# mRNA CAR T Cell Pilot Trial Triple Negative Breast Cancer

**Protocol #1110-1127**

**"Pilot Clinical Trial of Autologous Met Redirected T cells Administered Intratumorally and Intravenously in Patients with Operable Triple Negative Breast Cancer"**

December 14, 2011

Penn Medicine
Abramson Cancer Center

# Protocol 1127: Investigative Team

| | |
|---|---|
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| **Regulatory:** | Elizabeth Veloso |
| **Safety Monitoring Committee:** | Roger Cohen<br>David Porter<br>Wei-Ting Hwang |
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| **Correlative Biomarkers:** | Michael Kalos<br>Paul Zhang |
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| **Funding:** | NCI CCSG and BCRF<br>Commonwealth of PA |
| **MD Anderson Cancer Center:** | Laurence Cooper |

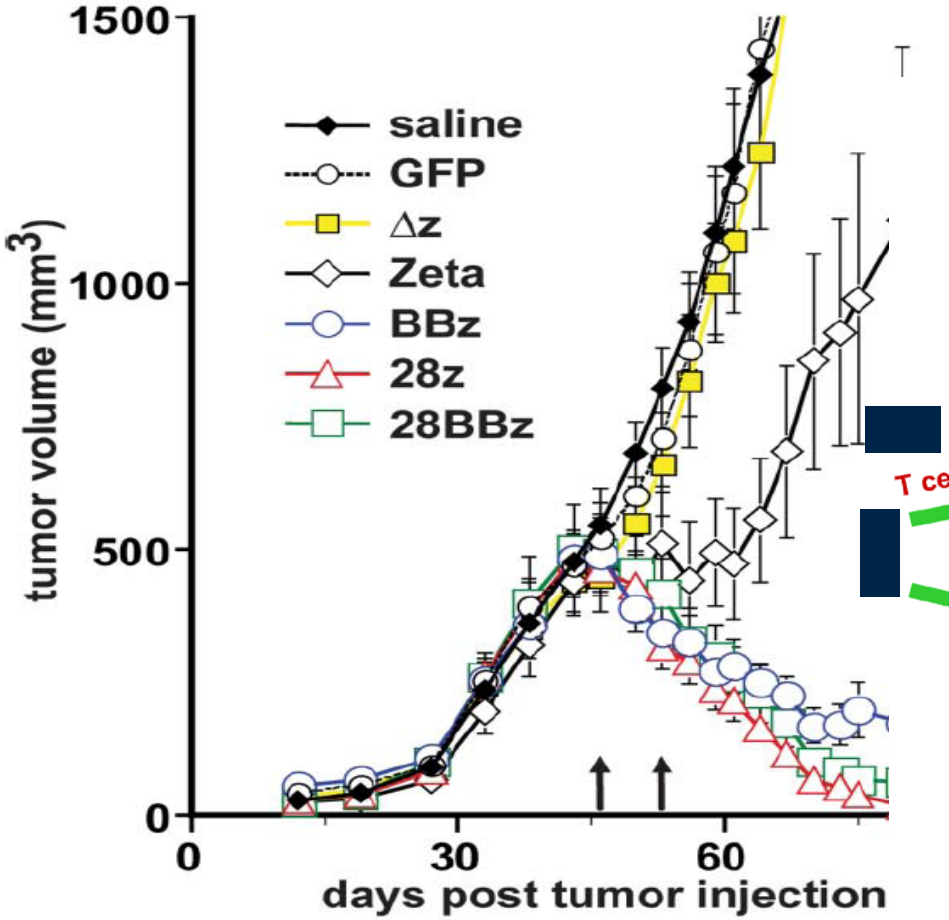
# Outline

- RNA CARs as a platform for pilot evaluation of new CAR targets
- pre-clinical and clinical data with RNA CARs
- Triple negative breast cancer
- Protocol design and safety features to minimize toxicity and maximize scientific integrity

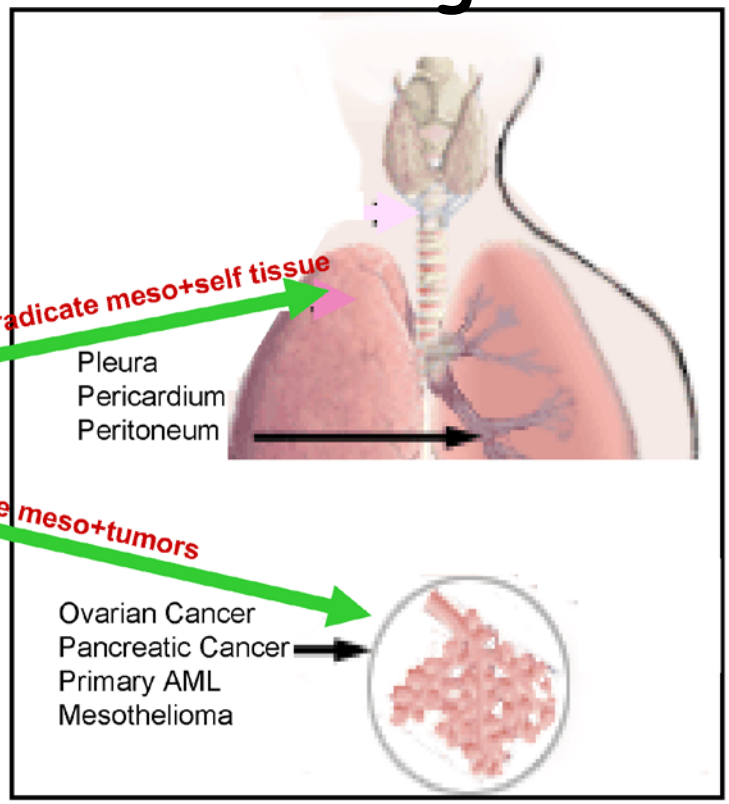
# Rationale for Transient CAR Expression

- **Safety concerns with CARs with additional signaling domains that render the T cells more potent and resistant to the tumor stroma and natural checkpoints (Tregs, etc).**
- **Pre-clinical animal models may not be predictive of toxicity**
- **First in human trials with new targets: transient CAR expression could be used as a safety feature.**
  - **If safety demonstrated with transient expression systems, then proceed with permanently modified cells**
  - **Short term expression may be sufficiently potent in some situations**
- **Approaches:**
  - **Suicide systems**
  - **Transient expression w protein or mRNA transduction**

# Anti-mesothelin and c-Met CARs engineered with lentiviral vectors have potent anti-tumor effects in pre clinical models




## The potential bad news: extravascular targets




Carpenito, PNAS 2009

# Approach to Optimize “RNA CARs”

- In non-dividing DC preparations: RNA optimization and electroporation is efficient (Javorovic et al. Molecular Therapy 12, 734, 2005; Holtkamp et al. Blood 108, 4009, 2006)
- TCRs were efficiently electroporated into T cells and shown to have function in vitro (Zhao et al. Molecular Therapy 13, 151, 2006)
- T cells will divide extensively, so that optimizing RNA stability, expression, combined with multiple infusions and dose escalation may be required
- Not previously tested in pre-clinical models or in patients

*Microenvironment and Immunology*

Cancer Research

# Multiple Injections of Electroporated Autologous T Cells Expressing a Chimeric Antigen Receptor Mediate Regression of Human Disseminated Tumor

Yangbing Zhao[1], Edmund Moon[2], Carmine Carpenito[1], Chrystal M. Paulos[1], Xiaojun Liu[1], Andrea L. Brennan[1], Anne Chew[1], Richard G. Carroll[1], John Scholler[1], Bruce L. Levine[1], Steven M. Albelda[2], and Carl H. June[1]

Zhao et al. Cancer Research, 2010

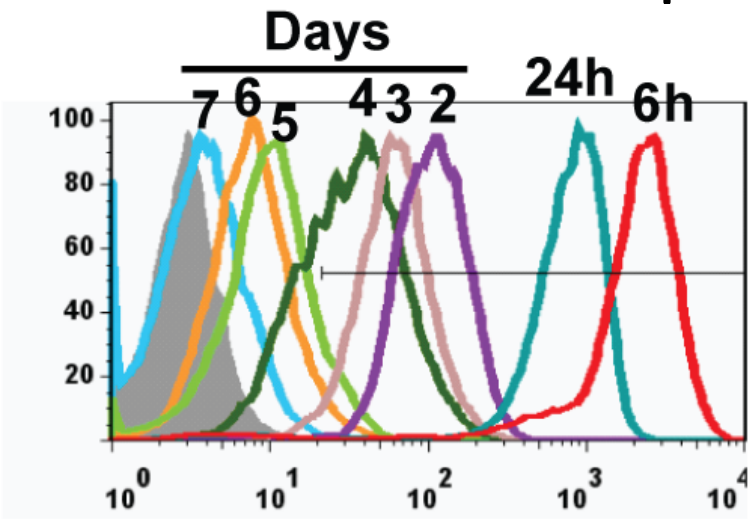
# Optimizing In Vitro Transcribed mRNA CAR Backbone

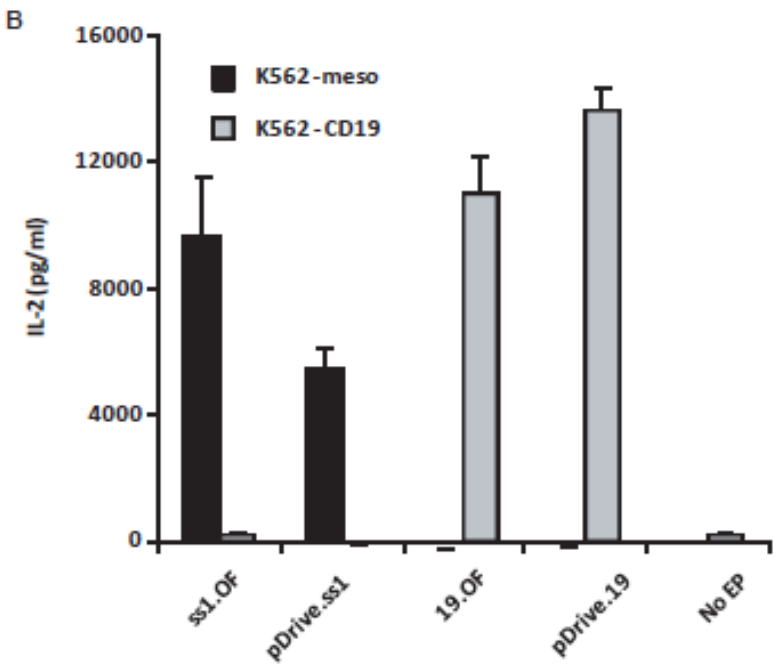
Modifications tested to optimize expression:

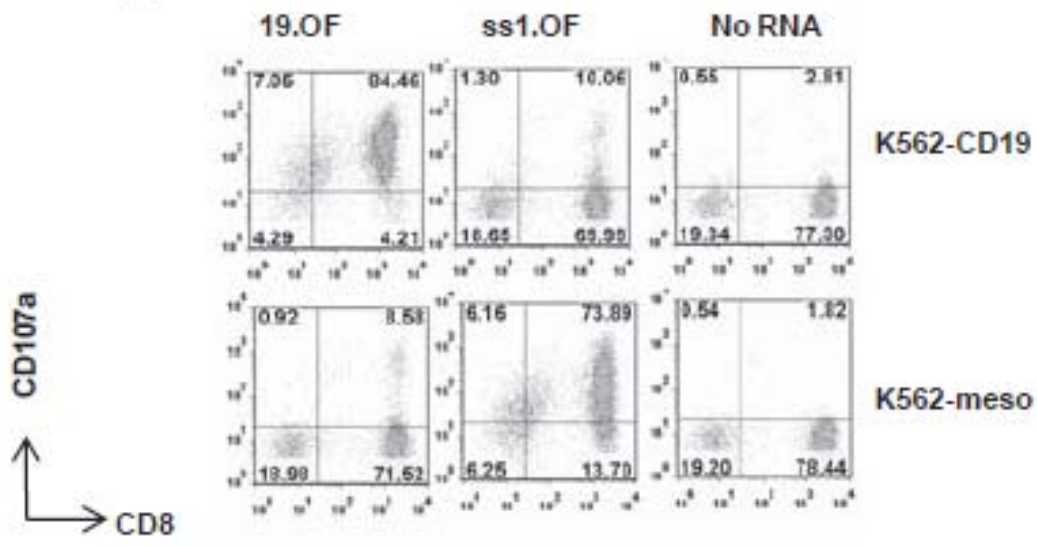
- 3’ repeats of untranslated regions (UTR) from beta globulin
- Longer poly(A) sequences
- 5’ SP163 translational enhancer
- CAP analog or CAP1 capping enzymes
- Codon optimization
- Removal of internal ORFs

**Yangbing Zhao**

# Expression and Function of RNA CAR on CD3/28 Activated T Cells Electroporation on Day 10




Yangbing Zhao

# In Vivo Function of Anti-mesothelin RNA CARs: Multiple Infusions Control Autologous Tumor

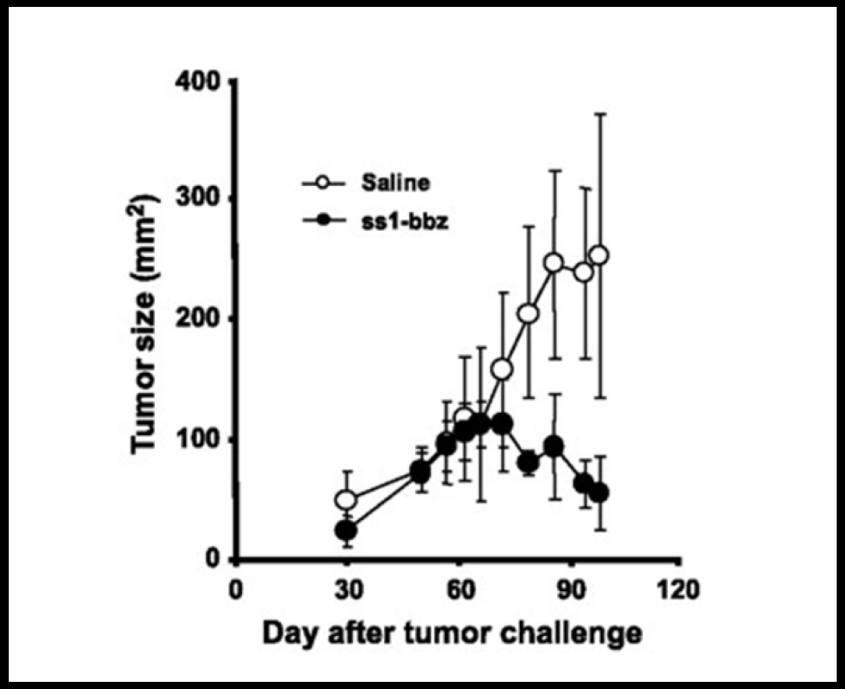
NOD/scid/$\gamma_c^{(-/-)}$

M108
Tumor cells s.c.

66 days

ss1-bbz RNA–electroporated
T cells injected intratumorally

every 4 days




M108: Human mesothelioma cell line

*Zhao Y et al. Cancer Res 2010*

# RNA CARs: Pre-Clinical Summary

- Multiple injections of RNA CAR T cells electroporated with optimized IVT mRNA provide potent anti-tumor effects in mouse tumor models with leukemia (ALL) and mesothelin (mesothlioma and ovarian) tumors, RNA CARs have potent treatment effects using CD19, meso and c-Met CARs.
- While not yet as effective as CARs using integrating viral vector technology, RNA CAR T cells have the potential to accelerate and decrease the cost of early stage CAR trials.
- RNA CARs are promising for 'risky' new CAR designs.
- Dose intensification and dose escalation of RNA CARs is important to achieve results similar to lentiviral CARs

# Phase I Clinical Trial Of Autologous Mesothelin RNA CAR T Cells Administered Intravenously In Patients With Progressive Malignant Pleural Mesothelioma

## Cohort #1

Enrollment

↓

Days -28 to 0
Apheresis and T cell manufacturing

↓

Day 0
CAR T Cell Infusion
Ongoing toxicity and safety assessments

↓

Days 1, 3
Ongoing toxicity and safety assessments

↓

Day 7
CAR T Cell Infusion
Outpatient toxicity and safety assessments

↓

Days 8, 10, 14, 21
Ongoing toxicity and safety assessments

↓

Day 42 and Month 3
Outpatient toxicity and safety

## Cohort #2

Enrollment

↓

Days -28 to 0
Apheresis and T cell manufacturing

↓

Days 0, 2, 4
CAR T Cell Infusions
Ongoing toxicity and safety assessments

↓

Days 7-11
Outpatient toxicity and safety assessments

↓

Days 14, 16, 18
CAR T Cell Infusions
Ongoing toxicity and safety assessments

↓

Day 21
Image-guided biopsy
Leukapheresis

↓

Days 23, 25, 28, 35 and Months 2, 3, 6
Outpatient toxicity and safety assessments

NIH OBA RAC #1010-1072

# Anti-mesothelin mRNA CARs: Status for Mesothelioma

**TCEL: Directed by Yangbing Zhao, MD PhD**

## National Cancer Institute

at the National Institutes of Health

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**Clinical Trials (PDQ®)**

**Autologous Redirected RNA Meso-CIR T Cells**

Basic Trial Information
Trial Description
- Summary
- Further Trial Information
- Eligibility Criteria

Trial Contact Information

**Basic Trial Information**

| Phase | Type | Status | Age | Sponsor | Protocol IDs |
|---|---|---|---|---|---|
| Phase I | Treatment | Active | 18 and over | Other | **UPCC 17510** NCT01355965 |

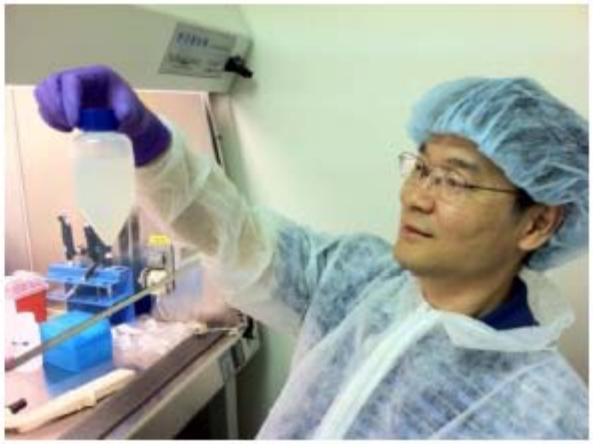
**Trial Description**

**Summary**

To determine the safety and manufacturing feasibility of IV autologous chimeric immune receptor (CIR) T cells transfected with anti-mesothelin messenger RNA (mRNA) expressing a single chain antibody variable fragment linked to the intracellular CD 3 zeta T cell receptor domain and the 4-1BB costimulatory domain.

**Daniel Sterman, MD**
**Andrew Haas, MD**

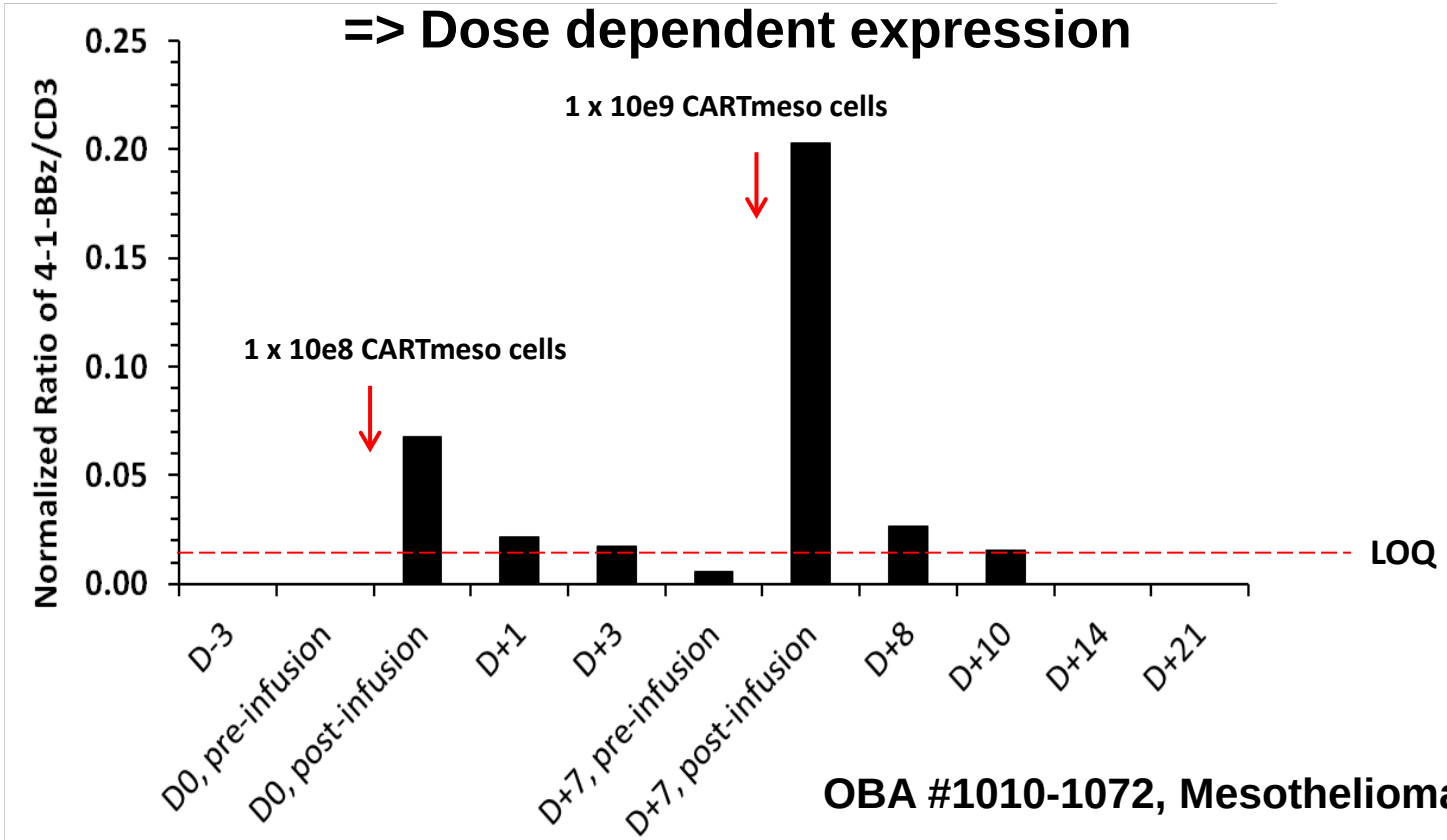
Meso RNA CAR produced for the first cancer patient treatment



First Patient to receive MesoCAR T cells with Dr. Andrew Haas (PI) (9/19/11)

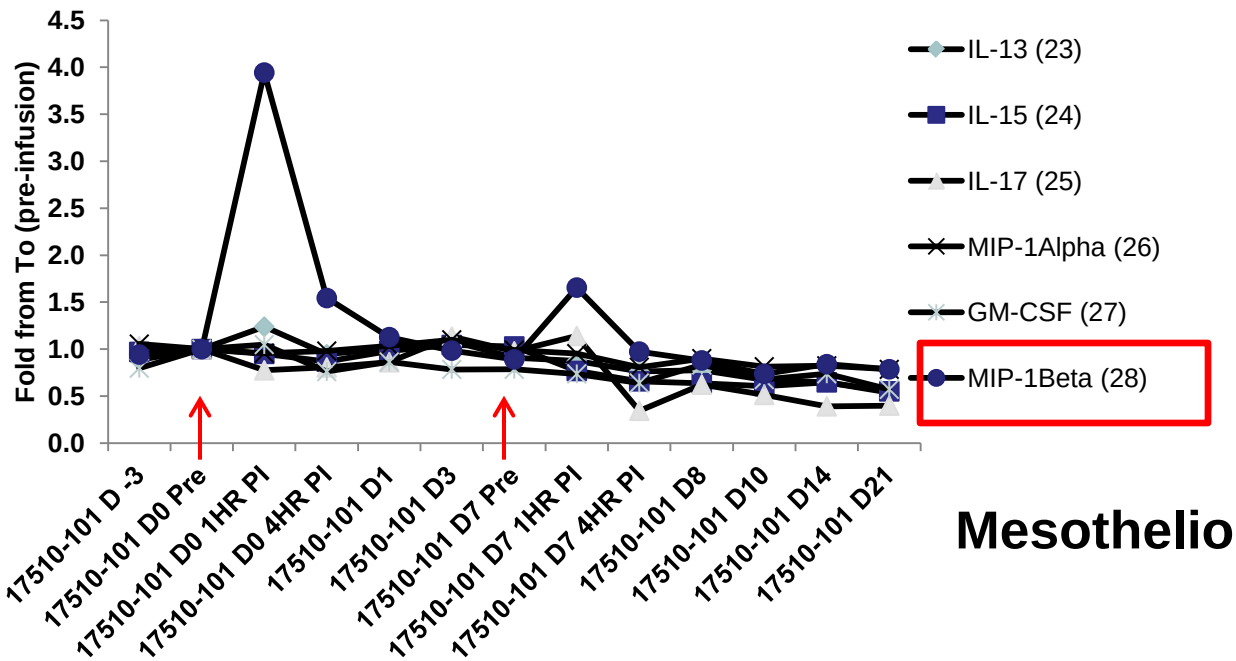
# Measurement of anti-mesothelin RNA CAR persistence by qRT-PCR

- Isolate mRNA from whole blood during treatment and synthesize cDNA
- Establish standard curve from infusion product
- Quantify transcripts for transgene using primers/probe to the transgene
- Normalize to housekeeping gene (input cDNA) and CD3 (% T cells)




OBA #1010-1072, Mesothelioma pt #1.

# Biomarkers following single infusions of anti-mesothelin RNA CARs: Luminex




**Mesothelioma pt #1.**

- **Luminex 30-plex from serum samples**
- **Each sample analyzed in duplicate**
- **Baseline values, pg/ml**
- **No cytokine release syndrome at MABEL dose**

TCSL

**OBA #1010-1072**

PENN UNIVERSITY of PENNSYLVANIA

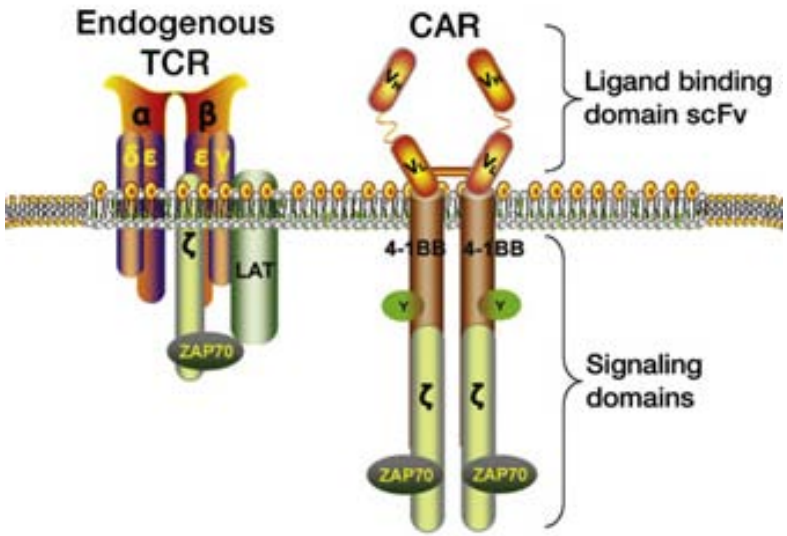
ABRAMSON family CANCER RESEARCH INSTITUTE

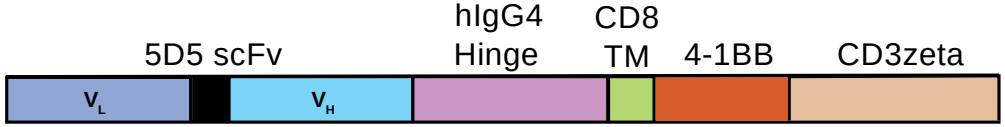
# Investigational product: *RNA c-Met CAR T cell*

1. Ex vivo expansion of autologous T cells using CD3/CD28 costimulation, using PENN's GMP CCVPF on campus

2. Electroporation of mRNA encoding anti-c-Met scFv. Construct based on 5D5 humanized mAb with 41BB and CD3zeta as signaling domains
(IND, pending; sponsor, Penn)

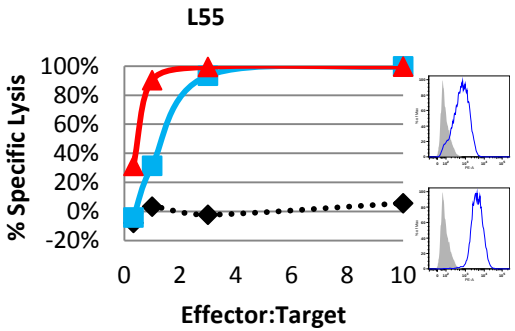
3. RNA CARs allow **phase 0** approach to optimize receptor design and improve safety for first in human targets with <u>extravascular</u> distribution

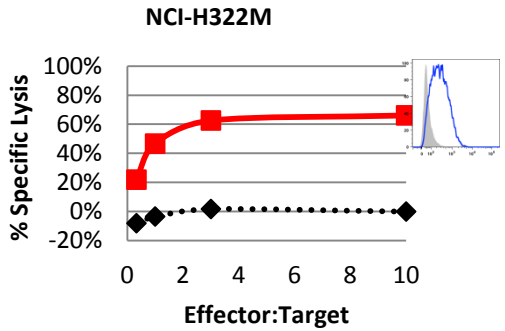


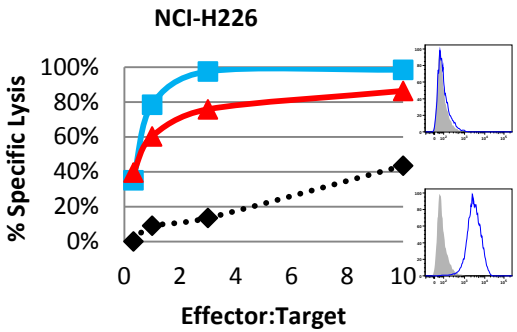



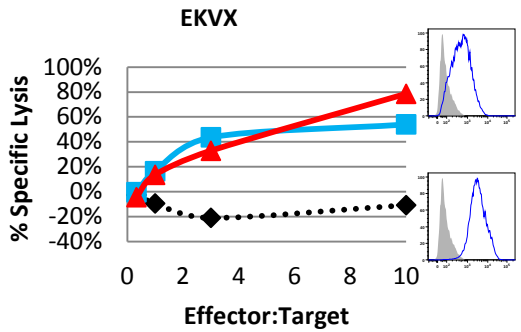
# c-met RNA CARs: lung cancer specific lysis curves

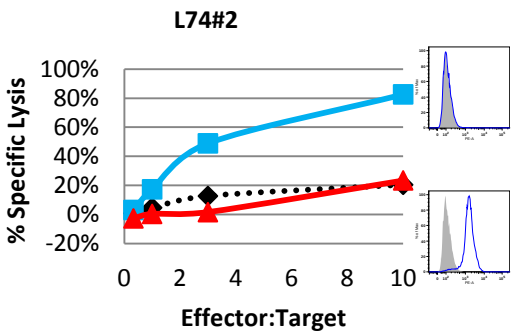
## Lung (NSCLC)

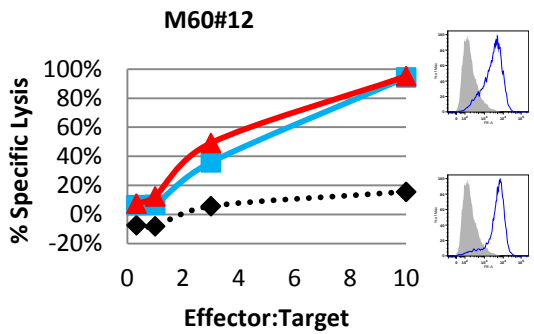


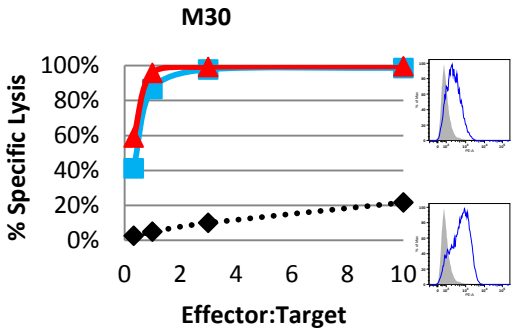



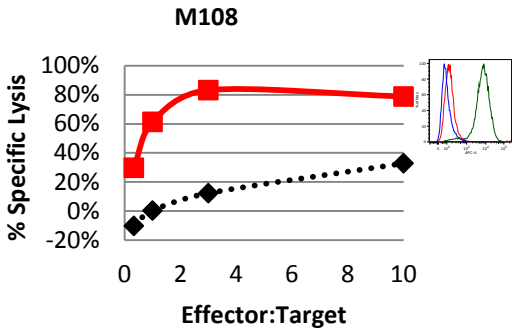




## Mesothelioma




Histograms: Top = Mesothelin
Bottom = cMet
Graphs: **Black** = CD19 CAR
**Red** = SS1 CAR
**Blue** = cMet CAR

**Shannon McGettigan**

# C-MET RNA CAR Experiment: in vivo

**Experimental design**

Establish large vascularized flank xenograft tumors with c-Met+ SKOV3/luc tumors on day 0 in NOD/Scid/$\gamma c^{-/-}$ mice

Treatment beginning at week 6, 7, 9 and 11: 1x10e7 CAR cells injected IT

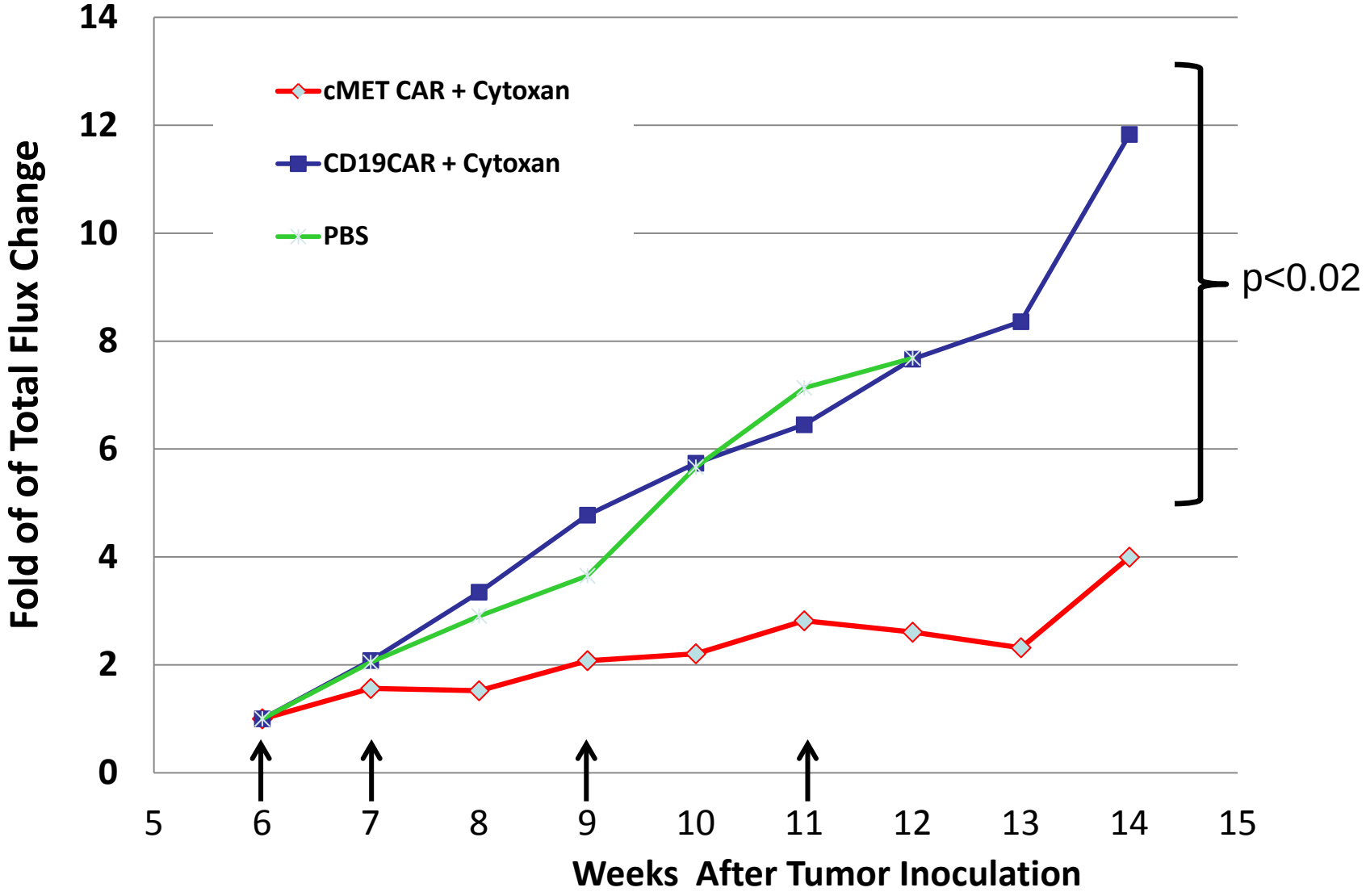
Cytoxan i.p. injection 24 hour before treatment at weeks 7, 9 and 11 to prevent xGVHD

Endpoints: survival and tumor burden (bioluminescence)

| Group | Mice (#) | Tumor Cells s.c. | Treatment i.t. Injection T cells |
|---|---|---|---|
| cMET CAR + Cytoxan | 9 | SKOV3-Luc | cMET CAR + Cytoxan |
| CD19 CAR + Cytoxan | 9 | SKOV3-Luc | CD19 CAR + Cytoxan |
| PBS | 7 | SKOV3-Luc | PBS |

**Shuguang Jiang**
**Yangbing Zhao**

## Multiple injections of c-met RNA CARs control advanced tumors in mice
## Normalized data of group average total flux

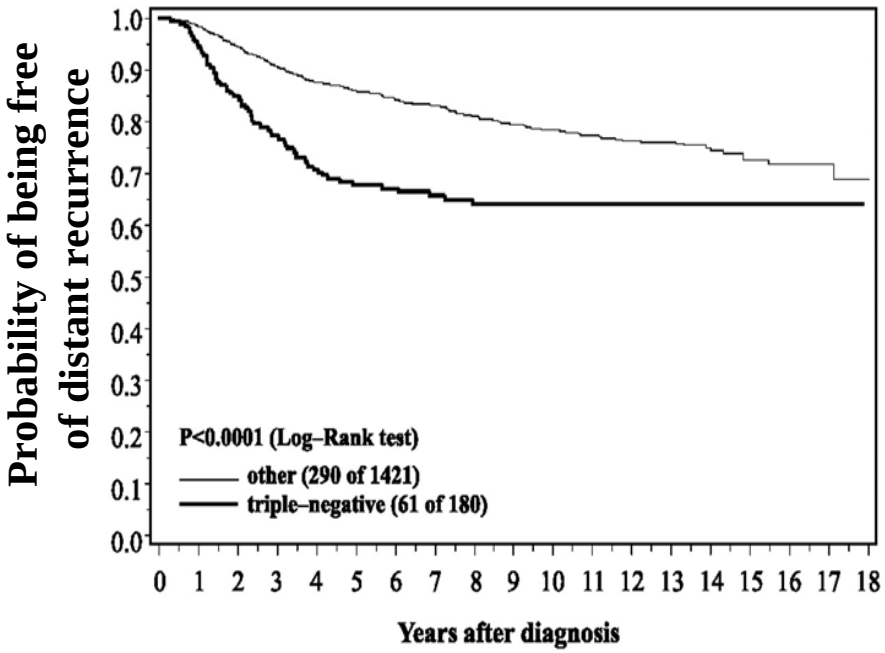


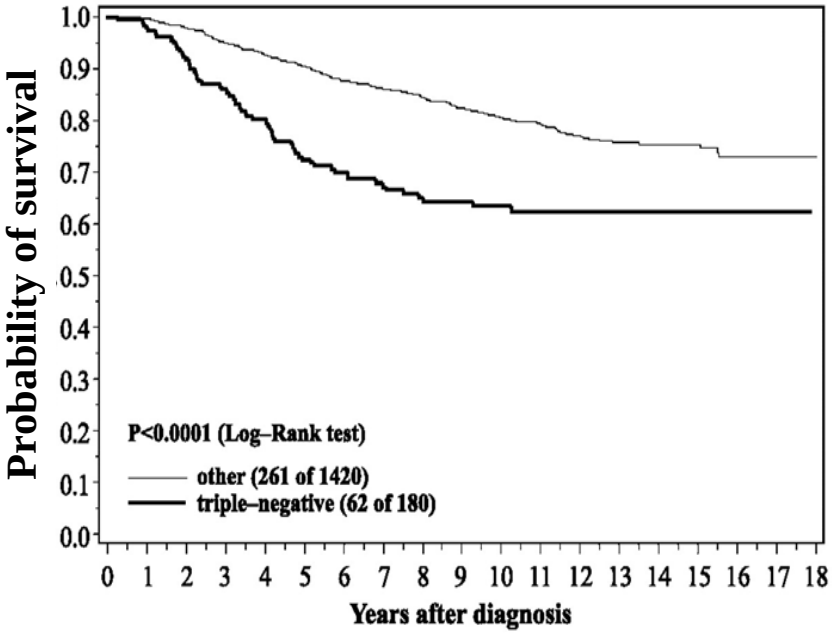
# Outline

- RNA CARs as a platform for pilot evaluation of new CAR targets
- pre-clinical and clinical data with RNA CARs
- **Triple negative breast cancer**
- **Protocol design and safety features to minimize toxicity and maximize scientific integrity**

# Rationale: Patients with TNBC have an inferior prognosis

**Cohort study of 1,600 patients with BC**

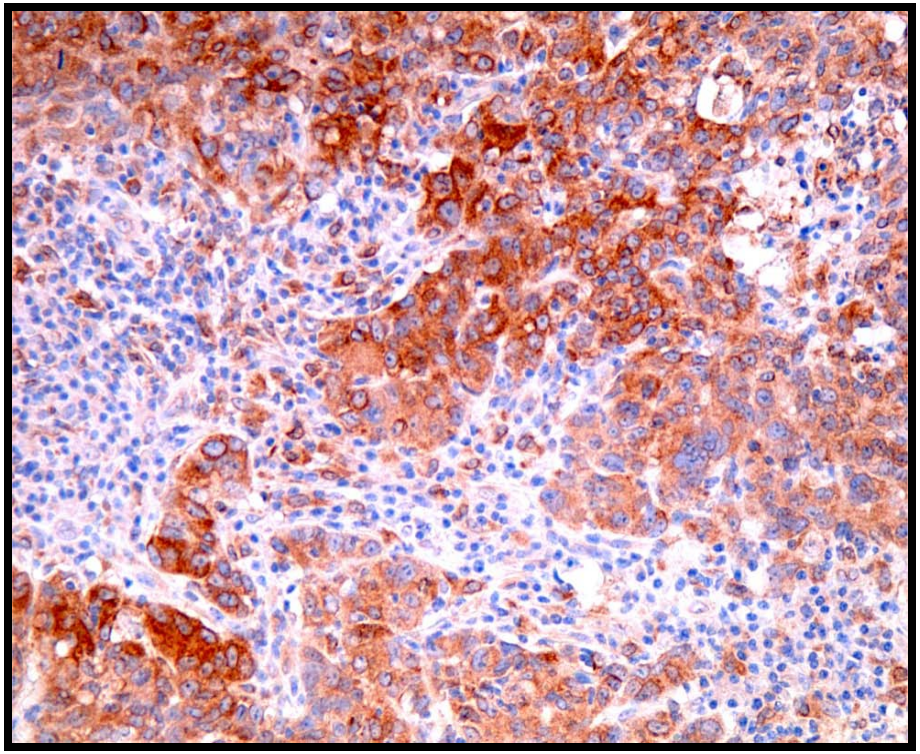



**Dent et al CCR 2007**

# TNBC and c-Met expression

| C-Met expression on tumor cells (%) | TNBC (n=38) | | ER+ BC (n=18) | |
|---|---|---|---|---|
| | n | % | n | % |
| 0 | 11 | 29 | 8 | 36 |
| 3-5 | 3 | 8 | 0 | 0 |
| 10-25 | 9 | 24 | 1 | 5 |
| 30-40 | 5 | 13 | 2 | 9 |
| 50-90 | 10 | 26 | 7 | 32 |



c-Met+ TNBC

* IHC performed on paraffin sections

**39% of TNBC are strongly c-Met+**

# TNBC among African American patients

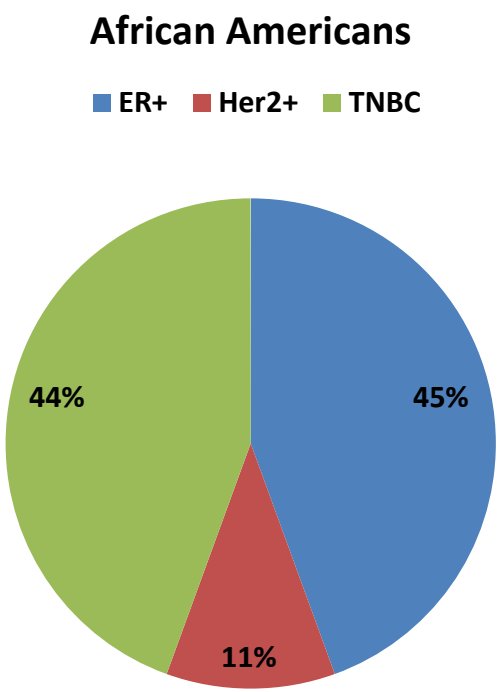
**Prevalence of BC subtypes according to race and menopausal status**

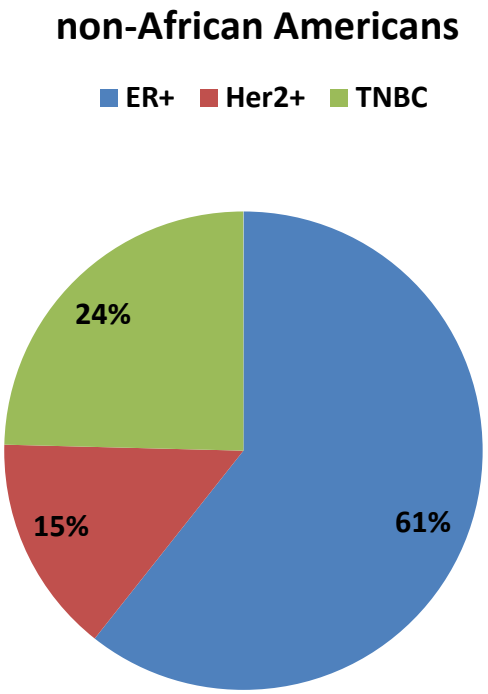
| | | No. (%) | | | |
|---|---|---|---|---|---|
| | | African American* | | Non–African American† | |
| Tumor Status | All Cases | Premenopausal (n = 97) | Postmenopausal (n = 99) | Premenopausal (n = 164) | Postmenopausal (n = 136) |
| Basal-like | 100 | 38 (39) | 14 (14) | 26 (16) | 22 (16) |
| HER2+/ER– | 33 | 9 (9) | 7 (7) | 9 (6) | 8 (6) |
| Luminal A | 255 | 35 (36) | 58 (59) | 83 (51) | 79 (58) |
| Luminal B | 77 | 9 (9) | 16 (16) | 30 (18) | 22 (16) |
| Unclassified | 31 | 6 (6) | 4 (4) | 16 (10) | 5 (4) |

*$P<.001$, $\chi^2$ test for basal-like vs other tumor types in premenopausal vs postmenopausal African American women.
†$P = .94$, $\chi^2$ test for basal-like vs other tumor types in premenopausal vs postmenopausal non–African American women.

**Carey LA NEJM 2006**

# Prevalence of TNBC in Penn cohort




**Julia Tchou, MD, PhD**
**Paul Zhang, MD**

**N=89**

# Protocol 1127

Pilot Clinical Trial of Autologous Met Redirected T cells Administered Intratumorally and Intravenously in Patients with Operable Triple Negative Breast Cancer

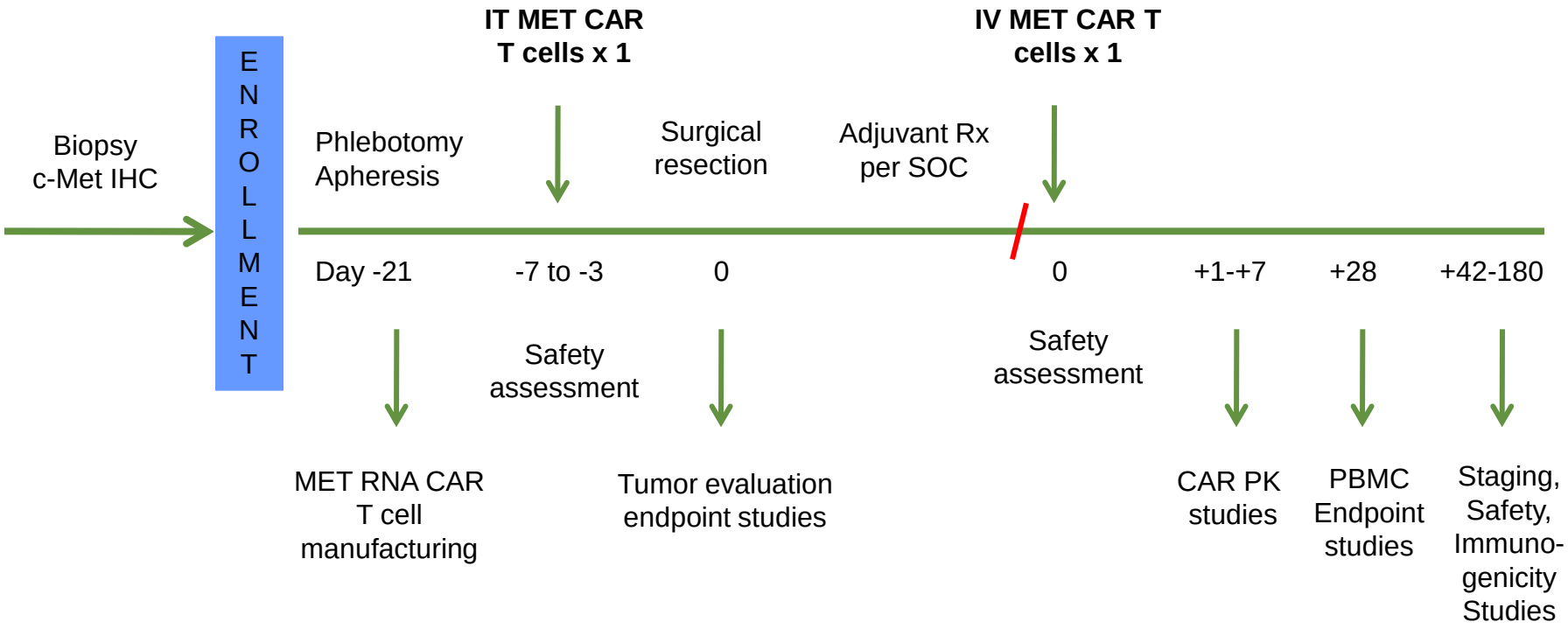
# Objectives of the Clinical Trial

- **Primary**
  - Determine safety of treating patients with newly diagnosed triple negative breast cancer with c-Met RNA CAR autologous T cells by IT and IV route of administration
- **Secondary**
  - Assess evidence of c-Met directed anti-tumor responses in resected tumor tissue
  - Determine immunogenicity of c-Met RNA CAR T cell product, including bioactivity, persistence, and induction of adaptive immunity

# Eligibility

- **Inclusion**
  - Adult patients newly diagnosed with operable, non-metastatic breast cancer
  - ER/PR-negative, Her2/neu-negative tumor which clinically measures <5cm
  - ECOG performance 0 or 1 and life expectancy >6 months
  - Adequate hematologic, renal, hepatic functions
- **Exclusion**
  - Prior therapy for breast cancer
  - History of autoimmune diseases, HIV, HBV, HCV
  - Pregnancy/lactation
  - Use of anticoagulants, immunosuppressive drugs, glucocorticoids, growth factors, or other experimental therapy
  - Significant psychiatric disorders
  - History of alcohol abuse or illicit drug use within 12 months

# Schema of the clinical protocol




UPCC# 13111
IRB# 813858
RAC# 1110-1127

# Dose cohorts

- **Intratumoral T cells (pre-op)**
  - Three dose levels (3x10e7, 1x10e8, 3x10e8 T cells)
  - 3+3 design; dose escalation if <1 DLT among 3 patients (or <2 DLT among 6 patients)
  - DLT-IT defined as treatment-related grade 3 or higher toxicity, or grade 2 or higher autoimmune or allergic reaction
  - Total of 9-18 patients, depending on toxicity
- **Intravenous T cells (after adjuvant Rx)**
  - Two doses (1x10e8/$m^2$ for cohort 1; 1x10e9/$m^2$ for cohorts 2 and 3)
  - Bayesian design; termination considered if 2 DLT in first 6 patients; 3 DLTs in the first 10 patients, etc.
  - DLT-IV uses same criteria as DLT-IT

# Clinical Trial Endpoints

- **Safety**
  - CTCAE v4.0 grading of toxicity from first IT dose to 6 months after completion of adjuvant therapy and a single IV dose
- **Met directed responses in resected tumor: mechanism of action**
  - Published pathological criteria for efficacy of pre-operative therapy in breast cancer (Symmans et al JCO 2007; Hatzis et al JAMA, 2011)
- **Pharmacology and PK of c-Met RNA CAR T cells**
  - Laboratory assessment of *in vivo* persistence and bioactivity
  - c-Met tumor expression after therapy
  - Induction of adaptive immunity after therapy
  - Anti-CAR antibody immune responses

**Michael Kalos**
**Paul Zhang**
**Robert Vonderheide**

# Risk Management Strategies

**T cell infusional toxicities**

- previous experience with CAR infusions in more than 40 patients: <grade 2 acute infusion reactions that resolve by day 2
- no IL-2 chase

**On target c-met toxicity**

- met is an extravascular antigen: on target acute CAR reactivity not expected based on target distribution
- antibody directed therapy in phase III trials: good safety record
- toxicity will be self-limited due to RNA expression

**Tumor distension / inflammation**

# Dose Rationale

**Goal is to test two doses: one below MABEL and one at the estimated MABEL. The approach is to first test a single IT injection, and if safe, then a single IV injection.**

**Flat dosing is most appropriate for IT injection, and BSA adjusted dosing for IV dosing of RNA CAR T cells.**

**Previous experience with CARs targeting extravascular antigens have <u>not</u> produced cytokine release syndromes:**

- **FRalpha (Kershaw): up to 1.6e11 retroviral CARs well tolerated**
- **CAIX (Lamers): up to 1.2e10 retroviral CARs with onset of biliary tract toxicity on day 5**
- **GD2 (Pule): 2e8/m2 retroviral CARs well tolerated**
- **Mesothelin (Haas/Sterman): 1e8 and 1e9 RNA CARs well tolerated**

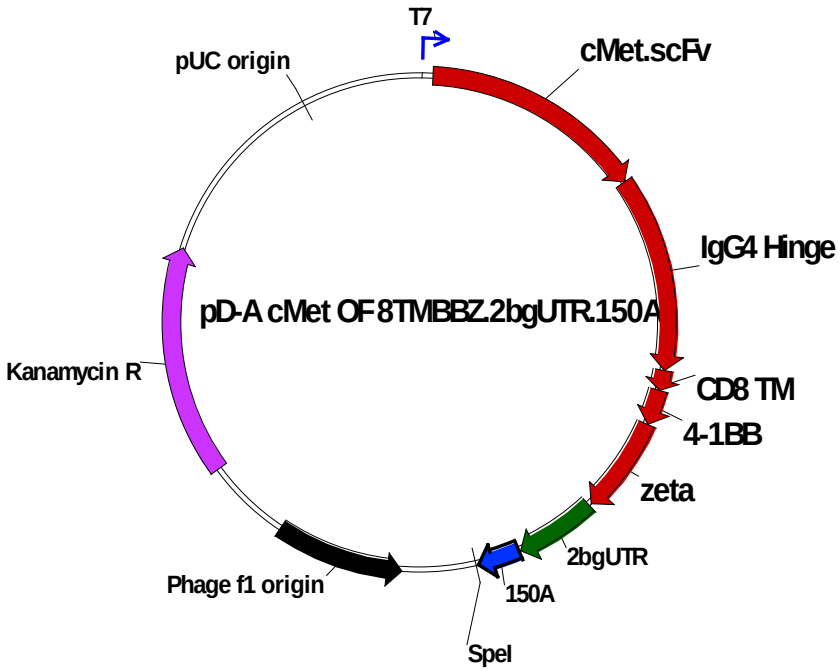
# Dose Rationale -2

**Given the previous successful dosing of patients with CARs targeting <u>extravascular</u> antigens, our proposed starting dose is 1.6e8 CARs IV for an average woman. This is a ~30 fold safety reduction compared to previous CAR infusions that have been well tolerated.**

**In addition to dose reduction, protocol #1127 incorporates two additional features that are expected to reduce the potential for toxicity:**

**1) "self limited" RNA CARs w <5 days expression in vivo**
**2) omission of high dose IL-2.**

# Clinical c-Met GMP IVT mRNA Production




*E Coli* Transformation
*SOP 1188-01*

Individual clone tested and selected for expansion to generate bacterial MCB

Production of Master Cell Bank (MCB)
*SOP 1189-02*
*Form 1189-02-A (Record of culture & storage)*

Safety, purity, and identity testing

Release Testing of MCB
*SOP 1190-01*
*Form 1190-01-A (Record of testing)*
*Form 1190-01-B (C of A)*

Large -scale Plasmid DNA Production & Purification
*SOP 1191-01*

DNA Linearization
*SOP 1192-01*

Safety, purity, and identity testing

Release Testing of Purified, Linearized DNA
*SOP 1193*
*Form 1193-10-A (Record of testing)*

**CVPF**

Optimization and validation of T cell electroporation process

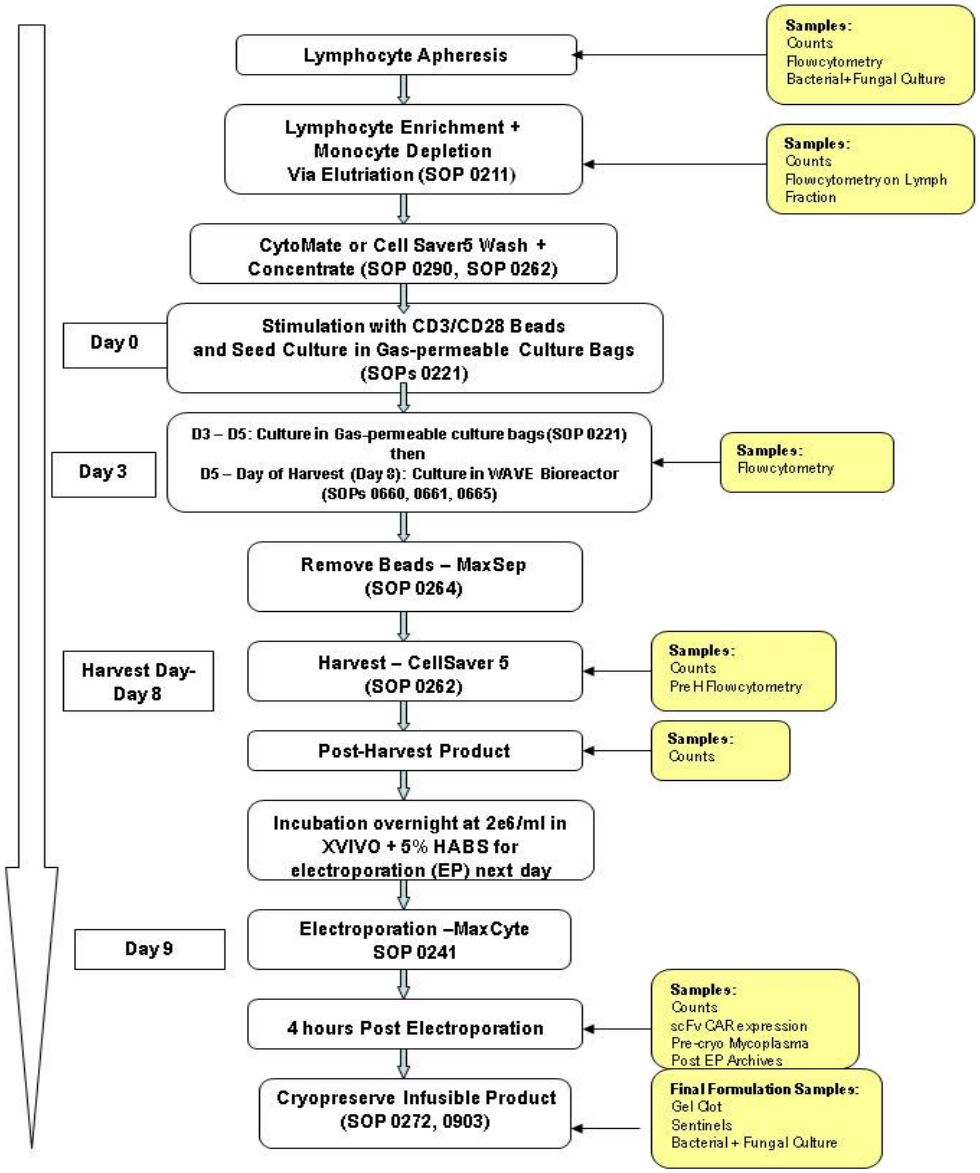
Production of mRNA (5'cap and 3'poly A)
*SOP 0409-01*

**TCEF**

**Anne Chew, PhD**
**Yangbing Zhao, MD PhD**

# Clinical c-Met RNA CAR T cell Production

More than 40 patients treated with this manufacturing process with no SAEs on related CAR T cell trials.




**Bruce Levine, PhD**

# Questions?

Thank you for your time and effort