Curative Potential of Cell Transfer Therapy for Cancer

SITC

November 6, 2015

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Disclosures

No personal disclosures to report.

DEVELOPMENT OF CELLULAR IMMUNOLOGY

1880's: Antibodies described
(dominated studies of immunology until 1960's)

1958: Journal of Immunology (137 papers)
"lymphocyte" not listed in index
Two papers on transfer of lymph node cells were the only papers dealing with lymphocytes

1960's: Importance of cellular immunology recognized as mediator of: allograft rejection
protection against transfer of mouse tumors

1970's: No convincing evidence for human lymphocytes reactive with cancer or the existence of human cancer antigens

No successful immunotherapies for cancer in humans

RECOMBINANT INTERLEUKIN-2

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1. **T-cell Growth Factor (IL-2) described by Morgan et al (Science 193:1007,1976).**

2. **DNA sequence of the gene coding for IL-2 was determined by Taniguchi, et al. (*Nature* 302:305, 1983).**

3. **IL-2 gene was expressed in E. coli; the biologic characteristics of this recombinant IL-2 were determined; Lymphokine Activated Killer (LAK) cells described (Rosenberg, S.A., et al., *Science* 223:1412, 1984).**

LABORATORY AND PRECLINICAL MURINE STUDIES THAT PRECEDED HUMAN CLINICAL TRIALS

Demonstration that cells grown in IL-2 maintain antigen specific functions in vitro	**Mouse:**	**Susan Schwarz**
		Paul Spiess
	Human:	**John Strausser**
Discovery of lymphokine activated killer (LAK) cells that recognize cancer cells	**Mouse:**	**Ilana Yron**
	Human:	**Michael Lotze**
		Elizabeth Grimm
Properties of anti-tumor lymphocytes in vitro (LAK and TIL cells)	**Mouse:**	**Douglas Mathisen**
		Maury Rosenstein
		James Yang
	Human:	**Amithabha Mazumder**
		Linda Muul
		Suzanne Topalian
Anti-tumor effects of IL-2 and cell transfer in murine models	**Mouse:**	**James Mule**
		Stephen Ettinghausen
		Timothy Eberlein
		Suyu Shu

(over 90 peer reviewed publications on these topics: 1978-1985)

EARLY EFFORTS TO DEVELOP HUMAN CANCER IMMUNOTHERAPY IN THE SURGERY BRANCH, NIH

Date	Administration to patients with metastatic cancer	
7/20/83	Natural mammalian IL-2 (Phase I)	16 patients
3/21/84	LAK cells produced with recombinant IL-2	27 patients
3/30/84	Recombinant IL-2 (Phase I)	23 patients
	Total	66 patients
11/29/84	High-dose bolus IL-2 plus LAK cells	

PATIENT: L.T.

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31 year old female referred to NCI with metastatic melanoma

June, 1982 **Excision level III melanoma of the scapula**

Dec., 1983 **Subcutaneous metastases**

March, 1983 **Treated with alpha-interferon; disease progressed**

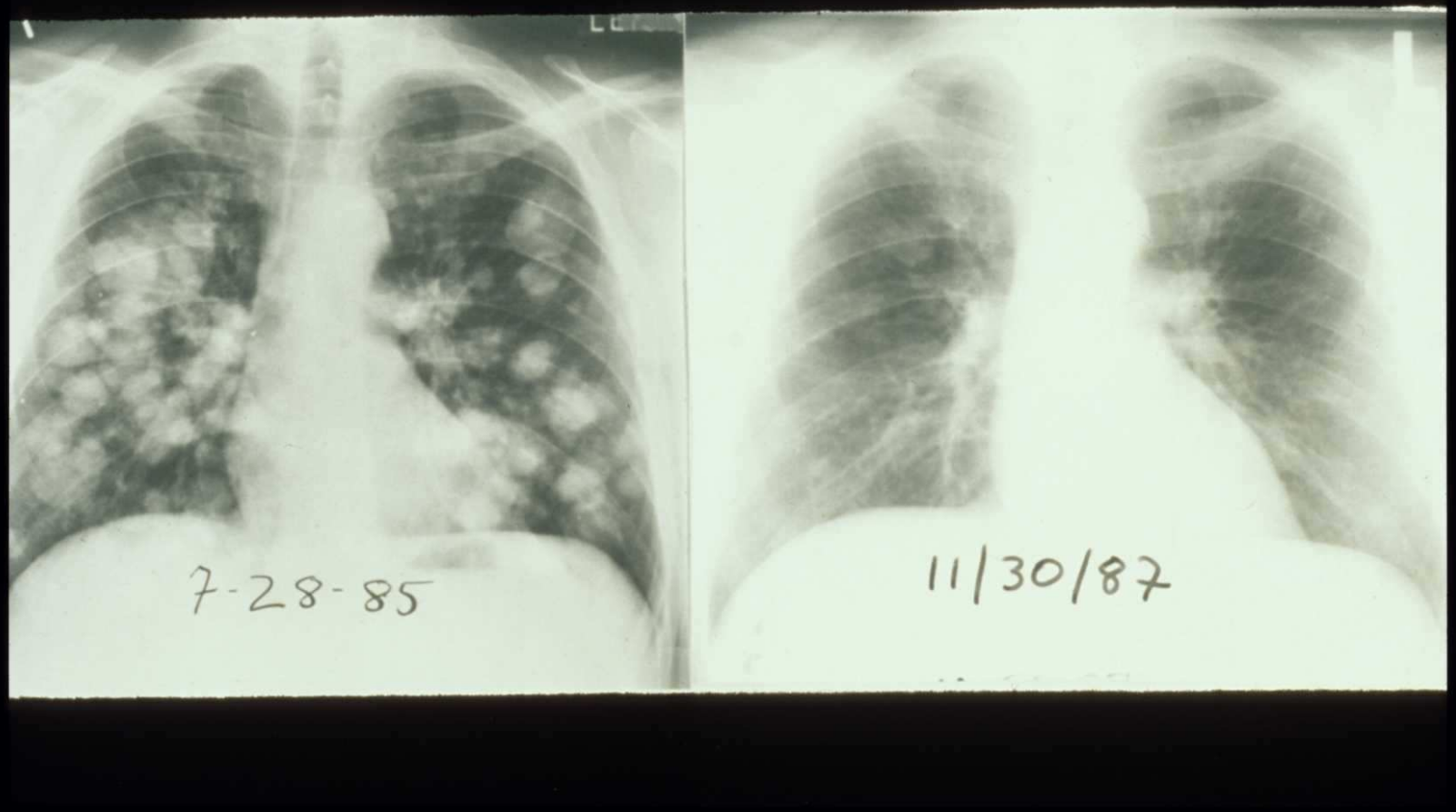
Nov., 1984 **To NCI, multiple subcutaneous metastases**
Treated with high dose bolus IL-2 plus LAK cells

Complete regression of metastases.

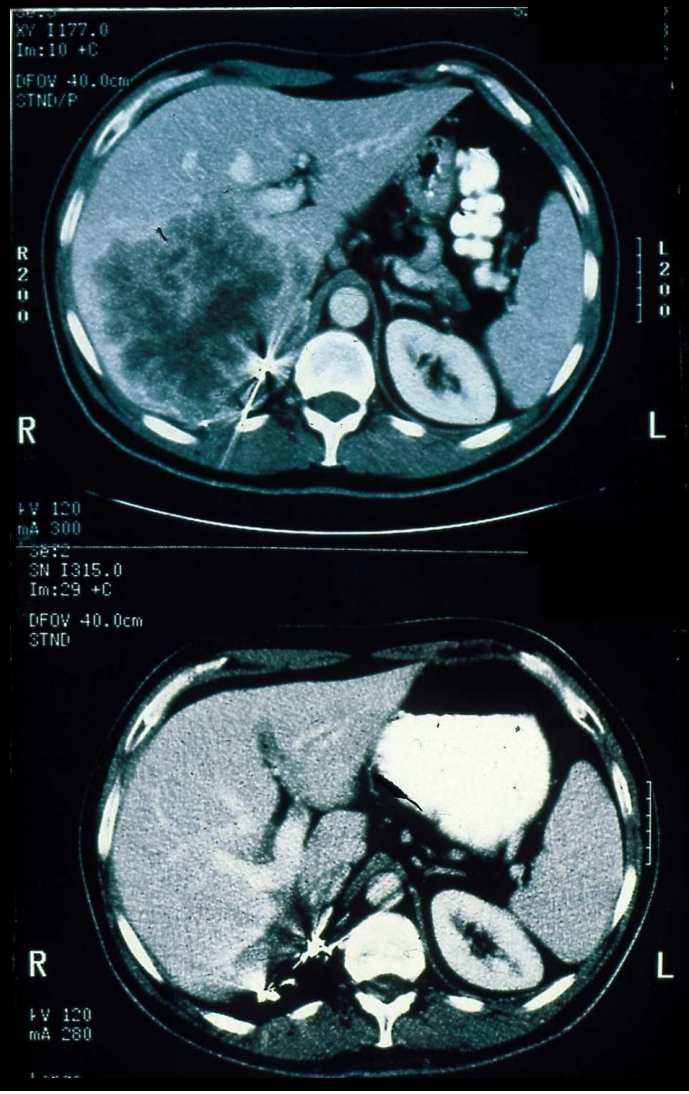
Remains disease free as of June, 2015.

(New Engl. J. Med. 313:1485, 1985)

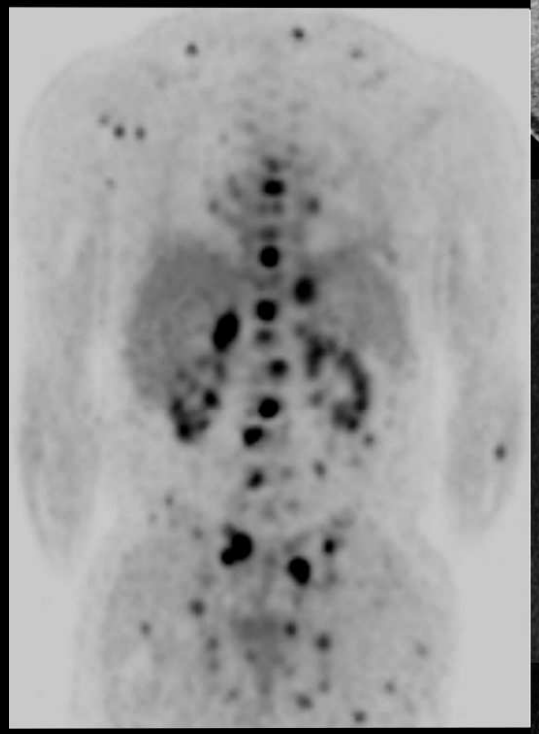
Metastatic melanoma: IL-2 administration

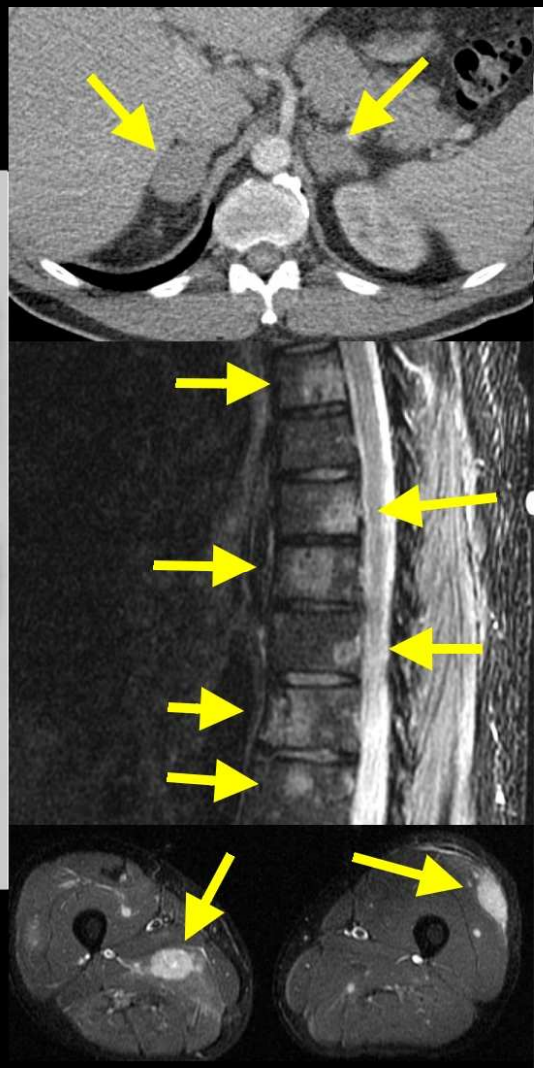


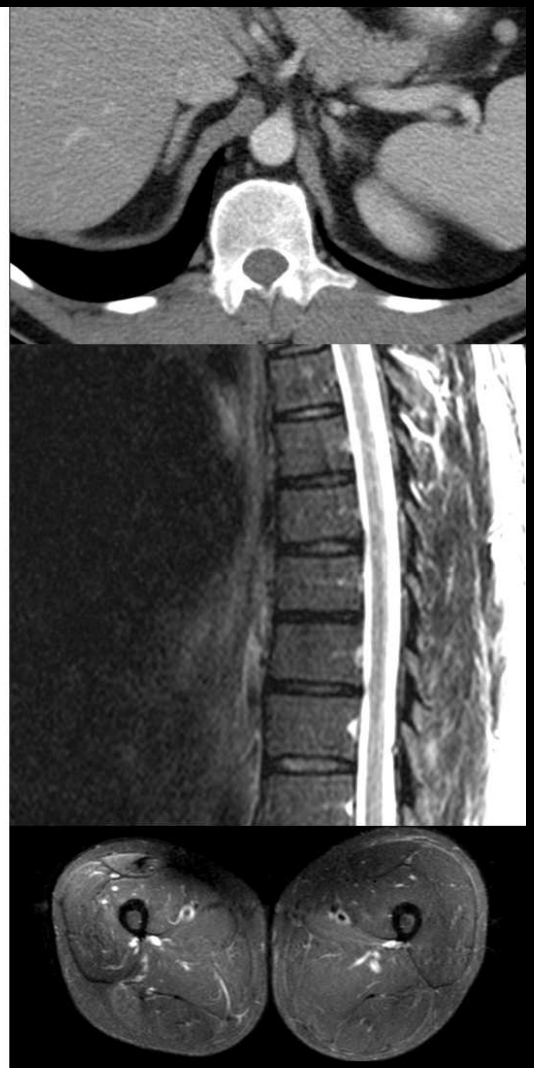
Metastatic renal cancer: IL-2 administration



W.R. Metastatic melanoma: IL-2 administration







Pre-Treatment

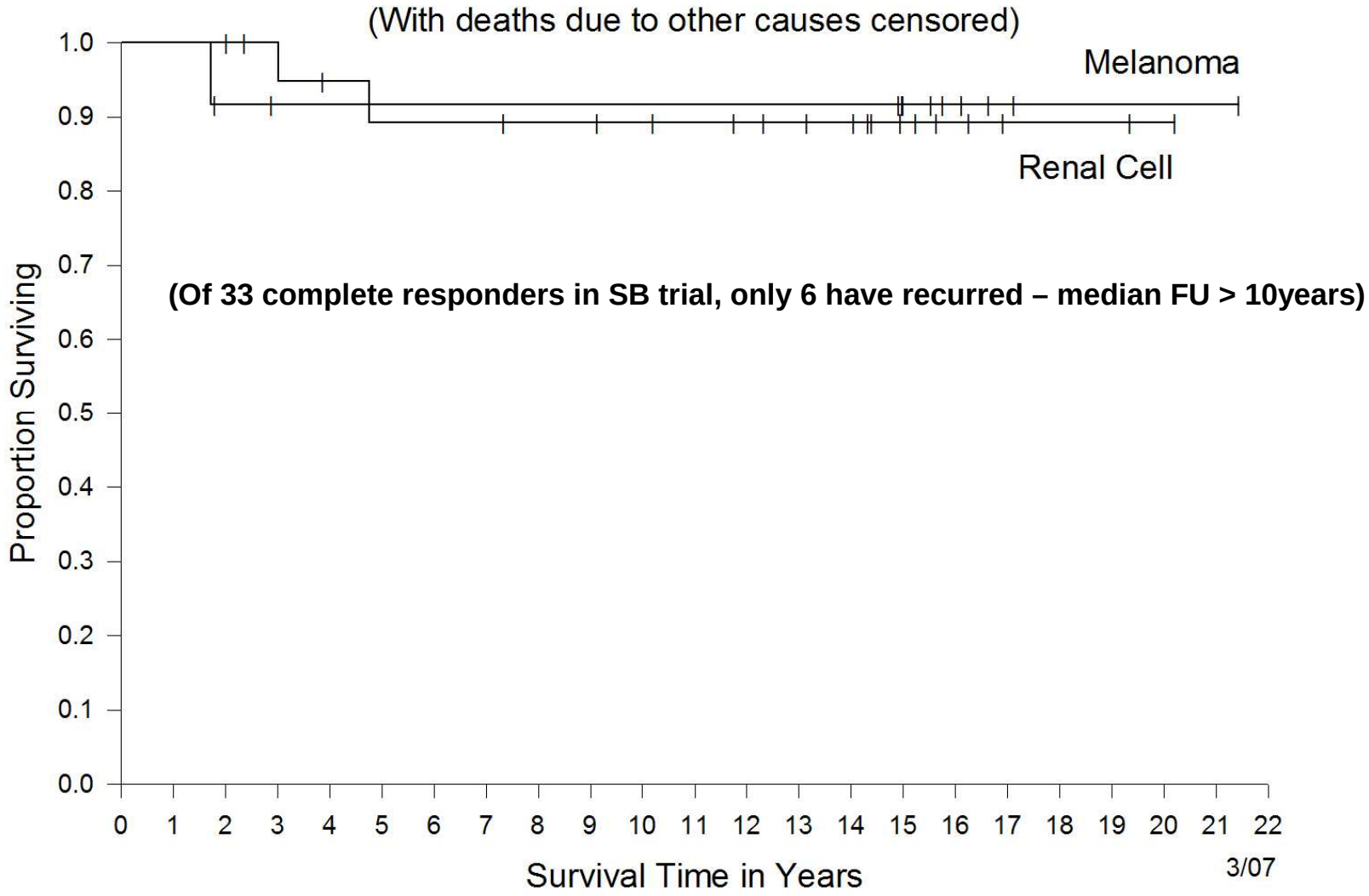
6 Months

RESPONSE OF PATIENTS WITH METASTATIC CANCER TREATED USING HIGH-DOSE BOLUS INTERLEUKIN-2

Diagnosis	Total	CR	PR	CR + PR
		Number of patients (%)		
Melanoma	182	12 (7%)	16 (9%)	28 (15%)
Renal Cell Cancer	227	21 (9%)	22 (10%)	43 (19%)
Total	409	33 (8%)	38 (9%)	71 (17%)

Patients accrued between Sept. 1985 and Nov. 1996.
Follow-up as of June 1, 2005 (median follow-up 14.3 yrs)

Complete Response to Treatment with High-Dose IL-2



(Of 33 complete responders in SB trial, only 6 have recurred – median FU > 10years)

Interleukin-2 was the first immunotherapy approved by the FDA

Approved by the U.S. Food & Drug Administration

- **1992 Metastatic renal cancer**
- **1998 Metastatic melanoma**

Licensing trial of IL-2 for melanoma

270 patients from 22 institutions

Objective responses:	**43 (16%)**
Partial:	**26 (10%)**
Complete:	**17 (6%)**

(Atkins et.al. J Clin Onco 17:2105-2116,1999)

TREATMENT RELATED MORTALITY OF ALL PATIENTS RECEIVING HIGH-DOSE, BOLUS IL-2 (720,000 IU/KG)*

1ST 155 patients	1/5/85 – 8/17/86	3.2%
next 310 patients	8/18/86 – 4/26/89	1.3%
next 1367 patients	7/28/89 – 9/1/03	0.4%

*Patients received IL-2 alone or in conjunction with cells, other cytokines or vaccines

QUESTION

What cells were responsible for the regression of melanoma in patients treated with IL-2 based immunotherapy?

TUMOR INFILTRATING LYMPHOCYTES (TIL)

TIL are immune cells that infiltrate into the stroma of growing tumors and can be grown in vitro in medium containing IL-2.

TIL can recognize autologous cancer cells based on assays of specific lysis or specific cytokine release when cocultured with cancer cells.

(Mouse: Science 233:1318, 1986)

(Human: NEJM 319:1676, 1988)

ADVANTAGES OF CELL TRANSFER THERAPY

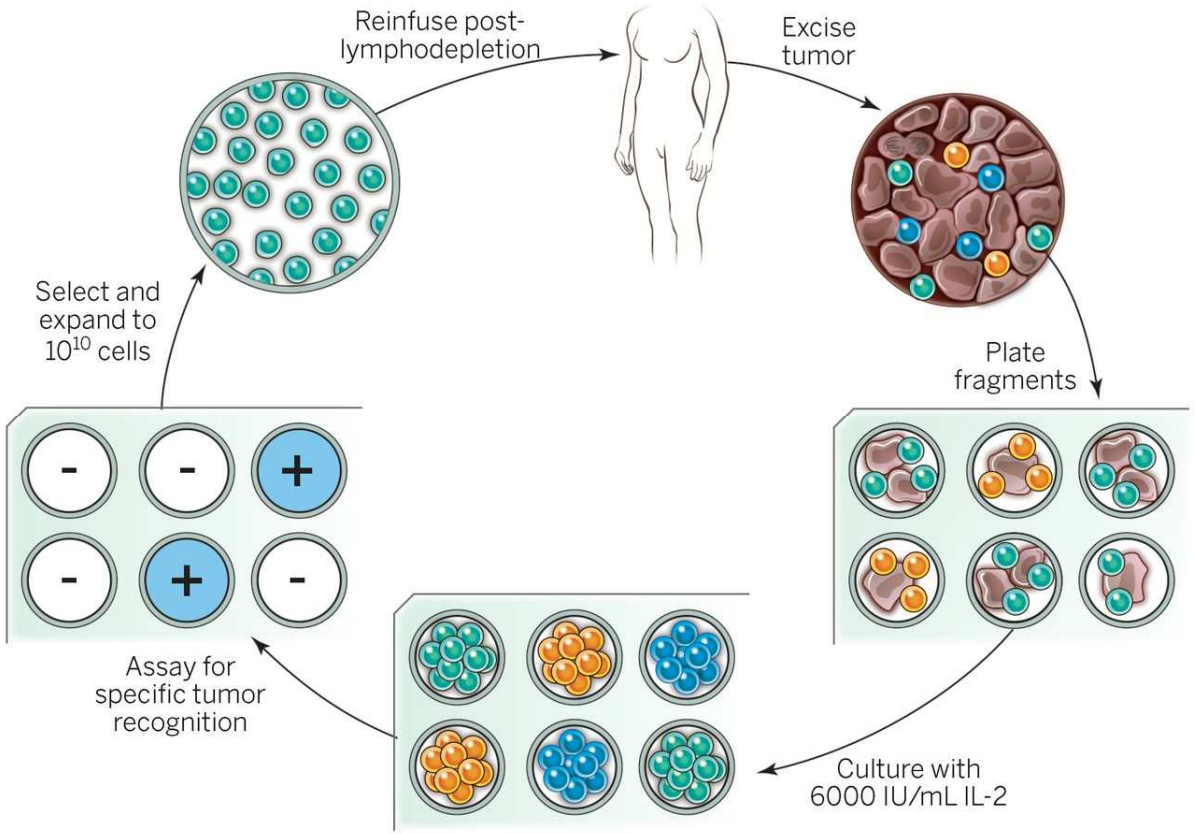
1. Administer large numbers of highly selected cells with high avidity for tumor antigens.

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

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

(First trials with autologous TIL reported in Dec. 1988 in NEJM.)

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



INITIAL RESULTS WITH CELL TRANSFER THERAPY FOLLOWING LYMPHODEPLETING CHEMOTHERAPY

Six of 13 (46%) patients with metastatic melanoma experienced objective cancer regression.

Four patients had mixed or minor responses.

All had previously been refractory to IL-2 administration and eight had prior chemotherapy.

(Dudley et al Science 298:850-854, 2002)

Summary of Cell Transfer Protocols for the Treatment of Patients with Metastatic Melanoma*

(median potential follow-up 9.8 years)

Total	PR	CR	OR
	number of patients (duration in months)		
93	32 (34%)	20 (22%)	52 (56%)
	84, 36, 29, 28, 21, 14, 14, 13, 12, 11, 9, 8, 7, 7, 7, 6, 6, 6, 6, 6, 5, 5, 4, 4, 4, 3, 3, 3, 2, 2, 2, 2	137+,135+,134+,124+,120+, 120+,116+,113+,104+,101+, 100+,95+, 94+, 94+, 94+, 93+, 82+, 64+, 63+, 19	

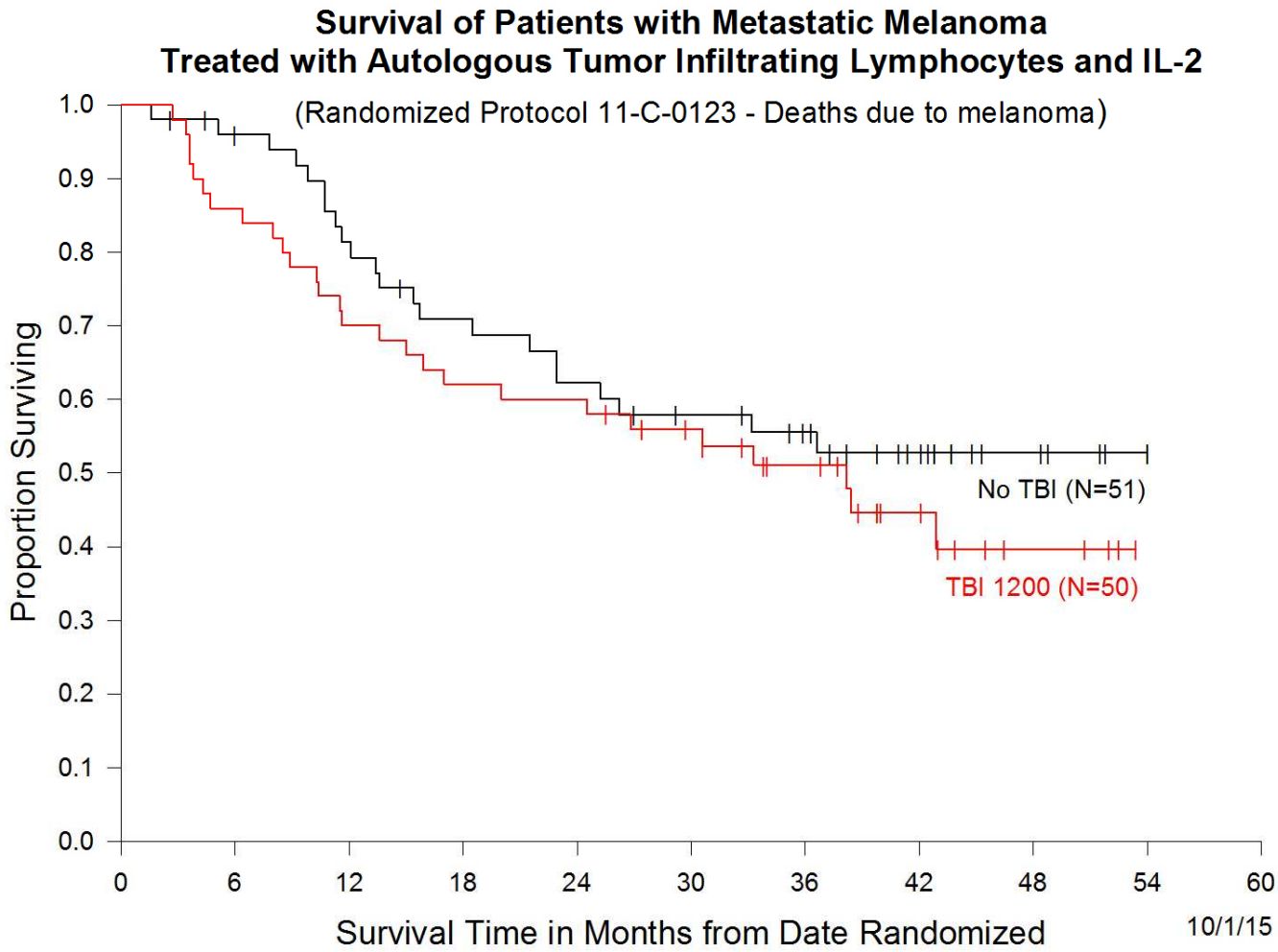
*from 3 consecutive trials using different lymphodepleting regimens

(Rosenberg et al, Clin Cancer Res 17:4550-7, 2011)

Randomized NMA vs NMA-TBI Study 10/1/15

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
NMA	51	11 (22%) 51+,43+,38+,37+,29+, 21, 19, 7, 6, 4, 4	12 (24%) 48+,45+,44+,42+,42+, 42+,41+,40+,39+,36+, 36+,32+	23 (45%)
NMA-TBI	50	19 (38%) 51+,50+,40+,25,19+, 14, 13, 11, 11,10, 9, 9, 9, 9, 8, 6, 5, 5, 4	12 (24%) 53+,46+,45+,42+,42+, 38+,37+,36+,27, 27+, 25+,14+	31 (62%)

(24 complete responses: 23 ongoing at 14 to 53 months; median potential followup 40.2 months)


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(Randomized Protocol 11-C-0123 - Deaths due to melanoma)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
No TBI (N=51)
TBI 1200 (N=50)
0
6
12
18
24
30
36
42
48
54
60
Survival Time in Months from Date Randomized
10/1/15

Summary of Cell Transfer Protocols for the Treatment of Patients with Metastatic Melanoma*

Total	PR	CR	OR
	number of patients (duration in months)		
194	62 (32%)	44 (23%)	106 (55%)
	84, 46+, 46+, 46+, 39+, 38+, 36, 36+, 34+, 29, 28, 25, 23+, 21, 21, 19, 19+, 14, 14, 14, 13, 13, 12, 11, 11, 11, 10, 9, 9, 9, 9, 9, 8, 8, 7, 7, 7, 7, 6, 6, 6, 6, 6, 6, 6, 5, 5, 5, 5, 4, 4, 4, 4, 4, 4, 3, 3, 3, 2, 2, 2, 2	137+,135+,134+,124+,121+, 120+,117+,114+,108+,101+, 100+, 98+, 95+, 94+, 94+, 93+, 82+, 64+, 63+, 51+, 43+, 42+, 42+, 41+, 41+, 40+, 40+, 40+, 39+, 38+, 38+, 38+, 36+, 36+, 36+, 35+, 32+, 30+, 29+, 27, 22+, 19, 19+, 14+	

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

(42 of 44 Complete Responders ongoing from 14 to 137 months)

Questions Guiding Current Efforts

What do TIL recognize that enables the in vivo control of the last cancer cell?

Can this supply clues to the immunotherapy of common epithelial cancers?

Clues to Identify the Antigen Recognized by TIL

Unlikely to be melanoma/melanocyte differentiation antigens

minimal to no eye and ear toxicity

major eye and ear toxicity when treating with MART-1 and gp100 gene-engineered cells

Unlikely to be cancer-germline antigens

minimal reactivity in TIL

no correlation with response

labor intensive cDNA cloning in a small number of patients showed reactivity against somatic mutations

Mining the Cancer Exome to Identify Immunogenic Cancer Mutations

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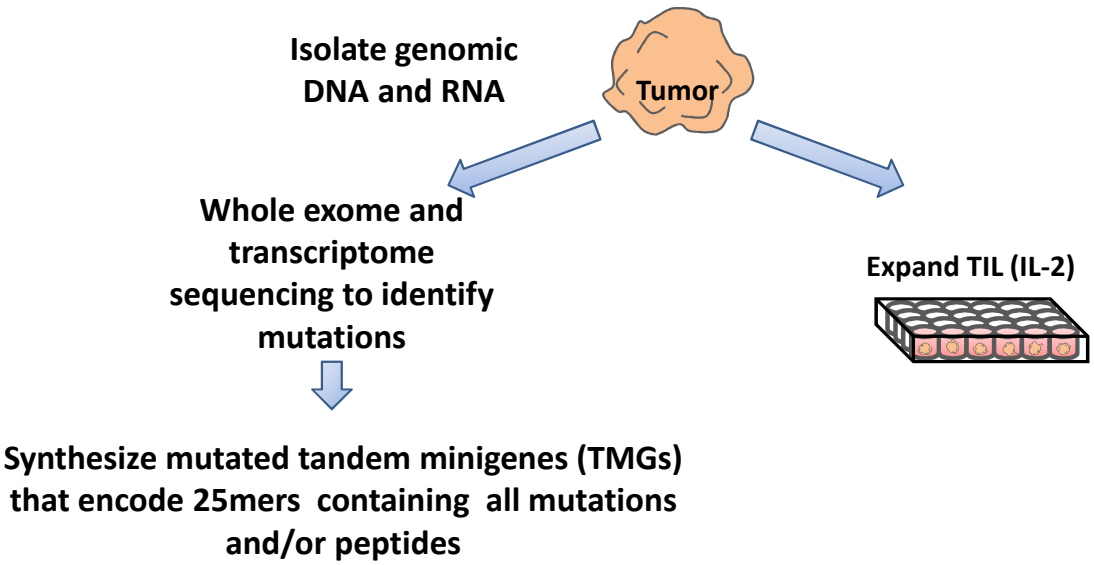
For a mutation to be a cancer antigen it has to:

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 some mutations will be antigenic.

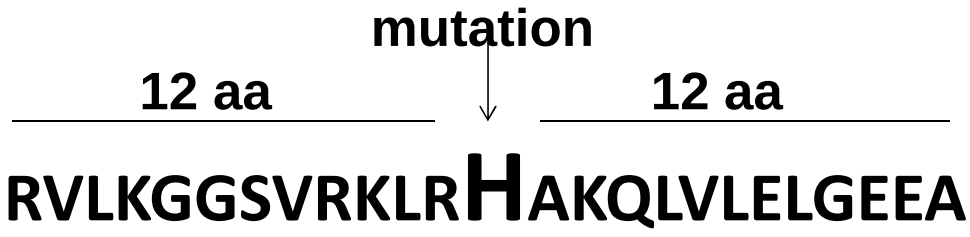
Blueprint for the generation of mutation-reactive T-cells in common cancers



Robbins et al, Nature Med 19:747-752, 2013
Lu et al, J Immunol 190:6034-42, 2013
Tran et al, Science 344:641-645, 2014
Tran et al, Science Oct. 29, 2015

Tandem minigene (TMG):

String of minigenes encoding the mutated AA flanked by 12 AA



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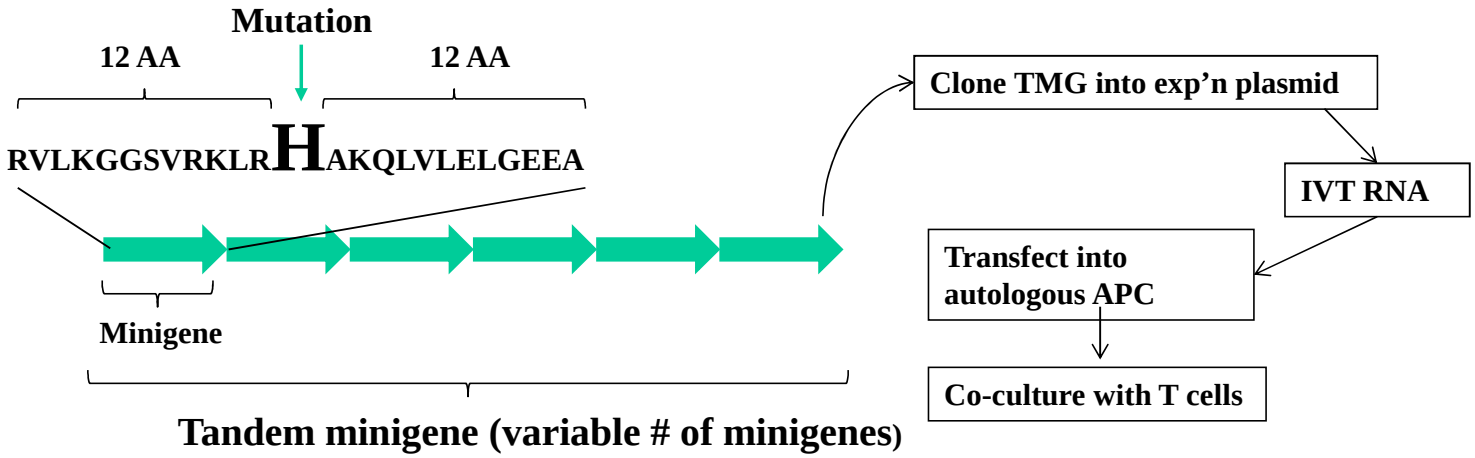


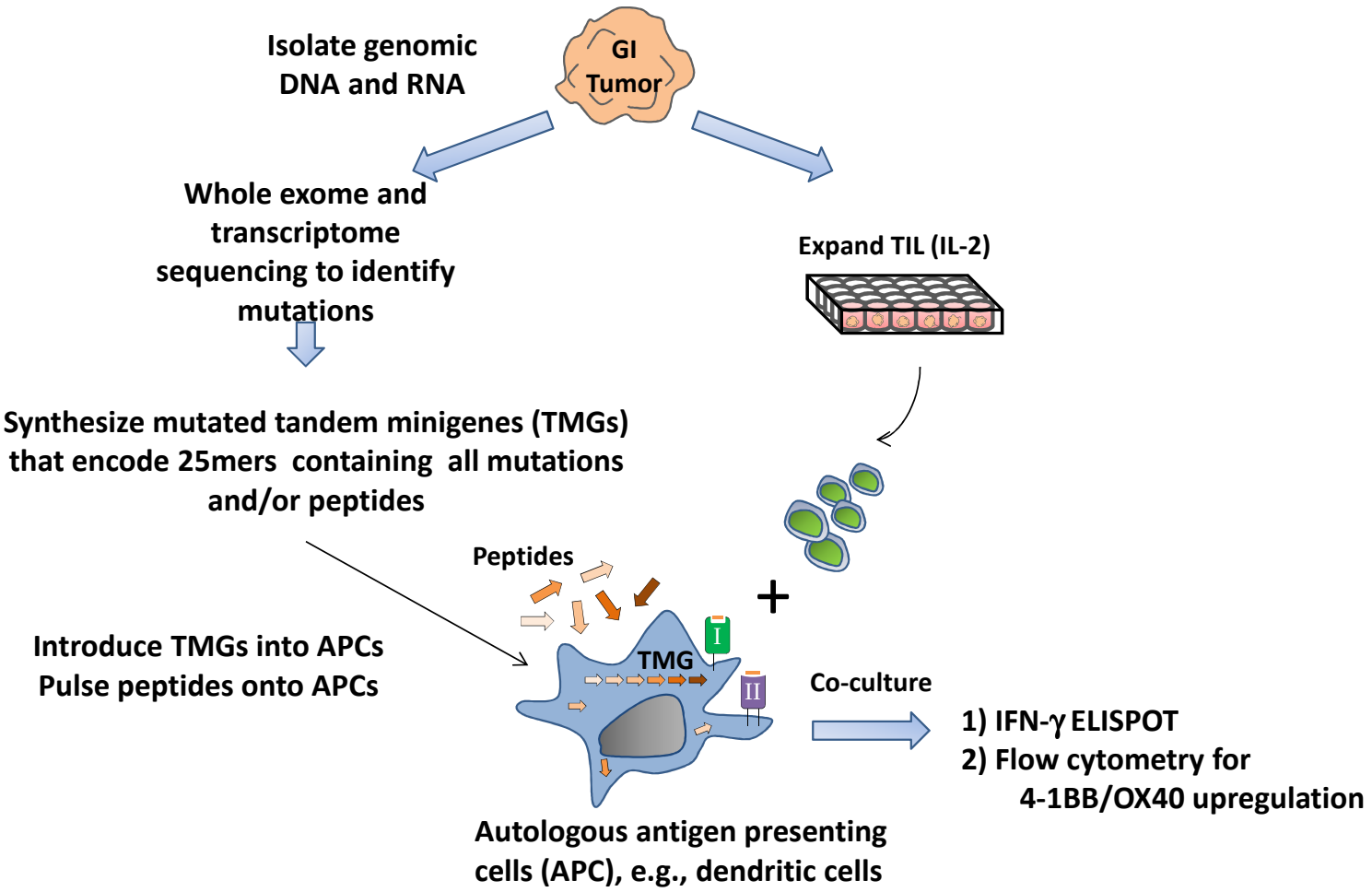
Advantages of this approach:

No need to predict peptide binding to MHC.

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

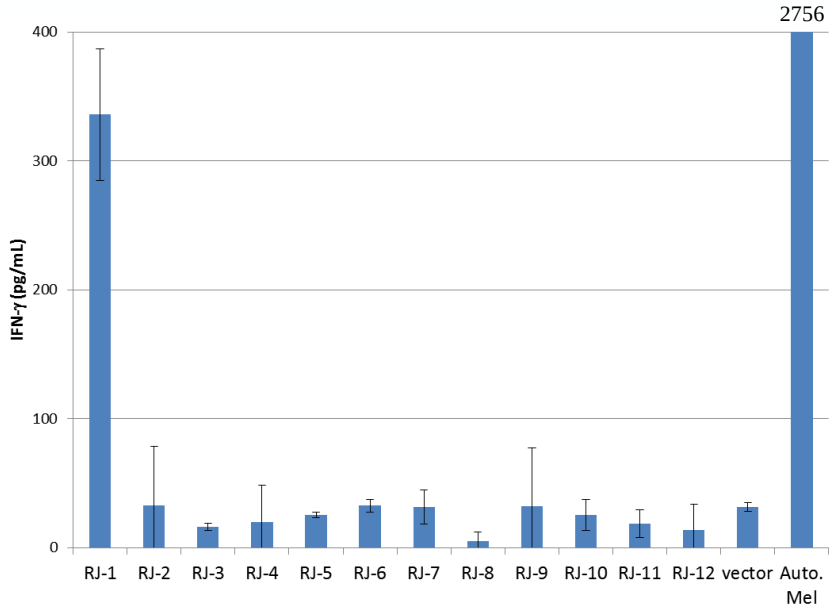
No tumor cell line necessary.

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



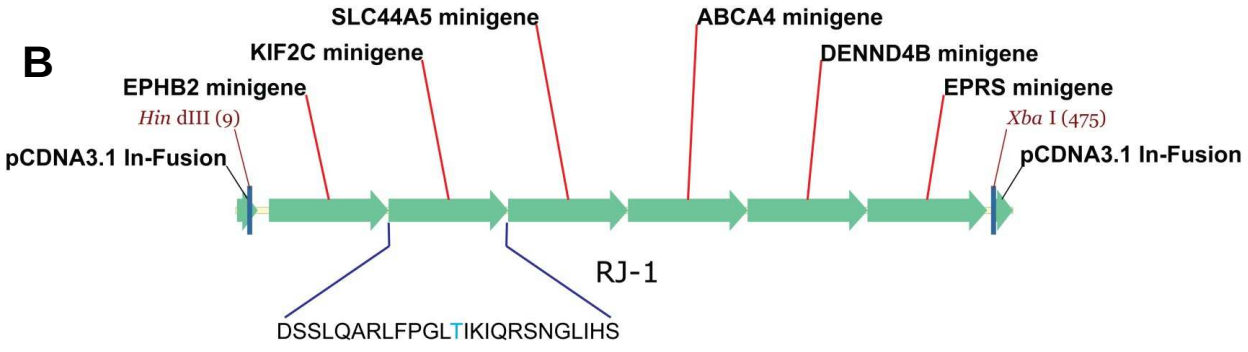
Minigene approach: J. bulk TILs recognize tandem minigene RJ-1

A

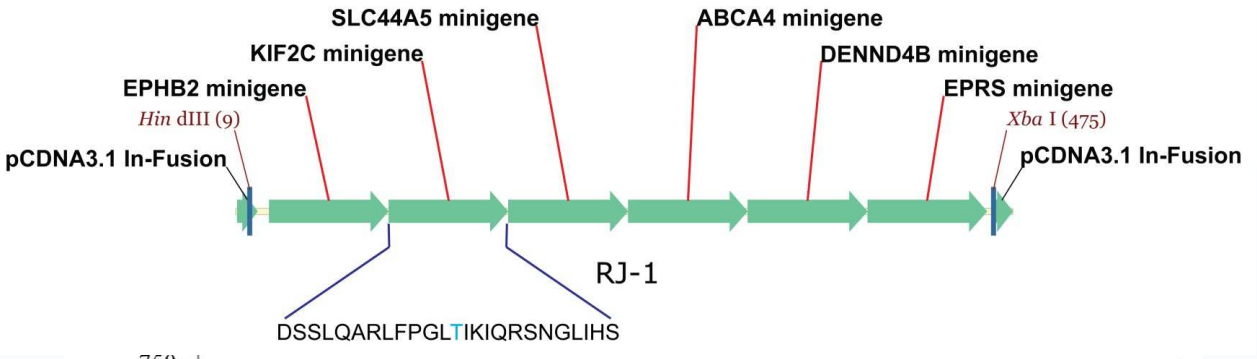


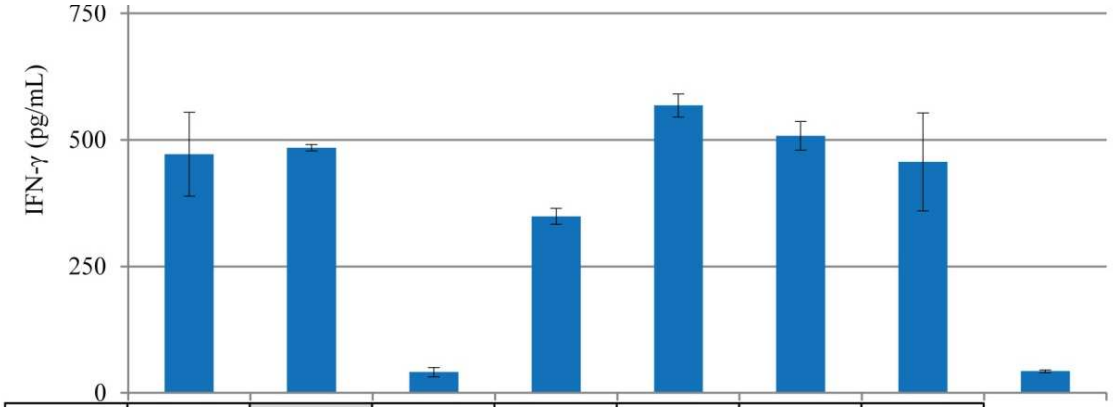
71 mutations
12 tandem minigenes
HLA-A*0205

B



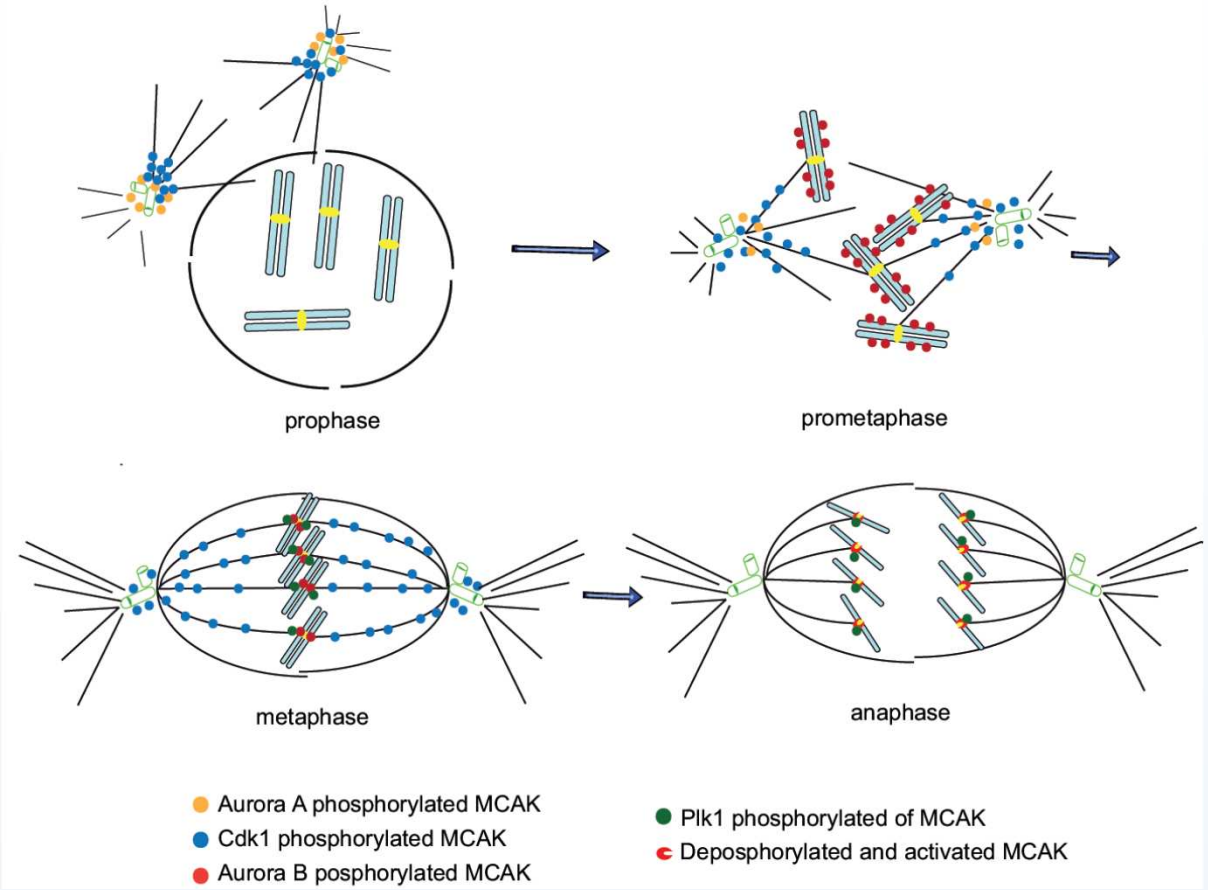
Mutated antigen KIF2C (kinesin family member 2C) recognized by R. J. TILs





EPHB2	mut	wt	mut	mut	mut	mut	mut	vector
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	

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



Sanhaji M et al, *Oncotarget* (2011)

Mutated antigens recognized by T cells from melanoma patients

Patient	Antigen	HLA RE	Patient	Antigen	HLA RE
164	ARCT1	DRβ1*0101	3713	WDR46	A*02:01
1290	CTNNB1	A*2402	3713	AHNAK	A*02:01
1290	Ki-67	DRβ1*1502	3713	SRPX	A*02:01
1359	CDC27	DRβ1*0401	3713	CENPL	A*29:02
1362	MART2	A*0101	3713	HELZ2	A*29:02
1363	LDLR-FUT	DRβ1*0101	3713	PRDX3	A*29:02
1558	TPI	DRβ1*0101	3713	GCN1L1	A*29:02
1700	NOP56	A*0201	3713	PLSCR4	A*29:02
1913	HLA-A11	-	3713	AFMID	A*29:02
1913	CDKN2A	A*11:01	3713	SEC22C	B*44:03
2098	CSNK1A1	A*02:01	3713	TPX2	B*44:03
2098	GAS7	A*02:01	3716	RXRB	Unknown class I
2098	HAUS3	A*02:01	3716	TFDP2	Unknown class I
2224	KPNA5	A*02:01	3784	FLNA	Unknown class I
2359	KIF2C	A*02:05	3784	GNB5	Unknown class I
2369	PPP1R3B	A*01:01	3784	KIF16B	Unknown class I
2369	PLEKHM2	A*01:01	3868	GANAB	A*02:01
2369	DOPEY2	A*26:01	3903	PHKA1	B*38:01
2556	MYH14	A*01:01	3903	KIAA1279	B*38:01
2556	RAC1	A*02:01	3919	TRIP12	A*01:01
2591	POLA2	C*07:01	3919	CFDP1	A*30:01
3309	MATN2	A*11:01	3919	TRIP12	Unknown class II
3309	CDK12	A*11:01	3998	MAGEA6	A*01:01
3466	COL18A1	A*02:01	3998	MED13	Unknown class I
3466	TEAD1	A*02:01	3998	PDS5A	Unknown class I
3466	ERBB2	A*02:01	3998	FAM20C	Unknown class I
3466	PDZD8	B*44:02	4000	GPD2	Unknown class I
3466	PXMP4	B*39:01	4000	AMPH	A*02:01
3466	KHSRP	B*39:01	4000	EVA1A	Unknown class I
3678	FBXO21	Unknown class I	4000	SETD1A	Unknown class I
3678	PDIA5	Unknown class II	4000	DBT	Unknown class I
3703	NSHDL	A*02:01	4000	HIVEP2	Unknown class I

64 somatic mutations identified using TIL from 25 patients with melanoma.
All were unique.

Questions Guiding Current Efforts

What do TIL recognize that enables the in vivo control of the last cancer cell?

Can this supply clues to the immunotherapy of common epithelial cancers?

Patient M.B.

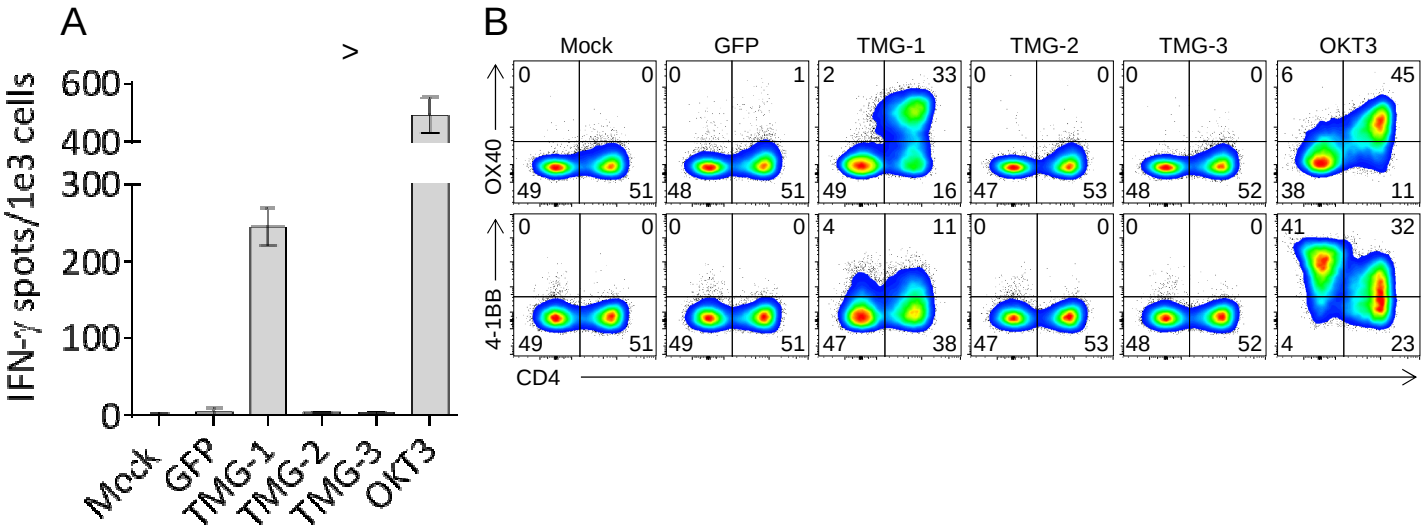
45 y.o. female with metastatic cholangiocarcinoma

12/2009	**Right hepatectomy for cholangiocarcinoma**
4/2010	**Multiple lung and liver metastases** **Received cisplatin and gemcitabine: PD**
5/2011	**Taxotere chemotherapy: PD**
3/2012	**Lung lesion resected for TIL; TIL infused; minimal response (not PR) then PD**
10/2013	**TMG approach to target unique cancer mutations (26 somatic mutations encoded in 3 TMG)**

(Tran et al, Science 344:641-645, 2014)

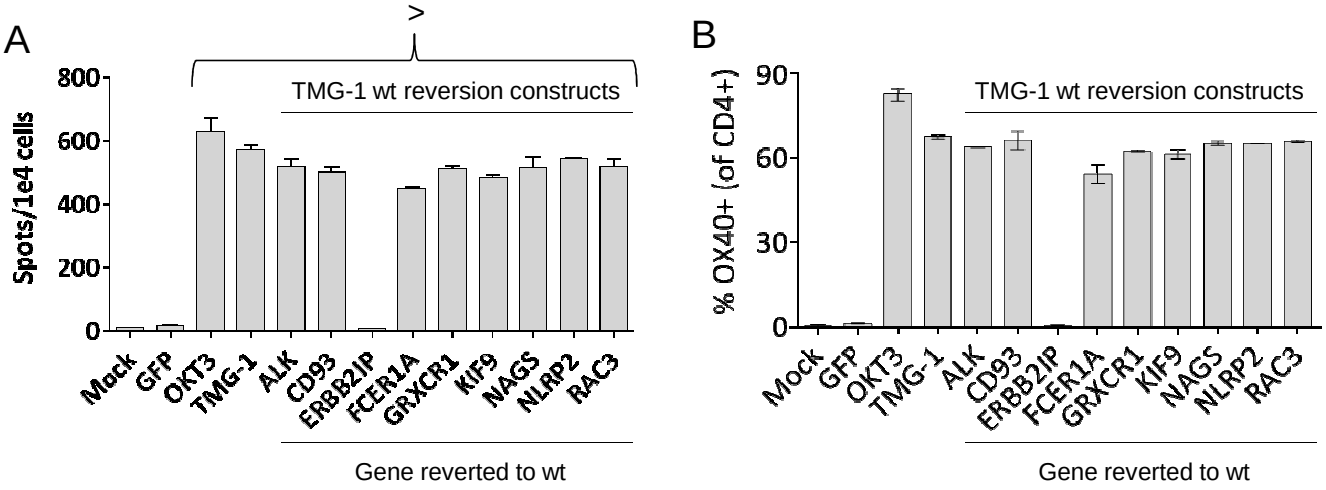
Only TMG-1 is recognized by CD4+ T cells in MB-3737 infusion bag

A) IFN-g ELISPOT assay; B) Flow cytometry



Only mutated ERBB2IP is recognized by CD4+ T cells in MB-3737 infusion bag

Co-culture exp't (transfected DCs + Infusion bagy;
A B) Flow cytometry

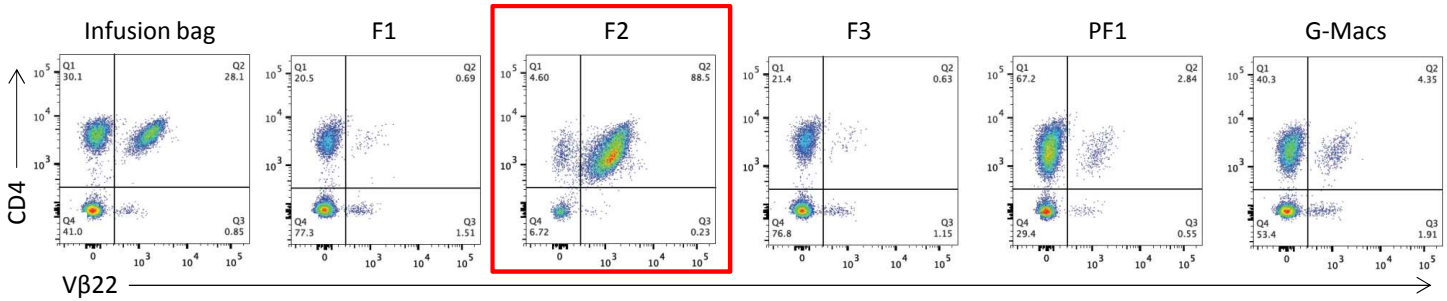


ERBB2IP

Tumor suppressor that binds to ERBB2

Attenuates downstream RAS/ERK signaling

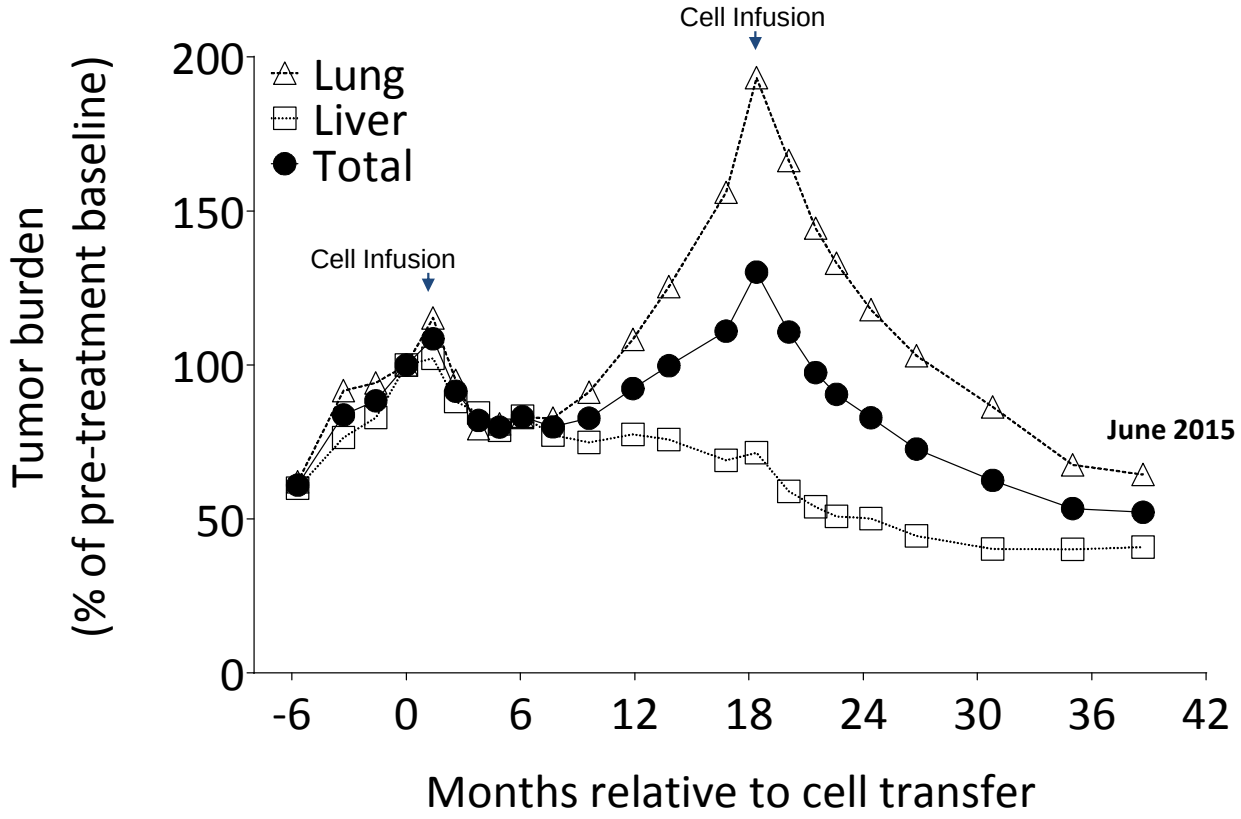
Isolation of ERBB2IP reactive cells



Use purified ERBB2IP autologous lymphocytes for treatment

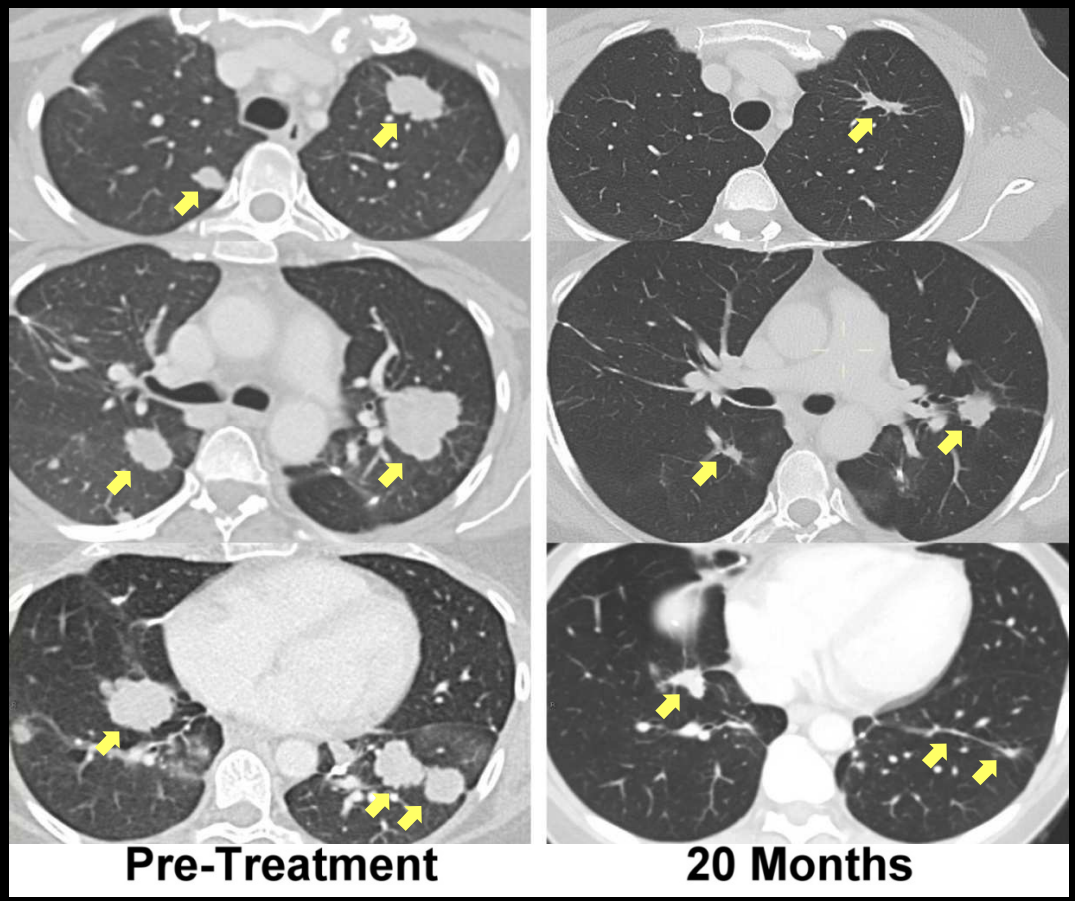
(Tran et al,Science 344:641-645, 2014)

Second Adoptive Therapy with Mutation-Specific Cells: 20 month FU



Tumor regression after ACT with ERBB2IP-mutation-reactive CD4+ cells

Lung CT



June 17, 2015

Mutated antigens recognized by T cells from patients with epithelial cancers

Patient	Histology	Antigen	HLA RE
3737	cholangio.	ERBB2IP	DQβ1*06:01
3978	cholangio.	ITGB4	Unknown class II
3569	colon	CSMD2	Unknown class I
3971	colon	CASP8	Unknown class I
3971	colon	MARK1	B*08:01
3971	colon	XYLT1	Unknown class I
3971	colon	HIST1H3B	A*02:01
3995	colon	KRAS	C*08:02
3995	colon	TUBGCP2	Unknown class I
3995	colon	RNF213	Unknown class I
4007	colon	SKIV2L	A*03:01
4007	colon	H3F3B	Unknown class I
4032	colon	API5	Unknown class I
4032	colon	RNF10	Unknown class I
4032	colon	PHLPP1	Unknown class I
4060	colon	SMC2A	Unknown class II
4071	colon	QSOX2	Unknown class I
4071	colon	MRPS28	Unknown class I
4071	colon	POR	Unknown class I
4077	colon	CPSF6	Unknown class I
4077	colon	HIST1H2BE	Unknown class I
4077	colon	FLII	A*02:01
4090	colon	USP8	Unknown class I
4090	colon	MRPL39	Unknown class II
4095	colon	KRAS	C*08:02
3942	rectal	NUP98	Unknown class I
3942	rectal	KARS	Unknown class I
3942	rectal	GPD2	Unknown class II
4081	rectal	ALDOC	Unknown class I
4081	rectal	RPL12	Unknown class I

Patient	Histology	Antigen	HLA RE
4069	pancreatic	ZFYVE27	Unknown class I
3948	esophageal	PLEC	Unknown class II
3948	esophageal	XPO7	Unknown class II
3948	esophageal	AKAP2	Unknown class II
4014	NSCLC	TGFBRAP1	Unknown class I
4014	NSCLC	USP11	B*57:01
4014	NSCLC	FDT1	Unknown class I
4014	NSCLC	HYAL2	Unknown class I
4014	NSCLC	ATRIP	Unknown class II
4014	NSCLC	CSNK2A1	Unknown class II
4037	NSCLC	NPM1	Unknown class I
4037	NSCLC	NPM1	Unknown class II
4037	NSCLC	ACOT7	Unknown class II
4037	NSCLC	RAD50	Unknown class II
4037	NSCLC	TNK1	Unknown class II
4037	NSCLC	KIAA1432	Unknown class II
4073	NSCLC	FAM83A	Unknown class I
4097	ovarian	HIST1H1B	Unknown class II
4097	ovarian	FLOT1	Unknown class II
4097	ovarian	DEF6	Unknown class II
4097	ovarian	GAK	Unknown class II
4097	ovarian	INPP5K	Unknown class II
4046	ovarian	USP9X	Unknown class I
4062	breast	RPBJ	Unknown class II
3775	cervical	SETDB1	Unknown class I
3775	cervical	METTL17	Unknown class I
3775	cervical	ALDH1A1	Unknown class I

57 somatic mutations from 22 patients with epithelial cancers recognized by autologous TIL.
All were unique (except one).

Mutated antigens recognized by T cells from patients with epithelial cancers

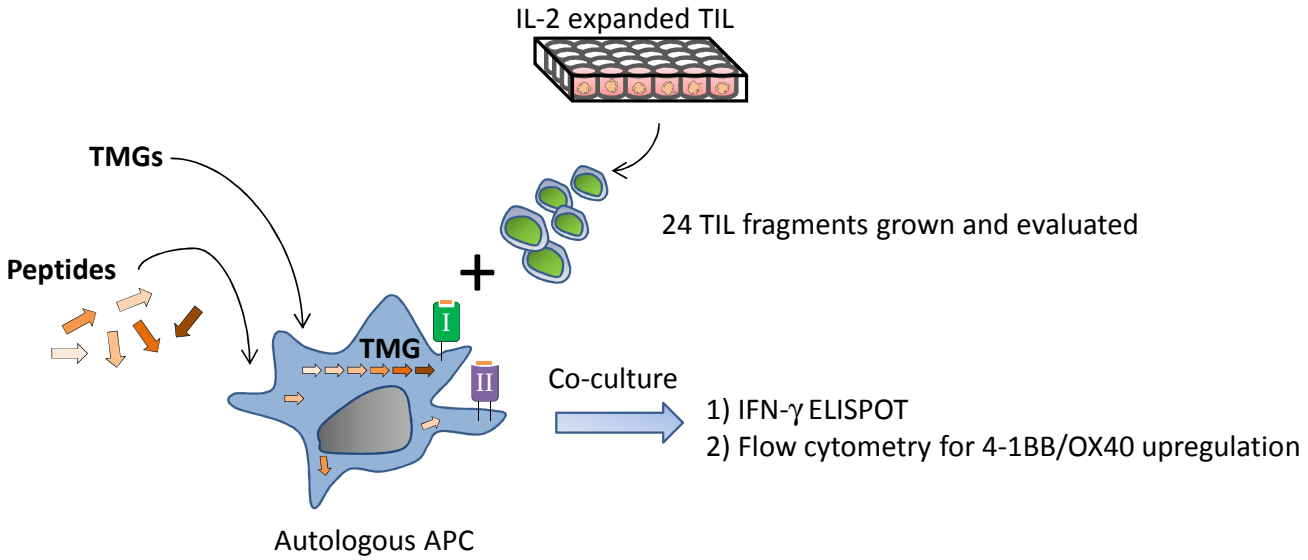
Patient	Histology	Antigen	HLA RE
3737	cholangio.	ERBB2IP	DQβ1*06:01
3978	cholangio.	ITGB4	Unknown class II
3569	colon	CSMD2	Unknown class I
3971	colon	CASP8	Unknown class I
3971	colon	MARK1	B*08:01
3971	colon	XYLT1	Unknown class I
3971	colon	HIST1H3B	A*02:01
3995	colon	KRAS	C*08:02
3995	colon	TUBGCP2	Unknown class I
3995	colon	RNF213	Unknown class I
4007	colon	SKIV2L	A*03:01
4007	colon	H3F3B	Unknown class I
4032	colon	API5	Unknown class I
4032	colon	RNF10	Unknown class I
4032	colon	PHLPP1	Unknown class I
4060	colon	SMC2A	Unknown class II
4071	colon	QSOX2	Unknown class I
4071	colon	MRPS28	Unknown class I
4071	colon	POR	Unknown class I
4077	colon	CPSF6	Unknown class I
4077	colon	HIST1H2BE	Unknown class I
4077	colon	FLII	A*02:01
4090	colon	USP8	Unknown class I
4090	colon	MRPL39	Unknown class II
4095	colon	KRAS	C*08:02
3942	rectal	NUP98	Unknown class I
3942	rectal	KARS	Unknown class I
3942	rectal	GPD2	Unknown class II
4081	rectal	ALDOC	Unknown class I
4081	rectal	RPL12	Unknown class I

Patient	Histology	Antigen	HLA RE
4069	pancreatic	ZFYVE27	Unknown class I
3948	esophageal	PLEC	Unknown class II
3948	esophageal	XPO7	Unknown class II
3948	esophageal	AKAP2	Unknown class II
4014	NSCLC	TGFBRAP1	Unknown class I
4014	NSCLC	USP11	B*57:01
4014	NSCLC	FDT1	Unknown class I
4014	NSCLC	HYAL2	Unknown class I
4014	NSCLC	ATRIP	Unknown class II
4014	NSCLC	CSNK2A1	Unknown class II
4037	NSCLC	NPM1	Unknown class I
4037	NSCLC	NPM1	Unknown class II
4037	NSCLC	ACOT7	Unknown class II
4037	NSCLC	RAD50	Unknown class II
4037	NSCLC	TNK1	Unknown class II
4037	NSCLC	KIAA1432	Unknown class II
4073	NSCLC	FAM83A	Unknown class I
4097	ovarian	HIST1H1B	Unknown class II
4097	ovarian	FLOT1	Unknown class II
4097	ovarian	DEF6	Unknown class II
4097	ovarian	GAK	Unknown class II
4097	ovarian	INPP5K	Unknown class II
4046	ovarian	USP9X	Unknown class I
4062	breast	RPBJ	Unknown class II
3775	cervical	SETDB1	Unknown class I
3775	cervical	METTL17	Unknown class I
3775	cervical	ALDH1A1	Unknown class I

57 somatic mutations from 22 patients with epithelial cancers recognized by autologous TIL.
All were unique (except one).

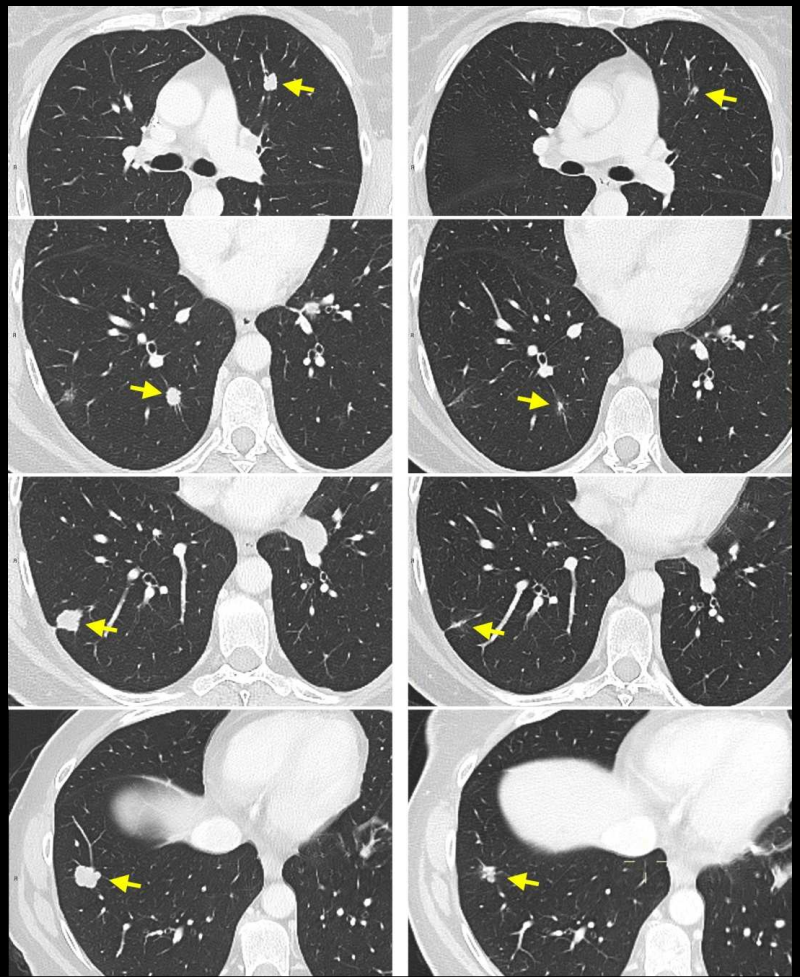
Patient CR (4095)

- **Whole-exome and transcriptome sequencing performed on lung lesions from colon cancer**
  - **61 putative mutations identified**
  - **5 TMGs constructed**
  - **4 long (25-amino acids) peptide pools**
  - **3 short (8-11 amino acids) peptide pools**



Tumor regression after ACT with KRAS-mutation-reactive CD4+ cells

Pt. C.R. PRE 2.5 months (9/15/15)



Detection of Immunogenic Mutations in Epithelial Cancers

Cancer type	# patients evaluated	# patients with immunogenic mutations
Ovary	5	4
Lung	8	7
Cervix	2	1
Head and Neck	1	0
Bladder	2	2
Breast	2	1
Bile Duct	7	5
Colorectum	12	12
Esophagus	2	2
Pancreas	1	1
	42	35

35 of 42 patients with metastatic epithelial cancers expressed immunogenic mutations (all unique except KRAS)

Cell therapy using autologous T-cells that target unique random somatic mutations is a possible treatment for all cancers with a sufficient mutational load.

Potential Improvements in Targeting Somatic Cancer Mutations

Develop methods for identifying tumor-reactive T-cells and TCRs against multiple somatic mutations

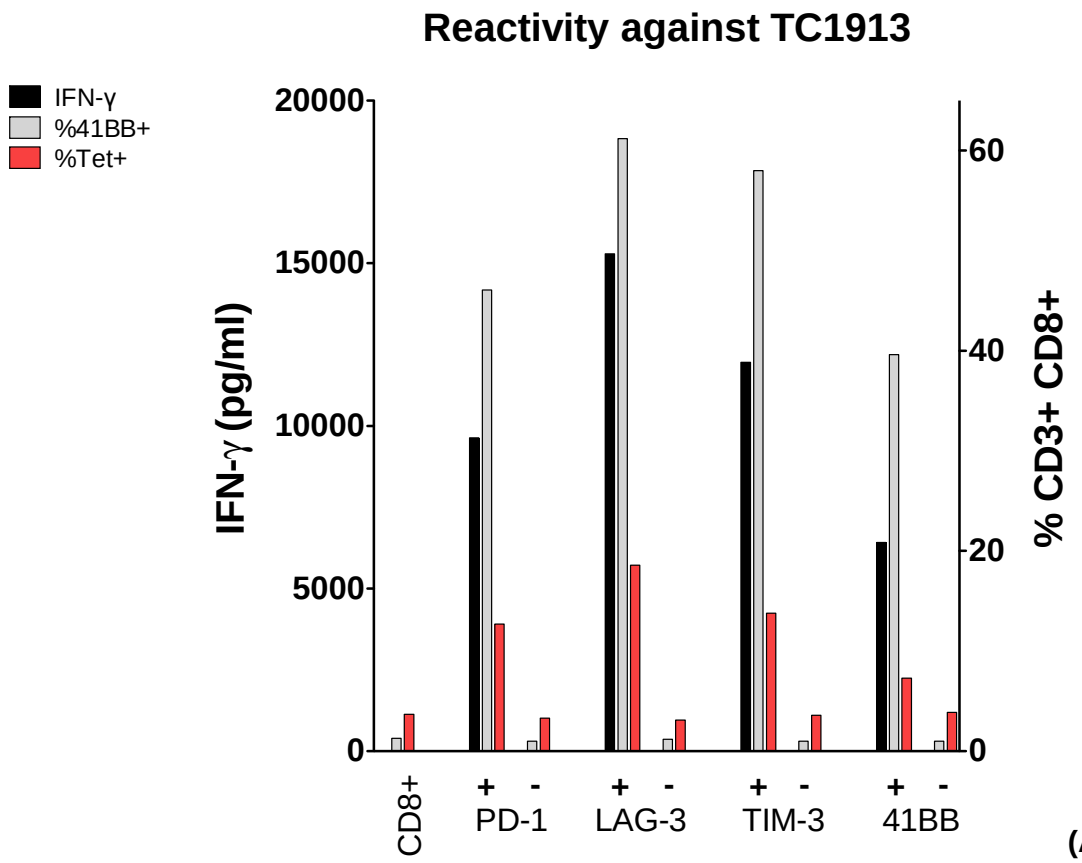
PD1+ cells in tumor and PBL recognize cancer (Alena Gros)

Rank frequency of TCRs in PD1+ cells is related to anti-tumor activity (Anna Pasetto)

Genetic Engineering of TCRs into autologous naïve T-cells

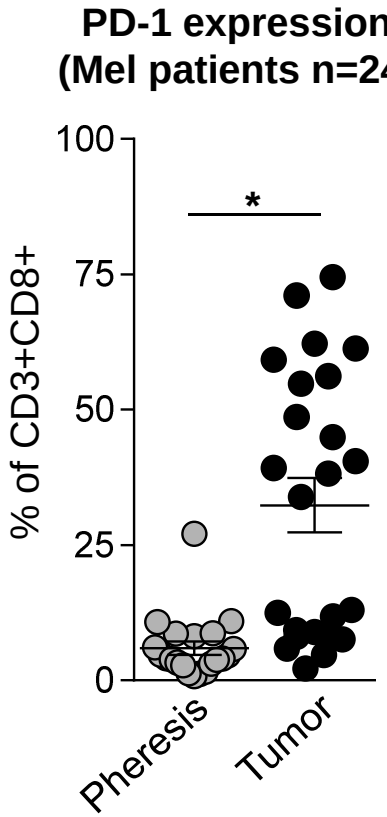
transient retroviral supernatant
CRISPR/CAS
transposon/transposase

Lymphocytes reactive with autologous tumor are highly enriched in subsets expressing PD-1, LAG-3, TIM-3 and 41BB



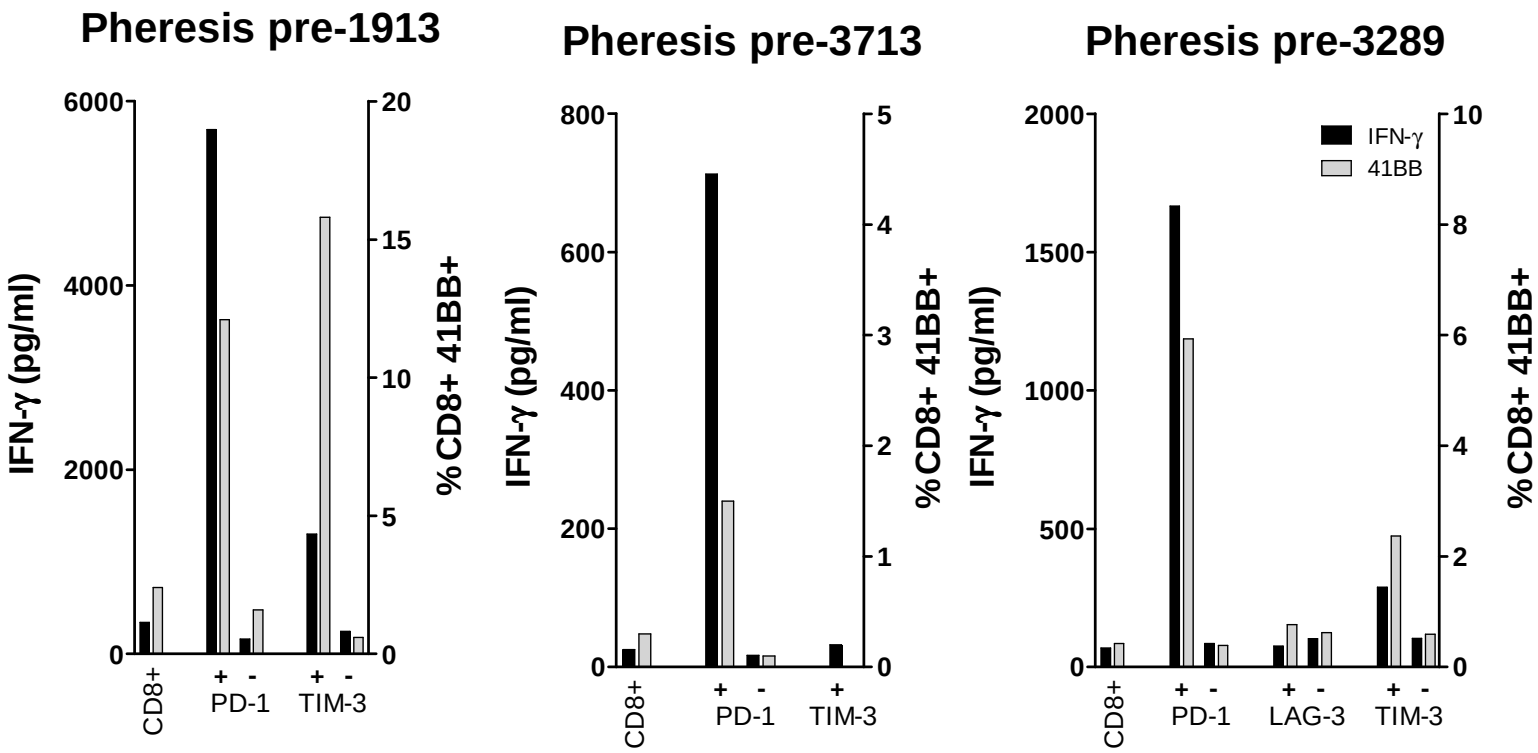
(Alena Gros)

PD-1 expression in peripheral blood lymphocytes is far less prevalent than in TIL



(Alena Gros)

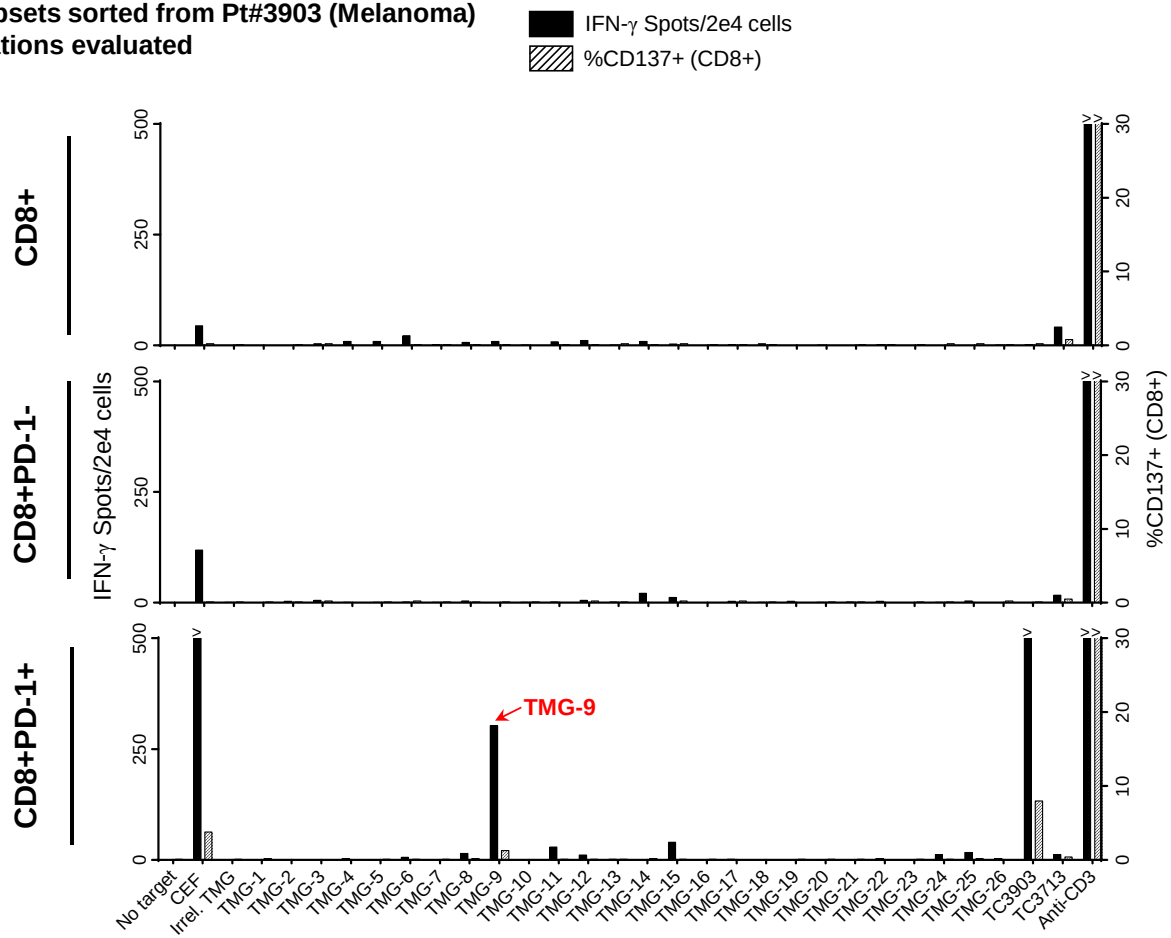
Enrichment of tumor-reactive cells from peripheral blood by selecting PD-1+ cells



(Alena Gros)

Selection of peripheral blood CD8+PD-1+ cells from enriches for mutation-reactive cells

T cell subsets sorted from Pt#3903 (Melanoma)
308 mutations evaluated



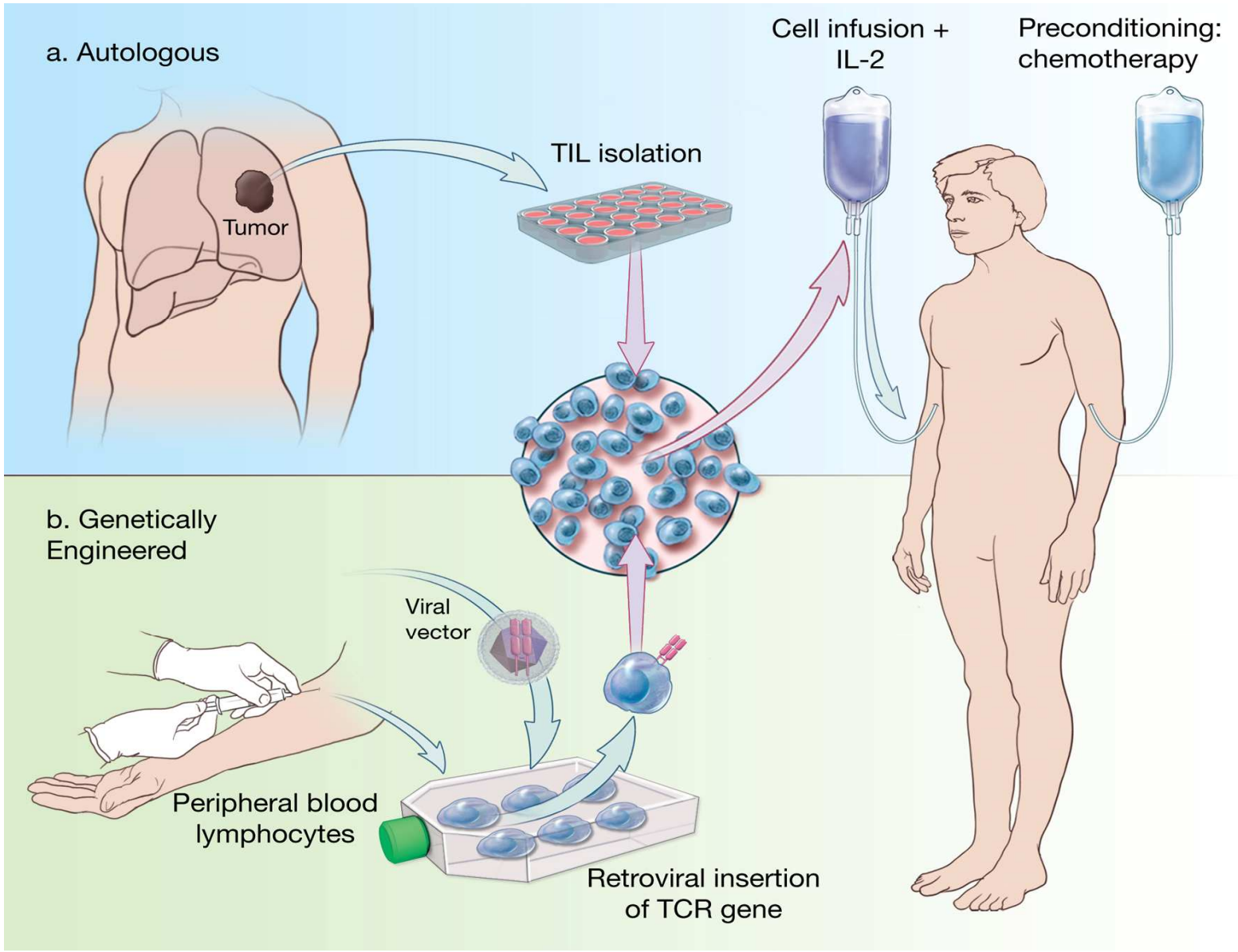
(Alena Gros)

In 10 of 12 melanoma patients the top 1 or 2 ranked TCRs recognize the cancer

Patient	Rank frequency of TCRs that recognize cancer (of top 10 most frequent TCRs)*
1913	2, 3, 4, 6
2650	1, 5, 6, 9
3678	4, 5
3713	2, 3, 5, 6, 8, 10
3759	1, 2, 3, 4, 6
3784	2, 8, 9,10
3903	1
3922	1
3926	1, 3, 5, 7
3977	1, 6
3992	none
3998	1, 2, 4, 5, 6, 7, 8

*not all of the top 10 TCRs tested because of ongoing work to match the α & β chains

(Anna Pasetto)


a. Autologous
Tumor
TIL isolation
Cell infusion +
IL-2
Preconditioning:
chemotherapy
b. Genetically
Engineered
Viral
vector
Peripheral blood
lymphocytes
Retroviral insertion
of TCR gene

Genetic Modification of Peripheral Lymphocytes with Retroviruses Encoding TCRs or CARs

Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Richard A. Morgan, Mark E. Dudley, John R. Wunderlich, Marybeth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Udai S. Kammula, Nicholas P. Restifo, Zhili Zheng, Azam Nahvi, Christiaan R. de Vries, Linda J. Rogers-Freezer, Sharon A. Mavroukakis, Steven A. Rosenberg*

Science 6 OCTOBER 2006 VOL 314

Tumor Regression in Patients With Metastatic Synovial Cell Sarcoma and Melanoma Using Genetically Engineered Lymphocytes Reactive With NY-ESO-1

Paul F. Robbins, Richard A. Morgan, Steven A. Feldman, James C. Yang, Richard M. Sherry, Mark E. Dudley, John R. Wunderlich, Azam V. Nahvi, Lee J. Helman, Crystal L. Mackall, Udai S. Kammula, Marybeth S. Hughes, Nicholas P. Restifo, Mark Raffeld, Chyi-Chia Richard Lee, Catherine L. Levy, Yong F. Li, Mona El-Gamil, Susan L. Schwarz, Carolyn Laurencot, and Steven A. Rosenberg

JOURNAL OF CLINICAL ONCOLOGY 2011 Mar 1;29(7):917-24.

Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19

James N. Kochenderfer,[1] Wyndham H. Wilson,[2] John E. Janik,[2] Mark E. Dudley,[1] Maryalice Stetler-Stevenson,[3] Steven A. Feldman,[1] Irina Maric,[4] Mark Raffeld,[3] Debbie-Ann N. Nathan,[1] Brock J. Lanier,[1] Richard A. Morgan,[1] and Steven A. Rosenberg[1]

blood 2010 116: 4099-4102
Prepublished online Jul 28, 2010

Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor

James N. Kochenderfer, Mark E. Dudley, Sadik H. Kassim, Robert P.T. Somerville, Robert O. Carpenter, Maryalice Stetler-Stevenson, James C. Yang, Giao Q. Phan, Marybeth S. Hughes, Richard M. Sherry, Mark Raffeld, Steven Feldman, Lily Lu, Yong F. Li, Lien T. Ngo, Andre Goy, Tatyana Feldman, David E. Spaner, Michael L. Wang, Clara C. Chen, Sarah M. Kranick, Avindra Nath, Debbie-Ann N. Nathan, Kathleen E. Morton, Mary Ann Toomey, and Steven A. Rosenberg

JOURNAL OF CLINICAL ONCOLOGY

2015 Feb 20;33(6):540-9. Epub 2014 Aug 25.

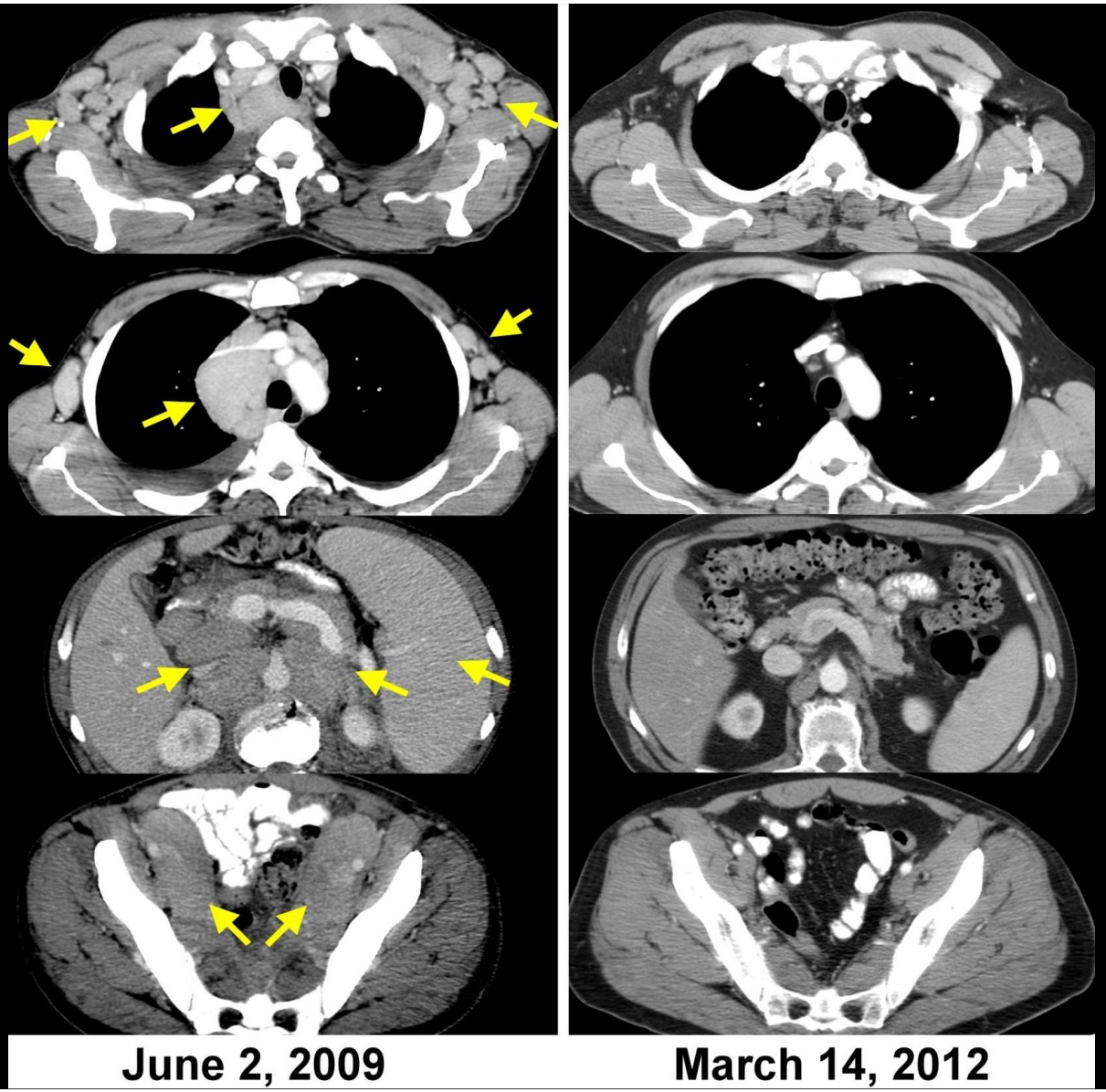
Five of nine large-cell lymphoma patients obtained CRs

Patient	Lymphoma type	Number of prior therapies	Infused CAR+ T cells/kg	Response (duration in months)
1	PMBCL	4	$5x10^6$	CR (35+)
2	PMBCL	3	$2.5x10^6$	NE, death
3	DLBCL, NOS	5	$2.5x10^6$	CR (25+)
4	PMBCL	10	$2.5x10^6$	CR (21+)
5	PMBCL	3	$2.5x10^6$	SD (1)
6	DLBCL, transformed from CLL	13	$1x10^6$	PR (1)
7	DLBCL, NOS	3	$1x10^6$	NE
8	DLBCL, NOS	2	$1x10^6$	CR (6)
9	DLBCL, NOS	3	$1x10^6$	CR (17+)

All but Patient 9 were refractory to their last chemotherapy regimen prior to enrollment, Patient 9 had relapsed after auto stem cell transplant. Patient 9 had a delayed conversion from PR to CR.

E.K.

Follicular lymphoma



Responses to Therapy with NY-ESO-1 TCR (4/15/14)

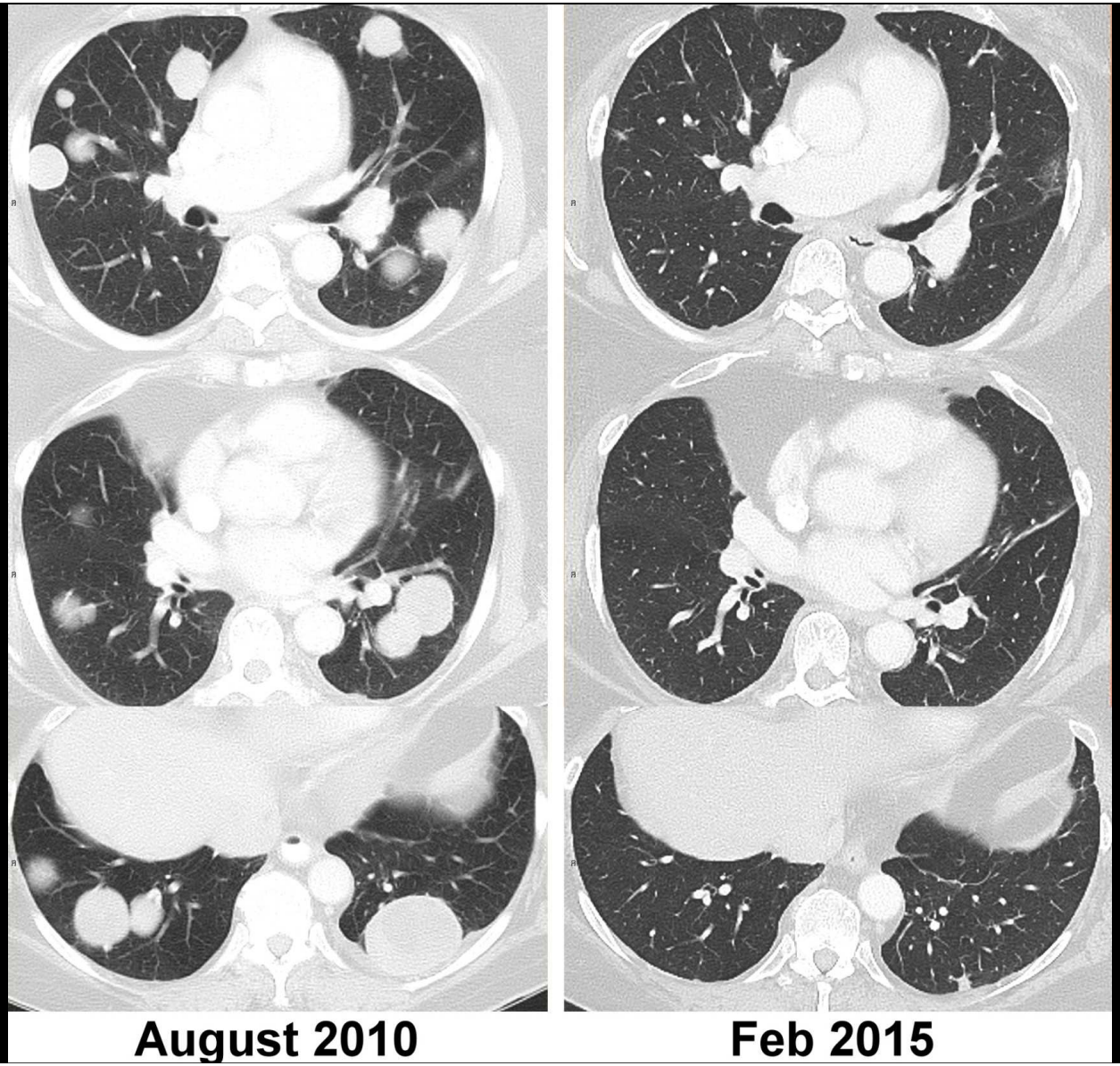
	Total	PR	CR	OR
	number of patients (duration in months)			
Melanoma	20	7 (35%) (28, 10+, 10**, 8, 5, 3, 3)	4 (20%) (58+, 48+, 31+**, 24)	11 (55%)
Synovial Cell Sarcoma	18	9 (50%) (42+**,14*, 8,7, 5, 4, 3**, 3, 2)	1(7%) (16+)	10 (56%)

*treated twice
**plus ALVAC vaccine

(Robbins et al J Clin Oncol 29:917-924, 2011)

A.R.
Synovial sarcoma

NY-ESO-1 TCR



Conclusions

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

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

Autologous peripheral lymphocytes genetically modified to express anti-tumor T cell receptors can mediate cancer regression in vivo.


DR. ROSENBERG


123


Day 10
Day 9
LIVE
Nudes
24 hrs/DAY
PEEP
SHOWS!
Tape $
MONEY
Here

Cell therapy using autologous T-cells that target random somatic mutations (or shared mutations) is a possible treatment of all cancers with a sufficient mutational load

---

Optimize identification of T-cells that recognize multiple antigens and/or "driver" mutations

Develop methods for purifying tumor reactive T-cells

- **PD1+ cells in tumor and PBL recognize cancer**
- **rank frequency of TCRs in PD1+ cells is related to anti-tumor activity**

Genetic Engineering of TCRs into autologous naïve T-cells

- **transient retroviral supernatant**
- **CRISPR/CAS**
- **transposon/transposase**

High-Dose Recombinant Interleukin 2 Therapy for Patients With Metastatic Melanoma: Analysis of 270 Patients Treated Between 1985 and 1993

By Michael B. Atkins, Michael T. Lotze, Janice P. Dutcher, Richard I. Fisher, Geoffrey Weiss, Kim Margolin, Jeff Abrams, Mario Sznol, David Parkinson, Michael Hawkins, Carolyn Paradise, Lori Kunkel, and Steven A. Rosenberg

Purpose: **To determine the short- and long-term efficacy and toxicity of the high-dose intravenous bolus interleukin 2 (IL-2) regimen in patients with metastatic melanoma.**

Patients and Methods: **Two hundred seventy assessable patients were entered onto eight clinical trials conducted between 1985 and 1993. IL-2 (Proleukin [aldesleukin]; Chiron Corp, Emeryville, CA) 600,000 or 720,000 IU/kg was administered by 15-minute intravenous infusion every 8 hours for up to 14 consecutive doses over 5 days as clinically tolerated with maximum support, including pressors. A second identical treatment cycle was scheduled after 6 to 9 days of rest, and courses could be repeated every 6 to 12 weeks in stable or responding patients. Data were analyzed through fall 1996.**

Results: **The overall objective response rate was 16% (95% confidence interval, 12% to 21%); there were 17 complete responses (CRs) (6%) and 26 partial responses (PRs) (10%). Responses occurred with all sites of disease and in patients with large tumor burdens. The median response duration for patients who achieved a CR has not been reached and was 5.9 months for those who achieved a PR. Twelve (28%) of the responding patients, including 10 (59%) of the patients who achieved a CR, remain progression-free. Disease did not progress in any patient responding for more than 30 months. Baseline performance status and whether patients had received prior systemic therapy were the only predictive prognostic factors for response to IL-2 therapy. Toxicities, although severe, generally reversed rapidly after therapy was completed. Six patients (2%) died from adverse events, all related to sepsis.**

Conclusion: **High-dose IL-2 treatment seems to benefit some patients with metastatic melanoma by producing durable CRs or PRs and should be considered for appropriately selected melanoma patients.**

J Clin Oncol 17:2105-2116.

EARLY EFFORTS TO DEVELOP HUMAN CANCER IMMUNOTHERAPY IN THE CLINICAL CENTER, NIH

Date	Administration to patients with metastatic cancer	
11/68	Blood transfusion from a patient with spontaneous regression	1 patient
11/15/77	Pig lymphocytes sensitized to human cancer	6 patients
1/29/80	Lymphokine Activated Killer (LAK) cells – (generated in mammalian IL-2; In-III labelled)	3 patients
7/20/83	Natural mammalian IL-2 (Phase I)	16 patients
3/21/84	LAK cells produced with recombinant IL-2	27 patients
3/30/84	Recombinant IL-2 (Phase I)	23 patients
		76 patients
11/29/84	High-dose bolus IL-2 plus LAK cells	

A PROGRESS REPORT ON THE TREATMENT OF 157 PATIENTS WITH ADVANCED CANCER USING LYMPHOKINE-ACTIVATED KILLER CELLS AND INTERLEUKIN-2 OR HIGH-DOSE INTERLEUKIN-2 ALONE

STEVEN A. ROSENBERG, M.D., PH.D., MICHAEL T. LOTZE, M.D., LINDA M. MUUL, PH.D., ALFRED E. CHANG, M.D., FRED P. AVIS, M.D., SUSAN LEITMAN, M.D., W. MARSTON LINEHAN, M.D., CARY N. ROBERTSON, M.D., ROBERTA E. LEE, M.D., JOSHUA T. RUBIN, M.D., CLAUDIA A. SEIPP, R.N., COLLEEN G. SIMPSON, R.N., AND DONALD E. WHITE, M.S.

Abstract We studied the effects of adoptive immunotherapy with lymphokine-activated killer (LAK) cells plus interleukin-2 or therapy with high-dose interleukin-2 alone in 157 patients with metastatic cancer for whom standard therapy had proved ineffective or no standard effective treatment was available. One hundred eight patients were treated with 127 courses of LAK cells plus interleukin-2, and 49 patients were treated with 53 courses of high-dose interleukin-2 alone.

Of 106 evaluable patients receiving LAK cells plus interleukin-2, 8 had complete responses, 15 had partial responses, and 10 had minor responses. The median duration of response was 10 months among those with complete responses and 6 months among those with partial responses; the patient with the longest complete response was still in remission 22 months after treatment. Of 46 evaluable patients treated with high-dose interleukin-2 alone, 1 had a complete response (remission >4 months), 5 had partial responses (2, >3, >5, 7, and >11 months), and 1 had a minor response. Seven of the total of nine complete responses still remain in remission. Hypotension, weight gain, oliguria, and elevation of bilirubin and creatinine levels were common, but these side effects resolved promptly after interleukin-2 administration was stopped. There have been four treatment-related deaths among these 157 patients.

This immunotherapeutic approach can result in marked tumor regression in some patients for whom no other effective therapy is available at present. Determining its ultimate role in cancer therapy awaits further attempts to increase the therapeutic efficacy of treatment and decrease its toxicity and complexity. (N Engl J Med 1987; 316:889-97.)

“It would be as difficult to reject the right ear and leave the left ear intact as it is to immunize against cancer.”

W. H. Woglum
Cancer Research

Cell therapy using autologous T-cells that target random somatic mutations (or shared mutations) is a possible treatment of all cancers with a sufficient mutational load

---

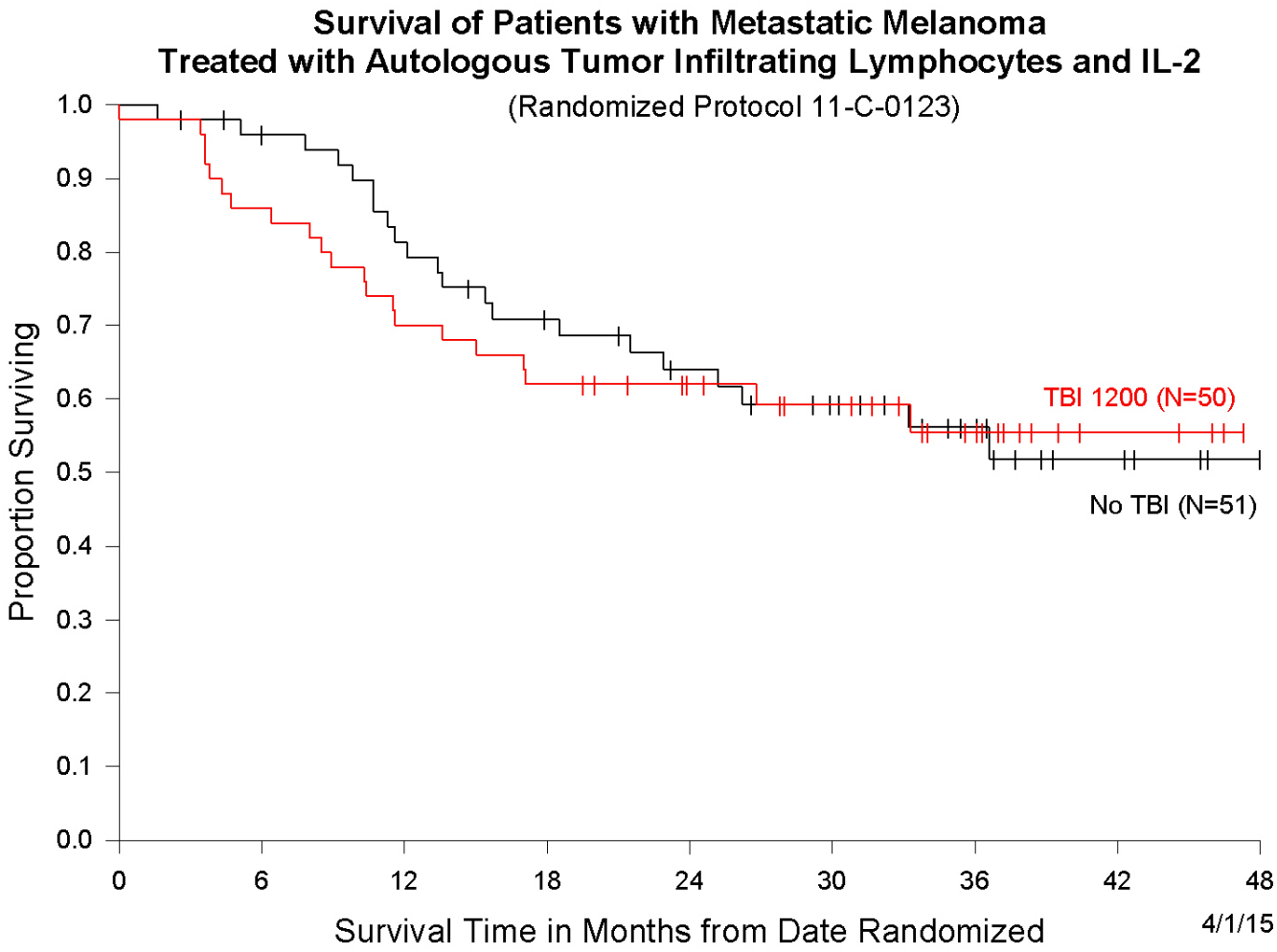
Optimize identification of T-cells that recognize multiple antigens and/or "driver" mutations

Develop methods for purifying tumor reactive T-cells

- **PD1+ cells in tumor and PBL recognize cancer**
- **rank frequency of TCRs in PD1+ cells is related to anti-tumor activity**

Genetic Engineering of TCRs into autologous naïve T-cells

- **transient retroviral supernatant**
- **CRISPR/CAS**
- **transposon/transposase**


Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(Randomized Protocol 11-C-0123)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TBI 1200 (N=50)
No TBI (N=51)
0
6
12
18
24
30
36
42
48
Survival Time in Months from Date Randomized
4/1/15

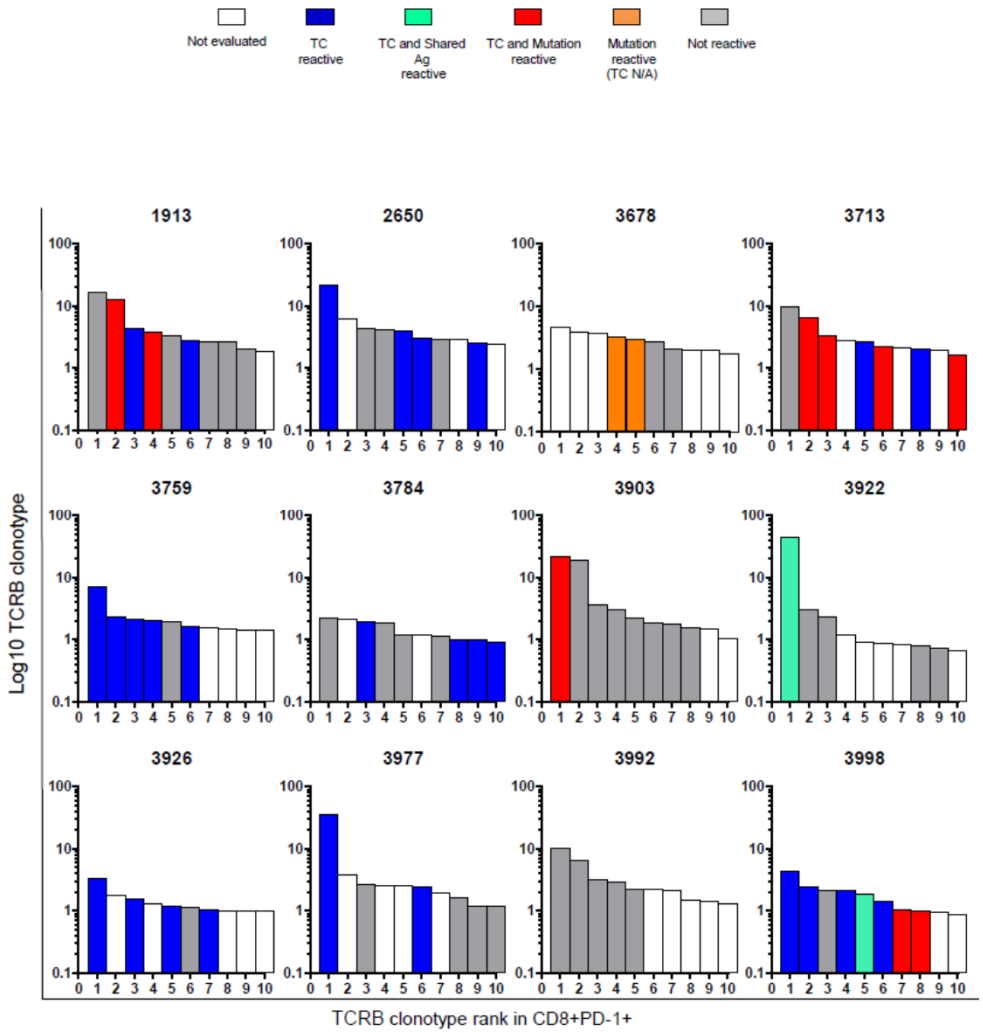
Whole exome sequencing identifies 26 mutations in a lung metastasis

Gene Symbol	Mutation Position Nucleotide (genomic)	Amino Acid (protein)	Mutation Type	Consequence
ALK	chr2_29996620-29996620_C_T	137R>H	Substitution	Nonsynonymous coding
AR	chrX_66858483-66858483__C	NA	Insertion	Frameshift
CD93	chr20_23012929-23012929_C_T	634R>Q	Substitution	Nonsynonymous coding
DIP2C	chr10_365545-365545_C_T	NA	Substitution	Splice site acceptor
ERBB2IP	chr5_65385316-65385316_A_G	805E>G	Substitution	Nonsynonymous coding
FCER1A	chr1_157544227-157544227_G_C	219D>H	Substitution	Nonsynonymous coding
GRXCR1	chr4_42590102-42590102_C_T	21A>V	Substitution	Nonsynonymous coding
HLA-DOA	chr6_33085209-33085209_C_T	NA	Substitution	Splice site donor
KIF9	chr3_47287859-47287859_T_C	155T>A	Substitution	Nonsynonymous coding
KLHL6	chr3_184692410-184692413_CAGA_	NA	Deletion	Frameshift
LHX9	chr1_196164923-196164923_A_	NA	Deletion	Frameshift
LONRF3	chrX_118007666-118007666_A_C	NA	Substitution	Splice site donor
NAGS	chr17_39440355-39440355_G_A	412R>H	Substitution	Nonsynonymous coding
NLRP2	chr19_60186650-60186650_G_T	591S>I	Substitution	Nonsynonymous coding

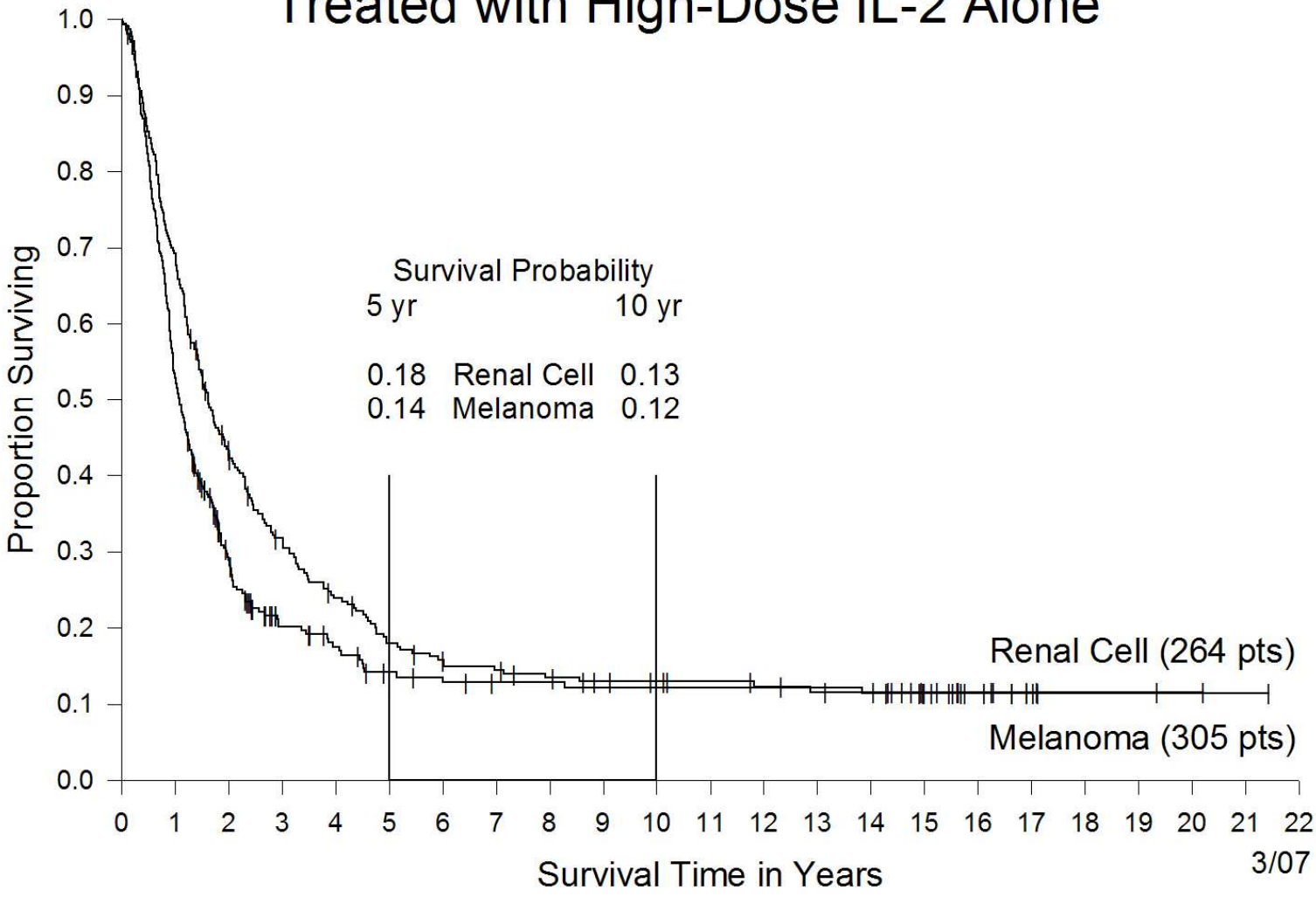
KRAS Mutations and Human Cancers

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


Not evaluated
TC reactive
TC and Shared Ag reactive
TC and Mutation reactive
Mutation reactive (TC N/A)
Not reactive
1913
2650
3678
3713
3759
3784
3903
3922
3926
3977
3992
3998
Log10 TCRB clonotype
TCRB clonotype rank in CD8+PD-1+

569 Patients with Metastatic Disease Treated with High-Dose IL-2 Alone



(N. Engl. J. Med. 313:1485-92, 1985)

SPECIAL REPORT

OBSERVATIONS ON THE SYSTEMIC ADMINISTRATION OF AUTOLOGOUS LYMPHOKINE-ACTIVATED KILLER CELLS AND RECOMBINANT INTERLEUKIN-2 TO PATIENTS WITH METASTATIC CANCER

Abstract We describe here the preliminary results of the systemic administration of autologous lymphokine-activated killer (LAK) cells and the recombinant-derived lymphokine interleukin-2 to patients with advanced cancer. This regimen was based on animal models in which the systemic administration of LAK cells plus interleukin-2 mediated the regression of established pulmonary and hepatic metastases from a variety of murine tumors in several strains of mice.

We treated 25 patients with metastatic cancer in whom standard therapy had failed. Patients received both 1.8 to 18.4×10^{10} autologous LAK cells, generated from lymphocytes obtained through multiple leukaphereses, and up to 90 doses of interleukin-2. Objective regression of cancer (more than 50 per cent of volume) was observed in 11 of the 25 patients: complete tumor regression occurred in one patient with metastatic melanoma and has been sustained for up to 10 months after therapy, and partial responses occurred in nine patients with pulmonary or hepatic metastases from melanoma, colon cancer, or renal-cell cancer and in one patient with a primary unresectable lung adenocarcinoma. Severe fluid retention was the major side effect of therapy, although all side effects resolved after interleukin-2 administration was stopped.

Further development of this approach and additional patient follow-up are required before conclusions about its therapeutic value can be drawn.

DURATION OF RESPONSE IN PATIENTS WITH METASTATIC CANCER TREATED USING HIGH-DOSE BOLUS INTERLEUKIN-2

Diagnosis	CR	PR
	Months	
Melanoma	186+,184+,182+,180+,178+, 171+,167+,158+,158+,157+, 16,12	35,31,19,10,10, 8,8,7,7,6, 5,5,5,4,4,2
Renal Cell Cancer	196+,186+,181+,181+,177+, 173+,173+,166+,157+,151+, 150+,140+,136+,126+,117+, 87+,46+,35,23,19,19	52,30,30,22,20, 17,16,15,14,14, 13,11,9,8,8, 7,7,6,4,4,4,4

"+" indicates ongoing response, as of June 1, 2005.
Of 33 patients with complete response, 27 remain in CR at 46 to 196 months.

NEJM Dec. 1988.

SPECIAL REPORT

USE OF TUMOR-INFILTRATING LYMPHOCYTES AND INTERLEUKIN-2 IN THE IMMUNOTHERAPY OF PATIENTS WITH METASTATIC MELANOMA

A Preliminary Report

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Genetic Engineering of Human Lymphocytes

GENE TRANSFER INTO HUMANS — IMMUNOTHERAPY OF PATIENTS WITH ADVANCED MELANOMA, USING TUMOR-INFILTRATING LYMPHOCYTES MODIFIED BY RETROVIRAL GENE TRANSDUCTION

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(N Engl J Med, 323:570-8, 1990)