Adoptive Immunotherapy

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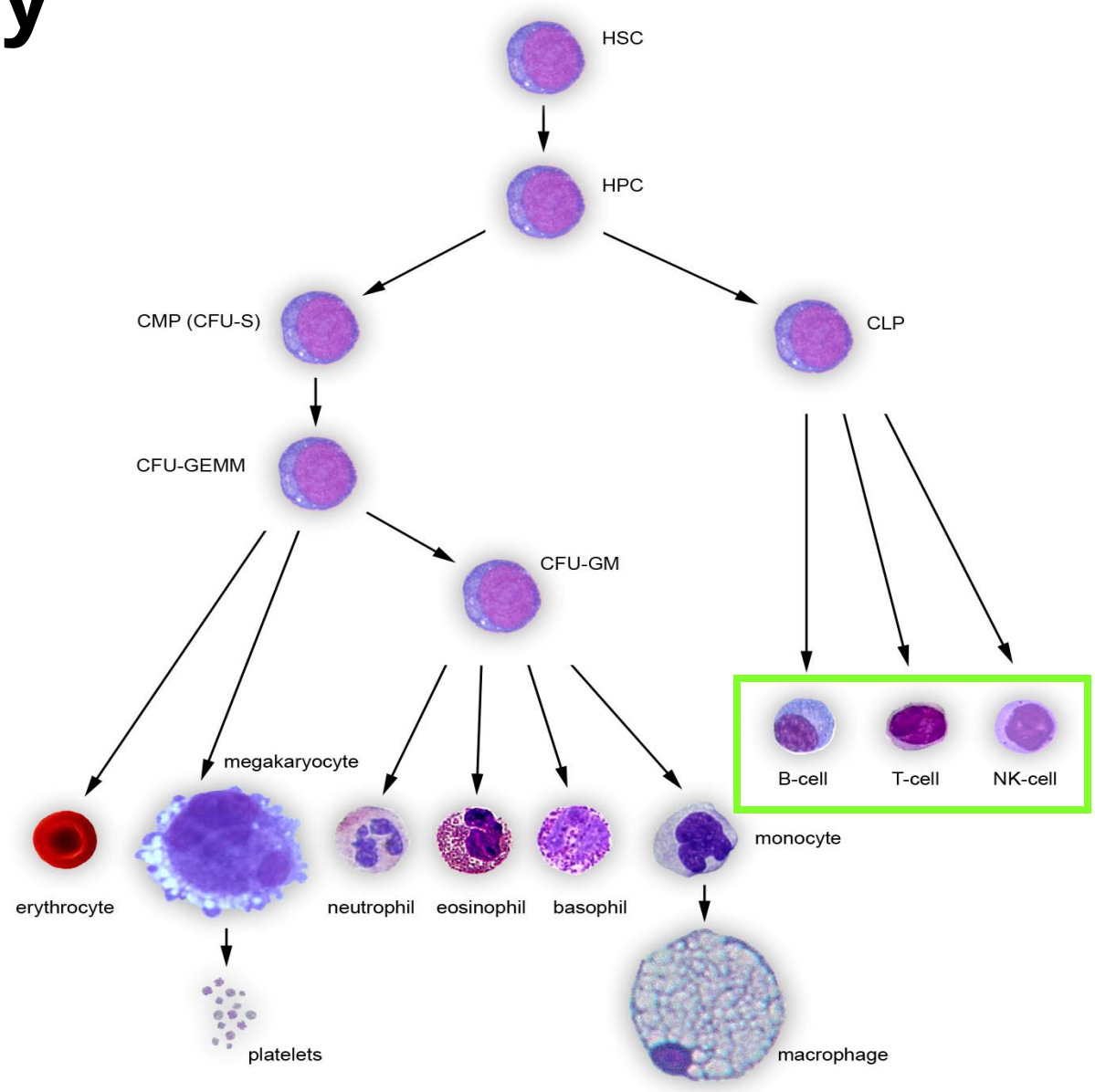
Cell Therapy

- Red cells
- Stem cells
- Platelets



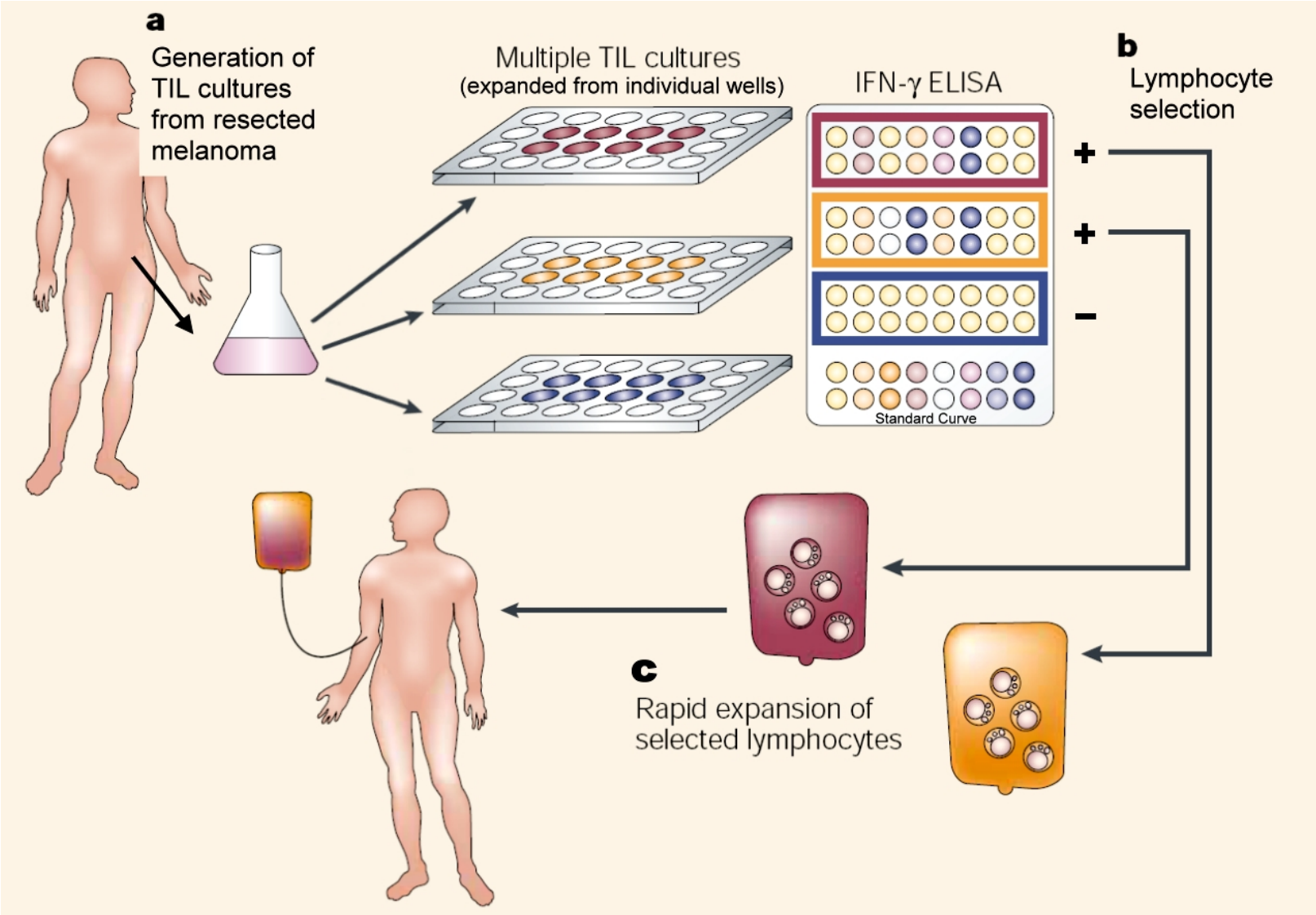
Adoptive T Cell Transfer

- Potent therapy
- Unique research tool
  - Host lymphodepletion
  - Lymphocyte attributes
  - Tumor antigen targets

Examples of Successful Adoptive Immunotherapy

Target	T cells	Reference
PTLD	Viral antigen specific	Heslop, 2009
Viral infection	Viral specific (uncultured)	Feuchtinger et al, 2010
B cell tumor	Genetically retargeted	Kalos et al 2011
Melanoma	Tumor infiltrating lymphocytes	Rosenberg et al 2011
Sarcoma	Genetically retargeted	Robbins et al 2011

Adoptive Cell Therapy with TIL for Melanoma



Dudley et al, Nat Rev Ca, 2003

ACT with Lymphodepletion

- Expand T cells to large numbers in the absence of potentially suppressive tumor environment
- Selection of optimal T cell attributes
- Manipulation of host immune system without damaging the effector T cells

Ensuring a Safe Product for ACT

All cells undergo extensive safety and quality testing to produce a certificate of analysis (COA) for each final product prior to infusion

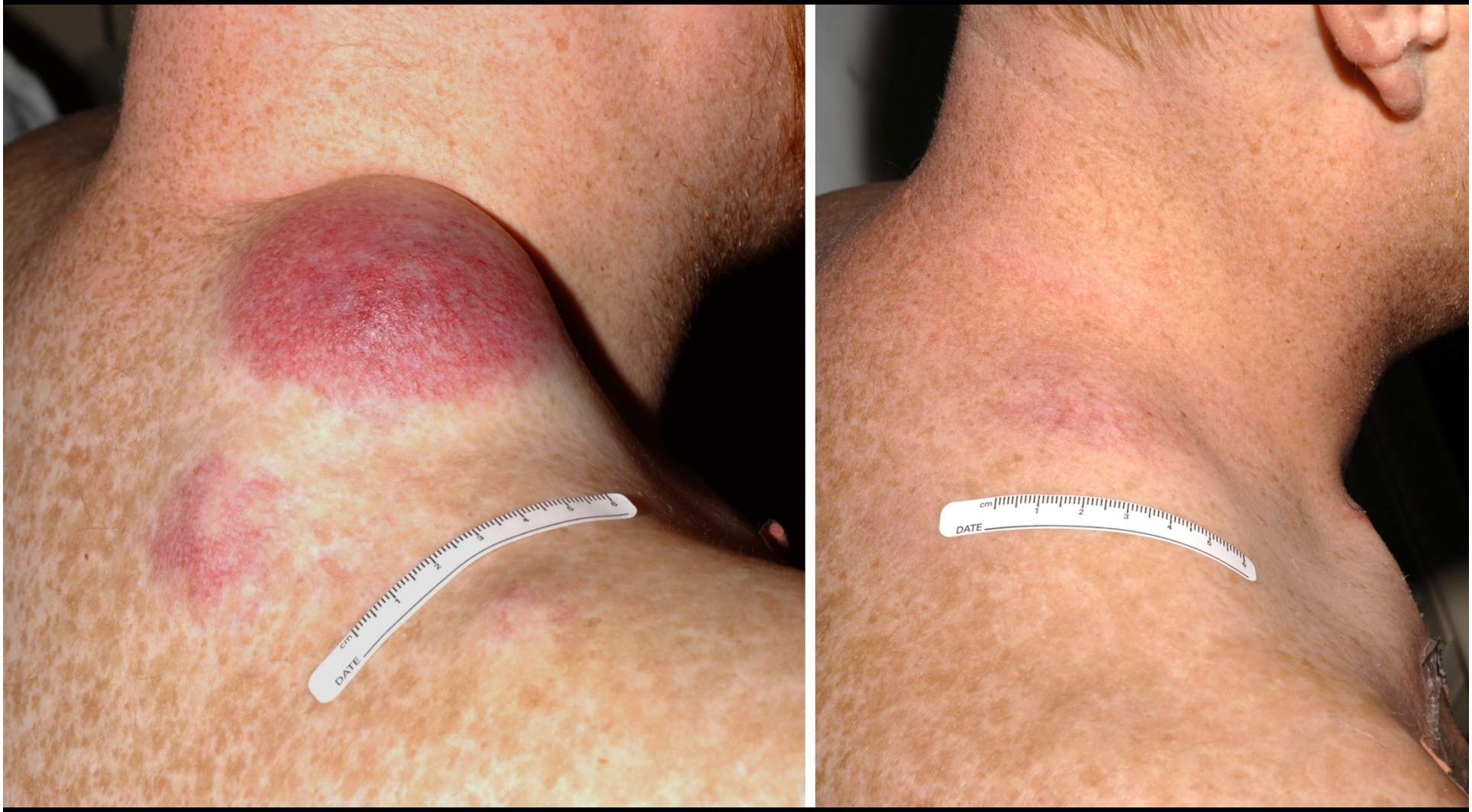
COA Criteria:

- Viable cell count
- Tumor recognition
- Gram stain
- Fungal testing
- Mycoplasma testing
- Bacterial testing
- Endotoxin testing
- Cytological analysis
- TCR expression
- RCR

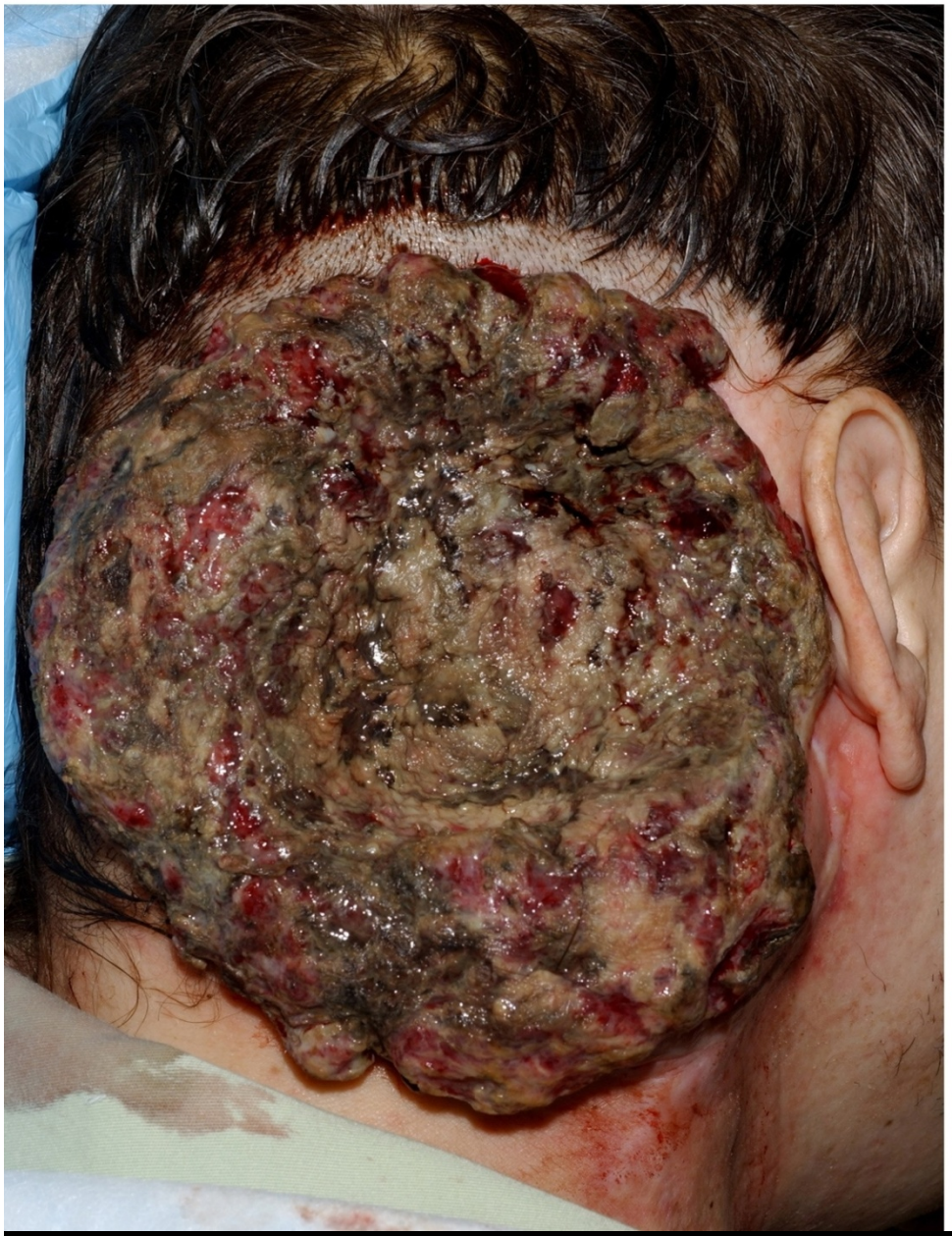
www.piercenet.com/media/cytokine_kit.gif
www.daigger.com http://isips.org/products/graphics/12a.gif

C.K. (200cGy) Pre

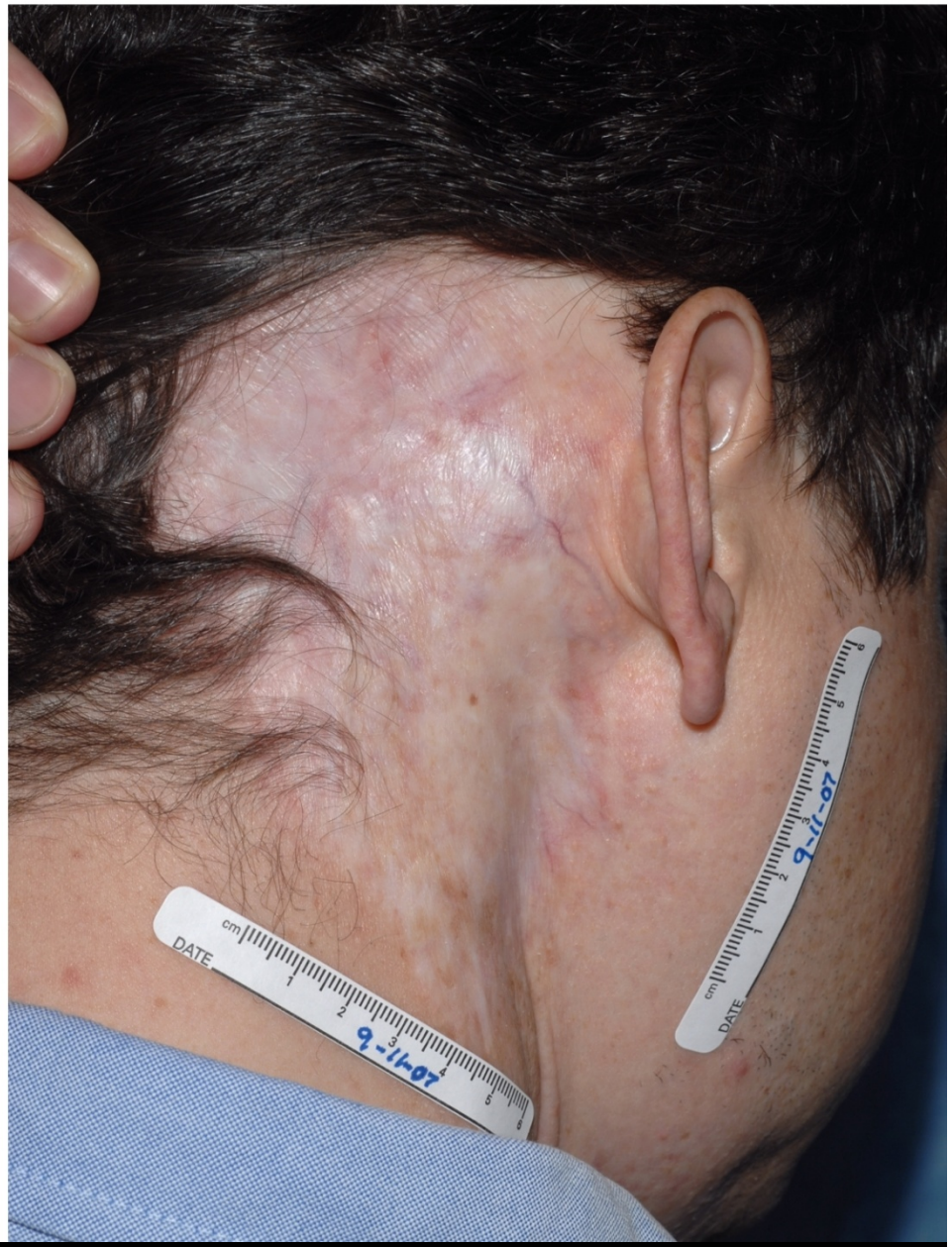
12 days

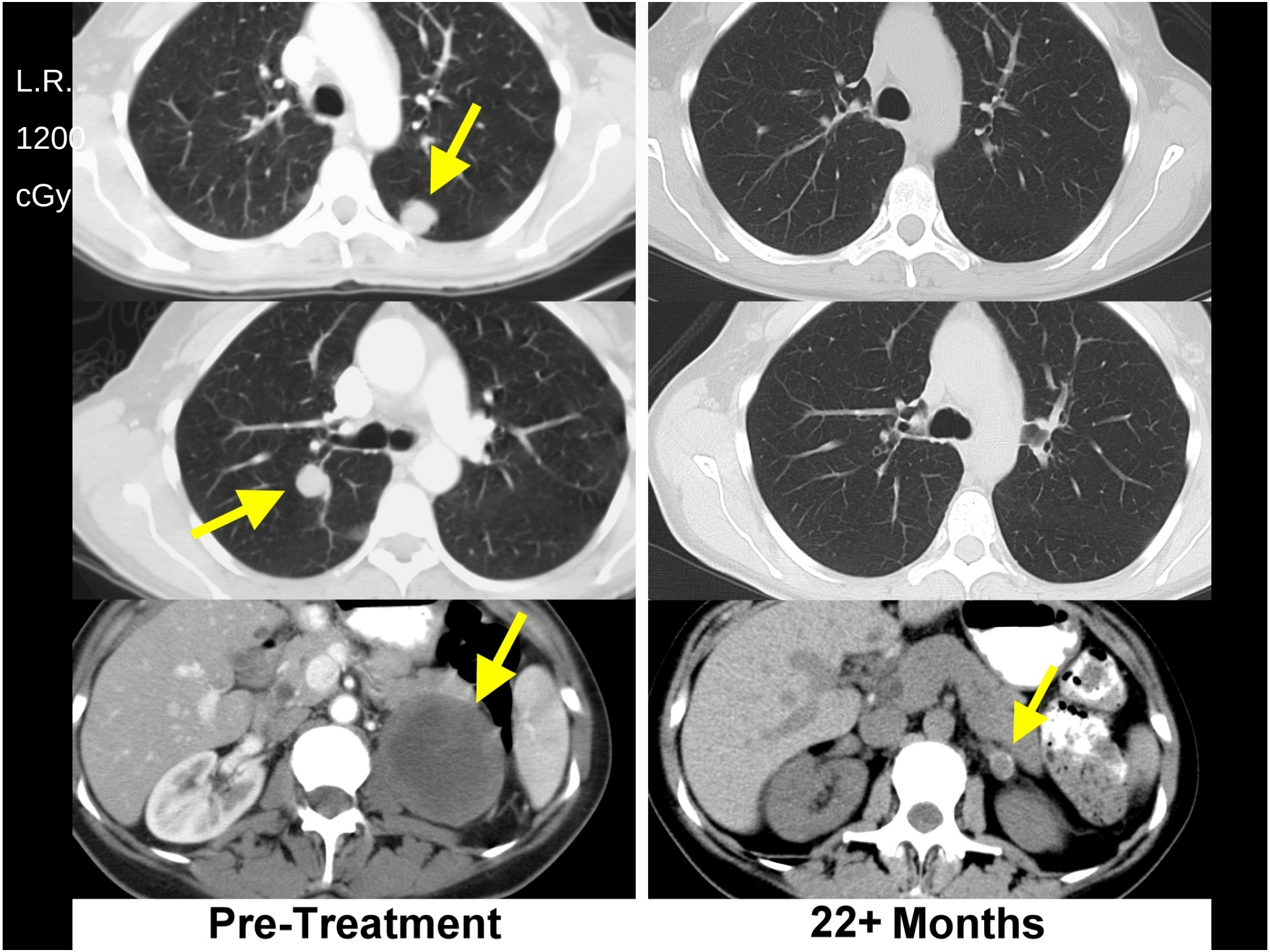


Pre-Treatment



30+ Months



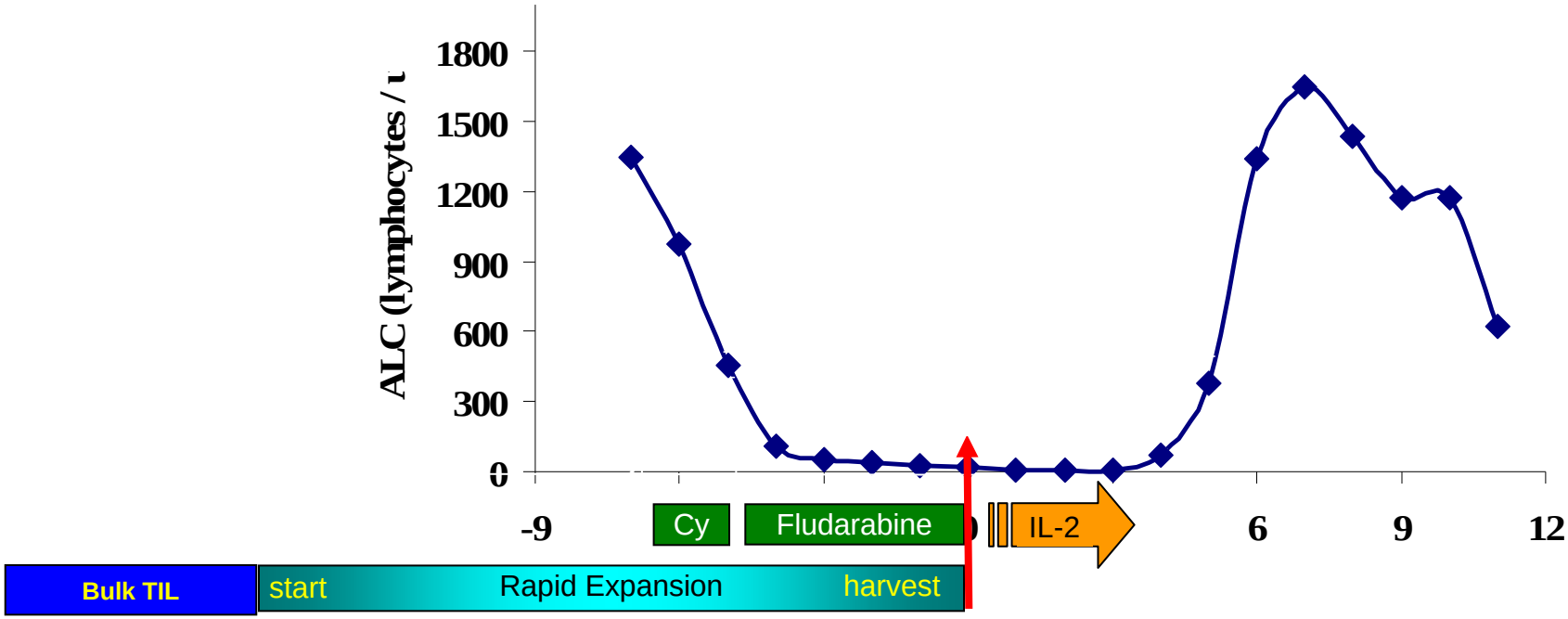

L.R.
1200
cGy
Pre-Treatment
22+ Months

- **ACT with lymphodepletion**
  - **Responses can be rapid**
  - **No relation between bulk of disease and response**
  - **Impact metastatic disease at any site**

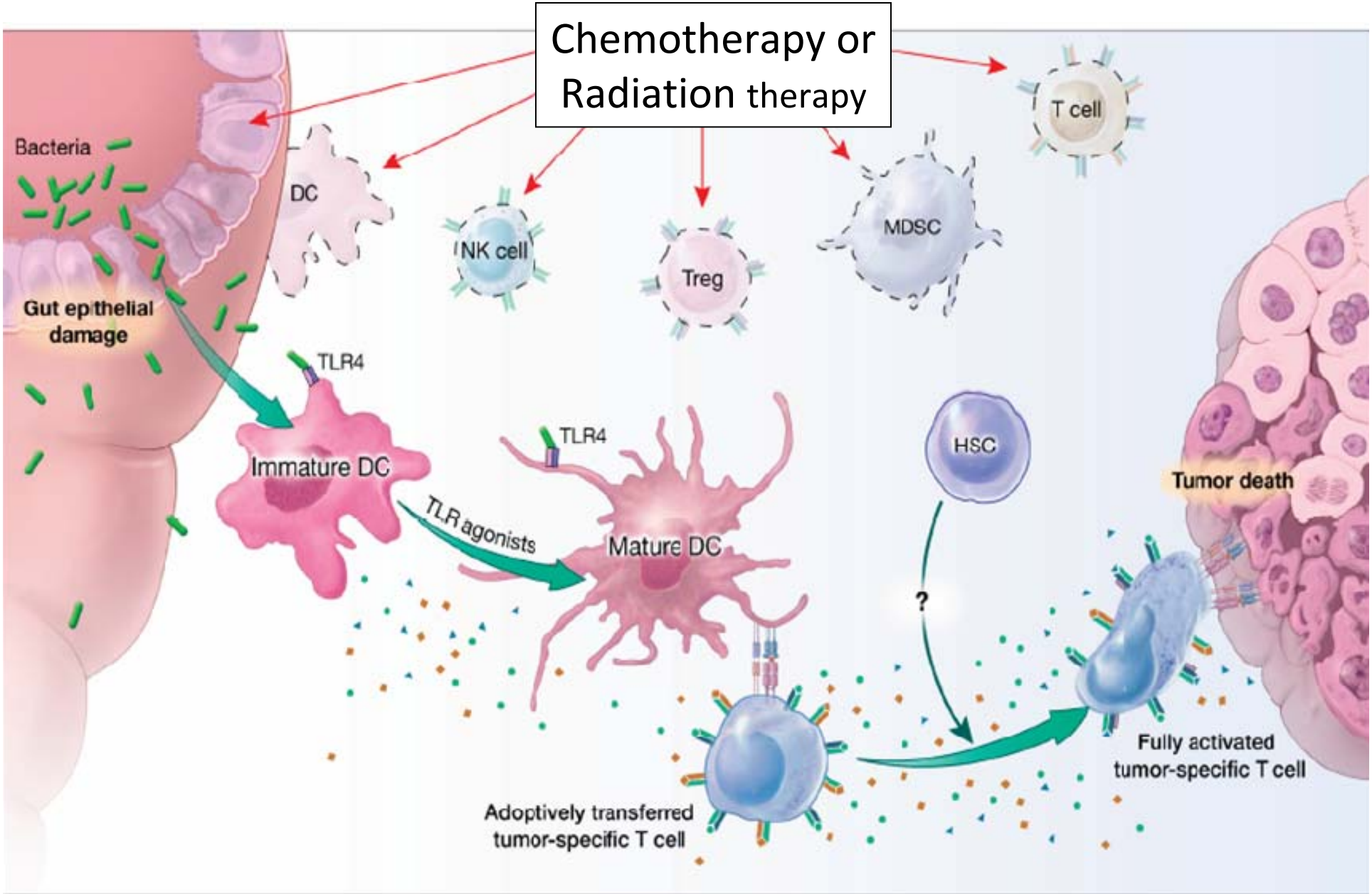
Adoptive T Cell Transfer

- **Potent therapy**
- **Unique research tool**
  - **Host lymphodepletion**
  - **Lymphocyte attributes**
  - **Tumor antigen target**

Adoptive Cell Therapy
Selected T cells
Lymphodepletion



Mechanisms underlying the impact of lymphodepletion on adoptively transferred T cells

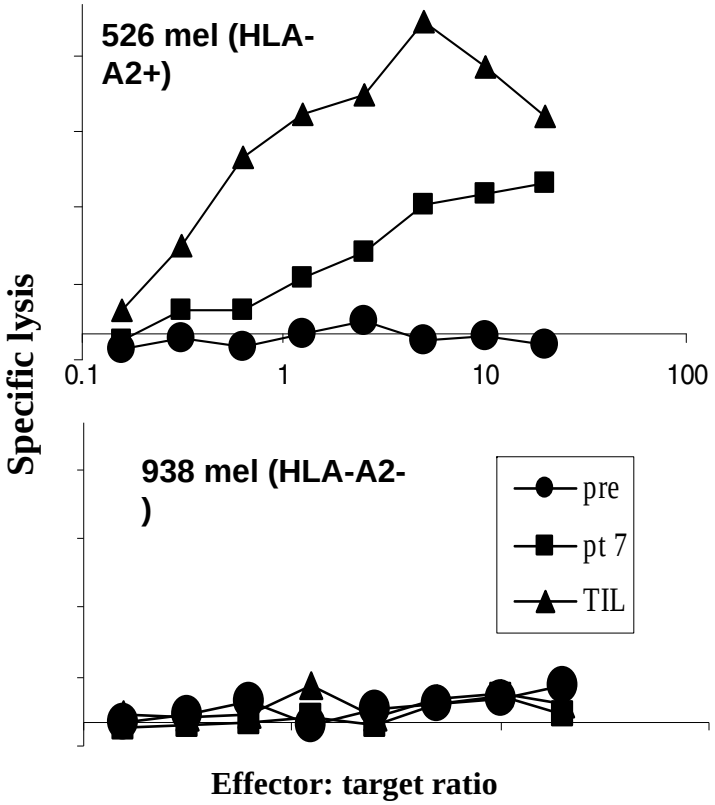


Lymphodepletion

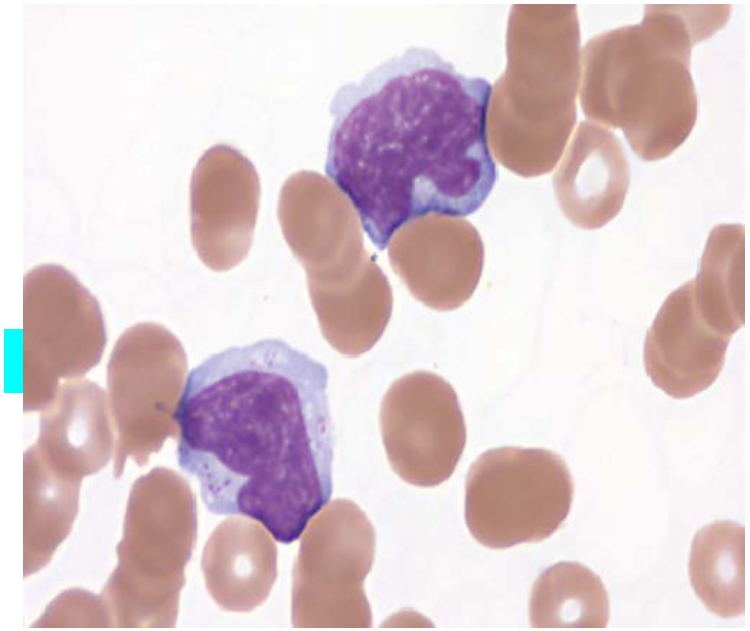
- Elimination of T_{REG}
- Increased homeostatic cytokines
- APC activation through TLR
- Stem cell “facilitation”

T cells are active *in vivo*

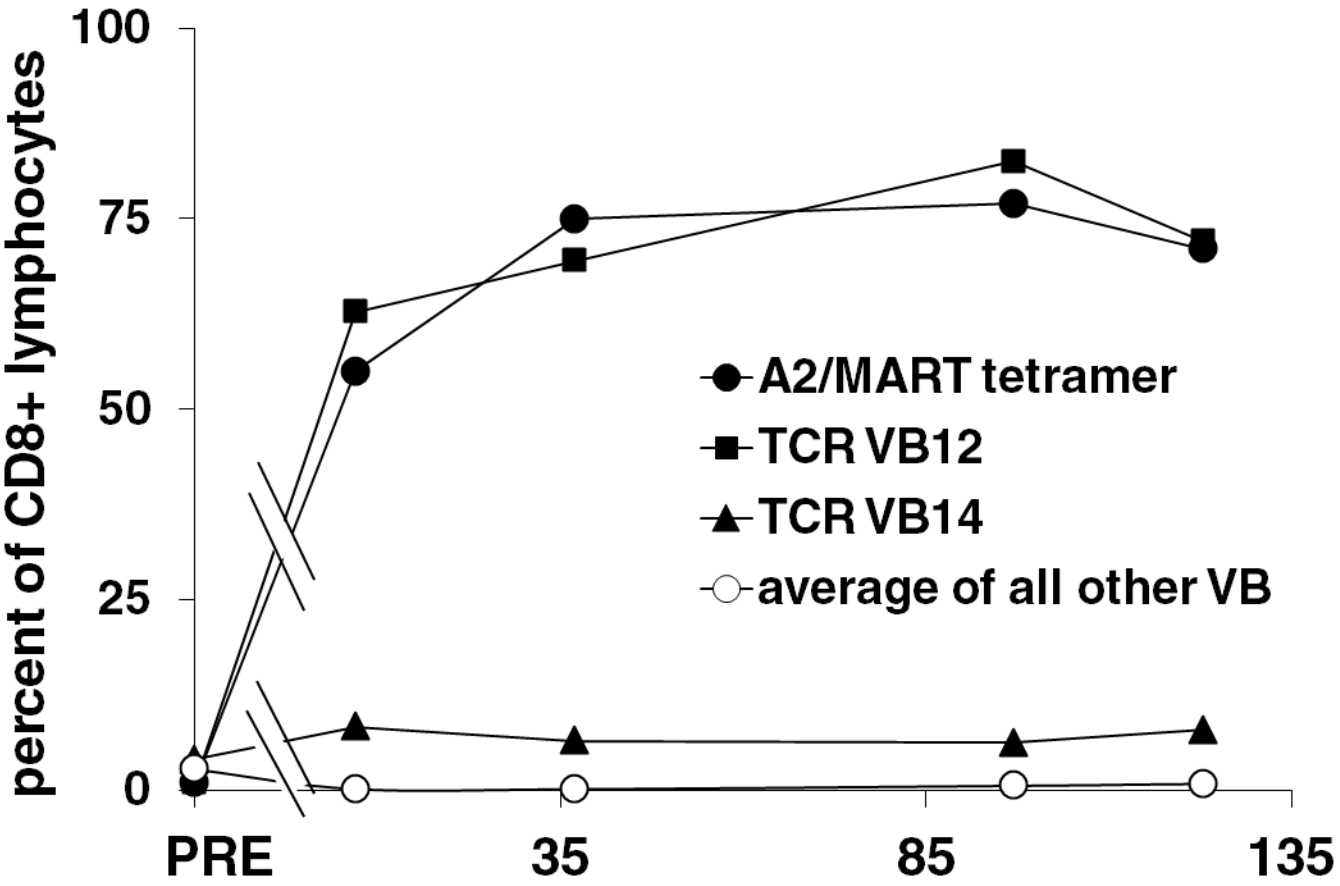
Specifically lytic



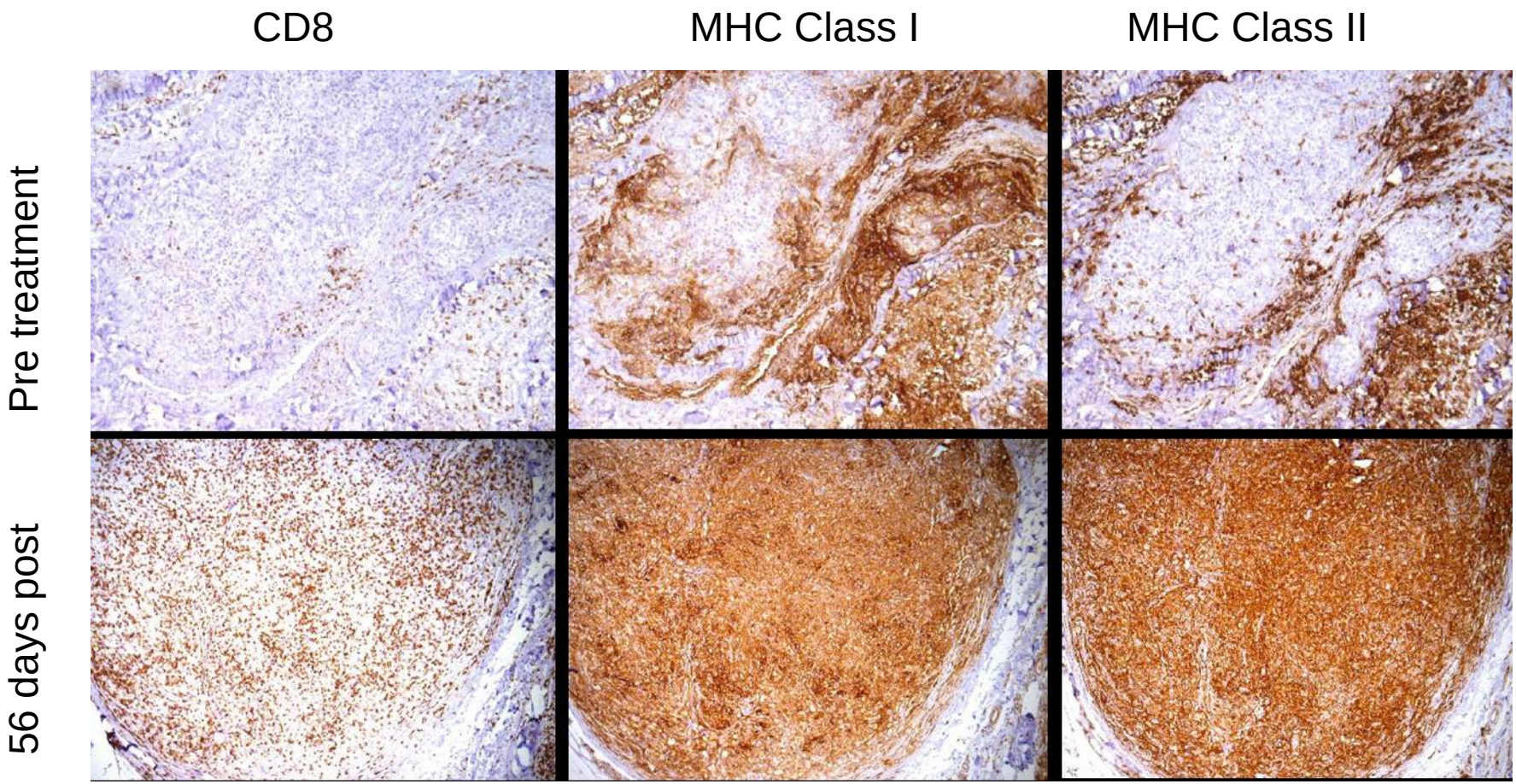
Phenotypically active



Persistence of Transferred TIL



Transferred T cells up-regulate expression of HLA by tumor cells *in vivo*



Impact of Lymphodepletion

- Functional and phenotypic activation of transferred T cells
- Proliferation and persistence
- Traffic to tumor and up-regulation of HLA on tumor cells
- Regression of bulky tumor masses

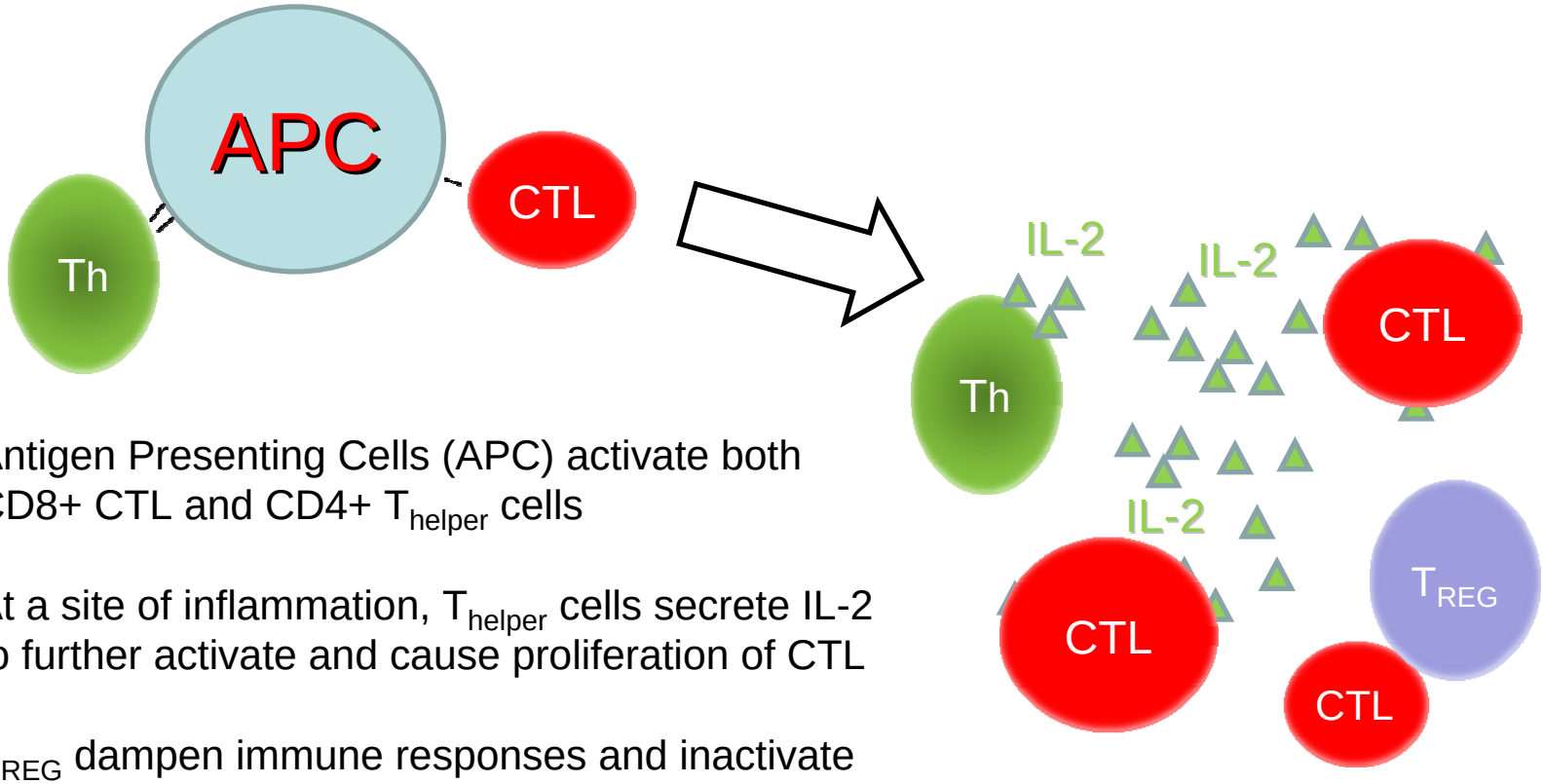
Adoptive immunotherapy – a unique research tool

- **Host Conditioning**
- **T cell attributes**
- **Target selection**

Attributes of T cells Associated with Response

- Minimum time in culture
- Persistence in vivo after transfer
- Replicative potential
  - Long telomeres
- Less differentiated phenotype
  - High expression of CD27, CD28

Potent immune responses require CD8+ and CD4+ cells

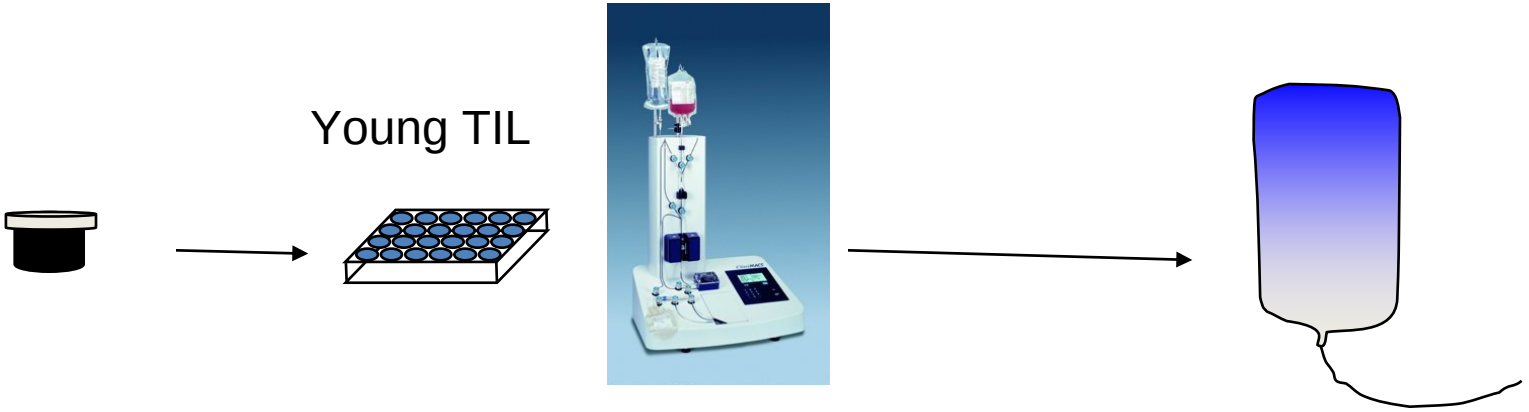


- Antigen Presenting Cells (APC) activate both CD8+ CTL and CD4+ T_{helper} cells
- At a site of inflammation, T_{helper} cells secrete IL-2 to further activate and cause proliferation of CTL
- T_{REG} dampen immune responses and inactivate CTL
- In ACT, chemotherapy eliminates endogenous T cells. Tumor reactive CTL are administered with exogenous IL-2

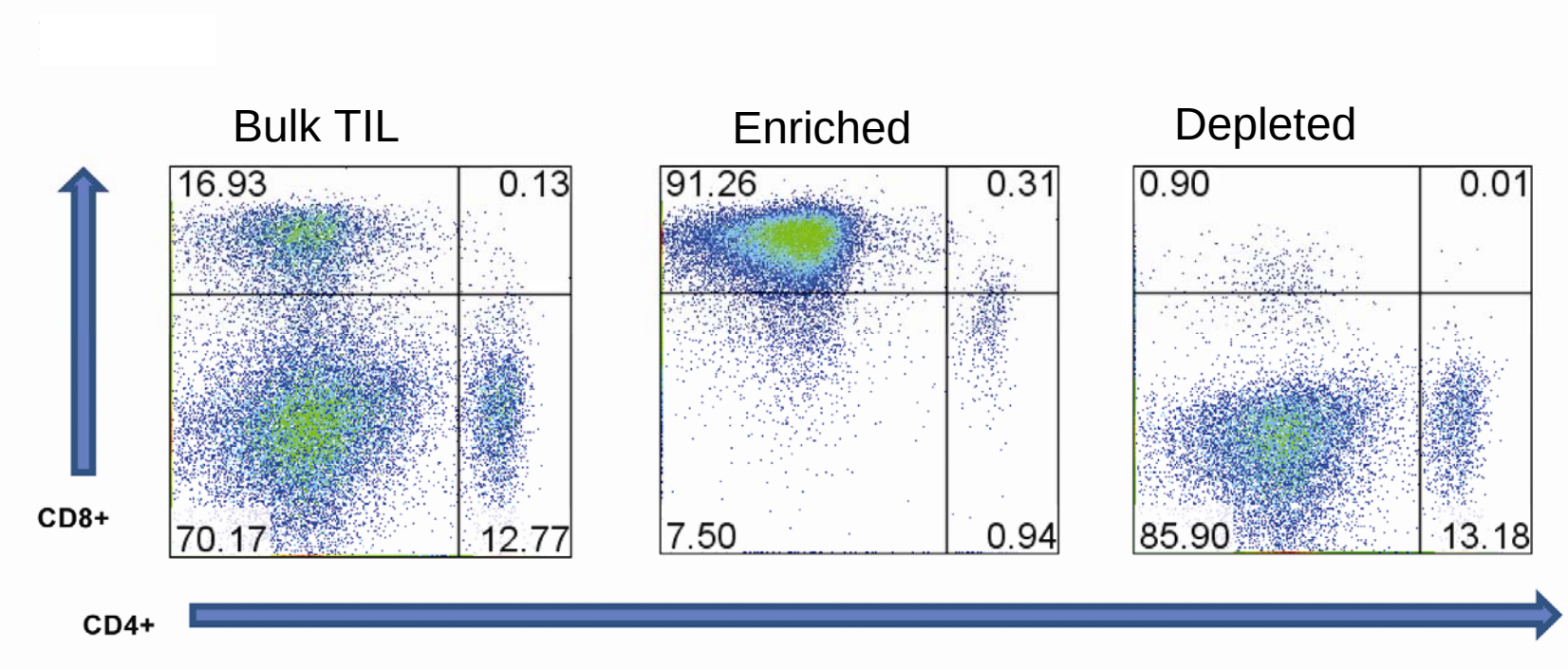
What is the role of CD4 cells

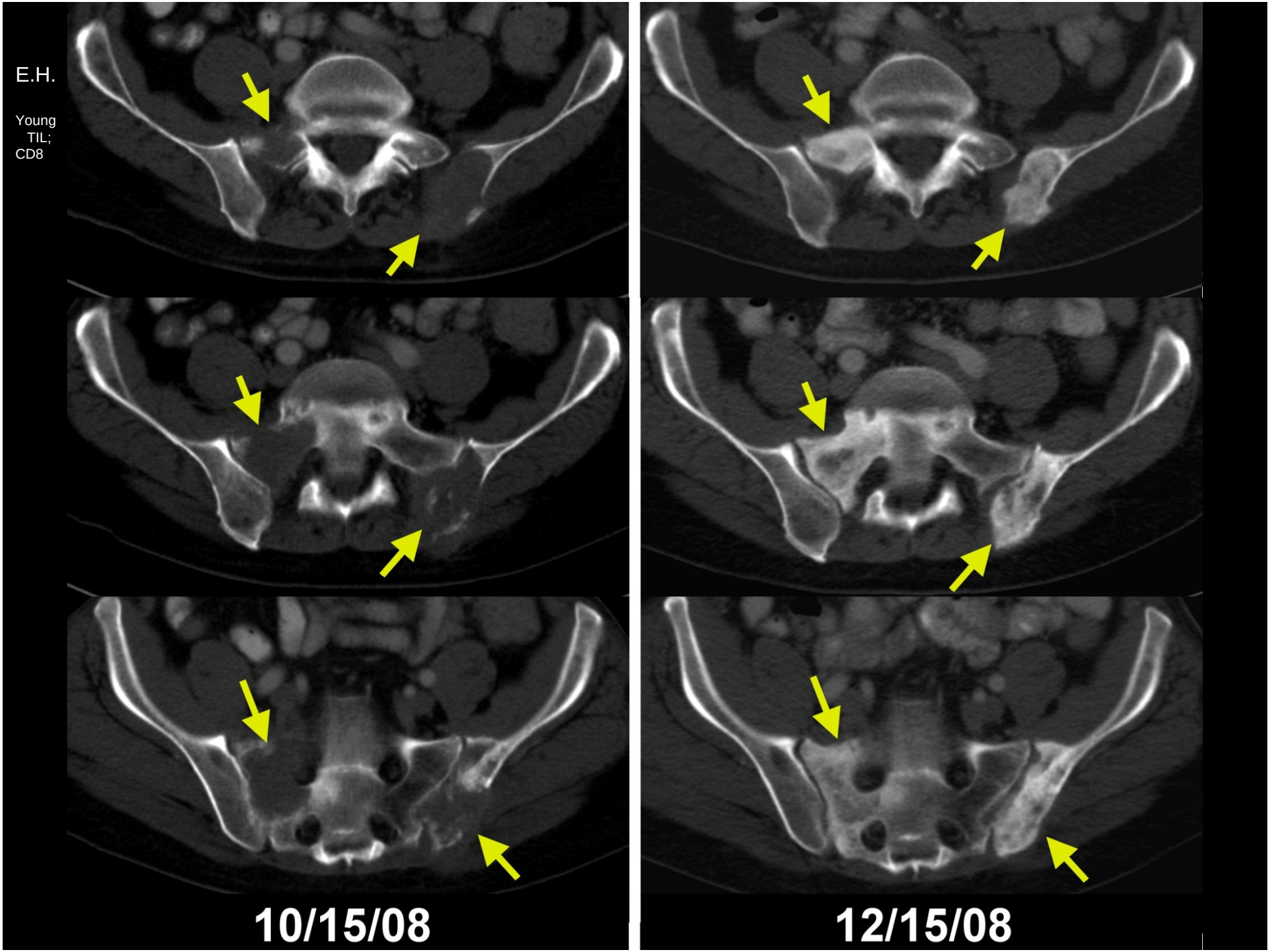
- CD4+ T-regs limit CD8+ ACT effectiveness in mouse models
- Anecdotal examples of CD4+ T cells correlated potent in vivo tumor rejection
- CD8+ TIL number is associated with response

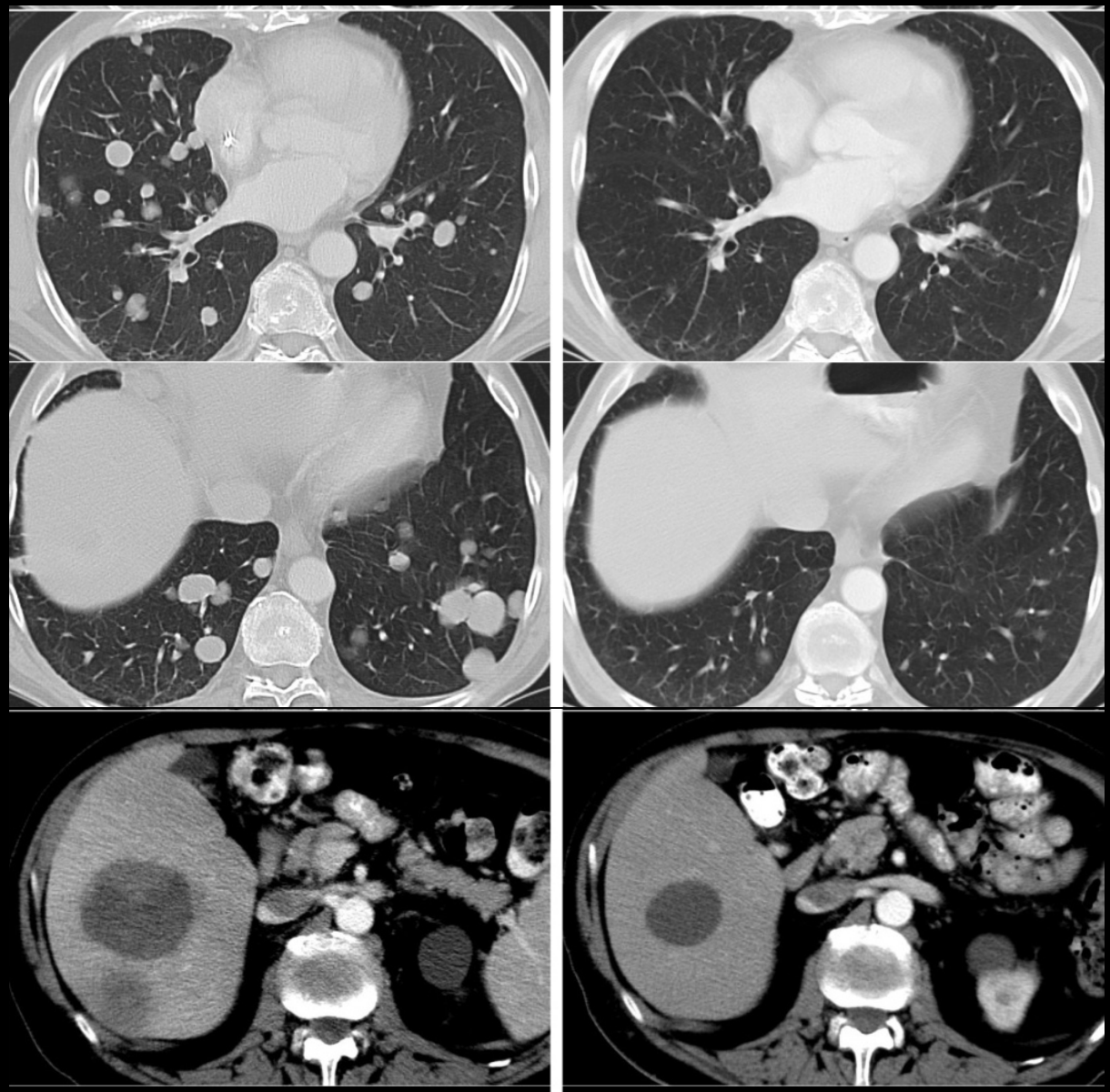
CD8 enrichment of TIL for Therapy



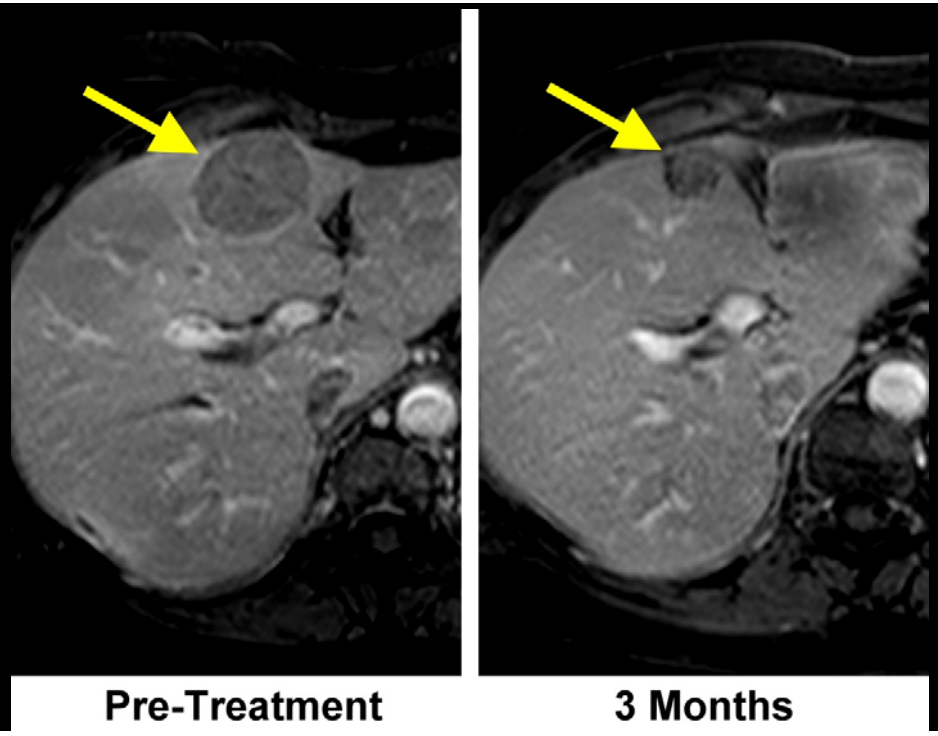
Optimized Clinical scale CD8+ enrichment of TIL using CliniMACS




E.H.
Young TIL; CD8
10/15/08
12/15/08

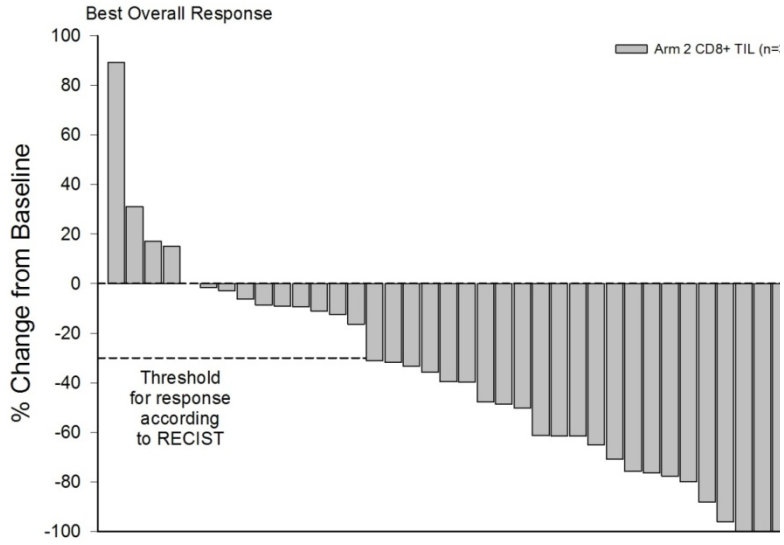

Pre-Treatment
+2 Months

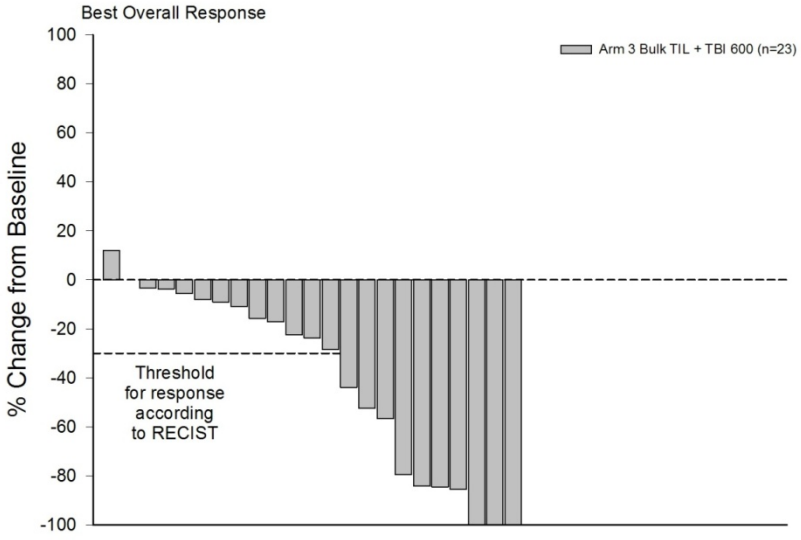

Day -9
Day +11
Day +27
Day +76


Pre-Treatment
3 Months

CD8+ Cells can Mediate Tumor Regression

- 56 patients treated
  - 33 with chemotherapy lymphodepletion
  - 23 with TBI and chemo lymhodepletion
- 29 (52%) objective responders



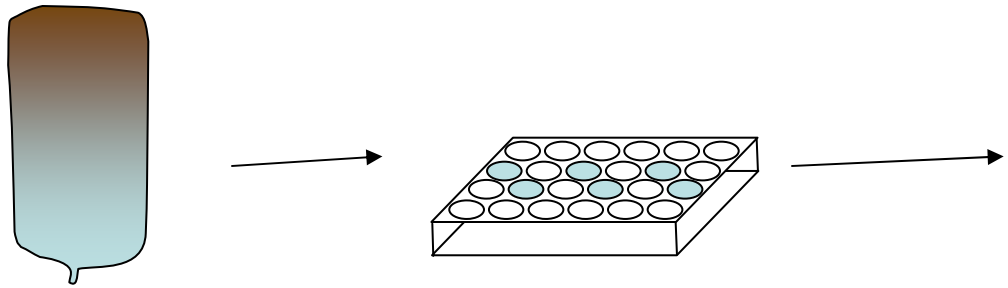


Adoptive immunotherapy – a unique research tool

- Host Conditioning
- T cell attributes
- Target selection

TCR-Transduced Peripheral Blood Lymphocytes (PBL)

- HLA-A2 patients only
- Uses PBMC pheresis prior to start
- Defective virus “infects” patient lymphocytes
- Engineered genes insert into lymphocyte genome
- New protein receptors are expressed (TCR)
- T cells start hunting tumor cells



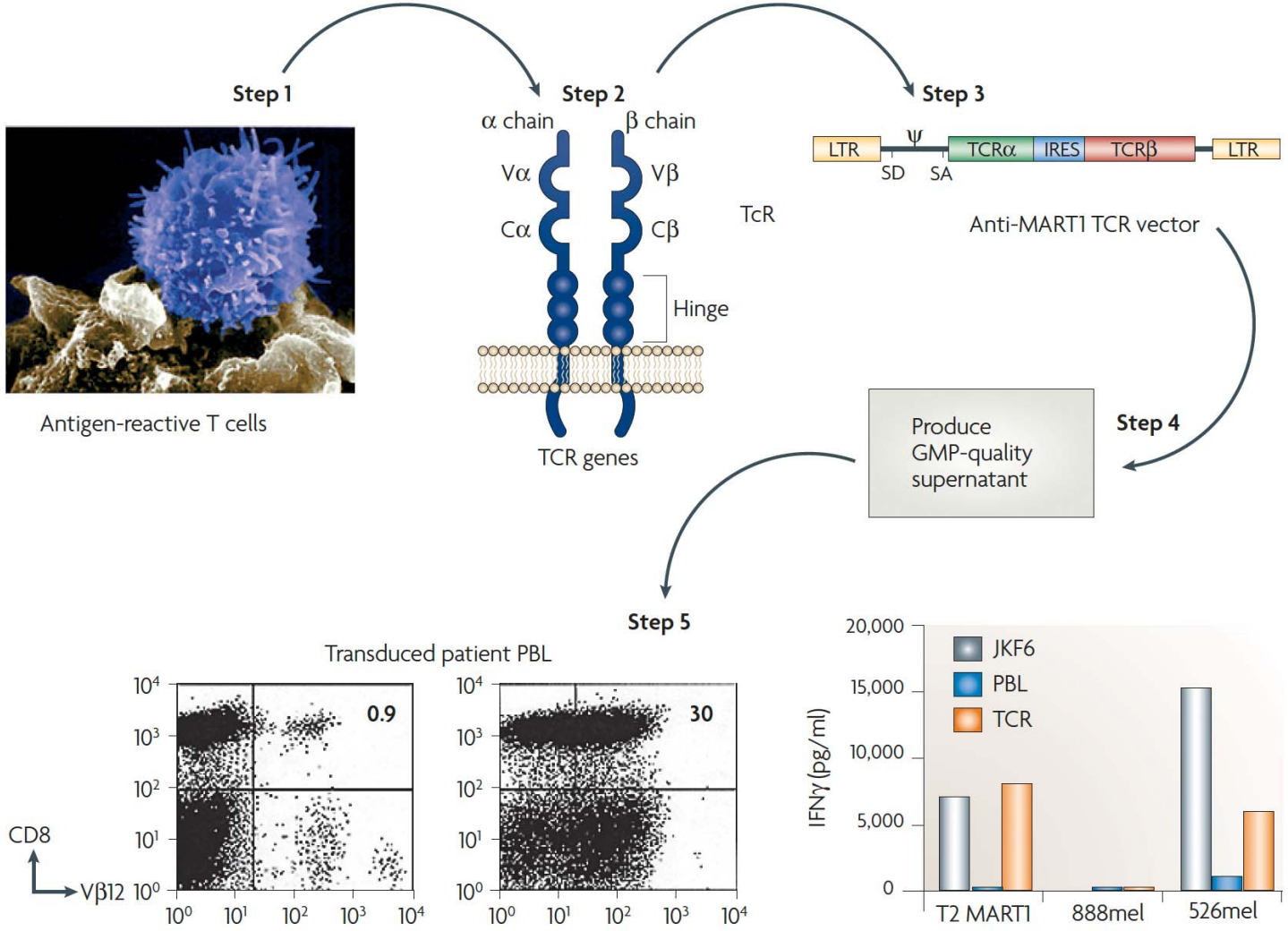
PBMC Pheresis

Transduce cells

Expand and test

http://flickr.com/photos/jepoirrier/385575933/

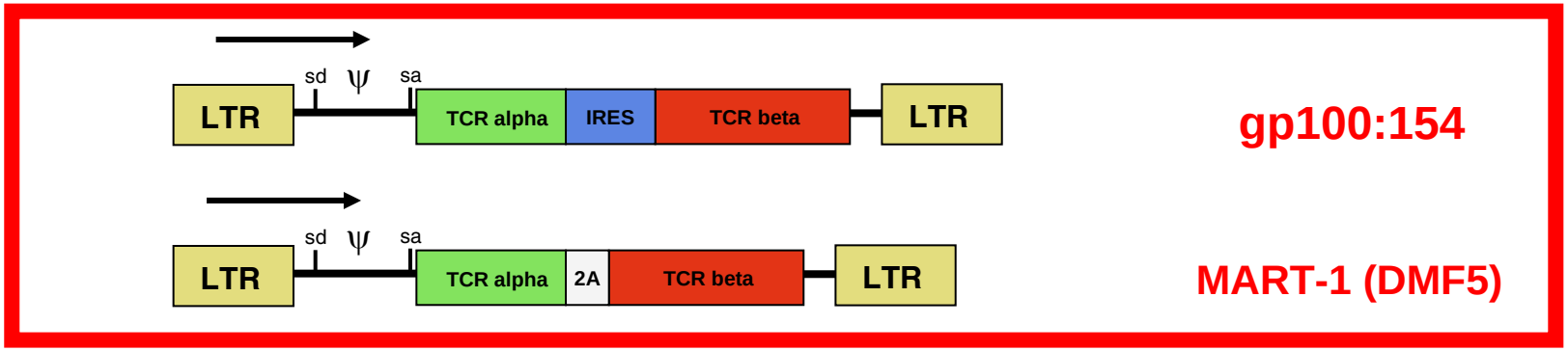
Development of TCR gene therapy



From, Rosenberg et al, Nat. Rev. Cancer April 2008

Anti-tumor antigen receptor containing retroviral vectors

Target Tumor Antigen



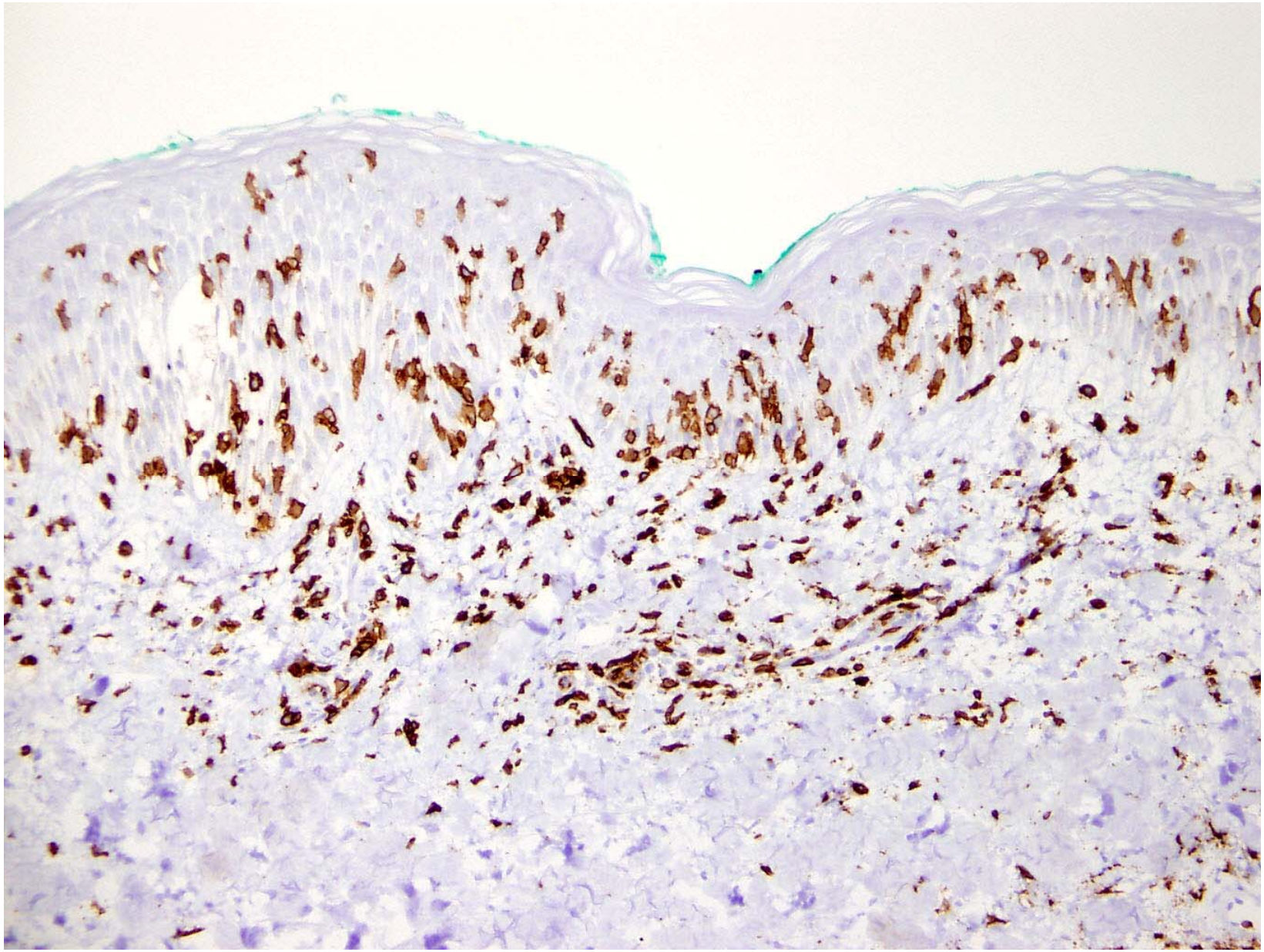
Johnson LA, et al 2009, Blood 114:535-46

NY-ESO-1

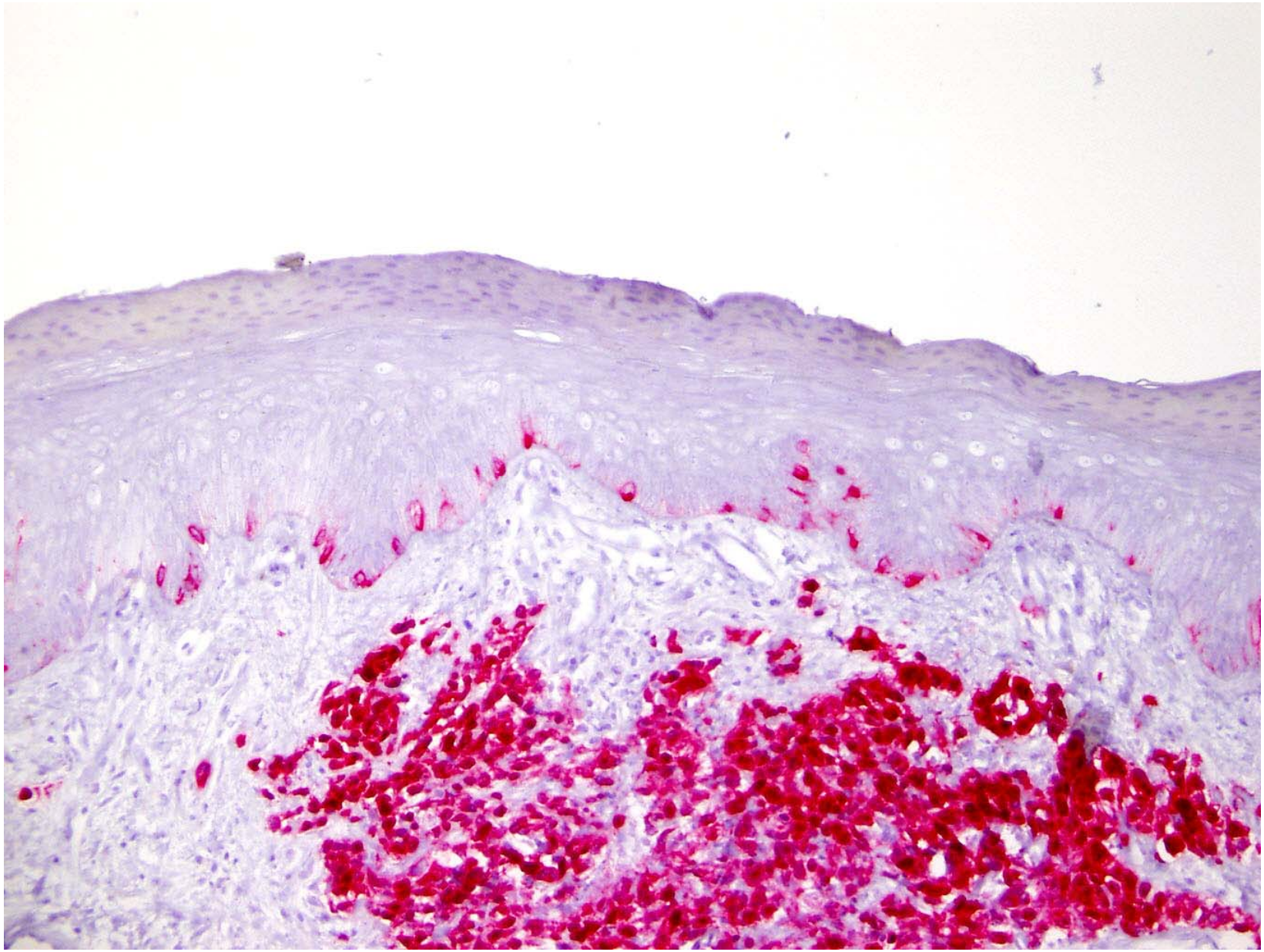
MART-1 and GP100

- Melanocyte differentiation antigens
- Expressed by tumors and normal melanocytes
  - Skin, eyes, ears
- TIL – low/no toxicity
- Moderate affinity “F4” TCR – no toxicity

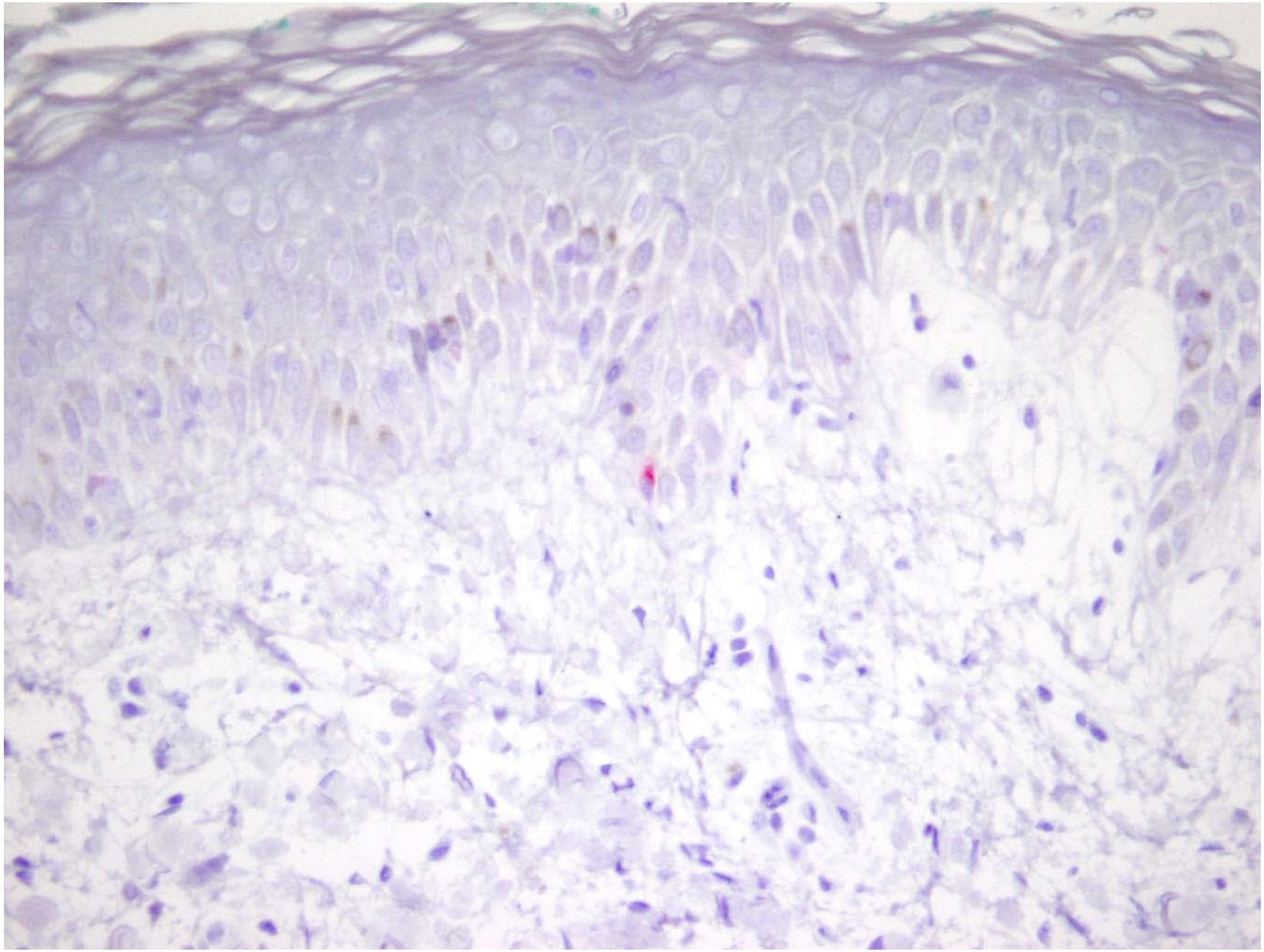
- “F5” and g154 TCRs selected for high affinity antigen recognition



Day 8: CD8 positive cells

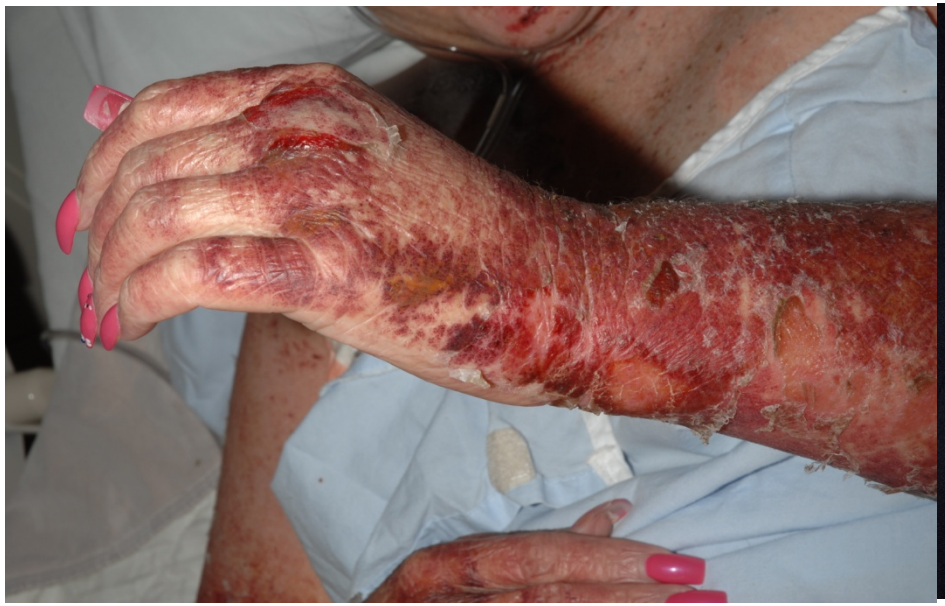


Melan-A positive control

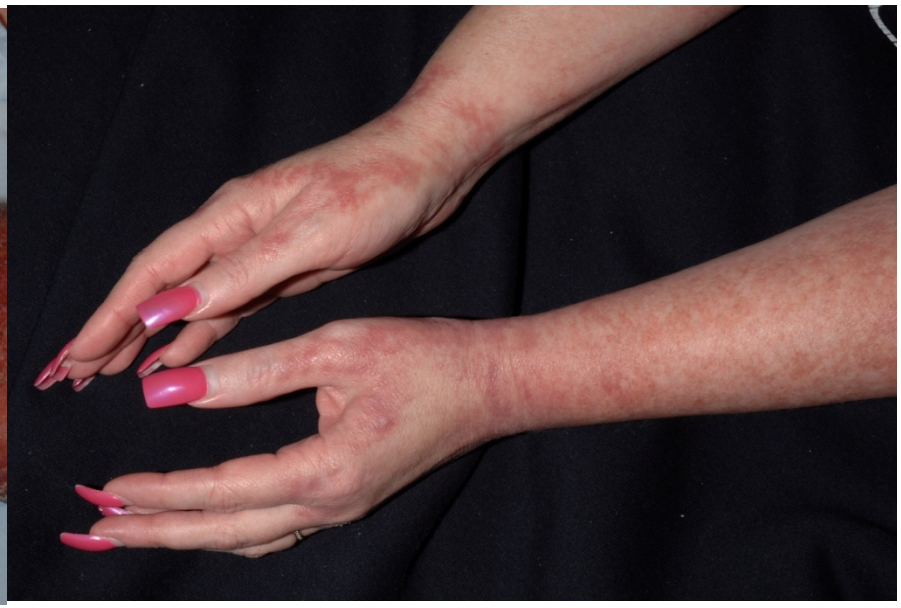


Day 8: Melan-A (rare specific staining)

Day +12



Day +26



SB Clinical Trials with MDA TCR

	Response (Number of Patients)		Toxicity (Grade 1/2/3)		
TCR	**Total**	**OR**	**Skin**	**Uveitis**	**Auditory**
MART-1TCR (DMF5)	20	6 (30%)	11/3/0	2/9/0	2/0/7
gp100TCR (gp154)	16	3 (19%)	11/4/0	0/4/0	2/2/3
Total	36	9 (25%)	22/7/0 (81%)	2/13/0 (42%)	4/2/3 (25%)

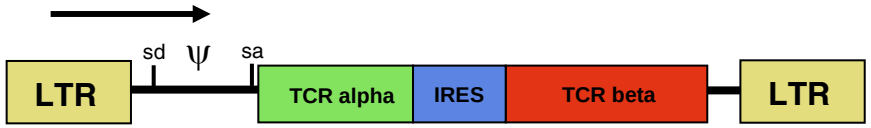
*Trials performed at the Surgery Branch, NCI. Response based on RECIST. Toxicity graded as shown below:

	Grade 1	Grade 2	Grade 3
Skin	Erythema	Desquamation <50%	Desquamation >50%
Eye	No symptoms	Anterior	Pan uveitis
Ear	15–25 dB, 2 freq.	>25 dB, 2 freq.	>25 dB, 3 freq.

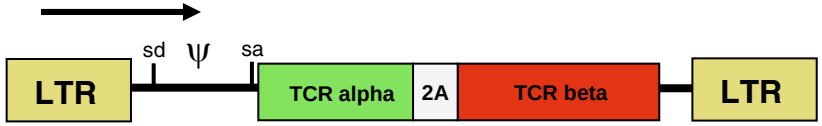
20-30% objective Response
~70% Grade 2-3 Toxicity

Anti-tumor antigen receptor containing retroviral vectors

Target Tumor Antigen



gp100:154



MART-1 (DMF5)

NY-ESO-1

Robbins PF et al, 2011, J Clin. Oncol. 29:917-24

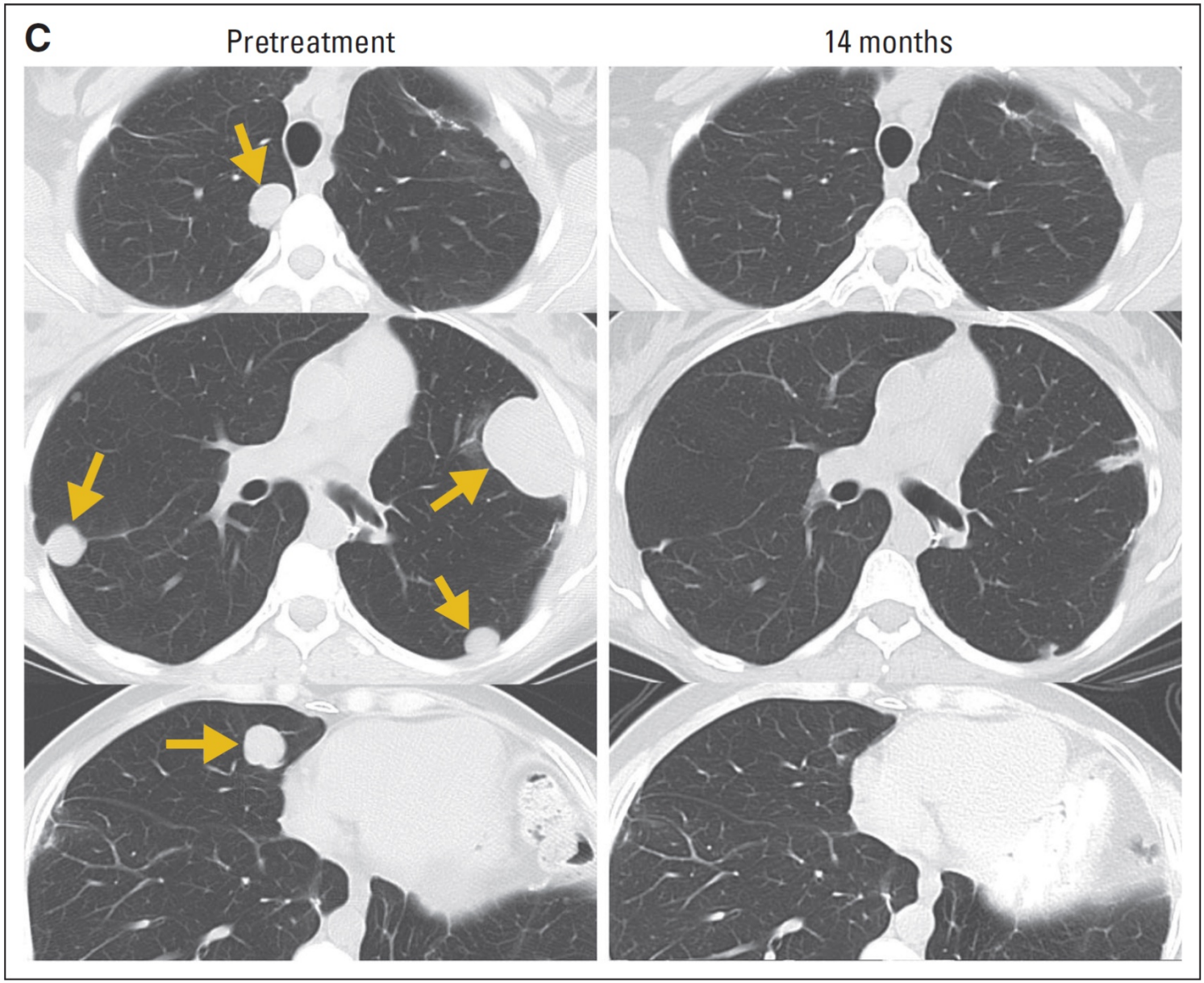
NY-ESO-1 TCR

- NY-ESO-1 – Cancer/Testes antigen
- Not expressed in normal adult tissues except testes (no class I expression)
- Expressed by 10-50% of tumors of multiple histologies including melanoma, breast, prostate, thyroid and ovarian.
- Expressed by ~90% of synovial cell sarcomas

- Eso TCR has an alpha chain CDR3 modification

Patients and outcomes for Eso TCR therapy

Patient No.	Age (years)	Sex	Sites of Disease	Response†
Melanoma				
1	52	M	ln	PR (8)
2	60	F	sc, lu	PD
3	30	F	bo, ln, panc, sb	PD
4	56	M	lu, ki	CR (22+)
5	32	M	ln	CR (20+)
6	38	M	ln	PR (3)
7	47	M	ln, lu	PD
8	39	F	ln, br, lu	PD
9	51	F	lu, ln, li	PD
10	61	M	ln, li, spl, lu, bo	PD
11	46	M	lu, li	PR (9+)
Synovial cell sarcoma				
12‡	20	M	lu, bo	PR (10)
13‡	37	F	lu	PR (18)
14‡	47	F	lu, ln	PR (5)
15‡	19	M	lu	PD
16	30	M	pl, hi	PR (8)
17	40	M	pl, hi	PD


C
Pretreatment
14 months

ESO TCR

- **5/11 responses in melanoma**
- **4/6 responses in synovial sarcoma**
- **No toxicity related to cells**

Adoptive T Cell Transfer

- **Potent therapy**
  - **Clinical responses in refractory disease**
  - **Break tolerance to self antigens**
- **Unique research tool**
  - **Patient, T cells, tumor**