

Use of MHC multimers to identify tumor reactive T cells

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Disclosures for this presentation

John Haanen

The following relationships exist related to this presentation:

Research grant received from BMS

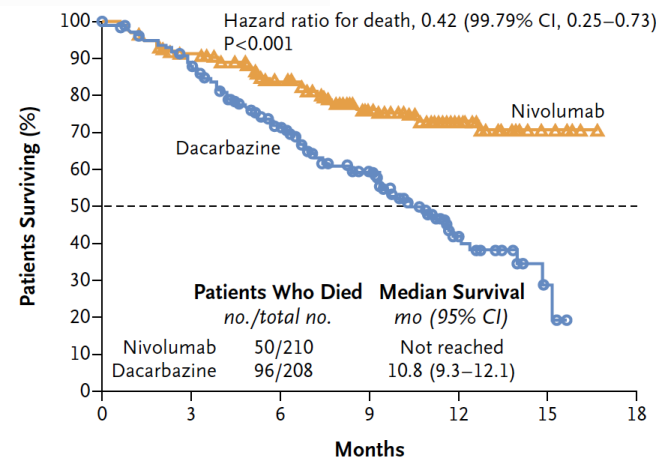
Clear value of mobilizing endogenous tumor-specific T cell responses

1. TIL therapy



J. Haanen, NKI-AVL

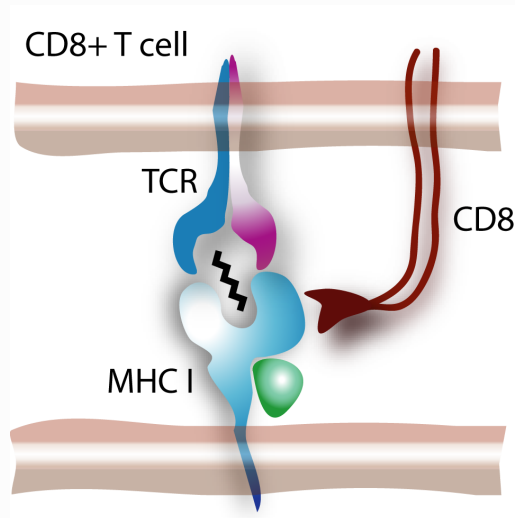
1. Checkpoint blockade



C. Robert, NEJM 2015



What could tumor-specific cytotoxic T cells detect on human cancer?

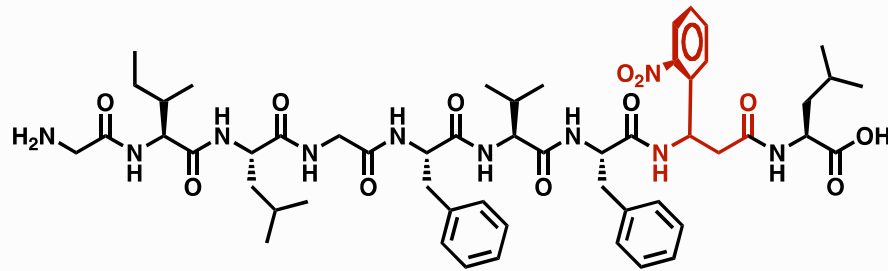


1. Self antigens (to which tolerance is incomplete)
Shared between patients

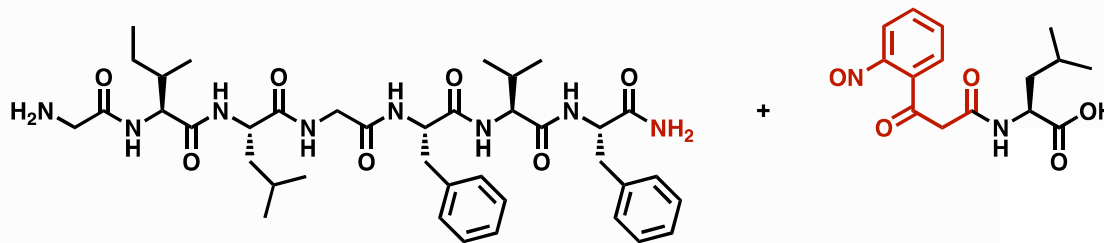
2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
Truly foreign, need to be monitored on a patient-specific basis

Each class, hundreds of (potential) T cell epitopes

UV-induced peptide exchange allows generation of 1,000s of pMHC multimers

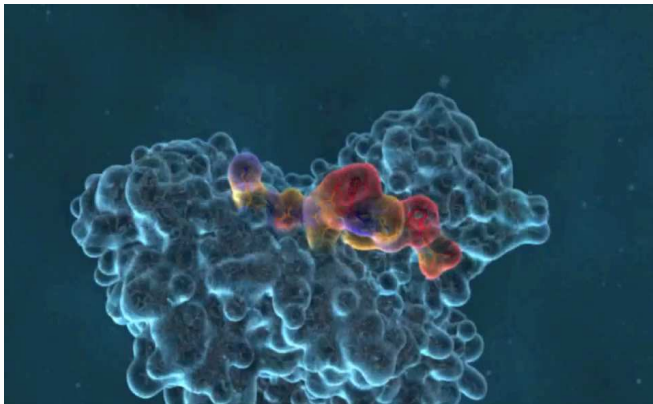


GILGFVLF(o-NO₂)L

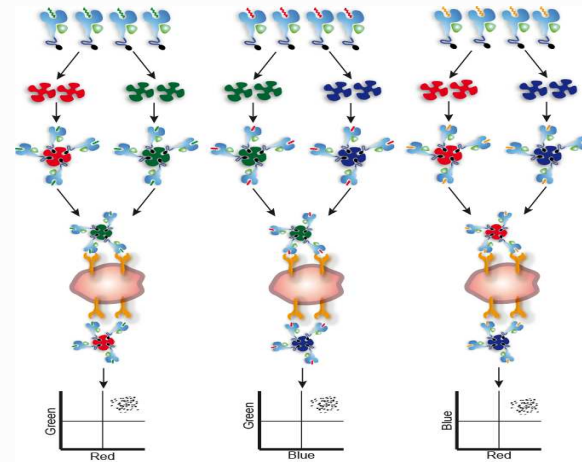


- HLA-A1, -A2, -A3, -A11, -B7, -B27, -B57 (Toebes *Nat Med* 2006, Bakker *PNAS* 2008)
- HLA-A24, -B40, -B58 (G. Grotenbreg, NUS, Singapore)

Tools for high-throughput analysis of neo-antigen specific CD8 T cell responses



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

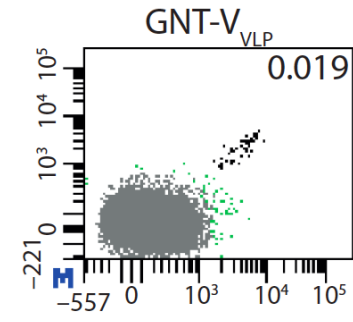
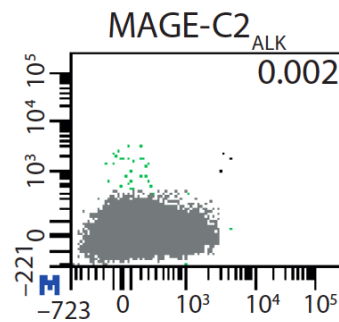
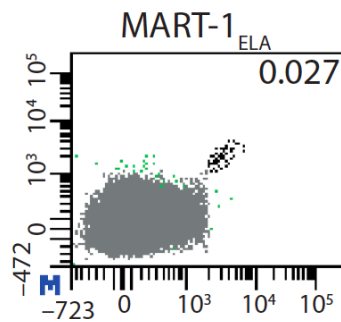


Hadrup *Nat Methods* 2009
Kvistborg *Science Transl Med* 2014

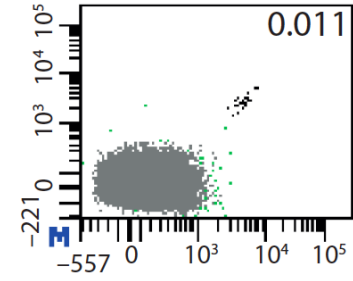
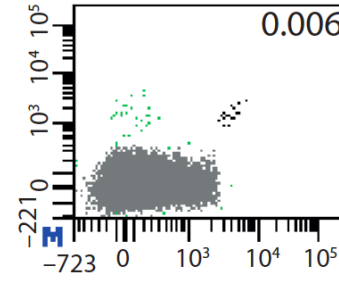
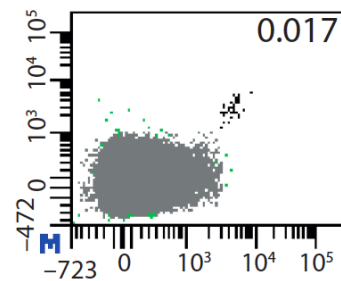
Allows analysis of T cell responses against 100s-1000s of (predicted) antigens

Flow results...

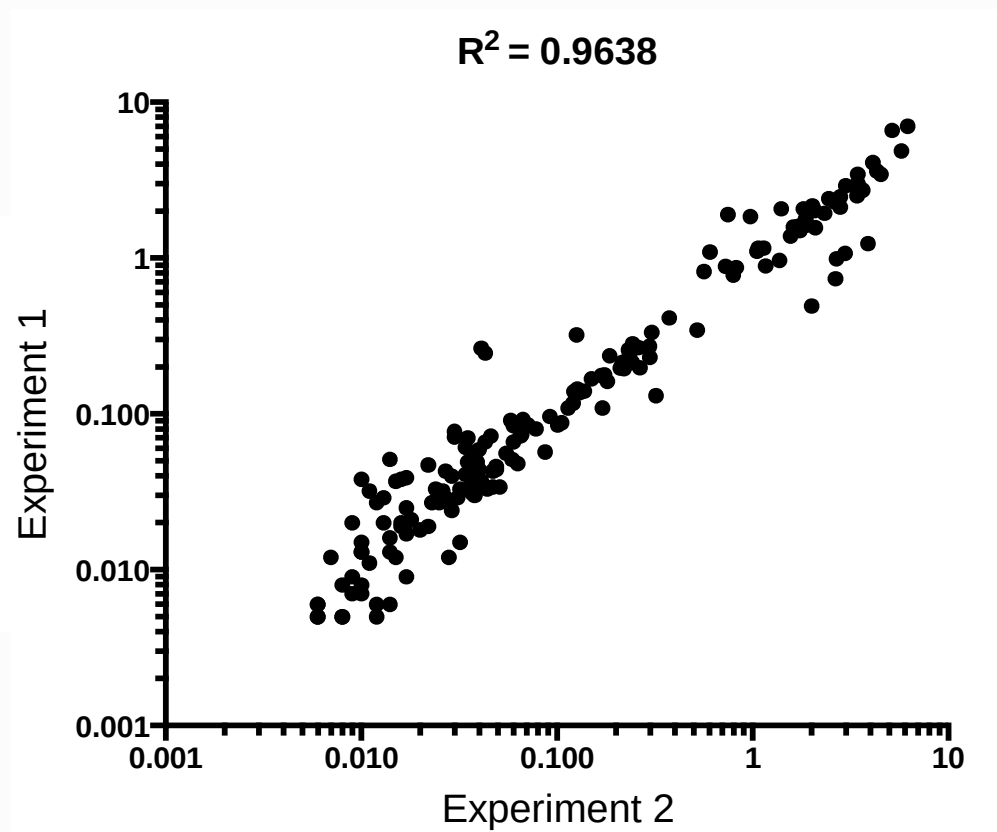
Pretherapy



Posttherapy

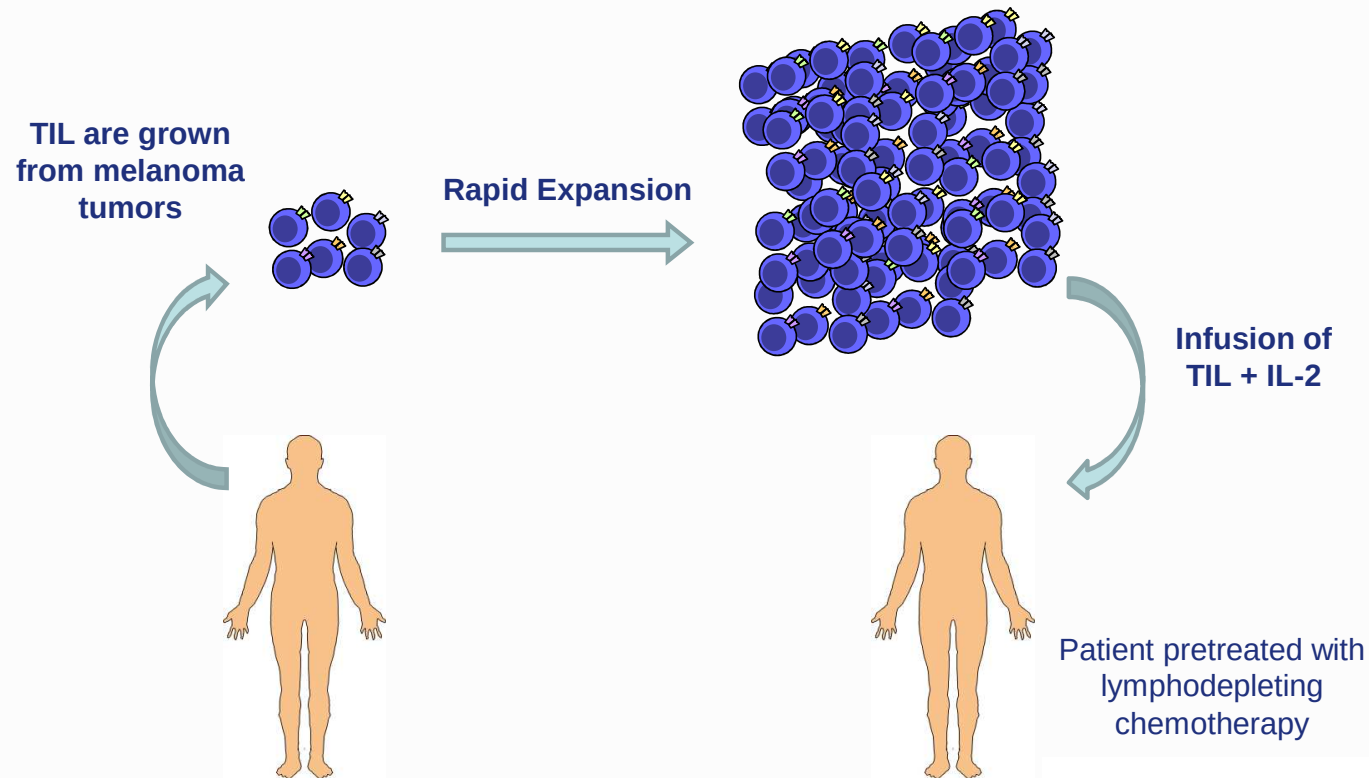


Reproducibility



Reproducibility between 2 independent experiments with changed color code is high

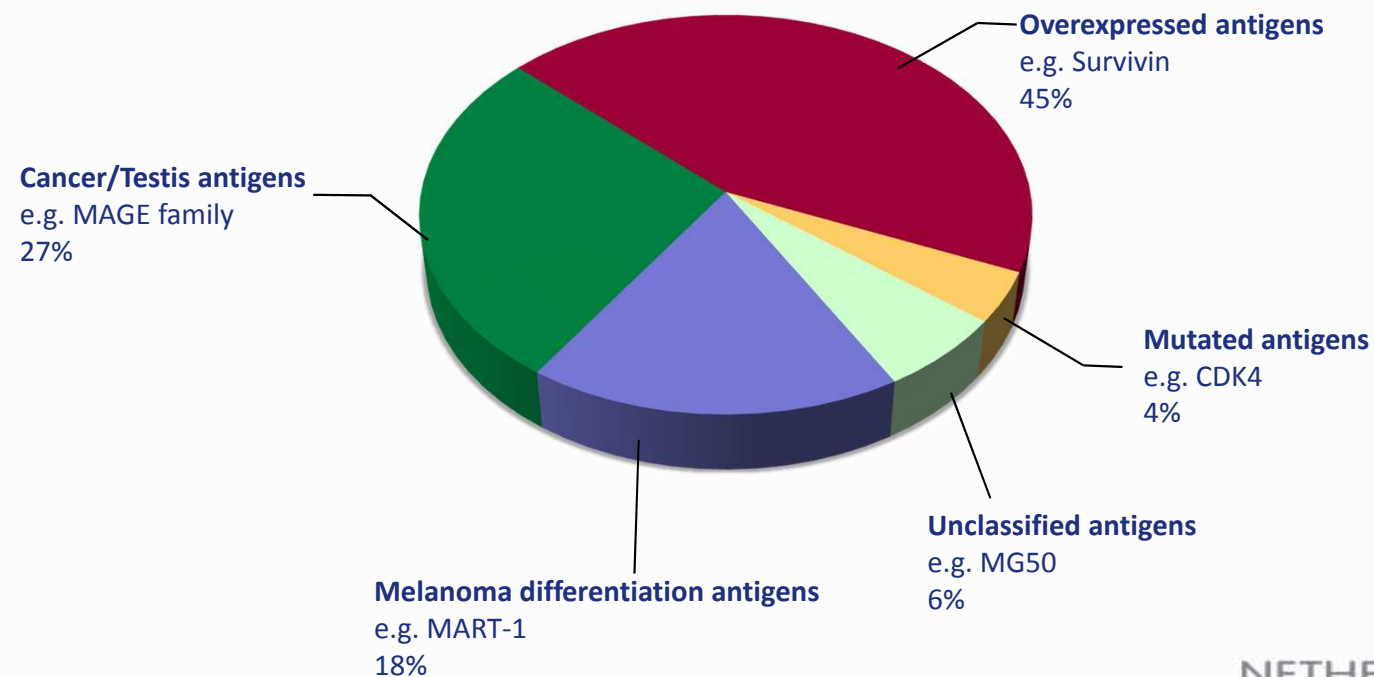
Tumor-infiltrating lymphocyte (TIL) therapy of melanoma



- 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?

Melanoma associated epitope panel

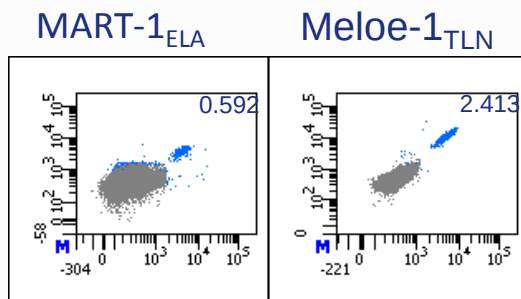
HLA-A2 restricted peptide panel includes 145 epitopes



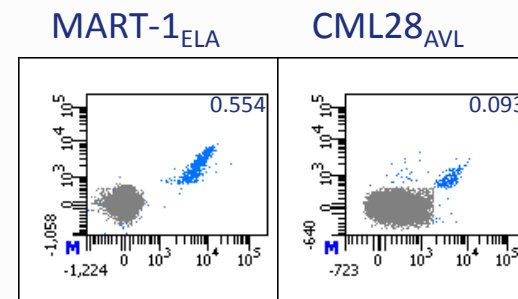
Kvistborg et al., Oncoimmunology 2012

Visualizing the composition of TIL

Pt. 1

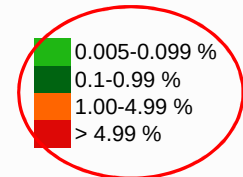


Pt. 2



Visualizing the composition of TIL

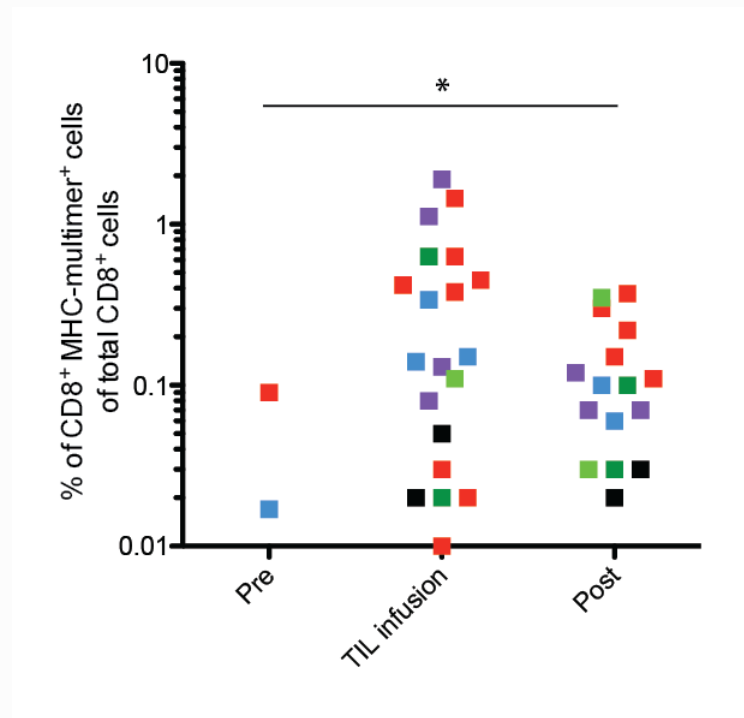
T cell responses are very low magnitude



		Young CD8 enriched TIL (NIH)																	Young TIL (Ella)						Selected TIL														
Patient		LR	LN	KS	MV	CR	ER	MA	LD	SW	HE	AS	RE	OJ	AS	MG	NJ	BJ(f)*	SC*	57SV	63SM	51VS	60SD	41BA	52SD	14PA	09BY	31YR	PS	OM	BJ(m)	SM	ER	CR	BM	CJ	AD		
MDA	Mart-1 _{ELA}	■	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	
	gp100 _{IMD}	■	■	■	■			■	■			■	■	■	■	■	■	■	■	■	■	■	■		■	■		■	■	■	■	■	■	■	■	■	■	■	
	gp100 _{YLE}			■	■				■	■			■	■	■	■									■	■		■		■			■	■					
	gp100 _{VLY}			■	■				■	■			■	■												■	■						■	■					
	gp100 _{AML}			■	■									■												■	■												
	gp100 _{KTW}			■	■									■												■	■												
	TRP2 _{VYD}			■										■											■	■							■						
	TRP2 _{SVY}																								■	■													
CT	NY-MEL 1 _{VLH}																									■	■												
	CML28 _{AVL}													■																									
	Mage A4 _{GVY}																										■	■											
	MAGE A10 _{GLY}				■							■					■											■	■										
	MageB1, B2 _{FLW}																								■	■													
	MAGE C2 _{LLF}																									■	■												
	MAGE C2 _{ALK}																									■	■												
	NY-ESO 1 _{SLL}																																						
OE	HERV K mel _{MLA}																■																						
	SSX-2 _{KAS}											■																											
	GnTV _{VLP}																								■	■	■												
	GnT-V _{VLP10mer}																								■	■										■			
	Meloe-1 _{TLN}			■	■																				■	■													
Telomerase _{RLF}																										■	■												
Bing 4 _{CQW}																									■	■								■					
Clinical response		NR	NR	NR	PR	PR	NR	NR	PR	PR	PR	NR	NR	NR	NR	PR	NR	NR	CR	PR	NR	PR	NR	NR	PR	CR	NR	NR	PR	PR	CR	PR	PR	NR	NR	CR	NR		

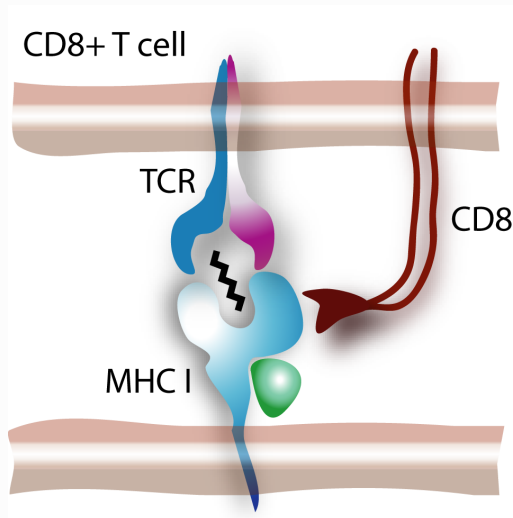
Kvistborg et al., Oncoimmunology 2012

TIL therapy broadens the melanoma-specific T cell repertoire



Kvistborg et al., Oncoimmunology 2012

What could tumor-specific cytotoxic T cells detect on human cancer?

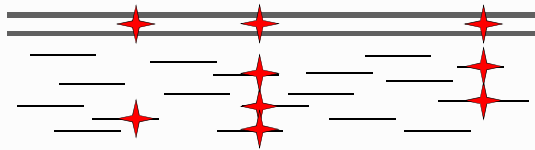


1. Self antigens (to which tolerance is incomplete)
Shared between patients
2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
In large part patient-specific, hence generally ignored

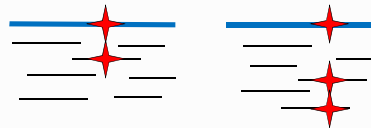
Specific questions to address

- ❖ Is CD8 T cell reactivity against neo-antigens common in human melanoma ?
- ❖ Do CD4 T cells also respond to neo-antigens in human melanoma ?
- ❖ Does this extend to other human malignancies ?

Analyzing the neo-antigen-specific CD8 T cell repertoire in human cancer?



Generate map of tumor-specific mutations (ExomeSeq)

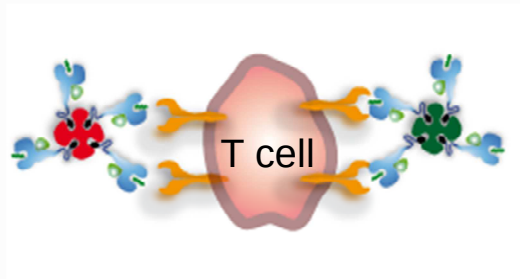


Determine which mutated genes are expressed (RNASeq)



MDLVLNELV**I**SLIVESKLLE
HLA-A2 _____
HLA-B7 _____
HLA-C2 _____

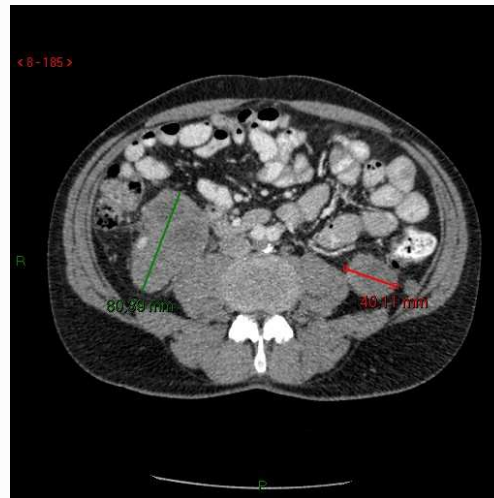
Predict epitopes for each mutation/ each HLA-allele *in silico*



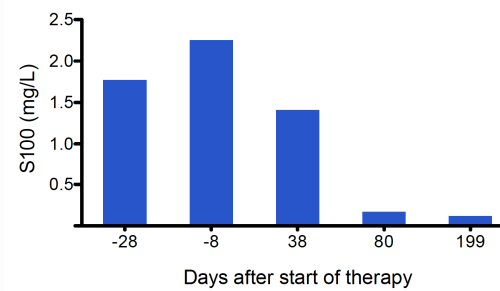
Screen for T cell recognition of mutated epitopes

Pt 002: Partial response upon anti-CTLA4 treatment

Aug 2010

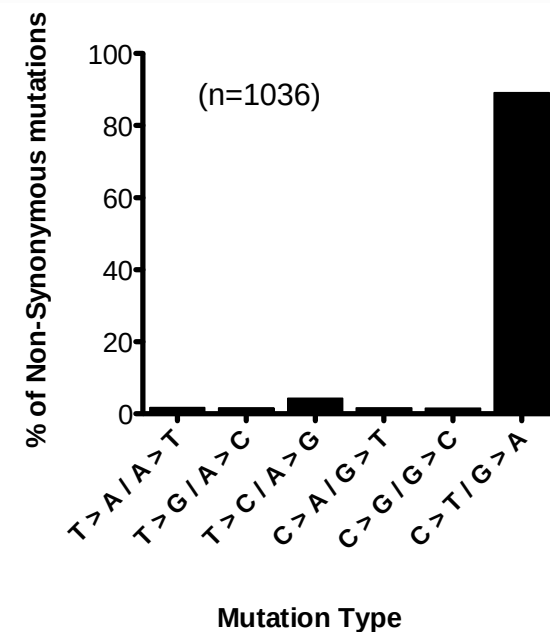
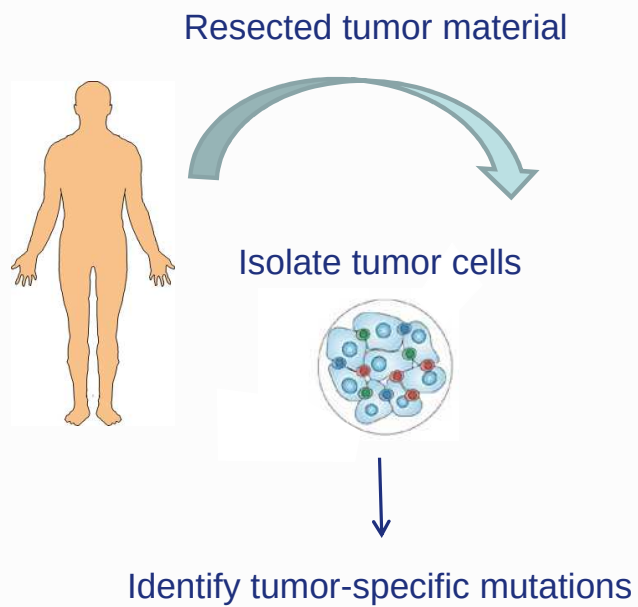


Dec 2010

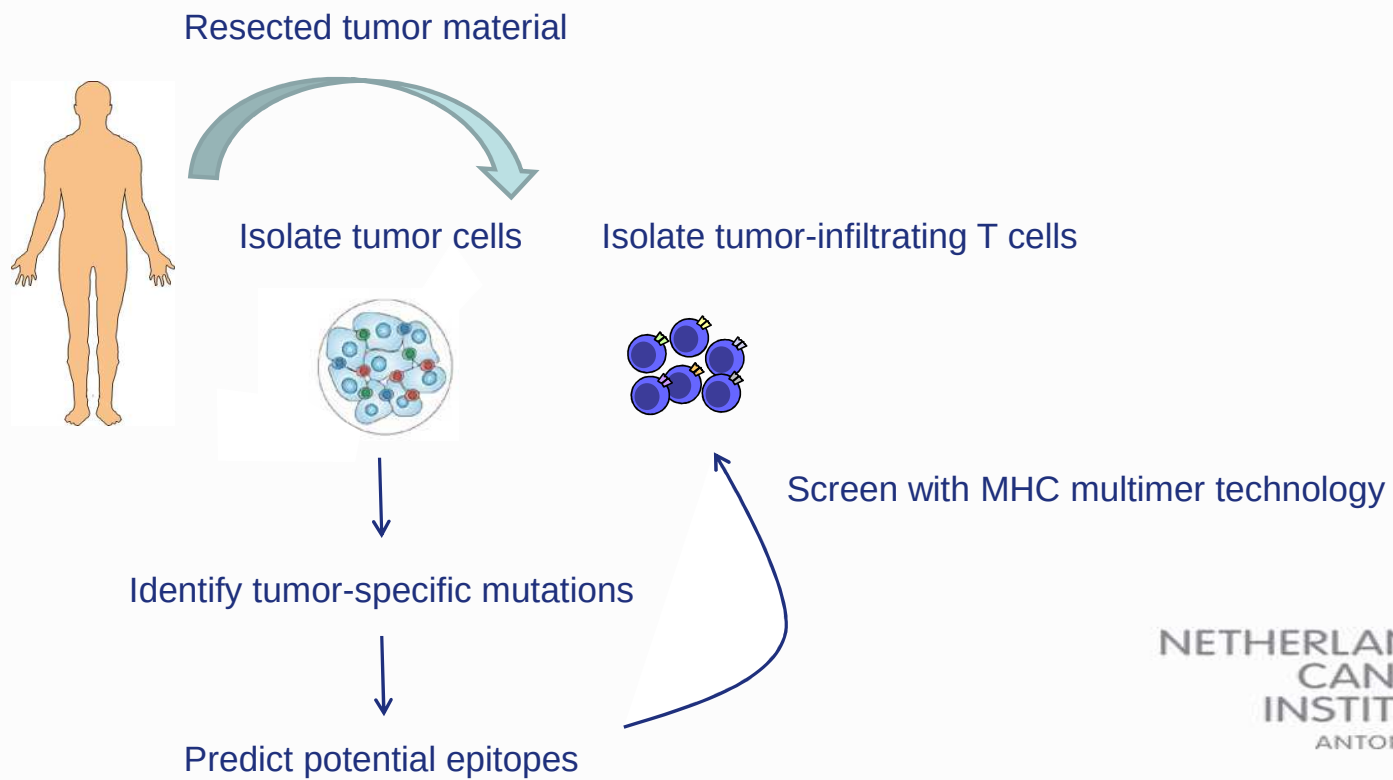


Van Rooij et al. J Clin Oncol 2013

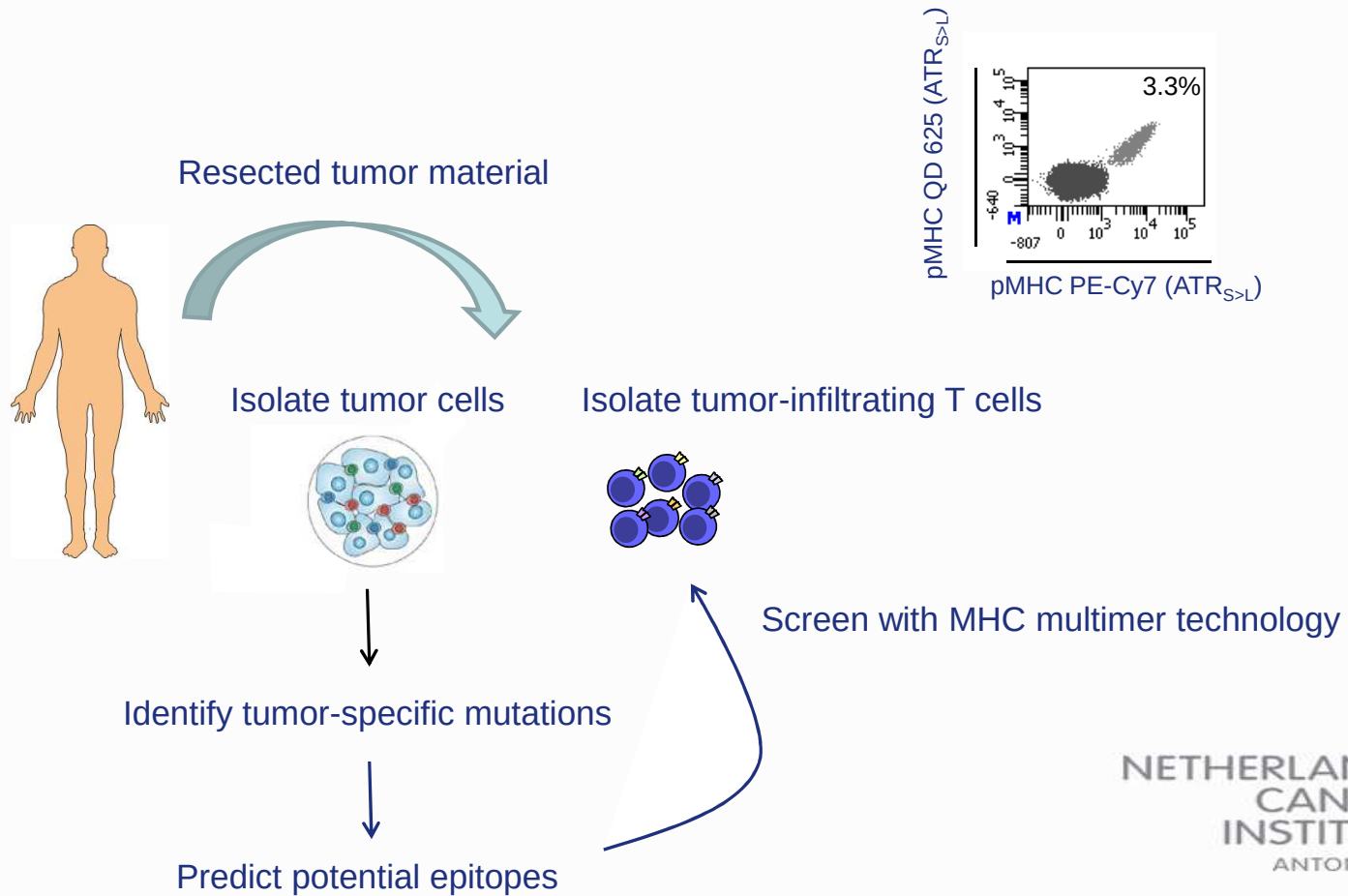
Analyzing the neo-antigen-specific T cell repertoire in human cancer?



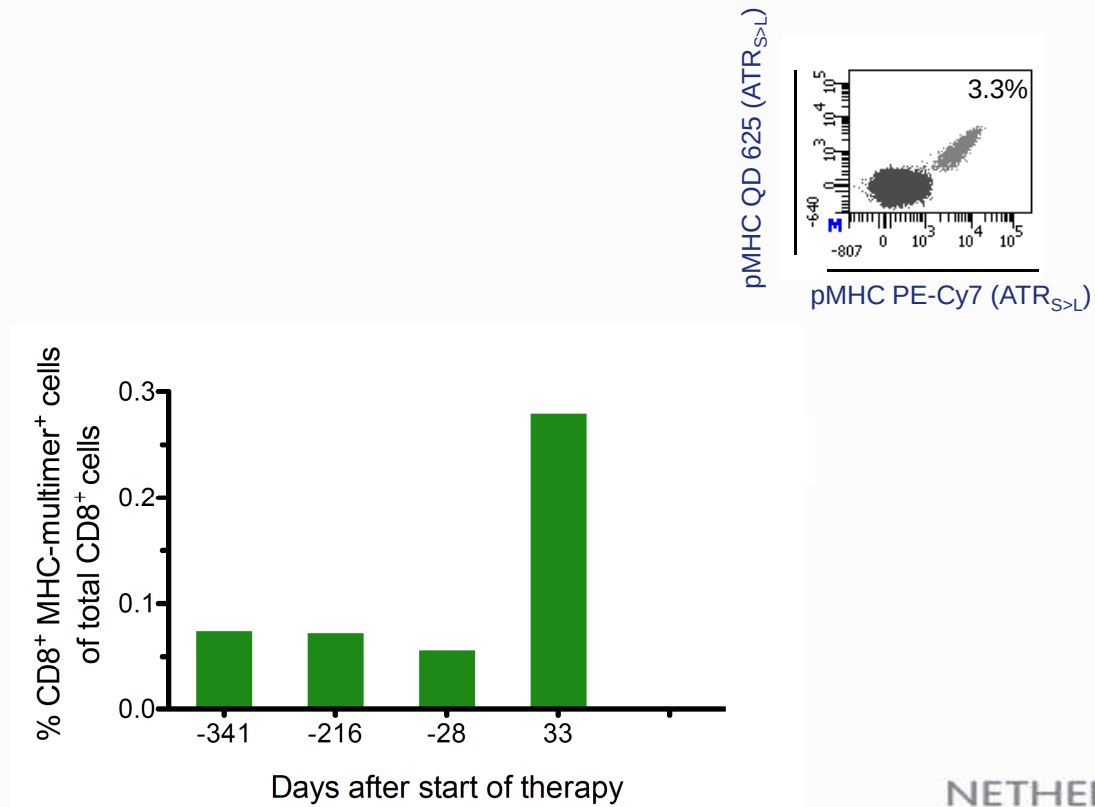
Analyzing the neo-antigen-specific T cell repertoire in human cancer?



Strong T cell response against an ATR_{S>L} neo-epitope within the tumor

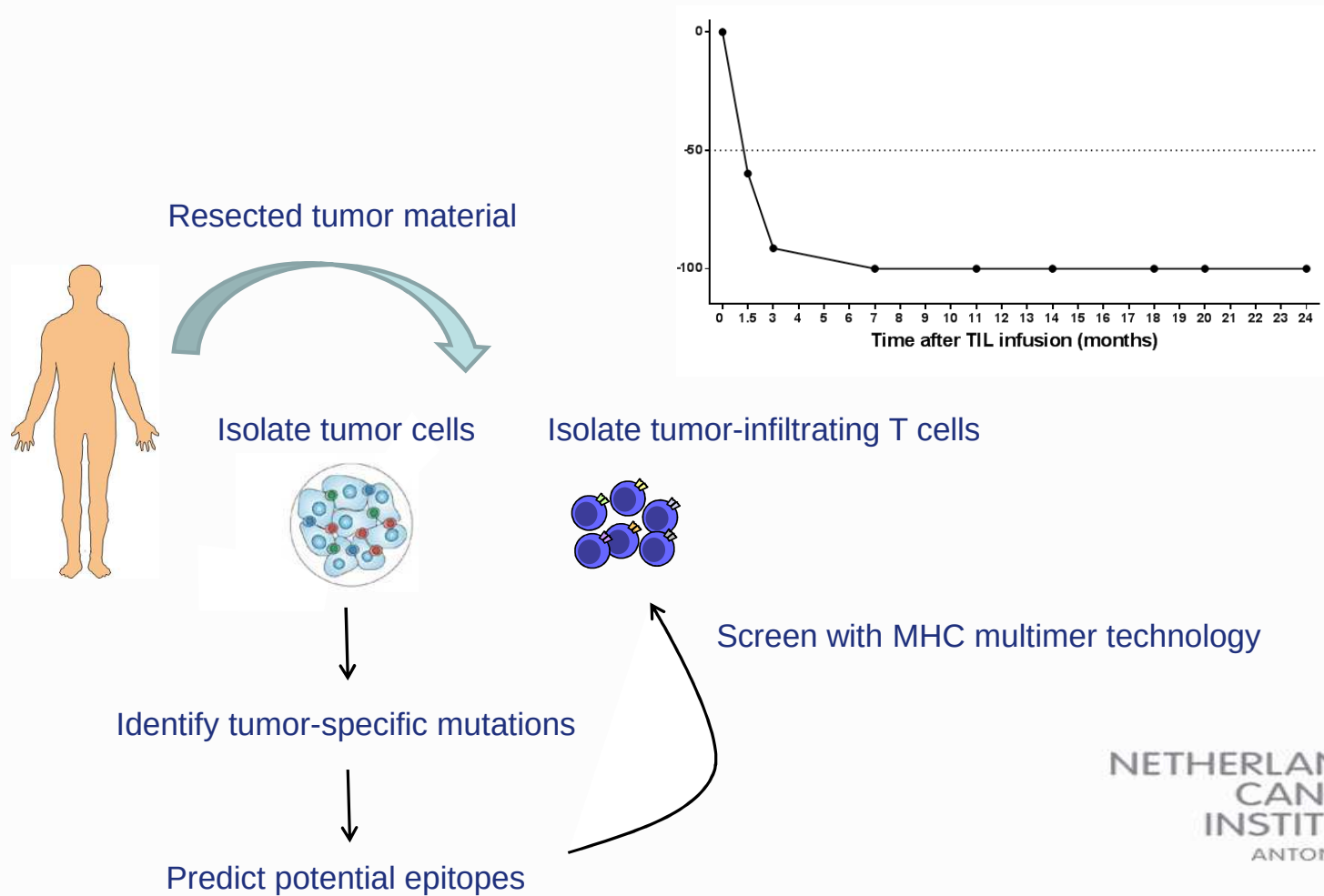


Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4



van Rooij et al., *J Clin Oncol* 2013

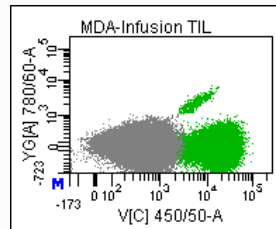
Pt 008: CR upon TIL therapy



Pt 008: CR upon TIL therapy

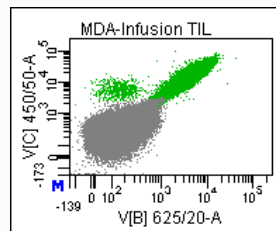
Infusion TIL product

0.172%



RASSF1_{R>C}

23%



DHX33_{R>W}

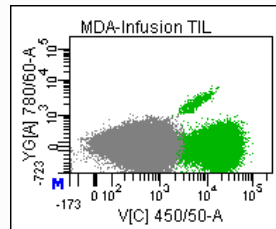
Pt 008: CR upon TIL therapy

Infusion TIL product

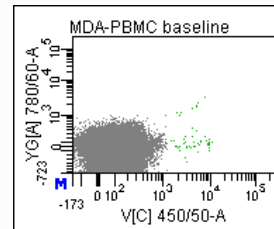
Pre-therapy PBMNC

D7 post-therapy

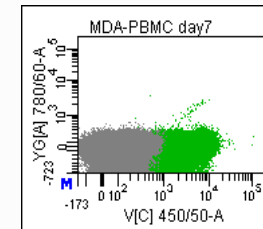
0.172%



0.002%

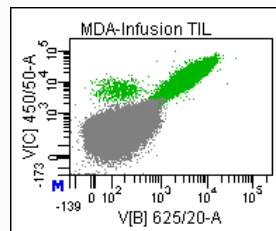


0.010%

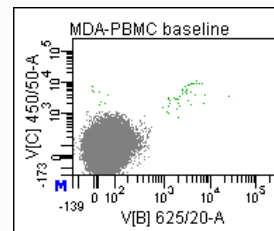


RASSF1_{R>C}

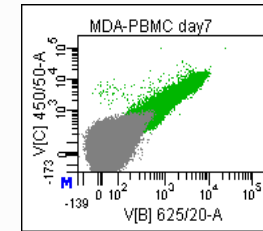
23%



0.009%



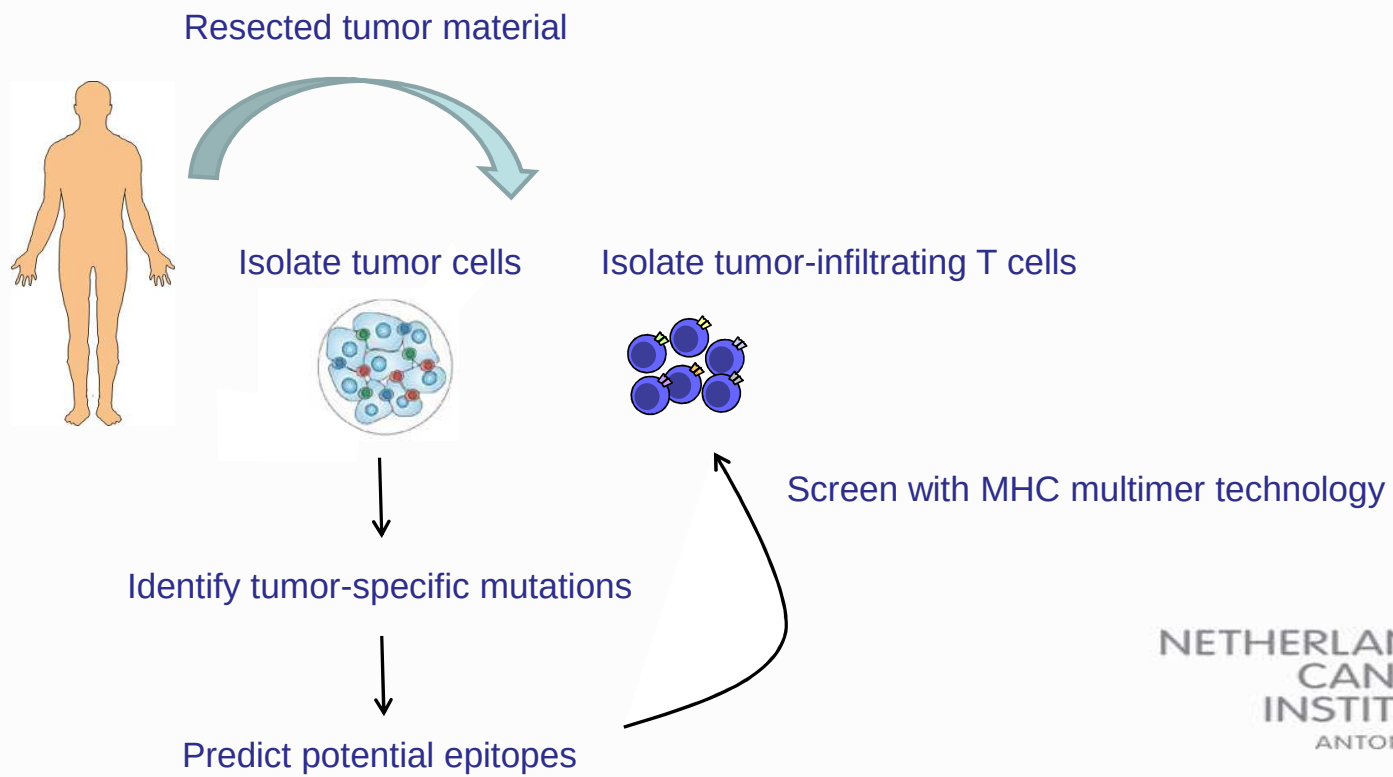
54%



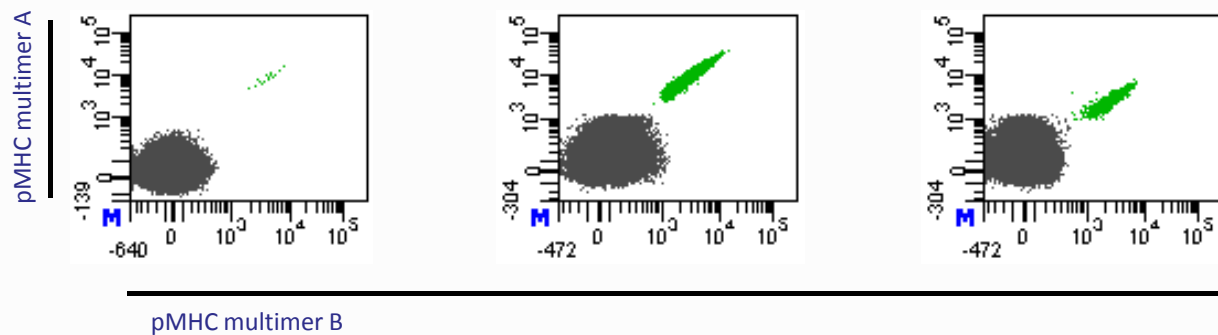
DHX33_{R>W}

Major (>5000 fold) increase in neo-antigen specific T cell reactivity upon TIL therapy

Pt 004:



Pt 004:

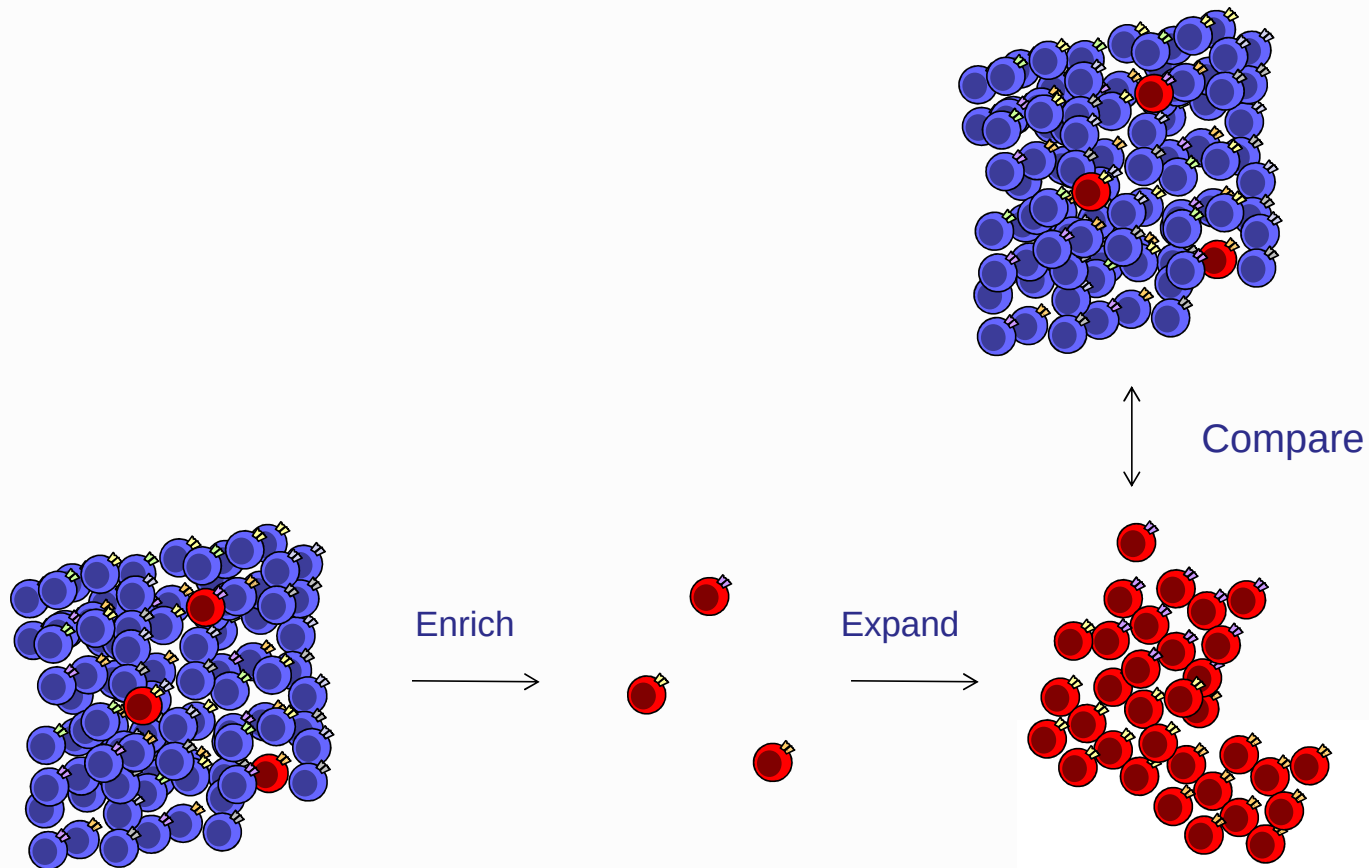


DNAH17_{H>Y} (0.003%)
 VLFEDAVAH_ > VLFEDAVAY_

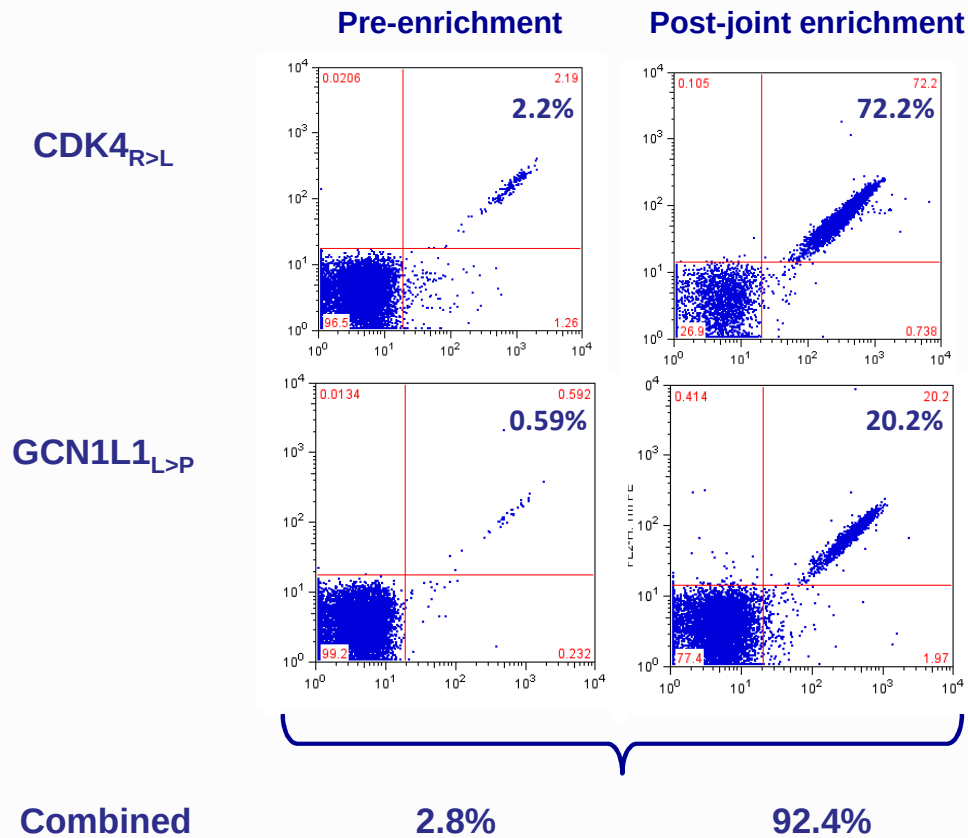
CDK4_{R>L} (1.604%)
 ARDPHSGHFV > ALDPHSGHFV

GCN1L1_{L>P} (0.407%)
 ALLETLSLLL > ALLETPSLLL

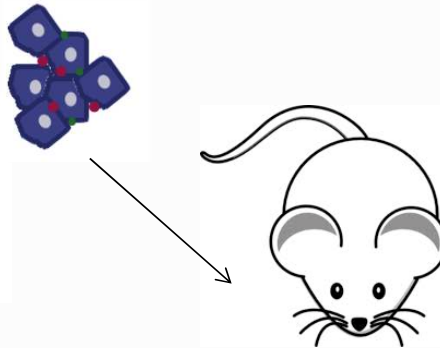
Are neo-antigens superior cancer rejection antigens?



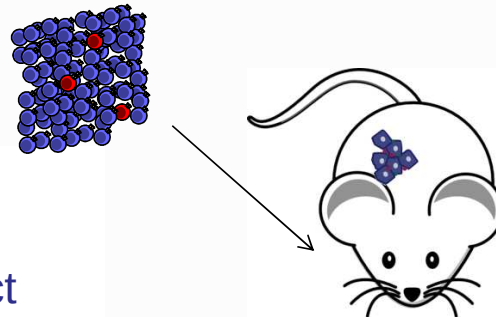
Are neo-antigens superior cancer rejection antigens?



1)
Inject human
melanoma
(NSG-mice)



2a)
Inject autologous
bulk T-cell product



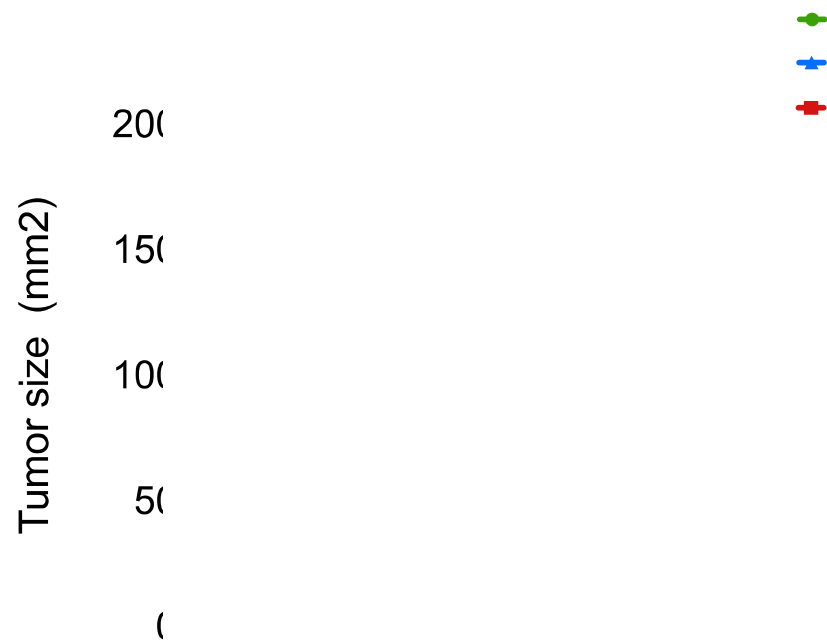
2b)
Inject autologous
neo-Ag enriched
T-cell product



3)
Monitor tumor growth



Neo-antigen enriched TIL can mediate superior tumor control



Summary of CD8 T cell analyses

11 patients analyzed, neo-antigen specific reactivity in 9

(Not all alleles covered, exome coverage incomplete, epitope predictions imperfect)

CD8 T cells frequently respond to the consequence of DNA damage in human melanoma

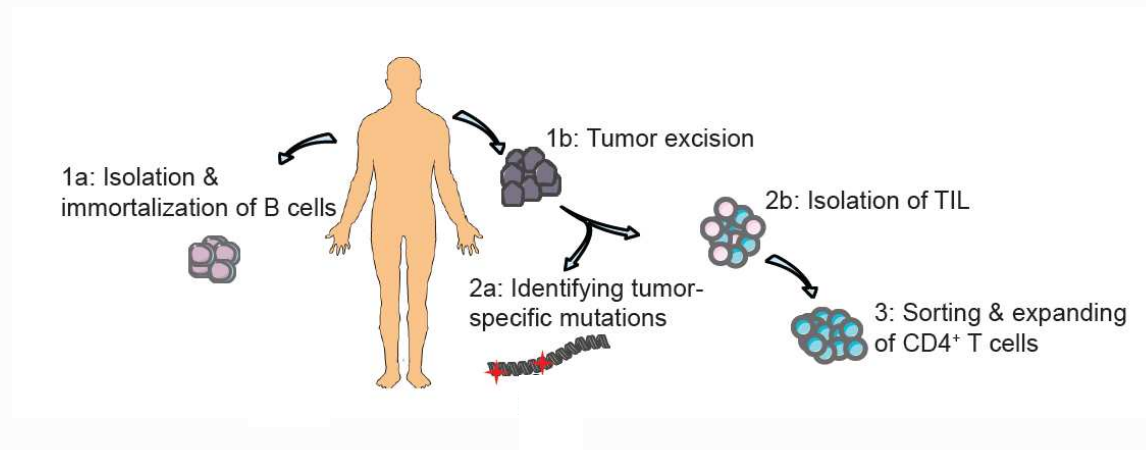
Evidence for neo-antigen reactive CD4 T cells?

Evidence for neo-antigen reactive CD4 T cells?

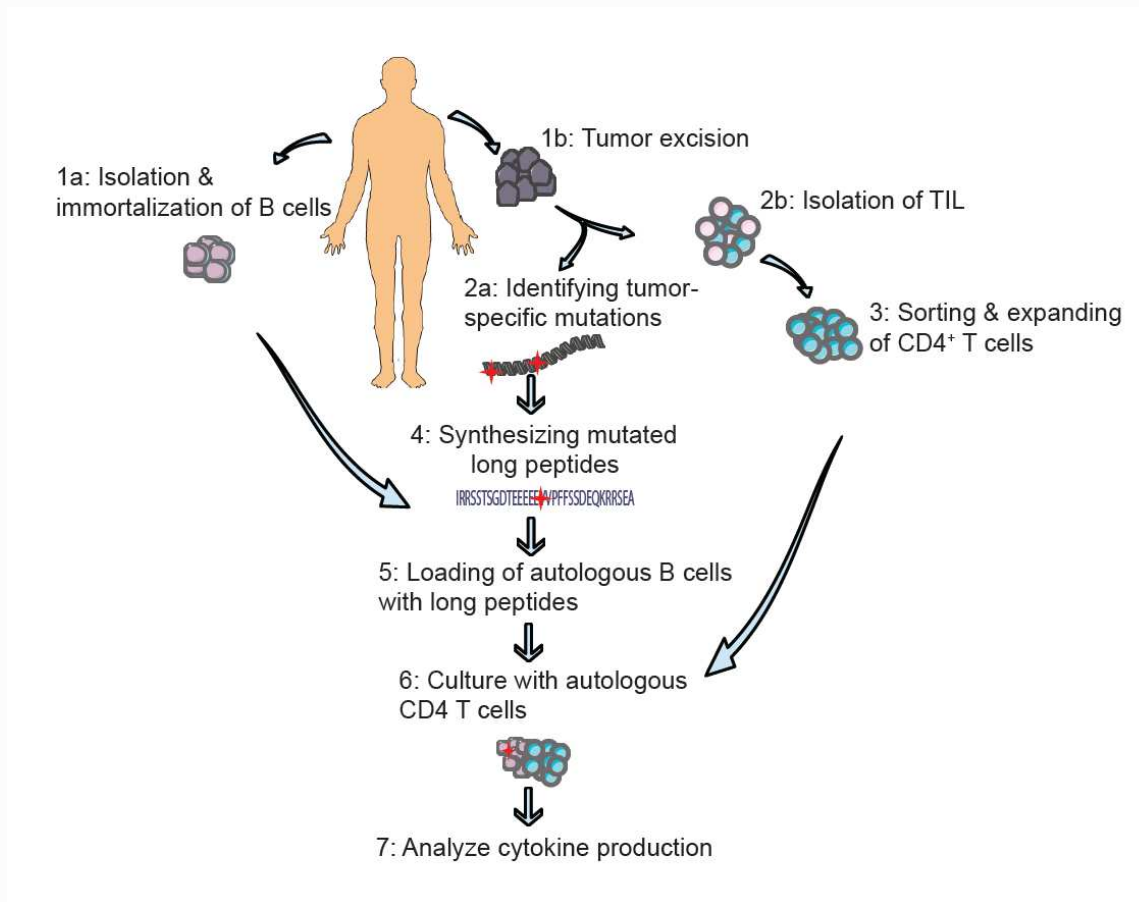
Rationale to assess existence of neo-antigen reactive CD4⁺ T cells:

- Tumor-specific CD4⁺ T cells contribute to tumor control in mouse models
- Human melanomas often express MHC class II
- A recent case report showed tumor regression upon infusion of CD4⁺ T cells specific for a mutated antigen (Tran et al. Science 2014)

Novel screening platform to detect neo-antigen specific CD4 T cells

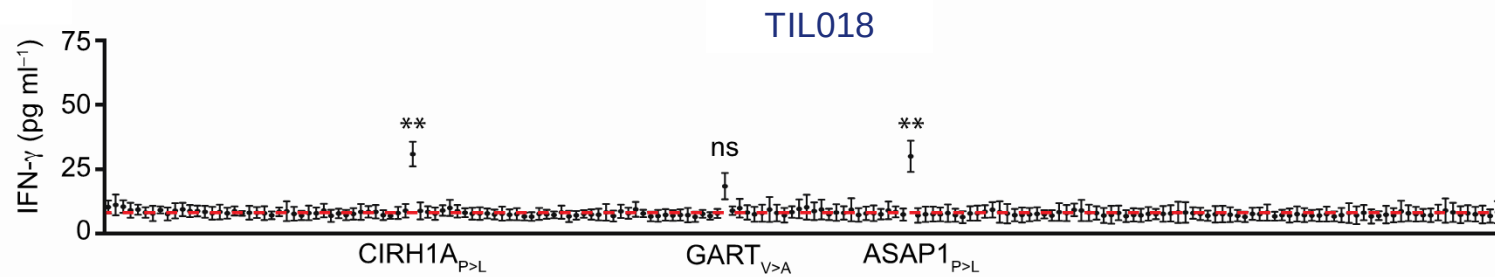


Novel screening platform to detect neo-antigen specific CD4 T cells

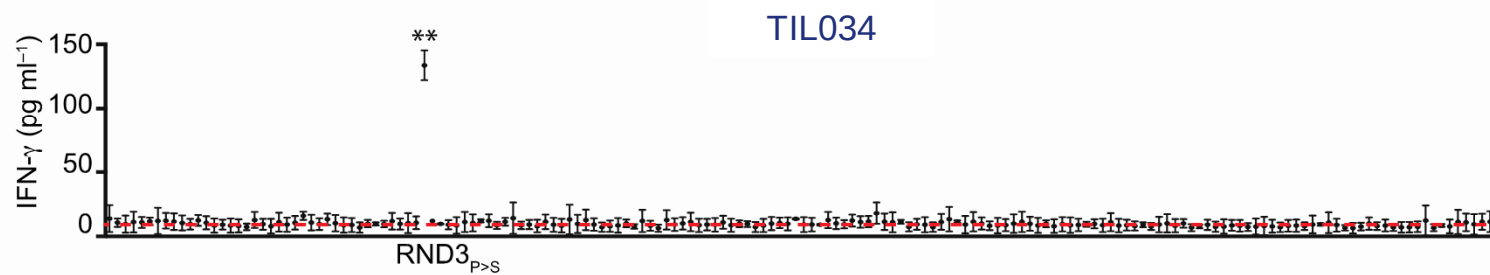
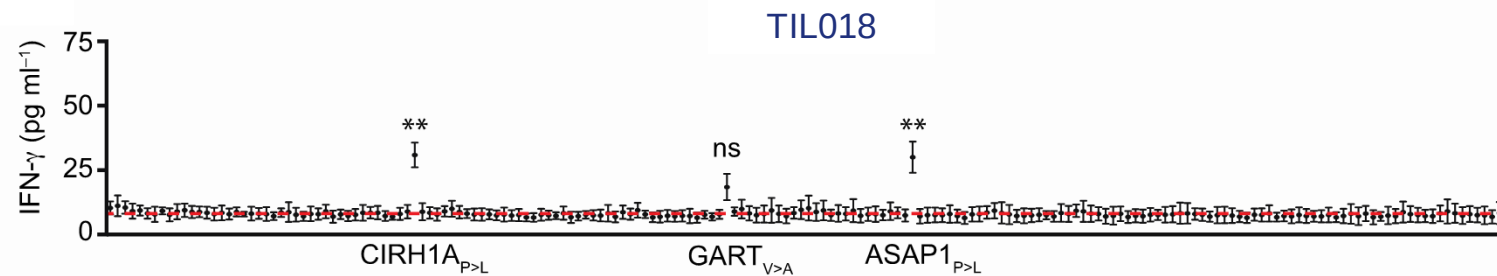


Linnemann, van Buuren, Bies et al, *Nat Med* 2014

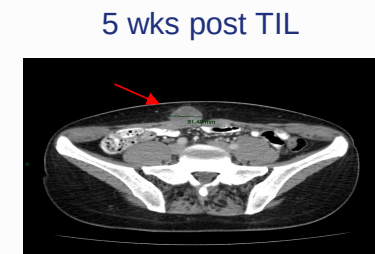
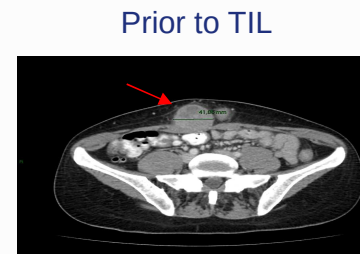
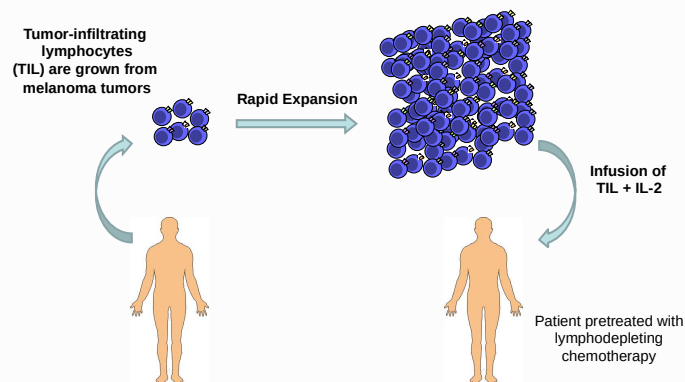
Evidence for neo-antigen reactive CD4 T cells?



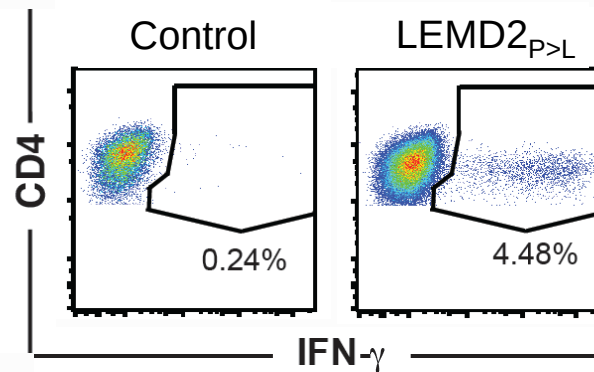
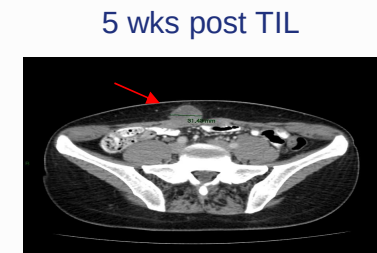
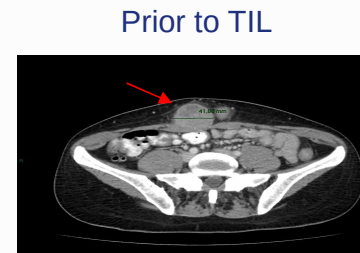
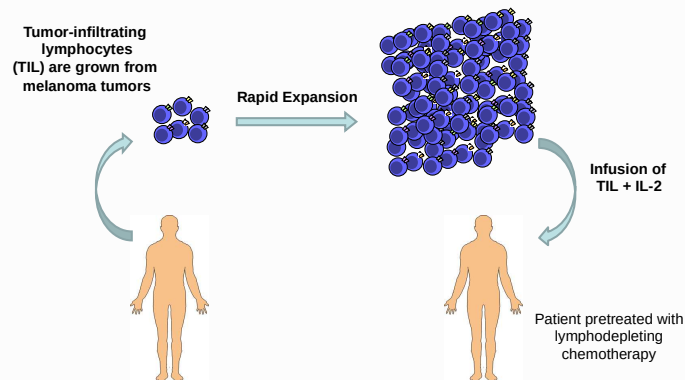
Evidence for neo-antigen reactive CD4 T cells?



Neo-antigen reactive CD4 T cells in clinically effective T cell products? (6 months PR upon TIL therapy)

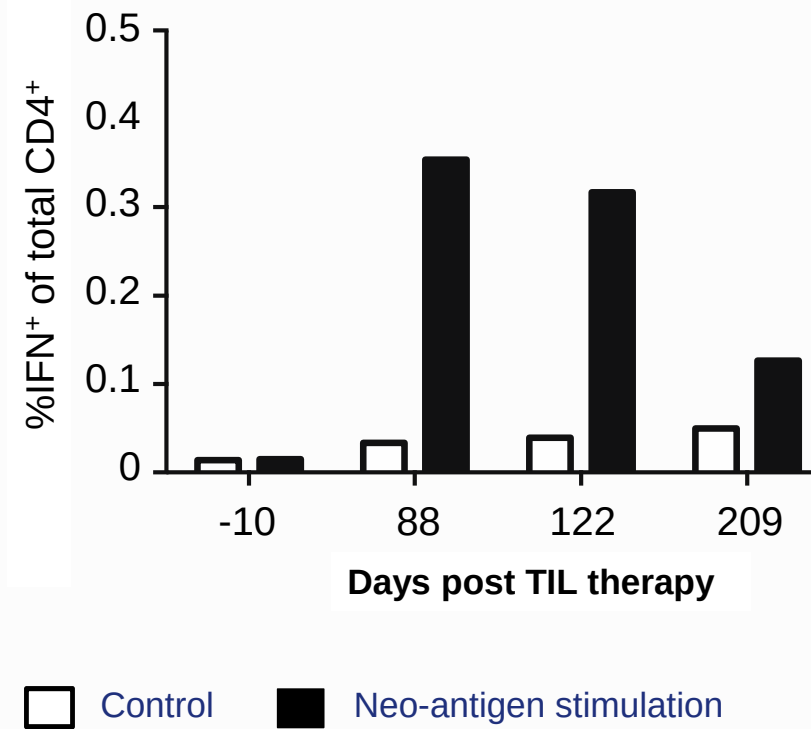


Neo-antigen reactive CD4 T cells in clinically effective T cell products? (6 months PR upon TIL therapy)

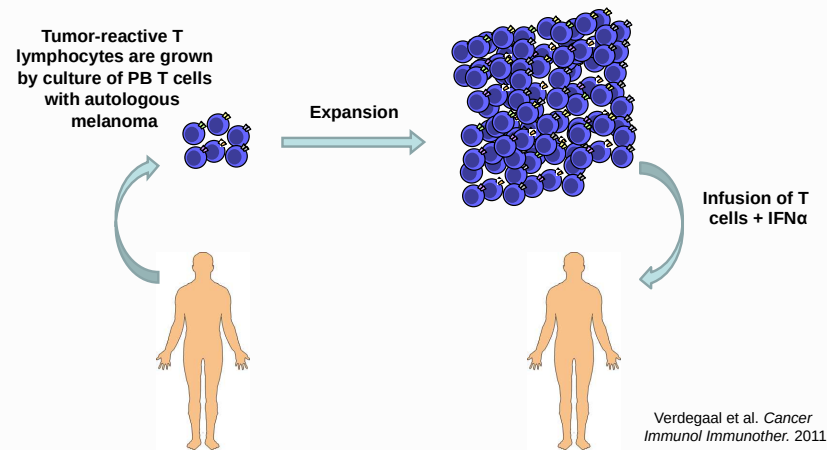


Neo-antigen reactive CD4 T cells in clinically effective T cell products? (6 months PR upon TIL therapy)

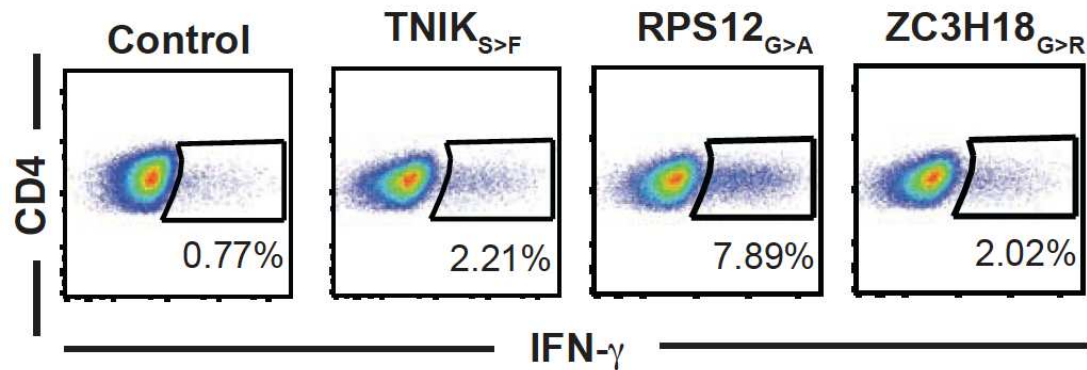
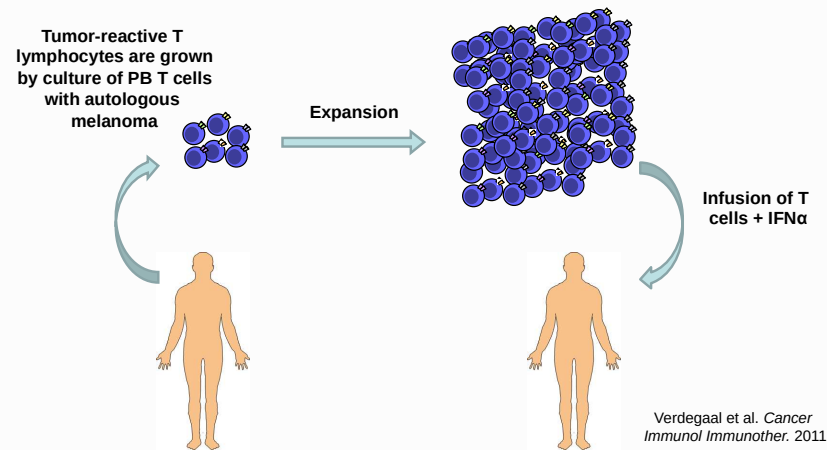
LEMD2_{P>L} specific CD4⁺ T cells persist after TIL therapy



Neo-antigen reactive CD4 T cells in clinically effective T cell products? (>7 years CR upon T cell therapy)



Neo-antigen reactive CD4 T cells in clinically effective T cell products? (>7 years CR upon T cell therapy)



Linnemann, van Buuren, Bies et al, *Nat Med* 2014

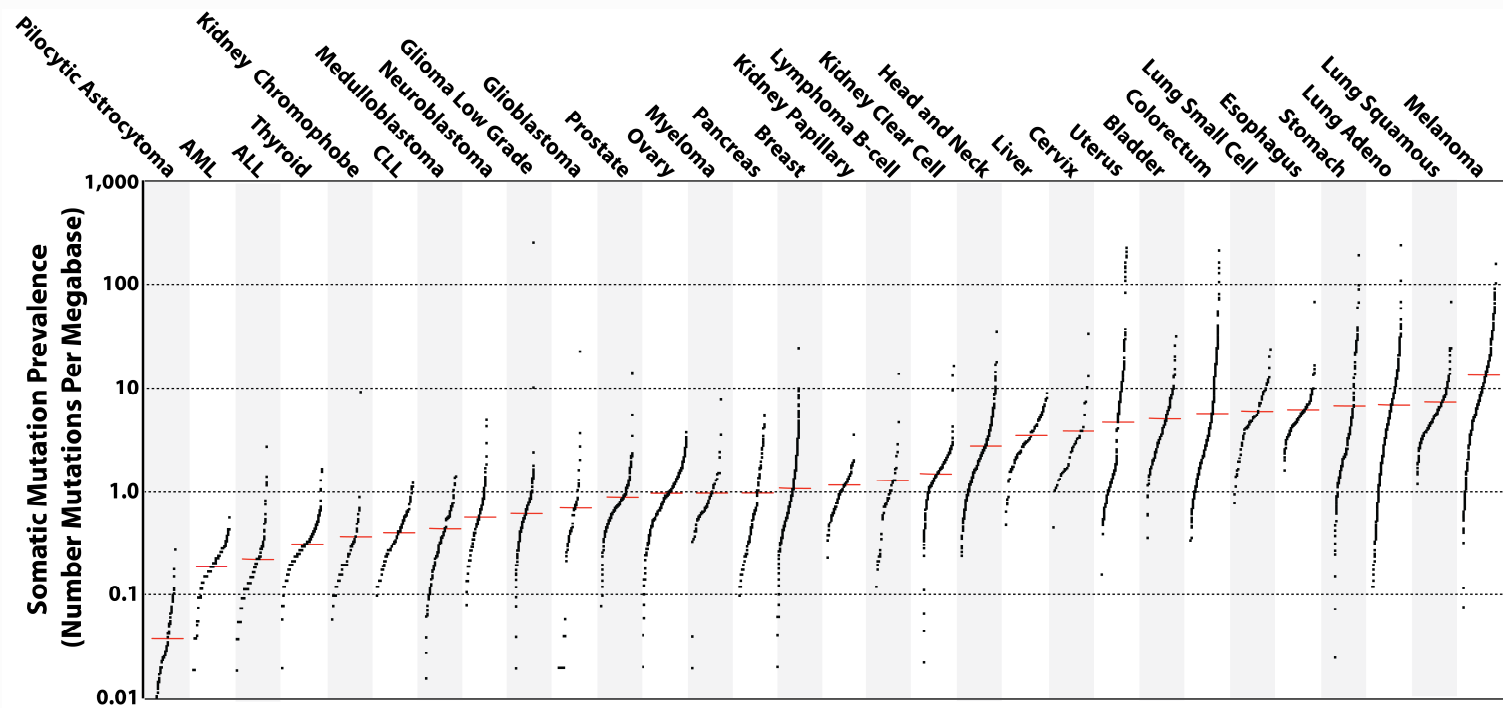


Summary of CD4 T cell analyses

5 patients analyzed, neo-antigen specific reactivity in 4

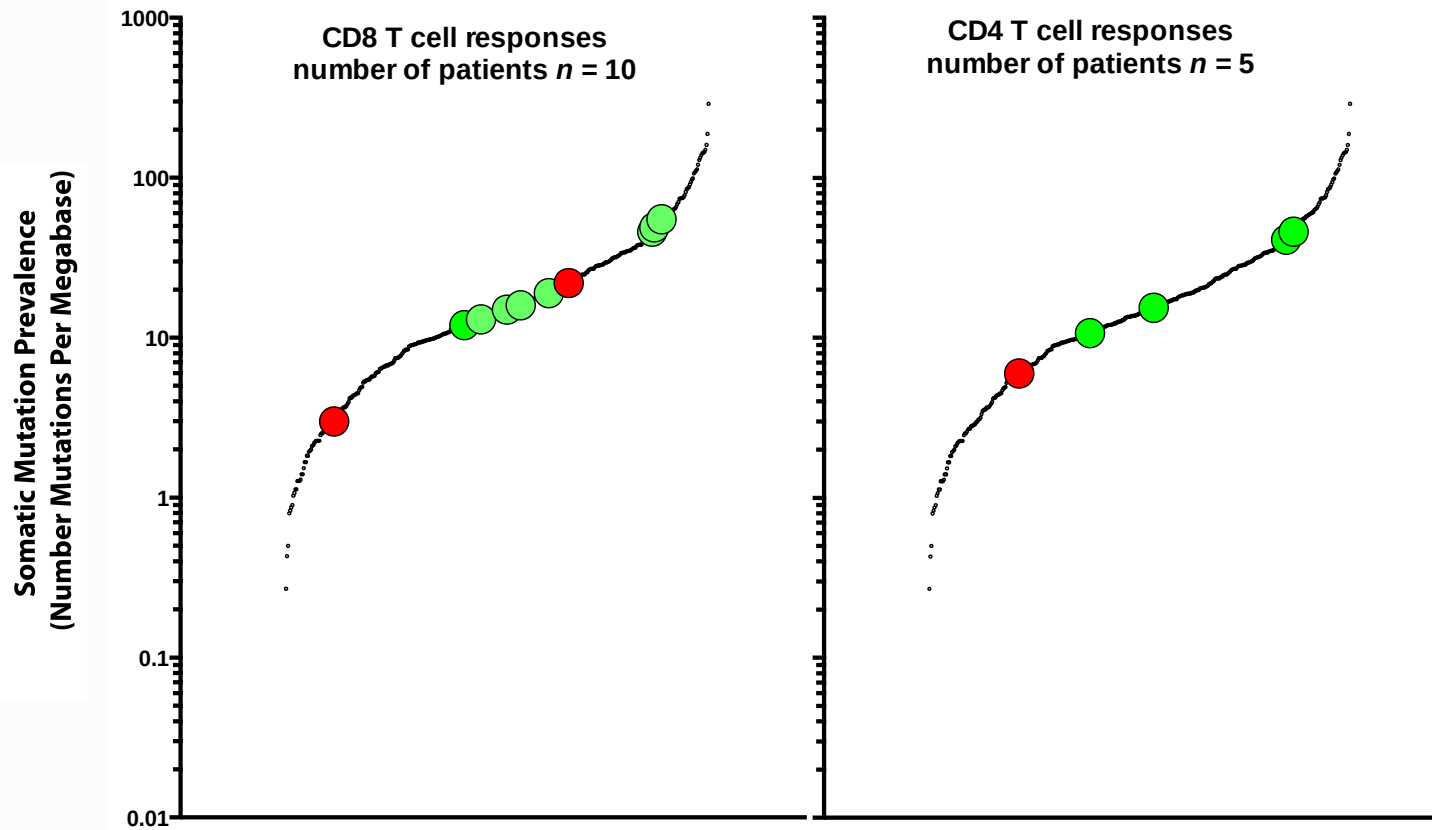
CD4 T cells also frequently respond to the consequence of DNA damage

Mutational load in human malignancies



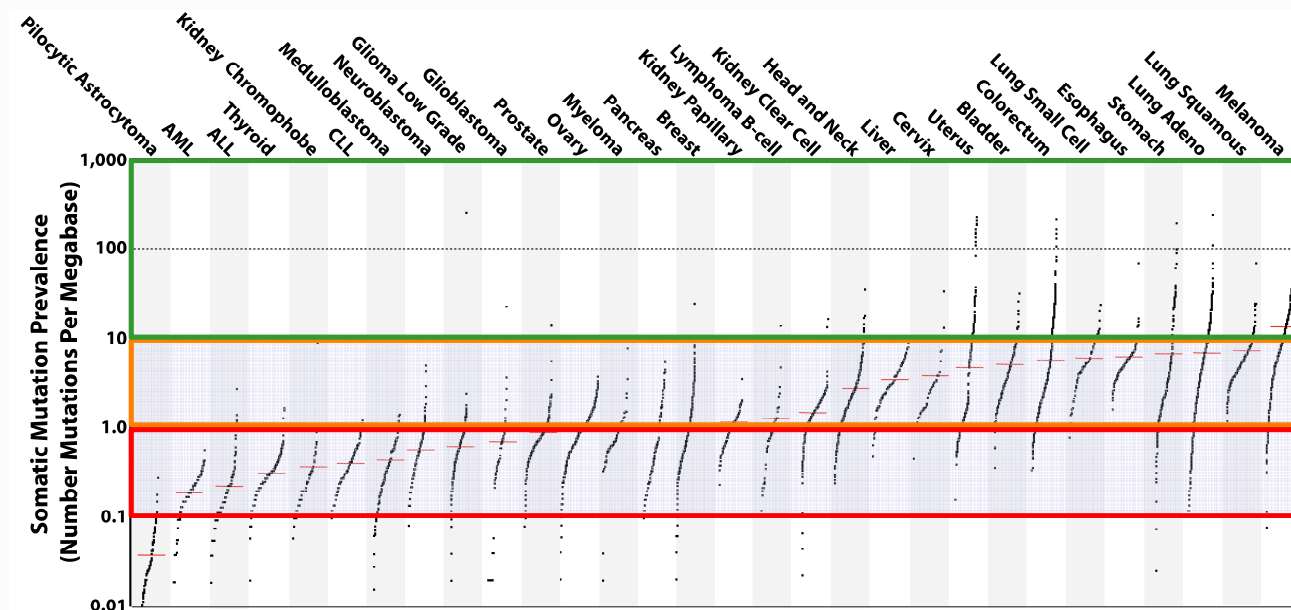
Adapted from Alexandrov et al, *Nature* 2013

Mutational load and T cell recognition of neo-antigen



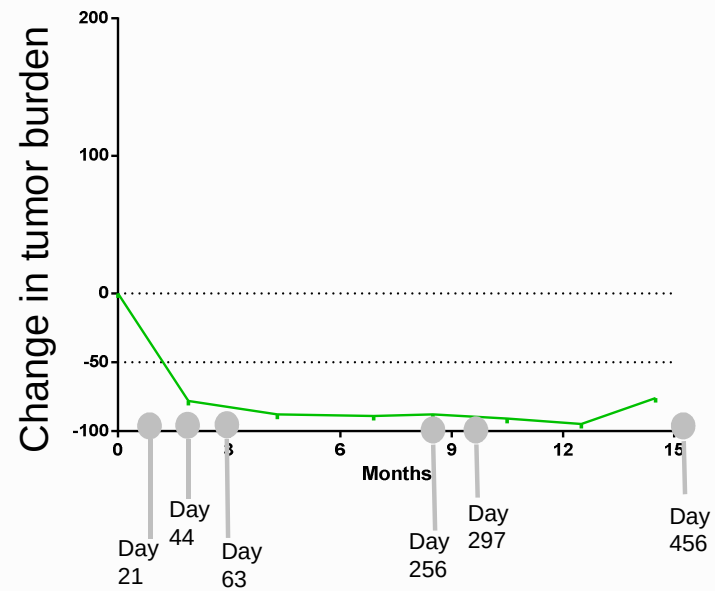
Melanoma data set from Alexandrov, *Nature* 2013 with positives ● and negatives ● superimposed

Can we expect a neo-antigen repertoire in other human cancers?

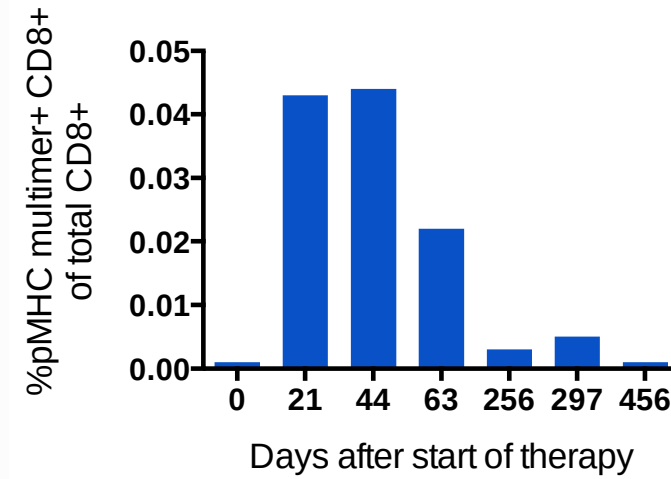
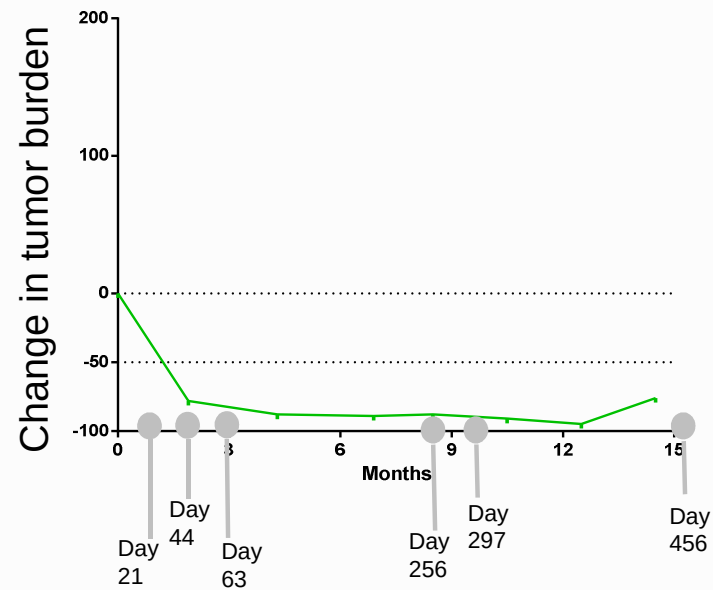


Adapted from Alexandrov et al, *Nature* 2013

NSCLC: Induction of neo-antigen specific T cell reactivity upon PD-1 blockade

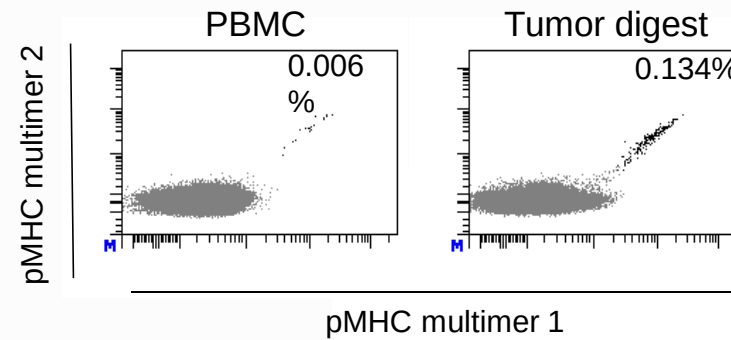
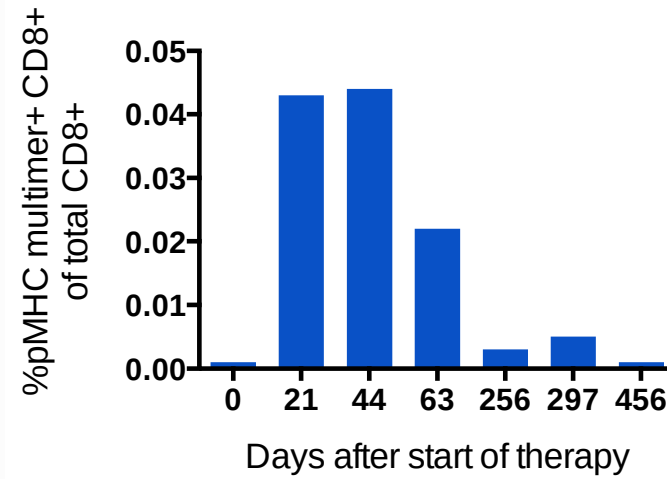
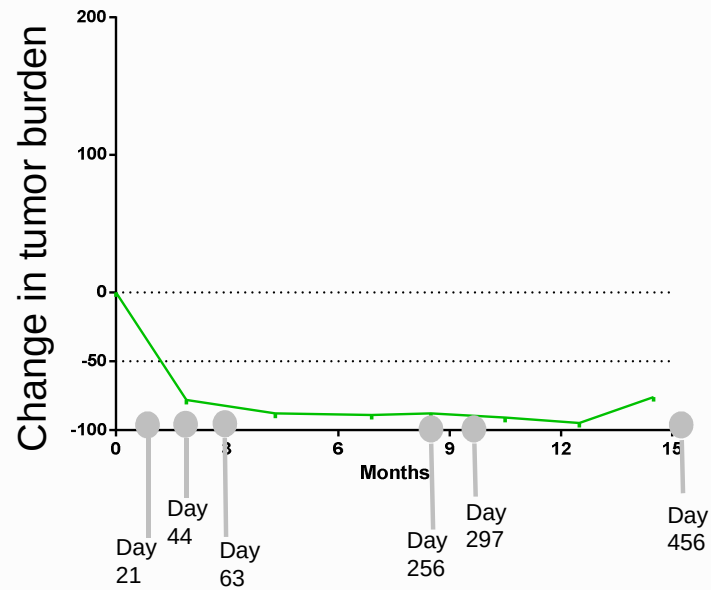


NSCLC: Induction of neo-antigen specific T cell reactivity upon PD-1 blockade



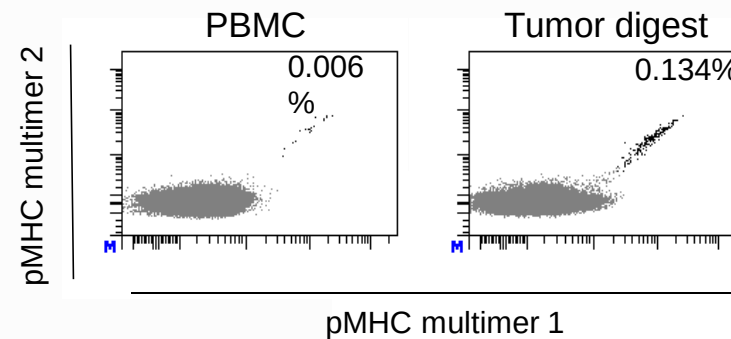
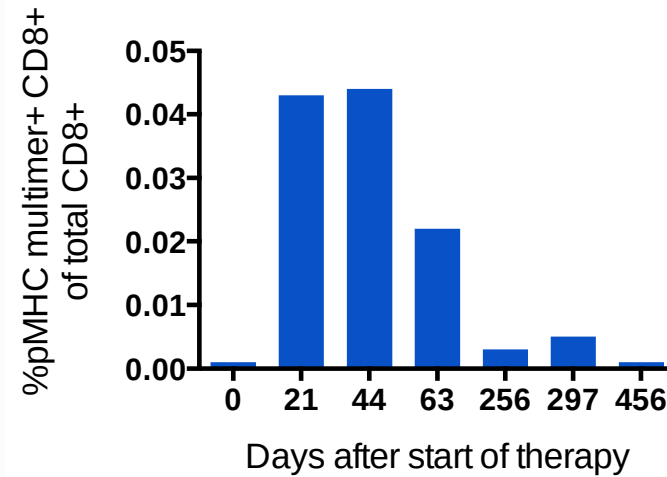
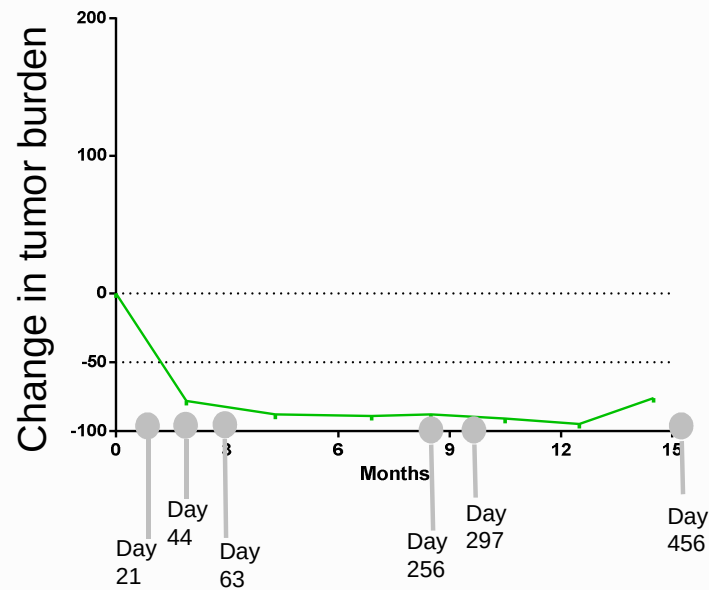
Rizvi et al, *Science* 2015

NSCLC: Induction of neo-antigen specific T cell reactivity upon PD-1 blockade



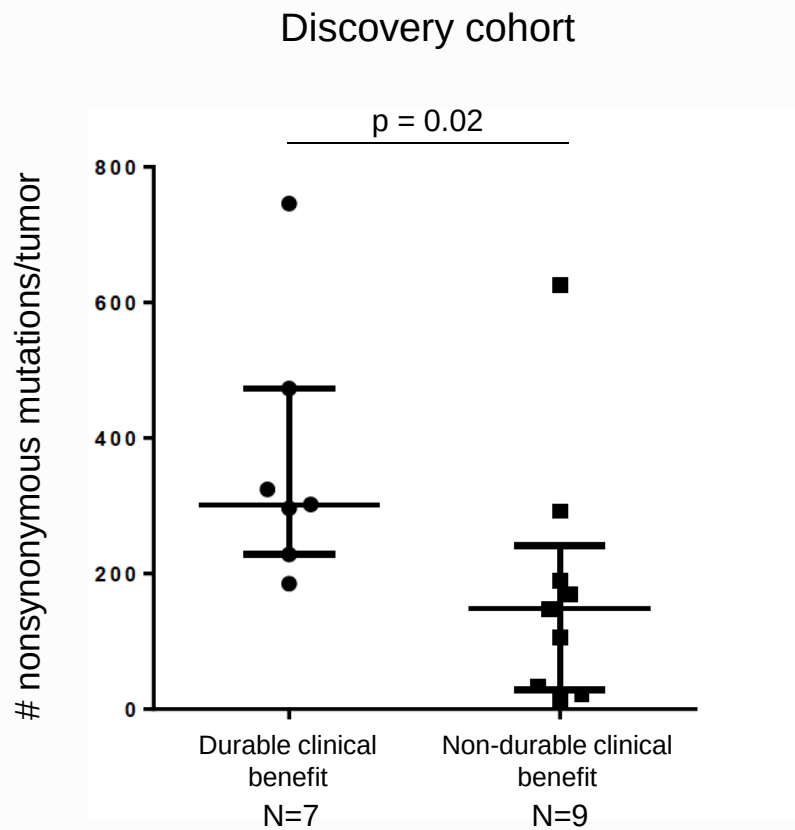
Rizvi et al, *Science* 2015

NSCLC: Induction of neo-antigen specific T cell reactivity upon PD-1 blockade

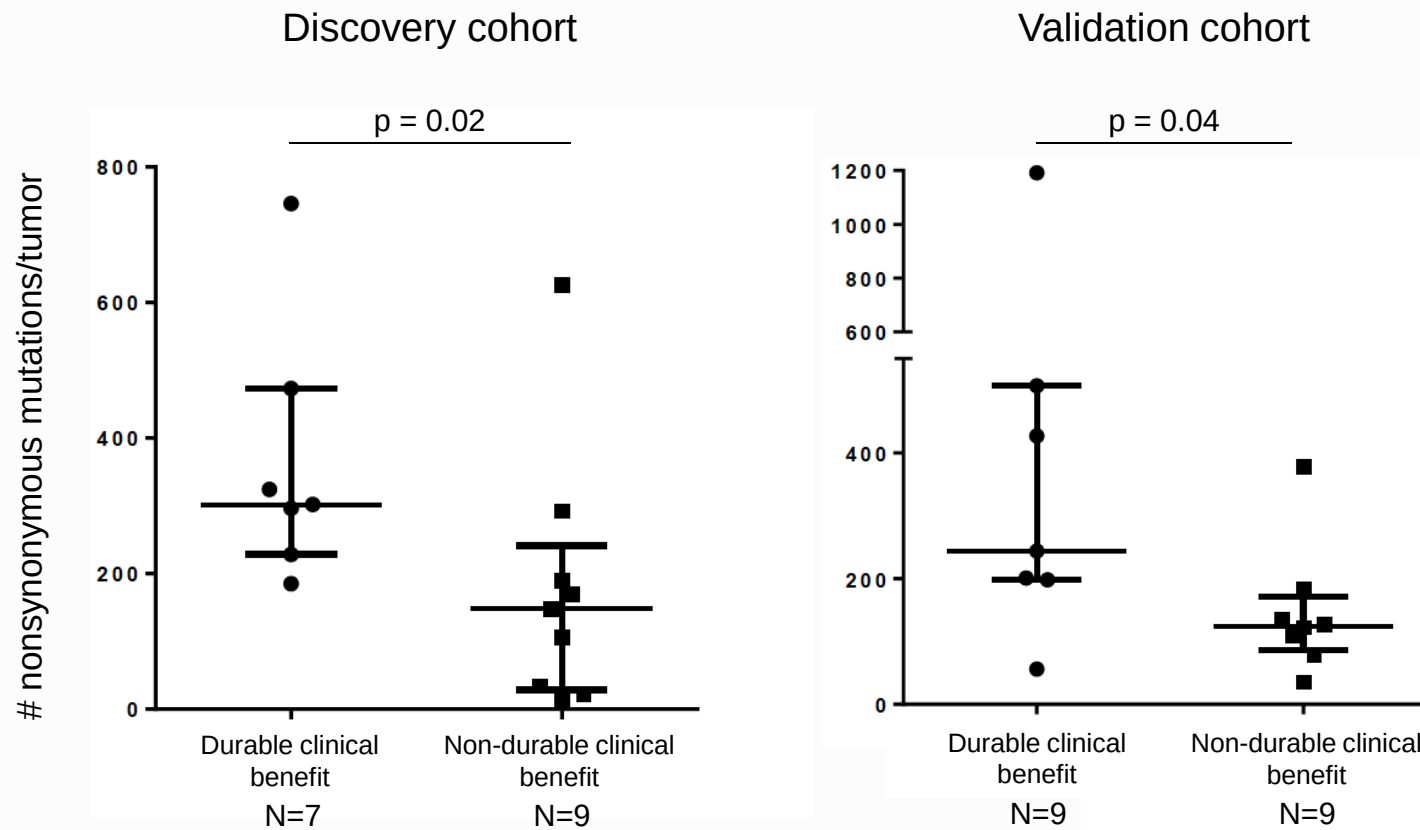


First evidence for neo-antigen specific CD8 T cell reactivity in lung cancer

NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)



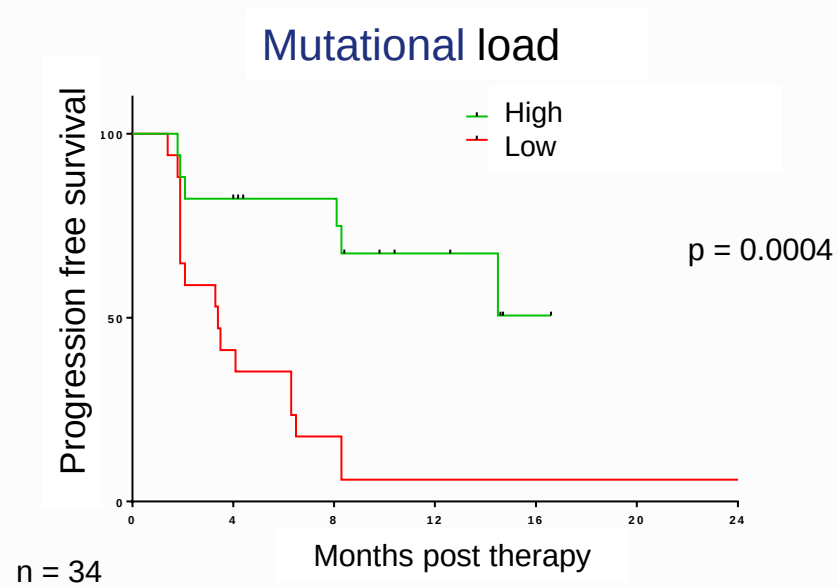
NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)



Rizvi et al, *Science* 2015



NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)



Rizvi et al, *Science* 2015

Conclusion and future directions

- ❖ The T cell based immune system commonly responds to the consequences of DNA damage in human melanoma
- ❖ Recognition of neo-antigens is likely to be a major component of clinically active immunotherapies
 - Tumor types with high mutational load are more likely to respond to immunotherapy

Note: NO clear threshold

Not (very) useful as a predictor of response for individual patients

Useful to understand biology of tumor control

Useful to identify tumor types that are attractive targets for immunotherapy

Incentive to develop therapies that boost neo-ag. specific T cell responses

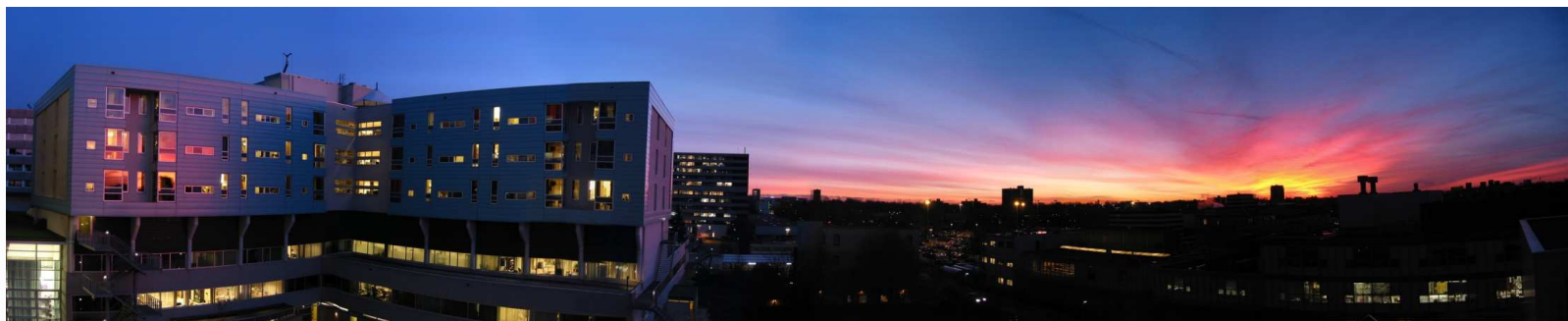


Conclusion and future directions

- ❖ Neo-antigen specific T cell reactivity can be enhanced by different types of immunotherapies (antiCTLA-4, antiPD-1, TIL therapy)
- ❖ Efforts to specifically enhance T cell reactivity against neo-antigens would be highly attractive

Lessons and Take Home Messages

- MHC multimers provide an excellent tool to study tumor-specific CD8 T cell in patients
- This technology allows to study tumor-specific T cell populations in all cancer types in peripheral blood and TIL in relation to cancer immunotherapy
- Neo-antigen-specific CD8 and CD4 T cells are common in melanoma and are likely to play a key role in cancer immunotherapy in melanoma and beyond



KG JEBSEN CENTER
for Cancer Immunotherapy



Cancer Immunotherapy
Dream Team

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AIMM Therapeutics

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Patrick Hwu

LUMC

Els Verdegaal

Sjoerd van der Burg

Ella Institute, Tel Aviv

Michal Besser

Jakob Schachter

Memorial Sloan Kettering

Naiyer Rizvi

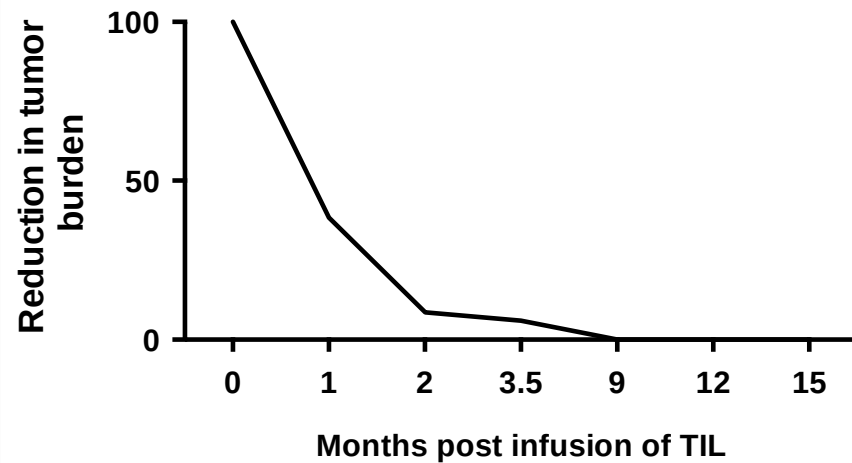
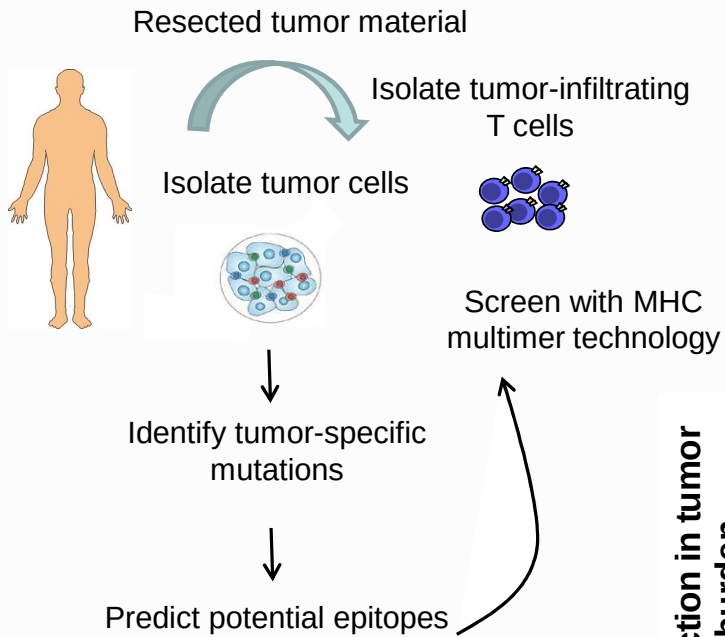
Matthew Hellman

Alexandra Snyder

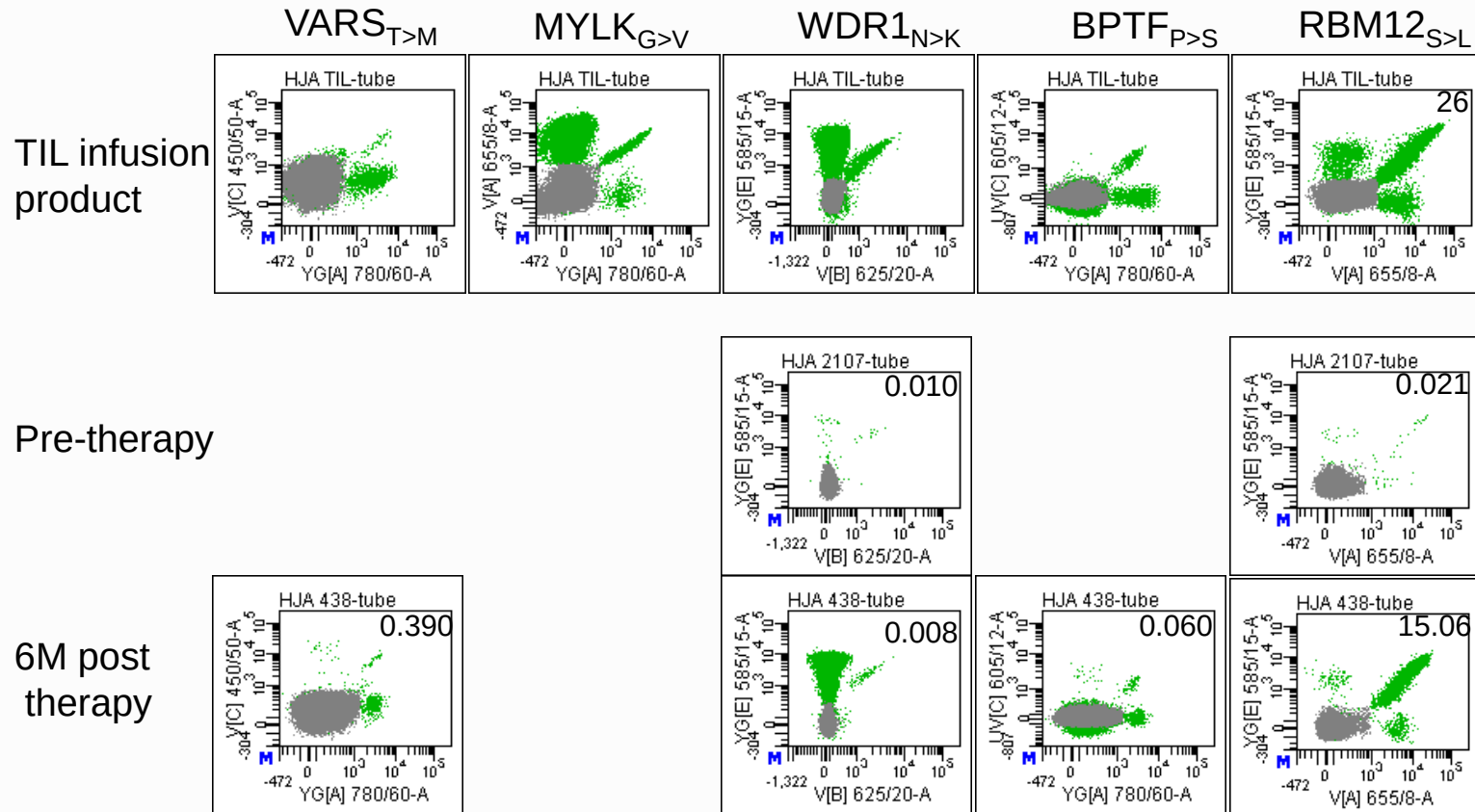
Timothy Chan



Pt 010: complete response upon TIL therapy

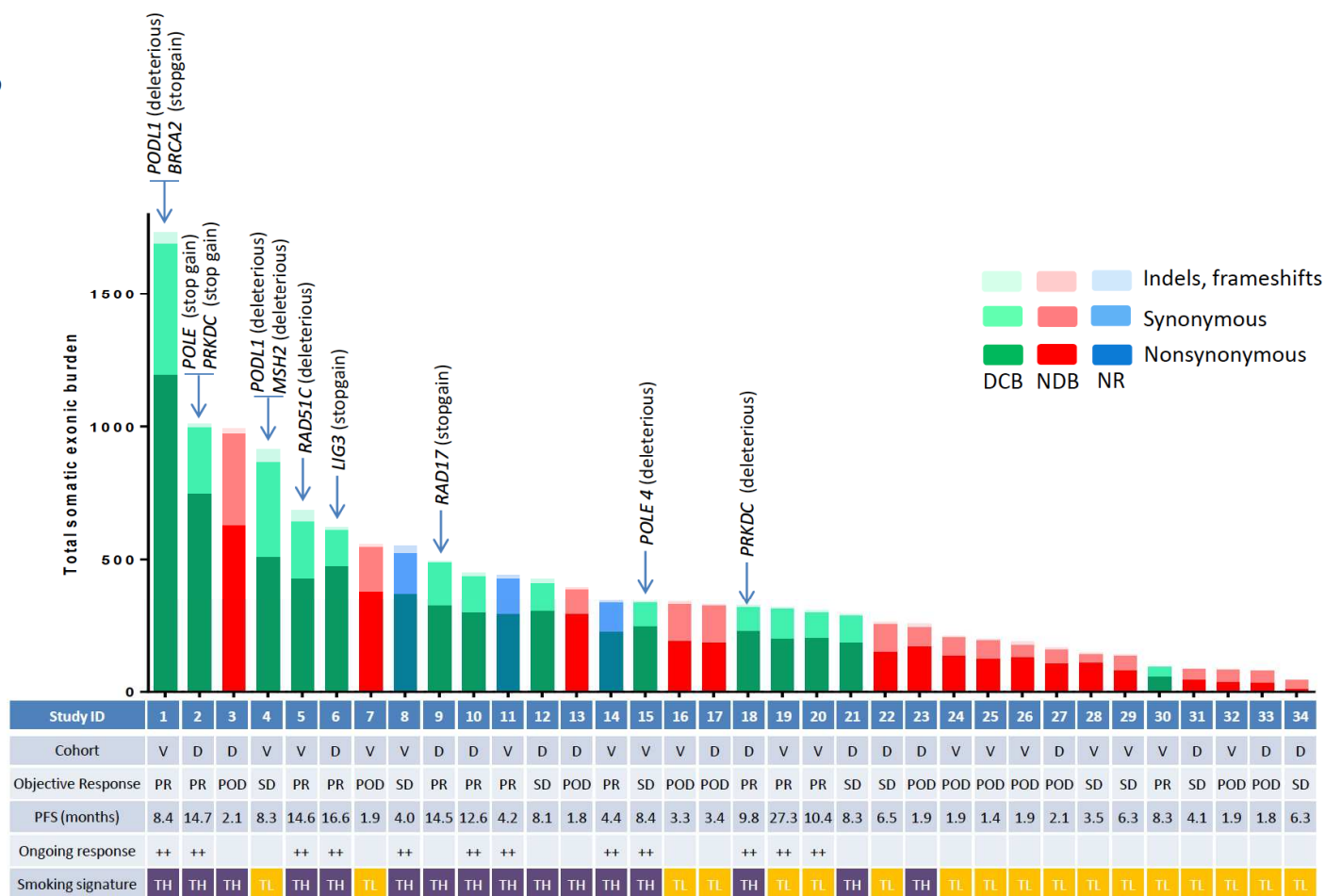


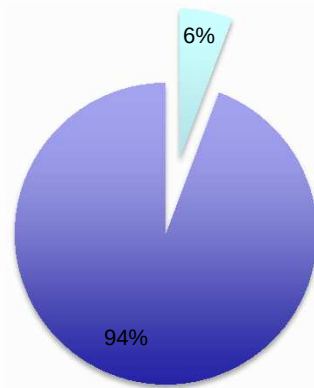
Pt 010: complete response upon TIL therapy



Major (500 fold) increase in neo-antigen specific T cell reactivity upon TIL therapy

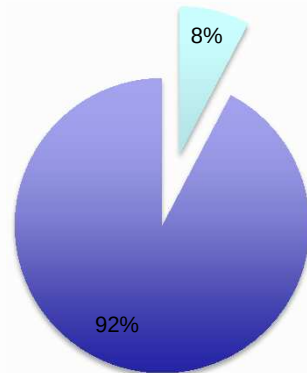
3





**9, 10 and 11 mers
Melanoma only**

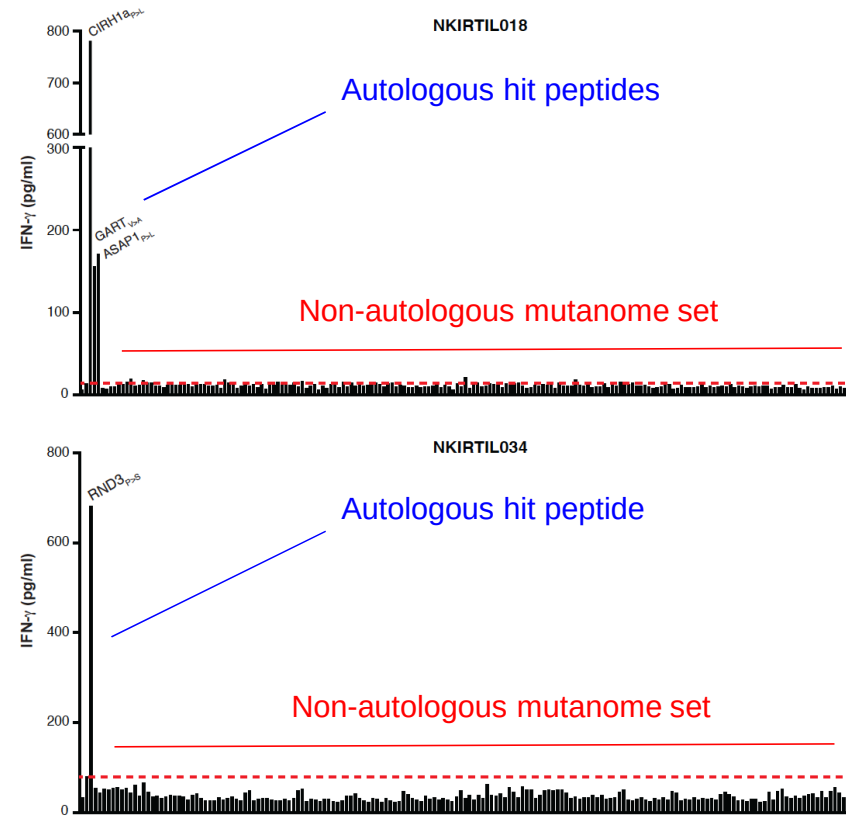
- epitopes with signature (n = 2)
- epitopes without signature (n = 31)



**9 mers
Melanoma only**

- epitopes with signature (n = 1)
- epitopes without signature (n = 13)

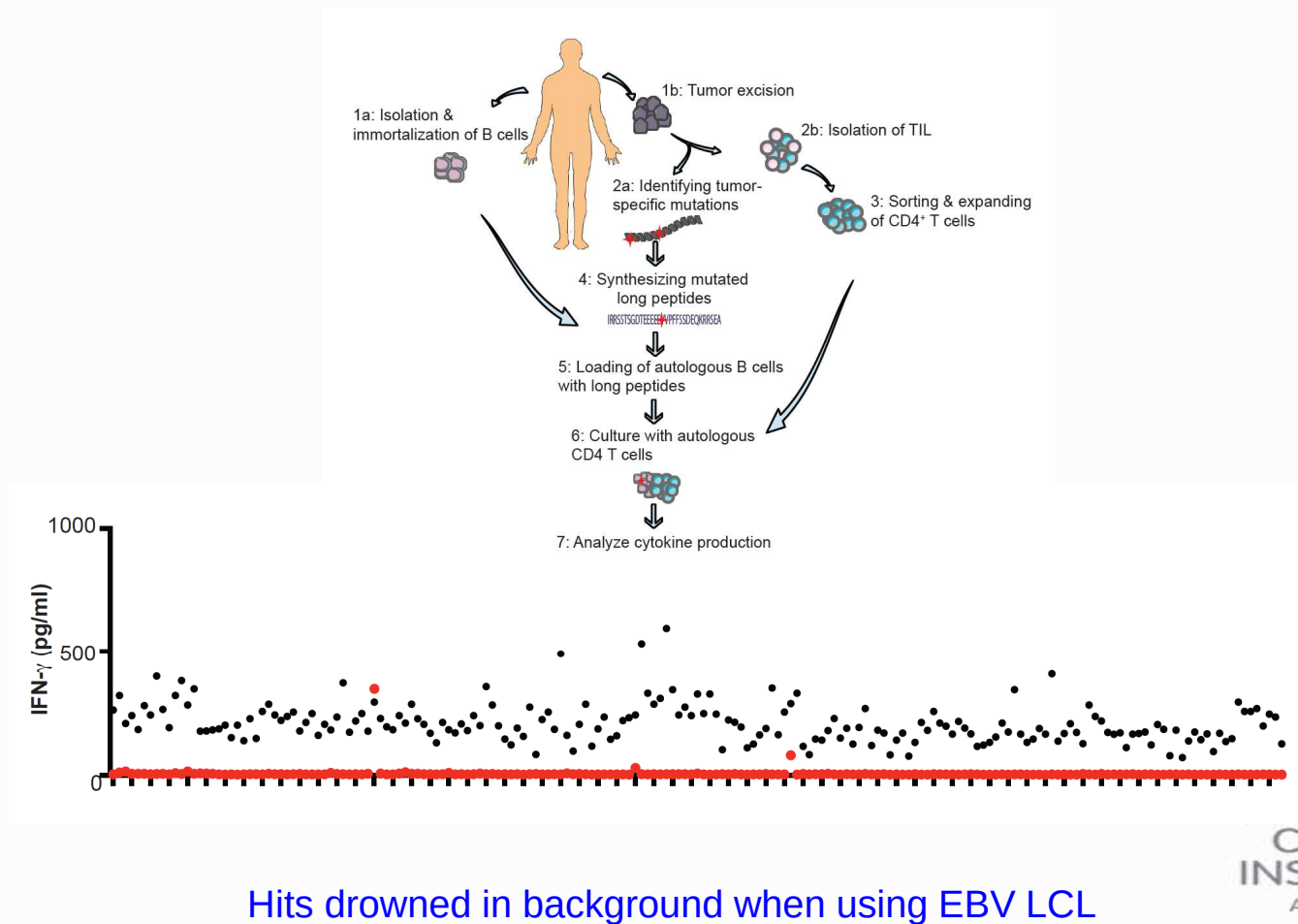
Evidence for neo-antigen reactive CD4 T cells?



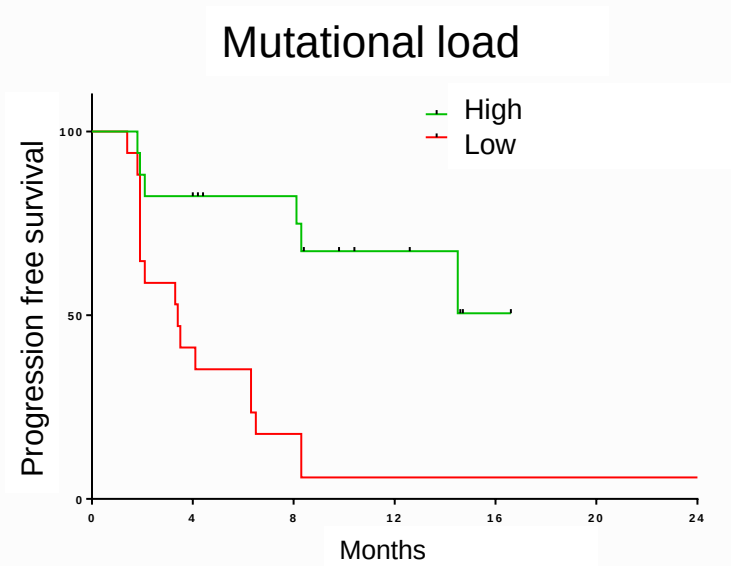
If True: Only reactivity against autologous mutanome set

If True: Reactivity against mutant peptides, reactivity against parental peptides

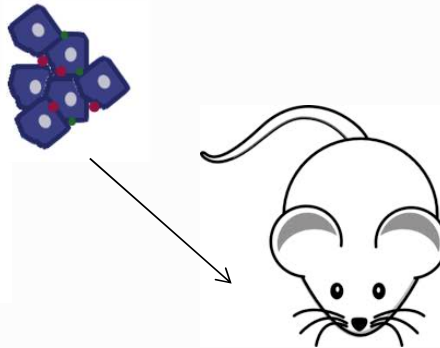
Side note: Oncogene-immortalized autologous B cells versus EBV B cells



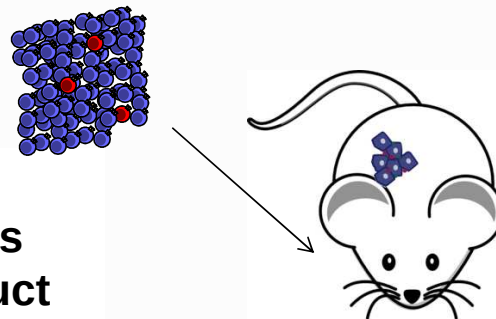
NSCLC: Mutational load and clinical outcome to antiPD-1 therapy (MSKCC)



1)
Inject human
melanoma
(NSG-mice)



2a)
Inject autologous
bulk T-cell product



2b)
Inject autologous
neo-Ag enriched
T-cell product



3)
Monitor tumor
growth



Neo-antigen enriched TIL can mediate superior tumor control

