Use of high-throughput sequencing methods to identify mutated antigens recognized by tumor-reactive T cells

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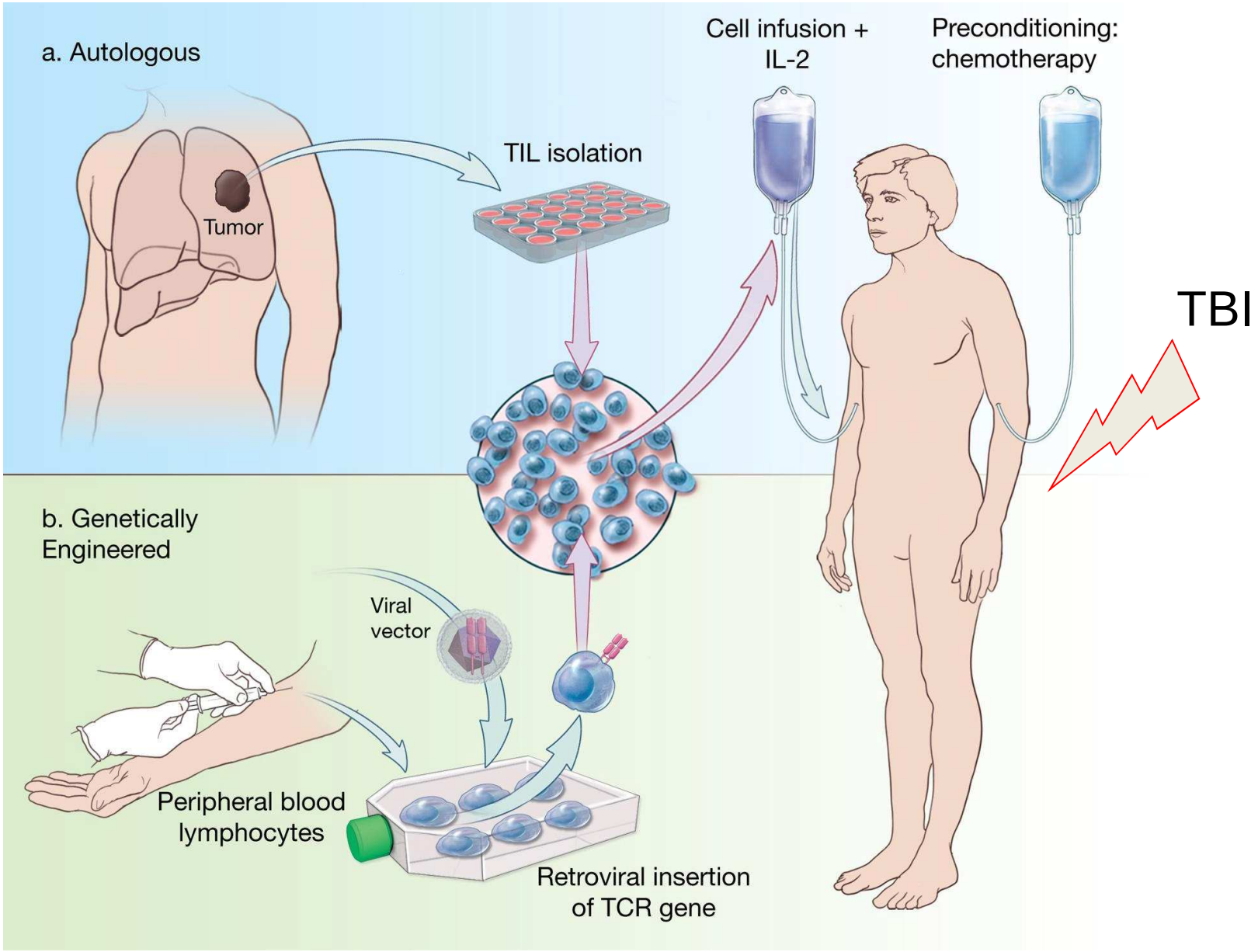
NATIONAL® CANCER INSTITUTE

Current Adoptive T cell-based immunotherapies

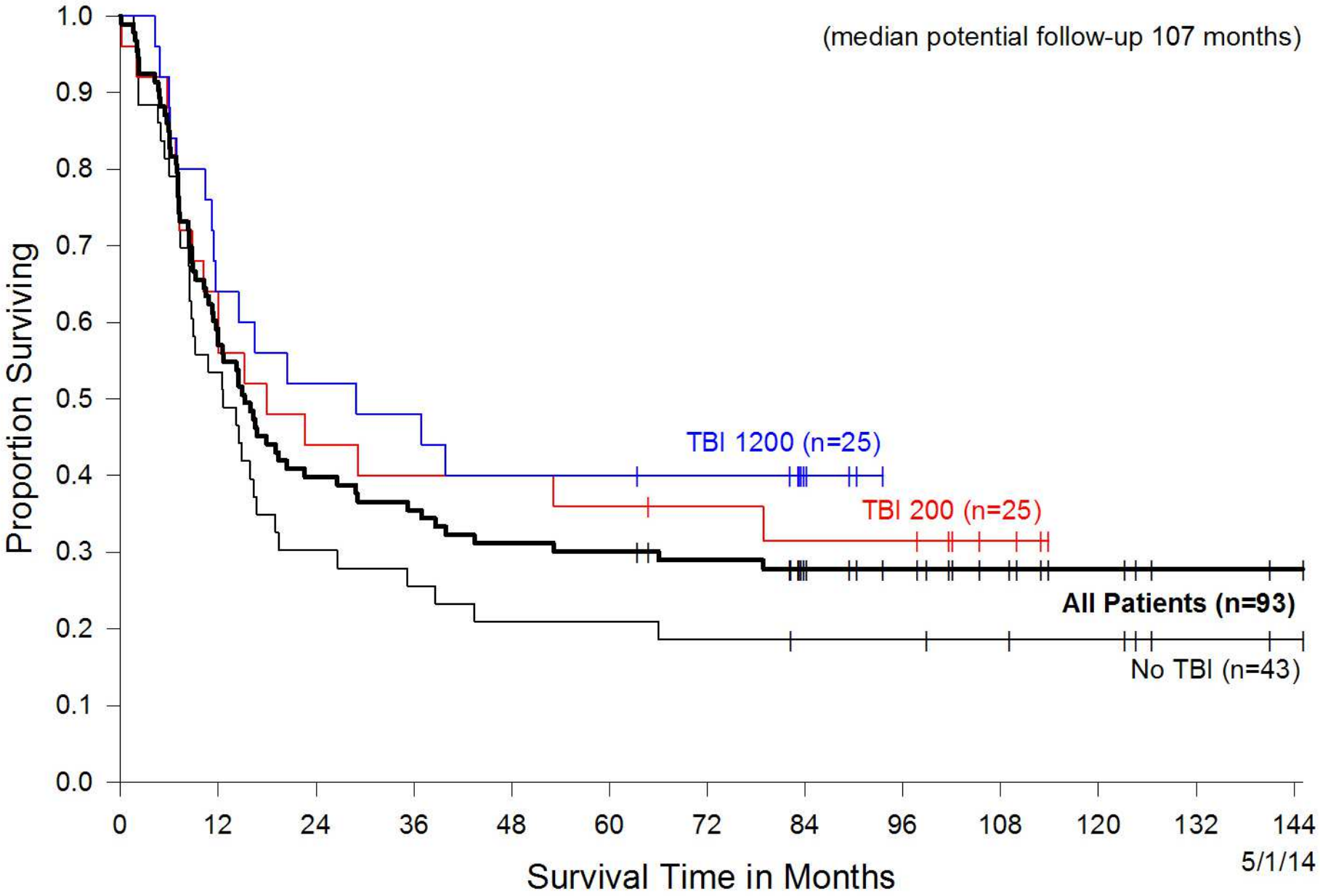


Response to Melanoma Adoptive TIL Transfer

Treatment	Total	PR	CR	OR (%)
		n(%) of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (126+, 124+, 123+, 109+, 82+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (113+, 109+, 105+, 102+, 64+)	13 (52%)
1200 TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (93+, 90+, 89+, 84+, 83+, 83+, 83+, 82+, 63+, 19)	18(72%)

(20 complete responses: 19 ongoing at 63 to 126 months) Updated 5/1/14

Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2



Antigen specificity and response to adoptive immunotherapy

- **Many factors influence response to TIL therapy including telomere lengths of administered T cells and in vivo persistence**
- **Antigen specificity may also be related to clinical response**
- **If antigen specificity important, what are distinguishing characteristics of potent tumor rejection antigens?**
- **How can these findings be translated into development of therapies for treatment of broader patient population?**

Trials targeting shared self antigens: Influence of normal tissue expression

Clinical responses observed in patients receiving PBMC transduced with MART-1 and gp100 reactive TCRs

- **However, targeting MART-1 and gp100 led to severe skin, eye and ear toxicity**

CEA targeted with high affinity TCR in colon cancer patients

- **Limited tumor regression observed along with severe dose limiting colitis**

Long term complete regression of leukemia and lymphoma observed in patients treated with anti-CD19 CAR

- **Normal B cells also depleted for extended periods**

Cancer/Germline Antigens

Antigens limited in expression in adults to germ cells

MAGEA3 HLA-A2 epitope targeted in melanoma patients

Unexpected neurological toxicity observed

No normal tissue toxicity observed with anti-NY-ESO-1 TCR

Response of Patients Treated with Anti-NY-ESO-1 TCR

Tumor type	n(%) of patients (duration in months)			
	Total	PR	CR	OR
Synovial cell sarcoma	18	10(55) 44+,18,11,10, 8,7,5,4,3,3	1(6) 13+	11(61)
Melanoma	20	7(35) 28,10,8,6+, 5,3,3	4(20) 58+,52+,36+ 24	11(55)

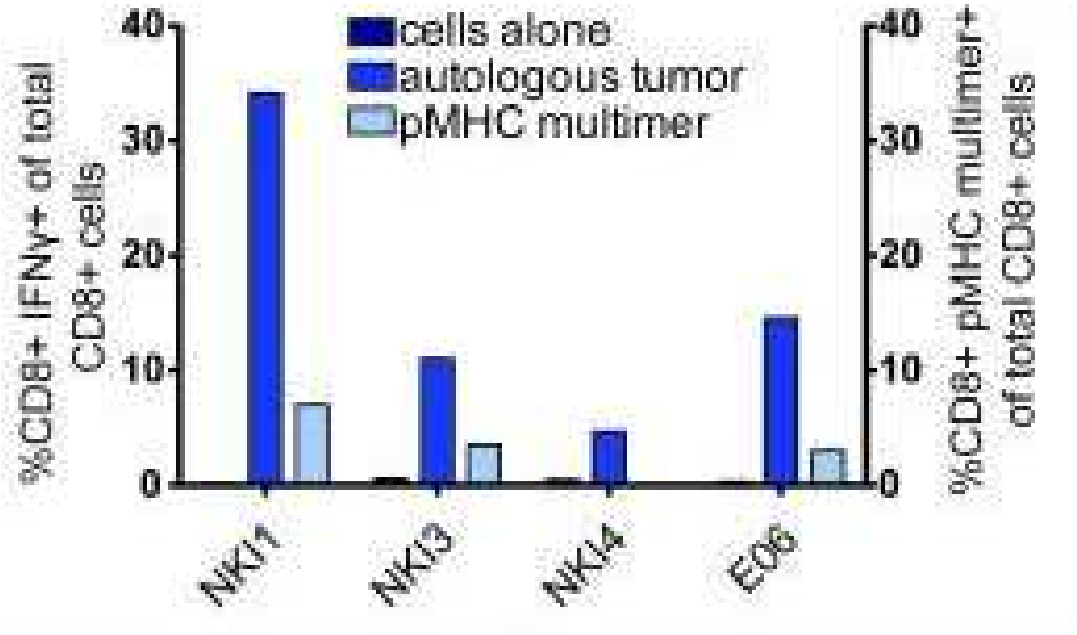
Response data updated as of May 1, 2014

High NY-ESO-1 expression only observed in low percentage of tumors

	Melanoma	Synovial Cell Sarcoma
	# positive/total (%)	
qRT-PCR	24/53(45)	ND
IHC (2-4+,>50%)	24/180(13)	9/14(64)

What are predominant targets recognized by clinically effective melanoma TIL?

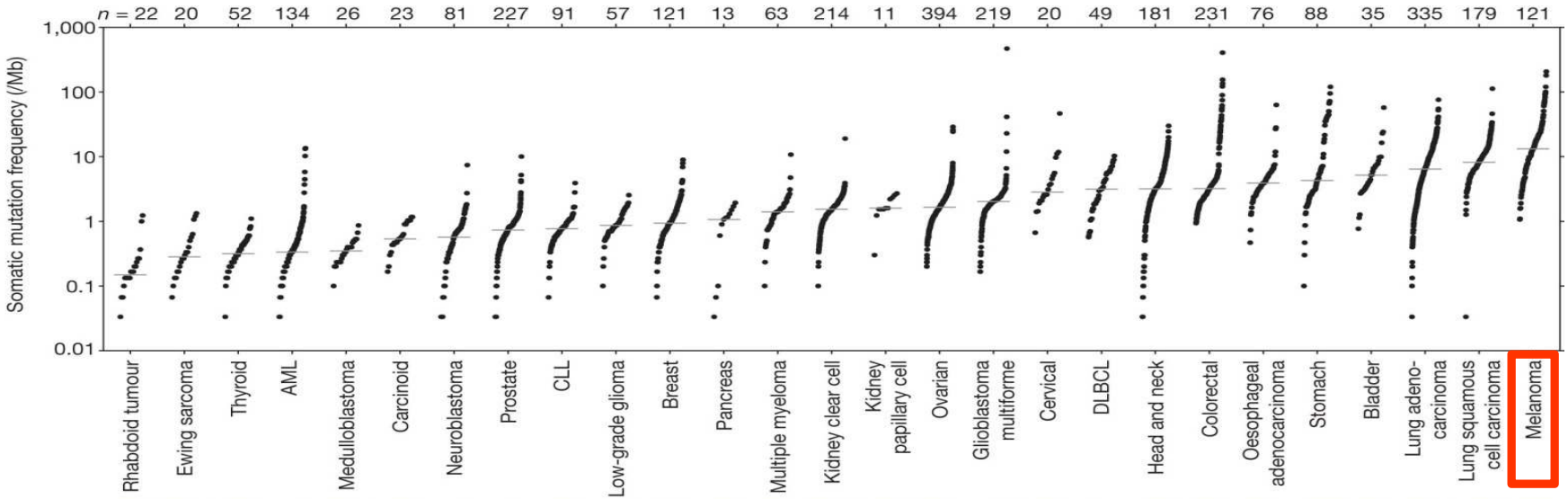
Tetramers generated from panel of 145 HLA-A*02:01 non-mutated epitopes



Total tetramer+ T cells : Median 0.2% Range <0.01-10%

Kvistborg et al. Oncoimmunology 2012

Somatic mutation frequencies observed in exomes from 3,083 tumour–normal pairs

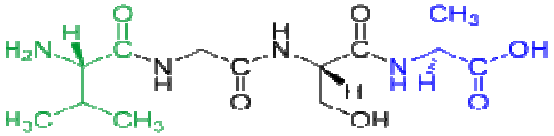


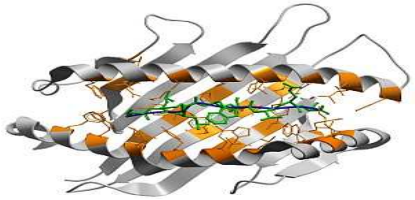
Lawrence et al. Nature 2013

Tumor Antigen Identification using Whole Exome Sequencing
Peptide approach

Sequence tumor and matched normal DNA to identify somatic mutations

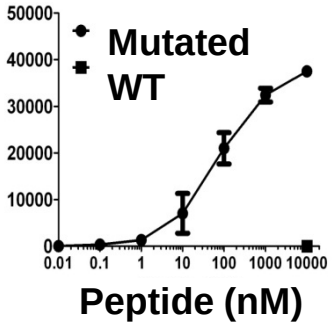
Utilize HLA binding algorithms to identify candidate epitopes





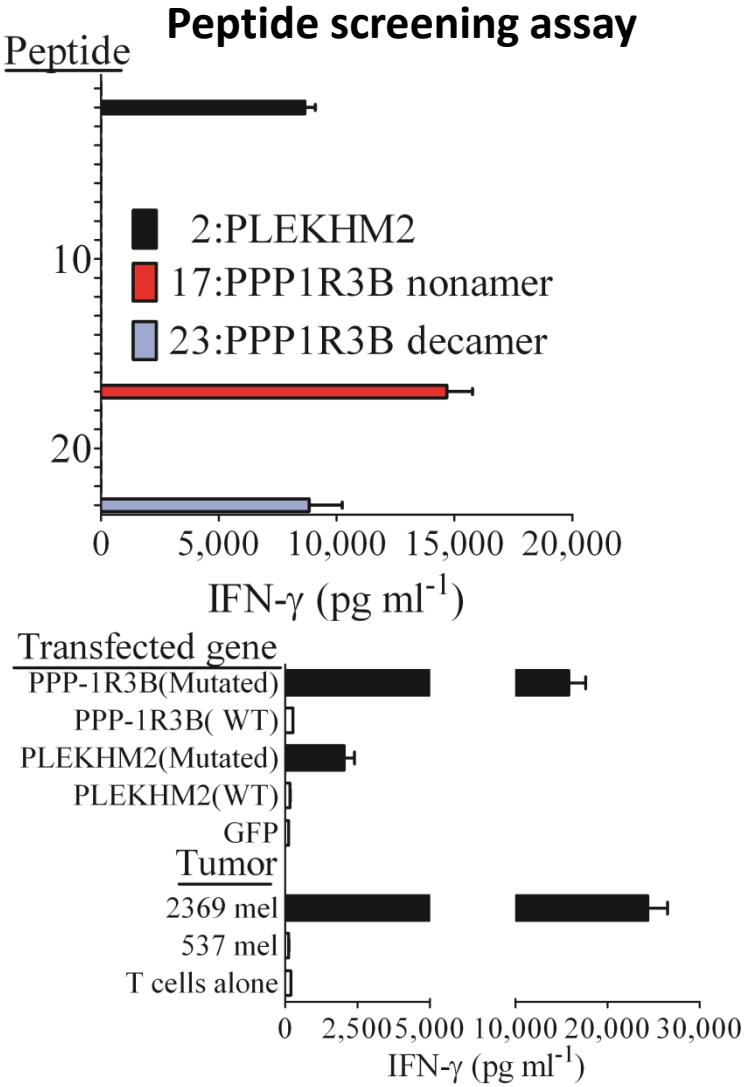
Co-culture TIL with targets pulsed with synthetic peptides

Evaluate response of positives to WT and mutated peptides



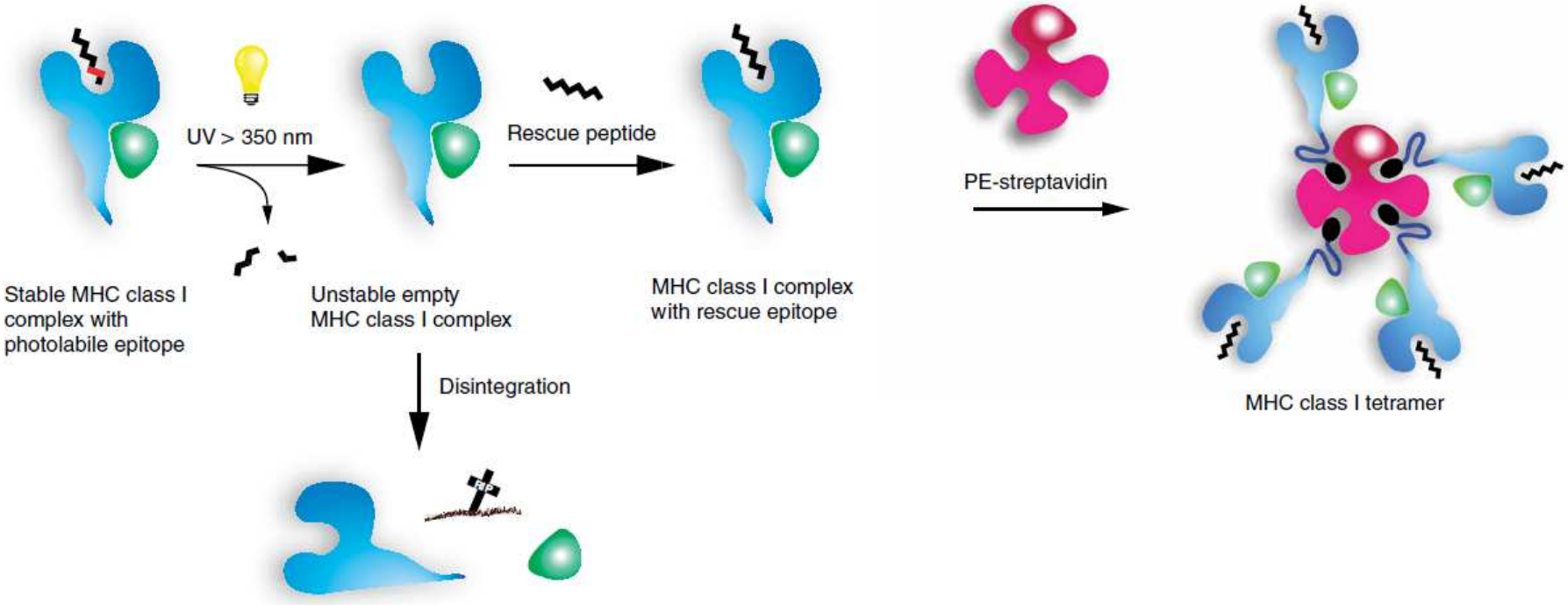
Screening of mutated candidate epitopes identified by exomic sequence for TIL recognition

	Mutated peptide	Predicted HLA-A1 affinity	Gene ID
1	FSDYYDLSY	2	TEX33
2	LTDDRLFTCY	3	PLEKHM2
3	YSSALDLCY	5	GRIN3B
4	FSDKKVGTY	5	PLCB1
5	HSEYSSFFY	6	HEG1
6	CSNFLLLAY	7	BAI3
7	ESDKEELVGY	7	MPP4
8	CTDTYMLELF	8	OR4C46
9	FTGTISVMY	12	UEVLD
10	QTQSVVFLY	13	COL9A1
11	MSSYIASFTY	14	SLCO1B7
12	ATALLEYLEY	20	TBRG4
13	CTDTYMLEL	22	OR4C46
14	LLDLMAYDRY	22	OR2T2
15	SSDSQEENY	23	MEOX2
16	LTSMAYDCY	31	OR8B3
17	YTDFHCQYV	49	PPP1R3B
18	WADWGHRTY	51	LRP2
19	FTMVILYVVY	54	LRRC3B
20	CVDSPPPLFF	71	NPAP1
21	VSDGFTAVM	85	RNPEP
22	WSCLGHLGY	86	RHOT2
23	YTDFHCQYVK	100	PPP1R3B



Robbins et al. Nat Med, 2013

Tetramer Screening Approach

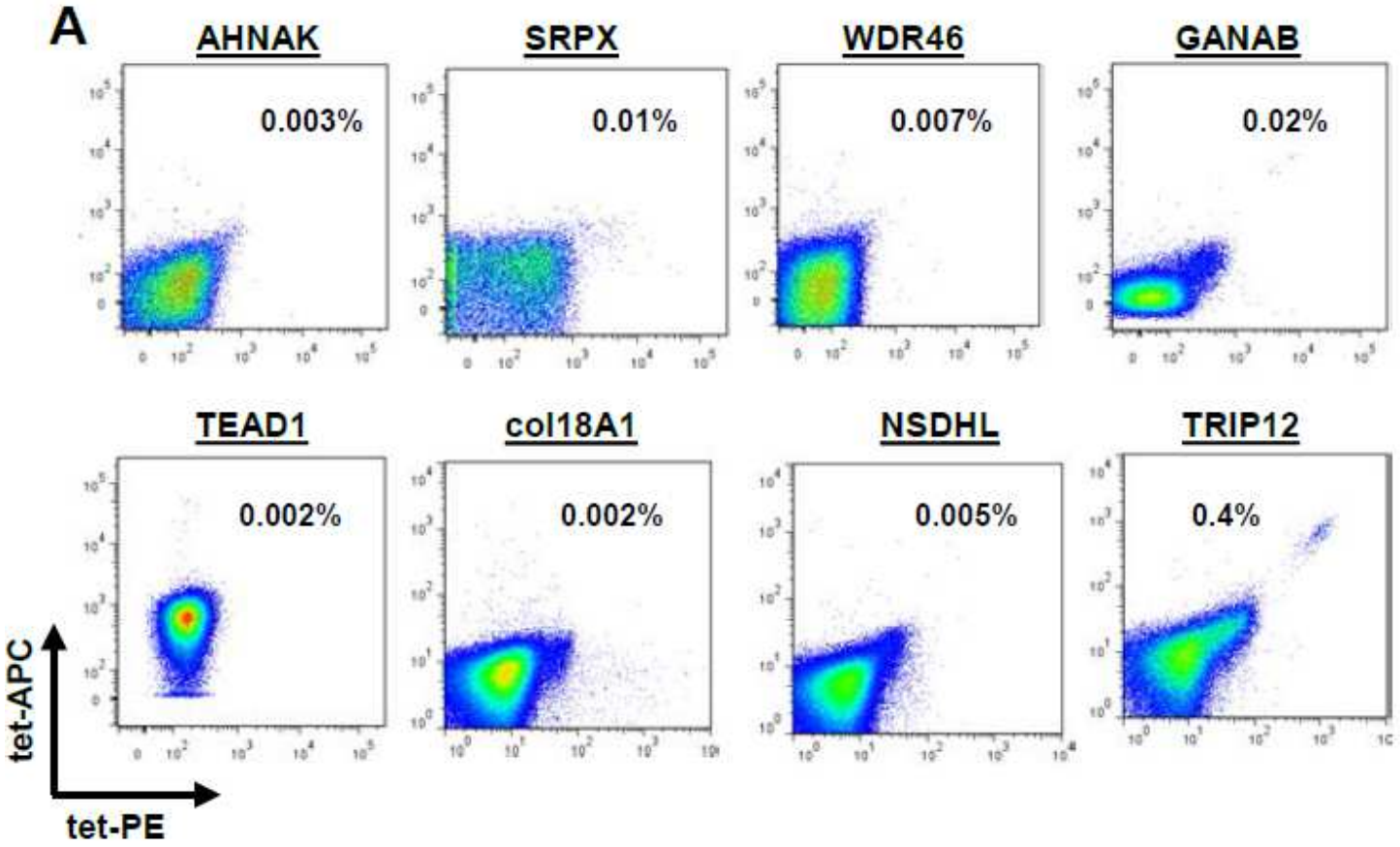


- **Large panels of MHC-multimers were generated from candidate mutated peptide using UV-mediated ligand exchange**

- **TIL, fresh tumor digests and PBMC were evaluated for binding**

Rodenko et al. Nature Protocols, 2006

Isolation of tetramer+ T-cells reactive withmutated antigens from peripheral blood



T cells reactive with 7 of 8 tetramer sorted population expanded *in vitro* following cell sorting

Tumor Antigen Identification using Whole Exome Sequencing
Mini-gene approach

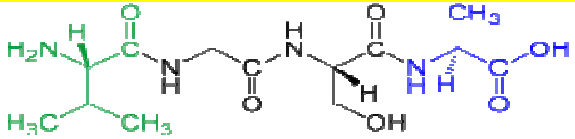
Sequence tumor and matched normal DNA, identify somatic mutations

Generate tandem mini-gene constructs encoding mutations

Transfect mutated mini-genes into:
Autologous normal cells (DC, B cells) OR
HLA-matched targets

Co-culture with TIL

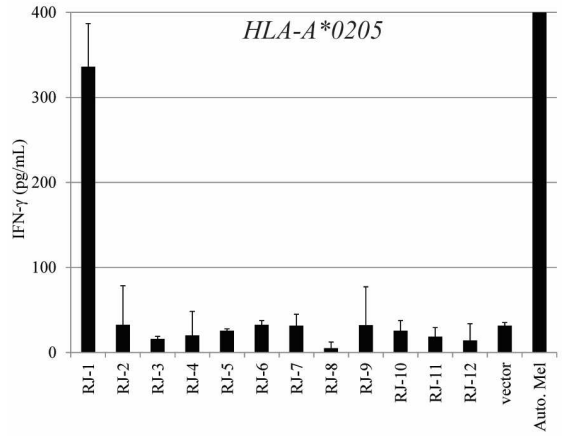
Predict high affinity peptides epitopes encoded by positive mini-gene

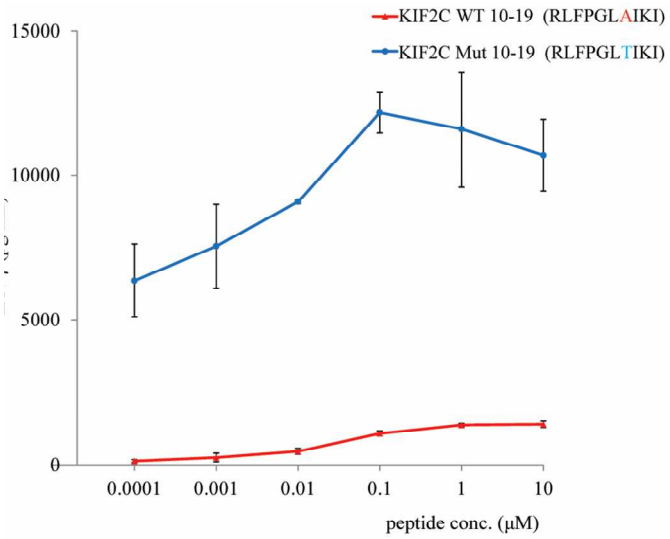


Identify peptides epitope recognized by TIL

Genetic screening approach:
Identification of a mutated epitope recognized by 2359 TIL

Mini-gene library screening assay
(71 non-synonymous mutations/
9 tandem mini-gene constructs)

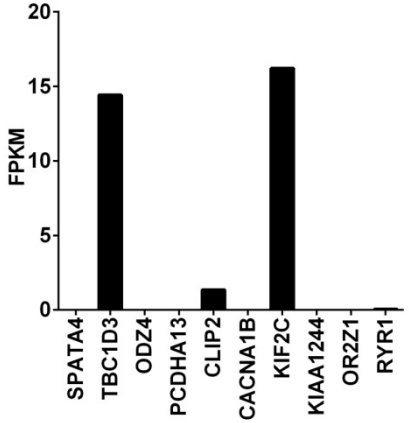




Predicted HLA-A*0205 binding peptides

	Gene ID	Mut peptide	HLA-*0205 Affinity nM
1	SPATA4	RLSELLSNL	8
2	TBC1D23	YLQQADQFFI	12
3	ODZ4	RLSSVTMSNV	21
4	CLIP2	GLMDNWKFKL	34
5	PCDHA13	KLYKISVEAV	37
6	CACNA1B	NVWNVMDFV	38
7	KIF2C	RLFPGLTIKI	55
8	KIAA1244	NLFAFPKEV	73
9	OR2Z1	YDAAVFMYMV	83
10	RYR1	LLYSHAILL	87

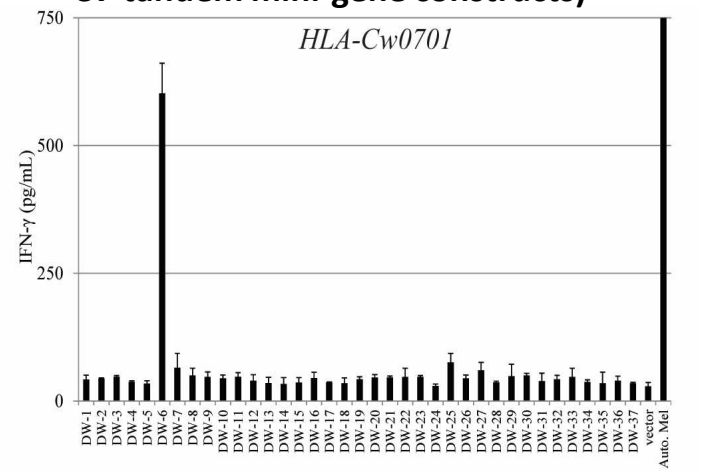
RNA-seq analysis – 2359 mel

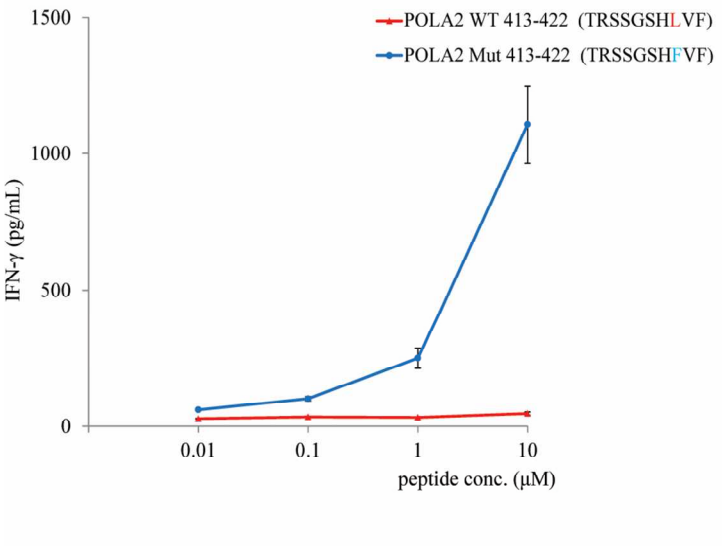


Genetic screening approach: Identification of a mutated epitope recognized by 2591 TIL

Mini-gene library screening assay

(218 non-synonymous mutations/ 37 tandem mini-gene constructs)





Predicted HLA-C*0701 binding predictions

	Gene ID	Mutated peptide	HLA-C*0701 Affinity nM
1	CYB5D2	FRFFIPEEL	58
2	ROS1*	FRIERNASSF	61
3	KIF13A	YRDSVFTWLL	61
4	IQCH*	SRRTIIHIL	64
5	KIF13A	YRDSVFTWL	97
6	DNHD1	VRLQRLHRL	188
7	HYDIN	KRNMGNVSM	244
8	SUOX*	TRNHLPVSNL	269
9	PRIM2*	FRAKLFKAL	273
10	IQCH*	RRTIIHILSL	280
11	CDKN2A*	LRRPRHSHL	293
12	GRIN3A	LRFLANTTF	457
13	BBOX1	KRMGFLCLTF	457
14	USP29	FYRDAKQLNM	570
15	MAS1	KRFKEFLKVV	598
16	BAGE2	FRILVTSSCF	662
17	MAS1	KRFKEFLKV	664
18	POLA2*	TRSSGSHFVF	686

*Genes with FPKM>1 in panel of 6 melanoma cell lines

Mutated Antigens Identified as Melanoma TIL Targets Using:

Conventional screening

Tumor type	TIL	Gene	HLA-RE
Melanoma	1290	β-catenin	A*2402
Melanoma	1290	Ki-67	DRβ1*1502
Melanoma	1700	NOP-56	A*0201
Melanoma	1913	HLA-A*11	-
Melanoma	1913	p14ARF/p16	A*11
Melanoma	1362	MART-2	A*0101
Melanoma	1558	TPI	DRβ1*0101
Melanoma	1363	LDLR-FUT	DRβ1*0101
Melanoma	1359	CDC-27	DRβ1*0401
Melanoma	1087	neo-PAP	DRβ1*0701
Melanoma	164	ARTC1	DRβ1*0101

Next generation sequencing approaches

Tumor type	TIL	Gene	HLA-RE
Melanoma	3713	CENPL	A*29:02
Melanoma	3713	HELZ2	A*29:02
Melanoma	3713	PRDX3	A*29:02
Melanoma	3713	GCN1L1	A*29:02
Melanoma	3713	AFMID	A*29:02
Melanoma	3713	PLSCR4	A*29:02
Melanoma	3713	SEC22C	A*29:02
Melanoma	3713	WDR46	A*02:01
Melanoma	3713	AHNAK	A*02:01
Melanoma	3713	SRPX	A*02:01
Melanoma	3466	COL18A1	A*02:01
Melanoma	3466	TEAD1	A*02:01
Melanoma	3466	ERBB2	A*02:01
Melanoma	3466	PDZD8	B*44:02
Melanoma	3466	PXMP4	B*39:01
Melanoma	3466	KHSRP	B*39:01
Melanoma	3868	GANAB	A*02:01
Melanoma	3903	PKHA1	B*38:01
Melanoma	3903	KIAA1279	B*38:01
Melanoma	3919	TRIP12	A*01:01
Melanoma	2556	MYH14	A*01:01
Melanoma	2556	RAC1	A*02:01
Melanoma	3703	NSHDL	A*02:01
Melanoma	2098	CSNK1A1	A*02:01
Melanoma	2098	GAS7	A*02:01
Melanoma	2098	HAUS3	A*02:01
Melanoma	2098	GAPDH	A*02:01
Melanoma	3309	MATN2	A*11:01
Melanoma	3309	CDK12	A*11:01
Melanoma	2369	PLEHHM2	A*01:01
Melanoma	2369	PPP1R3B	A*01:01
Melanoma	2359	KIF2C	A*02:05
Melanoma	2591	POLA2	C*07:01
Melanoma	2224	KPNA5	A*02:01

Relationship between mutation frequency and number of mutated antigens identified as targets of melanoma TIL

ID	Tumor type	# of mutated epitopes recognized	Screening approach	# nonsynon mutations
3713	mel	10	TMG transfection, mutated tetramers	6041
3466	mel	5	TMG transfection, mutated peptides/ tetramers	5718
3919	mel	5	TMG transfection, mutated tetramers	752
2369	mel	4	TMG transfection, cDNA library, mutated peptides	661
2098	mel	3	Mutated peptides, cDNA library	343
3903	mel	2	TMG transfection	464
2556	mel	2	cDNA library, TMG transfection	977
3309	mel	2	Mutated peptides	385
2224	mel	2	Mutated peptides	160
2359	mel	1	TMG transfection	131
3879	mel	1	Mutated tetramers	927
3703	mel	1	Mutated tetramers	304
2591	mel	1	TMG transfection, cDNA library	320
3926	mel	0	Mutated tetramers	176
3702	mel	0	Mutated tetramers	308
2133	mel	0	Mutated peptides	176

Whole Exome Sequencing Applied to Treatment of Non-melanoma Patients

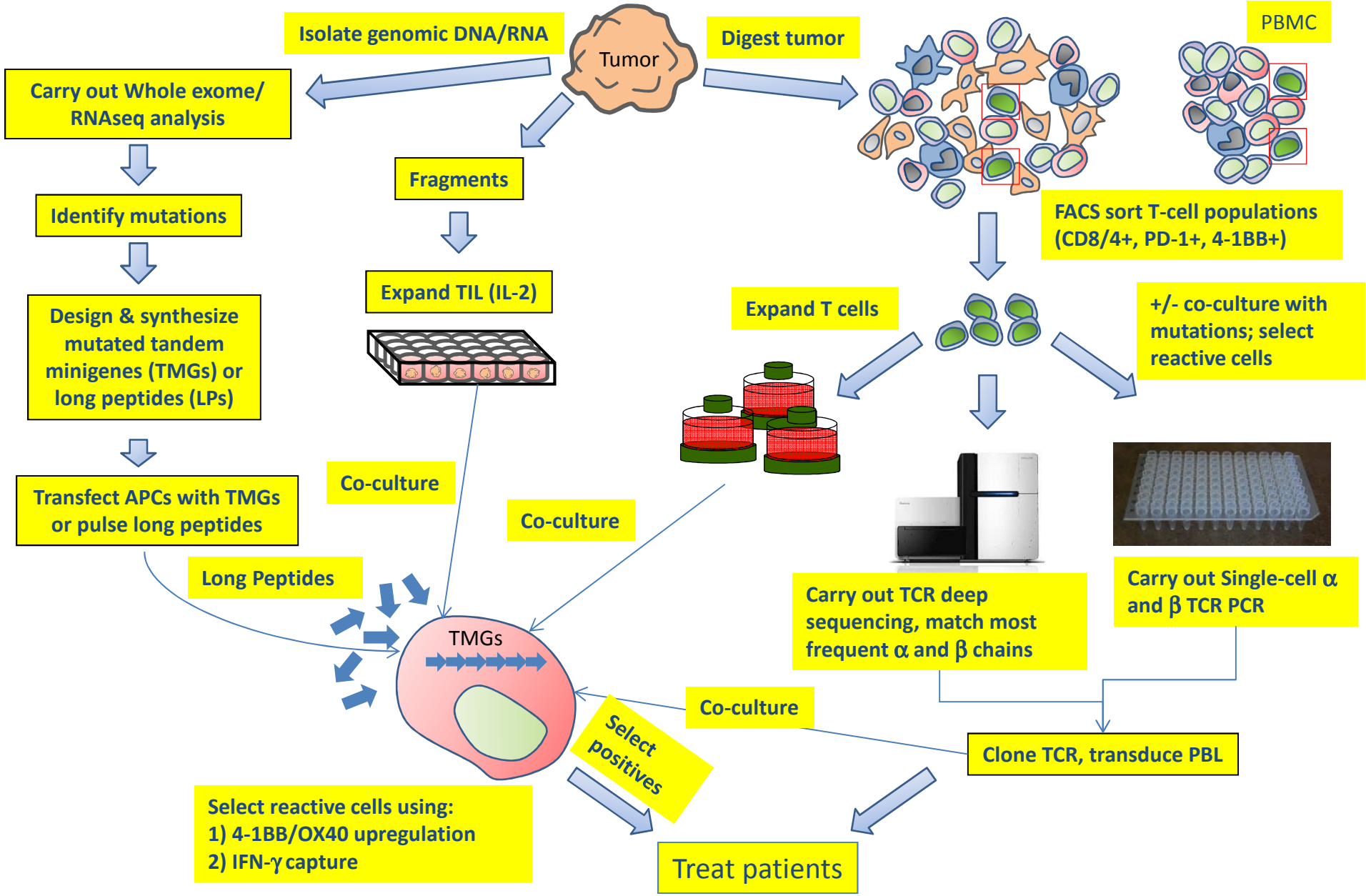
Gastrointestinal TIL evaluated for reactivity with mutated candidates

- **Mutated ERBB2 peptide identified as immunodominant CD4 T cell epitope recognized by cholangiocarcinoma TIL**
- **Reactivity with ERBB2 used to guide treatment**

Additional GI TIL being evaluated for reactivity with mutated candidate epitopes

Tran et al., Science, 2014

Application of High Throughput Sequencing to Development of Effective Cancer Immunotherapy



Future Directions

- **Develop pipeline for rapid identification of mutated T cell epitopes from sequence data**
  - **Integrate exomic and RNAseq data with peptide MHC binding and antigen processing algorithms**
- **Develop efficient methods for identifying and enriching tumor-reactive T cells from:**
  - **Fresh uncultured tumors**
  - **Peripheral blood**
- **Extend studies to additional tumor types including:**
  - **Lung, bladder, cervical and breast carcinomas**
- **Develop effective immunotherapies**

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Outline

- Adoptive Immunotherapy with TIL
- Adoptive Therapy with T cells Engineered to Express Tumor-reactive TCRs/CARs
- **Use of Whole Exome Sequencing to Identify Mutated Antigens for Therapy**