Assessment challenges in the non-clinical development of CAR and TCR modified effector cells

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Disclaimer

The upcoming presentation is not necessary the view of the agency, but rather a personal reflection on issues which normally arise during assessment of genetically modified T cells.

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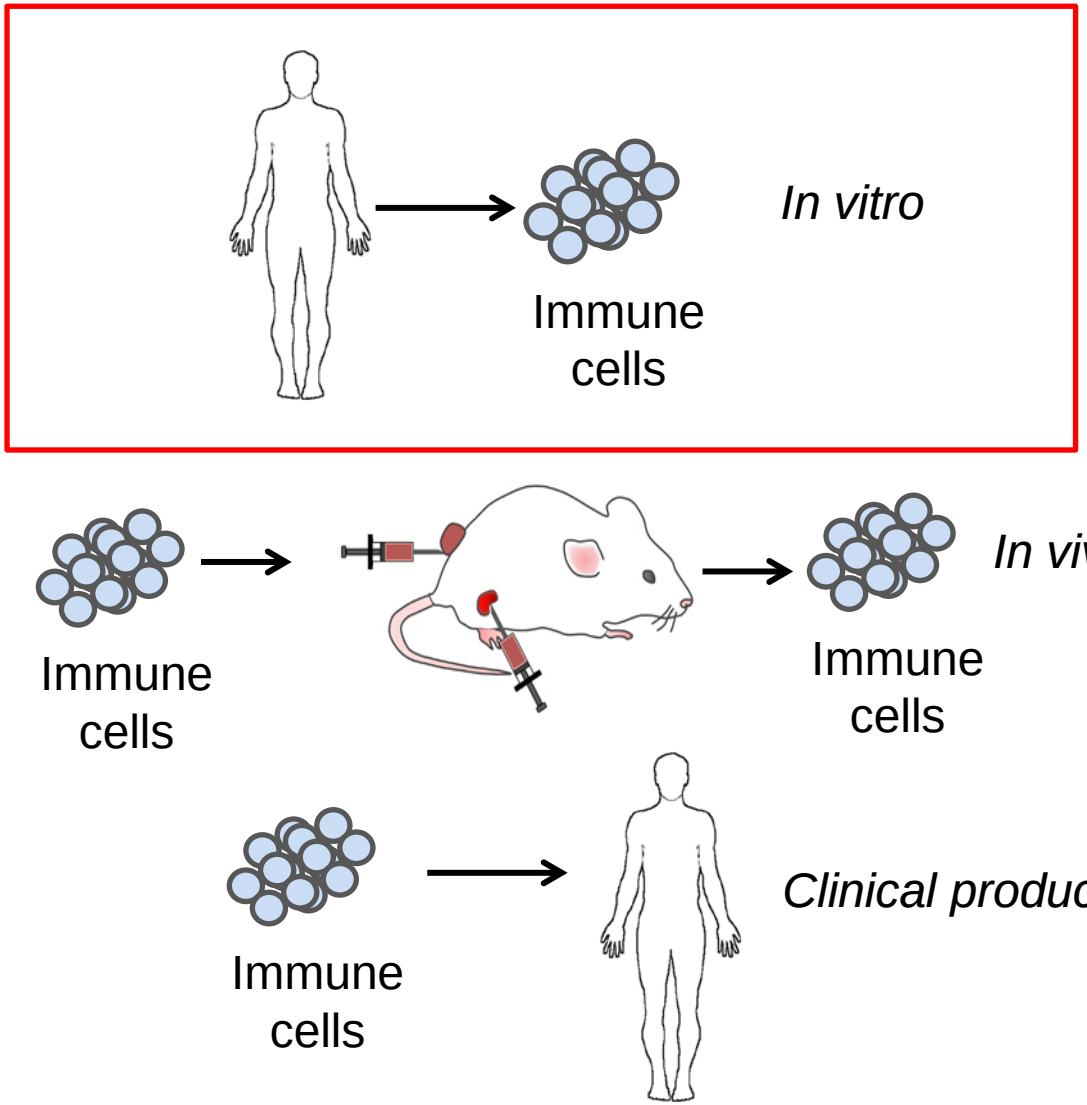
Non-clinical development

- **Pharmacodynamics (PD)**
  - Proof-of-concept
    - In vitro, specificity and reactivity
    - In vivo, tumor models (homologous systems)
- **Pharmacokinetics (PK)**
  - Biodistribution
  - Persistence
- **Toxicology/Safety studies**
  - In vitro
  - In vivo

PD – proof-of-concept assays and bridging to assays used in the clinic



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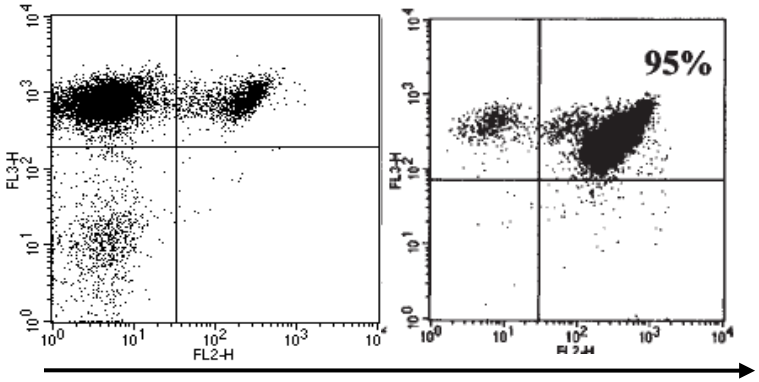
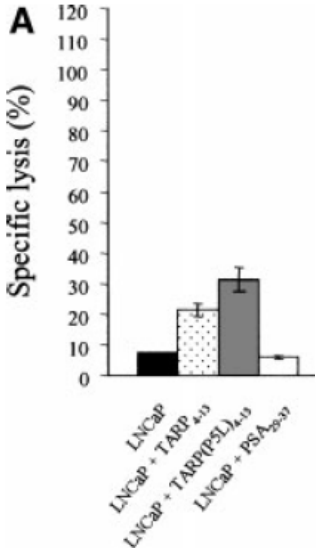
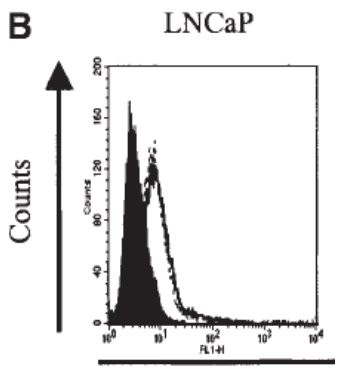
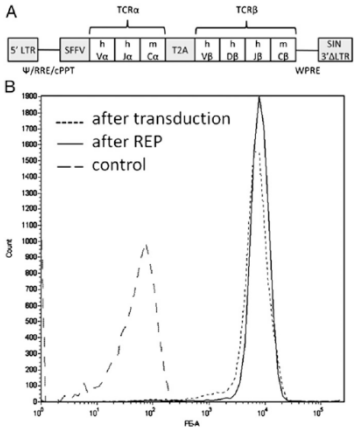


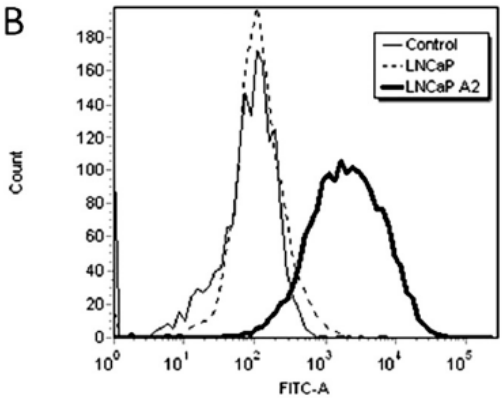
HLA-A*0201/TARP(P5L)$_{4-13}$ tetramer

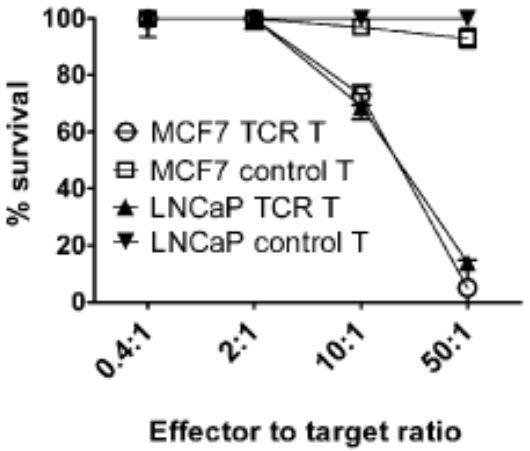
Interferon gamma











The Prostate 61:161-170 (2004)

Proc Natl Acad Sci U S A. 2012 Sep 25;109(39):15877-81.

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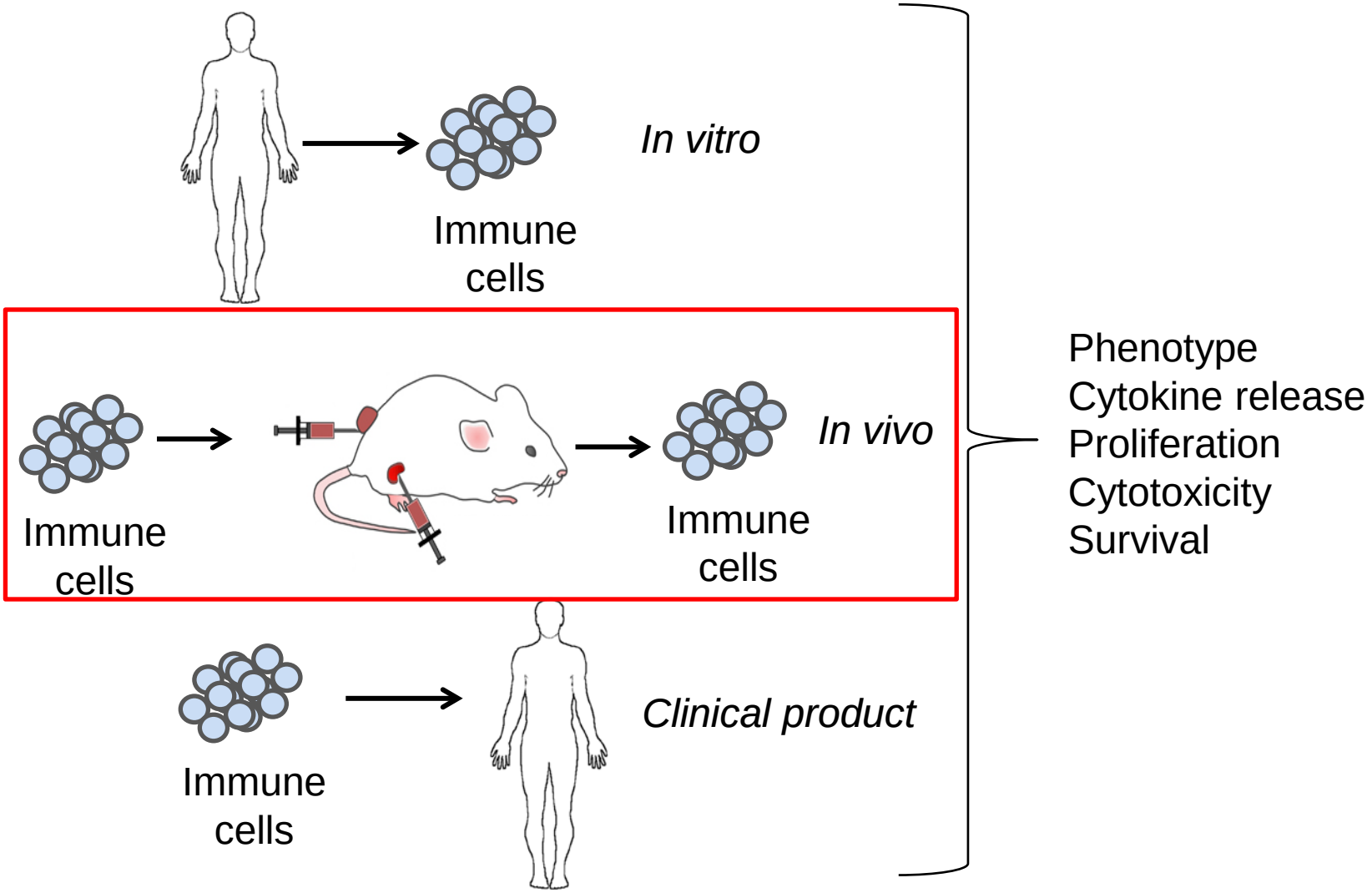
PD – proof-of-concept assays and bridging to assays used in the clinic



PD *In vivo* models - shortcomings

Tumour models using transplanted cell lines

Advantages

- Rapid and reliable tumour growth means treatment efficacy/altered tumour growth in different mouse strains easily determined
- Models for various cancer types available e.g. prostate, melanoma, breast cancer.
- Behaviour of cell lines able to be altered by modification of gene expression

Disadvantages

- Weaker model of natural tumour microenvironment (maybe improved by injection into orthotopic site)
- Injection and death of tumour cells may induce inflammation, altering tumour immune response
- Rapid tumour growth may prevent normal tumour: immune interaction to develop

Spontaneous tumour models

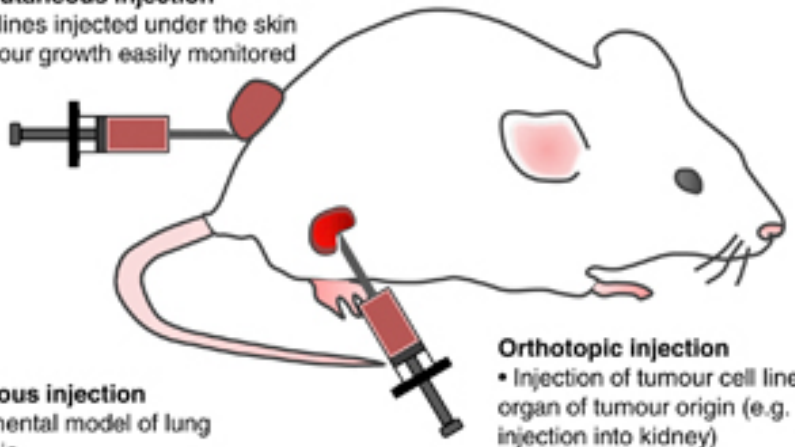
Advantages

- Heterogeneous tumour development more faithfully recapitulates human tumour development
- Tumour immune response, and immune escape may recapitulate clinical observations

Disadvantages

- Longer time required and higher cost compared to transplanted tumour models
- Tumour heterogeneity increases complexity of treatment, results can be more difficult to interpret

Subcutaneous injection
•Cell lines injected under the skin
•Tumour growth easily monitored



Intravenous injection
• Experimental model of lung metastasis

Orthotopic injection
• Injection of tumour cell line into organ of tumour origin (e.g. Renca injection into kidney)
• More faithful recreation of tumour microenvironment

Example of carcinogen induced cancer
• MCA induced fibrosarcoma
• DMBA/TPA induced skin papillomas
• DSS+AOM induced colon cancer

Genetically engineered tumour mouse models
• Strains of mice with systemic or organ specific expression of oncogenes which develop spontaneous tumours, generally between 3-12 months of age

PD *In vivo* models - Shortcomings

- **Species differences in immunology will be the same regardless of model.**

	MOUSE	HUMAN
Altered peripheral blood cell make up eg:		
Lymphocytes	~80%	~40%
Neutrophils	~20%	~60%
CD1 genes	One (CD1d)	Multiple (CD1a-e)
CD2-ligand interaction:		
T cell dependence	Low	High
Ligand	CD48	CD58 (LFA-3)
Affinity	Low	High
CD4 on macrophages	Absent	Present
EC present Ag to CD4+ T cells	No	Yes
CD5 and CD23 on B cells	Mutually exclusive expression	Co-expression
CD8 on DC	Present	Absent
CD28 expression on T cells	By 100% of $CD4^+$ and $CD8^+$ T cells	By 80% of $CD4^+$ and 50% of $CD8^+$ T cells
CD33 expression	Granulocytes	Monocytes
CD38 expression on B cells	Low on GC B cells, absent in plasma cells	High on GC B cells and plasma cells
CD40 on EC	Absent	Present
CD45 expressing cells	Purging extends graft survival	Purging does not extend graft survival
CD52 expression	Absent	Present
CD58 expression	Absent	Present
IL-10	Th2 cytokine	Th1 and Th2 cytokine
P-Selectin expression	Up-regulated by inflammatory mediators	Unresponsive to inflammatory mediators
TLR2 expression on PBL	Low (induced on many cells including T cells)	Constitutive (but not on T cells)
TLR3	Induced by LPS	Not induced by LPS
TLR10	Pseudogene	Highly expressed in lymphoid tissues
Hemotopoiesis in spleen	Continues into adulthood	Terminates prenatal
Hemotopoietic stem cells	c-kithigh	c-kitlow
Presence of Bronchus-associated Lymphoid Tissue (BALT)	Present	Absent in healthy tissue
Leukocyte defensins	Absent	Present on neutrophils
fMLP receptor affinity	Low	High
Fc RI	Absent	Present
Fc RIIA, C	Absent	Present
IL-13 effect on B cells	None	Induces switch to IgE
Thy 1 expression	Thymocytes, peripheral T cells	Absent from all T cells, yet expressed by neurones
Caspase 10	Absent	Present
IFN-α promotes Th1 differentiation	No	Yes
Th expression of IL-10	Th2	Th1 and Th2
GlyCAM	Present	Absent
MHC II expression on T cells	Absent	Present
Kv1.3 K channel on T cells	Absent	Present
MUC1 on T cells	Absent	Present
Granulysin	Absent	Present
Chemokine receptor CXCR1	Absent	Present
Chemokines:		
CXCL7, CXCL8, CXCL11, CCL13, CCL14, CCL15, CCL18, CCL23, CCL24/CCL26	All absent	All present
CCL6, CCL9, CCL12, CXCL15	All present	All absent
MRP-1/2, lungkine, MCP-5	Present	Absent
Passenger leukocytes	Account for graft immunogenicity	Do not account for graft immunogenicity

Ag = antigen, DC = dendritic cell, EC = endothelial cell, GC = germinal centre, LPS = lipopolysaccharide, N = neutrophil, PBL = peripheral blood leukocytes, Th = T helper cell, TLR = Toll-like receptor

Drug Discovery World Winter 2008/9

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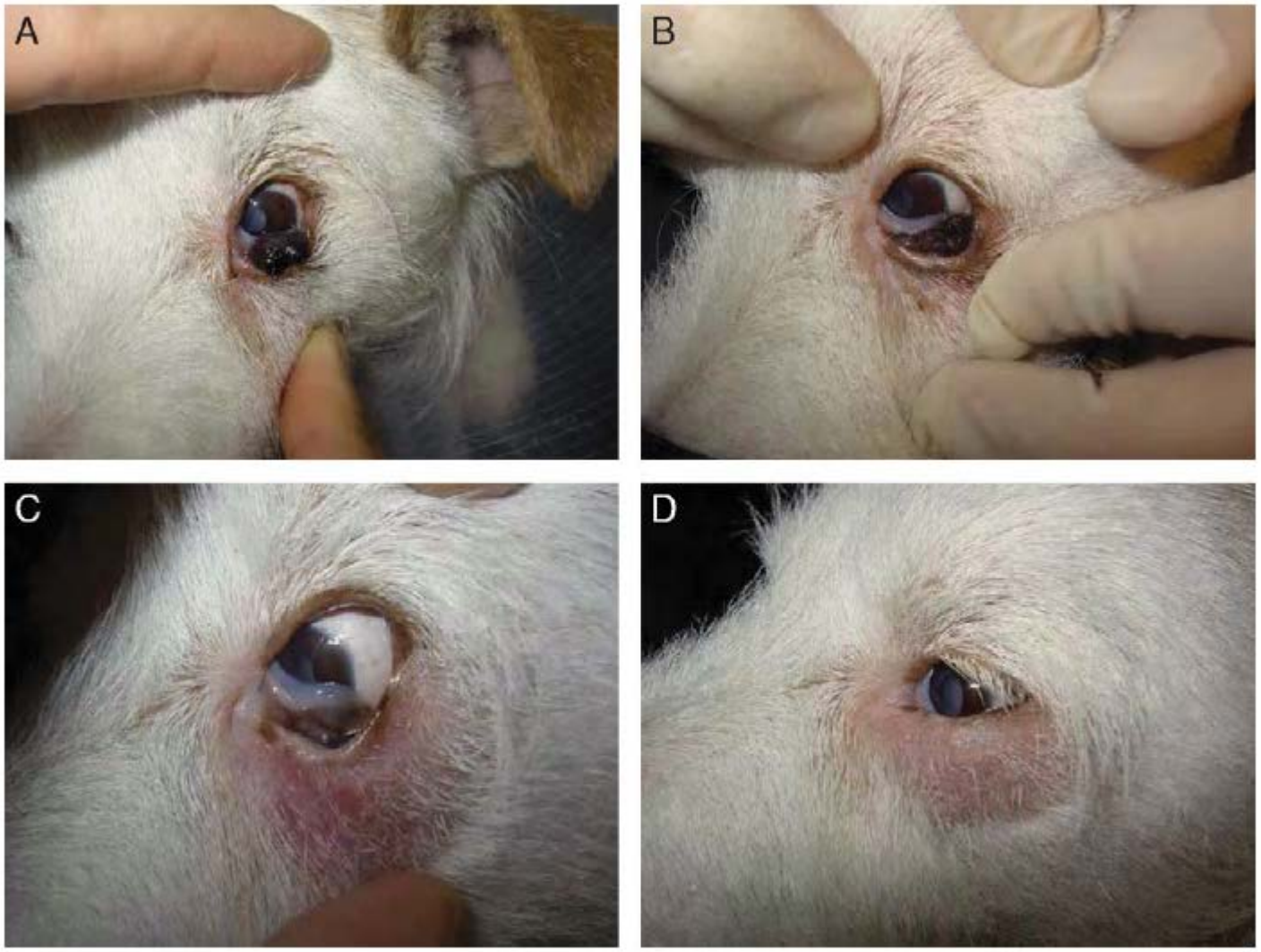
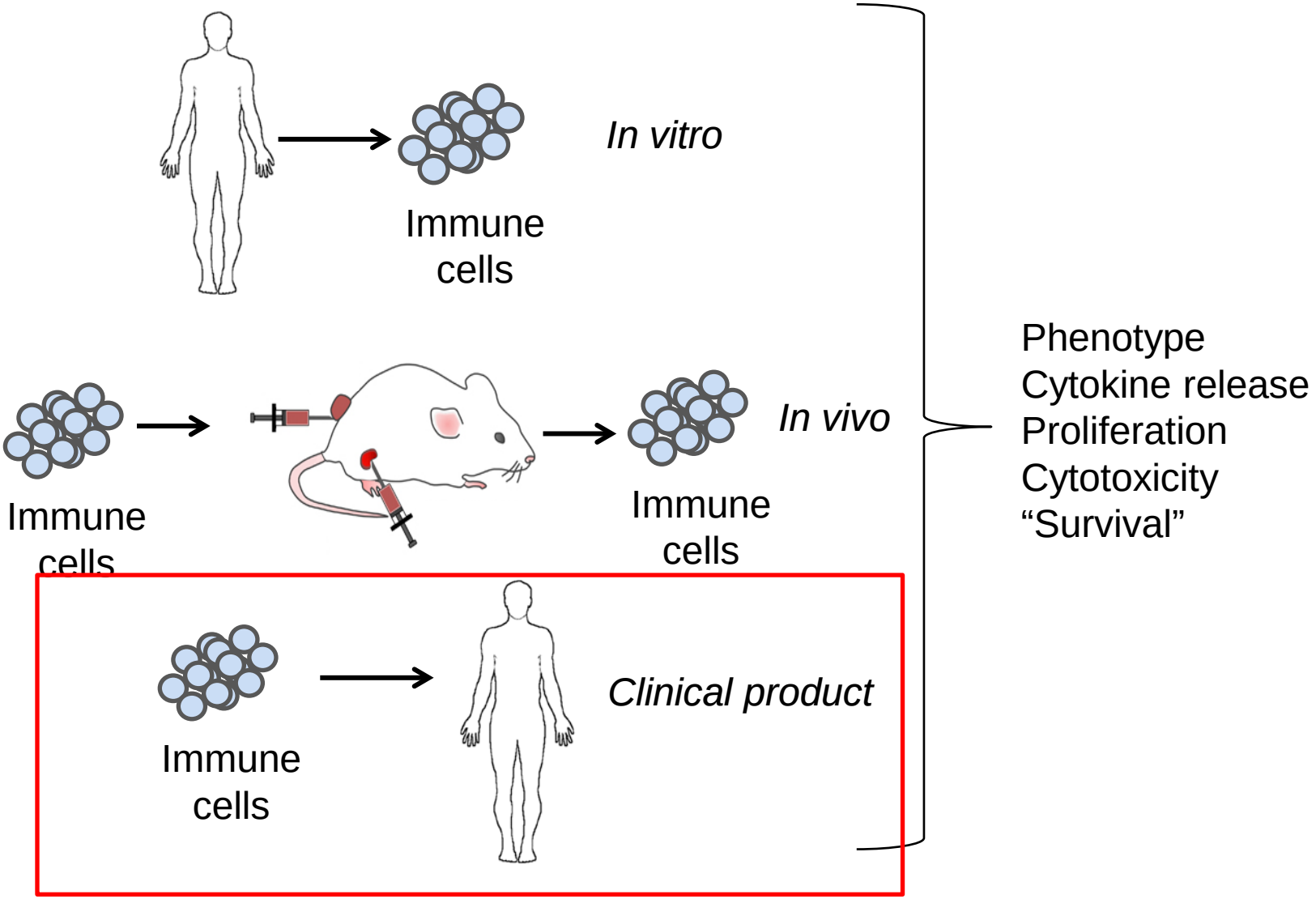
PD - Canine melanoma



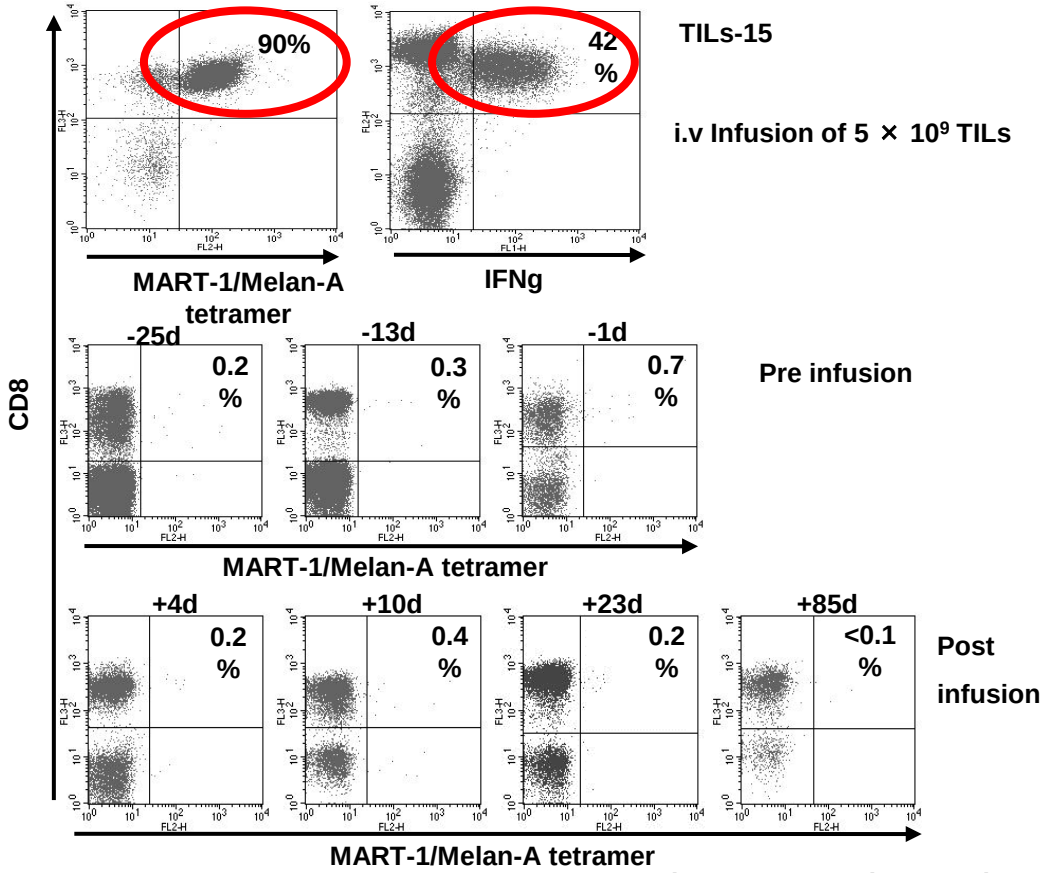
J Immunother 2008;31:377–384

PD models – proof-of-concept assays and bridging to assays used in the clinic



PD *In vitro* models – proof-of-concept assays and bridging to assays used in the clinic

Analysis of MART-1/Melan-A specific T cells, Pat 6



Carlsson B, Wagenius G and Tötterman TH J Immunother 2008

- Antigen-specific
- Reactive
- Patient pre-treated
- High cell dose

BUT

- Unable to detect after treatment
- No tumor response

ANALYSIS

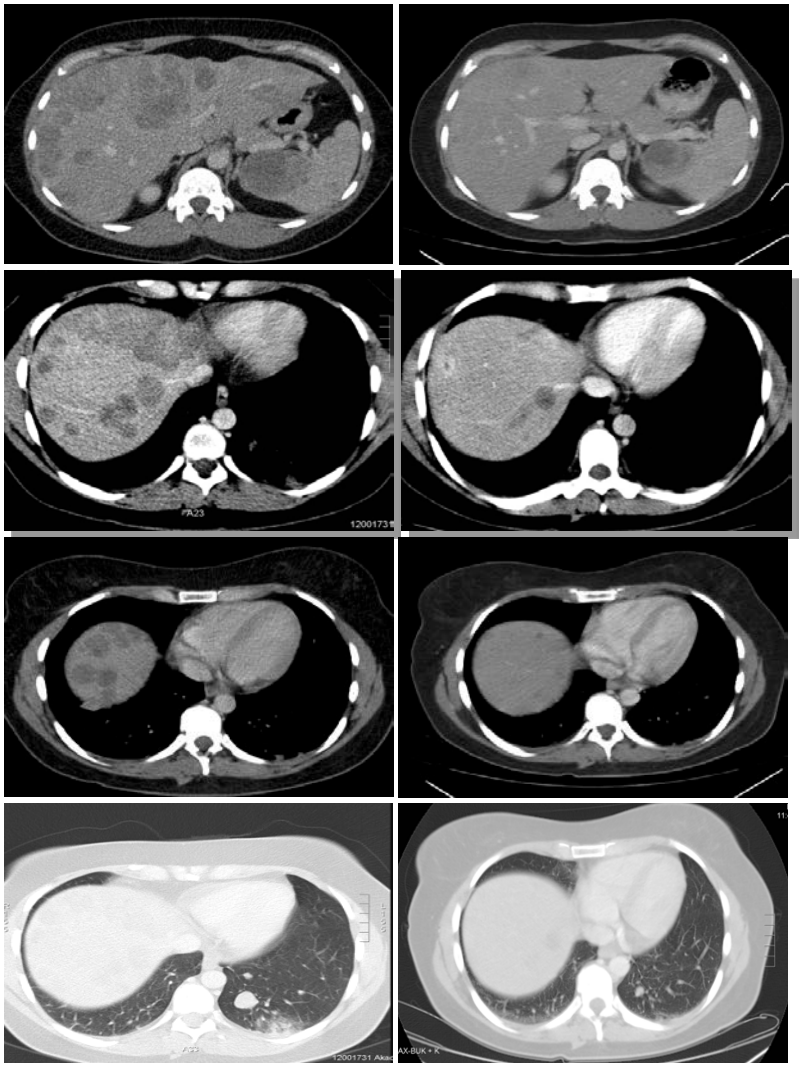
- Antigen expression in tumor unknown
- HLA-A02 expression in tumor unknown
- Tumor immune microenvironment unknown

CONSEQUENCE

- In vitro analysis is not truly predictable as to anti-tumor effects on a patient-basis
- Also TILs which are non-reactive in vitro might have clinical effect, they proliferate

PD *In vitro* models – proof-of-concept assays and bridging to assays used in the clinic

Patient 24



Non-antigen specific (with any available tool), i.e. “negative” potency assay

Patient pre-treated

High cell dose

Tumor-response

Ullenhag, JG. et al, Cancer immunology Immunotherapy, 2012

PD models – conclusions

- **Non-clinical models which generate clinically relevant PD data (in vitro and in vivo) are in many ways missing in comparison to models used for small molecules.**

  Ways forward;

  – Acknowledge the shortcomings and continue to develop products which have a probability of failing during clinical testing.
    - Such studies should be kept short and uncomplicated due to low predicted value.
  – Start using models which mimic the human disease more closely in regard to the tumor-immune system interactions.
  – Extend the clinical data in regard to “immune pathology” and efficacy (or lack thereof).

  Developers should consider, given the bureaucracy, cost and time associated with conducting clinical trials, utilizing preclinical in vivo models that can more accurately model tumor immunity and allow more informed assessment of intended therapies.

Pharmacokinetics

- **Biodistribution, extensive including the CNS.**
- **Persistence, cells will/can persist for a very long time.**

Risks - Immunogenicity - Safe CARs/TCRs?

Cancer Regression and Neurological Toxicity Following Anti-MAGE-A3 TCR Gene Therapy

Richard A. Morgan, Nachimuthu Chinnasamy,* Daniel Abate-*
Paul F. Robbins, Zhili Zheng,* Mark E. Dudley,* Steven A. Fela*
Richard M. Sherry, Giao Q. Phan,* Marybeth S. Hughes,* Udai S. Ka*
Crystal J. Hessman, Ashley A. Stewart,* Nicholas P. Restifo,**
Meghna Alimchandani,† Avi Z. Rosenberg,† Avindra Nath,‡
Bibiana Bielekova,‡ Simone C. Wuest,‡ Nirmala Akula,§ Francis J. M
*Barbara Mosetter,‖ Dolores J. Schendel,‖¶ Carolyn M. Laurencot,**

Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced With a Chimeric Antigen Receptor Recognizing *ERBB2*

Richard A Morgan[1], James C Yang[1], Mio Kitano[1], Mark E Dudley[1], Carolyn M Laurencot[1] and Steven A Rosenberg[1]

[1]*Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

Cardiovascular toxicity and titin cross-reacti T cells in myeloma and melanoma

Gerald P. Linette,[1] Edward A. Stadtmauer,[2] Marcela V. Maus,[2] Aaron P. Rapoport,[3] Bruce L. Levine,[2] Lyndsey Emery,[2] Leslie Litzky,[2] Adam Bagg,[2] Beatriz M. Carreno,[1] Patrick J. Cimino,[1] Gwendolyn K. Binder-Scholl,[4] Dominic P. Smethurst,[4] Andrew B. Gerry,[4] Nick J. Pumphrey,[4] Alan D. Bennett,[4] Joanna E. Brewer,[4] Joseph Dukes,[5] Jane Harper,[5] Helen K. Tayton-Martin,[4] Bent K. Jakobsen,[4,5] Namir J. Hassan,[5] Michael Kalos,[2] and Carl H. June[2]

[1]Siteman Cancer Center and Departments of Medicine and Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; [2]Abramson Cancer Center, Department of Medicine, and Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; [3]The Greenebaum Cancer Center, University of Maryland, Baltimore, MD; [4]Adaptimmune Ltd, Philadelphia and Abingdon, United Kingdom; and [5]Immunocore Ltd, Abingdon, United Kingdom

Toxicity

- CNS
- Cardiovascular
- Respiratory
- Cytokine storm *vs* anti-tumoral effect *vs* fatal toxicity *vs* off-target toxicity

Risks - Immunogenicity - Safe CARs/TCRs?

- **Toxicity/safety studies**
  - Using human immune cells in animals is irrelevant in terms of safety assessment due to;
    - MHC barrier
    - Xenogeneic barrier
    - Target specificity
  - Homologous products for *in vivo* testing
    - Always difficult to compare to the human product
    - Especially when using autologous products
  - Relevant in vitro safety assays?
    - Tissue reactivity screening?
    - HLA/TCR matching?
    - Sensitivity?

Discussion - Safety studies/methods

What methods (in vitro, in vivo) do we have available to gain more relevant safety data on genetically modified effector cells before first-time in man?

- In vivo?
  - Antibodies are normally safety-tested in NHP = NHP CAR?
  - Homologous murine CARs?
- In vitro
  - Tissue reactivity screening?
  - MHC/TCR matching?
  - Sensitivity?

For increased safety should all new products include a suicide construct?

- How fast can such a construct act in relation to the clinical fatalities (a few days after treatment)?

How can non-clinical data support a safe dose-selection?

- Activated cells will proliferate.