DET SUNDHEDSVIDENSKABELIGE FAKULTET
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REGION Herlev Hospital

SITC 29th annual meeting

National Harbor
November 6 – 9, 2014

Melanoma Therapy using Adoptive Transfer of Expanded Tumor Infiltrating T cells; Prospects and Pitfalls

Per thor Straten, professor, PhD,
Center for Cancer Immune Therapy (CCIT)
Copenhagen University Hospital, Herlev
Denmark

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First things first

Nothing to declare

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Outline of talk….

The CCIT experience

* Why initiate TIL therapy in melanoma ?
* Sum-up of our TIL trial incl clinical data
* Biological monitoring
* Next steps

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A case story

* Complete response and yet…..

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A case story

* Complete response and yet…..

Some more monitoring:

* A glance at CD4 T cells among TIL

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Acknowledgements

From CCIT, Herlev Hospital, Denmark

CCIT staff – but in particular:

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Manja Idorn

Collaborators

Barbara Seliger, Institute of Medical Immunology, Martin Luther University, Halle-Wittenberg, Germany

Göran Jönsson, Melanoma Genomics Unit, Lund university, Sweden
John Haanen (Amsterdam) & Robert Hawkins (Manchester)
Mark Dudley & Steve Rosenberg NIH, Washington, US

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Why initiate TIL therapy in melanoma

Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes

Mark E. Dudley,[1] John R. Wunderlich,[1] Paul F. Robbins,[1] James C. Yang,[1] Patrick Hwu,[1] Douglas J. Schwartzentruber,[1] Suzanne L. Topalian,[1] Richard Sherry,[1] Nicholas P. Restifo,[1] Amy M. Hubicki,[1] Michael R. Robinson,[2] Mark Raffeld,[3] Paul Duray,[3] Claudia A. Seipp,[1] Linda Rogers-Freezer,[1] Kathleen E. Morton,[1] Sharon A. Mavroukakis,[1] Donald E. White,[1] Steven A. Rosenberg[1]*

We report here the adoptive transfer, to patients with metastatic melanoma, of highly selected tumor-reactive T cells directed against overexpressed self-derived differentiation antigens after a nonmyeloablative conditioning regimen. This approach resulted in the persistent clonal repopulation of T cells in those cancer patients, with the transferred cells proliferating in vivo, displaying functional activity, and trafficking to tumor sites. This led to regression of the patients' metastatic melanoma as well as to the onset of autoimmune melanocyte destruction. This approach presents new possibilities for the treatment of patients with cancer as well as patients with human immunodeficiency virus–related acquired immunodeficiency syndrome and other infectious diseases.

9) and may depend on the destruction of regulatory cells, disruption of homeostatic T cell regulation, or abrogation of other normal tolerogenic mechanisms.

To determine whether prior lymphodepletion might improve the persistence and function of adoptively transferred cells, 13 HLA-A2$^+$ patients with metastatic melanoma received immunodepleting chemotherapy with cyclophosphamide and fludarabine for 7 days before the adoptive transfer of highly selected tumor-reactive T cells and high-dose interleukin-2 (IL-2) therapy (*10*) (Table 1). These patients all had progressive disease refractory to standard therapies, including high-dose IL-2, and eight patients also had progressive disease despite aggressive chemotherapy. The patients received an average of 7.8×10^{10} cells (range, 2.3×10^{10} to 13.7×10^{10}) and an average of nine doses of IL-2 (range, 5 to 12 doses). The T cells used for treatment were derived from tumor-infiltrating lymphocytes (TILs) and were rapidly expanded in vitro (*11*). All cultures were highly reactive when stimulated with an HLA-A2$^+$ melanoma or an autologous melanoma cell line (Table 1 and table S1).

Six of the 13 patients had objective clinical responses to treatment and four others demonstrated mixed responses, with significant shrinkage of one or more metastatic

Dudley et al. Science, 298, 2002

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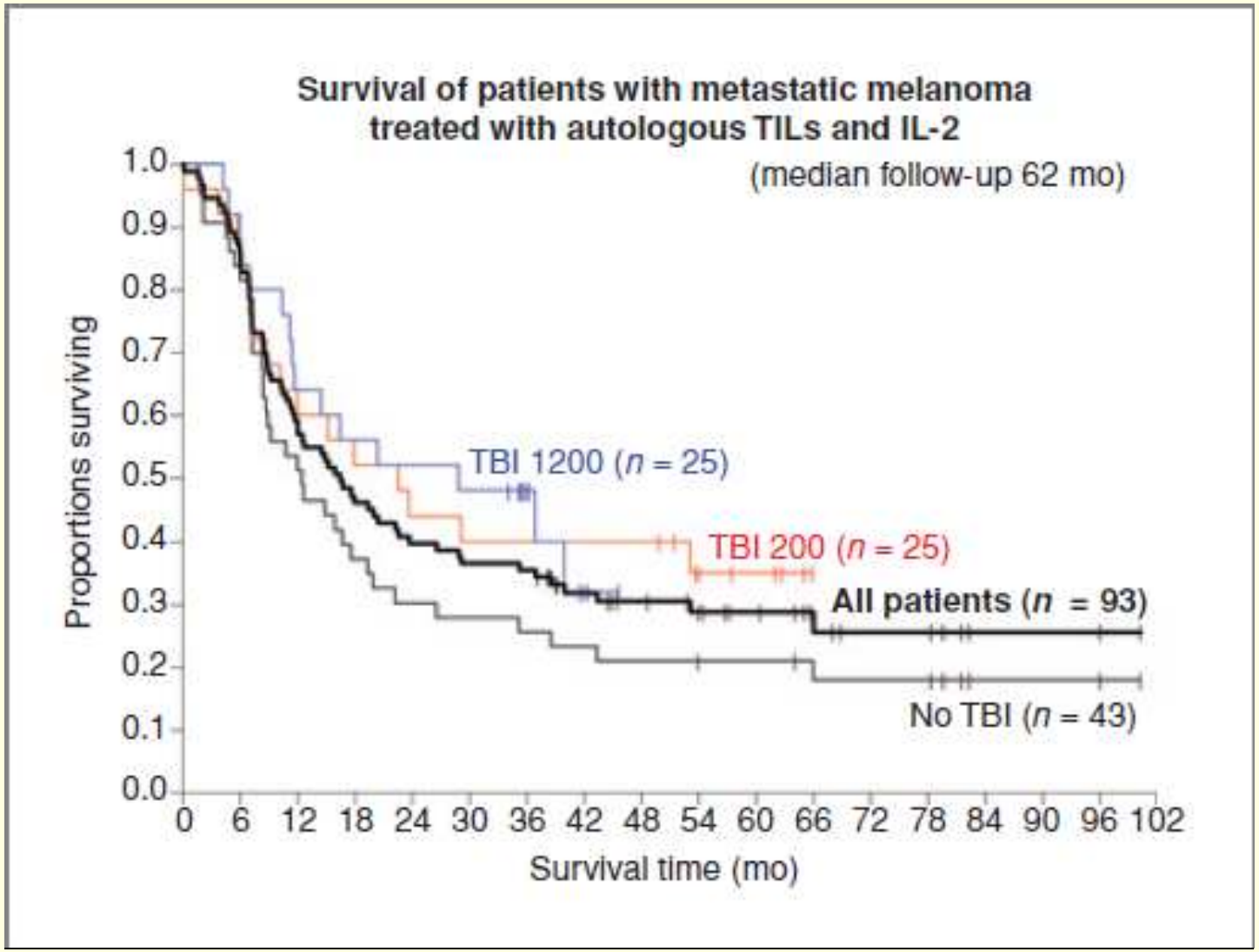
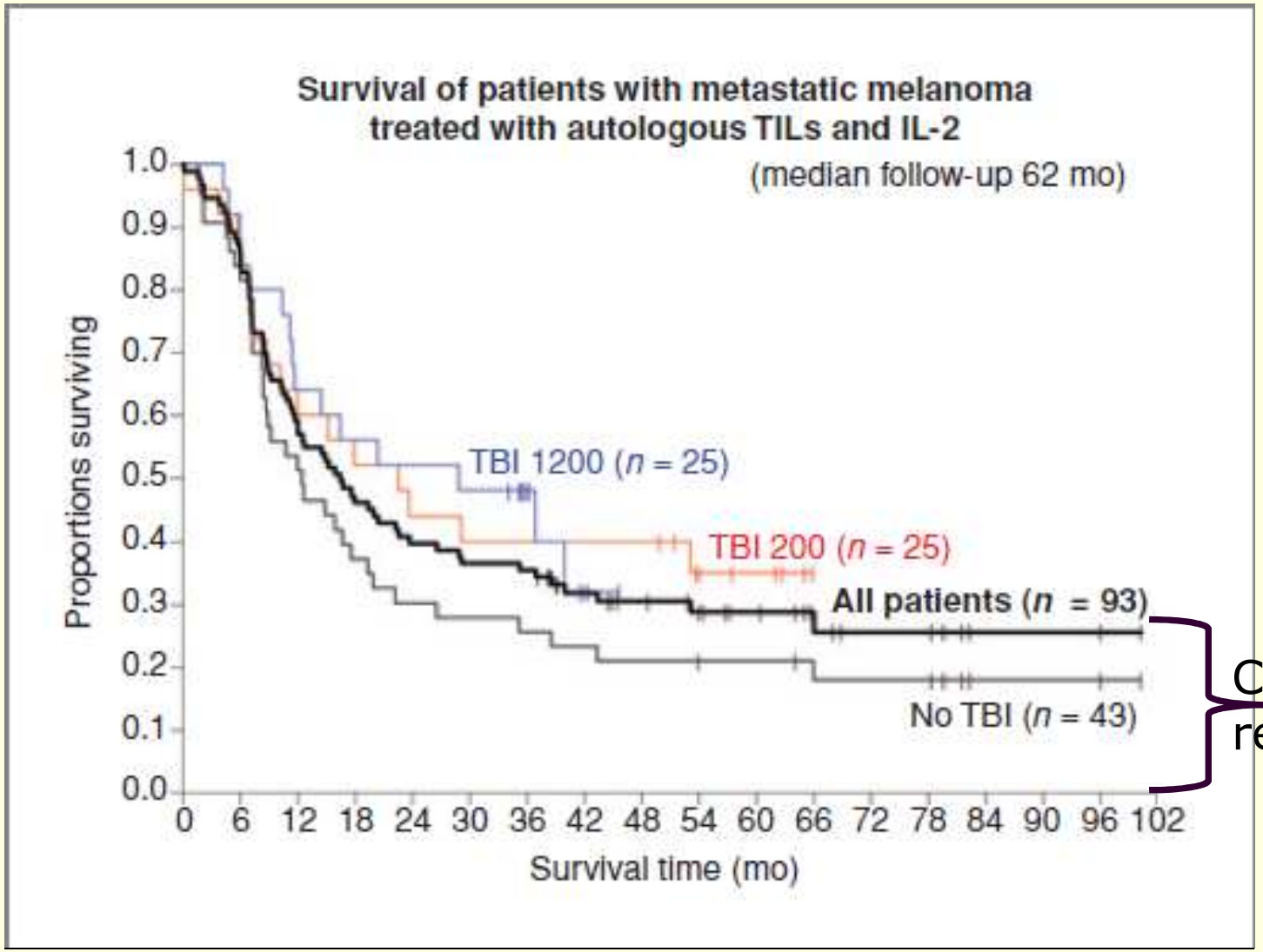
Why initiate TIL therapy in melanoma



Rosenberg et al., CCR, 2011, 17: 4550)

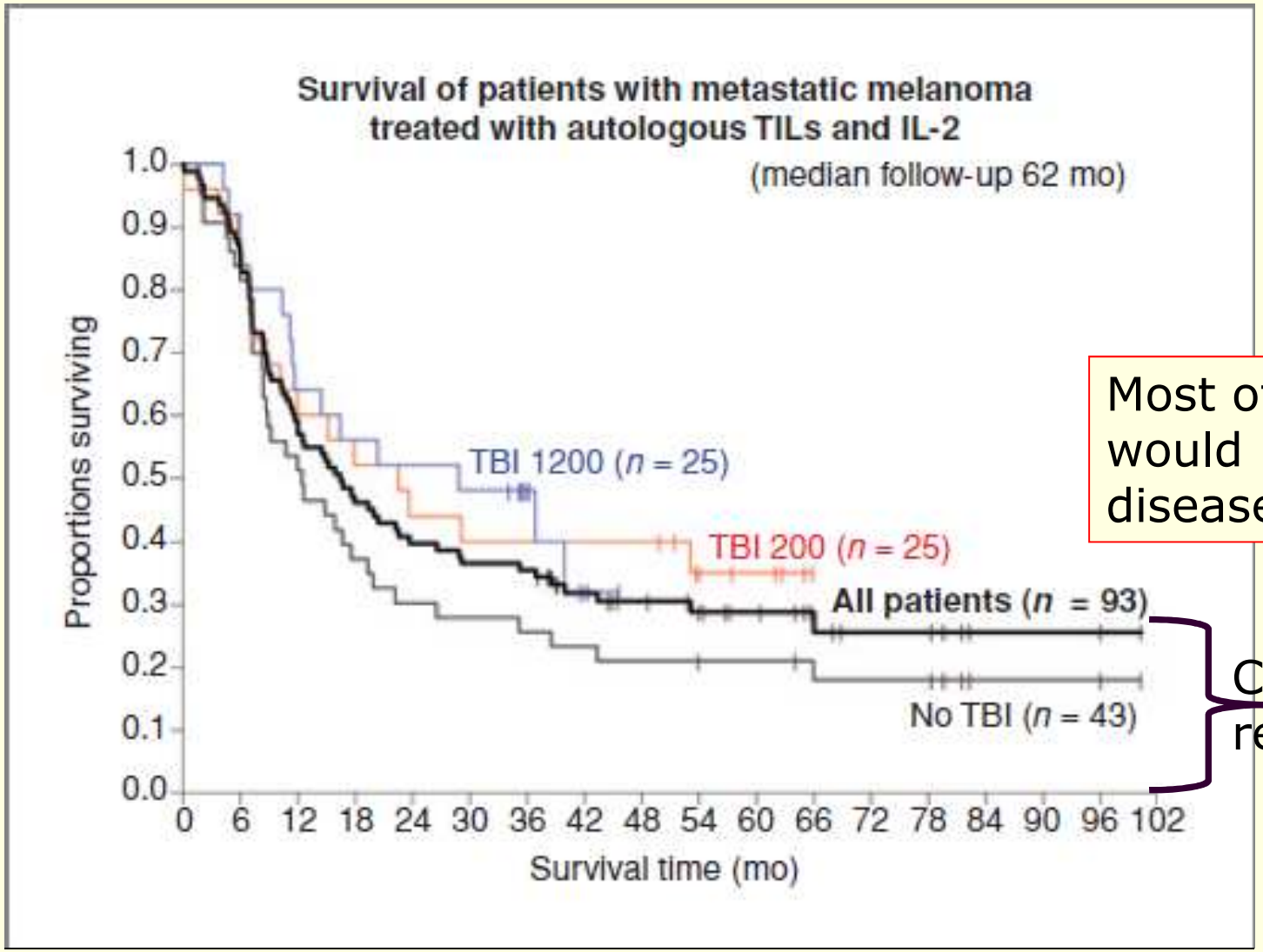
Why initiate TIL therapy in melanoma



Complete lasting responses

Rosenberg et al., CCR, 2011, 17: 4550)

Why initiate TIL therapy in melanoma

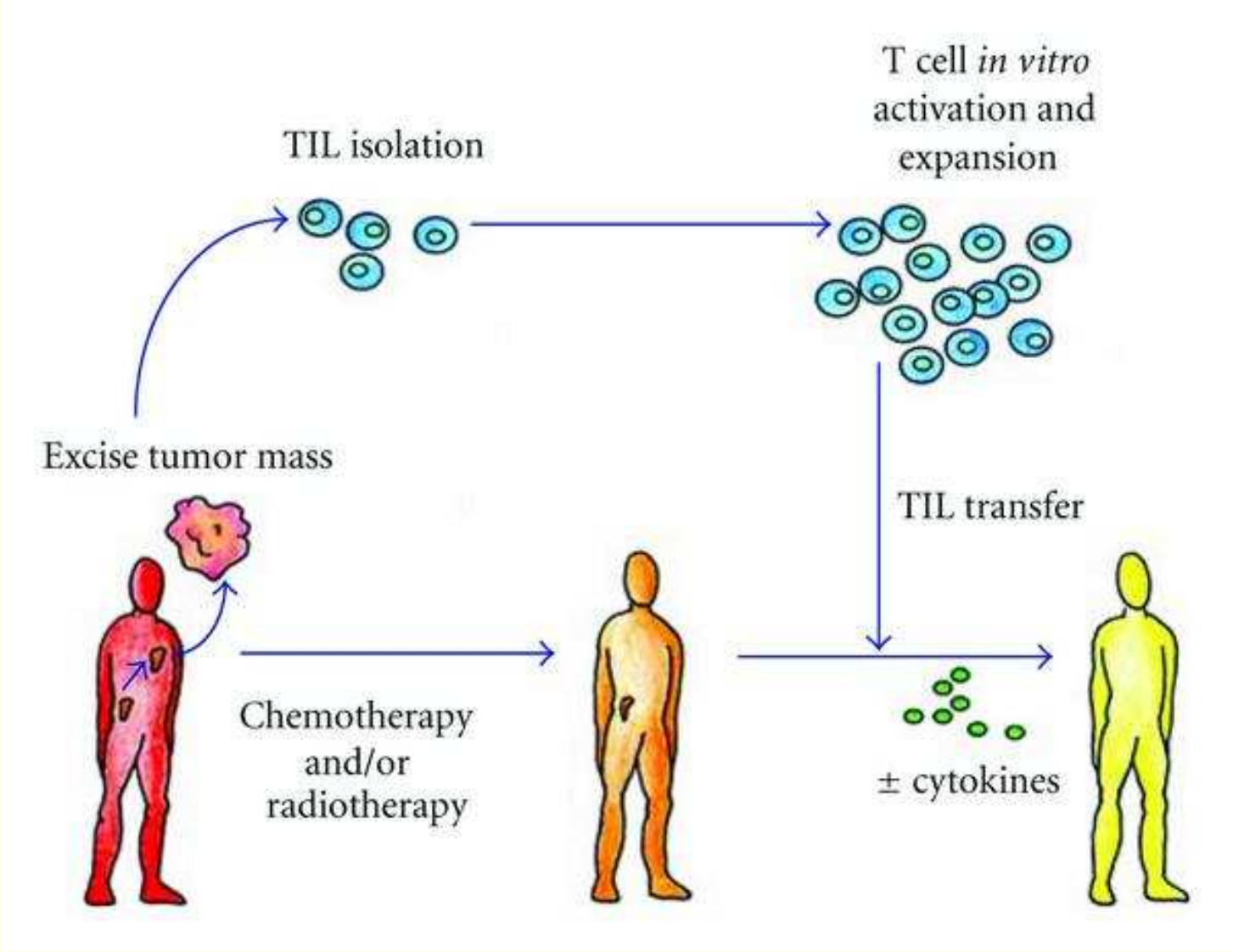


Most of these 20+ % would have died from disease !!!!!

Complete lasting responses

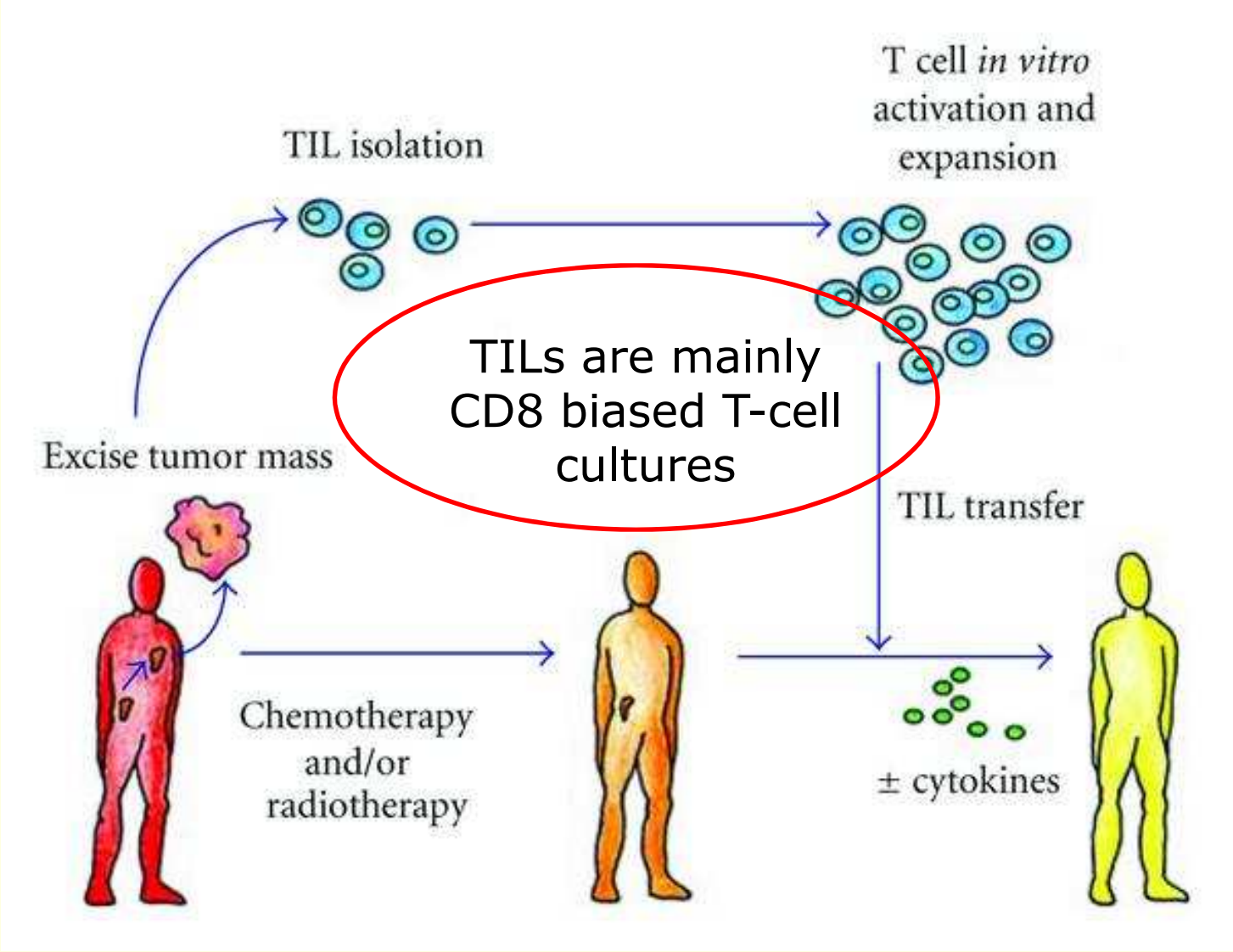
Rosenberg et al., CCR, 2011, 17: 4550)

TIL therapy: the short version



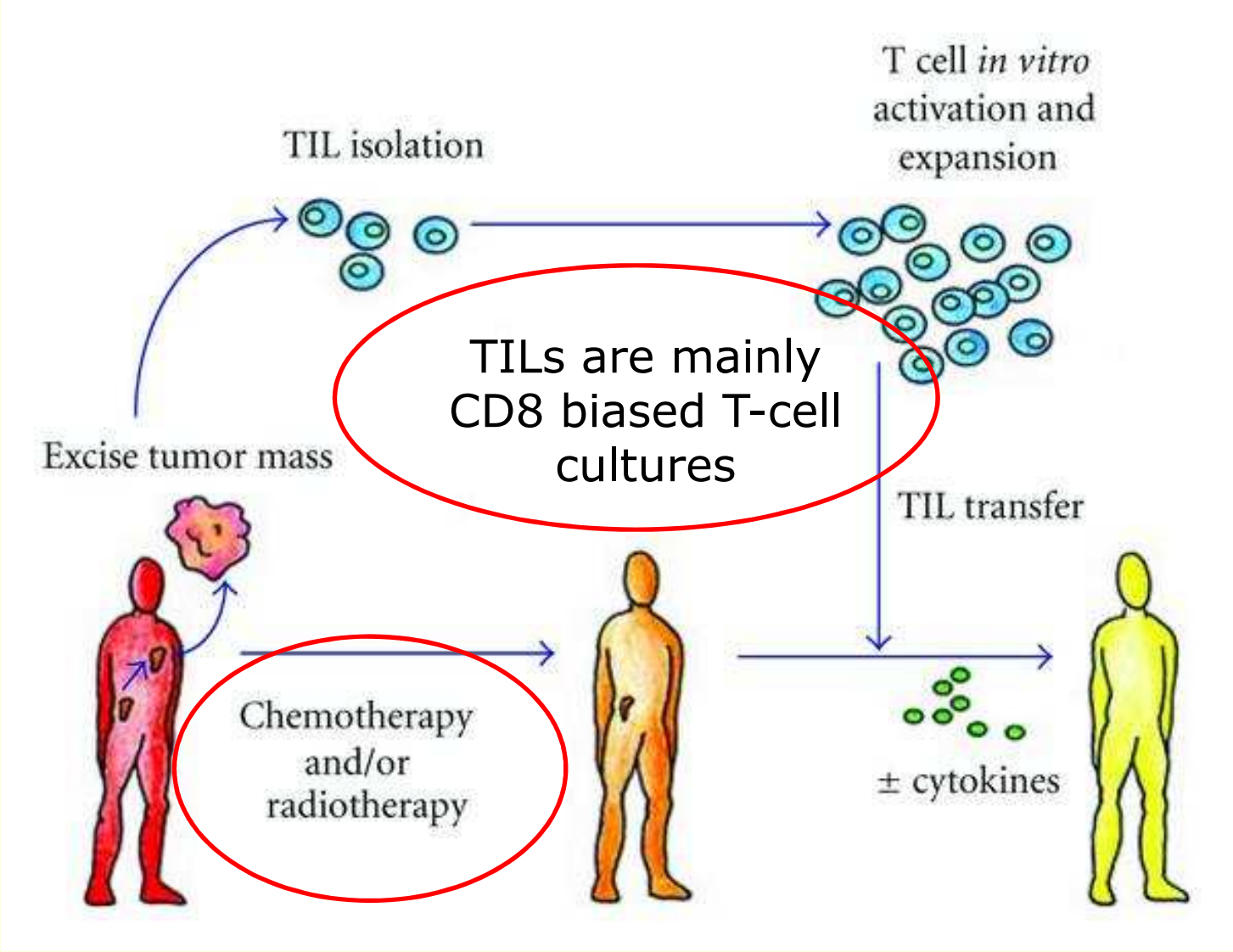
http://www.hindawi.com/journals/jir/2011/320571/fig3/

TIL therapy: the short version



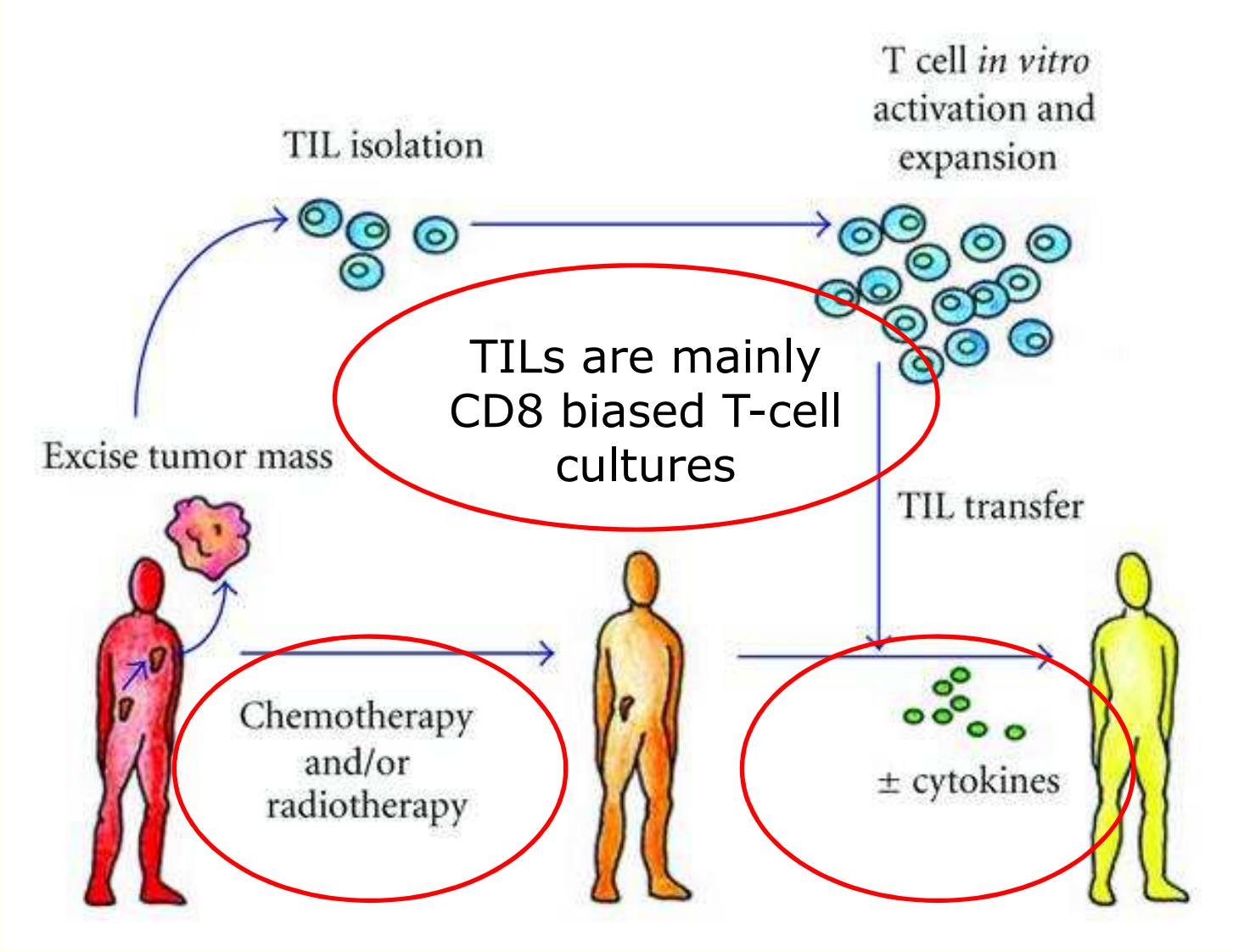
http://www.hindawi.com/journals/jir/2011/320571/fig3/

TIL therapy: the short version



http://www.hindawi.com/journals/jir/2011/320571/fig3/

TIL therapy: the short version



http://www.hindawi.com/journals/jir/2011/320571/fig3/

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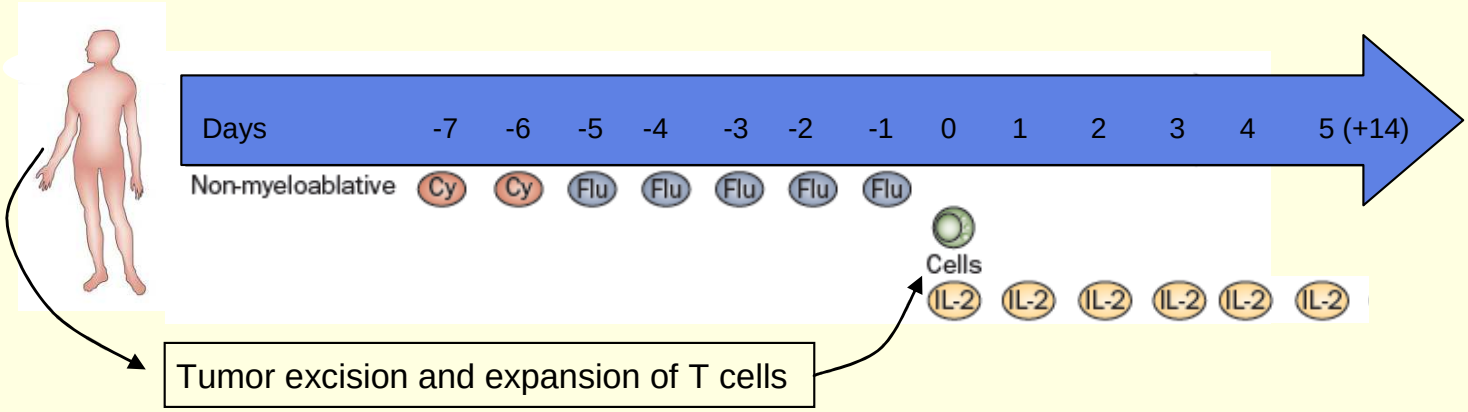
The CCIT experience



Interleukin-2

- **USA** → **High dose** (720.000 IU/kg i.v. every 8 hour)
- **CCIT Pilot study** (6 patients) → **low dose** (2 MIU s.c. daily for 14 days)
- **CCIT Amendment phase II** (recruiting – 25 treated) → **Intermediate dose** (iv decrescendo regimen: 18 MIU/m^2 over 6 h, 12 h and 24 h, 4.5 MIU/m^2 over 24 h for 3 days)*

***Keilholz et al. J Clin Oncol 1997**

Ellebaek *et al. Journal of Translational Medicine* 2012, **10**:169

Clinical trials.gov ID: NCT00937625

Clinical Response (RECIST 1.0)

31 patients treated

- 92% success rate for TIL-production
- 1 patient dead (CNS haemorrhage in brain metastasis)
- 2 patients evaluation pending

28 patients evaluated

- **4 CR** (49 (NED), 13 (NED),+33, +16 months)
- **7 PR** (+31 (NED), 12, +23 (NED), 12, +14, 8, +6 months)
- **12 SD** (4-6 months)
- **5 PD**

NED = No evidence of disease

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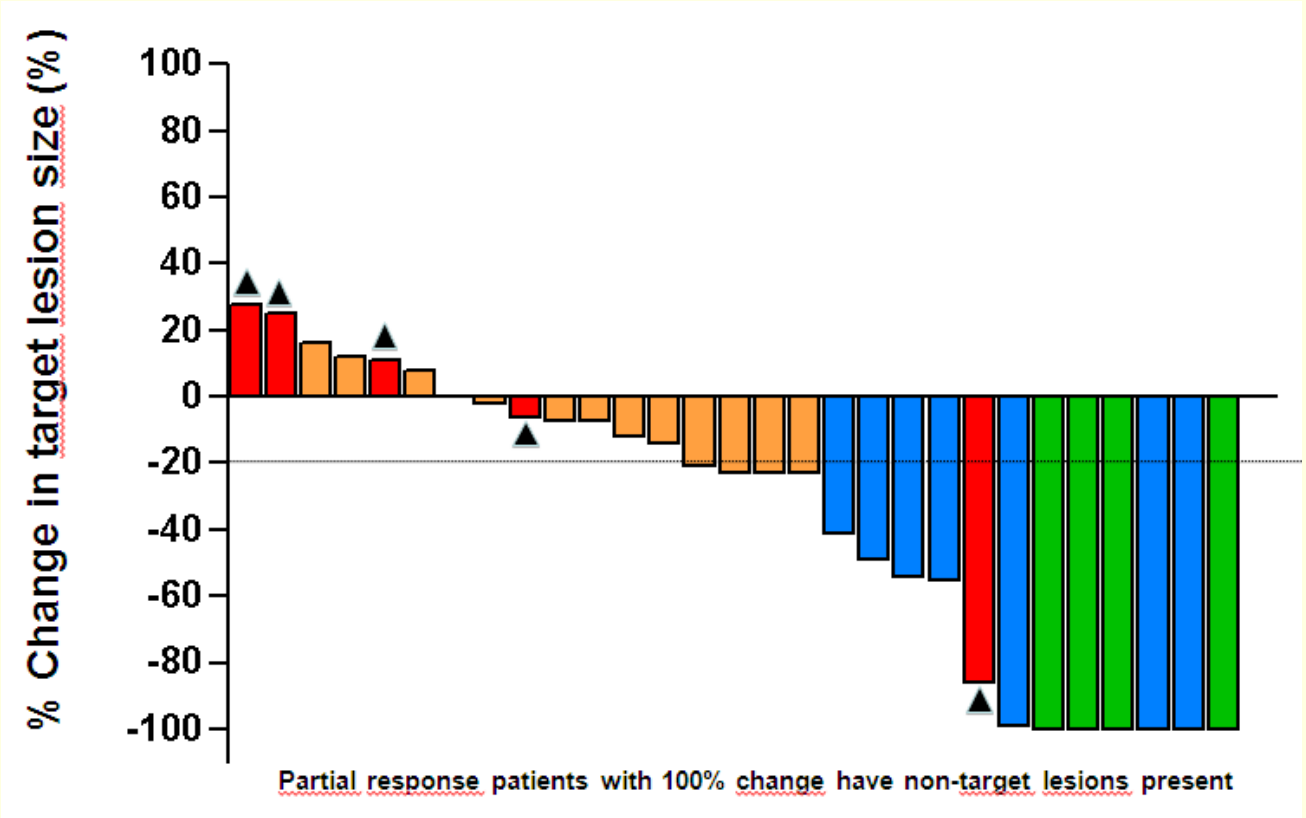
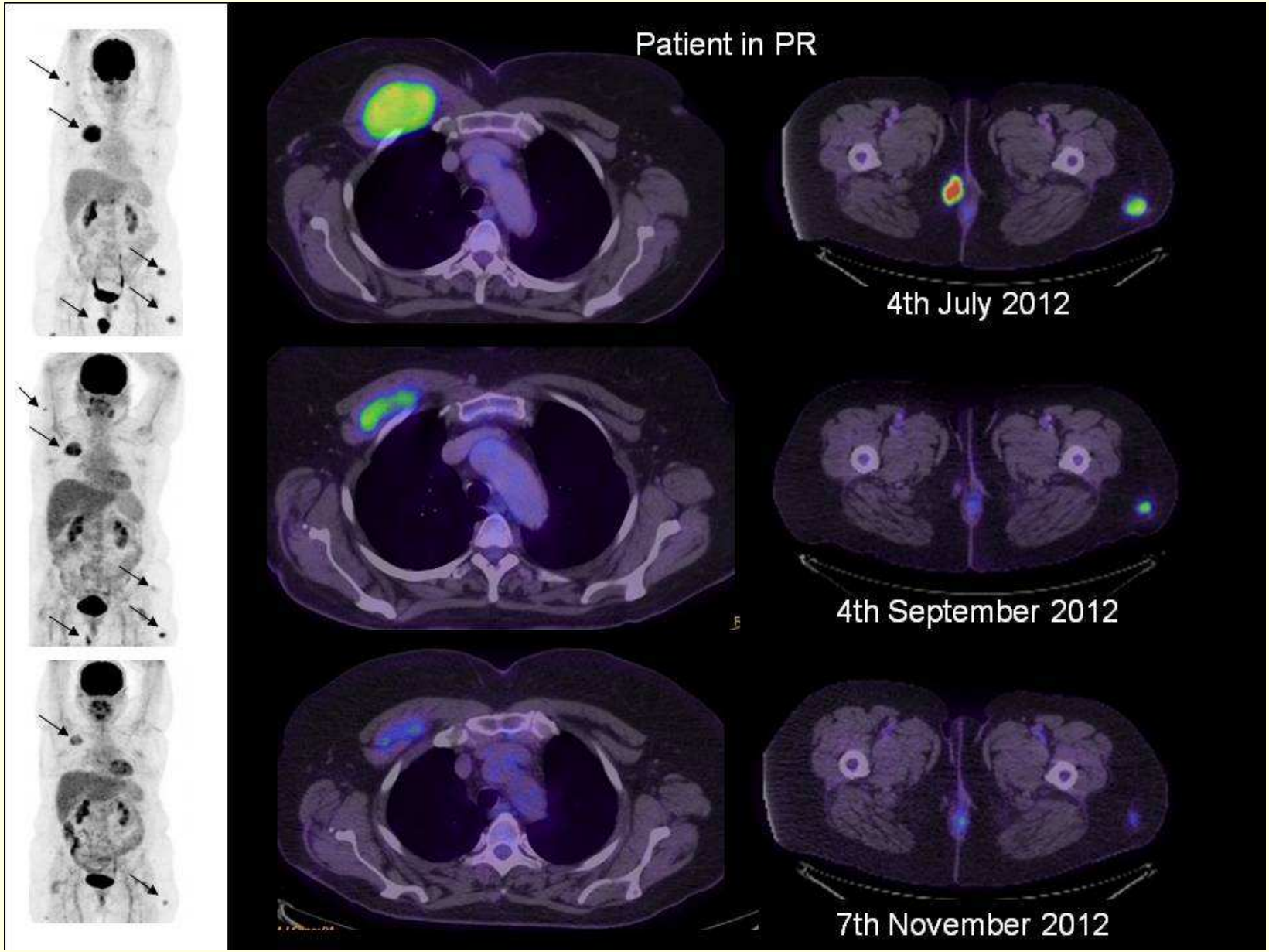
Clinical Response

Implementation of Lower Doses of IL-2

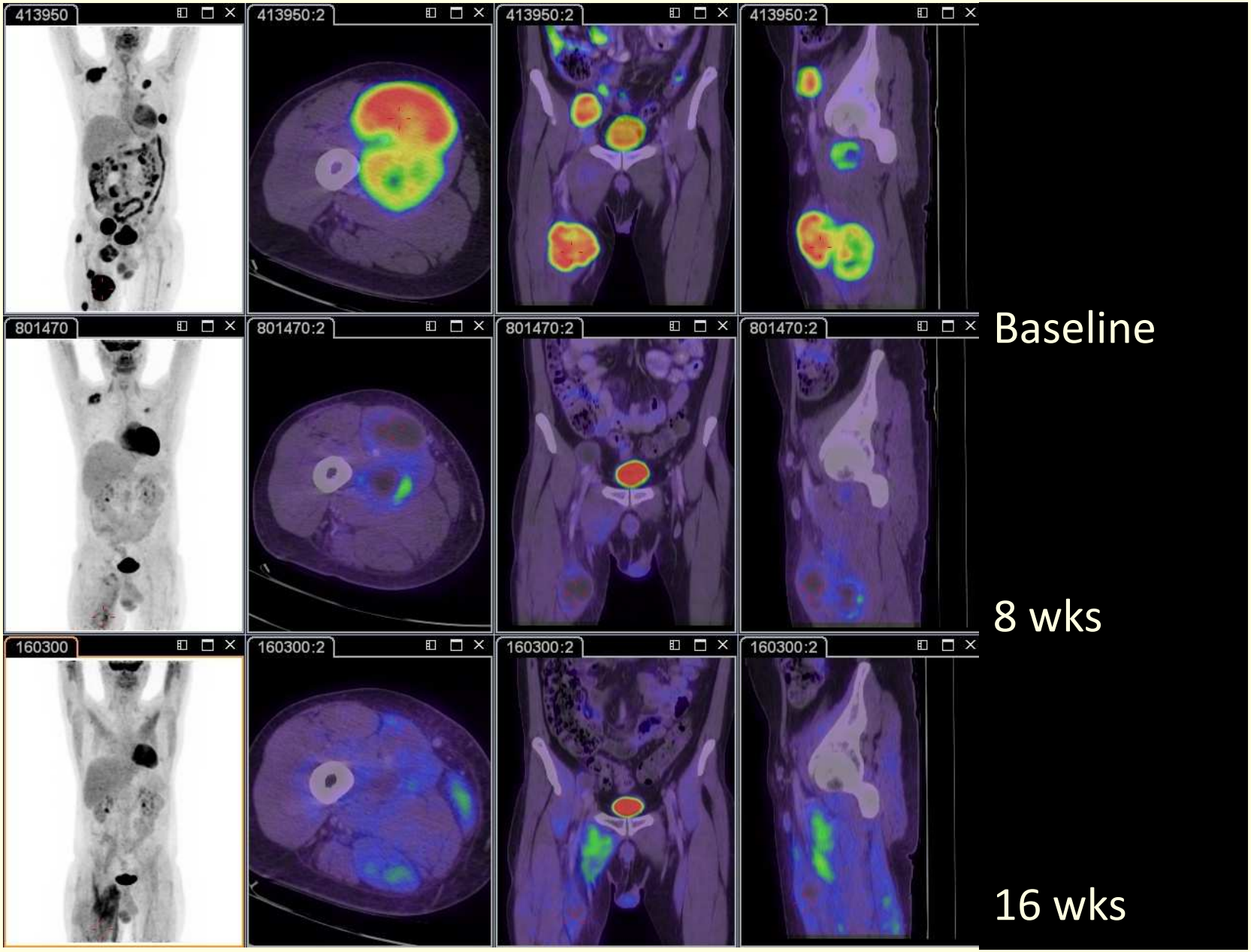
- **Reduced Toxicity**
- **Similar Clinical Results**



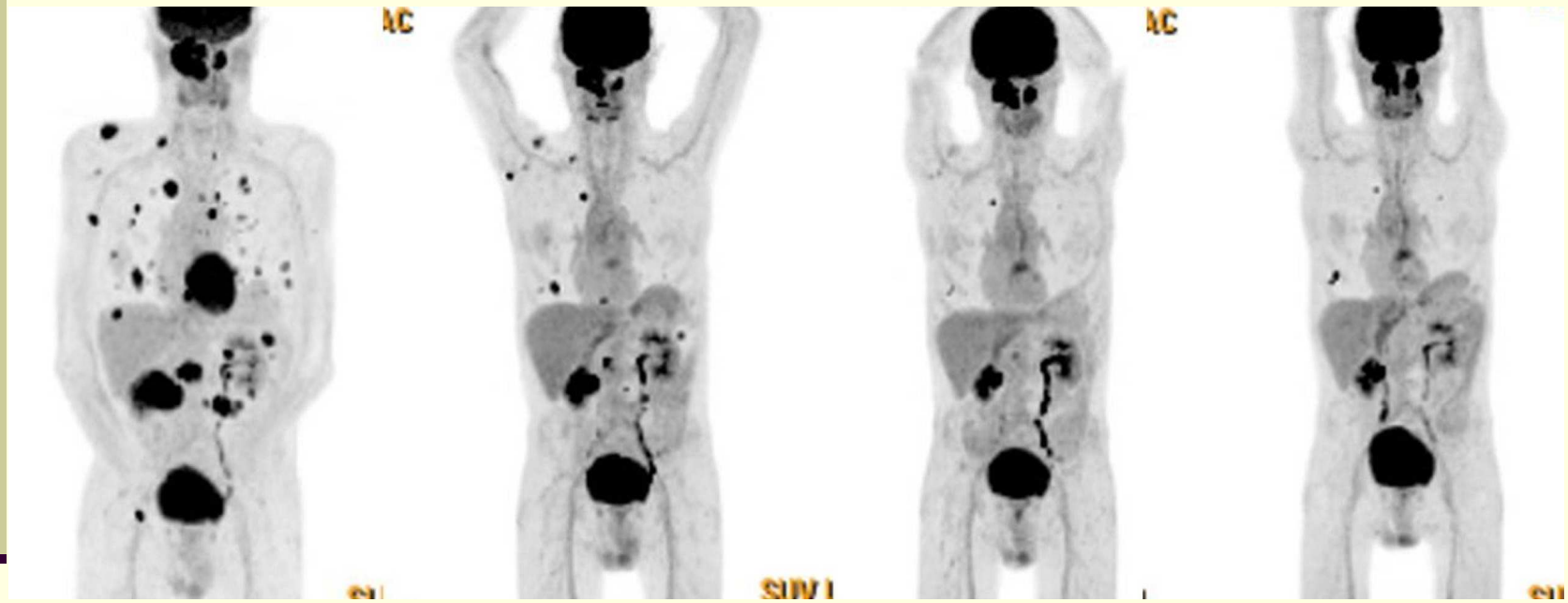
Patient MM0909.20 – PR/PMR (12 months)



Patient MM0909.26 – PR/PMR (+9 months)



Patient MM0909.31 – PR/PMR (+8 months)



Baseline 2 months 4 months 7 months

ACT using TIL in melanoma

Although phase III data are needed to make firm conclusions it seems that TIL therapy…..

* ***When it works*** **it can eradicate huge tumor masses….**

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ACT using TIL in melanoma

Although phase III data are needed to make firm conclusions it seems that TIL therapy…..

* ***When it works*** **it can eradicate huge tumor masses….(also with lower dose of IL-2)**

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Immune reactivity in TIL cultures

Monitoring of TIL cultures:

* Which antigens are recognized in the TIL lines ?
* Can they be followed over time upon treatment ?
* Correspondence between TAA recognition and clinical course ?

- What have we done ??

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Immune reactivity in TIL cultures

Monitoring of TIL cultures:

* Which antigens are recognized in the TIL lines ?
* Can they be followed over time upon treatment ?
* Correspondence between TAA recognition and clinical course ?

- What have we done ??
- Short answer – all we could !!!

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Immune reactivity in TIL cultures

This is where I skip some 40 slides (except a few…..)
based on published data and go to the conclusion…..

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Peptide specific T cells among TIL…?

Which peptide specificities to look for…?

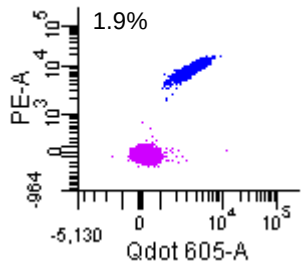
Aim ; To look for ***all*** known peptides restricted by HLA-A1, -A2, -A3, -A11, (A24), and -B7

Antigens; ***All*** published peptide antigens searched in
* Cancer Immunity database (van den Eynde & van der Bruggen),
* Cancer-testis antigen database (CTpedia) (Almeida et al.),
* published antigen list (Novellino et al, CII)
* pubmed search)

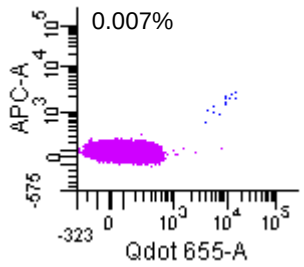
That gave us 174 peptides to study using tetramers (145/A2, 10/A1, 11/A3, 3/A11, and 5/B7)

Examples of T-cell responses detected

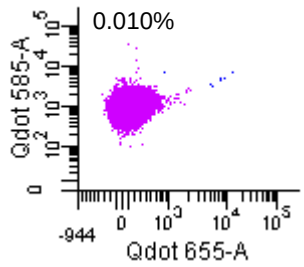
Differentiation antigens:



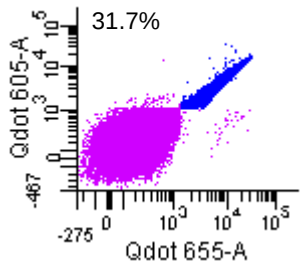
Mart-1$_{ELA}$ - HLA-A2



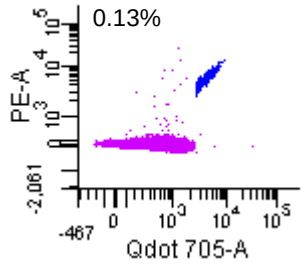
gp100$_{YLE}$ - HLA-A2



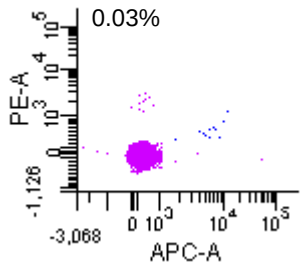
gp100$_{RLP}$ - HLA-A2



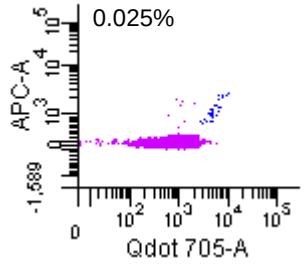
gp100$_{KTW}$ - HLA-A2



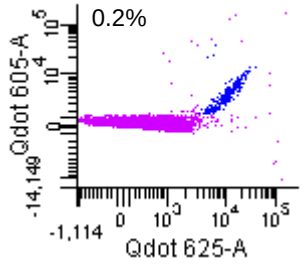
gp100$_{IMD}$ - HLA-A2



gp100$_{SSP}$ - HLA-B7

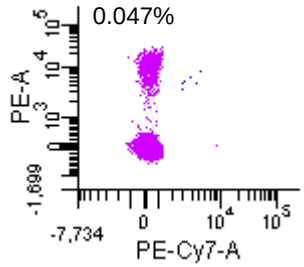


gp100$_{ALL}$ - HLA-A3

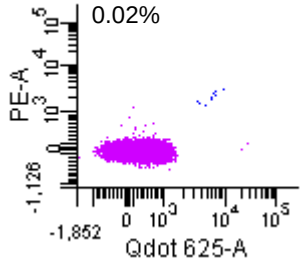


Tyrosinase$_{SSD}$ - HLA-A1

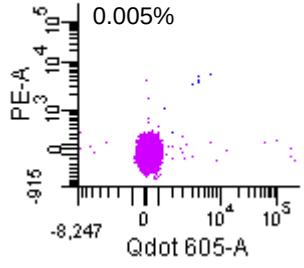
Cancer – testis antigens:



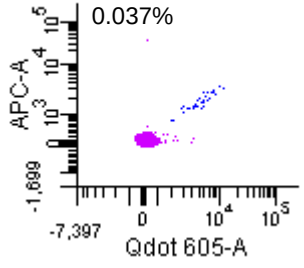
MAGE-A1$_{EAD}$ - HLA-A1



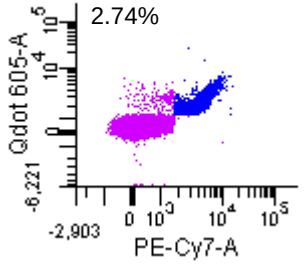
MAGE-A1$_{RVR}$ - HLA-B7



NY-ESO-ORF2$_{APR}$ - HLA-B7

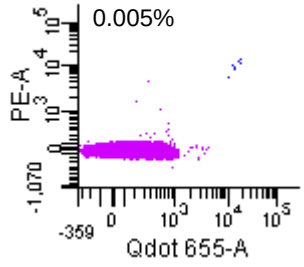


NY-ESO-ORF2$_{MLM}$ - HLA-A2

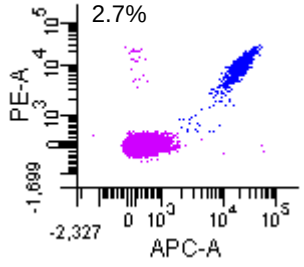


TAG$_{RLS}$ - HLA-A3

Overexpressed antigens:

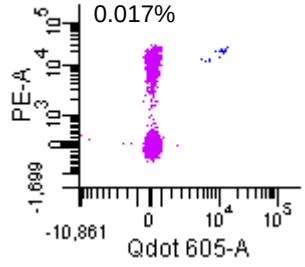


GnT-V$_{VLP-9}$ - HLA-A2



AIM-2$_{RSD}$ - HLA-A1

Mutation antigens:



N-ras$_{ILD}$ - HLA-A1

Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

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Andersen, R.S, Cancer Res. 72, 2012
Kvistborg, P., Oncoimmunology, 2, 2013
Andersen, R.S., Oncoimmunology, 7, 2013
Ellebaek, E., J. Translational Medicine, 10, 2012

Challenges for immune monitoring

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Could we be looking at the wrong peptides ???

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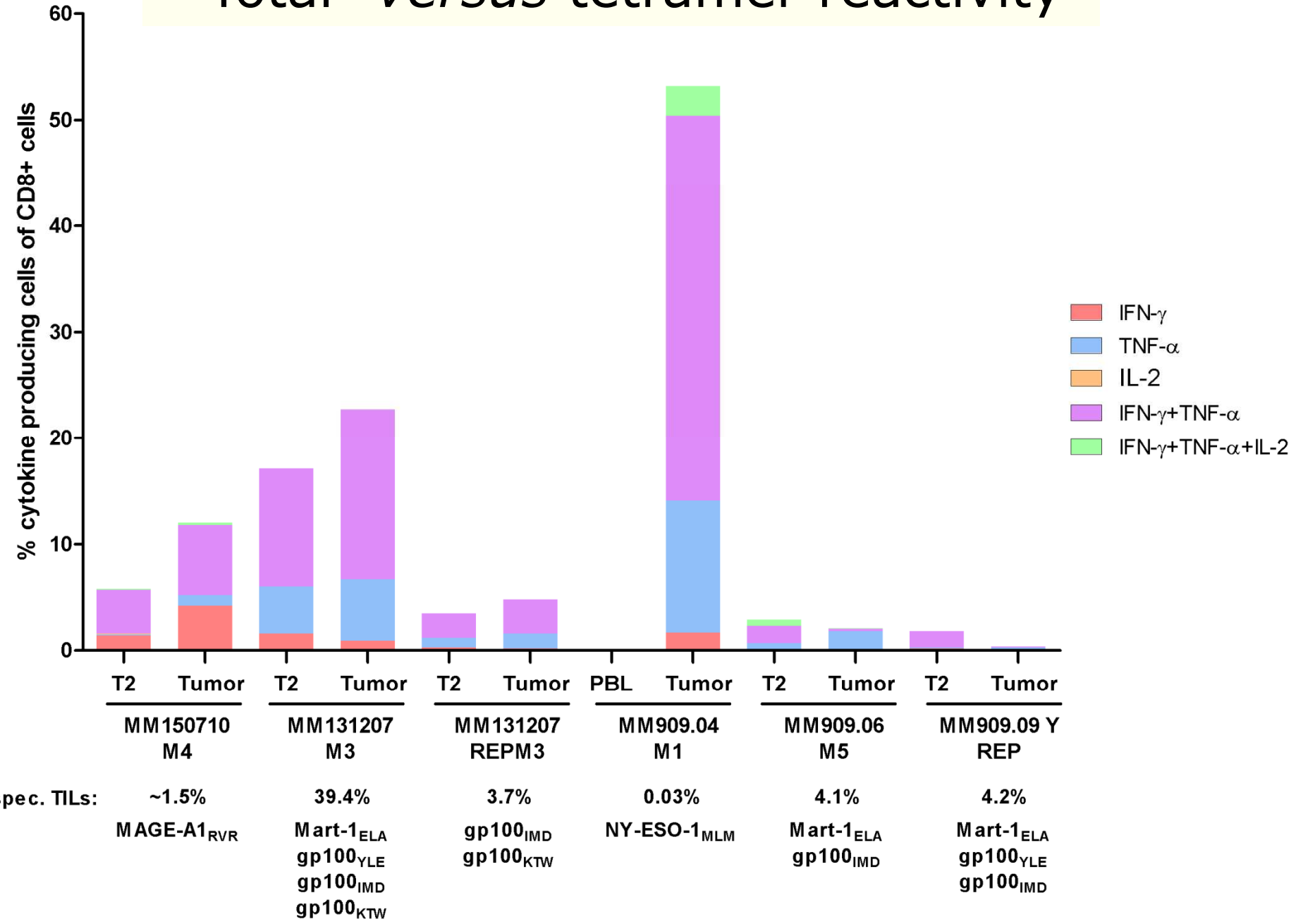
"Total" *versus* tetramer reactivity



Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

So… TIL cultures (seem) *not* to be dominated by the presence of cancer specific T cells – rather to the contrary…..

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Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration….

Also when considering global
T-cell reactivity
against autologous tumor cell lines

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Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

* Tetramer based monitoring after administration probably not feasible (in most cases……)

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Challenges for immune monitoring

ACT based on in vitro expanded TIL (as judged by tetramer analyses…) leads to….

* Quite low-frequency responses in TIL cultures (even…) prior to administration

* Tetramer based monitoring after administration probably not feasible (in most cases……)

So – we (i.e., Marco Donia) have done some old fasioned low-tech cellular monitoring instead!!

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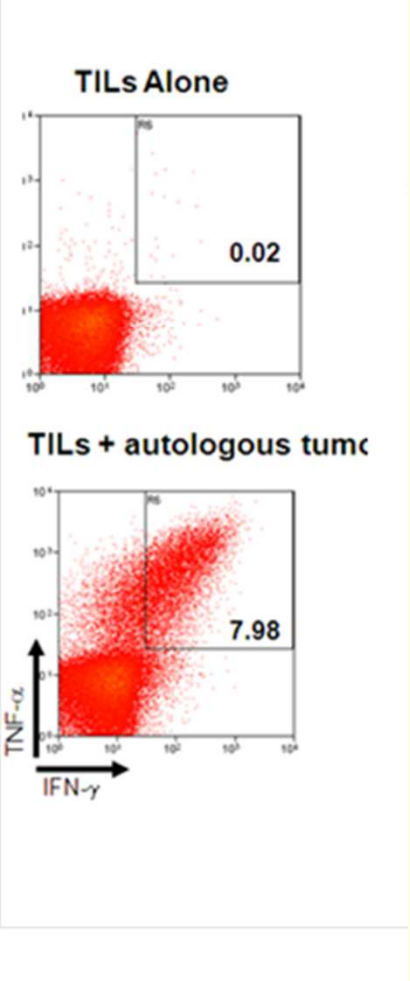
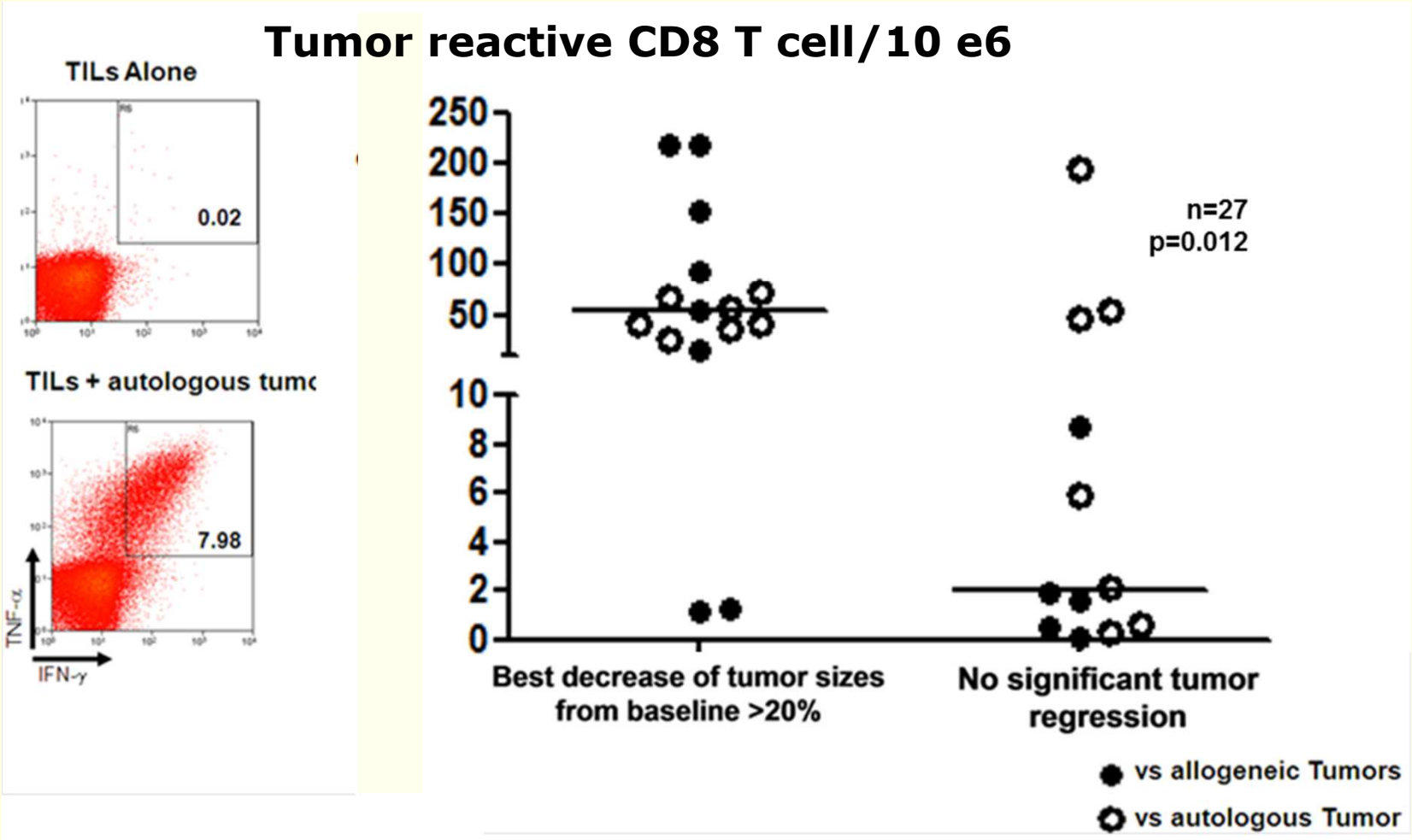
Andersen, R.S, Cancer Res. 72, 2012
Kvistborg, P., Oncoimmunology, 2, 2013
Andersen, R.S., Oncoimmunology, 7, 2013
Ellebaek, E., J. Translational Medicine, 10, 2012

Anticancer responses of TIL infusion products using a cellular assay



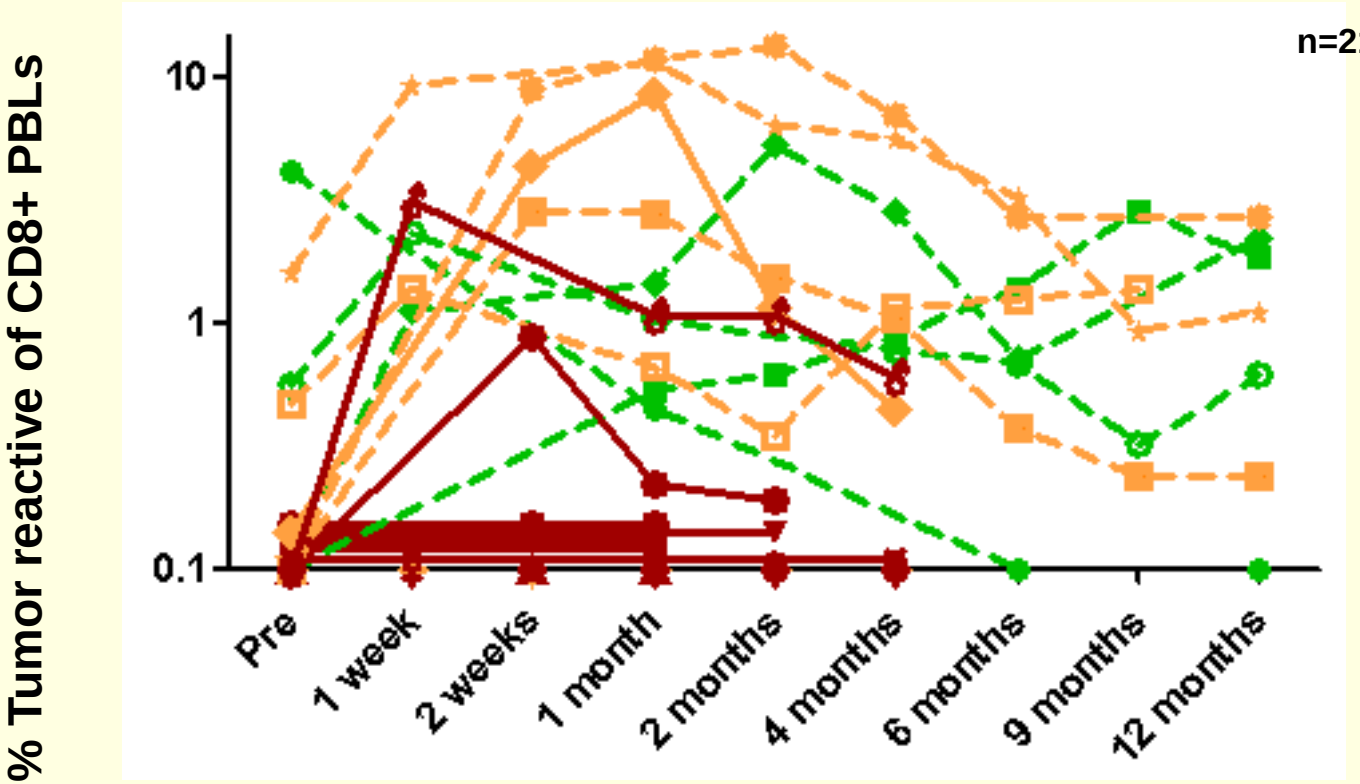
Donia M et al., J Invest Dermatol 2013
Donia M et al., Unpublished

Anticancer responses of TIL infusion products using a cellular assay



Donia M et al., J Invest Dermatol 2013
Donia M et al., Unpublished

Anticancer Responses in Peripheral Blood



Donia M et al., Unpublished

TIL characteristics which corresponds with clinical response

- (CD8 T) Cell numbers infused
- Tumor reactivity – in the culture and among PMBC
- Persistence in the patient
- “Young” T cells better than “old” (telomere/CD27)

TIL characteristics which corresponds with clinical response

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- Persistence in the patient
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<u>Lack of markers that would allow selection of patients before or even after treatment</u>

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Outline of talk….

The CCIT experience

* Why initiate TIL therapy in melanoma
* Sum-up of our TIL trial incl clinical data
* Biological monitoring
* Next steps

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Next steps for TIL based ACT

- Randomized phase III trial
  - Generate robust efficacy data
  - Approval of TIL therapy as standard treatment

    (Inge Marie Svane, PI, J. Haanen, Netherlands Cancer Institute, R. Hawkins, University of Manchester)

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)

Next steps for TIL based ACT

- Randomized phase III trial
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  - Approval of TIL therapy as standard treatment

    (Inge Marie Svane, PI, J. Haanen, Netherlands Cancer Institute, R. Hawkins, University of Manchester)

The hope is to establish the efficacy of TIL based ACT – and establish this treatment as a standart treatment of malignant melanoma in Europe !

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)

The CCIT experience; conclusions

- TIL therapy can eradicate huge tumor masses (even with lower dose IL-2)
- Reactivity againt auto (and allo) melanoma cells corresponds with clinical response
- Phase III study initiated (please join)

(Phase III; 162 patients, ippi against TIL, High dose IL-2, expected time: 2 years)

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A case story

* Complete response and yet…..

Some more monitoring:

* A glance at CD4 T cells among TIL

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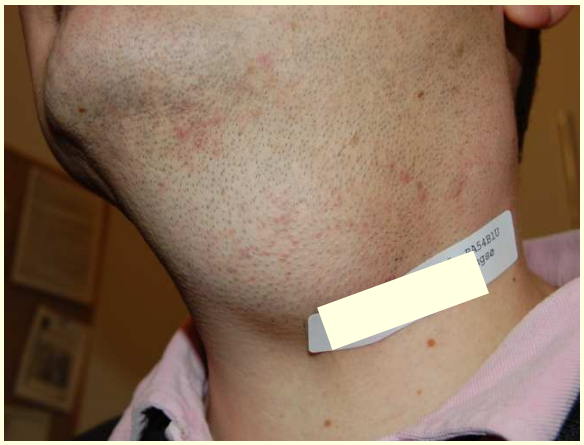
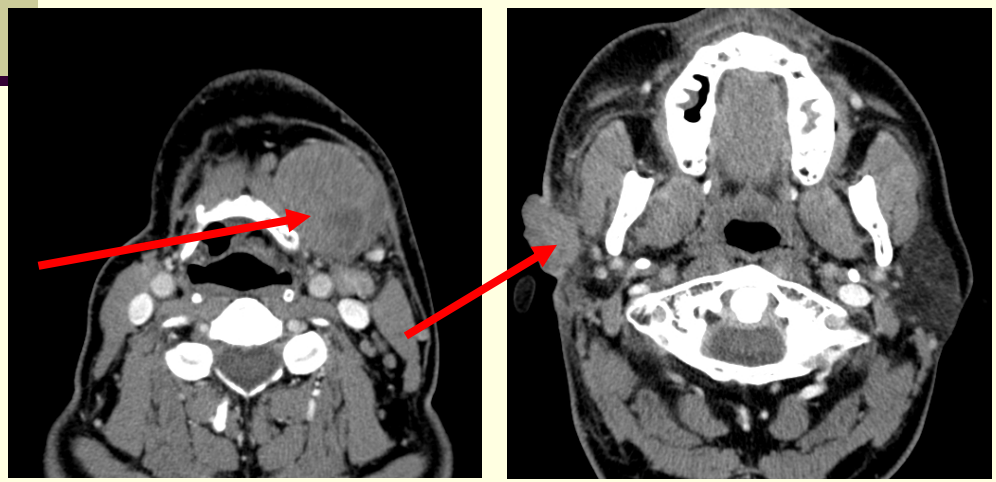
Case story; Complete responder

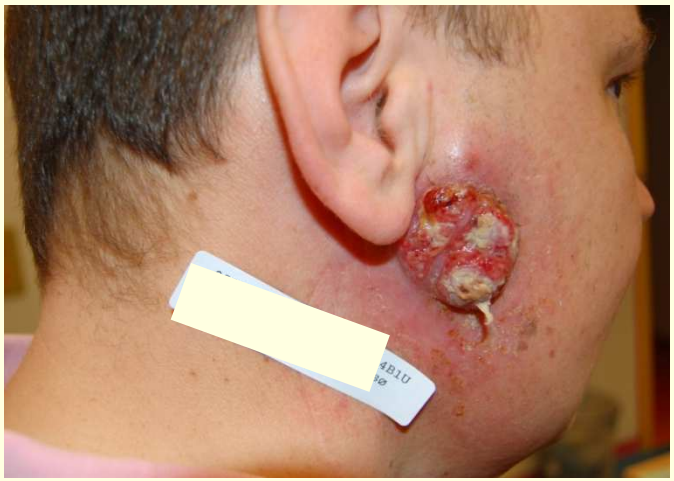
Patient no.909.11

- 42-year old male
- Good performance status



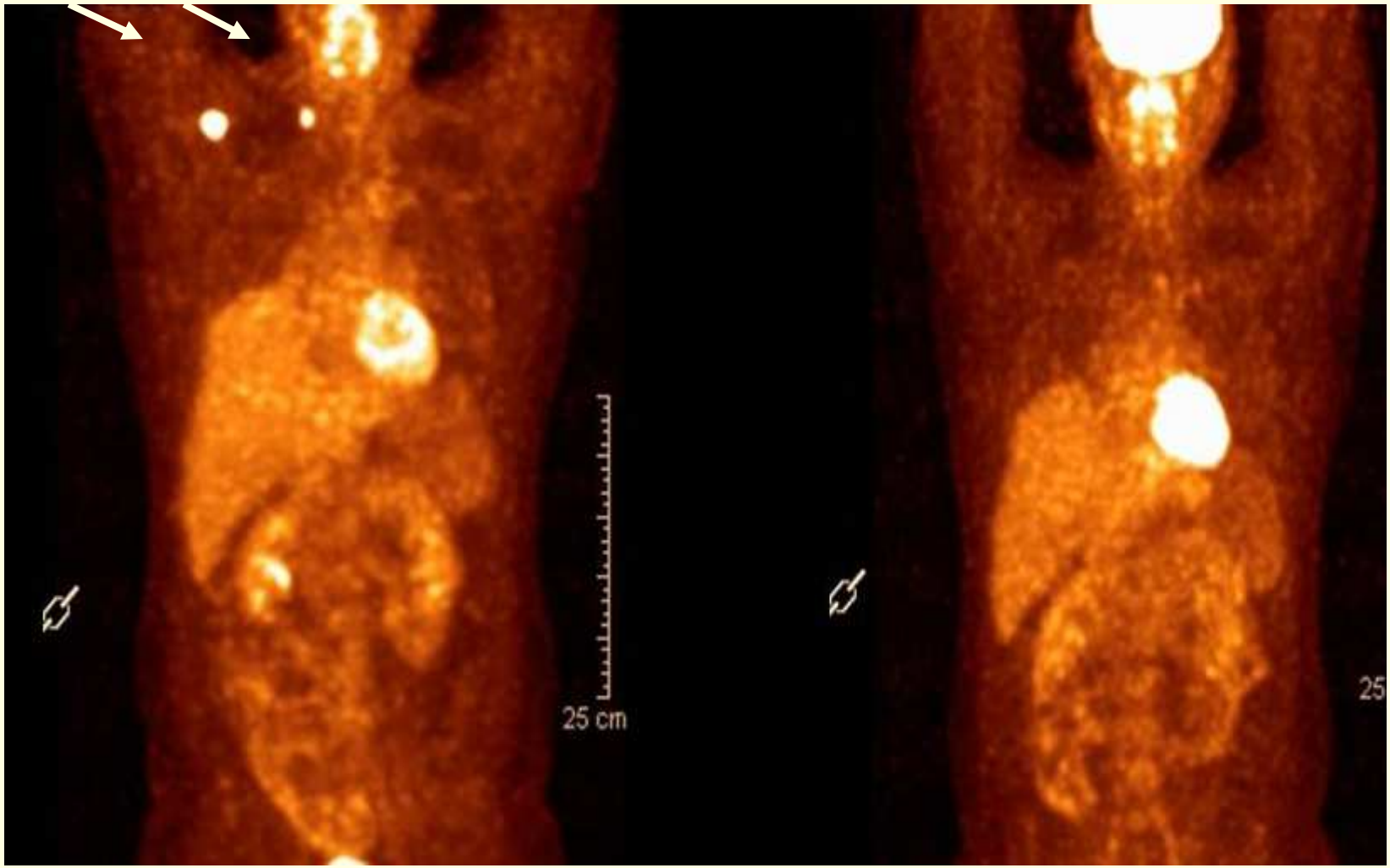
- Previous treatments:
  - IL-2/interferon
  - Ipilimumab
  - DC-vaccination
  - Resection of large metastases; left side of the neck and right cheek





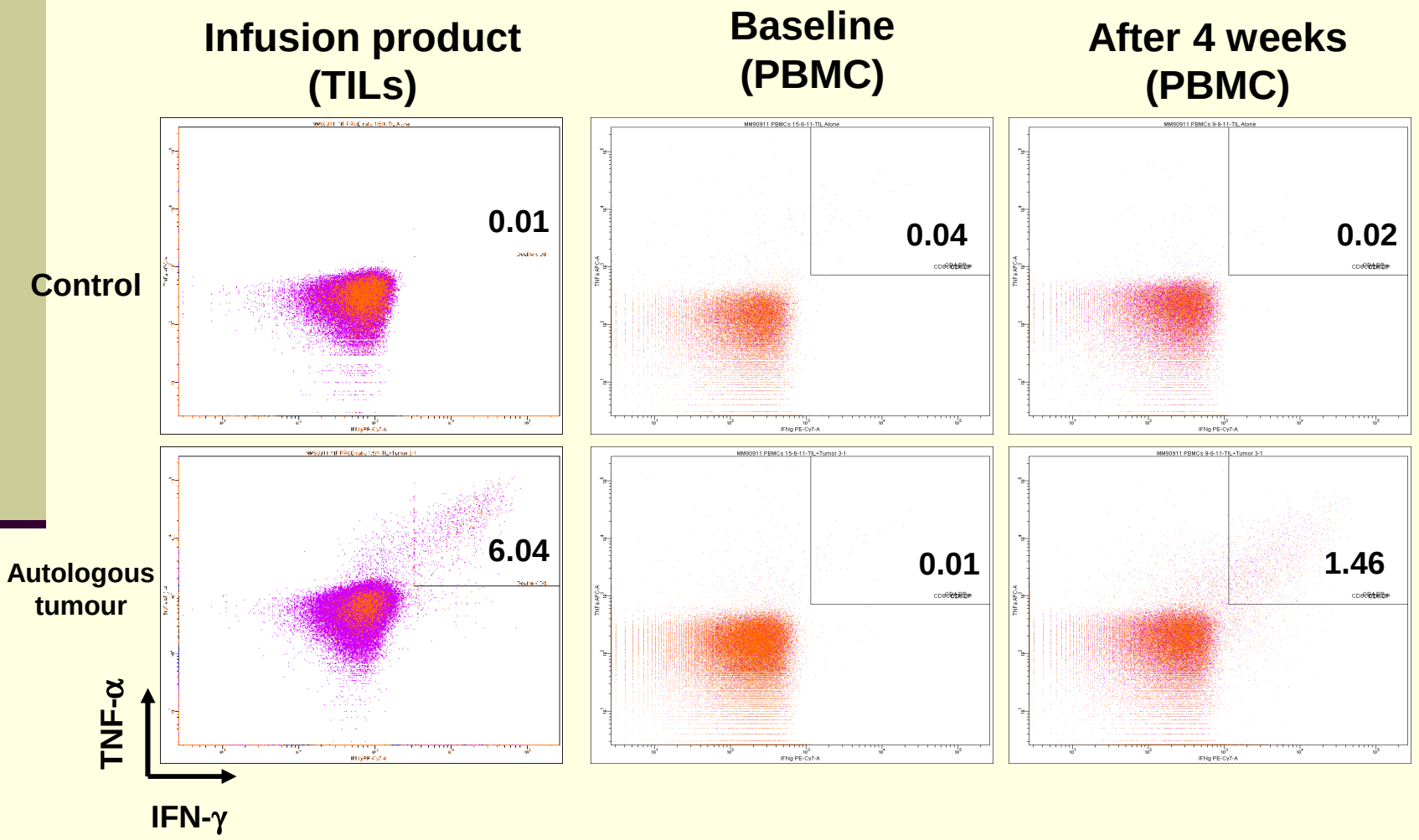
Administered TILs July 2011
Clinical Response - CR/CMR

Patient no.909.11



Case story; immune reactivity against autologous tumor

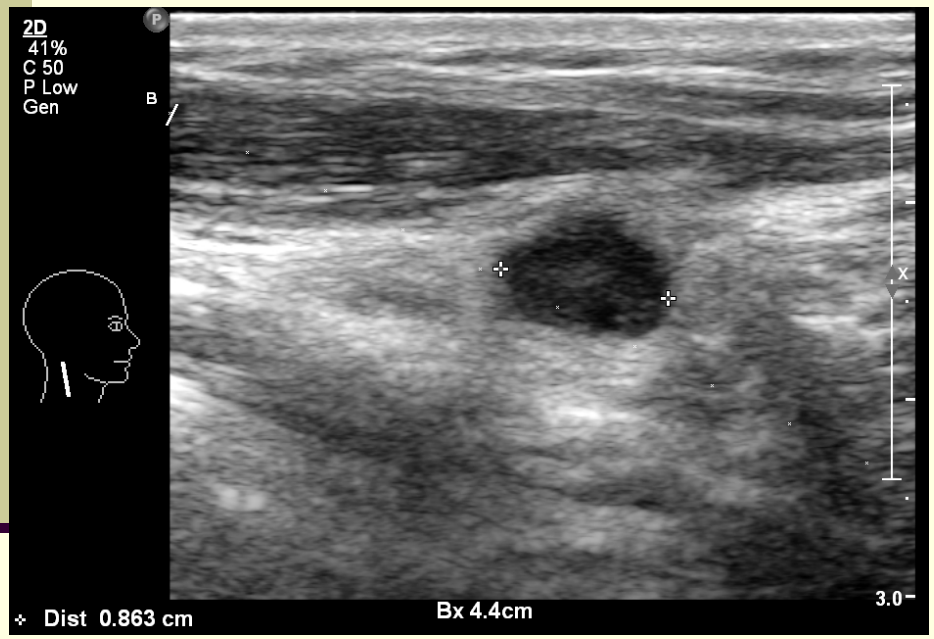
Patient no.909.11



Relapse in August 2012 – surgically resected -> NED+

Patient no.909.11

+ 13 months: Disease recurrence



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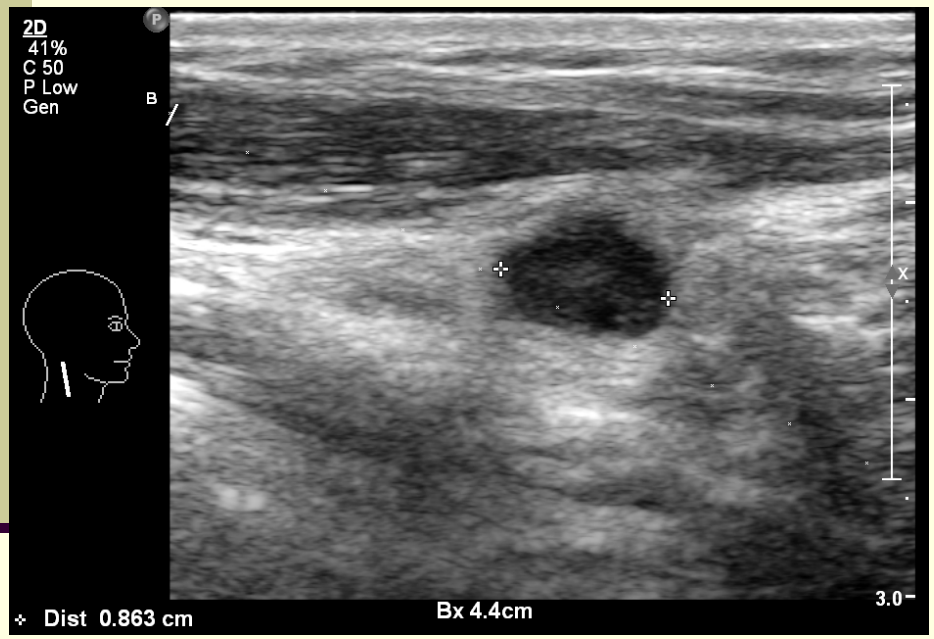
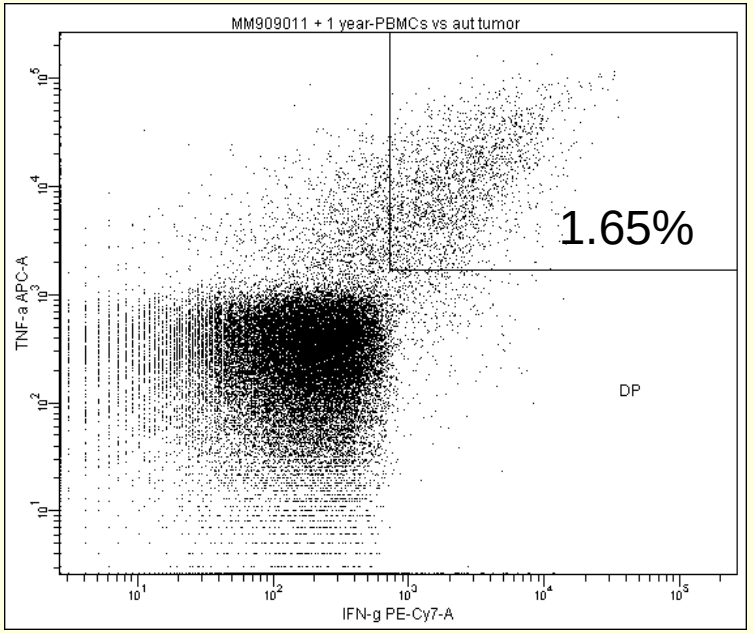
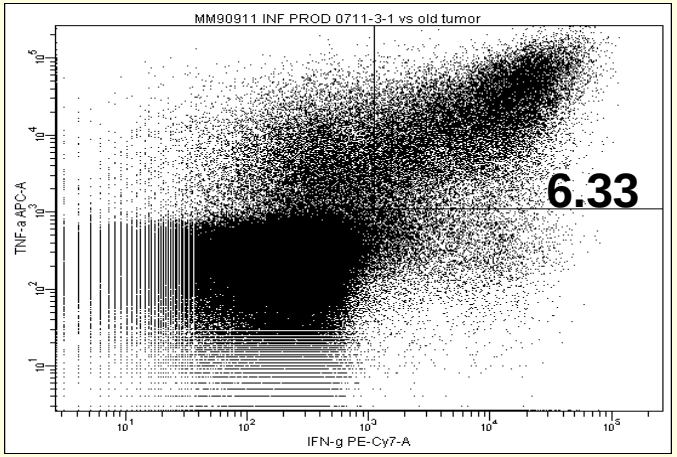
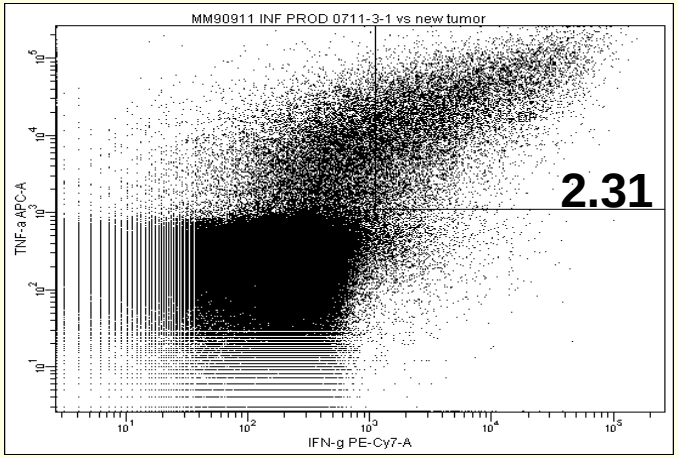


+13 months: PBMC reactivity



TIL reactivity against first and recurrent autologous tumor lines

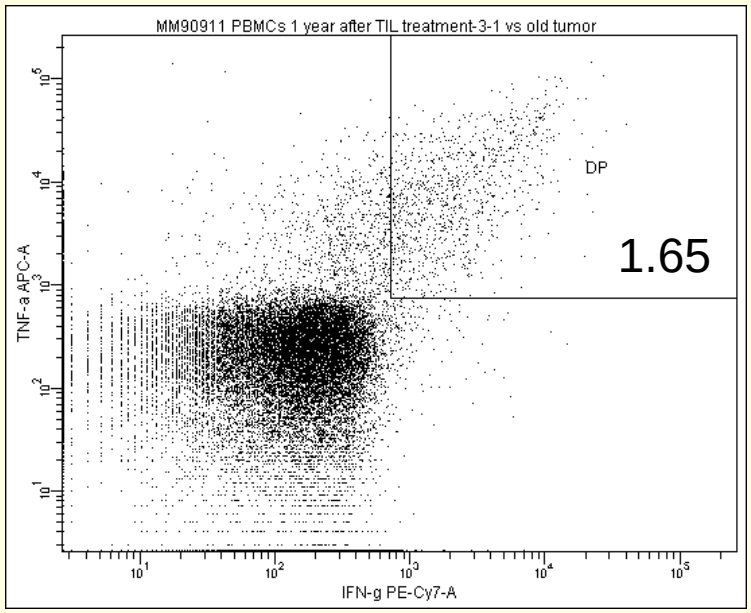
Patient no.909.11

	Old Tumor	New Tumor
TIL Culture		

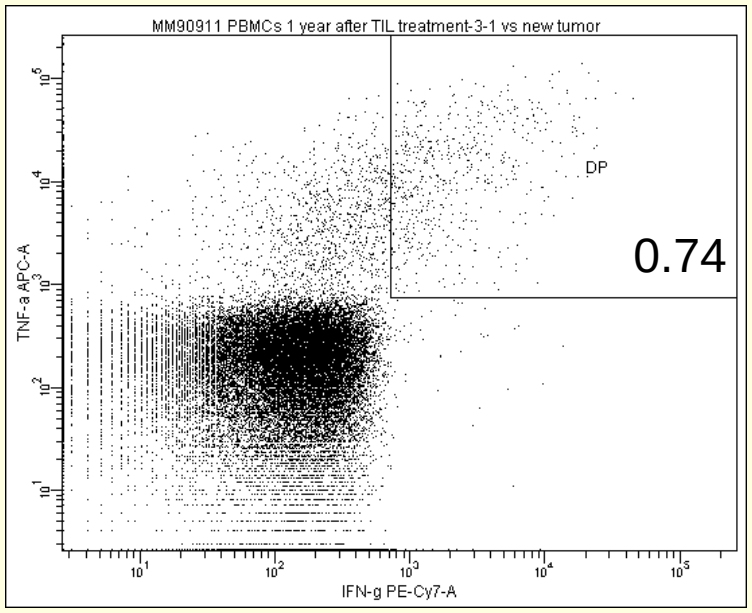
Reactivity in PBMC against first and recurrent autologous tumor lines

Patient no.909.11

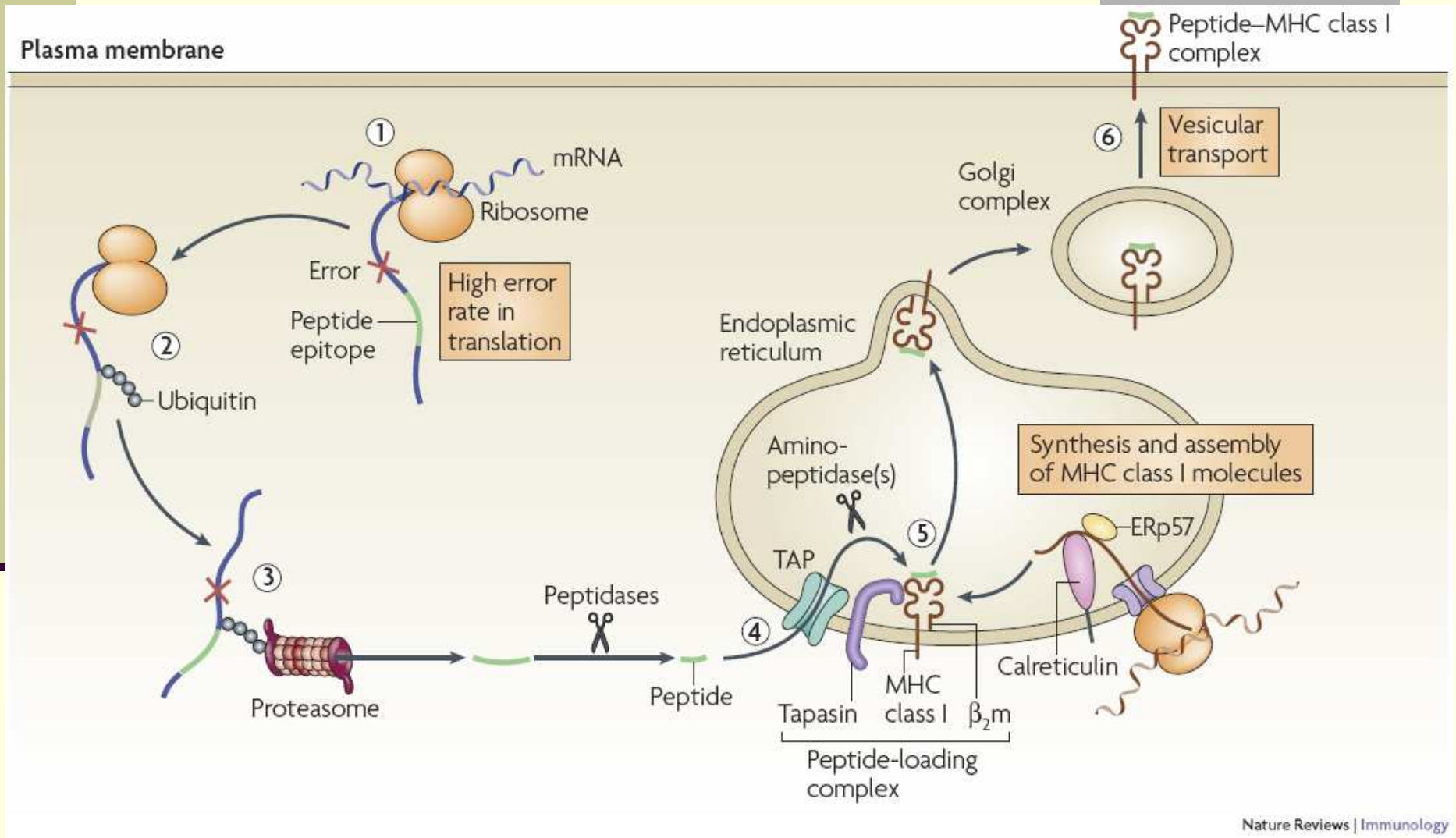
PBLs+13mo vs OLD Tumor



PBLs+13mo vs NEW Tumor

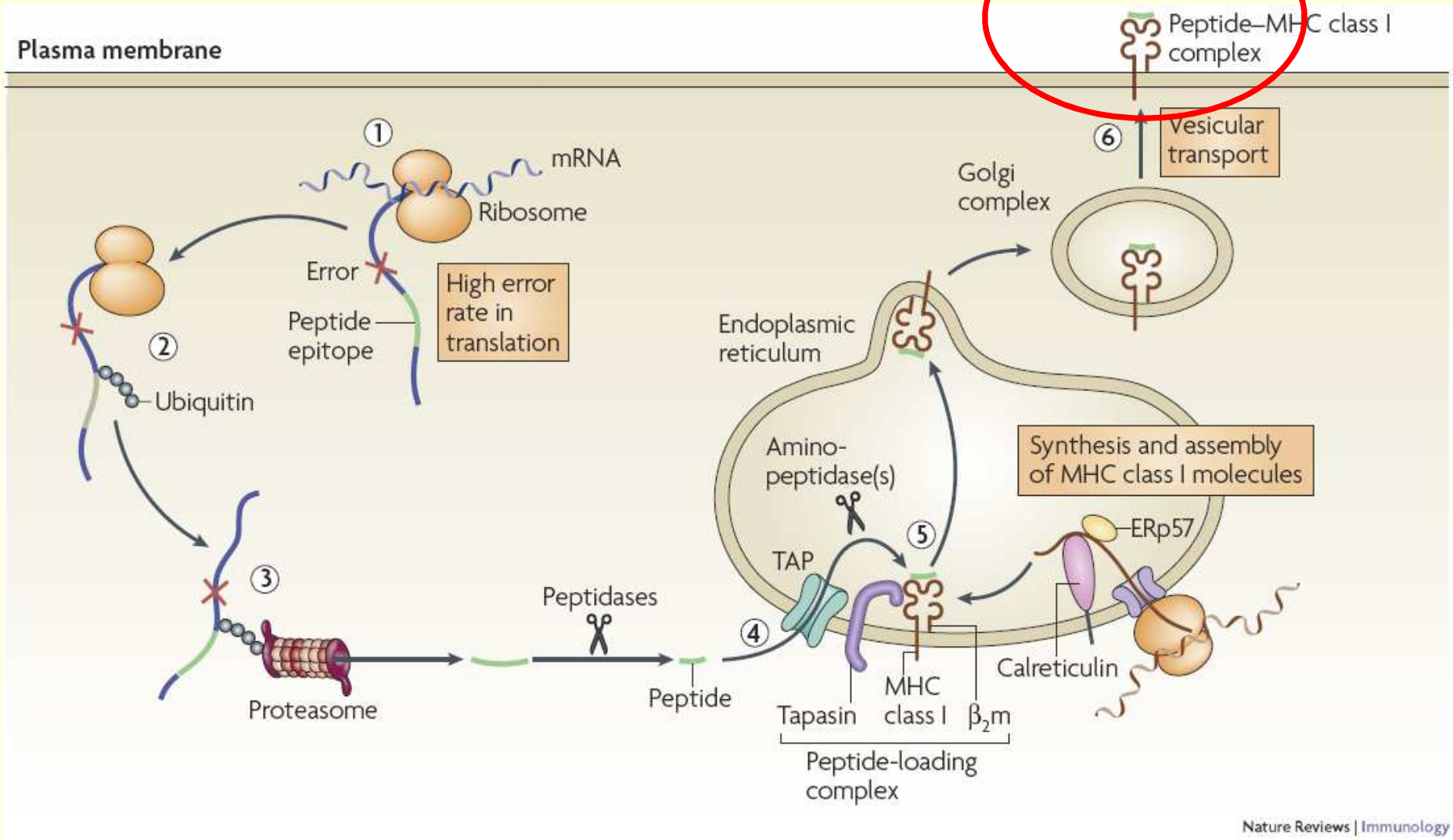


What T cell recognition rely on…



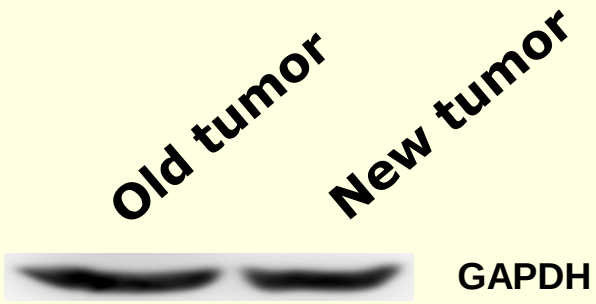
Vyas et al., Nature Rev Immunol 8, 607-618, 2008

What T cell recognition rely on…



Vyas et al., Nature Rev Immunol 8, 607-618, 2008

Down regulation of APM components the in recurrent tumor



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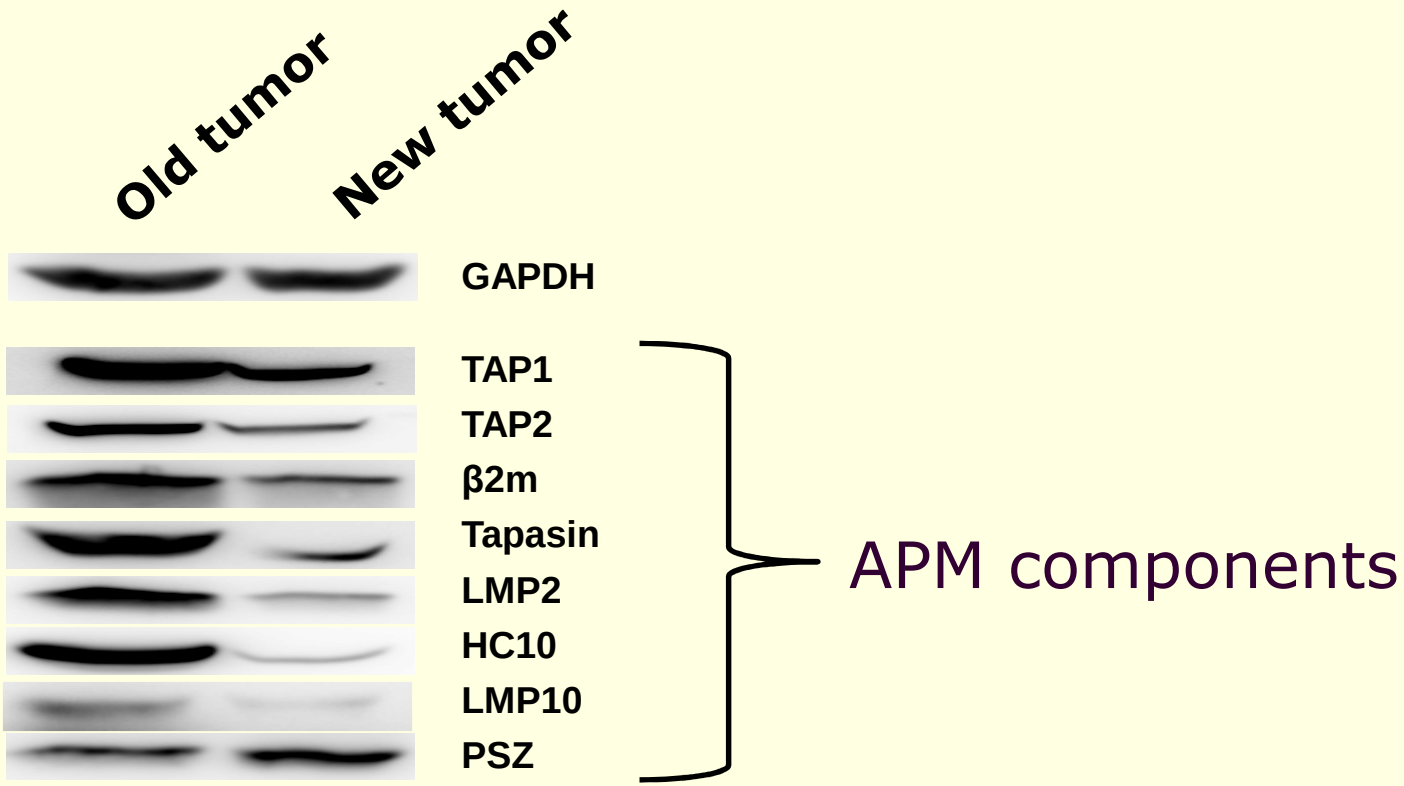

Decrease in the expression of APM components in relapse cancer cell line !!!

Case story conclusion

With more powerful responses we will see more frequent immune escape by cancer cells !!!

Case story conclusion

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In turn underscoring the need to study escape mechanisms and ways to counteract escape !!

Partially restored TIL recognition upon IFN-g upregulation of HLA…..

So Marco looked at whether down expression of APM could be adressed

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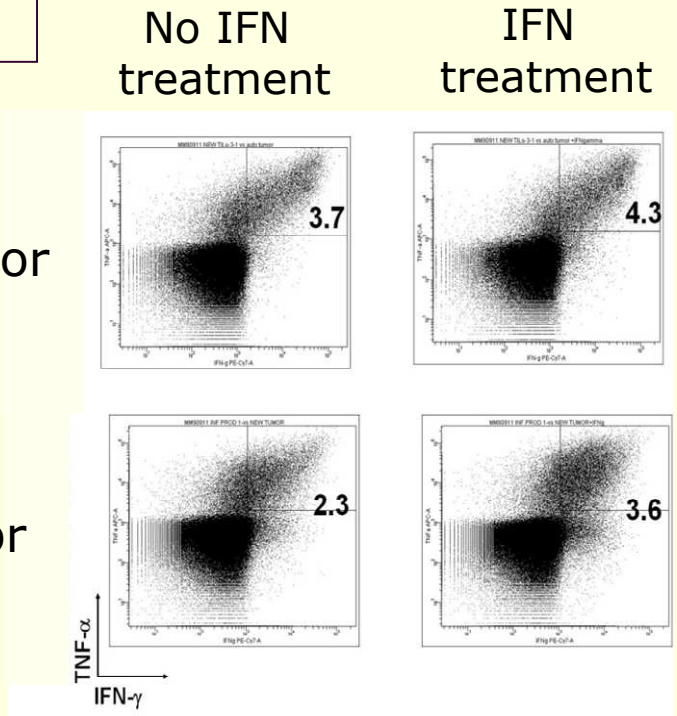
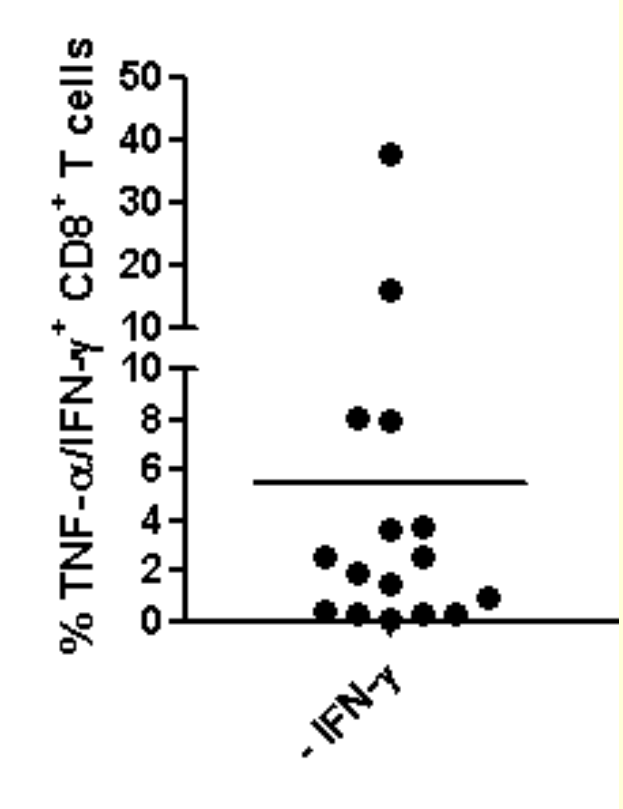


In patient no.909.11

Partially restored TIL recognition upon IFN-g upregulation of HLA…..a frequent phenomenon

Up-regulation of HLA molecules by IFN-g for increased recognition by autologous CD8 TILs



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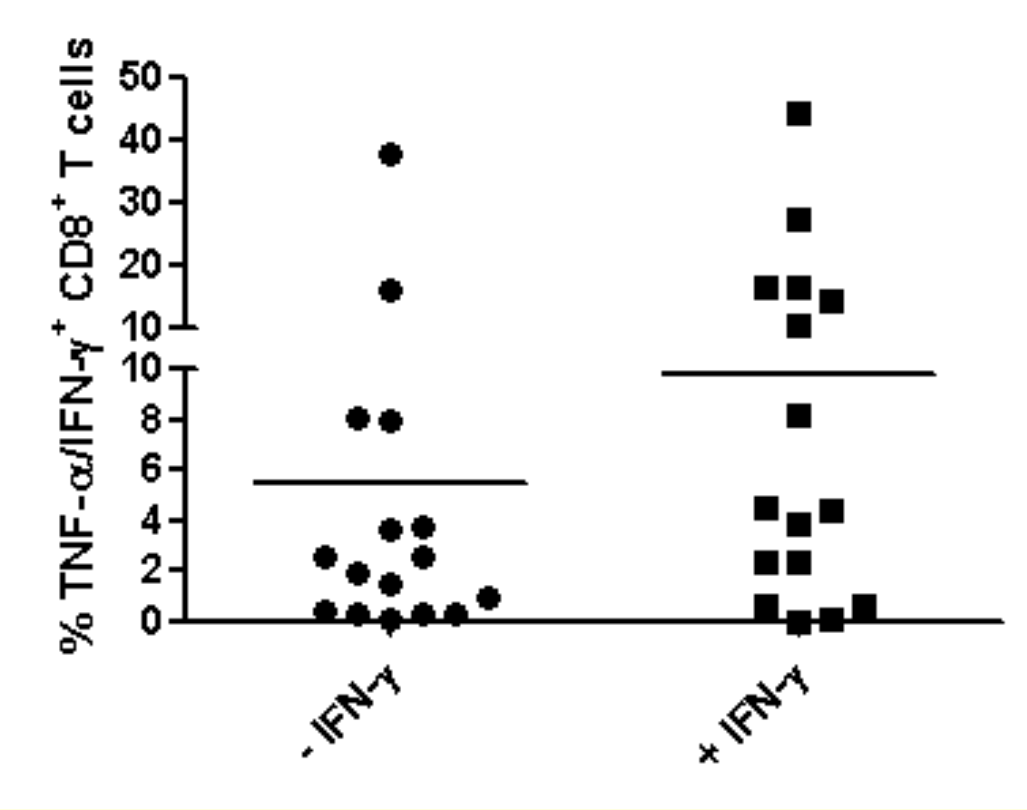
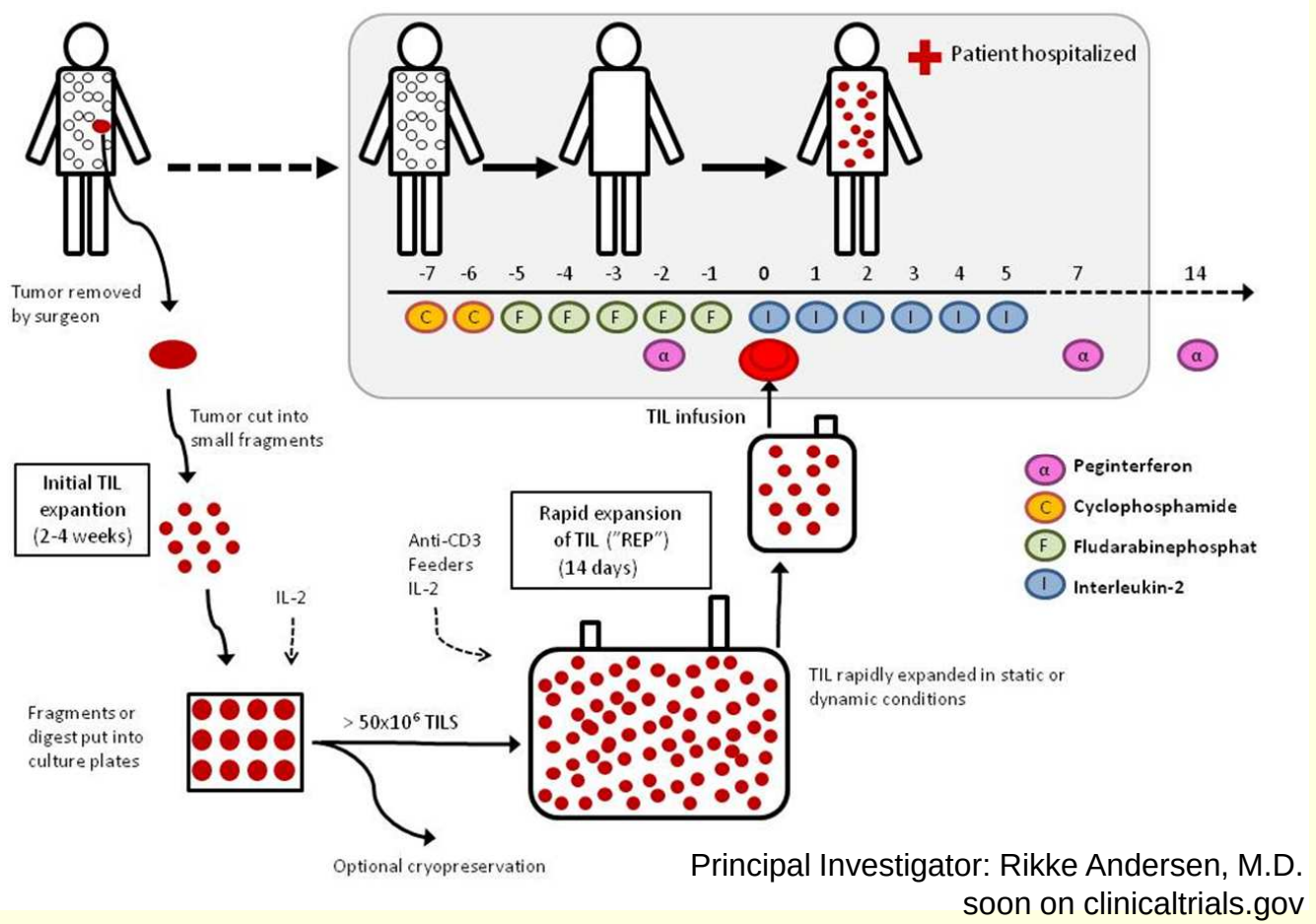


TIL in combination with IFN-α

Counteracting Immune Escape

- Combination with Interferons



Principal Investigator: Rikke Andersen, M.D.
soon on clinicaltrials.gov

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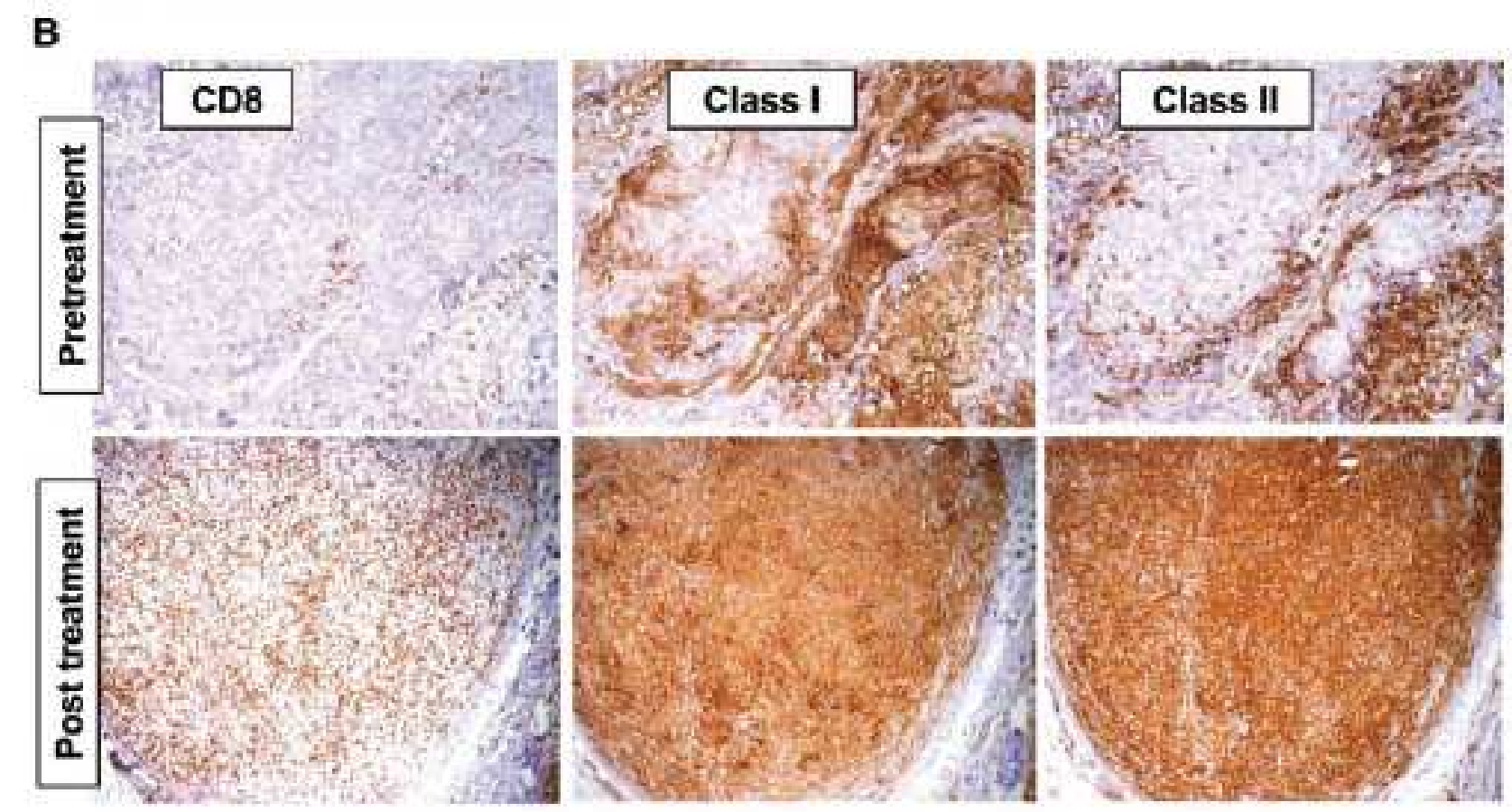
Up-regulation of HLA molecules…..

Cytotoxic CD8 T cells secrete IFN-γ which in turn lead to up-regulation of class I and II molecules



Dudley et al. Science, 298, 2002

MHC Class II in Melanoma

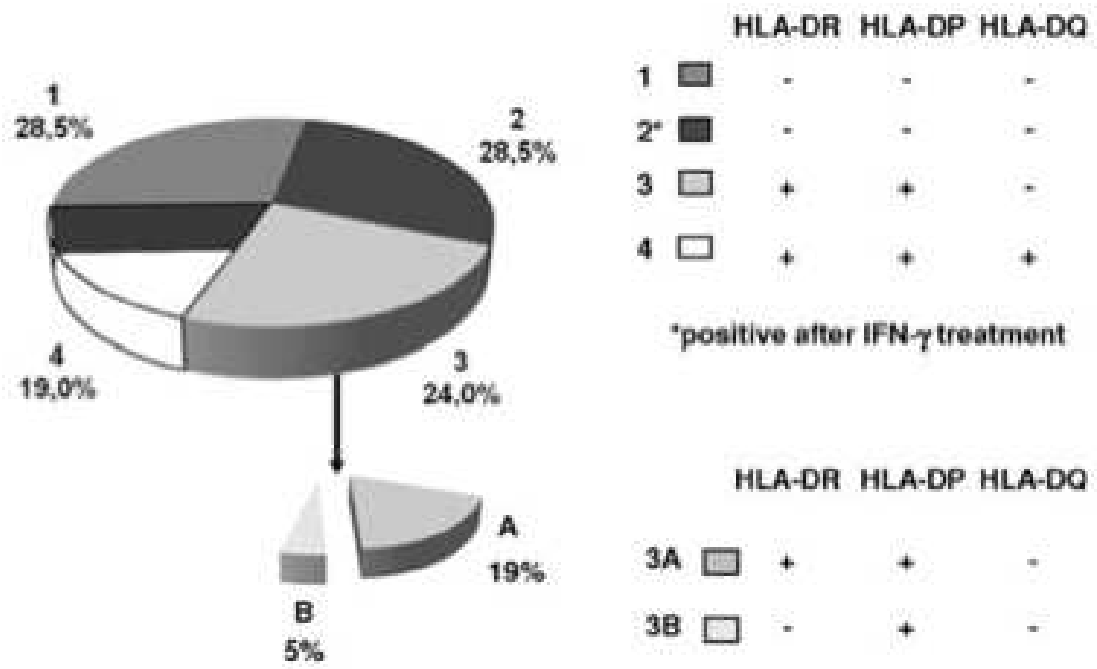


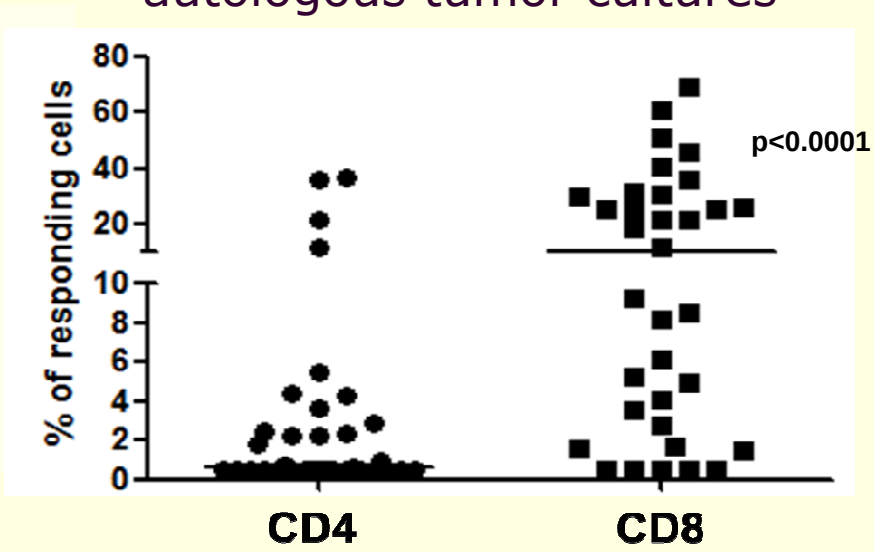
Fig. 2 Distribution of HLA class II phenotypes in 42 human melanoma cell lines (ESTDAB). Phenotypes 1–4 representing various patterns of HLA class II expression on the studied melanoma cell lines are presented. Surface expression of HLA class II molecules was determined by flow cytometry (mean fluorescence intensity, MFI) using a panel of HLA class II specific antibodies. To analyse induction with IFN-gamma the melanoma cells were treated with 800 U/ml for 48 h

Mendez et al, Cancer Immunol Immunother 2009

Tumor Reactive CD4+ T cells infiltrates melanoma

Most TIL cultures are CD8 biased….
but contain CD4 T cells as well…

% of T cells responding to autologous tumor cultures



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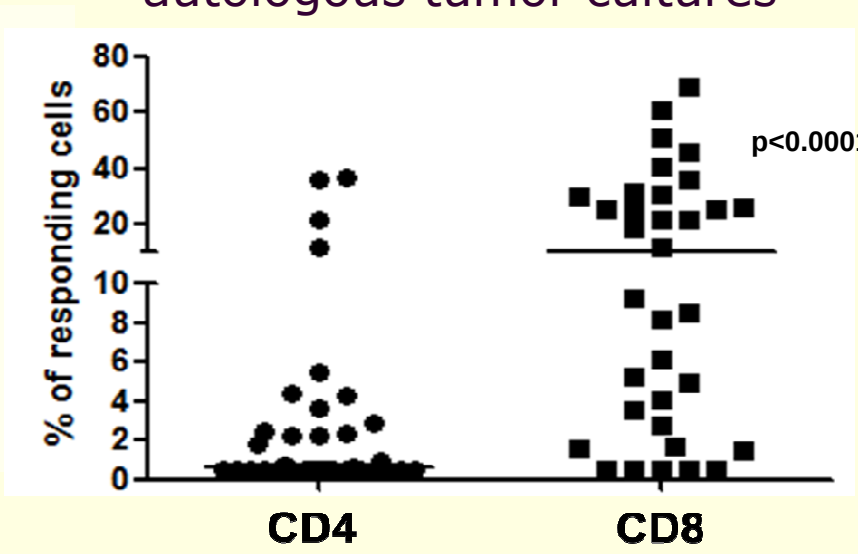
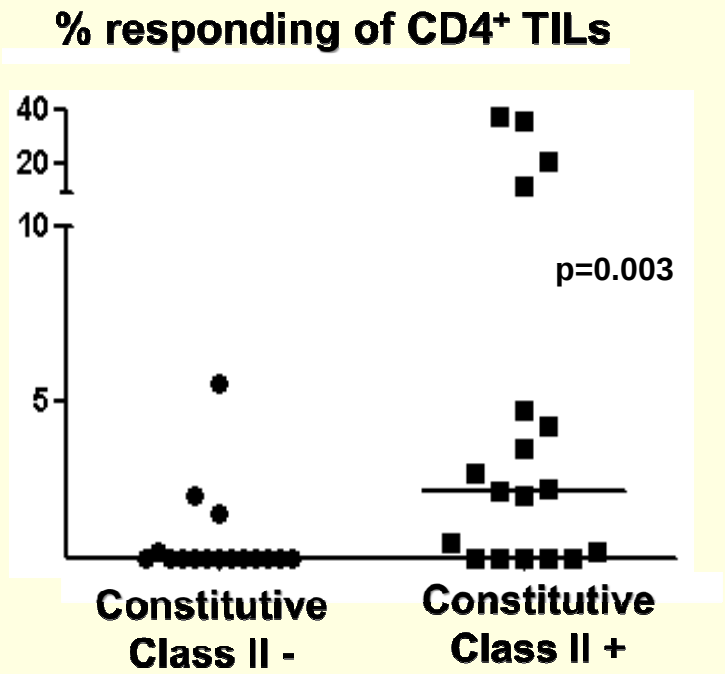


p=0.005

	With Response	No Response	
CD4	n=18	n=16	50 %
CD8	n=30	n=4	90 %

MHC Class II attracts Inflammatory CD4+ T cells

CD4 T cells are most prominantly present if cancer cells express class II molecules



MHC Class II attracts Inflammatory CD4$^+$ T cells

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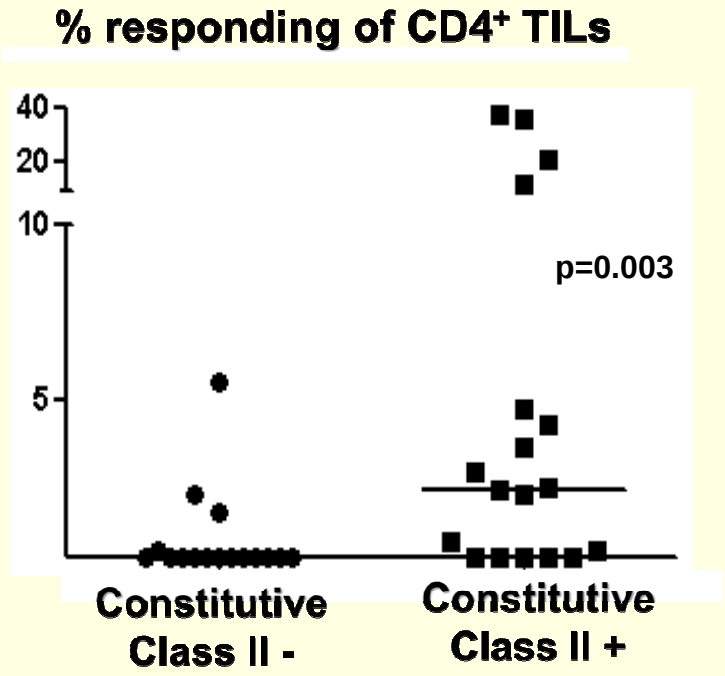
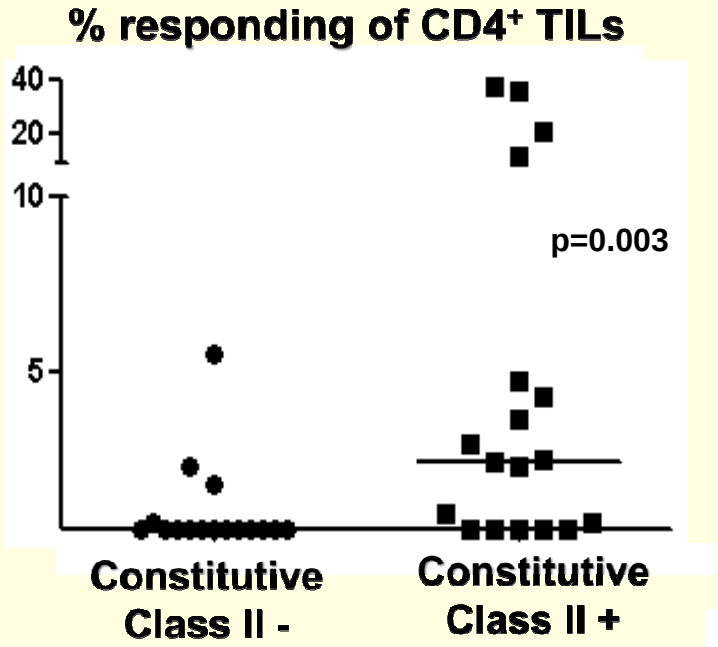
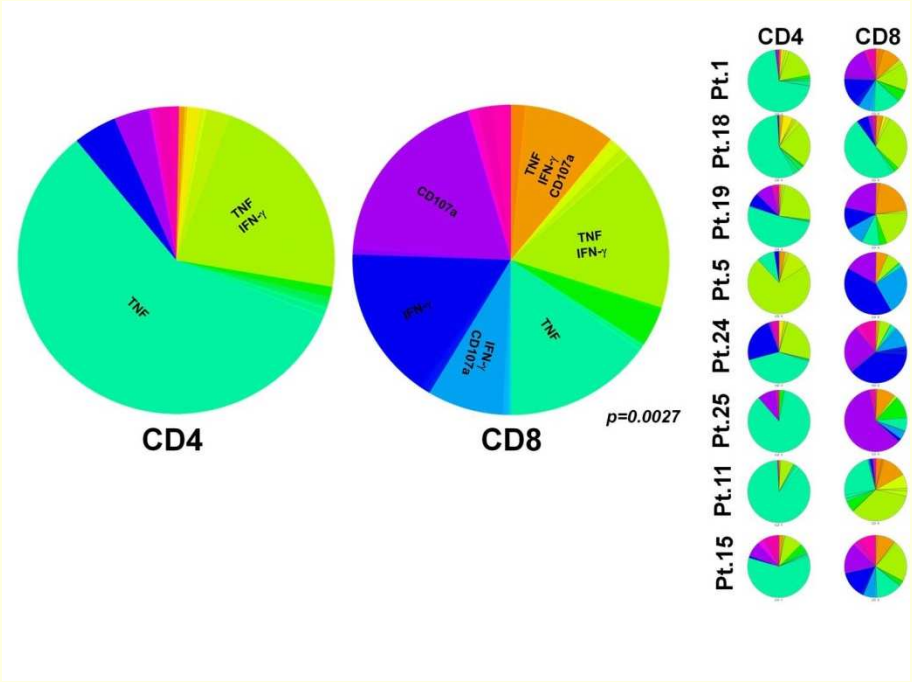
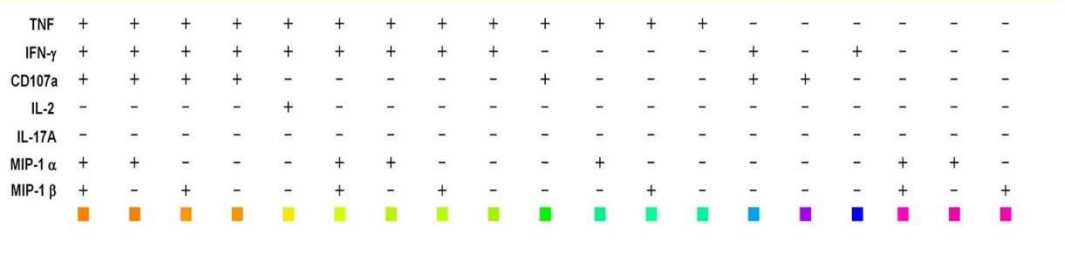


What do they do ??

Cytokine profiles of CD4+ and CD8 + T cells

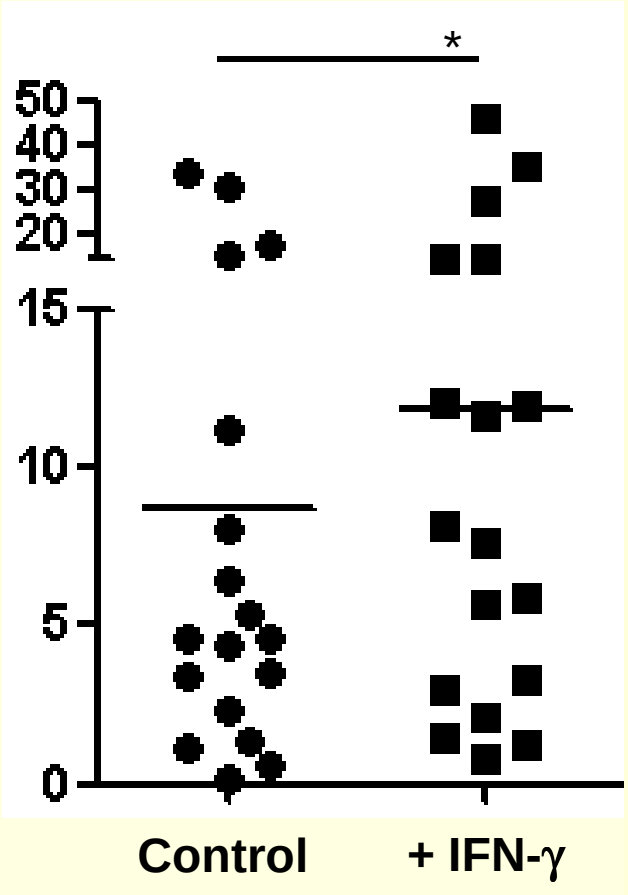






IFN increase recognition by CD8$^+$ TILs

% Tumor Reactivity of autologous CD8 TILs



CCIT, Confidential

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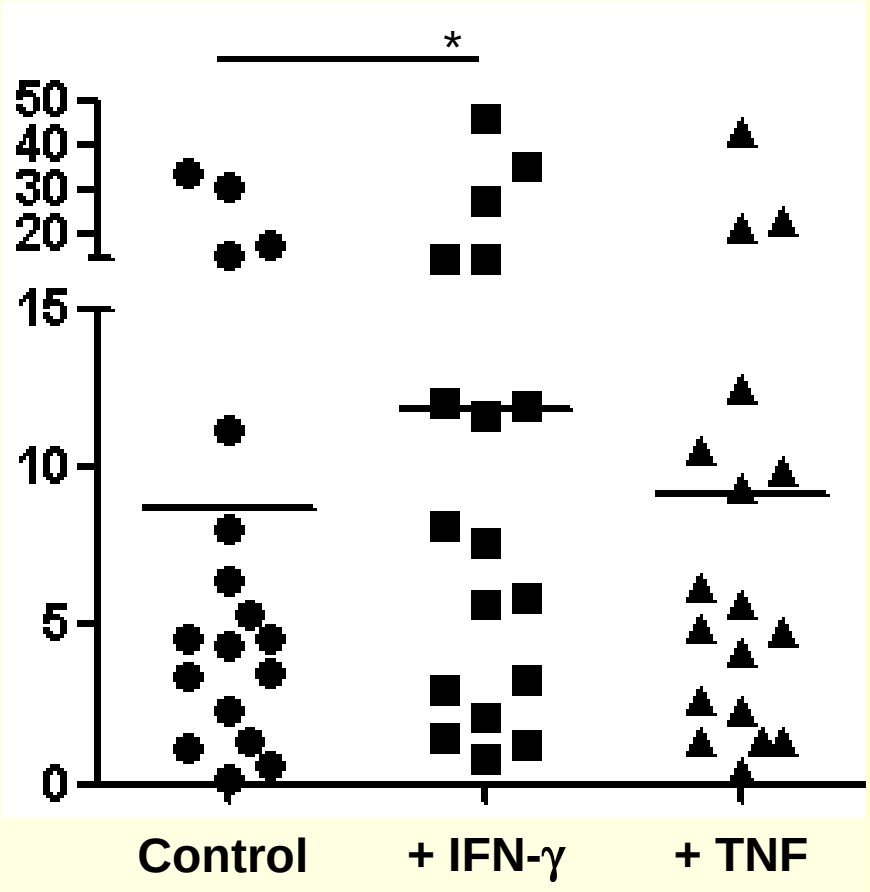

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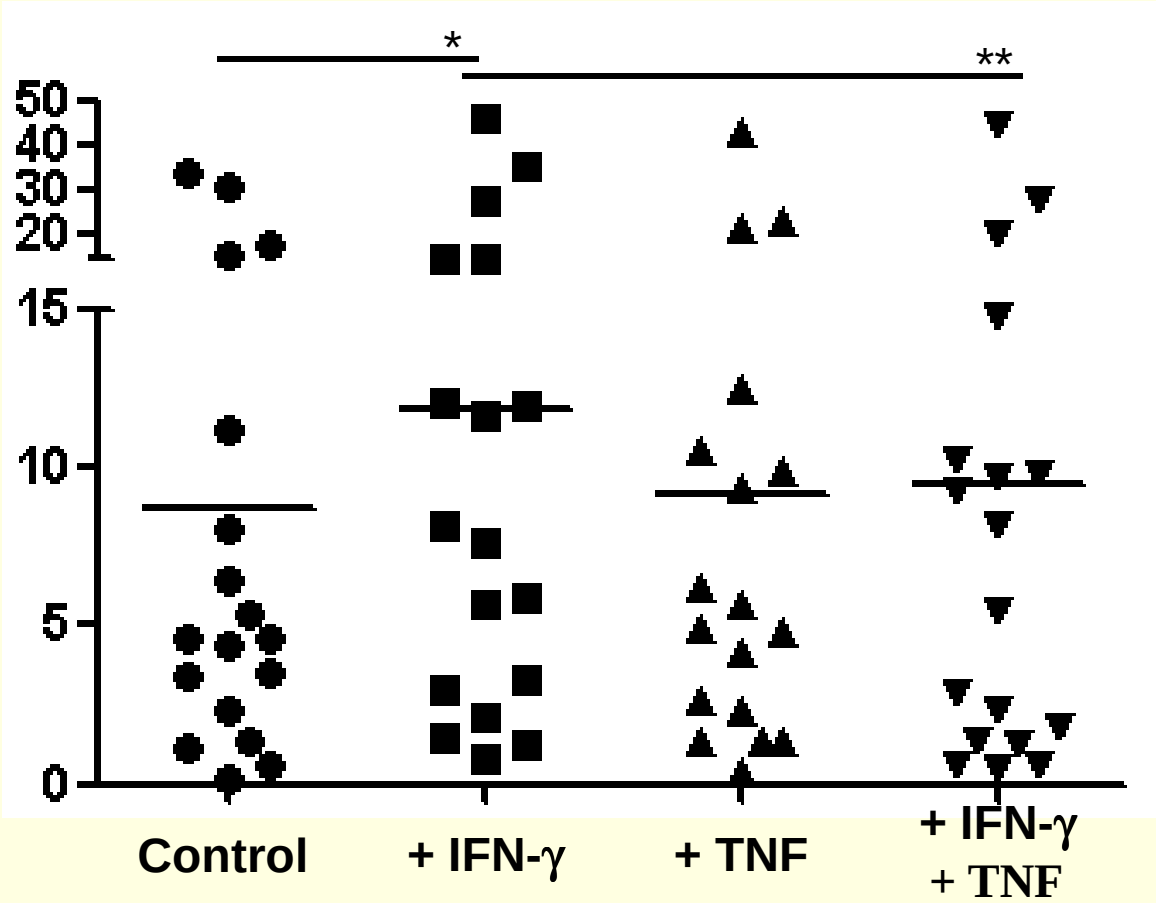
CD4+-derived TNF inhibits recognition by CD8+ TILs

% Tumor Reactivity of autologous CD8 TILs



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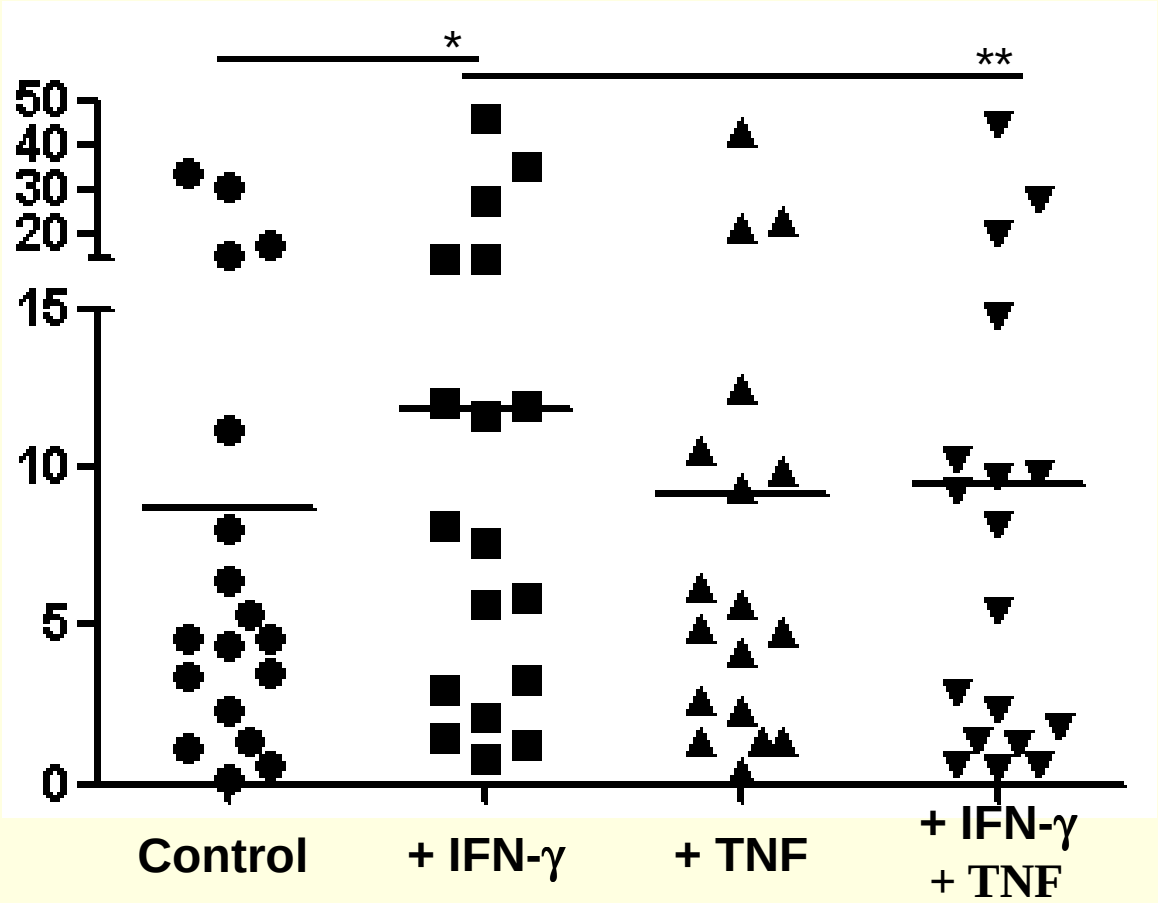
CD4$^+$-derived TNF inhibits recognition by CD8$^+$ TILs

IFN increase CD8 recognition -
addition of TNF abolish the effect of IFN



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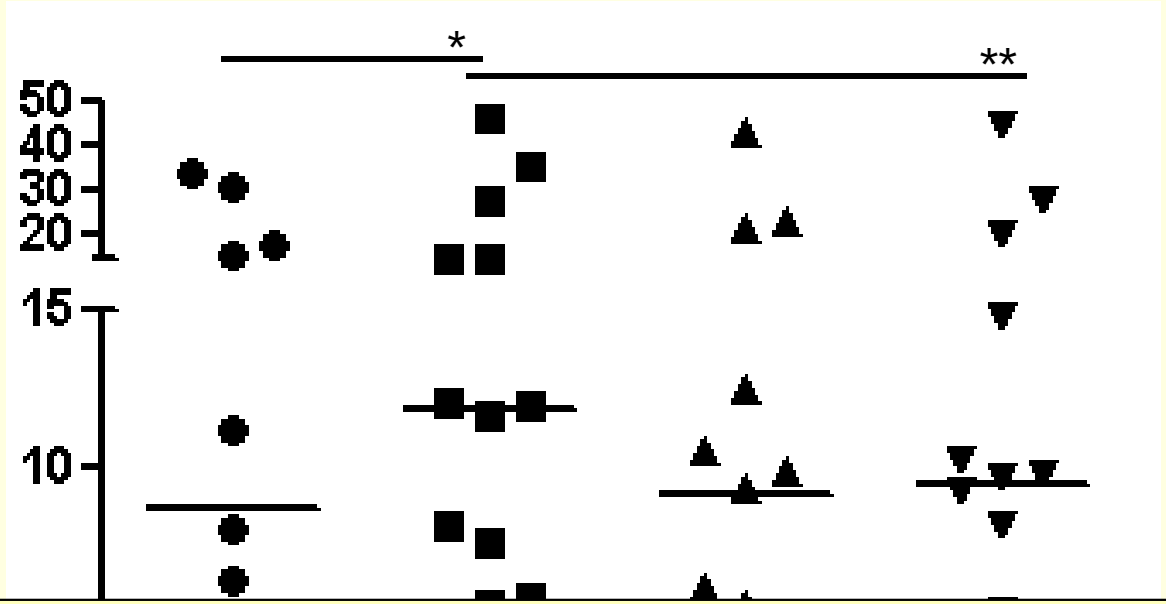
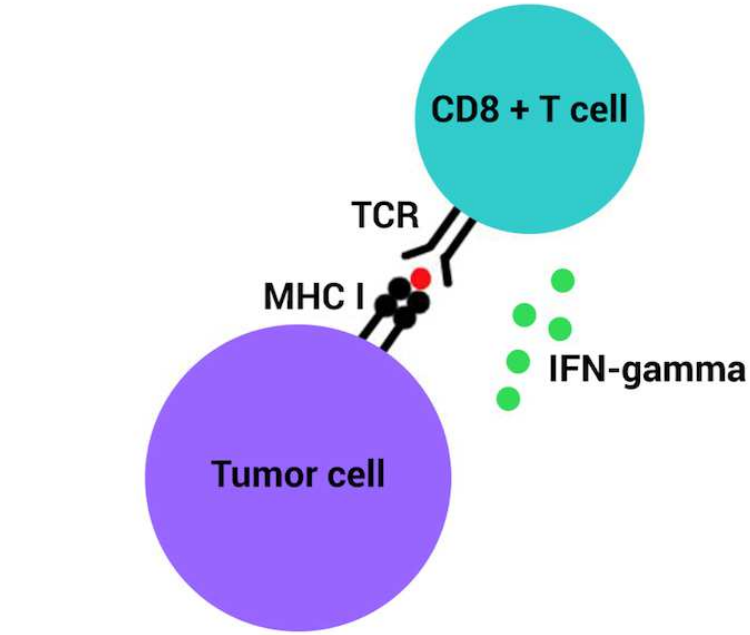


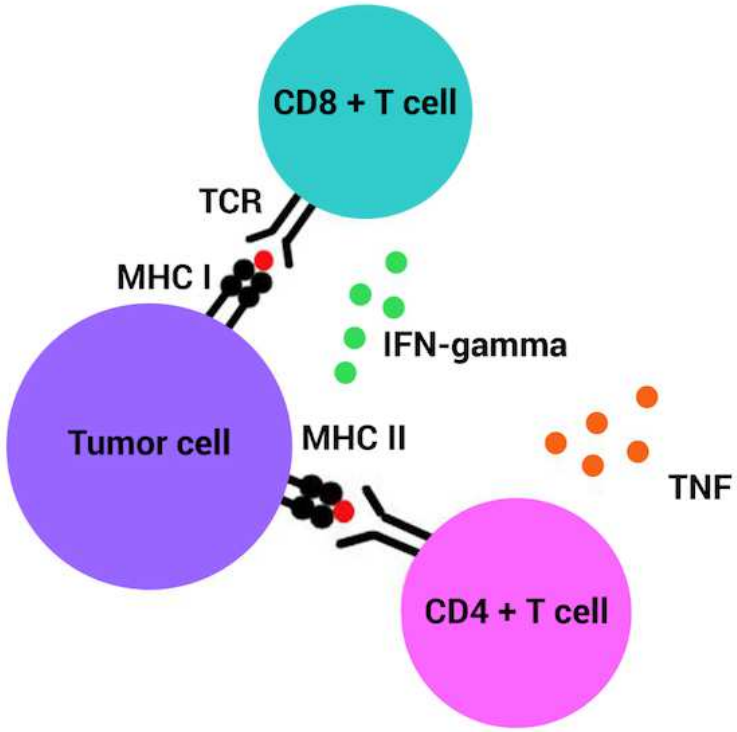
IFN from CD8 cells at the tumor site may increase MHC expression
But CD4 derived TNF may inhibit the increase in immune recognition

CIT, Confidential

CD4$^+$-derived TNF inhibits recognition by CD8$^+$ TILs



Activated CD8+ T cell producing IFN-gamma



Activated CD8+ T cell producing IFN-gamma and CD4+ T cell producing TNF

Increase in recognition by IFN

Back to baseline with added TNF

A glance at CD4 T cells: Conclusions

- Constitutive MHC class II^+ melanomas attract tumor reactive $CD4^+$ T cells

A glance at CD4 T cells: Conclusions

- Constitutive MHC class II^+ melanomas attract tumor reactive $CD4^+$ T cells
- Tumor reactive $CD4^+$ T cells show a marked inflammatory phenotype

A glance at CD4 T cells: Conclusions

- Constitutive MHC class II^+ melanomas attract tumor reactive $CD4^+$ T cells
- Tumor reactive $CD4^+$ T cells show a marked inflammatory phenotype
- **Tumor reactive $CD4^+$ T cells may dampen $CD8^+$ T cell recognition of melanoma cells**

Final Conclusions

- **TIL based ACT in melanoma – highly efficient in a significant fraction of patients – even with lower dose of IL-2**
- **These more powerful anti-cancer responses are likely to lead to more frequent excape of cancer cells from immune recognition**
- **CD4 T cells need further study – but may under certain conditions not be supportive of the cytotoxic response mediated by CD8 T cells**

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Thank you for your attention…..!!

Questions !!

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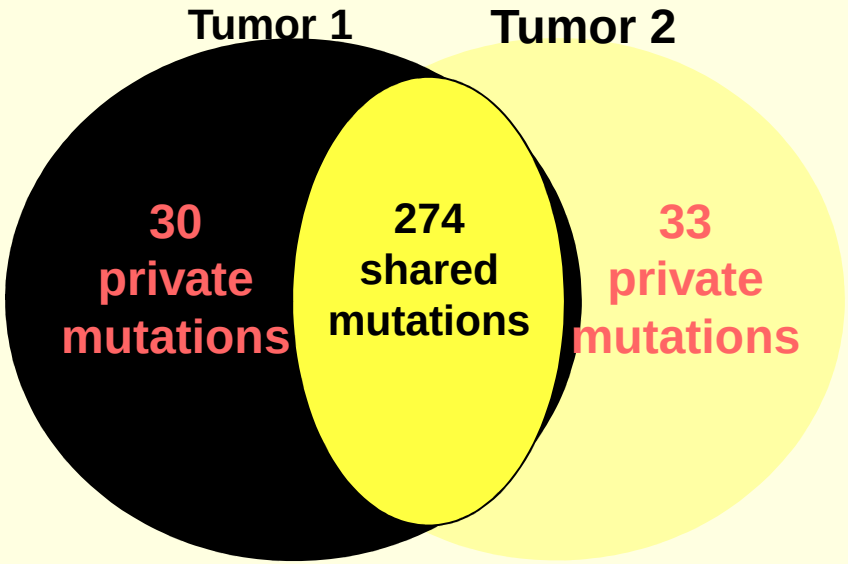
Genetic (whole-exome) Sequencing

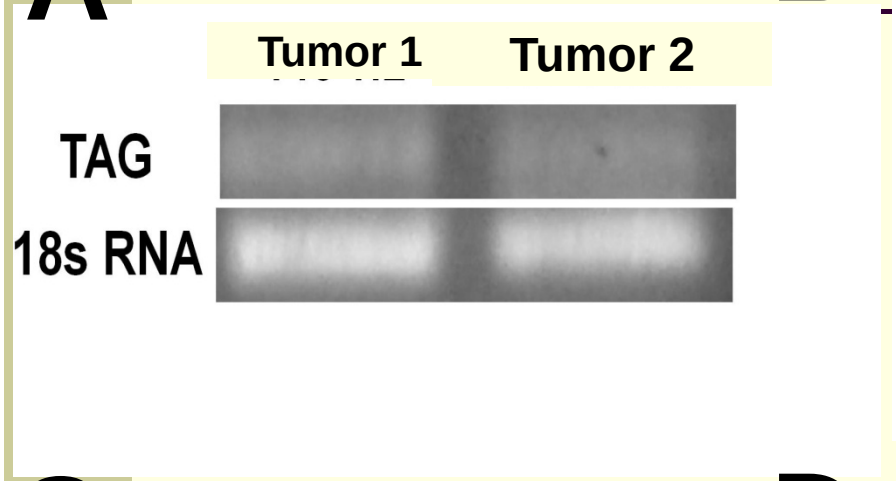


- Confirms the same origin of the tumors

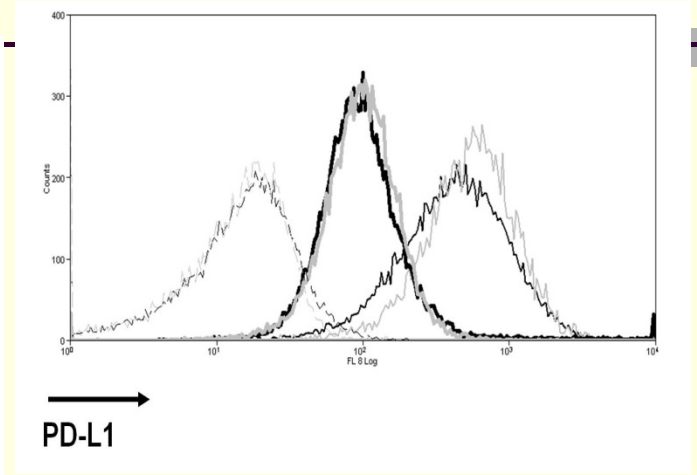
- Single Mutational events do not explain biological differences

Dr. Göran Jönsson
Melanoma Genomics Unit
University of Lund, Sweden

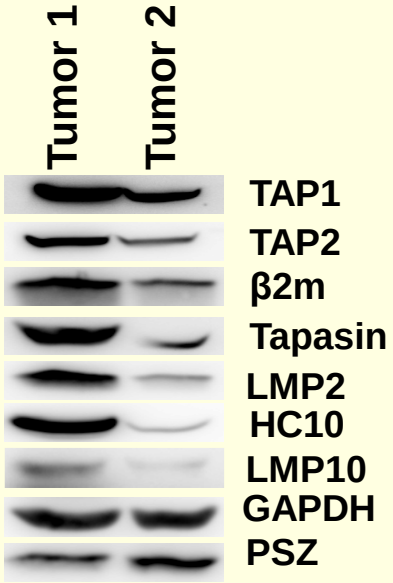
A


Tumor 1
Tumor 2
TAG
18s RNA

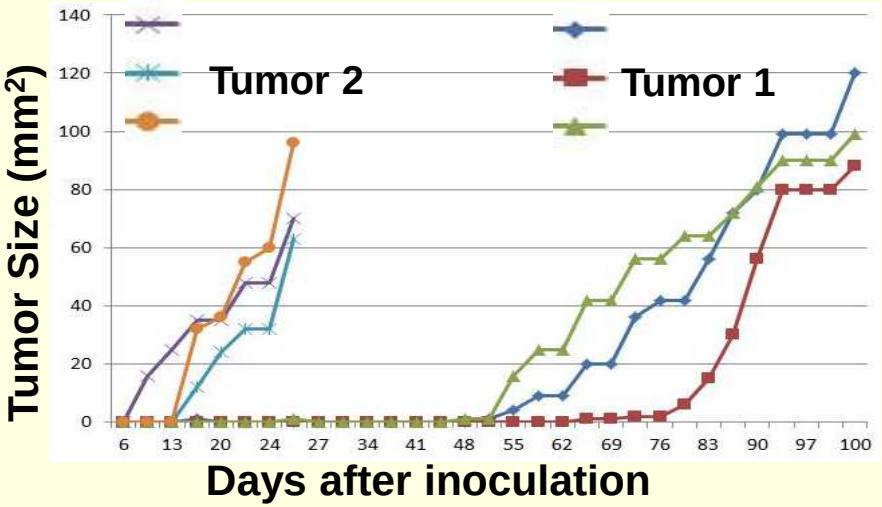
B


PD-L1

C

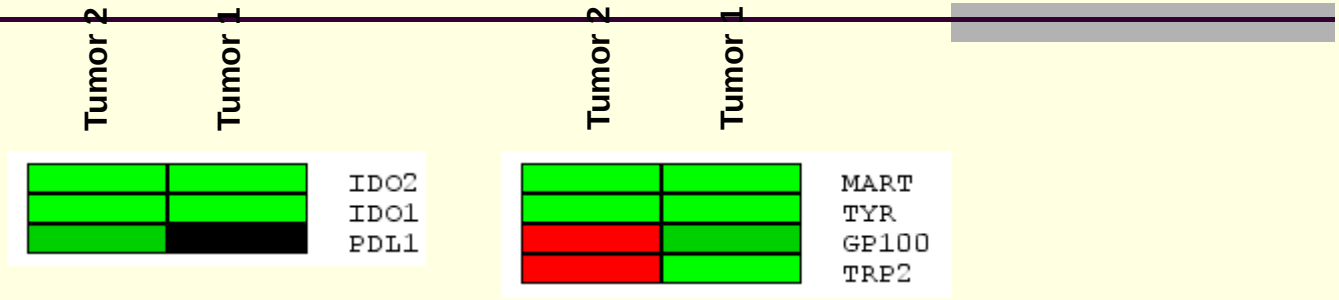

Tumor 1
Tumor 2
TAP1
TAP2
β2m
Tapasin
LMP2
HC10
LMP10
GAPDH
PSZ

D


Tumor 2
Tumor 1
Tumor Size (mm²)
Days after inoculation

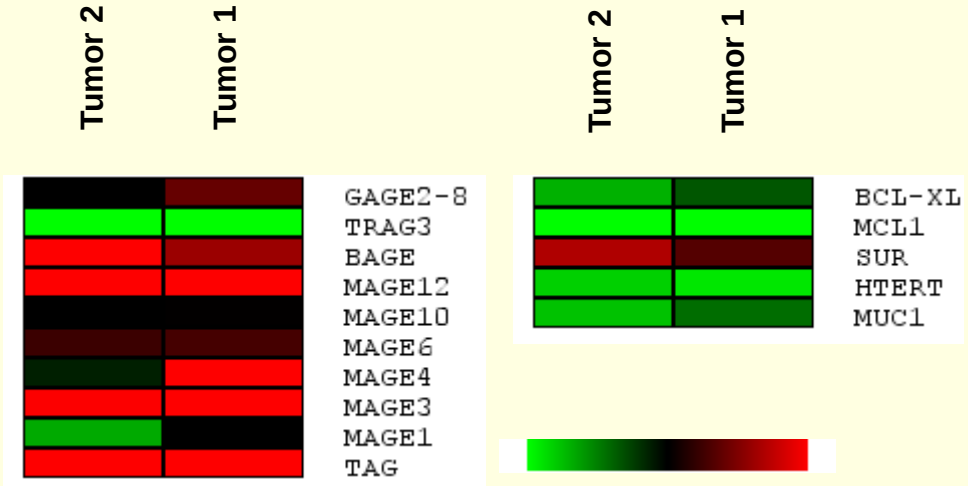
Immune suppressive

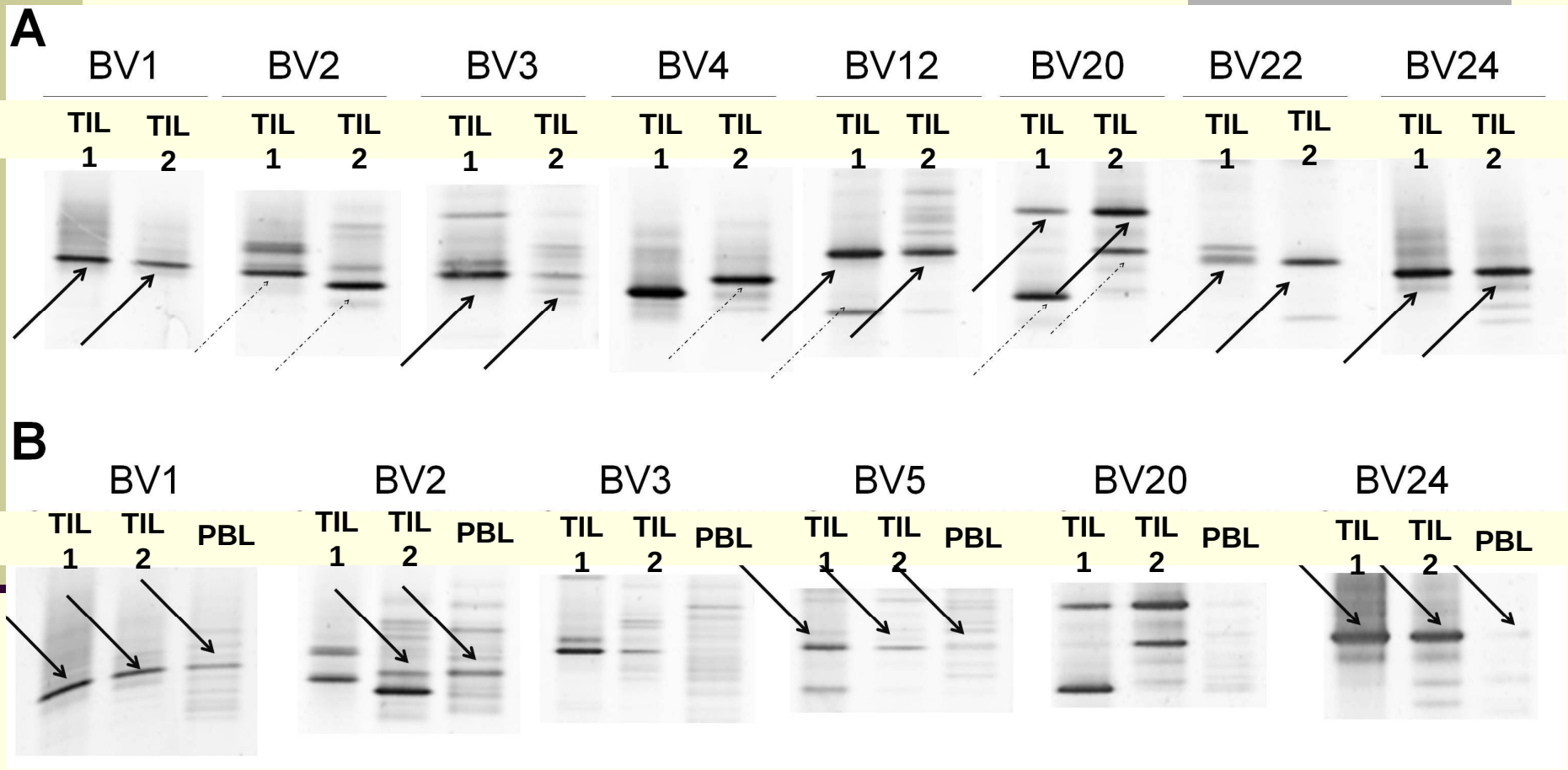
Melanocyte differentiation



Cancer-testis antigens

Survival/Malignancy




A
BV1
BV2
BV3
BV4
BV12
BV20
BV22
BV24
TIL 1
TIL 2
B
BV1
BV2
BV3
BV5
BV20
BV24
TIL 1
TIL 2
PBL