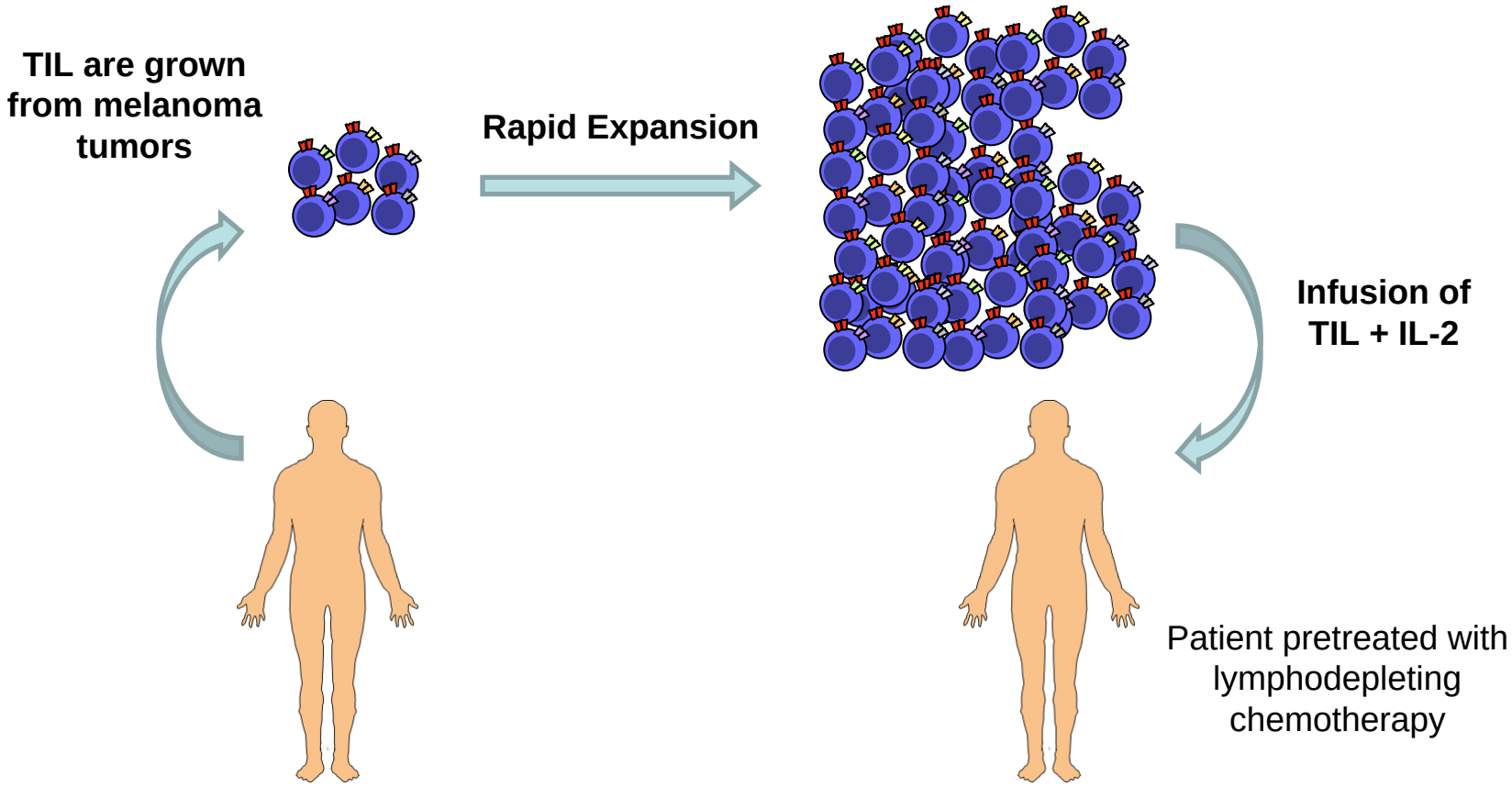
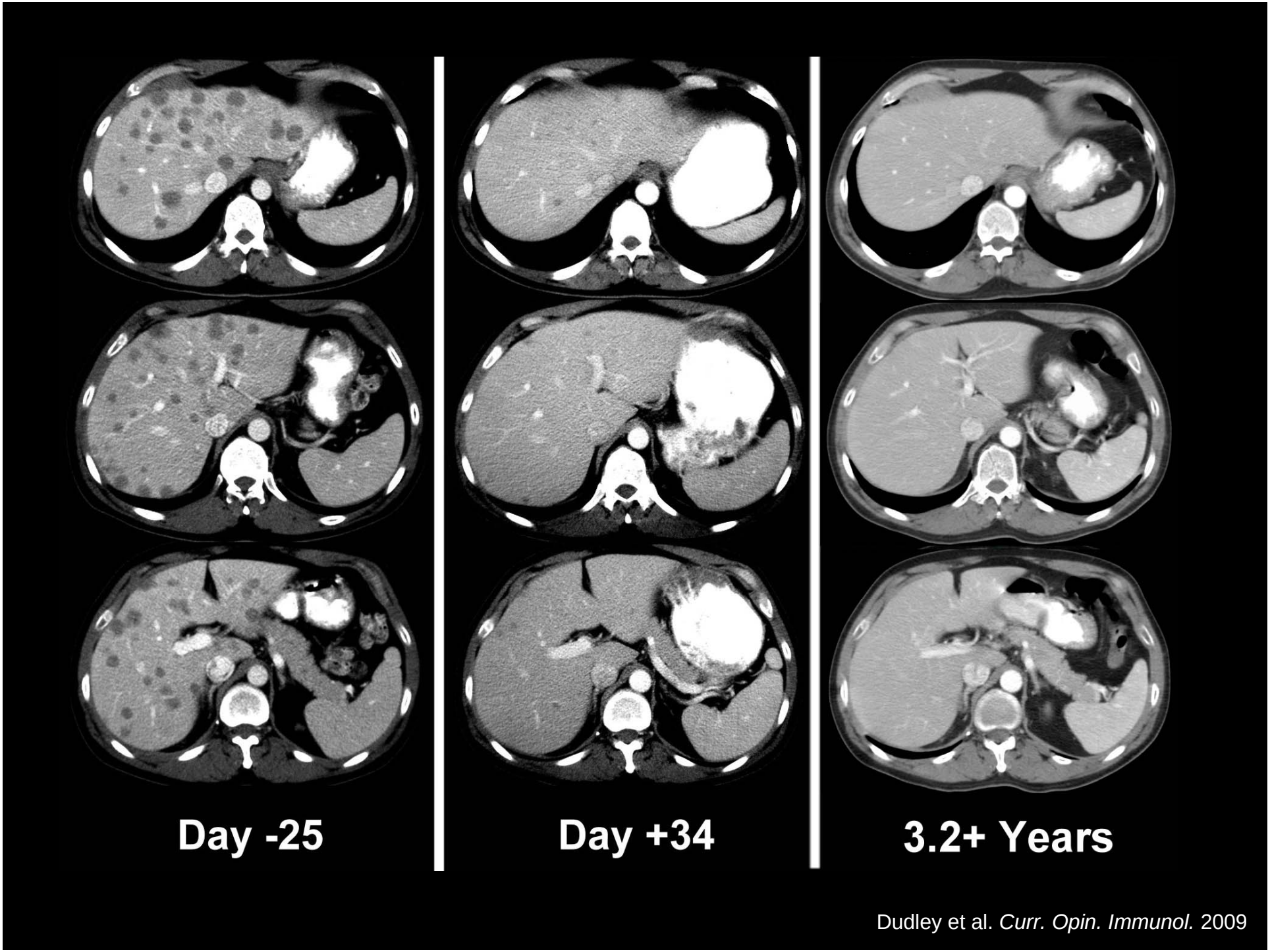
Dissecting therapy-induced T-cell responses in melanoma

NKI-AVL

Tumor-infiltrating lymphocyte (TIL) therapy of melanoma

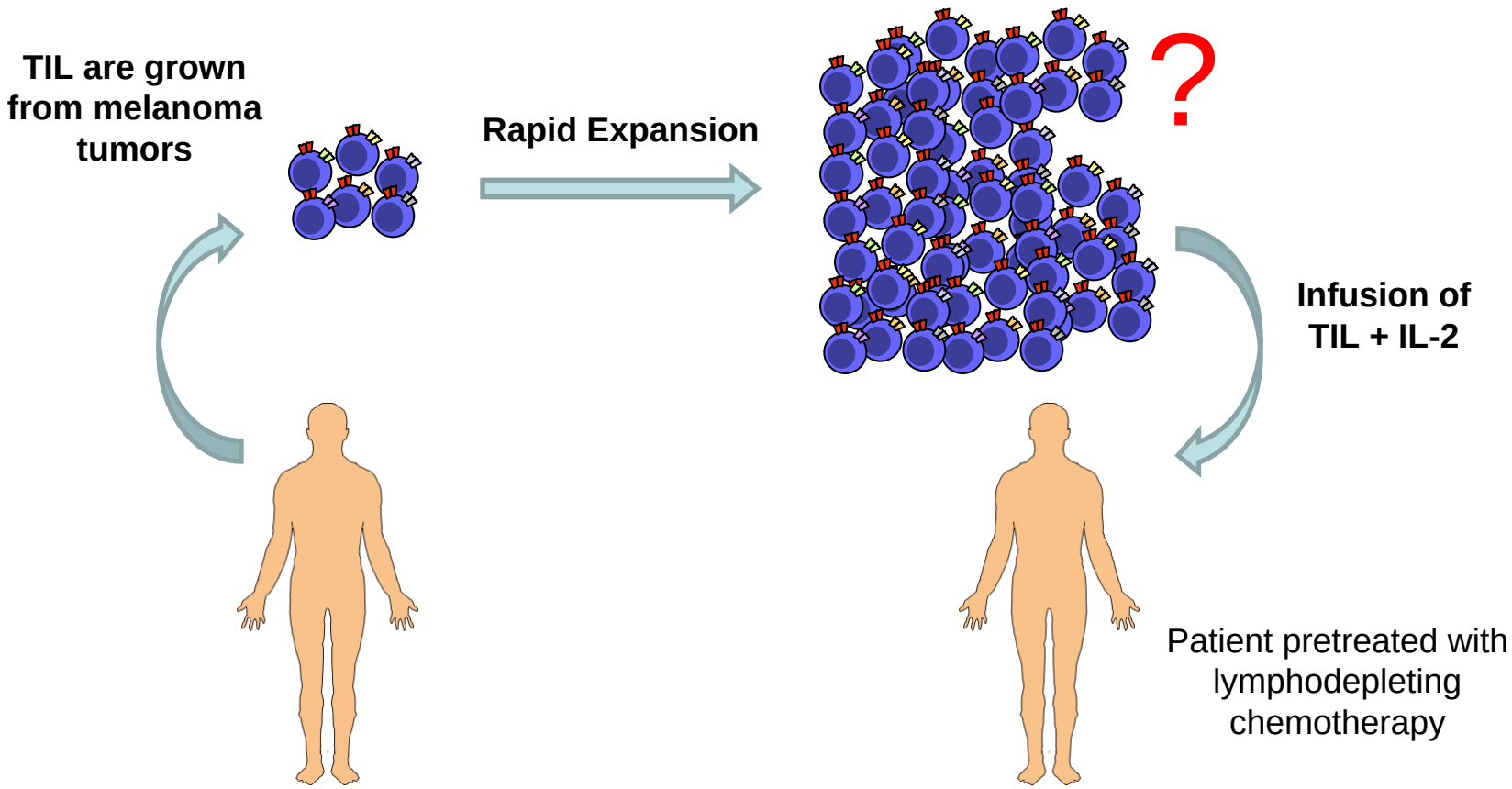


- 50% response rate in trials in multiple centers (US, Israel)
- Clinical effect at least partially mediated by cytotoxic T cells



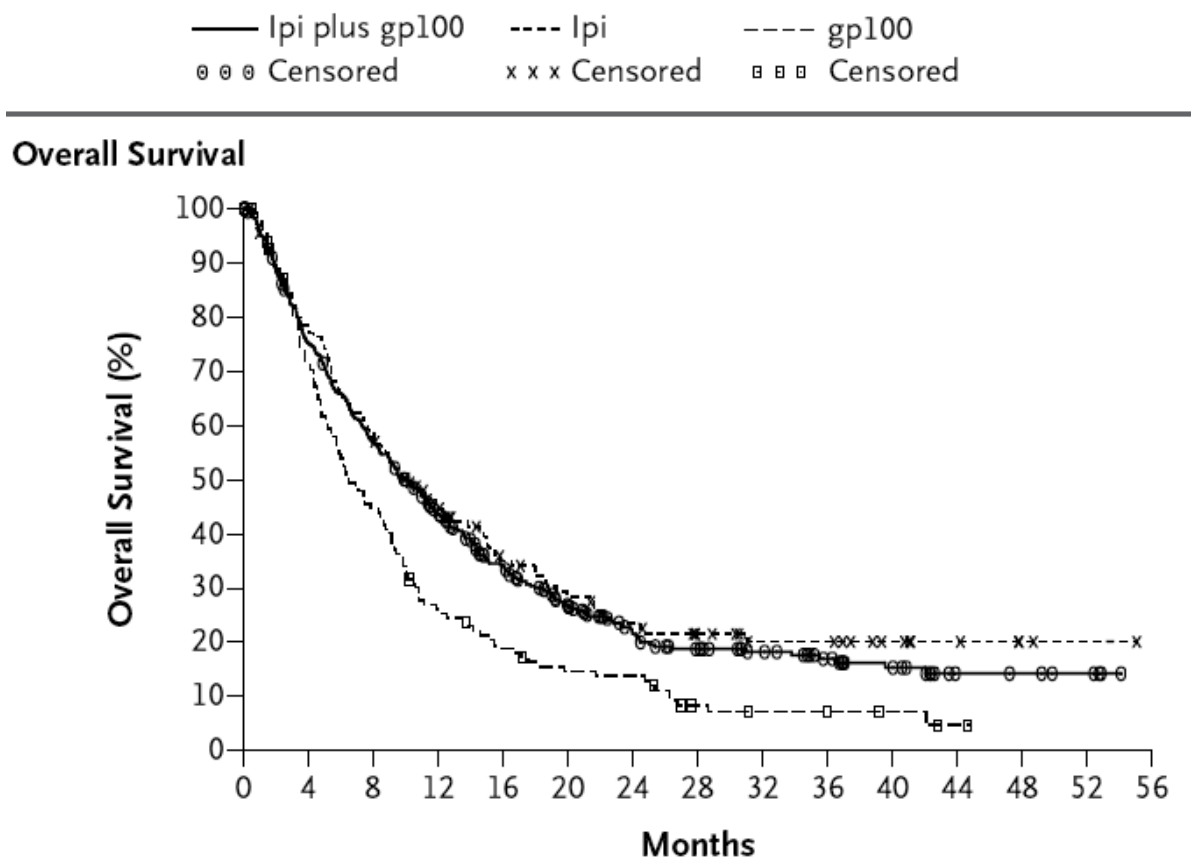
Dudley et al. *Curr. Opin. Immunol.* 2009

Tumor-infiltrating lymphocyte (TIL) therapy of melanoma



- Which cytotoxic T cells mediate cancer regression?
  - Could we specifically boost their numbers?

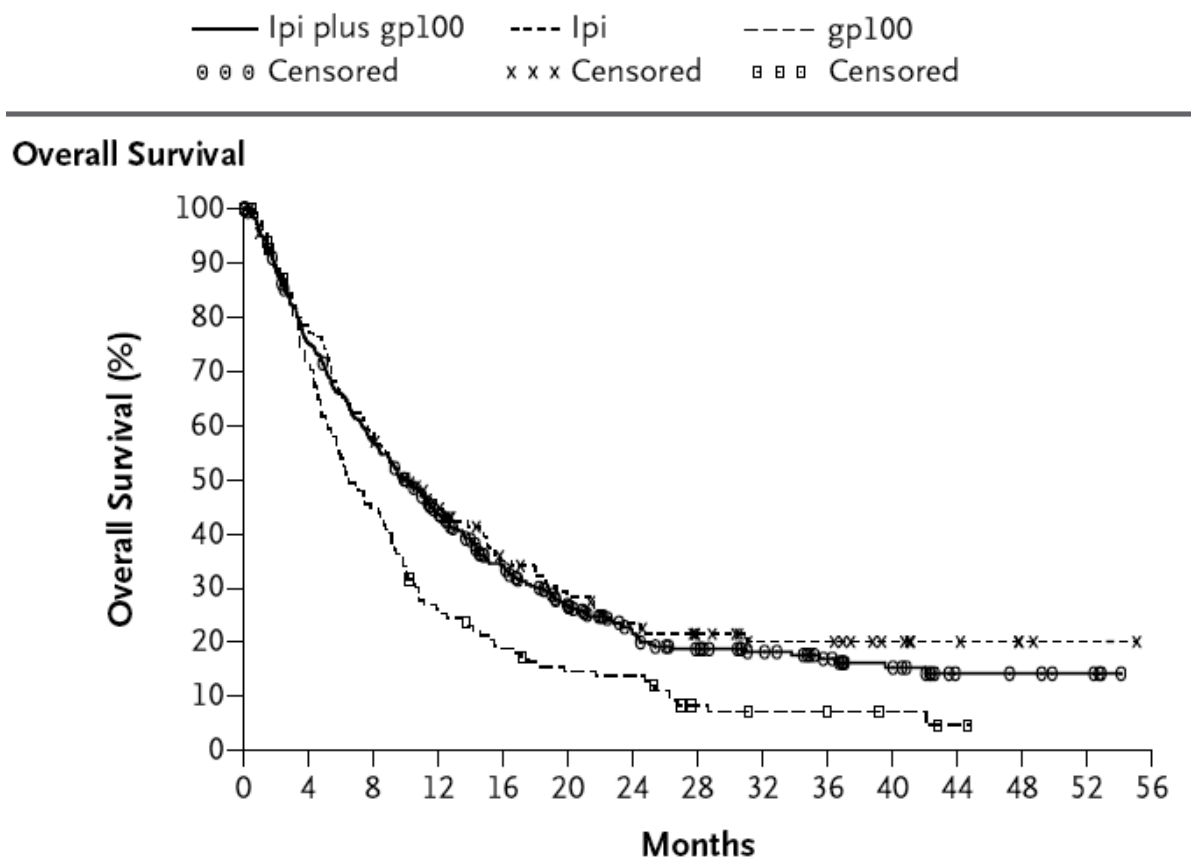
Anti-CTLA4 therapy of melanoma



- Survival benefit in metastatic disease
- Data from murine models suggest a role for cytotoxic T cells

Hodi et al. *NEJM* 2010

Anti-CTLA4 therapy of melanoma

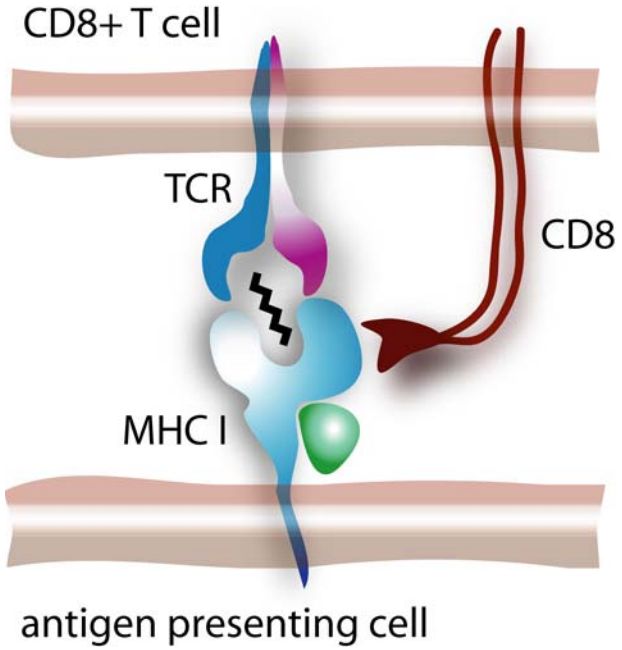


- Which cytotoxic T cells mediate cancer regression?
  - Could we specifically boost their numbers?
- Could we distinguish responders and non-responders?

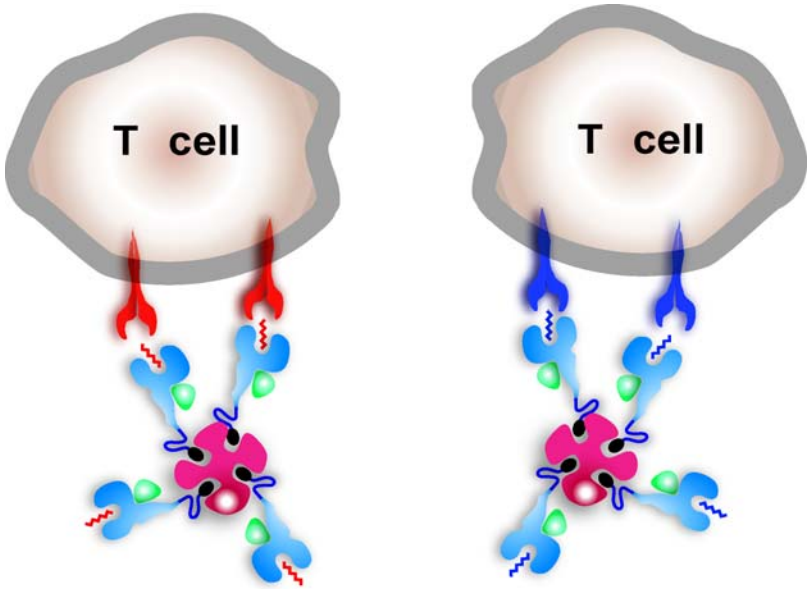
Hodi et al. *NEJM* 2010

Detecting tumor-specific cytotoxic T cells

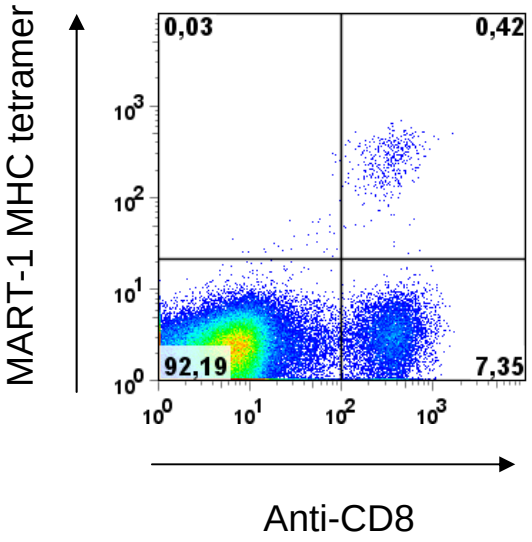
How a T cell sees a target cell



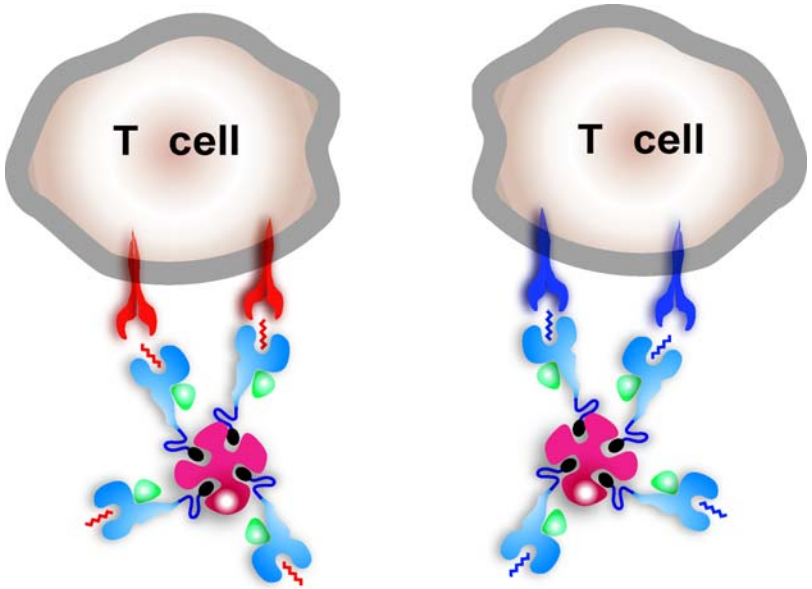
How an MHC tetramer sees a T cell



Detecting tumor-specific cytotoxic T cells

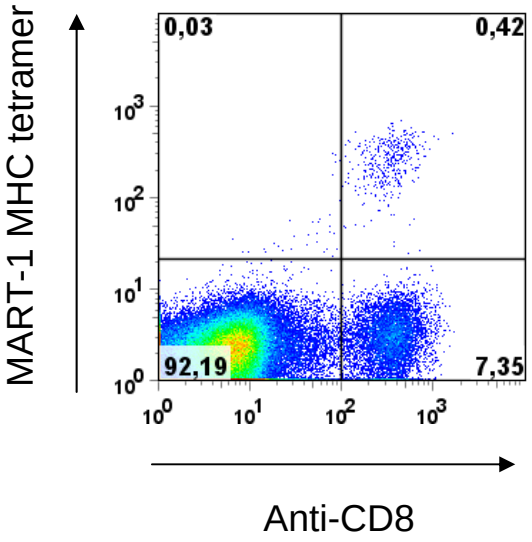


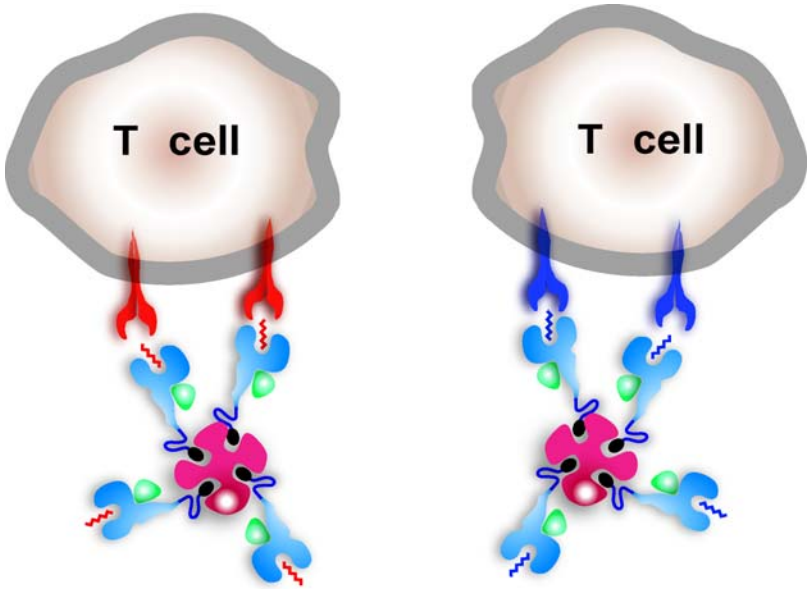
How an MHC tetramer sees a T cell



Detecting tumor-specific cytotoxic T cells

How an MHC tetramer sees a T cell



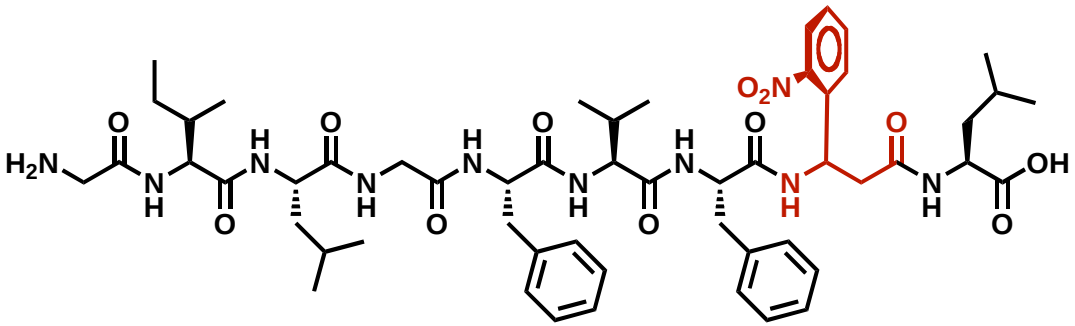


Hundreds of different melanoma-associated T cell antigens have been described

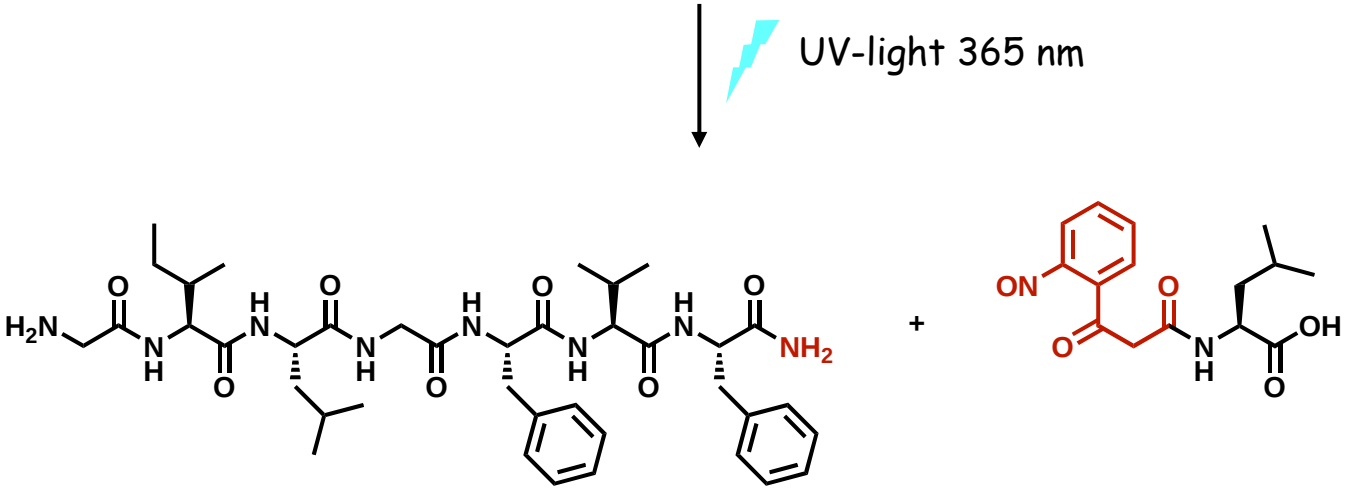
Two problems:

1. Generation of very large collections of MHC tetramer reagents is not possible with standard technology
2. Even if you would have such a collection, the amount of biological material is limiting

Generation of pMHC multimers by UV-induced peptide exchange

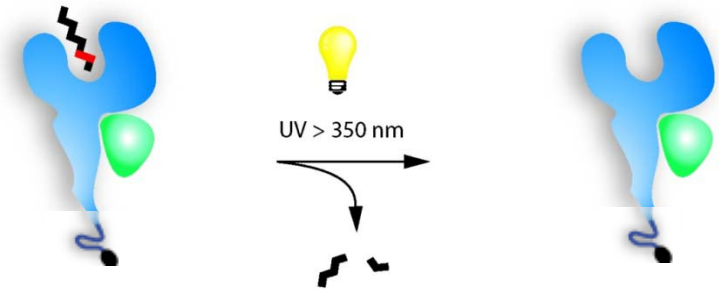


GILGFVF(*o*-NO_2)L



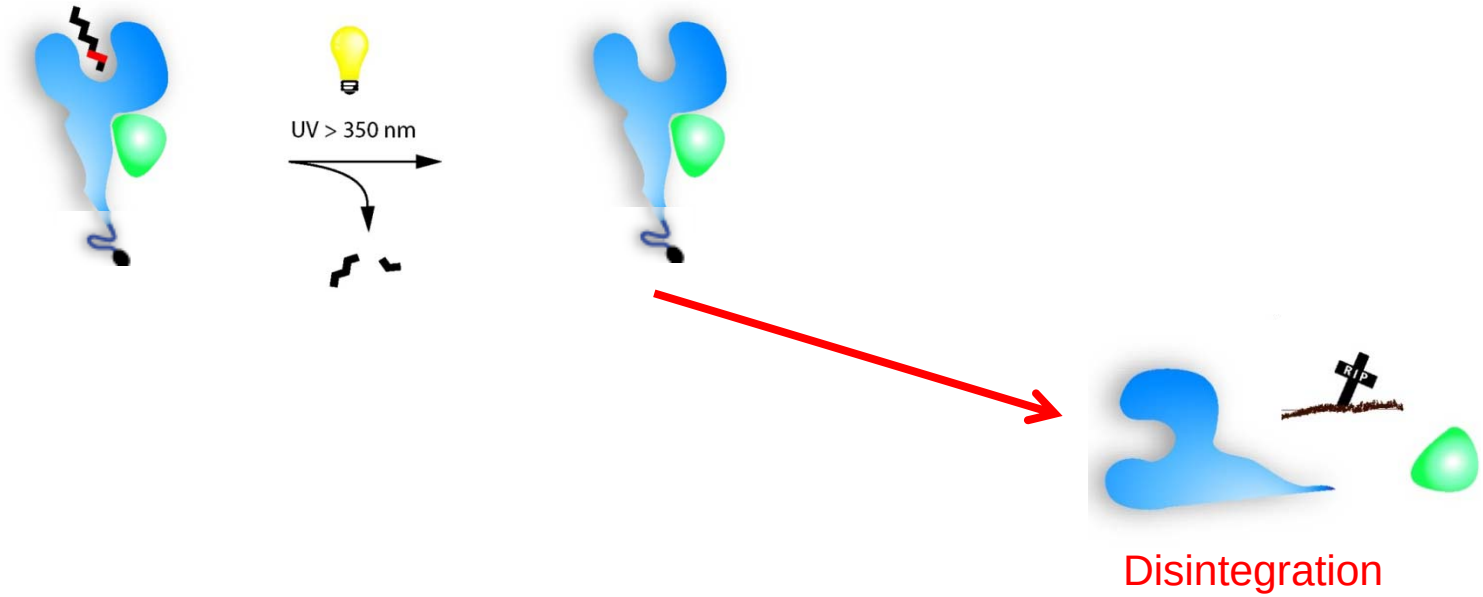
Toebes et al. *Nat. Med.* 2006
Bakker et al. *PNAS* 2008

Generation of pMHC multimers by UV-induced peptide exchange



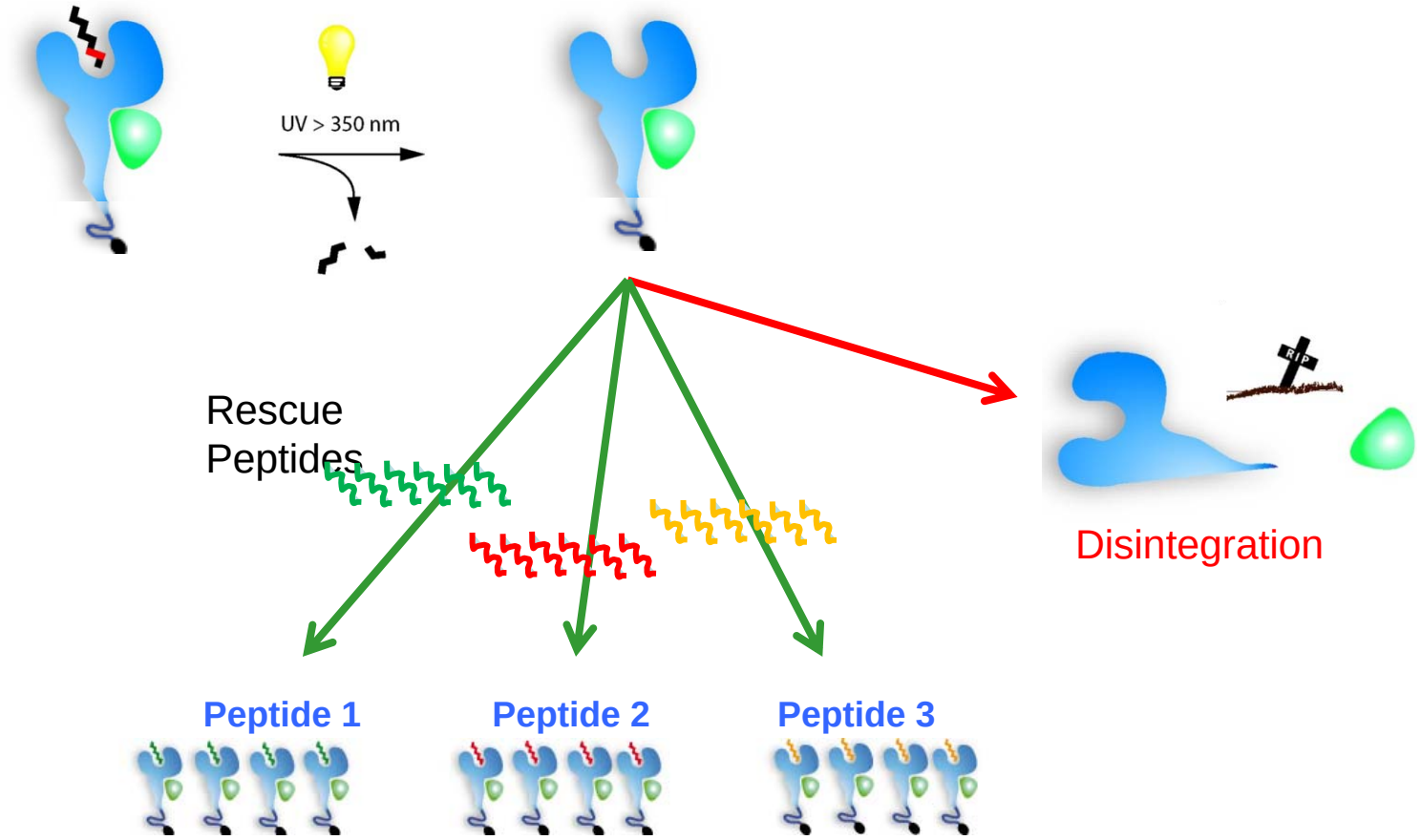
Toebes et al. *Nat. Med.* 2006
Bakker et al. *PNAS* 2008

Generation of pMHC multimers by UV-induced peptide exchange

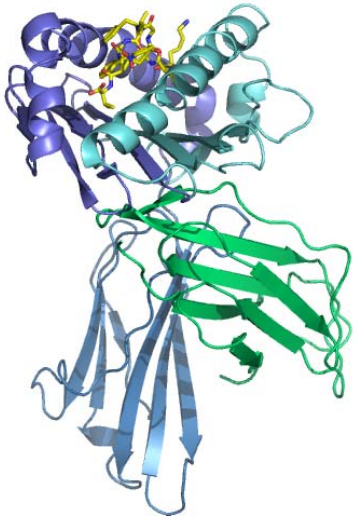


Toebes et al. *Nat. Med.* 2006
Bakker et al. *PNAS* 2008

Generation of pMHC multimers by UV-induced peptide exchange



In crystallo MHC ligand exchange

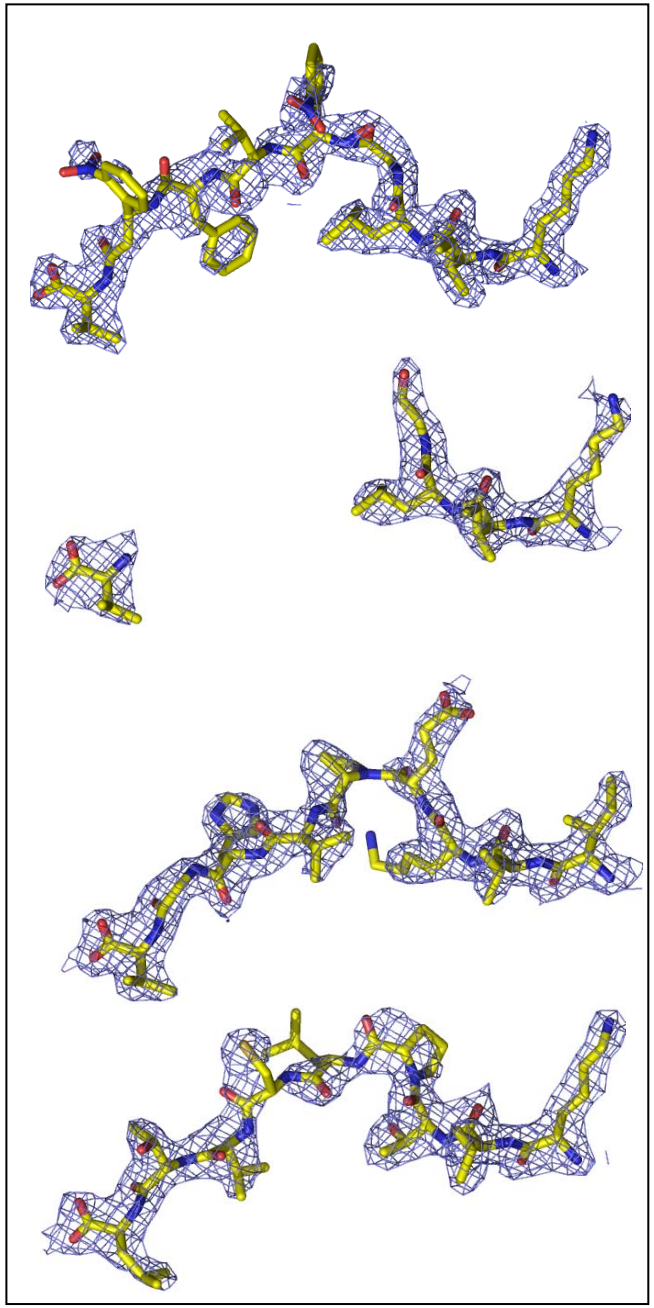


UV-sensitive ligand pre-cleavage
(KILGJ*VFJV)

UV-sensitive ligand post-cleavage
(visualization of the reaction intermediate)

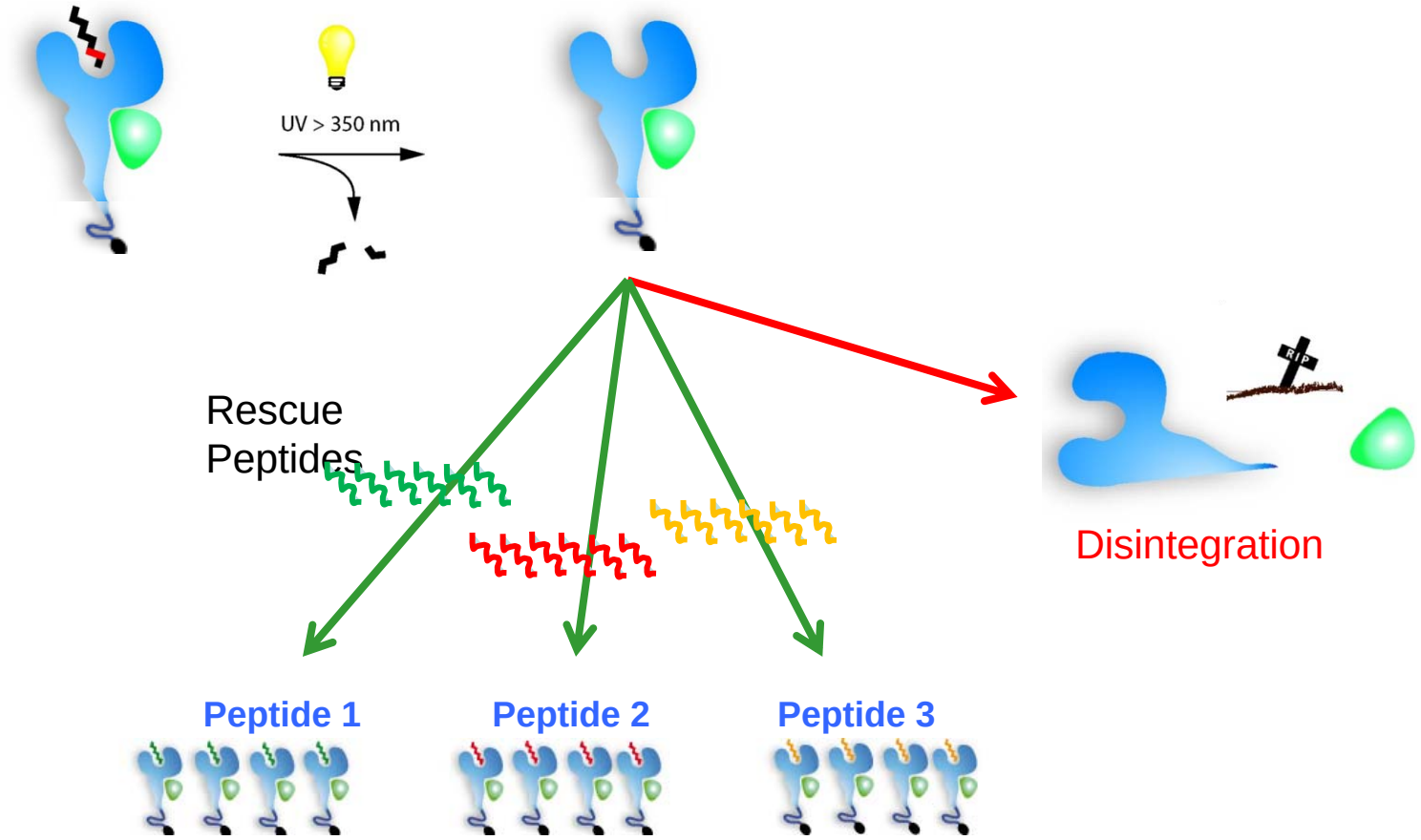
In crystallo exchange with HIV $RT_{468\text{-}476}$
(ILKEPVHGV)

In crystallo exchange with HIV $Env_{120\text{-}128}$
(KLTPLCVTL)



Celie et al. *JACS* 2009

Generation of pMHC multimers by UV-induced peptide exchange



Allows the creation of very large MHC multimer collections for all prevalent HLA class I alleles

- HLA-A1, -A2, -A3, -A11, -B7, -B57 (Toebes et al. *Nat. Med.* 2006, Bakker et al. *PNAS* 2008)
- HLA-A24, -B40, -B58 (G. Grotenbreg, NUS, Singapore)

How to monitor hundreds of T cell populations per patient?

Aim: Detection of low frequency T cell populations

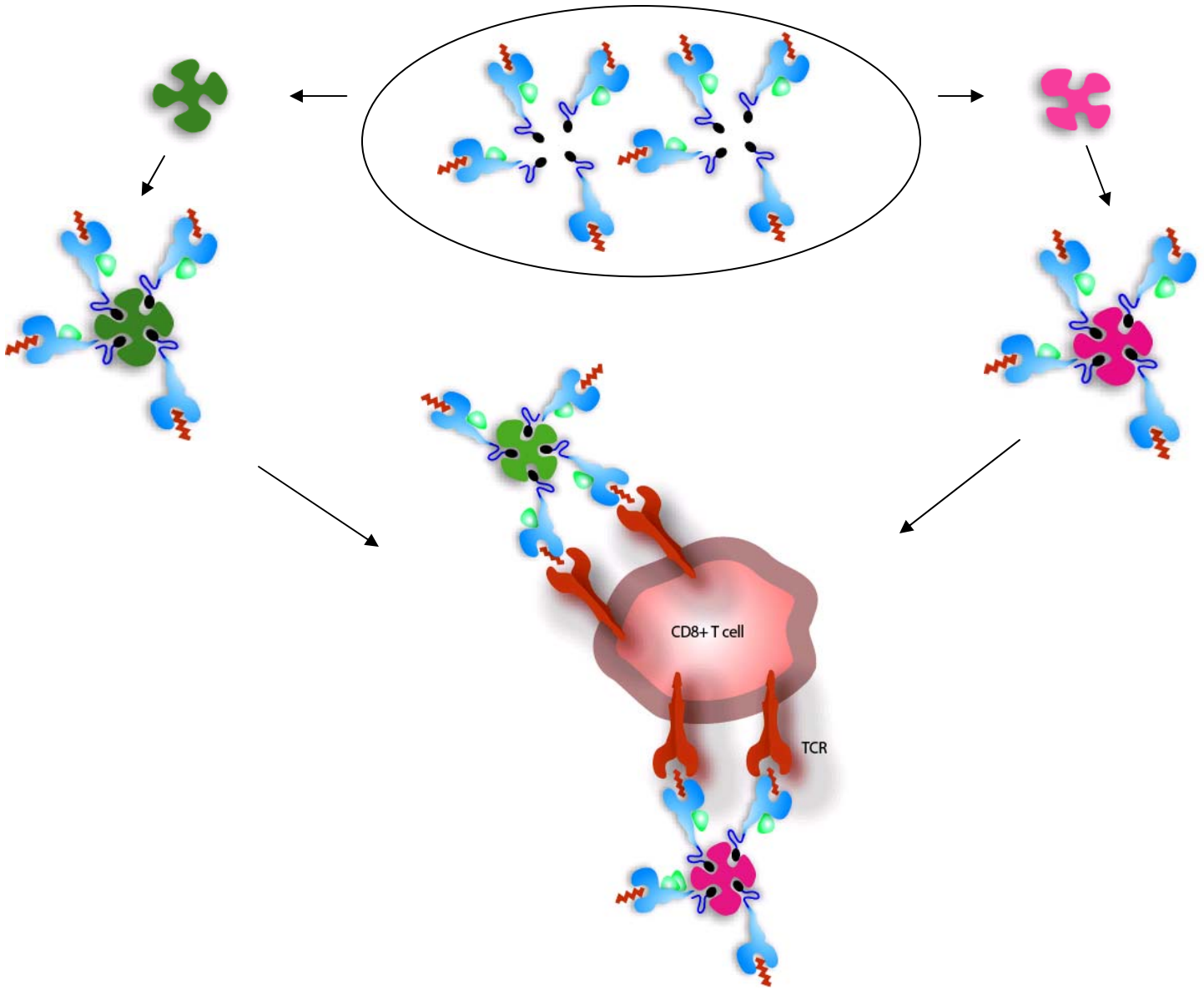
~ 0.02% of CD8 T cells

Required: record >50,000 CD8 T cells ~ 2 ml fresh blood

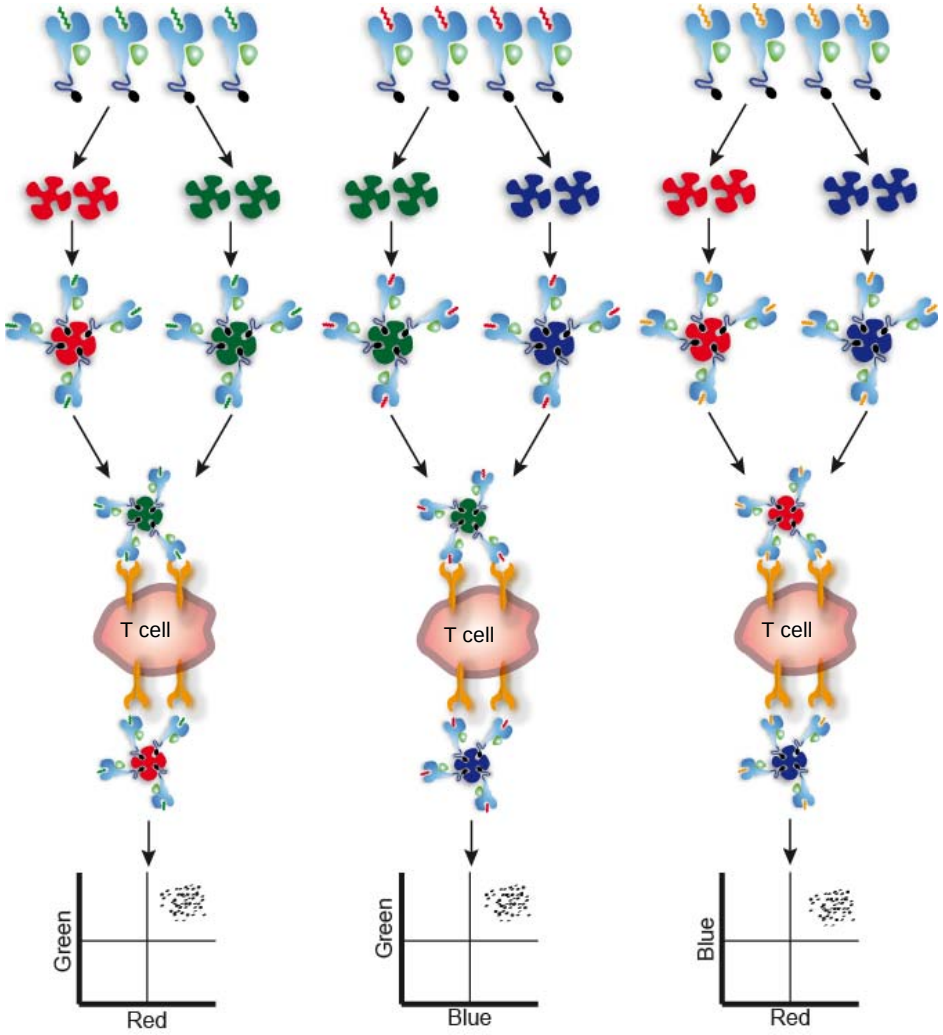
For 100 potential antigens: ~ 200 ml blood

25 parallel measurements per sample: ~ 8 ml blood

What if you couple the same pMHC to multiple fluorophores & start combining?



Self-assembling molecular codes



Generate fluorochrome conjugated MHC multimers

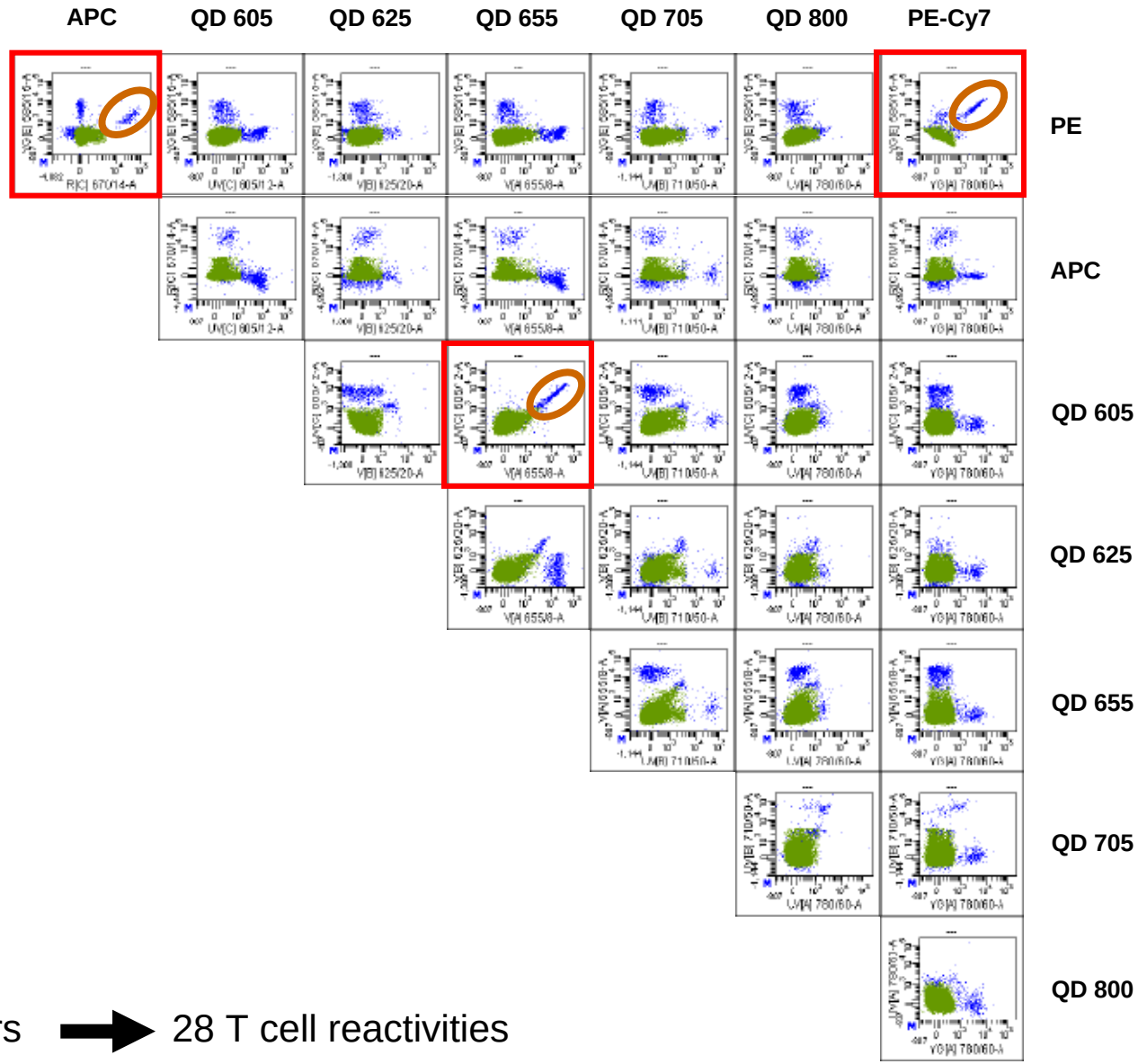
Mix to create a collection of differentially encoded MHC multimers

Assembly of combinatorial codes on T cell surfaces

Analysis by flow cytometry

Hadrup et al. *Nat. Methods* 2009

Example of a combinatorial coding read-out



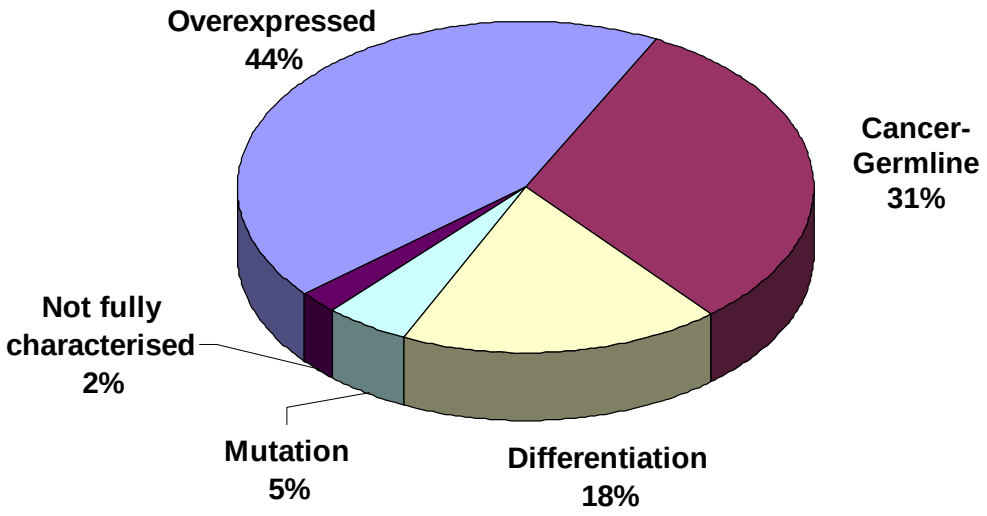
8 colors → 28 T cell reactivities

Selection of melanoma-associated epitope panel

Panel of 216 melanoma-associated peptides

- public databases
- literature analysis

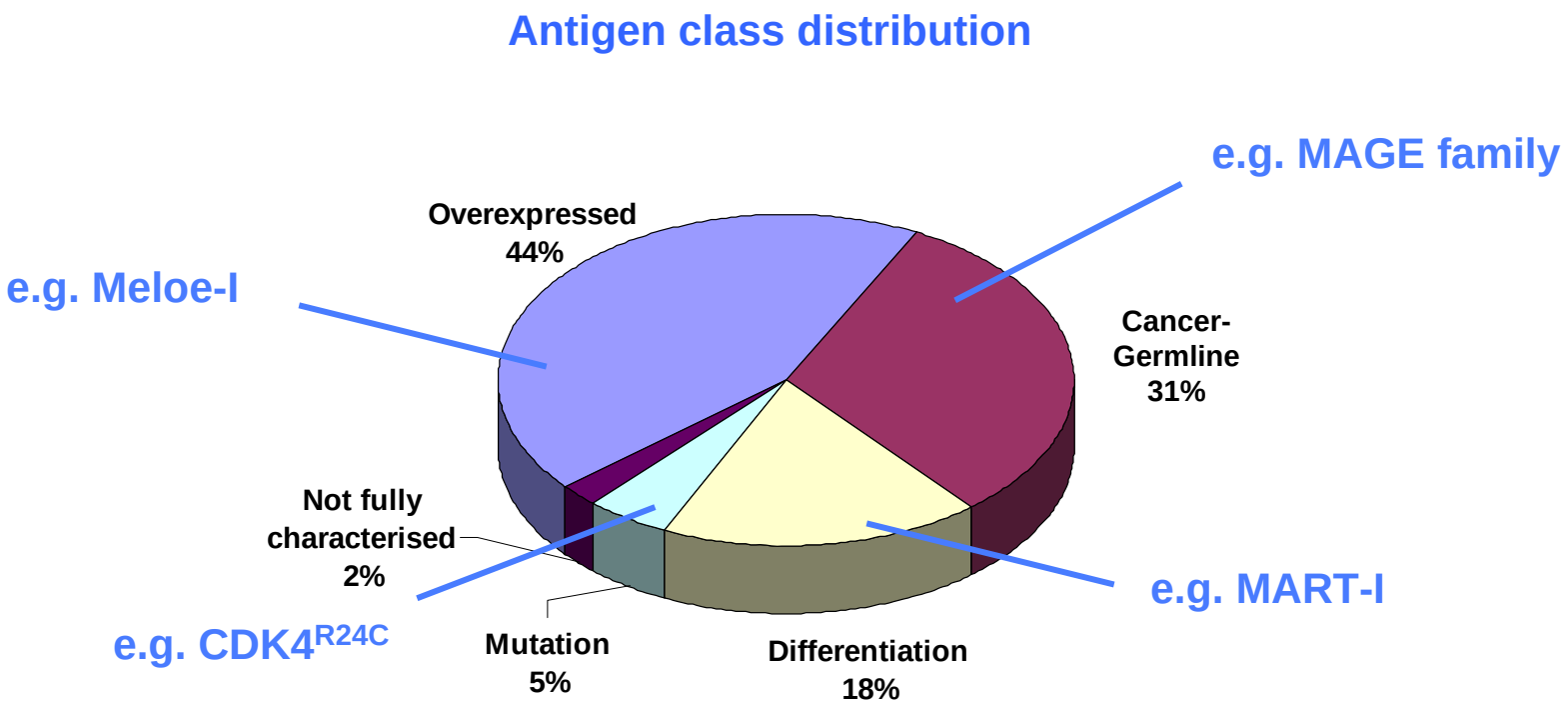
Antigen class distribution



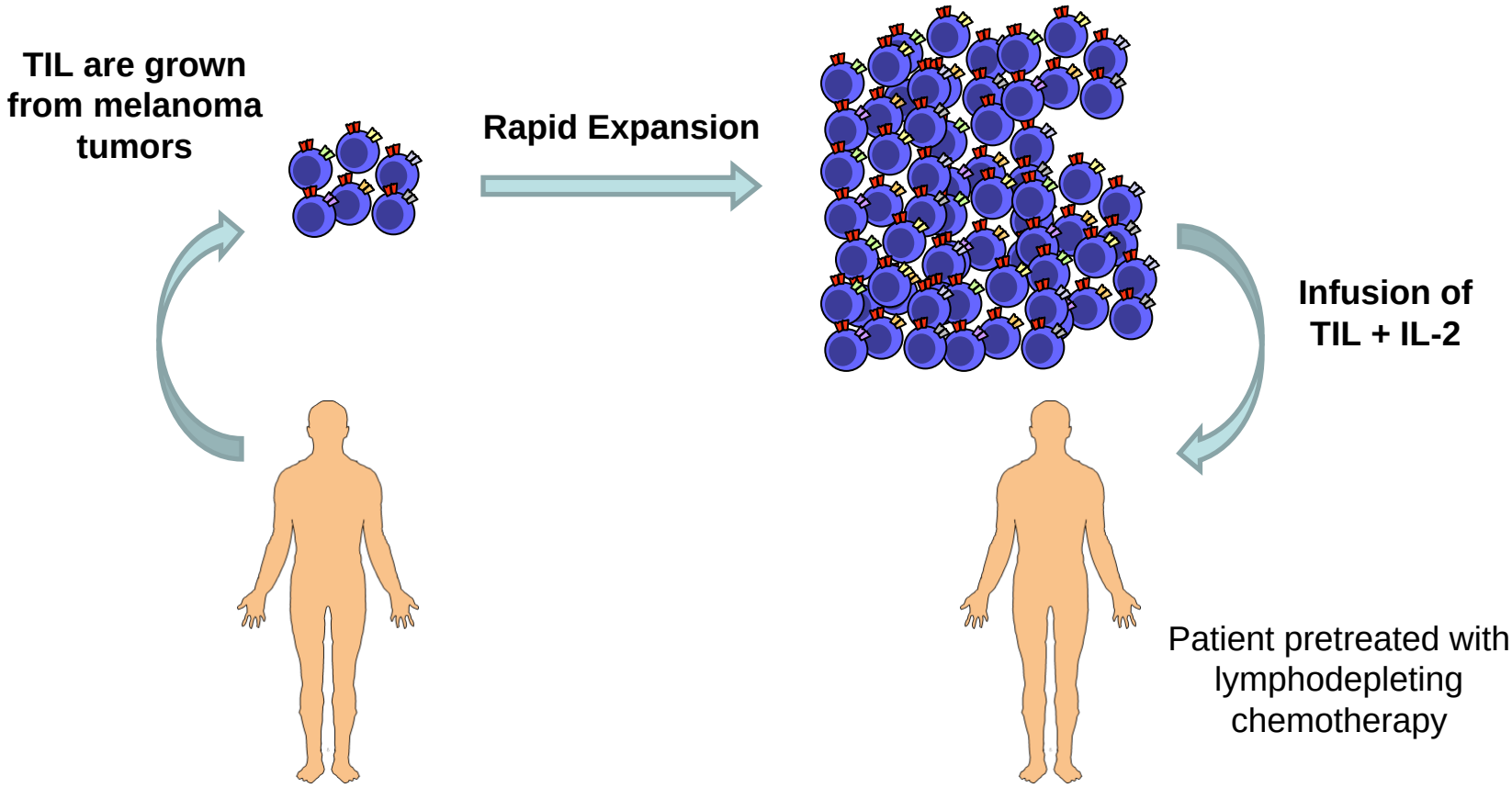
Selection of melanoma-associated epitope panel

Panel of 216 melanoma-associated peptides

- public databases
- literature analysis



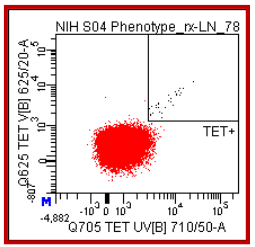
Dissecting therapy-induced T cell responses in melanoma: POC



- Do we detect tumor-specific T cell responses in the cell product?
- If so, what do these T cells recognize?
- Does composition of the cell product predict post-treatment immune reactivity?

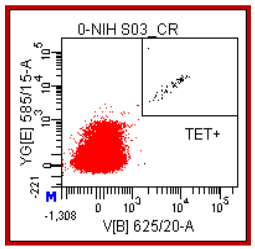
Identification of diverse T cell populations in HLA-A2^{+} TIL products

pt.LN



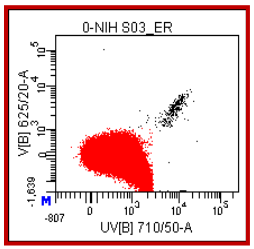
MART-1 (#78)
(0.087%)
ELAGIGILTV

pt.CR



MART-I (#78)
(0.113%)
ELAGIGILTV

pt.ER

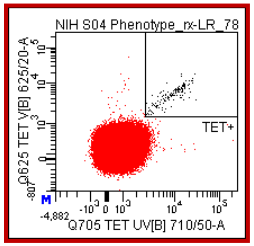


MART-I (#78)
(0.074%)
ELAGIGILTV

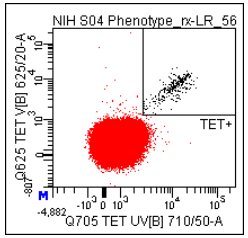
Numbers represent the % MHC tetramer^{+} T cells out of total CD8^{+} cells

Identification of diverse T cell populations in HLA-A2^{+} TIL products

pt.LR

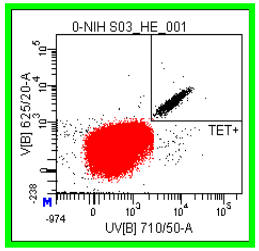


MART-1 (#78)
(0.018%)
ELAGIGILTV

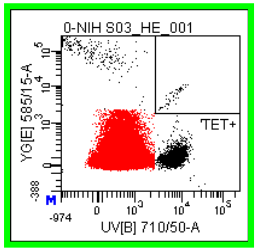


gp100 (#56)
(0.045%)
IMDQVPFSV

pt.HE

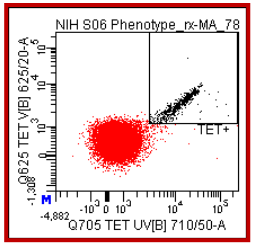


SSX-2 (#114)
(0.628%)
KASEKIFYV

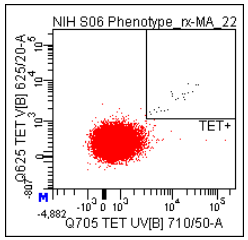


MAGE-A10 (#59)
(0.015%)
GLYDGMEHL

pt.MA

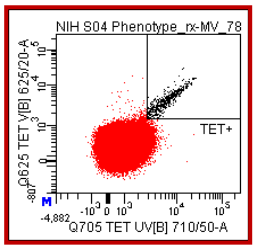


MART-1 (#78)
(4.581%)
ELAGIGILTV

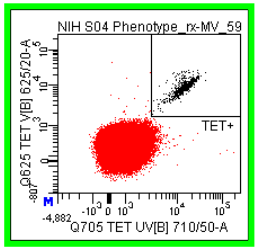


gp100 (#56)
(0.071%)
IMDQVPFSV

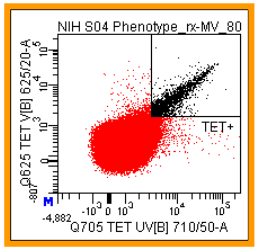
pt.MV



MART-1 (#78)
(0.150%)
ELAGIGILTV



MAGE-A10 (#59)
(0.137%)
GLYDGMEHL

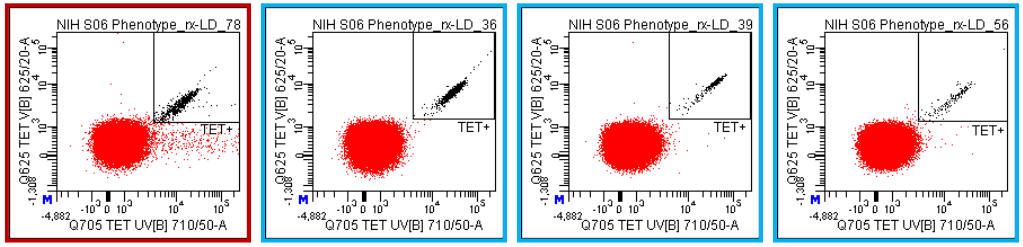


Meloe-1 (#80)
(0.344%)
TLNDECWPA

Numbers represent the % MHC tetramer^{+} T cells out of total CD8^{+} cells

Identification of diverse T cell populations in HLA-A2^{+} TIL products

pt.LD



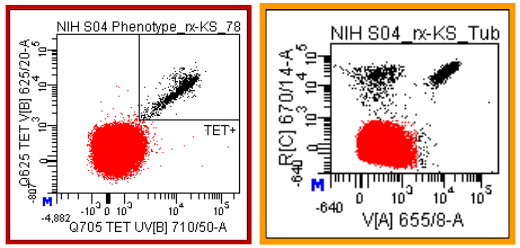
MART-1 (#78)
1.210%
ELAGIGILTV

Gp100 (#36)
1.897%
YLEPGPVTA

Gp100 (#39)
0.131%
VLYRYGSFSV

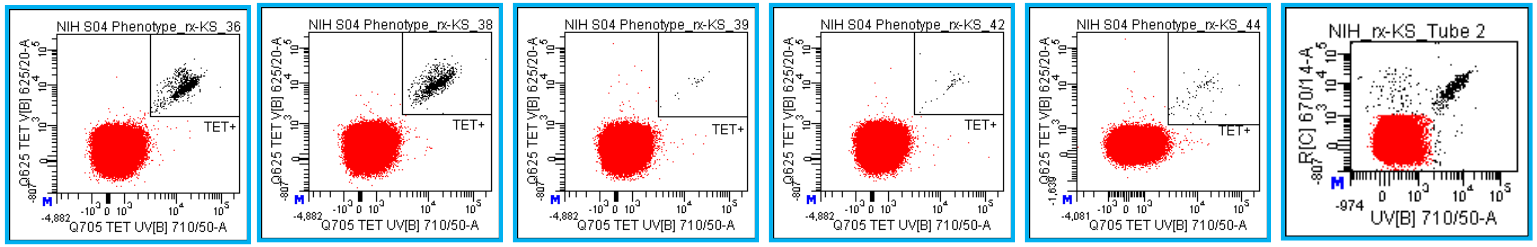
Gp100 (#56)
0.083%
IMDQVPFSV

pt.KS



MART-1 (#78)
0.633%
ELAGIGILTV

Meloe-1 (#80)
(1.45%)
TLNDECWPA



gp100 (#36)
0.453%
YLEPGPVTA

gp100 (#38)
0.376%
ITDQVPFSV

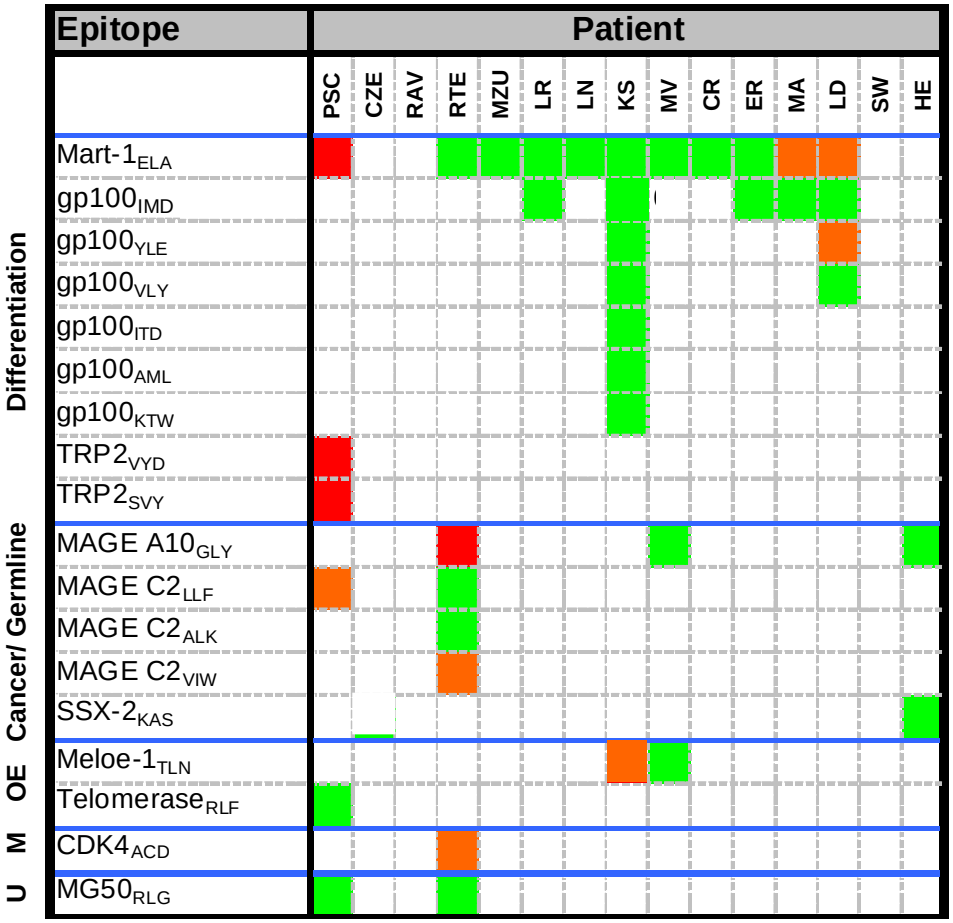
gp100 (#39)
0.010%
VLYRYGSFSV

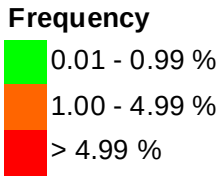
gp100 (#42)
0.016%
AMLGTHTMEV

gp100 (#44)
0.028%
KTWGQYWQV

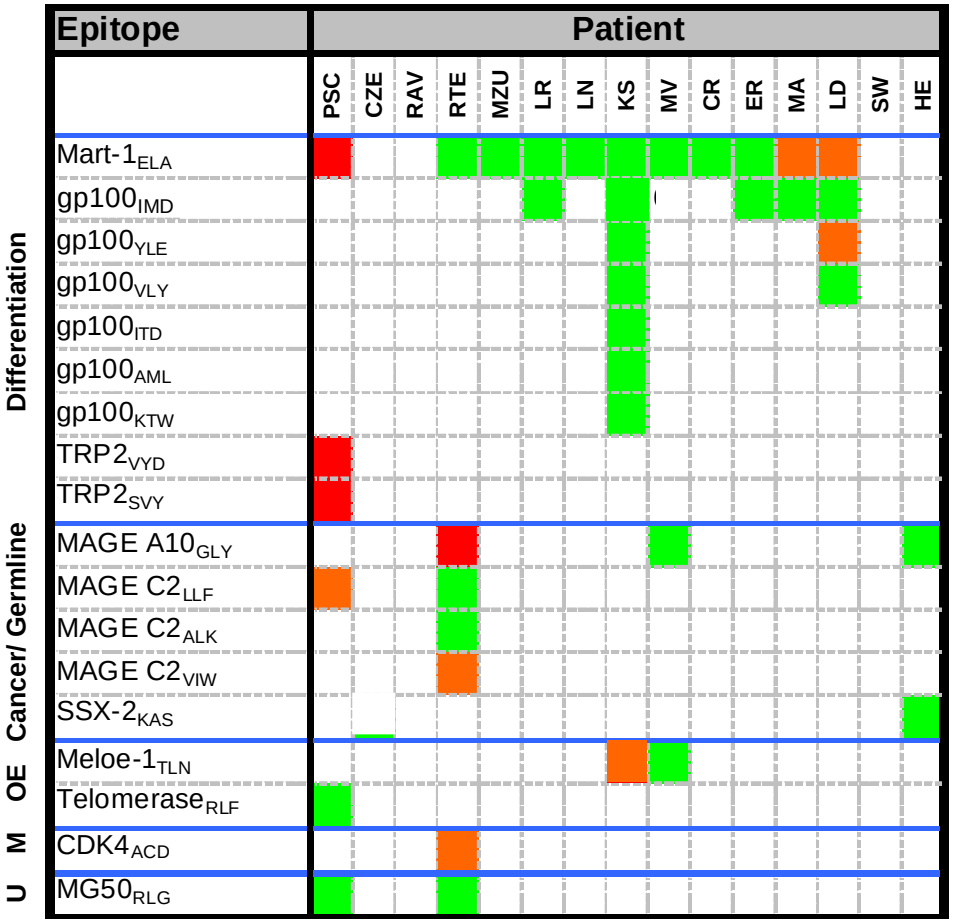
gp100 (#56)
(0.42%)
IMDQVPFSV

Summary of identified T cell responses in 15 T cell products



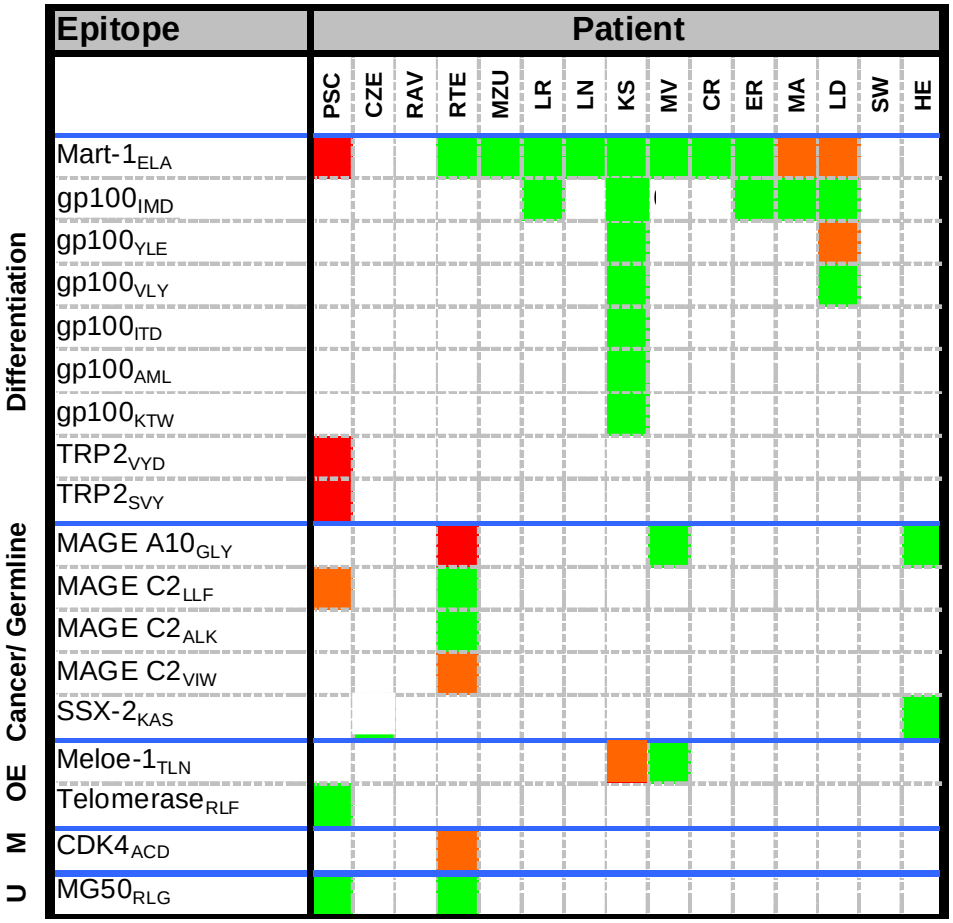


Summary of identified T cell responses in 15 T cell products



- Total of 39 melanoma-reactive T cell responses in 15 T cell products
- Number of responses ranges from 0 to 8
- Predominant recognition of MDA and C/G antigens
- T cell reactivity is *highly* patient-specific

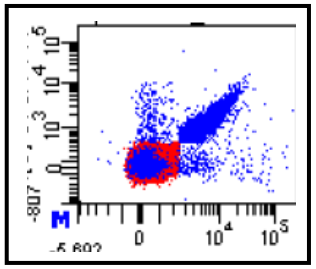
Summary of identified T cell responses in 15 T cell products



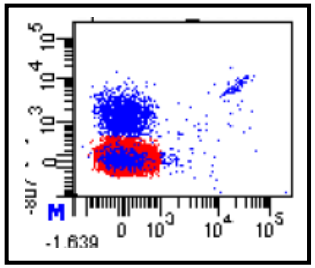
- Total of 39 melanoma-reactive T cell responses in 15 T cell products
- Number of responses ranges from 0 to 8
- Predominant recognition of MDA and C/G antigens
- T cell reactivity is *highly* patient-specific

- Are these T cell populations relevant for melanoma recognition?
- Does this analysis predict post-treatment immune reactivity?

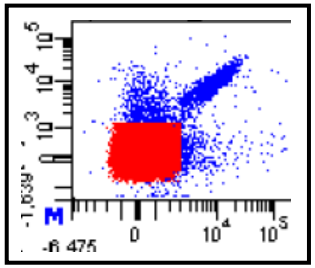
Isolation of a melanoma-reactive T cell populations for functional validation



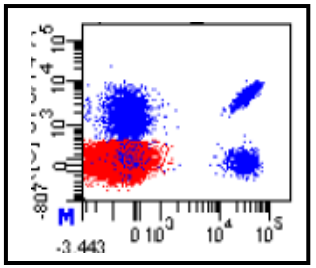
MAGE-A10
5.3%
GLYDGMEHL



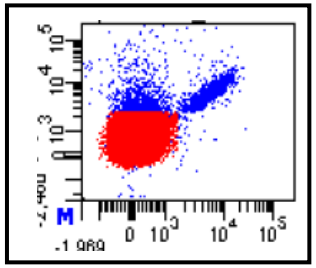
MAGE-C2
0.2%
ALKDVEERV



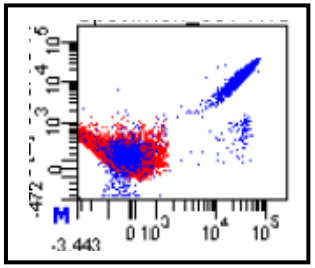
MAGE-C2
0.9%
LLFGLALIEV



MAGE-C2
1.5%
VIWEVLNAV



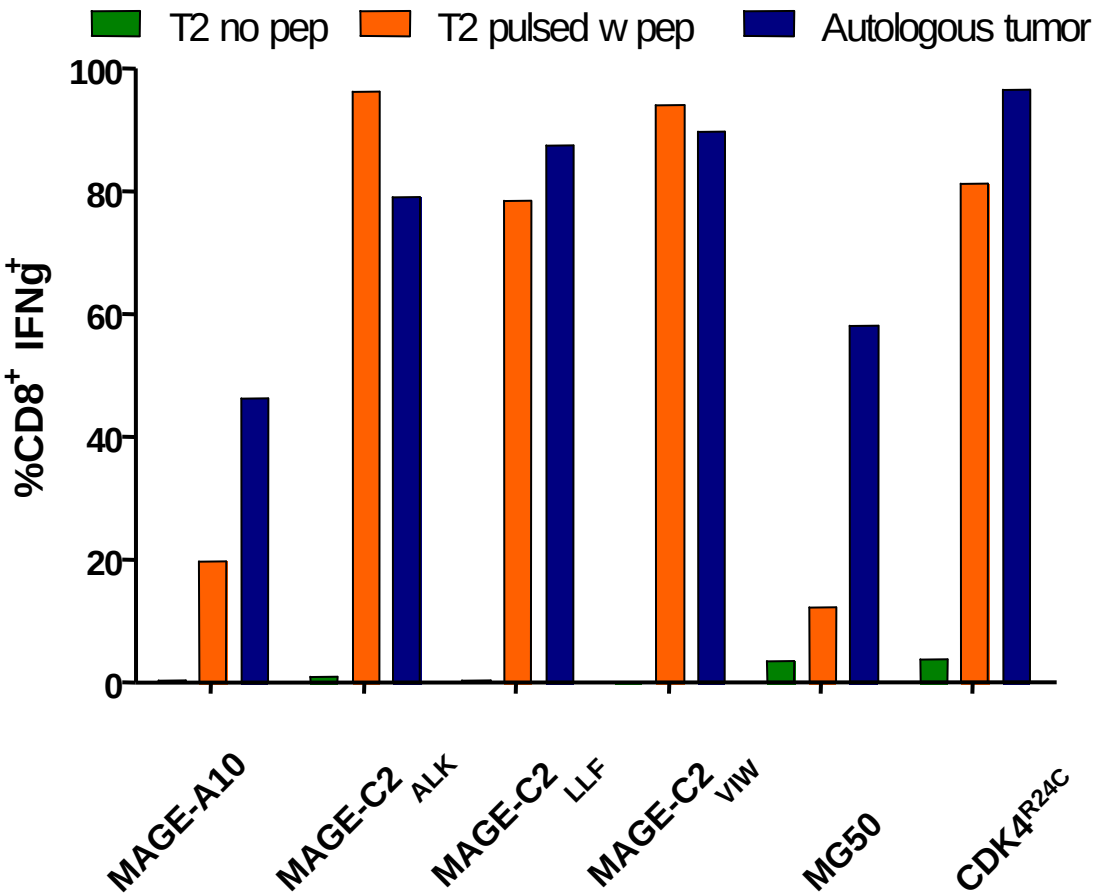
MG-50
0.8%
RLGPTLMCL



CDK4 R24C
2.3%
ACDPHSGHFV

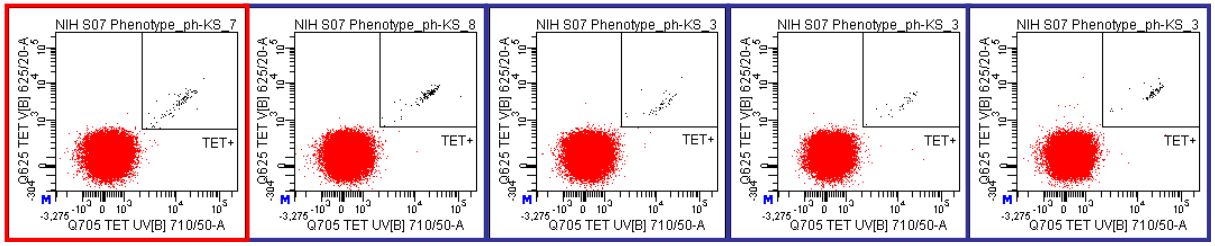
Numbers represent the % MHC tetramer^{+} T cells out of total CD8^{+} cells

Recognition of autologous melanoma by identified T cell populations



Detecting *post-therapy* T cell reactivity in patients treated with TIL

pt.KS
Post-Infusion PBMNC



MART-1 (#78)	**Meloe-1 (#80)**	**gp100 (#36)**	**gp100 (#38)**	**gp100 (#39)**
0.302%	(0.366%)	0.148%	0.106%	0.222%
ELAGIGILTV	TLNDECWPA	YLEPGPVTA	ITDQVPFSV	VLYRYGSFSV

- Total of 19 post-infusion responses in 10 HLA-A2^{+} TIL-treated patients
- 79% of pre-infusion responses is seen back in post-infusion PBMNC
- 95% of T cell responses in PBMNC post-therapy is predicted by in vitro reactivity

Conclusions

- We have developed and validated technology for the high-throughout detection of therapy-induced melanoma-specific T cell reactivity

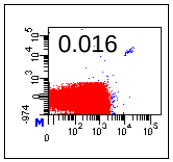
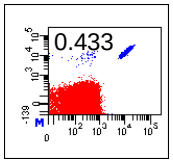
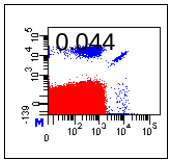
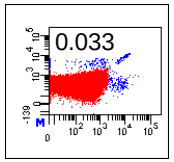
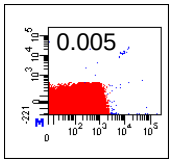
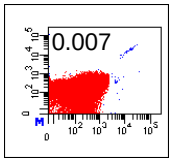
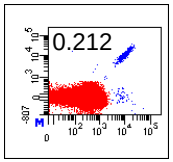
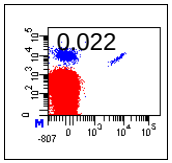
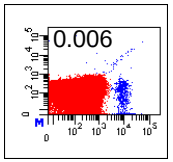
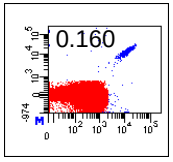
- T cell responses in TIL cell products are diverse, biased towards MDA and C/G antigens, and highly variable between patients

- In ‘young TIL’, individual T cell responses are generally of a (surprisingly) low magnitude

- T cell responses in TIL cell products predict immune reactivity post-therapy
  - No epitope spreading -

Is it possible to influence TIL cell product composition?

Individual tumor fragments yield distinct TIL products

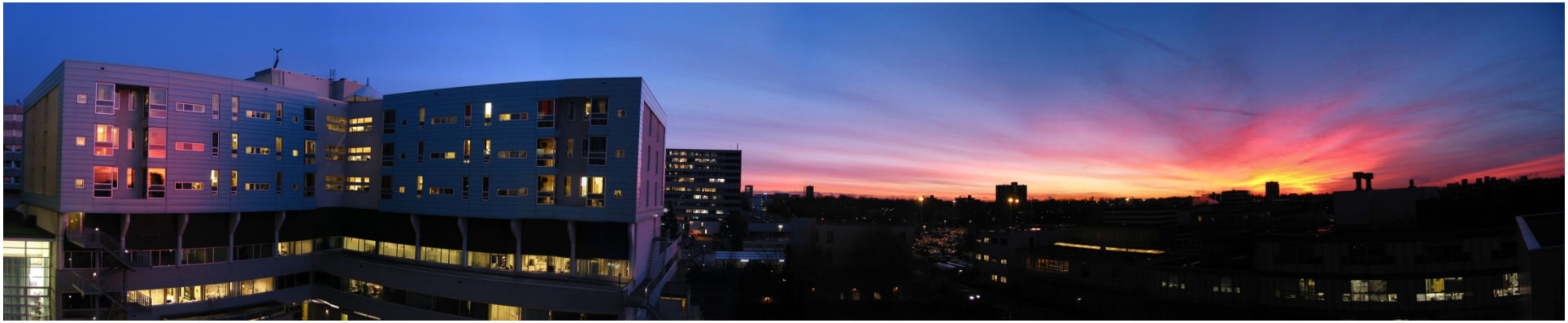
	CDK4^{R24C}	Mage A10	Mage C2 72	Mage C2 73	Cyclophilin B
F1					
F2					
F4					
F5	—				
F7					
F9					
F12	—				

Dissecting melanoma-specific T cell responses upon other immunotherapeutic treatments?

- Immune reactivity induced by anti-CTLA4 or anti-PD1 treatment?

- Immunological consequences of combination therapy?

  (e.g. BRAF inhibition plus anti-CTLA4)



Self-destructive MHC ligands & combinatorial coding

MHC-based monitoring

Jenny Shu

Manuel Fankhauser

Pia Kvistborg

Mireille Toebes

Arne Bakker

Carsten Linnemann

Chemical Biology

Boris Rodenko

Huib Ovaa

Clinical translation

Bianca Heemskerk

Annelies Jorritsma

Raquel Gomez

Nienke van Rooij

Bastiaan Nuijen

Christian Blank

John Haanen

CCIT, Copenhagen

Sine Hadrup

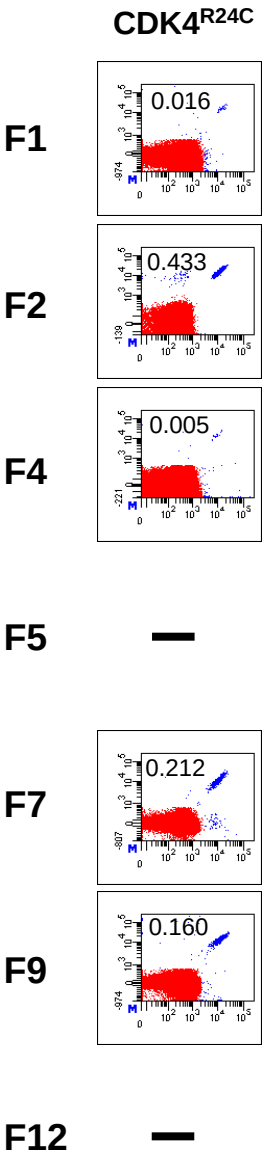
Charlotte Albæk Thrue

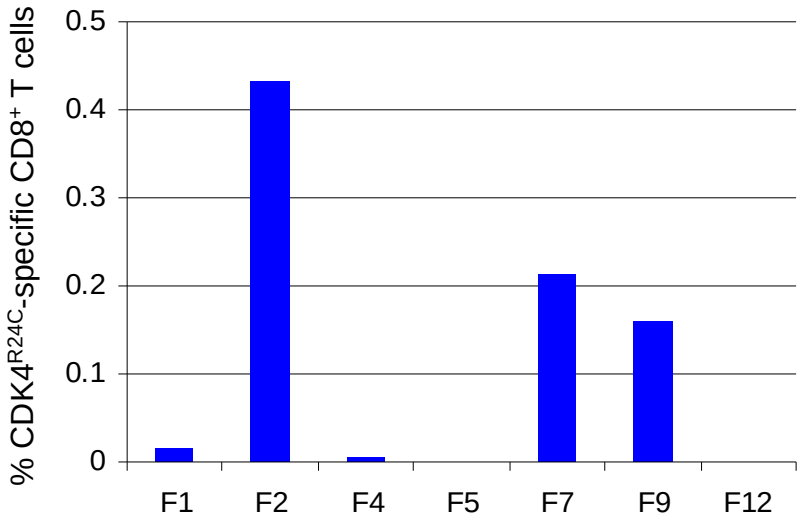
Surgery Branch, NIH

Steven Rosenberg

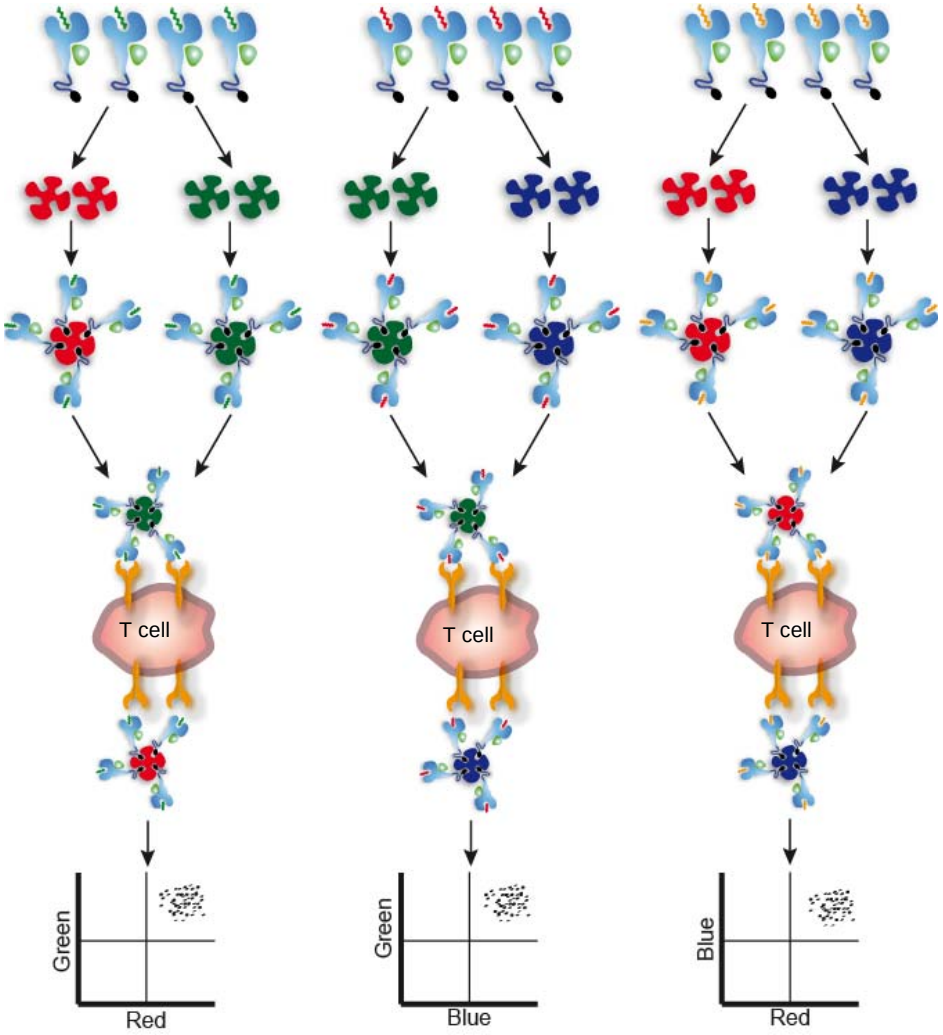
Mark Dudley

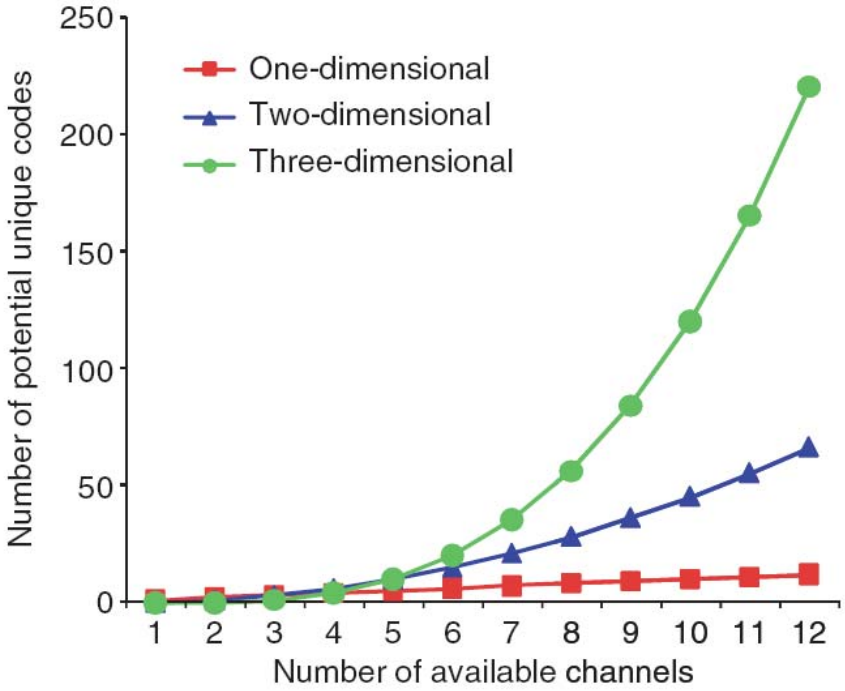
Individual tumor fragments yield distinct TIL products





Self-assembling molecular codes





Hadrup et al. *Nat. Methods* 2009