Dendritic cell-based cancer immunotherapy

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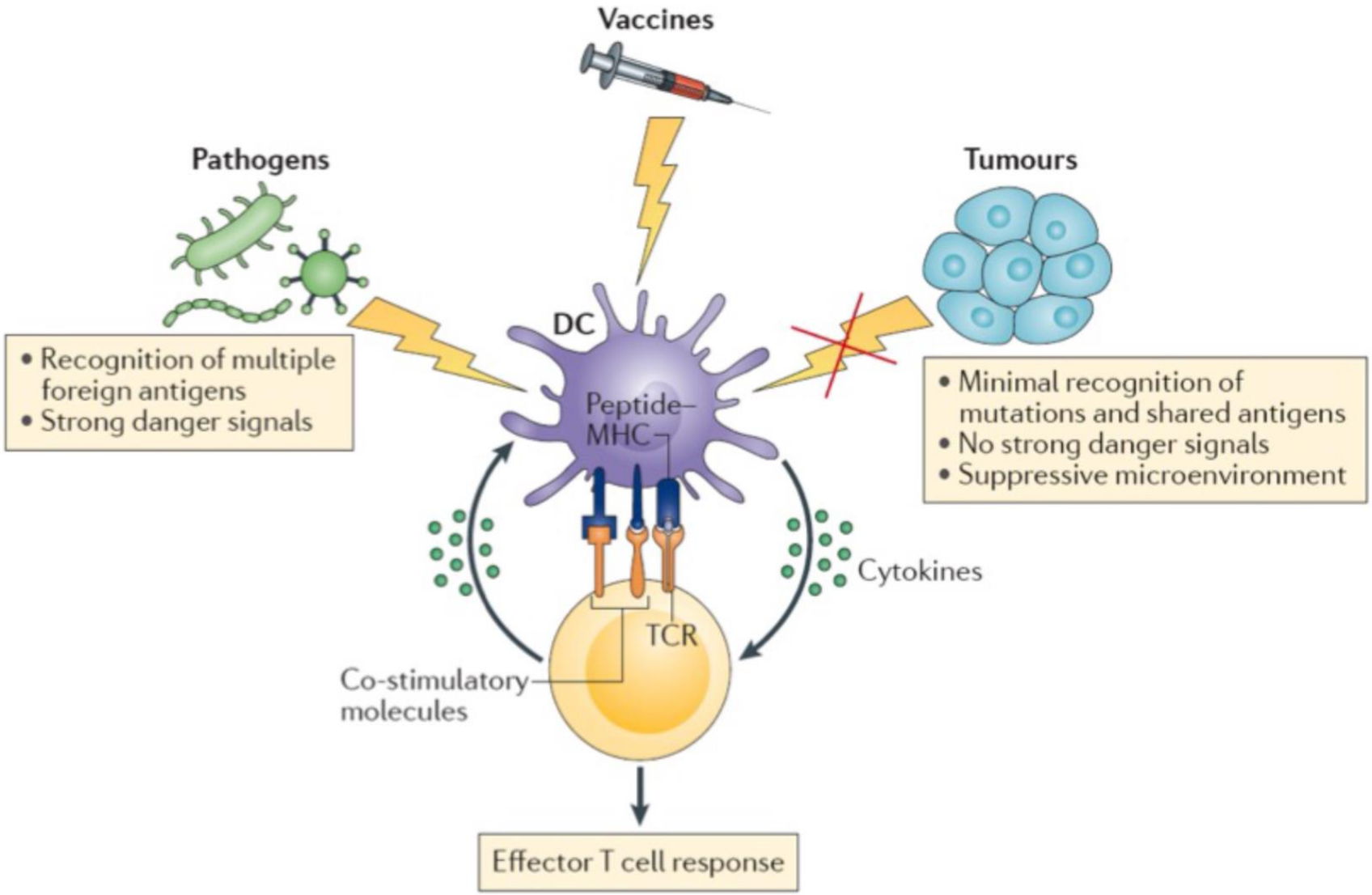
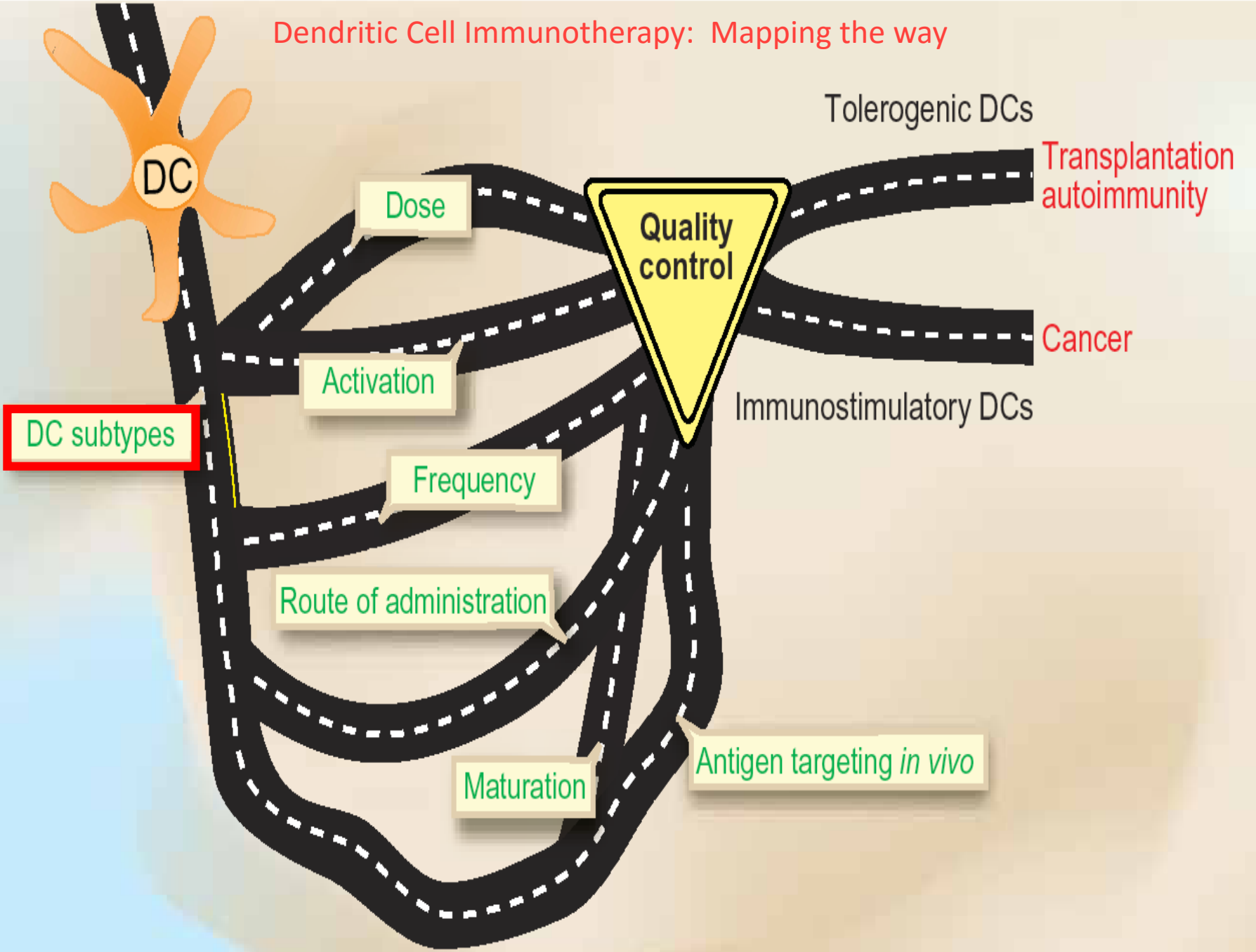
Why is vaccination against cancer so difficult?



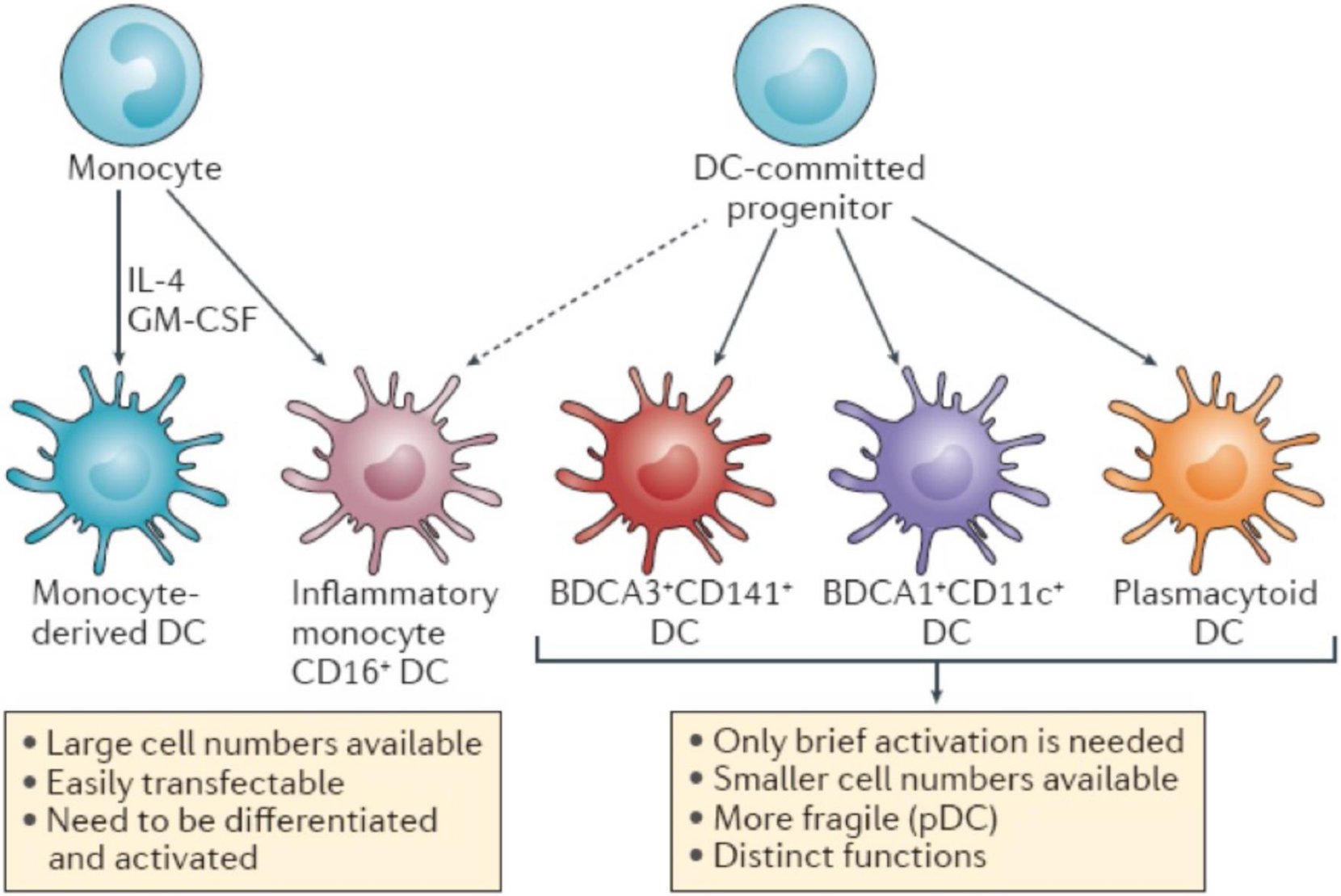
Exploitation of Dendritic Cells as a vaccine against cancer

Dendritic Cell Immunotherapy: Mapping the way

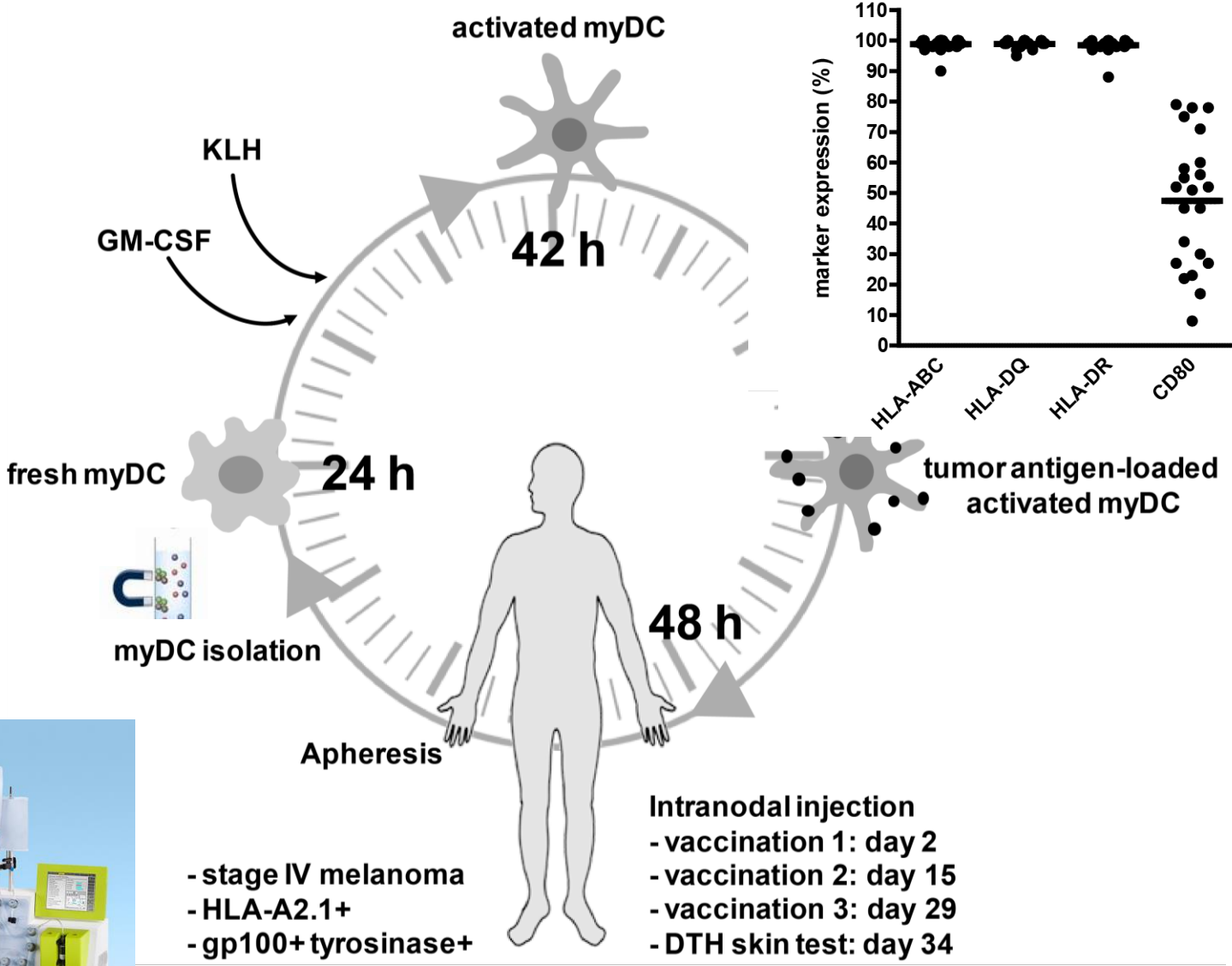


Figdor et al Nature Medicine 2004

Dendritic cell subsets

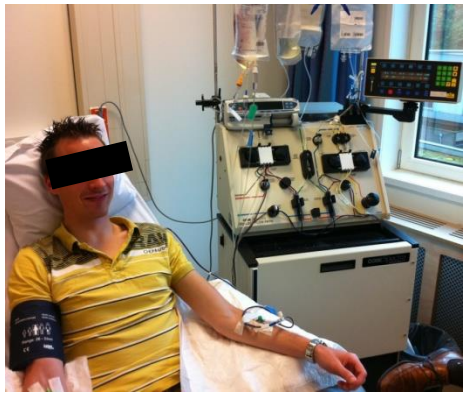


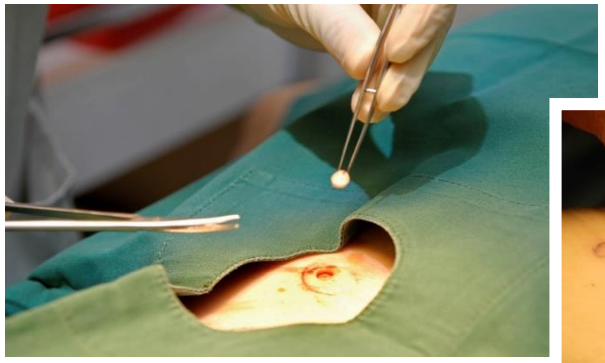
Rapid BDCA-1+ myDC vaccine preparation

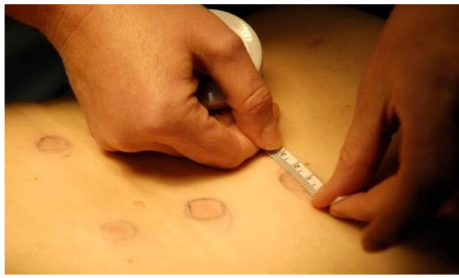


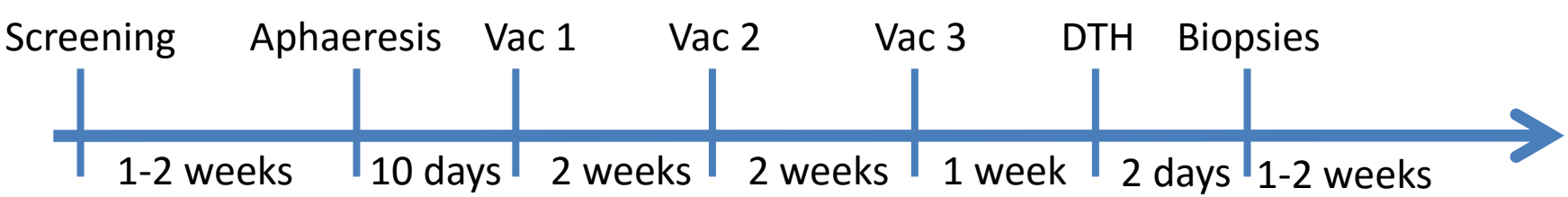
- stage IV melanoma
- HLA-A2.1+
- gp100+ tyrosinase+

Intranodal injection
- vaccination 1: day 2
- vaccination 2: day 15
- vaccination 3: day 29
- DTH skin test: day 34



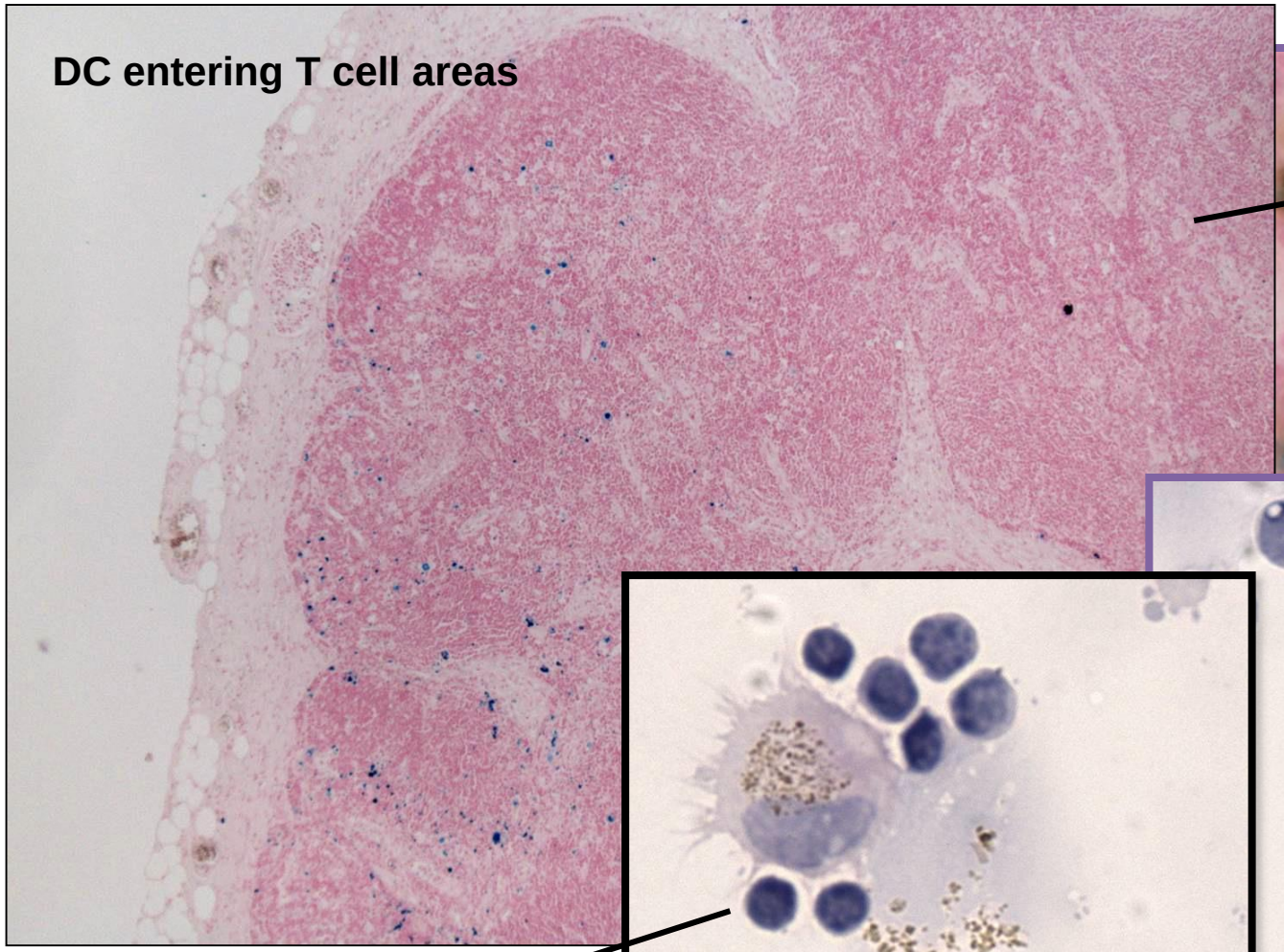


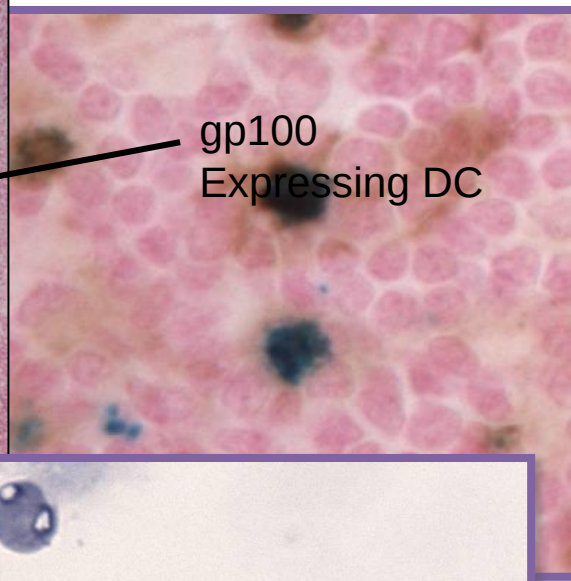


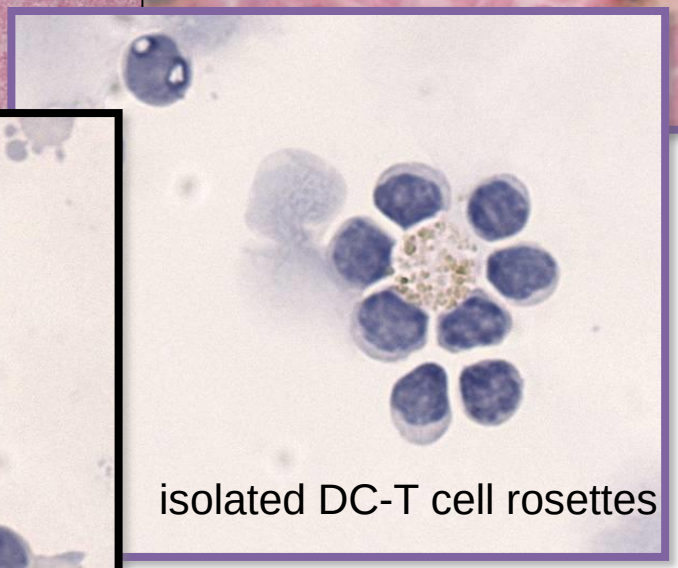

Screening
Aphaeresis
Vac 1
Vac 2
Vac 3
DTH
Biopsies
1-2 weeks
10 days
2 weeks
2 weeks
1 week
2 days
1-2 weeks

How effective are DC vaccines?

imaging to study function and fate of DCs infiltrating lymph nodes



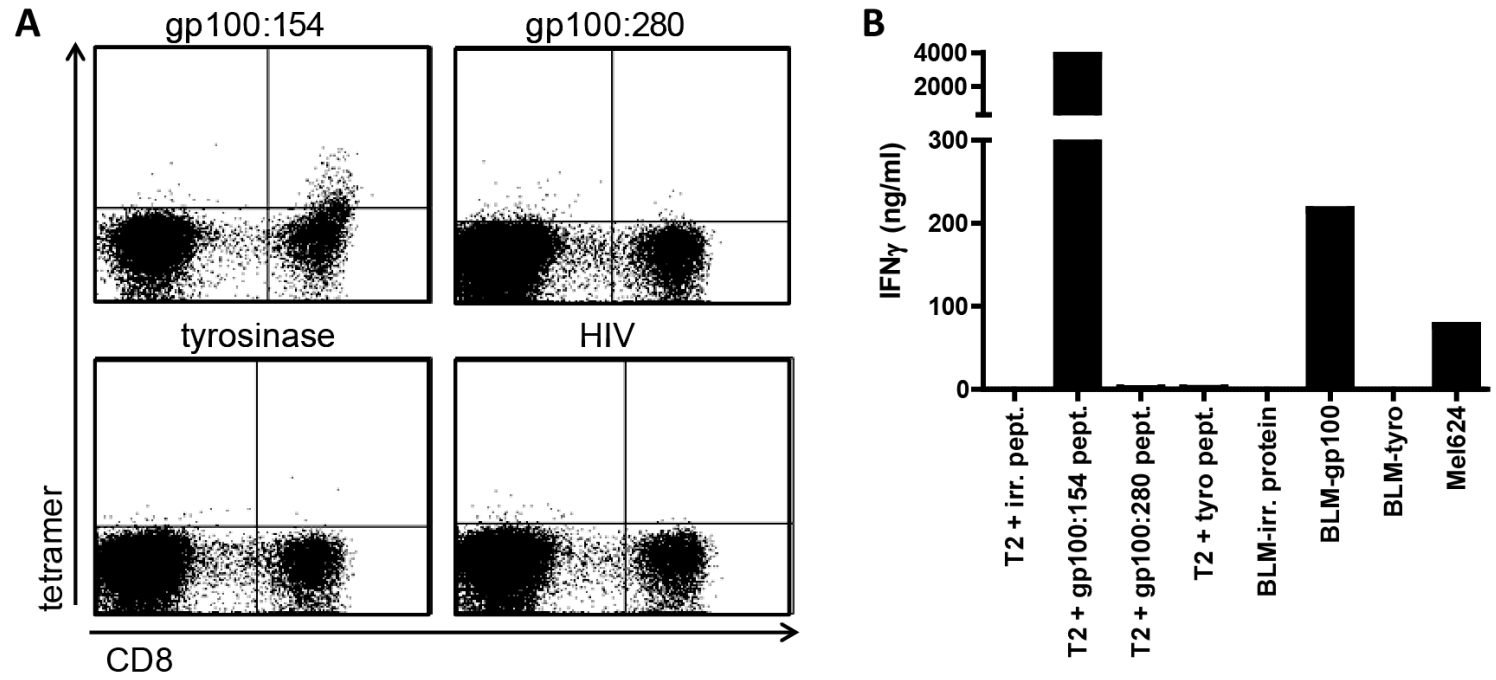




Antigen specific T cells

Danita Schuurhuis et al Cancer Res 2009

Complete remission



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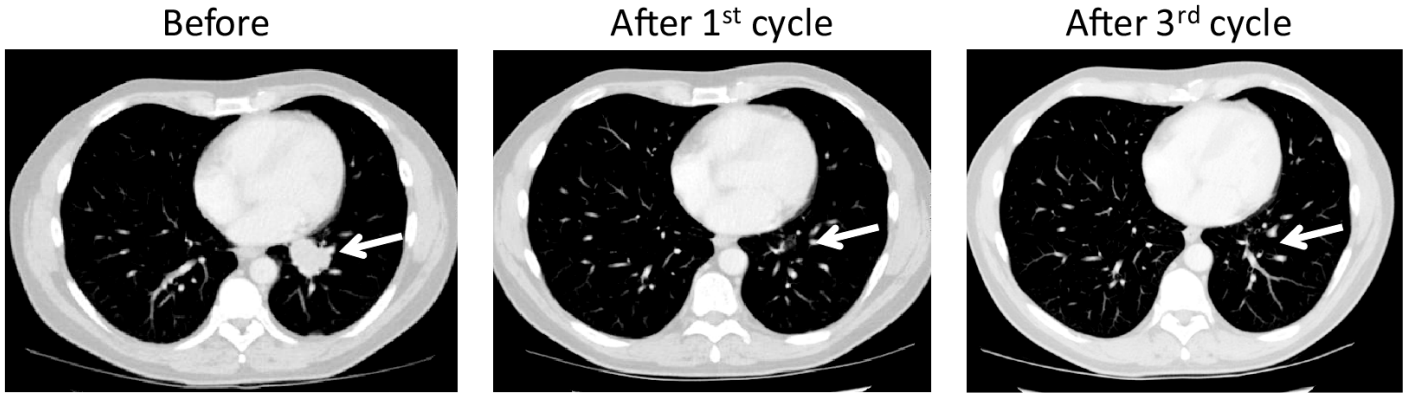

Patient VI-B-13

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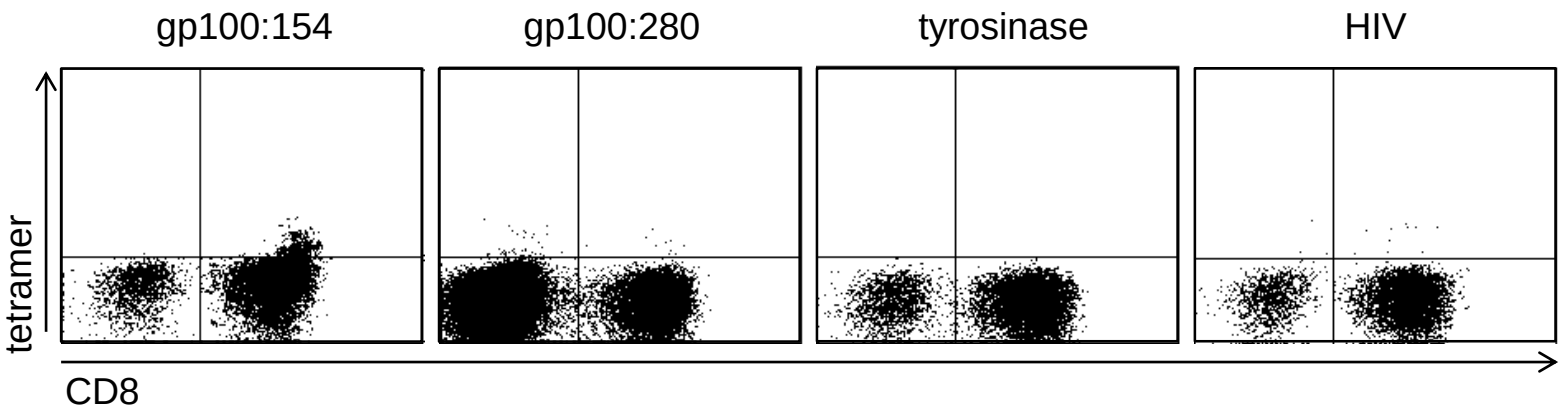
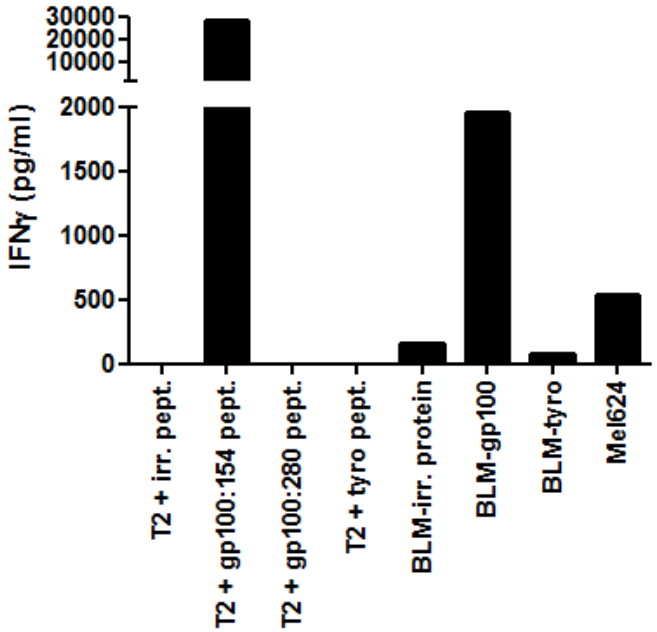
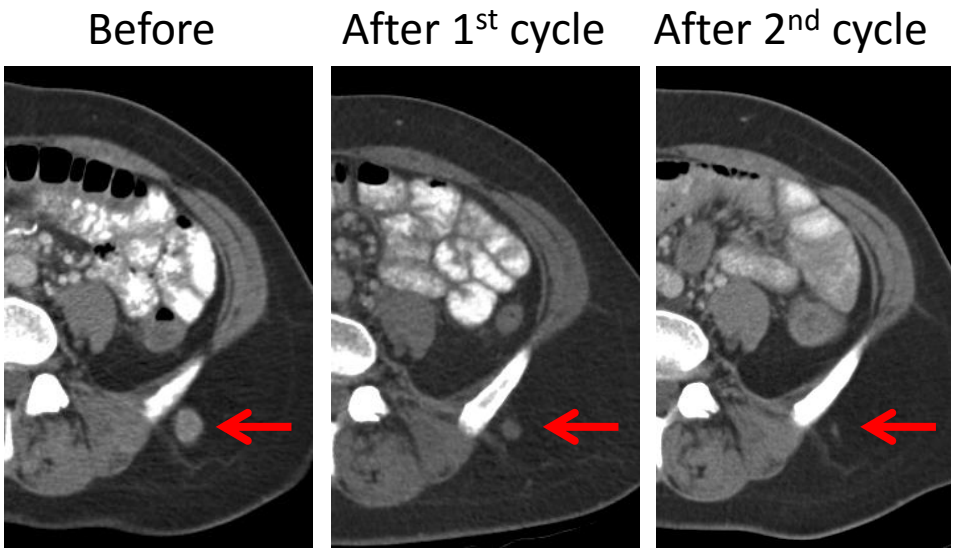
Mixed response







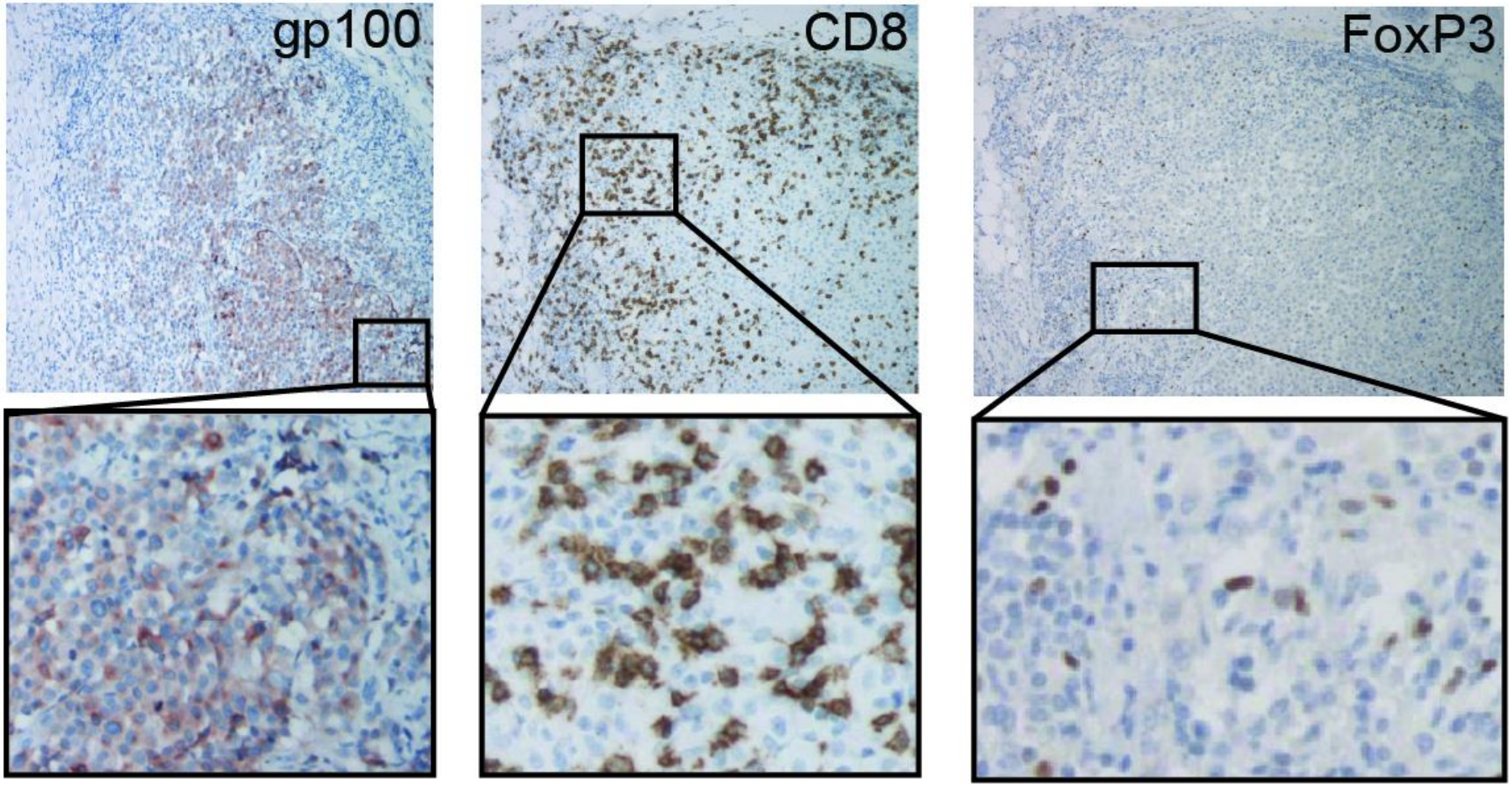
Patient VI-B-08

Histochemistry of progressive tumor

Tumor antigen

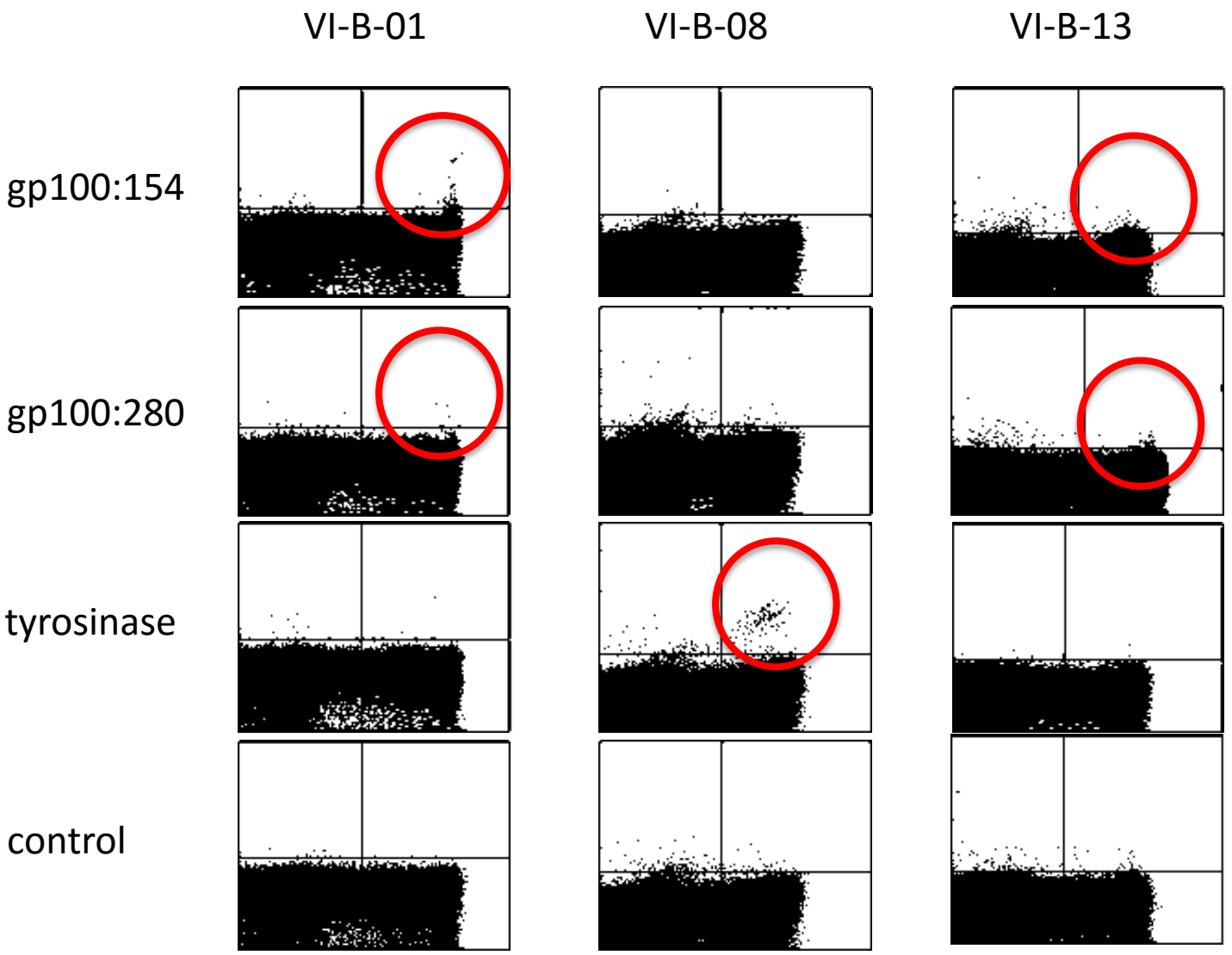
Cytotoxic T cells

Regulatory T cells



Patient VI-B-08

Tumor-specific T cells in peripheral blood



Schreibelt, Clinical Cancer Research 2015

Clinical responses in stage IV melanoma patients after vaccination with primary CD1c+ myeloid DCs

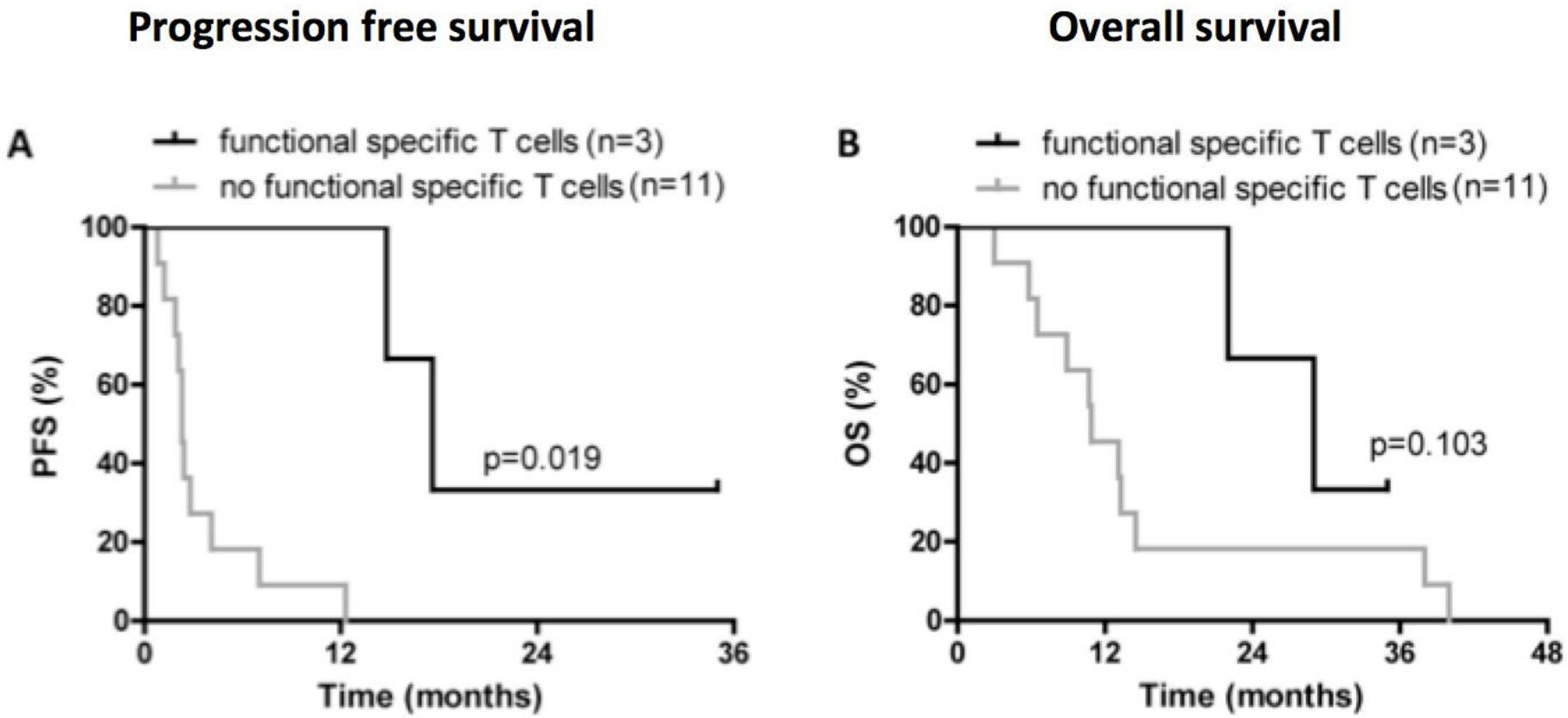
Patient	clinical response	Progression free survival (months	Overall survival (months)	T cells blood	T cells biopties
VI-B-01	SD	18	22	+++	+++
VI-B-02	PD	<4	7	-	-
VI-B-03	SD	7	40	-	-
VI-B-04	PD	<4	3	n.a.	n.a.
VI-B-05	PD	<4	9	-	+
VI-B-06	SD	4	13	-	-
VI-B-07	PD	<4	11	-	-
VI-B-08	MR	15	29	+++	+++
VI-B-09	SD	12	15	-	-
VI-B-10	PD	<4	38	-	-
VI-B-11	PD	<4	6	+	-
VI-B-12	PD	<4	11	n.t.	-
VI-B-13	CR	35+	35+	+++	+++
VI-B-14	PD	<4	13	-	-

SD = stable disease
PD = progressive disease
CR = complete remission
MR = mixed response

\+ = antigen-specific T cells present
+++ = functional specific T cells

Schreibelt, Clinical Cancer Research 2016

Clinical outcome and functional T cell response



Schreibelt, Clinical Cancer Research 2015

Vaccination with blood DCs

- **pDC and myDC vaccination is feasible and safe**
- **Induce strong de novo immune responses and objective clinical responses, even in advanced melanoma patients**
- **Clinical responses are associated with the presence of tumor-specific T cells**
- **pDC and myDC use different mechanisms to induce anti-tumor responses**

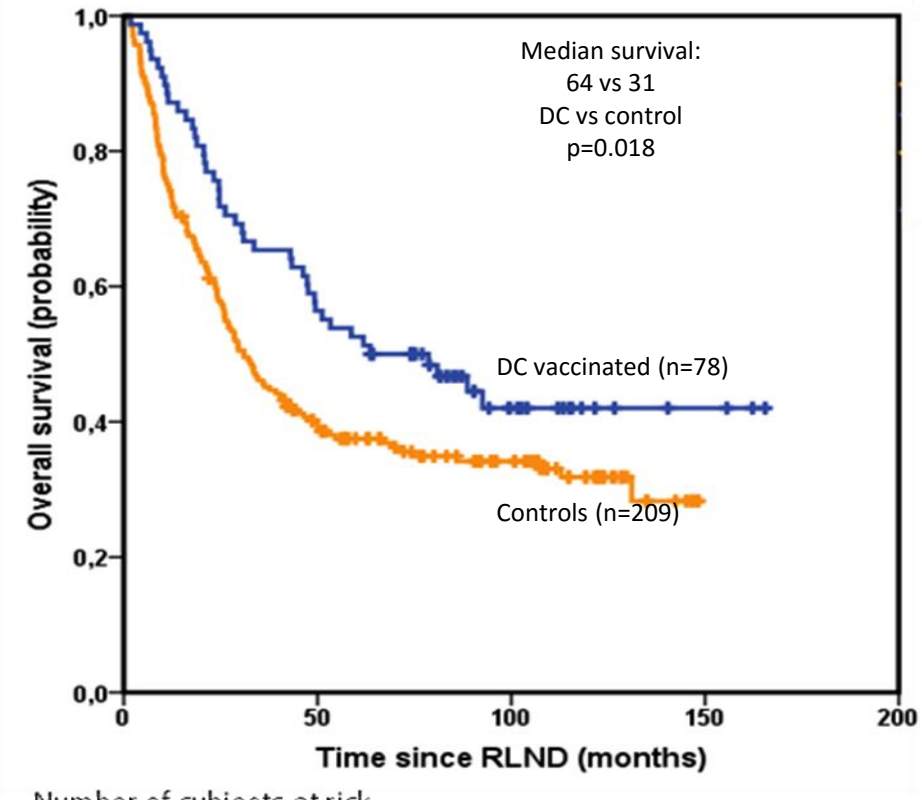
Towards less tumor burden…

Functional tumor-specific T cells after DC vaccination:

71% in patients with regional lymph node metastasis (st III)

23-30% in patients with distant metastasis (st IV)

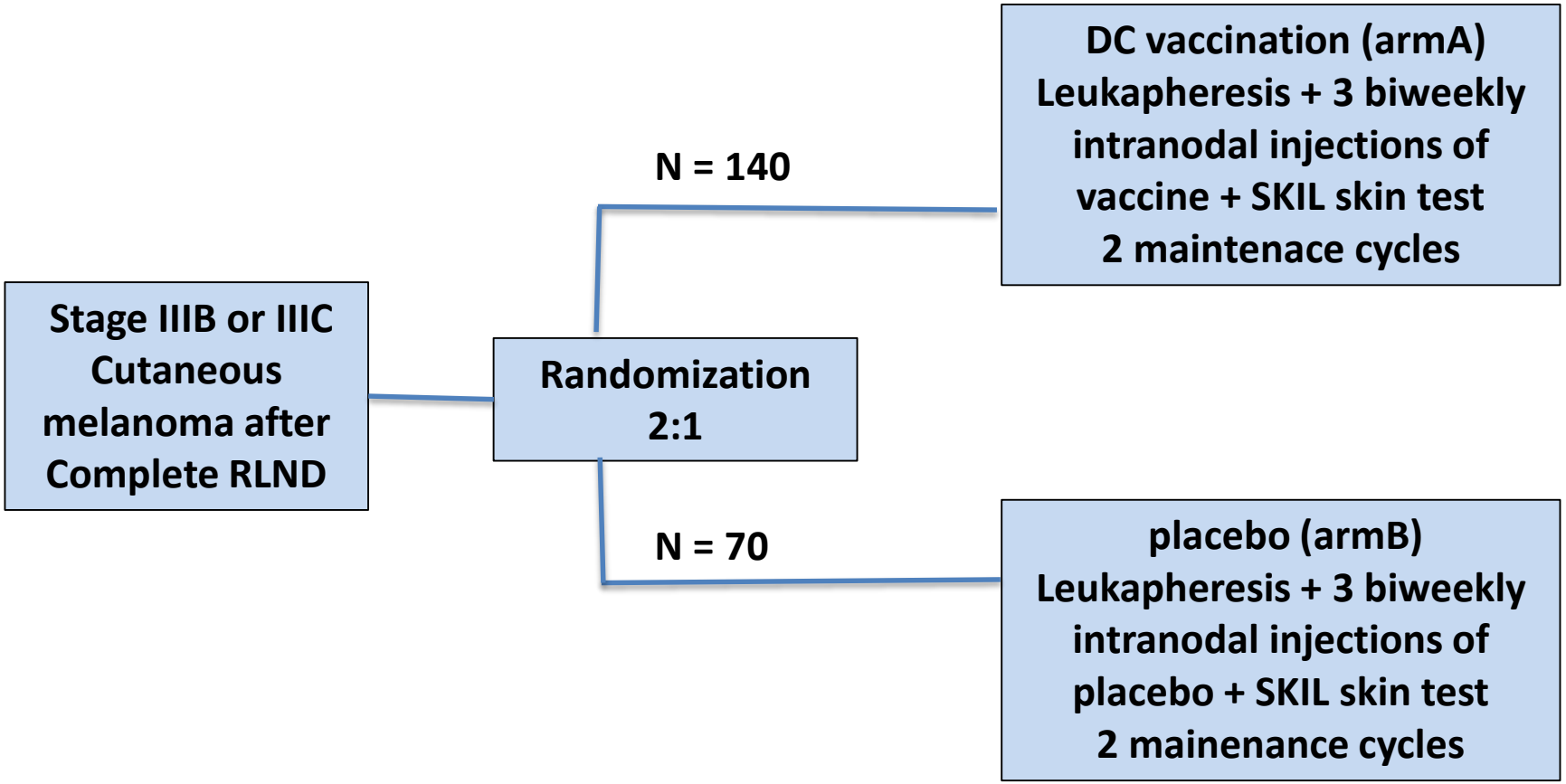
Overall survival of stage III melanoma patients



Number of subjects at risk

Co:	209	76	39	0
DC:	78	44	14	3

Phase III study (210pts) with combined pDC /myDC vaccine



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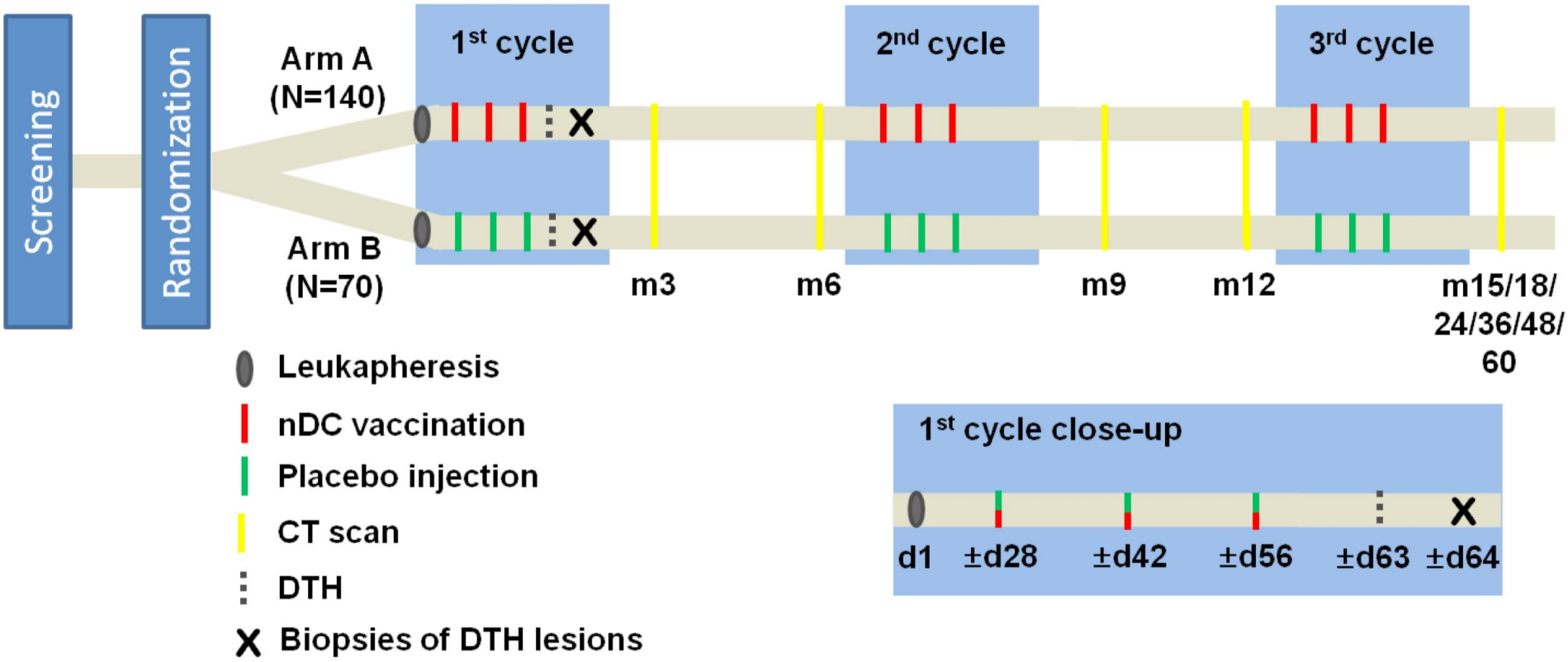


Phase III study (210pts) with combined pDC /myDC vaccine

7.1 Primary endpoint

The primary endpoint is 2-year RFS rate, defined as the percentage of patients who are alive and without recurrence of melanoma 2 years after randomization.

7.2 Secondary endpoints

-median RFS
-2-year and median OS
-adverse events profiles (safety)
-immunological responses
-quality of life and health economic aspects of nDC vaccination versus placebo

Preventive vaccination?

Antigens used in cancer vaccines

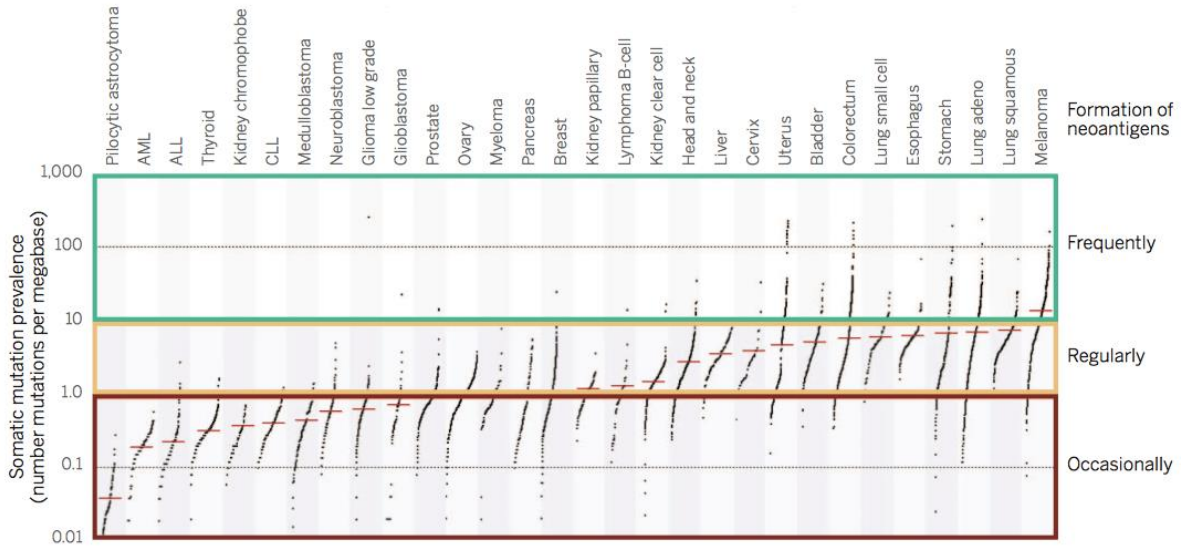
SHARED antigens

differentiation antigens: **gp100, tyrosinase, Melan A / Mart1**

Cancer-germline antigens: **Mage, NY-ESO-1, LAGE-1…….**

Neo-antigens:

Patient specific antigens



Alexandrov et al., Nature 2013

Lynch syndrome

- **Genetic cause: a germline mutation in mismatch repair genes**

  in particular *MLH1*, *MSH2, MSH6, EPCAM* and rarely *PMS2*

- **Lynch mutation carriers have increased risk for cancer**

  Colorectal cancer Life time risk 30-70%
  Endometrial cancer Life time risk 30-70%
  Ovarian, gastric, hepatobiliary, small bowel, urinary tract cancer
  Life time risk <10-15%
  Multiple primary cancers (synchronous and metachronous)
  (23% has a double tumor, LTR second carcinoma 90%)

- **Lynch syndrome accounts for up to 5% of CRC.**
- **Few adenomas (very fast progression from adenoma to cancer!)**
- **Young age at cancer diagnosis (mean 40-45 years)**
- **Colonoscopy to remove adenomas before cancer develops every 2 years starting at age 25 years**

Tumor-specific neo-antigens arise as a consequence of DNA mutations

Lynch syndrome

Defects in the mismatch repair system (MSI)

DNA damage

⇒ **frame shift mutation (prior to malignancy)**

⇒ **frame shift-derived neo-peptides**

⇒ **putative HLA binding epitopes**

⇒ **might be recognized by the immune system**

Mutations in Coding Microsatellites
- examples -

- ***TGFßRII*** Growth stimulation (epithelial) cells
- ***BAX*** Apoptosis inhibition
- ***OGT*** Protein modification (addition of N-acetyl glucosamine residues) to proteins involved in carcinogenesis
- ***Caspase 5*** Altered inflammatory response
- ***ß2M*** Stimulation of the immune surveillance

Saeterdal, I., et al., *Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer.* Proc Natl Acad Sci U S A, 2001. 98(23): p. 13255-60
Saeterdal, I., et al., *A TGF betaRII frameshift-mutation-derived CTL epitope recognised by HLA-A2-restricted CD8+ T cells.* Cancer Immunol Immunother, 2001. 50(9): p. 469-76
Schwitalle, Y., et al., *Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells.* Cancer Immun, 2004. 4: p. 14.

Antigens:
Frameshift peptides, TAA and KLH

Induction of (functional) antigen-specific CD8+ T cells

HLA-A2.1 binding peptides

Caspase-5	→	**FLIIWQNTM**
TGFBRII	→	**RLSSCVPVA**
CEA	→	**YLSGANLNL**

Immunogenic carrier protein

KLH →

Saeterdal, et al. Proc Natl Acad Sci U S A, 2001
Saeterdal, et al. Cancer Immunol Immunother, 2001
Schwitalle, et al Cancer Immun, 2004

Cancer vaccination: Can Lynch syndrome patients benefit from immunotherapy?

CRC with MSI is characterized by a strong infiltration of T cells
Philips et al. Br J Surg 2004

MMR-deficient tumors have a high mutational load and generate more protein truncations and the origin of neoantigens
Llosa et al. Cancer Discov 2015

Frameshift peptides are only expressed by tumor cells or premalignant counterparts
Woerner et al. Cancer Biomark 2006,Saeterdal, Glaudernack et al PNAS 2001

Antigens:
Frame-shift peptides and foreign protein

HNPCC

HLA-class I:

TGF-ßRII	**RLSSCVPVA**
Caspase-5	**FLIIWQNTM**

Colon Carcinoma

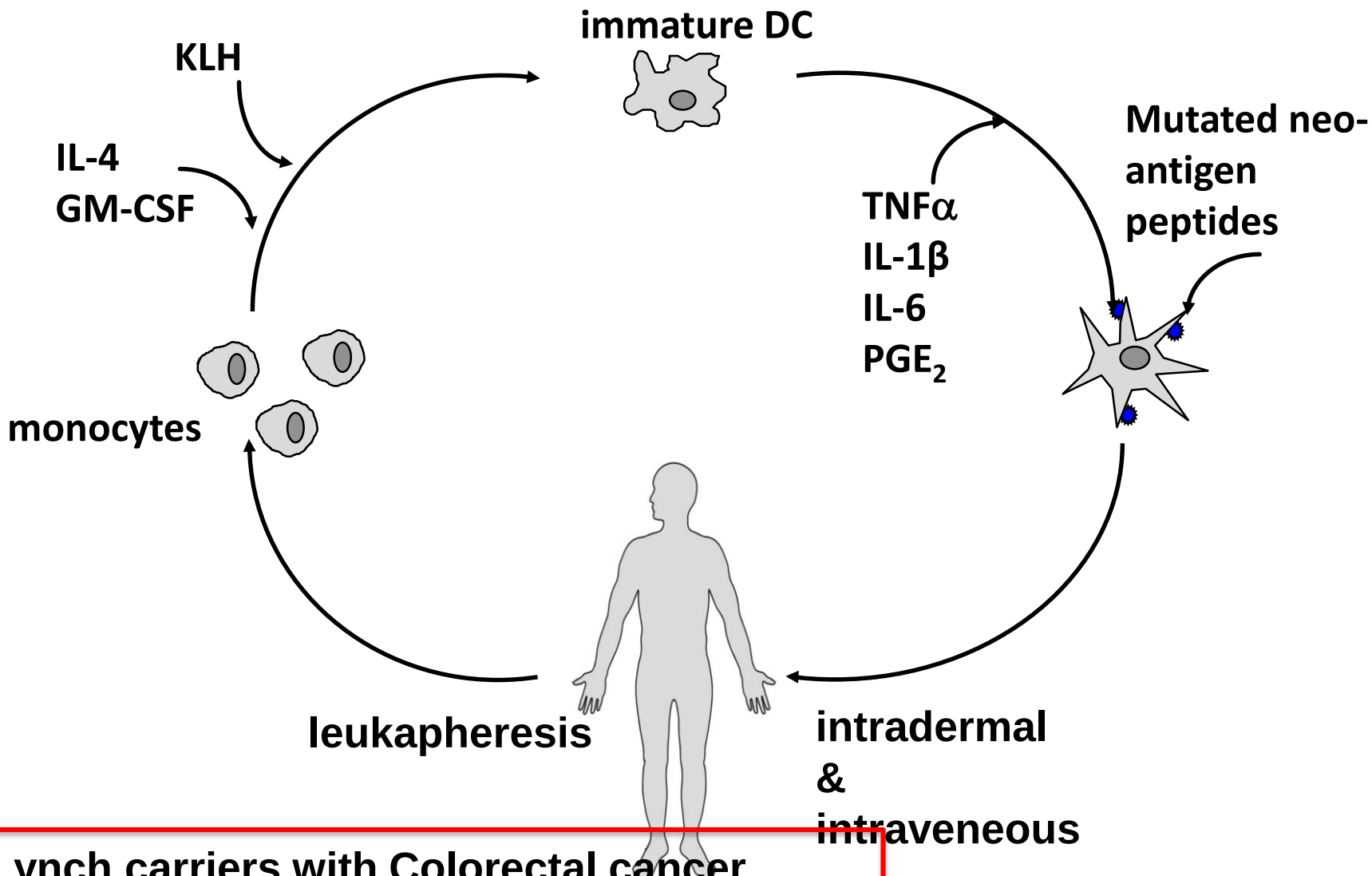
HLA-class I:

CEA	**YLSGANLNL**

Protein:

KLH (keyhole limpet hemocyanin) immunogenic protein T cell help

DC vaccination against mutated neo-antigens



A. Lynch carriers with Colorectal cancer
B. Lynch carriers with no cancer yet

Conclusions and Future prospective

- **DC vaccination against frameshift-derived neo-peptides is safe and can give rise to immune responses in Lynch syndrome carriers without any signs of autoimmunity**

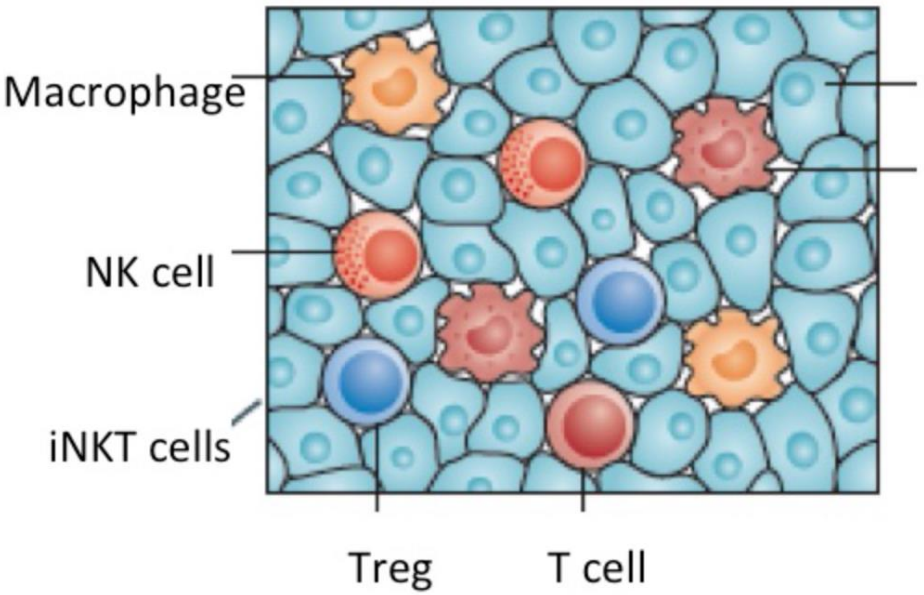
- **How to prove clinical efficacy?**
  - **Long term follow-up**
  - **Analyze expression of neo-antigens on adenoma's?**
  - **Investigate number of adenoma's/carcinoma's?**
  - **Subsequent trial: include patients in late 40ties**

Can we predict which patient will respond to immunotherapy?

How can we better understand the tumor-immune cell network?

tumor and immune system form a complex network

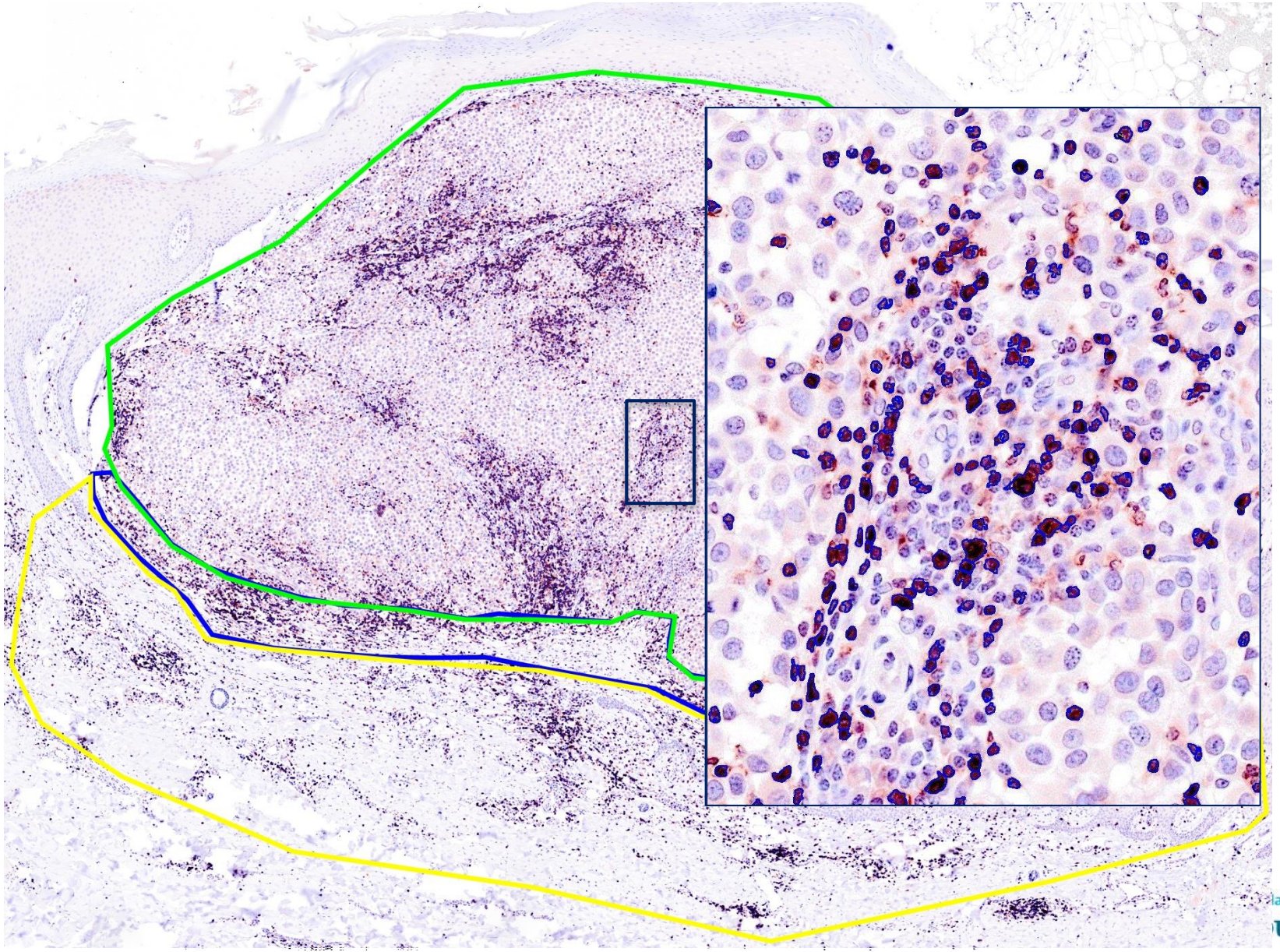
A better understanding of the tumor microenvironment is needed



- Down regulation of HLA molecules
- Failure to produce tumor antigens
- Failure effector cells to migrate into tumor
- Regulatory T cells (Treg)
- Myeloid derived suppressor cells (MDSC)
- Suppressive factors
  - TGF-β
  - IL-10
  - iNOS,
  - Arginase

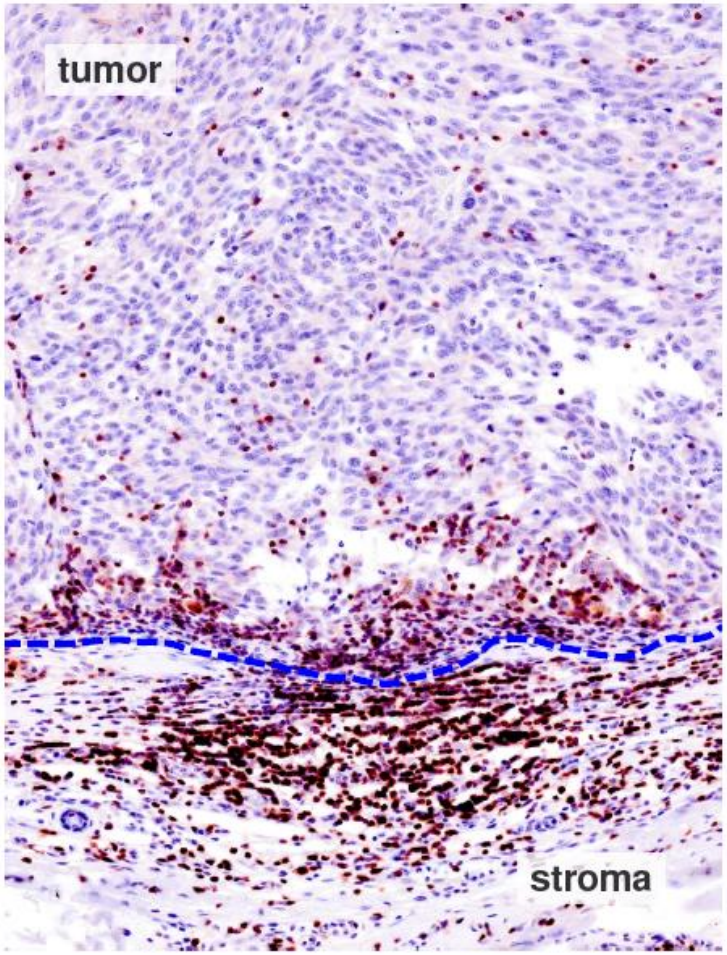
Co-evolution of the tumor – immune system network

Quantitative analysis of TILs density in primary melanoma

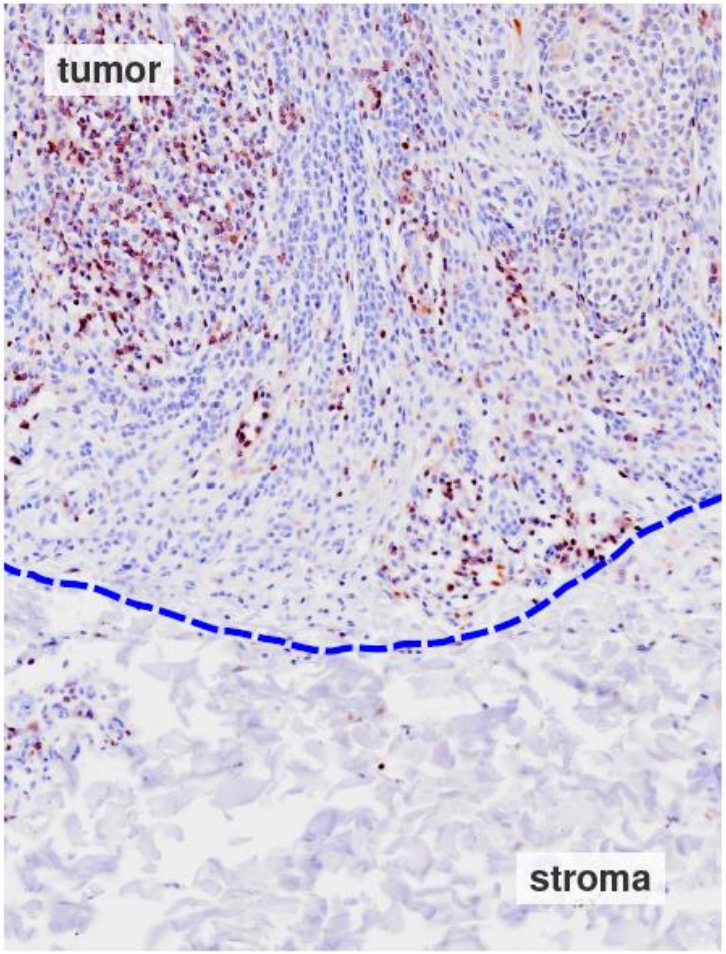


T cell infiltrates in primary melanoma and moDC therapy outcome

weak intramural infiltration

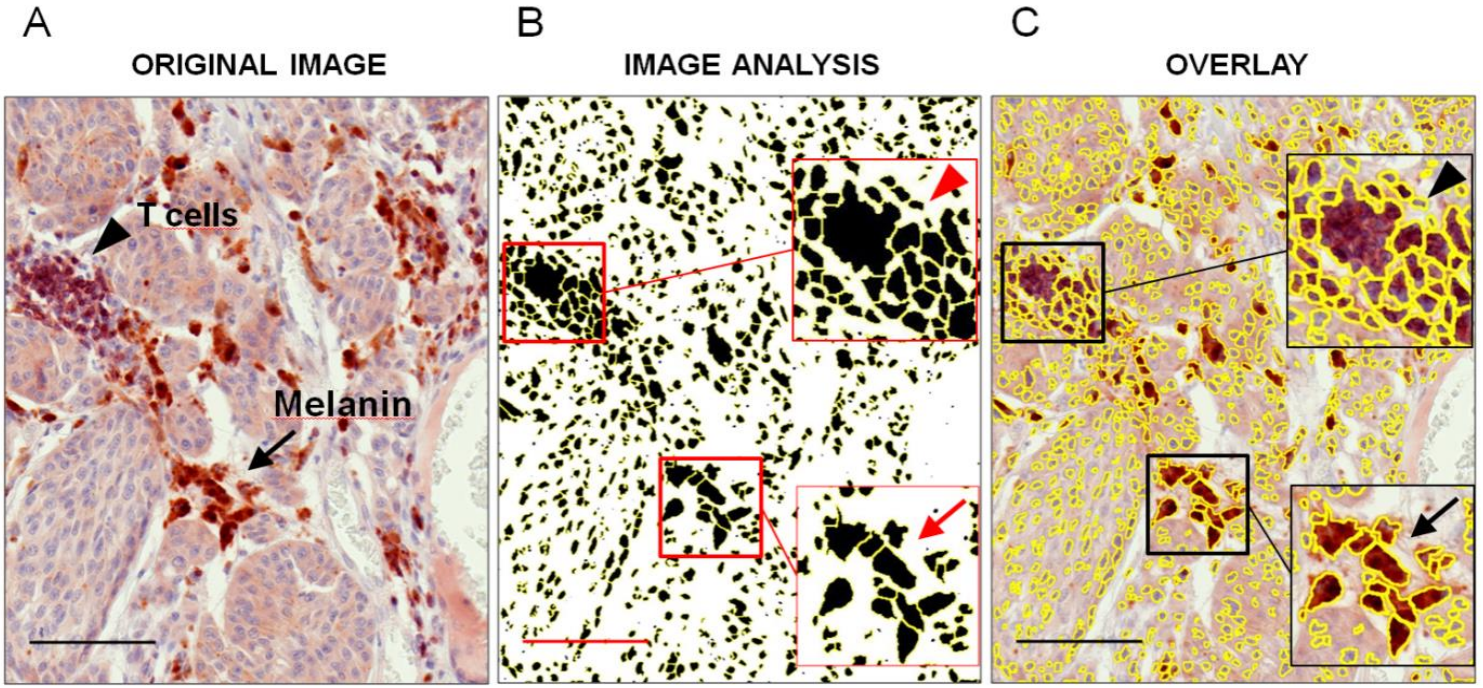


strong intramural infiltration

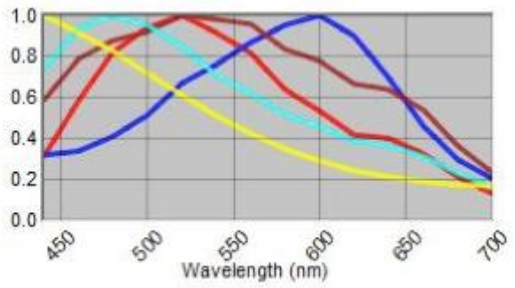


100 μm

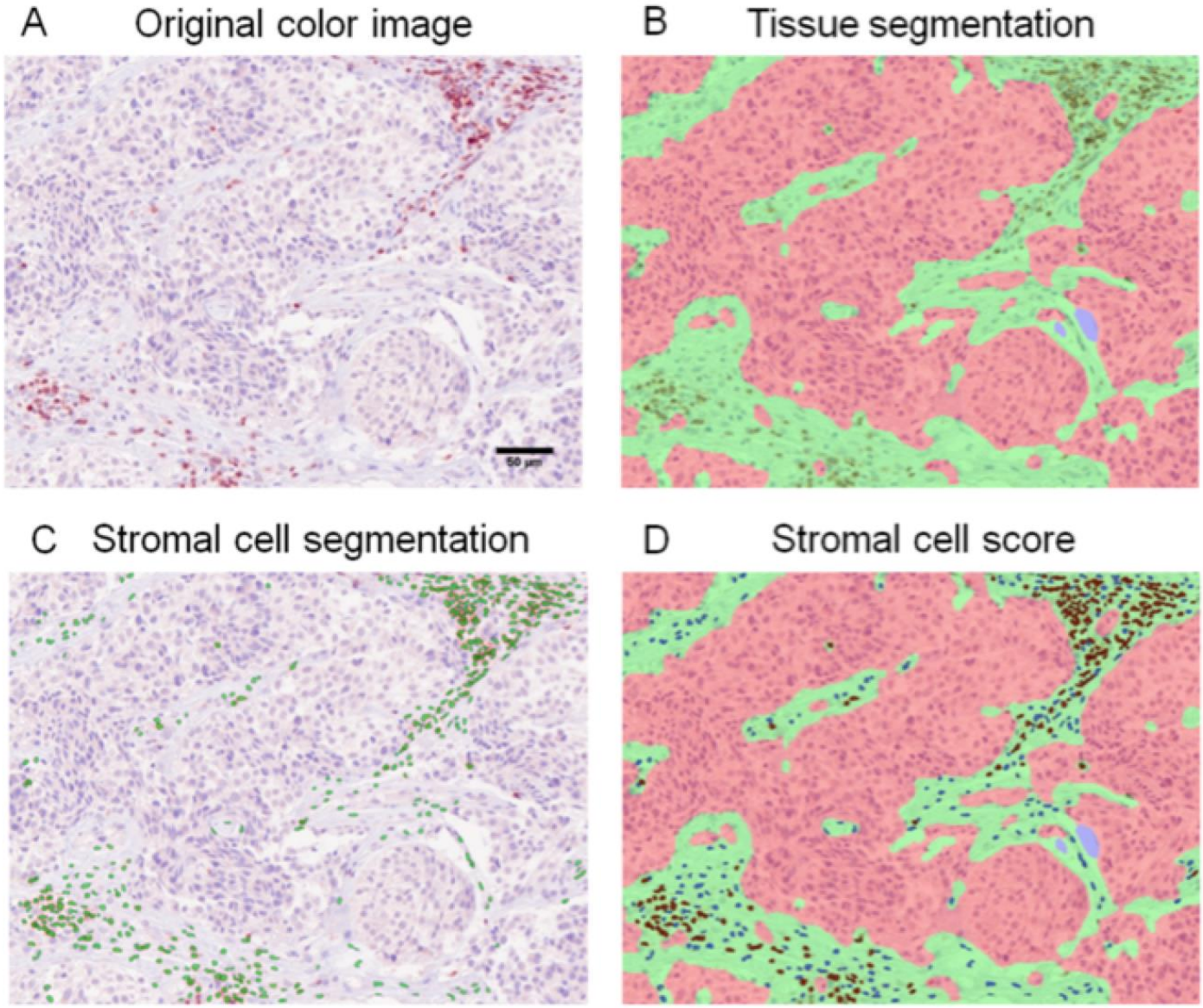
Multispectral image analysis



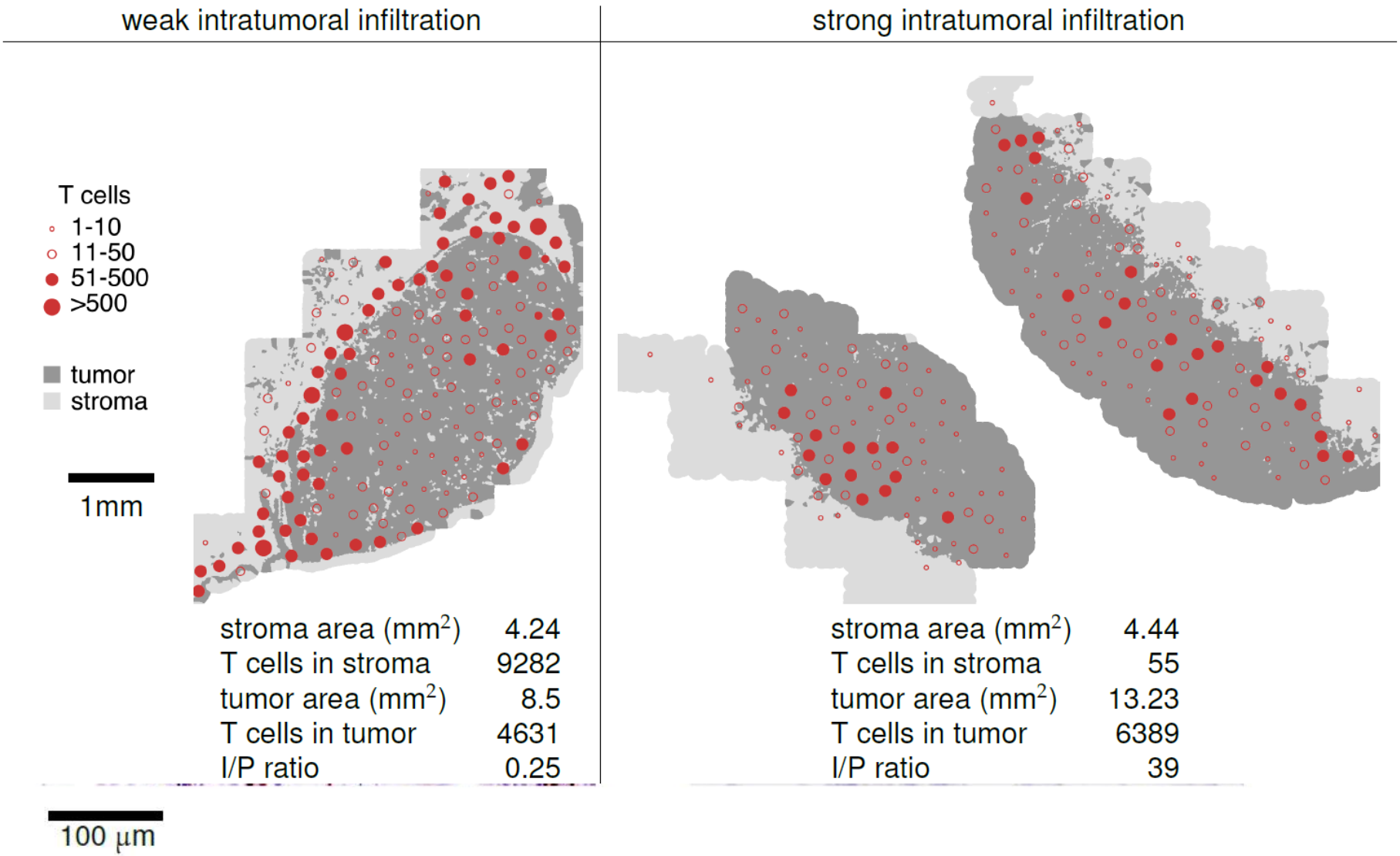
Vectra - Automated Multimodal Tissue Analysis



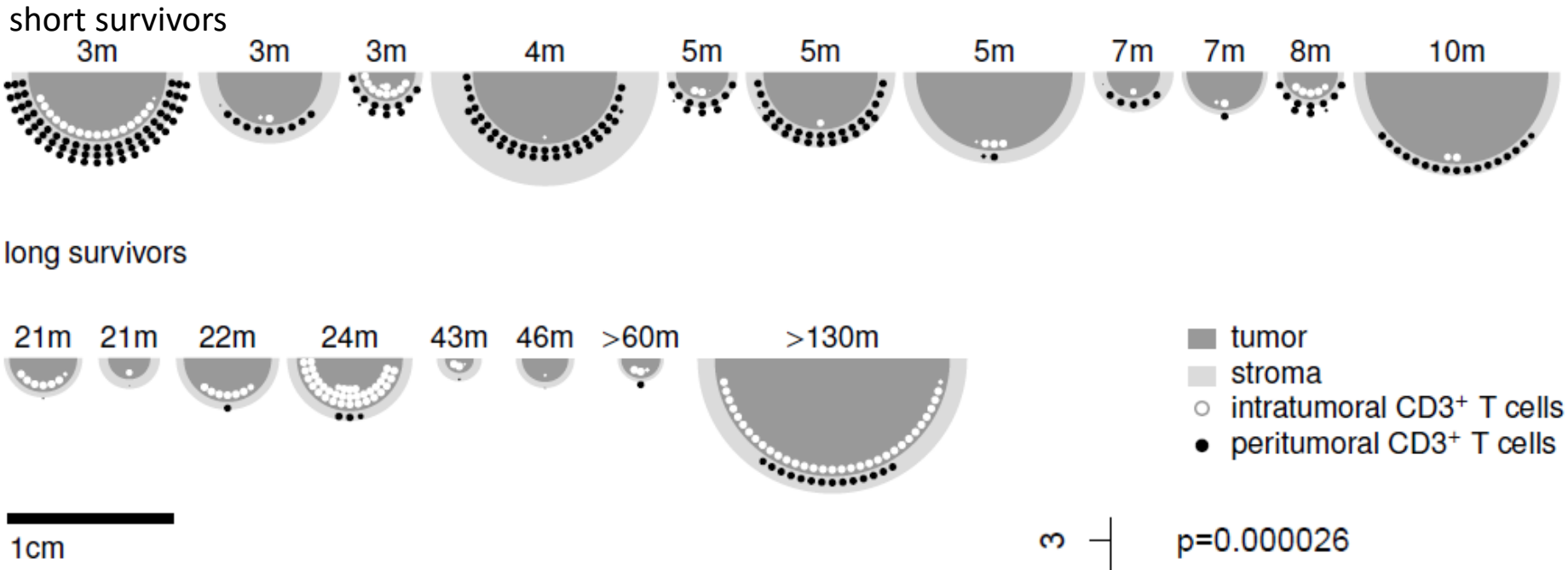
Tissue segmentation



T cell infiltrates in primary melanoma and moDC therapy outcome

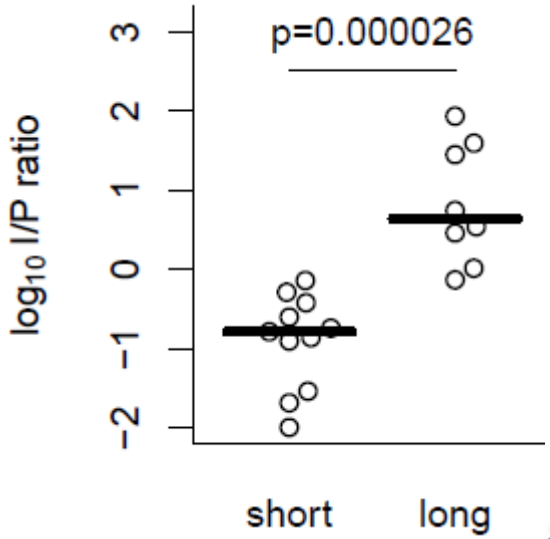


T cell infiltrates in primary melanoma and moDC therapy outcome



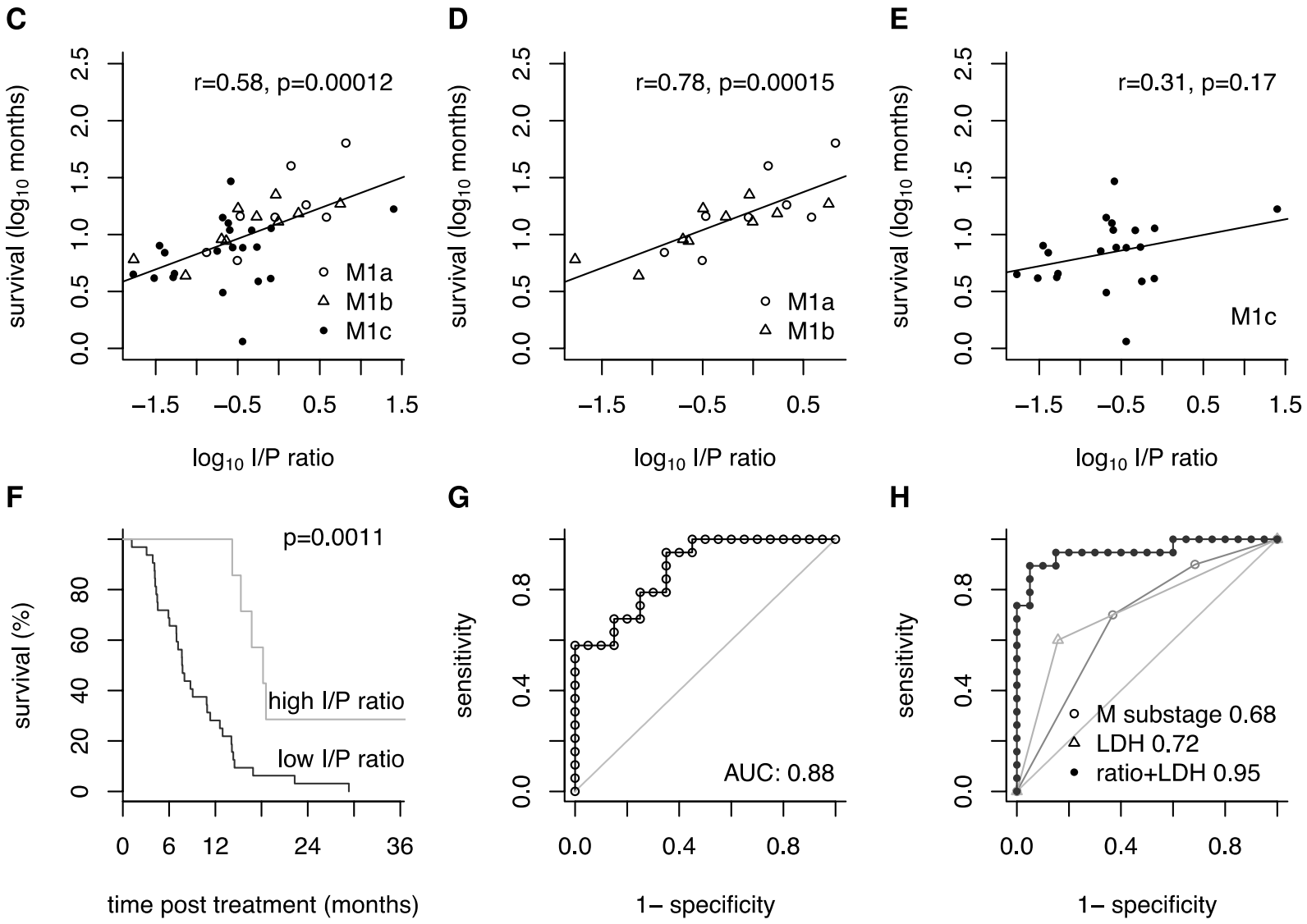
Intratumoral CD3+ T cells

Peritumoral CD3+ T cells



Vasaturo et al Cancer Research 2016

Peri/intratumoral T cell ratio in primary tumor strongly correlates with survival after DC-based immunotherapy



Vasaturo et al Cancer Research 2016

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Conclusion

- **The intra / peritumoral T cell ratio in primary melanomas predicts the outcome of DC-based vaccination of patients with metastatic disease ($P < 0.00026$).**

- **Already available at initial melanoma diagnosis, it can also be used when considering adjuvant immunotherapy and may help for the selection of patients that may benefit from the DCs immuno-therapy and to improve individualized therapy for patients with metastatic melanoma.**

- **Insight in the natural immune response in cancer patients is critical for the development of efficient cancer immunotherapies**

Making the most of dendritic cell-based immunotherapy

- **Dendritic cell vaccination (shared / neoantigens) is safe with minimal side effects. Some patients show long-lasting complete remissions**

- **Antigen specific T cells correlate with prolonged overall survival**
  - **SKILs, skin infiltrating lymphocytes**
  - **TILs, tumor infiltrating lymphocytes**

- **The immunosuppressive tumor microenvironment forms a major barrier for anti-cancer vaccines to effectively eradicate established tumors.**

Making the most of dendritic cell-based immunotherapy

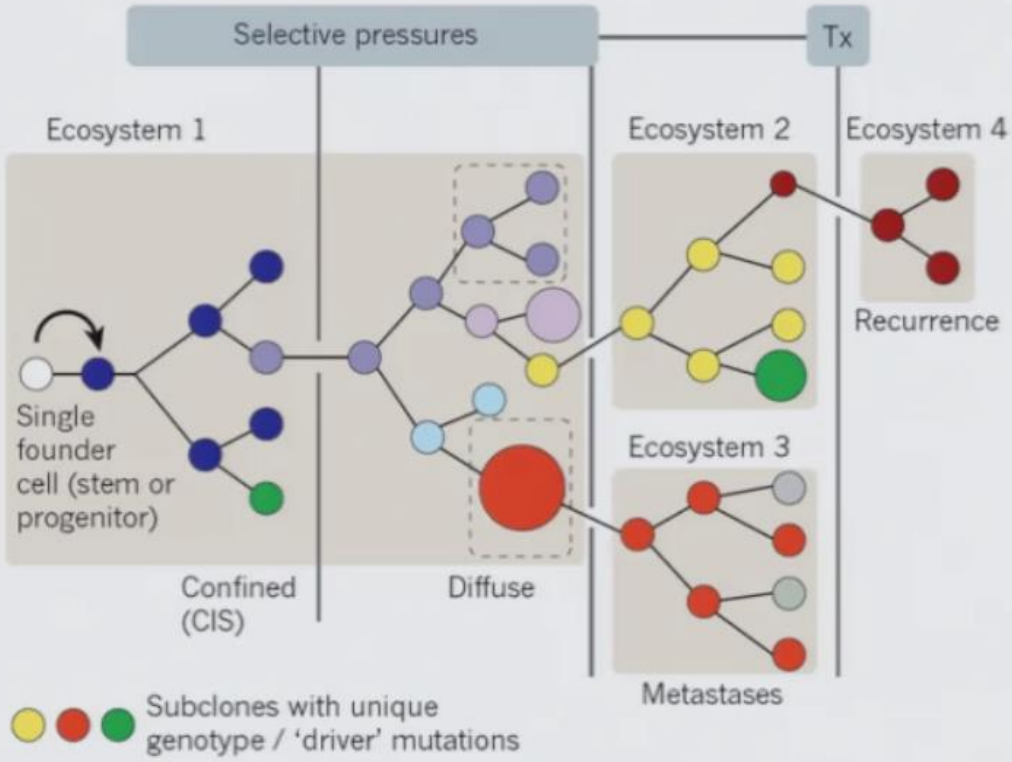
- **There is a good rationale for combination of immunotherapies therapies:**
  - **multi-antigen cancer vaccines [SPECIFICITY]**
  - **immune checkpoint blockade [RESET]**
  - **IDO/TDO/arginase inhibitors such as that manipulate immunosuppressive networks (Tregs, MDSC) [REVERSE IMMUNOSUPPRESSION]**

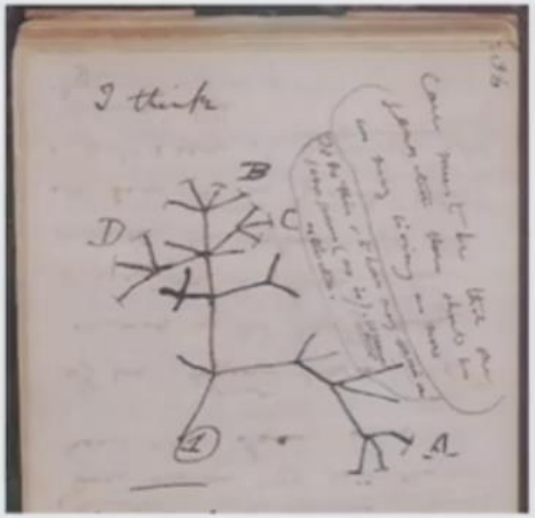
Making the most of dendritic cell-based immunotherapy

It will be extremely important to develop prognostic and predictive immune biomarker profiles by in vivo and ex vivo monitoring before, during, and after immunotherapy to make the right choices.

- **SKILS**
- **multifunctional T cells**
- **transcriptome signatures blood**
- **immune scoring of the tumor microenvironment**

The Darwinian evolution of cancer cells is under the pressure of the local ecosystem and the immune system

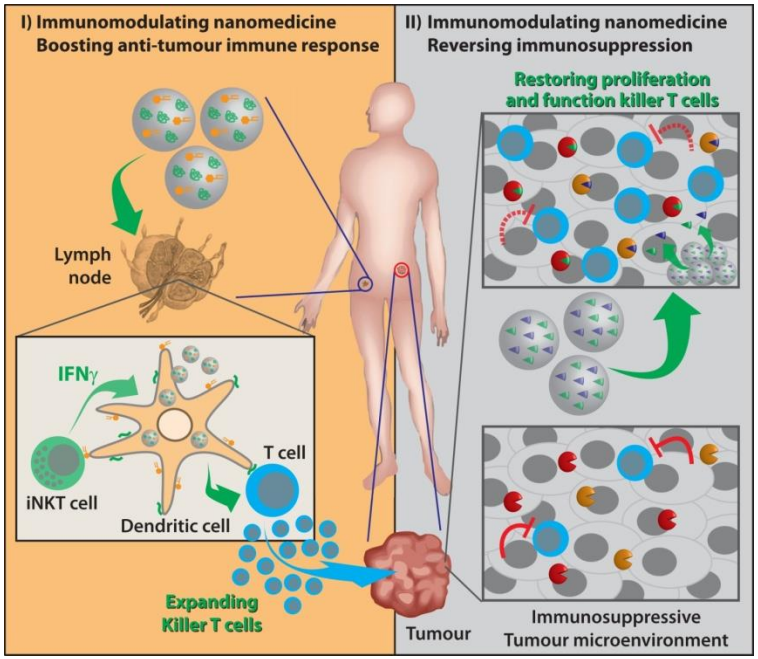


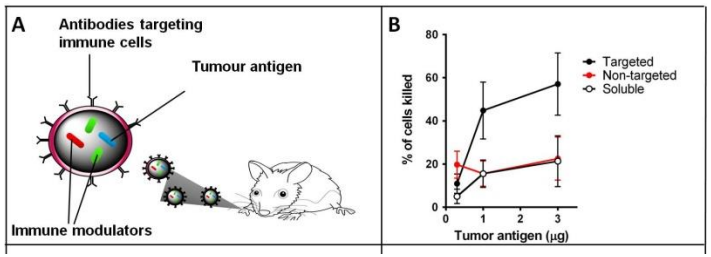


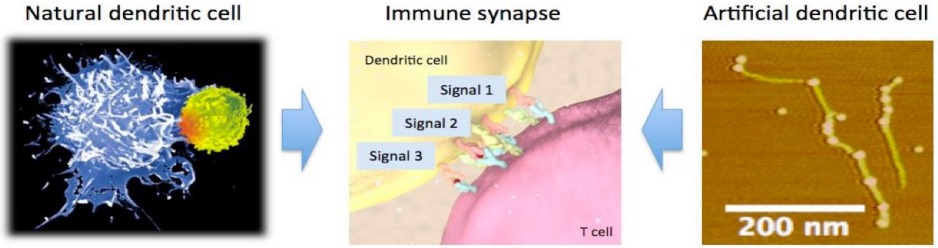
Greaves M et al. Nature 2012

Dendritic cell vaccines

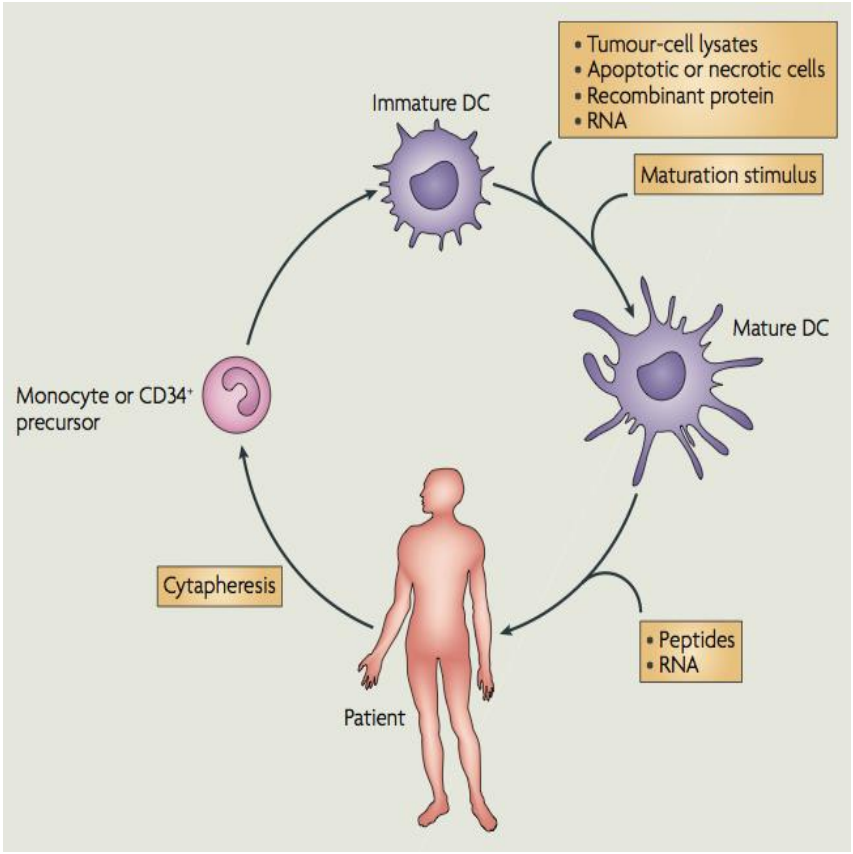
Preclinical nanomedicine program







Clinical DC vaccination program



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