Cancer Immunotherapy:
Toward adoptive T-cell therapy against cancer mutations

The Cholangiocarcinoma Foundation Webinar

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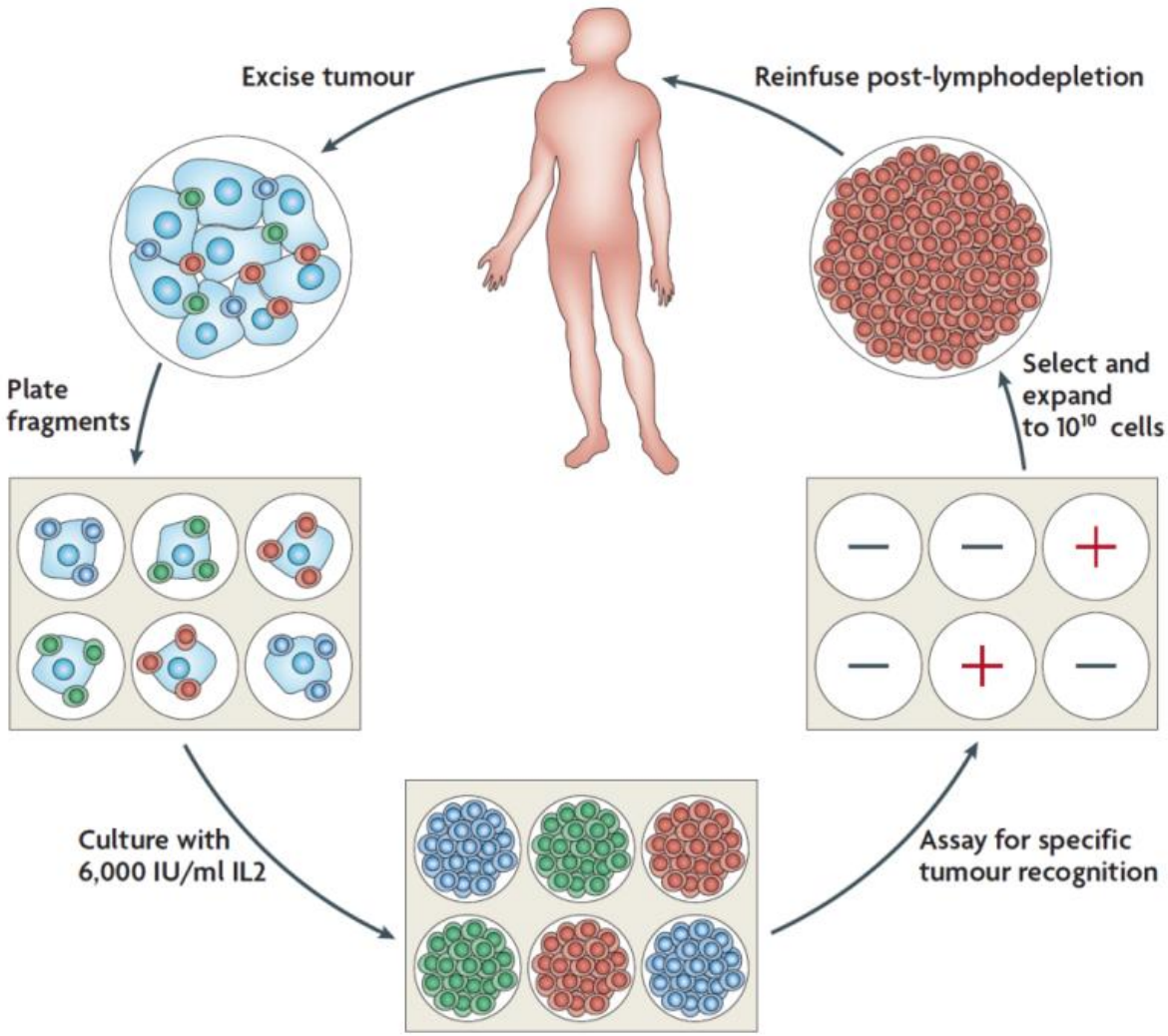
Adoptive cell therapy (ACT)

- A process by which immune cells are removed from a patient, grown/manipulated in the lab, and then reintroduced back into a patient

- In adoptive cell therapy for cancer, this ideally involves the transfer of activated immune cells, usually T cells, that can target the cancer

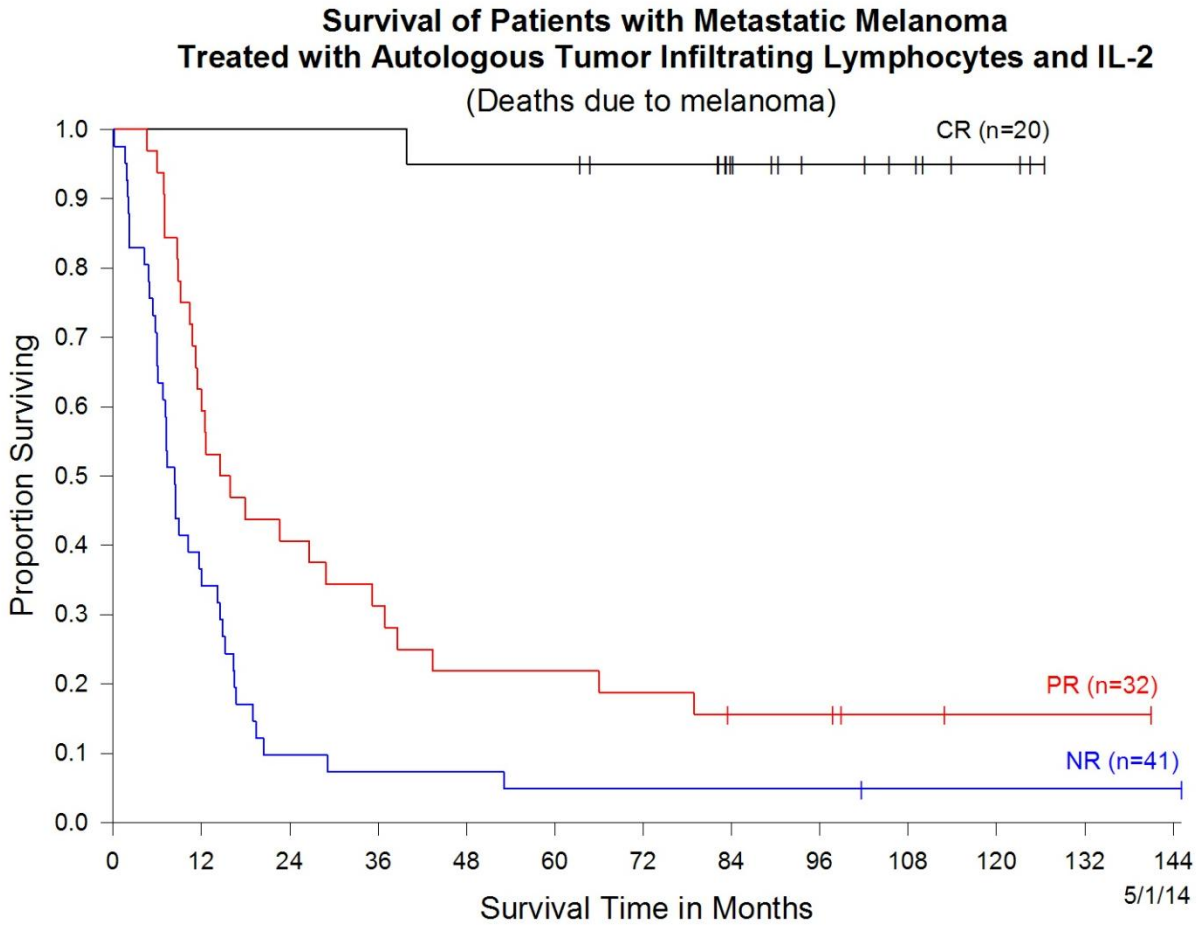
- Two major types of adoptive cell therapy:
    1. ACT using **t**umor-**i**nfiltrating **l**ymphocytes (TIL)
    2. ACT using T cells derived from blood that have been genetically modified with anti-tumor receptors

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

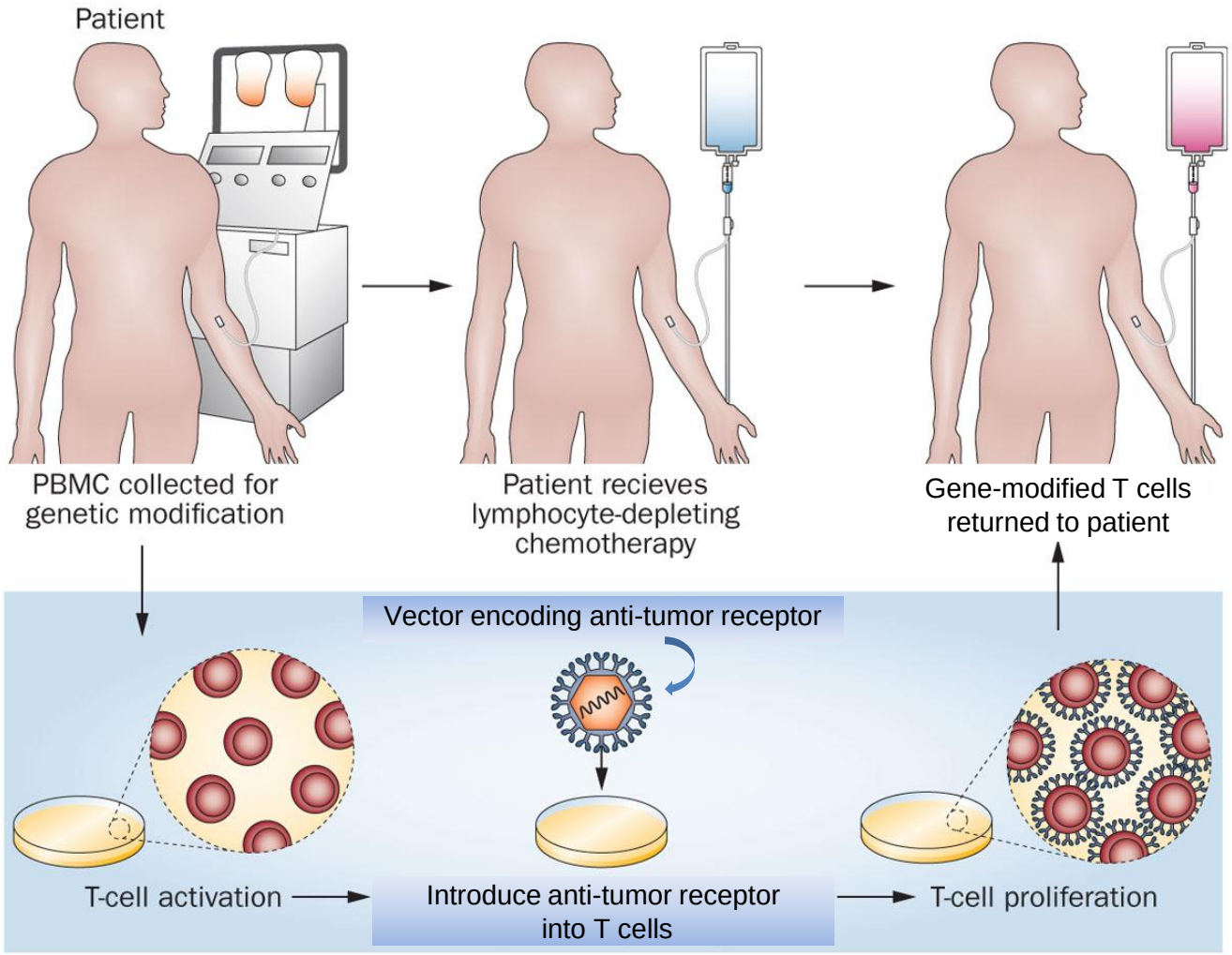


Rosenberg SA, et al, Nature Reviews Cancer 8, 299-308 (April 2008)

Adoptive transfer of TIL can cure some patients with metastatic melanoma

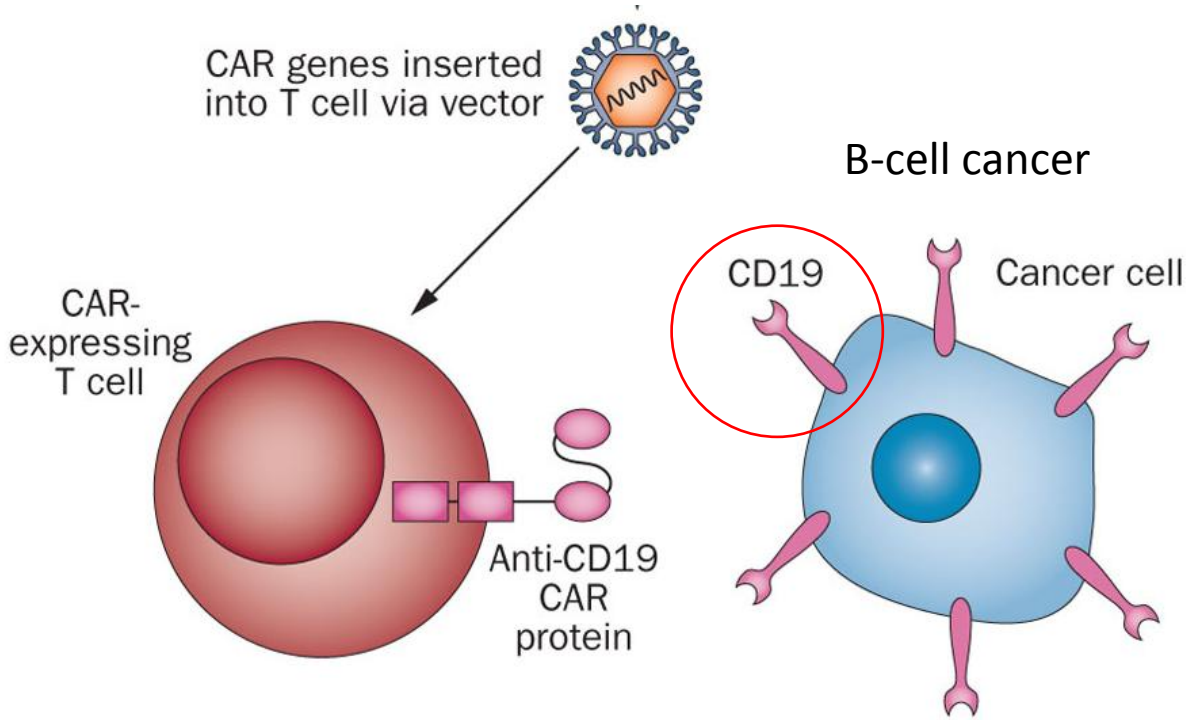


Adoptive cell therapy (ACT) with gene-modified T cells



Modified from Kochenderfer, J. N. & Rosenberg, S. A. (2013) . *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.46

Targeting B-cell leukemias and lymphomas with T cells genetically modified with receptors that target CD19



Modified from Kochenderfer, J. N. & Rosenberg, S. A. (2013) . *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.46

Adoptive transfer of T cells genetically modified with receptors targeting CD19 can mediate regression in patients with B-cell cancers

blood

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

Kochenderfer et al., 2010 Nov 18, 116, 20

First report demonstrating efficacy on a patient with follicular lymphoma

JOURNAL OF CLINICAL ONCOLOGY

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

Kochenderfer et al., 2014 Aug 25. pii: JCO.2014.56.2025

Response rate: 13/15 (8 complete, 4 partial, 1 stable)

The NEW ENGLAND JOURNAL of MEDICINE

Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Maude et al., 2014 Oct 16;371(16):1507-17

27/30 patients with ALL achieved a complete response

Efficacy has also been demonstrated in chronic lymphocytic leukemia (CLL): e.g., Kochenderfer et al., Blood, 22 March 2012, 119; 12, 2709; and Porter et al., N Engl J Med, August 25, 2011; 365:725-733

Why can't we use a similar approach to treat common solid cancers?

- Unlike B-cell leukemias and lymphomas where almost all these cancers express the CD19 molecule, so far we have been unable to find a suitable target that is frequently expressed in a large percentage of solid cancers

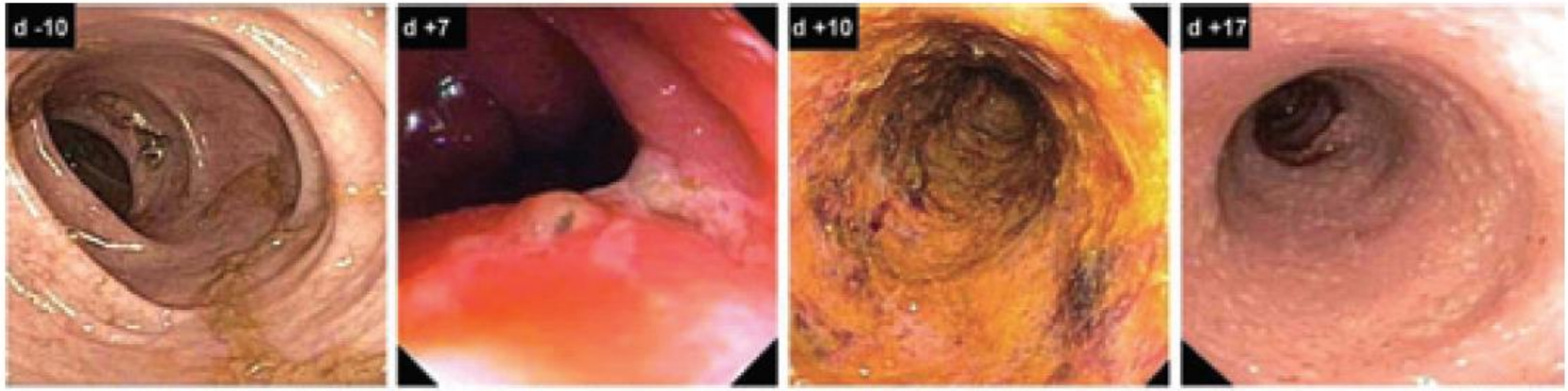
- Some solid cancers over-express certain proteins (e.g., carcinoembryonic antigen, CEA, in colorectal cancers)
  - Can we use T cells genetically engineered with receptors that target these over-expressed molecules to successfully treat cancer patients?

Why can't we use a similar approach to treat common solid cancers?

T Cells Targeting Carcinoembryonic Antigen Can Mediate Regression of Metastatic Colorectal Cancer but Induce Severe Transient Colitis

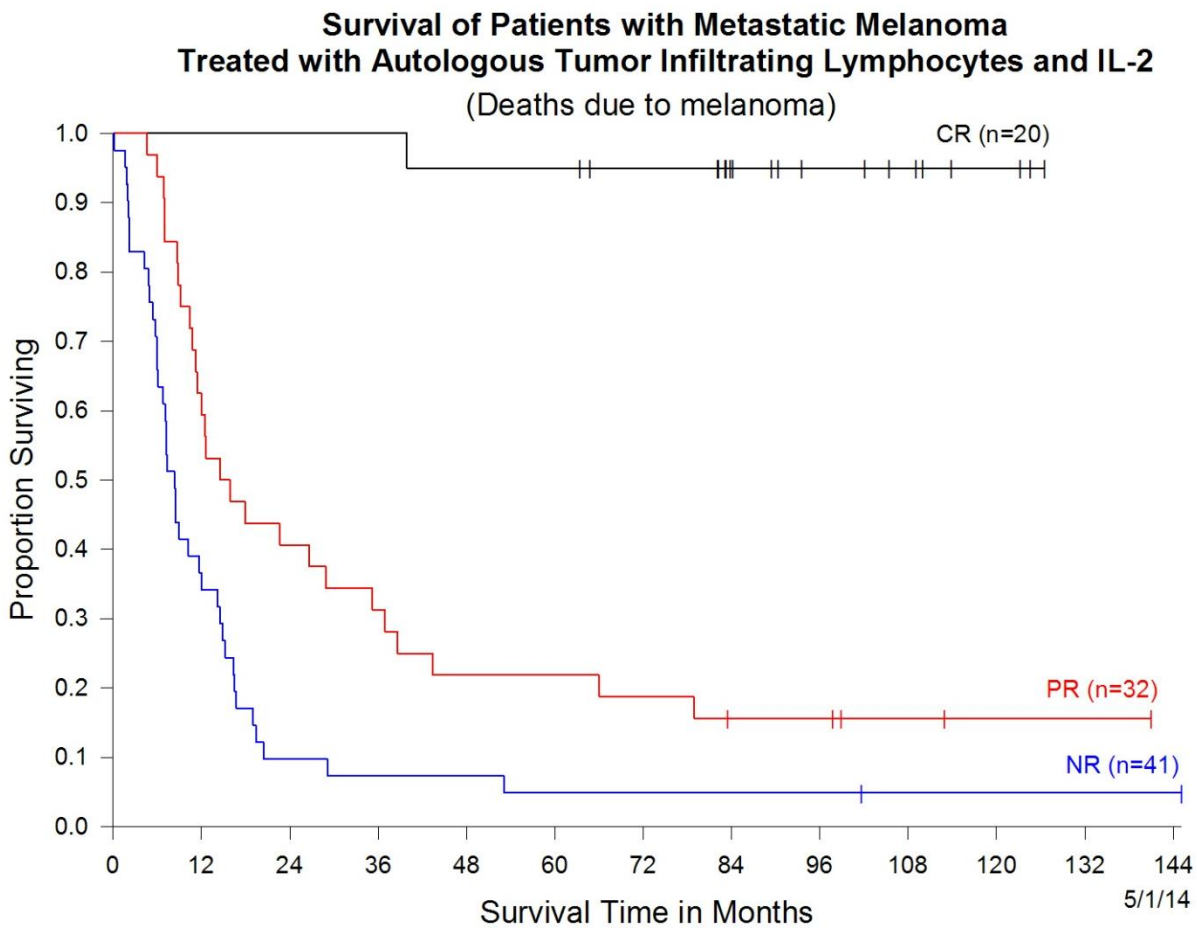
Parkhurst et al., Molecular Therapy (2011) 19 3, 620–626.

Colonoscopies prior to and after transfer of T cells targeting CEA



- T cells can destroy normal cells/tissues if they express the targeted molecule
- The current lack of common targets that are expressed by most solid tumors, **and not on normal essential tissues**, limits the use of genetically modified T cells to treat these cancers
- Are there other options?

Adoptive transfer of TIL can cure some patients with metastatic melanoma



Can TIL therapy be effective in other more common solid cancers?

The gastrointestinal (GI) cancer TIL clinical trial (NCT01174121)

- A phase II study using short-term cultured autologous tumor-infiltrating lymphocytes following a lymphodepleting regimen in metastatic digestive tract cancers (ClinicalTrials.gov identifier: NCT01174121)

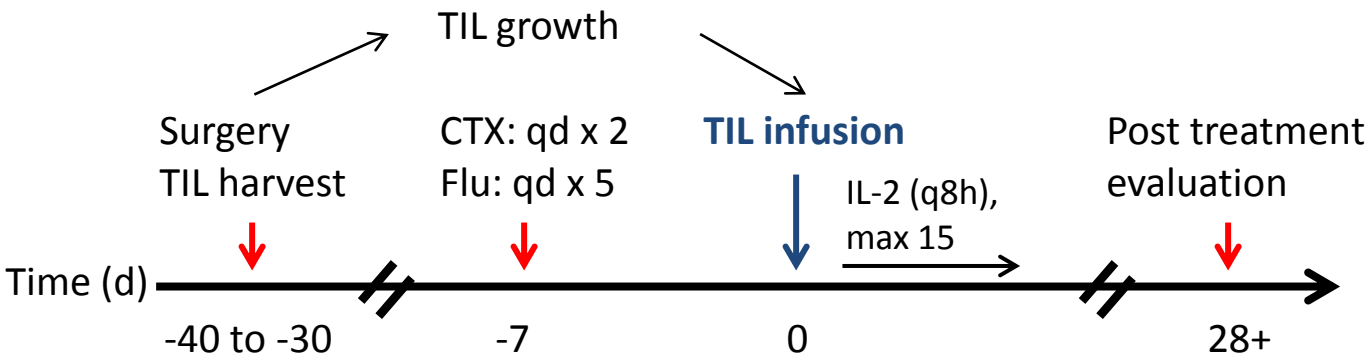
- Basic eligibility requirements
  - 18-66 years of age with metastatic cancer originating from the digestive tract (esophagus, stomach, pancreas, colon, rectum, liver or bile ducts)
  - Normal basic laboratory values
  - Refractory to approved standard systemic therapy
  - Good performance status (ECOG 0 or 1)

- AIM: To determine if autologous TIL infused in conjunction with high dose IL-2 following lymphodepletion can mediate tumor regression in patients with metastatic digestive tract adenocarcinomas

- (Note: Protocol was recently revised to include patients with metastatic breast, ovarian/endometrial, and urothelial cancers)

The gastrointestinal (GI) cancer TIL clinical trial (NCT01174121)

Trial overview



CTX: cyclophosphamide
Flu: fludarabine

Conventional TIL therapy is largely ineffective against metastatic GI cancers

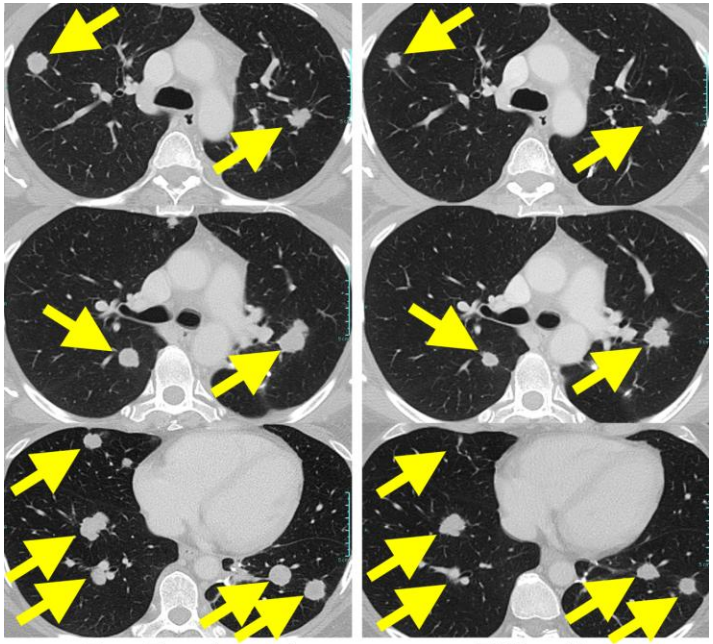
Patient	Primary	Cells ($x10^9$)	IL-2 doses	Response
3454	Colorectal	18.5	8	PD
3596	Colon	32.1	10	PD
3610	Rectal	20.0	3	PD
3671	Colon	30.3	3	PD
3674	Colorectal	69.5	1	PD
3690	Colon	50.0	7	PD
3717	Gastric	68.8	0	PD
3737	Cholangio	42.4	4	PD (13 mo SD)
3788	GE junction	98.1	3	PD
3812	Cholangio	45.2	3	PD
3894	Colon	67.8	3	PD
3942	Rectal	68.3	2	PD
3948	Esophageal	97.3	2	PR (unofficial)
3970	Colon (Lynch)	90	2	not evaluable
3971	Colon	40.8	4	PD
3978	Cholangio	78.5	4	PD

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

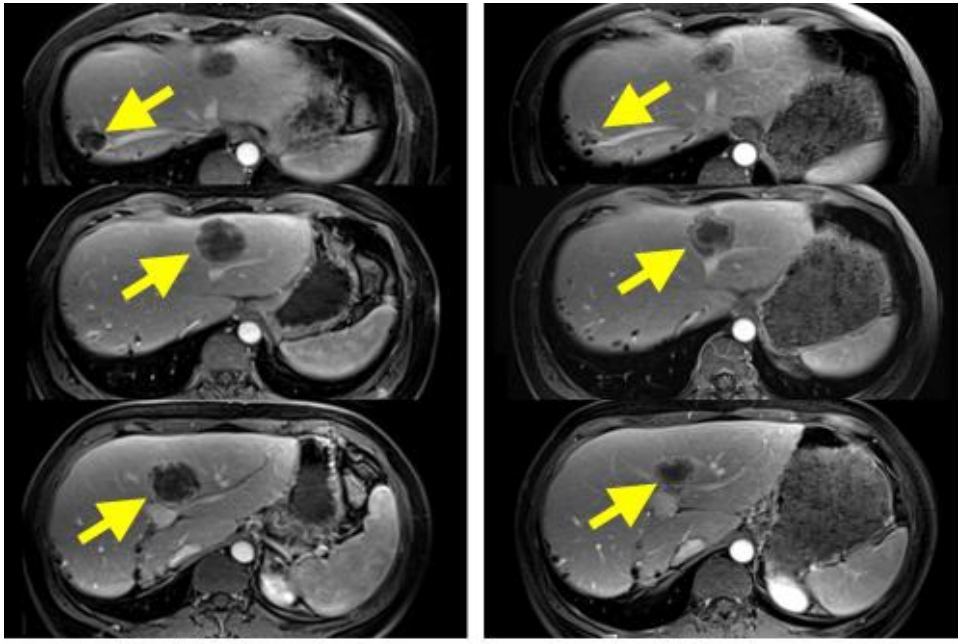
Case report: patient 3737

- Metastatic cholangiocarcinoma (liver, lung)
- Prior Tx: cisplatin + gemcitibine, gemcitibine, taxotere
- T-cell therapy with 42.4 billion TIL, 4 doses of IL-2
- Max. tumor regression: 30 % (for one month), stable disease for ~ 13 months

Lung CT



Liver MRI



- Did the TIL contribute to disease stabilization?
- What could the T cells be targeting?

T cells recognizing cancer mutations may play a major role in the efficacy of T-cell therapy in melanoma patients

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

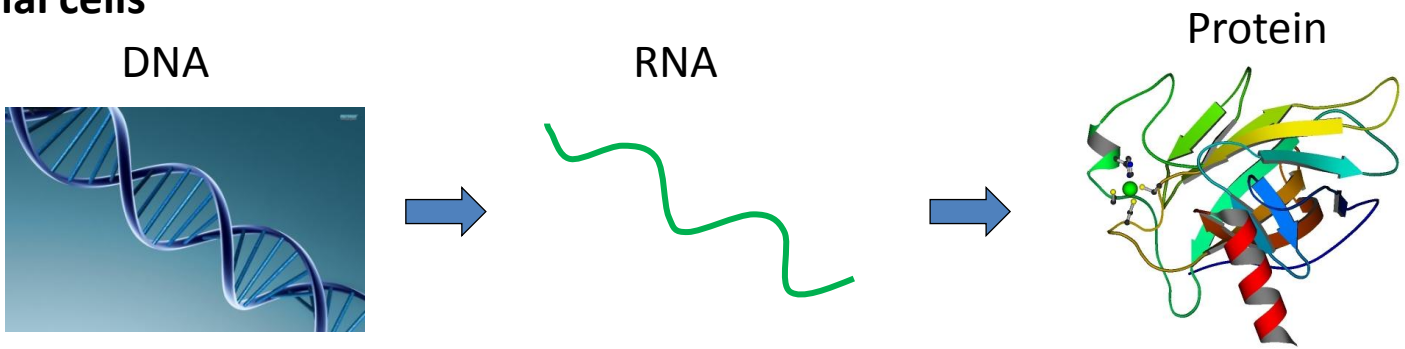
What is a mutation?

What is a mutation?

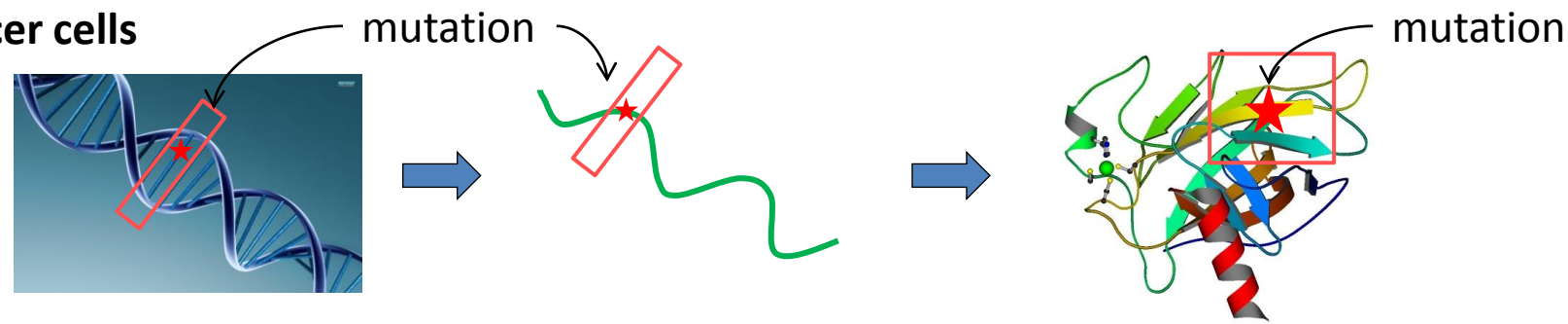
Mutation: a change in the DNA sequence in the genome of a cell

All cancers contain mutations or other genetic alterations

Normal cells

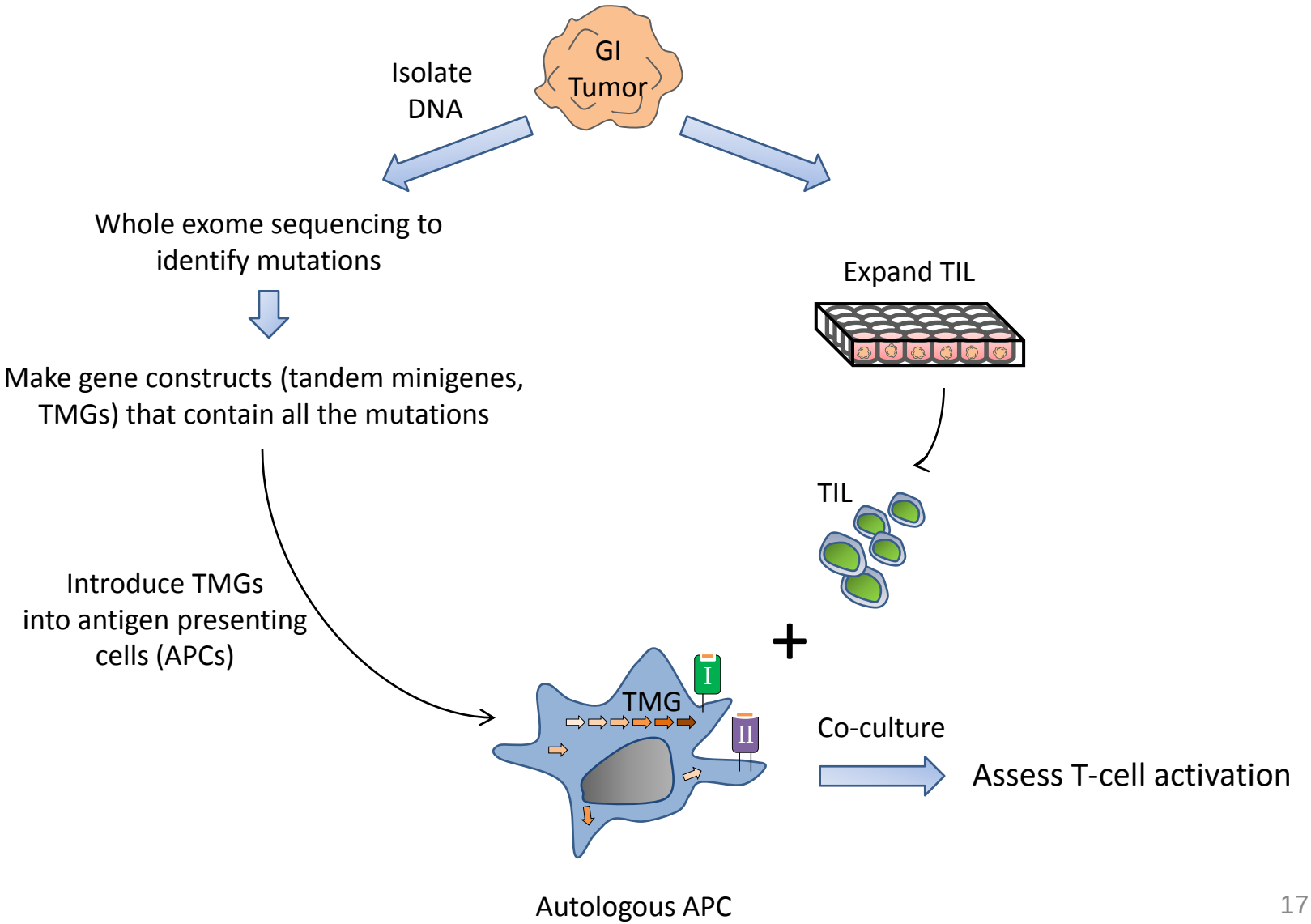


Cancer cells



Could TIL from patient 3737 recognize tumor mutations?

Assessing T-cell reactivity against mutated antigens



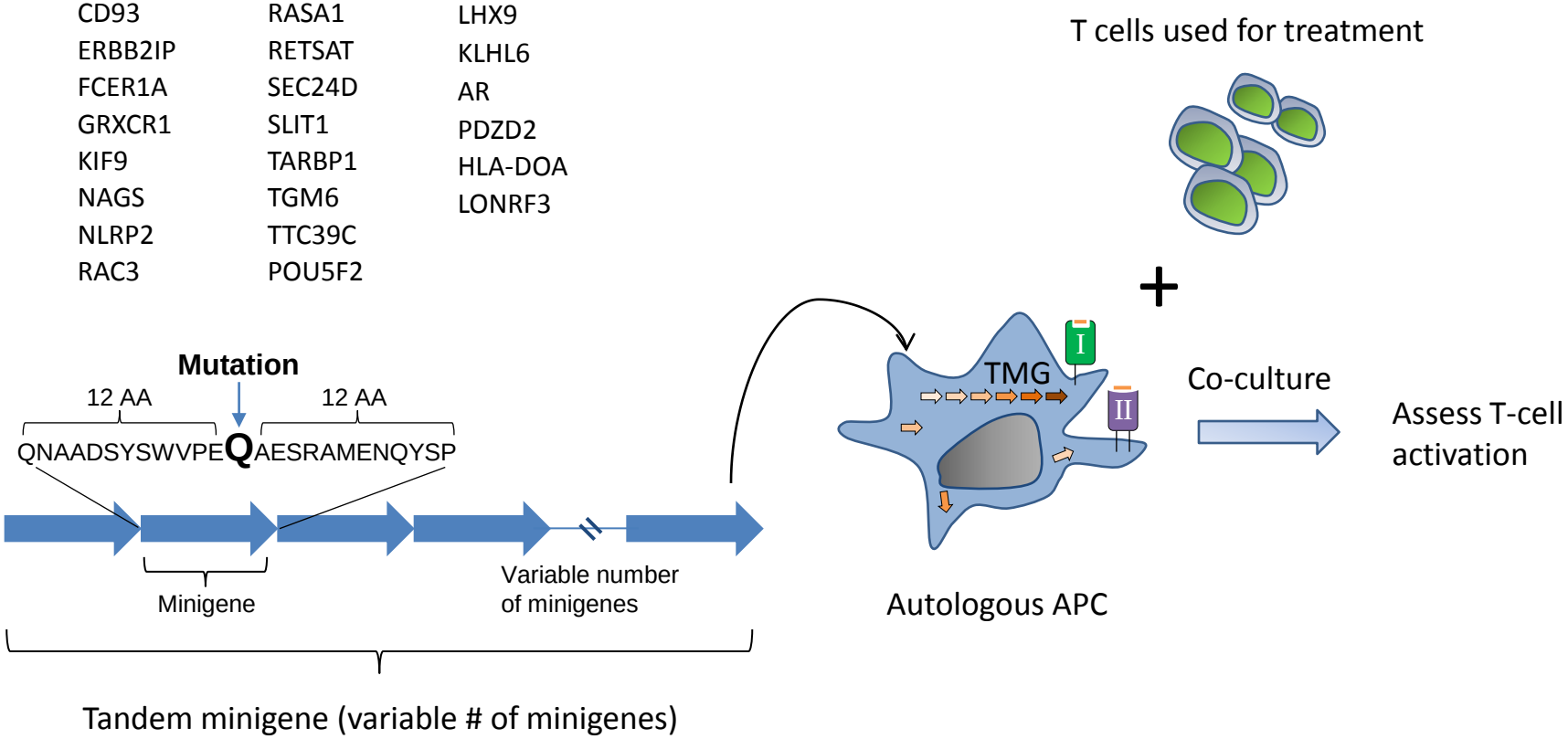
Whole exome sequencing identifies 26 mutations in a lung metastasis

Gene	Mutation Position	
	Nucleotide (genomic)	Amino Acid (protein)
ALK	chr2_29996620-29996620_C_T	137R>H
AR	chrX_66858483-66858483__C	NA
CD93	chr20_23012929-23012929_C_T	634R>Q
DIP2C	chr10_365545-365545_C_T	NA
ERBB2IP	chr5_65385316-65385316_A_G	805E>G
FCER1A	chr1_157544227-157544227_G_C	219D>H
GRXCR1	chr4_42590102-42590102_C_T	21A>V
HLA-DOA	chr6_33085209-33085209_C_T	NA
KIF9	chr3_47287859-47287859_T_C	155T>A
KLHL6	chr3_184692410-184692413_CAGA_	NA
LHX9	chr1_196164923-196164923_A_	NA
LONRF3	chrX_118007666-118007666_A_C	NA
NAGS	chr17_39440355-39440355_G_A	412R>H
NLRP2	chr19_60186650-60186650_G_T	591S>I
PDZD2	chr5_32124833-32124833_A_	NA
POU5F2	chr5_93102847-93102847_A_C	60V>G
RAC3	chr17_77584690-77584690_C_A	125T>N
RAP1GDS1	chr4_99532209-99532209_C_A	198L>I
RASA1	chr5_86703757-86703757_C_T	589R>C
RETSAT	chr2_85424308-85424308_C_T	553R>K
SEC24D	chr4_119872085-119872085_A_G	901M>T
SENP3	chr17_7408824-7408824_A_G	292M>V
SLIT1	chr10_98753840-98753840_G_C	1280N>K
TARBP1	chr1_232649342-232649342_C_A	655G>V
TGM6	chr20_2332325-2332325_G_A	398D>N
TTC39C	chr18_19966475-19966475_A_C	503N>T

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

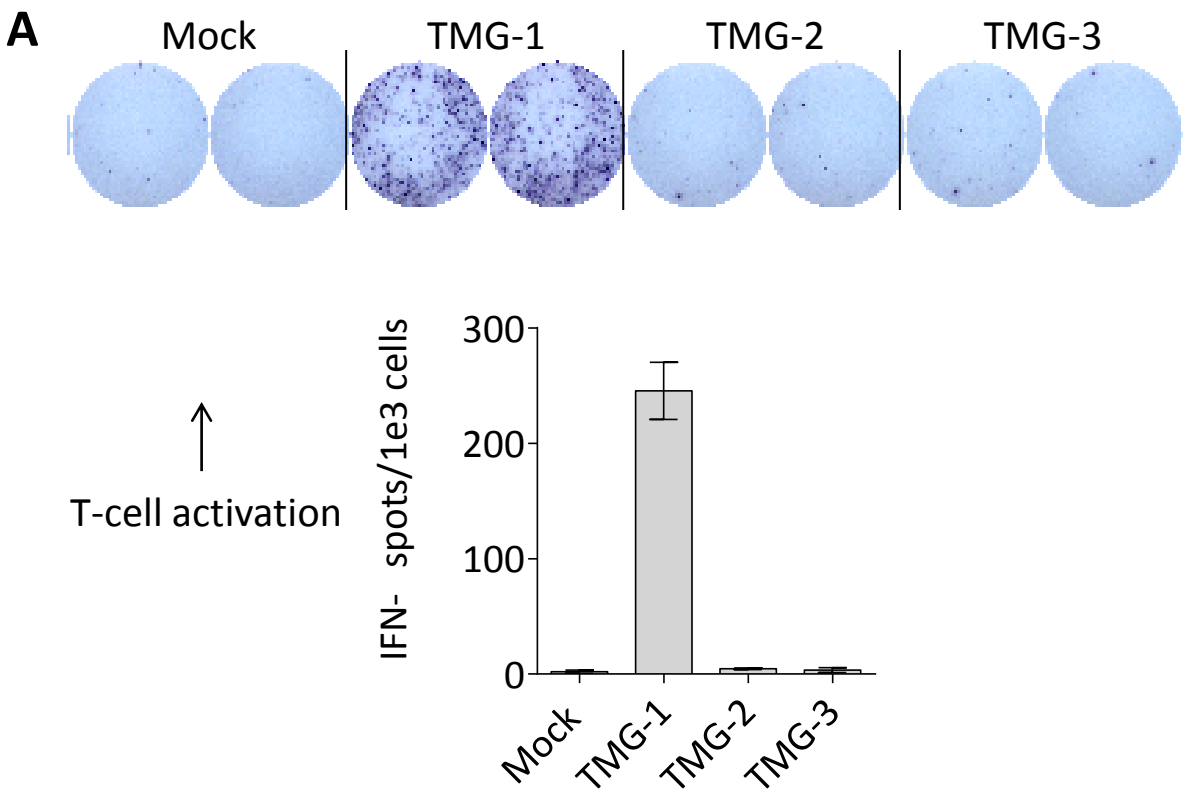
Three tandem minigenes (TMGs) were made containing all mutations

TMG-1	TMG-2	TMG-3
ALK	RAP1GDS1	SENP3
CD93	RASA1	LHX9
ERBB2IP	RETSAT	KLHL6
FCER1A	SEC24D	AR
GRXCR1	SLIT1	PDZD2
KIF9	TARBP1	HLA-DOA
NAGS	TGM6	LONRF3
NLRP2	TTC39C	
RAC3	POU5F2	



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

Co-culture exp't (transfected DCs + Infusion bag T cells):
A) IFN-γ ELISPOT assay

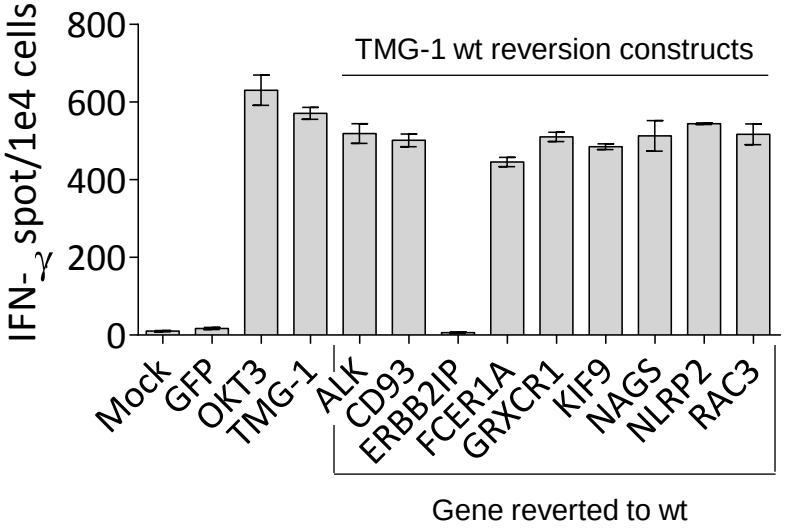


Only mutated ERBB2IP is recognized by T cells in 3737 infusion bag

Co-culture exp't (DCs expressing TMGs + Infusion bag):
IFN-γ ELISPOT assay

TMG-1
(mutated genes)
ALK
CD93
ERBB2IP
FCER1A
GRXCR1
KIF9
NAGS
NLRP2
RAC3

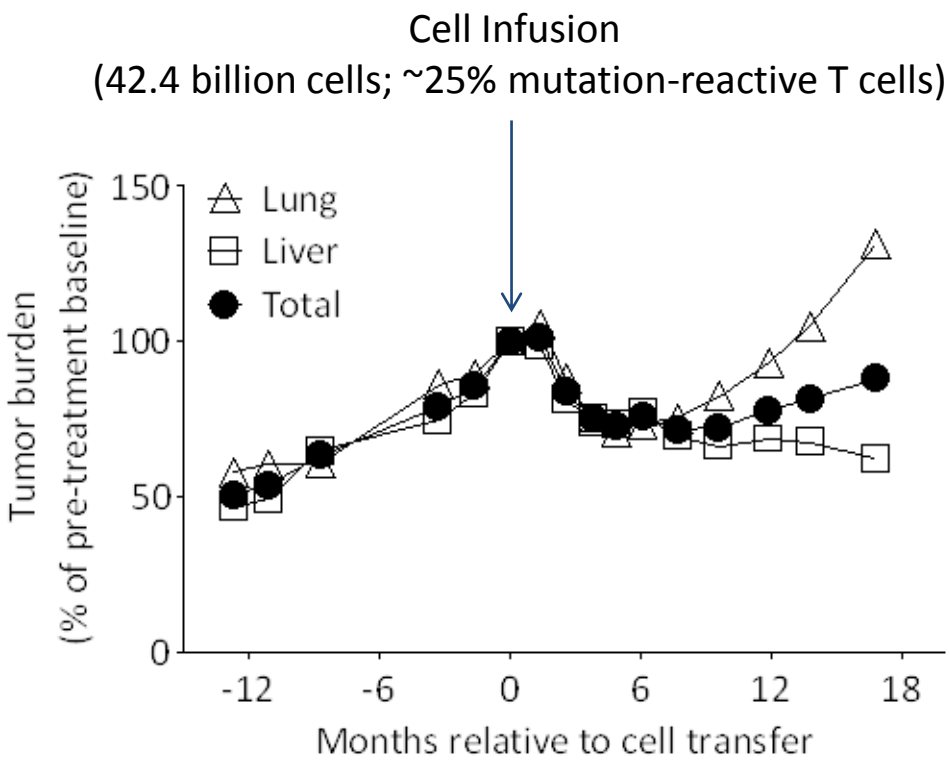
↑
T-cell activation



wt = wild-type (normal sequence)

~25 % of the infusion bag T cells were reactive against mutated ERBB2IP
These T cells **do not** react against wild-type (normal) ERBB2IP

Disease progression after about 1 year after first T-cell treatment



Can we do better?

Re-treatment with a highly pure population of mutation-reactive T cells

- 1st treatment infusion bag T cells were made up of 5 different T-cell cultures
  - F1, F2, F3, PF1, G-Macs

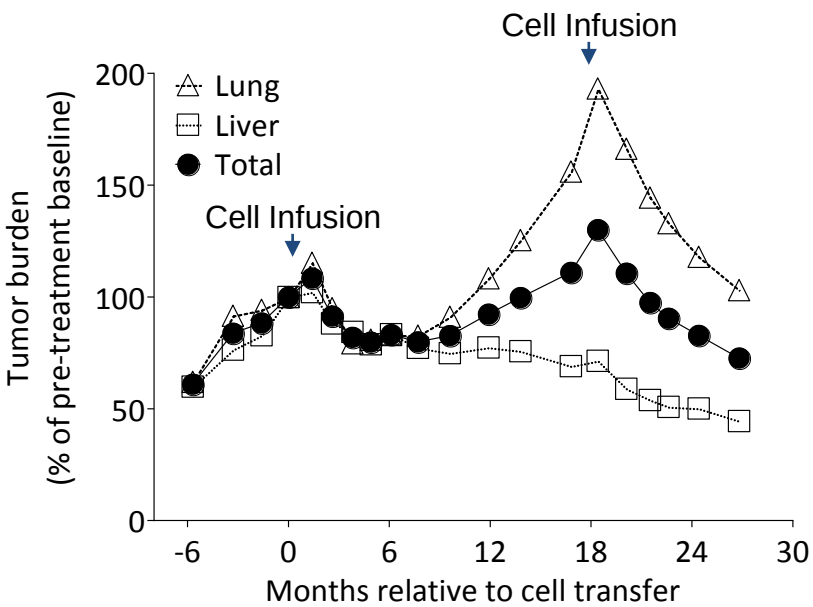
- Approximate % of ERBB2IP mutation-reactive T cells prior to expansion
  - F1: 0.7%
  - **F2: 89%**
  - F3: 0.6%
  - PF1: 2.8%
  - G-Macs: 4.4%

This T-cell culture was exclusively expanded for re-treatment

After expansion, ~95% of the cells were reactive to mutated ERBB2IP

2nd treatment: 126 billion T cells, ~95% (120 billion) ERBB2IP mutation reactive

Tumor regression after treatment with ERBB2IP-mutation-reactive T cells

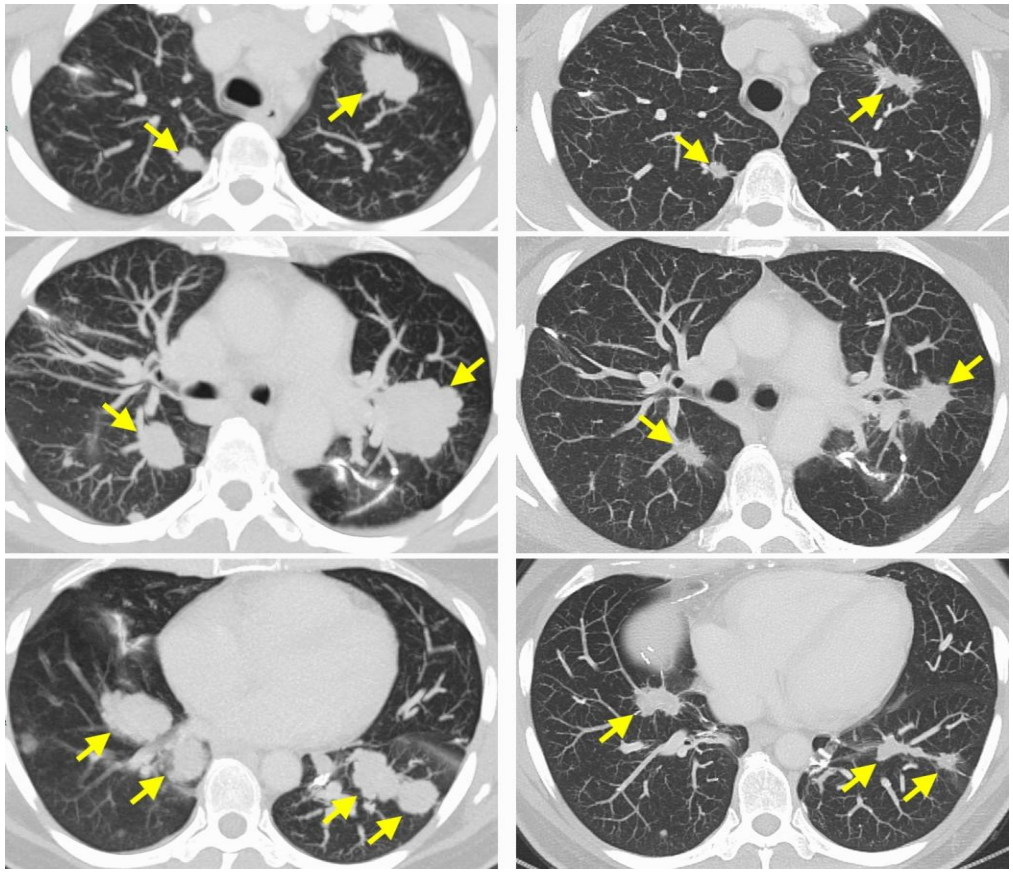


Official Partial Response
(first, and only, responder on our trial)

Lung CT

Pre-2nd ACT

6 months post



Mutation-reactive T cells identified in 6 out of 7 patients with metastatic gastrointestinal cancers

Patient	Cancer	# of mutations assessed	Mutation Reactive T cells detected?	Mutated gene recognized	T cell	Notes
3737	Cholangio	25	**Y**	ERBB2IP	CD4	Multiple clonotypes; TCRs isolated
3812	Cholangio	179	**N**	-------	-------	High background in TIL
3942	Rectal	140	**Y**	NUP98 KARS GPD2	CD8 CD8 CD4	TCRs isolated
3948	Esophageal	210	**Y**	PLEC ASTN2	CD4 CD4	
3971	Colon	119	**Y**	CASP8	CD8	TCR isolated
3978	Cholangio	37	**Y**	ITGB4	CD4	
4007	Colon	265	**Y**	SKIV2L H3F3B	CD8 CD8	Two clonotypes for SKIV2L; TCRs isolated; potential low freq. CD4

In most cases, the frequency of mutation-reactive cells in expanded TIL is relatively low

Conclusions

- Adoptive cell therapy with TIL can cure some patients (~20%) with metastatic melanoma

- Adoptive cell therapy using T cells engineered with receptors targeting CD19 can be highly effective against B-cell leukemias and lymphomas
  - However, most solid cancers do not express a similar/suitable molecule that we can target with gene-engineered T cells
    - ➢ Limits the use of this approach for these cancers

- Unlike melanoma, conventional TIL therapy is not very effective against metastatic gastrointestinal cancers

- In a patient with cholangiocarcinoma, the transfer of a highly pure population of T cells targeting a unique mutation expressed by a patient's tumors resulted in tumor regression

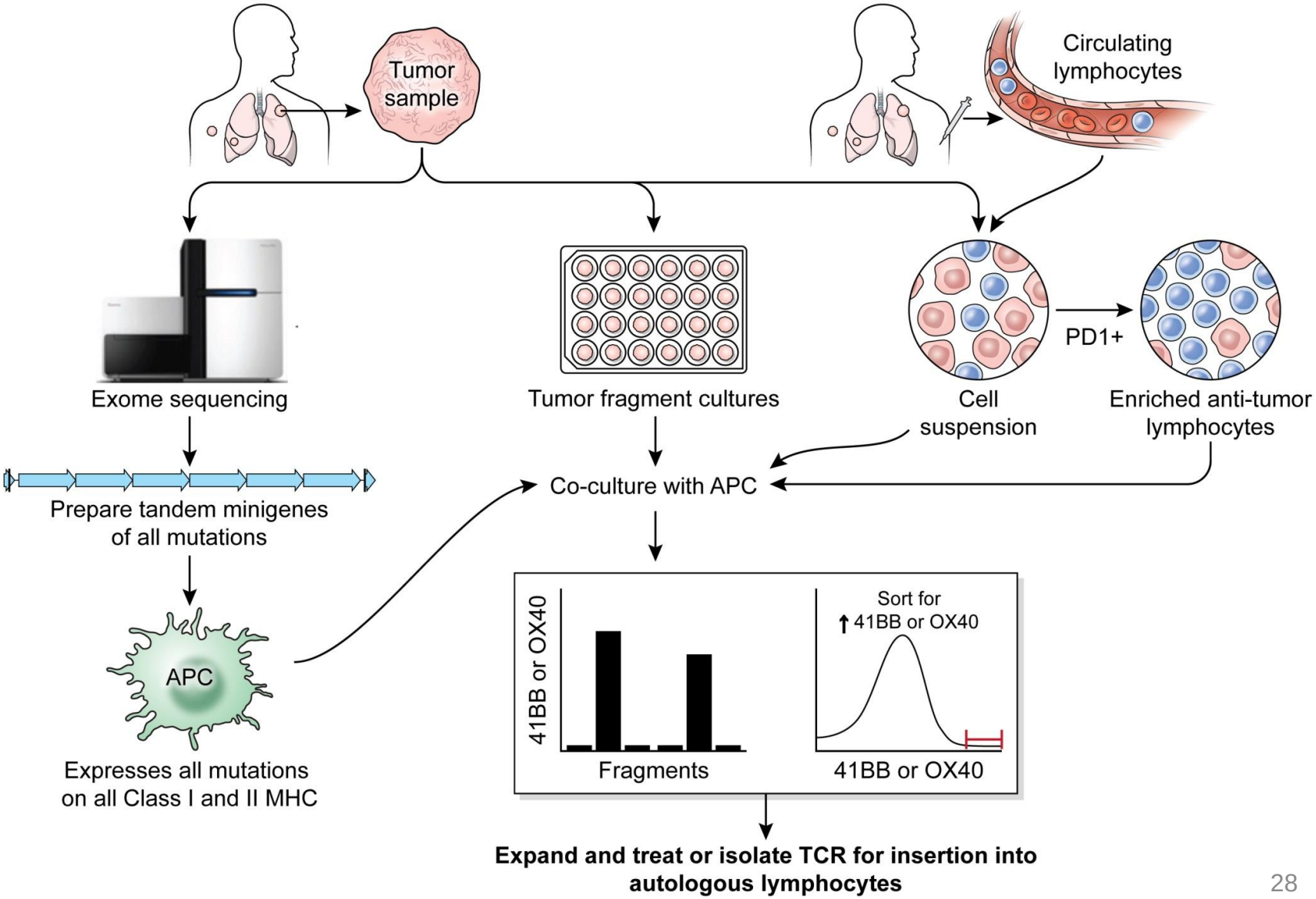
Conclusions

- Most patients with metastatic gastrointestinal cancers seem to mount a T-cell response against at least one mutation expressed by their tumors, although the overall frequency of mutation-reactive T cells appears to be relatively low

Conjecture

The identification and specific targeting of mutations unique to each patient's tumors may be a way to extend T-cell therapy to common epithelial cancers

The future of T-cell therapy for common solid cancers?



Using Patients' Own Immune System to Knock-out Cancer:

Adoptive Cell Therapy



Presenters:
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Melinda Bachini, CCF Patient Advocate and NIH Research Study Participant