

**Session III: Dilemmas and Challenges:
Approaches and Assessments**

**Session III
Current Status of Cancer Immunotherapy:
Trials, Results, and Challenges**

University of Pennsylvania

September 10, 2013



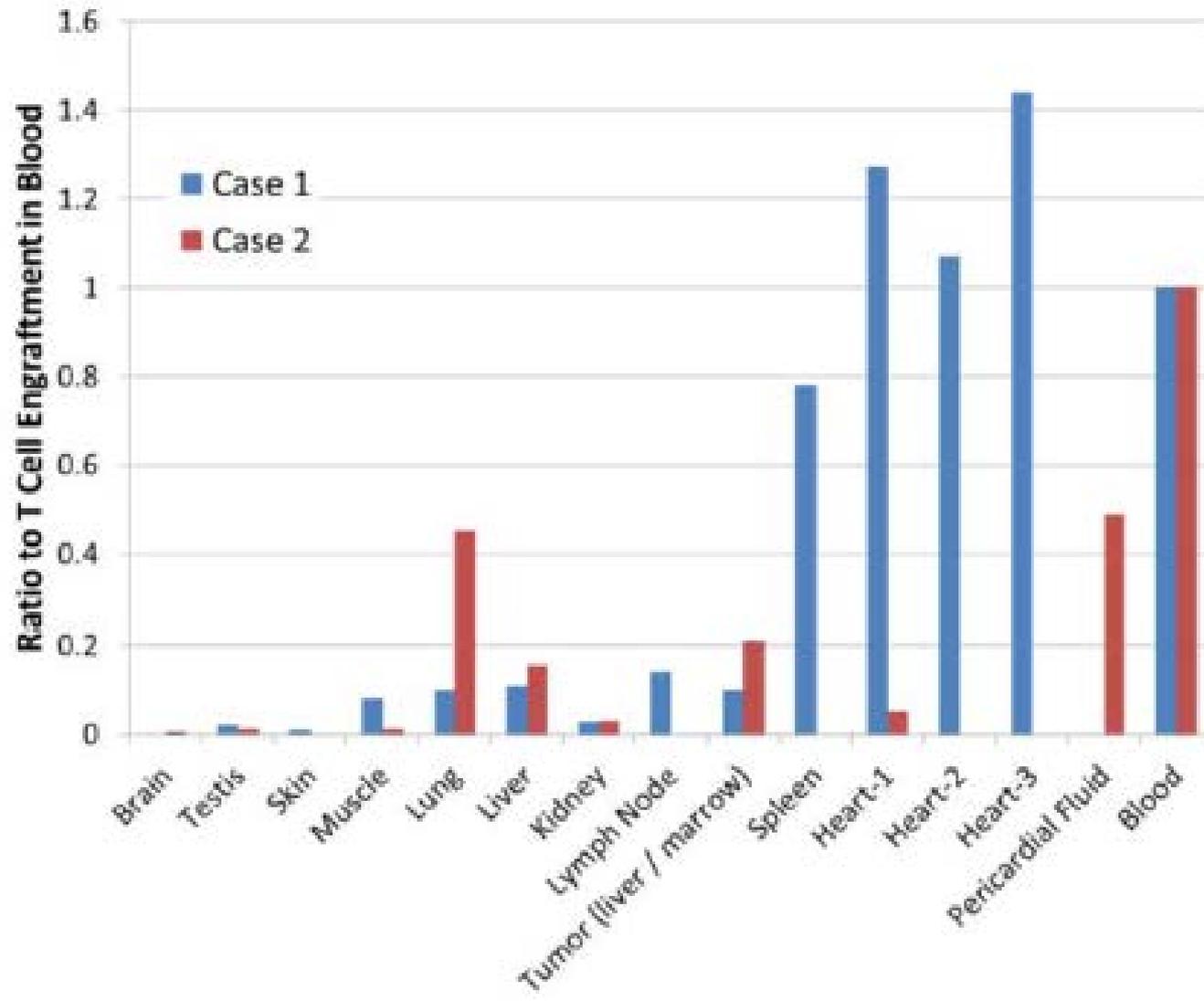
MAGE A3 TCR Protocols

- **Affinity enhanced HLA A1 restricted TCR for MAGE A3/A6 for melanoma (#1007-1056) and myeloma (#1007-1056)**
- **Product: affinity enhanced TCR originally isolated from a patient with melanoma. Resulting TCR had Kd of 6.5 uM; parental TCR was ~300 uM**
- **Events: onset of severe cardiogenic shock on day 4 (pt #1, melanoma) and day 5 (pt #1, myeloma) after T cell infusion**

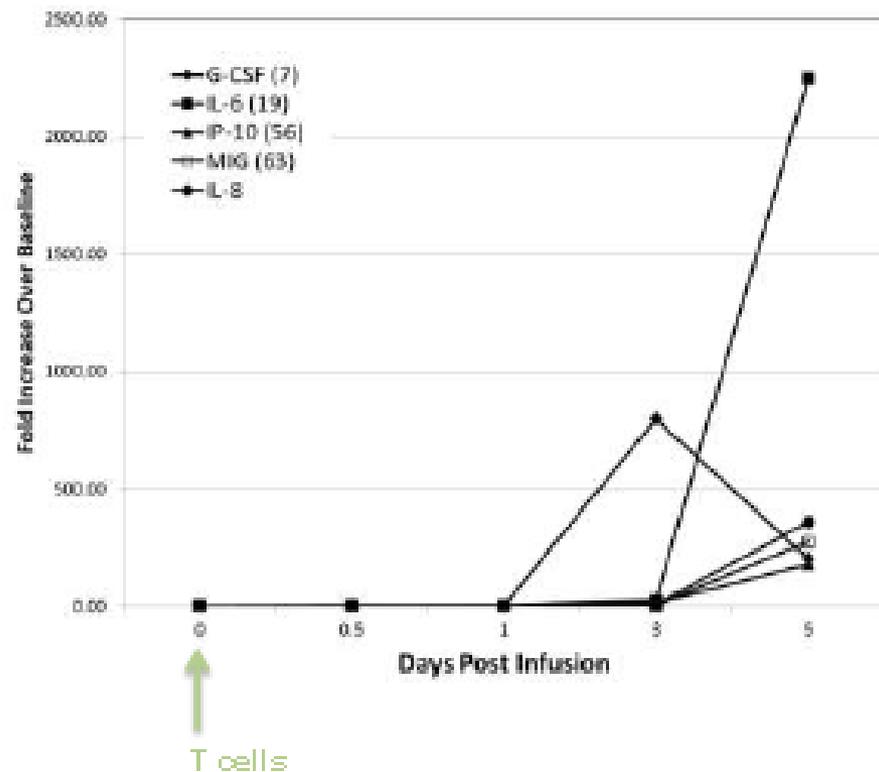
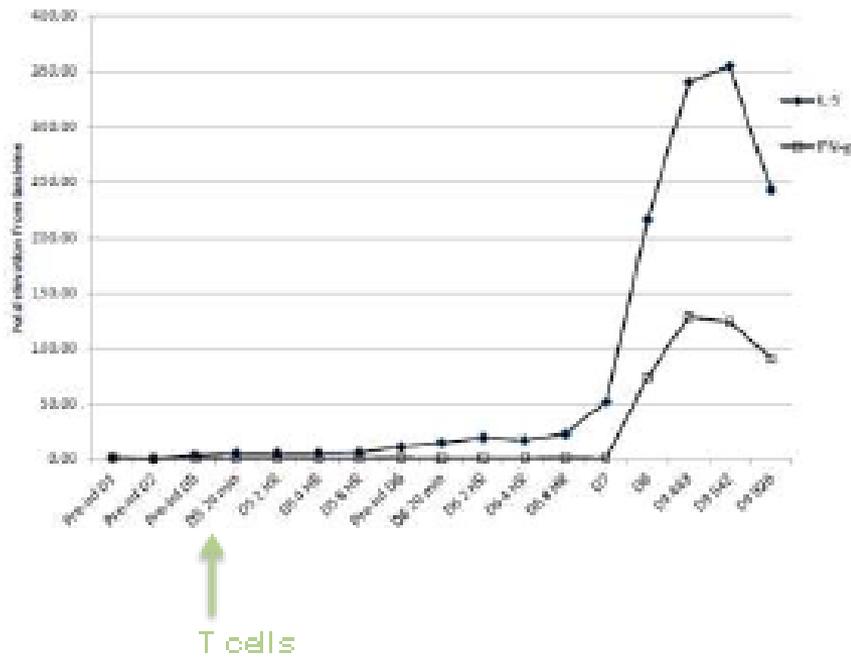
Pre-Clinical Evaluation of MAGE A3/A6 TCR Failed to Reveal Off Target Toxicity

- 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-1 α , MIP-1 β , IL-1 β , 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)
- In Vivo Efficacy
 - Demonstration of anti-tumor activity in vivo using clinical candidates in a NSG mouse model

MAGE A3 T Cells in Autopsy Tissues: qPCR



MAGE A3 T Cells: Cytokine Profile in Blood



- Case 1 – Melanoma
- IL-5 and IFN-gamma

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

Off Target Reactivity of the TCR for Titin

- **Summary of clinical course**

- Linette, G.P., E.A. Stadtmauer, M.V. Maus, A.P. Rapoport, B.L. Levine, L. Emery, L. Litzky, A. Bagg, B.M. Carreno, P.J. Cimino, G.K. Binder-Scholl, D.P. Smethurst, A.B. Gerry, N.J. Pumphrey, A.D. Bennett, J.E. Brewer, J. Dukes, J. Harper, H.K. Tayton-Martin, B.K. Jakobsen, N.J. Hassan, M. Kalos, and C.H. June. 2013. Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma. *Blood* 122:863-871.

- **Summary of laboratory investigation**

- Cameron, B.J., A.B. Gerry, J. Dukes, J.V. Harper, V. Kannan, F.C. Bianchi, F. Grand, J.E. Brewer, M. Gupta, G. Plesa, G. Bossi, A. Vuidepot, A.S. Powlesland, A. Legg, K.J. Adams, A.D. Bennett, N.J. Pumphrey, D.D. Williams, G. Binder-Scholl, I. Kulikovskaya, B.L. Levine, J.L. Riley, A. Varela-Rohena, E.A. Stadtmauer, A.P. Rapoport, G.P. Linette, C.H. June, N.J. Hassan, M. Kalos, and B.K. Jakobsen. 2013. Identification of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3–Directed T Cells. *Science Translational Medicine* 5:197ra103.

Lessons Learned: Engineered TCRs

- ❑ First example of off-target effects with TCR-engineered T cells
 - ❑ Affinity enhanced TCR engineered T cell therapy at risk for cross-reactivity
 - ❑ Biologically relevant preclinical screening of new TCRs is critical
- ❑ Dose reduction may not ameliorate risk and may only delay onset of toxicity (due to in vivo T cell expansion)
- ❑ Toxicity management: corticosteroids did not ablate toxicity in case #2. Would suicide systems or other forms abort toxicity?
- ❑ NY-ESO-1 TCRs are safe with encouraging results to date

Lessons Learned: Engineered TCRs II

- ❑ Preclinical screening of all available HLA A1 cell lines did not identify the reactivity
- ❑ Current in vitro studies are not predictive of in vivo outcomes with TCRs. CARs have a more predictable to pre-clinical evaluation
- ❑ Off target effects are established only in Phase I trials
- ❑ Mouse models currently available do not predict reactivity
- ❑ iPSCs have promise for preclinical screens