Phase I study of autologous T cells bearing fully-humanized chimeric antigen receptors targeting mesothelin in mesothelin-expressing cancers.

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No conflict of interest

Rationale for anti-mesothelin CARs

- **Mesothelin is an attractive target for CAR-based therapies given its known role as a tumor associated antigen and expression on pancreatic, ovarian cancer and mesothelioma cells[1,2,3]**

- **GPI anchored membrane protein (40 kDa) that can be shed from cells.**
- **Biological function remains unclear but has been shown to bind to CA-125 (MUC16) and may have a role in cellular adhesion, tumor invasion and metastasis.**
- **Normal expression is on mesothelial cells lining pleura, peritoneum, and pericardium.**
- **May have low expression on some other tissues, but anti-mesothelin antibodies or antibody-toxin conjugates have not shown much toxicity.**

[1]Chang K, et al 1992, [2]Arani P, et al 2001, [3]Hassan R, et al 2004.

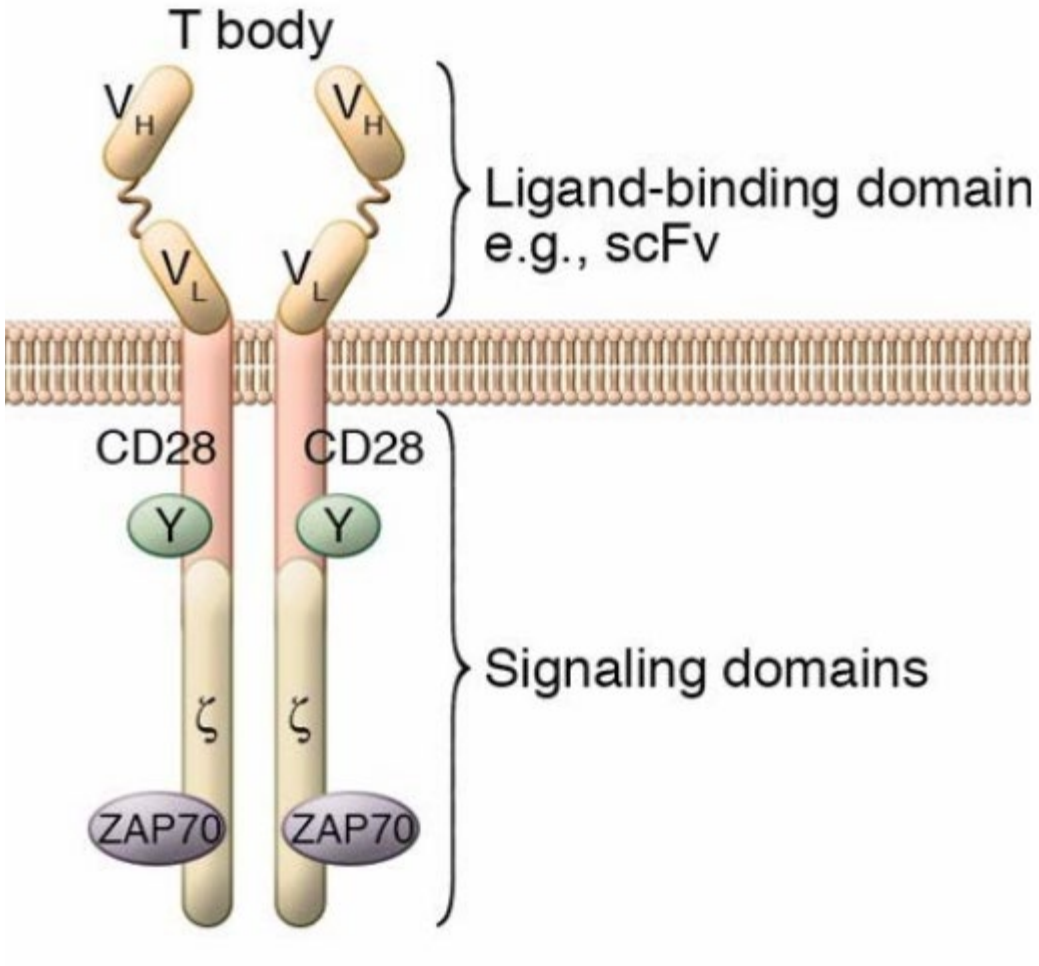
Rationale of M5 anti-mesothelin CAR

- **Prior studies of mesothelin-directed CAR T cells using a murine SS1 scFv delivered via mRNA transduced T cells or with lentiviral transduced T cells have been completed and demonstrated safety but limited efficacy[1,2]**
- **SS1 CARs in the blood peaked at Day 14 and were essentially gone by 28 days, perhaps due to removal by the host immune system.**
- **To improve these results, new CARs were created from a human phage library and screened with the goal of superior in vitro and in vivo activity in functional assays**

[1]Beatty GL, et al 2018, [2]Haas AR, et al 2019

M5 anti-mesothelin CAR

- The “M5 CAR” was selected. It binds a different epitope than the SS1 scFV.
- CAR consists the M5 single-chain variable fragment (scFv)
- The costimulatory CD137 (4-1BB) domain enhances T-cell mediated responses
- The intracellular T-cell receptor CD3-zeta chain signaling domain induces T-cell activation



M5 anti-mesothelin CAR Clinical Trials

UPCC 02916, Basket Study- Phase 1 dose escalation trial targeting patients with mesothelin-expressing refractory malignant mesothelioma, lung cancer, and ovarian.

Cohort 1 (3 patients)- IV injection of 1-3 x $10^7/m^2$ M5 CARTs
Cohort 2 (3 patients)- IV injection of 1-3 x $10^7/m^2$ M5 CARTs after cyclophosphamide pretreatment

Cohort 3 (3 patients)- IV injection of 1-3 x $10^8/m^2$ M5 CARTs
Cohort 4 (3 patients)- IV injection of 1-3 x $10^8/m^2$ M5 CARTs after cyclophosphamide pretreatment

Cohort 6 (3 patients)- Three repeated IV injection of 1-3 x $10^7/m^2$ M5 CARTs 21 days apart with cyclophosphamide pretreatment before 1st infusion

Inclusion & Exclusion Criteria

INCLUSION	EXCLUSION
i. Metastatic or recurrent lung adenocarcinoma. ii. Persistent or recurrent serous epithelial ovarian cancer iii. Malignant pleural and peritoneal mesothelioma (histologically confirmed epithelial)	HIV, HCV, HBV, HTLV I/II, or other active infections
Failure of at least one prior standard of care chemotherapy	Active autoimmune disease requiring immunosuppressive therapy
ECOG PS 0-1 Patients > 18 years of age	Clinically significant pericardial effusion; CHF (NY Heart Association Grade II-IV)
Adequate hematologic, renal, and hepatic function	Planned concurrent treatment with systemic high dose corticosteroids
Life expectancy > 3 months	Anticipated need for systemic chemotherapy within 2 weeks of aphaeresis and infusion
Understands experimental nature of therapy	Other active malignancy

Primary and Secondary Objectives

Primary objectives

- **Determine the safety and feasibility of intravenous administration and local delivery of lentiviral transduced huCART-meso cells, with and without lymphodepleting chemotherapy in the target population.**

Secondary Objectives

- **<u>Clinical objectives</u>:**
- **1. Assess the clinical anti-tumor effect by standard criteria (RECIST)**
- **2. Assess progression-free survival (PFS) and overall survival (OS)**

- **<u>Correlative objectives:</u>**
- **1. Evaluate huCART-meso cells engraftment and persistence in peripheral blood.**
- **2. Determine the bioactivity of huCART-meso cells in peripheral blood.**
- **3. Where tumor material can be obtained: Measure trafficking of CART-meso T cells**

Patient Characteristics: UPCC 02916, Basket Study

Count	Subject ID	Disease Type	Cohort	Sex (F/M)	Age at Consent
2	02916-02	M	Cohort 1	M	63
6	02916-06	O	Cohort 1	F	54
7	02916-07	O	Cohort 1	F	60
1	02916-01	O	Cohort 2	F	52
8	02916-08	M	Cohort 2	F	57
12	02916-12	M	Cohort 2	F	76
9	02916-09	M	Cohort 3	F	74
13	02916-13	O	Cohort 3	F	61

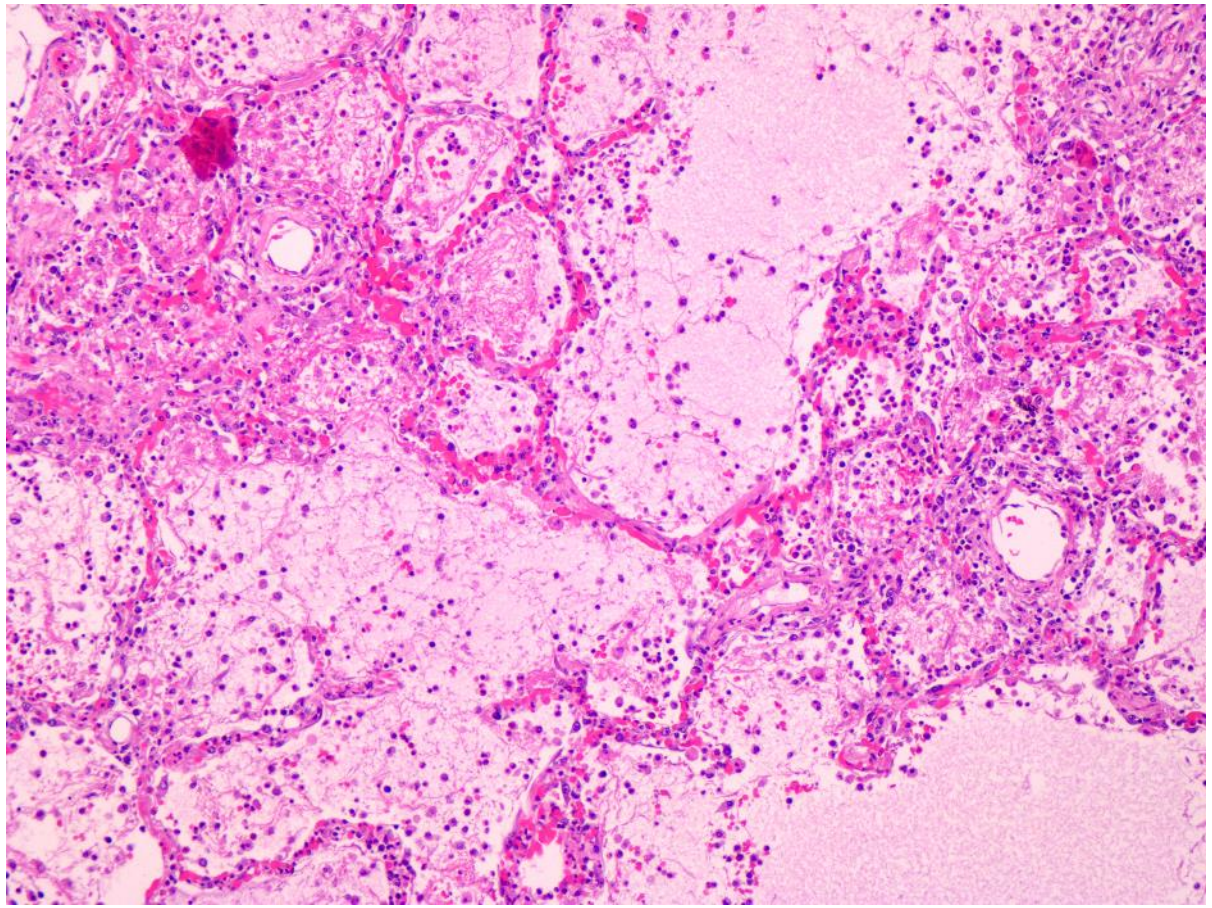
Abbreviations
Cohort 1
Cohort 2
Cohort 3
O : Recurrent Serous Ovarian Carcinoma
M : Malignant Epithelial Mesothelioma
L : Lung Adenocarcinoma

Primary Objective: Safety. UPCC 02916: Related SAEs

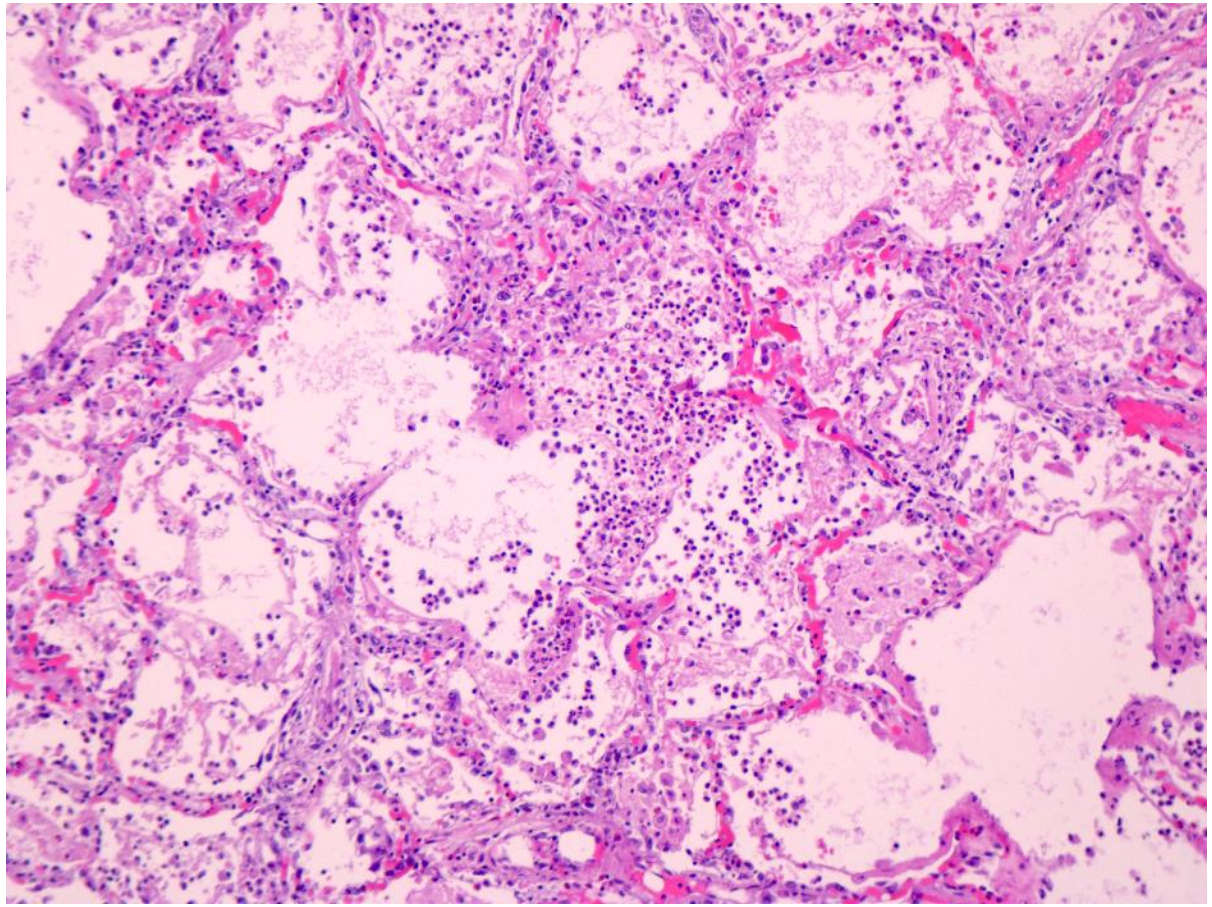
Subject ID#	CTCAE Category	Toxicity	Grade	Attribution	Start Date	Stop Date
02916-01	Vascular disorders	Hypotension	3	Possibly	8/15/2017	8/23/2017
	Metabolism and nutrition disorders	Hyponatremia	3	Possibly	8/17/2017	8/23/2017
	Blood and lymphatic system disorders	Febrile neutropenia	3	Possibly	8/19/2017	8/20/2017
02916-06	General disorders and administration site conditions	Fatigue	3	Possibly	7/8/2017	Ongoing at Study Discontinuation
	General disorders and administration site conditions	Edema limbs	2	Possibly	8/3/2017	Ongoing at Study Discontinuation
02916-09	Respiratory, thoracic and mediastinal disorders	Dyspnea	3	Probably	11/29/2017	12/3/2017
	Immune system disorders	Cytokine release syndrome	4	Probably	11/30/2017	12/3/2017
	Vascular disorders	Hypotension	3	Probably	11/30/2017	12/3/2017
	Respiratory, thoracic and mediastinal disorders	Hypoxia	4	Probably	11/30/2017	12/1/2017
	Respiratory, thoracic and mediastinal disorders	Respiratory failure	5	Probably	12/1/2017	12/3/2017
02916-13	Immune system disorders	Cytokine release syndrome	3	Definitely	11/20/2017	11/27/2017
	Vascular disorders	Hypotension	3	Definitely	11/21/2017	11/24/2017
02916-17	Immune system disorders	Cytokine release syndrome	3	Possibly	7/5/2019	7/6/2019
	General disorders and administration site conditions	Infusion related reaction	2	Definitely	7/23/2019	7/23/2019
02916-41	Immune system disorders	Cytokine release syndrome	3	Definitely	7/3/2020	7/7/2020
	Respiratory, thoracic and mediastinal disorders	Hypoxia	3	Definitely	7/4/2020	7/11/2020
	Respiratory, thoracic and mediastinal disorders	Pleuritic pain	2	Definitely	7/7/2020	7/10/2020

Cohorts 1 and 2 were well tolerated without dose limiting toxicity.
However, both patients in Cohort 3 (1-3 x $10^8/m^2$) had fevers and respiratory distress shortly after their infusions requiring ICU care. Patient 9 with mesothelioma died.

Lung tissue characterized by extensive polymorphous inflammatory infiltrates



H&E (100x)



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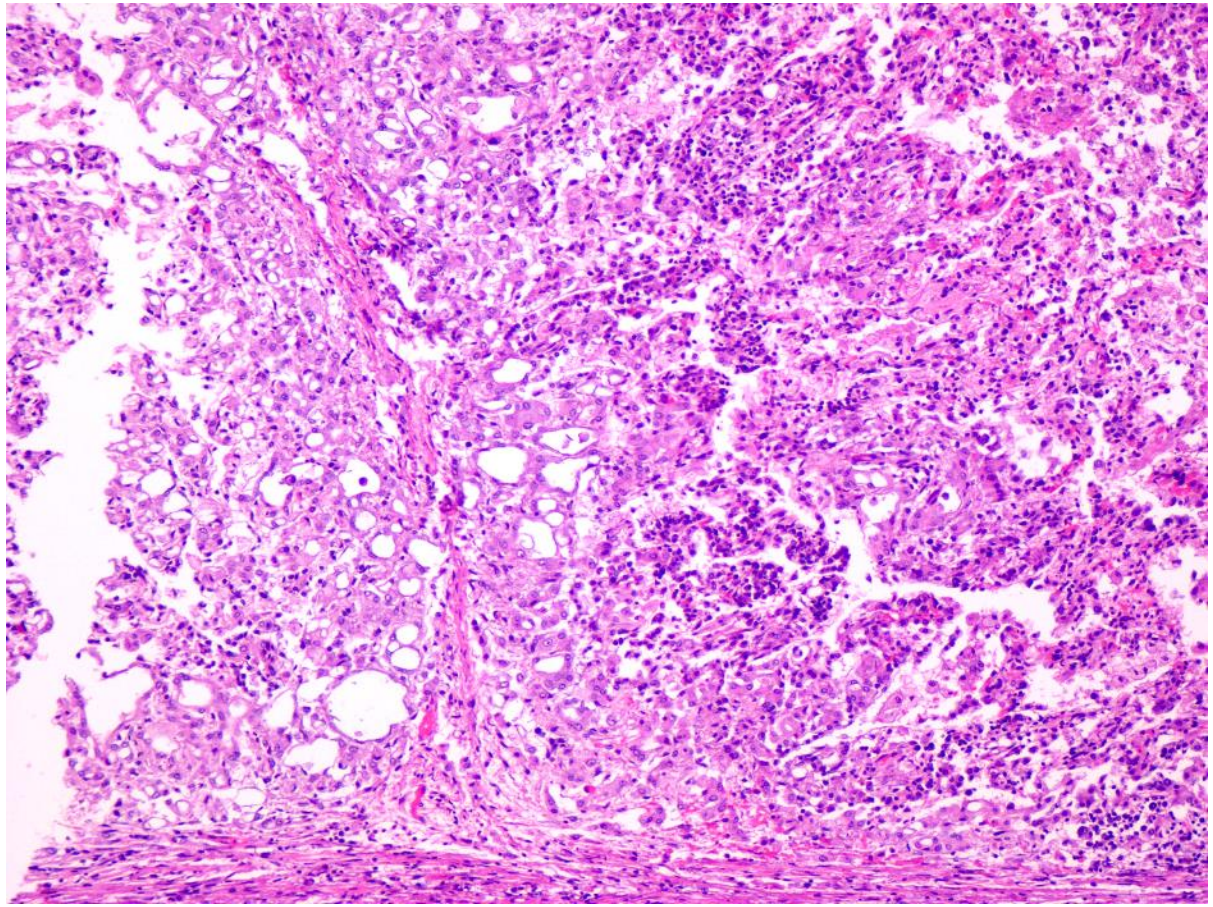
Metastatic deposits of mesothelioma seen in lung with surrounding lymphocytes



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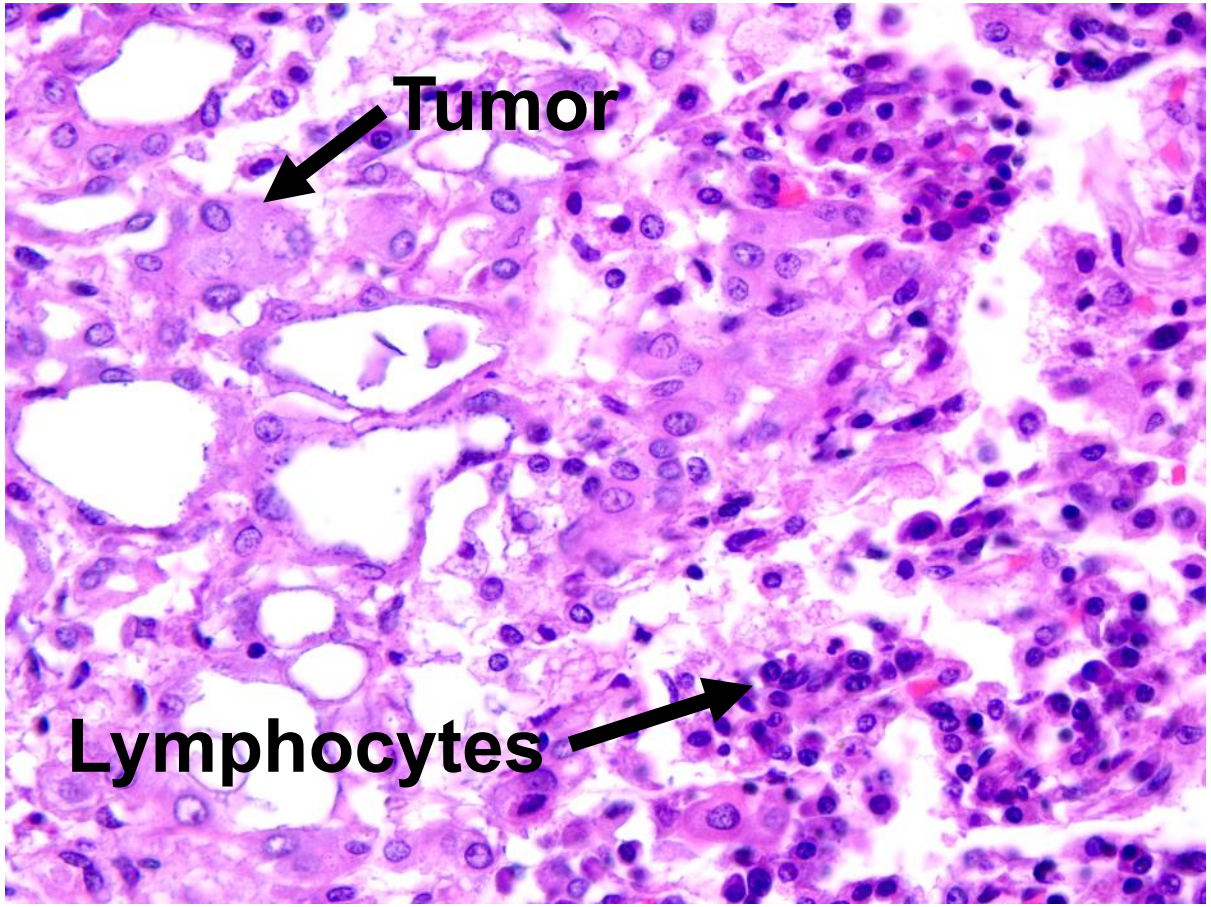

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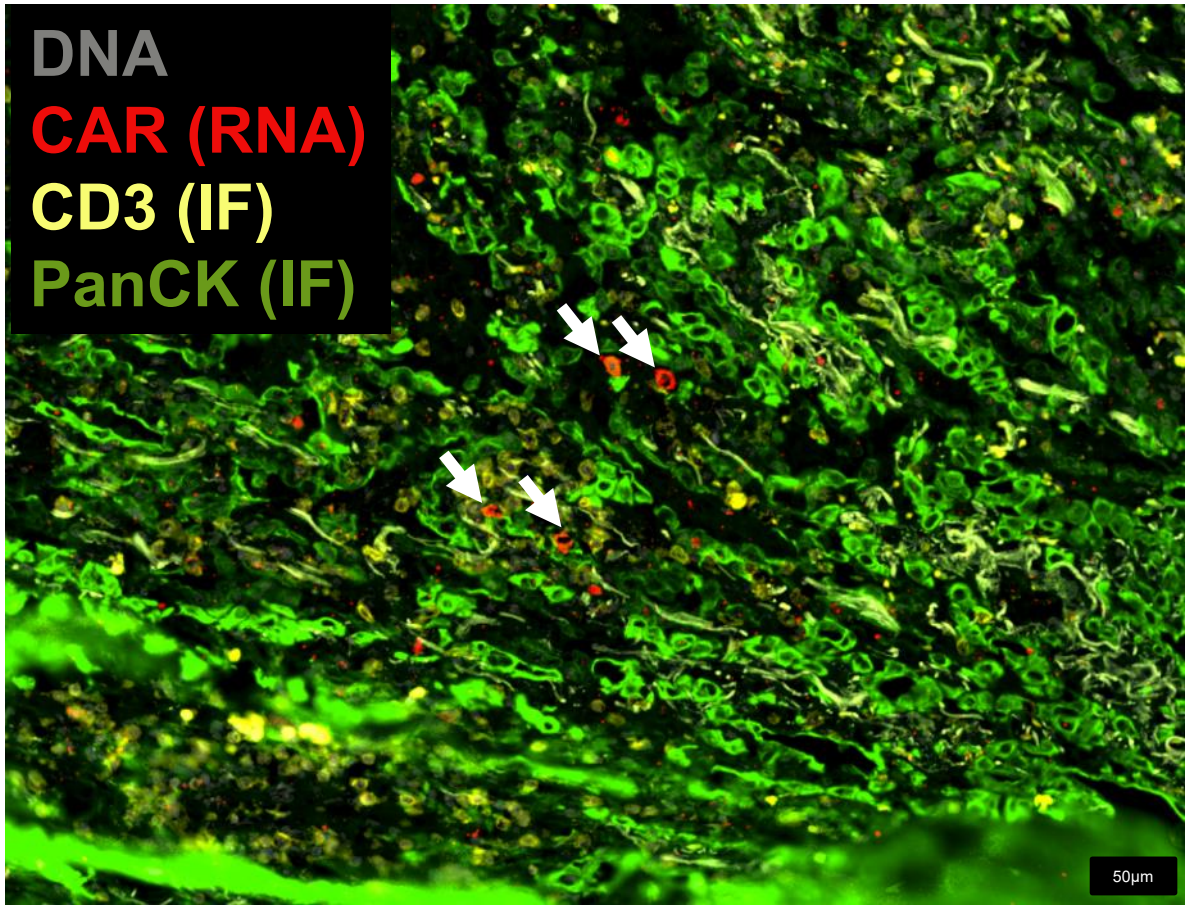
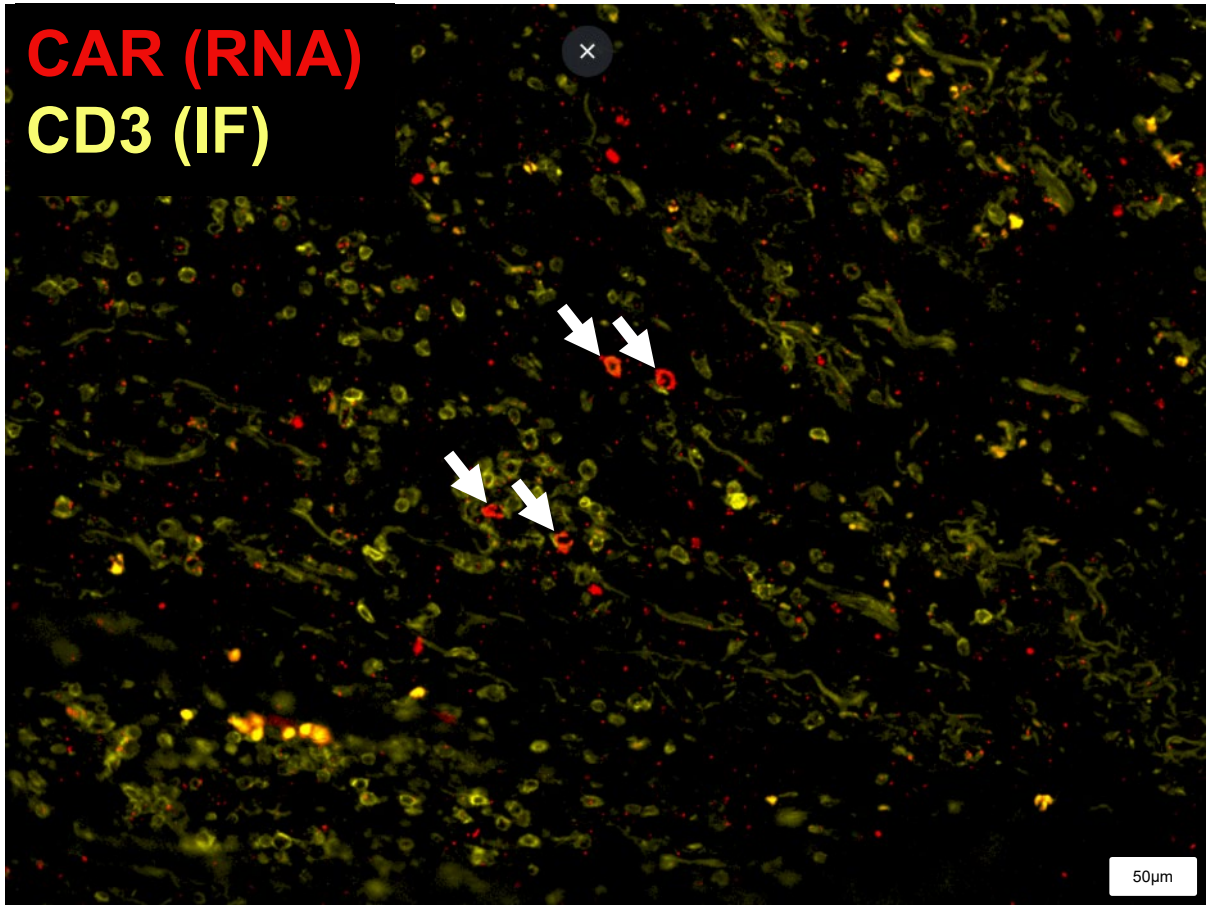
Large numbers of T cells and rare CAR T cells accumulate at the mesothelioma tumor edge



IF and RNAScope, 4 channels

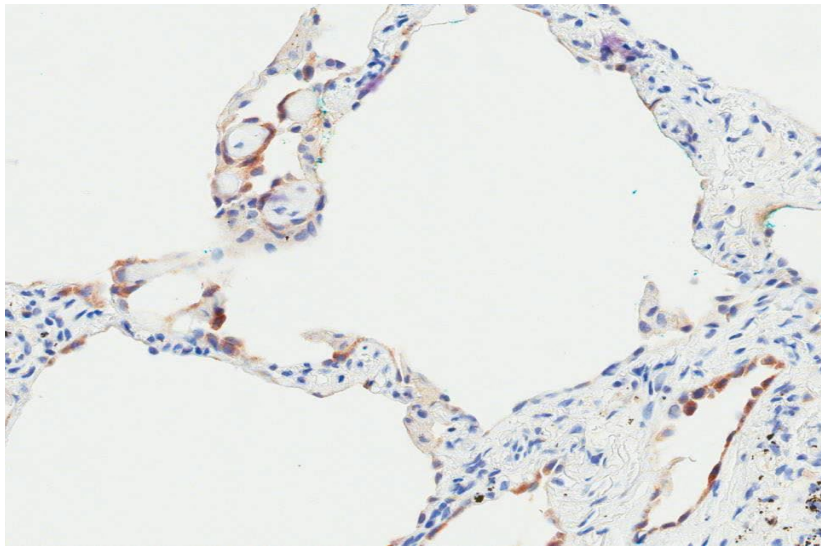


IF and RNAScope, 2 channels

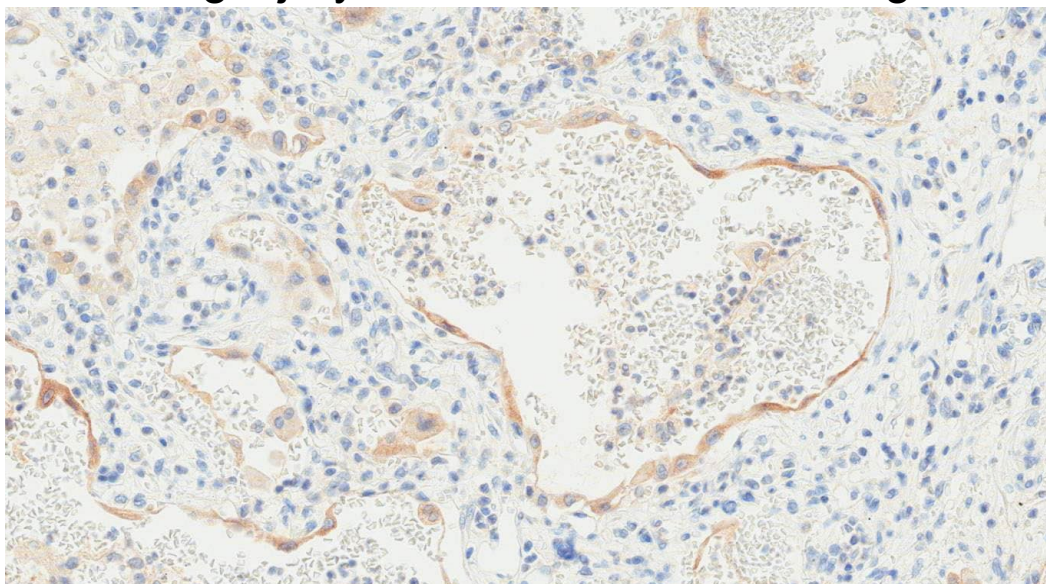
Cause of the Toxicity

- **Subsequent analyses showed that while mesothelin is normally not expressed on lung cells, there can be aberrant expression in areas of active inflammation and fibrosis.**

Interstitial lung disease



Acute lung injury on chronic interstitial lung disease



- **A final cause for the observed toxicity was not determined, however, our best hypothesis was that the highly active M5 CARTs given at high doses attacked lung epithelial cells that were expressing low levels of mesothelin- resulting in an off-tumor, on-target attack.**

Mitigating Toxicity

- Closed 3 x $10^8/m^2$ dose level and continue with 3 x $10^7/m^2$ dose level with repeat dosing to improve exposure/persistence of CART cells
- Require in-patient monitoring for 24 – 48 h
- Explore local infusion strategies to avoid first pass through lung and increase tumor exposure
  - Intrapleural, Intraperitoneal, Intra-arterial (hepatic, pancreatic) using Trisalus SureFire device
- Exclude patients with:
  - Radiographic evidence of extensive lung disease burden:
    - greater than lobar lymphangitic pulmonary involvement
    - greater than lobar bronchial wall thickening suggestive of peribronchial lymphatic disease extension
    - evidence of extensive bilateral parenchymal metastatic burden
  - Radiographic and/or clinical evidence of underlying interstitial lung disease of radiation pneumonitis
- Increase checkpoint washout to 4 months

UPCC 02916, Basket Study: new cohort

A new Cohort 6 (6 patients) was added with multiple infusions

First dose: IV injection of 1-3 x $10^7/m^2$ M5 CARTs after cyclophosphamide pretreatment , second dose on Day 21 (without CTX) and third dose on Day 42 (without Cytoxan)

Count	Subject ID	Disease Type	Cohort	Sex (F/M)	Age at Consent
17	02916-24	L	Cohort 6	F	57
24	02916-24	O	Cohort 6	F	62
30	02916-30	O	Cohort 6	F	62
32	02916-32	O	Cohort 6	F	51
33	02916-33	O	Cohort 6	F	59
41	02916-41	O	Cohort 6	F	59

Abbreviations
Cohort 6
O : Recurrent Serous Ovarian Carcinoma
M : Malignant Epithelial Mesothelioma
L : Lung Adenocarcinoma

No serious adverse events were noted in this new cohort!

UPCC 02916: Clinical Response Data

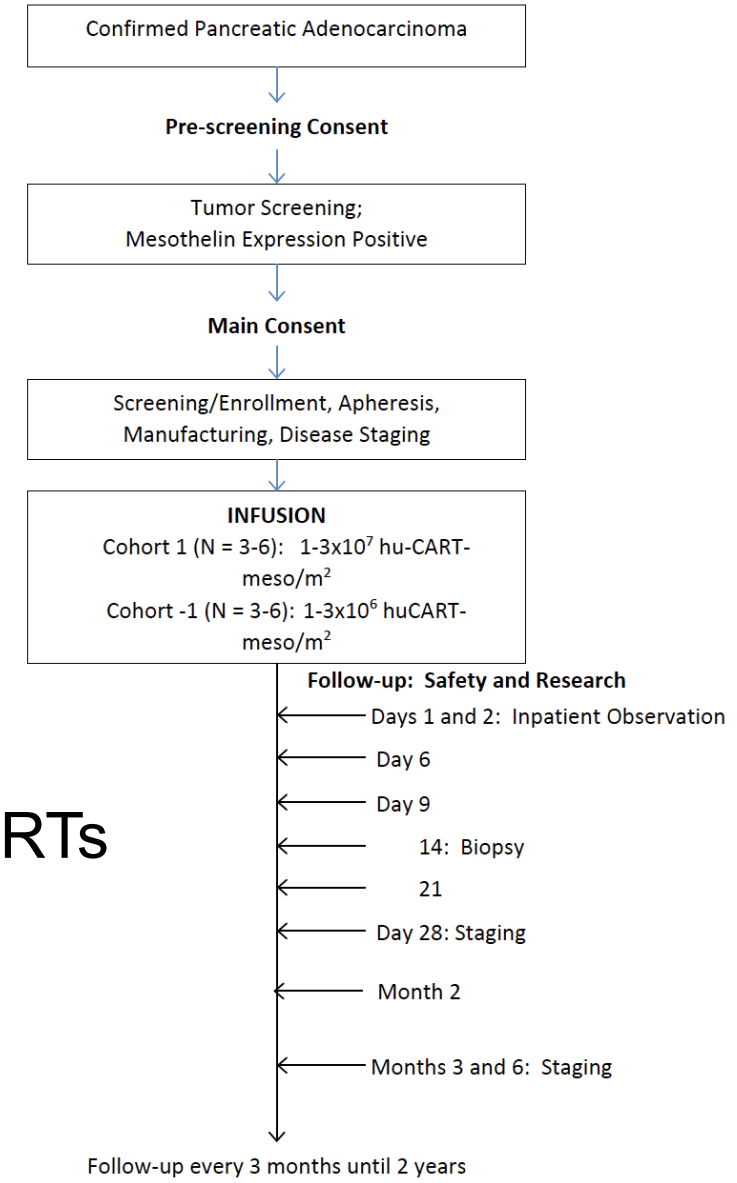
Subject Count	Cohort	Subject ID	Overall Tumor Response				
			Day 28	Day 14 (after 2nd infusion)	Month 2	Month 3	Month 6
1	1	02916-02	PD	NR	PD	Off-study	Off-study
2	1	02916-06	PD	NR	NR	Off-study	Off-study
3	1	02916-07	SD	NR	NR	PD	Off-study
4	2	02916-01	SD	NR	NR	Off-study	Off-study
5	2	02916-08	SD	NR	NR	Off-study	Off-study
6	2	02916-12	SD	NR	SD	SD	PD
7	3	02916-09	Off-study	NR	NR	Off-study	Off-study
8	3	02916-13	SD	NR	NR	PD	Off-study
9	6	02916-17	NR	SD	NR	SD	SD
10	6	02916-24	NR	PD	Off-study	Off-Study	Off-study
11	6	02916-30	NR	SD	NA	NA	PD
12	6	02916-32	NR	PD	Off-study	Off-Study	Off-study
13	6	02916-33	NR	PD	Off-study	Off-Study	Off-Study
14	6	02916-41	NR	SD	NA	PD	Off-Study

SD – Stable Disease; PD – Progressive Disease; NR – Not a required study time point

M5 anti-mesothelin CAR Clinical Trials

Another clinical trial was run in parallel

UPCC 14217, Pancreatic Adenocarcinoma- Phase 1 dose escalation trial targeting patients with mesothelin-expressing refractory pancreas cancer



Cohort 1 (3 patients)- IV injection of 1-3 x $10^7/m^2$ M5 CARTs

NCT03323944

UPCC 14217: Patients, Clinical Response Data and Related SEAs

Subject ID	Disease Type	Cohort	Sex (F/M)	Age at Consent
14217-14	P	Cohort 1	M	54
14217-20	P	Cohort 1	F	61
14217-27	P	Cohort 1	F	61

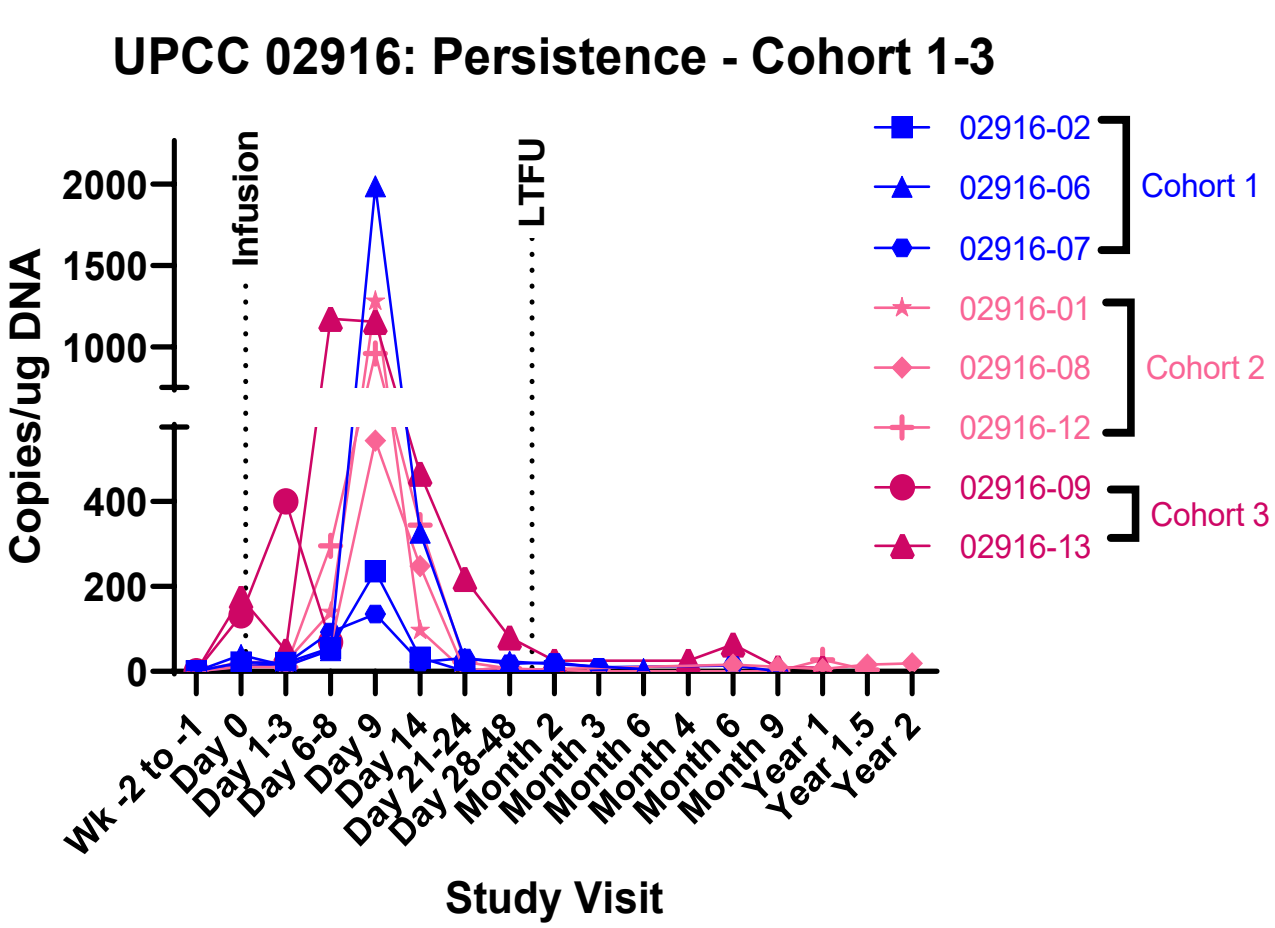
Subject Count	Cohort	Subject ID	Overall Tumor Response		
			Day 28	Month 3	Month 6
1	1	14217-14	PD	NA	Off-study
2	1	14217-20	PD	NA	Off-study
3	1	14217-27	SD	Off-study	Off-study

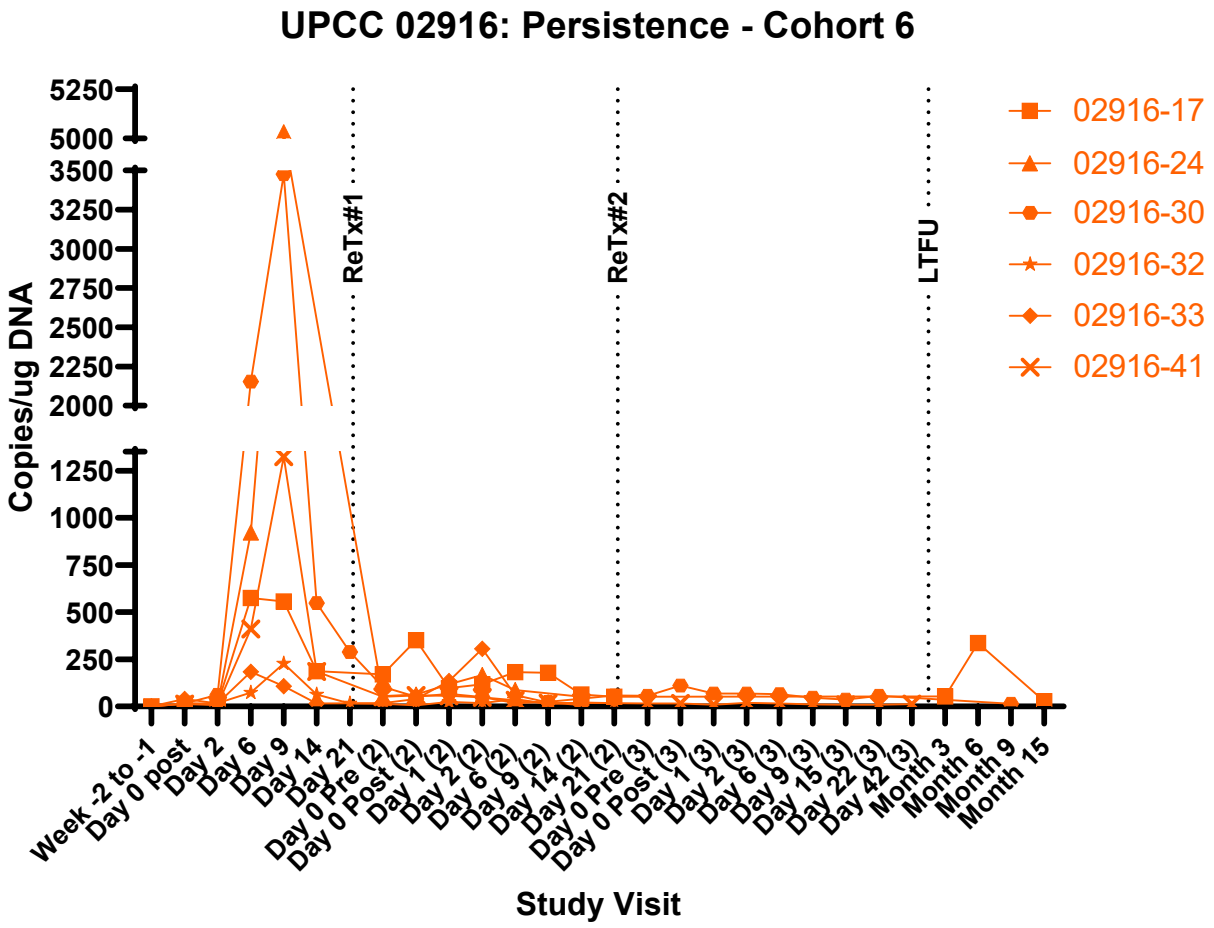
PD – Progressive Disease; NA – Not Assessed

Subject ID#	CTCAE Category	Toxicity	Grade	Attribution	Start Date	Stop Date
14217-20	Immune system disorders	Cytokine release syndrome	2	Definitely	8/5/2019	8/6/2019
	Respiratory, thoracic and mediastinal disorders	Dyspnea	3	Possibly	8/4/2019	8/11/2019
	Respiratory, thoracic and mediastinal disorders	Hypoxia	3	Probably	8/5/2019	8/11/2019
14217-27	Respiratory, thoracic and mediastinal disorders	Hypoxia	3	Probably	1/19/2020	Pending

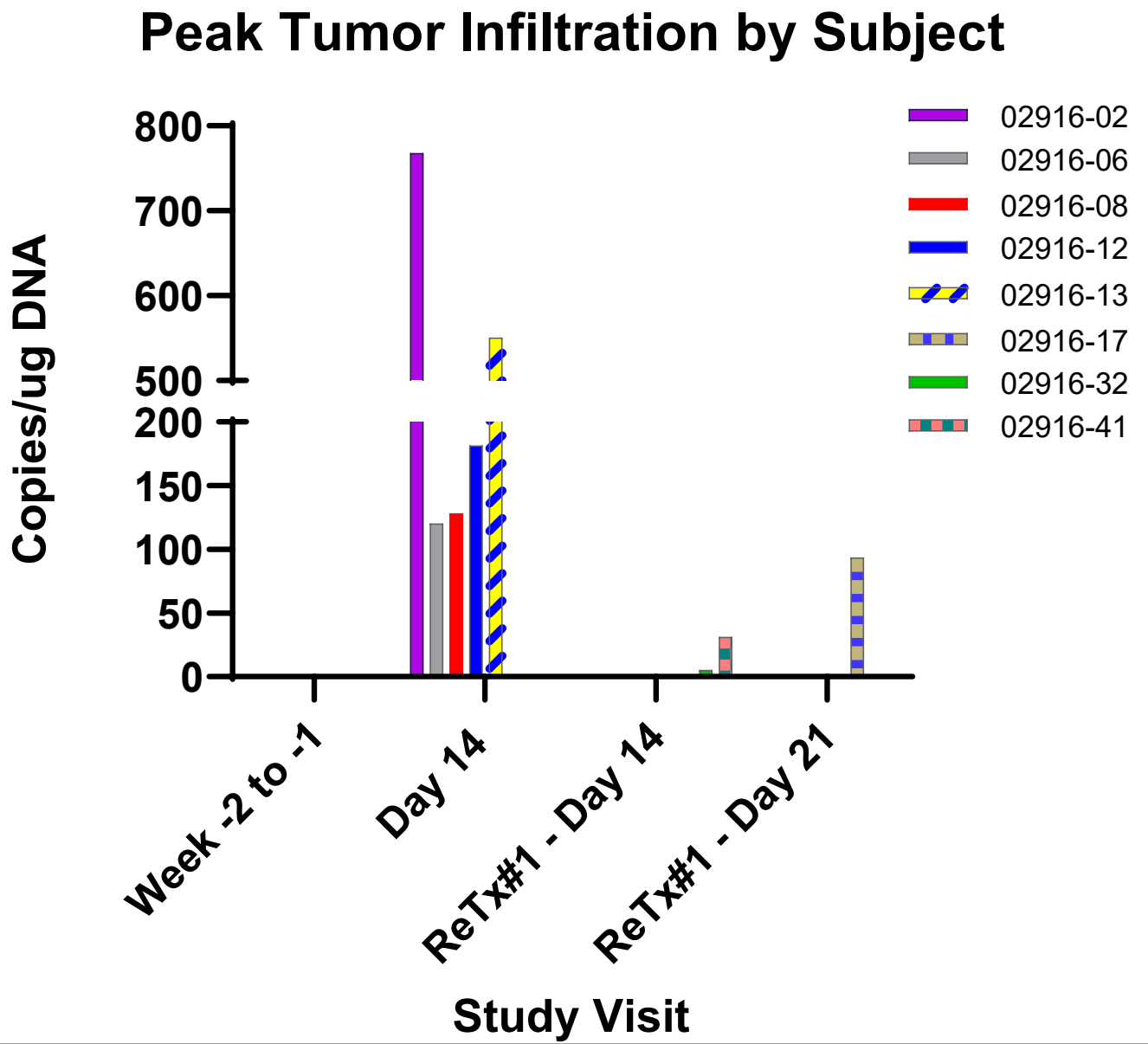
Bio-correlates

UPCC 02916: Persistence was limited in Peripheral Blood



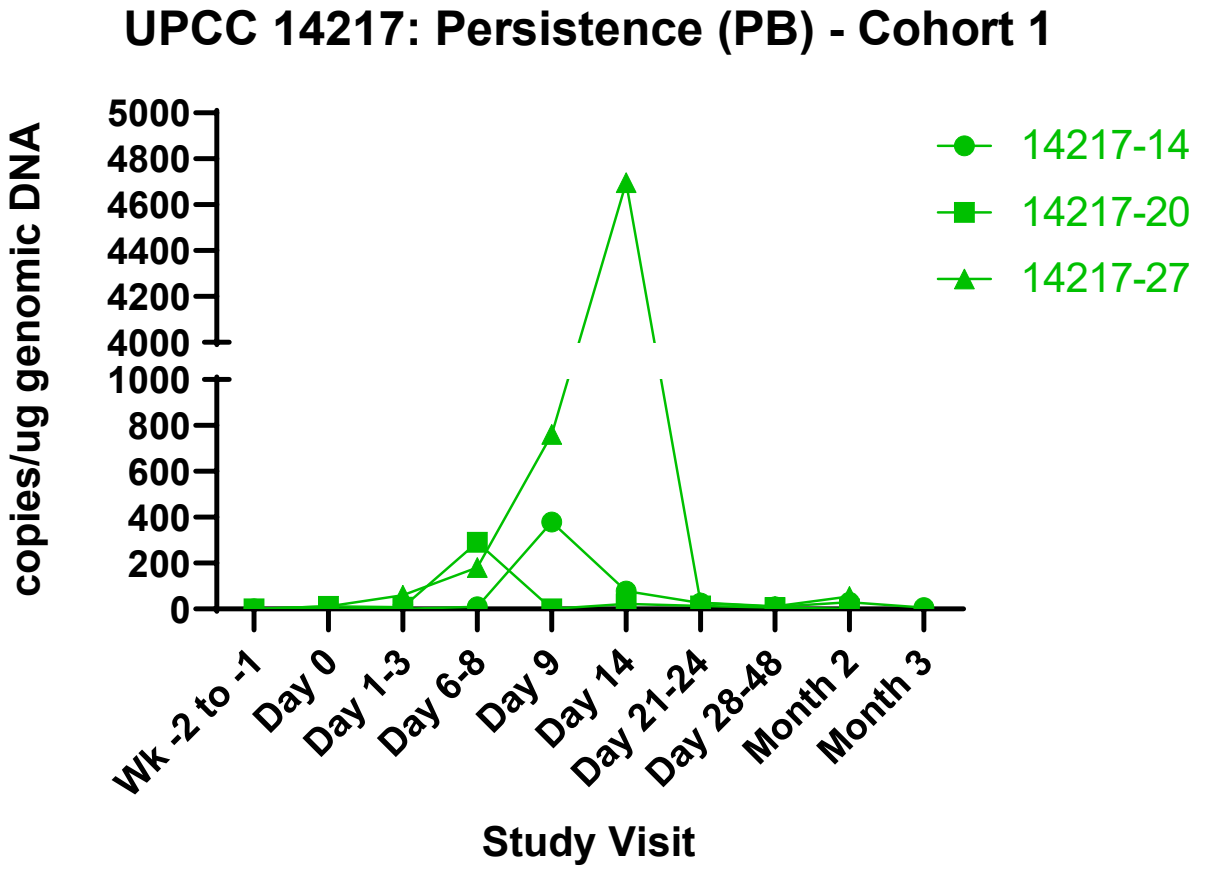


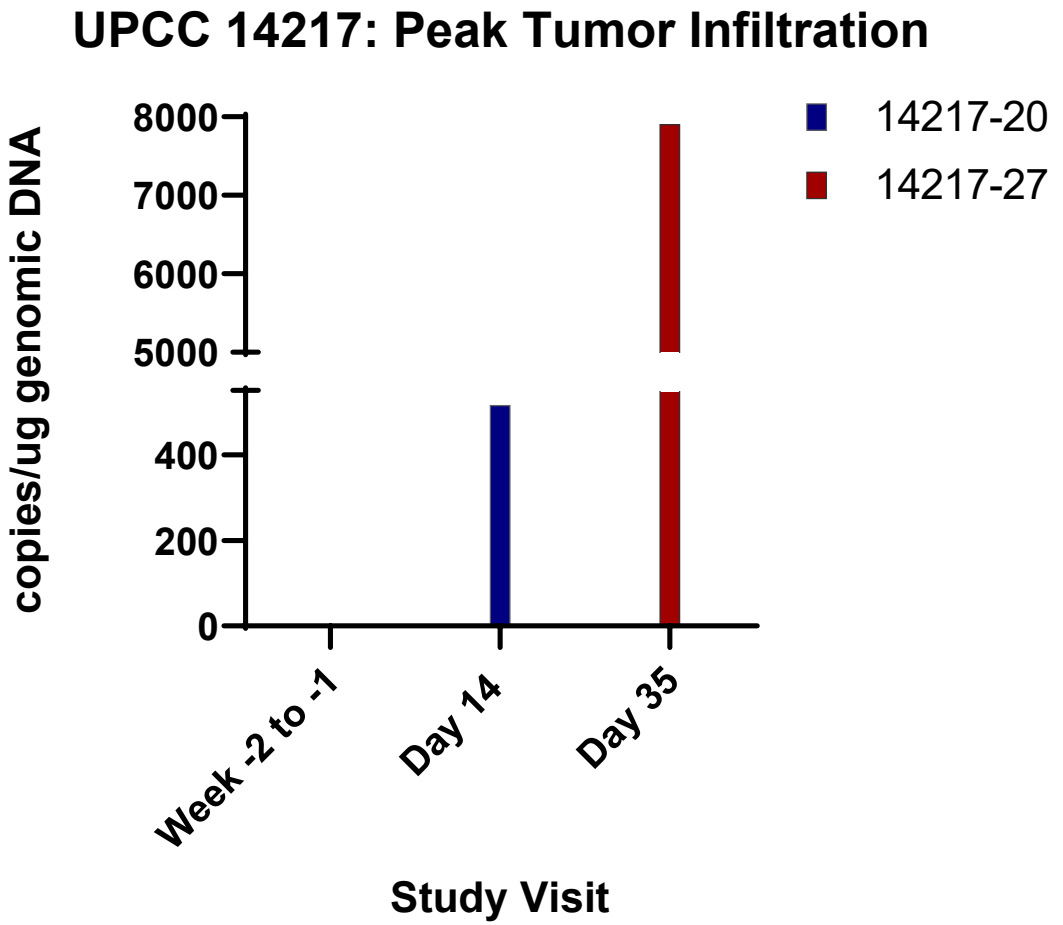
UPCC 02916: Some Tumor Infiltration was noted



- The persistence varies between Subjects
- All subjects were evaluated for intra-tumoral persistence of huCART-meso cells
- Only subjects who displayed some form of persistence are presented here

UPCC 14217: Persistence and Peak Tumor Infiltration





Summary

- The 1–3 x $10^7/m^2$ dose by IV route following cyclophosphamide LD is acceptable, whereas a 10-fold higher dose without cyclophosphamide is not
- CAR T cells expand in blood and reach tumors, with best response being stable disease
- Repeat dosing is feasible
- CART cells persistence was short and not improved from the murine scFV-based SS1 CAR
- Protocols modified to allow loco-regional administration following lymphodepletion, followed by IV delivery without lymphodepletion
  - Loco-regional delivery aims to improve E:T ratio at tumor and avoid the initial high exposure in the lungs
  - Adding fludarabine to the lymphodepletion in this context
- M5 MesoCART may be a suitable platform to explore combinations and next generation approaches

Future Directions

- Explore the use of local delivery techniques to enhance CART numbers and lower toxicity (intrapleural or intraperitoneal instillation for mesothelioma or ovarian cancers or intrahepatic delivery using the TriNav Infusion System)

- Explore combinations with oncolytic viruses, checkpoints, CD40 agonists

Study Team

- **Study PI:** Janos L. Tanyi MD, PhD and Mark O'Hara MD

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  Paul Hill MD (Biopsy)
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Thank you !