Identification of antigens recognized by T cells

Victor H. Engelhard, PhD
Carter Immunology Center
Department of Microbiology, Immunology, and Cancer Biology
University of Virginia School of Medicine
vhe@virginia.edu

Disclosures

Agenus Inc.

Consultant

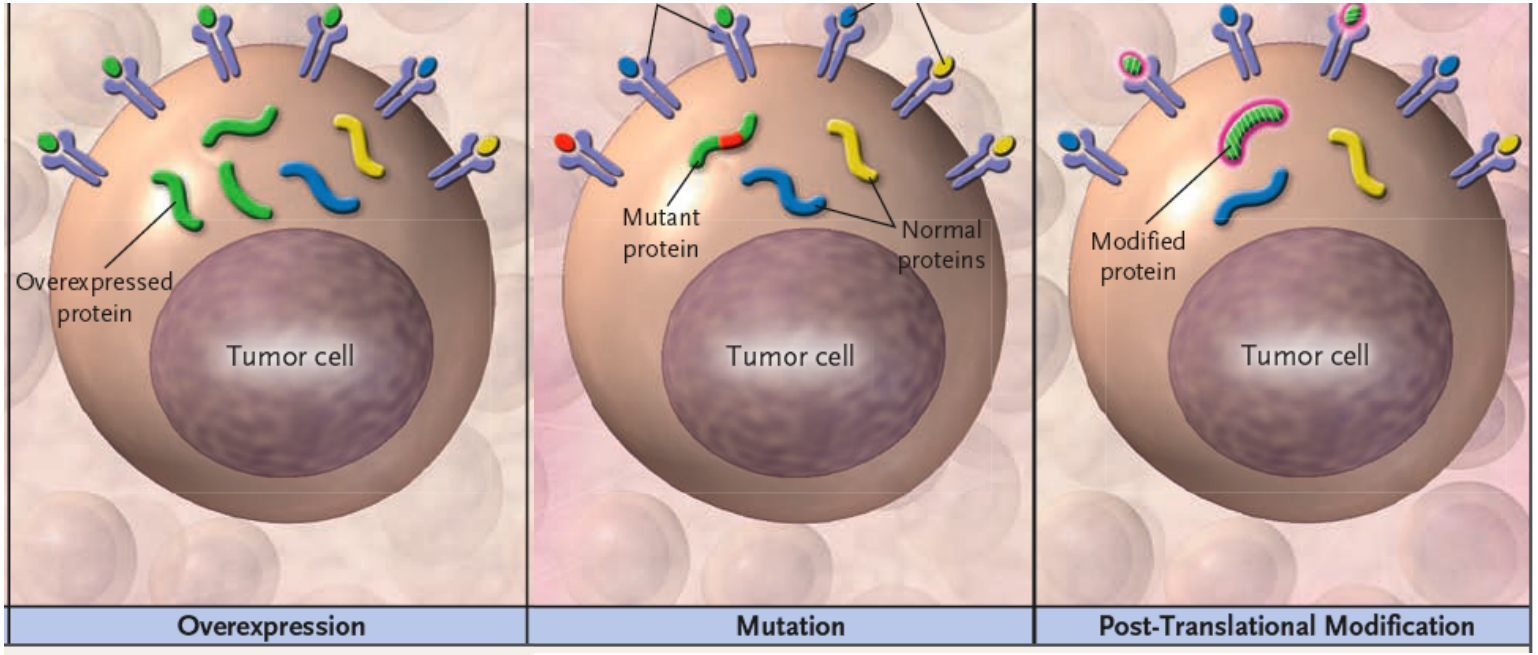
Shareholder

Characteristics of identified tumor antigens

Tissue-specific, cancer-testis antigens

Patient-specific neoantigens

Phosphopeptides



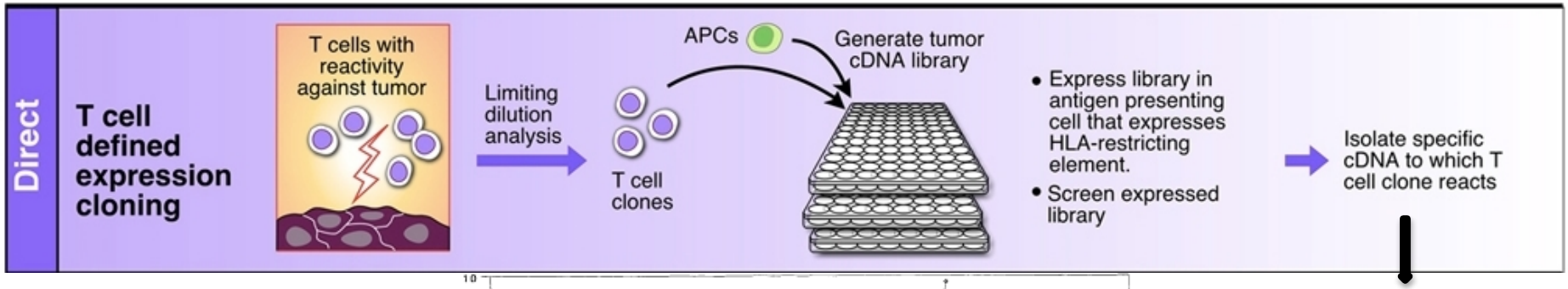
Finn N Engl J Med. 358:2704 (2008)

General approaches to antigen discovery

- Direct methodology
  - Isolate cancer reactive T cells from patients
  - Identify MHC and peptide that comprise the antigen recognized
- Reverse methodology
  - Identify genes, proteins, or peptides of interest expressed in/on cancer cells
  - Establish whether there are T cells in patients that recognize them

cDNA library approach

T. Boon/S. Rosenberg



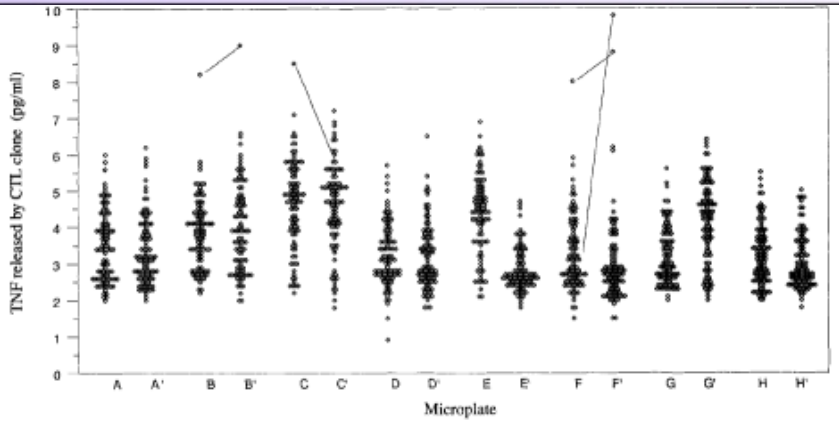


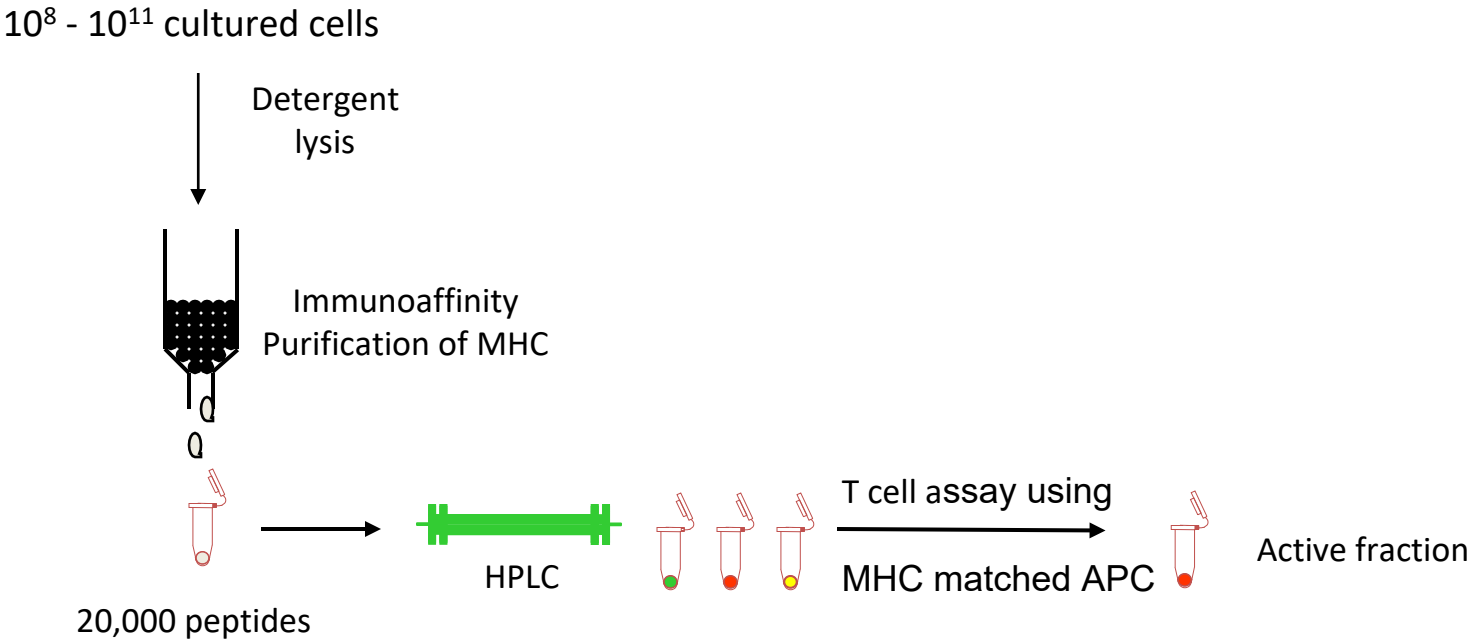
Figure 2. Stimulation of CTL 1/95 by COS cells cotransfected with HLA-A2 and pools of approximately 25 different cDNAs obtained from melanoma cell SK29-MEL. Each symbol represents the TNF content of one individual microculture, and the duplicate microplates are named A and A', B and B', etc. Duplicates of seemingly positive microcultures are connected with a line. Each pool of cDNAs was transfected into duplicate microcultures. Both the HLA-A2 gene and the cDNAs were cloned into expression vector pcDNAI/Amp. They were cotransfected with DEAE-Dextran into subconfluent COS cells. 48 h after transfection, CTL 1/95 (2,000 cells per well) was added. The culture supernatants were harvested 1 d later and tested on W13 cells for their TNF content.

Identify peptide using truncation cDNAs, synthetic peptides

Brusic & Wu Frontiers Biosci 17:635 (2012)
Coulie et al J Exp Med 180:35 (1994)

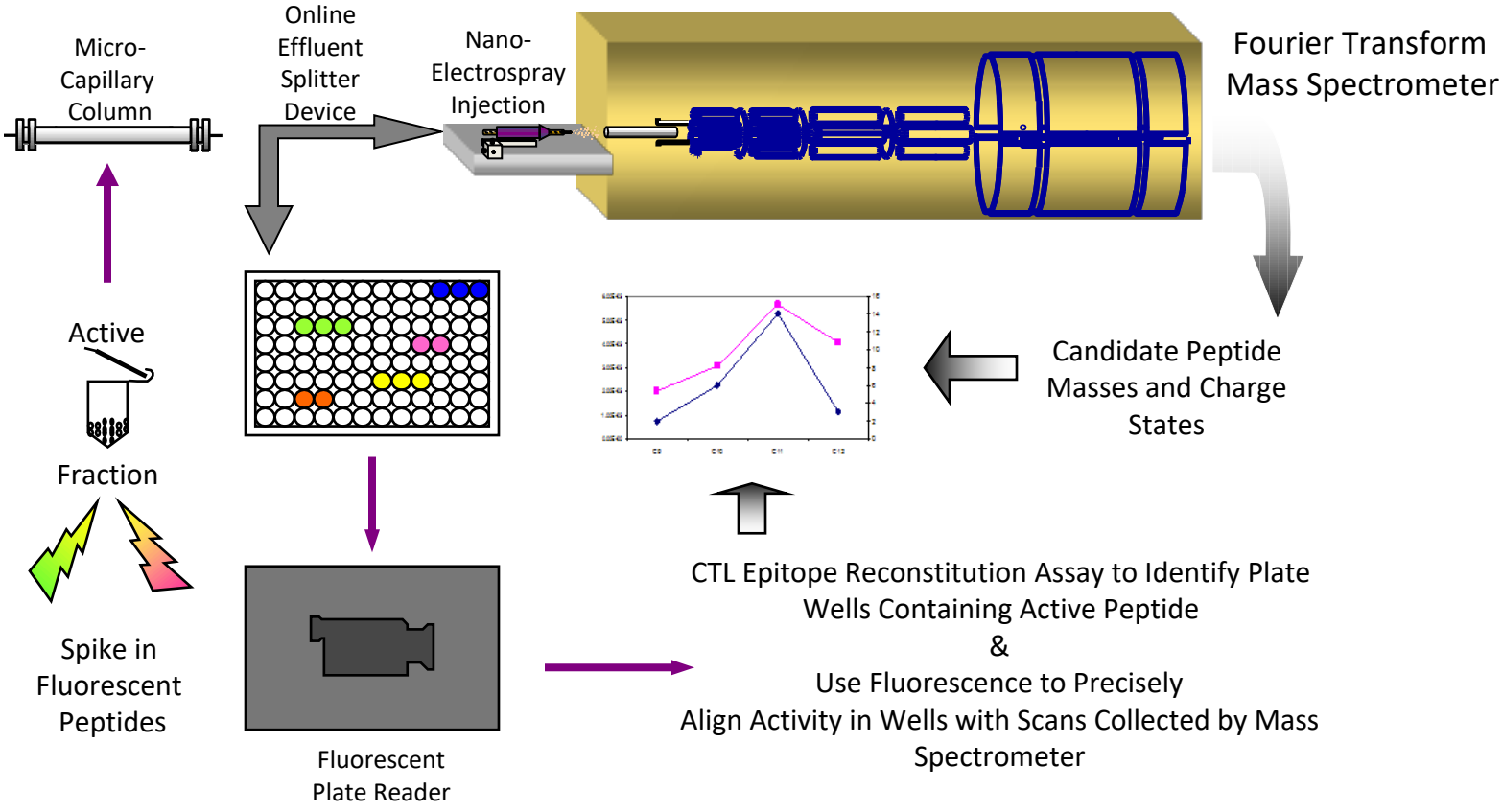
Mass spectrometry approach

V. Engelhard/D. Hunt

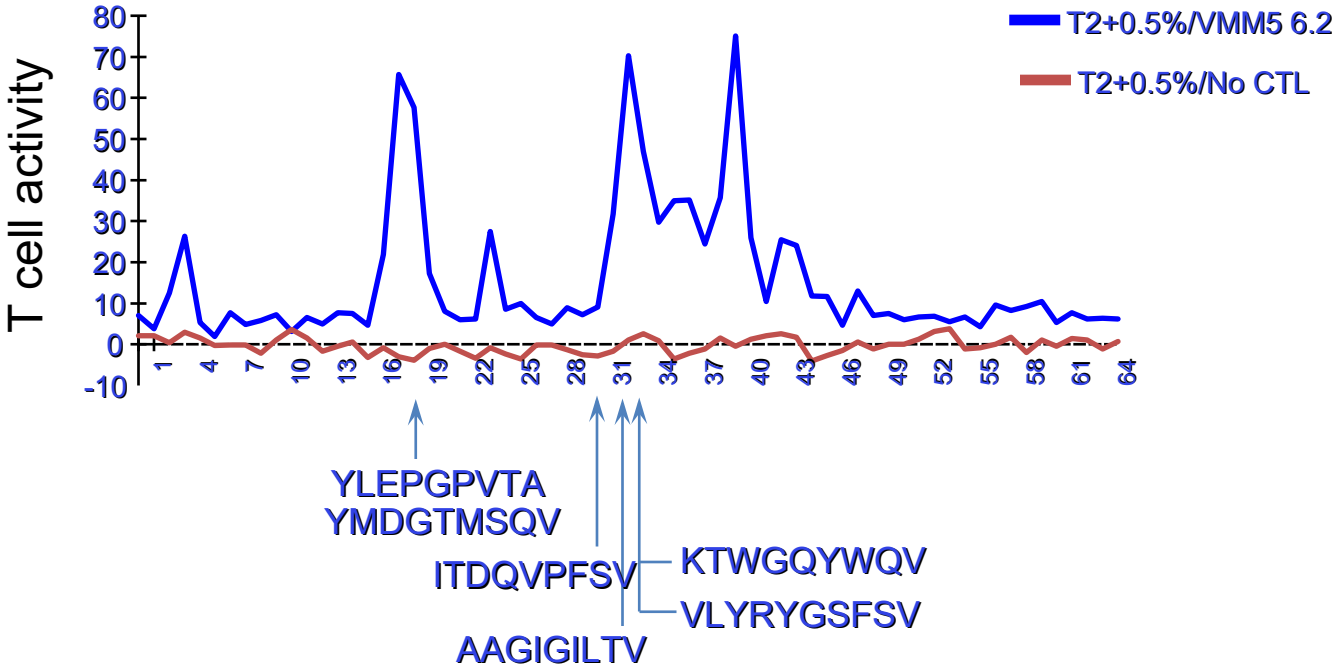


Mass spectrometry approach

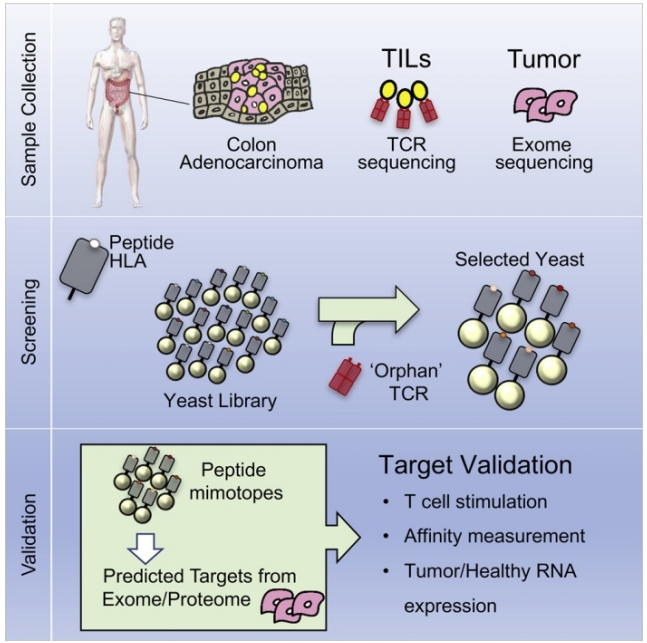
V. Engelhard/D. Hunt

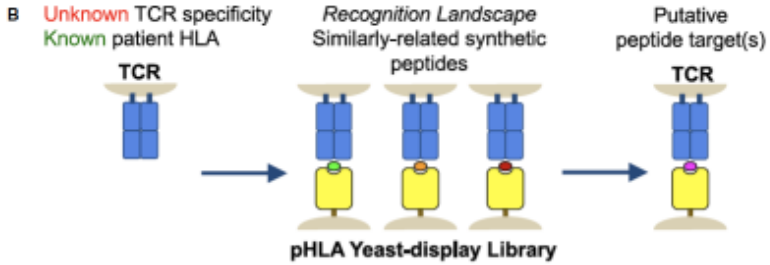


Antigens identified among naturally processed melanoma peptides



Approach to identification of antigens recognized by “orphan TCR” from TIL

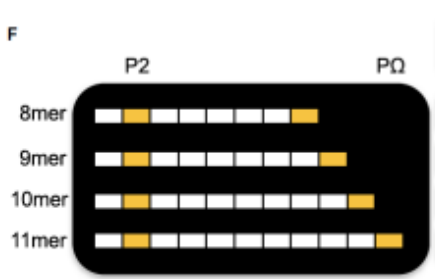




E

	P1	P2	P3	P4	P5	P6	P7	P8
Library	X	L M	X	X	X	X	X	L M V
Codon	NNK	MTG	NNK	NNK	NNK	NNK	NNK	NTG

F



Epitope Tag	Theoretical Nucleotide Diversity	Functional Diversity
V5	8.59×10^{9}	8.0×10^{7}
Myc	2.75×10^{11}	1.2×10^{8}
HA	8.80×10^{12}	1.3×10^{8}
VSV	2.81×10^{14}	7.0×10^{7}

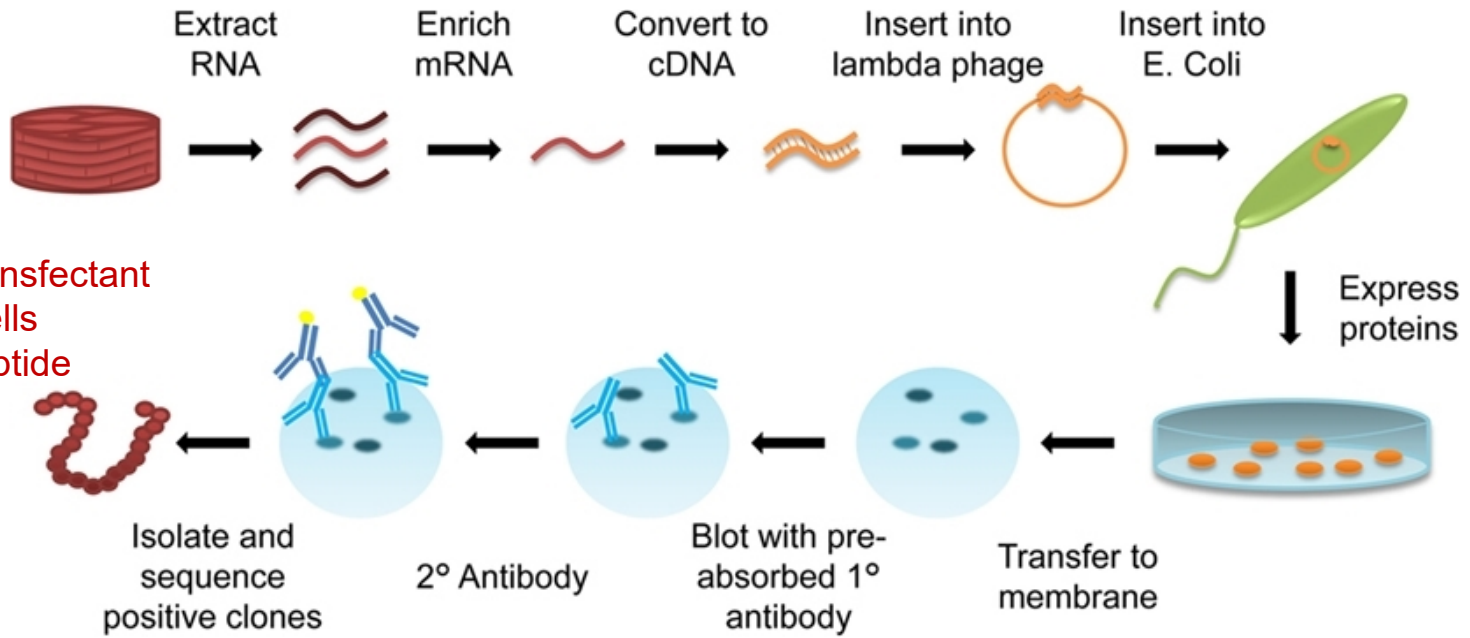
Gee et al. Cell 172:549 (2018)

Indirect Methods

Every method needs a hook…..

SEREX

L. J. Old



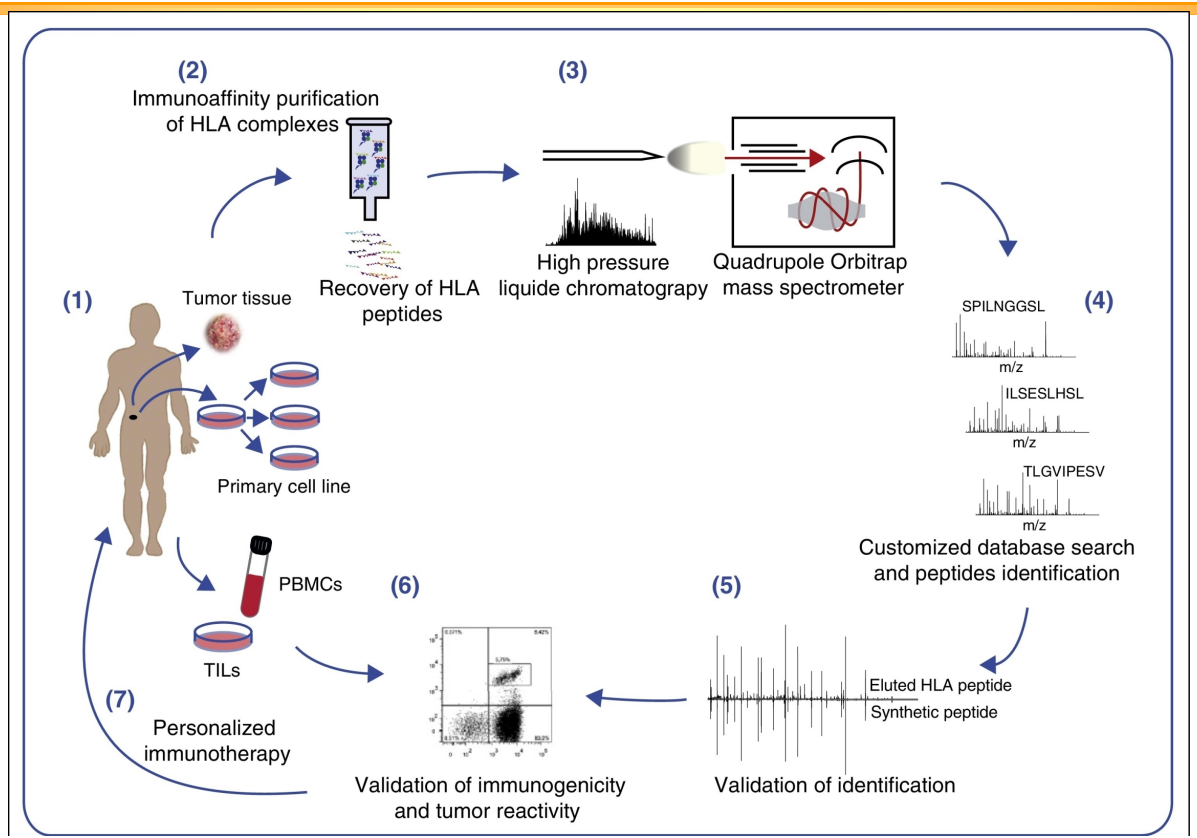
- Establish whether transfectant is recognized by T cells
- Identify MHC and peptide

From cancer patient

Success!: NY-ESO1

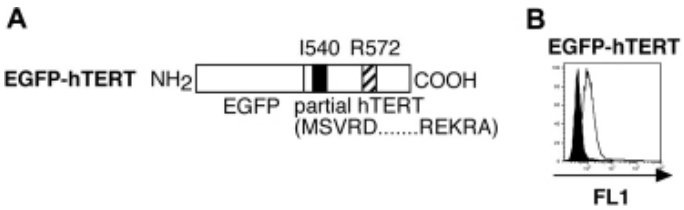
Gates et al. Front Immunol 8: 1332 (2017)

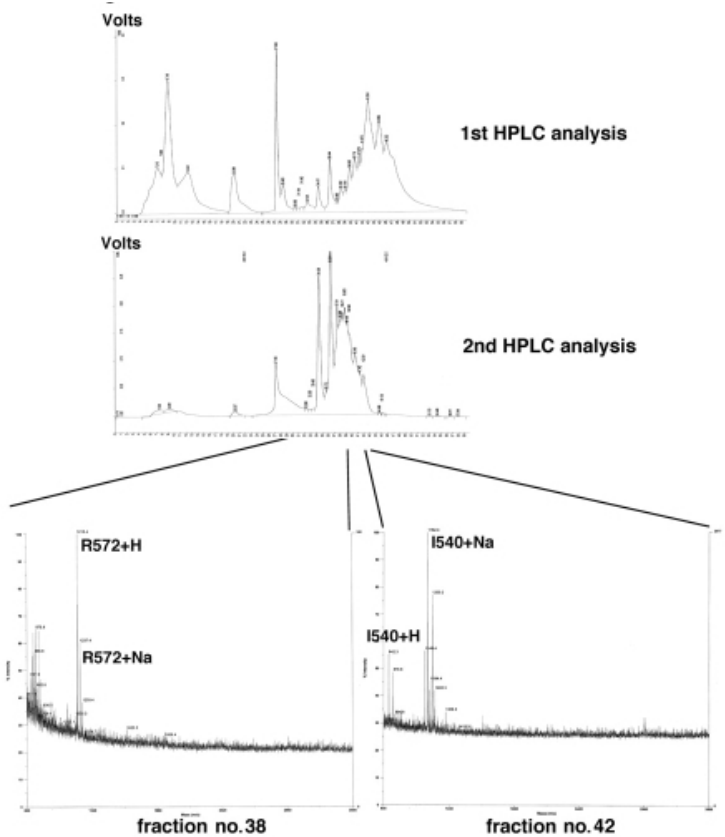
Mass spectrometry for general MHC peptide analysis and potential antigen identification



Bassani-Sternberg & Coukos. Curr Op Immunol 41:9 (2016)

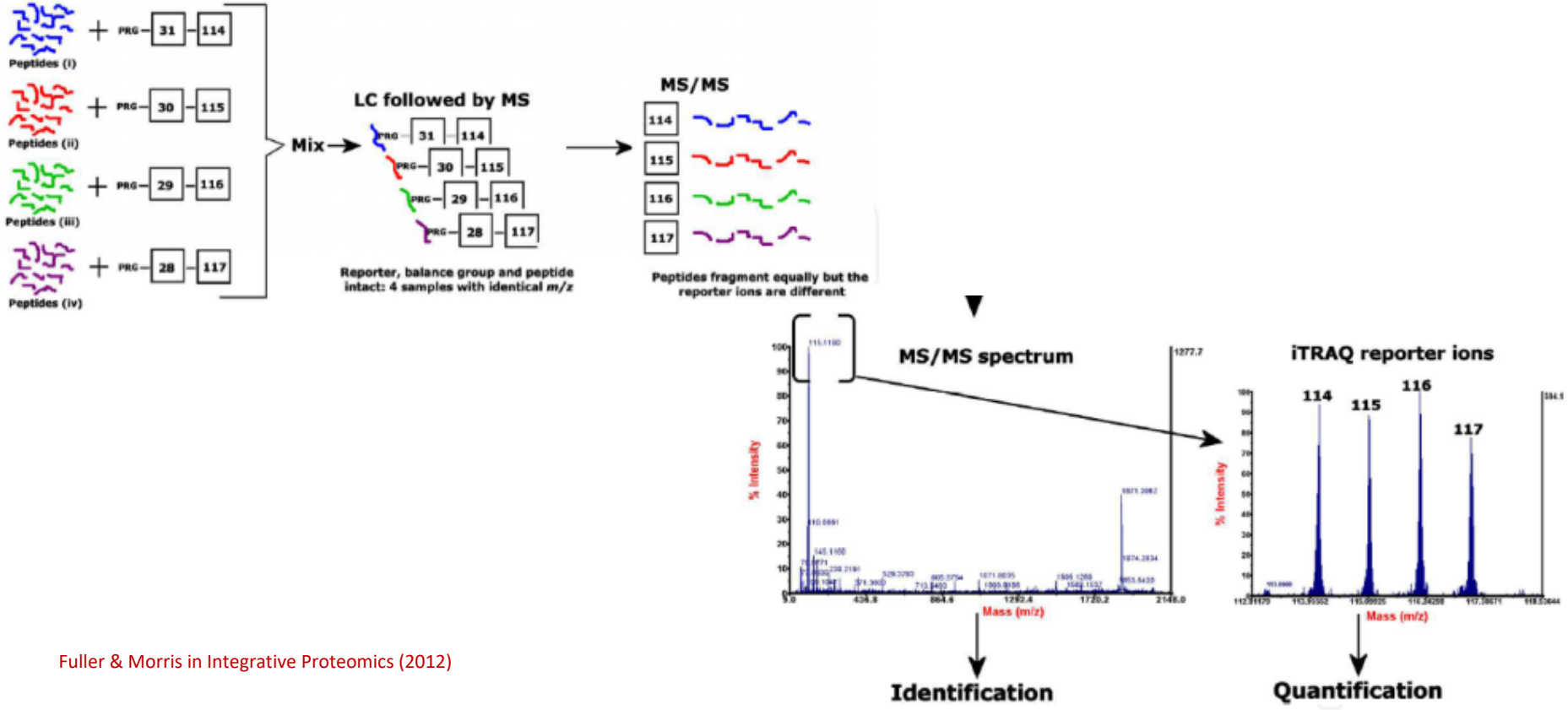
Targeted mass spectrometry: Finding what you are looking for





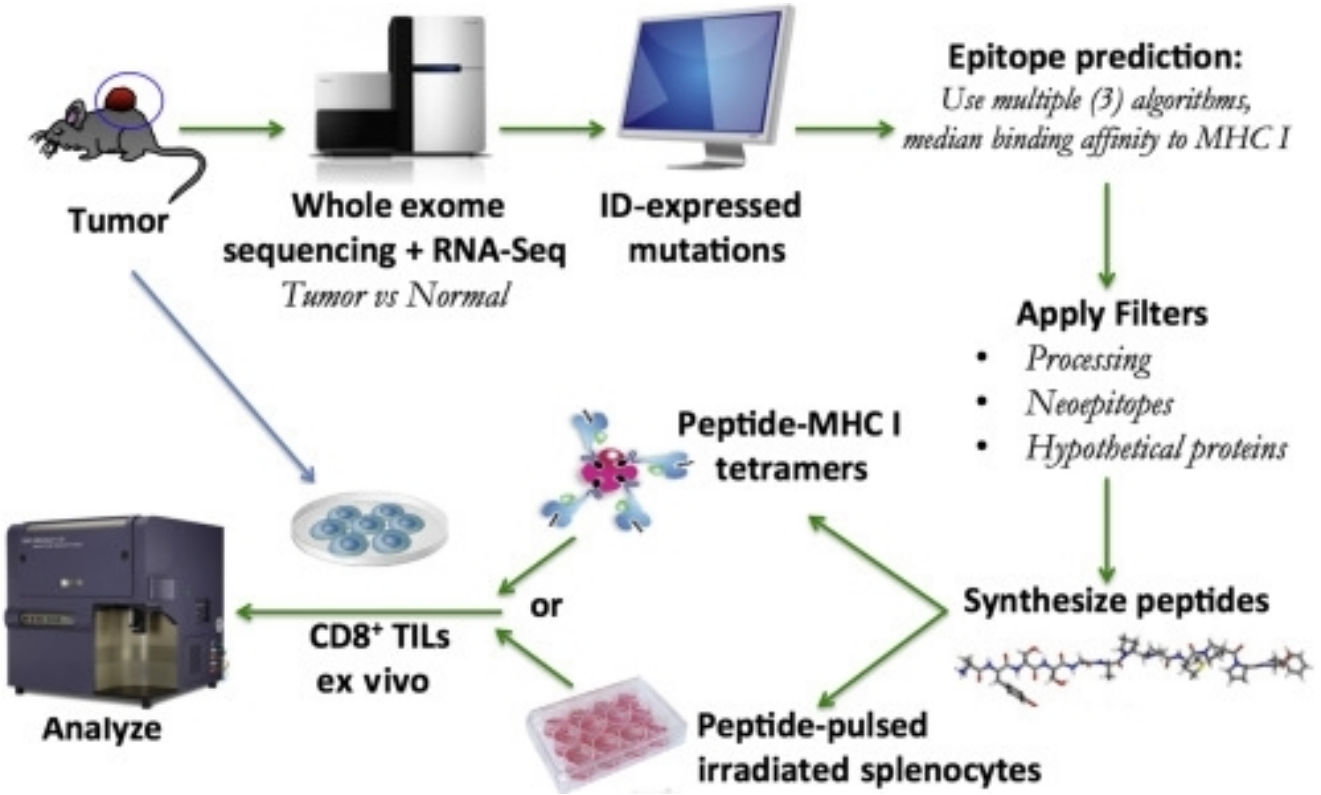
Hirano et al. Blood 2006;108:2662

iTRAQ enables quantitative comparison of the same peptide in different samples



Fuller & Morris in Integrative Proteomics (2012)

Rapid neoantigen identification enabled by NGS

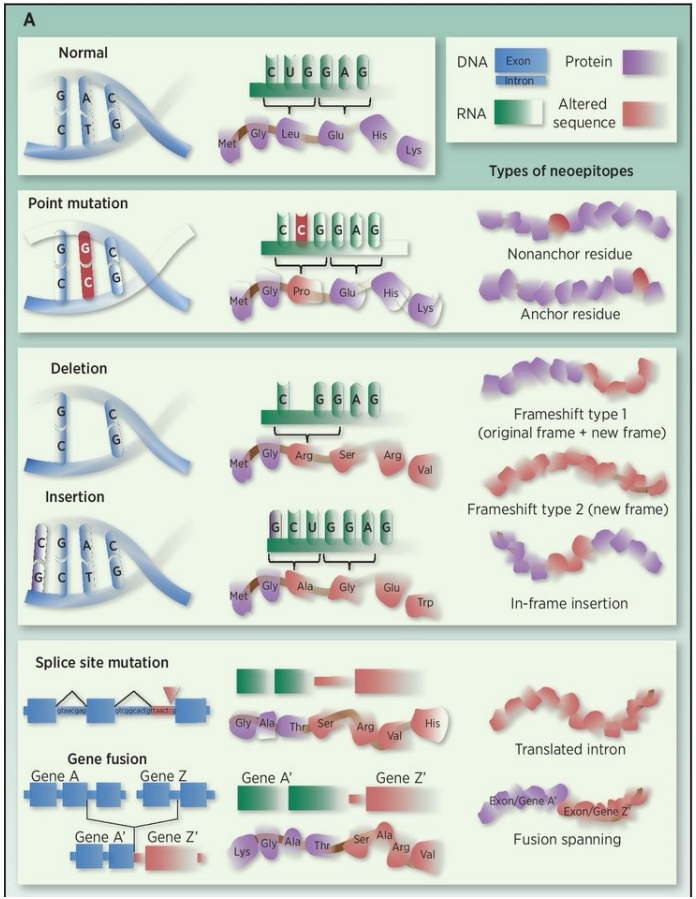


Sources of NeoAgs

Missense

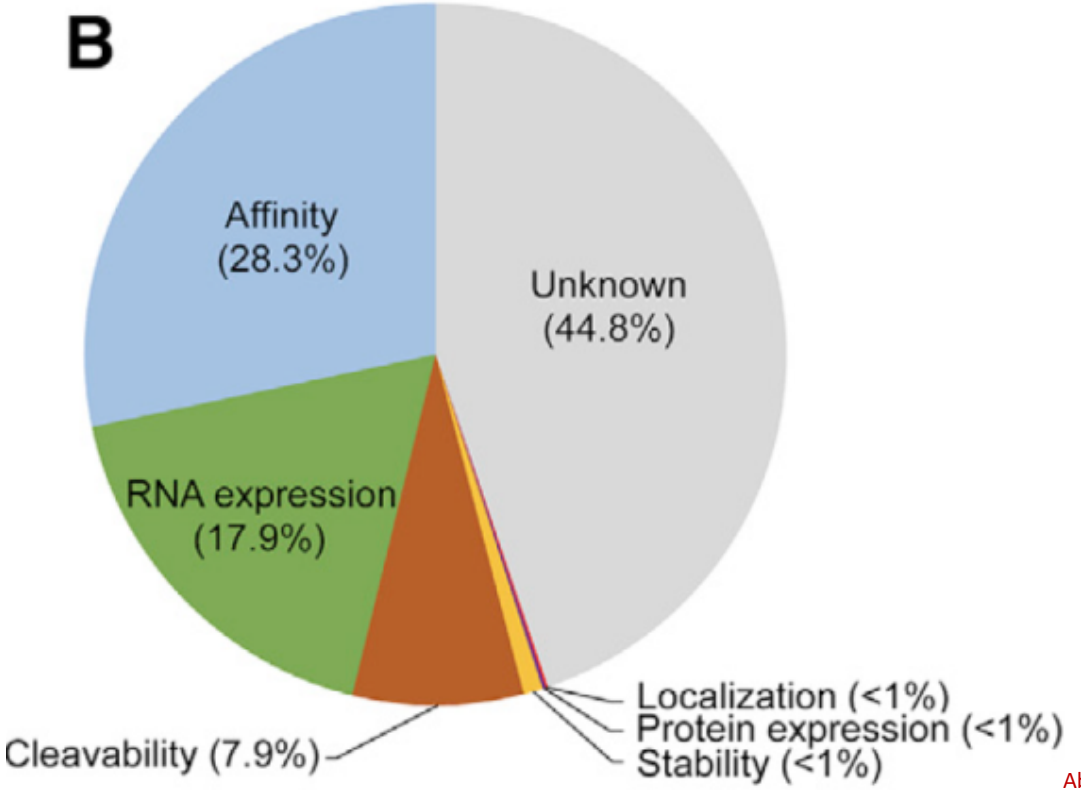
Frameshift

Splice variants



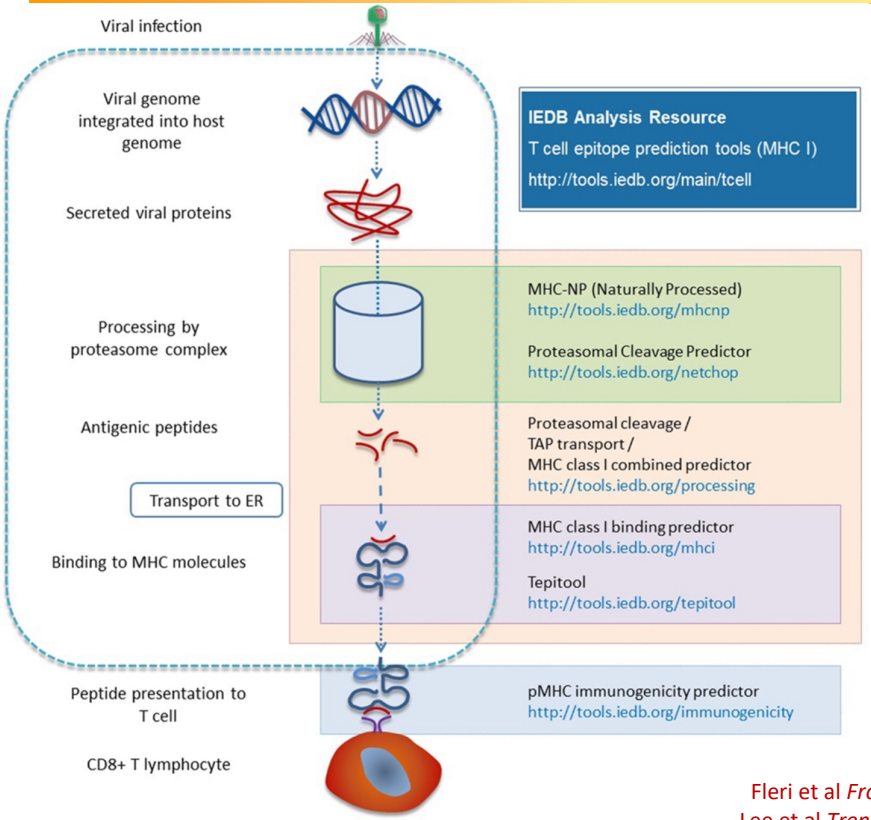
Tureci et al Clin Cancer Res 22:1885 (2016)

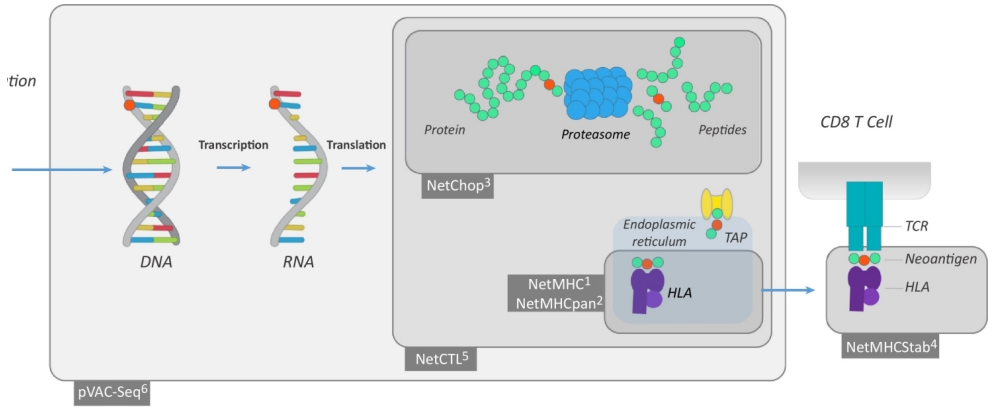
Analysis of factors predicting display of identified MHC-I associated peptides



Abelin et al Immunity 46:315 (2017)

Software tools to predict whether point mutations will lead to MHC-I presented peptides



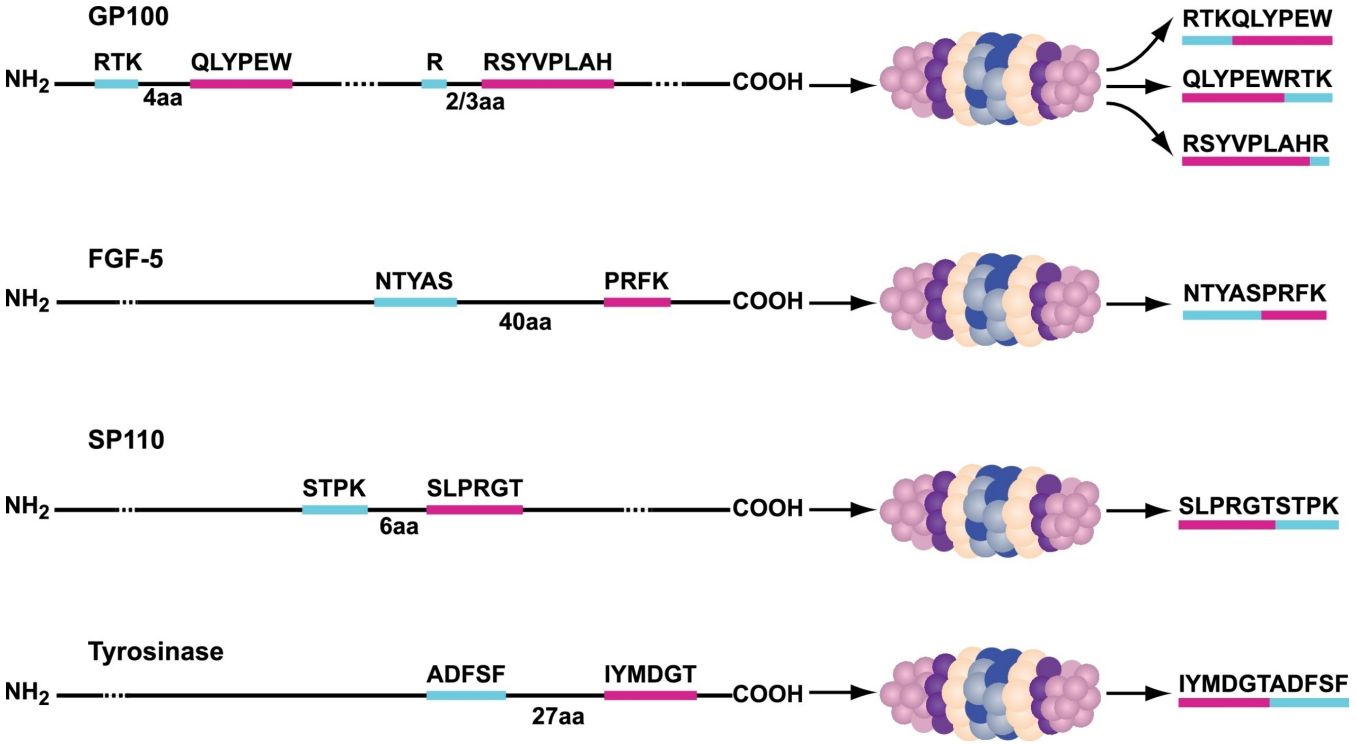


Ref. #	Tool	[Refs]	Elements of TSNA biology modeled
1	NetMHC	49	HLA binding
2	NetMHCpan	93	HLA binding across more HLA alleles
3	NetChop	94	Proteasomal peptide processing
4	NetMHCstab	54	HLA/peptide complex stability
5	NetCTL	53	Proteasomal processing, TAP transport, HLA binding
6	pVAC-Seq	61	DNA mutation, RNA expression, HLA binding

Trends in Immunology

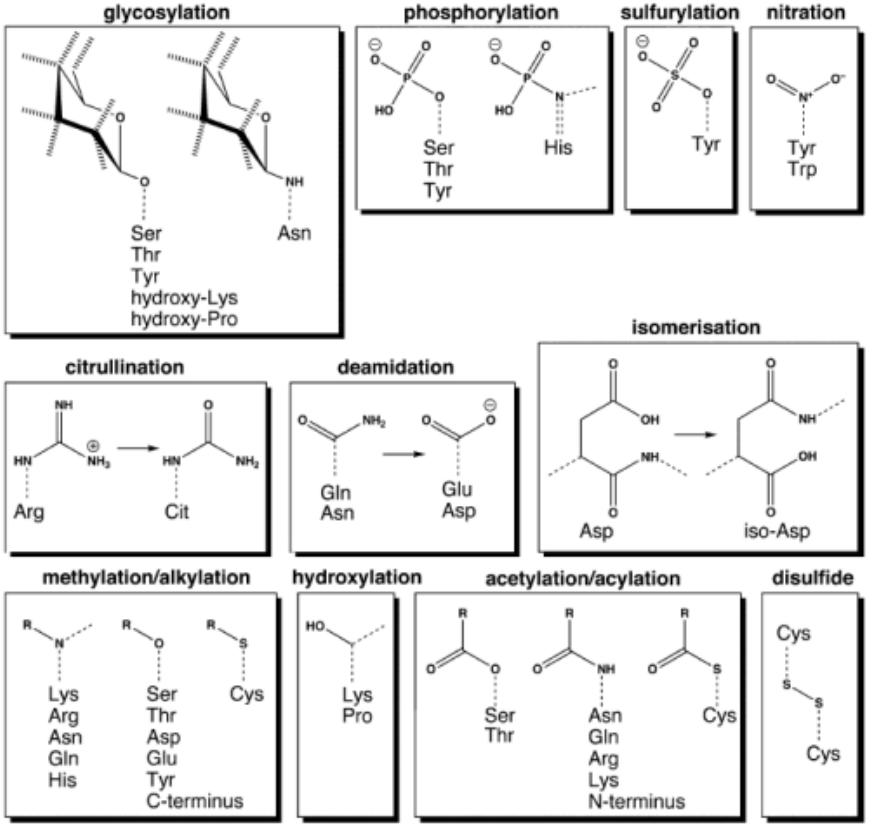
Fleri et al *Front Immunol* 8 278 (2017)
Lee et al *Trends Immunol* 39:536 (2018)

Proteasomal splicing during protein degradation creates new antigens



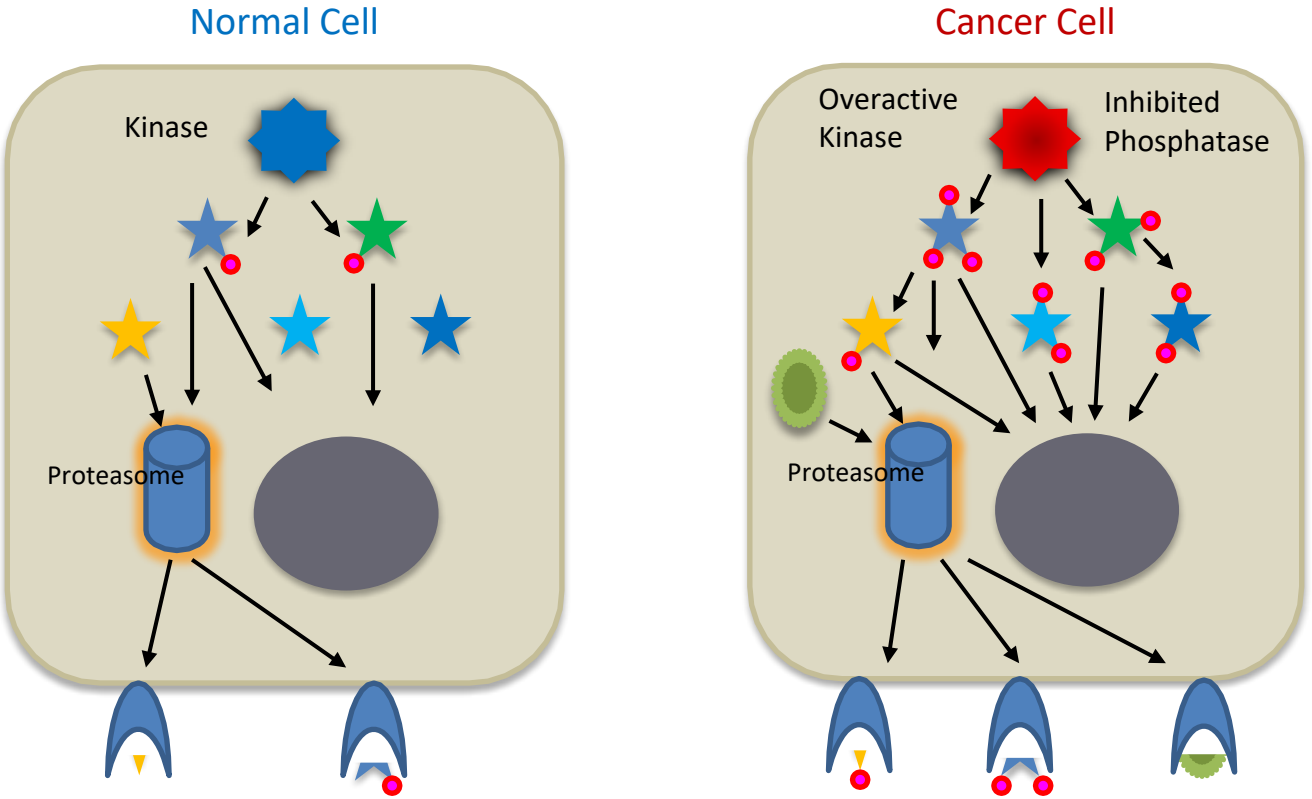
Vigneron et al Mol Immunol (2018)

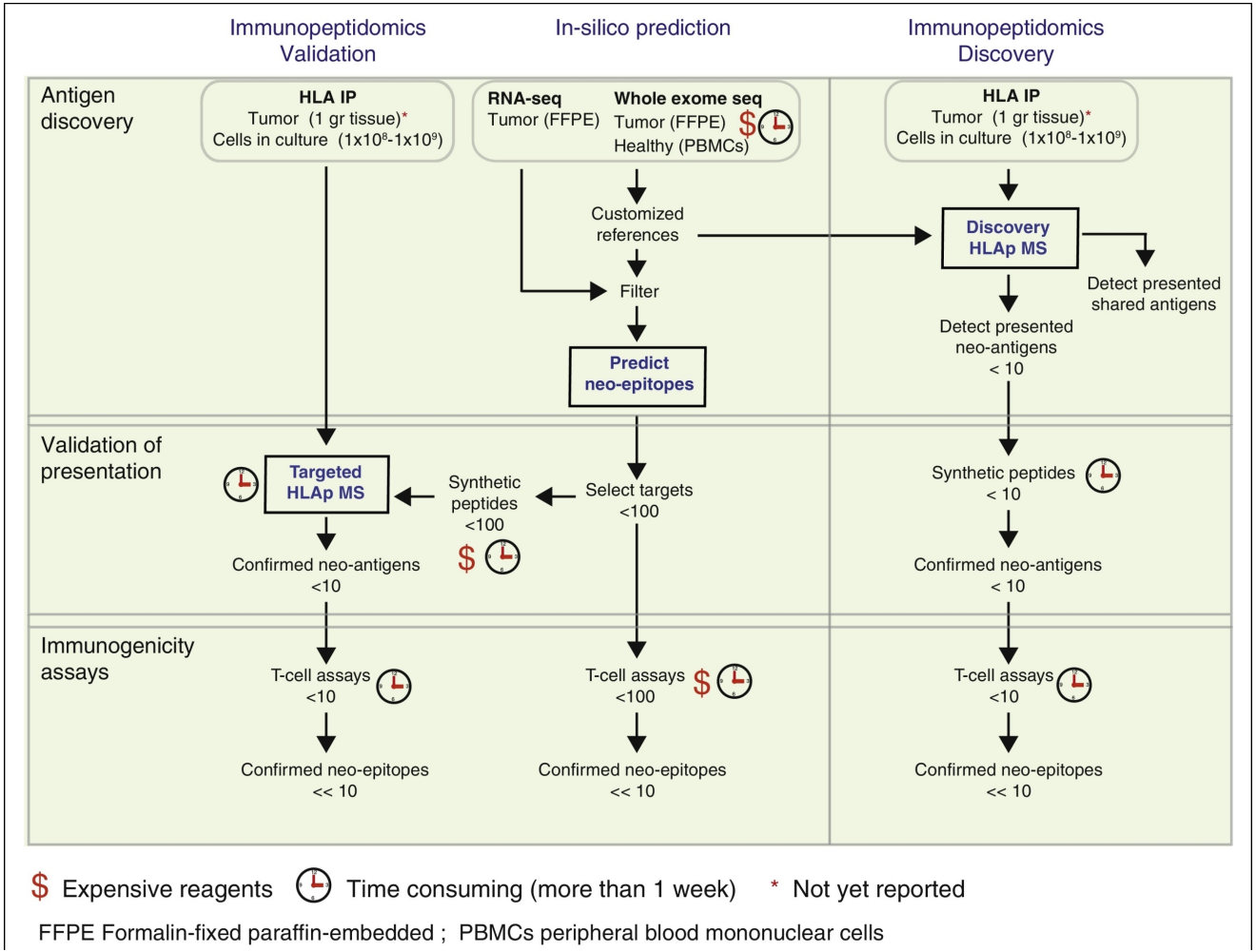
Post-translational modifications of proteins can survive to be presented on MHC peptides



Petersen et al J Mol Med 87:1045 (2009)

MHC-associated phosphorylated peptides
a better class of tumor antigen





Bassani-Sternberg & Coukos. Curr Op Immunol 41:9 (2016)