post-treatment period. Accordingly, adverse events that have onset in the post-treatment period will be assessed and followed as specified in 11.2. Specifically, the investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

Figure 1 Study Measurements and Visit Schedule

Assessments	Screening [1] (Baseline)	Treatment Period	Assessment Day				End of Study Early Withdrawal
		Day 1[1]	Day 2[2]	Day 3	Day 5 (±1 day)	Day 9 (±3 day)	Day 14 (±3 day) [8]
Informed Consent	X						
Rapid Antigen test for Influenza A & B	X						
Medical History/Physical Exam	X						
Influenza-related complications checklist[3]	X			X	X	X	X
Inclusion/Exclusion	X						
Clinical Chemistries[4]	X			X	X		X
Hematology[4]	X			X	X		X
Exposure Pharmacokinetic Sample		X		X[4]			
Serology (serum) Sample	X						X
Urinalysis[4]	X			X	X		X
Urine Pregnancy Test	X						X
Vital Signs[5]	X	X		X	X	X	X
ECG[6]	X						
Sample (nasopharyngeal swab) for Influenza Virus Culture/ PCR assay and for resistance studies		X		X	X	X	
Study Drug Administration		X					
Subject Diary Review[7]		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

Study Measurements and Visit Schedule Figure Legend on Next Page

Study Measurements and Visit Schedule Figure Legend

[1] It is expected that the date of Screening and Day 1 (date of administration of study drug) will be the same. Visits at Screening and on subsequent study days may occur in subject's home by the investigator (all visits) or appropriately trained study center staff (Day 3, 5, 9 visits).

[2] Day 2 will be a telephone contact with the subject to ensure compliance with diary card completion, concomitant medication and adverse event review.

[3] A targeted physical examination will be conducted at each visit to record the presence/absence of influenza related complications.

[4] Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subject may be enrolled and begin treatment with study drug prior to receiving results. A PK sample will be drawn 30-60 minutes following the second treatment administration injection. On Day 3 an extra tube will be included with the safety blood sample to collect the second PK sample for evaluation of peramivir concentrations.

[5] Vital sign measures will include blood pressure, pulse rate and respiration rate. Vital signs will be recorded at Screening, pre-dose and at 15 min following the study drug administration on Day 1, then once on remaining days as stipulated. The investigator will record oral temperature at baseline. Thereafter the subject will report oral temperature measurements twice daily in the Study Diary

[6] If the baseline ECG is interpreted by the conducting physician as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal and does not meet the exclusionary criteria (e.g. acute ischemia, medically significant dysrhythmia) then this subject may be enrolled If the conditions highlighted in section 10.1.5 are met for the subject.

[7] Subjects record symptom assessment in Study Diary, twice daily, beginning pre-dose on Day 1 through Day 9, then once daily through Day 14. Subjects record time lost from work or usual activities and rating of productivity compared to normal once daily through Day 14. Subjects record oral temperature twice daily throughout as well as all concomitant medication and adverse events.

[8] For any subject with unresolved moderate or severe intensity influenza symptoms a follow up assessment will be scheduled at Day 21 (± 3 days) and Day 28 (± 3 days) if required (See Section 10.4.1).

11 ADVERSE EVENT MANAGEMENT

11.1 Definitions

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

AEs may be designated as "nonserious" or "serious" (see Section 11.1.2).

Surgical procedures are not AEs but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) will be documented in a subject's study diary and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as AEs unless the symptom(s) worsen to the extent that the outcome fulfils the definition of an SAE, which then must be recorded as such (see Section 11.1.2). Likewise, a RAT for influenza is required at screening in order to determine eligibility for the study, and therefore a positive RAT is not considered an AE.

11.1.2 Serious Adverse Event

A SAE is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the

outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

In addition Suspected Unexpected Serious Adverse Reactions (SUSAR) may also be reported to competent authorities where this type of reporting is required (e.g. European Union Directives). See section 11.2.3.

11.2 Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of AEs are to be collected from the time of study drug administration through the follow-up period ending on Day 14. The Investigator or designee must completely and promptly record each AE on the appropriate CRF. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

The Investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

11.2.1 Definition of Severity

All AEs will be assessed (graded) for severity and classified into one of four clearly defined categories as follows:

- **Mild:** (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
- **Moderate:** (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- **Severe:** (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- **Life-threatening:** (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization.

11.2.2 Definition of Relationship to Study Drug

The blinded Principal Investigator must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication, is confirmed by improvement upon stopping the medication (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

11.2.3 Reporting Serious Adverse Events

Any SAE / SUSAR (Suspected Unexpected Serious Adverse Reaction) must be reported to BioCryst or its designee within 24 hours of the Investigator's recognition of the SAE by first notifying the Medical Monitor at the number listed below:

Telephone: **Europe: +44 1628 548000; North America: 1-888-724-4908**

Facsimile: **Europe: +44 1628 540028; North America: 1-888-887-8097 or 1-609-734-9208**

In addition to the telephone numbers listed above, local country-specific toll free numbers may be provided within the study reference manual.

The site is required to fax a completed SAE / SUSAR Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the SAE / SUSAR must be reported and sent by facsimile to BioCryst or its designee as soon as they are available.

The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (e.g., revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and for retaining a copy in their files.

If the Investigator becomes aware of any SAE / SUSAR occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify BioCryst or its designee.

Any SAEs / SUSARs considered possibly related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch / CIOMS reporting system in accordance with FDA and other applicable regulations. However, the Investigator is not obligated to actively seek reports of AEs in former study participants.

While pregnancy is not considered an AE, all cases of fetal drug exposure via parent as study participant (see Section 4.4) are to be reported immediately to BioCryst or its designee. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee.

11.2.4 Emergency Procedures

In the event of an SAE / SUSAR, the Principal Investigator may request the unblinding of the treatment assignment for the subject affected. If time allows (i.e., if appropriate treatment for the SAE is not impeded), the Principal Investigator will first consult with the Medical Monitor regarding the need to unblind the treatment assignment for the subject. At all times, the clinical well-being of any subject outweighs the need to consult with the Medical Monitor.

The Principal Investigator may contact the IVRS central randomization center and request the unblinding of the treatment assignment that corresponds to the affected subject. The IVRS center will record the name of the Investigator making the request, the date and time of the request, the subject number and date of birth. The Sponsor will be informed within 24 hours if unblinding occurred.

12 STATISTICAL METHODS

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and other selected efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. The term "treatment group" refers to randomized treatment assignment: peramivir 150 mg, peramivir 300 mg, or placebo. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date within each subject number.

Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted with the SAS® System, version 9.1.3 or higher.

12.1 Data Collection Methods

The data will be recorded on the CRF approved by BioCryst. The Investigator must submit a completed CRF for each subject who signs an informed consent form (ICF), regardless of duration. All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF.

Documents (including laboratory reports, hospital records subsequent to SAEs, etc.) transmitted

to BioCryst should not carry the subject's name. This will help to ensure subject confidentiality.

12.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be created and approved prior to the review of any data. This document will provide a more technical and detailed description of the proposed data analysis methods and procedures.

12.3 Study Hypothesis

The primary hypothesis for evaluating the primary objective may be stated as follows:

The null hypothesis (H_0) is that the time to alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir 150mg (H_{01}) or peramivir 300mg (H_{02}).

The alternative hypothesis (H_1) is that subjects treated with peramivir 150mg (H_{11}) or peramivir 300mg (H_{12}) have an improvement in time to alleviation of influenza symptoms over those treated with placebo.

12.4 Sample Size Estimates

A total of 750 evaluable subjects randomized in a 2:2:1 (300 subjects treated with peramivir 150mg: 300 subjects treated with peramivir 300 mg: 150 subjects treated with placebo) are estimated for this phase 3 study. Because results of clinic-based RAT tests may not precisely indicate presence of influenza infection, it is expected that at least 850 subjects will be randomized to treatment to ensure that 750 evaluable subjects are treated.

From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation for the 150 mg dose peramivir arm will be reduced by 30% compared to placebo (Table 5) yielding a hazard ratio of 0.70.

Table 5 Median time to alleviation of symptoms (30% reduction, 0.70 hazard ratio).

Median Time To Alleviation of Symptoms (Hours)		
Placebo	Peramivir 150mg	Difference (hours)
145.0	101.5	43.5
140.0	98.0	42.0
135.0	94.5	40.5
130.0	91.0	39.0
125.0	87.5	37.5
120.0	84.0	36.0
115.0	80.5	34.5
110.0	77.0	33.0
105.0	73.5	31.5
100.0	70.0	30.0

Using these assumptions, a sample size of 300 evaluable subjects per active treatment group and 150 evaluable subjects in the placebo group (a total of 750 evaluable subjects) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).

12.5 Analysis Populations

The populations for analysis will include the intent-to-treat (ITT), intent-to-treat infected (ITTI), per-protocol infected (PPI), and safety populations. Additional analysis populations may be defined to evaluate study results. Any additional analysis populations will be defined in the SAP.

Intent-To-Treat Population: The ITT population will include all subjects who are randomized. Subjects will be analyzed in the treatment group to which they were randomized. The ITT population will be used for analyses of accountability and demographics.

Intent-To-Treat Infected Population: The ITTI population will include all subjects who are randomized, received study drug, and have confirmed influenza by culture or PCR. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI population will be used for primary analyses of efficacy.

Per-Protocol Infected: The PPI population includes all subjects in the ITTI population who receive an adequate intramuscular injection. The definition of an adequate intramuscular injection will be further described in the SAP. The PPI population will be used as supportive of the primary analyses for efficacy completed with the ITTI population.

Safety Population: The safety population will include all subjects who received study drug. Subjects will be analyzed according to the treatment received. This population will be used for all safety analyses.

12.6 Interim and End of Study Analyses

Interim Analysis

An independent DMC will review safety data on an ongoing basis. Safety analyses will be presented in a manner consistent with the presentations intended for the final analysis.

End of Study Analysis

A final analysis is planned after the last subject completes or discontinues the study, and the resulting clinical database has been cleaned, quality checked, and locked.

12.7 Efficacy Analyses

12.7.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when a subject has Alleviation of Symptoms. A subject has Alleviation of Symptoms if all of the seven symptoms of influenza (nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, feeling feverish) assessed on his/her subject diary are either absent or are present at no more than mild severity level and at this status for at least 21.5 hours (24 hours - 10%).

Descriptive statistics for the primary efficacy endpoint will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized overall and for individual symptoms for each treatment group. Overall treatment differences will be assessed using a Cox Regression model with effects for BMI at screening, influenza type by PCR, treatment group, and influenza season at randomization (if necessary). Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing post-baseline assessment. Pairwise differences in time to alleviation of symptoms among the treatment groups will be evaluated using contrast statements from the final Cox model. In order to maintain the overall type I error in the presence of the planned comparisons between the two peramivir treatments and placebo, a Bonferroni correction will be applied to the primary efficacy endpoint analysis. P-values for the planned comparisons of each peramivir arm to placebo will be adjusted via a Bonferroni correction (i.e., if the unadjusted p-value for an active comparison versus placebo, p, is less than 0.05, then p^a=p*number of planned comparisons=p*2; otherwise, p^a=p). Superiority of peramivir to placebo will be established if the adjusted p-value is less than or equal to 0.05.

12.7.2 Secondary Efficacy Endpoints

All secondary endpoints will be summarized using descriptive statistics by treatment group, and study day/time, if appropriate. Statistical comparisons for each endpoint will be constructed without adjustment for multiple endpoints.

The reduction in viral shedding will be assessed as the change in viral titers defined as the time-weighted change from baseline in $\log_{10}$ tissue culture infective $dose_{50}$ ($TCID_{50}$/mL) and will be

summarized for each treatment group. The time-weighted average change from baseline will be calculated on a by-subject basis through Day 9 using the trapezoidal rule with all available post-baseline on-treatment data (data after initiation of study treatment) minus the baseline value. Specifically, the time-weighted area under the curve for time a (t_a) to time b (t_b) is given by the formula

$$TWAUC = \frac{AUC(t_a - t_b)}{(t_a - t_b)},$$

where $AUC(t_a - t_b) = \sum_{i=a}^{b-1} \frac{(y_i + y_{i+1})(t_{i-1} - t_i)}{2}$ and t_i represents the date of the i^{th} viral titer assessment and y_i represents the $\log_{10}$ value of the i^{th} viral titer assessment. If there is a baseline value and only one follow-up value, y_i then the time-weighted change from baseline is defined as the difference between y_i and baseline. If there is a baseline value and no follow-up value, the subject is excluded from analysis. The differences between each of the peramivir treatment groups and placebo will be evaluated using a van Elteren Test adjusting for BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary). Analyses of the PCR results will be analyzed in a similar manner.

Subject's oral temperature will be summarized by study visit and treatment group. Differences between the treatment groups will be assessed using the Wilcoxon Rank Sum Test controlling for BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary). A subject has Resolution of Fever if he/she has a temperature < 37.2°C (99.0°F) and no antipyretic medications have been taken for at least 12 hours. The time to resolution of fever will be estimated using the method of Kaplan-Meier using temperature and symptom relief medication information obtained from the subject diary data. Difference between the treatment groups will be assessed using the log rank statistic controlling for BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary). Subjects who do not have resolution of fever will be censored at the time of their last non-missing post-baseline temperature assessment.

The number and percentage of subjects experiencing influenza related complications will be summarized by complication preferred term and treatment group. The difference between the treatment groups will be assessed a logistic regression model with factors for treatment group, BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary). Pairwise differences between the treatment groups will be evaluated using contrasts from the final logistic regression model.

12.7.3 Exploratory Endpoint

The MRU, MRU-related direct costs, and indirect costs attributable to days missed of work and work productivity and/or performance losses will be summarized by treatment group and BMI. Methods for describing differences between treatment groups will be presented in the SAP.

Genotypic (including Hemagglutinin and Neuraminidase), phenotypic, viral culture and PCR data will be listed for each subject. These listings will be constructed in a manner consistent with the FDA June 2006 Guidance Document: *"Guidance for Submitting Influenza Resistance Data"*.[18] Additionally, the number and percentage of genotypic changes from wild-type amino acid will be summarized separately for treatment group, protein type, and study visit.

12.8 Safety Analyses

AEs will be mapped to a MedDRA-preferred term and system organ classification. The occurrence of TEAEs will be summarized by treatment group using MedDRA-preferred terms, system organ classifications, and severity. If a subject experiences multiple events that map to a single preferred term, the greatest severity and strongest Investigator assessment of relation to study drug will be assigned to the preferred term for the appropriate summaries. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to study drug will be generated.

Descriptive summaries of vital signs and clinical laboratory results will be presented by study visit. Laboratory abnormalities will be graded according to the DAIDS Table for Grading Adverse Events for Adults and Pediatrics (Publish Date: December 2004). The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized by treatment group. Laboratory toxicity shifts from baseline to Day 3, Day 5, and Day 14 will be summarized by treatment group.

Abnormal physical examination findings will be presented by treatment group. The number and percent of subjects experiencing each abnormal physical examination finding will be included.

Concomitant medications will be coded using the WHO dictionary. These data will be summarized by treatment group.

Subject disposition will be presented for all subjects. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

12.9 Sub-Study and Pharmacokinetic Analysis

A sub-study to collect pharmacokinetic samples in up to 60 peramivir treated subjects to examine exposure response will be conducted at selected sites. The data from the sub-study will be combined with the two PK samples (collected on all subjects at 30-60 minutes following administration of study drug and on study day 3) to perform a population exposure-response analysis. All analyses related to exposure-response will be completed as part of the sub-study. All statistical methods will be outlined as part of the sub-study protocol and exposure-response analysis plan. All sub-study analyses, and exposure-response analyses from PK samples obtained in this study and a companion study BCX1812-312, will be reported in a separate sub-study report.

12.10 General Issues for Statistical Analysis

12.10.1 Multiple Comparisons and Multiplicity

In order to maintain the overall type I error in the presence of the planned comparisons between the two peramivir treatments and placebo, a Bonferroni correction will be applied to the primary efficacy endpoint analysis. No other adjustments for multiple comparisons are planned.

12.10.2 Covariates

Primary and secondary efficacy analyses will be adjusted for BMI at screening, influenza type by PCR, and influenza season at randomization (if necessary).

12.10.3 Planned Sub-Groups

The primary efficacy endpoint will be summarized separately by BMI, influenza season at randomization (if necessary), and by viral subtype by PCR using descriptive statistics by treatment group and study day, if appropriate. No formal statistical testing will be utilized.

Additional analyses may be performed by country, if necessary, for submission to local regulatory authorities.

12.10.4 Missing Data

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been randomized. No attempt will be made retrospectively to obtain missing subject reported data (including influenza symptom severity assessments, temperature, ability to perform usual activities, missed days of work and impact of influenza on subject's work performance and/or productivity) that has not been completed by the subject at the time of return of the subject diary to the investigative site. In situations where it is not possible to obtain all data, it may be necessary to impute missing data.

In assessing the primary efficacy endpoint, for subjects who withdraw or who do not experience alleviation of symptoms, missing data will be censored using the date of subject's last non-missing assessment of influenza symptoms. Missing assessments of influenza symptoms conservatively will be imputed as having severity above absent or mild (as failures). For the subject diary data, the following data conventions will be utilized. Missing diary completion will be imputed as 11:59 for diary entries designated as morning and 23:59 for evening and daily reported values. Entries with values exceeding the 24-hour clock will be set to 23:59 of the day recorded. Select exploratory sensitivity analyses may be conducted to ascertain the effect, if any, of these methods. These sensitivity analyses are further described in the SAP. Secondary efficacy endpoints with time to event data will be censored using the date of subject's last non-missing assessment of the given endpoint.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1.1 Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; GCPs, including International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws

and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator should submit written reports of clinical study status to their Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) annually or more frequently if requested by the IRB/ IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the IRB/ IEC should be maintained in the study documents file. Copies of clinical study status reports (including termination) should be provided to BioCryst.

13.1.2 Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent form, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the investigational medicinal product is released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The Investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the Investigator will provide the IRB/IEC with a report of the outcome of the study.

13.1.3 Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by BioCryst and by the reviewing IRB/ IEC. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and BioCryst policy.

The Investigator must explain to potential subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the ICF. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1.4 Payment to Subjects

Reasonable compensation to study subjects may be provided if approved by the IRB/IEC responsible for the study at the Investigator's site.

13.1.5 Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his/her IRB/IEC as required.

13.2 Study Monitoring

During trial conduct, BioCryst or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow BioCryst monitors or its designees and appropriate regulatory authorities direct access to source documents to perform this verification.

13.3 Quality Assurance

The trial site may be subject to review by the IRB/IEC, and/or to quality assurance audits performed by BioCryst, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

13.4 Study Termination and Site Closure

BioCryst reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by BioCryst, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5 Records Retention

To enable evaluations and/or audits from regulatory authorities or BioCryst, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to BioCryst. The Investigator must obtain BioCryst's written permission before disposing of any records.

13.6 Study Organization

13.6.1 Data Monitoring Committee

BioCryst will assemble an independent Data Monitoring Committee (DMC) to assess safety parameters of the trial on a periodic, ongoing basis while the trial is in progress. The committee will include a statistician and three physicians, two of whom will be Infectious Disease / Clinical Virology specialists. Full details of the composition of the DMC and how the DMC is to operate will be described in a separate DMC charter.

13.7 Confidentiality of Information

BioCryst affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by BioCryst**.** However, in compliance with federal regulations, BioCryst requires the investigator to permit BioCryst's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study.

BioCryst will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule, where this rule is applicable. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of subjects participating in BioCryst-sponsored Clinical Trials. "Authorization" is required from each research subject, i.e., specified permission granted by an individual to a covered entity for the use or disclosure of an individual's protected health information. A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization may be combined in the Informed Consent document (approved by the IRB/IEC) or it may be a separate document, (approved by the IRB/IEC) or provided by the Investigator or Sponsor (without IRB/IEC approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appropriate individual. HIPAA authorizations are required for U.S. sites only.

13.8 Study Publication

All data generated from this study are the property of BioCryst and shall be held in strict confidence along with all information furnished by BioCryst. Independent analysis and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of BioCryst. Written permission to the Investigator will be contingent on the review by BioCryst of the statistical analysis and manuscript and will provide for nondisclosure of BioCryst confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

14 REFERENCES

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15 APPENDICES

15.1 NYHA Functional Classification Criteria: Heart Failure and Angina

NYHA Functional Classification of Heart Failure

Class I
No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea.
Class II
Symptoms with ordinary physical activity. Walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold weather, in wind, or when under emotional stress causes undue fatigue or dyspnea.
Class III
Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea.
Class IV
Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea.

NYHA Functional Classification of Angina

Class I
Angina only with unusually strenuous activity.
Class II
Angina with slightly more prolonged or slightly more vigorous activity than usual.
Class III
Angina with usual daily activity.
Class IV
Angina at rest.

15.2 Criteria for Severe COPD and Severe Asthma

The following guidelines are provided to assist in the evaluation of subjects who have a medical history for Chronic Obstructive Pulmonary Disease (COPD) and/or Asthma. Subjects with severe COPD or severe persistent Asthma are to be excluded from this study. (See section 8.1.2, exclusion criteria number 3).

Classification of Asthma from National Asthma and Education and Prevention Program

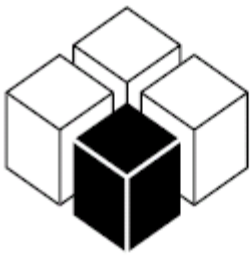
Classification	Days with Symptoms	Nights with Symptoms	For Adults and Children (> 5 yrs) who can use a spirometer or peak flow meter	
			FEV_1 or PEF % Predicted Normal	PEF Variability (%)
Severe persistent	Continual	Frequent	≤ 60	> 30
Moderate Persistent	Daily	> 1/ week	> 60 - < 80	> 30
Mild Persistent	> 2 / week but < 1 times / day	> 2/ month	≥ 80	20 – 30
Mild Intermittent	≤ 2 / week	< 2 / month	≥ 80	< 20
FEV_1: percentage predicted value for forced expiratory volume in 1 second. PEF: percentage of personal best for peak expiratory flow.				

Extracted from: Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program. HHS/NIH 2007

Spirometric Classification of COPD Severity based upon Post-Bronchodilator FEV1 (GOLD Criteria)

Stage	Characteristics
Mild COPD	$FEV_1/FVC < 70\%$ $FEV_1 \geq 80\%$ predicted
Moderate COPD	$FEV_1/FVC < 70\%$ $50\% \leq FEV_1 < 80\%$ predicted
Severe COPD	$FEV_1/FVC < 70\%$ $30\% \leq FEV_1 < 50\%$ predicted
Very Severe COPD	$FEV_1/FVC < 70\%$ $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure
FEV_1: percentage predicted value for forced expiratory volume in one second. FVC: forced vital capacity	

Extracted from: Rabe KF, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (GOLD Executive Summary). Am. J. Respir. Crit. Care Med. 2007:176;532-555.



BIOCRYST PHARMACEUTICALS, INC.

CLINICAL STUDY PROTOCOL

Protocol No. BCX1812-311

IND No. 76,350

A PHASE 3 MULTICENTER, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRAMUSCULAR PERAMIVIR IN SUBJECTS WITH UNCOMPLICATED ACUTE INFLUENZA

THE **IMPROVE I** STUDY

(**I**ntra**M**uscular **P**eramivir for the **R**elief **O**f symptoms and **V**irologic **E**fficacy)

Short title: Intramuscular Peramivir for the Treatment of Uncomplicated Influenza

Protocol Date(s):
Version 1.0: 04 September 2007
Version 2.0: 05 October 2007
Version 3.0 20 November 2007

BioCryst Pharmaceuticals, Inc.
2190 Parkway Lake Drive
Birmingham, AL 35244, USA
Phone: +1 919 859 1302
Fax: +1 919 851 1416

1 TITLE PAGE

Protocol Number:	BCX1812-311
Study Title:	A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza.
IND Number:	76, 350
Investigational Product:	Peramivir (BCX1812)
Indication Studied:	Uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive Birmingham, AL 35233
Development Phase:	3
Sponsor Medical Officer:	W. James Alexander, M.D., M.P.H. Senior Vice President, Clinical Development Chief Medical Officer Phone: +1 919 859 1302 Fax: +1 919 851 1416 Email Address: jalexander@biocryst.com
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Final Protocol Date:	Version 1.0: 04 September 2007
Amendment(s) Date(s):	Version 2.0: 05 October 2007 Version 3.0: 20 November 2007

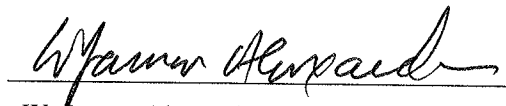
1.1 Protocol Approval Signature Page

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

BioCryst Pharmaceuticals, Inc.

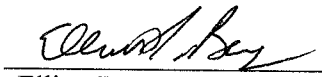
Reviewed and Approved by:



20 November 2007

W. James Alexander, M.D., M.P.H.
Senior Vice President, Clinical Development
Chief Medical Officer

Date



20 November 2007

Elliott Berger, PhD
Senior Vice President, Regulatory Affairs

Date

1.2 Clinical Study Protocol Agreement

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, random ized, double-blind, placebo-controlled study to evaluate the effic acy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

I have carefully read this protocol and a gree that it contains all of the necessary information required to c onduct this study. I agree to c onduct this study as described and according t o the Decl aration of Helsinki, Internationa l Conference on Har monization Guidelines for Good Clinical Practices, and all applicable regulatory requirements.

Investigator's Signature Date

Name (Print)

2 SYNOPSIS

Protocol No.	BCX1812-311
Protocol Title:	A phase 3, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza.
Sponsor:	BioCryst Pharmaceuticals, Inc.
Investigators/Study Sites:	Multinational
Development Phase:	3
Objectives:	
Primary:	To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza A.
Secondary:	1. To evaluate the safety and tolerability of peramivir administered intramuscularly 2. To evaluate secondary clinical outcomes in response to treatment 3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment
Exploratory:	1. To assess pharmacoeconomic measures as response to treatment 2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment
Number of Subjects:	Total enrollment: up to a total of 750 evaluable subjects will be randomized to treatment (150 subjects in the placebo treatment group, 300 subjects in the peramivir 300mg treatment group and 300 subjects in the peramivir 600mg treatment group). An evaluable subject is one who is randomized, receives study drug, and has confirmed acute influenza A by primary viral culture or PCR. A positive Rapid Antigen Test (RAT) for influenza A at screening will be required for enrollment. Because results of clinic-based RAT tests may not precisely indicate presence of influenza infection, it is expected that at least 850 subjects will be randomized to treatment to ensure that 750 evaluable subjects are treated.
Study Design:	This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in

	adults with uncomplicated acute influenza. Each subject's assignment to treatment will be stratified according to smoking status. All subjects will be centrally randomized to one of three treatment groups according to smoking status strata in a ratio of 2:2:1 such that 80% of subjects are randomized to one of the two single dose regimens of peramivir. Treatment Group 1: Peramivir 300mg Treatment Group 2: Peramivir 600mg Treatment Group 3: Placebo Study drug will be administered as bilateral 4mL intramuscular injections (total of 8mL injected in equally divided doses). Procedures for gluteal intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual. Subjects eligible for screening will have an anterior nasal swab collected for testing by rapid antigen testing (RAT) for influenza A, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/exclusion criteria may be enrolled into the study. All enrolled subjects will record the following information in a Study Diary: • Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14 • Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol) or other anti-pyretic medications. • Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14 • Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14 Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3,

	5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result on culture) as well as other virologic assessments (e.g. PCR, genotypic testing) All virologic assessments will be performed by a central laboratory. Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis. At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.
Study Population:	Male and female subjects, 18 years of age and older, with symptoms consistent with a diagnosis of uncomplicated acute influenza infection may be screened for enrollment. Subject eligibility will require the presence of two or more symptoms of at least moderate severity consistent with acute influenza as well as positive results obtained from a rapid antigen test (RAT) for influenza A at screening.
Inclusion Criteria:	1. Male and non-pregnant female subjects age ≥18 years. 2. A positive Influenza A Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one-hour. Subjects with a positive influenza B or mixed A and B RAT will be excluded. 3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening. 4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity. 5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity.

	6. Onset of symptoms no more than 48 hours before presentation for screening. 7. Written informed consent.
Exclusion Criteria:	1. Women who are pregnant or breast-feeding. 2. Presence of clinically significant signs of acute respiratory distress. 3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma. 4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the past 12 months. 5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia. 6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min). 7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza. 8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics. 9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening. 10. Currently receiving treatment for viral hepatitis B or viral hepatitis C. 11. Presence of known HIV infection with a CD4 count <350 cell/mm^3. 12. Current therapy with oral warfarin or other systemic anticoagulant. 13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening. 14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days. 15. Immunized against influenza with inactivated virus vaccine within the previous 14 days. 16. Receipt of any intramuscular injection within the previous 14 days. 17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study.

	18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study 19. Participation in a study of any investigational drug or device within the last 30 days.
Study Endpoints:	
Primary Endpoint:	*Clinical:* Time to alleviation of clinical symptoms of influenza.
Secondary Endpoint(s):	*Safety:* Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests. *Clinical:* Time to resolution of fever. Incidence of influenza related complications. *Virologic:* Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).
Exploratory Endpoint(s):	*Pharmacoeconomic*: Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity. *Virologic:* Quantitative change in influenza virus shedding, measured by PCR. Change in influenza virus susceptibility to neuraminidase inhibitors.
Investigational Product, Dose, and Mode of Administration:	
Peramivir (BCX-1812), 7 5mg/mL, 4mL (300m g) per injection, adm inistered as bilateral injections.	
Reference Therapy, Dose, and Mode of Administration:	
Matching Placebo (buffered diluent), 4mL per injection administered as bilateral injections.	
Duration of Treatment:	Following treatment on day 1, study duration for all subjects is expected to be up to 14 days (including all visits). Presence of unresolved adverse events and/or treatment-emergent laboratory findings at the Day 14 visit, or persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit, will require additional follow up.
Statistical Methods:	
Study Hypothesis:	The null hypothesis (H_0) for this study is that the time to alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir

<table>
<tr><td></td><td>300mg (H_{01}) or peramivir 600mg (H_{02}).

The alternative hypothesis (H_1) is that subjects treated with peramivir 300mg (H_{11}) or peramivir 600mg (H_{12}) have an improvement in time to alleviation of influenza symptoms over those treated with placebo.</td></tr>
<tr><td>Sample Size:</td><td>From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation for the 150 mg dose peramivir arm will be reduced by 30% compared to placebo (see table below) yielding a hazard ratio of 0.70.

<table>
<tr><td colspan="3">Median Time To Alleviation of Symptoms (Hours)</td></tr>
<tr><td>Placebo</td><td>Peramivir 150mg</td><td>Difference (hours)</td></tr>
<tr><td>145.0</td><td>101.5</td><td>43.5</td></tr>
<tr><td>140.0</td><td>98.0</td><td>42.0</td></tr>
<tr><td>135.0</td><td>94.5</td><td>40.5</td></tr>
<tr><td>130.0</td><td>91.0</td><td>39.0</td></tr>
<tr><td>125.0</td><td>87.5</td><td>37.5</td></tr>
<tr><td>120.0</td><td>84.0</td><td>36.0</td></tr>
<tr><td>115.0</td><td>80.5</td><td>34.5</td></tr>
<tr><td>110.0</td><td>77.0</td><td>33.0</td></tr>
<tr><td>105.0</td><td>73.5</td><td>31.5</td></tr>
<tr><td>100.0</td><td>70.0</td><td>30.0</td></tr>
</table>

Using these assumptions, a sample size of 300 evaluable subjects per active treatment group and 150 evaluable subjects in the placebo group (a total of 750 evaluable subjects) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).</td></tr>
<tr><td>Efficacy:</td><td>The intent-to-treat infected population (ITTI) will include all subjects who are randomized, received study drug, and have confirmed influenza A by primary viral culture or PCR. The primary efficacy variable is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when each of seven symptoms of influenza are either absent or are present at no more than mild severity level and remain at no worse than this severity status for a 21.5 hour (24 hours – 10%) period.

Descriptive statistics for the primary efficacy variable will be</td></tr>
</table>

	tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment differences between each active group and placebo will be assessed using a Wilcoxon-Gehan statistic stratified by smoking status at screening. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing assessment. The overall significance level will be maintained by utilization of Hochberg's method for the planned comparisons between the two active treatments and placebo. Time to resolution of fever will be analyzed in a similar manner without the adjustment for multiple comparisons. Efficacy analyses will be repeated for a Per-Protocol Infected population (PPI). This population will include those subjects in the ITTI population who received an adequate intramuscular injection. Details of this population will be described in the statistical analysis plan. The PPI population analysis will be used as supportive to the primary analysis with the ITTI. Changes in influenza virus $TCID_{50}$ (viral titers from nasopharyngeal specimens) will be compared using the van Elteren statistic controlling for smoking status at screening. Analyses of other continuous endpoints will be analyzed in a similar manner. The number and percentage of subjects experiencing influenza related complications (IRC) will be summarized by complication preferred term and treatment group. The difference between the treatment groups will be assessed using a logistic regression model with factors for treatment group and smoking status at screening. Pairwise differences between the treatment groups will be evaluated using contrasts from the final logistic regression model.
Safety:	Safety analyses will be presented for all subjects in the safety population, defined as all randomized subjects who receive at least one dose of study drug. Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. The occurrence of treatment-emergent AEs will be summarized using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs that are related to study medication will be generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms.

	Descriptive summaries of vital signs and quantitative clinical laboratory changes will be presented by study visit. Frequency and percentages of subjects with abnormal laboratory test results will be summarized by toxicity grade. Concomitant medications will be mapped to a WHO preferred term and drug classification. The number and percent of subjects taking concomitant medications will be summarized using preferred terms and drug classifications. The number and percent of subjects experiencing each abnormal physical examination finding will be presented. The number and percent of subjects discontinuing study as well as the reasons for discontinuation will be summarized by treatment group.
Date of Protocol:	Version 1.0: 04-September-2007
Amendment (Dates):	Version 2.0: 05-October-2007 Version 3.0: 20-November-2007

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3.1 List of Figures

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0–72}$	area under the curve from time 0 to 72 hours
$AUC_{0–\infty}$	area under the curve extrapolated from time 0 to infinity
BMI	Body Mass Index in kg/m^2
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CIOMS	Council for International Organizations of Medical sciences
C_{max}	maximum plasma concentration
CK	creatine kinase
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRF	Case Report Form
CV	coefficient of variation
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRC	influenza related complications
ITT	intent-to-treat
ITTI	intent-to-treat infected
IUD	intrauterine device
IVRS	interactive voice response system
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRU	medical resource utilization
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction
RAT	Rapid Antigen Test
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan

SD	standard deviation
SUSAR	Suspected Unexpected Serious Adverse Event
$t_{1/2}$	elimination half-life
$t_{1/2}$ λz	terminal half-life
$TCID_{50}$	tissue-culture infective $dose_{50}$
TEAEs	treatment-emergent adverse events
T_{max}	time to attain maximum plasma concentration
UPEP	Urine protein electrophoresis
WBC	white blood cell
WHO	World Health Organization

5 INTRODUCTION

5.1 Background

Influenza virus is a member of the *orthomyxovirus* family and causes an acute viral disease of the respiratory tract. Typical influenza illness is characterized by abrupt onset of fever, headache, myalgia, sore throat, and nonproductive cough.[1] The illness is usually self-limiting, with relief of symptoms occurring within 5 to 7 days. Nevertheless, it is an important disease for several reasons, including ease of communicability, short incubation time, rapid rate of viral mutation, morbidity with resultant loss of productivity, risk of complicating conditions, and increased risk of death, particularly in the elderly. During 19 of the 23 influenza seasons between 1972/1973 and 1994/1995, estimated influenza-associated deaths in the United States ranged from approximately 25 to more than 150 per 100,000 persons above 65 years of age, accounting for more than 90% of the deaths attributed to pneumonia and influenza.[2]

Presently, only a few measures are available that can reduce the impact of influenza: active immunoprophylaxis with an inactivated or live attenuated vaccine and chemoprophylaxis or therapy with an influenza-specific antiviral drug. Neuraminidase inhibitors are the current mainstay of antiviral treatment for influenza. Marketed neuraminidase inhibitors include zanamivir (Relenza®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche-Gilead), an oral prodrug of the active agent, oseltamivir carboxylate. Influenza neuraminidase is a surface glycoprotein that cleaves sialic acid residues from glycoproteins and glycolipids. The enzyme is responsible for the release of new viral particles from infected cells and may also assist in the spreading of virus through the mucus within the respiratory tract. The neuraminidase inhibitors represent an important advance in the treatment of influenza with respect to activity against influenza A and B viruses, with proven therapeutic value in reducing influenza lower respiratory complications,[3] and lower rates of antiviral drug resistance[4].

The use of currently available neuraminidase inhibitors has been limited by concerns including, the degree of effectiveness, the requirement for an inhaler device (zanamivir), and the emergence of resistant influenza virus variants in some treated populations.[5] In addition, there are risks of bronchospasm with zanamivir; and gastrointestinal side effects, with oseltamivir.

Peramivir is a neuraminidase inhibitor that represents a potentially promising addition to the armamentarium of drugs for the treatment of influenza infections due to its potential for parenteral administration and lower frequency of dosing.

5.2 Rationale for Study

An oral formulation of peramivir has previously been evaluated in a full range of safety, tolerability, pharmacokinetic, and efficacy studies. In a multinational phase 3 clinical trial conducted in 1999-2001, oral peramivir demonstrated antiviral activity against influenza A and B infections, and improvement in the relief of clinical symptoms. Because of the limited bioavailability of peramivir following oral administration (<5%), it was determined that the parenteral route of administration is more appropriate for the delivery of peramivir. Subsequent phase 1 studies of intravenous and intramuscular formulations of peramivir have confirmed that parenteral routes of administration result in plasma levels of drug that are as much as 100 times those achieved via the oral route. In a phase 2 study of intramuscular peramivir in subjects with acute uncomplicated influenza, subjects who received a single injection of 150mg or 300mg

peramivir had clinically meaningful reductions in the time to alleviation of symptoms of influenza, compared to subjects who received a placebo injection. Further details of these studies are provided below and in the Investigator Brochure.

Because of the previous demonstration of clinical efficacy of intramuscular peramivir in acute influenza in the phase 2 study, and the encouraging pharmacokinetic and preliminary safety profile of the intramuscular formulation of peramivir demonstrated to date, this phase 3 study will be conducted to evaluate the efficacy and safety profile of intramuscular peramivir and to determine the optimal single dose regimen.

5.3 Non-Clinical Experience with Peramivir

5.3.1 In vitro Assays

Peramivir is a selective inhibitor of viral neuraminidase, with 50% inhibitory concentrations (IC_{50}) for bacterial and mammalian enzymes of >300μM.[6] In an *in vitro* study, 42 influenza A and 23 influenza B isolates were collected from untreated subjects during the 1999–2000 influenza season in Canada.[7] These isolates were tested for their susceptibility to the neuraminidase inhibitors zanamivir, oseltamivir carboxylate, and peramivir using a chemiluminescent neuraminidase assay. Inhibition of Type A influenza neuraminidase by peramivir was approximately an order of magnitude greater than inhibition of neuraminidase from Type B viruses. IC_{50} values for the Type A enzymes ranged from <0.1 to 1.4nM, whereas the Type B enzymes ranged from <0.1 to 11nM, with three out of four values in the 5- to 11nM range. Peramivir was the most potent drug against influenza A (H3N2) viruses with a mean IC_{50} of 0.60nM as well as most potent against influenza B with a mean IC_{50} of 0.87nM.

In another *in vitro* comparison of peramivir, oseltamivir, and zanamivir, using a neuraminidase inhibition assay with influenza A viruses, the median IC_{50} of peramivir (approximately 0.34nM) was comparable to that of oseltamivir (0.45nM) and significantly lower than zanamivir (0.95nM). For influenza B virus clinical isolates, the median IC_{50} of peramivir (1.36nM) was comparable to that of zanamivir (2.7nM) and lower than that of oseltamivir (8.5nM).[8]

The potency of peramivir was evaluated against five zanamivir-resistant and six oseltamivir-resistant influenza viruses.[9] Peramivir remained a potent inhibitor against all oseltamivir-resistant viruses including the mutations H274Y, R292K, E119V, and D198N, with IC_{50} values ≤40nM. Peramivir also potently inhibited ($IC_{50} \leq 26$nM) the neuraminidase activity of zanamivir-resistant strains, which had the following mutations: R292K, E119G, E119A, and E119D. However, one zanamivir-resistant influenza B virus, B/Mem/96, with a mutation R152K isolated from cell culture, was relatively resistant to all neuraminidase inhibitors, including peramivir (IC_{50} = 400nM).

5.3.2 Animal Models

In a mouse model of influenza infection, a single intramuscular injection of peramivir (10mg/kg) given 4 hours prior to inoculation with an A/NWS/33 (H1N1) influenza strain resulted in 100% survival in contrast to 100% mortality in a control group injected with saline.[6] In the same mouse model, treatment of mice up to 72 hours after influenza infection using peramivir (20mg/kg) resulted in 100% survival, compared to 100% mortality in the control group injected with vehicle.[10]

Peramivir has also demonstrated activity in animal models utilizing a clinical H5N1 isolate as the

infecting virus strain. In a mouse model, a single intramuscular dose of peramivir (30mg/kg) injected 1 hour after inoculation with the highly pathogenic (H5N1) A/Vietnam/1203/04 strain, resulted in a 70% survival rate that was similar to that seen in mice treated with oseltamivir given orally at 10mg/kg/day for 5 days[11]. In similar experiments, mice inoculated with the same strain of H5N1 virus that were then treated for up to 8 days with intramuscular peramivir exhibited 100% survival[12]. This longer duration of peramivir treatment also prevented viral replication in the lungs, brain and spleen at days 3, 6 and 9 post inoculation.

5.4 Previous Phase 3 Clinical Experience with Oral Peramivir

An oral formulation of peramivir has previously demonstrated antiviral activity and preliminary clinical efficacy in challenge studies in human volunteers, as well as in treatment studies in patients with uncomplicated acute influenza infections during the influenza seasons of 1999-2001. A Phase 3 multinational study (BC-01-03) of oral peramivir was conducted. Two dose regimens of oral peramivir, 800mg QD for 5 days, or 800mg QD on Day 1, followed by 400mg QD for 4 days, were compared to a matched placebo treatment group. A total of 1246 subjects were randomized to treatment at sites in the USA, Western and Eastern Europe, South America, Australia and New Zealand. As presented in the Table 1 below, the primary end-point of time to relief of influenza symptoms in 694 subjects with confirmed influenza was not found to be significantly different (p=0.17) between the three treatment groups.[13] A sub-group analysis of the time to relief of symptoms by country or region demonstrated marked differences in the primary endpoint.. In the subset of influenza-infected subjects enrolled at sites in the US, clinically meaningful differences in time to relief of influenza symptoms between the placebo and the two peramivir arms were observed, however statistical significance (p=0.07) was not achieved. However, a number of secondary endpoints in this phase 3 study, such as time to overall well-being, time to normal activity, incidence of influenza related complications and quantity of viral shedding, achieved or approached statistically significant differences between the peramivir and placebo treatment groups (p=0.03-0.06).

Table 1 Results of study BC-01-03

Dose and Regimen	Median Time to Relief of Influenza Symptoms (Hours)	
	Overall Results (n=694)	US Sites (n=198)
Peramivir 800mg po x 5d	89.0	70.8
Peramivir 800mg po x 1d and 400mg po x 4d	91.7	88.8
Placebo x 5 days	104.4	106.8
p value	0.17	0.07

5.5 Previous Phase 1 Experience with Intramuscular Peramivir

Two phase 1studies evaluating the safety and pharmacokinetics of an intramuscular formulation of peramivir have been conducted in a total of 45 healthy volunteers receiving peramivir. An additional phase 1 study has recently been initiated to evaluate the pharmacokinetics and

tolerability of higher single doses (up to 600 mg) of intramuscular peramivir.

Study Peramivir-Him-06-111 evaluated the single dose pharmacokinetics and tolerability of 75mg, 150mg and 300mg doses of peramivir administered as intramuscular (i.m.) and intravenous (i.v.) injections in a crossover design (9 subjects per group). Peak plasma levels of i.m. peramivir generally occurred within 30 minutes following injection. Plasma pharmacokinetic parameters for i.m. peramivir are summarized in Table 2 below for the three intramuscular single dose regimens evaluated.

Table 2 Pharmacokinetic parameters from study Him-06-111.

Dose (mg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (hr·ng/mL)	$t_{1/2}$[a] (hr)
75 i.m.	4296 ± 812	11659 ± 1123	19.8 ± 7.9
150 i.m.	7612 ± 884	23952 ± 3804	24.3 ± 4.1
300 i.m.	15150 ± 2367	49649 ± 5619	22.8 ± 2.5
[a]terminal half life			

In a second phase 1 study, Peramivir-Him-06-112, the same dose levels of peramivir were administered as single i.m. injections on two consecutive days (6 subjects per group). This double-blind study also included a placebo arm. The pharmacokinetic parameters of i.m. peramivir following the second day of dosing were consistent with those seen following single doses of the drug.

An additional phase 1 study, BCX1812- 117, was initiated to evaluate the effect of needle length adjustment according to gender and body mass index on the pharmacokinetics and safety of peramivir following ventrogluteal intramuscular injection, and dorsogluteal intramuscular injection in a subgroup of subjects. Interim data are available for the first 40 subjects who received single peramivir doses of 600 mg, consisting of directly observed tolerability assessments, safety laboratory studies, reported adverse events, and pharmacokinetic data. Clinical laboratory results obtained 72 hours after dosing showed no abnormalities with regards to hematology or urinary analytes and the only chemistry analytes outside normal ranges (CK and AST) were related to receipt of intramuscular injection.

A majority of the first 40 subjects treated complained of acute pain immediately after injection at the ventrogluteal site and a number of these subjects reported that the pain was also associated with muscle cramping. Some subjects reported radiation of pain to the lower extremities. In most instances, these reactions abated after 15-30 minutes.

This protocol also evaluated the acute tolerability of doses of 450 mg and 600 mg of peramivir administered at the dorsogluteal site. Direct observations determined that the use of the dorsogluteal injection site resulted in an acute tolerability profile of the 600 mg dose of peramivir that was improved compared to that observed with ventrogluteal site injections. However, a number of these subjects also experienced acute pain and discomfort and some reported muscle cramping in the gluteal area which persisted for up to 30 minutes.

In summary, this study confirmed that the intramuscular injection of peramivir results in acute pain and discomfort after gluteal injection in the majority of subjects in whom total doses of up to 600mg are administered. In some subjects, the acute pain at the injection site may also be

associated with muscle cramping. Based on these findings, the dorsogluteal site will be utilized in future trials.

The safety data for i.m. peramivir administered as single doses ranging from 75 mg to 600mg at the dorsogluteal injection site in each of the 3 phase 1 studies conducted to date have been unremarkable. No serious adverse events were reported. The most commonly observed adverse events or laboratory abnormalities were injection site pain or discomfort, headache, and transient increases in muscle enzymes (CK). There have been several reports of signs and symptoms of self-limited vasovagal reactions following injections.. No consistent differences in frequency of adverse events or laboratory toxicities were observed between the active and placebo treatment groups in the controlled phase 1studies, with the exception that CK elevations appeared to be dose related in the peramivir treatment groups.

5.6 Phase 2 Experience with Intramuscular Peramivir

A phase 2 study BCX1812-211 was completed in 2007. This study was a randomized, double-blind placebo- controlled study to evaluate the efficacy and safety of two single dose regimens of peramivir. A total of 344 subjects were enrolled into this study with 115 subjects randomized to Placebo; 114 subjects randomized to peramivir 150 mg; and 114 subjects randomized to peramivir 300 mg. The primary endpoint of the study was the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza. Based on preliminary data, the primary endpoint of time to alleviation of clinical symptoms in BCX1812-211 did not achieve statistical significance in the pre-planned ITTI study population (Table 3).. Based on pre-planned and post hoc analyses, it appeared that a majority of subjects within this phase 2 study did not receive an adequate intramuscular injection.

In phase 1 studies (Him-06-111 and Him-06-112) of i.m. peramivir, significant increases in creatine kinase (CK) were observed at Day 3 compared with Baseline (Day 1) in all subjects who received active study drug or placebo. CK is a well established marker of muscle damage, and it was hypothesized that CK increase may act as a surrogate marker of an adequate i.m. injection.

Within the phase 2 study, an increase in CK between Baseline (Day 1) and Day 3 was not observed in a majority of subjects. In the phase 1 studies study drug was administered with a 1½ inch needle. In the phase 2 study a shorter needle (1 inch) was supplied with the study drug, with guidance that a longer needle (1½ inch) should be used for larger subjects. Based on the observed lack of CK increases at Day 3 compared to baseline, the Sponsor hypothesized that the needle used for injection failed to penetrate muscle and deliver intramuscular study medication in many subjects.

A sub group of subjects was identified in which a Day 3 CK increase of at least 50U/L was observed over baseline. Within this adequate intramuscular injection sub-group, notable improvements in the time to alleviation of symptoms were observed for both peramivir dose groups: 44.6 hours for peramivir 150 mg treatment and 64.8 hours for the peramivir 300 mg treatment

Table 3). A further sub-group analysis suggested that in subjects with an influenza B infection confirmed by PCR a single dose of peramivir had marginal activity (Table 3). These efficacy data support the further development of peramivir as a single dose, intramuscular treatment for acute influenza.

Table 3 Summary of Efficacy from BCX1812-211.

	Placebo	Peramivir 150mg	Peramivir 300mg
Intent-to-Treat Infected Population[1] (n=313) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=107 137.0 115.9-163.8	n=104 114.1 95.2-145.5 22.9	n=102 115.9 77.8-136.6 21.1
Adequate Injection Population[2] (n=101) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=40 152.2 103.8-183.9	n=32 107.6 76.8-175.1 44.6	n=29 87.4 40.8-163.8 64.8
ITTI Influenza A infected population (n=247) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=83 138.4 117.0-173.4	n=85 115.7 92.2-145.5 22.7	n=79 115.9 51.1-195.9 22.5
ITTI Influenza B infected population (n=66) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=24 117.1 100.3-162.3	n=19 100.8 68.2-162.0 16.3	n=23 123.3 67.5-178.7 -6.2

[1]: Intent-to-Treat Infected Population: PCR+ for either Influenza A and/or Influenza B at baseline/screening visit.

[2]: Adequate Injection Population: ITTI subjects in who study drug reached target muscle tissue, as evidenced by an increase in serum CK levels of ≥ 50 U/L over baseline at the Day 3 study visit.

An independent data monitoring committee reviewed grouped blinded safety data throughout study BCX1812-211. In the overall safety population (n=342), doses of peramivir 150 mg and 300 mg were both found to be well tolerated and no safety concerns were identified by the DMC. The three treatment groups were similar with respect to the frequency and severity of adverse events. Two serious adverse events were reported in the study, and neither was considered by the investigator to be related to treatment. One SAE (pyelonephritis) occurred 5-days after study treatment in a subject who received placebo, and one SAE (meningitis, resulting in death) occurred 10-days after study treatment in a subject who received 300 mg of peramivir. There were no meaningful differences among the three treatment groups with respect to the frequency or severity of graded laboratory toxicities. A summary of the adverse events and graded toxicities, together with a list of the most frequently reported adverse events, is presented in Table 4.

Table 4 Summary of Safety from BCX1812-211.

Safety Parameters	Placebo (N=114)	Peramivir 150 mg (N=113)	Peramivir 300 mg (N=115)
Any Clinical Adverse Event	49 (43%)	43 (38%)	44 (38%)
Any Graded Laboratory Toxicity	99 (87%)	93 (82%)	92 (80%)
Any Serious Adverse Event	1 (<1%)	0	1 (<1%)
Most Frequent Adverse Events Assessed as Study Drug-Related			
Diarrhea	5 (4%)	5 (4%)	6 (5%)
Nausea	7 (6%)	7 (6%)	9 (8%)
Vasovagal Reaction	4 (4%)	2 (2%)	0

5.7 Dose Rationale

Oseltamivir is approved for the treatment of uncomplicated acute influenza at a dosage of 75mg twice daily in adults[14]. Oseltamivir was shown to be clinically effective in a phase 3 study of oral oseltamivir versus placebo in naturally occurring seasonal influenza, and these data were sufficient for regulatory approval for marketing of oseltamivir. At least 75% of an oral dose of oseltamivir reaches the systemic circulation as oseltamivir carboxylate. When oseltamivir is administered orally at a dose of 75mg twice daily, the serum C_{max} of oseltamivir carboxylate is approximately 348ng/mL and the AUC_{0-48} is 10,876 h·ng/mL. The clinical data indicate that this level of exposure to oseltamivir was sufficient to provide clinical improvement in uncomplicated acute influenza.

The serum pharmacokinetic data (C_{max} and $AUC_{0-\infty,}$ respectively) following intramuscular doses of peramivir are approximately 7600ng/mL and 24,000 h·ng/mL for the 150mg dose and are approximately 15,000ng/mL and 49,000 h·ng/mL for the 300mg dose. Previous studies have assessed the concentrations of the neuraminidase inhibitor zanamivir in nasal and pharyngeal secretions after parenteral administration of this drug. . Within several hours after administration, the concentrations in secretions were approximately 100-fold lower than in serum or plasma. In theory, relatively high levels of a neuraminidase inhibitor in respiratory secretions are desirable in order to rapidly inactivate influenza virus and to delay or prevent the development of resistance in infecting virus strains. Intramuscular doses of peramivir, including doses of 300mg and 600mg have been shown to be well tolerated in previous Phase 1 studies. In the completed Phase 2 study, both doses of peramivir (150 mg and 300 mg) were well tolerated and no safety concerns were apparent. As evidence of a dose response between the 150mg and 300mg doses was observed in the phase 2 study, it is possible that a higher dose of 600mg of peramivir may further improve the treatment response observed in study 211. Therefore, it is appropriate to evaluate two dose regimens of 300mg and 600mg to undergo further evaluation in this Phase 3 study.

6 STUDY OBJECTIVES

6.1 Objectives

6.1.1 Primary Objective

To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza.

6.1.2 Secondary Objective(s)

The secondary objectives of this study are:

1. To evaluate the safety and tolerability of peramivir administered intramuscularly,
2. To evaluate secondary clinical outcomes in response to treatment,
3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment.

6.1.3 Exploratory Objective(s)

The following exploratory objectives have been identified for this study.

1. To assess pharmacoeconomic measures as response to treatment.
2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment.

6.2 Study Endpoints

6.2.1 Primary Endpoint

The primary clinical endpoint is the time to alleviation of clinical symptoms of influenza in subjects with influenza A.

6.2.2 Secondary Endpoint(s)

Secondary safety, clinical, and virologic endpoints will include evaluations in each subject of:

Safety: Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests.

Clinical: Time to resolution of fever; Incidence of influenza related complications.

Virologic: Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).

6.2.3 Exploratory Endpoints

Pharmacoeconomic and virologic evaluations in each subject for exploratory endpoints will also be assessed and include:

Pharmacoeconomic:	Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.
Virologic:	Quantitative change in influenza virus shedding, measured by PCR; Change in influenza virus susceptibility to neuraminidase inhibitors.

7 STUDY DESIGN

7.1 Overall Study Design and Plan

This is a multinational, randomized, double-blind study comparing the efficacy and safety of two single dose regimens of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza.

Up to a total of 750 evaluable subjects will be randomized to treatment (150 subjects in the placebo treatment group, 300 subjects in the peramivir 300mg treatment group and 300 subjects in the peramivir 600mg treatment group).

Each subject's assignment to treatment will be stratified according to smoking status. All subjects will be centrally randomized to one of three treatment groups in a ratio of 2:2:1 such that 80% of subjects are randomized to one of the two single dose regimens of peramivir.

Treatment Group 1: Peramivir 300mg
Treatment Group 2: Peramivir 600mg
Treatment Group 3: Placebo

Study drug will be administered as bilateral 4mL intramuscular injections (total of 8mL injected in equally divided doses). Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

Subjects eligible for screening will have an anterior nasal swab collected for testing by RAT for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/ exclusion criteria may be enrolled into the study.

All enrolled subjects will record the following information in a Study Diary.

- Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14.

- Oral temperature measurements will be taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol) or other antipyretic medication.

- Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14

- Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14

Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result) as well as other virologic assessments (e.g. PCR, genotypic testing). All virologic assessments will be performed by a central laboratory.

Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis.

At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.

Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in divided doses). Procedures for intramuscular injection, with a recommended needle length appropriate to the size and weight of the subject, are provided in the study drug administration manual.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1.1 Inclusion Criteria

Subjects must meet all of the following criteria for inclusion in this study:

1. Male and non-pregnant female subjects age ≥18 years.
2. A positive Influenza A Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one hour. Subjects with a positive influenza B or mixed A and B RAT will be excluded.
3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening.

4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity.
5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity.
6. Onset of symptoms no more than 48 hours before presentation for screening.
7. Written informed consent.

8.1.2 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

1. Women who are pregnant or breast-feeding.
2. Presence of clinically significant signs of acute respiratory distress
3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma. (See Section 15.2).
4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the past 12 months. (See Section 15.1).
5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.
6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min).
7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza.
8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics.
9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening.
10. Currently receiving treatment for viral hepatitis B or viral hepatitis C.
11. Presence of known HIV infection with a CD4 count <350 cell/mm^3.
12. Current therapy with oral warfarin or other systemic anticoagulant.
13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening.
14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days.
15. Immunized against influenza with inactivated virus vaccine within the previous 14 days.
16. Receipt of any intramuscular injection within the previous 14 days.
17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study.
18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study.
19. Participation in a study of any investigational drug or device within the last 30 days.

8.1.3 Removal of Subjects from Therapy or Assessment

All subjects are permitted to withdraw from participation in this study at any time and for any reason, specified or unspecified, and without prejudice. The Investigator or sponsor may terminate the subject's participation in the study at any time for reasons including the following:

1. Adverse event;
2. Intercurrent illness;
3. Non-compliance with study procedures;
4. Subject's decision;
5. Administrative reasons;
6. Lack of efficacy;
7. Investigator's opinion to protect the subject's best interest.

Any subject who withdraws because of an adverse event will be followed until the sign(s) or symptom(s) that constituted the adverse event has/have resolved or is determined to represent a stable medical condition.

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary, or if it is the desire of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject and determine the subject's medical condition. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data.

9 TREATMENTS

9.1 Treatments Administered

Peramivir is an investigational drug. Peramivir for intramuscular injection is a small-volume parenteral and will be supplied as a 75mg/mL solution in sodium citrate/ citric acid buffer. The pH is approximately 3.0.

A matched placebo solution of sodium citrate/ citric acid buffer with 1.2% sodium chloride at a pH of approximately 3.0 will be supplied.

The gluteal site of injection and the syringe needle length are to be recorded in the subjects CRF. Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

9.2 Identity of Investigational Product(s)

Peramivir and placebo peramivir will be supplied in clear 2mL vials. An individual study drug kit will contain 2 vials of blinded study drug (peramivir and/or placebo, depending upon the treatment group). Syringes and needles will be provided in which to draw up the solution for intramuscular injection. All study drug kits must be stored at 2-8^{o}C.

Each individual study drug kit will be labeled with some or all of the following information as required by local regulations:

- Sponsor name and contact information, study protocol number, kit number, description of the contents of the container, instructions for the preparation of the syringe and administration of the study drug, conditions for storage, statement regarding the investigational (clinical trial) use of the study drug and date for retest or expiry date.

Each vial of study drug will be labeled with some or all of the following information as required by local regulations:

- Sponsor name, study protocol number, description of the contents of the vial, instructions for the preparation of the syringe, statement regarding the investigational (clinical trial) use of the study drug and lot number.

9.3 Method of Assigning Subjects to Treatment Groups

Subjects will be centrally randomized according in a ratio of 2:2:1 to one of three treatment groups: single dose peramivir 300mg, single dose peramivir 600mg or placebo, in accordance with a computer-generated randomization schedule prepared by a non-study statistician. Eighty percent (80%) of subjects will be randomized to treatment with one of the two single dose regimens of peramivir, 20% will be randomized to treatment with placebo.

Each subject's assignment to treatment will be stratified according to smoking status.

Once a subject is eligible for randomization, he/she will be assigned two study drug kit numbers that will be obtained by study staff from the study interactive voice response system (IVRS). Once a study drug kit number has been assigned to a subject, it cannot be reassigned to any other subject.

9.4 Study Medication Accountability

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the sponsor, issued to the subject or directly administered to the subject (including date and time), and any drug accidentally destroyed. The sponsor will supply a specific drug-accountability form. At the end of the study, information describing study drug supplies (e.g., lot numbers) and disposition of supplies for each subject must be provided, signed by the Investigator or designee, and collected by the study monitor. If any errors or irregularities in any shipment of study medication to the site are discovered at any time, the Project Manager must be contacted immediately.

At the end of the study, all medication not dispensed or administered and packaging materials will be collected with supervision of the monitor and returned to the sponsor or destroyed on site as dictated by the appropriate Standard Operating Procedure at the participating institution.

9.5 Blinding/Unblinding of Treatments

This is a double-blind study. The treatment group assignment will not be known by the study subjects, the investigator, the clinical staff, the CRO or Sponsor staff during the conduct of the study.

Section 11.24 provides information regarding the process for unblinding the treatment assignment, if necessary, in the event of an SAE.

9.6 Prior and Concomitant Therapies

All medications, by any route of administration, used during this study must be documented on the Case Report Form (CRF). Prescription as well as non-prescription medications should be recorded. Medication used for the treatment of influenza-related symptoms will be captured by the subject in the diary card provided by BioCryst.

9.7 Overdose and Toxicity Management

To date there is no experience with overdose of intramuscular or intravenous peramivir. If overdose occurs, subjects should receive indicated supportive therapy and evaluation of hematologic and clinical chemistry laboratory tests should be conducted. The effect of hemodialysis on elimination of peramivir is unknown.

9.8 Dose Interruption

As this is a study of a single dose of peramivir or placebo, guidelines for treatment interruption for drug related SAEs or toxicities are not applicable.

10 STUDY CONDUCT

A study schedule of evaluations is presented in Figure 1. A detailed list of the evaluations and visits is also provided in the following sections.

10.1 Evaluations

All subjects enrolled in this study will undergo the following evaluations:

10.1.1 Informed Consent

Prior to any study-related procedure subjects will be administered informed consent. For further discussion of consent see section 10.21.

10.1.2 Medical History

Medical history, influenza vaccination status within the previous 12 months and demographic data (including smoking behavior) will be recorded at Screening/Baseline.

10.1.3 Rapid Antigen Test for Influenza

At Screening/Baseline, a commercially available, rapid antigen test (RAT) for influenza A will be performed on an adequate specimen collected by swabbing the anterior nose in accordance with the RAT manufacturer' instructions. A negative initial RAT should be repeated within one hour. Subjects with a positive influenza B or mixed A and B RAT will be excluded. Refer to the Study Manual for instructions regarding the use of the RAT kits provided for this study. Sites may use the kits provided by the Sponsor or any other commercially approved RAT available at their site to document a confirmed influenza infection.

10.1.4 Physical Examination and Influenza-related Complications Assessments

The Investigator will perform a physical examination at Screening/Baseline. Subject's height and weight, and BMI will be recorded at Screening/Baseline in the subjects CRF.

Study personnel will be provided with an influenza-related complications (IRC) checklist in the CRF to evaluate the subject for the presence of clinical signs and/or symptoms of the following influenza-related complications: sinusitis, otitis, bronchitis and pneumonia. Note that subjects with clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis, and/or pneumonia at screening are not eligible for enrollment in this study (See Section 8.1.2 exclusion criteria number 8).

A targeted physical examination will be conducted at each visit to record the presence/absence of influenza related complications (IRC). If the investigator determines that the subject experiences (or is presumed to experience) an IRC as noted above, he/she will record that assessment on the IRC CRF page and any medication used to treat the condition will be recorded on the concomitant medication page. The investigator will promptly provide appropriate treatment for any suspected or proven IRC. Such information describing IRC signs and/or symptoms should not be reported as adverse events. Any injection site reactions noted will be recorded in the CRFs as adverse events.

10.1.5 Vital Signs

Vital signs evaluations will include blood pressure, pulse rate, and respiration rate. The investigator will record oral or rectal body temperature at baseline. Thereafter the subject will record oral temperature twice daily in the study diary card.

Vital signs will be measured at Screening/Baseline, pre-dose, and at 15 minutes following the study drug injection on Day 1, then once daily on Days 3, 5, 9, and 14.

10.1.6 Electrocardiogram Measurements

A 12-lead electrocardiogram (ECG) will be obtained at Screening/Baseline. The principal investigator will be responsible for interpretation of the Screening ECG. This interpretation may be performed by the investigator or he/she may delegate this action to another physician and the investigator will acknowledge the interpretation. If this baseline ECG is interpreted as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal but does not meet the exclusionary criteria, the subject may be enrolled unless other exclusion criteria apply. The principal investigator is responsible to ensure that such an enrolled subject be informed of the nature of the abnormal ECG and that any medically indicated repeat ECG examinations and/or referral of the subject for further evaluation is made either during subject's participation in the study or immediately after the subject's discharge from the study.

10.1.7 Clinical Laboratories

Clinical chemistry profiles will include a Chemistry 20 panel (includes sodium, potassium, chloride, total CO_2 [bicarbonate], creatinine, glucose, urea nitrogen, albumin, total calcium, total magnesium, phosphorus, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, lactate dehydrogenase [LDH], total protein, total creatine kinase, and uric acid).

Hematology will include complete blood count (CBC) with differential.

Urinalysis will include dipstick tests for protein, glucose, ketones, blood, urobilinogen, nitrite, pH, and specific gravity and microscopic evaluation for RBCs and WBCs. For any subject with a Day 3 positive test for urine protein by dipstick test of 2+ or higher, who had a Baseline/ Day 1 protein dipstick of <2+, a 24 hour urine collection for assessment of protein and a simultaneous assessment by UPEP on the same specimen will be obtained.

Clinical laboratory studies (clinical chemistries, hematology, and urinalysis) will be completed at Screening/Baseline, and on Days 3, 5 and 14.

10.1.8 Urine Pregnancy Test

Females of childbearing potential will be evaluated for pregnancy at Screening/Baseline and Day 14 using a urine pregnancy test.

10.1.9 Serology for Influenza

Paired blood samples for determination of antibody to influenza A and B (serology) will be obtained with the clinical laboratory tests at Screening/Enrollment and at Day 14. These specimens will be stored at the central laboratory and will be analyzed if needed to confirm the diagnosis of influenza.

10.1.10 Samples for Virologic Laboratory Assessments

An adequate specimen will be collected by swabbing the anterior nose (bilateral) and posterior pharynx for virologic laboratory assessments including culture for the isolation of influenza virus and/or quantitative PCR assay at Screening/Baseline, and at Days 3, 5, and 9. Refer to the Laboratory Manual for instructions regarding the processing and shipment of these specimens.

10.1.11 Subject Self Assessments

Subject self assessments will be performed beginning pre-dose on Day 1 and recorded in the subject's Study Diary including the following:

- Oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol, provided) or other anti-pyretic medications. The times of each temperature determination will be recorded in the Study Diary. The baseline temperature will be recorded at the screening/Day 1 visit prior to dosing, regardless of whether the subject had recently taken an anti-pyretic; the time of anti-pyretic use pre-treatment will be recorded in the CRF, if applicable.

- Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily, beginning pre-dose on Day 1 and through Day 9, then once daily through Day 14.

- Assessment of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 visual analogue scale once daily through Day 14.

The subject's diary card will be reviewed by study staff at each visit for completion of the record

of all required items, with particular emphasis on alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms. Study staff will not attempt to ask subjects to retrospectively complete missing diary card data for any scheduled assessments that have not been completed prior to the clinic visit. Study staff should, however, remind the subject to complete the diary card at all scheduled times.

10.1.12 Concomitant Medications

All concomitant medications used during this study, with the exception of those medications taken for symptomatic relief of influenza symptoms, which will be recorded by the subject in their diary card, must be documented on the Case Report Form (CRF).

10.1.13 Adverse Events

AEs will be assessed from the time of administration of study medication through the final study visit.

10.1.14 Pharmacokinetic Exposure Samples

All subjects will have two pharmacokinetic (PK) samples drawn to assess peramivir drug levels. The first PK sample will be drawn on day 1 between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be drawn at the day 3 visit in all subjects. The sample will be drawn at the same time as the blood draw is completed for clinical laboratory investigations. The 30-60 minute sample (treatment day 1) and the day 3 PK sample will be analyzed for plasma concentrations of peramivir (ng/mL) and evaluated in a population exposure response analysis.

At selected sites a separate sub-study will also be conducted to collect additional PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK. Data from these two PK samples in all subjects will be combined with data from the PK sub-study (BCX1812-311PK) to perform a population based exposure-response analysis. This analysis will be described as part of the sub-study analysis plan.

All PK samples will be processed at a central bioanalytical laboratory. Refer to the instructions provided regarding the processing and shipment of these PK samples.

10.2 Screening Period

10.2.1 Informed Consent

The nature and purpose of the study and the expectations of a participating subject will be described to potential study subjects, their questions will be answered, and the subjects will then be asked to sign an informed consent document. Study subjects will then undergo the screening evaluation as noted in Section 10.22

10.2.2 Screening/Baseline Evaluation and Enrollment

Screening/baseline evaluation may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and

qualified staff.

Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subjects may be enrolled and receive treatment with study drug prior to receiving results of the laboratory assessments (with the exception of urine pregnancy test result, which must be known).

Eligible subjects will be enrolled and randomized to blinded study treatment. The randomization will be stratified by smoking status. The Investigator will prepare a request for blinded study drug assignment which includes the subject's screening number. The Investigator or designee at the clinical study center will contact the central randomization Interactive Voice System (IVRS call center). The IVRS call center will advise the study center of the two investigational study drug kit numbers that are assigned to that subject at enrollment.

Subjects that are determined to be ineligible will be advised accordingly, and the reason for ineligibility will be discussed. If desired by the subject the reason for ineligibility may be provided and/or discussed with their health-care provider by the Investigator or designee.

Ineligible subjects who have been screened for the study will also be entered on the IVRS. For such subjects, the screening number assigned, subject's date of birth and a reason for ineligibility will be entered on to the IVRS. **All ineligible subjects must be entered onto the IVRS within 24 hours of screening,** to assist with surveillance analysis during the course of the study.

10.3 Treatment Period—Study Day 1

Day 1 represents the only day of study drug dosing. Study drug administration should occur as soon as possible following informed consent, screening and randomization. Therefore, it is expected that the date of Screening/ Baseline and Day 1 will usually be the same date.

10.3.1 Pre-dose Evaluations-Study Day 1

Following an explanation of the Subject Self Assessment measures (Section 10.1.11), the subject shall complete the record of these assessments in their Study Diary prior to dosing. The subject will be counseled regarding the expectations for recording these assessments through Day 14.

Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) and a 12 lead ECG will be obtained prior to dosing.

A nasopharyngeal swab for influenza culture/ PCR assay will be obtained prior to dosing.

10.3.2 Post-dose Evaluations-Study Day 1

The blinded study drug will be administered (hour 0) as bilateral intramuscular injections within a period of ≤ 10 minutes. The calendar date and 24-hour clock time of the first and second injections will be recorded. The gluteal site of injection and the syringe needle length are also to be recorded in the subjects CRF. Sites are instructed to follow procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, provided in the study drug administration manual.

The following evaluations will be performed post-dose on Study Day 1:

- Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) 15 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the vital sign measurements in the subjects CRF.

- Draw a PK sample between 30 and 60 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the blood draw.

- Record any concomitant medications.

- Record any AEs.

10.4 Post-Treatment Assessment Period

10.4.1 Days 2, 3, 5, 9 and 14

Study evaluations will be performed on Days 2, 3, 5, 9 and 14 in accordance with the schedule of evaluations (Figure 1). Subjects with persistent moderate or severe influenza symptoms at day 14 will also complete a Day 21 visit, and if required a Day 28 visit.

Visits may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Study staff will attempt to contact the subjects on Day 2 by telephone to confirm their compliance with completion of the Subject Self Assessments, to note any concomitant medications and adverse events. Any adverse events reported by the subject during this telephone contact will be recorded on the adverse event form and verified during the visit on day 3.

For any subject with a Day 3 positive test for urine protein by dipstick test of 2+ or higher, who had a Baseline/ Day 1 protein dipstick of <2+, a 24 hour urine collection for assessment of protein and a simultaneous assessment by UPEP on the same specimen will be obtained.

At each visit it is important that the subject's Study Diary record be reviewed for completion of daily Subject Self Assessments. The subjects should be counseled as necessary regarding self assessments and Study Diary record requirements. The subject's diary card will be reviewed by study staff for alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms.

Day 3:

The second PK sample for all subjects will be obtained on Day 3 at the same time as the clinical laboratory blood specimen is obtained. The exact 24-hour clock time of the blood draw will be recorded in the subjects CRF.

Day 14:

If a subject has one or more persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit then the subject must be evaluated in further follow-up visits, at day 21 (± 3 days), and if required at day 28 (± 3 days) If a subject reports moderate or severe influenza symptoms then the investigator will

record the intensity of each of the influenza symptoms on a visit specific CRF page at the day 21 and day 28 visits. After day 14 the subject will not record symptoms in a diary.

Day 21 (if applicable):

The day 21 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 14. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at the Day 21 visit and such action(s) will be recorded on the Day 21 CRF page. The investigator will recall the subject for a further study visit at day 28 (± 3 days) if moderate or severe symptom(s) of influenza persist at Day 21.

Day 28 (if applicable):

The day 28 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 21. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at this visit and such action(s) will be recorded on the Day 28 CRF page. No further follow-up visits beyond day 28 are to be formally scheduled unless in the clinical judgment of the investigator further follow-up is required. The investigator will use his/her clinical judgment to manage the subject, referring the subject, if appropriate, for further medical care.

10.4.2 Adverse Events Reported at Post-treatment Visits

In this study, symptoms of influenza will be considered separately from adverse events reported during the post-treatment period. Accordingly, adverse events that have onset in the post-treatment period will be assessed and followed as specified in 11.2. Specifically, the investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

Figure 1 Study Measurements and Visit Schedule

Assessments	Screening [1] (Baseline)	Treatment Period	Assessment Day				End of Study Early Withdrawal
		Day 1[1]	Day 2[2]	Day 3	Day 5 (±1 day)	Day 9 (±3 day)	Day 14 (±3 day) [8]
Informed Consent	X						
Rapid Antigen test for Influenza A	X						
Medical History/Physical Exam	X						
Influenza-related complications checklist[3]	X			X	X	X	X
Inclusion/Exclusion	X						
Clinical Chemistries[4]	X			X	X		X
Hematology[4]	X			X	X		X
Exposure Pharmacokinetic Sample[9]		X		X[4]			
Serology (serum) Sample	X						X
Urinalysis[10]	X			X[4]	X		X
Urine Pregnancy Test	X						X
Vital Signs[5]	X	X		X	X	X	X
ECG[6]	X						
Sample (nasopharyngeal swab) for Influenza Virus Culture/ PCR assay and for resistance studies		X		X	X	X	
Study Drug Administration		X					
Subject Diary Review[7]		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

Study Measurements and Visit Schedule Figure Legend on Next Page

Study Measurements and Visit Schedule Figure Legend

[1] It is expected that the date of Screening and Day 1 (date of administration of study drug) will be the same. Visits at Screening and on subsequent study days may occur in subject's home by the investigator (all visits) or appropriately trained study center staff (Day 3, 5, 9 visits).

[2] Day 2 will be a telephone contact with the subject to ensure compliance with diary card completion, concomitant medication and adverse event review.

[3] A targeted physical examination will be conducted at each visit to record the presence/absence of influenza related complications.

[4] Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subject may be enrolled and begin treatment with study drug prior to receiving results. A PK sample will be drawn 30-60 minutes following the second treatment administration injection. On Day 3 an extra tube will be included with the safety blood sample to collect the second PK sample for evaluation of peramivir concentrations. For any subject with a Day 3 positive test for urine protein by dipstick test of 2+ or higher, who had a Baseline/ Day 1 protein dipstick of <2+, a 24 hour urine collection for assessment of protein and a simultaneous assessment by UPEP on the same specimen will be obtained.

[5] Vital sign measures will include blood pressure, pulse rate and respiration rate. Vital signs will be recorded at Screening, pre-dose and at 15 min following the study drug administration on Day 1, then once on remaining days as stipulated. The investigator will record oral temperature at baseline. Thereafter the subject will report oral temperature measurements twice daily in the Study Diary

[6] If the baseline ECG is interpreted by the conducting physician as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal and does not meet the exclusionary criteria (e.g. acute ischemia, medically significant dysrhythmia) then this subject may be enrolled If the conditions highlighted in section 10.1.5 are met for the subject.

[7] Subjects record symptom assessment in Study Diary, twice daily, beginning pre-dose on Day 1 through Day 9, then once daily through Day 14. Subjects record time lost from work or usual activities and rating of productivity compared to normal once daily through Day 14. Subjects record oral temperature twice daily throughout as well as all influenza related medications.

[8] For any subject with unresolved moderate or severe intensity influenza symptoms a follow up assessment will be scheduled at Day 21 (± 3 days) and Day 28 (± 3 days) if required (See Section 10.4.1).

[9] A PK sample will be drawn 30-60 minutes following the second treatment injection on Day 1, and on Day 3..

[10].For any subject with a Day 3 positive test for urine protein by dipstick test of 2+ or higher, who had a Baseline/ Day 1 protein dipstick of <2+, a 24 hour urine collection for assessment of protein and a simultaneous assessment by UPEP on the same specimen will be obtained.

11 ADVERSE EVENT MANAGEMENT

11.1 Definitions

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

AEs may be designated as "nonserious" or "serious" (see Section 11.1.2).

Surgical procedures are not AEs but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) will be documented in a subject's study diary and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as AEs unless the symptom(s) worsen to the extent that the outcome fulfils the definition of an SAE, which then must be recorded as such (see Section 11.1.2). Likewise, a RAT for influenza is required at screening in order to determine eligibility for the study, and therefore a positive RAT is not considered an AE.

11.1.2 Serious Adverse Event

A SAE is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the

outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

In addition Suspected Unexpected Serious Adverse Reactions (SUSAR) may also be reported to competent authorities where this type of reporting is required (e.g. European Union Directives). See section 11.2.3.

11.2 Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of AEs are to be collected from the time of study drug administration through the follow-up period ending on Day 14. The Investigator or designee must completely and promptly record each AE on the appropriate CRF. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

The Investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

11.2.1 Definition of Severity

All AEs will be assessed (graded) for severity and classified into one of four clearly defined categories as follows:

- **Mild:** (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
- **Moderate:** (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- **Severe:** (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- **Life-threatening:** (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization.

11.2.2 Definition of Relationship to Study Drug

The blinded Principal Investigator must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication, is confirmed by improvement upon stopping the medication (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

11.2.3 Reporting Serious Adverse Events

Any SAE / SUSAR (Suspected Unexpected Serious Adverse Reaction) must be reported to BioCryst or its designee within 24 hours of the Investigator's recognition of the SAE by first notifying the Medical Monitor at the number listed below:

Telephone:	**Europe: +44 1628 548000;**	**North America: 1-888-724-4908**
Facsimile:	**Europe: +44 1628 540028;**	**North America: 1-888-887-8097 or 1-609-734-9208**

In addition to the telephone numbers listed above, local country-specific toll free numbers may be provided within the study reference manual.

The site is required to fax a completed SAE / SUSAR Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the SAE / SUSAR must be reported and sent by facsimile to BioCryst or its designee as soon as they are available.

The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (e.g., revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and for retaining a copy in their files.

If the Investigator becomes aware of any SAE / SUSAR occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify BioCryst or its designee.

Any SAEs / SUSARs considered possibly related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch / CIOMS reporting system in accordance with FDA and other applicable regulations. However, the Investigator is not obligated to actively seek reports of AEs in former study participants.

While pregnancy is not considered an AE, all cases of fetal drug exposure via parent as study participant (see Section 4.4) are to be reported immediately to BioCryst or its designee. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee.

11.2.4 Emergency Procedures

In the event of an SAE / SUSAR, the Principal Investigator may request the unblinding of the treatment assignment for the subject affected. If time allows (i.e., if appropriate treatment for the SAE is not impeded), the Principal Investigator will first consult with the Medical Monitor regarding the need to unblind the treatment assignment for the subject. At all times, the clinical well-being of any subject outweighs the need to consult with the Medical Monitor.

The Principal Investigator may contact the IVRS central randomization center and request the unblinding of the treatment assignment that corresponds to the affected subject. The IVRS center will record the name of the Investigator making the request, the date and time of the request, the subject number and date of birth. The Sponsor will be informed within 24 hours if unblinding occurred.

12 STATISTICAL METHODS

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and other selected efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. The term "treatment group" refers to randomized treatment assignment: peramivir 300 mg, peramivir 600 mg, or placebo. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date within each subject number.

Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted with the SAS® System, version 9.1.3 or higher.

12.1 Data Collection Methods

The data will be recorded on the CRF approved by BioCryst. The Investigator must submit a completed CRF for each subject who signs an informed consent form (ICF), regardless of duration. All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF.

Documents (including laboratory reports, hospital records subsequent to SAEs, etc.) transmitted

to BioCryst should not carry the subject's name. This will help to ensure subject confidentiality.

12.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be created and approved prior to the review of any data. This document will provide a more technical and detailed description of the proposed data analysis methods and procedures.

12.3 Study Hypothesis

The primary hypothesis for evaluating the primary objective may be stated as follows:

The null hypothesis (H_0) is that the time to alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir 300mg (H_{01}) or peramivir 600mg (H_{02}).

The alternative hypothesis (H_1) is that subjects treated with peramivir 300mg (H_{11}) or peramivir 600mg (H_{12}) have an improvement in time to alleviation of influenza symptoms over those treated with placebo.

12.4 Sample Size Estimates

Up to a total of 750 evaluable subjects randomized in a 2:2:1 (300 subjects treated with peramivir 300mg: 300 subjects treated with peramivir 600 mg: 150 subjects treated with placebo) are estimated for this phase 3 study. Because results of clinic-based RAT tests may not precisely indicate presence of influenza infection, it is expected that at least 850 subjects will be randomized to treatment to ensure that 750 evaluable subjects are treated.

From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation for the 150 mg dose peramivir arm will be reduced by 30% compared to placebo (Table 5) yielding a hazard ratio of 0.70.

Table 5 Median time to alleviation of symptoms (30% reduction, 0.70 hazard ratio).

Median Time To Alleviation of Symptoms (Hours)		
Placebo	Peramivir 150mg	Difference (hours)
145.0	101.5	43.5
140.0	98.0	42.0
135.0	94.5	40.5
130.0	91.0	39.0
125.0	87.5	37.5
120.0	84.0	36.0
115.0	80.5	34.5
110.0	77.0	33.0
105.0	73.5	31.5
100.0	70.0	30.0

Using these assumptions, a sample size of 300 evaluable subjects per active treatment group and 150 evaluable subjects in the placebo group (a total of 750 evaluable subjects) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.025$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).

12.5 Analysis Populations

The populations for analysis will include the intent-to-treat (ITT), intent-to-treat infected (ITTI), per-protocol infected (PPI), and safety populations. Additional analysis populations may be defined to evaluate study results. Any additional analysis populations will be defined in the SAP.

Intent-To-Treat Population: The ITT population will include all subjects who are randomized. Subjects will be analyzed in the treatment group to which they were randomized. The ITT population will be used for analyses of accountability and demographics.

Intent-To-Treat Infected Population: The ITTI population will include all subjects who are randomized, received study drug, and have confirmed influenza A by culture or PCR. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI population will be used for primary analyses of efficacy.

Per-Protocol Infected: The PPI population includes all subjects in the ITTI population who receive an adequate intramuscular injection. The definition of an adequate intramuscular injection will be further described in the SAP. The PPI population will be used as supportive of the primary analyses for efficacy completed with the ITTI population.

Safety Population: The safety population will include all subjects who received study drug. Subjects will be analyzed according to the treatment received. This population will be used for all safety analyses.

12.6 Interim and End of Study Analyses

Interim Analysis

An independent DMC will review safety data on an ongoing basis. Safety analyses will be presented in a manner consistent with the presentations intended for the final analysis.

End of Study Analysis

A final analysis is planned after the last subject completes or discontinues the study, and the resulting clinical database has been cleaned, quality checked, and locked.

12.7 Efficacy Analyses

12.7.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the time to alleviation of symptoms of influenza in subjects diagnosed with influenza A, defined as the time from injection of study drug to the start of the time period when a subject has Alleviation of Symptoms. A subject has Alleviation of Symptoms if all of the seven symptoms of influenza (nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, feeling feverish) assessed on his/her subject diary are either absent or are present at no more than mild severity level and at this status for at least 21.5 hours (24 hours - 10%).

Descriptive statistics for the primary efficacy variable will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment comparisons between each active group and placebo will be assessed using a Wilcoxon-Gehan[19] statistic stratified by smoking status at screening Pairwise comparisons between each active group and placebo will be assessed using a Wilcoxon-Gehan test.. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing post-baseline assessment. For assessment of the primary efficacy endpoint, the overall significance level will be maintained by utilization of Hochberg's[20] method for the planned comparisons between the two active treatments and placebo.

12.7.2 Secondary Efficacy Endpoints

All secondary endpoints will be summarized using descriptive statistics by treatment group, and study day/time, if appropriate. Statistical comparisons for each endpoint will be constructed without adjustment for multiple endpoints.

The reduction in viral shedding will be assessed as the change in viral titers defined as the time-weighted change from baseline in $\log_{10}$ tissue culture infective $dose_{50}$ ($TCID_{50}$/mL) and will be summarized for each treatment group. The time-weighted average change from baseline will be calculated on a by-subject basis through Day 9 using the trapezoidal rule with all available post-baseline on-treatment data (data after initiation of study treatment) minus the baseline value. Specifically, the time-weighted area under the curve for time a (t_a) to time b (t_b) is given by the formula

$$TWAUC = \frac{AUC(t_a - t_b)}{(t_a - t_b)},$$

where $AUC(t_a - t_b) = \sum_{i=a}^{b-1} \frac{(y_i + y_{i+1})(t_{i-1} - t_i)}{2}$ and t_i represents the date of the i^{th} viral titer assessment and y_i represents the $\log_{10}$ value of the i^{th} viral titer assessment. If there is a baseline value and only one follow-up value, y_i then the time-weighted change from baseline is defined as the difference between y_i and baseline. If there is a baseline value and no follow-up value, the subject is excluded from analysis. The differences between each of the peramivir treatment groups and placebo will be evaluated using a van Elteren Test adjusting for smoking status at screening. Analyses of the PCR results will be analyzed in a similar manner.

Subject's oral temperature will be summarized by study visit and treatment group. Differences between the treatment groups will be assessed using the van Elteren test controlling for smoking status at screening. A subject has Resolution of Fever if he/she has a temperature < 37.2°C (99.0°F) and no antipyretic medications have been taken for at least 12 hours. The time to resolution of fever will be estimated using the method of Kaplan-Meier using temperature and symptom relief medication information obtained from the subject diary data. Differences between the treatment groups will be assessed using the Wilcoxon-Gehan statistic controlling for smoking status at screening. Subjects who do not have resolution of fever will be censored at the time of their last non-missing post-baseline temperature assessment.

The number and percentage of subjects experiencing influenza related complications will be summarized by complication preferred term and treatment group. The difference between the treatment groups will be assessed a logistic regression model with factors for treatment group and smoking status at screening. Pairwise differences between the treatment groups will be evaluated using contrasts from the final logistic regression model.

12.7.3 Exploratory Endpoints

The MRU, MRU-related direct costs, and indirect costs attributable to days missed of work and work productivity and/or performance losses will be summarized by treatment group and smoking status. Methods for describing differences between treatment groups will be presented in the SAP.

Genotypic (including Hemagglutinin and Neuraminidase), phenotypic, viral culture and PCR data will be listed for each subject. These listings will be constructed in a manner consistent with the FDA June 2006 Guidance Document: *"Guidance for Submitting Influenza Resistance Data"*.[18] Additionally, the number and percentage of genotypic changes from wild-type amino acid will be summarized separately for treatment group, protein type, and study visit.

12.8 Safety Analyses

AEs will be mapped to a MedDRA-preferred term and system organ classification. The occurrence of TEAEs will be summarized by treatment group using MedDRA-preferred terms, system organ classifications, and severity. If a subject experiences multiple events that map to a single preferred term, the greatest severity and strongest Investigator assessment of relation to study drug will be assigned to the preferred term for the appropriate summaries. All AEs will be

listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to study drug will be generated.

Descriptive summaries of vital signs and clinical laboratory results will be presented by study visit. Laboratory abnormalities will be graded according to the DAIDS Table for Grading Adverse Events for Adults and Pediatrics (Publish Date: December 2004). The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized by treatment group. Laboratory toxicity shifts from baseline to Day 3, Day 5, and Day 14 will be summarized by treatment group.

Abnormal physical examination findings will be presented by treatment group. The number and percent of subjects experiencing each abnormal physical examination finding will be included.

Concomitant medications will be coded using the WHO dictionary. These data will be summarized by treatment group.

Subject disposition will be presented for all subjects. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

12.9 Sub-Study and Pharmacokinetic Analysis

A sub-study to collect pharmacokinetic samples in up to 60 peramivir treated subjects to examine exposure response will be conducted at selected sites. The data from the sub-study will be combined with the two PK samples (collected on all subjects at 30-60 minutes following administration of study drug and on study day 3) to perform a population exposure-response analysis. All analyses related to exposure-response will be completed as part of the sub-study. All statistical methods will be outlined as part of the sub-study protocol and exposure-response analysis plan. All sub-study analyses, and exposure-response analyses from PK samples obtained in this study and a companion study BCX1812-312, will be reported in a separate sub-study report.

12.10 General Issues for Statistical Analysis

12.10.1 Multiple Comparisons and Multiplicity

In order to maintain the overall type I error in the presence of the planned comparisons between the two peramivir treatments and placebo, Hochberg's method will be applied to the primary efficacy endpoint analysis. No other adjustments for multiple comparisons are planned.

12.10.2 Covariates

Primary and secondary efficacy analyses will be adjusted for smoking status at screening.

12.10.3 Planned Sub-Groups

The primary efficacy endpoint will be summarized separately by smoking status at screening using descriptive statistics by treatment group and study day, if appropriate. No formal statistical testing will be utilized.

Additional analyses may be performed by country, if necessary, for submission to local regulatory authorities.

12.10.4 Missing Data

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been randomized. No attempt will be made retrospectively to obtain missing subject reported data (including influenza symptom severity assessments, temperature, ability to perform usual activities, missed days of work and impact of influenza on subject's work performance and/or productivity) that has not been completed by the subject at the time of return of the subject diary to the investigative site. In situations where it is not possible to obtain all data, it may be necessary to impute missing data.

In assessing the primary efficacy endpoint, for subjects who withdraw or who do not experience alleviation of symptoms, missing data will be censored using the date of subject's last non-missing assessment of influenza symptoms. Missing assessments of influenza symptoms conservatively will be imputed as having severity above absent or mild (as failures). For the subject diary data, the following data conventions will be utilized. Missing diary completion will be imputed as 11:59 for diary entries designated as morning and 23:59 for evening and daily reported values. Select exploratory sensitivity analyses may be conducted to ascertain the effect, if any, of these methods. These sensitivity analyses are further described in the SAP. Secondary efficacy endpoints with time to event data will be censored using the date of subject's last non-missing assessment of the given endpoint.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1.1 Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; GCPs, including International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator should submit written reports of clinical study status to their Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) annually or more frequently if requested by the IRB/ IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the IRB/ IEC should be maintained in the study documents file. Copies of clinical study status reports (including termination) should be provided to BioCryst.

13.1.2 Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent form, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the investigational medicinal product is released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The Investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the Investigator will provide the IRB/IEC with a report of the outcome of the study.

13.1.3 Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by BioCryst and by the reviewing IRB/ IEC. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and BioCryst policy.

The Investigator must explain to potential subjects or their legal representatives the aims, methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the ICF. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1.4 Payment to Subjects

Reasonable compensation to study subjects may be provided if approved by the IRB/IEC responsible for the study at the Investigator's site.

13.1.5 Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his/her IRB/IEC as required.

13.2 Study Monitoring

During trial conduct, BioCryst or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow BioCryst monitors or its designees and appropriate regulatory authorities direct access to source documents to perform this verification.

13.3 Quality Assurance

The trial site may be subject to review by the IRB/IEC, and/or to quality assurance audits performed by BioCryst, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

13.4 Study Termination and Site Closure

BioCryst reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by BioCryst, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5 Records Retention

To enable evaluations and/or audits from regulatory authorities or BioCryst, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to BioCryst. The Investigator must obtain BioCryst's written permission before disposing of any records.

13.6 Study Organization

13.6.1 Data Monitoring Committee

BioCryst will assemble an independent Data Monitoring Committee (DMC) to assess safety parameters of the trial on a periodic, ongoing basis while the trial is in progress. The committee will include a statistician and three physicians, two of whom will be Infectious Disease / Clinical Virology specialists. Full details of the composition of the DMC and how the DMC is to operate will be described in a separate DMC charter.

13.7 Confidentiality of Information

BioCryst affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by BioCryst**.** However, in compliance with federal regulations, BioCryst requires the investigator to permit BioCryst's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study.

BioCryst will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule, where this rule is applicable. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of subjects participating in BioCryst-sponsored Clinical Trials. "Authorization" is required from each research subject, i.e., specified permission granted by an individual to a covered entity for the use or disclosure of an individual's protected health information. A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization may be combined in the Informed Consent document (approved by the IRB/IEC) or it may be a separate document, (approved by the IRB/IEC) or provided by the Investigator or Sponsor (without IRB/IEC approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appropriate individual. HIPAA authorizations are required for U.S. sites only.

13.8 Study Publication

All data generated from this study are the property of BioCryst and shall be held in strict confidence along with all information furnished by BioCryst. Independent analysis and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of BioCryst. Written permission to the Investigator will be contingent on the review by BioCryst of the statistical analysis and manuscript and will provide for nondisclosure of BioCryst confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

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15 APPENDICES

15.1 NYHA Functional Classification Criteria: Heart Failure and Angina

NYHA Functional Classification of Heart Failure

Class I
No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea.
Class II
Symptoms with ordinary physical activity. Walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold weather, in wind, or when under emotional stress causes undue fatigue or dyspnea.
Class III
Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea.
Class IV
Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea.

NYHA Functional Classification of Angina

Class I
Angina only with unusually strenuous activity.
Class II
Angina with slightly more prolonged or slightly more vigorous activity than usual.
Class III
Angina with usual daily activity.
Class IV
Angina at rest.

15.2 Criteria for Severe COPD and Severe Asthma

The following guidelines are provided to assist in the evaluation of subjects who have a medical history for Chronic Obstructive Pulmonary Disease (COPD) and/or Asthma. Subjects with severe COPD or severe persistent Asthma are to be excluded from this study. (See section 8.1.2, exclusion criteria number 3).

Classification of Asthma from National Asthma and Education and Prevention Program

Classification	Days with Symptoms	Nights with Symptoms	For Adults and Children (> 5 yrs) who can use a spirometer or peak flow meter	
			FEV_1 or PEF % Predicted Normal	PEF Variability (%)
Severe persistent	Continual	Frequent	≤ 60	> 30
Moderate Persistent	Daily	> 1/ week	> 60 - < 80	> 30
Mild Persistent	> 2 / week but < 1 times / day	> 2/ month	≥ 80	20 – 30
Mild Intermittent	≤ 2 / week	< 2 / month	≥ 80	< 20
FEV_1: percentage predicted value for forced expiratory volume in 1 second. PEF: percentage of personal best for peak expiratory flow.				

Extracted from: Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program. HHS/NIH 2007

Spirometric Classification of COPD Severity based upon Post-Bronchodilator FEV1 (GOLD Criteria)

Stage	Characteristics
Mild COPD	$FEV_1/FVC < 70\%$ $FEV_1 \geq 80\%$ predicted
Moderate COPD	$FEV_1/FVC < 70\%$ $50\% \leq FEV_1 < 80\%$ predicted
Severe COPD	$FEV_1/FVC < 70\%$ $30\% \leq FEV_1 < 50\%$ predicted
Very Severe COPD	$FEV_1/FVC < 70\%$ $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure
FEV_1: percentage predicted value for forced expiratory volume in one second. FVC: forced vital capacity	

Extracted from: Rabe KF, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (GOLD Executive Summary). Am. J. Respir. Crit. Care Med. 2007:176;532-555.

CLINICAL STUDY PROTOCOL

Protocol No. BCX1812-311

IND No. 76,350

A PHASE 3 MULTICENTER, RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF INTRAMUSCULAR PERAMIVIR IN SUBJECTS WITH UNCOMPLICATED ACUTE INFLUENZA

THE **IMPROVE I** STUDY

(**I**ntra**M**uscular **P**eramivir for the **R**elief **O**f symptoms and **V**irologic **E**fficacy)

Short title: Intramuscular Peramivir for the Treatment of Uncomplicated Influenza

Protocol Date(s):
Version 1.0: 04 September 2007
Version 2.0: 05 October 2007
Version 3.0: 20 November 2007
Version 4.0: 18 December 2007

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1 TITLE PAGE

Protocol Number:	BCX1812-311
Study Title:	A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza.
IND Number:	76, 350
Investigational Product:	Peramivir (BCX1812)
Indication Studied:	Uncomplicated acute influenza
Sponsor:	BioCryst Pharmaceuticals, Inc. 2190 Parkway Lake Drive Birmingham, AL 35233
Development Phase:	3
Sponsor Medical Officer:	W. James Alexander, M.D., M.P.H. Senior Vice President, Clinical Development Chief Medical Officer Phone: +1 919 859 1302 Fax: +1 919 851 1416 Email Address: jalexander@biocryst.com
Compliance Statement:	This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and clinical research guidelines established by the Code of Federal Regulations (Title 21, CFR Parts 50, 56, and 312) and ICH Guidelines. Essential study documents will be archived in accordance with applicable regulations.
Final Protocol Date:	Version 1.0: 04 September 2007
Amendment(s) Date(s):	Version 2.0: 05 October 2007 Version 3.0: 20 November 2007 Version 4.0: 18 December 2007

1.1 Protocol Approval Signature Page

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

BioCryst Pharmaceuticals, Inc.

Reviewed and Approved by:

W. James Alexander, M.D., M.P.H.
Senior Vice President, Clinical Development
Chief Medical Officer

18 December 2007
Date

Elliott Berger, PhD
Senior Vice President, Regulatory Affairs

18 December 2007
Date

1.2 Clinical Study Protocol Agreement

Protocol No. BCX1812-311

Protocol Title: A phase 3 multicenter, random ized, double-blind, placebo-controlled study to evaluate the effic acy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practices, and all applicable regulatory requirements.

Investigator's Signature Date

Name (Print)

2 SYNOPSIS

Protocol No.	BCX1812-311
Protocol Title:	A phase 3, multicenter, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of intramuscular peramivir in subjects with uncomplicated acute influenza.
Sponsor:	BioCryst Pharmaceuticals, Inc.
Investigators/Study Sites:	Multinational
Development Phase:	3
Objectives:	
Primary:	To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza.
Secondary:	1. To evaluate the safety and tolerability of peramivir administered intramuscularly 2. To evaluate secondary clinical outcomes in response to treatment 3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment
Exploratory:	1. To assess pharmacoeconomic measures as response to treatment 2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment
Number of Subjects:	Total enrollment: approximately 600 subjects will be randomized. Subjects will be allocated to treatment using a 2:1 randomization schema. An evaluable subject is one who is randomized, receives study drug, and has confirmed acute influenza A or B by primary viral culture or PCR. A positive Rapid Antigen Test (RAT) for influenza A and B at screening will be required for enrollment. Because results of clinic-based RAT tests may not precisely indicate presence of influenza infection, it is expected that approximately 600 subjects will be randomized to treatment to ensure enrollment of approximately 450 subjects that are positive for influenza A.
Study Design:	This is a multinational, randomized, double-blind study comparing the efficacy and safety of a 300 mg dose of peramivir administered intramuscularly versus placebo in adults with

	uncomplicated acute influenza. All subjects will be centrally randomized to treatment with 300 mg of peramivir or placebo in a ratio of 2:1, and will be stratified according to smoking status and RAT test for influenza A or B. Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in equally divided doses). Procedures for gluteal intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual. Subjects eligible for screening will have an anterior nasal swab collected for testing by rapid antigen testing (RAT) for influenza A and B in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/exclusion criteria may be enrolled into the study. All enrolled subjects will record the following information in a Study Diary: • Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14. • Oral temperature measurements taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (Tylenol or paracetamol) or other anti-pyretic medications. • Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14. • Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14. Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result on culture) as well as other virologic assessments (e.g. PCR, genotypic testing). All virologic assessments will be performed by a central laboratory. Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The

	first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis. At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.
Study Population:	Male and female subjects, 18 years of age and older, with symptoms consistent with a diagnosis of uncomplicated acute influenza infection may be screened for enrollment. Subject eligibility will require the presence of two or more symptoms of at least moderate severity consistent with acute influenza as well as positive results obtained from a rapid antigen test (RAT) for influenza A and/or B at screening.
Inclusion Criteria:	1. Male and non-pregnant female subjects age ≥18 years. 2. A positive Influenza A and/or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one-hour. 3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening. 4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity. 5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity. 6. Onset of symptoms no more than 48 hours before presentation for screening. 7. Written informed consent.
Exclusion Criteria:	1. Women who are pregnant or breast-feeding. 2. Presence of clinically significant signs of acute respiratory distress. 3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma (See section 15.2). 4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the

	past 12 months. 5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia. 6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min). 7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza. 8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics. 9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening. 10. Currently receiving treatment for viral hepatitis B or viral hepatitis C. 11. Presence of known HIV infection with a CD4 count <350 cell/mm^3. 12. Current therapy with oral warfarin or other systemic anticoagulant. 13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening. 14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days. 15. Immunized against influenza with inactivated virus vaccine within the previous 14 days. 16. Receipt of any intramuscular injection within the previous 14 days. 17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study. 18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study. 19. Participation in a study of any investigational drug or device within the last 30 days.
Study Endpoints:	
Primary Endpoint:	*Clinical:* Time to alleviation of clinical symptoms of influenza.
Secondary Endpoint(s):	*Safety:* Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests.

	Clinical: Time to resolution of fever. Incidence of influenza related complications. *Virologic:* Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).
Exploratory Endpoint(s):	*Pharmacoeconomic*: Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity. *Virologic:* Quantitative change in influenza virus shedding, measured by PCR. Change in influenza virus susceptibility to neuraminidase inhibitors.
Investigational Product, Dose, and Mode of Administration:	
Peramivir (BCX-1812), 75mg/mL, 2mL (150mg) per injection, administered as bilateral intramuscular injections.	
Reference Therapy, Dose, and Mode of Administration:	
Matching Placebo (buffered diluent), 2mL per injection administered as bilateral intramuscular injections.	
Duration of Treatment:	Following treatment on day 1, study duration for all subjects is expected to be up to 14 days (including all visits). Presence of unresolved adverse events and/or treatment-emergent laboratory findings at the Day 14 visit, or persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit, will require additional follow up.
Statistical Methods:	
Study Hypothesis:	The null hypothesis (H_0) for this study is that the time to alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir 300mg. The alternative hypothesis (H_1) is that subjects treated with peramivir 300mg have an improvement in time to alleviation of influenza symptoms over those treated with placebo.
Sample Size:	From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation

	for the 300 mg dose peramivir arm will be reduced by 30% compared to placebo yielding a hazard ratio of 0.70. Data from the phase 2 study suggested that the activity of peramivir against influenza B was limited. Therefore, this study will be sized to ensure that a minimum number of influenza A subjects is enrolled to achieve study power. Using these assumptions, a sample size of 450 evaluable subjects with influenza A (300 in the 300 mg treatment group and 150 evaluable subjects in the placebo group) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.05$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).
Efficacy:	The primary efficacy analysis will be completed with the intent-to-treat infected population (ITTI). The ITTI population will include all subjects who are randomized, received study drug, and have confirmed influenza A and/or B by primary viral culture or PCR. The primary efficacy variable is the time to alleviation of symptoms, defined as the time from injection of study drug to the start of the time period when each of seven symptoms of influenza are either absent or are present at no more than mild severity level and remain at no worse than this severity status for a 21.5 hour (24 hours – 10%) period. Descriptive statistics for the primary efficacy variable will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group. Treatment differences between the 300 mg peramivir dose and placebo will be assessed using a Wilcoxon-Gehan statistic stratified by smoking status and positive PCR or viral culture for influenza A or B at screening. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing assessment. Efficacy analyses will be repeated for Intent To Treat Infected with influenza A (ITTI-A) population and Per-Protocol Infected population (PPI). The ITTI-A population includes all subjects who are randomized, received study drug, and have confirmed influenza A by primary viral culture or PCR. The PPI population will include those subjects in the ITTI population who received an adequate intramuscular injection. Details of this population will be described in the statistical analysis plan. Changes in influenza virus $TCID_{50}$ (viral titers from nasopharyngeal specimens) will be compared using the van Elteren statistic controlling for smoking status and positive PCR or viral culture for influenza A or B at screening. Analyses of other continuous endpoints will be analyzed in a similar manner.

	The number and percentage of subjects experiencing influenza related complications (IRC) will be summarized by complication preferred term and treatment group. The difference between the treatment groups (300 mg peramivir and placebo) will be assessed using a Cochran-Mantel-Haenszel (CMH) test statistic controlling for smoking status, and positive PCR or viral culture for influenza A or B at screening.
Safety:	Safety analyses will be presented for all subjects in the safety population, defined as all randomized subjects who receive at least one dose of study drug. Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ classification. The occurrence of treatment-emergent AEs will be summarized using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent SAEs and treatment-emergent AEs that are related to study medication will be generated. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Descriptive summaries of vital signs and quantitative clinical laboratory changes will be presented by study visit. Frequency and percentages of subjects with abnormal laboratory test results will be summarized by toxicity grade. Concomitant medications will be mapped to a WHO preferred term and drug classification. The number and percent of subjects taking concomitant medications will be summarized using preferred terms and drug classifications. The number and percent of subjects experiencing each abnormal physical examination finding will be presented. The number and percent of subjects discontinuing study as well as the reasons for discontinuation will be summarized by treatment group.
Date of Protocol:	Version 1.0: 04-September-2007
Amendment (Dates):	Version 2.0: 05-October-2007 Version 3.0: 20-November-2007 Version 4.0: 18-December-2007

3 TABLE OF CONTENTS

3.1 List of Figures

3.2 List of Tables

4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
$AUC_{0–72}$	area under the curve from time 0 to 72 hours
$AUC_{0–\infty}$	area under the curve extrapolated from time 0 to infinity
BMI	Body Mass Index in kg/m^2
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CIOMS	Council for International Organizations of Medical sciences
C_{max}	maximum plasma concentration
CK	creatine kinase
CMH	Cochran-Mantel-Haenszel
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRF	Case Report Form
CV	coefficient of variation
ECG	Electrocardiogram
GCP	Good Clinical Practice
HCG	human chorionic gonadotropin
HIV	Human immunodeficiency virus
IC_{50}	median inhibitory concentration
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRC	influenza related complications
ITT	intent-to-treat
ITTI	intent-to-treat infected (Includes ITTI and ITTI-A)
IUD	intrauterine device
IVRS	interactive voice response system
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MRU	medical resource utilization
NSAID	non-steroidal anti-inflammatory drug
PCR	polymerase chain reaction
PPI	per-protocol infected
RAT	Rapid Antigen Test
RBC	red blood cell

SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SUSAR	Suspected Unexpected Serious Adverse Event
$t_{1/2}$	elimination half-life
$t_{1/2}$ λz	terminal half-life
$TCID_{50}$	tissue-culture infective $dose_{50}$
TEAEs	treatment-emergent adverse events
T_{max}	time to attain maximum plasma concentration
UPEP	Urine protein electrophoresis
WBC	white blood cell
WHO	World Health Organization

5 INTRODUCTION

5.1 Background

Influenza virus is a member of the *orthomyxovirus* family and causes an acute viral disease of the respiratory tract. Typical influenza illness is characterized by abrupt onset of fever, headache, myalgia, sore throat, and nonproductive cough.[1] The illness is usually self-limiting, with relief of symptoms occurring within 5 to 7 days. Nevertheless, it is an important disease for several reasons, including ease of communicability, short incubation time, rapid rate of viral mutation, morbidity with resultant loss of productivity, risk of complicating conditions, and increased risk of death, particularly in the elderly. During 19 of the 23 influenza seasons between 1972/1973 and 1994/1995, estimated influenza-associated deaths in the United States ranged from approximately 25 to more than 150 per 100,000 persons above 65 years of age, accounting for more than 90% of the deaths attributed to pneumonia and influenza.[2]

Presently, only a few measures are available that can reduce the impact of influenza: active immunoprophylaxis with an inactivated or live attenuated vaccine and chemoprophylaxis or therapy with an influenza-specific antiviral drug. Neuraminidase inhibitors are the current mainstay of antiviral treatment for influenza. Marketed neuraminidase inhibitors include zanamivir (Relenza®, GlaxoSmithKline) and oseltamivir (Tamiflu®, Roche-Gilead), an oral prodrug of the active agent, oseltamivir carboxylate. Influenza neuraminidase is a surface glycoprotein that cleaves sialic acid residues from glycoproteins and glycolipids. The enzyme is responsible for the release of new viral particles from infected cells and may also assist in the spreading of virus through the mucus within the respiratory tract. The neuraminidase inhibitors represent an important advance in the treatment of influenza with respect to activity against influenza A and B viruses, with proven therapeutic value in reducing influenza lower respiratory complications,[3] and lower rates of antiviral drug resistance[4].

The use of currently available neuraminidase inhibitors has been limited by concerns including, the degree of effectiveness, the requirement for an inhaler device (zanamivir), and the emergence of resistant influenza virus variants in some treated populations.[5] In addition, there are risks of bronchospasm with zanamivir; and gastrointestinal side effects, with oseltamivir.

Peramivir is a neuraminidase inhibitor that represents a potentially promising addition to the armamentarium of drugs for the treatment of influenza infections due to its potential for parenteral administration and lower frequency of dosing.

5.2 Rationale for Study

An oral formulation of peramivir has previously been evaluated in a full range of safety, tolerability, pharmacokinetic, and efficacy studies. In a multinational phase 3 clinical trial conducted in 1999-2001, oral peramivir demonstrated antiviral activity against influenza A and B infections, and improvement in the relief of clinical symptoms. Because of the limited bioavailability of peramivir following oral administration (<5%), it was determined that the parenteral route of administration is more appropriate for the delivery of peramivir. Subsequent phase 1 studies of intravenous and intramuscular formulations of peramivir have confirmed that parenteral routes of administration result in plasma levels of drug that are as much as 100 times those achieved via the oral route. In a phase 2 study of intramuscular peramivir in subjects with acute uncomplicated influenza, subjects who received a single injection of 150mg or 300mg

peramivir had clinically meaningful reductions in the time to alleviation of symptoms of influenza, compared to subjects who received a placebo injection. Further details of these studies are provided below and in the Investigator Brochure.

Because of the previous demonstration of clinical efficacy of intramuscular peramivir in acute influenza in the phase 2 study, and the encouraging pharmacokinetic and preliminary safety profile of the intramuscular formulation of peramivir demonstrated to date, this phase 3 study will be conducted to evaluate the efficacy and safety profile of an intramuscular 300 mg peramivir dose compared to placebo.

5.3 Non-Clinical Experience with Peramivir

5.3.1 In vitro Assays

Peramivir is a selective inhibitor of viral neuraminidase, with 50% inhibitory concentrations (IC_{50}) for bacterial and mammalian enzymes of >300μM.[6] In an *in vitro* study, 42 influenza A and 23 influenza B isolates were collected from untreated subjects during the 1999–2000 influenza season in Canada.[7] These isolates were tested for their susceptibility to the neuraminidase inhibitors zanamivir, oseltamivir carboxylate, and peramivir using a chemiluminescent neuraminidase assay. Inhibition of Type A influenza neuraminidase by peramivir was approximately an order of magnitude greater than inhibition of neuraminidase from Type B viruses. IC_{50} values for the Type A enzymes ranged from <0.1 to 1.4nM, whereas the Type B enzymes ranged from <0.1 to 11nM, with three out of four values in the 5- to 11nM range. Peramivir was the most potent drug against influenza A (H3N2) viruses with a mean IC_{50} of 0.60nM as well as most potent against influenza B with a mean IC_{50} of 0.87nM.

In another *in vitro* comparison of peramivir, oseltamivir, and zanamivir, using a neuraminidase inhibition assay with influenza A viruses, the median IC_{50} of peramivir (approximately 0.34nM) was comparable to that of oseltamivir (0.45nM) and significantly lower than zanamivir (0.95nM). For influenza B virus clinical isolates, the median IC_{50} of peramivir (1.36nM) was comparable to that of zanamivir (2.7nM) and lower than that of oseltamivir (8.5nM).[8]

The potency of peramivir was evaluated against five zanamivir-resistant and six oseltamivir-resistant influenza viruses.[9] Peramivir remained a potent inhibitor against all oseltamivir-resistant viruses including the mutations H274Y, R292K, E119V, and D198N, with IC_{50} values ≤40nM. Peramivir also potently inhibited ($IC_{50} \leq 26$nM) the neuraminidase activity of zanamivir-resistant strains, which had the following mutations: R292K, E119G, E119A, and E119D. However, one zanamivir-resistant influenza B virus, B/Mem/96, with a mutation R152K isolated from cell culture, was relatively resistant to all neuraminidase inhibitors, including peramivir (IC_{50} = 400nM).

5.3.2 Animal Models

In a mouse model of influenza infection, a single intramuscular injection of peramivir (10mg/kg) given 4 hours prior to inoculation with an A/NWS/33 (H1N1) influenza strain resulted in 100% survival in contrast to 100% mortality in a control group injected with saline.[6] In the same mouse model, treatment of mice up to 72 hours after influenza infection using peramivir (20mg/kg) resulted in 100% survival, compared to 100% mortality in the control group injected with vehicle.[10]

Peramivir has also demonstrated activity in animal models utilizing a clinical H5N1 isolate as the

infecting virus strain. In a mouse model, a single intramuscular dose of peramivir (30mg/kg) injected 1 hour after inoculation with the highly pathogenic (H5N1) A/Vietnam/1203/04 strain, resulted in a 70% survival rate that was similar to that seen in mice treated with oseltamivir given orally at 10mg/kg/day for 5 days[11]. In similar experiments, mice inoculated with the same strain of H5N1 virus that were then treated for up to 8 days with intramuscular peramivir exhibited 100% survival[12]. This longer duration of peramivir treatment also prevented viral replication in the lungs, brain and spleen at days 3, 6 and 9 post inoculations.

5.4 Previous Phase 3 Clinical Experience with Oral Peramivir

An oral formulation of peramivir has previously demonstrated antiviral activity and preliminary clinical efficacy in challenge studies in human volunteers, as well as in treatment studies in patients with uncomplicated acute influenza infections during the influenza seasons of 1999-2001. A Phase 3 multinational study (BC-01-03) of oral peramivir was conducted. Two dose regimens of oral peramivir, 800mg QD for 5 days, or 800mg QD on Day 1, followed by 400mg QD for 4 days, were compared to a matched placebo treatment group. A total of 1246 subjects were randomized to treatment at sites in the USA, Western and Eastern Europe, South America, Australia and New Zealand. As presented in the Table 1 below, the primary end-point of time to relief of influenza symptoms in 694 subjects with confirmed influenza was not found to be significantly different (p=0.17) between the three treatment groups.[13] A sub-group analysis of the time to relief of symptoms by country or region demonstrated marked differences in the primary endpoint.. In the subset of influenza-infected subjects enrolled at sites in the US, clinically meaningful differences in time to relief of influenza symptoms between the placebo and the two peramivir arms were observed, however statistical significance (p=0.07) was not achieved. However, a number of secondary endpoints in this phase 3 study, such as time to overall well-being, time to normal activity, incidence of influenza related complications and quantity of viral shedding, achieved or approached statistically significant differences between the peramivir and placebo treatment groups (p=0.03-0.06).

Table 1 Results of study BC-01-03

Dose and Regimen	Median Time to Relief of Influenza Symptoms (Hours)	
	Overall Results (n=694)	US Sites (n=198)
Peramivir 800mg po x 5d	89.0	70.8
Peramivir 800mg po x 1d and 400mg po x 4d	91.7	88.8
Placebo x 5 days	104.4	106.8
p value	0.17	0.07

5.5 Previous Phase 1 Experience with Intramuscular Peramivir

Two phase 1 studies evaluating the safety and pharmacokinetics of an intramuscular formulation of peramivir have been conducted in a total of 45 healthy volunteers receiving peramivir. An additional phase 1 study has recently been initiated to evaluate the pharmacokinetics and

tolerability of higher single doses (up to 600 mg) of intramuscular peramivir.

Study Peramivir-Him-06-111 evaluated the single dose pharmacokinetics and tolerability of 75mg, 150mg and 300mg doses of peramivir administered as intramuscular (i.m.) and intravenous (i.v.) injections in a crossover design (9 subjects per group). Peak plasma levels of i.m. peramivir generally occurred within 30 minutes following injection. Plasma pharmacokinetic parameters for i.m. peramivir are summarized in Table 2 below for the three intramuscular single dose regimens evaluated.

Table 2 Pharmacokinetic parameters from study Him-06-111.

Dose (mg)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (hr·ng/mL)	$t_{1/2}$[a] (hr)
75 i.m.	4296 ± 812	11659 ± 1123	19.8 ± 7.9
150 i.m.	7612 ± 884	23952 ± 3804	24.3 ± 4.1
300 i.m.	15150 ± 2367	49649 ± 5619	22.8 ± 2.5
[a]terminal half life			

In a second phase 1 study, Peramivir-Him-06-112, the same dose levels of peramivir were administered as single i.m. injections on two consecutive days (6 subjects per group). This double-blind study also included a placebo arm. The pharmacokinetic parameters of i.m. peramivir following the second day of dosing were consistent with those seen following single doses of the drug.

An additional phase 1 study, BCX1812-117, was initiated to evaluate the effect of needle length adjustment according to gender and body mass index on the pharmacokinetics and safety of peramivir following ventrogluteal intramuscular injection, and dorsogluteal intramuscular injection in a subgroup of subjects. Interim data are available for the first 40 subjects who received single peramivir doses of 600 mg, consisting of directly observed tolerability assessments, safety laboratory studies, reported adverse events, and pharmacokinetic data. Clinical laboratory results obtained 72 hours after dosing showed no abnormalities with regards to hematology or urinary analytes and the only chemistry analytes outside normal ranges (CK and AST) were related to receipt of intramuscular injection.

A majority of the first 40 subjects treated complained of acute pain immediately after injection at the ventrogluteal site and a number of these subjects reported that the pain was also associated with muscle cramping. Some subjects reported radiation of pain to the lower extremities. In most instances, these reactions abated after 15-30 minutes.

This protocol also evaluated the acute tolerability of doses of 450 mg and 600 mg of peramivir administered at the dorsogluteal site. Direct observations determined that the use of the dorsogluteal injection site resulted in an acute tolerability profile of the 600 mg dose of peramivir that was improved compared to that observed with ventrogluteal site injections. However, a number of these subjects also experienced acute pain and discomfort and some reported muscle cramping in the gluteal area which persisted for up to 30 minutes.

In summary, this study confirmed that the intramuscular injection of peramivir results in acute pain and discomfort after gluteal injection in the majority of subjects in whom total doses of up to 600mg are administered. In some subjects, the acute pain at the injection site may also be

associated with muscle cramping. Based on these findings, the dorsogluteal site will be utilized in future trials.

The safety data for i.m. peramivir administered as single doses ranging from 75 mg to 600mg at the dorsogluteal injection site in each of the 3 phase 1 studies conducted to date have been unremarkable. No serious adverse events were reported. The most commonly observed adverse events or laboratory abnormalities were injection site pain or discomfort, headache, and transient increases in muscle enzymes (CK). There have been several reports of signs and symptoms of self-limited vasovagal reactions following injections. No consistent differences in frequency of adverse events or laboratory toxicities were observed between the active and placebo treatment groups in the controlled phase 1 studies, with the exception that CK elevations appeared to be dose related in the peramivir treatment groups.

5.6 Phase 2 Experience with Intramuscular Peramivir

A phase 2 study BCX1812-211 was completed in 2007. This study was a randomized, double-blind placebo- controlled study to evaluate the efficacy and safety of two single dose regimens of peramivir. A total of 344 subjects were enrolled into this study with 115 subjects randomized to Placebo; 114 subjects randomized to peramivir 150 mg; and 114 subjects randomized to peramivir 300 mg. The primary endpoint of the study was the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza. Based on preliminary data, the primary endpoint of time to alleviation of clinical symptoms in BCX1812-211 did not achieve statistical significance in the pre-planned ITTI study population (**Table 3**). Based on pre-planned and post hoc analyses, it appeared that a majority of subjects within this phase 2 study did not receive an adequate intramuscular injection.

In phase 1 studies (Him-06-111 and Him-06-112) of i.m. peramivir, significant increases in creatine kinase (CK) were observed at Day 3 compared with Baseline (Day 1) in all subjects who received active study drug or placebo. CK is a well established marker of muscle damage, and it was hypothesized that CK increase may act as a surrogate marker of an adequate i.m. injection.

Within the phase 2 study, an increase in CK between Baseline (Day 1) and Day 3 was not observed in a majority of subjects. In the phase 1 studies study drug was administered with a 1½ inch needle. In the phase 2 study a shorter needle (1 inch) was supplied with the study drug, with guidance that a longer needle (1½ inch) should be used for larger subjects. Based on the observed lack of CK increases at Day 3 compared to baseline, the Sponsor hypothesized that the needle used for injection failed to penetrate muscle and deliver intramuscular study medication in many subjects.

A sub group of subjects was identified in which a Day 3 CK increase of at least 50U/L was observed over baseline. Within this adequate intramuscular injection sub-group, notable improvements in the time to alleviation of symptoms were observed for both peramivir dose groups: 44.6 hours for peramivir 150 mg treatment and 64.8 hours for the peramivir 300 mg treatment (**Table 3**). A further sub-group analysis suggested that in subjects with an influenza B infection confirmed by PCR a single dose of peramivir had limited activity (**Table 3**). These efficacy data support the further development of peramivir as a single dose, intramuscular treatment for acute influenza.

Table 3 Summary of Efficacy from BCX1812-211.

	Placebo	Peramivir 150mg	Peramivir 300mg
Intent-to-Treat Infected Population[1] (n=313) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=107 137.0 115.9-163.8	n=104 114.1 95.2-145.5 22.9	n=102 115.9 77.8-136.6 21.1
Adequate Injection Population[2] (n=101) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=40 152.2 103.8-183.9	n=32 107.6 76.8-175.1 44.6	n=29 87.4 40.8-163.8 64.8
ITTI Influenza A infected population (n=247) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=83 138.4 117.0-173.4	n=85 115.7 92.2-145.5 22.7	n=79 115.9 51.1-195.9 22.5
ITTI Influenza B infected population (n=66) Median time to alleviation of symptoms (hrs) (95% Confidence Interval) Improvement over Placebo (hrs)	n=24 117.1 100.3-162.3	n=19 100.8 68.2-162.0 16.3	n=23 123.3 67.5-178.7 -6.2

[1]: Intent-to-Treat Infected Population: PCR+ for either Influenza A and/or Influenza B at baseline/screening visit.

[2]: Adequate Injection Population: ITTI subjects in who study drug reached target muscle tissue, as evidenced by an increase in serum CK levels of ≥ 50 U/L over baseline at the Day 3 study visit.

An independent data monitoring committee reviewed grouped blinded safety data throughout study BCX1812-211. In the overall safety population (n=342), doses of peramivir 150 mg and 300 mg were both found to be well tolerated and no safety concerns were identified by the DMC. The three treatment groups were similar with respect to the frequency and severity of adverse events. Two serious adverse events were reported in the study, and neither was considered by the investigator to be related to treatment. One SAE (pyelonephritis) occurred 5-days after study treatment in a subject who received placebo, and one SAE (meningitis, resulting in death) occurred 10-days after study treatment in a subject who received 300 mg of peramivir. There were no meaningful differences among the three treatment groups with respect to the frequency or severity of graded laboratory toxicities. A summary of the adverse events and graded toxicities, together with a list of the most frequently reported adverse events, is presented in **Table 4**.

Table 4 Summary of Safety from BCX1812-211.

Safety Parameters	Placebo (n=114)	Peramivir 150 mg (n=113)	Peramivir 300 mg (n=115)
Any Clinical Adverse Event	49 (43%)	43 (38%)	44 (38%)
Any Graded Laboratory Toxicity	99 (87%)	93 (82%)	92 (80%)
Any Serious Adverse Event	1 (<1%)	0	1 (<1%)
Most Frequent Adverse Events Assessed as Study Drug-Related			
Diarrhea	5 (4%)	5 (4%)	6 (5%)
Nausea	7 (6%)	7 (6%)	9 (8%)
Vasovagal Reaction	4 (4%)	2 (2%)	0

5.7 Dose Rationale

Oseltamivir is approved for the treatment of uncomplicated acute influenza at a dosage of 75mg twice daily in adults[14]. Oseltamivir was shown to be clinically effective in a phase 3 study of oral oseltamivir versus placebo in naturally occurring seasonal influenza, and these data were sufficient for regulatory approval for marketing of oseltamivir. At least 75% of an oral dose of oseltamivir reaches the systemic circulation as oseltamivir carboxylate. When oseltamivir is administered orally at a dose of 75mg twice daily, the serum C_{max} of oseltamivir carboxylate is approximately 348ng/mL and the AUC_{0-48} is 10,876 h·ng/mL. The clinical data indicate that this level of exposure to oseltamivir was sufficient to provide clinical improvement in uncomplicated acute influenza.

The serum pharmacokinetic data (C_{max} and $AUC_{0-\infty,}$ respectively) following intramuscular doses of peramivir are approximately 7600ng/mL and 24,000 h·ng/mL for the 150mg dose and are approximately 15,000ng/mL and 49,000 h·ng/mL for the 300mg dose. Previous studies have assessed the concentrations of the neuraminidase inhibitor zanamivir in nasal and pharyngeal secretions after parenteral administration of this drug. . Within several hours after administration, the concentrations in secretions were approximately 100-fold lower than in serum or plasma. In theory, relatively high levels of a neuraminidase inhibitor in respiratory secretions are desirable in order to rapidly inactivate influenza virus and to delay or prevent the development of resistance in infecting virus strains. Intramuscular doses of peramivir, including doses of 300mg and 600mg have been shown to be tolerated in previous Phase 1 studies. In the completed Phase 2 study, both doses of peramivir (150 mg and 300 mg) were well tolerated and no safety concerns were apparent. The evidence of a dose response between the 150mg and 300mg doses observed in study BCX1812-211 indicates that the 300 mg dose should be studied further. The results of study BCX1812-211 support advancing the 300 mg dose to undergo further evaluation in this Phase 3 study.

6 STUDY OBJECTIVES

6.1 Objectives

6.1.1 Primary Objective

To evaluate the efficacy of peramivir administered intramuscularly compared to placebo on the time to alleviation of clinical symptoms in adult subjects with uncomplicated acute influenza.

6.1.2 Secondary Objective(s)

The secondary objectives of this study are:

1. To evaluate the safety and tolerability of peramivir administered intramuscularly.
2. To evaluate secondary clinical outcomes in response to treatment.
3. To evaluate changes in influenza virus titer in nasopharyngeal samples (viral shedding) in response to treatment.

6.1.3 Exploratory Objective(s)

The following exploratory objectives have been identified for this study.

1. To assess pharmacoeconomic measures as response to treatment.
2. To assess changes in influenza viral susceptibility to neuraminidase inhibitors following treatment.

6.2 Study Endpoints

6.2.1 Primary Endpoint

The primary clinical endpoint is the time to alleviation of clinical symptoms of influenza.

6.2.2 Secondary Endpoint(s)

Secondary safety, clinical, and virologic endpoints will include evaluations in each subject of:

Safety: Incidence of treatment-emergent adverse events and treatment-emergent changes in clinical laboratory tests.

Clinical: Time to resolution of fever; Incidence of influenza related complications.

Virologic: Quantitative change in influenza virus shedding, measured by viral titer assay ($TCID_{50}$).

6.2.3 Exploratory Endpoints

Pharmacoeconomic and virologic evaluations in each subject for exploratory endpoints will also be assessed and include:

Pharmacoeconomic:	Medical resource utilization (MRU), missed days of work, and impact of influenza illness on subject's work performance and/or productivity.
Virologic:	Quantitative change in influenza virus shedding, measured by PCR; Change in influenza virus susceptibility to neuraminidase inhibitors.

7 STUDY DESIGN

7.1 Overall Study Design and Plan

This is a multinational, randomized, double-blind study comparing the efficacy and safety of a 300 mg dose of peramivir administered intramuscularly versus placebo in adults with uncomplicated acute influenza. Approximately 600 subjects will be randomized to treatment to ensure enrollment of approximately 450 subjects that are positive for influenza A. All subjects will be centrally randomized to 300 mg of peramivir or placebo in a ratio of 2:1 and will be stratified according to smoking status and RAT test for influenza A or B.

Study drug will be administered as bilateral 2mL intramuscular injections (total of 4mL injected in equally divided doses). Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

Subjects eligible for screening will have an anterior nasal swab collected for testing by RAT for influenza A and B, in accordance with the commercially available RAT kit instructions. If the initial RAT is negative, the test should be repeated within one hour. Subjects meeting the inclusion/ exclusion criteria may be enrolled into the study.

All enrolled subjects will record the following information in a Study Diary.

- Assessment of the presence and severity of each of seven symptoms of influenza on a 4-point scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily (AM, PM) through Day 9 following treatment, then once daily (AM) through Day 14.

- Oral temperature measurements will be taken with an electronic thermometer every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol) or other antipyretic medication.

- Assessment of subject's time lost from work or usual activities and rating of productivity compared to normal (rated as 0-10 on a visual analog scale) once daily through Day 14

- Doses of antipyretic, expectorant, and/or throat lozenges taken for symptomatic relief each day through Day 14

Anterior nose (bilateral) and posterior pharynx specimens (swabs) will be collected at Day 1 (pre-treatment) and at Days 3, 5, and 9, for quantitative virologic assessments. Specimens from all subjects yielding influenza virus will also be assessed for susceptibility to neuraminidase inhibitors (Day 1 and last specimen yielding positive result) as well as other virologic

assessments (e.g. PCR, genotypic testing). All virologic assessments will be performed by a central laboratory.

Two samples for pharmacokinetic (PK) testing for plasma levels of peramivir will be obtained from all subjects randomized. The first PK sample will be obtained between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be obtained at the day 3 visit in all subjects. The data from these PK samples will be utilized in a population exposure-response analysis.

At selected sites a separate sub-study will be conducted to collect additional PK samples between treatment and Day 3 for the purpose of conducting a separate exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

8.1.1 Inclusion Criteria

Subjects must meet all of the following criteria for inclusion in this study:

1. Male and non-pregnant female subjects age ≥18 years.
2. A positive Influenza A and/or B Rapid Antigen Test (RAT) performed with a commercially available test kit on an adequate anterior nasal specimen, in accordance with the manufacturer's instructions. A negative initial RAT should be repeated within one hour.
3. Presence of fever at time of screening of ≥38.0 ºC (≥100.4 ºF) taken orally, or ≥38.5 ºC (≥101.2 ºF) taken rectally. A subject self-report of a history of fever or feverishness within the 24 hours prior to screening will also qualify for enrollment in the absence of documented fever at the time of screening.
4. Presence of at least one respiratory symptom (cough, sore throat, or nasal symptoms) of at least moderate severity.
5. Presence of at least one constitutional symptom (myalgia [aches and pains], headache, feverishness, or fatigue) of at least moderate severity.
6. Onset of symptoms no more than 48 hours before presentation for screening.
7. Written informed consent.

8.1.2 Exclusion Criteria

Subjects to whom any of the following criteria apply will be excluded from the study:

1. Women who are pregnant or breast-feeding.
2. Presence of clinically significant signs of acute respiratory distress
3. History of severe chronic obstructive pulmonary disease (COPD) or severe persistent asthma. (See Section 15.2).
4. History of congestive heart failure requiring daily pharmacotherapy with symptoms consistent with New York Heart Association Class III or IV functional status within the past 12 months. (See Section 15.1).
5. Screening ECG which suggests acute ischemia or presence of medically significant dysrhythmia.

6. History of chronic renal impairment requiring hemodialysis and/or known or suspected to have moderate or severe renal impairment (actual or estimated creatinine clearance <50 mL/min).
7. Clinical evidence of worsening of any chronic medical condition (temporally associated with the onset of symptoms of influenza) which, in the investigator's opinion, indicates that such finding(s) could represent complications of influenza.
8. Current clinical evidence, including clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis and/or pneumonia, or active bacterial infection at any body site that requires therapy with oral or systemic antibiotics.
9. Presence of immunocompromised status due to chronic illness, previous organ transplant, or use of immunosuppressive medical therapy which would include oral or systemic treatment with > 10 mg prednisone or equivalent on a daily basis within 30 days of screening.
10. Currently receiving treatment for viral hepatitis B or viral hepatitis C.
11. Presence of known HIV infection with a CD4 count <350 cell/mm^3.
12. Current therapy with oral warfarin or other systemic anticoagulant.
13. Receipt of any doses of rimantadine, amantadine, zanamivir, or oseltamivir in the 7 days prior to screening.
14. Immunized against influenza with live attenuated virus vaccine (FluMist®) in the previous 21 days.
15. Immunized against influenza with inactivated virus vaccine within the previous 14 days.
16. Receipt of any intramuscular injection within the previous 14 days.
17. History of alcohol abuse or drug addiction within 1 year prior to admission in the study.
18. Participation in a previous study of intramuscular or intravenous peramivir or previous participation in this study.
19. Participation in a study of any investigational drug or device within the last 30 days.

8.1.3 Removal of Subjects from Therapy or Assessment

All subjects are permitted to withdraw from participation in this study at any time and for any reason, specified or unspecified, and without prejudice. The Investigator or sponsor may terminate the subject's participation in the study at any time for reasons including the following:

1. Adverse event;
2. Intercurrent illness;
3. Non-compliance with study procedures;
4. Subject's decision;
5. Administrative reasons;
6. Lack of efficacy;
7. Investigator's opinion to protect the subject's best interest.

Any subject who withdraws because of an adverse event will be followed until the sign(s) or symptom(s) that constituted the adverse event has/have resolved or is determined to represent a stable medical condition.

A subject should be withdrawn from the trial if, in the opinion of the Investigator, it is medically necessary, or if it is the desire of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject and determine the subject's medical condition. In any circumstance, every effort should be made to document subject outcome, if possible.

If the subject withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data.

9 TREATMENTS

9.1 Treatments Administered

Peramivir is an investigational drug. Peramivir for intramuscular injection is a small-volume parenteral and will be supplied as a 75mg/mL solution in sodium citrate/ citric acid buffer. The pH is approximately 3.0.

A matched placebo solution of sodium citrate/ citric acid buffer with 1.2% sodium chloride at a pH of approximately 3.0 will be supplied.

The gluteal site of injection and the syringe needle length are to be recorded in the subjects CRF. Procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, are provided in the study drug administration manual.

9.2 Identity of Investigational Product(s)

Peramivir and placebo peramivir will be supplied in clear 2mL vials. An individual study drug kit will contain 2 vials of blinded study drug (peramivir and/or placebo, depending upon the treatment group). Syringes and needles will be provided in which to draw up the solution for intramuscular injection. All study drug kits must be stored at 2-8^{o}C.

Each individual study drug kit will be labeled with some or all of the following information as required by local regulations:

- Sponsor name and contact information, study protocol number, kit number, description of the contents of the container, instructions for the preparation of the syringe and administration of the study drug, conditions for storage, statement regarding the investigational (clinical trial) use of the study drug and date for retest or expiry date.

Each vial of study drug will be labeled with some or all of the following information as required by local regulations:

- Sponsor name, study protocol number, description of the contents of the vial, instructions for the preparation of the syringe, statement regarding the investigational (clinical trial) use of the study drug and lot number.

9.3 Method of Assigning Subjects to Treatment Groups

Subjects will be centrally randomized in a ratio of 2:1 to a single dose peramivir 300mg or placebo, in accordance with a computer-generated randomization schedule prepared by a non-study statistician. Each subject's assignment to treatment will be stratified according to smoking status and RAT test for influenza A or B.

Once a subject is eligible for randomization, he/she will be assigned a study drug kit number that will be obtained by study staff from the study interactive voice response system (IVRS). Once a study drug kit number has been assigned to a subject, it cannot be reassigned to any other subject.

9.4 Study Medication Accountability

The Investigator/pharmacist must maintain accurate records of the disposition of all study drugs received from the sponsor, issued to the subject or directly administered to the subject (including date and time), and any drug accidentally destroyed. The sponsor will supply a specific drug-accountability form. At the end of the study, information describing study drug supplies (e.g., lot numbers) and disposition of supplies for each subject must be provided, signed by the Investigator or designee, and collected by the study monitor. If any errors or irregularities in any shipment of study medication to the site are discovered at any time, the Project Manager must be contacted immediately.

At the end of the study, all medication not dispensed or administered and packaging materials will be collected with supervision of the monitor and returned to the sponsor or destroyed on site as dictated by the appropriate Standard Operating Procedure at the participating institution.

9.5 Blinding/Unblinding of Treatments

This is a double-blind study. The treatment group assignment will not be known by the study subjects, the investigator, the clinical staff, the CRO, or Sponsor staff during the conduct of the study.

Section 11.24 provides information regarding the process for unblinding the treatment assignment, if necessary, in the event of an SAE.

9.6 Prior and Concomitant Therapies

All medications, by any route of administration, used during this study must be documented on the Case Report Form (CRF). Prescription as well as non-prescription medications should be recorded. Medication used for the treatment of influenza-related symptoms will be captured by the subject in the diary card provided by BioCryst.

9.7 Overdose and Toxicity Management

To date there is no experience with overdose of intramuscular or intravenous peramivir. If overdose occurs, subjects should receive indicated supportive therapy and evaluation of hematologic and clinical chemistry laboratory tests should be conducted. The effect of hemodialysis on elimination of peramivir is unknown.

9.8 Dose Interruption

As this is a study of a single dose of peramivir or placebo, guidelines for treatment interruption for drug related SAEs or toxicities are not applicable.

10 STUDY CONDUCT

A study schedule of evaluations is presented in **Figure 1**. A detailed list of the evaluations and visits is also provided in the following sections.

10.1 Evaluations

All subjects enrolled in this study will undergo the following evaluations:

10.1.1 Informed Consent

Prior to any study-related procedure subjects will be administered informed consent. For further discussion of consent see section 10.21.

10.1.2 Medical History

Medical history, influenza vaccination status within the previous 12 months and demographic data (including smoking behavior) will be recorded at Screening/Baseline.

10.1.3 Rapid Antigen Test for Influenza

At Screening/Baseline, a commercially available, rapid antigen test (RAT) for influenza A and B will be performed on an adequate specimen collected by swabbing the anterior nose in accordance with the RAT manufacturer' instructions. A negative initial RAT should be repeated within one hour. Refer to the Study Manual [or RAT test package insert(s)] for instructions regarding the use of the RAT kits provided for this study. Sites may use the kits provided by the Sponsor or any other commercially approved RAT available at their site to document a confirmed influenza infection.

10.1.4 Physical Examination and Influenza-related Complications Assessments

The Investigator will perform a physical examination at Screening/Baseline. Subject's height and weight, and BMI will be recorded at Screening/Baseline in the subjects CRF.

Study personnel will be provided with an influenza-related complications (IRC) checklist in the CRF to evaluate the subject for the presence of clinical signs and/or symptoms of the following influenza-related complications: sinusitis, otitis, bronchitis and pneumonia. Note that subjects with clinical signs and/or symptoms consistent with otitis, bronchitis, sinusitis, and/or pneumonia at screening are not eligible for enrollment in this study (See Section 8.1.2 exclusion criteria number 8).

If an IRC is suspected then a targeted physical examination will be conducted to record the presence/absence of the IRC. If the investigator determines that the subject experiences (or is presumed to experience) an IRC as noted above, he/she will record that assessment on the IRC CRF page and any medication used to treat the condition will be recorded on the concomitant medication page. The investigator will promptly provide appropriate treatment for any suspected or proven IRC. Such information describing IRC signs and/or symptoms should not be reported as adverse events. Any injection site reactions noted will be recorded in the CRFs as adverse events.

10.1.5 Vital Signs

Vital signs evaluations will include blood pressure, pulse rate, and respiration rate. The investigator will record oral or rectal body temperature at baseline. Thereafter the subject will

record oral temperature twice daily in the study diary card.

Vital signs will be measured at Screening/Baseline, pre-dose, and at 15 minutes following the study drug injection on Day 1, then once daily on Days 3, 5, 9, and 14.

10.1.6 Electrocardiogram Measurements

A 12-lead electrocardiogram (ECG) will be obtained at Screening/Baseline. The principal investigator will be responsible for interpretation of the Screening ECG. This interpretation may be performed by the investigator or he/she may delegate this action to another physician and the investigator will acknowledge the interpretation. If this baseline ECG is interpreted as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal but does not meet the exclusionary criteria, the subject may be enrolled unless other exclusion criteria apply. The principal investigator is responsible to ensure that such an enrolled subject be informed of the nature of the abnormal ECG and that any medically indicated repeat ECG examinations and/or referral of the subject for further evaluation is made either during subject's participation in the study or immediately after the subject's discharge from the study.

10.1.7 Clinical Laboratories

Clinical chemistry profiles will include a Chemistry 20 panel (includes sodium, potassium, chloride, total CO_2 [bicarbonate], creatinine, glucose, urea nitrogen, albumin, total calcium, total magnesium, phosphorus, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, lactate dehydrogenase [LDH], total protein, total creatine kinase, and uric acid).

Hematology will include complete blood count (CBC) with differential.

Urinalysis will include tests for protein, glucose, ketones, blood, urobilinogen, nitrite, pH, and specific gravity and microscopic evaluation for RBCs and WBCs. For any subject with a Day 3 positive test for urine protein of 2+ or higher, who had a Baseline/ Day 1 protein of <2+, a 24 hour urine collection for assessment of protein will be completed. All urinalysis tests will be completed by the central laboratory.

Clinical laboratory studies (clinical chemistries, hematology, and urinalysis) will be completed at Screening/Baseline, and on Days 3, 5 and 14.

10.1.8 Urine Pregnancy Test

Females of childbearing potential will be evaluated for pregnancy at Screening/Baseline and Day 14 using a urine pregnancy test.

10.1.9 Serology for Influenza

Paired blood samples for determination of antibody to influenza A and B (serology) will be obtained with the clinical laboratory tests at Screening/Enrollment and at Day 14. These specimens will be stored at the central laboratory and will be analyzed if needed to confirm the diagnosis of influenza.

10.1.10 Samples for Virologic Laboratory Assessments

An adequate specimen will be collected by swabbing the anterior nose (bilateral) and posterior pharynx for virologic laboratory assessments including culture for the isolation of influenza virus

and/or quantitative PCR assay at Screening/Baseline, and at Days 3, 5, and 9. Refer to the Laboratory Manual for instructions regarding the processing and shipment of these specimens.

10.1.11 Subject Self Assessments

Subject self assessments will be performed beginning pre-dose on Day 1 and recorded in the subject's Study Diary including the following:

- Oral temperature measurements with an electronic thermometer (provided by the Sponsor for the study) every 12 hours. With the exception of the baseline measurement, all temperature measurements will be obtained at least 4 hours after, or immediately before, administration of oral acetaminophen (paracetamol, provided) or other anti-pyretic medications. The times of each temperature determination will be recorded in the Study Diary. The baseline temperature will be recorded at the screening/Day 1 visit prior to dosing, regardless of whether the subject had recently taken an anti-pyretic.

- Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) on a 4-point severity scale (0, absent; 1, mild; 2, moderate; 3, severe) twice daily, beginning pre-dose on Day 1 and through Day 9, then once daily through Day 14.

- Assessment of the subject's time lost from work or usual activities and productivity compared to normal using a 0-10 visual analogue scale once daily through Day 14.

The subject's diary card will be reviewed by study staff at each visit for completion of the record of all required items, with particular emphasis on alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms. Study staff will not attempt to ask subjects to retrospectively complete missing diary card data for any scheduled assessments that have not been completed prior to the clinic visit. Study staff should, however, remind the subject to complete the diary card at all scheduled times.

10.1.12 Concomitant Medications

All concomitant medications used during this study, with the exception of those medications taken for symptomatic relief of influenza symptoms, which will be recorded by the subject in their diary card, must be documented on the Case Report Form (CRF).

10.1.13 Adverse Events

AEs will be assessed from the time of administration of study medication through the final study visit.

10.1.14 Pharmacokinetic Exposure Samples

All subjects will have two pharmacokinetic (PK) samples drawn to assess peramivir drug levels. The first PK sample will be drawn on day 1 between 30 and 60 minutes following study drug administration in all subjects. The second PK sample will be drawn at the day 3 visit in all subjects. The sample will be drawn at the same time as the blood draw is completed for clinical laboratory investigations. The 30-60 minute sample (treatment day 1) and the day 3 PK sample will be analyzed for plasma concentrations of peramivir (ng/mL) and evaluated in a population

exposure response analysis.

At selected sites a separate sub-study will also be conducted to collect additional PK samples for the purpose of conducting an exposure-response analysis. This sub-study will be conducted under a separate protocol, BCX1812-311PK. Data from these two PK samples in all subjects will be combined with data from the PK sub-study (BCX1812-311PK) to perform a population based exposure-response analysis. This analysis will be described as part of the sub-study analysis plan.

All PK samples will be processed at a central bioanalytical laboratory. Refer to the instructions provided regarding the processing and shipment of these PK samples.

10.2 Screening Period

10.2.1 Informed Consent

The nature and purpose of the study and the expectations of a participating subject will be described to potential study subjects, their questions will be answered, and the subjects will then be asked to sign an informed consent document. Study subjects will then undergo the screening evaluation as noted in Section 10.22

10.2.2 Screening/Baseline Evaluation and Enrollment

Screening/baseline evaluation may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subjects may be enrolled and receive treatment with study drug prior to receiving results of the laboratory assessments (with the exception of urine pregnancy test result, which must be known).

Eligible subjects will be enrolled and randomized to blinded study treatment. The randomization will be stratified by smoking status and RAT test for influenza A or B. The Investigator will prepare a request for blinded study drug assignment which includes the subject's screening number. The Investigator or designee at the clinical study center will contact the central randomization Interactive Voice System (IVRS call center). The IVRS call center will advise the study center of the investigational study drug kit number that is assigned to that subject at enrollment.

Subjects that are determined to be ineligible will be advised accordingly, and the reason for ineligibility will be discussed. If desired by the subject the reason for ineligibility may be provided and/or discussed with their health-care provider by the Investigator or designee.

Ineligible subjects who have been screened for the study will also be entered on the IVRS. For such subjects, the screening number assigned, subject's date of birth and a reason for ineligibility will be entered on to the IVRS. **All ineligible subjects must be entered onto the IVRS within 24 hours of screening,** to assist with surveillance analysis during the course of the study.

10.3 Treatment Period—Study Day 1

Day 1 represents the only day of study drug dosing. Study drug administration should occur as soon as possible following informed consent, screening and randomization. Therefore, it is

expected that the date of Screening/ Baseline and Day 1 will usually be the same date.

10.3.1 Pre-dose Evaluations-Study Day 1

Following an explanation of the Subject Self Assessment measures (Section 10.1.11), the subject shall complete the record of these assessments in their Study Diary prior to dosing. The subject will be counseled regarding the expectations for recording these assessments through Day 14.

Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) and a 12 lead ECG will be obtained prior to dosing.

A nasopharyngeal swab for influenza culture/ PCR assay will be obtained prior to dosing.

10.3.2 Post-dose Evaluations-Study Day 1

The blinded study drug will be administered (hour 0) as bilateral intramuscular injections within a period of ≤ 10 minutes. The calendar date and 24-hour clock time of the first and second injections will be recorded. The gluteal site of injection and the syringe needle length are also to be recorded in the subjects CRF. Sites are instructed to follow procedures for intramuscular injection, with a recommended needle length appropriate to the physical characteristics of the subject, provided in the study drug administration manual.

The following evaluations will be performed post-dose on Study Day 1:

- Vital sign measurements (blood pressure, pulse rate, respiration rate, and oral temperature) 15 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the vital sign measurements in the subjects CRF.

- Draw a PK sample between 30 and 60 minutes following the second intramuscular injection of blinded study drug; record the exact 24-hour clock time of the blood draw.

- Record any concomitant medications.

- Record any AEs.

10.4 Post-Treatment Assessment Period

10.4.1 Days 2, 3, 5, 9 and 14

Study evaluations will be performed on Days 2, 3, 5, 9 and 14 in accordance with the schedule of evaluations (Figure 1). Subjects with persistent moderate or severe influenza symptoms at day 14 will also complete a Day 21 visit, and if required a Day 28 visit.

Visits may be conducted in the investigator's office or clinic, or in the subject's home, in which case all evaluations must be conducted by appropriately trained and qualified staff.

Study staff will attempt to contact the subjects on Day 2 by telephone to confirm their compliance with completion of the Subject Self Assessments, to note any concomitant medications and adverse events. Any adverse events reported by the subject during this telephone contact will be recorded on the adverse event form and verified during the visit on day 3.

For any subject with a Day 3 positive test for urine protein of 2+ or higher, who had a Baseline/

Day 1 protein of <2+, a 24 hour urine collection for assessment of protein will be completed.

At each visit it is important that the subject's Study Diary record be reviewed for completion of daily Subject Self Assessments. The subjects should be counseled as necessary regarding self assessments and Study Diary record requirements. The subject's diary card will be reviewed by study staff for alleviation of symptoms as well as relapse of symptoms. Relapse is defined as the recurrence of at least one respiratory symptom and one constitutional symptom (both greater than mild in severity) for 24 hours and the presence of fever (unless influenced by antipyretic use). Relapse can only occur after the subject has met the endpoint criteria for alleviation of symptoms.

Day 3:

The second PK sample for all subjects will be obtained on Day 3 at the same time as the clinical laboratory blood specimen is obtained. The exact 24-hour clock time of the blood draw will be recorded in the subjects CRF.

Day 14:

If a subject has one or more persistent or recurrent symptoms of influenza (of the seven symptoms assessed) of either moderate or severe intensity at the Day 14 visit then the subject must be evaluated in further follow-up visits, at day 21 (± 3 days), and if required at day 28 (± 3 days) If a subject reports moderate or severe influenza symptoms then the investigator will record the intensity of each of the influenza symptoms on a visit specific CRF page at the day 21 and day 28 visits. After day 14 the subject will not record symptoms in a diary.

Day 21 (if applicable):

The day 21 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 14. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at the Day 21 visit and such action(s) will be recorded on the Day 21 CRF page. The investigator will recall the subject for a further study visit at day 28 (± 3 days) if moderate or severe symptom(s) of influenza persist at Day 21.

Day 28 (if applicable):

The day 28 visit is to be completed only if the subject reports symptoms of influenza of moderate or severe intensity at day 21. The investigator will make a clinical judgment as to the appropriate medical course of action for such subjects at this visit and such action(s) will be recorded on the Day 28 CRF page. No further follow-up visits beyond day 28 are to be formally scheduled unless in the clinical judgment of the investigator further follow-up is required. The investigator will use his/her clinical judgment to manage the subject, referring the subject, if appropriate, for further medical care.

10.4.2 Adverse Events Reported at Post-treatment Visits

In this study, symptoms of influenza will be considered separately from adverse events reported during the post-treatment period. Accordingly, adverse events that have onset in the post-treatment period will be assessed and followed as specified in 11.2. Specifically, the investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

Figure 1 Study Measurements and Visit Schedule

Assessments	Screening [1] (Baseline)	Treatment Period	Assessment Day				End of Study Early Withdrawal
		Day 1[1]	Day 2[2]	Day 3	Day 5 (±1 day)	Day 9 (±3 day)	Day 14 (±3 day) [8]
Informed Consent	X						
Rapid Antigen test for Influenza A and B	X						
Medical History/Physical Exam	X						
Influenza-related complications (IRC) checklist[3]	X			X	X	X	X
Inclusion/Exclusion	X						
Clinical Chemistries[4]	X			X	X		X
Hematology[4]	X			X	X		X
Exposure Pharmacokinetic Sample[9]		X		X[4]			
Serology (serum) Sample	X						X
Urinalysis[10]	X			X	X		X
Urine Pregnancy Test	X						X
Vital Signs[5]	X	X		X	X	X	X
ECG[6]	X						
Sample (nasopharyngeal swab) for Influenza Virus Culture/ PCR assay and for resistance studies		X		X	X	X	
Study Drug Administration		X					
Subject Diary Review[7]		X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X

Study Measurements and Visit Schedule Figure Legend on Next Page

Study Measurements and Visit Schedule Figure Legend

[1] It is expected that the date of Screening and Day 1 (date of administration of study drug) will be the same. Visits at Screening and on subsequent study days may occur in subject's home by the investigator (all visits) or appropriately trained study center staff (Day 3, 5, 9 visits).

[2] Day 2 will be a telephone contact with the subject to ensure compliance with diary card completion, concomitant medication and adverse event review.

[3] If an IRC is suspected then a targeted physical examination will be conducted to record the presence/absence of the IRC.

[4] Clinical laboratory assessments performed at Screening are for the purpose of establishing a baseline. Subject may be enrolled and begin treatment with study drug prior to receiving results. A PK sample will be drawn 30-60 minutes following the second treatment administration injection. On Day 3 an extra tube will be included with the safety blood sample to collect the second PK sample for evaluation of peramivir concentrations.

[5] Vital sign measures will include blood pressure, pulse rate and respiration rate. Vital signs will be recorded at Screening, pre-dose and at 15 min following the study drug administration on Day 1, then once on remaining days as stipulated. The investigator will record oral temperature at baseline. Thereafter the subject will report oral temperature measurements twice daily in the Study Diary

[6] If the baseline ECG is interpreted by the conducting physician as meeting the exclusionary criteria listed in section 8.1.2 the subject will not be enrolled in this study. If the ECG is interpreted as being abnormal and does not meet the exclusionary criteria (e.g. acute ischemia, medically significant dysrhythmia) then this subject may be enrolled If the conditions highlighted in section 10.1.5 are met for the subject.

[7] Subjects record symptom assessment in Study Diary, twice daily, beginning pre-dose on Day 1 through Day 9, then once daily through Day 14. Subjects record time lost from work or usual activities and rating of productivity compared to normal once daily through Day 14. Subjects record oral temperature twice daily throughout as well as all influenza related medications.

[8] For any subject with unresolved moderate or severe intensity influenza symptoms a follow up assessment will be scheduled at Day 21 (± 3 days) and Day 28 (± 3 days) if required (See Section 10.4.1).

[9] A PK sample will be drawn 30-60 minutes following the second treatment injection on Day 1, and on Day 3.

[10] For any subject with a Day 3 positive test for urine protein of 2+ or higher, who had a Baseline/ Day 1 protein of <2+, a 24 hour urine collection for assessment of protein will be completed.

11 ADVERSE EVENT MANAGEMENT

11.1 Definitions

11.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject. No causal relationship with the study drug or with the clinical study itself is implied. An AE may be an unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the clinical study. Clinically relevant abnormal results of diagnostic procedures including abnormal laboratory findings (e.g., requiring unscheduled diagnostic procedures or treatment measures, or resulting in withdrawal from the study) are considered to be AEs.

AEs may be designated as "nonserious" or "serious" (see Section 11.1.2).

Surgical procedures are not AEs but may constitute therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol and the conditions(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of study treatment. In the latter case the condition should be reported as medical history.

Assessment of seven influenza symptoms (cough, sore throat, nasal obstruction, myalgia [aches and pains], headache, feverishness, and fatigue) will be documented in a subject's study diary and analyzed as a measure of efficacy of the study treatment. These symptoms will not be reported as AEs unless the symptom(s) worsen to the extent that the outcome fulfils the definition of an SAE, which then must be recorded as such (see Section 11.1.2). Likewise, a RAT for influenza is required at screening in order to determine eligibility for the study, and therefore a positive RAT is not considered an AE.

11.1.2 Serious Adverse Event

A SAE is an adverse event that results in any of the following outcomes:

- Death
- Is life-threatening (subject is at immediate risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-subject hospitalization (formal admission to a hospital for medical reasons) or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., there is a substantial disruption of a person's ability to carry out normal life functions)
- Is a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the

outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse.

In addition Suspected Unexpected Serious Adverse Reactions (SUSAR) may also be reported to competent authorities where this type of reporting is required (e.g. European Union Directives). See section 11.2.3.

11.2 Method, Frequency, and Time Period for Detecting Adverse Events and Reporting Serious Adverse Events

Reports of AEs are to be collected from the time of study drug administration through the follow-up period ending on Day 14. The Investigator or designee must completely and promptly record each AE on the appropriate CRF. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. In such cases, the diagnosis should be documented as the AE and not the individual sign/symptom. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

The Investigator should attempt to follow all unresolved AEs and/or SAEs observed during the study until they are resolved, or are judged medically stable, or are otherwise medically explained.

11.2.1 Definition of Severity

All AEs will be assessed (graded) for severity and classified into one of four clearly defined categories as follows:

- **Mild:** (Grade 1): Transient or mild symptoms; no limitation in activity; no intervention required. The AE does not interfere with the participant's normal functioning level. It may be an annoyance.
- **Moderate:** (Grade 2): Symptom results in mild to moderate limitation in activity; no or minimal intervention required. The AE produces some impairment of functioning, but it is not hazardous to health. It is uncomfortable or an embarrassment.
- **Severe:** (Grade 3): Symptom results in significant limitation in activity; medical intervention may be required. The AE produces significant impairment of functioning or incapacitation.
- **Life-threatening:** (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization.

11.2.2 Definition of Relationship to Study Drug

The blinded Principal Investigator must review each AE and make the determination of relationship to study drug using the following guidelines:

Not Related: The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident, and no temporal relationship exists between the study drug and the event.

Unlikely: The event does not follow a reasonable temporal sequence from drug administration and is readily explained by the subject's clinical state or by other modes of therapy administered to the subject.

Possibly Related: There is some temporal relationship between the event and the administration of the study drug and the event is unlikely to be explained by the subject's medical condition, other therapies, or accident.

Probably Related: The event follows a reasonable temporal sequence from drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the subject's clinical state.

Definitely Related: The event follows a reasonable temporal sequence from administration of the medication, follows a known or suspected response pattern to the medication, is confirmed by improvement upon stopping the medication (dechallenge), and reappears upon repeated exposure (rechallenge, if rechallenge is medically appropriate).

11.2.3 Reporting Serious Adverse Events

Any SAE / SUSAR (Suspected Unexpected Serious Adverse Reaction) must be reported to BioCryst or its designee within 24 hours of the Investigator's recognition of the SAE by first notifying the Medical Monitor at the number listed below:

Telephone: **Europe: +44 1628 548000; North America: 1-888-724-4908**

Facsimile: **Europe: +44 1628 540028; North America: 1-888-887-8097 or 1-609-734-9208**

In addition to the telephone numbers listed above, local country-specific toll free numbers may be provided within the study reference manual.

The site is required to fax a completed SAE / SUSAR Report Form (provided as a separate report form) within 24 hours. All additional follow-up evaluations of the SAE / SUSAR must be reported and sent by facsimile to BioCryst or its designee as soon as they are available.

The Principal Investigator or designee at each site is responsible for submitting the IND safety report (initial and follow-up) or other safety information (e.g., revised Investigator's Brochure) to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and for retaining a copy in their files.

If the Investigator becomes aware of any SAE / SUSAR occurring within 30 days after a subject has completed or withdrawn from the study, he or she should notify BioCryst or its designee.

Any SAEs / SUSARs considered possibly related to treatment will be reported to the FDA and other Regulatory Competent Authorities as applicable via the MedWatch / CIOMS reporting system in accordance with FDA and other applicable regulations. However, the Investigator is not obligated to actively seek reports of AEs in former study participants.

While pregnancy is not considered an AE, all cases of fetal drug exposure via parent as study participant (see Section 4.4) are to be reported immediately to BioCryst or its designee. Information related to the pregnancy must be given on a "Pregnancy Confirmation and Outcome" form that will be provided by the Sponsor or its designee.

11.2.4 Emergency Procedures

In the event of an SAE / SUSAR, the Principal Investigator may request the unblinding of the treatment assignment for the subject affected. If time allows (i.e., if appropriate treatment for the SAE is not impeded), the Principal Investigator will first consult with the Medical Monitor regarding the need to unblind the treatment assignment for the subject. At all times, the clinical well-being of any subject outweighs the need to consult with the Medical Monitor.

The Principal Investigator may contact the IVRS central randomization center and request the unblinding of the treatment assignment that corresponds to the affected subject. The IVRS center will record the name of the Investigator making the request, the date and time of the request, the subject number and date of birth. The Sponsor will be informed within 24 hours if unblinding occurred.

12 STATISTICAL METHODS

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and other selected efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. The term "treatment group" refers to randomized treatment assignment: peramivir 300 mg or placebo. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date within each subject number.

Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05.

All statistical analyses will be conducted with the SAS® System, version 9.1.3 or higher.

12.1 Data Collection Methods

The data will be recorded on the CRF approved by BioCryst. All documentation supporting the CRF data, such as laboratory or hospital records, must be readily available to verify entries in the CRF.

Documents (including laboratory reports, hospital records subsequent to SAEs, etc.) transmitted to BioCryst should not carry the subject's name. This will help to ensure subject confidentiality.

12.2 Statistical Analysis Plan

A statistical analysis plan (SAP) will be created and approved prior to the review of any data. This document will provide a more technical and detailed description of the proposed data analysis methods and procedures.

12.3 Study Hypothesis

The primary hypothesis for evaluating the primary objective may be stated as follows:

The null hypothesis (H_0) is that the time to alleviation of influenza symptoms is the same for subjects treated with placebo and for subjects treated with peramivir 300mg.

The alternative hypothesis (H_1) is that subjects treated with peramivir 300mg have an improvement in time to alleviation of influenza symptoms over those treated with placebo.

12.4 Sample Size Estimates

From preliminary results of a phase 2 study evaluating peramivir treatment of uncomplicated influenza, it is expected that the median time to alleviation of symptoms will be 137.0 hours (95% CI: 115.9, 165.8) for subjects receiving placebo treatment. Additionally, it is expected that the median time to alleviation for the 300 mg dose peramivir arm will be reduced by 30% compared to placebo yielding a hazard ratio of 0.70. Data from the phase 2 study suggested that the activity of peramivir against influenza B was limited. Therefore, this study will be sized to ensure that a minimum number of influenza A subjects is enrolled to achieve study power.

Using these assumptions, a sample size of 450 evaluable subjects with influenza A (300 in the 300 mg treatment group and 150 evaluable subjects in the placebo group) is sufficient to provide at least 90% power to detect a hazard ratio of 0.70 using a log-rank statistic and $\alpha = 0.05$ (SAS version 9.1.3; total accrual time 7 months; total enrollment time 6 months).

12.5 Analysis Populations

The populations for analysis will include the intent-to-treat (ITT), intent-to-treat infected (ITTI and ITTI-A), per-protocol infected (PPI), and safety populations. Additional analysis populations may be defined to evaluate study results. Any additional analysis populations will be defined in the SAP.

Intent-To-Treat Population: The ITT population will include all subjects who are randomized. Subjects will be analyzed in the treatment group to which they were randomized. The ITT population will be used for analyses of accountability and demographics.

Intent-To-Treat Infected Population: The ITTI population will include all subjects who are randomized, received study drug, and have confirmed influenza by culture or PCR. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI population will be used for primary analyses of efficacy.

Intent-To-Treat Infected Population-A: The ITTI-A population will include all subjects who

are randomized, received study drug, and have confirmed influenza A by culture or PCR. Subjects will be analyzed according to the treatment randomized. If a discrepancy is noted in the final database for any subject, such that the drug differs from the randomized treatment assignment, efficacy analyses may be repeated with the subjects analyzed according to the treatment received. The ITTI-A population will be used for supportive analyses of efficacy.

Per-Protocol Infected: The PPI population includes all subjects in the ITTI population who receive an adequate intramuscular injection. The definition of an adequate intramuscular injection will be further described in the SAP. The PPI population will be used for supportive analyses of efficacy.

Safety Population: The safety population will include all subjects who received study drug. Subjects will be analyzed according to the treatment received. This population will be used for all safety analyses.

12.6 Interim and End of Study Analyses

Interim Analysis

An independent DMC will review safety data on an ongoing basis. Safety analyses will be presented in a manner consistent with the presentations intended for the final analysis.

End of Study Analysis

A final analysis is planned after the last subject completes or discontinues the study, and the resulting clinical database has been cleaned, quality checked, and locked.

12.7 Efficacy Analyses

12.7.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the time to alleviation of symptoms of influenza in subjects diagnosed with influenza, defined as the time from injection of study drug to the start of the time period when a subject has Alleviation of Symptoms. A subject has Alleviation of Symptoms if all of the seven symptoms of influenza (nasal congestion, sore throat, cough, aches and pains, fatigue (tiredness), headache, feeling feverish) assessed on his/her subject diary are either absent or are present at no more than mild severity level and at this status for at least 21.5 hours (24 hours - 10%).

Descriptive statistics for the primary efficacy variable will be tabulated by treatment group. Alleviation of symptoms will be determined by assessment of symptoms as reported on each subject's diary card. Time to alleviation of symptoms will be summarized for each treatment group (300 mg peramivir and placebo). Treatment comparisons between the 300 mg peramivir group and placebo will be assessed using a Wilcoxon-Gehan[19] statistic stratified by smoking status and positive PCR or viral culture for influenza A or B at screening for the ITTI and PPI populations. Subjects who do not experience alleviation of symptoms will be censored at the date of their last non-missing post-baseline assessment.

12.7.2 Secondary Efficacy Endpoints

All secondary endpoints will be summarized using descriptive statistics by treatment group, and study day/time, if appropriate. Statistical comparisons for each endpoint will be constructed without adjustment for multiple endpoints.

The reduction in viral shedding will be assessed as the change in viral titers defined as the time-weighted change from baseline in $\log_{10}$ tissue culture infective $dose_{50}$ ($TCID_{50}$/mL) and will be summarized for each treatment group. The time-weighted average change from baseline will be calculated on a by-subject basis through Day 9 using the trapezoidal rule with all available data (data after initiation of study treatment) minus the baseline value. Specifically, the time-weighted area under the curve for time a (t_a) to time b (t_b) is given by the formula

$$TWAUC = \frac{AUC(t_a - t_b)}{(t_a - t_b)},$$

where $AUC(t_a - t_b) = \sum_{i=a}^{b-1} \frac{(y_i + y_{i+1})(t_{i-1} - t_i)}{2}$ and t_i represents the date of the i^{th} viral titer assessment and y_i represents the $\log_{10}$ value of the i^{th} viral titer assessment. If there is a baseline value and only one follow-up value, y_i then the time-weighted change from baseline is defined as the difference between y_i and baseline. If there is a baseline value and no follow-up value, the subject is excluded from analysis. The differences between the 300 mg peramivir treatment group and placebo will be evaluated using a van Elteren Test adjusting for smoking status and positive PCR or viral culture for influenza A or B at screening.

Subject's oral temperature will be summarized by study visit and treatment group. Differences between the 300 mg peramivir treatment group and placebo will be assessed using the van Elteren test controlling for smoking status and positive PCR or viral culture for influenza A or B at screening. A subject has Resolution of Fever if he/she has a temperature < 37.2°C (99.0°F) and no antipyretic medications have been taken for at least 12 hours. The time to resolution of fever will be estimated using the method of Kaplan-Meier using temperature and symptom relief medication information obtained from the subject diary data. Differences between the treatment groups will be assessed using the Wilcoxon-Gehan statistic controlling for smoking status and positive PCR or viral culture for influenza A or B at screening. Subjects who do not have resolution of fever will be censored at the time of their last non-missing post-baseline temperature assessment.

The number and percentage of subjects experiencing influenza related complications will be summarized by complication preferred term and treatment group. The difference between the treatment groups (300 mg peramivir and placebo) will be assessed using a Cochran-Mantel-Haenszel (CMH) test statistic controlling for smoking status, and positive PCR or viral culture for influenza A or B at screening, if applicable.

12.7.3 Exploratory Endpoints

The MRU, MRU-related direct costs, and indirect costs attributable to days missed of work and work productivity and/or performance losses will be summarized by treatment group, smoking status, and positive PCR or viral culture for influenza A or B at screening, if applicable. Methods for describing differences between treatment groups will be presented in the SAP.

Genotypic (including Hemagglutinin and Neuraminidase), phenotypic, viral culture and PCR data will be listed for each subject. These listings will be constructed in a manner consistent with the FDA June 2006 Guidance Document: *"Guidance for Submitting Influenza Resistance Data"*.[18] Additionally, the number and percentage of genotypic changes from wild-type amino acid will be summarized separately for treatment group, protein type, and study visit.

12.8 Safety Analyses

AEs will be mapped to a MedDRA-preferred term and system organ classification. The occurrence of TEAEs will be summarized by treatment group using MedDRA-preferred terms, system organ classifications, and severity. If a subject experiences multiple events that map to a single preferred term, the greatest severity and strongest Investigator assessment of relation to study drug will be assigned to the preferred term for the appropriate summaries. All AEs will be listed for individual subjects showing both verbatim and preferred terms. Separate summaries of treatment-emergent SAEs and AEs related to study drug will be generated.

Descriptive summaries of vital signs and clinical laboratory results will be presented by study visit. Laboratory abnormalities will be graded according to the DAIDS Table for Grading Adverse Events for Adults and Pediatrics (Publish Date: December 2004). The number and percentage of subjects experiencing treatment-emergent graded toxicities will be summarized by treatment group. Laboratory toxicity shifts from baseline to Day 3, Day 5, and Day 14 will be summarized by treatment group.

Abnormal physical examination findings will be presented by treatment group. The number and percent of subjects experiencing each abnormal physical examination finding will be included.

Concomitant medications will be coded using the WHO dictionary. These data will be summarized by treatment group.

Subject disposition will be presented for all subjects. The number of subjects who completed the study and discontinued from the study will be provided. The reasons for early discontinuation also will be presented.

12.9 Sub-Study and Pharmacokinetic Analysis

A sub-study to collect pharmacokinetic samples in up to 60 peramivir treated subjects to examine exposure response will be conducted at selected sites. The data from the sub-study will be combined with the two PK samples (collected on all subjects at 30-60 minutes following administration of study drug and on study day 3) to perform a population exposure-response analysis. All analyses related to exposure-response will be completed as part of the sub-study. All statistical methods will be outlined as part of the sub-study protocol and exposure-response analysis plan. All sub-study analyses, and exposure-response analyses from PK samples obtained in this study and a companion study BCX1812-312, will be reported in a separate sub-study report.

12.10 General Issues for Statistical Analysis

12.10.1 Multiple Comparisons and Multiplicity

No adjustments for multiple comparisons are currently planned.

12.10.2 Covariates

Primary and secondary efficacy analyses will be adjusted for smoking status and positive PCR or viral culture for influenza A or B at screening, where appropriate.

12.10.3 Planned Sub-Groups

The primary efficacy endpoint will be summarized separately by smoking status and positive PCR or viral culture for influenza A or B at screening using descriptive statistics by treatment group and study day, where appropriate. No formal statistical testing will be utilized.

Additional analyses may be performed by country, if necessary, for submission to local regulatory authorities.

12.10.4 Missing Data

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been randomized. No attempt will be made retrospectively to obtain missing subject reported data (including influenza symptom severity assessments, temperature, ability to perform usual activities, missed days of work and impact of influenza on subject's work performance and/or productivity) that has not been completed by the subject at the time of return of the subject diary to the investigative site. In situations where it is not possible to obtain all data, it may be necessary to impute missing data.

In assessing the primary efficacy endpoint, for subjects who withdraw or who do not experience alleviation of symptoms, missing data will be censored using the date of subject's last non-missing assessment of influenza symptoms. Missing assessments of influenza symptoms conservatively will be imputed as having severity above absent or mild (as failures). For the subject diary data, the following data conventions will be utilized. Missing diary completion will be imputed as 11:59 for diary entries designated as morning and 23:59 for evening and daily reported values. Select exploratory sensitivity analyses may be conducted to ascertain the effect, if any, of these methods. These sensitivity analyses are further described in the SAP. Secondary efficacy endpoints with time to event data will be censored using the date of subject's last non-missing assessment of the given endpoint.

13 STUDY ADMINISTRATION

13.1 Regulatory and Ethical Considerations

13.1.1 Regulatory Authority Approvals

This study will be conducted in compliance with the protocol; GCPs, including International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines; FDA regulatory requirements and in accordance with the ethical principles of the Declaration of Helsinki. In addition, all applicable local laws and regulatory requirements relevant to the use of new therapeutic agents in the countries involved will be adhered to.

The Investigator should submit written reports of clinical study status to their Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) annually or more frequently if requested by the IRB/ IEC. A final study notification will also be forwarded to the IRB/IEC after the study is completed or in the event of premature termination of the study in accordance with the applicable regulations. Copies of all contact with the IRB/ IEC should be maintained in the study documents file. Copies of clinical study status reports (including termination) should be provided to BioCryst.

13.1.2 Ethics Committee Approvals

Before initiation of the study at each investigational site, the protocol, the informed consent form, the subject information sheet, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study must be obtained before the study center can be initiated or the investigational medicinal product is released to the Investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained, in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The Investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. On completion of the study, the Investigator will provide the IRB/IEC with a report of the outcome of the study.

13.1.3 Subject Informed Consent

Signed informed consent must be obtained from each subject prior to performing any study-related procedures. Each subject should be given both verbal and written information describing the nature and duration of the clinical study. The informed consent process should take place under conditions where the subject has adequate time to consider the risks and benefits associated with his/her participation in the study. Subjects will not be screened or treated until the subject has signed an approved ICF written in a language in which the subject is fluent.

The ICF that is used must be approved both by BioCryst and by the reviewing IRB/ IEC. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current ICH and GCP guidelines, and BioCryst policy.

The Investigator must explain to potential subjects or their legal representatives the aims,

methods, reasonably anticipated benefits, and potential hazards of the trial and any discomfort it may entail. Subjects will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told that refusal to participate in the study will not prejudice future treatment. They will also be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Subjects must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's dated signature. The subject should receive a signed and dated copy of the ICF. The original signed informed consent should be retained in the study files. The Investigator shall maintain a log of all subjects who sign the ICF and indicate if the subject was enrolled into the study or reason for non-enrollment.

13.1.4 Payment to Subjects

Reasonable compensation to study subjects may be provided if approved by the IRB/IEC responsible for the study at the Investigator's site.

13.1.5 Investigator Reporting Requirements

The Investigator will provide timely reports regarding safety to his/her IRB/IEC as required.

13.2 Study Monitoring

During trial conduct, BioCryst or its designee will conduct periodic monitoring visits to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow BioCryst monitors or its designees and appropriate regulatory authorities direct access to source documents to perform this verification.

13.3 Quality Assurance

The trial site may be subject to review by the IRB/IEC, and/or to quality assurance audits performed by BioCryst, and/or to inspection by appropriate regulatory authorities.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

13.4 Study Termination and Site Closure

BioCryst reserves the right to discontinue the trial prior to inclusion of the intended number of subjects but intends only to exercise this right for valid scientific or administrative reasons. After such a decision, the Investigator must contact all participating subjects immediately after notification. As directed by BioCryst, all study materials must be collected and all case report forms completed to the greatest extent possible.

13.5 Records Retention

To enable evaluations and/or audits from regulatory authorities or BioCryst, the Investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, case report forms and hospital records), all original signed informed consent forms, copies of all case report forms and detailed records of treatment disposition. The records should be retained by the Investigator according to local regulations or as specified in the Clinical Trial Agreement, whichever is longer.

If the Investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to BioCryst. The Investigator must obtain BioCryst's written permission before disposing of any records.

13.6 Study Organization

13.6.1 Data Monitoring Committee

BioCryst will assemble an independent Data Monitoring Committee (DMC) to assess safety parameters of the trial on a periodic, ongoing basis while the trial is in progress. The committee will include a statistician and three physicians, two of whom will be Infectious Disease / Clinical Virology specialists. Full details of the composition of the DMC and how the DMC is to operate will be described in a separate DMC charter.

13.7 Confidentiality of Information

BioCryst affirms the subject's right to protection against invasion of privacy. Only a subject identification number, initials and/or date of birth will identify subject data retrieved by BioCryst**.** However, in compliance with federal regulations, BioCryst requires the investigator to permit BioCryst's representatives and, when necessary, representatives of the FDA or other regulatory authorities to review and/or copy any medical records relevant to the study.

BioCryst will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule, where this rule is applicable. The Rule provides federal protection for the privacy of protected health information by implementing standards to protect and guard against the misuse of individually identifiable health information of subjects participating in BioCryst-sponsored Clinical Trials. "Authorization" is required from each research subject, i.e., specified permission granted by an individual to a covered entity for the use or disclosure of an individual's protected health information. A valid authorization must meet the implementation specifications under the HIPAA Privacy Rule. Authorization may be combined in the Informed Consent document (approved by the IRB/IEC) or it may be a separate document, (approved by the IRB/IEC) or provided by the Investigator or Sponsor (without IRB/IEC approval). It is the responsibility of the investigator and institution to obtain such waiver/authorization in writing from the appropriate individual. HIPAA authorizations are required for U.S. sites only.

13.8 Study Publication

All data generated from this study are the property of BioCryst and shall be held in strict

confidence along with all information furnished by BioCryst. Independent analysis and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of BioCryst. Written permission to the Investigator will be contingent on the review by BioCryst of the statistical analysis and manuscript and will provide for nondisclosure of BioCryst confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

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15 APPENDICES

15.1 NYHA Functional Classification Criteria: Heart Failure and Angina

NYHA Functional Classification of Heart Failure

Class I
No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea.
Class II
Symptoms with ordinary physical activity. Walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold weather, in wind, or when under emotional stress causes undue fatigue or dyspnea.
Class III
Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea.
Class IV
Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea.

NYHA Functional Classification of Angina

Class I
Angina only with unusually strenuous activity.
Class II
Angina with slightly more prolonged or slightly more vigorous activity than usual.
Class III
Angina with usual daily activity.
Class IV
Angina at rest.

15.2 Criteria for Severe COPD and Severe Asthma

The following guidelines are provided to assist in the evaluation of subjects who have a medical history for Chronic Obstructive Pulmonary Disease (COPD) and/or Asthma. Subjects with severe COPD or severe persistent Asthma are to be excluded from this study. (See section 8.1.2, exclusion criteria number 3).

Classification of Asthma from National Asthma and Education and Prevention Program

Classification	Days with Symptoms	Nights with Symptoms	For Adults and Children (> 5 yrs) who can use a spirometer or peak flow meter	
			FEV_1 or PEF % Predicted Normal	PEF Variability (%)
Severe persistent	Continual	Frequent	≤ 60	> 30
Moderate Persistent	Daily	> 1/ week	> 60 - < 80	> 30
Mild Persistent	> 2 / week but < 1 times / day	> 2/ month	≥ 80	20 – 30
Mild Intermittent	≤ 2 / week	< 2 / month	≥ 80	< 20
FEV_1: percentage predicted value for forced expiratory volume in 1 second. PEF: percentage of personal best for peak expiratory flow.				

Extracted from: Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program. HHS/NIH 2007

Spirometric Classification of COPD Severity based upon Post-Bronchodilator FEV1 (GOLD Criteria)

Stage	Characteristics
Mild COPD	$FEV_1/FVC < 70\%$ $FEV_1 \geq 80\%$ predicted
Moderate COPD	$FEV_1/FVC < 70\%$ $50\% \leq FEV_1 < 80\%$ predicted
Severe COPD	$FEV_1/FVC < 70\%$ $30\% \leq FEV_1 < 50\%$ predicted
Very Severe COPD	$FEV_1/FVC < 70\%$ $FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure
FEV_1: percentage predicted value for forced expiratory volume in one second. FVC: forced vital capacity	

Extracted from: Rabe KF, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (GOLD Executive Summary). Am. J. Respir. Crit. Care Med. 2007:176;532-555.