



Immunologie- Workshop

GBG
GERMAN
BREAST
GROUP
Heilung durch Innovation, Kompetenz und Partnerschaft



GBG Jahrestreffen 2017

Frankfurt am Main

02.-03. MÄRZ
Sheraton Frankfurt Congress Hotel

Tanja Fehm

*Klinik für Gynäkologie und Geburtshilfe
Heinrich Heine-Universität Düsseldorf*

Thomas Karn

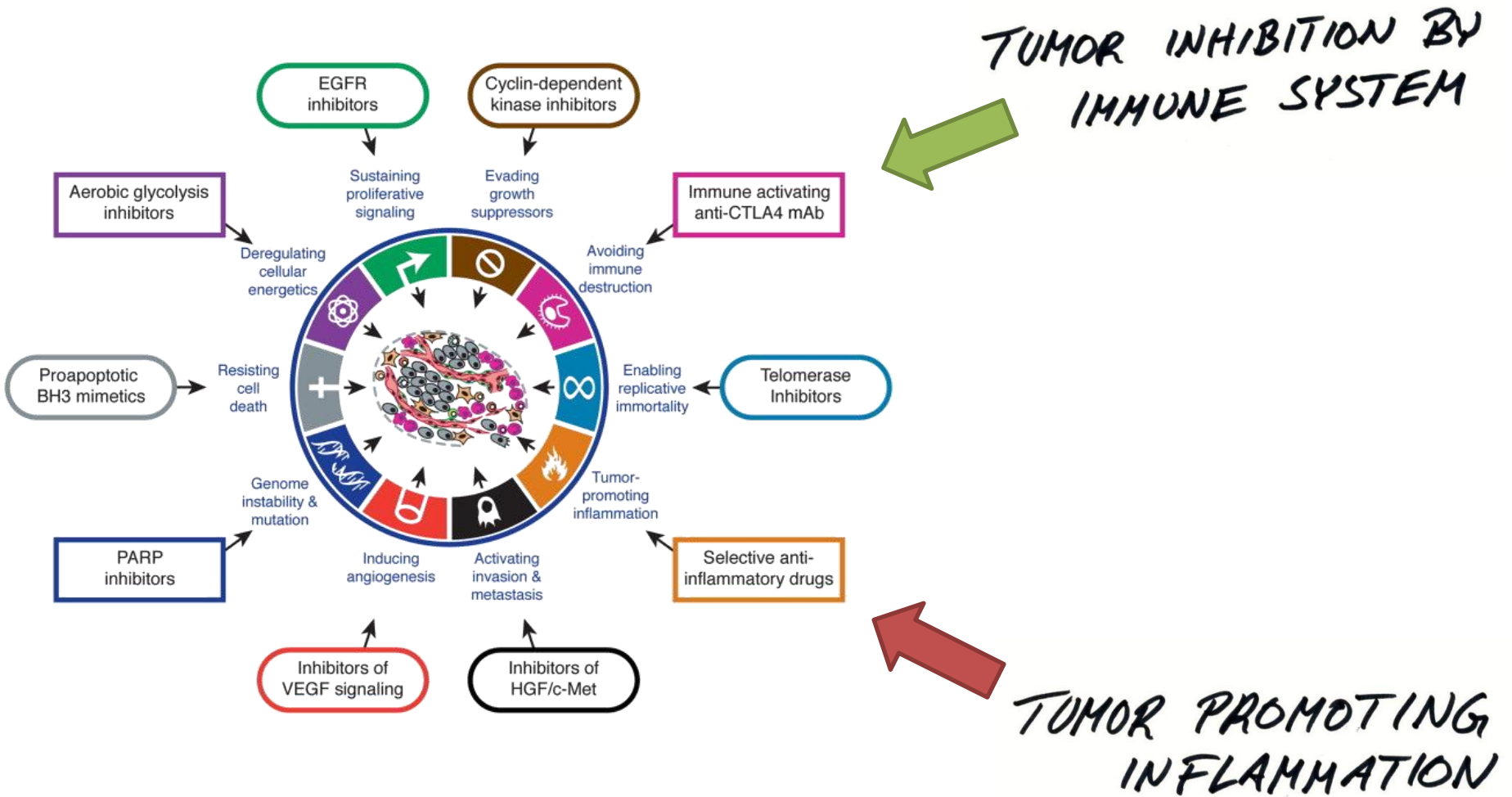
*Klinik für Gynäkologie und Geburtshilfe
J.W. Goethe-Universität Frankfurt*

Teil I:

- Das „Immunsystem im Tumorgeschehen“
- Immunology Basics
- Regulation der Immunantwort und „Immune Checkpoints“
- Immunzellen bei Brustkrebs
- Translationale Forschung und „Genomics“
- Klinische Rolle ...



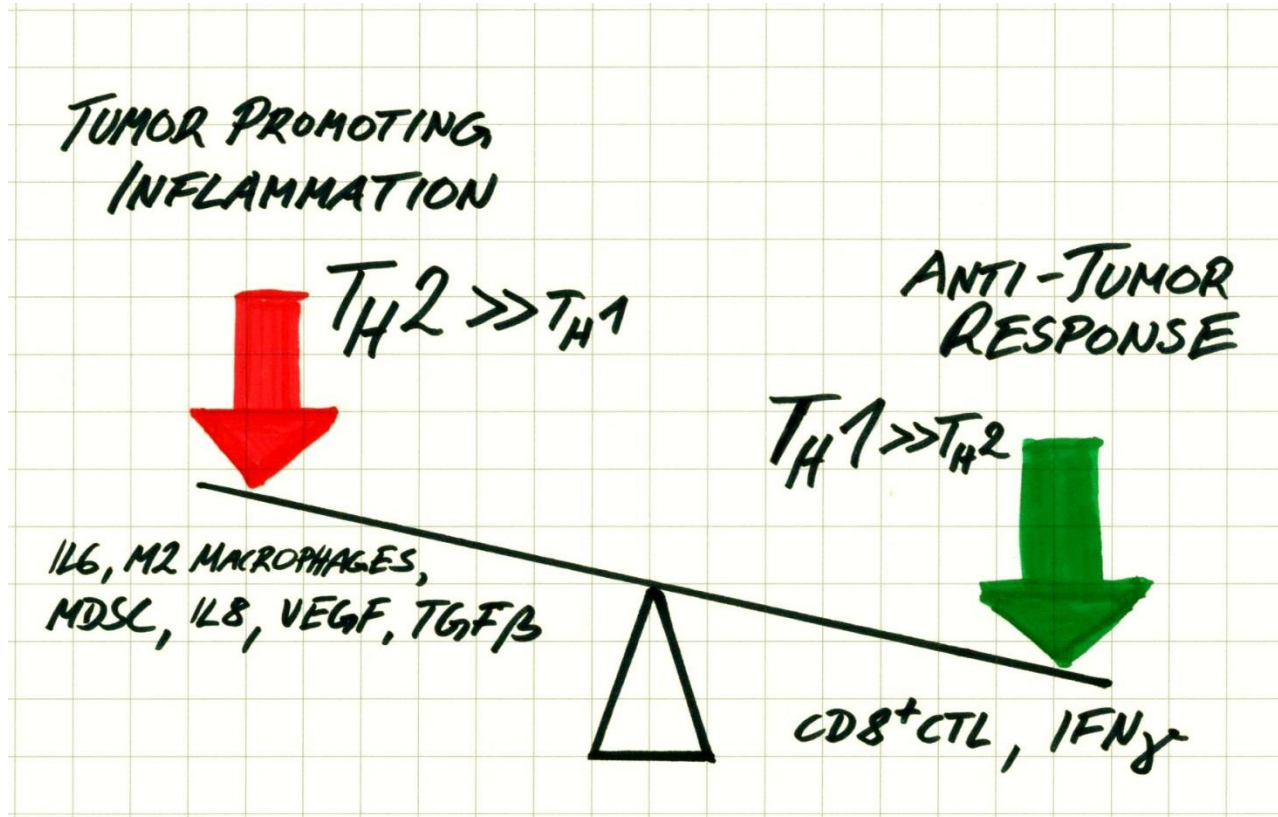
Immuno-oncology as a hallmark of cancer



Hanahan and Weinberg 2011, Cell 144, 5, 646

Dr. Thomas Karn - t.karn@em.uni-frankfurt.de

Balance of tumor immunity



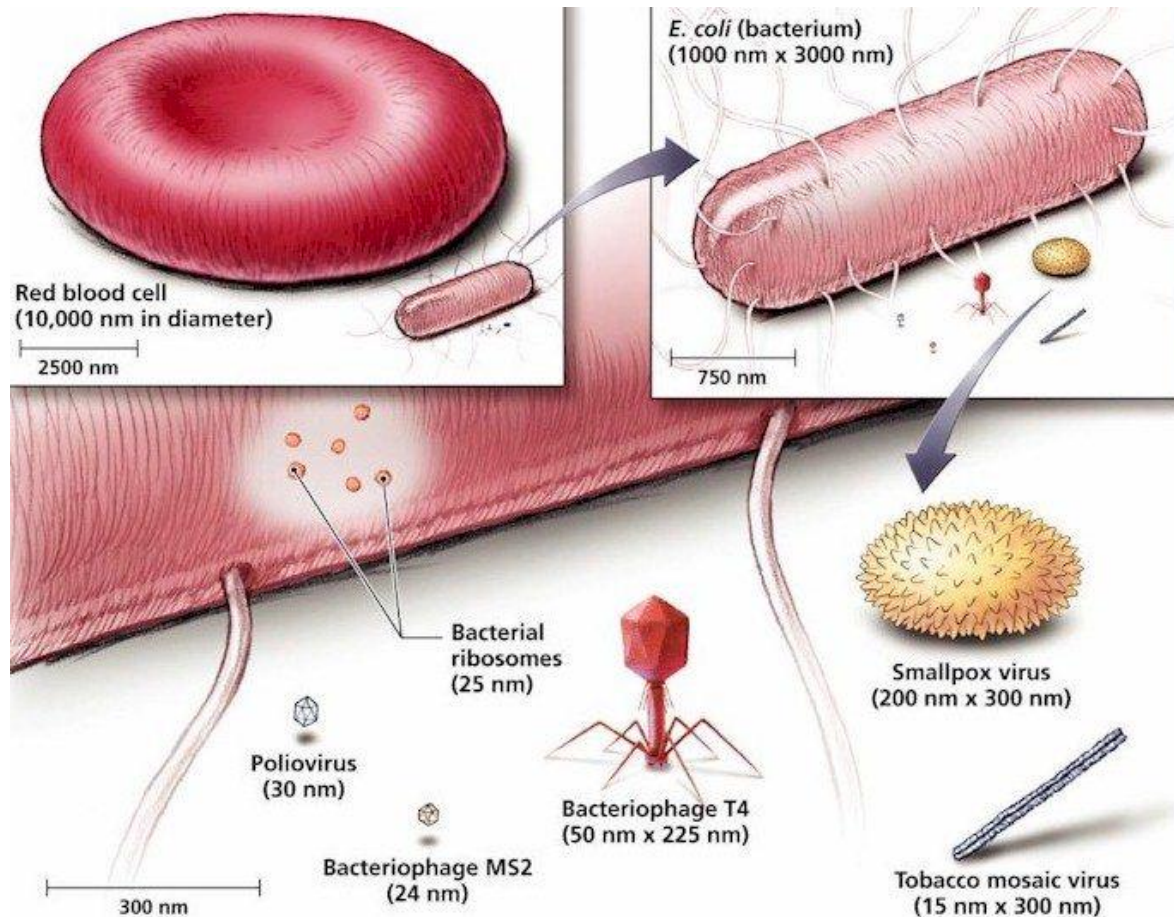
Immunsystem Grundlagen

- Zweck
- Mechanismen
- Steuerung



Zweck

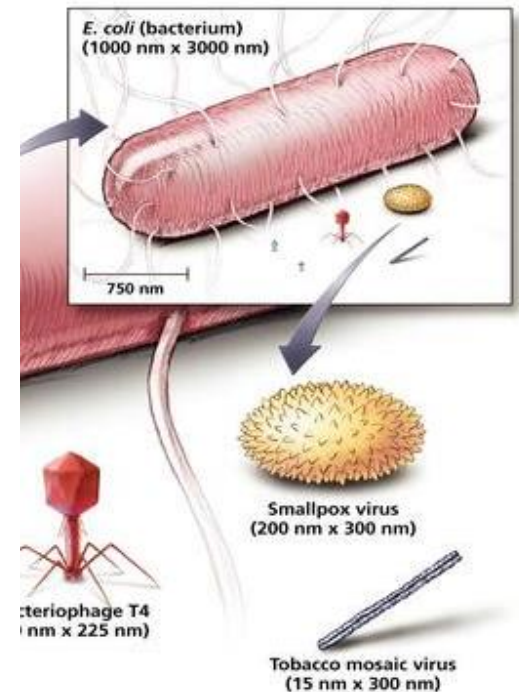
- Abwehr von (kleinen) Feinden



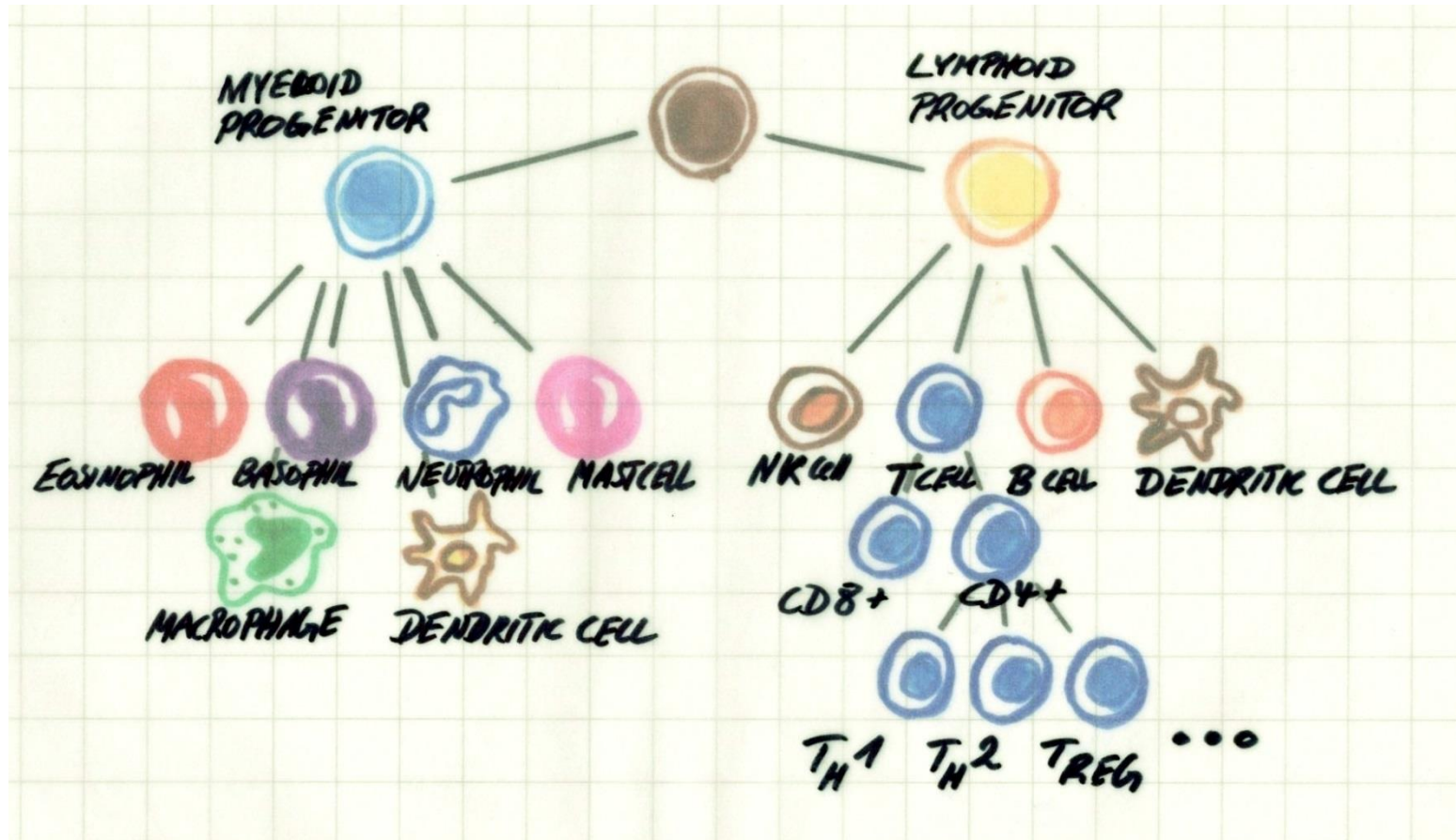
Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.

Zweck

- Abwehr von (kleinen) Feinden
- extrazellulär: Bakterien u.a. Mikroben
 - ➔ humorale Antwort
- intrazellulär: Viren (u.a.)
 - ➔ zelluläre Antwort



Immune cell types



Mechanismen

- Angeborenes Immunsystem:
 - Komplementsystem, Makrophagen und Granulozyten, Interferonsystem, NK-cells



- Erworbene Antwort auf neue Feinde:
 - Humoral: B-Zellen produzieren spezifische Antikörper (Ig) die extrazellulär wirken
 - Zellulär: T-Zellen entwickeln spezifische T-Cell-Receptors (TCR), mit denen sie Virus-befallene Zellen erkennen und daraufhin abtöten.



Beispiele des angeborenen Immunsystems

- Komplement-System erkennt Bakterien, markiert sie und löst Entzündung aus.
- Angelockte Makrophagen phagozytieren markierte Bakterien.
- Zellen mit Virenbefall aktivieren Interferon-System.

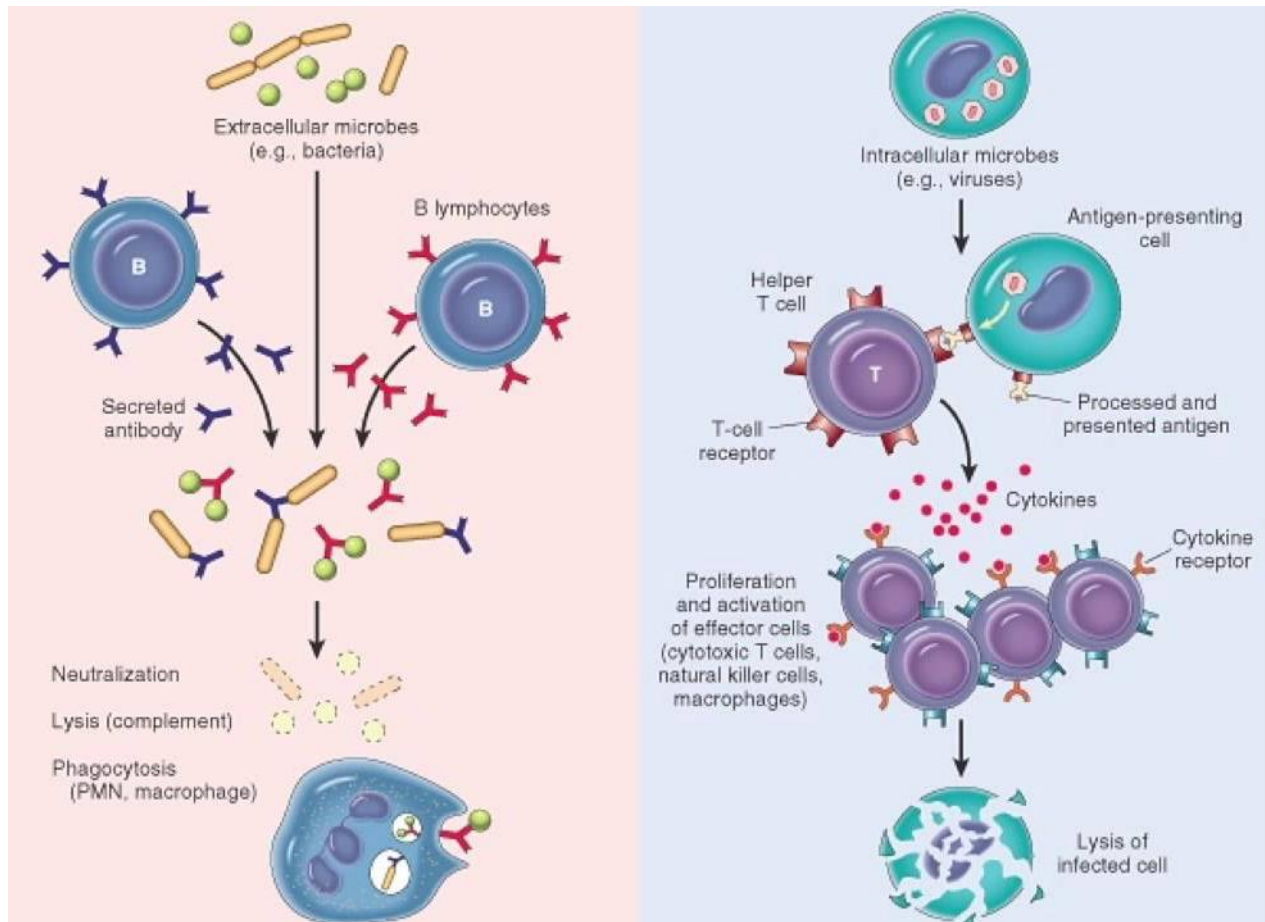


Erworbene Immunantwort

Humoral / Zellulär

B cells

T cells



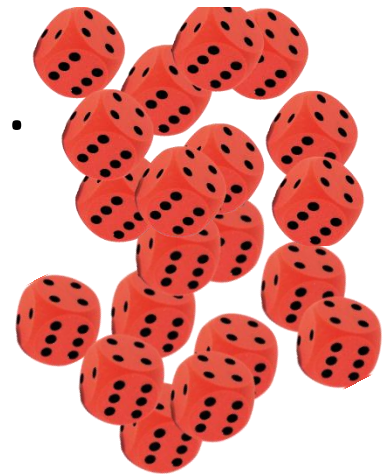
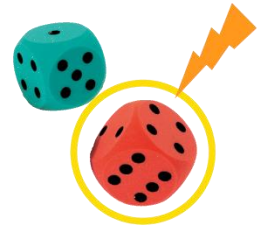
Die erworbene Immunantwort (I)

- Wie werden unbekannte Feinde erkannt?
- Bildung nahezu unendlich vieler T- und B-Zell Klone mit individuellen TCRs/BCRs.
- Autoreaktive T- und B-Zellen werden wieder eliminiert/inaktiviert (*negative selection*)
- Nicht-autoreaktive Zellen warten....

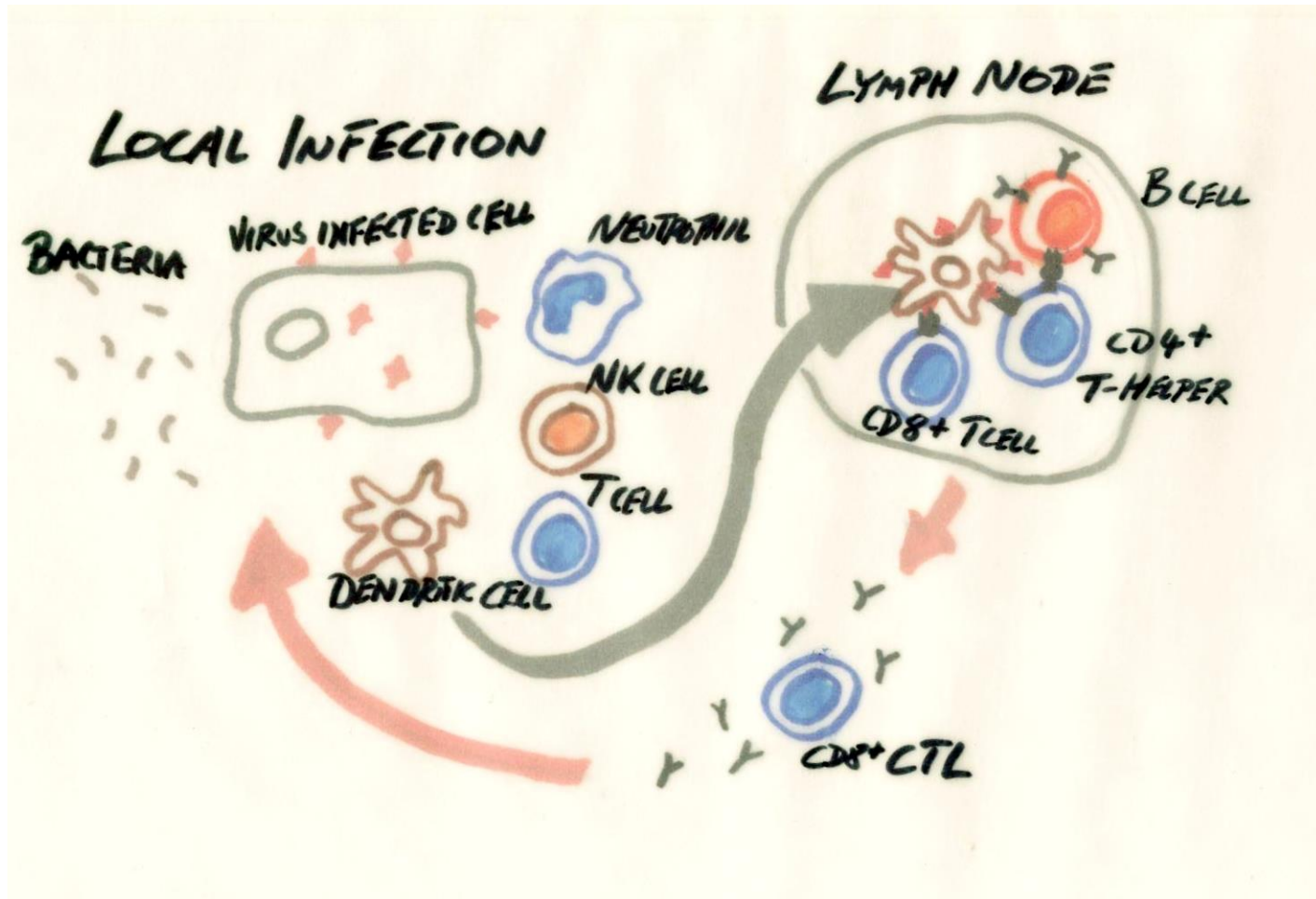


Die erworbene Immunantwort (II)

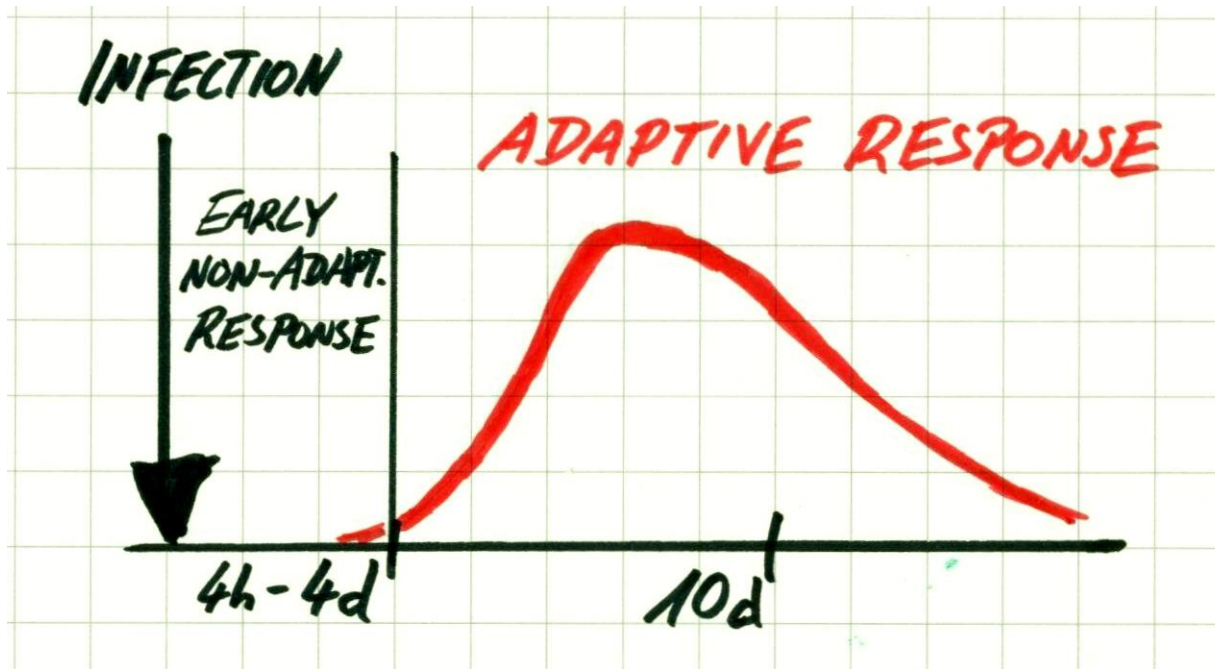
- Taucht ein Feind auf, auf den der Rezeptor passt, wird die T- oder B-Zelle aktiviert und klonal amplifiziert
- Die Rezeptoren werden durch weitere Mutationen optimiert (*positive selection*)
- Nach einigen Tagen ist ein Heer von spezifischen Immunzellen vorhanden....



Early and late adaptive response

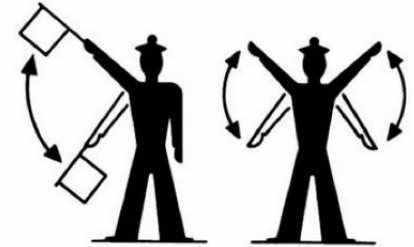


Timeline of immune response



Rapid on-/off-set of response

REGULAR INFECTION:

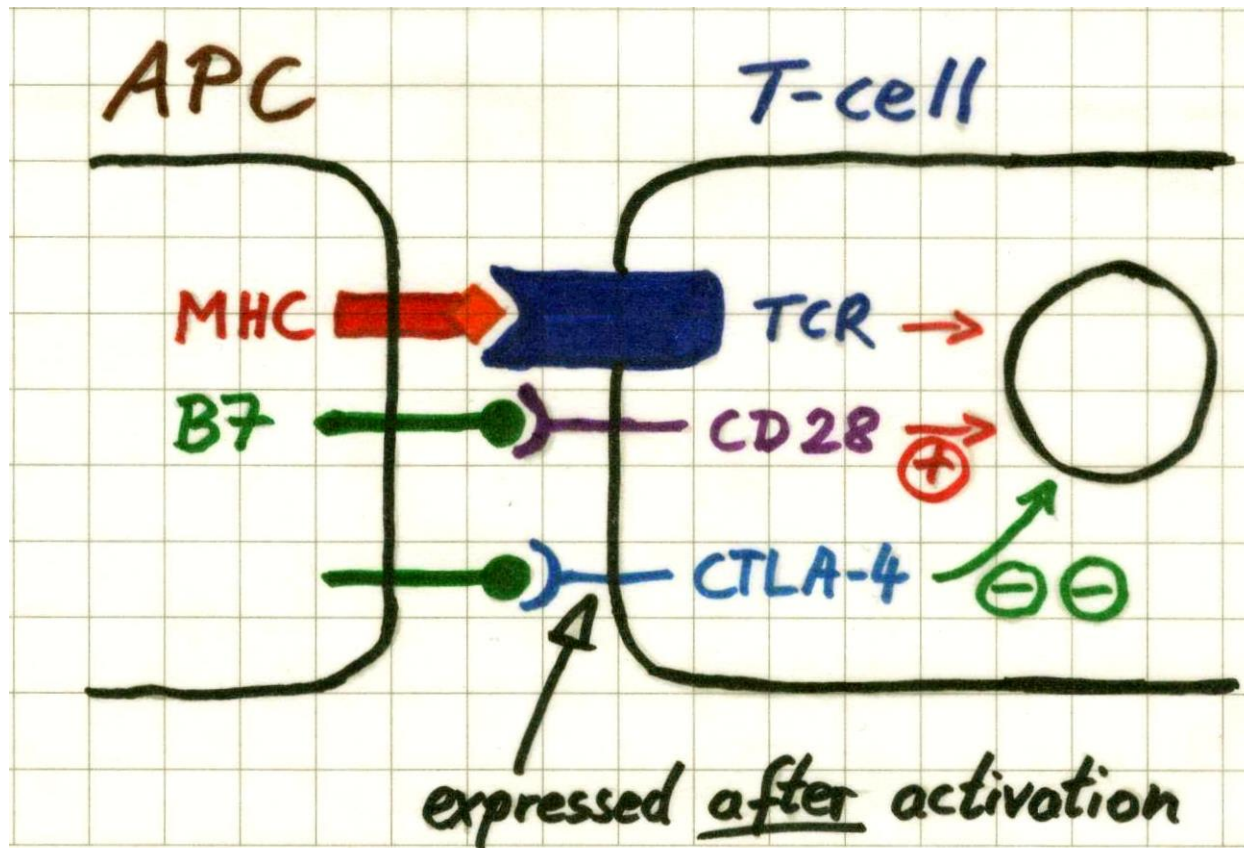


- POSITIVE SELECTION + RAPID ACTIVATION
- RESOLUTION OF INFECTION
- RAPID REPRESSION OF RESPONSE
- TIGHT FEEDBACK CONTROL
PREVENTS TISSUE DAMAGE

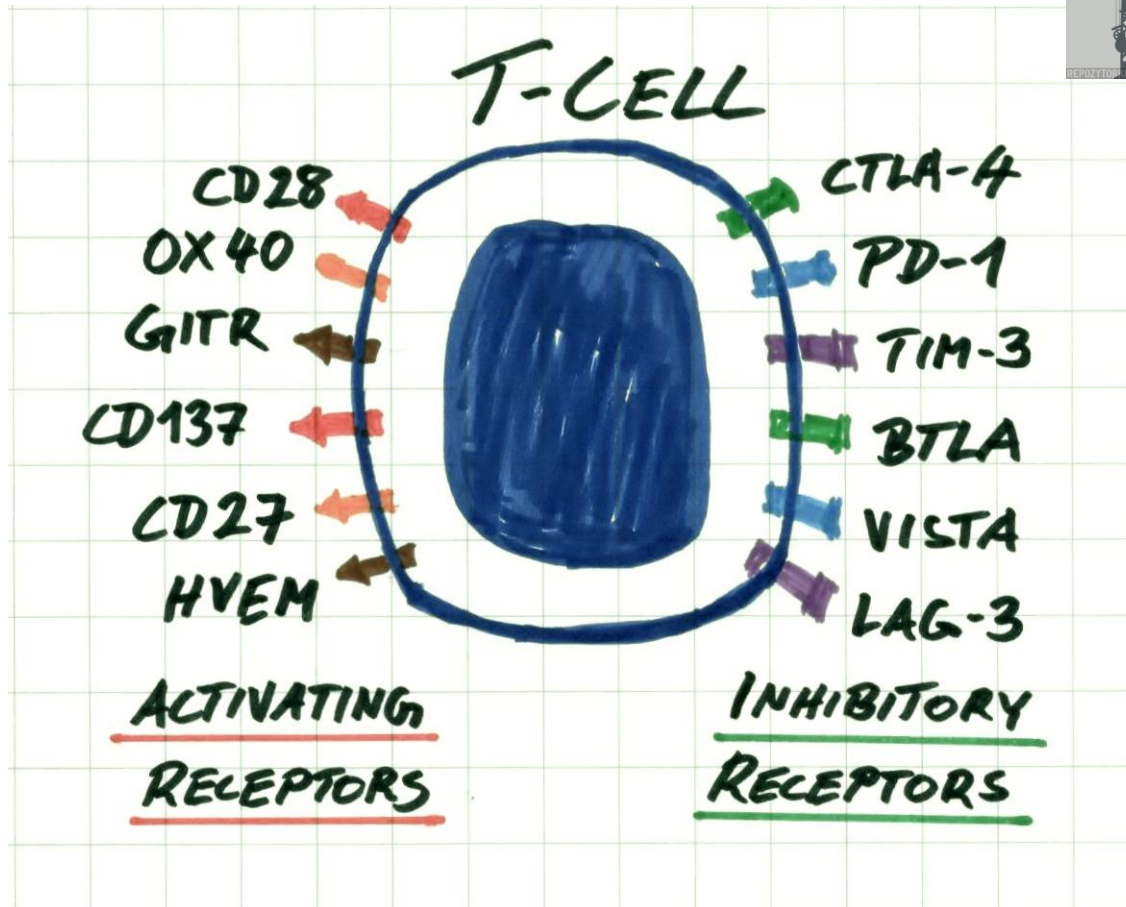
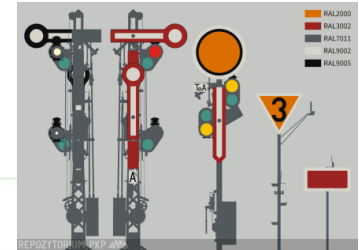
⇒ POSITIVE + NEGATIVE REGULATION



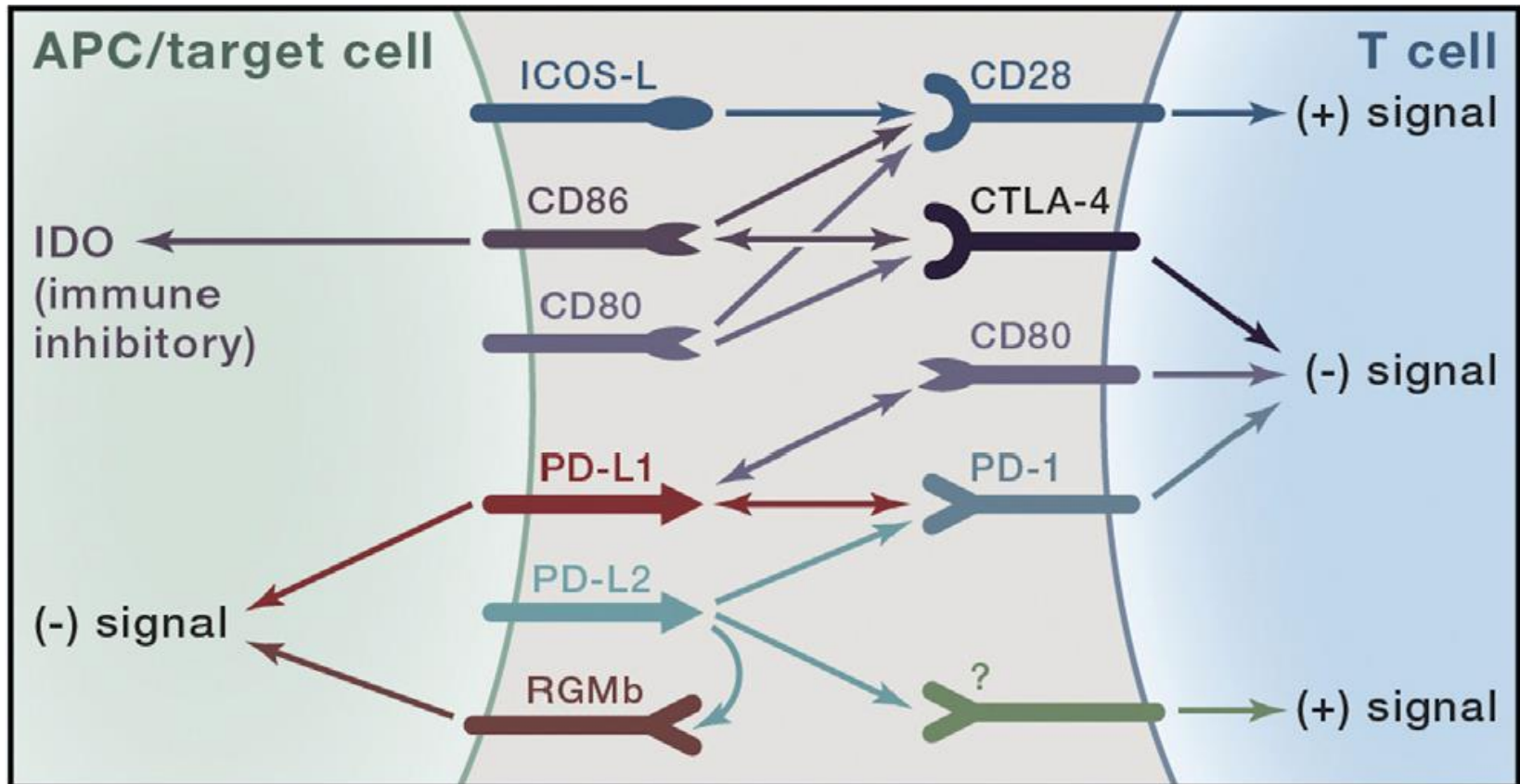
Co-stimulatory molecules



Co-stimulatory / co-inhibitory molecules

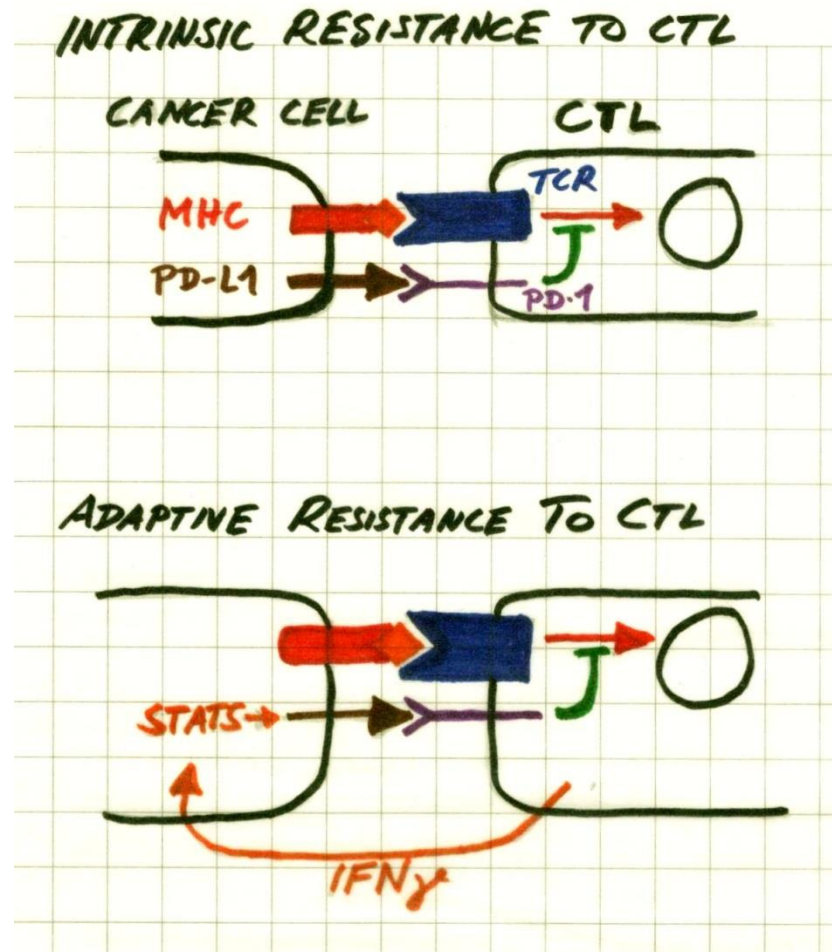


Complex interactions

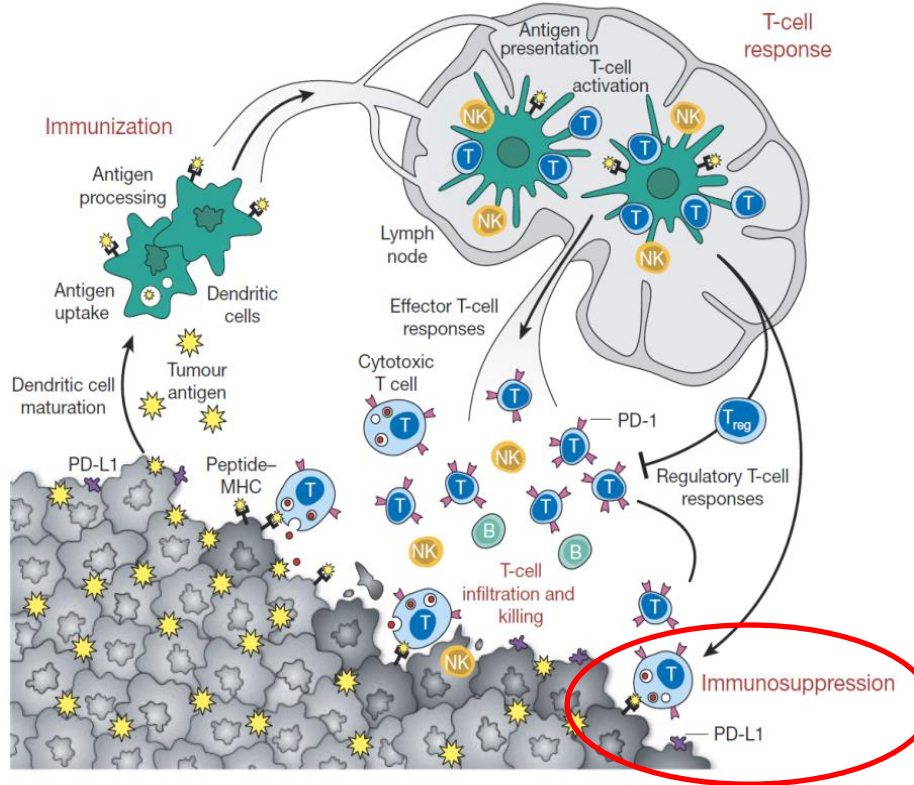
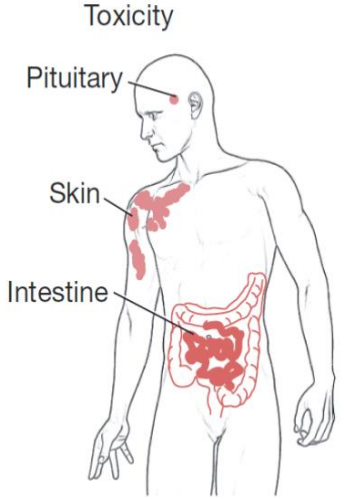
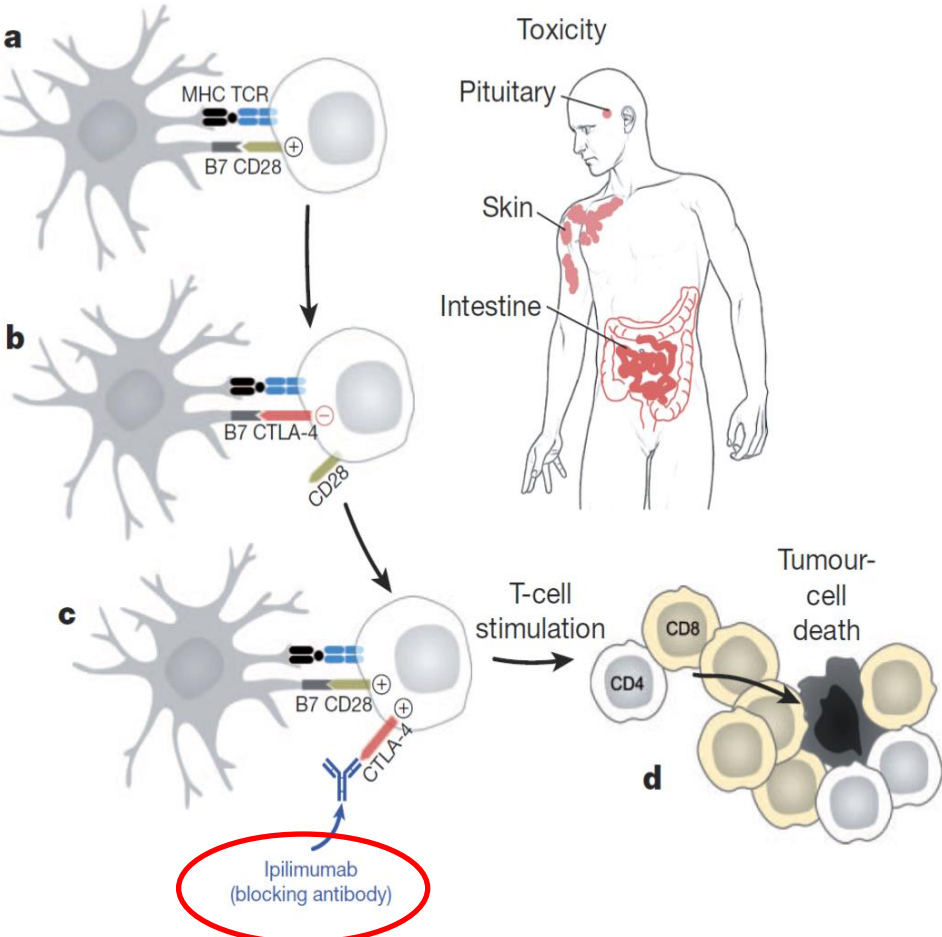


Topalian 2015 Cancer Cell, PMID 25858804

Tumor escape through PD-L1 expression



Therapieansätze von Chekpoint-Inhibitoren

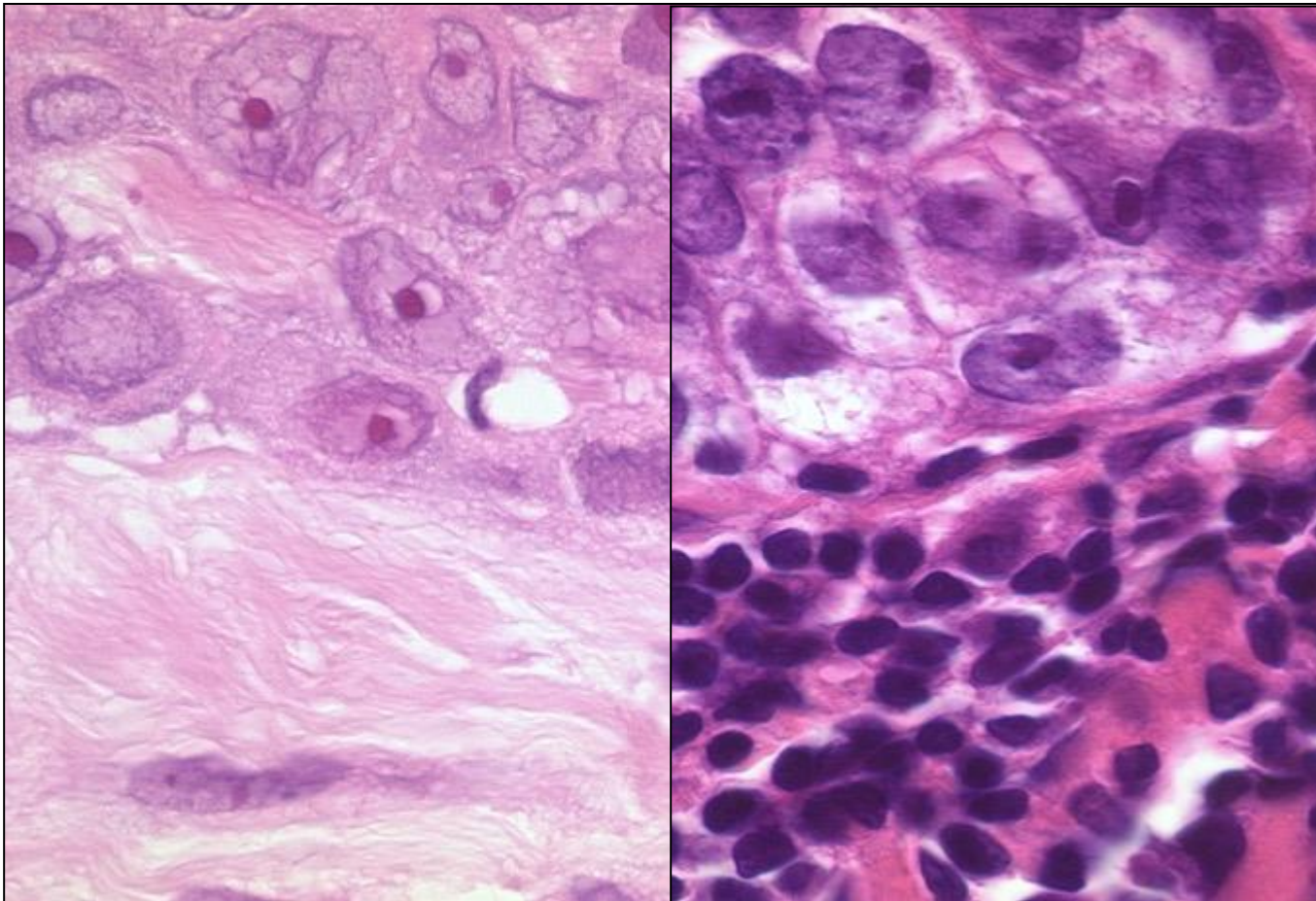


Mellman I, Coukos G, Dranoff G (2011) Cancer immunotherapy comes of age. Nature 480(7378): 480–489

Messung von Immunparametern im Mammakarzinom-Gewebe



Heterogenous immune infiltrate in breast cancer



Lymphocyte
predominant breast
cancer (LPBC)

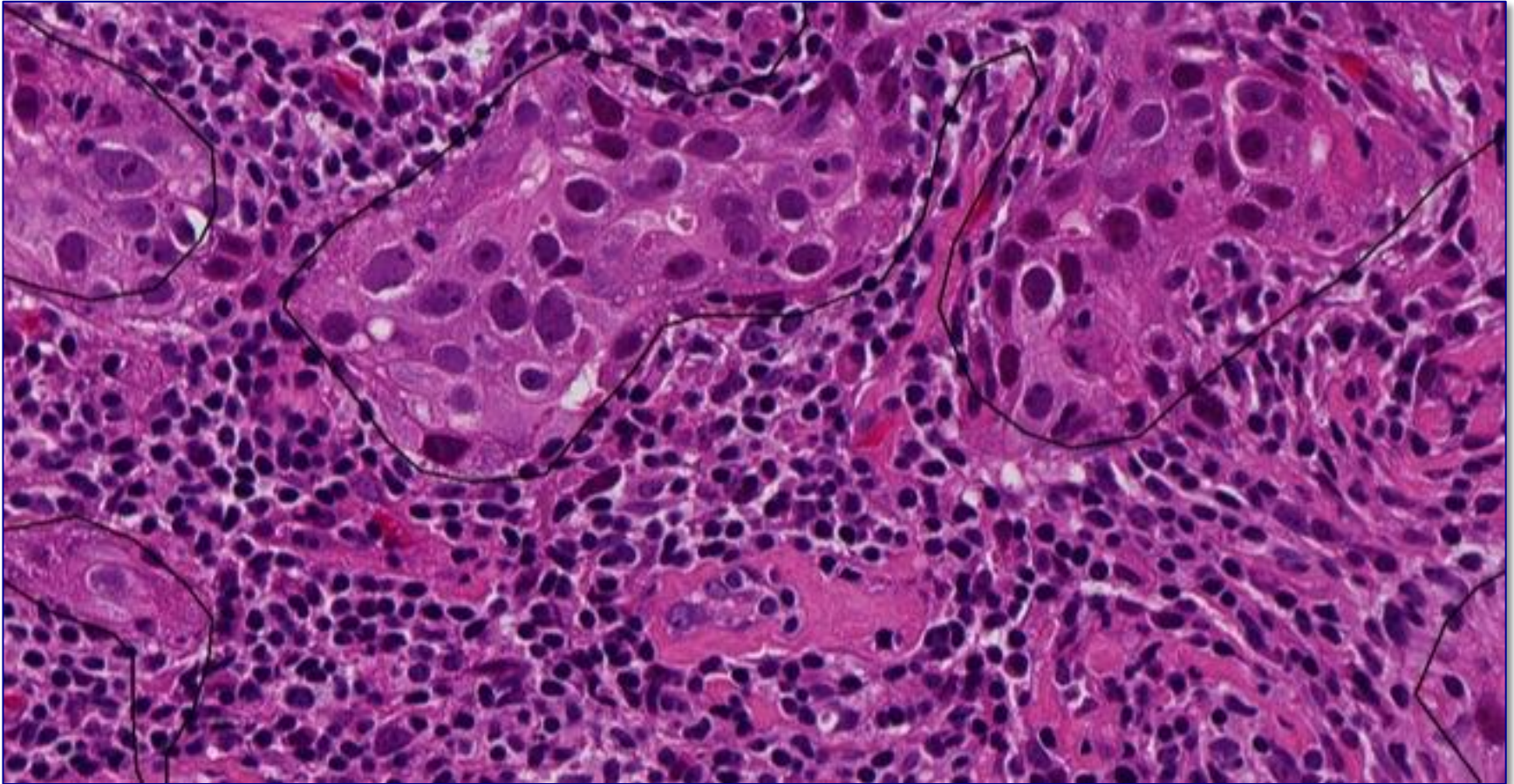
more lymphocytes
than tumor cells

10-25% of breast
cancers

San Antonio Breast Cancer Symposium, December 6 -10, 2016

Lymphocyte-predominant breast cancer (LPBC)

„more lymphocytes than tumor cells“ ($\geq 60\%$ TILs)



Denkert C, et al. J Clin. Oncol. 2010, J Clin. Oncol. 2015; SABCs 2013.

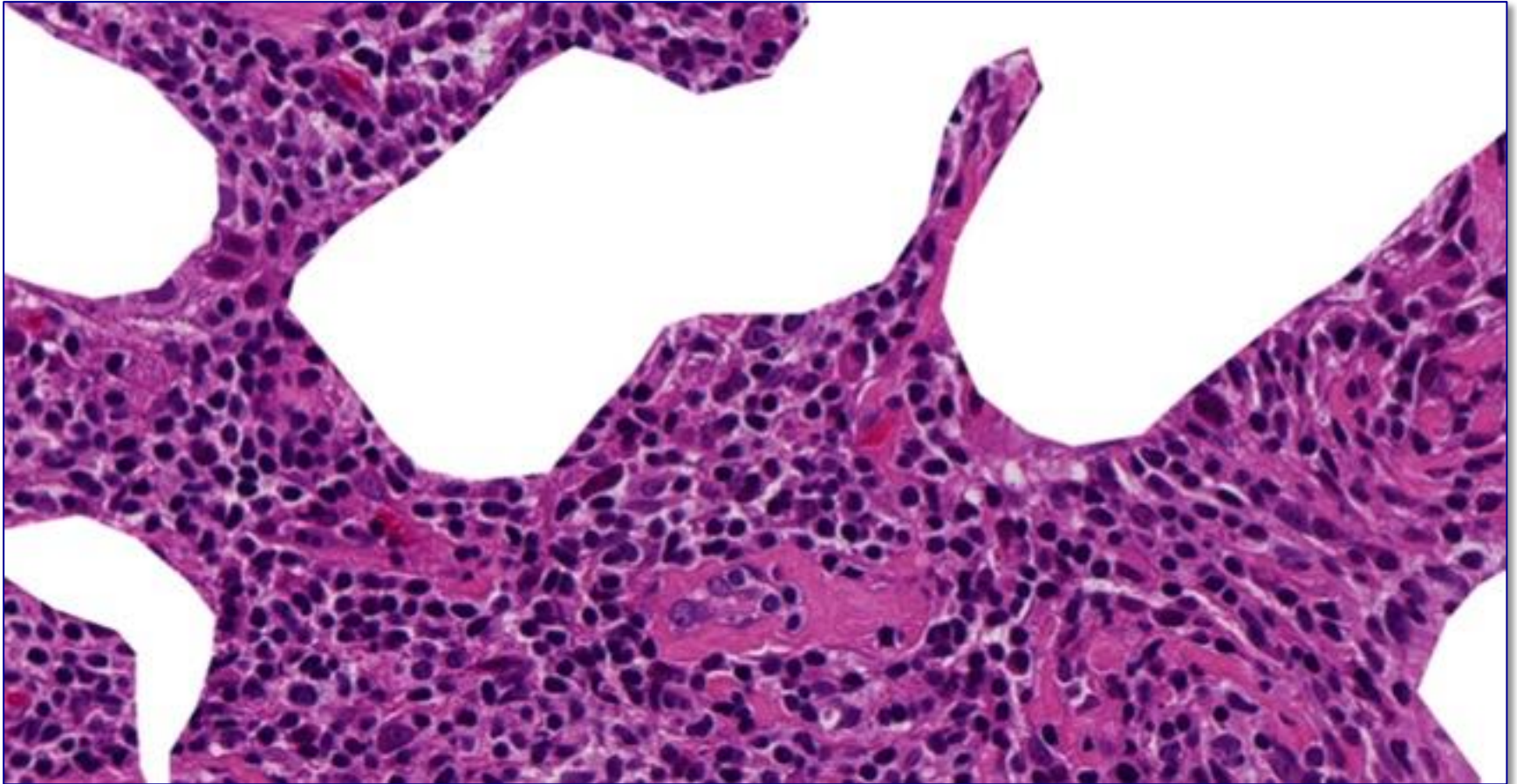
This presentation is the intellectual property of the author/presenter. Contact them at carsten.denkert@charite.de for permission to reprint and/or distribute.

intratumoral TILs – direkt contact with tumor cells



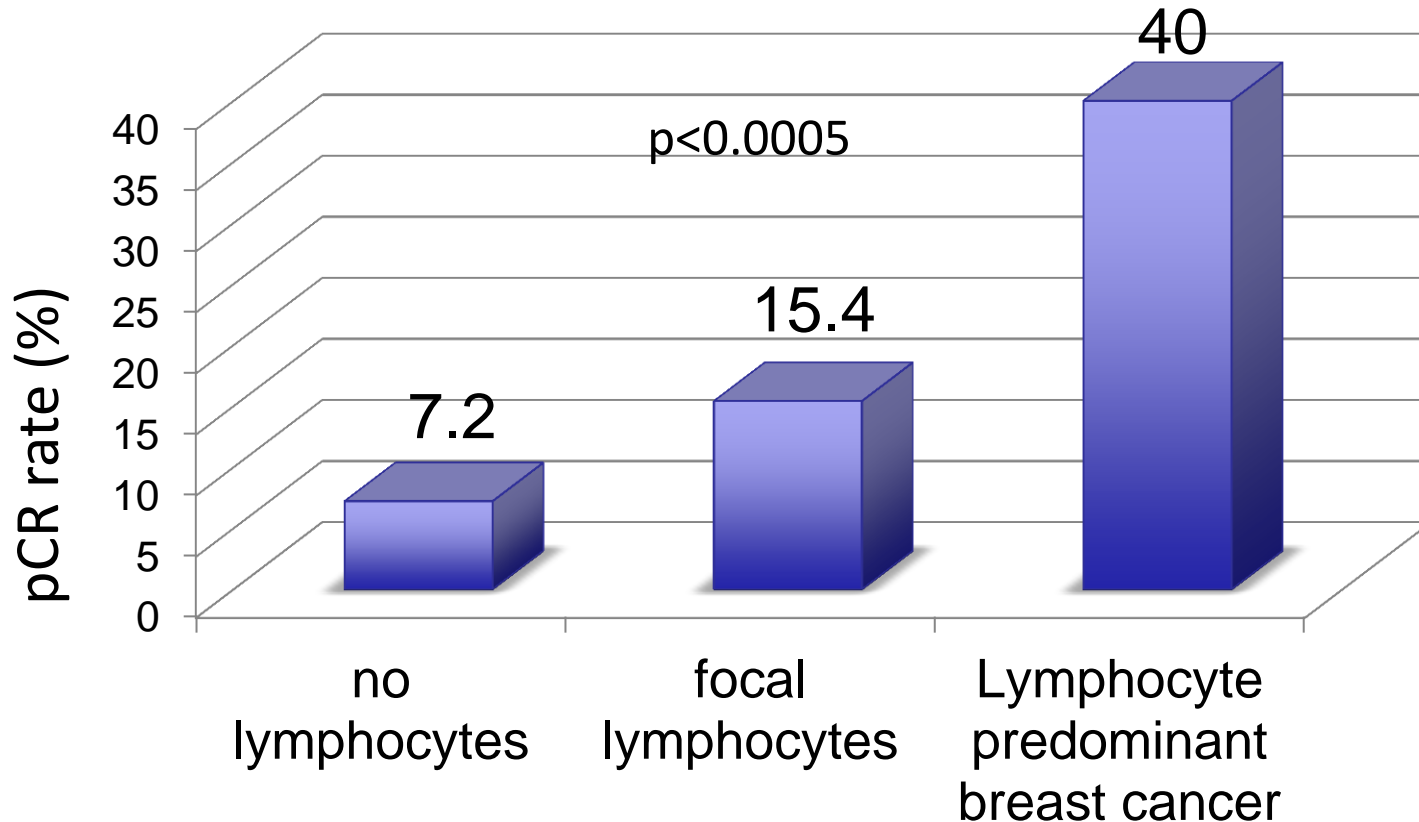
Denkert C, et al. J Clin. Oncol. 2010, J Clin. Oncol. 2015; SABCs 2013.

Patterns of tumor-infiltrating lymphocytes stromal TILs – between the tumor cells



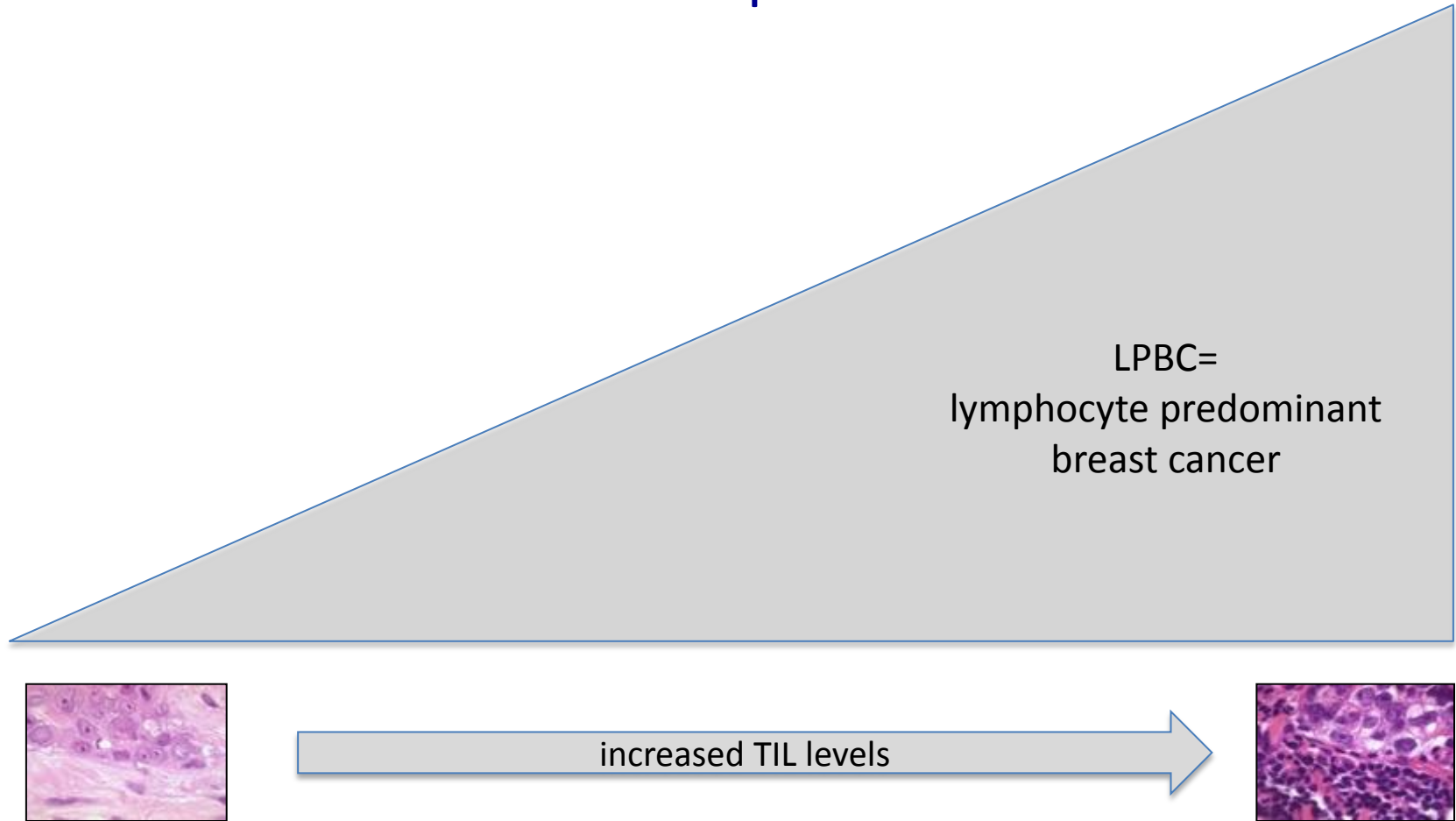
Denkert C, et al. J Clin. Oncol. 2010, J Clin. Oncol. 2015; SABCs 2013.

Clinical relevance – TILs are linked to response to neoadjuvant chemotherapy (GeparTrio, n=814)

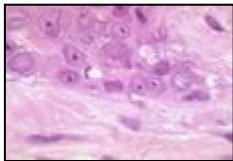
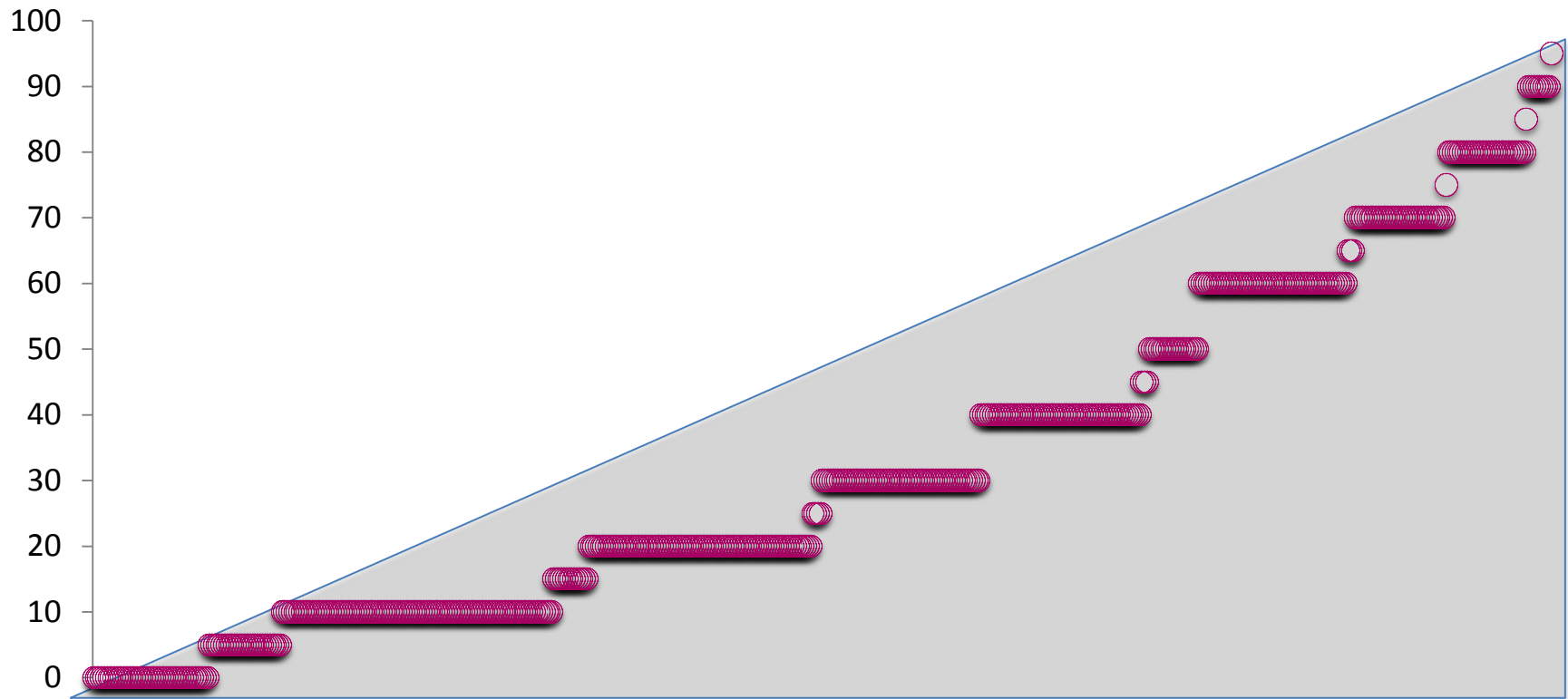


LPBC: n=100 (12%); Multivariat iTuLy: $p=0.001$, Denkert et al, JCO, 2010

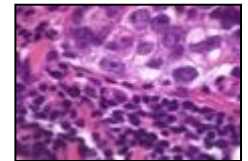
Tumor-associated lymphocytes are a continuous parameter



580 tumors from GeparSixto sorted by increasing TILs

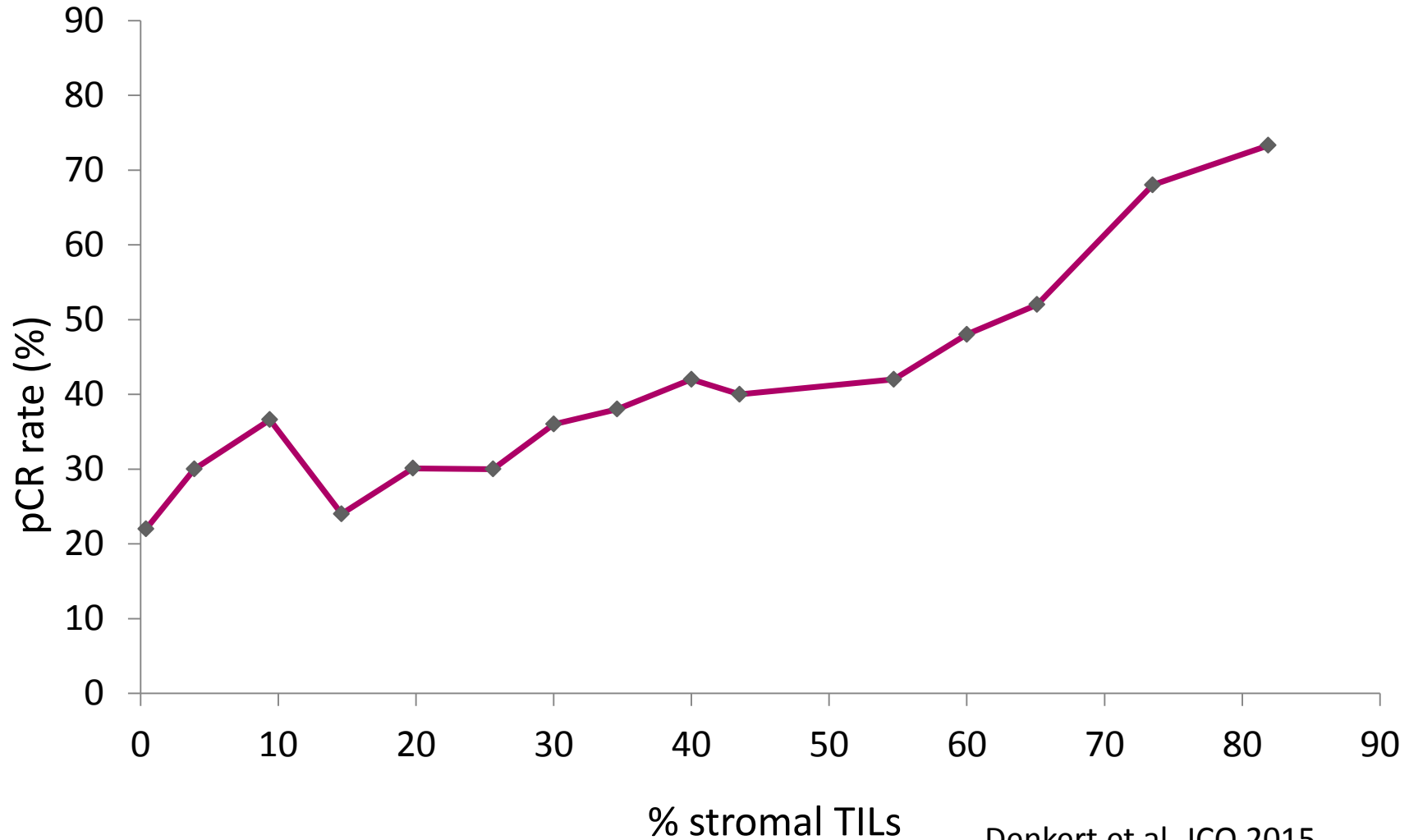


GeparSixto – sorted by increased TIL levels



TILs in GeparSixto are linked to increased pCR

STEPP analysis – pCR rate in GeparSixto



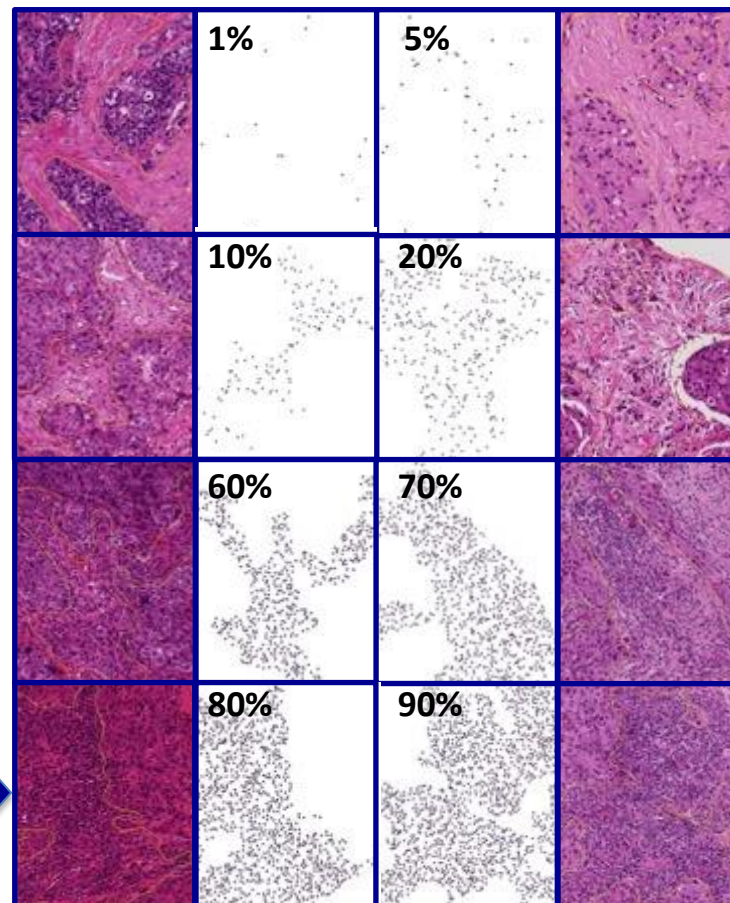
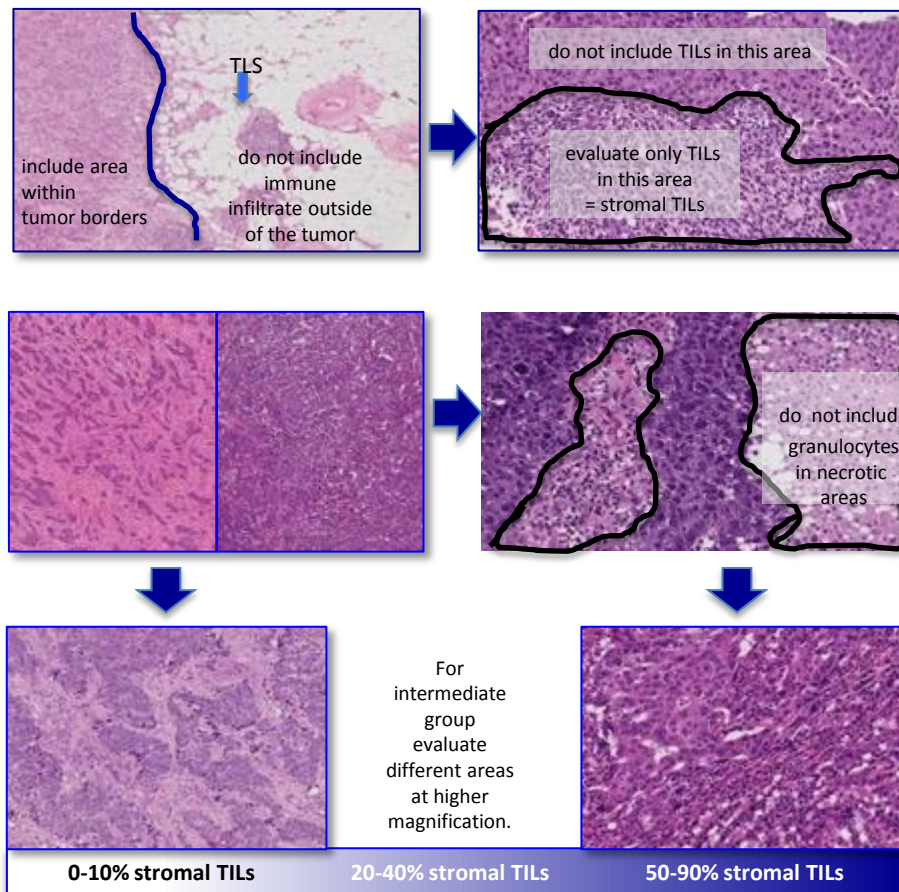
Denkert et al. JCO 2015

This presentation is the intellectual property of the author/presenter. Contact them at carsten.denkert@charite.de for permission to reprint and/or distribute.

The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TIL working group

2014

Salgado, Denkert et al., Annals of Oncology, 2015



Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: results of the ring studies of the international immuno-oncology biomarker working group

Denkert et al, Modern Pathology, 2016

32 pathologist, 2x 60 slides:

Sunil Badve, Zsuzsanna Bago-Horvath,

Anita Bane, Shahinaz Bedri,
Jane Brock, Ewa Chmielik,
Matthias Christgen, Cecile Colpaert,

Carsten Denkert, Sandra Demaria,

Gert Van den Eynden, Giuseppe Floris,

Stephen B Fox, Dongxia Gao,
Barbara Ingold Heppner, S. Rim Kim,

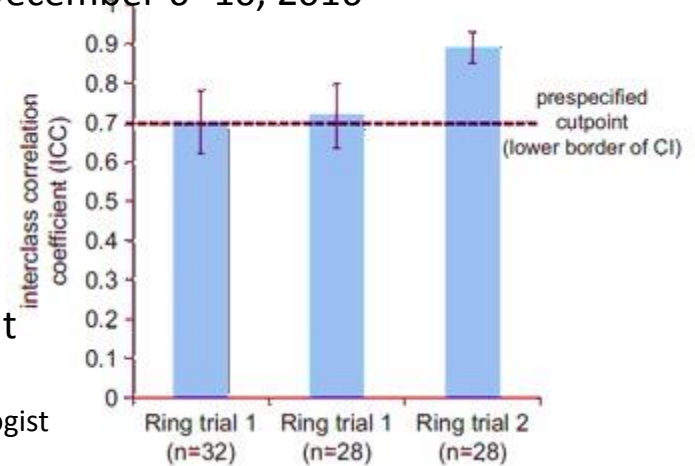
Zuzana Kos, Hans H. Kreipe,
Sunil R Lakhani, Frederique Penault-Llorca, Giancarlo Pruneri, Nina Radosevic-Robin,
David L. Rimm, Roberto Salgado
Stuart J. Schnitt, Bruno V. Sinn,

Peter Sinn, Nicolas Sirtaine,
Sandra A. O'Toole, Giuseppe Viale,

Koen Van de Vijver, Roland de Wind

intraclass correlation coefficient (ICC): 0.9

(90% true TIL measurement, 10% pathologist variation)



Virtual slide area
- will adjust to predefined area size when TIL evaluation is started

Rotary control for data entry.

Standardization area
Integrated visual feedback. Standardized images are shown automatically when TIL levels are entered by rotary control.

Slide 16/60

Start TIL evaluation

95%
100%
0%
5%

Back and go to next slide

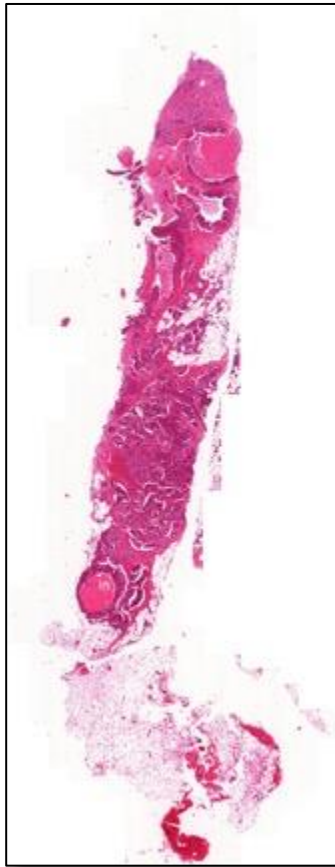
25%
30%
45%

Data for three different regions.

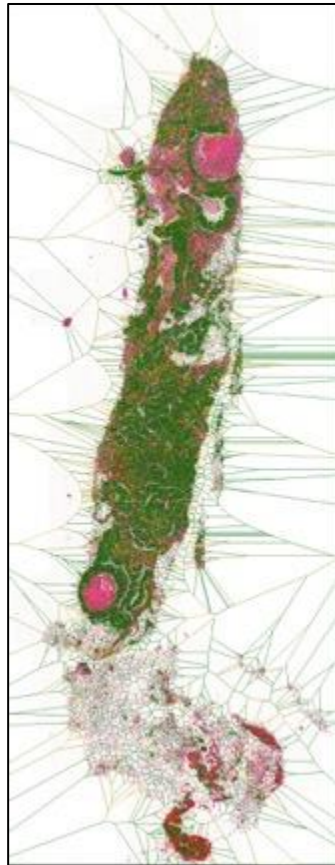
Automatic TIL counting by image analysis

Aim: fully automatic analysis of clinical study cohorts

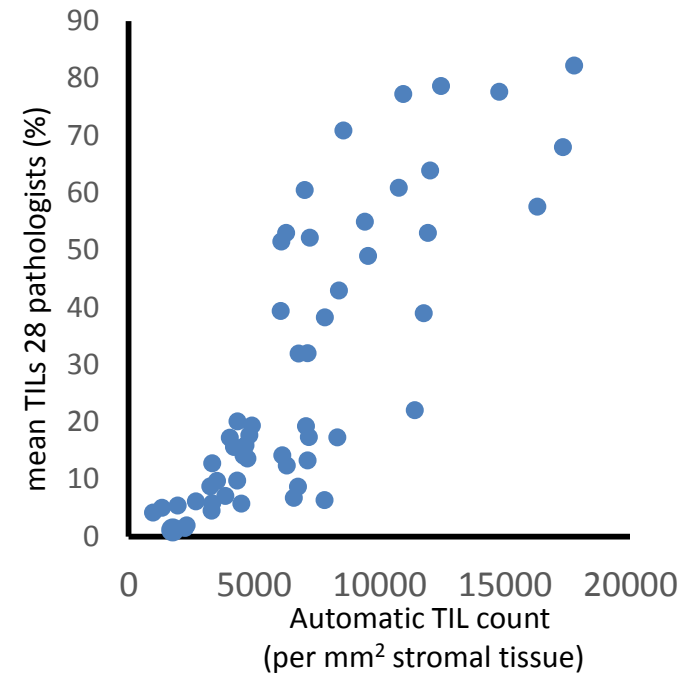
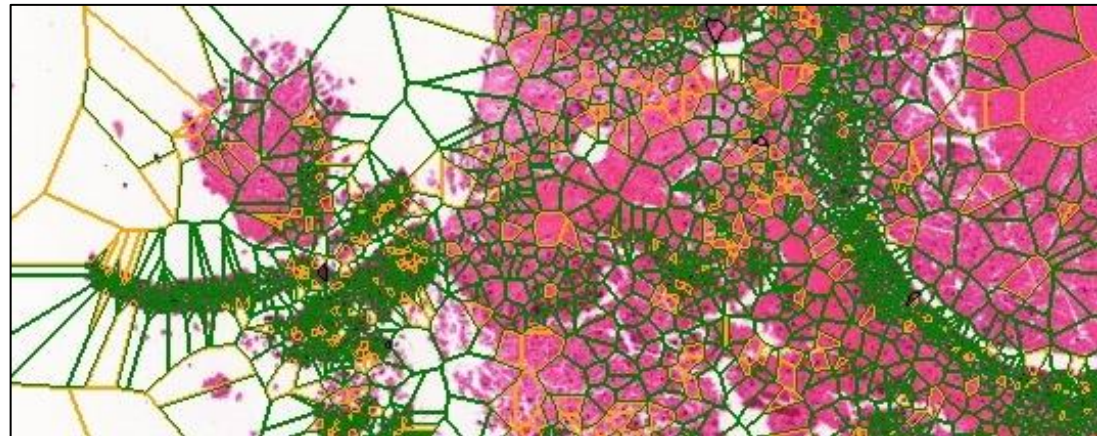
scanned digital slide
(core biopsy)



cell identification
(Voronoi diagram)

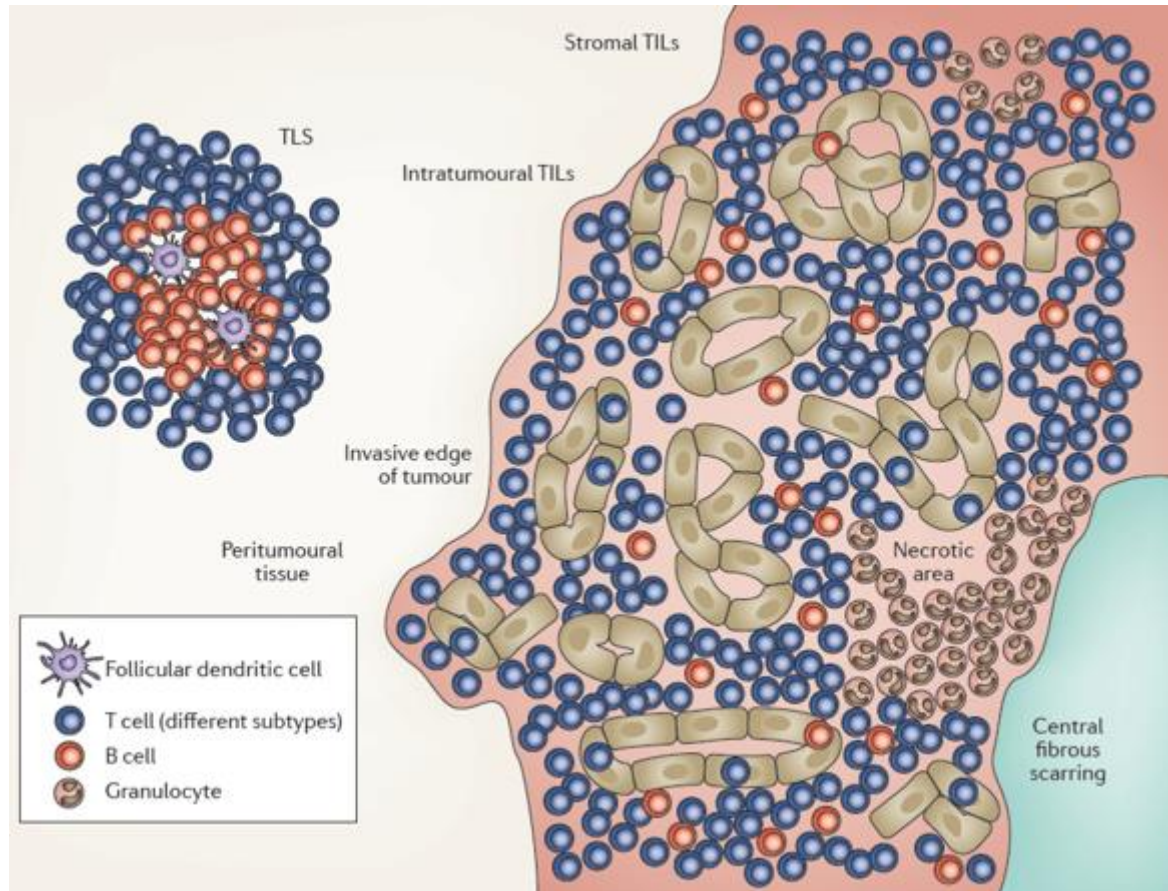


automatic counting of
stromal TILs



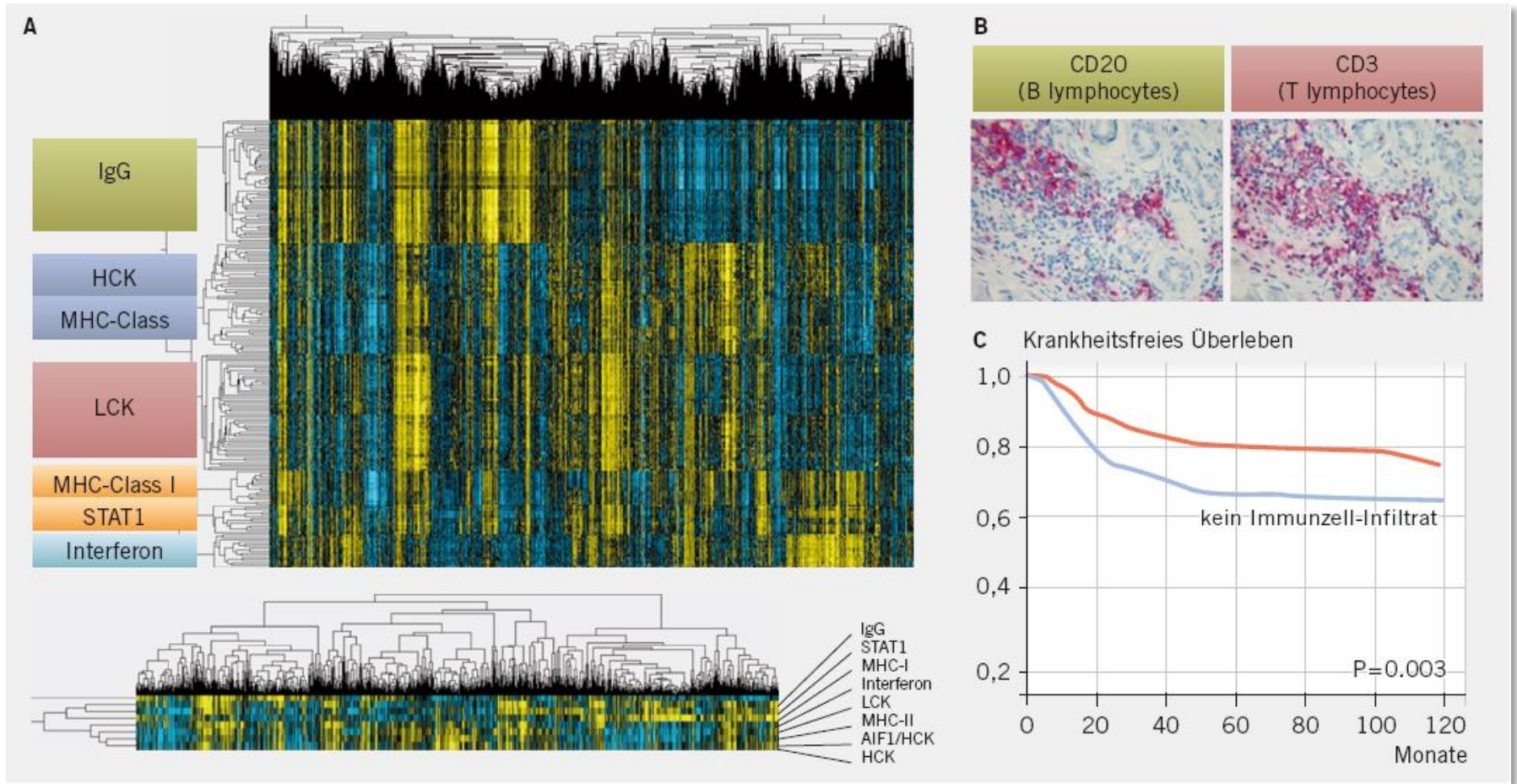
preliminary data, Stephan Wienert

Clinical relevance of host immunity in breast cancer: from TILs to the clinic



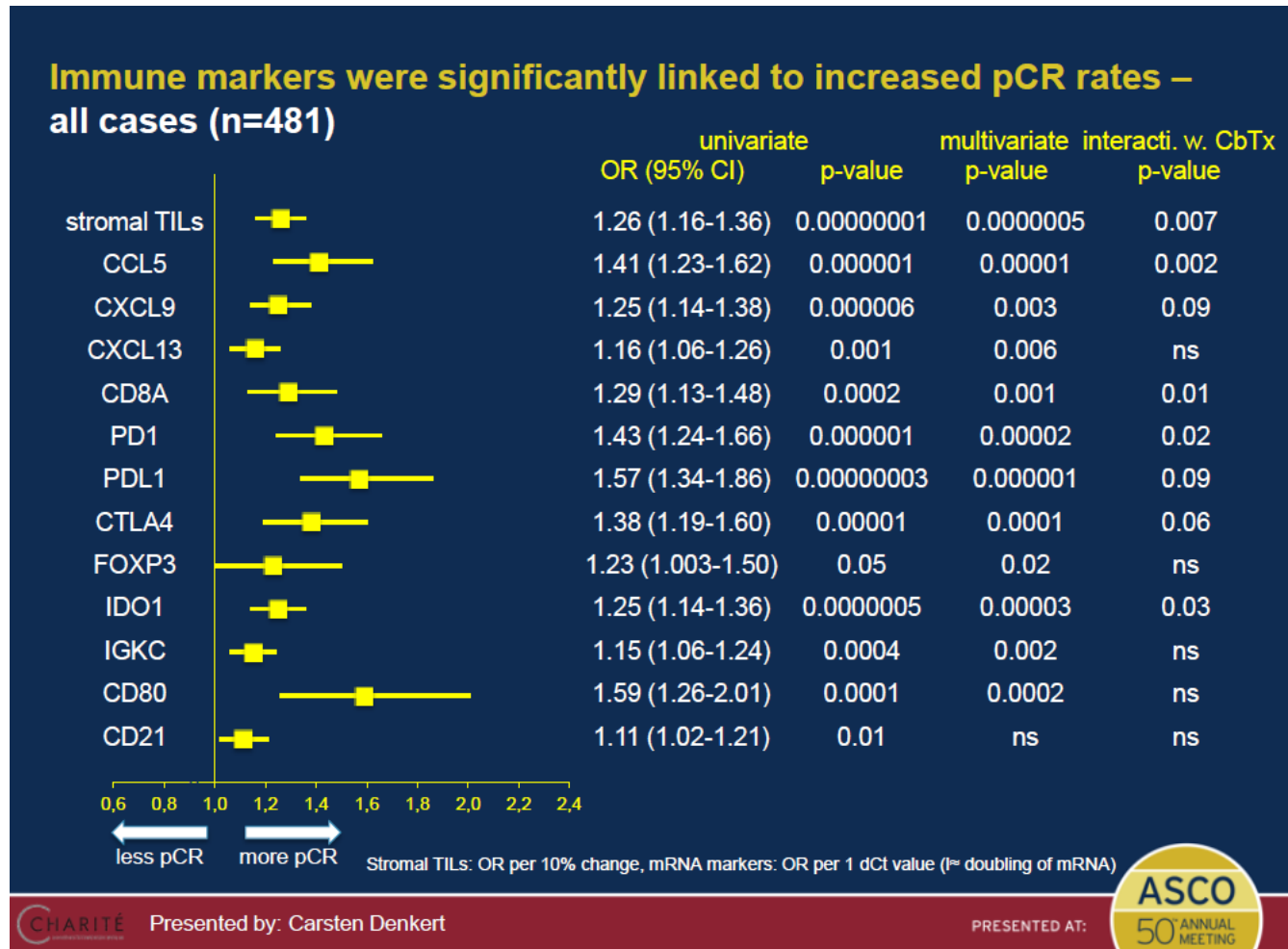
Savas... Denkert et al. Nature Clin. Oncol. 2016

Quantifying different immune cells within tumors



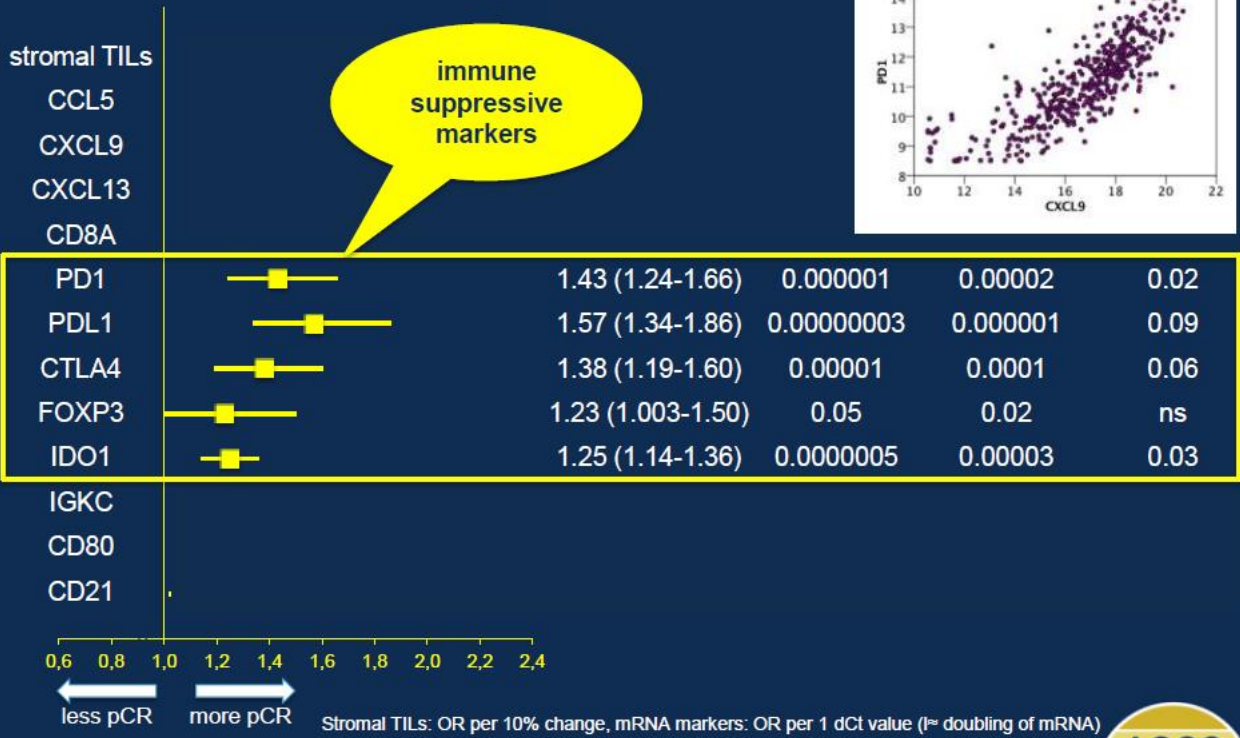
modified according to Rody et al. 2009, Breast Cancer Research, 11:R15

Strong correlation of ALL immune markers

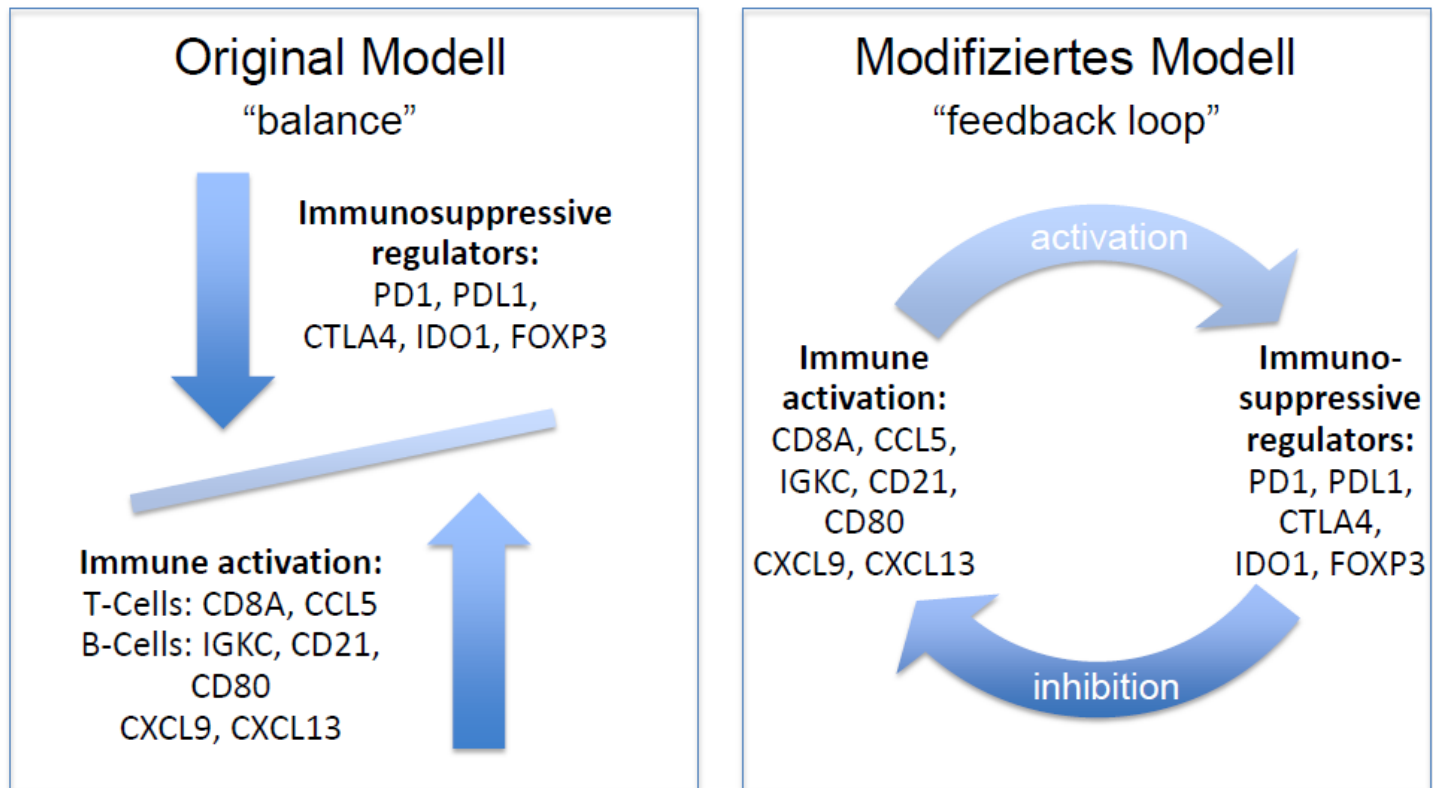


Strong correlation of ALL immune markers

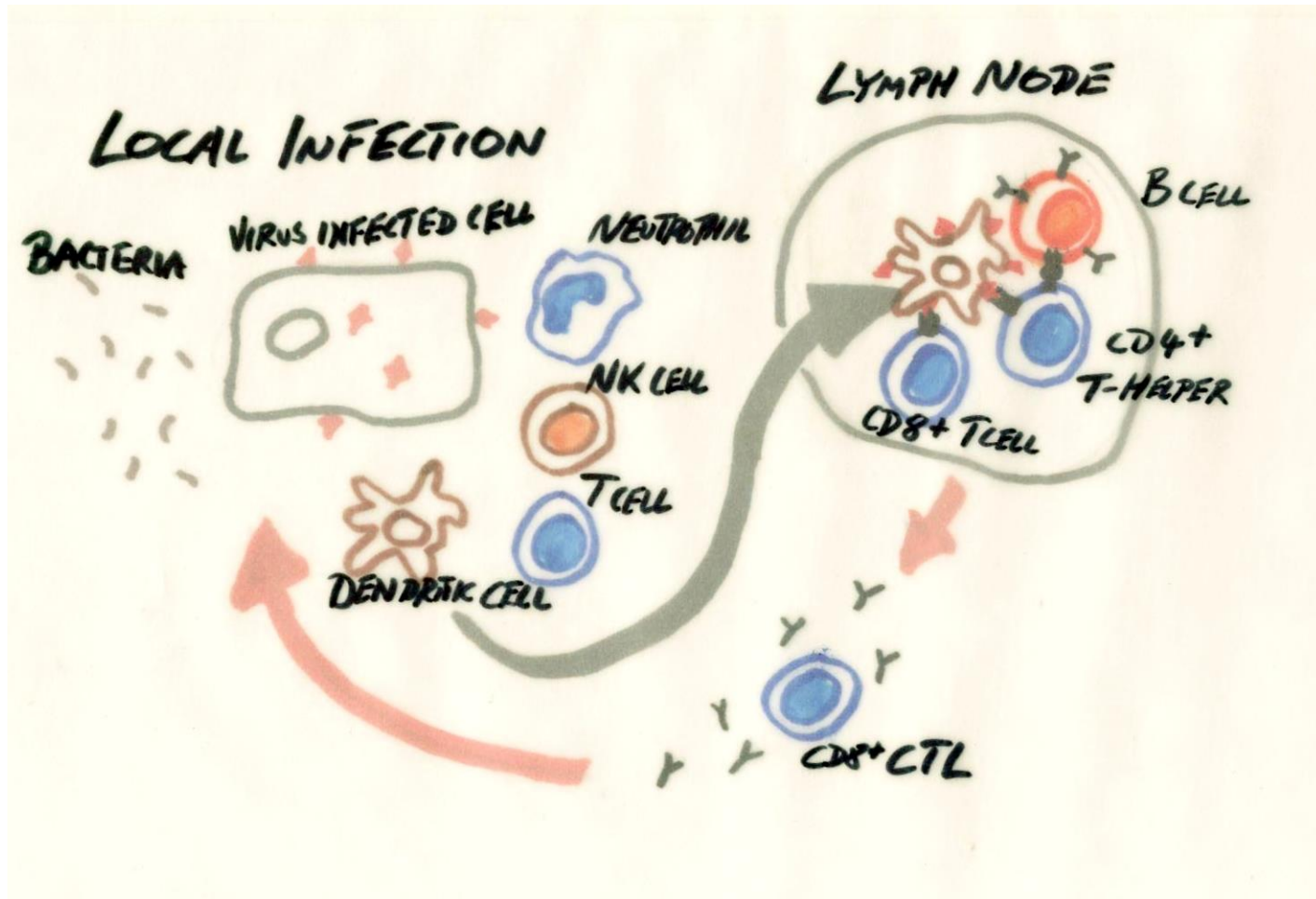
Immune markers were significantly linked to increased pCR rates – all cases (n=481)



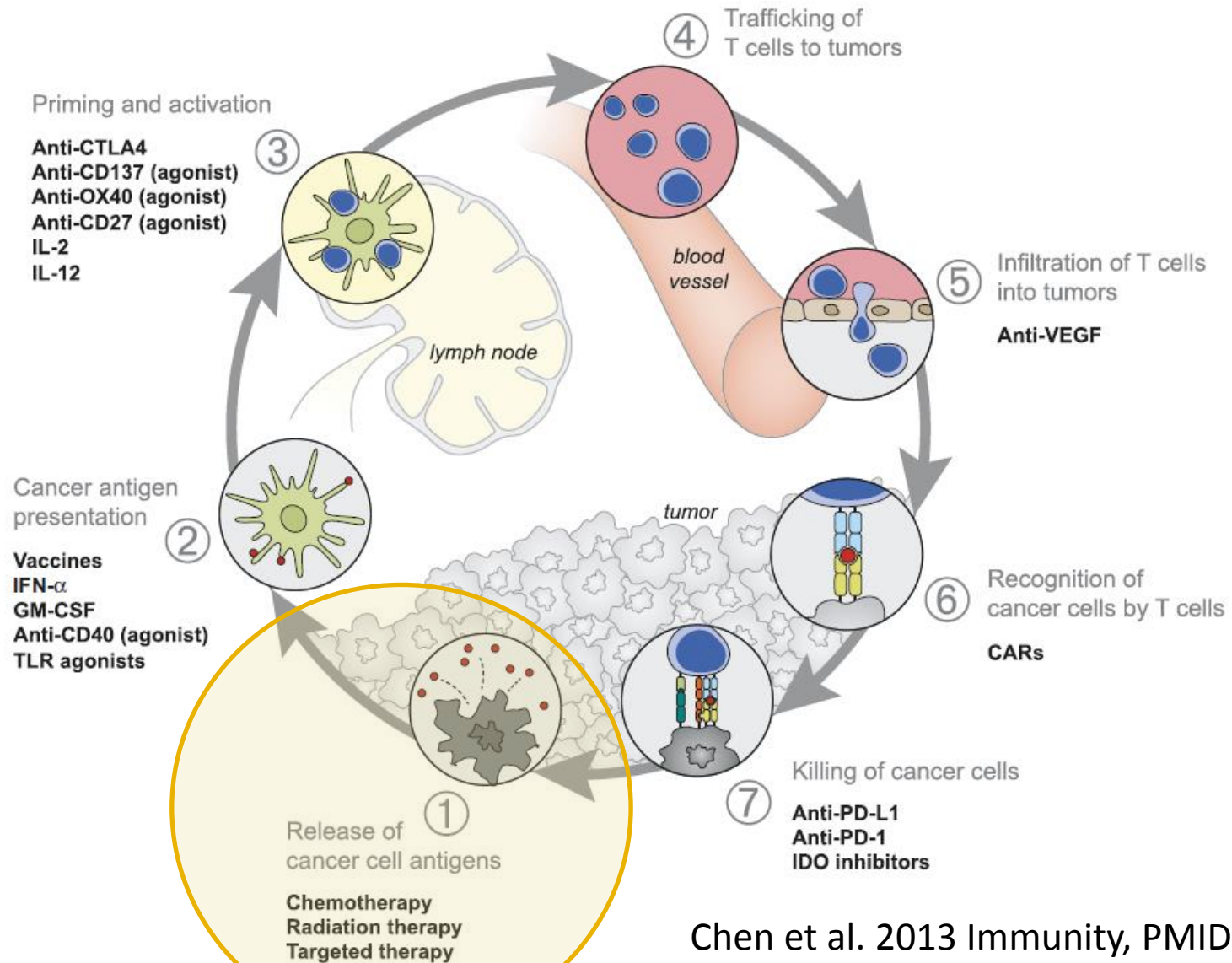
Modelle für immunologische Regulation beim Mammakarzinom



Woher kommen eigentlich die Antigene?



Cancer immunity cycle



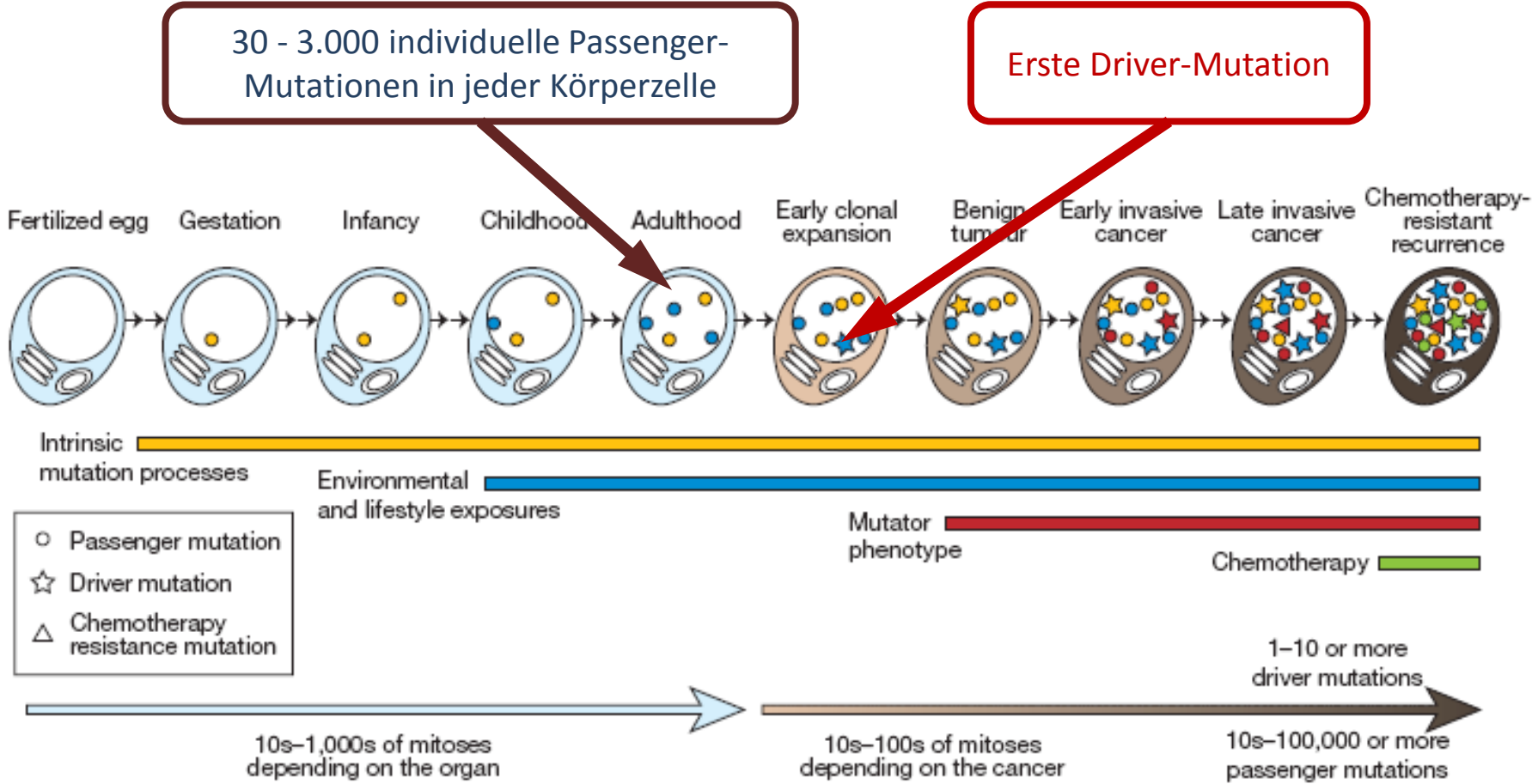
Chen et al. 2013 Immunity, PMID 23890059

Tumor-spezifische Antigene (TSA)

- Aberrante Expression von Genen, für die keine immunologische Toleranz besteht (z.B. Cancer-Testis-Antigene wie MAGE)
- Aberrante Überexpression (HER2)
- Neo-Antigene aufgrund von (Passenger)-Mutationen (z.B. durch Mutagene, UV-Schäden)

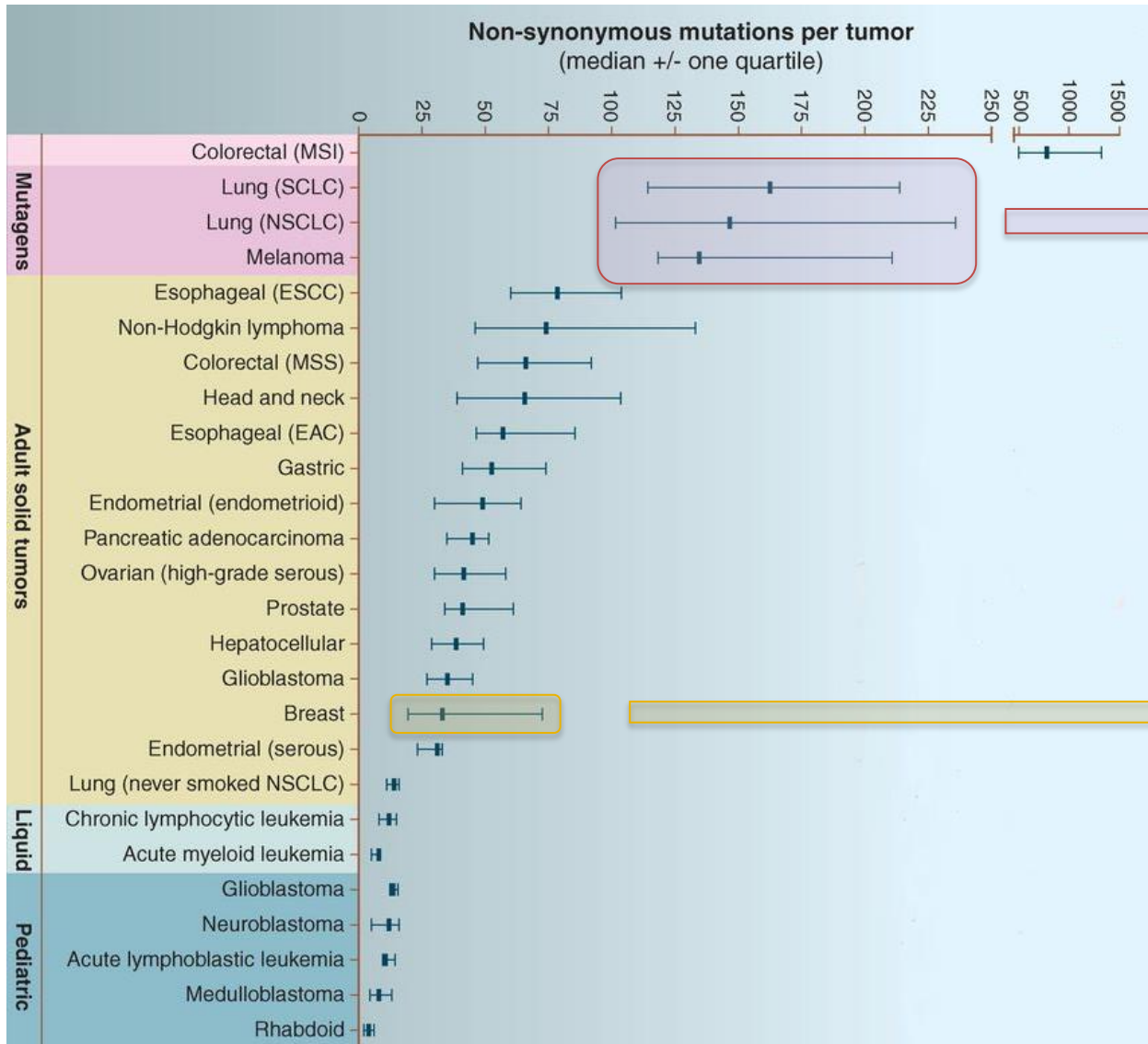


Neoantigene durch Passenger-Mutationen



Stratton et al. *Nature* 2009; **458(7239)**: 719-724.

Mutational Load



Potential neoantigens:
Melanoma: >100

Breast: 20-70

Vogelstein et al. 2013 Science, PMID 23539594

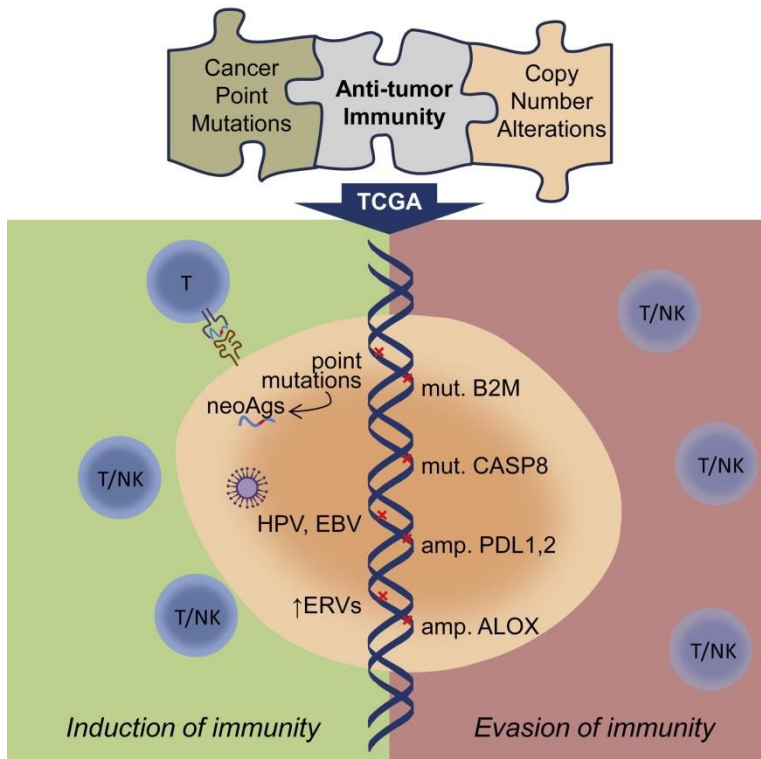
Predictive Factors for Immune Checkpoint Blockade

- Target expression (PD-1, PD-L1) problematic:
 - Antibodies & cutoffs (Kerr 2015 PMID 26134220, Patel 2015 PMID 25695955).
 - PD-L1 expression by infiltrating Lymphocytes (Herbst 2014 Nature PMID 25428504).
 - Negative cases may be strong responders (Postow 2015 JCO PMID 25605845, Topalian 2015 Cancer Cell PMID 25858804).
- Mutation/Neoantigen load:
 - Positive correlation with TIL in pan-cancer studies (Rooney 2015 Cell PMID 25594174).
 - Neoantigen load predictive for response (Snyder 2014 NEJM PMID 25409260, Rizvi 2015 Science PMID 25765070, Le 2015 NEJM PMID 26028255).

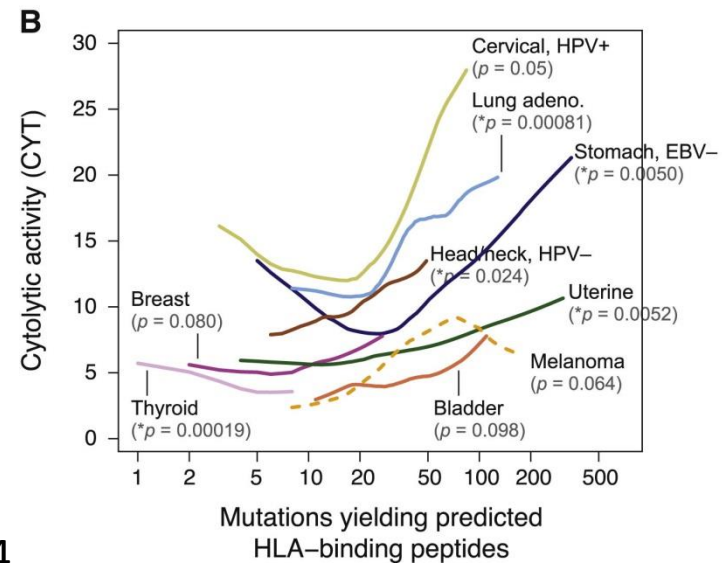
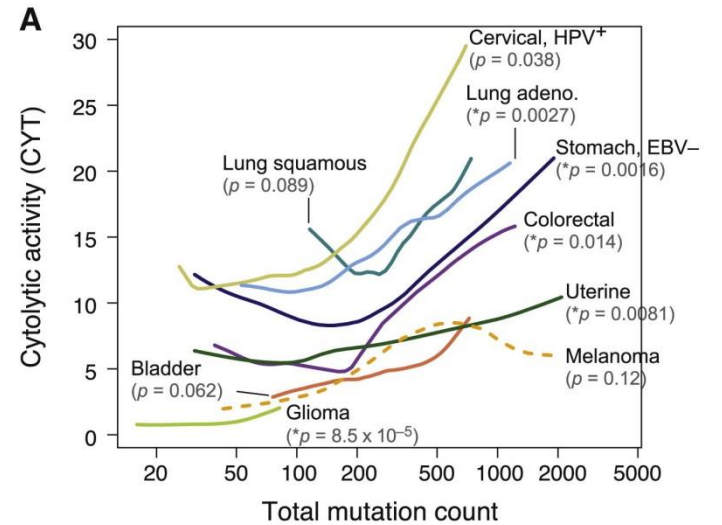


Genomics of TIL⁺ Cancer

- 8462 TCGA samples
- “CYT” metric (GZMA & PRF1 expression)



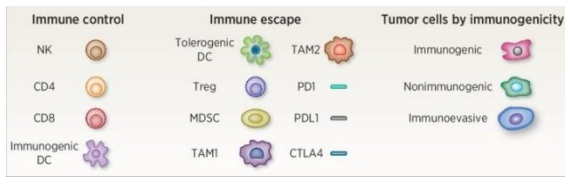
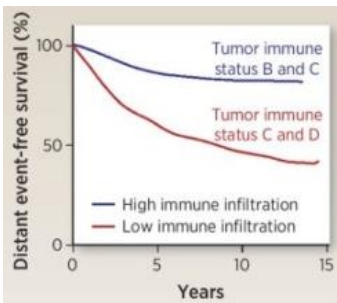
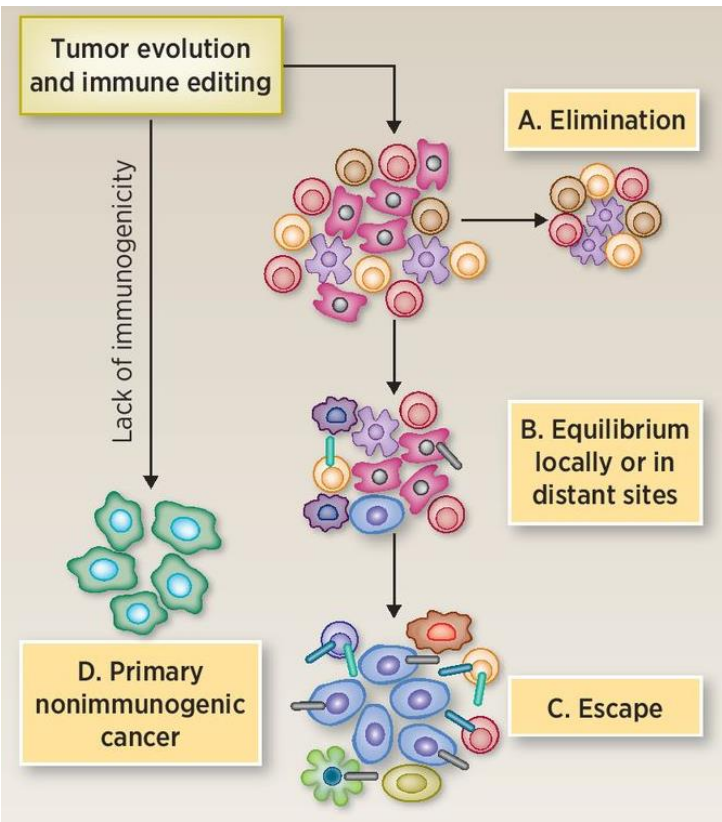
Rooney et al. 2015 Cell, PMID 25594174



Hat das Immunsystem auch eine Wirkung auf das Genom des Tumors?

Die Immunoediting Hypothese

Immunoediting



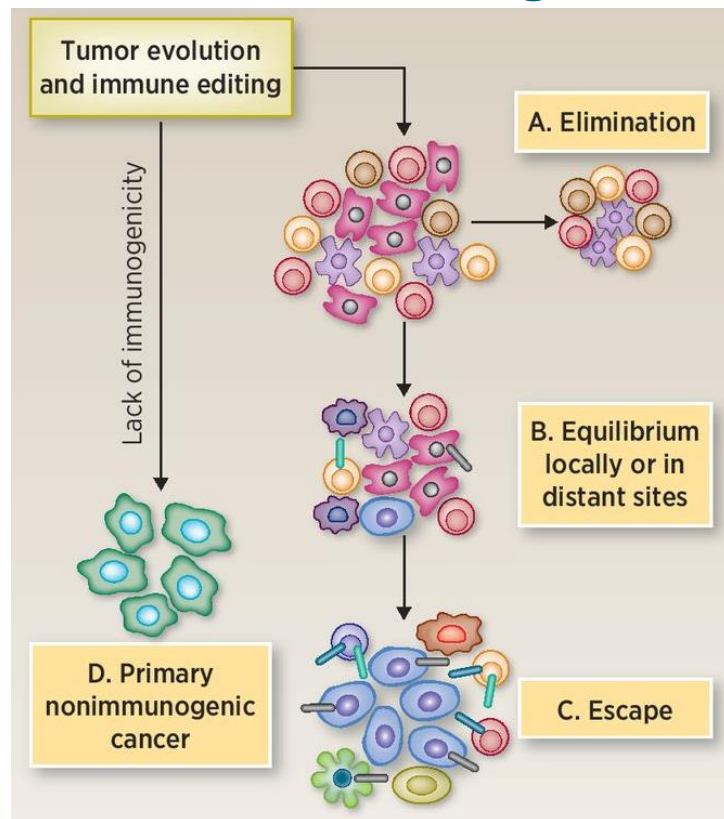
← Immune system edits tumor through elimination of sensitive tumor cells

← Surviving tumor cells become dominant

Pusztai, Karn et al. 2016 Clin Cancer Res 22(9):2105

Auswirkung auf Tumorerheterogenität?

Immunoediting



Effect on tumor heterogeneity:

➔ No clinically apparent tumors

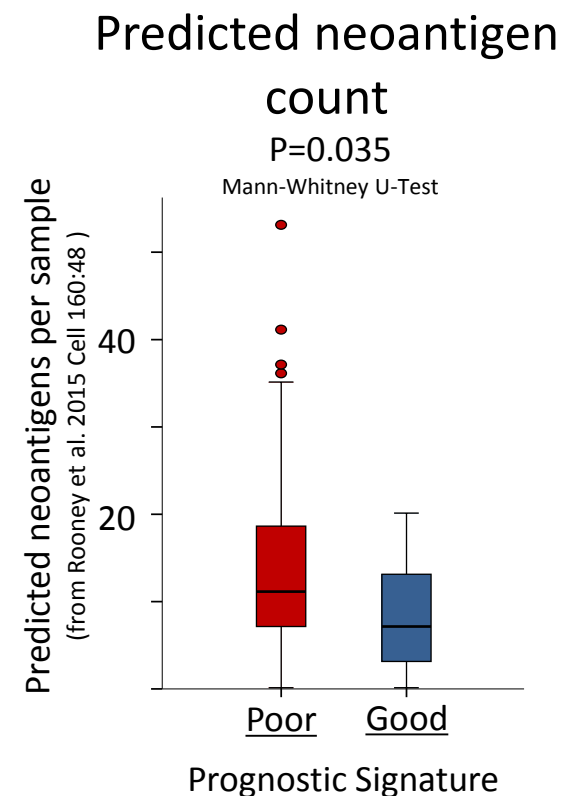
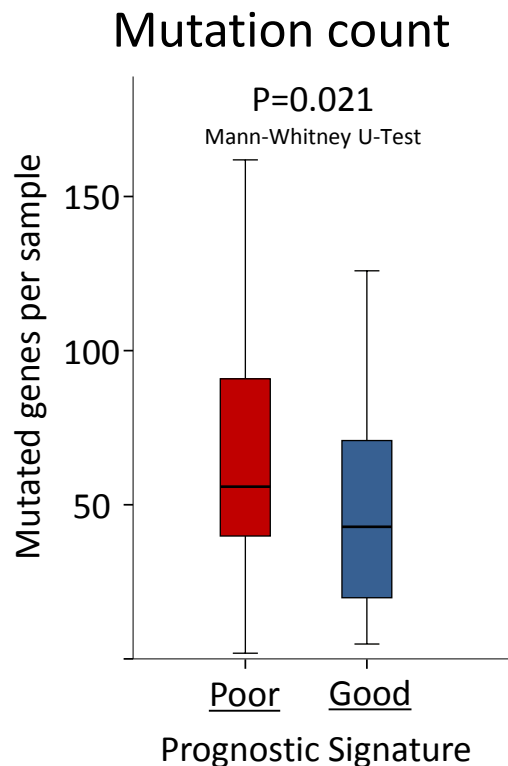
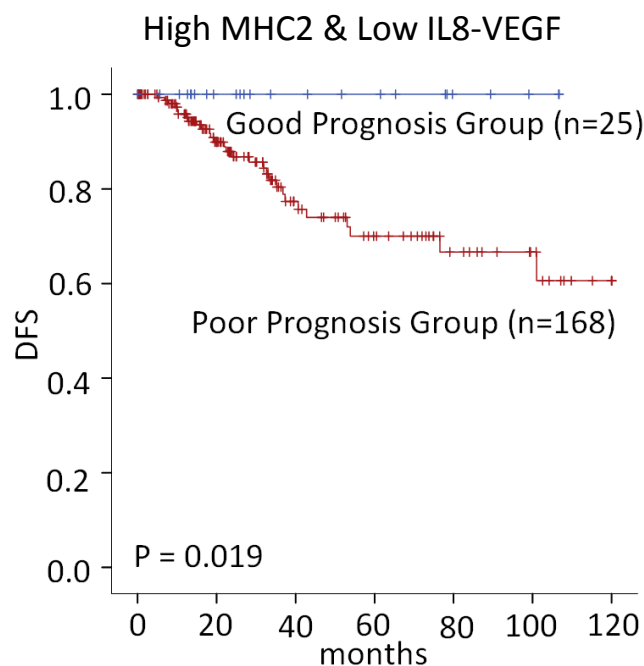
➔ Immune selection may prune clonal diversity

➔ Tumors that are 'invisible' to or have escaped immune control can clonally diversify

Pusztai, Karn et al. 2016 Clin Cancer Res 22(9):2105

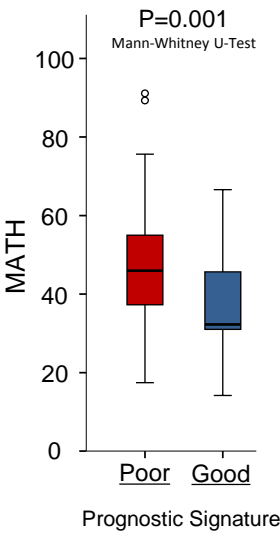
Prüfung an Daten aus TCGA

Good prognosis (i.e. immune rich / low inflammation) TNBC has low mutation and neoantigen loads

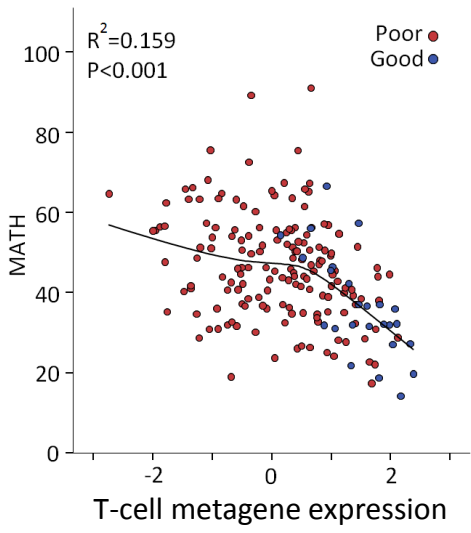


Good prognosis (i.e. immune rich / low inflammation) TNBC has low clonal heterogeneity

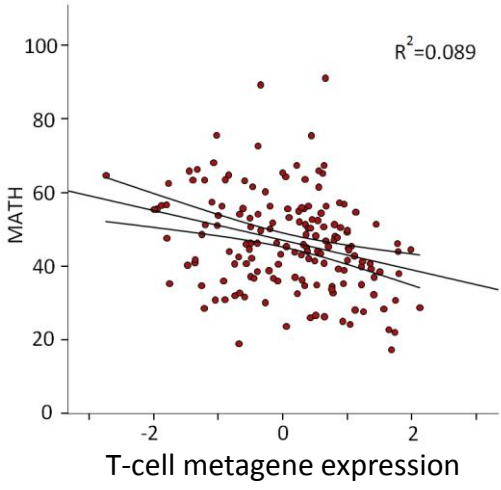
Clonal heterogeneity



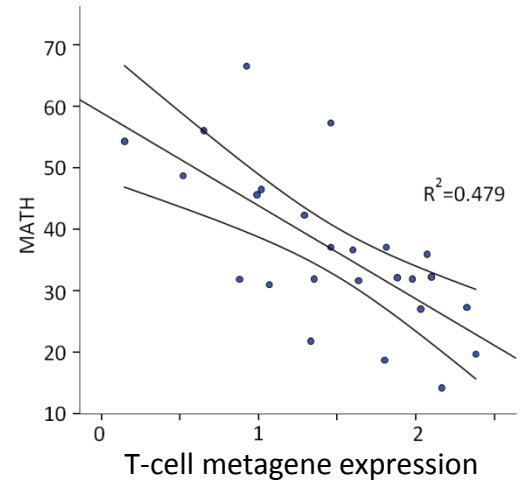
All TNBC



“Poor prognosis” TNBC



“Good prognosis” TNBC



MATH=Mutant-allele tumor heterogeneity score (Mroz et al. 2013; PMID 23079694).



Schlussfolgerung

| Tumor type | immune rich TNBC | immune poor TNBC |
|---|--|--|
| Prognosis | good | poor |
| Mutation load | low | high |
| Neoantigen load | low | high |
| Clonal heterogeneity | low | high |
| Negative association of heterogeneity and T-cell presence | strong | weak |
| Potential state of immuno editing hypothesis | equilibrium phase with pruning of clonal diversity | lack of immunogenicity / immune escape, clonal diversification |

Der Immune-Escape zeigt sich in der genomischen Heterogenität des Tumors.

Summary (I)

- Das Immunsystem enthält starke negative und positive Rückkopplungsmechanismen, um die Immunantwort streng zu regulieren
- Tumoren können sich dem Zugriff des Immunsystem z.T. durch Ausnutzung solcher Regulations-Mechanismen entziehen
- Gleichgewicht zwischen gegenläufiger T_H1 - und T_H2 -Antwort des Immunsystems auf die Tumorerkrankung.
- „Checkpoint-Inhibitoren“ greifen an solchen Regulationspunkten an und verschieben das Gleichgewicht wieder zur T_H1 -Antwort.



Summary (II)

- Bei Brustkrebs finden sich relativ häufig Tumor-infiltrierende Lymphozyten (TILs), besonders bei ER-negativen Subtypen
- TILs und verschiedene Immunmarker sind kontinuierliche Parameter und stark korreliert
- Sämtliche Immunmarker sind mit besserem Ansprechen auf neoadjuvante Chemotherapie wie auch mit besserer Prognose assoziiert
- Als Neoantigene werden hauptsächlich Passenger-Mutationen diskutiert



Teil II:

Kann man damit etwas vernünftiges anfangen?

