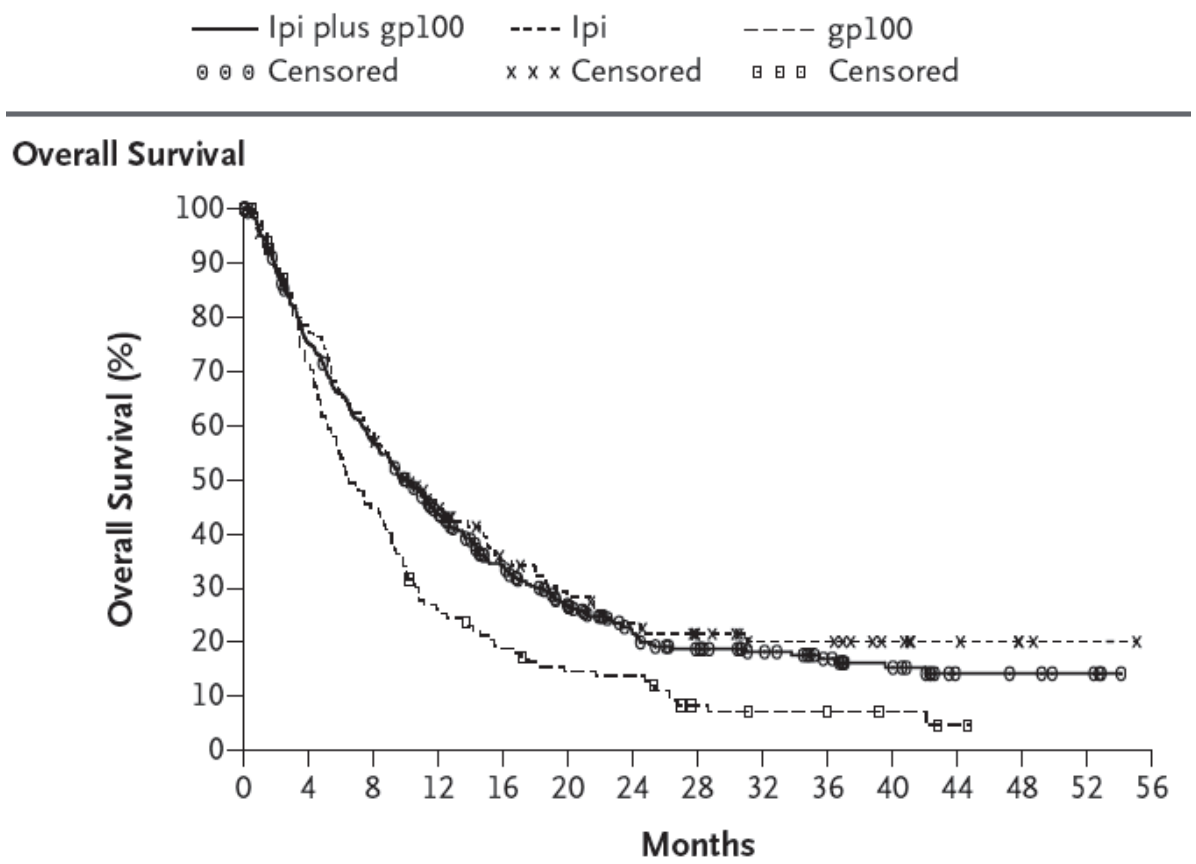
Dissection of anti-CTLA4-induced cytotoxic T cell responses in melanoma

Ipilimumab: clinical data phase III

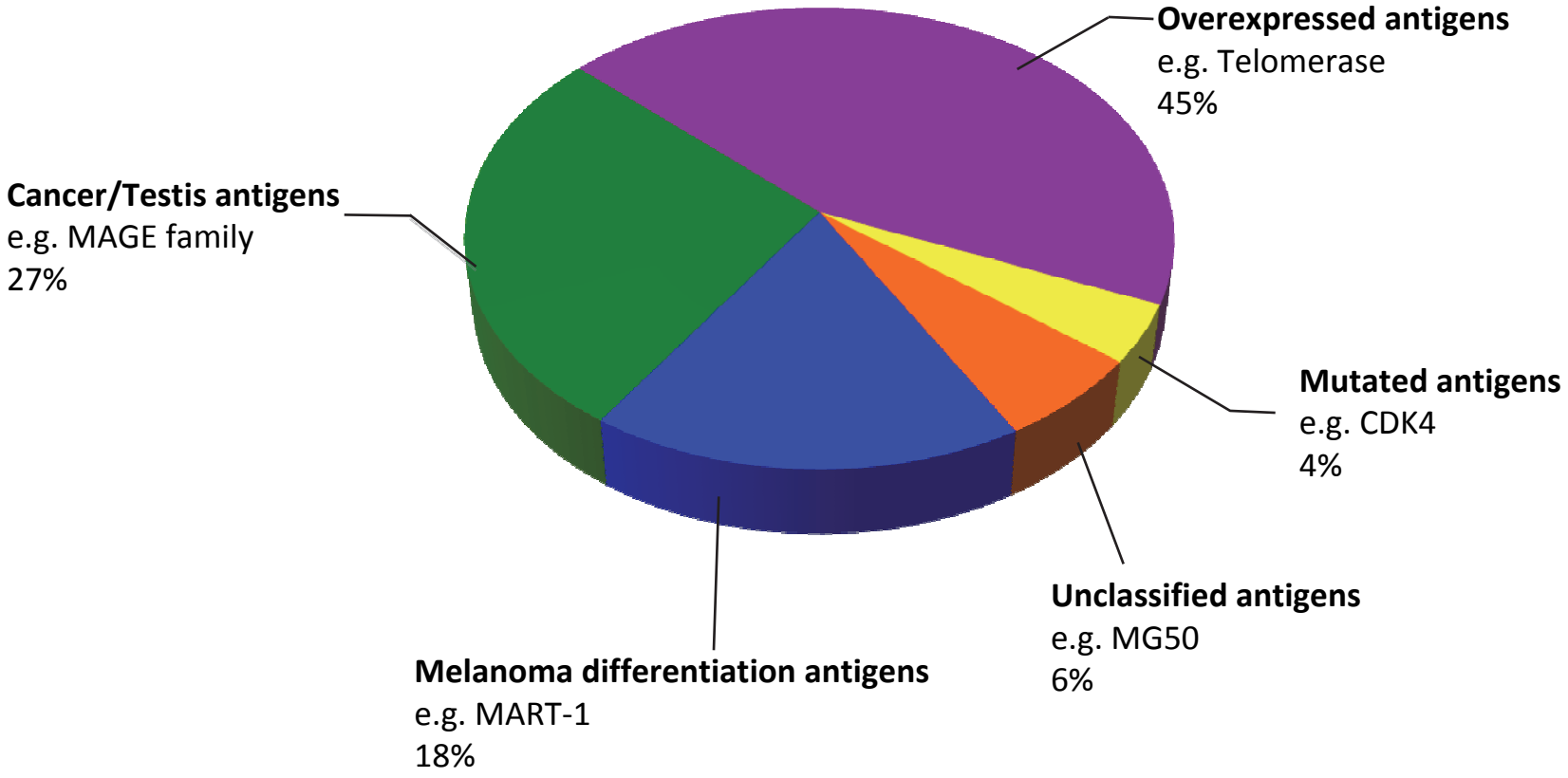


- Survival benefit in metastatic disease
- Data from murine models suggest a role for cytotoxic T cells

Hodi et al. *NEJM* 2010

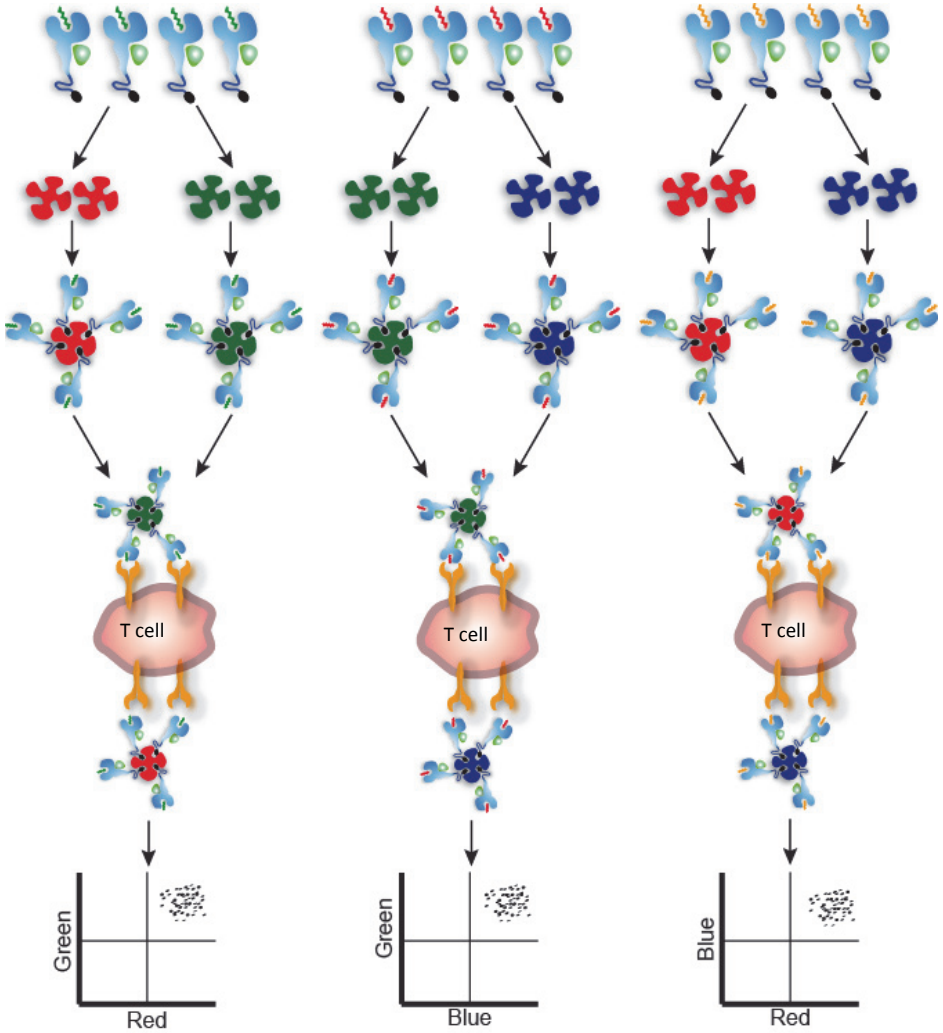
Melanoma associated epitope panel

HLA-A2 restricted peptide panel includes 145 epitopes



Andersen et al 2012, Kvistborg et al 2012

Self-assembling molecular codes



Generate fluorochrome conjugated MHC multimers

Mix to create a collection of differentially encoded MHC multimers

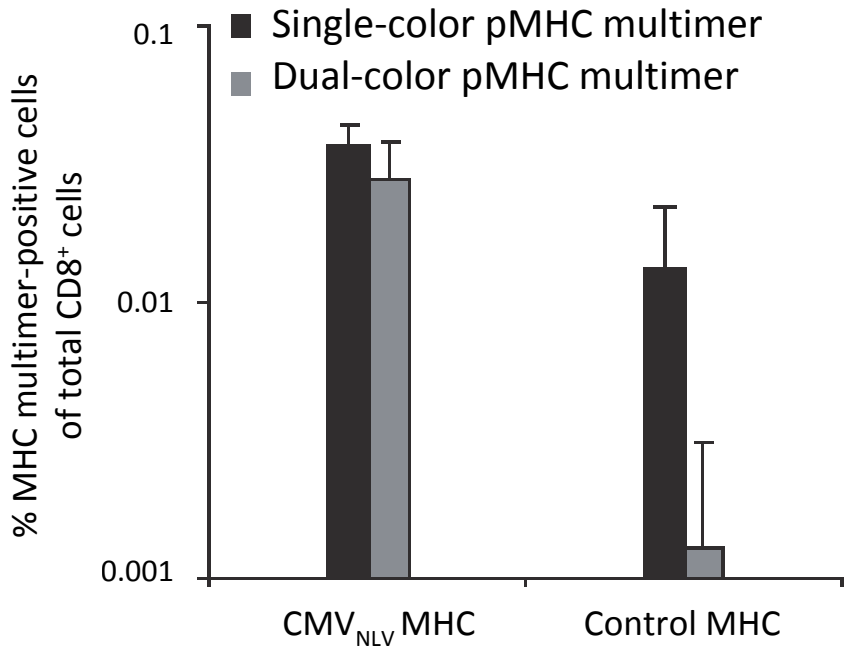
Assembly of combinatorial codes on T cell surfaces

Analysis by flow cytometry

Hadrup et al. *Nat. Methods* 2009

Increased sensitivity

Dual color staining of T cells result in reduced background signal

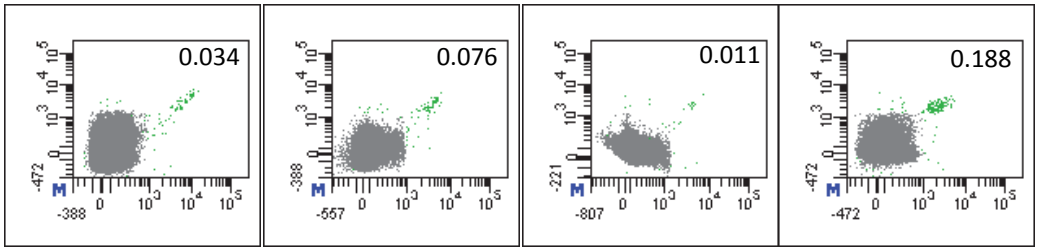


Hadrup et al. *Nat. Methods* 2009

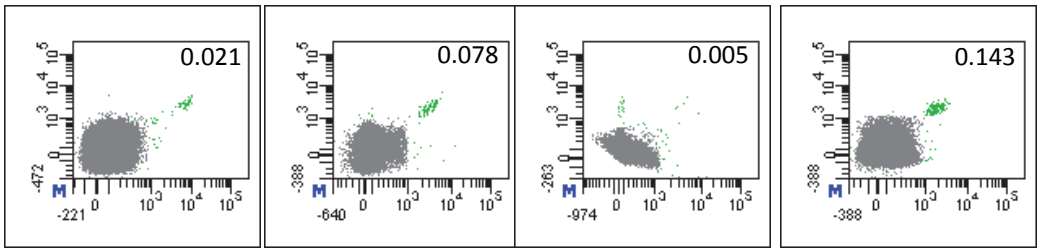
Example of T cell reactivities detected in Ipilimumab treated patients

Pre-treatment

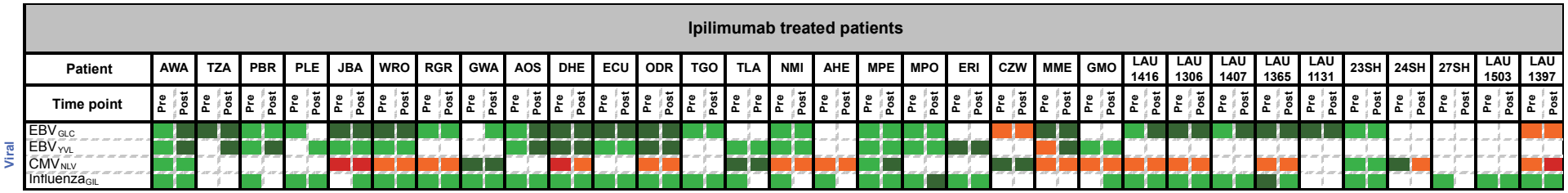
MAGE C2$_{KVL}$ MART-1$_{ELA}$ gp100$_{YLE}$ gp100$_{IMD}$

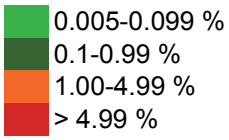


Post-treatment

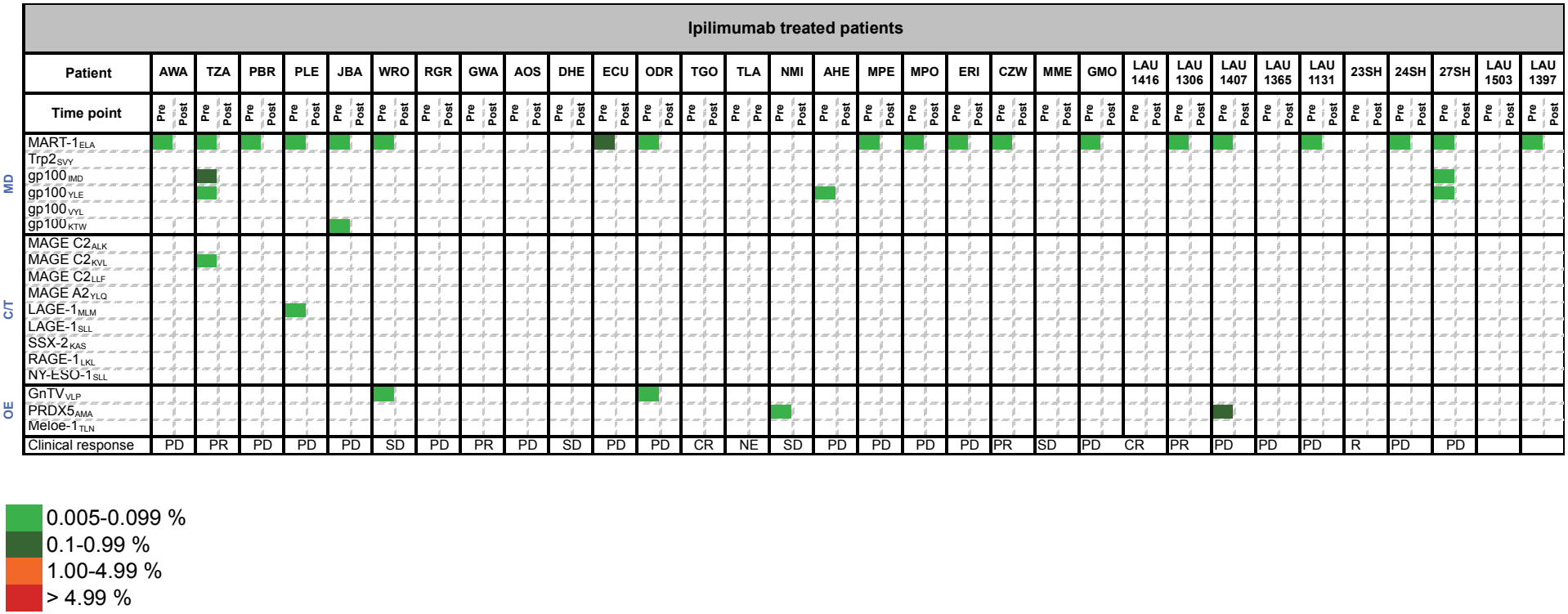


Viral responses can be detected in all patients

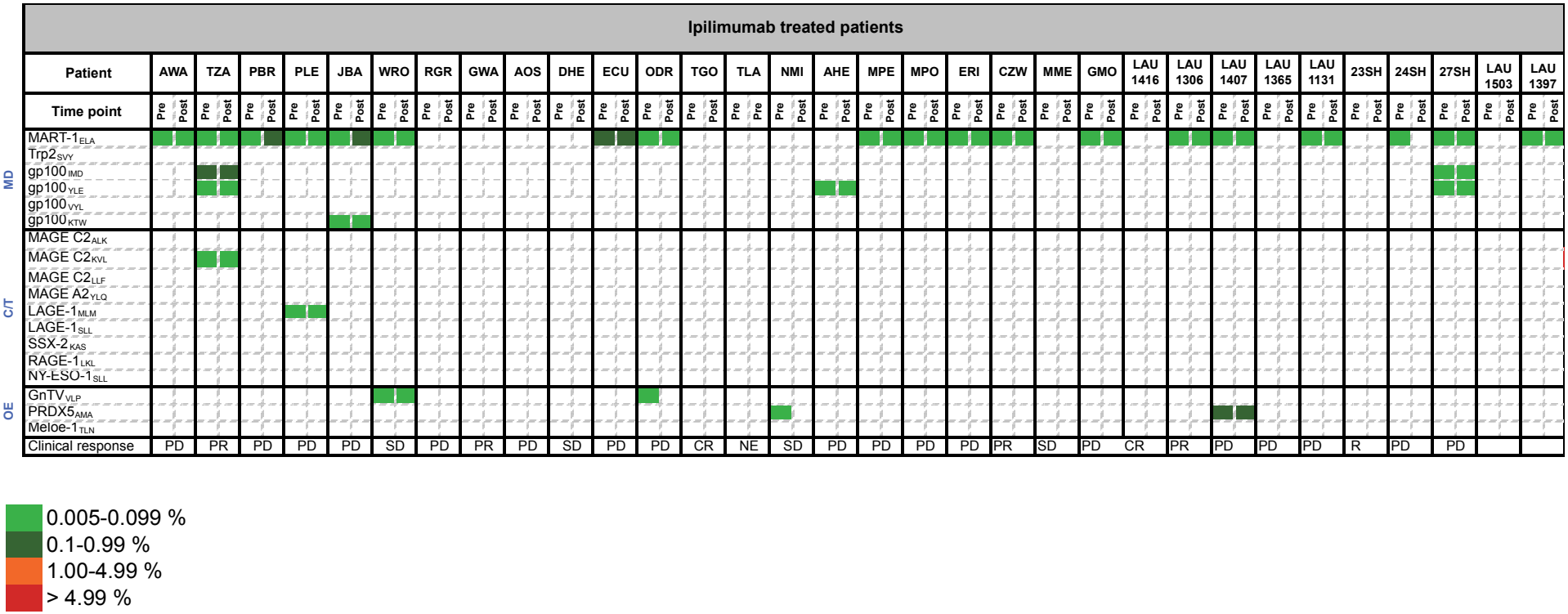




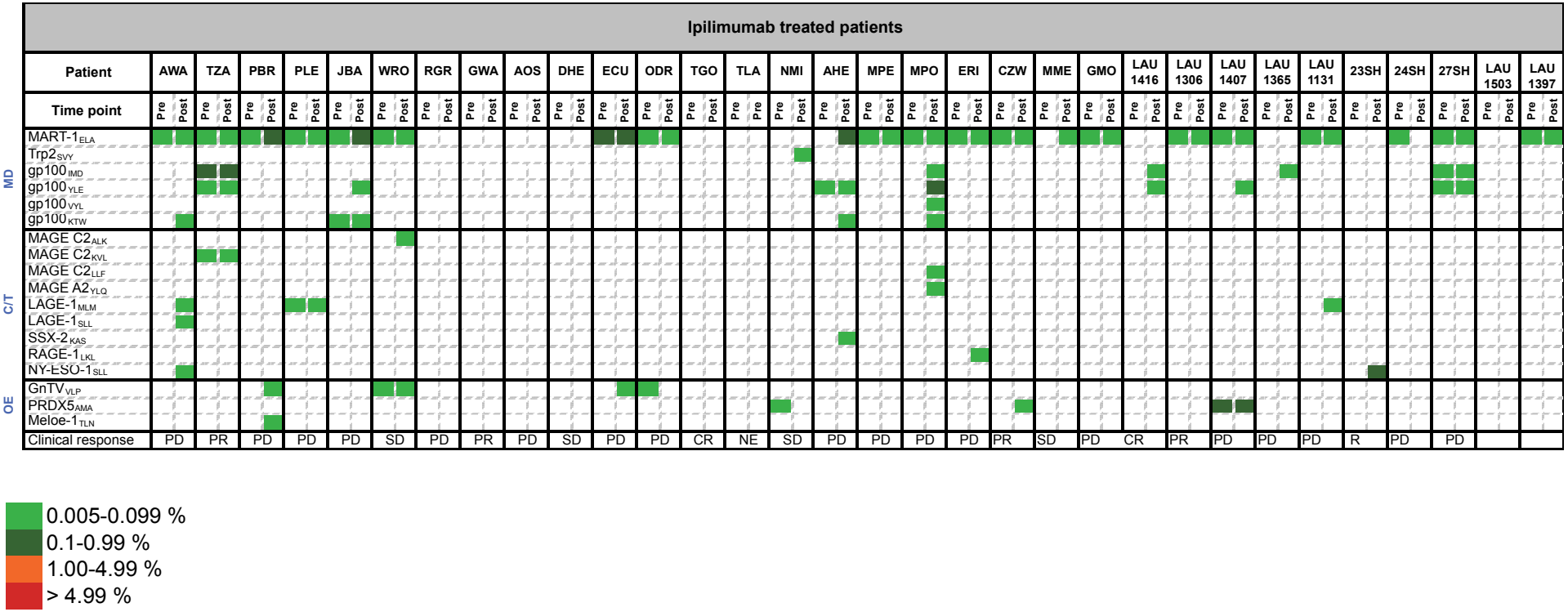
Melanoma-specific T cell responses detected pre-therapy:



Magnitude of these pre-existing responses is generally unaltered by Ipi treatment

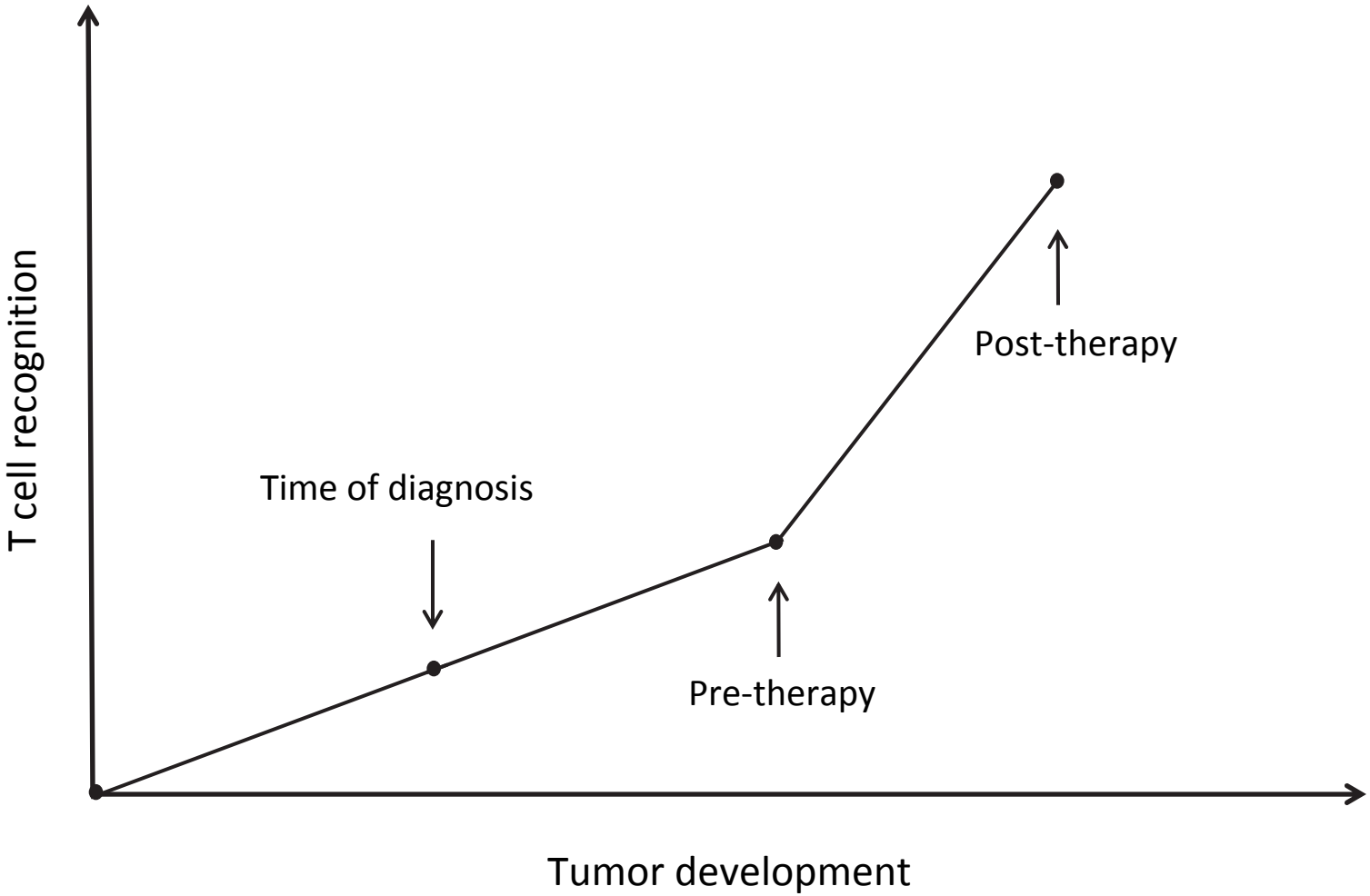


But a broadening of the T cell response is detected in many patients

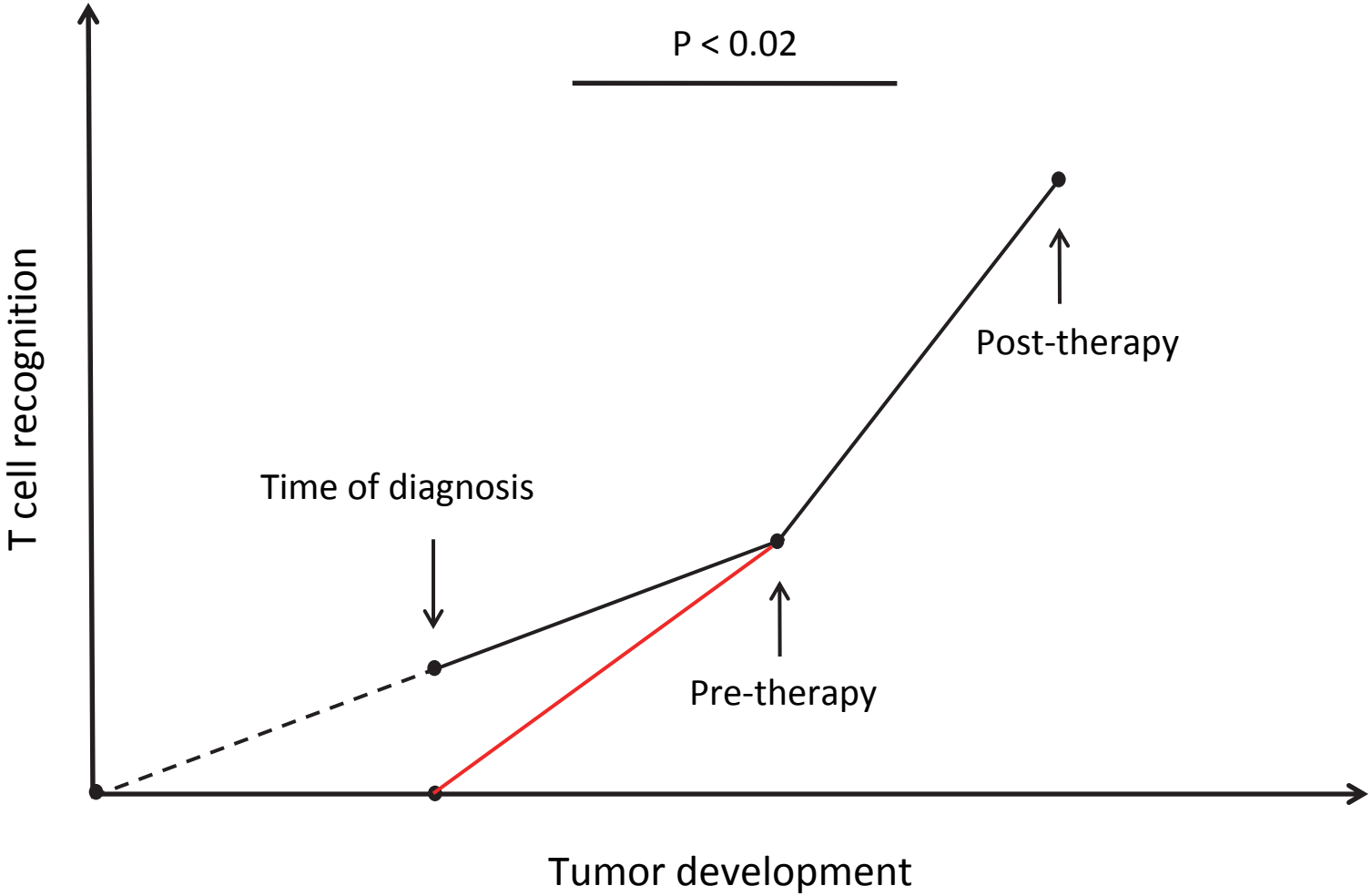


Broadening in the number of T cell responses in 16/32 patients (50%)

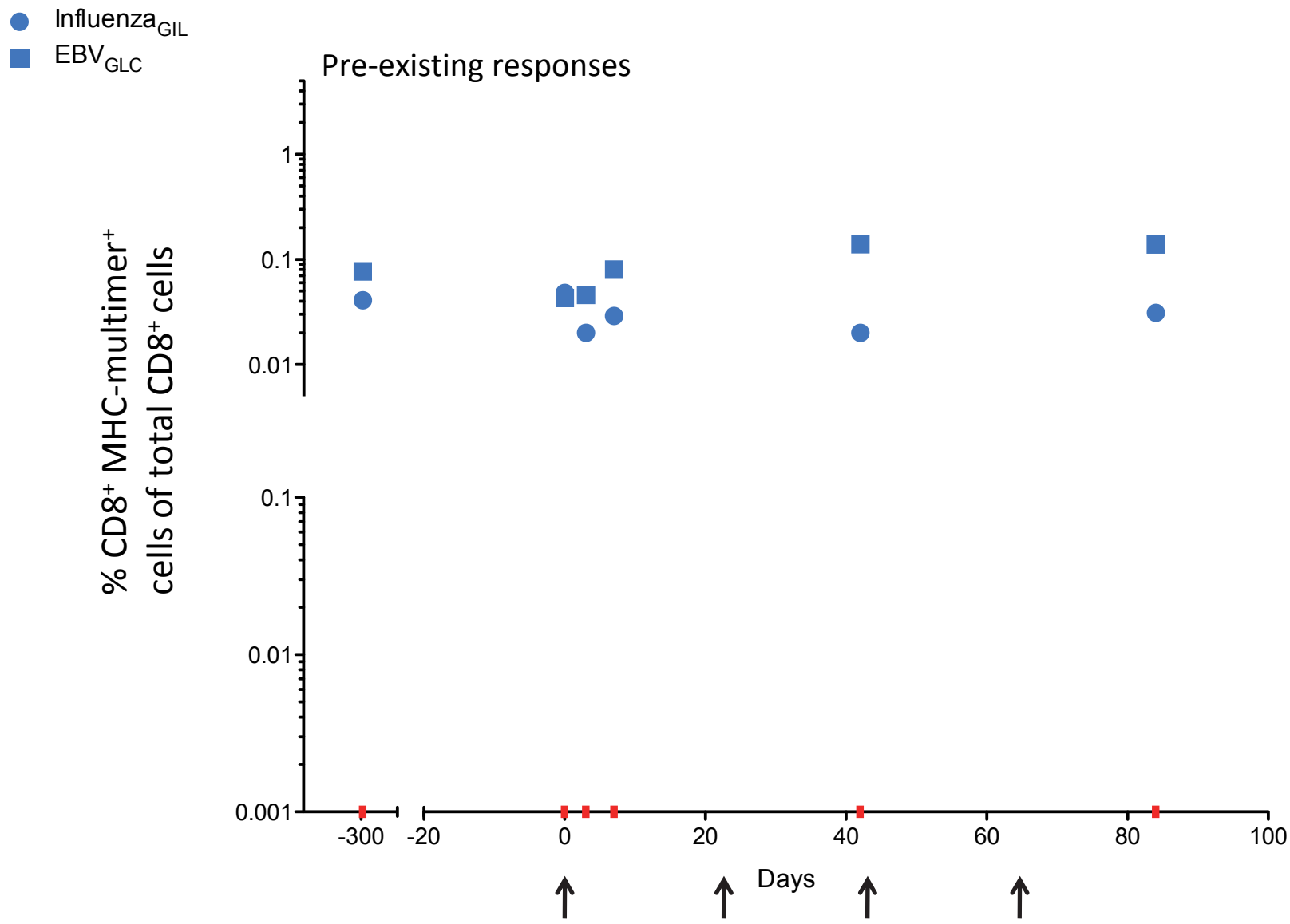
Broadening of T cell responses: therapy induced ?



Broadening of T cell responses: therapy induced ?



Kinetics of T cell responses during Ipilimumab treatment



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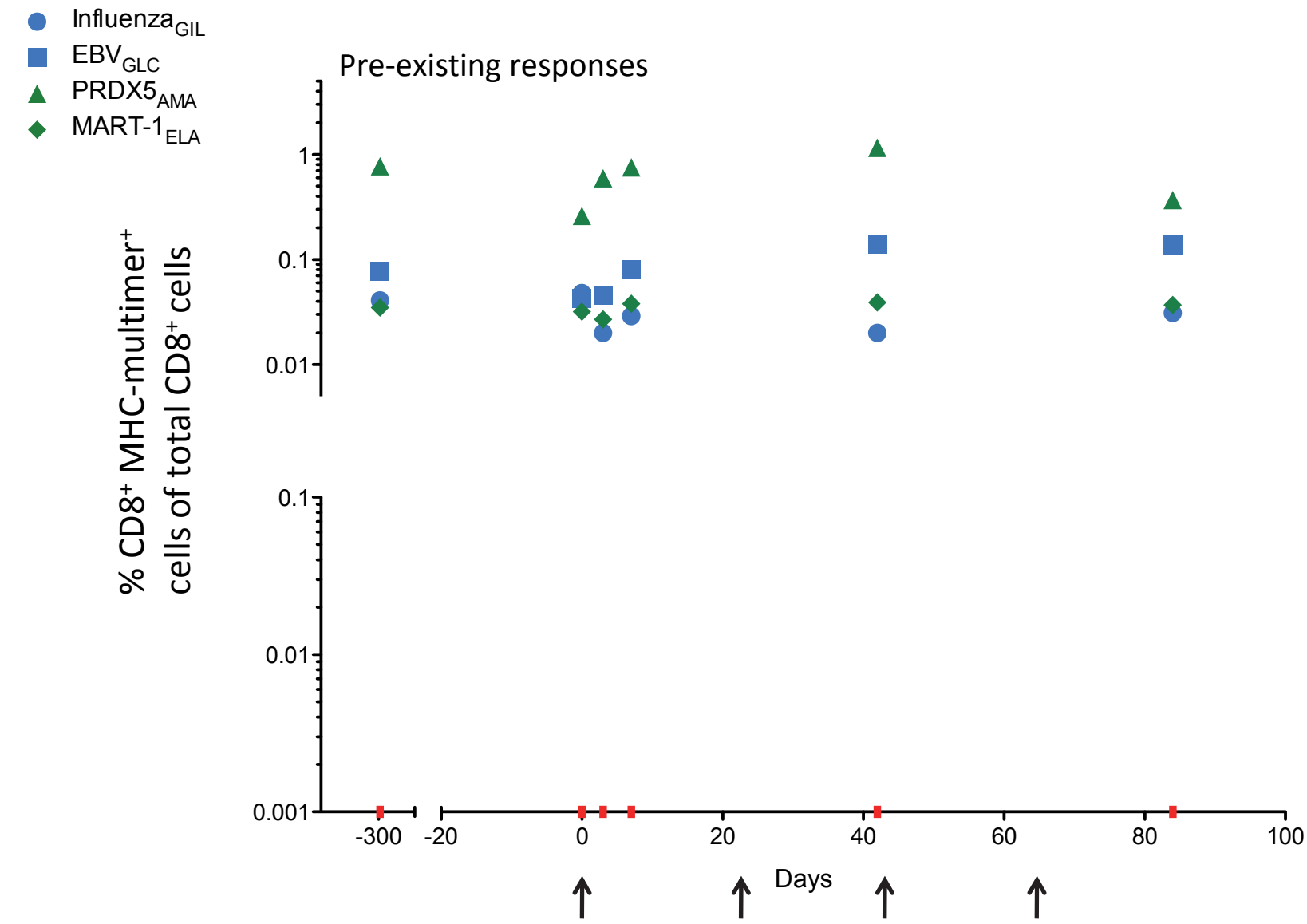

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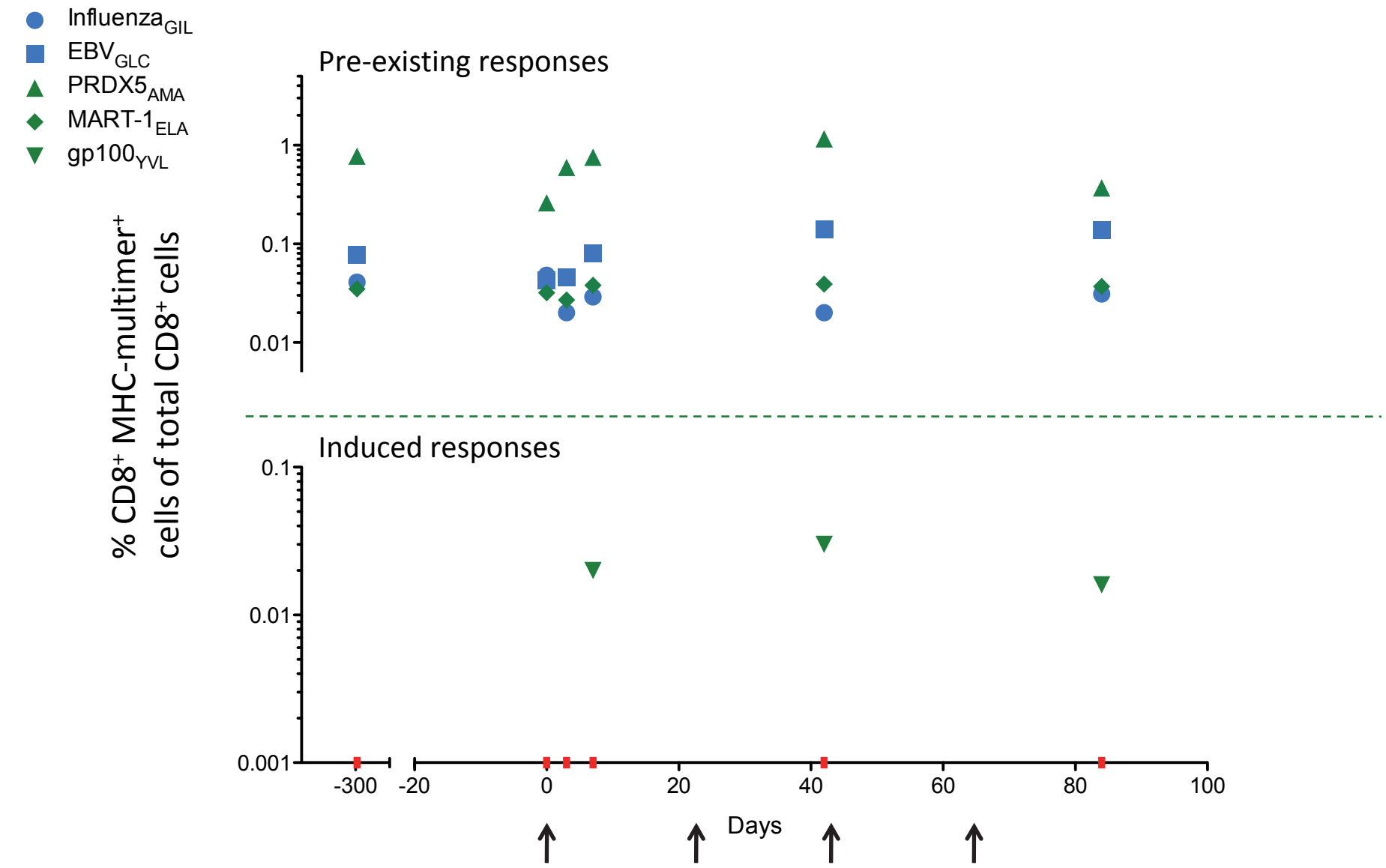

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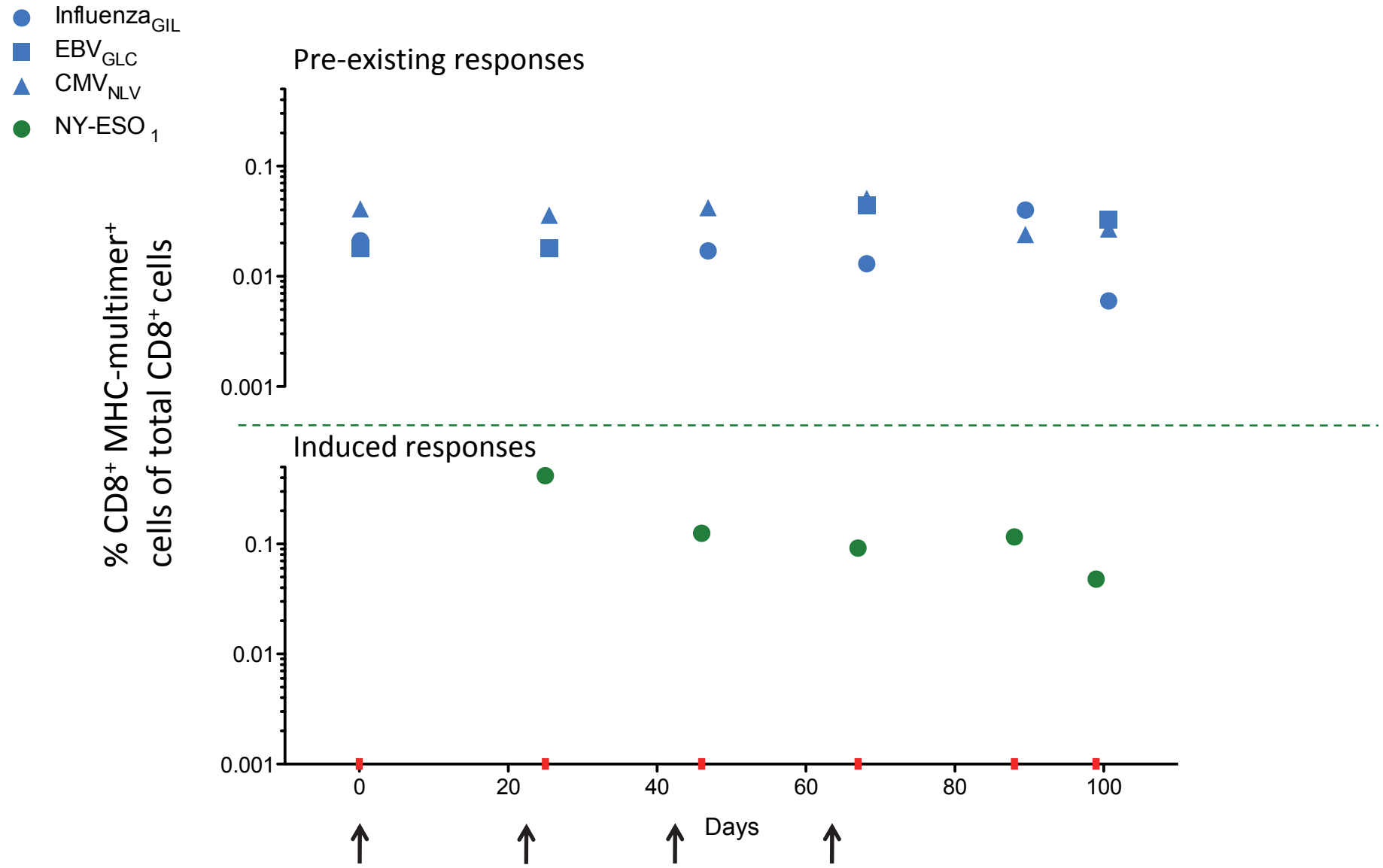
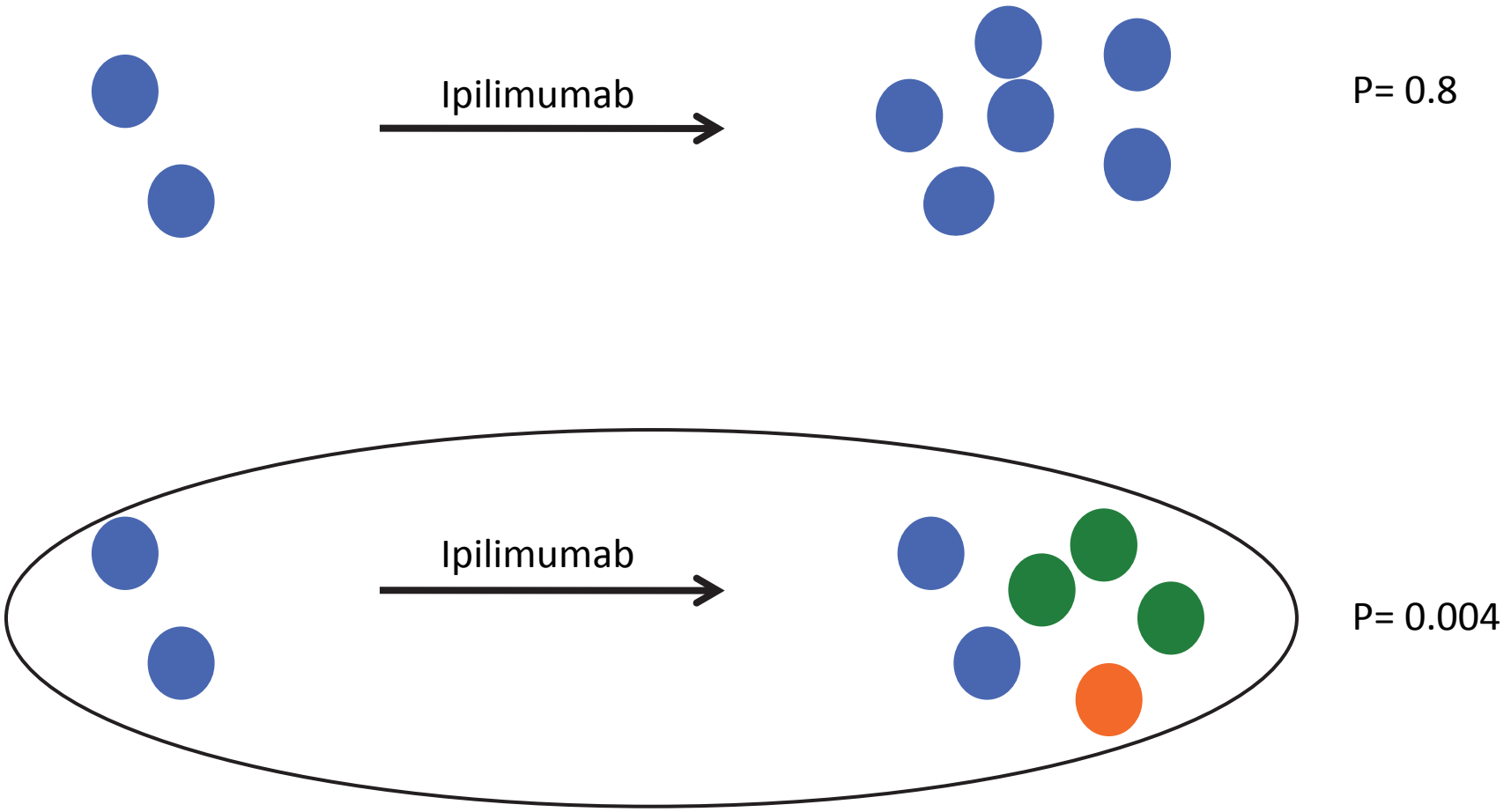


Two possible scenarios:

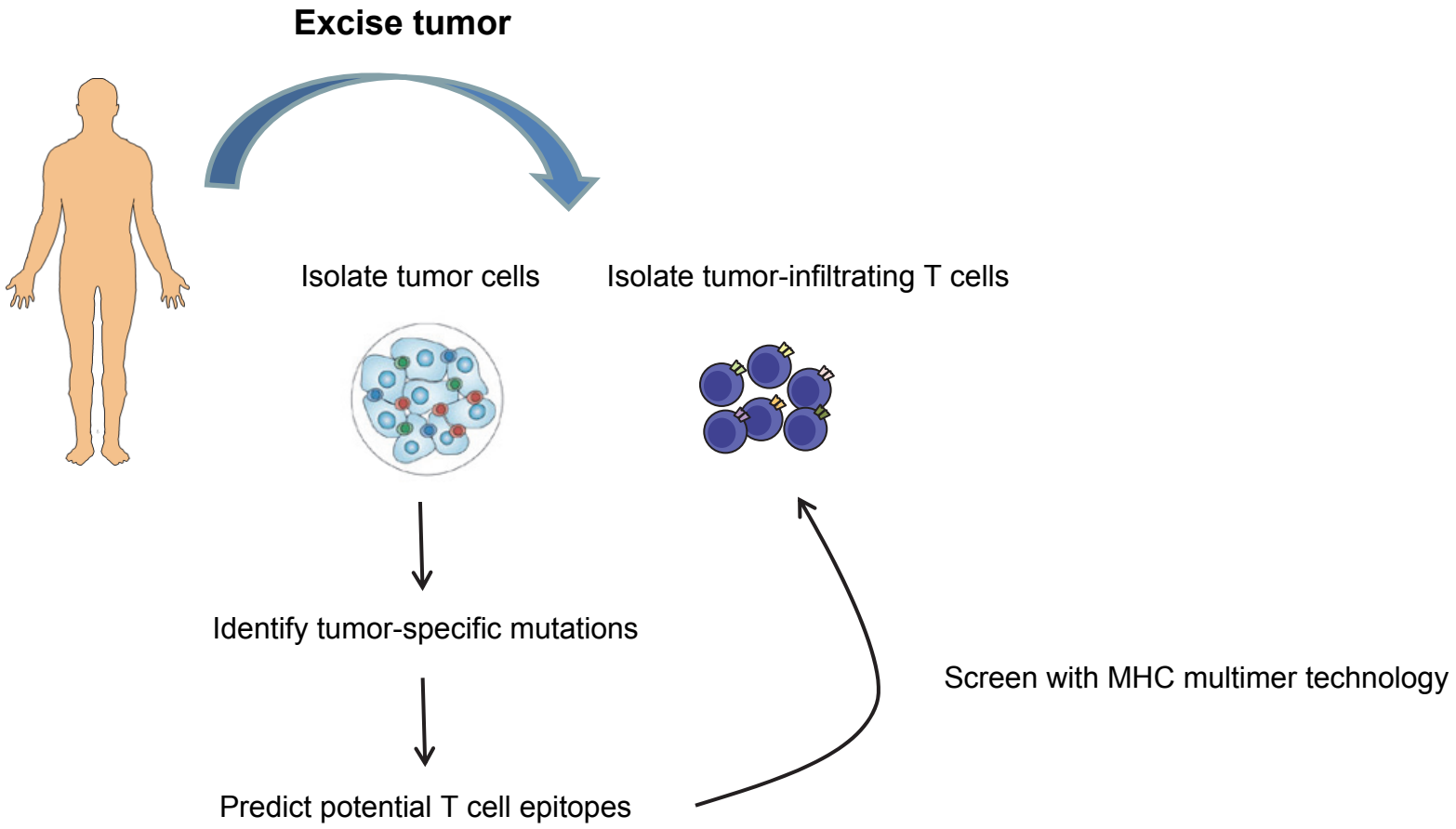


Summary

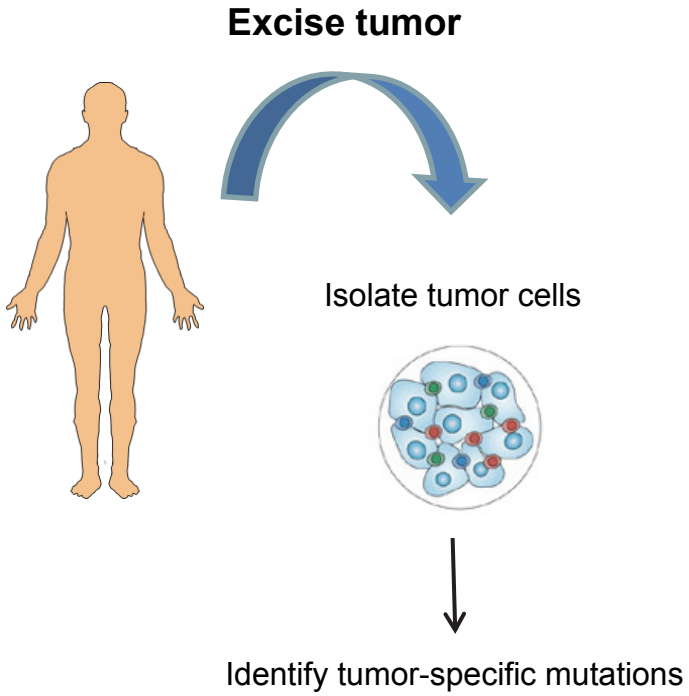
- Magnitude of pre-existing responses is in general unaltered by Ipilimumab therapy
- Broadening of T cell responses can be detected after Ipilimumab therapy
- Kinetic data strongly suggest post-therapy broadening is therapy-induced
- We have developed and validated technology for high-throughput detection of therapy-induced antigen-specific $CD8^+$ T cell reactivity

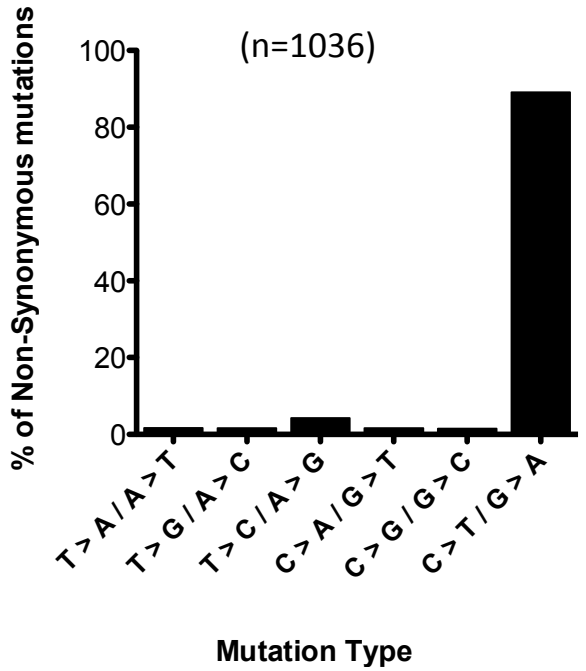
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

- Could we utilize this technology to analyze patient-specific tumor-reactive T cell responses?



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





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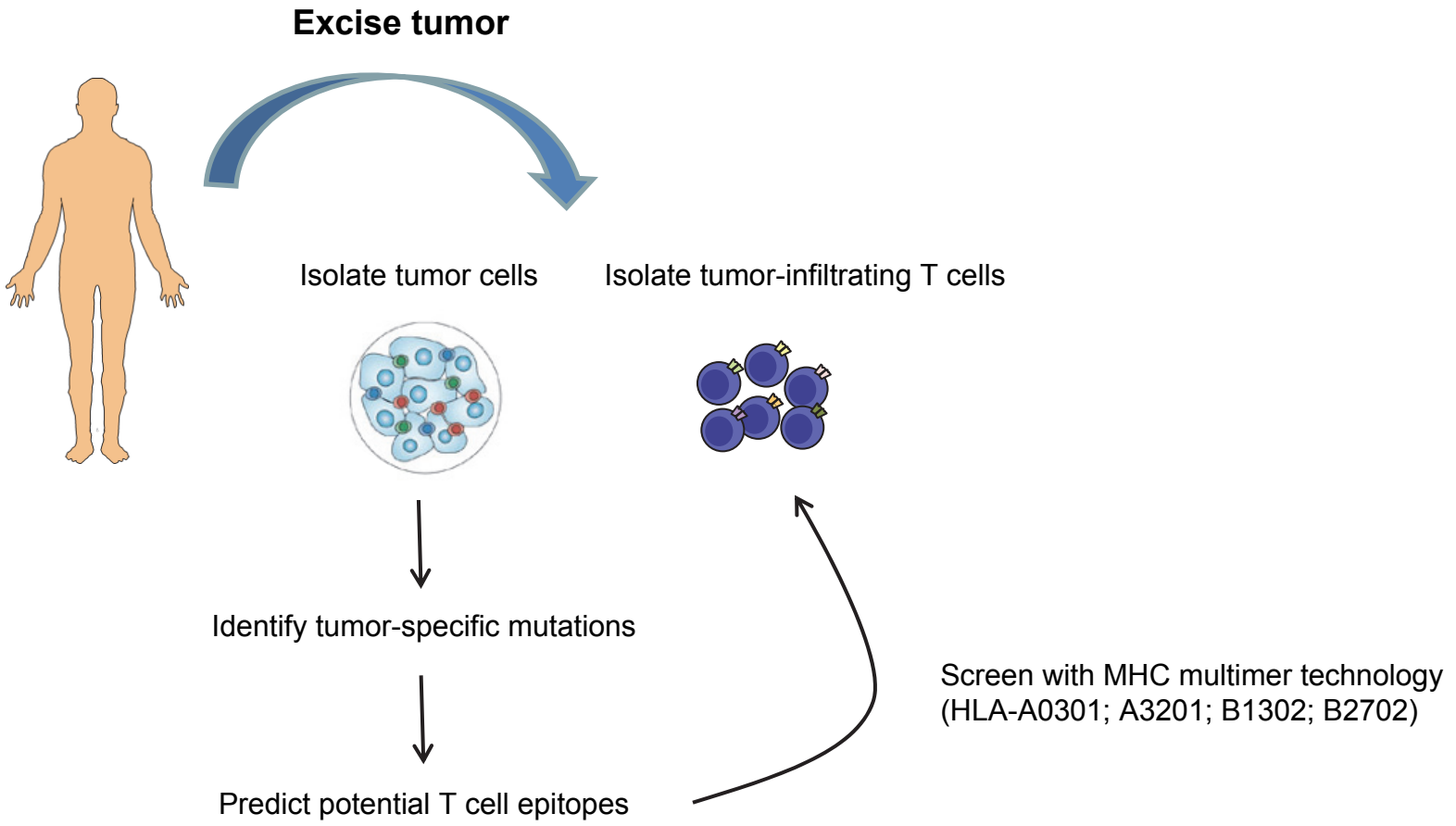
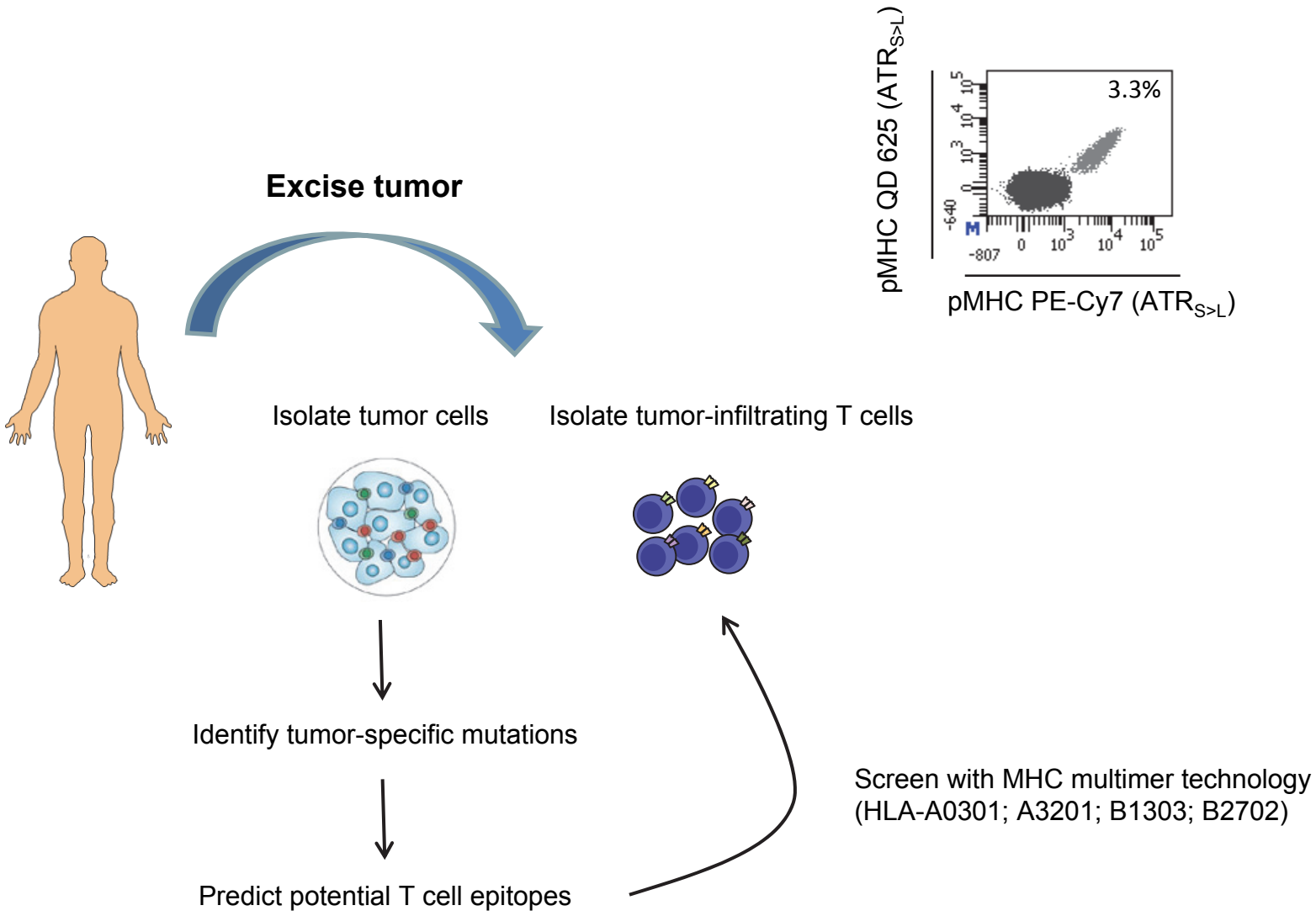
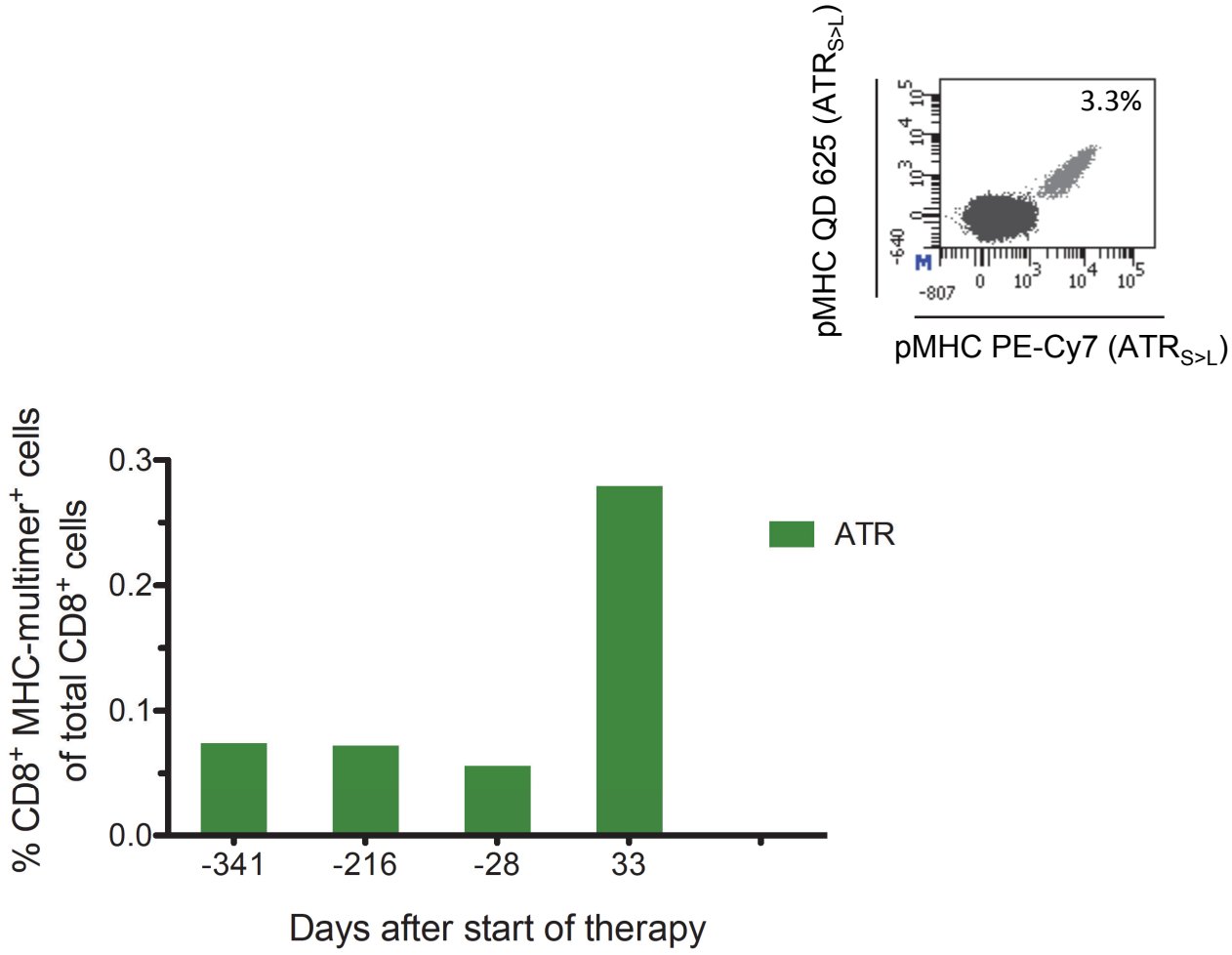


Strong T cell response against an $ATR_{S>L}$ epitope in TIL



Increased magnitude of neo-antigen-specific T cell response under Ipi



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- Analysis of neo-antigen specific T cell responses is possible using this approach

MHC-based monitoring

Daisy Philips

Marit van Buuren

Laura van Dijk

Mireille Toebes

Clinical translation

Nienke van Rooij

Bianca Heemskerk

Sander Kelderman

Christian Blank

CCIT, Copenhagen

Sine Reker Hadrup

Charlotte Albæk Thrue

PerThor Straten

Lausanne

Daniel Speiser

Immune monitoring

Willeke van de Kasteele

Trees de Jong

John Haanen

Ton Schumacher

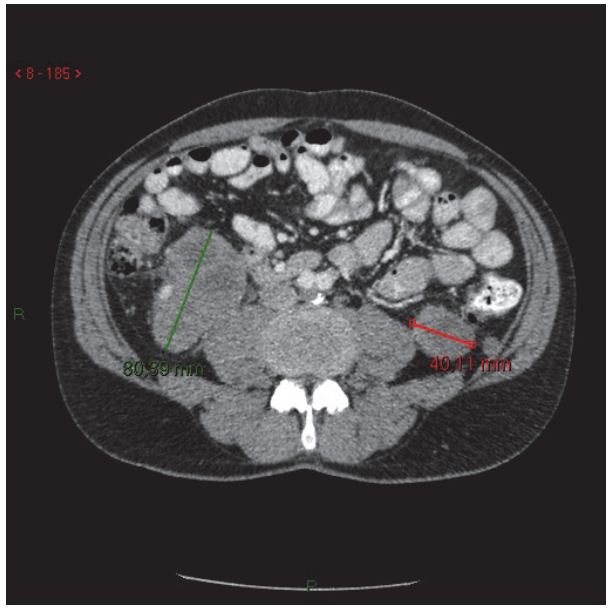
SH University Hospitals

Christian Ottensmeier

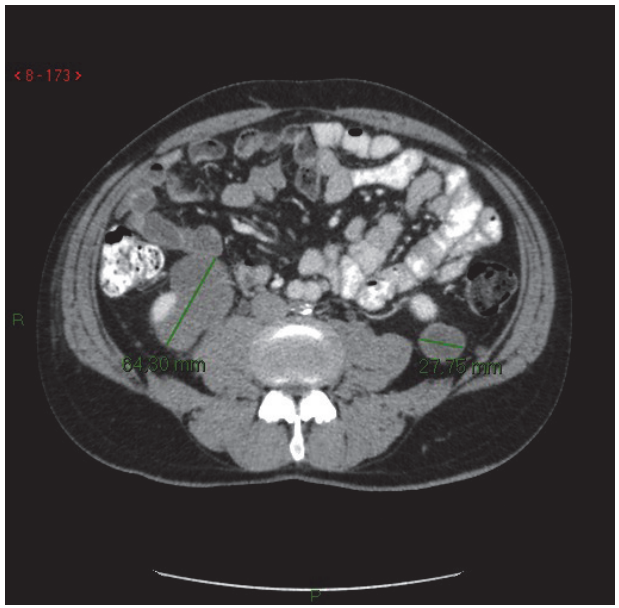
Bristol-Myers Squibb

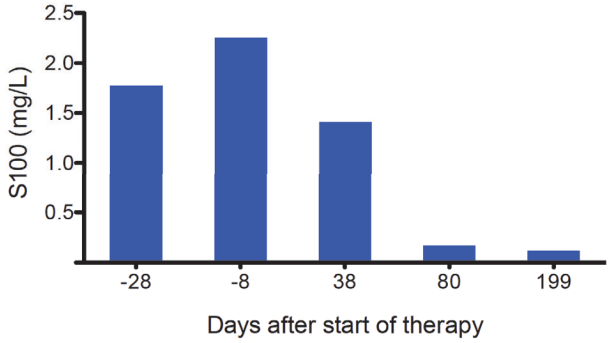
Pt 002: Partial response upon Ipilimumab

Aug 2010



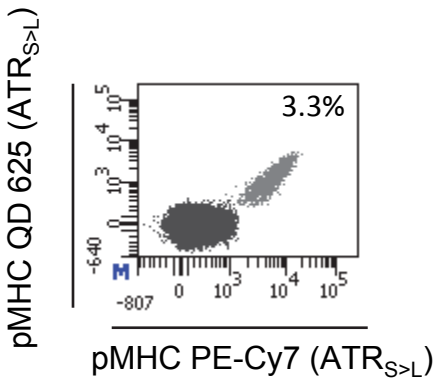
Dec 2010

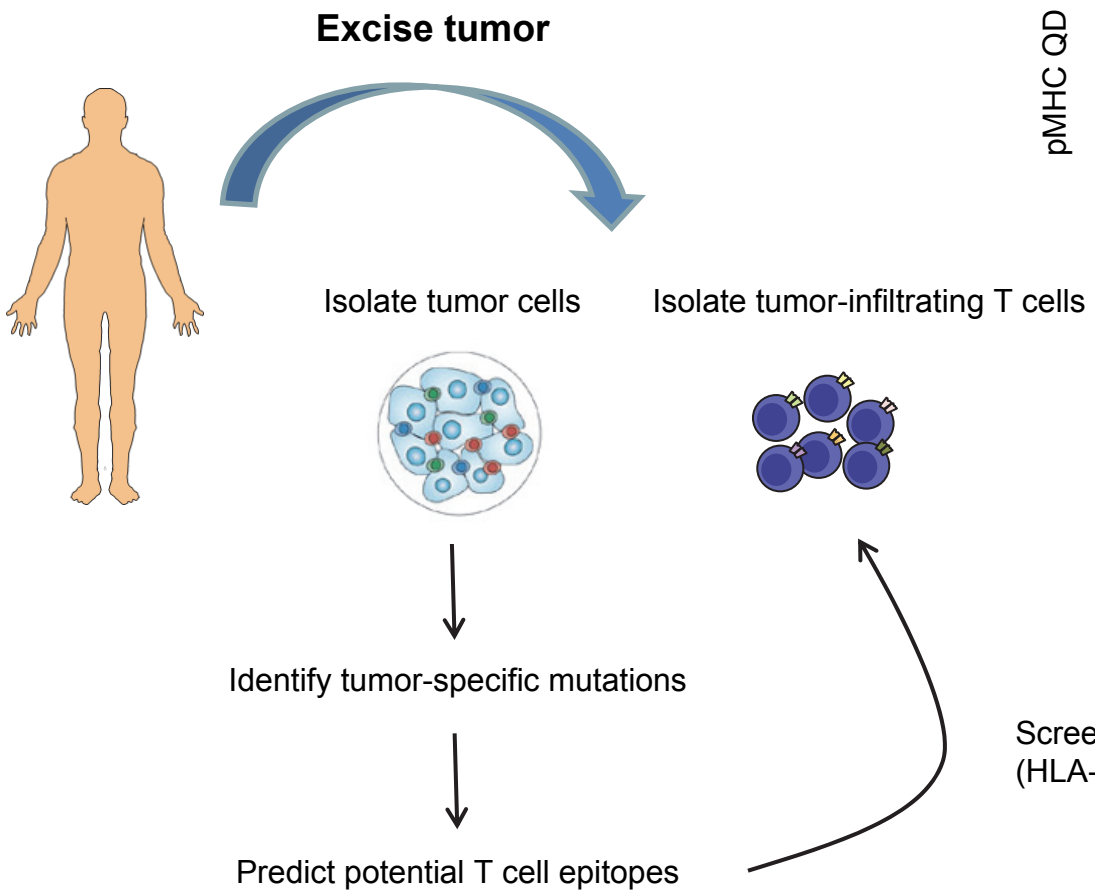




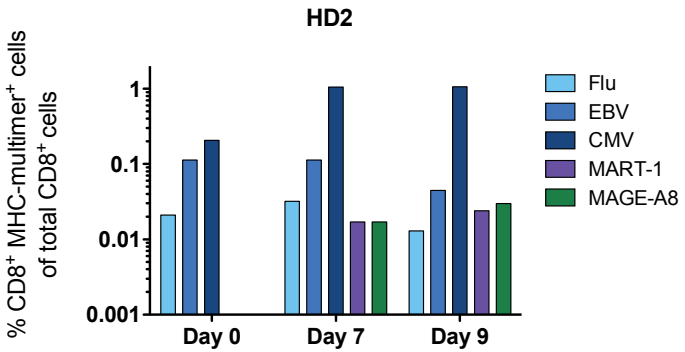
Strong T cell response against an $ATR_{S>L}$ epitope in TIL

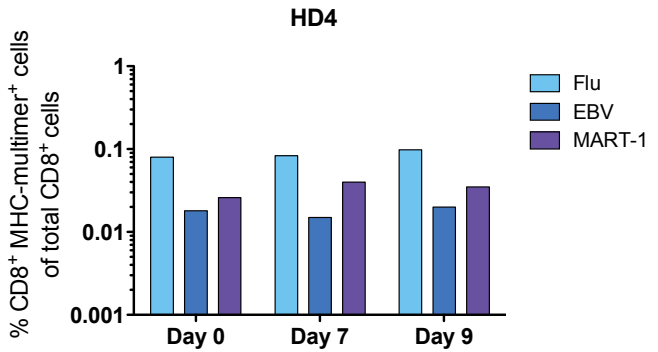
Reads tumor: WT 109 Mut 189
Reads matched healthy tissue: WT 274 Mut 0
Q score: 225, RNA^{+}

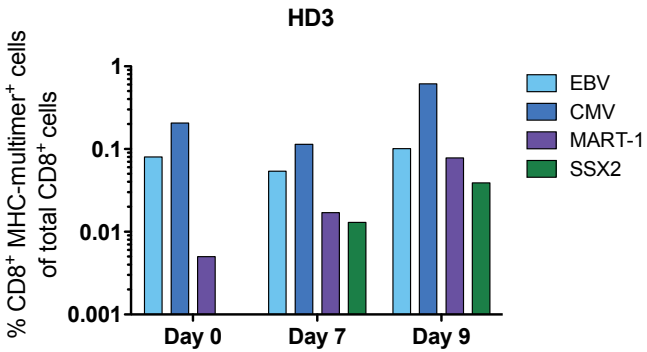


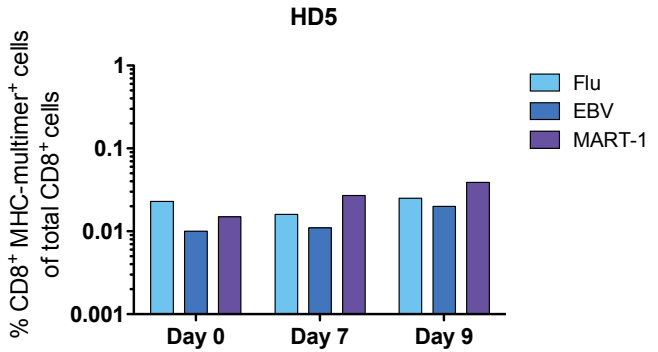


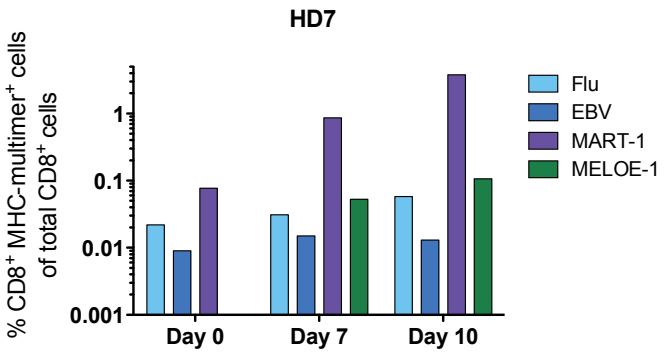
In vitro stimulation

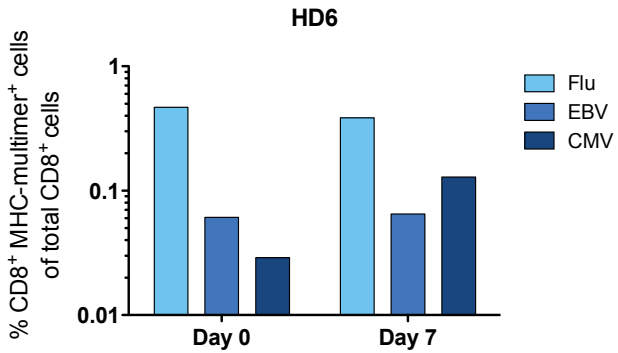




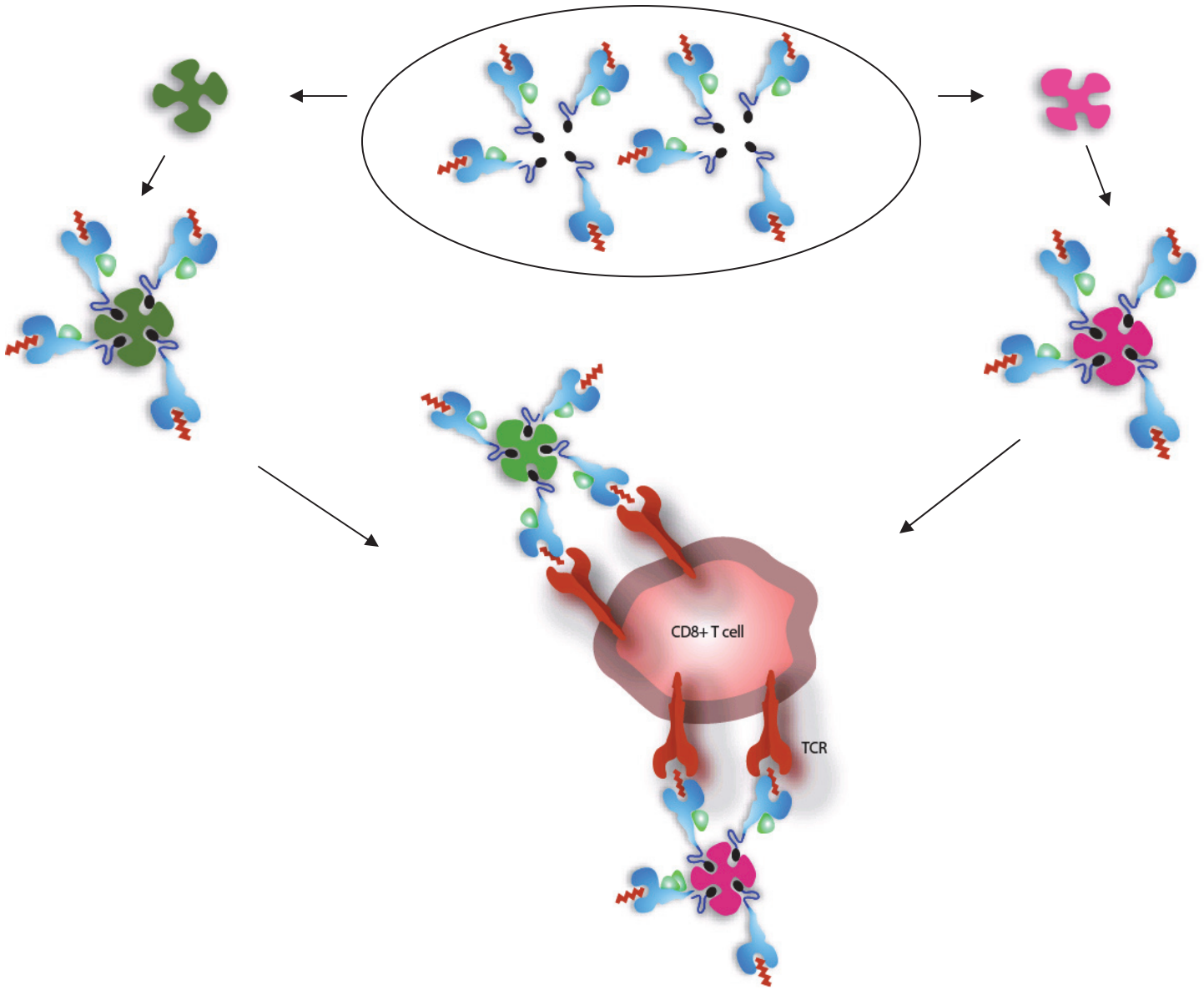




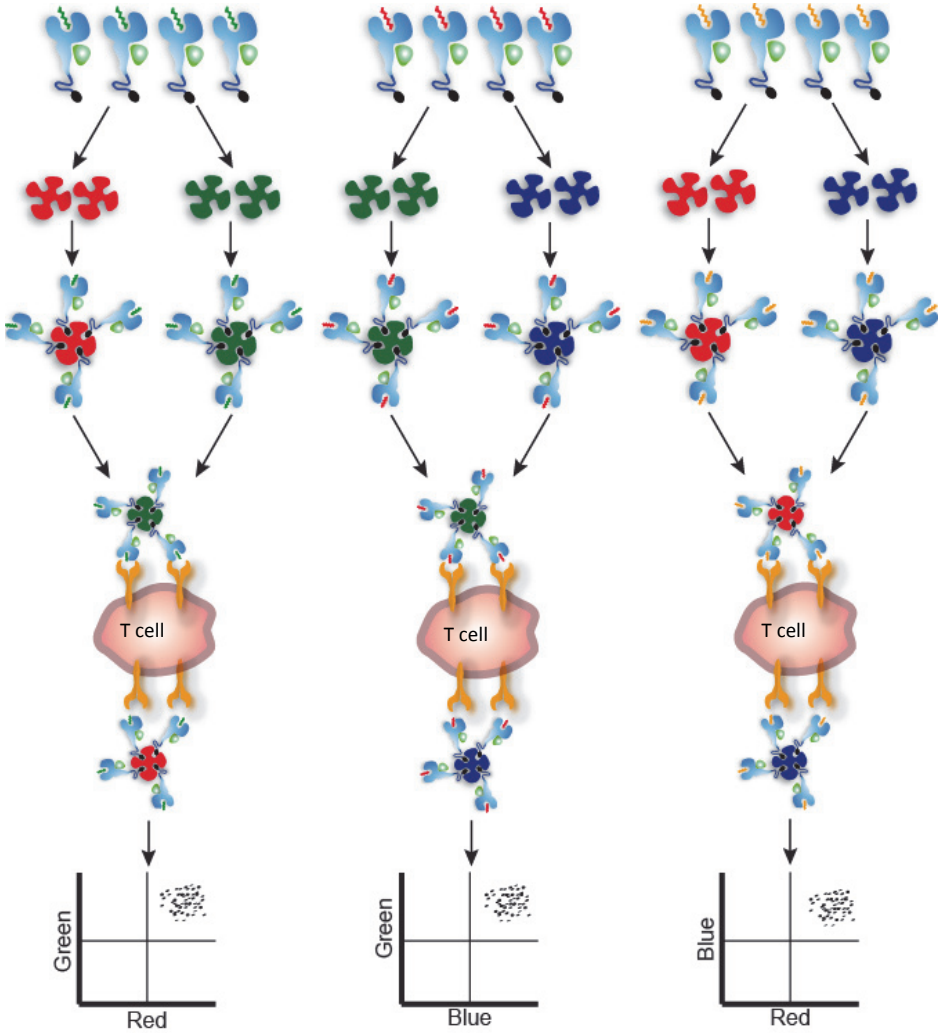




Self-assembling molecular codes



Dual color combinatorial coding



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 unique two-color codes

Combinatorial Coding Gating Strategy