Presenter Disclosure Information

Eric Tran

The following relationships exist related to this presentation:

No Relationships to Disclose

Identification of Antigens Targeted by Tumor Infiltrating Lymphocytes to Enhance Adoptive Immunotherapy

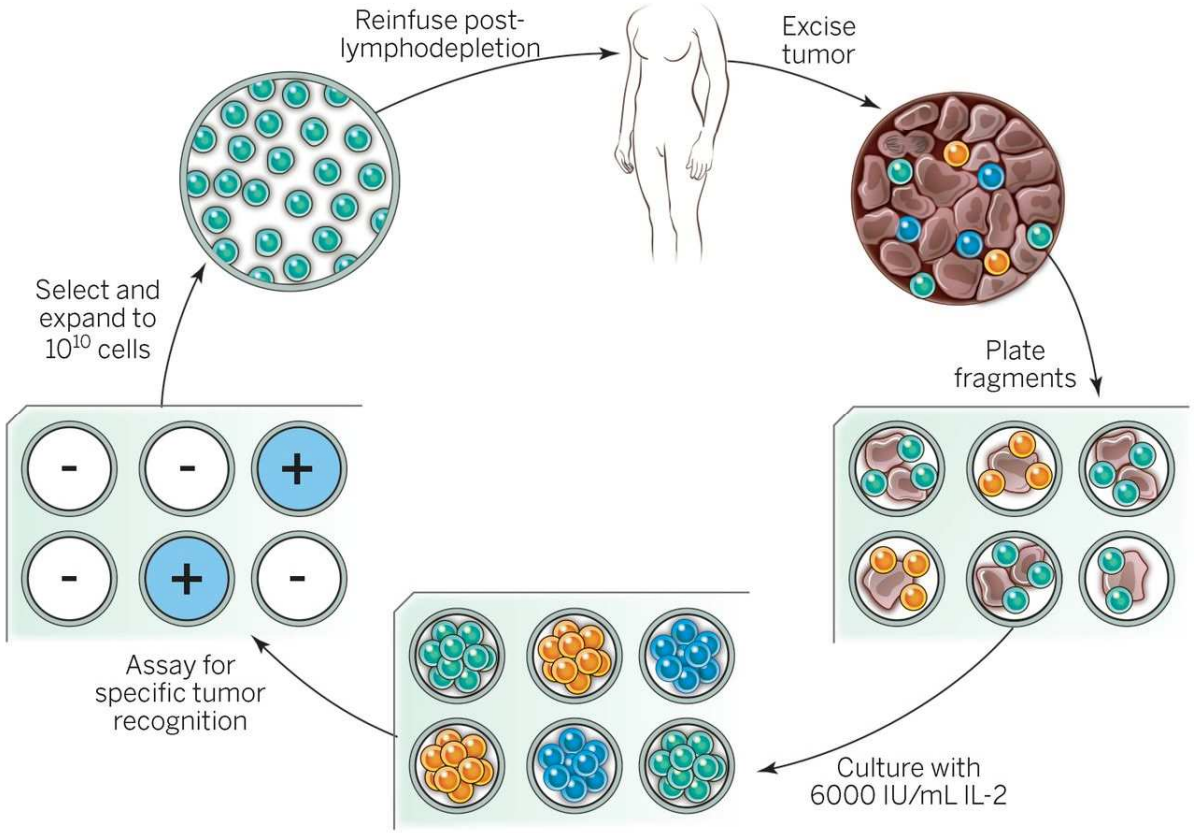
SITC Workshop

Nov. 5, 2015, National Harbor, MD

Eric Tran, PhD

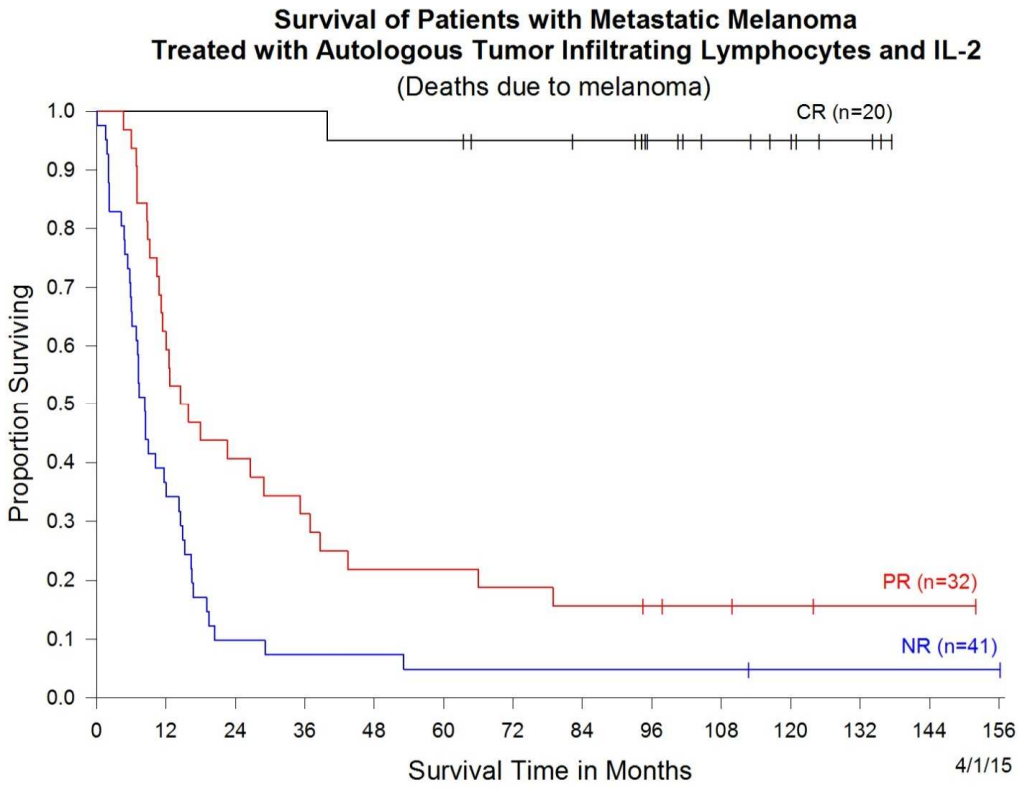
Rosenberg Lab, Surgery Branch, NCI, NIH

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



Rosenberg and Restifo, *Science*, Apr 3 2015, 348 (6230): 62-68

Adoptive transfer of TIL can cure some patients with metastatic melanoma



What antigens are recognized by TIL and are contributing to tumor regression?

T cells recognizing mutated antigens likely play a major role in the effectiveness of T-cell based therapies against melanoma

Efficient Identification of Mutated Cancer Antigens Recognized by T Cells Associated with Durable Tumor Regressions

Lu et al. Clin Cancer Res; 20(13) July 1, 2014

Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells

Robbins et al. NATURE MEDICINE VOLUME 19 | NUMBER 6 | JUNE 2013

Mutated PPP1R3B Is Recognized by T Cells Used To Treat a Melanoma Patient Who Experienced a Durable Complete Tumor Regression

Lu et al. The Journal of Immunology **2013, 190: 6034–6042**

VOLUME 31 · NUMBER 32 · NOVEMBER 10 2013

JOURNAL OF CLINICAL ONCOLOGY Van Rooij et al.

Tumor Exome Analysis Reveals Neoantigen-Specific T-Cell Reactivity in an Ipilimumab-Responsive Melanoma

The NEW ENGLAND JOURNAL of MEDICINE

Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma

N ENGL J MED 371;23 NEJM.ORG DECEMBER 4, 2014

Snyder et al.

Genomic correlates of response to CTLA-4 blockade in metastatic melanoma

Science 9 OCTOBER 2015 • VOL 350 ISSUE 6257

Van Allen et al.

Can TIL therapy be effective in epithelial cancers such as gastrointestinal cancers with lower mutation burdens?

Conventional TIL therapy is largely ineffective against metastatic GI cancers

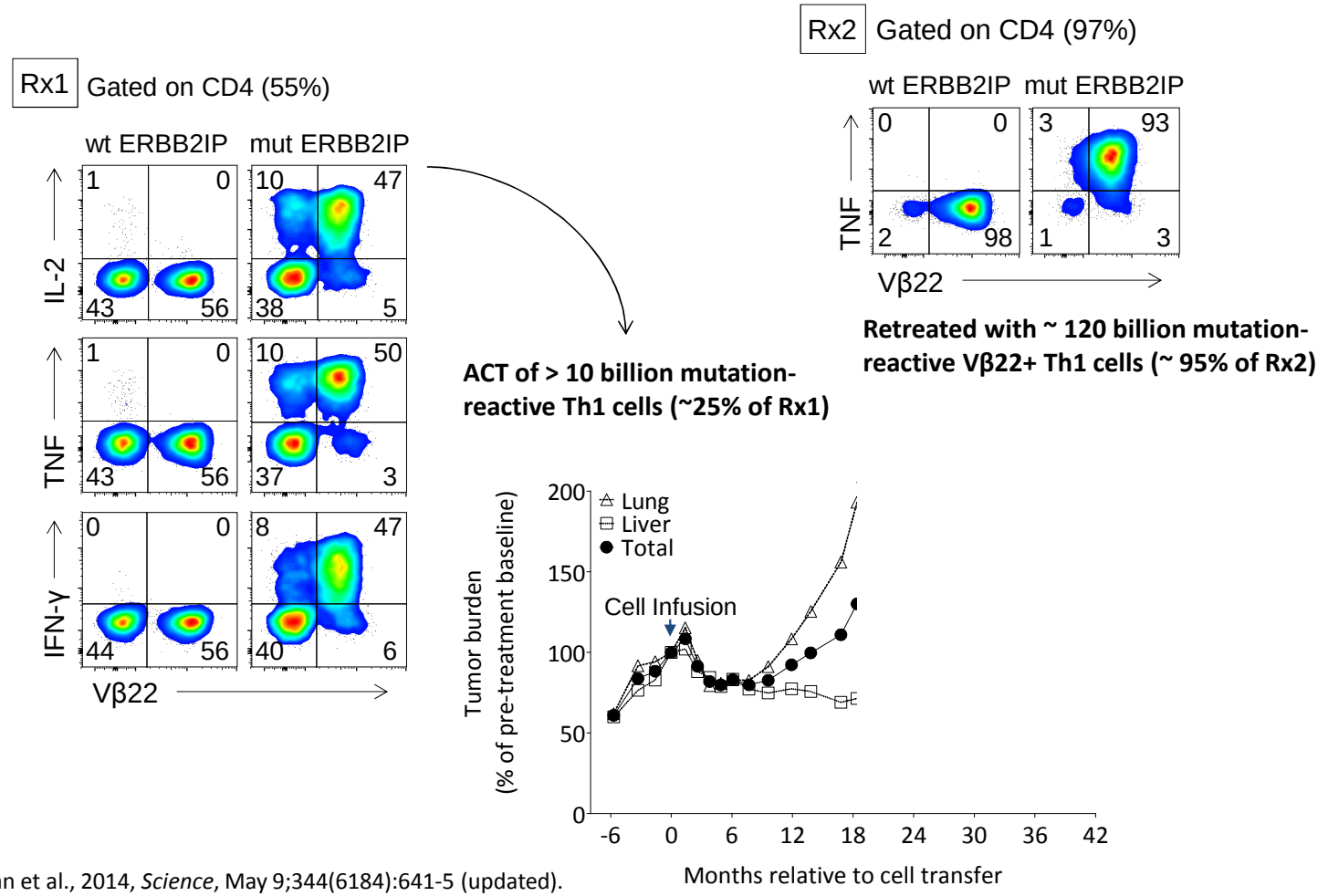
Patient ID	Tumor type	Response
3454	Colorectal	PD
3596	Colon	PD
3610	Rectal	PD
3671	Colon	PD
3674	Colorectal	PD
3690	Colon	PD
3717	Gastric	PD
3737	Cholangio	PD (13 mo SD)
3788	GE junction	PD
3812	Cholangio	PD
3894	Colon	PD
3942	Rectal	PD
3948	Esophageal	PD
3970	Colon (Lynch)	PD
3971	Colon	PD
3978	Cholangio	PD

PD: progressive disease
SD: stable disease
PR: partial response

Did T cells contribute to this response?

If so, what do they recognize? Mutations?

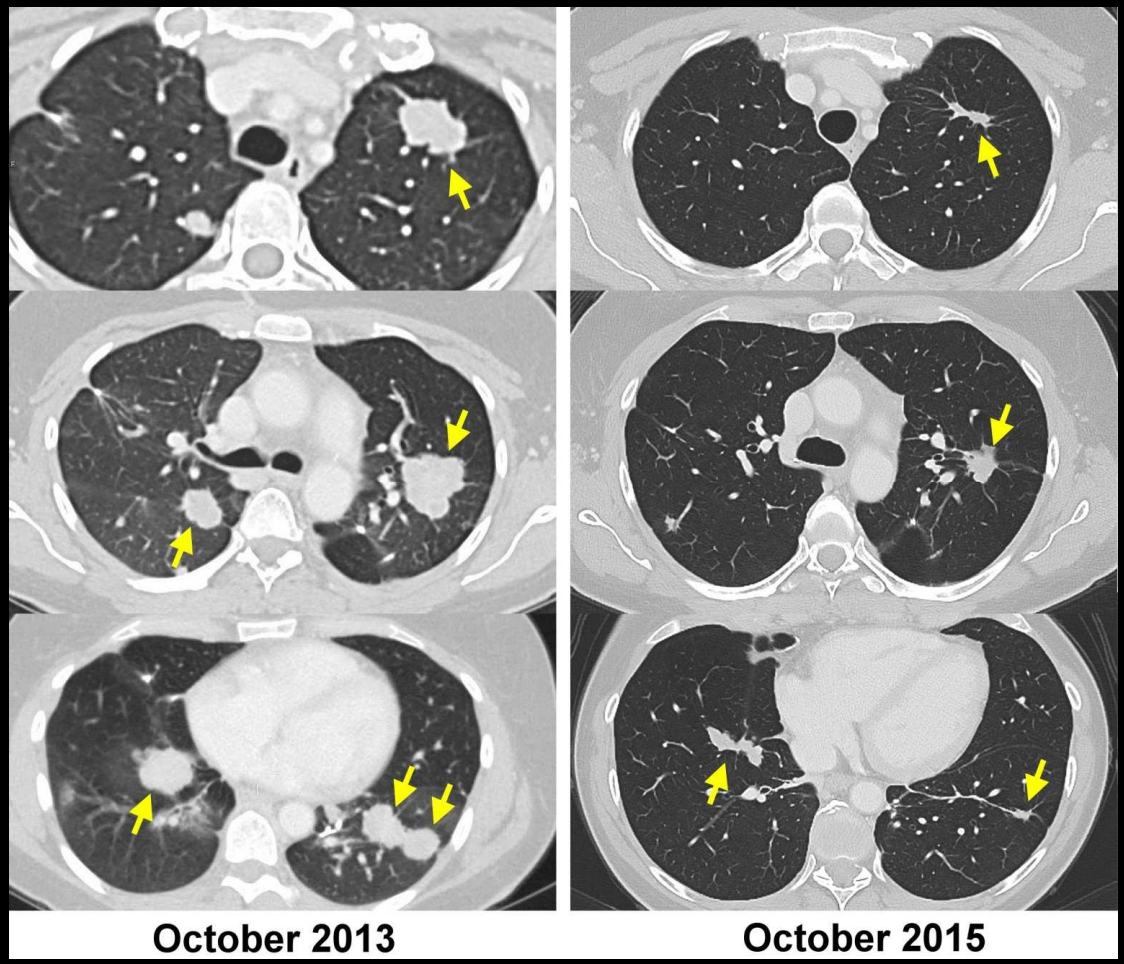
Mutation-reactive T cells can be found in a patient with cholangiocarcinoma and appear capable of mediating tumor regression



Tran et al., 2014, *Science*, May 9;344(6184):641-5 (updated).

Tumor regression after ACT with ERBB2IP-mutation-reactive Th1 cells

Lung CT

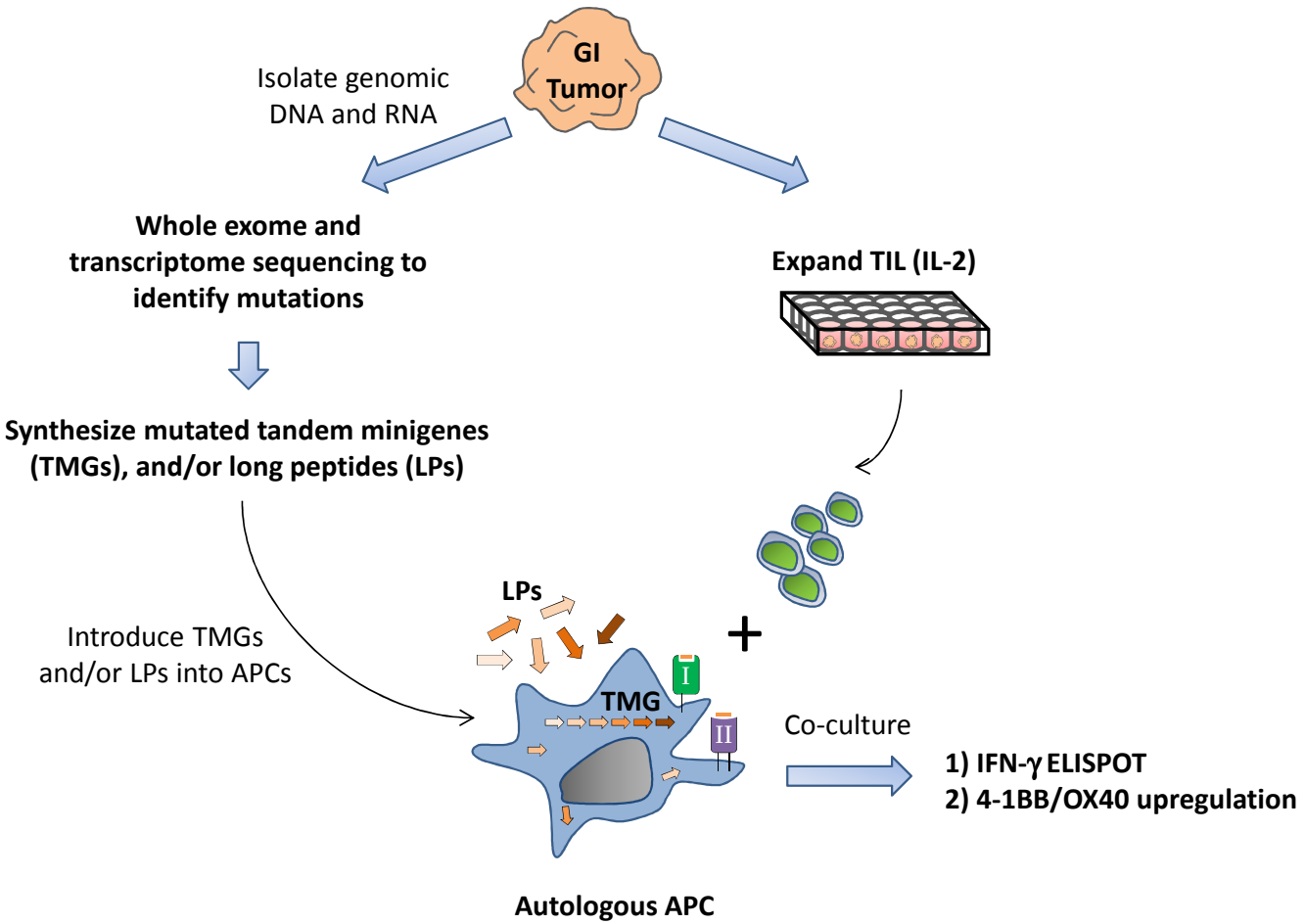


Main Questions

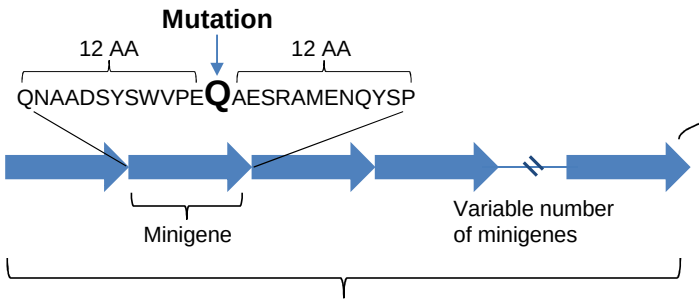
1. **Are mutation-reactive T cells frequently found in other patients with metastatic GI cancers?**

2. **Can we effectively harness the mutation-specific T-cell response to treat other patients with GI cancers?**

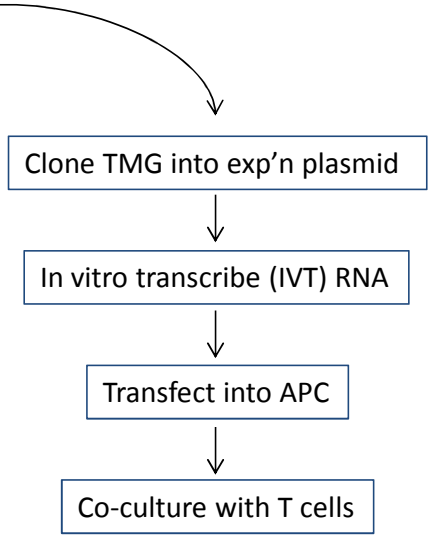
Assessing T-cell reactivity against mutated antigens



Tandem minigene (TMG):
String of minigenes encoding the mutated AA flanked by 12 AA on each side



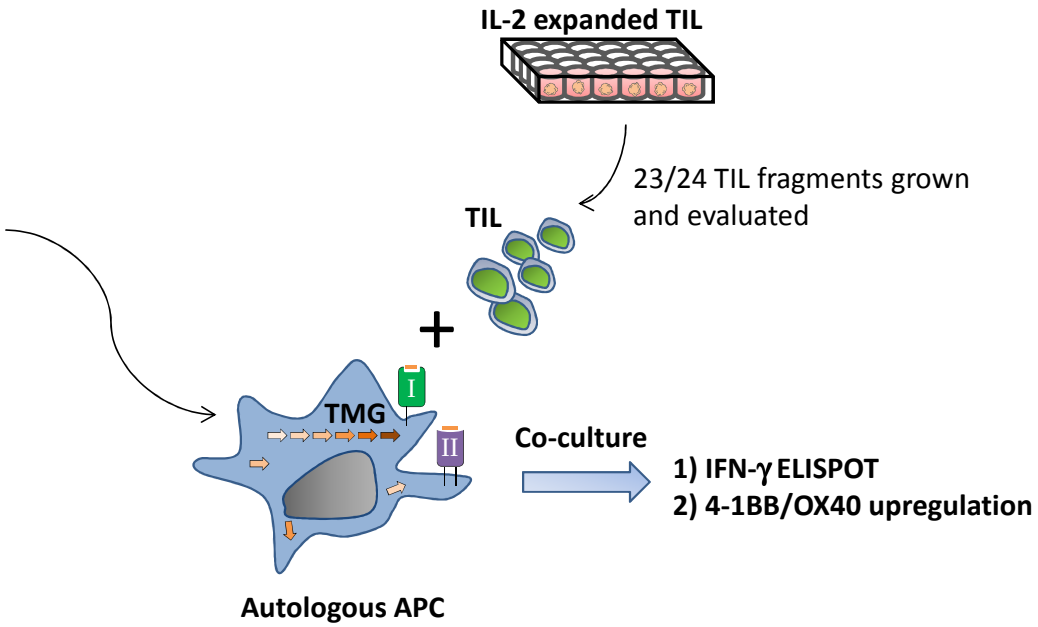
Tandem minigene (variable # of minigenes)



Patient LS (4007)

- 52-year old male with primary colon cancer metastatic to the liver and lung
- Hepatic wedge resection for GI-TIL and whole exome sequencing
- 134 mutations (PGDx, stringent), 264 mutations (in-house, relaxed)
  - 17 TMGs constructed

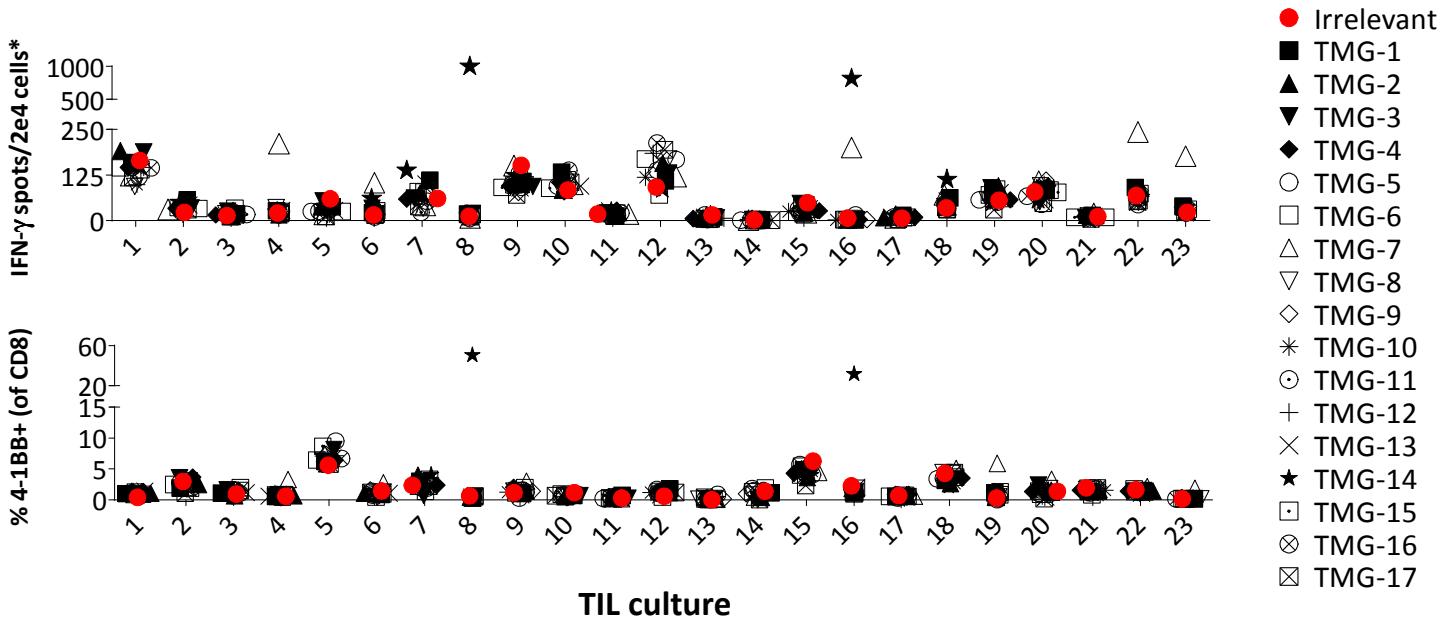
TMG	# minigenes
1	16
2	16
3	16
4	16
5	16
6	16
7	16
8	12
9	16
10	15
11	16
12	14
13	16
14	16
15	16
16	16
17	15



LS-4007: Several TIL cultures display reactivity against TMG-7 and TMG-14

Co-culture: TIL fragments with TMG RNA transfected DCs

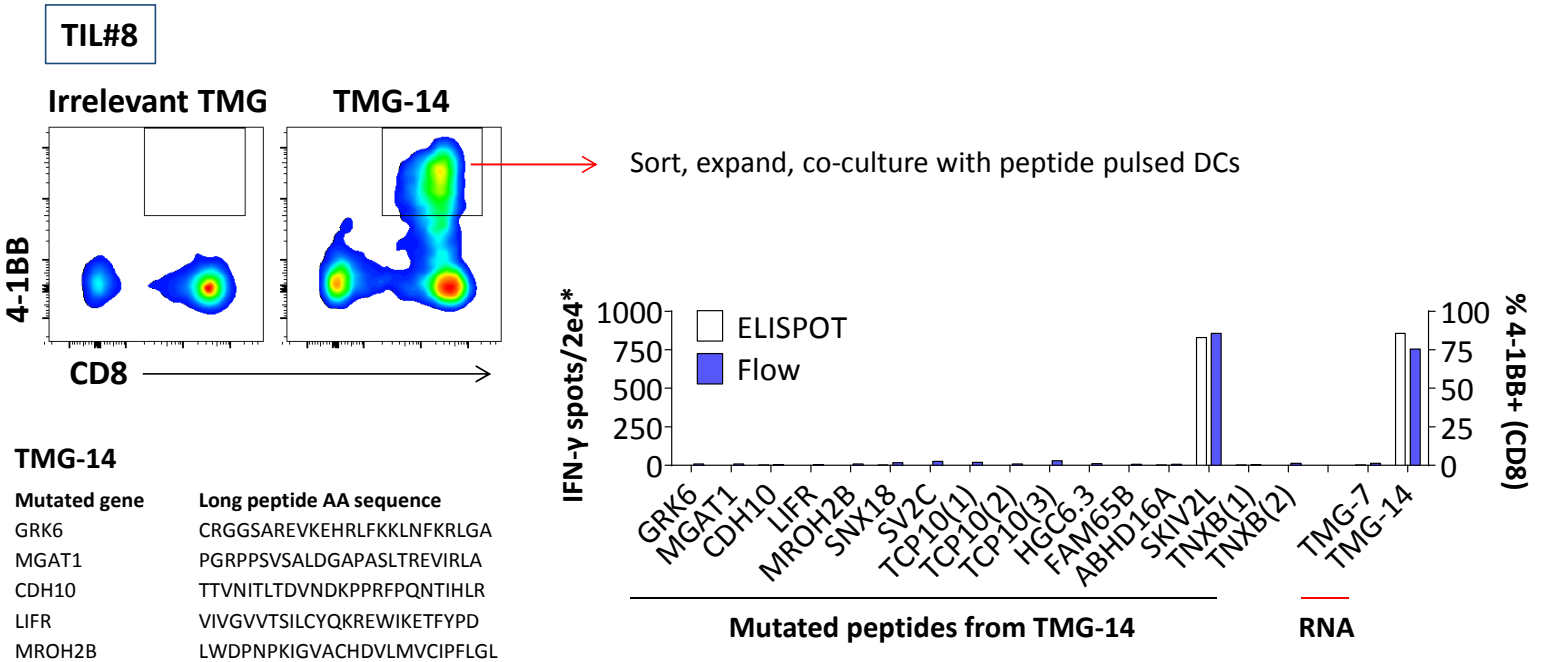
IFN-γ ELISPOT (top); 4-1BB upregulation by flow cytometry (bottom)



*above 500 spots not accurate

Tran et al., 2015, *Science*, October 29, 2015

LS-4007: What do TIL from culture #8 recognize?



TMG-14

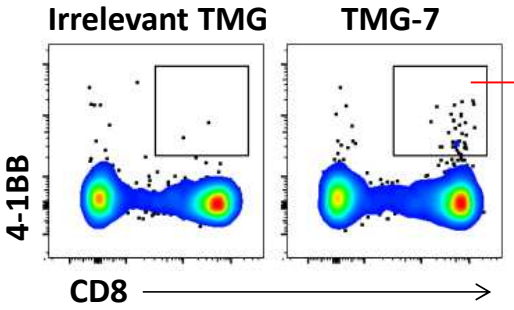
Mutated gene	Long peptide AA sequence
GRK6	CRGGSAREVKEHRLFKKLNFKRLGA
MGAT1	PGRPPSVSALDGAPASLTREVIRLA
CDH10	TTVNITLTDVNDKPPRFPQNTIHLR
LIFR	VIVGVVTSILCYQKREWIKETFYPD
MROH2B	LWDPNPKIGVACHDVLMVCIPFLGL
SNX18	YSTGEEASRDVDTWVFSLECKLDCS
SV2C	MEYDNGRFIGVKLKSVTFKDSVFKS
TCP10(1)	AFGKISHLSADEETTPKYAGRKSQS
TCP10(2)	TLALEPAFGKISPLSADEETTPKYAGRKSQS
TCP10(3)	LLALEPAFGKISPLSADEDTTPKYA
HGC6.3	VFSSPHTAGGMTSTVFSSPHTAGGM
FAM65B	MSDLAPSNLLAQHEVLRTLALLLTR
ABHD16A	NEIDTMFVDRRGAAEPQGQKLVICC
SKIV2L	PILKEIVEMLFSHGLVKVLFATETF
TNXB(1)	RETSAKVNWMPPRSRADSFKVSYQL
TNXB(2)	QFIPTASPLLCSSSPHSPAKAEAEI

SKIV2L: putative RNA helicase involved with altering RNA secondary structure

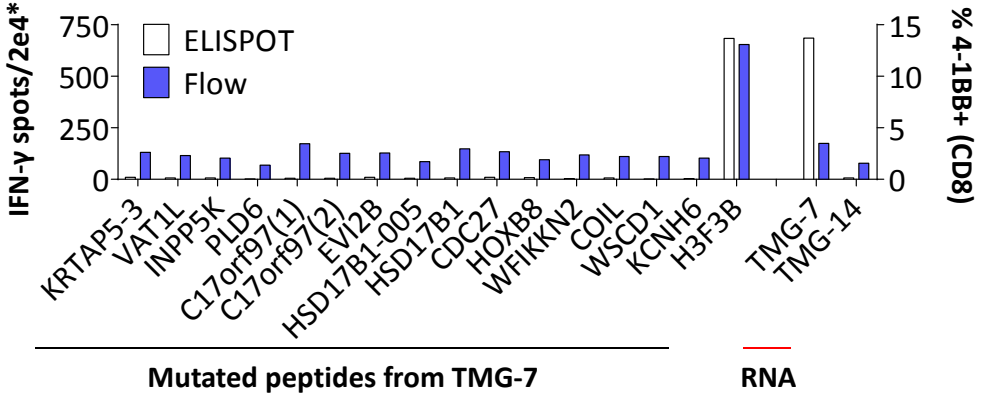
*above 500 spots not accurate

LS-4007: What do TIL from culture #23 recognize?

TIL#23



Sort, expand, co-culture with peptide pulsed DCs



TMG-7

Mutated gene	Long peptide AA sequence
KRTAP5-3	CGSCGGCKGGCGACGGSKGGCGSSC
VAT1L	IDNPPKTPLVPGYECSGIVEALGDS
INPP5K	PAWTDRILWRLKQQPCAGPDTPIPP
PLD6	DCDYMALNGSQIRLLRKAGIQVRHD
C17orf97(1)	RLDRRGGAGTMGDKDNDGEEEEREG
C17orf97(2)	FHIDPEALKGFHTDPKALKGFHPDP
EVI2B	TSTVKNSPRSTPPRSTPGFILDTTSNKQTP
HSD17B1-005	RRGSGRVLVTGSLGGLMA
HSD17B1	RRGSGRVLVTGSLGGLMGLPFNDVY
CDC27	LNTDSSVSYIDSA
HOXB8	PSSGGSFQHPSQTQEFYHGPSSLST
WFIKKN2	DRENVVMRPNHVCGNVVVTNIAQLV
COIL	NKATCGTVGDDNKEAKRKSPKKKEK
WSCD1	TICVKTHESGRRGIEMFDSAILLIR
KCNH6	VAAIPFDLLIFRNGSDETTTLIGLL
H3F3B	VKKPHRYRPGTVTLREIRRYQKSTE

H3F3B: histone protein of the H3 family

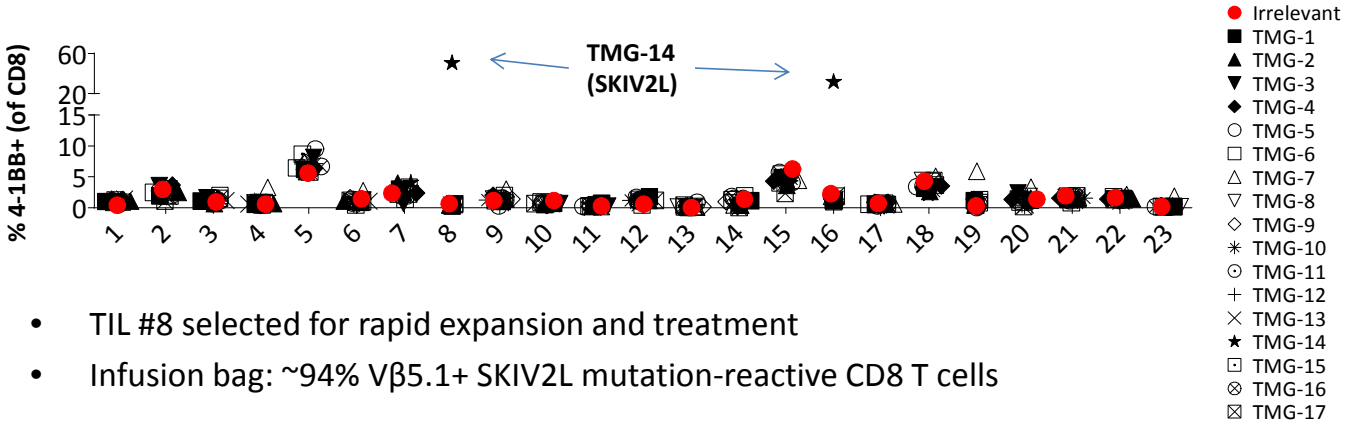
*above 500 spots not accurate

Evidence for mutation-reactive TIL in 20 out of 22 patients with metastatic GI cancers

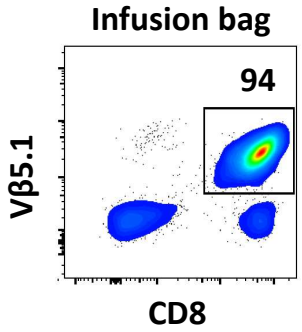
Patient ID	Tumor type	# of mutations assessed	Mutated gene recognized	T cell	Notes
3737	Cholangio	25	ERBB2IP	CD4	Multiple clonotypes
3812	Cholangio	179	-------	-------	High background in TIL
3942	Rectal	140	NUP98 KARS GPD2	CD8 CD8 CD4	
3948	Esophageal	210	PLEC XPO7 AKAP2	CD4 CD4 CD4	
3971	Colon	119	CASP8	CD8	
3978	Cholangio	37	ITGB4	CD4	Sorted PD-1+ T-cell subset
3995	Colon	154	TUBGCP2 RNF213 KRAS	CD8 CD8 CD8	KRAS-G12D
4007	Colon	264	SKIV2L H3F3B	CD8 CD8	Two clonotypes for SKIV2L
4032	Colon	222	API5 RNF10 PHLPP1	CD8 CD8 CD8	Two clonotypes for API5
4060	Colon	315	SMC1A	CD4	
4069	Pancreatic	97	ZFYVE27	CD8	
4071	Colon	285	QSOX2 POR MRPS28	CD8 CD8 CD8	
4077	Cholangio	127	HIST1H2BE FLII	CD8 CD8	
4078	Gastroesophageal	79	In progress	2xCD4 2xCD8	Sorted PD-1+ T-cell subset
4081	Colon	191	ALDOC RPL12	CD8 CD8	
4090	Colon	201	USP8 RPS15 MRLPL39	CD8 CD8 CD4	
4095	Colon	61	KRAS In progress	CD8 CD4/8?	KRAS-G12D; at least 2 clonotypes
4107	Cholangio	161	-------	-------	
4108	Colon	146	In progress	2xCD8	
4110	Cholangio	151	In progress In progress	CD8 CD4	
4112	Cholangio	215	In progress	4xCD8 CD4	
4115	Colon	123	In progress In progress	CD4 CD8	

Can we effectively harness the mutation-reactive T-cell response?

LS-4007: ACT with SKIV2L-mutation specific CD8+ T cells



- TIL #8 selected for rapid expansion and treatment
- Infusion bag: ~94% Vβ5.1+ SKIV2L mutation-reactive CD8 T cells



- **Infusion of 2.8e10 total cells (~2.6e10 mutation-reactive)**
  - **Third month follow up (05-18-15): Progressive disease (↑ ~22%)**
  - **NB: Cells expanded poorly during REP**

Patients treated with selected populations of mutation-reactive T cells

Patient ID	Tumor type	Cell number infused	% mutation reactive	T cell	Mutated gene targeted	Treatment date	Response
3737/3941	Cholangio	126e9	94.4	CD4+	ERBB2IP	10/25/13	PR (ongoing 24+ mo)
4032	Colon	37e9	74	CD8+	PHLPP1 (49%) API5 (22%) RNF10 (3%)	12/18/14	PD
4007	Colon	28e9	64.4	CD8+	SKIV2L	02/19/15	PD (3 mo)
4069	Pancreatic	90e9	91.9	CD8+	ZFYVE27	03/06/15	PD (2 ½ mo) (mixed response?)
4071	Colon	95.1e9	32.3	CD8+	QSOX2	03/26/15	PD
4077	Cholangio	65.8e9	38.3	CD8+	HIST1H2BE	05/08/15	PD (2 mo) (1 month, ↓ ~40%)
4081	Colon	152.4e9	72.1	CD8+	ALDOC	05/15/15	PD (2 mo)

Why are we not observing a higher response rate?

Potential reasons for lack of clinical efficacy with T-cell targeting of somatic mutations

- **T-cell properties:**
  - Dose
  - Type
  - Function/fitness/age
  - Number of antigens targeted
  - T-cell affinity/avidity toward antigen

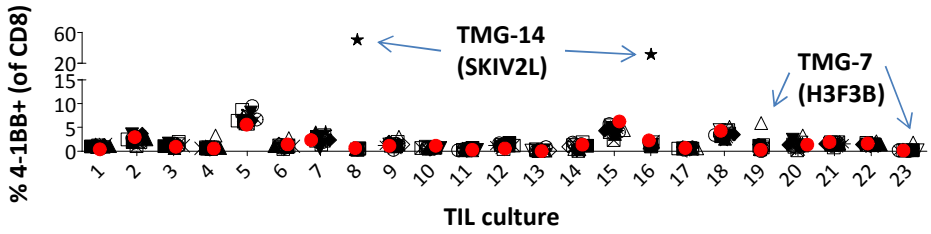
- **Tumor-intrinsic properties:**
  - Lack/heterogeneous expression of targeted mutation in metastases
  - Impaired MHC processing and presentation pathway

- **Other host (regulatory) factors**

- **Can we improve the efficacy of ACT against somatic mutations in epithelial cancers?**
  - T-cell properties → **Personalized TCR-gene therapy**
  - Tumor-intrinsic properties → **Study/evaluate tumor biopsies, target drivers if possible**
  - Other host factors → **Combine with other Rx** (e.g., checkpoint inhibitors, costimulatory antibodies, vaccines, oncolytic viruses, etc.)

Toward personalized TCR-gene therapy against somatic mutations

Patient LS (4007)



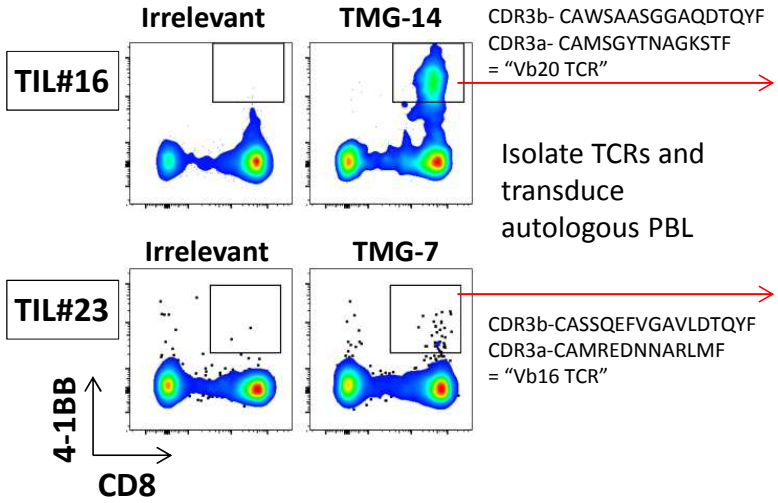
Are the mutations being targeted by TIL still expressed by a progressing lesion? (Tumor intrinsic properties)

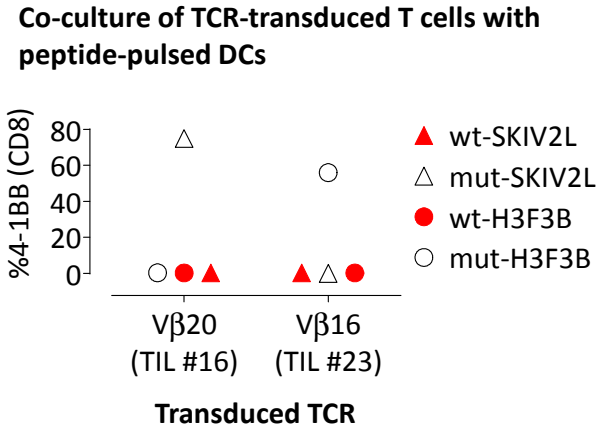
Biopsy: Progressing lung lesion
- Sequencing of RT-PCR products

Gene	Sequence	
	Wt	Mut
SKIV2L	0	3
H3F3B	0	3

Toward personalized TCR-gene therapy against somatic mutations

Patient LS (4007)



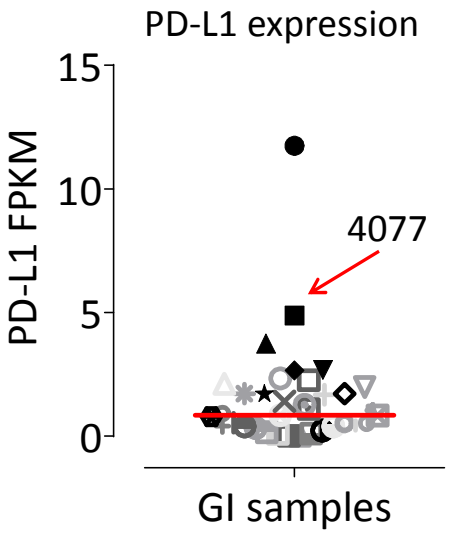


- **Clinical retroviral vectors encoding SKIV2L and H3F3B mutation-specific TCRs are in production, clinical protocol will soon be under internal review**
- **Patient is progressing, but we hope the protocol can be approved in time so we can treat the patient with two personalized mutation-reactive TCRs**

Combining mutation-reactive TIL and anti-PD-1 (pembrolizumab)

Patient LW-4077 (cholangiocarcinoma)

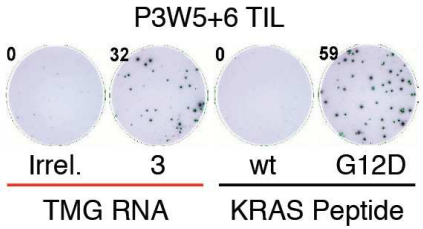
- Rx1 on 05/08/2015; 65.8e9 infused (~ 26e9 HIST1H2BE-mutation-reactive)
- First follow up on 06/17/2015: target lung lesions ↓ ~40%
- Second follow up, Progressive disease



	Rx1 (no pembrolizumab)	Rx2 + pembrolizumab
Treatment date	05/08/15	09/23/15
Cell #	65.8e9	59.5e9
TIL fragment #	F14	F14 (+20 days)
% HIST1H2BE-mutation-reactive (tetramer)	33%	36%
IL-2 doses	4	1
Response	PD (1 month, ↓ ~40%)	Follow-up on 11/05/15
Persistence, TRBV deep seq (~ 1 month)	0.49%	TBD

Toward T-cell targeting of hotspot driver mutations: Immunogenicity of KRAS-G12D

Patient 3995 (colorectal cancer)

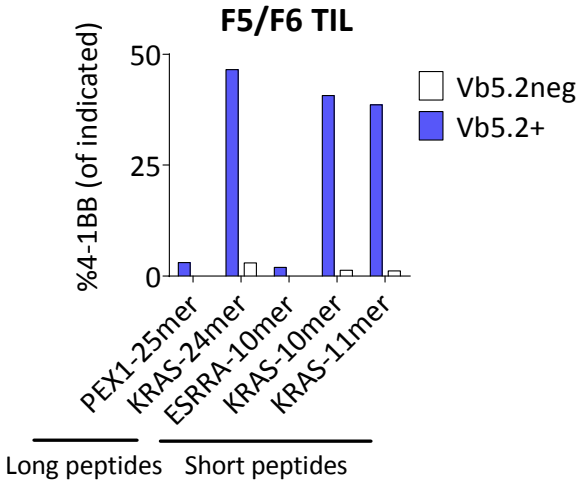


Tran et al., 2015, *Science*, October 29, 2015

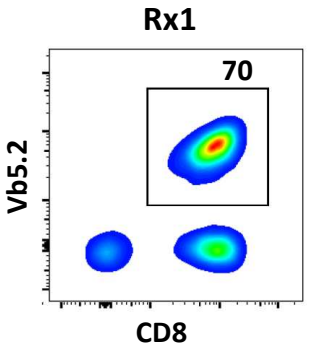
ACT with KRAS-G12D-mutation-reactive CD8+ TIL

Patient CR-4095 (colorectal cancer)

Co-culture: TIL fragment with peptide-pulsed DCs



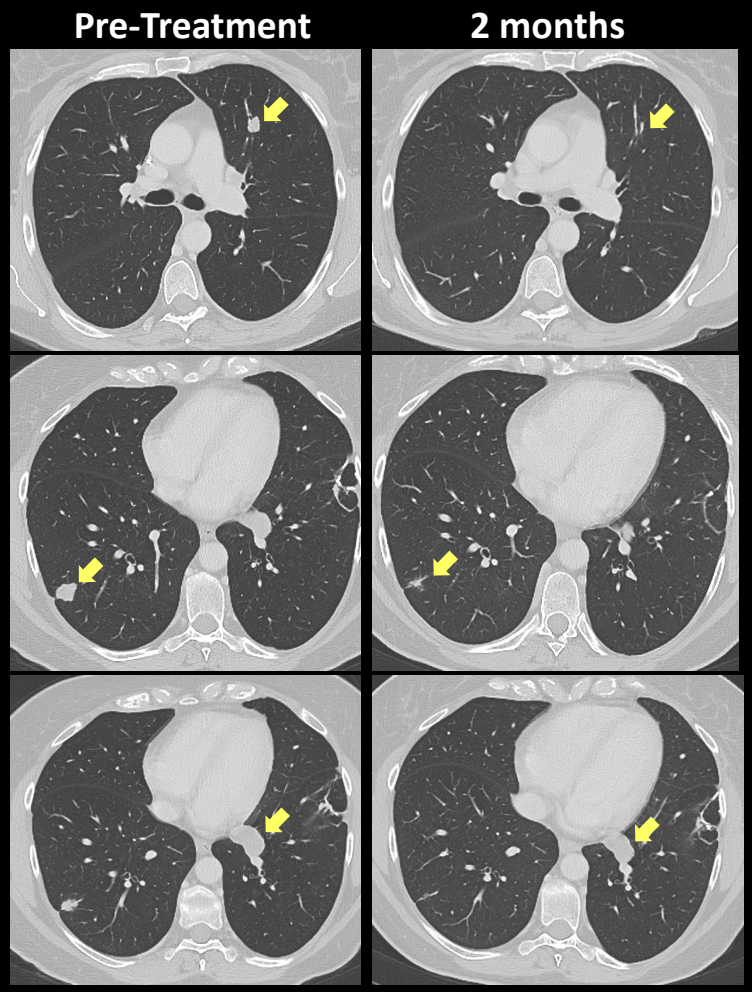
F6 TIL selected for treatment



Rx1 (07/01/15)
- 148e9 total cells
- Up to 70% KRAS-G12D reactive
- 5 doses of IL-2

ACT with KRAS-G12D-mutation-reactive CD8+ TIL

Lung CT



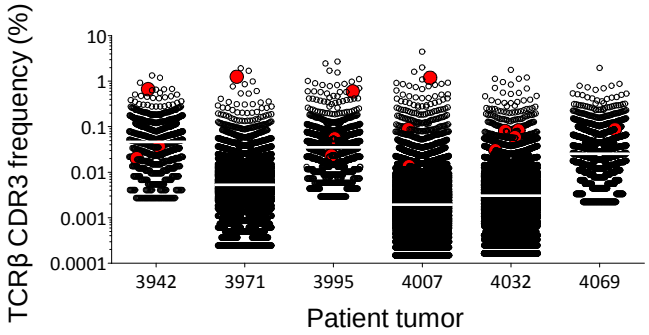
Ongoing stable disease (↓27% at 3 months)

Durability remains to be determined

TCR-isolated, also HLA-C*08:02 restricted

Conclusions

- Most patients (20/22) with metastatic gastrointestinal cancers mount a T-cell response against at least one somatic mutation expressed by their tumors
  - Highly variable frequency of mutation-reactive cells found in TIL fragments



  - In addition to unique mutations, the shared driver KRAS-G12D mutation can also be immunogenic

- Transfer of a highly pure population of TIL containing 120e9 mutation-reactive CD4+ T cells (95% pure) was capable of mediating ongoing tumor regression in a patient with cholangiocarcinoma

- Transfer of selected/enriched populations of TIL containing mutation-reactive T cells led to ongoing (3 mo) stabilization of disease for another patient, but was ineffective at mediating tumor regression in 6 other patients with metastatic GI cancers (response for 2 patients is pending)

- Mutation-reactive T cells and their T-cell receptors can be enriched and isolated for potential use in cell-based therapies

Current and future directions

- Continue to test whether mutation-reactive T cells can mediate regression in patients with metastatic gastrointestinal cancers
  - Current method: TIL fragment selection
  - In development: **Personalized TCR-gene therapy and combination therapies**
  - Target multiple mutations

- In patients where ACT of mutation-reactive cells is ineffective, when possible, evaluate expression of mutation in progressing tumors and other factors that may impact efficacy of T-cell therapy

- Develop the HLA-C*08:02-restricted TCR against KRAS-G12D for clinical use in TCR-gene therapy
  - Identify TCRs against other immunogenic hotspot driver mutations?

- Are we missing neo-epitope reactivities?
  - Probe TIL repertoire at the clonal level for mutation reactivity (Rami Yoseph)

- Major challenge, tumor heterogeneity?

- Immunologically targeting mutations may be the Ultimate Personalized Therapy
  - Unique patient-specific T cells/TCRs that specifically target mutations only expressed by their own cancers

Acknowledgments

Rosenberg Lab:
Mojgan Ahmadzadeh
Alena Gros
Yong-Chen Lu
Zhili Zheng
Satyajit Ray
Simon Turcotte (SB alumnus)

Robbins Lab:
Paul Robbins
Yong Li
Mona El-Gamil

Jared Gartner
Todd Prickett

TIL Lab:
Rob Somerville
John Wunderlich
Michelle Langhan
Tom Shelton

FACS Lab:
Arnold Mixon
Shawn Farid

Surgery Branch Immunotherapy and Surgical Teams

Jim Yang
Steve Feldman

Steven Rosenberg