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PROTOCOL TITLE:	**A Phase 2a, Multi-center, Placebo-controlled, Randomized Partially Blinded, Study of Infused METREXASSIST™ (Parenteral TK-112690) or Metrexassist™ Placebo Administered Along with Methotrexate Weekly for Four Consecutive Weeks to Patients with Recurrent or Residual SCCHN.**
PROTOCOL NUMBER:	**CLP-2690-0003**
VERSION	**2.0**
DRUG:	**METREXASSIST™**
SPONSOR NAME:	**Tosk, Inc.**
SPONSOR ADDRESS:	**2672 Bayshore Parkway, Suite 507** **Mountain View, CA 94043 USA**
CONTRACT RESEARCH ORGANIZATION	**Crystal Life Sciences** **Office Number: 812, 8th Floor** **Global Business Hub,Near Eon Free Zone** **Kharadi, Pune** **Maharashtra -411014** **INDIA**
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METREXASSIST™ (Parenteral TK-112690)
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SPONSOR/CRO SIGNATURE PAGE

STUDY TITLE:
A Phase 2a, Multi-center, Placebo-controlled, Randomized, Partially Blinded, Study of Infused METREXASSIST™ (Parenteral TK-112690) or Metrexassist™ Placebo Administered Along with Methotrexate Weekly for Four Consecutive Weeks to Patients with Recurrent or Residual SCCHN.

Signatures of the individuals below verify that all designated persons have accepted this version as final.

William Garland, Ph.D.
VP R&D, Tosk, Inc.

Tosk, Inc.	William Garland Representative (Signature)	26/Feb/2019 DD/ MMM/YYYY

Sahebrao K Pujari
Head Operations, Crystal Life Sciences

Crystal Life Sciences	Representative (Signature)	26/02/2019 DD/ MMM/YYYY

Dr. L. Krishnamurthy
Consultant Medical Monitor

Crystal Life Sciences	L.K. Murthy Representative (Signature)	26/02/2019 DD/ MMM/YYYY

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STUDY ACKNOWLEDGMENT / DISCLOSURE

Protocol number: CLP-2690-0003

Protocol title: A Phase 2a, Multi-center, Placebo-controlled, Randomized, Partially Blinded, Study of Infused METREXASSIST™ (Parenteral TK-112690) or Metrexassist™ Placebo Administered Along with Methotrexate Weekly for Four Consecutive Weeks to Patients with Recurrent or Residual SCCHN.

I have carefully read and understand the foregoing protocol and agree that it contains all the necessary information for conducting this study safely. I will conduct this study in strict accordance with this protocol, ICH guidelines for Good Clinical Practice, the Code of Federal Regulations, the Health Insurance Portability and Accountability Act (HIPAA), if applicable, the World Medical Association Declaration of Helsinki (Appendix I), and local regulatory guidelines. I will attempt to complete the study within the time designated.

I will ensure that the rights, safety and welfare, of study subjects under my care are protected. I will ensure control of the drugs under investigation in this study.

I will provide copies of the protocol and all other study-related information supplied by the Sponsor to all personnel responsible to me who participate in the study. I will discuss this information with them to assure that they are adequately informed regarding the drug and conduct of the study.

I agree to keep records on all study subject information (case report forms, shipment and drug return forms and all other information collected during the study) and drug disposition in accordance with DCGI and FDA regulations. I agree to retain and maintain strict accountability of the Investigational Products supplied to the study site.

I will not enroll any subjects into this study until applicable Regulatory approval, IEC/IRB approval and Sponsor approval are obtained.

_____

Principal Investigator's Signature **Date**_____

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
ADME	Absorption, Distribution, Metabolism and Excretion
ALB	Albumin
ALT	Alanine transaminase (SGPT)
ANC	Absolute neutrophil count
AP	Alkaline phosphatase
AST	Aspartate transaminase (SGOT)
AUC_{Last}	AUC calculated using last concentration measured
AUC_{Inf}	AUC_{Last} + last measured concentration/terminal elimination rate constant
AUMC	Area under moment curve
BMI	Body mass index
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CD40	Cluster of differentiation 40, CD40 is a costimulatory protein found on antigen presenting cells and is required for their activation.
CD40L	Ligand for CD40
CLS	Crystal Life Sciences (a clinical CRO)
CNS	Central nervous system
CR	Creatinine
CRF	Case report form
CRO	Contract Research Organization
CT	Computerized tomography
CTCAE	Common terminology criteria adverse events
dL	Deciliter
DLT	Dose limiting toxicity
ECG/EKG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ESF	Eligibility Screening Form
FDA	Food and Drug Administration
FGF-9	FGF-9 (fibroblast growth factor-9) is an approximately 26 kDa secreted glycoprotein of the FGF family. FGF family members possess broad mitogenic and cell survival activities
GCP	Good Clinical Practices
GLP	Good Laboratory Practice
GGT	Gamma-glutamyltransferase
GI	Gastrointestinal
Hct	Hematocrit
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL	Interleukin
IND	Investigational New Drug

IEC	Independent Ethics Committee
IRB	Institutional Review Board
iv	Intravenous
ip	Intraperitoneal
LC/MS	Liquid chromatography/mass spectrometry
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
LDH	Lactic Acid Dehydrogenase
LEU	Leucovorin
LLN	Lower limit of normal
LPS	Lipopolysaccharide
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mL	Milliliter
MRI	Magnetic resonance imaging
MRT	Mean residence time
MRT_{Last}	MRT calculated using last concentration measured
MRT_{Inf}	MRT calculated using last measured concentrationt
MTD	Maximum tolerated dose
MTX	Methotrexate
OMAS	Oral mucositis assessment scale
PBS	Phosphate buffered saline
PI	Principal Invesatigator
PK	Pharmacokinetics
PD	Pharmacodynamics
PROMS	Patient-reported oral mucositis symptom scale
PT	Prothrombin time
PTT	Partial thromboplastin time
RBC	Red blood cell
Rsq	Regression square
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious adverse event
SCCHN	Squamous cell carcinoma of the head and neck
SD	Standard deviation
SE	Standard Error
SGOT	Serum glutamic oxaloacetic transaminase (AST)
SGPT	Serum glutamic pyruvic transaminase (ALT)
TEAE	Treatment emergent adverse event
TK-112690	Tosk's anti-mucositis adjuvant
TNF	Tumor necrosis factor
ULN	Upper limit of normal
USP	United States Pharmacopoeia
WBC	White blood cell
WHO	World Health Organization
WMA	World Medical Association
µg	Micrograms

PROTOCOL REVISION HISTORY

Sr. No.	Revised version.	Revision date	Revision Summary
2	V2—	10 February 2019	1. Title of protocol simplified. 2. MTX dose reduced to 45 mg/m^2 3. Inclusion criteria modified to specifically include only patients who have failed at least one round of non-MTX chemotherapy 4. Exclusion criteria modified to specifically exclude patients who have not failed at least one round of non-MTX chemotherapy 5. Labels for placebo vials and the placebo storage carton updated

STUDY SYNOPSIS

Study
CLP-2690-0003

Title of the Study
A Phase 2a, Multi-center, Placebo-controlled, Randomized, Partially Blinded, Study of Infused METREXASSIST™ (Parenteral TK-112690) or Metrexassist™ Placebo Administered Along with Methotrexate Weekly for Four Consecutive Weeks to Patients with recurrent or residual SCCHN.

Principal Investigators
Approximately 3

Study Centers
Approximately 3

Clinical Phase
Phase 2a

Number of Subjects
The total number of individuals enrolled will be 22, 11 dosed TK-112690 and methotrexate and 11 patients dosed methotrexate and placebo. All patients will be dosed a nutritional supplement to assure adequate basal levels of uridine and uridine-related biochemicals.

Study Drug:TK-112690, an anhydrouridine inhibitor of uridine phosphorylase

Projected Subject Enrollment Duration
10 months

Objectives

Primary

Determine pilot efficacy of an iv infusion of TK-112690 to placebo as a mucositis preventive in subjects with locally advanced, residual, or recurrent or metastatic SCCHN scheduled to receive MTX as chemotherapy.

Secondary

Confirmation of the safety/tolerability of a continuous administered weekly for 4 weeks and lowering of plasma concentrations of surrogate markers of mucositis, e.g., CD40/CD40L, post TK-112690 in patients

suffering from local, advanced, residual or recurrent, metastatic SCCHN receiving sequential continuous iv infusions of TK-112690 and MTX or TK-112690 placebo and MTX.

Study Design

A Phase 2a, Multi-center, Placebo-controlled, Randomized, Partially Blinded, Study in Patients with recurrent or residual SCCHN.

Patients will receive MTX at a dose of 45 mg/m^2 administered weekly for **4 consecutive weeks** as an iv infusion along with a nutritional supplement containing uridine monophosphate (Fortasyn Connect™ / Souvenaid™, Nutricia NV) administered two hours before the MTX. One hour before the MTX treatment the patients will be administered the first infusion of the day of either TK-112690 or placebo depending on randomization infusion. Five hours after the MTX treatment the patients will be administered the second treatment of either TK-112690 or placebo depending on randomization. The TK-112690 dose will be 45 mg/kg.

- A total of 22 patients will be enrolled into one of 2 different dose groups: TK-112690 treated or placebo treated.
- Screening must be within 15 days of subject enrollment.
- Patients will remain for observation at the clinical site for a minimum of 25 hours post initial TK-112690 or placebo dose.
- Study follow-up will occur on Week 6, two weeks after the last dosing of MTX.
- Blinding: The study will be partially blinded. The patient and investigator will be blinded as to whether TK-112690 or placebo is administered. The CRO,sponsor, and site pharmacist will know whether the patient was administered active drug or placebo.

Main Study Endpoints/Outcomes

- **Mucositis Outcomes**

  Mucositis status in the patients will be evaluated using 4 different published and validated mucosit scales: OMAS/Sonis, PROMS, WHO, and CTCAE/mucositis.

- **Surrogate Marker Outcomes**

  Plasma samples will be obtained to determine surrogate markers of mucositis, eg, CD40/CD40 TNF-α, etc.

- **Safety Outcomes**

  Adverse events will be assessed throughout the study. Incidents of toxicity will be determined to at least possibly related to the study drug and graded according to CTCAE Version 4.0.

Study Procedures and Study Assessments

- A review of a patient's medical history including a detailed history of the patient's cancer and concomitant medications will be performed at screening.
- Screening for drugs of abuse will be performed at the time of patient screening and Day 0 (the day before start of study).
- Vital sign measurements will be performed at patient screening, on Day 0, and at 24 and 48

hours post each initial infusion of TK-112690/placebo as well as on Day 1 of Week 6.

- Physical examinations will be performed at screening, on Day 0, and 24 and 48 hours post each initial infusion of TK-112690/ placebo as well as on Day 1 of Week 6.
- ECOG performance status will be determined at patient screening, on Day 0, and at Day 1 of Weeks 6.
- For pre-menopausal females, a urine pregnancy test will be performed at screening, on Day 0, and at Day 1 of Week 6.
- 12-Lead ECG at screening and 24 hours post each initial infusion of TK-112690/ Placebo.
- CBC, Liver function test, Kidney function test determination at the clinical site prior to methotrexate treatment to ensure patient fit to dose.
- Serum chemistry, hematology, coagulation and urinalysis at screening, Day 0, and at 24 and 48 hours post each initial infusion of TK-112690/ placebo as well as on Day 1 of Week 6.
- Adverse events will be evaluated 5, 24 and 48 hours post each initial infusion of TK-112690/ Placebo as well as on Day 1 of Week 6.
- Blood samples will be obtained pre-initial infusion of TK-112690/ placebo and 5, 24 and 48 hours post each initial infusion as well as on Day 1 of Week 6. Plasma will be obtained from the blood samples in order to determine surrogate markers of mucositis, e.g., CD40/CD40L, TNF-α, etc.
- Patients will be evaluated for extent and severity of mucositis using established mucositis rating scales, e.g., OMAS/Sonis, PROMS, WHO, and CTCAE/mucositis on the day prior to study start (Day 0), and 24 and 48 hours post each initial infusion of TK-112690/ placebo as well as on Day 1 of Week 6. Examination will assess the lining of the mouth, throat, and other linings of the digestive tract. Incidence of oral mucositis, duration of severe oral mucositis, throat soreness, severity of pain and use of parenteral or transdermal opioid analgesics will be assessed.

Study Population

Patients at least 18 years old, diagnosed with locally advanced or residual or recurrent or metastatic SCCHN and scheduled to receive 45 mg/m^2 MTX to treat their cancer. The diagnosis must be confirmed by histopathology or cytology.

Inclusion Criteria

- Male and female subjects over 18 years old with a histologically or cytological confirmed diagnosis of locally residual, recurrent or metastatic SCCHN.
- Subject must have failed at least onecourses of non-MTX chemotherapy, orone course of non-MTX chemotherapyand chemo radiation for treating their SCCHN.
- No prior systemic treatments for cancer (chemotherapy and/or radiotherapy) 4 weeks prior to screening.
- No other concurrent, active, invasive malignancies.
- An Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2.
- Must have a life expectancy of at least 6 months.
- History of brain metastases allowed if disease has stabilized or improved after radiation and/or craniotomy.
- No active angina or uncontrolled arrhythmia.
- No detectable infection including hepatitis B/C and HIV.
- Not pregnant or nursing. Women of childbearing potential must have a negative urine pregnancy

test at screening and on the day before dosing and must use medically acceptable methods of birth control. Acceptable methods of birth control include oral or transdermal contraceptives, condoms, spermicidal foam, IUD, progestin implant or injection, abstinence, vaginal ring, or sterilization of partner. The reason for non-childbearing potential, such as bilateral tubal ligation, bilateral oophorectomy, hysterectomy, or post-menopausal for ≥ 1 year, must be specified in the patient's medical history file and CRF.

- Must have adequate organ and immune function as indicated by the following laboratory values:

Parameter	**Laboratory Values**
Serum creatinine	≤1.5 x ULN
Est. creatinine clearance	≥45 mL/min
Total bilirubin	≤2.0 mg/dL (≤34.2 µmol/L)
AST & ALT	≤3 x ULN
Absolute granulocytes	≥1.5 x 10^9 cells/L
Platelets	≥100,000/µL

- Be able to read orunderstand, and provide a signature or thumb impression on the Informed Consent Form (ICF) before entering the study.

Exclusion Criteria

- Subject has not failed at least one courses of non-MTX chemotherapy or one course of non-MTX chemotherapy and chemo radiation for treating their SCCHN.
- Uncontrolled active infection.
- Current mucositis (>Grade 1).
- Pregnant or nursing mother.
- Prior history of a cerebrovascular accident or hemorrhage.
- Congestive heart failure, as defined by New York Heart Association class III or IV.
- Uncontrolled hypertension.
- Active psychiatric/mental illness making informed consent or useful clinical follow-up unlikely.
- Subjects who have previously been enrolled into this study and subsequently withdrew.
- Subject receiving other investigational agent(s).
- Any systemic immunosuppressive medication/therapy (eg, other chemotherapy, steroids).
- Any significant systemic illness, unstable or severe medical condition(s) that could put the subject at risk during the study, interfere with outcome measures, or affect compliance with the protocol procedures such as intercurrent infection and/or autoimmune disease, ie, any condition that compromises the immune system.
- Known or suspected intolerance or hypersensitivity to the study materials (TK-112690 and/or excipients or closely related compounds).
- Subjects, who have received, or plan to receive, radiation or chemotherapy within 4 weeks of screening.
- Subjects that have a history of poor compliance in clinical research studies.
- Subjects that have participated in any other investigative clinical trial in the past 4 weeks.

Dose and Dosing Regimen

The dose of METREXASSIST™ (TK-112690) will be 45 mg/kg administered parenterally over one hour in a clear, neutral, aqueous formulation (no organic chemical excipients). METREXASSIST™ will be given one hour before and 5 hours after the MTX treatment. The nutrient drink will be given one hour before METREXASSIST™ infusion. The MTX formulation will that traditionally used as standard-of-

care at a study site.

Test Product, Dose and Mode of Administration

- METREXASSIST™ (TK-112690 for Infusion) is a clear, sterile, neutral (pH=7.4), aqueous solution containing 5 mL of 100 mg/mL TK-112690.
- METREXASSIST™ is packaged as a 5 mL solution in a 10 mL flint glass vial with an elastomeric stopper held in place by an aluminum seal and flip-off cap. The concentration of TK-112690 in the solution is 100 mg/mL
- METREXASSIST™ will be administered as a continuous iv infusion over a duration of one hour in a volume of 250 mL normal saline.
- The nutrient drink, Fortasyn Connect™ / Souvenaid™, is commercially available as a 125 mL, 125 kcal solution. The drink is administered orally.
- The placebo is isotonic saline.

Reference Therapy or Comparator

Placebo-Isotonic saline

Follow-up

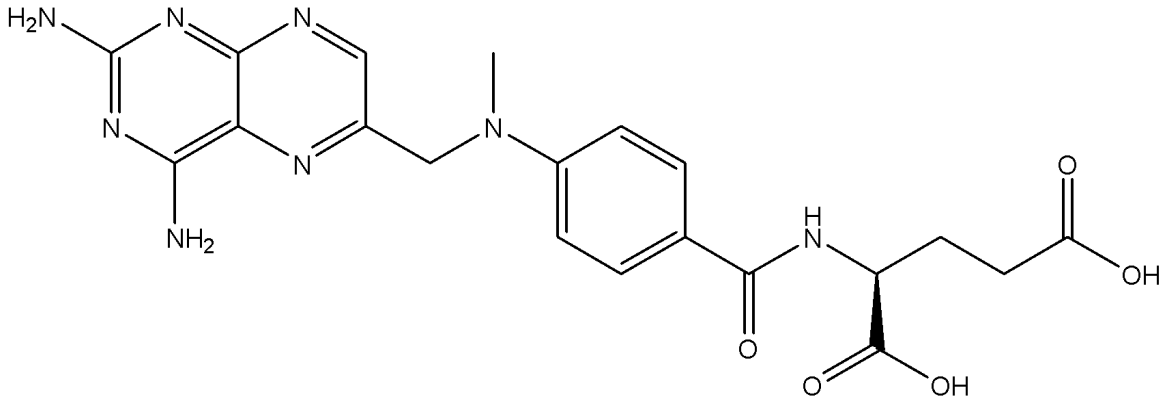
Subjects need to return at Day 1 of Week 6, which is two weeks from the last dose of the study drug.

Statistical Methods

- Safety: AEs, vital signs, physical examination data and clinical laboratory data will be analyzed using descriptive statistics with safety outcomes for patients in a dose group pooled & compared with patients in other groups.
- Mucositis: Scale data from individual doses groups will be analyzed using descriptive statistics.
- Surrogate Markers: Plasma concentration data evaluated for individual doses groups using descriptive statistics as well as compared with Surrogate marker data from the completed Phase 1bstudy.

INTRODUCTION

Methotrexate (MTX, structure below) was introduced in the early 1950s and remains the main antifolate in widespread use despite numerous attempts to replace or complement this chemotherapeutic.[1,2,3,4]MTX is used to treat a variety of cancers, such as head and neck cancer, leukemia, osteosarcoma, breast cancer, acute lymphoblastic, lymphocytic/meningeal leukemia, and non-Hodgkin lymphoma. It is also used to treat cancer of the uterus, breast, lung, gestational choriocarcinoma, chorioadenoma destruens, and hydatidiform mole. In these applications, MTX limits the metabolism of the cancer cell by inhibiting the enzyme responsible for folic acid metabolism, resulting in cellular folic acid deficiency,causingcell death. A particularly attractive application of MTX is as a relatively inexpensive, but useful, palliative treatment for advanced head and neck cancer.[5]



MTX is also used clinically in a variety of other pathogenic conditions, including rheumatoid arthritis, psoriasis, uveitis, and graft-versus-host disease.[6] In these applications, lower doses of MTX are used and the effect is generally attributed to an immunosuppression phenomenon.

Besides affecting cancerous tissue, MTX often produces unintended adverse effects in normal, non-cancerous, tissues, particularly tissues that have a high rate of proliferation, such as gut and bone marrow.[7] The side effects of MTX can threaten survival. Gastrointestinal mucosa, bone marrow, liver and hair are particularly vulnerable to the effects of MTX. Renal toxicity is also frequent with high doses of MTX.

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[1]Mager DR, Methotrexate, Home Health Now. 2015; 33:139-41.

[2] McGuire JJ. Anticancer antifolates: Current status and future directions. Curr Pharm Des. 2003; 9:2593-613.

[3] Visentin M, Zhao R, Goldman ID. The antifolates. Hematol Oncol Clin North Am. 2012; 26:629-48.

[4] Jolivet J, Cowan KW, Curt GA, Clendeninn NJ, Chabner BA. The pharmacology and clinical use of MTX. N Engl J Med. 1983; 309:1094–1104.

[5] Chakraborty S, Geetha M, Sujith KM, Biji MS, Sateeshan B. Palliative low dose fortnightly methotrexate in oral cancers: Experience at a rural cancer centre from India. South Asian J Cancer. 2014; 3:166-170.

[6]Hashkes PJ, Becker ML, Cabral DA, Laxer RM, Paller AS, Rabinovich CE, Turner D, Zulian F. Methotrexate: new uses for an old drug. J Pediatr. 2014; 164: 231-6.

[7]Gaies E, Jebabli N, Trabelsi S, Salouage I, Charfi R, Lakhal M, Klouz A, Methotrexate Side Effects: Review Article. J Drug MetabToxicol 2012. 3:125-129.

Mucositis describes the inflammation, lesions, and ulcers that develop in the oral and gastrointestinal tract because of treatment with chemotherapeutics such as MTX.[8] An effective adjuvant treatment that ameliorates MTX-induced mucositis would provide important relief to cancer patients undergoing chemotherapy.

Mucositis can affect up to 100% of people receiving high doses of chemotherapy.[9] The frequency of mucositis is responsible for increased patient hospitalization, resulting in a substantial financial burden for both patients and hospitals[10,11] In addition, mucosal damage increases the susceptibility of infection and sepsis in neutropenic patients. Pain, discomfort and the inability to eat or drink are the most frequent occurrences for patients undergoing chemotherapy. As a result, delays and suspensions in chemotherapy regimens are common for patients experiencing severe mucositis. The frequency, financial burden, and disruptions of effective cancer therapy all underscore the need for treatments that can mitigate mucositis.

To date, no product is available to effectively treat MTX-induced mucositis, especially mucositis outside the oral cavity:

- Several publications from animal studies have revealed evidence for compounds that can protect against MTX induced small intestinal damage. These potentially therapeutic compounds include various nutraceuticals, α-lipoic acid, lactoferrin, taurine, docosahexaenoic acid, prostaglandin E_1, N-acetylcysteine.[12,13,14,15,16[,17,18]] To date, none of these compounds are being widely explored in the clinic as potential side-effect reducing adjuvants for MTX-induced mucositis.

[8] Villa A, Sonis ST. Mucositis: pathobiology and management. CurrOpin Oncol. 2015; 27:159-64.

[9] Sonis ST, Perspectives on Cancer Therapy-Induced Mucosal Injury. Cancer. 2004;100:1995-2025.

[10] Carlotto A, Hogsett VL, Maiorini EM, Razulis JG, Sonis ST. The economic burden of toxicities associated with cancer treatment: review of the literature and analysis of nausea and vomiting, diarrhoea, oral mucositis and fatigue. Pharmacoeconomics. 2013; 31: 753-66.

[11] Elting, LS, The Burdens of Cancer Therapy. Cancer. 2003;98:1531-1539.

[12] Thomsen M, Vitetta L Adjunctive Treatments for the Prevention of Chemotherapy- and Radiotherapy-Induced Mucositis. Integr Cancer Ther. 2018 Aug 23:1534735418794885 (ePub prior to publication)

[13] Ahmed AA, Selim MA, El-Sayed NM. α-Lipoic acid ameliorates oral mucositis and oxidative stress induced by methotrexate in rats. Histological and immunohistochemical study. Life Sci. 2017;171: 51-59.

[14] Van'T Land B. Lactoferrin reduces MTX-induced small intestinal damage possibly through inhibition of GLP-2 mediated epithelial cell proliferation. Digestive Diseases and Sciences. 2004;49:425-433.

[15] Cetiner M. Taurine protects against MTX induced toxicity and inhibits leukocyte death. Toxicology and Applied Pharmacology. 2005;209;39-50.

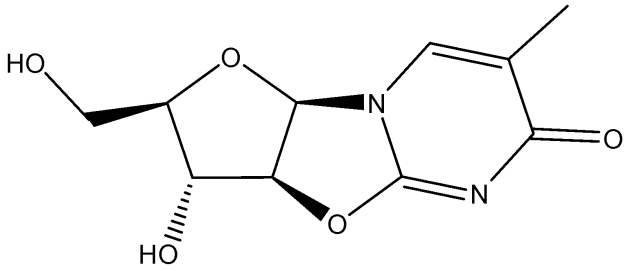
[16] Horie T. Docosahexaenoic acid exhibits a potent protection of small intestine from MTX-induced damage in mice. 1998; 62(15):1333-1338.

[17] Gao F. Protective effect of a synthetic analog of prostaglandin E1 on the small intestinal damage induced by the administration of MTX to rats. Journal of Pharmaceutical Sciences. 2001; 90:1040-1048.

[18] Ciralik H. Effects of N-Acetylcysteine on MTX-induced small intestinal damage in rats. The Mount Sinai Journal of Medicine. 2006;73:1086-1092.

- Epidermal growth factors are candidates for reducing radiation and chemotherapy induced mucositis. Palifermin (human recombinant keratinocyte growth factor-1) is approved by the FDA to decrease the incidence and duration of severe oral mucositis in patients with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell support. Palifermin is thought to maintain mucosal barrier integrity by stimulating basal cell proliferation and differentiation in the oral epithelia[19,20] The use of a protein that stimulates growth in cancer patients is problematic and concerning.

Preliminary preclinical data demonstrates the potential of TK-112690 (structure on the right) as a cytoprotectant against MTX induced mucositis.



- In a mouse model of human MTX-induced mucositis, TK-112690 protects from weight loss,[21] one of the cardinal features of human mucositis using a published weight model that mimics many of the features of "natural" mucositis.[22]
- TK-112690 limits the increase in systemic WBC concentration observed in mice post-MTX treatment,[23] and
- TK-112690 limits the increase in mucosal permeability observed in mice post-MTX treatment.[24]

Finally, TK-112690 appears unusually safe for clinical use. The compound does not affect the cytotoxicity of MTX toward three different cancer cell lines[25, 26] and two xenograft studies.[27, 28]

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[19] Nguyen DT, Shayani S, Palmer J, Dagis A, Forman SJ, Epstein J, Spielberger R.
Palifermin for prevention of oral mucositis in allogeneic hematopoietic stem cell transplantation: a single-institution retrospective evaluation. Support Care Cancer. 2015; 23: 3141-7.

[20] Blijlevens N and Sonis ST. Palifermin(recombinant keratinocyte growth factor-1): a pleiotropic growth factor with multiple biological activities in preventing chemotherapy and radiotherapy induced mucositis. Annals of Oncology. 2007;18:817-826

[21] Tosk Report: In vivo Response of Methotrexate (200 mg/kg Day 2, 100 mg/kg Day 3) and/or Dose Range of TK-112690 (15, 30, 45, 60, or 90 mg/kg) ip 3 Hours Before and After Methotrexate in Female C57Bl/6 Mice Pre-dosed with LPS (5 µg, Day 1, ip), FSR-PC-2690-0002

[22] deKoning BAE, Contributions of mucosal immune cells to MTX-induced mucositis. International Immunology. 2006;18: 941-949.

[23] Tosk Report: In vivo Measurement of Leukocytosis in Female C57Bl/6 Mice Treated with Methotrexate (50 mg/kg Days 1, 2, 3, 4, 6, and 8, ip) with or without TK-112690 (60 mg/kg 3 Hours Before and After Methotrexate, ip), FSR-PC-2690-0012

[24] Tosk Report: In Vivo Effect of 60 mg/kg TK-112690 (ip) on Increased Intestinal Permeability Induced by Methotrexate (100 mg/kg ip Days 2, 3 and 4) in C57Bl/6 Mice, FSR-PC-2690-0043

[25] Tosk Report: In Vitro Evaluation in U-937 and CCRF-CEM Cells of the Effect of a 72 Hour Treatment with TK-112690 (1.0, 10, 100 µM) or Leucovorin (1.0, 10, 100 µM) on the Antiproliferative Activity of Methotrexate (0.03 µM), FSR-PC-2690-0004.

[26] Tosk Report: In Vitro Effect of TK-112690 (1.0, 10, 100 µM) on the Cytotoxicty of Methotrexate (0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 100 µM) in AS283 Human Lymphoma Cells using CellTiter-Glo Assay, FSR-PC-2690-0014.

No untoward events were noted in studies in rats and dogs administered high doses of TK-112690 every day[29] for 28-days.[30,31] No CV effects were observed in a study with TK-11290 in instrumented monkeys. Further, no effects on the most common cytochrome p-450s were observed with TK-112690.[32] A study in human volunteers dosed once with up to 45 mg/kg of TK-112690 produced no untoward events,[33] and a predictable PK profile.[34] Recently, a Phase 1b study completed in 25 patients with head and neck cancer. No unanticipated safety findings were observed and promising anti-mucosal activity was found.

TK-112690 is an inhibitor of uridine phosphorylase (UPase). The clearance of uridine is controlled by UPase, and the inhibition of UPase leads to an increase of uridine (↑ uridine salvage).[35] In this regard, increased uridine is associated with protection from drug-induced toxicity in many situations, eg, zidovudine-induced mitochondrial myopathy/hyperlactatemia in mice,[36] cortical neuron damage from glucose deprivation-induced death,[37] glucose deprivation-induced death of immune-stimulated astrocytes,[38] zalcitabine-induced micro-vesicular steatohepatitis in mice,[39] in an animal model of lung inflammation,[40] cardinal features of asthmatic airway inflammation,[41] protective effect on cornea in a rabbit dry eye model,[42]

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[27] Tosk Study: In vivo Response of Subcutaneously Planted AS283 Human Lymphoma Xenografts in Male C.B.-17 SCID Mice to Treatment with Methotrexate (5.0 mg/kg q1d x 5, ip) and/or TK-112690 (30 mg/kg, 3 Hours Before and After Methotrexate, ip), FSR-PC-2690-0020

[28] Tosk Report: In Vivo Response of Subcutaneously Planted CCRF-CEM Leukemia Xenografts in Male Athymic NCr-nu/nu Mice to Treatment with Methotrexate (7.5 mg/kg for 5 Days, ip) and/or TK-112690 (30 mg/kg 3 Hours Before and After Methotrexate, ip), FSR-PC-2690-0003

[29] Tosk Report: A Cardiovascular Safety Pharmacology Study Using Radiotelemetry in Conscious Cynomolgus Monkeys Following Intravenous Injection, FSR-PC-2690-0028.

[30] Tosk Report: Toxicokinetics 0, 20, 60 and 180 mg/kg TK-112690 Administered iv Daily in a 28-day General Toxicity Study in Sprague-Dawley Rats, FSR-PC-2690-0009.

[31] Tosk Report: Toxicokinetics 0, 20, 60 or 180 mg/kg TK-112690 Administered iv Daily in a 28-Day General Toxicity Study in Beagle Dog, FSR-PC-2690-0010.

[32] Tosk Report: Determination of the Potential for TK-112690 to Inhibit the Enzyme Activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in Human Liver Microsomes, FSR-PC-2690-0026

[33] Tosk Report: TK90Clinical_Phase1a, FSR-CL-2690-0002

[34] Tosk Report: TK90PKPDUE_Phase1a FSR-CL-2690-0001.

[35] Pizzorno G, Cao D, Leffert JJ, Russell RL, Zhang D, Handschumacher RE.
Homeostatic control of uridine and the role of uridine phosphorylase: a biological and clinical update. BiochimBiophys Acta. 2002 Jul 18;1587(2-3):133-44.

[36] Lebrecht D et al., Uridine Supplementation Antagonizes Zidovudine-induced mitochondrial myopathy & hyperlactatemia in mice, Arthritis Rheum 2008; 58: 318.

[37] Choi JW et al., Uridine Protects Cortical Neurons from Glucose Deprivation-Induced Death: Possible Role of Uridine Phosphorylase. J Neurotrauma 2008; 25: 695.

[38] Choi JW et al., Uridine prevents the glucose deprivation-induced death of immune-stimulated astrocytes via the action of UPase. Neurosci Res. 2006; 56: 111.

[39] Lebrecht D et al., Uridine Supplementation Antagonizes Zalcitabine-induced Microvesicular Steatohepatitis in Mice. Hepatology 2007, 45:72.

[40] Evaldsson C et al., Anti-inflammatory effects of exogenous uridine in an animal model of lung inflammation. International Immunopharmacology 2007; 7: 1025.

[41] Muller et al., Local administration of uridine suppresses the cardinal features of asthmatic airway inflammation. Clinical & Experimental Allergy 2010; 40: 1552.

development of antigen-induced arthritis,[43] etc. Uridine is anti-apoptotic.[44] Significant with respect to mucosal toxicity, uridine protects from mucosal damage produced by dextran sulfate, an established IBD model.[45] Finally, uridine triacetate (VISTOGARD), an orally active prodrug of UR, recently received marketing approval by US FDA to treat an overdose of 5-FU. [46]

RATIONALE FOR THE CLINICAL DEVELOPMENT OF TK-112690

MTX, a structural analogue of folic acid, is widely used as a chemotherapeutic agent for various malignancies including head and neck cancer. One of the major toxic effects of MTX is intestinal injury, apparently mediated by both a direct effect of MTX, and an indirect effect produced by infiltration of gut bacteria and gut bacteria-associated toxins into the systemic circulation through the impaired mucosal barrier.

METREXASSIST™, TK-112690 for parenteral use, is a novel, non-toxic and specific therapy to treat mucositis caused by MTX treatment. In mouse models of human MTX-induced mucositis, TK-112690 protects from weight loss, one of the cardinal features of human mucositis, as well as limits the increase in systemic WBC concentration and mucosal permeability observed in mice post-MTX treatment. TK-112690 does not affect the cytotoxicity of MTX toward cancer cellsin*in vitro* and *in vivo* studies. Various other preclinical studies and a single dose study in human volunteers suggest no safety concerns with TK-112690.

The protocol provided in this document was designed further determine the tolerability of TK-112690 when administered weekly along with MTX as an intravenous infusion in patients with local, advanced, recurrent, metastatic SCCHN. Relevant mucositis endpoints will also be collected in the study. To increase the reliability of the results, the study is partially blinded- The patient and investigator will be blinded as to whether TK-112690 or placebo is administeredonlythe CRO, sponsor and site pharmacist will know whether the patient was administered active drug or placebo.

1.0 SUPPORTING INFORMATION

Methotrexate (MTX) is used to treat a wide range of cancers.[47]In addition to affecting cancerous tissue, MTX often produces unintended adverse effects in normal, non-cancerous, tissues, particularly tissues that have a high rate of proliferation, such as bone marrow and gut[47].

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[42] Ob et al., Protective Effect Uridine on Cornea in a Rabbit Dry Eye Model. Investigative Ophthalmology & Visual Science, 48; 1102.

[43] Narendra et al., Local but Not Systemic Administration of Uridine Prevents Development of Antigen- Induced Arthritis. PLos One 2015. 10: e0141863.

[44]Cansev M, Minbay Z, Goren B, Yaylagul EO, Cetinkaya M, Koksal N, Alkan T. Neuroprotective effects of uridine in a rat model of neonatal hypoxic-ischemic encephalopathy. Neurosci Lett. 2013;542:65-70.

[45]Jeengar MK, Thummuri D, Magnusson M, Naidu VGM, Uppugunduri S. Uridine Ameliorates Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice. Sci Rep. 2017; 7:3924..

[46] https://www.vistogard.com/Vistogard/media/Main-Media/final-labeling-text-vistogard.pdf

[47] Jolivet J, Cowan KW, Curt GA, Clendeninn NJ, Chabner BA, The pharmacology and clinical use of MTX, N Engl J Med 1983; 309:1094–1104.

Mucositis describes the inflammation, lesions, and ulcers that develop in the oral and gastrointestinal tract because of treatment with chemotherapeutics such as MTX[48,49] An effective adjuvant treatment that ameliorates chemotherapeutic-induced mucositis would provide important relief to cancer patients undergoing chemotherapy with MTX.

Mucositis can affect up to 100% of people receiving high doses of chemotherapy.[50] The frequency of mucositis is responsible for increased patient hospitalization, resulting in a substantial financial burden for both subjects and hospitals.[51] In addition, mucosal damage increases the susceptibility of infection and sepsis in neutropenic subjects[52,53,54] Pain, discomfort and the inability to eat or drink are the most frequent occurrences for subjects undergoing chemotherapy. As a result, delays and suspensions in chemotherapy regimens are common for subjects experiencing severe mucositis.[3, 4] The frequency, financial burden, and disruptions of effective cancer therapy all underscore the need for treatments that can mitigate mucositis.

- Several publications from animal studies have revealed evidence for compounds that can protect against MTX-induced small intestinal damage. These potentially therapeutic compounds include lactoferrin, taurine, docosahexaenoic acid, prostaglandin E_1, N-acetylcysteine.[55,56,57,58,59] To date, none of these compounds are being widely explored in the clinic as potential side-effect-reducing adjuvants for MTX-induced mucositis.
- Epidermal growth factors have become candidates for reducing radiation and chemotherapy induced mucositis. Palifermin (human recombinant keratinocyte growth factor-1) is approved by the FDA to decrease the incidence and duration of severe oral mucositis in subjects with hematologic malignancies receiving myelotoxic therapy requiring hematopoietic stem cell

[48] McGuire, DB, Mucosal Tissue Injury in Cancer Therapy, Cancer Practice 2002; 10: 170-191.
[49] Rubenstein EB, Clinical practice guidelines for the prevention and treatment of cancer therapy – induced oral and gastrointestinal mucositis, Cancer Supplement 2004; 100: 2026-2046.
[50]Sonis ST, Perspectives on Cancer Therapy-Induced Mucosal Injury, Cancer 2004; 100: 1995-2025.
[51]Elting, LS, The Burdens of Cancer Therapy Cancer 2003; 98: 1531-1539.
[52]Sonis, ST, Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation Journal of Clinical Oncology 2001; 19: 2201-2205.
[53]Wardley, AM, Prospective evaluation of oral mucositis in patients receiving myeloablative conditioning regimens and haemopoietic progenitor rescue, British Journal of Haematology 2001; 110: 292-299.
[54]Yuen, KY, Unique risk factors for bacteraemia in allogenic bone marrow transplant recipients before and after engraftment, Bone Marrow Transplantation 1998; 21: 1137-1143.
[55]Van'T Land, B, Lactoferrin reduces MTX-induced small intestinal damage possibly through inhibition of GLP-2 mediated epithelial cell proliferation. Digestive Diseases and Sciences 2004; 49: 425-433.
[56]Cetiner, M, Taurine protects against MTX induced toxicity and inhibits leukocyte death, Toxicology and Applied Pharmacology 2005; 209; 39-50.
[57]Horie, T., Docosahexaenoic acid exhibits a potent protection of small intestine from MTX-induced damage in mice. 1998 62(15) p. 1333-1338.
[58]Gao, F., Protective effect of a synthetic analog of prostaglandin E_1 on the small intestinal damageinduced by the administration of MTX to rats. Journal of Pharmaceutical Sciences. 2001. 90:p. 1040-1048.
[59]Ciralik, H., Effects of N-Acetylcysteine on MTX-induced small intestinal damage in rats. The Mount Sinai Journal of Medicine. 2006.73; 1086-1092.

support. Palifermin is thought to maintain mucosal barrier integrity by stimulating basal cell proliferation and differentiation in the oral epithelia[60,61]

One reason mucositis has remained a significant problem and limitation for the use of chemotherapy has been the lack of insight into the pathogenesis of the disease. In January 2002, the Multinational Association of Supportive Care in Cancer and the International Society for Oral Oncology assembled an expert panel to set guidelines for the evaluation, prevention, and treatment of mucositis.[3,4] From this meeting,[2] a five-step model emerged to explain the development and progress of mucositis:

Step 1: Initiation,
Step 2: Up-regulation of "message,"
Step 3: Signaling and amplification,
Step 4: Ulceration and inflammation, and
Step 5: Healing.

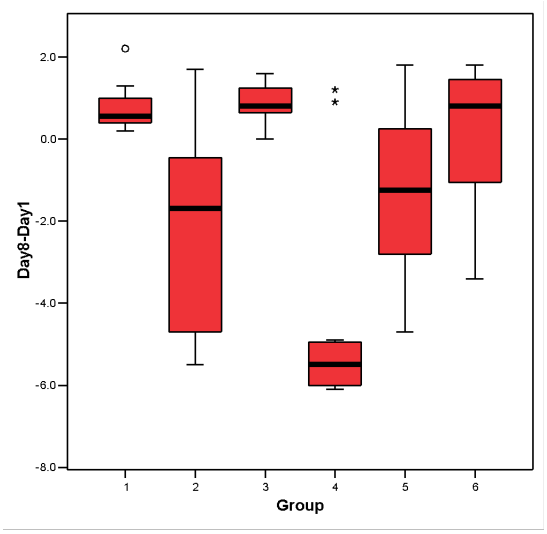
Using this model, arealistic animal model can be used to examine potential cytoprotectants for chemotherapeutic-induced mucositis.

TK-112690 Preliminary data demonstrating the potential of TK-112690 as a cytoprotectant against MTX induced mucositis was demonstrated at Tosk using a published weight model that mimics virtually all of the features of "natural" mucositis.[62] In this model, GI toxicity is induced in C57BL/6 mice by pre-dosing with lipopolysaccharide (LPS) on Day 1 followed by MTX injections on Days 2 and 3.

Typical results using this model are provided in the following **Figure.**

[60]Blijlevens, N and Sonis, S.T., Palifermin(recombinant keratinocyte growth factor-1): a pleiotropic growth factor with multiple biological activities in preventing chemotherapy and radiotherapy induced mucositis Annals of Oncology. 2007 18: 817-826.
[61] Duncan, M and Grant, G, Review article: oral and gastrointestinal mucositis-causes and possible treatments. Aliment PharmacolTher 2003; 18: p.853-874.
[62]deKoning, BAE, Contributions of mucosal immune cells to MTX-induced mucositis, International Immunology 2006, 18: 941-949.

Figure 1. Box plots showing prevention of weight loss by TK-112690 in a mouse model for MTX-induced mucositis



Animal weight change was calculated by subtracting Day 8-Day 1, and ten C57BL/6 mice per dose group were evaluated. Group 1=saline treatment, Group 2=MTX alone, Group 3=LPS alone, Group 4=MTX + LPS alone, Group 5=60 mg/kg TK-112690 qd with MTX or Group 6=60 mg/kg TK-112690 qd with MTX + LPS.

Why combine LPS and MTX in the model?

LPS, or endotoxin, is a polysaccharide that covers the outer membrane of most gram-negative bacteria[63,64]. The use of MTX as a chemotherapeutic agent is known to cause severe damage to the rapidly dividing crypt cells of the intestinal epithelium.[65] Diminished cell renewal and breakdown of the mucosal barrier can expose macrophages, CD4+T cells and other mucosal immune cells to the vast amounts of microbial pathogens within the intestinal lumen resulting in three of the hallmarks of mucositis: GI inflammation, weight loss, and sepsis.[66,67,68,69] Toll-like-receptors (TLR4) on the surface of innate immune cells, such as macrophages and monocytes, recognize the conserved patterns of endotoxins during the

[63]Raetz, CRH, Lipopolysaccharide Endotoxins, Annu. Rev. Biochem 2002; 71: 635-700.
[64]Beutler, B and Rietschel, ET, Innate immune sensing and its roots: the story of endotoxin, Nature Reviews Immunology, 2003; 3: 169-176
[65]deKoning, BAE, MTX-induced mucositis in mucin-2 deficient mice, Journal of Cellular Physiology 2007; 210; 144-152.
[66]Blijlevens, NMA, Mucosal barrier injury: biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview, Bone Marrow Transplantation 2000; 25: 1269-1278.
[67] Macdonald, TT, The mucosal immune system, Parasite Immunology 2003; 25: 235-246.
[68]Cheroutre, H, IELs: enforcing law and order in the court of the intestinal epithelium, Immunologic Reviews 2005; 206: 114-131.
[69]Strober, W, The immunology of mucosal models of inflammation.Annu Rev Immunol 2002; 20: 495-549

course of a bacterial infection.[70] Binding of LPS to TLR4 is the critical event that results in the activation of immune cells leading to their release of the pro-inflammatory cytokines TNF-α, IL-1 and IL-6.[71,72] Because LPS plays a central role in the inflammatory response and is a natural part of the intestinal microflora, its artificial injection prior to MTX treatment is meant to exacerbate the development of mucositis in the same way that bacteria perform this function in the pathology of natural mucositis.

1.1. NAME AND DESCRIPTION OF THE INVESTIGATIONAL DRUG

TK-112690 is a highly water soluble anhydrouridine that inhibits human and murine uridine phosphorylase (EC 2.4.2.3), an enzyme that catalyzes the chemical reaction of uridine + phosphate → uracil + α-D-ribose 1-phosphate. In this reaction, the two substrates are uridine and phosphate and the two products are uracil and α-D-ribose 1-phosphate.

METREXASSIST™ is TK-112690 for parenteral use provided as a 5 mL solution containing 100 mg/mL sterile TK-112690 packaged in a 10 mL glass vial sealed with an elastomeric stopper held in place with an aluminum seal.

1.2. SUMMARY PRE-CLINICAL EFFICACY RESULTS

In animal models of the human condition of mucositis, TK-112690 protects from three cardinal features associated with MTX-induced mucositis: weight loss, WBC increase (likely caused by infection), and increased mucosal permeability. The efficacy of TK-112690 in the weight loss model parallels the elevation in plasma uridine induced by inhibition of uridine phosphorylase. Findings in three *in vitro* studies and two *in vivo* studies with MTX and TK-112690 in human cancer cells suggest no effect of TK-112690 on the chemotherapeutic effect of MTX. Overall, the efficacy data suggest that treatment with TK-112690 may provide an innovative and useful approach to MTX-associated mucositis in man.

1.3. PHARMACOLOGY

1.3.1. IN VITRO

1. Effect of TK-112690 on U-937 and CCRF-CEM Human Cancer Cells.
   An *in vitro* study in CCRF-CEM and U-937 human cancer cells was performed to evaluate whether TK-112690 (1-100 μM) diminished the cytotoxicity of 0.03 μM MTX. Cell viability was measured using alamarBlue® and Leucovorin (LEU) was used as a positive control. The incubation was performed for 72 hours. For the study with U-937 cells, there was no statistical difference between cell viability in MTX (0.03 μM) test wells and test wells with MTX (0.03 μM) + TK-112690 (1, 10, 100 μM.). Therefore, the antiproliferative activity of MTX in this cancer cell line is not altered by the addition of TK-112690. In addition, the findings with the U-937 cells demonstrate that (i) TK-112690 does not interfere with MTX cytotoxicity, (ii) TK-112690 is not cytotoxic, and (iii) LEU (positive

[70]Beutler, B, Defective LPS signaling in C3H/HeJ and C57Bl/10 ScCr mice: Mutations in Tlr4 gene, Science 1998; 282: 2085-2088.
[71]Sonis, S.T. Mucositis as a biological process: a new hypothesis for the development of chemotherapy-induced stomatotoxicity, Oral Oncology 1998; 34: 39-43
[72]Tracey, KJ, and Cerami, A, Tumor Necrosis Factor, Other Cytokine and Disease,Annu.Rev. Cell Biol 1993 9: 317-343.

control) rescued cells from MTX cytotoxicity at concentrations equivalent to those studied with TK-112690.

For the study with CCRF-CEM cells, no statistical difference was noted between cell viability in MTX (0.03 μM) test wells and test wells with MTX + TK-112690 (1, 10, 100 μM). Therefore, the antiproliferative activity of MTX is not altered by the addition of TK-112690. In addition, there was no statistical difference between cell viability in untreated cells and cells treated with TK-112690 (1, 10, 100 μM) demonstrating that TK-112690 has no anti-proliferative activity in this cell line. Further, there was no statistical difference between cell viability in untreated cells and cells treated with MTX + LEU (1, 10, 100 μM). Therefore, LEU (positive control) completely rescued cells from MTX anti-proliferative activity at concentrations equivalent to those studied with TK-112690.

2. Effect of TK-112690 (1.0, 10, 100 μM) on the Cytotoxicity of MTX (0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 100 μM) in AS283 Human Lymphoma Cells using CellTiter-Glo Assay.
   An *in vitro* study in AS283 human cancer cells was performed to evaluate whether TK-112690 (1, 10, 100 μM) diminished the cytotoxicity of MTX (0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 100 μM). Cell viability was measured using CellTiter-Glo and DOX (10 μM) was used as a reference standard. The incubation was performed for 72 hours. There was no statistical difference between cell viability in MTX test wells and test wells with MTX + TK-112690 (1, 10, 100 μM). Therefore, the antiproliferative activity of MTX is not altered by the addition of TK-112690. Results from the study demonstrated that in the AS283 human lymphoma cancer cell line TK-112690 (1, 10, 100 μM) does not alter the IC_{50} of MTX (0.01-0.02 μM). In addition, TK-112690 is not cytotoxic in the AS283 cell line.

3. Effect of TK-112690 on the Inhibition of Uridine Phosphorylase Derived from Mouse and Human Intestinal Mucosa.
   Uridine phosphorylase (UPase) plays a key role in the regulation of pyrimidine metabolism by catalyzing the reversible phosphorolysis of uridine into uracil. 2, 2'Anhydrouridines such as TK-112690 are reported as potent inhibitors of UPase in both *in vitro* and *in vivo* systems[73,74] Inhibition of UPase can lead to the increased concentration of cellular uridine which is known to be a significant cell protectant.[75] Increasing the bioavailability of uridine by either UPase inhibition or exogenous uridine supplementation reduces adverse events associated with the use of fluropyrimidines[76,77] Additional animal studies have shown the ability of uridine to reduce the lethal effects of endotoxins.

[73]Veres Z, Szabolcs A, Szinai I, Dénes G, Kajtár-Peredy M, Otvös L. 5-Substituted-2,2'-anhydrouridines, potent inhibitors of uridine phosphorylase ,BiochemPharmacol. 1985; 34: 1737-40.

[74]Drabikowska AK, Lissowska L, Veres Z, Shugar D. Inhibitor properties of some 5-substituted uracil acyclonucleosides, and 2,2'-anhydrouridines versus uridine phosphorylase from E. coli and mammalian sources. BiochemPharmacol. 1987; 36: 4125-8

[75]Mazzola A, Amoruso E, Beltrami E, Lecca D, Ferrario S, Cosentino S, Tremoli E, Ceruti S, Abbracchio MP.
Opposite effects of uracil and adenine nucleotides on the survival of murine cardiomyocytes.J Cell Mol Med. 2008 Mar-Apr;12(2):522-36

[76]Pizzorno G, YeeL, Burtness BA, Marsh JC, Darnowski JW, Chu M, Chu SH, Chu E, Leffert JJ, Handschumacher RE, Calabresi P. Phase I clinical and pharmacological studies of benzylacycluridine, a uridine phosphorylase inhibitor. Clinical Cancer Research. 1998; 4: 1165-1160

[77] Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Young CW. High dose 5-flurouracil with delayed uridine "rescue" in mice. Cancer Research. 1982; 42; 3964-3970

[78, 79] To detect the activity of UPase, the reaction system was designed to measure the amount of uracil produced by the enzyme in the presence of uridine and phosphate.

Fresh mouse small intestine was obtained from sacrificed mice. The human intestine was commercially purchased and thawed at room temperature prior to use. The tissues were prepared in PBS fortified with EDTA and DTT. TK-112690 was added concentrations of 0, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000 and 10000 µM. The uridine phosphorylase preparation was 10% (w/v) with the precise concentration not determined. Uracil was measured by HPLC.

The activities of the enzyme decreased in the presence of TK-112690 demonstrating the inhibitory effect of the compound on the activity of human uridine phosphorylase. The IC_{50} was 20 µM. In summary, TK-112690 showed an inhibitory effect on the activity of uridine phosphorylases derived from both mouse and human tissues.

1.3.2 IN VIVO

Uridine Increase in Mice Post-TK-112690.
CD-1 female mice (n=3/treatment group) were injected ip with a range of TK-112690 doses (0, 15, 30 and 60 mg/kg) and plasma from the animals analyzed for concentration of uridine. Plasma uridine and TK-112690 concentrations were determined by HPLC from plasma samples collected 0.08, 0.50, 1, 2, 4 or 12 hours post TK-112690 injection. The HPLC assay used 5-fluorouracil as the internal standard for both uridine and TK-112690.

Results from the study at the two time points where uridine and TK-112690 were greatest are provided in **Table 1** The concentration of endogenous plasma uridine determined by the HPLC method described in this report is approximately 0.5 µg/mL.
Table 1. Mean plasma concentrations of TK-112690 and uridine for 0.08 and 0.5 time points

	Concentration (µg/mL)					
	15 mg/kg Dose		**30 mg/kg Dose**		**60 mg/kg Dose**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
TK-112690	10.33	7.66	38.27	14.70	77.88	49.52
Uridine	1.57	1.00	1.53	0.93	2.28	1.16

As expected, plasma concentrations of TK-112690 increased with increasing doses of TK-112690 administered either by ip or continuous infusion, which expected to be elevated through UPase inhibition by TK-112690.

[78]Galanos C, Freudenberg MA, Reutter W. Galactosamine-induced sensitization to the lethal effects of endotoxin.Proc. Natl. Acad. Sc. USA. 1979; 76: 5939-5943
[79] Rosenthal GJ, Lebetkin E, Thigpen JE, Wilson R, Tucker AN, Luster, MI. Characteristics of 2,3,7,8-teterachlorodibenzo-P-dioxin Induced Endotoxin Hypersensitivity: Association with Hepatotoxicity.Toxicology. 1989; 251: 239-251

1. LPS/MTX Weight Loss Model (See **Figure 1**).
   A published weight model[80] was employed to determine the ability of TK-112690 to protect against MTX-induced mucositis. Mice injected with MTX/LPS alone showed a severe, highly significant weight loss from day 5 to day 8 compared to animals treated only with saline. A significant reduction in MTX/LPS weight loss occurred in animals treated with 60 mg/kg TK-112690. In addition, dose ranging studies with TK-112690 in this model demonstrated that TK-112690 prevented MTX-induced weight loss at a dose as low as 15 mg/kg. The protection was dose-dependent.

2. Effect of TK-112690 on MTX-induced Intestinal Permeability of Iodixanol.
   A published model[81] was employed to determine the ability of TK-112690 to protect against MTX-induced intestinal permeability. Saline alone injected animals maintained a healthy mucosal barrier demonstrated by trace concentrations of plasma iodixanol. The MTX treatment produced a statistically significant ($p<0.005$) increase in plasma iodixanol compared to saline alone treated animals. Co-administration of TK-112690 with MTX provided plasma concentration similar ($p=1.00$) to those found in the saline treated animals supporting the hypothesis that TK-112690 protects from MTX-induced GI damage.

3. Effect of TK-112690 on Elevated Systemic WBC Concentration Produced in Mice by Treatment with MTX.
   A published model[82] was employed to determine the ability of TK-112690 to protect against MTX induced WBC elevation. MTX can have a cytotoxic effect on the rapidly-dividing cells of the mucosal lining leading to damage and increased permeability. This increase in intestinal permeability can lead to bacterial infiltration and infection. An elevation in the WBC of the MTX alone group is an indication of this bacterial infection and inflammation, possibly due to the MTX-induced intestinal damage. The MTX+TK-112690 did not exhibit this WBC increase supporting the hypothesis that TK-112690 protects against MTX-induced mucositis.

4. Effect of TK-112690 on *in vivo* Response to Treatment with MTX of Subcutaneously Planted CCRF-CEM Leukemia Xenografts in Male AthymicNCr-nu/nu Mice.
   An *in vivo* study of CCRF-CEM xenografts was performed to evaluate whether TK-112690 (30 mg/kg 3 hours before and after MTX, ip) diminished the antitumor efficacy of MTX (7.5 mg/kg for 5 Days, ip). The number of nonspecific deaths, the number of partial and complete tumor regressions, the number of tumor-free survivors, and the median number of days for the tumors in each group to reach two tumor mass doublings were calculated. The median time to reach two tumor mass doublings was used in the calculation of the overall delay in the growth of the median tumor. The study was terminated forty-eight days after tumor implantation.

   - Tumors in the vehicle-treated control group grew to the evaluation point in all ten mice. The median tumor reached two tumor mass doublings in approximately 10 days. There was an average body weight loss of 3% (0.8 g).

[80] de Koning BA, van Dieren JM, Lindenbergh-Kortleve DJ, van der Sluis M, Matsumoto T, Yamaguchi K, Einerhand AW, Samsom JN, Pieters R, Nieuwenhuis EE. Contributions of mucosal immune cells to methotrexate-induced mucositis, IntImmunol. 2006;18(6):941-9.
[81] Leblond J, Le Pessot F, Hubert-Buron A, Duclos C, Vuichoud J, Faure M, Breuillé D, Déchelotte P, Coëffier M. Chemotherapy-induced mucositis is associated with changes in proteolytic pathways.ExpBiol Med. 2008; 233: 219-28
[82] T. Robbins, D. Bowen, Q. Bui, M. Tran, Modulation of High-dose Methotrexate Toxicity by a Non-toxic Level of 5-Flourouracil. Toxicology1986; 41; 61-73

- Treatment with MTX was tolerated with a maximum average body weight loss of 9% (2.5 g). The MTX treatment delayed the growth of CCRF-CEM leukemia xenografts with a tumor doubling time of approximately 20 days.
- There was a single drug-related euthanasia out of ten animals following administration of TK-112690 at a dosage of 30 mg/kg/injection combined with MTX at a dosage of 7.5 mg/kg/injection with a corresponding maximum average body weight loss of 8% (2.3 g). Administration of TK-112690 combined with MTX delayed the growth of CCRF-CEM leukemia xenografts with a tumor doubling time of approximately 26 days. There was one complete tumor regression out of 10 animals which remained tumor-free at the conclusion of the study from the combined treatment.

Results from this study suggested that TK-112690 does not alter the antitumor efficacy of MTX against sc implanted CCRF-CEM human leukemia xenografts in male athymicNCr-nu/nu mice.

5. *In vivo* Response of Subcutaneously Planted AS283 Human Lymphoma Xenografts in Male C.B.-17 SCID Mice to Treatment with MTX (5.0 mg/kg q1d x 5, ip) and/or TK-112690 (30 mg/kg, 3 Hours Before and After MTX, ip).
   The purpose of this study was to determine whether TK-112690 effects MTX anti-tumor efficacy against subcutaneously (sc) implanted AS283 human lymphoma xenografts in male C.B.-17 SCID mice. MTX administered alone was used as a reference compound.

   Growth of AS283 human lymphoma xenografts was delayed by treatment with MTX (5.0 mg/kg/injected q1d x 5) alone or in combination with TK-112690 (30 mg/kg 3 hours before and after MTX). Groups receiving MTX with or without TK-112690 were statistically identical (p = 1.00). AS283 Xenograft Data Demonstrates that TK-112690 does not Interfere with MTX Anti-Tumor Efficacy. Growth of AS283 human lymphoma xenografts was delayed by treatment with MTX (5.0 mg/kg/injected q1d x 5) alone or in combination with TK-112690 (30 mg/kg 3 hours before and after MTX). Groups receiving MTX with or without TK-112690 were statistically identical (p = 1.00).

   AS283 Xenograft Data demonstrates that TK-112690 does not interfere with MTX anti-tumor efficacy.

   - Tumors in the vehicle-treated control group grew to the evaluation point in all ten mice. The median tumor reached 4,387 mg in 21 days. There was an average body weight gain of 14% (3.5 g) which correlates with tumor weight.
   - Treatment with MTX was tolerated with a maximum average body weight loss of 0.4 % (0.1 g). The MTX treatment delayed the growth of AS283 lymphoma xenografts with a median tumor weight value 2.8% of the control on Day 21 and a median tumor weight value 24.7% (40.0 mg) smaller than the median tumor weight value at the start of treatment (162 mg).
   - Treatment with TK-112690 at a dosage of 30 mg/kg/injection combined with MTX at a dosage of 5.0 mg/kg/injection was well tolerated with no average body weight (0.0 g). Administration of TK-112690 combined with MTX delayed the growth with a median tumor weight value 3.5% of the control on Day 21 and a median tumor weight value 5.6% (9.0 mg) smaller than the median tumor weight value at start of treatment (162 mg).

1.3.3. PRECLINICAL PHARMACOKINETIC (PK)/ADME STUDIES

Results from preclinical PK studies with TK-112690 can be found in **Table 2** below.

Table 2. Selected findings preclinical pharmacokinetic studies with TK-112690

Species	Sex	iv Treatment			Term. $t_{1/2}$ (h)	[a]MaxConc. (μg/mL)	[b]Vss (L/kg)	[c]CL (L/h/kg)	AUC (μg* h/mL)
		Duration	*Route*	*mg/kg*					
Cynomolgus monkey	2M 2F	Single dose	iv	100	13	220	0.64	0.19	540[d]
Beagle dog	2M 2F	28 days, qd	iv	60	36	240	2.20	0.25	253[e]
Sprague-Dawley rat	3M 3F	28 days, qd	iv	60	13	520	0.99	0.50	227[f]
CD-1 mice	F	Single dose	iv	50	21	221	1.72	1.2	43[f]
CD-1 mice	F	Single dose	ip	50	—	65	—	—	40[f]
CD-1 mice	F	Single dose	po	50	—	3	—	—	9[f]

[a]0 to 1 hours post-initiation of infusion, [b]Vss: volume distribution steady-state, [c]CL: clearance; [d]0 to 48 hours; [e]0 to 120 hours; [f]0 to72 hours; M=male, F=female;

In addition, TK-112690 at concentrations up to 300 μg/mL did not inhibit CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 in human liver microsomes and TK-112690 at concentrations ranging from 30-300 μg/mL did not induce CYP1A2, CYP2B6, or CYP3A4 enzyme activities.

Finally, the potential drug interaction of TK-112690 and MTX was assessed *in vivo* by pre-treating CD-1 mice for 3 days with 50 mg/kg TK-112690 prior to administration of MTX (50 mg/kg). Due to sensitivity limits in the HPLC analytical test method used, only MTX plasma concentrations 30 minutes post-initiation of infusion were measurable, and a complete pharmacokinetic evaluation was not possible because of this limitation. However, the limited data obtained suggested no difference as determined by an independent sample t-test ($p>0.5$) in the MTX plasma concentrations with the TK-112690 pre-treatment compared to plasma concentrations from the MTX control group.

These studies demonstrated that TK-112690:

- Exhibited approximate dose proportional increases in systemic exposure in animals following iv administration.
- Has an elimination half-life of approximately 13, 35 and 13 hours in rats, dogs and monkeys, respectively.
- Has a volume of distribution in all animals that greatly exceeds vascular volume suggesting considerable tissue distribution.
- Neither inhibits or induces the most common CYP450s.
- Appears to not affect the clearance of MTX in mice.

1.4. TOXICOLOGY/SAFETY

1.4.1. PRE-CLINICAL TOXICOLOGY.

Findings from the principal safety studies performed with TK-112690 are provided in **Table 3**.

Table 3. Safety studies with TK-112690

Study Description	GLP	Species	Route	Treatment Duration	Dosing Regimen	Results
Acute Safety in Mice	No	Female CD-1 Mice	ip	Single dose Observation up to 7 days post-initiation of infusion	0, 500, 1000 and 2000 mg/kg qd	No significant findings re weight &clinical chemistry measures
Exploratory Safety in Mice	No	Female C57BL/6 Mice	ip	7 Days	0, 10, 30 & 100 mg/kg q7d	No significant findings with weight, clinical chemistry and hematology measures
14-Day Dose Ranging Rodent	No	Male/Female Sprague-Dawley Rats	iv	14 Days	0, 10, 30, 90 or 270 mg/kg q14d	No significant findings with animal weight, organ weight, gross pathology, clinical chemistry and hematology; treatment related increases in TBIL in male and female animals at 30, 90 and 270 mg/kg/day, but all values still within normal range
14-Day Dose Ranging Canine	No	Male/Female Beagle Dogs	iv	14 Days	0, 3.3, 10, 30 or 90 mg/kg	No significant findings with animal weight, organ weight, gross pathology, clinical chemistry and hematology
28-Day Safety + 14-Day Recovery Rodent	Yes	Male/Female Sprague-Dawley Rats	iv	28 Days + 14-Day Recovery	0, 20, 60 or 180 mg/kg q28d	NOAEL=180 mg/kg/day

Study Description	GLP	Species	Route	Treatment Duration	Dosing Regimen	Results
28-Day Safety + 14-Day Recovery Canine	Yes	Male/Female Beagle Dogs	iv	28 Days + 14-Day Recovery	0, 20, 60 or 180 mg/kg q28d	NOAEL=180 mg/kg/day
Cardiovascular Safety in Telemetered Non-human primate	Yes	Cynomolgus Monkeys	iv	Single dose	0, 10, 30 and 100mg/kg qd	No effect on body temperature, respiratory rate, arterial blood gases and Lead II ECG variables (RP, PR, QT, QTcF, QTcQ and QRS intervals).
Ames Genotoxicity	Yes	*In vitro;S.typhimurium*strains TA97, TA98, TA100, TA1535 and *E. coli*strain uvrA-pKM101			8-5000 µg/plate with, and without, metabolic activation.	No revertants

These studies demonstrated that TK-112690:

- Does not produce toxic effects at doses of up to 180 mg/kg delivered iv for 28-days to rats and dogs.
- An iv dose of 100 mg/kg of TK-112690 to male, telemetered, Cynomolgus monkeys, produces no effects on cardiac function.
- No apparent effect of gender on disposition.

1.4.2 CLINICAL TOXICOLOGY

As further described in the following sections, single and multiple doses of METREXASSIST™ (TK-112690) for parenteral use have been studied in healthy human volunteers (Study CLP-2690-0001) and cancer patients (Study CLP-2690-0002) without any apparent drug-related adverse effects.

1.5. SUMMARY OF CLINICAL DATA

METREXASSIST™ was studied in a single escalating dose, Phase 1a study in normal, healthy, male volunteers (dose levels of 5, 15, 25, 35 or 45 mg/kg administered as an intravenous infusion over 4 hours). METREXASSIST™ was also studied in a multiple escalating dose, Phase 1b study in SCCHN patients (dose levels of 15, 30, 45, or 60 mg/kg, bid, administered as an intravenous infusion over 1 hours) weekly for 4 weeks. The Phase 1a study was performed under GCP conditions in South Africa. The Phase 1b studies was performed under GCP conditions in India.

1.5.1 Phase 1a Study[83]

1.5.1.1 Study Design

Subjects (3 subjects/cohort/15 subjects total) were admitted to the study center the day prior to dosing (Day 0). Study drug was administered iv over 4 hours on the morning of Day 1. Subjects remained confined for 48 hours and returned for a follow-up visit at Day 7 (± 24 hours) post dosing. Blood samples were obtained for determination of plasma concentrations of TK-112690 and uridine prior to dosing (baseline, and specified hours post-initiation of infusion). No subject participated in more than one cohort. Dose escalation was permitted if no dose-limiting toxicity (DLT) was observed by Day 7 post-infusion to the last subject in the previous dose cohort. Safety was assessed by monitoring adverse events (AEs), conducting physical examinations and 12-lead electrocardiograms (ECGs), measuring vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) and conducting laboratory tests (clinical chemistry, hematology and coagulation) and physical examinations before dosing, specified time post-dosing, and at Day 7 ± 1 day post dosing.

1.5.1.2 Overall Safety

Administration of METREXASSIST™ to the volunteers was well tolerated. Only two adverse events were reported as either possibly (headache in Subject 2, 5 mg/kg dose group) or definitely (burning at infusion site experienced by Subject 3, 5 mg/kg dose group) drug related. In the 15 subjects receiving METREXASSIST™, there were no ≥ Grade 3 AEs which were considered related to study drug. There were no serious adverse events (SAEs) and therefore no IND Safety Reports. No actions were taken for safety reasons, and there were no protocol modifications based on safety concerns associated with the TK-112690 administration. Apparent polyuria was noted in Subjects 1, 3-8 and 10-15. However, the greater than expected urine volumes were not considered excessive given the relatively large volume of fluids concurrently consumed by the volunteers and was, therefore, considered not drug related.

1.5.1.3 Clinical Chemistry

Blood samples were obtained from the volunteers at Screening, Day 0, Pre-Dose (Day 1), 4 hour (Day 1), 12 hours (Day 1), 24 hours (Day1), 48 hours (Day 2) and 7 Days post-dose. The blood was analyzed for electrolytes such as sodium, potassium, chloride, and calcium (total), enzymes such as aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, and biochemical such as CO_2, urea, creatinine, uric acid, phosphate, protein (total), albumin, globulin, bilirubin (total), bilirubin (direct), cholesterol, triglyceride, glucose, and C- reactive protein. Values for all analytes were within the normal laboratory range at all METREXASSIST™ doses.

1.5.1.4 Hematology

Blood samples were obtained from the volunteers at Screening, Day 0, Pre-Dose (Day 1), 4 hours (Day 1), 12 hours (Day 1), 24 hours (Day1), 48 hours (Day 2) and 7 Days post-dose. The blood was analyzed for hemoglobin, erythrocyte count, hematocrit, mean cell volume, MCH, MCHC, platelet count, leucocyte count, neutrophils, neutrophils (absolute), lymphocytes, lymphocytes (absolute), monocytes, monocytes (absolute), eosinophils, eosinophils (absolute), basophils and basophils (absolute). Values for all analytes were within the normal laboratory range at all METREXASSIST™ doses.

[83] Tosk FSR-CL-2690-0002: TK90 Clinical_Phase1a

1.5.1.5 Coagulation

Blood samples were obtained from the volunteers at Screening, Day 0, Pre-Dose (Day 1), 4 hours (Day 1), 12 hours (Day 1), 24 hours (Day1), 48 hours (Day 2) and 7 Days post-dose. The samples were analyzed for coagulation times. No effects at any METREXASSIST™ dose on PT or PTT coagulation times were observed.

1.5.1.6 Urinalysis

Urine samples were collected from each volunteer on Day 0, and on Days 1-2. No effect at any METREXASSIST™ dose was noted on urine appearance, color, or concentration of glucose, ketones, proteins or occult blood.

1.5.1.7 Physical Examination

Physical examinations, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, extremities, and body weight were performed at screening, Day0, pre-dose and at 4, 12, 24, 48 hours, and 7 Days post-dose.

1.5.1.8 Vital Signs

Vital signs [supine blood pressure (systolic and diastolic), heart rate, respiratory rate and oral body temperature] were performed at screening, Day0, pre-dose and 4, 6, 8, 12, 24, 48 hours, and 7 Days post-dose. No effect of the drug at any METREXASSIST™ dose was observed on any vital sign measurement.

1.5.1.9 EKGs

12-Lead EKGs were obtained at screening and at 4, 12, and 24 hours post-dose. No drug-induced changes were noted.

1.5.1.10 Pharmacokinetics

Mean TK-112690 measured plasma concentrations are provided in **Table** 4 below. Mean pharmacokinetic parameter estimates derived from plasma concentrations for 15-45 mg/kg doses are provided in **Table** 5.

Table 4. *Mean plasma concentrations TK-112690 in volunteers (3 volunteers/dose group)***.**

		Hours Post Initiation of 4 Hour Infusion									
Dose (mg/kg)	**#Values**	**4**		**6**		**8**		**12**		**24**	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
5	3	13.16	3.27	5.53	0.91	2.95	0.76	0.71	0.19	nm	–
15	3	24.22	1.94	17.06	3.95	8.81	2.32	2.57	0.01	0.26	0.05
25	3	38.40	0.58	18.48	3.80	10.49	2.77	4.60	1.42	1.37	1.54
35	3	58.69	2.50	21.51	2.06	12.01	1.34	4.60	0.54	0.60	0.24
45	3	79.60	4.59	29.31	1.79	16.08	1.36	6.40	0.72	1.17	0.05
Mean=µg/mL ± SD											

Table 5. *Mean pharmacokinetic parameter estimates derived from plasma concentrations for 15-45 mg/kg doses*

Parameter Estimates	Mean	SD
Dosing time	0	0
Rsq	0.97	0.04
Peak Concentration	50.27	22.03
No._points_Lambda_z	4.00	0.00
Tlast	24.00	0.00
Clast	0.89	0.80
AUClast	257.02	93.10
Lambda_z	0.19	0.04
$t_{1/2}$_Lambda_z	3.92	1.06
AUCall	257.02	93.10
AUCINF(observed)	263.13	95.62
AUCINF(observed)/D	0.0091	0.0012
AUC_%Extrap(observed)	2.13	2.84
Vz(observed)	632.28	129.70
Cl(observed)	113.32	14.21
AUMClast	1678.50	598.78
AUMCINF(observed)	1871.16	765.97
AUMC_%Extrap(obs.)	8.14	8.53
MRTlast	4.55	0.50
MRTINF(observed)	5.07	1.23
Vss(observed)	563.87	81.97
Units: Dosing time=Hours , Rsq=Dimensionless, Tmax=Hours, Cmax=µg/mL, Points_Lambda_z=Number, Tlast= Hours, Clast= µg/mL, AUClast=µgxHour/mL; Lambda_z=$Hour^{-1}$, t1/2_Lambda_z=Hours, AUCall, µg∗Hour/mL, UCINF(observed), AUCINF(observed)/D=Dimensionless, AUC_%Extrap(obs.), %, Vz(observed), mL/kg, Cl(observed), mL∗hour/kg, AUMClast, ng x $hour^2$ /mL, AUMCINF(observed) , ng x $hour^2$ /mL, AUMC_%Extrap(obs.), %, MRTlast, Hours, MRTINF(observed), Vss(observed), mL/kg		

Measurement of intact TK-112690 and uridine in blood and urine samples obtained during the Phase 1 study demonstrated that:

- The LC/MS/stable isotope dilution assay developed to measure both TK-112690 and uridine in plasma and urine appears adequate to measure relevant concentrations of both analytes in plasma and urine of humans treated with METREXASSIST™.
- Peak TK-112690 concentrations achieved at doses of 35 or 45 mg/kg were approximately 50 µg/mL
- The terminal elimination half-life of TK-112690 appears to be approximately 4 hours based on alambda_Z calculation and approximately 5 hours based on medium residence time (MRT) calculation.
- The volume of distribution appears to be approximately 600 mL/kg.
- Excellent proportionality of systemic exposure with dose was observed (plasma AUC increased from approximately 70 µg∗h/mL at a 5 mg/kg dose to approximately 40 µg∗h/mL at the 45 mg/kg dose).

- At doses ≥15 mg/kg, the increase in uridine at 6 hours post start of the infusion over pre-dose values appears to be approximately 50%.
- The mean amount of TK-112690 excreted into urine as intact drug was approximately 41% at the lowest dose evaluated, 5 mg/kg, and 17% at the highest dose evaluated, 45 mg/kg.

1.5.1.11 Overall Summary

The findings above suggest:

- No safety concerns are apparent.
- Variability in blood levels for TK-112690 is modest, ≤25%.
- The MRT for TK-112690 is consistent with bid dosing.
- The $AUC_{0\text{-}24h}$ for TK-112690 at a 25 mg/kg dose of TK-112690, approximately 200 µg* h/mL, is more than twice that needed to achieve efficacy in the MTX-induced weight loss model, 80 µg* h/mL, (**Figure 1,** bid dosing).

1.5.2 Phase 1b Study

1.5.2.1 Study Design

Patients were admitted to the study center the day prior to dosing (Day 0). Study drug was administered iv over 1 hour on the morning of Day 1 one hour after the patients drank a nutritional supplement. MTX was administered 45 mg/m^2 by iv infusion over one hour one hour after the TK-112690 treatment. A second TK-11690 dose was administered 6 hours after the first. Subjects remained confined for 48 hours. This sequence was repeated for three additional weeks. Patients returned for a follow-up visits 5 and 8 weeks (± 24 hours) post TK-112690 treatment. Blood samples were obtained for determination of uridine (Biomarker) prior to dosing (baseline, and 5 and 24 hours post TK-112690 treatment). Blood samples for Surrogate Marker measurements were obtained pre-dose of TK-112690, and 5, 24, 48 hours and 5 and 8 weeks post the TK-112690 dose. Dose cohorts were 15, 30, 45 and 60 mg/kg, bid. Each of the cohorts (cohorts 1-5) consisted of three patients. An additional 9 patients were dosed at 60 mg/kg bid, the MTD determined from dosing to cohorts 1-5.

No subject participated in more than one cohort. All patients histologically confirmed SCCHN. Safety was assessed by monitoring adverse events (AEs), conducting physical examinations and 12-lead electrocardiograms (ECGs), measuring vital signs (blood pressure, heart rate, respiratory rate, and oral body temperature) and conducting laboratory tests (clinical chemistry, hematology and coagulation) and physical examinations before dosing, at specified time post-dosing, and at weeks 5 and 8 (± 1 day) post dosing. Mucositis status was evaluated pre-dose, at 24 and 48 hours post dose, and at weeks 5 and 8 post dose.

1.5.2.2 Patient Characteristics, Dropouts, and Reason for Dropouts

A total of 25 patients at 5 centers signed the informed consent and were enrolled in the study. 06 females were enrolled and 19 males. ECOG scores ranged from 0 to 1 except for a score of 2 for patient 12. Sixteen patients completed the protocol. Eighteen patients completed 4 weeks of

dosing. Of the nine patients who did not complete the protocol two died. Following investigation, both deaths were attributed to causes other than dosing with TK-112690.[84,85]

Patient characteristics along with dropouts are listed in the following **Table**.

Table 6. ***Patient characteristics, Dropouts and Reason for Dropouts***

Patient	Gender	Dose (mg/kg)	Cohort	Age	BMI (kg/m^2)	Initial ECOG	Disposition
1	Male	15	1	37	16.40	1	Completed
2	Male	15	1	52	15.20	1	Completed
3	Male	15	1	52	20.10	1	Completed
4	Male	30	2	62	22.50	1	Completed
5	Male	30	2	31	23.20	1	Completed
6	Male	30	2	63	14.70	1	Completed
7	Male	45	3	55	15.60	0	Completed
8	Male	45	3	80	15.80	0	Completed
9	Female	45	3	83	19.00	0	Dead
10	Male	45	3	66	19.20	0	Weeks 1, 2, 3 and 4 completed, Patient did not report for week 5. Lost to Follow up
11	Male	60	4	45	15.97	1	Completed
12	Male	60	4	38	14.60	2	Week 1, 2 and 3 completed. Consent self-withdrawal.
13	Female	60	4	72	15.20	1	Completed
14	Female	60	5	49	25.60	1	Week 1 Completed. Withdrawal following SAE.
15	Female	60	5	47	20.70	0	Completed
16	Female	60	5	70	17.50	0	Dead
17	Female	60	5	60	16.20	0	Week 3 completed followed by self-withdrawal.
18	Male	60	5	52	25.10	1	Completed
19	Male	60	5	45	25.30	0	Completed
20	Male	60	5	32	17.14	0	Completed

[84] Government of India Directorate General of Health Services Central Drugs Standard Control Organization (Medicines Safety Monitoring Division), Order re CT/SAE-D-334/2017, Patient DDR/TK/004/009, 9 September 2018. `

[85] Government of India Directorate General of Health Services Central Drugs Standard Control Organization (Medicines Safety Monitoring Division), Order re CT/SAE-O-90/2018), Patient MNB/TK/005/016, 5 October 2018.

21	Male	60	5	56	15.82	0	Week 3 completed followed by self-withdrawal
22	Male	60	5	49	17.40	1	Completed
23	Male	60	5	67	28.20	0	Week 2 completed followed by withdrawal due to an SAE
24	Male	60	5	41	18.10	0	Week 5 completed followed by self-withdrawal
25	Male	60	5	50	19.30	1	Completed

No relationship among dose, age, BMI, or ECOG and disposition is apparent. Male patients completed the trial at a higher rate (14 of 19) than female patients (2 of 6).

1.5.2.3 Overall Safety

Administration of METREXASSIST™ plus a relatively high dose of methotrexate to the cancer patients resulted in a total of 35 treatment-emergent adverse events (TEAEs) were reported in 21 (84.0%) patients; the majority were mild to moderate in severity and resolved with or without treatment. However, all events were assessed to be not related to study drug but attributed to the disease under study and/or concomitant disease conditions. A total of 6 CTCAE Grade 3/4 were reported in 5 (20%) patients.

Treatment-emergent serious adverse events (TESAEs) were reported in 5 (20%) patients. All TESAEs were assessed to be not related to the study drug but attributed to the disease under study and/co-morbidities. A total of 4 (16.0%) patients discontinued the study due to AEs. All were cases of anemia (n=4). A total of 2 (8%) patients died due to AEs. Febrile neutropenia caused one death (n=1) and pancytopenia the other (n=1).

No actions were taken for safety reasons, and there were no protocol modifications based on safety concerns associated with the TK-112690 administration. No dose limiting toxicity (DLT) was reported in any of the patients. The observed AEs, SAEs, and deaths are described or listed in the sections below.

1.5.2.4 SAEs

SAEs in the study are provided in the following **Table**.

Table 7. Reported SAEs in Study.

Patient	SAE	TK-112690 (mg/kg)	Causality by PI	Outcome
9	Febrile neutropenia resulting in to death	45	Not related	Death
14	Stomatitis	60	Not related	Recovered
15	Upper respiratory tract infection	60	Not related	Recovered
16	Pancytopenia	60	Not related	Death
23	Febrile neutropenia converted into pancytopenia	60	Not related	Recovered

No SAEs occurred in 15 or 30 mg/kg, bid, dose groups. No increase in SAEs with increase in TK-112690 dose from 45 to 60 mg/kg, bid, is apparent. An SAE occurred in 25% (1/4) of the patients administered 45 mg/kg, bid, and 26% (4/15) of the patients dosed 60 mg/kg TK-112690, bid.

1.5.2.5 CTCAE Grade 3/4 AEs

Table 8. CTCAE Grade 3/4 Adverse events

A total of 5 CTCAE Grade 3/4 were reported in 5 (20%) patients. These events included Increase WBC count (n=1), Increase Absolute neutrophils count (ANC, n=1), febrile neutropenia (n=1), pancytopenia (n=1), febrile neutropenia converted into pancytopenia (n=1), and anemia (n=1). All events were assessed to be not related to METREXASSIST™ but attributed to the disease under study and/ or concomitant disease conditions.

Patient	AEs Grade 3/4 Severity	TK-112690 (mg/kg)	Causality Determined by PI	Outcome
3	Increased WBC	15	Unrelated to TK-112690	Recovered
3	Increase ANC	15	Unrelated to TK-112690	Recovered
9	Febrile neutropenia	45	Unrelated to TK-112690	Death
16	Pancytopenia	60	Unrelated to TK-112690	Death
23	Pancytopenia	60	Not related	Recovered
24	Anemia	60	Not related	Lost to follow-up

1.5.2.6 Clinical Chemistry

Blood samples were obtained from each patient at Screening, Day 024 hour (Day1), 48 hours (Day 2), at equivalent times on weeks 2, 3 and 4, and at weeks 5 and 8 post-dose. The blood was analyzed for electrolytes such as sodium, potassium, chloride, and calcium (total), enzymes such as aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, and biochemical such as creatinine, uric acid, phosphate, protein (total), albumin, globulin, bilirubin (total), bilirubin (direct), cholesterol, triglyceride, glucose, and C- reactive protein.

Selected, but typical, hematology findings from the study are provided in the **Figures** below.

Figure 2. Plasma glucose concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

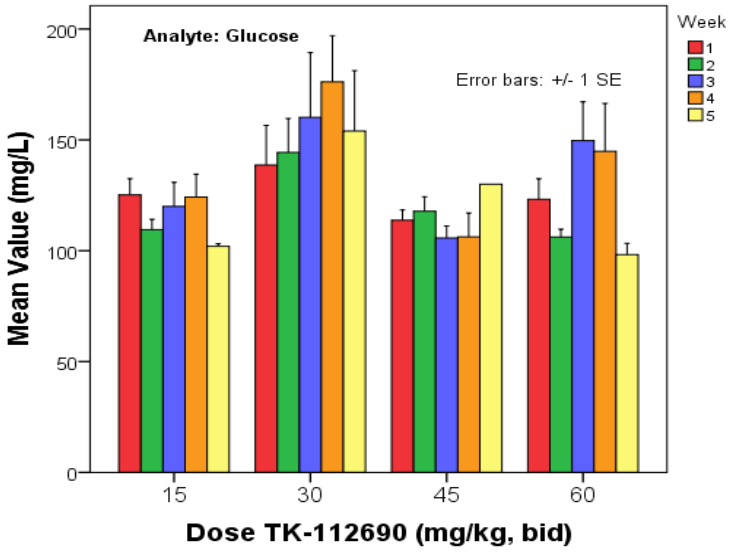


Figure 3. Plasma creatinine concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

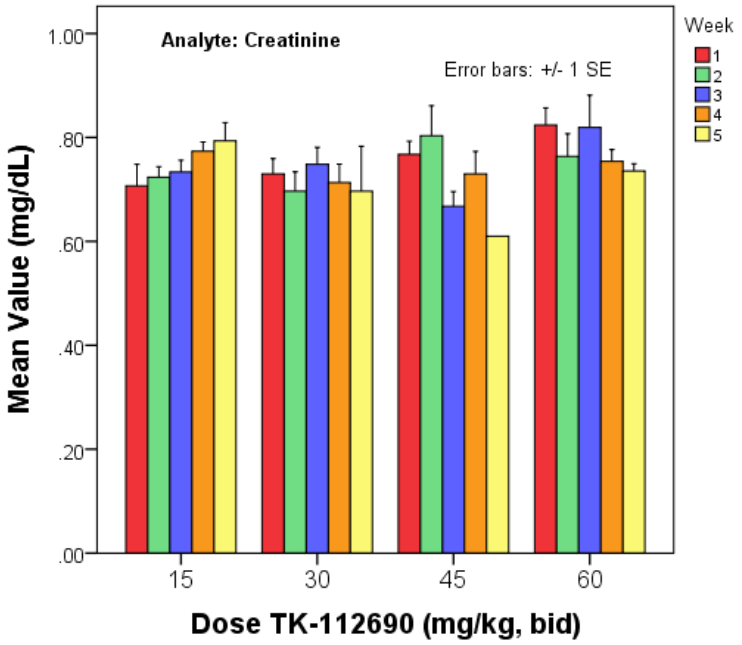


Figure 4. Plasma AP (alkaline phosphatase) concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

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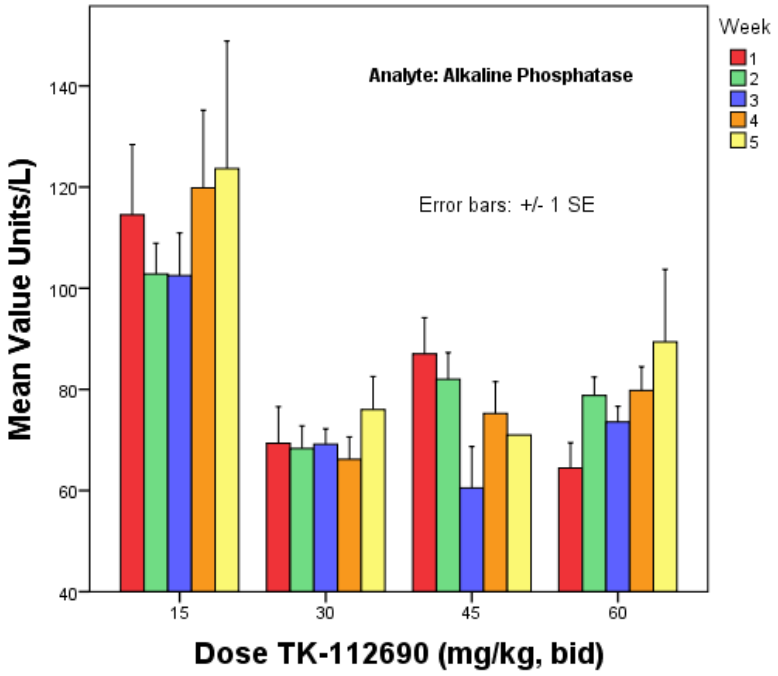


Figure 5. Plasma AST concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

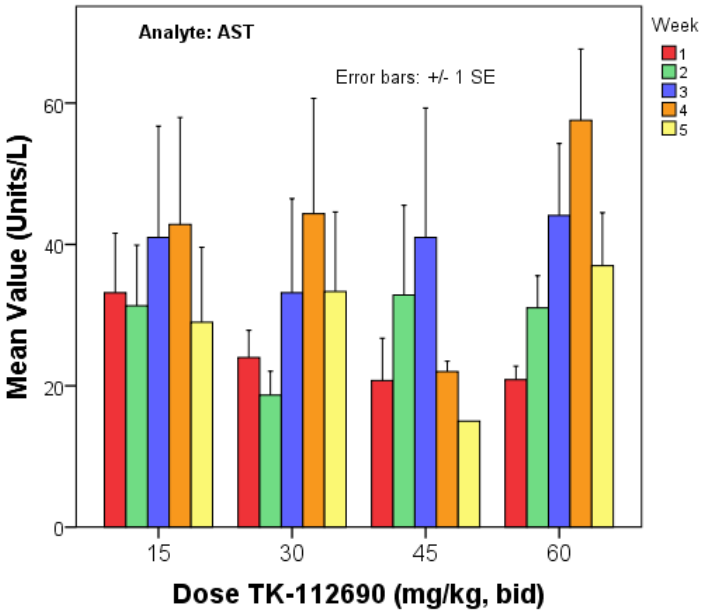
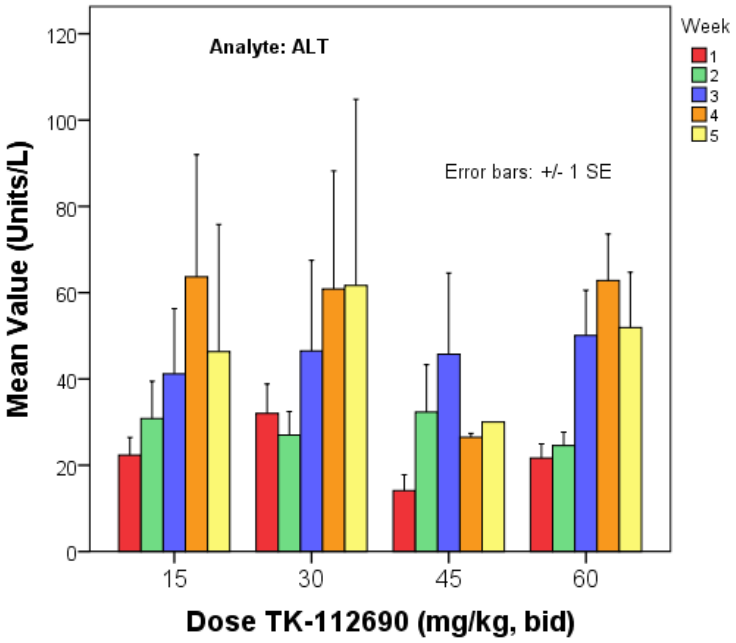


Figure 6. Plasma ALT concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined).Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.



Like the glucose measurements and creatinine measurements (**Figures 2** and **3**), the measurement of albumin, sodium, potassium, chloride, calcium, phosphorus, triglyceride,

creatine kinase, total protein, uric acid, BUN. and cholesterol showed no clinically significant changes across treatment groups. One liver-related measures, alkaline phosphatase (**Figures 4**, respectively), show a potentially protective effect of TK-112690 treatment. Other liver function measurements such as AST/ALT (**Figures 5** and **6**), bilirubin and direct bilirubin show much less change with TK-112690 treatment compared to pre-dose.

CRP levels, Positive or Negative determination only, provided no useful information's

.

1.5.2.7 Hematology

Blood samples were obtained from each patient at Screening, Day 0, 24 and 48 hours Week1, and at equivalent times on weeks 2, 3 and 4, and at weeks 5 and 8 post-dose. The blood was analyzed for hemoglobin, erythrocyte count, hematocrit, mean cell volume, MCH, MCHC, platelet count, leucocyte count, neutrophils, neutrophils (absolute), lymphocytes, lymphocytes (absolute), monocytes, monocytes (absolute), eosinophils, eosinophils (absolute), basophils and basophils (absolute).

Selected, but typical, hematology findings from the study are provided below.

Figure 7.WBC concentrations ± SE in subjects providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour values combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

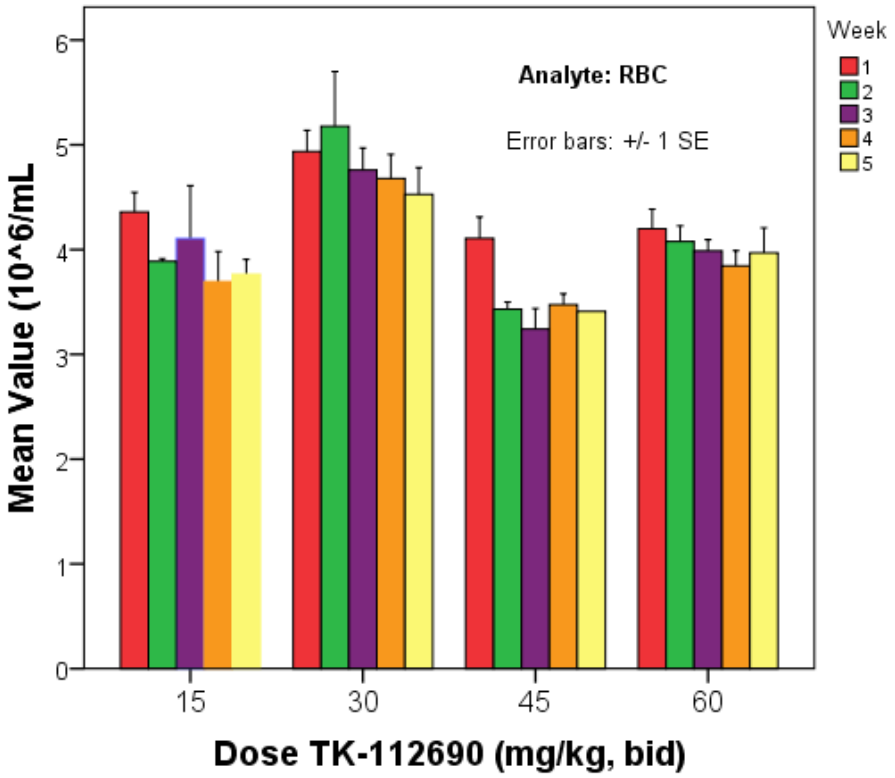


Figure 8.WBC concentrations for every subject providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour samples combined).Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

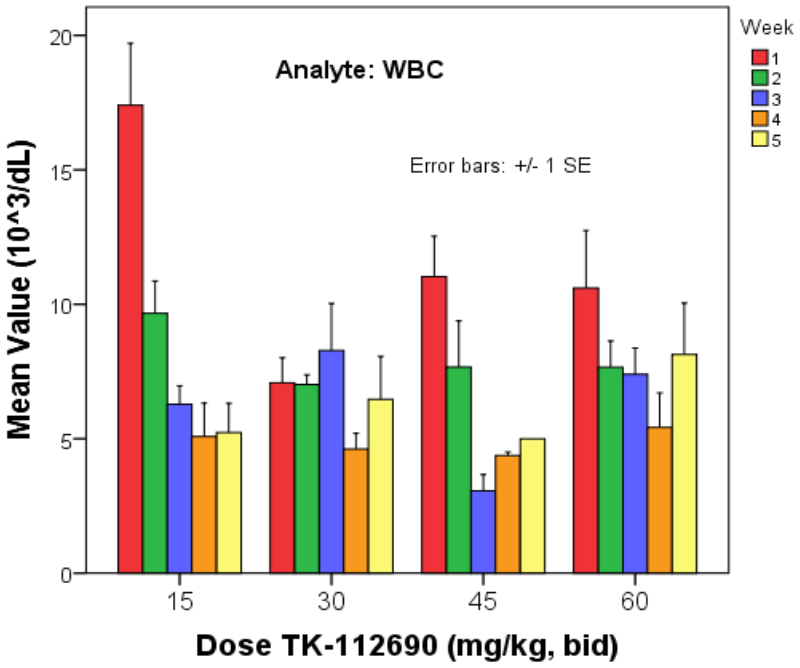


Figure 9.WBC concentrations for every subject providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour samples combined).Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

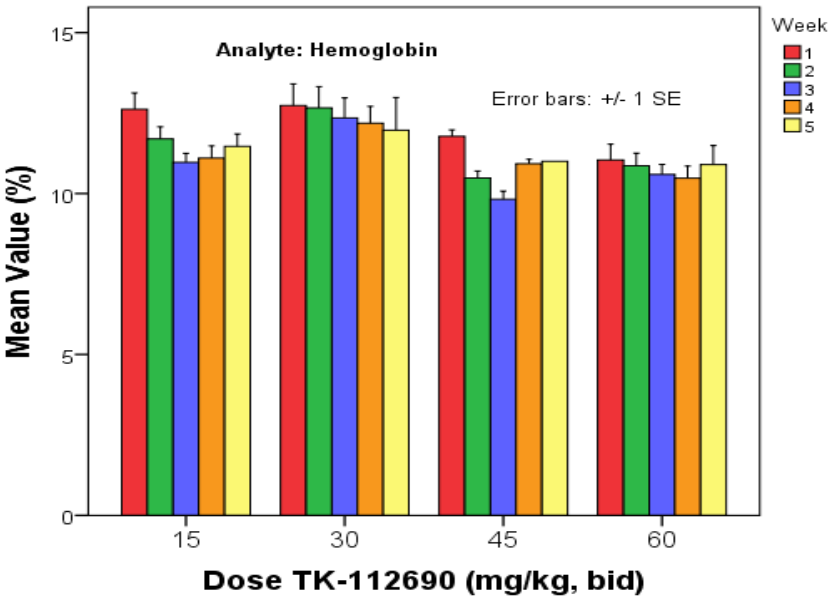


Figure 10. Lymphocyte concentrations for every subject providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour samples combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.

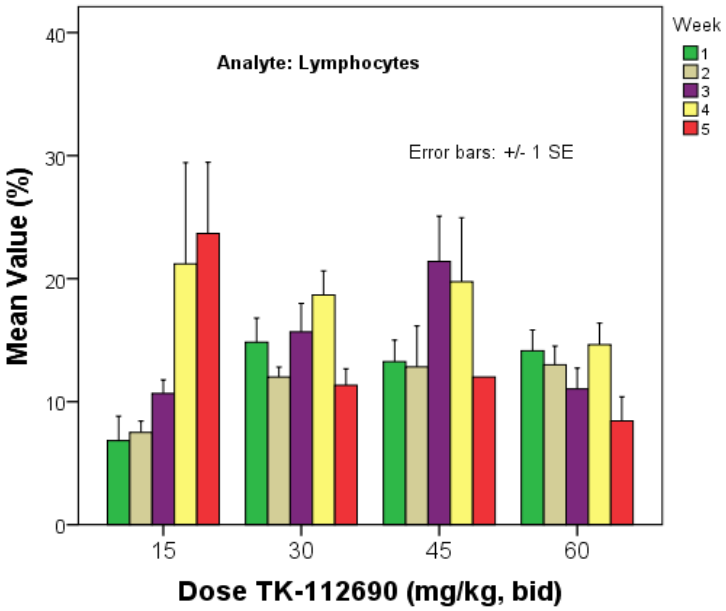
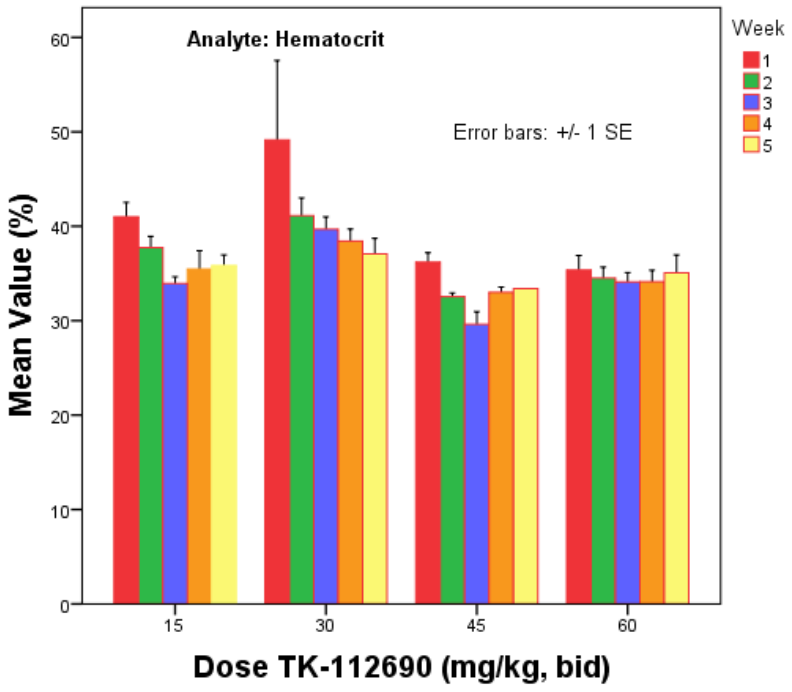


Figure 11. Hematocrits for every subject providing a blood sample pre-dose and at Weeks 1, 2, 3, and 4 (24-hour and 48-hour samples combined). Week1=Pre-dose, Week2=Study Week1, Week3=Study Week2, Week4=Study Week3, Week5= Study Week4.



Although Table 8 reports five, CTCAE Grade 3/4 hematology-related adverse events, a more general evaluation of hematology findings for all patients, do not suggest an overall concern.

Table 9. Other Safety Tests

Measure	Test	Results
Coagulation	Prothrombin (PT) tests performed at screening, Day0, 24 and 48 hours post-dosed on Weeks 1-4, and 5 and 8 Weeks post-dose.	Prothombrin (PT). No apparent effect of TK-112690 on PT. All mean values at pre-dose and at Week1, 2, 3, 4, 5 and 8 between 13 and 15 seconds. Partial Thromboplastin Time (PTT). No apparent effect of TK-112690 on PTT. All mean values at pre-dose and at Week1, 2, 3, 4, 5 and 8 between 29 and 40 seconds.
Urinalysis	Urine analyses tests performed at screening, Day0, 24 and 48 hours post-dosed on Weeks 1-4, and 5 and 8 Weeks post-dose..	pH No apparent effect of TK-112690 on urine pH. Mean values for dose cohorts ranged from 5.8 to 7.2. Positive/Negative Nitrite No apparent effect of TK-112690 on urinary nitrite. Vast majority of sample either negative, or, in some cases, no measurement. Positive finding for only two samples: One patient in 15 mg/kg, bid, dose group at 24 hours/Week 1, and one patient in 15 mg/kg, bid, dose group at 48 hours/Week 4. Occult Blood No apparent effect of TK-112690 on urinary occult blood. All samples either negative for occult blood, or, in some cases, no measurement. No positive findings for occult blood. Appearance (Clear/Hazy) Data too variable for any conclusion, but no apparent effect of TK-112690 on urine appearance. Color (Pale Yellow/Yellow) Data too variable for any conclusion, but no apparent effect of TK-112690 on urine color. Glucose (+++, ++, +, None) No apparent effect of TK-112690 on urine glucose. Some scattered findings. Single +++ finding in 30 mg/kg, bid, dose cohort at Week 3/48 hours. Single ++ findings in 30 mg/kg, bid, dose cohort pre-dose, 30 mg/kg, bid, dose group at Week 5, and 60 mg/kg, bid, dose cohort at Week 3/24 hours. Single + findings in 60 mg/kg, bid, dose cohort, at Week 1/24 hours, Week 3/ 48 hours, and Week 4/48 hours. All other findings none, or no measurements. Protein (+++, ++, +, Trace or None)

<table>
<tr><td></td><td></td><td>No apparent, consistent effect of TK-112690 on urinary protein. Some scattered findings. Single +++ findings in 60 mg/kg, bid, dose cohort at Week 4/48 hours and Week 5. Single ++ findings in 30 mg/kg, bid, dose cohort at Week 2/24 hours, Week 2/48 hours and Week 8, and 60 mg/kg, bid, dosed cohort at Week 8. Single + findings in 15 mg/kg, bid, dose cohort pre-dose, 30 mg/kg, bid, dose cohort at Week3/24 hours, Week 3/48 hours, and Week 5 (two findings), 45 mg/kg, bid, dose cohort at Week 5, and 60 mg/kg, bid, at Week 2/ 24 hours, Week 2/48 hours, Week 3/48 hours (two findings), Week 4/48 hours, and Week 5. All other findings none, trace or no measurements.</td></tr>
<tr><td>Vital Signs</td><td>Vital signs measurements were performed on each patient at Screening, Day 0, 12 hours (Day 1), 24 hour (Day1), 48 hours (Day 2), at equivalent times on weeks 2, 3 and 4, and at weeks 5 and 8 post-dose. Vital signs measured included supine blood pressure (systolic and diastolic), heart rate, respiratory rate and oral body temperature. were performed at screening, Day 0, pre-dose and 4, 6, 8, 12, 24, 48 hours and 5 and 8 Days post-dose.</td><td>No apparent effect of TK-112690 dosing on blood pressure, pulse, respiratory rate, body temperature, or BMI

Diastolic BP (mmHg)
<table>
<tr><th>Week</th><th>Dose</th><th>Mean</th><th>n</th><th>SD</th></tr>
<tr><td>Pre-dose</td><td>15</td><td>115</td><td>3</td><td>13</td></tr>
<tr><td></td><td>30</td><td>115</td><td>3</td><td>8</td></tr>
<tr><td></td><td>45</td><td>123</td><td>4</td><td>10</td></tr>
<tr><td></td><td>60</td><td>119</td><td>15</td><td>8</td></tr>
<tr><td>1</td><td>15</td><td>122</td><td>6</td><td>2</td></tr>
<tr><td></td><td>30</td><td>124</td><td>6</td><td>8</td></tr>
<tr><td></td><td>45</td><td>117</td><td>8</td><td>8</td></tr>
<tr><td></td><td>60</td><td>117</td><td>30</td><td>7</td></tr>
<tr><td>2</td><td>15</td><td>122</td><td>6</td><td>5</td></tr>
<tr><td></td><td>30</td><td>127</td><td>6</td><td>6</td></tr>
<tr><td></td><td>45</td><td>114</td><td>6</td><td>5</td></tr>
<tr><td></td><td>60</td><td>118</td><td>28</td><td>7</td></tr>
<tr><td>3</td><td>15</td><td>122</td><td>6</td><td>7</td></tr>
<tr><td></td><td>30</td><td>125</td><td>6</td><td>3</td></tr>
<tr><td></td><td>45</td><td>113</td><td>4</td><td>5</td></tr>
<tr><td></td><td>60</td><td>118</td><td>26</td><td>8</td></tr>
<tr><td>4</td><td>15</td><td>121</td><td>6</td><td>1</td></tr>
<tr><td></td><td>30</td><td>127</td><td>6</td><td>4</td></tr>
<tr><td></td><td>45</td><td>114</td><td>4</td><td>5</td></tr>
<tr><td></td><td>60</td><td>118</td><td>20</td><td>7</td></tr>
</table>
Diastolic BP (mmHg)
<table>
<tr><th>Week</th><th>Dose</th><th>Mean</th><th>n</th><th>SD</th></tr>
<tr><td>Pre-dose</td><td>15</td><td>75</td><td>3</td><td>14</td></tr>
<tr><td></td><td>30</td><td>79</td><td>3</td><td>3</td></tr>
<tr><td></td><td>45</td><td>78</td><td>4</td><td>5</td></tr>
<tr><td></td><td>60</td><td>80</td><td>15</td><td>5</td></tr>
<tr><td>1</td><td>15</td><td>78</td><td>6</td><td>4</td></tr>
<tr><td></td><td>30</td><td>79</td><td>6</td><td>5</td></tr>
</table>
</td></tr>
</table>

			45	75	8	4
			60	78	30	4
		2	15	77	6	4
			30	79	6	3
			45	74	6	5
			60	76	28	7
		3	15	80	6	6
			30	82	6	2
			45	73	4	5
			60	78	26	5
		4	15	79	6	3
			30	81	6	2
			45	73	4	5
			60	76	20	4

Respiratory Rate (Per-minute)

Week	Dose	Mean	n	SD
Pre-dose	15	17	3	1
	30	17	3	1
	45	17	4	1
	60	17	15	2
1	15	18	6	2
	30	15	6	3
	45	17	8	1
	60	17	30	2
	Total	17	50	2
2	15	18	6	1
	30	17	6	1
	45	17	6	1
	60	17	28	2
3	15	18	6	2
	30	17	6	2
	45	17	4	1
	60	17	26	2
4	15	17	6	1
	30	18	6	1
	45	17	4	1
	60	18	20	2

Heart Rate (Per Minute)

Week	Dose	Mean	n	SD
Pre-dose	15	76	3	2
	30	87	3	10
	45	80	4	2
	60	80	15	4

		1	15	80	6	7
			30	85	6	10
			45	79	8	2
			60	80	30	5
		2	15	81	6	7
			30	85	6	5
			45	78	6	2
			60	79	28	5
		3	15	80	6	6
			30	79	6	3
			45	78	4	2
			60	80	26	4
		4	15	77	6	6
			30	78	6	2
			45	80	4	2
			60	79	20	4
		BMI (kg/m^2)				
		Week	Dose	Mean	n	SD
		0	15	18	3	2
			30	20	3	5
			45	17	4	2
			60	19	15	4
		1	15	18	3	2
			30	20	3	5
			45	17	4	2
			60	19	15	5
		2	15	18	3	2
			30	20	3	5
			45	18	3	2
			60	19	14	4
		3	15	18	3	1
			30	20	3	5
			45	17	2	2
			60	18	13	3
		4	15	18	3	2
			30	20	3	5
			45	17	2	2
			60	19	10	4
		Body Temperature (°C)				
		Week	Dose	Mean	n	SD
		0	15	115	3	13
			30	115	3	8
			45	123	4	10
			60	119	15	8

		1	15	98	6	0
			30	98	6	0
			45	98	8	0
			60	98	30	1
		2	15	98	6	0
			30	98	6	0
			45	98	6	0
			60	98	28	1
		3	15	98	6	0
			30	98	6	0
			45	98	4	0
			60	98	26	1
		4	15	98	6	0
			30	98	6	0
			45	98	4	0
			60	98	20	1
Physical Examination	A physical examination was performed on each patient at Screening, Day 0, 12 hours (Day 1), 24 hours (Day1), 48 hours (Day 2), at equivalent times on weeks 2, 3 and 4, and at weeks 5 and 8 post-dose. The physical examinations included assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, and extremities.	Overall no apparent effect of TK-112690 dosing on findings from the Physical Examination. Physical exam results were scored as either change (C) or no change (NC). NC was recorded for all eye, head/neck/thyroid, blood/lymphatics, immune system, nervous/psychiatric, general and site of administration, respiratory, cardiovascular, GI/Hepatobiliary, musculoskeletal, infections/infestations, renal, and reproductive. C was noted: ● In skin of Patient 7 at Week1/48 hours. ● In ears/nose/throat of Patient 1 at Week 3/24 hours, Week 4/24 hours, Week 4/48 hours, and Week 8. ● In ears/nose/throat of Patient 14 at Week1/24 hours, and Week 1/48 hours. ● In ears/nose/throat of Patient 19 at Week 5 and Week 8. ● In ears/nose/throat of Patient 19 at Week 8. ● In endocrine/metabolism/nutrition at Week 5, and Week 8.				
EKG	A 12-Lead ECG was performed on each patient at Screening and at 24 hours on Day1 as well as at equivalent times on Weeks 2, 3 and 4.	No apparent effect of TK-112690 dosing on ECG. All ECGs normal except for Patient 1 (15 mg/kg TK-112690, bid) at Weeks 1, 2, 3 and 4, and Patient 3 (15 mg/kg TK-112690, bid) at Weeks 1 and 4.				
Disease Progression	CT scan for size of target lesion at screening and Week 8.	Complete data available only for Patients 1, 2, 3, 5, 6, 8, 11, 19, 20, and 22. Given the limited data no analysis by RECIST criteria was possible. However, for the sum of				

		longest tumor diameter measure, 7 lesions decreased and 3 increased, although the changes were not large.

1.5.2.11 TK-112690 Pharmacokinetics (PK)

Blood samples for PK were obtained on each patient at Pre-dose (Day 1), 5 hours (Day 1), and 24 hour (Day1) post-dose, and TK-112690 measured by LC/MSMS in the harvested plasma. Reports on the PK[86] and LC/MS/MS assay[87] are available. Except for a few scattered measurements, TK-112690 concentrations in pre-dose samples were nm. The two following **Figures** provide the mean TK-112690 concentration data observed with dose and dosing interval.

Figure 12. Bar chart showing mean concentration of TK-112690, pg/mL ± SE, for individual dosing intervals at 5 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients who completed 4 doses.Interval 1=Study Week 1, Interval 2=Study Week 2, Interval 3=Study Week 3, and Interval 4=Study Week 4.

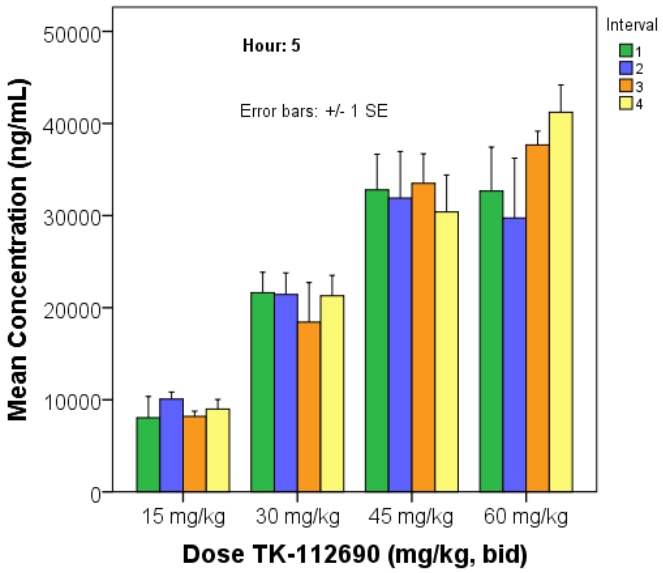
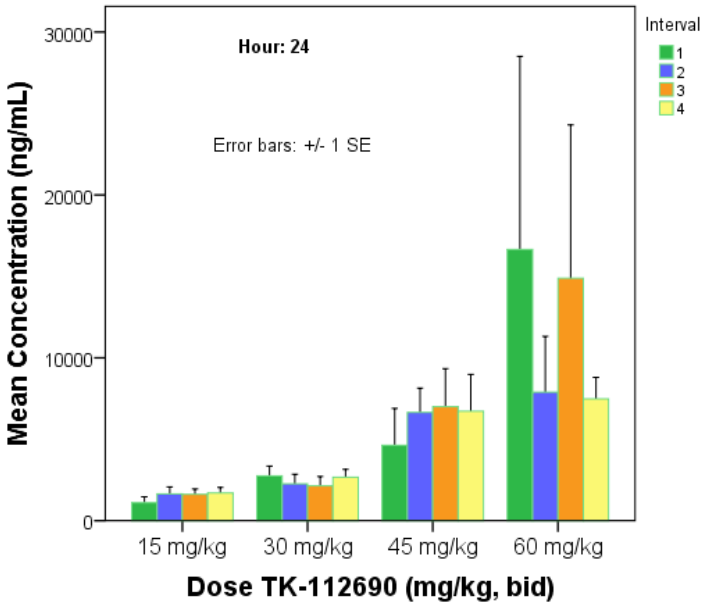


Figure 13. Bar chart showing mean concentration of TK-112690, pg/mL ± SE, for individual dosing intervals at 24 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690.Analysis includesdata from all patients who completed 4 doses.Interval 1=Study Week 1, Interval 2=Study Week 2, Interval 3=Study Week 3, and Interval 4=Study Week 4.

[86] Tosk FSR-CL-2690 0015: PK/PD of TK-11290 Administrated to Cancer Patients in Clinical Study CLP-2690-0002.
[87] Tosk FSR-CL-2690-0014: Method Validation for "Determination of TK-112690 in Human Plasma by LC/MS/MS"



The TK-112690 blood level data suggests:

- Plasma concentrations increase with increasing dose.
- The increase in concentration observed with the 60 mg/kg dose is somewhat less than expected.
- Comparing the 5-hour and 24-hour data, the terminal elimination half-life appears to be somewhat longer than the approximately 4 hours observed in the Phase 1a study. The estimated terminal elimination half-life in this study is approximately 6 hours.

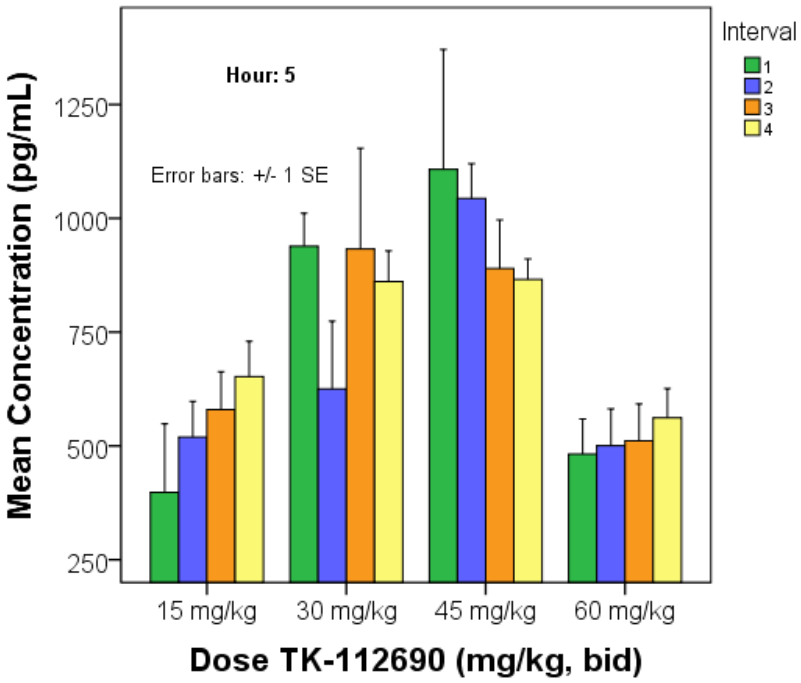
1.5.2.12 Biomarker (Uridine)

Uridine was also measured in the same samples used for PK measurement using a pilot LC/MS/MS assay.[88] The uridine data can be found in the same report providing the TK-112690 concentration data.[86]

Uridine data from the study are provided in the **Figure** below.

Figure 14. Bar chart showing mean concentration of uridine, pg/mL ± SE, for individual dosing intervals at 5 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690.Analysis includes data from all patients who completed 4 doses. Interval 1=Study Week 1, Interval 2=Study Week 2, Interval 3=Study Week 3, and Interval 4=Study Week 4.

[88] Tosk ATM-2690-0002: An LC/MS/MS Test Method for Uridine in Human Plasma Post Administration of TK-112690.



Compared to the 15 mg/kg dose,[89] the 30 and 45 mg/kg, bid, doses of TK-112690 provided the expected increase in uridine (TK-112690 is an inhibitor of an enzyme responsible for the clearance of uridine, uridine phosphorylase. The reason for the less than expected increase in uridine with the 60 mg/kg, bid, dose of TK-112690 is unknown.

1.5.2.13 Surrogate Markers (CD40, CD40L, TNF-α, FGF-9)

Blood samples were obtained from each patient for surrogate marker determination at, pre-dose (Day 1), and 5-hours (Day 1), 24-hour (Day1), 48-hours (Day 2) post dosing with TK-112690 as well as at equivalent times on weeks 2, 3 and 4. Complete data for the Surrogate Markers are available in a Tosk report.[90]

No specific trend emerged as no dose related changes has been observed on scheduled time points in these biomarkers. This could be due to the high variability in the marker levels and few subjects per group. FGF9 measurements were almost all non- measurable.

Virtually all FGF9 concentrations were found non-measurable.

[89]Pre-dose uridine measurements in the study were found very variable and unreliable likely because of a mistake in the protocol positioning the pre-dose sample one hour before the TK-112690 dose which was after treatment with the nutraceutical containing uridine monophosphate. The nutraceutical treatment would be expected to initially disturb the many components of the "uridine" biochemical system. This disturbance would not be expected to measurements taken at 5 and 24 hours post the TK-112690.

[90] Tosk FSR-CL-2690-0016: Plasma Concentrations CD40, CD40L, FGF9, and TNF-(Post TK-11290 Administration to Cancer Patients in Clinical Study CLP-2690-0002.

1.5.2.14 Mucositis Status

Patients were evaluated for mucositis status on Day 0, pre-dose (Day 1), 24 hours (Day1), 48 hours (Day 2), at equivalent times on 2, 3, and 4 weeks post-dose. The mucositis evaluation included four standard scales: CTCAE/mucositis, WHO/Oral Mucositis, PROMS, and OMAS/Sonis.

Data for the scales are provided below. In general, the mucositis experience experienced by the patients was low, and many low values were recorded by the Scales. Much higher values would be expected in patients dosed with methotrexate but with no TK-112690.

Figure 15. Bar chart showing mean score NCI mucositis scale for individual dosing intervals at 24 and 48 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients evaluated.

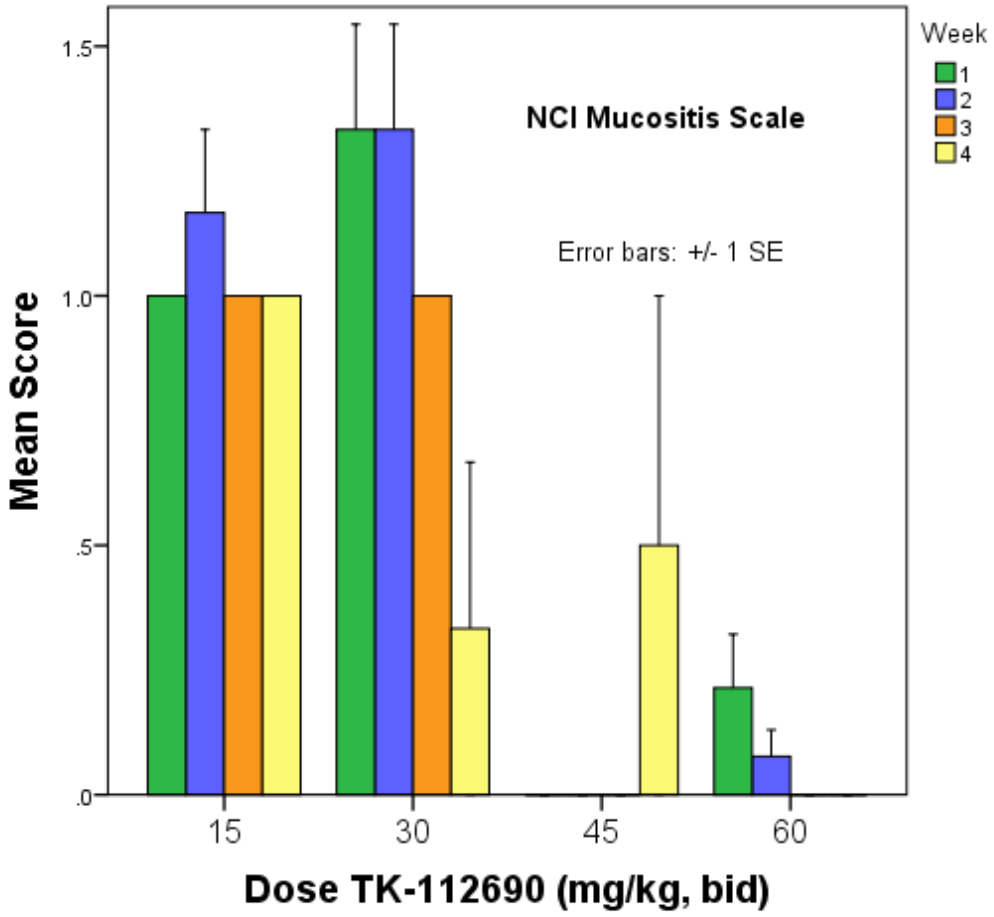


Figure 16. Bar chart showing mean score WHO oral mucositis scale for individual dosing intervals at 24 and 48 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients evaluated.

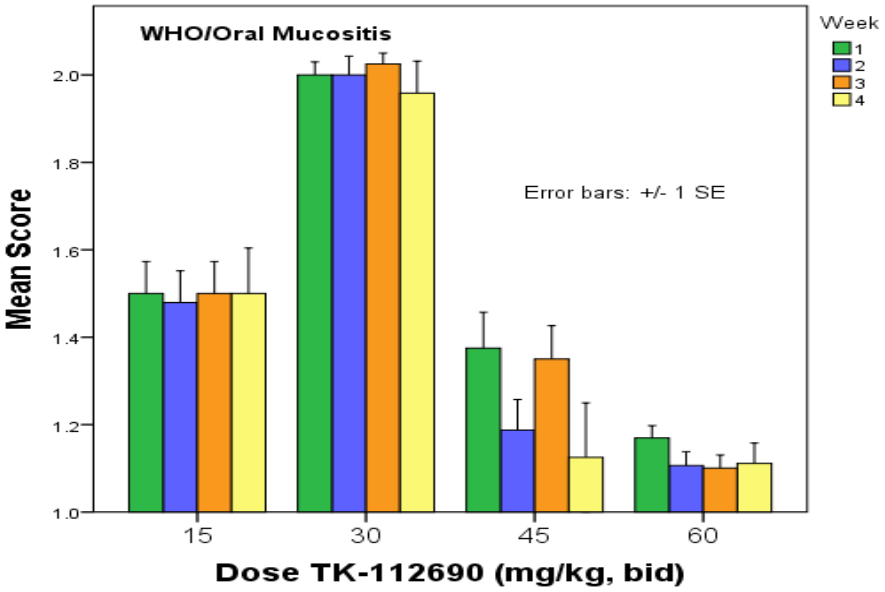


Figure 17. Bar chart showing mean score PROMS scale for individual dosing intervals at 24 and 48 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients evaluated.

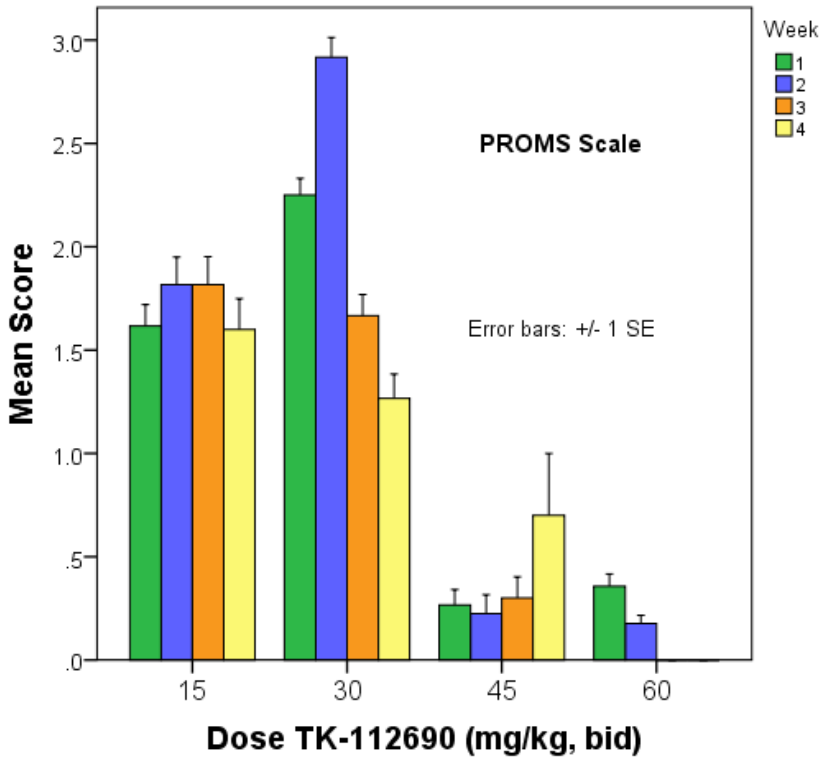


Figure 18. Bar chart showing mean OMAS mouth pain score for individual dosing intervals at 24 and 48 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients evaluated.

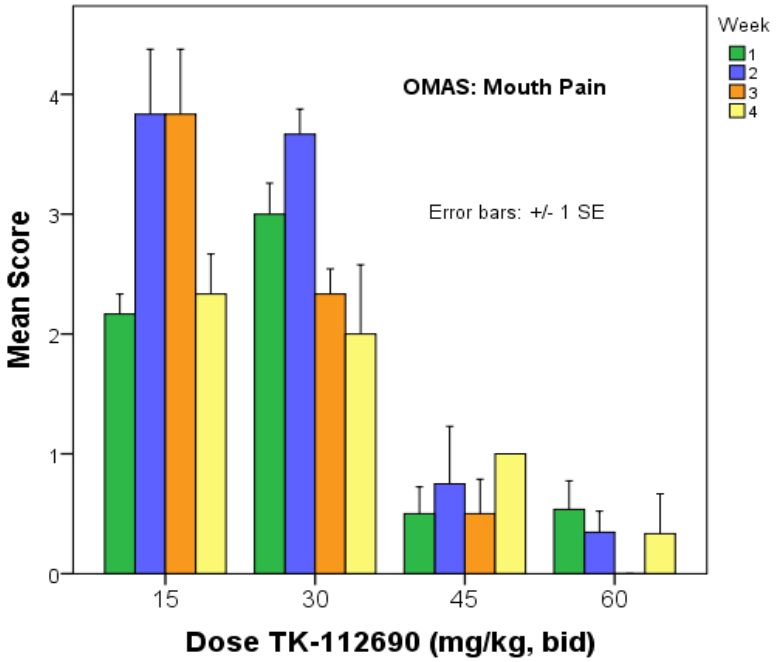
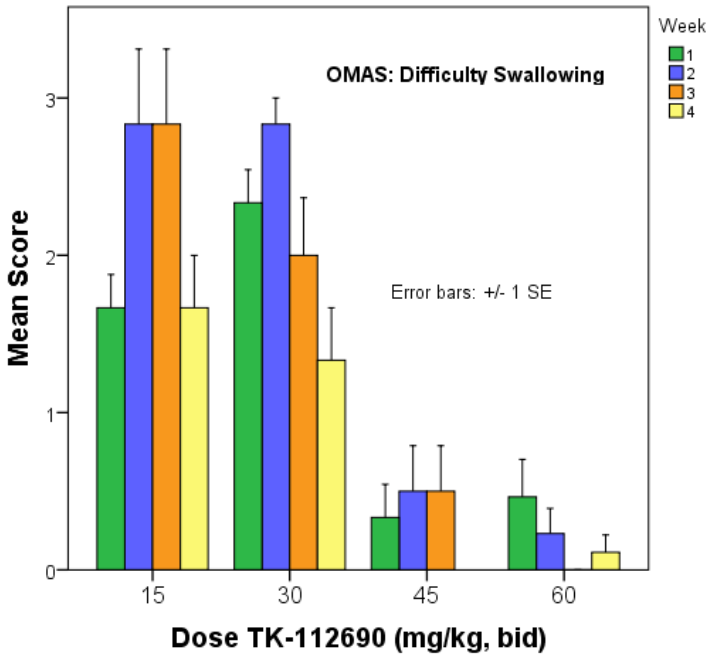


Figure 19. Bar chart showing mean OMAS difficulty swallowing score for individual dosing intervals at 24 and 48 hours post 15, 30, 45 or 60 mg/kg dose of TK-112690. Analysis includes data from all patients evaluated.



The scores in the mucositis scale tests suggest that TK-11290 at doses of 45 and 60 mg/kg, bid, mitigated mucositis produced by a relatively high methotrexate dose of 45 mg/m^2.

1.5.2.15 Overall Summary

The findings above from the Phase 1b study suggest:

- Although numerous adverse events, some serious including two deaths, occurred during the study, none appear associated with the TK-112690 treatment. Instead, the adverse events appear associated with the methotrexate treatment or the patient's illness. None of the adverse events appears related to the dose of TK-112690 employed. All the PIs in the study agree that none of the adverse events can be attributed to the TK-11290 treatment.
- The PK of TK-112690 in patients appears like that observed in the Phase 1a study in volunteers, except the terminal elimination half-life in patients appears slightly longer. Plasma concentrations of TK-112690 increased with increasing dose of drug, although the increase at 60 mg/kg, bid, was somewhat less than expected.
- The increase in plasma uridine compared to the lowest dose administered expected by the TK-122690 treatment was achieved with the 30 mg/k, bid, and 45 mg/kg, bid, doses but not the 60 mg/kg, bid, dose..
- The suppression of CD40 levels, a measure of immune activation, with TK-112690 doses > 15 mg/kg, bid, supports the notion that TK-112690 acts to diminish the immune response which Sponsorbelieves underlies chemotherapy-induced mucositis
- The finding that TK-112690 treatment at doses > 15 mg/kg, bid, appears to decrease two liver damage markers, GGT and alkaline phosphatase needs further clinical verification and study in animals.
- The protective effect of TK-112690 on mucositis post methotrexate observed in animal studies was confirmed in the Phase 1b study. but this confirmation needs further verification in a placebo controlled clinical trial.

1.6. PURPOSE OF STUDY

METREXASSIST™, TK-112690 for parenteral use, is a novel, non-toxic and specific therapy to treat mucositis, a major side-effect of MTX. This study is designed primarily to establish, in a placebo-controlled trial, pilot efficacy compared to placebo and secondarily to confirm the safety and tolerability of TK-112690 when administered weekly for 4 weeks along with MTX as an intravenous infusion in subjects with local, advanced, recurrent, metastatic SCCHN.

1.7. RATIONALE FOR DOSAGE

1.7.1. TK-112690

The dose of TK-112690 to be tested in patients in this study will be 45 mg/kg, bid. This dose was found tolerable and likely efficacious in the Phase 1b study in patients with SCCHN. The drug will be given iv over one hour.

1.7.2. Placebo

Placebo would be an identical a volume of isotonic saline in 10 mL vials as used for the TK-112690 dose.

1.7.3. MTX

The dose of MTX will be 45 mg/m^2. This dose was used in the Phase 1b study in patients with SCCHN.

1.7.4 Nutritional Supplement

TK-112690 is a uridine phosphorylase (UPase) inhibitor. Inhibition of UPase leads to increased uridine which is hypothesized to be the active protecting agent. The nutritional supplement, Fortasyn Connect™ (Nutricia NV), contains uridine monophosphate and is being dosed to ensure that all the cancer patients have enough uridine in the body for TK-112690 to have the desired effect. In multiple preclinical studies at Tosk, uridine alone, without TK-112690, had no protective effect on MTX-induced mucositis.

1.8. CONDUCT OF TRIAL

This clinical trial will be conducted according to the current revision which has its origin in the Declaration of Helsinki (Revised 59th WMA General Assembly, Seoul, October 2008 -Appendix 1). The study will be conducted in compliance with this protocol, CFR parts 50, 56 and 3112, Good Clinical Practice (CPMP/ICH/135/95), Schedule **Y** of the Indian Drugs and Cosmetics Act, and designated standard operating procedures.

2.0. STUDY OBJECTIVES

2.1. PRIMARY OBJECTIVE

The primary objective of this study is to establish pilot efficacy compared to placebo for TK-112690 as an anti-mucositis agent when administered weekly for 4 weeks by iv infusion to subjects with locally advanced, residual or recurrent or metastatic SCCHN scheduled to receive MTX as chemotherapy.

2.2. SECONDARY OBJECTIVES

The secondary objective of this study is to confirm the safety and tolerability of a continuous iv infusion of TK-112690 demonstrated in the Phase 1b study in patients suffering from local, advanced, residual recurrent, metastatic SCCHN receiving sequential continuous iv infusions of TK-112690 and MTX.

3.0. STUDY DESIGN AND TREATMENT

3.1 STUDY OUTCOMES/ENDPOINTS

Key study outcomes/endpoints are listed below:

- Efficacy. Mucositis scores in 4 standard mucositis scales.
- PD: Evaluation potential relationships among TK-112690 dose, mucositis scores, and surrogate markers of mucositis, e.g., CD40/CD40Land TNF-α.

3.1.2. DOSE MODIFICATIONS

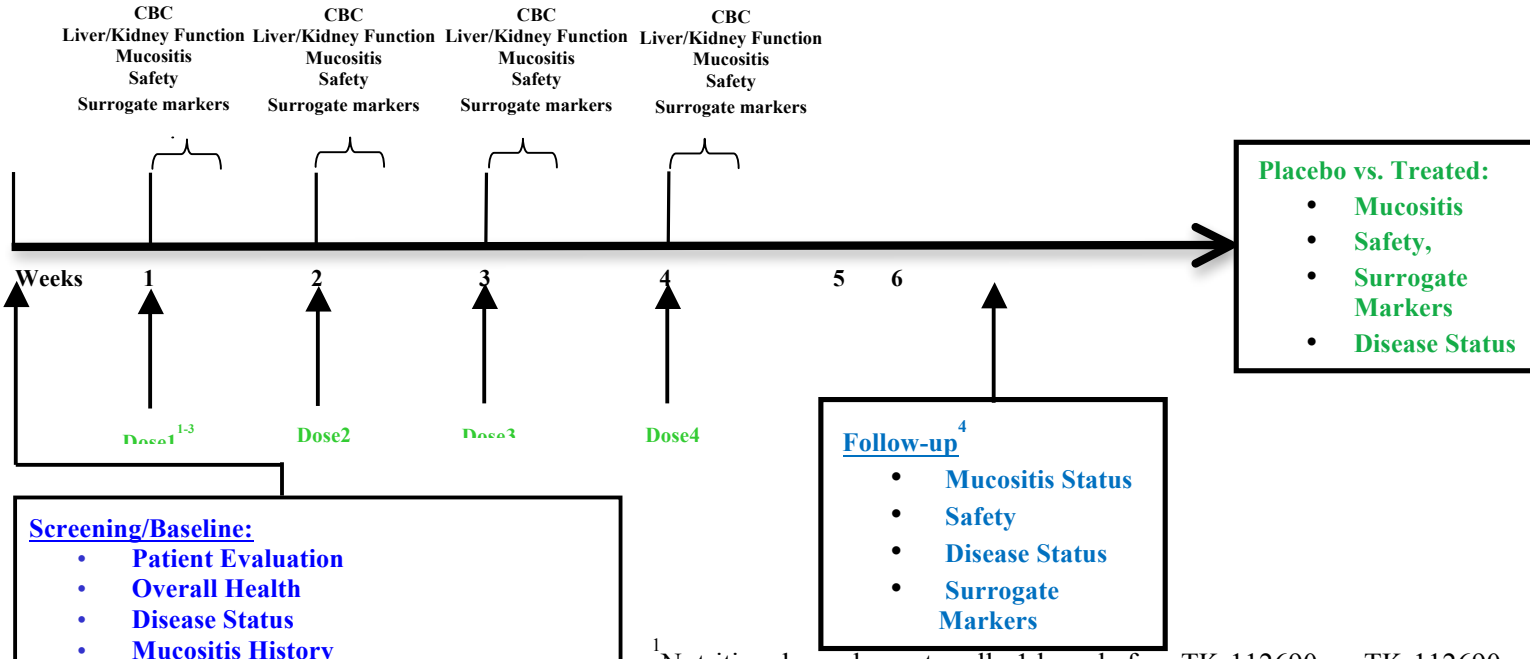
Treatment will be withheld following grade 3 or 4 adverse events. Treatment may be restarted when the adverse events have recovered to ≤ grade 2. Subjects in whom treatment is withheld for > 2 weeks will have treatment discontinued permanently.

3.2. OVERVIEW OF STUDY DESIGN AND DOSING REGIMEN

This is a multi-center, randomized, partially blinded, placebo controlled, Phase 2a, pilot efficacy/safety study of METREXASSIST™ (parenteral TK-112690) administered weekly for 4 weeks in combination with MTX in subjects with SCCHN.

Figure 20 below provide, respectively, an overview of the study design.

Figure 20. Overview of Study Design and Dosing Regimen.



[1]Nutritional supplement orally 1 hour before TK-112690, or TK-112690 placebo.

[2]TK-112690 (45 mg/kg) or TK-112690 placebo 1 hour before, and 5 hours after, MTX (45 mg/m^2).

[3]Patient can continue treatment after fourth dose with agreement of the PI.

3.3 CONCOMITANT MEDICATION AND TREATMENT

Subjects should continue at study initiation with their concomitant medications, as directed by their physicians. Any medication or therapy received by the patient within 4 weeks of baseline, during the study, including: over-the-counter, herbal preparations, supplements, prescription and non-prescription drugs, vaccinations or discontinued medications must be reported on the appropriate concomitant

medication page(s) of the case report form (CRF). The information on the concomitant medication/therapy CRFs will include the name of the medication/therapy, dose, frequency, route of administration, dates of use (start and finish date), and indication for use. Subjects should be instructed not to take any medications including over-the-counter products, without first consulting with the PI. Any adverse event that results from taking a concomitant medication/therapy should be recorded on the CRF adverse event page. Additionally, any diagnostic, therapeutic or surgical procedure performed during the study period, should be recorded including date, indication, description of the procedure and any clinical findings.

3.3.1. Palliative and Supportive Care

Palliative and supportive care for disease-related symptoms will be offered as needed to all subjects in this study per the caring physician's judgment. These may be anti-emetics, anti-diarrhea agents, anti-abdominal cramping agents and blood products and blood transfusions as appropriate. Stomatitis may be treated at the PI's discretion

3.4. RESTRICTIONS

3.4.1. Prior Therapy

Participation in another clinical trial within 4 weeks of dosing is not allowed.

3.4.2. Fluid and Food Intake

There are no protocol-mandated restrictions with respect to ingested fluids and food.

3.4.3. Subject Activities and Restrictions

There are no specific restrictions in physical activities.

3.5. RANDOMIZATION AND BLINDING

The Phase 2a study will be partially blinded. The patient and investigator will be blinded as to whether TK-112690 or placebo is administered. The CRO, sponsor, and site pharmacist will know whether the patient was administered active drug or placebo.

A list providing randomization, active drug and placebo, for each of the 22 subjects will be prepared (List A). Another list will provide randomization for 8 additional subjects if additional subjects are enrolled (List B). Both lists will be kept by the CRO.

A designated person or Pharmacist will prepare the TK-112690 treatments by adding either active drug or placebo into 250 ml 0.9% sodium chloride iv bottle (**Appendix 3**). Whether active drug or placebo is added will reflect the subject number and the randomization suggestion made in the Randomization List for that subject number. The CRO will provide the designated person or pharmacist with vials re-labelled to maintain the partial blind. The randomization will be done by the CRO or a third-party vendor.

3.6. NUMBER OF SUBJECTS IN THE STUDY

A total of 22 subjects will be recruited over a period of 10 months.

3.7. CENTERS

This is a multi-center study conducted within India across various geographical regions in India. At least 3 centers are planned.

4.0. STUDY POPULATION

4.1. TARGET POPULATION

Subjects considered for enrollment will be selected from subjects presenting with a confirmed diagnosis of local, advanced, recurrent, metastatic SCCHN for which MTX at a dose of 45 mg/m^2 is the scheduled therapy.

4.2. INCLUSION CRITERIA

- Male and female subjects over 18 years old with a histologically or cytologically confirmed diagnosis of locally residual, recurrent or metastatic SCCHN.
- Subjects must have a histologically confirmed diagnosis SCCHN (any stage).
- Subject must have failed at least onecourses of non-MTX chemotherapy or one coursenon-MTX chemotherapy and chemo radiation for treating their SCCHN.
- No prior systemic treatments for cancer (chemotherapy and/or radiotherapy) within 4 weeks of dosing.
- Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2.
- Must have a life expectancy of at least 6 months.
- History of brain metastases allowed if disease has stabilized or improved after radiation and/or craniotomy.
- No active angina or uncontrolled arrhythmia.
- No other concurrent, active, invasive malignancies.
- No detectable infection including hepatitis B/C and HIV.
- No pregnant or nursing females. Women of childbearing potential must have a negative serum ß-hCG pregnancy test at screening and must use medically acceptable methods of birth control. Acceptable methods of birth control include oral or transdermal contraceptives, condoms, spermicidal foam, IUD, progestin implant or injection, abstinence, vaginal ring, or sterilization of partner. The reason for non-childbearing potential, such as bilateral tubal ligation, bilateral oophorectomy, hysterectomy, or post-menopausal for ≥ 1 year, must be specified on patient's source documents (medical history file) and CRF.
- Must have adequate organ and immune function as indicated by the following laboratory values:

Parameter	***Laboratory Values***
Serum creatinine	≤1.5 x ULN

Estimated creatinine clearance	≥45 mL/min
Total bilirubin	≤2.0 mg/dL (≤34.2 µmol/L)
AST (SGOT) & ALT (SGPT)	≤3 x ULN
Absolute granulocyte count	≥1.5 x 10^9 cells/L
Platelets	≥ 100,000/µL

- Be able to read or understand, and provide a signature/thumb impression on the Informed Consent Form (ICF) before entering into the study.

4.3. EXCLUSION CRITERIA

- Subject has not failed at least one courses of non-MTX chemotherapy or one course of non-MTX chemotherapy and chemoradiation for treating their SCCHN.
- Uncontrolled active infection.
- Current mucositis (> Grade 1).
- Pregnancy or nursing mother.
- Prior history of a cerebrovascular accident or hemorrhage.
- Congestive heart failure as defined by New York Heart Association class III or IV.
- Uncontrolled hypertension.
- Active psychiatric or mental illness making informed consent or careful clinical follow-up unlikely.
- Subjects who have previously been enrolled into this study and subsequently withdrew.
- Subject receiving other investigational agent(s).
- Any systemic immunosuppressive medication/therapy (eg, chemotherapy, steroids).
- Any significant systemic illness, unstable or severe medical condition(s) that could put the subject at risk during the study, interfere with outcome measures or affect compliance with the protocol procedures such as autoimmune disease, ie, any condition that compromises the immune system and/or intercurrent infection
- Known or suspected intolerance or hypersensitivity to the study materials [TK-112690 and/or excipients or closely related compounds].
- Subjects who have received radiation or chemotherapy within 4 weeks of projected enrollment.
- Subjects that have a history of poor compliance in clinical research studies.
- Subjects who have participated in any other investigative clinical trial in the past 4 weeks.

4.4. WAIVERS

No waivers can be granted for inclusion in the study.

5.0. STUDY TREATMENT

5.1. DESCRIPTION OF TREATMENT

5.1.1. Study Drug

METREXASSIST™ (TK-112690 for Injection) is a clear, sterile, neutral, aqueous solution containing 100 mg/mL TK-112690. TK-112690 is a water soluble anhydrouridine that inhibits uridine phosphorylase. METREXASSIST™ is an aqueous 5 mL solution, pH 7.4 in 50 mM sodium phosphate,

packaged in a 10 mL flint glass vial with an elastomeric stopper held in place by an aluminum seal and flip-off cap. METREXASSIST™ is supplied for administration by continuous iv infusion over one hour in a final of volume of 250 mL Saline for Injection (USP). METREXASSIST™ is to be re-suspended for infusion in Saline for Injection (USP). The vials must be stored frozen (-15 to -25°C).. Once opened, solution for infusion containing must be used within 8 hours of preparation.

5.1.2. Study Drug Placebo

10 mL glass vials of isotonic saline equipped with an elastomeric stopper held in place by an aluminum seal and flip-off cap.

5.1.3. MTX

MTX drug product traditionally used at the clinical site,

5.1.4. Nutritional Supplement

The nutritional supplement containing uridine monophosphate (Fortasyn Connect™ / Souvenaid™) is a commercial, over the counter, product available from Nutricia NV. The supplement will be given to all dosed patients one hour prior to the TK-112690/placebo infusion (2 hours before the MTX infusion).

5.2. STUDY THERAPY ADMINISTRATION

The study therapy will be administered as an infusion via a peripheral vein in the forearm delivered using a catheter. **This access vein for the infusion must not be used for blood sampling**. Replacement of the intravenous catheter will be done according to local practice and the discretion of PI or designee, e.g., in the event of phlebitis. If a catheter is replaced, it should be flushed with saline prior to removal unless there are signs of phlebitis. TK-112690/placebo will initially be administered as a one-hour infusion. **In case of immediate adverse reaction to the infusion, please follow management of reaction (Appendix 4: Infusion Reaction Criteria).**

Epinephrine (1:1000) for subcutaneous injection, diphenhydramine (12.5 mg to 50 mg) for iv injection, and any other medications and resuscitation equipment for the emergency management of anaphylactic reactions must be available in the room where the infusions are being performed.

All patients will be evaluated at a follow-up visit on Day 1 of Week 6 post after initial drug treatments.

METREXASSIST™ (TK-112690 for Injection) should be prepared for infusion by a healthcare professional using aseptic technique. METREXASSIST™ will be diluted into 250 mL of 0.9% sodium chloride in a iv bag/bottle. Please refer to **Appendix 3** for the directions on how to calculate and dilute for the different doses to be administered to the subjects.

For the placebo will be prepared as described above for the TK-112690 except isotonic saline in 10 mL glass vials will be substituted for the 10 mL METREXASSIST™ vials.

Dosing will be calculated on a mg/kg basis and rounded to the closest 10 mg. The TK-112690/placebo dose will be 45 mg/kg.

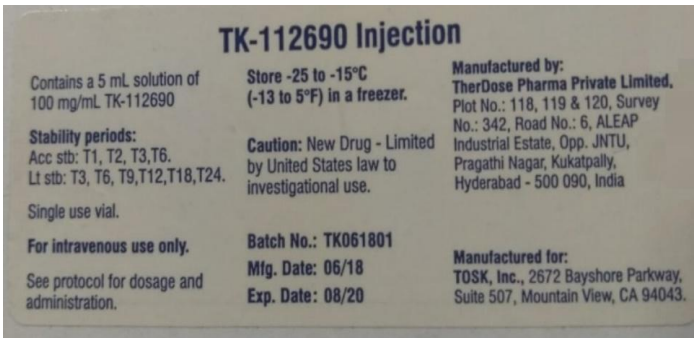
5.3. SELECTION AND TIMING OF DOSE FOR EACH SUBJECT

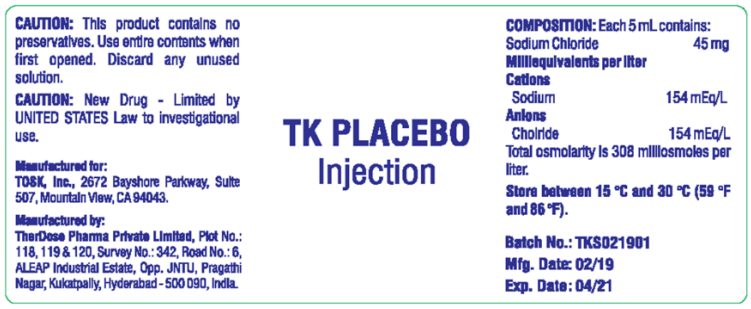
Dosage will be administered to enrolled subjects as defined in the protocol ± one day

5.4. PACKAGING AND LABELING

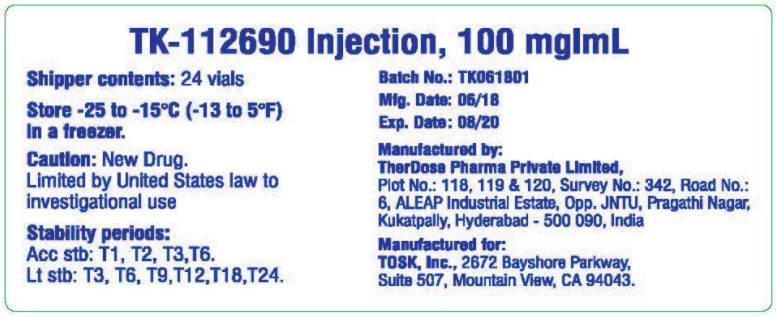
METREXASSIST™ and placebo will be supplied by Tosk in labeled, sealed 10 ml vials.

The vials will be labeled as follows:





The boxes will be labeled as follows:



5.5. BLIND\CODE BREAK

Phase 2a is a partially blinded study. The patient and investigator will be blinded as to whether TK-112690 or placebo is administered, and the CRO, Sponsor,and site pharmacist (unblinded) will know whether TK-112690 or placebo. was administered. The Pharmacist will be receiving all the vials from CRO, on randomization to a particular group the unblinded pharmacist will take the IP\Placebo and mask it with a white sticker so that the blind is maintained at all times. At the request of the investigator, the code can be broken. A Subject's treatment assignment will not be broken until the end of the study, unless medical treatment of the subject depends on knowing the study treatment the subject received. In the event that the blind needs to be broken because of a medical emergency, the investigator may unblind an individual subject's treatment allocation. As soon as possible, the investigator should first contact the medical monitor to discuss the medical emergency and the reason for revealing the actual treatment received by that subject. It should be documented in the case record form. For unblinding, investigator may also ask the medical monitor, who will in turn ask the unblinded statistician to provide the code to the investigator for the particular subject. The date on which the code was broken, together with the identity of the person responsible, must also be documented. When the investigator unblinds a patient treatment for emergency reasons, he/she must note the date, time, and reason for doing it and record this information in the source notes.

5.6. STORAGE AND ACCOUNTABILITY

All study therapy must be kept in a secure place under adequate storage conditions. The TK-112690infusion concentrate must be frozen (-20˚C ±5˚C) and protected from light.
Placebo must be stored between 15 ˚C to 30 ˚C.

The PI has overall responsibility for ensuring that study therapy is stored in a safe limited access location under the specified appropriate storage conditions. Limited responsibility may be delegated to a nominated pharmacy representative, but this delegation must be documented.

Study therapy will be dispensed by the pharmacist or designated member of the study team. The PI or designee will record dispensing of the study medication on a study medication accountability record. These records should include dates, quantities, batch/serial numbers, updated expiration dates (if applicable), and the unique code number assigned to the investigational product and trial subject. This record will be made available to clinical monitoring personnel for the purpose of accounting for the

clinical study medication supply. A study medication supply inspection for inventory purposes and assurance of proper storage will be conducted as necessary. Any significant discrepancy will be recorded and reported to the sponsor and a plan for resolution will be documented.

5.7. INVESTIGATIONAL PRODUCT RETENTION AT STUDY SITE AND DESTRUCTION

All study therapy required for completion of this study will be provided by Tosk (Metrexassist™, placebo and the nutritional supplement,).

The recipient will acknowledge receipt of the drug indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all study drug dispensed, used, and returned will be maintained. At the conclusion of the trial, all unused and used vials of will be returned to the sponsor or designee. Alternately, drug may be destroyed on site as dictated by the standard operating procedures at the participating institution (if appropriate). Vials should only be destroyed or returned after drug accountability has been performed by the clinical research associate and all vials were accounted for. If there is an error or perceived error, vials should be returned to Tosk.

5.8. DURATION

Phase 2a:

Start Date:	First Quarter 2019
End of Recruitment:	3rd Quarter 2019
End of Treatment:	Fourth Quarter 2019

6.0. STUDY PROCEDURES

6.1. INFORMED CONSENT

All subjects are to give informed consent in accordance with the origins of the Declaration of Helsinki (Appendix 1) and CFR part 50. The informed consent form as well as any advertisements used to recruit patients must be approved by the IRB/IEC.

The subject will provide the sign/thumb impression on the Informed Consent Form (ICF) before she/he enters the study, ie, before screening blood samples, screening assessments or any other study-related activity. The subject will be given sufficient time to consider the study's implications before deciding whether to participate. The informed consent form as well as any advertisements used to recruit patients must be approved by the IRB/IEC. Participants must sign/thumb impression the IRB/IEC-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the research file.

Should there be any amendments to the final protocol, the subject must agree to sign/thumb impression the amended ICF indicating that they re-consent to participate in the trial.

6.2. SUBJECT IDENTIFICATION

6.2.1. Subject Registration

Prior to registration and any study-specific evaluations being performed, all subjects must have given written informed consent for the trial and must have completed the screening evaluations (see Section 7.1). Subjects must meet all of the eligibility requirements listed in Section 4.0.

6.2.2. Subject Number

When the subject has completed the screening process and enters into the trial, he/she will be assigned a unique subject number composed of a site identification number and unique subject identification sequence number. The subject number and the subject's initials will be used as identification for the whole duration of the study.

6.3. MEDICAL HISTORY

Medical history will be performed at screening and will establishing age, sex, race, history of smoking/alcohol consumption/drugs and including subject's disease history. The review of previous/ongoing medications should cover a period of time that is 180 days prior to enrollment.

6.4. PHYSICAL EXAMINATION

A complete physical examination will be conducted at screening, on Day 0, 24 and 48 hours post each TK-112690/placebo dosing and on Day 1 of Week 6. Physical examination will be performed by site personnel who are experienced and routinely conduct physical examinations.

6.5. VITAL SIGNS, WEIGHT DETERMINATIONS AND CARDIOVASCULAR ASSESSMENTS

Body temperature will be recorded by the oral, rectal or tympanic route at screening and during weekly visits. The same route should be used for all measurements, if possiblesupine blood pressure (systolic and diastolic), respiratory rate and pulse will be recorded at screening (defined as baseline) and at Day 0, and 24 and 48 hours post TK-112690 /placebo infusions as well as on Day 1 of Week6. The time of the measurements will be noted. All measurements will be made with the subject in the supine position. Blood pressure should be determined by cuff (manual or automated is acceptable, although the same method should be used throughout the study).

Weight will be determined concurrent with vital sign measurements.

A standard 12 lead ECG will be recorded at screening (defined as baseline) and at 24 post each TK-112690/placebo dosing. The PI will provide an overall judgment of the ECG.

6.6. DISPENSING STUDY DRUG

Study drugs must be used only as directed in the protocol. The PI will be provided with forms to enable accurate, written records of all medication received from Tosk. These records will be kept by the PI or pharmacist/nominated person. The PI or pharmacist/nominated person will also keep accurate records of the quantities dispensed and used of the investigational product.

At the end of the study, all unused trial medication (ie, vials) will be returned by the pharmacist/ nominated person to a nominated contractor on behalf of Tosk. All certificates of delivery and returns must be signed by the pharmacist/nominated person. Based on entries in the drug accountability forms, at the end of the trial, it must be possible to reconcile delivery records with those of usage and returned stocks. All study medication must be accounted for and all discrepancies documented appropriately.

6.7. CLINICAL LABORATORY TESTS

6.7.1. Laboratory Parameters

Besides the Central Laboratory Tests, an in-house (at site) CBC, Kidney function test, Liver function test will be performed to qualify the subject for dosing with MTX, .Coagulation: (prothrombin time, PT, and activated partial thromboplastin time, PTT,tests will be performed at the Local laboratory associated with the clinical site as per schedule of events.

Subjects will be in a seated or supine position during blood collection. Clinical laboratory tests to be performed are included in **Table 10**.

Table 10. Central Laboratory Tests

Hematology:	**Serum Chemistry:**
Hematocrit (Hct)	Albumin (ALB)
Hemoglobin (Hb)	Alkaline phosphatase (ALK-P)
Mean corpuscular hemoglobin (MCH)	Alanine aminotransferase (ALT or SGPT)
Mean corpuscular hemoglobin concentration (MCHC)	Aspartate aminotransferase (AST or SGOT)
Mean corpuscular volume (MCV)	Blood urea nitrogen (BUN)
Platelet count	C-reactive protein
Red blood cell (RBC) count	Calcium (Ca)
White blood cell (WBC) count with differential	Chloride (Cl)
	Creatinine
	Creatinine kinase
	Direct bilirubin
	Total bilirubin
	Total cholesterol (non-fasting)
	Total protein
	Triglycerides (non-fasting)
	Uric acid
	Gamma-glutamyltransferase (GGT)
	Globulin
	Glucose (Random)
	Lactate dehydrogenase (LDH)
	Phosphorus
	Potassium (K)
	Sodium (Na)

Urinalysis :(will be performed by dipstick)
Appearance
Color
Glucose
Protein
Occult blood
Nitrite
pH

Urine human chorionic gonadotropin: Females of childbearing potential only

Coagulation:

- Prothrombin time (PT)
- Activated partial thromboplastin time (PTT)

The laboratory measurements of clinical chemistry, hematology and urinalysis will be performed during screening (defined as baseline), on Day 0 and at 24 and 48 hours post each initial infusion of TK-112690 or Placeboas well as on Day 1 of Week6.

All laboratory measurements will be performed at a central laboratory. The laboratory will provide the PI and Tosk with test results including units, relevant reference ranges and updates as necessary. Laboratory measurements can also be performed at local sites for medical or informational reasons However; these measurements will not be considered part of the study record.

6.7.2. Pharmacokinetics (PK)

No blood samples will be collected for PK. If necessary, to establish correct dosing of an individual patient at a particular time, an aliquot of blood sample relevant to the suspect dosing from either the **(6.7.3)** surrogate marker **(6.7.4)** blood samples obtained from the patient.

6.7.3. Surrogate Markers

Blood samples will be collected for the surrogate marker measurements which shall include relevant inflammatory and immune markers, e.g., CD40/CD40L. **Note: these samples must be collected from the arm not used for drug infusion.** A total of17 blood samples will be collected for surrogate marker measurements: Pre-initial infusion of TK-112690 or placebo, at 5 and 24 hours and 48 hours post each initial infusion of TK-112690 or placebo as well as on Day 1 of Week 6. The blood samples will be collected in a 10 mL heparinized Vacutainers.

6.7.4. Blood Samples

A blood sample for CBC, Kidney function test, Liver Function test determination will be obtained 24 hours or so before each MTX dose to assure the patient is suitable for treatment with this chemotherapeutic. The sample will be processed at the local laboratory associated with the clinical site, and the data provided to the PI, the CRO and Tosk.

6.7.5 Summary of Blood Samples to be obtained.

A summary of the blood samples to be obtained in the study is provided in **Table 11**.

Table 11. Nature and total volume of blood samples

Purpose	Size (mL)	Time	Total Volume (mL) / Patient
CBC, Kidney function test, Liver Function test	7	Pre-dose Weeks 1-4	28

Laboratory Test	14	Screening Day 0 Weeks 1-4 (24 and 48 hours) Week 6	154
Surrogate Markers	5	Weeks 1-4 (pre-dose, 5, 24 and 48 hours) Week 6	85
		Total	267

6.7.6 Processing Blood Samples.

Blood sample each will be collected per **Table 11** above and Schedule of Events (**Table 12**) for Laboratory, Surrogate Marker measurements in a heparinized tube and immediately placed on ice. Within 30 minutes of collection, plasma will be isolated by centrifugation at 4°C/1000∗g for 10 minutes.

- CBC, Kidney function test, Liver Function test (7 mL): This is a test prior to dosing to demonstrate that the patient can safely be treated with methotrexate. The test will be performed at the laboratory used by the clinical site.
- Laboratory Test samples (14 mL): The plasma for the laboratory tests will be split into two equal parts, transferred into polypropylene freezer vials and then frozen at -20°C to -70°C. One of the two vials will be used for the Laboratory Tests performed at the site and the other vial will be sent to the central laboratory.
- Surrogate Marker samples (5mL). The plasma for sample) will be transferred to a polypropylene freezer vial and then frozen at -20°C to -70°C.

Specific shipping instructions will be issued by the analytical laboratory performing the analyses. The heparinized tube and freezer vial will be labeled with study code, subject number, subject initials, date and sampling time points. The plasma samples will be stored at the center until the study coordinator arranges transport to the analytical sites.

Laboratory test will be performed both at the central laboratory except . Assays used to measure surrogate markers will be performed at Tosk, or a CRO engaged by Tosk.

6.6 MUCOSITIS ASSESSMENT

Mucositis status in subjects will be assessed using scoring systems described in more detail in **Appendix 20.6**.

7.0. STUDY ACTIVITIES

Study visits should occur on the indicated visit day.

7.1. SCHEDULE OF EVENTS

Schedule of events are provided in **Table 12 below**.
Table 12. Schedule of events (weekly cycle for 4 weeks and follow up at 6 week)

Evaluation	**Screening**	**Day 0**	**Weeks 1, 2, 3, and 4**					**Week 6 Follow-up**
			0h	*5h*	*6h*	*24h*	*48h*	

Informed Consent[1]	X							
I/E Criteria	X							
Medical history[2]	X							
Test for drugs of abuse	X	X						
Physical examination[3,4]	X	X				X[5]	X[5]	X
Vital signs and weight	X	X				X[6]	X[6]	X[6]
ECOG	X	X						X
Pregnancy test (females)	X	X						X
12-Lead ECG	X					X		
Laboratory tests[7]	X	X				X	X	X
Randomization		X						
CBC, Kidney function test, Liver Function test		X[8]						
Nutritional supplement			X[9]					
TK-112690/placebo treatments[10]			X[10]		X[13]			
MTX treatment			X[11]					
Surrogate marker sample			X[12]	X		X	X	X
Mucositis evaluation[14]		X				X	X	X
Disease Status	X							X
AEs assessment				X		X	X	X

[1] To be obtained before any study-related activities are performed.
[2] Include a review of previous/ongoing medications for 180 days prior to enrollment.
[3] Complete examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, extremities, and body weight.
[4] Height will be measured at screening only.
[5] Partial examinations to update findings from the examination performed at screening.
[6] Include blood pressure, pulse, respiratory rate and temperature.
[7] Includes serum chemistry, hematology (including coagulation), urinalysis; Hepatitis B/C & HIV only at screening
[8] CBC, Kidney function test, Liver Function test at clinical site can be performed anytime within 24 hours of a methotrexate treatment (NEEDED FOR ALL MTX TREATMENTS, EG, TREATMENTS ON WEEKS 1, 2, 3, AND 4, TO VERIFY OK TO DOSE MTX)
[9] One hour before TK infusion/placebo
[10] Beginning of TK-112690/placebo infusions is 0 hours. Dose is 45 mg/kg.
[11] [45] mg/m^2 one hour after TK-112690/placebo infusion.
[12] Pre-dose control samples for surrogate marker measurements.
[13] 6 hours after TK-112690/placebo treatment, ie, 5 hours post MTX treatment
[14] See Section 20.6 for mucositis assessment scales.

7.1.1 Screening Visit

To be accomplished within 15 days of expected initial dosing and ONLY AFTER THE ICF IS SIGNED.

An Eligibility Screening Form (ESF) documenting the subject's fulfillment of the entry criteria for all subjects considered for the study is to be completed by the PI. Subjects who are considered for study entry, but who fail to meet the eligibility requirements, will also have an ESF completed with the reason for ineligibility. These subjects will not be entered into the trial database. The ESFs for subjects who fail to meet the eligibility criteria requirements should be kept in the files at the sites.

During this visit the following activities will be performed:

- Eligible subjects meeting the inclusion/exclusion criteria for this study will be asked to read, comprehend, and sign/thumb impression an informed consent form.
- The subject medical history and concomitant medication use will be recorded as well as a history of drug abuse, alcohol consumption, and tobacco use.
- Perform a urine screen for drugs of abuse.
- A complete physical examination will be performed assessing the following: Skin, eyes, ear, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, extremities, and body weight.
- Vital signs and weight will be measured as well as an AE assessment.
- ECOG performance status (Section 20.2 **Appendix 2**)
- A pregnancy test will be performed on women of childbearing potential.
- A 12-lead ECG will be performed.
- The following laboratory test will be performed: hematological panel, infective agent panel, serum chemistry, coagulation and urinalysis (Laboratory tests, see **Table 10** for complete list of tests).
- Disease (tumor) assessment by CT scanning will be performed within 7 days post screening but before the first dosing.

7.1.2 Day before start of study (Day 0)

Prior to study start, all subjects will undergo the following:

- Randomization
- Perform urine screen for drugs of abuse.
- A physical examination to update findings from the examination performed at screening.
- Perform vital sign and weight measurements.
- ECOG performance status.
- A pregnancy test will be performed on women of childbearing potential.
- Perform laboratory tests (hematology, chemistry, coagulation and urinalysis).
- Perform a mucositis assessment (Section 20.6**, Appendix 6**).

7.1.3. Days 1 and 2 (Weeks 1-4)

Patients will be hospitalized on Day 1 and observed until after the 25-hour evaluations and plasma sample collection are obtained. All subjects will undergo the following on Days 1 and 2 of Weeks 1-4:

- An in-house CBC, Kidney function test, Liver Function test to be performed within 24 hours of expected methotrexate dosing to verify patient Fit to receive methotrexate.
- The nutritional supplement is ingested 2 hours prior to initiation of the MTX treatment, one hour prior to the TK-112690/placebo infusion.
- TK-112690/placebo is administered as a one hour infusion pre-MTX and 5 hours post-MTX (±15mins window period).
- One hour after the initial infusion of TK-112690 or placebo, MTX will be administered by continuous infusion for one hour (±15mins window period).
- Physical examination, vital signs, weight, mucositis evaluation at 24 and 48 hours post the initialTK-112690/placebo infusion.
- 12-lead ECG at 24 hours post initial TK-112690/placebo infusion.
- Laboratory tests (hematology, chemistry, coagulation and urinalysis) 24 and 48 hours post initial TK-112690/placebo infusion.

- Plasma samples will be collected for surrogate marker measurements pre-dose of the nutraceutical and 5, 24 and 48 hours initial infusion of TK-112690 orplacebo.
- Mucositis assessments (Section 20.6, **Appendix 6**) at 24 and 48 hours post initial infusion TK-112690 or placebo.
- AEs to be recorded at 5, 24 and 48 hours post initial infusion of TK-112690 or placebo.
- ±15mins of window period is considered acceptable for schedule of events.

7.1.4. Follow-Up Evaluations

All patients will undergo the following on Day 1 of Week 6:

- Perform a physical examination and determine vital signs, weight, and AE assessment.
- Determine ECOG performance status.
- For females of childbearing age, a pregnancy test will be performed.
- Perform laboratory tests (hematology, chemistry, coagulation and urinalysis).
- Obtain a blood sample for surrogate marker evaluation.
- Disease (tumor) assessment by CT scanning will be performed.
- Mucositis assessment (Section 20.6, **Appendix 6**).

8.0. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. PREGNANCY

Subjects who become pregnant during the study should discontinue the study immediately. Subjects should be instructed to notify the PI if it is determined after completion of the study that they became pregnant during the treatment phase of the study.

Whenever possible, a pregnancy should be followed to term, any premature terminations reported, and the status of the mother and the child should be reported to Tosk after delivery.

8.2. SUBJECT WITHDRAWAL CRITERIA

A subject may voluntarily discontinue study participation at any time. At any time, the PI may also at his/her discretion discontinue a subject's study participation. Subjects may be withdrawn from this study for the following reasons:

- At the PI's discretion in situations where the subject is unable to comply, or complies poorly with the protocol.
- Disease progression and/or experiences intolerable toxicity.
- Major protocol violation.
- Lost to follow-up.
- If TK-112690/placebo treatment is delayed due to toxicity for more than 2 weeks.
- Inter-current event preventing administration of study medication for more than 2 weeks.
- Subject withdraws consent.
- Subject becomes pregnant.
- Subject starts any other chemotherapy agent or anti-cancer treatment other than study therapy for refractory solid tumor, e.g., radiation therapy.

- Death.

If there is an ongoing toxicity due to the study agent, subjects must be followed with appropriate medical management until resolution or stabilization, regardless of evidence of progressive disease.

The subject will be advised in the Informed Consent Forms that he/she has the right to withdraw from the study at any time without prejudice and may be withdrawn at the discretion of the PI or Tosk at any time. In the event that the subject drops out of the study or is withdrawn from the study, the withdrawal CRF should be completed. On the withdrawal page the PI should record the date of the withdrawal, the person who initiated withdrawal and the reason for withdrawal.

Reasonable effort should be made to contact any subject lost to follow-up during the course of the study in order to complete assessments and retrieve any outstanding data.

At the time of study withdrawal, following will be recorded for these premature removals:

- Update medical history including any additional cancer therapies, including starting date (immunotherapy, targeted therapy, radiation and surgical interventions) if known.
- Physical examination and determination of vital signs, weight and AEs.
- Laboratory tests (hematology, chemistry, coagulation and urinalysis).
- Mucositis assessment (Section 20.6, **Appendix 6**).

Subjects with an ongoing apparently drug-induced toxicity will continue to be seen at monthly intervals until the toxicity resolves or stabilizes to a level acceptable to the medical monitor and the PI. If the subject starts new cancer therapy, the relationship to study drug should be re-assessed.

9.0. ASSESSMENT OF SAFETY

9.1. SPECIFICATION OF SAFETY PARAMETERS

9.1.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical Investigational subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an Investigational product, whether or not related to the Investigational product. Pre-existing conditions which worsen during a study are to be reported as adverse events.

All adverse events, regardless of causal relationship, encountered during the clinical study will be reported on the AE page of the CRF (please see section 9.3). Intensity of AEs will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) and reported in detail as indicated on the CRF. If an adverse event occurs which is not contained in the NCI-CTCAE, the four-point scale (mild, moderate, severe, life-threatening) below will be used.

Mild	discomfort noticed but no disruption of normal daily activity
Moderate	discomfort sufficient to reduce or affect daily activity
Severe	severe discomfort, inability to work or perform normal daily activity
Life threatening	represents an immediate threat to life

9.1.2. Laboratory Test Abnormalities

Laboratory test results will be recorded on the laboratory results pages of the CRF. Laboratory test value abnormalities as such should not be reported on the AE page of the CRF as adverse events, unless there is an associated clinical condition for which the subject is given treatment or concomitant treatment is altered, study treatment is interrupted/delayed or the dose of study drug is modified, or the subject is permanently discontinued from the study drug because of the abnormal test value.

9.1.3. Serious Adverse Event

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (subject is at immediate risk of death from the event as it occurred).
- Requires subject hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Other medically important condition.

All of the above criteria apply to the case as a whole and should not be confused with the outcomes of individual reactions/events. More than one of the above criteria can be applicable to the one event.

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

The definition and reporting requirements of the ICH Guideline for Clinical Safety Data management, Definitions and Standards for Expedited Reporting Topic E2 will be adhered to (see **Appendix 5**).

All SAEs, whether or not deemed drug-related or expected, must be reported to Tosk and to the trial clinical research associate (clinical monitor).

9.1.4. Follow-up of Adverse Events

All adverse events (and treatments for them) must be documented throughout the Study Treatment Phase starting after the administration of the first dose of study medication and for 28 days after the last intake of study medication, and followed up/medically managed until the event is either resolved or adequately explained, even after the subject has completed his/her study treatment. Unrelated, mild or moderate events must be followed for 28 days after the last study drug administration. Serious, life threatening or related events must be followed until resolution, the subject's death, the start of a new cancer therapy or the relationship is re-assessed.

9.1.5. Follow-up of Abnormal Laboratory Values

In the events of unexplained abnormal laboratory test values, the tests should be repeated and followed up until they have returned to normal range or to the baseline value, and/or an adequate explanation of the

abnormality is found. If a clear explanation is established it should be recorded on the CRF.

9.1.6. Pregnancy

Pregnancy, in and of itself, is not regarded as an adverse event, unless there is suspicion that study medication may have interfered with the effectiveness of a contraceptive medication or method. The procedures that will be followed based on whether a pregnancy is confirmed by a positive serum or urine test result are listed below:

- Pregnancy confirmed by a positive serum test result, then:
  - PI and subject must notify each other immediately
  - PI must notify the sponsor immediately
  - Discontinue study medication immediately
  - Withdraw the subject from the study
  - Perform the required Early Termination visit study evaluations
    PI must complete and submit the initial and follow-up report to the sponsor
- Pregnancy confirmed by a positive urine test result, then:
  - PI and subject must notify each other immediately
  - PI must notify the sponsor immediately
  - Study medication must be discontinued immediately
  - A serum pregnancy test must be performed to confirm the urine test result. (The serum test should be performed at the investigative site to ensure the test will be performed promptly and the result available immediately for review)
- If a positive serum test confirms the urine test result, then:
  - Withdraw the subject from the study
  - Perform the required Early Termination visit study evaluations
  - PI must complete and submit the required initial and follow-up report to the sponsor
- If a negative serum test does not confirm the urine test result, then:
  - The PI will use his/her expert judgment, based on an assessment of the potential benefit/risk to the subject, to determine if it is in the subject's best interest to resume study medication and continue participation in the study

Any pregnancy diagnosed during the study, or that occurs within 28 days after stopping study medication, must be reported immediately to the PI. The PI will notify the sponsor or designee. The outcome of all such pregnancies (i.e., spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be documented and followed-up on a form that will be provided by the sponsor. The pregnancy will be followed to term and the outcome, including any premature termination, must be reported to the sponsor. All live births must be followed for a minimum of 30 days or to the first well-baby visit. All reports of congenital abnormalities/birth defects and spontaneous abortions/miscarriages should be reported as an SAE for this study. Elective abortion procedures, without complications, should not be considered as adverse events.

9.2. ASSESSING, RECORDING, AND ANALYZING SAFETY PARAMETERS

Safety lab assessments will be performed by the central laboratory and will be collected and processed according to standard investigative site procedures. Normal value ranges and laboratory certification will be collected from all laboratories prior to study initiation.

Safety laboratory assessments will include:

- Complete Blood Count with differential
- Enzyme panel including AST, ALT, LDH, creatinine kinase, alkaline phosphatase
- Chemistry panel including total protein, albumin, total bilirubin, uric acid, phosphate, triglycerides, cholesterol, calcium, BUN, creatinine, sodium, potassium, chloride, bicarbonate, glucose, and phosphorus; and
- Urinalysis including pH, glucose, protein, ketones and blood

9.2.1. ECG Parameters

ECG parameters will be analyzed including ECG abnormalities and PI's assessment of clinical significance analyzing above parameters, rhythm abnormalities, and conduction abnormalities. Any abnormalities will be reported on the CRF with a copy of the ECG recording.

9.3. RECORDING AND REPORTING ADVERSE EVENTS/INTERCURRENT ILLNESSES

The PI will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. For each AE, the PI should note the start and resolution dates, the severity, whether it meets the definition of an SAE, the relationship of the event to the study drug, the action taken regarding study drug, and the outcome of the event. Data should be transcribed from the source documents to the case report forms as per the case report form instructions, so that all changes in intensity and seriousness will be captured on the AE case report form.

The PI must report in detail all adverse signs and symptoms which are either volunteered by subjects or observed during or following the course of Investigational product administration on the appropriate CRF page.

All AEs will be monitored until resolution or, if the AE is determined to be chronic, a cause is identified. If an AE is considered possibly related to study treatment and remains unresolved at the conclusion of the study, this event will be followed until resolution, stabilization, or initiation of treatment that confounds the ability to assess the event.

9.3.1. Relationship of AE to Study Medication

The relationship of an adverse event to study medication is to be assessed according to the following definitions:

Definitely Related: Strong evidence exists that the study drug caused the adverse event. There is a temporal relationship between the event onset and administration of the study drug. There is strong therapeutic and pharmacologic evidence that the event was caused by the study drug. The subject's clinical state or concomitant therapies have been ruled out as a cause. In the case of cessation or reduction of the dose, the event abates or resolves, and reappears upon re-challenge.

Probably Related: A temporal relationship exists between the event onset and the administration of study drug, and appears with some degree of certainty to be related based on the known therapeutic and pharmacologic actions of the study drug. It cannot be readily explained by the subject's clinical state or

concomitant therapies. In the case of cessation or reduction of the dose, the event abates or resolves.

Possibly Related: A temporal relationship exists between the event onset and the administration of study drug. Although the adverse event may appear unlikely to be related to the study drug, it cannot be ruled out with certainty; and/or the event cannot be readily explained by the subject's clinical state or concomitant therapies.

Not Related: Evidence exists that the adverse event definitely has an etiology other than the study drug (eg, pre-existing condition or underlying disease, intercurrent illness, or concomitant medication) and does not meet any criteria listed above.

Included in the description should be the nature of the sign or symptom; the date of onset; date of resolution (duration); the severity; the relationship to study treatment or other therapy; the action taken (if any), and the outcome.

9.3.2. Unexpected Adverse Event

An unexpected adverse event is one not previously reported (in nature, severity or incidence) in the current PI's Brochure, in the clinical plan, or elsewhere. Unexpected, as used in this definition, refers to an adverse drug experience that has not been previously observed (eg, included in the PI Brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

9.3.3. Reporting of Serious Adverse Events

Adverse events classified as serious require expeditious handling and reporting to Dr. Shanna Stopatschinskaja at Tosk to comply with regulatory requirements. For any serious adverse event (SAE) that occurs while a subject is on-study; within 30 days of the last study drug administration, regardless of any opinion as to the relationship of the SAE to the study drug; or if any SAE that the PI feels is related to the study drug occurs later than 30 days after the last study drug administration, Tosk must be notified by telephone within 24 hours of becoming aware of the event. During both business and non-business hours, the telephone number listed below should be used to notify Tosk.

All SAEs require that, in addition to telephone notification, a Serious Adverse Event Report Form be completed and forwarded via facsimile to Tosk at the number listed below within 24 hours of becoming aware of the event.

All SAES (not clearly related to disease progression) whether considered to be drug-related or not should be considered unexpected in this first-in-human study, and must rapidly be communicated to the institutional review board (s) and all other investigators.

SAEs will be reported to:

Sponsor:

William Garland, Ph.D.
VP R&D, TOSK, INC.

2672 Bayshore Parkway, #507
Mountain View, CA 94043
+1 408-348-5164 (Cell)
+1 408-245-6838 (Landline)
+1 408-245-6808 (Fax)

All SAEs will be reviewed by both Dr. Garland and the Tosk Safety Monitor:

Shanna Stopatschinskaja, MD
Dolores Medical Services
1073 Dolores St, San Francisco, CA 94110
+1 908-230-9327 (cell)
stopats@gmx.net

CRO:

Dr.L.Krishnamurthy
Consultant Medical Monitor
+91 9603662465
dr_murthy130475@yahoo.com

Intuitions / Hospital as applicable.

Drug Control General of India

FDA Bhavan, ITO, Kotla Road, New Delhi - 110002
Phone: +91-11-23216367(CDSCO)/ 23236960
Fax: +91-11-23236973
E-mail:- dci@nic.in

All adverse events must be recorded in the case report form (CRF). To avoid colloquial expressions, the adverse event should be reported in standard medical terminology and will be coded in MedDRA. Whenever possible, the adverse event should be evaluated and reported as a diagnosis rather than as individual signs or symptoms. If a definitive diagnosis is not possible, the individual symptoms and signs should be recorded. Any laboratory abnormalities deemed clinically significant by the PI should be reported on the adverse event CRF. Whenever possible, the etiology of the abnormal findings will be documented on the CRF. Any additional relevant laboratory results obtained by the PI during the course of this study will be supplied to the sponsor and recorded on the CRF. The required SAE information should also be completed on the CRFs and the data reported within 24 hours from the time that the event was identified. This will ensure that the written documentation is transmitted to the appropriate pharmacovigilance contact person within the required reporting time period.

The IRB/regulatory authority must be informed if the SAE or AE, in the opinion of Tosk or the PI, is likely to affect the safety of the subjects or the conduct of the study.

All Investigators participating in the trial will also be notified of any unexpected SAEs determined to be related to study treatment.

For any questions about adverse events or serious adverse events contact Sponsor's medical monitor

9.4. WARNINGS AND PRECAUTIONS

Subjects receiving study therapy (TK-112690) should be monitored by a physician experienced in the use of cancer chemotherapy agents. Use of these agents in pregnant or breast-feeding women is contraindicated.

9.4.1. Precautions

Infusion reactions with the first dose of TK-112690 may be observed. TK-112690 administration should be interrupted in all subjects with severe infusion reactions and appropriate medical therapy administered.

9.4.2. Treatment Failure

In case of treatment failure, patients will be treated using the Standard Guidelines for Management of Mucositis.[91]

9.5 EFFICACY ASSESSMENTS

9.5.1 Mucositis Assessment Scales

The scales described in **Section 20.6** of this protocol will be used to assess/rate mucositis. The scales are OMAS/Sonis,[92,93] PROMS,[94] WHO and CTCAE/mucositis.

Response

Response will be determined according to the RECIST methodology. It will be the best response recorded from the start of the treatment until disease progression/recurrence. To be assigned a status of partial response or complete response, changes in tumor measurements must be confirmed by repeat assessments performed at least 4 weeks after the criteria of response are first met.

Target lesions

- Complete response is defined as the disappearance of all target lesions.

[91] Rubenstein EB, Peterson DE, Schubert M, Keefe D,McGuire D, Epstein J, EltingLS,Fox PC, Cooksley C, Sonis ST. Clinical Practice Guidelines for Mucositis. Cancer 2004;100 (9 Suppl):2026–2046.

[92] Sonis ST, Eilers JP, Epstein JB, LeVeque FG, Liggett WH Jr, Mulagha MT, Peterson DE, Rose AH, Schubert MM, Spijkervet FK, Wittes JP. Validation of a new scoring system for the assessment of clinical trial research of oral mucositis induced by radiation or chemotherapy. Mucositis Study Group. Cancer.1999; 85: 2103-13.

[93] Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. Sonis ST, Oster G, Fuchs H, Bellm L, Bradford WZ, Edelsberg J, Hayden V, Eilers J, Epstein JB, LeVeque FG, Miller C, Peterson DE, Schubert MM, Spijkervet FK, Horowitz M. J ClinOncol.2001 ;19:2201-5.

[94] Kushner JA, Lawrence HP, Shoval I, Kiss TL, Devins GM, Lee L, Tenenbaum HC, Development and validation of a Patient-Reported Oral Mucositis Symptom (PROMS) scale. J Can Dent Assoc. 2008; 74: 59.

- Partial response is defined as at least a 30% decrease in the sum of the longest diameter of the target lesions, taking as a reference the baseline sum longest diameter.
- Progressive disease is defined at least a 20% increase in the sum of the longest diameter of target lesions, taking as a reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions.
- Stable disease is defined as shrinkage insufficient to qualify for partial response or insufficiently increased to qualify for progressive disease, taking as a reference the smallest sum longest diameter since the treatment started.

Non-target lesions

- Complete response is defined as the disappearance of all non-target lesions.
- Non-complete response /non-progressive disease is defined as persistence of 1 or more non-target lesion.
- Progressive disease is defined as the appearance of 1 or more new lesions and/or unequivocal progression of existing non-target lesions.
- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease and progressive disease.

Confirmation of No Response on Tumor

To be assigned a best tumor response status of partial response or complete response, changes in tumor measurements in subjects with responding tumors must be confirmed by repeat studies that should be performed no less than 4 weeks after the criteria for response are first met. In the case of stable disease, follow-up measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 8 weeks.

Determination of Overall Best Tumor Response by RECIST (Version 1.1) Methodology

The overall assessment of best tumor response will involve all parameters as depicted in **Table 13**below.

Table 13. Tumor response criteria

Target lesions*	**Non-Target lesions****	**New Lesions*****	**Overall Response**
Complete Response	Complete Response	No	Complete response
Complete Response	Non-complete Response /Non-progressive disease	No	Partial response
Partial Response	Non-progressive disease	No	Partial response
Stable disease	Non-progressive disease	No	Stable disease
Partial disease	Any response	Yes or No	Progressive disease
Any response	Progressive disease	Yes or No	Progressive disease
Any response	Any response	Yes	Progressive disease
*Measurable lesions only **May include measurable lesions not followed as target lesions or non-measurable lesions ***Measurable or non-measurable lesions			

The overall best tumor response is the best response recorded from the start of the treatment until disease progression/recurrence, taking as reference for tumor progression the smallest measurements recorded since the treatment started. The subject's best tumor response assignment will depend on the achievement of both measurement and confirmation criteria.

Subjects will be defined as being not evaluable for response (NE) if there is no post-baseline lesion assessment, unless the subject terminates from the study for disease progression.

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration" on the Study Termination CRF. Every effort should be made to document the objective progression even after discontinuation of treatment. It should also be noted that a tumor marker increase does not constitute adequate objective evidence of tumor progression. However, such a tumor marker increase should prompt a repeat radiographic evaluation to document whether or not objective tumor progression has occurred. In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of a CR depends upon this determination, it is recommended that the residual lesion be investigated by fine needle aspirate or biopsy before confirming the CR status.

9.5.3. Surrogate Markers

The effect of TK-112690 treatment on mucositis-related inflammatory markers such as CD40/CD40L will be studied. The results of the exploratory assessments will not be recorded in the CRF but will be reported separately in an addendum.

9.5.4. Surrogate Markers

Plasma samples for establishing the surrogate marker profiles will be obtained at times specified in the protocol. The results of the exploratory assessments will not be recorded in the CRF but will be reported separately in an addendum.

9.6 APPROPRIATENESS OF MEASUREMENTS

Study measurements are those associated with a standard evaluation of a new modality for modifying side-effects associated with chemotherapy.

10.0. STATISTICAL CONSIDERATIONS

10.1. GENERAL CONSIDERATIONS

All analyses will be performed. and all **Tables**, **Figures,** and data listings will be prepared using a validated statistical program. Summary statistics for continuous variables will include the mean, standard deviation, median, minimum, and maximum value; categorical variables will be presented as counts and percentage.

10.1.1. Sample Size and Associated Power

The primary objective of this Phase 2a trial is to evaluate the pilot efficacy and the safety/tolerability of Metrexassist™ administered intravenously to subjects with cancer also receiving 45 mg/m^2 MTX. This is

an exploratory study and is may not be powered to demonstrate statistical significance. The sample size is based not on statistical calculations but on the outcome in the Phase 1b study where all patients received TK-112690 experienced relatively little mucositis. Without the TK-112690 considerably more mucositis is expected, perhaps enough to achieve statistical significance. At a minimum, sufficient data will be obtained to accurately power the next study with TK-112690 and MTX.

10.1.2. Randomization of the Trial

The clinical CRO or a third-party provider will alternatively assign patients as per the randomization list. If a patient drops out of the study before completing first dosing, he/she will be replaced by someone with the same assigned treatment.

10.2. POPULATIONS

10.2.1. Efficacy and Safety Population

The efficacy population will consist of all subjects who received at least four doses of TK-112690. The safety population will consist of all subjects who receive at least one dose of TK-112690.

10.3. ANALYSIS OF SAFETY

Subjects in study will be evaluated for safety at Week 1 and 2. Subjects with tolerable toxicity will be allowed to continue study therapy for an additional 2 weekly treatments and possibly more at the discretion of the investigator.

Vital signs (systolic and diastolic blood pressure, and pulse), weight determinations and physical examination results will be summarized by treatment group using appropriate descriptive statistics. Continuous variables will be summarized using the number of observations, mean, standard deviation, minimum, median and maximum values. Categorical values will be summarized using number of observations and percentages.

Withdrawals from the study will be summarized by dose group.

10.3.1. Incidence of Adverse Events

Adverse events will be classified and graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

The primary safety analysis using descriptive statistics will include summaries of incidence rates, severity, and type of adverse events; summary of changes in subjects' clinical laboratory results; and summaries of the number of toxicity grades for laboratory and non-laboratory data.

Adverse events will be coded using the MedDRA adverse event dictionary. Frequency of treatment emergent adverse events will be calculated for each body system, by preferred term, by treatment group, for number of subjects and proportion reporting the event. The severity of the adverse events and the relationship to study medication will be summarized for each body system and preferred term by treatment group. Withdrawals due to adverse events will be summarized for each body system and preferred term by treatment group.

10.3.2. Laboratory Data

The number and percentage of subjects with laboratory abnormalities at the screening visit and the final visit (or early withdrawal) will be tabulated.

Descriptive statistics (number of observations, mean, standard deviation, minimum, median and maximum values) will be calculated for clinical laboratory tests (hematology, serum chemistry, and urinalysis) at applicable visits.

10.4. SUBJECT ACCOUNTABILITY AND MISSING DATA

Data from subjects lost to follow-up will be tabulated and any difference between those subjects and subjects not lost to follow-up will be noted.

10.5. INTERIM ANALYSIS

No interim analyses will be performed.

11.0. DIRECT ACCESS TO SOURCE DATA / DOCUMENTS AND INVESTIGATOR RESPONSIBILITY

It is understood that the term "PI" as used in this protocol and on case report forms refers to the Principal PI or a member of the staff that the Principal PI designates to perform a certain duty. However, on specific CRFs the investigator him/herself is required to sign where indicated. The Principal PI is ultimately responsible for the conduct of all aspects of the study.

The PI will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice, Hong Kong, Somerset West, and Edinburgh) and with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

It is the responsibility of the PI to obtain written informed consent from each individual participating in this study, after adequate explanation of the aims, methods, objectives and potential hazards of the study. The PI must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time. The document must contain all the elements described in 21 CFR, Part 50 and ICH Guidelines.

The PI shall make accurate and adequate reports to Tosk and/or its representative and to the EC/IRB on the progress of the study at appropriate intervals. Additionally, the PI shall make accurate and adequate final status reports, through completed CRFs, to the monitors and to the IRB within three weeks after the completion, termination, or discontinuation of the study. The final report must include all case report forms that were not previously provided to the monitors.

Qualified representatives of the sponsor or sponsor designees ("study monitors") will monitor the study according to a predetermined monitoring plan. Monitoring visits provide the sponsor with the opportunity to:

- Evaluate the progress of the study.

- Verify the accuracy and completeness of CRFs.
- Assure that all protocol requirements, applicable laws and/or regulations, and PI's obligations are being fulfilled.
- Resolve any inconsistencies in the study records.

The PI must allow the study monitors to periodically review, at mutually convenient times during the study and after the study has been completed, all CRFs and office, hospital, and laboratory records supporting the participation of each subject in the study. The CRFs and other documentation supporting the study must be kept up-to-date by the PI and the research staff at the investigative site. These study materials must be available for review by the study monitor, and/or other qualified representatives of the sponsor, at each monitoring visit.

The study monitor will regularly inspect the various records of the study (CRFs, subject medical and laboratory records, and other pertinent data) provided that subject confidentiality is maintained in accordance with local institution, state, country, and federal requirements. The study monitor will verify the data against other source documentation in order to verify its accuracy and completeness. The study monitor will identify data discrepancies and collaborate with the PI and research staff to resolve the discrepancies in the CRF in a timely manner. The study monitor will generate queries as required using the CRF system. Protocol deviations will also be identified and recorded on a "Protocol Deviation Log". The study monitor will follow an "Issue Escalation" plan in order to ensure that each issue identified during a monitoring visit is appropriately documented, reported, and resolved in a timely manner in accordance with the plan's requirements.

11.1. SUBJECT INFORMATION AND CONSENT

The PI will ensure that the subject is given full and adequate verbal and written information about the nature, purpose, possible risk and benefit of the study. Subjects, legal guardian or responsible relative must also be notified that they are free to discontinue their participation in the study at any time. The subject legal guardian or responsible relative should be given the opportunity to ask questions and, when possible, time for consideration. The PI is responsible for obtaining signed informed consent from all subjects before enrollment. In subjects unable to consent to treatment, consent must be obtained from a close family member or legal guardian in writing before enrollment.

A copy of the subject information including the signed Consent Form should be retained by the subject.

11.2. SUBJECT CONFIDENTIALITY

All data computer-processed at Tosk or by its designee will be identified by subject number only, thereby ensuring that the subject's identity remains unknown to the sponsor. The subjects should be informed in writing that the data will be stored and analyzed in a computer, maintaining confidentiality in accordance with national data legislation. The subjects should also be informed in writing that authorized representatives of the company and/or Regulatory Authorities, may require access to those parts of the hospital/practice records relevant to the study, including medical history, for verification of data. The Principal PI is responsible for keeping a subject identification list or form of all subjects enrolled, including enrollment code, subject number, full name and address.

11.3. SOURCE DATA

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

11.4. SOURCE DOCUMENTS

Source documents are defined as original documents, data and records (eg, hospital records, clinical and office charts, laboratory notes, memoranda, subject diaries or evaluation check lists, pharmacy dispensing records, recorded data from automated instruments, copies or manuscripts certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, records kept at pharmacy, at the laboratories and at medico technical departments involved in the clinical trial).

11.5. DIRECT ACCESS

Direct access is defined as the permission to examine, analyze, verify and reproduce any records and reports that are important to evaluation of a clinical trial. Any party (eg, domestic and foreign regulatory authorities, Tosk monitors and auditors) with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subject identities and sponsor proprietary information.

11.6. STUDY MONITORING

In accordance with applicable regulations and Good Clinical Practice, Tosk / CRO monitors will periodically contact the site, including conducting on-site visits. During these contacts, the monitor will check and assess the progress of the study, review the data collected, conduct source document verification, and identify any issues and address their resolution. These activities are performed in order to verify that the data are authentic, accurate, and complete; the safety and rights of the subjects are being protected; and the study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

12.0. QUALITY CONTROL/ QUALITY ASSURANCE

An independent audit at the study site may take place at any time during or after the trial. The independent audit can be conducted by the Quality Assurance (QA) Department at of Tosk or a regulatory authority.

12.1. QUALITY CONTROL

Quality Control is defined as the operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial related activities have been fulfilled. Quality Control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

12.2. QUALITY ASSURANCE

Quality Assurance is defined as the planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded) and reported in compliance with

Good Clinical Practice (GCP) and the applicable regulatory requirements.

12.3. INSPECTION

An Inspection is defined as the act by a regulatory authority of conducting an official review of documents, facilities, records and any other resources that are deemed by the authorities to be related to the clinical trial and that may be located at the site of the trial, or at the sponsor's and/ or clinical research organization facilities or at any other establishments deemed appropriate by the regulatory authorities.

12.4. AUDIT

An audit is a systematic and independent review of trial-related activities and documents to determine whether the validated trial-related activities were conducted and the data were recorded, analyzed and accurately reported according to the protocol, designated Standard Operating Procedure (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirements.

13.0. ETHICS APPROVALS

Before initiating a trial, the PI should have written and dated approval/favorable opinion from the relevant IRB or IEC for the trial protocol (and any amendments), written informed consent form, consent form updates, subject recruitment procedures (eg, advertisements), and any other written information to be provided to subjects. Approval will be indicated in writing with reference to the final protocol number and date. Details of the IRB's or IEC's constitution including names of its members and what function they perform on the committee (eg, chairman, specialist, lay-member) should be made available to Tosk. During the trial the PI should provide to the IRB all documents that are subject to review.

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the IRB/IEC. Prior to obtaining approval, the protocol must be approved by the Sponsor. The initial protocol and all protocol amendments must be approved by the IRB/IEC prior to implementation.

13.1. INDEPENDENT ETHICS COMMITTEE (IEC)

An independent body (a review board or a committee, institutional, regional, national, or supranational), constituted of medical professionals and non-medical members, whose responsibility it is to ensure the protection of the rights, safety and well-being of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, reviewing and approving/providing favorable opinion on, the trial protocol, the suitability of the investigator(s), facilities, and the methods and material to be used in obtaining and documenting informed consent of the trial subjects.

13.2. INSTITUTIONAL REVIEW BOARD (IRB)

This is an independent body constituted of medical, scientific, and non-scientific members, whose responsibilities are to ensure the protection of the rights, safety and well-being of human subjects involved in a trial by, among other things, reviewing, approving, and providing continued review of trial protocol and amendments and of the methods and material used in obtaining and documenting informed consent of the trial subjects.

14.0. MODIFICATION OF PROTOCOL

Once the protocol has been approved by the IRB/IEC, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the PI and approved by Tosk and the IRB/IEC prior to implementation.

The PI should not implement any deviation from, or changes to, the protocol without agreement by Tosk. Any changes which may affect a subject's treatment or informed consent, especially those increasing potential risks, must receive prior review and documented approval/favorable opinion from the IRB or IEC. The only exceptions are when necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial [eg, change in monitor(s), change of telephone number(s)].

As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

- To the IRB or IEC for review and approval/favorable opinion.
- To the sponsor for agreement and, if required.
- To the regulatory authority (ies).

The party initiating an amendment must confirm it clearly in writing and it must be signed and dated by Tosk and the Principal PI. Tosk will ensure that the Investigators submit necessary protocol amendments to the appropriate IRBs or IECs.

All agreed protocol amendments must be clearly documented using standard procedures as defined by Tosk, and must be signed and dated by Tosk and the PI.

15.0. DATA HANDLING AND RECORD KEEPING

15.1. COMPLETION OF CASE REPORT FORMS

Site personnel will collect study data using CRFs compliant with FDA 21 CFR Stage 11 regulations. The PI must sign and date a declaration on the CRF attesting to his/her responsibility for the quality of all data recorded and that the data represents a complete and accurate record for each subject's participation in the study.

Data reported on the CRF that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. Any data to be recorded directly on the CRFs (to be considered as source data) will be identified at the start of the trial.

15.2. ARCHIVING

As described in the ICH GCP Guidelines, "essential documents," including CRFs, source documents, consent forms, laboratory test results, and medication inventory records, should be retained by the PI until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by

an agreement with Tosk. The PI should obtain written permission from Tosk prior to the destruction of any study document.

These records will be made available at reasonable times for inspection and duplication, if required, by a properly authorized representative of the US FDA in accordance with 21 CFR 312.68 or other National Regulatory Authorities.

16.0. FINANCIAL DISCLOSURE

The PI will be required to disclose any financial arrangement whereby the value of the compensation for conducting the study could be influenced by the outcome of the study following information; any significant payments of other sorts from Tosk such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoria; any proprietary interest in METREXASSIST™ [parenteral (TK-112690)] or any significant equity interest in Tosk as defined in the US Code of Federal Regulations [21 CFR 54 2(b)].

In consideration of participation in the study, Tosk will reimburse the PI or nominated payee according to schedule attached to the PI Agreement.

17.0. FINANCING AND INSURANCE

The costs necessary to perform the study will be agreed to with each PI and will be documented in a separate financial agreement that will be signed by the PI and Crystal Life Science prior to the trial commencing.

Subjects will be reimbursed for reasonable study-related travel expenses.

With respect to any liability directly or indirectly caused by the study drugs in connection with this Clinical Trial, Tosk assumes liability by law on behalf of the PI and his/her assistants for possible injury to the subject provided the PI and his/her assistants have followed the instructions of Tosk in accordance with this protocol and any amendments thereto, that supplied by Tosk, and that the PI and his/her assistants have in general performed this Clinical Trial in accordance with scientific practice and currently acceptable techniques and know-how.

18.0. PUBLICATION POLICY

18.1. PUBLICATION POLICY

It is intended that the results of the study may be published as scientific literature. Results may also be used in submissions to regulatory authorities. The following conditions are to protect commercial confidential materials (patents, etc.), not to restrict publication. However, any proposed oral or written use of such results must be submitted to Tosk for review and written approval at least 60 days prior submission for publication, presentation, or use.

All information concerning TK-112690—such as patent applications, formulae, manufacturing processes, basic scientific data, or formulation information supplied to the PI by Tosk and not previously

published—is considered confidential by Tosk and shall remain the sole property of Tosk. The PI agrees not to use it for other purposes without the written consent of Tosk.

It is understood by the PI that Tosk will use the information developed in this clinical study in connection with the development of TK-112690 and therefore may be disclosed as required to other Tosk Investigators or any appropriate international Regulatory Authorities. In order to allow for the use of information derived from this clinical study, the PI understands that he/she has an obligation to provide Tosk with complete test results and all data developed during this study. In accordance with generally recognized principles of scientific collaboration, co-authorship with any Tosk personnel will be discussed and mutually agreed upon before submission of a manuscript to a publisher.

18.2. CONFIDENTIALITY

The principal PI and any other study personnel involved in this study shall not disclose, or use for any purposes (other than for the performance of this study), any data, records, or other information (hereinafter collectively "Information") disclosed to the Principal PI or other study personnel. Such information shall remain the confidential and proprietary property of Tosk and shall be disclosed only to the Principal PI or other designated personnel. The obligation of non-disclosure shall not apply to the following:

- Information after such time that it is or becomes publicly available through no fault of the PI or other study personnel.
- Information after such time that it is disclosed to the Principal PI by a third party entitled to disclose such information.

The PI must assure that subject anonymity will be maintained and that identities are protected from unauthorized parties. Subjects should not be identified by their names but by their initials and an identification code on CRFs or other documents submitted to the sponsor. Documents that will not be submitted to Tosk (eg, written informed consent form), should be maintained by the PI in strict confidence.

19.0. REFERENCE LIST

20.0. APPENDICES:

20.1. APPENDIX 1: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1960
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added) 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations, the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

20.2. APPENDIX 2: ECOG PERFORMANCE STATUS

ECOG PERFORMANCE STATUS

0 Fully active, able to carry on all pre-disease performance without restriction.

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work).

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

5 Dead.

20.3. APPENDIX 3: METREXASSIST™ (TK-112690 FOR INJECTION) DOSE PREPARATION

OVERVIEW

TK-112690 must be administered under the supervision of a physician experienced in the use of anti-neoplastic medicinal products. Solutions of formulated TK-112690 should not be co-administered or mixed with any other solution.

TK-112690 is provided as a concentrate for solution for infusion in a single-use vial, which contains a nominal amount of 500 mg of TK-112690 in 5 mL (concentration of 100mg/mL).

METREXASSIST (TK-112690) is formulated with 50 mM sodium phosphate pH 7.4. The drug product is a clear, colorless, sterile liquid solution that has to be diluted in 0.9 % sodium chloride solution prior to administration.TK-112690**IS NOT TO BE ADMINISTERED AS AN IV PUSH OR BOLUS**. TK-112690 should be administered as a one-hour iv infusion.

HOW SUPPLIED

TK-112690 is supplied as 5 mL of a sterile solution in single-use glass vials to deliver 500 mg of METREXASSIST per vial as follows:

- Single unit 500 mg vial: Contains 5 mL of TK-112690 (100 mg/mL).

METREXASSIST™ vials must be stored frozen (-15 to -25°C). TK-112690 vials should be protected from light. Store in the original carton until time of use.

DOSE PREPARATION

TK-112690 should be diluted for infusion by a healthcare professional using aseptic technique. Withdraw the necessary amount of TK-112690 to obtain the required dose and dilute in a total volume of 250 mL of 0.9% Sodium Chloride Injection, USP.

Calculation for the amount ofTK-112690 for dilution into the iv bag is presented on the following page. Calculation is based on patient's weight (in kilograms) and desired Dose Level.

The parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Diluted TK-112690 solutions for infusion may be stored at 2-8°C (36-46°F) for up to 8 hours. No incompatibilities between TK-112690 and polyvinylchloride bags have been observed.

CALCULATION OF METREXASSIST FOR DILUTION INTO 250 ML 0.9% SODIUM CHLORIDE IV BAG

Part A. Calculation of mL (milliliters) needed to dilute into iv bag for dose level based on patient's weight in kg (kilograms)

For Dose Levels 45 mg/kg, use supplied 100 mg/mL METREXASSIST (TK-112690 for injection):

Dose Level	*Multiply*	Weight Patient	*Divide*	Concentration of TK-112690	*Equals*	Total mL for Dose Level
45 mg/kg	*x*	____*kg*	*/*	*100 mg/mL*	=	_____*mL*

Example : 85 kg patient

Dose Level	*Multiply*	*Weight of Patient*	*Divide*	*Concentration of* TK-112690	*Equals*	*Total mL for Dose Level*
45 mg/kg	*x*	*85 kg*	*/*	*100 mg/mL*	=	*38.25 mL*

Part B. Calculation of number of vials needed to obtain required mL for 45 mg/kg dose levels

mL Required	*Divide*	mL/ vial	*Equals*	Number of Vials Required
_mL	/	5 mL	=	____ # Vials

Example: 85 kg patient

38.25 mL	*/*	*5 mL*	=	*7.65 Vials*

DOSE ADMINISTRATION

DO NOT ADMINISTER AS AN IV PUSH OR BOLUS. TK-112690 dose should be delivered over 1 hour by IV infusion.

20.4. APPENDIX 4: INFUSION REACTION CRITERIA

Epinephrine (1:1000) for subcutaneous injection, diphenhydramine (12.5 mg to 50 mg) for iv injection, and any other medications and resuscitation equipment for the emergency management of anaphylactic reactions must be available in the room where the infusions are being performed.

Section 1
The subject's study treatment must be discontinued *immediately* if a Grade 4 AE occurs during the infusion of TK-112690, and the subject must be withdrawn from the study. The subject's study treatment must be discontinued *immediately* if a Grade 3 AE occurs during the infusion of TK-112690 and should not be completed even if the subject recovers. Subsequent administration of TK-112690should be preceded by prophylactic premedication as outlined in Section 2. If another Grade 3 infusion reaction occurs during a subsequent treatment day, treatment will again be discontinued for the rest of that infusion. **If the investigator wishes to continue treatment in this subject, there should be discussion with the medical monitor about the possibility of reduced infusion rates for subsequent infusions.**

If Grade 1 or 2 AEs occur during infusion of TK-112690, the subject may complete the treatment at half the previous infusion rate (180 minutes or shorter based on the acceleration of the infusion allowed after the first dose). Subsequent administration of TK-112690 should be preceded with prophylactic treatment as outlined in Section 2. To assess the events, please refer to the NCI CTCAE in the Study Reference Manual.

Treatment Recommendations Prior to, During, and Immediately After Infusion
Section 2 Prophylactic Treatment Prior to Infusion

Prophylactic measures prior to the administration of should not be initiated unless a previous infusion reaction has occurred. Subjects who have previously experienced a reaction to must receive prophylactic treatment prior to subsequent infusions. Prophylactic treatment with acetaminophen (1 g) and diphenhydramine HCl (12.5 to 50 mg; or equivalent dose of a similar agent) by mouth 30 to 60 minutes prior to the start of the infusion is recommended. **Steroids should not be used as pre-medication in this study**.

Section 3 Treatment of Infusion Reactions
The use of acetaminophen plus an antihistamine, such as diphenhydramine (or similar agent), is recommended for the treatment of an infusion reaction. For adjustment to the subject's infusion see Section 1.

Modification of the Subject's Treatment during Therapy

Acetaminophen and diphenhydramine should be repeated as clinically indicated for the infusion reaction. Vital sign measurements will be recorded on the appropriate CRF. Any infusion reaction will be recorded as an AE on the AE CRF. Management of Grade 3 or Grade 4 infusion reaction is outlined in Section

These recommendations do not address life-threatening events, including anaphylaxis, for which study treatment should be discontinued and all appropriate standard measures, including full resuscitation medicine and equipment, must be available and should be used as clinically indicated.

20.5. APPENDIX 5: ICH GUIDELINES, TOPIC E2

ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2

A serious adverse event is any experience that suggests a significant hazard, contraindications, side effect or precaution. It is any adverse event that at any dose fulfils at least one of the following criteria:

- Is fatal (results in death).
  (Note: death is an outcome, not an event).
- Is life-threatening.
  (Note: the term "life-threatening" refers to an event in which the subject was at immediate risk of death at the time of the event, it does not refer to an event which could hypothetically have caused a death it been more severe).
- Required in subject hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Is medically significant or requires intervention to prevent one or more of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor is appropriate on other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

An unexpected adverse event is one, the nature or severity of which is not consistent with the applicable product information.

Causality is initially assessed by the investigator. For SAEs, causality can be one of the two possibilities:

- No (unrelated, equals not drug related)
- Yes (possibly, probably or definitely drug related; or relationship not provided)

The term severe is a measure of intensity, thus a severe adverse event is not necessarily serious. For example, nausea of several hours duration may be rated as severe, but may not be clinically serious.

A serious adverse event occurring during the study or which comes to the attention of the investigator within 28 days after stopping the treatment or during the protocol defined follow-up period, if this is longer, whether considered treatment related or not, must be reported. In addition, a SAE that occurs after this time, if considered related to test "drug," should be reported.

Progression of neoplasia should not be reported as an AE or SAE. Findings that are clearly consistent with the expected progression of the underlying cancer should not be reported as an AE, and hospitalizations due to the progression of cancer do not necessarily qualify for a SAE. If there is any

uncertainty about a finding being due solely to progression of neoplasia, the finding should be reported as an AE or SAE as appropriate.

Such preliminary reports will be followed by detailed descriptions later, which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

For SAEs, the following must be assessed and recorded on the adverse event page of the CRF: intensity, relationship to test substance, action taken, and outcome.

The investigator must notify the Ethics Review Committee/Institutional Review Board of a SAE in writing as soon as is practical and in accordance with international and local laws and regulations.

20.6. APPENDIX 6: MUCOSITIS SCORING

Mucositis scoring will be performed using the following scales and other determinations.

OMAS Scale

Location	Ulceration/pseudomembrane* (circle)				Erythema** (circle)		
Upper lip	0	1	2	3	0	1	2
Lower lip	0	1	2	3	0	1	2
Right cheek	0	1	2	3	0	1	2
Left cheek	0	1	2	3	0	1	2
Right ventral and lateral tongue	0	1	2	3	0	1	2
Left ventral and lateral tongue	0	1	2	3	0	1	2
Floor of mouth	0	1	2	3	0	1	2
Soft palate/fauces	0	1	2	3	0	1	2
Hard palate	0	1	2	3	0	1	2

*Ulceration/Pseudomembrane:
0 = no lesion
1 = < 1 cm^2
2 = 1 cm^2–3 cm^2
3 = > 3 cm^2

**Erythema:
0 = none
1 = not severe
2 = severe

PROMS Scale

Questionnaire for Patient-reported Oral Mucositis Symptom Scale

Presence of infection: Yes No (circle) If yes, circle: local non-oral systemic

Mouth pain

Please indicate by a vertical line on the scale line below how severe the pain in your mouth is NOW

No pain — Most severe pain

Impact on swallowing:

Please indicate by a vertical line on the scale below how well you can swallow.

No trouble in swallowing — Cannot swallow anything at all (even saliva)

Please indicate how well you can swallow foods or liquids by checking below:

Function:

- Normal_____
- Only soft, solid foods_____
- Only liquids_____
- No foods or liquids_____

This questionnaire asks you to evaluate some situations you may have experienced in the past week. All of the situations refer to the condition of your mouth. You can indicate the severity of the situation by placing a vertical mark along the lines below.

First, we will use this type of line to rate temperature as an example.

On a hot day in the middle of the summer, if we asked you to rate how warm it was today, you would probably mark the line as follows:

not warm at all _____|_____ extremely warm

On a cool day in the fall, you might indicate:

not warm at all _____|_____ extremely warm

On a cold day in the winter, you might indicate:

not warm at all _____|_____ extremely warm

To practice: Please tell me how warm it is outside today by placing a mark on the line below.

not warm at all _____ extremely warm

Now that you know how to use this scale, please indicate to what degree these situations have affected you in the past week.

Mouth pain

no pain _____ worst possible pain

Difficulty speaking because of mouth sores

no trouble speaking _____ impossible to speak

Restriction of speech because of mouth sores

no restriction of speech _____ complete restriction of speech

Difficulty eating hard foods (hard bread, potato chips, etc.) because of mouth sores

no trouble eating hard foods _____ impossible to eat hard foods

Difficulty eating soft foods (Jello, pudding, etc.) because of mouth sores

no trouble eating soft foods _____ impossible to eat soft foods

Restriction of eating because of mouth sores

no restriction of eating _____ complete restriction of eating

Difficulty drinking because of mouth sores

no trouble drinking _____ impossible to drink

Restriction of drinking because of mouth sores

no restriction of drinking _____ complete restriction of drinking

Difficulty swallowing because of mouth sores

not difficult to swallow _____ impossible to swallow

Change in taste

no change in taste _____ complete change in taste

WHO Scale for Oral Mucositis

Grade 0 = No oral mucositis
Grade 1 = Erythema and soreness
Grade 2 = Ulcers, able to eat solids
Grade 3 = Ulcers, requires liquid diet (due to mucositis)
Grade 4 = Ulcers, alimentation not possible (due to mucositis)

Mucositis per CTCAE version 4.0

Grade 1 = Asymptomatic or mild symptoms; intervention not indicated.
Grade 2 = Moderate pain; not interfering with oral intake; modified diet indicated
Grade 3 = Severe pain; interfering with oral intake
Grade 4 = Life-threatening consequences; urgent intervention indicated
Grade 5 = Death