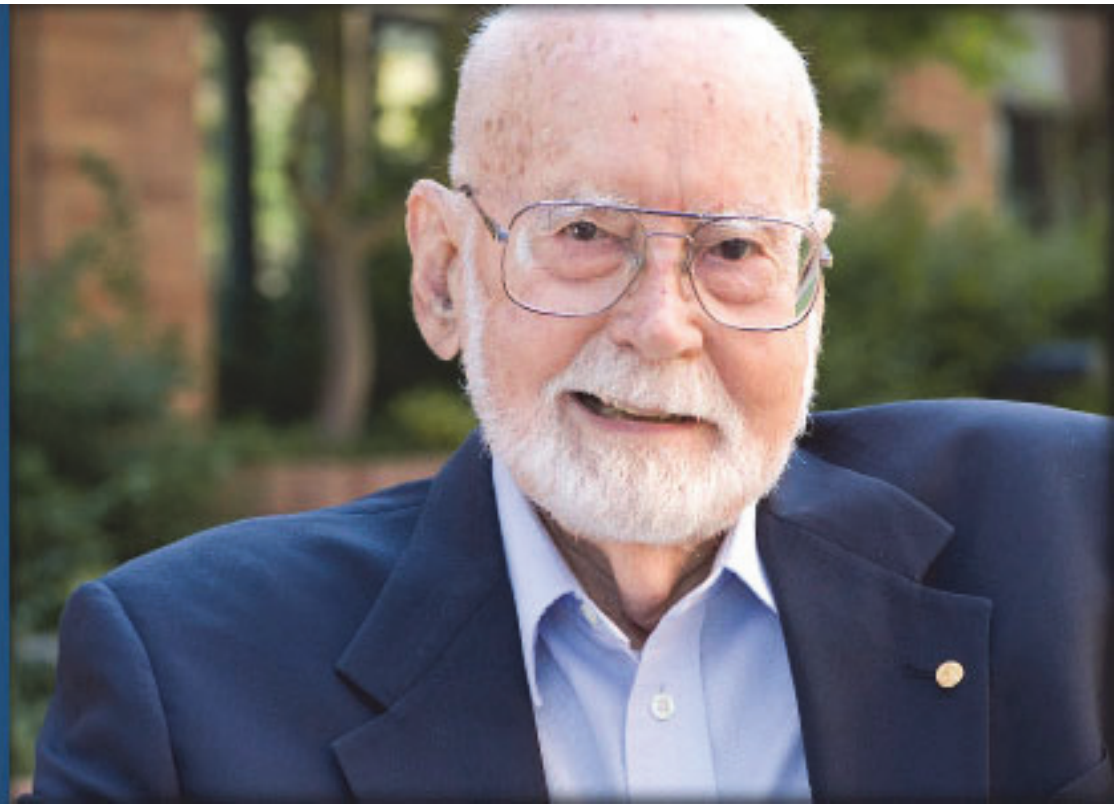
REMEMBERING

DR. E. DONNALL THOMAS

1920 - 2012

1990 Nobel Laureate
Father of Bone Marrow Transplantation



Adoptive T Cell Therapy:

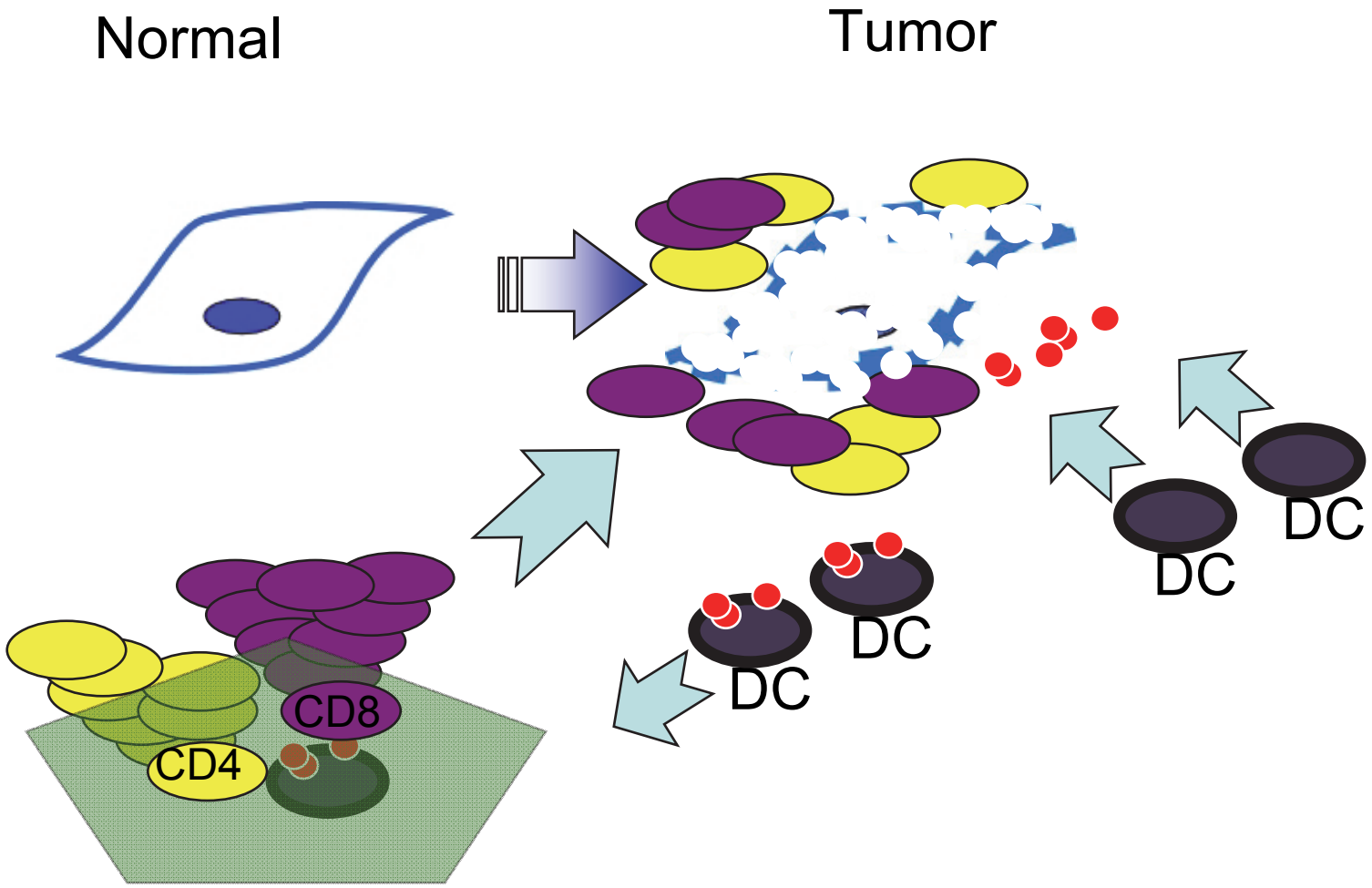
Faster, Higher, Stronger

Cassian Yee MD
Member
Fred Hutchinson Cancer Research Center
Program in Immunology
Clinical Research Division

Professor
University of Washington
Division of Oncology
Department of Medicine

c*yee@fhcrc.org*

Tumor Immune Surveillance



Possible Reasons For Failure of Tumor Immune Surveillance

Immune evasion

Low numbers

Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Immune evasion

Low numbers

Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells

Low numbers

Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells

More of them

Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells - *stronger*

More of them - *higher*

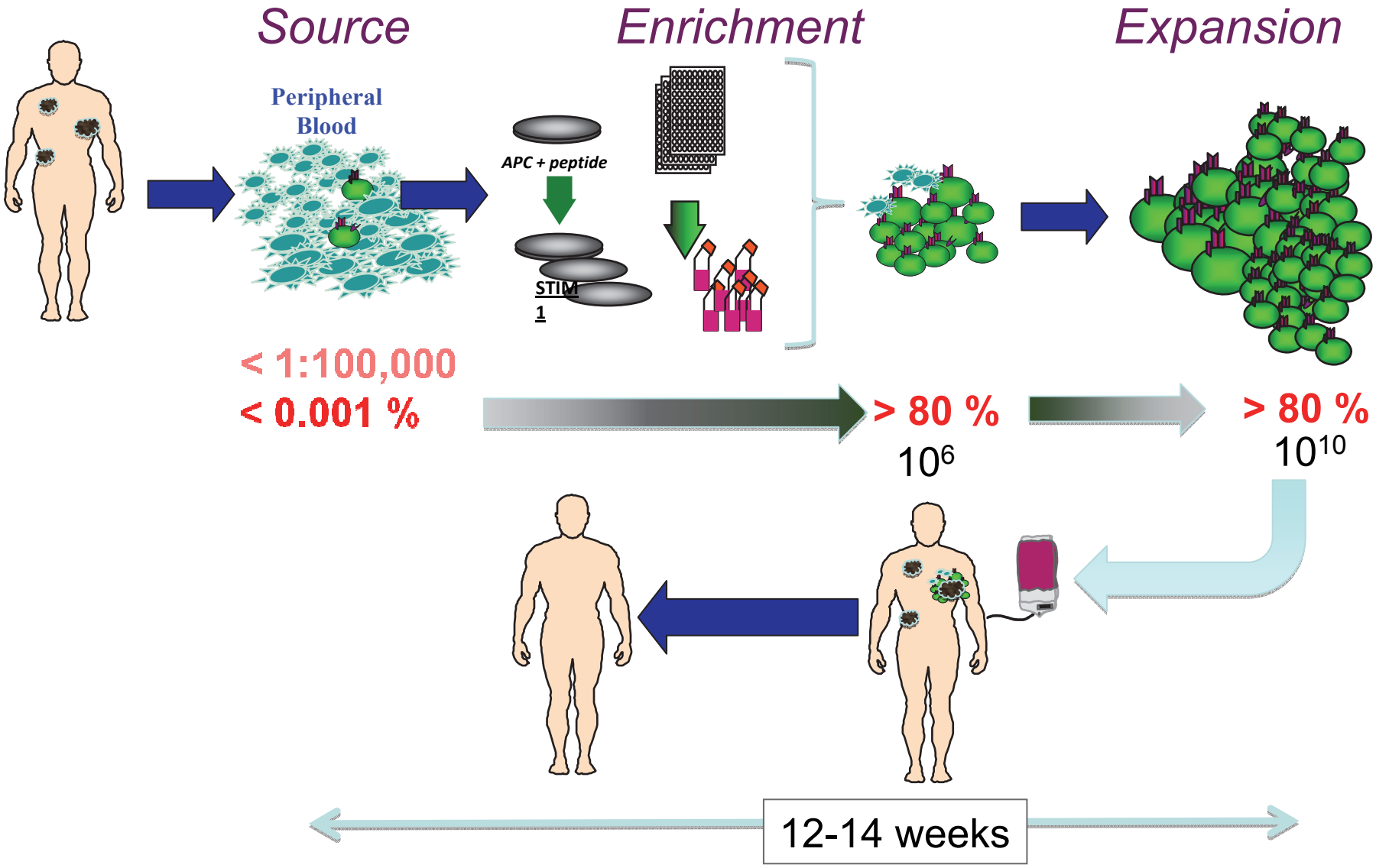
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells - *stronger*

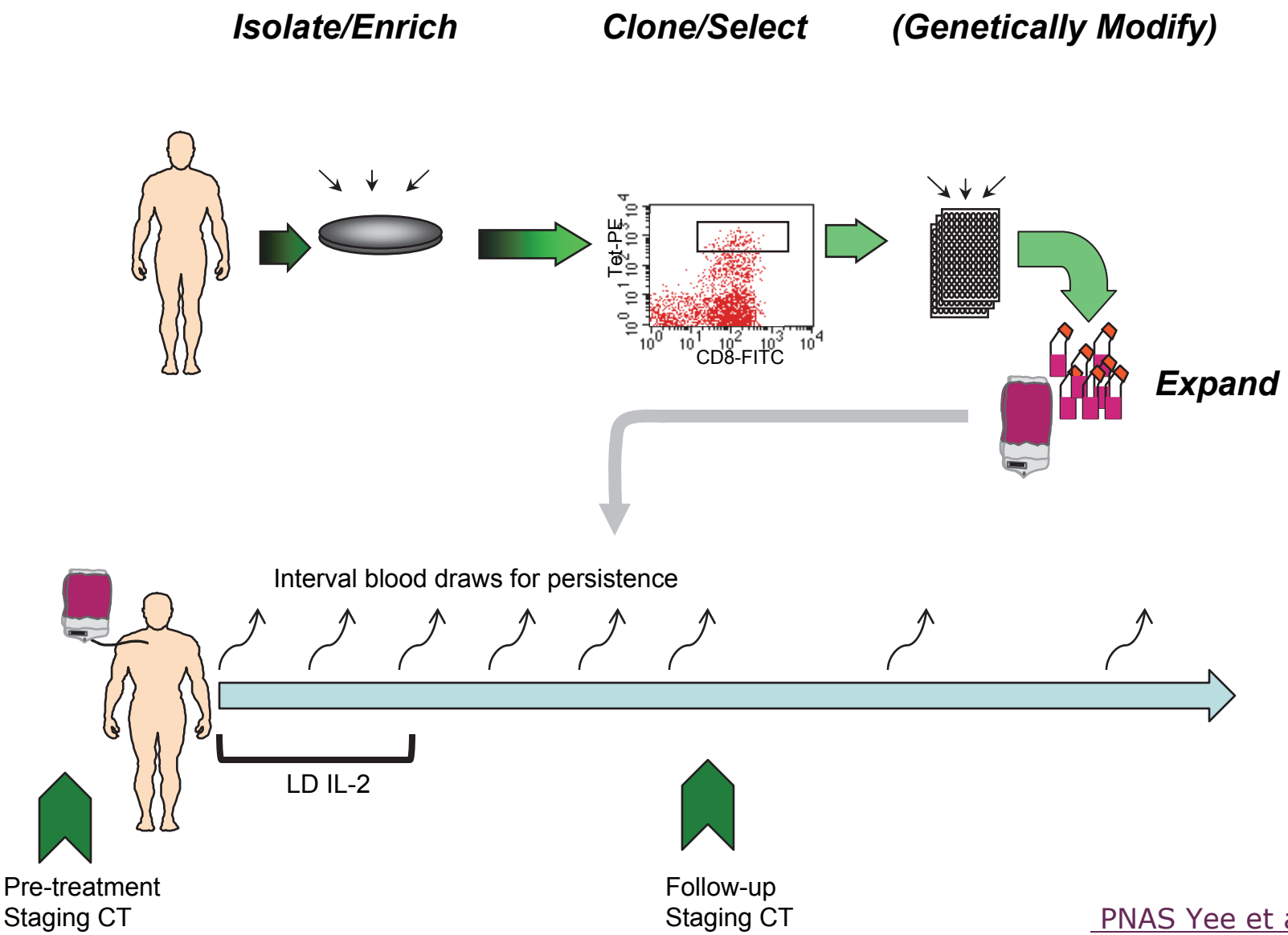
More of them - *higher*

Expedience - *faster*

T Cell Therapy



Adoptive T Cell Therapy: Basic Protocol



PNAS Yee et al. 2002

Adoptive CD8 T Cell Therapy for Melanoma

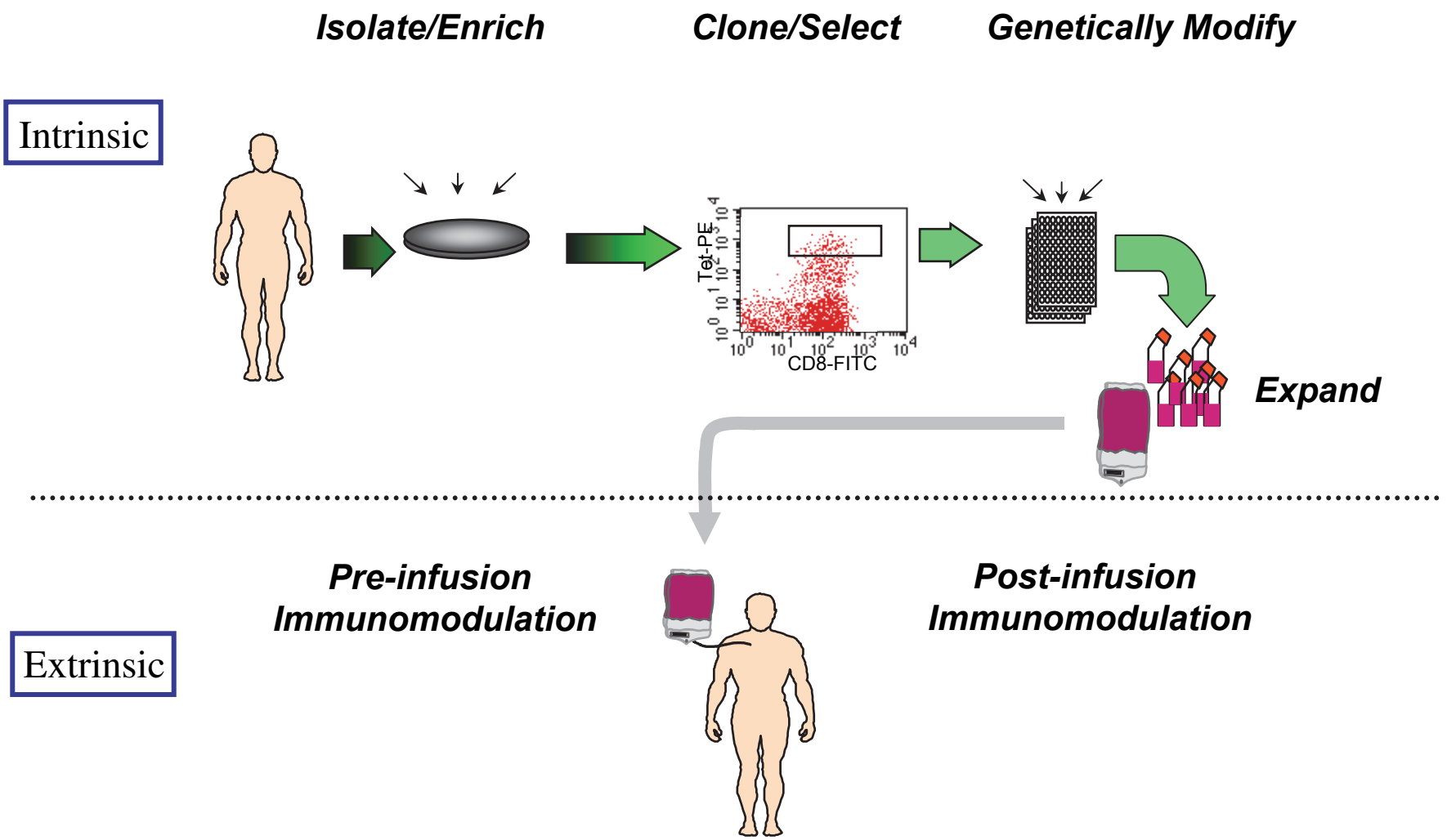
Safety

Persistence

Efficacy

PNAS 2002

Adoptive T Cell Therapy: Basic Protocol



PNAS Yee et al. 2002

Adoptive T Cell Therapy: Extended Protocol

Intrinsic

Isolate/Enrich

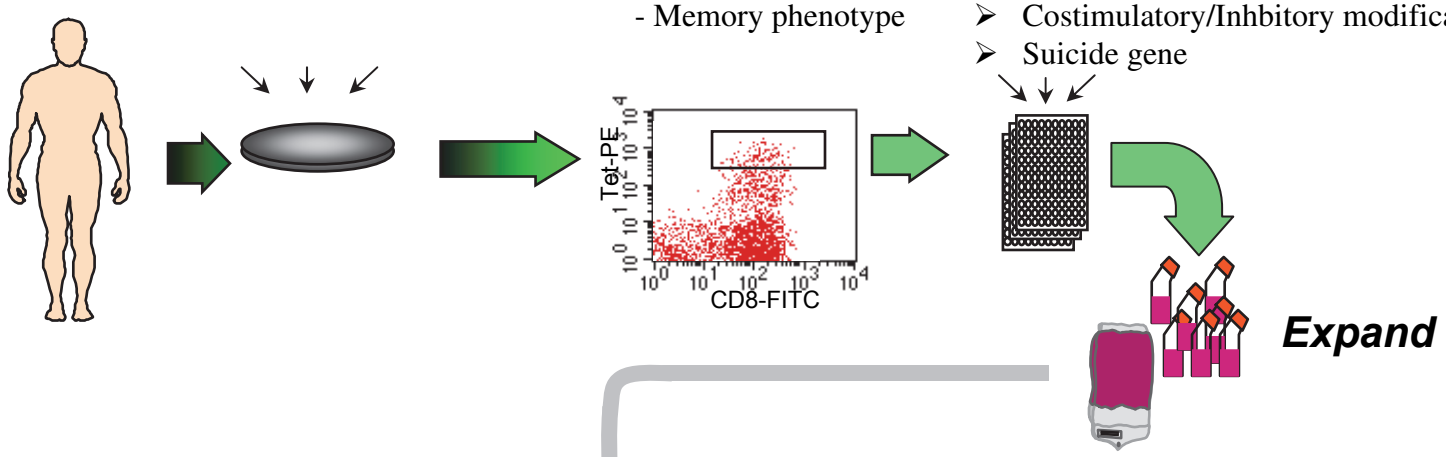
- Cytokine modulation

Clone/Select

- Phenotype
  - CD8/CD4
  - Memory phenotype

Genetically Modify

- TCR
- Chimeric receptor
- Costimulatory/Inhbitory modification
- Suicide gene

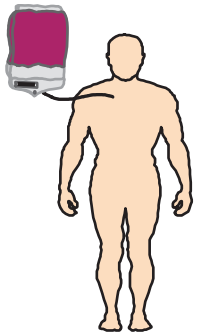


Expand

Extrinsic

Pre-infusion Immunomodulation

- Lymphodepletion
  - Chemotherapy/TBI

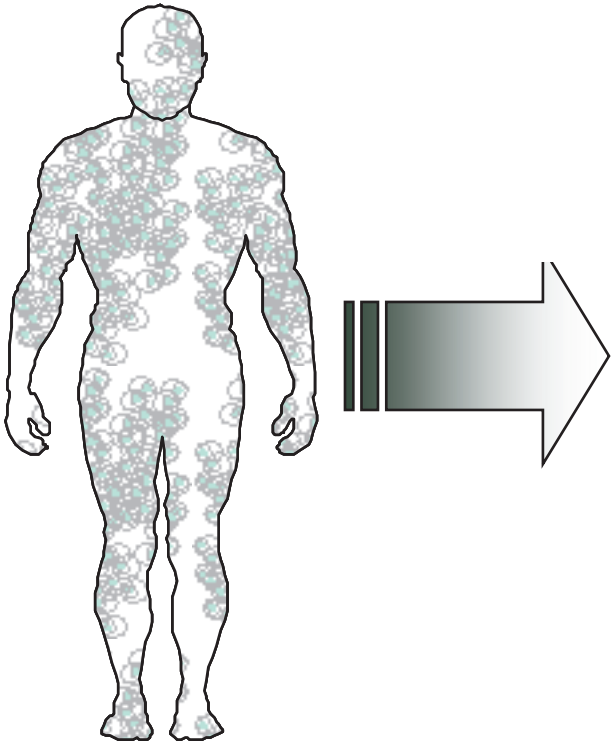


Post-infusion Immunomodulation

- Cytokine help
  - Low-dose IL-2
  - High-dose IL-2
  - Other γ-chain receptor cytokines
- Anti-CTLA4, Anti PD-1
- Vaccine + adoptive therapy

Hematol Oncol Clin North Am. 2006 Jun;20(3):711-3

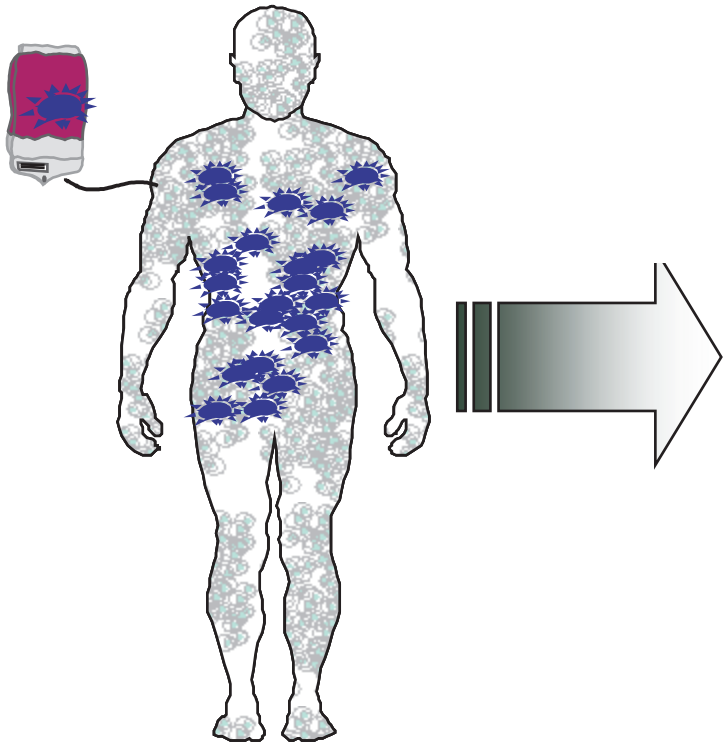
Lymphoid Homeostasis



other growth signals
other cytokines
IL-15
IL-7

Lymphodepletion

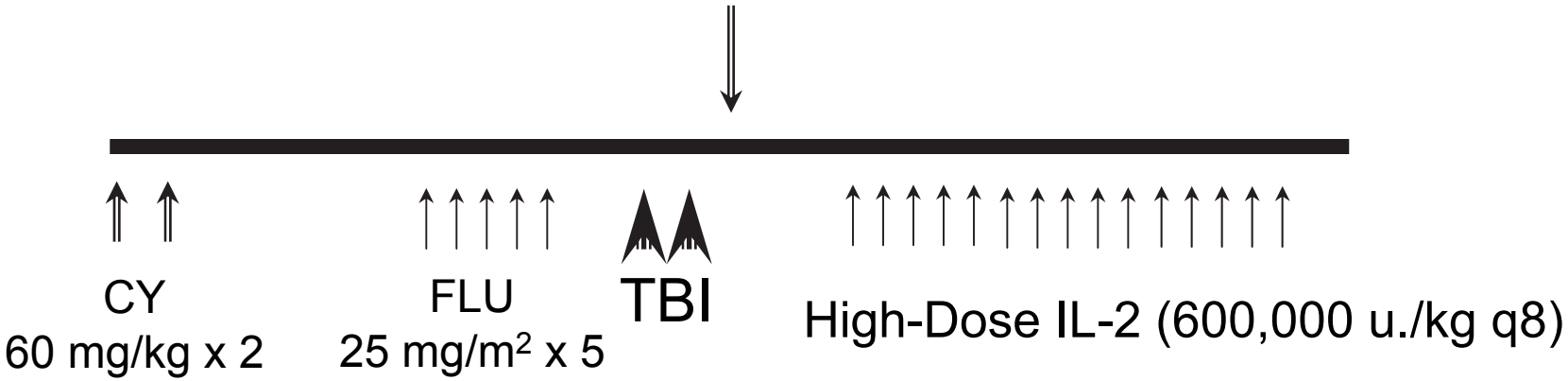
building a better environment



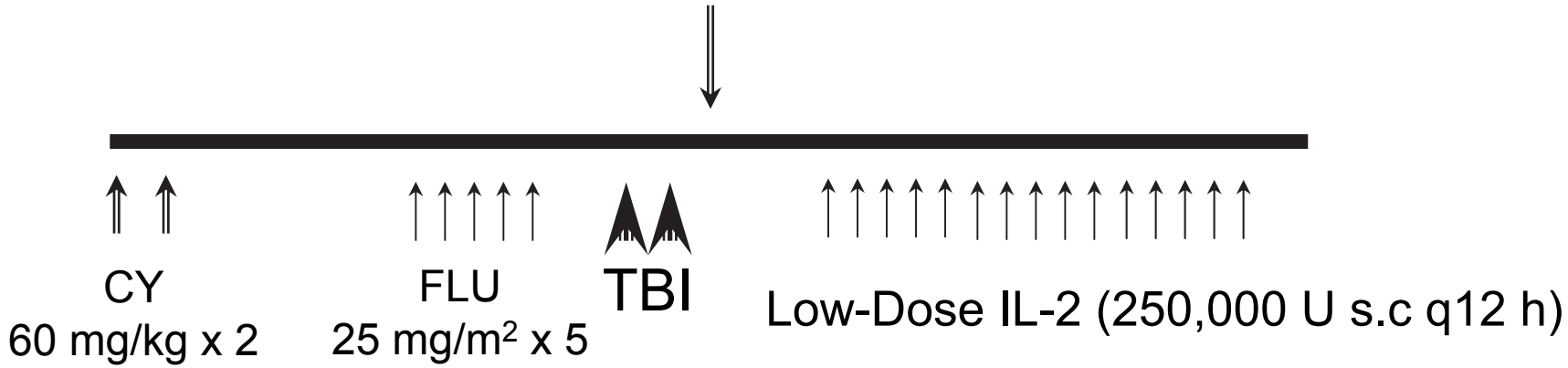
Increase 'space' for transferred T cells
Eliminate 'suppressor cells'
Supply Growth Factors

Increase 'space' for transferred T cells
Eliminate 'suppressor cells'
Supply Growth Factors

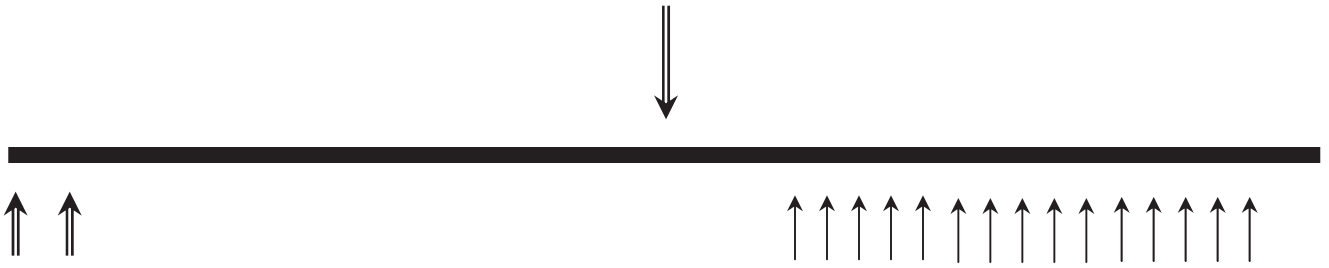
Adoptive Therapy following Lymphodepletion



Adoptive Therapy following Lymphodepletion



Adoptive Therapy following Lymphodepletion



CY
60 mg/kg x 2

Low-Dose IL-2 (250,000 U s.c q12 h)

Objectives :
- Evaluate Safety
- Evaluate T Cell Persistence
- Evaluate anti-tumor efficacy

T Cell Infusion:
- Antigen-specific CD8+ T cell clones
- Targeting MART-1, gp100
- Dose: 10^{10} cells / m^2

Eligibility Criteria :
- Stage IV (Metastatic)
- HLA-A2

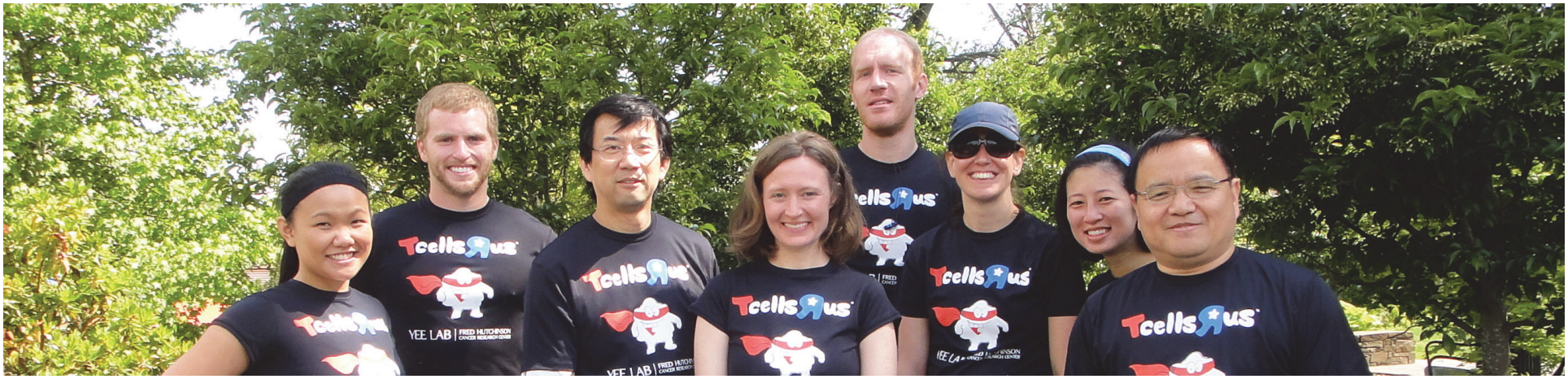
PNAS

Transferred melanoma-specific CD8$^+$ T cells persist, mediate tumor regression, and acquire central memory phenotype

Aude G. Chapuis[a], John A. Thompson[b], Kim A. Margolin[b], Rebecca Rodmyre[a], Ivy P. Lai[a], Kaye Dowdy[a], Erik A. Farrar[a], Shailender Bhatia[b], Daniel E. Sabath[c], Jianhong Cao[a], Yongqing Li[a], and Cassian Yee[a,1]

[a]Program in Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; [b]General Oncology and Hematology, Seattle Cancer Care Alliance and University of Washington, Seattle, WA 98109; and [c]Department of Laboratory Medicine, University of Washington, Seattle, WA 98195

Proc Natl Acad Sci USA
March 5, 2012



Ivy Lai

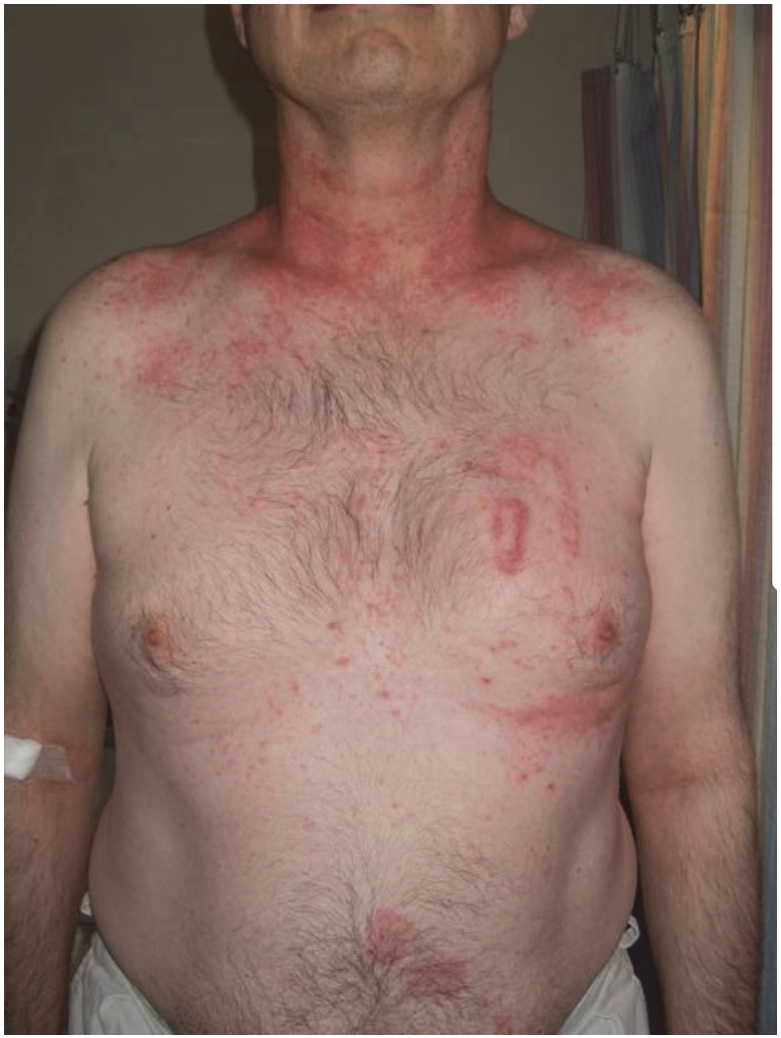
Erik Farrar

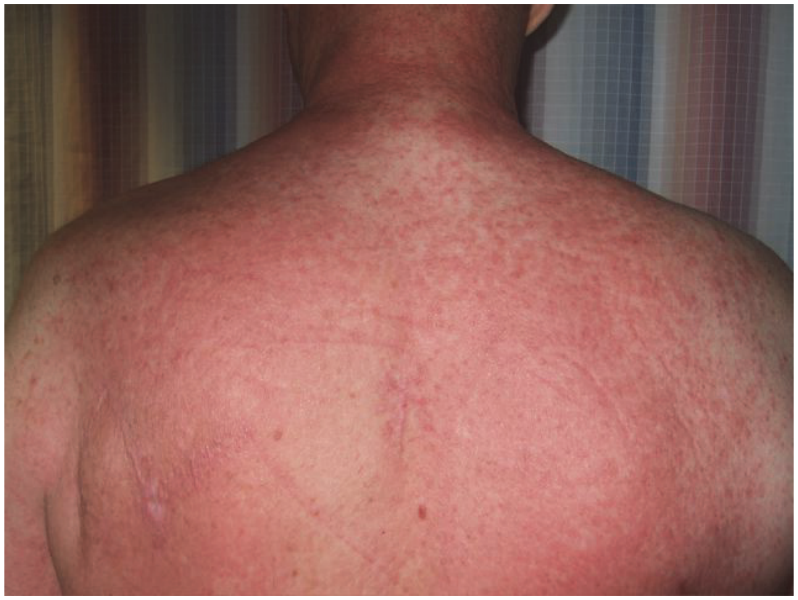
Aude Chapuis

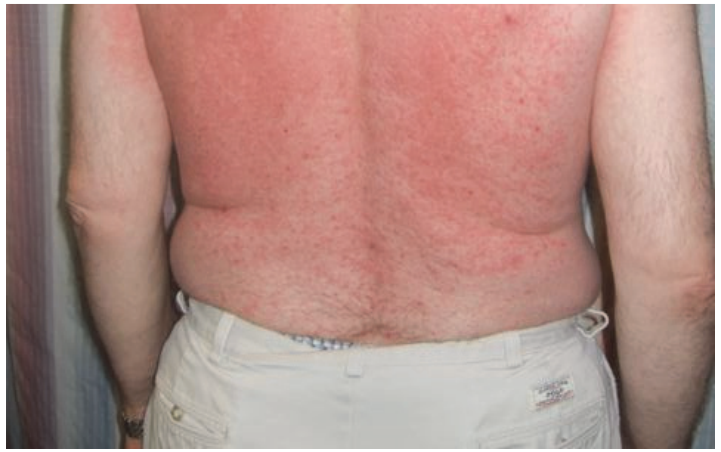
Target and Disease Sites

Patient	Target	Toxicity	Persistence	Disease Sites	Response
2140-1	Tyrosinase			Cervical,supraclavicular LN, Chest Wall, Breast Pulmonary nodules	
2140-2	Tyrosinase			Mediastinal, Pulmonary nodules	
2140-3	gp100			Mesenteric LN, scapular subcutaneous dz	
2140-4	MART-1			Pulmonary, inguinal, subcutaneous	
2140-5	MART-1			Right and left kidneys, adrenal, liver	
2140-6	MART-1			Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.	

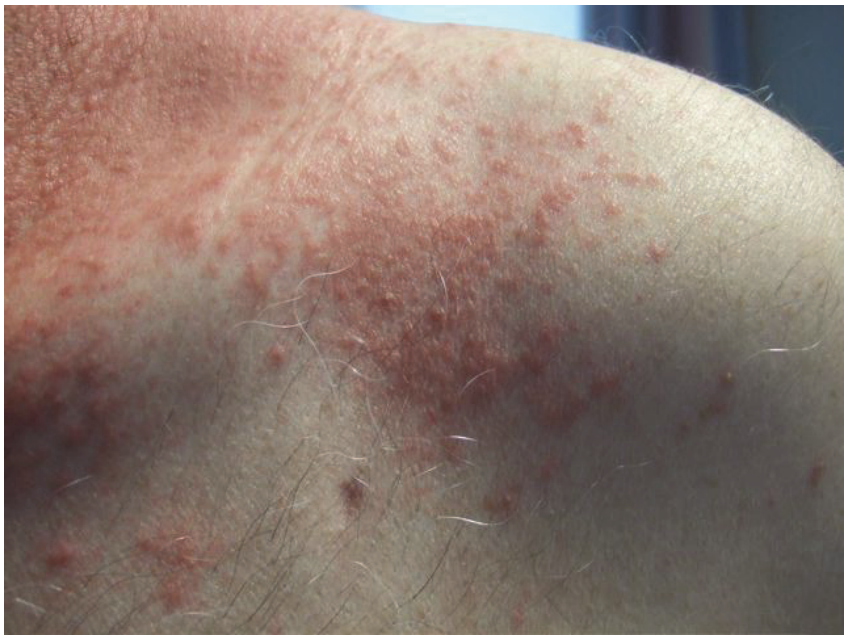
Toxicity







Toxicity



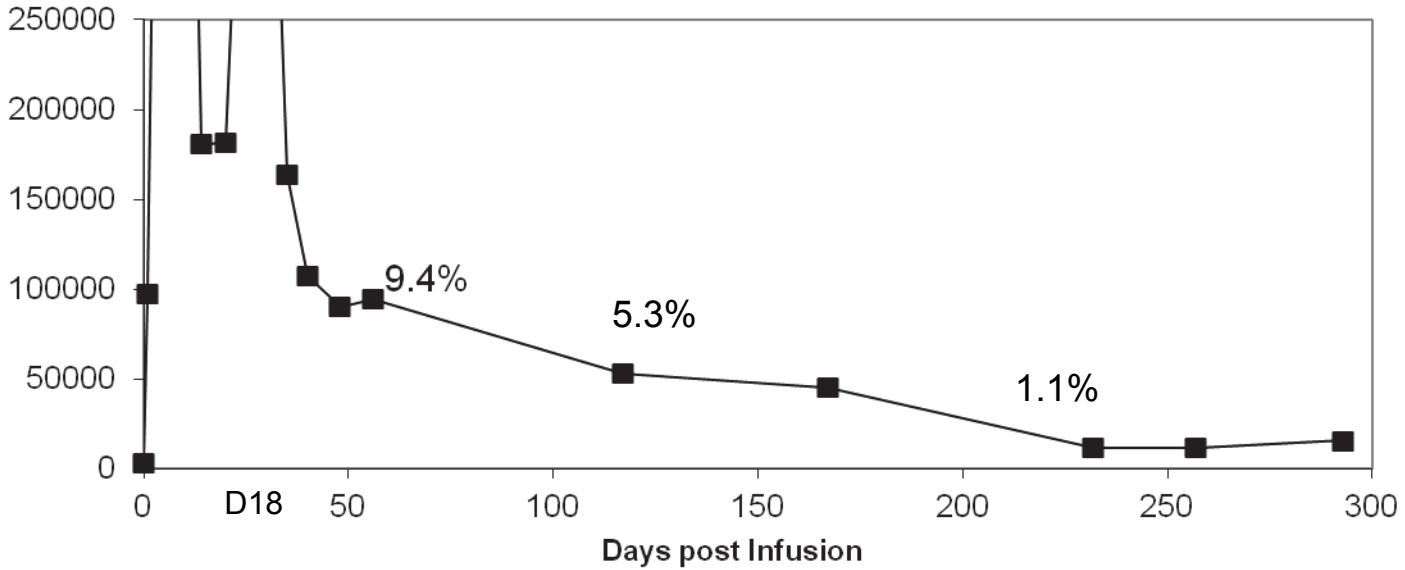
- "interstitial inflammatory infiltrate consisting predominantly of lymphocytes and plasma cells with admixed eosinophils, focal neutrophils and ***melanophages***."
- CD20 CD20 [L26] Rare positive cells
- CD3 CD3 [LN10] Positive, 3+ (> 75% of cells)
- CD4 CD4 [1F6] Minority of lymphocytes in epidermis
- CD8 CD8 [4B11] Positive, 2+ (25 - 75% of cells) Majority of lymphocytes in epidermis

Toxicity

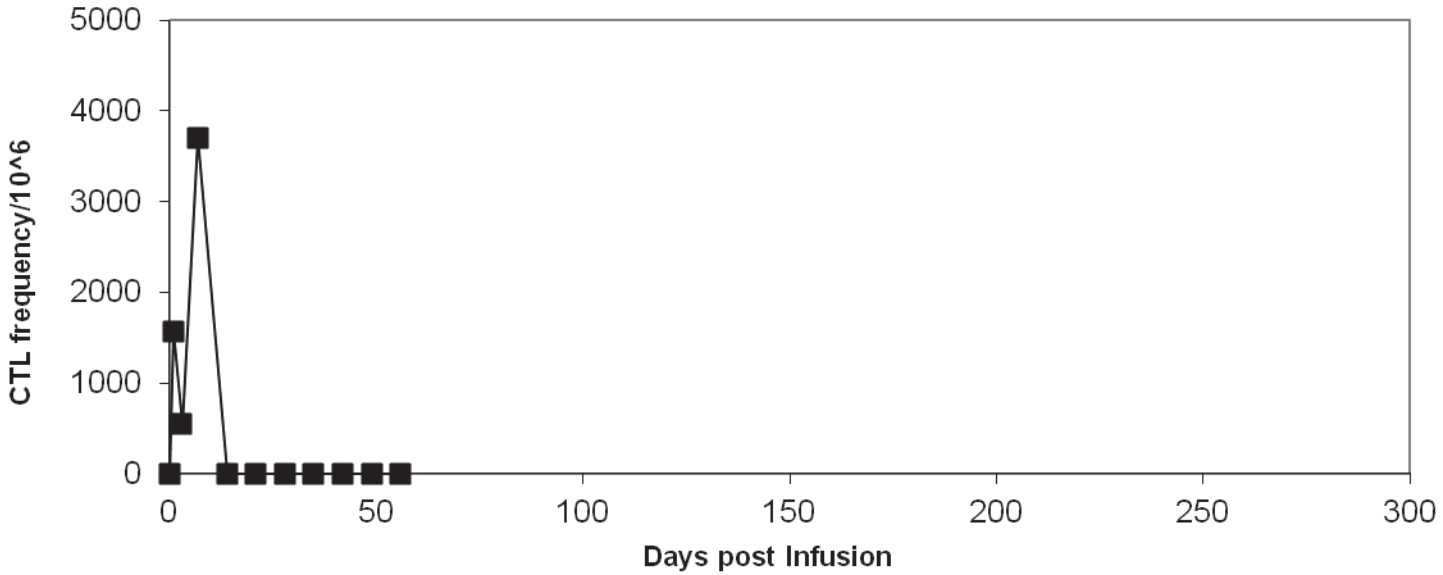
Patient	Target	Toxicity	Persistence	Disease Sites	Response
2140-1	Tyrosinase	F,N,R		Cervical,supraclavicular LN, Chest Wall, Breast Pulmonary nodules	
2140-2	Tyrosinase	F		Mediastinal, Pulmonary nodules	
2140-3	gp100	F,N,R		Mesenteric LN, scapular subcutaneous dz	
2140-4	MART-1	F, N, R		Pulmonary, inguinal, subcutaneous	
2140-5	MART-1	F, N,R		Right and left kidneys, adrenal, liver	
2140-6	MART-1	F, N, R		Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.	

Persistence

2140-1

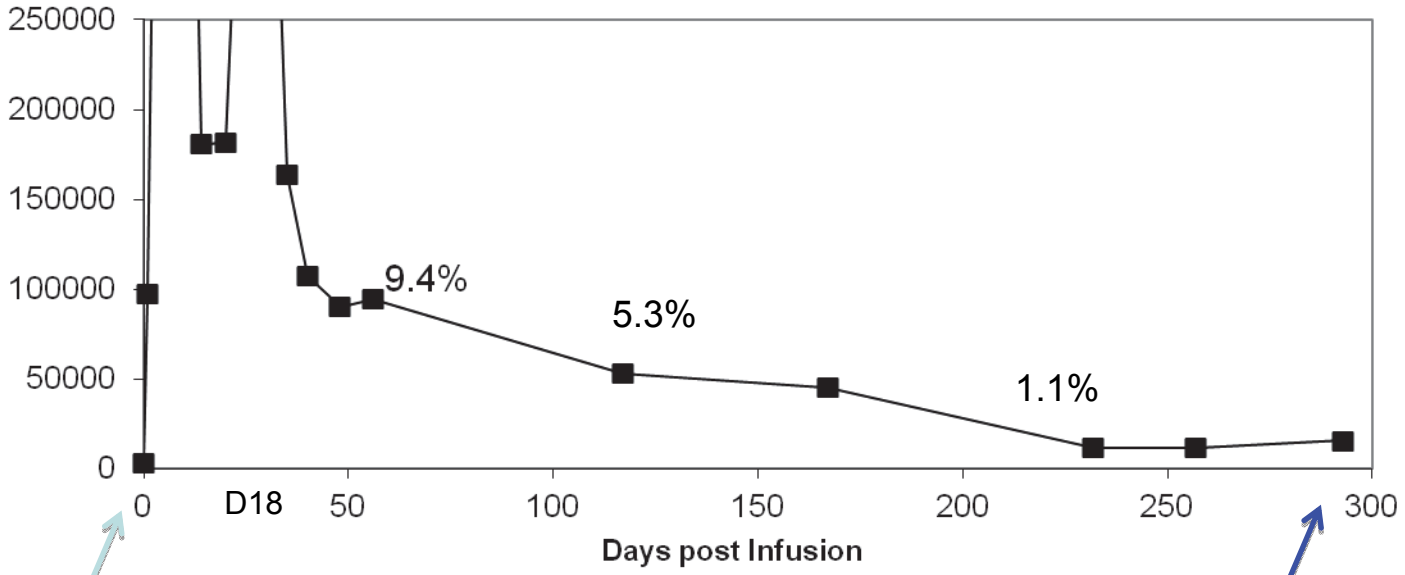


2140-2



Persistence

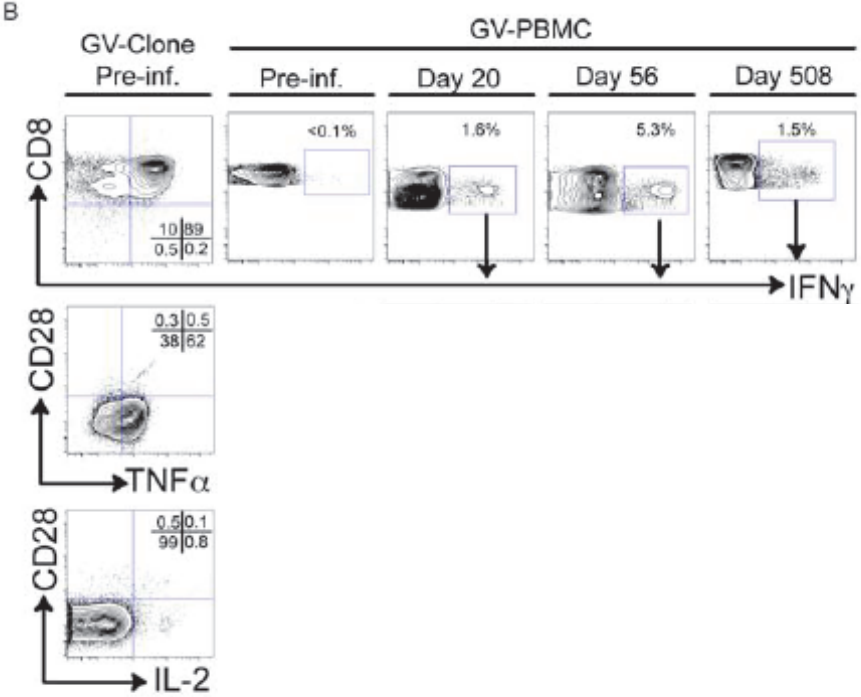
2140-1



CD45 RO+
CD28-
CD127-lo

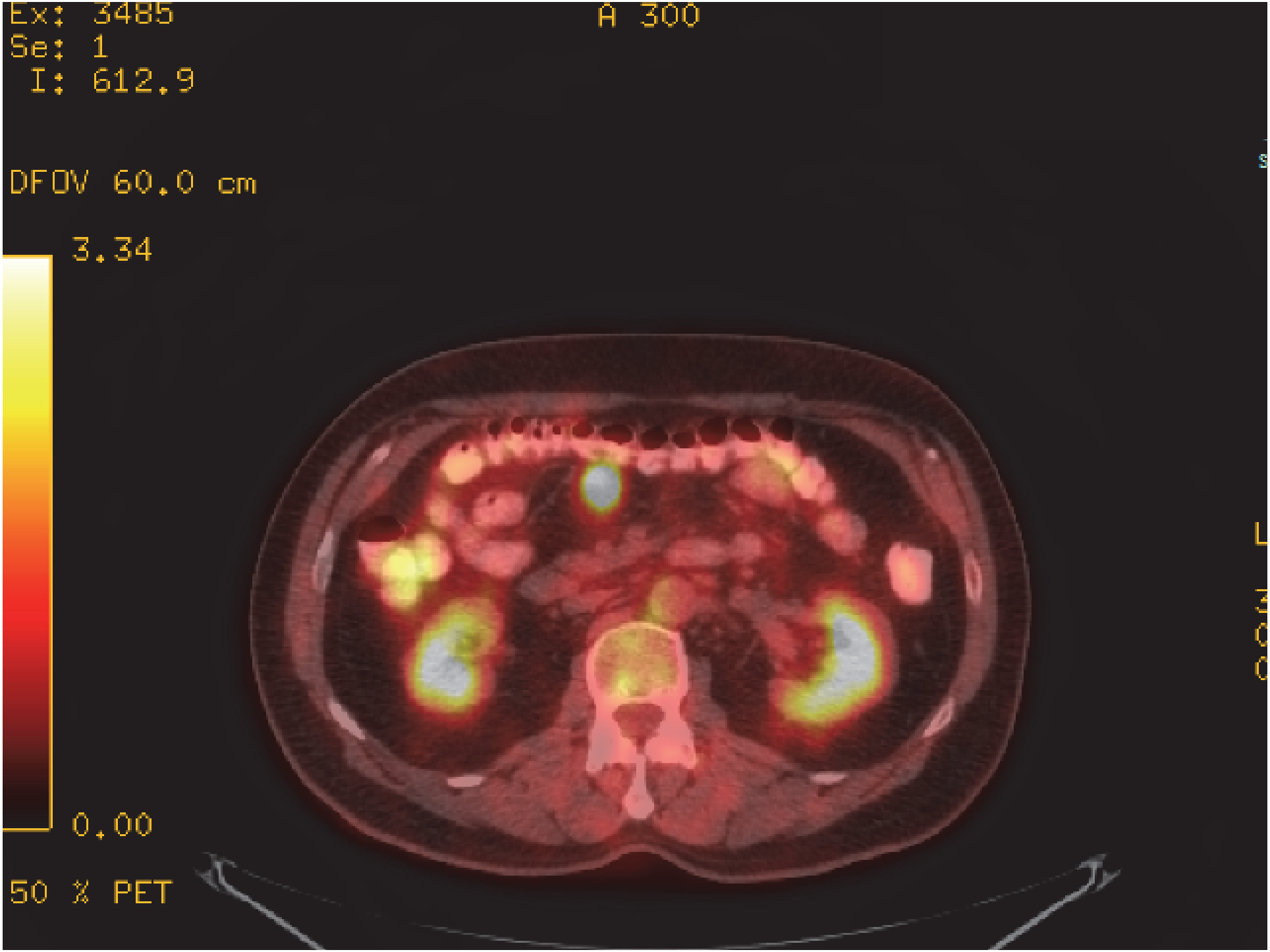
CD45 RO+
CD28++
CD127-hi

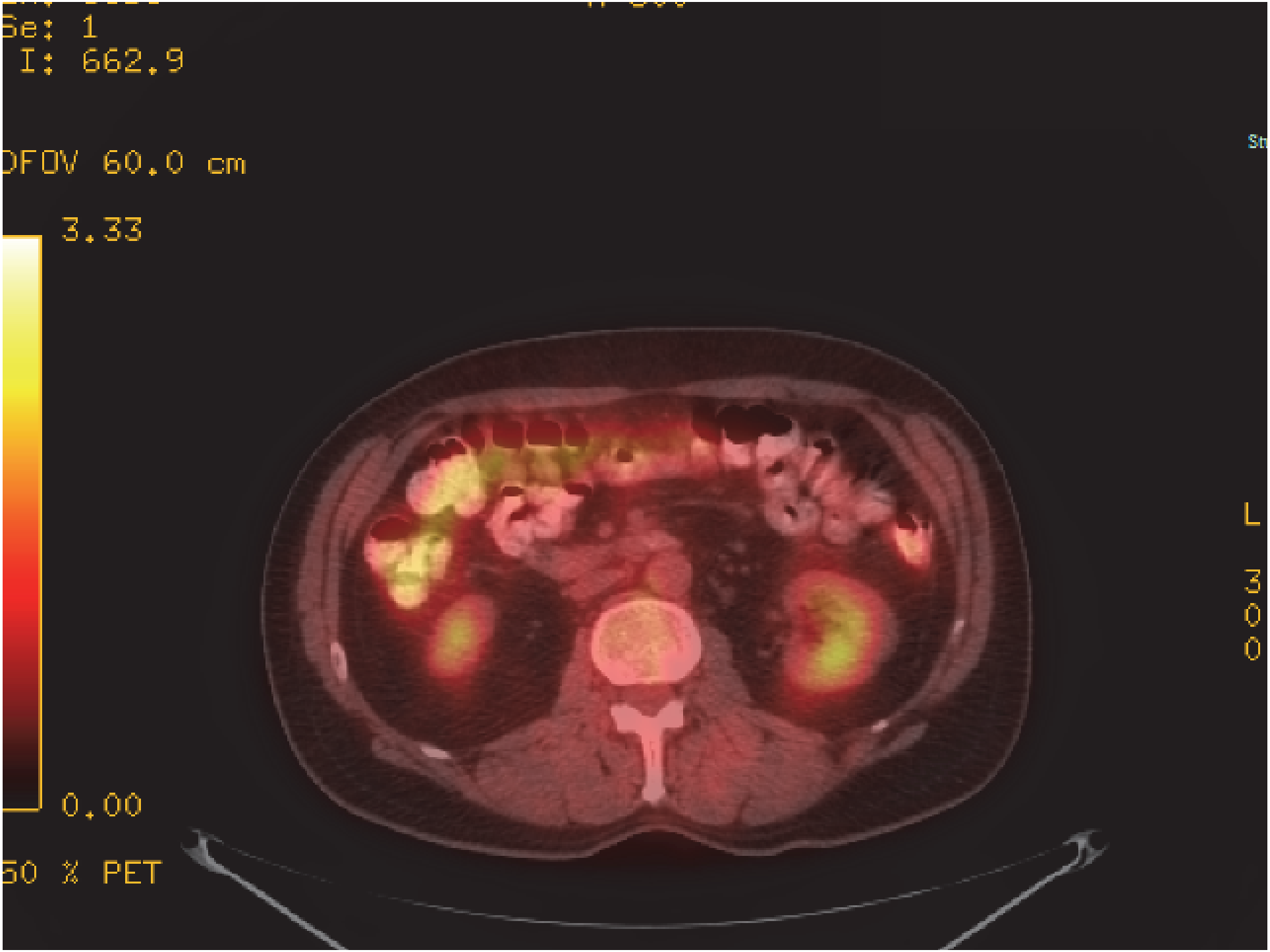
B


GV-Clone
Pre-inf.
GV-PBMC
Pre-inf.
Day 20
Day 56
Day 508
<0.1%
1.6%
5.3%
1.5%
CD8
10 89
0.5 0.2
IFNγ
CD28
0.3 0.5
38 62
TNFα
CD28
0.5 0.1
99 0.8
IL-2

Clinical Response

Patient	Target	Toxicity	Persistence	Disease Sites	Response
2140-1	Tyrosinase	F,N,R		Cervical,supraclavicular LN, Chest Wall, Breast Pulmonary nodules	
2140-2	Tyrosinase	F		Mediastinal, Pulmonary nodules	
2140-3	gp100	F,N,R		Mesenteric LN, scapular subcutaneous dz	
2140-4	MART-1	F, N, R		Pulmonary, inguinal, subcutaneous	
2140-5	MART-1	F, N,R		Right and left kidneys, adrenal, liver	
2140-6	MART-1	F, N, R		Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.	


Ex: 3485
Se: 1
I: 612.9
A 300
DFOV 60.0 cm
3.34
0.00
50 % PET


Se: 1
I: 662.9
DFOV 60.0 cm
3.33
0.00
50 % PET
L
0.00

Clinical Response

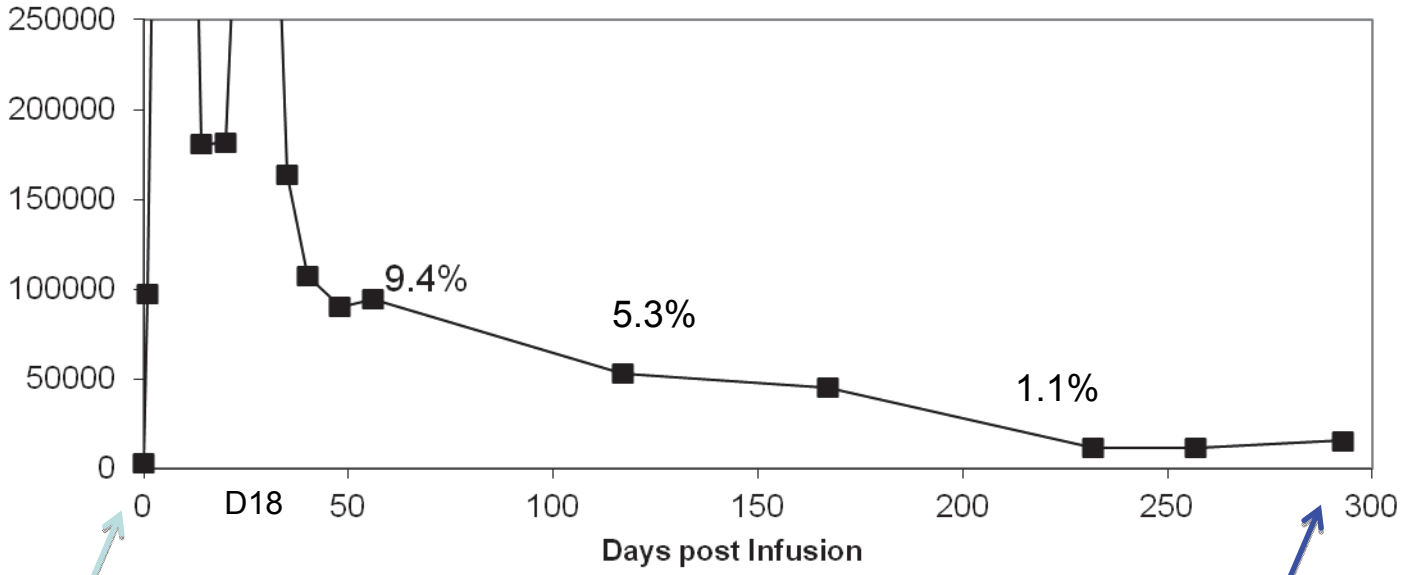
Patient	Target	Toxicity	Persistence	Disease Sites	Response
2140-1	Tyrosinase	F,N,R	>290 days	Cervical,supraclavicular LN, Chest Wall, Breast Pulmonary nodules	MR
2140-2	Tyrosinase	F	16 days	Mediastinal, Pulmonary nodules	PD
2140-3	gp100	F,N,R	>85 days	Mesenteric LN, scapular subcutaneous dz	CR (> 12 mths)
2140-4	MART-1	F, N, R	> 30 days	Pulmonary, inguinal, subcutaneous	SD
2140-5	MART-1	F, N,R	> 30 days	Right and left kidneys, adrenal, liver	PR
2140-6	MART-1	F, N, R	> 30 days	Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.	PR

CD8 T Cell Therapy Following Cytoxan Conditioning

- Extended duration of in vivo persistence
  - High-dose Cytoxan
  - CD8+ T cell clones
  - IL-2
- Durable Clinical Responses ?

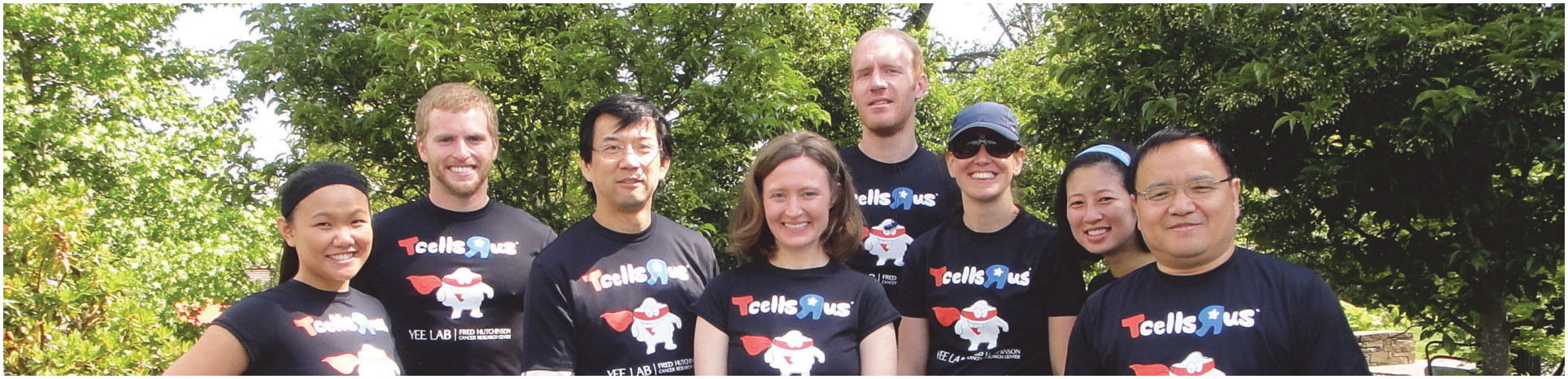
Persistence

2140-1



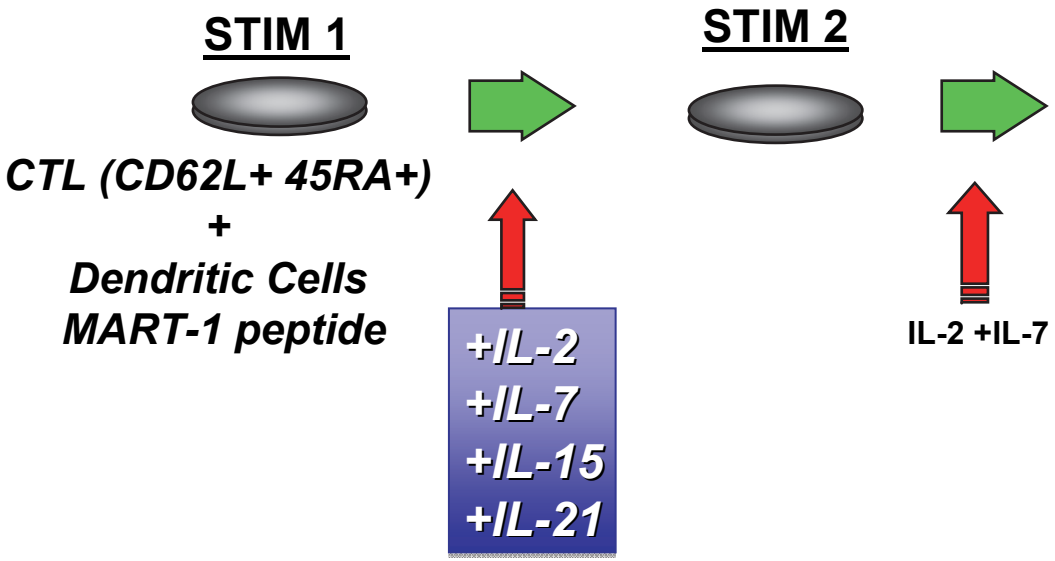
CD45 RO+
CD28-
CD127-lo

CD45 RO+
CD28++
CD127-hi



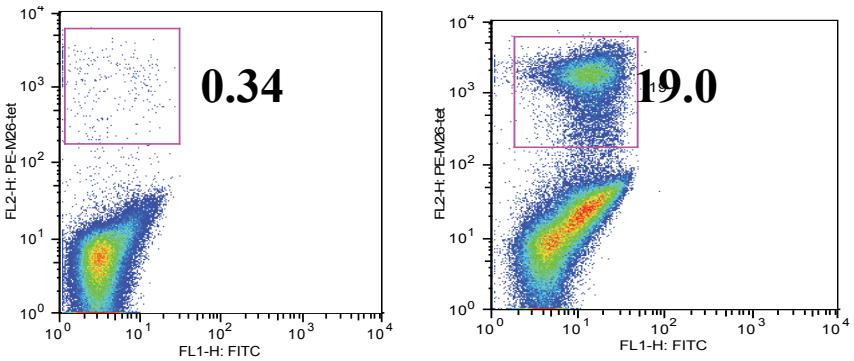
Yong Qing Li

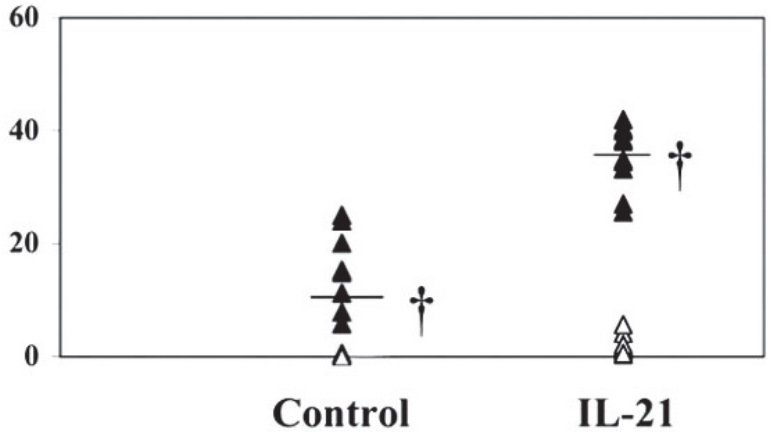
Can we enrich for or modulate the phenotype of antigen-specific CTL during in vitro priming?



- Frequency
- Surface Phenotype
- Functional Assays
- Affinity

CTRL* IL-21





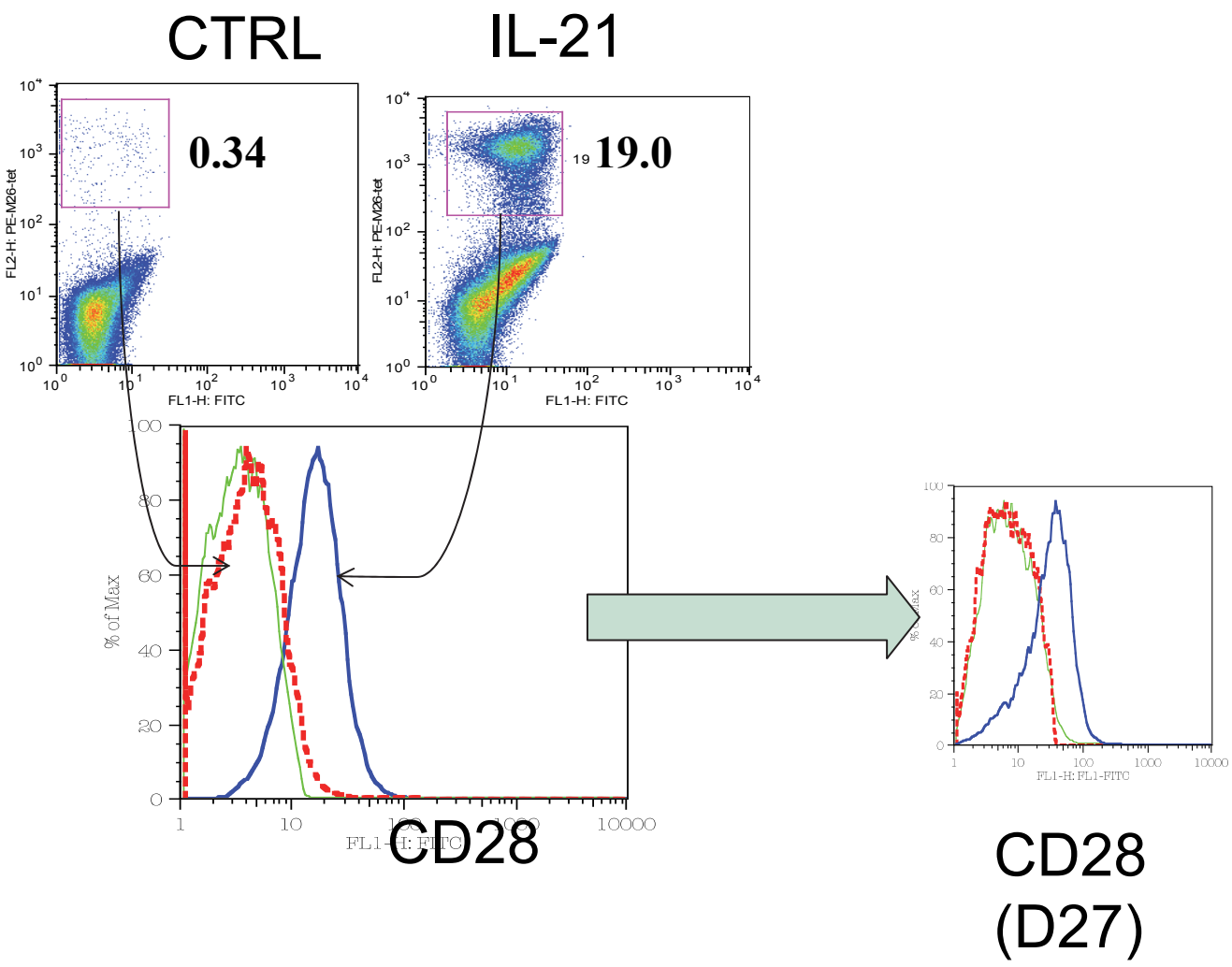
Greater Frequency

(ABS# 20-30-fold)

Improved Function

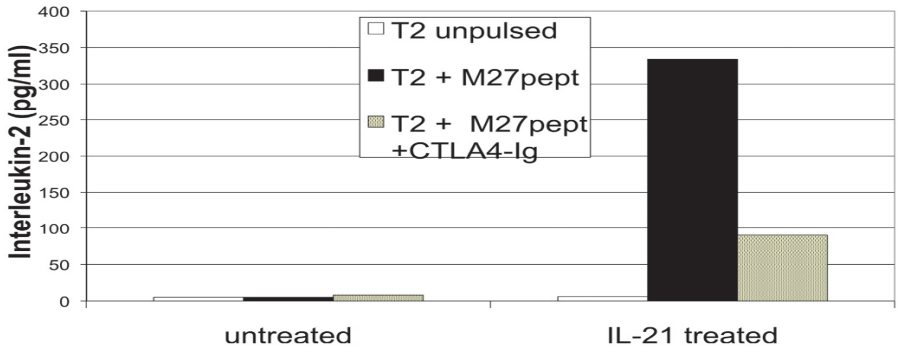
(by peptide titration and Tetramer dissociation)

Li et al, J Immunol 2005
Li et al, Blood 2008


CTRL
IL-21
0.34
19.0
FL2-H: PE-M26-tet
FL1-H: FITC
% of Max
CD28
CD28
(D27)

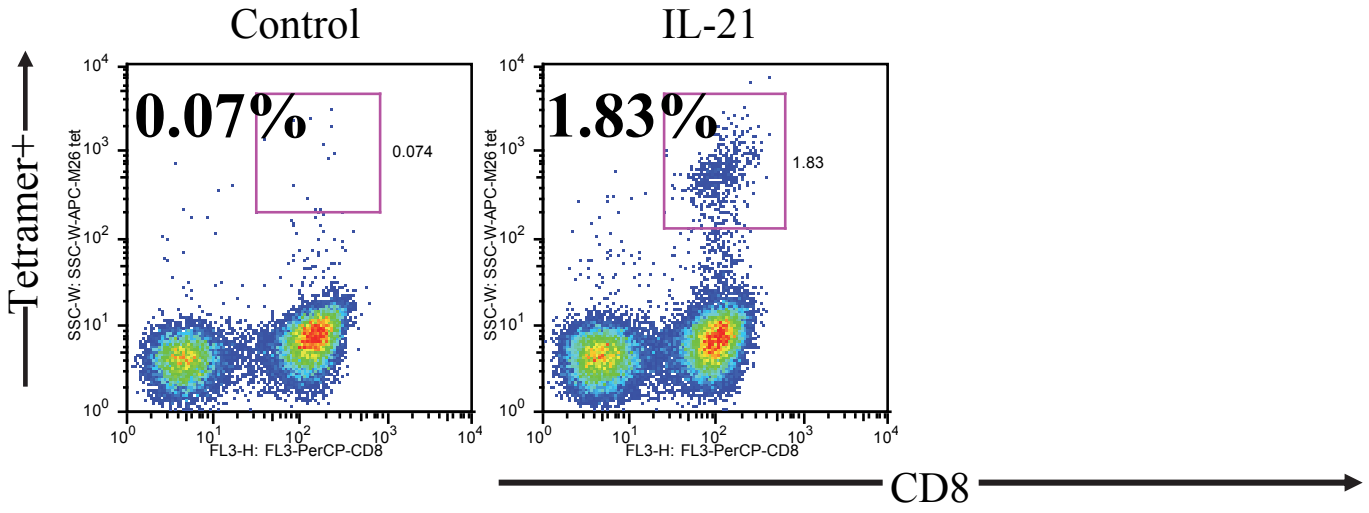
Helper-Independence

	CD45R0	CD62L	CD127	CD28
Naïve	-	+	+	+
Central M.	+	+	++	+
Effector M.	+	-	-	-



Synergism with CD25 depletion

A

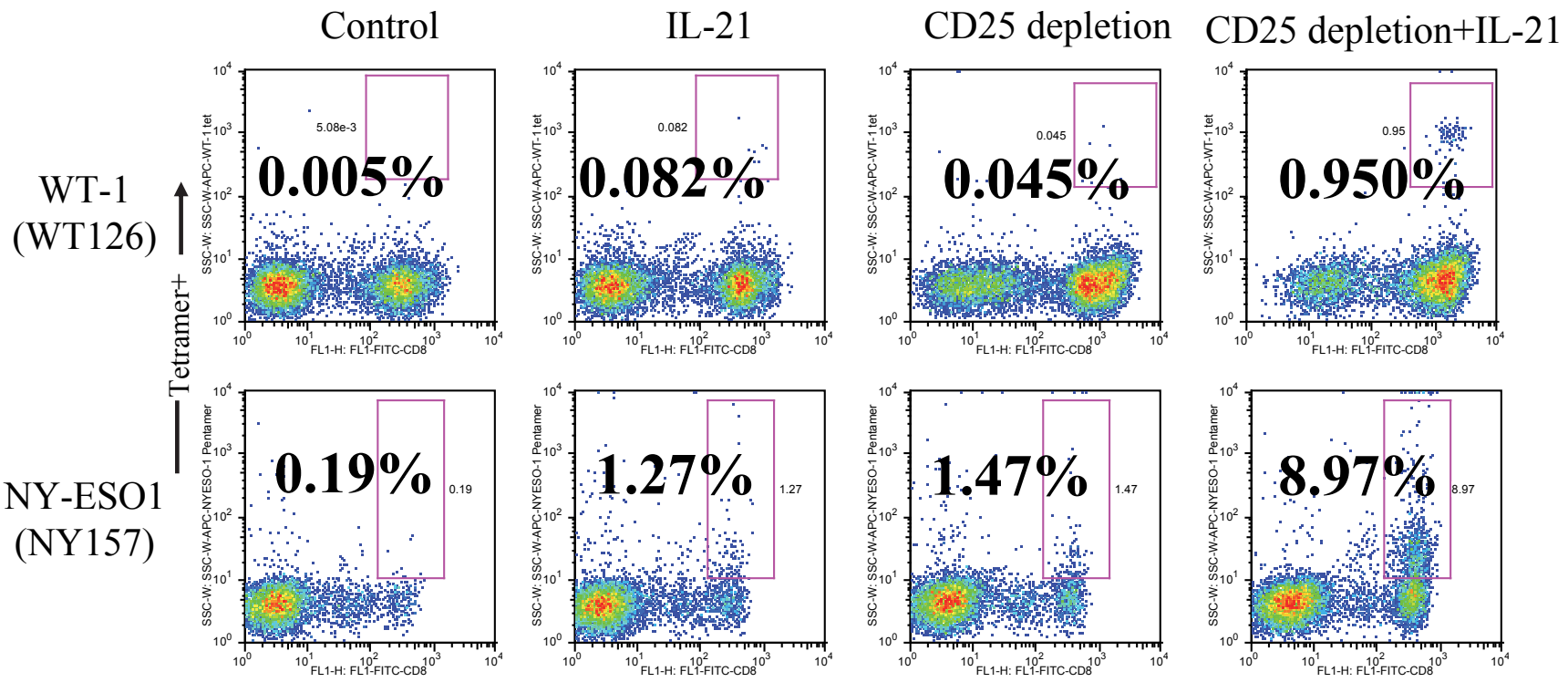


B

	Experiment. 1 Absolute number (x 10^6) / Fold increase (vs control)	Experiment. 2 Absolute number (x 10^6) /Fold increase (vs control)	Experiment. 3 Absolute number (x 10^6) / Fold increase (vs control)
Control	1.45 / 1	1.68 / 1	0.45 / 1
IL-21	17.14 / 11.82	23.17 / 13.79	8.37 / 18.60
CD25 depletion	8.20 / 5.66	12.64 / 7.52	4.35 / 9.67
CD25 depletion + IL-21	244.94 / 168.92	462.65 / 275.38	141.00 / 313.33

Figure 2

A



B

	WT-1(WT126) Absolute number (x 10^6) / Fold increase (vs control	**NY-ESO1(NY157)** Absolute number (x 10^6) /Fold increase (vs control)
Control	0.023 / 1	0.558 / 1
IL-21	0.413 / 17.96	5.144 / 9.21
CD25 depletion	0.203 / 8.83	6.659 / 11.93
CD25 depletion + IL-21	5.928 / 257.74	56.78 / 101.76

IL-21 modulation of CTL

- higher in vitro frequency of rare TAA-specific CTL
  - Synergistic increase with in vitro CD25 depletion
- enhanced affinity/cytolytic function
- central memory/ helper-independent phenotype
- active on naïve CTL → program stable phenotype/early exposure, dose-dependent

Addressing Possible Reasons For Failure of Tumor Immune Surveillance

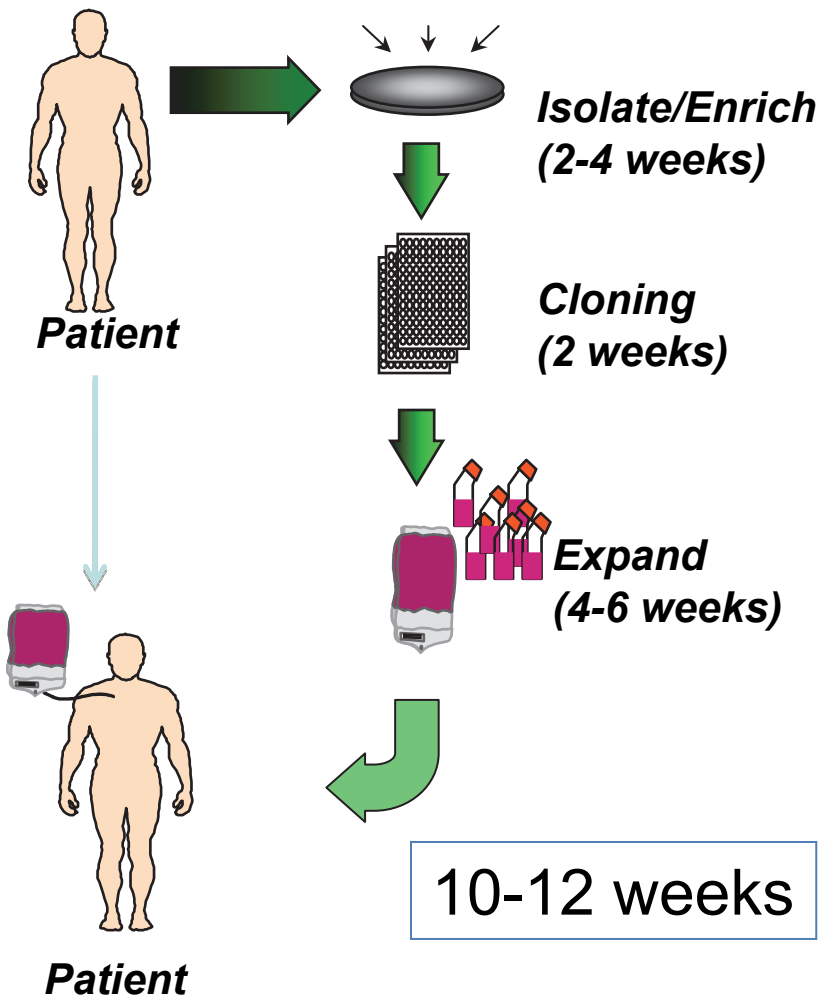
Highly functional T cells - *stronger*

More of them - *higher*

Expedience - *faster*

Expedience

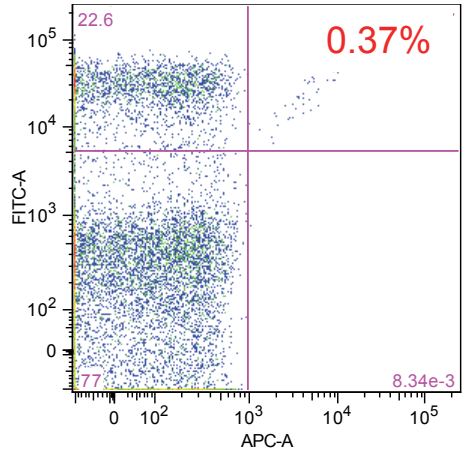
Clones

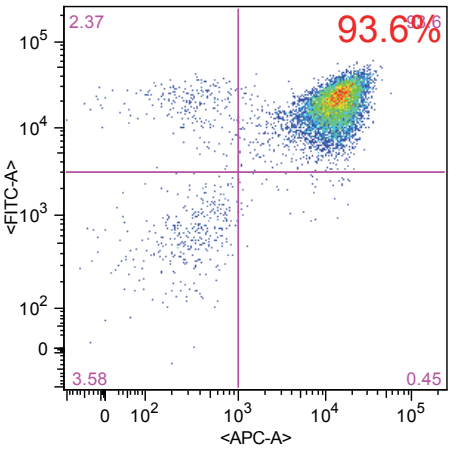


Patient

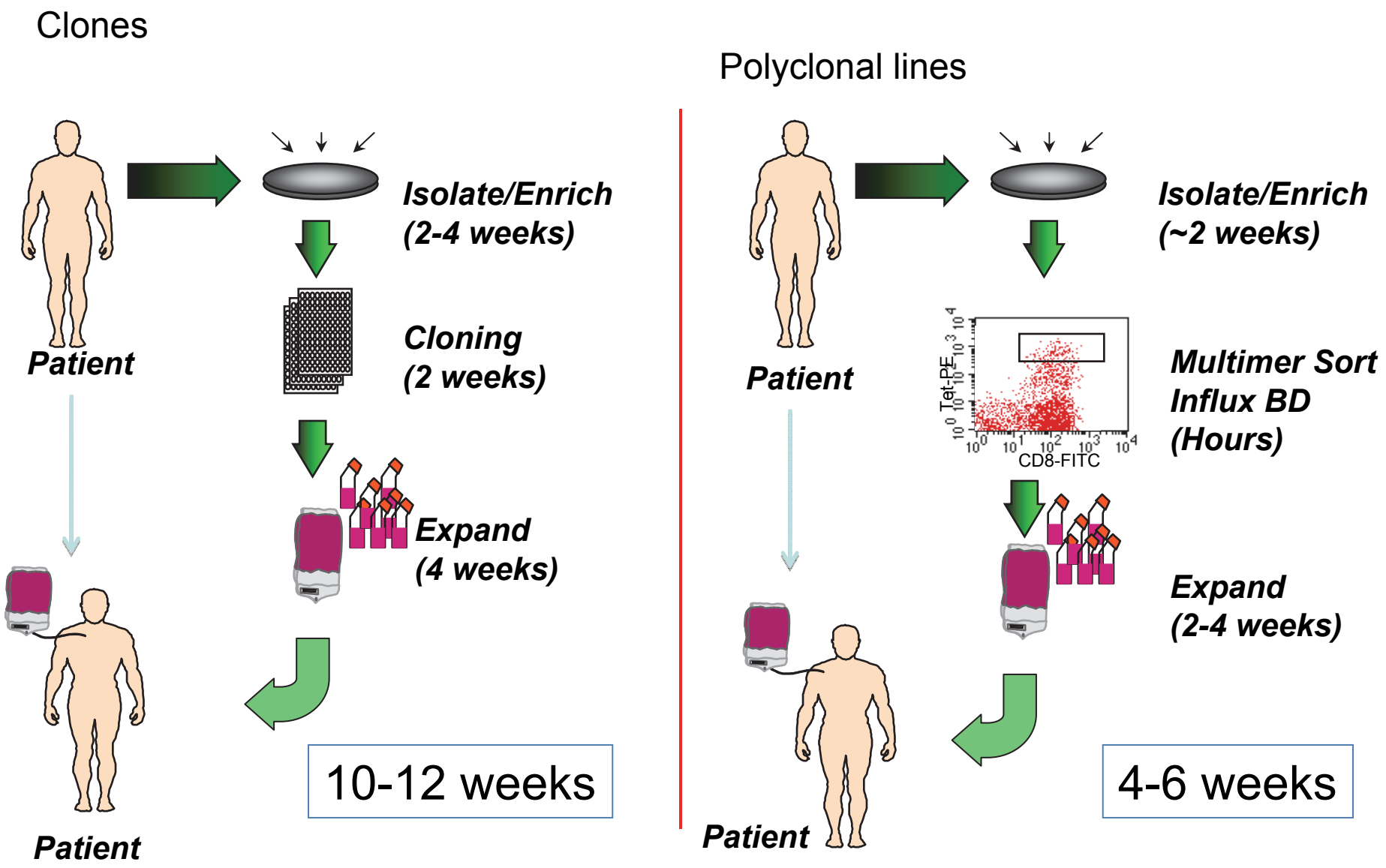
Clinical Grade pMHC-multimer-based Sorting

S

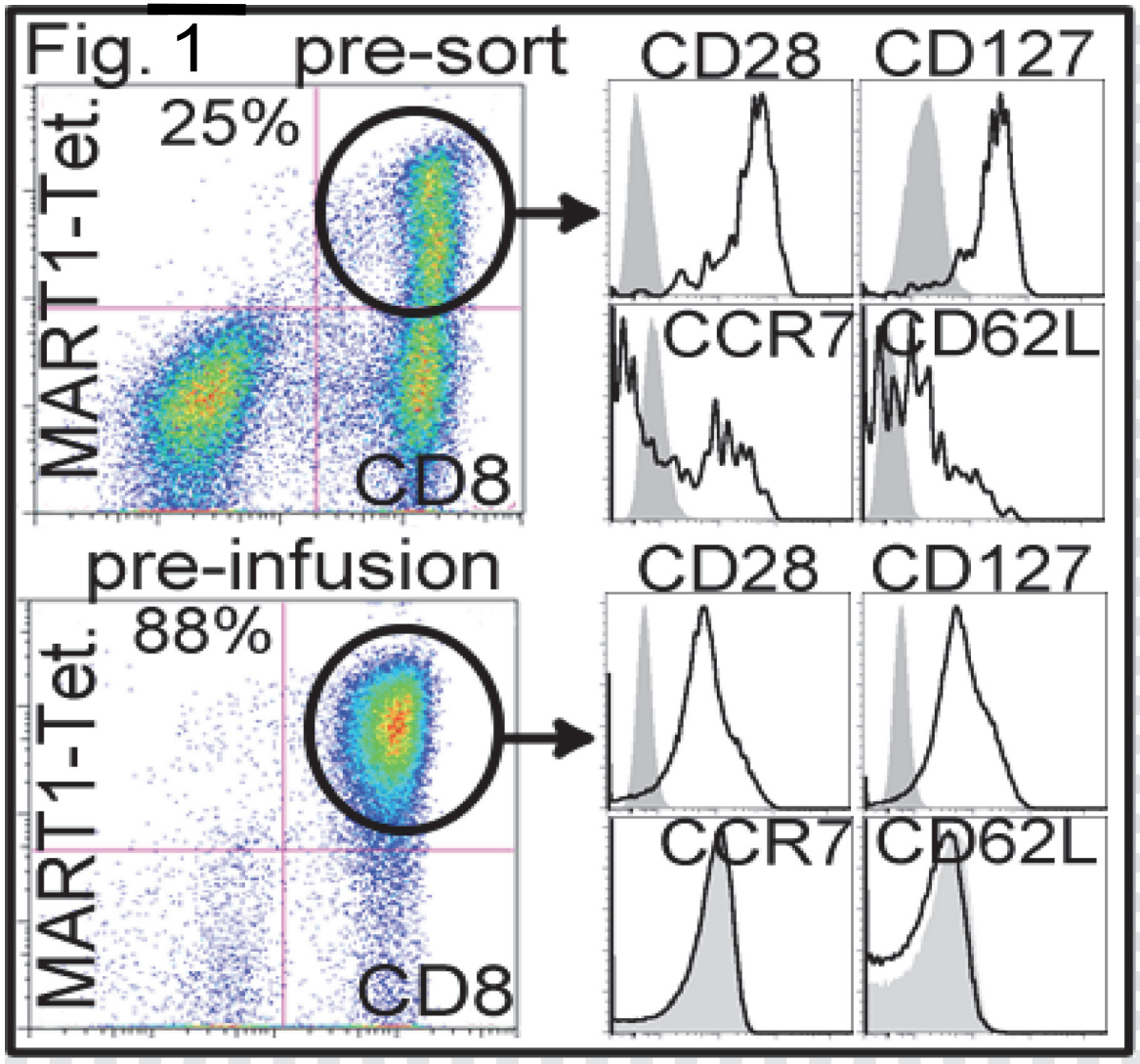




Expedience


Clones
Isolate/Enrich
(2-4 weeks)
Cloning
(2 weeks)
Expand
(4 weeks)
Patient
Patient
10-12 weeks
Polyclonal lines
Isolate/Enrich
(~2 weeks)
Multimer Sort
Influx BD
(Hours)
Tet-PE
CD8-FITC
Expand
(2-4 weeks)
Patient
Patient
4-6 weeks

IL-21 modulation generates population of TAA-specific Tcm



Adoptive T Cell Therapy: Extended Protocol

Intrinsic

Isolate/Enrich

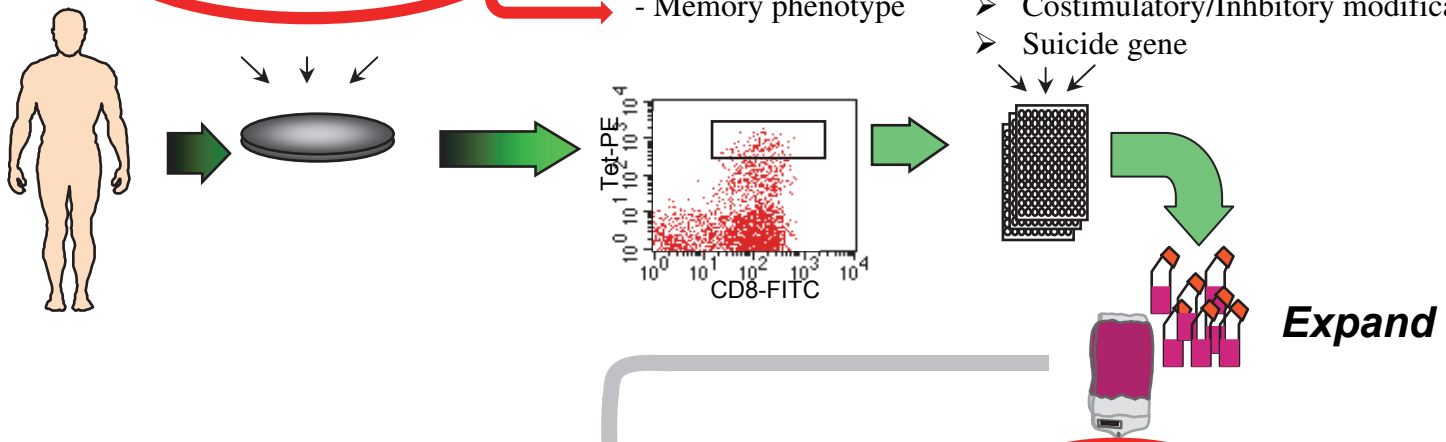
- Cytokine modulation

Clone/Select

- Phenotype
  - CD8/CD4
  - Memory phenotype

Genetically Modify

- TCR
- Chimeric receptor
- Costimulatory/Inhbitory modification
- Suicide gene

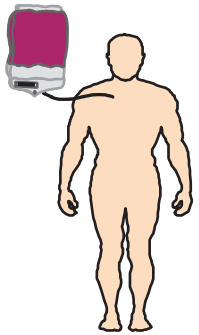


Expand

Extrinsic

Pre-infusion Immunomodulation

- Lymphodepletion
  - Chemotherapy/TBI



Post-infusion Immunomodulation

- Cytokine help
  - Low-dose IL-2
  - High-dose IL-2
  - Other γ-chain receptor cytokines
- Anti-CTLA4, Anti PD-1
- Vaccine + adoptive therapy

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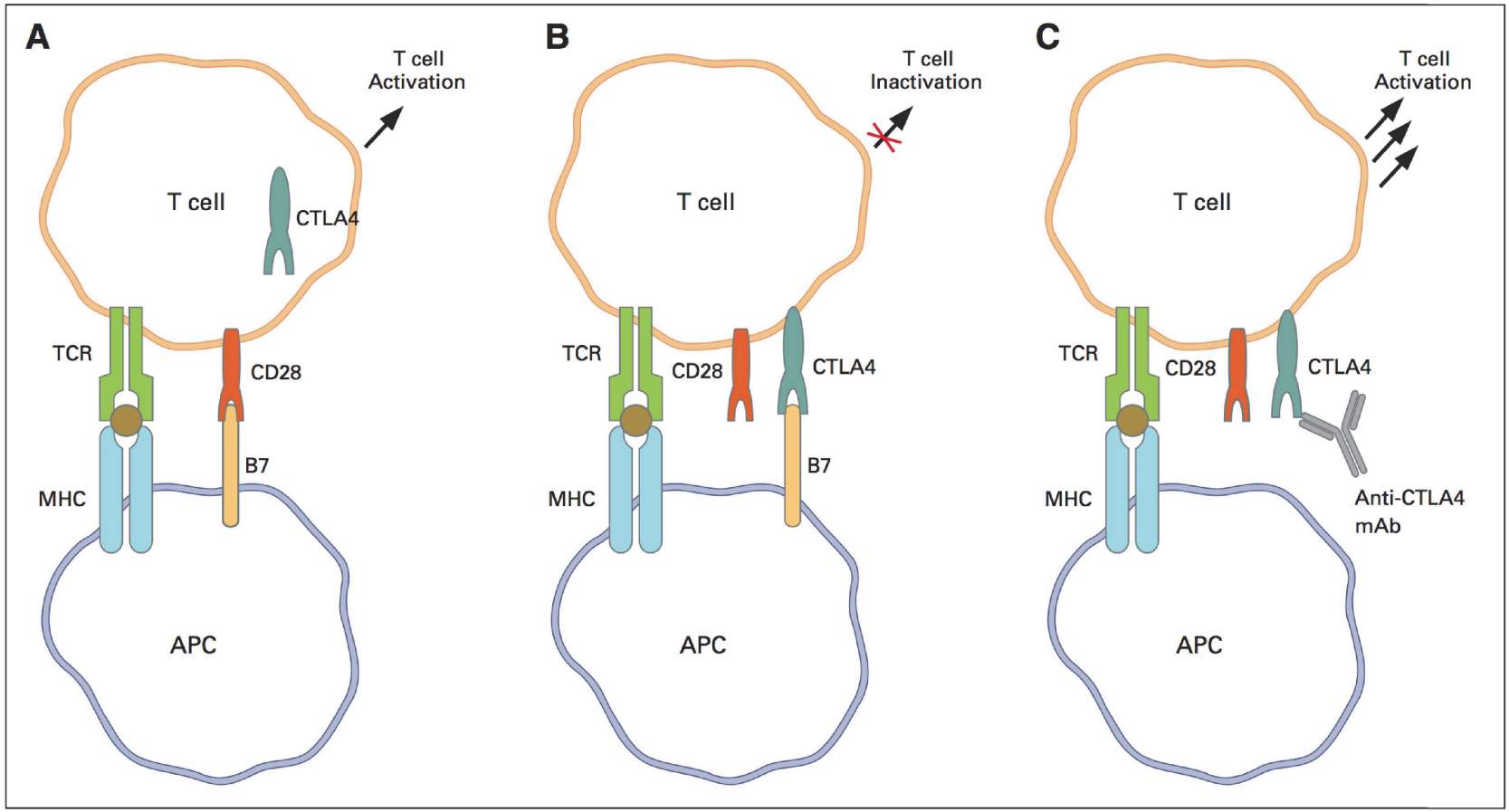
2. Anti-CTLA4 (Ipilimumab)

Mechanism of action of anti-CTLA-4 on CD8 T cells:



Phase I/II Clinical Trial of Adoptive Therapy using **IL-21-treated Antigen-specific CTL** In combination with **anti-CTLA4 Antibody Treatment**

Primary Aims

•Safety of anti-CTLA4 and CTL

•Influence of anti-CTLA4 and CTL on persistence and anti-tumor efficacy

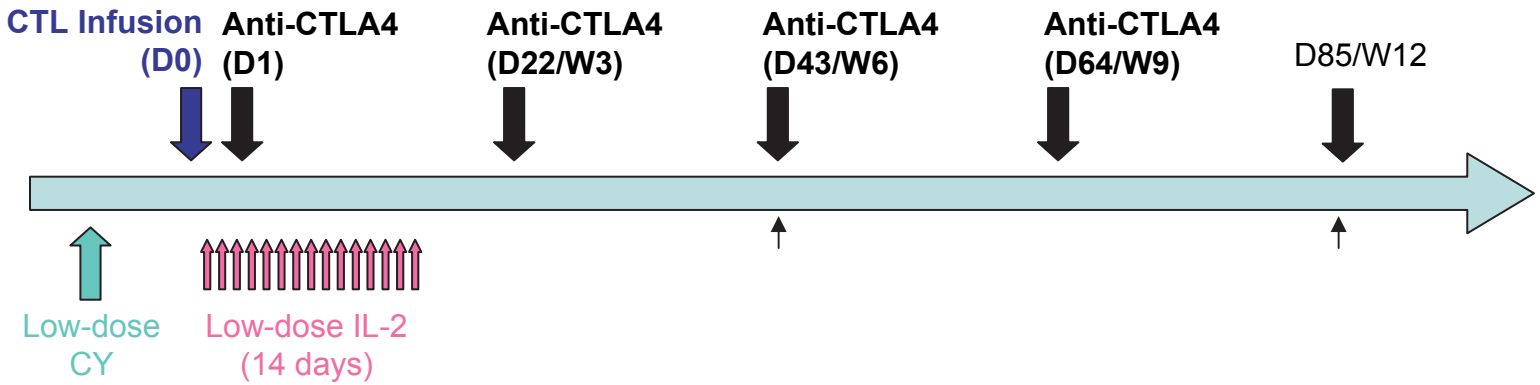
Secondary Aim

•Influence of anti-CTLA4 and adoptively transferred CTL targeting melanoma on epitope-spreading

Eligibility

•Metastatic melanoma – measurable disease

•Expression of HLA A*0201



IL-21- CTL + anti-CTLA4

Patient	Age, Sex	Previous Treatments
1	59 M	Surgery, IFN, HD IL-2, T-cell clones (#2179), **ipilimumab**.
2	66 F	Surgery, HD IL-2, Cisplatin + ALT 801 (anti-p53 antibody linked to IL2), **ipilimumab**.
3	33 M	Surgery, HD IL-2, **ipilimumab** x 2.
4	39 M	Surgery, HD IL-2.
5	46 M	Surgery x 2.
6	63 F	Surgery x 2.
7	68 F	Surgery x 2

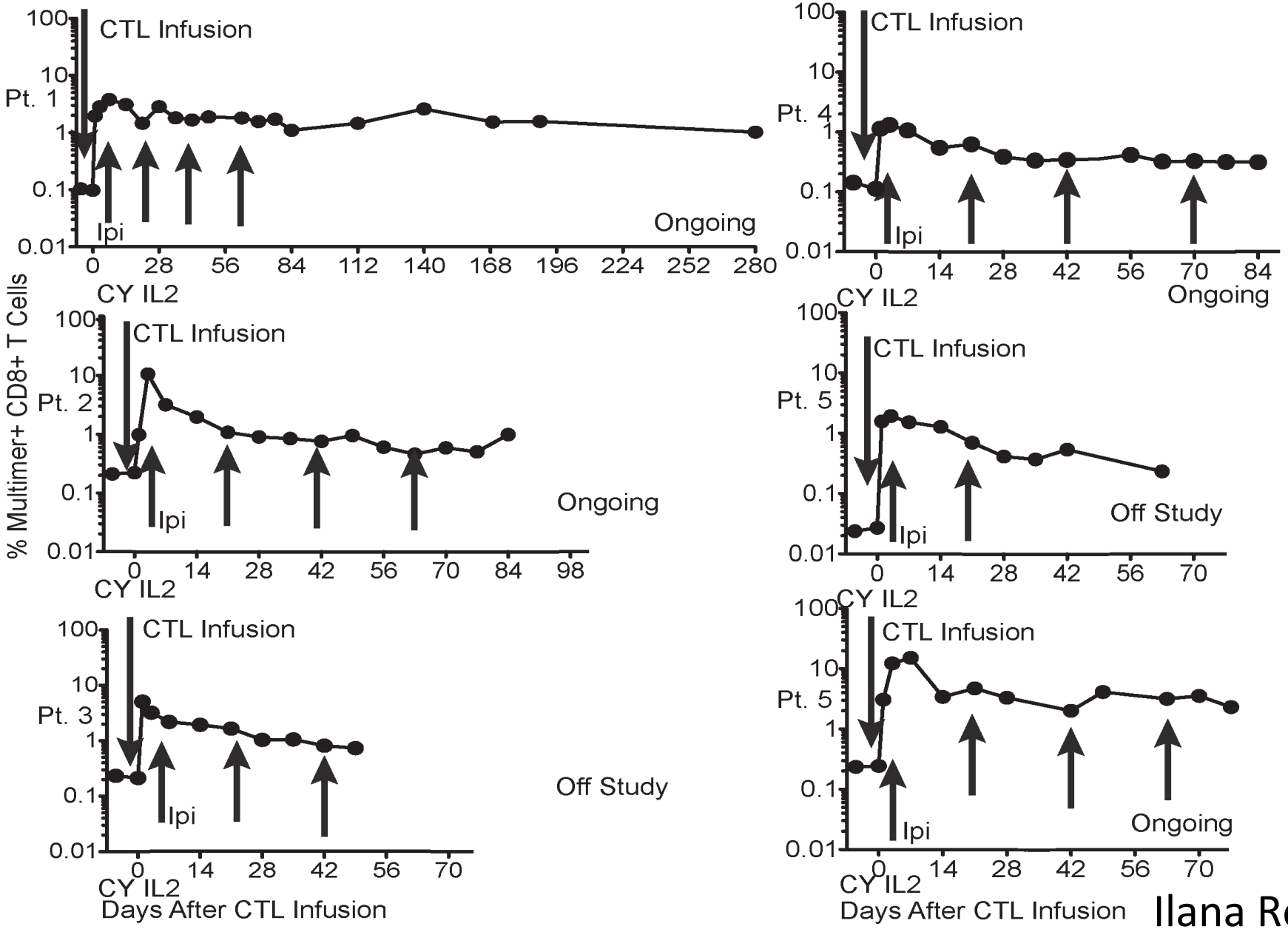
IL-21- CTL + anti-CTLA4

Patient	Age, Sex	Previous Treatments	Disease Site	Prior Ipilimumab Failure
1	59 M	Surgery, IFN, HD IL-2, T-cell clones (#2179), **ipilimumab**.	Subcarinal, hilar, paratracheal LAD.	YES
2	66 F	Surgery, HD IL-2, Cisplatin + ALT 801 (anti-p53 antibody linked to IL2), **ipilimumab**.	Pulmonary nodes	YES
3	33 M	Surgery, HD IL-2, **ipilimumab** x 2.	Pre-tracheal, hilar, pulmonary, liver, pancreatic, pelvic LAD.	YES
4	39 M	Surgery, HD IL-2.	Sucutaneous neck, gluteus, left flank and lung nodes.	NO
5	46 M	Surgery x 2.	Cutaneous lower left extremity	NO
6	63 F	Surgery x 2.	Lung nodes, pelvic LAD.	NO
7	68 F	Surgery x 2	Left inguinal LAD, cutaneous nodes.	NO

IL-21- CTL + anti-CTLA4

- Treatment has been overall well tolerated
- 5/6 patients developed mild rashes within 1 week of CTL infusion and 1 patient developed vitiligo at ~12 weeks.
- Main side effects observed have been related to ipilimumab (dry skin, GI symptoms, elevated liver enzymes).
- 1 patient received Vemurafenib for progressive disease 2 weeks after last dose of ipilimumab and developped fevers/rash necessitating hospitalization.

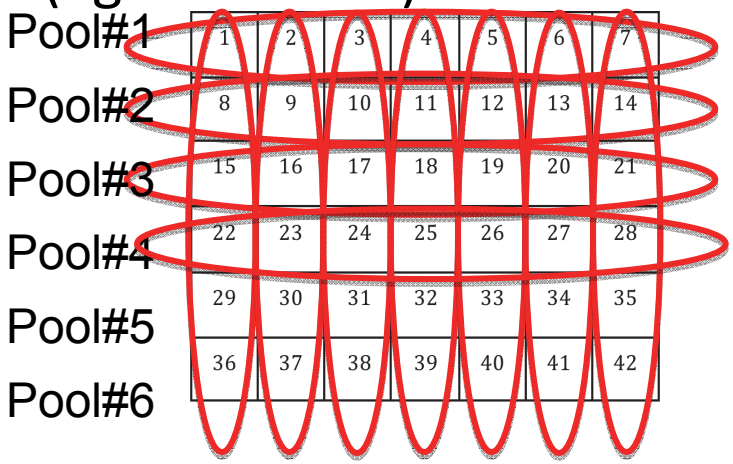
IL-21-derived polyclonal CTL persist in vivo ≥ 42 days (multimer)



Ilana Robe

Assessment of epitope spreading: method

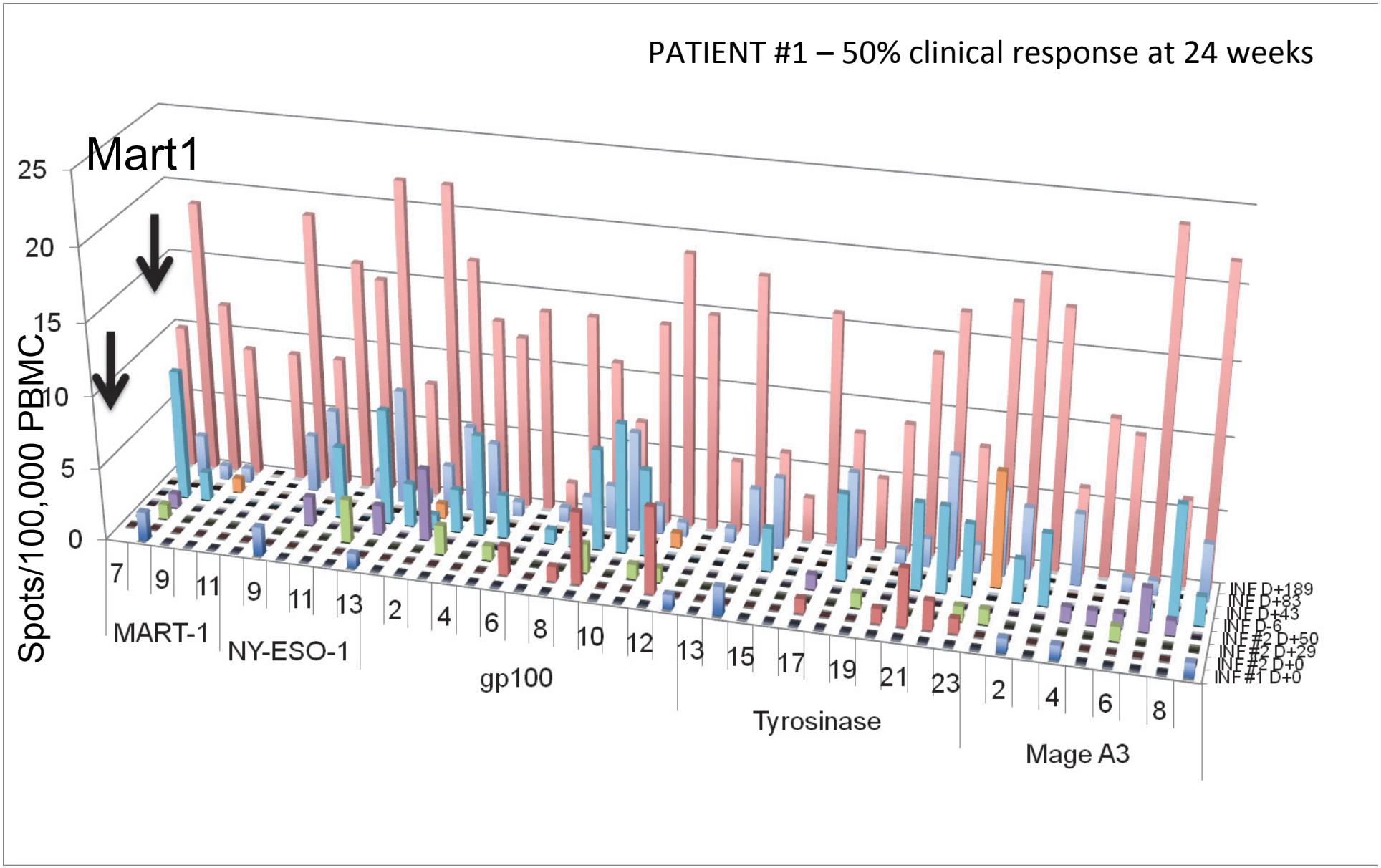
- Designed peptide libraries spanning MART-1, NY-ESO1, Gp100, Tyrosinase, Mage A3
- 15mers with 4 amino-acid offset: Long peptide sequence with short offset number = most chances of multiple epitope hits.
- Arranged in matrix (Eg. NY-ESO-1):



- PBMC reactivity to individual peptide pools tested by IFNγ Elispot.
- Results expressed spots/100,000 PBMC

Ilana Roberts

Assessment of epitope spreading: results pt 1



Conclusions/Future Directions

- No immediate toxicities were observed with LD CY, CTL, low-dose IL-2 and Ipilimumab (not enough patients to reliably establish safety).
- Polyclonal CTL generated in the presence of IL-21 and infused in patients receiving anti-CTLA4 persist and upregulate/acquire characteristics associated with the establishment of long-lived memory T-cells.
- Persistent tumor-specific CTL (with characteristics of long-lived memory) may not be sufficient to induce tumor regression in all patients.
- Evidence of epitope spreading was observed in patients with tumor regression/stable disease. Results need to be compared to patients receiving ipilimumab alone.

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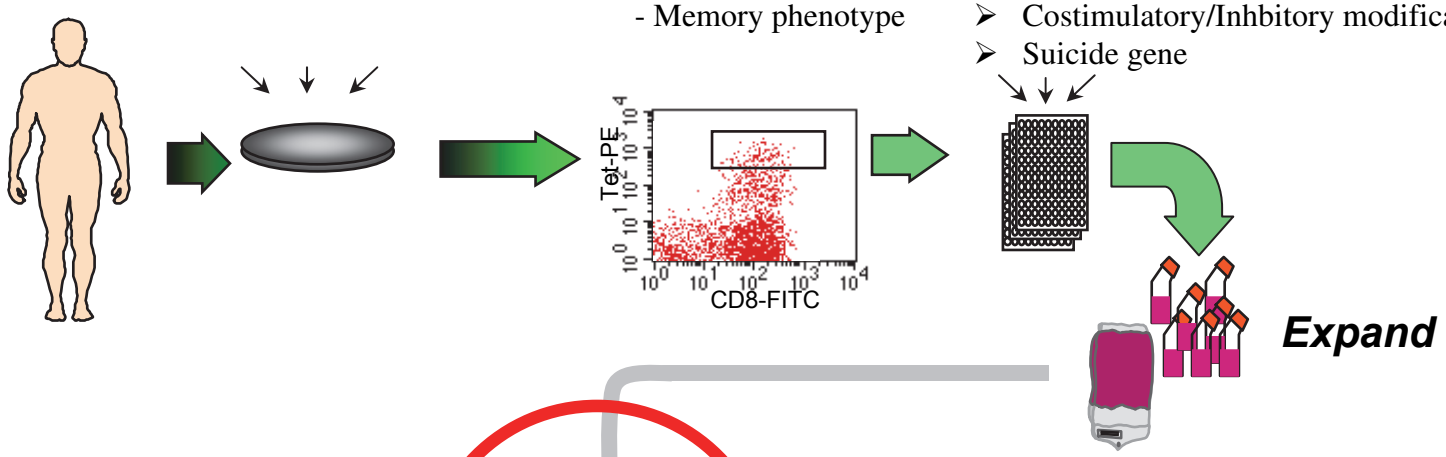

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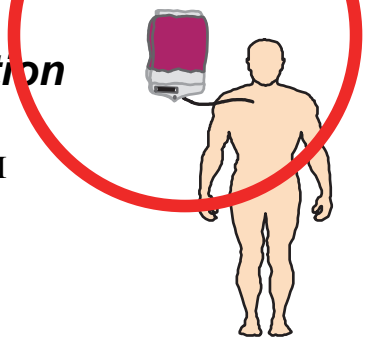

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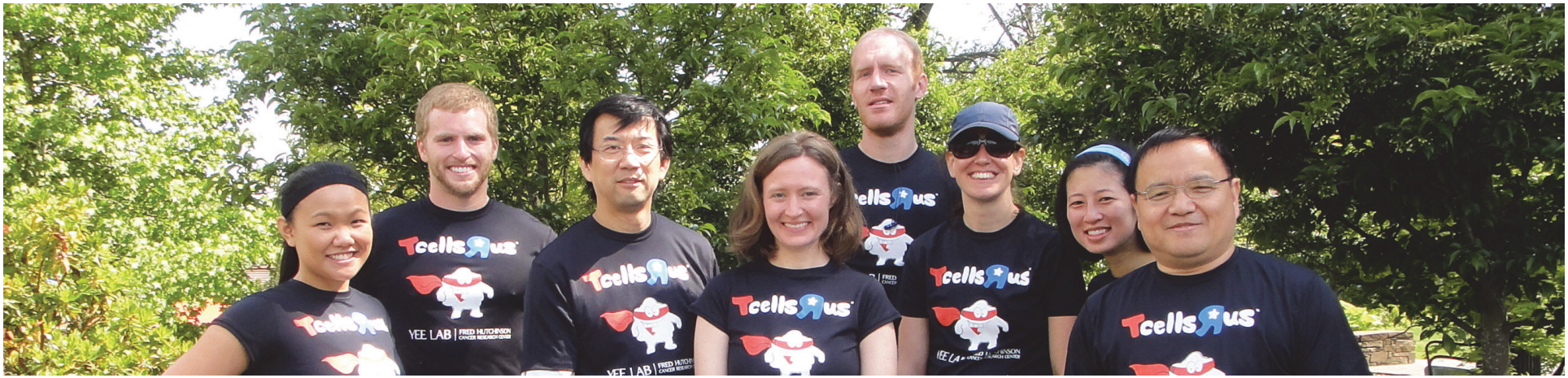

- Therapy Lab
  - Erik, Ivy, Rebecca
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  - Jianhong Cao
  - Andrew Berger

- YongQing Li (IL-21)
- Aude Chapuis (CTL + aCTLA4)
- Sylvia Lee (CD25 depletion)
- Erik Farrar (Cell Sorting)

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- Edson Foundation
- Bezos Immunotherapy Fund
- Walker Fellowship
- GCRC/ITHS

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