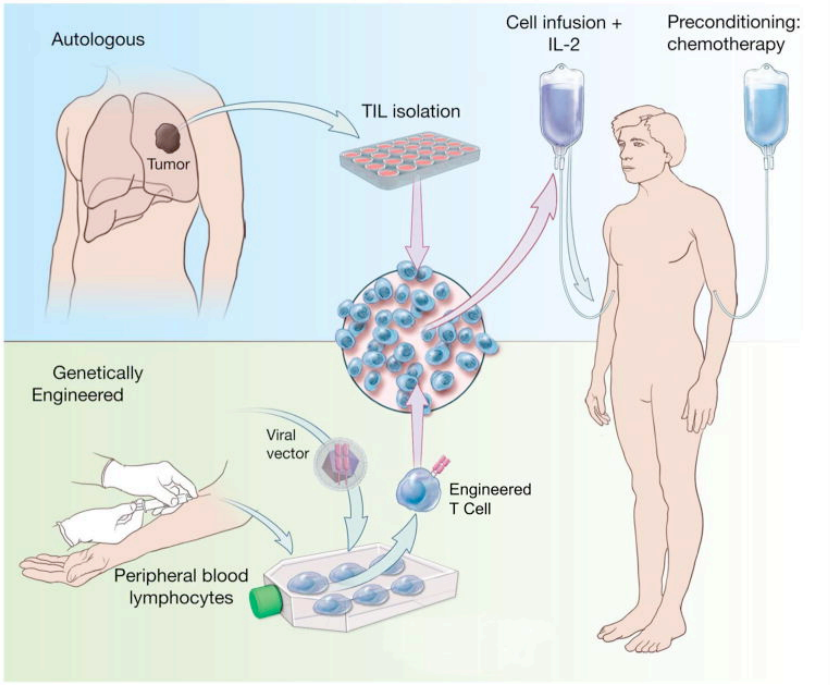
TCR gene therapy: clinical experience, toxicity and future perspectives

John Haanen



Park et al., Trends in Biotechnology 2011

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Adoptive T-cell therapy

- Adoptive T-cell therapy for melanoma has proven efficacy
  - TIL (Dudley et al. Science 2002; Besser et al., Clin Cancer Res 2013; Joseph et al., Clin Cancer Res 2011; Pilon-Thomas et al., J Immunotherapy 2012; Donia et al., J Invest Dermatol 2013)
  - Peripheral blood derived T-cells Mackensen et al., J Clin Oncol 2006; Yee et al., PNAS 2002; Hunder et al., NEJM 2008; Wallen et al., Plos One 2009; Khammari et al. J Invest Dermat 2009; Verdegaal et al., Cancer Immunol Immunother 2011)

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Why TCR gene therapy?

Alternative strategies for adoptive cell transfer as TIL therapy has limitations

- Presence of resectable metastasis is required
- TIL grow from ~80-90% of resected tumor samples
- Very patient-specific treatment
- Laborious and time-consuming
- Very difficult to obtain tumor-specific TIL from tumors other than melanoma

Aim to generate off-the-shelf reagents for many cancer patients

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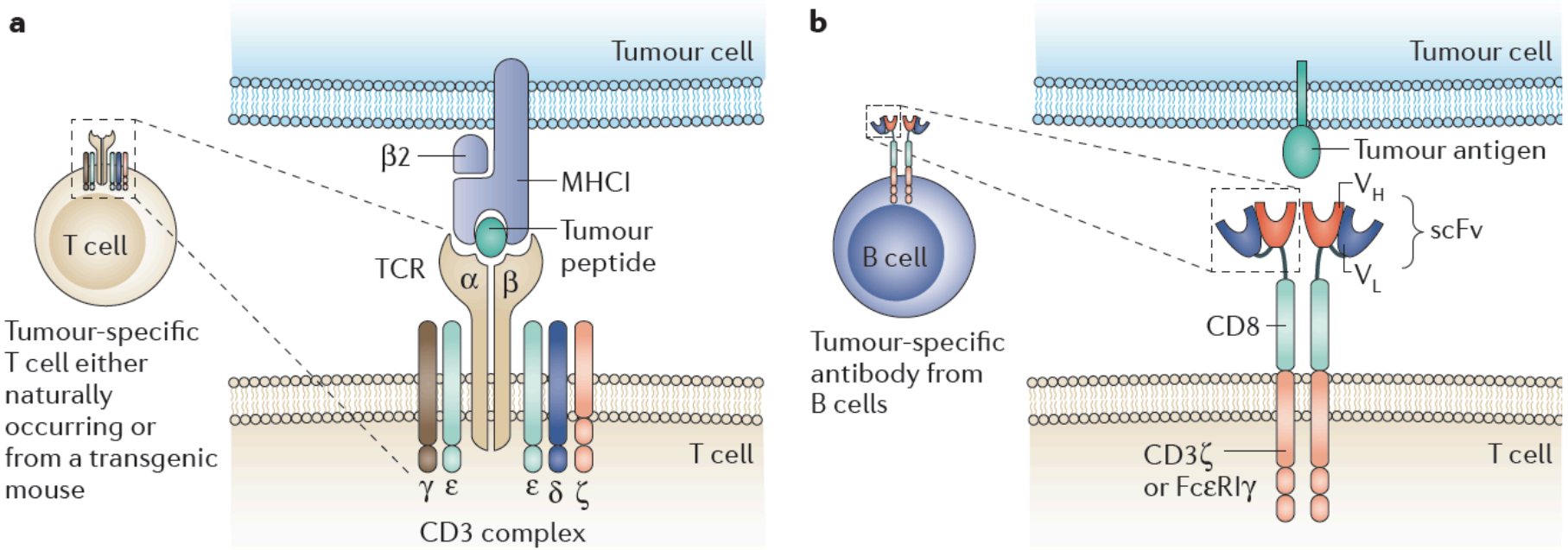
Gene-modified T cells



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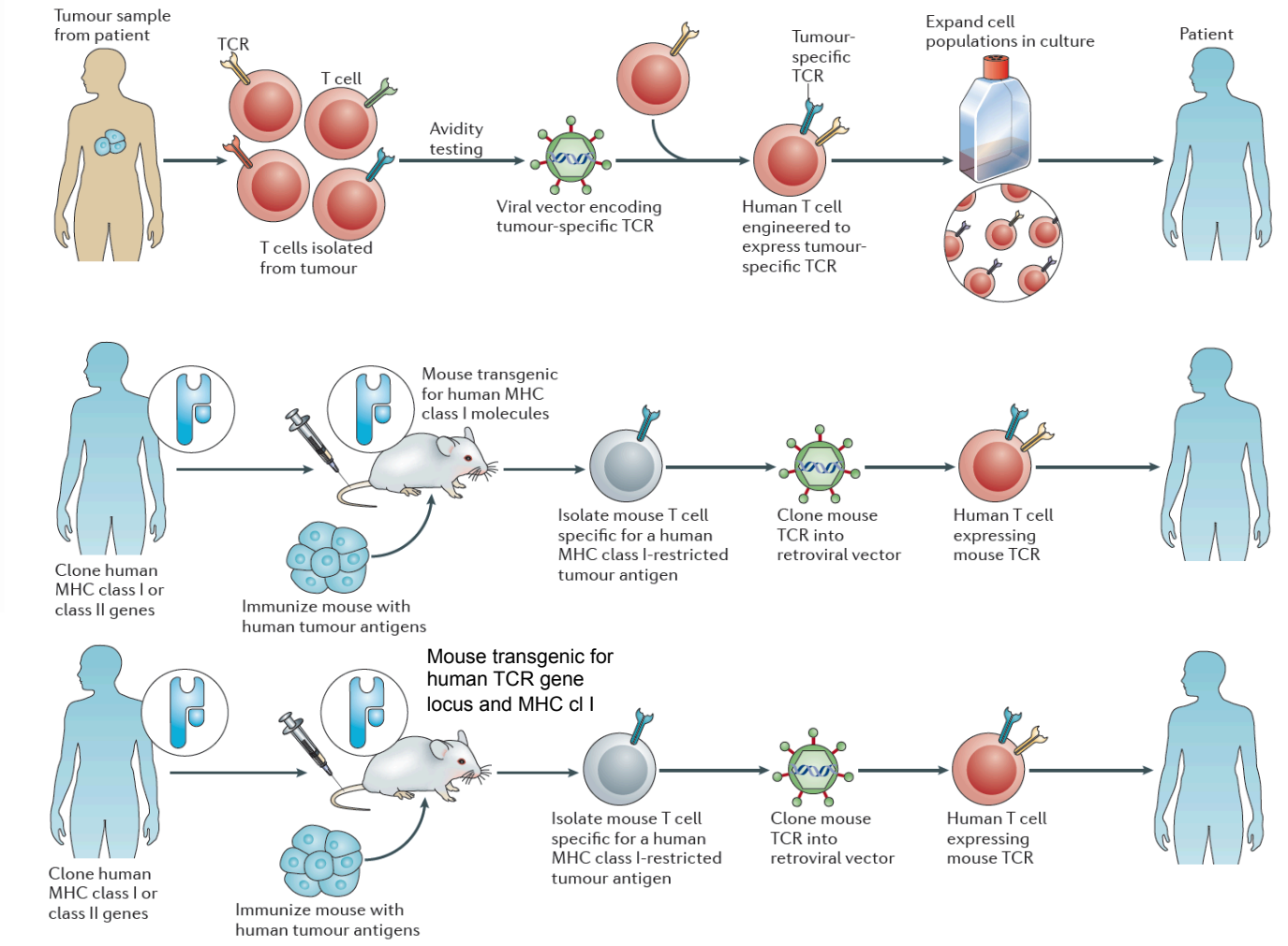
Kershaw et al. Nat Rev Cancer 2013

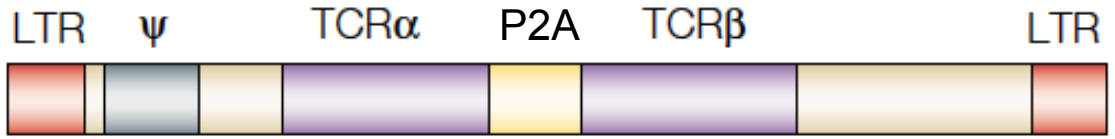
Genetically modified peripheral blood lymphocytes



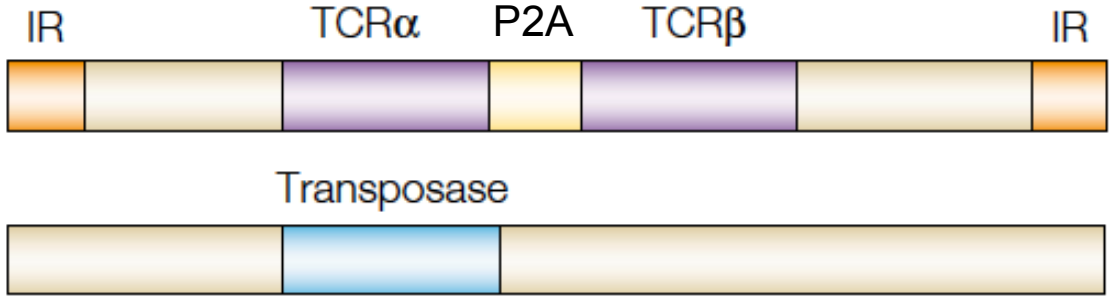
Modified from: Restifo et al., Nature Rev Immunol (2012)

Transduction platforms for gene transfer

a Retro- or lentiviral vector



b Transposon-based vector



Schumacher. Nat Rev Immunol 2002

Clinical experience with TCR gene therapy

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

  (Morgan et al., Science 2006)

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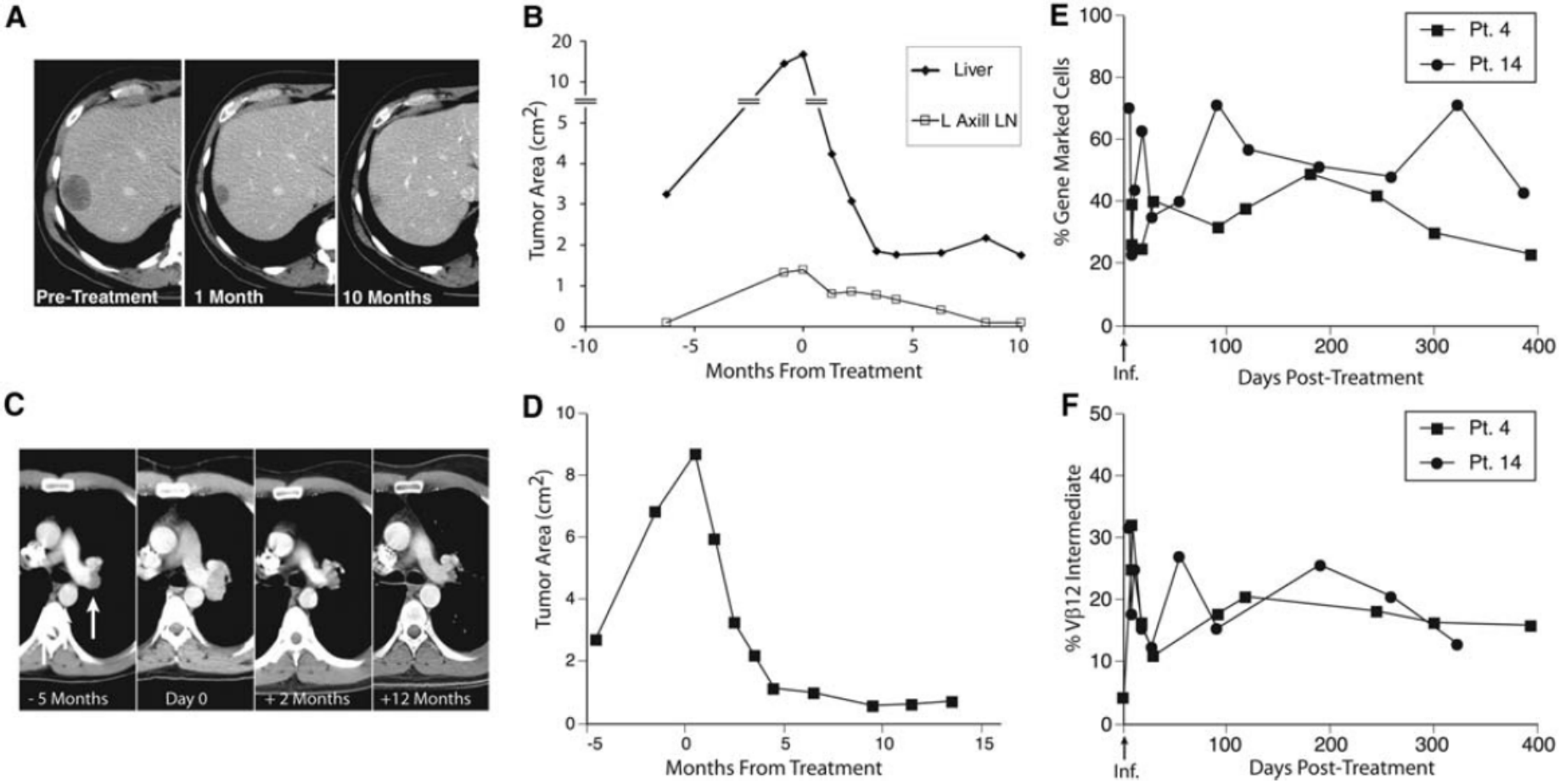
Cancer regression upon transfer of MART-1 TCR redirected T cells



Morgan et al., Science 2006

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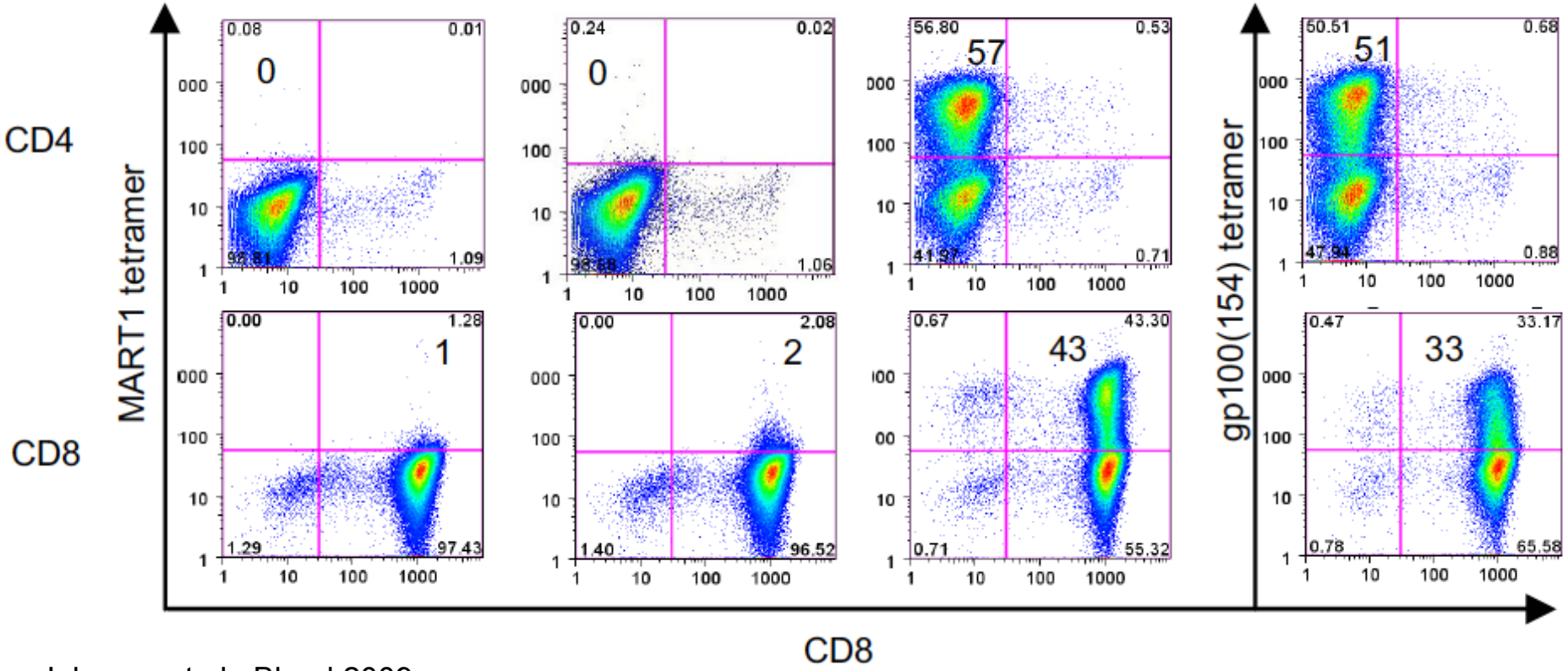
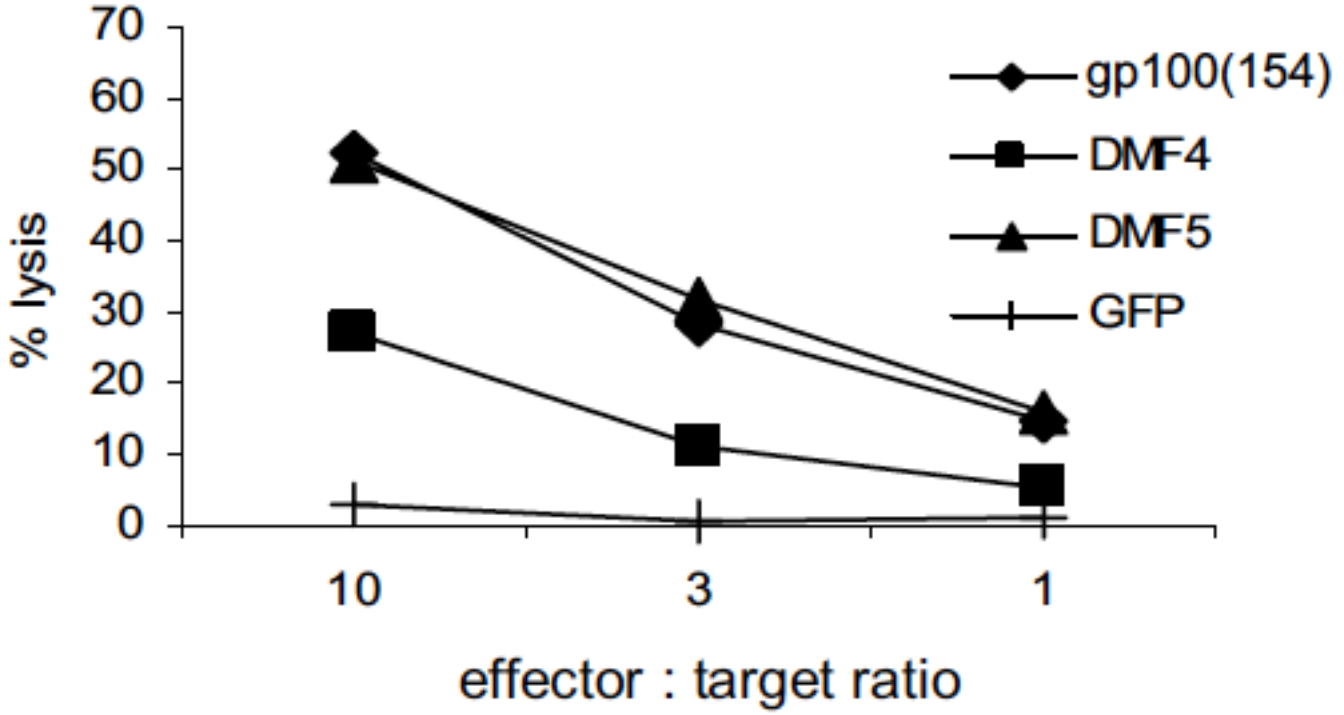
DMF5 and gp100 specific TCR were highly expressed by transduced CD4 and CD8 T cells



Johnson et al., Blood 2009

Higher cytolytic activity of 2nd generation MDA-specific TCRs



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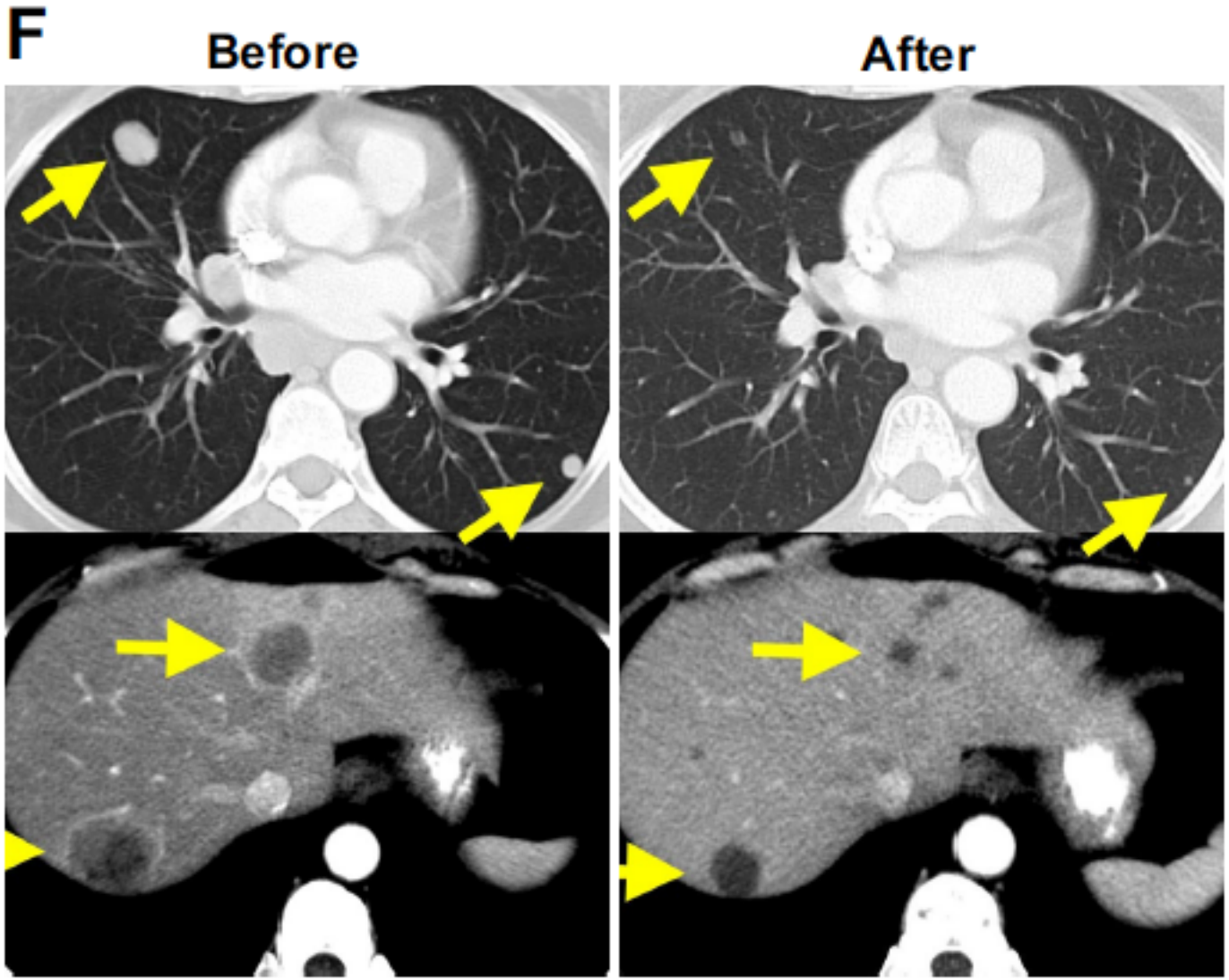
Johnson et al., Blood 2009

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



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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)

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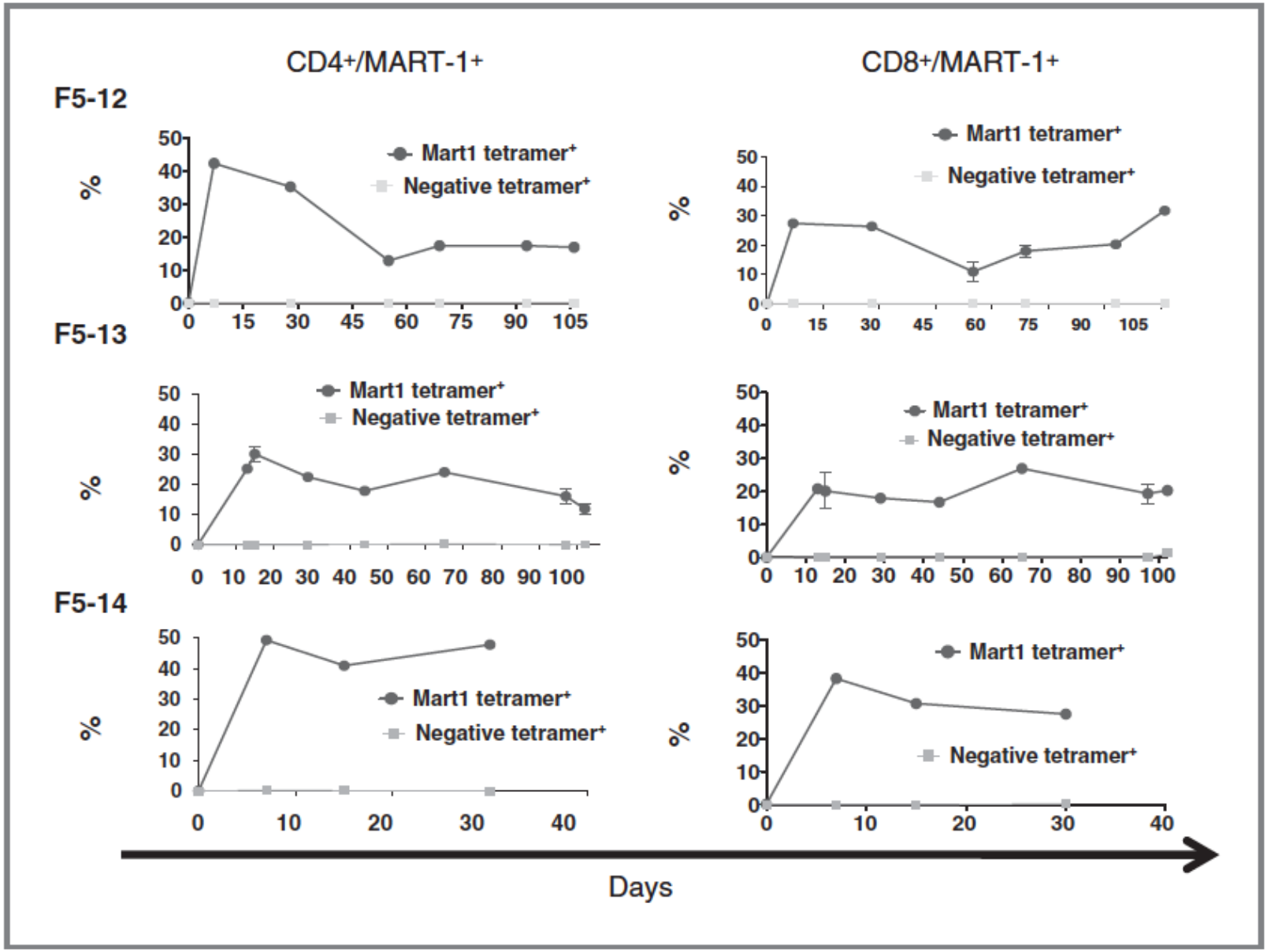
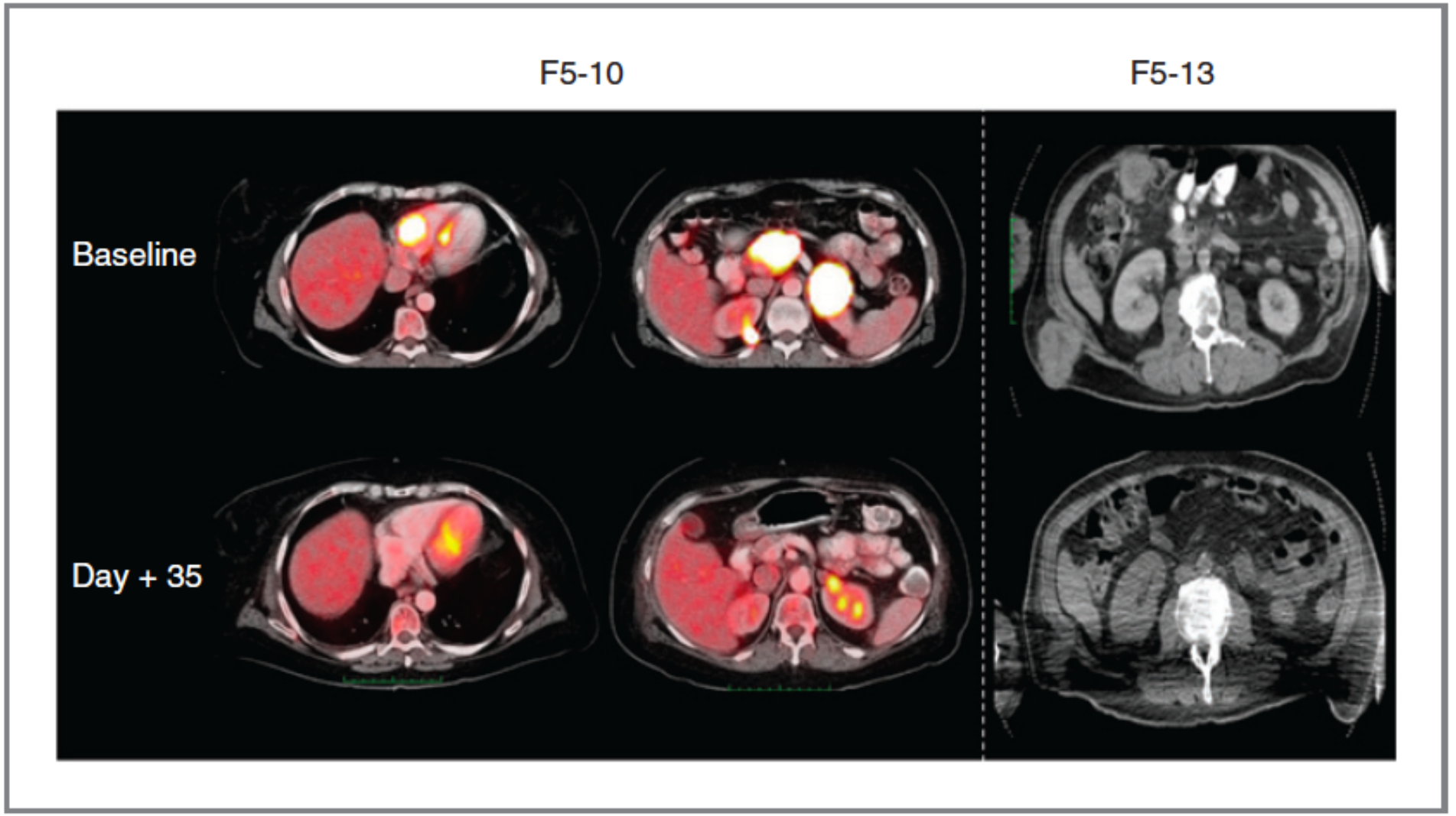
Persistence of gene modified (non-cryopreserved) cells after infusion

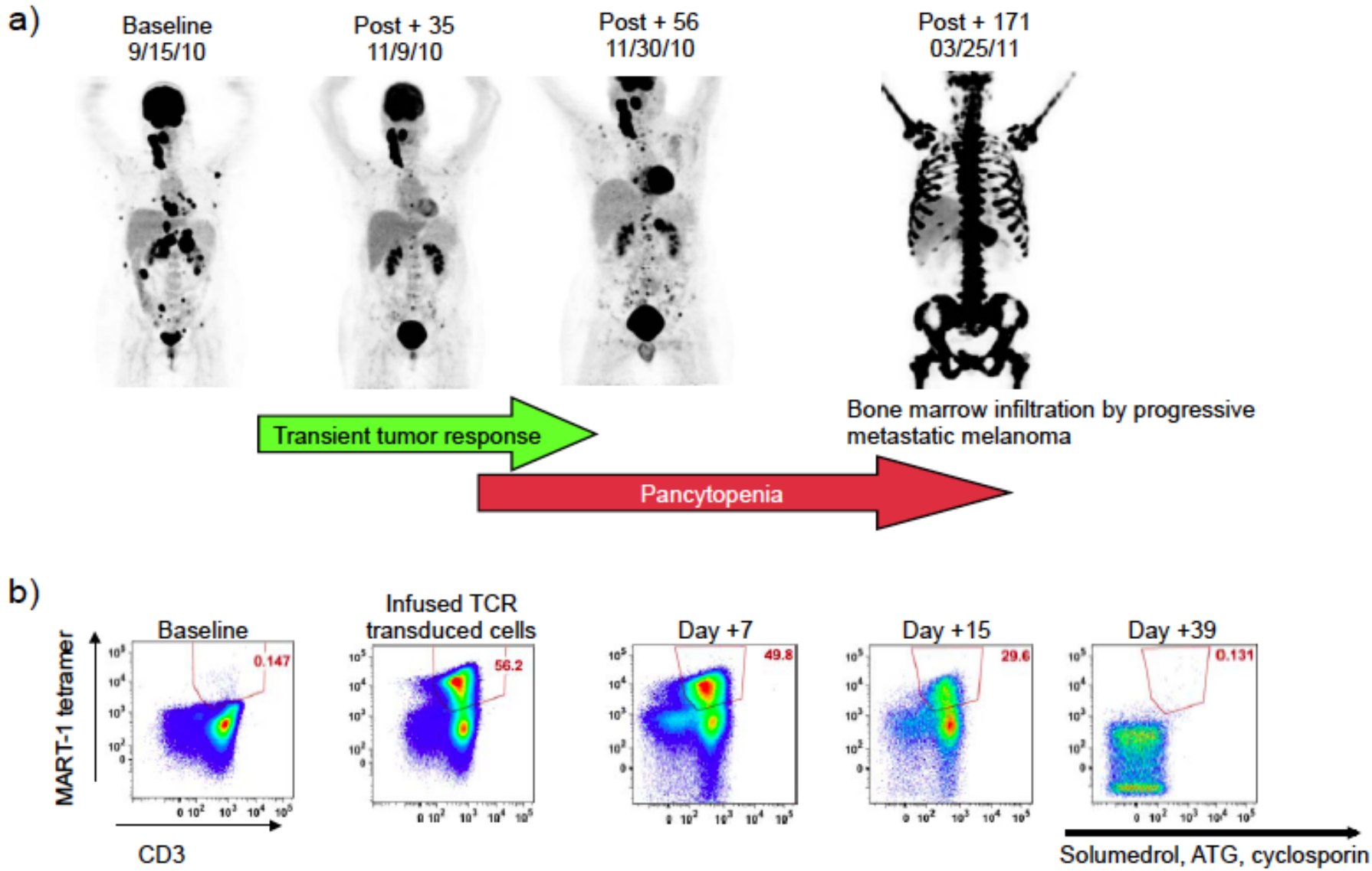


Clinical responses upon adoptive T-cell transfer



Chodon et al., Clin Cancer Res 2014

Pre- and post-treatment PET scans showing evidence of major tumor response and MART-1-specific TCR transgenic cell levels in patient F5-10



TCR gene therapy for melanoma

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  - 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 ACT program NKI-AVL

- TIL treatment melanoma
  - Current status: 10 patients treated in phase I/II feasibility trial
  - Future: Randomized multicenter phase III trial
    - TIL treatment vs ipilimumab as first or second line treatment
    - In collaboration with:
      - Herlev Hospital, Copenhagen (Inge Marie Svane & Marco Donia)
      - University of Manchester (Robert Hawkins & Ryan Guest)

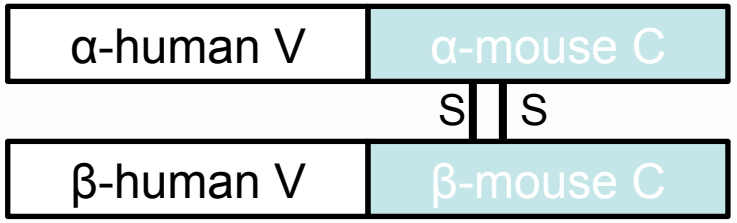
- MART-1 TCR transduced T cells
  - Phase I/II trial in collaboration with Univ of Lausanne

TCR choice and expansion protocol

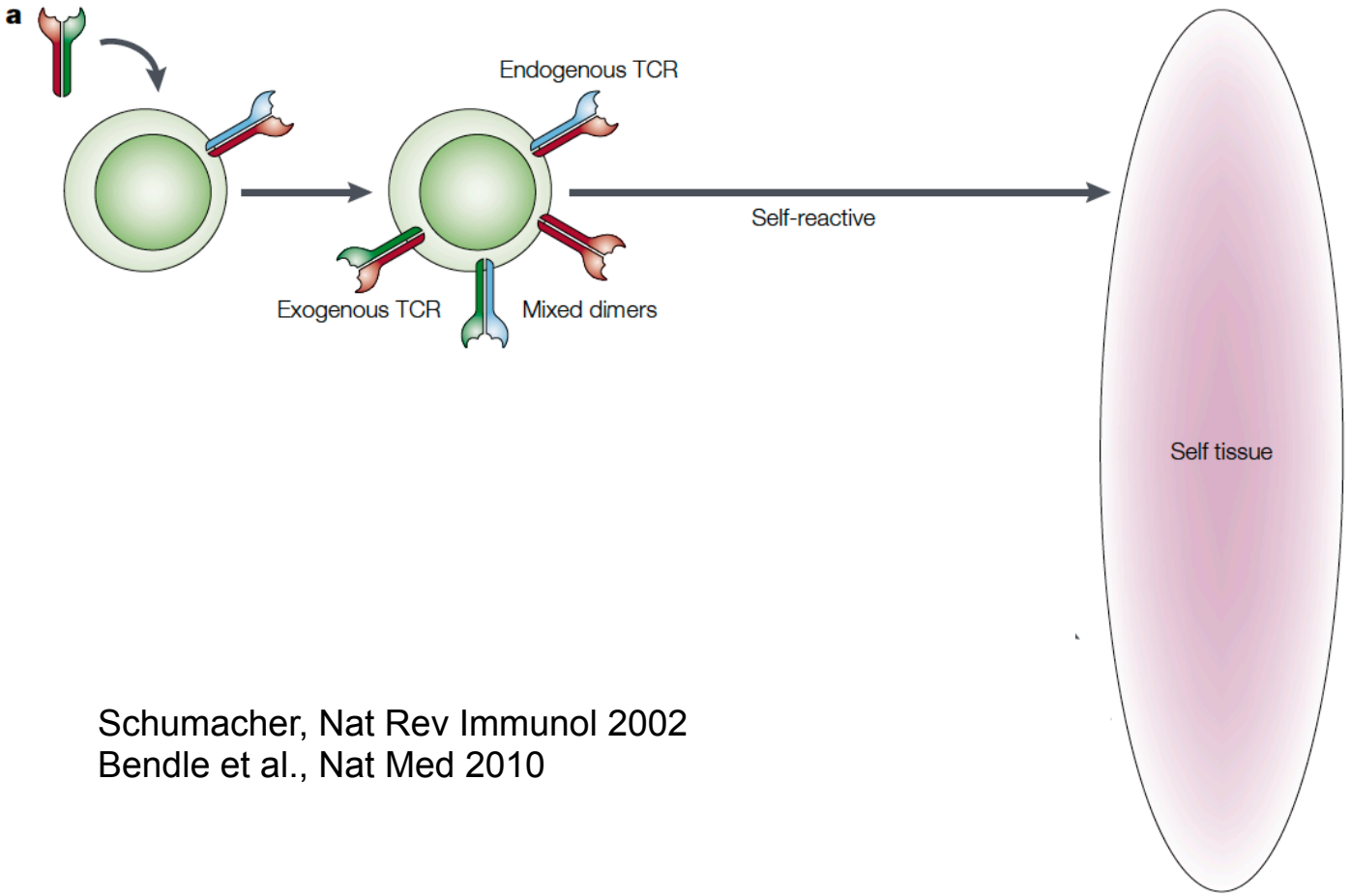
Design 1D3$_{opt}$HMCys TCR construct:

- 1D3 TCR recognizes MART-I 26-35 epitope (not affinity-matured)
- MP71 retroviral vector

(Jorritsma & Gomez-Eerland et al. Blood 110(10):3564-72, 2007)



Potential toxicities of TCR gene therapy (I)



Schumacher, Nat Rev Immunol 2002
Bendle et al., Nat Med 2010

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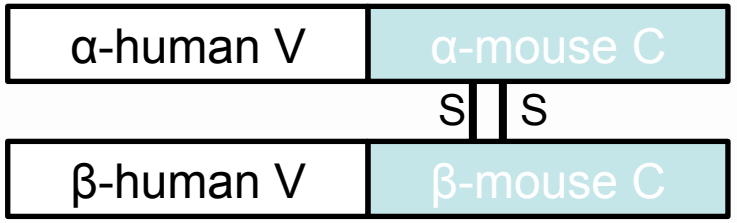
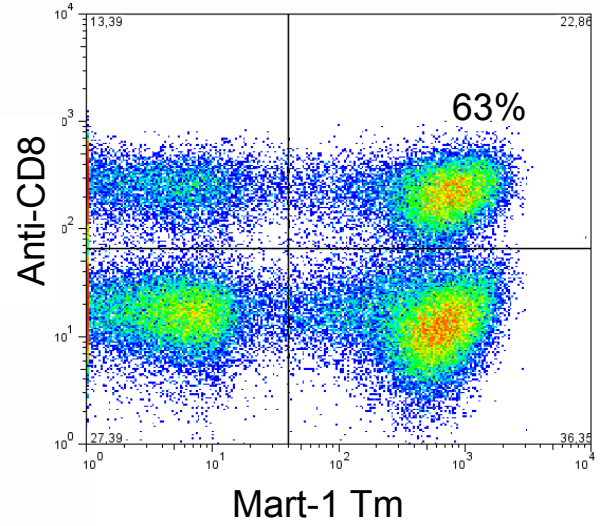


Expansion IL-7/IL-15 + aCD3/aCD28 beads

- "Less differentiated" phenotype compared with IL-2 + aCD3 mAb
- Better engraftment in humanized mouse model

(Kaneko et al. Blood 113(5): 1006-15, 2009)



Trial design

Patient group: Stage IIIc/IV melanoma

Clinical protocol: Simon 2-stage design phase I/II study

- Non-myeloablative chemotherapy
  cyclophosphamide/fludarabine
- T cell infusion (5x 10^9 cells)
- Low-dose interleukin-2
  (2x10^6 IU/once daily up to two weeks)

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Clinical results so far…

- 3 patients have been treated
  - Patient 1 died at day 9 following T cell infusion

- Protocol was put on hold and was amended (100x fewer cells to be infused)
  - Two patients were treated
    - Patient 2: mixed response (PD)
    - Patient 3: 1st evaluation CT in 1 week

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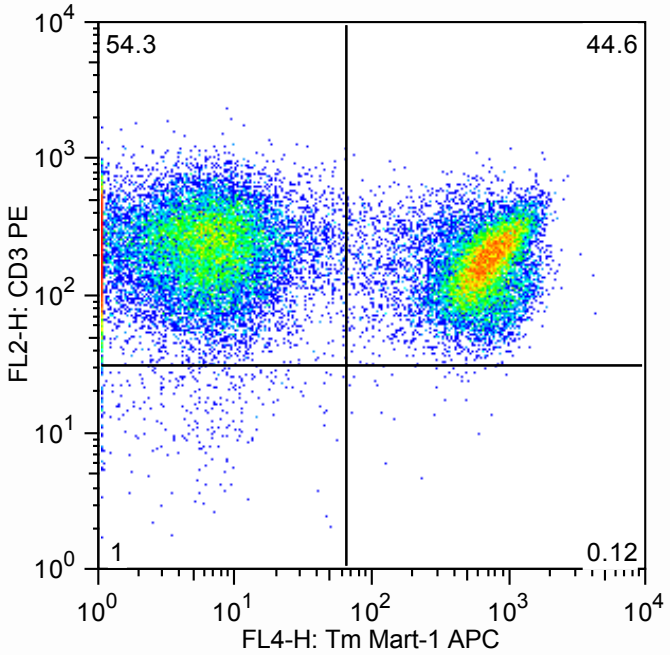
Patient 2

- 48 year old man
- Liver, cutaneous, kidney , lung and bone metastasis
- Heavily pretreated
- 5 x 10^7 transduced cells



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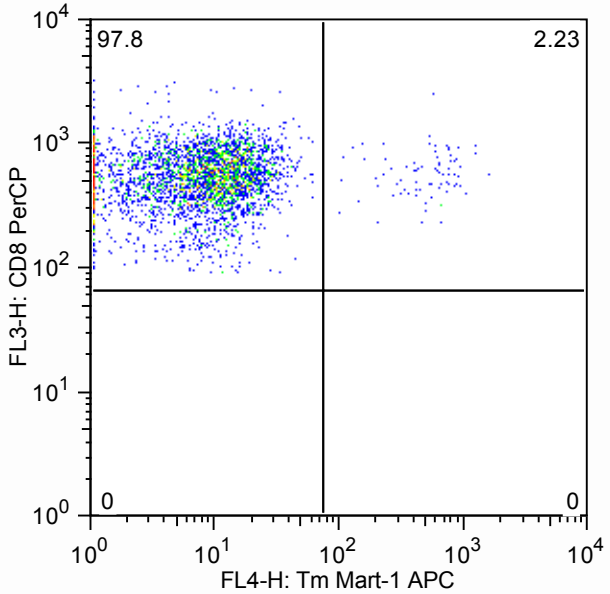
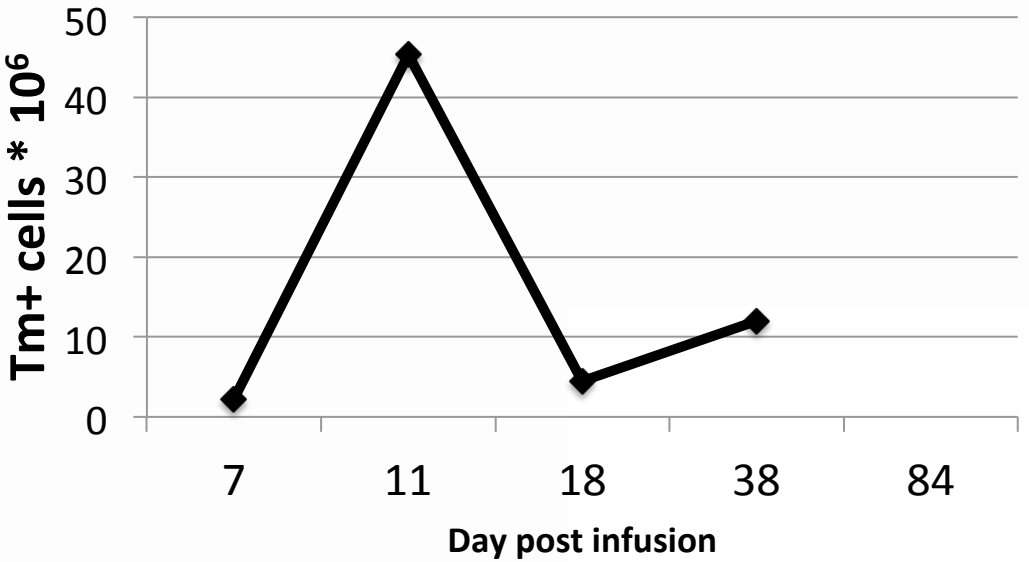
Patient 2

- No severe toxicity
- Stable levels of IL-6, LDH, CRP, pro-calcitonin (inflammation markers)
- Blood samples showed the presence of modified T cells





Calculation based on the amount of lymphocytes measured by the AKL/ml and for ~ 5 L blood per person

Patient 2

- Regression of some subcutaneous metastases
- On CT-scan: progression of LN and bone metastases at 4 weeks after infusion
- 2 months after infusion: surgery because of pathological fracture: tumor material was negative for MART-1

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Patient 3

- 75 year old woman
- Metastasis in lymph node, long, spleen and liver (positive for MART-1)
- History:
  - 2009: Diagnosed with metastatic melanoma
  - 2010: Dacarbazine
  - 2011: MART-1 DNA vaccine
  - 2011 stable disease
  - 2012 progressive disease – MEK162
  - 2012 progressive disease – inclusion M11TCR trial
  - TCR trial on hold because of patient 1
  - 2013 ipilimumab
  - 2014 progressive disease – re-inclusion M11TCR trial

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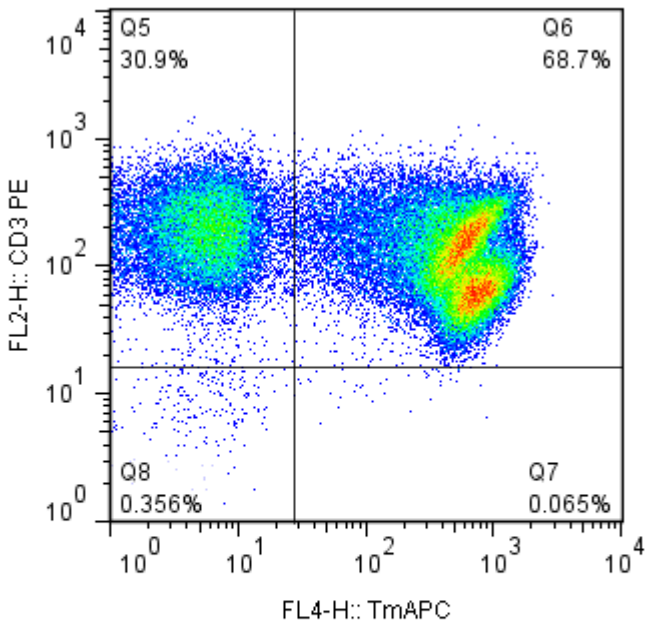
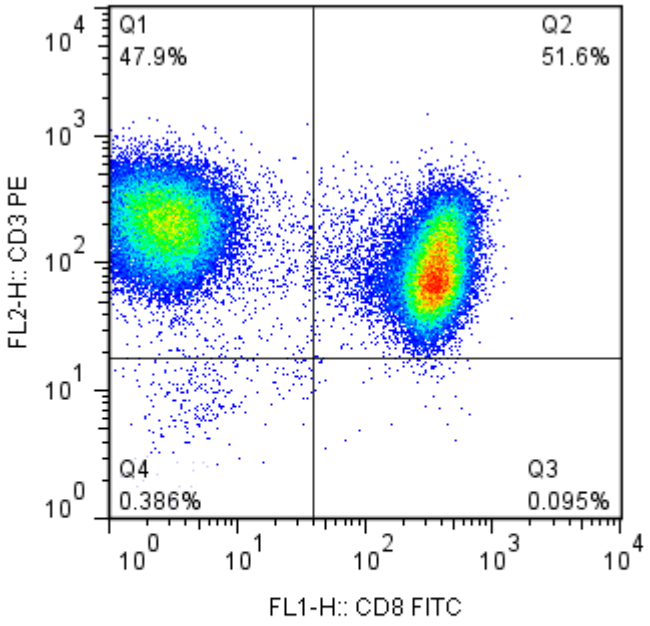
Infusion product

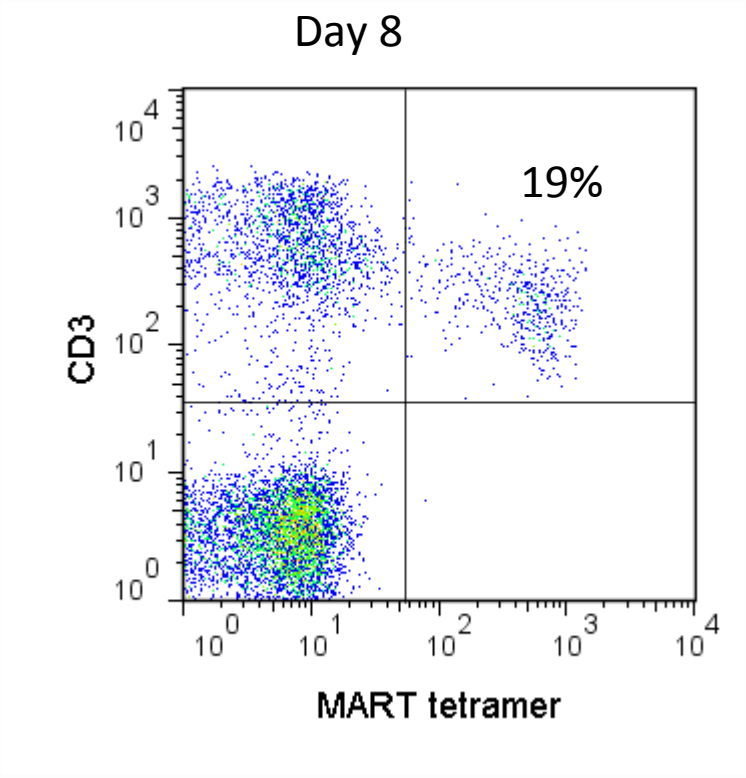
- 67% transduction efficiency
- $5x10^7$ transduced T cells
- 52% CD8

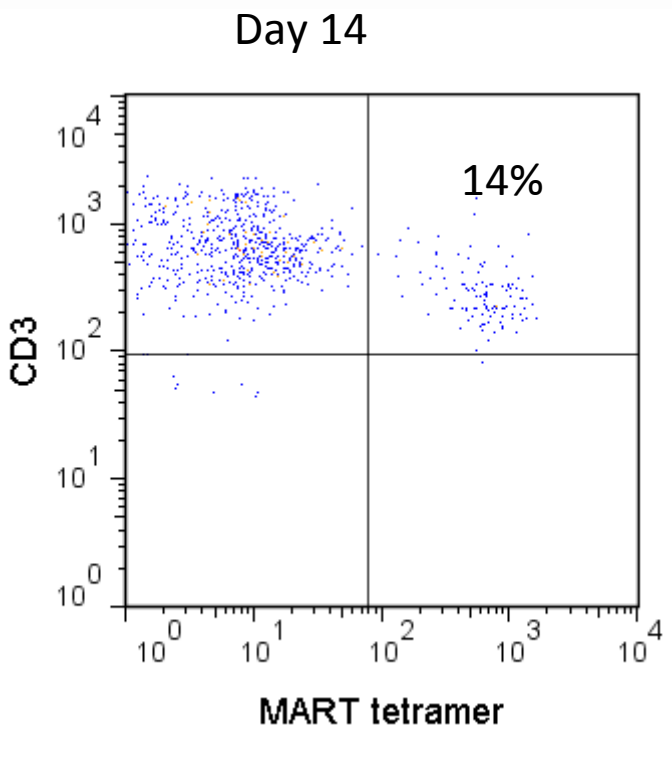




Expansion T cells in circulation

- Absolute number in blood (5L) is estimated to be $8x10^7$ on day 8





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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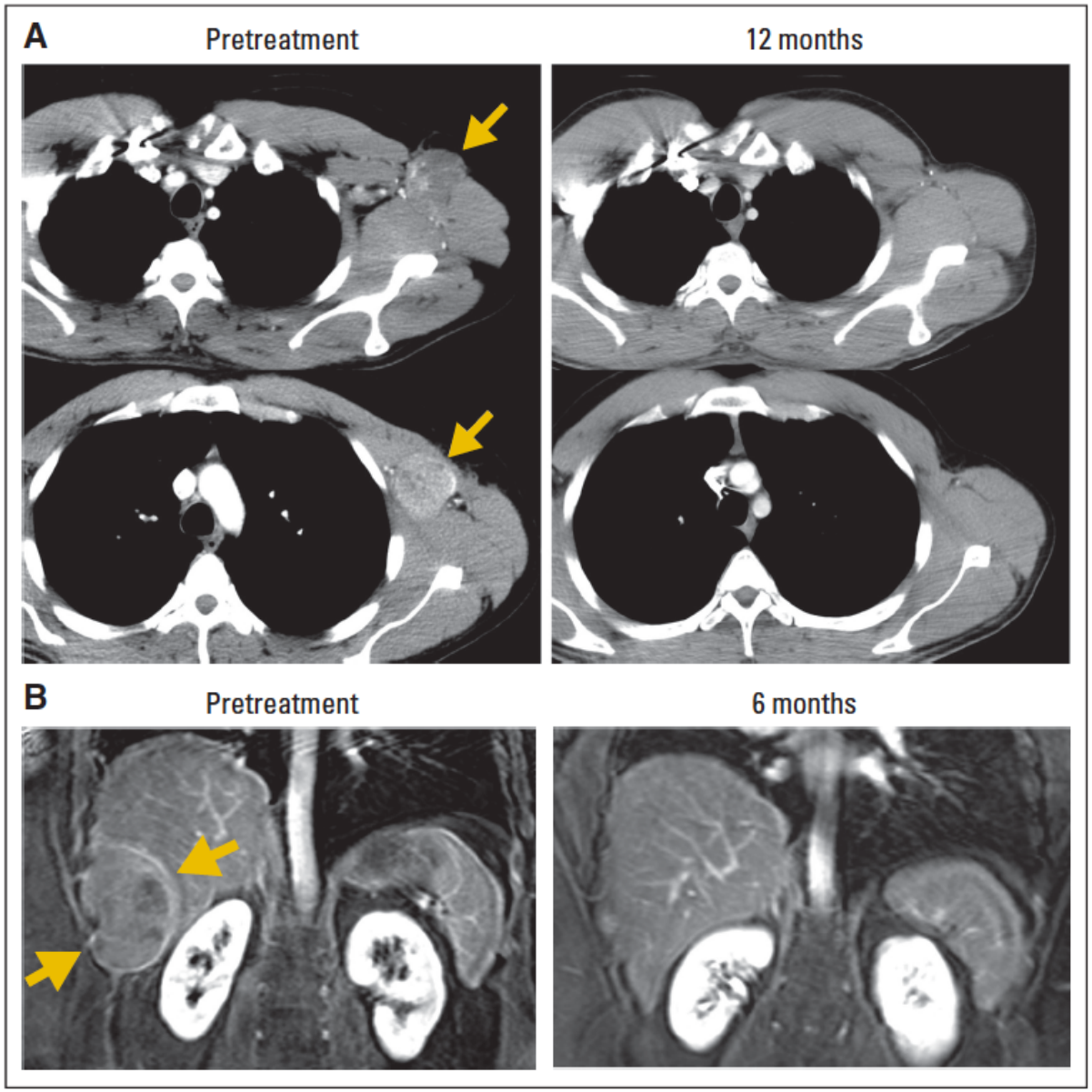
Patient characteristics and outcome

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+)
Synovial cell sarcoma														
12‡	20	M	lu, bo	R, S, C, I	83	5	82	8	77	64	91	10,065	117	PR (10)
13‡	37	F	lu	R, S, C	50	8	90	5	78	78	93	11,656	94	PR (18)
14‡	47	F	lu, ln	R, S, C	56	8	89	11	81	76	91	10,836	50	PR (5)
15‡	19	M	lu	R, S, C, I	16	5	46	40	67	63	89	5,371	< 30	PD
16	30	M	pl, hi	S, C	59	5	92	8	74	57	88	6,512	199	PR (8)
17	40	M	pl, hi	R, S, C	52	5	81	18	78	69	92	8,098	< 30	PD

Robbins et al., J Clin Oncol 2011

Clinical responses



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  (Robbins et al., J Clin Oncol 2011)
- 2009: CEA TCR gene therapy in colorectal cancer
  - Response in 2/3 patients (1 according RECIST)

  (Parkhurst et al., Mol Therapy 2009)

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- 2009: CEA TCR gene therapy in colorectal cancer
  - Response in 2/3 patients (1 according RECIST)

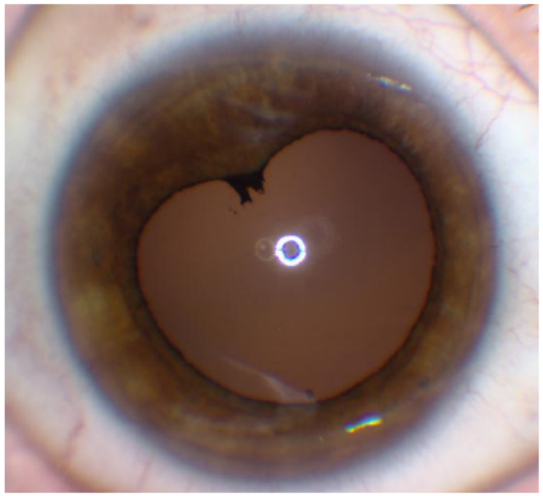
  (Parkhurst et al., Mol Therapy 2009)
- 2012 MAGE-A3 TCR gene therapy
  - Two independent trials were aborted due to unexpected toxicity

  (Morgan et al., J Immunother 2013; Linette et al., Blood 2013)

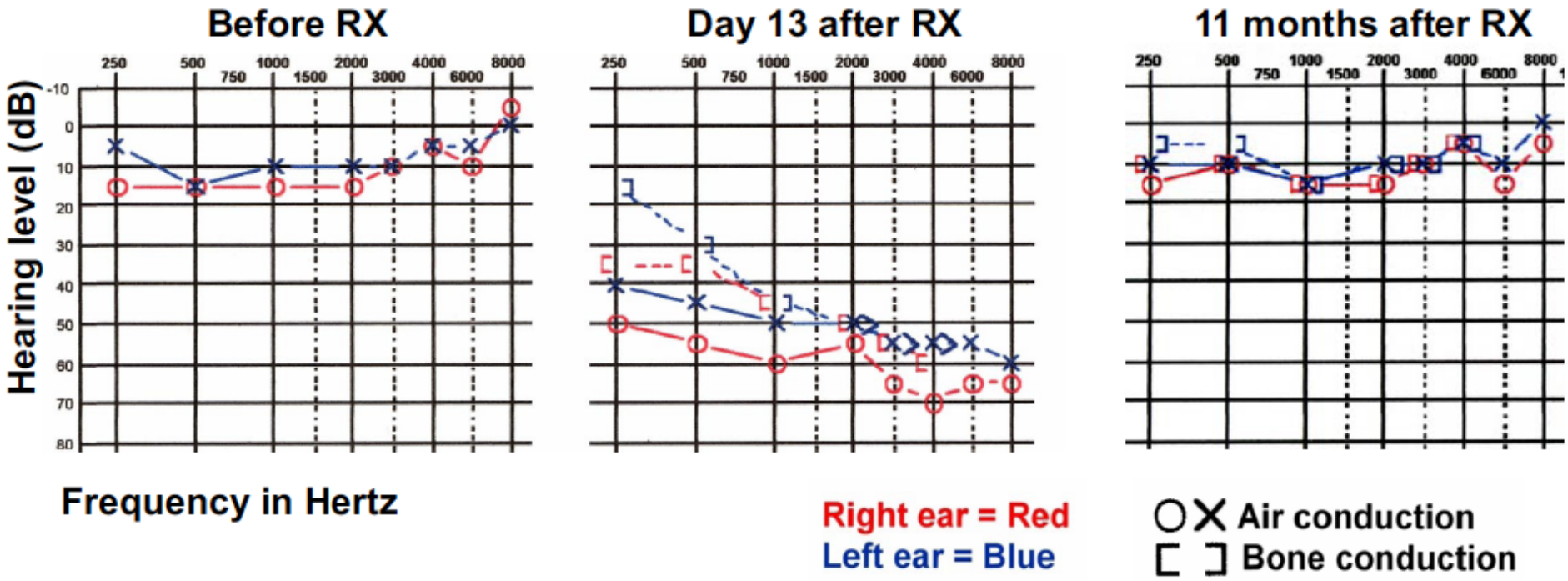
Toxicity observed in TCR gene therapy trials

- DMF4: no toxicity reported
- DMF5 + gp100 TCR:
  - Skin rash (100%)
  - Uveitis (50% of DMF5 treated pts)
  - Hearing loss (Vogt-Koyanagi-Harada) 25%
- DMF5 + DC vaccination
  - Skin rash and cytokine release syndrome
- 1D3 TCR
  - Cytokine release syndrome (multi-organ failure)
  - Skin rash

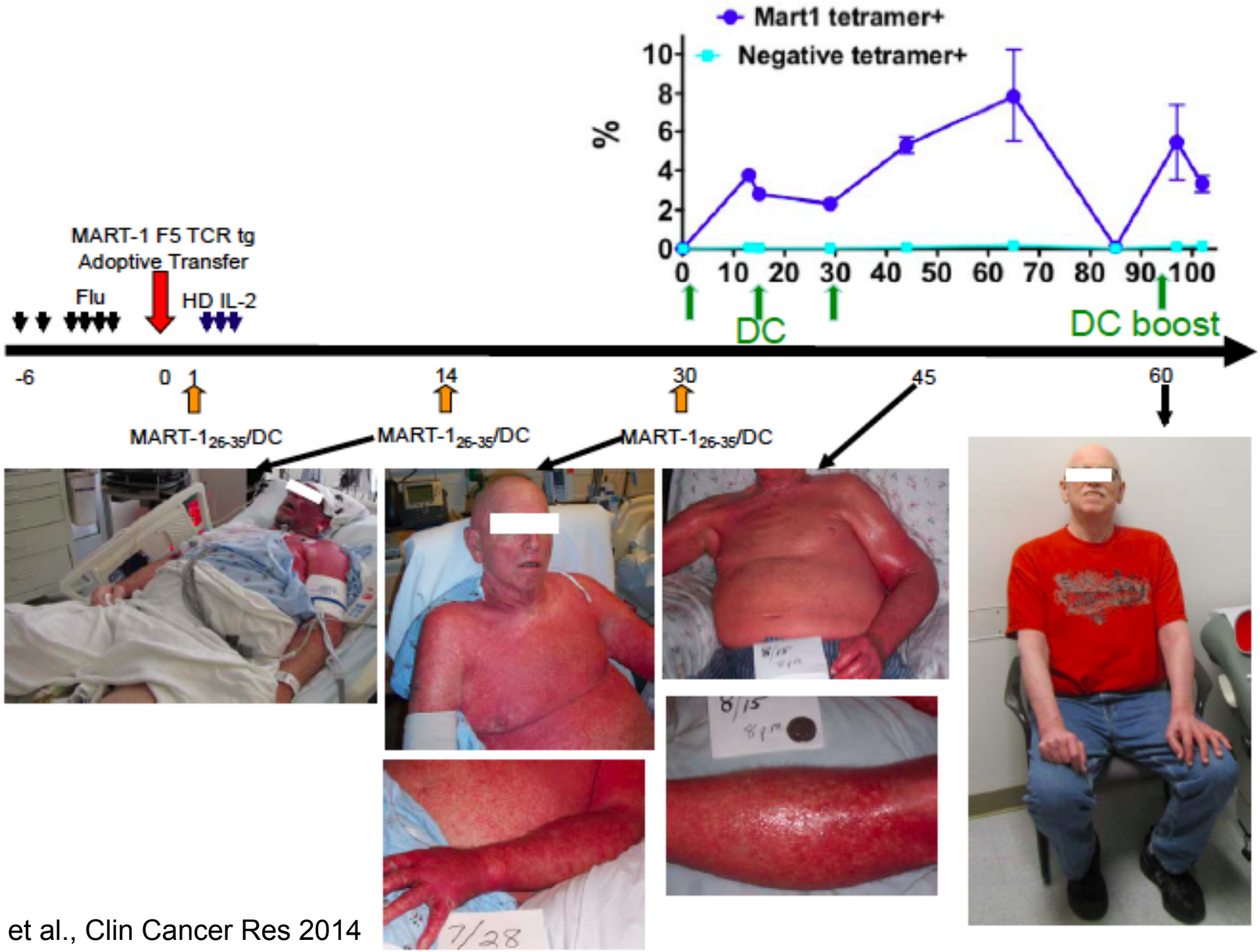
On target toxicity in eye and inner ear



Johnson et al., Blood 2009

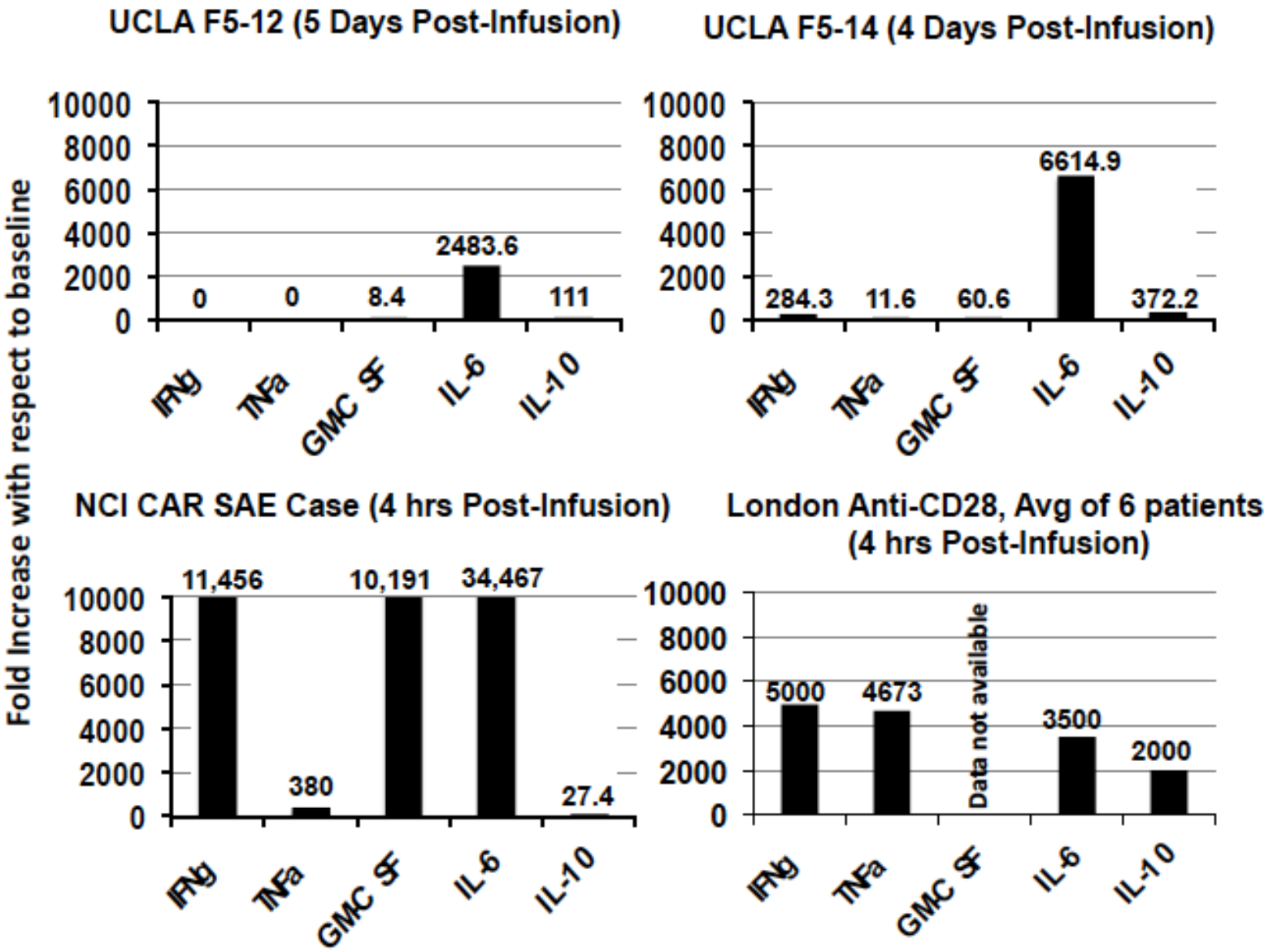


Recall whole body rash and re-expansion of the TCR transgenic cells in peripheral blood of patient F5-13 with subsequent MART-1/DC vaccinatio



Chodon et al., Clin Cancer Res 2014

Cytokine production by multiplex assay in plasma from patients F5-12 and F5-14 to study the potential development of a cytokine storm



Patient 1

- 43 year old female
- Bulky disease with two large abdominal metastases (16 and 18 cm)
- Multiple pulmonary, subcutaneous and lymph node metastases of 1-3 cm
- One small brain metastasis of 8mm
- Large volume (~10L) ascites

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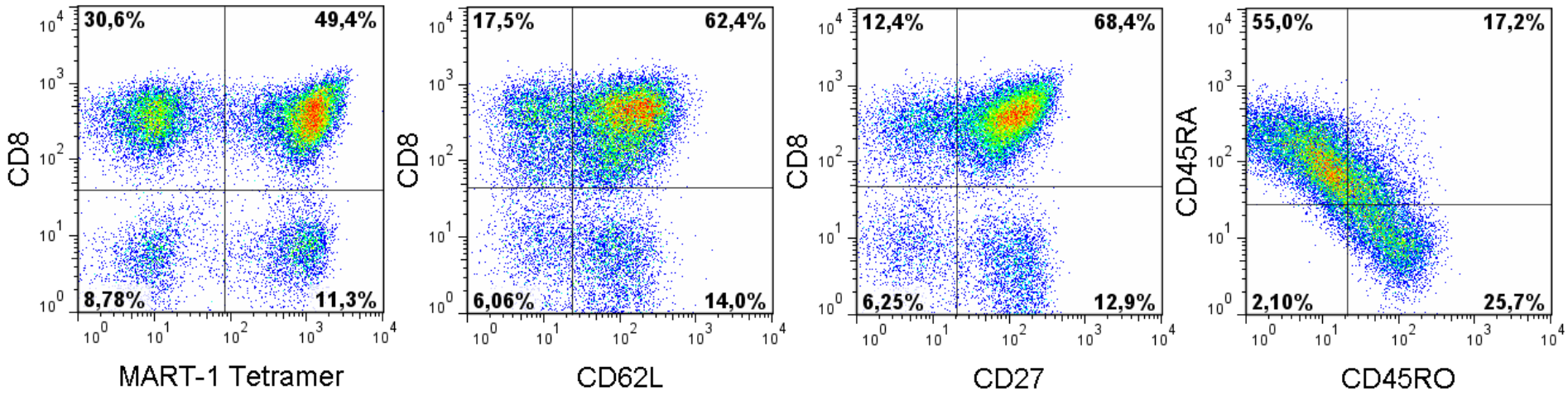
Infusion product: characteristics

- Transduction efficiency 61%
- $4.56x10^9$ transduced cells
- $CD8^+/CD4^+$ ratio 80/20
- Sterile
- Functional *in vitro*



Clinical course

- T cell infusion at day 0
- At day 1, patient developed high fever (>40°C)
- Blood positive for ESBL at day 1
- Patient treated with imipenem and vancomycin
- Stabilized during following days, no signs of sepsis
- Vancomycin discontinued at day 4
- Patient showed fluid retention
- No sign of on-target toxicity against MART-1

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Clinical course

- Morning of day 6, not responding to verbal stimuli
- Fever of 39.5°C, high pulse (110/min)
- Antibiotic regimen changed to meropenem, vancomycin and amoxicillin
- Transport to ICU. Generalized tonal clonal convulsion.
- Cardiac arrest
- Intubated and resuscitated for 10 minutes
- Kept in coma
- CT scan showed brain edema and bleeding of lesion
- All cultures remained sterile (sputum, BAL, ascites, liquor)
- No meningitis
- No tumor lysis syndrome
- After 48 hours, sedation stopped, no neurological improvement → patient died on day 9 from multiple organ failure

Possible explanations

1. Bacterial sepsis
2. Cardiac arrest induced by heart failure/ hemorrhage
3. T cell related
   a) Recognition other MHC-peptide complex
   b) Cytokine release

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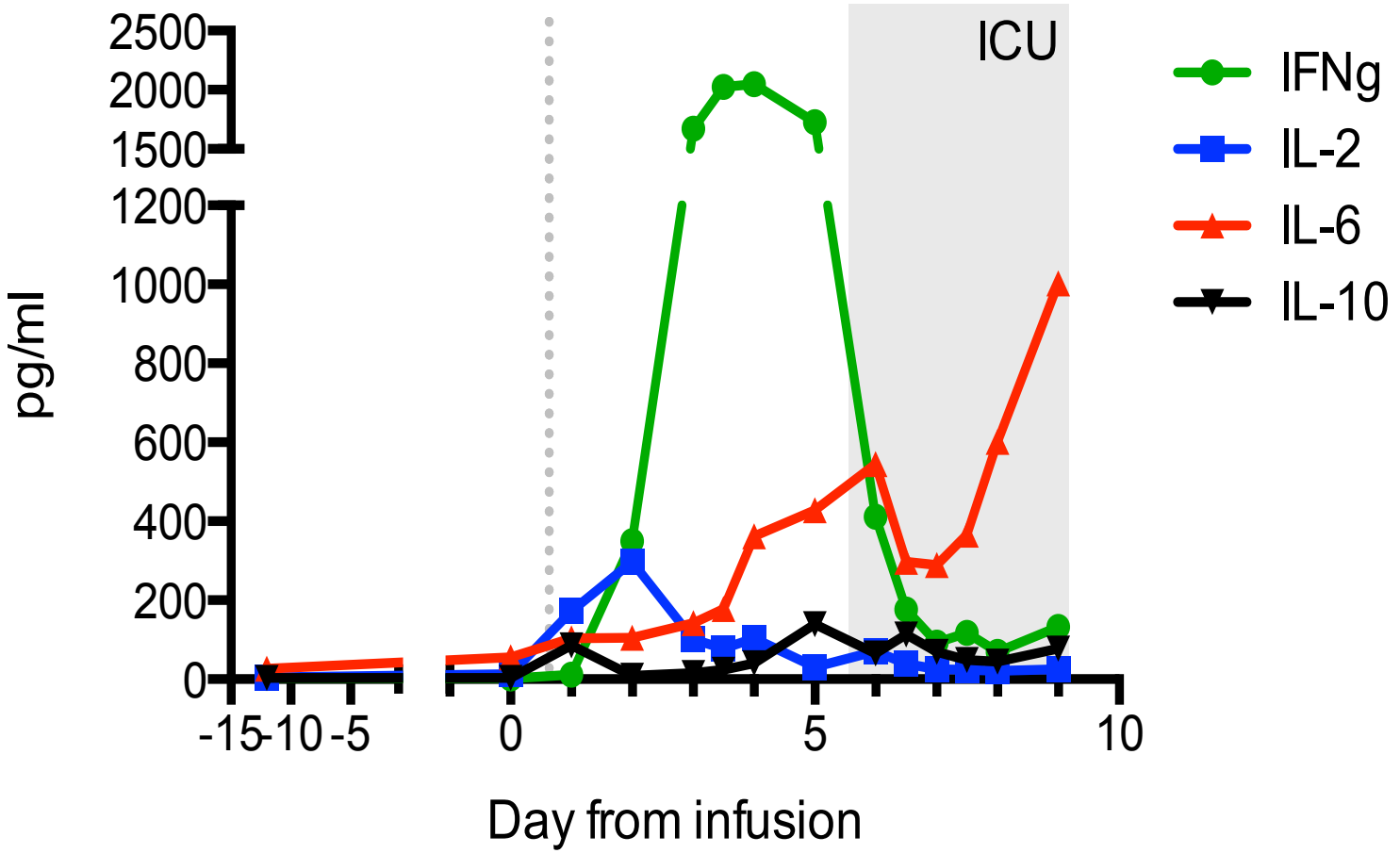
Cytokine release in multiple organ failure patient 1 at NKI

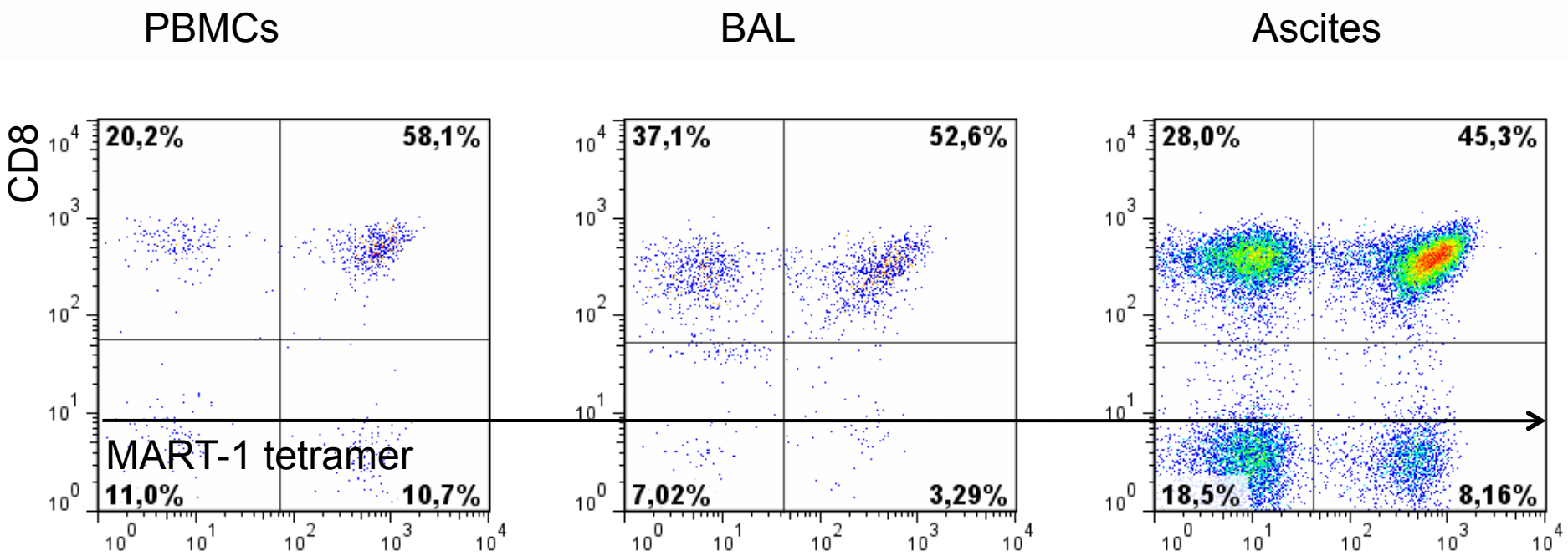
- Consecutive rise of IFN-γ and IL-6



- Same profile BAL and ascites

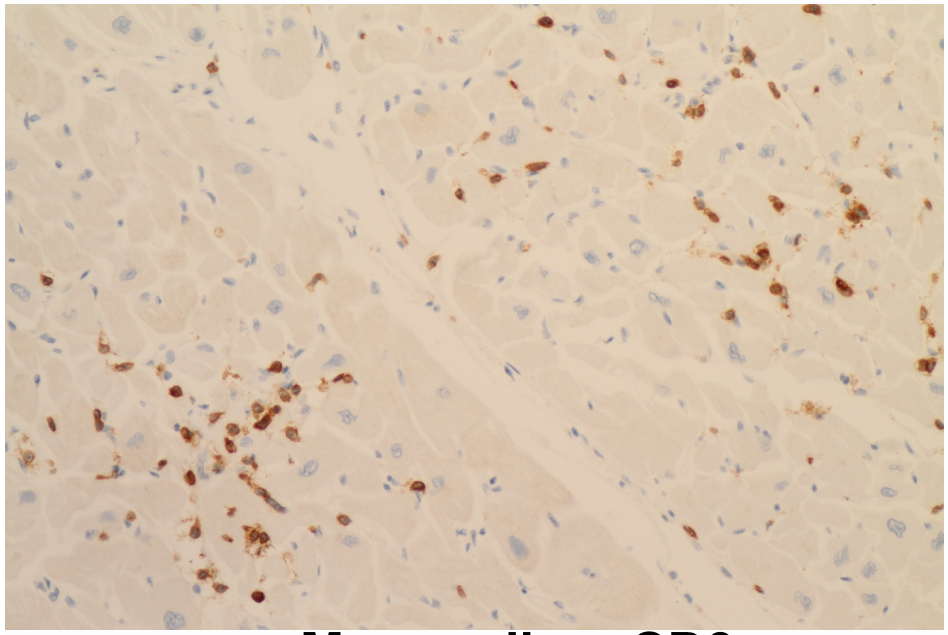
1D3 TCR modified cells present in body fluids

Day 7

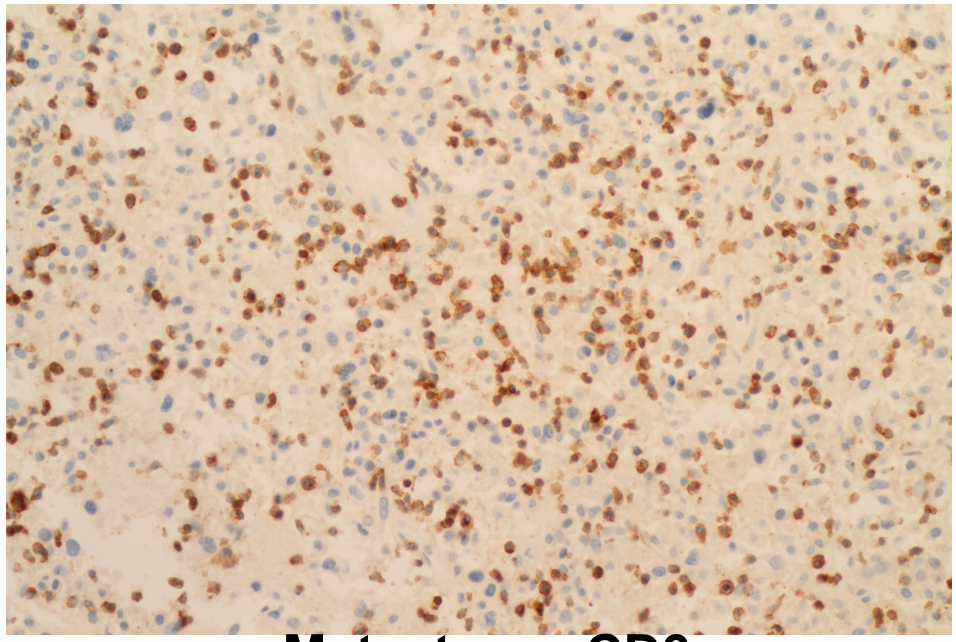


Post mortem examination

- Lymphocytic myocarditis with influx of CD3/CD8/ Granzyme B/MART-1 TCR^+ cells
- Extensive infiltration of CD3/CD8/Granzyme B/MART-1 TCR^+ cells in peritoneal metastases
- Minor T cell infiltration in other organs



Myocardium CD3



Metastases CD3

Patient 2: On-target activity

- Inflamed moles and a macular rash
- Vitiligo (depigmentation) of the skin

→Biopsy of skin showed T cell infiltration

→Local loss of melanocytes at inflammation site

→Vitiligo confirmed by Woods lamp

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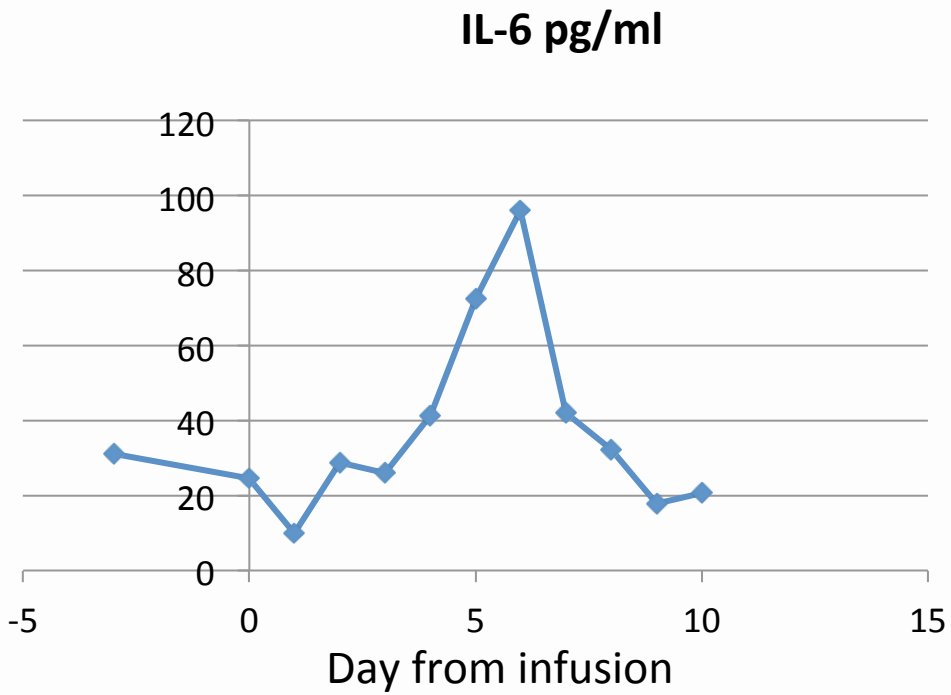
Small peak in IL-6 at time of skin rash and fever



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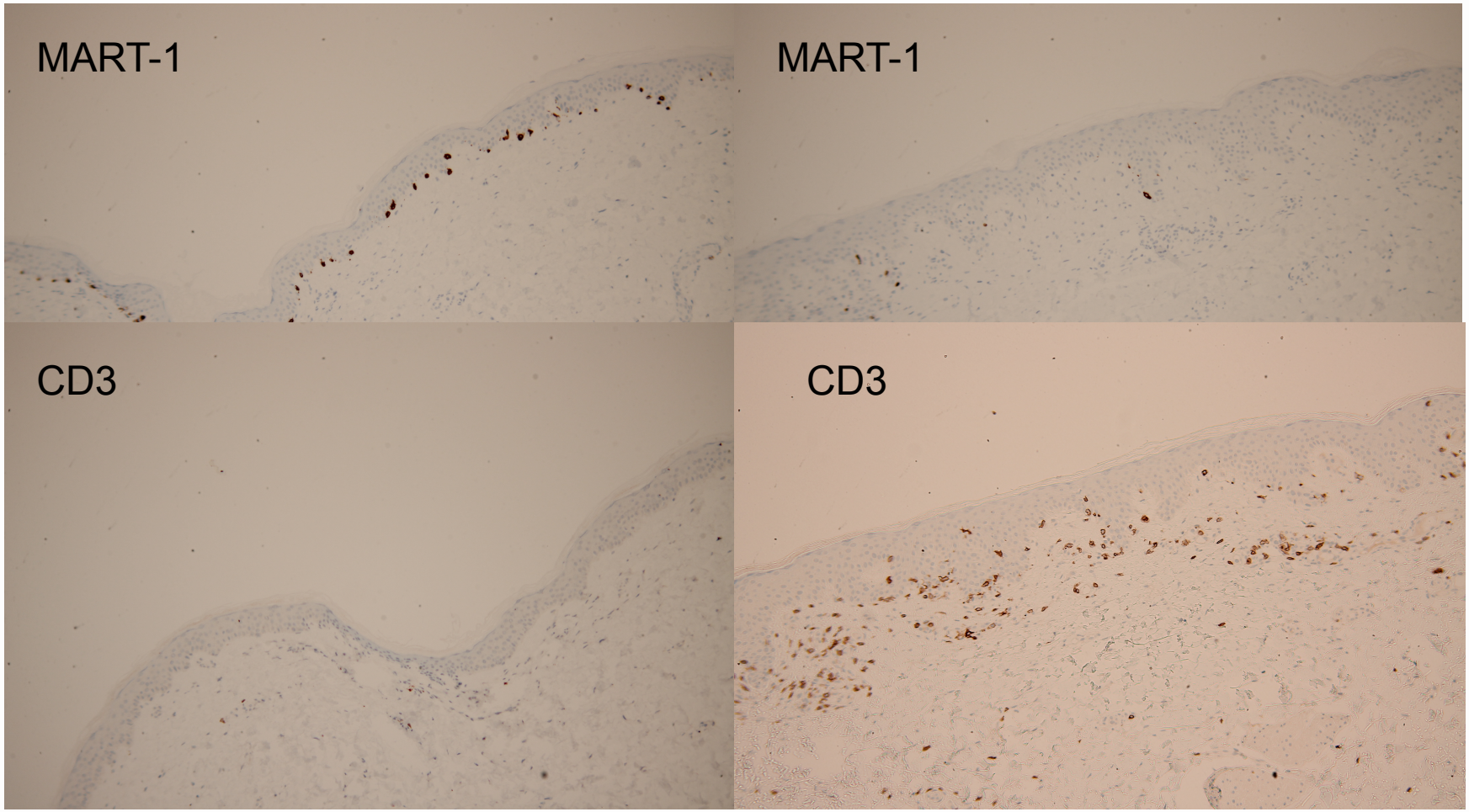
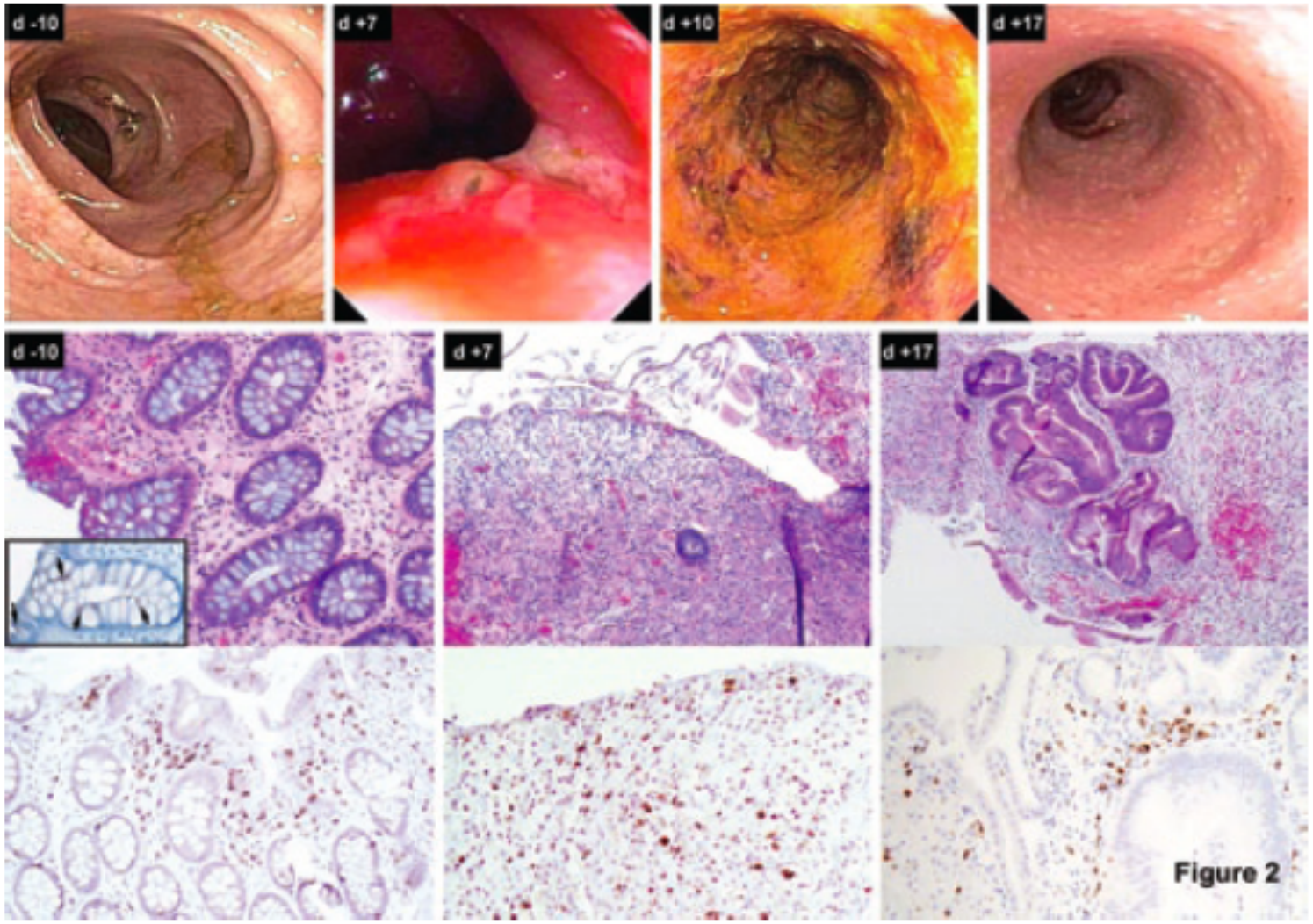
Skin reaction

- Skin rash
- Biopsy from normal skin inflamed skin



Severe colitis in CEA TCR gene therapy



Parkhurst et al., Molecular Therapy 2009

MAGE-A3 TCR gene therapy trials

- TCR recognizing HLA-A1 restricted MAGE-A3 peptide
  - Melanoma and multiple myeloma patient
  - Affinity of original TCR was improved (CDR3 region)
  - Both patients developed hypotension and severe cardiac problems leading to death within 5 days after adoptive therapy

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MAGE-A3 TCR gene therapy trials

- TCR recognizing HLA-A2 restricted MAGE-A3 peptide KVAELVHFL (shared with MAGE-A9
  - 9 cancer patients expressing MAGE-A3
  - Affinity modified TCR from murine origin
  - 3 out of 9 patients developed severe neuological symptoms
    - Epileptic seizures, mental disturbances
    - Infarcted areas on brain MRI
    - 2 patients died, 1 fully recovered

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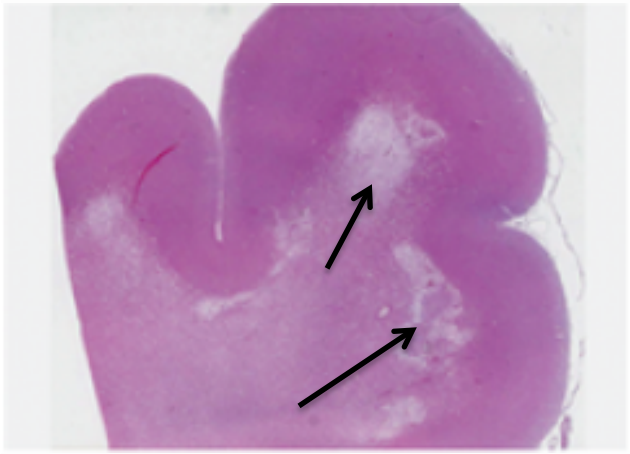
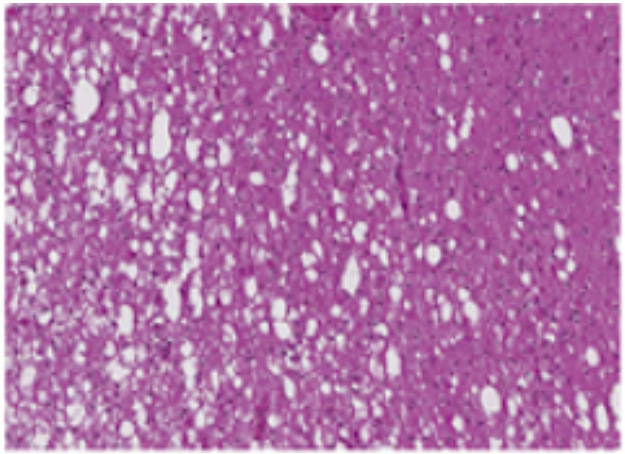
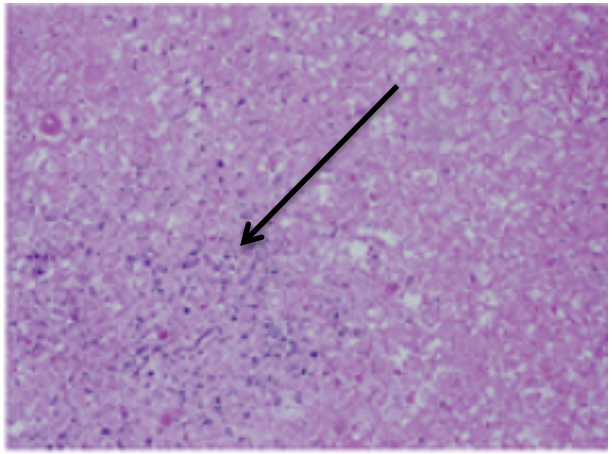
Brain damage on autopsy



White matter changes



Vacuolization



Areas of infarction

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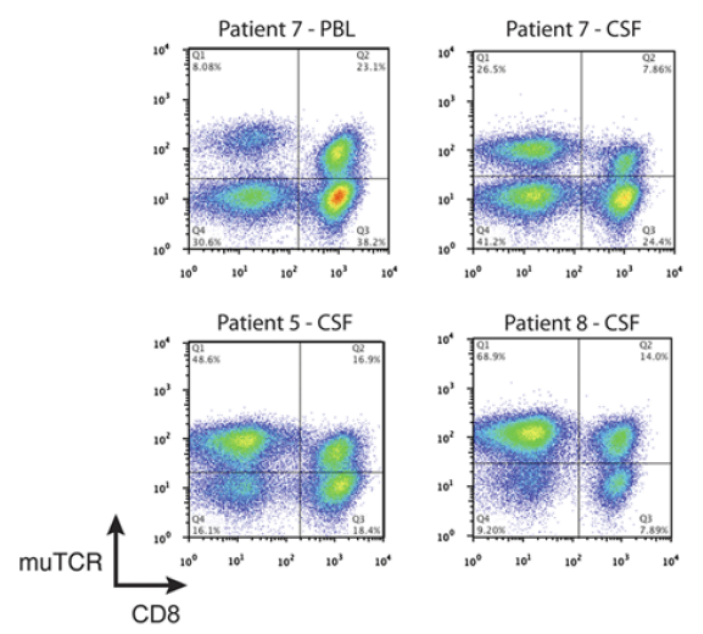
Morgan et al., J Immunotherapy 2013

Cross-reactivity of HLA-A2/MAGE-A3 peptide specific TCR with low level MAGE-A12 expression in brain



- MAGE-A12 is expressed at low level in the brain, whereas MAGE-A3 is not
- MAGE-A3 TCR recognizes MAGE-A12 peptide
- Neurological toxicity may be caused by cross-reactivity of T cells with MAGE-A12 peptide in brain

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Morgan et al., J Immunotherapy 2013

Future of TCR gene therapy

- Target choice?
- Which viral platform?
- Affinity modification?
- Suicide switch?
- Which cell type (T cell? Which T cell? NK cell?)
- How to activate and to expand?
- How many cells should we infuse?
- Deletion of endogenous TCR?
- Deletion of inhibitory receptors?
- Improve trafficking?

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Acknowledgements

Department of Immunology

Raquel Gomez-Eerland
Annelies Jorritsma
Nienke van Rooij
Samira Michiels
Bianca Kaiser-Heemskerk
Pia Kvistborg
Sander Kelderman
Willeke van den Kasteele
Trees de Jong
Christian Blank
Adriaan Bins

Ton Schumacher

Pathology

Bart van der Wiel

CRA and NP

Loes Pronk
Henk Mallo
Sandra Adriaansz

ICU

Lenie Hulshoff

Centre Pluridisciplinaire d'Oncologie Lausanne, Switzerland

Serge Leyvraz
Pedro Romero
Emanuela Romano
Alexandre Harari

Immunocore

Namir Hassan
Dominque Smethurst
Bent Jakobsen

Sheba Medical Insitute

Michal Besser
Jakob Schachter

Surgery Branch, NIH

Mark Dudley
Steven Rosenberg

Slotervaart Pharmacy/AmBTU

Susanne Quaak
Edith Vermeij
Bas Beerman
Denise Serras Geraldes
Roel van Gijn
Joost van den Berg
Bastiaan Nuijen
Jos Beijnen

Floor 4B with nursing staff
Clinical laboratory
FACS facility

Sanquin

NKI-KWF Research Fund