Cover Page

Release Date: August 27, 2024

ClinicalTrials.gov ID: NCT02403505

Unique Protocol ID: IND 172259

Brief Title: Early Phase Clinical Trial About Therapeutic Biological Product Mix for Treating CEA Positive Rectal Cancer (CEA+RC-BCG)

Official Title: Conducting an Early Phase Clinical Trial to Assess for CEA Antigen Presentation Therapeutic Biological Product Mix Activity That Suggests the Potential for Clinical Benefits of CEA Positive Rectal Cancer Patients.

Secondary IDs: FWA00015357 [Registry ID: HHS, Human Protections Administrator]
IRB00009424 [Registry ID: HHS, IRB]
IORG0007849 [Registry ID: HHS, IORG]
NPI-1831468511 [Registry ID: HHS, Health Care Provider Individual]
NPI-1023387701 [Registry ID: HHS, Health Care Provider Organization]
IND 172259 [Registry ID: FDA, Investigational New Drug Application (IND)]

Human APCs treat CEA protein into small fragments, and then kill CEA (+) RC cells in vivo.

4. General Investigational plan *[21 CFR 312.23(a)(3)]*

Contents

21 CFR 312.23 (a)(3)

Introductory statement and general investigational plan.

(iv) A brief description of the overall plan for investigating the drug product for the following year. The plan should include the following: …… (including **Statistical Analysis Plan**)

My investigation will use commercially available lawfully marketed prescription biological products by US Pharmacy like as following:

Millipore - https://www.sigmaaldrich.com/US/en/product/mm/219369
Millipore-Sigma CEA Antigen Protein
Product name: Carcinoembryonic Antigen, Human Colon Adenocarcinoma Cell Line
Pack Size: 0.1 mg
Product Number: 219369
Catalogue No.: D44022
Brand: Millipore
Company: Sigma-Aldrich Inc.

- Add 2 mL distilled water into above 0.1 mg CEA protein Antigen.

Before 5 minutes for the percutaneous route with the multiple puncture device, above biologic 1 mL - 0.05 mg CEA will be added into following biologic:

DailyMed - https://dailymed.nlm.nih.gov/dailymed/
LABEL: BCG VACCINE - bacillus calmette-guerin substrain tice live antigen injection, powder, lyophilized, for solution
***NDC** 0052-0603-02*
***BLA** 103050*
Packager: Merck Sharp & Dohme Corp.

- BCG VACCINE contains live bacteria. BCG VACCINE for percutaneous use is an attenuated, live culture preparation of the Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis. The TICE® strain used in this BCG VACCINE preparation. The TICE® BCG organism is grown for preparation of freeze-dried cake.
- Bacillus of Calmette and Guerin (BCG) strain of Mycobacterium bovis
- 1 to 8 × 10^8 colony forming units (CFU) of BCG (equivalent to approximately 50 mg wet weight)
- TICE® BCG organism 50 MG

- Mix above biological products to take the percutaneous use and treat CEA (+) Rectal Cancer.
- The IND 172259 Phase 1 clinical investigation NCT02403505 will use 1 mL - 0.05 mg of the biological product, **CEA Protein** add into the biological product, **BCG VACCINE** and mix above them to take the percutaneous use and treat CEA positive rectal cancer.

(*a*) The rationale for the drug or the research study.

The rationale for the clinical research study of CEA Protein Antigen plus BCG Vaccine Mix to treat the CEA positive rectal cancer via following three processes: 1. Trained Immunity. 2. Antigen Presentation Therapy **(It is Key).** 3. Innate immune memory.

1. Activate the Trained Immunity Reaction to CEA Protein Antigen (Pharmacology)

- Trained immunity - https://en.wikipedia.org/wiki/Trained_immunity

Trained immunity is the modification of cells in the innate immune system (the one with which an organism is born) to create a "memory" of a pathogen. Trained immunity creates no antibodies in preparation for a second encounter. Instead, the immunity is mediated mostly by epigenetic modifications -- alterations in gene expression and cellular function without changes to the original DNA sequence. The resulting immunity lasts up to several months and is usually unspecific because there is no production of specific antibodies or receptors. The term "innate immune memory" can be used as a synonym for the term “trained immunity”. Monocytes/macrophages can undergo epigenetic modifications after a ligation of their pattern recognition receptors (PRRs). This ligation with CEA protein antigen may prepare these cells for a second encounter with the training pathogen CEA protein antigen. The secondary response may be heightened not only against the training pathogen CEA, but also against different pathogens **(Rectal Cancer Cells)** whose antigens can be recognized by the same PRRs. This effect has also been observed when stimulating cells by vaccination against tuberculosis with a vaccine containing **BCG**.

2. Activate the Antigen Presentation Reaction to CEA Proteins (Pharmacology)

- Antigen presentation - https://en.wikipedia.org/wiki/Antigen_presentation

BCG can activate macrophages, a kind of antigen presenting cells (APCs), treat CEA protein antigen into small peptide fragments, and then kill CEA positive rectal cancer cells in vivo. In the antigen presentation process, these CEA protein antigen protein molecules are mainly degraded into small peptides by cytosolic proteases in the proteasome, but there are also other cytoplasmic proteolytic pathways. APCs can internalize exogenous CEA protein antigen by endocytosis, but also by pinocytosis, macro-autophagy, endosomal micro-autophagy or chaperone-mediated autophagy. After internalization, the CEA protein antigen can be enclosed in vesicles called endosomes. There are three compartments involved in this antigen presentation pathway: early endosomes, late endosomes or endolysosomes and lysosomes, where the CEA protein antigen can be hydrolyzed by lysosome-associated enzymes (acid-dependent hydrolases, glycosidases, proteases, lipases). This process is favored by gradual reduction of the pH. The main proteases in endosomes are cathepsins and the result is the degradation of the antigens into oligopeptides. The CEA positive rectal cancer cells will be similar to the CEA above.

3. Produce the Innate immune memory to CEA protein antigen (Pharmacology):

- Innate immune memory - https://en.wikipedia.org/wiki/Immunological_memory

Trained Immunity is also Innate Immune Memory for unspecific. Innate immune memory (trained immunity) is defined as a long-term functional reprogramming of innate immune cells evoked by exogenous or endogenous insults and leading to an altered response towards a second challenge after returning to a non-activated state. When innate immune cells receive an activation signal, for example, through recognition of BCG with Pattern recognition receptors (PRRs), they start the expression of proinflammatory genes, initiate an inflammatory response, and undergo epigenetic reprogramming. After the second stimulation, the transcription activation is faster and more robust. Innate immunological memory was reported in monocytes, macrophages, and some others, change their epigenetic state and respond differently after priming insult. Additionally, cellular metabolism doesn't return to the state before stimulation, and trained cells remain in a prepared state. This status can last from weeks to several months and can be transmitted into daughter cells. Secondary stimulation induces a new response, which is faster and stronger. The secondary response may be heightened not only against the training pathogen CEA, but also against different pathogens (Rectal Cancer Cells) whose antigens can be recognized by the same PRRs.

4. Produce the Type IV hypersensitivity as side effect risk (Toxicology)

- Type IV hypersensitivity - https://en.wikipedia.org/wiki/Type_IV_hypersensitivity

Type IV hypersensitivity, often called delayed-type hypersensitivity, is a type of hypersensitivity reaction that takes several days to develop. It is a type of cell-mediated response. This response involves the interaction of T cells, monocytes, and macrophages. This reaction is caused when CD4+ Th1 cells recognize foreign antigen **(including CEA protein antigen mix BCG)** in a complex with MHC class II on the surface of antigen-presenting cells. These can be macrophages that secrete IL-12, which stimulates the proliferation of further CD4+ Th1 cells. CD4+ T cells secrete IL-2 and interferon gamma (IFN-γ), inducing the further release of other Th1 cytokines, thus mediating the immune response. Activated macrophages produce hydrolytic enzymes and, on presentation with certain intracellular pathogens, transform into multinucleated giant cells. The overreaction of the helper T cells and overproduction of cytokines damage tissues, cause inflammation, and cell death. An example of a tuberculosis (TB) infection that comes under control: M. tuberculosis organisms are engulfed by macrophages after being identified as foreign pathogens, but due to an immuno-escape mechanism peculiar to mycobacteria, TB bacteria are able to block the fusion of their enclosing phagosome with lysosomes which would destroy TB bacteria. Thereby TB organisms can continue to replicate within macrophages. After several weeks, the immune system ramps up and, on stimulation with IFN-gamma, the macrophages become capable of killing M. tuberculosis organisms by forming phagolysosomes and nitric oxide radicals. The hyper-activated macrophages secrete TNF-α which recruits multiple monocytes to the site of infection. These monocyte-macrophage system cells differentiate into epithelioid cells which wall off the infected cells but result in significant inflammation and local damage. **BCG can produce similar but lighter damage.** Type IV hypersensitivity can usually be resolved with trigger avoidance. In the IND 172259 Phase 1 clinical investigation NCT02403505, **IGRA blood test negative participant will be negative IGRA blood test with TB antigens.**

(*b*) the indication(s) to be studied.

The indications for CEA protein antigen plus BCG Vaccine Mix for Percutaneous Use to treat CEA positive rectal cancer like as following:

1. Treat CEA positive rectal cancer.
2. Activate human trained immunity reaction via the pattern recognition receptors (PRRs) of immune cells litigated with CEA protein antigen.
3. Activate human CEA protein antigen presentation reaction.
4. The human antigen presenting cells (APCs) treat CEA target protein antigen into small peptide fragments, and then kill CEA positive rectal cancer cells in vivo.
5. BCG activates APCs presenting CEA protein antigen to Memory T Cells and the Trained Immunity is also Innate Immune Memory.
6. The secondary response of trained immunity may be heightened not only against the training pathogen CEA, but also against different pathogens (Rectal Cancer Cells) whose antigens can be recognized by the same PRRs.

(*c*) the general approach to be followed in evaluating the drug; …

My investigation will use commercially available lawfully marketed prescription biological products by US Pharmacy like as following:

Millipore - https://www.sigmaaldrich.com/US/en/product/mm/219369

- **Millipore-Sigma CEA Antigen Protein**
  Product name: Carcinoembryonic Antigen, Human Colon Adenocarcinoma Cell Line
  Pack Size: 0.1 mg
  Product Number: 219369
  Catalogue No.: D44022
  Brand: Millipore
  Company: Sigma-Aldrich Inc.
- Add 2 mL distilled water into above 0.1 mg CEA protein antigen

Before 5 minutes for the percutaneous route with the multiple puncture device, above biologic 1 mL - 0.05 mg CEA will be added into following biologic BCG:

DailyMed - https://dailymed.nlm.nih.gov/dailymed/
LABEL: BCG VACCINE - bacillus calmette-guerin substrain tice live antigen injection, powder, lyophilized, for solution
NDC *0052-0603-02*
BLA *103050*
Packager: Merck Sharp & Dohme Corp.

- **Study Type:** Interventional
- **Primary Purpose:** Treatment
- **Study Phase:** Phase 1 / Clinical Biological Pharmacology
- **Interventional Study Model:** Single Group Assignment / Single Usage / Single Dosage
- **Number of Arms:** 1 / single-arm study
- **Masking:** None (Open Label)
- **Allocation:** N/A
- **Enrollment:**
  - ✓ 20 CEA Positive Rectal Cancer Patients
  - ✓ Clinical Rectal Cancer Diagnosis Stage 0 - IIA
  - ✓ Clinical Rectal Cancer Diagnosis without symptoms
  - ✓ Clinical Rectal Cancer Diagnosis without metastasis
  - ✓ Positive testing CEA by blood-drawing
  - ✓ TB negative participant is negative IGRA blood test with TB antigens.
- **Dose:** CEA Protein Antigen 0.05 MG plus BCG Vaccine 50 MG Mix
- **Route:** Percutaneous Use with Multiple Puncture Device
- **Duration:** Our trial duration will be 12-week duration.
- **Endpoint:** Positive IGRA blood test with CEA protein antigen after percutaneous 21 days

(*d*) the kinds of clinical trials to be conducted in the first year following the submission (if plans are not developed for the entire year, the sponsor should so indicate);

Our planned duration will be 4-week duration.

(*e*) the estimated number of patients to be given the drug in those studies; and

20 CEA (+) RC Patients [Positive testing by blood-drawing]

(*f*) any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs.

- **The safety exclusions:**
  - ✧ Pregnant
  - ✧ Thrombosis
  - ✧ Allergy
  - ✧ TB participant is positive IGRA blood test with TB antigens.
  - ✧ Symptoms of rectal cancer
  - ✧ Metastasis of rectal cancer
  - ✧ Evidence of critical illness

BCG should **not** be given to individuals previously infected with M. tuberculosis. A person given the IGRA blood test with TB antigens. The TB positive is the positive IGRA blood test with TB antigens. The TB negative is the negative IGRA blood test with TB antigens.

Type IV hypersensitivity, often called delayed-type hypersensitivity, is a type of hypersensitivity reaction that takes several days to develop. It is a type of cell-mediated response. This reaction can be caused by antigen-presenting cells. These monocyte-macrophage system cells differentiate into epithelioid cells which wall off the infected cells but result in significant inflammation and local damage. **BCG can produce similar but lighter damage.**

Table of procedures: Month-1

Procedure	Online Enrollment	M 1 W 1 D 1	M 1 W 1 D 3	M 1 W 4 D 3	M 1 W 4 D 5	Online Notification
Medical history *	√	√	√	√	√	√
Test CEA by blood-drawing **		√				√
IGRA blood test with TB antigens ***		√		√		√
IGRA blood test with CEA protein antigen ****				√		√
Review Results			√		√	√
Biological Product Mix (Percutaneous Use)			√			√
Online Interview & Online Questionnaire For every day in 3 months	√	√	√	√	√	√

* This includes online reporting on symptoms every day for up to 90 days.
** Test CEA protein antigen by blood-drawing.
*** BCG should not be given to individuals previously infected with M. tuberculosis, if a person got the Positive Interferon-gamma release assay blood test (IGRA) with TB antigens.
**** Take Interferon-gamma release assay blood test (IGRA) with CEA protein antigen.

If participants agree, can go ahead following:

Table of procedures: Month-2

Procedure	Online Enrollment	M 2 W 1 D 1	M 2 W 1 D 3	M 2 W 4 D 3	M 2 W 4 D 5	Online Notification
Medical history *	√	√	√	√	√	√
Test CEA by blood-drawing **		√				√
IGRA blood test with TB antigens ***		√		√		√
IGRA blood test with CEA protein antigen ****				√		√
Review Results			√		√	√
Biological Product Mix (Percutaneous Use)			√			√
Online Interview & Online Questionnaire For every day in 3 months	√	√	√	√	√	√

* This includes online reporting on symptoms every day for up to 90 days.
** Test CEA protein antigen by blood-drawing.
*** BCG should be given to individuals previously injected with BCG vaccine. A person given the Interferon-gamma release assay blood test (IGRA) with TB antigens.
**** Take Interferon-gamma release assay blood test (IGRA) with CEA protein antigen.

Table of procedures: Month-3

Procedure	Online Enrollment	M 3 W 1 D 1	M 3 W 1 D 3	M 3 W 4 D 3	M 3 W 4 D 5	Online Notification
Medical history *	√	√	√	√	√	√
Test CEA by blood-drawing **		√				√
IGRA blood test with TB antigens ***		√		√		√
IGRA blood test with CEA protein antigen ****				√		√
Review Results			√		√	√
Biological Product Mix (Percutaneous Use)			√			√
Online Interview & Online Questionnaire For every day in 3 months	√	√	√	√	√	√

* This includes online reporting on symptoms every day for up to 90 days.
** Test CEA protein antigen by blood-drawing.
*** BCG should be given to individuals previously injected with BCG vaccine. A person given the Interferon-gamma release assay blood test (IGRA) with TB antigens
**** Take Interferon-gamma release assay blood test (IGRA) with CEA protein antigen.

If it is possible, please take clinical test like as following:

- A person given the tuberculin skin test [10 tuberculin units (10 TU) purified protein derivative (PPD) by Intradermal Injection (ID)] must return within 48 hours. The positive is skin test reading > 5 mm induration at 48 hours. The negative is skin test reading < 5 mm induration at 48 hours.
- A person given the CEA protein skin test [0.01 mg CEA protein antigen by Intradermal Injection (ID)] must return within 48 hours. The positive is skin test reading > 5 mm induration at 48 hours. The negative is skin test reading < 5 mm induration at 48 hours.

It is my **commitment** that our Institutional Review Board (IRB) (IRB00009424) that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of each of the studies in my proposed clinical investigation and that the sponsor-investigator (me i.e. Han Xu, M.D., Ph.D.) will report to our IRB (IRB00009424) the proposed changes in my research activity in accordance with the requirements of part 56.

I (**Han Xu, M.D., Ph.D., FAPCR, Sponsor-Investigator, Medical Director, IRB Chair)** write the **statement** with respect to each clinical study involving human subjects that it either will be conducted in compliance with the institutional review board regulations in part 56 or will not be subject to the regulations under §56.104 or §56.105; and that it either will be conducted in compliance with the informed consent regulations in part 50 or will not be subject to the regulations under §50.23 and §50.24.

Sponsor (**Han Xu, M.D., Ph.D., FAPCR, Sponsor-Investigator)** does **not** transfer any obligations for the conduct of any clinical study to a Contract Research Organization (CRO).

Institutional Review Board (IRB) will be to review, to approve the initiation of, and to conduct periodic review of, biomedical research involving human subjects. My investigation will be conducted in compliance with the requirements for institutional review (21 CFR Part 56) and informed consent (21 CFR Part 50). Our IRB's review of the investigator's qualifications or the sponsor's qualifications, including any institutional requirements for sponsor-investigator studies, will surely abide by 21 CFR Part 56 and 21 CFR Part 50. After ***IRB approval,*** an IND covering the investigations will be **in effect**.

21 CFR § 50.3 (f)

I (sponsor-investigator) will actually conduct, with online referral clinical investigators, the clinical investigation, i.e., under whose immediate direction the test article is administered or dispensed to, or used involving, a subject.

21 CFR § 56.102 (k)

I (sponsor-investigator) will actually conduct, with online referral clinical investigators, the clinical investigation, i.e., under whose immediate direction the test article is administered or dispensed to, or used involving, a subject.

Han Xu, M.D., Ph.D., FAPCR, Sponsor-Investigator, IRB Chair, Medical Director
Health Care Provider - Individual (NPI - 1831468511)
Health Care Provider - Clinical Ethicist (Code - 174V00000X)
Health Care Provider - Pharmacist - Clinical Pharmacy Specialist (Code - 1835P0018X)
Medicine Invention Design Incorporation (MIDI) IRB #1 (IRB00009424)
Medicine Invention Design Incorporation (IORG0007849)
Medicine Invention Design Incorporation (FWA00015357)
CAQH Approved Maryland State License (D11379922)
Health Care Provider - Group/Organization (NPI - 1023387701)
Health Care Provider - Health Maintenance Organization - (Code - 302R00000X)
Health Care Provider - Multi-Specialty Group - (Code - 193200000X)
Health Care Provider - Pharmacist - Clinical Pharmacy Specialist (Code - 1835P0018X)

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