The Development of Cellular Immunotherapy for Patients with Cancer

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No personal disclosures.

GOAL

Develop effective immunotherapies for patients with metastatic cancer based on the adoptive transfer of immune cells with anti-cancer activity

Cancer Deaths 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

1 in 2-3 Americans will develop an invasive cancer

1 in 5 Americans will die of cancer

Major Challenge Confronting Cancer Immunotherapy

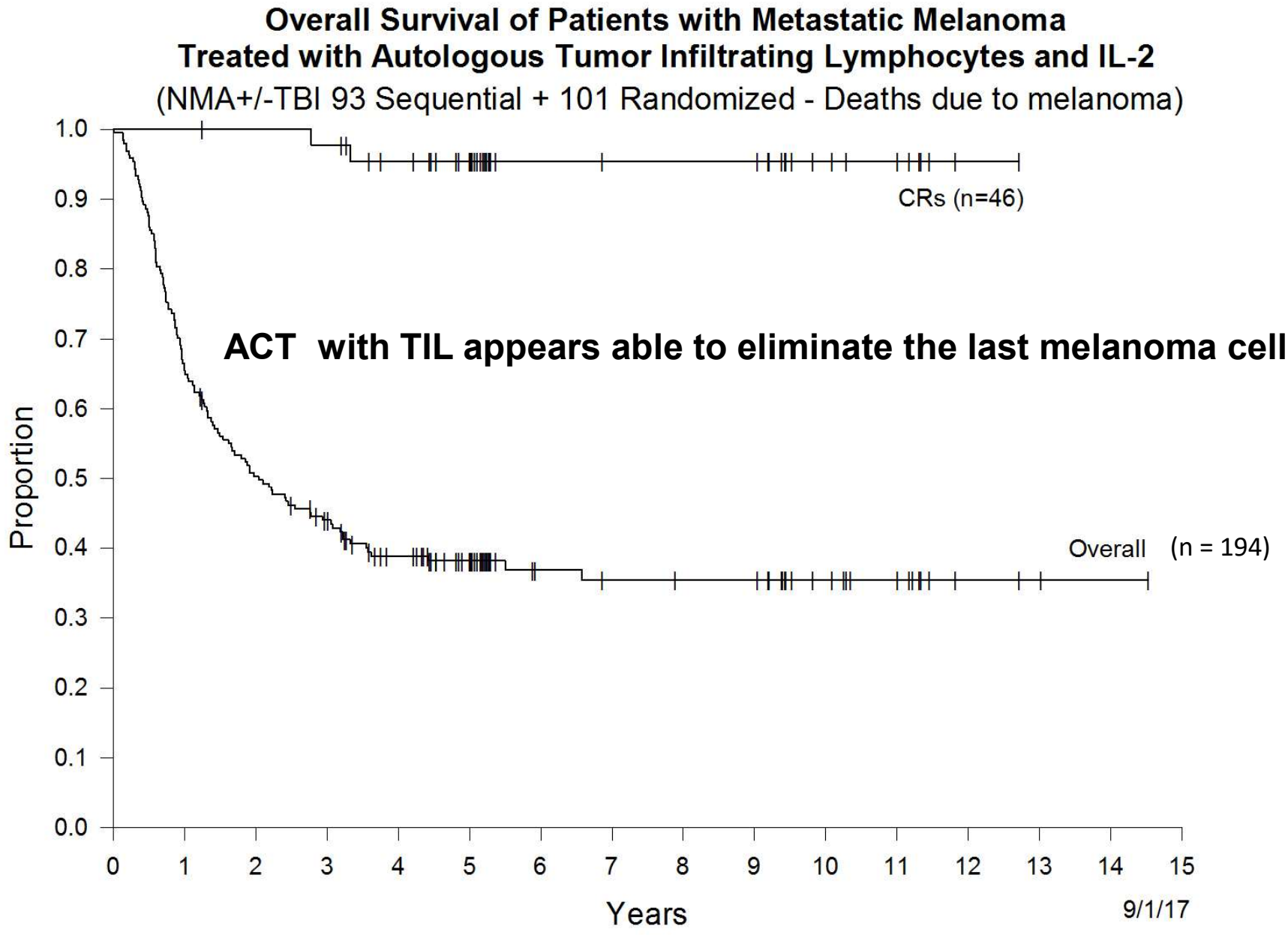
The development of effective immunotherapies for patients with metastatic epithelial solid cancers that cannot be cured by any available treatment and result in 90% of cancer deaths.

Summary of Cell Transfer Protocols for the Treatment of Patients with Metastatic Melanoma* (median f/u over 8 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: 8.6 years)

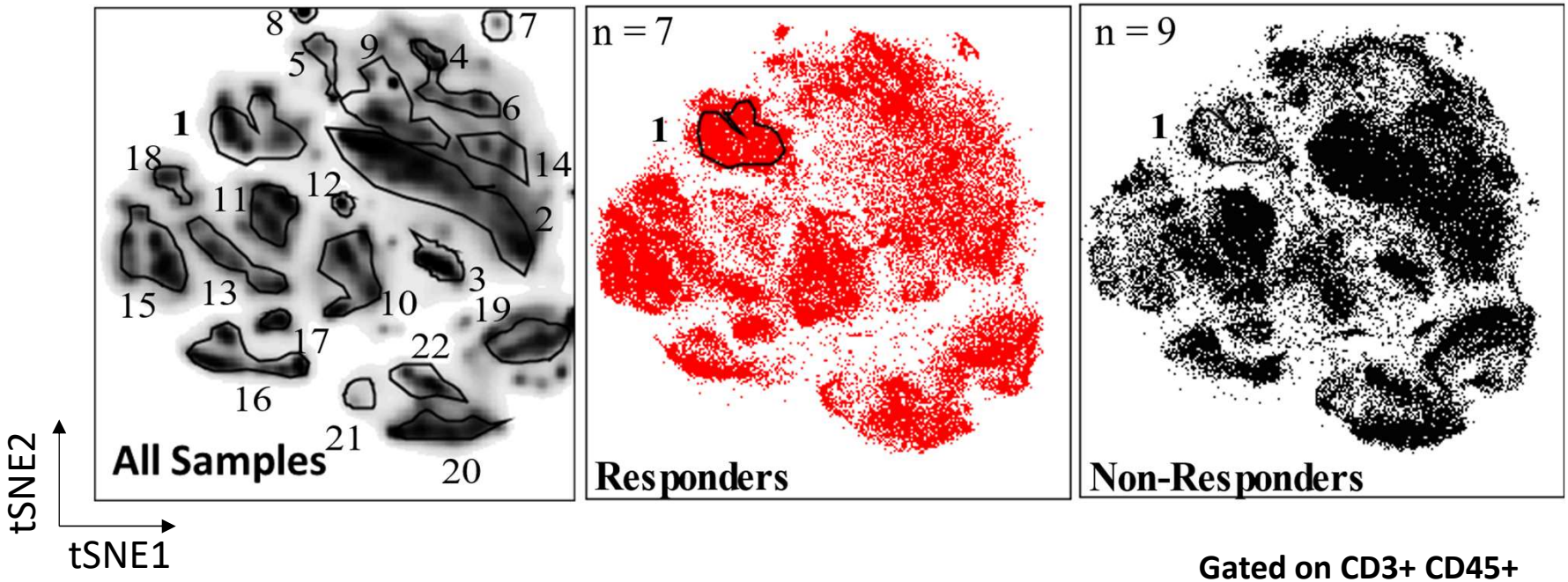
Question

What are the phenotypic characteristics of the cells that mediate cancer regression in vivo?

<u>Approach:</u>
High dimensional single cell transcriptome analysis of up to 10,000 cells

(tSNE analysis: t-distributed stochastic neighbor embedding)

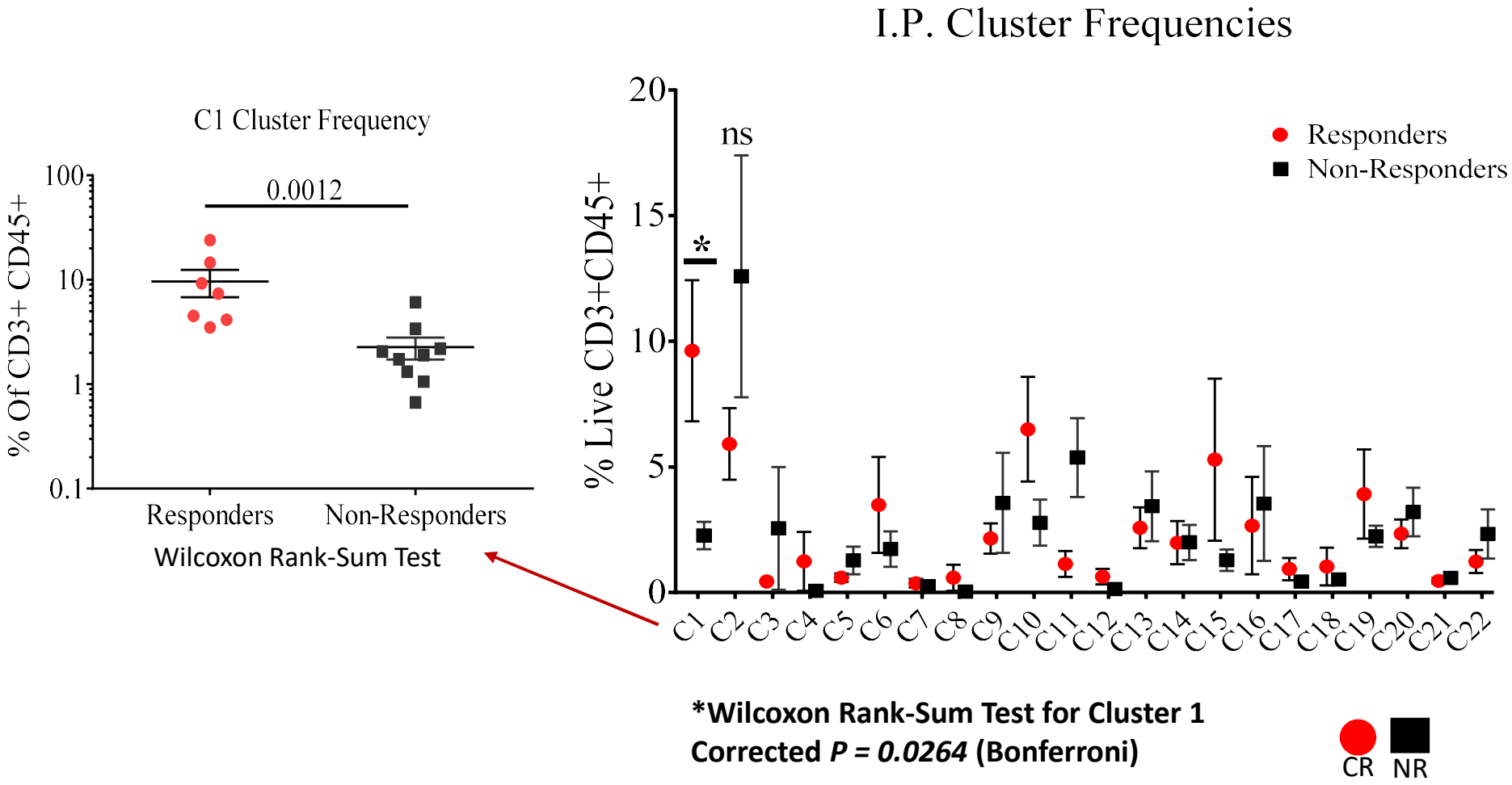
Pilot study of 16 melanoma Rx1 I.P samples by CyTOF



Gated on CD3+ CD45+
22 Clusters were defined

(Sri Krishna, Frank Lowery)

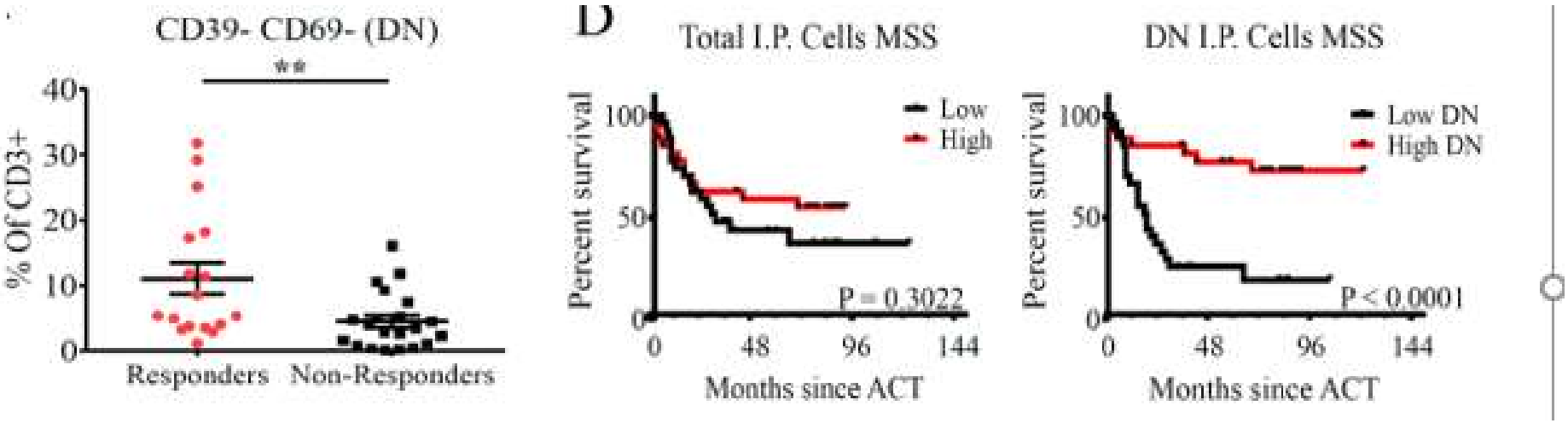
CyTOF analyses of 16 pilot Rx1 I.P. samples indicated that Cluster 1 is significantly higher in CR Rx1 relative to NR Rx1



Cluster 1 was highly enriched in CD39neg,CD69neg cells.

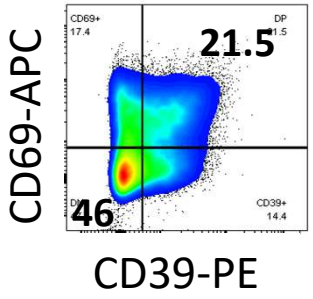
(Sri Krishna, Frank Lowery)

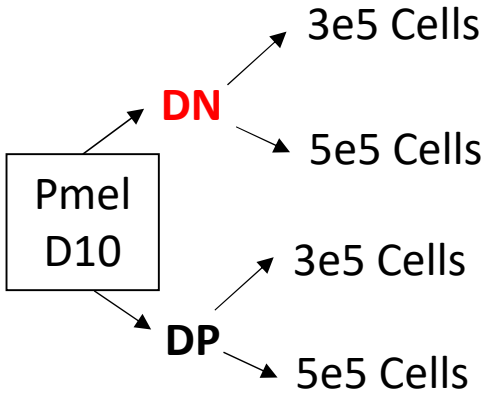
CD39neg,CD69neg "stem-like" lymphocytes appear to be the effector cells responsible for melanoma treatment



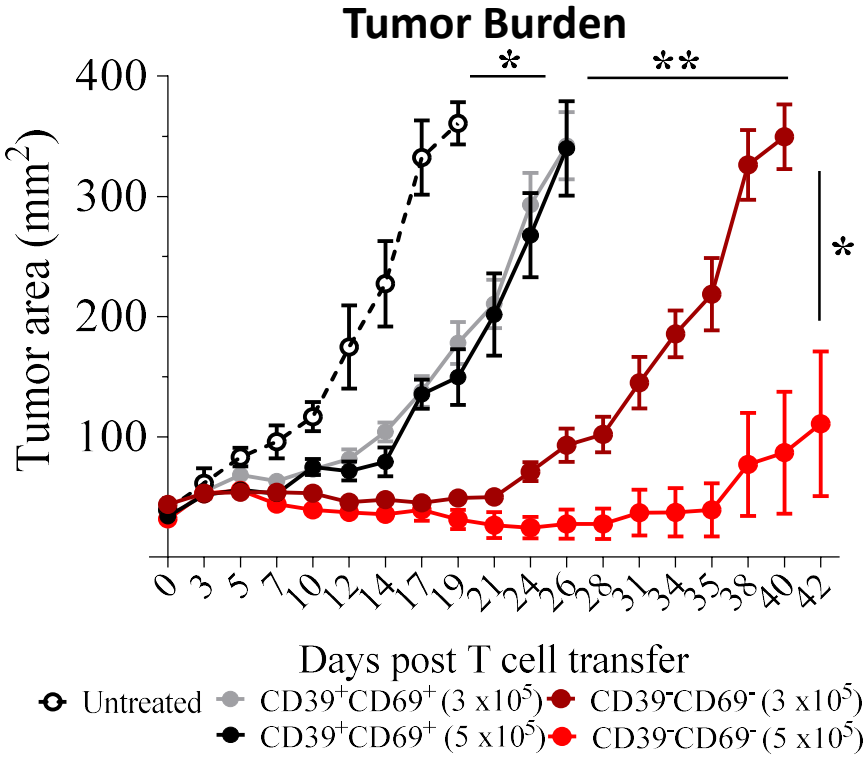
(Sr Krishna, Frank Lowery

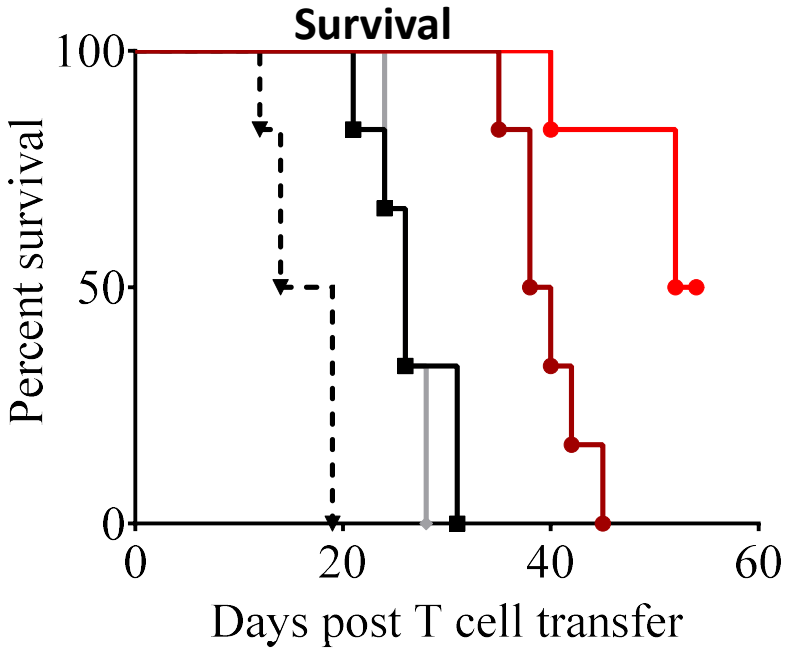
CD8+ DN T-cells are curative in murine tumor ACT models





(No Vaccine)
N = 6 mice per group





Devikala Guruswamy, Zhiya Yu)

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 product 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.

Screen all Cancer Mutations to Test their Immunogenicity

Whole exome sequencing and RNA-Seq to identify all cancer mutations

Introduce every cancer mutation into the patient's antigen presenting cells (APCs) as a 25mer with the mutated amino acid in the middle

Coculture APCs with patient TIL that mediated cancer regression

If TIL recognize the mutation interferon-gamma is secreted thus identifying the cancer antigen

<u>Advantages</u>

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.

Immunogenic Mutations in Patients with Metastatic Melanoma

Patients evaluated (#)	Median (# of mutations)	Total	Screened (of total)	Immunogenic Epitopes (#)
76	318	44381	13400 (30%)	180

Patients with mutation-reactive T cells in TIL: 55/76 = 72%

Immunogenic mutations (of # screened): 180/13400 = 1.3%

8% CD4
92% CD8

All neoantigens were unique, none shared

Immunogenic Mutations in Patients with Gastrointestinal Cancers

Patients Evaluated	Patients with Neoantigens	Median	Total	Screened	Immunogenic
(number)		(number of mutations)			
130	104/80%	123	20046	15256 (76%)	210

Immunogenic mutations of number screened: 210/15256 = 1.3%

47% CD8
53% CD4

All neoantigens were unique except 2 patients shared the same KRAS mutation restricted by Cw*0802

Updated 10/8/20

Mutated Antigens Recognized by TIL from 195 Patients with Epithelial Cancers

Cancer	# of patients screened	# of patients with neoantigen reactivity	Total # of neoantigens recognized
Colorectal	95	80 (84%)	166
Anal	1	1 (100%)	1
Cholangiocarcinoma	14	10 (71%)	17
Pancreatic	11	7 (64%)	11
Esophageal	5	4 (80%)	7
Endometrial	3	3 (100%)	4
Breast	43	29 (67%)	100
NSCLC	11	8 (73%)	34
Ovarian	7	6 (86%)	16
Stomach	4	2 (50%)	6
Prostate	1	1 (100%)	1
Total	195	151 (77%)	363

All neoantigens were unique except for 2 KRAS antigens.

An advantage of targeting mutations is its applicability to target multiple cancer types.

Responses in Patients with Chemorefractory Metastatic Solid Epithelial Cancers

TIL	**0/20**	**0**
Selected TIL	**3/25**	**12 %**
Selected TIL + Pembro	**6/26**	**23 %**

Patient M.B.

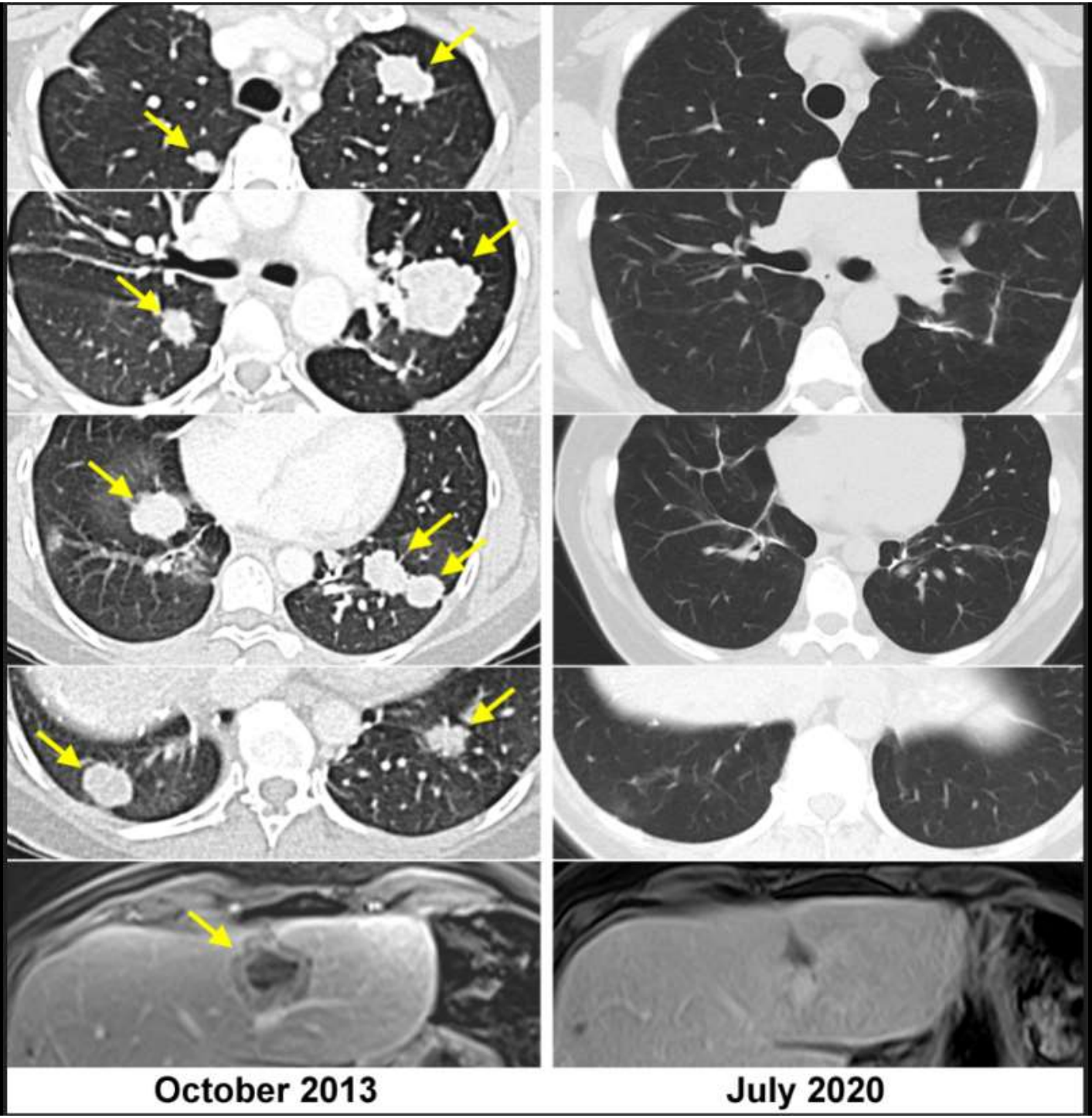
45 y.o. female with metastatic cholangiocarcinoma

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 with cells selected to target a unique ERBB2IP cancer mutation (of 26 mutations)**

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

M.B.

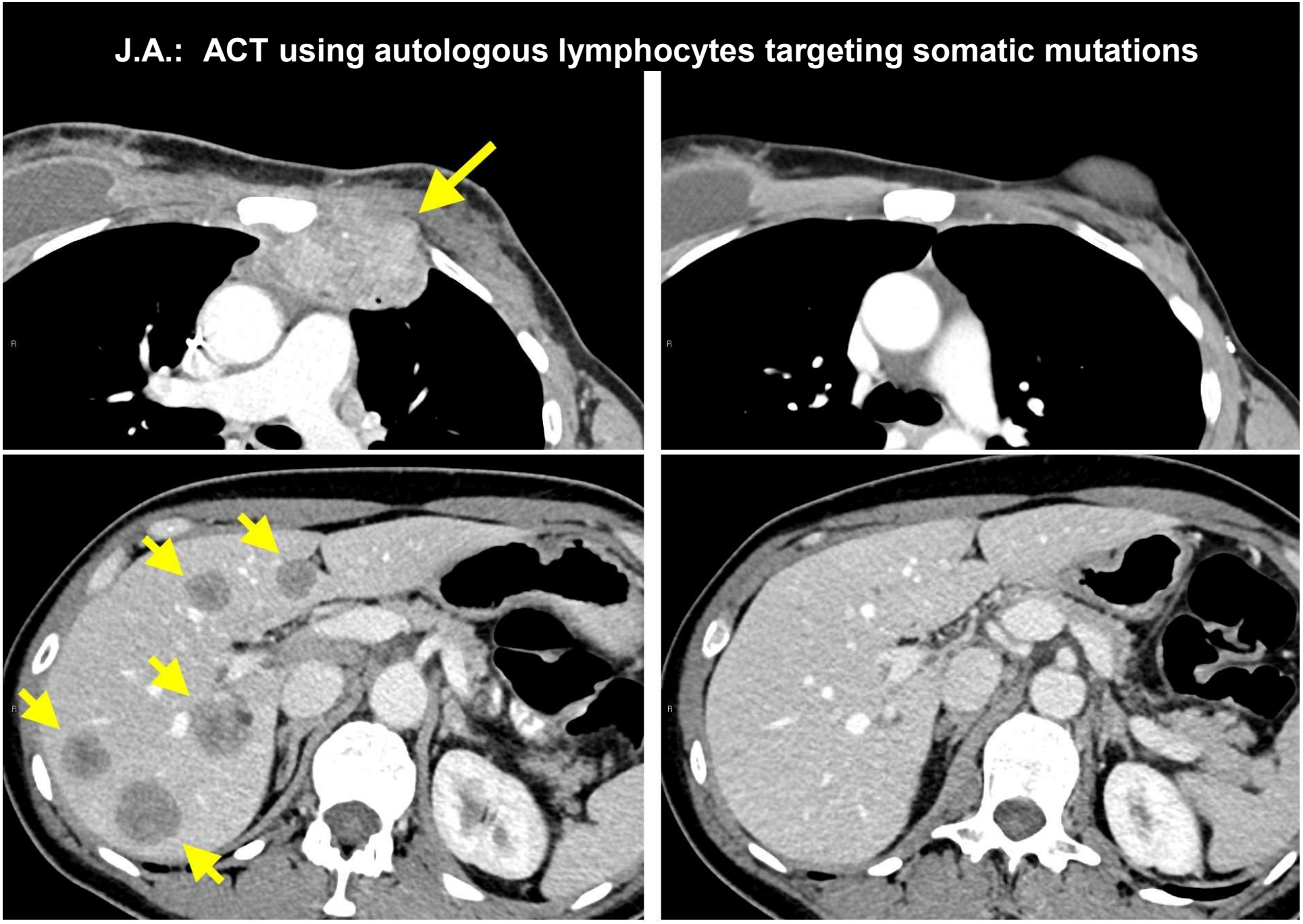
Selected TIL



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 She is now in an ongoing complete response of multiple nodal, chest wall, and liver metastases 58 months after treatment	

(Zacharakis et al, Nature Med, June 2018)


J.A.: ACT using autologous lymphocytes targeting somatic mutations
Pre-Treatment
14 Months

Apparently Random Somatic Mutations wereTargeted in this Patient with Metastatic Breast Cancer

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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

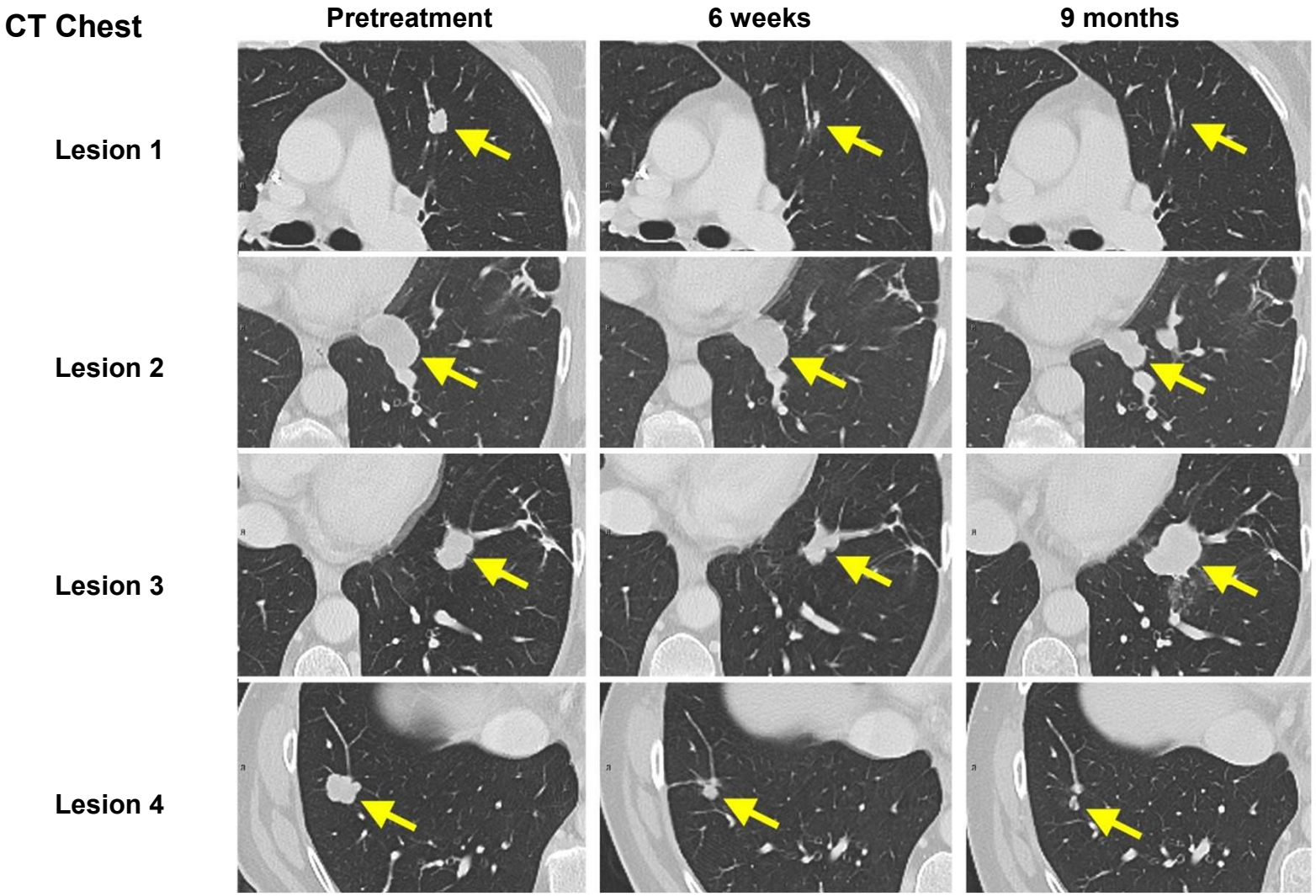
23% of infused cells contained neoantigen reactivity. All 8 neoantigen TCR present in PBL at 6 weeks.

Patient C.R. Treated with anti-Kras Reactive Lymphocytes

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)**

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 over 5 years after treatment

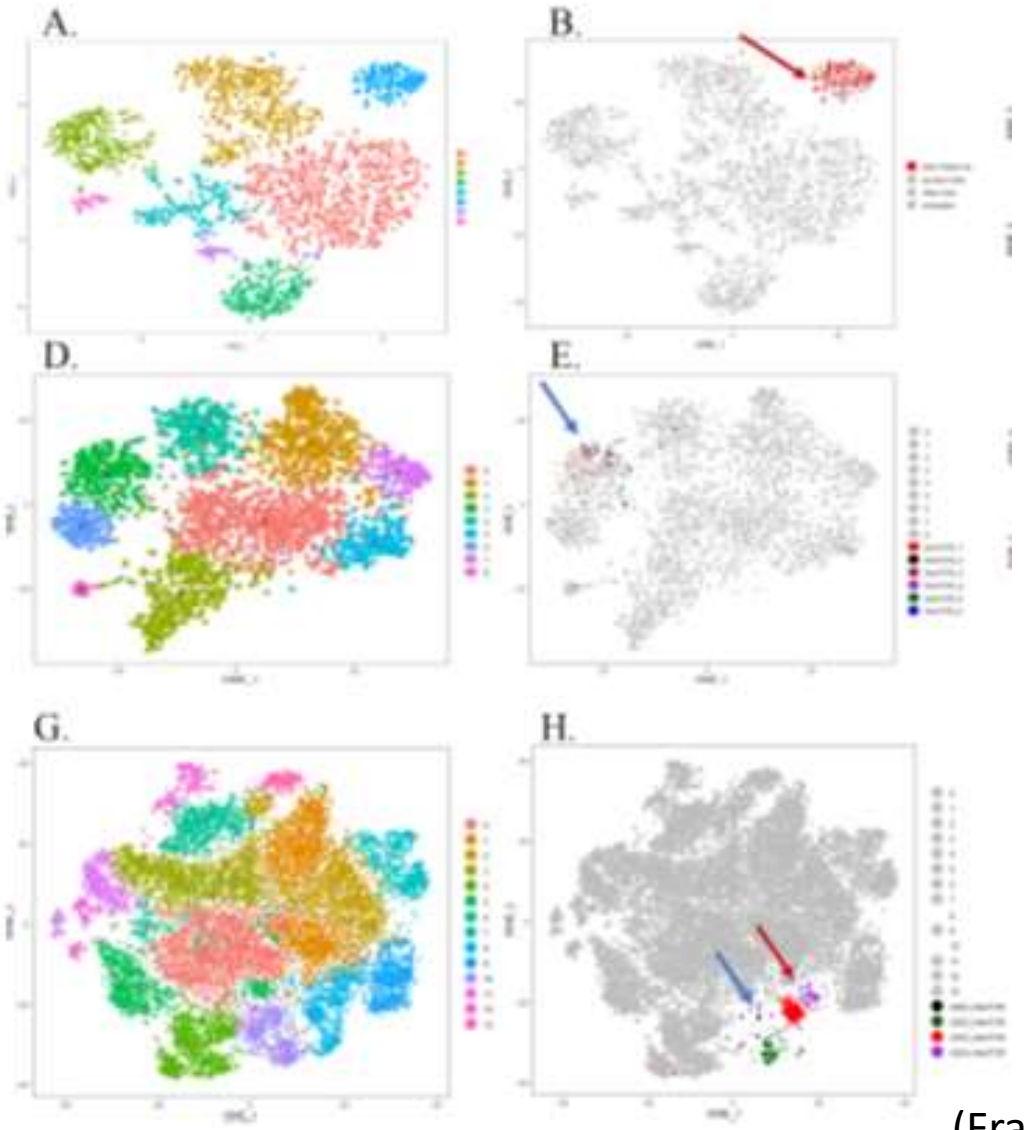
RNA analysis of freshly isolated TIL from metastatic cancer

gene expression | TCR overlay

Rectal cancer

Breast cancer

9 cancer types



(Frank Lowery, Sri Krishna)

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

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.

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”

TCRs available for the treatment of patients with p53 mutations

using “off-the-shelf” anti p53 TCRs

TCR source	p53 mutation	p53 mutation frequency (%) *	HLA restriction	HLA frequency (%)†	Potentially treatable patient (%)
4141; 4196	R175H	4.406	A*02:01	47.40	2.088
4259	Y220C	1.521	A*02:01	47.40	0.721
4273	R248W	2.527	DPB1*02:01	27.30	0.690
4149; 4343	Y220C	1.521	DRB3*02:02	32.80	0.499
4127	G245S	1.494	DRB3*02:02	32.80	0.490
4285	R175H	4.406	DRB1*13:01	10.00	0.441
4259	Y220C	1.521	DRB1*04:01	17.30	0.263
4304	M237I	0.411	DRB1*01:01	20.00	0.206
4266	R248W	2.527	A*68:01	2.80	0.071
4324	T211I	0.076	C*06:02	11.20	0.009
4293	Y236S	0.018	DRB3*02:01	0.33	0.006
4350	L111R	0.02	DRB1* 08:03	7-20% in Chinese populations	0.004
4350	L111R	0.02	A*11:01	14.00	0.003
4114	C135R	0.08	Class II	na	na
Total					**5.5%**

(P. Kim)

iTCR THERAPY OF BREAST CANCER: PATIENT M.K.

48 yo female with breast cancer

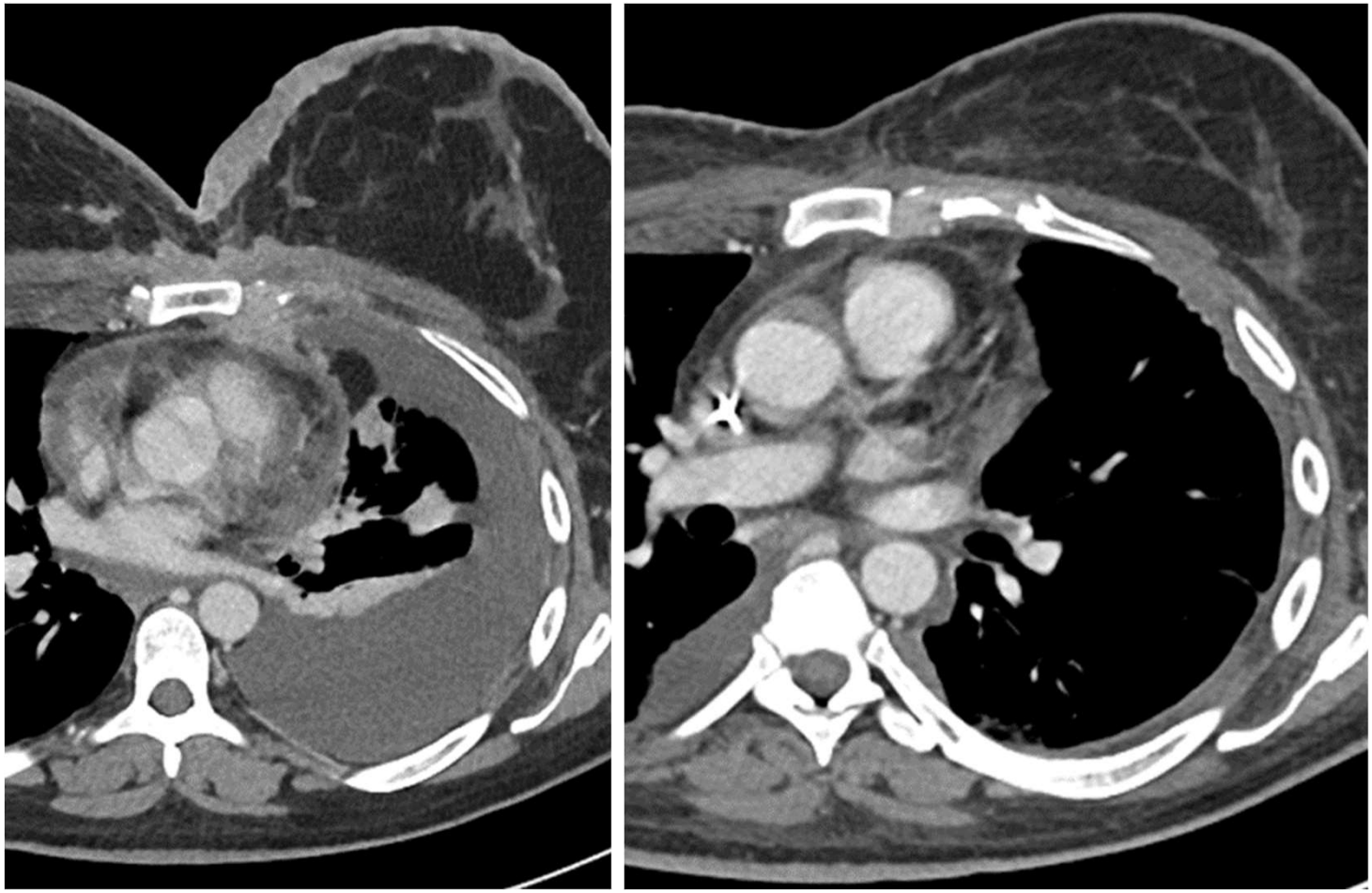
Feb. 2012	**Lumpectomy, l.n., dissection, local radiotherapy & tamoxifen for ER^-, PR^-, $Her2^+$ & breast cancer**	
Sept. 2014	**Local recurrence** **Doxataxel, Carboplatin, Herceptin**	**Progressed**
March 2015	**Navelbine, Herceptin, Pertuzumeb**	**Progressed**
Aug. 2015	**Kodcyla**	**Progressed**
July 2016	**Xeloda, Herceptin**	**Progressed**
Feb. 2017	**Lapatimid, Herceptin**	**Progressed**
Dec. 2017	**Ibrance, Anastrazole, Herceptin**	**Progressed**
Dec. 2018	**Doxil**	
May 2019	**Taxol, Herceptin**	
Sept. 2019	**NCI** **Pericardial window for tamponade** **ACT with $5.3x10^{10}$ (p53 cells)**	

90% response: Recurred at 6 months

Breast cancer: Treatment with anti-p53 (R175H) TCR

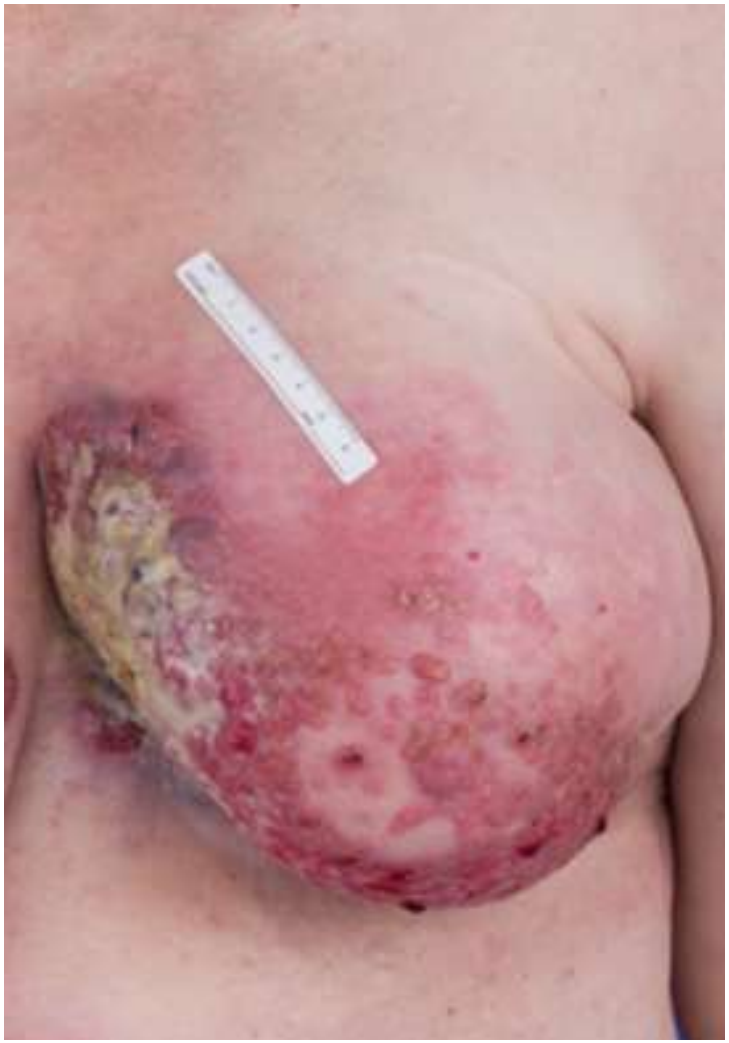
PRE

Day 60

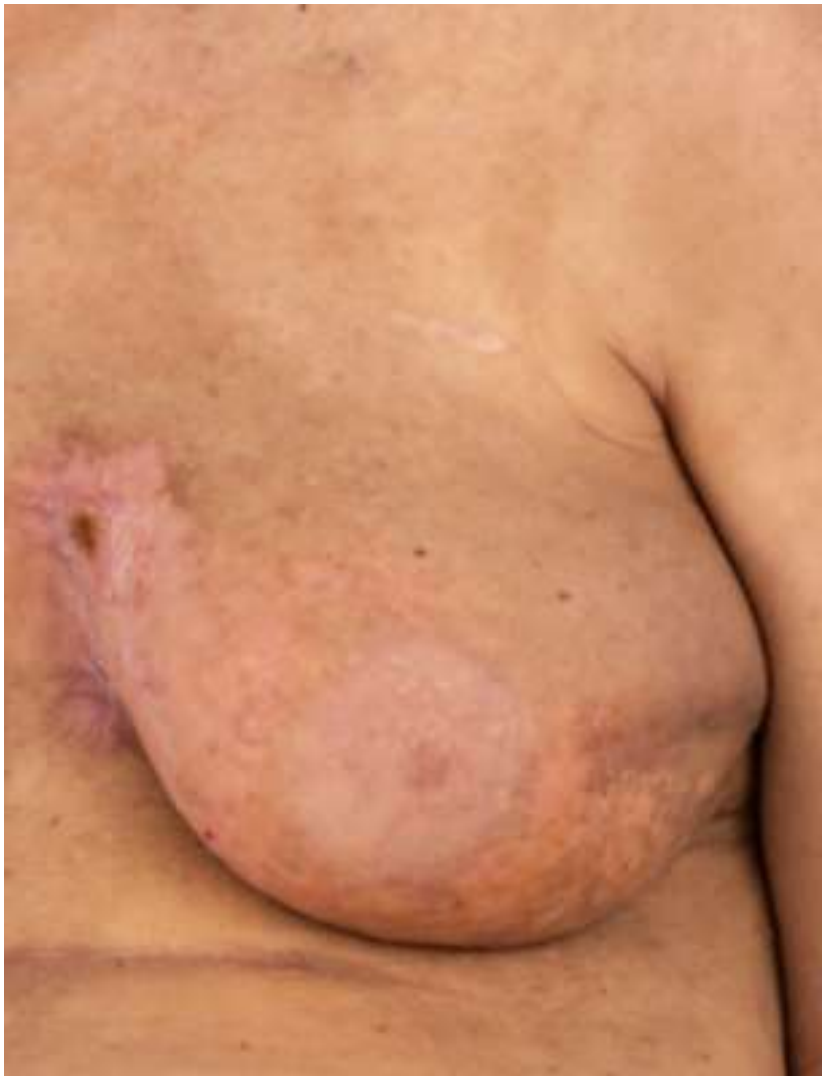


Breast cancer: Treatment with allogeneic anti-p53 (R175H) TCR

9-18-19 Pre



1-6-20 Day 60



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)

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.

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)

KRAS Mutations in Human Cancers

Kras protein is a GTPase essential for normal tissue signaling.

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

Eight “hotspot” mutations in the KRAS gene at amino acid positions 12, 13, and 61 account for greater than 80% of all KRAS mutations.

Library of TCRs Available for Treatment of Patients with KRAS Mutations

KRAS Mutation	Mutation Frequency (%)	HLA Restriction (%)	HLA Frequency (%)	Potentially Treatable Patients (%)
G12D	35 %	C*0802	8 – 14 %	2.8 %
		A*1101	14 – 30 %	4.9 – 10.5 %
		DRB3*02	32 %	11.2 %
G12V	24 %	A*1101	14 – 30 %	3.4 – 10.5 %
		C*0102	1.3 – 7.2 %	1.3 – 7.2 %
		DRB3*02	32 %	11.2 %
		DRB1*0701	26 %	6.1 %
		DRB1*0301	20 %	4.8 %
		DRB1*0101	18 %	4.3 %
G12R	3 %	DQA1*0505	35 %	1 %
		DRB5*01	35 %	1 %
TOTAL				**29 %**

(Noam Levin, 2020)

Immunogenic Mutations in Patients with Metastatic Breast Cancer

Patients evaluated	Median	Total	Screened	Immunogenic Epitopes
(#)		(# of mutations)	(of total)	(#)
43	119	7936	4722 (60%)	100

Patients with mutation-reactive T cells in TIL: 29/43 = 67%

Immunogenic mutations (of # screened): 100/4722 = 2.1%

74% CD4
26% CD8

All neoantigens were unique, none shared

(updated 10/8/20)

CAR-T cells for the Treatment of Solid Epithelial Cancers

CAR-T cells require the use of monoclonal antibodies (MoAb) that recognize molecules on the cell surface.

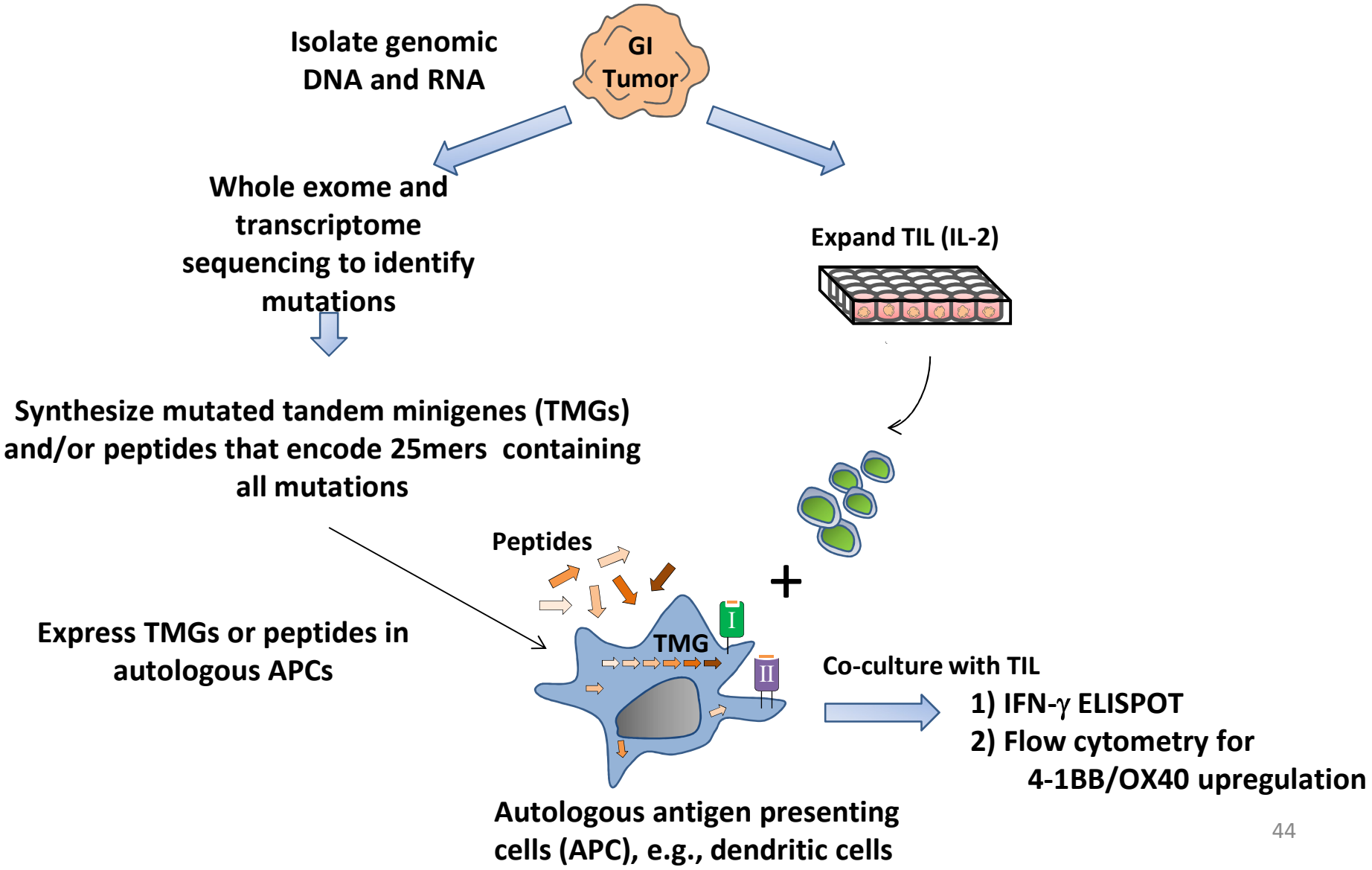
MoAb were first described by Kohler and Milstein 45 years ago. (Nature 256:495-7, 1975)).

Two major problems:

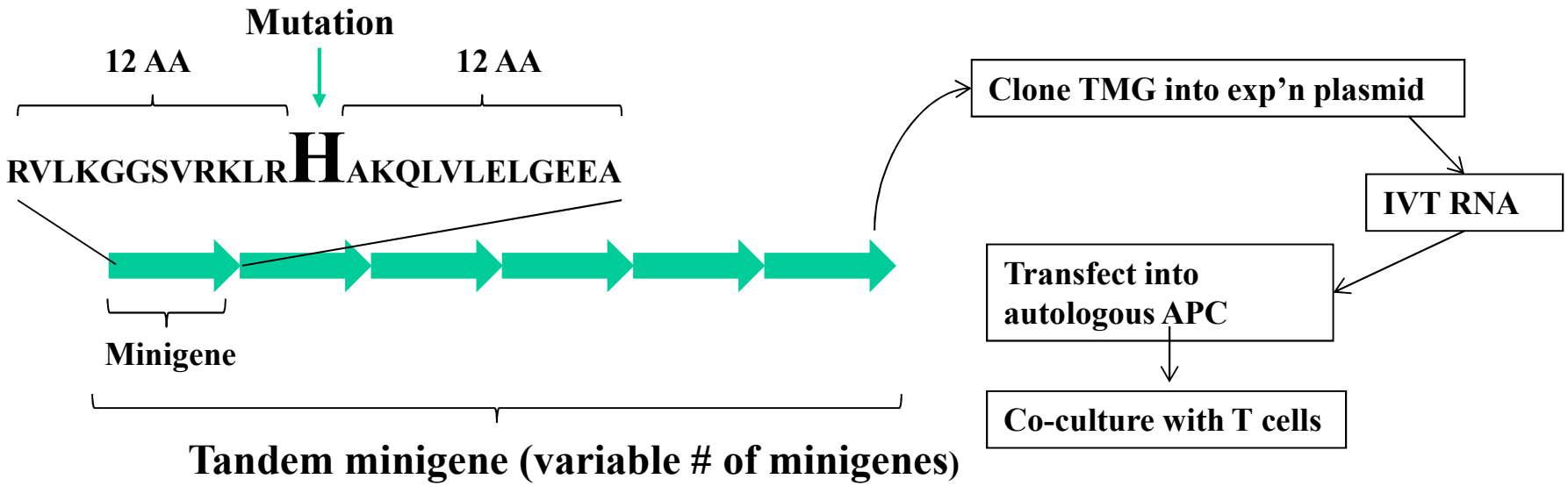
1) No MoAb have been identified that recognize cell surface molecules unique to epithelial cancers.

2) Normal cells expressing the target of MoAb are highly sensitive to destruction.

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



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



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)

Two Critical Issues in the Development of Cancer Immunotherapies

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Identify the antigenic targets on the cancer cell

Identify the characteristics of the immune cells that recognize and destroy cancer cells

Potential improvements in targeting of somatic mutations in epithelial cancers

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Increase the frequency and number of mutation reactive cells in the infusion product

- **Enrich the mutation-reactive cells using markers in the fresh cell suspension or upregulated after activation (41BB)**
- **Transduce mutation-reactive TCRs into naïve or CM cells (FDA approval 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

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

ADVANTAGES OF CELL TRANSFER THERAPY

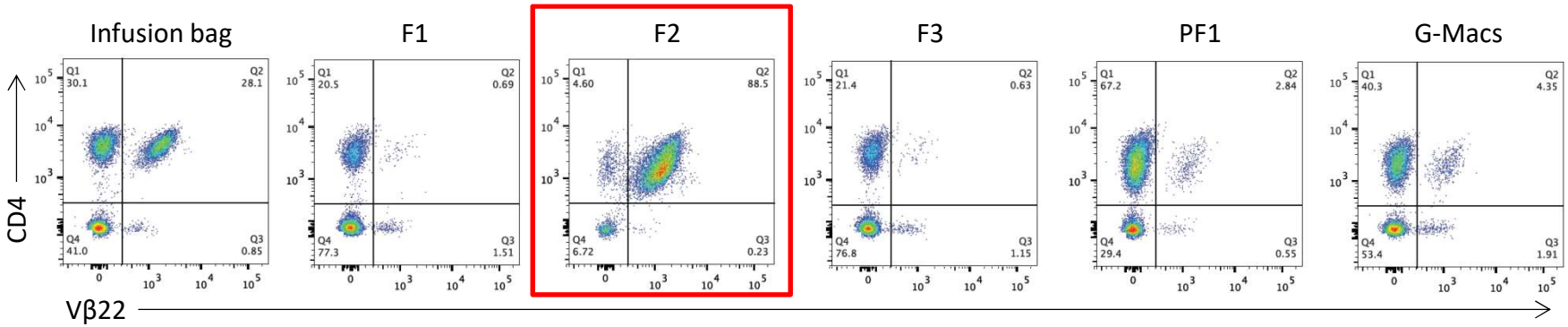
1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

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.

Isolation of ERBB2IP reactive cells



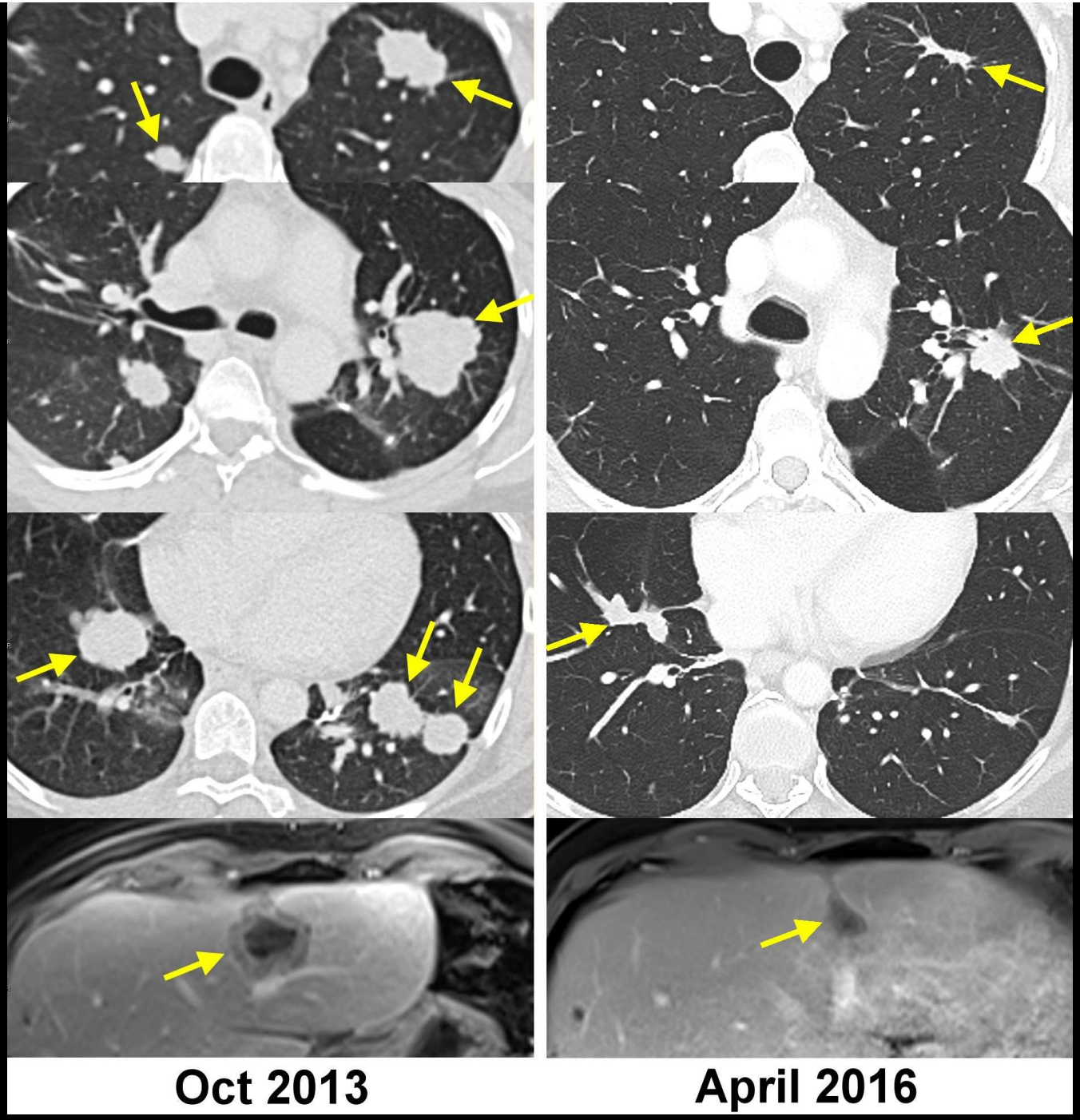
Use enriched ERBB2IP autologous lymphocytes for treatment

Objective response of lung and liver metastases ongoing for over 7years

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

M.B.

Selected TIL



P53 Mutations in Human Cancer

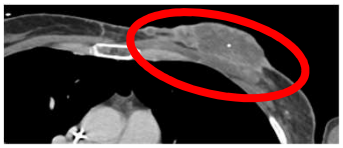
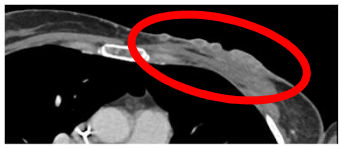
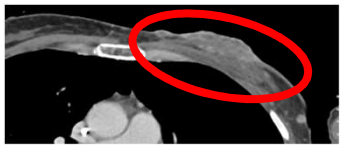
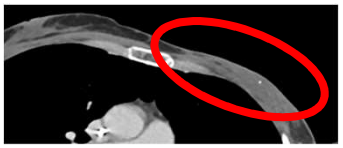
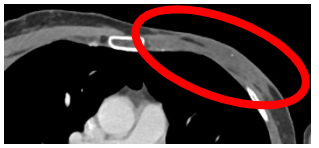
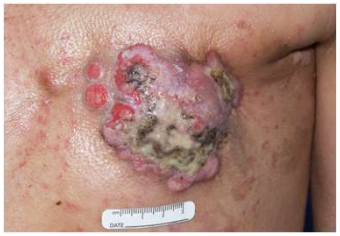
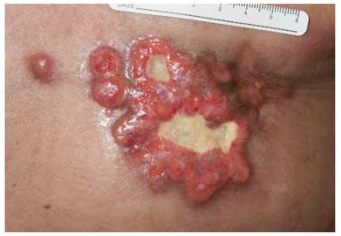
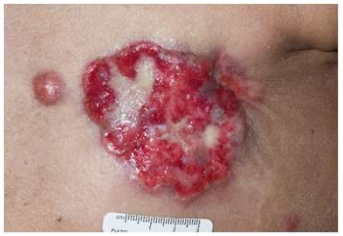
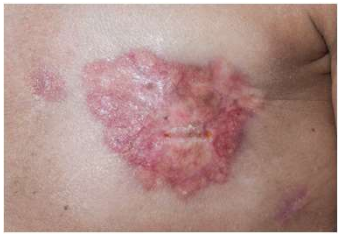
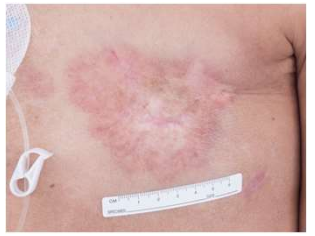
Tumor suppressor gene

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”

Pt. [4355] Breast cancer course following ACT

Day -9	+40	+82	+105	+151	
					L breast
					L breast

CAR-T cells for the Treatment of Solid Epithelial Cancers

CAR-T cells require the use of monoclonal antibodies (MoAb) that recognize molecules on the cell surface.

MoAb were first described by Kohler and Milstein 45 years ago. (Nature 256:495-7, 1975)).

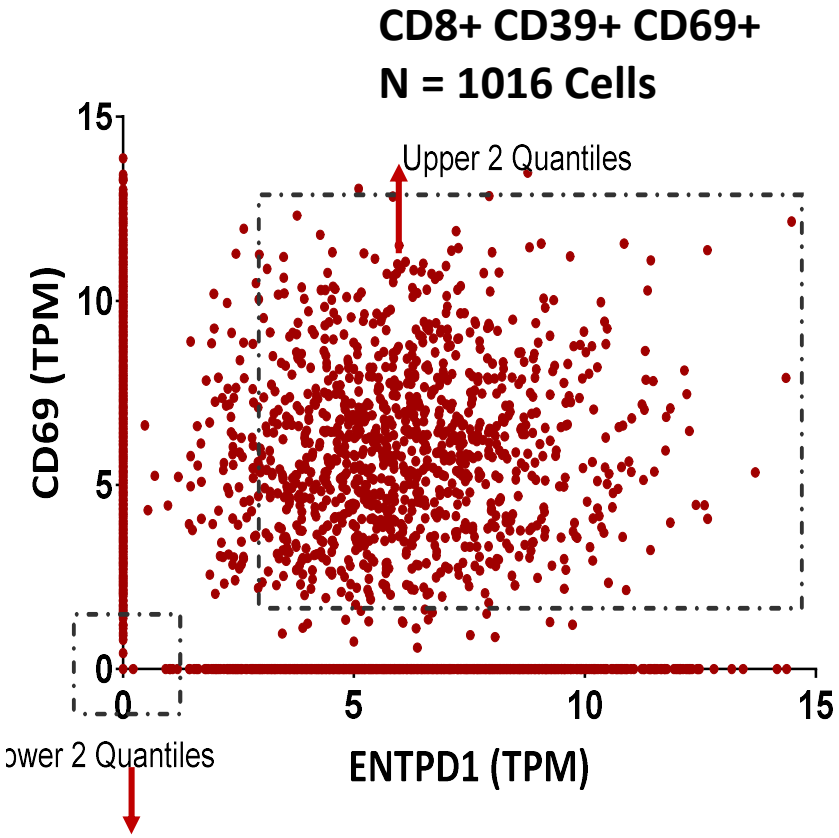
Two major problems:

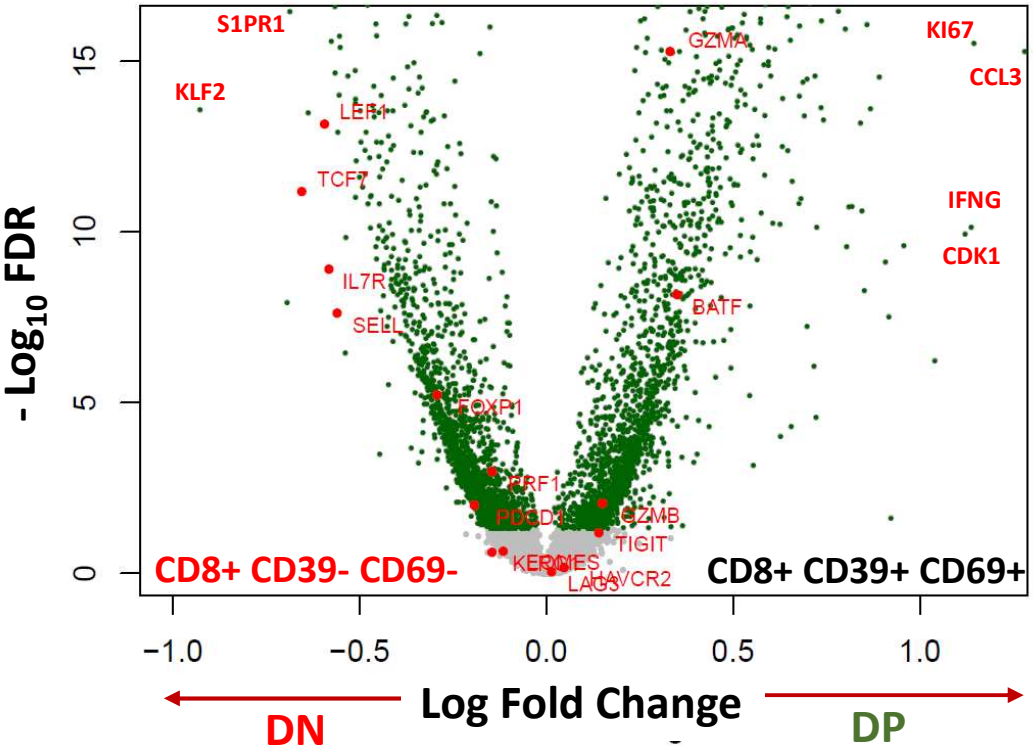
1) Very few MoAb exist that recognize cell surface molecules unique to cancer (e.g. EGFRvIII).

2) Normal cells expressing the target of MoAb are highly sensitive to destruction.

(Most MoAb in clinical use target growth factors, such as EGFR or Her2, that are expressed on the surface of normal cells.)

Gene signature of CD39- CD69- cells by single cell transcriptome analysis shows enrichment of memory markers





Thanks to Li Jia

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

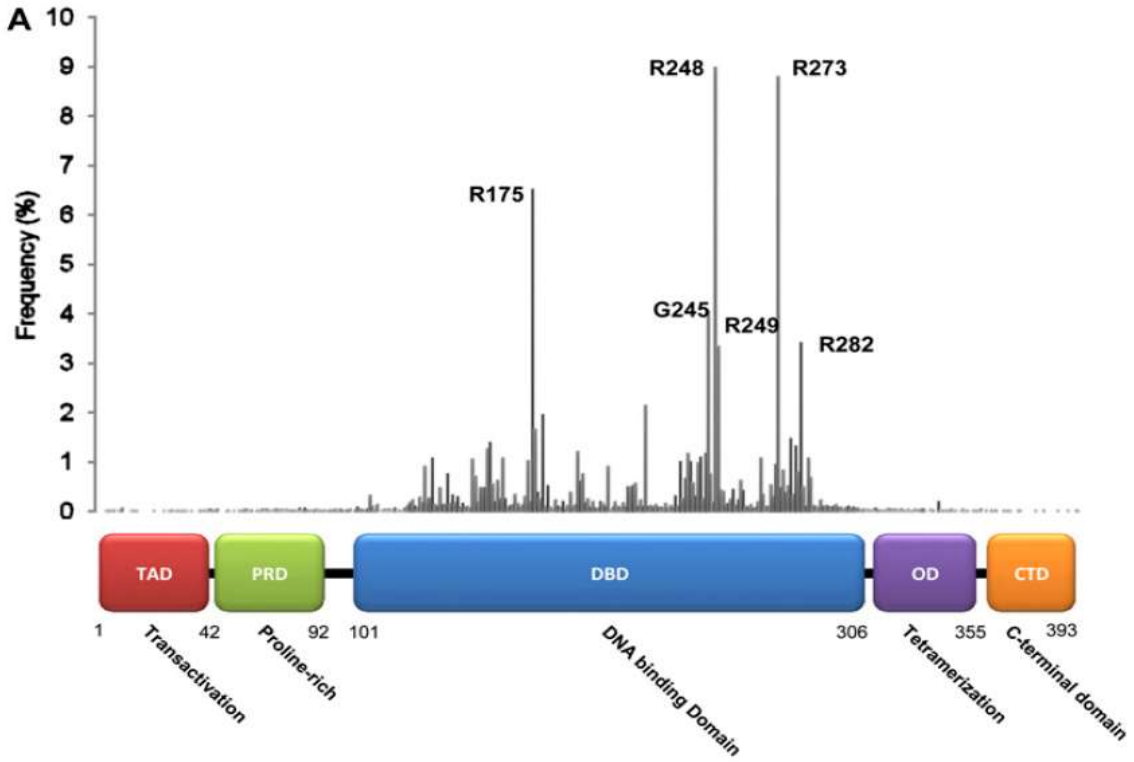
3. **Non-mutated proteins on the surface of cells that are not essential for survival and can be recognized by antibodies or specific ligands**
   e.g.: CD19 (CAR: αCD19 antibody)

Library of TCRs Available for Treatment of Patients with KRAS Mutations

KRAS Mutation	Mutation Frequency (%)	HLA Restriction (%)	HLA Frequency (%)	Potentially Treatable Patients (%)
G12D	35 %	C*0802	8 – 14 %	2.8 %
		A*1101	14 – 30 %	4.9 – 10.5 %
		DRB3*02	32 %	11.2 %
G12V	24 %	A*1101	14 – 30 %	3.4 – 10.5 %
		C*0102	1.3 – 7.2 %	1.3 – 7.2 %
		DRB3*02	32 %	11.2 %
		DRB1*0701	26 %	6.1 %
		DRB1*0301	20 %	4.8 %
		DRB1*0101	18 %	4.3 %
G12R	3 %	DQA1*0505	35 %	1 %
		DRB5*01	35 %	1 %
TOTAL				**29 %**

(Noam Levin, 2020)

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.

ADVANTAGES OF CELL TRANSFER THERAPY

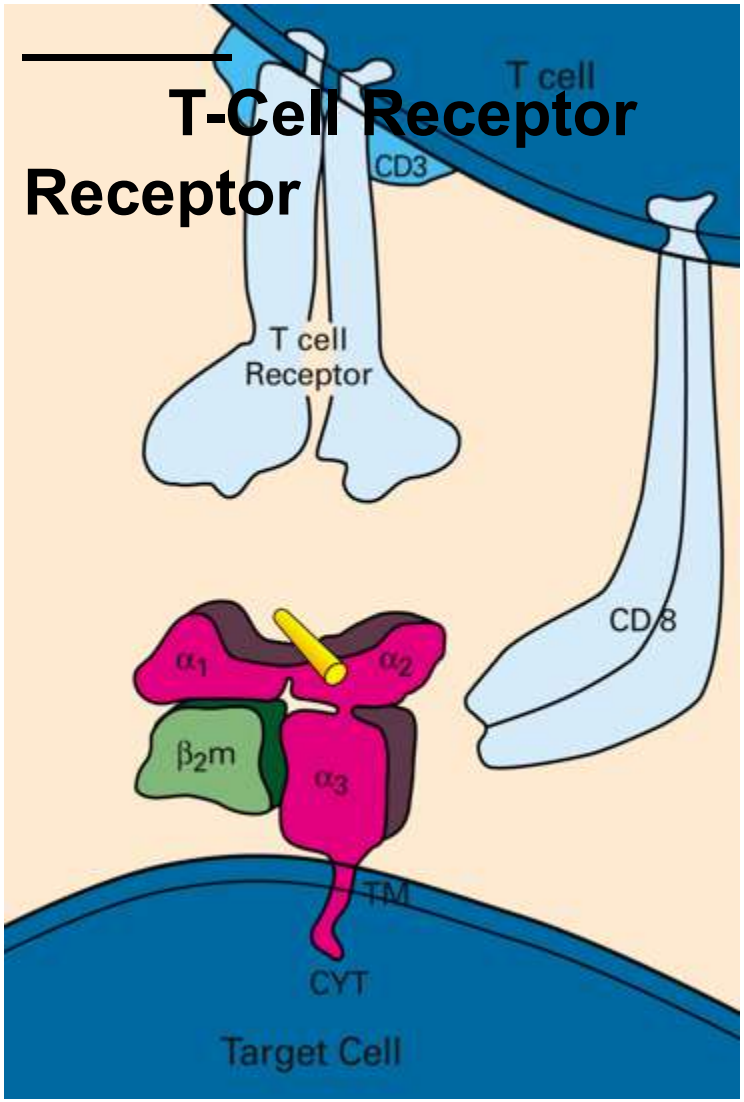
1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

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

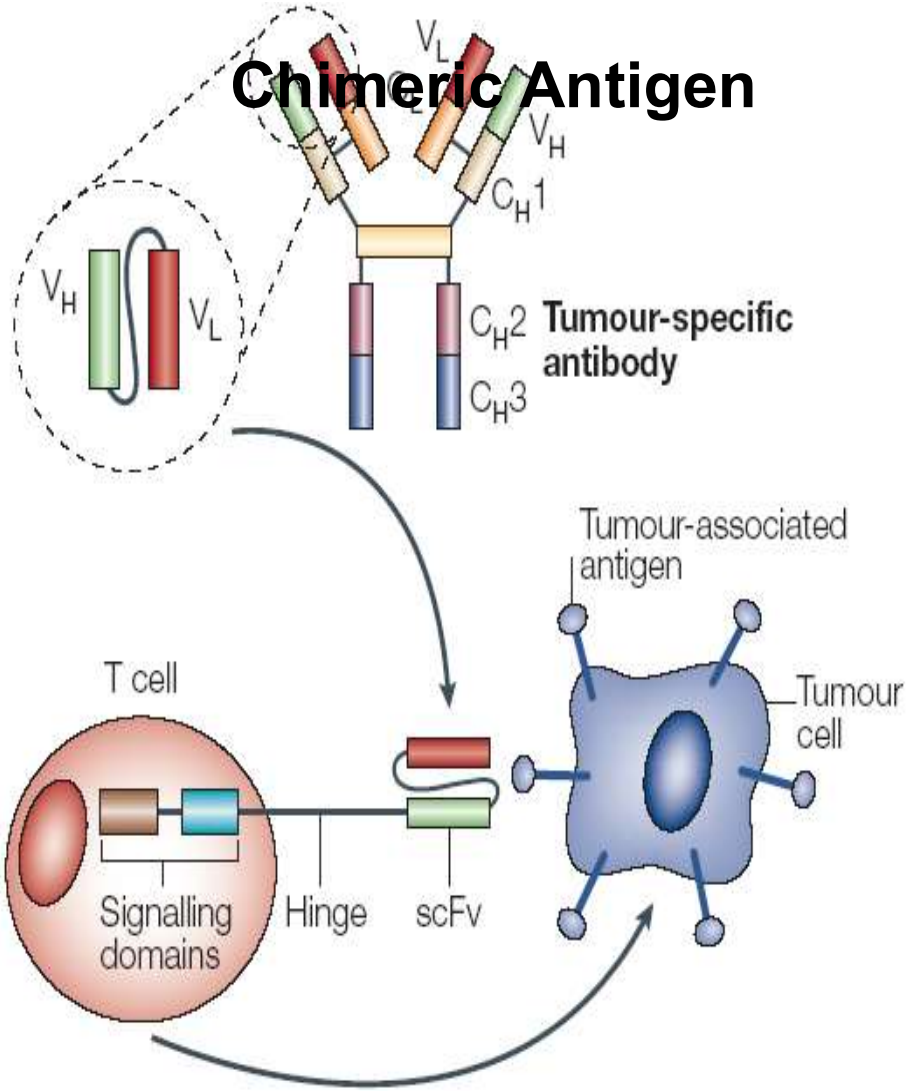
3. Manipulate host prior to cell transfer to provide altered environment for transferred cells.

Antigen Recognition by TCRs and CARs

T-Cell Receptor



Chimeric Antigen Receptor



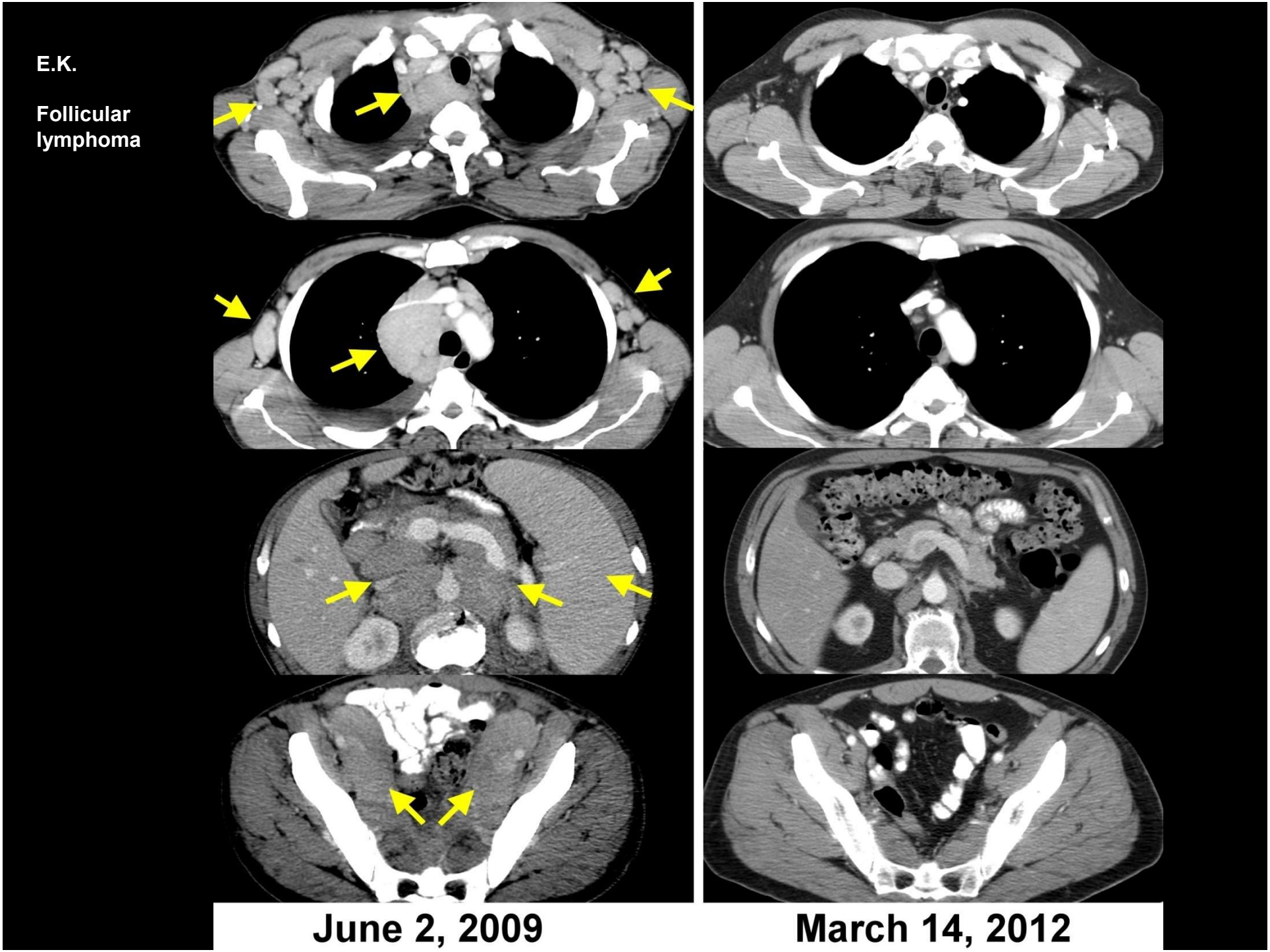
Patient E.K. Treated with Autologous Anti-CD19 CAR T-Cells

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**

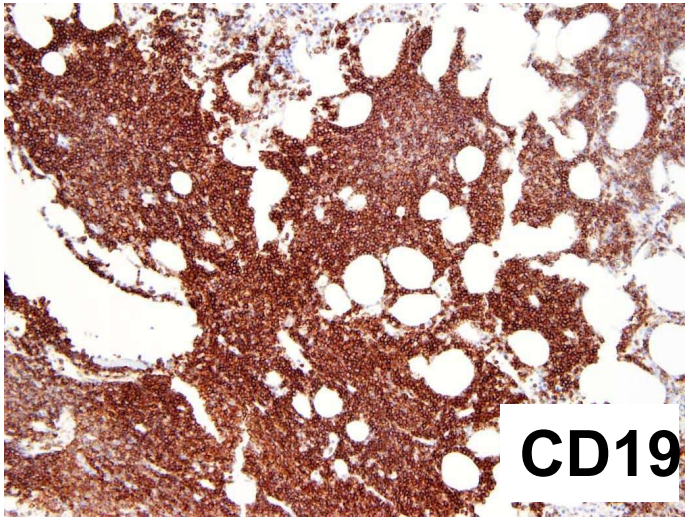
In ongoing progression-free regression over 10 years later.

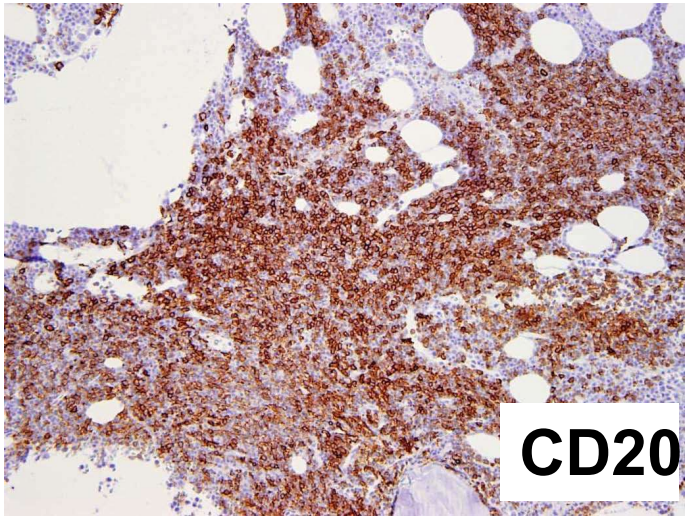
(Blood 116:3875-86, 2010; 119:2709-20, 2012)


E.K.
Follicular lymphoma
June 2, 2009
March 14, 2012

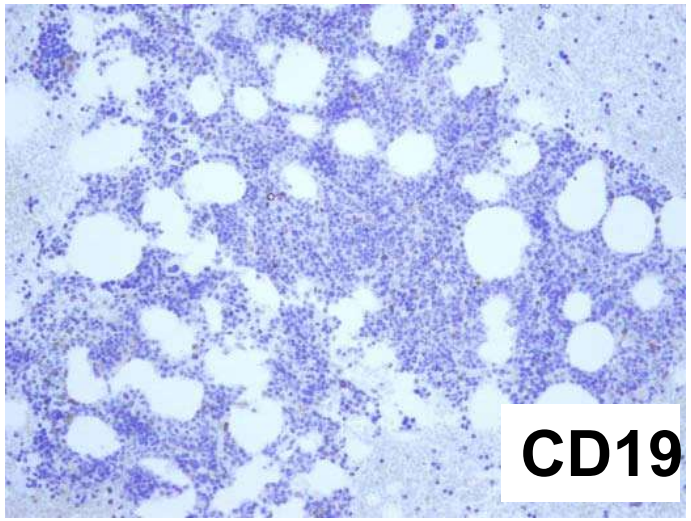
Bone marrow biopsies showed extensive CLL before treatment and nearly absent B-lineage cells after treatment

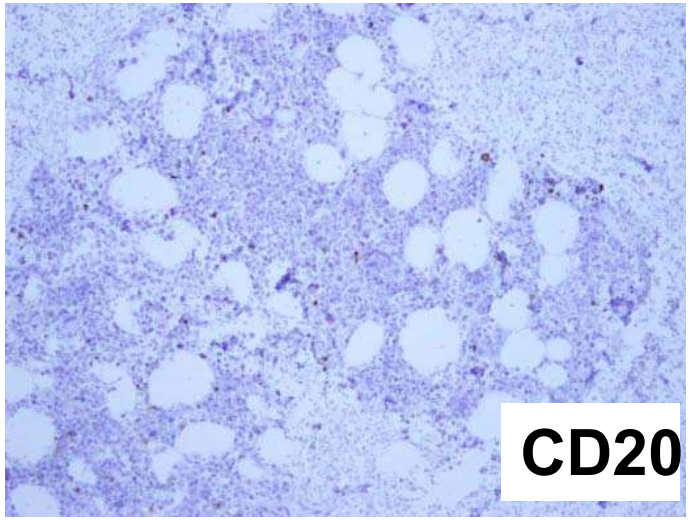
Before treatment



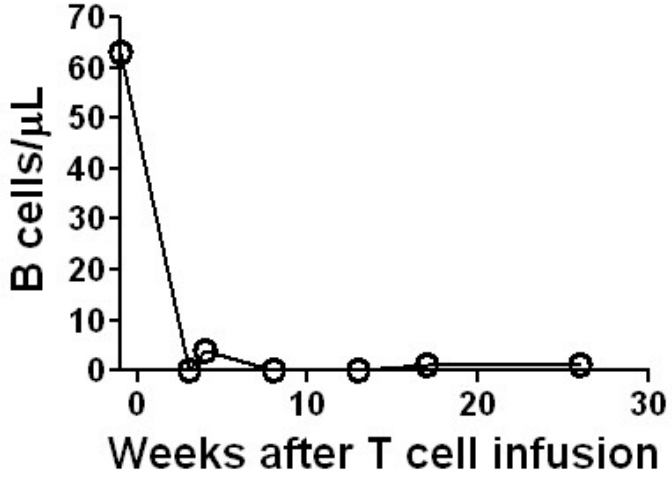


3 months after treatment

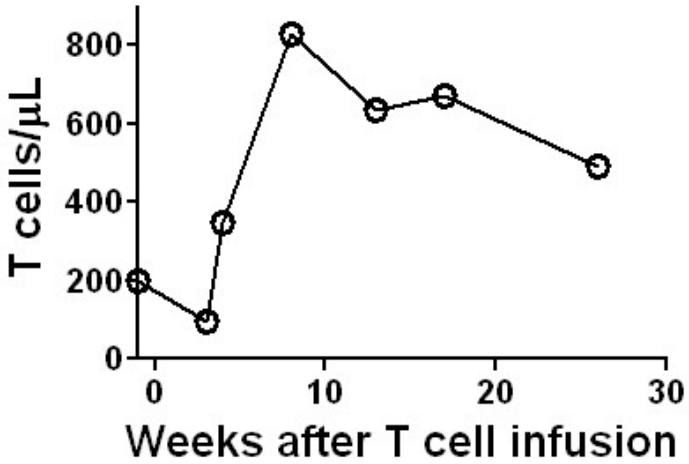


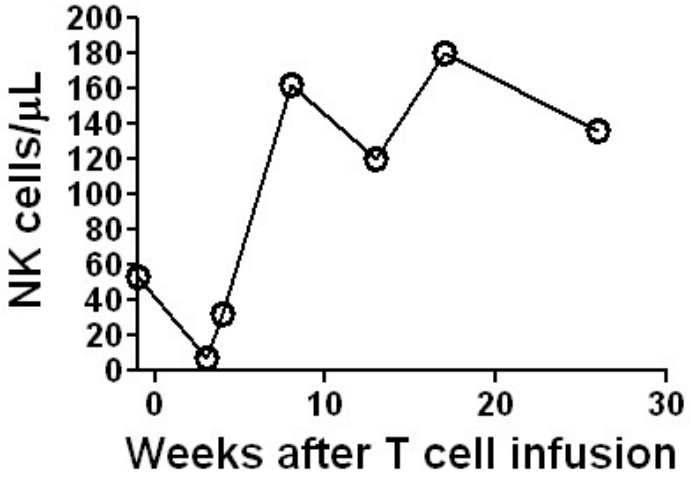


In Patient 8, normal blood B cells were eliminated after CAR-transduced T cell infusion



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





Treatment of Patients with Refractory Diffuse Large B-cell Lymphoma

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

In 2012 SB/NCI signed a CRADA to transfer our technology to Kite Pharma.
In 2017 Kite received FDA approval.
In October 2017 Kite was sold to Gilead Sciences, Inc. for $11.9 billion.

Patient S.S.

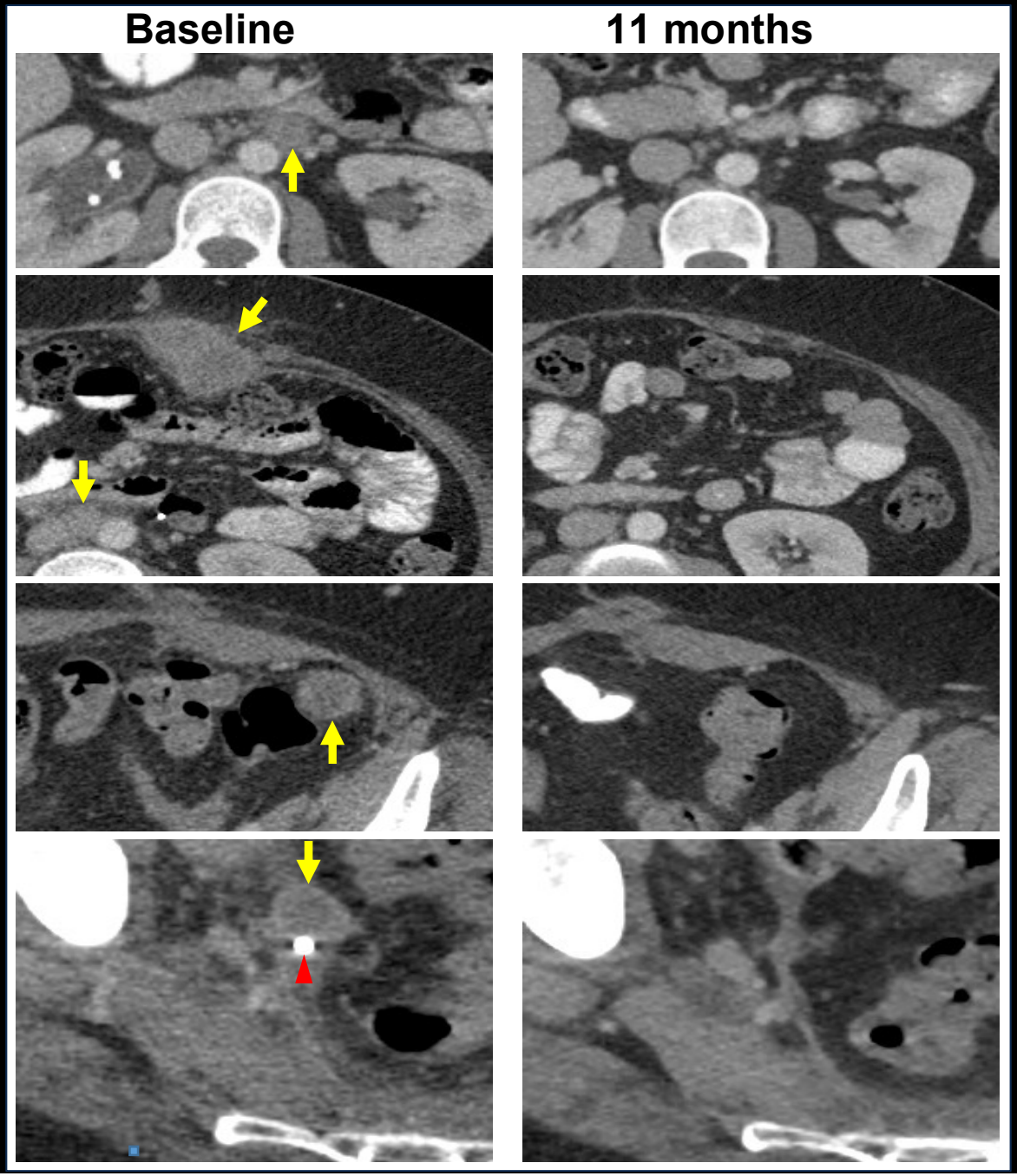
36 y.o. female with metastatic cervical cancer

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)**

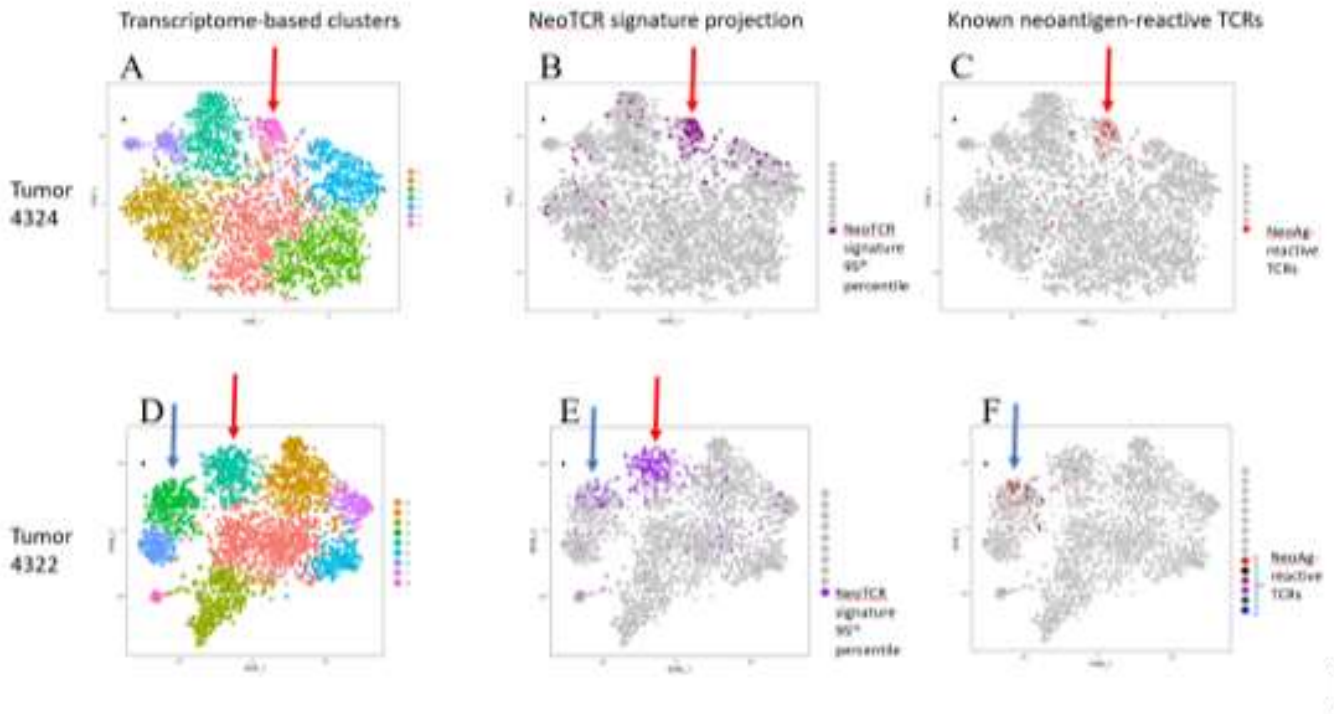
Experienced complete regression of all disease including relief of urinary obstruction and remains disease-free over 7 years later.

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

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



NeoTCR signature projection onto T-cell transcriptome maps



G

Genes in NeoTCR signature					
CXCL13	LRRN3	ASB2	ENTPD1	GALNT2	TIGIT
NSMCE1	HMOX1	GZMB	CLIC3	NELL2	CXCR6
AC092580.4	DUSP4	LAG3	NDFIP2	LAYN	PTMS
GPR25	ACP5	CAR5	IL4R	CD9	TOX