Primer on Adoptive Cell Therapy

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UNIVERSITY OF PENNSYLVANIA
Abramson Cancer Center

CELEBRATING 250 YEARS 1765–2015 Perelman School of Medicine UNIVERSITY of PENNSYLVANIA

the cure is within
ABRAMSON CANCER CENTER

Presenter Disclosure Information

Carl June

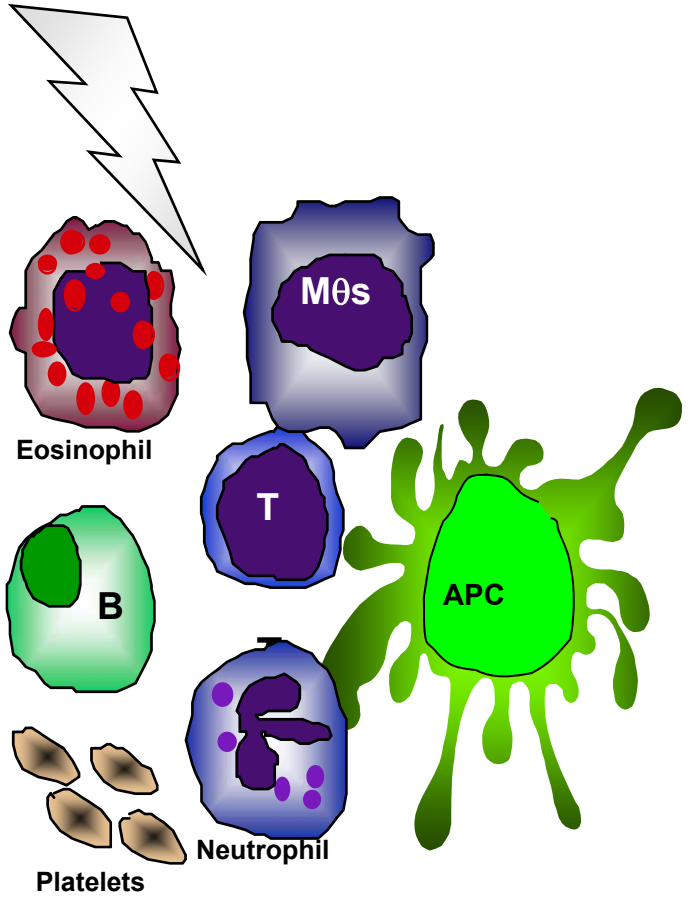
The following relationships exist related to this presentation:

Novartis

- Sponsored research grant
- Intellectual Property
- Royalties

Outline: Engineered T Cells

- **Synthetic biology: approaches to overcome tumor immune suppression and tolerance**
- **TCR engineered T cells**
- **CAR T cells**
- **TILs**



The Three Laws of Immunology

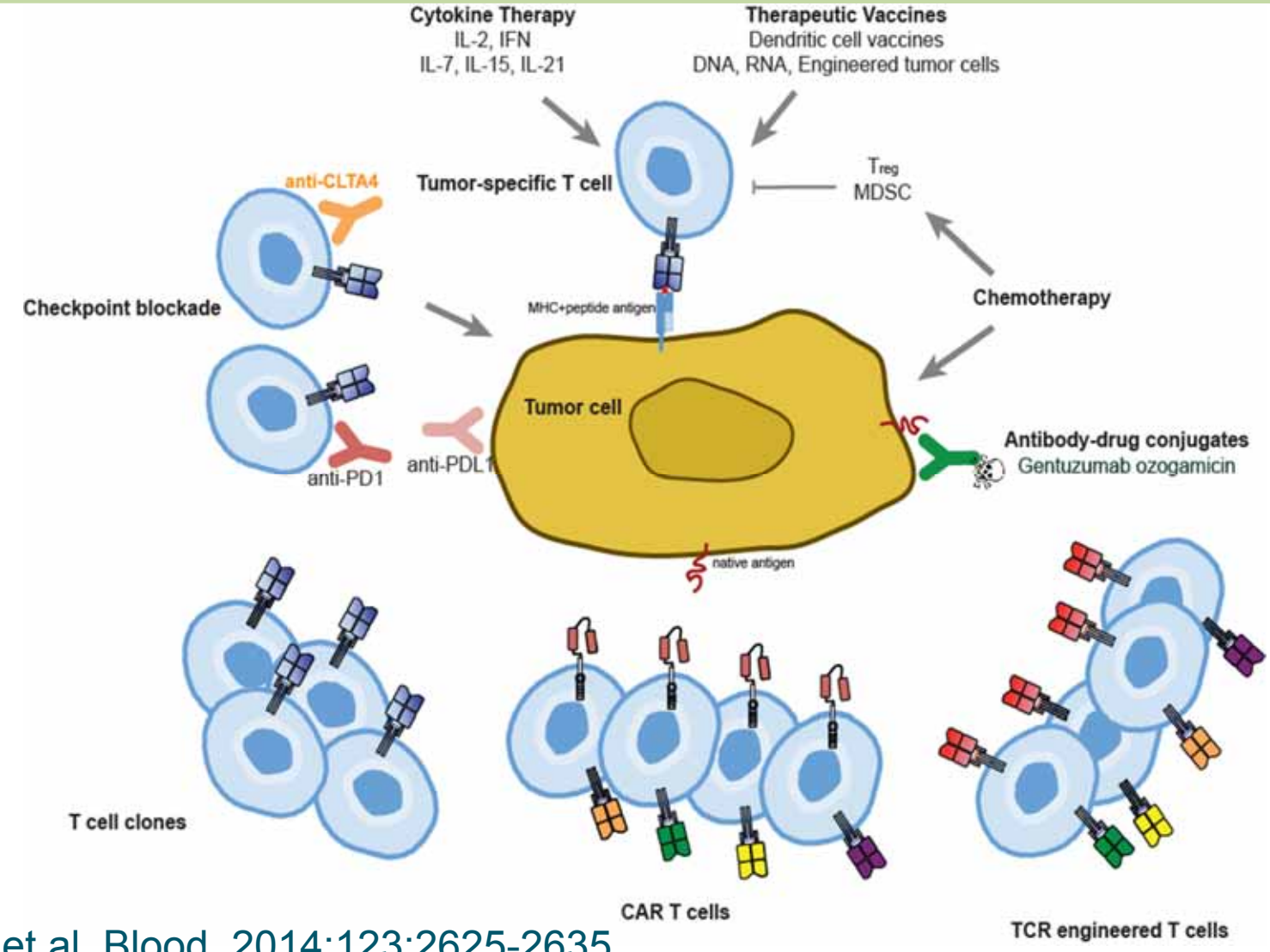
1. **The Immune System is Capable of Recognizing a Virtually Unlimited Array of Specific Structures or "Antigen" (*Universality*)**

2. **The Response to Self-Antigens is Eliminated or Controlled (*Tolerance*)**

3. **The Response is Appropriate to the Inducing Pathogen (*Appropriateness*)**

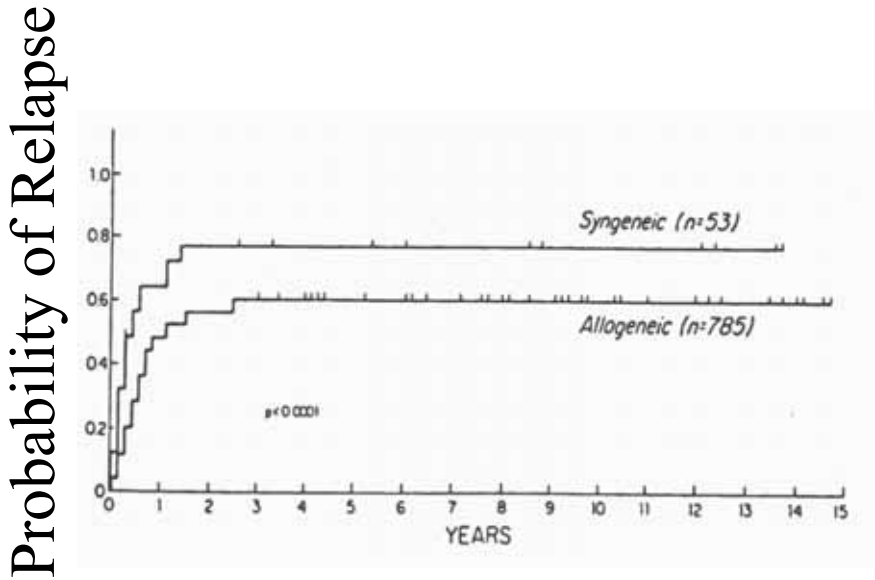
Adapted from W. E. Paul, M.D., Editor, Fundamental Immunology

Approaches to Overcome Tolerance: Immune Tolerance to Cancer



Maus MV et al. Blood. 2014;123:2625-2635.

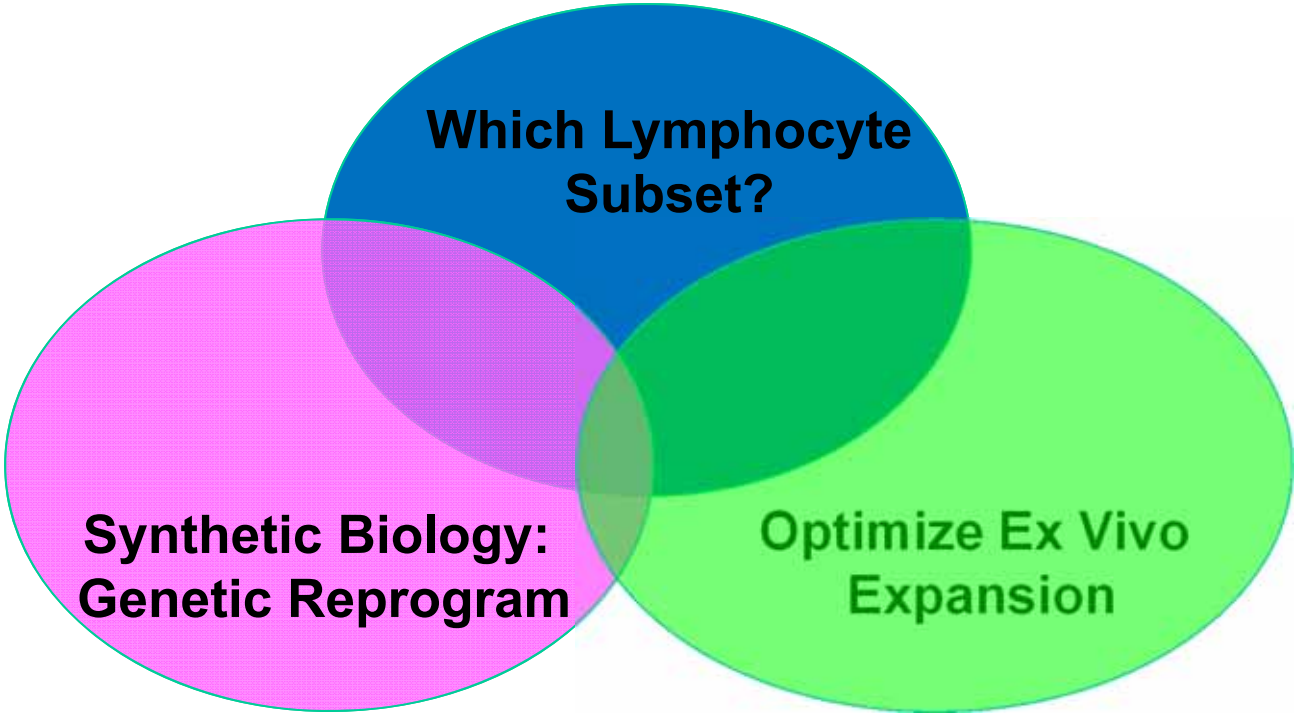
Immune Responses Can Cure Chemotherapy Resistant Human Tumors



Weiden and Thomas, 1970s

- Allogeneic but not syngeneic bone marrow transplants shown to cure childhoood leukemia
- The allogeneic immune response is the most potent antitumor effect known, but can not be routinely used in adults due to limited donors and graft versus host disease.

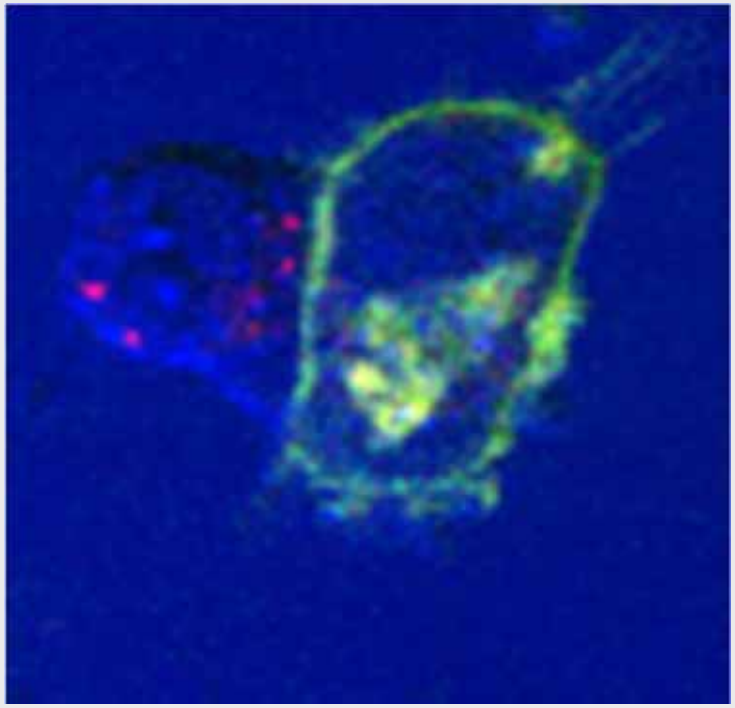
Essential factors for augmenting adoptive immunotherapy



CTLs (Killer) T Cells: Primary Weapons for Cancer Gene Therapy

- **CTLs can be "serial" killers: One T cell can kill many tumor cells**
- **T cells evolved to kill cells with new RNA or DNA, i.e. viruses (and tumors)**
- **Non-cross resistant killers: Because T cells have many killing mechanisms, they can be more effective than any single drug**
- **T cells can be self replicating, unlike drugs**

Example of CTL killing a tumor cell: rapid induction of apoptosis



Stinchcombe J, et al. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 2001;15:751-61.

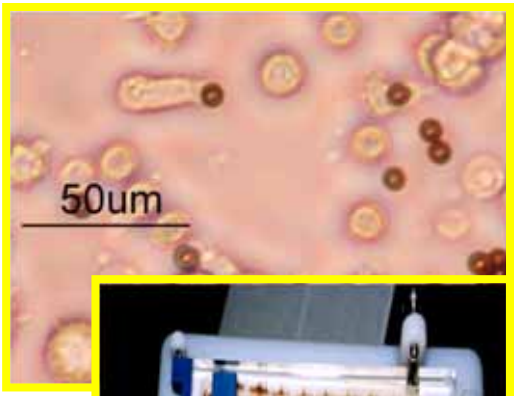
Considerations for T Cell Therapy

- 1 kg of tumor = 10^12 cells
- Our first 3 patients had 3 to 7 lbs of tumor!
- It is not realistic to expect tumor eradication unless the killing machinery (T, NK, macrophage) is equivalent to tumor burden. i.e. "E:T" ratio ~= 1
- Failure to achieve critical mass of T cells explains previous trials with disappointing results
- Two potential solutions:
  - Infuse huge numbers of T cells (TILs)
  - Infuse small numbers of T cells programmed to divide

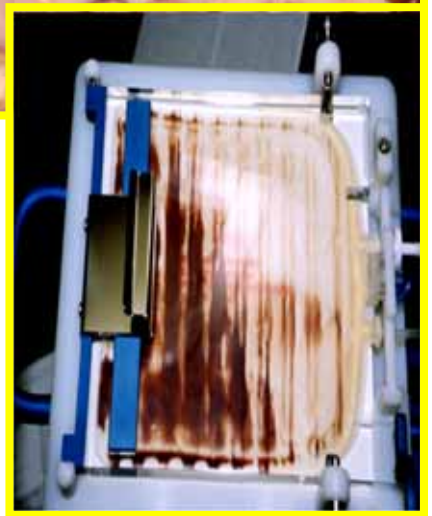
Development of Clinical Scale T Cell Manufacturing Process

- 1987: Discovery that CD28 is 'gatekeeper' for T cell proliferation (Mol Cell Biol, 1987)
- 1993: CD3/CD28 beads first produced
- 1996: First HIV patients treated
- Research was funded by the Office of Naval Research.
- Patents owned by US Government.

Bead addition



Bead removal



T cell infusion

Translational lessons:

1. **Basic science discoveries**
2. **Unrestricted funding from government**
3. **Long time frame: patience!**

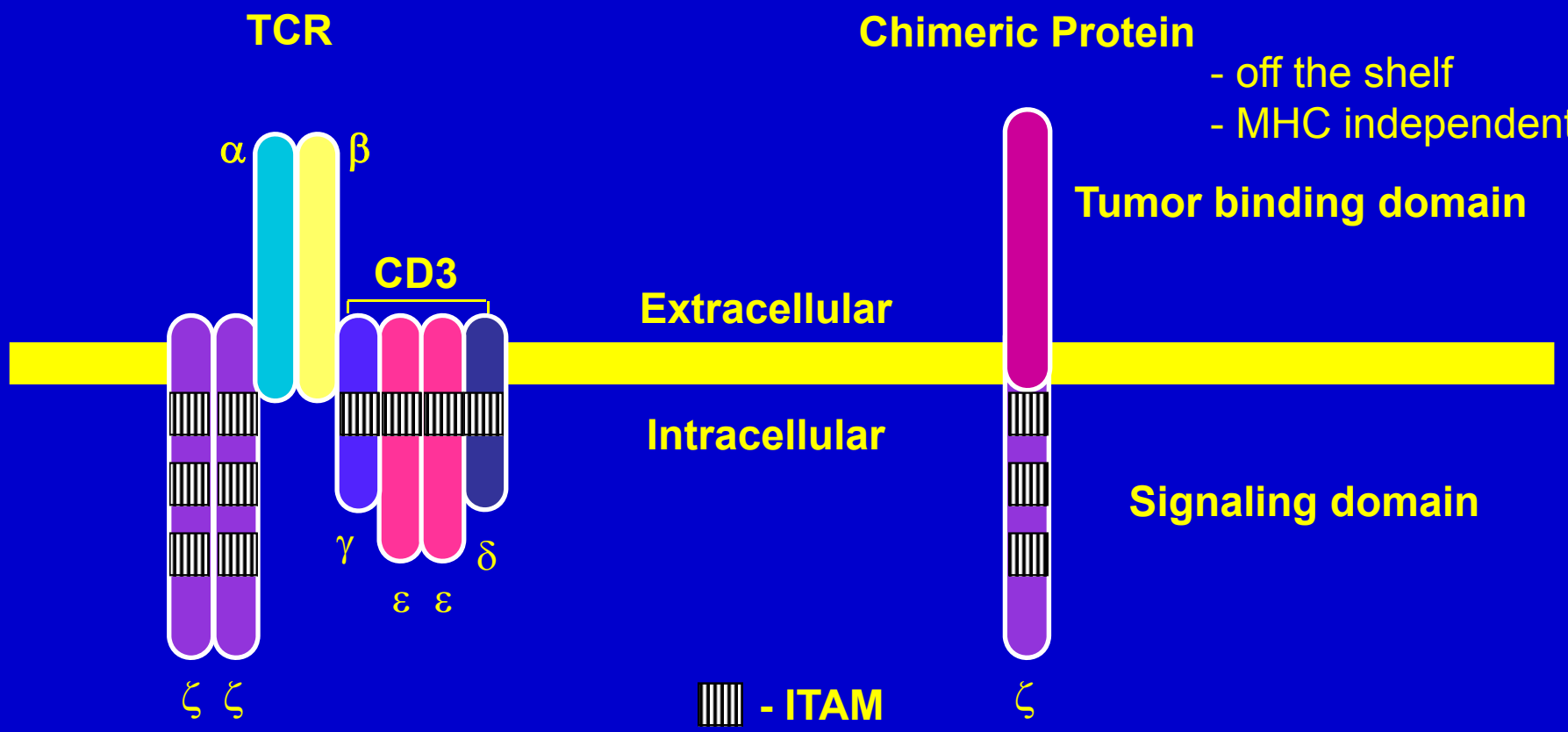
Synthetic Biology:
Cell therapy and gene therapy at the crossroads

- **"Synthetic biology is a new area of biological research that combines science and engineering. Synthetic biology encompasses a variety of different approaches, methodologies and disciplines, and many different definitions exist. What they all have in common, however, is that they see synthetic biology as the design and construction of *new biological functions and systems not found in nature*."**

Wikipedia

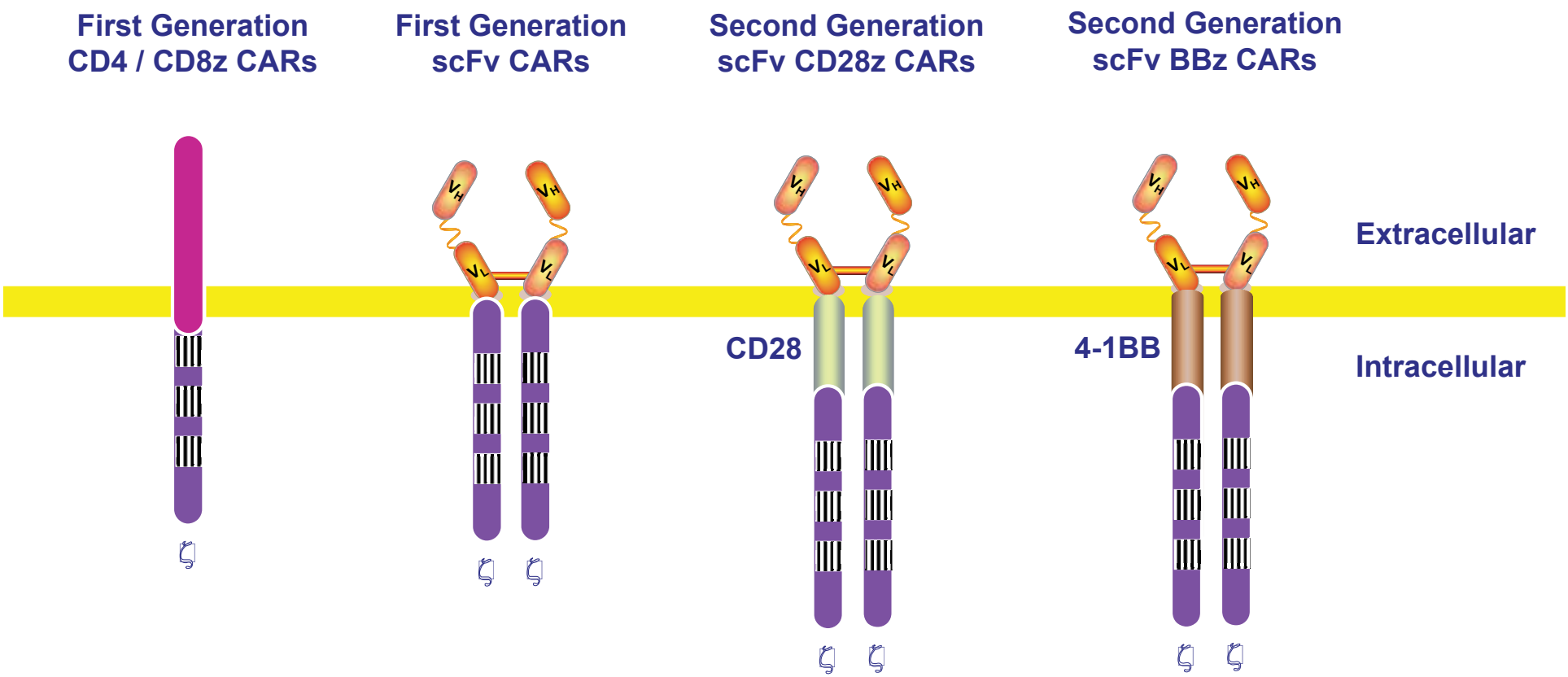
Using Synthetic Biology to Overcome Tolerance
Creation of Bi-specific T cells

TCR heterodimer approach "CAR" or T body approach



Kalos & June. Immunity. 2013;39:49-60

Design of CAR T Cells



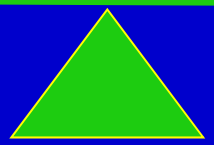
Irving & Weiss, 1991
Letourneur, 1991
Romeo, 1991

Eshhar, 1993

Roberts, 1995
Finney, 1998
Maher, 2002

Imai, 2004
Milone, 2009
Carpenito, 2009

Engineered CARs and TCRs: which is "better"?



TCR

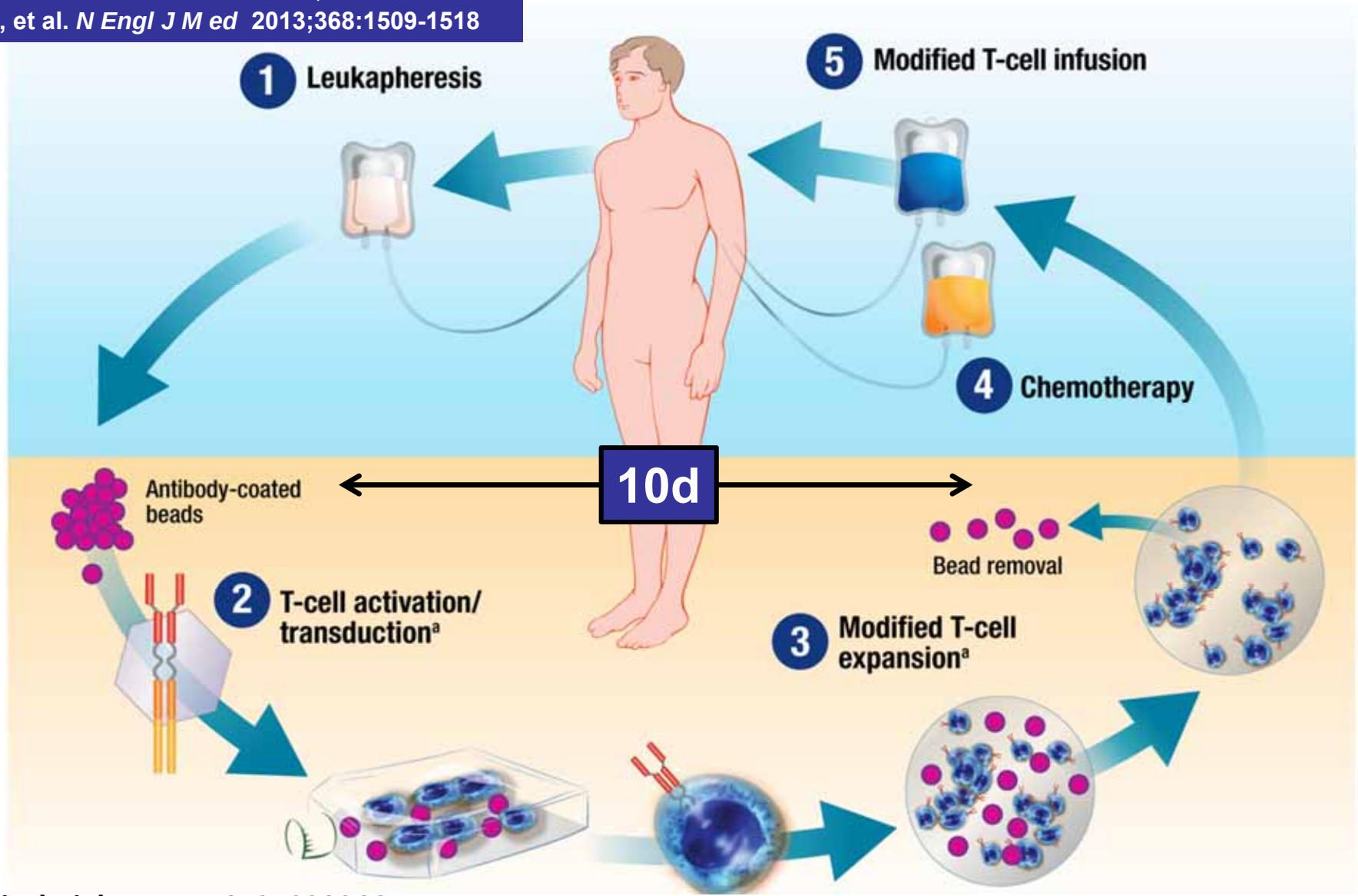
1. Sensitive signal amplification derived by evolution
2. Low avidity
3. Targets intracellular proteome
4. Requires MHCI expression and HLA matching on tumor cell
5. Life long persistence

CAR

1. Signal amplification derived by synthetic biology
2. Avidity controllable
3. Targets only surface structures
4. MHC independent: "off the shelf"
5. Shorter in vivo persistence?

Second Generation CAR CLL Study Overview*

Porter DL, et al. *N Engl J Med*. 2011;365(8):725-733
Kalos M, et al. *Sci Transl Med.* 2011;3:95ra73
Grupp S, et al. *N Engl J M ed* 2013;368:1509-1518



* ClinicalTrials.gov #NCT01029366

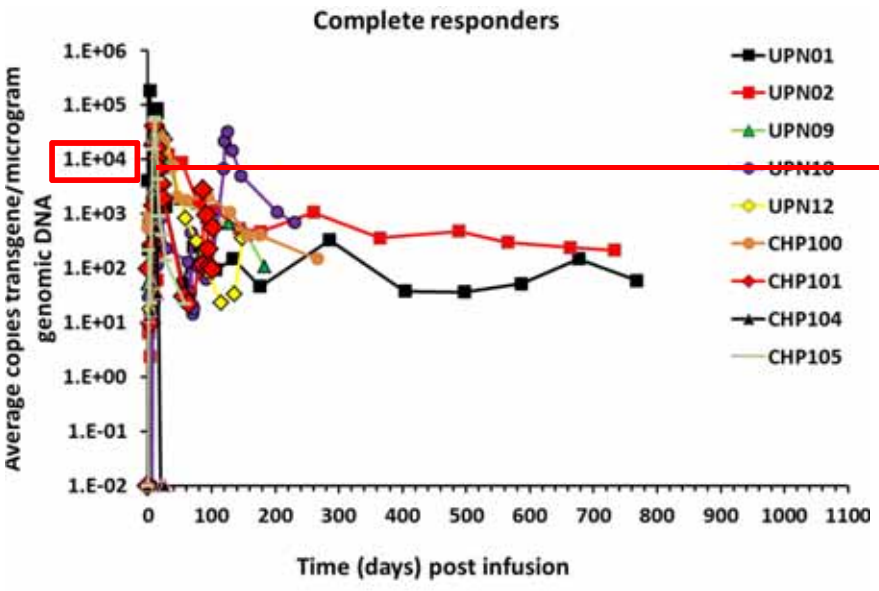
CART19 CLL: Generalities on First 3 Treated Patients

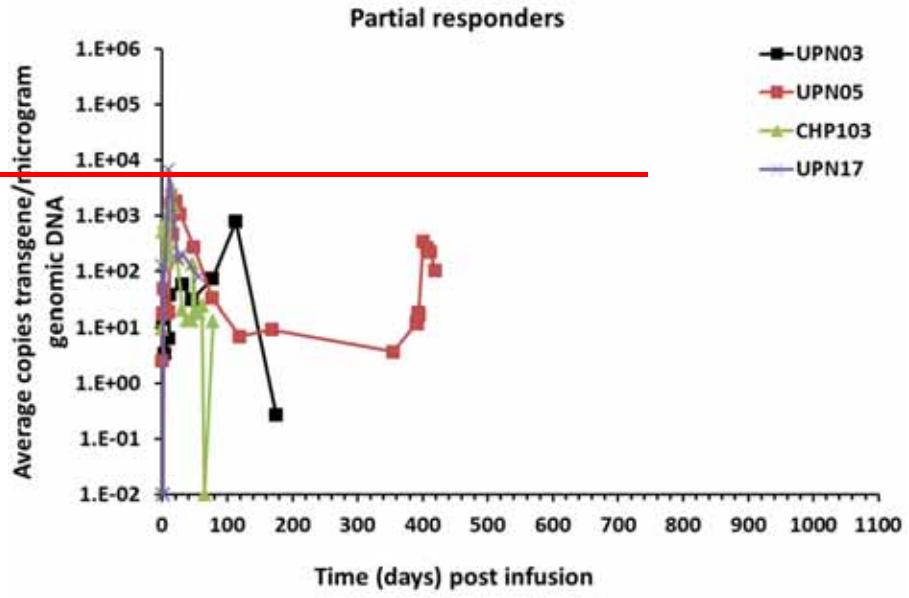
- All 3 patients had Chronic Lymphocytic Leukemia (CLL)
  - ✓ Late stage incurable leukemia
  - ✓ 3.5-7 pounds of tumor/patient
- Each infused CAR T cell or its progeny killed more than 1000 tumor cells: CARs are "Serial Killers"
- Remissions durable to date
- Sustained antibody delivery with a single infusion of engineered T cells (beyond 3+ yrs)

Porter, D.L. et al.. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia New England Journal of Medicine 365:725-733.

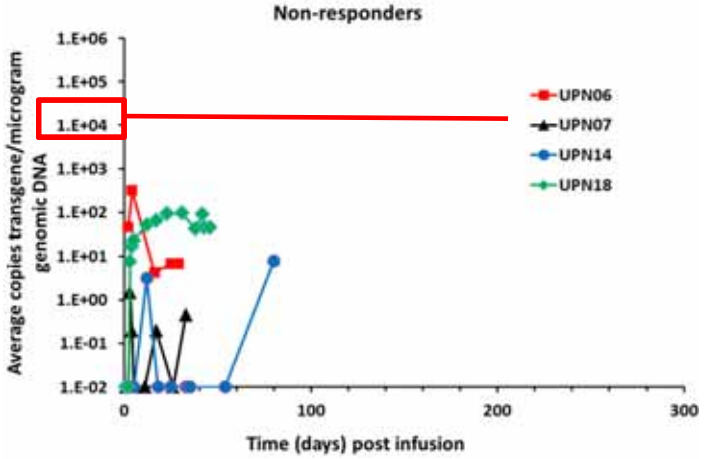
Kalos, M., et al . 2011. T cells expressing chimeric receptors establish memory and potent antitumor effects in patients with advanced leukemia. Science Translational Medicine 3:95ra73.

Predictive Biomarker: Magnitude of peripheral CTL019 cell expansion distinguishes responders





c.a. 6% of total mononuclear cells



04409, 959 cohorts

Q-PCR analysis
CTL019 cells/microgram genomic DNA

Micheal Kalos, PhD

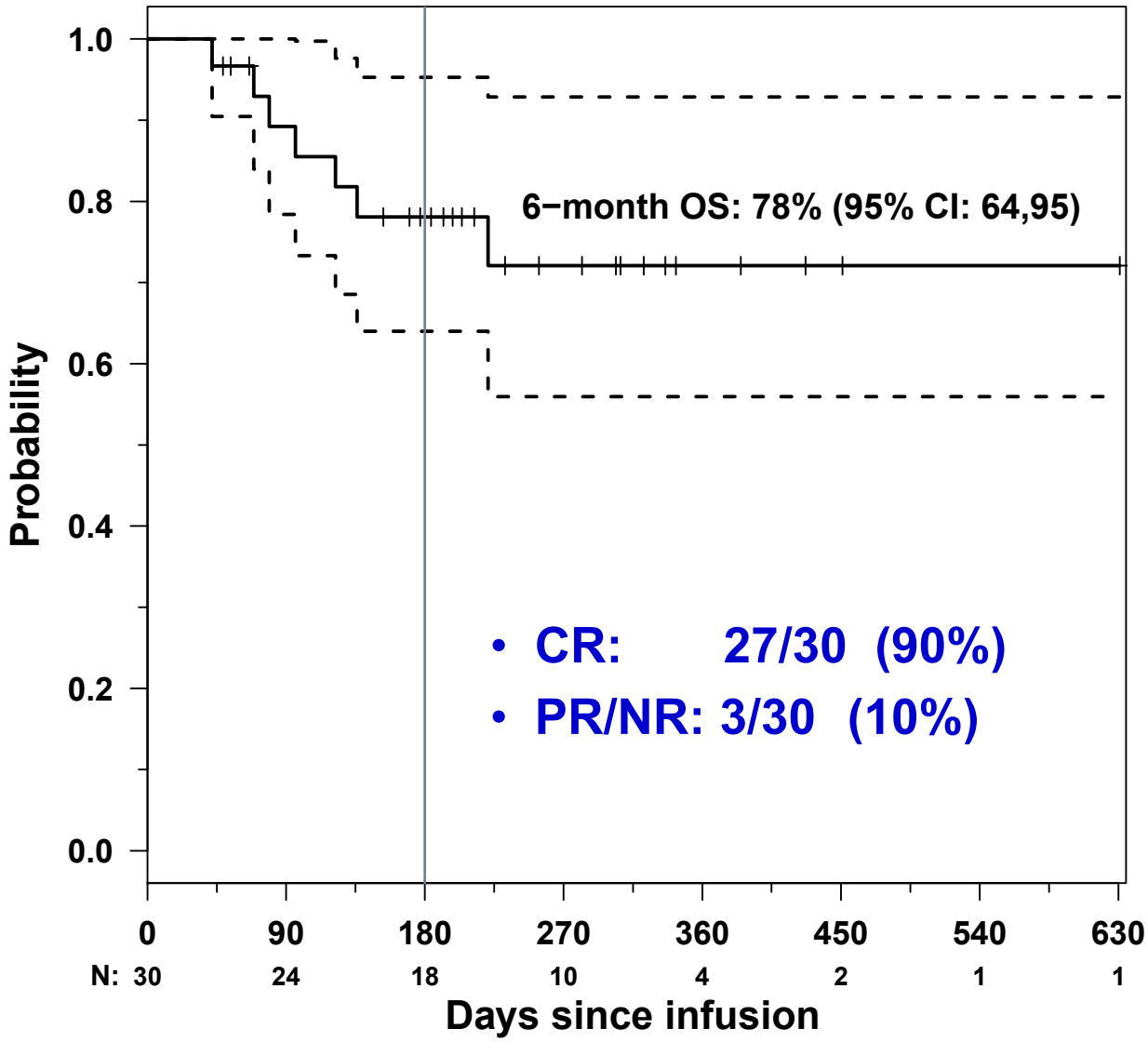
Perelman School of Medicine
University of Pennsylvania

Clinical Update of Pediatric and Adult ALL Patients Treated with CART19

	Pediatric Cohort N=25	Adult Cohort N=5	Total N=30
Sex			
Female	11 (44%)	1 (20%)	12 (40%)
Male	14 (56%)	4 (80%)	18 (60%)
Age at Infusion Median (range)	11 (5, 22)	47 (26, 61)	14 (5 ,61)
Race			
African American	1 (4%)	1 (20%)	2 (6.7%)
Asian	2 (8%)		2 (6.7%)
Caucasian	21 (84%)	4 (80%)	25 (83.3%)
Pacific Islander	1 (4%)		1 (3.3%)
Post Allogeneic Transplant			
Yes	18 (72%)	0 (0%)	18 (60%)

Maude et al, NEJM 2014

Summary of CART19 Efficacy in ALL (n=30)
Case mix on phase I: 25 pediatric and 5 adult



Allogeneic CART19 for Relapsed CD19+ Disease

***Kochenderfer et al. Blood 2013 :*122(25):4129**

- All pts with disease after allo-HSCT and prior DLI
  - N=10 (4 CLL, 4 MCL, 2 DLBCL)
- CARs manufactured from each patient's allo-HSCT donor
  - Matched sibling donor (6), Unrelated donor (4)
  - Cell dose: 0.4 – 7.8 x 10^6 CAR T/kg
- Results
  - 1 CR (CLL, URD) 9+ mo
  - 1 PR (MCL, URD) 3+ mo
  - 6 SD (1-11+ mo); 2 PD

  => No GVHD. Towards universal donors for CAR T cells?

B Cell Aplasia: Is it necessary and for how long?

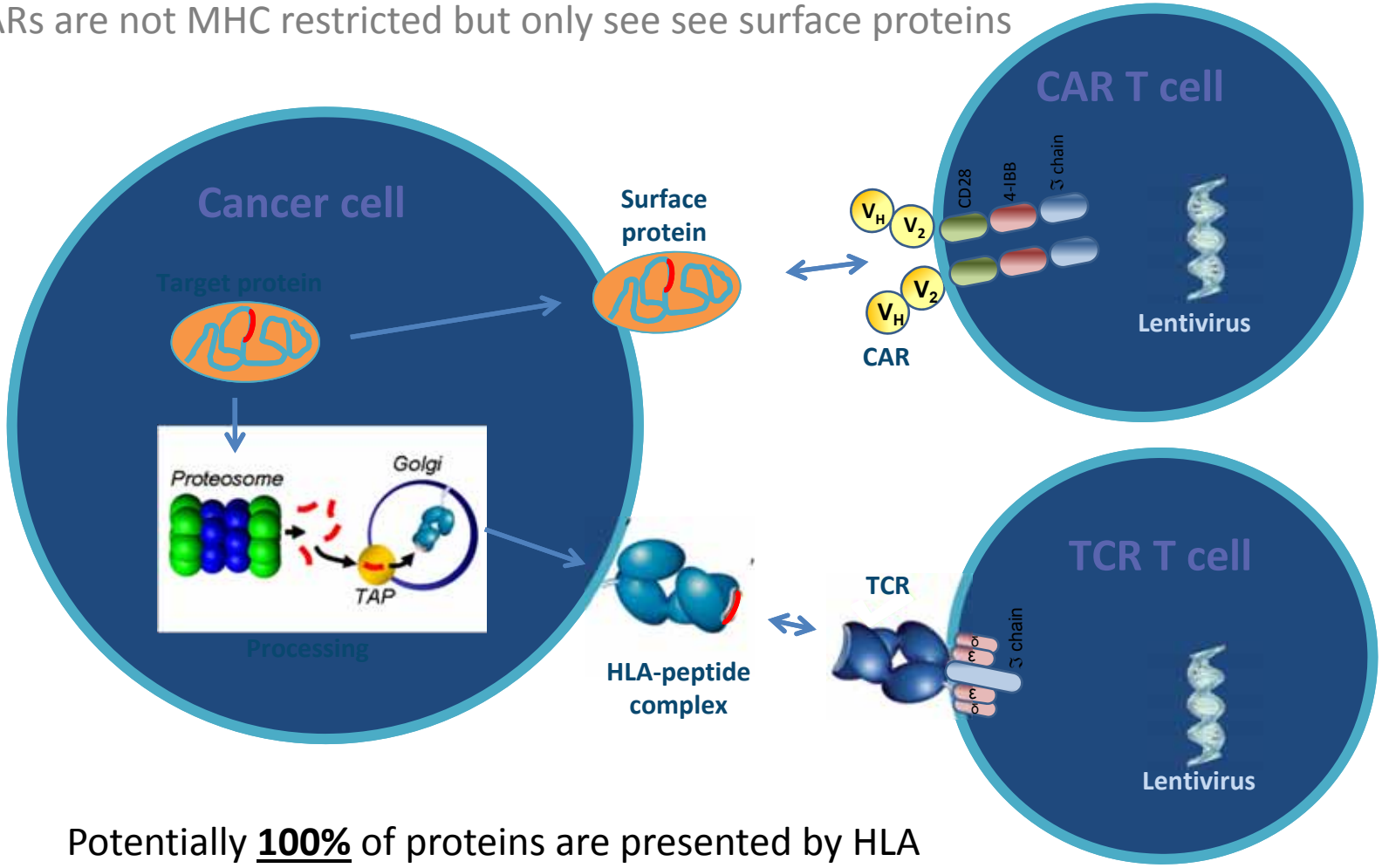
- Do CD19 CARs kill all leukemic “stem cells”?
- Cancer stem cells can persist more than a decade!
  - MacKie et al. Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. N Engl J Med. 2003;348:567-568.
- Strategies to mitigate or terminate B cell aplasia:
  - Conditional suicide systems to eliminate CARs
  - Target B cell subsets to preserve repertoire
    - Anti-lambda or anti-kappa CARs
    - Anti-idiotype CARs

CARs in Development

Commercial CARs: Celgene, Juno, Kite, Novartis, Opus, Takara	
Academic Institute (US)	**Target(s)**
Fred Hutchinson Cancer Center	CD20
Baylor College of Medicine	GD-2, Her2, CD30, kappa Ig
National Cancer Institute (NCI)	CD19, EGFRvIII , mesothelin
Roger Williams Medical Center (RI)	CEA, PSMA
University of Pennsylvania	CD19, mesothelin, BCMA, EGFRvIII PSMA
Children's Mercy Hospital Kansas City	GD-2
Academic Institute (non-US)	**Target(s)**
Chinese PLA General Hospital	CD19, CD20, CD33, CD138, HER2
Christie Hospital NHS Foundation Trust	CD19
Peter MacCallum Cancer Centre, Australia	LewisY
University of Zurich	FAP

TCRs can recognize intracellular proteins

CARs are not MHC restricted but only see see surface proteins

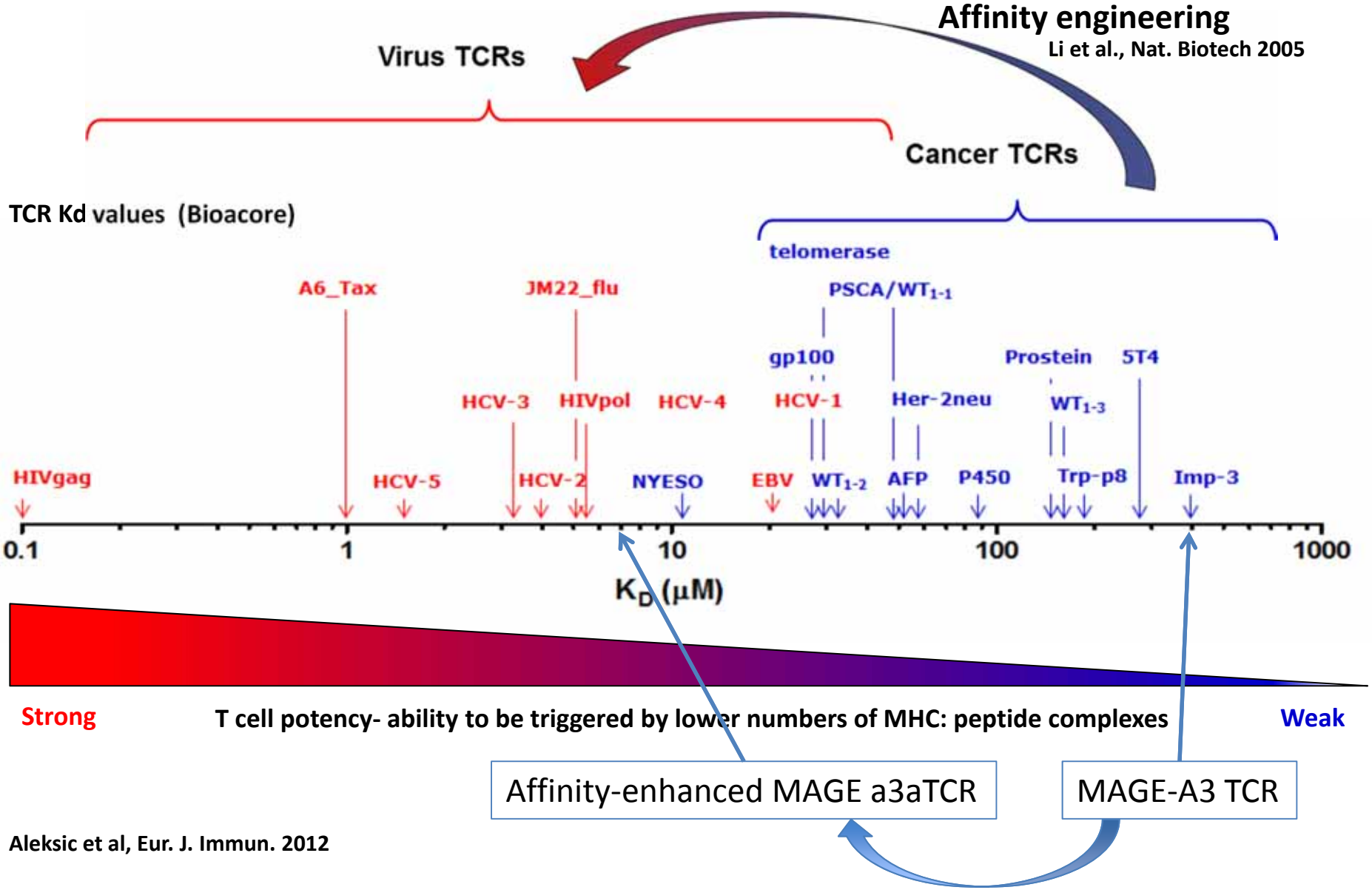


Potentially **<u>100%</u>** of proteins are presented by HLA

2013: Highly potent engineered T cells show tumor specificity is required. Cross reactivity can be lethal!

1. Linette, G.P. et al. 2013. Cardiovascular toxicity and titin cross-reactivity of affinity enhanced T cells in myeloma and melanoma. Blood 122:863-871.
2. Cameron, B.J., et al. 2013. Identification of a Titin-Derived HLA-A1–Presented Peptide as a Cross-Reactive Target for Engineered MAGE A3–Directed T Cells. Science Translational Medicine 5:197ra103.
3. Morgan, R.A., et al. 2013. Cancer Regression and Neurological Toxicity Following Anti-MAGE-A3 TCR Gene Therapy. J Immunother 36:133-151.

TCR Affinity Engineering Can Impart Viral-like TCR Affinity to Cancer-specific TCRs

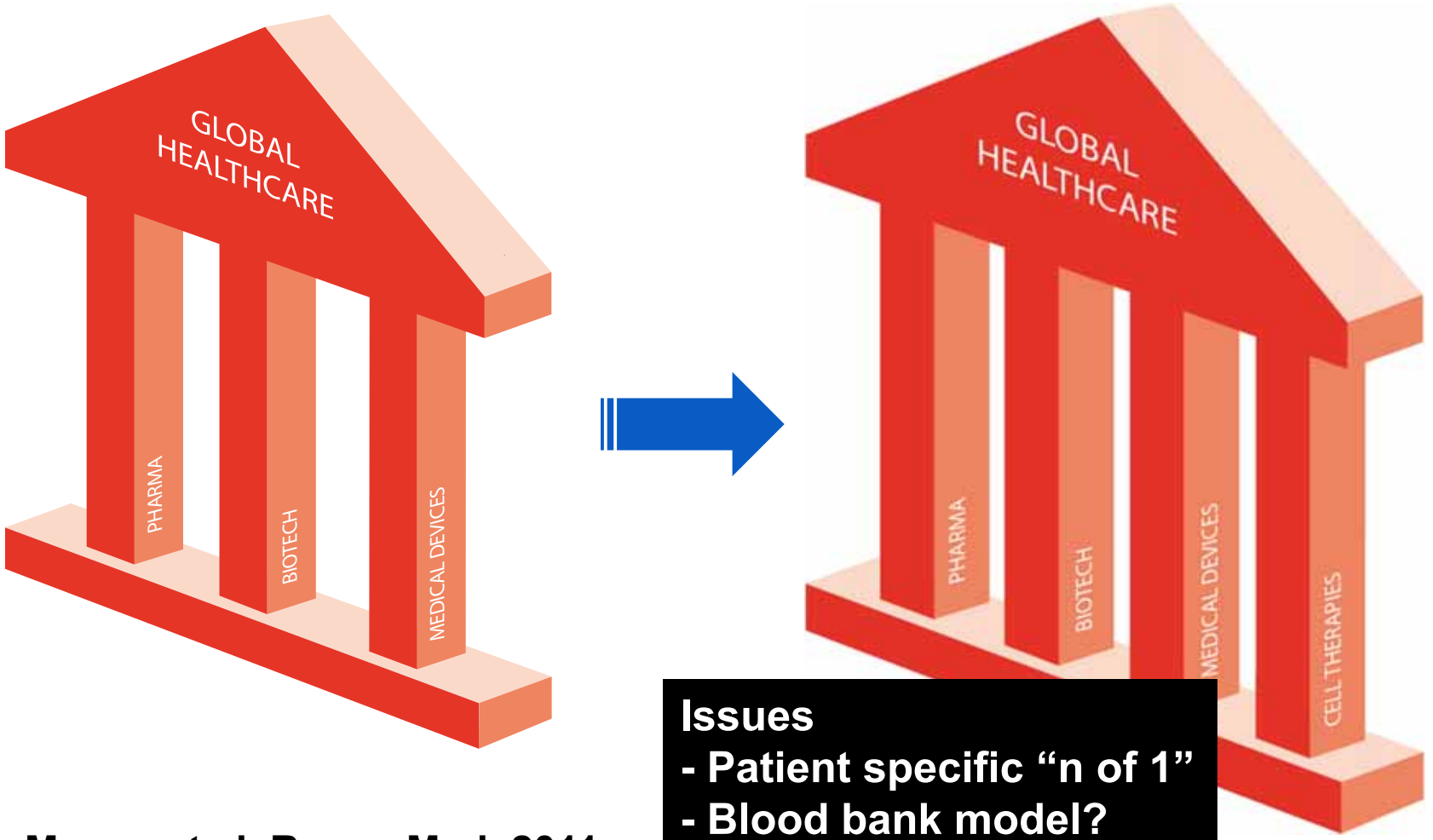


Aleksic et al, Eur. J. Immun. 2012

Conclusions and Implications MAGE A3 Enhanced TCR

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

Health Care Challenges



Issues
- **Patient specific "n of 1"**
- **Blood bank model?**
- **Central manufacturing?**

Chris Mason et al, Regen Med. 2011
Levine and June, Nature. 2013

TILs vs CAR T Cells

"When you come to a fork in the road, take it"

Yogi Berra

TIL Manufacturing in Blood Banks
Improved Culture Process

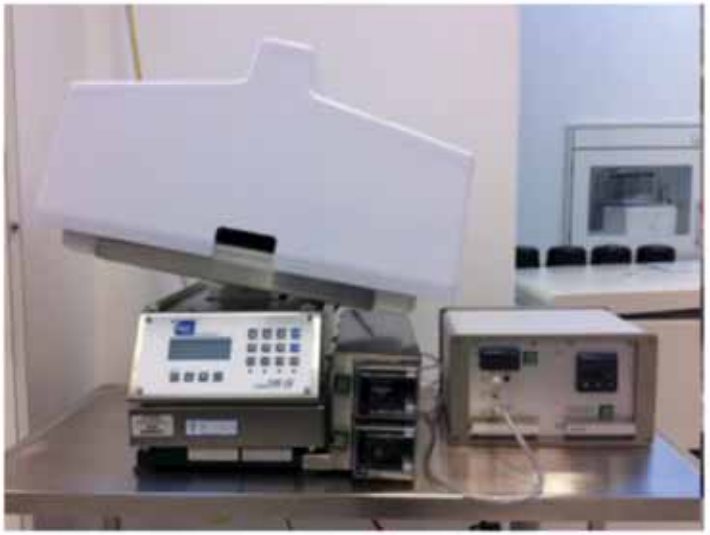
From Static to Dynamic culture (REP)

Introduction of a Practical Protocol for semi-automated TIL expansion

Anti-CD3
Feeder Cells
IL-2

REP

Day 7



Wave® Bioreactor

- Less manipulations
- Operator dependent actions x6 only during weekdays
- Scalable to Blood Banks

Inge-Marie Svane, Copenhagen

Melanoma Phase III TIL Trial: Europe

- **Randomized phase III trial**
  - Generate robust efficacy data
  - Approval of TIL therapy as standard treatment

  (Collaborators: J. Haanen, Netherlands Cancer Institute, R. Hawkins, University of Manchester)

- **Combination therapies**
  - BRAF inhibitor
  - Interferon-α

Inge Marie Svane
CCIT, Herlev, DK

John Haanen
NKI, Amsterdam, NL

Robert Hawkins
The Christie, Manchester, UK

ATTACK
ADOPTIVE ENGINEERED T-CELL TARGETING TO ACTIVATE CANCER KILLING

Lessons and Take Home Messages

- Adoptive cell therapy (ACT) is currently served with 3 flavors:
  - ✓ CAR T cells
  - ✓ TCR engineered T cells
  - ✓ TILs and CTL lines/clones

- After many years ACT is heading towards FDA approval:
  - ✓ CD19 CARs for leukemia
  - ✓ CD19 CARs for lymphoma
  - ✓ TCR T cells for sarcoma and melanoma

- Issues in the field:
  - ✓ how to combine with checkpoint inhibitors?
  - ✓ Manufacturing scale up: robotics, automation and engineering
  - ✓ Target identification