Adoptive T-Cell Immunotherapy

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Surgery Branch, NCI
November 21, 2014

Adoptive T-Cell Therapy: Concept and Principles

- Identifying and transferring tumor-reactive T-cells activated and expanded in vitro is a direct way to achieve the same goal as vaccines, cytokines, etc
- This permits the use of reagents and methods perhaps not tolerated in vivo
- This also allows independent manipulation of the recipient to optimize conditions for the infused T-cells

T-Cell Adoptive Therapy: Concept and Principles

- Components of adoptive cellular immunotherapy:
  - T-cell repertoire recognizing tumor associated antigens
  - Host immunosuppression
  - Cytokine support
  - Antagonists of immune inhibition in the tumor microenvironment

T-Cell Adoptive Therapy: Concept and Principles

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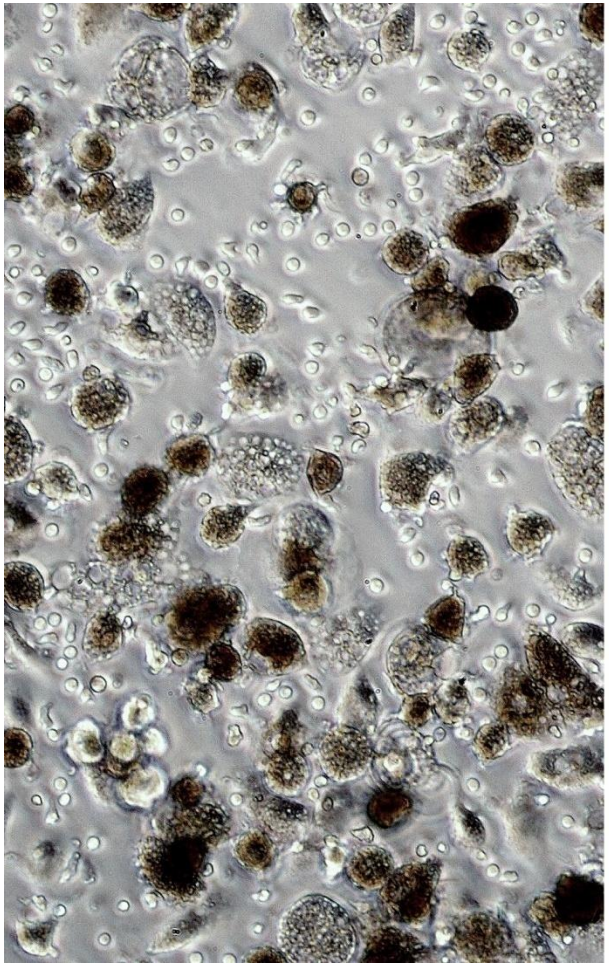
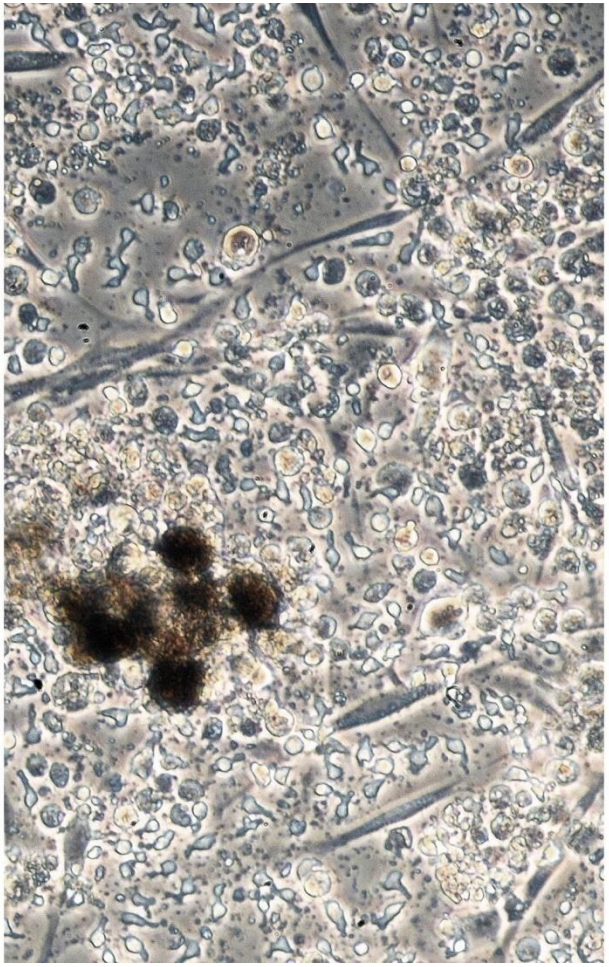
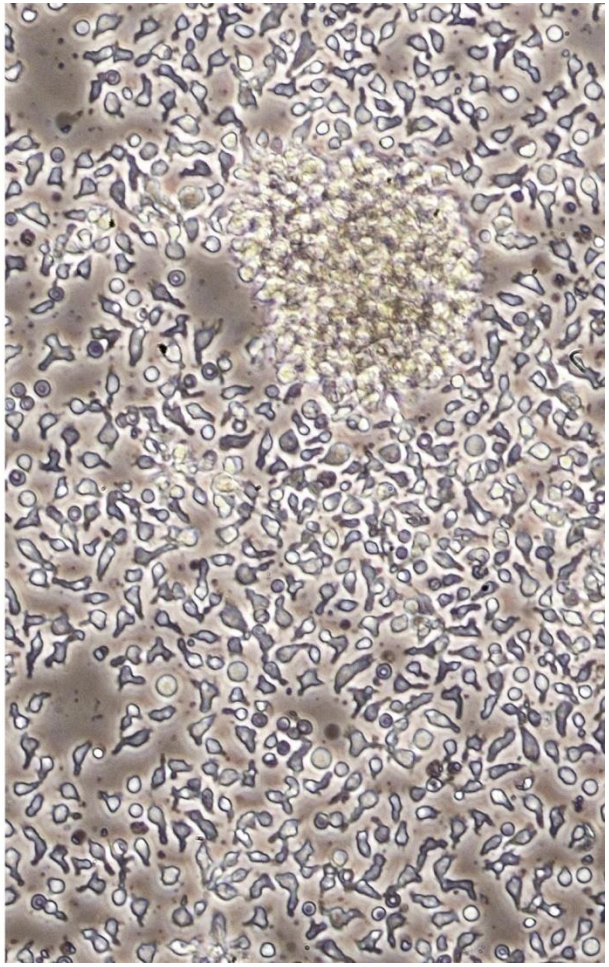
Melanoma Tumor Infiltrating Lymphocytes (TIL)

- Human melanomas frequently contain resident T-cells that can recognize the autologous tumor
- These can be expanded in vitro using IL-2 and anti-CD3
- This consistent source of tumor-reactive T-cells allowed the development of the field of adoptive cell transfer

Melanoma TIL
(Tumor Infiltrating Lymphocytes)

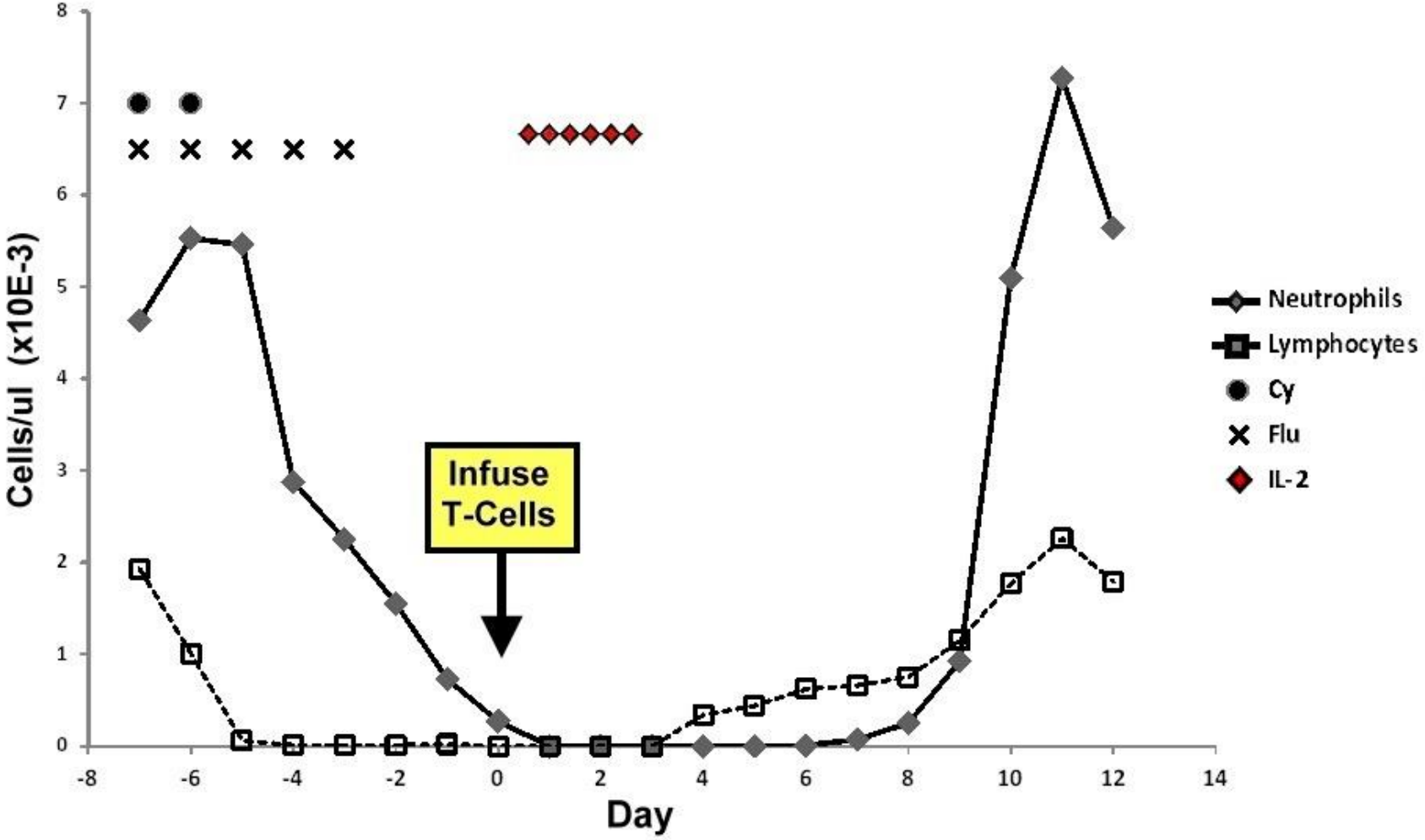






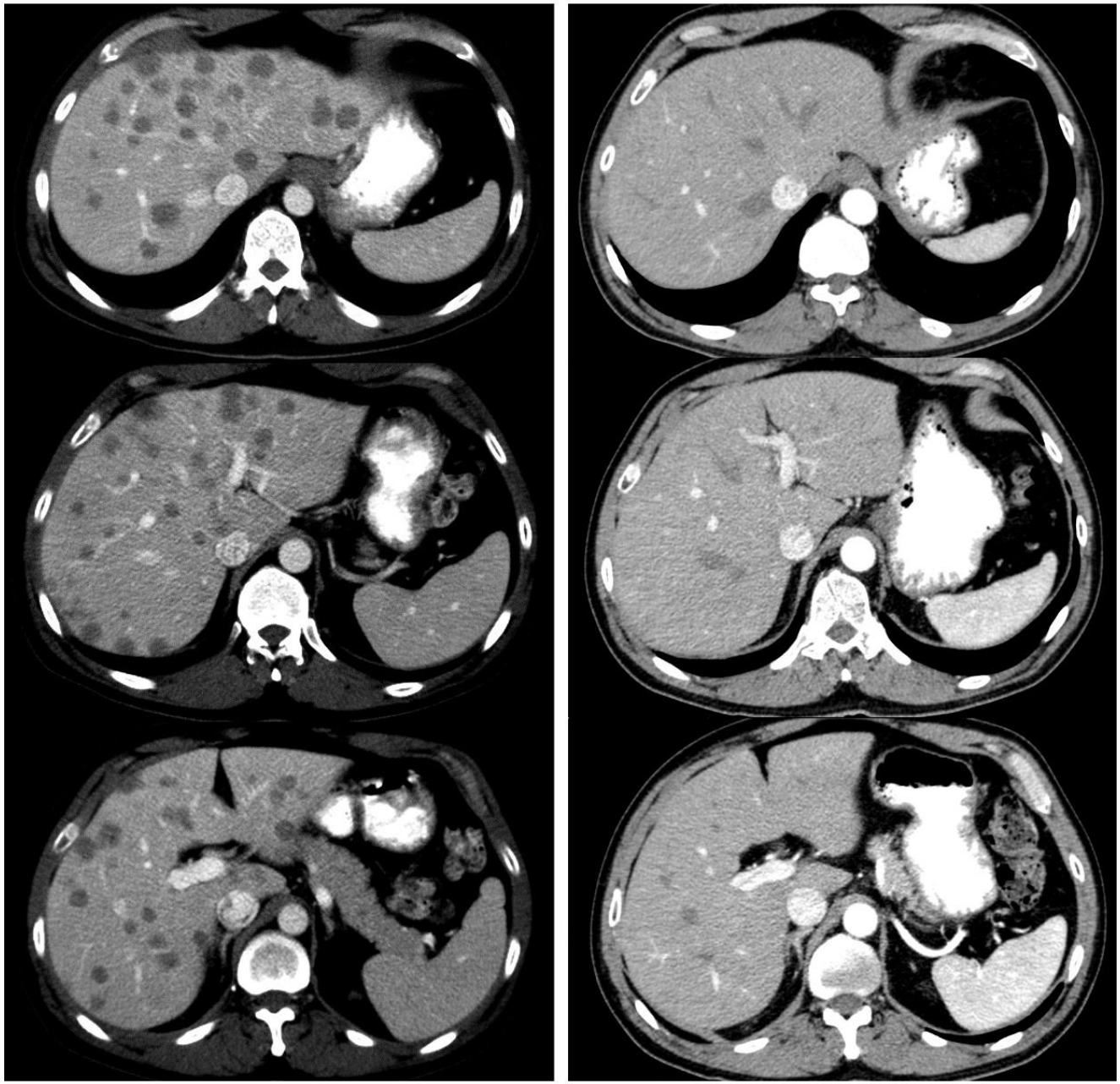
Fresh digest **One week** **Two weeks**

Cyclophosphamide + Fludarabine
Non-Myeloablative Chemotherapy



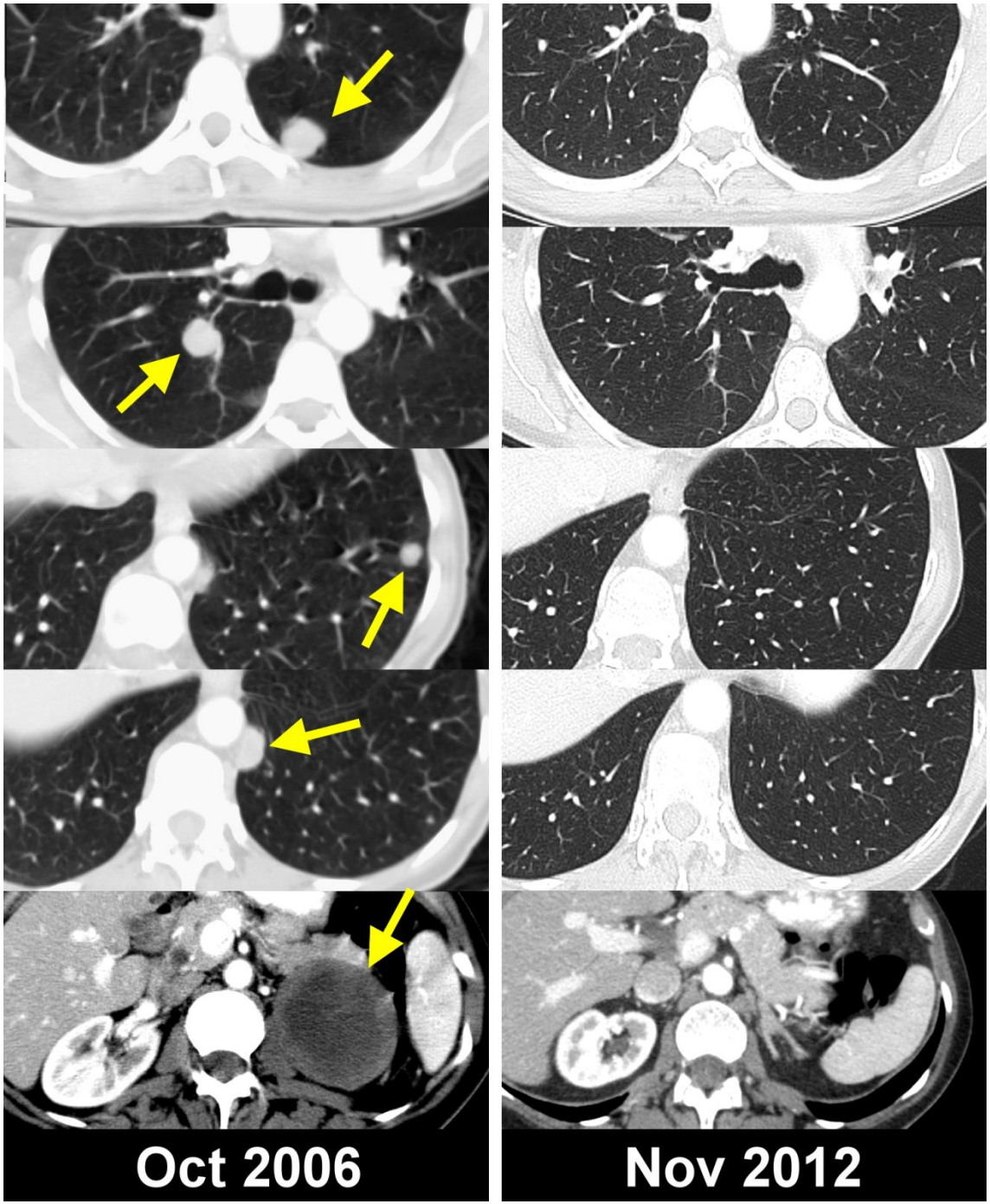
Other Sites: Lung

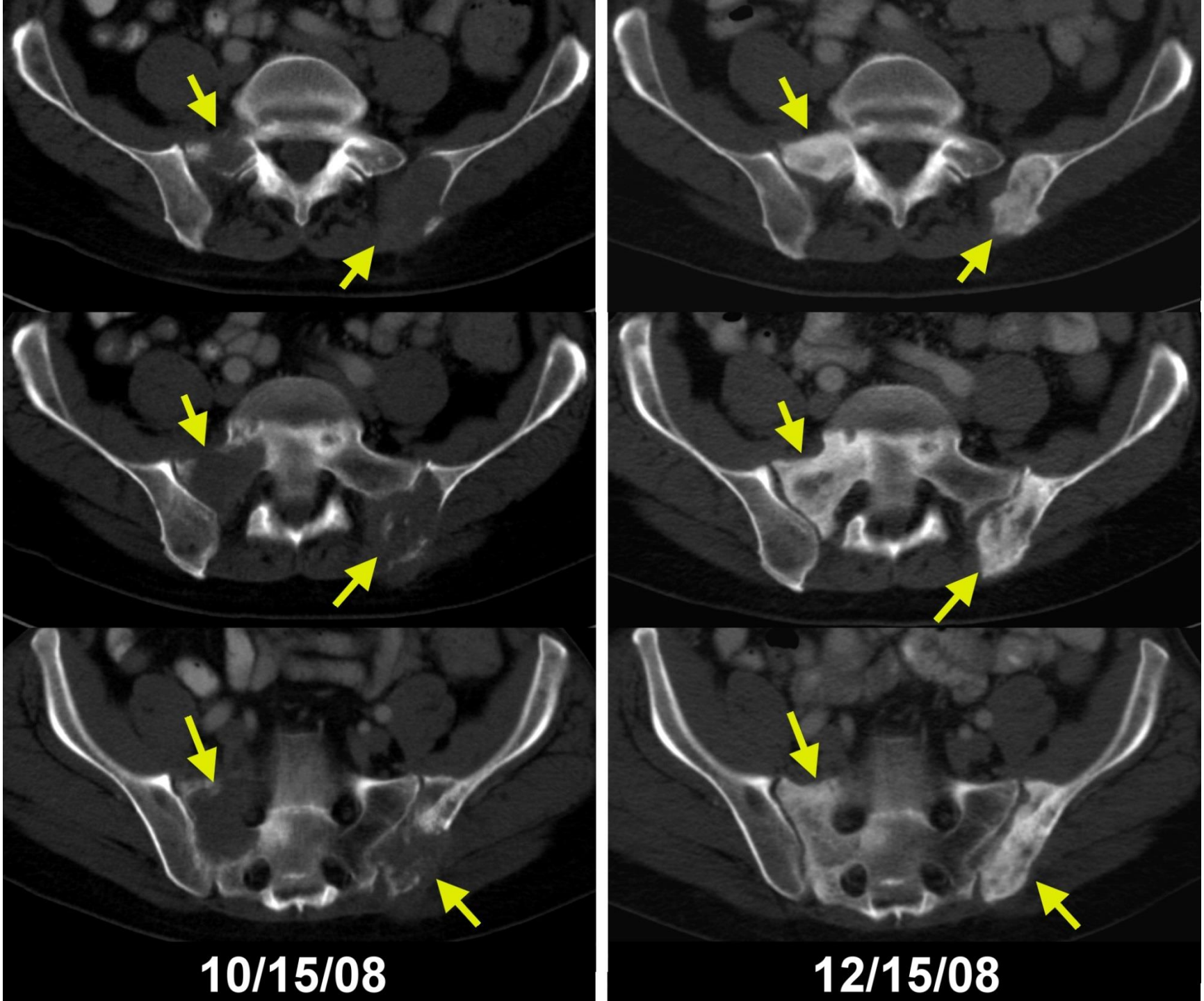
CR 99+ months



Nov 10, 2003

Feb 9, 2012


Oct 2006
Nov 2012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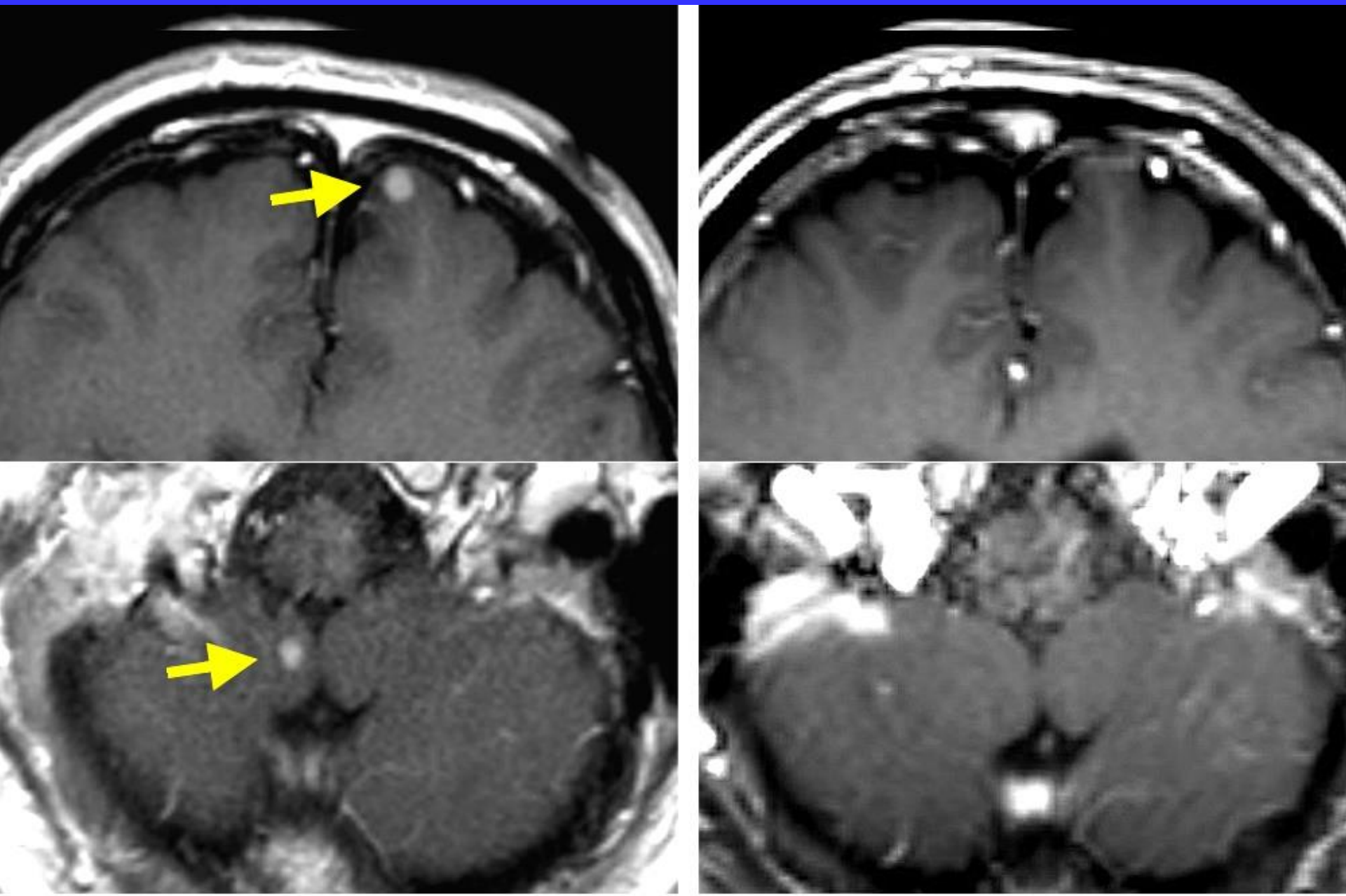
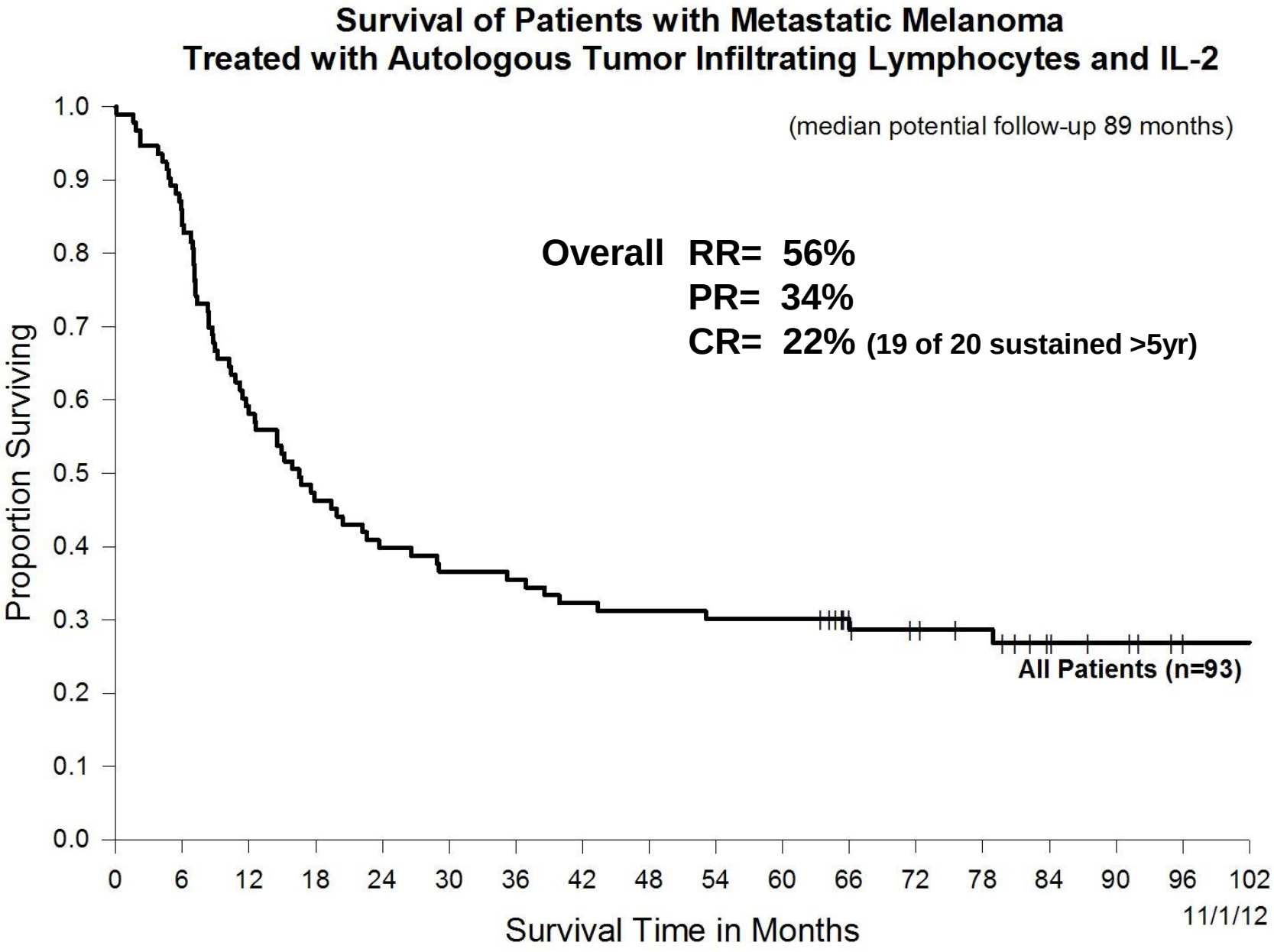


Pre-Treatment

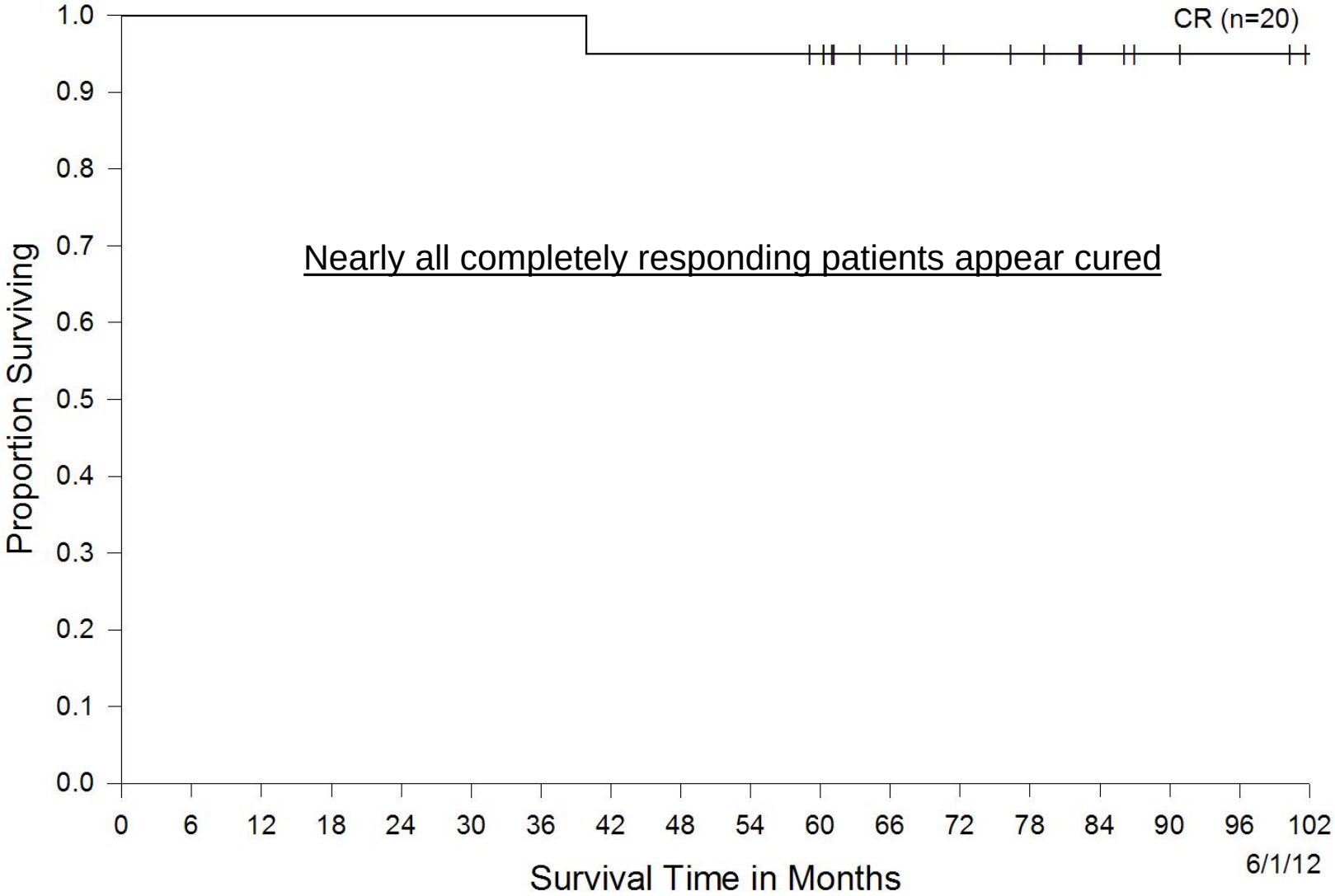
11 Months

TIL for Metastatic Melanoma

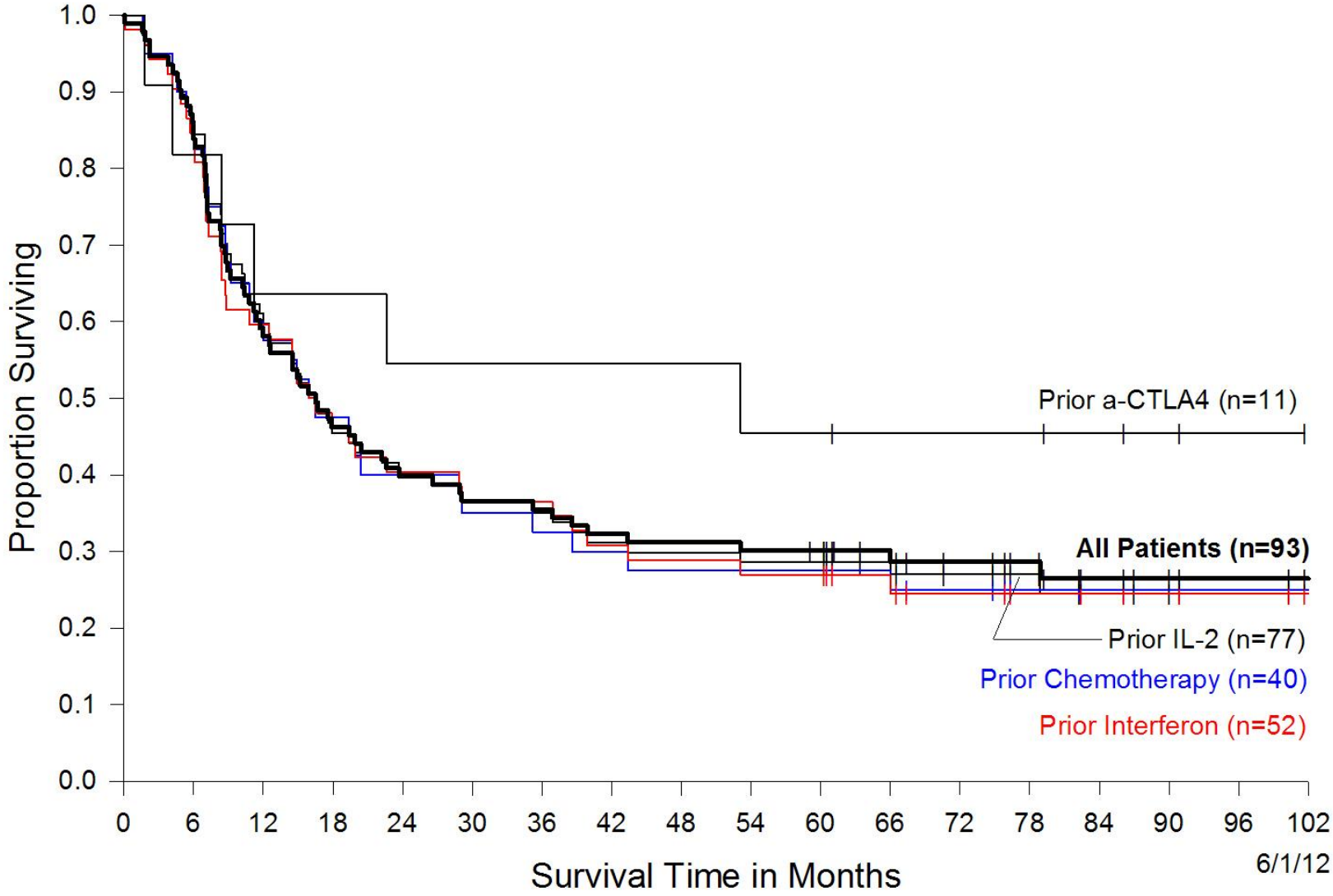
- Between 2000 and 2007, 93 patients with measurable metastatic melanoma were treated with a preparative lymphodepleting regimen followed by TIL and IL-2
- 86% had visceral metastases
- 83% had prior IL-2
- Only 2 patients were treated twice and there was one patient death during treatment due to sepsis

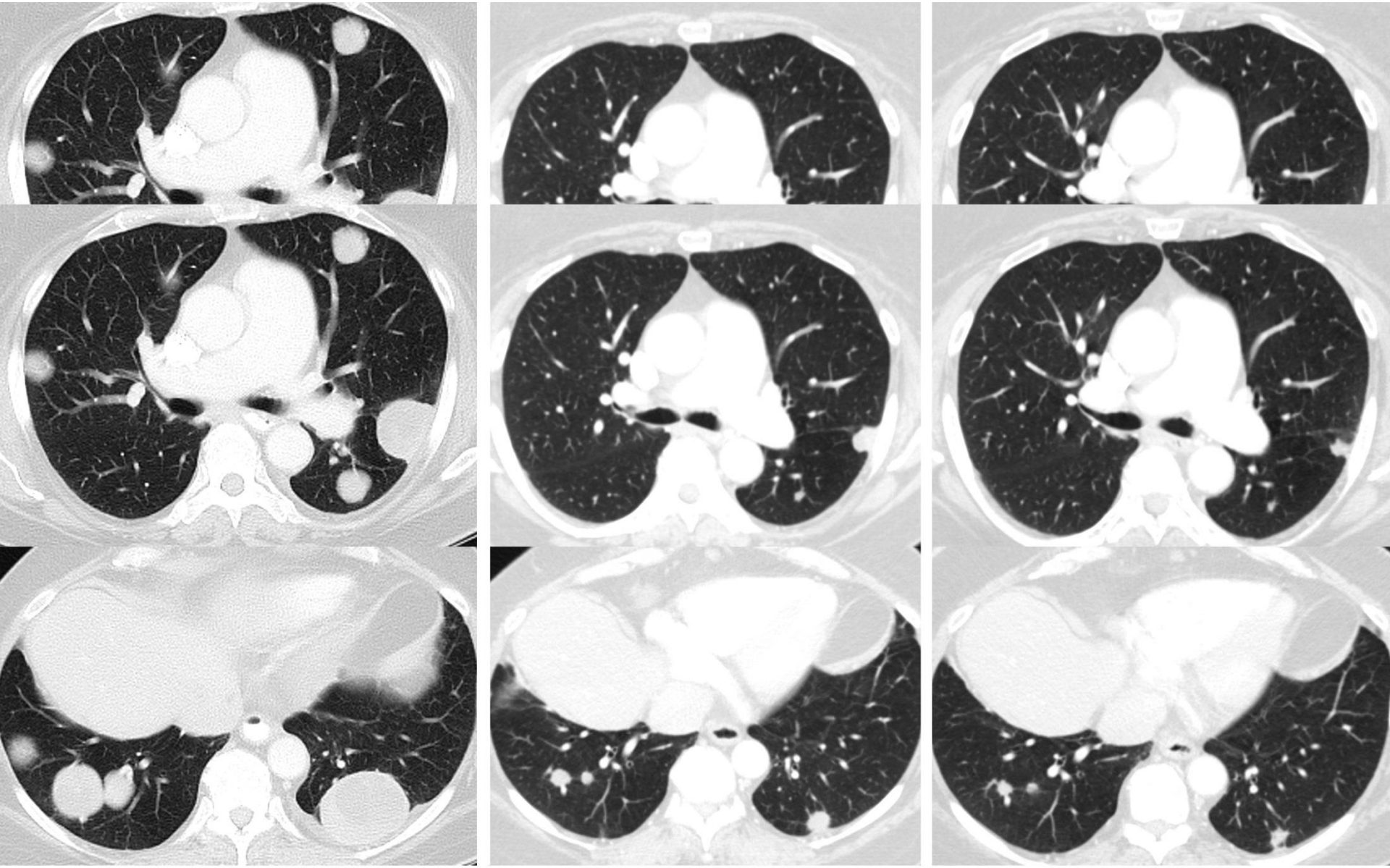

Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
(median potential follow-up 89 months)
Overall RR= 56%
PR= 34%
CR= 22% (19 of 20 sustained >5yr)
All Patients (n=93)
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Survival Time in Months
11/1/12

Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

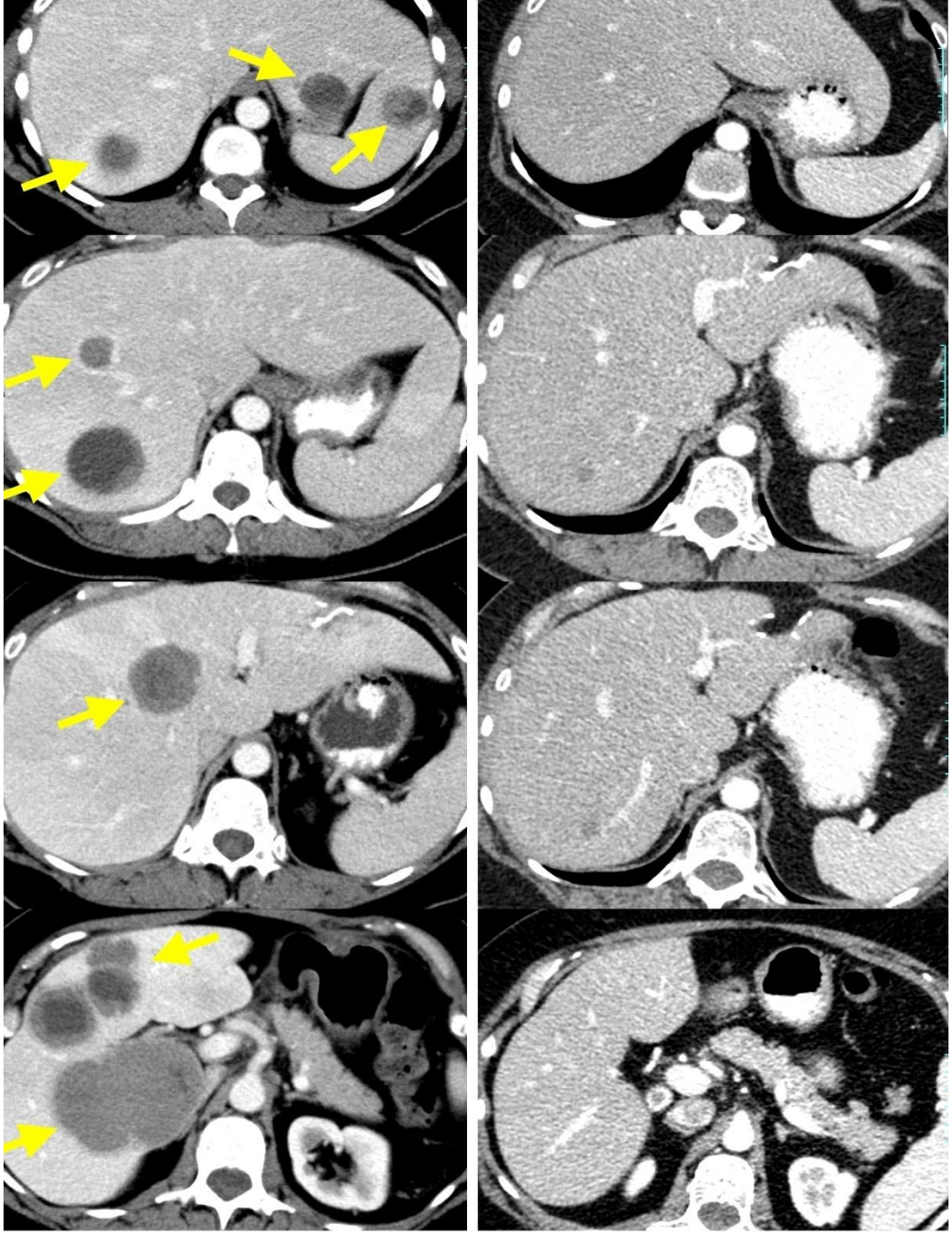


Impact of prior therapy on the response to ACT using selected TIL





Aug 2010 May 2011 Oct 2012



Sept 2010 Sept 2012

New TIL Protocol

- Beginning March 2011, 101 patients were randomized to receive TIL and IL-2 after either Cy-Flu or Cy-Flu-TBI
- No treatment related mortality
- Long-term results pending and responses still evolving
- Interim overall RR for all pts is 54% with 17% CR

Tumor Associated Antigens

- Using tumor-reactive T-cells and expression cloning, over 100 tumor-associated antigens recognized by T-cells have been identified by multiple investigators

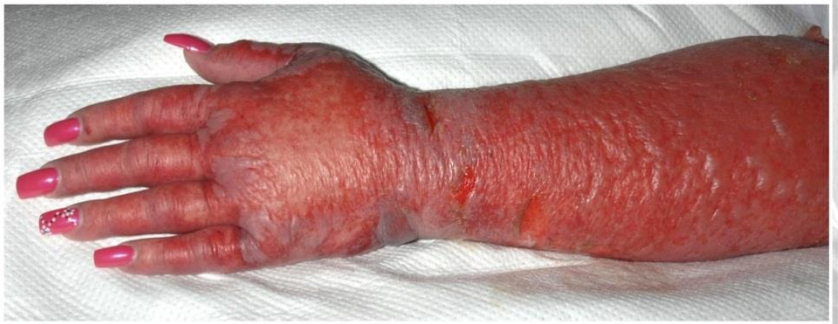
Tumor-Associated Antigens Recognized by T-Cells

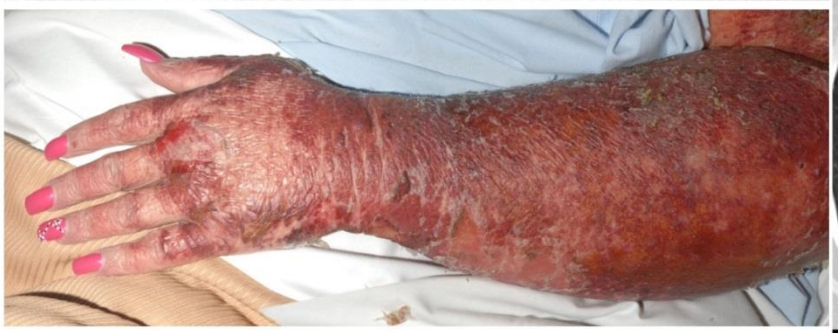
- Melanocyte/melanoma differentiation antigens (MDA; MART1, gp100, tyrosinase, TRP 1/2)
- Tumor testis antigens (NY-ESO1, MAGE, PRAME, SSX families)
- Overexpressed normal proteins (TERT, mesothelin)
- Viral oncoproteins (E6/E7, Merkel's)
- Proteins containing tumor specific mutations (B-catenin, PPP1R3B)

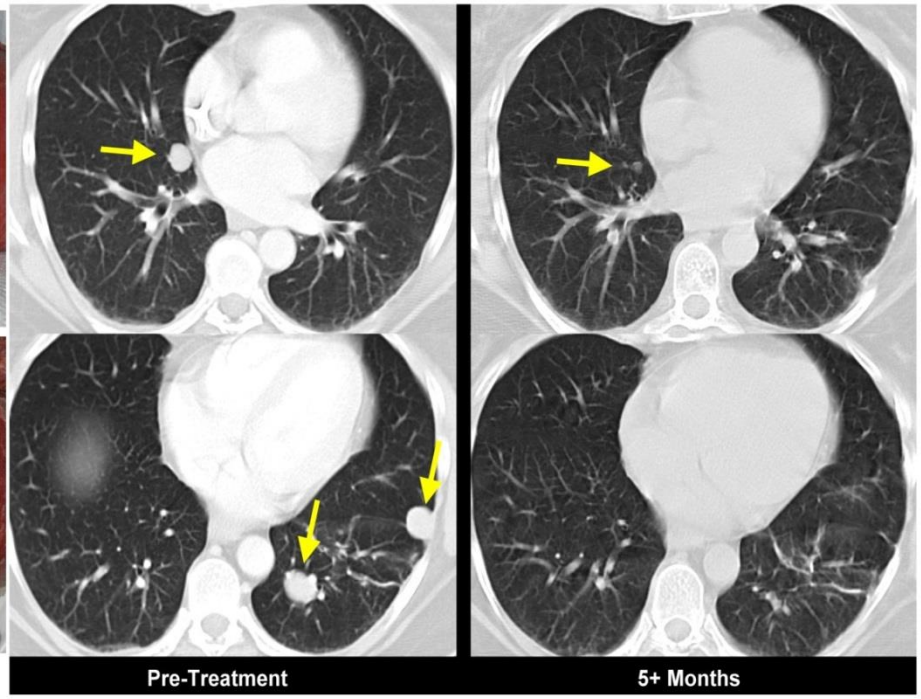
Responses to TCR-Engineered PBL Targeting MDA

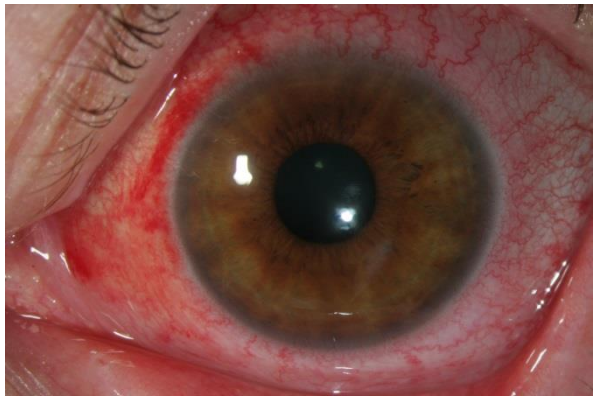
- Two protocols targeting MART-1 and gp100 with TCR-transduced PBL showed response rates of 30% and 19% respectively
- 81% of patients had rashes with melanocyte destruction, 42% developed uveitis and 42% experienced impaired hearing, indicative of MDA-mediated autoimmunity

Targeting Melanocytic Proteins:
Anti-MART1 TCR-Engineered PBL

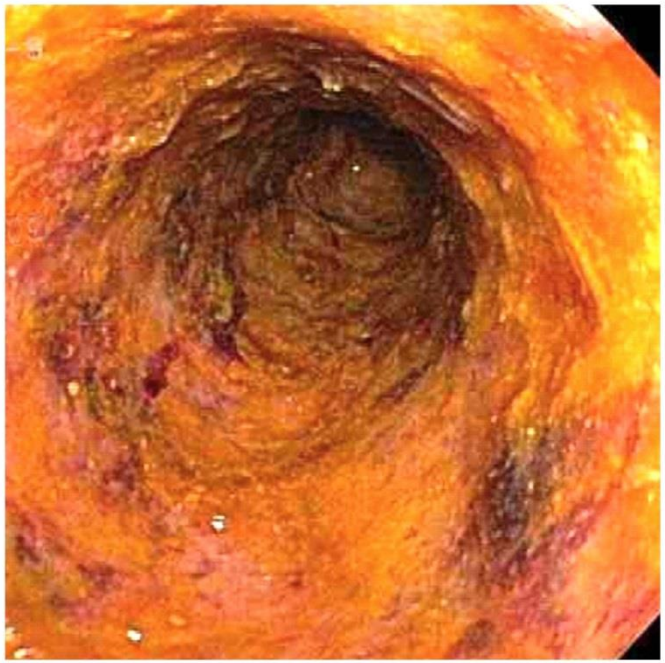


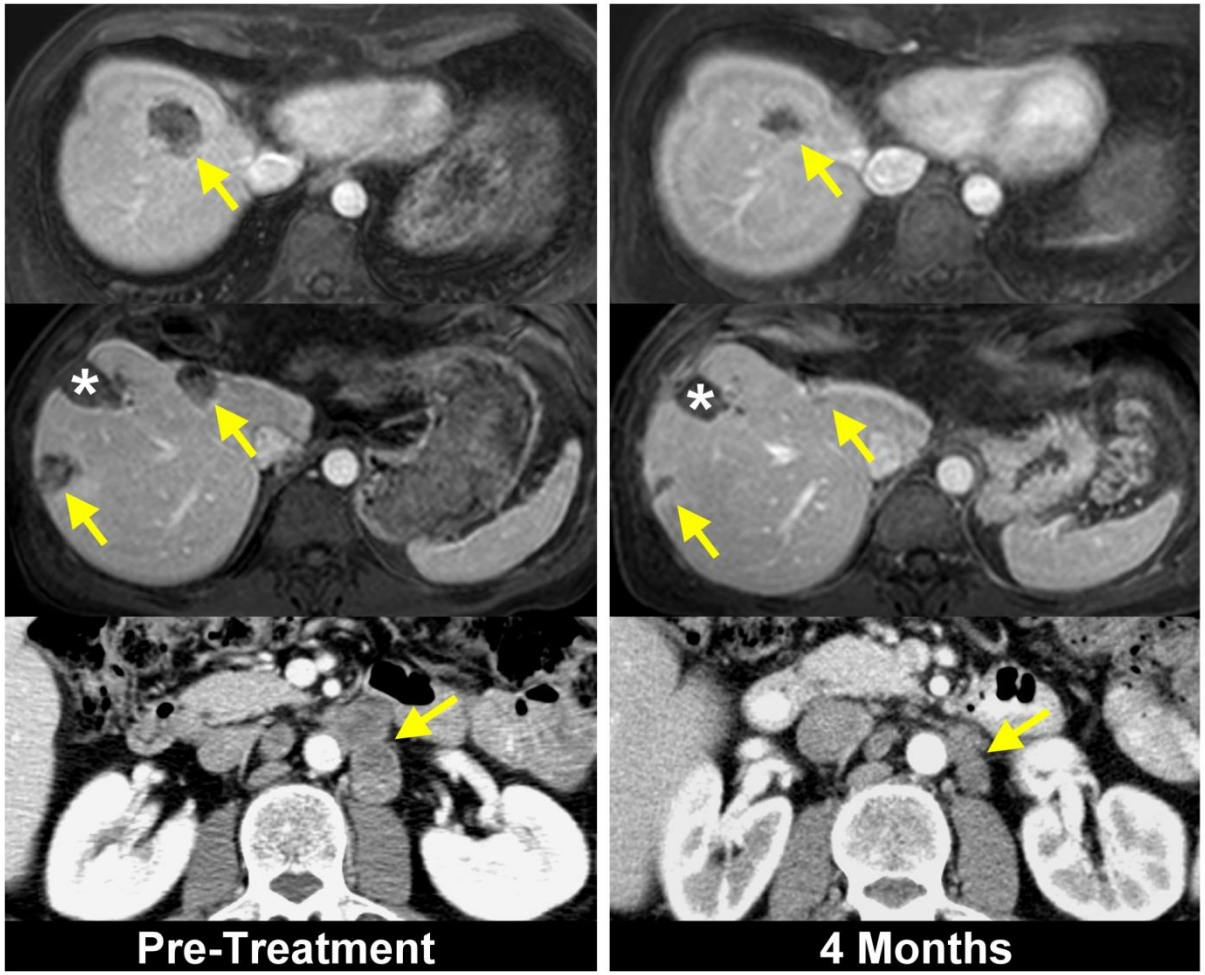






PBL with TCR Targeting CEA





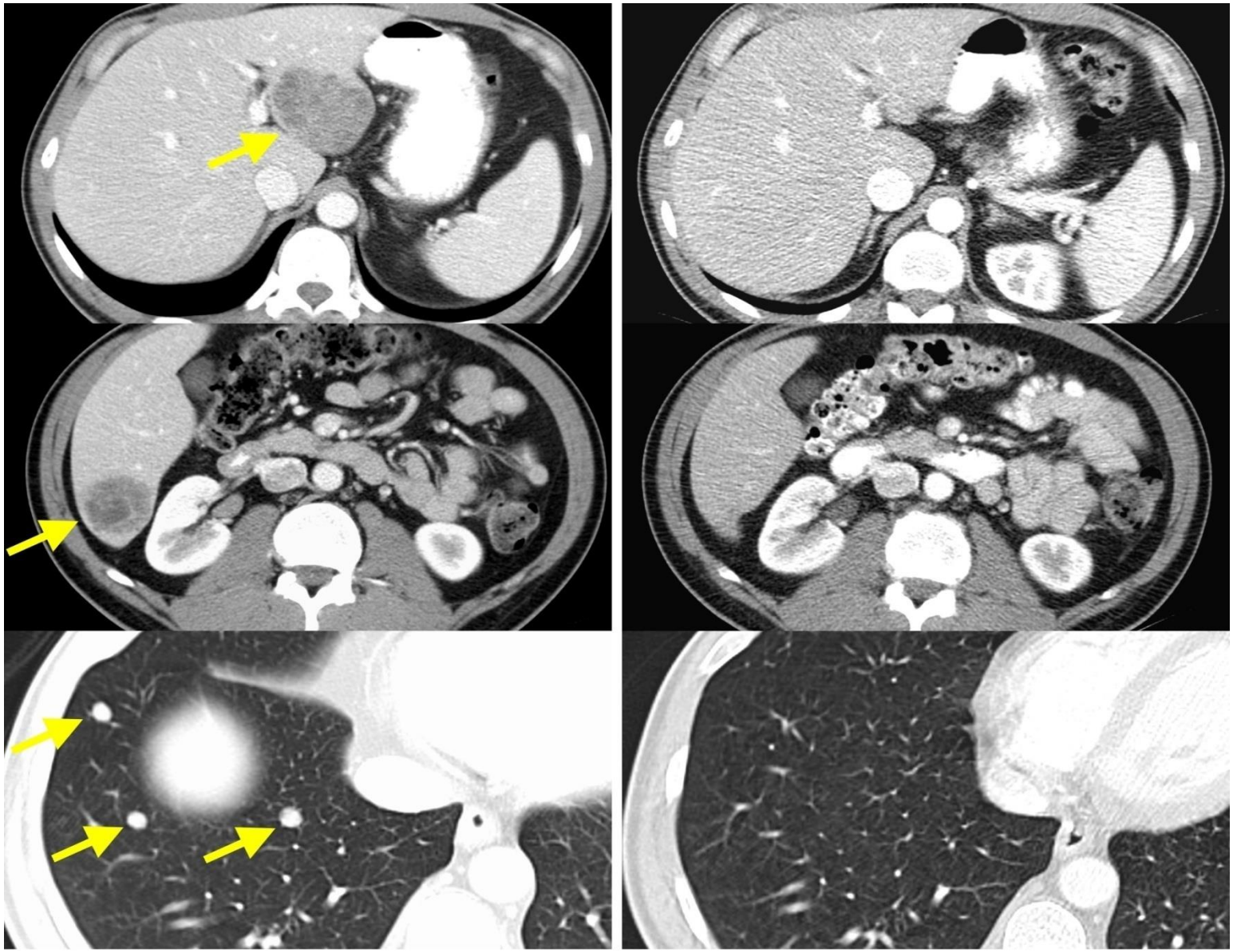
* Post RFA

Effective attack on normal self-antigens may cause unacceptable autoimmunity

Can Other Defined Melanoma Antigens Mediate Complete Regressions?

- 22 patients with metastatic melanoma were given PBL transduced with a TCR recognizing NY-ESO-1 (with Cy-Flu conditioning and IL2)
- The overall RR was 50% with:
  - 4 CRs: durations 51+,41+,26+,25mo
  - 7 PRs: durations 22+,10,8,5+,5,4,3mo
- No autoimmune toxicities were seen

Gene Therapy with Anti-NY ESO1 TCR (Melanoma)



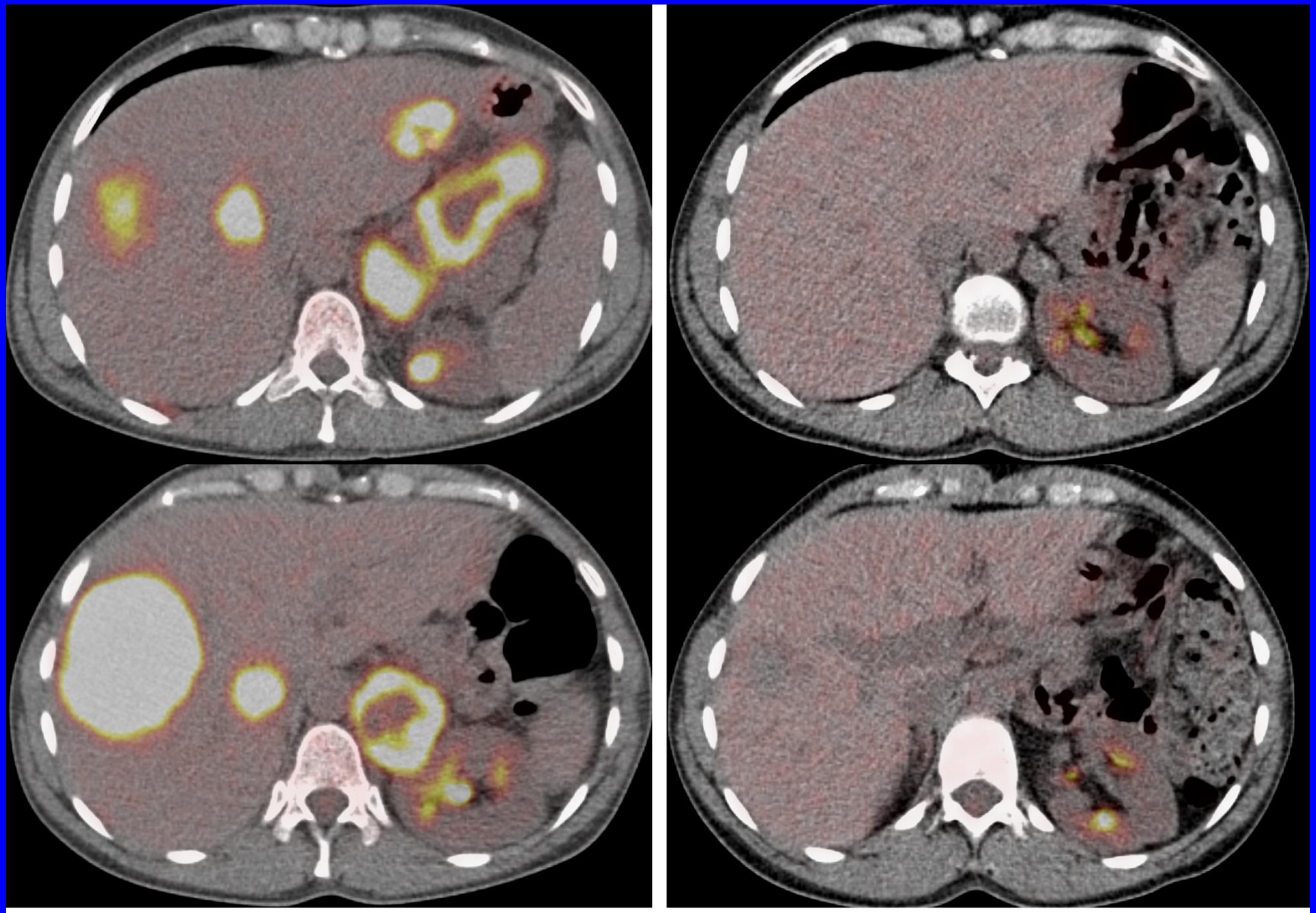
December 2009 March 2012

Targeting CD19

- Autoimmune B-cell destruction may be an acceptable toxicity when treating B-cell malignancies
- Anti-CD19 chimeric antigen receptor (CAR) used with preparative Cy-Flu but no IL-2
- Eight evaluable pts with heavily pre-treated DLBCL or PMBCL
- 4 CR and 2 PR

Kochenderfer, JCO 2014

Primary Mediastinal B-Cell Lymphoma



Pre-Treatment

22 Months

Future Targets for Receptor Gene Therapy

- Chimeric Antigen Receptors
  - Mesothelin
  - EGFR vIII
  - VEGFR2
  - GD2
  - Other CD Ags for hematological CA
- T-Cell Receptors
  - WT-1
  - MAGE family
  - Thyroglobulin
  - ???

Clinical Observations on Melanoma Patients Responding to TIL

- The vast majority of melanoma TIL contain anti-MDA reactivity
- Of the 93 patients given TIL and followed long term, there were 52 objective responders and 20 of these responses were ongoing at 5 years
- Only one of these patients developed uveitis and auditory symptoms indicative of MDA-mediated autoimmunity

What are the Cogent Tumor-Associated Antigens Recognized by TIL?

- X Melanocyte/melanoma differentiation antigens (MDA; MART1, gp100, tyrosinase, TRP 1/2)
- X Tumor testis antigens (NY-ESO1, MAGE, PRAME, SSX families)
- X Overexpressed normal proteins (TERT)
- Proteins containing tumor specific mutations (B-catenin, PPP1R3B)

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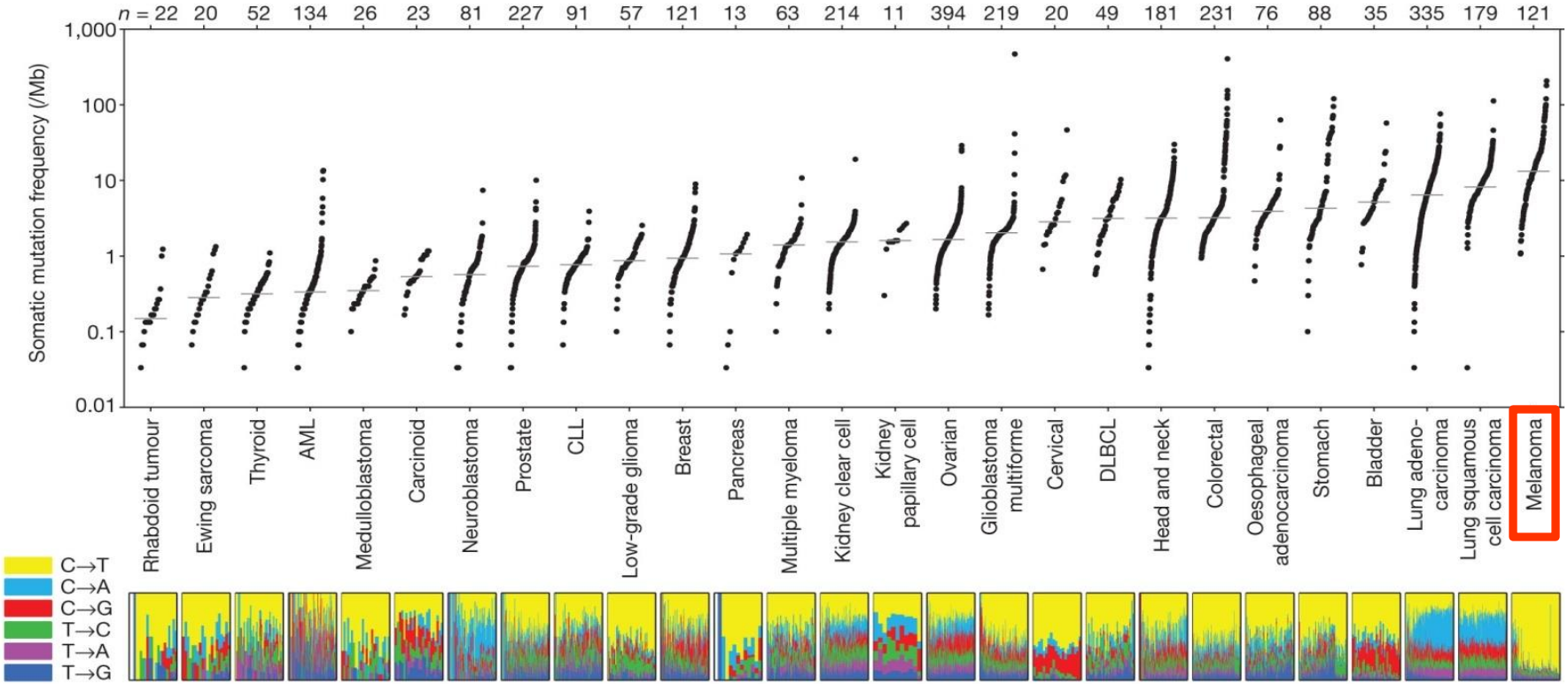
- Overexpressed normal proteins (TERT)
- **Proteins containing tumor specific mutations (B-catenin, PPP1R3B)**

Somatic mutation frequencies observed in exomes from 3,083 tumour–normal pairs.



MS Lawrence *et al. Nature* 1-5 (2013) doi:10.1038/nature12213

Strategy:

Grow reactive TIL, identify MHC restriction

Whole exome sequence autochthonous tumor

Identify all potential 9-mers and 10-mers that could contain each mutated AA-residue

Rank for predicted peptide-MHC binding by algorithm

Synthesize top binding candidate peptides

Screen for recognition by TIL

ref AA	var AA
KWSAPQQNFLQGKTSSVVEM	KWSAPQQNFLLGKTSSVVEM
NRGPAYMFSDRSTSLSIEEER	NRGPAYMFSDCSTSLSIEEER
NNIGDNIFFSSLSLLYALSMV	NNIGDNIFFSLLSLLYALSMV
AVTDFLVAVLVMPFSIVYIVR	AVTDFLVAVLMMPFSIVYIVR
LFSCSVVDVFSQLNQSFEIIK	LFSCSVVDVFFQLNQSFEIIK
RQAGDDFVESSILDAVQRVDS	RQAGDDFVESFILDAVQRVDS
GNVAPKTPAGRLFCVFYGLFG	GNVAPKTPAGHLFCVFYGLFG
SLSLAPLSPRAPSEDVFAFDL	SLSLAPLSPRVPSEDVFAFDL
QMIAVILGSLFLIASVTWLLW	QMIAVILGSLLLIASVTWLLW
ICLPGTSSDYNLMDGDLGLIS	ICLPGTSSDYHLMDGDLGLIS
FPDTLLGDPGRRVRFFDPLRN	FPDTLLGDPGWRVRFFDPLRN
ICFAPYHFSFPLDFLVKSNEI	ICFAPYHFSFSLDFLVKSNEI
CMEKFDKKRFRYIILKQKFLE	CMEKFDKKRFCYIILKQKFLE
KKSLADEAEVHLKFSAKLHSE	KKSLADEAEVYLKFSAKLHSE
NILSAFPFLQEYTVKLSNIPT	NILSAFPFLQKYTVKLSNIPT
LQLPLFCLFLGIYTVTVVGNL	LQLPLFCLFLEIYTVTVVGNL
MFFKDIEEQYPTSLACVEIVN	MFFKDIEEQYLTSLACVEIVN
GGKELLADQNLKFIDPFCKIA	GGKELLADQNFKFIDPFCKIA
QSTLHSLSTSSSSVTLLLALM	QSTLHSLSTSLSSVTLLLALM
QSQGILNAMITKISNELQALT	QSQGILNAMIAKISNELQALT
RALGTLLHTNLRIKRDSQGEL	RALGTLLHTNVRIKRDSQGEL
FPDTLLGDPGRRVRFFDPLRN	FPDTLLGDPGWRVRFFDPLRN
LFSCSVVDVFSQLNQSFEIIK	LFSCSVVDVFFQLNQSFEIIK
CEMLSILALVGVLHPFYRSNN	CEMLSILALVRVLHPFYRSNN
QSQGILNAMITKISNELQALT	QSQGILNAMIAKISNELQALT
KNLTEEMAGLDETIAKLTKEK	KNLTEEMAGLNETIAKLTKEK
NGIPAEYTAPHPHPAPEYTGQ	NGIPAEYTAPYPHPAPEYTGQ
VDLAITICIVLNTLFMAMEHY	VDLAITICIV*NTLFMAMEHY
LQLPLFCLFLGIYTVTVVGNL	LQLPLFCLFLEIYTVTVVGNL
YQRGHLPTGGHLAVCHFPCLL	YQRGHLPTGGRLAVCHFPCLL

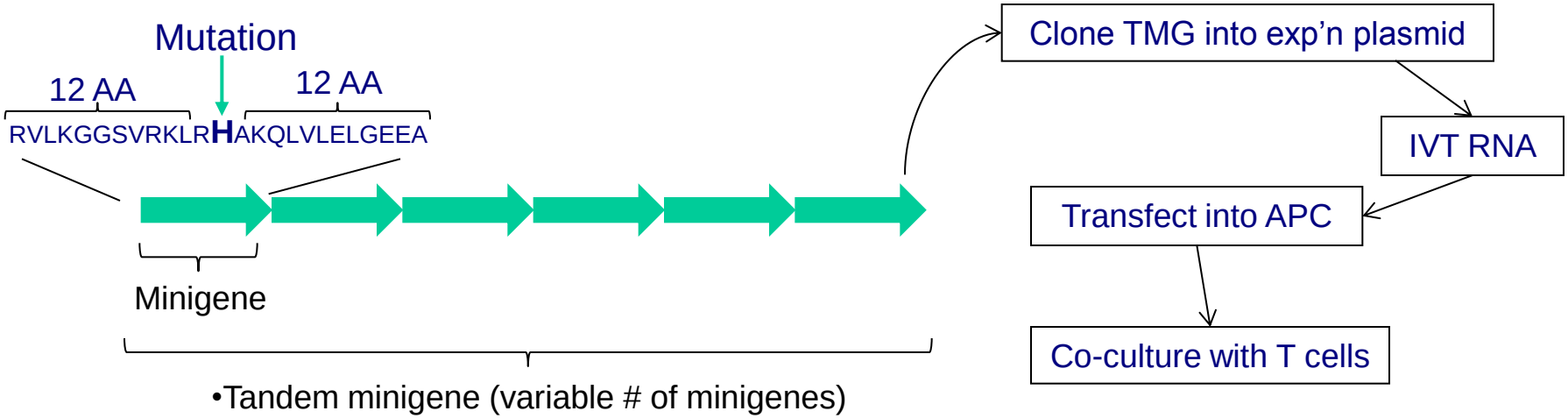
Exomic sequencing of melanoma 2369
(570 mutations; 10830 Candidate Peptides)

Rank	Peptide	Mutation	Affinity (nM)	Gene	IFN-γ (pg/ml)
1	FSDYYDLSY	117 G to S	2	C22orf33	<30
2	LTDDRLFTCY	1005 H to Y	3	PLEKHM2	10400
3	YSSALDLCY	621 N to D	5	GRIN3B	<30
4	FSDKKVGTY	688 L to F	5	PLCB1	<30
5	HSEYSSFFY	603 H to Y	6	HEG1	<30
6	CSNFLLLAY	84 S to L	7	BAI3	<30
7	ESDKEELVGY	332 F to L	7	MPP4	<30
8	CTDTYMLELF	191 H to Y	8	OR4C46	<30
9	FTGTISVMY	60 P to S	12	UEVLD	<30
10	QTQSVVFLY	156 S to L	13	COL9A1	<30
11	MSSYIASFTY	356 L to F	14	LST-3TM12	<30
12	CTDTYMLEL	191 H to Y	22	OR4C46	<30
13	LLDLMAYDRY	117 G to D	22	OR2T2	<30
14	SSDSQEENY	117 G to E	23	MEOX2	<30
15	LTSMAYDCY	122 R to C	31	OR8B3	<30
16	YTDFHCQYV	176 P to H	49	PPP1R3B	13400
17	WADWGHRTY	3344 A to T	51	LRP2	<30
18	FTMVILYVVY	219 S to L	54	LRRC3B	<30
19	CVDSPPPLFF	528 S to F	71	C15orf2	<30
20	VSDGFTAVM	198 P to S	85	RNPEP	<30

Limitations of Peptide Prediction Algorithms

- Need to know presenting MHC allele
- Many candidate peptides are not processed by the protoeasome
- Labor intensive to synthesize and test all possible candidate peptides
- (Insertion and deletions not considered)

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



All potential mutated epitopes (up to 13-mers) and all MHC alleles are tested for processing, presentation and recognition

Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer

Science, May 2014

Eric Tran,[1] Simon Turcotte,[1*] Alena Gros,[1] Paul F. Robbins,[1] Yong-Chen Lu,[1] Mark E. Dudley,[1†] John R. Wunderlich,[1] Robert P. Somerville,[1] Katherine Hogan,[1] Christian S. Hinrichs,[1] Maria R. Parkhurst,[1] James C. Yang,[1] Steven A. Rosenberg[1‡]

43 yo F with cholangiocarcinoma refractory to chemotherapy and metastatic to lungs and liver

Treated with bulk TIL empirically with minimal response

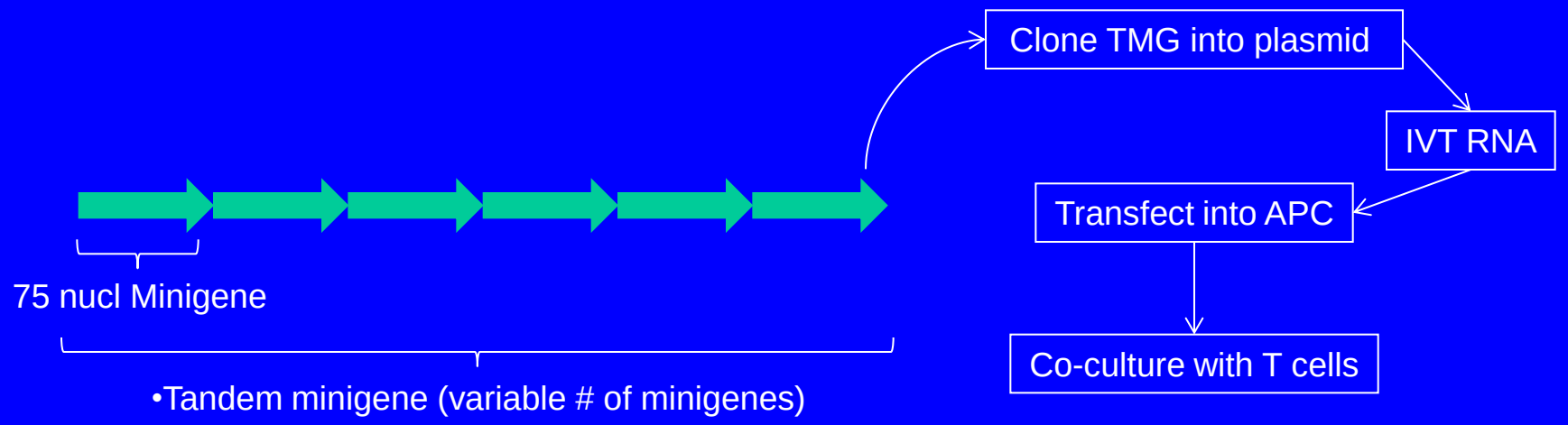
Tumor WES showed 26 non-synonymous mutations

Screened TIL against tandem minigenes and found a mutation (*mut*-ERBB2IP) reactive T-cell culture

Retrospectively, first TIL culture contained 10 billion of these T-cells (+ 30 billion unreactive T-cells)

New culture with 120 billion of these CD4 cells (95% pure) grown and given

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



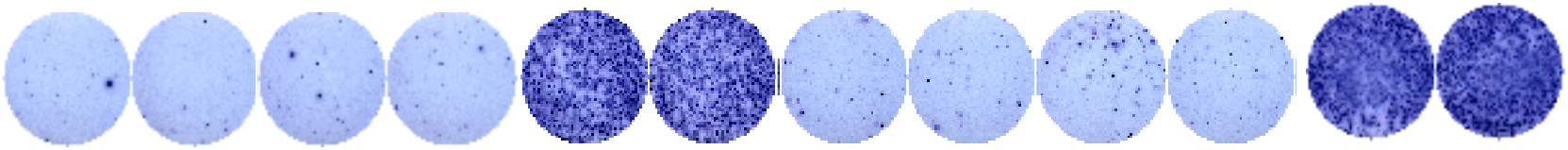
- Three Tandem Mini-Genes (TMGs) generated for Pt. MB

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 induces IFN-g secretion and upregulation of the CD4+ T-cell activation marker OX40

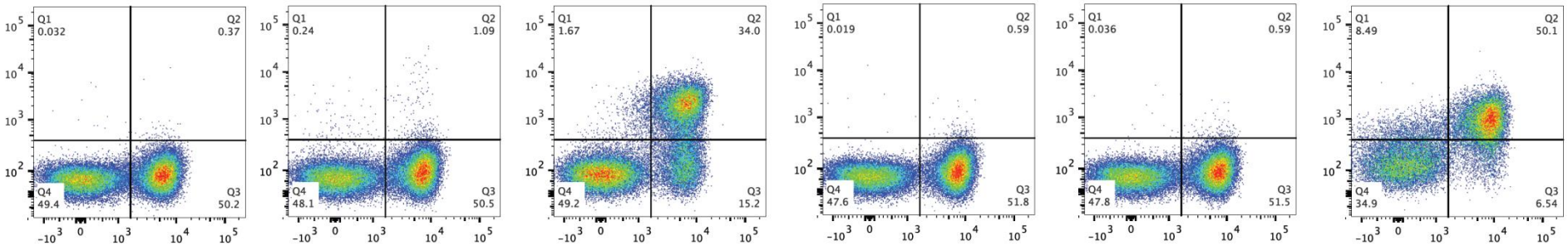
•**Co-culture TIL + TMG-APC: IFN-g ELISPOT assay**

•Mock •GFP •TMG-1 •TMG-2 •TMG-3 •OKT3



•**Flow cytometry**

•Mock •GFP •TMG-1 •TMG-2 •TMG-3 •OKT3

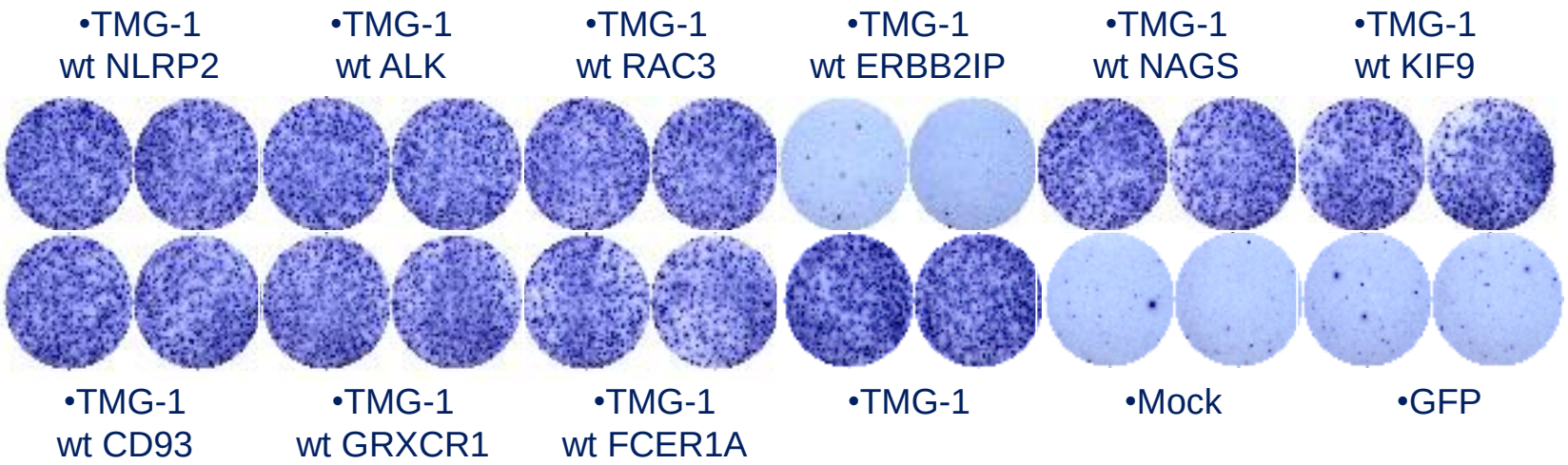


•OX40

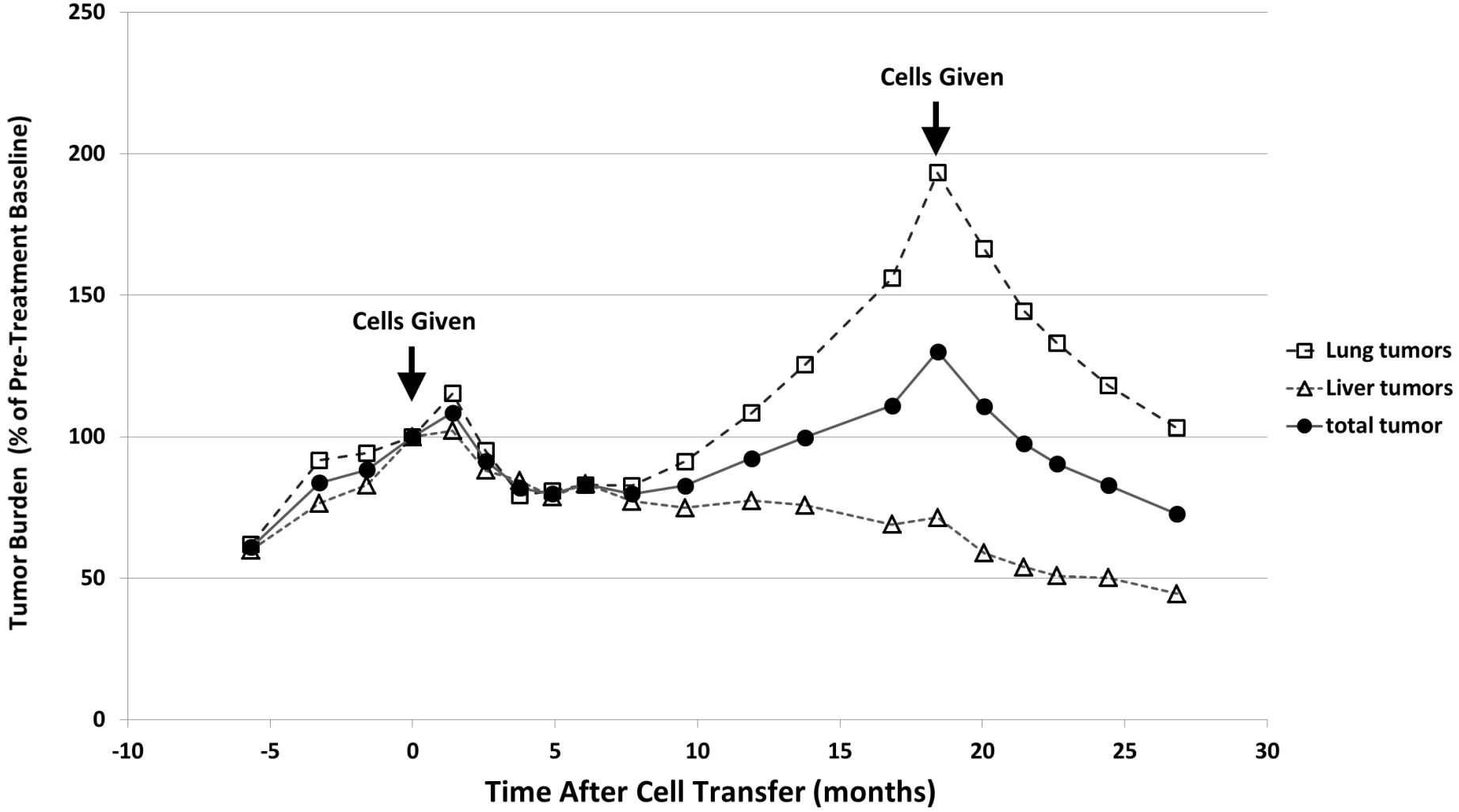
•CD4

•Only the mutated minigene ERBB2IP is recognized by Pt. MB infusion TIL

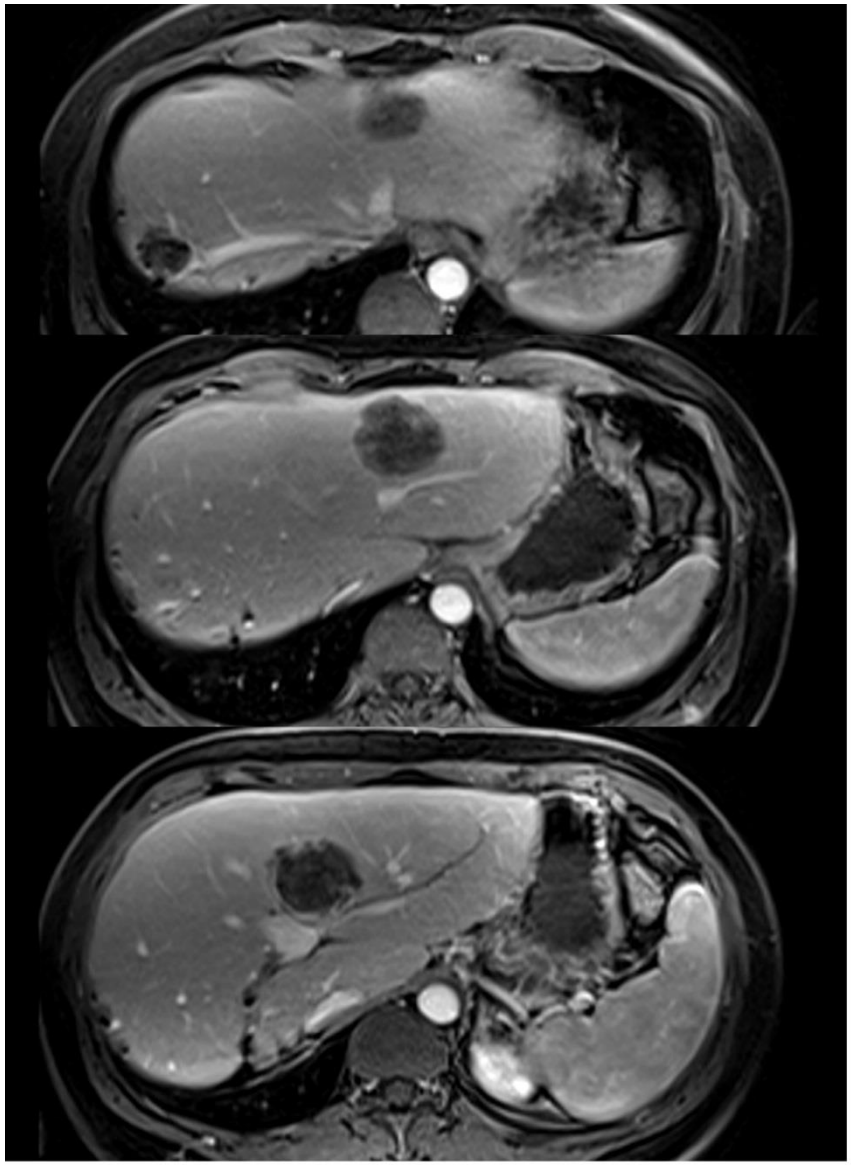
IFN-g ELISPOT assay: Minigenes in TMG individually 'back-mutated' to w.t.



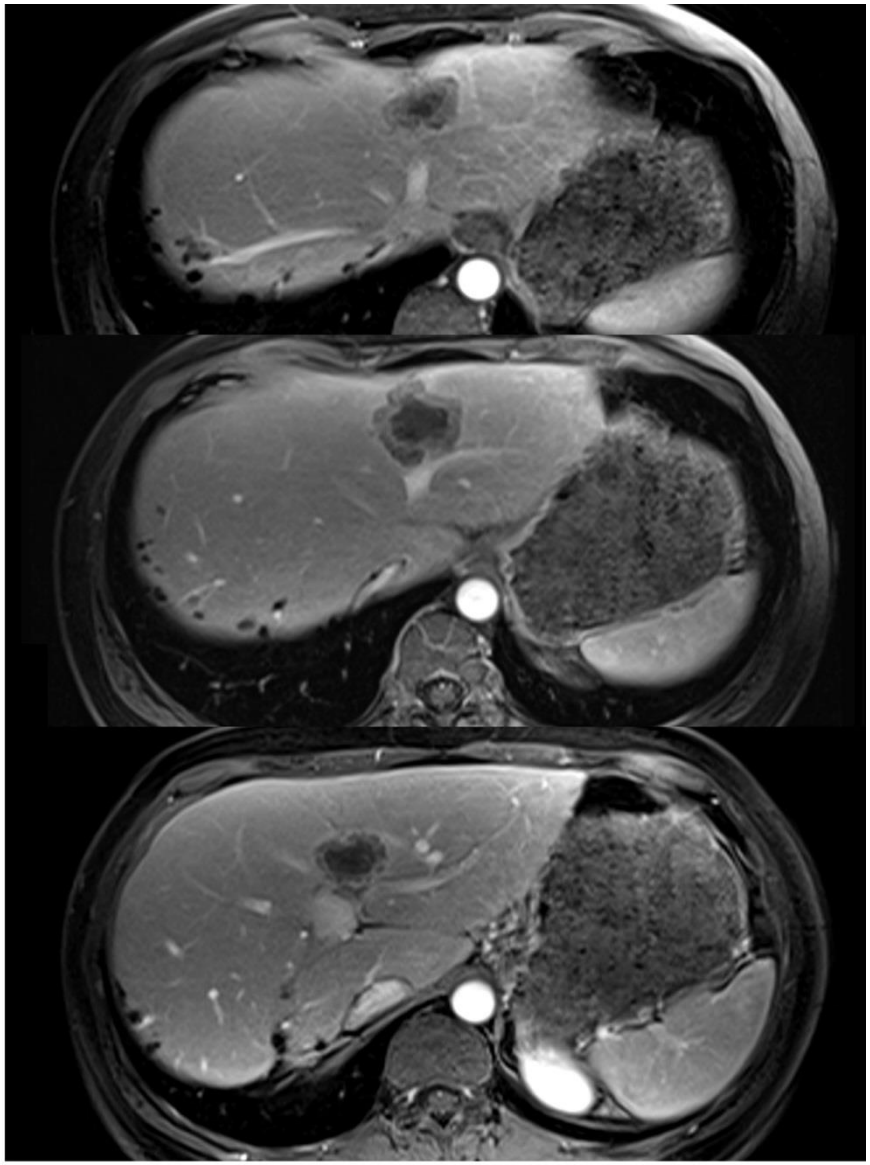
Tumor Burden (RECIST)



Treatment #1: Liver

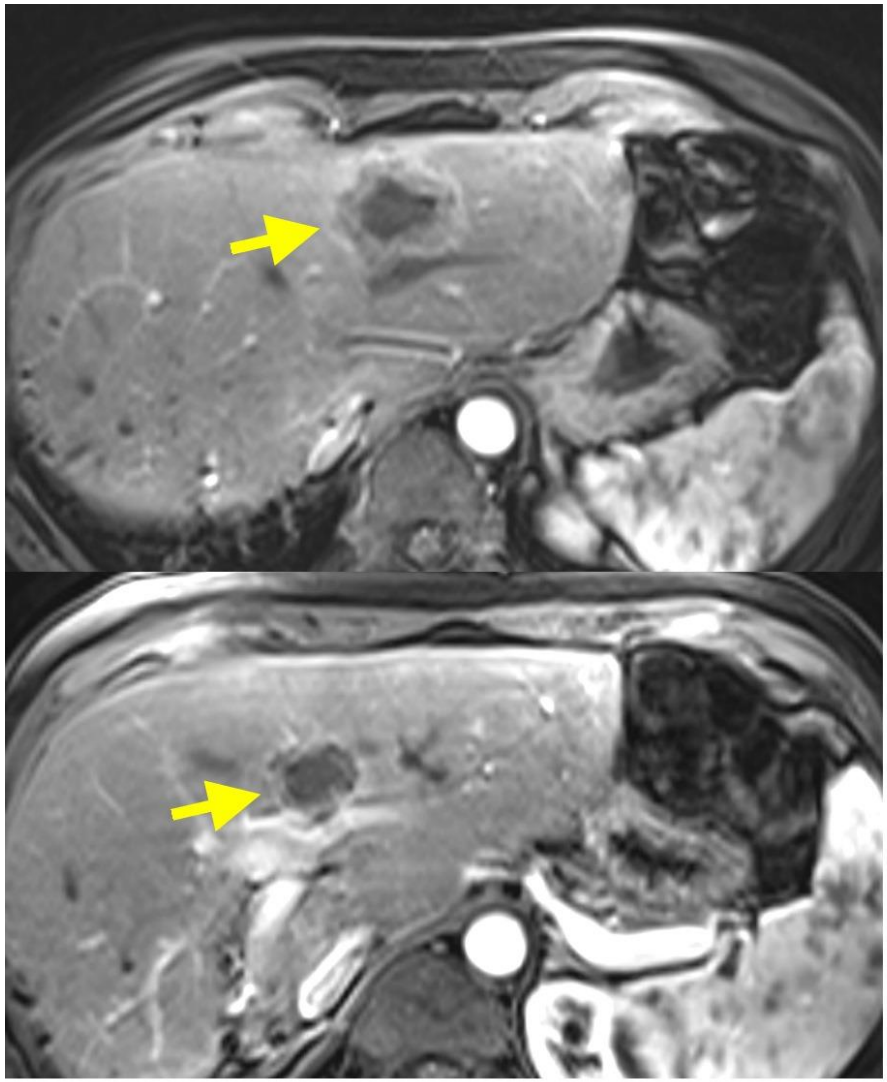


Pre-Treatment

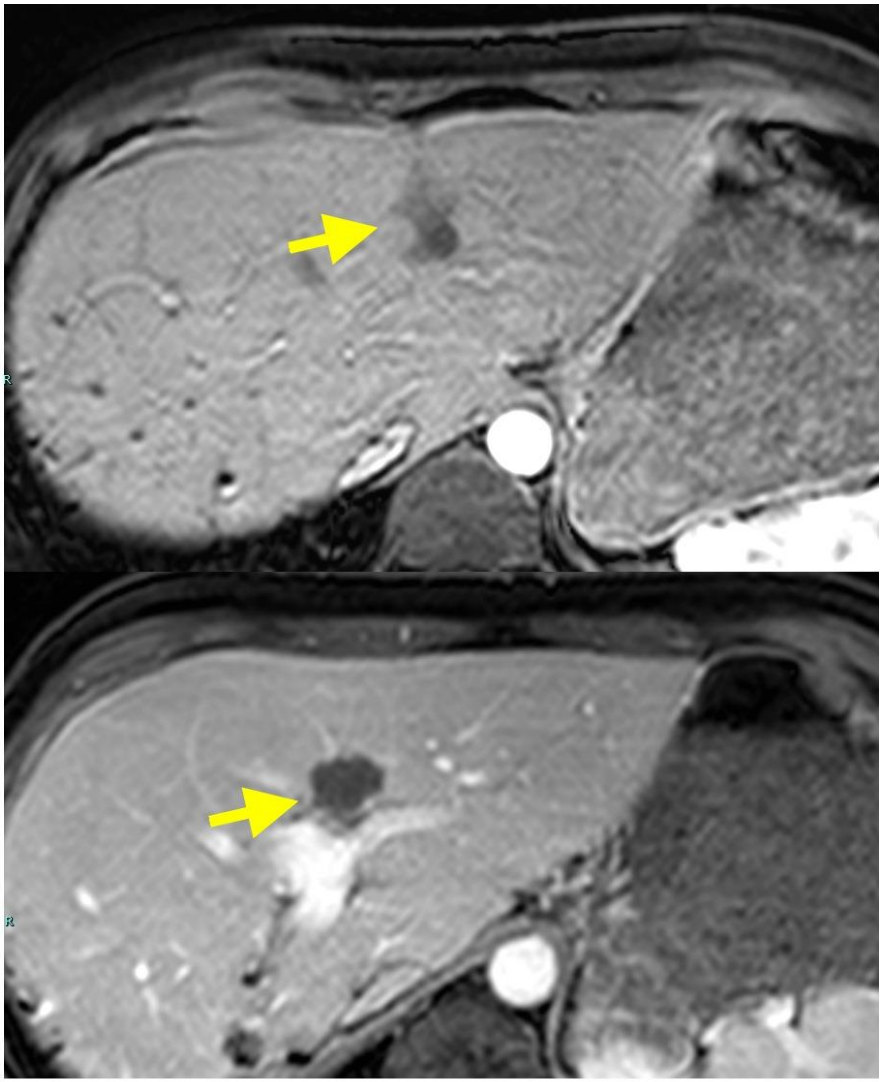


7 Months

Treatment #2: Liver

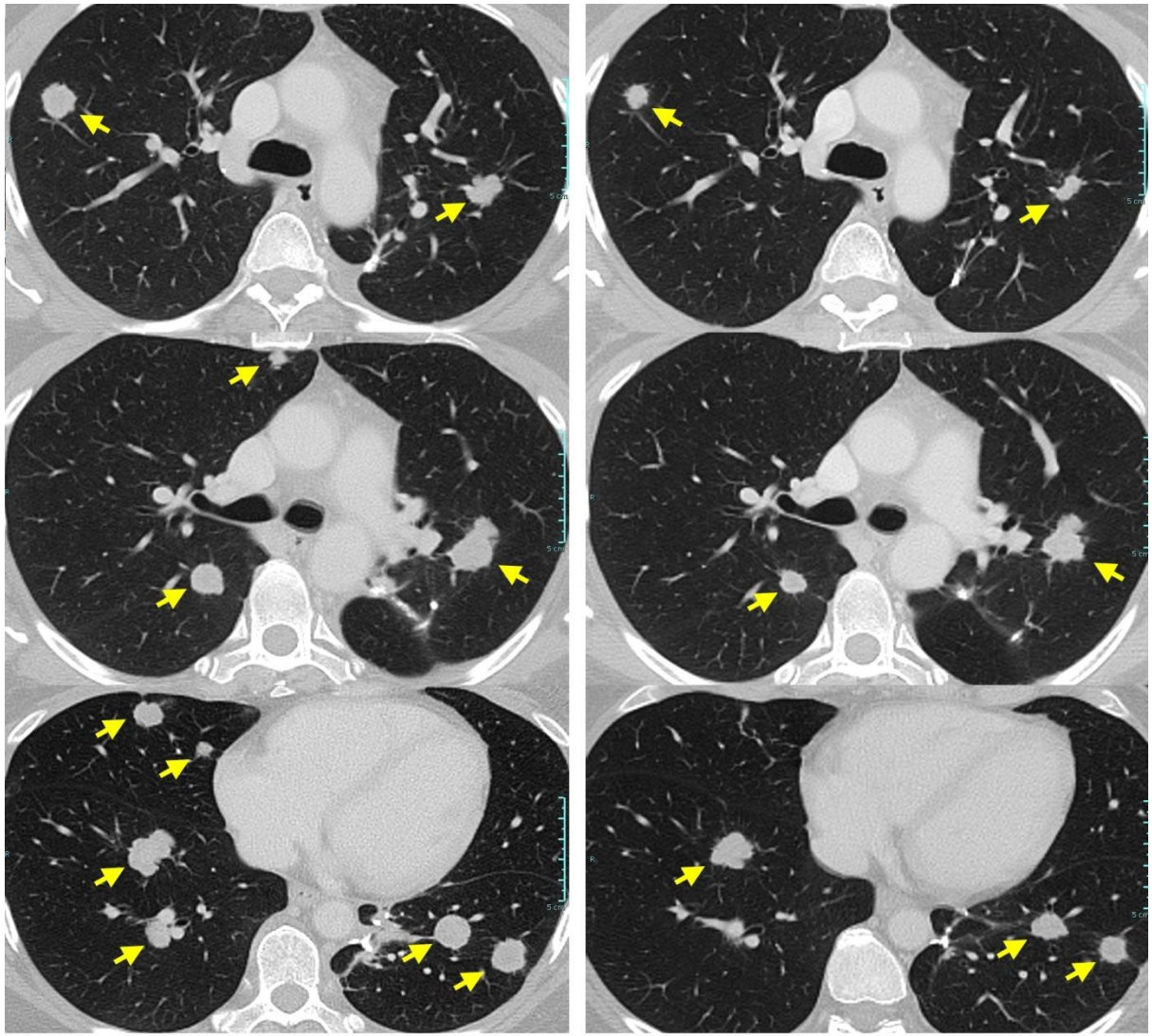


Pre-Treatment



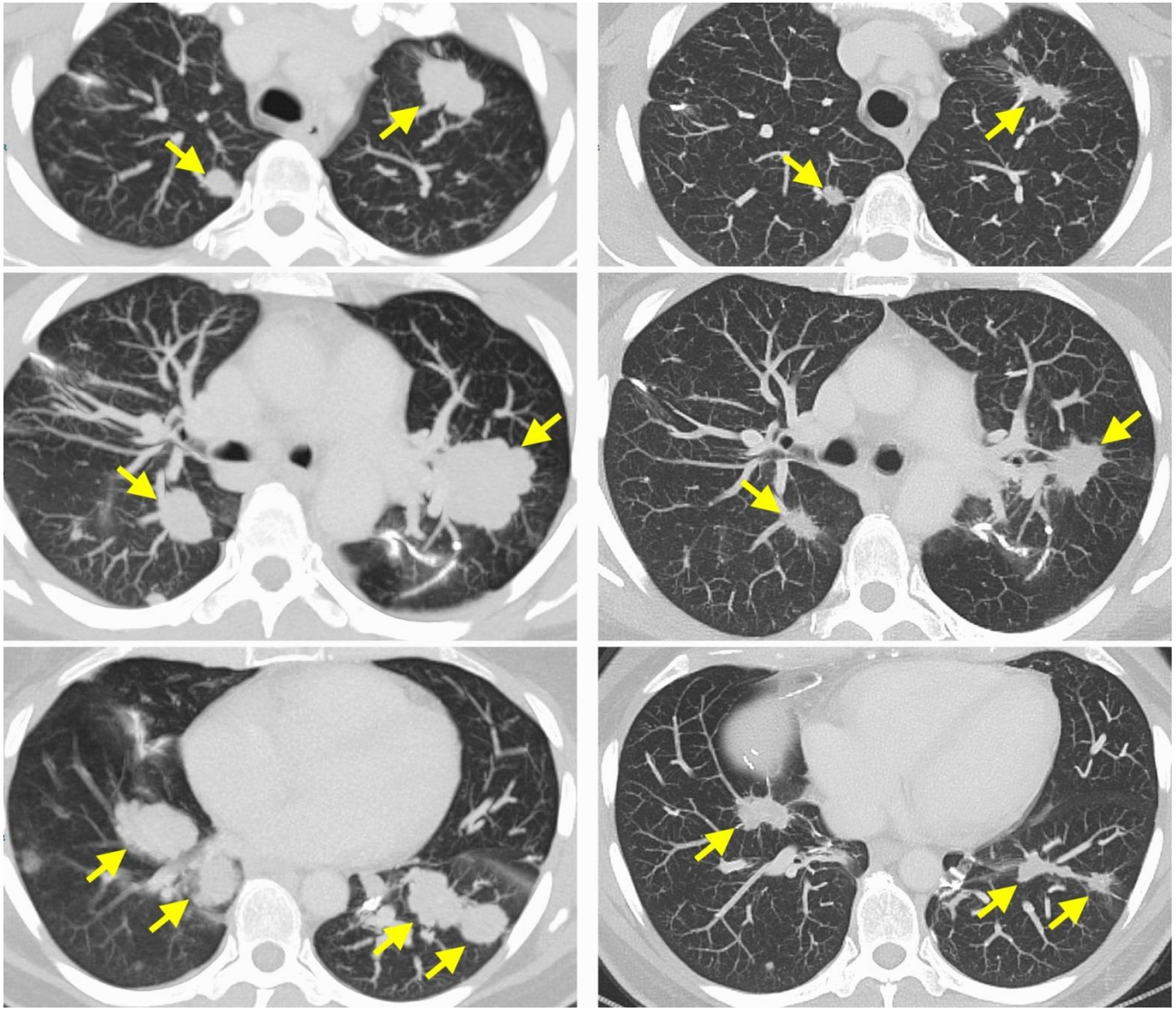
8 months

Treatment #1: Lungs



Pre-Treatment 7 Months

Treatment #2: Lungs



TIL From Colon Cancer

- **Metastatic colon cancer (liver, lung, spleen, peritoneum)**
- **Prior Tx: FOLFOX + bevacizumab, FOLFIRI, FOLFOX + panitumumab, RFA, regorafenib**
- **Whole genome sequencing of liver lesion: 119 non-synonymous mutations**

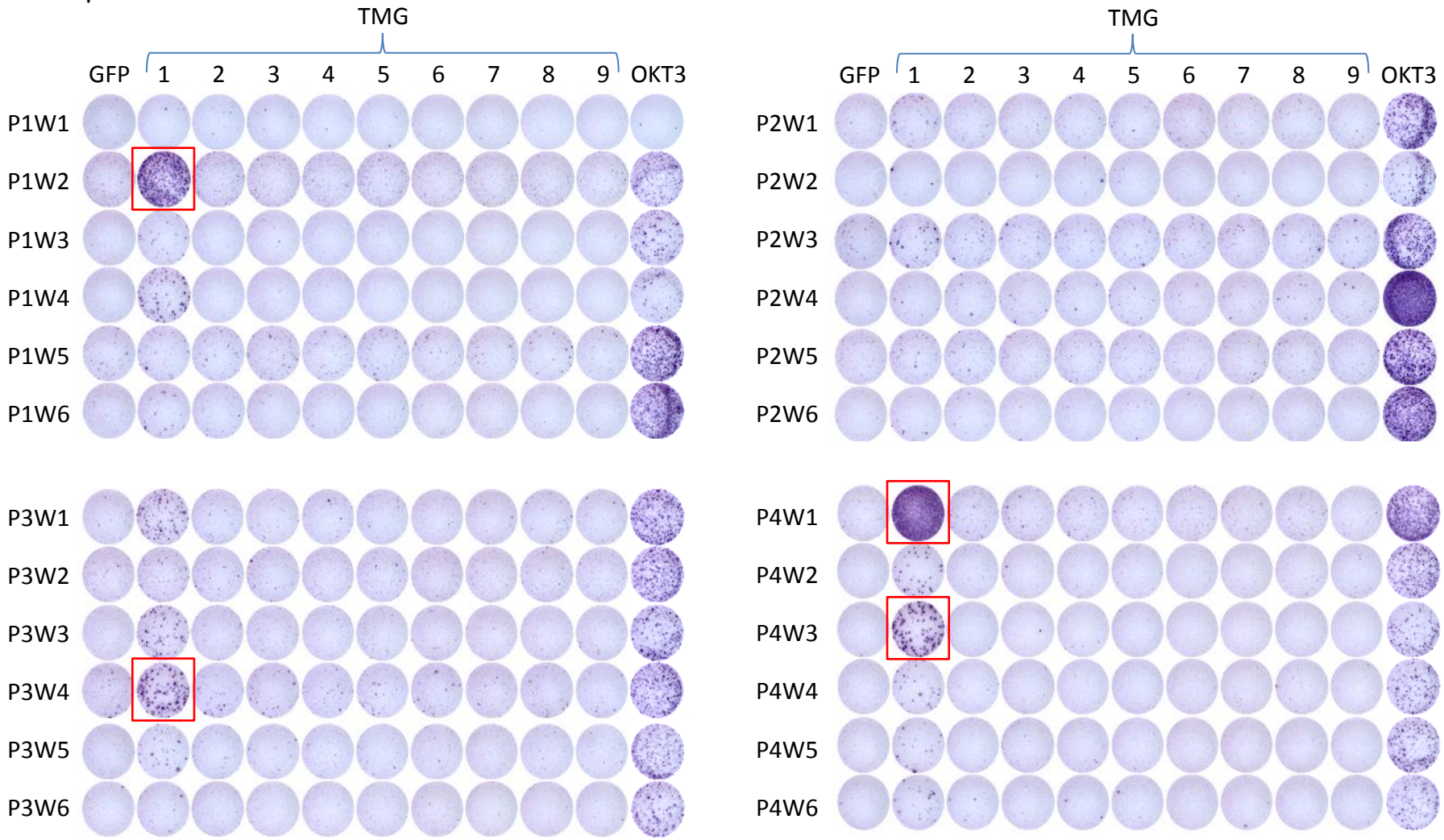
1. **24 fragment cultures of TIL grown**
2. **Nine TMGs synthesized encoding mutations**

TMG	# minigenes
1	16
2	16
3	16
4	15
5	16
6	15
7	15
8	3
9	7

Multiple TIL Cultures Display Reactivity Against TMG-1

Co-culture of TIL fragments with RNA transfected DCs

IFN-γ ELISPOT



Eric Tran

Summary

- Mutation-reactive T cells can be detected in patients with metastatic gastrointestinal cancers

Patient	Cancer	# of mutations assessed	Mutation Reactive T cells detected?	Mutated gene recognized	T cell	Notes
(3737)	Cholangio	26	Y	ERBB2IP	CD4	Multiple mutation-reactive CD4 T-cell clones
(3942)	Rectal	140	Y	NUP98 KARS GPD2	CD8 CD8 CD4	
(3971)	Colon	119	Y	CASP8	CD8	
(3978)	Cholangio	37	N	N/A	N/A	Lowering mutation call threshold
(4007)	Colon	265	Y	TBD	CD8	Potentially two reactivities

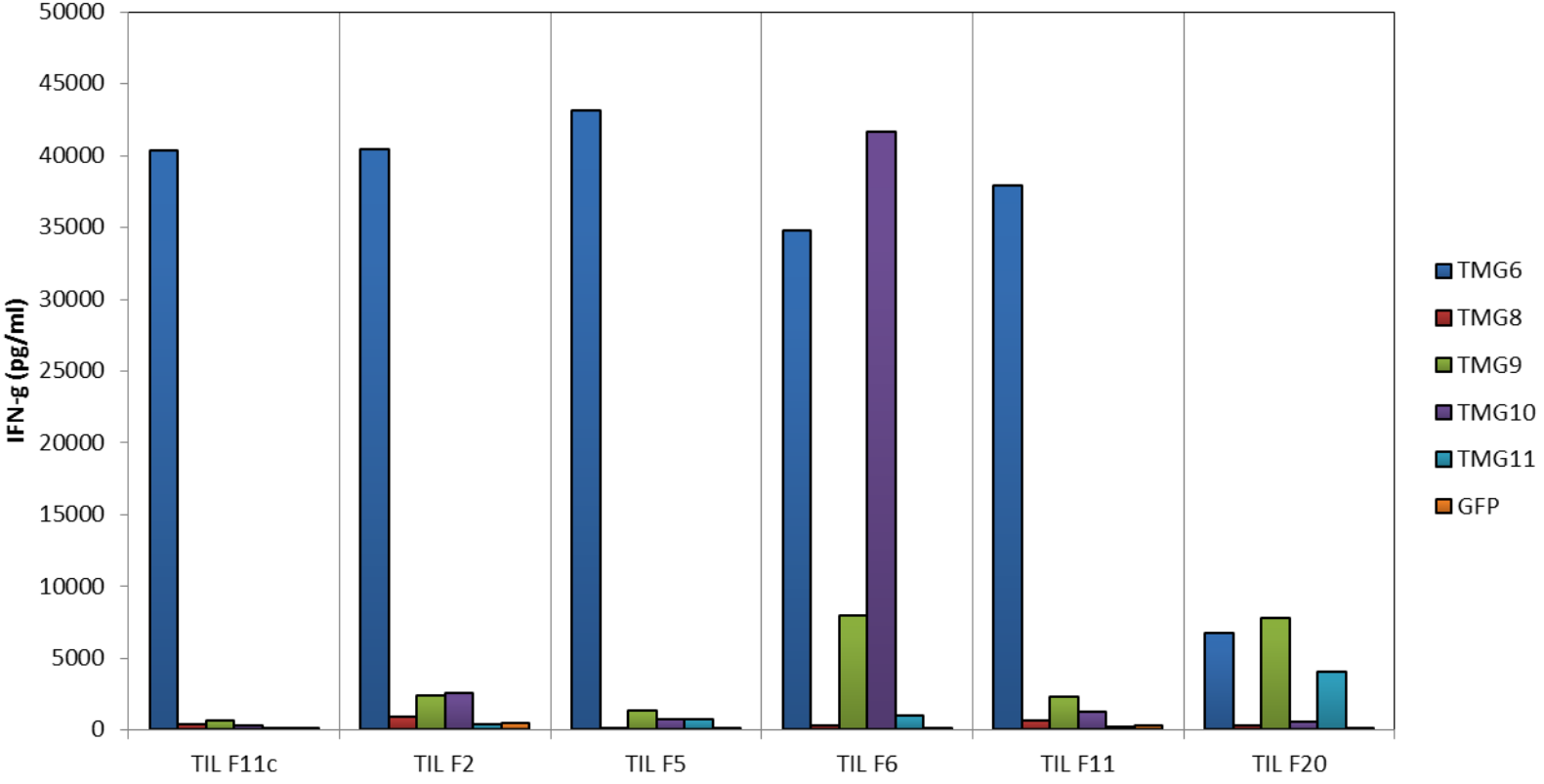
Number of mutated T cell antigens identified in patients with Melanoma by whole exome sequencing

Patient	Tumor type	ID	# of mut. antigens identified	# nonsynon mutations
DA	mel	3713	10	5549
TQ	mel	3466	5	4477
MB	mel	3919	5	1252
DS	mel	2369	4	740
BC	mel	2098	3	448
DD	mel	3903	2	499
KT	mel	2556	2	908
RC	mel	3309	2	1674
AH	mel	2224	2	1812
RJ	mel	2359	1	299
RR	mel	3868	1	1167
SN	mel	3703	1	540
DW	mel	2591	1	379
IG	mel	3926	0	411
KA	mel	3702	0	574
EW	mel	2133	0	1215

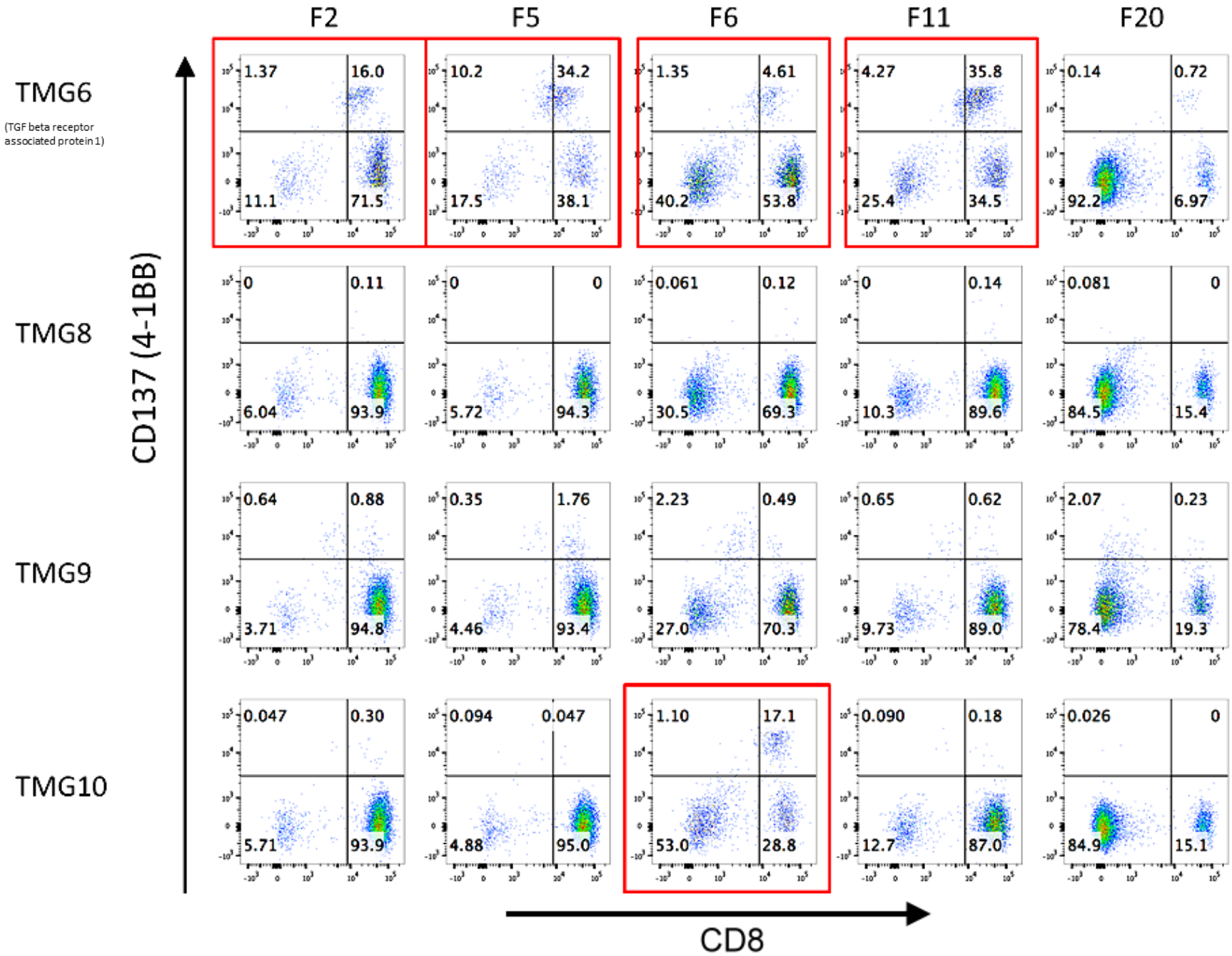
Lung Cancer TIL

- TIL grown from intramuscular metastasis
  - 24 fragment-derived cultures
- WES showed approximately 270 non-synonymous mutations
- 18 TMGs synthesized and expressed in autologous DCs
- TIL cultures vs TMG assay performed

IFN-g Secretion



TIL Fragment Cultures


F2
F5
F6
F11
F20
TMG6
(TGF beta receptor associated protein 1)
TMG8
TMG9
TMG10
CD137 (4-1BB)
CD8
1.37
16.0
11.1
71.5
10.2
34.2
17.5
38.1
1.35
4.61
40.2
53.8
4.27
35.8
25.4
34.5
0.14
0.72
92.2
6.97
0
0.11
6.04
93.9
0
0
5.72
94.3
0.061
0.12
30.5
69.3
0
0.14
10.3
89.6
0.081
0
84.5
15.4
0.64
0.88
3.71
94.8
0.35
1.76
4.46
93.4
2.23
0.49
27.0
70.3
0.65
0.62
9.73
89.0
2.07
0.23
78.4
19.3
0.047
0.30
5.71
93.9
0.094
0.047
4.88
95.0
1.10
17.1
53.0
28.8
0.090
0.18
12.7
87.0
0.026
0
84.9
15.1

Isolation of Reactive T-Cells by Sorting for Activation Markers

- 4-1BB (CD137) is a short-lived marker for recent T-cell activation
- Separation of fresh TIL by 4-1BB sorting can enrich for tumor reactivity

  (Gros, J Clin Invest 2014)

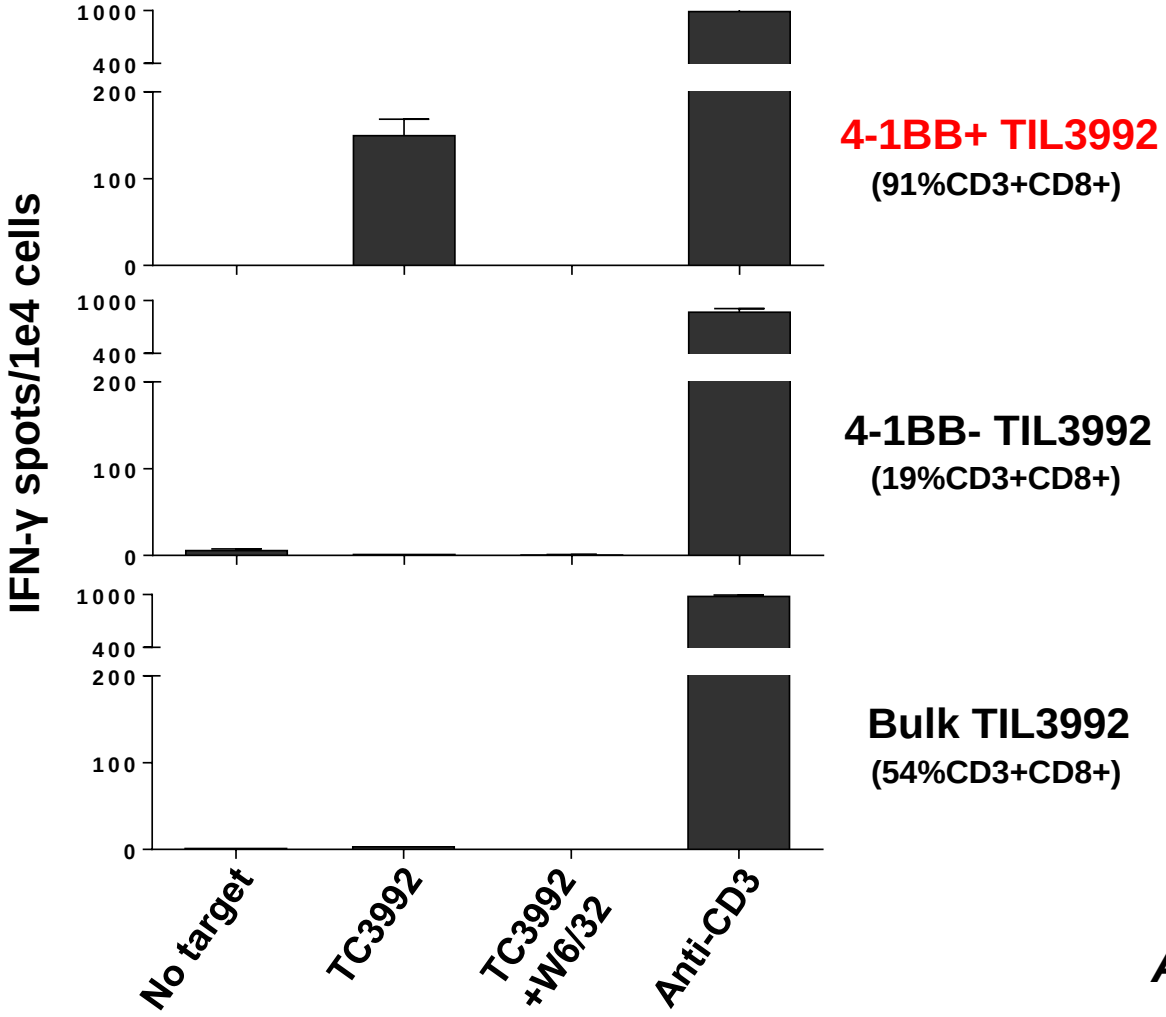
- FACS for clinical use have become available

4-1BB+ TIL3992 appear to be enriched in tumor-reactive cells

Coculture *(TIL3992)*

1e4 effectors:1e5 Target cells

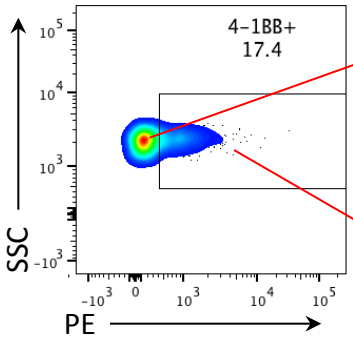
Target = Established Tumor Line 3992



A. Gros

Ongoing clinical study evaluating the efficacy of 4-1BB+ selected TIL in melanoma patients

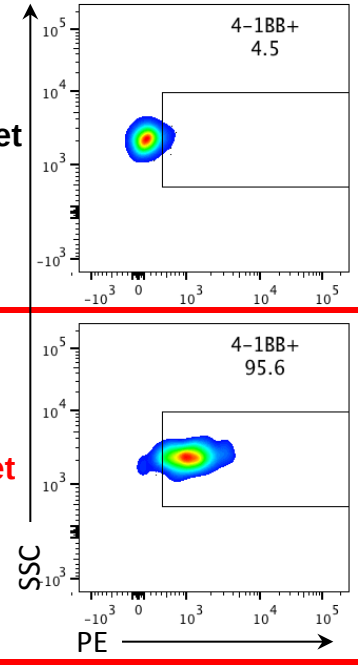
FrTu#4011 labeled
4-1BB-PE



4-1BB neg subset

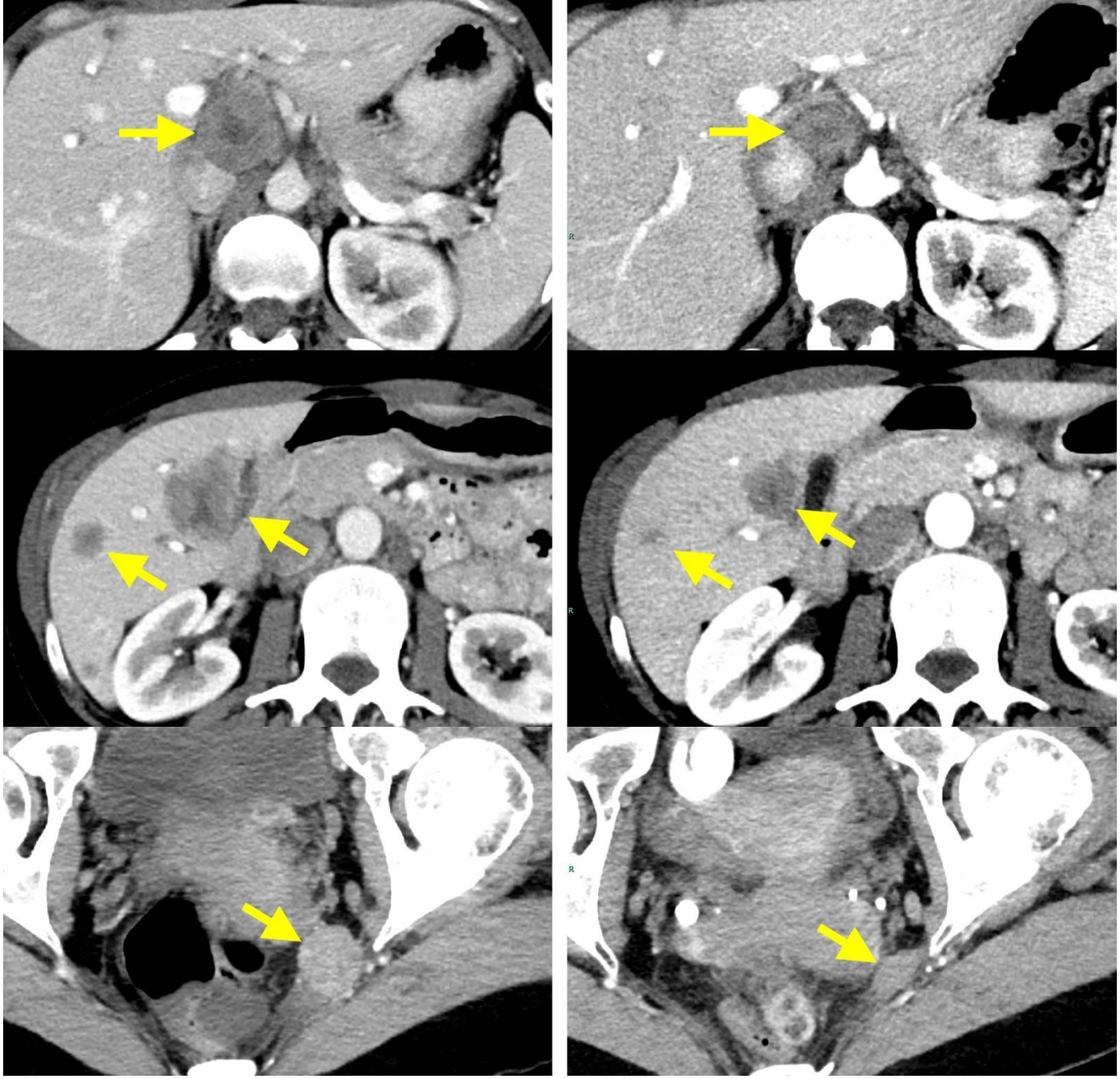
4-1BB pos subset

Post-sort purity

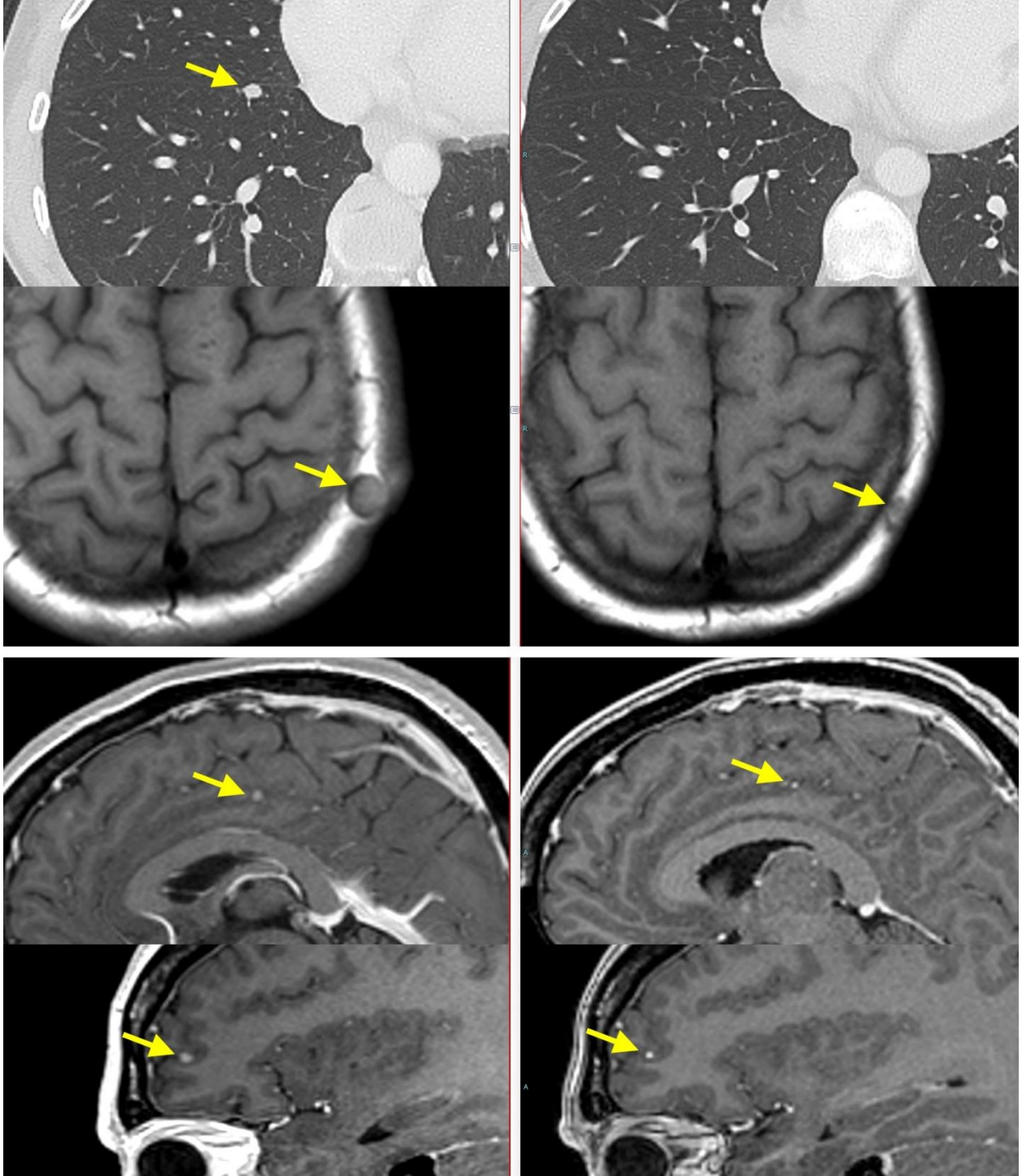


Total FrTu cells sorted	4-1BB+ cell yield	Viability Post-sort
~7e6 cells	2e5 cells	95.3%

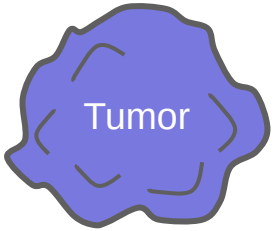
→ Rapid expansion protocol (15 days) → 13e9 infused cells



Pre-Treatment **2 Months**



Schematic for Rapid Generation of Mutation-Specific TIL by Cell Sorting

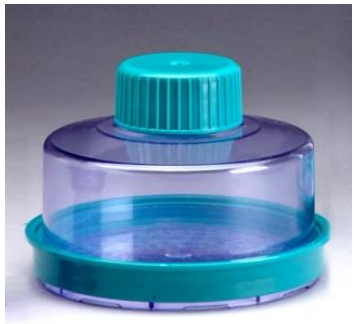


(WES & TMGs)

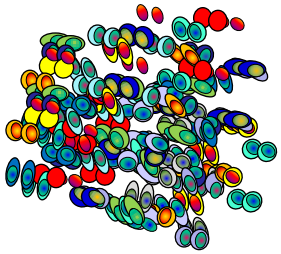
•Establish initial TIL culture from fragments (2-4 weeks)

ELISA vs TMG to detect reactive wells

Rapid ExPansion with anti-CD3 and feeder cells



2 Weeks



Bulk TIL Culture

Co-culture with TMG-DC

Sort 4-1BB+ T-cells

Cell Infusion

Conclusions

- T-cell transfers can cure some patients of widespread metastatic melanoma
- Peptide and minigene approaches have both shown that many of these tumor-reactive TIL are recognizing tumor-specific mutations
- This finding may allow not only improvements in melanoma T-cell therapy, but may lead to adoptive cell therapy for nearly any cancer
- New TIL protocols, selecting for mutation reactivity, are open for lung, ovarian, breast, bladder and GI cancers

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