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University Medical Center Rotterdam

Immune monitoring in cancer patients following treatment with anti-PD-1 antibody

NSCLC patients not responding to nivolumab show lowered frequency of co-stimulatory receptor-deficient CD8 T cells

Cor Lamers
Laboratory of Tumor Immunology
Department of Medical Oncology

NVC-SKMI, Zwolle, 21 November 2108

PD-1, programmed cell death protein 1, aka CD279

- Cell surface receptor
- Expressed mainly on T cells and is induced by T cell activation
- Two ligands: PD-L1 and PD-L2
- Down-regulates T cell activation
- Acts as **checkpoint** to guard against autoimmunity

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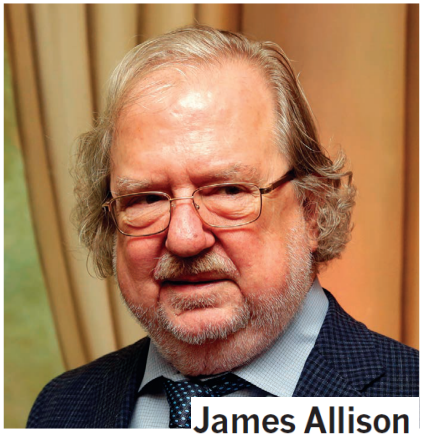
Cancer immunologists scoop medicine Nobel prize

One of the hottest areas in cancer research, immunotherapy can dramatically extend lives



James Allison

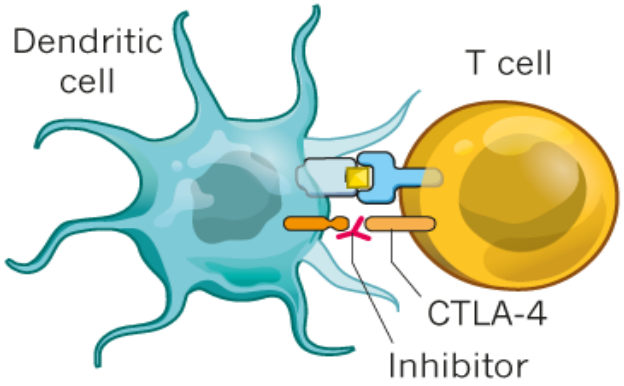
Tasuku Honjo

IMMUNE BOOST

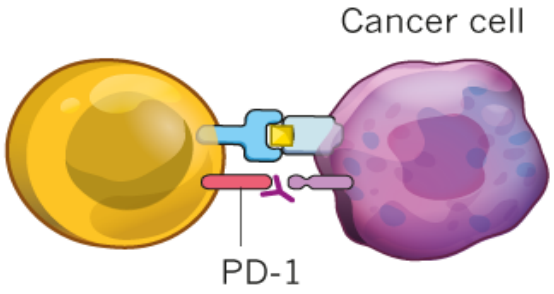
Several methods are showing promise in helping immune sentinels called T cells to attack cancer.

CHECKPOINT INHIBITOR DRUGS

'Checkpoint' proteins block T-cell activity. Inhibitor drugs can release the brakes on T cells at different stages.



The CTLA-4 checkpoint protein prevents dendritic cells from priming T cells to recognize tumours. Inhibitor drugs block the checkpoint.



The PD-1 checkpoint protein prevents T cells from attacking cancer cells. The inhibitor drug allows T cells to act.

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Anti-PD-1 Immune Therapy – The Principle

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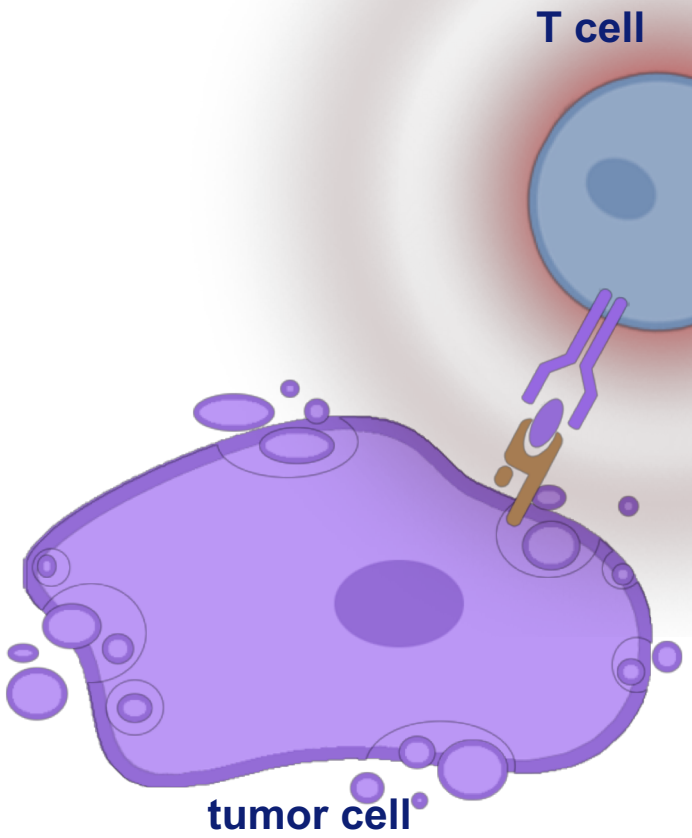
Anti-PD-1 Immune Therapy – The Principle



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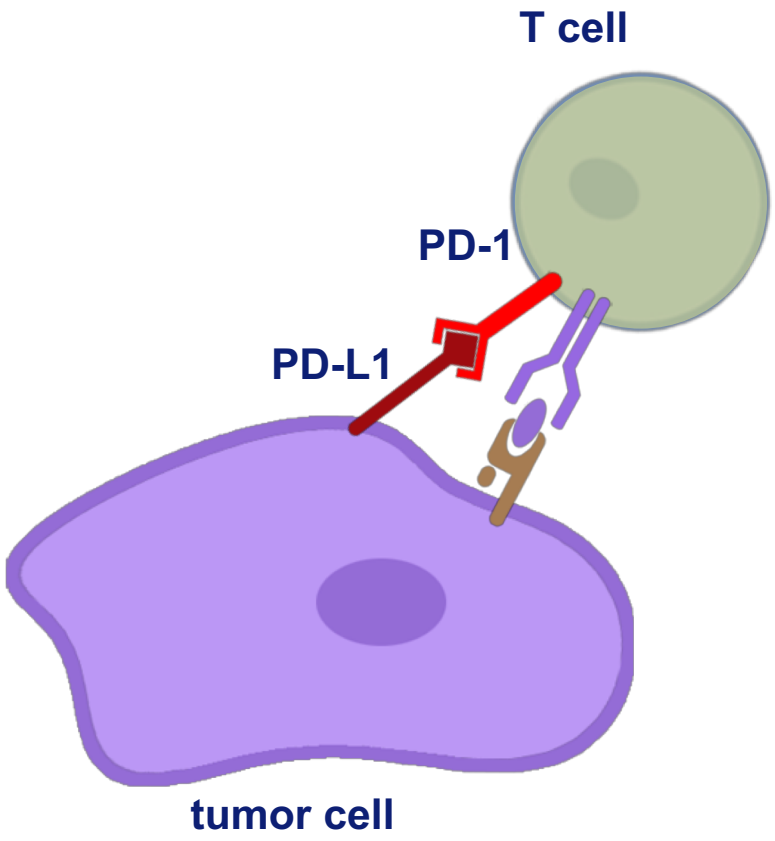
Anti-PD-1 Immune Therapy – The Principle



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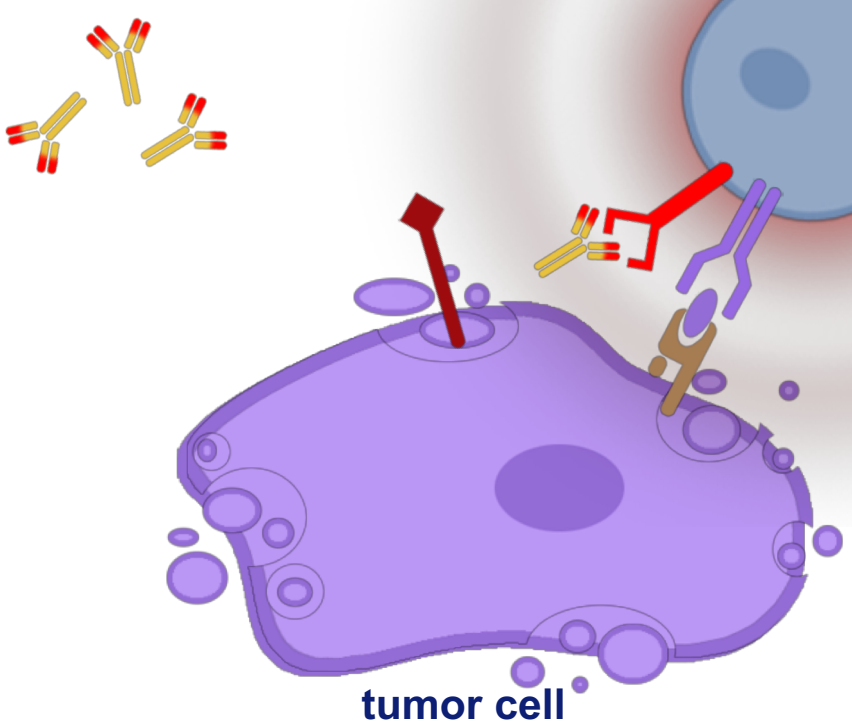
Anti-PD-1 Immune Therapy – The Principle

αPD-1 antibody
(i.e. nivolumab, pembrolizumab)



αPD-L1 antibody
(i.e. Atezolizumab, Durvalumab, Avelumab)

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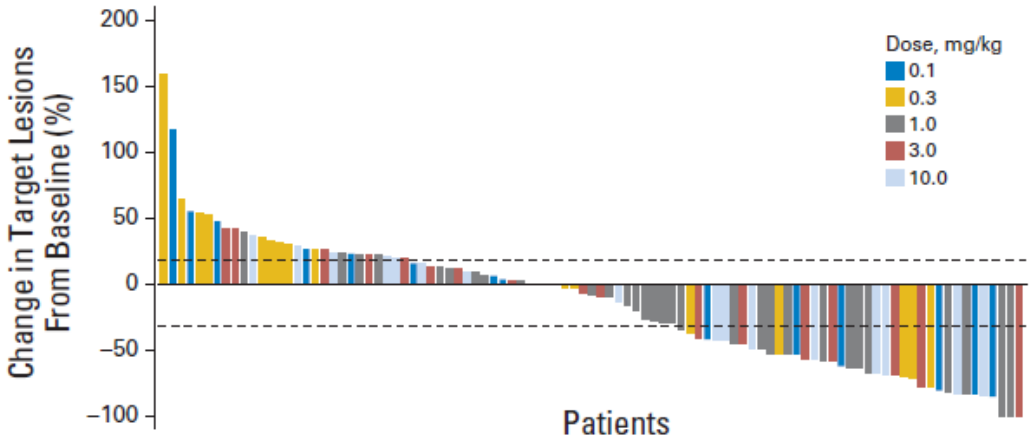
Nivolumab – first clinical data in advanced melanoma

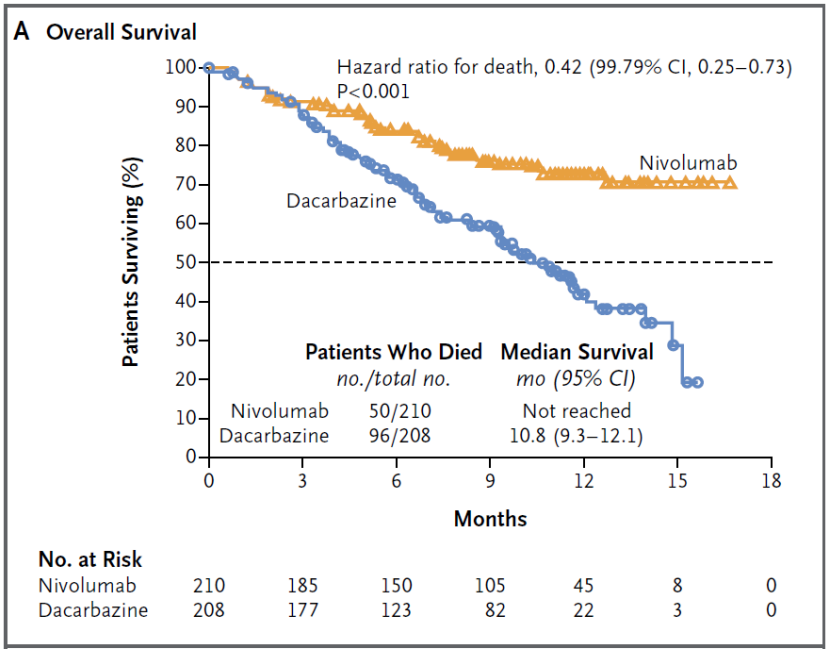
Survival, Durable Tumor Remission, and Long-Term Safety in Patients With Advanced Melanoma Receiving Nivolumab



Overall response rate after ipilimumab: 31%*

Topalian et al. JCO, 2014

Nivolumab in Previously Untreated Melanoma without *BRAF* Mutation



Overall response rate as frontline therapy 40%

Robert et al. NEJM, 2015

* anti-CTLA4 mAb (BMS)

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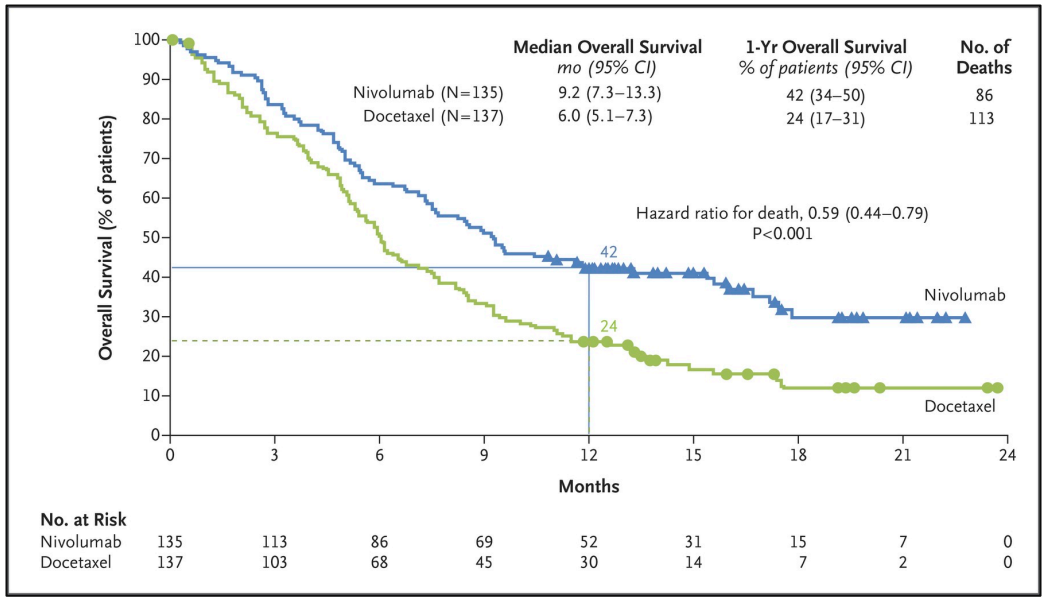
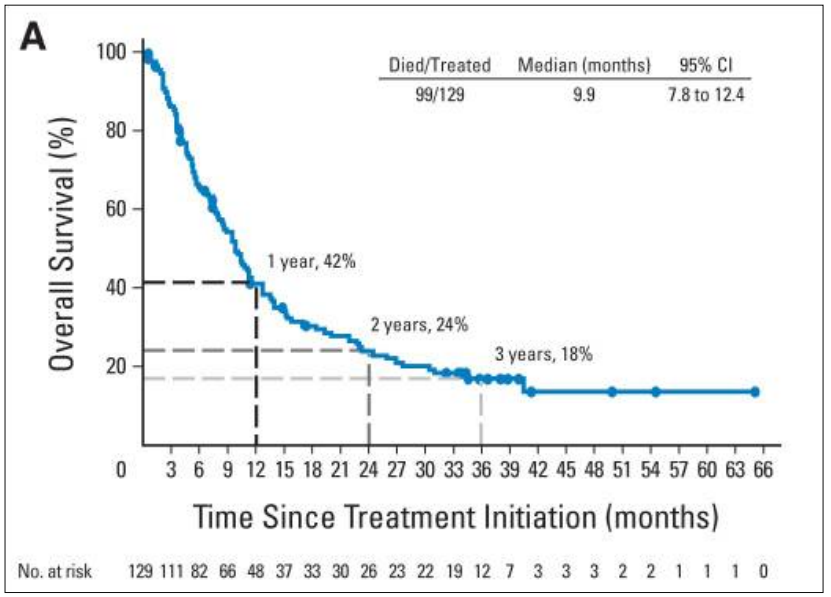
Anti-PD-1 Immune Therapy in NSCLC – Promises and Challenges



Brahmer, N Engl J Med, 2015



Gettinger, J Clin Oncol, 2015

- increased overall survival compared to standard treatment
- approved by FDA and EU in cancer types of multiple origins

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FDA and EU-approved Immune Checkpoint Inhibitors (ICI) Available in The Netherlands

Anti-CTLA4	Yervoy® **Ipilimumab** (Bristol-Myers Squibb)
Anti-PD-1	Opdivo® **Nivolumab** (Bristol-Myers Squibb)
	Keytruda® **Pembrolizumab** (Merck)
Anti-PDL-1	Tecentriq® **Atezolizumab** (Roche)
	Imfinzi® **Durvalumab** (Astra Zeneca)
	Bavencio® **Avelumab** (Merck Pfizer)

These drugs are increasingly gaining first-line indications

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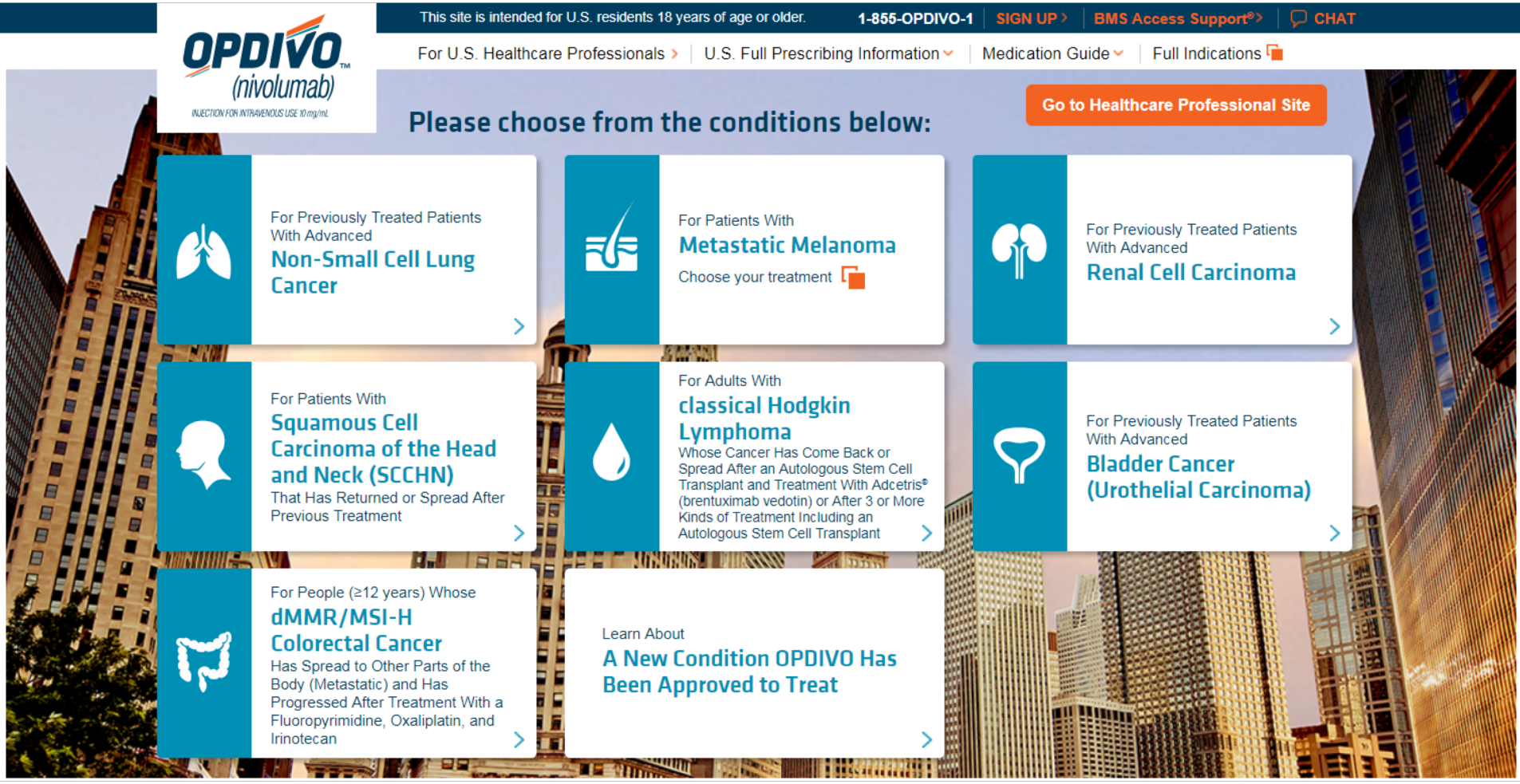
PD-1 inhibitor therapy has become big business



Bristol-Myers Squibb website

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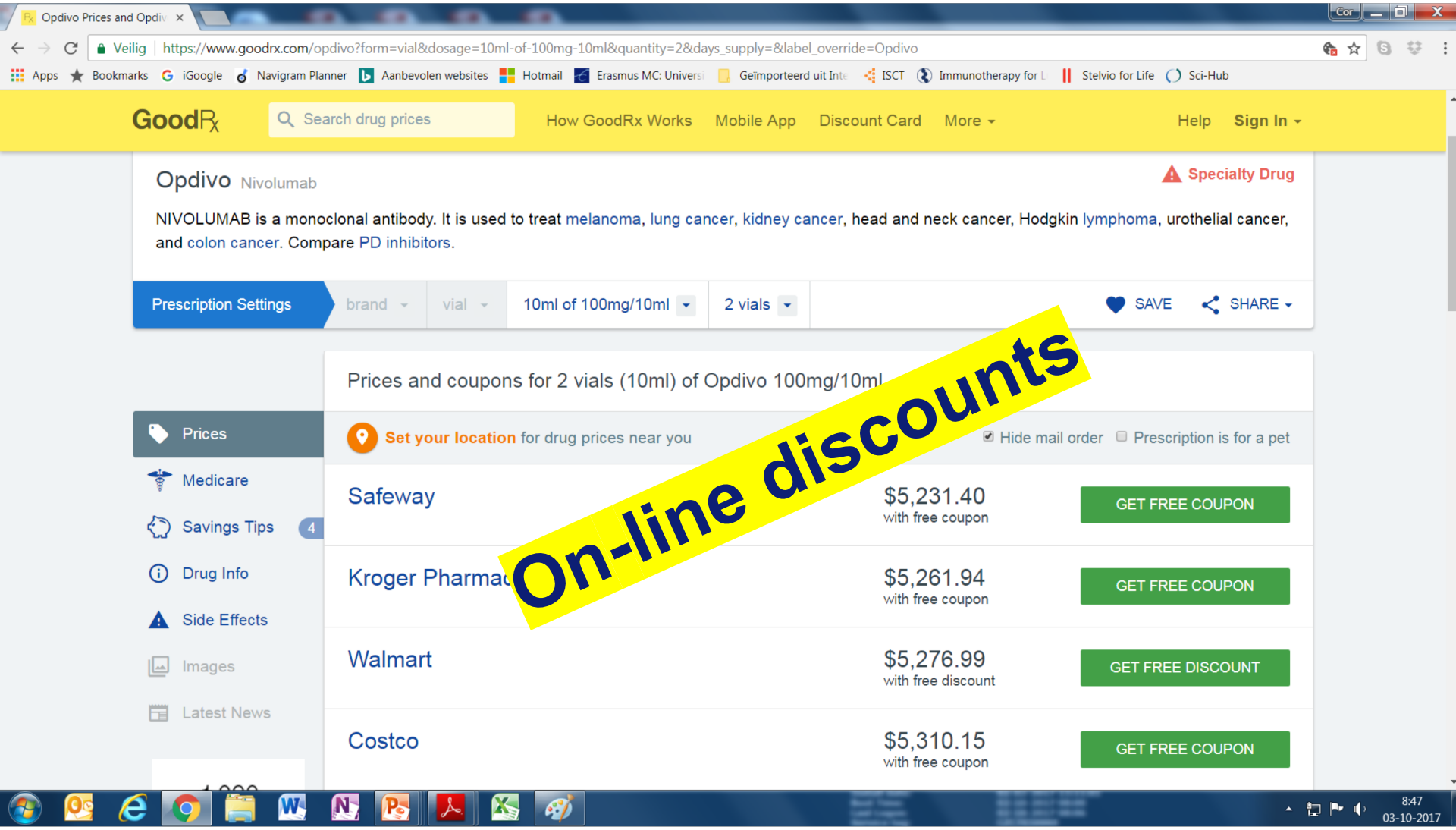
Opdivo Prices and Opdiv
Veilig | https://www.goodrx.com/opdivo?form=vial&dosage=10ml-of-100mg-10ml&quantity=2&days_supply=&label_override=Opdivo
Apps Bookmarks iGoogle Navigram Planner Aanbevolen websites Hotmail Erasmus MC: Universi Geïmporteerd uit Inte ISCT Immunotherapy for L Stelvio for Life Sci-Hub
GoodRx
Search drug prices
How GoodRx Works
Mobile App
Discount Card
More
Help
Sign In
Opdivo Nivolumab
Specialty Drug
NIVOLUMAB is a monoclonal antibody. It is used to treat melanoma, lung cancer, kidney cancer, head and neck cancer, Hodgkin lymphoma, urothelial cancer, and colon cancer. Compare PD inhibitors.
Prescription Settings
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vial
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2 vials
SAVE
SHARE
Prices and coupons for 2 vials (10ml) of Opdivo 100mg/10ml
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Medicare
Savings Tips 4
Drug Info
Side Effects
Images
Latest News
Set your location for drug prices near you
Hide mail order
Prescription is for a pet
Safeway
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with free discount
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Costco
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with free coupon
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Current challenges of PD-1 treatment

- Many patients do not demonstrate clinical benefit
- Many patients show clinical toxicities
- Costs of patient treatment are high

Hypothesis

Frequencies of immune cells and their subset distributions in blood predict response to PD-1 treatments and facilitate treatment stratification

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Blood Parameters are Associated with Clinical Response to Immune Checkpoint Inhibitors

Marker	ICI therapy	Cancer	N	Study results	Reference
Lymphocyte count	Ipilimumab	Melanoma	51, 73	⩾1000 per μl at week 6 → ↑ OS	(Delyon *et al*, 2013; Ku *et al*, 2010)
	Ipilimumab	Melanoma	82, 40	↑ At 2–8 weeks *vs* baseline → ↑ response	(Bjoern *et al*, 2016; Martens *et al*, 2016b)
	Ipilimumab	Melanoma	95	↑ At week 12 *vs* baseline → ↑ OS	(Simeone *et al*, 2014)
	Nivolumab	Melanoma	98	⩾1000 per μl at week 3–6 → ↑ OS	(Nakamura *et al*, 2016)
Relative lymphocyte count	Ipilimumab	Melanoma	209	↑ Baseline → ↑ OS	(Martens *et al*, 2016a)
	Pembrolizumab	Melanoma	616	↑ Baseline → ↑ OS	(Weide *et al*, 2016)
Total leucocyte count	Ipilimumab	Melanoma	59	↓ Baseline → ↑ response	(Gebhardt *et al*, 2015)
Eosinophil count	Ipilimumab	Melanoma	209	↑ Baseline → ↑ OS	(Martens *et al*, 2016a)
	Ipilimumab	Melanoma	59	↑ At week 3 *vs* baseline → ↑ response	(Gebhardt *et al*, 2015)
	Ipilimumab	Melanoma	73	↑ At week 6 *vs* baseline → ↑ OS	(Delyon *et al*, 2013)
Relative eosinophil count	Pembrolizumab	Melanoma	616	↑ Baseline → ↑ OS	(Weide *et al*, 2016)
Neutrophil count	Ipilimumab	Melanoma	59	↓ Baseline → ↑ response	(Gebhardt *et al*, 2015)
	Ipilimumab	Melanoma	720	↓ Baseline → ↑ PFS and OS	(Ferrucci *et al*, 2016)
	Nivolumab	Melanoma	98	<4000 per μl at week 3–6 → ↑ OS	(Nakamura *et al*, 2016)
Neutrophil/lymphocyte ratio	Ipilimumab	Melanoma	58, 185	↓ Baseline → ↑ OS	(Khoja *et al*, 2016; Zaragoza *et al*, 2016)
	Ipilimumab	Melanoma	187	↓ Baseline → ↑ PFS and OS	(Ferrucci *et al*, 2015)
	Nivolumab	NSCLC	175	↓ Baseline → ↑ OS	(Bagley *et al*, 2017)
Derived neutrophil/ lymphocyte ratio	Ipilimumab	Melanoma	720	↓ Baseline → ↑ PFS and OS	(Ferrucci *et al*, 2016)
Monocyte count	Ipilimumab	Melanoma	209	↓ Baseline → ↑ OS	(Martens *et al*, 2016a)

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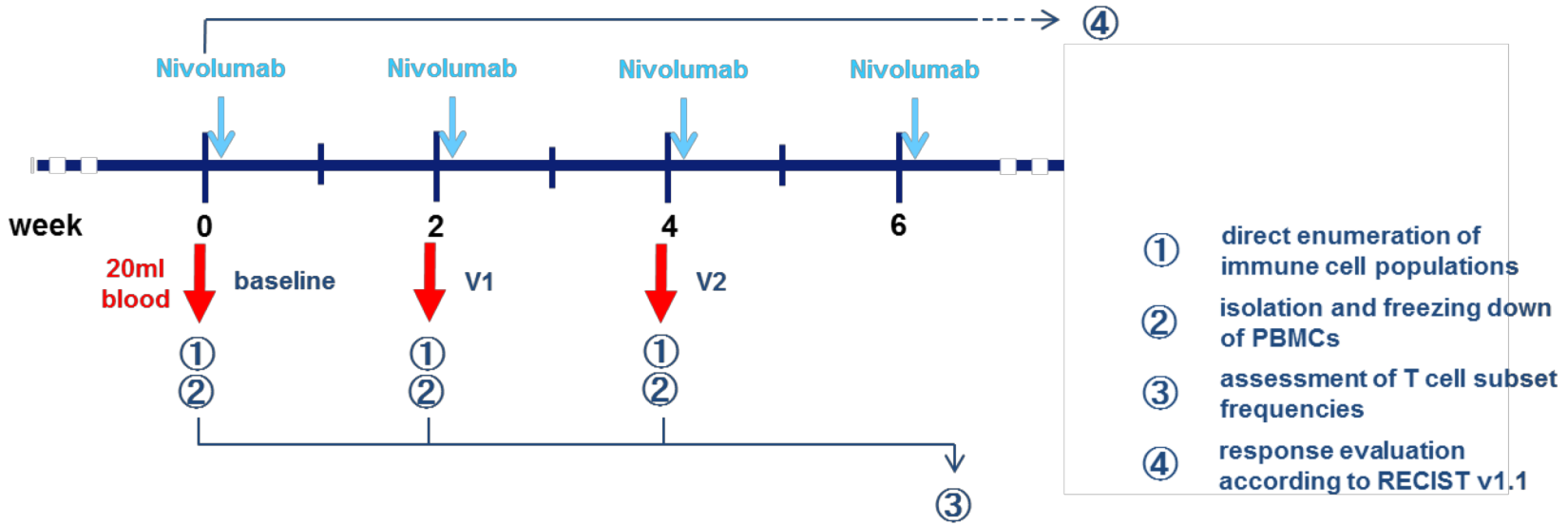
MULTOMAB

(prospective saMpling in intravenoUsLy Treated Oncology patients: Monoclonal AntiBodies)



Collaborations:

Translational Pharmacology (group Mathijssen)

Pulmonary Diseases (group Aerts)

Patient numbers per October 2018:

Melanoma:	>150
NSCLC:	>250

Also other tumor types

Efforts:

- Collection of patient blood pre- and post-treatment
- Processing/storage of whole blood, serum, PBMC, DNA/RNA
- Measurement of antibody levels in sera (pharmacokinetics)
- Measurement of immune cells in whole blood /PBMC (immune profiling)
- Clinical patient evaluation (tumor burden, toxicity, response)

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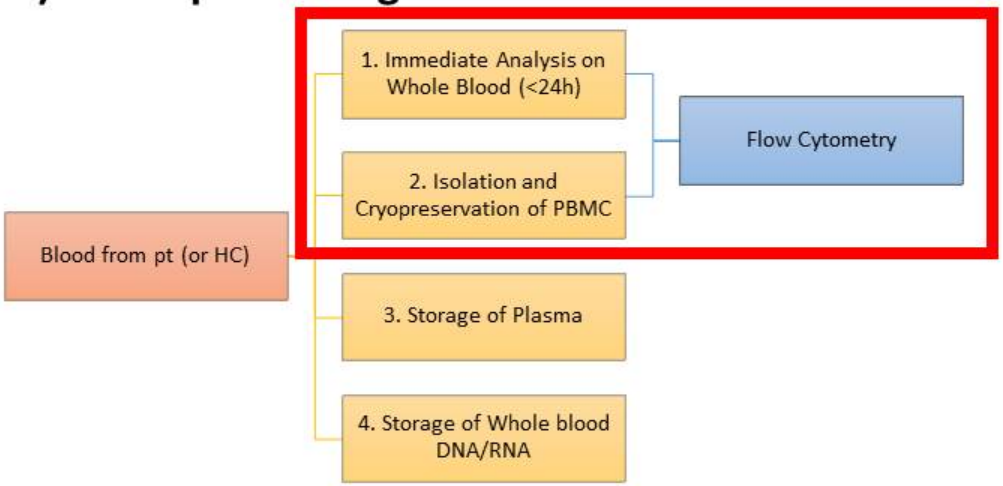
Immune monitoring – methods applied

A) Blood processing

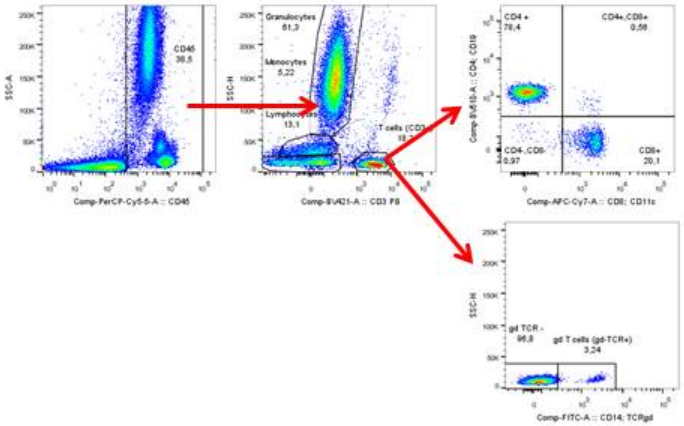


B) Multiplex flow cytometry (12colors)

FACS Celesta

- **Enumeration of 18 immune cell subsets**
- **Assess expression of T cell markers for:**
  - **Maturation**
  - **Co-stimulation**
  - **Co-inhibition**
  - **Chemokines**
  - **Total of 300 combinations**

C) Absolute numbers and T cell markers



Lymphocytes:

- B cells
- NK cells
- T cells
- γδ T cells

Granulocytes:

- Eosinophils
- Immature neutrophils
- Mature neutrophiles
- PMN-MDSCs

Monocytes:

- Classical monocytes
  - M-MDSCs
- Intermediate monocytes
- Non-classical monocytes
- Dendritic cells

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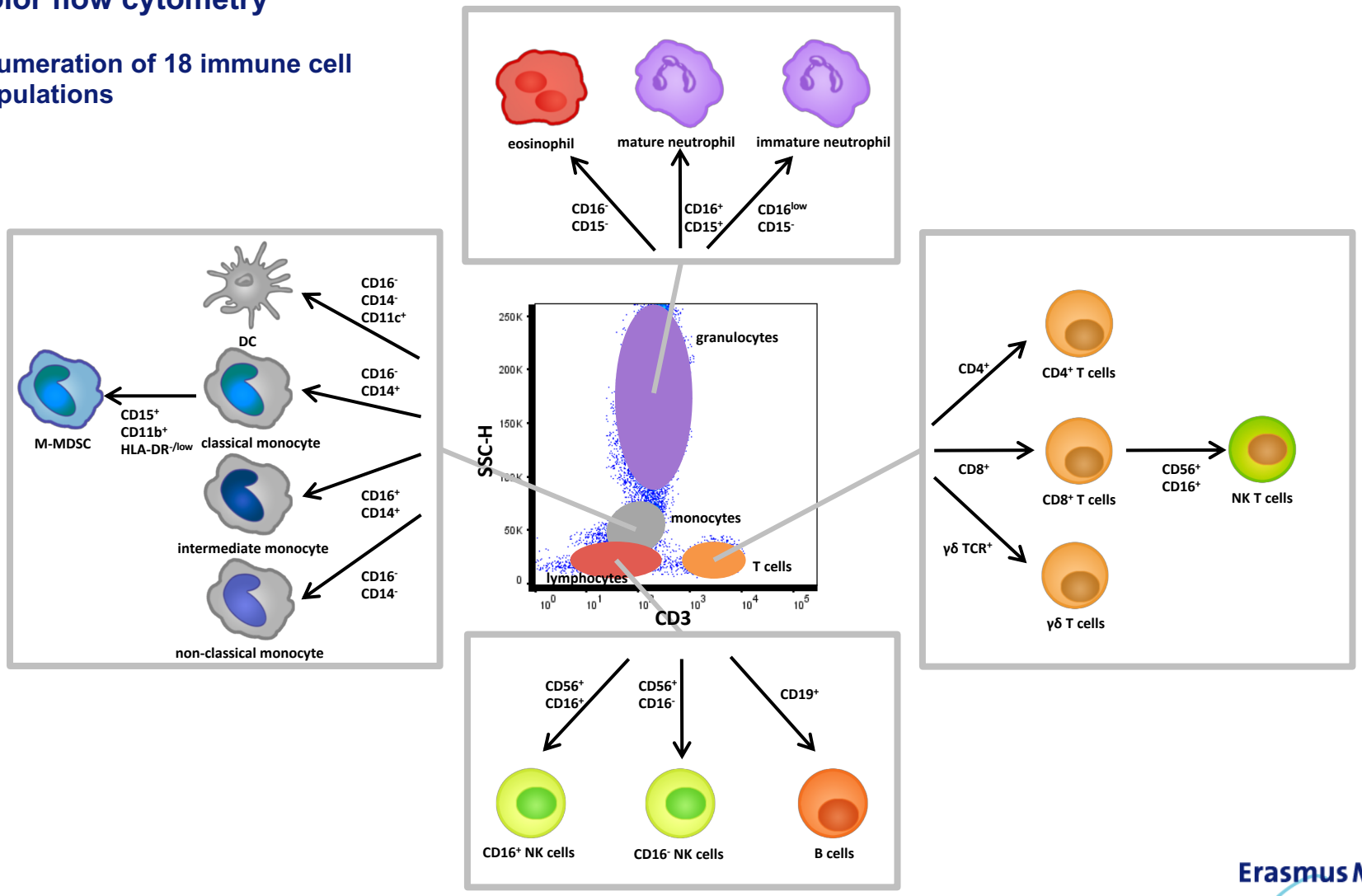
Flow Cytometric Analysis of Blood

12-color flow cytometry

- enumeration of 18 immune cell populations



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Exploratory Cohort

Tumor type:		NSCLC
Treatment*:		Nivolumab, Q2W, 3mg/kg
Median age in years (range):		65 (35-79)
Sex: - female - male		30 (42.3%) 41 (57.7%)
BOR: - progressive disease (PD) - stable disease (SD) - partial response (PR)		**32 (45.1%) 25 (35.2%) 14 (19.7%)**
Median follow-up in days (range):		242 (35-544)
WHO performance status:	0 1 unknown	16 (22.5%) 37 (52.1%) 18 (25.4%)
Histology of primary lung tumor:	adenocarcinoma squamous cell carcinoma great cell carcinoma	48 (67.6%) 21 (29.6%) 2 (2.8%)

* all patients received platinum-based pre-treatment

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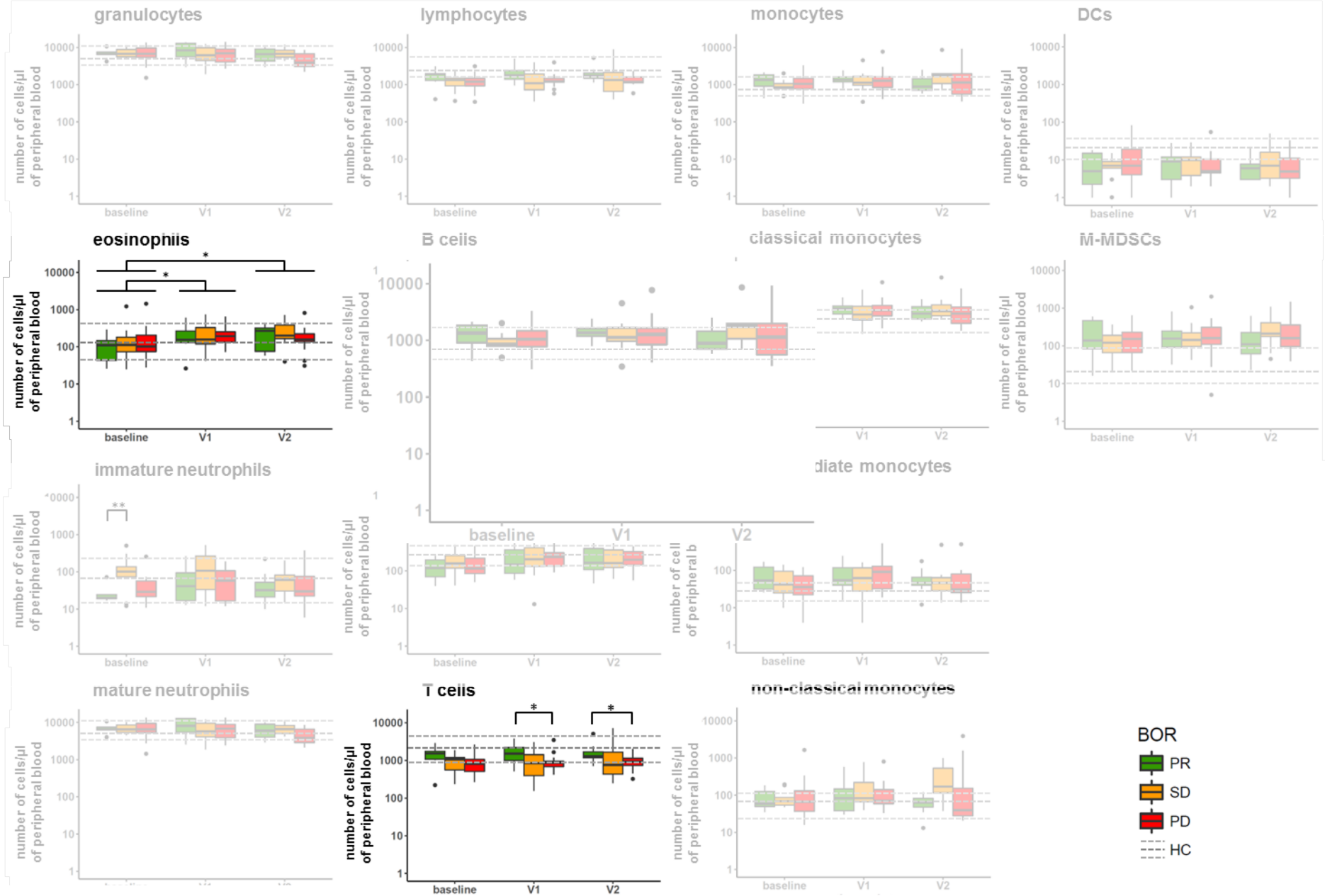
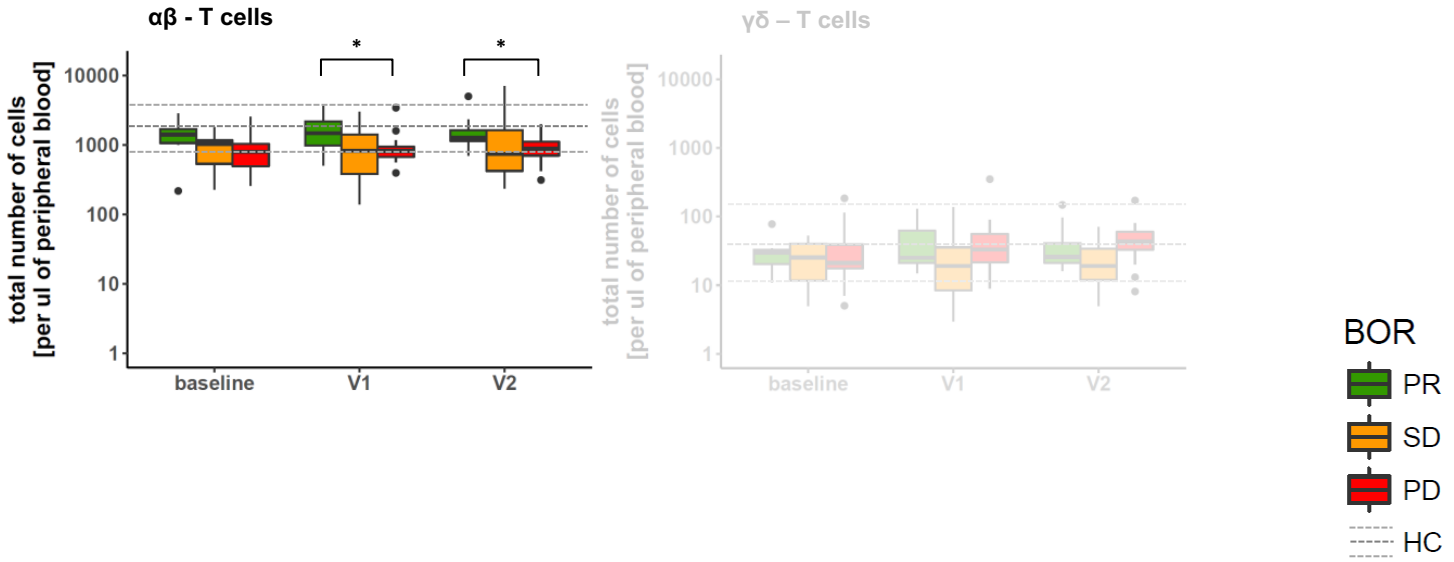
Nivolumab treatment in general does not result in changed numbers of peripheral immune cell populations



Patients responding to nivolumab show high numbers of CD8 T cells



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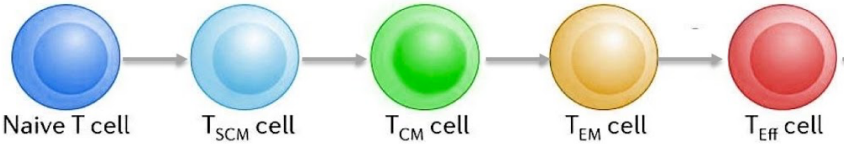
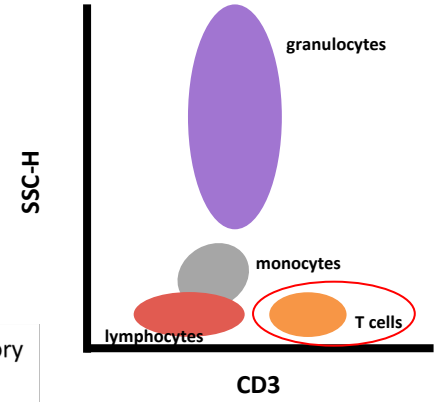
Flow Cytometric Analysis of Blood

12-color flow cytometry

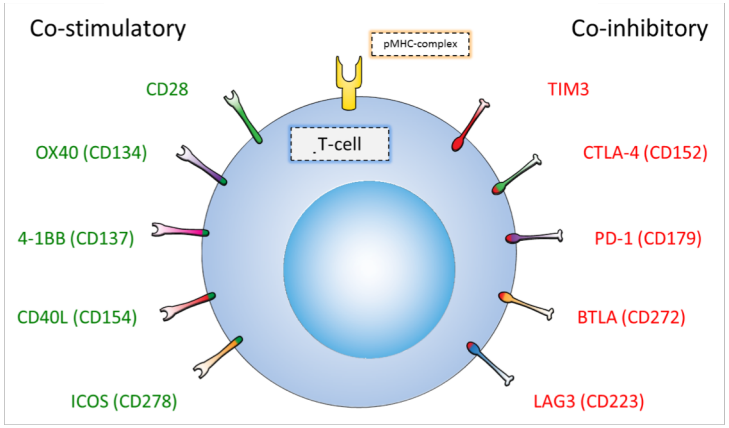
- enumeration of 18 immune cell populations
- assess expression of T cell markers for: maturation, co-stimulation, co-inhibition and chemokines (>300 combinations)

Differentiation

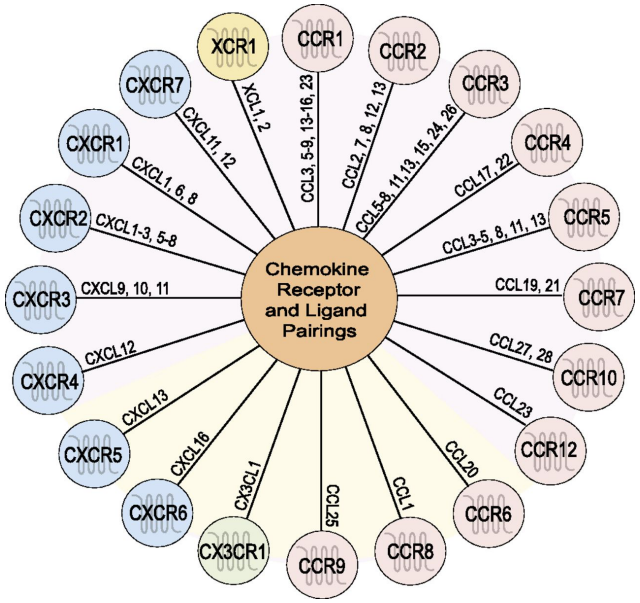




Co-signaling receptors



Chemokine receptors



Multiplex flow cytometry – panels 2-6

2[b]	T cell proliferation/ regulatory T cell markers	Ki67, CD25, FOXP3, PD-1
3[b]	T cell maturation markers	CCR7, CD45RA, CD95, CD69, CD27, CD103
4[b]	T cell co-inhibitory receptors	CD57, LAG3, BTLA, PD-1, TIM3
5[b]	T cell co-stimulatory receptors	CD28, OX40, 4-1BB, CD40L, ICOS
6[b]	T cell chemoattractant receptors	CXCR3, CXCR4, CCR1, CCR4, CCR5

[b] assessment of T cell subset frequencies in PBMC samples

Multiplex flow cytometry – analysis work scheme

1	2 →	3 →	4
immune cell populations (panels 1)	**defined CD4/CD8 T cells subsets (panels 2,3)**	**CD4/CD8 T cells subsets according to single and combination of markers (panels 4-6)**	**CD4/CD8 T cells subsets according to cumulation of markers (panels 4-6)**

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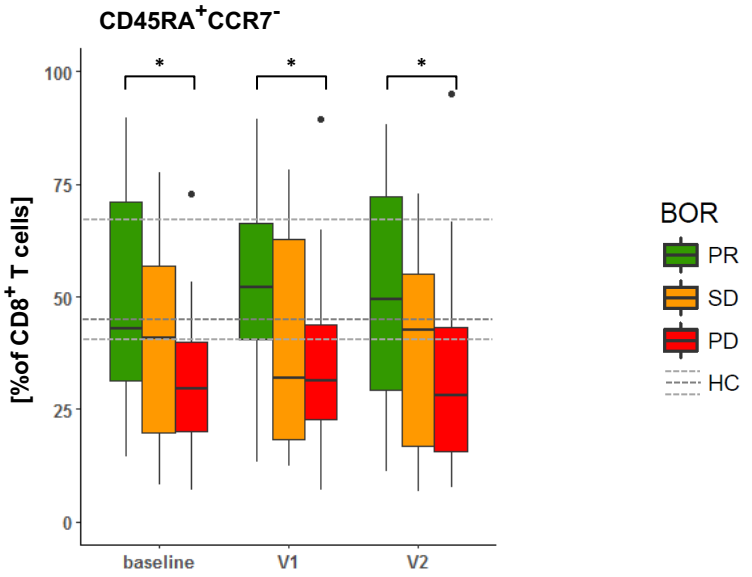
Patients with PR show enhanced frequencies of CD8 T cells with CD45RA^{+}CCR7^{-} and CD95^{+}CD69^{-} phenotypes



These observations suggest a role for differentiated/tissue-egressed (possibly antigen experienced) CD8 T cells

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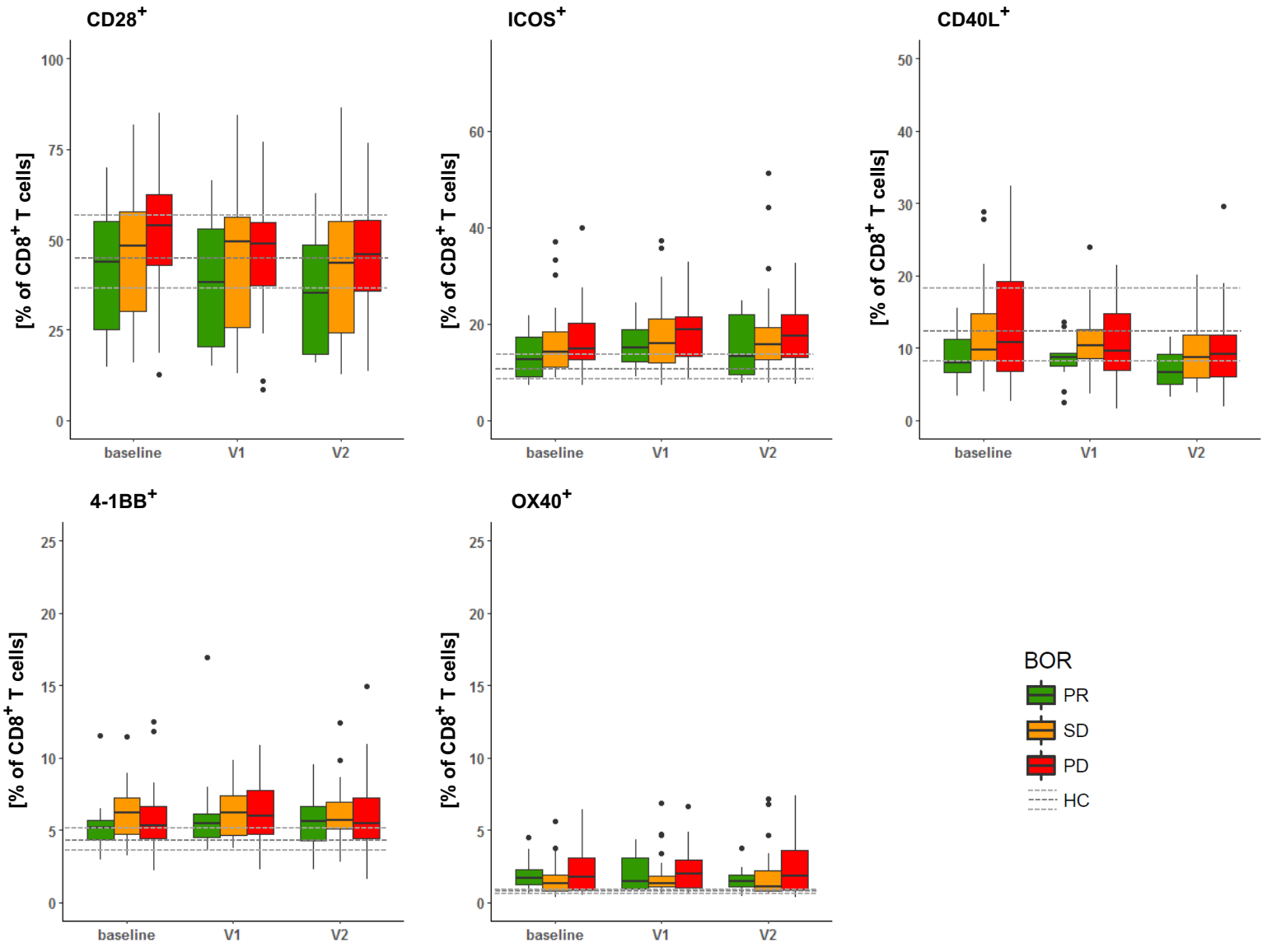
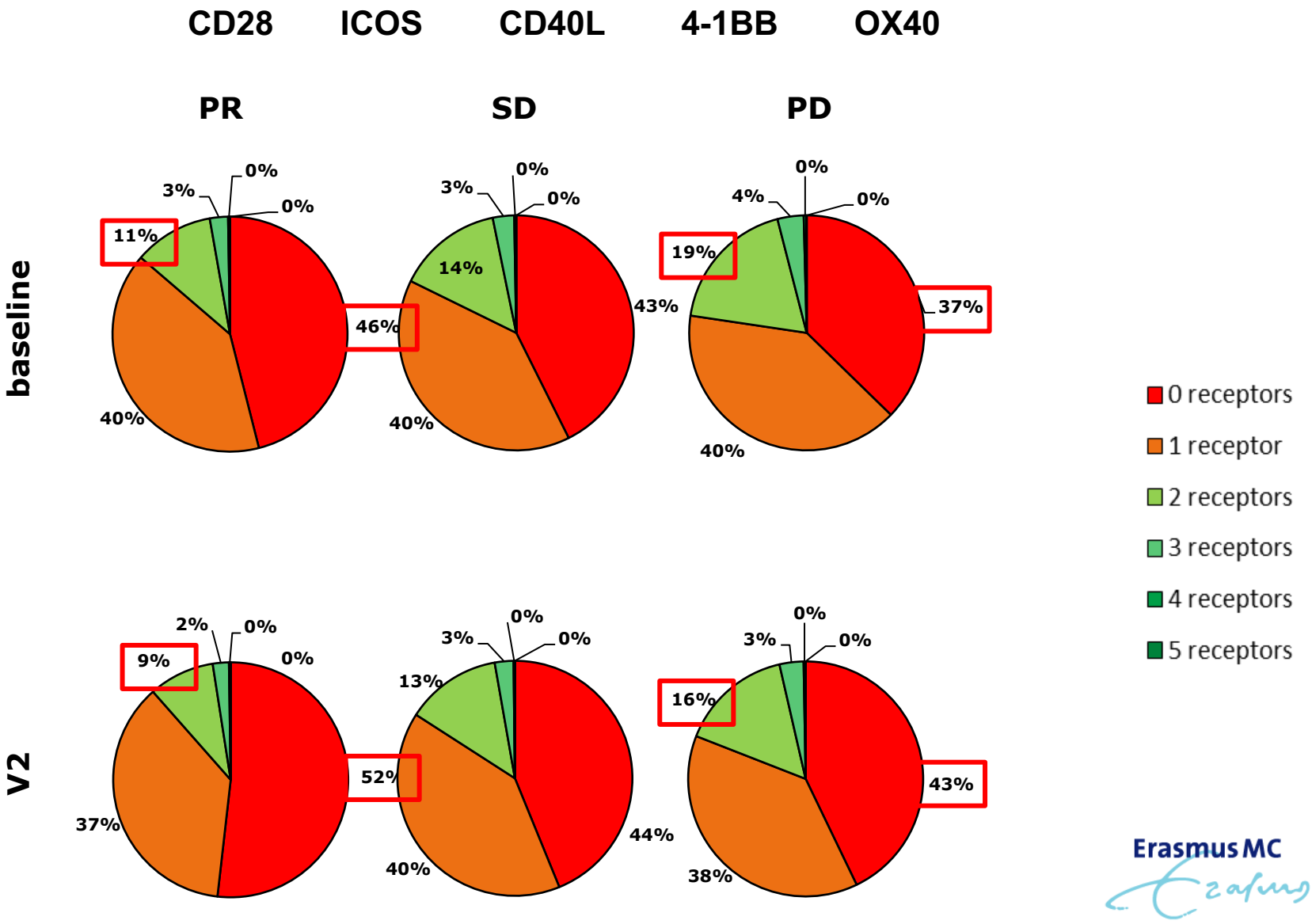
Individual co-signaling receptors do not predict response



Patients with PR display reduced frequencies of CD8 T cells co-expressing CD28 and CD40L, ICOS or ICOS

Patients showing response to therapy are characterized by higher frequencies of CD8 T cells lacking multiple co-stimulatory receptors



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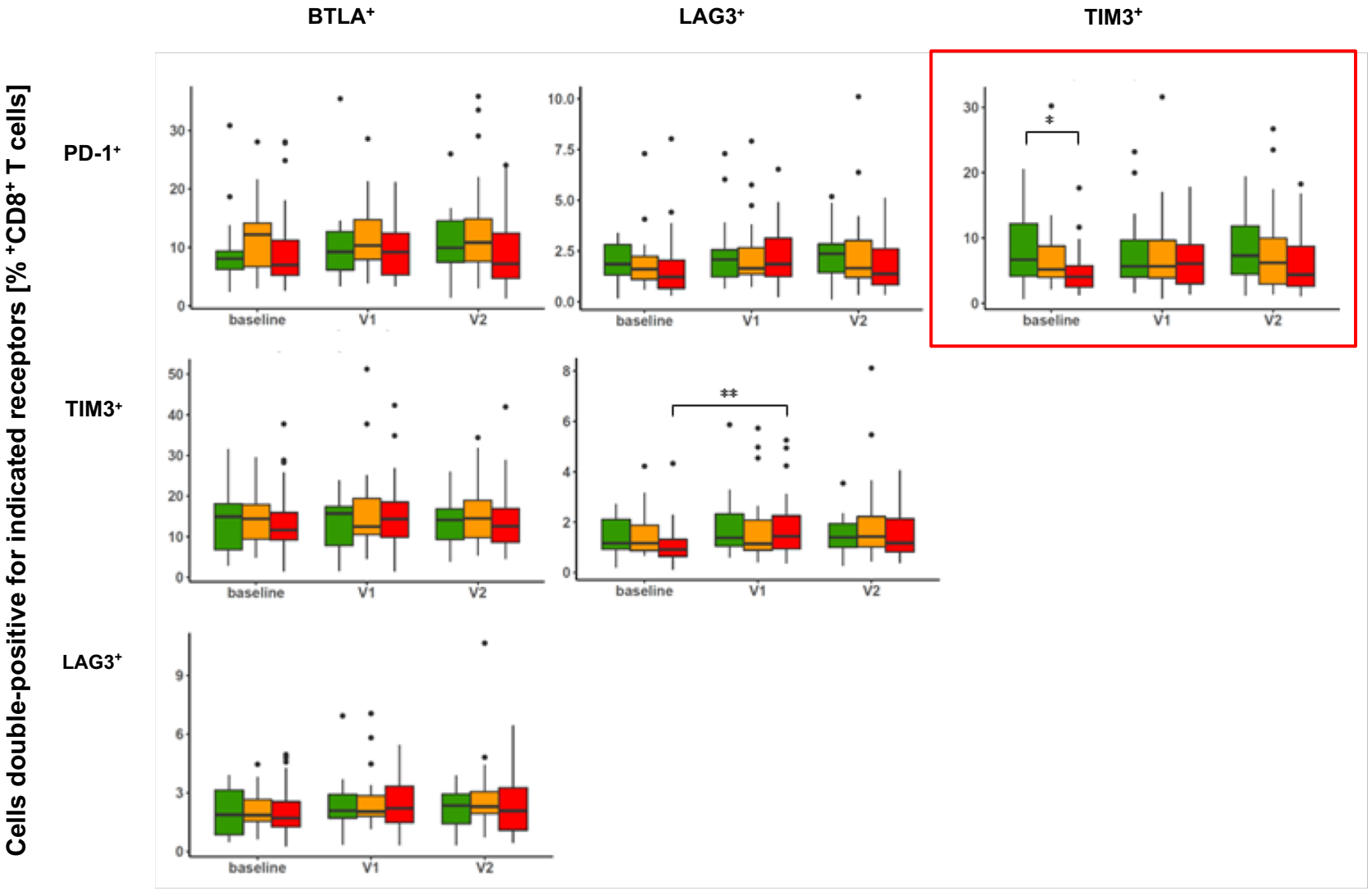
Patients with PR display enhanced frequencies of CD8 T cells co-expressing PD-1 and TIM-3



Observations suggest a decrease function of CD8 T cells

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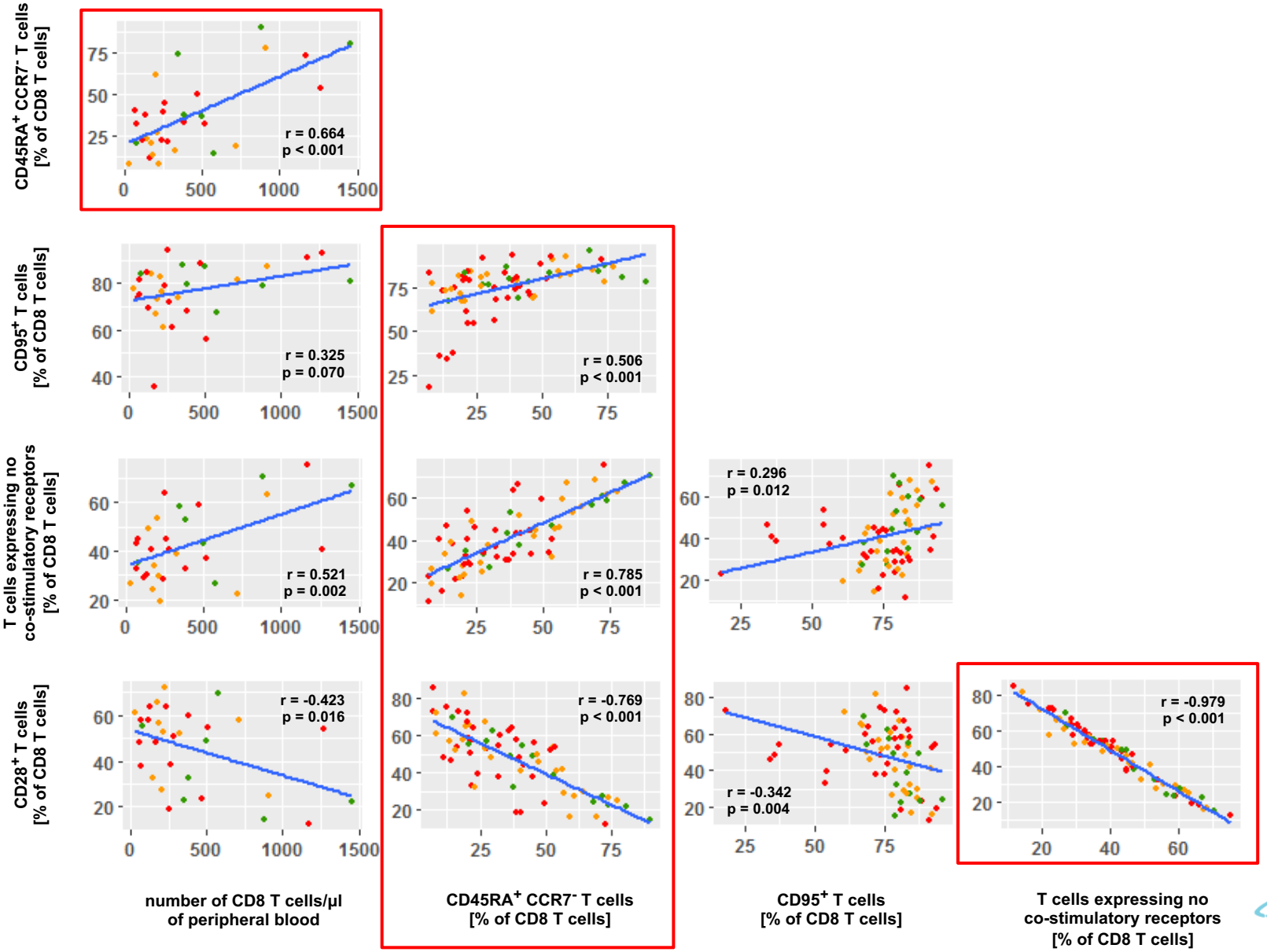
Number and differentiation phenotypes of CD8 T cells in PR patients are correlated to lack of co-stimulatory receptors



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Summary:

NSCLC patients with partial response to nivolumab display at baseline:

- higher numbers of $CD8^+$ T cells
- enhanced frequency of terminally differentiated $CD8^+$ T cells
  ($CD45RA^+CCR7^-$ and $CD95^+CD69^-$
- enhanced frequency of $CD8^+$ T cells devoid of co-stimulatory receptors
  ($CD28^-$ $ICOS^-$ $CD40L^-$ 4-1BB$^-$ $OX40^-$)
- enhanced frequency of $CD8^+$ T cells co-expressing co-inhibiting receptors PD1 and TIM3

→ ***NSCLC patients responding to nivolumab have enhanced frequency of $CD45RA^+CCR7^-$ CD8 T cells lacking co-stimulatory receptors***

→ ***NSCLC patients responding to nivolumab appear to possess more antigen-experienced T cells***

Next steps:

- Correlate findings with overall and progression free survival
- Investigate whether this profile is also found in other tumor types
- Correlate immune profiles with treatment-related toxicity
- Correlate immune profiles with nivolumab pharmacokinetics

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Acknowledgements

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Department of Medical Oncology

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