Abbreviated Title: The BrEAsT Trial
NIH Protocol #: 20C0056
IBC #: RD-19-IX-02
RSC #: 2746
NCT #: NCT04296942
Version Date: June 01, 2021

NCI Supplement

Title: A Phase Ib Trial of Sequential Combinations of BN-Brachyury, Entinostat, ado-trastuzumab emtansine and M7824 in Advanced Stage Breast Cancer (BrEAsT)

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Investigational Agents:

Drug Name:	BN-Brachyury	Entinostat	M7824	Ado-trastuzumab emtansine (also known as T-DM1 or Kadcyla)
IND Number:	19165	19165	19165	19165
Sponsor:	NCI, CCR	NCI, CCR	NCI, CCR	NCI, CCR
Manufacturer:	Bavarian Nordic	Syndax	EMD Serono, Inc.	CC Pharmacy

Coordinating Center: Center for Cancer Research

Responsible Safety Monitoring Committee: NCI Safety Monitoring Committee

PRÉCIS

Background:

- Current $\geq 2^{nd}$ line treatments for metastatic breast cancer provide modest response rates, and modest improvement in progression-free survival but no treatments are curative.
- Bavarian-Nordic (BN)-Brachyury vaccine is a recombinant poxvirus vaccine against the transcription factor brachyury, which plays an important role in the epithelial-to-mesenchymal transition in breast cancer. A recently completed phase 1 study of BN-Brachyury vaccine showed the vaccine was well tolerated and generated an immune response.
- M7824 is a novel bifunctional fusion protein composed of a monoclonal antibody against human PD-L1 fused to the soluble extracellular domain of human TGF-β receptor II (TGF-βRII), which functions as a TGF-β "trap."
- Ado-trastuzumab emtansine (T-DM1 or Kadcyla) is an antibody drug conjugate used in second- and third- line treatment of metastatic HER2+ breast cancer (HER2+BC). T-DM1 activates ADCC, dendritic cell maturation, increases TILs, increased PD-L1 expression, and increased immunomodulatory cytokines.
- Entinostat is a class 1 histone deacetylase inhibitor (HDACi) which suppresses tumor initiating cells, regulatory T-cells and myeloid-derived suppressor cells (MDSCs), as well as enhances cytotoxic T-cell mediated lysis, direct natural killer (NK) lysis, NK cell activation, increases PD-L1 expression and antibody-dependent cellular cytotoxicity (ADCC). In addition, entinostat may also be able to overcome HER2 resistance.
- We propose a Phase 1b trial to evaluate the safety and efficacy of the stepwise combination of the BN-Brachyury vaccine, M7824, T-DM1 and entinostat in metastatic breast cancer.
    - Arm 1 – Triple Negative Breast Cancer (TNBC); M7824 + BN-Brachyury
    - Arm 2 – ER-/PR-/HER2+ Breast Cancer; M7824 + BN-Brachyury + T-DM1
    - Arm 3 - ER-/PR-/HER2+ Breast Cancer; M7824 + BN-Brachyury + T-DM1+ Entinostat

Objectives:

Primary Objectives:

- Arms 1-3: Overall response rate (ORR; PR+CR)
- Arms 1-3: Safety for each of the three combinations of agents explored in the arms

Eligibility:

Selected Inclusion Criteria

- Histologically confirmed metastatic breast cancer with appropriate IHC testing by a certified lab:
    - For Arm 1: Triple negative breast cancer. Hormone receptor negative is defined by estrogen receptor < 10% and progesterone receptor < 10%. HER2 negative breast cancer is defined as HER2 per IHC 0 or 1+ or 2+ with negative FISH.
    - For Arms 2 and 3: Hormone receptor negative, HER2+ breast cancer as defined by estrogen receptor < 10% and progesterone receptor < 10%. HER2 positive as per IHC 3+ or 2+ with positive FISH.

- Prior treatment:
    - For Arm 1: ≥1 prior therapy in the metastatic setting. Patients with known PD-L1 positive tumors must have received prior treatment with atezolizumab + nab-paclitaxel. Patients with ER 1-9% must have received treatment with at least two lines of endocrine treatment (SERM, AI, fulvestrant) with one prior treatment including a CDK4/6 inhibitor + endocrine therapy for their metastatic cancer and should be considered endocrine therapy resistant.
    - For Arms 2 and 3: ≥ 1 prior treatment in the metastatic setting with a taxane (docetaxel or paclitaxel), herceptin and pertuzumab.
- Females or males ≥ 18 years old
- ECOG 0 or 1
- Measurable metastatic disease per RECIST 1.1.
- For Cohort 3, Arms 2 and 3: At least one biopsiable lesion and willingness to undergo up to three research biopsies.
- Adequate hematopoietic, hepatic, renal and cardiac (EF ≥ 50%) function.

Selected Exclusion Criteria

- Patients who have received chemotherapy, including trastuzumab and pertuzumab in the previous 3 weeks; other investigational agents within 4 weeks or a PD-1/PD-L1 antibody within 4 weeks prior to study enrollment; radiotherapy ≤ 4 weeks of study entry.
- Symptomatic CNS metastases and leptomeningeal disease are excluded but treated brain metastases (no radiotherapy within 6 weeks) or asymptomatic brain metastasis are allowed.
- History of invasive malignancy ≤ 3 years prior to enrollment.
- History of congestive heart failure (CHF) as defined as NYHA class 3 or 4 or hospitalization for CHF (any NYHA class) within 6 months of trial start.
- Concurrent use of chronic systemic steroids except for physiologic systemic steroids for replacement defined as 10mg of prednisone or an equivalent dose.

Design:

This multicenter study contains three separate, single arm phase 1b trials.

- Arm 1 will evaluate M7824 and BN-Brachyury in patients with TNBC.
- If this doublet is determined to have acceptable toxicity (0-1 DLTs of the first 6 patients), up to 19 patients will be enrolled on Arm 2 in which BN-Brachyury, M7824 and T-DM1 will be evaluated in patients with advanced HR-/HER2+ BC with disease progression after treatment with THP or intolerance to THP.
- If this triplet is determined to have acceptable toxicity (0-1 DLTs of the first 6 patients), 19 patients will be enrolled on Arm 3 in which BN-Brachyury, entinostat, M7824 and T-DM1 will be evaluated in patients with advanced HR-/HER2+ BC with disease progression after treatment with THP or intolerance to THP.
- Up to 51 evaluable patients will be recruited for this study, with an accrual ceiling set at 65 patients.

Trial Drugs

- BN-Brachyury vaccine every 3 weeks until cycle 9, then every 12 weeks:

    - Recombinant MVA-BN-Brachyury (R2PD): 4 injections of vaccines with 1 given SC in each extremity on Day 1 of Cycles 1 and 2. Each injection of MVA-BN-Brachyury consists of 2.0 x 10^8 infectious units (Inf.U).
    - Recombinant FPV-Brachyury: 1 injection given SC in one extremity on Day 1 of Cycles 3 and beyond. Each injection of FPV-Brachyury consists of 1.0 x 10^9 Inf.U.
- T-DM1 3.6mg/kg via IV infusion q3 weeks on Day 1 of each cycle.
- M7824 2,400mg via IV infusion q3 weeks on Day 1 of each cycle.
- Entinostat 5mg by mouth weekly (RP2D) administered by patient on Days 1, 8, and 15 of each cycle.

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1 NUMBER OF PATIENTS TO BE SEEN AT NIH

We plan to enroll up to 65 patients on this study.

We plan to enroll 35 participants at the NIH.

2 NCI RECRUITMENT AND SCREENING ACTIVITIES

2.1 RECRUITMENT STRATEGIES

Please refer to Section 2.3 of the Sponsor Protocol for recruitment strategies.

2.2 SCREENING EVALUATION

2.2.1 Screening activities prior to the obtaining informed consent

Please refer to Section 2.4.1 of the Sponsor Protocol for screening activities prior to the obtaining informed consent.

2.2.2 Screening activities performed after a consent for screening has been signed

The following screening procedures will be performed only after the subject has signed the study consent or the consent for study # 01C0129 (provided the procedures are permitted on that study) on which screening evaluations or tests will be done within 14 days prior to enrollment, except when specified differently. Assessments performed at outside facilities or on another NIH protocol within these timeframes and those below may also be used to determine eligibility once a patient has signed the consent.

2.2.2.1 Pathologic Confirmation

Pathological confirmation of diagnosis and ER, PR and HER2 expression in the Laboratory of Pathology at NIH Clinical Center or Walter Reed National Military Medical Center at Bethesda.

However, if no pathologic specimen is available, patients may enroll with a pathologists report showing a histologic diagnosis of TNBC or HER2-positive breast cancer in a College of American Pathologists (CAP) accredited laboratory (or other accrediting entity) and a clinical course consistent with the disease.

2.2.2.2 Screening Visit: Physical Exam, Labs and Imaging

Please refer to Section 2.4.2.2 of the Sponsor Protocol for the screening visit procedures.

3 NCI REGISTRATION PROCEDURES

3.1 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

3.2 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure

participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a transient lab abnormality may be rescreened.

4 NCI TREATMENT ASSIGNMENT PROCEDURES

Please refer to Section 2.5 of the Sponsor Protocol for Cohorts, Arms, and Arm Assignment procedures.

5 COST AND COMPENSATION

5.1.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

5.1.2 Compensation

Participants will not receive compensation on this study.

5.1.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

6 CORRELATIVE STUDIES FOR RESEARCH / PHARMACOKINETI STUDIES

6.1 BIOSPECIMEN COLLECTION

Please refer to Section 5.1 of the Sponsor Protocol for the biospecimen collection procedures.

Biopsy Acquisition

Samples will be picked up and delivered for processing and storage by the contacts listed below:

- Primary contact: Ryan Joyce, 240-760-6180, pager 11609
- Backup contact: Trung Pham, 301-760-6180, pager 11609

6.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

6.2.1 Sample Management and Storage at Clinical Services Program – Leidos Biomedical Research, Inc. (CSP)

Clinical Services Program - Leidos Biomedical Research, Inc.
Attn: Theresa Burks
1050 Boyles Street
Bldg. 469/Room 121
Frederick, MD 21702

On days samples are drawn, Jen Bangh at CSP (part of NCI Frederick Central Repositories) should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens.

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80ºC freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing patient specimens. The subcontractor does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only coded, linked samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the

withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input, and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

6.2.2 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

6.2.2.1 BPC contact information

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

6.2.2.2 Sample Data Collection, Sample Storage, Procedures for Storage of Tissue Specimens in the Laboratory of Pathology, Protocol Completion/Sample Destruction

Please refer to Section 5.2.2.2, 5.2.2.3, 5.2.3, 5.2.4 of the Sponsor Protocol for these procedures.

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

Please refer to Section 7 of the Sponsor Protocol for reporting requirements and monitoring plan procedures.

7.1 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.2 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.2.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of BN-Brachyury as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the BN-Brachyury, but are not fatal or life-

threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.2.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.2.3 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial;
- clinical site;
- the Principal Investigator;
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons;
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed;
- if the trial has been completed, a brief description of any study results.

7.2.4 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death

- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.3 NIH Required Data and Safety Monitoring Plan

Please refer to Section 7.4 of the Sponsor Protocol for the study team and safety monitoring committee procedures.

8 COLLABORATIVE AGREEMENTS

8.1 Cooperative Research and Development Agreement (CRADA)

Through a collaborative research and development agreement (CRADA), three study drugs have been secured for use in this clinical trial.

The BN-Brachyury vaccines will be provided by Bavarian Nordic through CRADA 05261 with LTIB.

Entinostat will be provided by Syndax through CRADA 03121 with LTIB.

M7824 will be provided by EMD Serono, Inc. through CRADA 02666 with LTIB.

9 HUMAN SUBJECTS PROTECTIONS

Please refer to Section 12.1 and 12.2 of the Sponsor Protocol for the rationale for subject selection and participation of children.

9.1 Evaluation of Benefits and Risks/Discomforts

Please refer to Section 12.4 of the Sponsor Protocol for the risks and benefits of participation on this study.

9.2 Consent Process and Documentation

Please refer to Section 12.6 of the Sponsor Protocol for the consent process and documentation.

Note: For the NIH site, the initial consent process or re-consent may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant.

Manual (non-electronic) signature on electronic document:

> When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the Adobe platform (which is not 21 CFR Part 11 compliant) to obtain the required signatures.
>
> During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations.
>
> Both the investigator and the subject will sign the document using a finger, stylus or mouse.

Electronic signature on electronic document:

When permitted by the NIH Clinical Center, an electronic signature may be obtained using the iMedConsent platform to obtain the required signatures once it is designated as 21 CFR Part 11 compliant.

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations.

The identity of the participant will be determined by a prompt which will require the provision of information from a form of government-issued identification prior to obtaining the signature. If participant does not have such identification available, security questions will be used to confirm identity.

Both the investigator and the subject will sign the document electronically per system prompts.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

Please refer to Section 13 of the Sponsor Protocol for study discontinuation and closure, quality assurance and quality control, and conflict of interest procedures.

Abbreviated Title: The BrEAsT Trial
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Phone: 301-480-7174
E-mail: fatima.karzai@nih.gov

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Sponsor:	NCI, CCR	NCI, CCR	NCI, CCR	NCI, CCR
Manufacturer:	Bavarian Nordic	Syndax	EMD Serono, Inc.	Will be purchased from commercial sources.
Supplier:	Bavarian Nordic	Syndax	EMD Serono, Inc.	Local site

Coordinating Center: Center for Cancer Research

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PRÉCIS

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

- Arm 1: M7824 + BN-Brachyury in TNBC
  - To determine if the addition of BN-Brachyury and M7824 improves overall response rates (ORR) in triple negative breast cancer (TNBC).
  - To determine the clinical safety of BN-Brachyury and M7824 in breast cancer
- Arm 2: M7824 + BN-Brachyury + T-DM1 in HER2+BC.
  - To determine if the addition of BN-Brachyury and M7824 to T-DM1 improves ORR in HER2+ breast cancer (HER2+BC) over T-DM1 alone.
  - To determine the clinical safety of BN-Brachyury, M7824 and T-DM1 in breast cancer.
- Arm 3: M7824 + BN-Brachyury + T-DM1 + Entinostat in HER2+BC
  - To determine if the addition of BN-Brachyury, M7824, and Entinostat to T-DM1 improves ORR in HER2+BC over T-DM1 alone.
  - To determine the clinical safety of BN-Brachyury, M7824, Entinostat and T-DM1 in breast cancer.

1.1.2 Secondary Objectives

- Arm 1: M7824 + BN-Brachyury in TNBC
  - To determine if adding BN-Brachyury to M7824 improves progression-free survival (PFS) in TNBC.
- Arm 2: M7824 + BN-Brachyury + T-DM1 in HER2+BC
  - To determine if adding BN-Brachyury, M7824 to T-DM1 increases the PFS in patients with metastatic HER2+ metastatic breast cancer who progressed on initial treatment with THP (or intolerant to THP).

  - To determine if adding BN-Brachyury, M7824 to T-DM1 increases the absolute percentage of stromal TILs (as measured by the Salgado method) in metastatic deposits in patients with metastatic HER2+ metastatic breast cancer.
- Arm 3: M7824 + BN-Brachyury + T-DM1 + Entinostat in HER2+BC
  - To determine if adding BN-Brachyury, entinostat, M7824 to T-DM1 increases PFS in patients with metastatic HR-/HER2+ metastatic breast cancer who progressed on initial treatment with THP (or intolerant to THP).
  - To determine if adding BN-Brachyury, entinostat, M7824 to T-DM1 increases the absolute percentage of stromal TILs (as measured by the Salgado method) in metastatic deposits in patients with metastatic HER2+ metastatic breast cancer.

1.1.3 Exploratory Objectives

Studies may be performed on selected patients if adequate samples are available:

- Peripheral blood mononuclear cells (PBMCs):
  - Changes in immune cell subsets.
  - Changes in brachyury-specific, MUC1-specific, and CEA-specific immune cell subsets.
  - Histone acetylation as surrogate marker for entinostat pharmacodynamics (Arm 3 only).
- Plasma/Serum:
  - Changes in brachyury-specific, MUC1-specific, and CEA-specific antibodies.
  - Changes in soluble factors like sCD27 and sCD40.
  - Changes in cell free DNA.
  - Changes in TGF-beta levels.
  - Changes in pharmacokinetics of M7824.
- Tissue Biopsy (Cohort 3, Arms 2 and 3 only):
  - Brachyury expression on tumor.
  - T-cell clonality score.
  - Immunohistochemistry/multispectral imaging.
  - Changes in PD-L1 on tumor.
  - Neoepitopes derived from tumor biopsies.
  - HER2 expression on tumor biopsies.
  - Tumor mutational burden.

1.2 Background and Rationale

Invasive breast cancer remains the most common malignancy in women in the United States (US) with an annual incidence of 123.1 cases per 100,000 women. (1, 2) Treatment of invasive breast cancer involves local control with surgery and/or radiation where appropriate, as well as systemic control with endocrine therapy, HER2 targeted therapy, and/or chemotherapy. Despite advances in early detection and effective treatments, breast cancer is the second most common cause of US cancer deaths in women with an annual rate of 21.9 deaths per 100,000 women. (1) It is estimated that in 2018, there will be 266,120 new cases of breast cancer with 40,920 deaths. (3) Triple negative breast cancer (TNBC) comprises approximately 10-15% of all breast cancers and HER2+ breast cancer (HER2+BC) also accounts for approximately 15-20% of breast cancers. (4) The majority of breast cancer is hormone receptor positive.

1.2.1 Current Standards of Care for Advanced Breast Cancer

Recurrent/Refractory Advanced Triple Negative Breast Cancer

TNBC is defined by lack of estrogen (ER) and progesterone (PR) receptor staining on immunohistochemistry (IHC) as well as absence of HER2 amplification. (5) Thus, patients with TNBC do not benefit from known targeted therapies to endocrine receptors or HER2 amplification. (6) Standard therapy for metastatic TNBC is single agent chemotherapy and, in metastatic patients, ORRs are around 30-35%, with median PFS 4.5-6 months, and median OS around 12 months. (7) Clearly, new approaches to the treatment of TNBC are needed to improve outcomes.

While recent guidelines by the American Society of Clinical Oncology and the American College of Pathologists recommend that ER or PR status should be considered positive if 1% or more of the tumor cells demonstrate positive nuclear staining with IHC, it is becoming clearer that tumors with low ER or PR staining, defined as 1 to 9% on IHC, often behave more like TNBC than like hormone receptor positive breast cancers. (8, 9) Only about 6% of breast cancers are low ER or PR 1 to 9%. (10) Molecular analysis shows similar ER gene signature scores in ER negative tumors and 1 to 9% IHC ER+ tumors. Furthermore, among patients with 1 to 9% ER+ tumors, 48% of these tumors had gene expression signatures that were basal-like, with only 8% of these tumors being identified as luminal B. (8) Clinically these tumors gain likely little benefit from endocrine therapy. Due to the molecular profile and clinical course, many clinical trials including TNBC now have relaxed the definition of TNBC from <1% on IHC to < 10% ER or PR staining on IHC.

Recurrent/Refractory Advanced HER2+ Breast Cancer

HER2+BC is defined by IHC staining of 3+ in 10% or more of tumor cells or HER2 gene amplification by an in situ hybridization (ISH) method (average HER2 copy number $\geq$ 6.0 signals/cell or HER2/chromosome enumeration probe [CEP]17 $\geq$ 2.0 ratio). (11) Historically, HER2+BC is an aggressive tumor and carried a very poor prognosis prior to the advent of HER2 targeted therapy. (12) With the addition of trastuzumab, and more recently pertuzumab, to a taxane backbone, survival has improved but no therapies are curative.

First line treatment of metastatic HER2+BC with docetaxel, trastuzumab, and pertuzumab (THP) carries a median progression free survival (PFS) of 18.7 months and a median overall survival (OS) of 56.6 months. (13) Patients remain on trastuzumab +/- pertuzumab maintenance until disease progression at which patients switch to another HER2-tagerted therapy like T-DM1, lapatinib + capecitabine, or patients can continue with trastuzumab plus another agent (i.e., capecitabine, lapatinib or chemotherapy).

After disease progression on initial HER2-targeted therapy, subsequent HER2-targeted therapies carry a median PFS of 6 to 9.6 months and a median OS of 15-30 months (**Table 1**). (14-19) The EMILIA study demonstrated a survival

Table 1: Clinical benefit of current standard of care metastatic HER2+ breast cancer regimens.

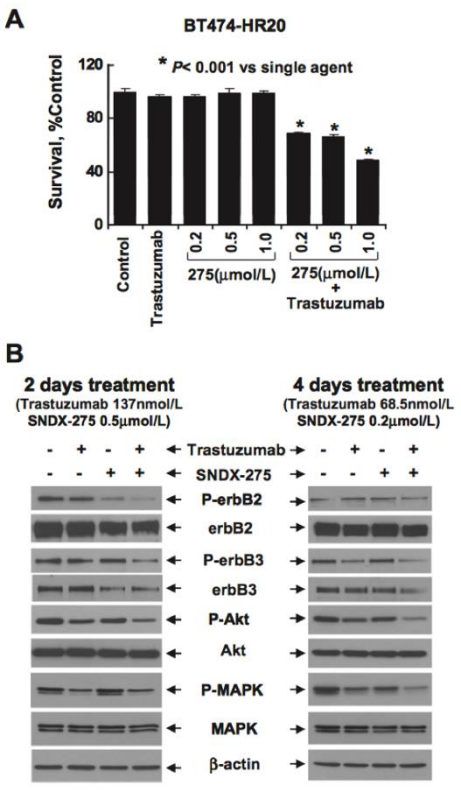
Lapatinib + Capeciabine	7-8.3 months	15-17 months	22 to 33%
T-DM1	9.6 months	29.9 months	44%
Trastuzumab + Capecitabine	8 months	24-25 months	20 to 48%
Trastuzumab + Lapatinib	3 months	15.1 months	10%
Trastuzumab + Chemotherapy	5-9 months	15-31 months	47 to 78%

advantage with second-line ado-trastuzumab emtansine (T-DM1 or Kadcyla) compared to lapatinib + capecitabine in patients who were previously treated with a taxane plus trastuzumab. (20) However, second-line studies involving T-DM1 have mainly been performed in patients who did not receive pertuzumab in the first line metastatic setting, as these studies were designed before a survival advantage was seen with THP in metastatic HER2+BC. Recent retrospective, population-based analyses of patients treated with T-DM1 after first line treatment with THP shows decreased "real world" efficacy of T-DM1. Patients with HER2+BC who received second line T-DM1 after first-line treatment with THP had a response rate of 18% and duration of treatment of 4 to 5 months. (21, 22) This is consistent with other reported clinical data and the clinical experience of the primary investigator.

1.2.2 Immune Modulation and Overcoming HER2 Resistance

HER2-Targeting Agents

Trastuzumab is a humanized monoclonal antibody directed against domain IV of the HER2 extracellular domain. (23) It binds to FcyRIII on immune effector cells and is a potent mediator of antibody-dependent, cell-mediated cytotoxicity (ADCC), increases immunomodulatory cytokines and cross-presentation of tumor antigens. (24, 25)



T-DM1 is an antibody drug conjugate that is composed of humanized antibody trastuzumab and DM1, a maytansinoid derivative, linked with a non-reducible thioether linker. Maytansoid are antimitotic agents and prevent microtubule assembly. (26) Like trastuzumab, T-DM1 binds to FcyRIII and activates ADCC. (27) T-DM1 also induces dendritic cell and NK cell maturation, increases TILs, and increased immunomodulatory cytokines like type 1 and type 2 interferons, increases cross-presentation of tumor antigens and increases PD-L1 on tumor cells. (24, 27, 28)

Histone Deacetylase Inhibitors

Histone deacetylase inhibitors (HDACi) are important enzymes in epigenetic regulation of gene expression. For example, HDAC1 is a key determinant in reversal of carcinoma immune escape. Multiple studies have demonstrated HDACi have anti-cancer activity in multiple tumor types through dysregulating cell cycle progression, causing apoptosis and interrupting tumor angiogenesis. (29, 30)

Figure 1: Entinostat (SNDX-275) synergizes with trastuzumab and overcomes HER2 resistance (A) through downregulation of (B) HER2 and HER3 as well as decreased AKT signaling. *Image from Huang et al., 2011.*

Entinostat is a class I-selective HDACi that has shown activity in multiple subtypes of breast cancer. (31) Monotherapy with weekly entinostat is well tolerated based on the Phase 1 study in which there were no grade 4 toxicities and the only dose-limiting grade 3 toxicities included hypophosphatemia, hyponatremia and hypoalbuminemia – all of which were reversible. To date, there is an acceptable toxicity profile with > 900 patients treated with entinostat. The most common adverse events reported are fatigue, nausea and vomiting.

A phase II study called ENCORE-301 found that when added to exemestane, weekly entinostat (5mg) prolonged median PFS and reduced the risk of disease progression. (32) In this trial, there was a similar serious adverse event rate between the two arms (16% exemestane + entinostat vs 12% exemestane + placebo) with most common AEs reported being neutropenia (13%), fatigue (11%), and nausea/vomiting (5%). Entinostat with exemestane is currently being evaluated in a Phase III study (NCT02115282).

Preclinical data has also indicated that entinostat can reverse acquired HER2 resistance (**Figure 1**). When combined with trastuzumab, entinostat downregulated HER2 and HER3 and inactivated P13K/AKT signaling – the two main mechanisms of trastuzumab resistance. (31, 33) There is an on-going trial involving entinostat in combination with trastuzumab +/- lapatinib (NCT01434303). Preliminary reports of efficacy demonstrated no benefit but showed an appropriate safety profile. (34) When used in combination with trastuzumab and/or lapatinib, 10mg every 2 weeks resulted in no reported grade 3 or 4 toxicities and no dose reductions (per Syndax, 5mg weekly is equivalent to 10 mg every 2 weeks based on PKs).

In addition, HDACi are known to have immunomodulatory effects. HDACi upregulate MHC class I and II, resulting in enhanced antigen-mediated cancer cell killing through a HLA-restricted mechanism. (30, 35, 36) They also suppress tumor-initiating cells, regulatory T-cells and myeloid-derived suppressor cells. Entinostat and vorinostat (a pan HDACi) both have been shown to increase the sensitivity of prostate cancer and breast cancer cells to cytotoxic T-cell mediated lysis, direct NK lysis and ADCC. (30, 37) An immune subset analysis from the ENCORE-301 (exemestane +/- entinostat) trial demonstrated an increase in HLA-DR-positive monocytes and a decrease in granulocytic and monocytic myeloid-derived suppressor cells. (38) Furthermore, in preclinical studies immunogenic modulation was seen against a broad range

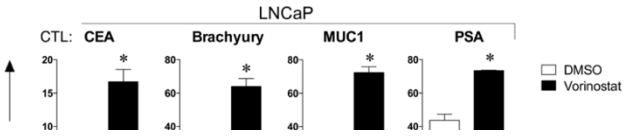


Figure 2: Carcinoma cells exposed to vorinostat are significantly more sensitive to CTL-mediated killing. Human prostate (LNCaP) and breast (MDA-MB-231) carcinoma cerlls were exposed to vorinostat (3 µM, black bars) or to vehicle (DMSO, open bars) prior to being used as tarets for antigen-specific CTL lysis using CAE-, brachyury-, MUC1-, or PSA-specific CD8+ cells as effector cells (E:T = 30:1). To verify the effector T cells were HLA-restricted, CTLs were incubated with HLA-A2 negative AsPC-1 pancreatic carcinoma cells exposed to vehicle (DMSO) or vorinostat. Results are presented as mean +/- S.E.M. from 3-6 replicate wells, and are representative of 1-4 independent experiments. Asterisks denote statistical significance relative to controls. *Image from Gameiro et al., 2016.*

of tumor-associated antigens including brachyury, CEA, MUC1 and PSA (**Figure 2**) and this was associated with increased exposure of multiple proteins involved (39) in antigen processing and tumor recognition (30), making it an ideal companion for a vaccine.

Entinostat also increases cell-surface PD-L1 expression in carcinoma in vitro and in vivo. Entinostat promotes an inflamed tumor signature and has the potential to convert a non-inflamed "cold" tumor to an inflamed "hot" tumor signature through immunogenic modulation. (39)

HDAC inhibitors synergize with immunotherapy through increased CD8+ T cells and NK cell trafficking. Studies have also demonstrated the generation of long-term immunologic memory. (40) The Phase 1 study ENCORE-601 evaluated the safety of Entinostat 5mg weekly (*starting dose in this study*) with Pembrolizumab in patients with NSCLC or with melanoma. (41, 42) Patients tolerated treatment well with no increase in immune related adverse events (irAEs) over what is expected with checkpoints. Furthermore, clinical efficacy is seen in patients with prior resistance to checkpoints.

1.2.3 Checkpoint Inhibitor Experiences in Breast Cancer

PD-1/L1 antibodies have demonstrated promise in multiple malignancies. However, the results of clinical trials evaluating single agent anti-PD-1/L1 antibodies in breast cancer have been underwhelming to date with objective response rates (ORR) of 2-10% in unselected breast cancers and ORR of 5-44% in patients with PD-L1 positive breast tumors. (43-45) TNBC have responded better to anti-PD-1/L1 antibodies than other breast cancer subtypes (TNBC = 15-20% in PD-L1+ tumors; TNBC = 8-10% in unselected PD-L1+ tumors; ER+ = 12% in PD-L1+ tumors; HER2+ = 3.8% in unselected tumors). Due to lack of efficacy seen in early trials (43), as well as initial concerns about combining anti-PD-1/L1 antibodies with HER2-targeted therapies, few checkpoint trials have been conducted in HER2+BC. Loi et al. recently demonstrated a good safety profile for the combination of pembrolizumab and trastuzumab in the PANACEA study for patients with trastuzumab-resistant breast cancer. Furthermore, there was suggestion of increased clinical benefit with an ORR of 15% and a durable clinical response (defined as complete response, partial response or stable disease $\geq$ 6 months) of 24% in PD-L1 positive tumors. (46) Atezolizumab + T-DM1 compared to T-DM1 + placebo was evaluated in the KATE2 study. There was no improvement in the primary trial endpoint, progression free survival, between the two arms and overall survival data is still maturing. There were similar grade 3 to 5 AEs reported in the two arms (43.1% Atezolizumab + T-DM1 vs 41.2% Atezolizumab + placebo); however, there were more patients with adverse events (mostly grade 1 or 2) reported in the Atezolizumab + T-DM1 arm (32.6% vs 19.1%). (47)

1.2.4 M7824: A bifunctional fusion protein with anti-TGF-β and anti-PD-L1

Transforming growth factor β (TGF-β) is a pleiotropic cytokine. In the premalignant state, it has tumor-suppressive effects and suppresses tumorigenic inflammation. However, in patients with advanced cancers including breast cancer, TGF-β is associated with malignant progression, evasion of immune surveillance, invasion, and metastasis. (48-50)

The role of TGF-β1 signaling differs based on breast cancer subtype. Hormone receptor positive breast cancer cell lines are not as sensitive to TGF-β inhibition as hormone receptor negative breast cancer cell lines. (51) Higher levels of TGF-β are associated with greater invasive and metastatic potential and tamoxifen resistance. (51, 52) Furthermore, a functional synergy between TGF-β and HER2 has been described. Higher levels of TGF-β1 and TGF-β3 have been

identified as potential contributors to HER2 resistance and blockade of TGF-β:HER2 interaction may reverse HER2 resistance leading to improve efficacy of HER2-targeted therapy. (53, 54) The direct effects of TGF-β on T cells include decreases in perforin, granzymes, interferon gamma, FAS ligand, and natural killer group 2D (NKG2D). It can also decrease NKG2D and major histocompatibility complex (MHC) class I polypeptide sequence A (MICA) in NK cells. (55) Elevated levels of TGF-β have been found to correlate with poor outcomes in many different human cancers. (56-59) Prompted by these observations, antibodies (e.g., fresolimumab) and small-molecule inhibitors (e.g., galunisertib) targeting the TGF-β pathway have entered clinical development, where they have demonstrated initial signs of efficacy in breast cancer, hepatocellular carcinoma, pancreatic cancer, and melanoma. (60, 61)

PD-L1 expression on tumor cells has also been associated strongly with poor prognosis in a variety of human cancers. (62-65) In recent years, a number of agents targeting the PD-1/PD-L1 pathway have received regulatory approval, demonstrating impressive durations of response for multiple tumor types, including melanoma, non-small cell lung cancer, renal cell cancer, and head and neck cancer. (66-74) Notably, atezolizumab, durvalumab and avelumab are all anti–PD-L1 antibodies with proven efficacy and regulatory approval. (75, 76) Unfortunately, not all cancer types seem to respond to these agents, and, even among susceptible cancer types, the percentage of responding patients is usually < 20%. (77)

In an effort to increase the rate of response to these therapies, many ongoing trials are evaluating anti–PD-1/PD-L1 agents in combination with other immunotherapies. (78) Importantly, combined inhibition of PD-L1 and TGF-β is a promising therapeutic strategy because these key pathways have independent and complementary immunosuppressive functions; therefore, their dual inhibition may result in synergistic antitumor activity.

M7824 is a novel bifunctional fusion protein composed of a fully human IgG1 monoclonal antibody against human PD-L1 fused, via a flexible glycine-serine linker, to the soluble extracellular domain of human TGF-β receptor II (TGF-βRII), which functions as a TGF-β "trap." Preclinical studies of M7824 have shown its ability to simultaneously bind PD-L1 and TGF-β, as well as appropriately block PD-L1 signaling and TGF-β signaling in vitro. M7824 was shown to have better antitumor efficacy than anti-PD-L1 or TGF-β trap control (a mutated antibody that doesn't bind to PD-L1, linked with the TGF-β trap) monotherapies in both MC38 and EMT-6 tumor models, performed in both wild-type mice and B cell deficient mice. Because M7824 is very immunogenic in mice, due to the fully human antibody and its immune stimulatory mechanism of action, host anti-drug antibodies preclude continued dosing. In an orthotopic EMT-6 breast cancer model using B cell-deficient Jh mice M7824 showed much better antitumor activity than either anti-PD-L1 antibody or TGF-β trap control alone (**Figure 3A**). At tumor re-challenge, 13/13 mice previously cured with M7824 treatment were completely protected (resistant to tumor; **Figure 3B**). Furthermore, M7824 therapy conferred a long-term protective immunity that extended survival.(79)

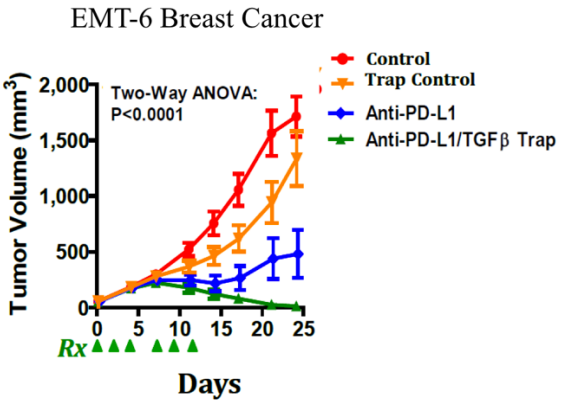


Figure 3a : Preclinical studies using B cell-deficient Jh mice with subcutaneous EMT-6 breast cancer tumors showing significantly reduced tumor volume with M7824 (anti-PD-L1/TGFβ) compared with either anti-PDL1 antibody or TGF-β trap alone.

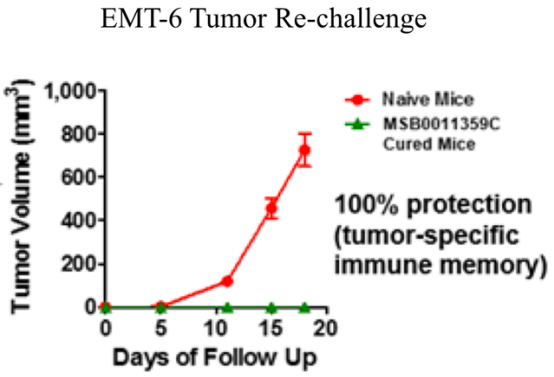


Figure 3b: Mice previously cleared of their EMT-6 breast cancer tumors after treatment with M7824 were resistant to tumor re-challenge with EMT-6 cells

Figure 3: Data from preclinical studies of EMT-6 Breast Cancer tumors and M7824 rechallenge.

Furthermore, LTIB has shown that M7824 (a) mediates antibody-dependent cellular cytotoxicity (ADCC), (b)increases tumor cell gene expression of molecules involved in T-cell trafficking to the tumor (e.g., CXCL-11), (c) enhances TRAIL- and antigen-specific CD8+ T-cell lysis of tumor cells and (d) reduces TGF-β-induced signaling in the TME. (80, 81)

Based on these preclinical data, a Phase I 3+3 dose-escalation study was completed to evaluate the pharmacokinetics (PK), safety, tolerability, and biological and clinical activity of M7824 in patients with advanced solid tumors (NCT02517398).

Sixteen heavily pretreated patients received M7824 at 1, 3, 10, or 20 mg/kg once-every-2-weeks. M7824 was shown to saturate peripheral PD-L1 at doses ≥ 3 mg/kg and sequester plasma TGF-β1, -β2, and -β3 throughout the dosing period at ≥1 mg/kg. The only DLT observed was colitis with associated anemia (20 mg/kg). No MTD was reached. Grade 3 treatment-related adverse events occurred in 3 patients (skin infection secondary to localized bullous pemphigoid (3 mg/kg), asymptomatic lipase increase (20 mg/kg), and colitis (20 mg/kg) with associated anemia). These toxicities are on par with other PD-1/PD-L1 inhibitors. The only added toxicity seen over traditional PD-1/PD-L1 inhibitors was the occurrence of keratoacanthomas which have been described previously with TGFβ inhibitors in 10-15% of patients. (82, 83) There were no treatment-related grade 4–5 events. (84)

An expansion cohort in TNBC was recently completed by EMD Serono. This data is not published to date, but there are 2 reported responses in the 22 patients on the TNBC expansion cohort (ORR = 10%). The ORR of single agent M7824 in TNBC is similar to that of other single agent checkpoint inhibitors.

1.2.5 Brachyury Expression in Breast Cancer and Brachyury Vaccine Development

Brachyury is a member of the T-box family of transcription factors. It is overexpressed in cancer cells (including breast) compared with normal tissue and has been linked to chemotherapy resistance and metastatic potential. Brachyury induces the phenomenon of mesenchymalization in human carcinoma cells which is associated with more invasive metastatic tumor behavior and with resistance to chemotherapy, radiation, small molecule-targeted therapies and immunotherapies (**Figure 4**). (85, 86)

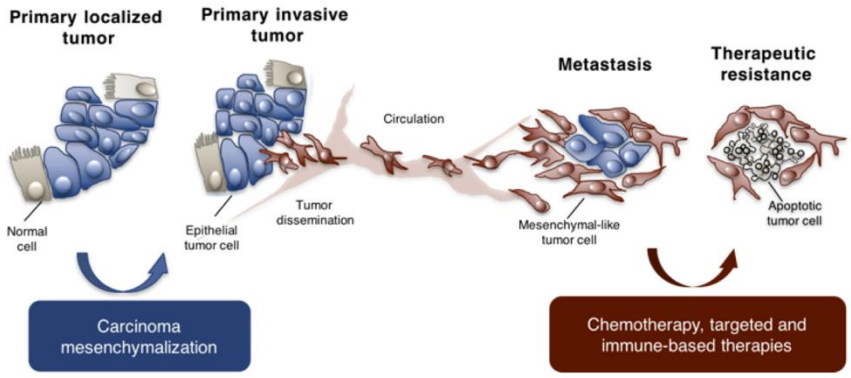


Figure 4: Role of the phenomenon of mesenchymalization in tumor metastasis and acquisition of therapeutic resistance. Adapted from *Palena and Hamilton, 2015.*

Analysis of expression of indicated transcripts in the breast cancer TCGA (The Cancer Genome Atlas) dataset showed that all breast cancer subtypes have some degree of brachyury expression. (87) Brachyury expression correlates most closely with hormone receptor negativity (**Figure 5**). HER2 expression does not appear to have a significant impact on brachyury expression. Furthermore, brachyury expression is higher in tumor-positive lymph nodes than in the primary tumor. (85, 87) In addition, metastatic lesions (pleura, bone and brain) also exhibited high levels of brachyury expression.

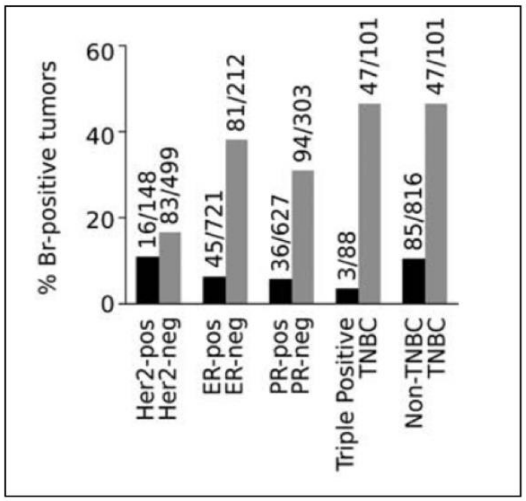


Figure 5: Percentage of tumors in the TCGA database that are positive for brachyury mRNA expression in samples classified based on their hormone receptor classification. *Image from Hamilton et al., 2016.*

High brachyury expression has been correlated with worse clinical outcomes in small sample of TNBC patients (n =58). Even after adjusting for clinical-pathologic variables like stage and p53 expression, patients with higher brachyury expression experienced significantly shorter relapse-free survival compared with patients with low brachyury expression (HR 3.92; CI 1.02 to 15.1; p = .047). (87)

This association with worse clinical outcome was confirmed in another larger study. (85) Breast tumor gene expression data (n = 4010) derived from 23 datasets from the Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI) was compiled. Each sample was assigned into low (first quartile, lowest 25%), intermediate (second quartile, intermediate 50%) and high (third quartile, highest 25%) subgroups according to brachyury

mRNA levels. A Kaplan-Meier estimate of survival was used to evaluate differences in prognosis among the 3 subgroups. Among 357 breast cancer patients treated with tamoxifen monotherapy as adjuvant therapy for 5 years post-diagnosis, high brachyury expression significantly correlated with higher risk of recurrence (p = 0.0283, n = 357, **Figure 6A**) and distant metastasis (p = 0.0150, n = 332; **Figure 6B**). To minimize the effect of clinical confounding factors such as tumor size, grade, nodal status, age, HER2, ER and PR status that might cause false positive association between gene expression and poor prognosis, a Cox Proportional-Hazards Regression (COXPH) survival analysis was conducted (n = 270). When mRNA expression signal was used as a continuous variable, increased expression of brachyury was significantly associated with higher risk of recurrence (p = 2. 270x10^{-5}, n=270) and distant metastasis (p = 0.0001, n = 270).

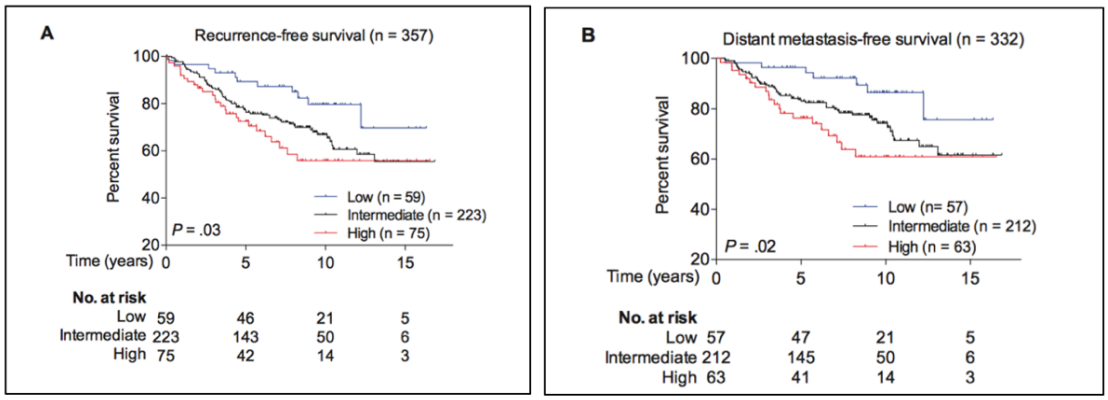


Figure 6: Brachyury expression and prognosis. Kaplan-Meier estimated of recurrence-free survival (A) and distant metastasis-free (B) according to brachyury mRNA levels among 357 patients who received adjuvant tamoxifen monotherapy. The low (lowest 25%), intermediate, and high (highest 25%) were defined based on the level of brachyury mRNA in a cohort of 4010 breast cancer samples that included 357 patients analyzed here. All statistical tests were two-sided. *Figure from Palena et al. 2014.*

Brachyury Immunogenicity

By using a major histocompatibility complex (MHC)-peptide binding prediction algorithm, a 9-mer HLA-A2 binding epitope of the brachyury protein has been identified, (88) which was successfully used *in vitro* to expand human brachyury-specific CD8+ T-cell lines from peripheral blood of both normal donors and cancer patients. Functional assays demonstrated that these brachyury-specific T-cell lines are able to efficiently lyse, in an MHC-restricted manner, human carcinoma cell lines (breast, lung, colon) endogenously expressing the brachyury protein.

In vitro, MVA-BN-Brachyury-infected human dendritic cells activated CD8+ and CD4+ T-cells specific against the self-antigen brachyury. (89) A recent dose escalation phase 1 study confirmed the development of brachyury-specific CD4+ and/or CD8+ T-cell responses after vaccination in over 80% of patients vaccinated with the MVA-BN-Brachyury vaccine. The induction of brachyury-specific T-cell responses was rapid with responses developing in 17 of 28 patients after a single vaccination and in 9 of 28 patients after two vaccinations. (89)

Brachyury has also been incorporated into a recombinant *Saccharomyces cerevisiae* vaccine. Prior studies demonstrated that this vaccine can be taken up by and induce maturation of human

dendritic cells, which in turn activate brachyury-specific CD4+ and CD8+ T-cells. (90) A dose-escalation phase 1 study demonstrated brachyury-specific immune responses in the majority of evaluable patients on trial with 54% developing brachyury-specific CD4+ and/or CD8+ T-cell responses after vaccination. (91)

Furthermore, utilizing the identified HLA-A2-binding epitope of brachyury, CD8+ brachyury-specific T-cell responses were detected in the blood of cancer patients following vaccinations with either CEA- or prostate-specific antigen (PSA)-based vaccines, likely due to the phenomenon of antigen cascade. (92) All together, these results demonstrated that brachyury is an immunogenic protein in humans.

Poxviral Vectors

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. The virus actively replicates in human cells, resulting in the presentation of high levels of antigen to the immune system over a period of 1–2 weeks, substantially increasing the potential for immune stimulation. The immune response specific to vaccinia then eliminates the virus. As a result of its safety profile and ability to elicit both humoral and cell-mediated immunity in humans, the vaccinia virus was chosen as one of the vectors to deliver MUC-1, CEA, and Triad of Costimulatory Molecules (TRICOM) in previous NCI-sponsored trials.

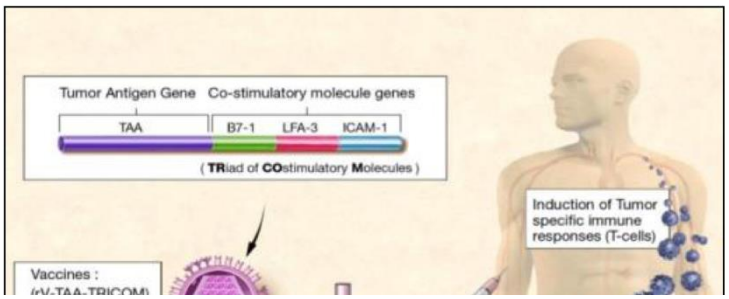


Figure 7 BN-Brachyury recombinant poxvirus vaccine platform.

Immunization with live recombinant poxviral vectors that have been genetically engineered to express one or more antigens allows expression of Tumor-Associated Antigens (TAAs) and subsequent co-presentation of antigenic peptides with host histocompatibility antigens, a strategy that favors the induction of cell-mediated immune responses (**Figure 7**). Recombinant poxviruses can infect antigen-presenting cells, including dendritic cells and macrophages, resulting in efficient expression of TAAs simultaneously with costimulatory molecules required to elicit T cell responses. TAAs expressed by recombinant poxviruses are presented to the immune system together with highly immunogenic viral pathogen associated molecular patterns, which may act as adjuvants to enhance immune responses to the TAAs. Thus, the use of recombinant poxviral vectors to present TAAs to the immune system results in the generation of killer T cells that specifically destroy the selected tumor with little incremental toxicity.

Overview of Modified Vaccinia Ankara (MVA)

MVA-BN-Brachyury is a novel recombinant vector-based therapeutic cancer vaccine designed to induce an enhanced immune response against brachyury, which is overexpressed in many solid tumor types, such as lung, breast, ovarian, chordoma, prostate, colorectal, and pancreatic adenocarcinoma. Modified vaccinia Ankara (MVA) is a replication-deficient, attenuated derivative of vaccinia. It is used in the smallpox vaccination and is now being developed by

Bavarian Nordic (BN) as a recombinant viral vector to produce vaccines against infectious diseases and cancer.

Many MVA vector-based trials conducted in patients with cancer have demonstrated its safety and the immunogenicity of its transgenes. In total, for MVA-BN and MVA-BN-based recombinant vaccines, the exposure sums up to more than 9,600 subjects (more than 7,700 with MVA-BN, more than 400 with recombinant vaccines other than MVA-BN Filo, and more than 1,500 with MVA-BN-Filo), having received more than 15,000 single doses of vaccine.

In all completed and ongoing clinical trials, vaccinations with MVA-BN have shown to be generally safe and well tolerated. No cases of death, assessed as being even possibly related, have been reported for a subject in a clinical trial using MVA-BN. Results obtained from completed Phase 1 and 2 trials and ongoing trials with several recombinant MVA-BN based vaccines in healthy adults and children, HIV infected, and cancer subjects demonstrate a similar safety profile as MVA-BN alone. No pattern regarding Serious Adverse Drug Reactions (SADRs) could be detected. (See **Appendix D**: Clinical and Safety Profile of MVA-BN and Recombinant MVA-Based Vaccines for complete list of reported adverse effects and serious suspected adverse drug reactions possibly related to the vaccine.)

MVA-BN was tested for safety and immunogenicity among healthy volunteers in 3 Phase 1 and 2 dose finding trials. (93-95) Across these trials a linear dose relationship was observed between the vaccine doses and both vaccinia ELISA and Plaque Reduction Neutralization Test (PRNT) titers. Maximum ELISA seroconversion rates and peak titers were reached 2 weeks after the second vaccination, with 100% seroconversion after the second dose for all dose groups receiving at least 2 x 10^7 $TCID_{50}$ per 0.5 mL dose of MVA- BN. Statistical analysis indicated lower doses to be inferior to the standard dose tested throughout all dose ranging trials, whereas the standard dose achieved ELISA seroconversion rates between 81% and 100% already after the first dose. For the PRNT, the same trend was observed with about 77% seroconversion rates 2 weeks after the second MVA-BN administration in all groups receiving the highest dose.

The early onset of seroconversion and the higher titers of total and neutralizing antibodies combined with an excellent safety profile qualified the dose of at least 5 x 10^7 $TCID_{50}$ as the most suitable human dose. The final optimal (standard) dose and schedule for the general population was decided to be 2 doses of at least 5 x 10^7 $TCID_{50}$ MVA-BN administered subcutaneously (s.c.) 2-4 weeks apart.

Overview of the Fowlpox Vaccine

Fowlpox virus (FPV) is a member of the genus Avipox, which is evolutionarily divergent from vaccinia virus. (96) Immune responses to vaccinia are essentially non-cross-reactive with fowlpox and do not block infection and immunization with fowlpox vectors. Unlike vaccination with vaccinia, fowlpox vectors are replication incompetent with early viral and transgene expression but late gene expression is blocked. There is minimal surface antigen made and therefore, minimal neutralizing antibody immune responses are induced with fowlpox vectors. This allows for multiple boosting efforts with the fowlpox-based vectors. Prior work performed within the LTIB has demonstrated the production of neutralizing antibodies with repeated vaccinations with vaccinia vectors. The recently reported PROSTVAC phase 3 trial used a similar prime-boost regimen with the recombinant vaccine virus prime (PROSTVAC-V) followed by multiple recombinant fowlpox virus boosts (PROSTVAC-F).

1.2.6 Tumor Infiltrating Lymphocytes and Clinical Outcomes in Breast Cancer

Standardized methodology for assessing tumor infiltrating lymphocytes (TILs) in H&E sections has been validated by an international working group.(97) TILs are classified as stromal or intratumoral and are graded on a continuous scale. TILs are a well-established prognostic and predictive marker in breast cancer. Pathological evaluation of pre-treatment breast cancers consistently show low numbers of TILs in the tumor, with most breast tumors having 25% or less of the cells within the tumor microenvironment (TME) being lymphocytes. TNBC has the highest TILs with median 20-25% followed by HER2+BC median 15-20% and hormone receptor positive breast cancer (HR+BC) median 5-10%. (98-101) TILs are prognostic in TNBC and HER2+BC, with higher levels correlating with a better prognosis. Several large meta-analyses have consistently found that for each absolute 10% increase in TILs per high power field, the risk of relapse decreases by 15-20% and the risk of death decreases by 20-25%. (99, 100, 102, 103) This association has not been found in hormone receptor positive (HR+BC) breast cancer. (99, 100, 104)

Breast cancer is generally not considered a "hot" or lymphocyte-predominant (LPBC; defined as ≥ 50 to 60% lymphocyte infiltration) tumor with only 10-20% of breast cancers being lymphocyte predominant. (98) LPBCs have significantly improved pathologic complete response (pCR; 40% LPBC vs 5% in breast cancers with fewer TILs) with neoadjuvant chemotherapy. (98, 105) Recently, the CLEOPATRA trial evaluated baseline TILs in patients with advanced HER2+BC treated with THP and confirmed that higher TIL values are associated with better overall survival in metastatic HER2+BC. (106)

1.2.7 Combination Therapy Rationale

As described above, checkpoint inhibitor monotherapy does not seem to work well in breast cancer despite multiple efforts in the clinical setting. In tumors like breast cancer, it is hypothesized that intrinsic resistance to immunotherapy may be due to too few neoantigens being present, defective antigen presentation, or suppressive signals (like TGF-β) in the TME that exclude T cells and NK cells in the TME. (107-110) Together, these defects create an immunosuppressive TME that does not allow for an effective tumor immune response.

The three key components of a successful anti-tumor immune response are:

1) presence of effector cells in the form of antigen specific T lymphocytes and/or natural killer cells
2) the ability of those cells to traffic to the tumor
3) effective cytotoxicity of those cells within the TME

The use of a multimodality approach may help to shift the TME back to an immunopermissive TME and allow engagement of the immune system to eradicate the tumor. In this study, the reasoning for trial drug selection is as follows:

A) Brachyury Vaccine:

Given the known expression of the TAA brachyury on breast tumors, (87) the BN-brachyury vaccine was chosen to help generate brachyury-specific T cells, as well as T cells against the cascade antigens CEA and MUC1.

Clinical data produced by LTIB with various brachyury vaccines have consistently demonstrated generation of a brachyury-specific T cell response in breast cancer patients.

- Phase 1 trial with MVA-BN-Brachyury (*used in this trial*): 5 of 5 breast cancer patients generated a brachyury-specific T cell response. (111)
- Phase 1 trial with yeast-Brachyury: 2 of 5 breast cancer patients generated a brachyury-specific T cell response. (91)
- Prolonged responder to PANVAC: metastatic ER+/PR+/HER2+ breast cancer patient generated a robust brachyury-specific T cell response to PANVAC (MUC1, CEA pox virus vaccine) at the time of her complete response, where brachyury was a cascade antigen generated from the vaccine (*data not yet published*). This complete response is on-going at 9 years.

	Yeast Brachyury Vaccine Trial *Heery et al 2015. Cancer Immunol Res.*(91)	**Brachyury-TRICOM Vaccine Trial** *Heery et al. 2017. Clin Cancer Res.*(111)
Study Size	34 patients , heavily pretreated	38 patients , heavily pretreated
Study Design	Phase 1, 3+3 dose escalation	Phase 1, 3+3 dose escalation
Primary Outcome	Safety and Brachyury-specific T-cell responses	Safety and Brachyury-specific T-cell responses
Safety *(G = grade)*	No DLTs. No G3 reactions. G1/2 injection site reactions common	No DLTs. G3 diarrhea (n=1) G1/2 injection site reactions and G1/2 flu-like symptoms common
Immune Responses	17 of 31 evaluable patients (54%) developed Brachyury-specific CD4 and/or CD8 T-cell responses; 78% had responses at highest dose level	28 of 34 evaluable patients (82%) developed Brachyury-specific CD4 and/or CD8 T-cell responses; 87% had responses at highest dose level
Immune Responses in Breast Cancer Patients	2 of 5 breast cancer patients (40%) developed Brachyury-specific CD4 and/or CD8 T-cell responses	**5 of 5 breast cancer patients (100%)** developed Brachyury-specific CD4 and/or CD8 T-cell responses

In addition to the increased immune responses seen with the poxviral-based vaccine compared to yeast-based vaccines, unpublished data from the LTIB suggest that vaccinating with a poxviral vector containing a tumor associated antigen, like brachyury, leads to upregulation of PD-L1 in T-cell poor murine tumor models. We suspect this is likely due to trafficking of activated T-cells to the tumor and subsequent release of IFN-γ. This may explain why therapeutic vaccines alone rarely lead to objective responses and provides reasoning for combining PD-1/L1 inhibitors with poxviral-based vaccines.

Clinically, we have seen the benefits of adding poxviral-based vaccines to PD-L1 inhibitors in immunogenically cold tumors, like breast cancer. In the BN-CV301 phase 1 study (BN-CV301 is a poxviral vaccine that contains the TAA MUC1 and CEA), two of 12 patients enrolled had prolonged stable disease when treated with anti-PD-L1 antibody following administration of BN-CV301. Both patients had KRAS mutated, colorectal cancer, progressed on the single agent vaccine, were transitioned to a anti-PD-L1 antibody and then had a decrease in tumor markers by ≥35%. Both patients had prolonged stable disease and remained on the checkpoint inhibitor for 41 weeks and on-going at 85 weeks (*data unpublished*). Their time on anti-PD-L1 with stable disease far exceeded expectations given that prior trials have found a median progression-free

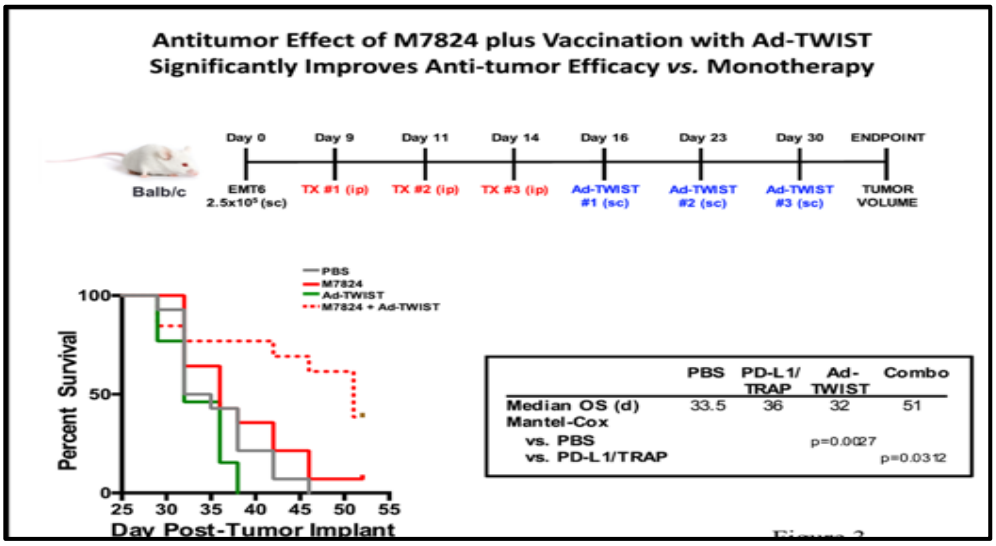
survival of 10 weeks (2.2 months) in patients with MSS, mCRC who receive an anti-PD-L1 antibody. (112)

The BN-Brachyury vaccine will help to generate more brachyury-specific T-cell responses in these breast cancer patients and increase the presence of effector cells in the tumor/TME. Due to the stronger immune responses seen with poxviral-based vaccines, the preclinical data that demonstrated upregulation of PD-L1 in the tumor, and the clinical experiences of benefit seen with checkpoint inhibitor after poxviral-based vaccine, BN-Brachyury was chosen as the vaccine backbone for this trial.

B) M7824

Preclinical data of M7824 in the EMT-6 breast cancer model in B-cell deficient mice was extremely promising with extended survival in a dose-dependent manner and 10 of 10 cures (See **Figure 3**). Furthermore, when M7824 was combined with an Ad-Twist vaccine (murine tumor associated antigen TWIST1) in a syngeneic model, there was improvement in survival (**Figure 8**) compared to monotherapy and increased CD8+ T cell and NK cell activation.(79)

(A)



(B)

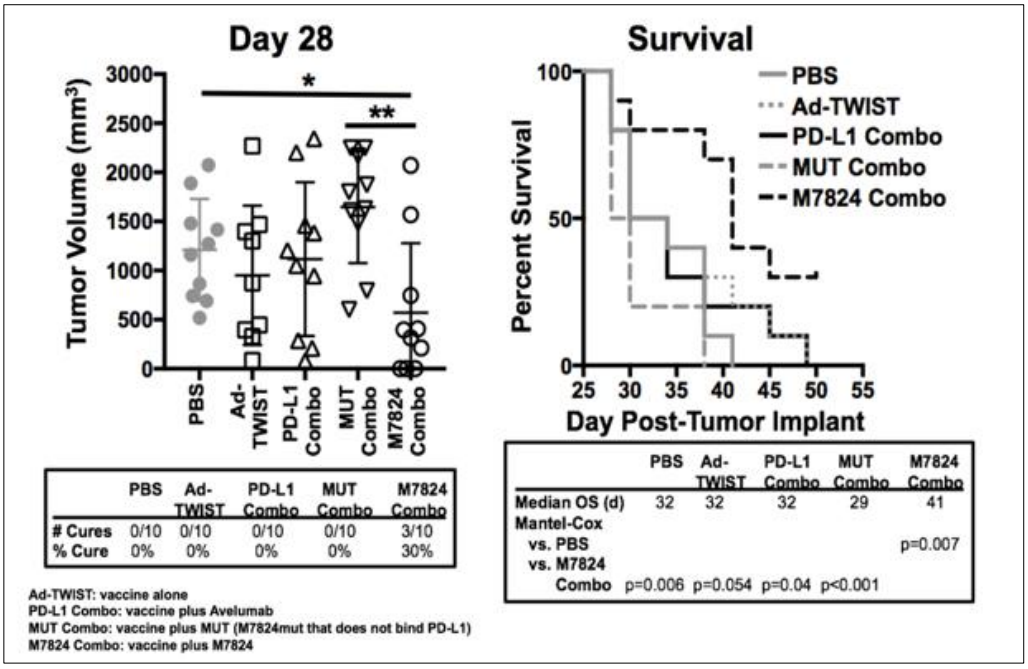


Figure 8: Improved anti-tumor activity with M7824 combination (A) M7824 plus vaccination with Ad-TWIST significantly improves anti-tumor efficacy verse M7824 monotherapy in a syngeneic mouse model (B) M7824 plus Ad-TWIST has better anti-tumor efficacy than Avelumab plus Ad-TWIST or Ad-TWIST alone in a syngeneic mouse model.

Clinical activity of single agent M7824 in breast cancer appears to be equivalent to other checkpoint inhibitors (10% response rate in TNBC patients; *data unpublished, personal communication with EMD Serono*). Preclinical data shows that M7824 may be better than anti-PD-L1(**Figure 8A**) and when combined with Ad-TWIST vaccine, M7824 was better than avelumab (**Figure 8B**). LTIB has extensively evaluated M7824 preclinically and has demonstrated that compared to other anti-PD-L1 antibodies, M7824 has unique immune effects. As previously mentioned, M7824 mediates ADCC, increases gene expression of molecules involved T-cell trafficking, and enhances antigen-specific CD8+ T-cell lysis of tumor cells. (80, 81) Furthermore, TGF-β sequestration in the TME not only leads to improved CD8+ T-cell lysis and NK cell trafficking, the blockade of TGF-β:HER2 interaction may also reverse HER2 resistance leading to improve efficacy of HER2-targeted therapy. (53, 54)

M7824 was chosen as the PD-L1 checkpoint inhibitor backbone of this regimen due to its synergy with the BN-Brachyury vaccine as well as the added benefits of TGF-β sequestration in the TME (increased CD8+ trafficking, increased NK cell infiltration, reversal of HER2 resistance, reversal of mesenchymalization, etc). In the GMB/LTIB branch at the NCI, one ongoing trial currently employs this combination – The QuEST trial (NCT03493945; IND 17851; PI J. Gulley). To date, 15 patients have received one or more doses of BN-Brachyury + M7824 (10 patients BN-Brachyury + M7824; 5 patients

BN-Brachyury + M7824 + ALT803). There has been one grade 3 pancreatitis (elevation in pancreatic enzymes in the absence of clinical symptoms but with imaging concerning for pancreatic inflammation; grade 3 due to use of steroids) which was attributed to M7824 and is known immune related adverse event of anti-PD-L1 antibodies. There have been no other grade 3 or higher adverse events or immune related adverse events attributed to the combination of M7824 and BN-Brachyury +/- ALT803. The BrEAsT trial uses a higher dose of M7824 than that of the QuEST trial (2400mg flat dose every 3 weeks vs 1200mg flat dose every 2 weeks) which is why the safety evaluation is being conducted in Arm 1. However, the 2400mg dose every 2-week regimen as well as a similar regimen of 30mg/kg dose every 2 weeks have been evaluated in several patients. In the phase 1 trial of M7824, which was conducted at the NCI in this branch, there was no evidence of more SAEs or irAEs with higher doses of M7824 (i.e., 20mg/kg or 30mg/kg dose levels).(113) There is also an ongoing, unpublished dose expansion cohort of the phase 1 trial with the 2400mg flat dose being given every 2 weeks and to date, there has not an increase in severe adverse events or irAEs over what is expected with the 1200mg every 2-week dose (unpublished data).

C) Entinostat

As demonstrated in **Figure 2**, LTIB has demonstrated that treatment with entinostat makes breast cancer cells more sensitive to T-cell mediated lysis in vitro. This immunogenic modulation was observed against a broad range of TAA including brachyury and was associated with increase expression of antigen processing and tumor immune recognition. HDAC inhibitors restore protein expression of MHC class I and antigen processing and presentation molecules. (30) Entinostat exposure also increases the sensitivity of breast cancer cells (MDA-MB-231) to NK-mediated attack through direct lysis (increased NK activation and function) and through anti-PD-L1 mediated ADCC. (114)

Furthermore, the LTIB has also demonstrated that entinostat increases PD-L1 expression in mice implanted with PC-3 (prostate) carcinoma cells (**Figure 9A**). (114) Others have shown upregulation of PD-L1 on tumors with entinostat treatment. This has been attributed to the epigenetic modulation that is seen with HDAC inhibitors. Preliminary results of the ENCORE-601 trial involving entinostat with pembrolizumab in checkpoint refractory NSCLC demonstrated clinical efficacy in multiple patients regardless of prior checkpoint treatment or PD-L1 status. (41) The addition of entinostat to checkpoint refractory tumors or to cold tumors is promising. There are currently multiple on-going clinical trials involving HDAC inhibitors with checkpoint inhibitors (*NCT02915523; NCT02909452; NCT02708680; NCT02437136; NCT02453620; NCT02697630; NCT03250273; NCT01928576).*

Preclinical data performed by Christmas et al, demonstrates synergy with the combination of entinostat, an anti-PD-1 antibody and a HER2-targeted in HER2/neu transgenic breast cancer models. This combination significantly reduced tumor size and improved overall survival in mice (**Figure 9B, C**; see 5th line from top). (115) In addition, the triplet significantly decreased suppression of granulocytic MDSCs in the TME, increased CD8+ effector T cells and reduced Tregs compared to single agent HER2-targeted therapy.

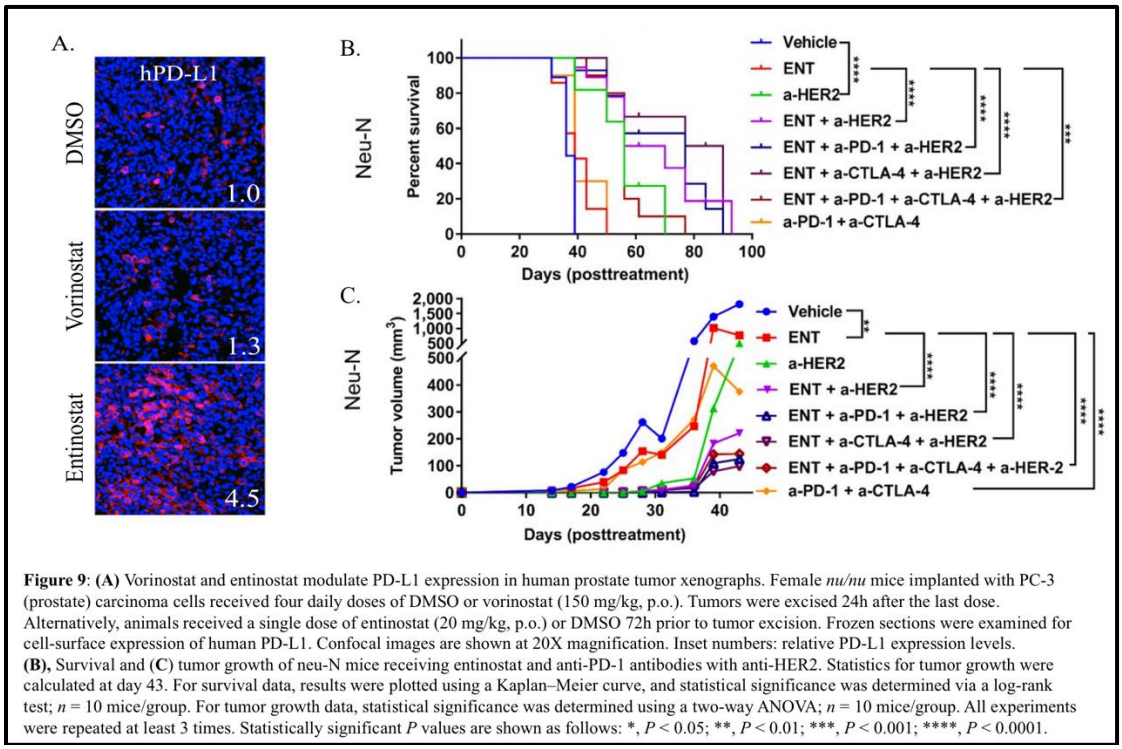


Figure 9: **(A)** Vorinostat and entinostat modulate PD-L1 expression in human prostate tumor xenographs. Female *nu/nu* mice implanted with PC-3 (prostate) carcinoma cells received four daily doses of DMSO or vorinostat (150 mg/kg, p.o.). Tumors were excised 24h after the last dose. Alternatively, animals received a single dose of entinostat (20 mg/kg, p.o.) or DMSO 72h prior to tumor excision. Frozen sections were examined for cell-surface expression of human PD-L1. Confocal images are shown at 20X magnification. Inset numbers: relative PD-L1 expression levels. **(B),** Survival and (**C**) tumor growth of neu-N mice receiving entinostat and anti-PD-1 antibodies with anti-HER2. Statistics for tumor growth were calculated at day 43. For survival data, results were plotted using a Kaplan–Meier curve, and statistical significance was determined via a log-rank test; $n = 10$ mice/group. For tumor growth data, statistical significance was determined using a two-way ANOVA; $n = 10$ mice/group. All experiments were repeated at least 3 times. Statistically significant P values are shown as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Figure 9

As above, preclinically entinostat improves T-cell mediated cytotoxic killing of breast cancer cells through increased MHC expression, improved NK activation and function, improved NK-mediated lysis and improved antigen processing and trafficking, making it an ideal companion for a therapeutic cancer vaccine like BN-Brachyury. Furthermore, epigenetic modulation leading to increased PD-L1 expression makes it an ideal companion for a checkpoint inhibitor.

D) T-DM1

As discussed above, T-DM1 is a standard of care regimen for metastatic HER2+BC patients. While many of its immune effects are similar to trastuzumab (ADCC, increased immunomodulatory cytokines, and increased antigen presentation), T-DM1 also has the added benefit of increased PD-L1 expression on tumor cells. Furthermore, a maytansine-containing drug antibody conjugate (similar to T-DM1) induces immunogenic cell death in tumor cells.(116) The on-going KATE2 trial (*NCT02924883)* is evaluating T-DM1 with Atezolizumab. Preliminary evidence showed no improvement in PFS but survival data is still maturing. Other on-going trials with checkpoints and T-DM1 include a phase 1b trial combining pembrolizumab and T-DM1 (*NCT03032107)* and the phase 2 trial, PembroMab, which is evaluating pembrolizumab and T-DM1 in patients with metastatic HER2+BC (*NCT02318901).* Efficacy data from these trials have not been reported to date.

T-DM1 was chosen as the HER2+BC due to the added benefits of increased PD-L1 and immunogenic cell death. This will help to increase presence of effector cells in the form of antigen specific T lymphocytes and/or natural killer cells, increase immune cell trafficking and improve cytotoxicity of these cells within the TME.

Summary Statement

We hypothesize that combining these four agents will lead to a robust immune response against HR-/HER2+BC with improved response rates. The immune effects of the standard of care therapy T-DM1 may be enhanced through combination with entinostat, M7824 and the BN-Brachyury vaccine. In a tumor that generally does not respond to checkpoint monotherapy, this combination of agents will address the three key components of a successful anti-tumor immune response. Furthermore, the use of novel combination approaches is in keeping with the Cancer Moonshot Taskforce's mandate which called for the use of innovative strategies to rapidly translate new agents from bench to bedside. Rational combination of immune therapies is a plausible strategy to achieve this aim and is especially warranted in treating patients who have exhausted most, if not all, therapeutic options. Enhancing immunity via several different mechanisms is a promising means to produce objective responses in a substantially increased portion of patients. Preclinical data in an orthotopic HER2+ TuBO model demonstrates a significant tumor reduction in mice who received the four agents (*red box in* **Figure 10**) proposed in this trial (entinostat, M7824 ,vaccine and T-DM1; **Figure 10**). For anti-tumor studies, TuBo tumor cells (4×10^5, s.c.) were orthotopically implanted into the mammary fat pad of female Balb/c mice on day 0. Mice were randomized based on tumor size and treatment initiated when tumors reached $200mm^3$ (n=10/group) based on tumor size and treatment initiated when tumors reached $200mm^3$ (day 18), as per schematic (Figure 10). Tumors were measured twice weekly using calipers, with tumor volume being determined as length x length x width/2. Animals were monitored by the veterinarian staff for signs of toxicity, including weight loss. No toxicity was reported in this study. Animals were sacrificed on day 32 and immune populations in the tumor were examined.

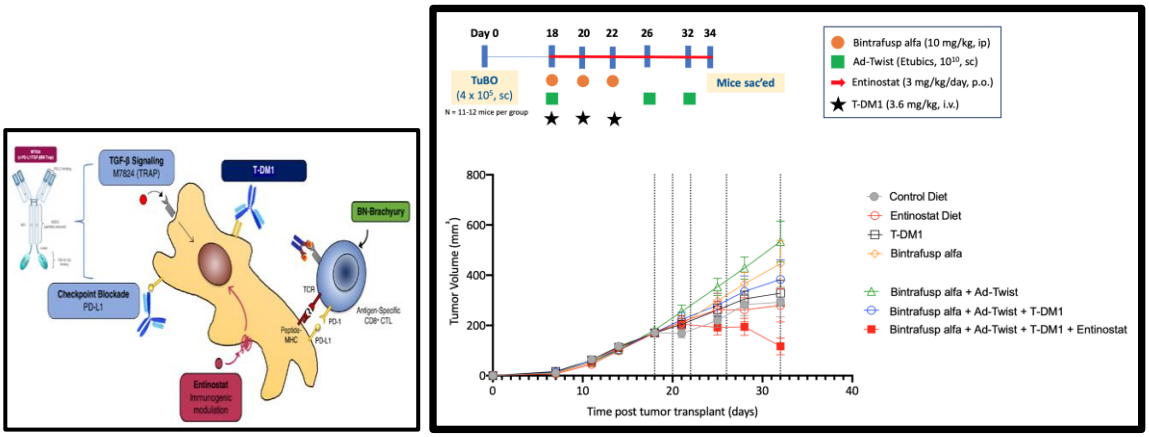


Figure 10: Combination Immunotherapy Reduces Tumor Volume in Orthotopic HER2+ Breast Cancer Mouse Model

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA FOR ARM 1: M7824 + BN-BRACHYURY IN TNBC

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed metastatic TNBC, defined as ER < 10%, PR < 10% per immunohistochemistry (IHC) and HER 2 negative. HER2 negative or unamplified breast cancer is defined as IHC 0 or 1+ or IHC 2+ with FISH average HER2 copy number < 4.0 signals per cell or HER2/CEP17 < 2.0 with average HER2 copy number < 4.0 signals per cell. (117) HER2 testing must have been performed in a laboratory accredited by the College of American Pathology (CAP) or another accrediting entity.

2.1.1.2 Patients must have measurable disease, per RECIST 1.1. See Section **6.3** for the evaluation of measurable disease.

2.1.1.3 Patients must have received at least one prior treatment for metastatic disease and progressed on treatment or been intolerant to treatment. Patients with known PD-L1 positive tumors must have received prior treatment with atezolizumab + nab-paclitaxel. Patients with ER 1-9% must have received treatment with at least two lines of endocrine treatment (SERM, AI, fulvestrant) with one line including a CDK4/6 inhibitor + endocrine therapy for their metastatic cancer and should be considered endocrine therapy resistant.

2.1.1.4 Female or male ≥18 years.

2.1.1.5 ECOG performance status 0 or 1 (see **Appendix A**).

2.1.1.6 Patients must have adequate bone marrow function as defined below:

- absolute neutrophil count ≥1,500/mcL (≥ 1.5X 10^6/L)
- platelets ≥100,000/mcL
- hemoglobin ≥ 9 mg/dL (transfusion to obtain hemoglobin ≥ 9 mg/dL within 24 hours prior to dosing is allowed)

2.1.1.7 Patients must have adequate renal function, defined as:

- serum creatinine ≤ 1.5 X upper limit of normal (ULN) OR
- measured or calculated creatinine clearance ≥ 60 mL/min for participant with creatinine levels > 1.5 X institutional ULN (GFR can also be used in place of creatinine or CrCl).

2.1.1.8 Patients must have adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dL will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dL, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study. Adequate hepatic function for patients with known liver metastases is defined as AST and ALT levels ≤ 5 X ULN.

2.1.1.9 The effects of BN-Brachyury, entinostat, M7824 and T-DM1 on the developing human fetus are unknown. However, the two components of T-DM1 are known to have negative fetal effects including oligohydramnios and oligohydramnios sequence (pulmonary hypoplasia, skeletal malformations and neonatal death). For this reason:

- Women of child-bearing potential must agree to use adequate contraception (see **Table 17**) at study entry, for the duration of study participation and for 7 months after the last dose of study medication. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
- Men should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of study medications.

2.1.1.10 Patients with well-controlled HIV infection are eligible for trial as long as:

- On an effective anti-retroviral therapy (ART) > 4 weeks and with evidence of viral suppression as defined as HIV viral load < 400 copies/mL within the last 3 months;
- CD4 ≥ 200 cells/μL within the last 3 months; and
- No reported opportunistic infections within 6 months prior to enrollment, except for the following which will be allowed:

  i. Esophageal candidiasis treated within last 6 months or currently improving with antifungal treatment.

  ii. Oral and/or genital HSV treated within last 6 months or currently improving with antiviral treatment.

  iii. Mycobacterium avium infection in last 6 months or that has been treated for at least 1 month.

2.1.1.11 Patients with evidence of chronic hepatitis B virus (HBV) infection are eligible for trial as long as the HBV viral load is undetectable on suppressive therapy, if indicated.

2.1.1.12 Patients with history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible

if they have an undetectable or unquantifiable HCV RNA 12 weeks or longer after definitive treatment completion.

2.1.1.13 Patients must be able to understand and willing to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who have received chemotherapy in the prior 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents or a PD-1/L1 agent within 4 weeks prior to study enrollment.

2.1.2.2 Patients who have received radiotherapy within 4 weeks prior to study entry.

2.1.2.3 Patients with active brain metastases or leptomeningeal metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. However, patients with treated brain metastases are eligible if there is no magnetic resonance imaging (MRI) evidence of progression for 6 weeks after treatment is complete and within 28 days prior to the first dose of trial drug. Patients requiring immunosuppressive doses of systemic corticosteroids (> 10mg/day prednisone equivalent) for palliation are excluded.

2.1.2.4 Patients with a history of another invasive malignancy $\leq$ 3 years prior to enrollment (patients with non-melanoma skin cancers, carcinoma in situ of the breast or cervix are eligible).

2.1.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to agents used in study. For example, reaction to prior vaccination with vaccinia virus or prior hypersensitivity reaction to fully humanized monoclonal antibodies (Grade $\geq$ 3 NCI-CTCAEv5).

2.1.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing active infection that requires systemic treatment with ongoing antibiotics (*eligible if can stop antibiotics on day of enrollment*), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that in the opinion of the primary investigator would prohibit the patient from complying with study requirements.

2.1.2.7 Patients with bone metastases who have initiated denosumab or a bisphosphonate therapy within 28 days prior to or after Cycle 1 Day 1. Continuation of prior therapy is allowed.

2.1.2.8 Patients with inherited bleeding disorders, a history of bleeding diathesis such as vWF deficiency or recent (within 3 months prior to enrollment) clinically significant bleeding events that, in the judgment of the investigator, would interfere with patient's ability to carry out the treatment program.

2.1.2.9 Participants unwilling to accept blood products as medically indicated.

2.1.2.10 Patients should have no evidence of being immunocompromised as listed below:

- Active, known or suspected autoimmune disease. Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to an autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment or conditions not expected to recur in the absence of an external trigger in the opinion of the primary investigator.

- Altered immune function that in the judgement of the PI that may affect a patient's ability to adequately engage the immune system and respond to the immunotherapy agents being administered, including but not limited to: inflammatory bowel disease; active infectious enteritis; eosinophilic enteritis; lupus erythematous; ankylosing spondylitis; scleroderma; multiple sclerosis. These criteria do not include all disease with an immune-related component but are not autoimmune in nature or have a primary alteration in the general immune function that may interfere with the vaccine mechanism of action, for example celiac disease.
- Immunosuppressive therapy post-organ transplant.

2.1.2.11 Concurrent use of chronic use of systemic steroids, except for physiologic doses of systemic steroids for replacement, defined as 10mg of prednisone per day or equivalent, or local (topical, nasal, ophthalmic or inhaled) steroid use or prior concomitant use with chemotherapy. Systemic steroids must have been discontinued >2 weeks prior to trial start. Prior use of corticosteroids in short-term schemes (duration shorter than 3 days) for indications such as prophylaxis of reactions to intravenous contrast for imaging studies or chemotherapy-related AEs are not considered part of this exclusion. Prior use of corticosteroids for brain metastasis ending at least 14 days prior to enrollment is not considered part of this exclusion criteria.

2.1.2.12 Pregnant and breastfeeding women are excluded from this study because of the potential for teratogenic or abortifacient effects with all of the agents involved in this trial.

2.1.2.13 Clinically significant cardiomyopathy, coronary disease, chronic heart failure (CHF; New York Heart Association class III or IV or hospitalization for CHF), or cerebrovascular accident within 6 months prior to enrollment.

2.1.2.14 Patients with a history of myocarditis are excluded due to the potential of myocarditis with anti-PD-L1 antibodies.

2.1.2.15 Patients with pulse oximetry < 92% on room air will be excluded due to the potential of pneumonitis with anti-PD-L1 antibodies.

2.1.2.16 Any other condition, which would, in the opinion of the Principal Investigator or Medical Monitor, indicated the subject is a poor candidate for the clinical trial or would jeopardize the subject or the integrity of the data obtained.

2.2 Eligibility Criteria for Arms 2 and 3: M7824, BN-Brachyury, T-DM1 +/- Entinostat in HER2+BC

2.2.1 Inclusion Criteria

2.2.1.1 Patients must have histologically or cytologically confirmed metastatic HER2+ breast cancer. HER2 positive or amplified breast cancer is defined as IHC 3+ or FISH average HER2 copy number ≥ 6 signals per cell or HER2/CEP17 ≥ 2.0. (117) HER2 testing

must have been performed in a laboratory accredited by the College of American Pathology (CAP) or another accrediting entity.

2.2.1.2 Patients must have hormone receptor negative, HER2+ breast cancer. Hormone receptor negative is defined as estrogen receptor < 10% by IHC and progesterone receptor < 10% by IHC.

2.2.1.3 Patients must have measurable disease, per RECIST 1.1. See Section **6.3** for the evaluation of measurable disease.

2.2.1.4 Patients in Cohort 3 must have at least one lesion deemed safe to biopsy and be willing to undergo up to three biopsies while on trial.

2.2.1.5 Patients must have received front-line treatment for metastatic disease with a taxane, trastuzumab and pertuzumab (THP; docetaxel or paclitaxel allowed) and progressed on treatment or were intolerant to treatment. Patients must have received at least one prior therapy in the metastatic setting. Prior T-DM1 therapy is allowed.

2.2.1.6 Female or male ≥18 years.

2.2.1.7 ECOG performance status 0 or 1 (see **Appendix A**).

2.2.1.8 Patients must have adequate bone marrow function as defined below:

– absolute neutrophil count	≥1,500/mcL (≥ 1.5X 10^6/L)
– platelets	≥100,000/mcL
– hemoglobin	≥ 9 mg/dL (transfusion to obtain hemoglobin ≥ 9 mg/dL within 24 hours prior to dosing is allowed)

2.2.1.9 Patients must have adequate renal function, defined as:

- serum creatinine ≤ 1.5 X upper limit of normal (ULN) OR
- measured or calculated creatinine clearance ≥ 60 mL/min for participant with creatinine levels > 1.5 X institutional ULN (GFR can also be used in place of creatinine or CrCl).

2.2.1.10 Patients must have adequate hepatic function, defined as AST and ALT levels ≤ 3 X ULN and total bilirubin < 1.5 X ULN, unless known diagnosis of Gilbert's syndrome, where bilirubin ≤ 5 mg/dL will be permitted. Gilbert's syndrome will be defined as elevated unconjugated bilirubin, with conjugated (direct) bilirubin within the normal range and less than 20% of the total. Total bilirubin will be permitted up to 5 mg/dL, if patients have historical readings consistent with the definition of Gilbert's syndrome prior to entering study. Adequate hepatic function for patients with known liver metastases is defined as AST and ALT levels ≤ 5 X ULN.

2.2.1.11 Patients must have adequate cardiac function as defined by an ejection fraction ≥ 50%.

2.2.1.12 The effects of BN-Brachyury, entinostat, M7824 and T-DM1 on the developing human fetus are unknown. However, the two components of T-DM1 are known to have negative fetal effects including oligohydramnios and oligohydramnios sequence (pulmonary hypoplasia, skeletal malformations and neonatal death). For this reason:

- Women of child-bearing potential must agree to use adequate contraception (see **Table 17**) at study entry, for the duration of study participation and for 7 months after the last dose of study medication. Should a woman become pregnant or

suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

- Men should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of study medications.

2.2.1.13 Patients with well-controlled HIV infection are eligible for trial as long as:

- On an effective anti-retroviral therapy (ART) > 4 weeks and with evidence of viral suppression as defined as HIV viral load < 400 copies/mL within the last 3 months;
- CD4 $\geq$ 200 cells/μL within the last 3 months; and
- No reported opportunistic infections within 6 months prior to enrollment, except for the following which will be allowed:

  i. Esophageal candidiasis treated within last 6 months or currently improving with antifungal treatment.

  ii. Oral and/or genital HSV treated within last 6 months or currently improving with antiviral treatment.

  iii. Mycobacterium avium infection in last 6 months or that has been treated for at least 1 month.

2.2.1.14 Patients with evidence of chronic hepatitis B virus (HBV) infection are eligible for trial as long as the HBV viral load is undetectable on suppressive therapy, if indicated.

2.2.1.15 Patients with history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable or unquantifiable HCV RNA 12 weeks or longer after definitive treatment completion.

2.2.1.16 Patients must be able to understand and willing to sign a written informed consent document.

2.2.2 Exclusion Criteria

2.2.2.1 Patients who have received chemotherapy, including herceptin and/or pertuzumab in the prior 3 weeks (6 weeks for nitrosoureas or mitomycin); other investigational agents or a PD-1/L1 agent within 4 weeks prior to study enrollment.

2.2.2.2 Patients who have received radiotherapy within 4 weeks prior to study entry.

2.2.2.3 Patients with active brain metastases or leptomeningeal metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. However, patients with treated brain metastases are eligible if there is no magnetic resonance imaging (MRI) evidence of progression for 6 weeks after treatment is complete (no radiotherapy within 6 weeks) and within 28 days prior to the first dose of trial drug or asymptomatic brain metastasis. Patients requiring

immunosuppressive doses of systemic corticosteroids (> 10mg/day prednisone equivalent) for palliation are excluded.

2.2.2.4 Patients with a history of another invasive malignancy ≤ 3 years prior to enrollment (patients with non-melanoma skin cancers, carcinoma in situ of the breast or cervix are eligible).

2.2.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to agents used in study. For example, reaction to prior vaccination with vaccinia virus or known hypersensitivity reaction to fully humanized monoclonal antibodies (Grade ≥ 3 NCI-CTCAEv5).

2.2.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing active infection that requires systemic treatment with ongoing antibiotics (*eligible if can stop antibiotics on day of enrollment*), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situation that in the opinion of the primary investigator would prohibit the patient from complying with study requirements.

2.2.2.7 Known history of a gastrointestinal illness that in the investigator's opinion would prevent the absorption of entinostat, which is an oral agent. (Arm 3 ONLY)

2.2.2.8 Patients with bone metastases who have initiated denosumab or a bisphosphonate therapy within 28 days prior to or after Cycle 1 Day 1. Continuation of prior therapy is allowed.

2.2.2.9 Patients with inherited bleeding disorders, a history of bleeding diathesis such as vWF deficiency or recent (within 3 months prior to enrollment) clinically significant bleeding events that, in the judgment of the investigator, would interfere with patient's ability to carry out the treatment program.

2.2.2.10 Participants unwilling to accept blood products as medically indicated.

2.2.2.11 Patients should have no evidence of being immunocompromised as listed below:

- Active, known or suspected autoimmune disease. Patients are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to an autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment or conditions not expected to recur in the absence of an external trigger in the opinion of the primary investigator.
- Altered immune function, that in the judgement of the PI that may affect a patient's ability to adequately engage the immune system and respond to the immunotherapy agents being administered, including but not limited to: inflammatory bowel disease; active infectious enteritis; eosinophilic enteritis; lupus erythematous; ankylosing spondylitis; scleroderma; multiple sclerosis. These criteria do not include all disease with an immune-related component, but are not autoimmune in nature or have a primary alteration in the general immune function that may interfere with the vaccine mechanism of action, for example celiac disease.
- Immunosuppressive therapy post-organ transplant.

2.2.2.12 Concurrent use of chronic use of systemic steroids, except for physiologic doses of systemic steroids for replacement, defined as 10mg of prednisone per day or equivalent, or local (topical, nasal, ophthalmic or inhaled) steroid use or prior concomitant use with chemotherapy. Systemic steroids must have been discontinued >2 weeks prior to trial start. Prior use of corticosteroids in short-term schemes (duration shorter than 3 days)

for indications such as prophylaxis of reactions to intravenous contrast for imaging studies or chemotherapy-related AEs are not considered part of this exclusion. Prior use of corticosteroids for brain metastasis ending at least 14 days prior to enrollment is not considered part of this exclusion criteria.

2.2.2.13 Pregnant and breastfeeding women are excluded from this study because of the potential for teratogenic or abortifacient effects with all of the agents involved in this trial.

2.2.2.14 Clinically significant cardiomyopathy, coronary disease, chronic heart failure (CHF; New York Heart Association class III or IV or hospitalization for CHF), or cerebrovascular accident within 6 months prior to enrollment.

2.2.2.15 Patients with a history of myocarditis are excluded due to the potential of myocarditis with anti-PD-L1 antibodies.

2.2.2.16 Patients with pulse oximetry < 92% on room air will be excluded due to the potential of pneumonitis with anti-PD-L1 antibodies.

2.2.2.17 Any other condition, which would, in the opinion of the Principal Investigator or Medical Monitor, indicated the subject is a poor candidate for the clinical trial or would jeopardize the subject or the integrity of the data obtained.

2.3 RECRUITMENT STRATEGIES

This study will be listed on www.clinicaltrials.gov, on NIH social media platforms, and on OSU websites and social media platforms. We will also engage in local outreach to local hospitals and breast cancer advocacy groups (such as SHARE, Koman, and breast cancer research foundation) with IRB-approved recruitment materials.

2.4 SCREENING EVALUATION

2.4.1 Screening activities prior to the obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

2.4.2 Screening activities performed after a consent for screening has been signed

The following screening procedures will be performed only after the subject has signed the study consent within 14 days prior to enrollment, except when specified differently. Assessments performed at outside facilities or at the local site, but off-protocol within these timeframes and those below may also be used to determine eligibility once a patient has signed the consent.

2.4.2.1 Pathologic Confirmation

Pathological confirmation of diagnosis and ER, PR and HER2 expression will be obtained at the local-site or participating facilities.

However, if no pathologic specimen is available, patients may enroll with a pathologists report showing a histologic diagnosis of TNBC or HER2-positive breast cancer in a College of American Pathologists (CAP) accredited laboratory (or other accrediting entity) and a clinical course consistent with the disease.

2.4.2.2 Screening Visit: Physical Exam, Labs and Imaging

History and Physical Exam:

A thorough medical history and physical examination with height, weight and vital signs including pulse oximetry will be conducted at screening. Results of the physical examination including any abnormalities will be documented and can be used for the baseline examination if conducted within 3 days prior to initiating therapy. Abnormal findings will be reassessed at subsequent visits. On subsequent visits, any newly diagnosed or worsening conditions, signs and symptoms, whether related or unrelated to the trial, will be reported as adverse events.

Performance Status:

The ECOG performance status (**Appendix A**) will be assessed at screening and documented in the clinical record.

Laboratory Assessment at Screening:

- CBC with differential;
- PT/INR/PTT;
- Biochemical profile for Screening: 14 comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST (SGOT), ALT (SGPT), total bilirubin, serum creatinine, reflex Free T4 and TSH;
- CD4 if clinically indicated (within 3 months prior to enrollment);
- HBV, HCV, HIV testing including viral load if clinically indicated (within 3 months prior to enrollment).

Pregnancy Testing:

For female subjects of childbearing potential, urine or serum beta-HCG will be performed on initial screening.

Patients who are postmenopausal (age-related amenorrhea for 12 or more consecutive months, or documented FSH > 40 mIU/mL / Estradiol < 20mIU/mL), or who had undergone hysterectomy or bilateral oophorectomy are exempt from pregnancy testing. Patients who experienced chemotherapy-induced menopause within the past 2 years and are considered to be of childbearing potential must undergo pregnancy screening.

Imaging for Disease Evaluation at Screening: *May be completed within 28 days*

- CT of chest/abdomen /pelvis (MRI may be substituted if clinically indicated). An attempt to be consistent with imaging modality for evaluation throughout trial must be made.
- Brain MRI (preferred) in patients with known CNS disease or concern for CNS metastasis as described in Sections **2.1.2.3**, **2.2.2.3** or head CT with contrast, if clinically indicated.

Additional Screening

- A 2D Echocardiogram will be performed within 28 days prior to prior to enrollment for all patients in Arms 2 and 3 due to the use of T-DM1.
- An EKG will be performed within 28 days prior to enrollment for all patients.

2.5 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.5.1 For Participating Site Registration

Registration will be a two-part process as participants are screened on this protocol. A protocol registration form will be supplied by the CCR study coordinator and updates will be provided as needed. To initially register a subject, after the participant has signed consent, complete the top portion of the form and send to CCR study coordinator. Once eligibility is confirmed, after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the participant is being registered for treatment and send to CCR study coordinator. In addition, source documents supporting the eligibility criteria must be sent to the CCR study coordinator. The CCR study coordinator will notify you either by e-mail or fax that the protocol registration form has been received which will include the unique patient/subject ID number. Questions about eligibility should be directed to the CCR study coordinator or PI. Questions related to registration should be directed to the CCR study coordinator.
When a participant has a status change (e.g., subject screened on the study, does not meet eligibility criteria and is removed from the study, participant is taken off protocol therapy or off study, etc.), the Participant Status Update Form will be supplied by the CCR study coordinator. Send the completed form to the CCR study coordinator.

2.5.2 Screen Failure

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a transient lab abnormality may be rescreened.

2.6 TREATMENT ASSIGNMENT PROCEDURES

Cohorts

Number	Name	Description
1	First Cohort	Patients with metastatic triple negative breast cancer.
2	Second Cohort	Direct assignment of patients with metastatic hormone receptor negative, HER2 positive breast cancer
3	Third Cohort	Alternating direct assignment of patients with metastatic hormone receptor negative, HER2 positive breast cancer.

Arms

Number	Name	Description
1	M7824 + BN-Brachyury	Bifunctional fusion molecule involving PD-L1 with TGF-b sequestering agent added to a vaccine for the tumor associated antigen called brachyury.
2	M7824 + BN-Brachyury + T-DM1	Bifunctional fusion molecule involving PD-L1 with TGF-b sequestering agent added to a vaccine for the tumor associated antigen called brachyury. These investigational agents will be added to standard of care treatment called T-DM1.
3	M7824 + BN-Brachyury + T-DM1 + Entinostat	Bifunctional fusion molecule involving PD-L1 with TGF-b sequestering agent added to a vaccine for the tumor associated antigen called brachyury as well as to an oral HDAC inhibitor called entinostat. These investigational agents will be added to standard of care treatment called T-DM1.

Arm Assignment

- Patients in Cohort 1 will be directly assigned to Arm 1.
- Following the completion of the DLT period for Arm 1, patients in Cohort 2, will be directly assigned to Arm 2. Six patients will be assessed for safety. Patients in Arm 1 will continue to enroll on this arm until 8 – 13 patients are accrued.
- Following completion of the DLT period for Arm 2, 6 additional patients in Cohort 2 will be directly assigned to Arm 3 to be assessed for safety.
- Following completion of the DLT period for Arm 3, patients in Cohort 3 will be directly assigned on an alternating basis between Arms 2 and 3.

2.7 BASELINE EVALUATION

Baseline evaluation is the C1D1 evaluation. Data collected during screening may count for the baseline evaluation if conducted within the below specified time periods and do not need to be repeated prior to first treatment:

- History and physical examination with vital signs (including pulse oximetry), ECOG evaluation (**Appendix A**: Performance Status Criteria), skin assessment, history of prior therapy, review of concurrent medications, and review of baseline symptoms. Must be completed within 3 days of C1D1.

- Laboratory Assessment. Must be completed within 7 days of C1D1 except where noted below.
    - CBC with differential
    - Sodium, potassium, chloride, CO2, creatinine or measured creatinine clearance, glucose, BUN
    - Mineral panel (magnesium, calcium, phosphorus and albumin)
    - Hepatic panel (alkaline phosphatase, AST/ALT/total bilirubin) with total protein
    - PT/INR/PTT – Must be completed < 72 hours prior to biopsy if being performed
    - Urine or serum β-HCG test in women of childbearing potential (or endocrine evaluation with LH, FSH and estradiol) – Must be completed on C1D1.
    - Troponin, CK, CK-MB
    - Tumor Markers: – CEA, CA15-3, CA 27.29
    - Urinalysis
- Assessment of disease by imaging (may include CT chest, abdomen and pelvis or MRI). Must be completed within 28 days prior to C1D1.
- EKG. Must be completed within 7 days prior to C1D1.
- Research Blood. Refer to tables in Section **5.1** for additional information.
- Tumor Biopsy. Required in Cohort 3 only, optional in others. Refer to tables in Section **5.1** for additional information. Must be collected within 7 days prior to C1D1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label, multi-center, phase 1b trial with three arms to evaluate BN-Brachyury, entinostat, M7824 +/- T-DM1 in advanced breast cancer. Arm 1 will involve BN-Brachyury and M7824 in TNBC. Arms 2 and 3 will involve BN-Brachyury, M7824, T-DM1 +/- entinostat in ER-/PR-/HER2+ breast cancer that have previously progressed on or was intolerant to a taxane plus trastuzumab and pertuzumab (THP; paclitaxel can have been used in place of docetaxel). Up to 51 patients will be treated on this study with an accrual ceiling set at 65 patients to allow for a small number of inevaluable patients and up to 10 screening failures. Each cycle is 21 days in duration.

Arm 1: TNBC

Since M7824 and BN-Brachyury have not previously been used together in breast cancer, an eight – thirteen patient phase 1b (Arm 1) study will be conducted to evaluate safety of the combination as well as to evaluate efficacy. Patients in Arm 1 must have measurable disease but are not required to have biopsiable disease. Since no HER2-targeted agent will be used, this arm is limited to TNBC patients only.

On C1D1, patients will receive M7824 2,400mg IV every 3 weeks (a higher dose of M7824 than used in the ongoing QuEST trial referenced above). Patient will receive the first MVA-BN-Brachyury dose on C1D1 and return to clinic for the 2nd MVA-BN-Brachyury dose on C2D1. On C3D1, patients will then transition to the FPV-Brachyury vaccine every 21 days until Cycle 9. After that, FPV-Brachyury will be administered every 12 weeks. No biopsies are required. Patients will undergo staging with imaging every 9 weeks (i.e., every 3 cycles).

After the first 6 patients are enrolled and evaluated for safety, this arm will continue to accrue patients until 8 patients have received at least 2 cycles of treatment. If there are 2 or more objective responses, this arm will be expanded to a total of 13 patients. If there is an additional 1 response (total 3 responses in 13 patients), this arm may be further expanded.

If there are 0 to 1 DLTs in the first 6 patients on Arm 1, then Arms 2 and 3 of the trial will open. Patients in Cohort 2 will be assigned to Arm 2. Following the completion of the DLT period for Arm 2, additional patients will be assigned to Arm 3. The first six patients in both arms will be evaluated for DLTs. Following the completion of the DTL period for Arm 3, patients in Cohort 3 will be directly assigned in an alternating fashion to Arms 2 and 3.

Arm 2: HER2+

Fourteen patients will be enrolled in Arm 2. All patients in Cohort 2, Arm 2 must have measurable disease based on RECIST criteria. All patients in Cohort 3, Arm 2 must have measurable disease based on RECIST criteria as well metastatic deposits that can be safely biopsied for the study secondary objective of TIL evaluation. Patients in Arm 2 may have an optional tumor biopsy at baseline and an optional biopsy after 6 weeks on treatment. The study will require a baseline biopsy and a biopsy after 6 weeks on treatment for patients in Cohort 3. There will also be an optional biopsy at the time of progression.

On C1D1, patients will receive T-DM1 3.6mg/kg IV every 3 weeks per standard of care. Patients will also receive M7824 2,400mg IV every 3 weeks (see Section **3.2**).

Patients will receive the first MVA-BN-Brachyury on C1D1 and return to clinic for the 2nd MVA-BN-Brachyury dose on C2D1.

On C3D1, patients will undergo the 2nd biopsy (Optional in Cohort 2, Required in Cohort 3). Patients will then transition to the FPV-Brachyury vaccine q 21 days until cycle 9. After that, FPV-Brachyury will be administered every 12 weeks. Patients will undergo staging every 9 weeks (i.e., every 3 cycles).

Arm 2 will continue to accrue patients until 14 patients have received at least 2 cycles of treatment to evaluate for efficacy of the combination. If there are 4 or more objective responses, this arm will be expanded to a total of 19 patients. If there are an additional 2 responses (total of at least 6 responses of 19 patients), this arm may be further expanded.

Arm 3: HER2+

Fourteen patients will be enrolled in Arm 3. All patients in Cohort 2, Arm 3 must have measurable disease based on RECIST criteria. All patients in Cohort 3, Arm 3 must have measurable disease based on RECIST criteria as well metastatic deposits that can be safely biopsied for the study secondary objective of TIL evaluation. Patients in Cohort 2 can undergo an optional biopsy at baseline and an optional biopsy after 6 weeks on treatment. The study will require a baseline biopsy and a biopsy after 6 weeks on treatment for patients in Cohort 3. There is also be an optional biopsy at the time of progression.

On C1D1, patients will receive T-DM1 3.6mg/kg IV every 3 weeks per standard of care. Patients will also receive M7824 2,400mg IV q 3 weeks (see Section **3.2**).

Patients will receive the first MVA-BN-Brachyury on C1D1 and return to clinic for the 2nd MVA-BN-Brachyury dose on C2D1. Patients will start entinostat 5mg weekly on C1D1 and continue with self-administration on a weekly basis.

On C3D1, patients will undergo the 2nd biopsy (Optional in Cohort 2, Required in Cohort 3). Patients will then transition to the FPV- Brachyury vaccine every 21 days until cycle 9. After that, FPV-Brachyury will be administered every 12 weeks. Patients will undergo staging every 9 weeks (i.e., every 3 cycles).

Arm 3 will continue to accrue patients until 14 patients have received at least 2 cycles of treatment to evaluate for efficacy of the combination. If there are 4 or more objective responses, this arm will be expanded to a total of 19 patients. If there are an additional 2 responses (total of at least 6 responses of 19 patients), this arm may be further expanded.

Patients will be dispensed enough entinostat by the local site pharmacy to complete 1 cycle (i.e., three entinostat 5mg will be dispensed at the start of each cycle; if dose reduced then nine entinostat 1mg tablets will be dispensed at the start of each cycle). See Section **3.2.4.1** for the specific administration procedures of entinostat.

NOTE: Participating sites will be made aware of participant arm/cohort assignment at the time of study registration.

3.1.1 Study Stopping Rule, All Arms

Accrual will be halted if there is an occurrence of a grade 5 toxicity (patient death) within 30 days of receiving any study treatment that is not due to disease progression. Prior to resumption of the study, an expedited safety report will be sent to and reviewed by the FDA. Any SAE also must be evaluated by the clinical investigator.

In each arm, for toxicity that is not a DLT and does not require discontinuation of the IND agent, these agents may be held at the discretion of the investigator and may be reinitiated after toxicity has improved at the same or lower dose level (if applicable). In the even that one drug is discontinued due to toxicity, patients may continue treatment with the other drug(s) at the discretion of the investigator.

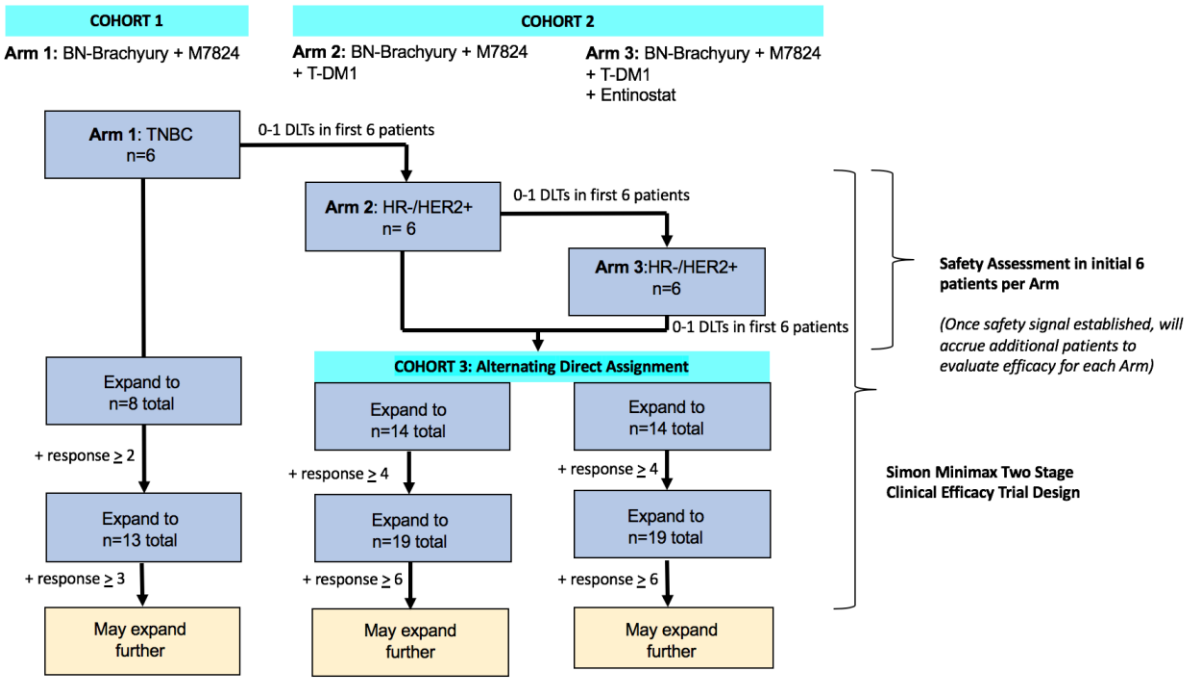


Figure 11: Trial Schema

3.1.2 Dose Limiting Toxicity

For the pre-specified safety monitoring plans below, a dose-limiting toxicity (DLT) is defined as an adverse event or abnormal laboratory value assessed as suspected to be trial treatment related (possible, probable or definite) and unrelated to disease or disease progression that occurs within the 30 days of the first treatment. Toxicities and lab values will be categorized and graded according to CTCAE v5.0.

1. Any grade ≥3 non-hematologic, non-hepatic adverse event with the following exceptions:
    a. Nausea and/or vomiting that persists for 48 hours despite supportive care
    b. Electrolyte imbalances that persist for 48 hours despite supportive care
2. Grade ≥ 3 fatigue lasting ≥ 7 days
3. Grade ≥ 4 neutropenia (ANC < 500uL) lasting > 7 days
4. Grade ≥ 4 thrombocytopenia
5. Grade ≥ 3 bleeding events
6. Grade ≥ 3 febrile neutropenia
7. Grade ≥ 4 elevation in AST or ALT
8. Grade ≥ 4 elevation in bilirubin

<u>Monitoring of dose-limiting toxicities</u>

Up until the time the first 3 patients complete at least one cycle of treatment on each arm, accrual will proceed slowly (Arm 1: no more than 1 patient per 6 days; Arms 2 and 3: no more than 1

patient per 28 days) to more closely monitor safety, and after 3 patients receive at least 1 cycle of drugs, a safety review will be conducted before additional patients are enrolled. If there is one or more DLT in the first 3 patients on a given arm, accrual of the next 3 patients on the associated arm will proceed slowly with no more than 1 patient per 21 days (1 cycle) to continue closely monitor safety. On the basis of the monitoring criteria, if 2 of the first 2 patients or if 3 patients experience DLT within the first 6 patients, then the trial will halt accrual and the treatment regimen may be modified. The review will include a per-patient listing of all reported AEs to date, including actions required for dosing, to more fully review the nature, frequency, severity and timing of the events. This information combined with fewer DLTs may also result in modification of the treatment regimen. These reviews and decisions will be made by the Principal Investigator/ clinical research team as outlined in Section **7.4.1**.
Throughout enrollment of all arms, DLTs within the 30 days of treatment will be summarized with pre-specified criteria based on sequential boundaries to pause enrollment to more fully review safety if excessive numbers of DLTs are observed. The criteria are such that the probability of crossing the boundary is at most 0.05 if the true DLT rate is equal to 10%, and the probabilities of pausing are 0.67, 0.90 and 0.98 if the true DLT rate is equal to 30%, 40% or 50%.

The accrual will be paused if excessive numbers of DLTs are seen, that is, if the number of DLTs is equal to or exceeds b_n out of n patients in the population evaluable for toxicity. **Table 2** gives the criteria for pausing enrollment to more fully evaluate safety.

Table 2. Criteria for pausing enrollment because of DLTs to more fully review safety, for sample size up to 19 patients.

Number of patients	n	1-2	3-6	7-12	13-19
Number of patients for whom DLT is reported	b_n	2	3	4	5

3.2 DRUG ADMINISTRATION AND PRE-MEDICATIONS

The BN-Brachyury vaccines will be given on the day of treatment with M7824 and T-DM1 (Arms 2 & 3 only). The preferred order of drug administration is vaccine, then M7824 administration, then T-DM1 if applicable; When possible, administer the vaccine within 15 minutes prior to the M7824 administration; however, the exact timing of vaccine administration is flexible. Patients in Arm 3 will be provided with a take-home supply of entinostat and will self-administer entinostat around bedtime on the day of the infusions. Patients will also self-administer entinostat on subsequent designated days of the cycle (Days 8 and 15; may be taken up to 24 hours late before considered a missed dose).

A window of -1 or +3 days for every 3 week cycle is allowed in the event of scheduling issues (i.e., holiday, bad weather, family responsibilities, security alerts or other scheduling issues). This can also extend to complications of disease not attributable to disease progression or protocol therapy. These delays will not be considered protocol deviation. Any dose that cannot be accommodated within this window will be skipped and the dose not made up.

3.2.1 MVA-BN-Brachyury and FPV-Brachyury Vaccines

MVA-BN-Brachyury is administered via subcutaneous injection. The preferred injections sites include the left upper arm, the right upper arm, the left outer thigh, and the right outer thigh. One

dose consists of 4 separate injections: one injection in each of the listed injection sites. Each 0.5mL injection of MVA-BN-Brachyury consists of a nominal virus titer of 2.0 x 10^8 infectious units (Inf.U).

FPV-Brachyury is administered via subcutaneous injection as a single injection per dose. Options for administration sites include the upper arm or outer thigh. When possible, each subsequent dose should be administered at the same injection site as the first dose. Each injection of FPV-Brachyury consists of a nominal virus titer 1.0 x 10^9 Inf.U.

Ideally, the BN-Brachyury vaccines will be administered prior to the start of the M7824 infusion.

3.2.2 M7824

Subjects will be scheduled to receive M7824 at a flat dose of 2,400 mg IV once every 3 weeks. M7824 can be administered through a central line (preexisting) or a peripheral IV. There is no need to prime the line and purge the air prior to administration in this protocol. A filter is required for administration of M7824. The preference is for M7824 to be given via peripheral line. However, if the research team documents that the main material of the port is titanium, M7824 can be administered via mediport. It is the responsibility of the research team to confirm and document this in CRIS. Once documented, the ordering physician can change the route of administration to mediport.

Please refer to the pharmacy manual for a list of acceptable materials known to be compatible with M7824.

As T-DM1 is dosed every 3 weeks, a q3 week dosing regimen for M7824 was selected based on PK modeling and Phase 1 trial safety data. Subjects will receive an IV infusion of M7824 over 1 hour (-10 minutes / +20 minutes, that is, 50 to 80 minutes). As a routine precaution, subjects enrolled in this trial must be observed for 60 minutes post end of infusion, in an area with resuscitation equipment and emergency agents. During cycle 1, vital signs will be monitored every15 minutes during infusion and until completion of observation period. Starting with cycle 2, vital signs will be monitored every 30 minutes during the infusion and until the completion of the observation period.

Current experience revealed that infusion related reactions (IRRs) to M7824 seldom occur and are generally mild to moderate in severity. Therefore, administration of a premedication is generally not required.

If an Investigator deems it necessary to administer a premedication, an antihistamine (for example, 25-50 mg diphenhydramine) and acetaminophen 500-650 mg intravenously or equivalent oral dose is recommended approximately 30 to 60 minutes prior to each dose of M7824. If Grade ≥ 2 infusion reactions are seen during the first two infusions, premedication should not be stopped. Steroids as premedication are not permitted.

Management of symptoms should follow the guidelines shown in **Table 10**.

3.2.2.1 Immediate Hypersensitivity Reaction

Hypersensitivity reactions may require immediate intensive care. M7824 should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1: 1,000 dilution), allergy

medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

A complete guideline for the emergency treatment of anaphylactic reactions according to the Working Group of the Resuscitation Council United Kingdom and can be found at https://www.resus.org.uk/pages/reaction.pdf

3.2.2.2 Flu-Like Symptoms

For prophylaxis of flu like symptoms, a nonsteroidal anti-inflammatory drug (NSAID), e.g., ibuprofen 400 mg or comparable NSAID dose, may be administered 2 hours before and 8 hours after the start of each IV infusion.

Patients can receive M7824 for up to 12 months should they remain on trial.

3.2.3 Ado-trastuzumab emtansine (T-DM1)

Patients in Arms 2 and 3 will receive T-DM1 3.6 mg/kg by IV infusion every 3 weeks. The first infusion will be administered over 90 minutes and subsequent infusions will be administered over 30 minutes, if the first infusion was well tolerated. If the first infusion is not well tolerated, then the next infusion will be administered over 90 minutes (as per the T-DM1 package insert). Infusion times may be adjusted for patients experiencing any infusion-related reaction. There does not need to be an observation time between the completion of M7824 and the start of T-DM1, however, vital signs should be recorded following the completion of M7824 and immediately prior to the start of T-DM1. As routine and as described in the FDA package insert for T-DM1, subjects who receive T-DM1 should be observed following completion of T-DM1 infusion.

Per the FDA package insert for T-DM1,

- First infusion: Patients should be observed during the infusion and for at least 90 minutes following the initial dose for fever, chills, or other infusion related reactions.
- Subsequent infusions: Patients should be observed during the infusion and for at least 30 minutes after infusion if the first infusion was well tolerated or for 90 minutes if not well tolerated.

During cycle 1, vital signs will be monitored every15 minutes during the infusion and until completion of observation period. Starting with cycle 2, vital signs will be monitored every 30 minutes during the infusion and until the completion of the observation period.

Conventional trastuzumab and ado-trastuzumab emtansine products are NOT interchangeable. T-DM1 should be prepared according to the FDA-approved prescribing information using an infusion bag containing 250mL of 0.9% sodium chloride injection (do NOT use 5% dextrose). Infusions must be administered through a 0.2 or 0.22 micron nonprotein adsorptive polyethersulfone filter. Diluted T-DM1 may be stored in the refrigerator (2 to 8℃) for up to 24 hours prior to use.

Pre-medications

In order to mitigate potential infusion-related reactions, premedication with an antihistamine and with paracetamol (acetaminophen) (for example, 25-50 mg diphenhydramine and 500-650 mg paracetamol [acetaminophen] IV or oral equivalent) approximately 15 to 60 minutes prior to dosing of M7824 is optional and at the discretion of the Investigator. If Grade ≥ 2 infusion

reactions are seen during the first two infusions, premedication should not be stopped. Premedication can be given to the patient at the same time as the BN-Brachyury vaccine.

Since T-DM1 is considered a drug with low emetic risk by both ASCO and NCCN, the use of a prophylactic single dose of a 5-HT3 antagonist or dopamine blocker (i.e., prochlorperazine) is encouraged. The antiemetic regimen may be modified based on patient specific factors or standard institutional practices.

3.2.4 Self-Administered Study Drugs

The entinostat used in this study is a self-administered investigational agent. Such agents are dispensed from the pharmacy to a participant, participant's representative or to a Patient Care Unit for self-administration and a record of the dispensed investigational agent is generated and kept by the dispensing pharmacy.

3.2.4.1 Entinostat (Arm 3 only)

At the start of each cycle, patients will be dispensed enough entinostat by the local site pharmacy to complete 1 cycle (i.e., three entinostat 5mg will be dispensed at the start of each cycle; if dose reduced then nine entinostat 1mg tablets will be dispensed at the start of each cycle). Entinostat is to be administered on an empty stomach, at least 2 hours after a meal and 1 hour before the next meal on Days 1, 8 and 15 of each cycle. If a patient forgets to take a dose on the assigned day, the dose can be taken up to 1 day (24 hours) later. If a dose is more than 1 day late, that dose will be skipped. Do NOT split, crush or chew entinostat tablets. In case of vomiting after the entinostat tablet is swallowed, the dose should not be taken again unless the expelled tablet is visualized in the vomitus and is entirely intact. Patients should discuss with the study team before attempting to re-take the dose.

For Cycle 1 Day 8, patients will be asked to take the entinostat tablet in the morning of the day of the clinical visit in order to assess for pharmcokinetic data 4-6 hours after taking the medication. (118) There is also an optional research lab visit on Cycle 1 Day 15 where patients will be instructed to do the same.

Patients will be asked to keep a medication diary and bring it with them on each study visit (See **Appendix B**). Patients will also bring any remaining pills to each study visit. The research nurse will review and validate the completeness and accuracy of the participant's diary with the participant.

If a participant goes off study while at home, the research nurse will document and ensure the return of the unused oral investigational agents from the participant. Unused investigational agent will be destroyed per dispensing pharmacy procedure.

3.3 Study Intervention Compliance

Participants in Arm 3 will be provided with a medication diary (**Appendix B**: Oral Medication Diary *(Arm 3 only)*) and asked to complete this at the time of self-administration of entinostat. Research nurses will collect this medication diary at the start of each cycle and will verify that entinostat was taken within the appropriate time period specified by the protocol. Medication diaries will be used to calculate study intervention compliance.

3.4 DOSE MODIFICATIONS

- In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).
- Once a patient has a dose reduction for toxicity, the dose will not be increased.
- Any of the trial drugs may be stopped for toxicity per the discretion of the primary investigator if is it felt that the toxicity can be directly linked to a specific drug or drugs. However, if 2 drugs or more must be stopped, the patients will be taken off treatment.
- Participants continuing to experience toxicity at the off-treatment visit will be contacted for additional assessments until the toxicity has resolved or is deemed irreversible. Patients must remain on the study to have additional assessments completed.
- For AEs that are unrelated to the study drugs, study drug may be held for up to 28 days at the discretion of the PI. Any dose that would have occurred within this window will be skipped and the dose not made up.
- If any treatment related AE observed was grade > 2, treatment should be delayed by 1 week (except for hyperbilirubinemia where drug will be held for grade 2 – see **Table 7**; except for electrolyte abnormalities that can be easily replenished or other lab abnormalities that have no clinical consequence) for up to 4 consecutive weeks and possibly patients should be reduced by 1 dose level (See **Table 3**, **Table 4**, **Table 5**, **Table 6**, **Table 7**).
- If, after 4 weeks of delay, all treatment related AEs have still not resolved (to grade ≤ 1), then any further treatment should be stopped.
- If any treatment-related AE observed is grade 4, then all treatments should be stopped. The one exception is grade 4 thrombocytopenia in which the drug will be held and then reduce one dose level.

Table 3A: Recommended Dose Reduction Schedule for Adverse Events for Arm 2

Dose Level	Dose Reduction Schedule	T-DM1 Dose
1	Starting dose	3.6 mg/kg IV q3 weeks
-1	First dose reduction	3.0 mg/kg IV q3 weeks
-2	Second dose reduction	2.4mg/kg IV q3 weeks
-3	Third dose reduction	Discontinue treatment

* There will be no dose reductions of the BN-Brachyury vaccines.
** M7824 may be reduced at any time after the DLT period in the study from 2,400mg to 1,800mg at discretion of the PI for any side effects possibly attributed to the TGF-beta component of the drug (i.e., mucosal bleeding, excessive keratoacanthomas).

Table 3B: Recommended Dose Reduction Schedule for Adverse Events for Arm 3

Dose Level	Dose Reduction Schedule	T-DM1 Dose	Entinostat
1	Starting dose	3.6mg/kg IV q3 weeks	5mg po q7 days
-1	First dose reduction	3.6mg/kg IV q3 weeks	3mg po q7 days
-2	Second dose reduction	3.0mg/kg IV q3 weeks	3mg po q7 days
-3	Third dose reduction	2.4mg/kg IV q3 weeks	2mg po q7 days
-4	Requirement for further dose reduction	Discontinue treatment	

* There will be no dose reductions of the BN-Brachyury vaccines.
** M7824 may be reduced at any time after the DLT period in the study from 2,400mg to 1,800mg at the discretion of the PI for any side effects possibly attributed to the TGF-beta component of the drug (i.e., mucosal bleeding, excessive keratoacanthomas).

3.4.1 Dose Modification of Entinostat

Table 4: Non-hematologic Toxicity

Toxicity	**Dose Modifications**
Grade 4	Administer symptomatic remedies/ start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions: 1. If recovered within 4 weeks of onset (i.e., ≤3 missed doses), resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg. • If receiving 3 mg, restart study drug at same dose or reduce to 2 mg (see **Table 3B**). • If receiving 2 mg, discontinue study treatment. 2. If not recovered within 4 weeks, permanently discontinue study drug.
Grade 3	Administer symptomatic remedies / start prophylaxis. Hold dose until recovery to Grade 1 or baseline under the following directions: 1. If recovered within 1 week, resume study drug at prior dose. If not recovered within 1 week, continue to hold dose. 2. If recovered within 2-4 weeks, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg. • If receiving 3 mg, restart study drug at same dose or reduce to 2 mg (see **Table 3B**). • If receiving 2 mg, permanently discontinue study drug. 3. If not recovered within 4 weeks, permanently discontinue study drug.
Recurrence of the **same** ≥ Grade 3 toxicity despite dose reduction	If the **same** ≥ Grade 3 event **recurs:** 1. Administer symptomatic remedies/ start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline. 2. If recovered within 2 weeks, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg. • If receiving 3 mg, restart study drug at same dose or reduce to 2 mg (see **Table 3B**). • If receiving 2 mg, permanently discontinue study drug. 3. If the **same** ≥ Grade 3 event **recurs** (i.e., third occurrence) despite entinostat dose reduction to 2 mg, as described above, discontinue study drug.
≤Grade 2	Administer symptomatic remedies / start prophylaxis. Dosing of study drug may be interrupted at the Investigator's discretion. • If dose is held for 4 consecutive weeks, permanently discontinue study drug. • If toxicity resolves, resume entinostat at the original dose.

[1] *If greater than 50% of doses are missed during any 6 week period, discontinue from study drug treatment.*

Table 5: Hematologic Toxicity	
Toxicity	**Dose Modifications**
≥ Grade 3 neutropenia, ≥ Grade 3 uncomplicated thrombocytopenia, or Grade 2 complicated thrombocytopenia	Administer symptomatic remedies / start prophylaxis. Hold dose[1] until recovery to Grade 1 or study baseline under the following direction: 1. If not recovered by next scheduled dose, skip the dose. If recovered by next scheduled dose, resume study drug at prior dose. 2. If receiving 2 mg dose, and not recovered by either of the next 2 scheduled doses, permanently discontinue study treatment. Otherwise, skip each dose. If recovered for either of these doses, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg. • If receiving 3 mg, restart study drug at 2 mg (see **Table 3B**). 3. If not recovered within 4 weeks, permanently discontinue study drug.
Recurrence of the **same** hematologic toxicity	If the same hematologic toxicity recurs: 1. Administer symptomatic remedies/ start prophylaxis. Hold[1] dose until recovery to Grade 1 or baseline. 2. If recovered within 2 weeks, resume study drug as follows: • If receiving 5 mg, restart study drug at 3 mg • If receiving 3 mg, restart study drug at 2 mg. (See **Table 3B**) • If receiving 2 mg, permanently discontinue study drug 3. If the same ≥ Grade 3 event recurs (i.e., third occurrence) despite entinostat dose reduction to 2 mg, as described above, permanently discontinue study drug.

[1] *If greater than 50% of doses are missed during any 6 week period, discontinue from study drug treatment.*

3.4.2 Dose Modification of T-DM1

Dose modifications for T-DM1 according to published FDA recommendations. Please see https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf for full details.

Once the dose of T-DM1 is reduced, the dose should not be re-escalated.

If a planned dose is delayed or missed, it should be administered at the next planned cycle. The infusion may be administered at the dose and rate the patient tolerated in the most recent infusion.

The infusion rate of T-DM1 should be slowed or interrupted if the patient develops an infusion-related reaction. Permanently discontinue T-DM1 for life-threatening infusion-related reactions.

Management of increased serum transaminases, hyperbilirubinemia, left ventricular dysfunction, thrombocytopenia, pulmonary toxicity or peripheral neuropathy may require temporary

interruption, dose reduction or treatment discontinuation of T-DM1 as per guidelines provided in **6, Table 7, Table 8, Table 9**.

Hepatotoxicity

Table 6: Dose Modification Guidelines for Increased Serum Transaminase (AST/ALT)

Grade 2 (> 2.5 to ≤ 5x ULN)	**Grade 3 (> 5 to ≤ 20x ULN)**	**Grade 4 (> 20 x ULN)**
Treat at same dose level.	Do not administer T-DM1 until AST/ALT recovers to Grade ≤ 2 and then reduce one level.	Permanently discontinue T-DM1.

ALT = alanine transaminase; AST = aspartate transaminase; ULN = upper limit of normal.

Table 7: Dose Modification for Hyperbilirubinemia

Grade 2 (> 1.5 to ≤ 3x ULN)	**Grade 3 (> 3 to ≤ 10x ULN)**	**Grade 4 (> 10 x ULN)**
Do not administer T-DM1 until total bilirubin recovers to Grade ≤1 and then treat at same dose level.	Do not administer T-DM1 until total bilirubin recovers to Grade ≤ 1 and then reduce one level.	Permanently discontinue T-DM1.

Permanently discontinue T-DM1 treatment in patients with serum transaminases > 3x ULN and concomitant total bilirubin > 2x ULN.

Permanently discontinue T-DM1 in patients diagnosed with nodular regenerative hyperplasia (NRH).

Left Ventricular Dysfunction

Table 8: Dose Modification for Left Ventricular Dysfunction

Symptomatic CHF	**LVEF < 40%**	**LVEF 40% to ≤45% AND decrease is ≥ 10% points from baseline**	**LVEF 40% to ≤45% AND decrease is < 10% points from baseline**	**LVEF > 45%**
Discontinue T-DM1.	Do not administer T-DM1. Repeat LVEF assessment within 3 weeks. If LVEF < 40% is confirmed, then discontinue T-DM1.	Do not administer T-DM1. Repeat LVEF assessment within 3 weeks. If the LVEF has recovered to within 10% points from baseline, discontinue T-DM1.	Continue with T-DM1. Repeat LVEF assessment within 3 weeks.	Continue with T-DM1.

CHF = congestive heart failure; LVEF = left ventricular ejection fraction

Thrombocytopenia

A reduction in dose is recommended in the case of Grade 4 thrombocytopenia (platelets < 25,000/mm^3).

Table 9: Dose Modification Guidelines for Thrombocytopenia

Grade 3 **PLT 25,000/mm^3 to 50,000/mm^3**	**Grade 4** **PLT < 25,000/mm^3**
Do not administer T-DM1 until PLT recovers to Grade ≤ 1 (≥ 75,000/mm^3) and then treat at same dose level.	Do not administer T-DM1 until PLT recovers to Grade ≤1(≥ 75,000/mm^3), and then reduce one dose level.

PLT = platelets

Pulmonary Toxicity

T-DM1 should be permanently discontinued in patients with interstitial lung disease (ILD) or pneumonitis.

Peripheral Neuropathy

T-DM1 should be temporarily discontinued in patients experiencing Grade 3 or 4 peripheral neuropathy until resolution to ≤ Grade 2.

3.4.3 Treatment Discontinuation of BN-Brachyury Vaccines

No dose modification. If unable to tolerate due to side effects, the vaccine will be removed from the treatment regimen.

3.4.4 Treatment discontinuation of M7824

- Any Grade 4 adverse drug reactions (ADRs), as defined by CTCAE v5 and assessed as related to M7824 by the Investigator, except for laboratory values that are determined to not be clinically significant or single laboratory valued that resolve to Grade ≤ 1 or baseline grade within 7 days with adequate medical management.
- Any Grade 3 ADRs possibly, probably or definitely related to M7824 except for any of the following:
    - Transient (≤ 48 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management.
    - Transient (≤ 48 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis which is controlled with medical management.
    - Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor.
    - Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis.

- Grade 3 Hemoglobin decrease (< 8.0 g/dL) that is clinically manageable with blood transfusions or erythroid growth factor use does not require treatment discontinuation.
- Keratoacanthoma and squamous cell carcinoma of the skin.
- Any endocrinopathy that can be medically managed with hormone replacement.
- Any grade 3 adverse drug reaction which in the opinion of the investigator is not clinically relevant or can be medically managed with minimal risk to the patient (e.g., placement of a pleural catheter for recurrent inflammatory pleural effusions).

3.4.5 Suggested Evaluation of Suspected Myocarditis

If a patient has symptoms concerning for a diagnosis of myocarditis or myopericarditis, cardiology will be consulted immediately. In addition, the following clinical evaluation may be performed depending on clinical symptoms and clinical suspicion of the investigator:

- Troponin-I and CK, CK-MB x 1
- EKG in triplicate
- 2E Echo to evaluate ejection fraction and TDI-derived longitudinal strain
- Cardiac MRI if applicable

3.4.6 Suggested Evaluation of Suspected Bleeding or Hemorrhage Events and Treatment Modification

For anemia or hemorrhage events assessed as treatment-related, items queried may include but are not limited to detailed relevant past medical and treatment history, bruising tendency, history of blood transfusions and/or dependency, and a request for an updated patient history including details such as concomitant medications, all laboratory data, updated dosing information and recent tumor evaluation scans.

In this protocol, anemia may be due to M7824 (documented in 29% of patients) and/or T-DM1 (documented in approximately 10 to 15% of patients). M7824 treatment-related anemia is an AESI (see Investigators' Brochure). Notably, there are many reasons for hemorrhage and/or anemia in patients with advanced cancer, and Hb level of at least 9 g/dl is required for this study. A thorough investigation of new anemia cases of unspecified etiology is recommended.

General Guidance for anemia management and evaluation:

- Participants must enter the study with Hgb values at least 9 g/dL and baseline anemia evaluation is conducted per recommendations below.
- Consider hematology consult for severe and or refractory anemias.
- All relevant hematologic testing for treatment related anemias should be done prior to blood transfusion, if clinically feasible.
- Transfusion should be performed at the discretion of the investigator, based on clinical assessment and considered when participant experiences significant anemia.
- Guidance for evaluation of baseline anemia or suspected treatment-related anemias is provided below

  - Hb and CBC with differential (e.g., MCV, RDW, ANC, hematocrit, reticulocytes counts)
  - Peripheral blood smear for cell morphological assessment
  - Complete metabolic panel including liver panel-LFTs, bilirubin, LDH, renal function, and serum folate, B12 values and other chemistries
  - Coagulation factors (PT, PTT, INR)
  - Urinalysis including culture
  - Iron panel (TIBC, ferritin, Fe)

- Discuss further management with Principal Investigator for clinically significant treatment related anemias.

Multiple protocols using M7824, several mucosal bleeding events ranging from low grade gingival bleeding and epistaxis to more serious hemoptysis, GI bleeding and hematuria have been observed. Some of these events can be attributed to bleeding events related to cancer directly and others bleeding events can be attributed to an inflammatory process (e.g. colitis) which is a known toxicity of anti-PD-L1 agents including M7824. However, there remains the possibility that M7824 may increase the overall risk of bleeding in ways that may not be directly related to direct tumor bleeding or inflammatory bleeding events described with checkpoint inhibitors like M7824. However, there is no evidence of a negative effect on coagulation or platelet number or function. It is hypothesized that this possible increased mucosal bleeding risk may be due to the known mucoprotective effects of TGFβ. Accordingly, patients will be closely monitored for mucosal bleeding (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool).

- If Grade 3 mucosal bleeding occurs in the absence of thrombocytopenia ($\geq$ 75,000), liver enzyme abnormalities (< Grade 3 AST or ALT elevation +/- abnormal bilirubin) or coagulation abnormalities (INR. < 2.5), M7824 will be held with BN-Brachyury, T-DM1 +/- entinostat being allowed to continue per PI discretion. See Tables 5, 6, 7 and 9 for specific dosing instructions for agents. M7824 can be resumed once mucosal bleeding is $\leq$ Grade 1 and the dose may be reduced to 1800mg q3 weeks per PI discretion.
- If Grade 3 non-mucosal bleeding or hemorrhage occurs in the absence of thrombocytopenia ($\geq$75,000), liver enzyme abnormalities (< Grade 3 AST or ALT elevation +/- abnormal bilirubin) or coagulation abnormalities (INR < 2.5), T-DM1 will be held with BN-Brachyury, M7824 +/- entinostat being allowed to continue per PI discretion. See Tables 5, 6, 7 and 9 for specific dosing instructions for agents.
- If Grade 3 bleeding or hemorrhage occurs in the presence of thrombocytopenia (<75,000), liver enzyme abnormalities ($\geq$ Grade 3 AST or ALT elevation +/- abnormal bilirubin) or coagulation abnormalities (INR $\geq$ 2.5) T-DM1, M7824 and entinostat will be held until a formal workup is completed with BN-Brachyury being allowed to continue per PI discretion.
- If a drug is held for hemorrhage or bleeding and the hemorrhage or bleeding is attributed to a trial drug, a dose reduction should be considered if the drug is to be reintroduced once AEs resolve ($\leq$ Grade 1 within 2 cycles).
  - If bleeding or hemorrhage is attributed to a specific drug, then that drug should be reduced by 1 dose level if is it resumed.

- If bleeding or hemorrhage cannot be attributed to a specific drug, then the PI should consider reducing all suspected drugs by 1 dose level if the patient is to remain on treatment.
- If mucosal bleeding occurs, the PI should consider reducing M7824 to 1800mg q3 weeks.

3.4.7 Toxicity Management

Table 10: Treatment Modification Guidance for Symptoms of Infusion-Related Reactions including Immediate Hypersensitivity

Guidelines below are merely suggestions. If an immune-mediated reaction occurs, the subject should be treated according to the best available medical practice.

Infusion-Related Reactions (IRR) are an important risk for M7824.

NCI-CTCAE Grade	Treatment Modification for M7824
Grade 1 – mild Mild transient reaction; in general, infusion interruption not indicated; intervention not indicated.	• Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • Hold infusion if deemed necessary by the investigator.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, nonsteroidal anti inflammatory drugs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	• Stop the infusion of the study intervention. • Increase monitoring of vital signs as medically indicated as participants are deemed medically stable by the attending Investigator. • If symptoms resolve quickly, resume infusion at 50% of original rate with close monitoring of any worsening signs and symptoms, otherwise dosing held until resolution of symptoms with mandated premedication for the next scheduled visit. • If not improving, consider administration of glucocorticoids and stop the infusion for that day. • If the participant has a second IRR Grade ≥ 2 on the slower infusion rate despite premedication, the infusion should be stopped, and the investigator may consider withdrawal of this participant from the study.

NCI-CTCAE Grade	Treatment Modification for M7824
Grade 3 or Grade 4 – severe or life-threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	• Stop the infusion of study intervention immediately and disconnect infusion tubing from the participant with additional appropriate medical measures and closely monitor until deemed medically stable by the attending Investigator. Hospitalization and/or close monitoring is recommended. • Administration of glucocorticoids may be required. • For Grade 3 or 4 IRRs, permanent discontinuation of study intervention is mandated.
Once the infusion is interrupted or rate reduced to 50% of previous infusion rate, it must remain decreased for all subsequent infusions. For all types and grades of infusion reactions, details about drug physical constitution, method of preparation, and infusion must be recorded. Participants should be instructed to report any delayed reaction immediately.	
Once the infusion is interrupted or rate reduced to 50% of previous infusion rate, it must remain decreased for all subsequent infusions. For all types and grades of infusion reactions, details about drug physical constitution, method of preparation, and infusion must be recorded. Participants should be instructed to report any delayed reaction immediately.	

Table 11 Immune-related adverse events (irAEs)

Immune-related AEs are specific to immunotherapies and vary by organ system.
The following immune-related AEs are important identified risks for M7824:

- Immune-related pneumonitis
- Immune-related hepatitis
- Immune-related colitis
- Immune-related nephritis and renal dysfunction
- Immune-related endocrinopathies
- (thyroid disorders, adrenal insufficiency, type 1 diabetes mellitus, pituitary disorders)
- Immune related rash
- Other immune-related events (myositis, myocarditis, encephalitis)

The following immune-related AEs are important potential risks for M7824:

- Guillain-Barré syndrome
- Uveitis
- Pancreatitis
- Myasthenia gravis/myasthenic syndrome

Recommended guidance and management for specific irAEs are provided in the current NCCN (guideline available at http://www.nccn.org).

Requirements in addition to NCCN guidelines:

- Permanent treatment discontinuation is required in case of immune-related Grade 4 rash/inflammatory dermatitis, nephritis, autoimmune hemolytic anemia, hemolytic uremic syndrome, aplastic anemia, immune thrombocytopenia, acquired thrombotic thrombocytopenic purpura inflammatory arthritis, myositis and polymyalgia-like syndrome.
- For Grade 4 immune-related lymphopenia, permanent treatment discontinuation will be required, if lymphopenia is considered immune-related in nature, no clear alternative explanation exists for the event, and it does not resolve within 14 days. Permanent treatment discontinuation is not required when the AE is manifested by a single laboratory value out of normal range without any clinical correlates. In this case, treatment should be held until the etiology is determined. If the event is not considered immune-related and resolves to Grade ≤ 1, restarting treatment may be considered.
- For Grade 1 immune-related pneumonitis: continue treatment. If clinically indicated, monitor participants weekly or more frequently as needed with history, physical examination and pulse oximetry. If symptoms appear and/or changes in the physical exam are noted, treat as Grade 2.
- For myositis: in case of management with rituximab, treatment should be discontinued.
- For Grade 3 or 4 endocrinopathies: withhold until clinically stable or permanently discontinue depending on severity.
- For hepatitis with no tumor involvement of the liver: withhold if total bilirubin increases to more than 1.5 and up to 3 times ULN, permanently discontinue if more than 3 times ULN.
- Hepatitis with tumor involvement of the liver: permanently discontinue if total bilirubin increases to more than 3 times ULN.

Table 12 Management of M7824 mediated Skin Reactions

Skin reactions are considered important identified risk for M7824.

- Hyperkeratosis
- Keratoacanthoma
- Cutaneous squamous cell carcinoma (cSCC)
- Basal cell carcinoma

• Actinic keratosis
Management

• Discontinuation or termination not required in most cases. Continuation of treatment should be evaluated by the Investigator.
• Emollients may be used
• Develop diagnostic and treatment plan in collaboration with Investigator and dermatologist
• Treatment follow-up will depend on number and localization of lesions.
  o Single lesion: full excision may be recommended
  o Multiple lesion or location not suitable for full excision: Mohrs surgery, cryotherapy or other standard treatment options depending on pathology. Retinoids may be used after discussion with Investigator.
• Close clinical follow-up for re-evaluation, resolution and potential recurrence should be implemented
• In general, treatment of skin lesions should be based on local guidelines/standard of care.

Additional consideration: Keratoacanthoma lesions may resolve spontaneously without surgical intervention within weeks after discontinuing M7824. Consult with Medical Monitor as needed for management of skin lesions.

Table 13 Management of Treatment-Related Anemia

Anemia is considered an important identified risk for M7824. • All relevant hematological testing for treatment-related anemias should be done prior to a blood transfusion, if clinically feasible
Basic Anemia Evaluation

• CBC with emphasis on red cell indices
• If indicated and at clinical discretion, the following should be considered:
  o Iron studies
  o Serum Folate and Vit B12 values
  o Coagulation factors
  o Fecal occult blood
  o Urinalysis
  o Hormone panel: TSH, Erythropoietin
  o Peripheral blood smear

Further Recommendation Based on Suspected Etiology (in Addition to Basic Anemia Testing)
• Suspected Hemolysis   o bilirubin, LDH, Coombs test, haptoglobin • Suspected bleeding:

○ Consider imaging/interventional radiology consultation as indicated ○ Consider imaging and/or endoscopy as clinically indicated • Suspected aplastic anemia: ○ Hematology consultation ○ Consider bone marrow aspiration/morphologic evaluation
Additional consideration: In general, blood transfusions and erythroid growth factors are permitted as clinically indicated.

Table 14 Management of Bleeding Adverse Events

Bleeding Adverse Events	
• Bleeding adverse events are considered important identified risk for M7824. • In general, mild and moderate mucosal bleedings resolve without discontinuation of treatment. • These events may include, but are not limited to the following: ○ Epistaxis ○ Hemoptysis ○ Gingival bleeding ○ Hematuria	
Non-tumor Bleeding	
Grading	Management
Grade 2	• If resolves to Grade ≤ 1 by the day before the next infusion, study intervention may be continued • If not resolved to Grade ≤ 1 by the day before the next infusion, but is manageable and /or not clinically relevant, assessment if clinically reasonable to administer the following infusion will be per PI discretion.
Grade 3	• Permanently discontinue treatment unless an alternative explanation can be identified (such as concomitant use of antithrombotic agents, traumatic events, etc.) • In case of alternative explanations, hold study treatment until the event recovers to Grade ≤ 1
Grade 4	• Treatment must be permanently discontinued if no alternative explanation is identified.
Tumor Bleeding	

Grade ≥ 2	• Study treatment must be held till the event recovers to Grade ≤ 1 • Permanently discontinue treatment if the Investigator considers the participant to be at risk for additional severe bleeding.

Table 15 Impaired Wound Healing

• Impaired wound healing is considered important potential risk for M7824. • Elective surgery on study will be allowed per PI discretion. • It is recommended to hold study intervention for approximately 4 weeks post major surgery for observation. Post-operative wound healing should be closely monitored.

3.5 STUDY CALENDAR

1 cycle = 21 days

Procedure	**Screening**	**Cycle 1**			**Cycle 2**	**Cycle 3 Cycle 5 Cycle 7**	**Cycle 4 Cycle 6 Cycle 8**	**Cycle 9 And beyond**	**End of treatment visit[13]**	**Safety visit[13]**	**Long term follow up[14]**
		Day 1	**Day 8**	**Day 15**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**(≤ 30 days from last dose)**	**(28 days after removal from study therapy)**	
Informed consent	X	X[1]									
History and PE[2]	X	X (≤ 3 days)	X		X	X	X	X	X	X	
Vital signs[15]	X	X	X		X	X	X	X	X	X	
Performance Score	X										
Skin assessment[16]		X	X		X	X	X	X	X	X	
Clinical Labs	X	X[4] (≤ 7 days)	X[4]		X[4]	X[4]	X[4]	X[4]	X[4]	X	
Pathology confirmation[5]	X										
Tumor block		X[6]									
Tumor biopsy[7]		X (≤ 7 days)				X (Cycle 3 only)			X *(optional)*		
Research Blood[8]			See Table below								
Urine or serum pregnancy testing in women of childbearing potential[9] (2.4.2.2)	X	X			X	X	X	X	X	X	
Advance Directives Form	X *(optional)*										
Radiological Assessments[10]	X	X (≤ 28days)				X (q9 wks)		X (q9 wks)			X
Adverse Events		X	X		X	X	X	X	X	X	X
Concomitant Medications	X	X			X	X	X	X	X	X	
Electrocardiogram[11]	X	X (< 7 days)	X								

Procedure	**Screening**	**Cycle 1**			**Cycle 2**	**Cycle 3 Cycle 5 Cycle 7**	**Cycle 4 Cycle 6 Cycle 8**	**Cycle 9 And beyond**	**End of treatment visit[13]**	**Safety visit[13]**	**Long term follow up[14]**
		Day 1	**Day 8**	**Day 15**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**Day 1 (-1/+3days)**	**(≤ 30 days from last dose)**	**(28 days after removal from study therapy)**	
2D Echo every ~84 days *Arms 2 and 3 only*	X (< 28 days)					X every 84 days or 4 cycles → → →					
MVA-BN-Brachyury[12]		X			X						
FPV-Brachyury[12]						X	X	X Every 12 wks			
Entinostat weekly[12] *Arm 3 only*		X	X	X	Take on Days 1, 8, and 15 of each cycle. Can be taken up to 1 day late.						
T-DM1[12] *Arms 2 and 3 only*		X			X	X	X	X			
M7824[12]		X			X	X	X	X			

[1] Registration must be completed within 24 hours of signing consent.
[2] History including prior treatments and review of baseline adverse events and concurrent medications. Physical examination includes breast and lymph node exam. Height (at screening only) and weight are also included. Full history and physical to be performed at initial clinical visit. On subsequent visits, abbreviated exam, review of symptoms and review of medications.
[3] CBC with differential, PT/INR/PTT, comprehensive metabolic panel (see **2.4.2.2**), and anti-HIV, anti-HCV, and Hepatitis B Surface Ag (within 3 months prior to enrollment). If applicable, CD4 T cell count within 3 months.
[4] Labs must be obtained ≤ 7 days of initiating treatment: CBC with differential, PT/INR/PTT (will be repeated if ≥ 72 hours prior to biopsy for Arms 2 and 3), sodium, potassium, chloride, carbon dioxide, BUN, creatinine or measured creatinine clearance, glucose, AST, ALT, bilirubin, calcium, total protein, albumin, alkaline phosphatase, phosphorus, magnesium, urinalysis. Troponin-I and CK, CK-MB will be evaluated at, C1D1, C1D8, C2D1 and C3D1. Tumor Markers (CEA, CA15-3, CA27.29). TSH and reflexed Free T4 (if TSH abnormal) will be performed at screening and at restaging visits (approximately every 9 weeks) unless there is a clinical concern that warrants evaluation on a more frequent basis.

[5] Pathological confirmation of diagnosis of either TNBC (Arm 1) or ER-/PR-/HER2+ (Arms 2 and 3). Patients may enroll with a pathologist's report showing a histologic diagnosis of TNBC or HER+BC in a College of American Pathologists (CAP) accredited laboratory and a clinical course consistent with the disease. If report not available, disease will be confirmed at the local site.

[6] Primary tumor paraffin block will be requested at C1D1 to perform comparative genomic analysis as part of correlative studies planned for this clinical trial.

[7] Planned biopsy after consent but within 7 days prior to first dose of drug on Cohort 3 as well as a planned biopsy on C3D1. There is also an optional biopsy at the time of progression on all arms. Patients in Cohorts 1 and 2 may also have optional biopsies at baseline and at C3D1.

[8] Where feasible, research blood for all study assessments will be collected as per **Table 16**. The exact collection time for PK samples will be recorded.

[9] For female subjects of childbearing potential, urine or serum beta-HCG will be performed on initial screening. A urine or serum beta-HCG will be performed before each administration of the trial drugs during the treatment phase (must be performed on C1D1), at the end-of-treatment visit, and at the post-treatment follow-up visit.

[10] Baseline imaging at screening (or within 28 days prior to enrollment): CT of chest/abdomen /pelvis (MRI may be used when CT scan is not an option to follow the disease clinically). Brain MRI may be ordered in patients with known CNS disease or if there is concern for CNS metastasis. Gadolinium will be used with MRI. Head CT with contrast may be done instead of MRI. Other imaging on study: CT of chest/abdomen/pelvis to be repeated every 9 weeks +/- 3 days after start of treatment. Bone scan and additional imaging assessments may also be performed as clinically indicated at screening or during the study.

[11] Baseline12-lead EKG at screening (within 28 days prior to enrollment), at baseline, and repeat EKG on Cycle 1 Day 8 with repeats only for abnormal baseline EKG, Cycle 1 Day 8 EKG or if patient is symptomatic.

[12] Day 1 Dosing Schedule for each Cycle: BN-Brachyury vaccine is preferred to be given prior to the M7824 infusion, which will be followed by T-DM1 administration. Patients will be provided with a take-home supply of entinostat and will self-administer entinostat after the completion of the T-DM1 infusion on day 1 of each cycle, preferably prior to bed. On C1D8, patients will take entinostat at the time of their clinical appointment. If patients return for the optional C1D15 labs, patients will self-administer entinostat upon arrival at the clinical center. Patients will self-administer entinostat on Days 1, 8, and 15 of each subsequent cycle.

Allowance for scheduling changes: brief interruption and delay in the 21-day cycle may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, and government holidays, etc. This can also extend to complications of disease not attributable to disease progression or protocol therapy. These delays will not be considered protocol deviation.

[13] End of treatment visit: Where feasible or logistically possible, on the day of or within 30 days of the decision to discontinue treatment prematurely before completion of one year of treatment. Does not need to be completed if drug is withheld after one year of treatment.

[14] Long term follow up: Every 3 months for a year followed by every 6 months for an additional year. During this time: patients who have progressed on treatment will be followed by phone or email for survival, adverse events and further tumor therapy. Patients who have not progressed on treatment will be scanned until progression.

[15] Vital signs will be monitored every 15 minutes (Cycle 1) and every 30 minutes (Cycle 2 and beyond) during M7824 infusion and until completion of the observation period in all Arms. Vital signs should also be recorded immediately prior to the start of T-DM1 in Arms 2 and 3. (See **3.2.2** and **3.2.3** for more detail.)

[16] Skin assessment must be performed at baseline, every 6 weeks, and at the end of treatment or at the 28 (±7 days) day safety follow-up (if not performed in the previous 6 weeks), if feasible.

Table 16 **Research Blood Draws**

Week (Day)	**Time**	**M7824**			**Entinostat**	**Immune and Other Analyses**			
		PK	**ADA**	**TGFβ1 levels**	**Histone Deacetylation**	**Immune phenotyping, Antigen specific responses, immune subsets**	**T cell clonality**	**Soluble Factors**	**CfDNA**
					Accounted for in the 60-80ml for PBMCs				
Cycle 1 Day 1	predose	X	X	X	X	X	X	X	X
	EOI	X							
Cycle 1 Day 8				X	X	X	X	X	
Cycle 1 Day15	*Optional*			X	X	X	X	X	
Cycle 2 Day 1	predose	X		X[1]	X[1]	X[1]	X[1]	X[1]	
Cycle 3 Day 1	predose	X	X	X		X	X	X	X
	EOI	X							
Cycle 6 Day 1	predose	X	X						
End of Treatment		X	X		X	X	X	X	X

[1]Cycle 2 Day 1 labs to be drawn only if patient did not do Cycle 1 Day 15 optional labs. Do not need both timepoints

3.6 COST AND COMPENSATION

3.6.1 Costs

Subjects costs will be based on local guidelines as described in the site-specific consent.

3.6.2 Compensation

Participants will not receive compensation on this study.

3.6.3 Reimbursement

Reimbursement will be provided per local guidelines as indicated in the site consent document.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Regardless of reason for removal from study therapy, patients will be asked to have a 28 day follow-up safety visit. Patients who refuse to return for this visit will be asked to review any safety concerns by phone within this time period.

3.7.1 Criteria for removal from protocol therapy

- Clinical or radiographic progression of disease except when the investigator feels the subject is still benefiting from treatment. (It is generally preferable for patients to remain on treatment past initial radiographic progression in case there is pseudo - progression, except when the investigator feels that the clinical picture warrants changing therapy at initial progression.);
- Participant requests to be withdrawn from active therapy;
- Unacceptable toxicity as defined in Sections **3.1.2** and **3.3**;
- Start of another systemic anticancer treatment or participation in another investigational therapeutic trial;
- Use of restricted medication (Section **4.3**);
- Investigator discretion;
- Positive pregnancy test;
- Permanent loss of capacity to give consent.

3.7.2 Off-Study Criteria

- Screen failure;
- PI decision to end the study;
- Participant requests to be withdrawn from study;
- Completion of follow up period;
- Participant lost to follow-up;
- Investigator discretion;
- Disease progression;
- Permanent loss of capacity to give consent;
- Death.

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 4 clinic visits scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within the next 4 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS / MEASURES

Any medications (other than those excluded by the clinical trial protocol) that are considered necessary to protect subject welfare or alleviate symptoms and will not interfere with the trial medications may be given at the Investigator's discretion.

4.1 ANTIEMETICS

Due to low rates of nausea with M7824, BN-Brachyury vaccine and entinostat, no antiemetic prophylaxis will be given. However, if a patient does experience nausea/vomiting, antiemetics may be used.

Since T-DM1 is considered a drug with low emetic risk by both ASCO and NCCN, the use of a single dose of a 5-HT3 antagonist or dopamine blocker (i.e., prochlorperazine) is encouraged. Due to the effect of CYP3A4 inhibitors on T-DM1, avoid aprepitant, fosaprepitant, and netupitant, as well as other CYP3A4 inhibitors. The use of steroids for antiemetic effect is allowed per the discretion of the primary investigator. The antiemetic regimen may be modified based on patient specific factors or standard institutional practices.

4.2 GROWTH FACTOR SUPPORT

Significant neutropenia is uncommon with single agent T-DM1, M7824 or entinostat. However, growth factor support is allowed for grade 3 neutropenia or at the discretion of the primary investigator after the completion of the DLT period for each trial arm. If neutropenia or a neutropenic fever requiring growth factor support occurs during the DLT period, this will be counted as a DLT.

4.3 RESTRICTED MEDICATIONS

The following medications should **NOT** be administered during the trial:

- Other immunotherapies or immunosuppressive drugs for example, chemotherapy or systemic corticosteroids **except** for prophylaxis or treatment of allergic reactions, endocrine replacement therapy at low dose prednisone [≤ 10 mg daily] or equivalent, for the treatment of irAEs, or for short courses (≤ 14 days) as appropriate medical therapy for

unrelated medical conditions (e.g., asthma). Steroids with no or minimal systemic effect (topical, inhalation) are allowed.

- Prophylactic use of corticosteroids for infusion related reactions. Corticosteroid administration prior to CT scans in patients with intravenous contrast allergy is allowed.
- Any live vaccine therapies for the prevention of infectious disease. Administration of inactivated vaccines is allowed (for example, inactivated influenza vaccines or locally approved COVID vaccines).
- Systemic anticancer treatment other than the investigational agents in this trial.
- Herbal supplements.

No formal drug-drug interaction studies with the BN-Brachyury-vaccines, M7824 or entinostat have been performed. Use caution when administering entinostat concurrently with medications that have a narrow therapeutic index.

No formal drug-drug interaction studies with T-DM1 have been conducted. *In vitro* studies indicate that DM1, the cytotoxic component of T-DM1, is metabolized mainly by CYP3A4 and to a lesser extent by CYP3A5. Concomitant use of strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole) with T-DM1 should be avoided due to the potential for an increase in DM1 exposure and toxicity. Consider an alternate medication with no or minimal potential to inhibit CYP3A4. If concomitant use of strong CYP3A4 inhibitors is unavoidable, consider delaying T-DM1 treatment until the strong CYP3A4 inhibitors have cleared from the circulation (approximately 5 elimination half-lives of the inhibitors) when possible. If a strong CYP3A4 inhibitor is co-administered and T-DM1 treatment cannot be delayed, patients should be closely monitored for adverse reactions (https://www.accessdata.fda.gov).

4.4 CONTRACEPTION

The effects of BN-Brachyury, entinostat, M7824 and T-DM1 on the developing human fetus are unknown. However, both components of T-DM1 are known to cause fetal defects including oligohydramnios and oligohydramnios syndromes. For this reason, women of childbearing potential must agree to use two methods of adequate contraception (see **Table 17**) at study entry, for the duration of study participation and for at least 7 months for women and 4 months for men after the final dose of any study-related medications (T-DM1 has a prolonged half-life). Cessation of birth control after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method and the withdrawal method are not acceptable methods of birth control. Women with hormone receptor positive breast cancer should avoid hormonal contraceptive agents.

Table 17: Effective methods of contraception (two methods must be used for men and women of child-bearing potential)

Barrier Methods	*Intrauterine Device Methods*	*Hormonal Methods**
Male condom plus spermicide	Copper T	Implants
Cap plus spermicide	Progesterone T[a]	Hormone shot or injection
Diaphragm plus spermicide	Levonorgestrel-releasing intrauterine system (e.g., Mirena)	Combined pill
		Minipill
		Patch

[a]This is also considered a hormonal method of birth control
*Women with hormone receptor positive breast cancer should avoid hormonal agents; however, since all participants in this study will have hormone receptor negative (or low hormone receptor positivity) breast cancer, these agents can be used.

4.5 BREAST FEEDING

It is not known whether BN-Brachyury, entinostat, M7824 or T-DM1 is excreted in breast milk. It is recommended that women do not breastfeed during treatment on trial and for at least 7 months after the last dose due to the prolonged half-life of T-DM1 and M7824.

4.6 BLOOD DONATION

Patients should not donate blood while participating in this study or for 7 months following the last treatment on trial due to the prolonged half-life of T-DM1 and M7824.

5 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1 BIOSPECIMEN COLLECTION

Test/assay	**Volume (approx.) per timepoint**	**Type of tube**	**Collection point**	**Location of specimen analysis* (processing/storage location)**
M7824 Pharmacokinetics	4 mL blood for serum	Serum Separator tubes (SST)		EMD Serono (BPC)
ADA by ELISA	4mL blood for serum			
Standard and 123 immune cell subsets by FACS				

Test/assay	**Volume (approx.) per timepoint**	**Type of tube**	**Collection point**	**Location of specimen analysis* (processing/storage location)**
Functional Analysis of immune cell subsets by FACS	60-80 mL blood for PBMCs	Sodium heparin (green top) tubes	See Section **3.5**	LTIB (CSP)
Antigen Specific Immune Response by cytokine staining assay				
Histone acetylation				
T cell clonality by immunoSeq platform				NCI Frederick Genomic Core Facility, Nanostring (CSP)
RNA expression by Nanostring				
Soluble Factors by ELISA	8 mL blood for serum	Serum Separator tubes (SST)		LTIB (CSP)
TGFβ1-3 levels by ELISA or bead based multiplex assays	6mL blood for plasma	EDTA (lavender top) tubes		LTIB, Dr. Liang Cao's Lab (CSP)
Circulating free tumor DNA (cfDNA) by PCR system	10 mL blood for plasma	EDTA (lavender top) tubes		Dr. Liang Cao's Lab (CSP)
Immune Markers by IHC	Tumor samples	N/A		NCI – GMB TIME Lab (LP)
RNA expression by Nanostring	Tumor samples	N/A		NCI Frederick Genomic Core Facility, Nanostring (LP)
T cell clonality by immunoSeq platform	Tumor samples	N/A		LTIB and NCI Frederick Genomic Core Facility (LP)

*Research samples will be sent to the Clinical Services Program – Leidos Biomedical Research, Inc. (CSP) (Section **5.2.1**), the Blood Processing Core (BPC) (Section **5.2.2**), or Laboratory of Pathology (LP) (Section **5.2.3**) for barcoding, initial processing and storage. From these facilities, coded, linked samples will be sent to the designated labs for analysis in batch shipments or upon request.

5.1.1 Peripheral Blood Collection and Assays

The following tests may be performed on selected patients' peripheral blood samples if there are adequate samples. If collected samples are not sufficient, the correlative studies will be performed in order of priority as detailed below. These correlative studies will be done in an exploratory fashion. The amount of blood that may be drawn from adult patients and volunteers (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

Pharmacokinetics

To evaluate M7824 PK, 4ml of serum will be collected as per Section **3.5**. Serum samples will be analyzed by a validated immunoassay to quantitate M7824 concentration. Samples may be further tested in qualified or validated PK characterization assays. Coded, linked samples will be shipped to EMD Serono for analysis.

ADA

To evaluate ADA (anti-drug antibody), 4ml of blood will be collected as per Section **5.1.1**. Serum samples will be analyzed by a validated electrochemiluminescence immunoassay (ECLA) to detect the presence of anti-M7824. Samples that screen positive will be subsequently tested in a confirmatory assay. Those confirmed positive will be titered for a quasi-quantitative result. Samples may be further tested in qualified or validated immunogenicity characterization methods. The investigation will be done by EMD Serono using ELISA. Coded, linked samples will be shipped to EMD Serono for analysis.

Immune Phenotyping

Exploratory immunologic studies will be conducted to evaluate the study drug's effect on the immune response before and after treatment, to gain insight into potential biomarkers, and help improve the administered therapy. Blood will be collected as per Section **5.1.1**. The following immune assays may be performed at the Laboratory of Tumor Immunology and Biology (LTIB) at the NCI's Center for Cancer Research (CCR) in select patients where adequate samples are available:

1. Pre- and post-therapy PBMCs, separated by Ficoll-Hypaque density gradient separation, will be analyzed for antigen-specific immune responses using an intracellular cytokine staining assay. PBMCs will be stimulated in vitro with overlapping 15-mer peptide pools encoding the tumor-associated antigen Brachyury; if sufficient PBMCs are available, the cascade antigens CEA, and MUC1 will be included. Control peptide pools will involve the use of human leukocyte antigen peptide as a negative control and CEFT peptide mix as a positive control. CEFT is a mixture of peptides of CMV, Epstein-Barr virus, influenza, and tetanus toxin. Post-stimulation analyses of CD4 and CD8 T cells will involve the production of IFN-γ, IL-2, tumor necrosis factor, and CD107a. If sufficient PBMCs are available, assays may also be performed for the development of T cells to other tumor-associated antigens. A detailed description of this assay has been previously reported. (119)

2. If sufficient PBMCs are available, PBMCs from selected subjects may be analyzed for changes in standard immune cell types (CD4 and CD8 T cells, NK cells, Tregs, myeloid-derived suppressor cells [MDSCs], and dendritic cells) as well as 123 immune cell subsets, as described elsewhere (120) and for function of specific immune cell subsets, including CD4 and CD8 T cells, NK cells, Tregs, and MDSCs.

Analyses of soluble factors:

1. Sera may be analyzed pre- and post-therapy for the following soluble factors: sCD27, sCD40 ligand, and selected patients may be analyzed for cytokines and antibodies to human tumor antigens such as Brachyury, CEA, or MUC1.

Additional assays:

Blood samples may be used for additional research studies, which may include phenotypic and functional analysis of immune-cell subsets, TCR clonality, and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor-associated antigens, and/or other markers.

Plasma analysis for TGFβ1 levels and circulating free DNA:

Plasma may be analyzed pre- and post-therapy in Dr. Liang Cao's lab for TGFβ1-3 levels using ELISA or bead based multiplex assays and circulating free tumor DNA using PCR-based techniques.

Exploratory pharmacodynamics for entinostat (Arm 3 only):

1. Histone acetylation has been evaluated as a surrogate for entinostat pharmacodynamics. Protein lysine acetylation will be measured by multiparameter flow cytometry in PBMCs (CD19 B-cells, CD3T-cells, and CD14 monocytes) to explore the association with PFS. (121) This will be collected at baseline, Cycle 1 Day 8 (within 4-6 hours of taking entinostat) and Cycle 1 Day 15 (optional; within 4-6 hours of taking entinostat). This will be performed on PBMCs collected for the immune analyses. No additional blood collection is needed.

5.1.2 Tumor Biopsy Collection and Assays

Biopsy Acquisition

Biopsies will be performed as per the Study Calendar **3.5**. Attempts will be made to obtain up to four cores (18G or 20G preferred) if safe and feasible. These tumor core biopsies will be obtained percutaneously by interventional radiology as long as considered minimal surgical risk. Two 3-millimeter punch biopsies of skin will be acceptable in lieu of 18-gauge core biopsies for patients with skin involvement. Inability to get tissue with a reasonable attempt will not preclude treatment and the patient will remain eligible for all other translational components.

- Cores 1 and 2: Formalin-Fixed Paraffin-Embedded (FFPE)
- Cores 3 and 4: Fresh frozen

Tissue samples will be sent to Laboratory of Pathology for disease evaluation first, remaining samples will be processed and stored for research (Section **5.2.3**). If fresh-frozen cores are obtained, they will be processed and stored in CSP (Section **5.2.1**). See site specific instructions for collection shipping procedures.

Immune Markers by Immunohistochemistry

Sections of formalin-fixed, paraffin-embedded (FFPE) tissues from before and after therapy may be stained using antibodies for multiple immune cell and tumor cell markers (e.g. CD4, CD8, FOXP3, PD-1, PD-L1) and analyzed using the Vectra Polaris platform/multispectral imaging in selected patients where tumor samples are adequate.

HER2/Brachyury Expression

HER2 testing may be performed and if so, will be performed according to the recommendations made by the College of American Pathologists (CAP). (122) FFPE samples may be evaluated for brachyury expression by using a rabbit monoclonal anti-brachyury antibody MAb 54-1 at a 1:500-1:2000 dilution, as previously described. (123, 124) Nuclear, cytoplasmic and total brachyury staining will be independently scored, with brachyury being observed either in the nucleus, the cytosol or both compartments of the tumor cells. The relative staining intensity will be scored as weak (+) for pale intensity, moderate (++) for intermediate intensity, and strong (+++) for intense, dark immunoprecipitate. Immunoreactivity index will be calculated by multiplying the percentage of positive cells by the staining intensity.

5.1.3 Genetic Assays

T-cell Clonality

DNA may be extracted from cryopreserved PBMC or tumor tissue. TCR Vb CDR3 sequencing will be performed at the CCR Genomics Core using the Immunoseq kit from Adaptive Biotechnologies at the survey (tumor) or deep (PBMC) resolution. In this assay, a multiplex PCR system amplifies and quantifies the rearranged CDR3b sequences from sample DNA.

RNA Expression

RNA may be extracted from cryopreserved PBMC or tumor tissue. Sequencing of select gene panels may be performed at the CCR Genomics Core in collaboration with Nanostring.

5.2 SAMPLE STORAGE, TRACKING, AND DISPOSITION

See site specific instructions for collection and shipping procedures for research blood samples.

5.2.1 Sample Management and Storage at Clinical Services Program – Leidos Biomedical Research, Inc. (CSP)

All data associated with the patient samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80ºC freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI Frederick Central Repositories (managed under a subcontract) store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited-access facilities with sufficient security, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance with applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role limited to clinical research databases and repositories containing patient specimens. The subcontractor does not conduct or have any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of the subcontractor to accept only coded, linked samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input, and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.2.2 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.2.2.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.2.3 Sample Storage

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80□C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times. Access to stored clinical samples is restricted.

Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.2.3 Procedures for Storage of Tissue Specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. Specimens/recuts can then be requested with the permission of the appropriate Principal Investigator using the Tissue Resource Committee request for Human Biological Materials for Research form.

5.2.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The site PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of Section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The CCR PI and all site PIs will be responsible for data collection. Site PIs will be trained on use of the CCR remote data capture system C3D. The CCR PI and the site PIs will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 28 days after the study intervention was last administered. Beyond 28 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study.
- Is associated with clinical signs or symptoms.
- Requires treatment or any other therapeutic intervention.
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact.
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per Section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository. Clinicaltrials.gov
- BTRIS (automatic for activities in the Clinical Center).
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.

- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

6.3.1 Antitumor Response

For the purposes of this study, patients should be re-evaluated for response every 9 weeks +/- 3 days. In addition to a baseline scan, confirmatory scans can also be obtained 3 to 6 weeks following initial documentation of objective response per the discretion of the primary investigator.

At baseline, tumor lesions will be selected and categorized as target or non-target lesions. Target lesions include those lesions that can be accurately measured in at least 1 dimension as ≥ 20 mm with conventional techniques or ≥ 10 mm with CT scan. Malignant lymph nodes with a short axis diameter ≥ 15 mm can be considered target lesions. Up to a maximum of 2 target lesions per organ and 5 target lesions in total will be identified at baseline. These lesions should be representative of all involved organs and selected based on their size (those with the longest diameter) and their suitability for accurate repeated measurements. A sum of the longest lesion diameter (LLD) for all target lesions will be calculated and reported as the baseline sum LLD. For malignant lymph nodes identified as target lesions, the short axis diameter will be used in the sum of LLD calculation. All other lesions (or sites of disease) should be identified as non-target lesions (including bone lesions).

All post-baseline response assessments should follow the same lesions identified at baseline. The same mode of assessment (e.g., CT) used to identify/evaluate lesions at baseline should be used throughout the course of the study unless subject safety necessitates a change (e.g., allergic reaction to contrast media).

For the primary endpoint antitumor activity will be evaluated with target and/or non-target lesions according to the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (Version 1.1). (125)

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥20 mm;
- By CT scan:
  - Scan slice thickness 5mm or under: as ≥10 mm;
  - Scan slice thickness >5mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement.

Tumor markers: Tumor markers alone cannot be used to assess response.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

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 (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.4.3 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 3 to 6 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥3 to 6 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients 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."* Every effort should be made to document the objective progression even after discontinuation of treatment.

As an exploratory endpoint antitumor activity will also be evaluated according to iRECIST (REF). Using iRECIST criteria the following will be incorporated into the assessment:

- An increase in the sum of target lesions of more than 20%, unequivocal increase in the non-target lesions or new lesions result in iUPD (unconfirmed progression disease); iUPD can be assigned multiple ties as long as iCPD (confirmed progressive disease) is not confirmed at the next assessment.
- Progression is confirmed in the target lesion category if the next imaging assessment after iUPD (4-8 weeks later) confirms a further increase in sum of measures of target disease from iUPD, with an increase of at least 5mm. Progression is confirmed in the non-target lesion category if subsequent imaging, done every 4-8 weeks after iUPD shows a further unequivocal increase in non-target lesions. Progression is confirmed in the new lesions category if at next assessment additional new lesions appear or an increase in the size of previously seen new lesions is seen ($\geq$5 mm for sum of new target lesion).
- However, the criteria for iCPD (after iUPD) are not considered to have been met if complete response, partial response or stable disease criteria (compared with baseline and as defined by RECIST 1.1) are met at the next assessment after iUPD. The status is reset (unlike RECIST 1.1, in which any progression precludes later complete response, partial response, or stable disease). iCR, iPR, or iSD should then be assigned; and if no change is detected, then the timepoint response is iUPD.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.4.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.4.5 Progression-Free Survival

Progression Free Survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. In the absence of progression or death, PFS is censored at the date of last disease evaluation

6.3.5 Response Review

All imaging studies will be reviewed by an independent radiologist and will be assess according to RECIST 1.1.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs.

7.3 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS FOR MULTI-CENTER TRIALS

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-compliance in Human Subjects Research, found here. Until such time as direct electronic reporting mechanisms are available to participating sites, the site PI must immediately

report to the coordinating center PI any deaths possibly related to the research within 24 hours of PI awareness of the event. The Site PI must also report any other events required by Policy 801 to the coordinating center PI within 7 days of PI awareness.

The participating site Reportable Event Form is attached as **Appendix E**: CCR Reportable Event Form (REF).

Once direct electronic reporting mechanisms are available, these will be utilized. Please also notify the coordinating center PI and study coordinator of your submission at the time you make it.

For IND studies, the site PI will also directly submit reports to the CCR as IND sponsor per Section **8.3**, **8.6** and **8.8**.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (approximately weekly) when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timeline.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee comprised of physicians, biostatisticians and a lay member selected based on experience, area of expertise, reputation for objectivity, absence of conflicts of interest and knowledge of or experience with clinical trial research. Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC will operate under the rules of an approved charter that will be written and reviewed at the organization meeting of the SMC. Each review will focus on unexpected protocol safety issues that are identified during the conduct of the clinical trial.

Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death;
- A life-threatening adverse event (see Section **8.1.3**);
- Inpatient hospitalization or prolongation of existing hospitalization;
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death (21CFR312.32).

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.6 Adverse Events of Special Interest (AESI)

Adverse events of special interest (AESIs) are serious or nonserious AEs that are of clinical interest and should be closely followed.
AESIs include following:

- Infusion-related reactions including immediate hypersensitivity.
- Immune-related adverse events.
- TGFβ inhibition/M7824 mediated skin reactions.
- Anemia.
- Bleeding AEs.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.

- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets a protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in Section **8.4**

All SAE reporting must include the elements described in Section **8.1.6**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to CCR

As death due to disease progression is part of the study objectives, and captured as an endpoint in this study, they will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to Section **8.3**.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal Exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known. Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.2**) should be reported as SAEs.
The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of study drugs.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or

congenital abnormality) occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL NON-ADHERENCE REPORTING

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month over the duration of the study**. In addition, any non-adherence to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol non-adherence reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Monitoring based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. The intensity and frequency of monitoring will be based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. OSRO Monitoring visits and related activities will be conducted throughout the life cycle of each protocol, with the first activity being before study start to conduct a Site Assessment Visit (SAV) (as warranted),

followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

If protocol non-adherence is identified by the Monitor (i.e., any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the site Staff) the Monitor will note the observation, review with site Staff and if unresolved, request that the Staff document the non-adherence on the protocol-specific OSRO Site Protocol Non-Adherence Log (see Section **8.8**).

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

We hypothesize that sequentially evaluated combinations of BN-Brachyury, M7824, T-DM1 and entinostat will enhance the clinical and immunologic response in patients with advanced breast cancer, specifically for HER2+BC patients who previously progressed after treatment (or were intolerant to) with a taxane, trastuzumab and pertuzumab. The trial will enroll patients initially to BN-Brachyury + M7824, and then add TDM-1 and finally entinostat.

- **Primary Endpoints:**
  - Objective response rate (ORR; PR+CR), as defined in Section **6.3.3**, for each of the three treatment arms enrolled sequentially as described below.
  - Safety for the combinations explored in the arms.
- **Secondary Endpoints:**
  - Progression free survival, as designed in Section **6.3.4.5.**
  - Change in absolute percentage of stromal TILs quantified according to the Salgado method in serial tumor biopsies collected at baseline and on C3D1 (Arms 2 and 3 only).
- **Exploratory Endpoints** (studies may be performed in selected patients if adequate samples are available):
  - Peripheral blood mononuclear cells (PBMCs):
    - Changes in immune cell subsets.
    - Changes in brachyury-specific, MUC1-specific, and CEA-specific T cells measured by intracellular cytokine staining (IFNγ, IL2, TNF, CD107a) .
    - Histone acetylation as surrogate for entinostat pharmacodynamics (Arm 3 only).
  - Plasma/Serum:
    - Changes in brachyury-specific , MUC1-specific, and CEA-specific antibodies.
    - Changes in soluble factors like sCD27 and sCD40.
    - Changes in cell free DNA.
    - Changes in TGF-beta levels.

- Changes in pharmacokinetics of M7824.

- Tissue Biopsy (Cohort 3, Arms 2 and 3):
  - Brachyury expression on tumor.
  - T-cell clonality score.
  - Immunohistochemistry/multispectral imaging.
  - Changes in PD-L1 on tumor.
  - Neoepitopes derived from tumor biopsies.
  - HER2 expression changes.
  - Tumor mutational burden.

10.2 SAMPLE SIZE DETERMINATION

The primary objective of this trial , on which the sample size is based, is to determine the overall clinical response rate of BN-Brachyury, entinostat, M7824 and T-DM1 (PR+CR) evaluated in arms with increasing numbers of agents as follows:

Arm 1:

In similar subjects with TNBC (unselected for PD-L1 expression) who received a checkpoint inhibitor, the clinical response rate was approximately 8-10%. (43-45) The goal would first be to determine if using BN-Brachyury plus M7824 could be shown in a small pilot arm to improve upon this by a modest amount. Accrual of the first 3 patients on trial in this arm will be slow (no more than 1 patient per 6 days) in order to allow for monitoring of toxicity. If there is one or more DLT in the first 3 patients on this arm, accrual of the next 3 patients on the associated arm will proceed slowly with no more than 1 patient per 21 days (1 cycle) to continue to closely monitor safety. If there are 0-1 patients with DLTs among the 6 patients enrolled on this arm, to do so, accrual to this arm will be continue by using a Simon optimal two-stage phase II trial design (126) to rule out an unacceptably low PR+CR rate of 10% (p0=0.10) in favor of an improved response rate of 35% (p1=0.35). With 1-sided alpha=0.10 (probability of accepting a poor treatment=0.10) and beta=0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 8 evaluable subjects, and if 0 to 1 of the 8 have a clinical response, then no further subjects will be accrued. If 2 or more of the first 8 subjects have a response, then accrual would continue until a total of 13 evaluable subjects have been enrolled. As it may take 3 to 6 months to determine if a subject has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 2 subjects with a response out of 13 subjects, this would be an uninterestingly low response rate. If there were 3 or more of 13 (23.1%) who experienced a response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (10% response rate), the probability of early termination is 81.3%.

Arms 2 and 3:

Following determination of safety in arm 1 (0-1 with DLT among first 6 patients enrolled), patients who are HER-2 positive will undergo an initial safety evaluation and then will be assigned in an alternating fashion (1:1) to the second and third arms: BN-Brachyury, M7824 and T-DM1, or BN-Brachyury, M7824, TDM-1, and entinostat. Provided that there are 0-1 patients with DLTs among the 6 patients enrolled on Arm 2, this arm will be temporarily closed and patients will be enrolled on Arm 3 for a safety evaluation. Provided that there are 0-1 patients with DLTs among the 6 patients enrolled on Arm 3, patients with HER2 positive breast cancer will be directly allocated1:1 to Arms 2 and 3. In Arms 2 and 3, accrual of the first 3 patients in each arm will proceed slowly

with no more than 1 patient enrolled every 28 days in order to allow for safety monitoring. If there is one or more DLT in the first 3 patients on any arm, accrual of the next 3 patients on the associated arm will proceed slowly with no more than 1 patient per 21 days (1 cycle) to closely monitor safety. If there are 2 or more patients with a DLT among the first 6 treated on either arm, the trial will halt accrual pending an amendment to detail how the study will proceed from that point forward.

The primary objective is to determine if either arm could improve upon the 18% response rate by a modest amount. Each arm will be conducted using a Simon minimax two-stage phase II trial design (126) to rule out an unacceptably low PR+CR rate of 18% (p0=0.18) in favor of an improved response rate of 40% (p1=0.40). With 1-sided alpha=0.10 (probability of accepting a poor treatment=0.10) and beta=0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll 14 evaluable subjects in each arm, and if 0 to 3 of the 14 have a clinical response, then no further subjects will be accrued. If 4 or more of the first 14 subjects have a response in an arm, then accrual would continue until a total of 19 evaluable subjects have been enrolled in that arm. As it may take 3 to 6 months to determine if a subject has experienced a response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 4 to 5 subjects with a response out of 19 subjects in an arm, this would be an uninterestingly low response rate. If there were 6 or more of 19 (31.6%) who experienced a response, this would be sufficiently interesting to warrant further study that combination in later trials. If accrual ends to one arm because of insufficient activity, the other arm will remain open to enroll patients directly. Under the null hypothesis (18% response rate), the probability of early termination is 76.5%.

There will be no adjustment for the multiplicity of the three arms.

To allow for a small number of inevaluable patients, up to 42 patients may be enrolled in Cohort 2 (21 per Arm, if necessary) to explore this primary objective.

With arms requiring up to 13+19+19=51 evaluable patients and allowing for up to 4 total inevaluable patients and up to 10 screening failures, the accrual ceiling will be set at 65 patients.

Safety will also be assessed as a primary objective. All patients will be assessed for safety as described in Sections **10.3.1** and **10.4.4**.

Secondary Objectives:

Progression Free Survival (PFS) will be evaluated as a secondary objective. This will be estimated using a Kaplan-Meier curve and the median PFS will be reported along with a 95% confidence interval, separately by arm, based on the patients who are enrolled in the evaluation of clinical response.

Change in TILs will also be evaluated as a secondary objective. Data from 678 patients with evaluable TILs enrolled on the CLEOPATRA trial in HR-/HER2+ breast cancer indicated a mean baseline stromal TIL value of 21% (SD=22.4%). In the present study with HR-/HER2+ patients, it is anticipated that the baseline TIL value will be assumed to be approximately 25%, with the same 22% standard deviation. A very conservative estimate of the SD associated with a change between two time points is the baseline SD x square root (2), or 22 x 1.41 =31%, based on an assumption of no correlation between the values at baseline and at the time of biopsy. An assumption of the correlation=0.5 would result in the SD of the change being baseline SD x 1.0, or 22%, but we will assume SD=31% to be conservative.

The goal will be to estimate if the absolute change in TIL percentage from baseline is meaningfully large, as well as noting the magnitude and reporting that with appropriate descriptive statistics and graphical presentation. With 15 evaluable patients for TILs in the two larger arms, there will be 82% power to detect a difference between a mean baseline TIL value of 25% (0.25) and mean post-biopsy TIL value of 50% (0.50), assuming a standard deviation of the change of 31% (effect size= 25/31=0.806), using a paired t-test with a two-sided 0.05 significance level. More conservatively if only 13 or 11 patients have paired samples assessed (from any of the three arms), then there will be 82% and 84% power to detect a mean difference of 27% (effect size=0.871) or 31% (effect size=1.0), respectively. In practice, the changes in the TIL% may not be normally distributed, so the study will plan to test the effect using a Wilcoxon signed rank. In practice, the changes in the TIL% may not be normally distributed, so the study will plan to test the effect using a Wilcoxon signed rank test instead of a paired t-test.

Progression Free Survival (PFS) will be evaluated as a secondary objective. This will be estimated using a Kaplan-Meier curve and the median PFS will be reported along with a 95% confidence interval, separately by arm. based on the patients who are enrolled in the evaluation of clinical response.

10.3 POPULATIONS FOR ANALYSES

10.3.1 Evaluable for toxicity:

All patients who are treated with any amount of the combination of agents will be evaluable for toxicity from the time of their first treatment; thus, all patients will be evaluable for toxicity from the time of their first treatment with the arm-specific combinations of BN-Brachyury, entinostat, M7824 and T-DM1. Any patient who receives some but not all treatments in each given arm will be summarized separately.

10.3.2 Evaluable for objective response:

Only those patients who have measurable disease present at baseline, have received at least two cycles of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. Patients who have measurable disease at baseline, have received at least 1 cycle of therapy and have clinical/objective disease progression prior to the first restaging will be considered evaluable.

10.4 STATISTICAL ANALYSES

10.4.1 General Approach

Data will be summarized descriptively and graphically, with reporting the outcome measures indicated along with two-tailed 95% confidence intervals (CIs), as well as two-tailed 80% CIs (corresponding to the design with one-sided α=0.10) for median PFS.

10.4.2 Analysis of the Primary Efficacy Endpoint

The ORR will be estimated in each arm using two-sided 80% and 95% CI about the observed percentages of response (PR+CR).

10.4.3 Analysis of the Secondary Endpoints

Stromal TILs will be summarized descriptively and graphically by time point and as absolute and relative change from baseline. The absolute change in TILs values from baseline following two cycles of treatment will be compared via Wilcoxon signed rank test.

The distribution of PFS will be estimated using the Kaplan-Meier (KM) method, among patients evaluable for efficacy, and appropriate confidence intervals (80% as well as 95% two-sided confidence intervals) at the median and selected time points will be provided to help interpret results relative to the expected results. This will be done by arm and may be evaluated by combining patients in arms 2 and 3 if they are sufficiently similar ($p>0.30$ by global two-tailed log-rank test).

The fraction of patients who experience a DLT during the single dose level safety phases of the trial will be reported, along with a summary, confidence interval, and description of any toxicities noted of grade 3 or higher.

10.4.4 Safety Analyses

Adverse events (AE) recorded throughout treatment will initially be summarized as the maximum grade, for each AE type. The number and frequency of patients experiencing each AE type and grade will tabulated, among patients evaluable for toxicity. The number (%) of patients experiencing at least one grade 3 to 5 AE will be reported with 95% CI. This will be done separately for the initial safety cohort and for the patients in the primary efficacy cohort. Patients who receive at least 1 dose of all drugs in their cohort will be evaluable for safety analyses.

10.4.5 Baseline Descriptive Statistics

Continuous variables will be summarized as mean±SD, median and interquartile range, and range of values. Categorical variables will be summarized as number (%) of patients.

10.4.6 Sub-Group Analyses

Analyses will be performed separately by arm.

10.4.7 Tabulation of individual Participant Data

Given the sample size, graphical summaries of individual data will often be used, for example scatter plot TILs according to time point with the paired values connected by lines.

10.4.8 Exploratory Analyses

Blood sample collection (as specified in Section **5.1.1**), as well as biopsy specimens at baseline and cycle 3 will yield a set of immune subsets. Values will be summarized descriptively and graphically over time. Given the sample size we cannot investigate association of baseline or change from baseline to cycle 1 or cycle 2 in relation to PFS using Cox modeling; therefore, these investigations would focus on KM plots according to dichotomized values of biomarker values, without statistical hypothesis testing. In order to avoid guarantee-time bias, the assessments of changes to cycle 1 or cycle 2 may need to take a landmark analysis approach because patients will need to be alive and progression free at cycle 1 or cycle 2 in order to have a blood sample and be part of the analysis. The changes in the biomarkers will also be summarized descriptively and graphically for responders and non-responders.

11 COLLABORATIVE AGREEMENTS

11.1 MULTI-INSTITUTIONAL GUIDELINES

Until an electronic submission system is available to participating sites, documents requiring submission to the reviewing IRB per reliance agreement, including local consent documents generated from an approved model consent, should be provided to the coordinating center for submission to the IRB. Thereafter, consents may be submitted directly to the IRB using iRIS.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Both men and women are eligible for this trial if they meet eligibility criteria. Subjects from all racial and ethnic groups are eligible for this trial if they meet the eligibility criteria. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to racial or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

As there is a risk of severe bleeding with this study drug, participants must be willing to receive blood transfusions if medically necessary for their own safety. Participants must be able to receive blood transfusions in order to minimize the risks of receiving M7824. Another reason for this is that including these patients could compromise the scientific validity of the study. For example, death from blood loss could make it difficult to assess other aspects of the investigational immunotherapy's safety—a primary scientific goal in this early-phase immunotherapy trial, which are carried out in small numbers of participants.

12.2 PARTICIPATION OF CHILDREN

The age group for enrollment on this trial is 18 or more years of age. Because no dosing or adverse event data are currently available on the use of BN-Brachyury, entinostat, M7824 or T-DM1 in participants < 18 years of age, children are excluded from this study.

12.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

BN-Brachyury, entinostat, and M7824 are investigational agents for use in metastatic breast cancer. Furthermore, BN-Brachyury, entinostat, M7824, and T-DM1 have never been used in combination. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity. Toxicity data will be collected and reviewed to ensure that there were no severe toxicities that would preclude further patient enrollment. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored.

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document and in Section **12.4.1**. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4 RISK/BENEFIT ASSESSMENT

12.4.1 Known Potential Risks

Some of the procedures performed on this study are not known to be associated with risk. These include urine tests and EKGs. Below are a list of procedures and study interventions that are associated with risk.

12.4.1.1 Risk of MRI

If there is concern for CNS metastasis or history of CNS metastasis, the PI may request a brain MRI.

People are at risk for injury from the MRI magnet if they have some kinds of metal in their body. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss.

There are no known long-term risks of MRI scans.

12.4.1.2 Risk of Gadolinium Enhanced MRI

The gadolinium infusion may cause mild symptoms such as coldness in the arm during the injection, a metallic taste, headache, and nausea. There are risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Procedure-related risks from MRI and gadolinium enhanced MRI will be explained fully during informed consent.

12.4.1.3 Risks of Exposure to Ionizing Radiation

This research study involves the possibility of 7 CT CAP scans, 4 CT guided biopsies, and 1 head CT collected for research purposes per year.

The amount of radiation exposure received from these procedures is equal to approximately 11 rem. The CT scans and CT guided biopsies in this study will expose the research participant to 36.7 years' worth of background radiation. This level of exposure results in an increased risk of cancer.

12.4.1.4 Risk of Biopsy

CT-guided biopsies will be performed as clinically indicated. Up to 3 CT-guided biopsies may be performed on this study for research purposes per year.

Patients in all cohorts may be required to undergo a biopsy at screening in order to confirm their diagnosis

Patients in Cohort 3: 2 required biopsies while on treatment, 1 optional biopsy at progression.

Patients in Cohort 1 and 2: 3 optional biopsies (2 while on treatment and 1 at progression).

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.4.1.5 Additional Risks of CT Scans

In addition to the radiation risks addressed above, CT scans that employ contrast may cause allergic reactions, injection site reactions abdominal discomfort and fainting.

12.4.1.6 Research Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.4.1.7 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.2 Known Potential Benefits

The potential benefits from this therapy are stabilization or shrinkage of the tumor and a reduction in chances of developing new lesions with decrease of symptoms caused by progressive disease.

12.4.3 Assessment of Potential Risks and Benefits

Metastatic or refractory/recurrent breast cancers are in need of improved therapy options. No current therapies are curative. Preclinical studies suggest that the use of a combination of multiple immunotherapy agents may have improved anti-tumor efficacy.

A number of clinically appropriate strategies to minimize risks to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefits of the combination of a therapeutic vaccine against brachyury (BN-brachyury), a bifunctional fusion protein targeting PD-L1 and TGF beta (M7824), an immune and epigenetic modifier (entinostat) and a standard of care antibody-drug conjugate (T-DM1) in subjects with advanced breast cancer for patients retaining the ability to consent and those who lose capacity to consent during the course of the trial outweigh the risks associated with this combination immunotherapy proposed in this study.

The potential benefit to a patient that goes onto this study is a reduction in the bulk of their tumor which may or may not have favorable impact on symptoms and/or survival.

Potential adverse reactions attributable to the administration of the study drug utilized in this trial are discussed in Section **14**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity.

12.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other permissible secure remote platforms per policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of

the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Note: When required, witness signature will be obtained similarly as described for the investigator and participant as described below.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or on the electronic document. Signatures on electronic documents are described below. Note: FDA only regulates electronic signatures (i.e., an electronic timestamp is generated at the time of signature) in FDA regulated research.

Please see site specific supplement for electronic signature requirements for participating site.

For the optional biopsies in the protocol, the patient will be asked to sign a separate consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute and other participating sites has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research

data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 BN-BRACHYURY (IND # *19165*)

14.1.1 Source / Acquisition and Accountability

BN-Brachyury is an active cancer immunotherapy. This phase 1b study is being conducted under an IND held by the Center for Cancer Research (CCR), National Cancer Institute (NCI). Bavarian Nordic, Inc. is the manufacturer of BN-Brachyury and will supply product for this phase 1b study.

MVA-brachyury and FPV-brachyury will be provided to each clinical trial site by the manufacturer. The investigator or designee (e.g. pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the site and the manufacturer or IND holder.

14.1.2 Toxicity

MVA-BN-derived vectors that encode heterologous (non-vaccinia virus) antigens are being developed for the treatment of cancer. In GLP studies, MVA-BN and MVA-BN-derived vectors have been administered to 5 different animal species, including primates. In addition, MVA-BN and MVA-BN-derived vectors have been administered in clinical trials to over 7800 human subjects, including immunodeficient individuals. No marked toxicity and no drug-related serious AEs have occurred in any of these studies.

14.1.3 Formulation and Preparation

MVA-BN-Brachyury is supplied as a frozen aqueous suspension in 2 mL clear borosilicate glass, single use vials. The closure is a sterile bromobutyl rubber stopper, crimped with an aluminum cap and covered with a polypropylene closure. Each 0.5mL injection will contain a nominal virus titer of 2.0×10^8 Inf.U. Each vial must be thawed at room temperature for approximately 10–15 minutes prior to preparation, and should not be re-frozen after thawing. The thawed product will be a slightly turbid to milky suspension which may contain clumps or aggregates. To ensure homogeneity, the vial should be swirled vigorously, but not shaken, for approximately 10–20

seconds immediately (within 3 minutes) prior to use. The thawed drug product is to be drawn into a syringe with an appropriately sized safety-shielded needle suited to patient comfort (e.g., 22- to 28-gauge).

FPV-Brachyury is a liquid-frozen, highly attenuated, live recombinant virus. FPV-Brachyury is a clear to milky suspension. The product needs to be free from visible extraneous particles considering that very small particles or clumps/aggregates are product related. A turbid appearance including the tendency towards sedimentation is based on the product-inherent characteristics. Additional details can be found in the Pharmacy Manual.

Packages and vials are labeled according to the respective product specification. One FPV-Brachyury vial has sufficient volume to deliver 0.5 mL and contains a nominal virus titer of 2 x 10^9 Inf.U per mL.

Detailed trial-specific instructions on the preparation and administration of MVA-BN-Brachyury and FPV-Brachyury are provided in the clinical trial protocol and/or a separate Pharmacy Manual supplied to each clinical trial site.

14.1.4 Stability and Storage

MVA-BN-Brachyury and FPV-Brachyury are stable at -80°C ± 10°C and -20°C ± 5°C, respectively. MVA-BN-Brachyury and FPV-Brachyury should be stored frozen and remain frozen until use. After the completion of preparation, the injection must be administered within 9 hours. Avoid exposure to direct light and product may not be refrozen

14.1.5 Administration Procedures

Administration of BN-Brachyury is described in Section **3.2.1**.

14.1.6 Incompatibilities

There are no known drug interactions associated with the use of MVA-BN and MVA-BN-derived vectors.

14.2 ENTINOSTAT (IND # *19165*)

14.2.1 Source / Acquisition and Accountability

Entinostat is a class 1-selective histone deacetylase inhibitor. This phase 1b study is being conducted under an IND held by the CCR, NCI. Syndax is the manufacturer of entinostat and will supply product for this phase 1b study.

Entinostat will be provided to each clinical trial site by the manufacturer. The investigator or designee (e.g. pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the site and the manufacturer or IND holder.

14.2.2 Metabolism

Th exact mechanism of metabolism is unknown at this time. However, initially a renal and biliary clearance of entinostat was suspected. Subsequent studies have shown that hepatic metabolism seems to be a minor component of drug elimination in humans. (118, 127)

The pharmacokinetics of entinostat were linear over the dose range of 2 to 12 mg/m2 given weekly. Considerable inter-patient variation in absorption was observed. The elimination half-life of entinostat ranged from 54 to 161 hours. The median elimination half-life of entinostat under fasted conditions is 140 hours. Data from *in vitro* experiments showed that, while entinostat inhibited cytochrome P-450 (CYP) enzymes 2B6 and 3A4, the degrees of the inhibition make it unlikely that any *in vivo* systemic interactions would occur. Intestinal CYP 3A4 may be inhibited by entinostat. However, entinostat did not inhibit any tested P glucuronosyltransferase (UGT) enzymes. In addition, entinostat was found to induce CYP 1A2, CYP 2C6, and CYP 2B8 as well as UGT 1A4. Finally, entinostat is a substrate for P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) transporters.

In pharmacodynamic studies, entinostat-dependent protein lysine hyperacetylation was observed at all doses tested to date, with preliminary data suggesting that hyperacetylation may be associated with improved clinical outcome when entinostat is administered in combination with exemestane in breast cancer. PK and PD data will be evaluated on cycle 1 Day 8 and cycle 1 Day 15 (*optional*).

14.2.3 Toxicity

The most common AEs (>20%) for entinostat based on 217 patients who received monotherapy were fatigue (48%), nausea (47%), thrombocytopenia (45%), anemia (40%), hypoalbuminemia (37%), hypophosphatemia (32%), neutropenia (32%), vomiting (28%), anorexia (25%), headache (24%), diarrhea (21%), hyponatremia (21%). Grade 3 and 4 events (>10%) included thrombocytopenia (22%), anemia (19%), neutropenia (16%), and hypophosphatemia (15%). Serious adverse events (>5%) included fatigue (9%), dyspnea (7%), anorexia (6%), neutropenic infection (6%) and dehydration (6%).

The safety profile of entinostat when given in combination was similar to that seen when given as monotherapy. Overall, 97% of the 740 patients who received entinostat in combination with another agent experienced at least 1 TEAE. In general, the AEs reported most frequently among patients receiving entinostat in combination, regardless of indication, were fatigue, nausea, anemia, thrombocytopenia, leukopenia, diarrhea, vomiting, and neutropenia.

As would be expected, the AE profiles of entinostat when given in combination varied somewhat based on the agent with which it is given and the corresponding patient population. Entinostat in combination with 5-azacitidine was generally associated with an increased number of AEs relative to its use in combination with an aromatase inhibitor (AI), erlotinib, or other agents. Consistent with the overall AE profile of entinostat, nausea with or without vomiting, fatigue, and anemia were the most prevalent AEs regardless of the patient population or the agent given in combination.

Overall, among the 535 patients with solid tumors receiving entinostat in combination, the AEs reported most frequently were as follows: fatigue (66%); nausea (58%); anemia (44%); vomiting and diarrhea (each 34%); thrombocytopenia and anorexia (each 30%); leukopenia (25%); dyspnea (22%); and injection site reaction (20%).

Overall, among the 205 patients with hematologic malignancies receiving entinostat in combination, the SAEs reported most frequently were: fatigue (70%); thrombocytopenia (67%); anemia (65%); neutropenia (62%); leukopenia (60%); nausea (58%); anorexia (41%); diarrhea (29%); hypoalbuminaemia (36%); injection site reaction (35%); febrile neutropenia (34%); vomiting (33%); constipation (32%); hyperglycemia; hypocalcaemia and hyponatraemia (each 31%); edema peripheral and dyspnea (each 30%); and headache and hypophosphataemia (each 20%).

14.2.4 Formulation and Preparation

Entinostat is supplied in two strengths of film-coated tablets containing 1 mg (pink to light red) or 5 mg (yellow) of entinostat, produced using the polymorph B form of the drug. The film coating is derived from an aqueous suspension consisting of hypromellose, talc, titanium dioxide, and ferric oxide pigments (red and yellow) as coloring. Each tablet also contains mannitol, sodium starch glycolate, hydroxypropylcellulose, potassium bicarbonate, and magnesium stearate as inert fillers.

14.2.5 Stability and Storage

Entinostat tablets are to be stored at up to 25°C (77°F); excursions are permitted from 15°C to 30°C (59°F to 86°F). Entinostat tablets are to be stored in a secure, locked storage area to which access is limited.

14.2.6 Administration Procedures

Administration of Entinostat is described in Section **3.2.4.1**.

14.2.7 Incompatibilities

At this time, there are no known drug interactions or incompatibilities with entinostat.
It is thought that entinostat does inhibit CYP450 enzymes but at higher concentrations than clinically achievable. (127) However, no formal drug-drug interactions have been completed. It is recommended to use caution when administering entinostat concurrently with medication that have a narrow therapeutic index.

14.3 M7824 (IND # *19165*)

14.3.1 Source / Acquisition and Accountability

M7824 is a novel bifunctional fusion protein composed of a monoclonal antibody against human PD-L1 fused to the soluble extracellular domain of human TGF-β receptor II (TGF-βRII), which functions as a TGF-β "trap." This phase 1b study is being conducted under an IND held by the CCR, NCI. EMD Serono is the manufacturer of M7824 and will supply product for this phase 1b study.

M7824 will be provided to each clinical trial site by the manufacturer. The investigator or designee (e.g., pharmacist) will maintain an ongoing inventory of the investigational product supply according to standard site procedures. The investigational product will be dispensed at the direction of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired or unused product will be returned to the manufacturer or disposed of according to standard site procedures based on agreement between the site and the manufacturer or IND holder.

14.3.2 Metabolism

This preliminary PK analysis was based on data from 12 subjects (3 subjects each at 1, 3, 10, and 20 mg/kg) from EMR200647-001 as of the data cutoff date of 04 May 2016. Additional PK data from EMR200647-001 and MS200647-0008 will be reported at a later time. Samples for measurement of M7824 serum concentrations in Study EMR200647-001 were collected and mean M7824 serum concentrations were plotted against time after dosing by dose group. The PK profiles at the 3, 10, 20 mg/kg dose groups indicate a one-phase elimination with no obvious distribution phase after 1 hour iv infusion of M7824. Similar PK profile characteristics were observed at the lowest dose groups (1 mg/kg) with a slightly faster elimination rate.

Non-compartmental analyses were performed for each dose group with PK parameters summarized. Based on data from 12 patients in the dose escalation part of EMR200647-001, M7824 demonstrated approximately dose-proportional PK from 3 to 20 mg/kg. The mean apparent terminal half-life (t1/2) after the 1st dose was about 6 to 7 days for dose levels above 3 mg/kg. In the 1 mg/kg dose group, a mean terminal t1/2 of about 5 days was observed. When compared to the exposure at NOAEL in the 13-weeks repeat dose toxicity study in cynomolgus monkeys (140 mg/kg biweekly), the exposure at the highest clinical dose tested to date in humans (20 mg/kg biweekly) is 6.6-fold lower in Cmax and 4.3-fold lower in AUC, which indicate a safety margin of at least 4-fold.

As T-DM1 is dosed every 3 weeks, a dosing interval of every 3 weeks was preferred to the standard every 2 week dosing for M7824. The dose-level was informed by extensive population PK modeling performed by the EMD Serono pharmacokinetics team after reviewing data from the ongoing M7824 -001 and -0008 trials.

For M7824 monotherapy, 1200 mg every 2 weeks was selected as a recommended phase 2 dose. (128) In combination studies in which concomitant therapies are administered q3w, the same dosing interval for M7824 is preferred for convenience and compliance. The steady-state C_{trough} ($C_{trough,ss}$) at 1200 mg q2w is considered the target efficacious $C_{trough,ss}$ for q3w dosing of M7824 in combination studies with q3w dosing. Based on population PK modeling, median $C_{trough,ss}$ achieved with 2400 mg q3w dosing is similar to that projected with 1200 mg q2w dosing. In addition, for >90% of the participants to be dosed with 2400 mg q3w, $C_{trough,ss}$ is estimated to be above the lower bound of 95% CI for $C_{trough,ss}$ projected with 1200 mg q2w. Since Ctrough,ss is sim2400 mg q3w is expected to achieve target $C_{trough,ss}$ and full PD-L1 target occupancy and TGFβ trapping in blood in > 90% of the participants.

Safety data to support 2400 mg q3w dosing: The highest dose for M7824 tested in EMR200647-001 was 30 mg/kg once every 2 weeks and the maximum tolerated dose was not reached. Three (3) participants in EMR200647-001 dose escalation cohorts received the actual dose of at least 2400 mg q2w: 2 in 30 mg/kg cohort and 1 in 20 mg/kg. All 3 participants who received ≥ 2400 mg q2w had no DLTs. For the 2400 mg q3w dosing, both the maximum and average concentrations at steady state are not expected to exceed those at 2400 mg q2w. The overall safety profile of M7824 in Phase I was considered well tolerated, can be adequately managed and consistent across various tumor types. Refer to Investigator's Brochure for more details.

14.3.3 Toxicity

In a phase 1, open-label 3+3 dose-escalation study of M7824 in 16 patients, 3 patients experienced grade 3 drug-related adverse events including skin infection secondary to grade 2 bullous pemphigoid, lipase increased, and colitis with associated anemia. There were no grade 4 – 5 treatment related adverse events. Please see table below for details:

Treatment-related adverse events

	3 mg/kg (n = 3)		*10 mg/kg (n = 3)*		*20 mg/kg (n = 7)*		*Total (n = 16)*	
	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3	Any Grade	Grade 3
Patients with any event**	**2 (66.7)**	**1 (33.3)**	**1 (33.3)**	**0 (0.0)**	**4 (57.1)**	**2 (28.6)**	**7 (43.8)**	**3 (18.8)**
Anemia					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Bullous pemphigoid	1 (33.3)						1 (6.3)	
Colitis					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Dermatitis acneiform			1 (33.3)				1 (6.3)	
Dyspnea exertional***					1 (14.3)		1 (6.3)	
Hyperthyroidism					1 (14.3)		1 (6.3)	
Hypophosphatemia					1 (14.3)		1 (6.3)	
Hypothyroidism			1 (33.3)		1 (14.3)		2 (12.5)	
Infusion-related reaction					1 (14.3)		1 (6.3)	
Keratoacanthoma					1 (14.3)		1 (6.3)	
Lipase increase					1 (14.3)	1 (14.3)	1 (6.3)	1 (6.3)
Nausea	1 (33.3)						1 (6.3)	
Pruritus	1 (33.3)						1 (6.3)	
Rash maculo-papular	1 (33.3)		1 (33.3)				2 (12.5)	
Skin infection	1 (33.3)	1 (33.3)					1 (6.3)	1 (6.3)
Vomiting	1 (33.3)						1 (6.3)	

**There were no treatment-related AEs in the 3 patients treated with 1 mg/kg M7824.
***The differential for this dyspnea was pneumonitis vs. lymphangitic spread of disease (disease progression).

The emergent immune-related AE (irAEs) associated with M7824 in more than 670 treated patients with different tumor types in Phase I studies is consistent with PD-(L)1 agents. The other important identified risks for M7824 are dermatological AEs related to TGFβ inhibition and include keratoacanthomas and cutaneous squamous cell carcinoma. These AEs were similar to those observed in Ferguson-Smith Syndrome, in which TGFβ is genetic mutated, as well as phase I studies with fresolimumab (a pan TGFβ inhibitor). (129, 130) In the EMR200647-001/ MS200647-0008 studies, these skin AEs were observed in approximately 7% of participants and were adequately managed via excision and some spontaneously regressed without requiring

treatment discontinuation. In the context of encouraging clinical activity in various advanced cancer types, these skin lesions were overall manageable and no new safety signals emerged in the EMR200647-001/MS200647-0008 studies compared with established therapies targeting PD-(L)1 or TGFβ blockade. These lesions have not been a criterion for treatment discontinuation, but thus far have all spontaneously regressed following treatment discontinuation.

After discussion among NCI investigators on multiple protocols using M7824, multiple bleeding events ranging from low grade gingival bleeding and epistaxis to more serious hemoptysis, GI bleeding and hematuria have been observed. Some of these events can be attributed to bleeding events related to cancer directly and others bleeding events can be attributed to colitis or cystitis which is a known toxicity of anti-PD-L1 agents including M7824. However, there remains the possibility that M7824 may increase the overall risk of bleeding in ways that may not be directly related to direct tumor bleeding or inflammatory bleeding events described with checkpoint inhibitors like M7824. It is hypothesized that this possible increased bleeding risk may be due to TGF beta inhibition which has an effect on angiogenesis; bleeding has also been observed in patients receiving M7824 and may be drug-related (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool). Accordingly, patients will be closely monitored for mucosal bleeding (e.g., gum bleeding, nose bleeds, coughing up blood, blood in their urine, or blood in the stool).

A preliminary analysis of QT data from about 130 triplicate ECGs in 19 subjects from EMR200647-001 dose escalation cohorts up to a dose of 20 mg/kg showed that M7824 has no effect on QTcF intervals or heart rate, with QT-concentration slope close to 0 (0.002 ms per μg/mL) with 90% CI including zero (-0.01 to 0.01). The predicted upper bound of the 2-sided 90% CI of delta QTc at the average Cmax after the first 20 mg/kg dose (493 μg/mL) was 1.35 msec. while the predicted upper bound of 90% CI of delta QTcF change from baseline at Cmax value was less than 5 ms. Additionally, nonclinical safety study revealed no identified proarrhythmic risk.

14.3.4 Formulation and Preparation

M7824 drug product is provided as a sterile liquid formulation and packaged at a 10mg/ml concentration in USP/Ph Eur type I 50R vials that are filled with drug product solution to allow an extractable volume of 60ml (600mg/60ml). The vials are closed with rubber stoppers in serum format complying with USP and Ph Eur with an aluminum crimp seal closure. Each single-use vial contains 600mg of M7824, formulated as 10mg/mL of active, 6% (w/v) Trehalose, 40mM NaCl, 5mM Methionine, 0.05% (w/v) Tween 20, 10 mM L Histidine at pH 5.5.

The liquid formulation is diluted directly with 0.9% sodium chloride solution for injection. The estimated volumes of delivery are anticipate to be no more than 250mL. The verified concentration range in the infusion solution is 0.16mg/mL to 9.6 mg/mL.

14.3.5 Stability and Storage

M7824 drug product must be stored at 2°C to 8°C until use. The storage condition is based on data from ongoing long term stability studies with M7824. M7824 drug product stored at room (23°C to 27°C) or higher temperatures for extended periods of time might be subject to degradation.

The chemical and physical in-use stability for the infusion solution of M7824 in 0.9% saline solution has been demonstrated for a total of 72 hours at room temperature. However, from a

microbiological point of view, the diluted solution should be used immediately and is not intended to be stored unless dilution has taken place in controlled and validated aseptic conditions. If not used immediately, in-use storage times and conditions prior to administration are the responsibility of the user. Do not freeze or shake the diluted solution. No other drugs should be added to the infusion containers containing M7824.

14.3.6 Administration Procedures

Administration of M7824 is described in Section **3.2.2**.

14.3.7 Incompatibilities

No formal drug interaction studies have been conducted with M7824 in humans.

14.4 T-DM1

Please see package insert for T-DM1 (also known as Kadcyla and Ado-Trastuzumab Emtansine) available at the FDA website: www.accessdata.fda.gov

14.4.1 Source / Acquisition and Accountability

Ado-trastuzumab emtansine (also known as T-DM1 or Kadcyla) will be purchased by the site pharmacy from commercial sources.

T-DM1 will be purchased from commercial sources by the site pharmacy and stored according to product labeling and standard site procedures. T-DM1 will be dispensed at the discretion of an investigator for administration to a study participant enrolled on the clinical trial. Disposal of expired product will be according to standard site procedures.

14.4.2 Administration Procedures

Administration of T-DM1 is described in Section **3.2.3**.

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16 APPENDICIES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 APPENDIX B: ORAL MEDICATION DIARY *(ARM 3 ONLY)*

Oral Medication Diary for Entinostat

Patient's Name: _____ Date: _____

Patient Study ID: _____ Cycle #: _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle (21 days).
2. You will take Entinostat by mouth each Tuesday (white spaces). You may take the capsules one hour before or two hours after eating, as you prefer. If you forget to take the pills on your assigned day, you can take the missed dose up to 1 day after (light gray space). Do not take the weekly dose if more than 1 day late after scheduled dose (dark gray spaces).
3. Record the date, the number of pills you took, and when you took them.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring your pill bottle and this form to your physician when you go for your next appointment.

General Comments:

_____

_____

Day	# pills and when taken: Entinostat			Comments	Day	# pills and when taken: Entinostat			Comments
	Date	# Capsules	Time			Date	# Capsules	Time	
1					15				
2					16				
3					17				
4					18				
5					19				
6					20				
7					21				
8					22	Next Cycle			
9					23				
10					24				
11					25				
12					26				
13					27				
14					28				

Patient's Signature: _____Date: _____

Study Team will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study _____
3. Patient's planned daily dose_____
4. Total number of pills taken this cycle _____

Physician/Nurse's Signature _____

16.3 APPENDIX C: LTIB SOP ON HUMAN CORE BIOPSY PROCESSING FOR IHC/IF PROCESSING

1. Using sterile forceps and blade, process the tissue immediately after being collected by Interventional Radiology by cutting it in different pieces, as needed. Your time frame prior to freezing is maximum of 20 min.
2. Immediately embed each tissue piece in a labeled & dated plastic capsule containing OCT medium (TissueTek #4583), free of air bubbles. Cover the entire tissue with extra OCT.
3. Transfer the OCT/core samples to a liquid nitrogen bath and cover with a lid. After 10-15 min, transfer the frozen specimens to the vapor phase of a liquid nitrogen tank.

16.4 APPENDIX D: CLINICAL AND SAFETY PROFILE OF MVA-BN AND RECOMBINANT MVA-BASED VACCINES

MVA-BN Vector Backbone

To date, 19 clinical trials (13 sponsored by Bavarian Nordic [BN] and 6 sponsored by the Division of Microbiology and Infectious Diseases [DMID], National Institute of Allergy and Infectious Diseases [NIAID], the National Institutes of Health [NIH]) evaluating the safety and immunogenicity of MVA-BN have been completed. Currently 2 BN sponsored clinical trials are ongoing.

In total, for MVA-BN and MVA-BN-based recombinant vaccines, the exposure sums up to more than 9,600 subjects (more than 7,700 with MVA-BN, more than 400 with recombinant vaccines other than MVA-BN Filo, and more than 1,500 with MVA-BN-Filo), having received more than 15,000 single doses of vaccine. For more details, refer to the current IB of MVA-BN-BRACHYURY vaccine.

16.4.1 Safety Overview of MVA-BN and Recombinant MVA-based Vaccines

In all completed and ongoing clinical trials, vaccinations with MVA-BN have shown to be generally safe and well tolerated. No cases of death, assessed as being even possibly related, have been reported for a subject in a clinical trial using MVA-BN. Results obtained from completed Phase 1 and 2 trials and ongoing trials with several recombinant MVA-BN based vaccines in healthy adults and children, HIV infected, and cancer subjects demonstrate a similar safety profile as MVA-BN alone. Additional information on the safety profile of MVA-BN and recombinant MVA-based vaccines is provided in the Investigator's Brochure.

Adverse Drug Reactions

The following **Table 18** summarizes the pooled Adverse Drug Reaction (ADR) data of all completed MVA-BN trials. The safety profile of each of the trials with recombinant MVA-BN-based vaccines is comparable to the safety profile observed with MVA-BN trials as displayed in **Table 19** as the occurrence of the ADRs is considered to be a reaction to the vector rather than the insert, based on previous experience with recombinant MVA-BN vaccine candidates.

Table 18: **Suspected Adverse Drug Reactions Reported by ≥ 1% of Subjects in Completed MVA-BN Clinical Trials*** (N = 7,535 subjects)

Preferred Term (PT)	**No. of reports by subjects**	**Frequency (%)**
Injection site pain	6,201	82.3
Injection site erythema	4,920	65.3
Injection site swelling	3,401	45.1
Injection site induration	3,253	43.2
Injection site induration (solicited)	*3,248 out of 7,013*	*46.3*
Injection site induration (unsolicited)	*5 out of 521*	*1.0.*
Injection site pruritus	2,856	37.9
Injection site pruritus (solicited)	*2,645 out of 6,339*	*41.7*

Preferred Term (PT)	**No. of reports by subjects**	**Frequency (%)**
Injection site pruritus (unsolicited)	*211 out of 1,213*	*17.4*
Fatigue	2,121	28.1
Fatigue (solicited) +	*2,121 out of 6,339*	*33.5*
Myalgia	2,444	32.4
Myalgia (solicited)	*2,443 out of 7,448*	*32.8*
Myalgia (unsolicited)	*1 out of 86*	*1.2*
Headache	2207	29.3
Headache (solicited)	*2,176 out of 7,013*	*31.0*
Headache (unsolicited)	*31 out of 521*	*6.0*
Nausea	1,073	14.2
Nausea (solicited)	*1,072 out of 7,448*	*14.4*
Nausea (unsolicited)	*1 out of 86*	*1.2*
Rigors/chills	664	8.8
Rigors/chills (solicited)	*663 out of 6,732*	*9.8*
Rigors/chills (unsolicited)	*1 out of 802*	*0.1*
Body temperature increased	269	3.6
Injection site discoloration	190	2.5
Appetite disorder	218	2.9
Appetite disorder (solicited)++	*218 out of 1,801*	*12.1*
Injection site nodule	149	2.0
Pain in extremity	147	2.0
Pain in extremity (solicited)	*139 out of 1,346*	*10.3*
Pain in extremity (unsolicited)	*8 out of 6,188*	*0.1*
Arthralgia	206	2.7
Injection site hematoma	107	1.4
Pyrexia	94	1.2
Axillary pain	91	1.2

* POX-MVA-001, -002, -004, -005, -007, -008, -009, -010, -011, -013, -023, -024, -027, -028, -029, -030, -036, HIV-NEF-004 and HIV- POL-002; 7 subjects in POX-MVA-009 received Dryvax® either on the same day or within 7 days after MVA-BN administration and were therefore not included to avoid a potential bias in the adverse event reporting. + not in POX-MVA-001; ++ only in NIH trials

Looking only at the events that were reported by at least 1% of subjects, the majority of ADRs represented local vaccination site reactions as well as common systemic reactions typical for modern injectable vaccines and were classified as being mild to moderate in intensity and resolved completely without intervention within the first 7 days following vaccination. To date, no trends have been identified suggesting the occurrence of any particular unexpected adverse reactions or classes of adverse reactions following vaccinations with MVA-BN.

Cardiac Signs and Symptoms

Based on observations with replicating smallpox vaccines particular attention has been placed on monitoring for cardiac signs and symptoms in all clinical trials using MVA-BN. Despite close cardiac monitoring, no confirmed event indicating a case of myo-/pericarditis has been observed in any completed MVA-BN trial.

Serious Suspected Adverse Drug Reactions

As of 31 July 2016, a total of 7 (7 out of 7,758 vaccinated subjects = 0.09%) serious suspected ADRs have been reported for MVA-BN smallpox vaccine in completed and ongoing trials (**Table 18**).

All of them have been thoroughly reviewed by BN and the trial specific Data Safety Monitoring Board who concluded that the continued use of MVA-BN in a clinical setting presented no special risks to the subjects. No pattern regarding Serious Adverse Drug Reactions (SADRs) could be detected.

Table 19: **Serious Suspected Adverse Drug Reactions (Assessed by the Investigator to Be At Least Possibly Related to MVA-BN)**

Trial Code	Age/ Gender	Days After Vaccination	Event	Outcome	Underlying Diseases/ Circumstances	PI Assess-ment	BN Opinion
POX-MVA-005	30/ Male	70 days after second vaccination	Sarcoidosis	Stable and asympto-matic	Urinary tract infection with Chlamydia trachomatis at time of first symptoms (arthralgia)	Possibly related	Possibly related
POX-MVA-005	31/ Female	26 months after second vaccination	Crohn's disease	Stable and asympto-matic under therapy	Abnormal lab results (elevated alkaline phosphatase, absolute neutrophils and platelet counts) at screening for 2-year follow-up POX-MVA-023 (excluded)	Possibly related	Possibly related
POX-MVA-008	28/ Female	8 days after second vaccination	Transitory ocular muscle paresis	Resolved without sequelae	No relevant medical history	Probably related	Possibly related
POX-MVA-010	30/ Female	133 days after second vaccination	Congestive heart failure due to cardio-myopathy	Stable under cardiac medi-cations	Surgery for ventricular septal defect as child. HIV infection. Concomitant (denied, therefore previously unknown to BN) participation in a Growth-Hormone Releasing Hormone (GH- RH) trial; event also assessed as possibly related to GH-RH	Possibly related	Unlikely related
POX-MVA-011	39/- Female	1 day after second vaccination	Simple pneumonia and pleurisy	Resolved without sequelae	HIV infection (CD4 count 4 weeks prior to second vaccination was 299 cells/µL). History of chronic obstructive pulmonary disease. Acute sinusitis and nasal congestion due to swimmer's ear which triggered hospital admittance.	Possibly related	Unlikely related
POX-MVA-036	27/ Female	0 days after second vaccination	Throat tightness and other hypersensitivity symptoms such as hives, pruritus, tender vaccination site, swollen axilla,	Resolved without sequelae	The subject received her second dose of MVA-BN 21 days after the first dose and after 2 hours developed symptoms such as skin reactions and throat tightness which was responsive to epinephrine treatment. She had no wheezing and was not	Possibly related	Possibly related

Trial Code	Age/ Gender	Days After Vaccination	Event	Outcome	Underlying Diseases/ Circumstances	PI Assess-ment	BN Opinion
			angioedema of forearms		hypotensive. Symptoms subsided after several days under prednisone and diphenhydramine treatment. She has a family history of allergies and a medical history of shingles. She has received multiple vaccines before but never had previous hives or other problems with vaccines.		
POX-MVA-036	30/Male	117 days after first vaccination	Non-ST-segment elevation myocardial infarction	Resolved without sequelae	Positive family history for cardiovascular diseases (both grandfathers had myocardial infarctions in their 50ies, father had blood clots), as well as overweight with a BMI above 33. A few days before event onset, subject returned from a trip to India with diarrhea and was started on ciprofloxacin treatment (which per US prescribing information is associated with angina pectoris and myocardial infarction). He showed chest pain and increased troponin I, but no ST segment changes in the ECG and no coronary artery disease in cardiac catheterization. A post-infectious myocarditis (published case reports exist for campylobacter, shigella, and salmonella) was considered as alternative etiology for the reported event.	Possibly related	Unlikely related

16.4.2 Safety Profile of MVA-BN Smallpox Vaccine in Healthy Population Compared to Special Populations

BN has evaluated the safety and immunogenicity of MVA-BN-based recombinant vaccines for several indications such as cancer, HIV and measles in more than 1,900 subjects including healthy and HIV infected populations. In recombinant MVA vaccine trials, doses up to 5 x 10e8 $TCID_{50}$ were administered applying varying schedules of repeated vaccinations, e.g. a 3-dose schedule was used for recombinant HIV vaccines and multiple vaccinations have also been performed in subjects receiving a recombinant therapeutic breast cancer vaccine (MVA-BN-HER2). Results obtained from these Phase 1 and 2 trials demonstrate a similar safety profile and vector immunogenicity as compared to MVA-BN alone.

Two of the recombinant vaccine trials allowed for a direct comparison with MVA-BN as an active control. These trials (HIV-POL-002 and HIV-NEF-004) were performed to evaluate 2 different recombinant MVA-based HIV vaccine candidates in HIV-infected subjects. In both

trials, a total of 3 vaccinations with either the recombinant HIV vaccine or MVA-BN were performed according to a 0, 8 and 16 week regimen.

16.4.3 Immunogenicity Overview of MVA-BN

MVA-BN was tested for safety and immunogenicity among healthy volunteers in 3 Phase 1 and 2 dose finding trials. (93-95) Across these trials a linear dose relationship was observed between the vaccine doses and both vaccinia ELISA and Plaque Reduction Neutralization Test (PRNT) titers. Maximum ELISA seroconversion rates and peak titers were reached 2 weeks after the second vaccination, with 100% seroconversion after the second dose for all dose groups receiving at least 2 x 10^7 $TCID_{50}$ per 0.5 mL dose of MVA- BN. Statistical analysis indicated lower doses to be inferior to the standard dose tested throughout all dose ranging trials, whereas the standard dose achieved ELISA seroconversion rates between 81% and 100% already after the first dose. For the PRNT, the same trend was observed with about 77% seroconversion rates 2 weeks after the second MVA-BN administration in all groups receiving the highest dose.

The early onset of seroconversion and the higher titers of total and neutralizing antibodies combined with an excellent safety profile qualified the dose of at least 5 x 10^7 $TCID_{50}$ as the most suitable human dose. The final optimal (standard) dose and schedule for the general population was decided to be 2 doses of at least 5 x 10^7 $TCID_{50}$ MVA-BN administered subcutaneously (s.c.) 2-4 weeks apart.

16.5 APPENDIX E: CCR REPORTABLE EVENT FORM (REF)

NCI Protocol #: Click or tap here to enter text.	
Protocol Title: Click or tap here to enter text.	
Report version: *(select one)* ☐ Initial Report ☐ Follow-up	
Site Principal Investigator: Click or tap here to enter text.	
Date site PI was notified of the problem: Click or tap to enter a date.	**Date of problem:** Click or tap to enter a date.
If delay in reporting to the coordinating center, please explain: Click or tap here to enter text.	
Location of problem: *(e.g., patient's home, doctor's office)* Click or tap here to enter text.	
Description of Subject Does this problem apply to a subject? ☐ yes ☐ not applicable (more than one subject is involved) **If yes, enter details below:** Subject ID: Click or tap here to enter text. (*do not use medical record number)* Sex: ☐Male ☐ Female Age: Click or tap here to enter text. Diagnosis: Click or tap here to enter text.	
Name the problem: *(select all that apply)* ☐ Specimen collection issue ☐ Informed consent issue ☐ Ineligible for enrollment ☐ Breach of PII ☐ Other, briefly state the nature of the problem: Click or tap here to enter text. **Detailed Description of the problem:** (*Include any relevant treatment, outcomes or pertinent history*): Click or tap here to enter text.	

What are you reporting? ☐ **unanticipated problem** ☐ **death** ☐ **non-compliance (other than a protocol deviation)** ☐ **protocol deviation** ☐ **new information that might affect the willingness of subjects to enroll or continue participation in this study**
If interventional or expanded access study, please answer the following questions about your site: How many participants are still receiving the study intervention? Click or tap here to enter text. How many participants completed study interventions but remain in follow up? Click or tap here to enter text. How many participants are enrolled but not yet receiving study interventions? Click or tap here to enter text.
Have similar problems occurred on this protocol at your site? ☐ **Yes** ☐ **No**
Describe what steps you have already taken or will be taking as a result of this problem: Click or tap here to enter text.

INVESTIGATOR'S SIGNATURE:	**DATE:**