Durable Complete Response in a Patient with Metastatic Melanoma Following Adoptive Transfer of Autologous T cells Recognizing 10 Mutated Tumor Antigens

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Presenter Disclosure Information

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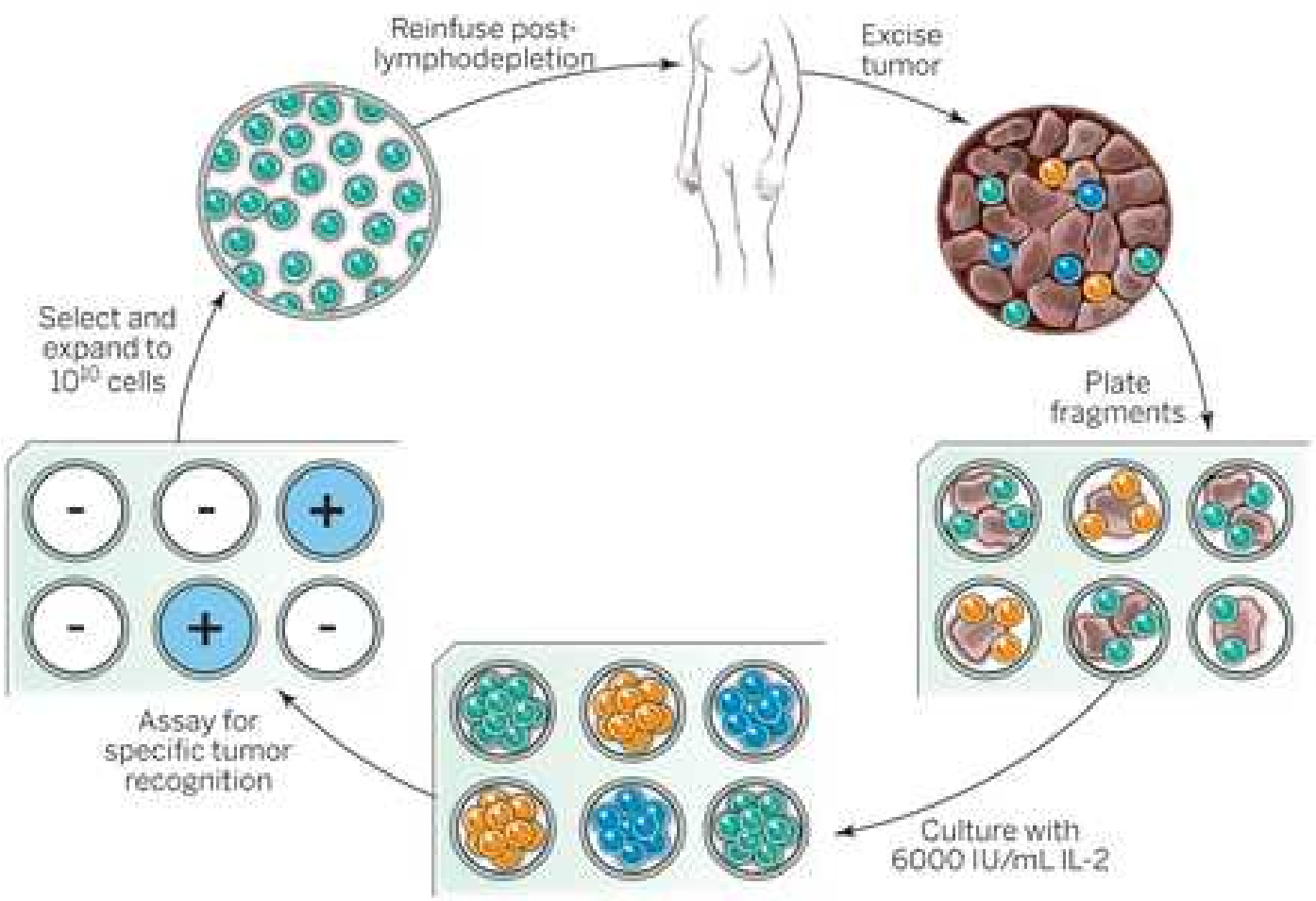
Todd D. Prickett, Ph.D.

I have no financial relationships to disclose.

- and -

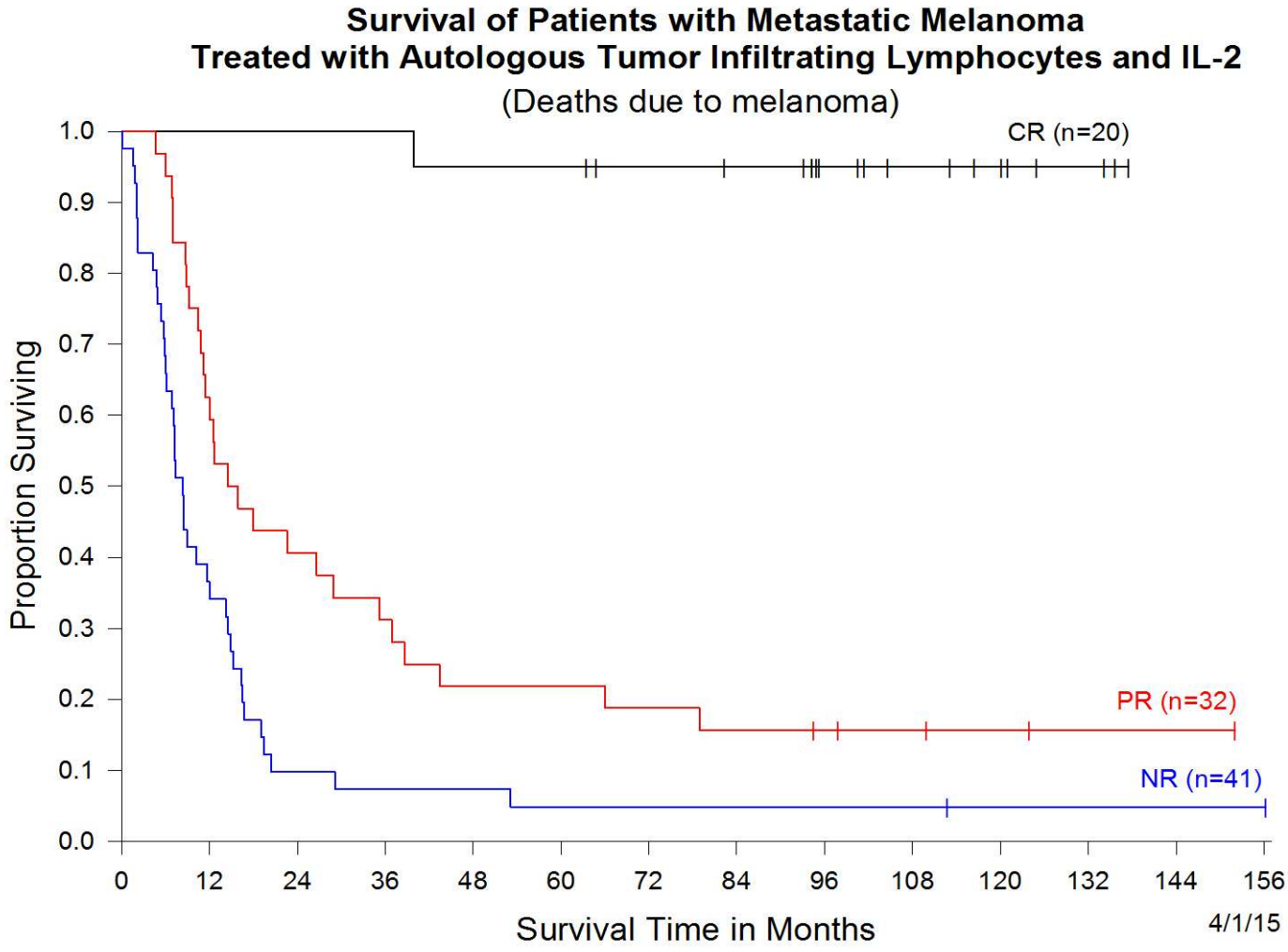
I will not discuss off label use and/or investigational use in my presentation.

Adoptive Cell Therapy (ACT) for Treatment of Metastatic Melanoma: Schematic of Treatment



Rosenberg, SA et al., Science, 2015

Adoptive Cell Transfer is an Effective Treatment for Patients with Metastatic Melanoma

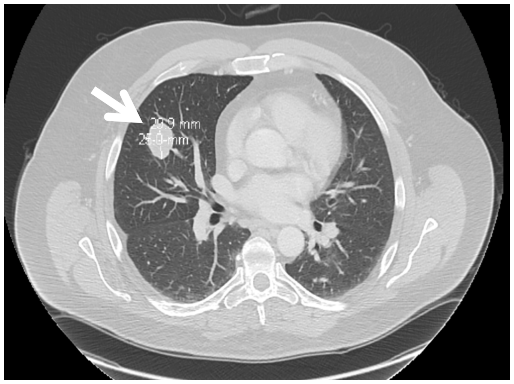


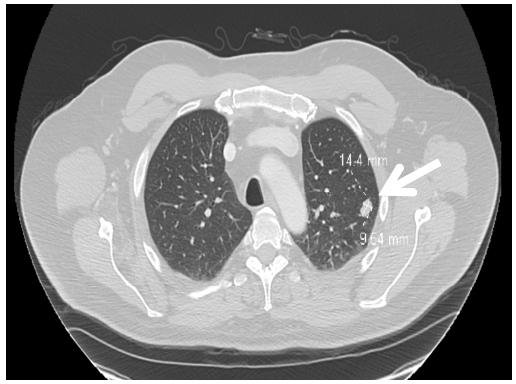
Kind gift from Dr. Rosenberg

Patient with Metastatic Melanoma Exhibited a Durable Complete Regression upon Treatment with Bulk TIL Therapy

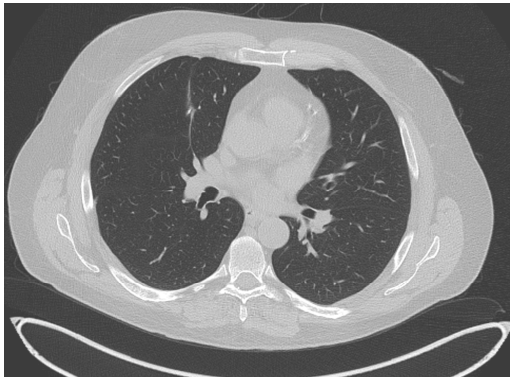
- Pt.3713 contained >4,000 somatic non-synonymous mutations upon whole-exome and RNA-Seq sequencing of fresh tumor

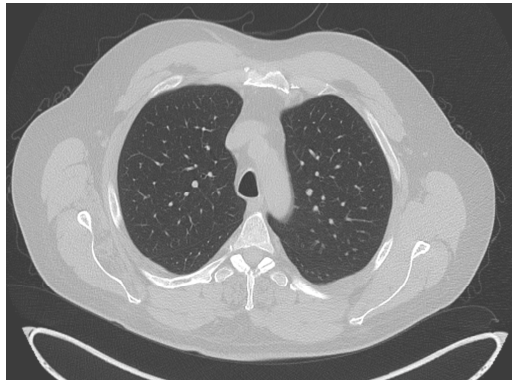
- Using this approach we focused on 720 gene products to test in the TMG screening.





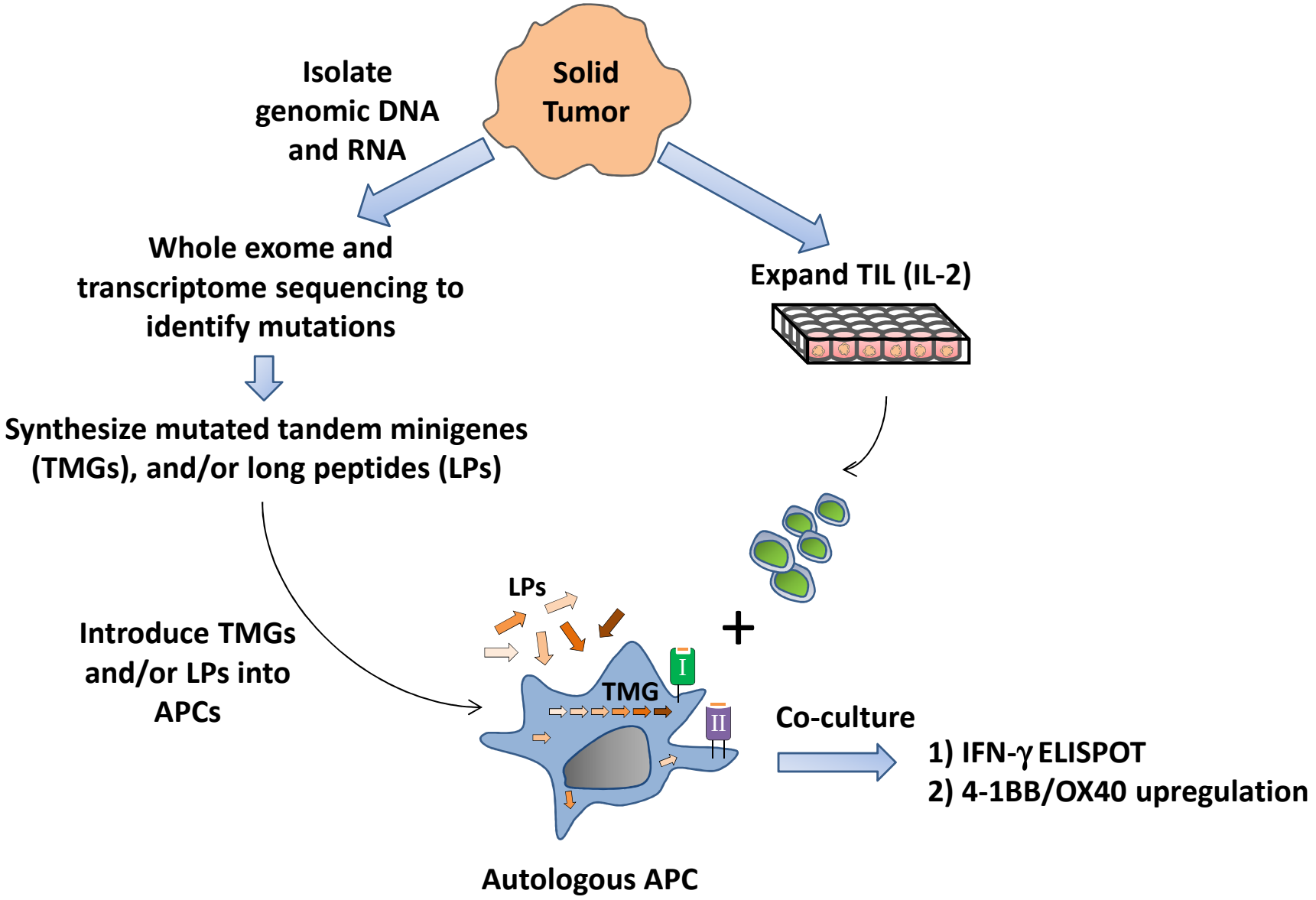
Prior to TIL therapy





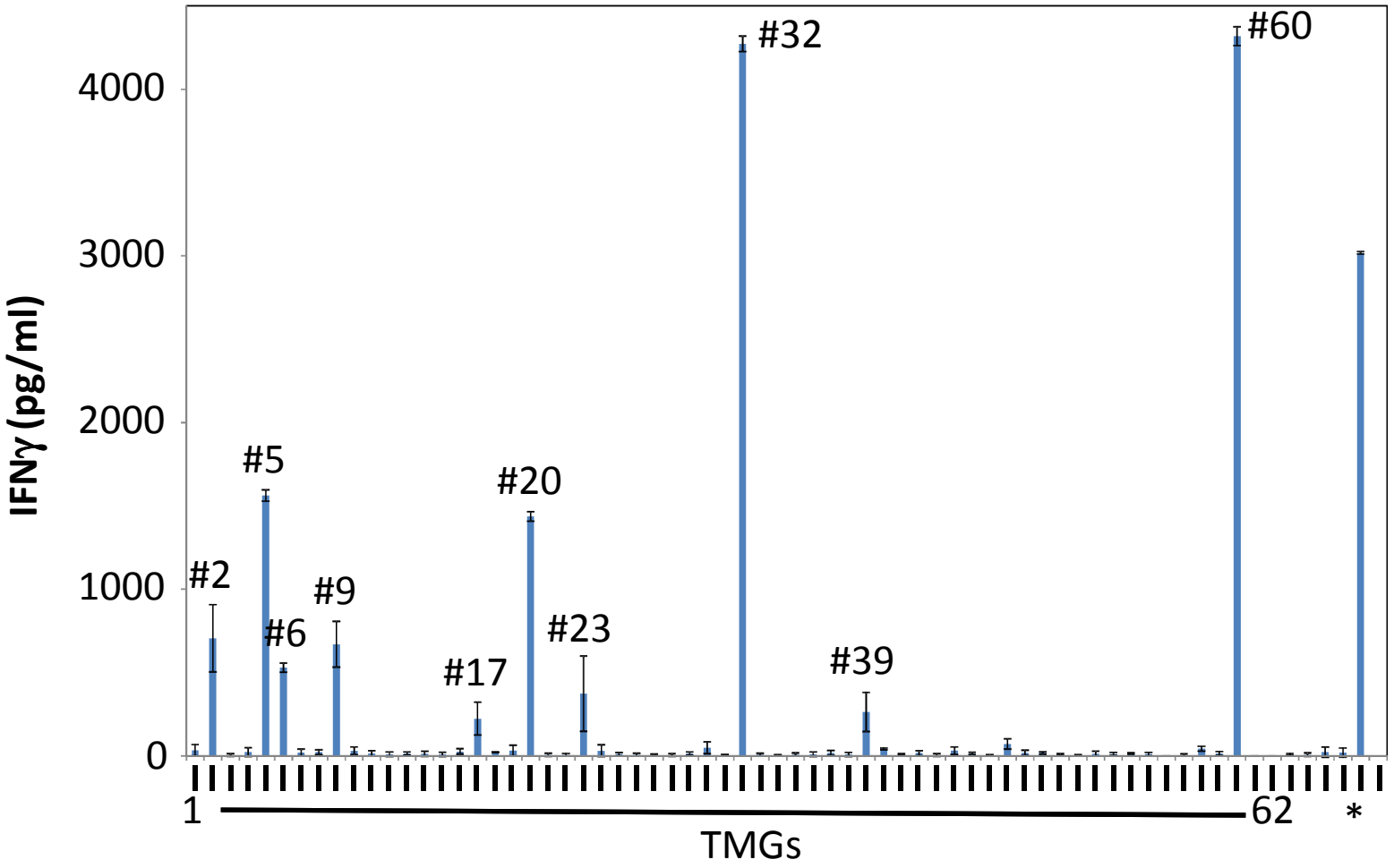
Post-TIL therapy

Assessing T-cell Reactivity against Mutated Antigens



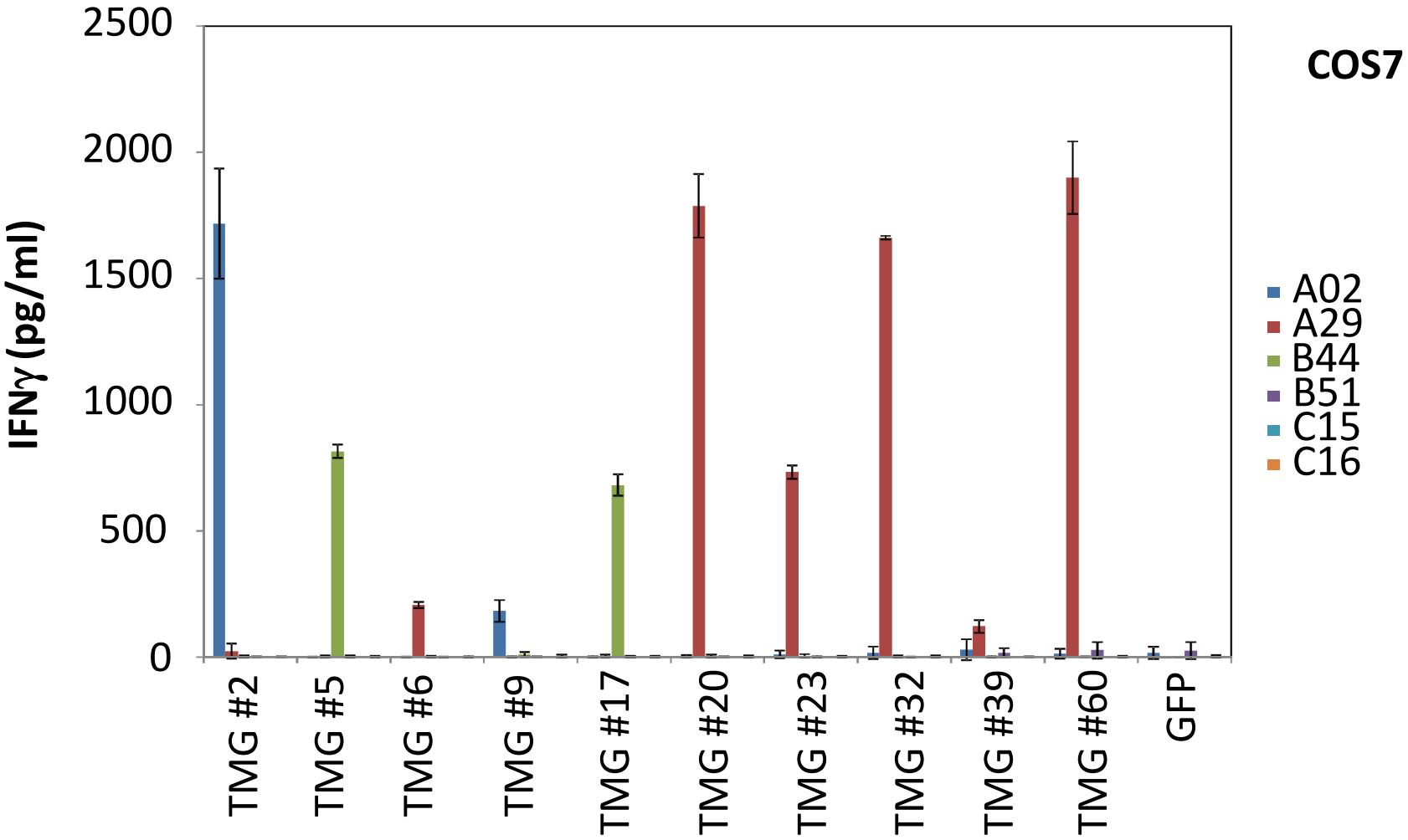
Kind gift from E. Tran, SB/NCI

Tandem Minigene (TMG) Screening of 62 TMGs Reveals 10 Independent TMGs Recognized by Autologous Tumor Infiltrating Lymphocytes (TIL)



* Autologous melanoma cell line

Mutated Novel Antigens Presented to pt.3713 TIL using Three Different Class I HLA Molecules

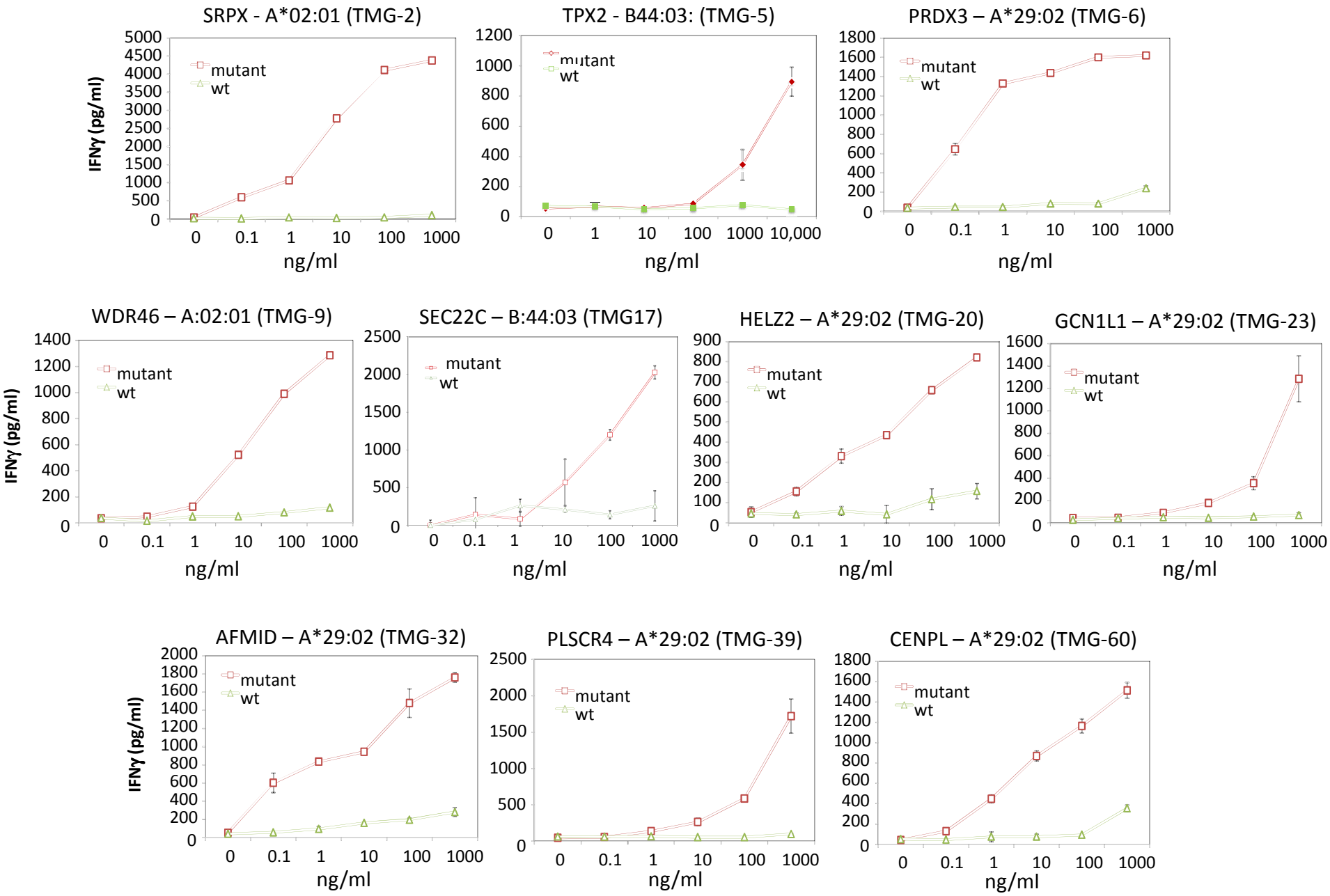


Minimal Epitopes of the 10 TMGs Recognized by pt.3713 TIL

Gene	Mutated amino acid sequence	Wild type amino acid sequence	HLA restriction element	RPKM
SRPX	TLWCSPIKV	TPWCSPIKV	A*02:01	133
WDR46	FLIYLDVSV	FLTYLDVSV	A*02:01	40
PRDX3	FFYLLDFTF	FFYPLDFTF	A*29:02	49
HELZ2	QTNPVTLQY	QTDPVTLQY	A*29:02	20
GCN1L1	IMQTLAGELY	IMQTPAGELY	A*29:02	18
AFMID	EVLPFFLFF	EALPFFLFF	A*29:02	11
PLSCR4	RVCGPCSTY	RVRGPCSTY	A*29:02	9
CENPL	TLYSLTLLY	TLYSLTPLY	A*29:02	4
TPX2	TEDEHFEFY	TEDEHFEFH	B*44:03	55
SEC22C	AEHSLQVAY	AEHSLQVAH	B*44:03	23

Using the consensus IEDB peptide/MHC binding algorithm minimal mutated peptide epitopes were identified and tested via peptide pulsing on either EBV B cells or dendritic cells and co-cultured with infusion TIL-3713.

Peptide Dose Curves Show Several Different Avidities for Mutated Neo-epitopes



Sequence and Reactivity of Dominant T Cell Clonotypes in 3713 TIL

Infused TIL				Pre-PBMC		Post 1 month		Post 1 year	
Rank‡	Freq.(%)	TR-BV	Antigen reactivity	Rank	Freq.(%)	Rank	Freq.(%)	Rank	Freq.(%)
1	4.5	15-1	CENPL	52466	0.0005	7	2.8	8	1.2
2	3.9	6-1	SRPX	ND†	<0.0001	1	4.5	4	1.9
4	2.8	11-1	SRPX	49395	0.0005	2	4.5	ND	<0.0003
5	2.6	5-6	SRPX	ND	<0.0001	3	4.3	6	1.6
6	2.6	10-2	SRPX	27655	0.0012	4	4.1	7	1.4
7	2.5	10-2	SRPX	ND	<0.0001	5	4.1	14	1
10	1.8	27-1	HELZ2	15120	0.0017	9	2.2	12	1
13	1.6	29-1	SEC22C	ND	<0.0001	17	1.3	28	0.5
18	1.4	7-9	AFMID	ND	<0.0001	41	0.5	56	0.3

Among the top 20 T cell clonotypes in the infused TIL, 5 recognized the mutated SRPX epitope, and single clonotypes recognized the mutated CENPL,HELZ2, SEC22C and AFMID mutated epitopes.

None of the Somatically Mutated Genes are Known to be Involved in Oncogenesis

- **SRPX** – (Sushi-repeat containing protein, X-linked) gene is abundantly expressed in retina and contains domains that resemble those found in selectins and complement proteins (Meindl et al. 1995).

- **CENPL –** Targets CENPA to centromeres and is required for proper kinetochore function and mitotic progression (Okada et al. 2006).

- **HELZ2** - (also known Peroxisomal proliferator-activated receptor alpha-interacting cofactor complex, 285 kDa subunit) is a nuclear transcriptional coactivator for PPAR-α and PPAR-γ (Tomaru et al. 2006).

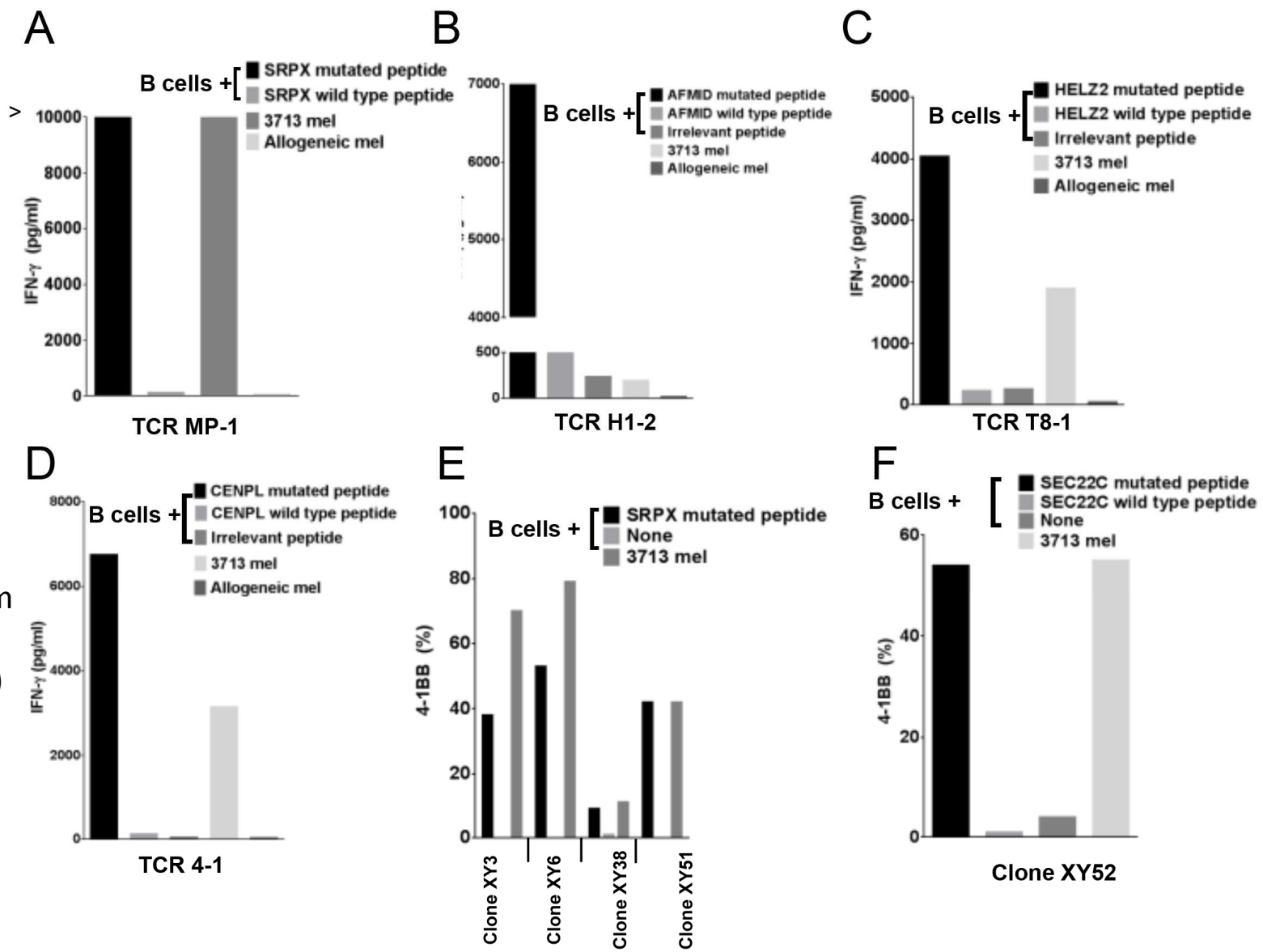
- **SEC22C** – Secretion deficient 22 (SEC22C) gene overexpression causes disruption of SNARE-dependent vesicle transport from the ER to the Golgi apparatus

- **AMFID** - converts N-formyl-L-kynurenine into formate.

Recognition of mutated novel antigens by dominant T cell clonotypes (Clones and TCRs) in Infused TIL

Cloned TCR (retroviral)

T cell clones from Infusion bag TIL (limited dilution)



Summary

- Bulk TIL that mediated a durable complete regression recognize 10 different mutated neo-epitopes

- Deep sequence analysis of Bulk TIL revealed multiple clonotypes
  - 9 of 20 top clonotypes recognized mutated epitopes as well as autologous 3713 TC cells
  - 5 of the top 10 clonotypes recognize mutated SRPX epitope

Future Directions

- Study additional patients to evaluate the diversity of neo-epitopes
- Identify mutation-reactive TCRs for adoptive immunotherapy
- Evaluate recognition of mutated and previously described non-mutated antigens in an attempt to identify the targets of effective TIL
- Evaluate TIL and fresh tumors in an attempt to identify shared signaling pathways and/or genetic aberrations that may distinguish complete responders from non-responders to adoptive immunotherapy

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Also being presented on **Poster 48** this evening and tomorrow!

Lessons and Take Home Messages

Key points:

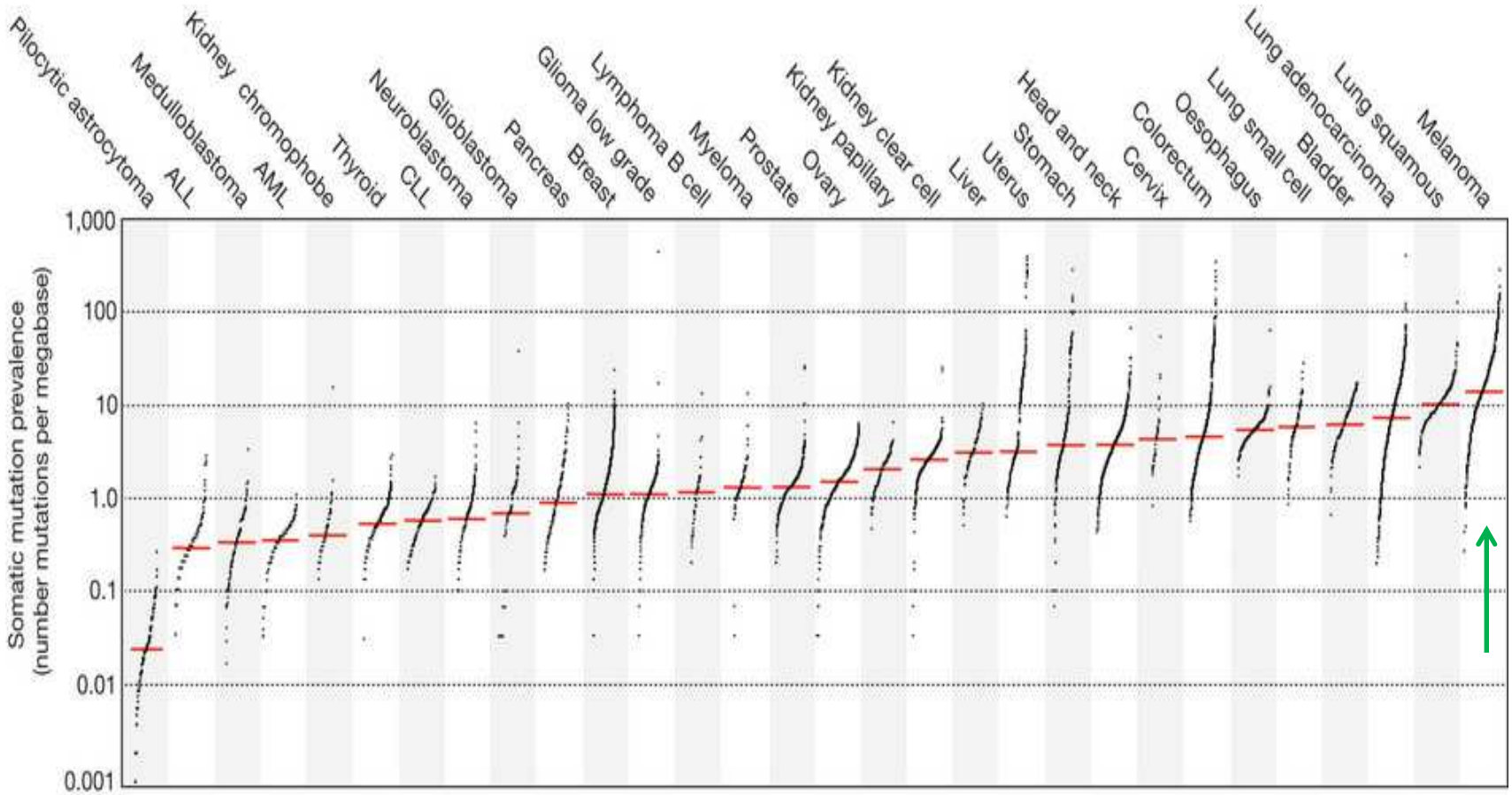
- WES and RNA-Seq sequencing coverage (150-200X)
- Screening using both TMGs and long peptides helps find class I and class II restricted neo-epitopes
- TCR α/β testing for proper neo-epitope recognition

Potential impact on the field: A screening approach that can be used to determine the best subset of tumor-specific TIL via neo-epitope(s) recognition for rapid expansion and infusion back into patients. Truly a personalized medicine approach.

Lessons learned: Next generation sequencing and bioinformatic pipeline needs to be fine tuned.

- It's okay to screen more genes (potentially high false positive rate) compared to not screening enough genes (potentially high negative rate).

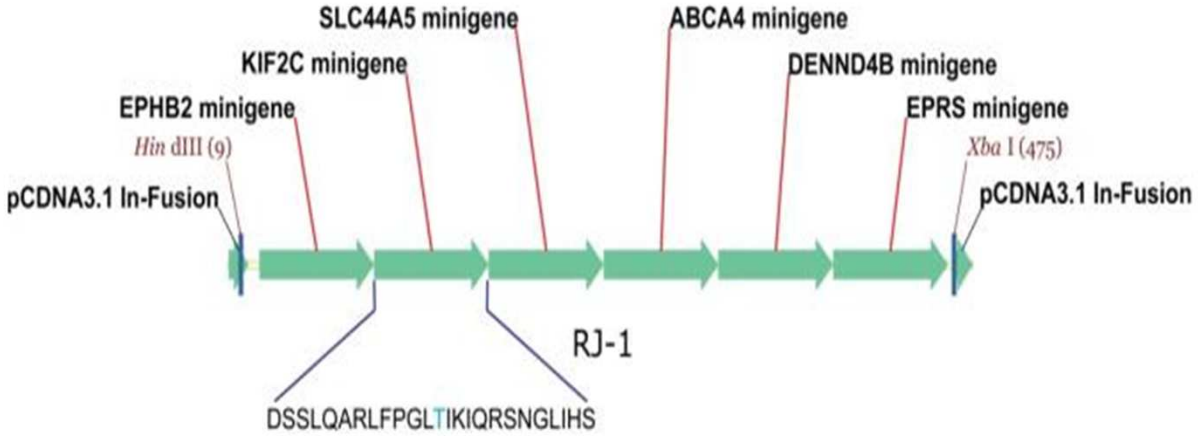
Somatic Mutation Prevalence Among Different Cancer Histologies



Every dot represents a sample whereas the red horizontal lines are the median numbers of mutations in the respective cancer types.

Alexandrov LB, et al. Nature, 2013.

Exome Sequencing-based Antigen Discovery: Minigene Based Approach



Utilizing Invitrogen or IDT DNA to generate 1kbp genestrings for direct cloning into mammalian expression and IVT RNA plasmid for studies in antigen presenting cells including EBVs, DCs, and TC lines.

Lu et al. Clin. Can. Res 2014