Update on Immunotherapy Trials for HIV and Cancer

Protocol #9912-365: CD4-zeta CAR T in HIV Patients

Protocol #1007-1056: MAGE A3 TCR for Myeloma

Protocol #1007-1057: MAGE A3 TCR for Melanoma

June 19, 2012

Penn Medicine
Abramson Cancer Center

Adaptimmune
Transforming T Cell Therapy

Overview

- Studies with CD4zeta CAR: long term follow up

- Adverse events with MAGE A3 TCR in melanoma and myeloma
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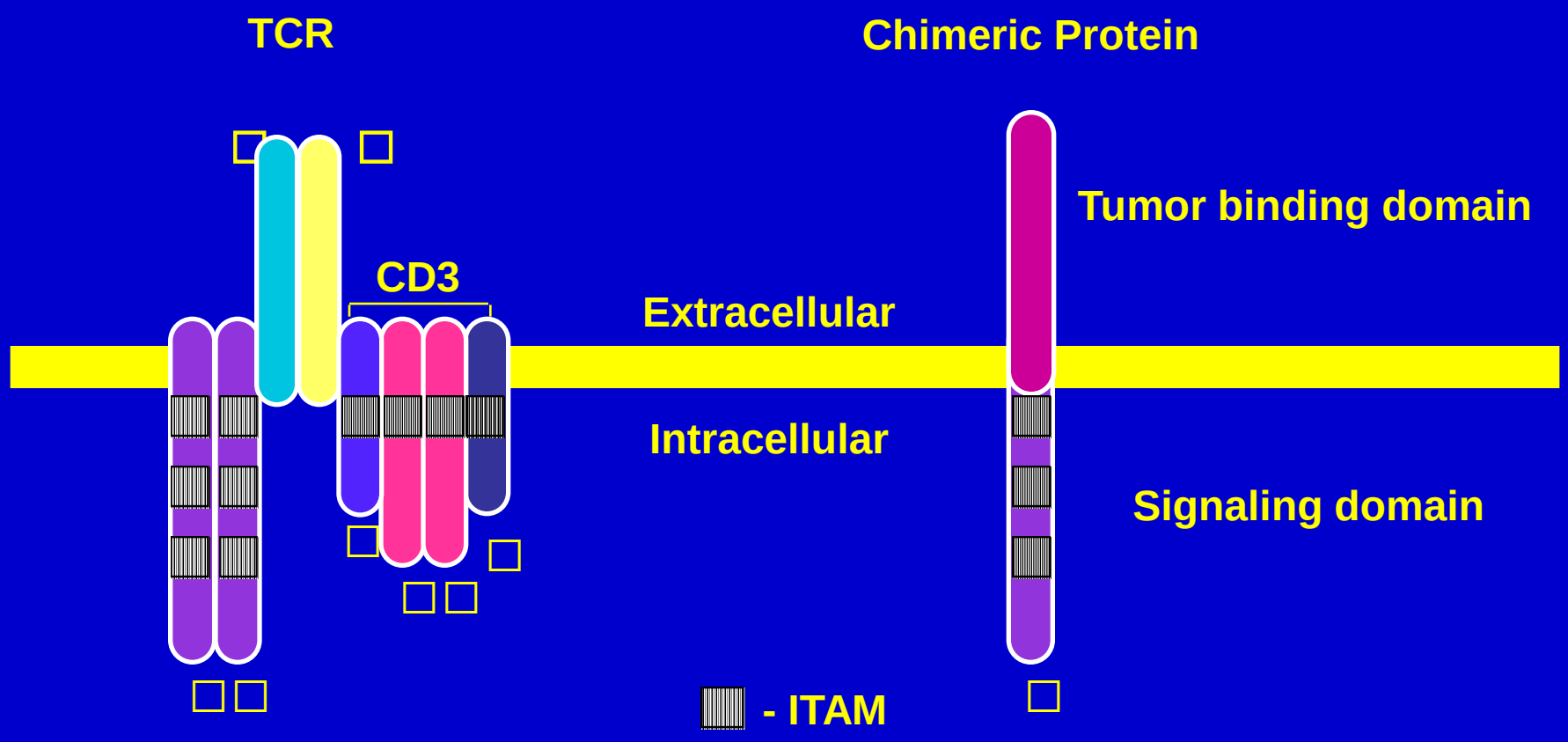
Two Basic Approaches to Overcome Tolerance
Creation of Bi-specific T cells

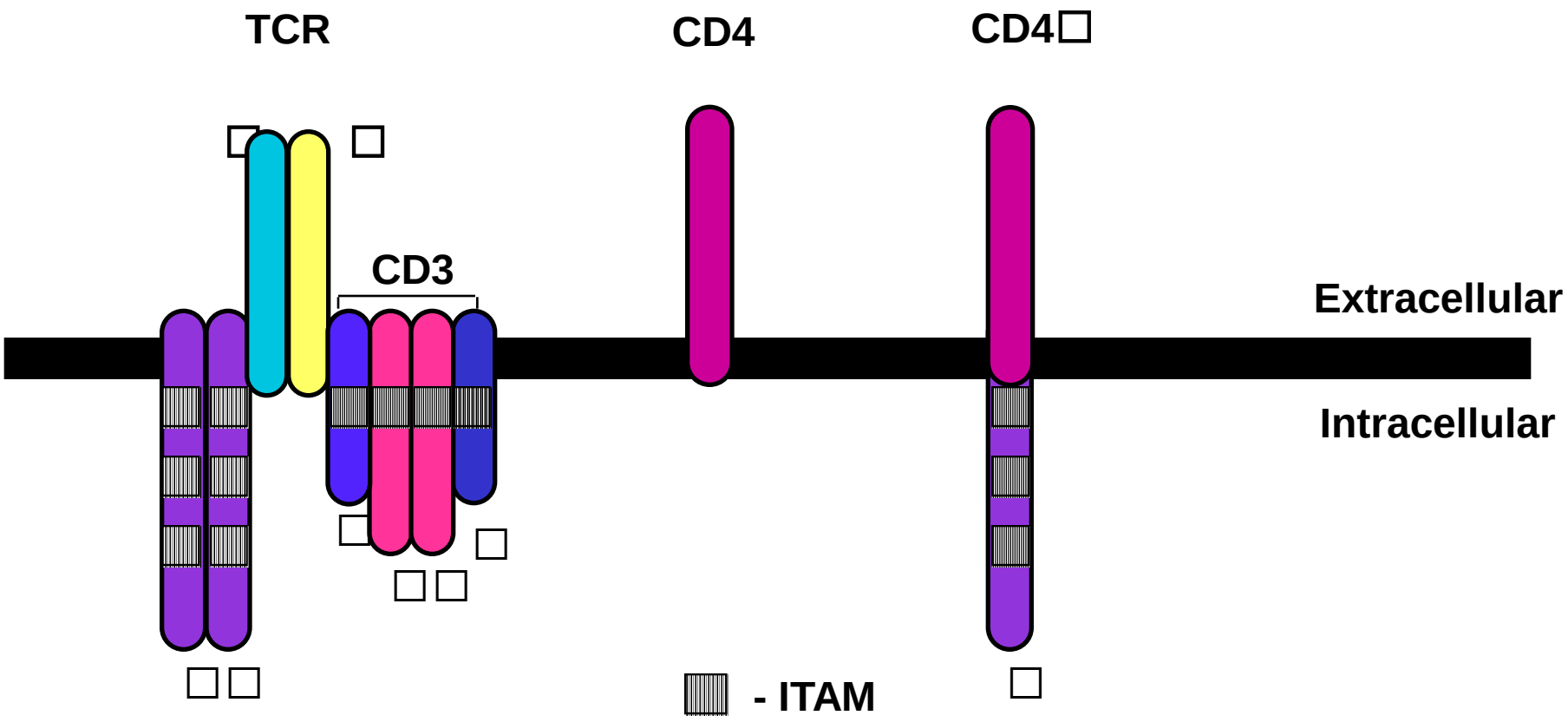
TCR heterodimer approach "CAR" or T body approach



Chimeric Antigen Receptors (CARs) Background

- 1989 Redirected T cell concept pioneered in vitro by Eshhar and colleagues: (Gross et al, PNAS 86: 10024, 1989)
- Despite strong pre-clinical rationale, technical difficulties have prevented clinical translation until recently:
  - Efficient T cell culture systems
  - Efficient gene transfer systems
- First clinical experiences in cancer:
  - Kershaw et al. *Clin Cancer Res*. 12: 6106, 2006
  - Lamers et al. *J Clin Oncol. 24: e20,* 2006
  - > Disappointing efficacy due to poor T cell engraftment?

Decade Long Engraftment and Persistence of Genetically Modified T Cells: Studies with CD4□ Chimeric Antigen Receptor (CAR)



Scholler et al. ScienceTM, in press

The First CAR Studies
CD4ζ Sponsored Clinical Trials for HIV

Cell Genesys Phase I open label (Mitsuyasu, et. al.)

Patients: controlled measurable HIV infection with ARV. Two groups, infusions of transduced CD4ζ with or without IL2.

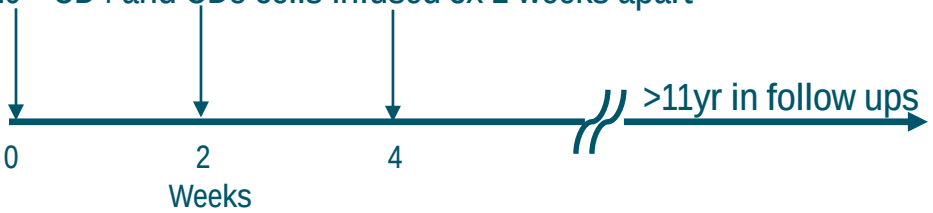
A single infusion of 2-3x10^{10} CD4 and CD8 cells

6 million units of IL2 infused continuously for 4 days beginning 4hrs prior to T cell infusion.

Cell Genesys Randomized Phase II Trial (Deeks, et. al.)

Patients: controlled HIV infection with ARV. Two groups, infusion with or without CD4ζ.

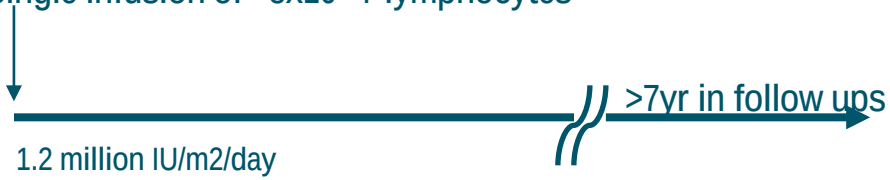
10^{10} CD4 and CD8 cells infused 3x 2 weeks apart



ONR Sponsored Randomized Phase IIa Trial (Aronson, et. al.)

Patients: controlled measurable HIV infection with ARV. Three groups: IL-2 , CD4ζ T cells or CD4ζ T cells with IL2.

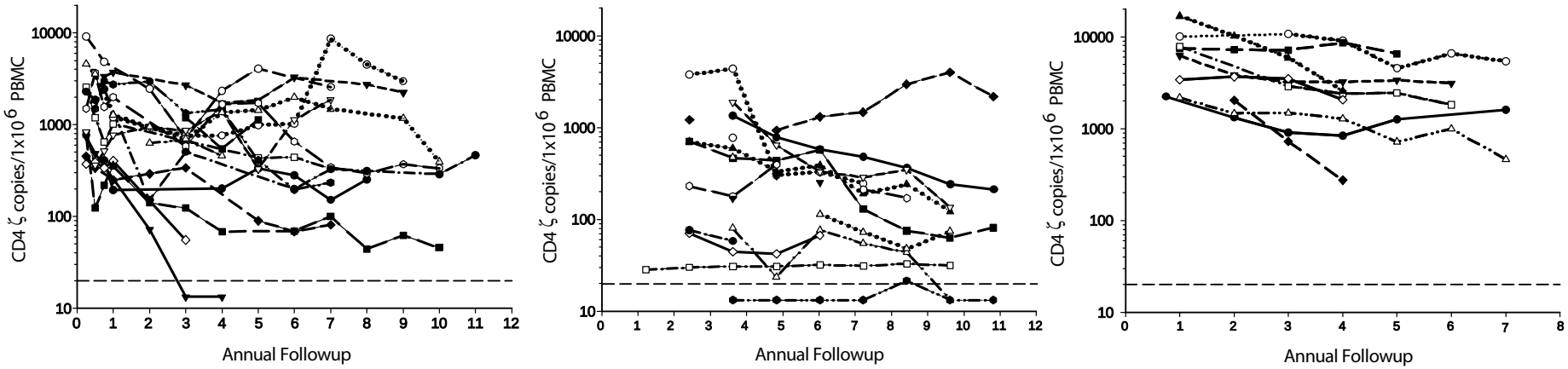
Single infusion of ~8x10^{9} T lymphocytes



1.2 million IU/m2/day subcutaneous IL-2 for 56 days

Long Term Follow Up: CD4zeta CARs

Stable levels of persistence



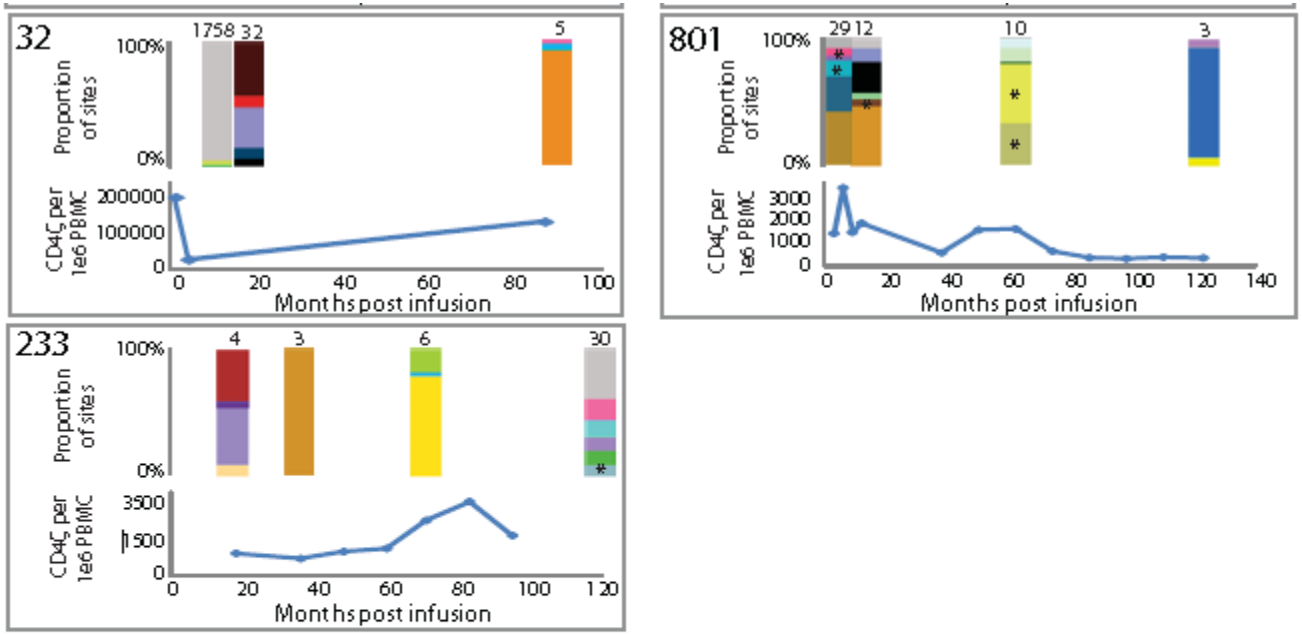
Half-life >16 years

Study	No. of subjects in followup	Decay rate (log10 copies/yr)		Half-Life (yrs post infusion)	
		Estimate	P -value	Estimate	95% CI
Deeks	17	-0.06	0.034	24.5	13.3, 235.9
Mitsuyasu	17	-0.085	0.005	16.5	10.5, 50.1
Aronson	9	-0.112	0.013	17.5	10.2, 80.2
Combined	43	-0.074	0.001		

Scholler et al. ScienceTM, 2012

Long Term Follow Up: CD4zeta CARs
Integration Sites

Annual	1	2	3	4	5	6	7	8	9	10	11	Total
Detected	*20*	*25*	*33*	*31*	*28*	*25*	*24*	*13*	*8*	*4*	*1*	*212*
Tested	*20*	*25*	*35*	*33*	*29*	*26*	*24*	*15*	*9*	*4*	*1*	221



Top five abundant sites marked by different colors

Low abundance sites colored grey

* Within 50kb of cancer-associated gene 5'end

Scholler et al. ScienceTM, 2012

Summary CD4z CARs: persistence and safety

Infusions of CD4z modified T cells results in long term (>decade) persistence at stable levels of ~0.5% of T cells. Lymphodepleting conditioning regimen was not required.

Persistence of CAR T cells indistinguisable from natural CTLs

41 of 43 patients have CD4z CAR persistence in PBMC up to 11 years post infusion.

No SAE in >540 years of patient followup

- Integrating murine gamaretroviruses are safe in human T cells

Experience with TCRs: Overview

- Development of affinity enhanced TCRs to cancer-testes antigens
- Clinical trial study designs
- Safety data for affinity enhanced TCRs targeting NY-ESO-1 / LAGE-1
- SAEs following administration affinity enhanced Mage-A3 TCR T cells
- Interim conclusions and implications

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Cancer – Testes Antigens: Shared tumor specific antigens

- Normal expression restricted to male germ cells in the testes and excluded in adult somatic tissues
- Expression up-regulated in a variety of cancers
- NY-ESO-1 family: small family of X-linked genes that includes NY-ESO-1 and LAGE1
- MAGE family: large family of >45 X-linked genes
- Spontaneous humoral and cellular responses demonstrated against NY-ESO-1 and MAGE-A family
- Late-stage cancer vaccine trials are in progress

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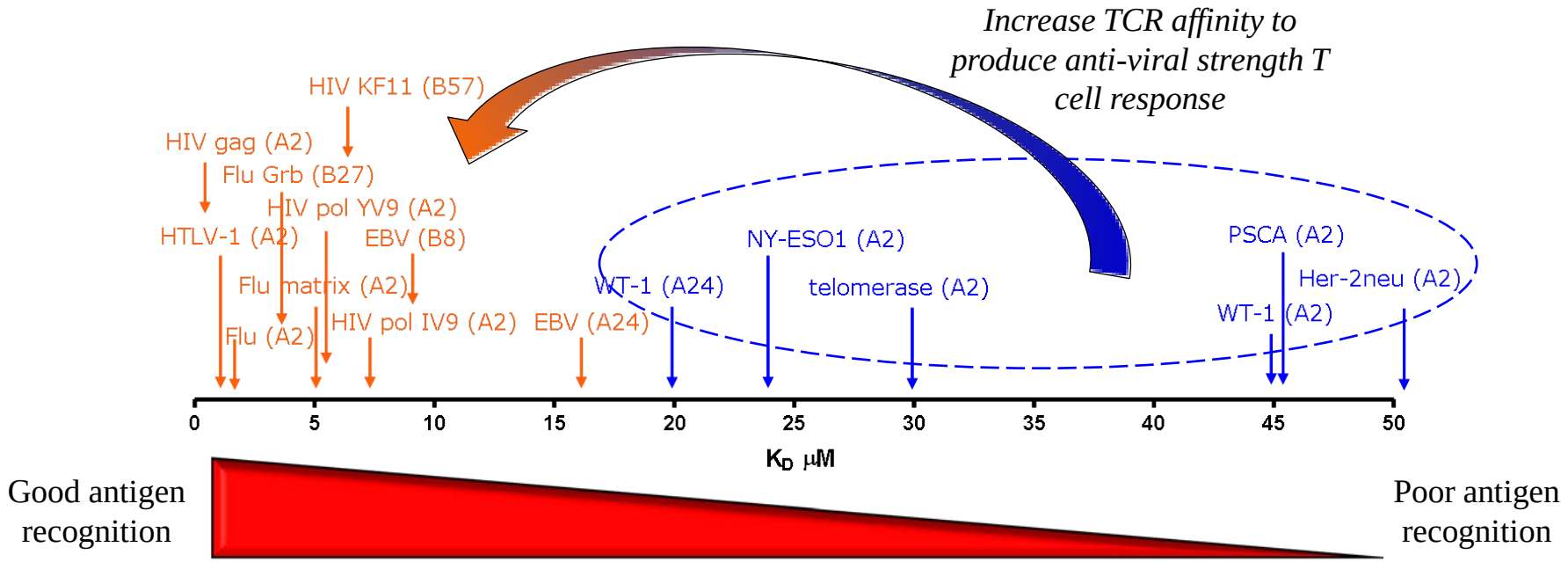
Affinity Enhancement of TCRs: Rationale

- Tumor antigen-specific TCRs in the circulating repertoire have undergone thymic selection which deletes high affinity TCRs
- High affinity TCRs are required to trigger a T cell response to low levels of cognate antigen
- Tumors have been immunologically selected for low level antigen expression
- Generation of affinity-enhanced TCRs addresses this potential roadblock to efficacious TCR-mediated engineered T cell therapy

TCR Affinities for Viral versus Self Antigens



- Jakobsen et al, unpublished data
- Concept supported in Cole *et al*, J Immunol, 2007; Stone *et al*, Immunology 2009

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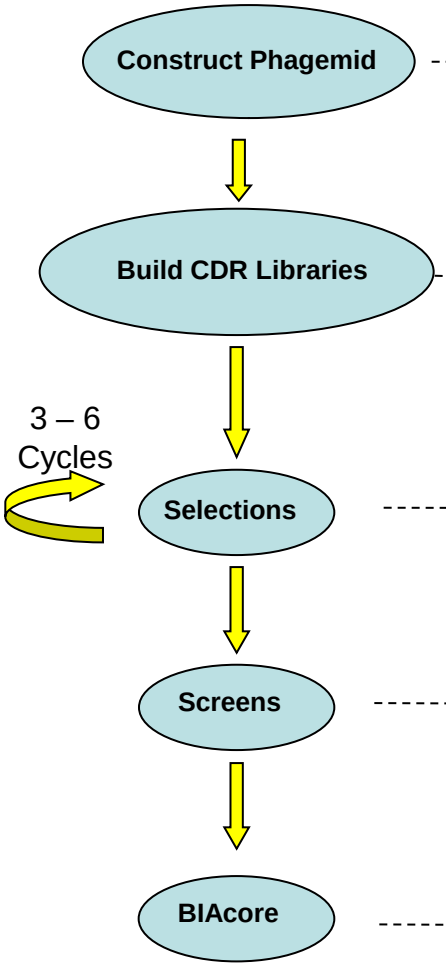
Generation of Affinity Optimized TCRs by Adaptimmune



- Ligate both wildtype TCR variable chain genes into proprietary bacteriophage TCR display vector
- Test TCR display by ELISA and immunoblotting

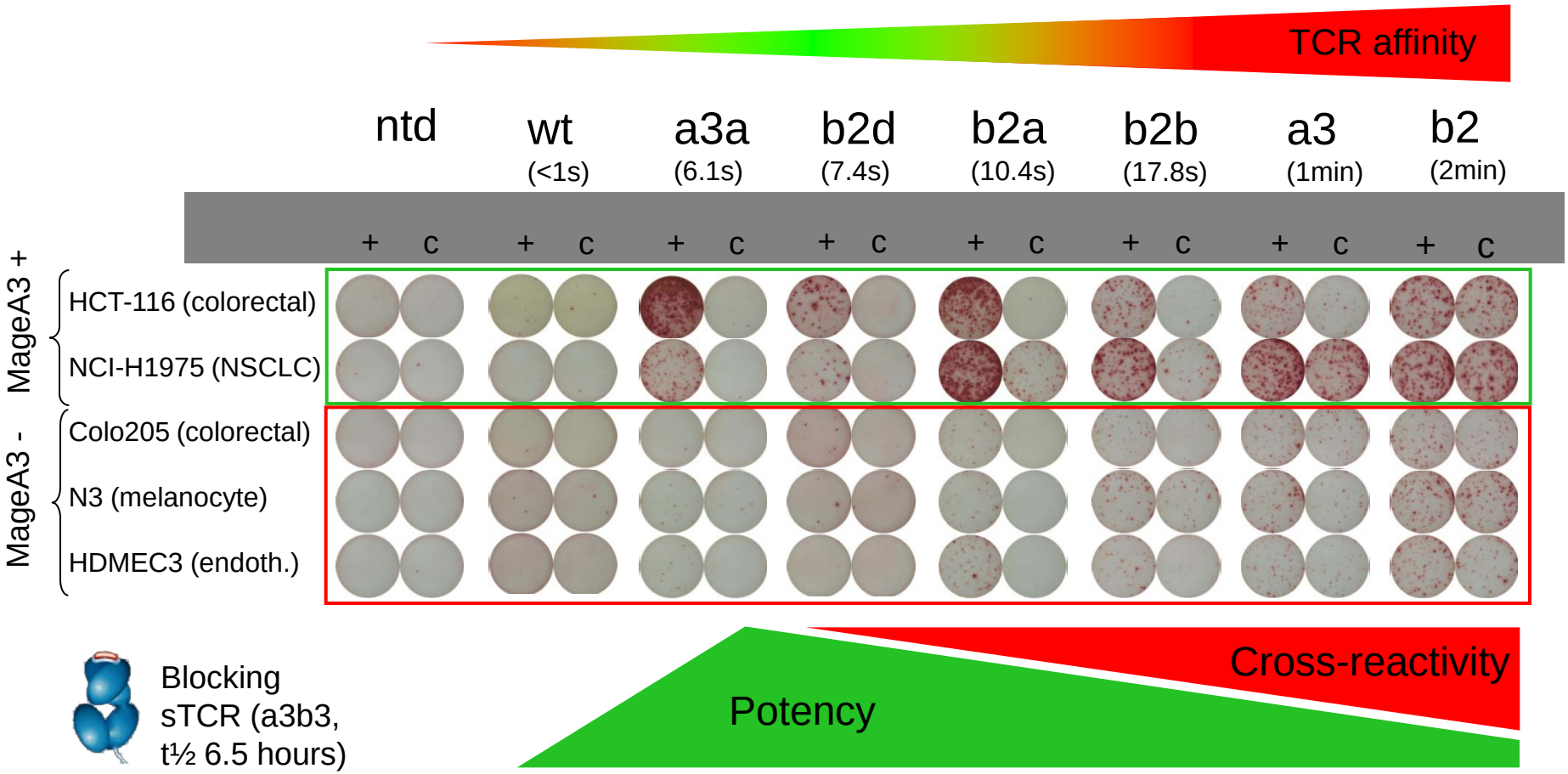
- Separate oligonucleotide –directed mutagenesis of the CDR2 and CDR3 regions of each TCR variable chain
- NNK codon mutagenesis in blocks of 5 overlapping CDR codons
- Electroporation and helper phage infection to produce four bacteriophage libraries for each TCR

- In vitro selections using biotinylated peptide/HLA antigen
- Selected bacteriophage used to re-infect bacteria to produce affinity-enriched TCR libraries.

- ELISA studies to measure antigen specificity and estimate TCR affinities
- DNA sequence analysis to determine if convergence of amino acid sequence to putative high affinity TCR sequence is achieved

- Mutate soluble wildtype TCR chain expression constructs to the CDR sequences of selected putative high affinity TCRs
- Test refolded putative “first generation” high affinity soluble TCRs for affinity and specificity by BIAcore
- Combine successful high affinity TCR chain mutations to obtain further “second generation” increases in affinity

Development of Mage-A3 TCR - Affinity Enhancement Panel



+ = Target cells & TCR transduced T cells
c = Target cells, TCR transduced T cells & pico-molar affinity, soluble TCR masking antigen

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Development of Mage-A3 TCR - Affinity Enhancement

TCR chosen for clinical evaluation:

Affinity of Mage A3 specific TCRs			
TCR	**awt/bwt ('wt')**	**a3a/bwt ('a3a')**	**awt/b2a ('b2a')**
T1/2	**<1s**	**6.1s**	**10.4s**
Kd	**300 μM**	**6.55 μM**	**9.4 μM**

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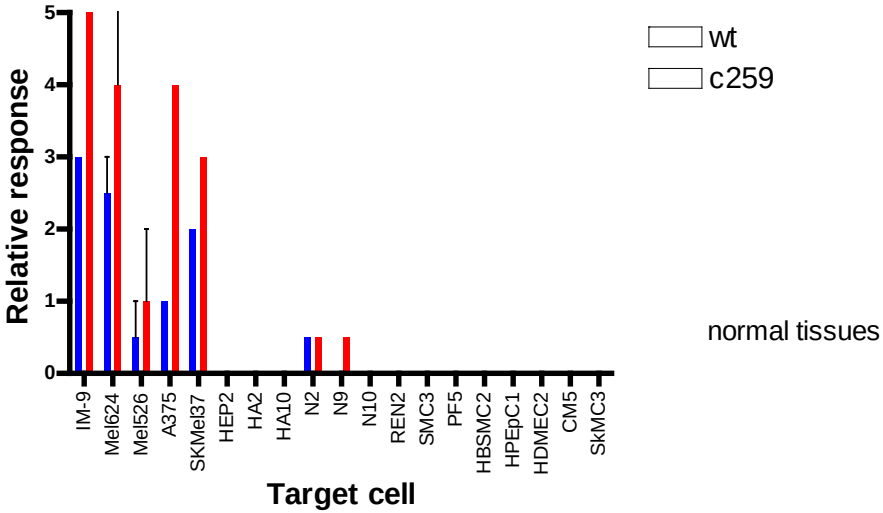
Overview of Preclinical Safety Studies (Mage-A3 TCR)

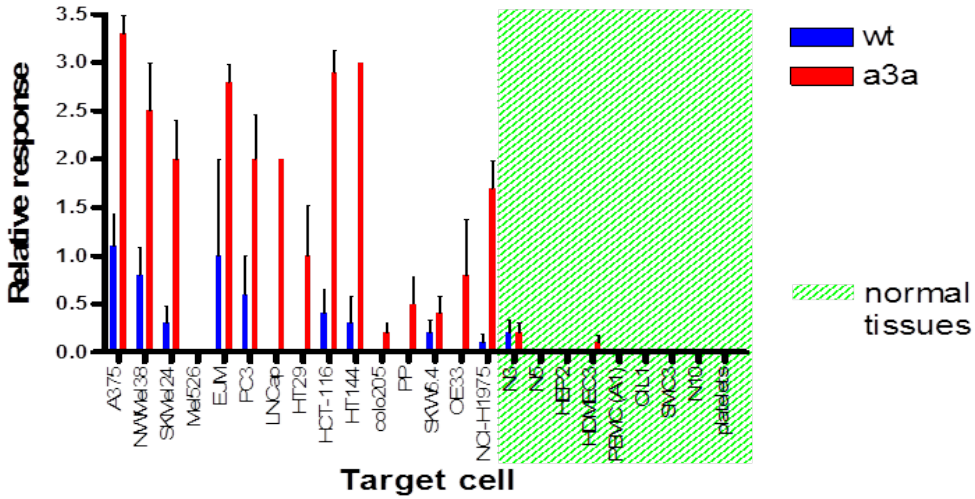
- ELISPOT analysis against tumor cell lines and primary cells for specificity and activity
  - Primary cells: Epidermal melanocytes, hepatocytes, dermal microvascular endothelial cells, ciliary epithelial cells, PBMC and platelets
  - Readouts: IFN-γ, GrB
- Luminex to measure cell function in presence of HLA-A1 + Mage-A3 tumor cells & PBMC
  - IFN-α, IFN-γ, MIP-1α, MIP-1β, IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, GM-CSF, TNF-α, RANTES, MCP-1, IP-10, Eotaxin, MIG.
- Cytotoxicity assays
  - LDH assays (normal and tumor cells)
  - Degranulation assays (tumor cells)
- Activity with mispaired TCR containing affinity enhanced chain
  - Evaluated in NY-ESO-1 TCR as an example
  - Activity only seen in cells transduced with a single very high affinity chain not used in clinical studies
- In Vivo Efficacy
  - Demonstration of anti-tumor activity in vivo using clinical candidates in a NSG mouse model

ELISPOT Analysis (IFNγ) of Mage-A3 and NY-ESO-1 Transduced Cells Against Normal and Tumor Cell Lines

NY-ESO-1



MAGE-A3



- Derivation of TCR described in Robbins *et al*., J Immunol, 2008
- HLA-A2 : SLLMWITQC
- 2 mutations in the α CDR3 chain; wild type β
- Previously tested in clinic: efficacy and no safety concerns (Robbins *et al*, JCO, 2011)

- Derivation not yet published (in preparation)
- HLA-A1: EVDPIGHLY
- 4 substitutions in the α CDR2 chain; wild type sequences in TCR β chain
- First in human trials

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Production of Engineered Autologous T Cell Product for NY-ESO-1 and MAGE A3 Trials

Day 0

- Lymphocyte Apheresis

- *Bacterial and fungal*
- *Cell number and viability*

Day 1

- Elutriation
- Wash
- CD25 depletion
- Wash

- *Phenotyping*

- Activation/Expansion (CD3/CD28 beads)
- Lentiviral vector transduction

Day 3

- Vector wash out
- Expansion (static)

- *Cell pellet for VSV-G (**day 3 and 5**)*

Optional Day 5/6-12

- Optional Perfusion Culture
- Dependent on target dose

Day 7

- *Cell pellet for VSV-G*
- *Viability*
- *Transduction and phenotyping*

Cell Harvest

- Bead Removal
- Harvest
- Final Formulation
- Controlled rate freeze

Pre Harvest

- *Cell pellet for VSV-G*
- *Cell and sup for mycoalert*
- *Viability*
- *Biologic RCL (post infusion ok)*

Post Harvest

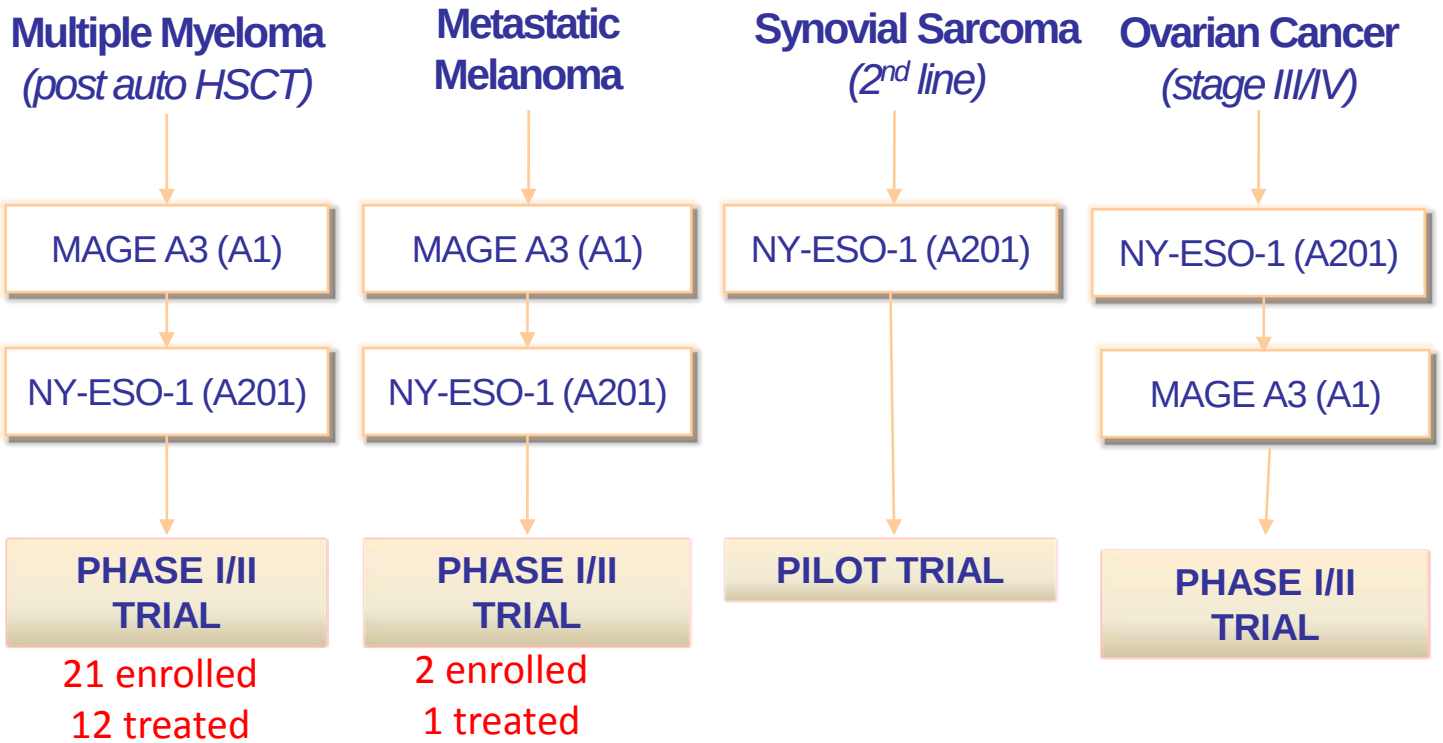
- *Viability*

Final Formulation

- *Sentinel vials*
- *Bactec and fungal (day 7 interim ok)*

Cell and Vaccine Production Facility, University of Pennsylvania

Overview of Clinical Studies: Design and Enrollment Status



- Eligibility criteria for all studies captures patients who have limited options
- Split infusion: 30%/70% dose on days 1 and 2 to mitigate unexpected infusion reactions

  (none yet observed; myeloma trial uses single infusion due to tumor location in bone marrow, and sarcoma trial due to safety data available for the NY-ESO-1 TCR)
- Daily follow-up in first 1-2 weeks with cytokine monitoring and correlative analysis

Update on Safety of NY-ESO-1 TCR

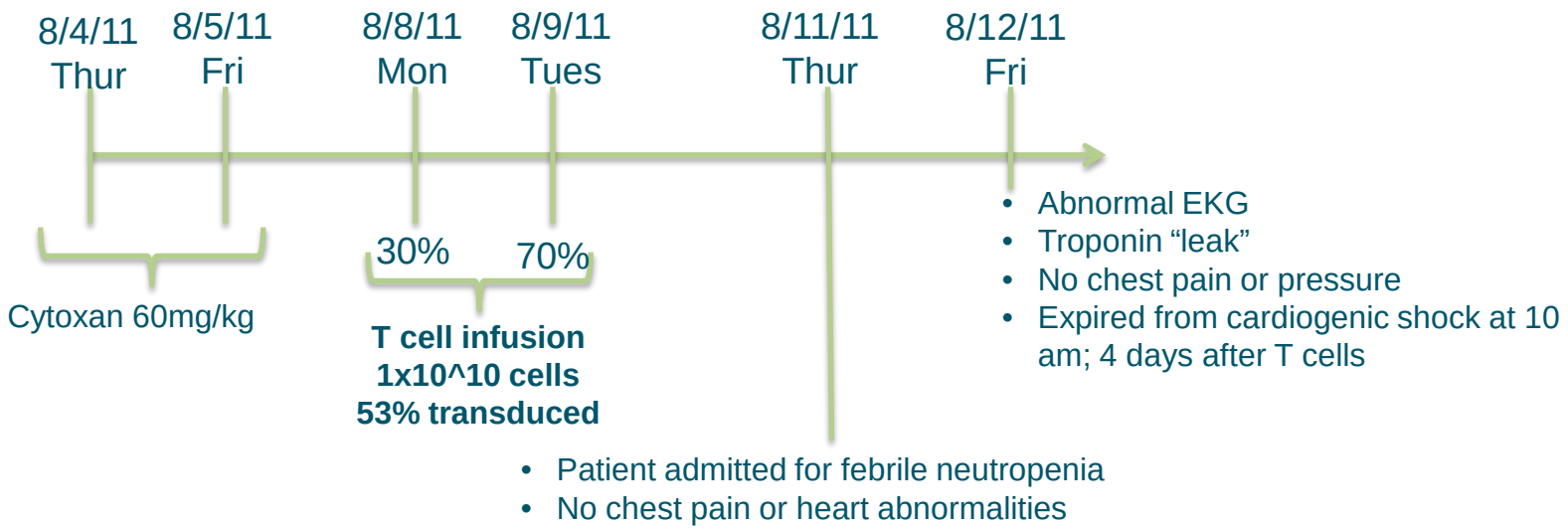
- Eleven patients treated to date with NY-ESO-1 T cells. All enrolled on the myeloma study
- No reportable AEs related to the study treatment
- Manageable GI toxicity observed
  - Known low-frequency side effect following auto-HSCT (Kline *et al*, Bone Marrow Transplant, 2008; Lazarus *et al,* BBMT, 2011)
  - Previously reported at a higher frequency (13%) in trials evaluating transfer of non-gene modified autologous T cell trials post auto-HSCT in myeloma (Rapoport, Stadtmauer *et al,* CCR, 2009)
- 4 SAEs (reversible) on the study
  - 3 unrelated
  - 1 possibly related to T cells but not the NY-ESO-1 TCR
- No safety concerns identified regarding the affinity enhanced NY-ESO-1 TCR

SAEs Following Infusion with Cells Expressing the Mage-A3 TCR

- Two patients have been treated with cells expressing the Mage-A3 affinity enhanced TCR
  - One on the melanoma study (August 8 and 9, 2011)
  - One on the myeloma study (May 11, 2012)
- Both patients died 4-5 days following T cell infusion with cardiac related events:
  - Melanoma patient: investigation concluded “demand ischemia, and severe underlying silent ASCVD”
  - Myeloma patient: initial investigation implicates direct T cell mediated cardiac myonecrosis
- Mage-A3 TCR protocols have been suspended pending investigation

Case 1: Mage-A3 SAE

- 63 year old man with metastatic melanoma, initially diagnosed in 2005 as a stage IIIB (T3b N1a M0) melanoma, with metastatic disease diagnosed in March 2007
- Prior treatments included:
  - Interferon started in September 2005
  - Dendritic cell vaccine in 2007-2008 timeframe with clinical response
  - Ipilimumab started in Feb 2008 and ended April 2011



Case 1: Mage-A3 SAE

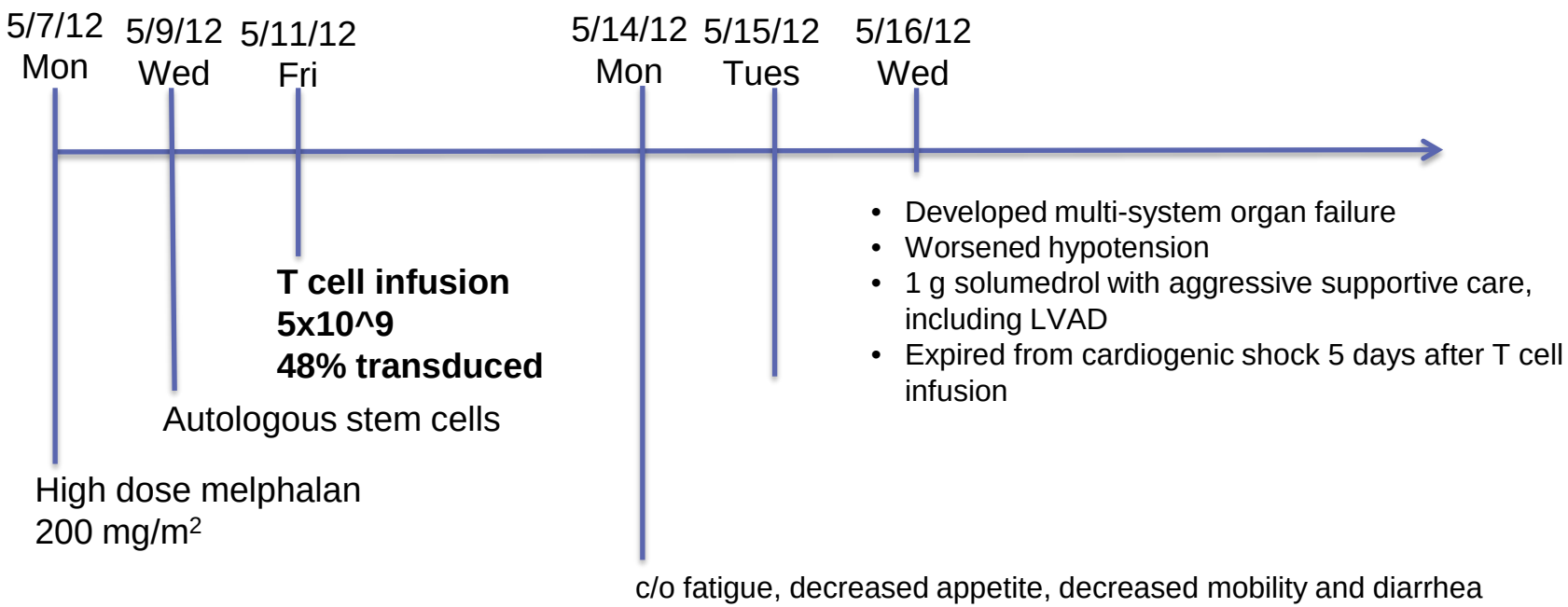
- Autopsy revealed:
  - Extensive acute MI and prior ischemic damage to the left ventricle with two prior “silent” transmural MI (despite normal screening EKG and echocardiogram)
  - Patient had 95% blockage of proximal LAD, minimal occlusion in other coronary arteries. Neutrophils in tissue.
- Additional investigation revealed:
  - No expression of Mage-A3 in patient heart tissue by RT-PCR
  - No expression of Mage-A3 in additional screen of heart tissue from 5 additional donors
  - Similar marking of Mage-A3 T cells in heart, blood and spleen; thought to reflect the hemorrhagic nature of the MI
  - Cytokine levels in the patient were elevated on the day of the patient’s death, but were similar to those observed on other adoptive T cell therapy trials (Kalos *et al*, 2011): contribute to “demand ischemia”?

Case 1: Mage-A3 SAE

- Conclusions and outcome from the event:
  - The patient had prior undetected and significant heart disease which was potentiated by anemia and metabolic demands from FUO. Potential contribution of cyclophosphamide (known to induce transient cardiac toxicity)
  - No evidence that the Mage-A3 specificity of the cells contributed to the event
  - Event underscored the importance of screening out patients with underlying ischemic cardiac disease:
    - Implemented nuclear cardiac stress tests in all studies (except sarcoma) as a screening procedure
    - Added explicit language regarding the death and the additional cardiac screen to ICFs on all trials containing the Mage arm
  - Mage arms in all trials were placed on hold immediately following the event; after FDA review of the final report and protocol modification, the Mage arms were reopened in spring of this year

Case 2: Mage-A3 SAE

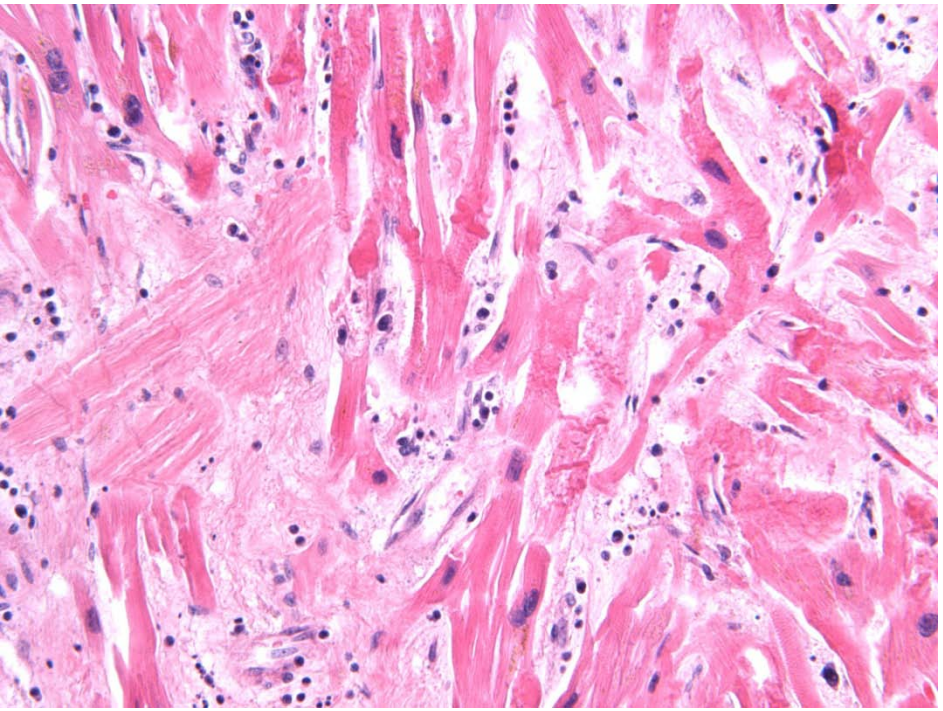
- 53 year old man initially diagnosed with multiple myeloma in 2009.
- Prior treatments included:
  - Radiation to thoracic spine May – June 2009
  - Velcade and decadron August – December 2011 (PD)
  - Revlamid and decadron August – September 2012 (PD)
  - D-PACE in February 2012, and again in April 2012 (PR)



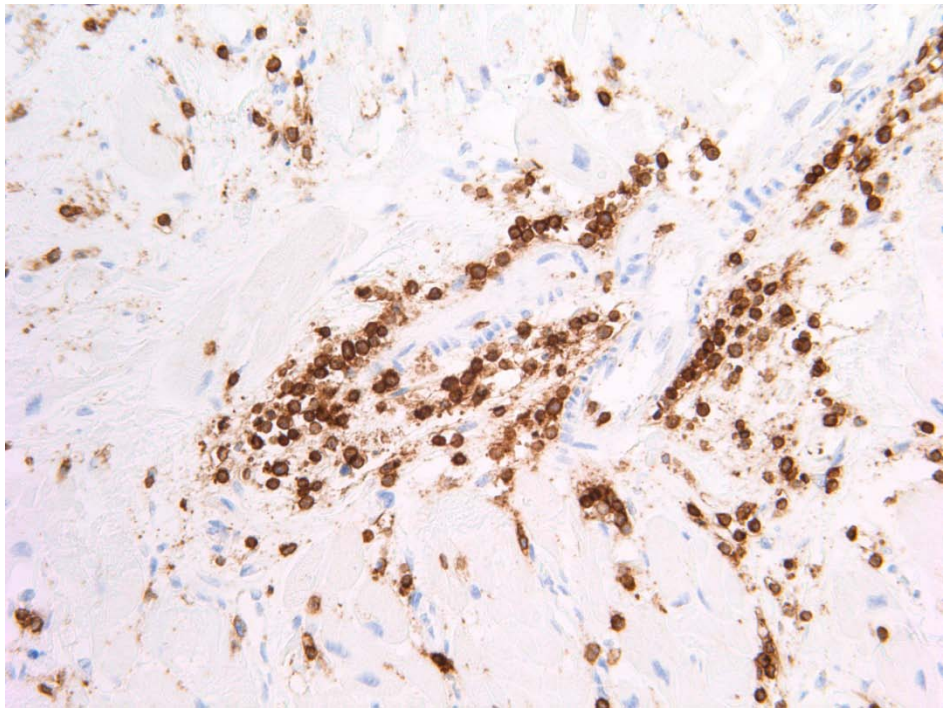
Case 2: Mage-A3 SAE interim results of investigation

- Patient had past medical history of hypertrophic obstructive cardiomyopathy (diagnosed at age 18) and atrial fibrillation
  - Normal EF of 64%
  - Nuclear cardiac stress test revealed no evidence of ischemia, no arrythmias, normal wall motion
  - Patient was enrolled following cardiologist evaluation that past Hx posed no additional risk
- Provisional autopsy have revealed
  - 500ml serous pericardial fluid
  - No evidence of acute ischemic MI. Extensive myonecrosis similar to “allograft cardiac rejection”
  - Organs appear as expected following cardiogenic shock, except the heart which contains significant hematopoietic infiltrate comprised of CD3+ cells
- Additional investigations revealed
  - High levels of IFN-γ in pericardial effusion, no TNF-α
  - Other serum cytokines present at similar levels to CD19 CAR or NY-ESO-1 arm
  - T cell infiltrate in cardiac tissue sections
  - No reactivity of patient cell product to cardiomyocyte cell lines

Case 2: Mage-A3 SAE
Preliminary autopsy histology findings



- H&E of LV heart tissue section from Case 2
- 20x
- Additional sections show similar infiltrate



- CD3 IHCstain of same heart tissue section
- 20x
- Additional sections show similar infiltrate

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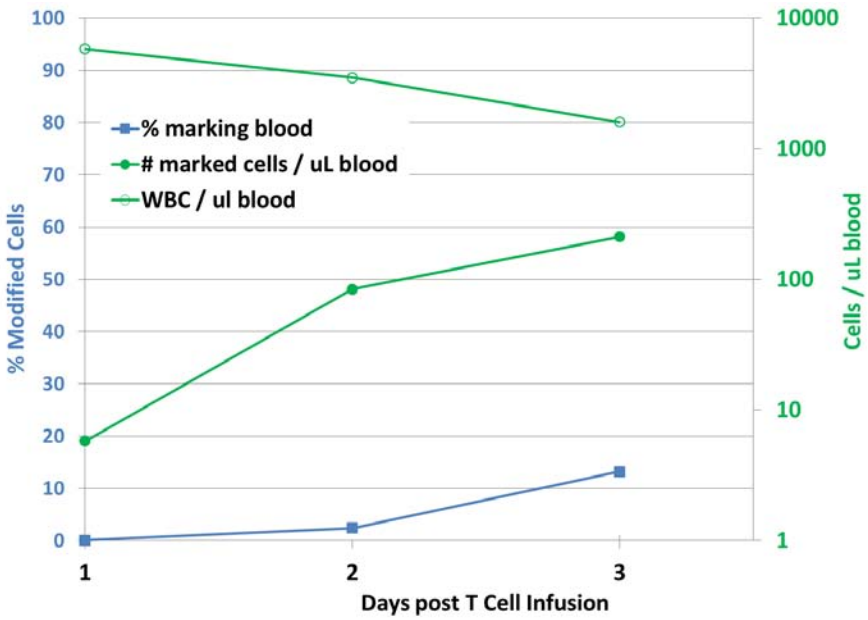
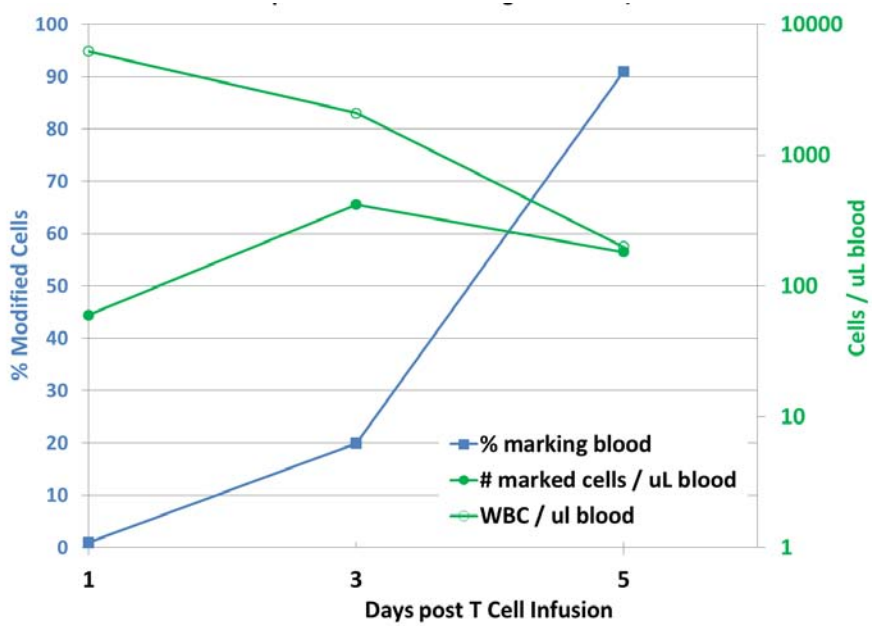
Engraftment and Expansion of Gene Modified Cells in Each Case PCR

Case 1



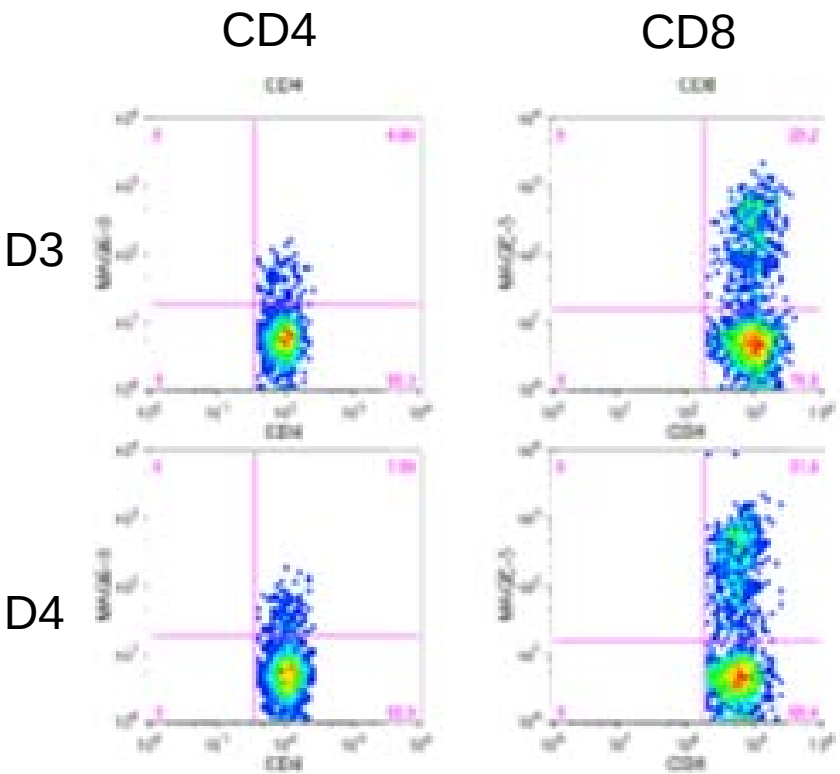
Case 2

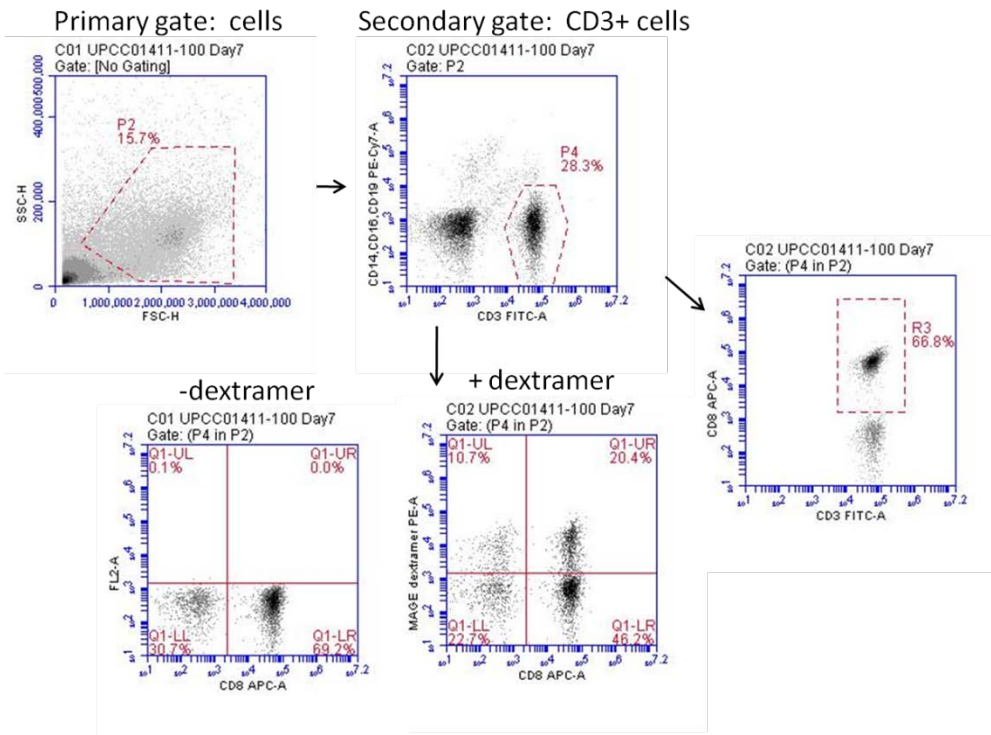


- Case 1 – Melanoma
- Total number of persisting cells = 1.3e9 td cells on day 3 post T cells
- 35% of CD8 and 30% of CD4 cells at day 3 post T cells expressed the TCR by Vβ5.1 stain

- Case 2 – Myeloma
- Total number of persisting cells similar to NY-ESO-1 arm (9.1 e8 td cells on day 5)
- Prelim PCR data show higher % marking on day 5 than in NY-ESO-1 patients (~60% or lower in NY-ESO-1 arm)

Engraftment and Expansion of Gene Modified Cells in Each Case
Tetramer or dextramer staining by flow cytometry





- Case 1 – Melanoma
- tetramer staining day 3 and 4
- Case 2 – Myeloma
- dextramer staining day 5

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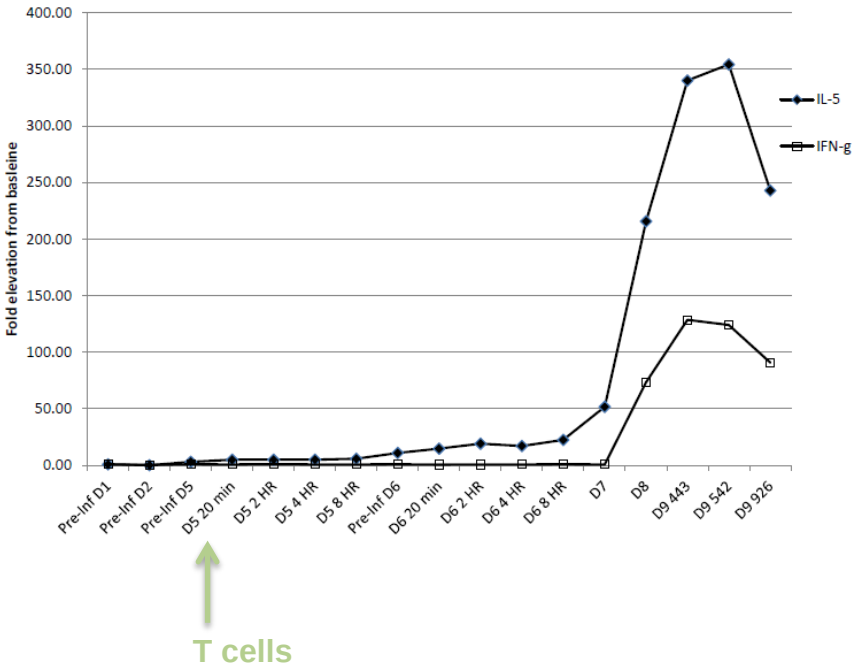
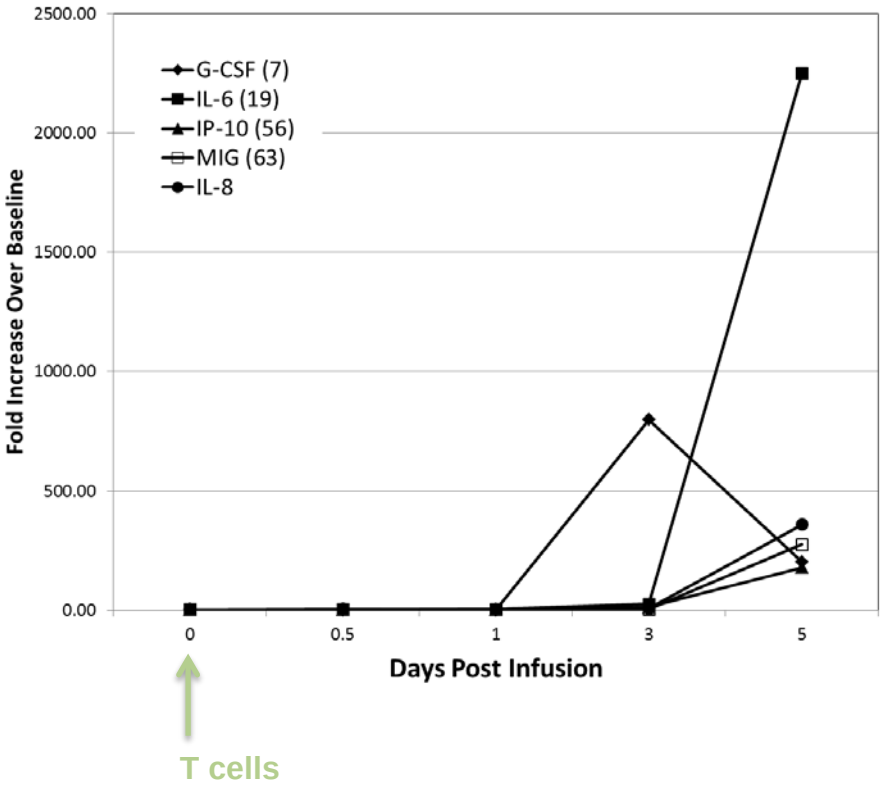
Cytokine Profile: Cytokines Increased > 100-fold

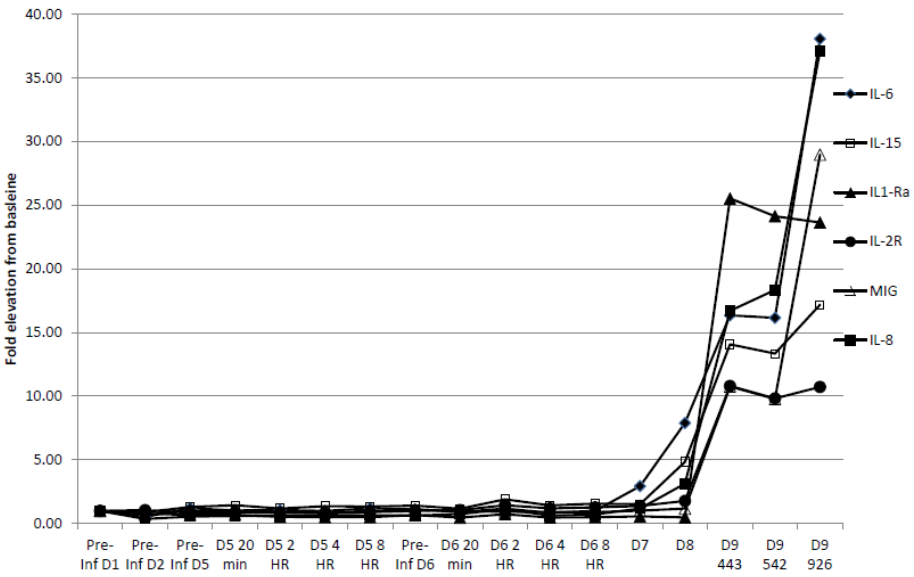


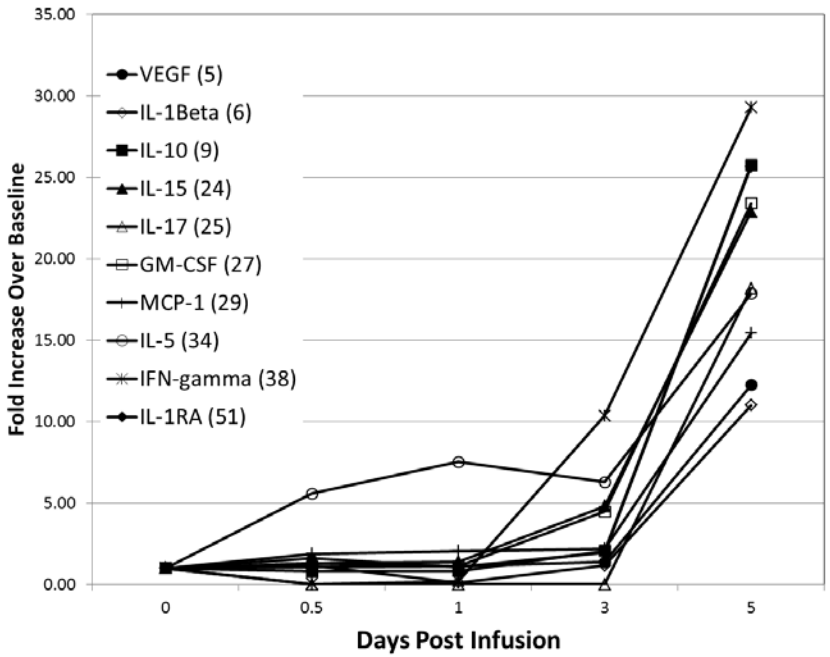


- Case 1 – Melanoma
- IL-5 and IFN-γ

- Case 2 – Myeloma
- IL-6, G-CSF, IP-10, MIG, IL-8

Cytokine Profile: Cytokines increased 10 to 100 –fold

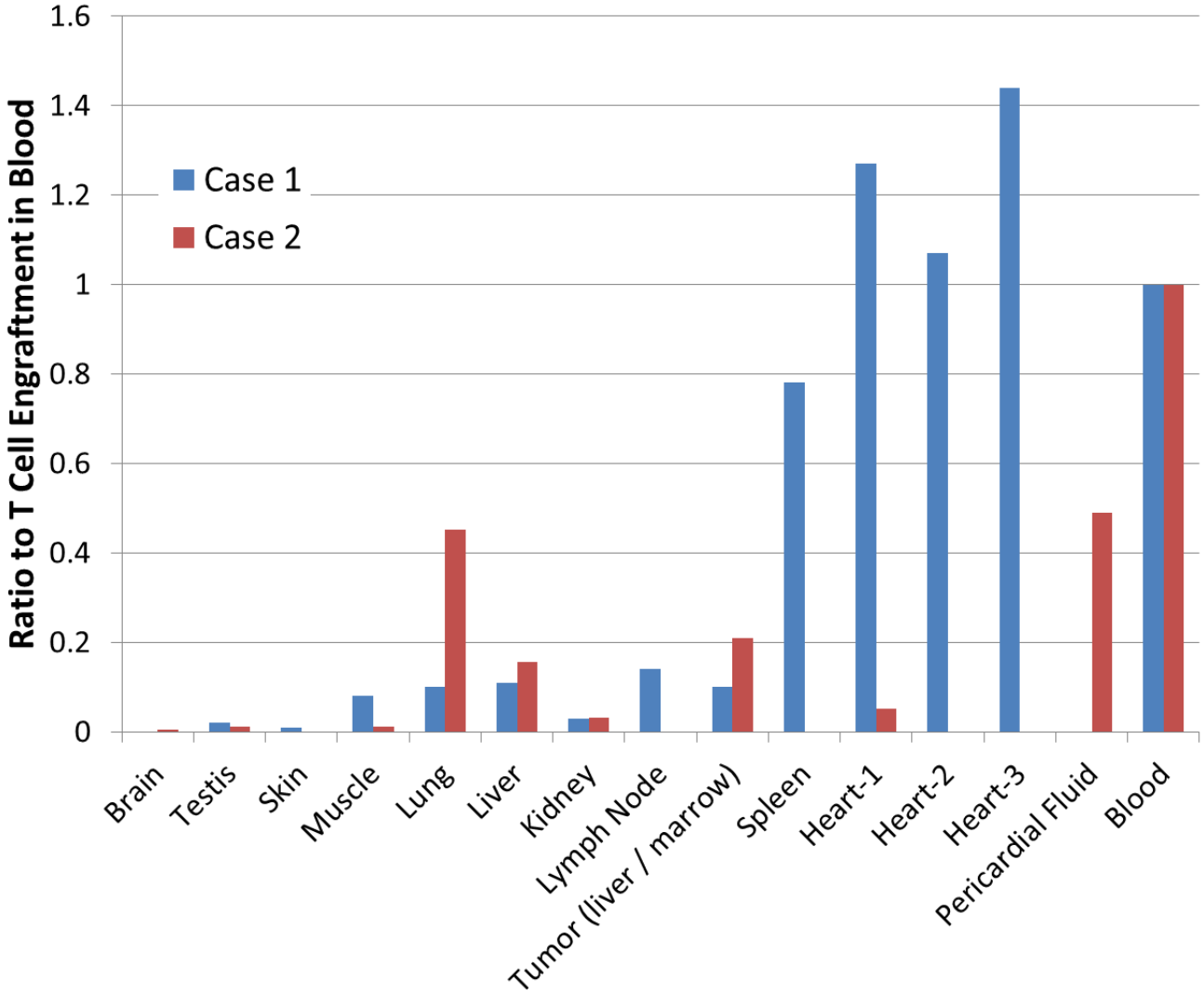




- Case 1 – Melanoma
- IL-6, IL-15, IL1-RA, IL-2R, MIG, IL-8

- Case 2 – Myeloma
- VEGF, IL-1β, IL-10, IL-15, IL-17, GM-CSF, MCP-1, IL-5, IFN-γ, IL-1RA

Mage-A3 T Cells in Autopsy Tissues: qPCR



Mage-A3 SAE – Planned Studies

- Evaluate the effect of IFN-γ and chemotherapy on Mage-A3 expression in heart and other tissues
  - Panel of primary cells (including cardiac myocytes, aortic endothelial cells, coronary artery smooth muscle cells, aortic adventitial fibroblasts, cardiac microvascular endothelial cells, coronary artery endothelial cells, cardiac fibroblasts)
  - Primary tissue as possible from autopsy
- Screen panel of similar epitopes which may potentially be cross reactive
- Investigate the possibility of allo-reactivity
- Investigate the possibility of mispairing
  - Dextramer versus Vβ and Vα stain
  - Deep sequencing

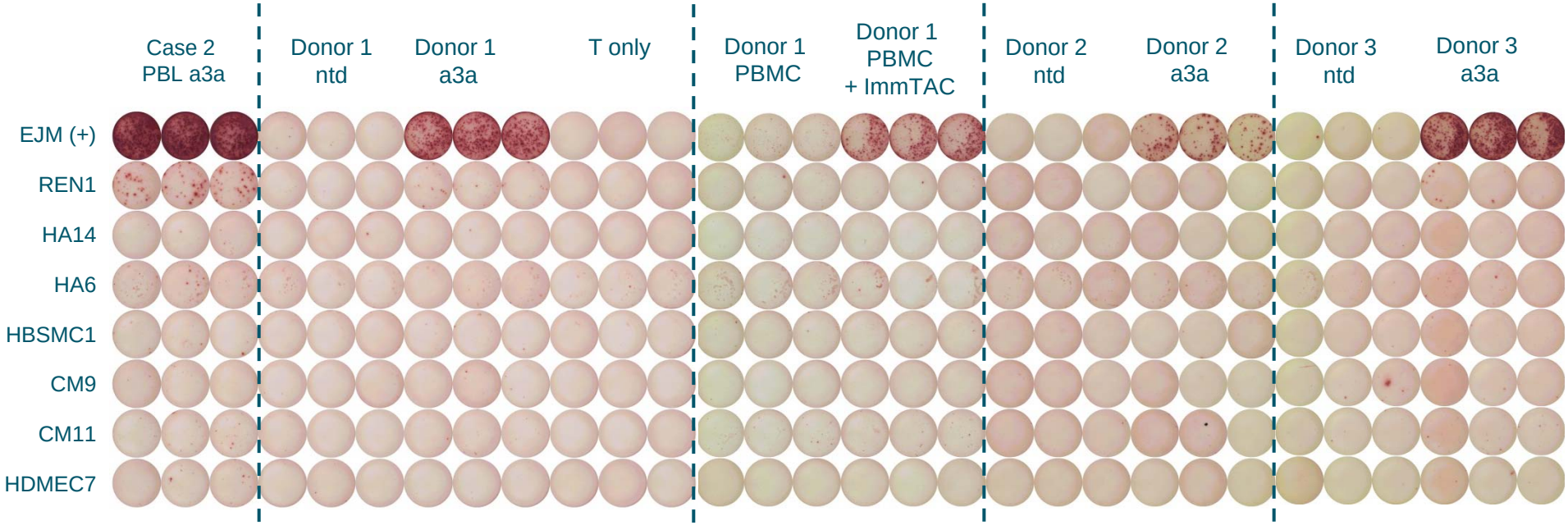
Mage-A3 IFN-γ ELISpot

- **Effectors:**
  - **Donor 1 (HLA-A1+) mixed CD8/4 transduced with MageA3 a3a or (non-transduced), expanded to d12 for cryopreservation. Defrosted for 2h prior to assay.**
  - **Donors 2 and 3 (A1+) PBL transduced and expanded to day 13 used fresh.**
  - **Case 2 pre-infusion a3a T cell product. 15,000/well. Defrosted for 2h prior to assay**
  - **Donor 1 (A1+) PBMC 25,000/well +/- 0.1nM IMCmage1 (mTCR)**
- **Targets**
  - **EJM multiple myeloma from defrost (HLA-A1+), 50,000/well**
  - **Variety of primary normal cells (all HLA-A1+), 50,000/well, defrosted for approx 1hr prior to assay**
  - **Case 2 autopsy tissues (single cell suspension 50,000/well where possible)**

EJM	Multiple myeloma (positive control)
REN1	Renal epithelial cells
HA14	Astrocytes
HA6	Astrocytes
HBSMC1	Bronchial smooth muscle cells
CM9	Cardiac myocytes
CM11	Cardiac myocytes
HDMEC7	Dermal microvascular endothelial cells
HEP1	Hepatocytes
HEP4	Hepatocytes
N5	Melanocytes
N3	Melanocytes
N10	Melanocytes
HEP2	Hepatocytes
HDMEC3	Dermal microvascular endothelial cells
HDF9	Dermal fibroblasts
HDF11	Dermal fibroblasts
Heart	01411-100 autopsy
Kidney	01411-100 autopsy
Liver	01411-100 autopsy
Skeletal Muscle	01411-100 autopsy
Brain	01411-100 autopsy

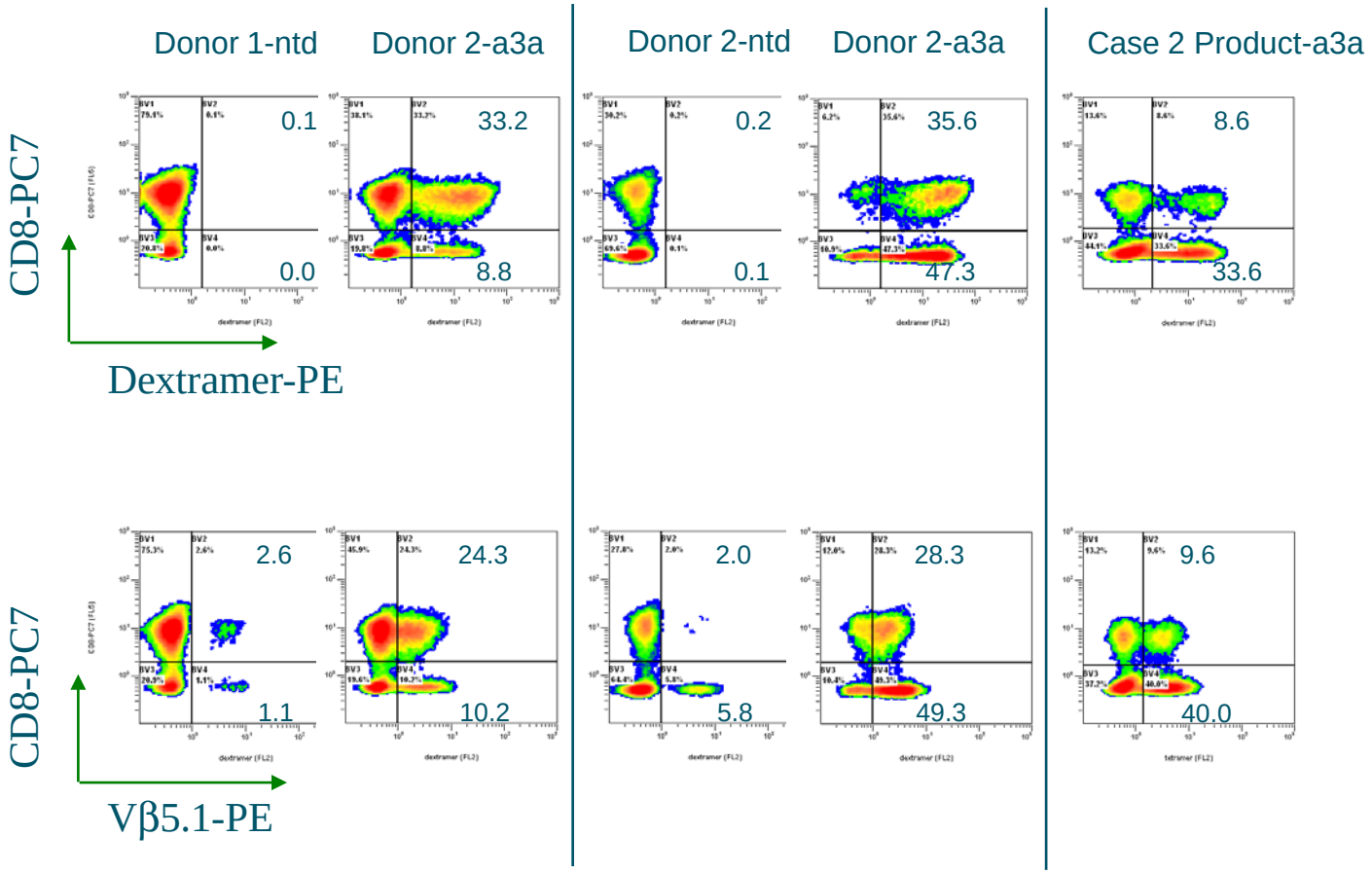
Mage-A3 IFN-γ ELISpot

- Evaluate the effect of IFN-γ and chemotherapy on Mage-A3 expression in heart and other tissues



Mage-A3 SAE – Initial Mixed Dimer Studies

- Comparison of Mage-A3 dextramer staining vs Vbeta5.1 staining



Interim Summary: Mage-A3 SAE

- Program on hold and will be stopped unless “cleared”
- HLA A2 restricted MAGE A3 TCR trial at NCI stopped due to unexpected CNS toxicity (clinicaltrials.gov NCT01273181)
- Case 1 and Case 2 have similarities and differences
  - Mechanisms appear to differ
  - Demand ischemia and unrecognized severe ASCVD in Case 1, while direct cardiac toxicity implicated in Case 2
- What we know
  - TCR transduced T cells expanded
  - T cells expressed MAGE A3 TCR and trafficked to tissues, including heart
  - Cytokine storm not the cause of death
  - Target is not expressed on normal heart
- What we don’t know
  - Is this an on target effect?
  - Is the target upregulated in response to IFN-γ or chemotherapy?
  - Is the etiology of each event similar?
  - Is this a “class effect” with MAGE A3 TCRs?

Conclusions and Implications

- NY-ESO-1 TCRs are safe with encouraging results to date
- Consider revision of protocol exclusion criteria for future trials that employ potent T cell Rx compared to standard chemotherapy regimens
- Current in vitro studies are <u>not</u> predictive of in vivo outcomes
- Off target effects are established only in Phase I trials
- Dose reduction may not ameliorate risk and may only delay onset of toxicity
- Toxicity management: corticosteroids did not ablate toxicity in case #2. Would suicide systems or other forms of abort toxicity?