

Defining the role of mutated antigens

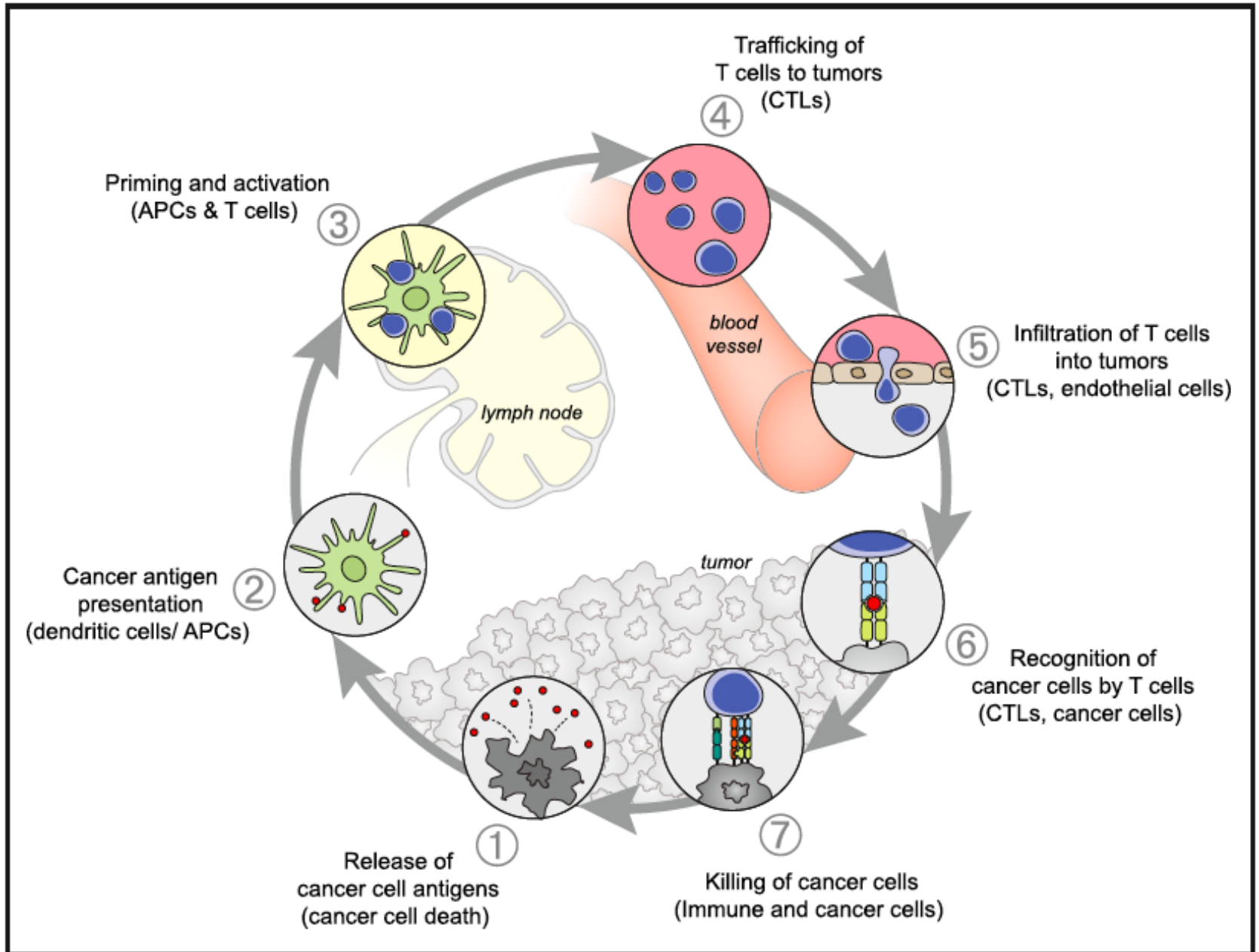
John Haanen

NETHERLANDS
CANCER
INSTITUTE

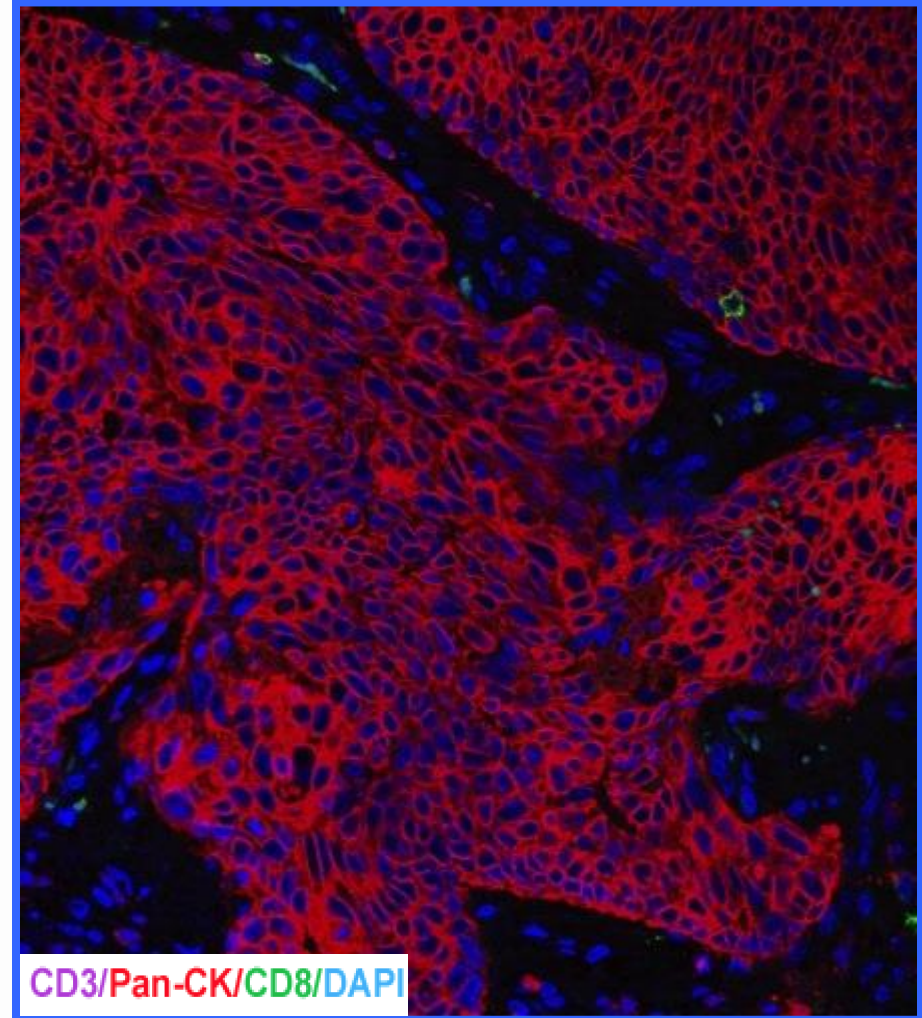
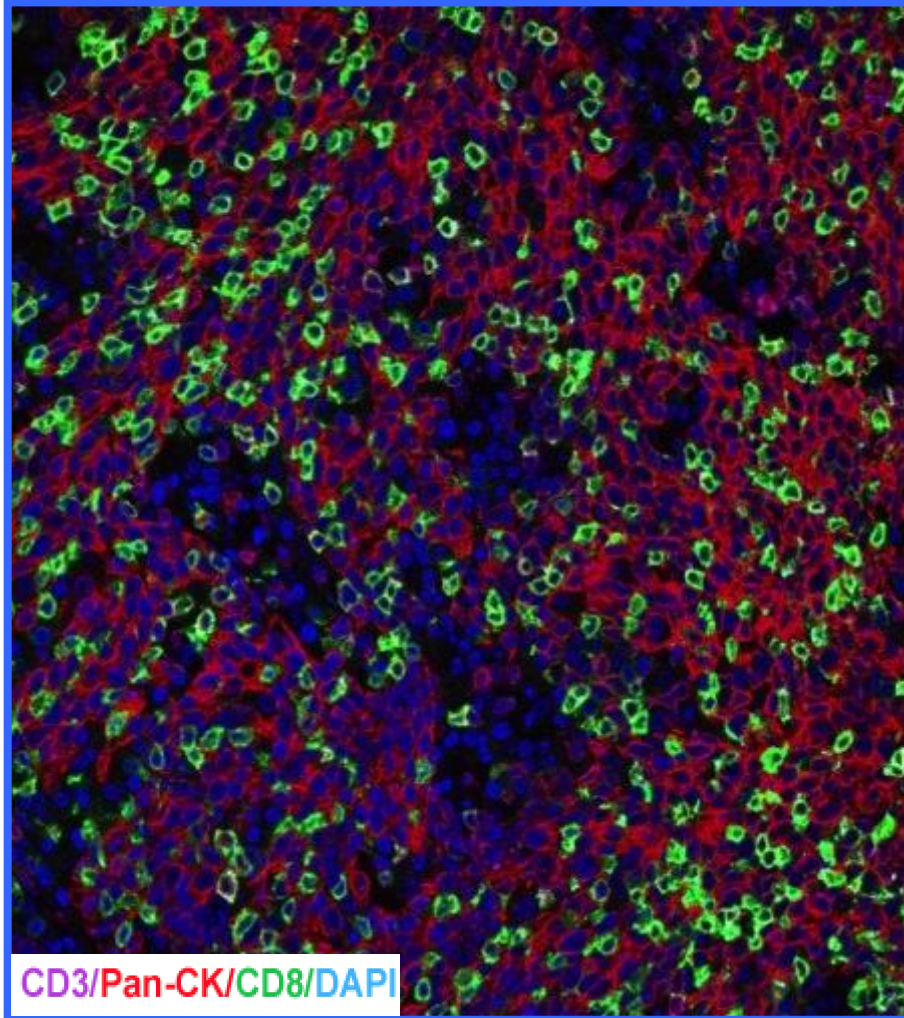
ANTONI VAN LEEUWENHOEK



Cancer immunity cycle



HNSCC commonly triggers Immune Responses: Tumor Infiltrating Lymphocytes



Role for T cells in cancer

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D.,
Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D.,
Marco Massobrio, M.D., Giorgia Regnani, M.D.,
Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D.,
Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D.,
Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

PERSPECTIVES

OPINION

The immune contexture in human tumours: impact on clinical outcome

Wolf Herman Fridman, Franck Pagès, Catherine Sautès-Fridman and
Jérôme Galon



Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, *et al.*
Science **313**, 1960 (2006);
DOI: 10.1126/science.1129139

Cancer Research

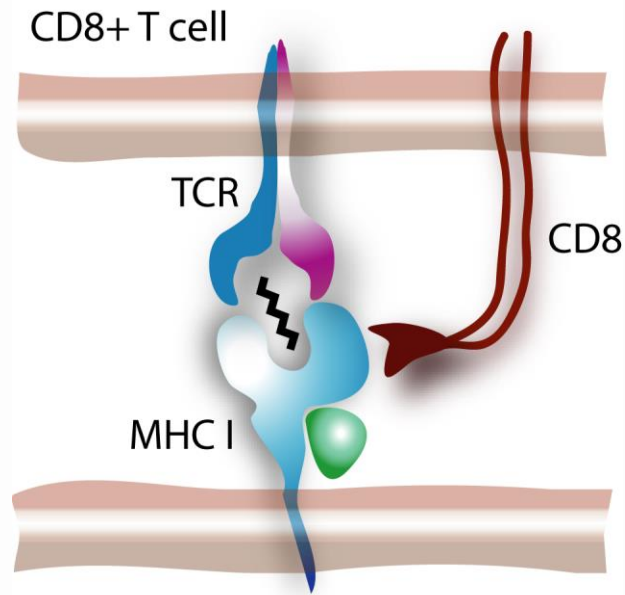


Immunotype and Immunohistologic Characteristics of Tumor-Infiltrating Immune Cells Are Associated with Clinical Outcome in Metastatic Melanoma

Gulsun Erdag, Jochen T. Schaefer, Mark E. Smolkin, et al.

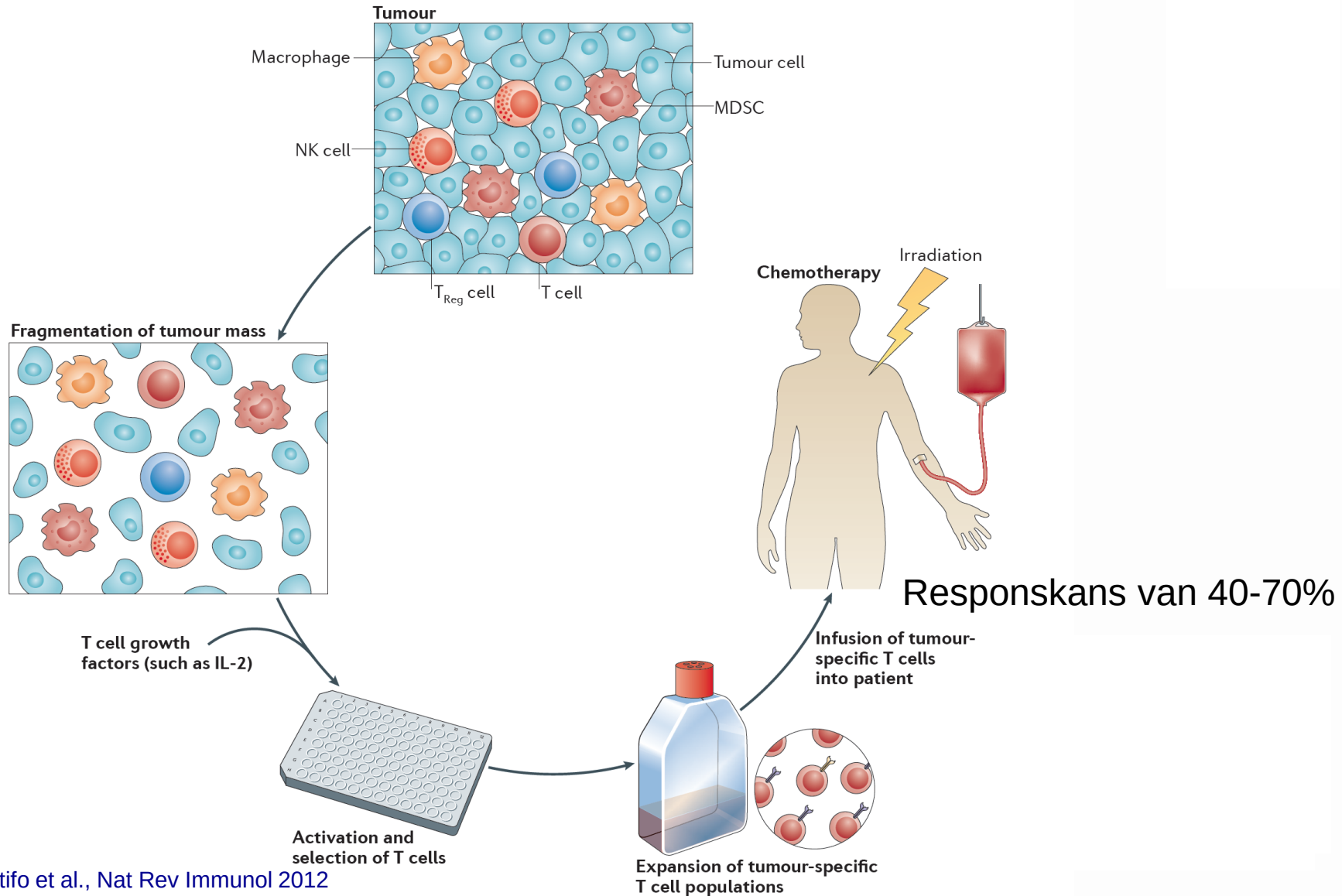
Cancer Res 2012;72:1070-1080. Published OnlineFirst January 19, 2012.

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



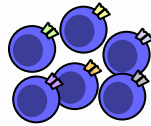
1. Self antigens (to which tolerance is incomplete)
Shared between patients
2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
In large part patient-specific, hence generally ignored

Tumor infiltrating lymphocytes: TIL therapy in melanoma

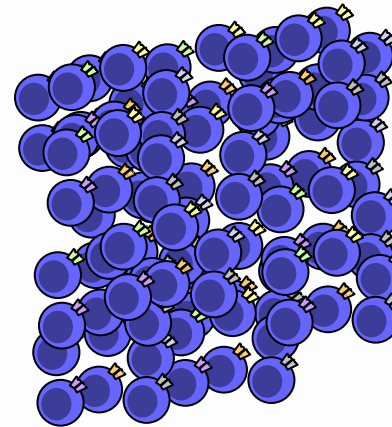


The big unknown

Tumor-infiltrating lymphocytes (TIL) are grown from melanoma tumors



Rapid Expansion



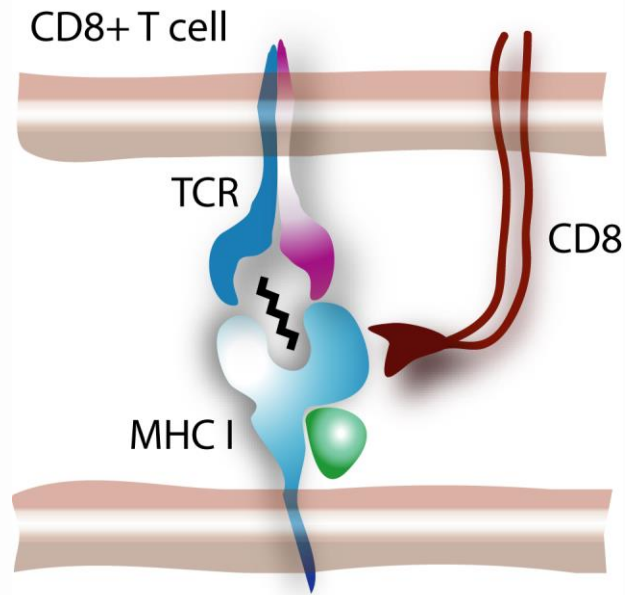
?

Infusion of TIL + IL-2

Patient pretreated with lymphodepleting chemotherapy

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

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

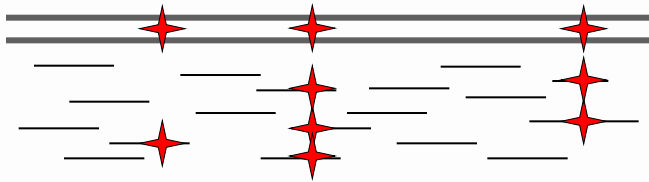


1. Self antigens (to which tolerance is incomplete)
Shared between patients (Kvistborg et al. Oncoimmunol 2013)
2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations

Develop technology for high-throughput monitoring of T cell responses

- Problem I: Many antigens (Toebe et al., Nat Med 2006)
- Problem II: Limitations in patient sample size (Anderson et al. Nat Protoc 2012)

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



Generate map of tumor-specific mutations (ExomeSeq)



Determine which mutated genes are expressed (RNASeq)

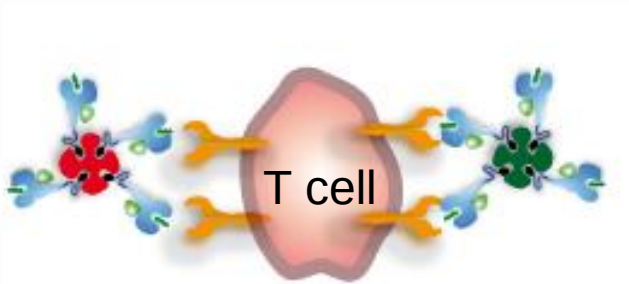


Predict epitopes for each mutation/ each HLA-allele *in silico*

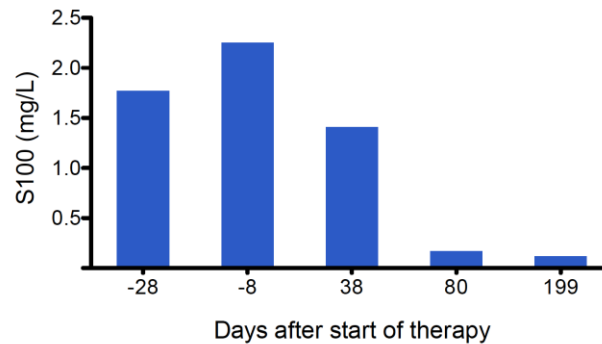
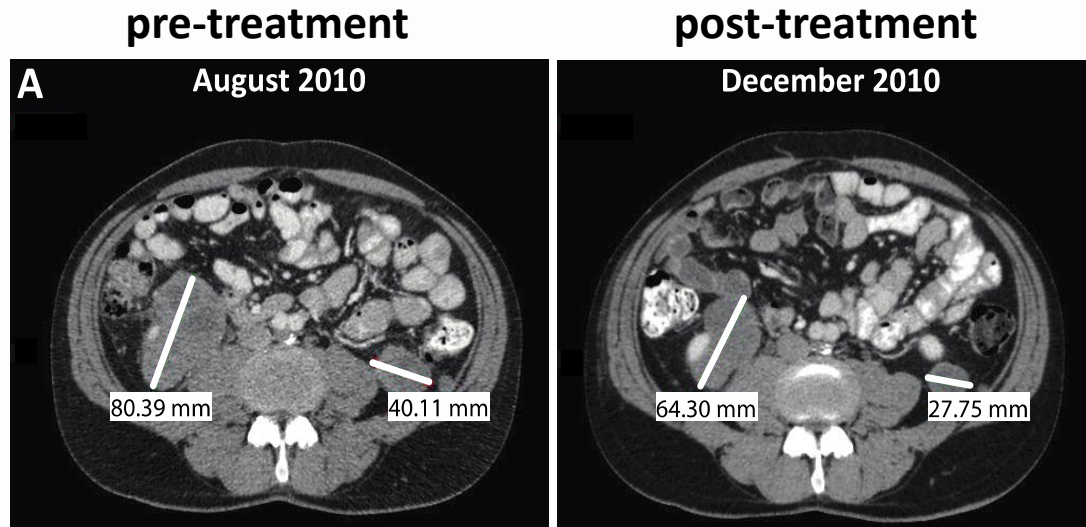


Screen for T cell recognition of mutated epitopes

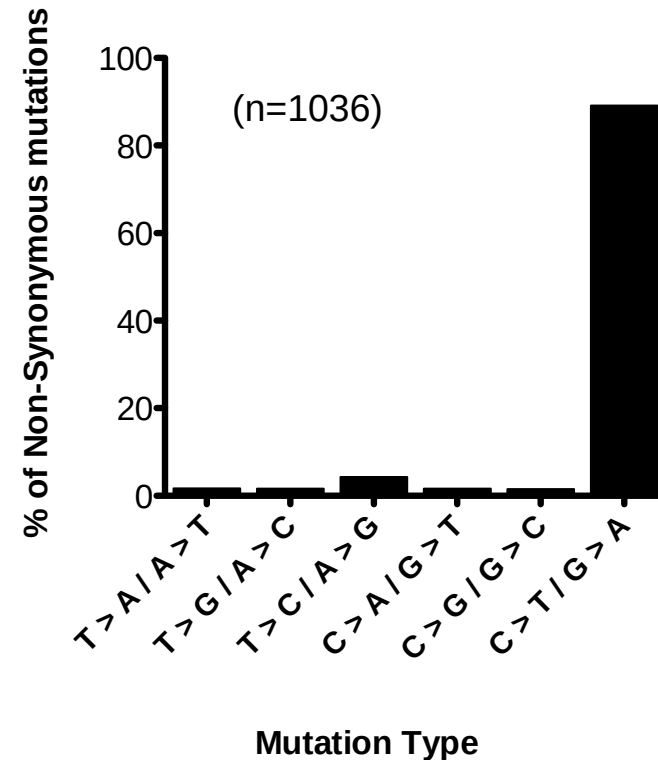
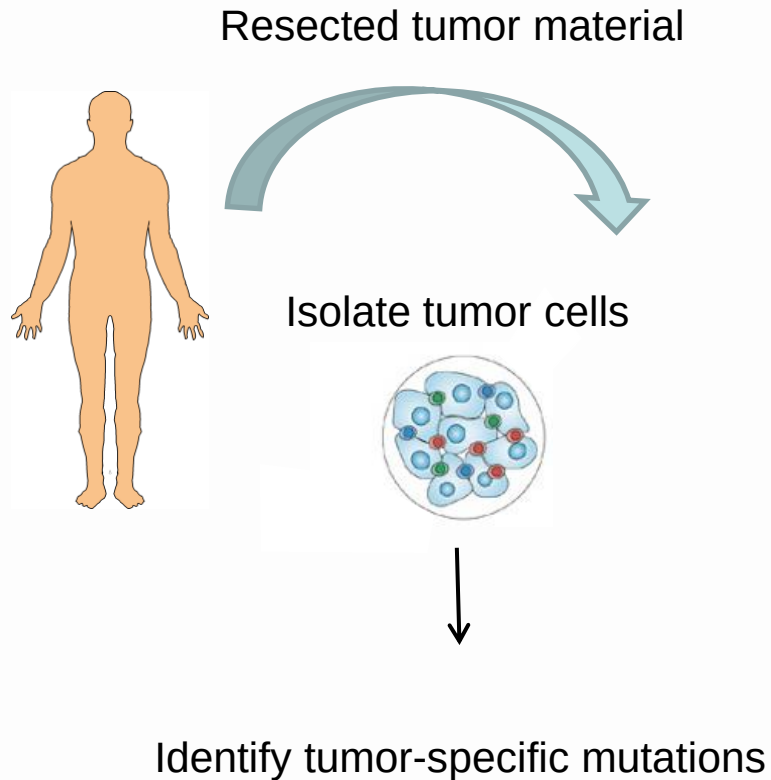
MDLVLNELV**I**SLIVESKLLLE
HLA-A2 _____
HLA-B7 _____
HLA-C2 _____



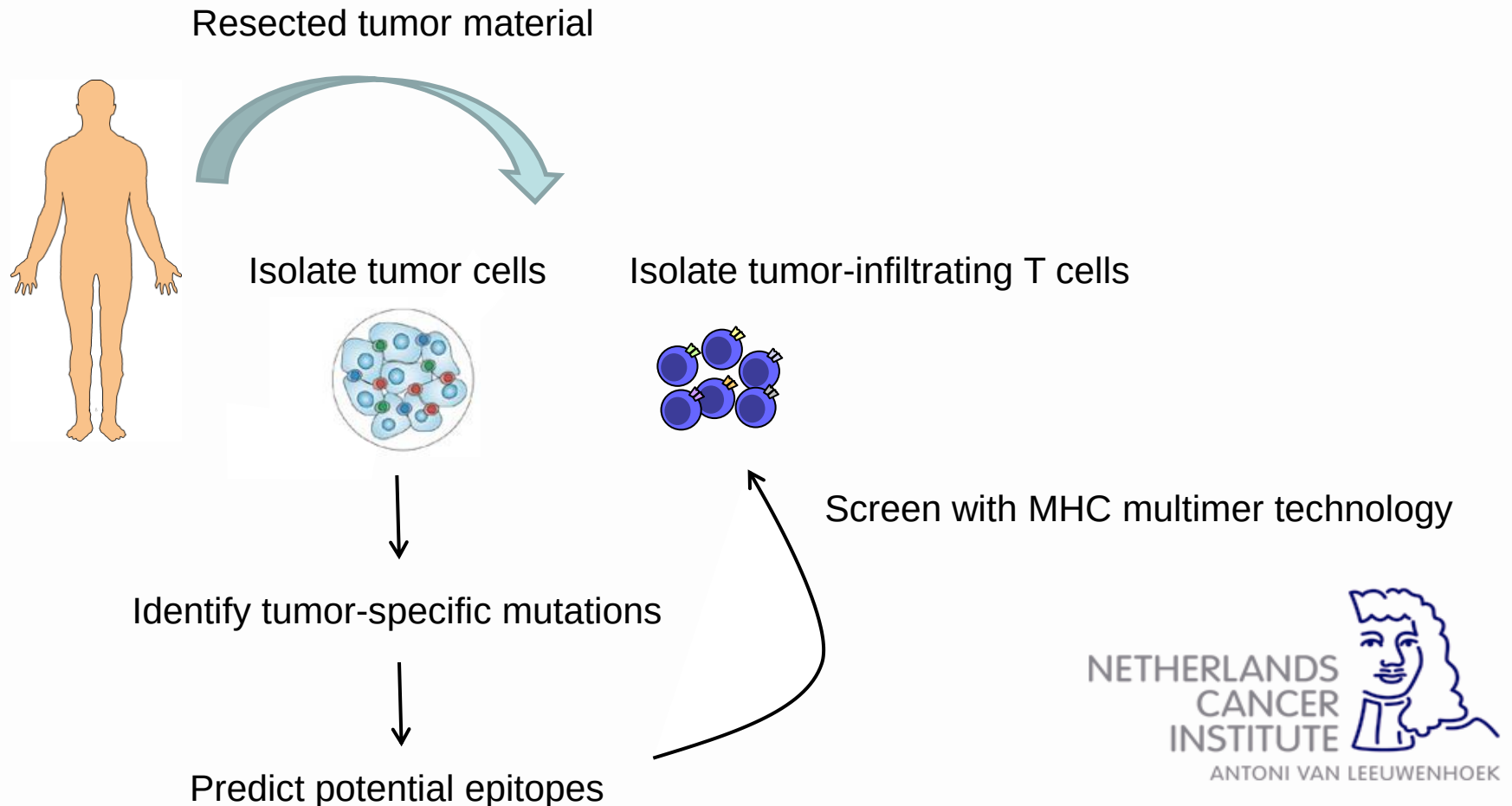
Pt 002: Partial response upon anti-CTLA4 treatment



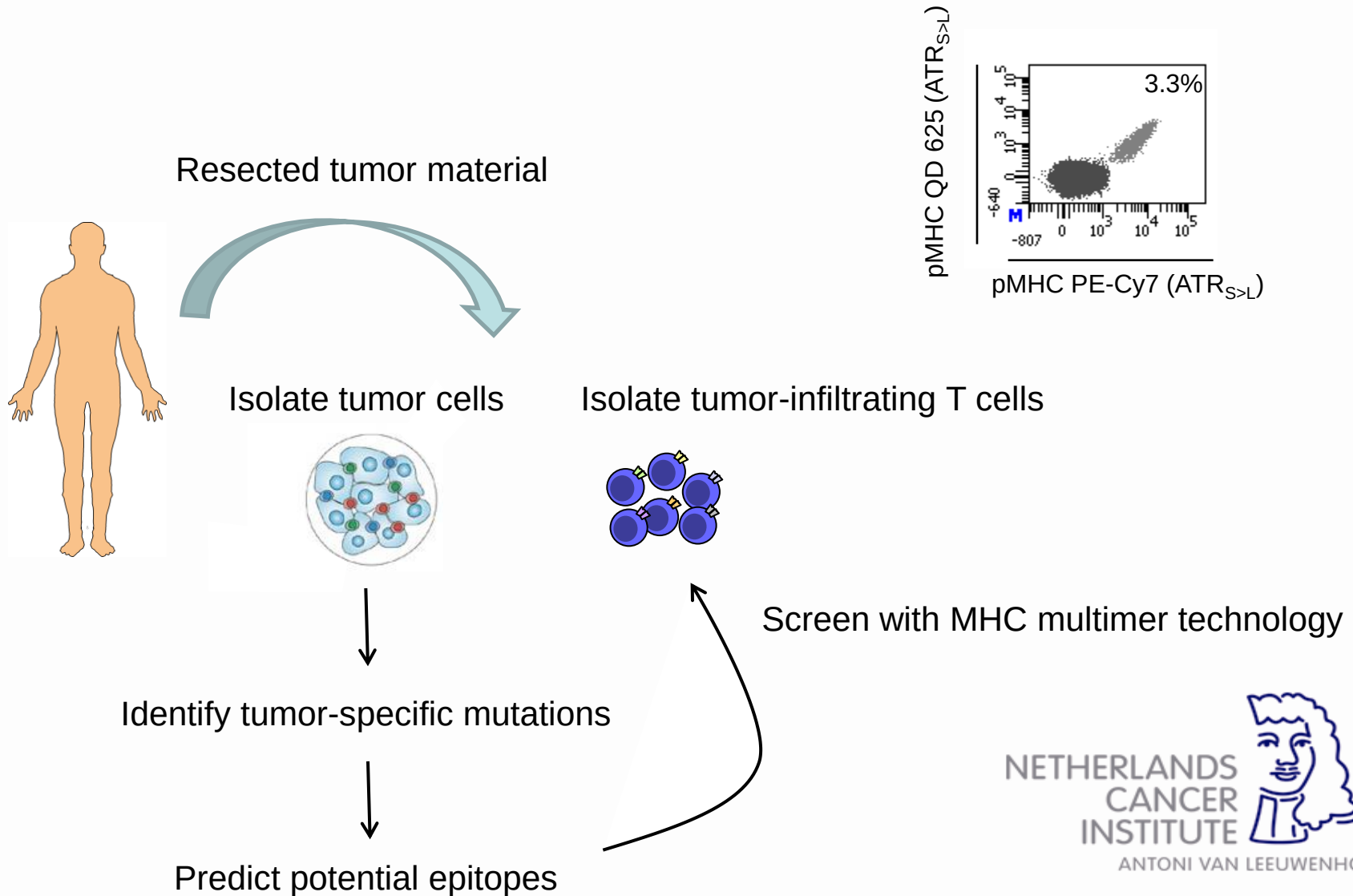
Analyzing the neo-antigen-specific T cell repertoire in human cancer?



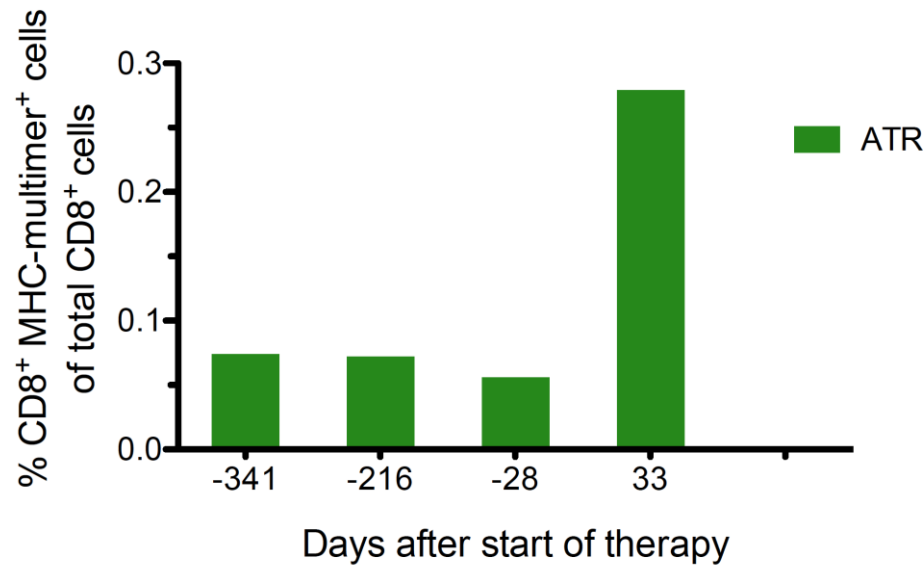
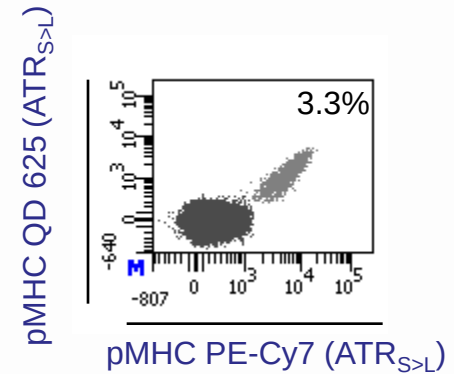
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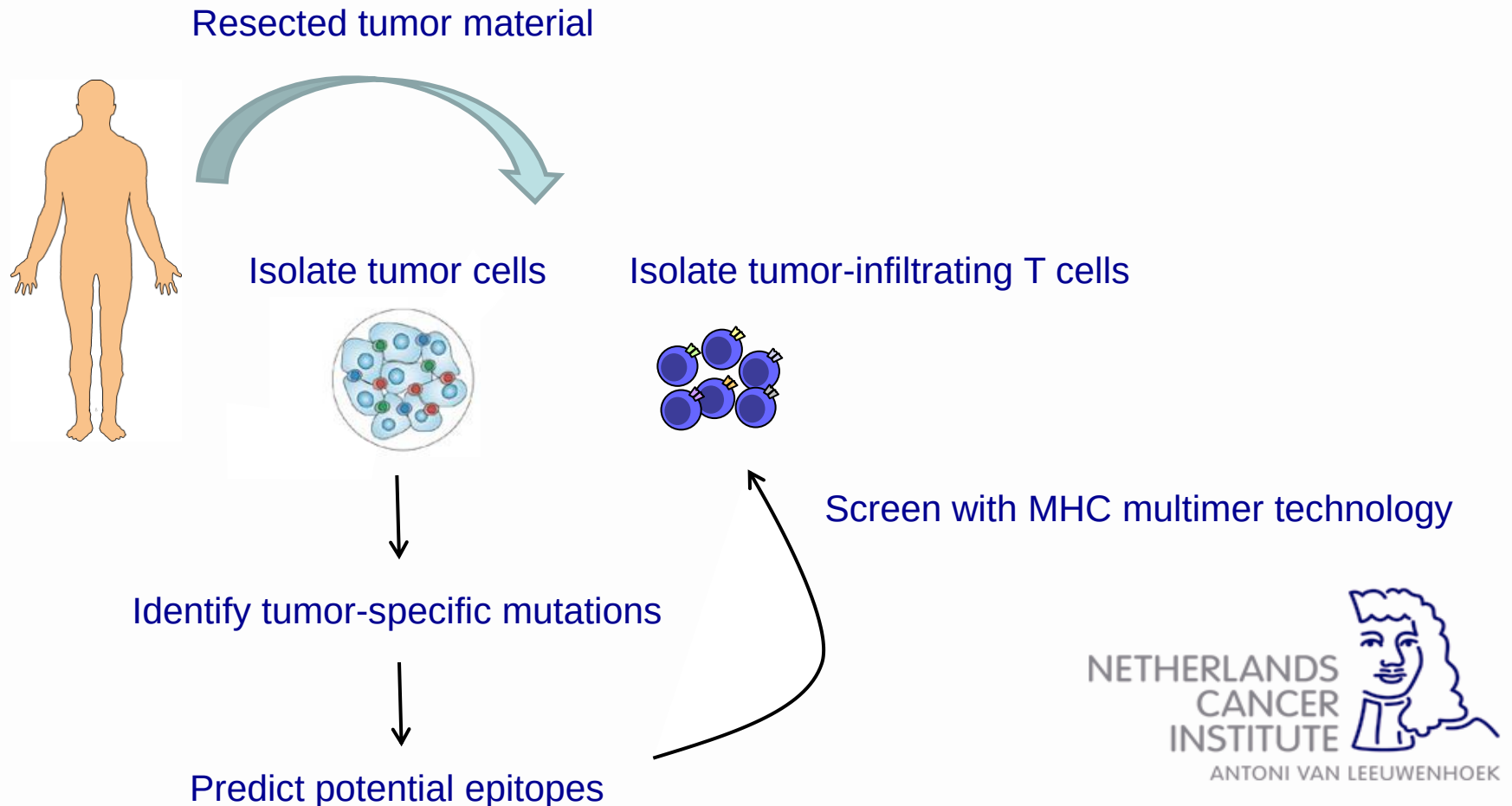
Strong T cell response against an ATR_{S>L} neo-epitope within the tumor



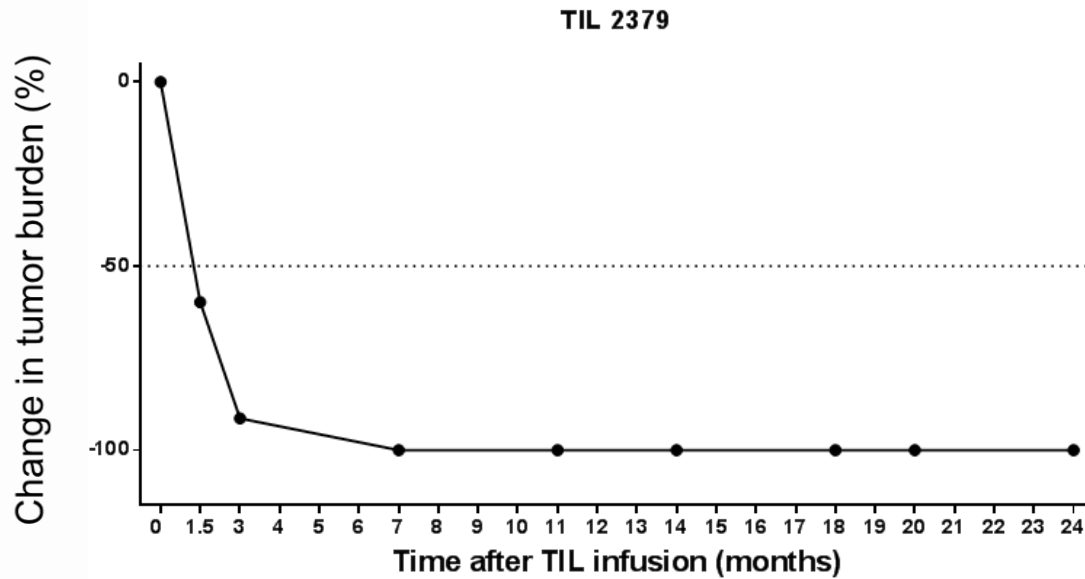
Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4



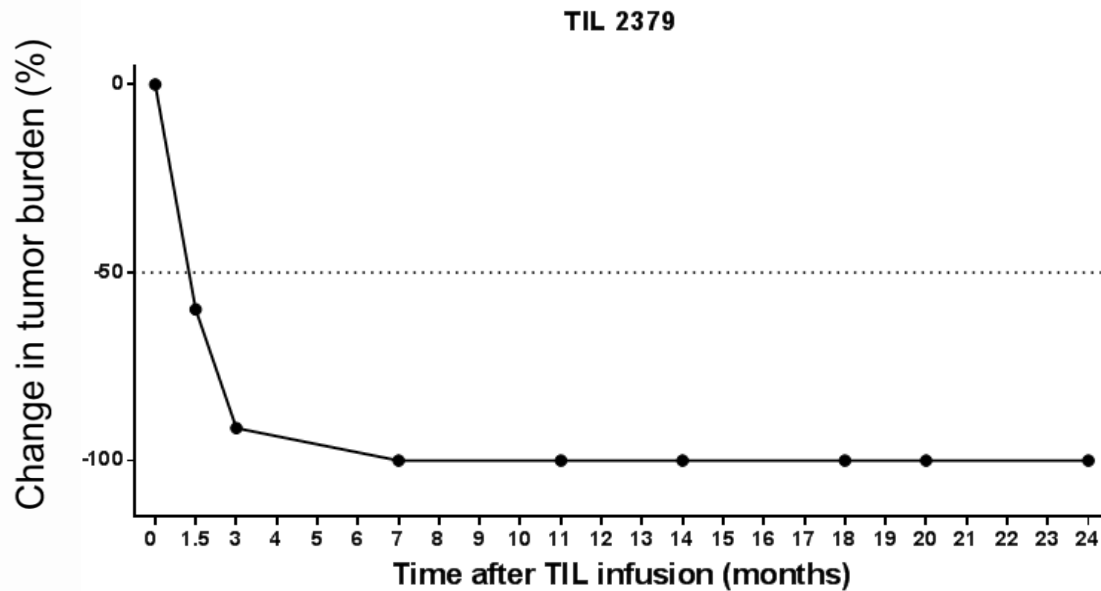
Pt 008: CR upon TIL therapy



Pt 008: CR upon TIL therapy



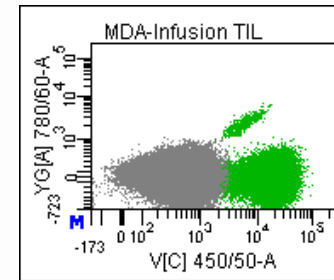
Pt 008: CR upon TIL therapy



Profound neo-antigen reactivity in TIL product

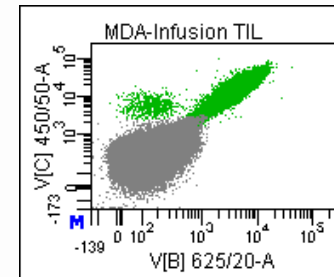
Infusion TIL product

0.172%



RASSF1_{R>C}

23%



DHX33_{R>W}

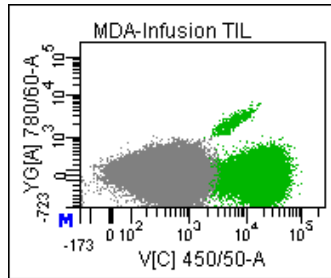
Pt 008: CR upon TIL therapy

Infusion TIL product

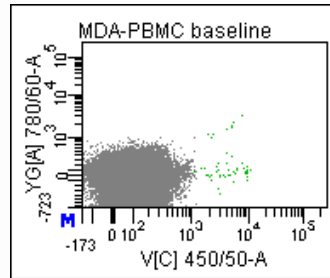
Pre-therapy PBMC

D7 post-therapy

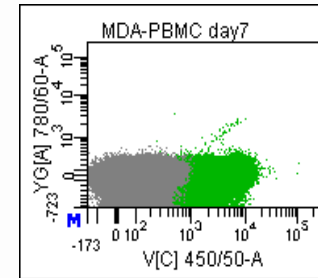
0.172%



0.002%

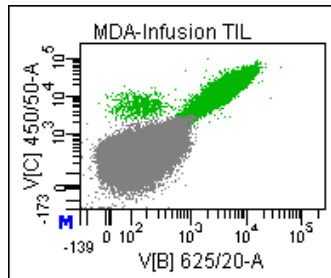


0.010%

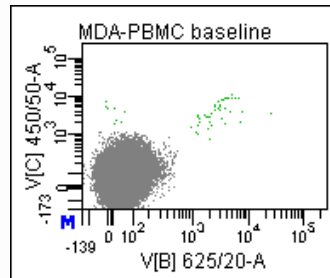


RASSF1_{R>C}

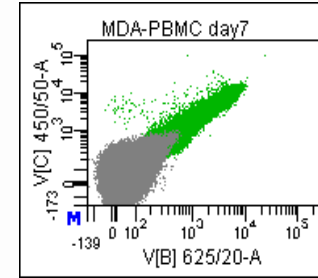
23%



0.009%



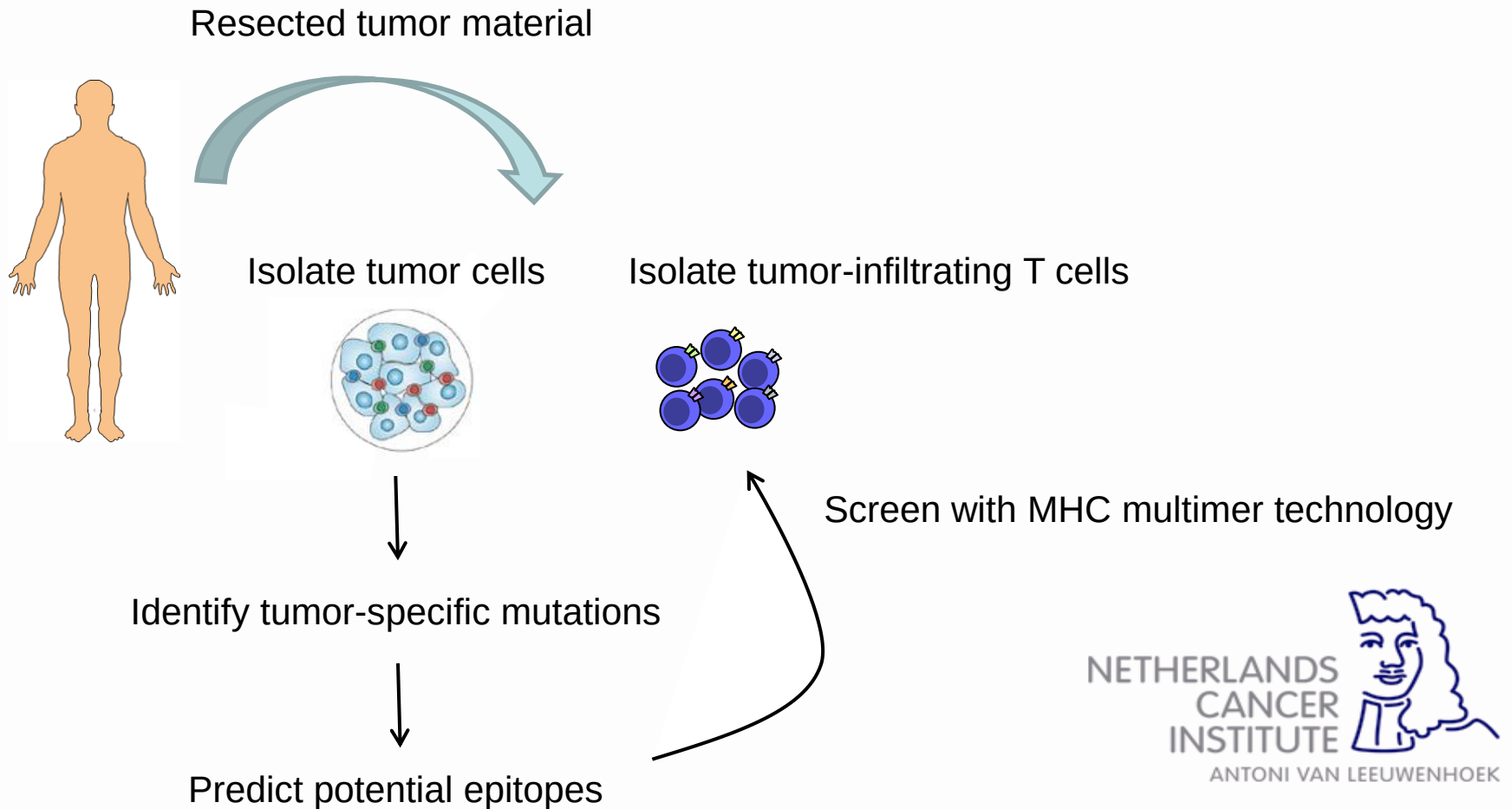
54%



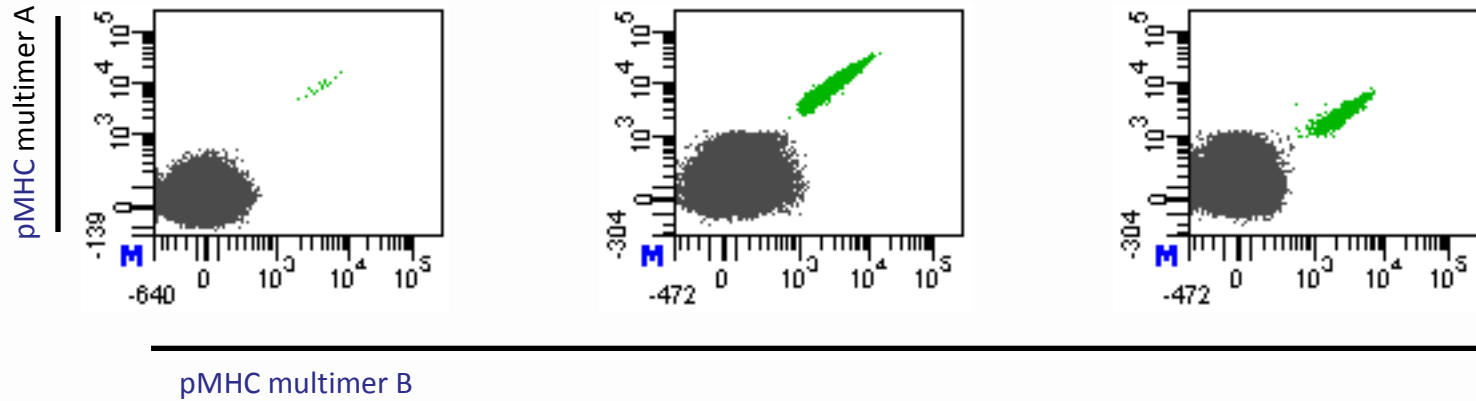
DHX33_{R>W}

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

Pt 004:



Pt 004:

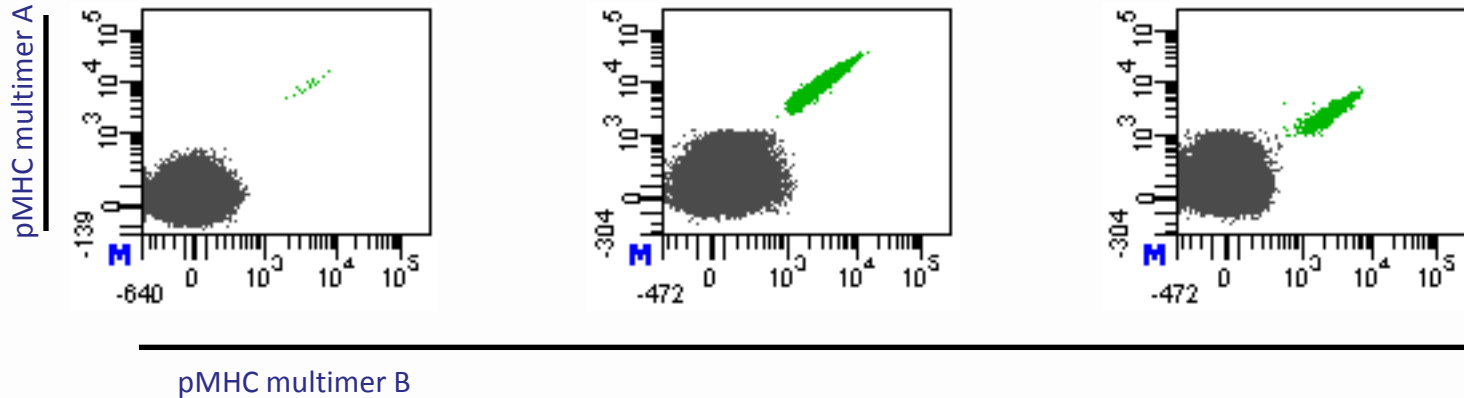


DNAH17_{H>Y} (0.003%)
VLFEDAVAH > VLFEDAVAY

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

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

Pt 004:



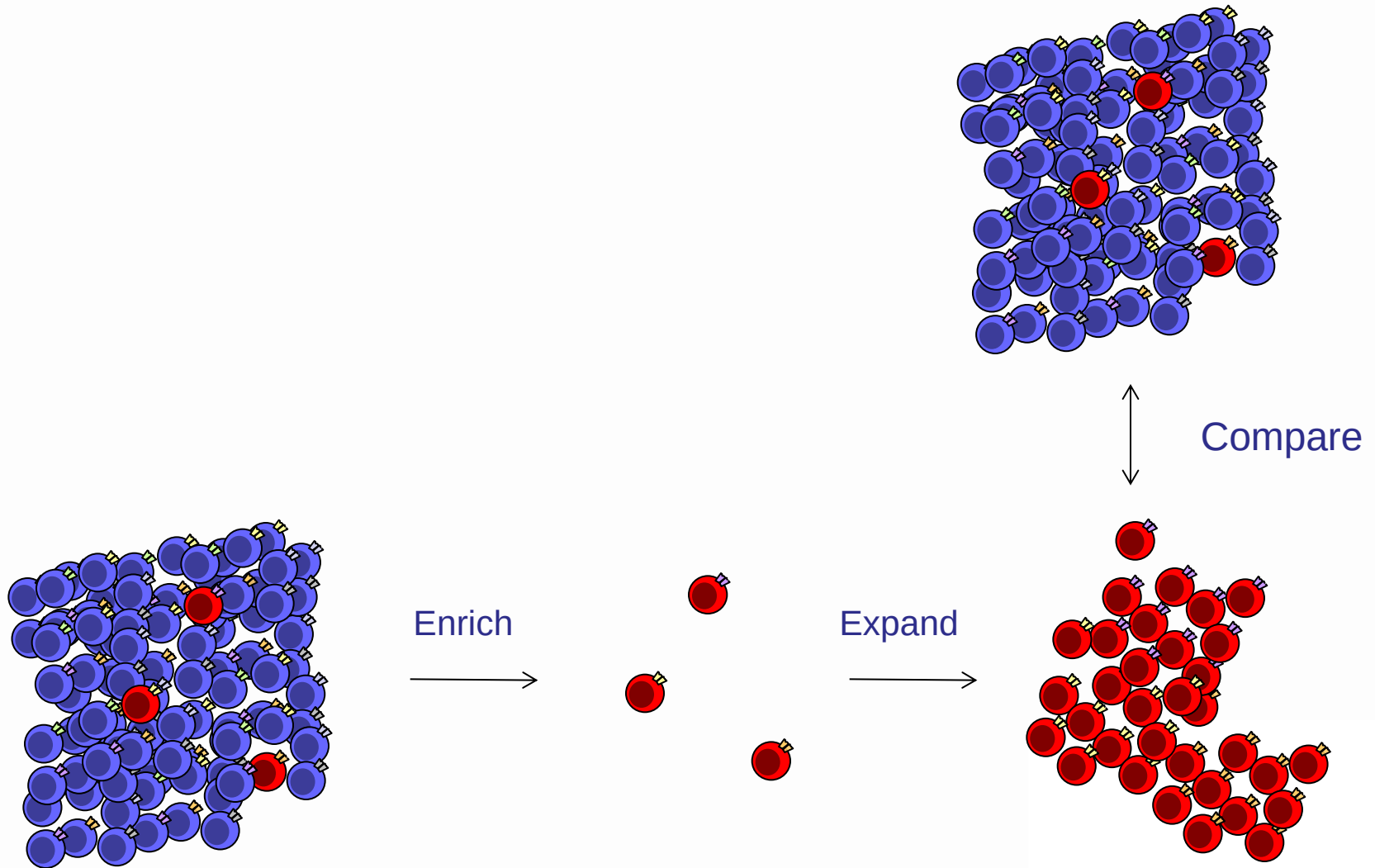
DNAH17_{H>Y} (0.003%)
 VLFEDAVAHH > VLFEDAVAYY

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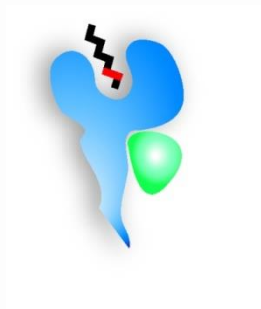
GCN1L1_{L>P} (0.407%)
 ALLETLSLLL > ALLETPSLLL

**Mutations can result in neo-antigens derived from oncogenes and
 (presumed) passenger genes**

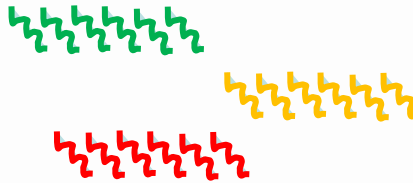
Are neo-antigens superior cancer rejection antigens?



Are neo-antigens superior cancer rejection antigens?



+



+

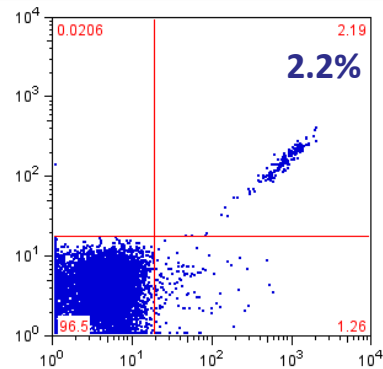


- Develop peptide exchange MHC streptamers to create defined TIL products

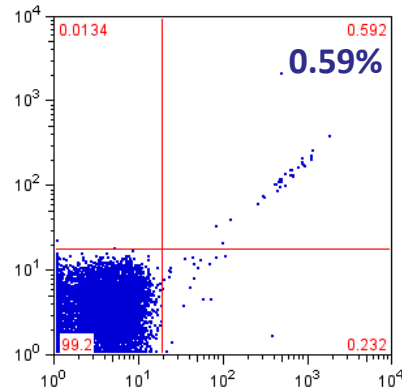
Are neo-antigens superior cancer rejection antigens?

CDK4_{R>L}

Pre-enrichment



GCN1L1_{L>P}



Combined

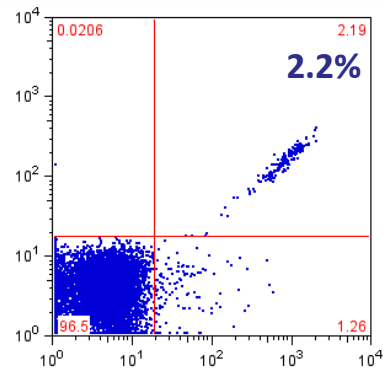
2.8%



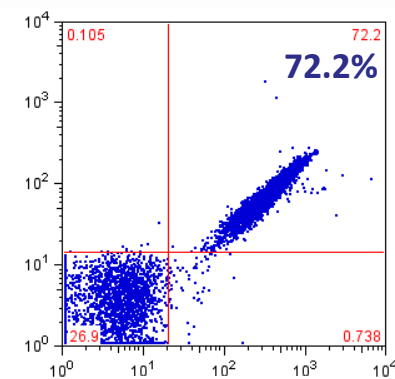
Are neo-antigens superior cancer rejection antigens?

CDK4_{R>L}

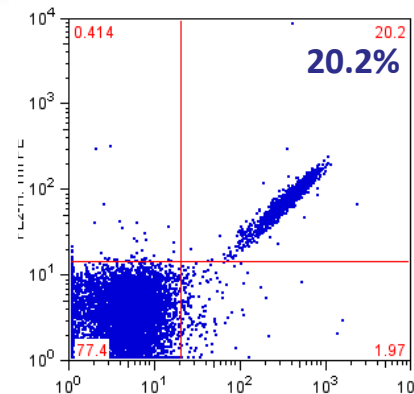
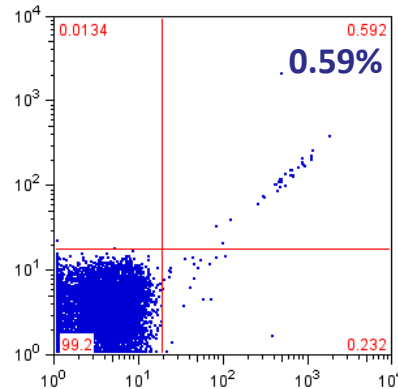
Pre-enrichment



Post-joint enrichment



GCN1L1_{L>P}

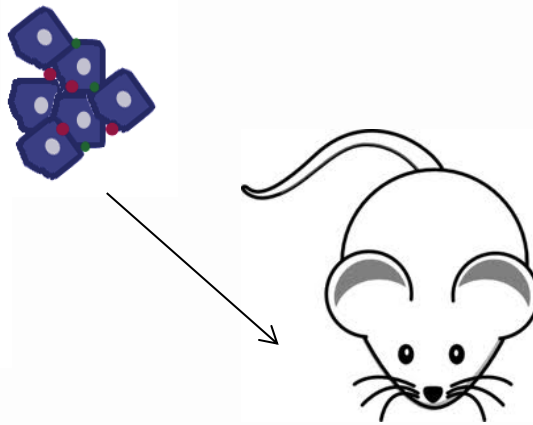


Combined

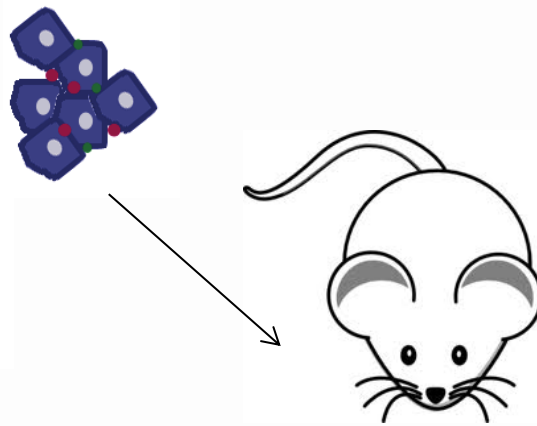
2.8%

92.4%

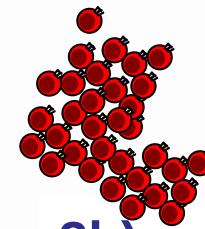
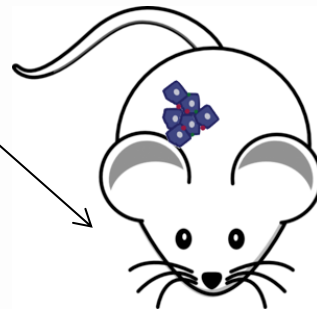
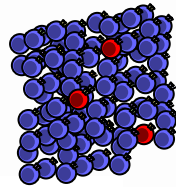
1)
**Inject human
melanoma**
(NSG-mice)



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melanoma
(NSG-mice)**

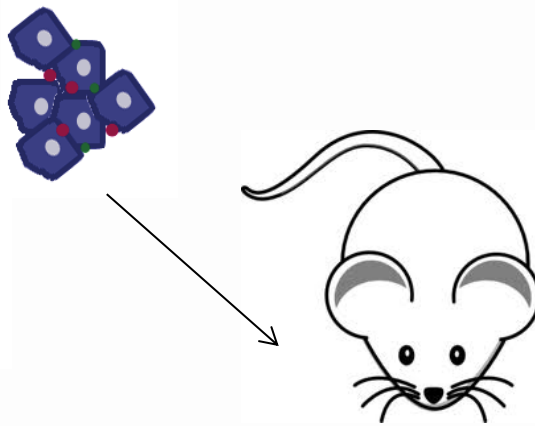


2a)
**Inject autologous
bulk T-cell product**

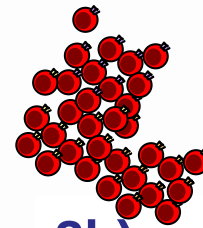
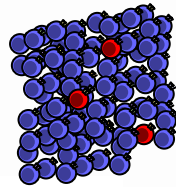


2b)
**Inject autologous
neo-Ag enriched
T-cell product**

1)
**Inject human
melanoma
(NSG-mice)**



2a)
**Inject autologous
bulk T-cell product**

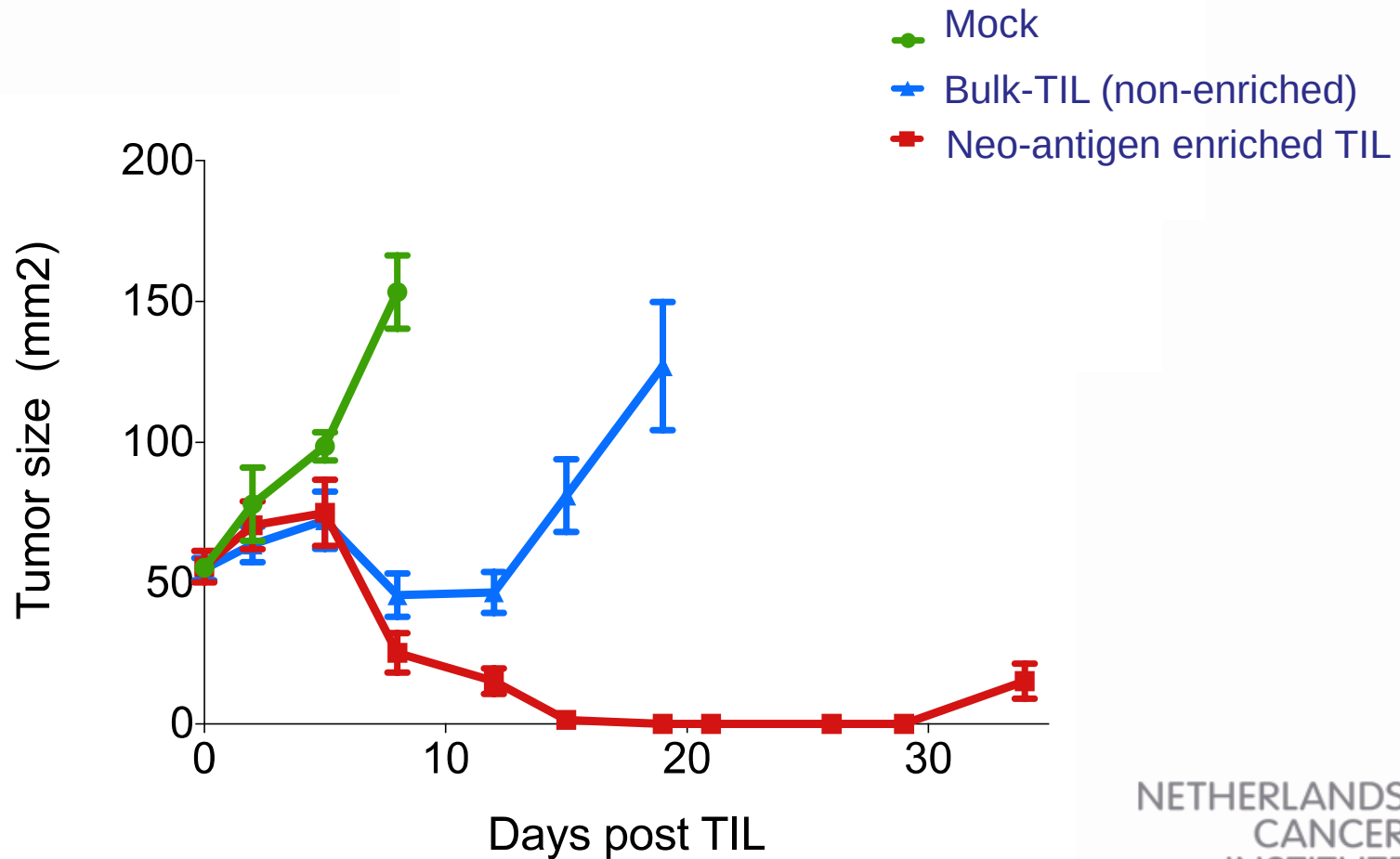


2b)
**Inject autologous
neo-Ag enriched
T-cell product**

3)
**Monitor tumor
growth**

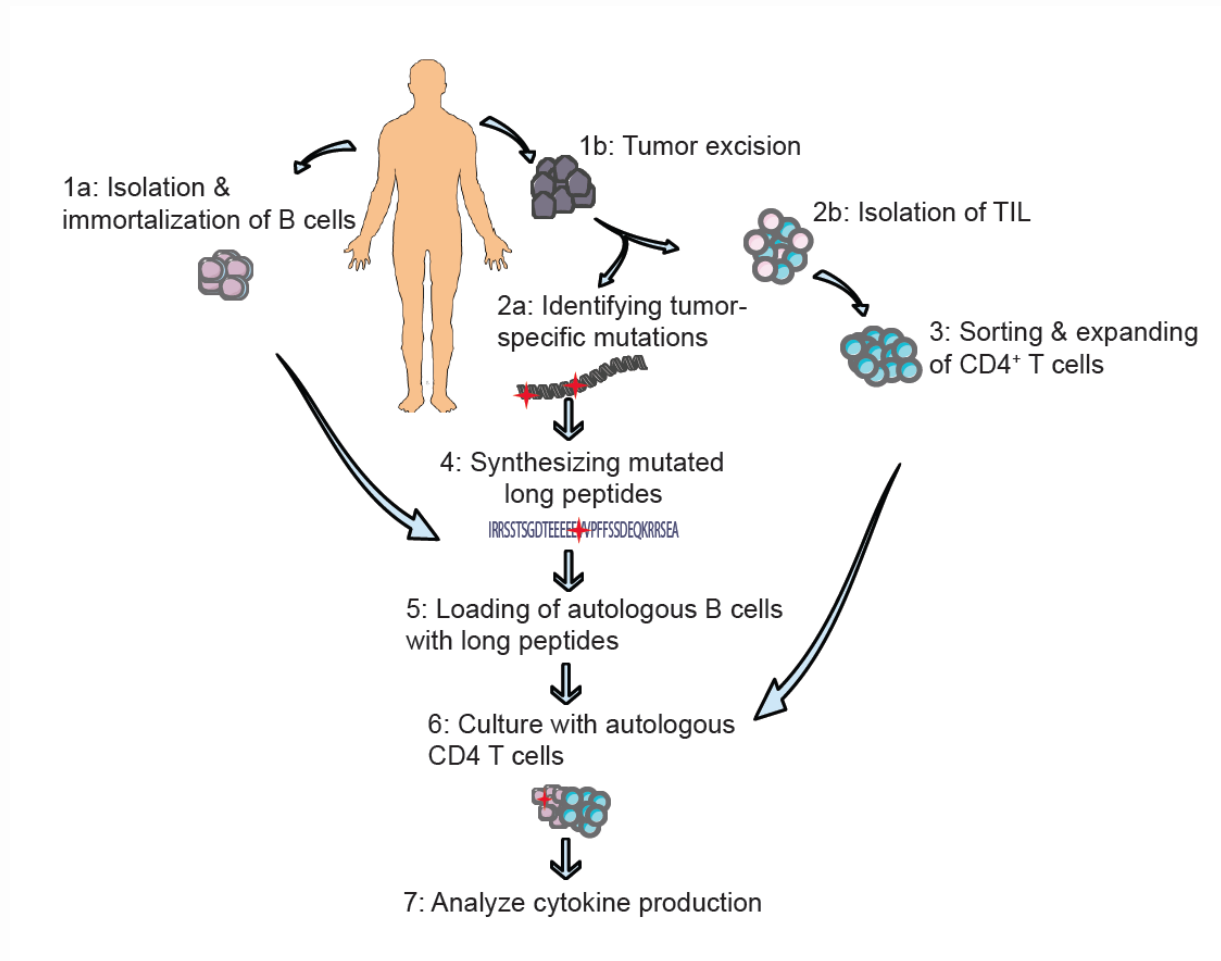


Neo-antigen enriched TIL can mediate superior tumor control



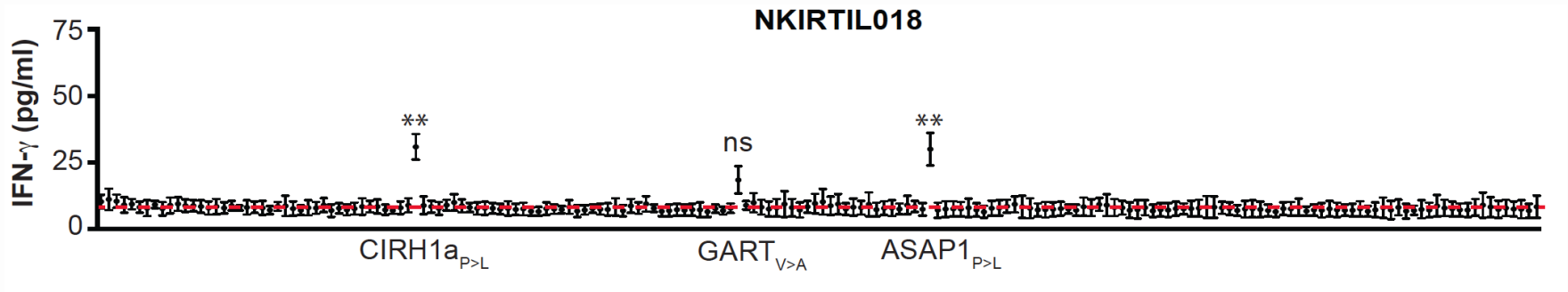
Evidence for neo-antigen reactive CD4 T cells?

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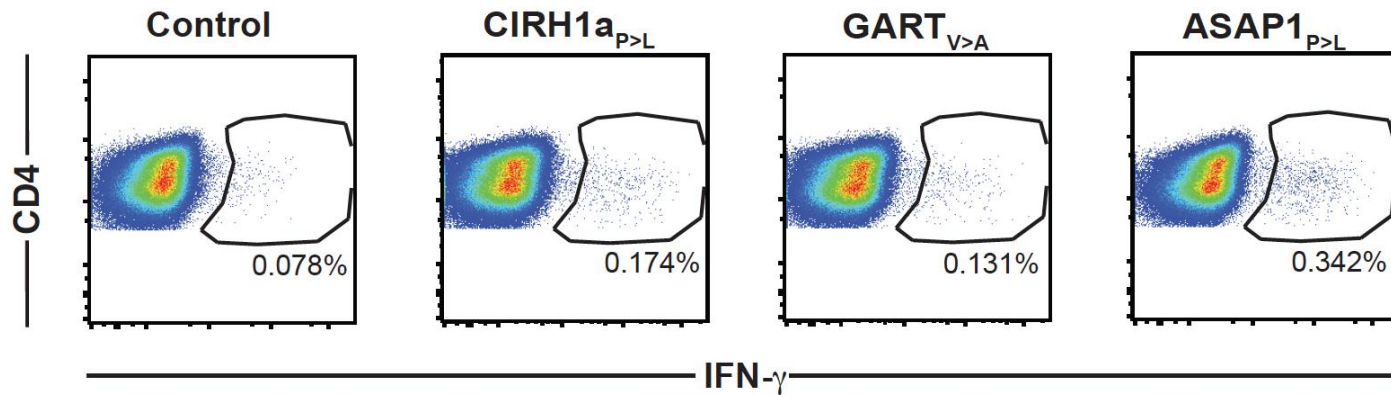
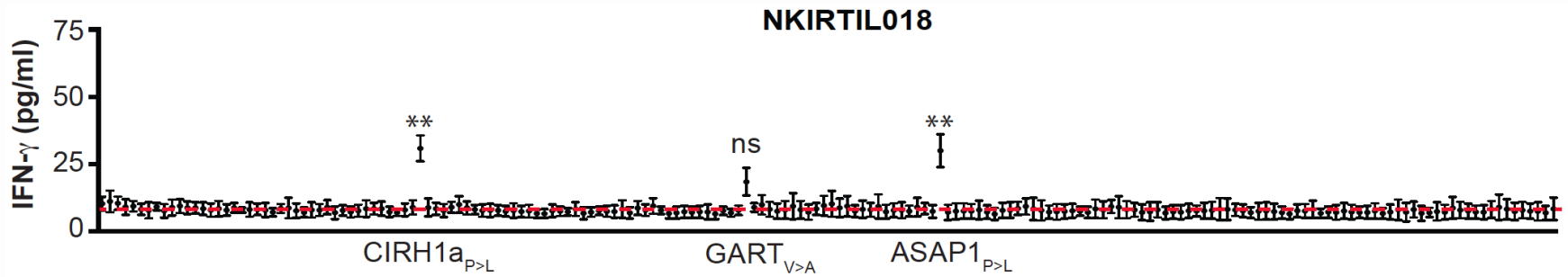


Independent of MHC tools – but does require functional activity

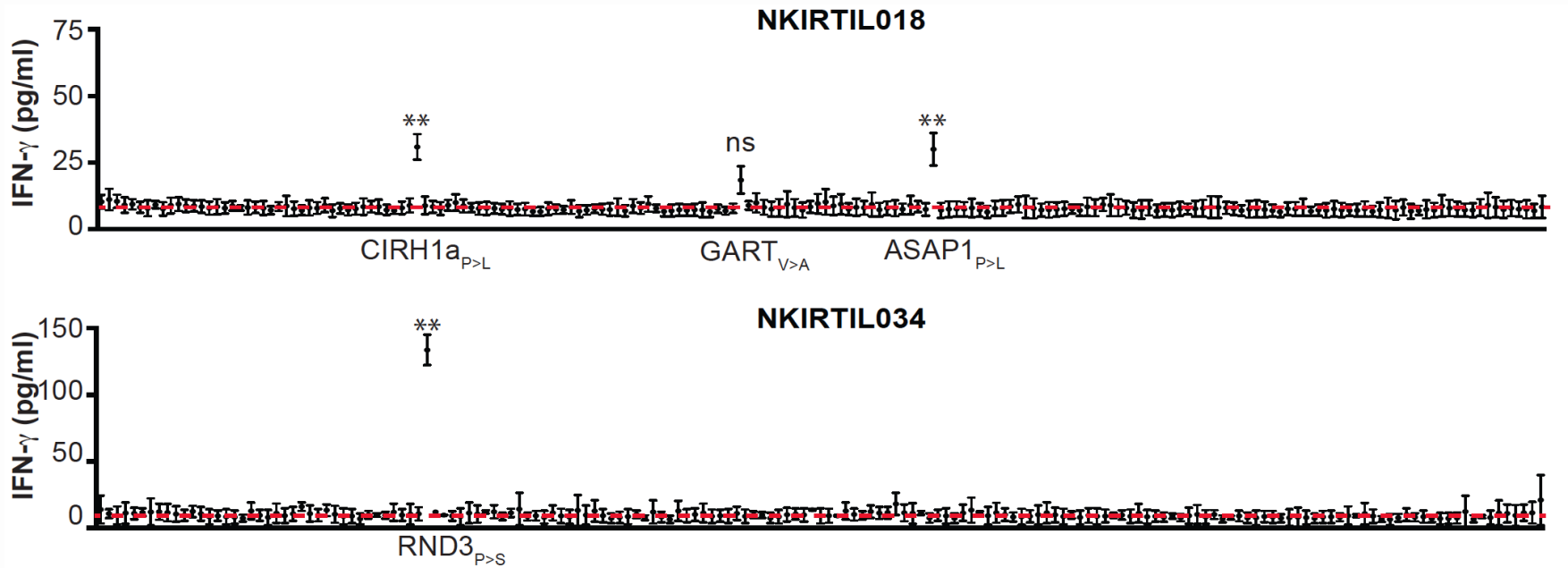
Evidence for neo-antigen reactive CD4 T cells?



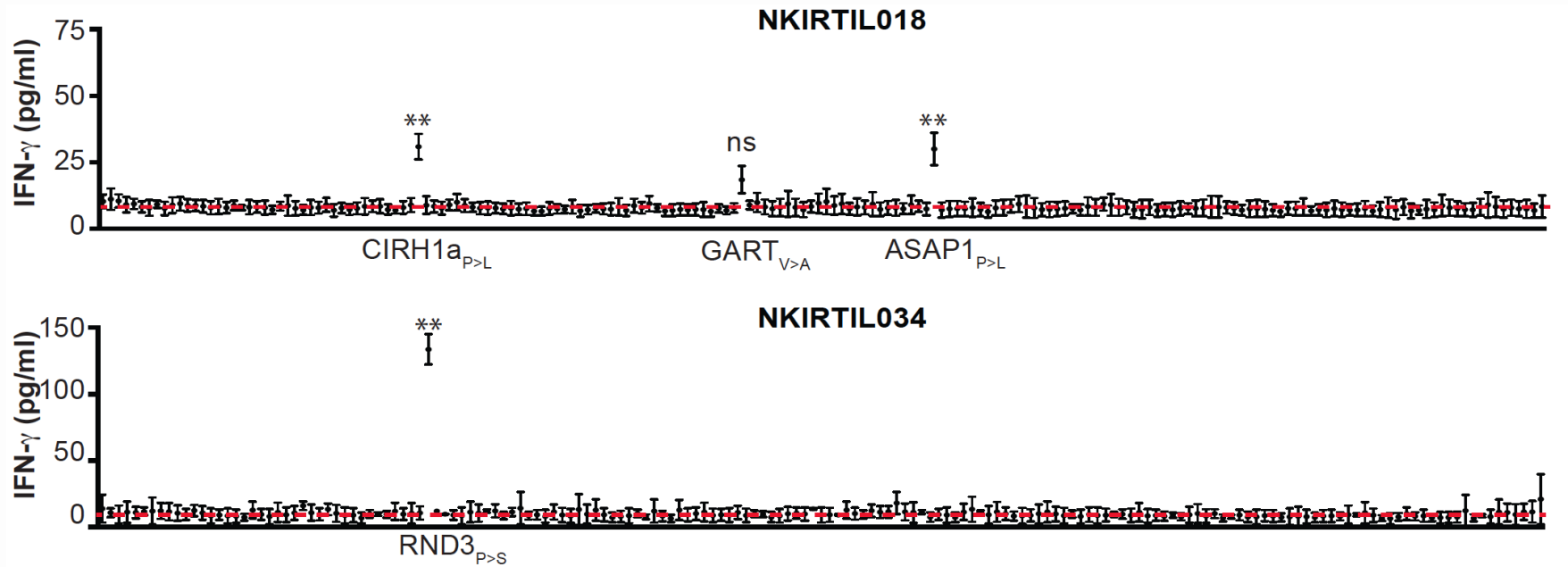
Evidence for neo-antigen reactive CD4 T cells?



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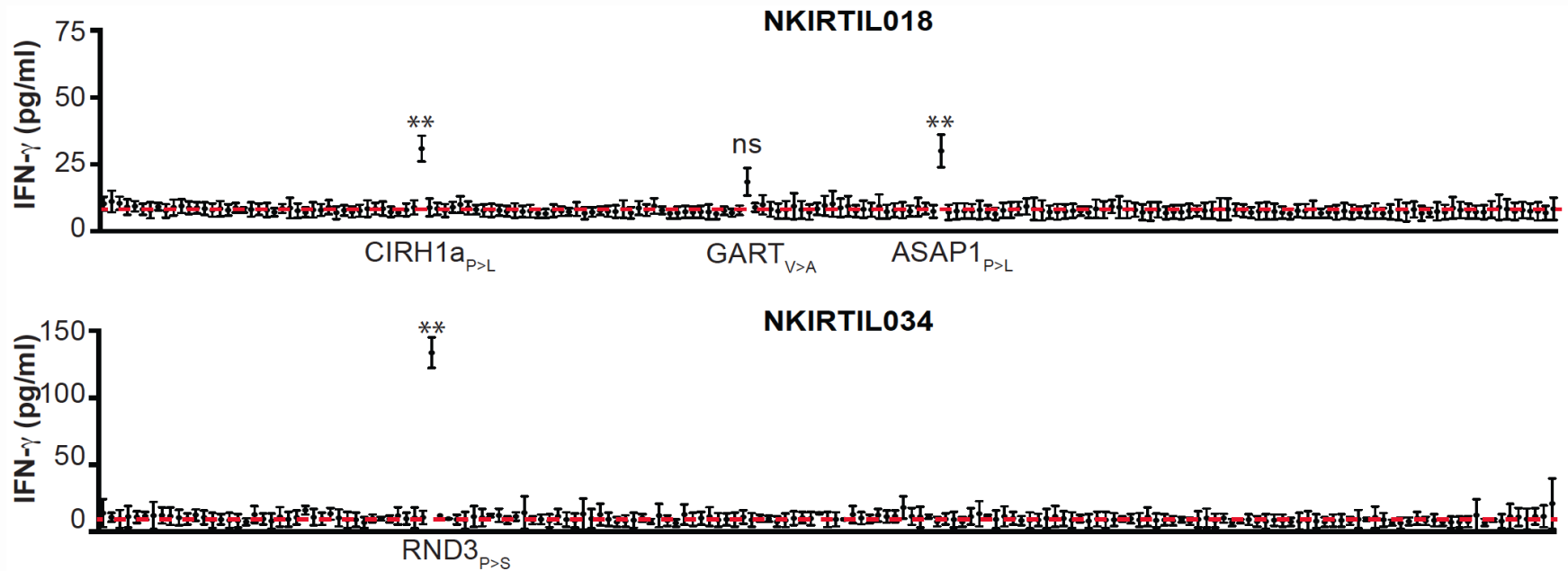


Evidence for neo-antigen reactive CD4 T cells?



Evidence for CD4 reactivity against mutant peptides
True neo-antigen specific T cell responses or haphazard cross reactivity?

Evidence for neo-antigen reactive CD4 T cells?



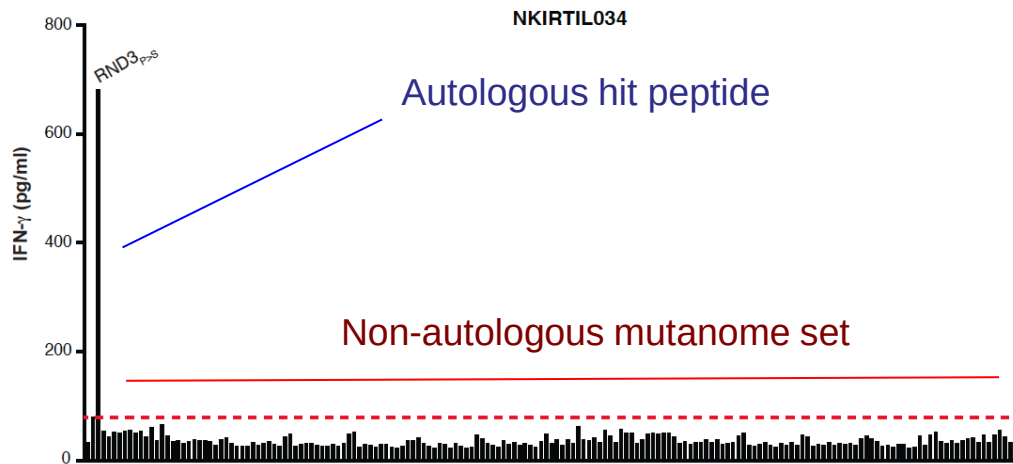
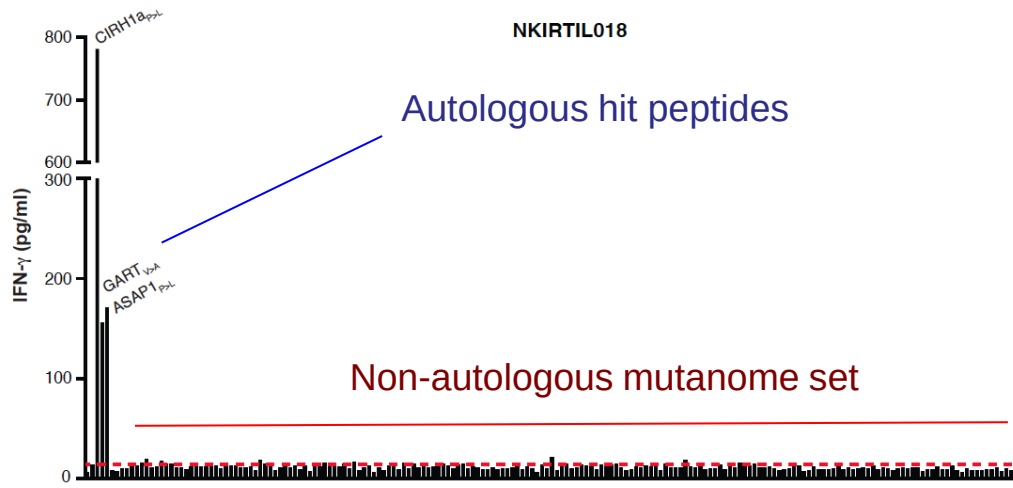
Evidence for CD4 reactivity against mutant peptides

True neo-antigen specific T cell responses or haphazard cross reactivity?

If True: Only reactivity against autologous mutanome set

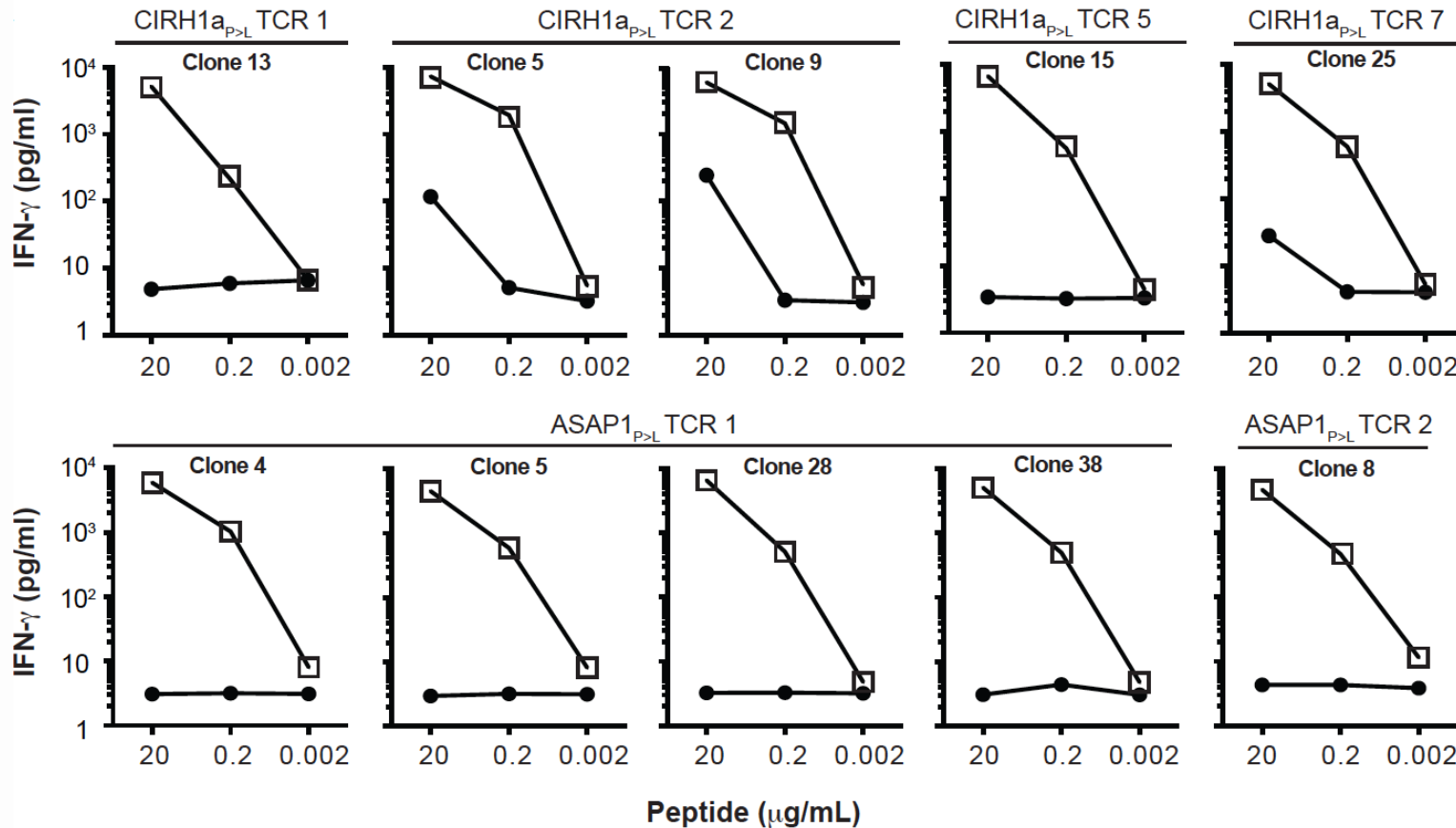
If True: Reactivity against mutant peptide > reactivity against parental peptide

Evidence for neo-antigen reactive CD4 T cells?



If True: Only reactivity against autologous mutanome set

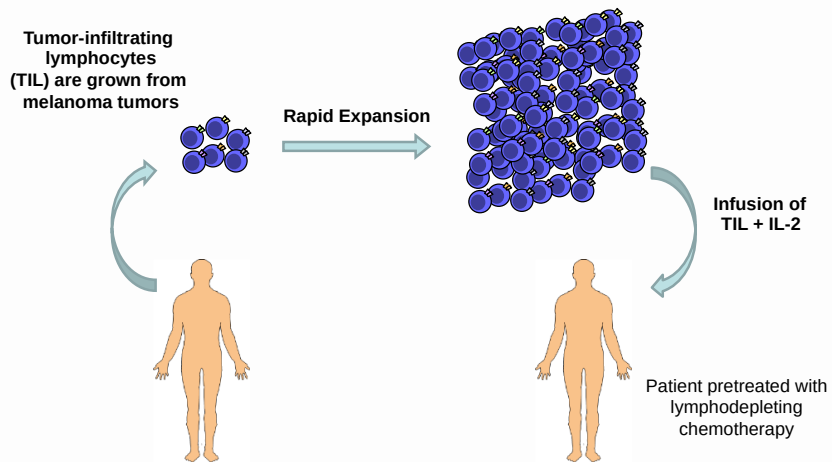
Evidence for neo-antigen reactive CD4 T cells?



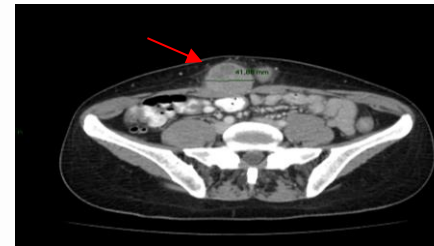
If True: Reactivity against mutant peptide > reactivity against parental peptide

Neo-antigen reactive CD4 T cells in clinically effective ACT products?

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



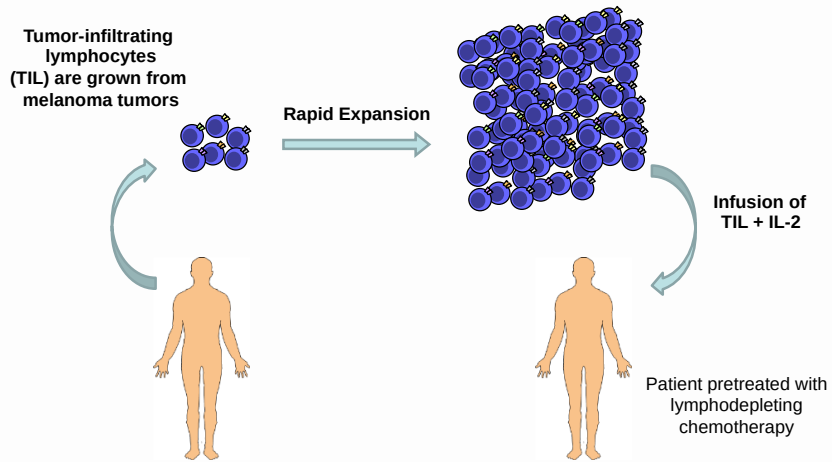
Prior to TIL



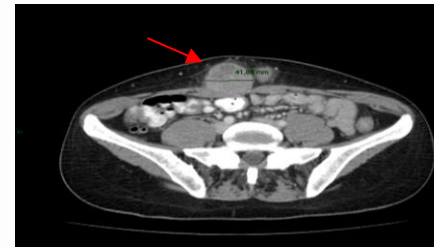
5 wks postTIL



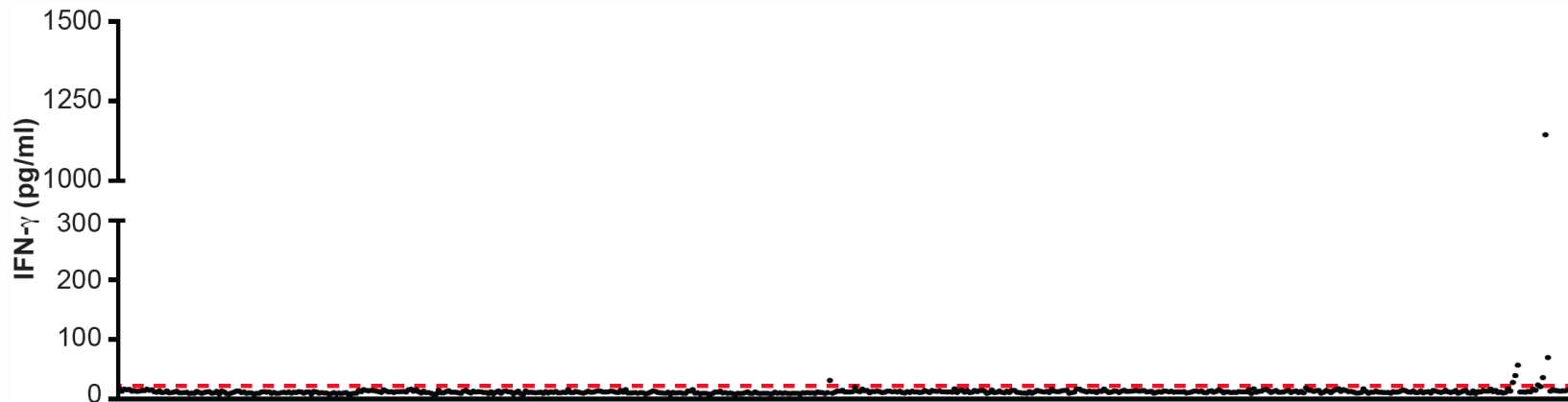
Neo-antigen reactive CD4 T cells in clinically effective ACT products? (6m PR upon TIL therapy)



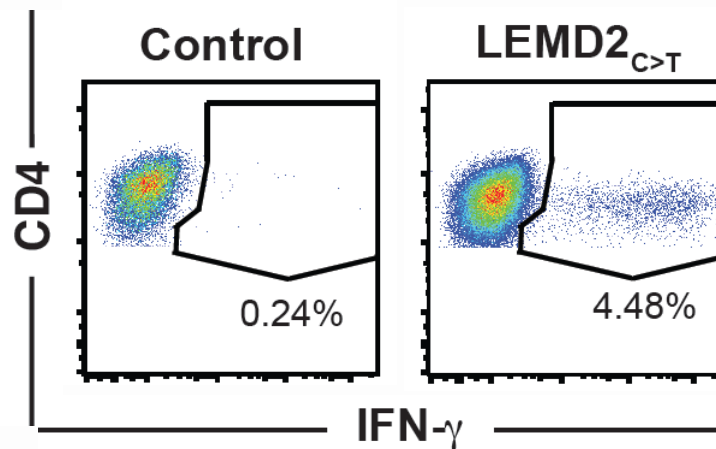
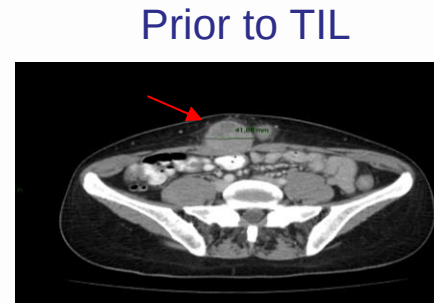
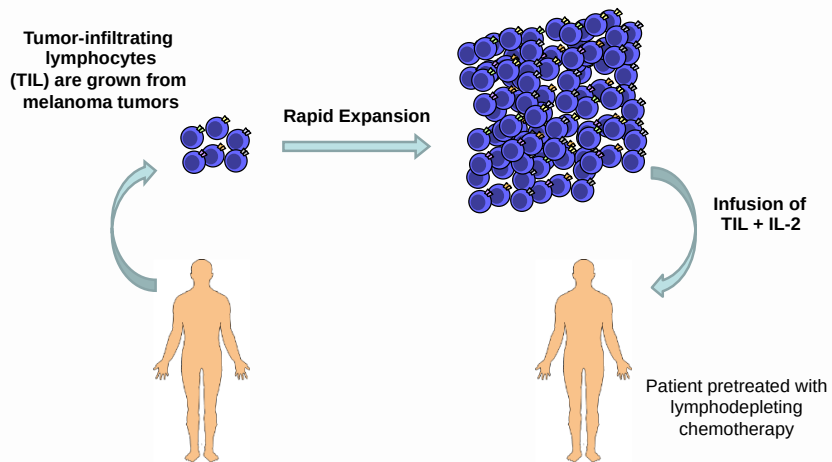
Prior to TIL



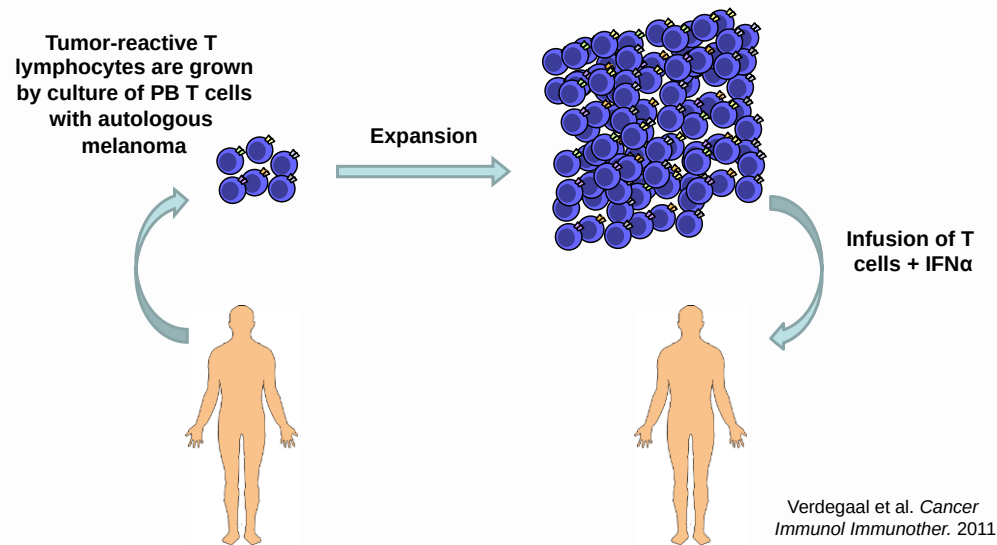
5 wks postTIL



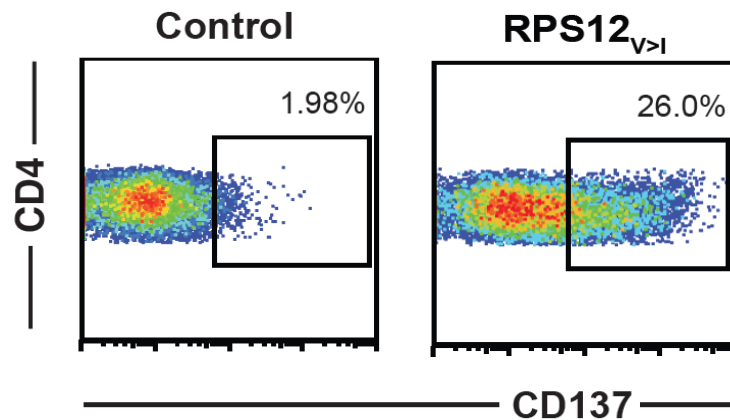
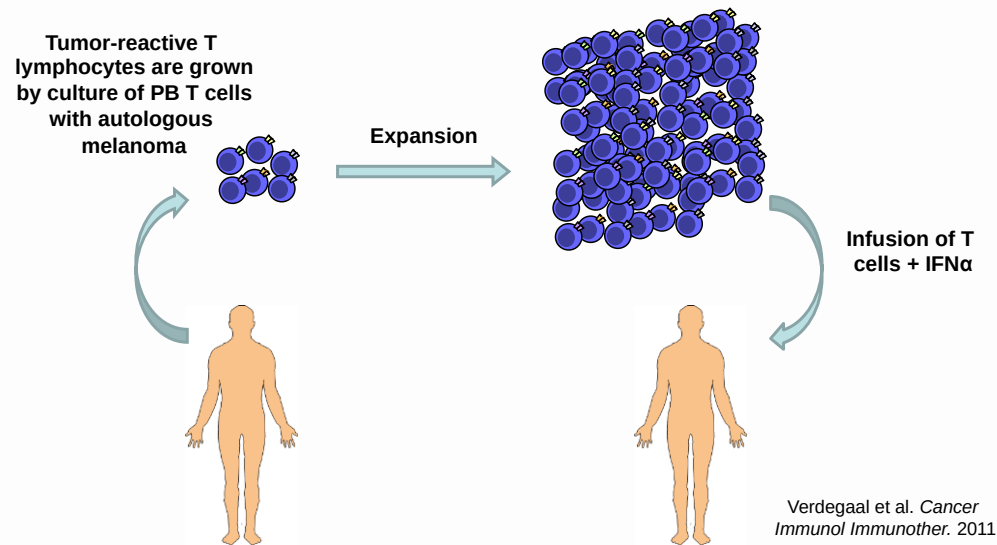
Neo-antigen reactive CD4 T cells in clinically effective ACT products? (6m PR upon TIL therapy)



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



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



Cancer exome-guided immunomonitoring

- ❖ Exome-based analysis of neo-antigen specific T cell responses in human cancer is feasible

Cancer exome-guided immunomonitoring

- ❖ Exome-based analysis of neo-antigen specific T cell responses in human cancer is feasible
 - *Dissect the role of neo-antigen specific T cell reactivity in melanoma immunotherapy (TIL therapy, anti-CTLA4, anti-PD1 etc.)*

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 - *Dissect the role of neo-antigen specific T cell reactivity in melanoma immunotherapy (TIL therapy, anti-CTLA4, anti-PD1 etc.)*
- ❖ The T cell based immune system commonly interacts with the consequences of DNA damage in human melanoma
 - CD8 T cells:** 8 pts analyzed, neo-antigen specific reactivity in 6. Not all alleles covered, exome coverage incomplete, epitope predictions imperfect....)
 - CD4 T cells:** 5 pts analyzed, neo-antigen specific reactivity in 4

Big questions ahead:

1). Should we develop **personalized immunotherapies** to target these antigens?

> Antigen-selected TIL

> Anti-CTLA4 or anti-PD1 plus neo-epitope vaccines

Big questions ahead:

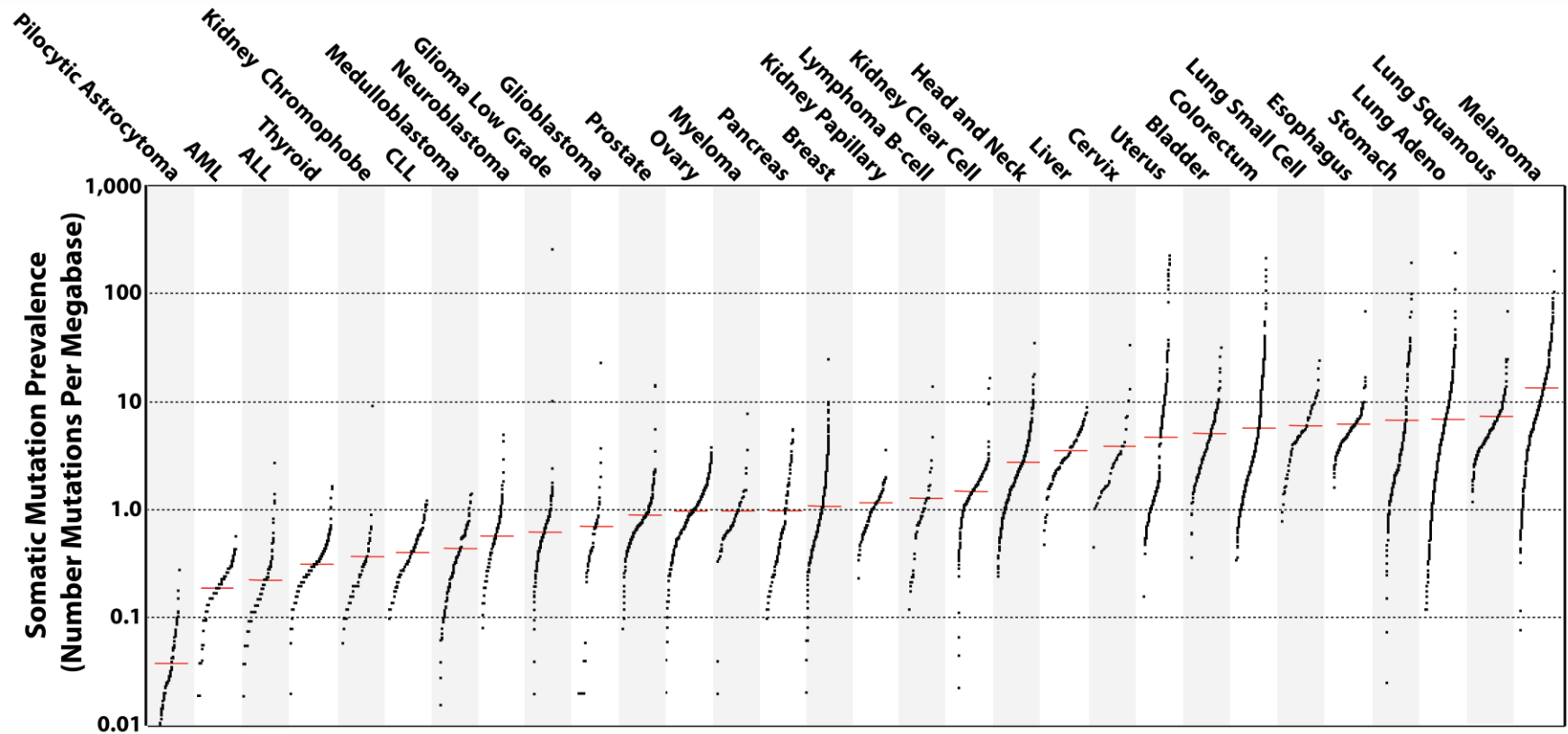
1). Should we develop **personalized immunotherapies** to target these antigens?

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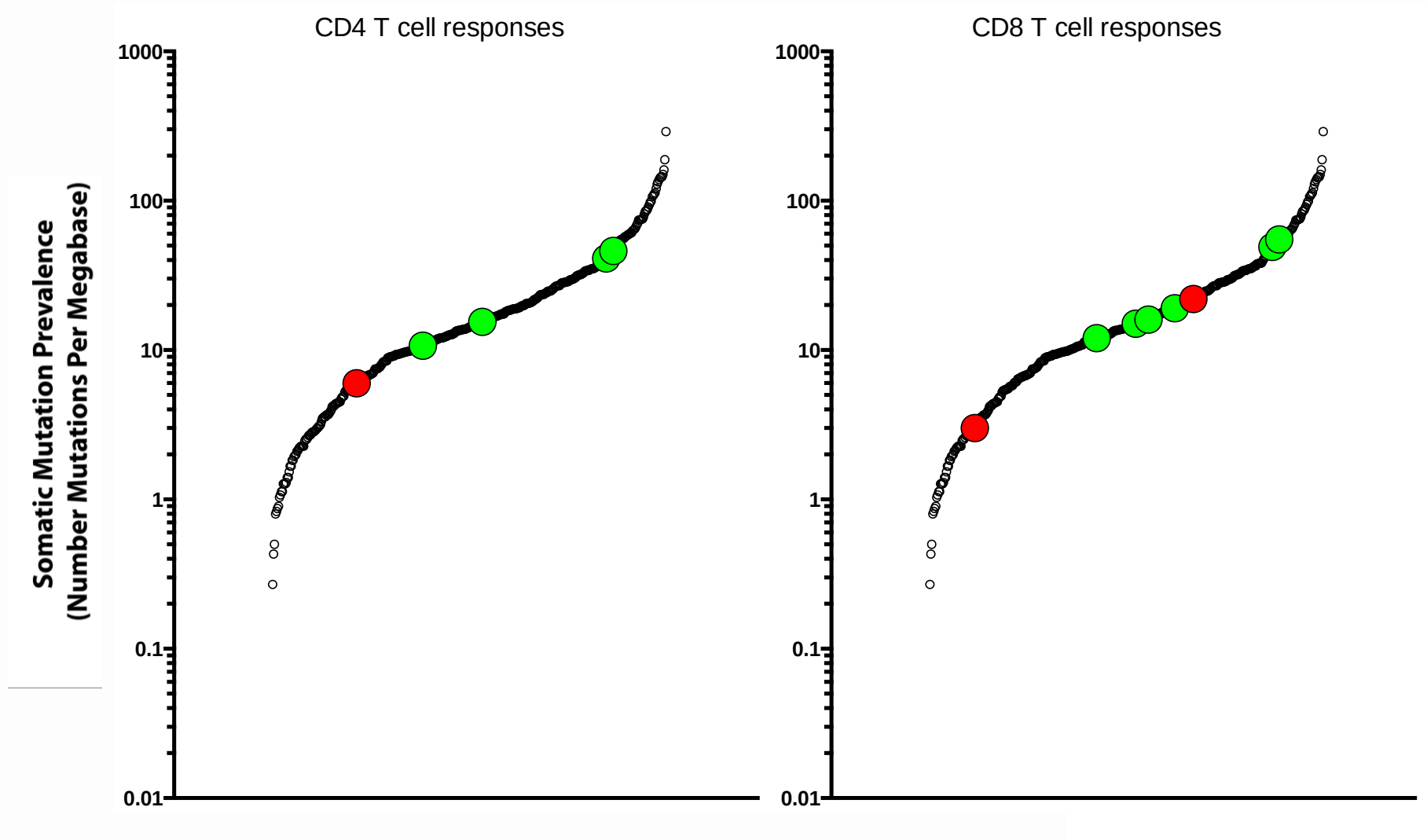
> Anti-CTLA4 or anti-PD1 plus neo-epitope vaccines

2). Can we expect a neo-antigen repertoire that can be exploited in **other human malignancies**?

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

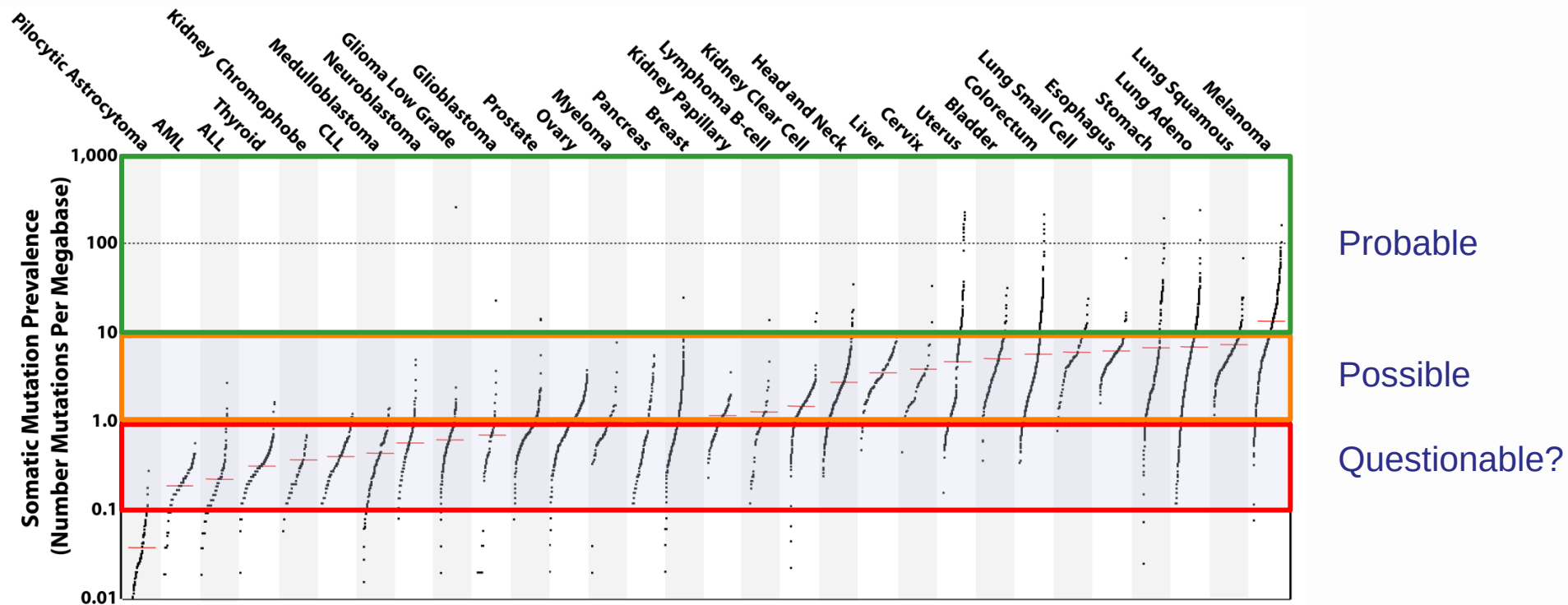


Can we expect a neo-antigen repertoire in other human cancers?



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

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





Cancer exome-guided personalized immunotherapy



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