T cells as a Drug for the Personalized Immunotherapy of Cancer

BSA/NCAB Meeting

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Major Challenge Confronting Cancer Immunotherapy

The development of effective immunotherapies for patients with metastatic epithelial solid cancers that result in over 80% of cancer deaths.

ADVANTAGES OF CELL TRANSFER THERAPY

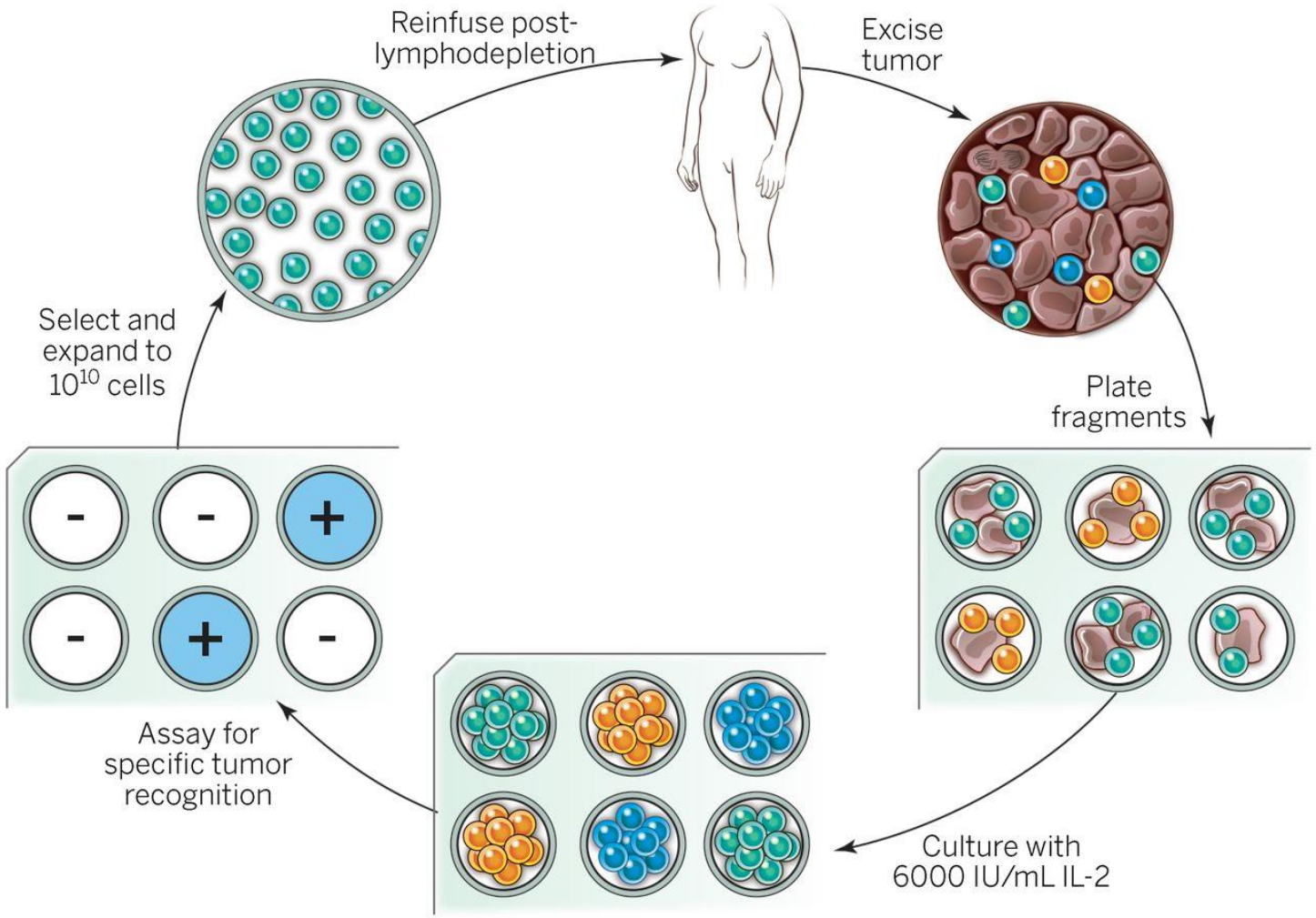
1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

2. Administer cells activated ex-vivo to exhibit anti-tumor effector function.

3. Potentially identify exact cell subpopulations and effector functions required for cancer regression in vivo.

4. Manipulate host prior to cell transfer to provide altered environment for transferred cells.

Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL)

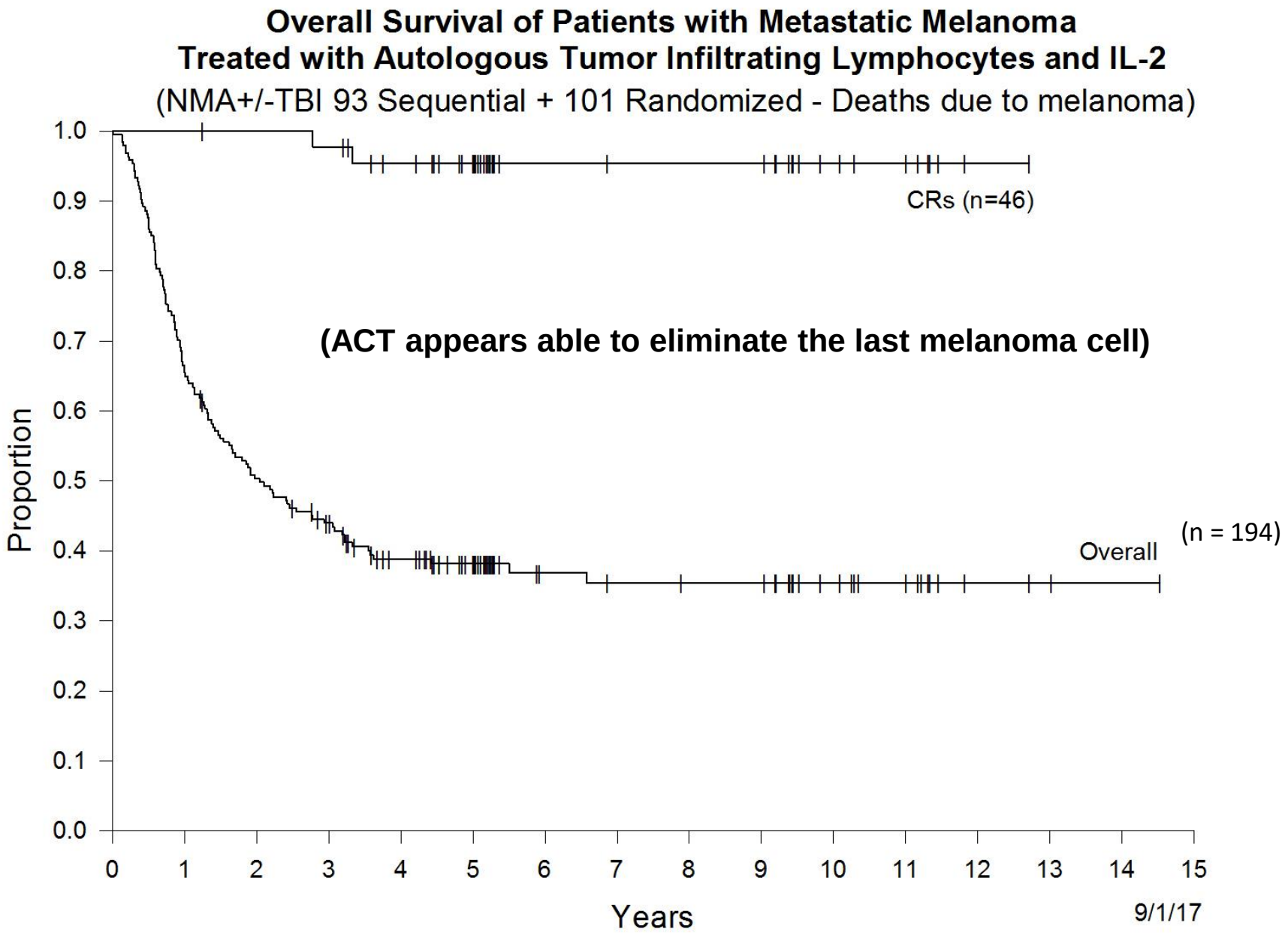


Summary of Cell Transfer Protocols for the Treatment of Patients with Metastatic Melanoma* (median f/u 6.3 years)

Total	PR	CR	OR
	number of patients (duration in months)		
194	60 (31%)	46 (24%)	106 (55%)
	84, 71+,70+,63+,58+,55+,51+,37, 36, 28, 25, 22, 21, 19, 19+,14, 14, 14, 14, 13, 12+,11, 11, 11, 10, 10, 9, 9, 9, 9, 8, 8, 7, 7, 7, 7, 7, 6, 6, 6, 6, 6, 6, 5, 5, 5, 5, 5, 4, 4, 4, 4, 4, 4, 3, 3, 3, 3, 3, 2	152+,142+,137+,136+,136+, 134+,132+,123+,121+,118+, 114+,113+,113+,112+,110+, 110+,108+, 64+, 63+, 63+, 63+, 62+, 62+, 62+, 62+, 61+, 61+, 60+, 60+, 60+, 60+, 59+, 58+, 57+, 54+, 53+, 53+, 50+, 45+, 45+, 43+, 39+, 38+, 27, 19, 14+	

*from four trials (5 groups) using different lymphodepleting regimens

(44 of 46 Complete Responders ongoing from 14 to 152 months)
(44 of 46 Complete Responders received a single treatment)



(Median followup: 6.3 years)

Question

What do TIL recognize that enables the in vivo destruction of the last melanoma cell?

(Specific cancer regression in the absence of off-tumor on-target, toxicities in patients led us to explore the role of specific cancer mutations as the targets of TIL.)

Mining the Cancer Exome to Identify Immunogenic Cancer Mutations

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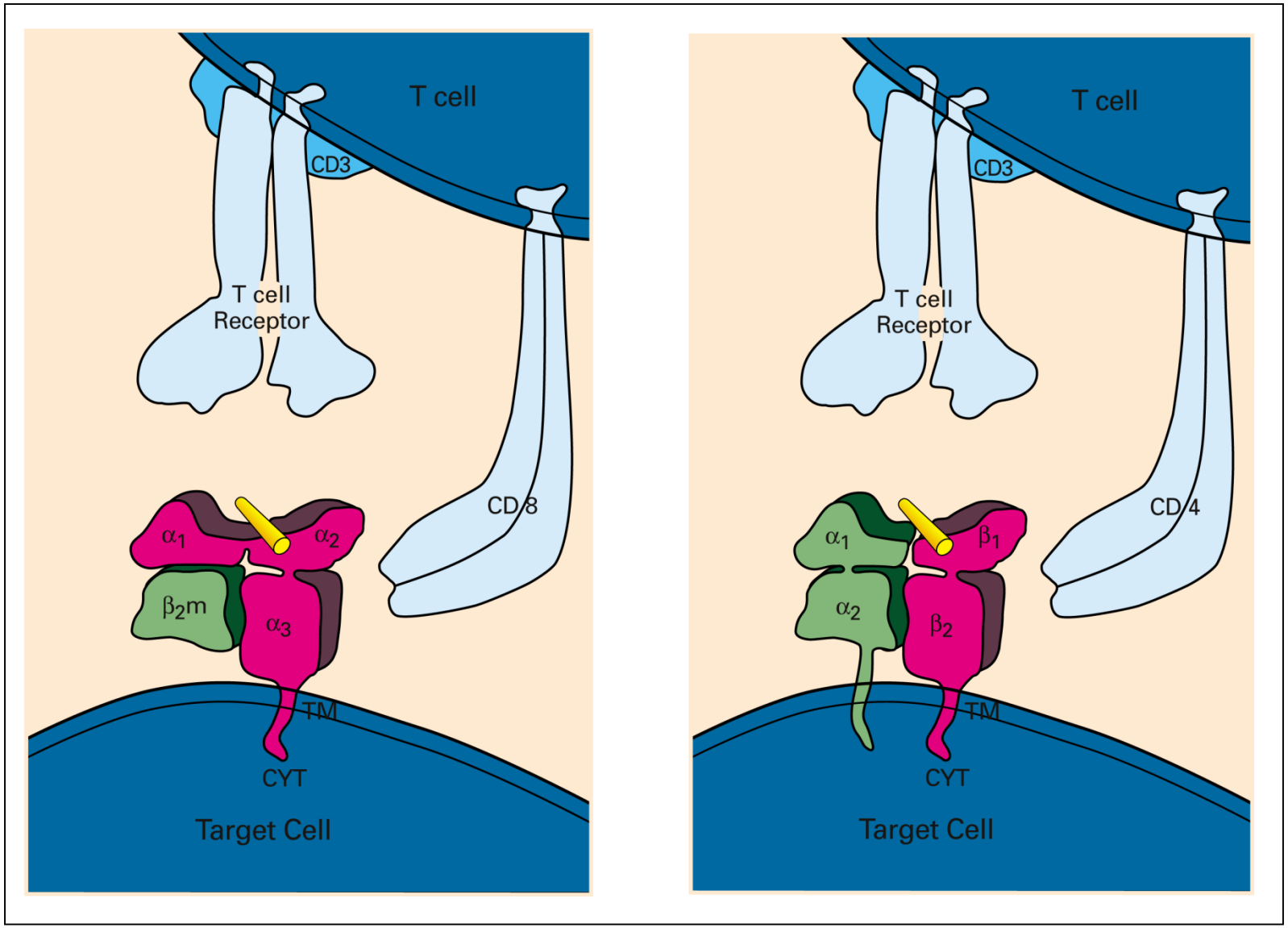
For a mutation to be a cancer antigen it has to:

1) be processed intracellularly into a 9-11 amino acid peptide

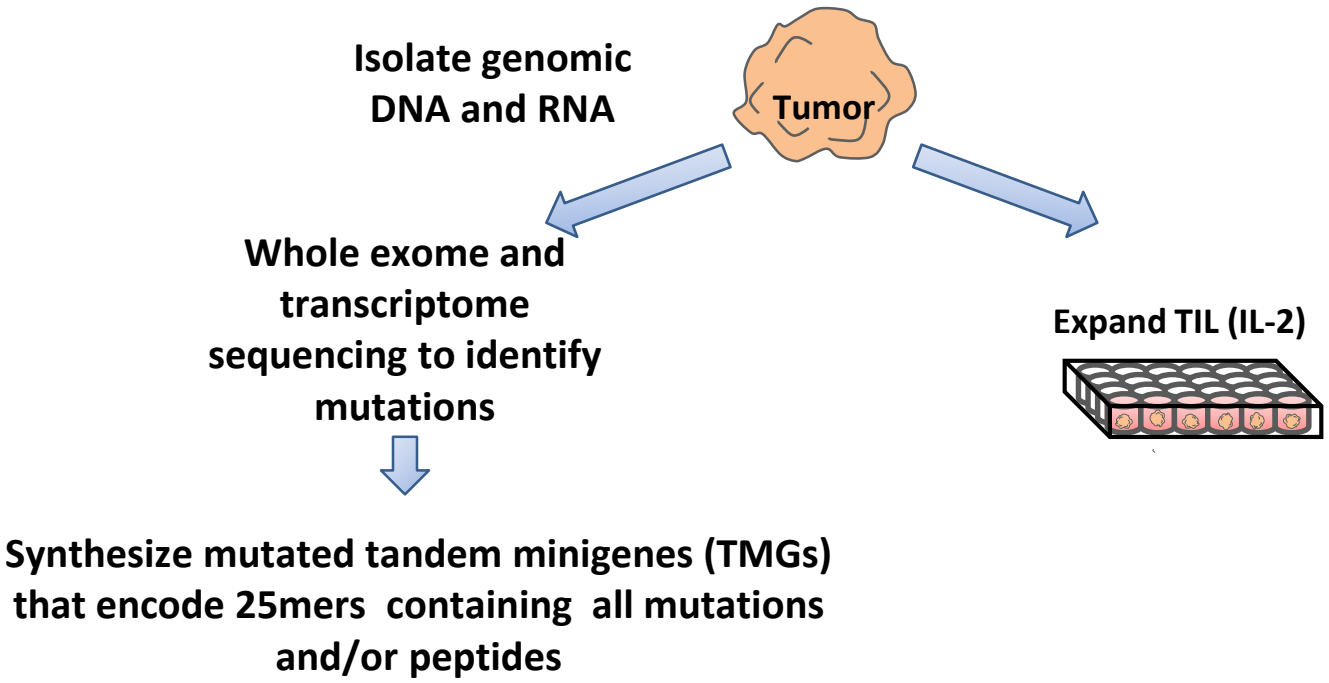
2) the peptide must fit and be presented in the groove of on one of the patient's surface MHC molecules

Thus, only rare mutations will be antigenic.

Antigen recognition by $CD4^+$ and $CD8^+$ T lymphocytes



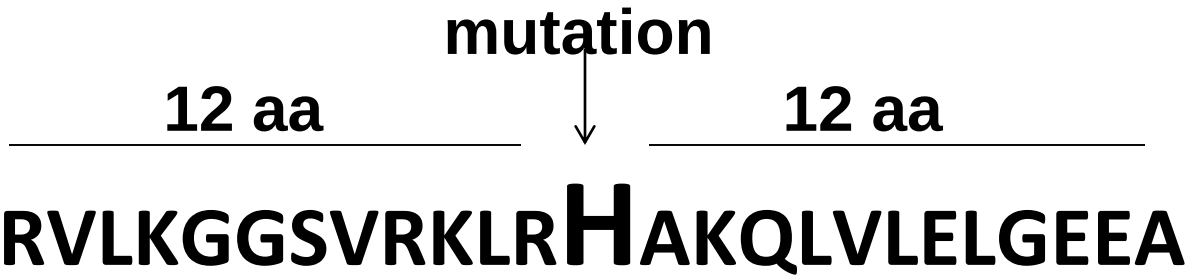
Blueprint for the generation of mutation-reactive T-cells in common cancers



(Nature Med 19:747-752, 2013; Science 344:641-645, 2014)

Tandem minigene (TMG):

String of minigenes encoding the mutated AA flanked by 12 AA



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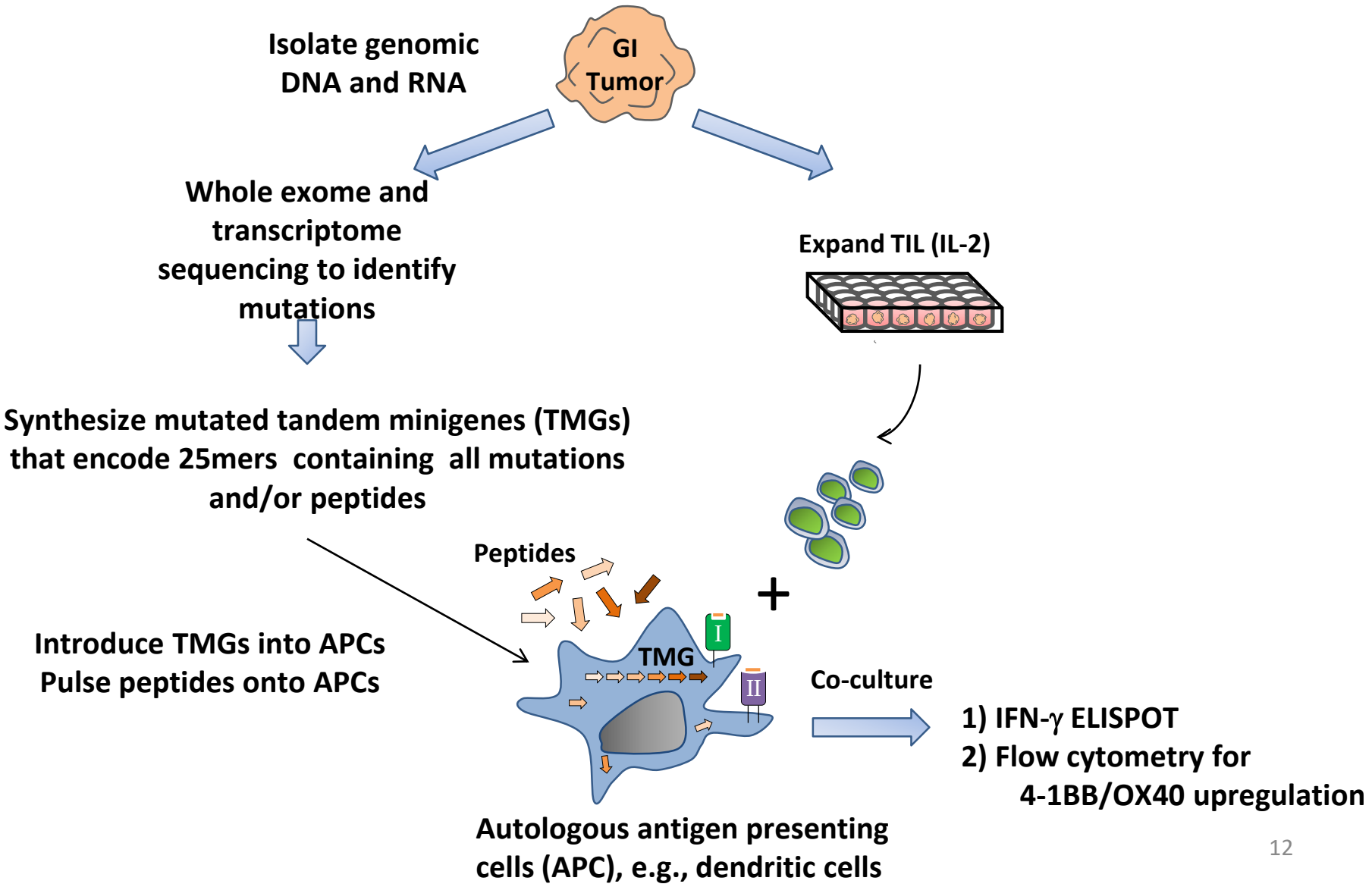

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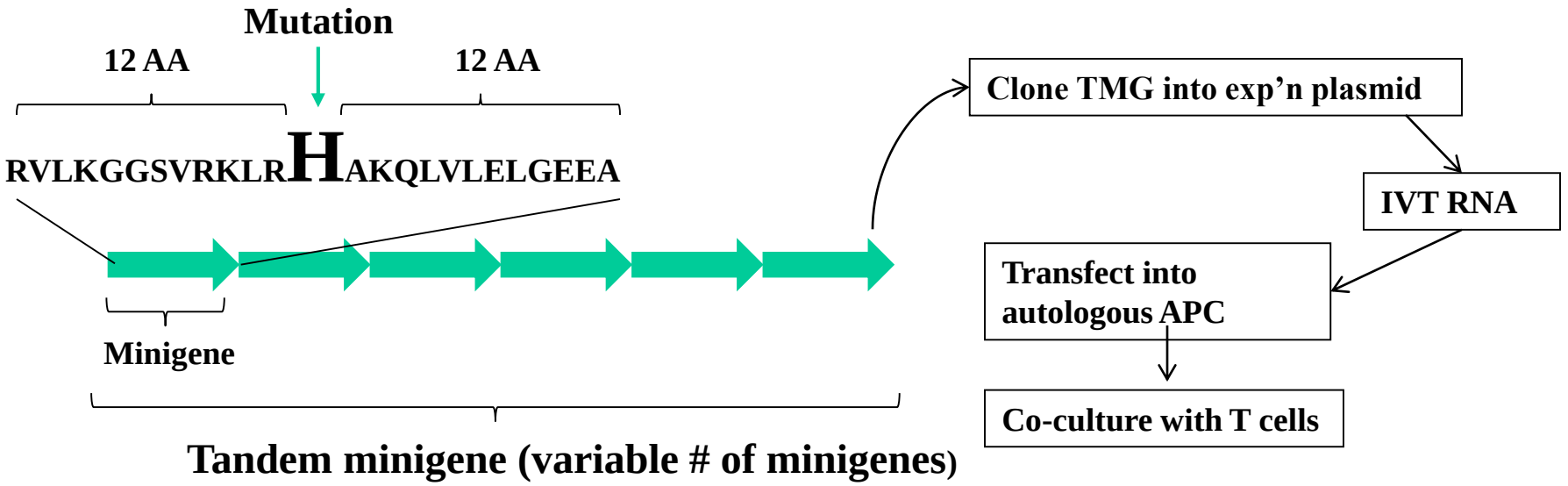


Advantages of this approach:

No need to predict peptide binding to MHC.

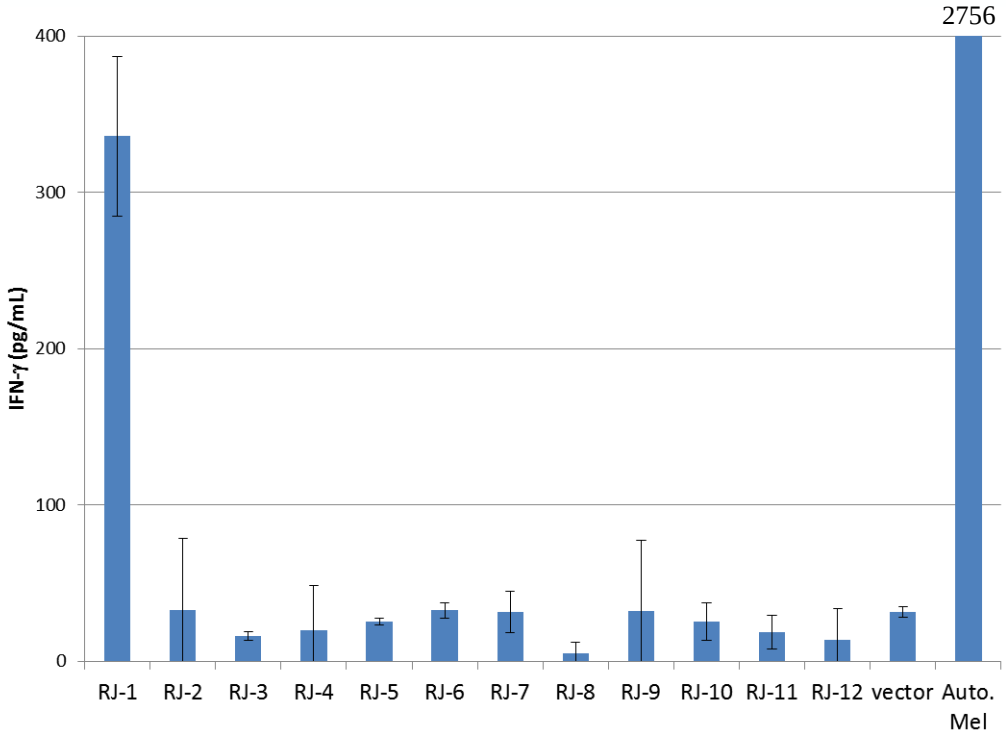
All candidate peptides and all MHC loci are included in the screen.

No tumor cell line necessary.

(Nature Med 19:747-752, 2013; Science 344:641-645, 2014)

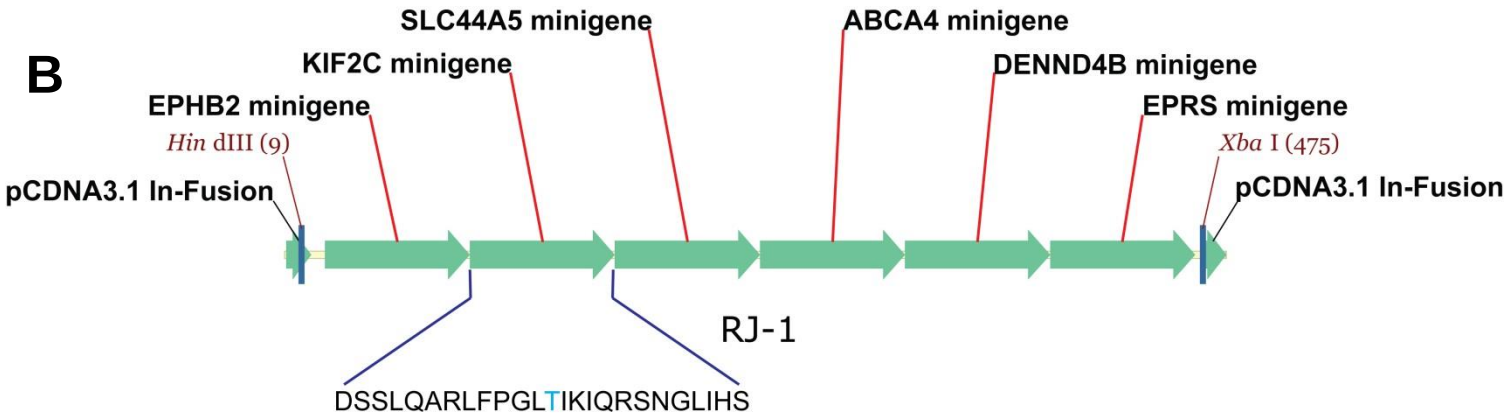
Minigene approach: J. bulk TILs recognize tandem minigene RJ-1

A

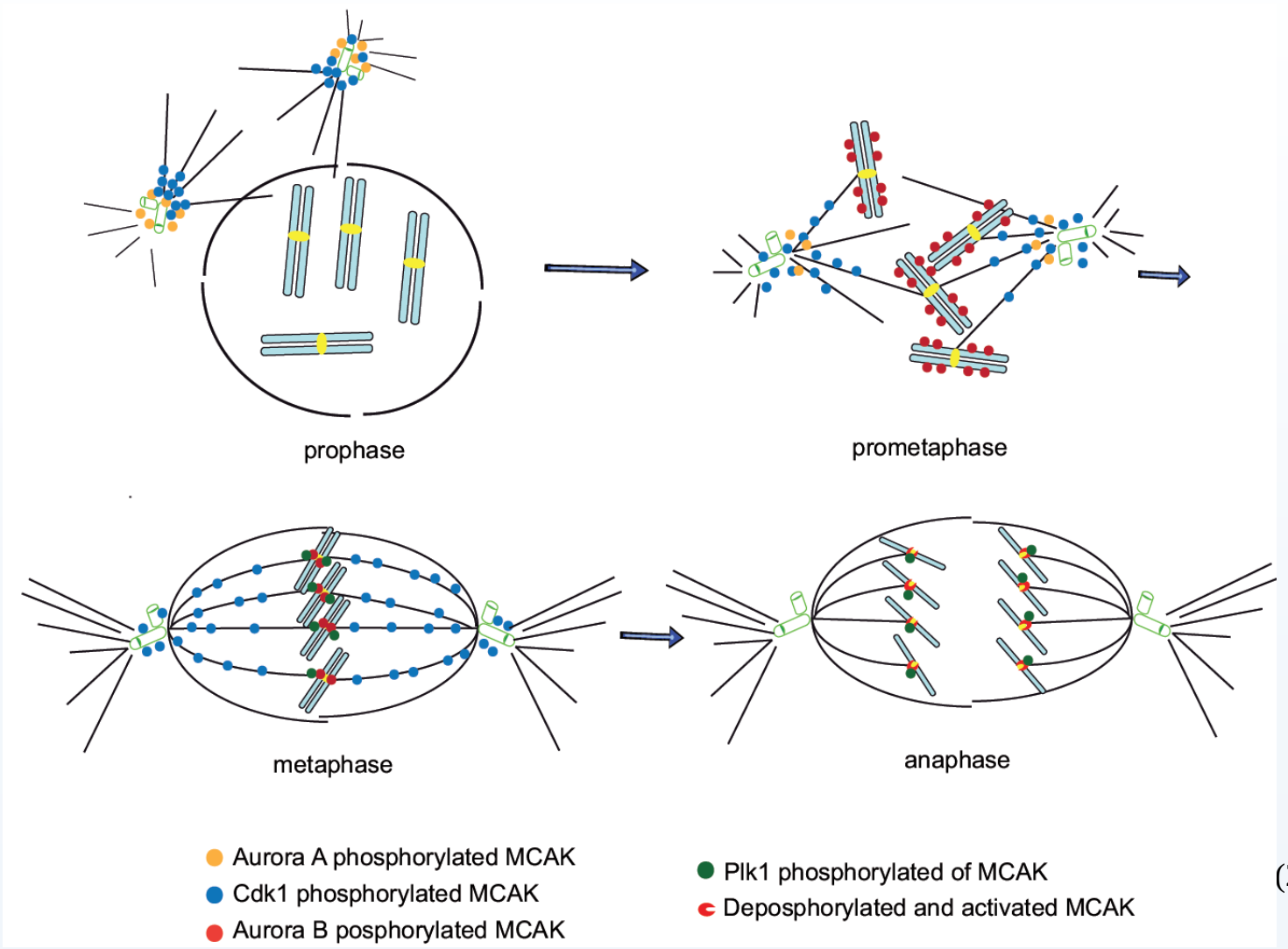


71 non-synonymous mutations
12 tandem minigene constructs
HLA-A*0205

B



Kinesin family member 2C (KIF2C)
also known as mitotic centromere-associated Kinesin (MCAK)



(2011)

Immunogenic Mutations in Patients with Melanoma

Patients evaluated	Median	Total	Screened	Immunogenic neoepitopes
(number)	(number of mutations)			
22	318	13664	3938	54

Patients with mutation reactive T cells in TIL: **18/22 = 82%**

Immunogenic mutations of number screened: **54/3938 = 1.4%**

6% CD4

94% CD8

All neoantigens were unique, none shared.

(updated 6/18)

63% of Patients with Melanoma Recognized Two or More Immunogenic Mutations

Immunogenic mutations 54/3938 = 1.4%

# neoantigens per patient	# patients
0	4 (18%)
1	4 (18%)
2	6 (27%)
3	4 (18%)
>3	4 (18%)

(updated 6/18)

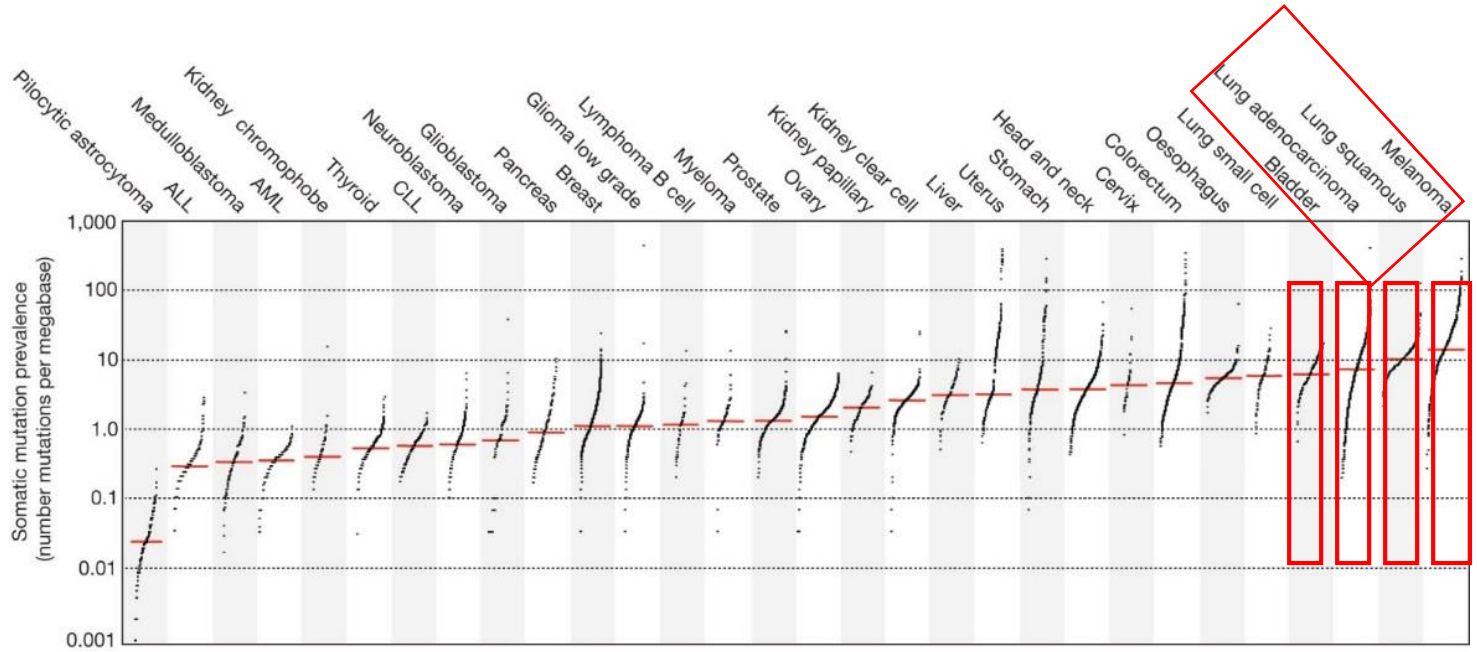
Preliminary Conclusion

Adoptive cell therapy mediates complete, durable, and likely curative, regressions of metastatic melanoma based on the recognition of immunogenic cancer mutations.

Can this insight be used to develop a "blueprint" for the treatment of common epithelial cancers?

Immunotherapy for Cancer Using Checkpoint Modulators

(**Alexandrov et al, *Nature* 2013**)



Limitations of Current Immunotherapies

The common epithelial cancers such as those arising in the colon, liver, stomach, pancreas, prostate, ovary, etc very rarely respond to current immunotherapies and account for over 80% of cancer deaths.

Are there antigens on the common epithelial human cancers that can be targeted by cell-based immunotherapy?

Patient M.B.

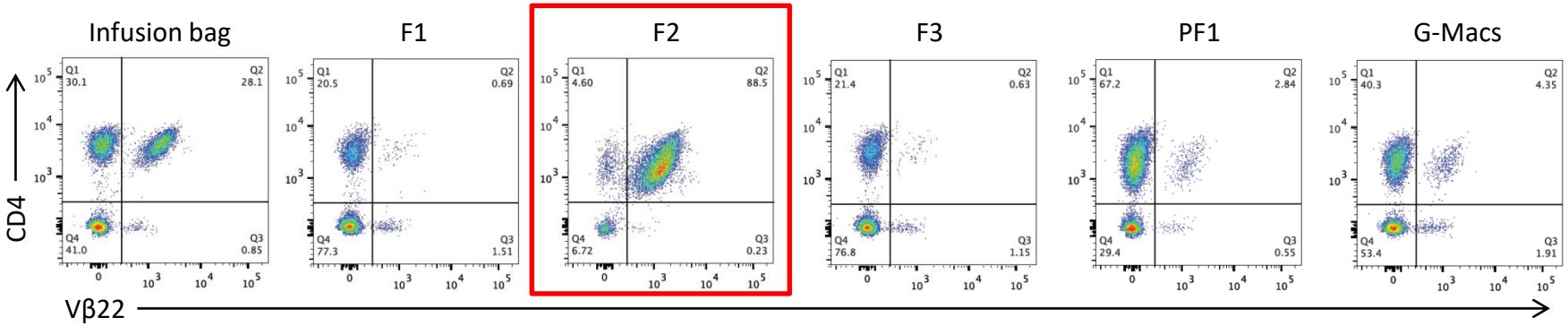
45 y.o. female with metastatic cholangiocarcinoma

12/2009	**Right hepatectomy for cholangiocarcinoma**
4/2010	**Multiple lung and liver metastases** **Received cisplatin and gemcitabine: PD**
5/2011	**Taxotere chemotherapy: PD in lung and liver**
3/2012	**Unselected TIL from resected lung lesion infused; PD**
10/2013	**TMG approach to target unique cancer mutations (26)**

Ongoing response and living normally 56 months later.

(Tran et al, Science 344:641-5, 2014)

Isolation of ERBB2IP reactive cells



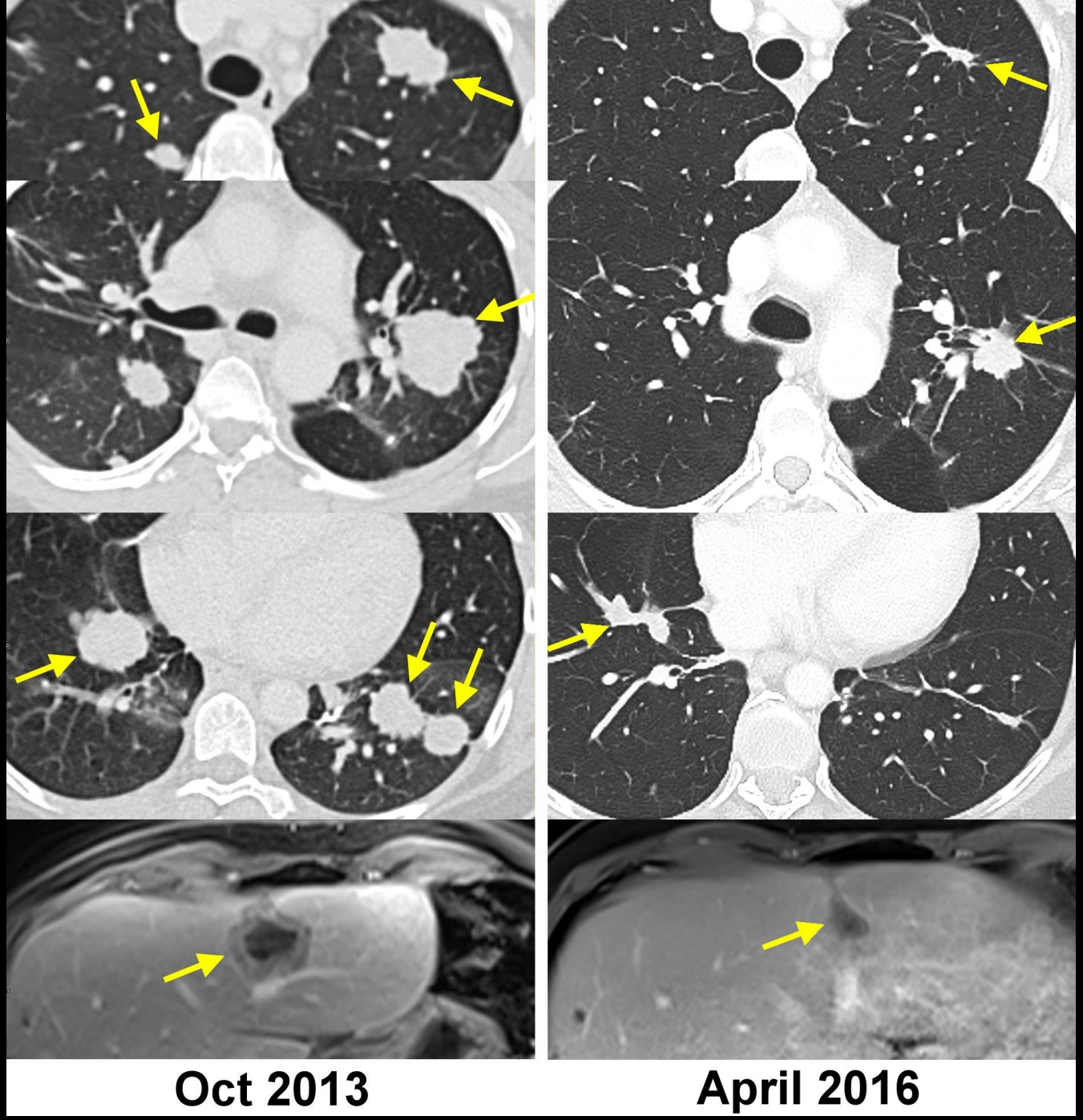
Use enriched ERBB2IP autologous lymphocytes for treatment

Objective response of lung and liver metastases ongoing for 53 months

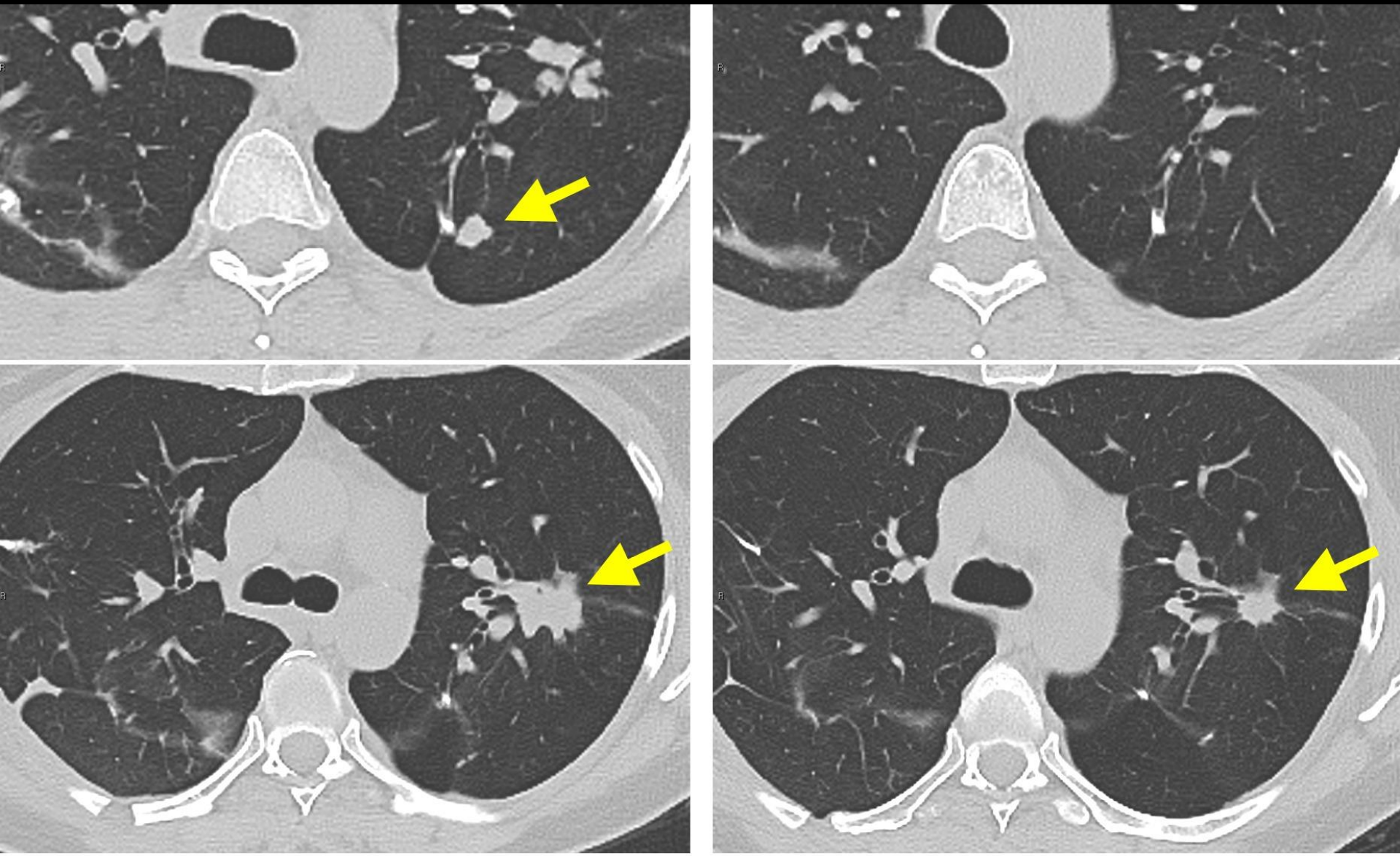
(Tran et al, Science 344:641-5, 2014)

M.B.

Selected TIL



Response to Pembrolizumab after ACT Targeting of ERB2IP; Ongoing overall response at 4 years



Pre-Treatment

6 Months

Immunogenic Mutations in Patients with Gastrointestinal Cancers

Patients evaluated	Median	Total	Screened	Immunogenic
(number)	(number of mutations)			
72	113	10261	7496	120

Immunogenic mutations of number screened: 120/7496 = 1.6%

49% CD8

51% CD4

All neoantigens were unique except 2 patients shared the same KRAS mutation.

(updated 6/18)

50% of Patients with GI Cancers Recognized Two or More Immunogenic Mutations

Immunogenic mutations 120/7496 = 1.6%

# neoantigens per patient	# patients
0	13 (19%)
1	22 (31%)
2	22 (31%)
3	9 (13%)
>3	6 (9%)

Mutated Antigens Recognized by TIL from 99 Patients with Epithelial Cancers

Cancer	# of patients screened	# of patients with neoantigen reactivity	Total # of neoantigens recognized
Colorectal	45	39 (87%)	95
Cholangiocarcinoma	12	9 (75%)	20
Pancreatic	6	5 (83%)	7
Esophageal	2	2 (100%)	3
Endometrial	3	3 (100%)	4
Breast	10	7 (70%)	22
NSCLC	11	8 (73%)	34
Ovarian	7	6 (86%)	16
Stomach	3	2 (67%)	5
TOTAL	99	81 (81.8%)	197

All neoantigens were unique except for 2 KRAS antigens.

Hypothesis (1)

Recognition of random somatic mutations is the "final common pathway" explaining cancer regression from most immunotherapies for solid cancers.

IL-2

anti-CTLA4

anti-PD1

anti-CD40

Tumor infiltrating lymphocytes

J.A. 51 year old female with metastatic breast cancer

2003	Localized Ductal Carcinoma in Situ; underwent mastectomy	
Aug. 2013	ER+, PR+ invasive breast cancer metastatic to multiple nodal groups, chest wall, bone	
Sept. 2013	Pacitaxel chemotherapy	Progressed
Feb. 2014	Arimidex	Progressed
Sept. 2014	Xeloda chemotherapy	Progressed
Oct. 2014	Navelbine chemotherapy	Progressed
Nov. 2014	Taxotere, Adriamycin, Cytoxan chemotherapy	Progressed
Jan. 2015	Lucitanib (TKI inhibitor)	Progressed
Sept. 2015	Everolimus (mTOR inhibitor)	Progressed
Dec. 2015	NCI for cell transfer immunotherapy targeting mutations expressed by her cancer (62 mutations) Received 80e9 cells plus 7 doses of IL-2 and 4 doses of Pembrolizumab She is now in an ongoing complete response of multiple nodal, chest wall, and liver metastases 30 months after treatment	

(Zacharakis et al, Nature Med, June 2018)

Mutations Targeted in Patient with Metastatic Breast Cancer

SL3A2: 4F2 cell-surface heavy chain

Function: Required for the function of light chain amino-acid transporters

KIA0368: Proteasome-associated protein ECM29 homolog

Function: Adapter/scaffolding protein that binds to the 265 proteasome

CADPS2: Calcium-dependent secretion aviator 2 Calcium-binding protein

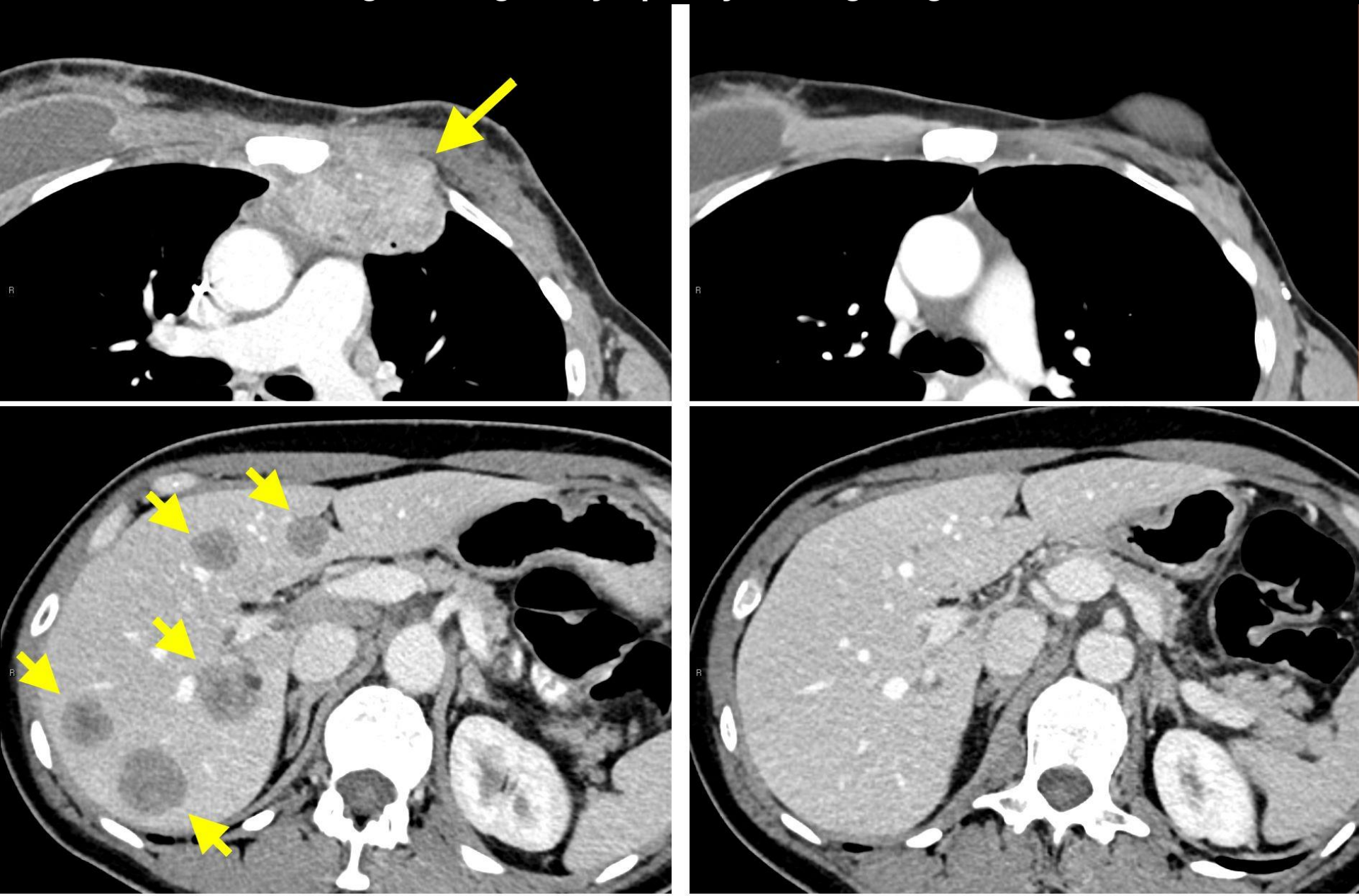
Function: Involved in exocytosis of vesicles filled with neurotransmitters and neuropeptides

CTSB: Cathepsin B. Thiol protease

Function: Which is believed to participate in intracellular degradation and turnover of proteins

23% of infused cells contained neoantigen reactivity. All 8 neoantigen TCR present in PBL at 6 weeks.

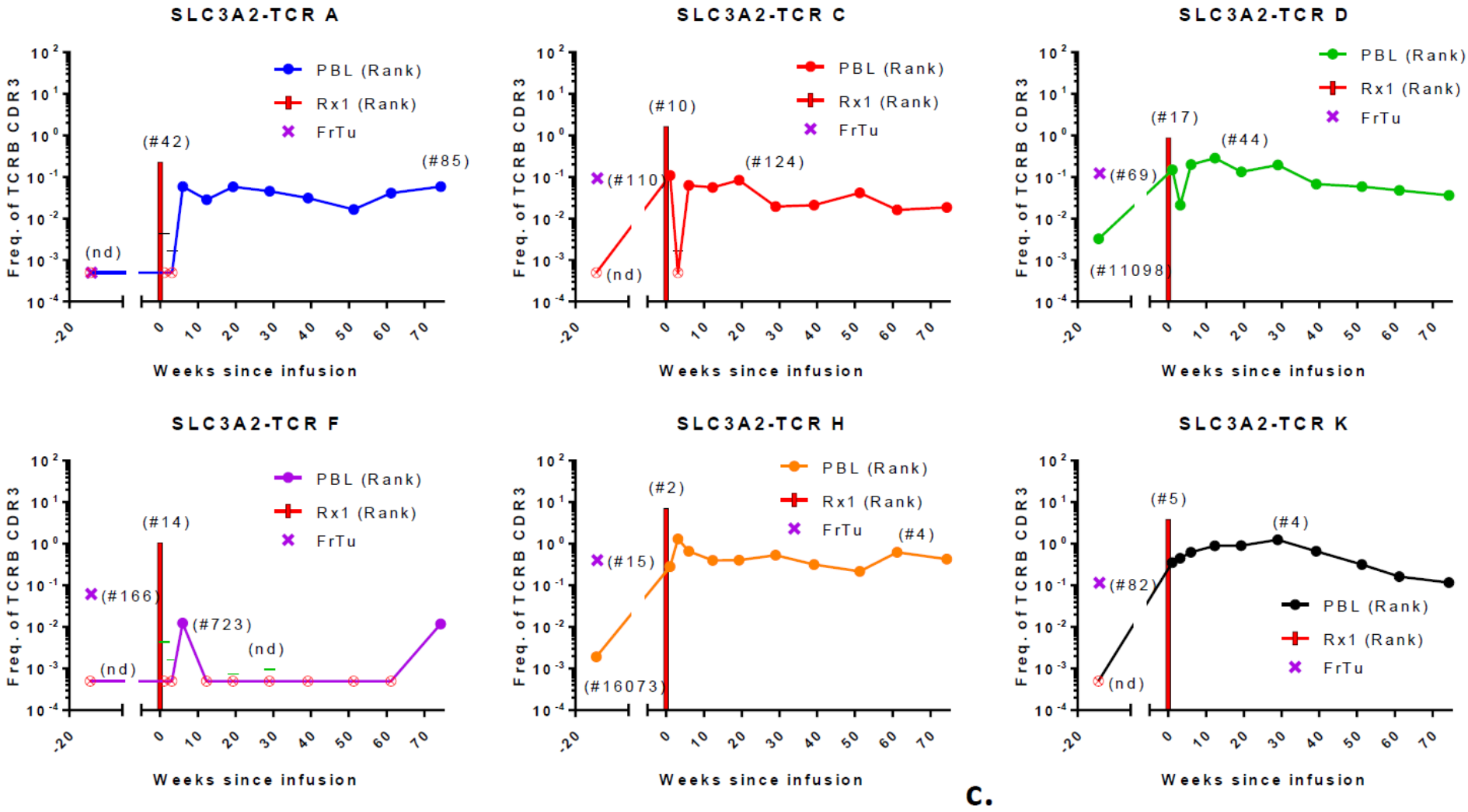
J.A.: ACT using autologous lymphocytes targeting somatic mutations



Pre-Treatment

14 Months

Isolation and in vivo Persistence of Mutation-reactive Cells in a Patient with Breast Cancer



C.

Patient S.S.

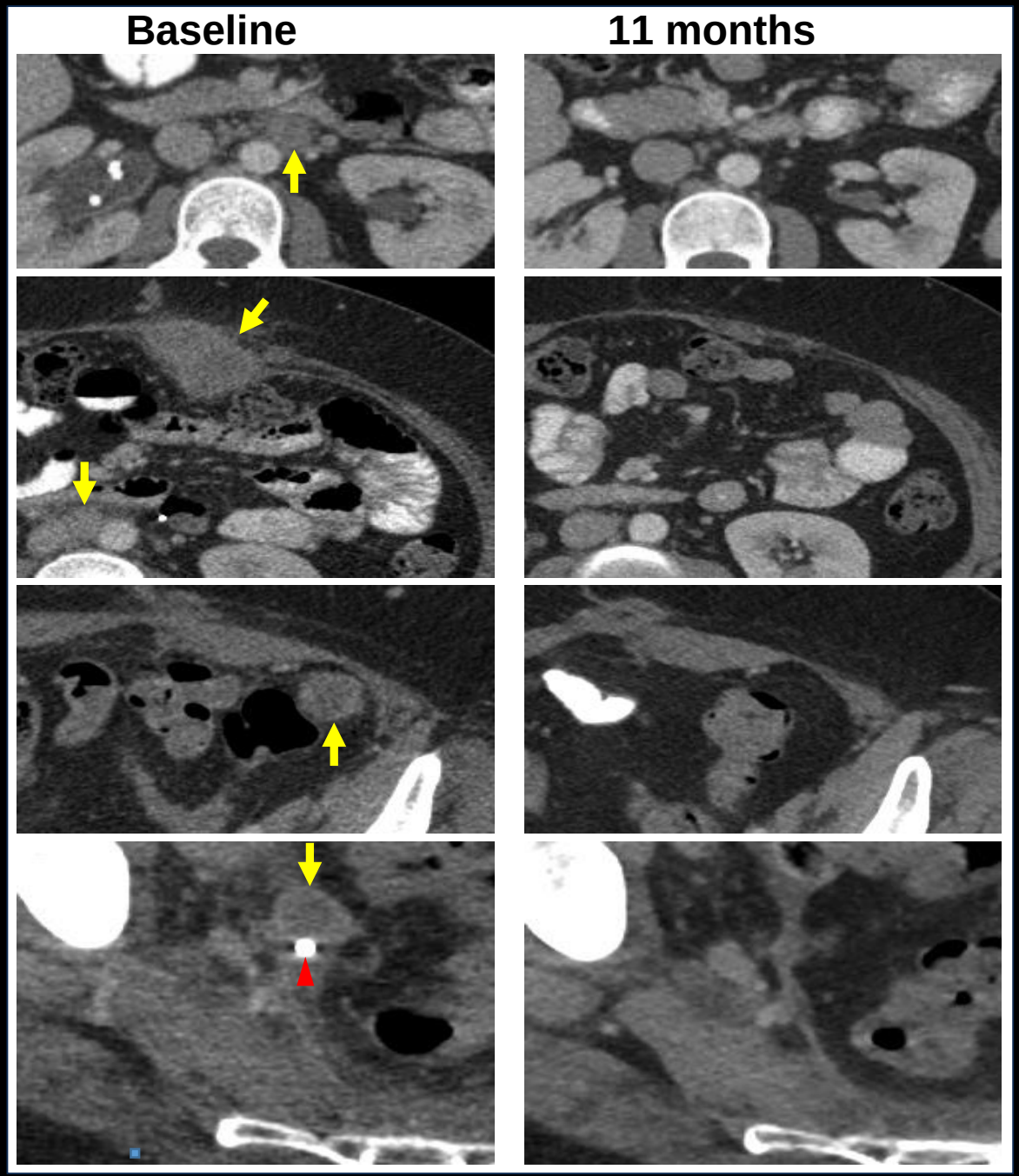
36 y.o. female with metastatic cervical cancer

10/2011	**Presented with fungating cervical mass, lung and intraperitoneal metastases**
11/29/11	**Radiation therapy and cisplatin chemotherapy**
10/06/12	**Cancer progressed. She underwent hysterectomy and excision of both ovaries**
11/2012 to 1/2013	**Developed liver, lymph node, intra-abdominal metastases and urinary tract obstruction requiring a stent**
3/15/13	**At NCI/Surgery Branch treated with cell transfer immunotherapy (75 billion of her own tumor infiltrating lymphocytes and IL-2)**

Experienced complete regression of all disease including relief of urinary obstruction and remains disease-free 4 years later.

(Stevanovic et al, Science 356:200, 2017)

Patient S.S. with metastatic cervical cancer treated with cell transfer immunotherapy



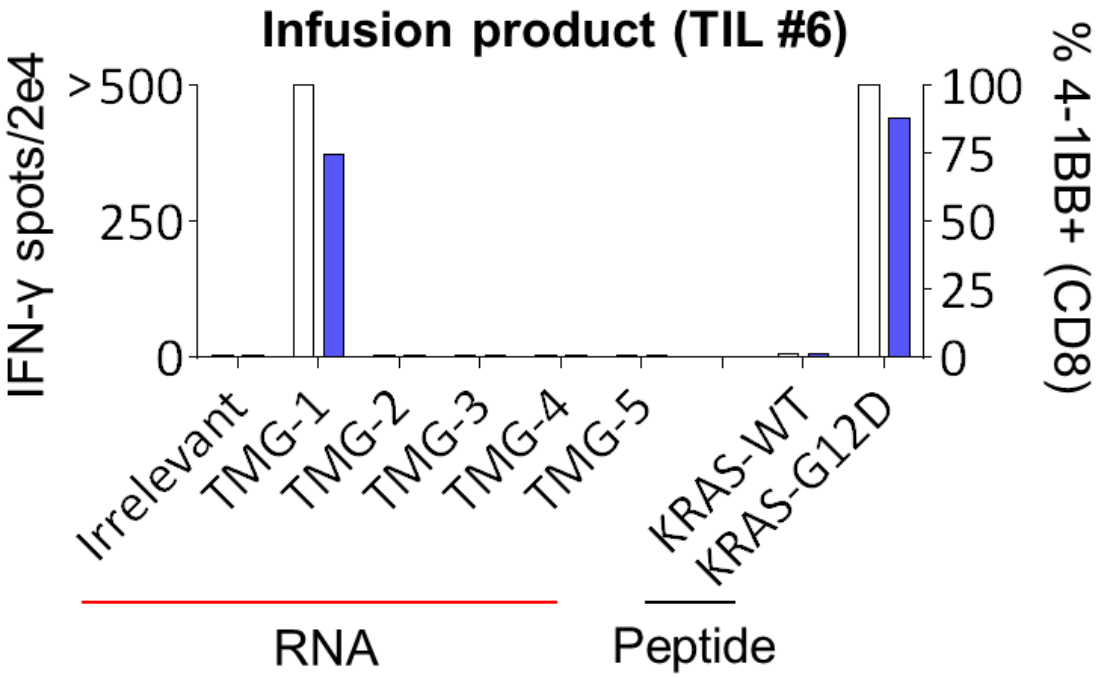
Patient C.R.

49 y.o. female with metastatic colon cancer

9/5/13	**Sigmoid colectomy, partial cystectomy** **Multiple lung metastases**
5/14/14	**Radiotherapy to bladder suture line**
9/13/14	**FOLFOX chemotherapy: PD**
3/29/15	**Two lung metastases resected for TIL**
7/1/15	**TMG approach to target unique cancer mutations** **(61 somatic mutations including KRAS-G12D)**

Patient CR (4095) with treatment refractory metastatic colorectal cancer

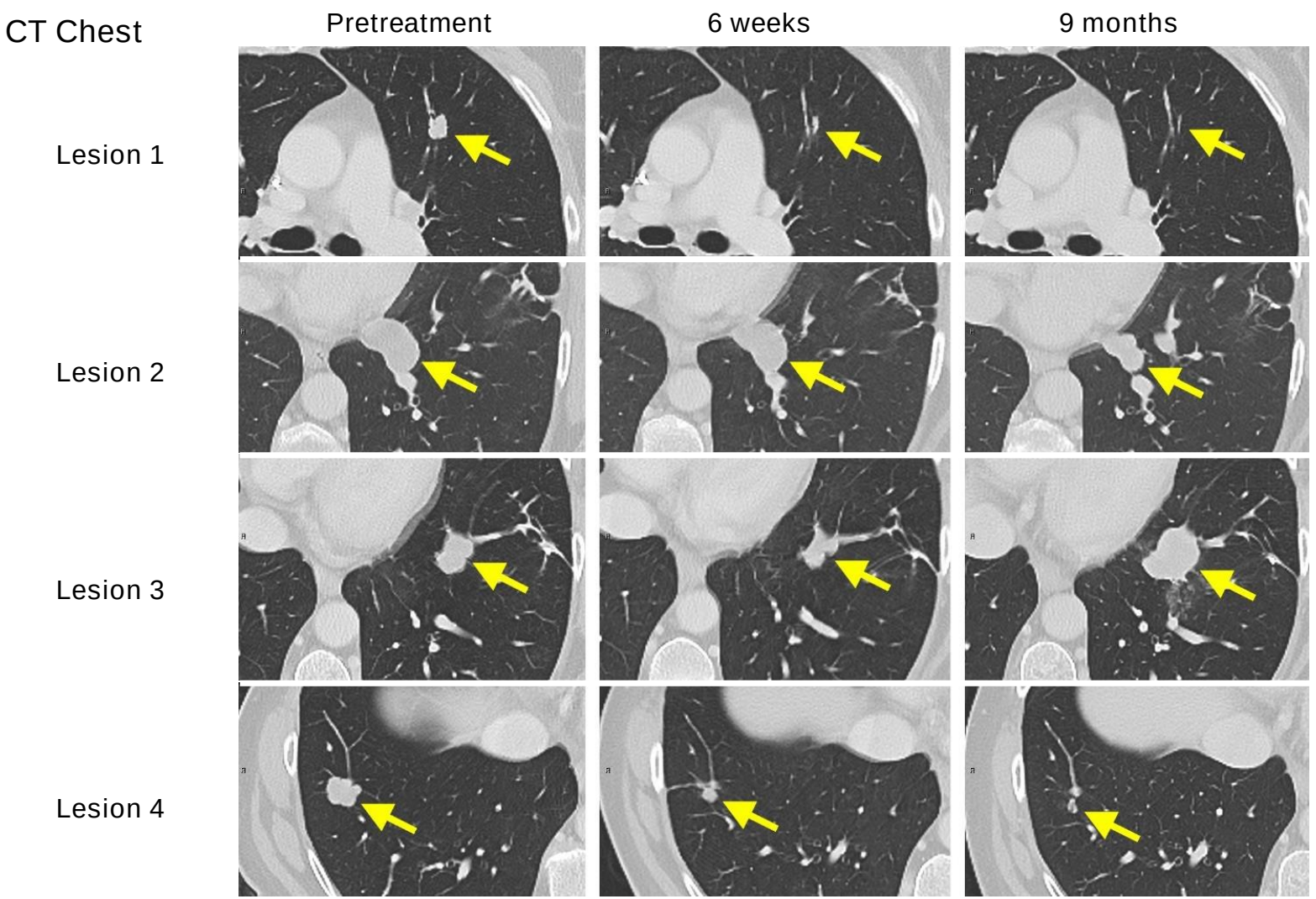
- **Whole-exome and transcriptome sequencing performed on lung lesions**
  - **61 putative mutations identified**
  - **5 TMGs constructed**



Rx:
- 1.48 x 10^{11} cells, ~75% $KRAS^{G12D}$-reactive
- 5 doses IL-2

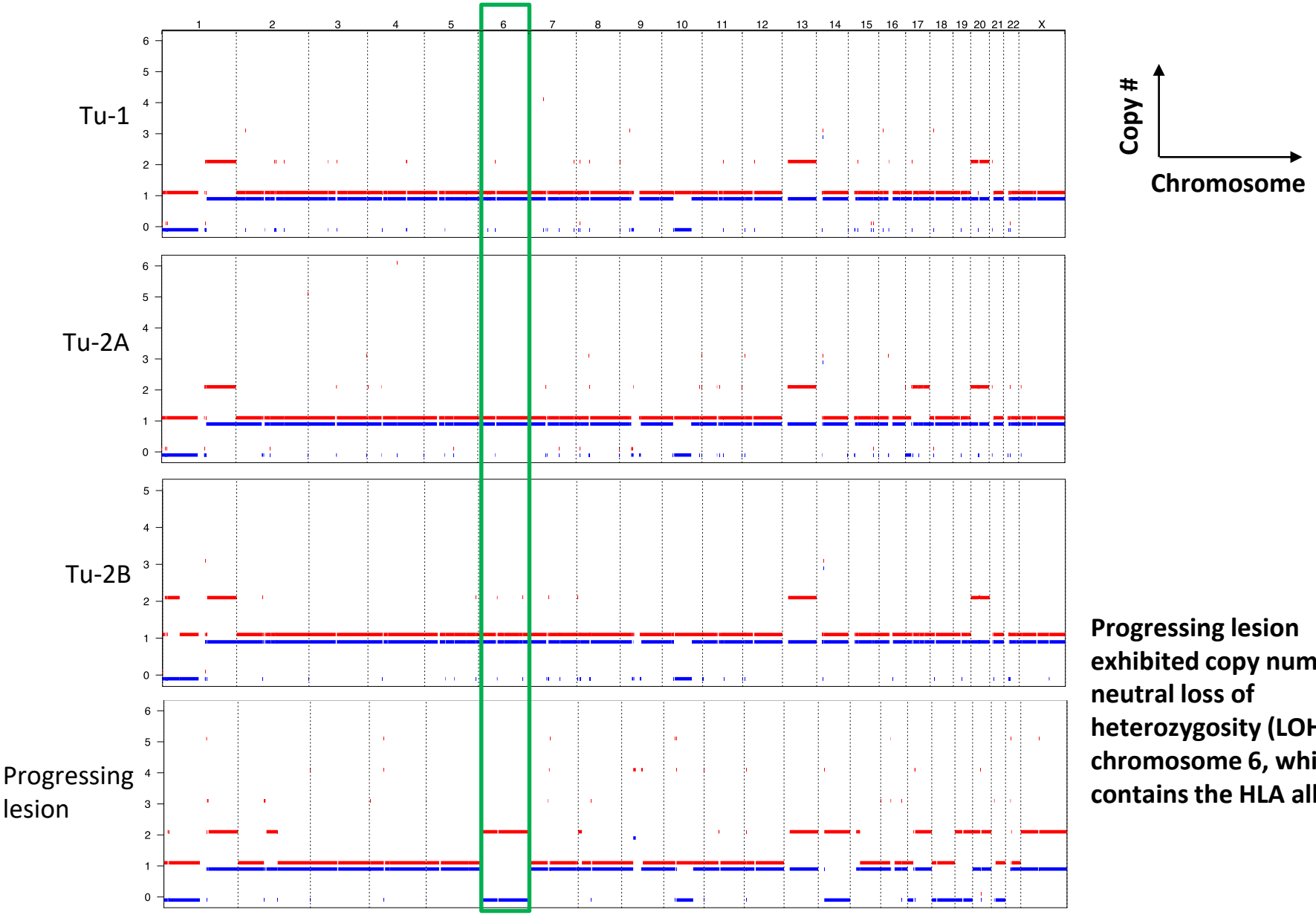
(Tran et al, New Engl J Med, 375, 2016)

Response after infusion with KRAS^{G12D}-reactive TIL



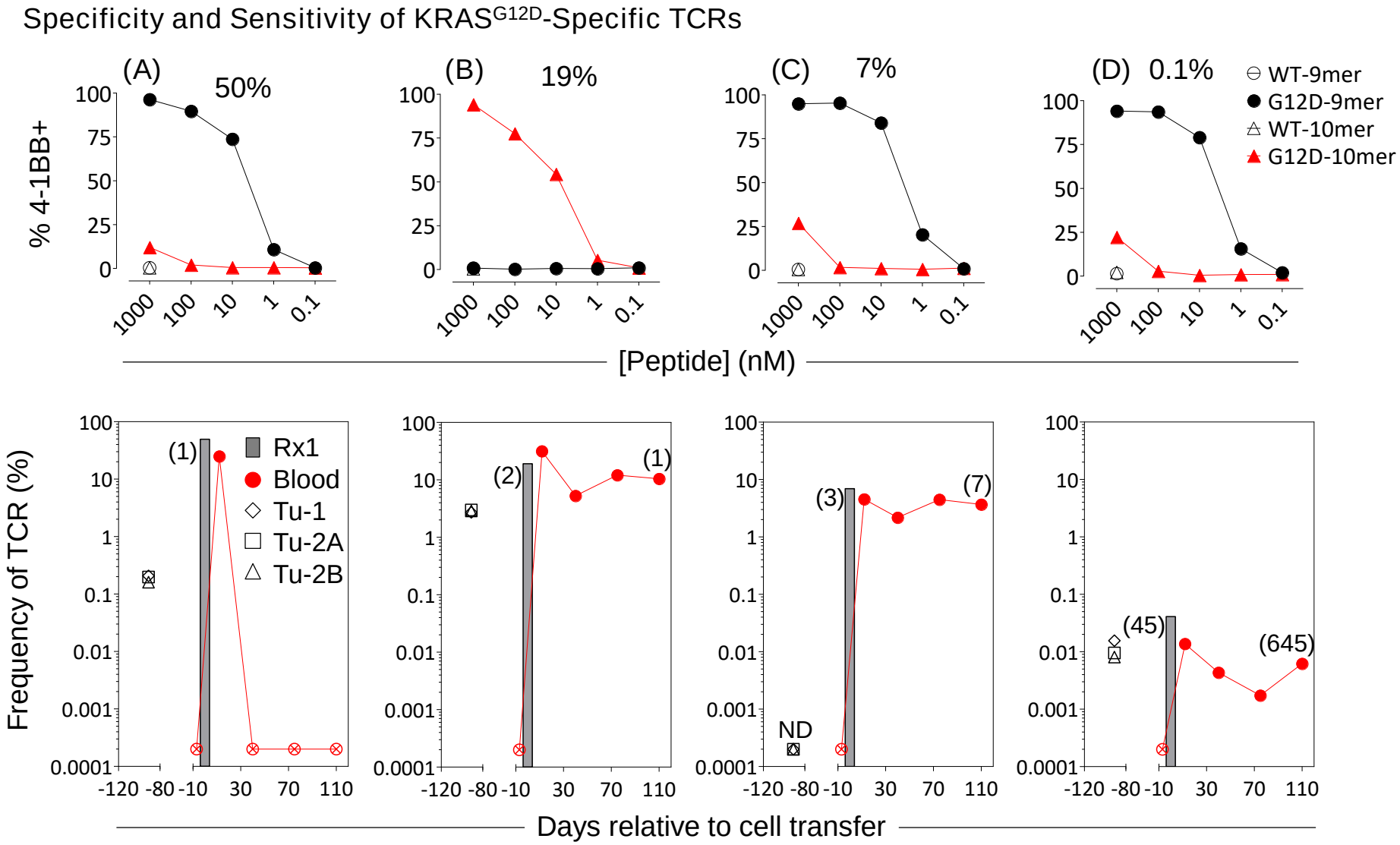
- **6/7 lesions regressed at 9 months post ACT**
- **1 lesion (#3) progressed at 9 months; excised; patient NED 35 months after treatment**

Why did one lesion progress? Chromosome copy number analysis



Progressing lesion exhibited copy number neutral loss of heterozygosity (LOH) at chromosome 6, which contains the HLA alleles

Four different KRAS^{G12D}-reactive TCRs in patient infusion TIL



Blueprint for Cancer Immunotherapy Directed Against the Common Epithelial Cancers

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Target the immunogenic somatic mutations unique to the autologous patient's cancer.

Raise a library of T-cell receptors against shared cancer mutations (e.g. Kras, p53)

Hypothesis (2)

Any intracellular protein can potentially be a "cancer antigen" if mutated and processed intracellularly to a peptide that can bind to the autologous MHC.

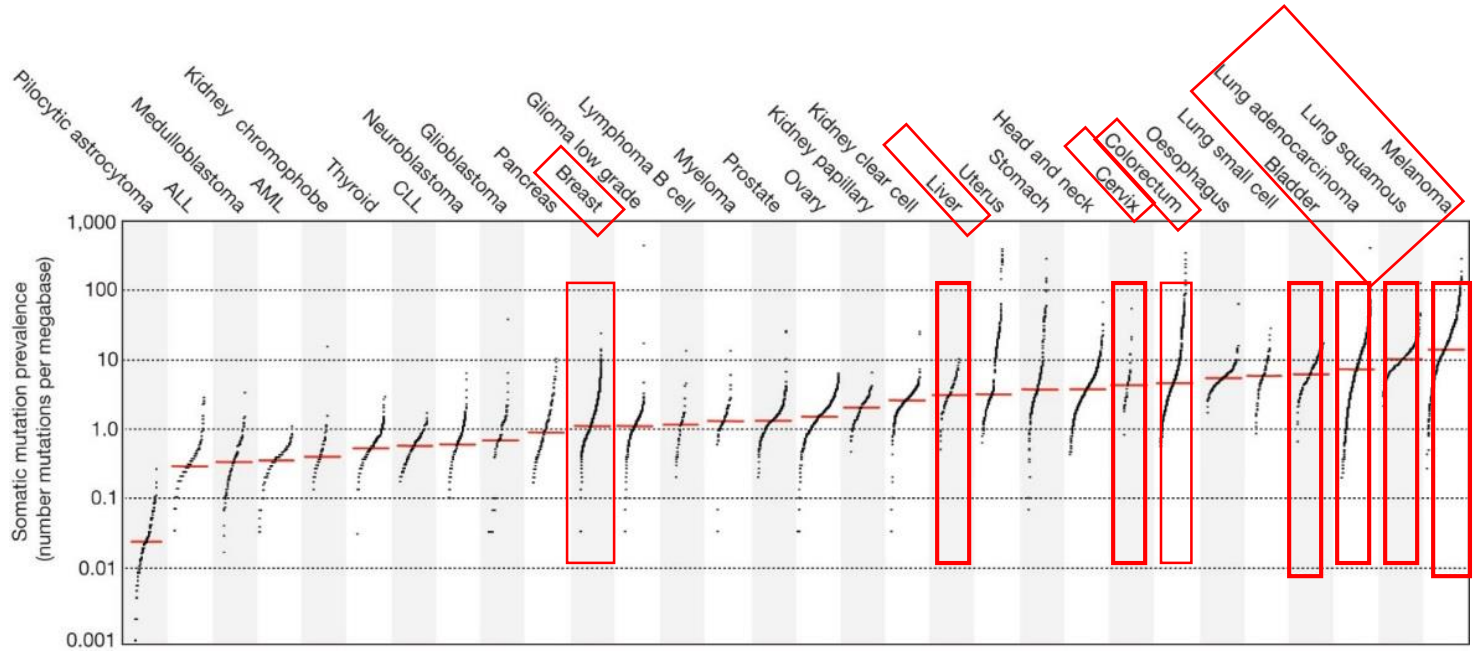
(About 1 in 70 mutated neoepitopes are neoantigens.)

Bad news: Treatment will be highly individualized and thus complex.

Good news: Virtually all cancer patients are potentially eligible.

Adoptive cell transfer for patients with cancer

(**Alexandrov et al, *Nature* 2013**)



Potential improvements in targeting of somatic mutations in epithelial cancers

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Improve methods to identify multiple mutation targets expressed by tumor (robotics)

Develop rapid methods for identifying mutation-reactive TCRs (robotics)

Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17 to use a GMP 293GP line to produce transient vectors with minimal testing)

Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM

Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells

Vaccinate with mutations recognized by transferred cells

Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection

Cancer Antigens

1. **Unique somatic mutations in an intracellular protein**

2. **Mutations in driver oncogenes or tumor suppressor genes that can be shared among patients.**
   e.g.: Kras
   p53
   PIK3CA

3. **Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands**
   e.g.: CD19 (CAR: αCD19 antibody)
   CD70 (CAR: CD27 ligand)

KRAS Mutations and Human Cancers

KRAS protein is a GTPase essential for normal tissue signaling

Activating mutations are essential steps in the development of many cancers.

Tumor	Frequencies of KRAS mutation	% of All KRAS Mutations						
		G12A	G12D	G12R	G12C	G12S	G12V	G13D
Pancreatic CA	**70%**	2	**51**	12	3	2	**30**	1
Colorectal	36%	7	**34**	1	9	5	**24**	19
Lung Adeno CA	20%	7	**17**	2	42	5	**20**	2
Endometrial	18%	11	**36**	0	9	2	**24**	15
Ovarian (EOC)	14%	4	**41**	2	5	0	**37**	5
Prostate	7%	2	**22**	1	10	3	**35**	23

Modified from Cosmic database by James Yang

Anti-Kras T-cell Receptors Isolated from Patients with Metastatic Cancer

KRAS Mutation	Patient #	Cancer Diagnosis	CD4/8	HLA-restriction	Method
G12D	4095	Colon	CD8	C*08:02 (8%)*	TMG
	4238	Colon	CD4	DR3*02 (16%)	IVS
			CD4	DRB1*08:02 (5%)	IVS
G12V	4148	Endometrial	CD4	DRB1*07:01 (25%)	TMG
			CD8	A*11:01 (14%)	IVS
G12C	4173	Ovarian	CD4	DRB1*11.01 (10%)	IVS
G12R	4268	Colon	CD4	CLASS II	TMG

*allele frequency

(Gal Cafri, Rami Yoseph, submitted)

P53 Mutations in Human Cancer

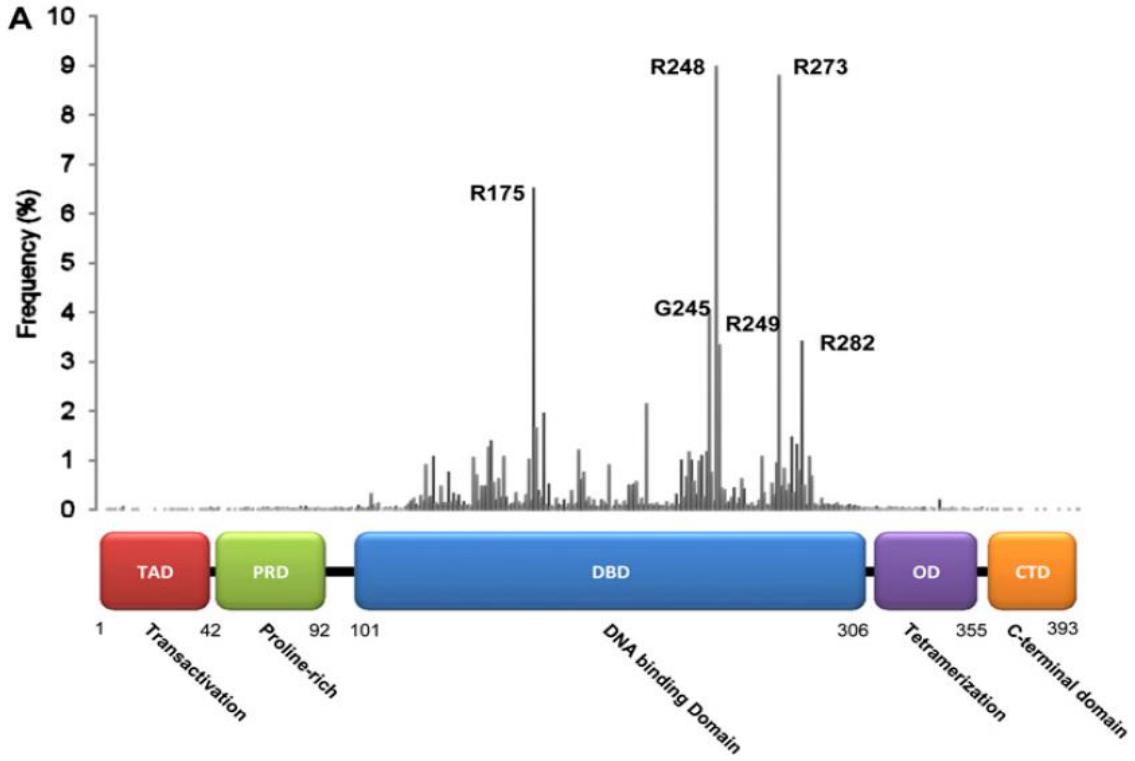
Tumor suppressor gene

50% of human cancers contain a p53 mutation

Most frequently mutated gene in human cancers

Mutations occur throughout the gene but there are up to 10 major “hot spots”

A novel method to screen for T cell responses to p53 “hotspot” mutations



Synthesize one TMG (ten 25mers) encoding the top ten p53 “hotspot” mutations.

Synthesize the top 10 25mer peptides.

Coculture patient TIL with TMGs and peptides.

50% of p53 “hotspot” Mutations are Immunogenic

Mutation	# Pt	# tested	# immunogenic	% reactive of screened
R175H	5	4	2	50.0%
Y220C	3	3	2	66.7%
G245D	0	0	0	-
G245S	2	1	1	100.0%
R248Q	6	6	3	50.0%
R248W	5	4	3	75.0%
R249S	0	0	0	-
R273C	2	1	0	0.0%
R273H	5	4	0	0.0%
R282W	4	3	2	66.7%
Total	**32**	**26**	**13**	**50.0%**

T-cell receptors against common p53 “hotspot” mutations can potentially be used to treat multiple patients whose cancers express these mutations.

(P.Malekzadeh, submitted)

Generation of a T-Cell Receptor Library Targeting p53 Mutations

Patient	Age / Sex	Cancer Type	TP53 mutation	T cell type	HLA restriction
1	52M	Colon	R175H	CD8	A*02:01
2	36M	Colon	R175H	CD8	A*02:01
3	55M	Colon	R175H	CD4	Class-II
4	46M	Colon	R175H	CD4	DRB1*13:01
5	44F	Colon	Y220C	CD4 CD8	DRB1*04:01 A*02:01
6	39F	Ovary	Y220C	CD4	DRB3*02:02
7	58F	Ovary	G245S	CD4	DRB3*02:02
8	62M	Colon	R248Q	CD8	Class-I
9	69F	Colon	R248Q	CD4 CD8	Class-I and -II
10	41F	Colon	R248W	CD8	A*68:01
11	49M	Rectal	R248W	CD4	DPB1*02:01
12	66F	Pancreas	R282W	CD4	Class-II

Cancer Antigens

1. **Unique somatic mutations in an intracellular protein**

2. **Mutations in oncogenes or tumor suppressor genes that can be shared among patients.**
   e.g.: Kras
   p53

3. **Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands**
   e.g.: CD19 (CAR: αCD19 antibody)
   CD70 (CAR: CD27 ligand)

Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL in patients with common epithelial cancers.

Identification and targeting of mutations unique to each cancer or shared mutations such as KRAS or p53 has the potential to extend cell therapy to patients with common epithelial cancers.

Treatment of Patients with Diffuse Large B-cell Lymphoma

	Objective Response (%)	Complete Reponse	
		Total	Ongoing
Surgery Branch	73%	47%	42%
Kite Pharma	82%	54%	40%

(FDA approval October, 2017)

Blueprint for Cancer Immunotherapy Directed Against the Common Epithelial Cancers

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Target the immunogenic somatic mutations unique to the autologous patient's cancer.

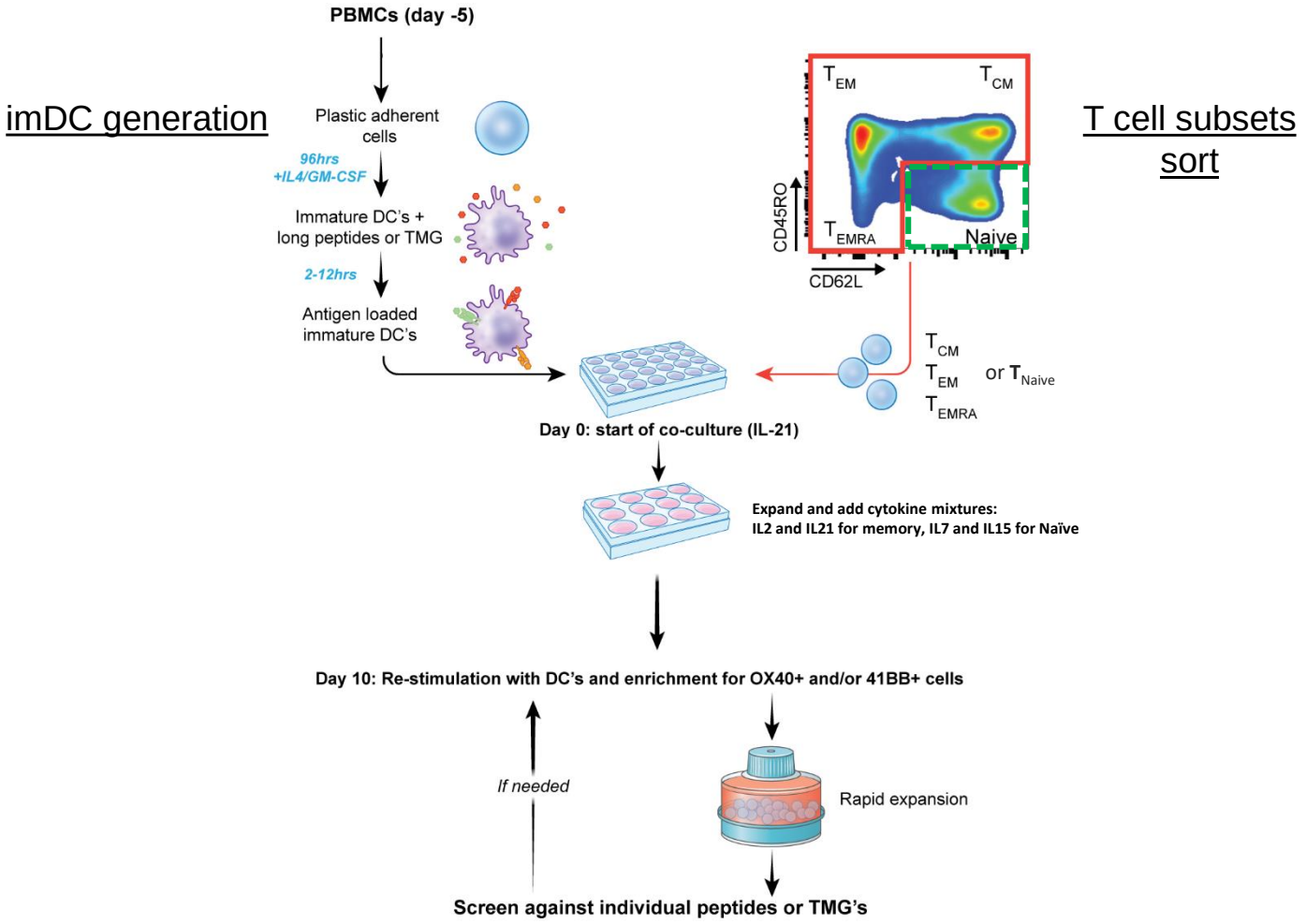
Raise a library of T-cell receptors against shared cancer mutations (e.g. Kras, p53)

Mutation specific TCRs in Peripheral Blood Lymphocytes are Present in the Memory Subpopulations

Cancer	Patient	T_N	T_{CM}	T_{EM}	T_{EMRA}
GI	4213	None	None	SMAD5, (0.000812)	None
Ovarian	4097	None	None	None	None
	4046	None	USPX, (0.001392)	USPX, (0.000776)	None
NSCLC	4014	None	None	None	None
	4134	None	None	GRB7, (0.023095)	None

Isolation of *KRAS* mutation-reactive TCRs from cancer patients' blood samples using IVS

- **Approach overview**

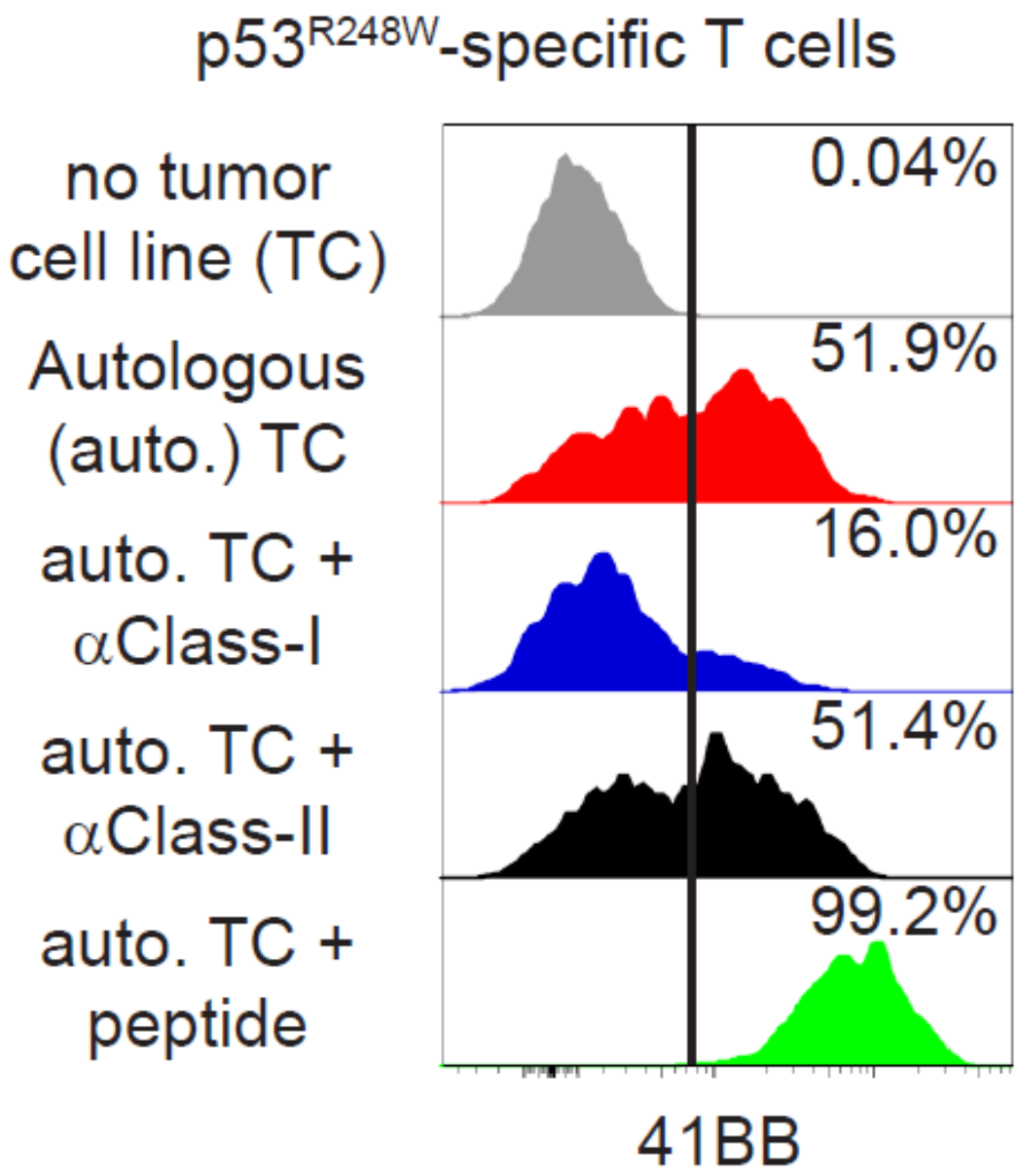


Major Challenge Confronting Cancer Immunotherapy

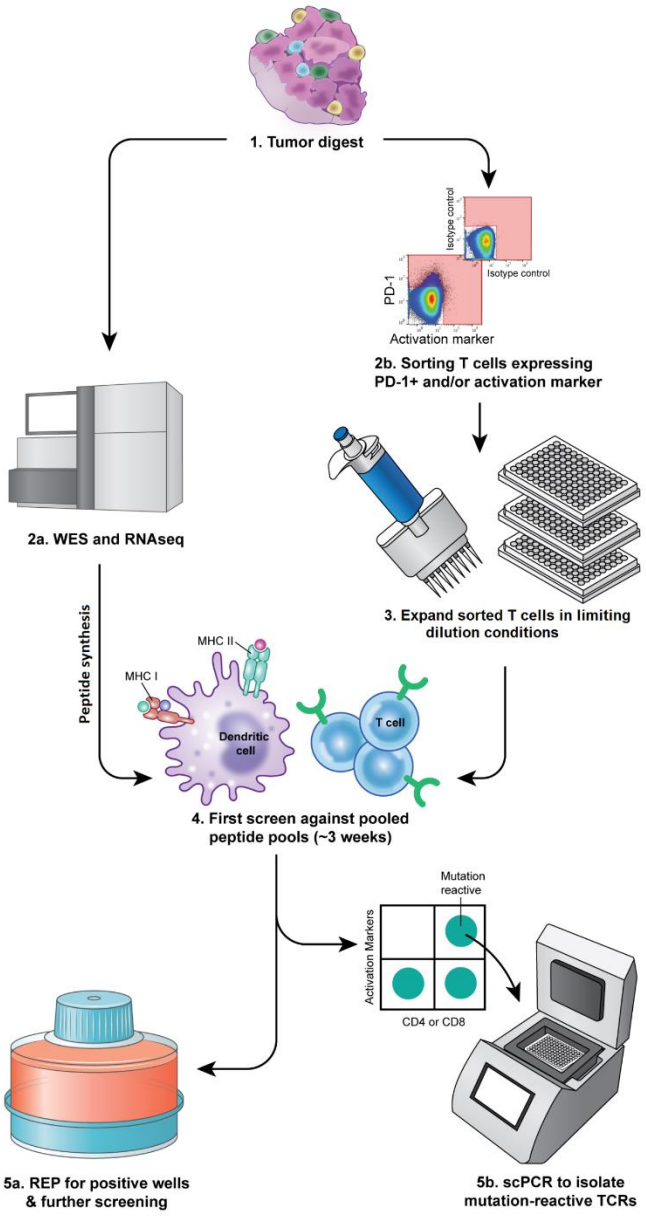
The development of effective immunotherapies for the 80% of patients with metastatic epithelial solid cancers that cannot be cured by any available treatment.

Are there antigens on solid human cancers that can be targeted by cell-based immunotherapy?

Recognition of colon cancer by T-cells expressing a natural p53 mutation



Limiting dilution culturing of sorted TILs for enhanced detection of neoantigen-reactive cells



Summary of neoantigen-reactive T cells identified by limiting dilution

Patient ID	Age*/Sex	Tumor histology	No. of mutation assessed	Reactivities found in TIL fragments screen†	T cell type	Reactivities found in LD cultures†	T cell type	No. of reactive TCRs found using LD ‡
4078	48/M	Gastroesophageal junction adenocarcinoma	104	None		$GBAS^{E207K}$	CD8	**1**
						$PLXNB3^{W609G}$	CD4	**1**
						$DLAT^{G294L}$	CD4	**1**
						$TMPRSS4^{H233Y}$	CD4	**1**
						$PSMD2^{G644A}$	CD4	**1**
4097	59/F	Ovarian	317	$HIST1H1B^{A71D}$	CD4	$HIST1H1B^{A71D}$	CD4	7
				$INPP5K^{L176V}$	CD4	$HYAL4^{R94S}$	CD4	1
						$HSPG2^{H3568L}$	CD4	1
4148	68/F	Endometrial	108	None		$KRAS^{G12V}$	CD4	**1**
4217	49/M	Colon	176	$MAP3K2^{S153F}$	CD4	$MAP3K2^{S153F}$	CD4	**1**
				$UEVLD\text{-}1/2^{F191V}$	CD4	$UEVLD\text{-}1/2^{F191V}$	CD4	**3**
				$RAD51B^{L202R}$	CD4	$RAD51B^{L202R}$	CD4	3 (**2**+1)
				$MUC4^{R4435S}$	CD8	$TBCK^{R747S}$	CD4	1
4127	58/F	Ovarian	180	$TP53^{G245S}$	CD4	$TP53^{G245S}$	CD4	**3**
						$HIST1H2BM^{E77V}$	CD4	**1**
						$GORASP^{L248FS§}$	CD4	**3**
						$TUBA1B^{S287T}$	CD4	**1**
4166	40/M	Pancreatic	156	$NPLOC4^{I312V}$	CD8	$ZNF727^{H163Q}$	CD4	1
						TNC^{E743D}	CD4	4
			Total:	8	CD8: 2	19	CD8: 1	
					CD4: 6		CD4: 18	

* At the time of admission

† Neoantigen specificity was determined by testing against WT peptides

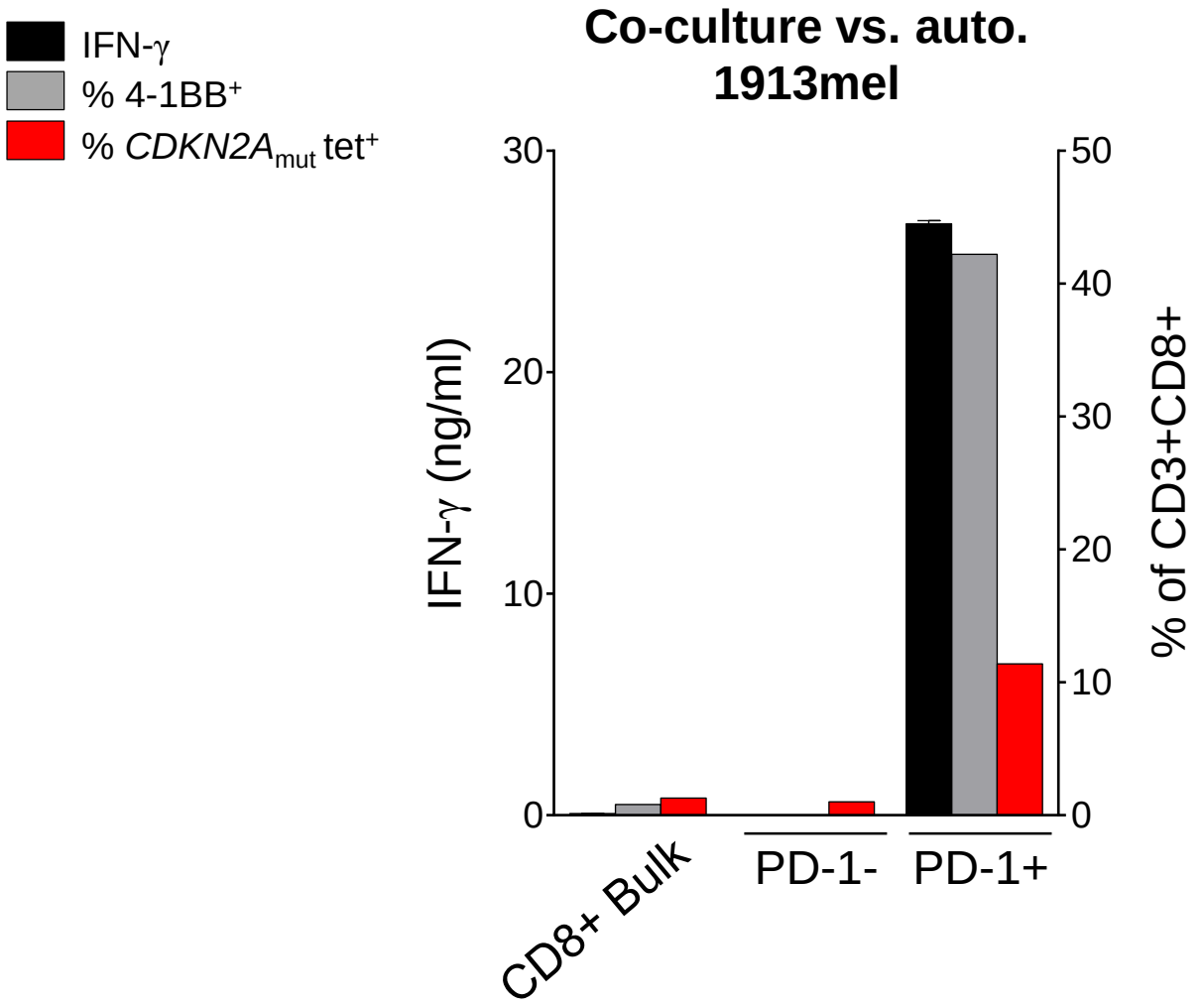
‡ TCR that were constructed and tested are bolded

§ FS Frame shift mutation

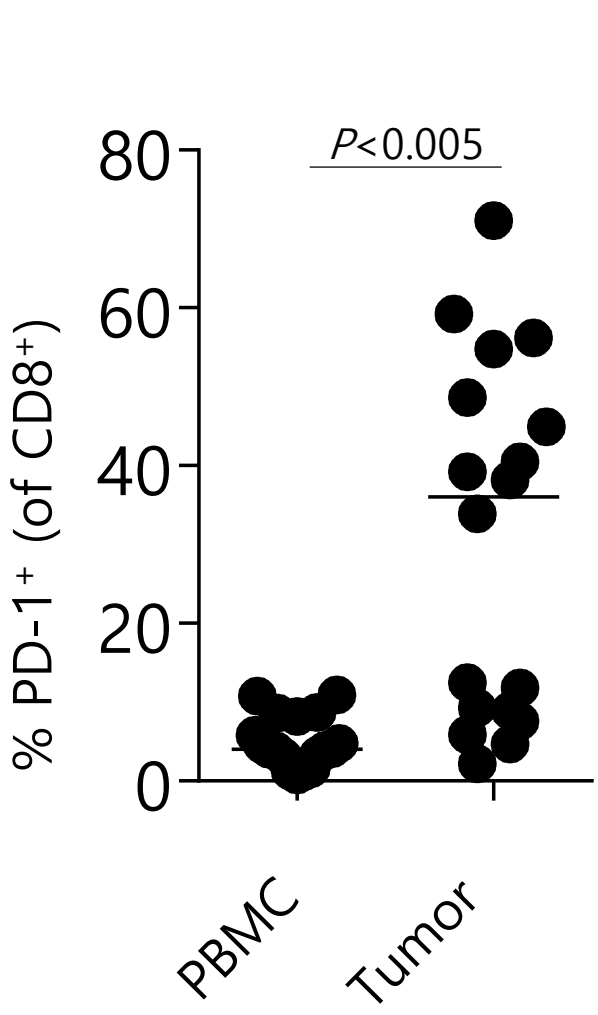
- We were able to rediscover most of the neo-antigen reactivities found in fragment screenings
- We identified substantial additional number of new reactivities that were missed when TIL fragments were screened
- Limiting dilution cultures are clonal or highly oligoclonal, that makes the isolation faster and more reliable using scPCR techniques

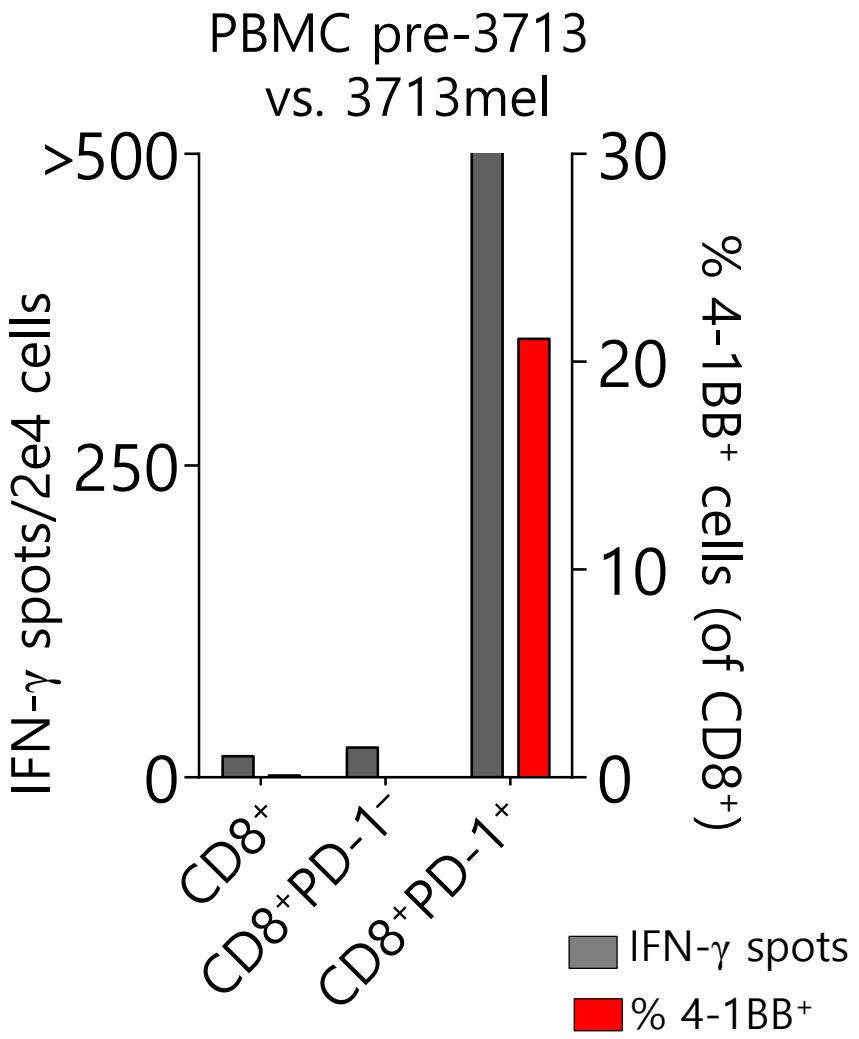
Tumor biopsies represent the main source for the isolation of tumor-reactive and mutation-specific lymphocytes

PD-1 expression in the fresh tumor can guide the identification of tumor-reactive cells (*Gros et al. JCI 2014*)



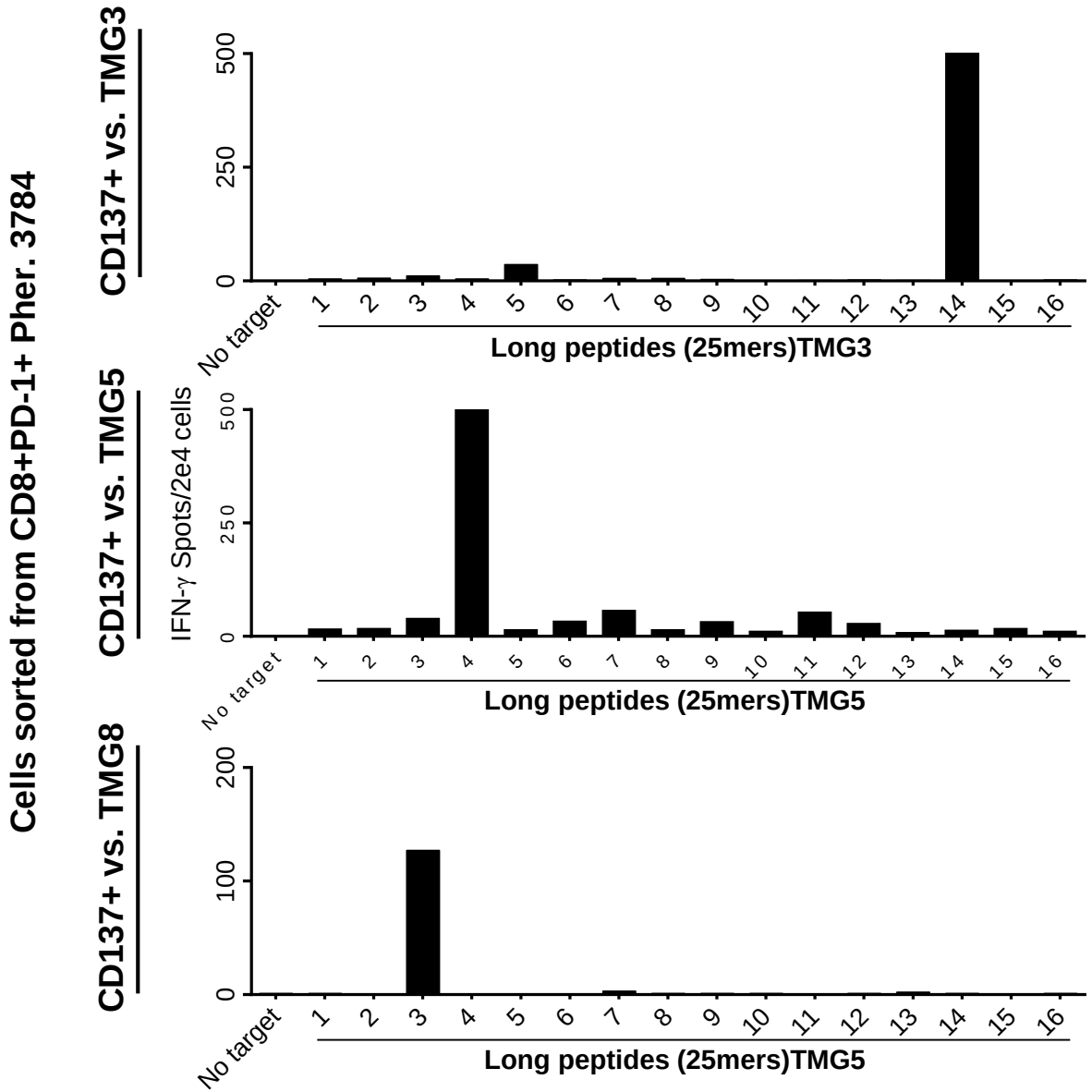
Enrichment of tumor and mutation-reactive cells by sorting for CD8+PD1+ cells





(Gros et al, J Clin Invest 124:2246-2259, 2014; Nat Med 22:433-438, 2016)

CD8+PD-1+ cells isolated from peripheral blood of Pt#3784 recognize three unique mutated antigens



FLNAmut: Filamin A
(cytoskeleton remodeling)
VVISQSEIGDASCVRVSGQGLHEGH

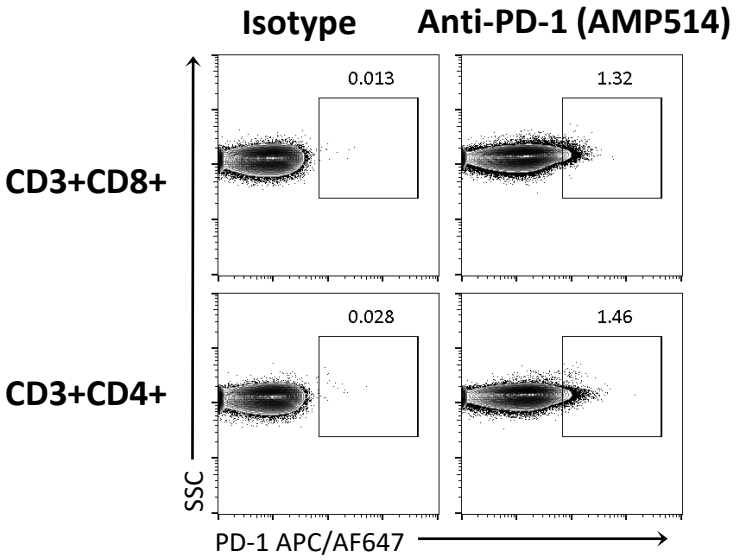
KIF16Bmut: Kinesin-like protein
(intracellular trafficking)
REKQQREALERAPARLERRHSALQR

SONmut: DNA binding protein
(promotes pre-mRNA splicing)
RKTVRARSRTPSCRSRSHTPSRRRR

Can mutation-specific cells be identified in peripheral blood of patients with gastrointestinal cancers?

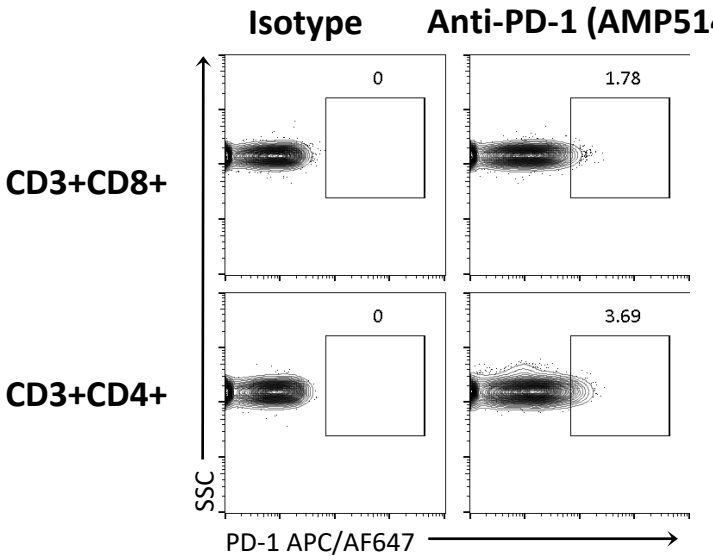
Metastatic gastric cancer: 175 mutations

Populations sorted from Pheresis:



1)CD8+
2) CD8+PD-1-
3) CD8+PD-1+
4) CD8+PD-1hi
5) CD4+
6) CD4+PD-1-
7) CD4+PD-1+
8) CD4+PD-1hi

Populations sorted from FrTu:



1)CD8+
2) CD8+PD-1-
3) CD8+PD-1+
4) CD4+
5) CD4+PD-1-
6) CD4+PD-1+

Evaluated recognition of 175 total putative mutations

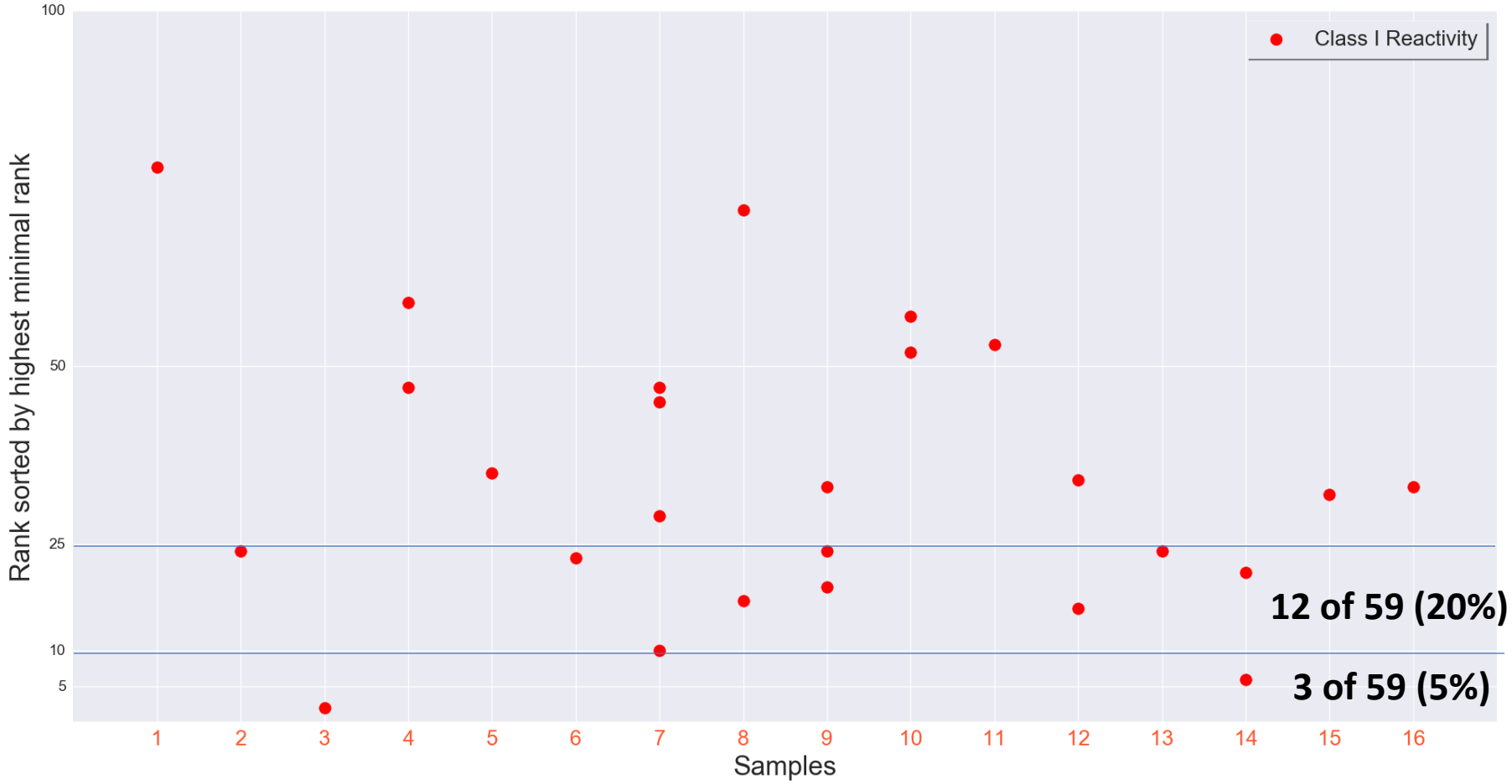
Summary of neoantigen reactivities detected in the blood and tumor subsets derived from Pt.4078

Patient	Tumor	mutations evaluated	Pheresis		FrTu		TIL fragments	
			CD8+PD-1+/hi	CD4+PD-1+/hi	CD8+PD-1+	CD4+PD-1+	CD8+	CD4+
4078	GE	175	2 CD8+ (DLAT, GBAS)	3 CD4+ (TMPRSS4, TCF25, PSMD2)	2 CD8+ (DLAT, GBAS)	High background	0	0

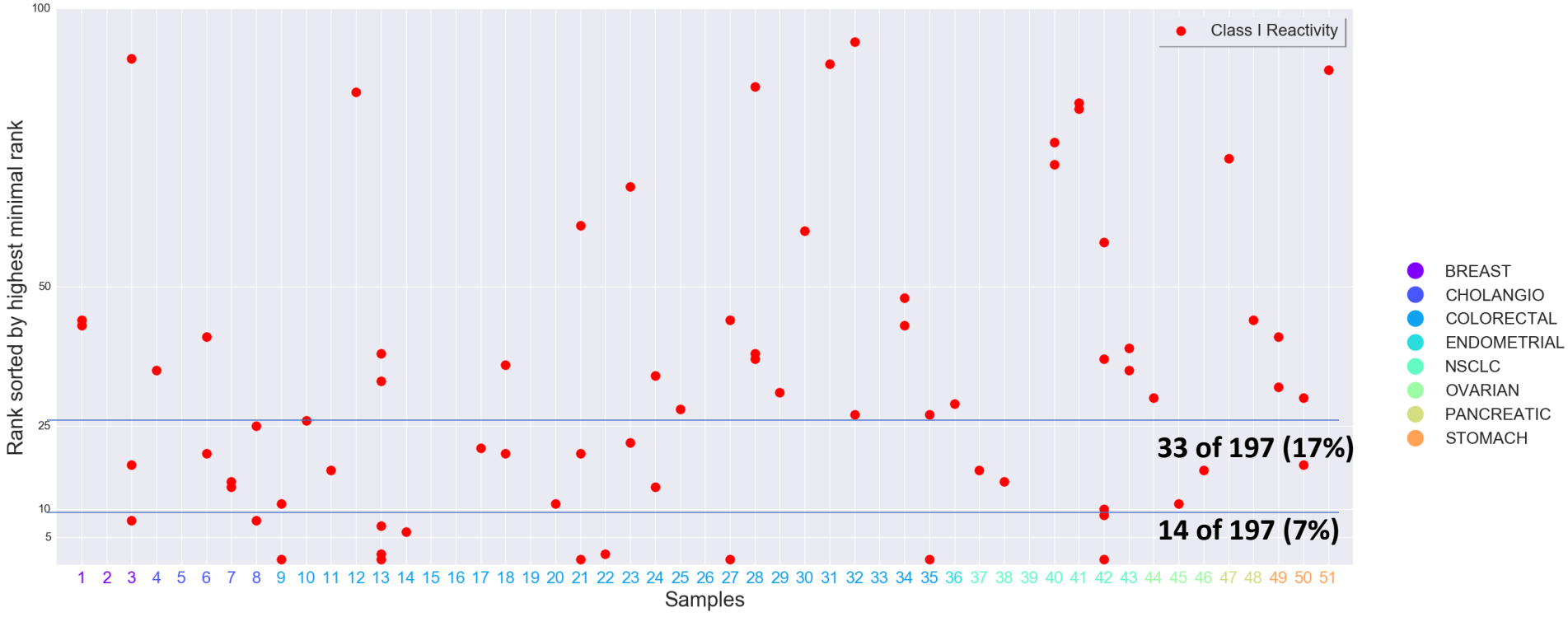
Circulating CD8+PD-1+ cells AND intratumoral CD8+PD-1+ cells recognized DLAT and GBAS but only circulating lymphocytes recognized TM PRSS4, TCF25, PSMD2.

Thus improved techniques have the potential to identify increased T cells reactive with mutated antigens.

NetMHCpan3.0 ranked immunogenic 25mers (Melanoma)

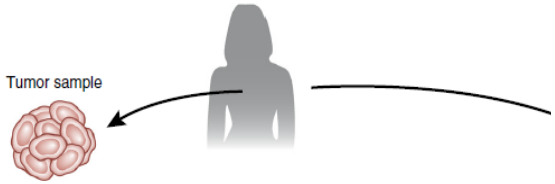


NetMHCpan3.0 ranked immunogenic 25mers (Epithelial cancers)



Top 10% of predicted epitopes included 14 of 187 (7%) identified immunogens.
Top 25% of predicted epitopes included 33 of 187 (18%) identified immunogens.

A novel method to screen for T cell responses to p53 "hotspot" mutations



T cells from tumor or blood

Tumor infiltrating lymphocytes

Isolate DNA and RNA

Whole-exome and transcriptome sequencing to identify TP53 "hotspot" mutations

p53 "hotspot" mutation

1. TMG-IRR
2. p53wtTMG
3. p53mutTMG

1. DMSO
2. p53 LP wt
3. p53 LP mut

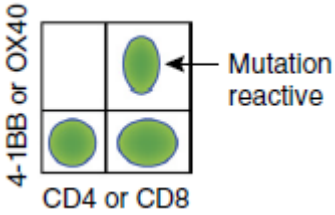
Co-Cultured with autologous antigen presenting cells

Readout

1. IFNγ
2. 41BB/OX40

Y= reactive, interferon-gamma ELISPOT and/or 41BB expression higher than controls.

N= no reactivity, interferon-gamma ELISPOT and/or 41BB expression similar to or lower than controls.



p53 "hotspot" mutation TMG

YKQSQHMTEVVR**H**CPHHERCSDSDGSGNLLGRNSFEV**H**VCACPGRDRRTEYMCNSSCMGGMN**L**RPILTIITLEDS

R175H R273H R248L

FEVRVCACPGRD**W**RTEEENLRKKGESGNLLGRNSFEV**C**VCACPGRDRRTEHYNYMCNSSCMG**S**MNRRPILTIITL

R282W R273C G245S

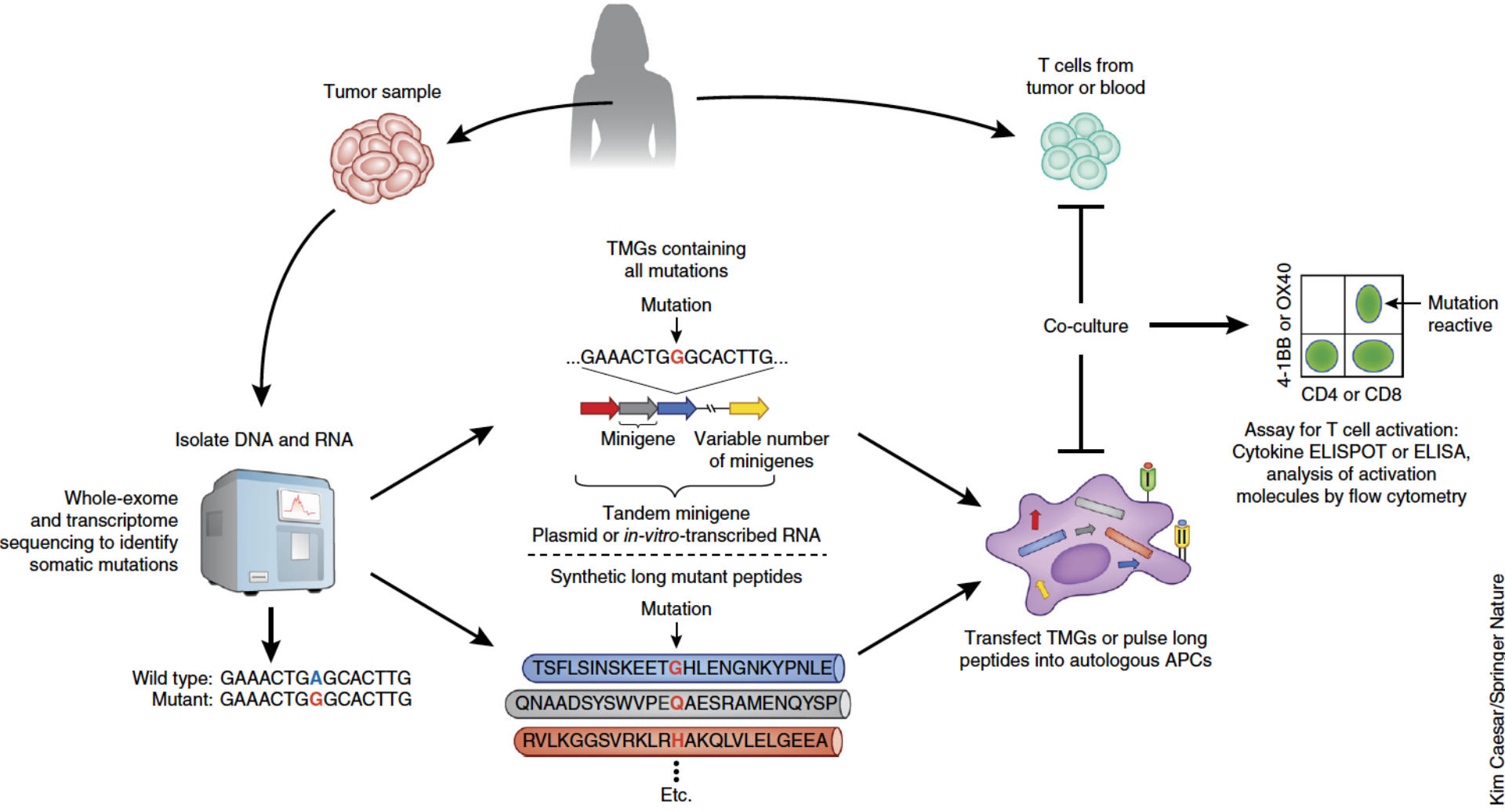
YMCNSSCMGGMN**Q**RPILTIITLEDSHYNYMCNSSCMG**D**MNRRPILTIITLSGNLLGRNSFEV**L**VCACPGRDRRTE

R248Q G245D R273L

YMCNSSCMGGMN**W**RPILTIITLEDSDRNTFRHSVVVP**C**EPPEVGSDCTTIMCNSSCMGGMNR**S**PILTIITLEDSS

R248W Y220C R249S

“Blueprint” for Identification of Neoantigen-reactive T cells from Patients with Cancer



Kim Caesar/Springer Nature

Cancer Antigens

1. **Unique somatic mutations in an intracellular protein**

2. **Mutations in oncogenes or tumor suppressor genes that can be shared among patients.**
   e.g.: Kras
   p53

3. **Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands**
   e.g.: CD19 (CAR: αCD19 antibody)
   CD70 (CAR: CD27 ligand)

Progress on generating a TCR library targeting p53 mutations

p53 mutation	HLA	TCRs
R175H	A*02:01 DRB1*13:01	3 1
Y220C	A*02:01 DRB1*04:01 DRB3*02:02	pending 1 1
G245S	DRB3*02:02	4
R248Q	pending	pending
R248W	A*68:01 DPB1*02:01	3 pending
R273H	pending	pending
R282W	pending DRB4*01:01	1 Pending

Summary - Human TCRs targeting *KRAS*^{G12} mutations identified in the Surgery Branch

Mutation	CD4/CD8	HLA restriction (*)	Minimal epitopes
KRAS p.G12V	CD4	HLA-DRB1*07:01 (25%)	
	CD8	HLA-A*11:01 (14%)	VVGAVGVGK VVVGAGGVGK
KRAS p.G12D	CD4	HLA-DR3*02 (16% **allele freq.**)	
	CD8 - 4 TCRs (E. Tran)	HLA-C*08:02 (11.7%)	GADGVGKSA (3 TCRs) GADGVGKSAL (1 TCR)
	CD4	HLA-DRB1*08:01 (~4.5%)	
KRAS p.G12C	CD4 (G. Cafri)	HLA-DRB1*11*01 (10%)	
KRAS p.G12R	CD4 (M. Parkurst, A Sachs)	TDB	

* % US Caucasians individuals that have the allele in - Allelefrequencies.net

Identify additional TCRs targeting *KRAS* mutations from other potential candidates

Dr. James Yang laboratory has isolated G12V and G12D HLA-A11 restricted KRAS TCRs.

Limitations of Current Immunotherapies

Treatment using checkpoint modulators or adoptive cell therapy with unselected TIL can be effective in treating tumors with high mutation rates (melanoma, smoking-induced lung cancer, some bladder cancers).

The common epithelial cancers such as those arising in the colon, liver, stomach, pancreas, prostate, ovary, etc rarely respond to current immunotherapies.

Are there antigens on the common epithelial human cancers that can be targeted by cell-based immunotherapy?

Major Challenge Confronting Cancer Immunotherapy

The development of effective immunotherapies for the 80% of patients with metastatic epithelial solid cancers that cannot be cured by any available treatment.

Estimated Cancer Deaths in 2017 in the U.S.

	New Cases	Deaths
Total	1,688,780	600,920
Solid cancers	1,515,870	542,620
Hematologic	172,910	58,300

(American Cancer Society, 2017)

Systemic Treatments that Can Cure Metastatic Solid Cancers

1958	**methotrexate for choriocarcinoma (Min Chiu Li, NCI)**
1977	**cis-platin combination chemotherapy for germ-cell testicular cancers (Lawrence Einhorn, U. Indiana)**
1985	**interleukin-2 for melanoma and renal cancer**

Potential improvements in targeting of somatic mutations in epithelial cancers

---

Enrich cancer mutation-reactive cells

- **PD1+ cells in tumor and circulating lymphocytes**
- **41BB+ after antigen stimulation**

Improve methods to identify multiple mutation targets expressed by tumor (robotics)

Develop rapid methods for identifying mutation-reactive TCRs (robotics)

Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17)

Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM

Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells

Vaccinate with mutations recognized by transferred cells

Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection

Potential improvements in targeting of somatic mutations in epithelial cancers

---

Improve methods to identify multiple mutation targets expressed by tumor (robotics)

Develop rapid methods for identifying mutation-reactive TCRs (robotics)

Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 11-1-17 to use a GMP 293GP line to produce transient vectors with minimal testing)

Add anti-PD-1 (reexpressed by infused cells in vivo) or other CPM

Knockout CISH or PD-1 (or other inhibitory molecules) on transferred cells

Vaccinate with mutations recognized by transferred cells

Raise libraries of TCRs reactive with shared KRAS or P53 mutations (?other driver genes)

Obtain mutation-reactive TCRs from circulating lymphocytes and mutations from paraffin sections or liquid DNA) – eliminate need for tumor resection

NCI/Kite-Gilead CRADA

NCI Principal Investigator – Steven Rosenberg, M.D., Ph.D.
Kite-Gilead Principal Investigator – Alessandro Riva, M.D.

Cooperative Research and Development Agreement for the Development of T cell Therapy Using Neoantigen Reactive T Cell Receptors Retrovirally Transduced into Autologous Peripheral Blood Lymphocytes

<u>Experimental Plan</u>

1. **To generate clinical proof-of-concept data for personalized neoAg TCR-engineered T-cell therapy using retroviral insertion.**

2. **To develop a streamlined, high-throughput process for neoAgTCR isolation from peripheral blood lymphocytes (PBL).**

3. **To evaluate the safety and efficacy of KRAS mutation targeting TCR therapy**

Conclusions

Peripheral blood CD8+PD-1+ lymphocytes represent a small subset of all the circulating CD8+ cells

The CD8+PD-1+ lymphocyte subset in peripheral blood can contain multiple mutation-specific cells capable of recognizing tumor

Each immunogenic mutation detected was unique to the autologous tumor

Circulating CD8+PD-1+ lymphocytes from melanoma patients also contain T cells recognizing shared tumor antigens

Mutated antigens recognized by TIL from patients with melanoma

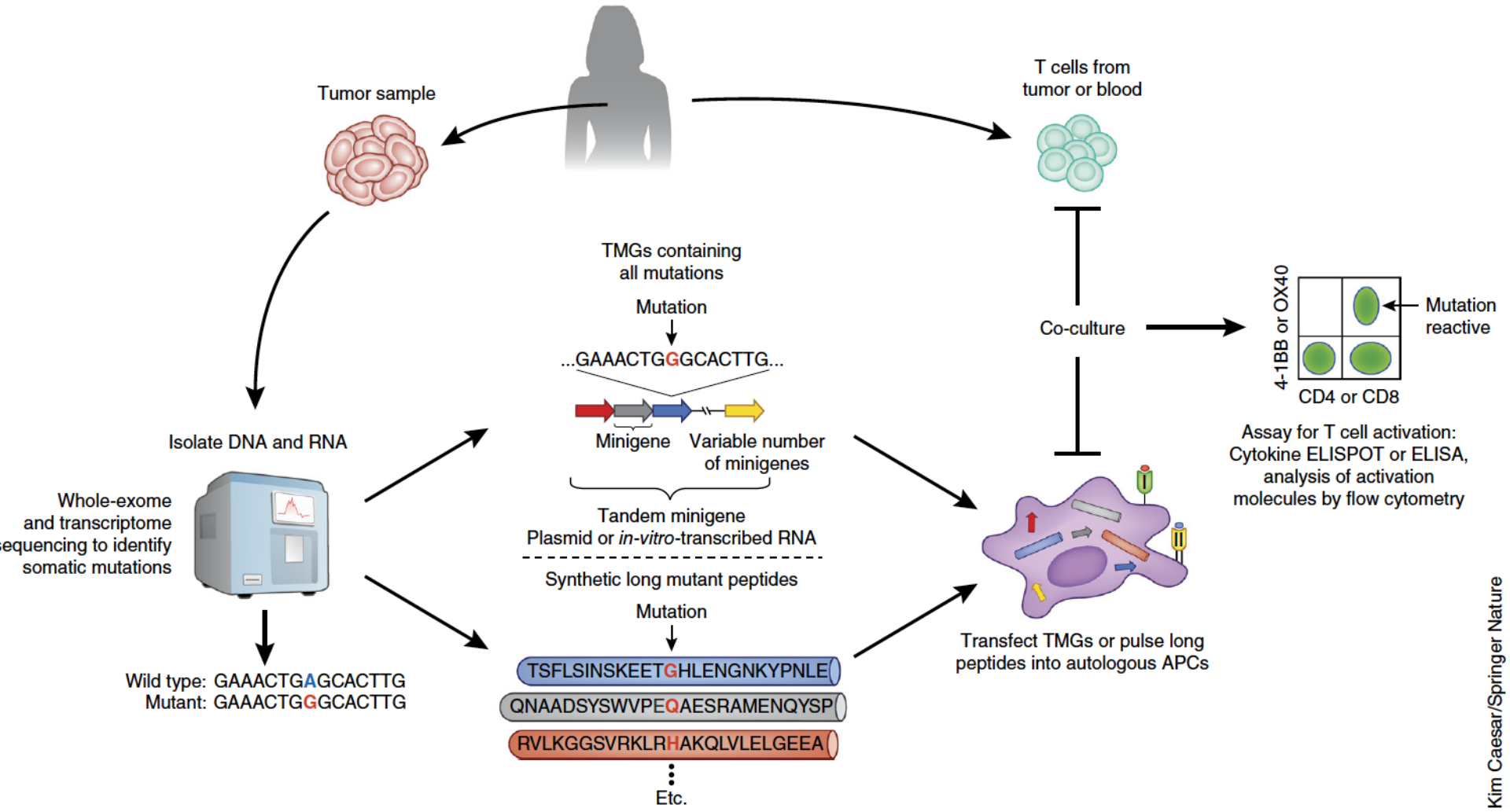
Tumor	Antigen	AA change	HLA RE
164	ARCT1	altORF	DRB1*0101
1290	CTNNB1	p.S37F	A*2402
	MKI67	p.S1242L	DRB1*1502
1359	CDC27	P.S711L	DRB1*0401
1362	MART2	p.G448E	A*0101
1363	LDLR-FUT	gene fusion	DRB1*0101
1558	TPI	p.T28I	DRB1*0101
1700	NOP56	p.G124E	A*0201
1913	HLA-A11	p.S11F	-
	CDKN2A	p.V59fs	A*11:01
2098	CSNK1A1	p.S27L	A*02:01
	GAS7	p.H229Y	A*02:01
	HAUS3	p.T160A	A*02:01
2224	KPNA5	p.P384S	A*02:01
2359	KIF2C	p.A16T	A*02:05
2369	PPP1R3B	p.P176H	A*01:01
	PLEKHM2	p.H902Y	A*01:01
	DOPEY2	p.P2168L	A*26:01
2556	MYH14	p.A600V	A*01:01
	RAC1	p.P29S	A*02:01
2591	POLA2	p.L420F	C*07:01
3107	ANXA1	p.E87K	class II
3309	MATN2	p.E226K	A*11:01
	CDK12	p.E928K	A*11:01

Tumor	Antigen	AA change	HLA RE
3466	COL18A1	p.S306F	A*02:01
	TEAD1	p.L388F	A*02:01
	ERBB2	p.H458Y	A*02:01
	PDZD8	p.I311N	B*44:03
	PXMP4	p.S176C	B*39:01
	KHSRP	p.P592L	B*39:01
3678	CORO7	p.S33L	B*51:01
	FBXO21	p.S250Y	C*14:02
	RECQL5	p.E558G	B*44:02
	UGGT2	p.P882L	A*02:01
	XPNPEP1	p.S663T	A*03:01
	PMVK	p.R78C	class II
3703	NSHDL	p.A290V	A*02:01
3713	WDR46	p.T300I	A*02:01
	AHNAK	p.S4460F	A*02:01
	SRPX	p.P55L	A*02:01
	CENPL	p.P79L	A*29:02
	HELZ2	p.D614N	A*29:02
	PRDX3	p.D614N	A*29:02
	GCN1L1	p.P769L	A*29:02
	PLSCR4	p.R247C	A*29:02
	AFMID	p.A52V	A*29:02
	SEC22C	p.H218Y	B*44:03
	TPX2	p.H458Y	B*44:03
3716	TFDP2	p.A406T	B*35:01
	ZMYM4	p.H203Y	B*15:01

Tumor	Antigen	AA change	HLA RE
3784	FLNA	p.R2049C	B*07:02
	GNB5	p.P377L	B*07:02
	KIF16B	p.L1020P	B*07:02
	SON	p.R1927C	B*07:02
3795	NRAS	p.Q61K	A*01:01
	RBBP6	p.R660C	A*01:01
3868	GANAB	p.S320F	A*02:01
3881	NDUFS2	p.G21R	class I
	MFI2	p.D503N	class I
3903	PHKA1	p.P34L	B*38:01
	KIAA1279	p.P246S	B*38:01
	CCAR2	p.H227Y	B*38:01
3919	TRIP12	p.F1577S	A*01:01
	CFDP1	p.P128S	A*30:01
	TRIP12	p.F1577S	class II
3998	MAGEA6	p.E168K	A*01:01
	MED13	p.P1691S	A*30:02
	MED13	p.P1691S	B*15:01
	PDS5A	p.Y1000F	C*03:03
	TVP23B	p.S148F	class I
4000	GPD2	p.A332V	class I
	AMPH	p.G247A	A*02:01
	EVA1A	p.A23V	class I
	DBT	p.A2V	class I
	HIVEP2	p.P1682L	class I
4087	SF3B1	p.R625H	class II

78 neoepitopes identified as targets of autologous TIL from 34 patients with melanoma.
31 of 34 (91%) expressed neoantigens. All were unique. (unpublished)

Identification of neoantigen-reactive T cells from patients with cancer



Kim Caesar/Springer Nature

Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL.

Identification and targeting of mutations unique to each cancer has the potential to extend cell therapy to patients with common epithelial cancers.

Cancer Antigens

1. **Unique somatic mutations in an intracellular protein**

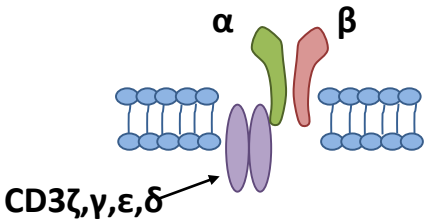
2. **Mutations in oncogenes or tumor suppressor genes that can thus be shared among patients.**
   e.g.: Kras
   p53

3. **Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands**
   e.g.: CD19 (CAR: αCD19 antibody)

Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

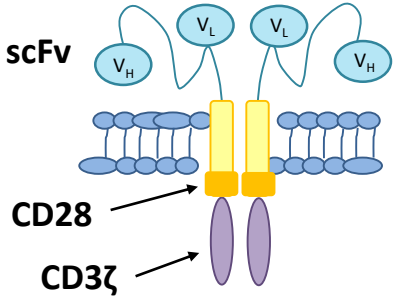
TCR Vector (eg, MART1, NY-ESO)

TCR receptor



CAR Vector (eg, CD19)

CAR receptor



Patient E.K.

48 year old male with follicular non-Hodgkin lymphoma

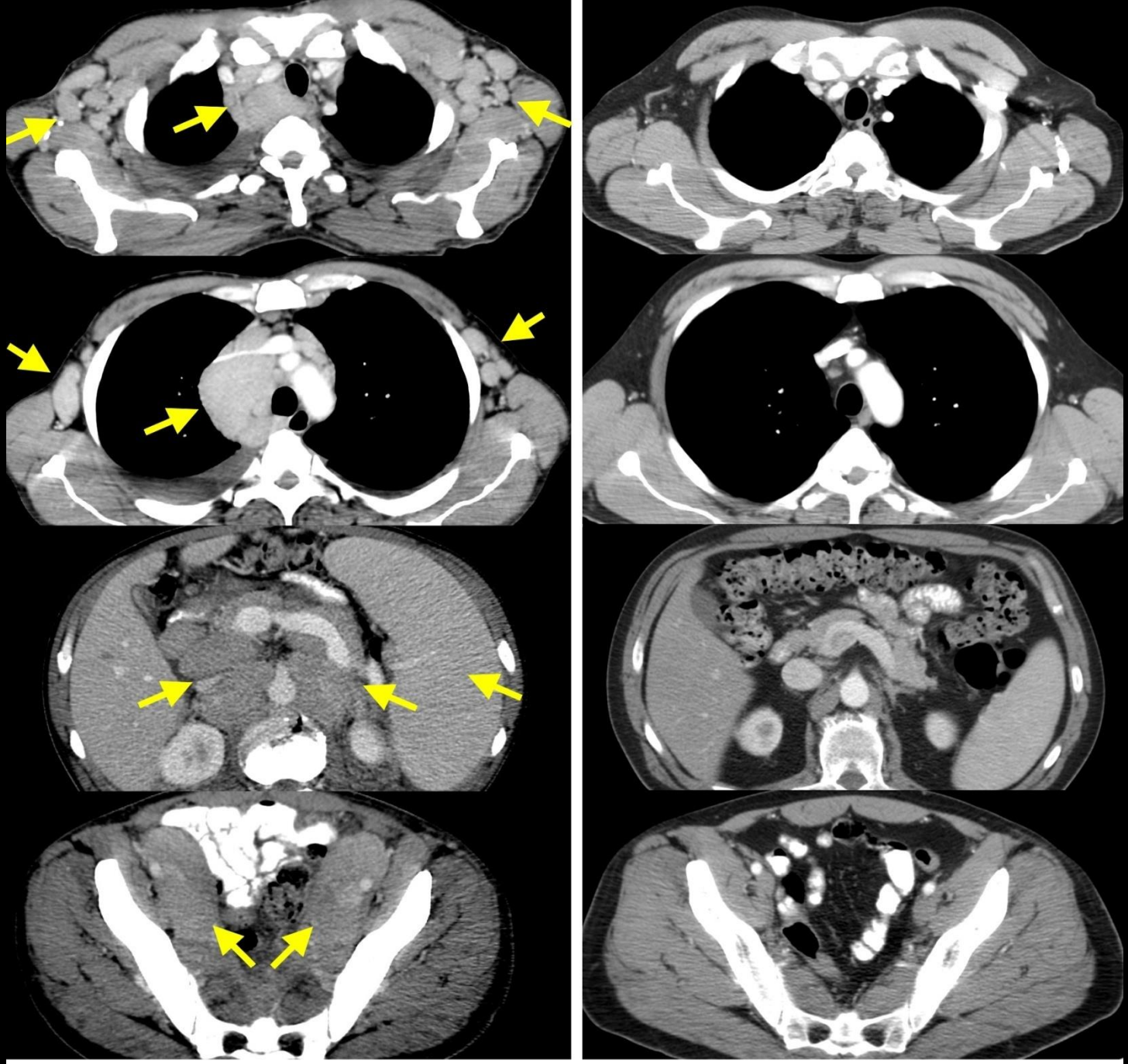
Aug. 2002	**diagnosed with stage IV lymphoma** **7 cycles PACE chemotherapy (cisplatin, doxorubicin, cyclophosphamide, etoposide)**
April 2004	**idiotypic/KLH vaccine (5 doses)**
Sept. 2007	**ipilimumab**
Nov. 2007	**6 cycles EPOCH-R chemotherapy (etoposide, predisone, vincristine, cyclophosphamine, rituximab)**
May 2009	**To NCI for treatment with autologous anti-CD19 CAR transduced T cells**

In ongoing progression-free regression as of February, 2014 (57+ months).

(Blood 116:3875-86, 2010; 119:2709-20, 2012)

E.K.

Follicular lymphoma

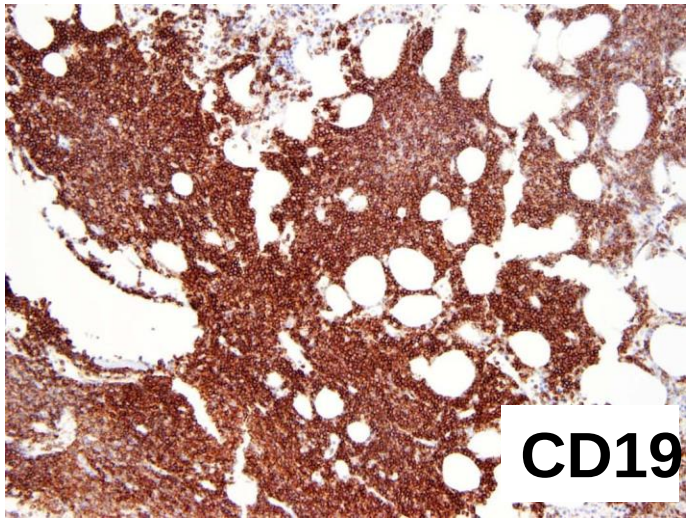


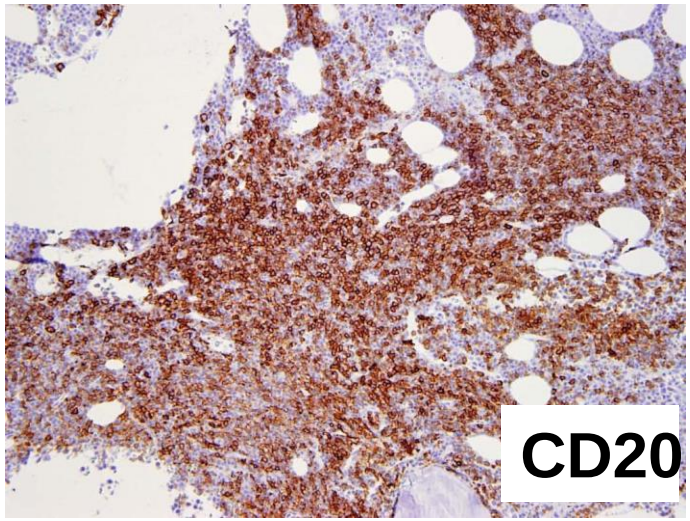
June 2, 2009

March 14, 2012

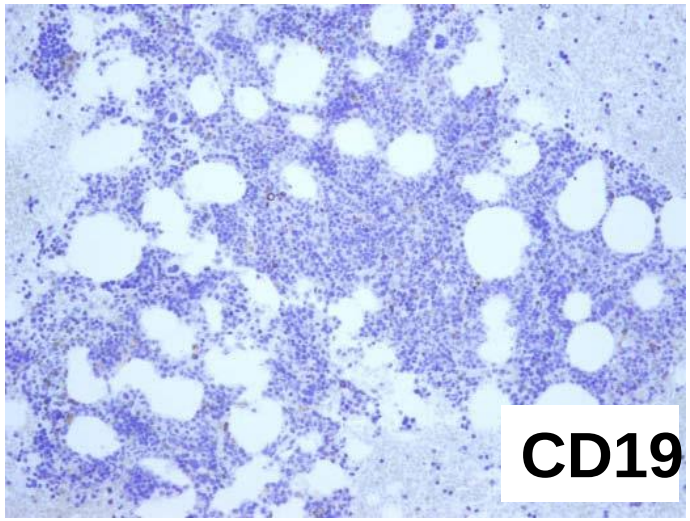
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

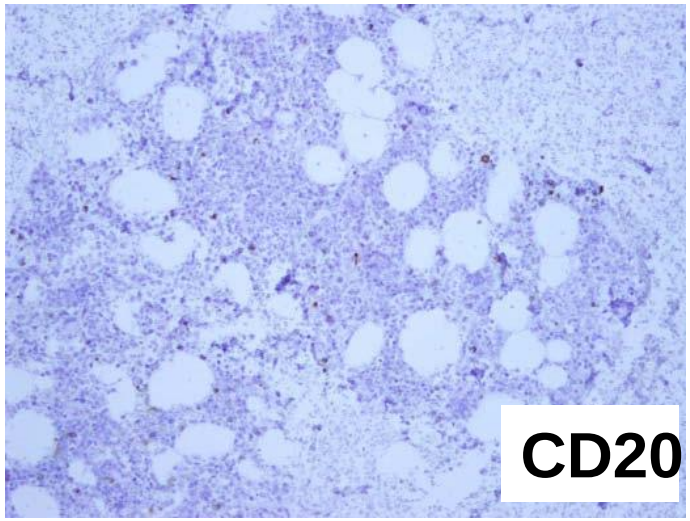
Before treatment



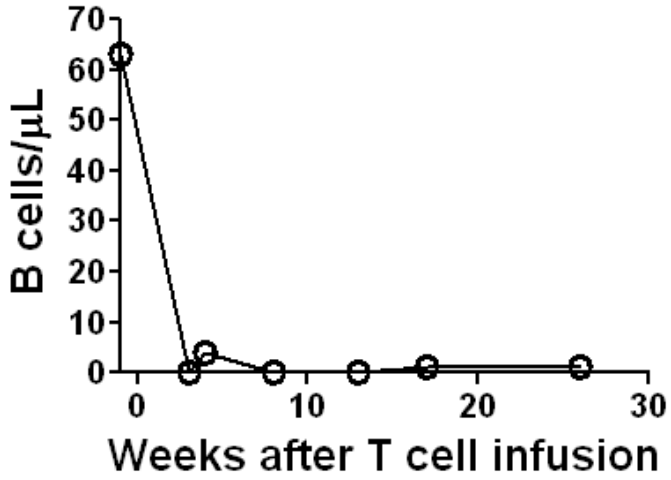


3 months after treatment

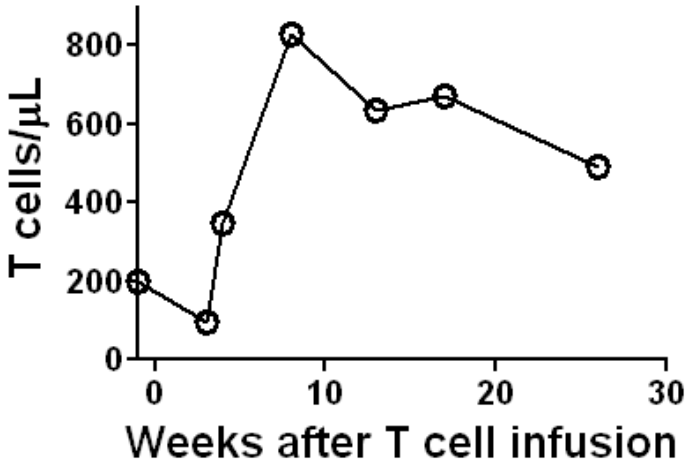


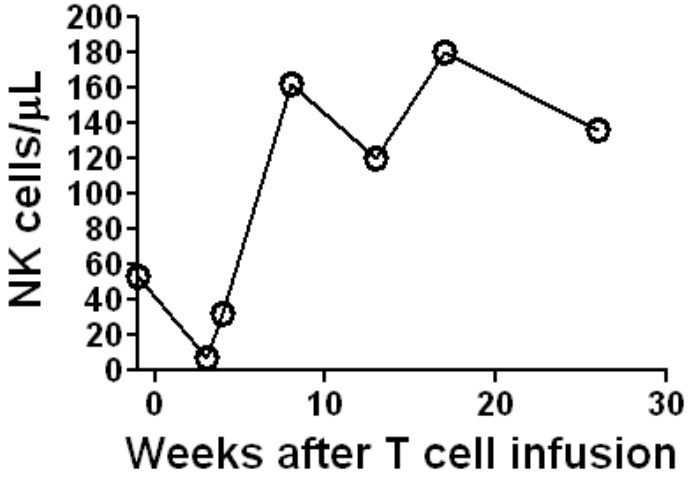


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion

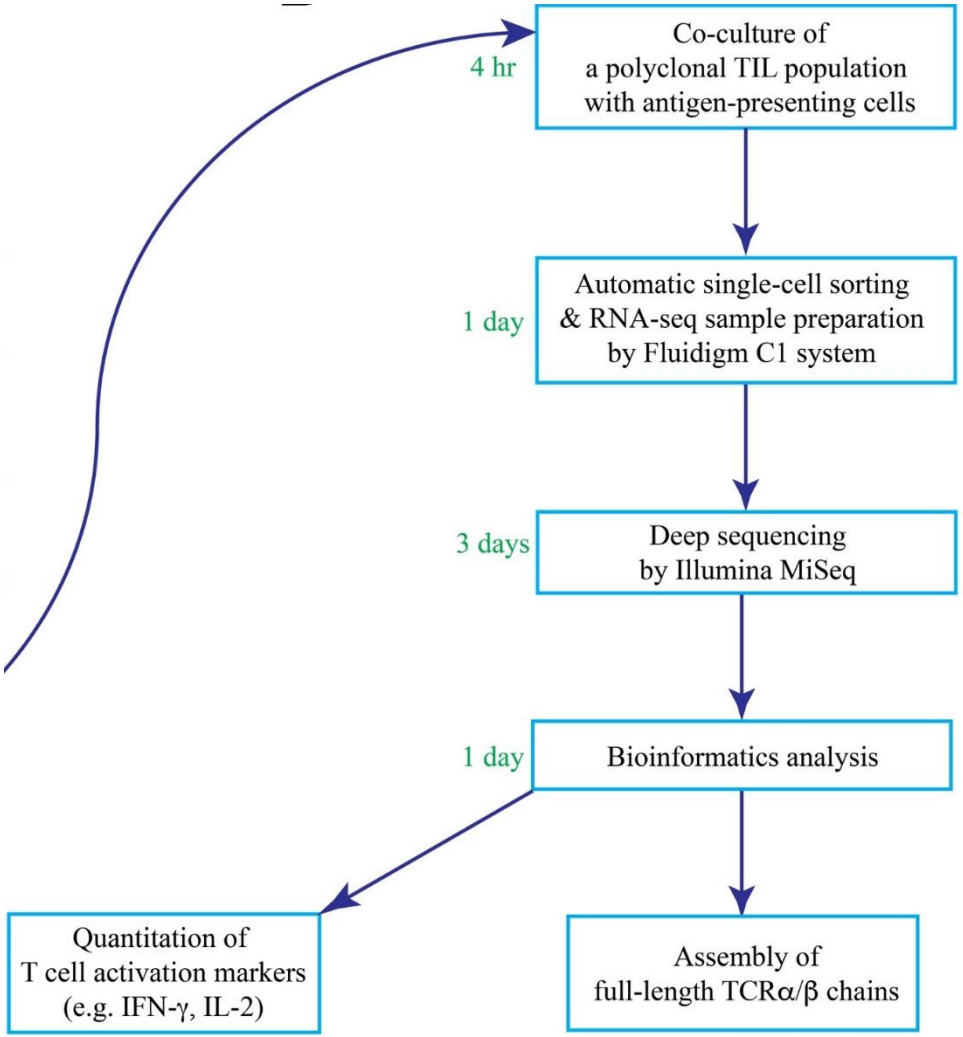


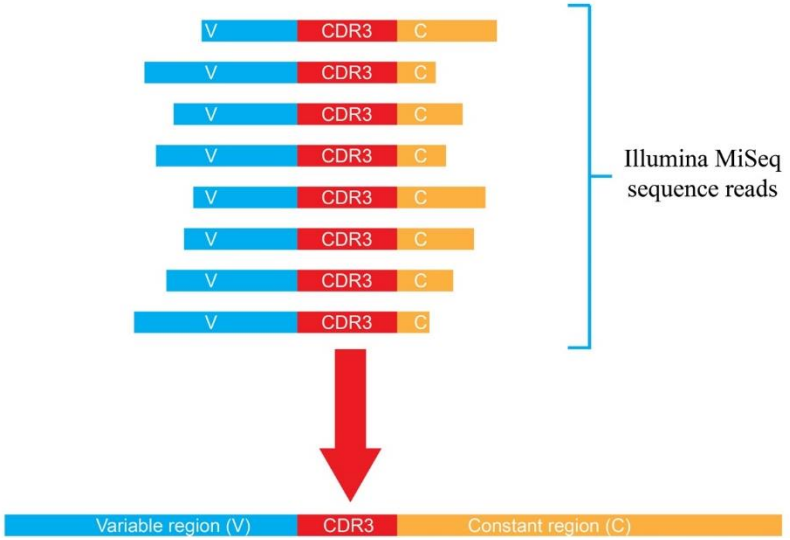
In contrast, T and NK cell counts rapidly recovered after treatment





Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

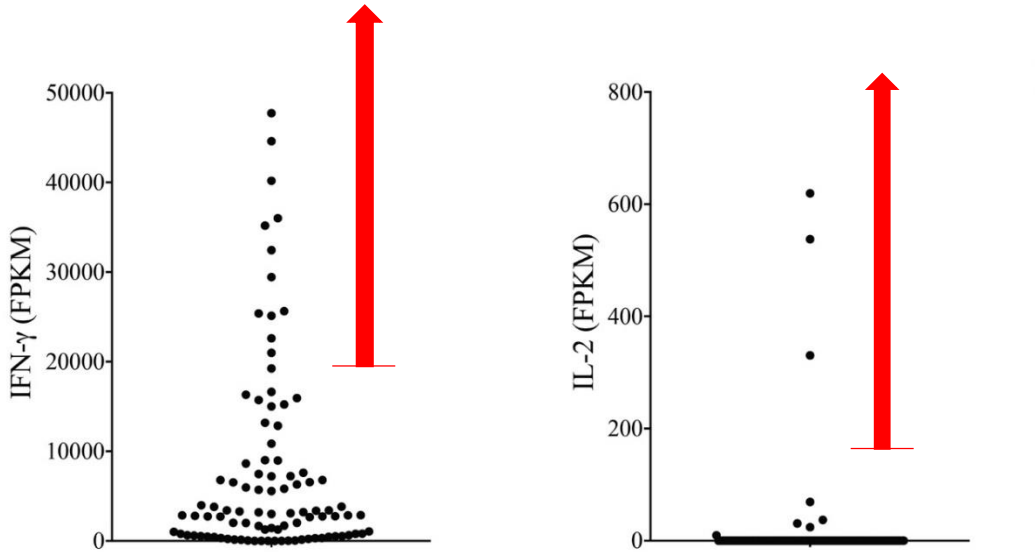




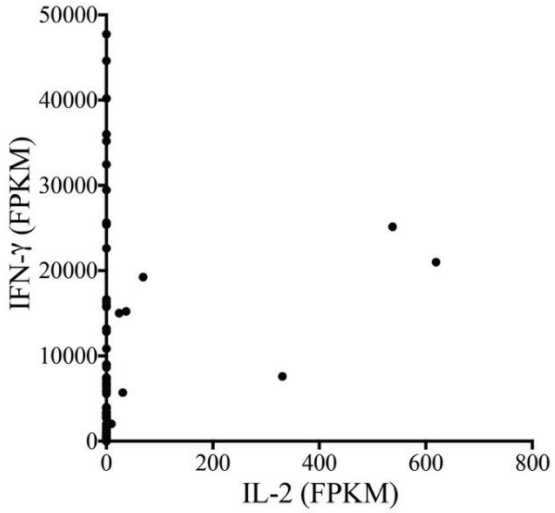
(William Lu, Anna Pasetto)

Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

A



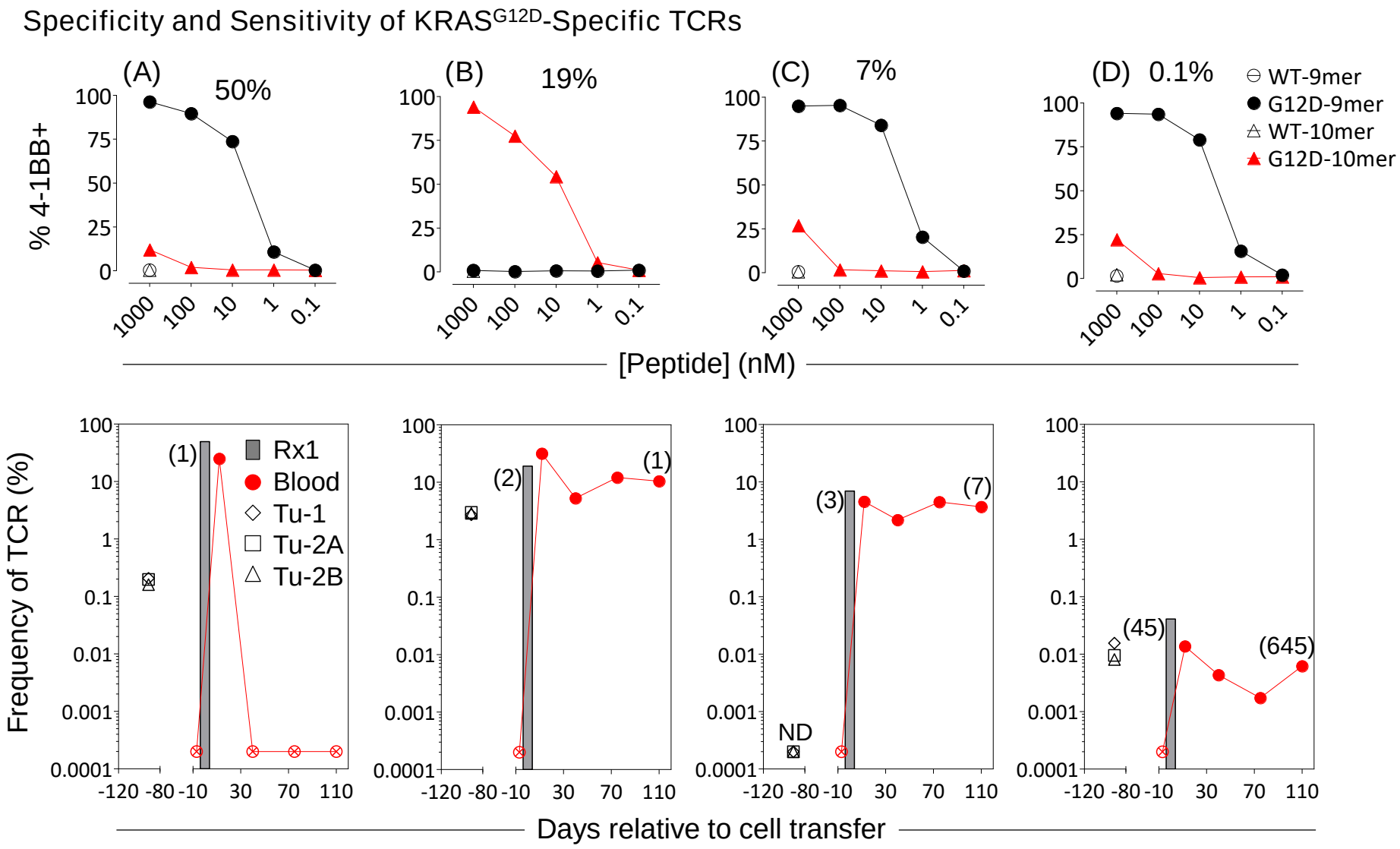
B



C

TCR variable region	CDR3 (nucleotide sequence)	CDR3 (amino acid sequence)
AV38-1	TGTGCTTTCATGTGGGGATTAGGTCAGAATTTTGTCTTT	CAFMWGLGQNFVF
BV28	TGTGCCAGCAGTGTGGAGCGGGAGAACACCGGGGAGCTGTTTTTT	CASSVERENTGELFF

Four different KRAS^{G12D}-reactive TCRs in patient infusion TIL



Why the differences in the persistence in blood?
T-cell differentiation state? Avidity for antigen?

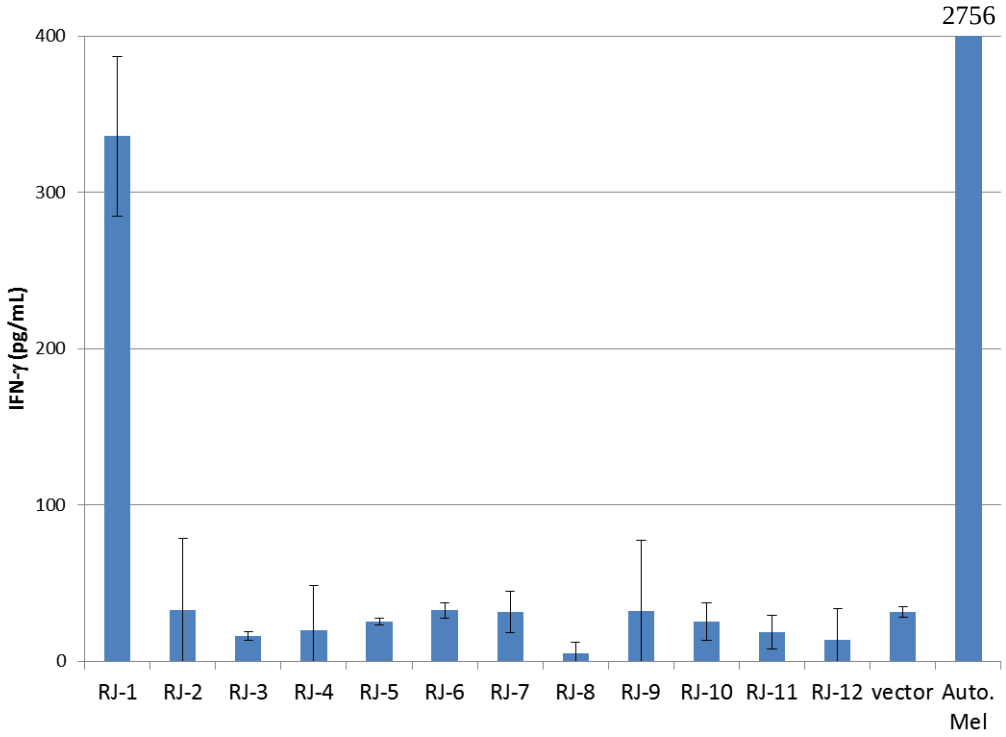
Half of Patients with Melanoma Recognize Three or More Immunogenic Mutations

Immunogenic mutations 56/3961 = 1.4%

# neoantigens per patient	# patients
0	1 (6%)
1	4 (22%)
2	4 (22%)
3	4 (22%)
4	1 (6%)
>4	4 (22%)

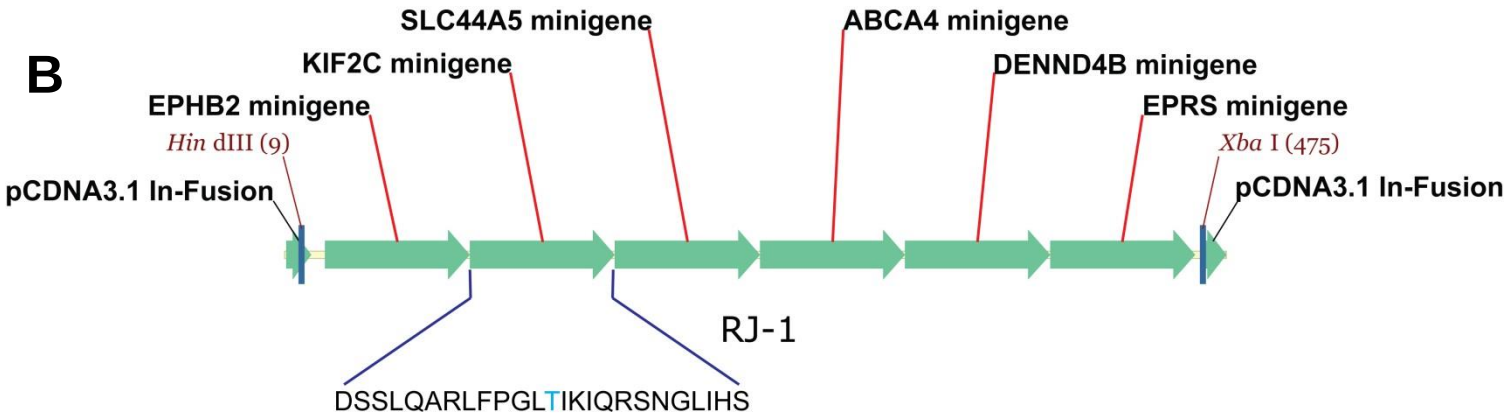
Minigene approach: J. bulk TILs recognize tandem minigene RJ-1

A

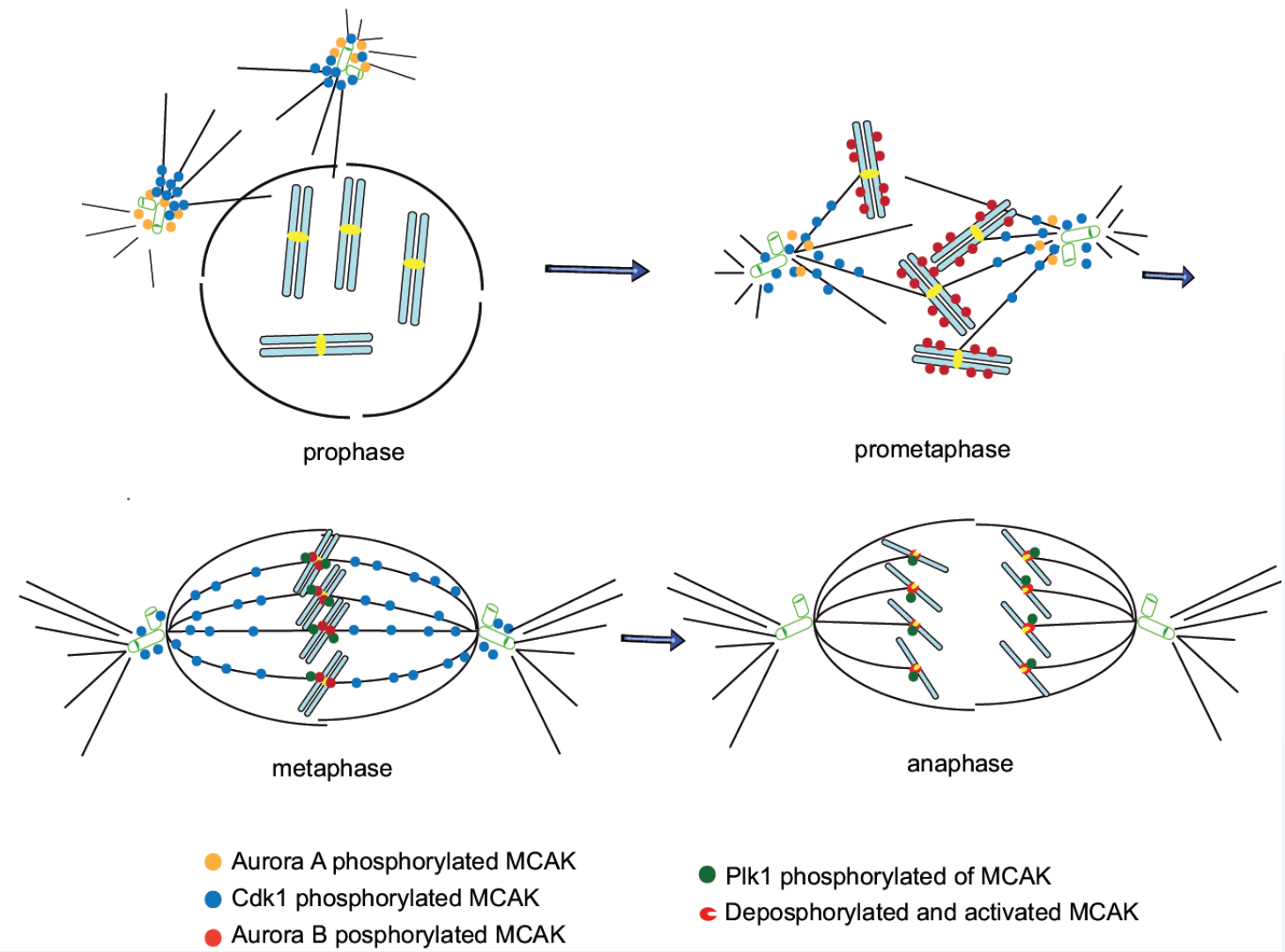


71 non-synonymous mutations
12 tandem minigene constructs
HLA-A*0205

B

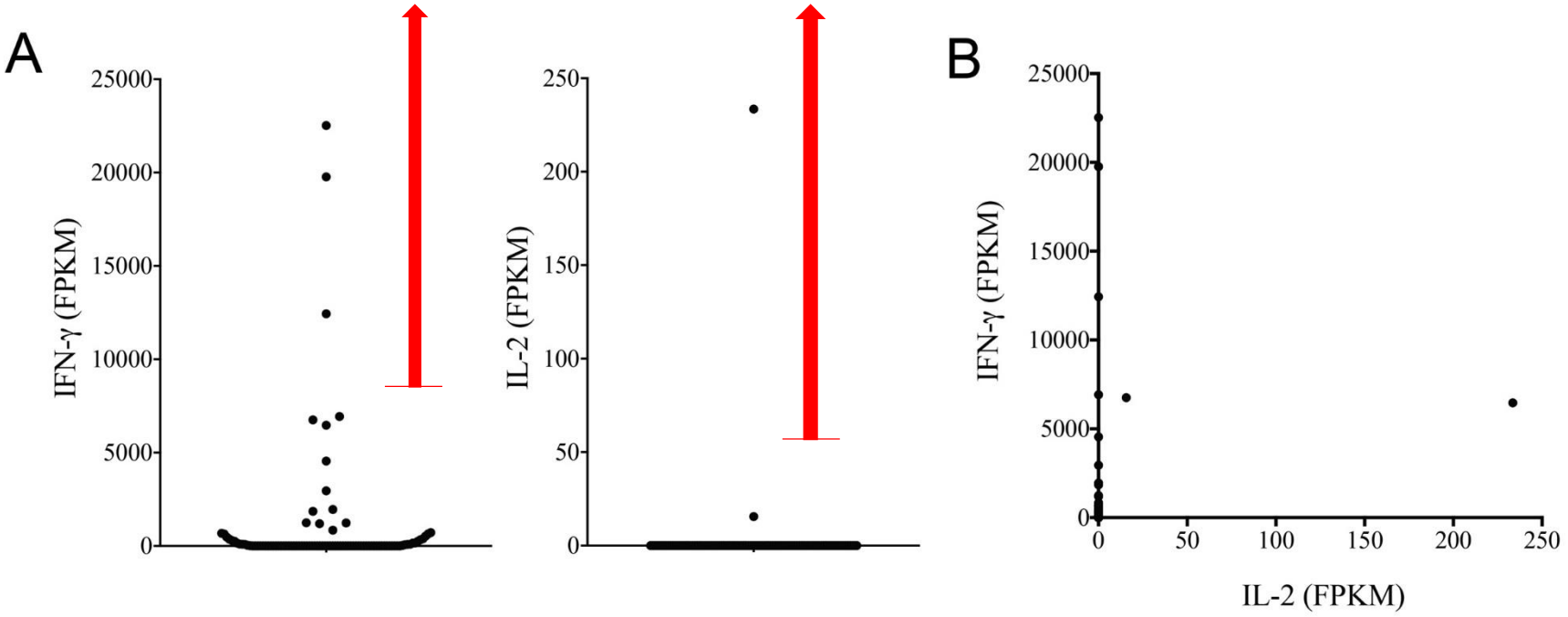


Kinesin family member 2C (KIF2C)
also known as mitotic centromere-associated Kinesin (MCAK)



Sanhaji M et al, *Oncotarget* (2011)

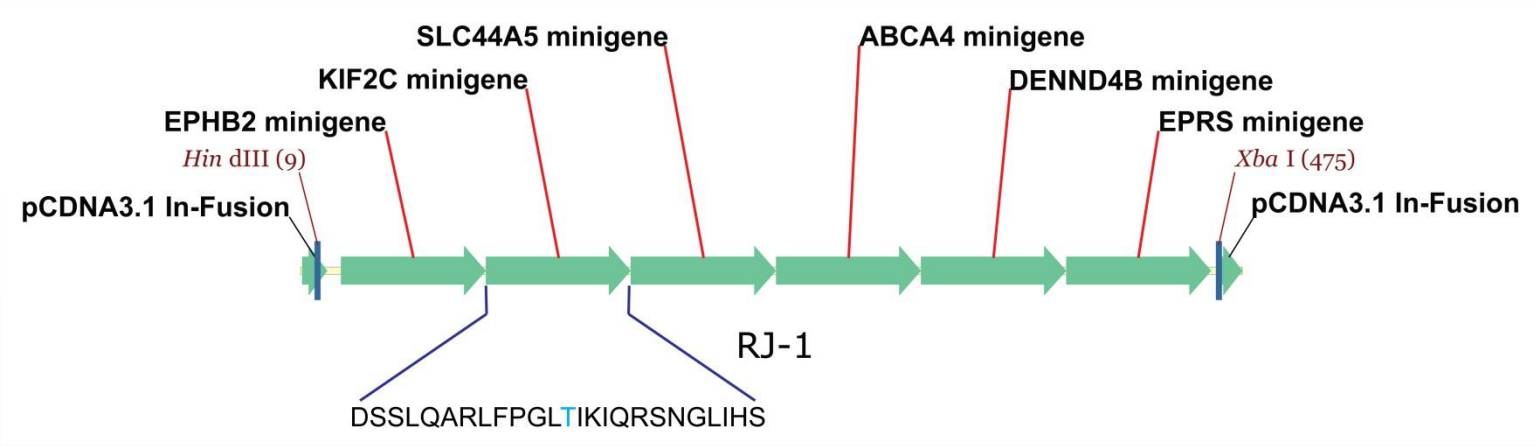
Rapid Identification of the TCR Sequence from a Polyclonal Population Using Single Cell RNA/Seq

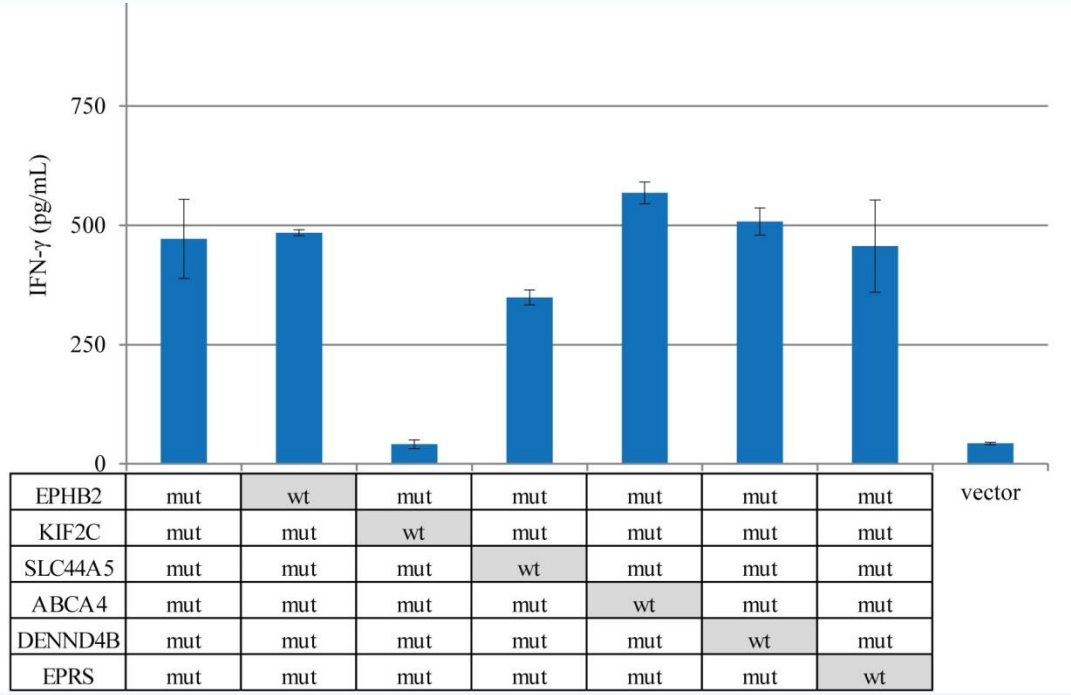


C

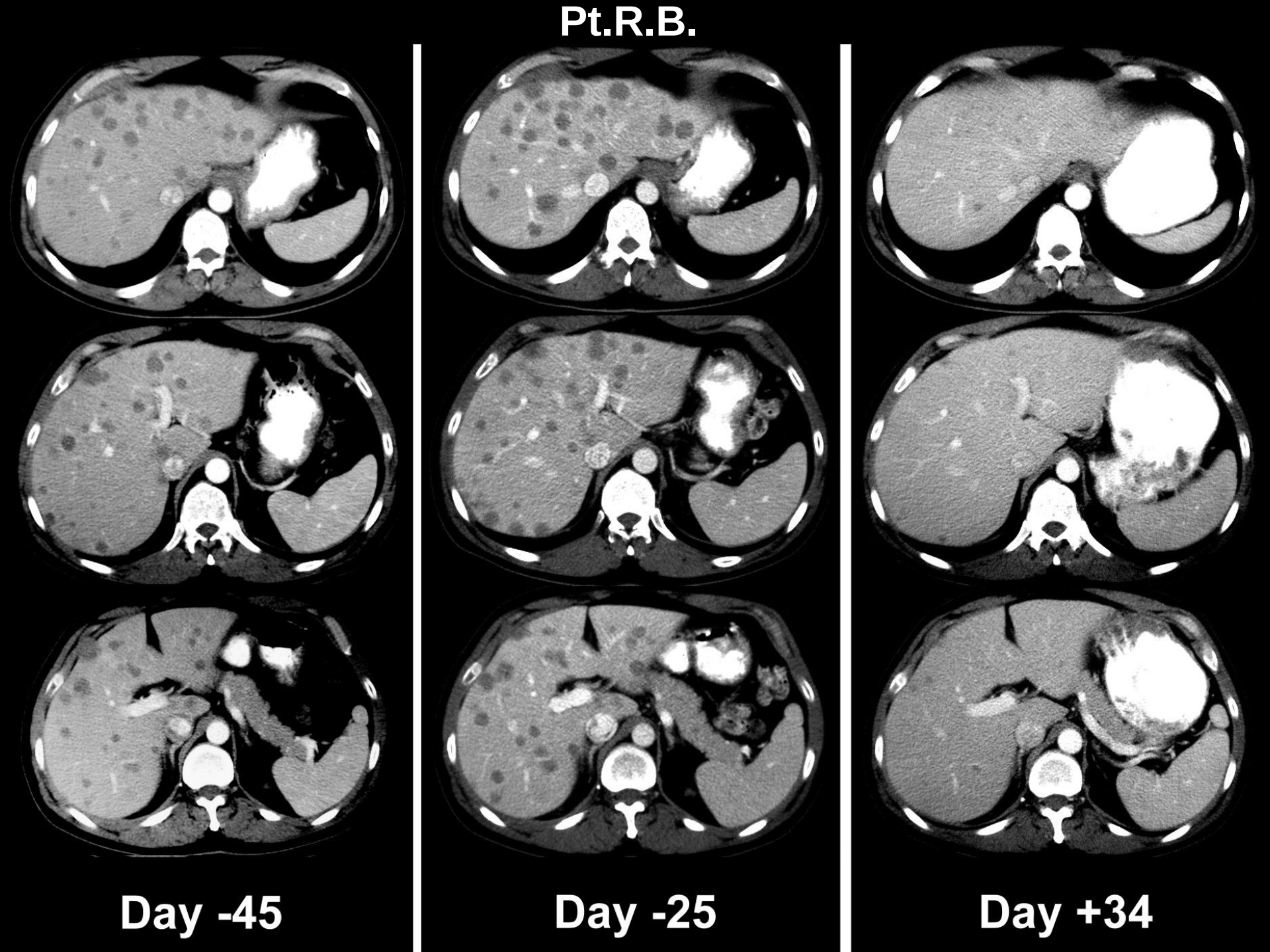
TCR variable region	CDR3 (nucleotide sequence)	CDR3 (amino acid sequence)
AV4	TGCCTCGTGGGTGACATGGACCAGGCAGGAACTGCTCTGATCTTT	CLVGDMDQAGTALIF
BV5-6	TGTGCCAGCAGCTTGGGGAGGGCAAGCAATCAGCCCCAGCATTTT	CASSLGRASNQPQHF

Mutated antigen KIF2C (kinesin family member 2C) recognized by R. J. TILs



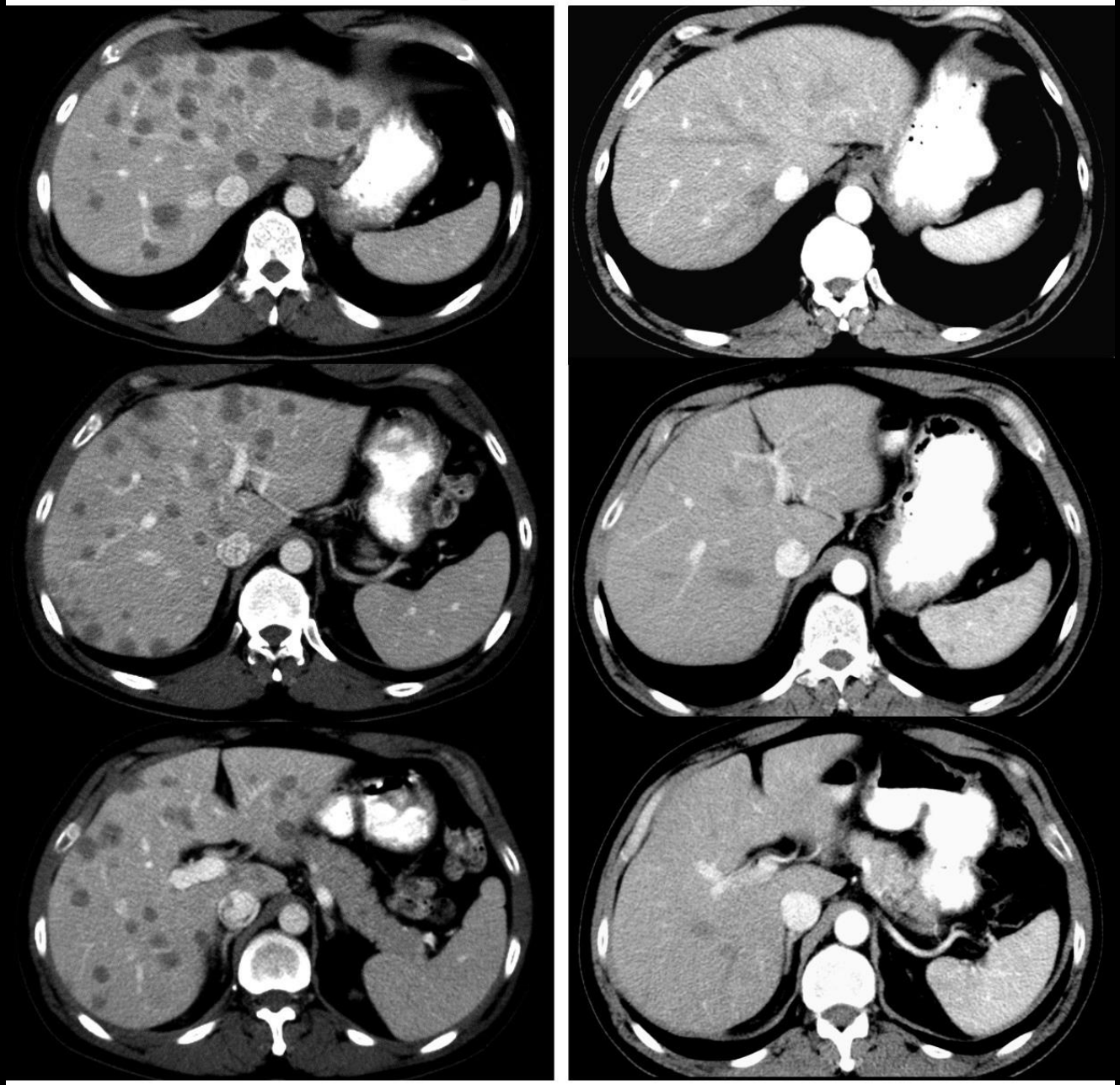


EPHB2	mut	wt	mut	mut	mut	mut	mut
KIF2C	mut	mut	wt	mut	mut	mut	mut
SLC44A5	mut	mut	mut	wt	mut	mut	mut
ABCA4	mut	mut	mut	mut	wt	mut	mut
DENND4B	mut	mut	mut	mut	mut	wt	mut
EPRS	mut	mut	mut	mut	mut	mut	wt


Pt.R.B.
Day -45
Day -25
Day +34

Other Sites: Lung

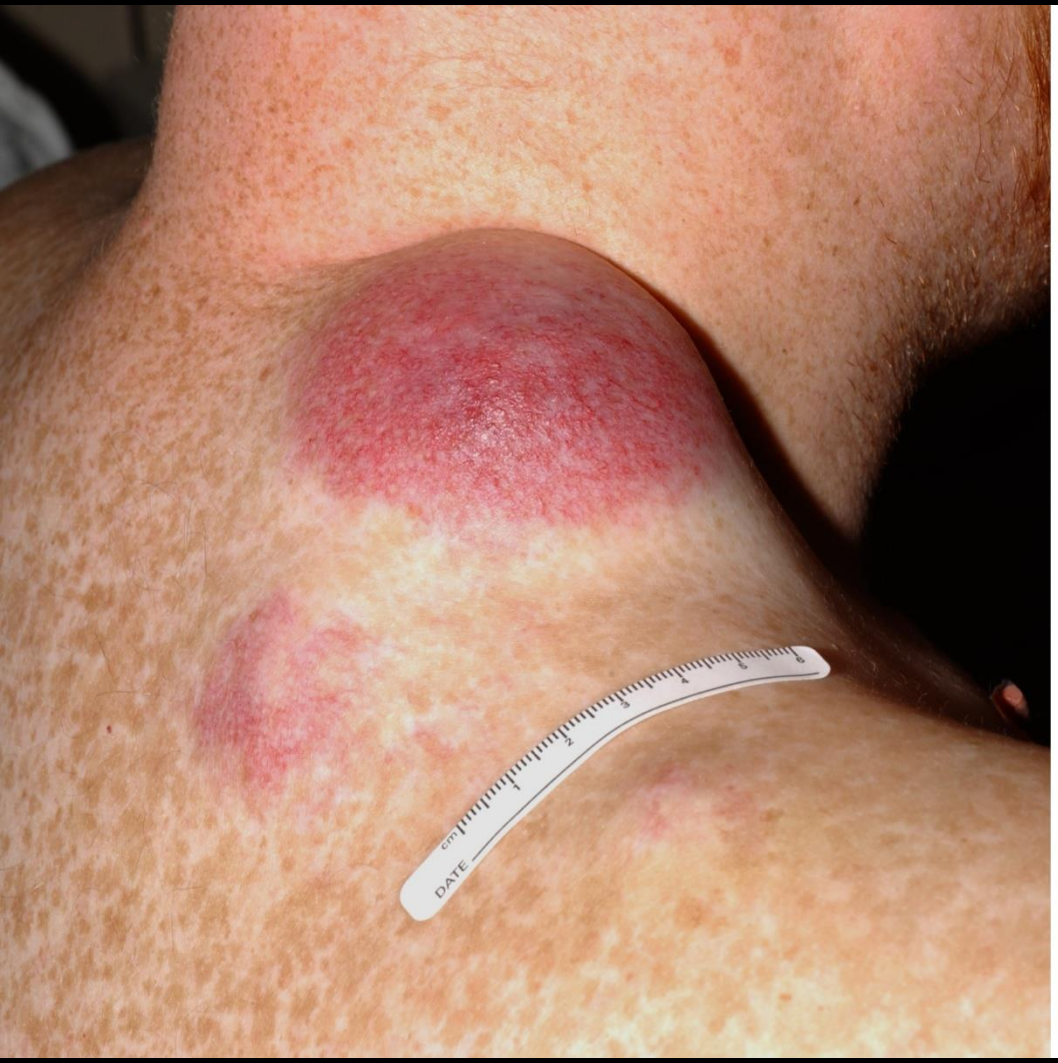
CR 75+ mo.

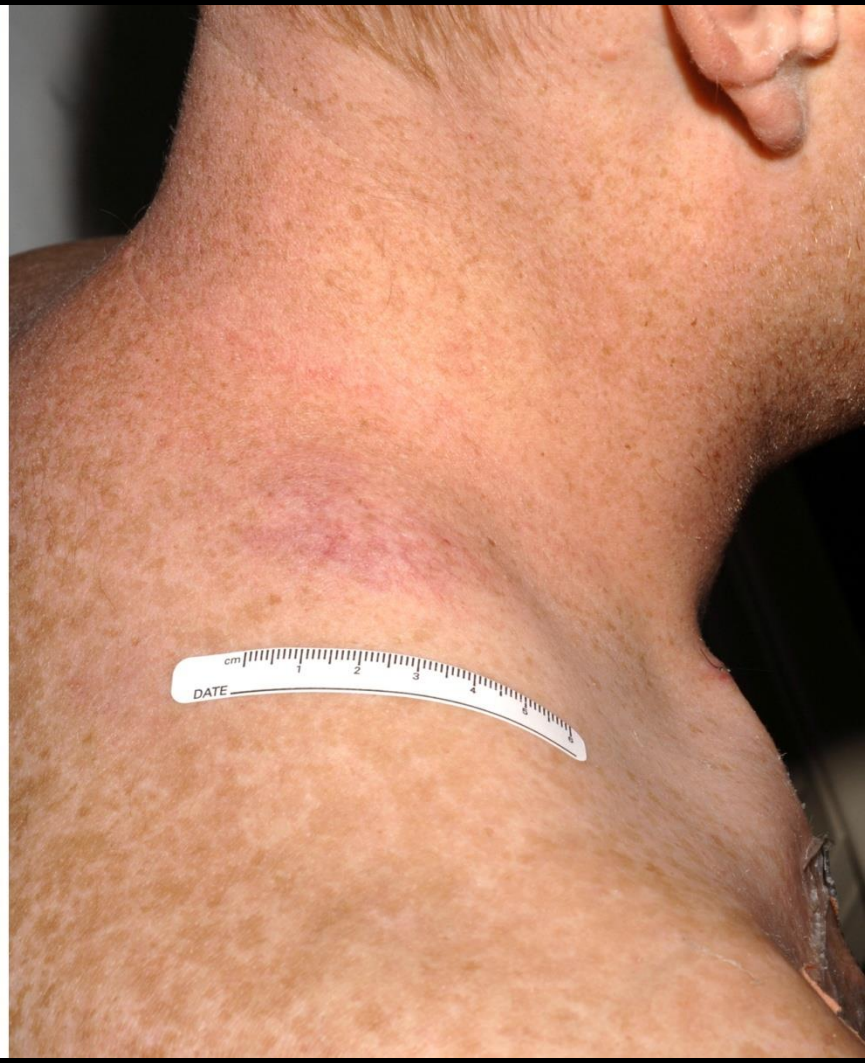


Nov 10, 2003

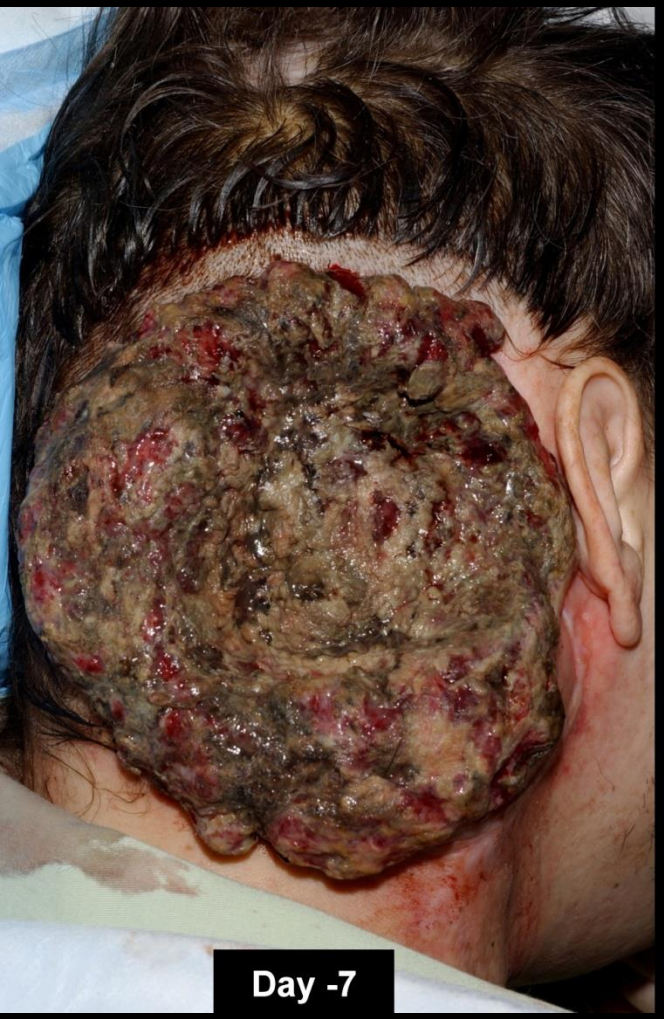
Feb 17, 2010

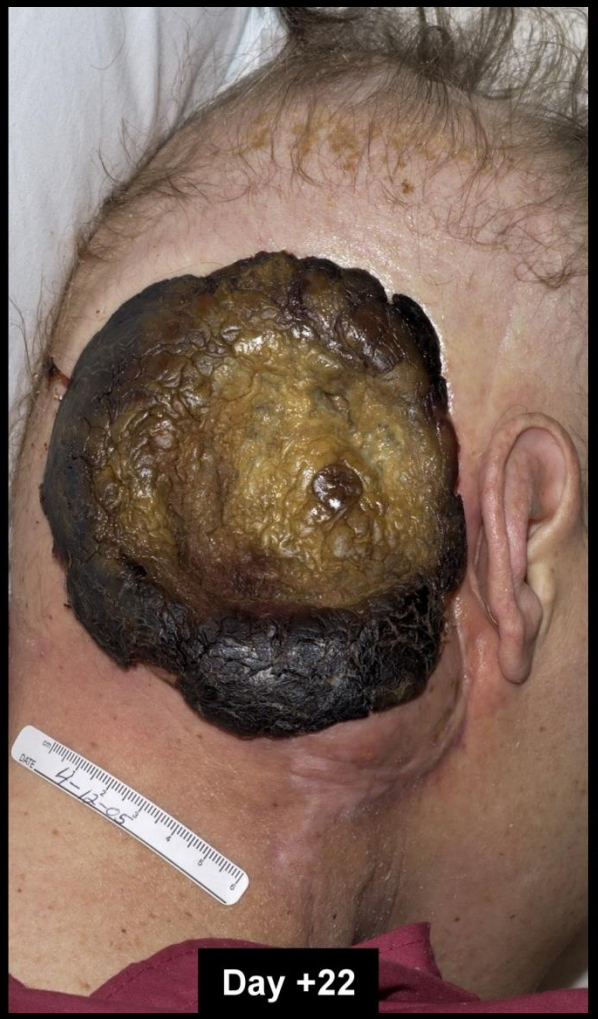
C.K. (200cGy) Pre 12 days

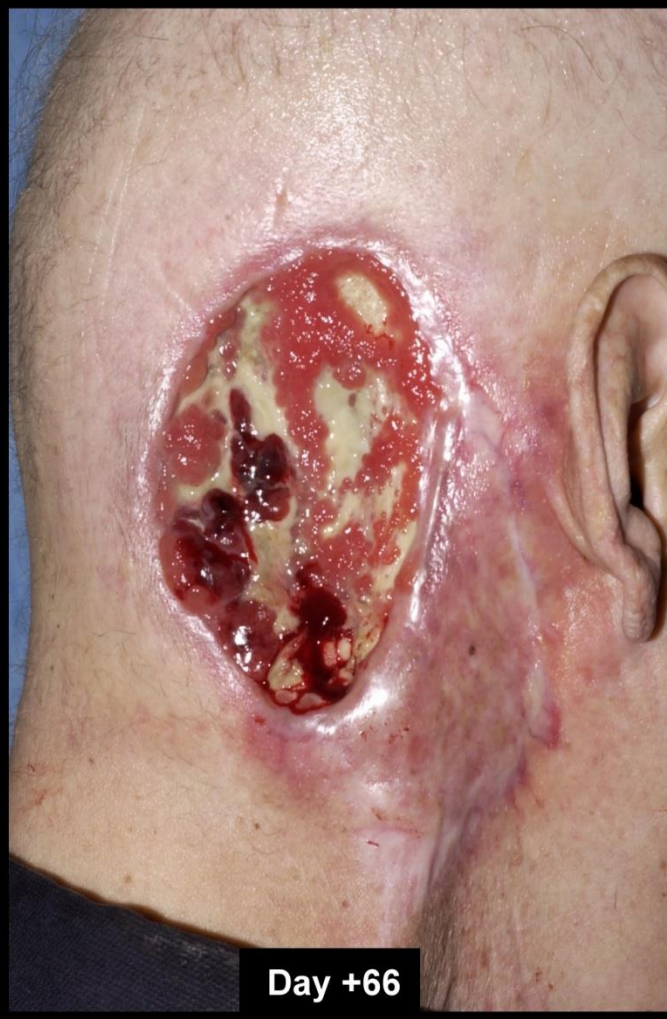




A.H.: N-M cell transfer

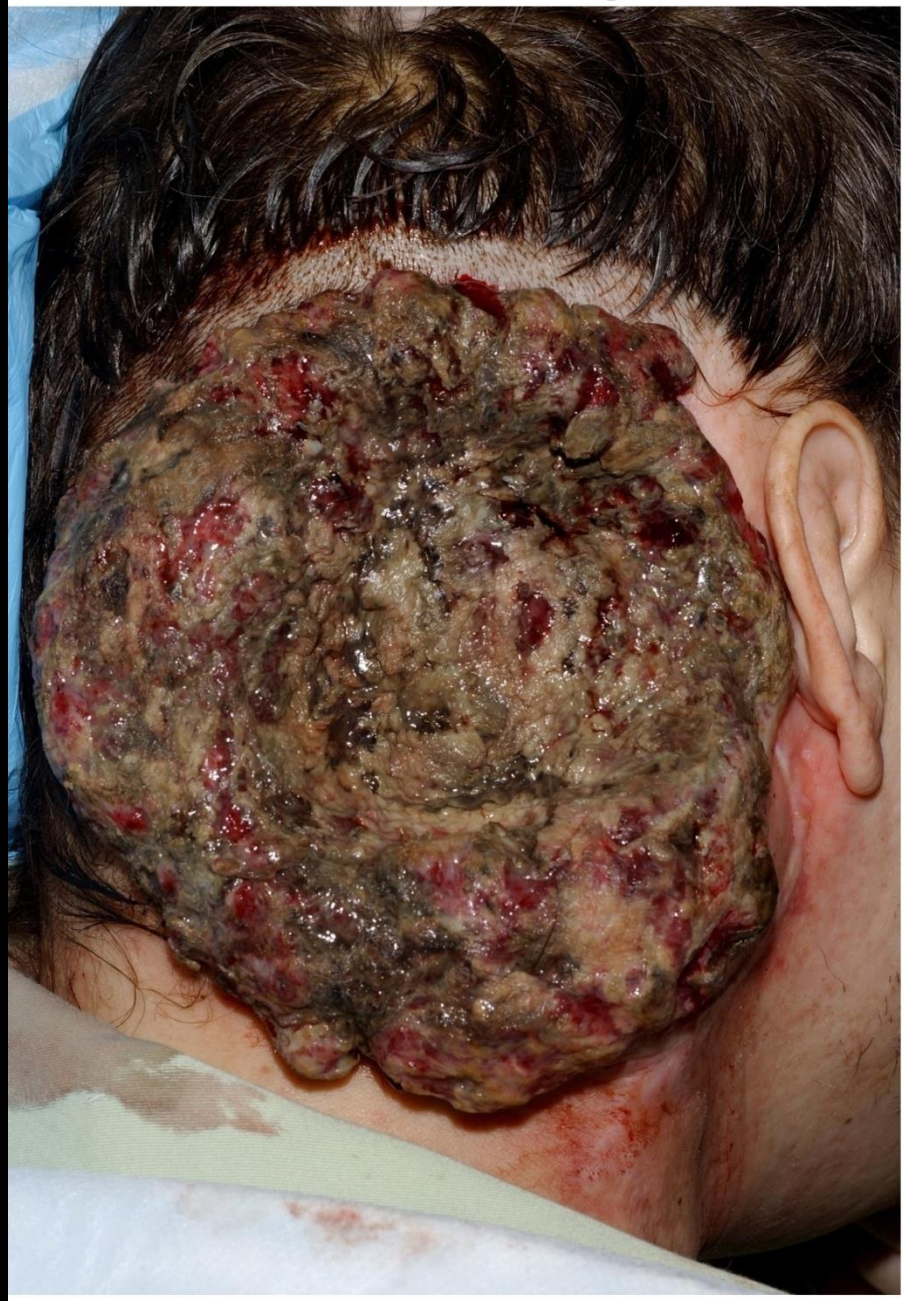


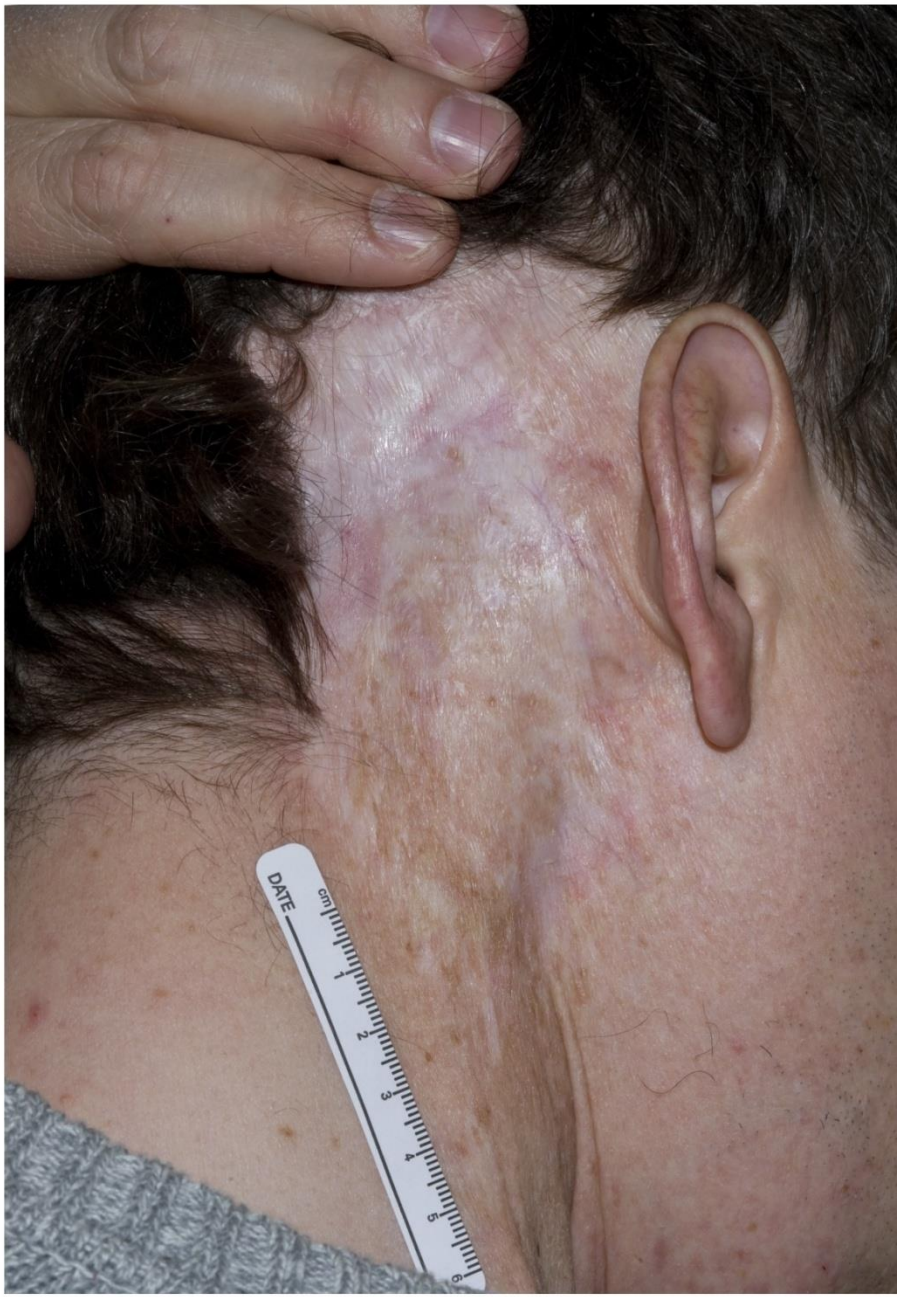




Other Sites: Lung

CR 59+ mo.

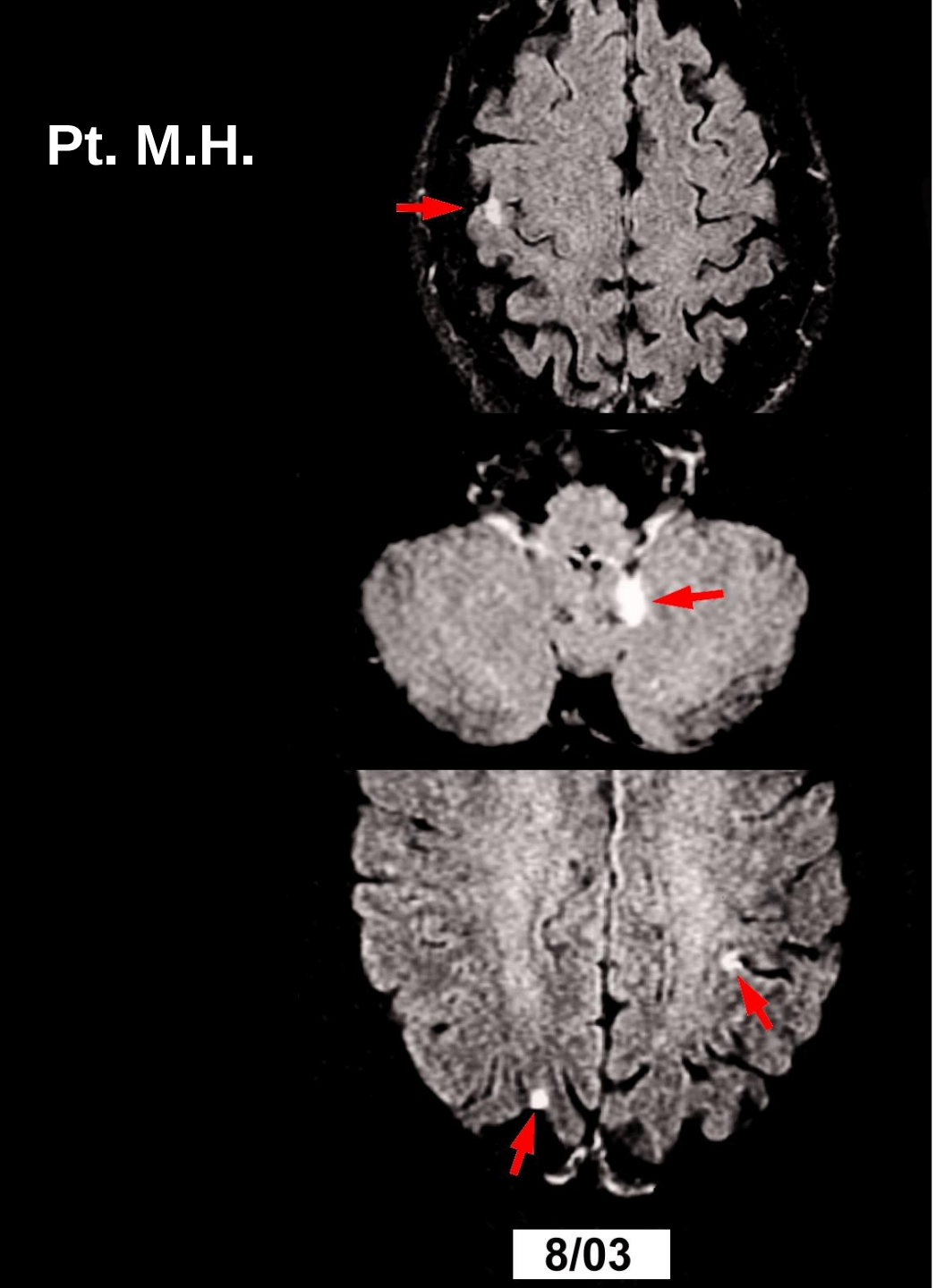




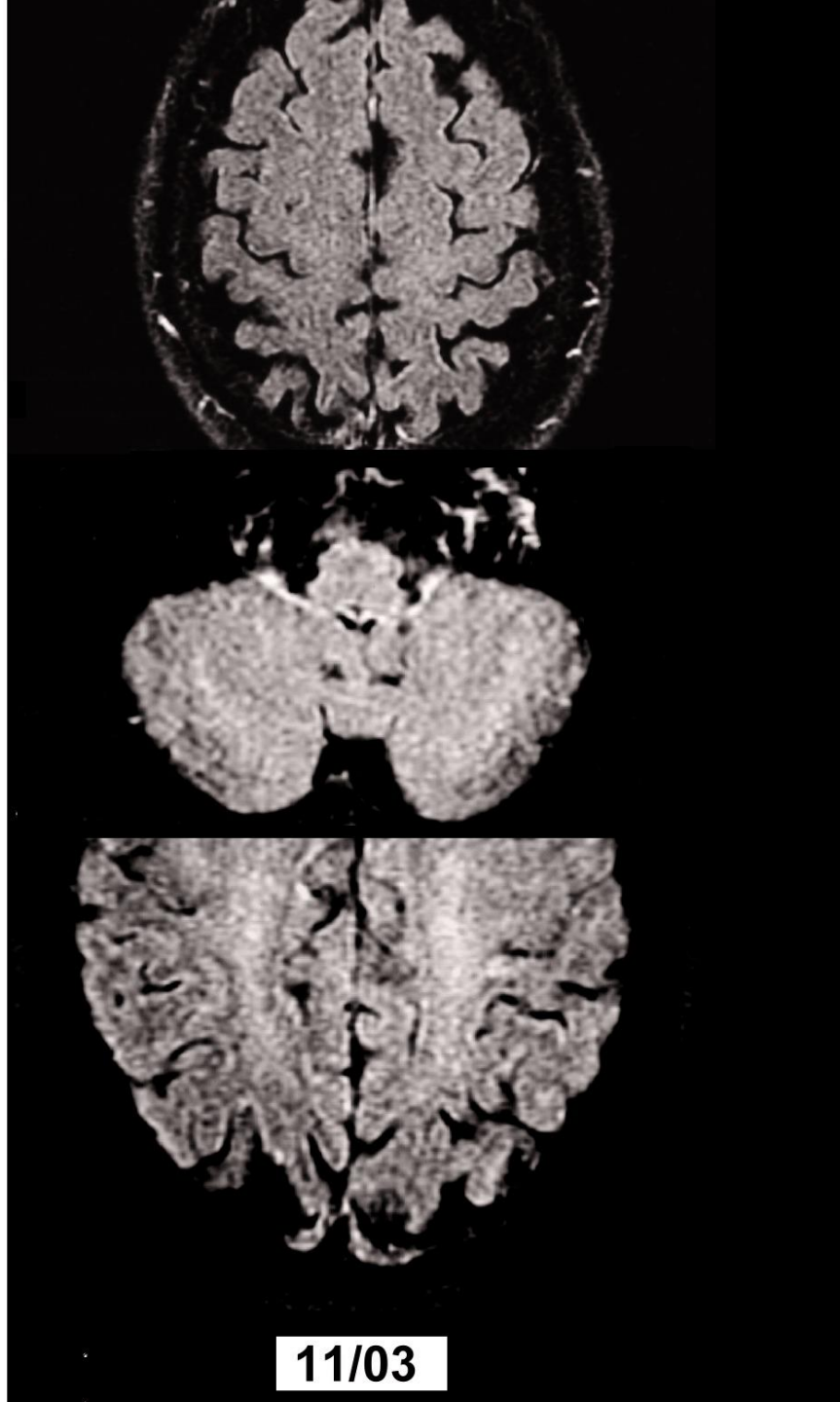
March 21, 2005

Feb 23, 2010

Pt. M.H.



8/03



11/03

Potential improvements being explored to improve targeting of somatic mutations in epithelial cancers

---

Purify tumor reactive cells

- **PD1+ cells in tumor and circulating lymphocytes**
- **41BB+ after antigen stimulation**

Identify multiple mutation targets expressed by tumor

Add anti-PD-1 (reexpressed by infused cells in vivo)

Transduce mutation-reactive TCRs into naïve or CM cells

Knockout CISH or PD-1 on transferred cells

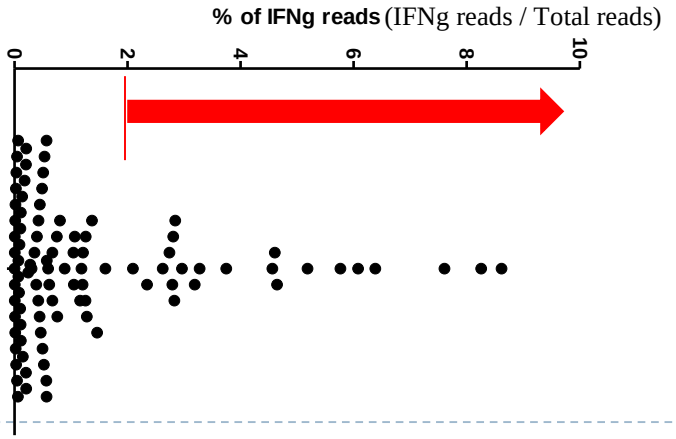
Vaccinate with mutations recognized by transferred cells

Rapid Selection and Identification *of TCRs Recognizing Cancer Mutations*

1. **4112 TIL fragments were screened against TMG library, and Fragment 5 recognized TMG-9**

2. **Fragment 5 T cells co-cultured with TMG-9-pulsed DCs for 4 hr and single-cell RNAseq performed on 41BB positive cells**

				Read 1				Read 2			
Single-cell ID	Total reads (R1)	IFNg reads	% of IFNg reads	TCR variable region	CDR3 (nucleotide)	CDR3 (amino acid)	# of reads	TCR variable region	CDR3 (nucleotide)	CDR3 (amino acid)	# of reads
26	163002	4829	2.96	TRBV28	TGTGCCAGCAGTGTGGAGCGGGAGAACACCGGGGAGCTGTTTTTT	CASSVERENTGELFF	125	TRBV28	TGTGCCAGCAGTGTGGAGCGGGAGAACACCGGGGAGCTGTTTTTT	CASSVERENTGELFF	98
				TRAV38-1	TGTGCTTTCATGTGGGGATTAGGTCAGAATTTTGTCTTT	CAFMWGLGQNFVF	14	TRAV38-1	TGTGCTTTCATGTGGGGATTAGGTCAGAATTTTGTCTTT	CAFMWGLGQNFVF	8
62	176886	52	0.029	TRBV19	TGTGCCAGTAGCCTGACCTTCCCAAGCGAATACTACGAGCAGTACTTC	CASSLTFPSEYYEQYF	53	TRBV19	TGTGCCAGTAGCCTGACCTTCCCAAGCGAATACTACGAGCAGTACTTC	CASSLTFPSEYYEQYF	46
				TRAV24	TGTGCCTTTATGGACAGAGATGACAAGATCATCTTT	CAFMDRDDKIIF	19	TRAV24	TGTGCCTTTATGGACAGAGATGACAAGATCATCTTT	CAFMDRDDKIIF	14



>2% IFNg reads: **23** single-cells with identical TCR(BV28) sequences
1~2% IFNg reads: **6** single-cells with identical TCR(BV28) sequences
6 sample without any TCR sequences

All single-cell samples

The whole-transcriptome of 4112 F5 after 4 hr co-culture with TMG-9 pulsed DC

Single-cell ID	Sequencer	IFNg	TNF	TNFRSF9 (4-1BB)	PDCD1 (PD-1)	LAG3	CD244 (2B4)	CD160	HAVCR2 (Tim-3)	CD4	CD8A	CD8B	GAPDH
26	MiSeq	15736	1093	536	0	223	0	0	376	0	34	0	3517
26	HiSeq	34685	2011	221	0	388	0	0	434	0	71	0	8327
62	MiSeq	150	40	81	0	0	0	0	152	0	33	87	3353
62	HiSeq	305	102	230	0	21	0	0	266	0	76	89	7441

Unit: FPKM (Fragments Per Kilobase of transcript per Million mapped reads)

Sequenced by
(1) MiSeq v3: 2 X 250 b.p. X ~200,000 reads / single-cell sample
(2) HiSeq 2500 rapid mode: 2 X 100 b.p. X ~1,500,000 reads / single-cell sample

Analyzed by Partek Flow

Potential improvements being explored to improve targeting of somatic mutations in epithelial cancers

---

Purify tumor reactive cells

- **PD1+ cells in tumor and circulating lymphocytes**
- **41BB+ after antigen stimulation**

Identify multiple mutation targets expressed by tumor

Add anti-PD-1 (reexpressed by infused cells in vivo)

Transduce mutation-reactive TCRs into naïve or CM cells

Knockout CISH or PD-1 on transferred cells

Vaccinate with mutations recognized by transferred cells

Categories of antigens to target using cell therapy

1. **Mutations unique to each individual cancer**

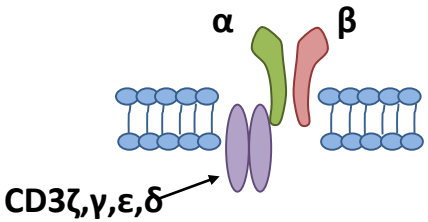
2. **Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)**

3. **Shared antigens unique to cancer (cancer-testes antigens)**

Construction of T-cell Receptors (TCR) and Chimeric Antigen Receptors (CAR)

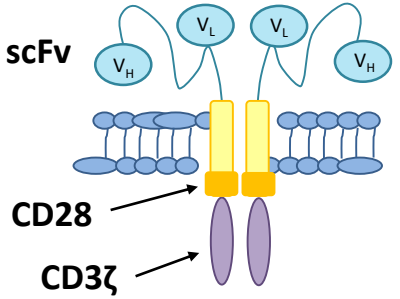
TCR Vector (eg, MART1, NY-ESO)

TCR receptor



CAR Vector (eg, CD19)

CAR receptor



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April 2004	**idiotypic/KLH vaccine (5 doses)**
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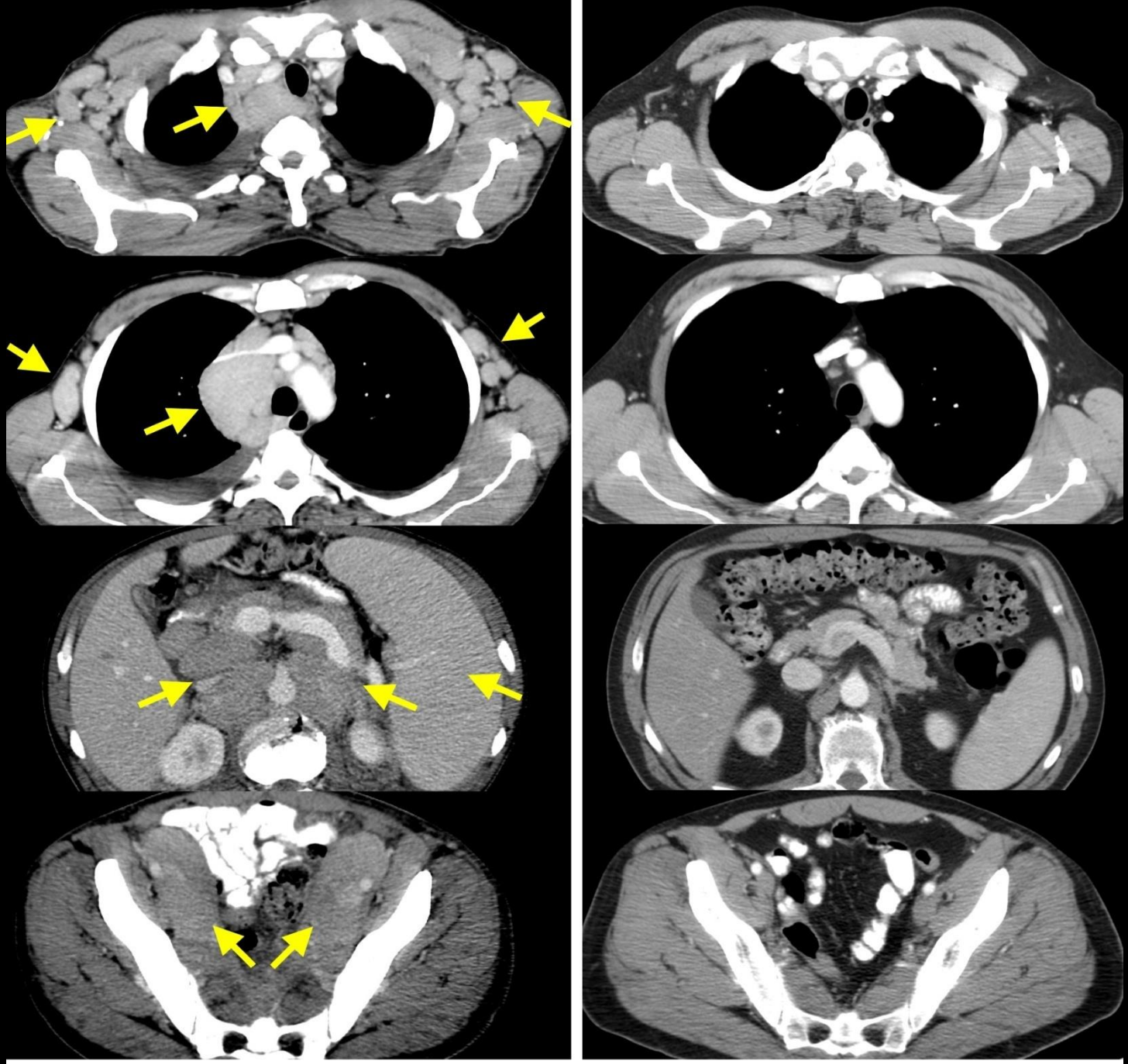
In ongoing progression-free regression as of October, 2017 (101+ months).

(Blood 116:3875-86, 2010; 119:2709-20, 2012)

E.K.

Follicular lymphoma

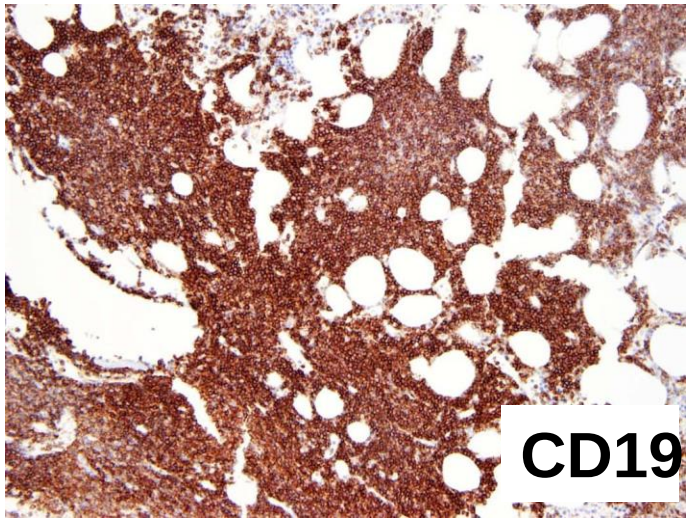


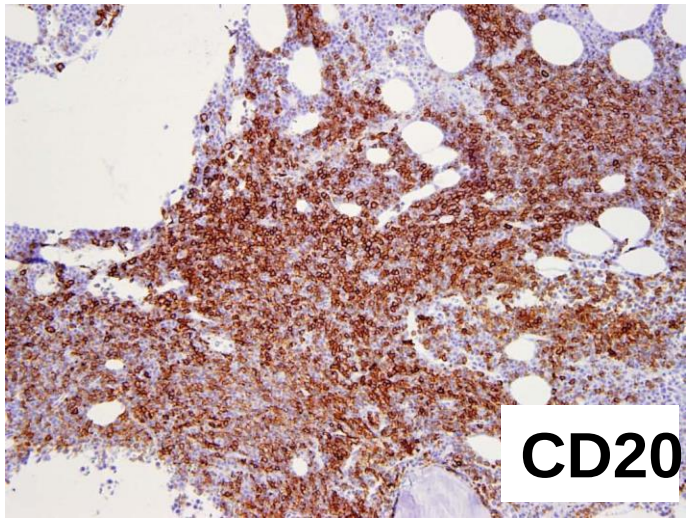
June 2, 2009

March 14, 2012

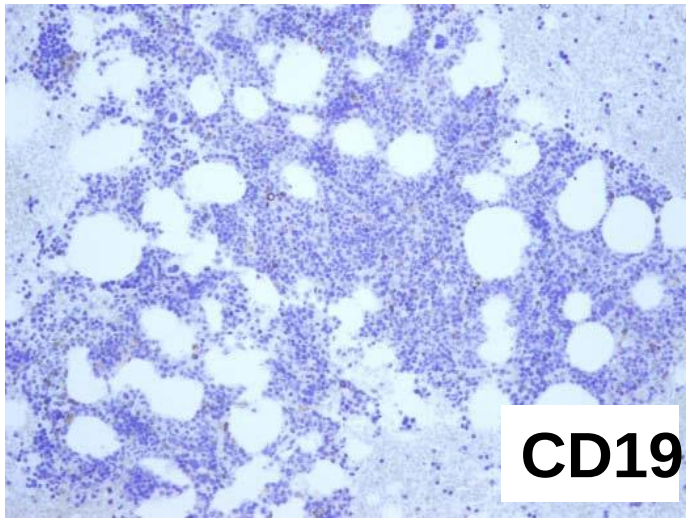
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

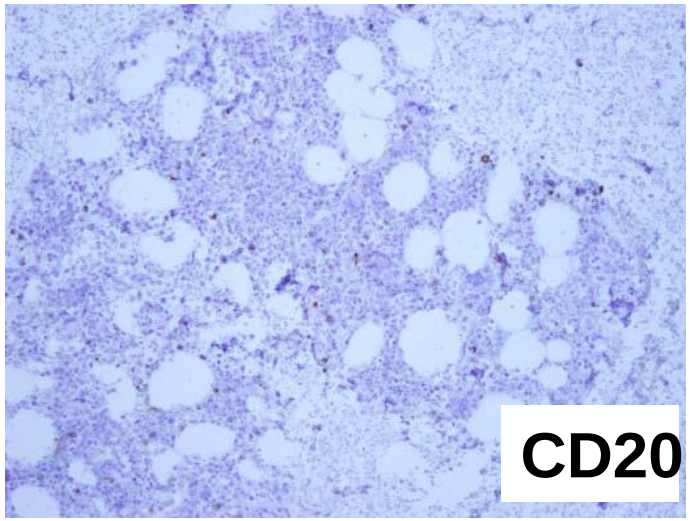
Before treatment



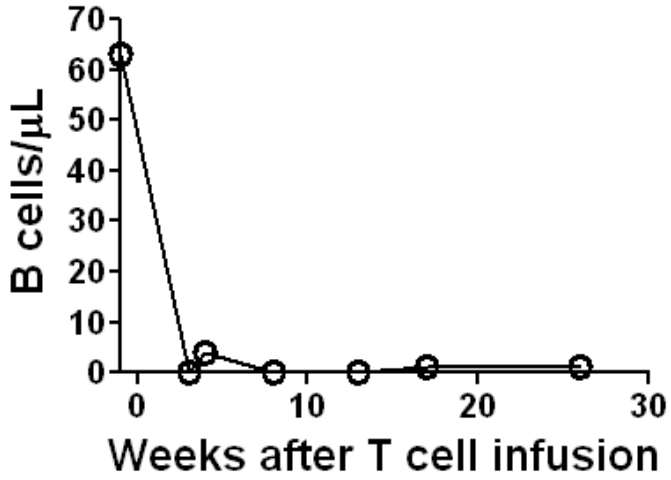


3 months after treatment

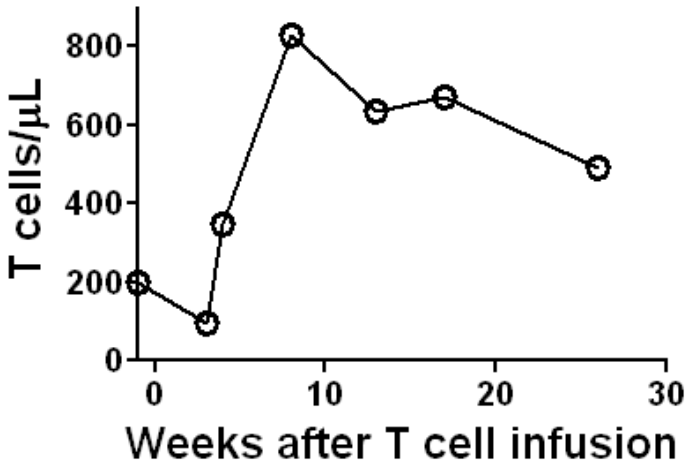


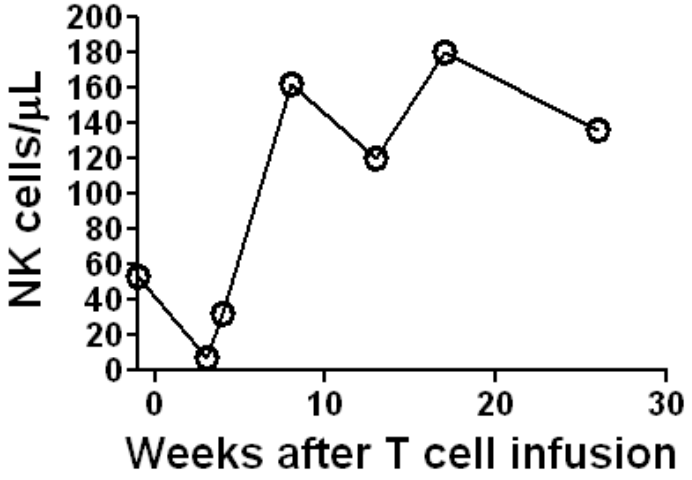


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion



In contrast, T and NK cell counts rapidly recovered after treatment





Patients with Refractory Lymphomas Treated with Anti-CD19 CAR in the Surgery Branch, NCI

Lymphodepleting chemotherapy:

300-500 mg/m^2 cyclophosphamide qd x 3

30mg/m^2 fludarabine qd x 3

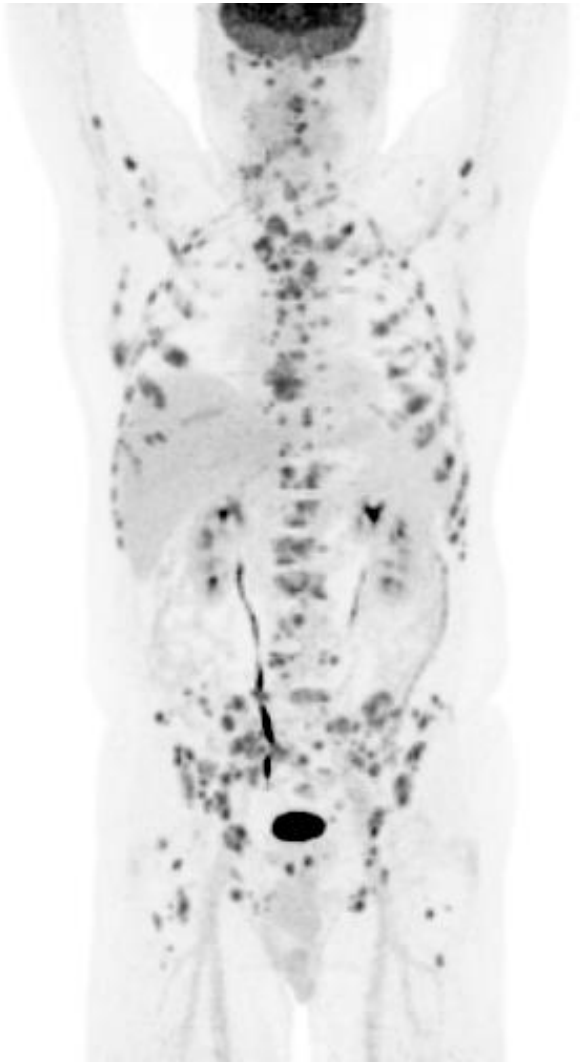
	Total	CR	PR	OR
			(number, duration months)	
DLBCL	19	9 (47%) (20+, 15+, 11+, 9+, 8+, 7+, 7+, 6+, 6+)	5 (26%) (14, 13+, 7, 3**, 1)	14 (73%)
Follicular	2	2 (19, 8+)	---	---
Mantle cell	1	1 (13+)	---	---

***LTFU**

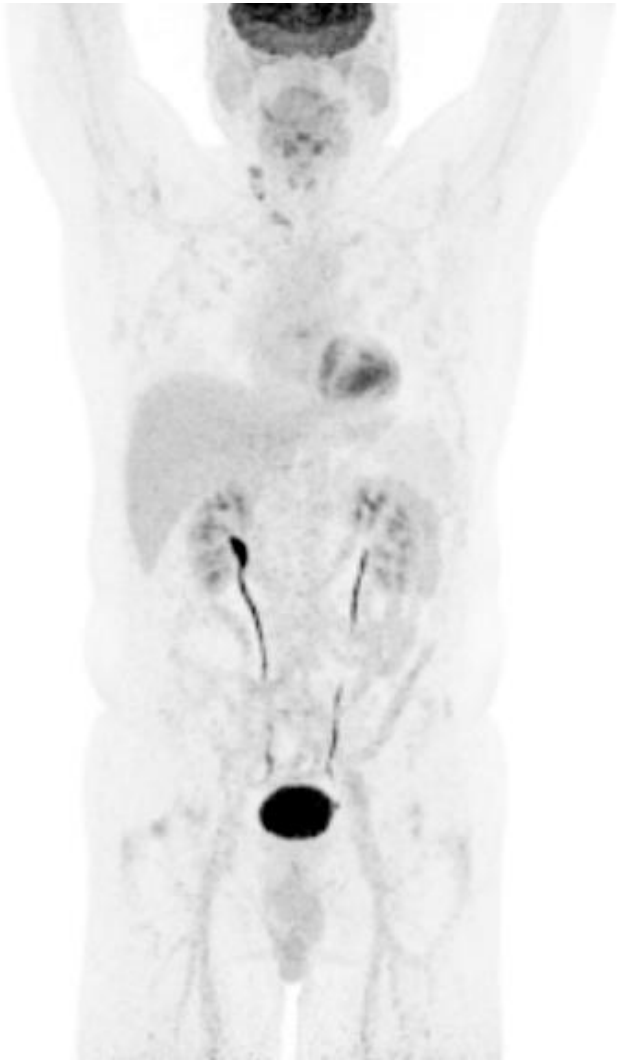
**** allotransplant in PR**

Patient with DLBCL after anti-CD19 T-cell infusion

Before treatment

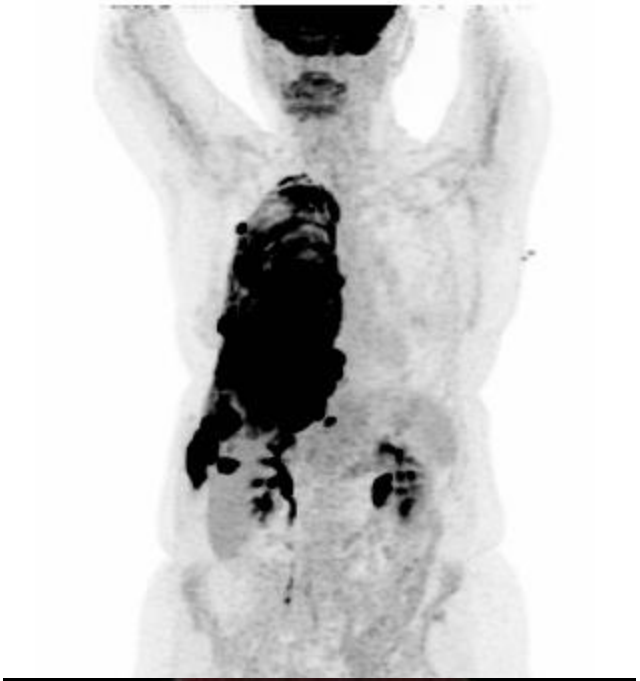


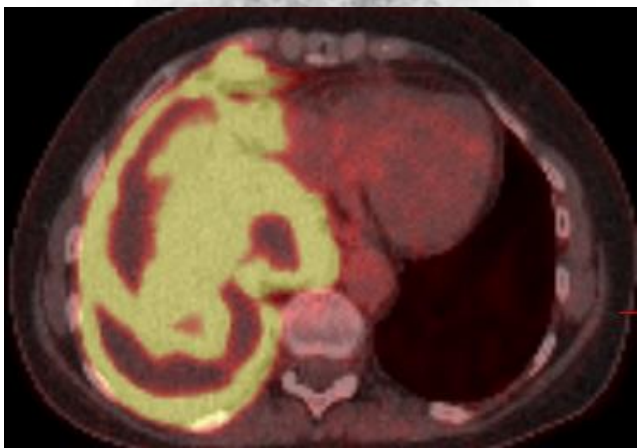
24 weeks after treatment



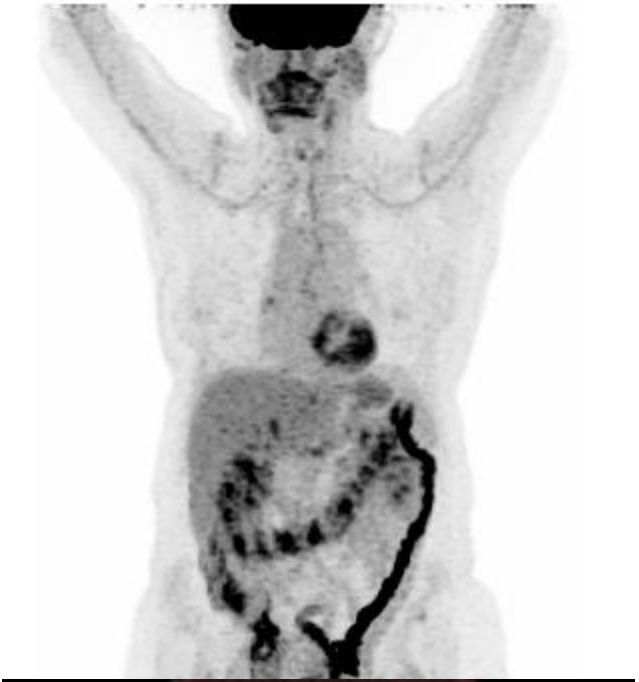
Patient with DLBCL after infusion of anti-CD19 CAR T cells

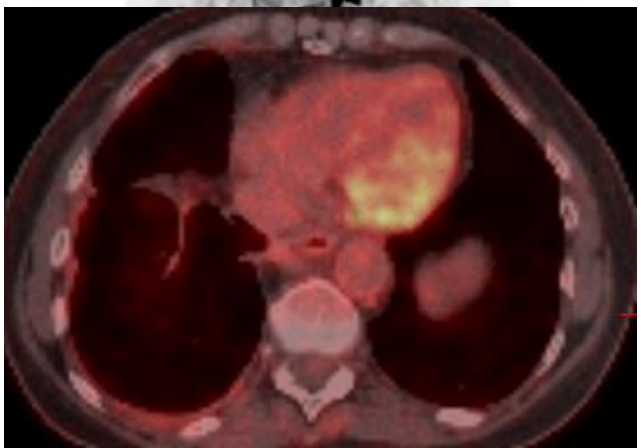
Before treatment





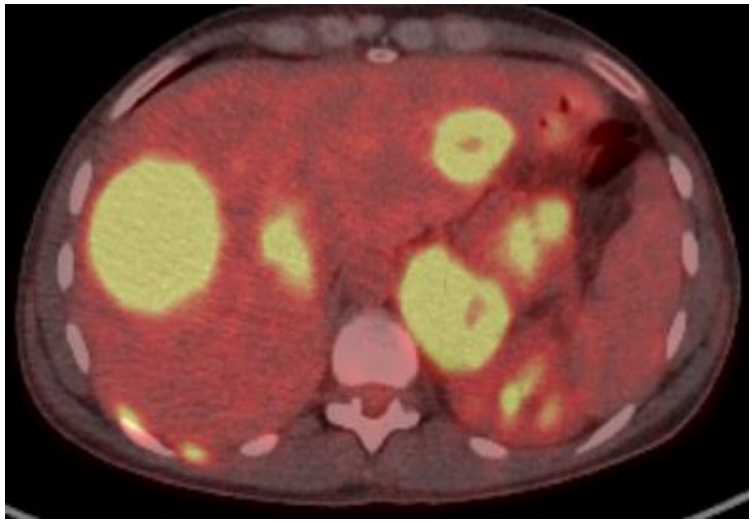
14 weeks after treatment



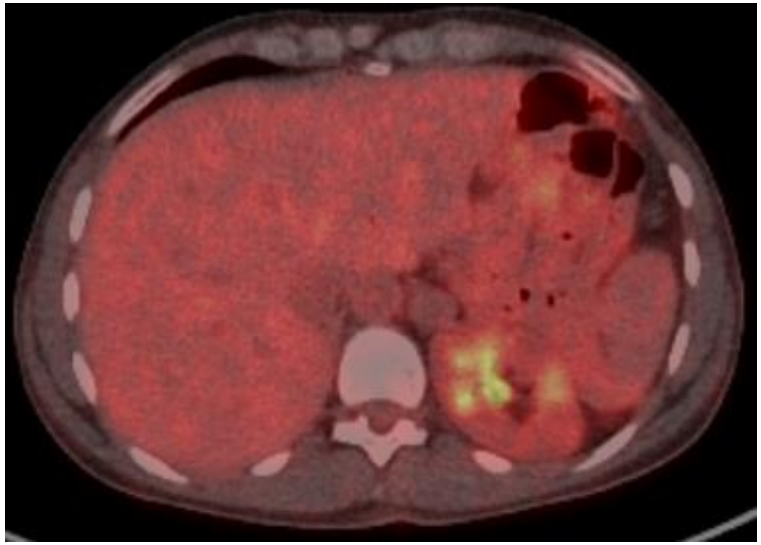


Complete remission of chemo-refractory primary mediastinal B-cell lymphoma ongoing 13 months after treatment

Before treatment



9 months after treatment



Categories of antigens to target using cell therapy

1. **Mutations unique to each individual cancer**

2. **Antigens expressed on cancers and on non-essential normal tissues (CD19, thyroglobulin)**

3. **Shared antigens unique to cancer (cancer-testes antigens)**

Cancer/Testes Antigens - Shared Tumor Specific Antigens

Expressed during fetal development

Restricted in their expression in adult normal tissues to germ cells

Up-regulated in 10-80% of cancers from multiple tissues

NY-ESO-1 Family

- **Small family of X-linked genes that includes NY-ESO-1 and LAGE-1**

MAGE Family

- **Family of ~ 45 X-linked genes**

Responses to Therapy with NY-ESO-1 TCR

	Total	PR	CR	OR
		number of patients (duration in months)		
Melanoma	19	6 (32%) (10**, 28, 8, 6+, 3, 3)	4 (21%) (58+, 54+, 28, 40+**)	10 (53%)
Synovial Cell Sarcoma	15	9 (60%) (47+**,18*, 12**,10, 8, 7, 5, 4, 3**)	1(7%) (20+)	10 (67%)

*treated twice

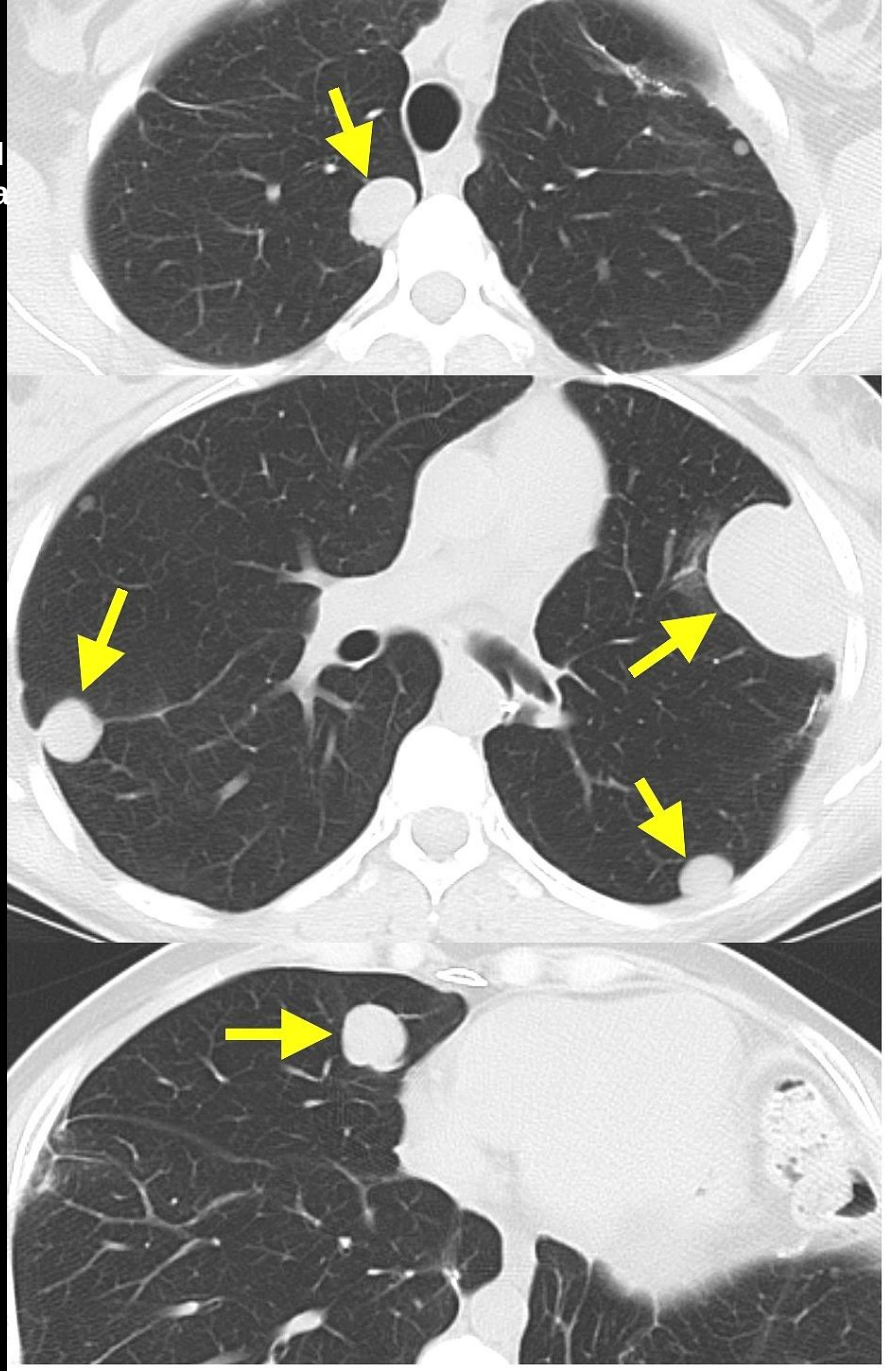
**plus ALVAC vaccine

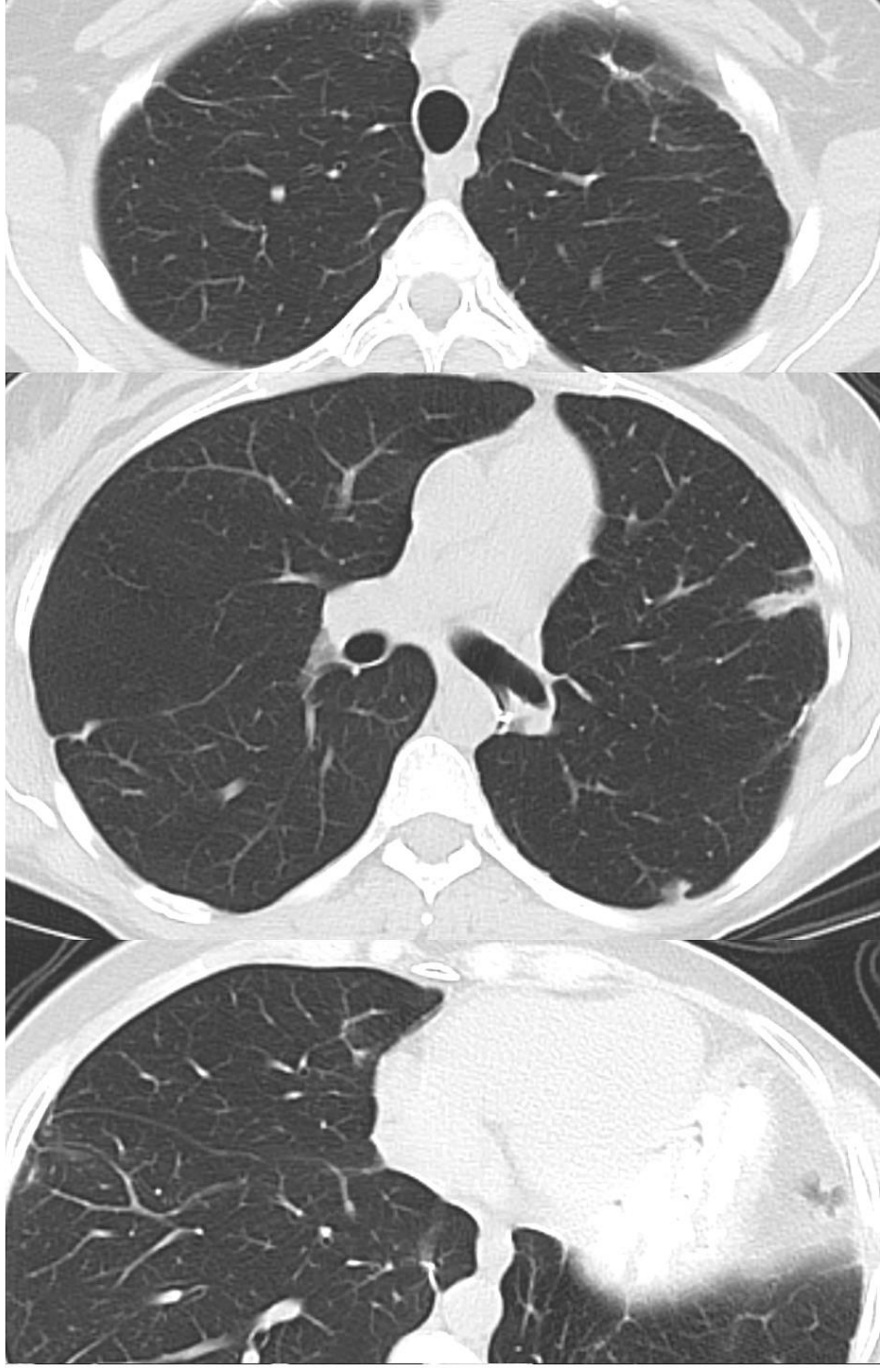
(Robbins et al J Clin Oncol 29:917, 2011; Clin Cancer Res 21:1022,2015)

H.K.

Synovial Sarcoma

ESO TCR



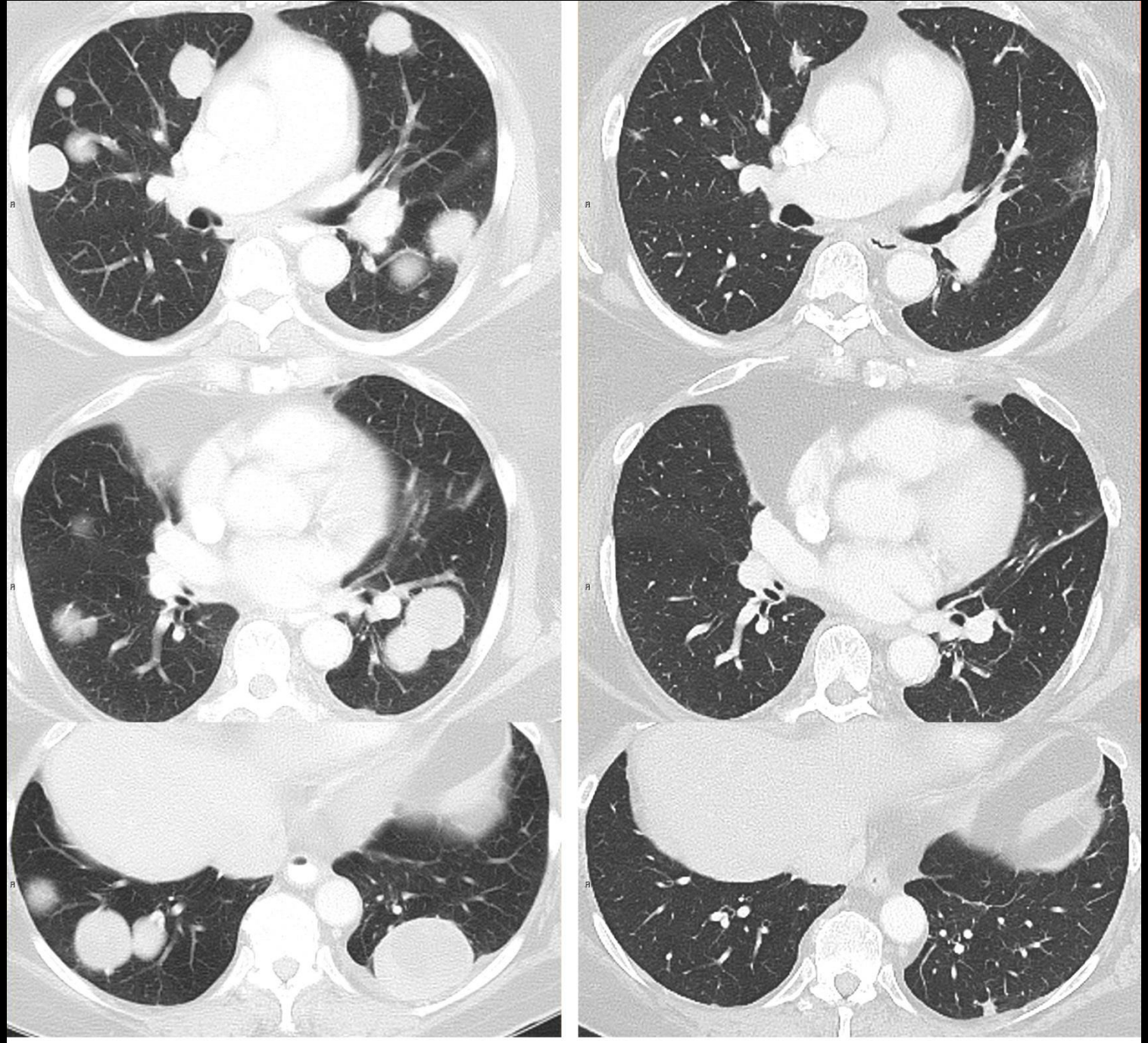


Pre-Treatment

14 Months

A.R.
Synovial sarcoma

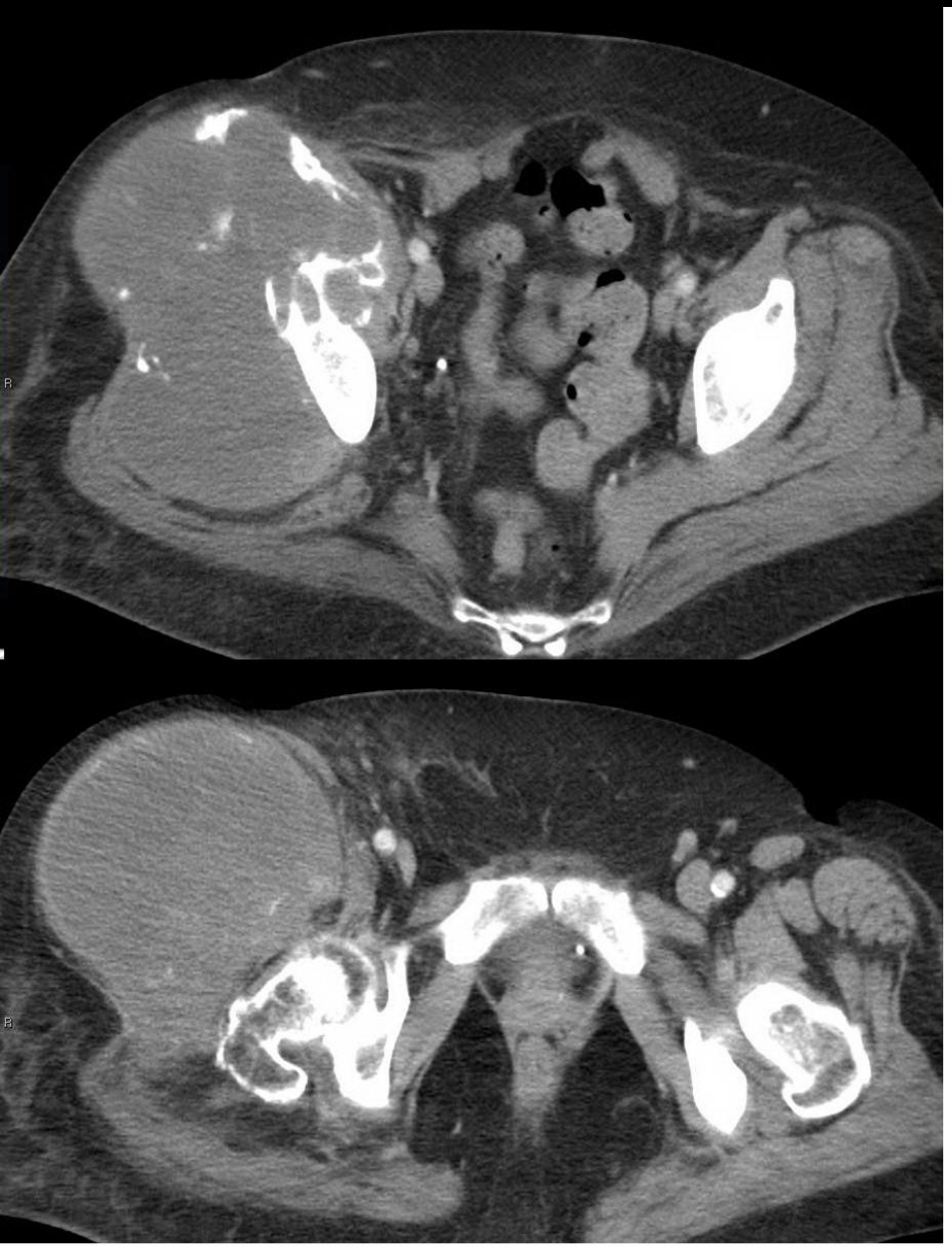
NY-ESO-1 TCR

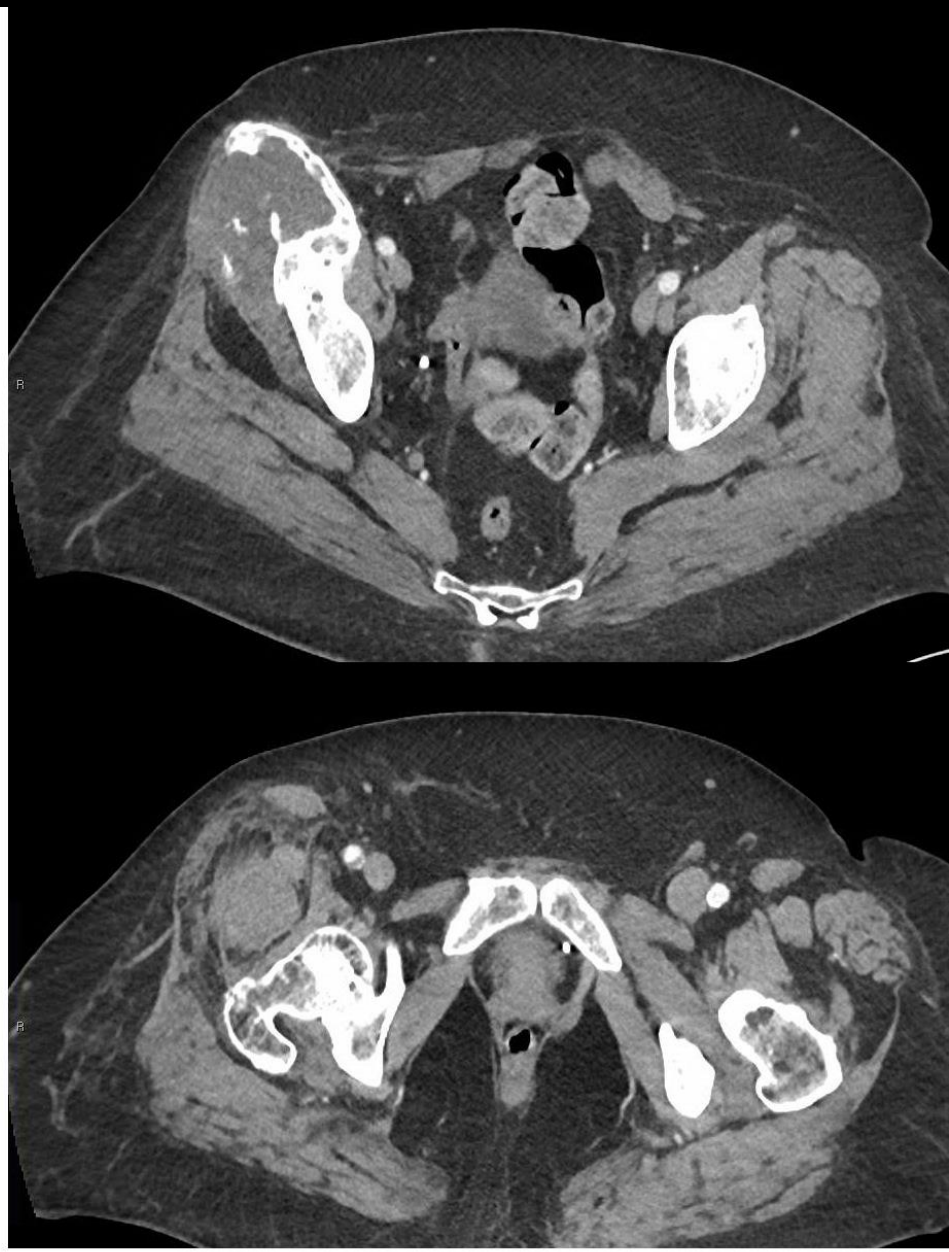


August 2010

Feb 2015

A.R. Synovial sarcoma NY-ESO-1 TCR





August 2010

Feb 2015

Conclusions

Cell transfer therapy can mediate durable regressions in patients with metastatic cancer refractory to other treatments.

T-cells that recognize unique somatic mutations can be found in TIL and PBL.

Identification and targeting of mutations unique to each cancer has the potential to extend cell therapy to patients with common epithelial cancers.

Autologous lymphocytes genetically engineered to express TCRs or CARs can mediate the regression of metastatic cancers.