Adoptive Cell Transfer (ACT)

John Haanen

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Aim of this presentation

- Basic aspects of ACT
- How does it work and how well does it work?
- Is there still a place for ACT in the era of checkpoint inhibitors?

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What is ACT?

- Infusion of an immune cell product with the aim to induce or augment an anti-tumor immune response

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Which cells are transferred?

- Mostly CD3+ T cells
  - Tumor-infiltrating lymphocytes
  - Antigen-receptor gene modified T cells (blood derived)
    - TCR gene modified T cells
    - Chimeric antigen receptor (CAR) gene modified T cells
  - T cell clones/lines (oligoclonal population) from blood
- Other cell types: NK cells, DC

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How does ACT work?

How effective is ACT?

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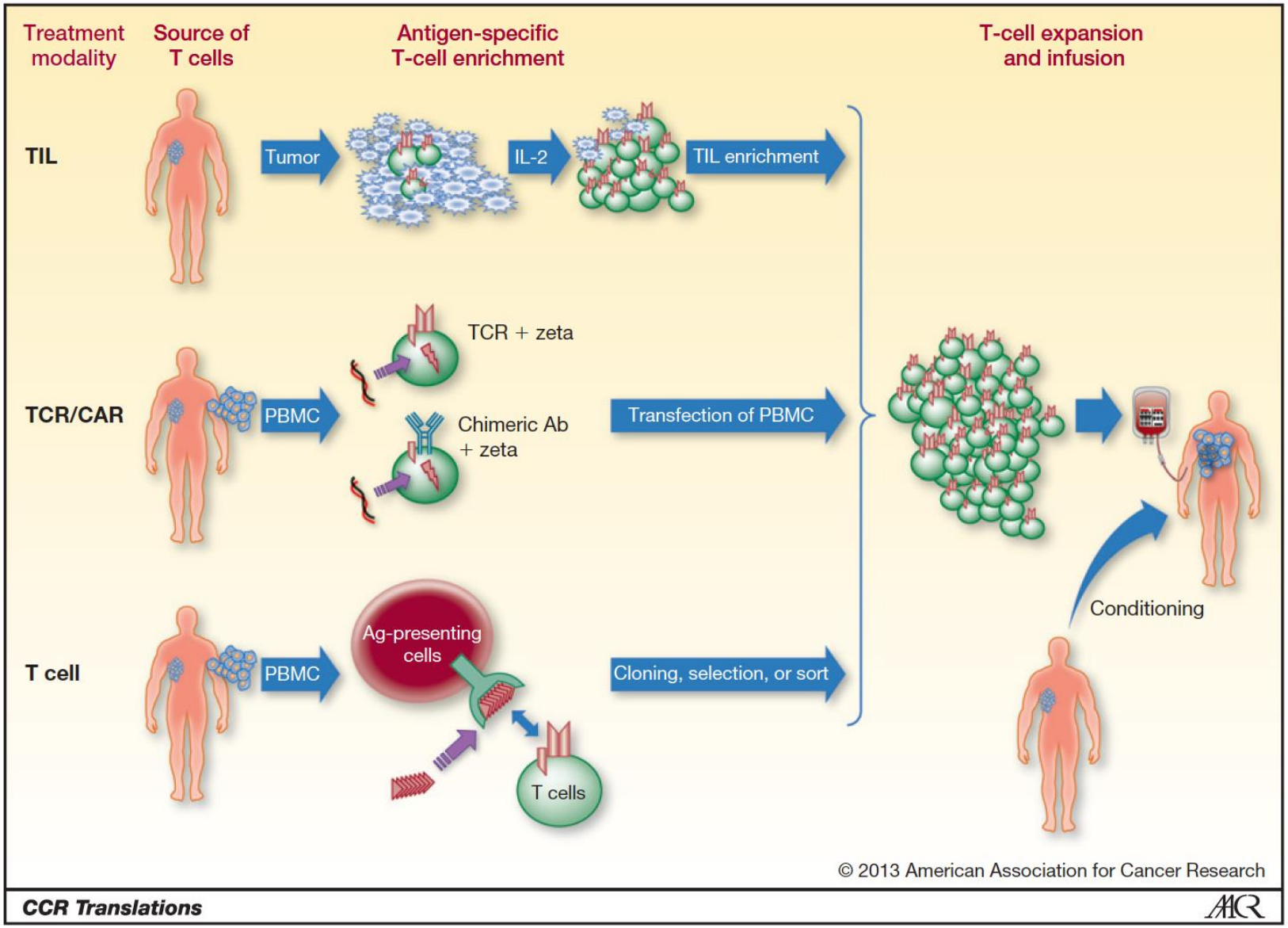
ACT with CD3+ T lymphocytes



Yee C Clin Cancer Res 2013

How effective is ACT?

- Infusion of peripheral blood derived T cells
- Infusion of TCR gene modified T cells
- Infusion of TIL

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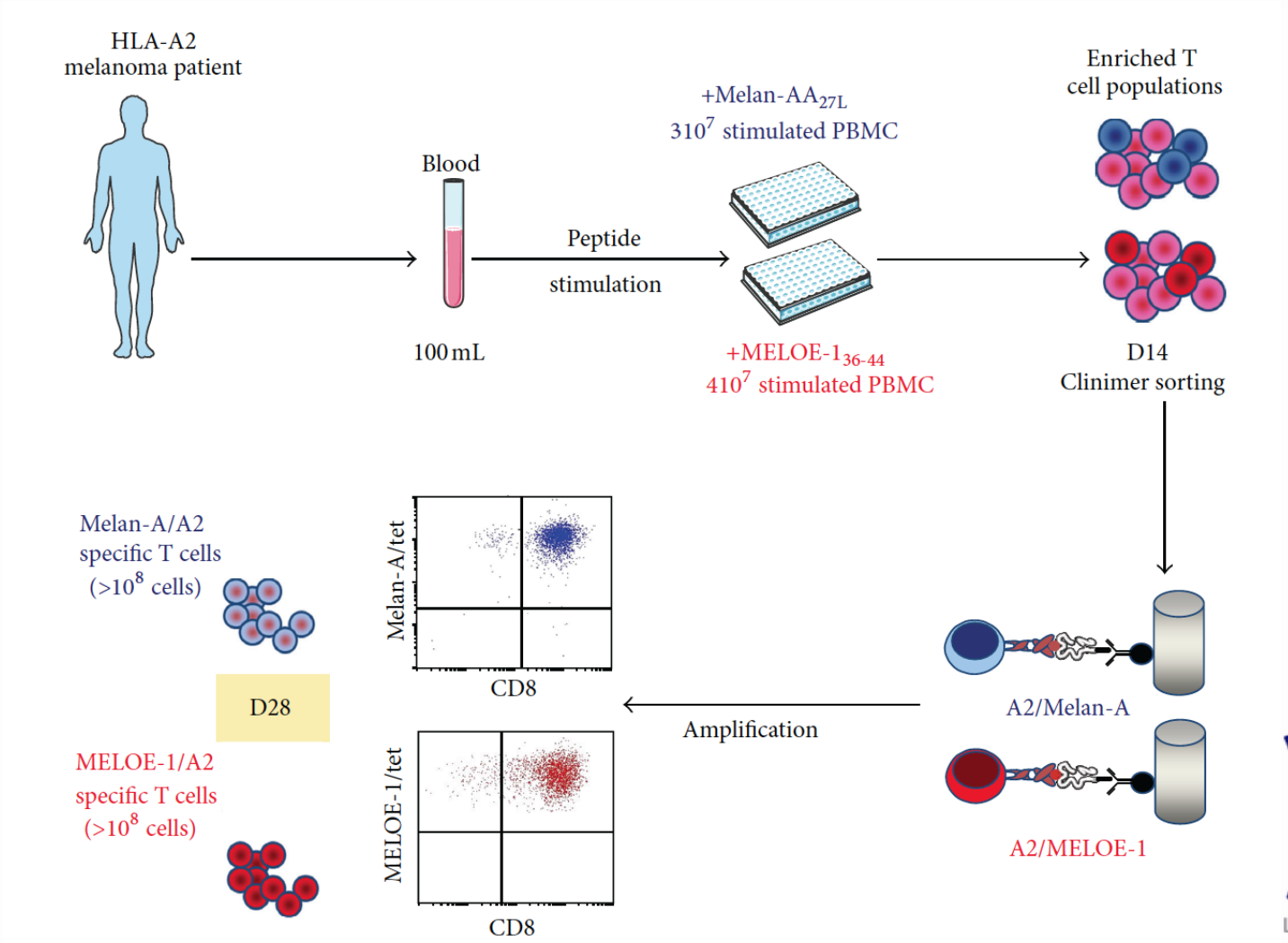
Isolation of melamoma-specific CD8 T cells from peripheral blood



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Labarriere et al. Clin Dev Immunol 2013

Infusion of MART-1 specific T cells

Table 1. Patient Demographics and Treatment Characteristics

Patient No.	Age (years)	Sex	KPS (%)	Prior Therapy	Disease Sites	Melan-A Expression*	No. of T-Cell Infusions	Adverse Effects	Eosinophilia (%)†	Clinical Course	Duration of Clinical Course (months)
1	50	F	80	Chemo/immuno	Sk/Lu	3+	3	Fever I°	13	PD	
2	50	M	80	Chemo/immuno	Lu/Li/B/Sk	2+	3	Fever I°	6	PD	
3	39	F	100	Chemo/immuno	Lu/Li/B	3+	3	Fever I°	12	PD	
4	35	M	100	Chemo/immuno	Lu/LN	3+	3	No	No	PD	
5	62	M	100	Chemo/immuno	LN	2+	10	Fever II°, chills	51	PR	11
6	60	M	90	Chemo/immuno	LN	3+	5	Fever II°, chills	23	SD	3
7	58	F	90	Chemo/immuno	LN/Sk	2+	3	No	No	PD	
8	63	M	80	IFN/chemo	Sk	3+	7	Fever II°, chills	26	MR	14
9	41	F	100	IFN/chemo	Li/LN	3+	3	No	No	PD	
10	68	F	90	Chemo/immuno	LN/Sk	3+	3	No	No	PD	
11	59	F	100	IFN/chemo	LN	3+	9	Fever II°, chills	14	CR	24+

Abbreviations: KPS, Karnofsky performance status; F, female; Chemo, chemotherapy; Immuno, immunotherapy; Sk, skin; Lu, lung; Fever I°, WHO grade I, < 38°C; PD, progressive disease; M, male; Li, liver; B, bone; LN, lymph node; Fever II°, WHO grade II, 38-40°C; PR, partial regression; SD, stable disease; MR, mixed response; IFN, interferon; CR, complete regression.

*Staining of tumor specimens was performed with an anti-Melan-A (A103; Novocastra, Newcastle, United Kingdom) monoclonal antibody; 2+, 50-75% of cells reactive; 3+ > 75% of cells reactive.

†Maximum peak eosinophil levels after T-cell transfer; eosinophils % of total leukocytes.

Mackensen et al. J Clin Oncol 2006

Infusion of MART-1 and gp100-specific T cell clones

Table 1. Patient demographics and clinical summary

			Pretreatment status			No. of infusions			Response	
ID no.	Age	Sex	Previous Tx*	Disease sites†	Target antigen	No IL-2	+IL-2	Toxicity‡	Type	Duration§
1017-1	45	F	IFN	Skin, LN	MART1	4	3	F, M	Stable disease	21.3
1017-2	46	F	Chemo	Lu, LN, CW	MART1	1	3	F, M, R	Minor¶	2.0
1017-3	50	F	Bio-chemo, IFN	Lu, liver	MART1	1	3	None	Progressive disease	—
1017-4	55	M	IFN	Lu	gp100	1	3	F, M	Stable disease	3.6
1017-5	56	F	Chemo	Skin, LN	MART1	1	2	F, M	Stable disease	15.2
1017-6	53	F	Bio-chemo	Lu	gp100	1	3	F, M	Mixed‖	6.8
1017-7	50	M	Bio-chemo	Lu, LN	gp100	1	3	F	Stable disease	14.7
1017-8	47	F	Chemo, IFN	Lu, BrPl	MART1	1	3	F, M	Minor**	15.3
1017-9	59	M	Bio-chemo	Lu, liver	gp100	1	5	F, M	Stable disease	7.0
1017-10	38	F	Bio-chemo	Lu, liver	gp100	1	2	F	Progressive disease	—
	49.9					13	30			10.9

Yee et al. PNAS 2002

PNAS

Transferred melanoma-specific CD8$^+$ T cells persist, mediate tumor regression, and acquire central memory phenotype

Aude G. Chapuis[a], John A. Thompson[b], Kim A. Margolin[b], Rebecca Rodmyre[a], Ivy P. Lai[a], Kaye Dowdy[a], Erik A. Farrar[a], Shailender Bhatia[b], Daniel E. Sabath[c], Jianhong Cao[a], Yongqing Li[a], and Cassian Yee[a,1]

[a]Program in Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; [b]General Oncology and Hematology, Seattle Cancer Care Alliance and University of Washington, Seattle, WA 98109; and [c]Department of Laboratory Medicine, University of Washington, Seattle, WA 98195

Edited by Tak W. Mak, The Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute at Princess Margaret Hospital, University Health Network, Toronto, ON, Canada, and approved February 3, 2012 (received for review August 30, 2011)

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Cancer Immunol Immunother (2011) 60:953–963
DOI 10.1007/s00262-011-1004-8

ORIGINAL ARTICLE

Successful treatment of metastatic melanoma by adoptive transfer of blood-derived polyclonal tumor-specific CD4+ and CD8+ T cells in combination with low-dose interferon-alpha

Els M. E. Verdegaal · Marten Visser · Tamara H. Ramwadhdoebé · Caroline E. van der Minne · Jeanne A. Q. M. J. van Steijn · Ellen Kapiteijn · John B. A. G. Haanen · Sjoerd H. van der Burg · Johan W. R. Nortier · Susanne Osanto

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Treatment of Metastatic Melanoma with Autologous CD4+ T Cells against NY-ESO-1

Naomi N. Hunder, M.D., Herschel Wallen, M.D., Jianhong Cao, Ph.D., Deborah W. Hendricks, B.Sc., John Z. Reilly, B.Sc., Rebecca Rodmyre, B.Sc., Achim Jungbluth, M.D., Sacha Gnjatic, Ph.D., John A. Thompson, M.D., and Cassian Yee, M.D.

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Conclusion

- Infusion of peripheral blood derived melanoma-specific T cells is feasible
- Time consuming (4-16 weeks)
- Few but sometimes lasting responses are seen
- How to improve?
  - Are we targeting the right antigens?
  - Are we infusing the right T cells?
  - Combination therapy?

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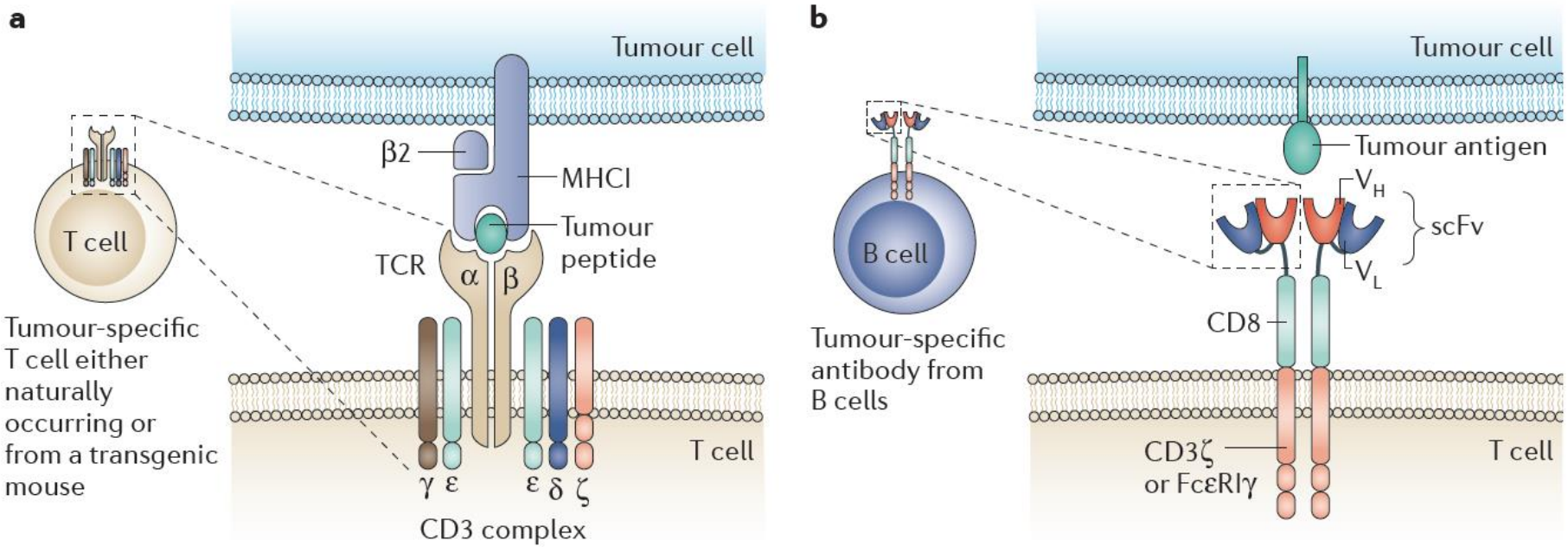
Infusion of gene-modified T cells



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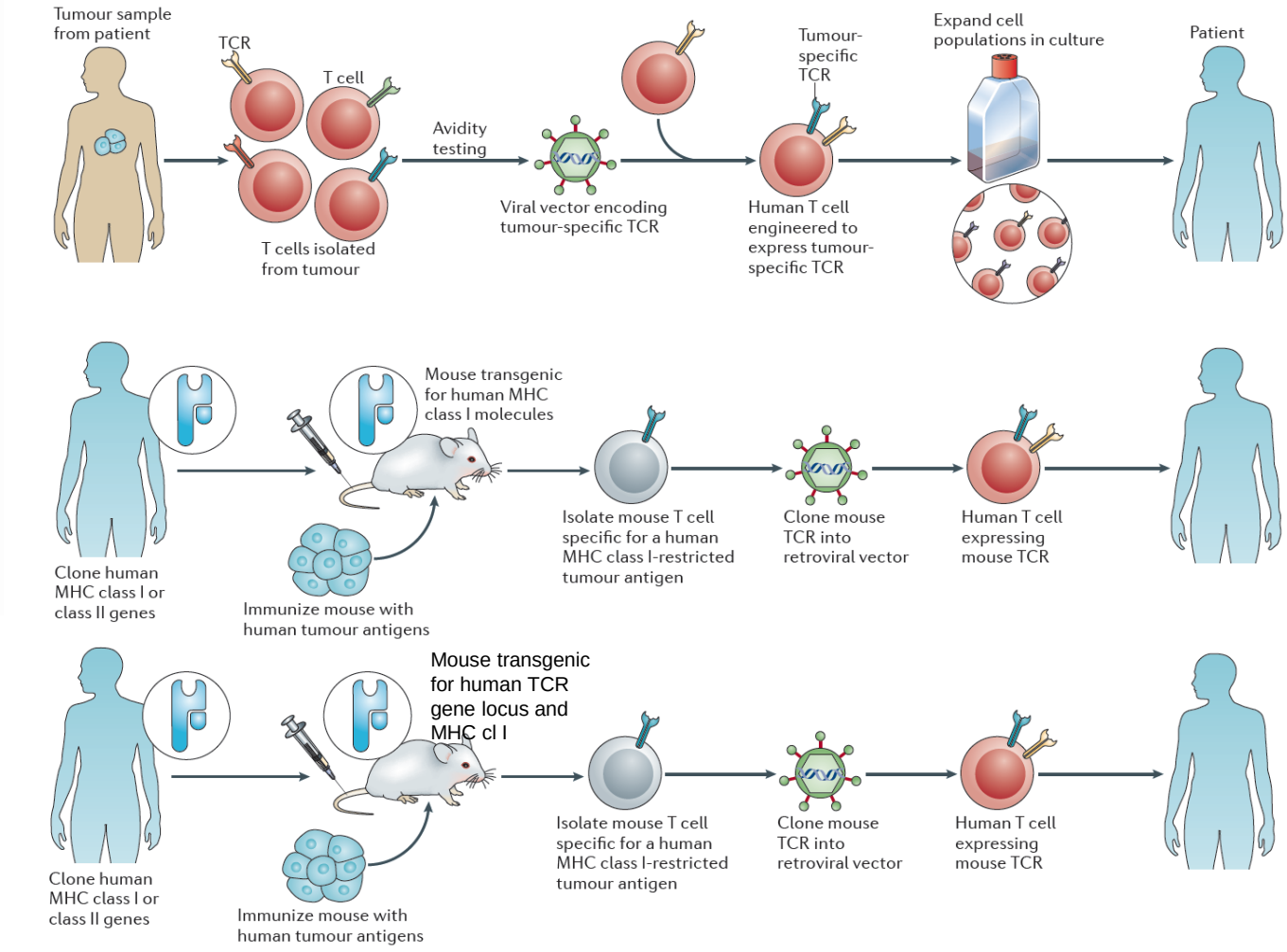
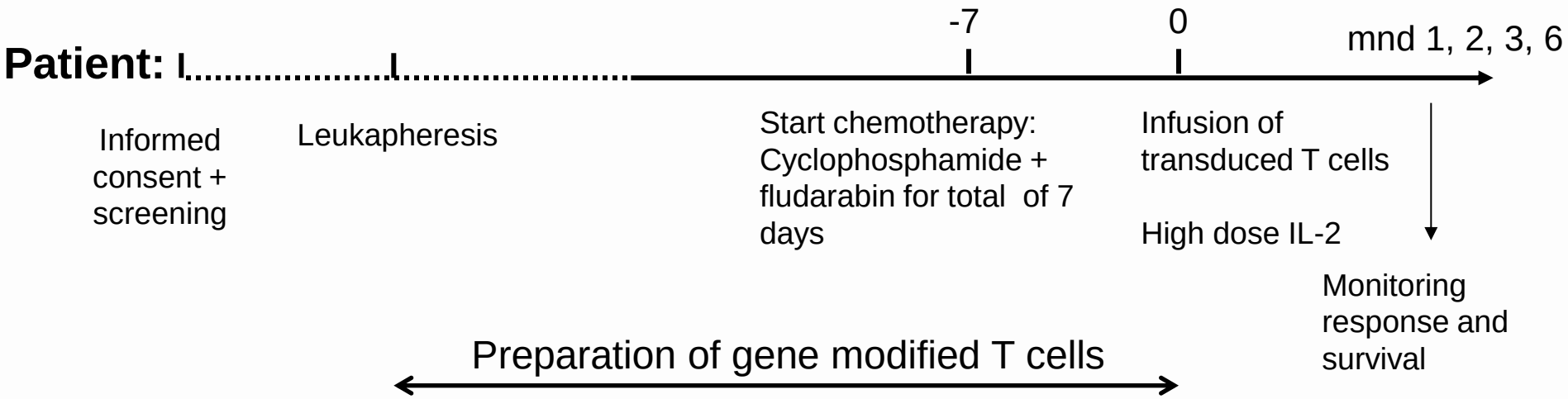
Kershaw et al. Nat Rev Cancer 2013

Genetically modified peripheral blood lymphocytes



Modified from: Restifo et al., Nature Rev Immunol (2012)

Schedule of treatment



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Clinical experience with TCR gene therapy

- 2006: MART-1 TCR gene therapy
  - RR 13% (n=15)

  (Morgan et al., Science 2006)
- 2009: MART-1 and gp100 TCR gene therapy
  - RR 30% (MART-1 TCR; n=20)
  - RR 19% (murine gp100 TCR; n=16)

  (Johnson et al., Blood 2009)

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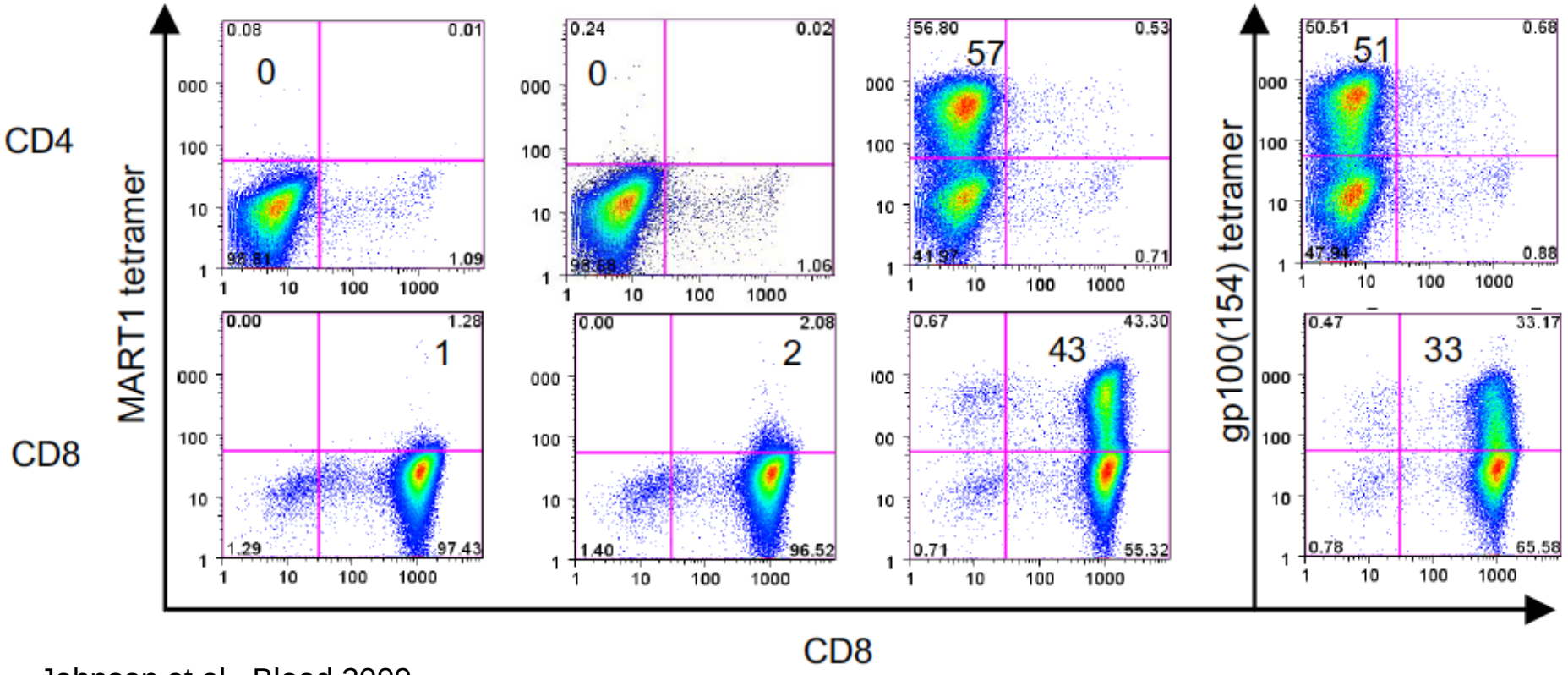
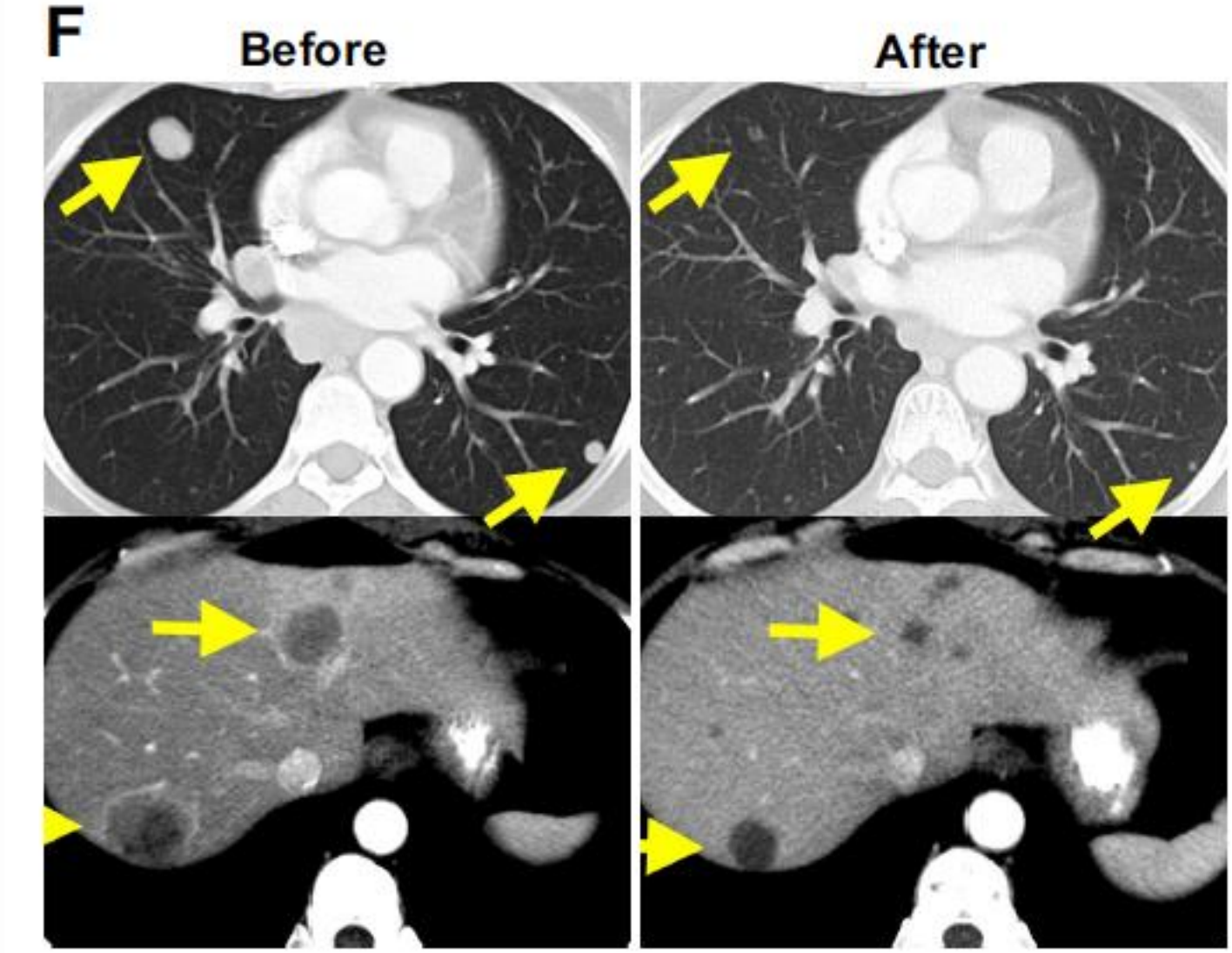
DMF5 and gp100 specific TCR were highly expressed by transduced CD4 and CD8 T cells



Johnson et al., Blood 2009

Clinical activity of MART-1 and gp100-specific TCR gene therapy



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- 2014: MART-1 TCR gene therapy + DC vaccination
  - Response in 11/14 (not according RECIST)
  - SD at 90 days in 50%

  (Chodon et al. Clin Cancer Res 2014)

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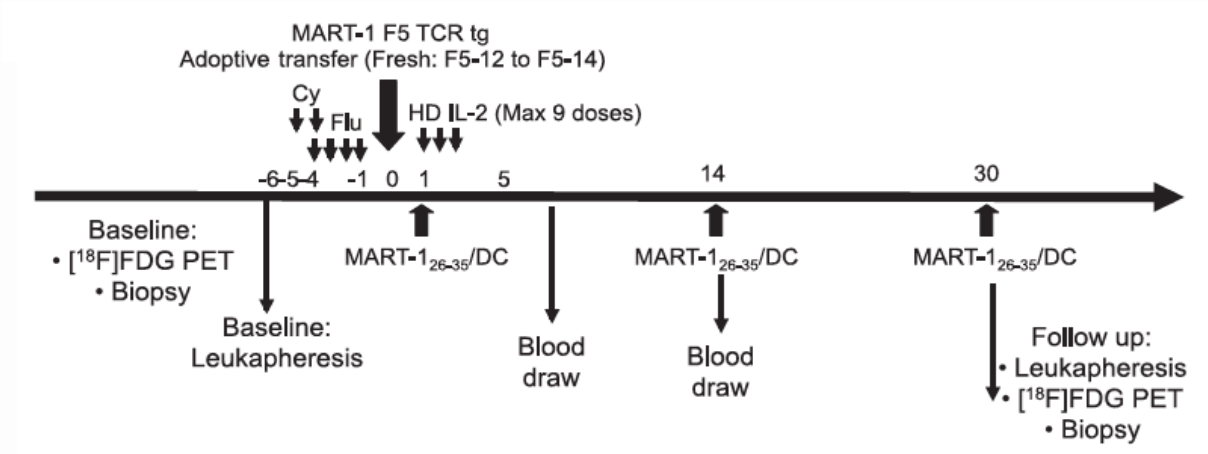
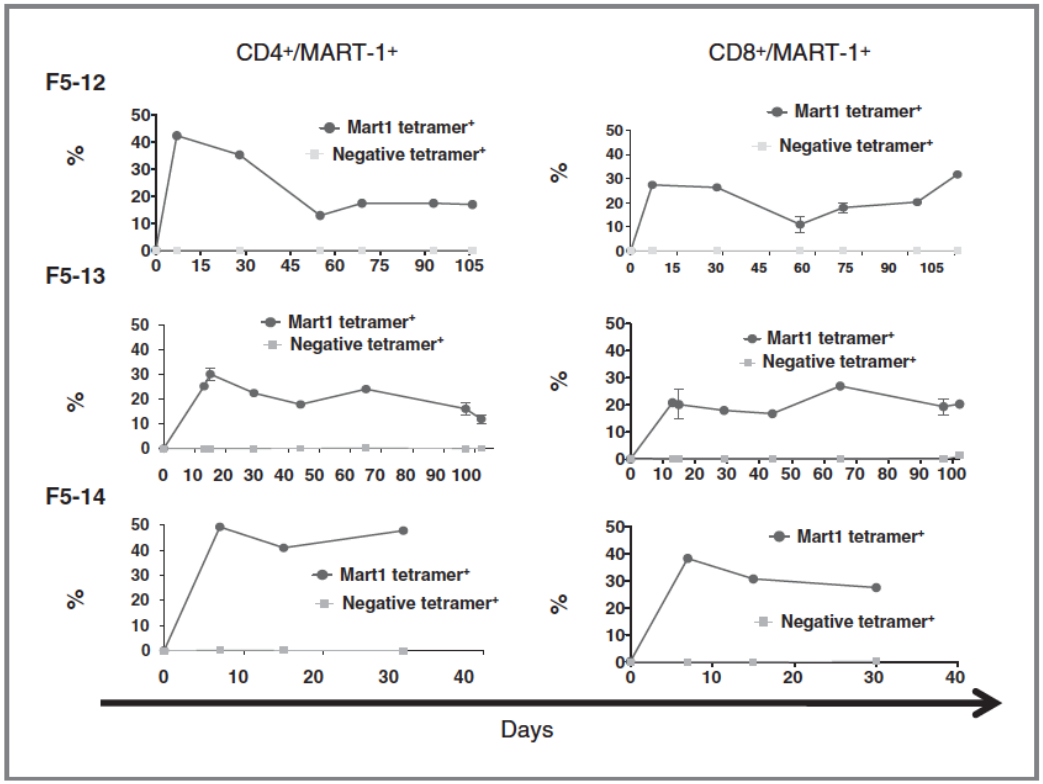
Schedule and persistence of gene modified T cells after infusion





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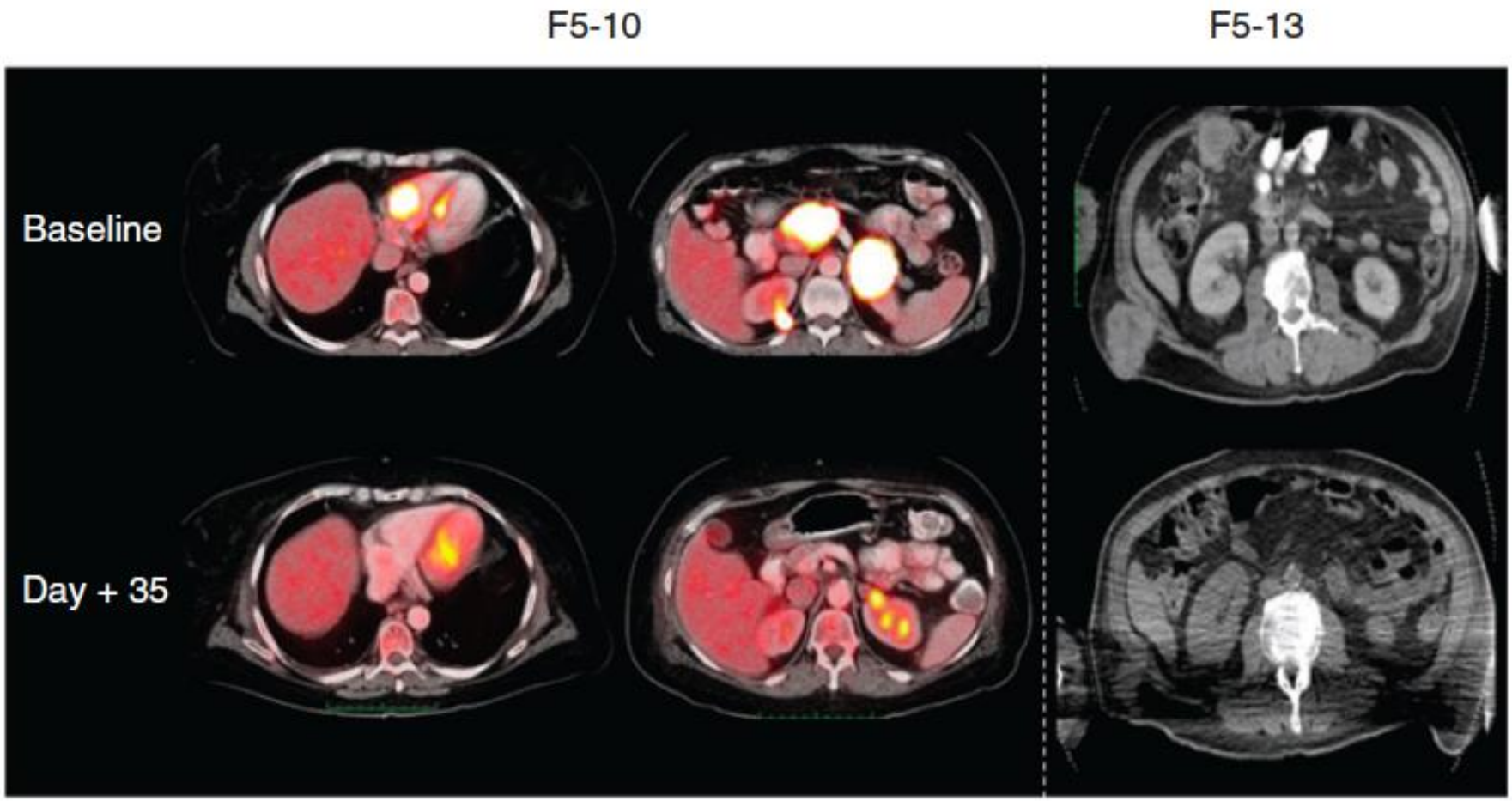
Chodon et al., Clin Cancer Res 2014

Clinical responses upon adoptive T-cell transfer



Chodon et al., Clin Cancer Res 2014

TCR gene therapy for melanoma

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- 2009: MART-1 and gp100 TCR gene therapy
  - RR 30% (MART-1 TCR; n=20)
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  (Johnson et al., Blood 2009)
- 2014: MART-1 TCR gene therapy + DC vaccination
  - Response in 11/14 (not according RECIST)
  - SD at 90 days in 50%

  (Chodon et al. Clin Cancer Res 2014)
- 2012: MART-1 TCR gene therapy (Haanen et al. unpublished)

Clinical experience with TCR gene therapy

- 2006-2014: MART-1 and gp100 TCR gene therapy

- 2011: NY-eso-1 TCR gene therapy in melanoma and synovial sarcoma
  - RR 45% (n=11) and 67% (n=6)

  (Robbins et al., J Clin Oncol 2011)

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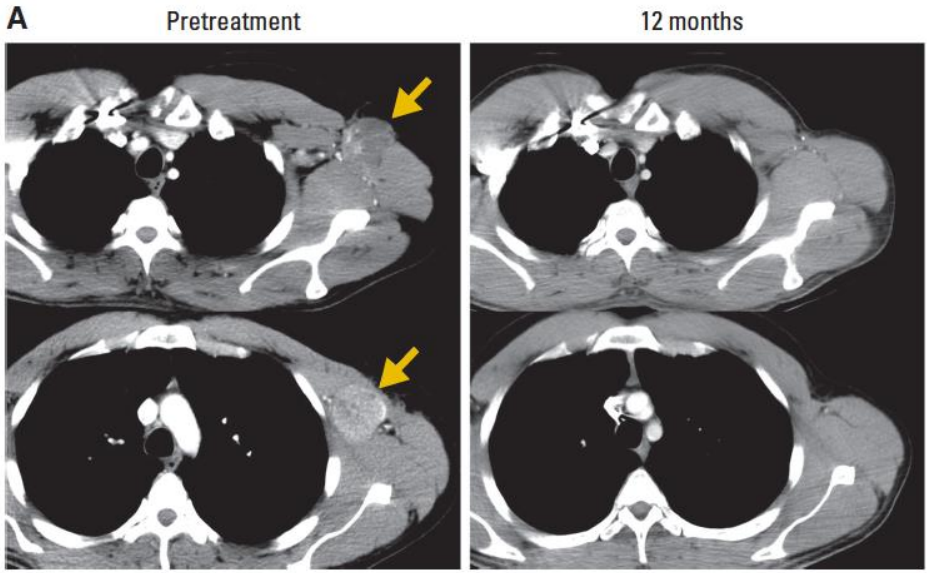
Patient characteristics and outcome

Patient No.	Age (years)	Sex	Sites of Disease	Prior Treatment	No. of Cells ($\times 10^9$)	No. of IL-2 Doses	% of CD3		NY-ESO-1 Tetramer Positive		Vβ13.1 Positive (% of CD3)	Tumor Cell Targets (pg/mL IFN-γ)*		Response†
							CD8	CD4	% of CD8	% of CD4		NY-ESO-1 Positive	NY-ESO-1 Negative	
Melanoma														
1	52	M	ln	R, S, I	130	6	97	2	86	64	94	515	< 30	PR (8)
2	60	F	sc, lu	S, I	71	6	82	17	76	53	90	3,890	< 30	PD
3	30	F	bo, ln, panc, sb	R, S, I	47	1	98	1	80	65	91	11,978	130	PD
4	56	M	lu, ki	R, S, I	50	7	91	9	80	74	94	11,230	< 30	CR (22+)
5	32	M	ln	S, C, I	64	4	98	2	85	76	94	26,019	288	CR (20+)
6	38	M	ln	S, I	51	7	93	7	87	79	94	28,907	536	PR (3)
7	47	M	ln, lu	R, S, I	23	7	96	4	70	58	90	9,577	178	PD
8	39	F	ln, br, lu	R, S, C, I	38	8	68	32	78	70	94	ND	ND	PD
9	51	F	lu, ln, li	S, C, I	31	10	94	6	83	69	96	11,952	35	PD
10	61	M	ln, li, spl, lu, bo	R, S, C, I	16	8	84	16	79	56	92	16,063	49	PD
11	46	M	lu, li	R, S, I	37	6	93	7	63	58	85	5,795	< 30	PR (9+)



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Robbins et al., J Clin Oncol 2011

Conclusion

- Infusion of TCR gene modified T cells is feasible and can result in objective responses
- Infused T cell can persist for months
- Can be very toxic!
- Finding the right target is key
- Is one target enough?
- How to improve?
  - Combination with other IT?

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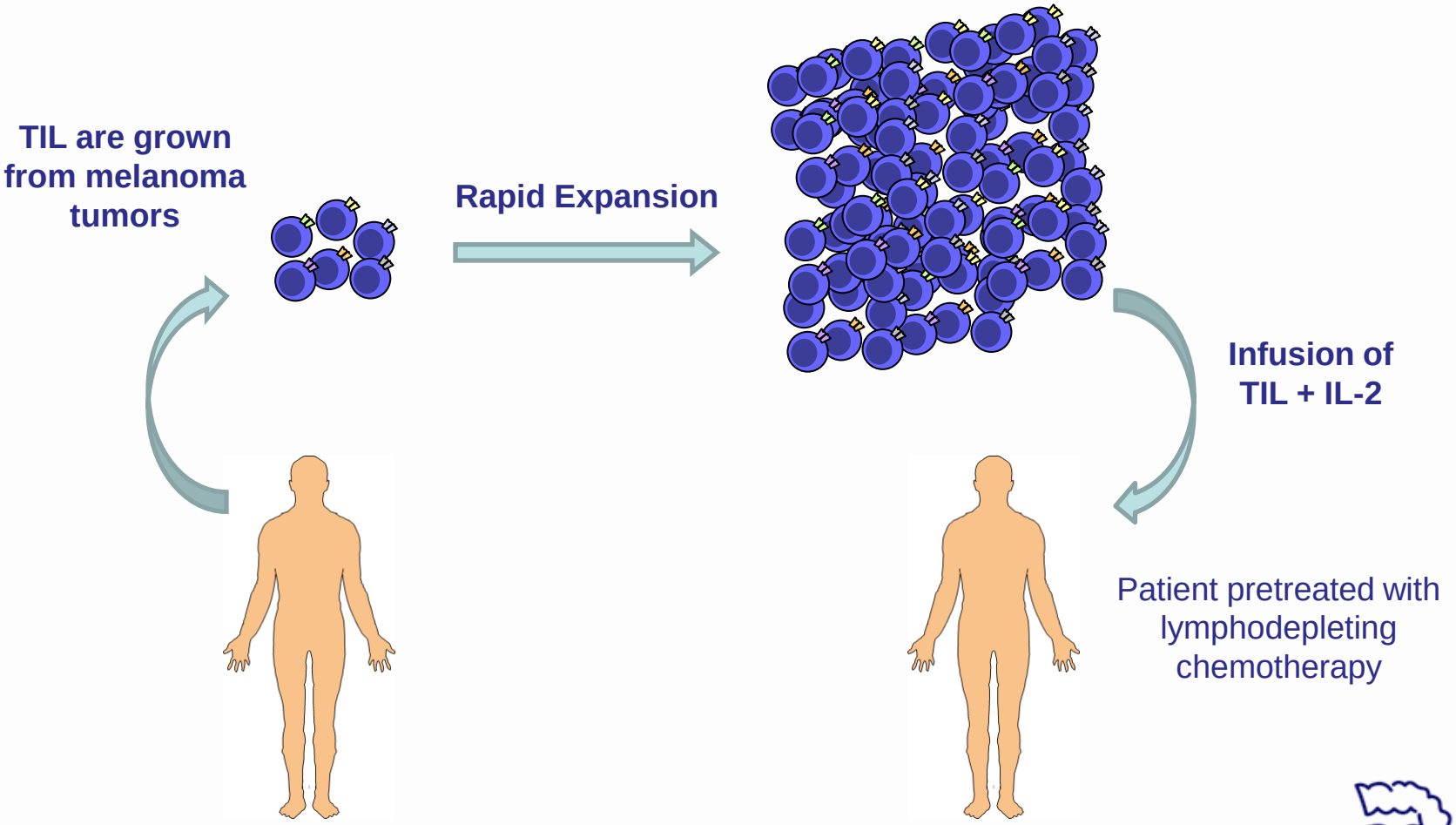
Infusion of tumor Infiltrating Lymphocytes



- High response rate in phase II trials in multiple centers (US, Israel, NL, UK, DK)
- Clinical effect at least partially mediated by CD8 T cells

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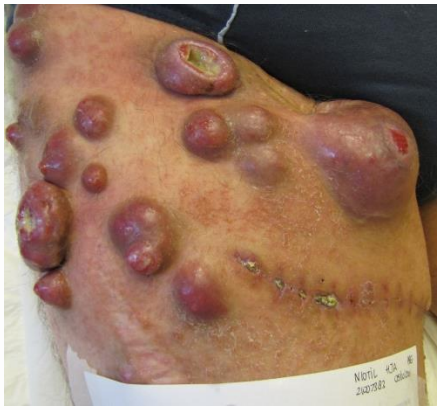
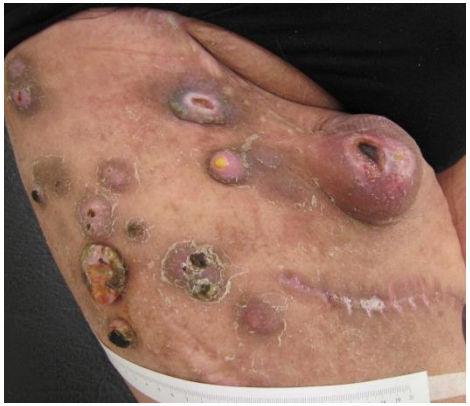
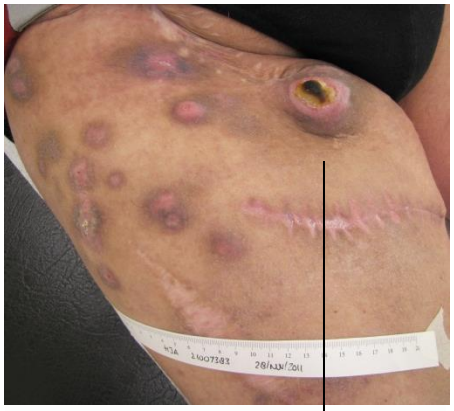
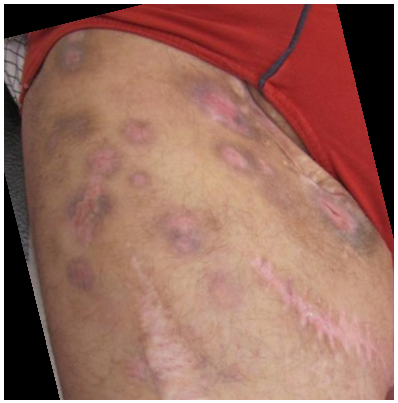
Clinical data N10TIL003: ongoing CR at 24 months

Prior to TIL | 3 wks post TIL | 8 wks post TIL | 12 wks post TIL









Biopsy at wk 7 showed no viable tumor cells

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TIL therapy

- > 300 metastatic melanoma patients have been treated world wide in at least 8 centers
- Objective responses observed in 38-72% of treated patients
- In ITT analysis (n=80): ORR 29%
- Median survival of treated patients: ± 16 m
- Long-term CRs

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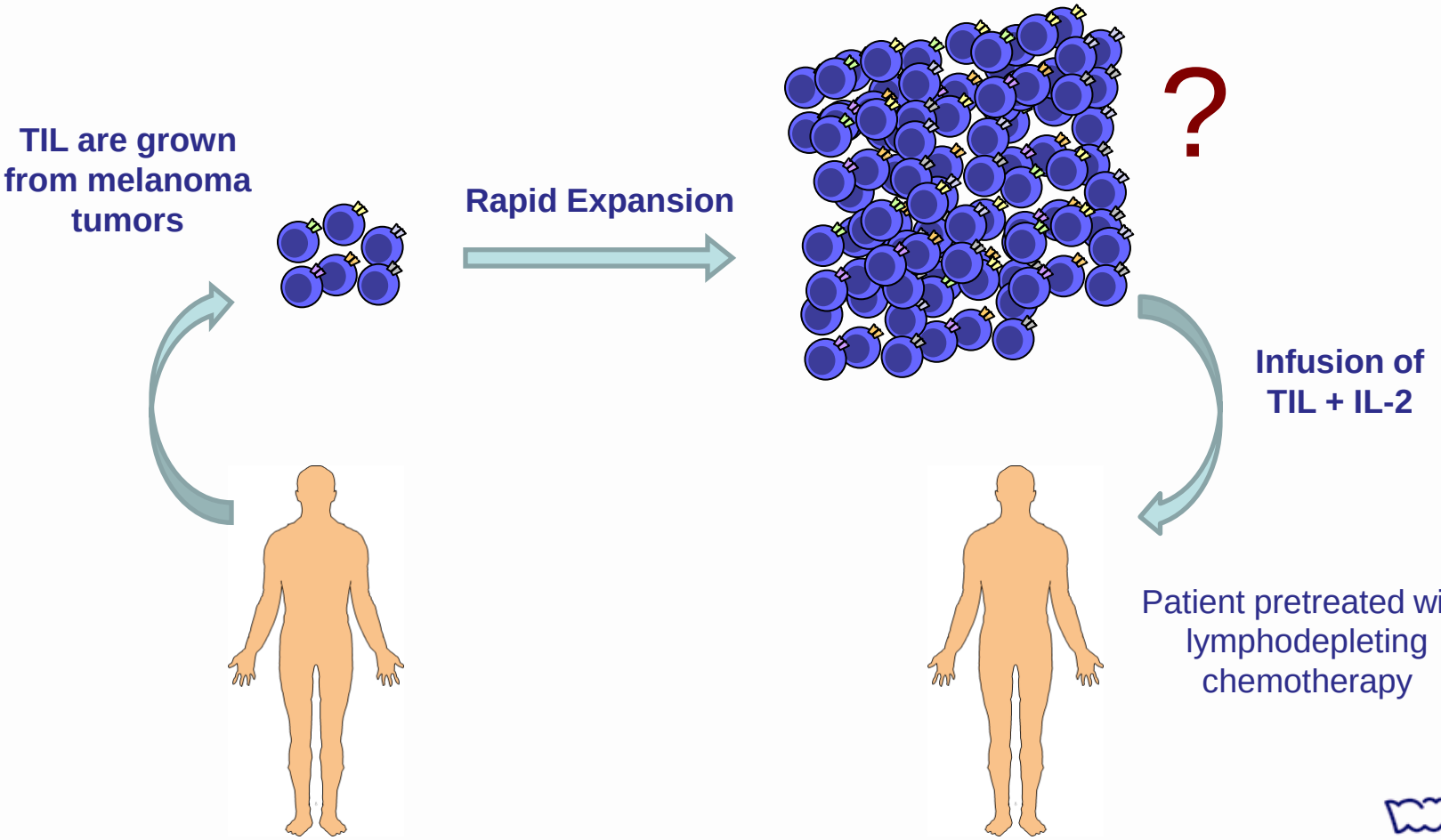
The big unknown



- Which cytotoxic T cells mediate cancer regression?
- Could we specifically boost their numbers?

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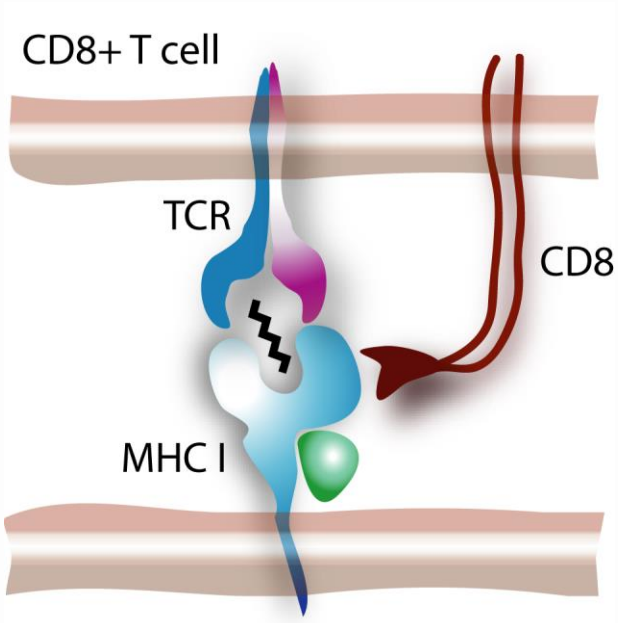
What could tumor-specific cytotoxic T cells detect on human cancer?



1. Self antigens (to which tolerance is incomplete)
   Shared between patients

2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
   In large part patient-specific, hence generally ignored

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TILs against shared tumor antigens

- In the majority of TILs T cells specific for shared antigens can be found
  - Melanocyte differentiation Ags (Mart-1, gp100, etc)
  - Cancer/Testis gene products (NY-eso-1, MAGE, SSX-2, etc
  - Overexpressed Ags (Meloe etc.)
- Low frequency (mostly below 1%)
- No correlation with response

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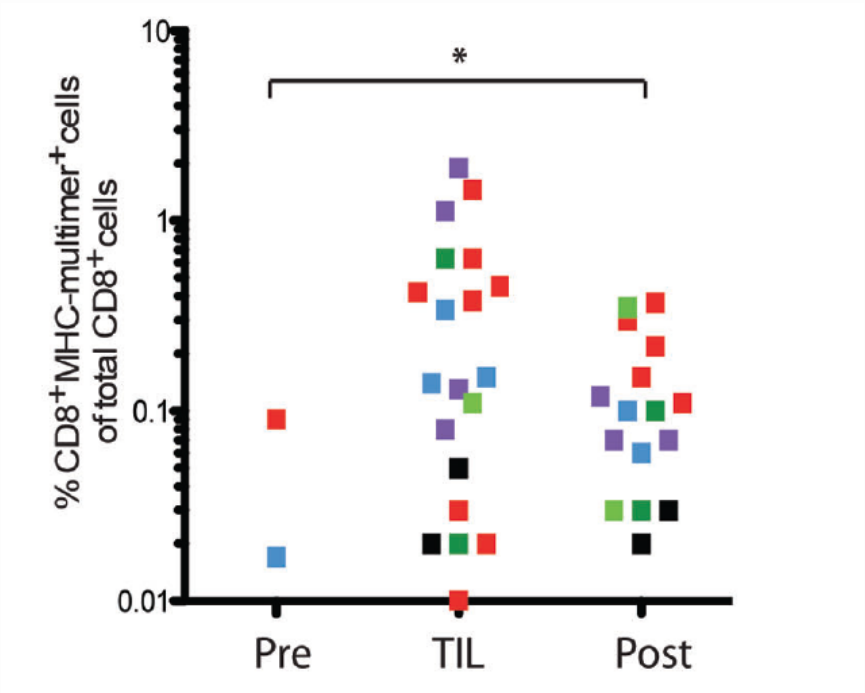
Kvistborg et al., Oncoimmunology 2012

RESEARCH PAPER

OncoImmunology 1:4, 409–418; July 2012; © 2012 Landes Bioscience

TIL therapy broadens the tumor-reactive CD8+ T cell compartment in melanoma patients

Pia Kvistborg,[1,†] Chengyi Jenny Shu,[1,†] Bianca Heemskerk,[1] Manuel Fankhauser,[1] Charlotte Albæk Thrue,[2] Mireille Toebes,[1] Nienke van Rooij,[1] Carsten Linnemann,[1] Marit M. van Buuren,[1] Jos H.M. Urbanus,[1] Joost B. Beltman,[3] Per thor Straten,[2] Yong F. Li,[4] Paul F. Robbins,[4] Michal J. Besser,[5,6] Jacob Schachter,[5] Gemma G. Kenter,[7] Mark E. Dudley,[4] Steven A. Rosenberg,[4] John B.A.G. Haanen,[1] Sine Reker Hadrup[2] and Ton N.M. Schumacher[1,*]



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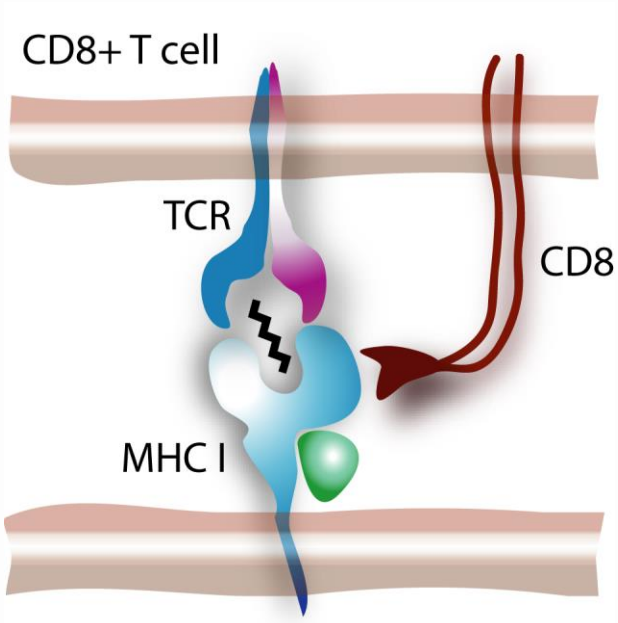
What could tumor-specific cytotoxic T cells detect on human cancer?



1. Self antigens (to which tolerance is incomplete)
   Shared between patients

2. 'Neo-antigens', epitopes that arise as a consequence of tumor-specific mutations
   In large part patient-specific, hence generally ignored

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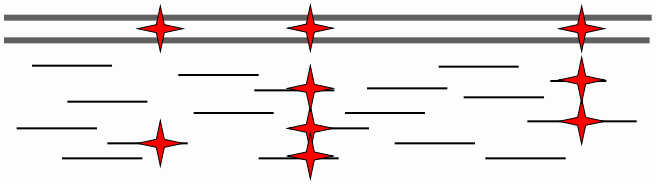
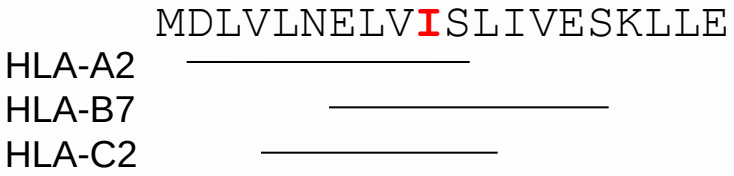
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

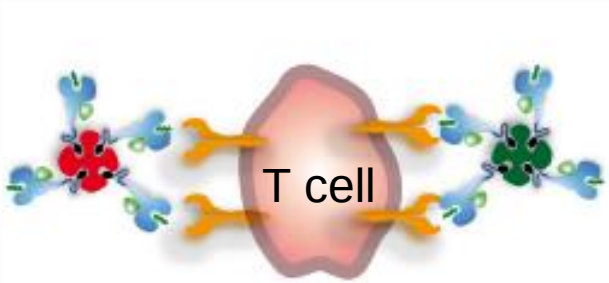


Generate map of tumor-specific mutations (ExomeSeq)

Determine which mutated genes are expressed (RNASeq)



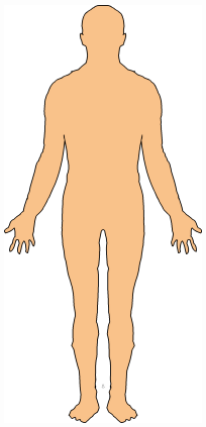
Predict epitopes for each mutation/ each HLA-allele *in silico*



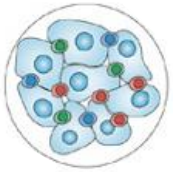
Screen for T cell recognition of mutated epitopes

Pt 008: CR upon TIL therapy

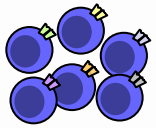
Resected tumor material



Isolate tumor cells



Isolate tumor-infiltrating T cells



Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes

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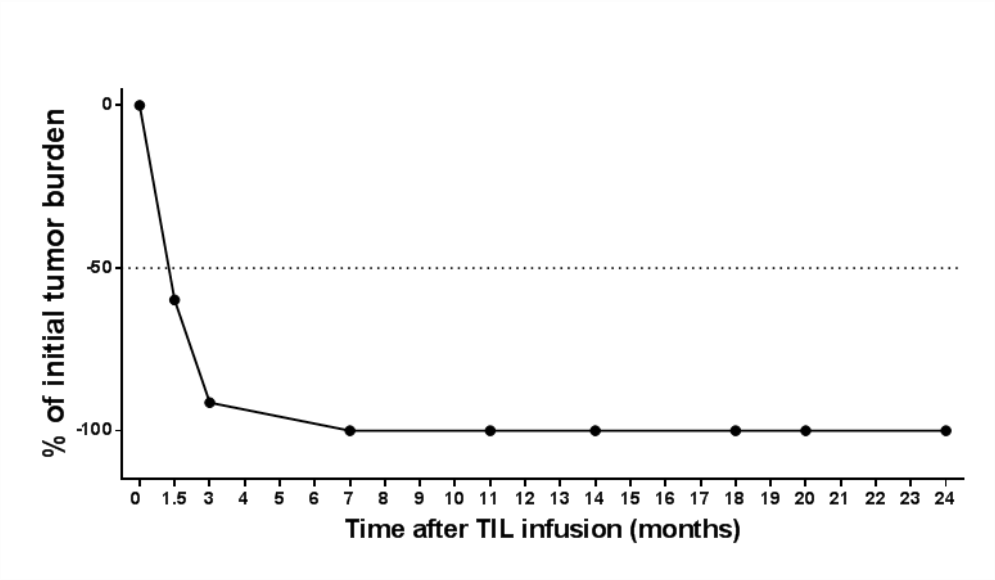
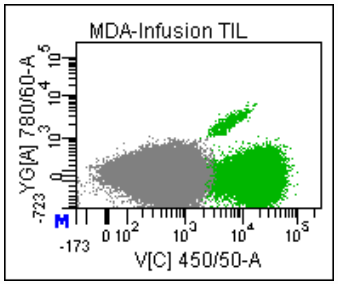
Pt 008: CR upon TIL therapy

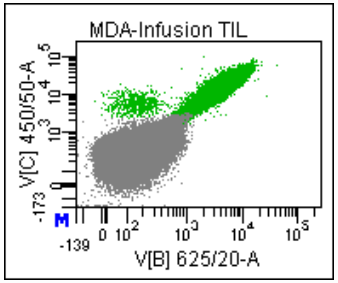


Infusion TIL product

0.172%



23%



Profound neo-antigen reactivity in TIL product

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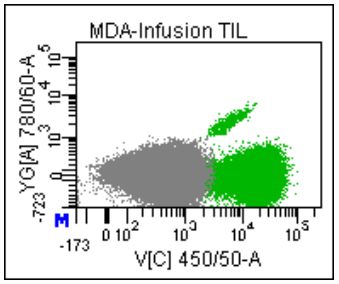
Pt 008: CR upon TIL therapy

Infusion TIL product

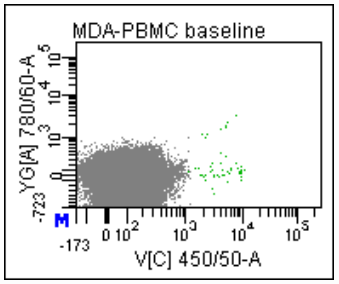
Pre-therapy PBMNC

D7 post-therapy

0.172%



0.002%



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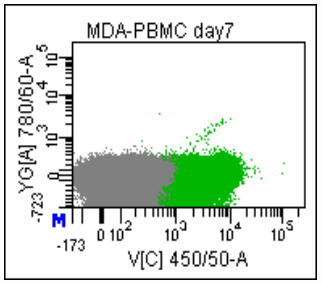

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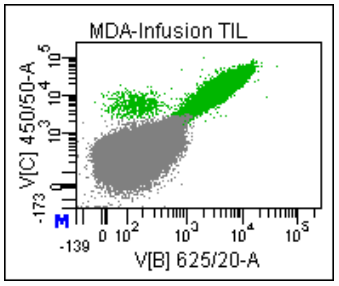

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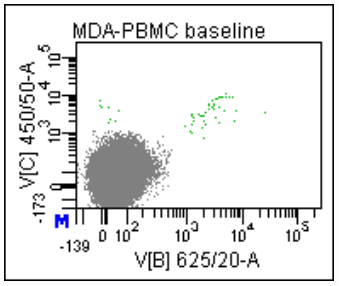

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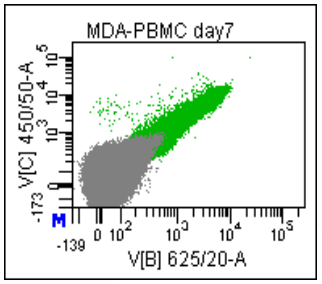
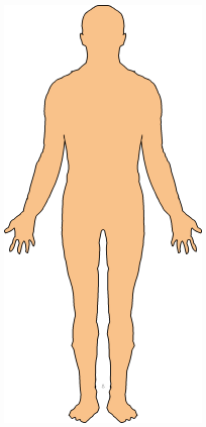


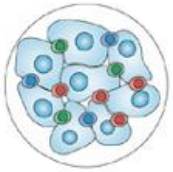
Major increase in neo-antigen specific T cell reactivity upon TIL therapy

Pt 004:

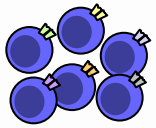
Resected tumor material



Isolate tumor cells



Isolate tumor-infiltrating T cells



Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes

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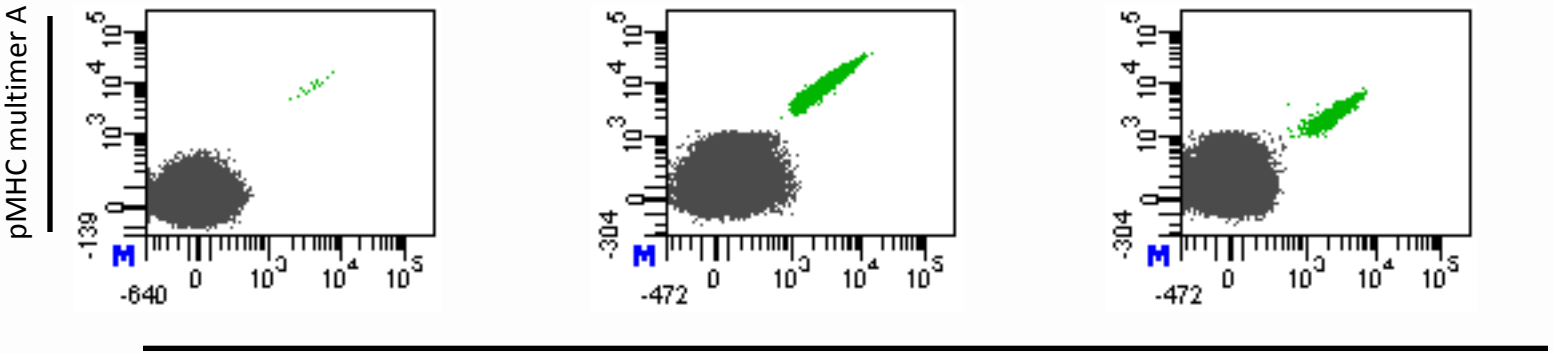
Pt 004:



DNAH17$_{H>Y}$ (0.003%)
VLFEDAVA**H** > VLFEDAVA**Y**

CDK4$_{R>L}$ (1.604%)
A**R**DPHSGHFV > A**L**DPHSGHFV

GCN1L1$_{L>P}$ (0.407%)
ALLET**L**SLLL > ALLET**P**SLLL

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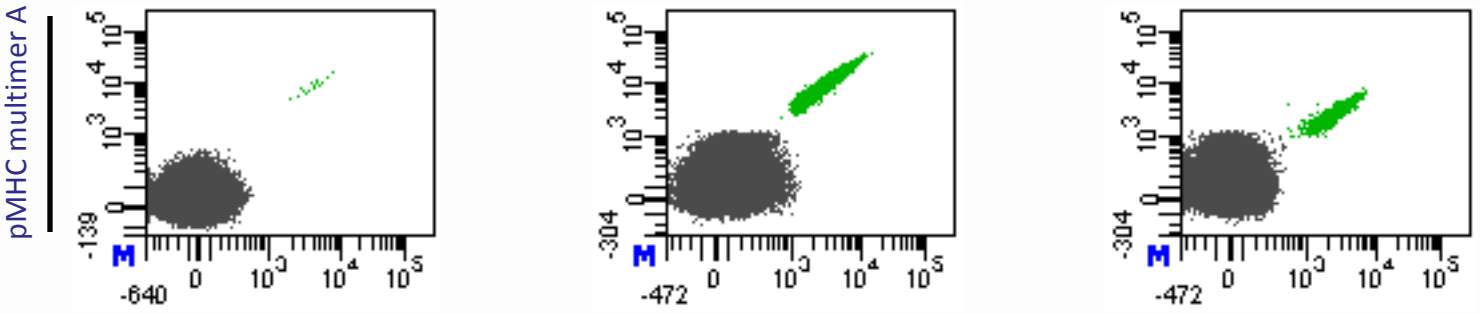
Pt 004:



$DNAH17_{H>Y}$ (0.003%)
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A**R**DPHSGHFV > A**L**DPHSGHFV

$GCN1L1_{L>P}$ (0.407%)
ALLET**L**SLLL > ALLET**P**SLLL

Mutations can result in neo-antigens derived from oncogenes and (presumed) passenger genes

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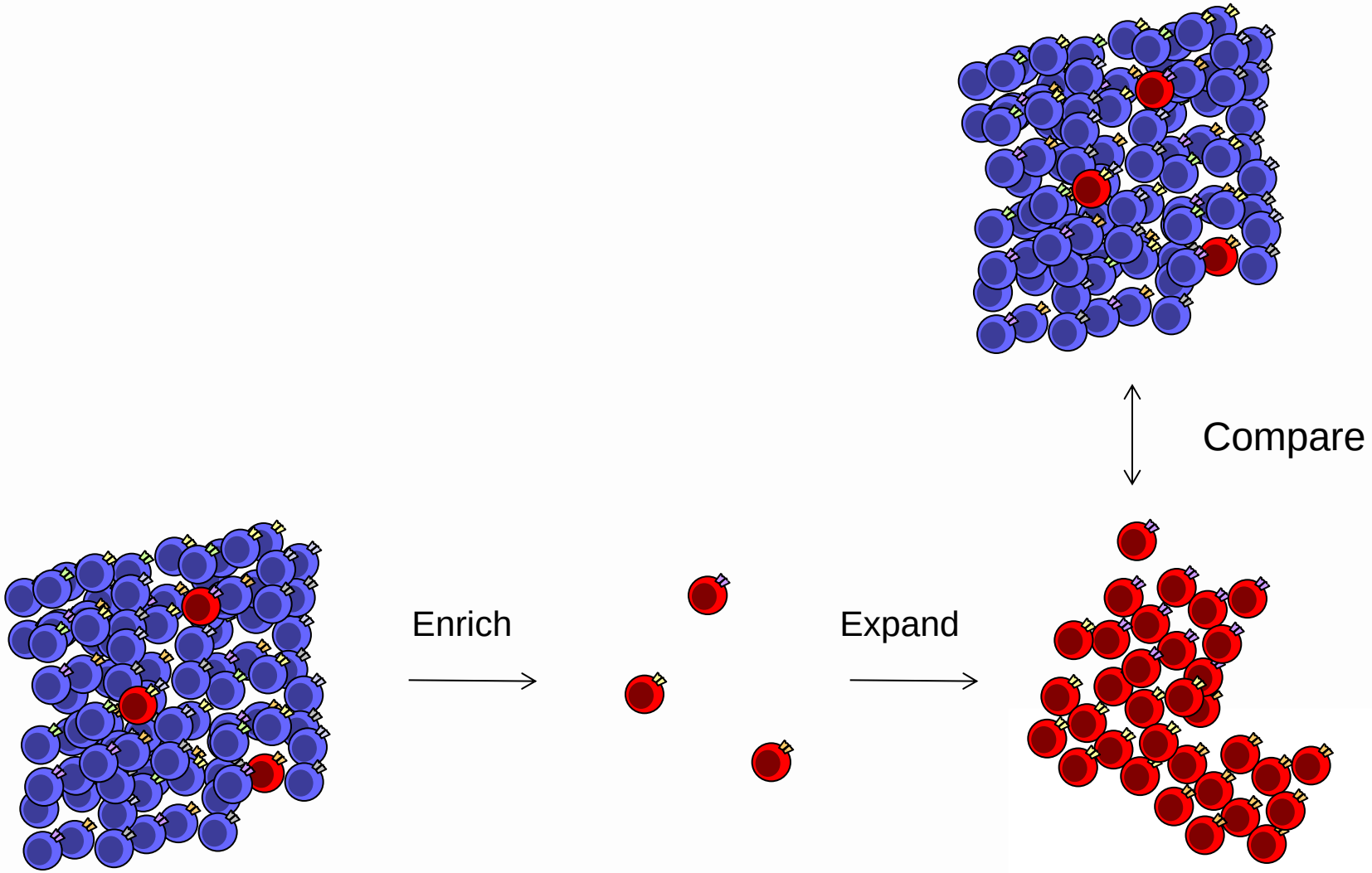
Are neo-antigens superior cancer rejection antigens?



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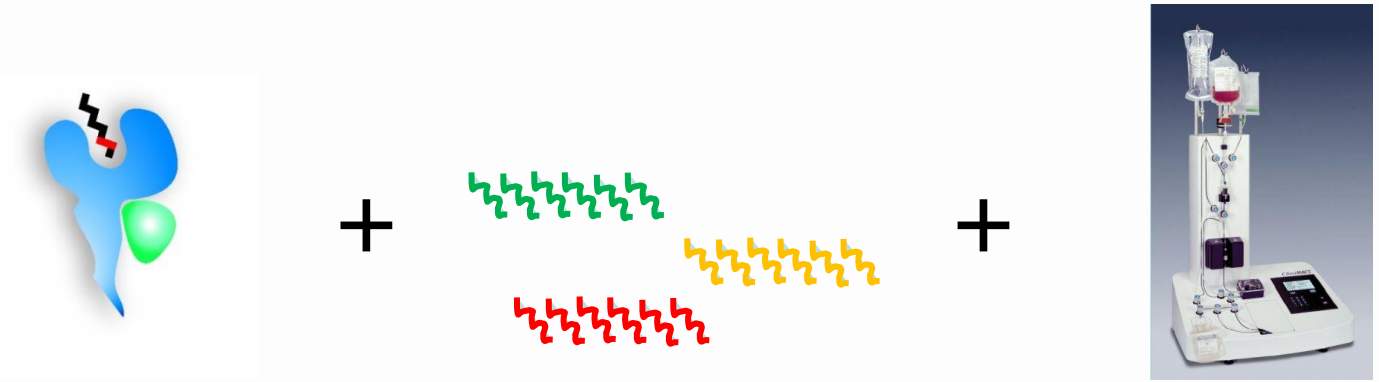

- Develop peptide exchange MHC streptamers to create defined TIL products

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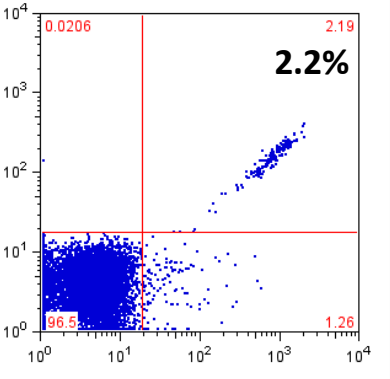
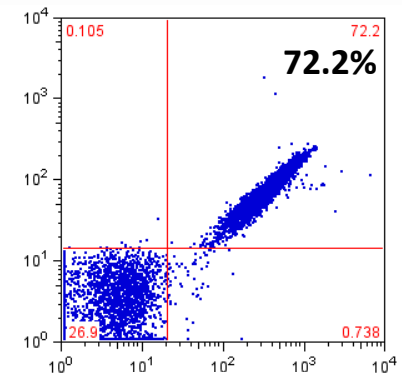
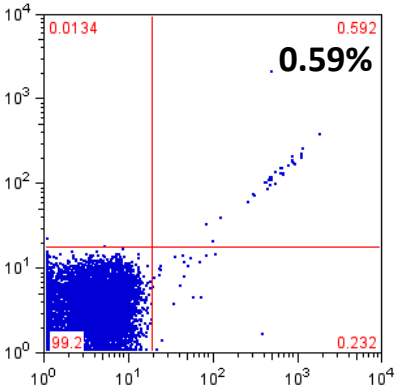
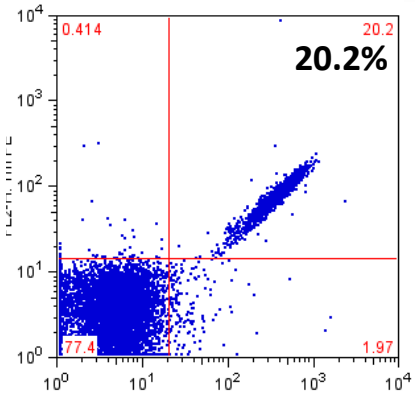
Are neo-antigens superior cancer rejection antigens?

	Pre-enrichment	Post-joint enrichment
$CDK4_{R>L}$		
$GCN1L1_{L>P}$		
Combined	2.8%	92.4%

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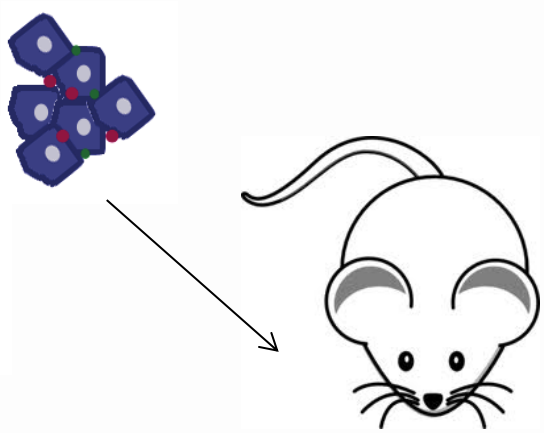
1)
Inject human melanoma
(NSG-mice)



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Inject human melanoma
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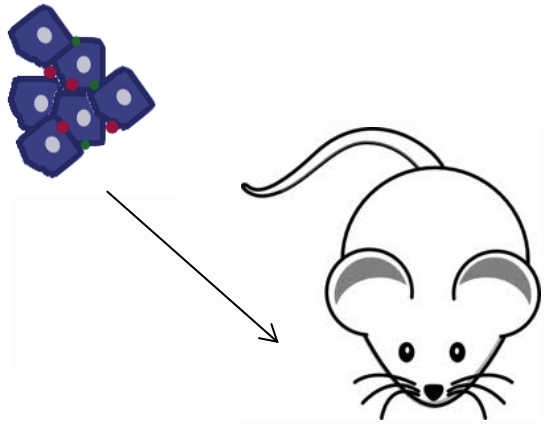

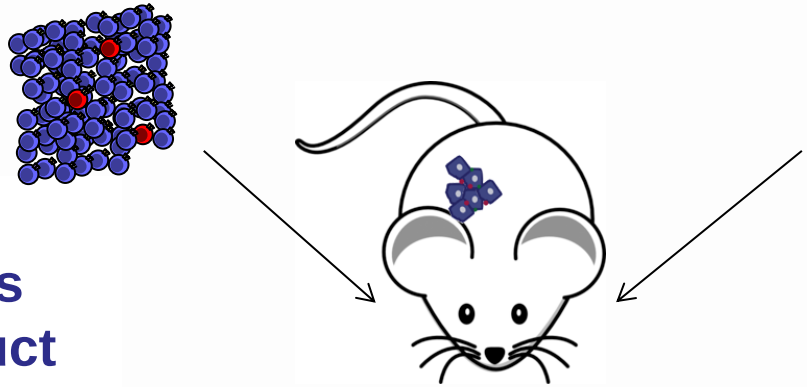

2a)
Inject autologous bulk T-cell product

2b)
Inject autologous neo-Ag enriched T-cell product

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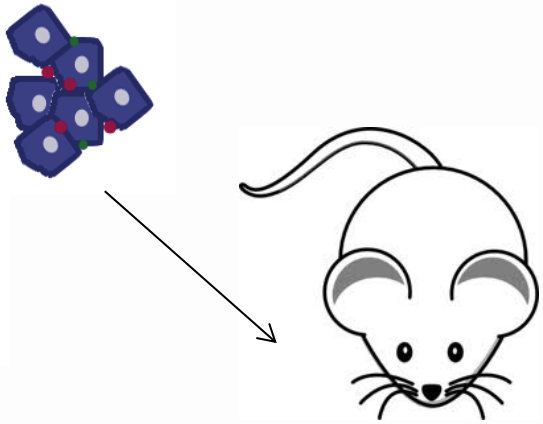
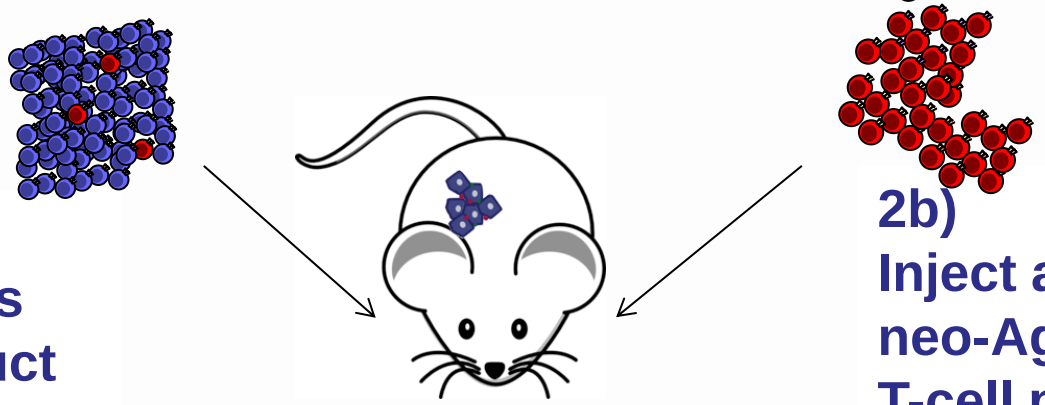
1)
Inject human melanoma
(NSG-mice)

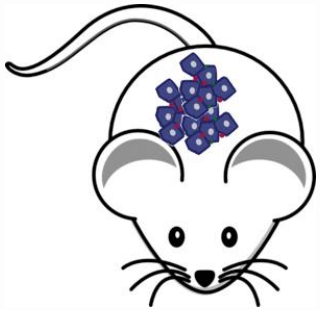




2a)
Inject autologous bulk T-cell product

2b)
Inject autologous neo-Ag enriched T-cell product

3)
Monitor tumor growth



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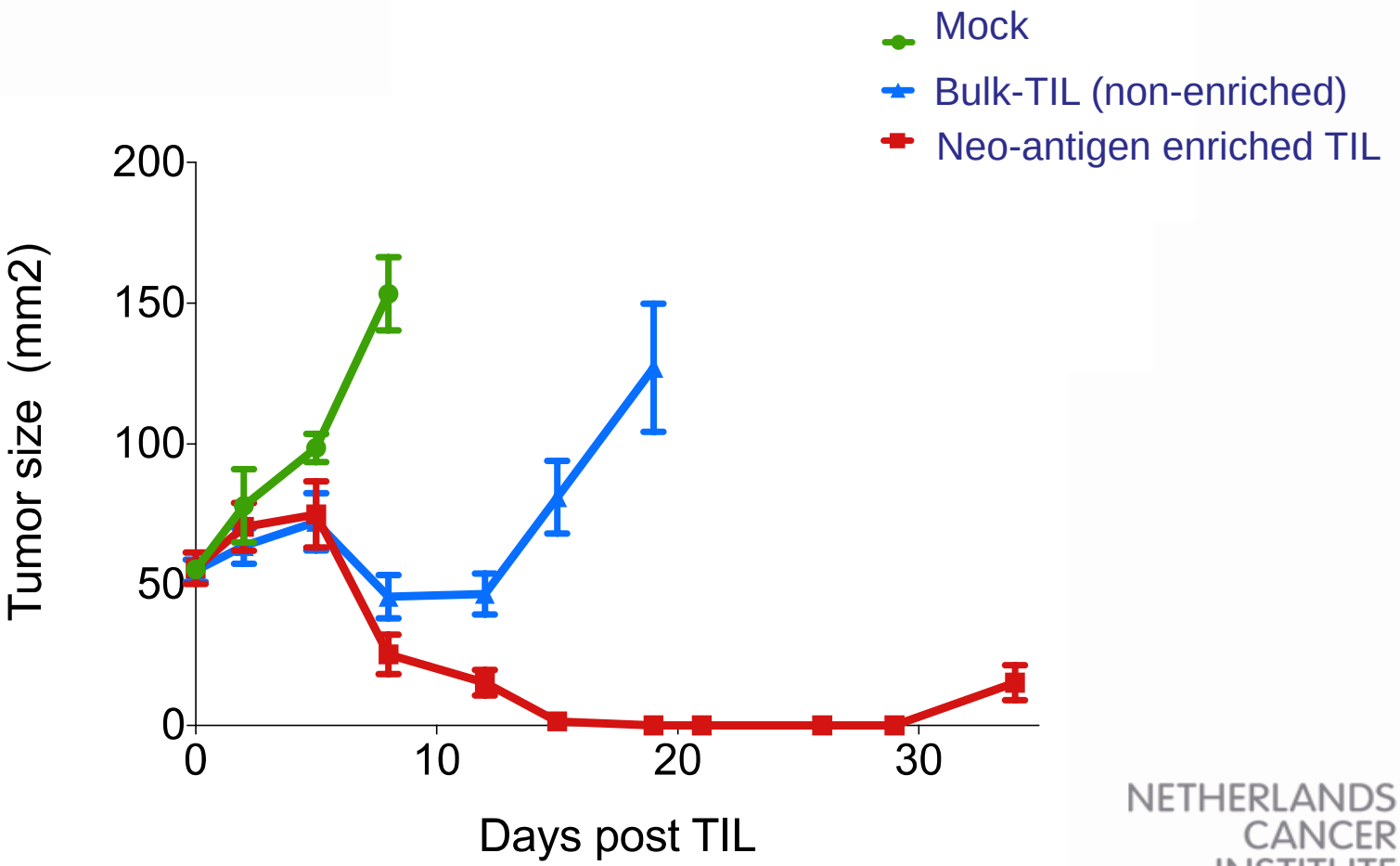
Neo-antigen enriched TIL can mediate superior tumor control



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Conclusion

- TIL infusion is feasible and can result in objective responses including durable CRs
- Neo-antigen-specific T cells are present in the majority of melanoma TIL
- Neo-antigen specific TIL play a (superior) role in tumor rejection

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Is there a place for ACT in the immune checkpoint blockade era?

- Unresolved Q:
  - We need a RCT comparing TIL with standard of care
  - Can TIL be combined with checkpoint inhibitors? (anti-PD1)
  - Can TIL be improved by selection of tumor-reactive T cells (CD137 or PD1 enrichment)
  - Can TIL be improved by knock-down of PD1 or Ppp2r2d?
  - Can we boost the neo-antigen specific cells by vaccines?

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European TIL trial consortium

- **NL:**
  - **John Haanen**: NKI-AVL, Amsterdam, The Netherlands
  - Joost van den Berg: TIL production by AmBTU and Sanquin
- **DK:**
  - **Inge Marie Svane**: Herlev Hospital, Copenhagen,
  - Marco Donia: TIL production
- **UK:**
  - **Robert Hawkins**: University of Manchester and the Christie NHS Foundation Trust, UK
  - Ryan Guest: TIL production by CTL

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Taking the next step for TIL based ACT

Randomized phase III study comparing TIL based ACT to standard ipilimumab treatment in metastatic melanoma

To obtain EMA approval of ‘classical’ TIL therapy as an ATMP

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TIL preparation harmonization procedure

- Three different production sites at blood supply units
- Establishment of uniform production methods and common SOP
- Validation procedure finalized

Procedure and procotol approved by

VHP European Committee

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Study design

Patients: 168 patients with metastatic (stage IV) melanoma and a resectable metastasis will be randomized 1:1 between arm A, standard treatment (ipilimumab) and arm B, TIL treatment.

Arm A: standard ipilimumab (3 mg/kg x 1 day i.v., q3w, 4 treatments).

Arm B: non-myeloablative chemotherapy (cyclophosphamide 60 mg/kg/day x 2 days i.v., fludarabine 25 mg/m^2/day x 5 days i.v.) followed by intravenous adoptive transfer of at least 5 x 10^9 TIL followed by high dose interleukin-2 (600.000 IU/kg/dose every 8 hours for up to 15 doses).

Stratification: Patients will be stratified for BRAF V600 mutation, 1st or 2nd line treatment, and treatment center

Study endpoint

Primary endpoint: PFS at 6 months by RECIST 1.1

Secondary endpoints:

- PFS according to RECIST 1.1 and irRC.
- ORR according RECIST 1.1 and irRC
- CR rate
- Overall survival
- Safety
- Constructive technology assessment (CTA) will be performed to evaluate the impact on patient, organizational and economic consequences

Cancer exome-guided immunomonitoring

Nienke van Rooij
Marit van Buuren
Daisy Philips
Mireille Toebes
Laura van Dijk
Pia Kvistborg

Ton Schumacher

Cancer Immunotherapy Dream Team

MHC-based technologies

Chemical Biology
Boris Rodenko
Huib Ovaa

CCIT, Copenhagen
Sine Hadrup

STAGE Therapeutics
Lothar Germeroth

PDX models
Kristel Kemper
Daniel Peeper

Sanger Institute
Sam Behjati
Mike Stratton

Utrecht University
Can Kesmir

Clinical translation

Bianca Heemskerk
Sander Kelderman
Raquel Gomez
Joost van den Berg
Samira Michels
Bastiaan Nuijen
Christian Blank