Adoptive T Cell Therapy

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Immunotherapy : A Renaissance in Cancer Treatment

Adoptive T Cell Therapy

T cells taken from a leukaemia patient and multiplied in culture are ready for infusion.

ADOPTIVE CELL THERAPY

Honing that killer instinct

Genetically altered immune cells are helping to push life-threatening cancers into remission and generating a buzz.

BY COURTNEY HUMPHRIES

A few years ago, when Michel Sadelain spoke about adoptive cell transfer (ACT) therapy at cancer meetings, his colleagues were dubious about what seemed a drastic and unconventional approach: harvesting and genetically altering his patient's immune cells to train them to attack her cancer. "I can't tell you how many nearly empty rooms I've spoken to about this technique," says Sadelain, director of the Center for Cell Engineering at Memorial Sloan-Kettering Cancer Center in New York.

The technique harnesses the power of the immune system by recruiting the body's own T cells — immune cells that recognize and marshal an attack against foreign invaders and diseased cells. T cells travel through the body, using their receptors to scan for small bits of protein called antigens on the surface of foreign cells. If an antigen matches the receptors, the T cell activates and launches an attack. In theory, malignant cells should be ideal targets for T cells, but tumours have ways of shielding themselves from an immune attack. With ACT, scientists tweak the T cells to give them a fighting chance. Sadelain calls them "living drugs".

Pilot studies in the past couple of years have had promising results, leading to increased interest and dozens more clinical trials investigating the technique. Success stories — albeit involving small numbers of patients — tell of people with aggressive cancers whose tumours melted away in days or weeks. In a field where extending life by a few weeks or months is considered a breakthrough, the complete remission of even a few patients is stunning. Sadelain is no longer speaking to empty rooms. Suddenly, he says, ACT has captured the imagination of scientists and pharmaceutical companies as if it were a new approach, rather than a field that has been developing for twenty years.

But there are both scientific and logistic challenges to expanding the use of this therapy. Researchers are still learning to control the cells' potency to ensure they can vanquish cancer without damaging normal tissue — an issue complicated by the fact that many cancer antigens are also found on normal cells. Another problem is that it's not yet clear how to turn ACT into a profitable business model, as harvesting and growing living cells requires much more time and skill than prescribing a drug. So while pharmaceutical companies are licensing proprietary receptors and looking into ways to scale up the process, that's just the start of the endeavour. As with any therapy, the companies still need to embark on large, multicentre clinical trials to test the effectiveness of the therapies on a broader group of patients. But large trials also require a way to engineer and distribute large quantities of cells, so they will only happen if companies are confident of long-term profitability.

Proponents of the approach say that the possibility of eradicating life-threatening tumours makes these challenges worth tackling. And recent progress in designing ACT therapies that are surprisingly effective is causing many in the field to sit up and take notice.

BOOSTING THE BODY'S CELLS

There are three strategies for ACT therapies (see 'Cellular attack'); the most-developed of which is the simplest. The tissue surrounding a tumour is likely to contain immune cells with antitumour activity, so doctors take a sample of this tissue and select those T cells that have been primed to attack the cancer. They culture these cells in the lab until they have enough, and re-infuse the cells back to patients along with the T-cell growth factor interleukin-2 (IL-2), which promotes the proliferation of antigen-specific T cells. However, the endogenous immune system has suppressive mechanisms that keep the immune response in check, and these mechanisms also prevent the newly transferred cells from working effectively. So patients must also be treated with drugs or radiation

Immune Checkpoint Blockade

DRUG DEVELOPMENT

Releasing the brakes

Tumours can put a brake on the immune system, but new therapies work by removing these brakes. Now, researchers have to figure out how to use them most effectively.

BY KAREN WEINTRAUB

First it was one melanoma patient, a woman named Sharon, who should have died but didn't. Then, several more outlived their prognoses — not just surviving but seeing their tumours shrink dramatically or even disappear. As the successes accumulated, in both individual patients and larger clinical trials, oncologist Antoni Ribas slowly began to accept that the immune treatments he was giving to his cancer patients were making a profound difference. Initially only about one in ten patients improved, but that fraction increased as he and his colleagues tested newer versions of the therapy. Ribas, a tumour immunology researcher, now has dozens of patients, like Sharon, whom he had expected to succumb cancer years ago. His patient load at the Jonsson Comprehensive Cancer Center at the University of California, Los Angeles (UCLA) used to stay about the same from one year to the next, with new melanoma patients roughly equaling the number who didn't make it. Now, the number of patients is growing.

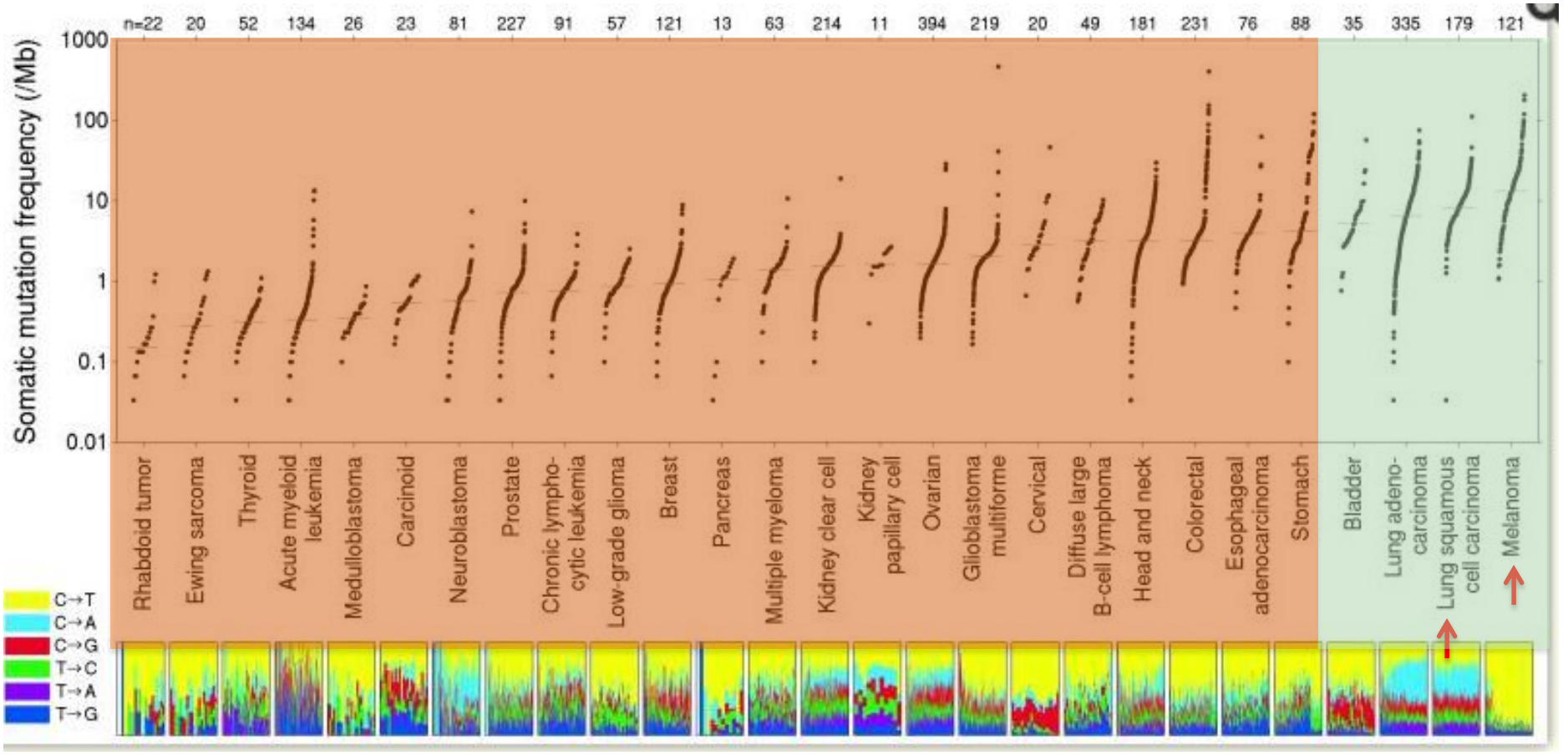
The drugs he uses are known as immune checkpoint blockades and they are designed to circumvent one of the insidious ways in which cancer staves off an immune response. The immune system has a number of checkpoints — mechanisms that help to prevent it from getting out of control and attacking the body's own cells. The checkpoints act much like the brakes on a car: even if the immune system is trying to prompt its T cells into action, the checkpoints suppress the activation. Tumours can turn on these checkpoints and prevent a T-cell attack, but immune checkpoint blockades take the brakes off the T cells, freeing them to fight the malignancy.

When other researchers saw the results of clinical trials of checkpoint blockades in melanoma, they dismissed them as too narrow to be of much use in other cancers. Melanoma was different, they said, and has a known immune component. Then, in 2012, everything changed. In one study, a checkpoint blockade caused a measurable improvement in 31% of renal cancer patients, and in 18% of patients with lung cancer, which kills more people every year than colon, breast and pancreatic cancers combined[1]. Researchers and drug companies realized that these blockades, also called checkpoint inhibitors, might be as effective in patients with any type of solid tumour as they were in those with melanoma. Jedd D. Wolchok, a medical oncologist at Memorial Sloan-Kettering Cancer Center in New York City, says the lung

NATURE.COM
For some of the latest research on immune therapies: go.nature.com/kzzctc

Clinical Outcomes following Immune Checkpoint Inhibitor Therapy

Correlation with Endogenous Tumor-reactive Population?



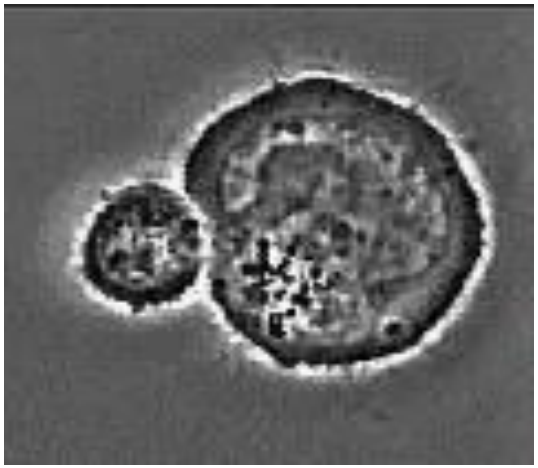
Hodi et al, NEJM 2010
Topalian S L et al. JCO 2014
Lawrence et al, Nature. 2013

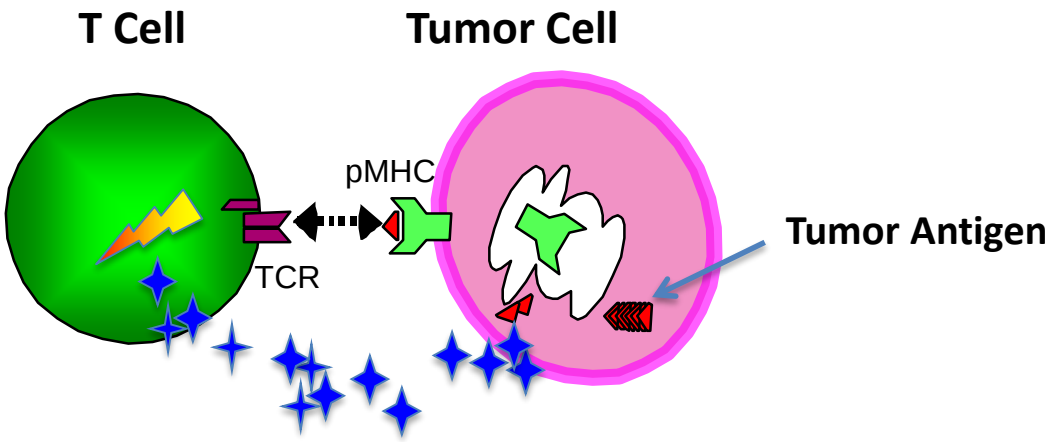
Endogenous Immune Response

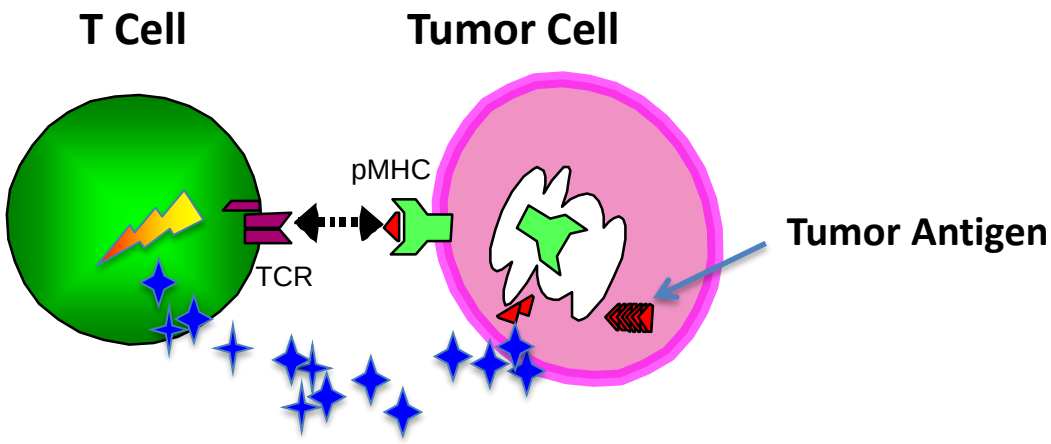
- Endogenous population of tumor reactive T cells

- Transfer of such T cells to patients with low endogenous frequency will lead to more effective combined therapy

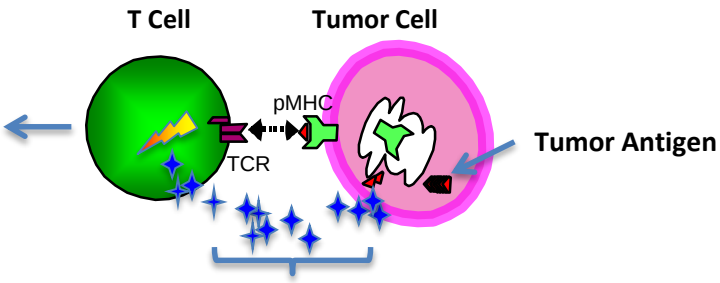
Renaissance in Immunotherapy Through Basic Research






T Cell
Tumor Cell
pMHC
TCR
Tumor Antigen

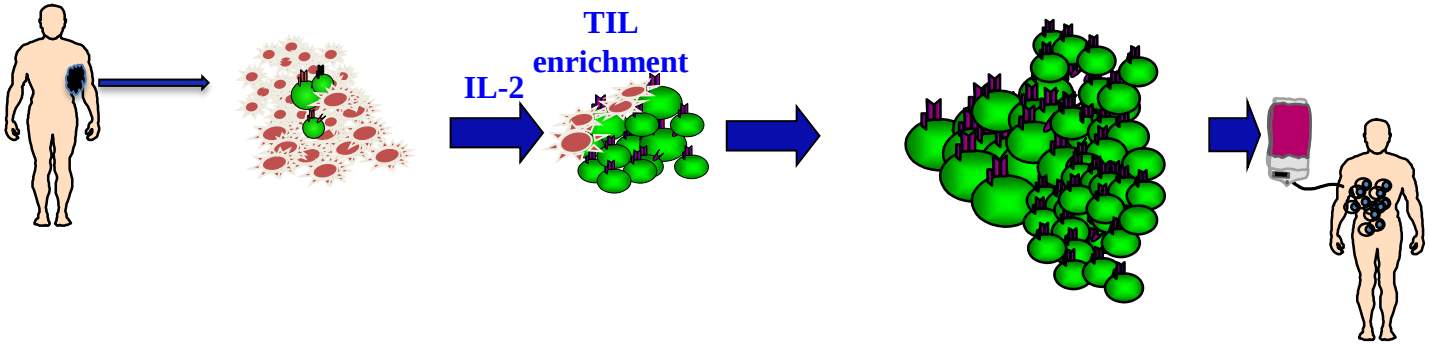
Immunotherapy vs. Conventional Therapy



- Non-cross-resistance to chemotherapy, radiation therapy
- Multiple killing mechanisms
- Potential for ‘memory’ = longterm protection = remission
- Single vs multiple lifelong administration

How to make T cells?

TIL
Tumor-infiltrating
Lymphocytes

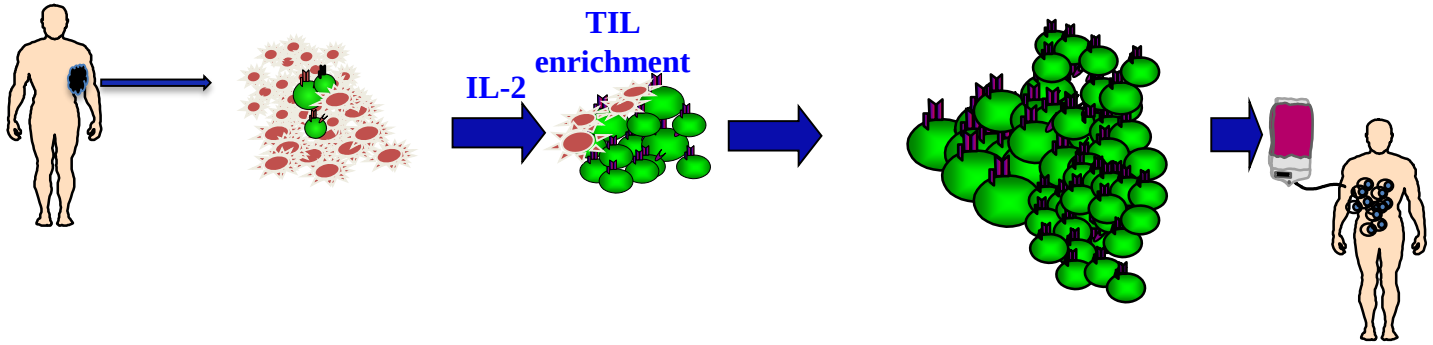


TCR/CAR
Engineered
T cells

ETC
Endogenous
T cell Therapy

How to make T cells?

TIL
Tumor-infiltrating Lymphocytes



Myelo-Ablative

Conditioning Lymphodepletion

Cytoxan (60 mg/kg)
Fludarabine (25 mg/m2)

TBI (1200)

HD IL-2

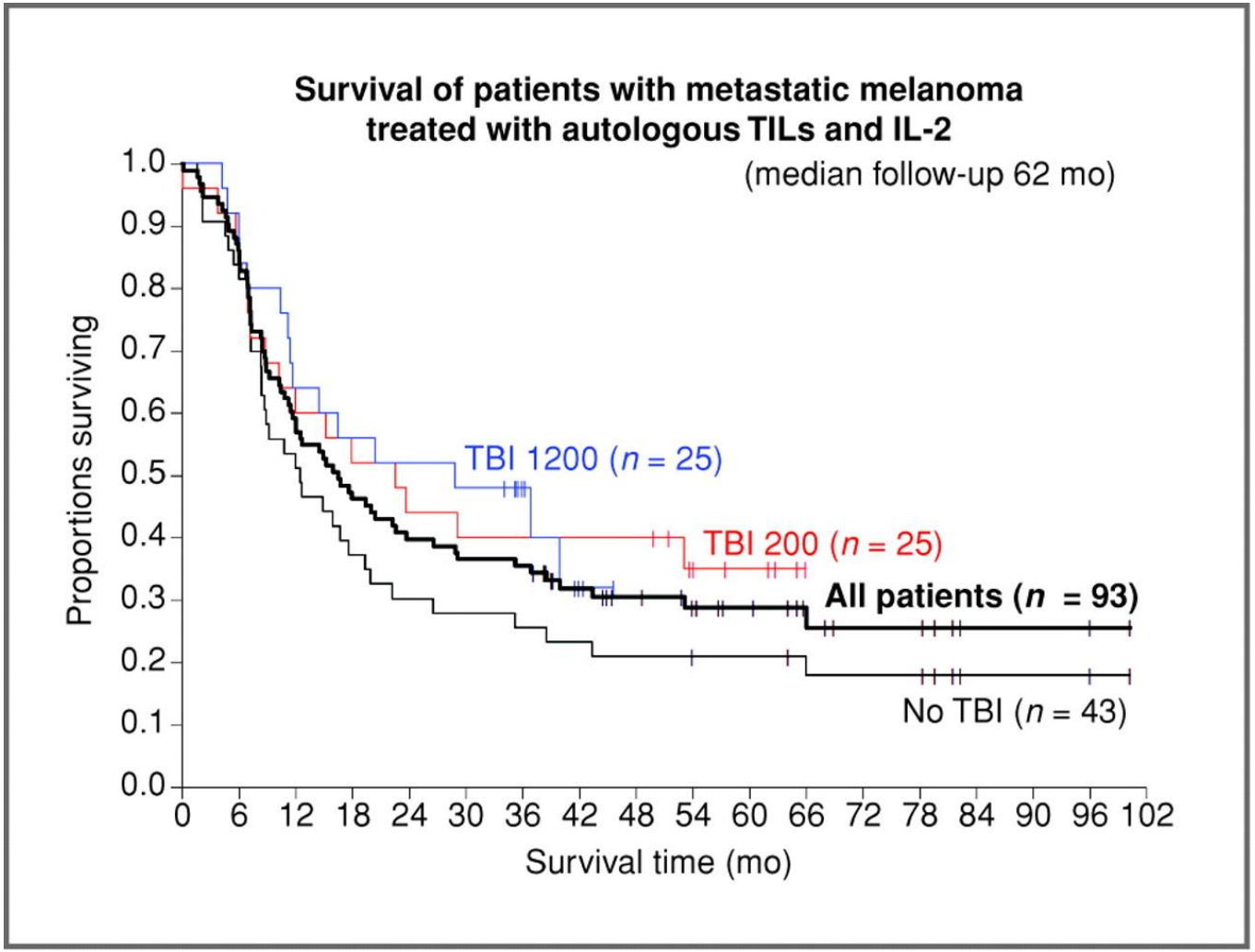
Tumor Infiltrating Lymphocyte

Treatment	*n* (%) of patients (duration in mo)			OR (%)
	Total	PR	CR	
No TBI	43	16 (37)	5 (12)	21 (49)
		84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2	82+, 81+, 79+, 78+, 64+	
200 TBI	25	8 (32)	5 (20)	13 (52)
		14, 9, 6, 6, 5, 4, 3, 3	68+, 64+, 60+, 57+, 54+	
1,200 TBI	25	8 (32)	10 (40)	18 (72)
		21, 13, 7, 6, 6, 5, 3, 2	48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19	
Total	93	32 (34)	20 (22)	52 (56)

Dudley, JCO, 2008

Tumor Infiltrating Lymphocyte

Overall survival of patients receiving TILs with the chemotherapy preparative regimen alone (no TBI) or plus 2 or 12 Gy TBI.



Rosenberg S A et al. Clin Cancer Res 2011;17:4550-4557

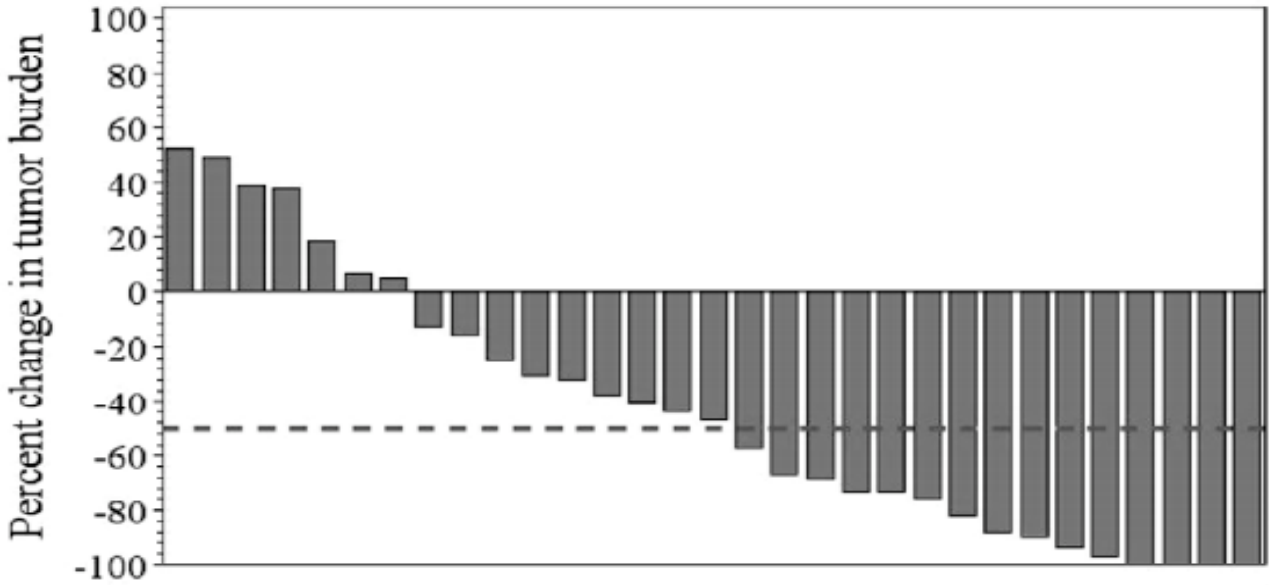
Complete Responses in Patients who Failed Prior Immunotherapy

	n (%)*		
	Total	CR	PR
All patients	93	20 (22)	32 (34)
Prior treatment			
None	5 (5)	2 (40)	1 (20)
IL-2	77 (83)	14 (18)	28 (36)
Chemotherapy	40 (43)	7 (18)	16 (40)
IFN	52 (56)	11 (21)	17 (33)
Anti-CTLA4	11 (12)	5 (45)	2 (18)
IL-2 chemotherapy	37 (40)	6 (16)	16 (43)
IL-2 anti-CTLA4	8 (9)	3 (38)	1 (13)
IL-2 anti-CTLA4 chemotherapy	6 (7)	2 (33)	1 (17)

Rosenberg, CCR, 2012

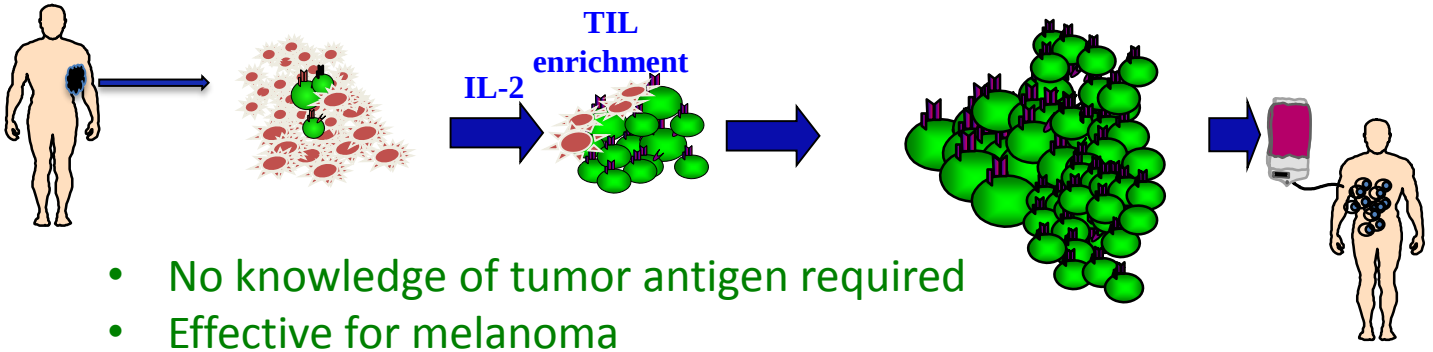
TIL Response Rates can be reproduced at other institutions

Institution	Patient #	RR	CR
NCI (Rosenberg, 2011)	43	49%	12%
Sheba Medical Center (Itzhaki, 2011)	31	48%	13%
MD Anderson (Radvanyi, 2012)	31	48%	6.5%



How to make T cells?

TIL
Tumor-infiltrating Lymphocytes



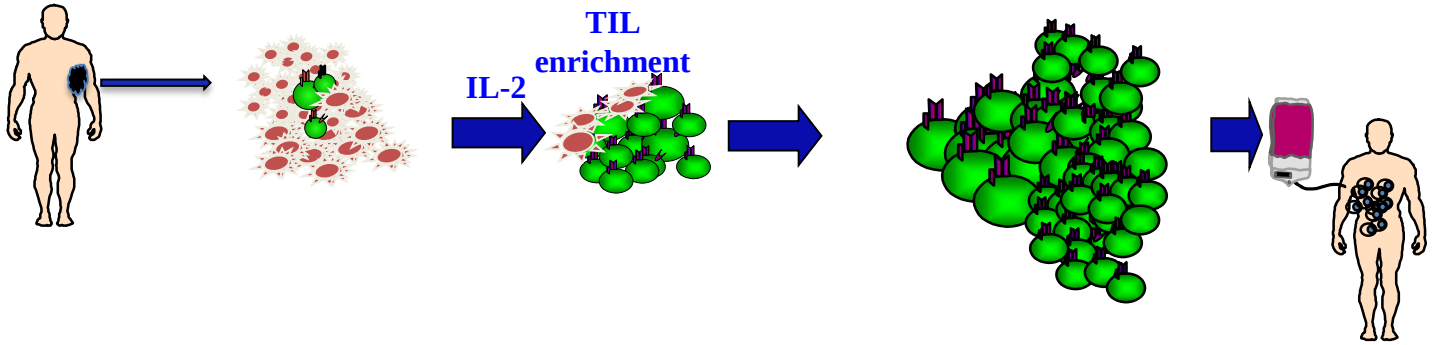
- No knowledge of tumor antigen required
- Effective for melanoma
- Possible other TIL+ tumors
- Serious toxicities, Selection bias

- Limit Conditioning
- In vitro Selection
- Enhance Trafficking
- Overcome Immune Resistance

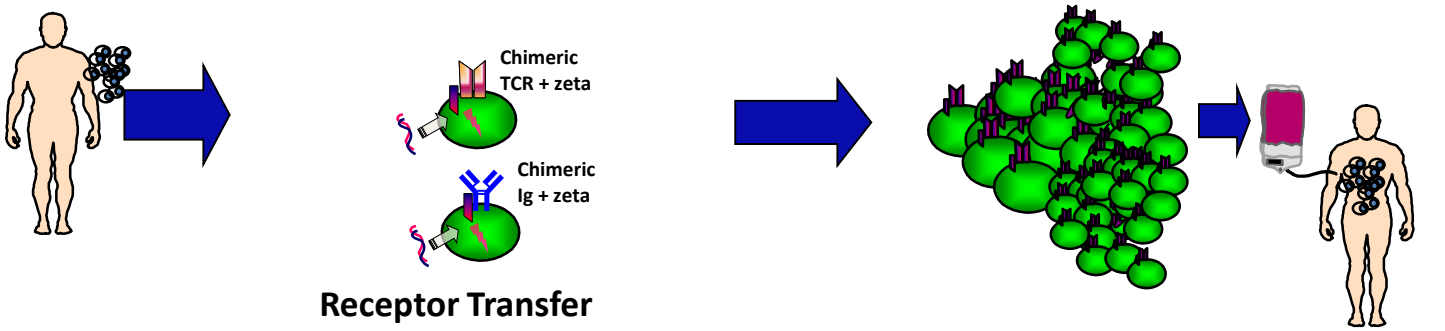
- Low-dose lymphodepletion/ Post-infusion IL2/other
- CD137 / PD1
- Chemokine / TME modulation
- TGF-B

How to make T cells?

TIL
Tumor-infiltrating
Lymphocytes



TCR/CAR
Engineered
T cells



How to make T cells?

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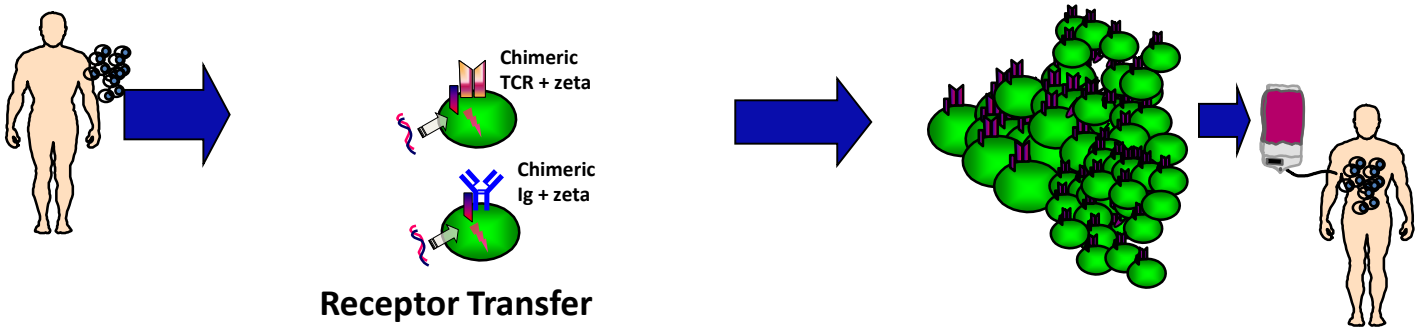
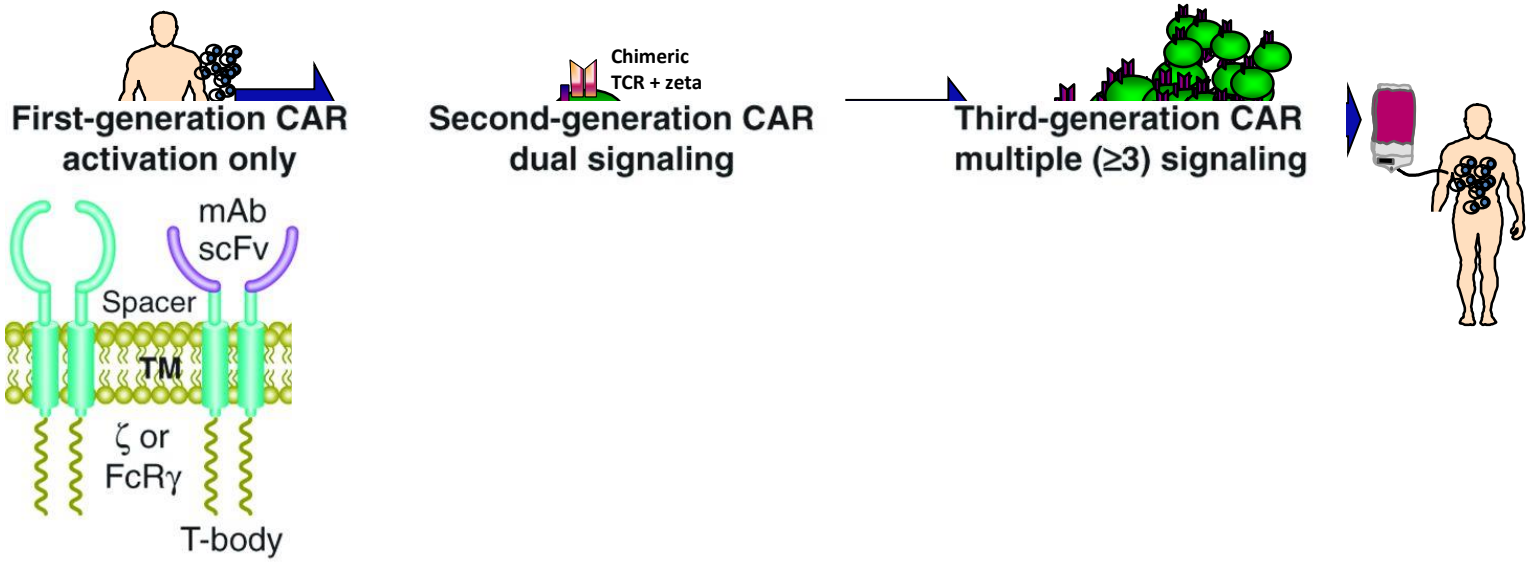


- Potential off-the-shelf
- Genetic enhancement possible
- Leukemia
- Potentially other malignancies
- Serious toxicities
- Regulatory/ Safety

- Subset, Dose
- Unique to B cell targets?
- Synthetic Biology and Regulation
- Multivalent targeting

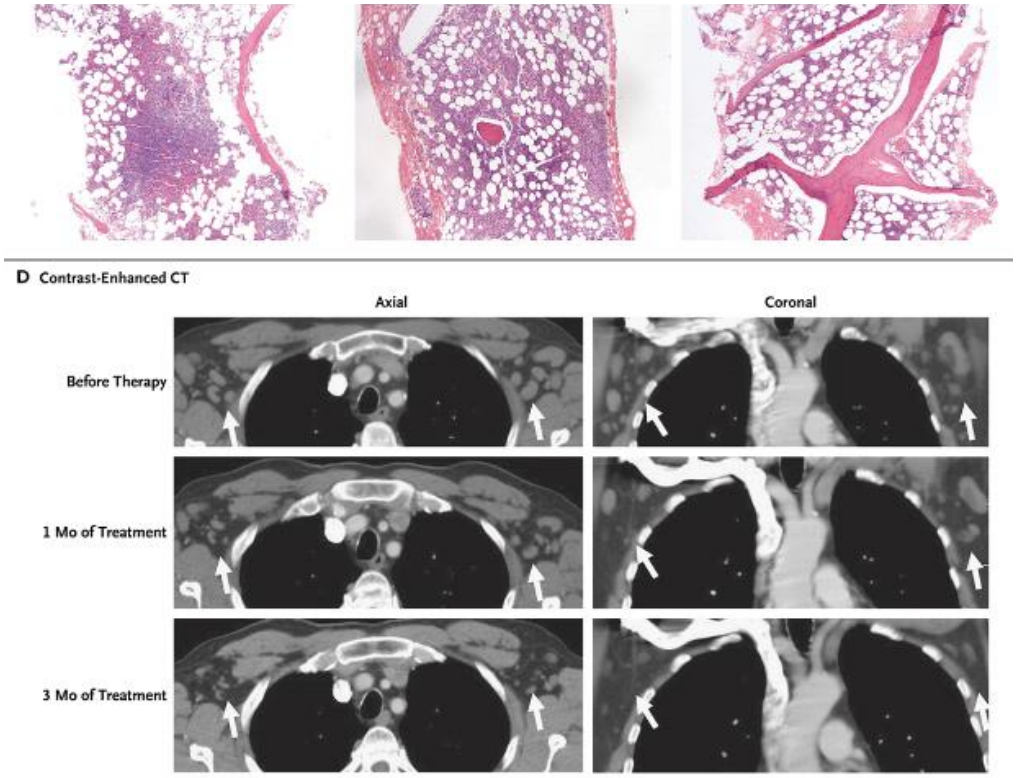
How to make T cells?

TCR/CAR
Engineered
T cells



Sadelain M et al. Cancer Discovery 2013;3:388-398

Targeting CD19 for B cell malignanices with CAR T cells



Persist > 6 months,
>1000-fold expansion
>1000:1 killing,
> 1 kg tumor
No immunogenicity

Porter et al N Engl J Med. 2011 365(8): 725–733. (CLL)

Brentjens et al Sci Transl Med. 2013 5(177). (ALL)

Kochenderfer et al Blood 2010 11:4099 (NHL)

Targeting CD19 for B cell leukemias with CAR T cells

Table 1 Summary of reported CD19-negative relapse in trials of anti-CD19 CAR-T cells for B-ALL

Treating institute	Patient populations	Construct (scFv-Hinge-TM-CD-SD)	Gene transfer method	Conditioning therapy	Infused cell dose	Responses observed	Reported relapse
MSKCC [26]	Adult 33 32 evaluable for response	SJ25C1-CD28-CD3ζ	Retrovirus	Cy or Cy/Flu	1–3 × 10^6 CAR^+ T cells/kg	CR: 29/32 (91%)	14 relapse with 2 CD19– relapse
Upenn [24]	Pediatric and young adult 59	FMC63-CD8α-4-1BB-CD3ζ	Lentivirus	Investigator's choice	10^7–10^8 cells/kg with a transduction efficiency of 2.3–45%	CR: 55/59 (93%)	20 relapse with 13 CD19– relapse
NCI [25]	Young adult 38	FMC63-CD28-CD3ζ	Retrovirus	Cy/Flu or FLAG or IE	1 or 3 × 10^6 CAR^+ T cells/kg	CR: 23/38 (61%)	2 CD19– relapse
FHCRC [5]	Adult 30 29 evaluable for response	FMC63-IgG4 CD28-4-1BB-CD3ζ	Lentivirus	Cy ± etoposide or Cy/Flu	2 × 10^5 or 2 × 10^6 or 2 × 10^7 CAR^+ T cells/kg (1:1 CD4+:CD8+)	CR: 27/29 (93%)	9 relapse with 2 CD19– relapse

MSKCC Memorial Sloan Kettering Cancer Center, *Upenn* University of Pennsylvania, *NCI* US National Cancer Institute, *FHCRC* Fred Hutchinson Cancer Research Center, *scFv* single-chain variable fragment, *B-ALL* B cell acute lymphoblastic leukemia, *Cy* cyclophosphamide, *Flu* fludarabine, *FIAG* fludarabine + Ara-c + G-CSF, *IE* ifosfamide/etoposide, *CR* complete remission, *CAR-T* chimeric antigen receptor-modified T cell

19/ 45 are CD19- relapses (> 30 %)

Wang et al, J. Hemat. & Onc. V 10, N 53, pp 1-11, 2017.

T-cell therapy at the threshold

Carl June, Steven A Rosenberg, Michel Sadelain & Jeffrey S Weber

$ 475,000

$ 373,000

NOVARTIS FOR B CELL LEUKEMIAS

GILEAD (KITE) FOR B CELL LYMPHOMAS

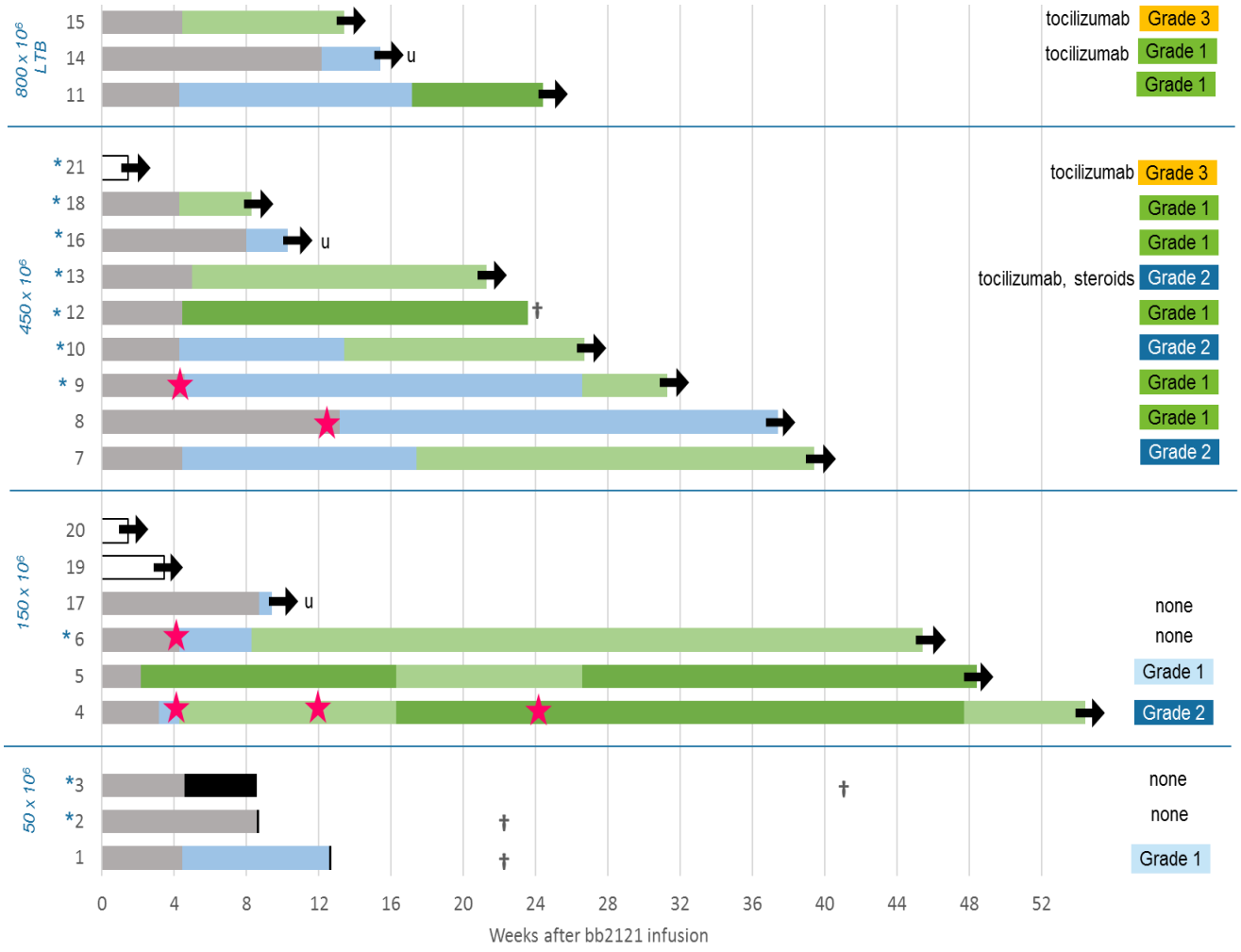
Weber is at the Donald A. Adam Comprehensive Melanoma Research Center at H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, USA.
e-mail: cjune@exchange.upenn.edu, m-sadelain@ski.mskcc.org, sar@nih.gov, jeffrey.weber@moffitt.org

patients with melanoma who receive c... transfers. So dealing with the tumor microenvironment is critical.

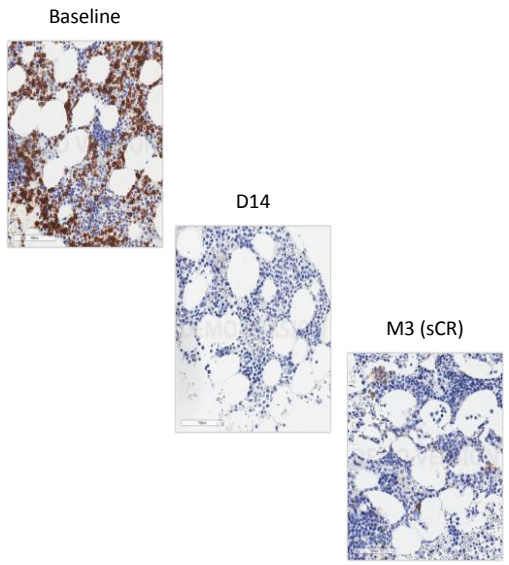
Carl June: Our data show that replicative capacity of the transferred T cells may be a key factor that is required for efficacy of the procedure.

they tend to appear or in all cells of positive tumors. CD19 is a great target for CAR therapy, but few other cell-surface molecules possess such a favorable profile—high expression on most tumor cells and expression in normal cells restricted to a dispensable cell type. Target identification remains a major research goal.

Targeting BMCA for Multiple Myeloma with CAR T cells



Clearance of Myeloma in the Bone Marrow by IHC as Early as Day 14



Stable Disease PR VGPR CR/sCR PD

★ MRD- † deceased

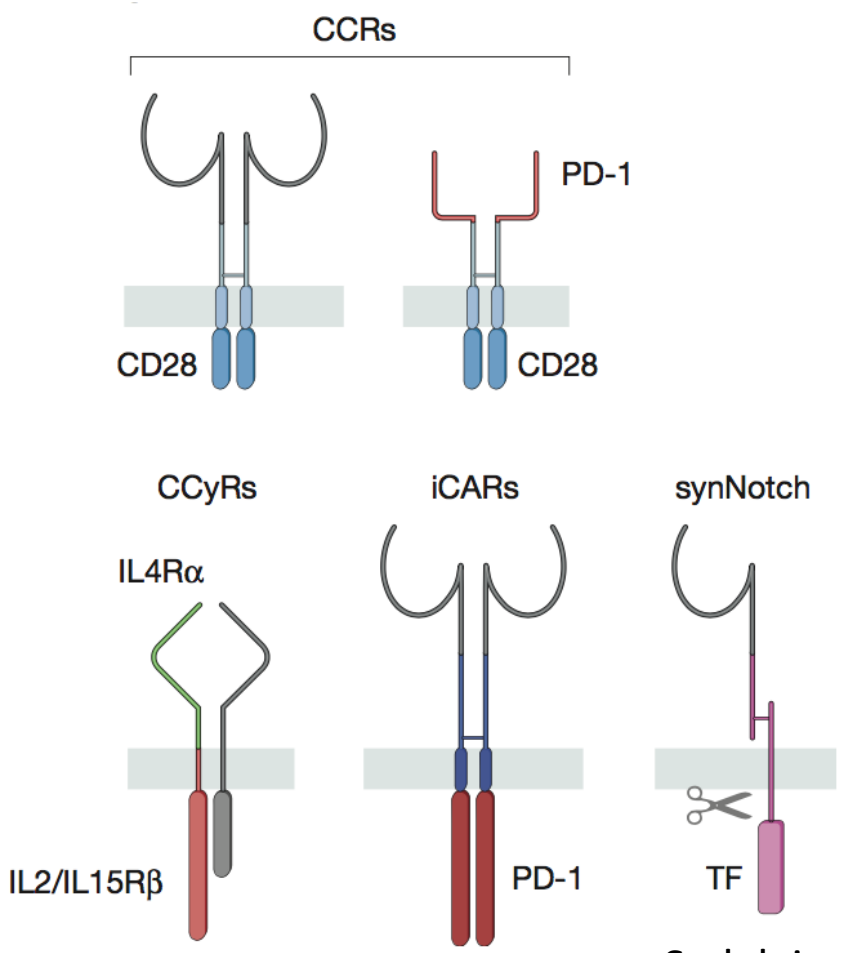
u = unconfirmed response

* High tumor burden (>50% bone marrow involvement)

Includes unscheduled assessments.

Jesus G. Berdeja, MD[1], et al. *ASCO 2017*
Ali et al Blood 128:1688, *2016*

- RNA-CAR therapy
- Bispecific CAR therapy
- Synthetic CAR:



Sadelain M et al Nature 2017 545:423

How to make T cells?

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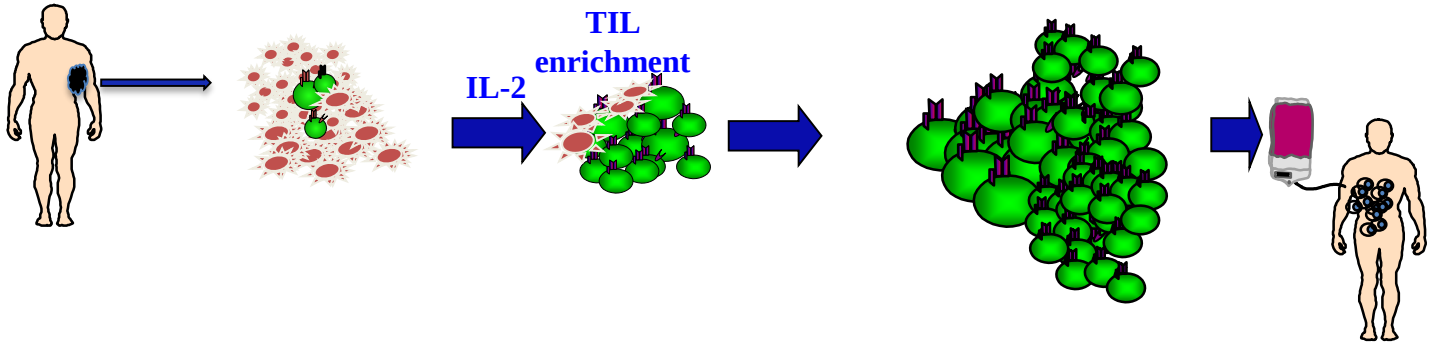
Lymphocytes



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Engineered
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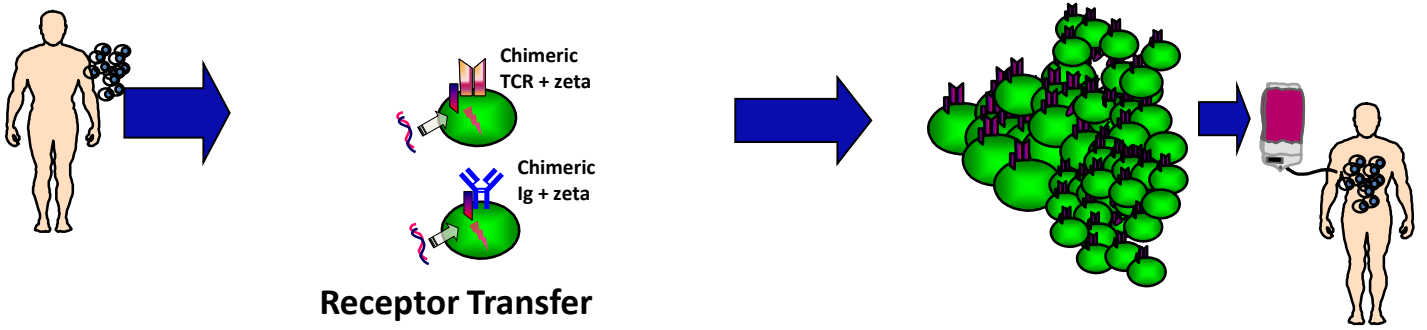
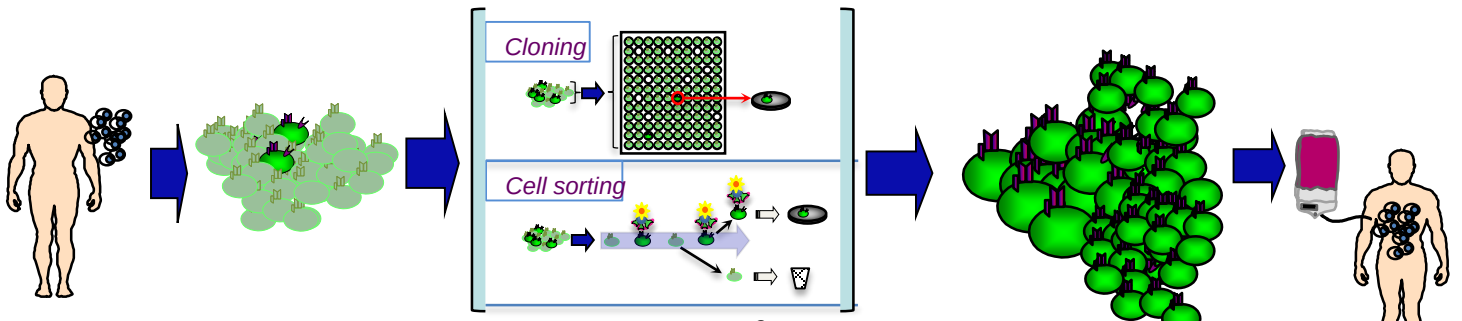


Receptor Transfer

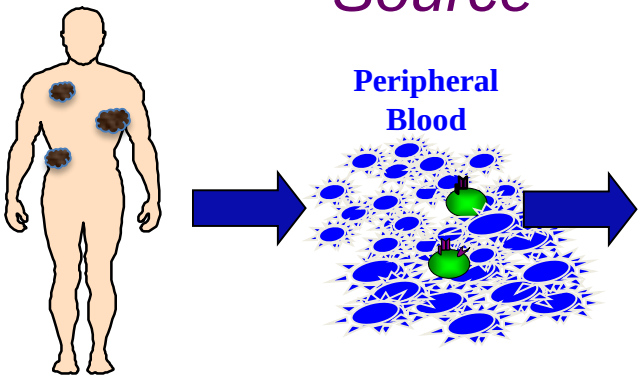
ETC
Endogenous
T cell Therapy



Antigen-specific
T Cell Enrichment

Endogenous T Cell Therapy (ETC)

Source



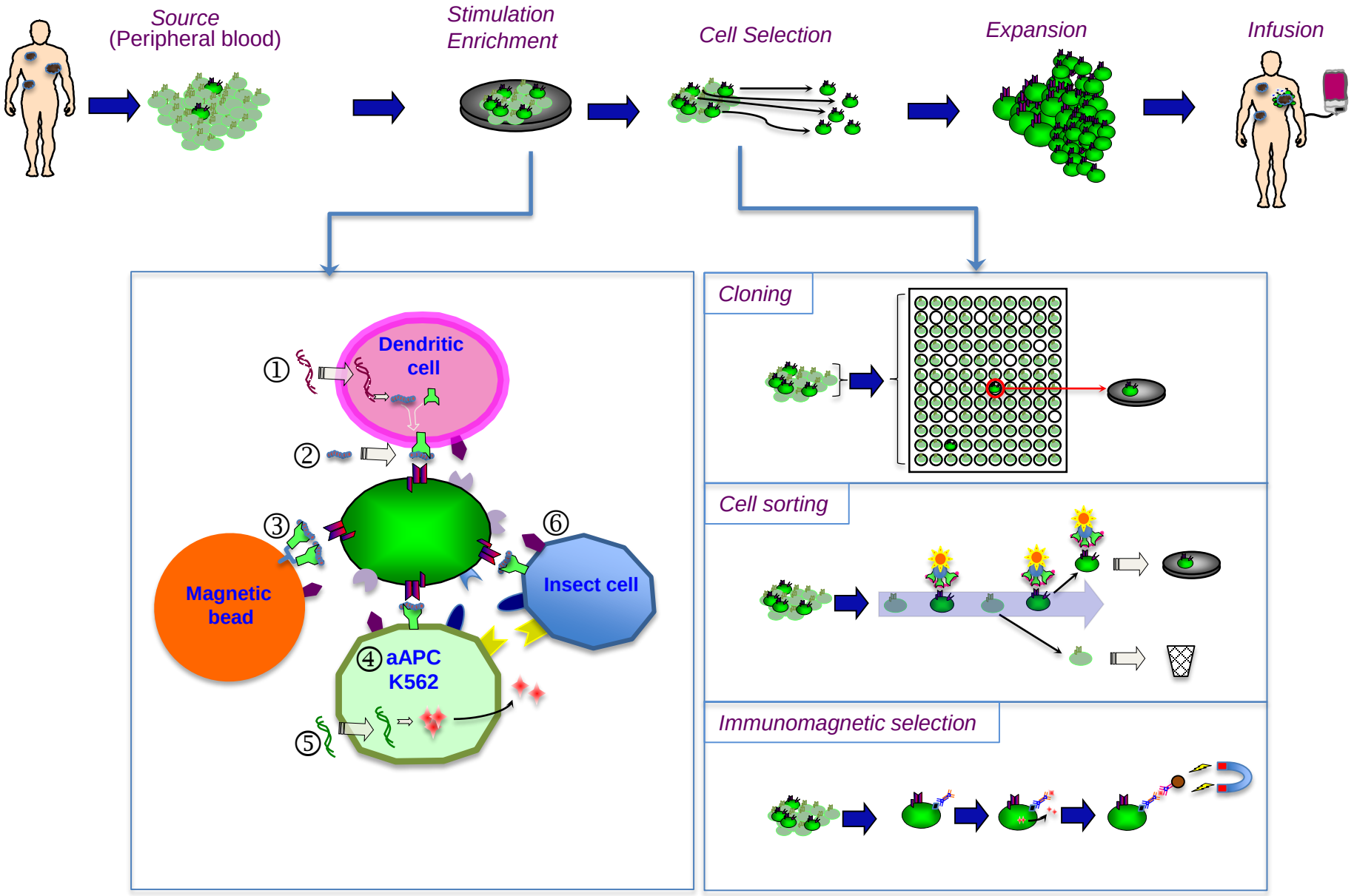
< 1:100,000

< 0.001 %

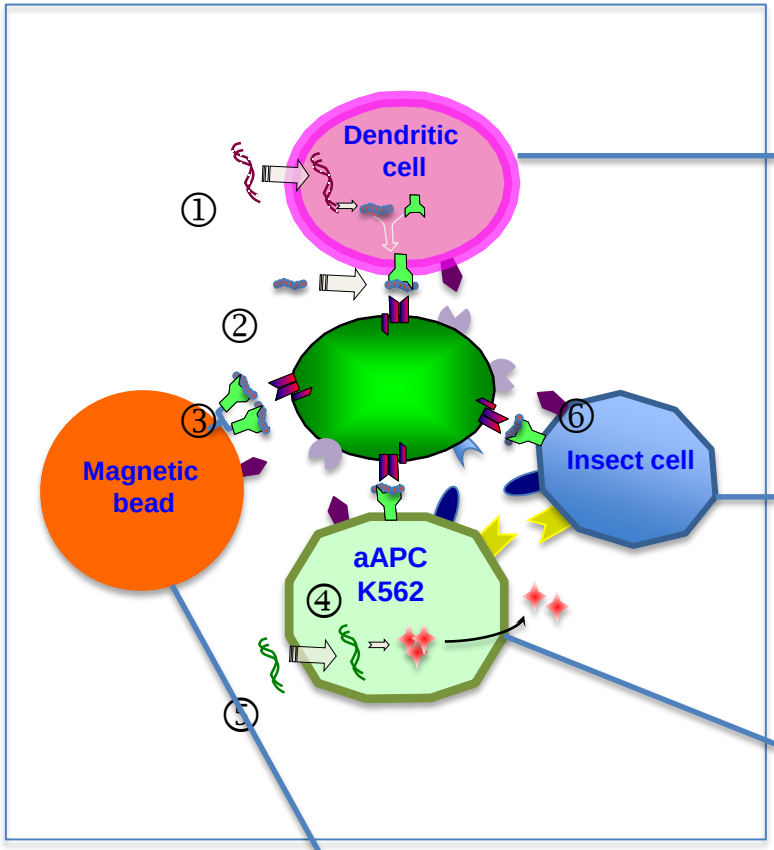
- TCR repertoire
  - "self-selected" affinity
  - Unbiased from TIL
- Accessible
  - Peripheral blood
  - Low morbidity/Outpatient
- Regulatory simplicity
  - Genetic modification
- Rapid deployment
  - Discovery → Implementation
  - Flexibility

- Time and labor-intensive
- "technically challenging"

Antigen-specific T Cell Therapy



Endogenous T Cell Therapy



Yee et al PNAS 2002
Hunder et al NEJM 2008
Chapuis et al STM 2013
Chapuis et al JCO 2016

Melanoma
AML
Breast
MCC

Khammari et al, JID, 2009

Melanoma

Bollard, Rooney, Heslop, Brenner
JEM 2004. Blood 2005

NPC
HD
EBV-LPD

Sun S et al, Immunity 1996

Mitchell MS et al JCO 2002

Melanoma

Butler MO et al STM 2011

Melanoma

Maus MV and June CH, Clin Immunol et al 2003

Oelke M and Schneck JP et al Nat Med 2003

Melanoma

Endogenous T Cell Therapy

Science Translational Medicine
AAAS

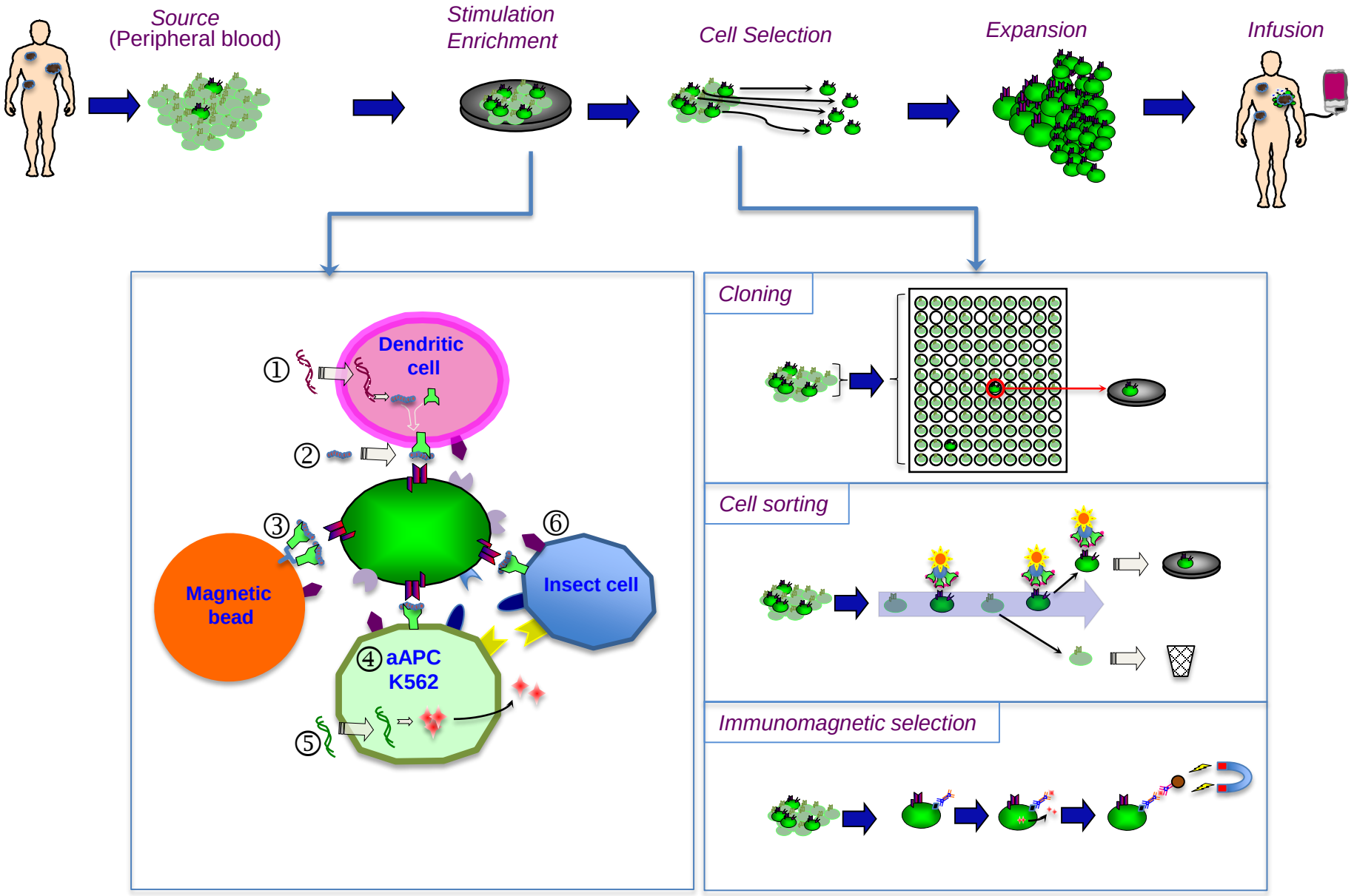
Establishment of Antitumor Memory in Humans Using in Vitro–Educated CD8 + T Cells
Marcus O. Butler *et al.*
Sci Transl Med **3**, 80ra34 (2011);
DOI: 10.1126/scitranslmed.3002207

- aAPCs (K562, CD80, CD83, HLA-A2)
- MART-1 specific CTL + IL-2/ IL-15
- Treatment plan:
  - CTL alone (no conditioning or IL-2)

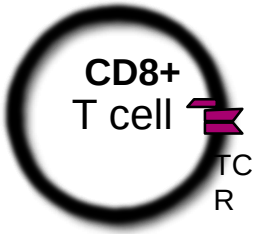
Endogenous T Cell Therapy

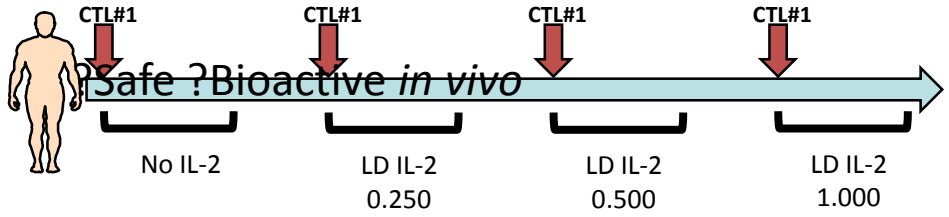
No.	Age/sex	Metastatic disease at study entry	Previous therapy	Total cells infused		Status on day 70	Time to next therapy	Outcome after CTL or next therapy	Duration of response (months)
				Graft 1	Graft 2				
1	74/M	Liver, adrenal, spleen, lung, skin	LND; carboplatin, paclitaxel, sorafenib; gp100 vaccine	4.0×10^8	None	Death on day 51	—	Died without therapy	—
2	69/M	Lung, skin	WLE; LND; temozolomide; melphalan limb perfusion	4.0×10^8	4.0×10^8	PD	Day 103 ipilimumab (10 mg/kg)	PR	16
3	49/F	Lung, adrenal	WLE; LND; RT; HD IL-2	4.3×10^8	4.3×10^8	MR	Day 146 ipilimumab (10 mg/kg)	PR	31+
4	68/M	Skeletal muscle, lung, mediastinum, cardiac	Small-bowel resection; HD IL-2; ipilimumab versus gp100 versus both	3.8×10^8	3.8×10^8	SD	Day 140 RAF265	SD	3
5	66/M	Lymph nodes	WLE; LND	4.4×10^9	2.5×10^9	PR	No other therapy	CR to CTL day 140	25+
6	55/M	Lung	WLE; LND; pulmonary nodule resection	1.8×10^9	3.4×10^9	SD	Day 287 HD IL-2	Death due to line sepsis	—
7	70/F	Lung, skin	WLE; LND; adjuvant IFN	4.0×10^9	4.0×10^9	PD	Day 335 ipilimumab (3 mg/kg)	SD	6
8	80/M	Lung, mediastinum	LND; RT; temozolomide	3.6×10^9	3.6×10^9	SD	Day 372 ipilimumab (3 mg/kg)	SD	5
9	64/M	Lung, skin	WLE; LND; adjuvant IFN	4.4×10^9	4.4×10^9	PD	Day 146 ipilimumab (10 mg/kg) +	PR	13+

Antigen-specific T Cell Therapy

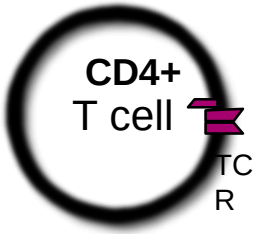


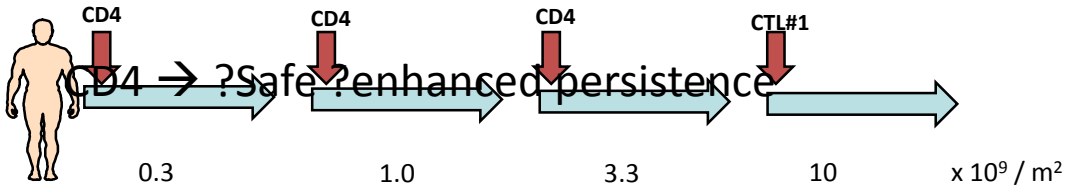
Endogenous T Cell Therapy



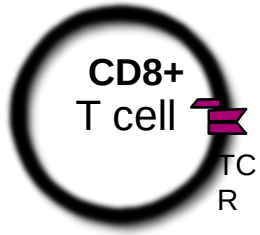


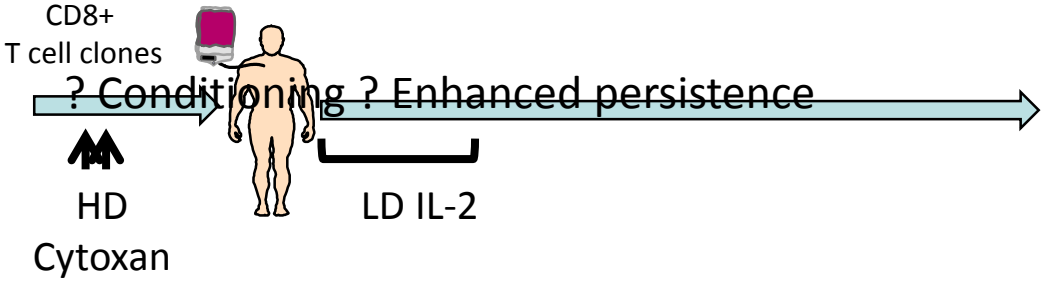
2002
PNAS





2008
NEJM

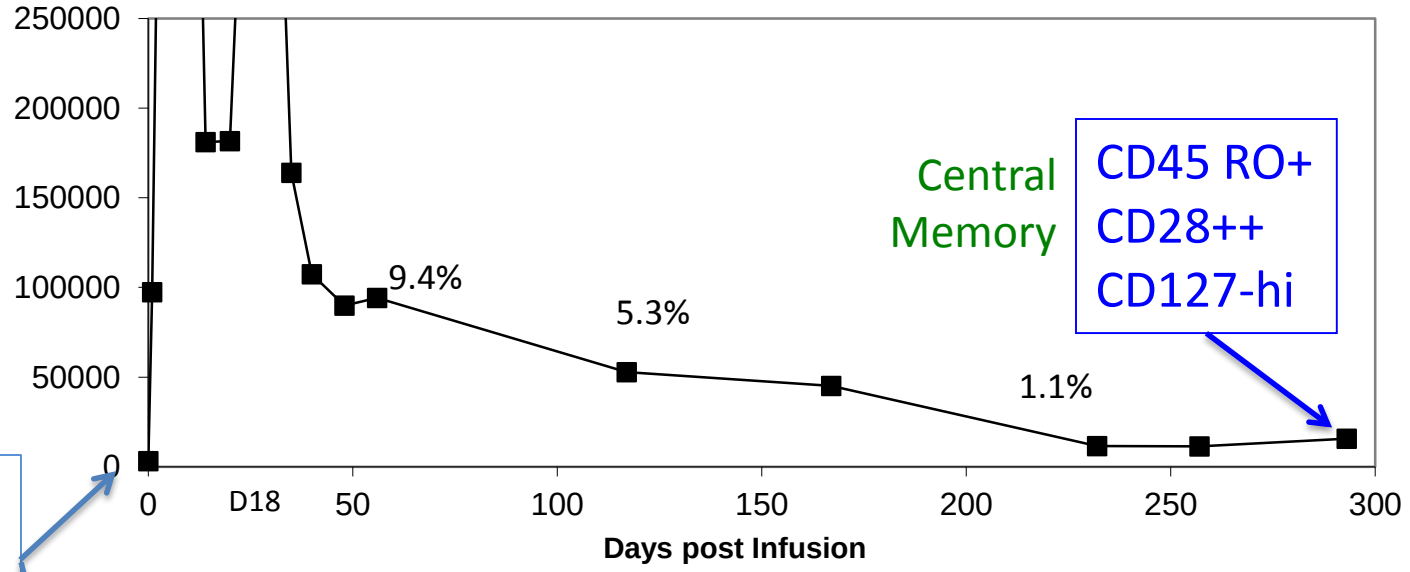




2012
PNAS

Persistence

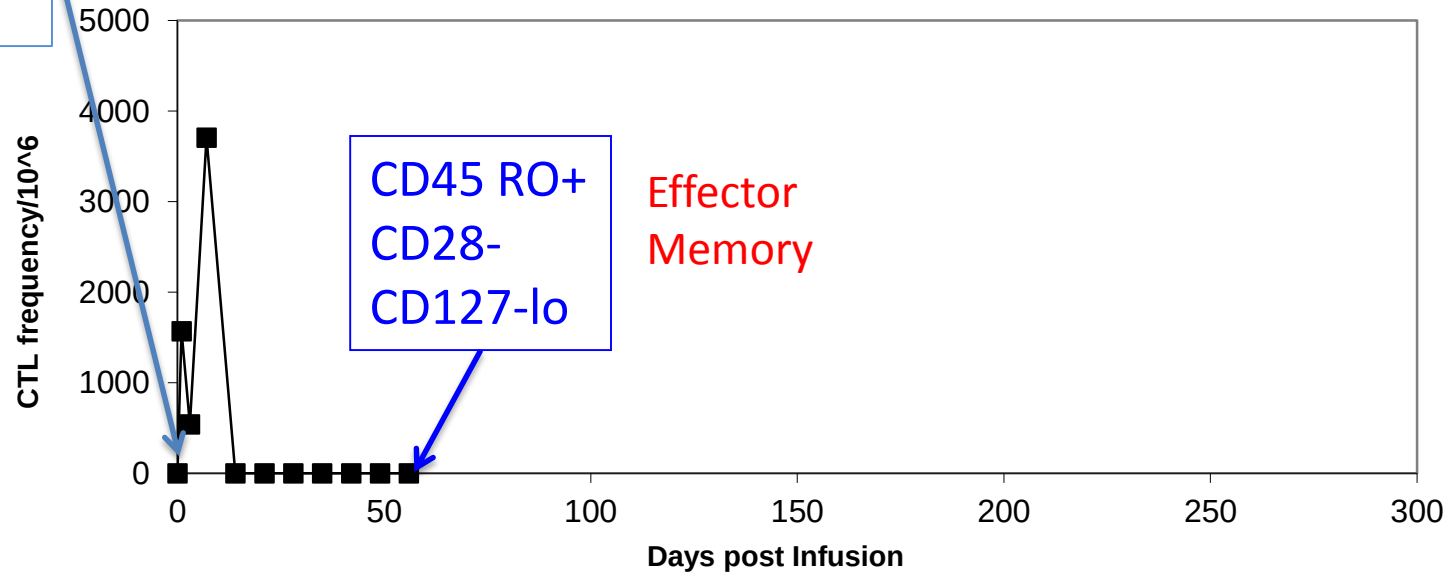
2140-3 Complete Response



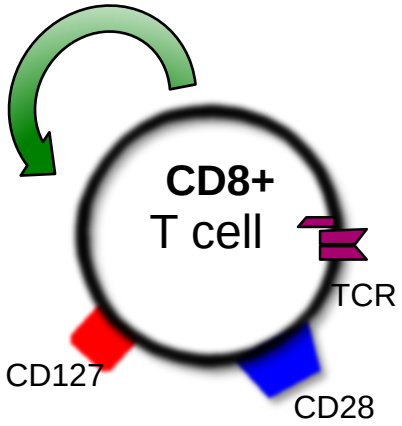
Effector Memory

CD45 RO+
CD28-
CD127-lo

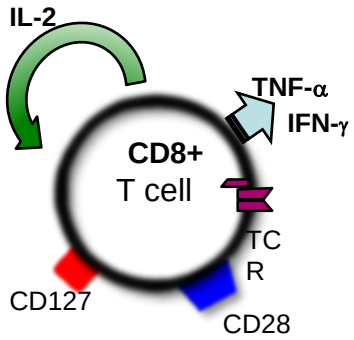
2140-2 Progressive Disease



A ‘more equal’ T cell



What Flavor of T cell?



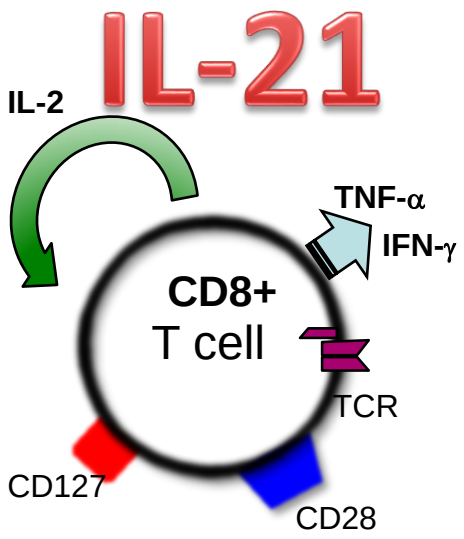
Naïve	Central Memory	Effector Memory	Terminal Effector

cytolytic

proliferative

CD28+	CD28++	CD28 -	CD28 -
CD127+	CD127+	CD127-	CD127-
CD45RA+	CD45RO+	CD45RO+	CD45RA+

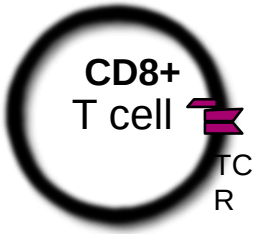
Priming with IL-21 Generates Central Memory-type T cell

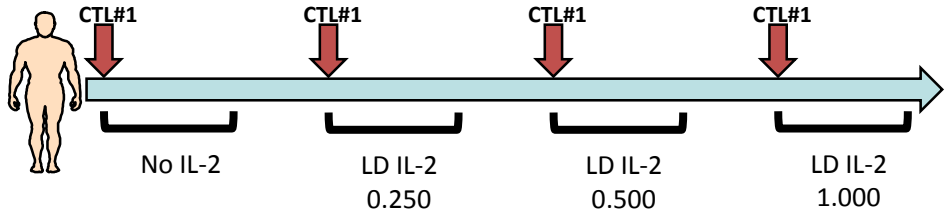


CD28++
CD127+
CD45RO+

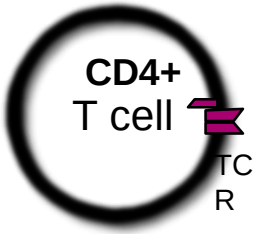
'helper-independent CTL (Tcm) '

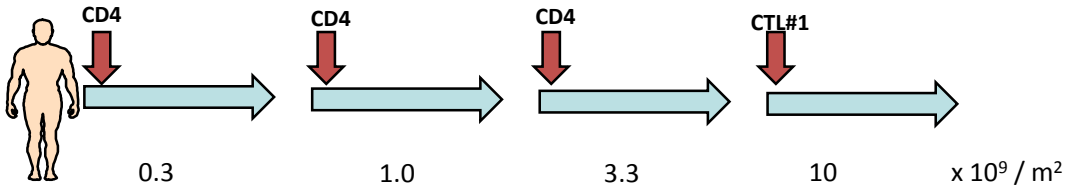
J Immunol 2005
Blood 2008
Science Transl Med 2013



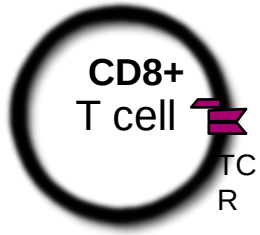


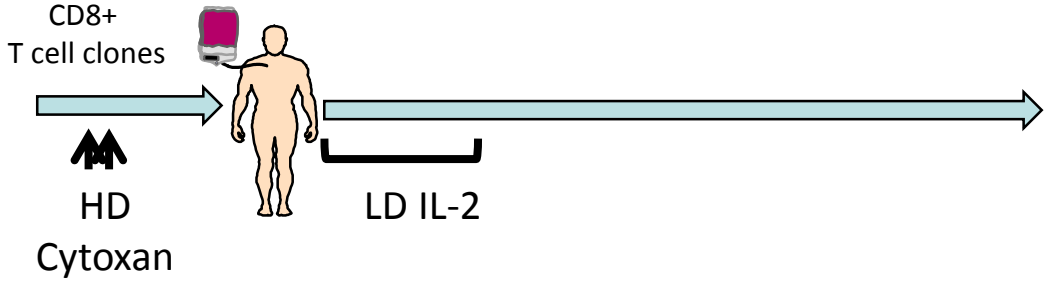
2002
PNAS



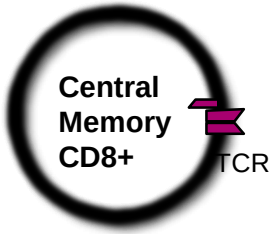


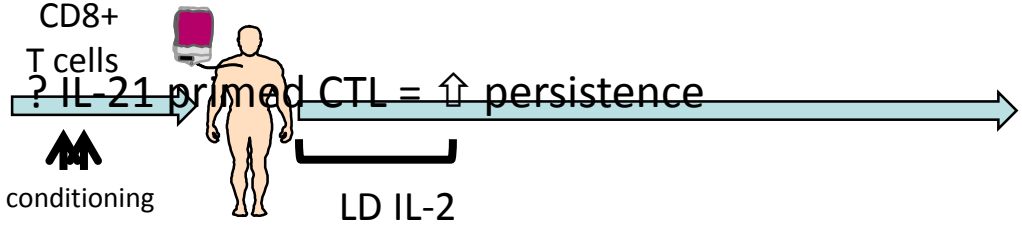
2008
NEJM





2012
PNAS

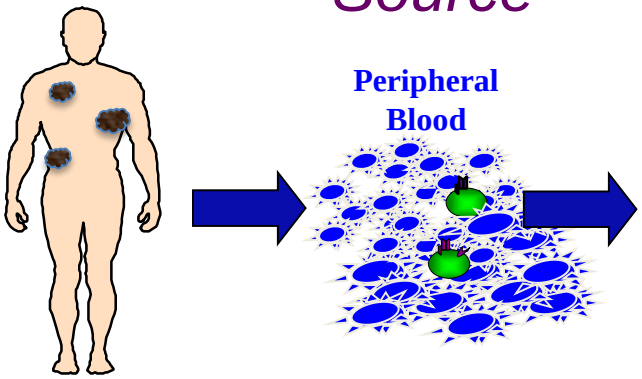




2013
STM

Endogenous T Cell Therapy (ETC)

Source



< 1:100,000

< 0.001 %

- TCR repertoire
  - "self-selected" affinity
  - Unbiased from TIL
- Accessible
  - Peripheral blood
  - Low morbidity/Outpatient
- Regulatory simplicity
  - Genetic modification
- Rapid deployment
  - Discovery → Implementation
  - Flexibility

- Time and labor-intensive
- "technically challenging"

Beer Margarita paradigm

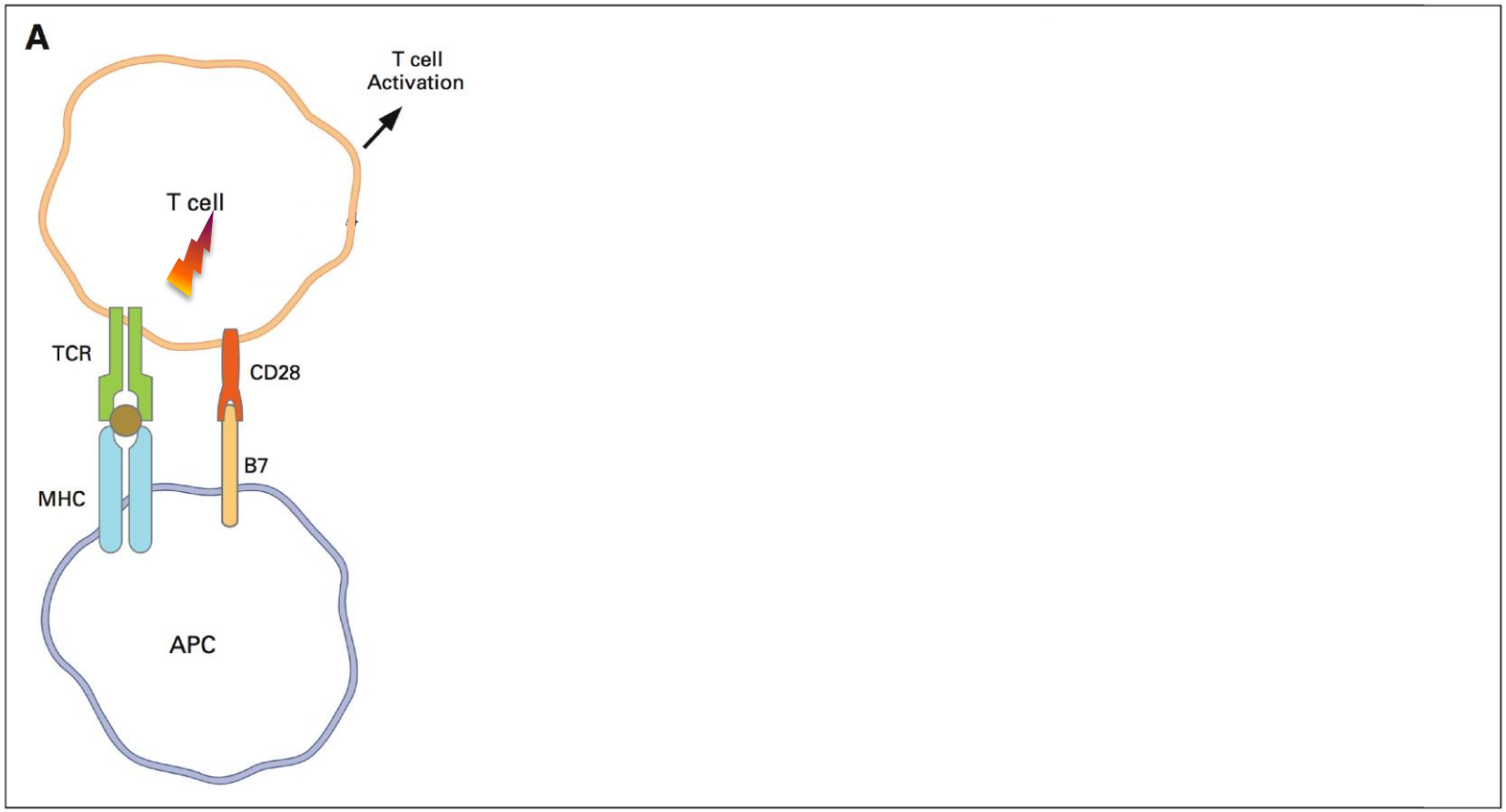
The Beer

CD8
CD4
T_{cm}
T_{eff}
T_{fh}
$Th_{1\text{-}50}$
NK
NKT

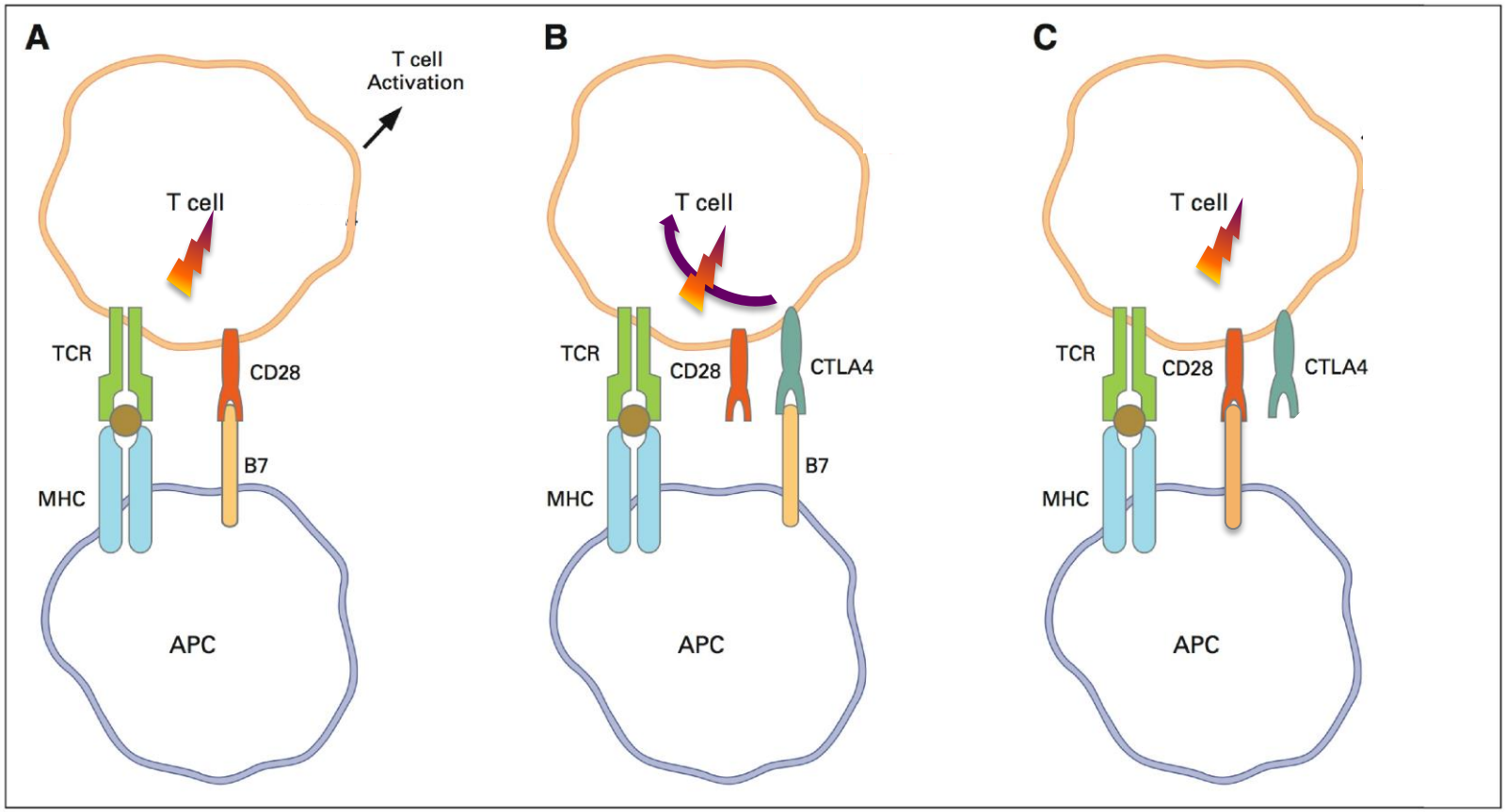
The Margarita

CTLA4
PD1/PD-L1
GITR
OX40
CD40
CD137
Vaccine Therapy
Oncolytic Virotherapy
Radiation Therapy
Targeted Therapy
Chemotherapy

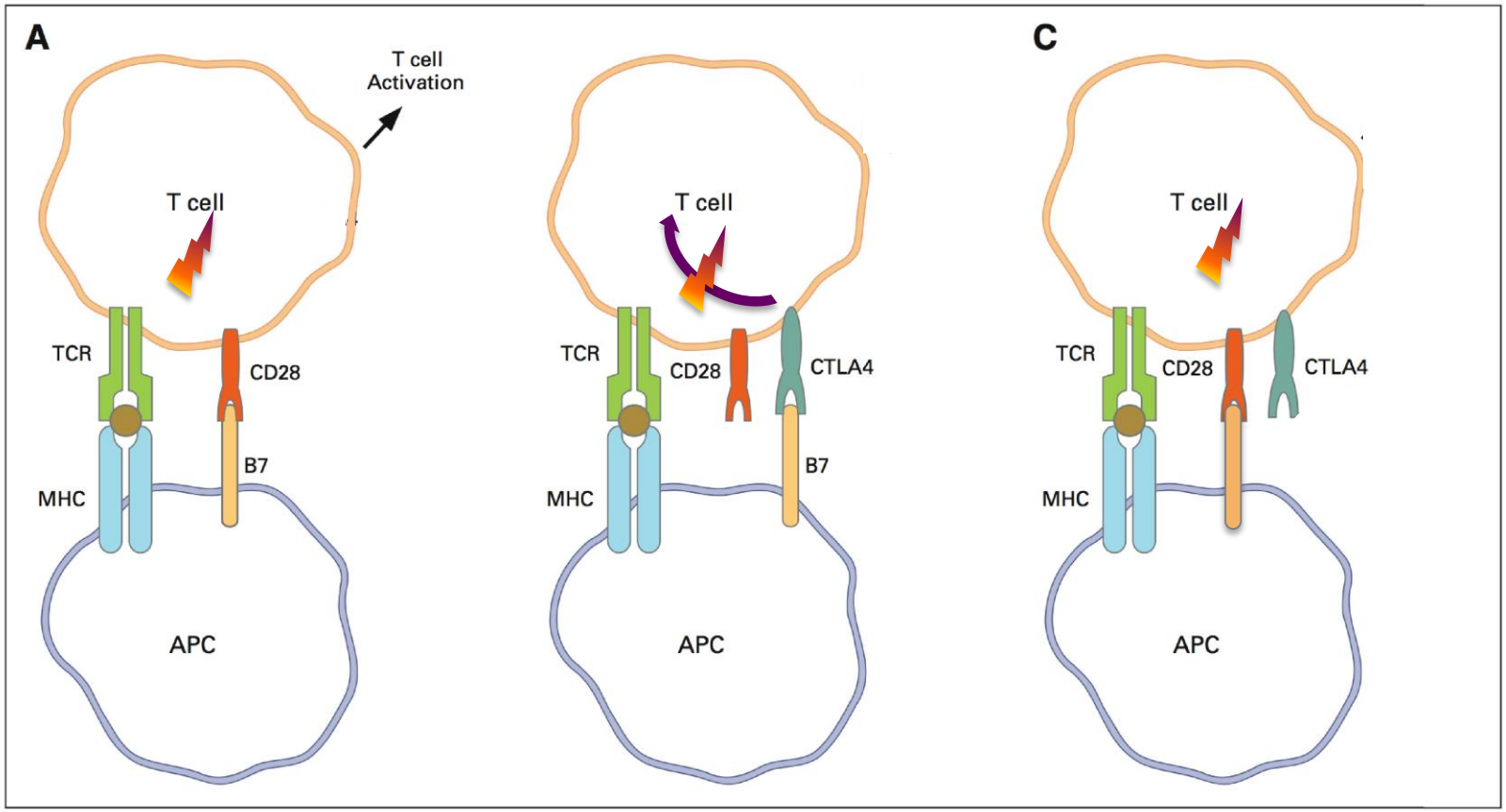
Costimulatory and Inhibitory Receptors
Rationale for CTLA4 Blockade



Blockade of Immune Checkpoint Inhibitor CTLA4



Blockade of Immune Checkpoint Inhibitor CTLA4



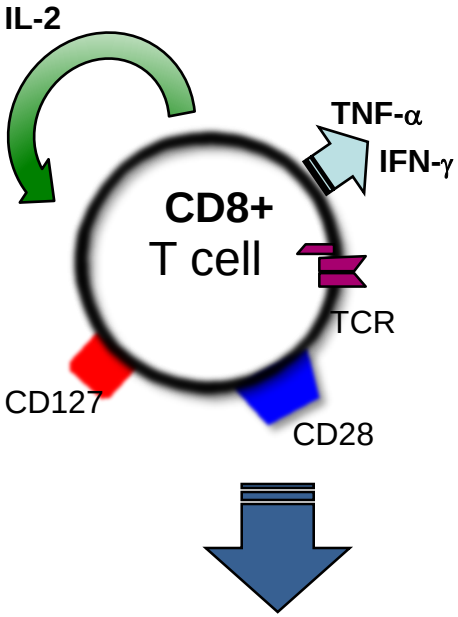
Blockade of Immune Checkpoint Inhibitor CTLA4

- Transferred tumor antigen-specific T cells enhanced proliferative potential

- Endogenous tumor antigen-specific T cells – lower threshold for activation
  - Leads to antigen-spreading
  - Multivalent response

- Eradicate / Modulate function CTLA4+ Tregs

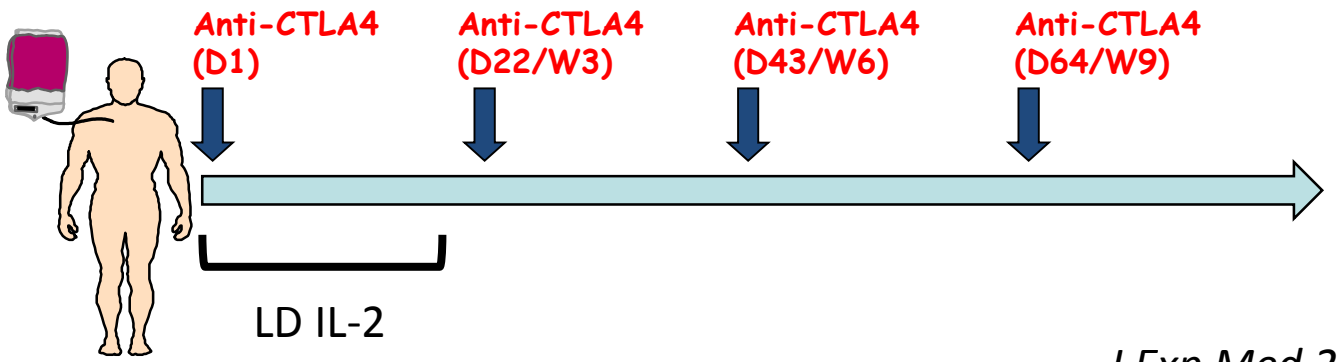
Phase I/II Trial of Adoptive T Cell Therapy in Combination with Immune Checkpoint Blockade



- Metastatic Melanoma
- HLA-A2+
- MART-1
- 10^{10} cells/m^2

Aude Chapuis

IL-21-CD8+
T cells



J Exp Med 2016
J Clin Onc 2016

Clinical Responses In Patients with Metastatic Melaonma Receiving Adoptive T Cell Therapy And Concurrent Anti-CTLA4

Patient	Prior Ipilimumab treatment failure	6 weeks	12 weeks	16/19 weeks	28 weeks	40 weeks	84 weeks
1	YES	SD (-2)	SD (-34)		PR (-80)	PR (-90)	CR (-100)
2	YES	SD (-2)	SD (-6)	SD (-7)			
3	NO	SD (0)	SD (+19)		PD (+25)		
4	YES	PD (69)					
5	NO	PD (+30)					
6	NO	PD (+31)	SD (+17)				
7	NO	SD (-45)	PR (-90)		CR (-100)		
8	NO	SD (+8)	SD (+21)	PD (+27)			
9	NO	SD (-41)		PR (-71)			
10	NO	PR (-76)	PR (-79)				

J Exp Med 2016
J Clin Onc 2016

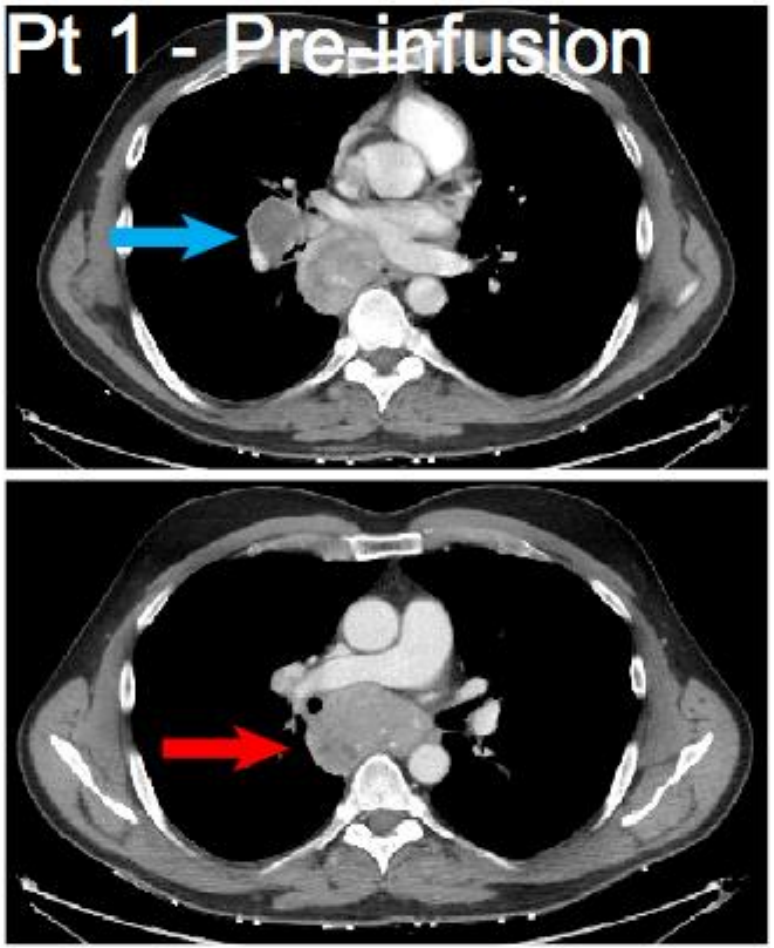
Clinical Responses In Patients Receiving Adoptive T Cell Therapy And Concurrent Anti-CTLA4

Patient	Prior Ipilimumab treatment failure	6 weeks	12 weeks	16/19 weeks	28 weeks	40 weeks	84 weeks
1	YES	SD (-2)	SD (-34)		PR (-80)	PR (-90)	CR (-100)
2	YES	SD (-2)	SD (-6)	SD (-7)			
3	NO	SD (0)	SD (+19)		PD (+25)		
4	YES	PD (69)					
5	NO	PD (+30)					
6	NO	PD (+31)	SD (+17)				
7	NO	SD (-45)	PR (-90)		CR (-100)		
8	NO	SD (+8)	SD (+21)	PD (+27)			
9	NO	SD (-41)		PR (-71)			
10	NO	PR (-76)	PR (-79)				

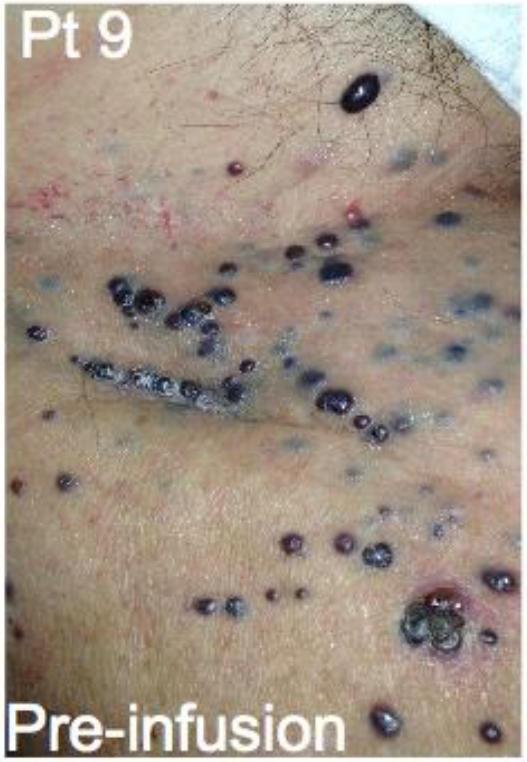
WHO RECIST Criteria

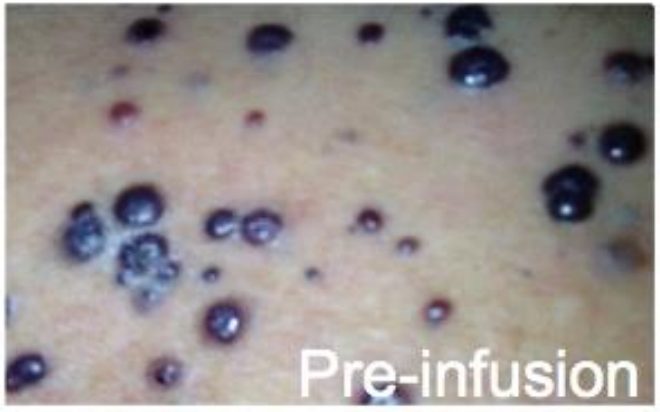
J Exp Med 2016
J Clin Onc 2016

Clinical Response
Adoptive CTL therapy + anti-CTLA4

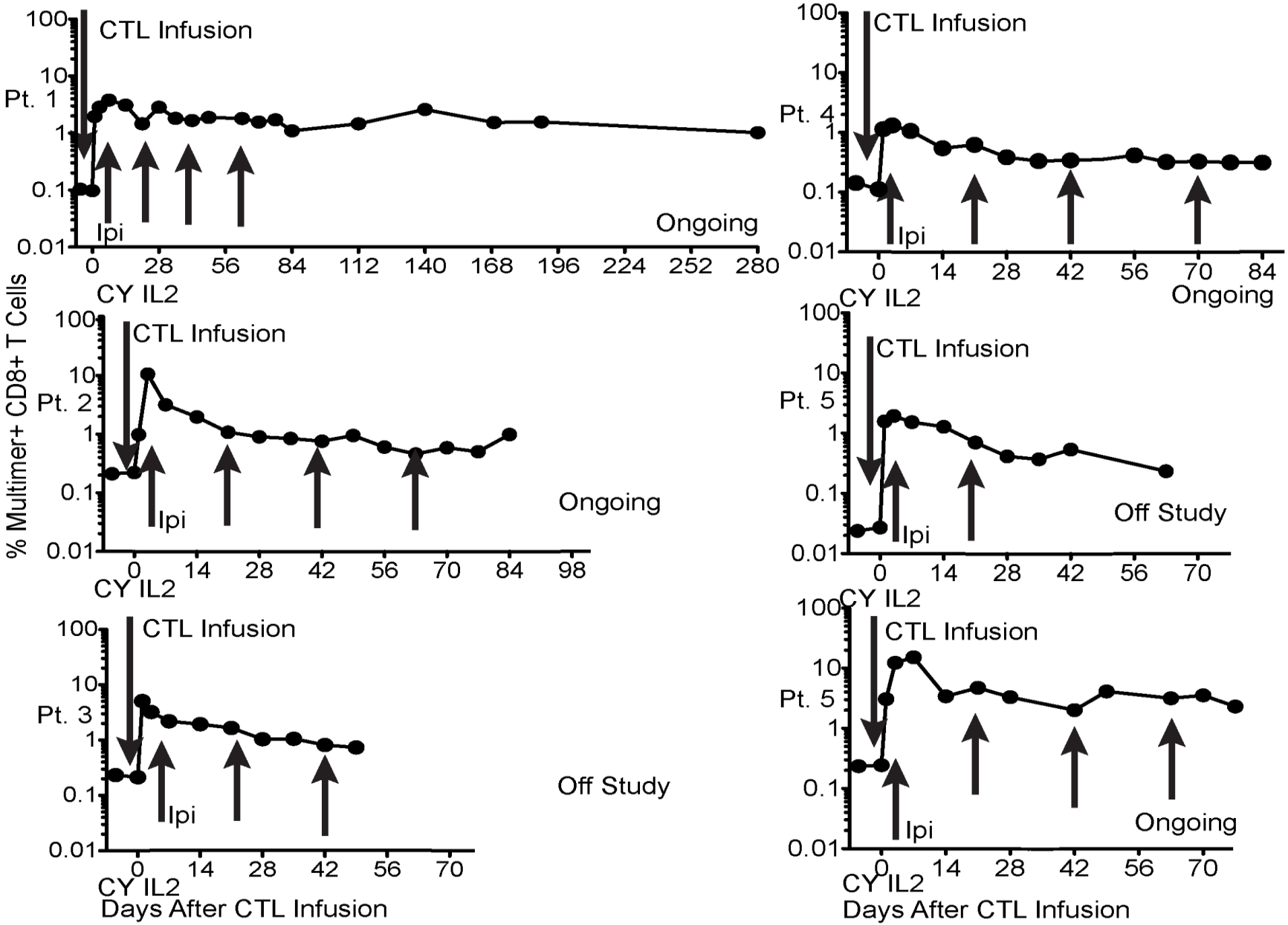


J Exp Med 2016
J Clin Onc 2016

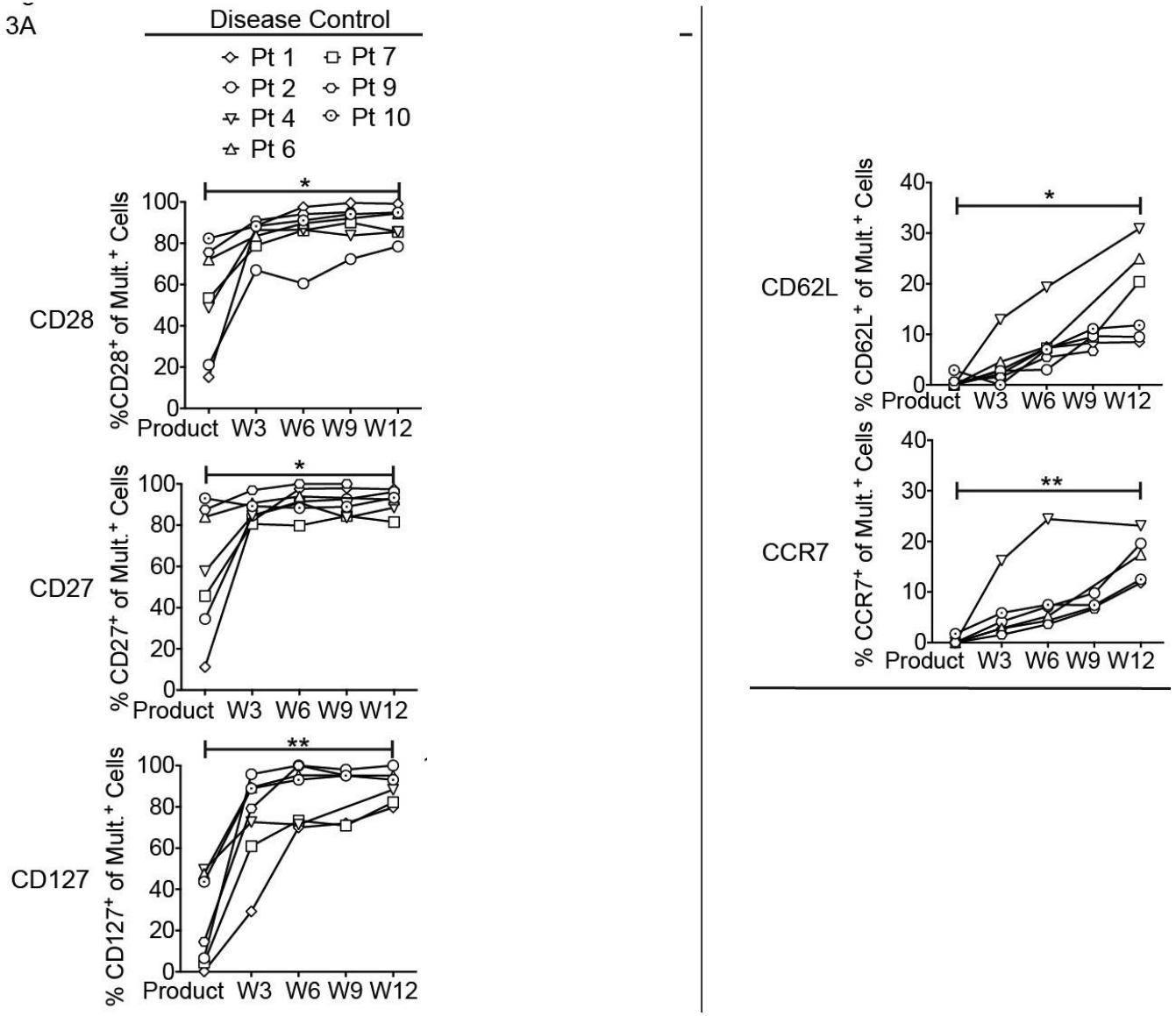

Pt 9
Pre-infusion


Pre-infusion

Persistence

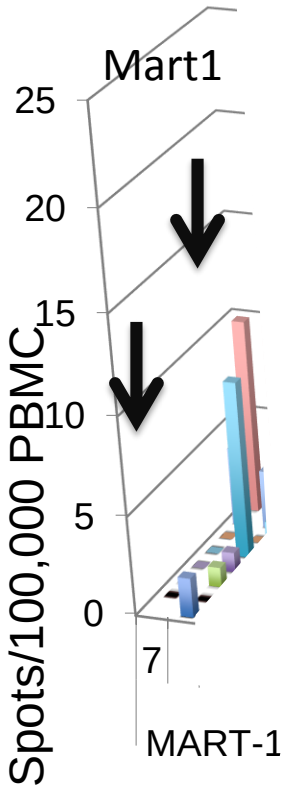


Acquisition of Central Memory Markers Correlates with Clinical Response



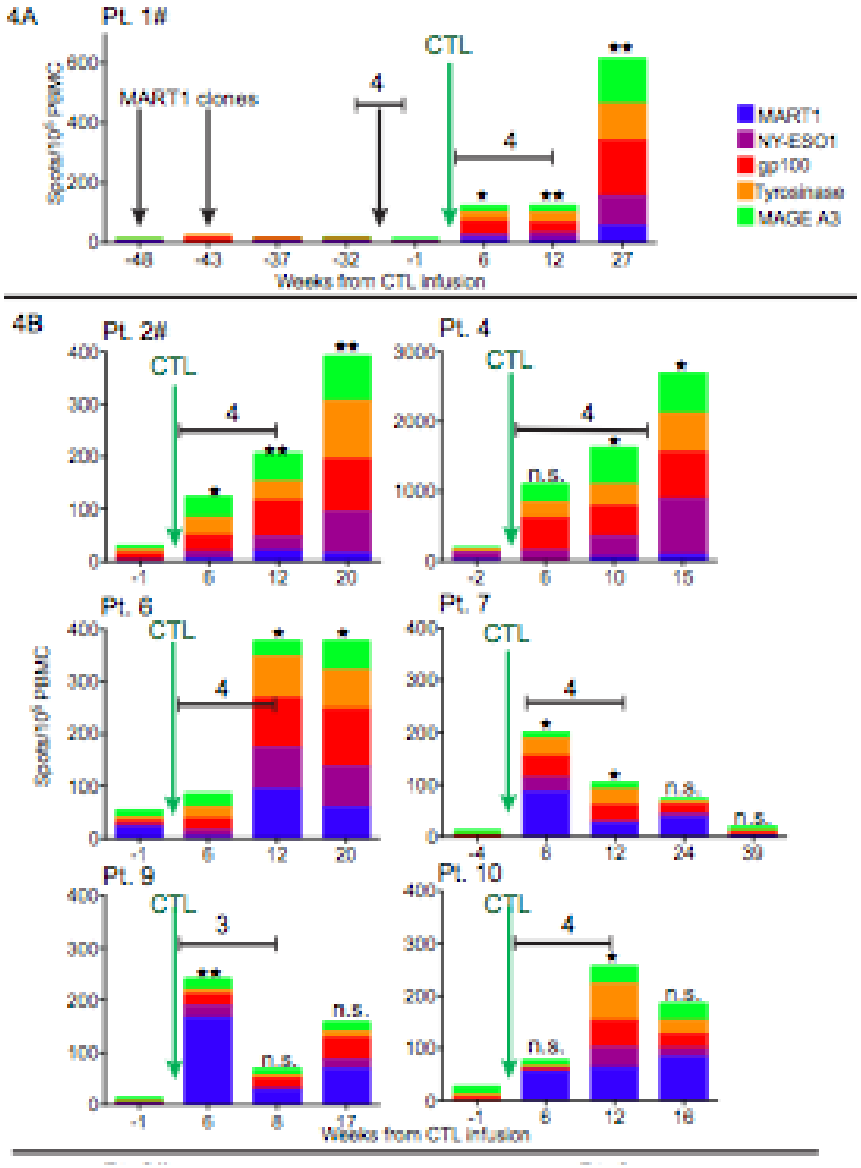
Antigen-spreading

PATIENT #1 – 50% clinical response at 24 weeks



INF D+189
INF D+83
INF D+43
INF D-6
INF #2 D+50
INF #2 D+29
INF #2 D+0
INF #1 D+0

Responders



Chapuis and Yee et al
J Clin Onc 2016
J Exp Med 2016

Conclusions

- Combination of T cell therapy and anti-CTLA4 leads to establishment of long-lived central memory T-cells.
- Evidence of epitope spreading was observed in patients with tumor regression/stable disease.
- Established a **highly effective outpatient strategy**
  - > 60% disease control in patients with metastatic dz
  - Phase II study -> 30 patients (MD Anderson Cancer Center)

Challenges

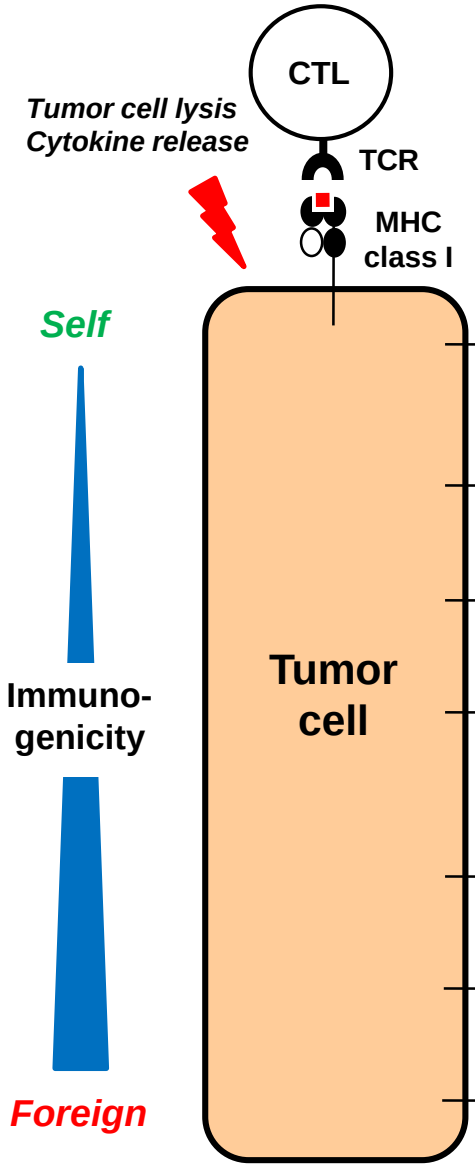
- **Non-melanoma solid tumor malignancies**
- Streamline process.

Considerations for Antigen Selection

- **Tumor-Essential**
  - Driver mutation (K-ras)
  - Tumorigenicity (HPV, Polyoma T Ag)
- **Tumor-Supportive**
  - Proliferation (WT1, Suvivin)
  - Receptor (EphA2, Her2)
  - Metastatic/Stroma (FAP, CT antigens, VEGF)
- **Tumor-Non-Essential**
  - Differentiation antigens (MART-1, PSMA)
  - Tissue-specific (BCMA, B cell)

Specificity:

Tumor-associated Antigens



Non-mutated antigens (normal)

→ Self Ags - ubiquitous, not tumor-specific (eg. actin, vimentin) > *98%*

→ Tissue differentiation Ags (eg. tyrosinase, MART-1)

→ Cancer / testis Ags (eg. MAGE, GAGE, NY-ESO-1)

→ Over-expressed in tumors (eg. KIT, HER2, MDM2)

Viral Antigens

→ Single point mutations

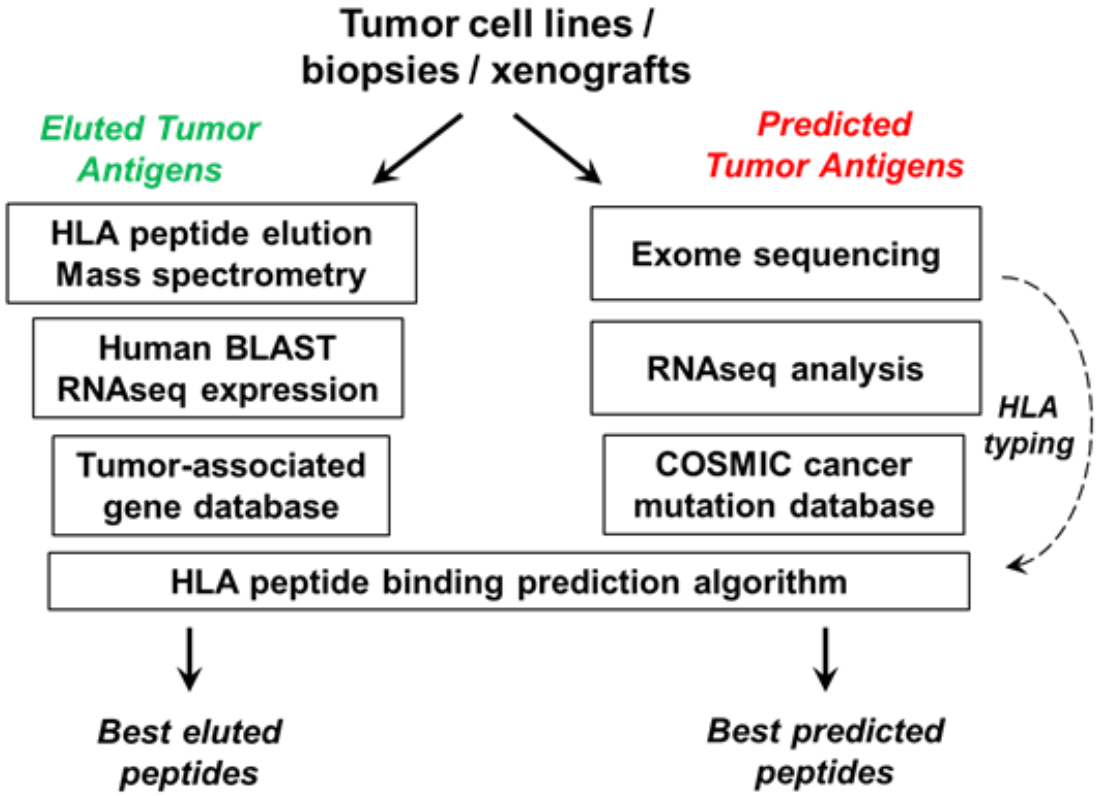
→ Deletions / insertions / frameshifts

→ Fusion peptides / mis-spliced / intron translation

Mutated antigens (aberrant)

G. Lizee

Antigen Discovery by MS/MS and RNAseq/Exome Sequencing



MELANOMA, LUNG CANCER, OVARIAN CANCER, COLORECTAL CANCER, PANCREATIC CANCER, SARCOMA

Rammensee H-G, Schreiber R, Mardis E, Wu C, Hacohen N, Schumacher T, Hinrichs C, Rosenberg SA, Sagin U

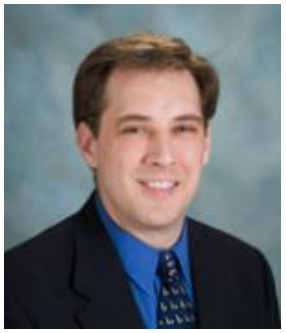
(”Targeted Neoantigens in Cancer Immunotherapy” Cancer J 2017;23)

Discovery of Novel Pancreatic Antigen by MS/MS and RNAseq/Exome Sequencing

Diagnosed with Stage IV pancreatic adenocarcinoma (lung mets).

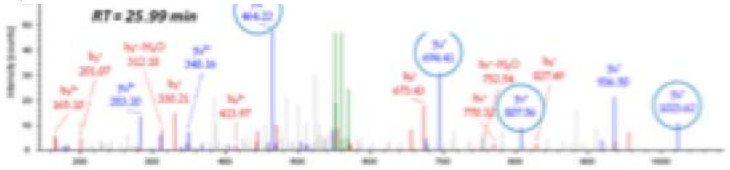
Cell line derived and analyzed by MS

Epitope derived antigen confirmed by RNAseq

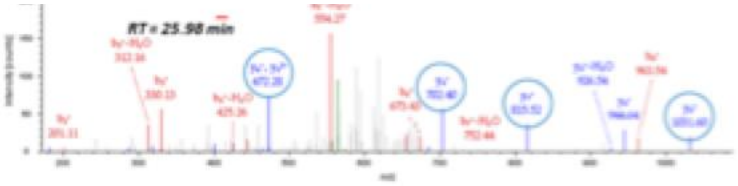


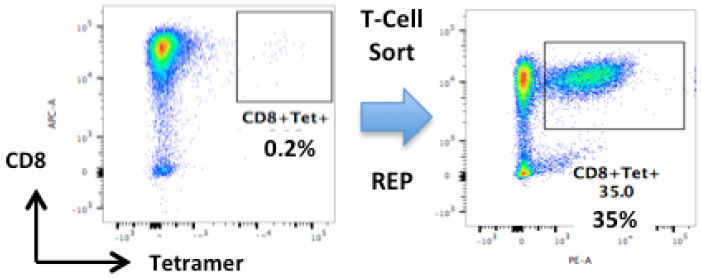
Greg Lizee

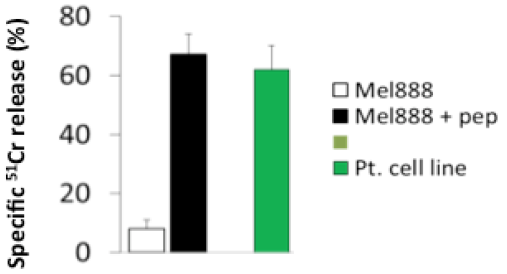
Natural Eluted Peptide



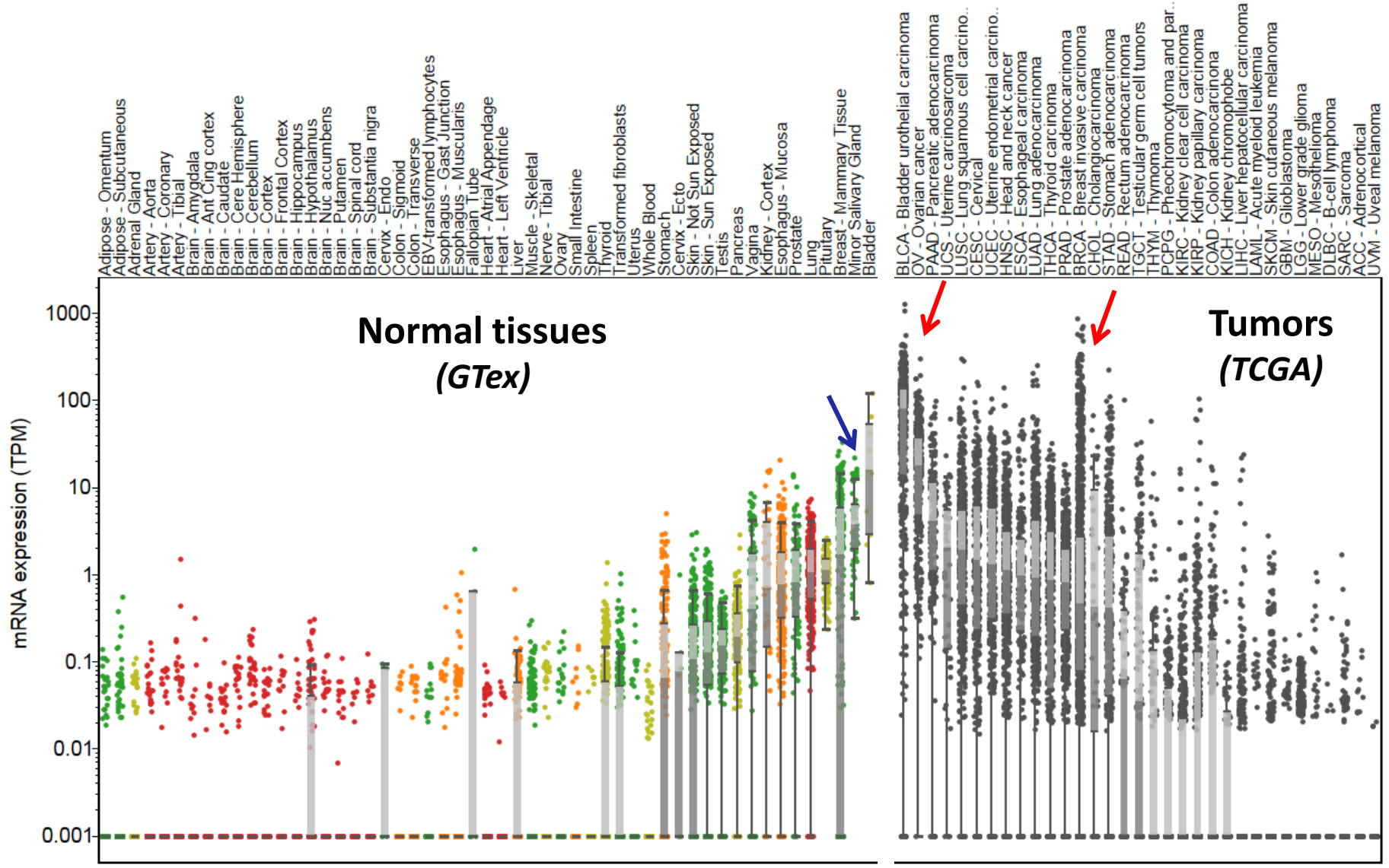
Synthetic Isotope-labeled Peptide

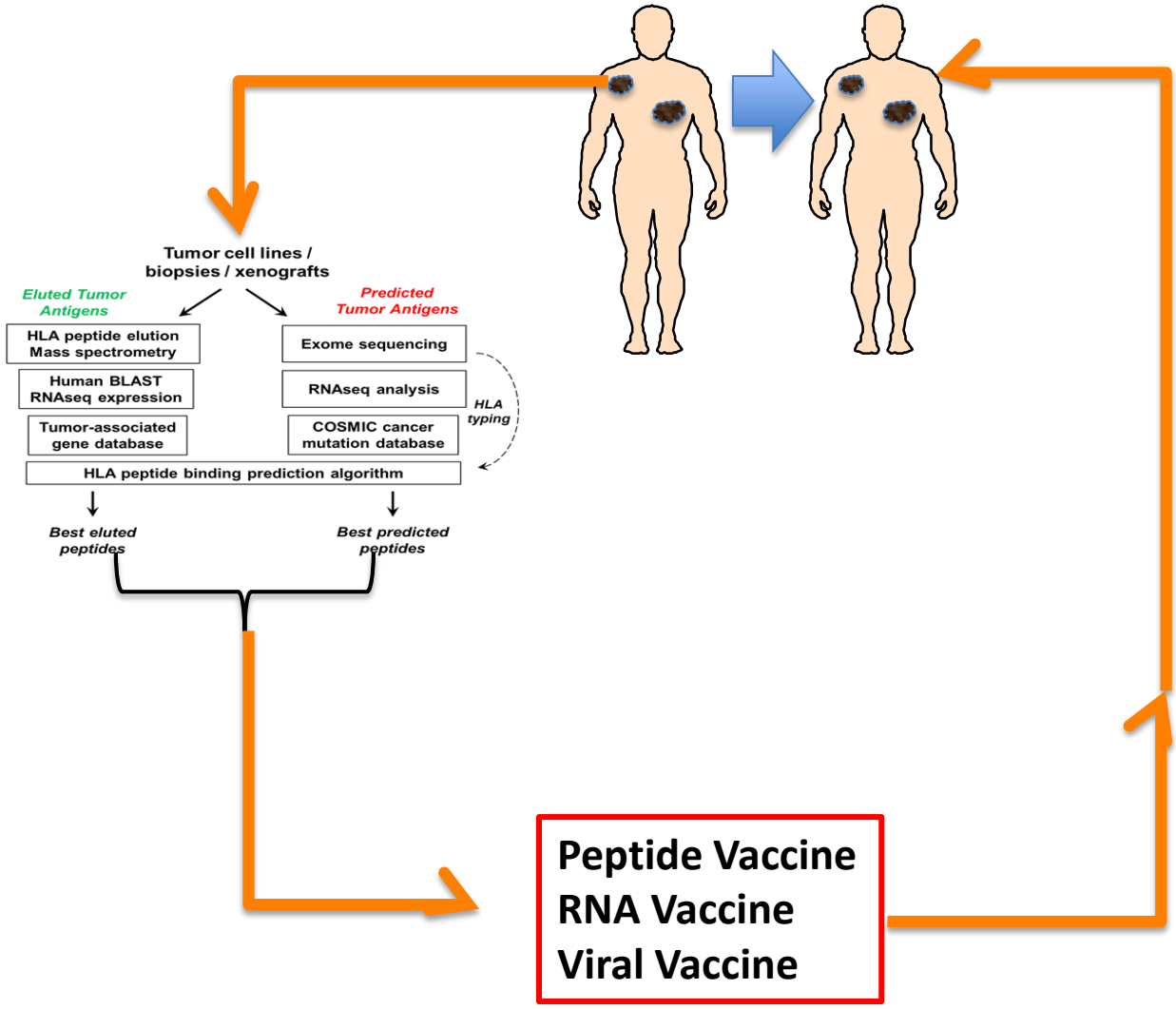




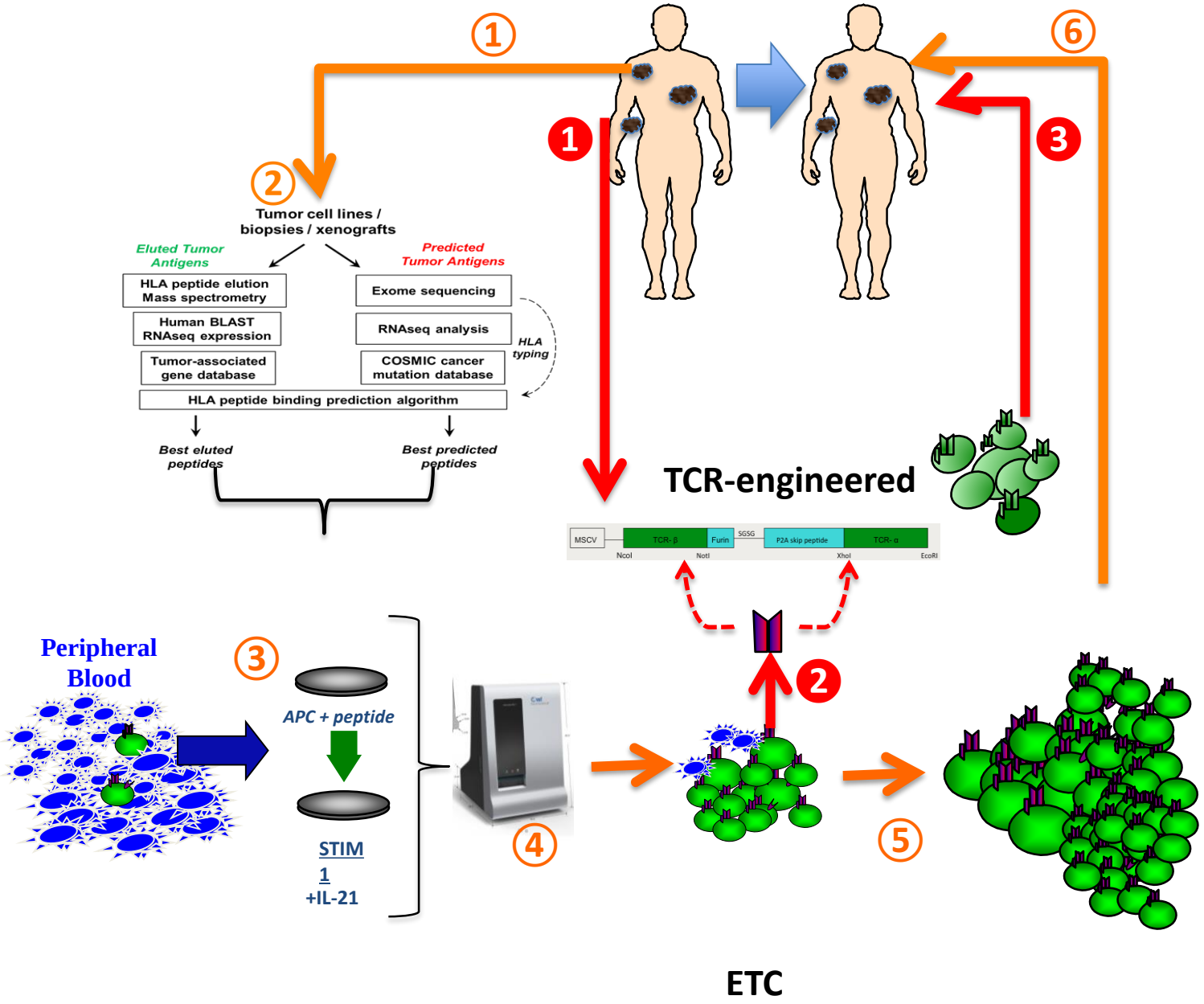


PANCX demonstrates a favorable tumor overexpression profile





?formulation
?adjuvant
?schedule/dosing
?tumor burden


1
6
1
3
2
Tumor cell lines / biopsies / xenografts
Eluted Tumor Antigens
Predicted Tumor Antigens
HLA peptide elution Mass spectrometry
Human BLAST RNAseq expression
Tumor-associated gene database
Exome sequencing
RNAseq analysis
COSMIC cancer mutation database
HLA typing
HLA peptide binding prediction algorithm
Best eluted peptides
Best predicted peptides
TCR-engineered
MSCV
TCR- β
Furin
SGSG
P2A skip peptide
TCR- α
NcoI
NotI
XhoI
EcoRI
Peripheral Blood
3
APC + peptide
STIM
1
+IL-21
4
2
5
ETC

Conclusions- II

- Non-melanoma solid tumor malignancies can be targeted by ETC
- MS/exome/RNA seq analysis yield ‘new’ epitopes that can elicit peptide-specific T cells
- Such T cells are capable of recognizing tumor cells presenting endogenous antigen
- Peptide epitopes identified by this approach are immunogenic and may represent ***shared*** potential tumor rejection antigens

Challenges

- Non-melanoma solid tumor malignancies
- Streamline process.

Challenges

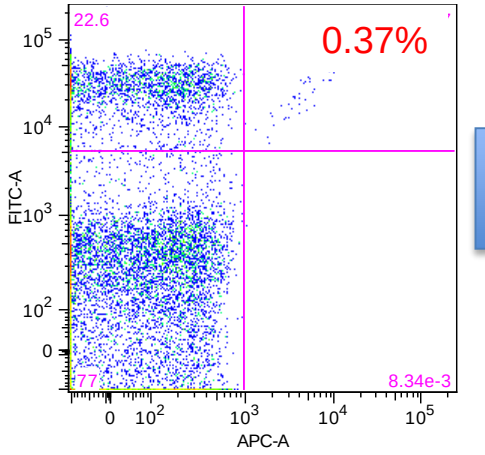
- Non-melanoma solid tumor malignancies

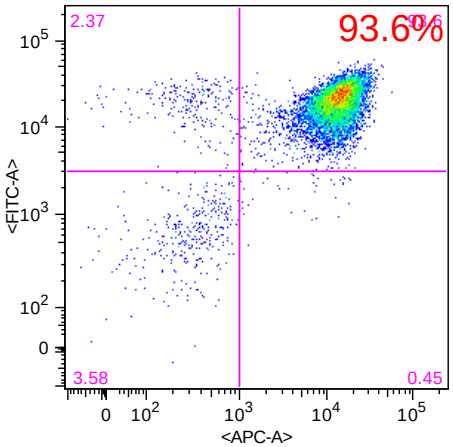
- **Streamline process**.

Streamline Process

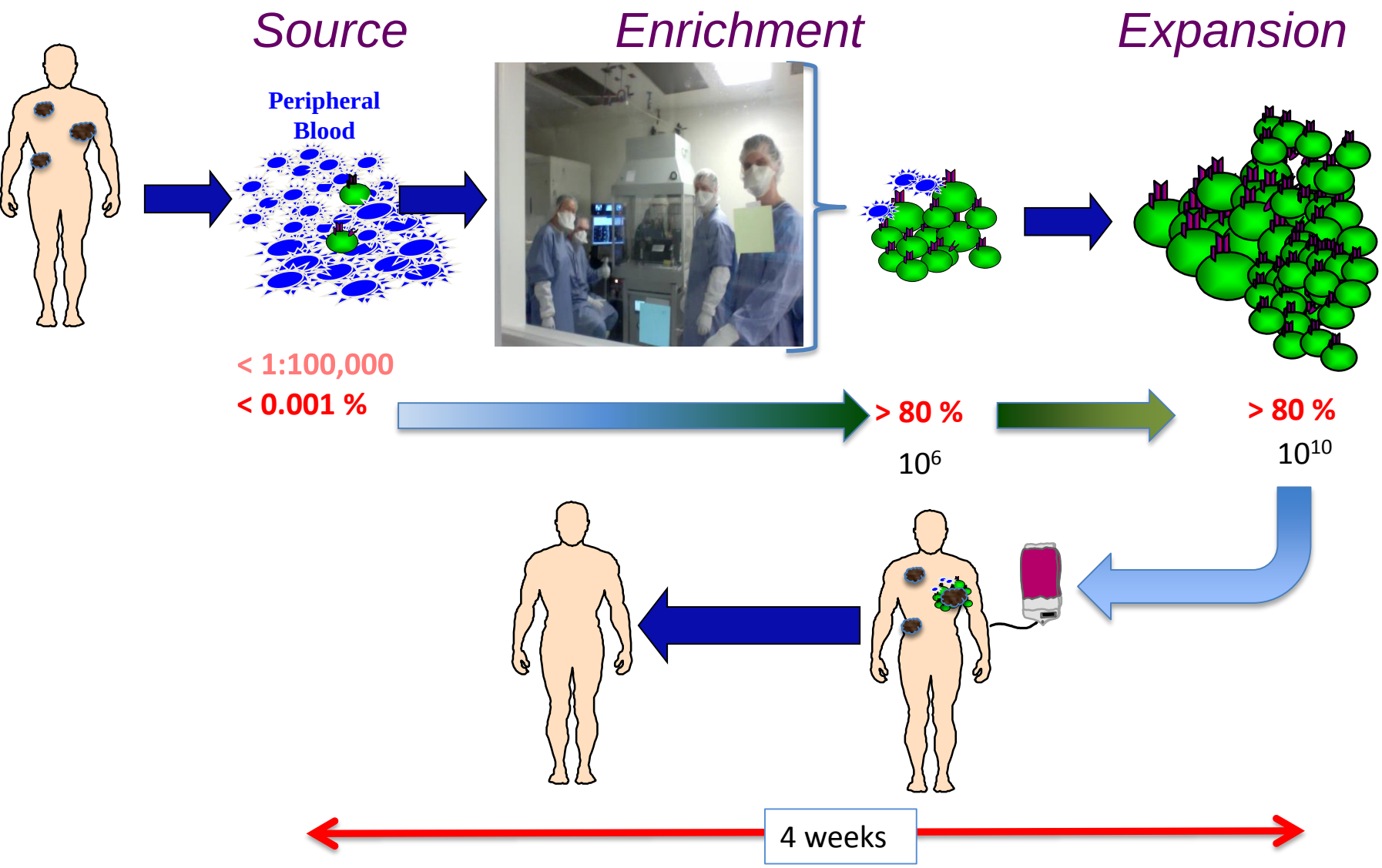
Clinical Grade pMHC-multimer-based Sorting

S

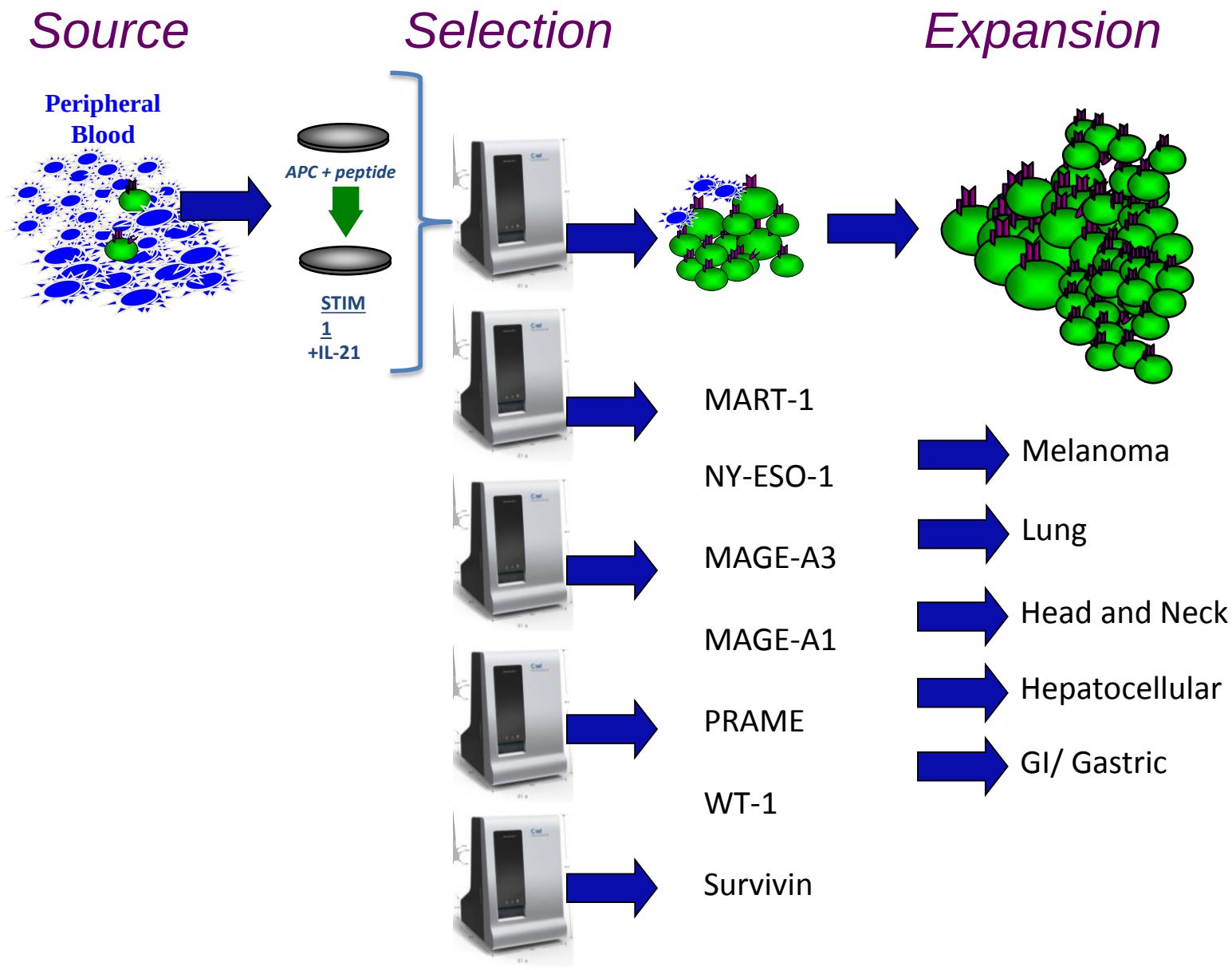


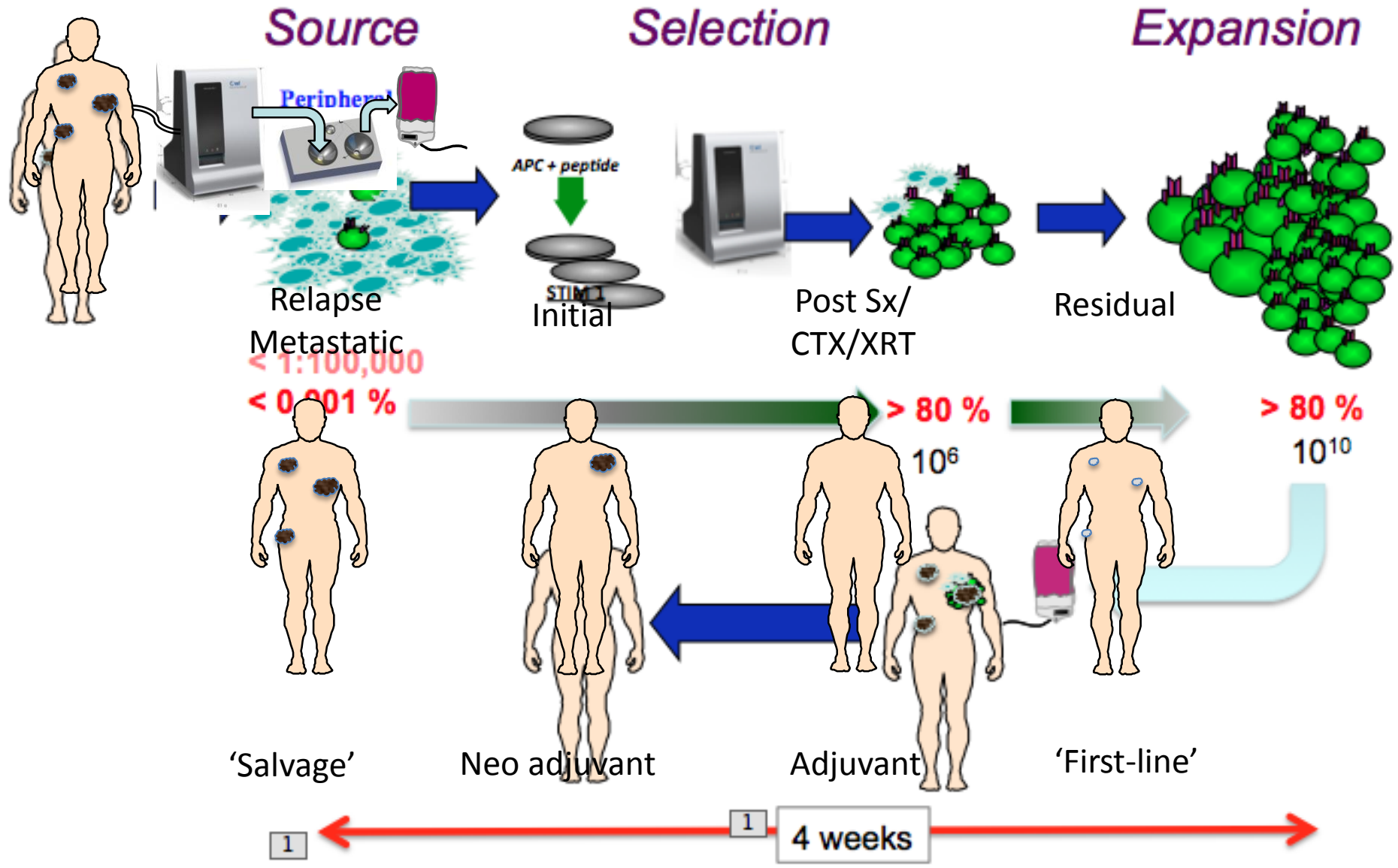


Streamline Process



T Cell Therapy: Enabling Technologies
Turnkey Operation




Source
Selection
Expansion
Peripheral
APC + peptide
STIM 1
Relapse
Metastatic
Initial
Post Sx/
CTX/XRT
Residual
< 1:100,000
< 0.001 %
> 80 %
10^6
> 80 %
10^10
'Salvage'
Neo adjuvant
Adjuvant
'First-line'
1
1
4 weeks

Beer Margarita paradigm

The Beer

Cytokines
Chemokines

The Margarita

CD8
CD4
T_{cm}
T_{eff}
T_{fh}
$Th_{1\text{-}50}$
NK
NKT

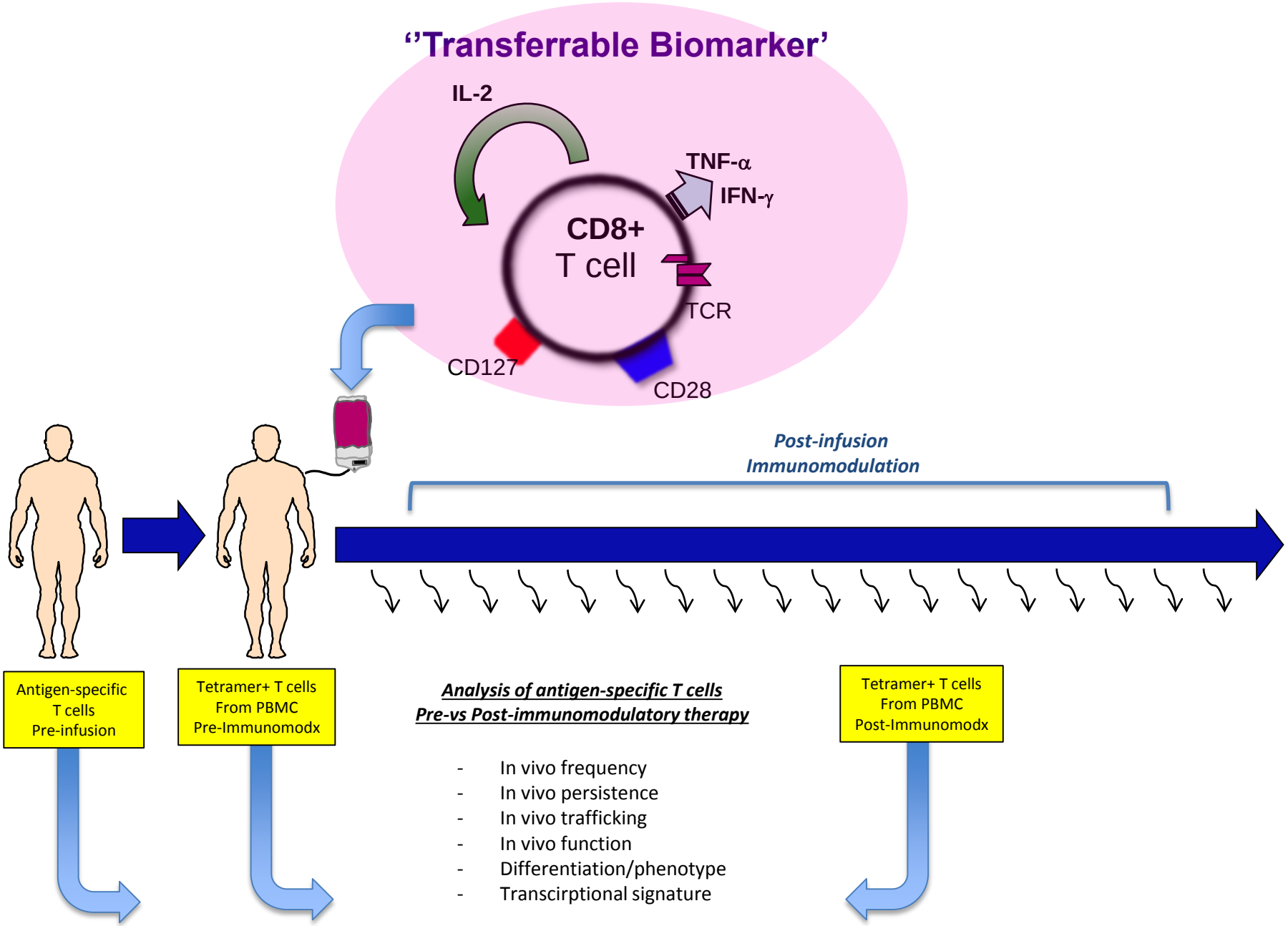
Genetic Modification

-safety
-knockdown
-conditional expression

CTLA4
PD1/PD-L1

GITR
OX40
CD40
CD137

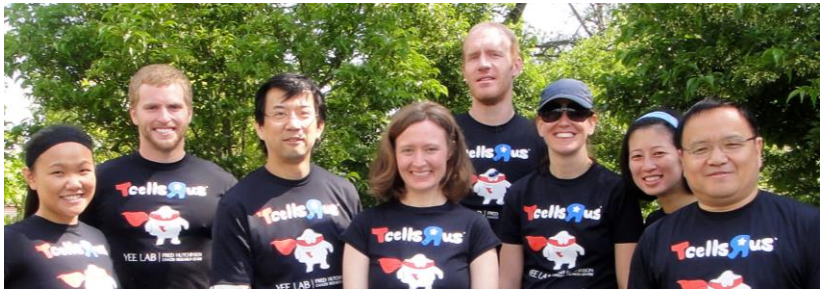
Vaccine Therapy
Oncolytic Virotherapy
Radiation Therapy
Targeted Therapy
Chemotherapy


''Transferrable Biomarker'
IL-2
TNF-α
IFN-γ
CD8+
T cell
TCR
CD127
CD28
Post-infusion
Immunomodulation
Antigen-specific
T cells
Pre-infusion
Tetramer+ T cells
From PBMC
Pre-Immunomodx
Analysis of antigen-specific T cells
Pre-vs Post-immunomodulatory therapy
- In vivo frequency
- In vivo persistence
- In vivo trafficking
- In vivo function
- Differentiation/phenotype
- Transcirptional signature
Tetramer+ T cells
From PBMC
Post-Immunomodx

Future Challenges

1. Conditioning
   - Engineered vs Endogenous T Cell therapy
2. Targets
   - Personalized vs Impersonalized
   - Antigen-spreading vs Multivalency
   - Tumorigenic?
3. Effector Cell
   - Gene-editing/Backpacking/Modification
     - Survival
     - Safety
     - Efficacy
   - Innate vs Adaptive
4. Combination

Where the money came from and People who did all the work



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