

Current Status of T-Cell Adoptive Transfer for Advanced Cancer

James Yang
Surgery Branch, CCR, NCI

Adoptive T-Cell Therapy

- The goal is to generate the largest number of tumor-reactive T-cells in patients with cancer
- This approach transfers cultured tumor-reactive T-cells, expanded in vitro, into a properly prepared recipient
- Allows manipulation of the host to minimize inhibitory factors and to support the transferred T-cells

Adoptive T-Cell Therapy: Two Basic Flavors

Naturally occurring T-cells: Tumor Infiltrating Lymphocytes (TIL)

- Melanoma

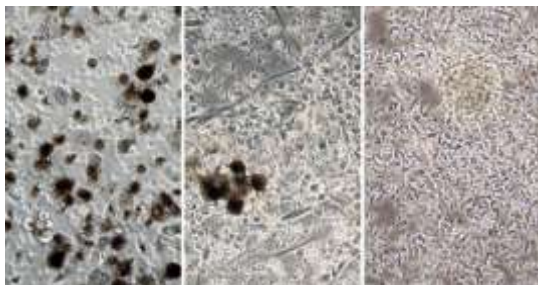
Genetically engineered T-cells: Modifying peripheral blood lymphocytes to introduce new functions and specificities

- Classical MHC-restricted T-cell receptors
- Chimeric (antibody-based) antigen receptors (CARs)

Lessons from Melanoma

- Melanoma is unique in that most patients with melanoma harbor tumor-reactive infiltrating lymphocytes in their metastatic lesions
- These tumor infiltrating lymphocytes (TIL) are a consistent source of T-cells to expand in culture and administer
- Therefore most of the principles known about autologous T-cell transfer were learned from giving TIL to patients with melanoma

Melanoma TIL (Tumor Infiltrating Lymphocytes)



Fresh digest

One week

Two weeks

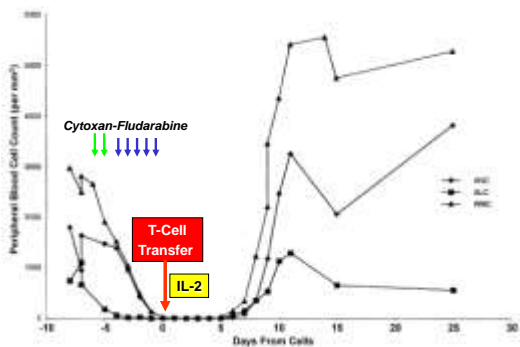
TIL History

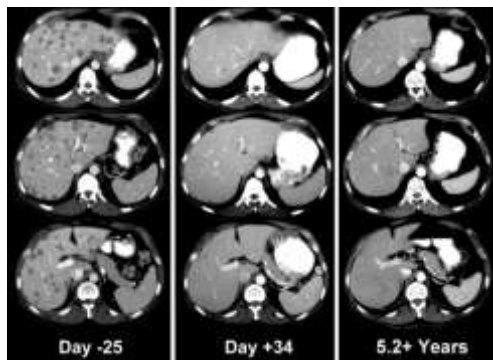
- Simply infusing large numbers of TIL expanded in vitro along with supportive IL2 caused a third of patients with metastatic melanoma to respond, but responses were often brief
- Survival of transferred T-cells in vivo was extremely low
- Mouse models showed that efficacy was reduced by:
 - Regulatory (inhibitory) T-cells
 - Low endogenous levels of supportive cytokines
 - Competition for those cytokines by other T-cells

Lesson #1

- Immunosuppressing the host immediately before adoptive T-cell transfer improves survival of transferred cells and increases efficacy
- This is because:
 - Endogenous regulatory T-cells are deleted
 - Lymphopenia stimulates IL-7 and IL-15 to be produced from non-lymphoid sources to 'restore' lymphocytes to normal
 - There are fewer bystander immune cells competing for and consuming those cytokines

Cyclophosphamide + Fludarabine Non-Myeloablative (NMA) Chemotherapy

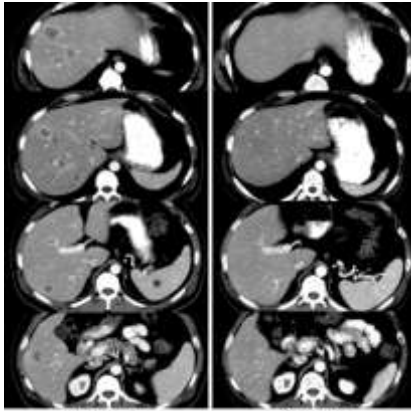






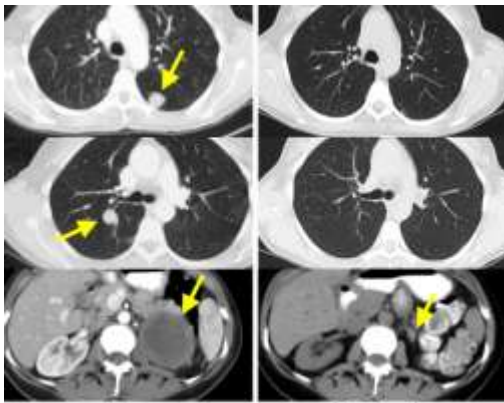
Pre-Treatment

12 Days after Cell Transfer



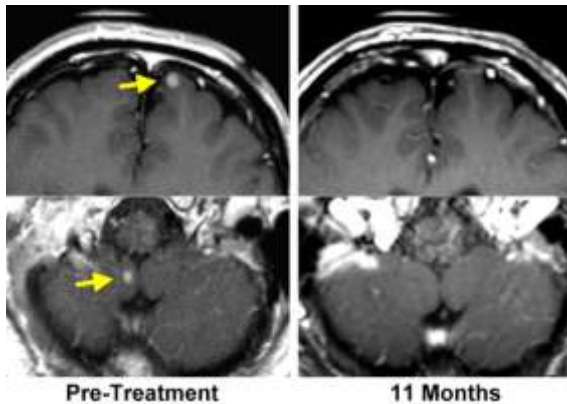
Pre-Treatment

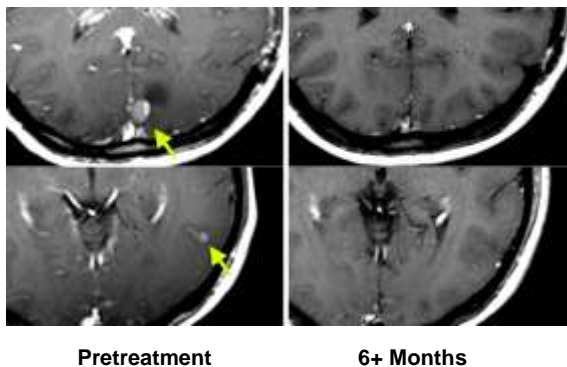
27+ Months



Pre-Treatment

32+ Months



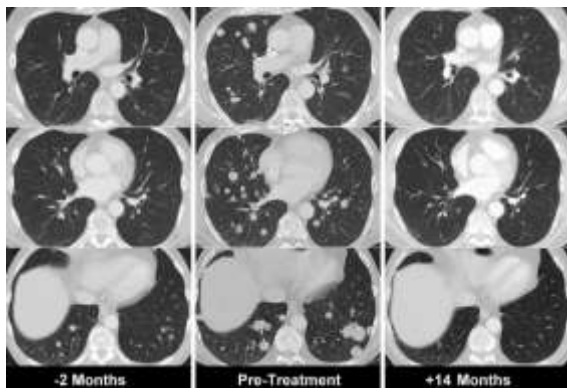


TIL Transfer Therapy
(Response Duration- Months)

Pre-Treatment	Total	PR	CR	OR (%)
Cy/Flu	43	16	5	21 (49%)
		(84, 36, 29, 28, 14, 13, 11, 8 8, 7, 4, 3, 3, 2, 2, 2)	(95+, 82+, 79+, 78+, 64+)	
C/F + 200 TBI	25	8	5	13 (52%)
		(14, 9, 6, 6 5, 4, 3, 3)	(68+, 64+, 60+, 57+, 54+)	
C/F + 1200TBI	25	8	10	18 (72%)
		(21, 13, 7, 6, 6, 5, 4, 3)	(48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19)	

Newer TIL Trials

- Rapidly growing TIL without in vitro reactivity testing (and treating all patients) is an approach called "Young TIL"
 - Can also cause durable major regressions
 - Marginally lower RR, but much easier
 - Other institutions have been able to begin this (Ella Institute, Israel, MDACC, Moffitt Cancer Center)
 - Randomized Phase II trial enriching Young TIL for CD8 cells did not improve efficacy



Newest TIL Protocol

- Intensifying preparative host immunosuppression
 - The best outcome in Phase II TIL studies was from adding 1200 cGy TBI (and CD34 ASCS) to Cy-Flu
 - The overall RR was 72% with 40% CRs and only one of these 10 CRs relapsed (all with >4 yrs F/U)
 - Murine models show that intensifying preparative TBI increases ACT efficacy and CD34 BMSC also contribute
- **Accruing: Randomized trial of TIL with Cy-Flu versus 1200 TBI + Cy-Flu**

Questions

- What about adoptive therapy for melanoma patients who cannot grow TIL?
- What about adoptive therapy for patients with non-melanoma tumors?

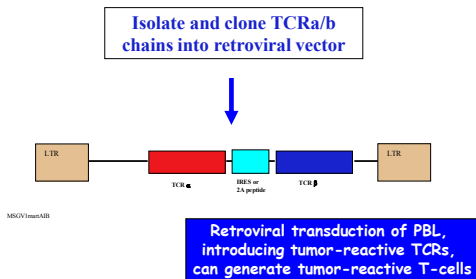
Applying Adoptive Cell Transfer to Other Patients/Cancers

- Target other cancers which fortuitously express melanoma-associated antigens
- Capture rare T-cells which react with other cancers and clone their T-cell receptors (TCR) for engineering into any patient's T-cells
- Introduce chimeric antigen receptors (CAR) into PBL which bind tumors with antibodies but activate using normal T-cell signaling machinery

T-Cell Gene Engineering

Redirecting T-Cell
Recognition and Improving
T-Cell Capabilities

T-Cell Receptor (TCR) Gene Therapy



REPORTS

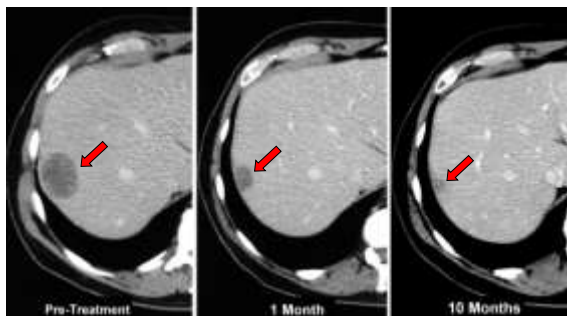
Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

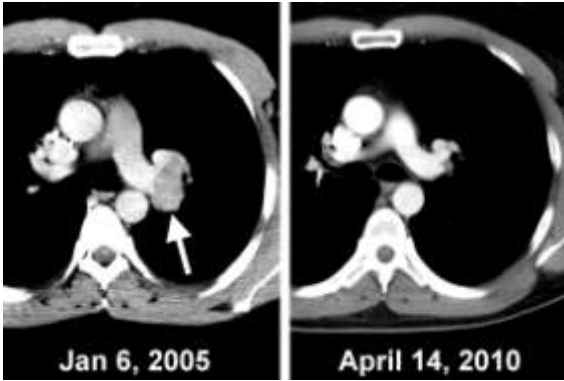
Richard A. Morgan, Mark E. Dudley, John H. Wunderlich, Margareth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Uday S. Kamnitsas, Nicholas P. Restifo, Zhili Zheng, Azam Wahvi, Christiana R. de Vries, Linda J. Rogers-Freer, Sharon A. Mansueti, Steven A. Rosenberg*

Through the adoptive transfer of lymphocytes after host immunodepletion, it is possible to mediate objective cancer regression in human patients with metastatic melanoma. However, the generation of tumor-specific T cells in this mode of immunotherapy is often limiting. Here we report the ability to specifically confer tumor reactivity by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a T cell receptor. Adoptive transfer of these transfected cells in 33 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. The observed high sustained levels of circulating, engineered cells at 2 year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions. This study suggests the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

Science, Oct 2006

Tumor Regression After Receiving TCR-Transduced T Cells Against MART-1





Objective RR in 32 pts= 13%

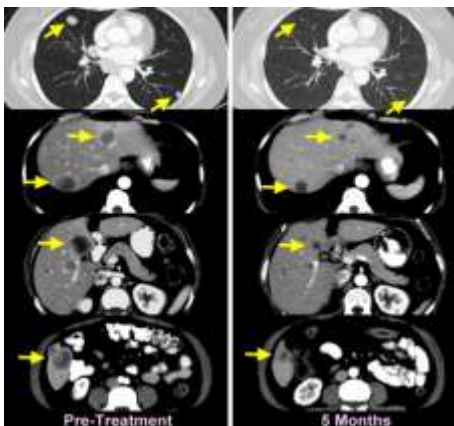
F5 High-Avidity Anti-MART1 TCR



F5 High-Avidity Anti-MART-1 TCR



PBL
With High
Avidity
Murine
Anti-
GP100
TCR



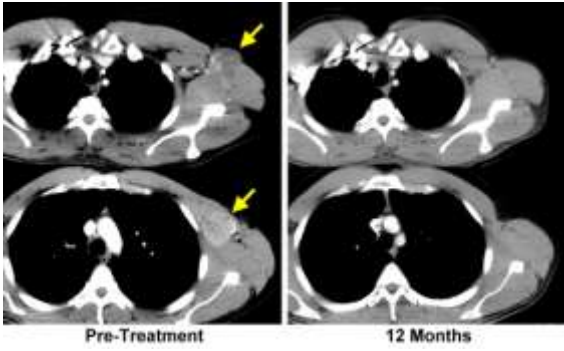
NY-ESO-1

- Tumor-testis antigen originally found in an esophageal cancer
- Expressed in 10-20% of melanomas
- Also found in myeloma and many common adenocarcinomas (eg. breast, pancreas)
- Expressed in 80% of synovial sarcomas
- The TCR from an HLA-A2-restricted T-cell recognizing NY-ESO-1 was cloned

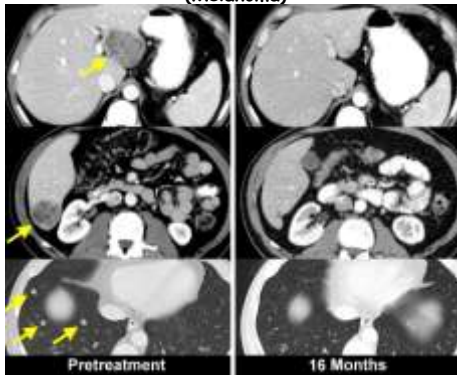
Adoptive Therapy with Gene-Engineered Lymphocytes: Anti-NY-ESO-1 TCR

- 12 patients with melanoma treated with 11 evaluable
 - 2 CR (15+, 17+ mo)
 - 3PR (8, 4+, 3 mo)
- 6 patients with synovial sarcoma treated with all evaluable
 - 4 PR (14+, 10, 7+, 5 mo)

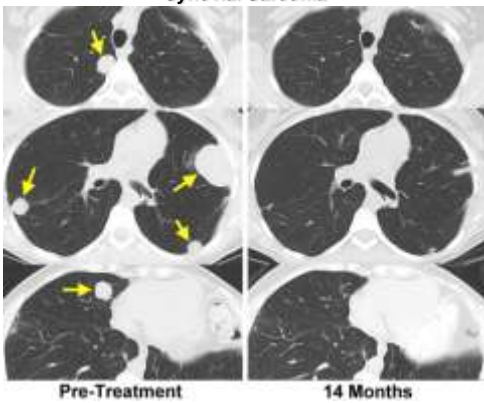
**Gene Therapy with Anti-NY ESO-1 TCR
(Melanoma)**



**Gene Therapy with Anti-NY ESO1 TCR
(Melanoma)**



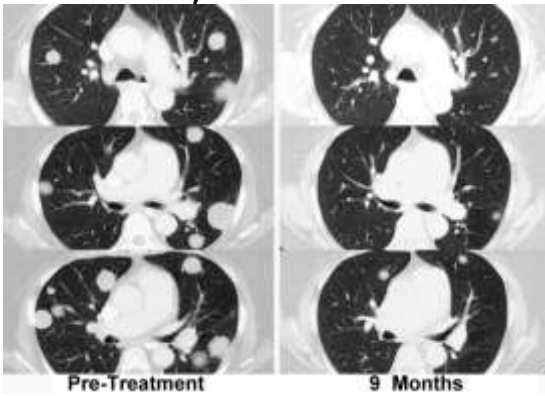
Synovial Sarcoma



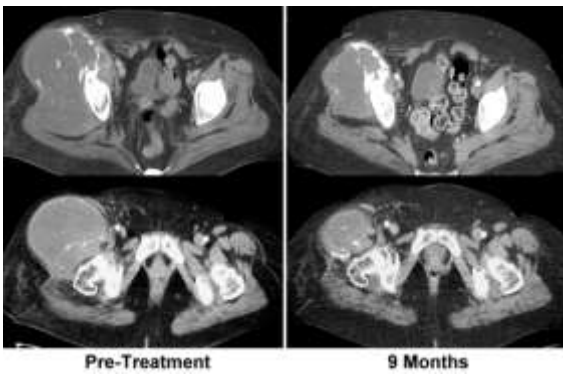
Patient #6 with Synovial Sarcoma



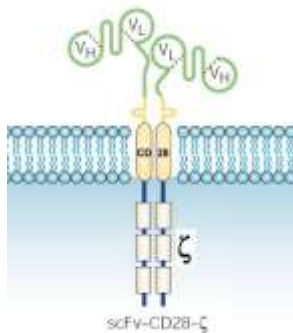
Synovial Sarcoma



Synovial Sarcoma

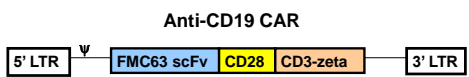


Chimeric Antigen Receptor (CAR)



T cells genetically engineered to express an anti-CD19 chimeric antigen receptor

Retroviral vector combining an extracellular domain from an anti-CD19 antibody with the CD3-zeta signaling protein



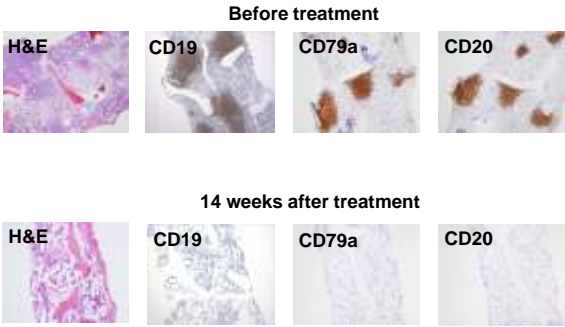
CD19 is expressed on CLL cells and most follicular lymphomas

Pre-infusion IFN-gamma ELISA

Effector cells	CD19-expressing targets			CD19-negative targets		Effectors alone
	Toledo	Nalm6	CD19-K562	NGFR-K562	CCRL-CEM	
anti-CD19 CAR-transduced	2180	4765	48050	581	193	110
Not transduced	63	70	59	66	66	31

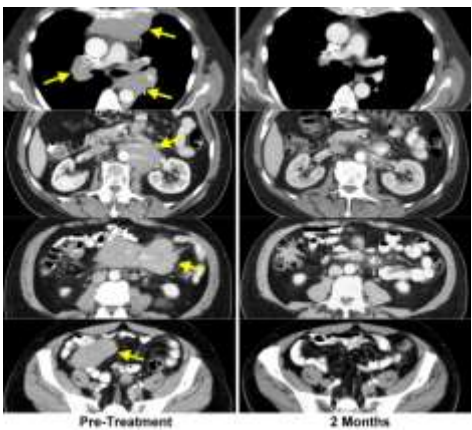
(units pg/mL)

Bone marrow biopsies were performed before and 14 weeks after treatment



B-Cell Lymphoma: Chimeric Receptor Fusing Anti-CD19 and CD3-zeta





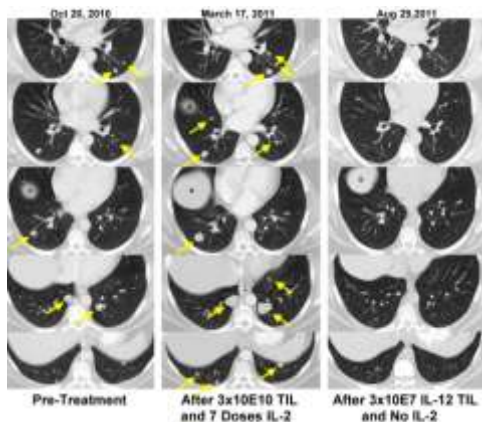


Summary

- Three alternative approaches to engineering tumor-reactive T-cells for adoptive therapy have been tested in very early clinical trials and all have shown evidence of anti-tumor activity
 - Cross-targeting melanoma-associated Ags
 - Transducing patient PBL with TCRs raised in HLA-transgenic mice
 - Using MoAb-based chimeric antigen receptors

Future Directions

- Another protocol using a CAR against VEGF-R2 (KDR) has also started accruing patients
- A protocol to use systemic IL-15 to support T-cell transfer has been approved
- Engineering a T-cell to secrete IL-12 enhances its function in mice (fewer cells are equally effective)



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