

Genexpression und weitere Prognosemarker in Stanzbiopsien und Residualtumoren – bereit für die klinische Anwendung?

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**Marburg
University**

COI statement

Advisory Board: MSD Oncology, Daiichi Sankyo, Astra Zeneca, Biontech

Invited Speaker: Astra Zeneca

Licencing Fees: VmScope digital pathology software

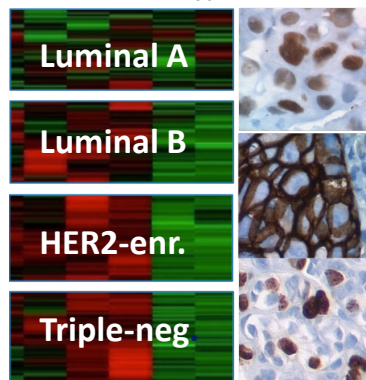
Research grants (institutional) Myriad, German Breast Group

Patents: WO2020109570A1; Patent WO2015114146A1; Patent WO2010076322A1



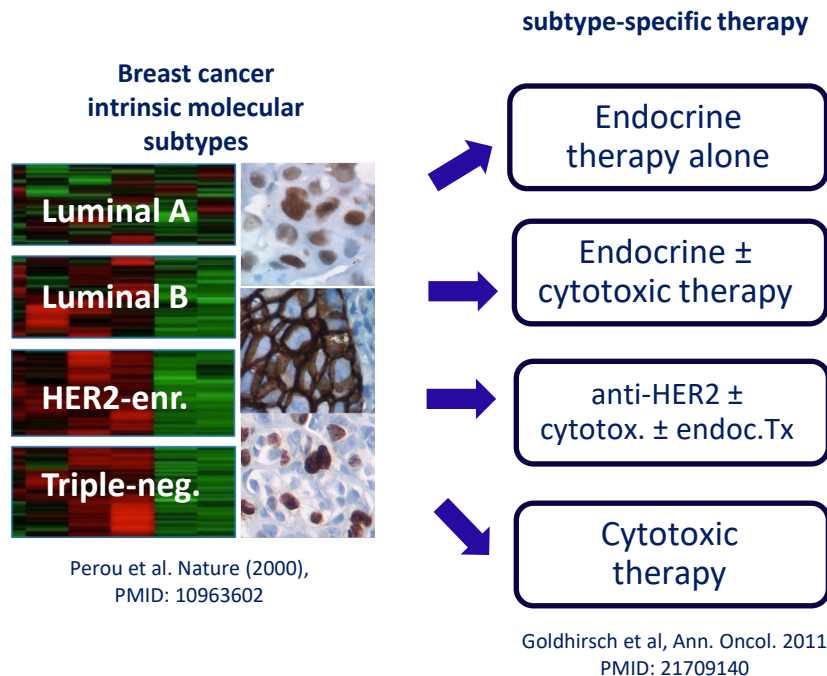
Intrinsische Subtypen – seit 2000

Breast cancer intrinsic molecular subtypes



Perou et al. Nature (2000),
PMID: 10963602

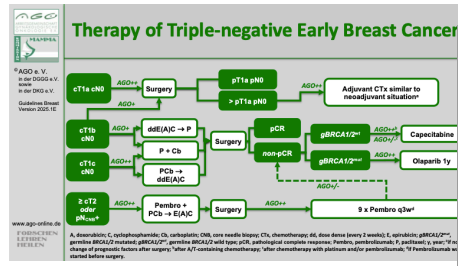
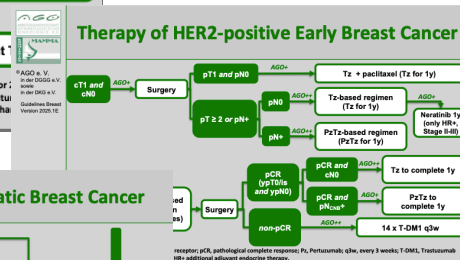
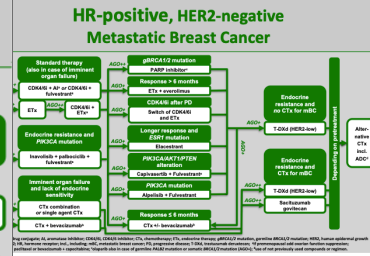
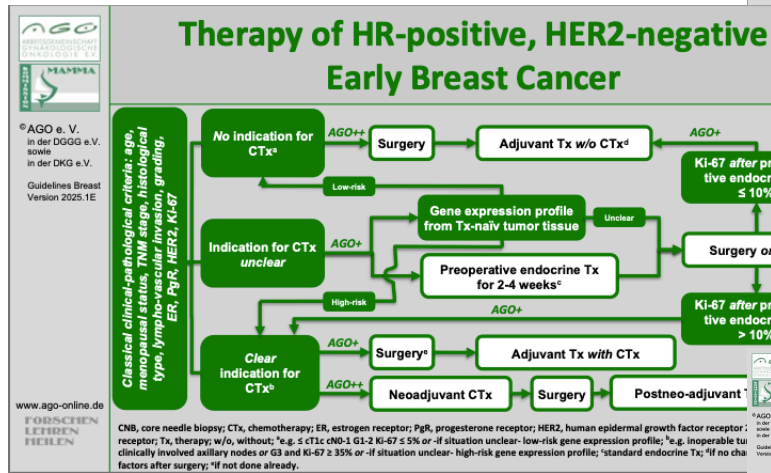
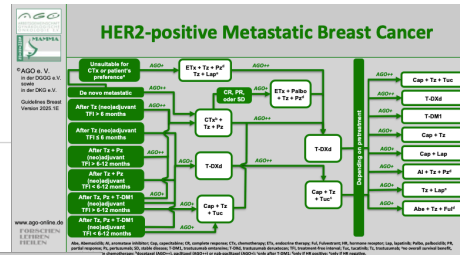
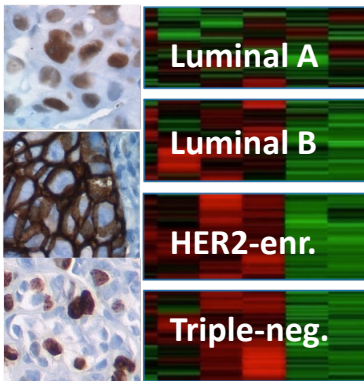
Subtyp-spezifische Therapie (St. Gallen 2011)



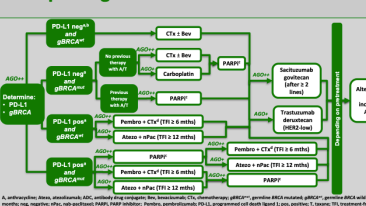
Seit 2021 – immer komplexere Therapiealgorithmen

German Clinical Guideline 2025/26

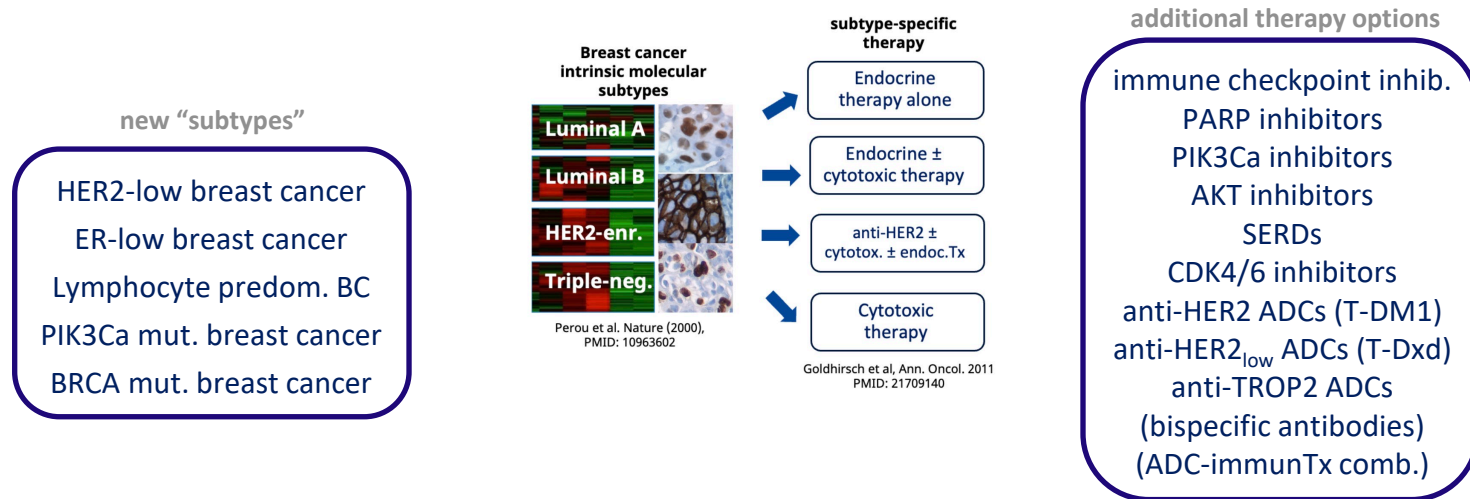
Breast cancer
molecular subtypes



Triple-negative Metastatic Breast Cancer



Increased diversity: complex therapy + new „subtypes“

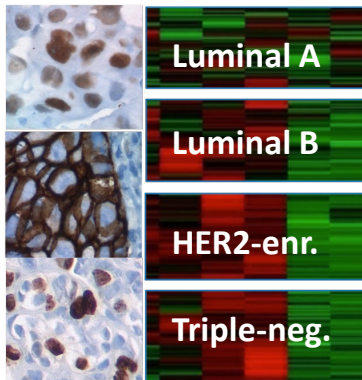


Denkert et al, Lancet Oncol. 2021; Denkert et al. Lancet Oncol. 2018;
Villegas et al. Eur. J. Cancer 2021; Jank et al., Clin. Canc. Res, 2024

Loibl, et al. Lancet 2021;
Loibl, et al. Ann Oncol. 2024
Loibl et al, NEJM 2025

TNBC – molekulare Subtypen, z.B. Burstein Subtypes

Breast cancer
molecular subtypes



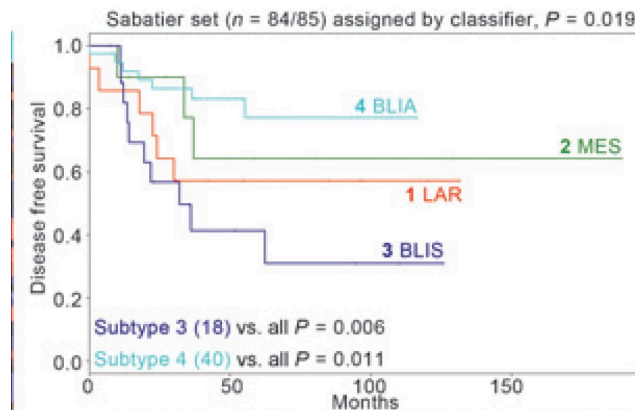
Biology of Human Tumors

Clinical
Cancer
Research

Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer

Clin. Cancer Res, 2015

Matthew D. Burstein¹, Anna Tsimelzon², Graham M. Poage³, Kyle R. Covington², Alejandro Contreras^{2,4}, Suzanne A.W. Fuqua², Michelle I Savage³, C. Kent Osborne², Susan G. Hilsenbeck², Jenny C. Chang⁵, Gordon B. Mills⁶, Ching C. Lau⁷, and Powel H. Brown³



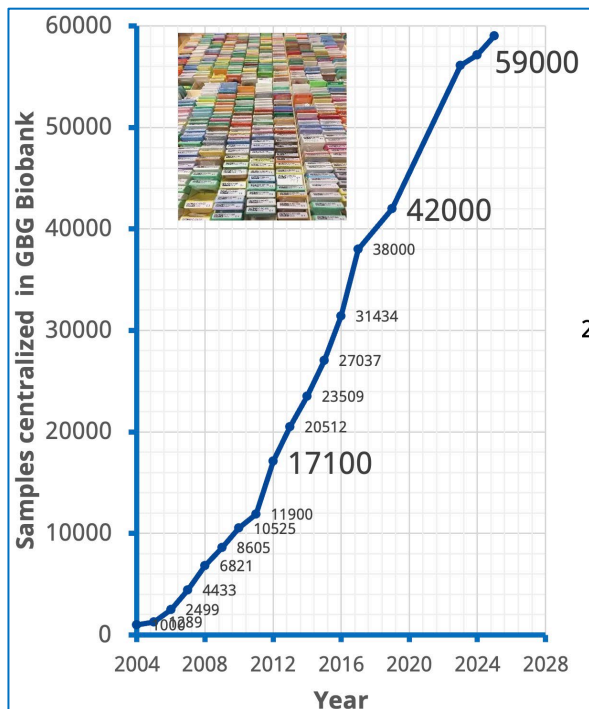
BLIA = immun-aktiviert

BLIS = immun-supprimiert

GBG 2025/26 – Update: Subtypisierung des Mammakarzinoms

- HRpos/HER2neg Mammakarzinom - Adaptive Subtypen
- TNBC – Kombination von TILs und Immun-Subtypen
- Residualtumor – Kombination von Proliferationsmarkern und TILs

GBG Biobank – Grundlage der translationalen Forschung



44 clinical trials

GeparDo	GAIN-2
GeparDuo	GeparSepto
GeparTrio	DAFNE
TBP	NeoPheobe
React	Genevieve
BCP	InSeMa
ICE	Evelyn
GAIN	Katherine
IBIS-II	Penelope
GeparQuattro	BMBC
Radar	BrightNess
NNBC3	GeparOcto
TABEA	DESIREE
GeparQuinto	PALLAS
Sofia	GeparX
LEA	GeparNuevo
ICE II	GeparOLA
Panther	PADMA
MALE	GeparDouze
E-Vita	AMICA
TIFFANY	SASCIA
GeparSixto	GeparPiPPa

- >59.000 FFPE samples from
- >30.000 patients in 44 clinical trials
- therapy-response data + survival data
- all samples centralized at UMR; 3.500 RNA datasets;
- clinical trial network of GBG – ongoing new trials

immune checkpoint inhibitors

PARP inhibitors

PIK3Ca inhibitors

CDK4/6 inhibitors

anti-HER2 ADCs (T-DM1)

adjuvant CTX

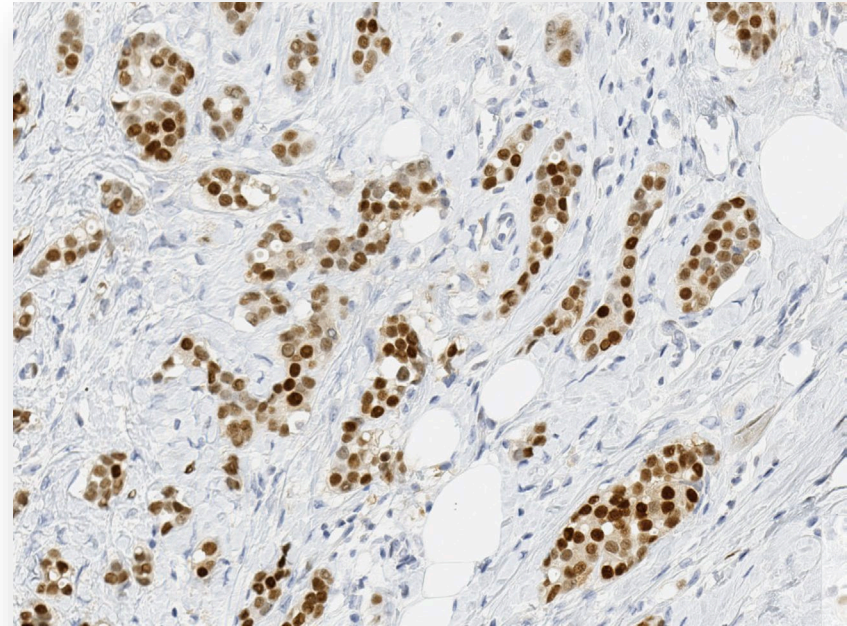
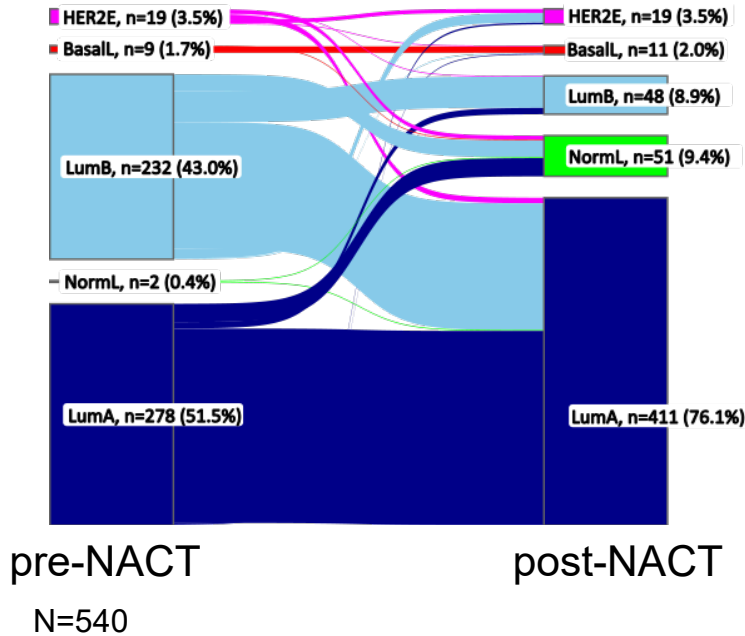
neoadjuvant CTX

anti-TROP2 ADCs

Forschungsanträge:
Trafo-Board der GBG

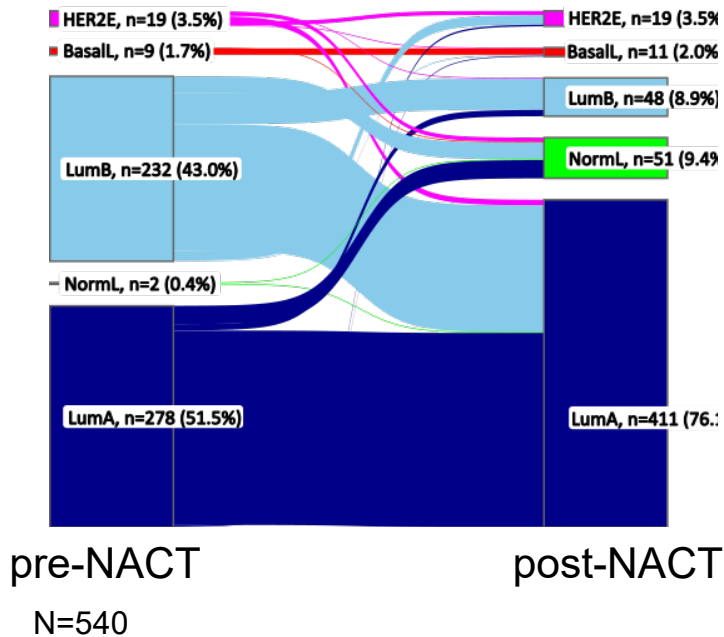
Changes of breast cancer subtypes during therapy

During chemotherapy
“lumB to lumA”

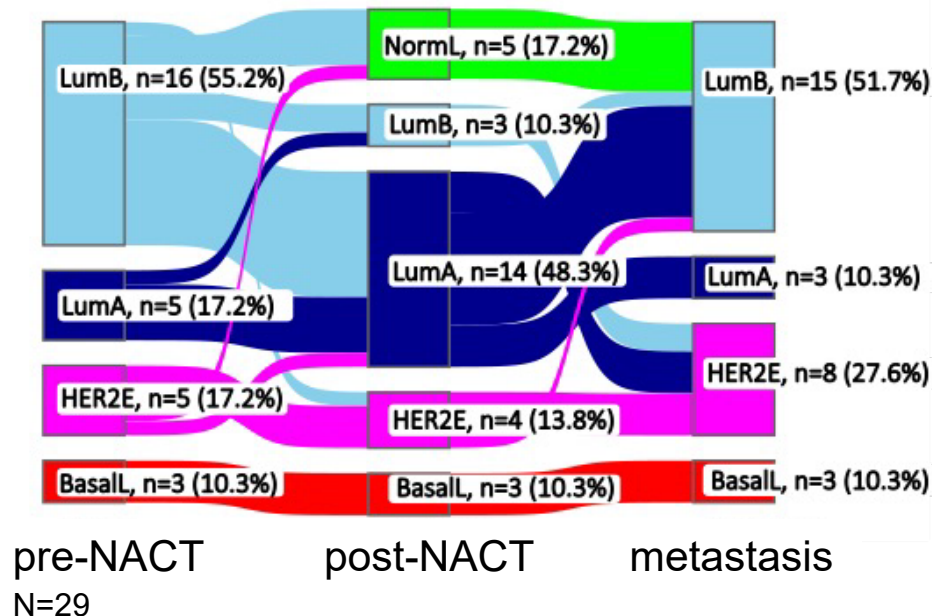


Changes of breast cancer subtypes during therapy

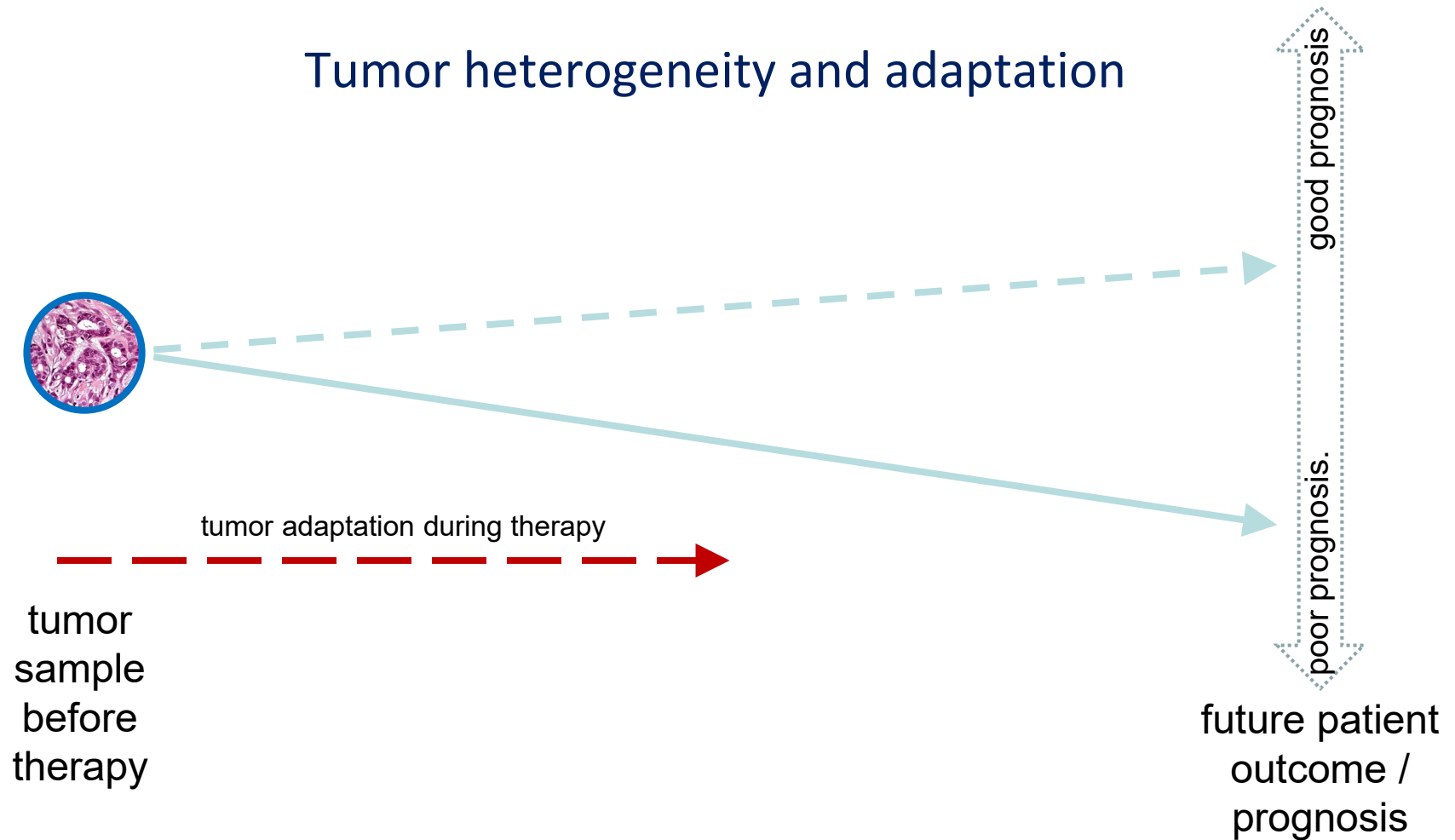
During chemotherapy
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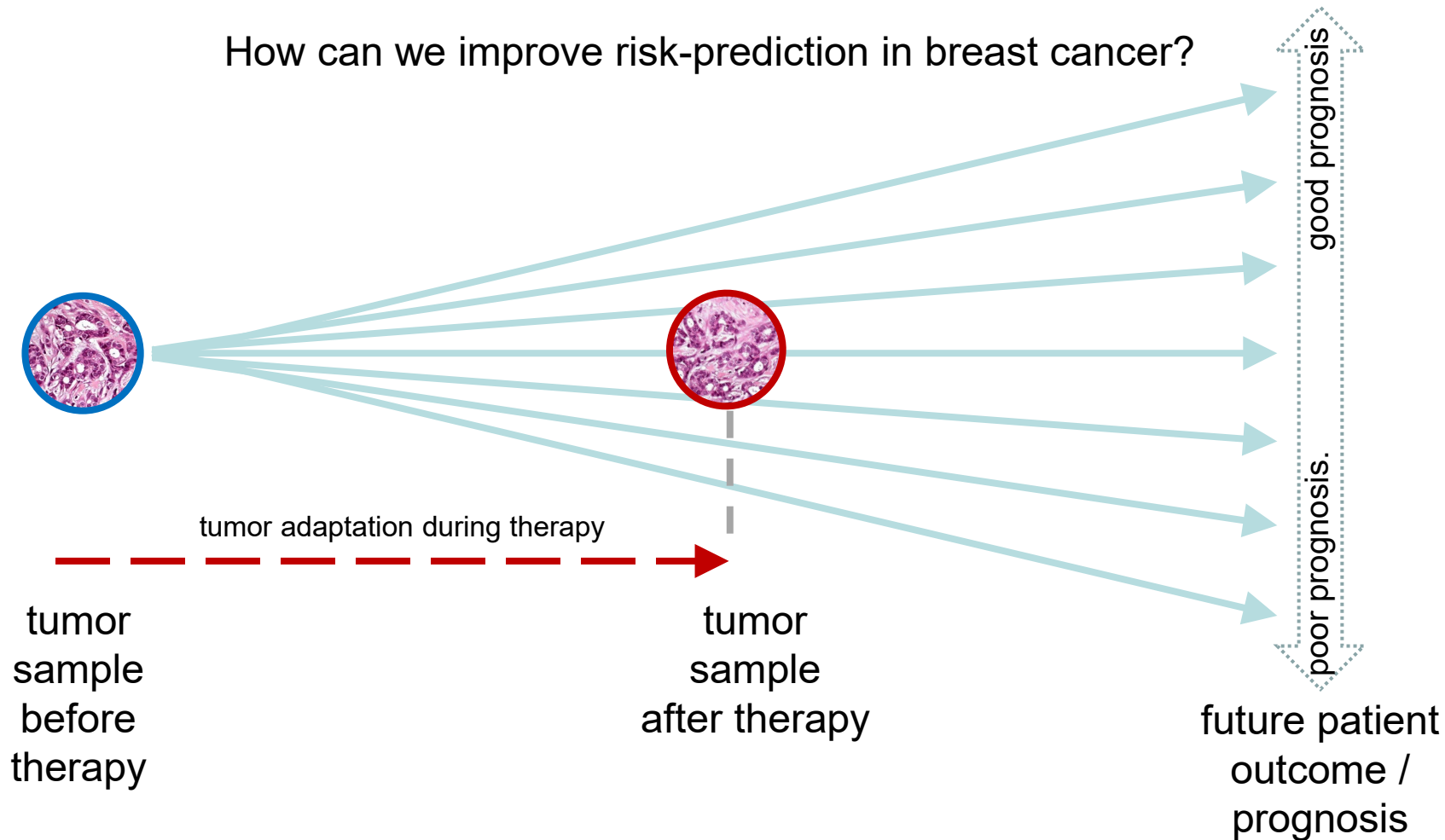
In metastatic disease
“back to lumB”



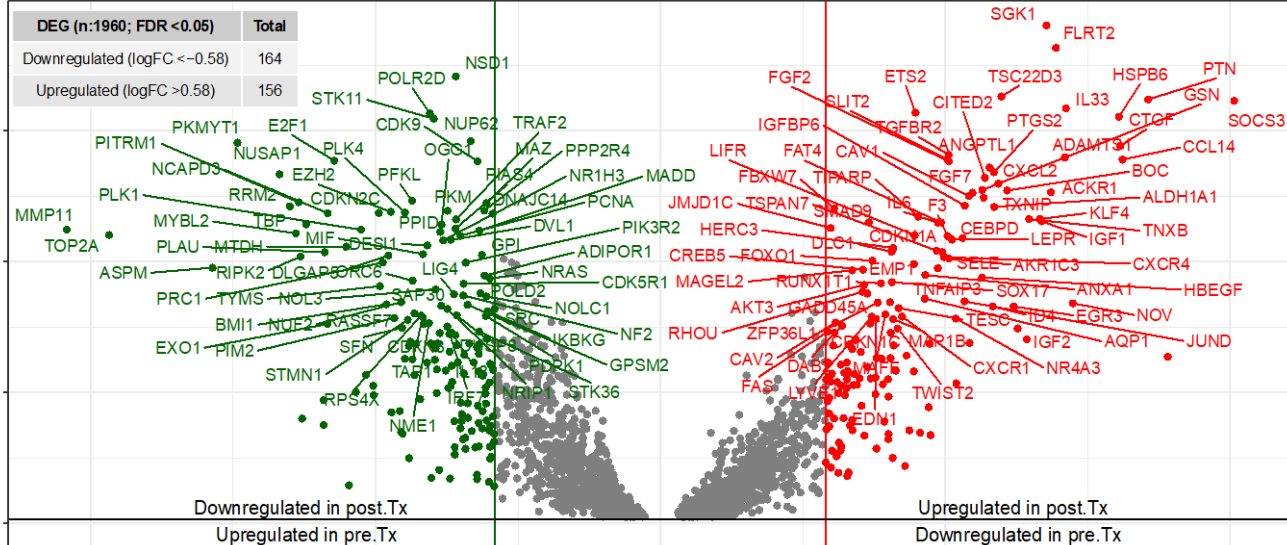
Tumor heterogeneity and adaptation

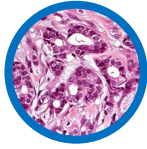


How can we improve risk-prediction in breast cancer?

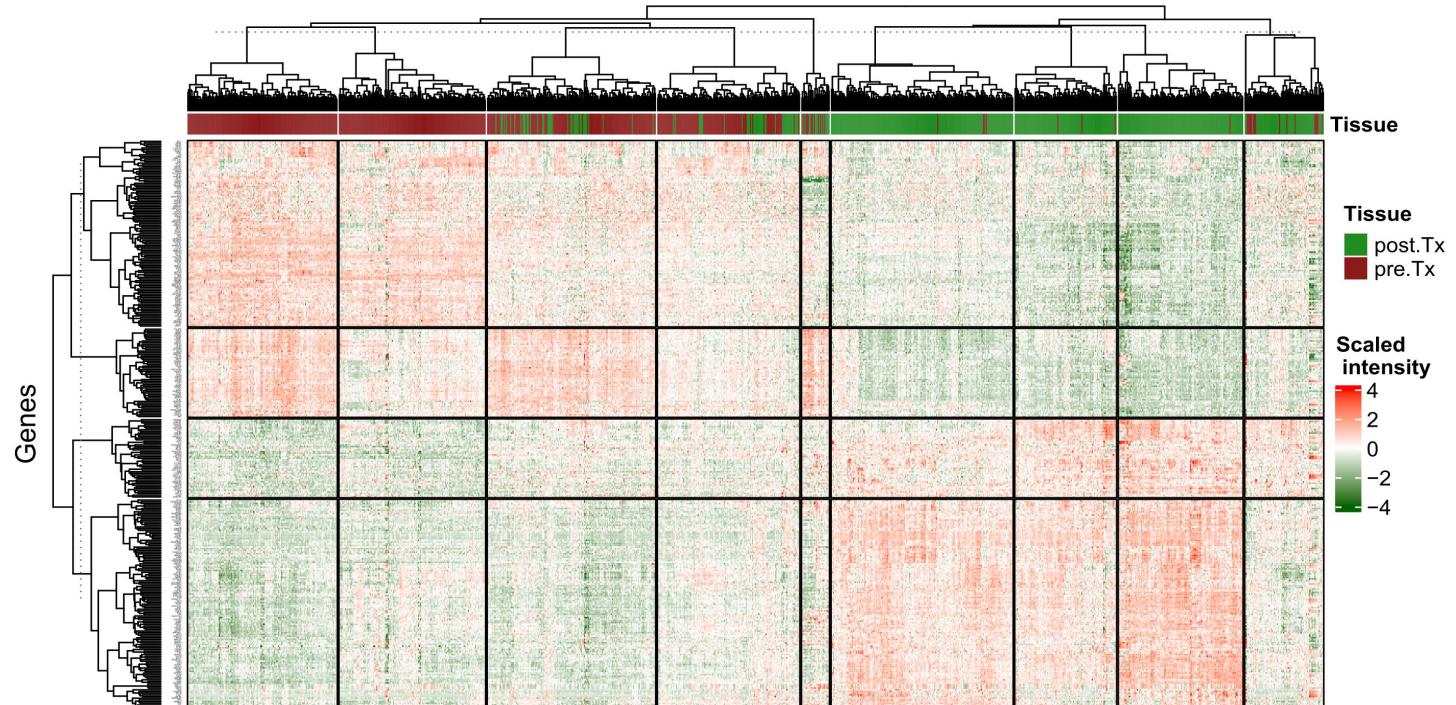
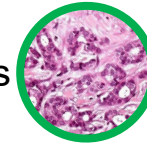


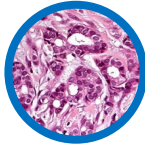
335 DEGs



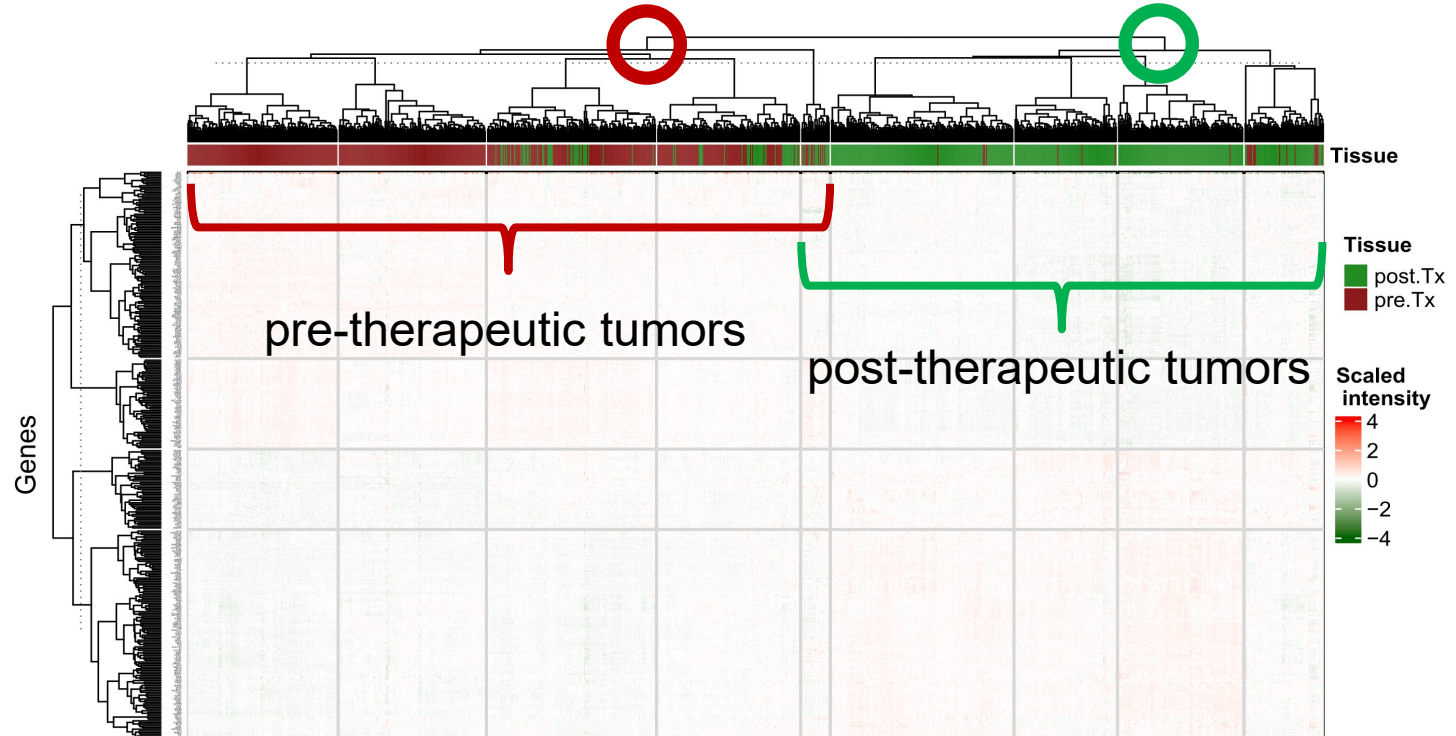
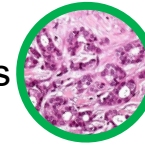


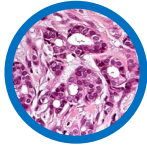
clustering based on 335 differentially expressed genes



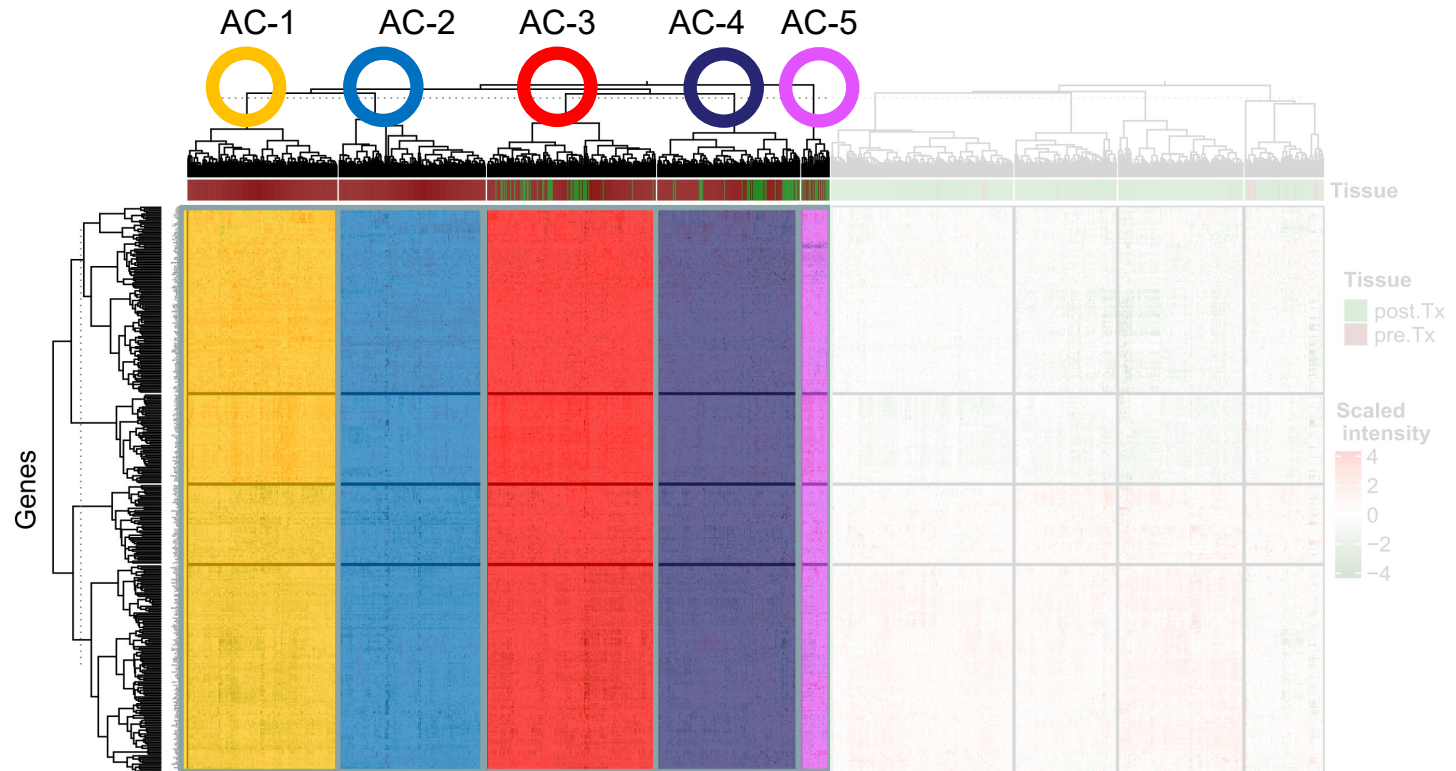


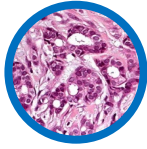
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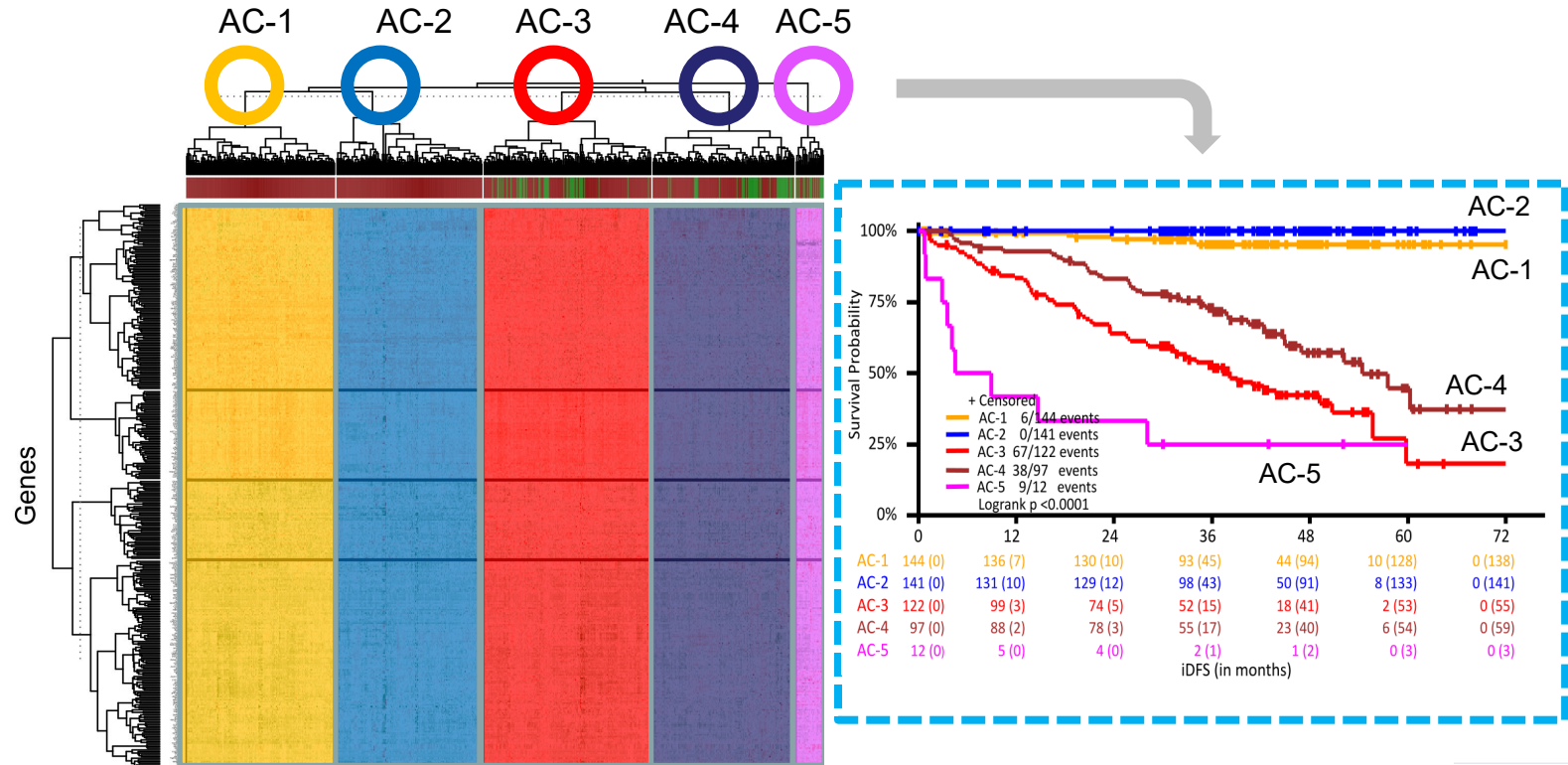


pre-therapeutic tumors: five adaptive clusters AC-1 to AC-5



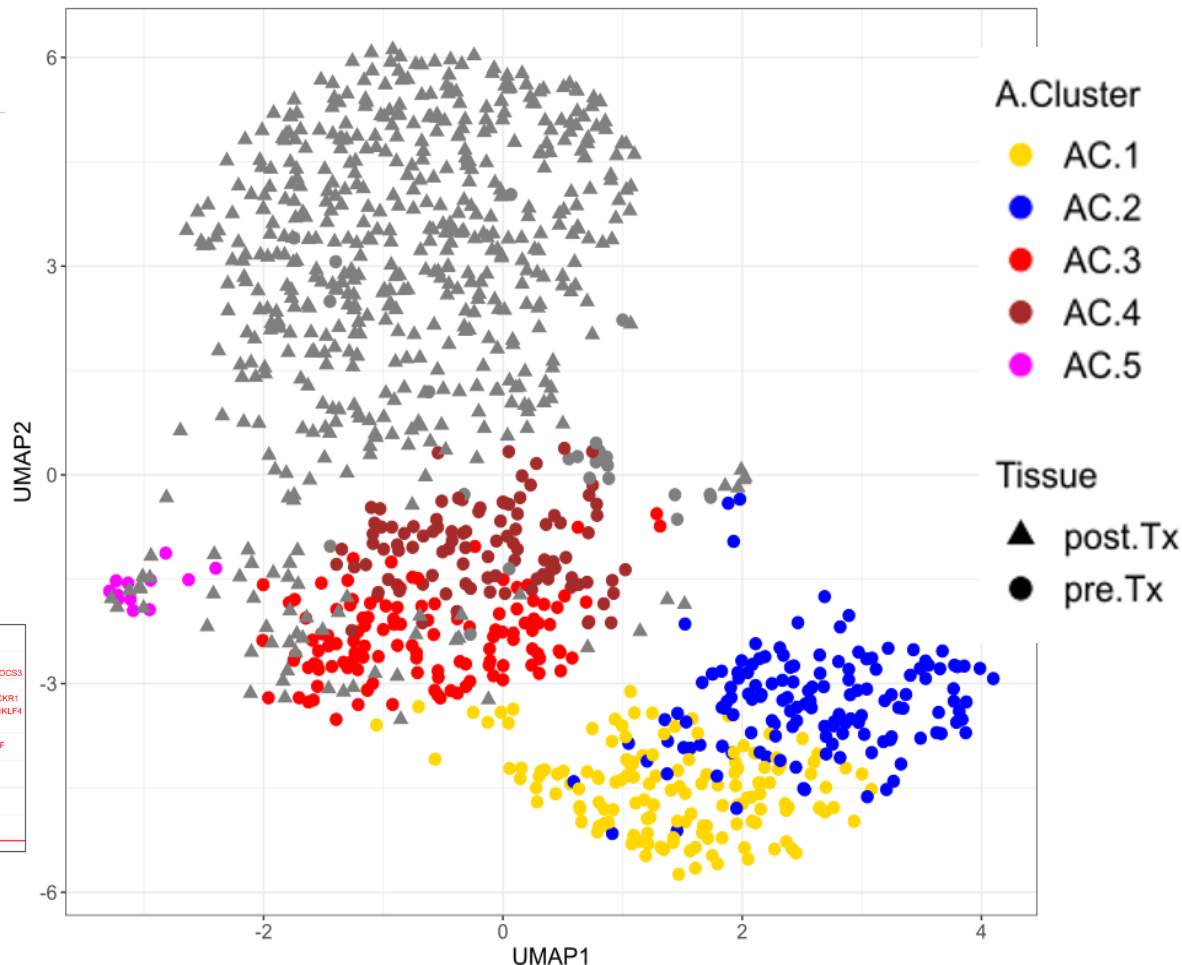
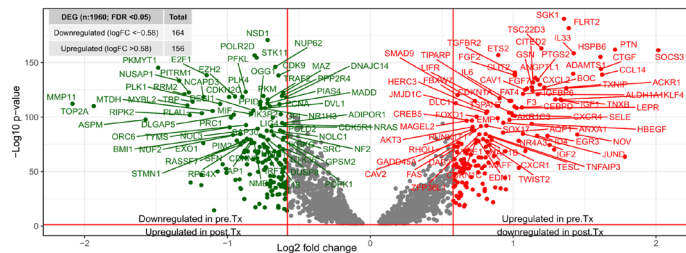


pre-therapeutic tumors: prognostic relevance of AC-clusters

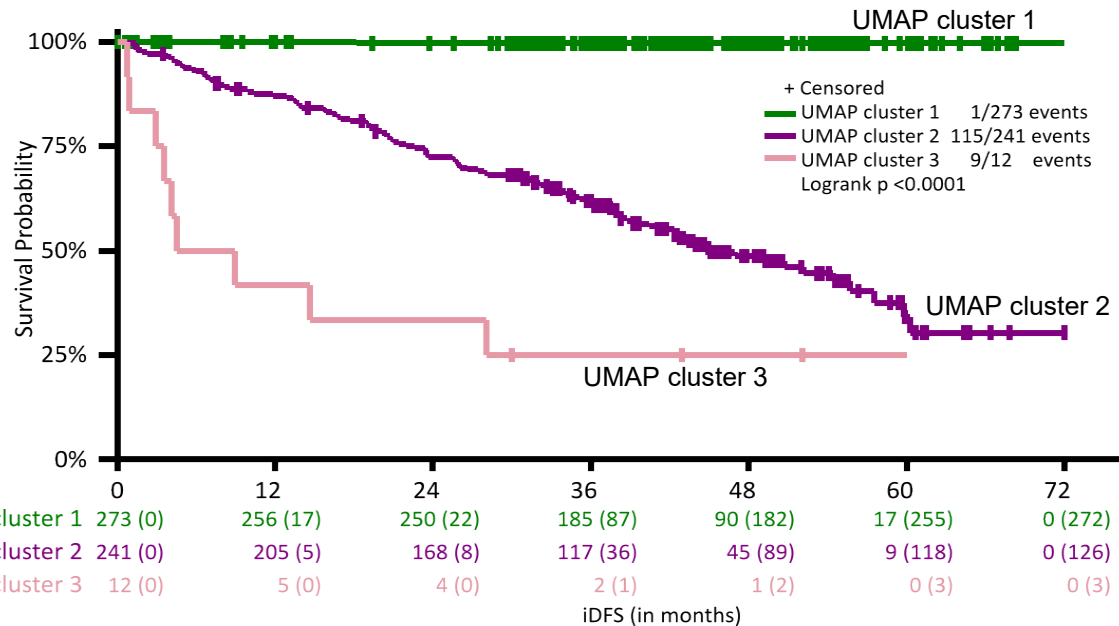
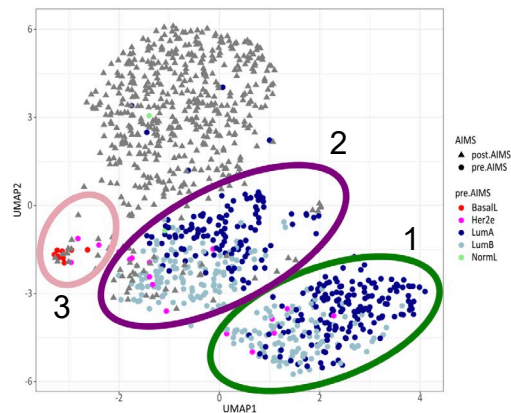


Similarity of UMAP and AC clusters

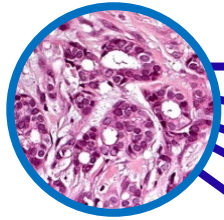
335 genes
1080 tumors (540 pairs)



UMAP clusters are prognostic in Penelope – similar to AC clusters



Adaptive Subtypen beim Mammakarzinom



AC-1

AC-2

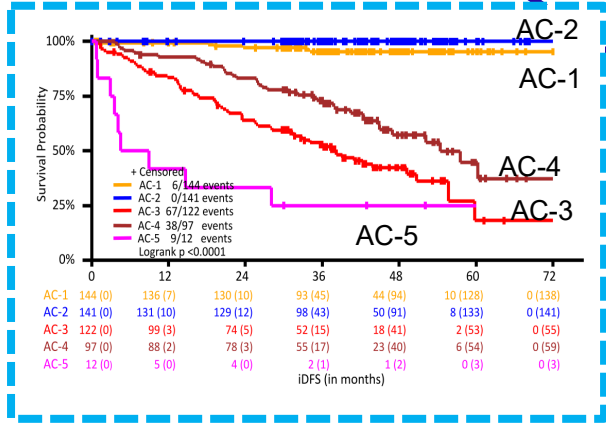
AC-4

AC-3

AC-5

good prognosis
poor prognosis

1. Tumoren verändern sich unter der Therapie
2. Wenn wir diese Veränderungen kennen, können wir die Prognose besser vorhersagen.
3. AC-subtypes als neue Option für eine prognostische Klassifikation beim luminalen Mammakarzinom.

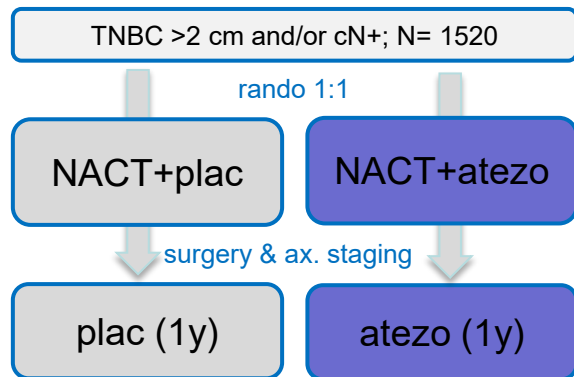


GBG 2025/26 – Update der Subtypisierung des Mammakarzinoms

- HRpos/HER2neg Mammakarzinom - Adaptive Subtypen
- GeparDouze Immuntherapie – Kombination von TILs und Immun-Subtypen
- Residualtumor – Kombination von Proliferationsmarkern und TILs

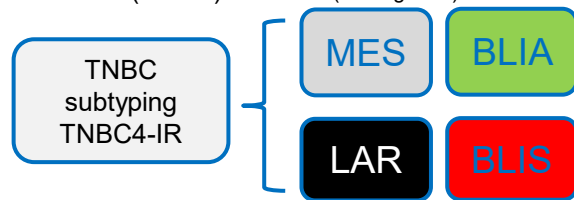
GeparDouze translational research

clinical trial



Gene expression analysis

(n=482) HTG OBP (2549 genes)



TILs in complete trial cohort (n=1543)

- 3 stromal TIL groups: low <10%, intermed. 10-29%, high ≥30%
- pCR, EFS, atezolizumab response

TNBC subtypes for immunotherapy response (TNBC4-IR, n=482)

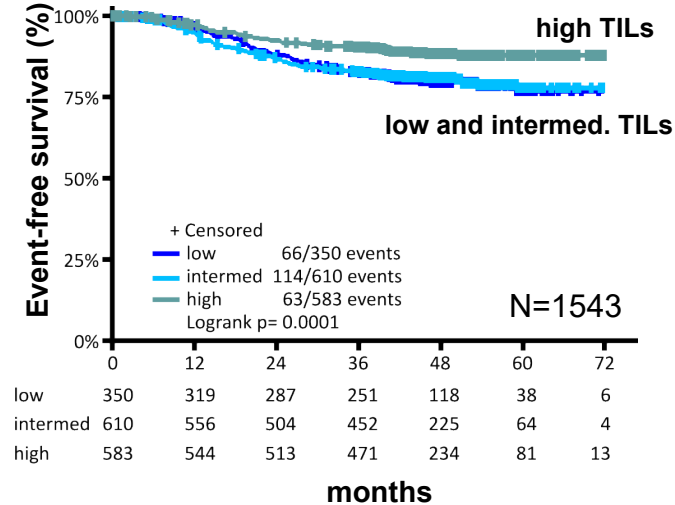
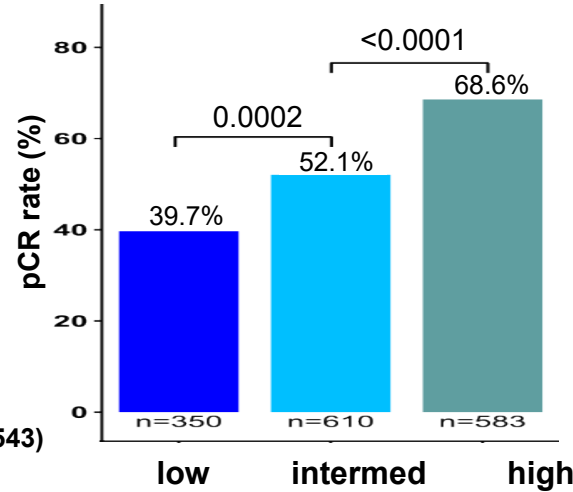
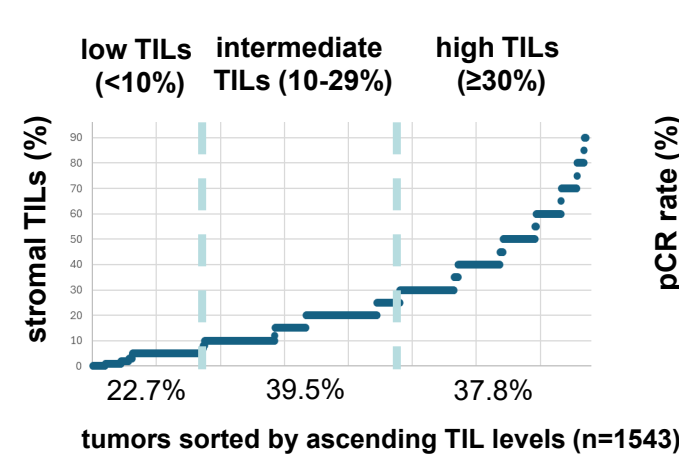
- **BLIA**: basal-like immune-activated,
- **BLIS**: basal-like immunosuppressed,
- **LAR**: luminal androgen receptor,
- **MES**: mesenchymal
- modified subtyping (Burstein et al.¹, Bissanum et al.²)

TNBC6: BL1, BL2, IM, MES, M, LAR³ as well as gene expression analysis: will be reported separately

1. Burstein et al. Clin Cancer Res. 2015, PMID: 25208879;
2. Bissanum et al. J Pers Med. 2021 PMID: 34575658;
3. Lehmann et al, J Clin Invest. 2011, PMID: 21633166.

TILs: increased pCR and improved EFS

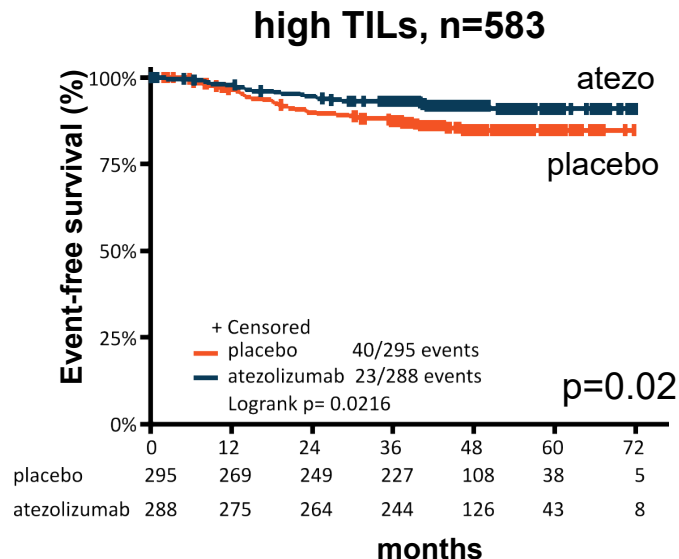
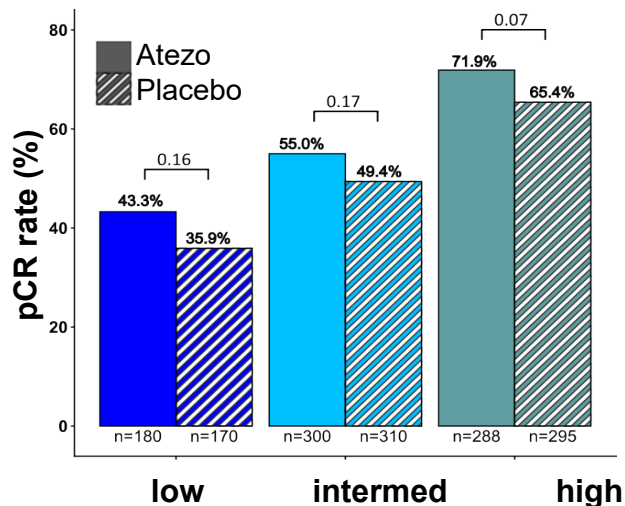
(complete cohort, n=1543)*



- increased TILs: improved pCR rate (p=0.0002 and p<0.0001)

- high TILs: improved EFS (p=0.0001), no difference for low and intermediate TILs

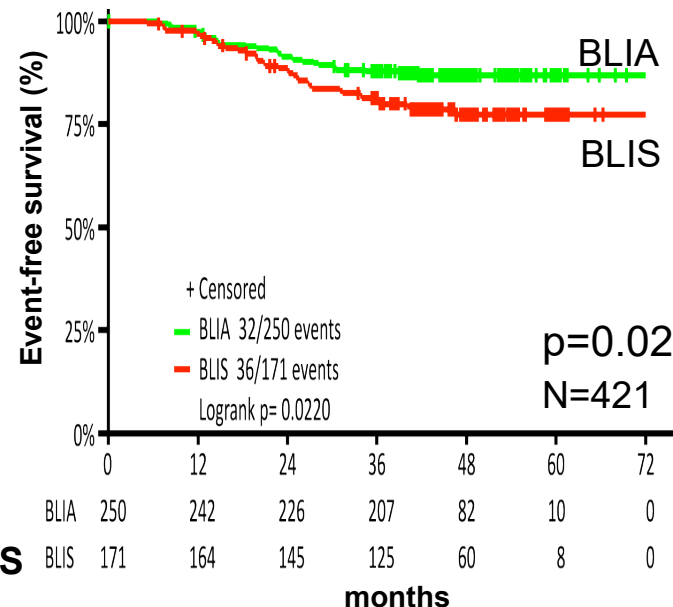
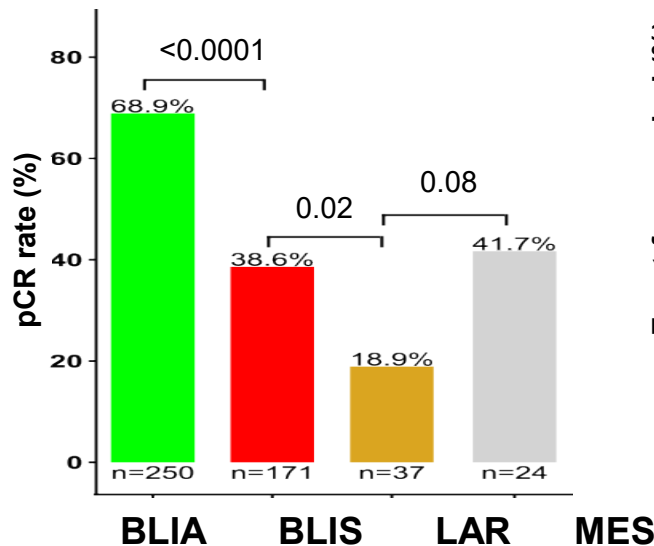
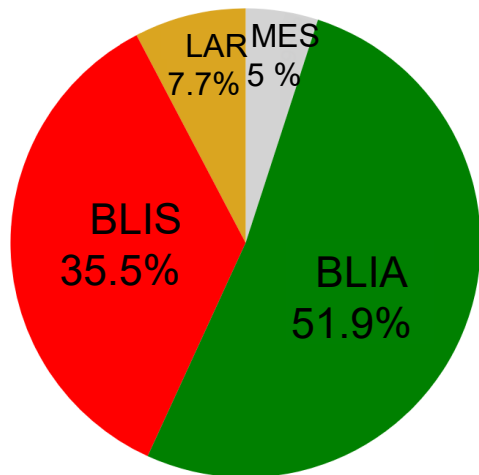
TILs and atezolizumab treatment



- atezolizumab: numerically increased pCR rate in all 3 TIL groups

- improved EFS with atezolizumab in pts with high TILs ($\geq 30\%$, $p=0.02$)
- no significant difference for low & intermediate TILs

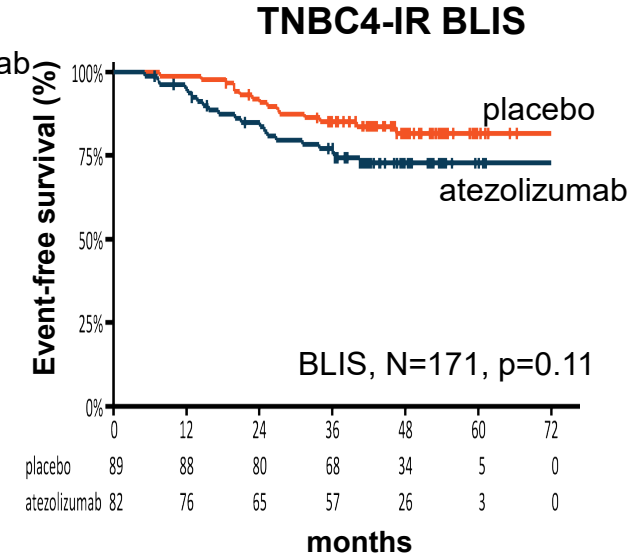
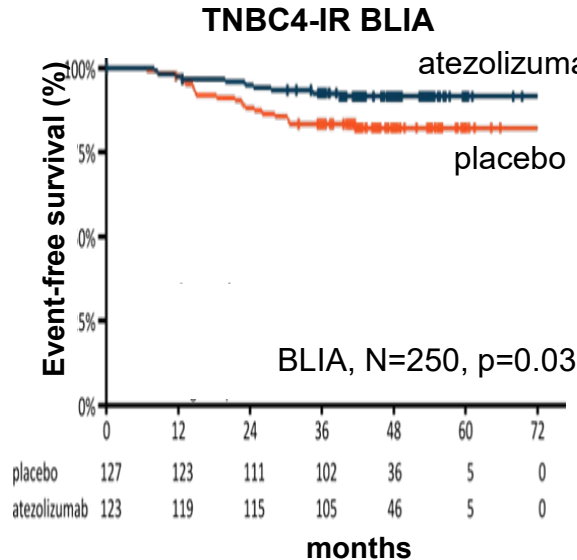
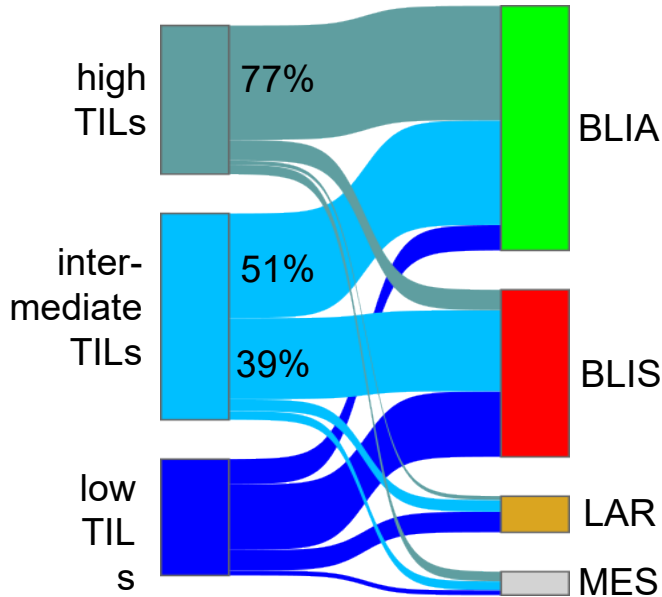
BLIA and BLIS as main subtypes



- Highly significant differences in pCR rate for TNBC4-IR, complete cohort
- improved EFS for BLIA compared to BLIS (p=0.02), complete cohort

Comparison of TILs and TNBC-subtypes

heterogeneity of intermediate TILs



- intermed. TILs: heterogeneous mixture of BLIA and BLIS

- improved EFS with atezolizumab only in BLIA tumors

GeparDouze translationale Forschung - Ergebnisse

- Positive Wirkung von Atezolizumab auf EFS bei immunaktivierten Tumoren
 - definiert als entweder TILs $\geq 30\%$ oder BLIA-Subtyp
- Tumoren mit intermediären TILs sind heterogen (gemischt BLIA & BLIS)
 - Kombination aus Immunaktivierung und -erschöpfung
- Ziel: TNBC-Subtypisierung als Standard etablieren

- Neue Optionen für Immuntherapie-Biomarker:
 - Identifizierung von Tumoren mit hohen TILs: Die meisten davon sind BLIA-Tumoren
 - Bewertung des BLIA-Subtyps bei Tumoren mit intermediären TILs

Prognostic Markers in Residual Tumors after Neoadjuvant Chemotherapy (NACT) for Early Triple-Negative Breast Cancer (TNBC) – a Pooled Analysis from nine Neoadjuvant GBG/AGO-B Trials

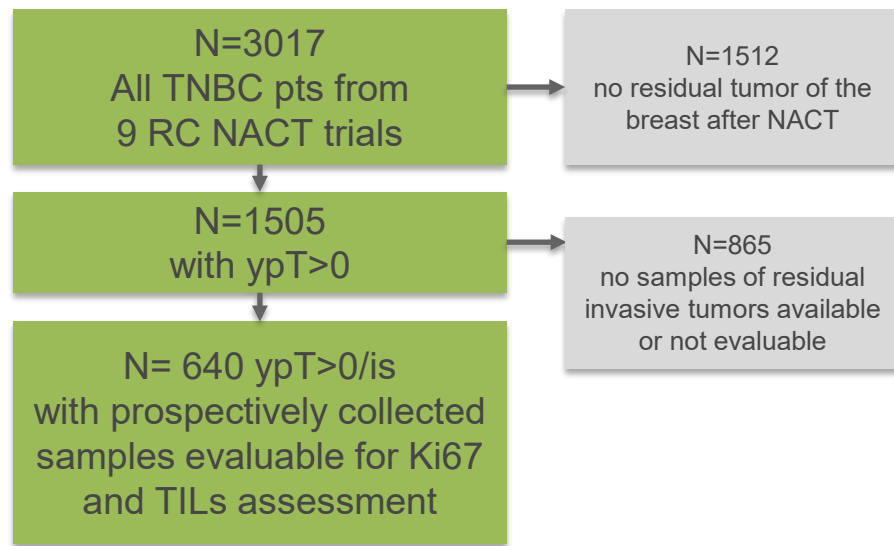
Johannes Holtschmidt¹, Andreas Schneeweiss², Nadine Frickel³, Natalie Filmann¹, Michael Untch⁴, Andreas Hartkopf⁵, Volkmar Müller⁶, Christine Solbach⁷, Marion van Mackelenbergh⁸, Jens-Uwe Blohmer⁹, Kristina Lübke¹⁰, Thomas Karn¹¹, Frank Holms¹², Peter A. Fasching¹³, Paul Jank³, Maren Darsow¹⁴, Frederik Marmé¹⁵, Michael Braun¹⁶, Elmar Stickler¹⁷, Rudolf Weide¹⁸, Tanja Fehm¹⁹, Christian Schem²⁰, Annika Eidmann³, Bärbel Felder¹, Sibylle Loibl^{1,21}, Carsten Denkert^{3,22}

1 GBG Forschungs GmbH, Neu-Isenburg, Germany; 2 Division of Gynaecological Oncology, National Center for Tumor Diseases, University Hospital and German Cancer Research Center, Heidelberg, Germany; 3 Institute of Pathology, Philipps-University Marburg, Marburg, Germany; 4 Department of Obstetrics and Gynecology, Interdisciplinary Breast Cancer Center, Medical School Berlin, Helios Kliniken Berlin-Buch, Berlin, Germany; 5 Department of Women's Health, University Hospital Tübingen, Tübingen, Germany; 6 Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 7 Breast Unit, University Medical Center, Goethe-University Frankfurt, Frankfurt am Main, Germany; 8 Department of Gynecology and Obstetrics, University Hospital of Schleswig-Holstein, Campus Kiel, Kiel, Germany; 9 Department of Gynecology with Breast Center, Charité - Universitätsmedizin Berlin, Berlin, Germany; 10 Breast center, Diakovere Henriettenstift, Breast Center, Hannover, Germany; 11 Department of Gynecology and Obstetrics, University of Frankfurt, UCT Frankfurt-Marburg, Frankfurt am Main, Germany; 12 Department of Obstetrics and Gynecology, St. Barbara-Klinik Hamm-Hessen GmbH, Germany; 13 Department of Gynecology and Obstetrics, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany; 14 Competence Centre for Breast Surgery and Breast Health, Luisenkrankenhaus Düsseldorf, Düsseldorf, Germany; 15 University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany; 16 Breast Cancer Center, Department of Gynecology, Red Cross Hospital, Munich, Germany; 17 Breast Center, Department of Gynecology and Obstetrics, RWTH University Hospital Aachen, Aachen, Germany; 18 Department of Hematology and Oncology, Praxis für Haematologie und Onkologie, Koblenz, Germany; 19 Department of Gynecology and Obstetrics, University Hospital Düsseldorf, Düsseldorf, Germany; 20 Mammazentrum Hamburg, am Krankenhaus Jerusalem, Hamburg, Germany; 21 Goethe University Frankfurt, Germany; 22 University Hospital Marburg (UKGM), Marburg, Germany

-This is a joint study by GBG and AGO-B-

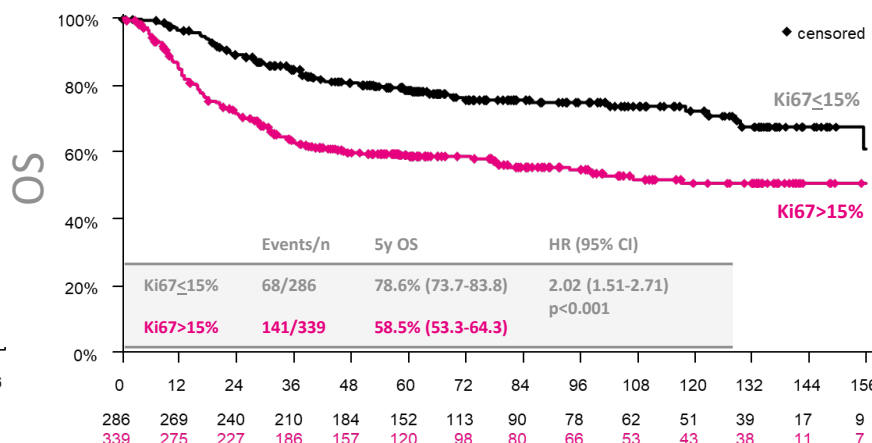
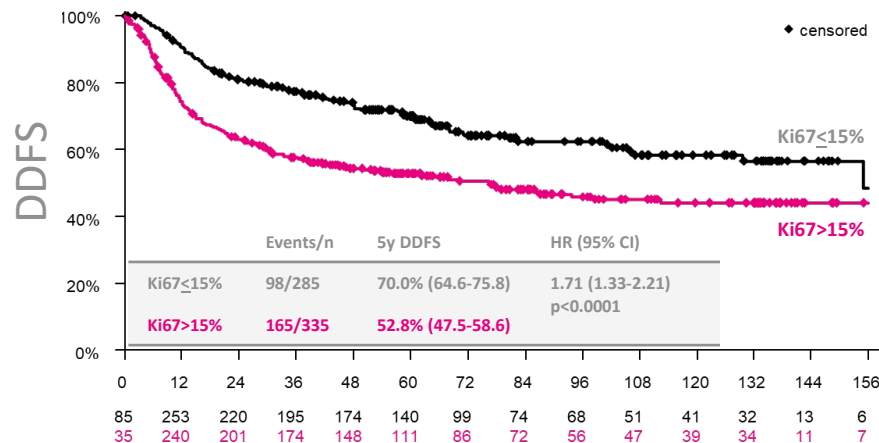
Consort Flow Diagram & Methods

Data from 3017 pts with early TNBC (ER/PR<1%), who were enrolled in nine neoadjuvant randomized controlled (RC) GBG /AGO-B trials (GeparTrio, GeparQuattro, GeparQuinto, GeparSixto, GeparSepto, GeparOcto, GeparNuevo, GeparOla, and GeparX) were included.



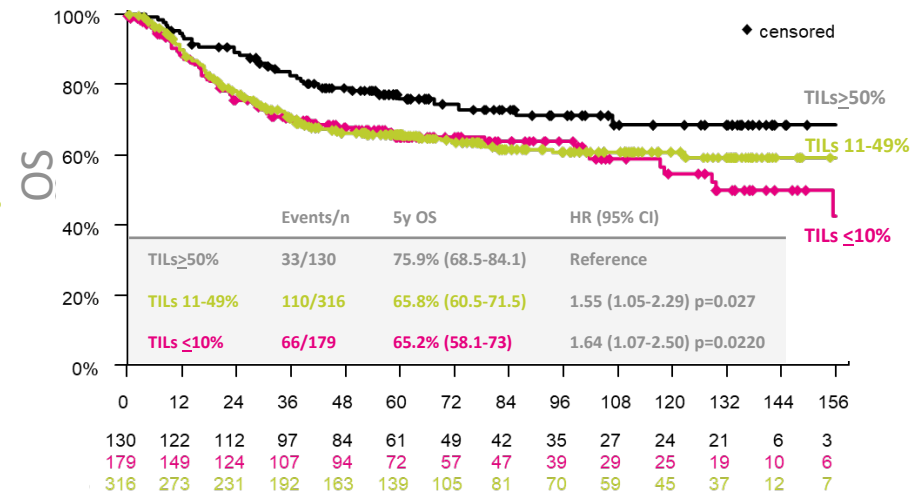
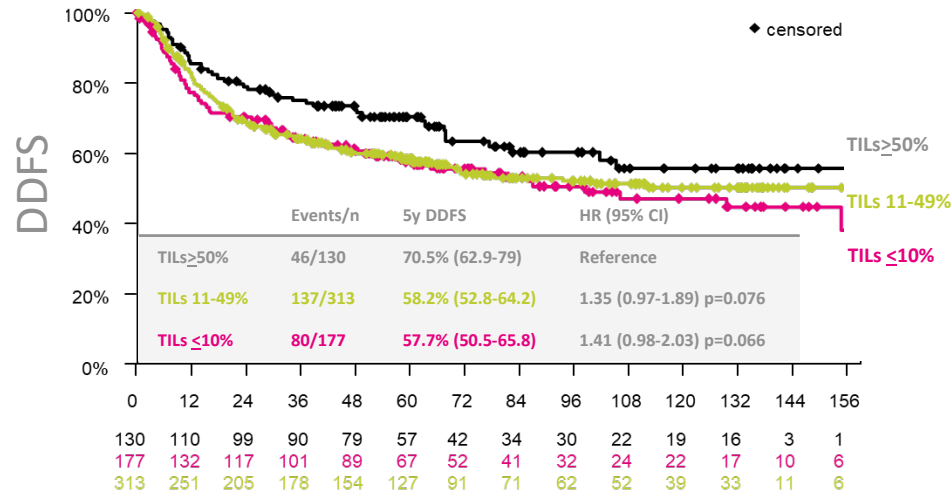
- Central assessment for Ki67 and TILs
- Landmark analyses for survival were performed with the landmark set at the time of surgery (Log-rank stratified by study/Cox PH regression with study as random effect)
 - DDFS (Primary endpoint)
 - OS

Ki67 >15% vs ≤15% in Residual Tumors



- Ki67 >15% vs ≤15% in residual tumors was associated with significantly worse DDFS and OS
- A significant increase in relative risk (HR) for DDFS/OS events by 9.4%/10.7% for each 10% increase in Ki67 in residual tumors was observed

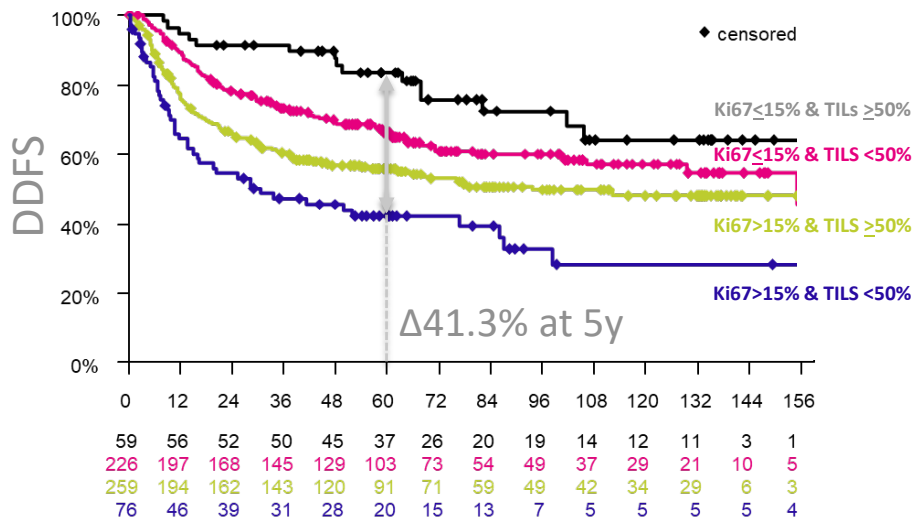
TILs $\geq 50\%$ vs 11%-49% vs $\leq 10\%$ in Residual Tumors



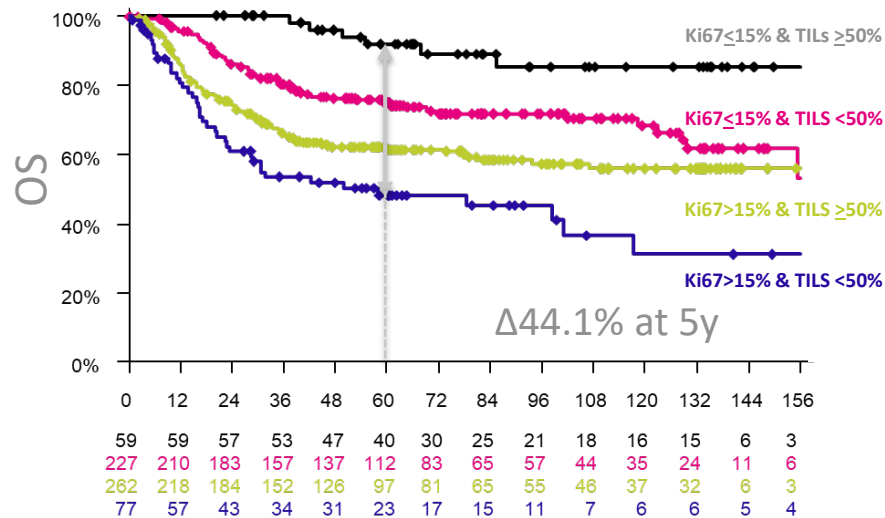
- TILs $\geq 50\%$ in residual tumors were associated with a significantly improved OS and a clear trend towards improved DDFS
- TILs in residual tumors were associated with a significant relative reduction of 6.3%/8.7% in risk (HR) for DDFS/OS events for each 10% increased TILs level at surgery.

Combined Ki67 & TILs in Residual Tumors

	Events/n	5y DDFS	HR (95% CI)
Ki67≤15% & TILs ≥50%	15/59	83.7% (74.5-94.1)	Reference
Ki67≤15% & TILs <50%	83/226	66.3% (60.1-73.1)	1.66 (0.96-2.89) p=0.07
Ki67>15% & TILs ≥50%	120/259	55.8% (49.9-62.4)	2.35 (1.37-4.03) p=0.002
Ki67>15% & TILs <50%	45/76	42.4% (32.2-55.8)	3.62 (2.00-6.52) p<0.0001



	Events/n	5y OS	HR (95% CI)
Ki67≤15% & TILs ≥50%	6/59	92.1% (84.9-99.9)	Reference
Ki67≤15% & TILs <50%	62/227	75.0% (69.3-81.2)	3.12 (1.35-7.21) p=0.008
Ki67>15% & TILs ≥50%	101/262	61.5% (55.6-67.9)	4.77 (2.09-10.91) p=0.0002
Ki67>15% & TILs <50%	40/77	48.0% (37.4-61.7)	7.57 (3.19-17.94) p<0.0001



The next generation of breast cancer subtyping

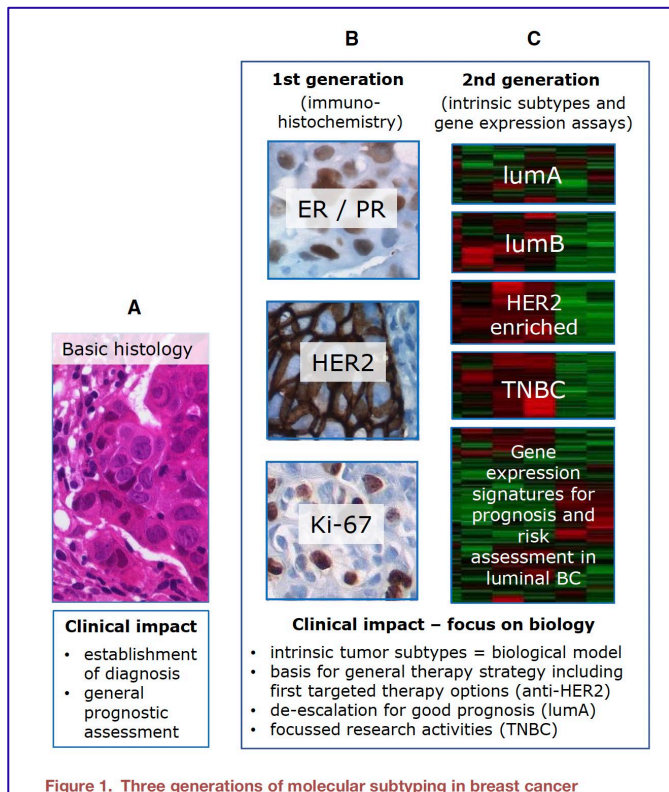


Figure 1. Three generations of molecular subtyping in breast cancer

Denkert, Loibl, Cancer Cell 2022

■ Adaptiv:

- Subtypen verändern sich unter Therapie
- aktuell: Validierung an SASCIA

■ Molekular:

- zusätzliche Genassays zur Subtypisierung beim TNBC
- TNBC4-IR als “GBG Standard” zur Subtypisierung entwickeln

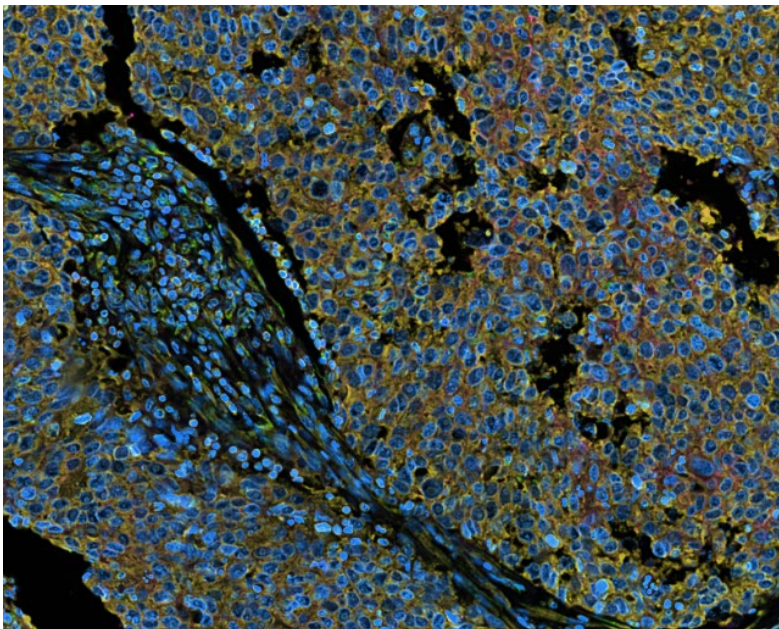
■ Integrativ:

- Kombination von TILs und TNBC-Subtypes
- Kombination von TILs und KI67

■ Räumlich:

- Fokus auf verschiedenen Zellen im Tumorgewebe

The next generation of breast cancer subtyping



10X Xenium GeparDouze

Marcella Carausu, Christos Sotiriou, Thomas Karn

- **Adaptiv:**
 - Subtypen verändern sich unter Therapie
 - aktuell: Validierung an SASCIA
- **Molekular:**
 - zusätzliche Genassays zur Subtypisierung beim TNBC
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- **Integrativ:**
 - Kombination von TILs und TNBC-Subtypes
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- **Räumlich:**
 - Fokus auf einzelne Zellen im Tumorgewebe

= Schwerpunkt für 2026

GBG

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Keyur Mehta
Valentina Nekljudova
Karsten Weber
Krishna Rachakonda
Julia Rey
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Bärbel Felder
Christiane Rothhaar
Stefanie Lettkemann
Sascha Wendt
Katrin Greß

Subboard Trafo of GBG

Selected research partners

Sherene Loi
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Fabrice André
Roberto Salgado
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TIL working group



Vielen Dank an alle Patientinnen, Studienzentren und Pathologen, die Gewebeproben zur Verfügung stellen!
Vielen Dank an alle Kooperationspartner!

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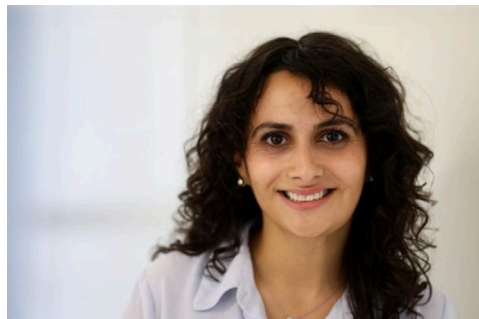
Vielen Dank an alle Patientinnen, Studienzentren und Pathologen, die Gewebeproben zur Verfügung stellen!
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bis 2025: Paul Jank



ab 2026: Sofia Born



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Vielen Dank für die Aufmerksamkeit!

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