

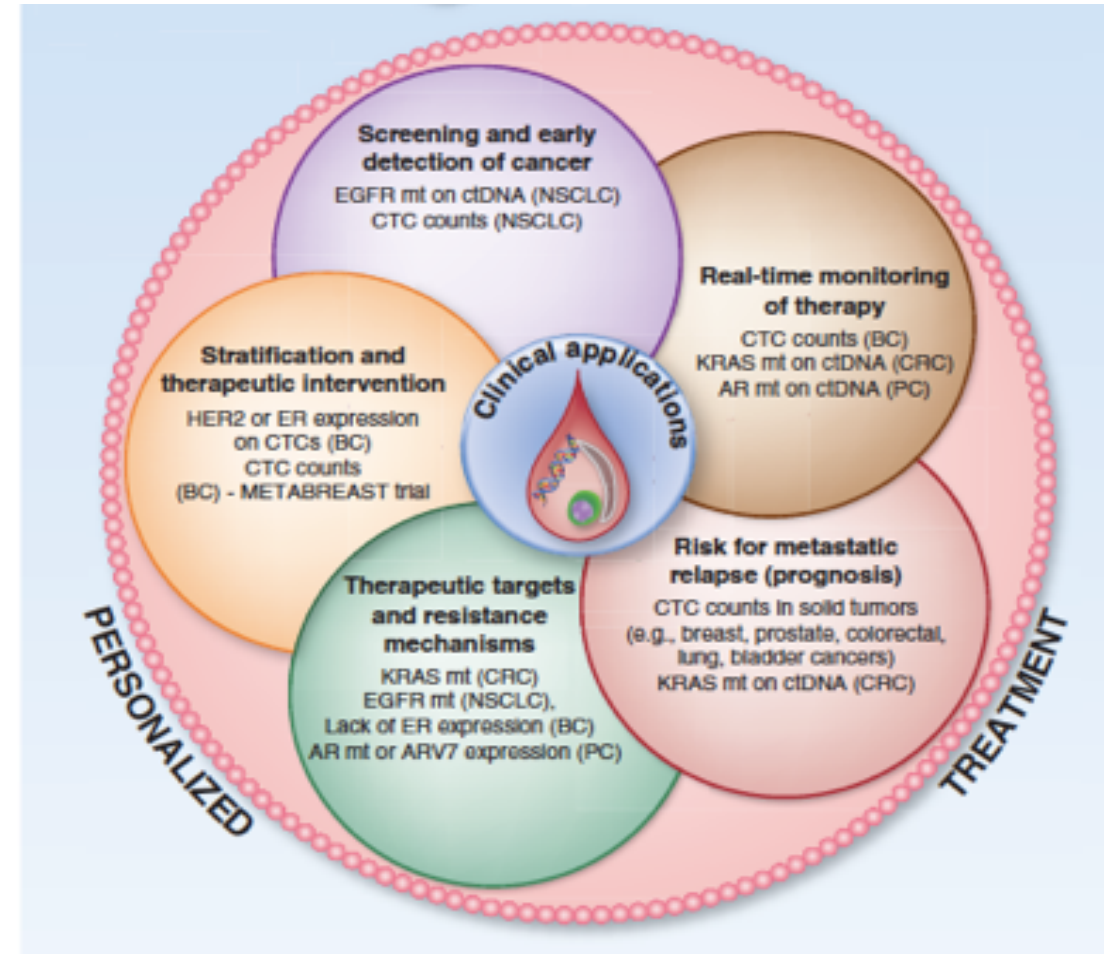
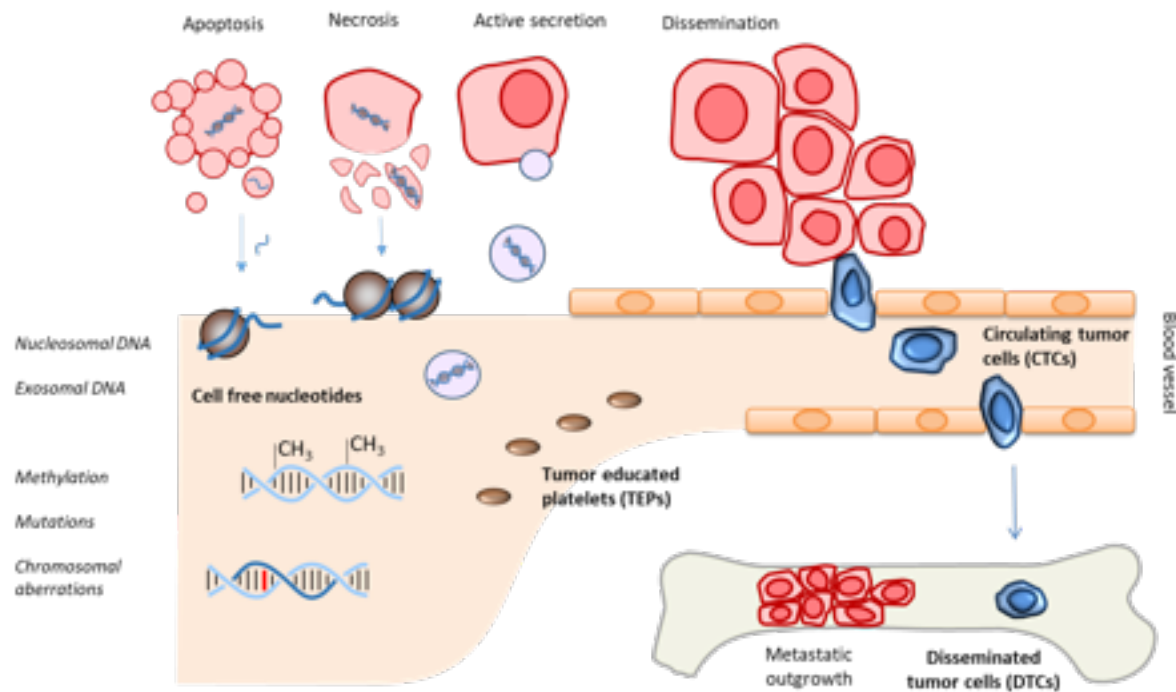
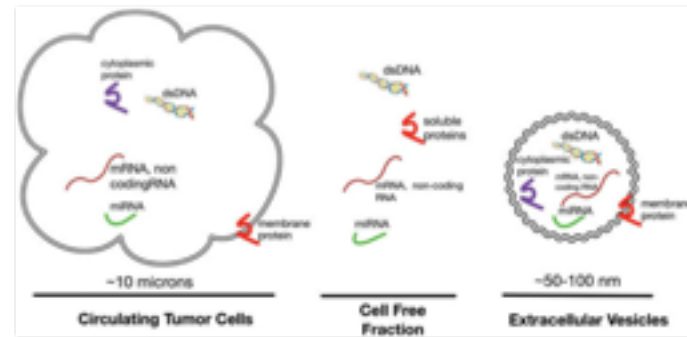
Clinical relevance of liquid biopsies (in brain metastases patients)

Anna Sophie Berghoff, MD, PhD

Department of Medicine I, Clinical Division of Oncology

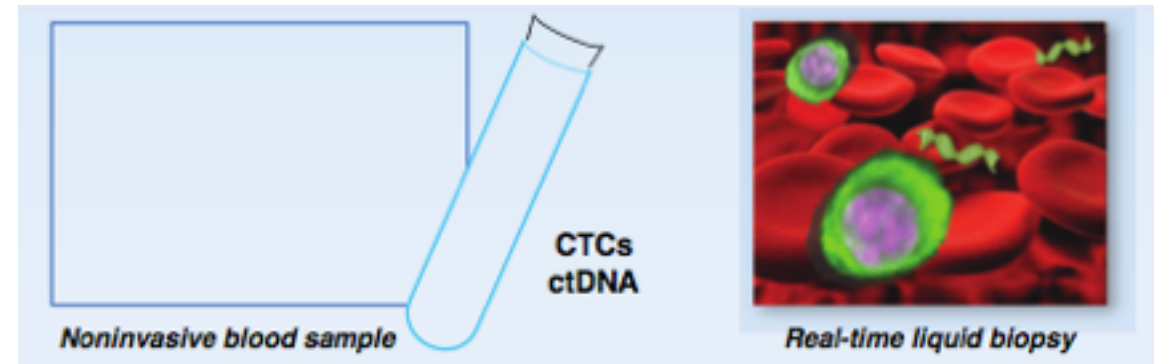
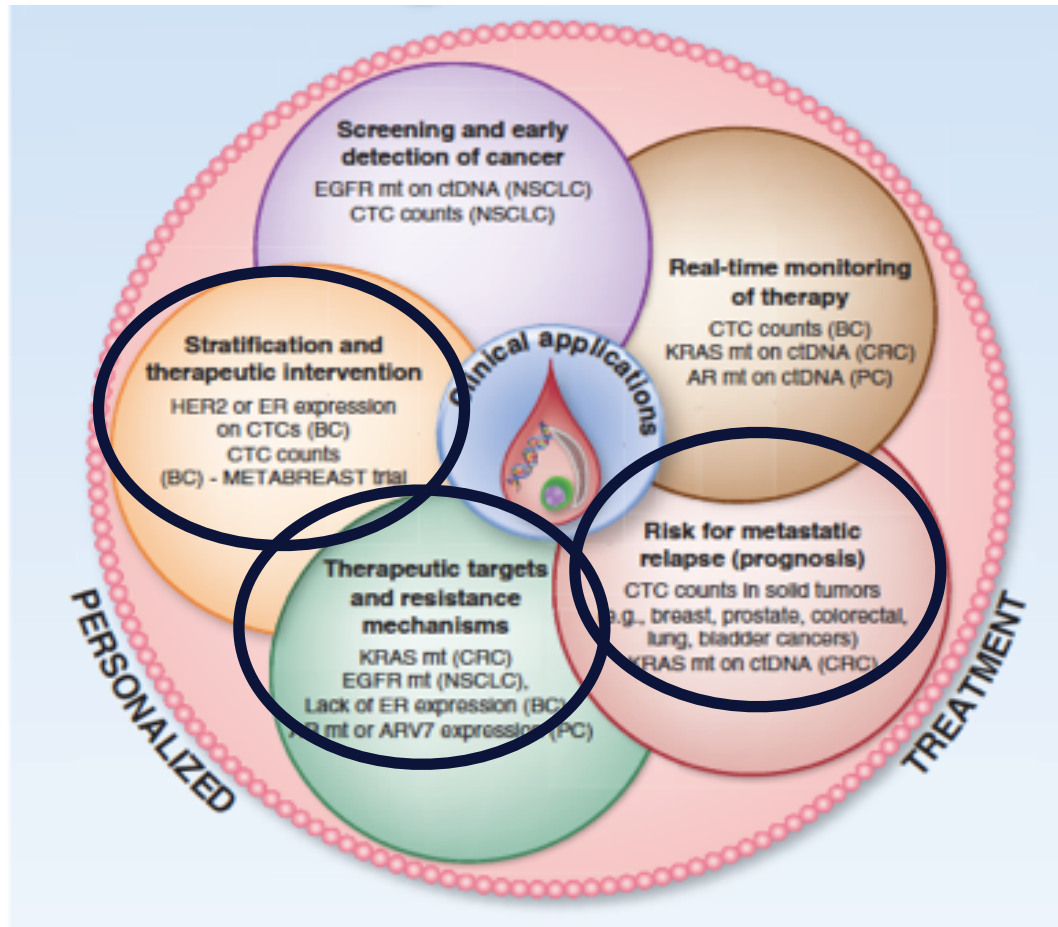
Medical University of Vienna

Liquid biopsies as the basis for personalized cancer treatment



Alix-Panabières & Pantel (2016) *Cancer Discovery*

Liquid biopsies as the basis for personalized cancer treatment in brain metastasis patients



Blood based tests in BM patient:

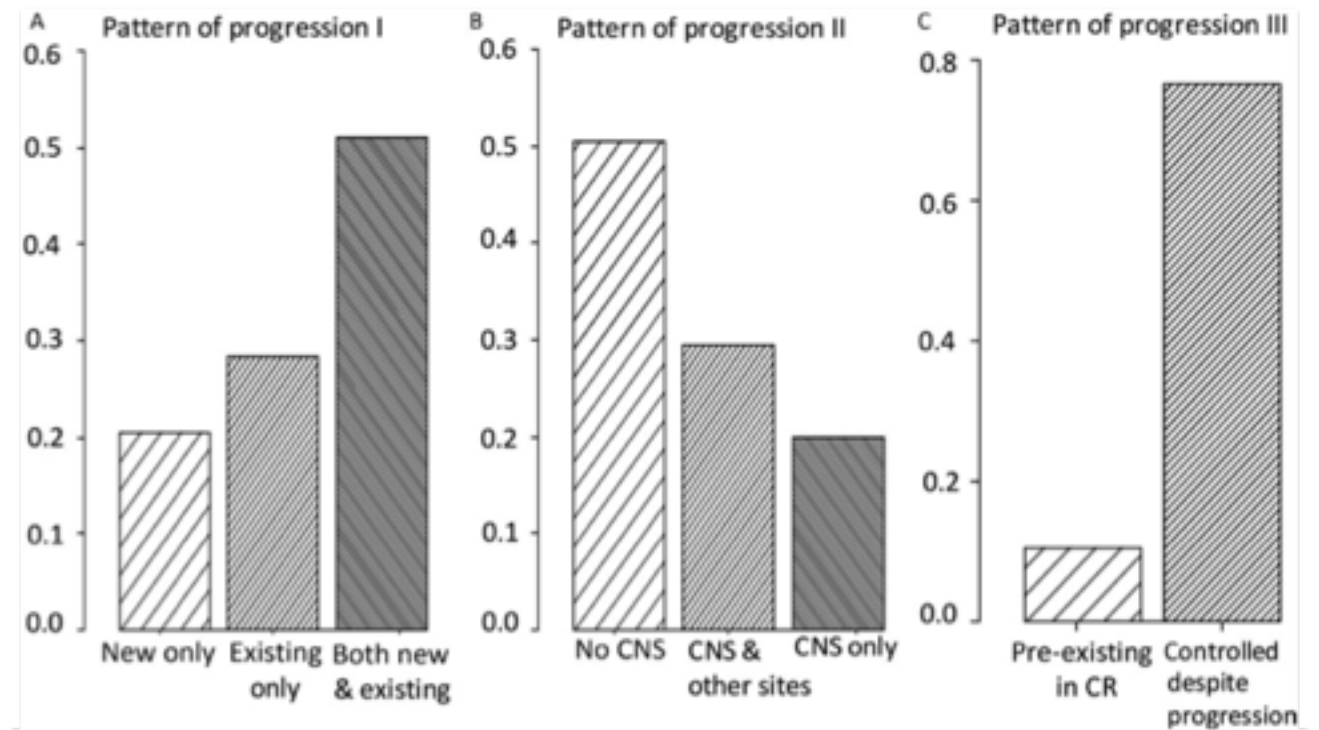
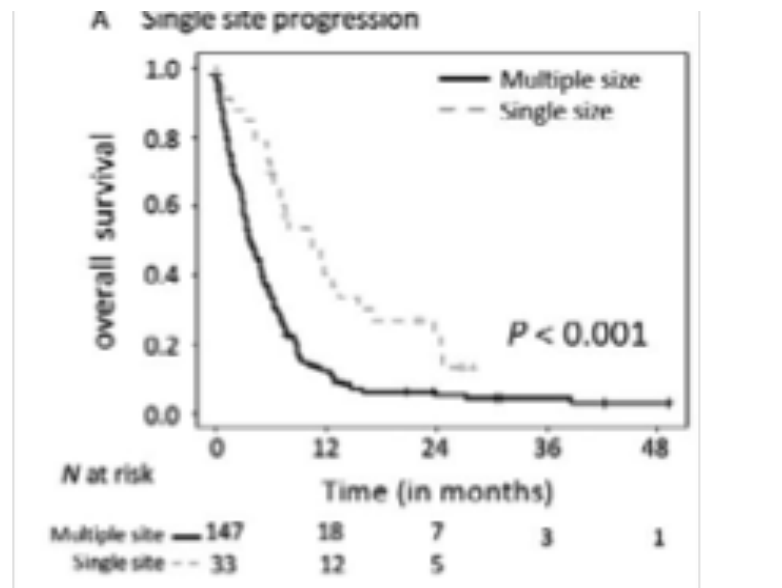
- Liquid biopsy: cancer cell related
- Liquid biomarker: not cancer cell related

Alix-Panabières & Pantel (2016) *Cancer Discovery*

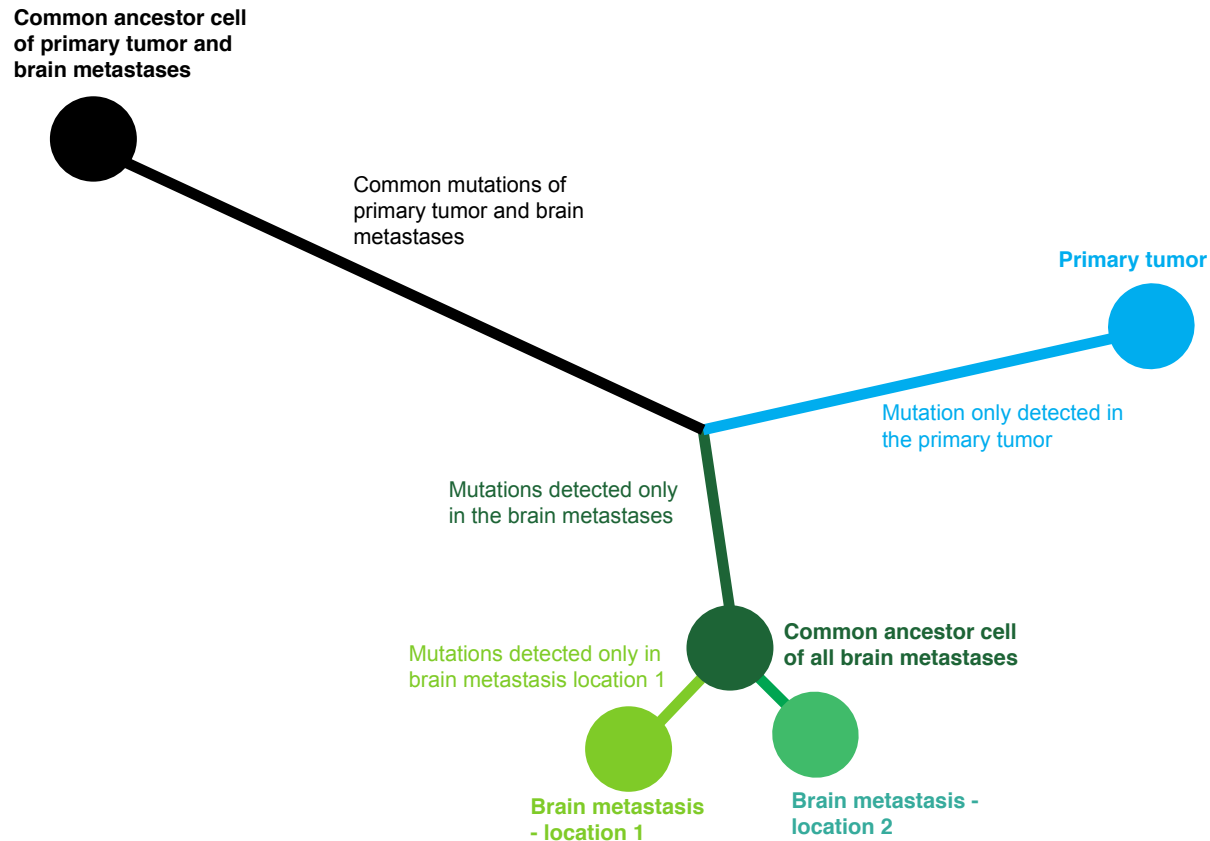
Frequent intracranial failure of (effective) systemic therapies

Progression patterns under BRAF inhibitor treatment and treatment beyond progression in patients with metastatic melanoma

Jessica C. Hassel^{1,2}, Kristina Buder-Bakhaya^{1,2}, Carolin Bender¹, Lisa Zimmer², Benjamin Weide³, Carmen Loquai⁴, Selma Ugurel², Alla Slynko⁵ & Ralf Gutzmer⁶ On behalf of the German Dermatocology Group (DeCOG/ADO)

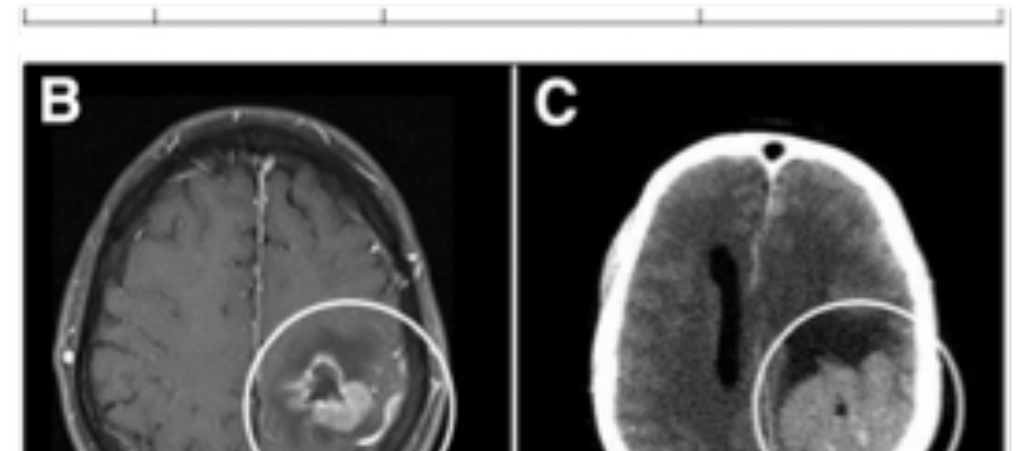


Mixed intra- and extracranial responses due to branched evolution



A Retrospective Evaluation of Vemurafenib as Treatment for BRAF-Mutant Melanoma Brain Metastases

JAMES J. HARDING,^{a,c} FEDERICA CATALANOTTI,^d RODRIGO R. MUNHOZ,^a DONAVAN T. CHENG,^d AMIN YAQUBIE,^a NICOLE KELLY,^a GREGORY C. McDERMOTT,^c ROMONA KERSELLIUS,^c TAHAR MERGHOUB,^a MARIO E. LACOUTURE,^a RICHARD D. CARVAJAL,^a KATHERINE S. PANAGEAS,^b MICHAEL F. BERGER,^d NEAL ROSEN,^{a,c,f} DAVID B. SOLIT,^{a,c,d} PAUL B. CHAPMAN^{a,c}



Predictive biomarkers for targeted therapies in brain metastases

Acta Neuropathol

Table 1 Overview of biomarker tests that should be considered for therapy planning in patients with brain metastases

| Cancer type | Biomarker | Test method | Discordance rate between primary tumor and brain metastasis | Approved targeted drugs |
|-------------------------|----------------------|---------------------------------------|---|---|
| NSCLC | EGFR mutation | Gene sequencing | 0–32 % | Gefitinib, Erolitinib |
| | ALK rearrangement | FISH | 0–12.5 % | Crizotinib |
| Breast cancer | HER2 amplification | IHC, FISH | 0–14 % | Lapatinib, Trastuzumab, Pertuzumab, T-DM1 |
| | ER and PR expression | IHC | 30–50 % | Tamoxifen |
| Melanoma | BRAF mutations | Gene sequencing, IHC, COBAS V600 test | 0 % | Vemurafenib, Dabrafenib, Trametinib |
| Colorectal cancer | RAS mutations | Gene sequencing | Unknown | Cetuximab, Panitumumab |
| Gastroesophageal cancer | HER2 amplification | IHC, FISH | 0 % | Trastuzumab |

ALK anaplastic lymphoma kinase gene, *BRAF* v-RAF murine sarcoma viral oncogene homolog B1 gene, *EGFR* epidermal growth factor receptor gene, *ER* estrogen receptor, *FISH* fluorescent in situ hybridization, *HER2* human epidermal growth factor receptor 2, *IHC* immunohistochemistry, *NSCLC* non-small-cell lung cancer, *PR* progesterone receptor, *RAS* rat sarcoma gene

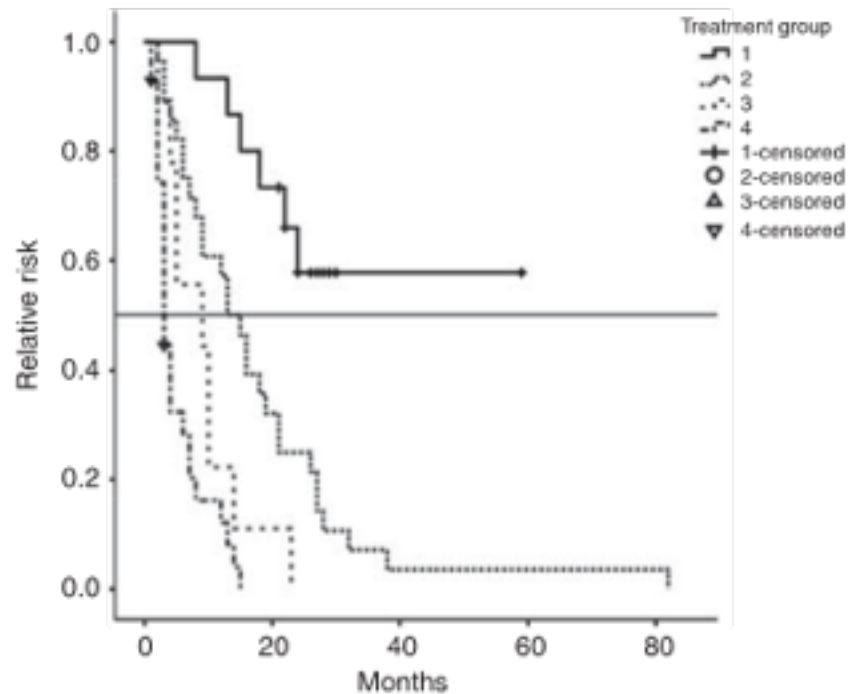
Berghoff et al, Acta Neuropathol 2014

Optimized systemic therapy makes the difference!

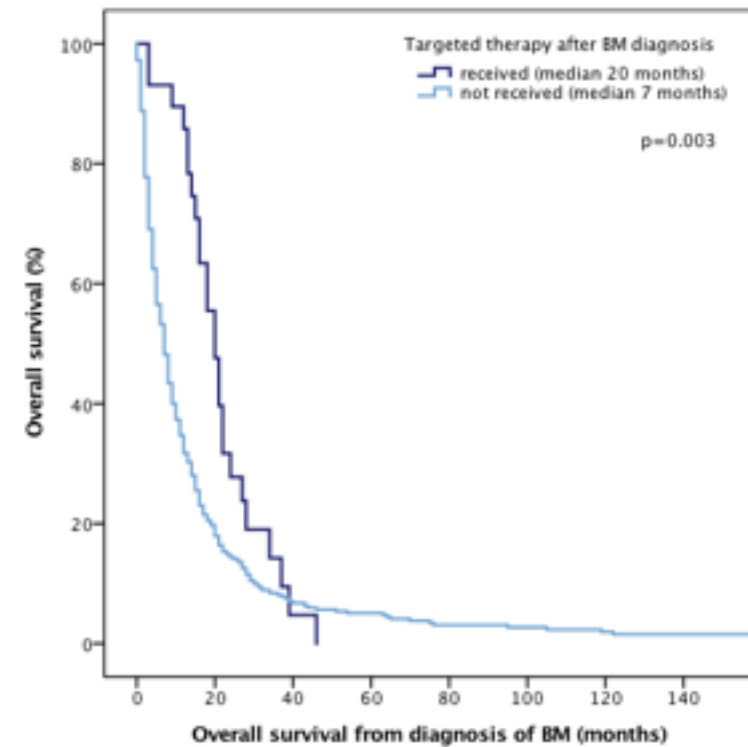
Impact of anti-HER2 therapy on overall survival in HER2-overexpressing breast cancer patients with brain metastases

R Bartsch^{1,2}, A Berghoff^{1,2}, U Pluschnig^{1,2}, Z Bago-Morvath^{3,4}, P Duboky^{1,4}, A Rottenfusser^{1,5}, C DeVries^{1,2}, M Rudas^{1,2}, F Fitzal^{1,2}, K Dieckmann^{1,2}, RM Mader^{1,2}, M Gnant^{1,4}, CC Zielinski^{1,2} and GG Steger^{1,2,3}

¹Clinical Division of Oncology, Department of Medicine I, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria; ²Comprehensive Cancer Centre, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria; ³Department of Pathology, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria; ⁴Department of Surgery, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria; ⁵Department of Radiotherapy, Medical University of Vienna, Währinger Gürtel 18-20, Vienna 1090, Austria

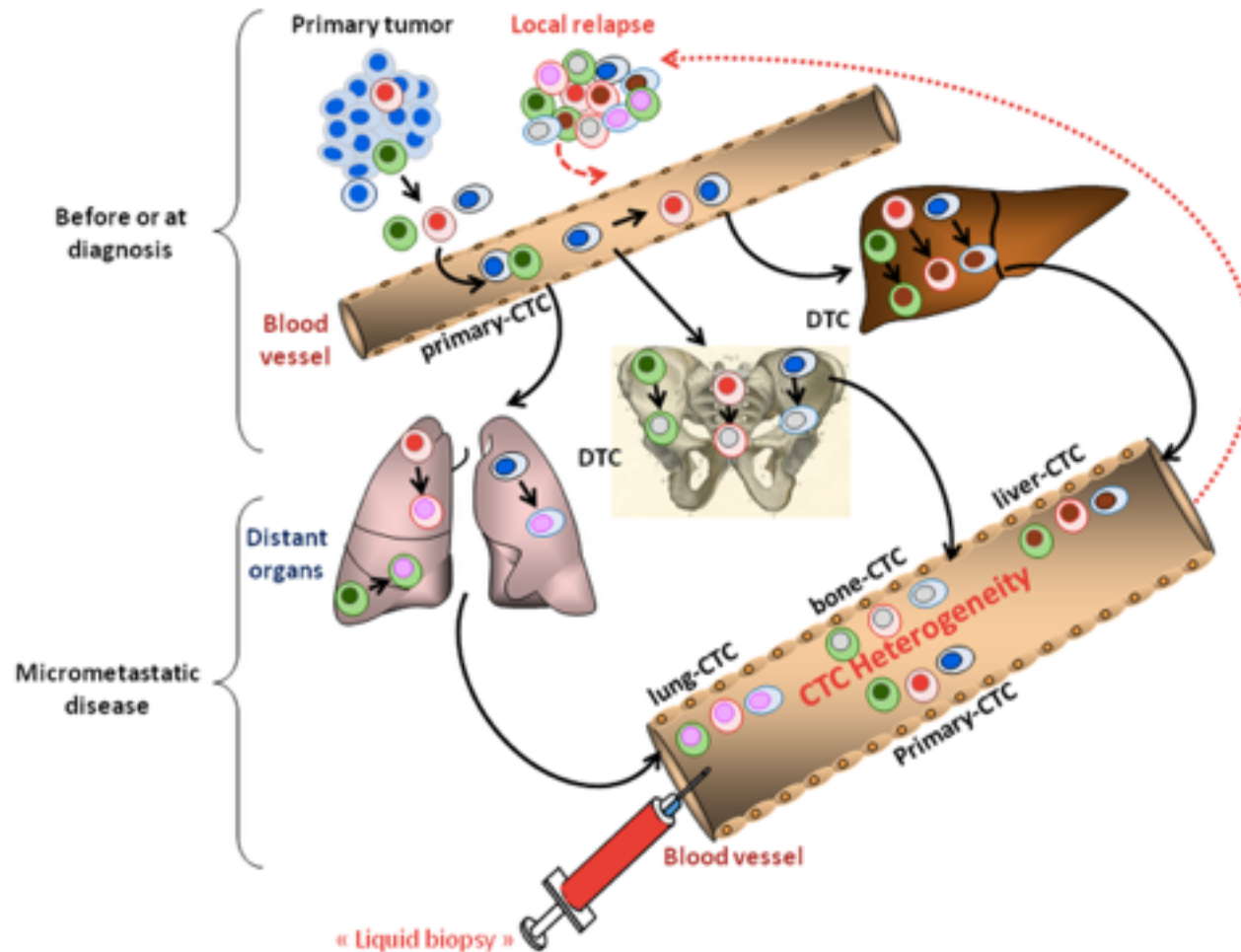


Cancer of unknown primary BM - Targeted therapies after molecular analysis

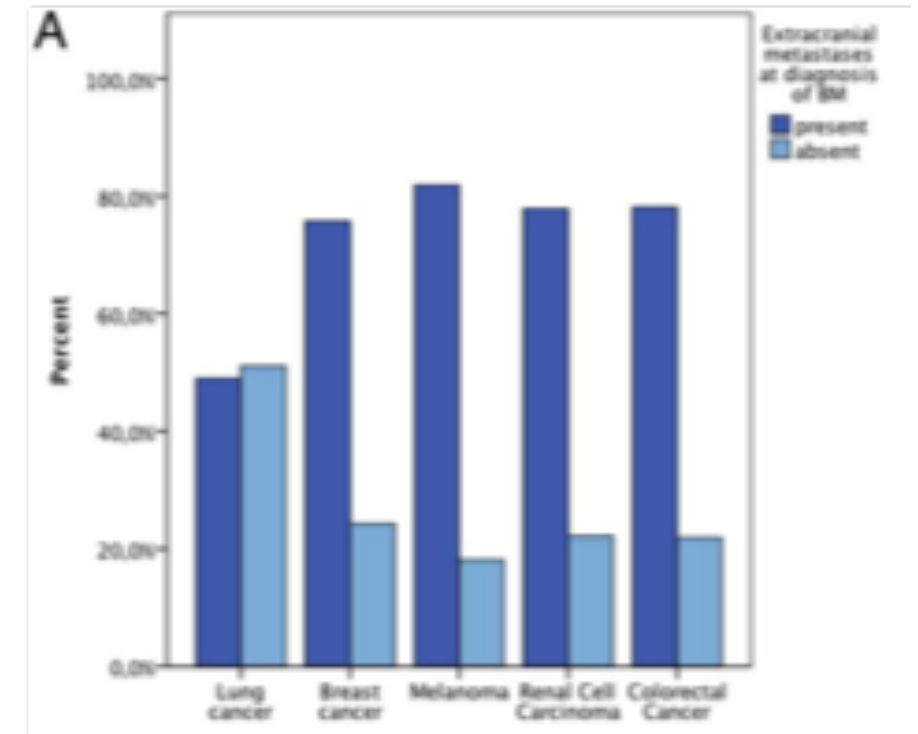


Füreder L,... Berghoff AS; in revision

Liquid biopsies – a surrogate for genetic characteristics of the cancer disease?



Intracranial location: Challenge in BM?

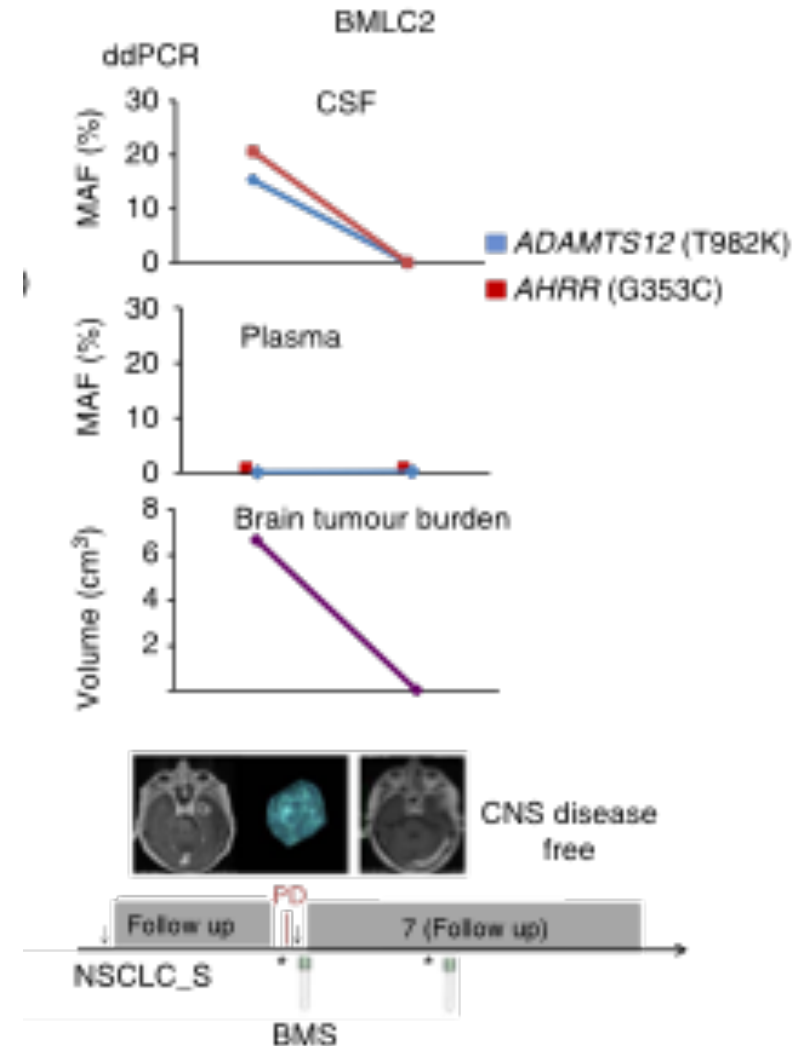
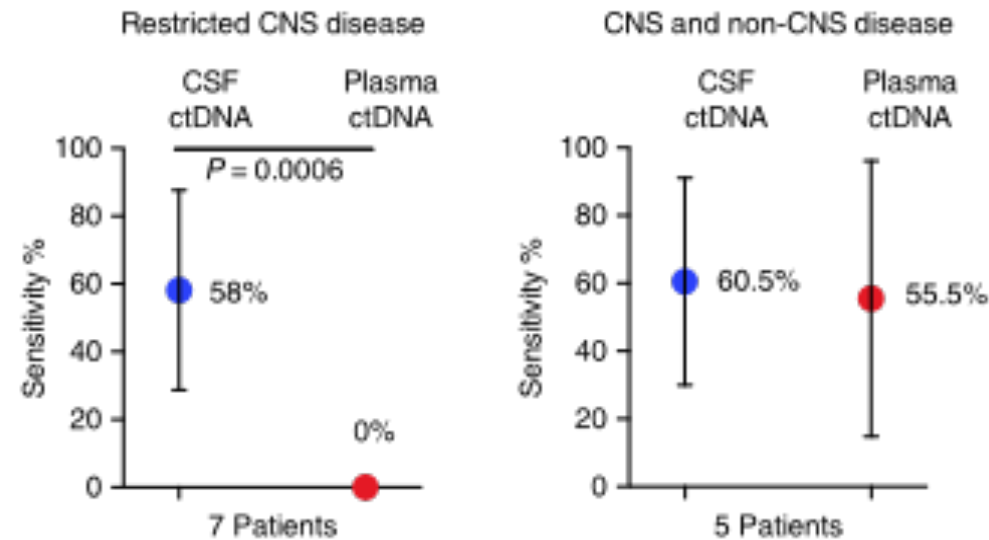


Alix-Panabières & Pantel (2010) Clin Cancer Res
Berghoff et al. ESMO open 2016

Analytical challenge of liquid biopsy in brain metastases patients – blood vs. CSF

Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma

Leticia De Mattos-Arruda^{1,2,3}, Regina Mayor¹, Charlotte K.Y. Ng², Britta Weigelt², Francisco Martinez-Ricarte^{3,4}, Davis Torrejon¹, Mafalda Oliveira¹, Alexandra Arias¹, Carolina Raventos¹, Jiabin Tang⁵, Elena Guerini-Rocco², Elena Martinez-Sáez⁴, Sergio Lois⁴, Oscar Marin⁴, Xavier de la Cruz^{4,6}, Salvatore Piscuoglio², Russel Towers⁷, Ana Vivancos¹, Vicente Peg⁴, Santiago Ramon y Cajal^{3,4}, Joan Carles¹, Jordi Rodon¹, Maria González-Cao⁸, Josep Tabernero^{1,3}, Enriqueta Felip^{1,3}, Joan Sahuquillo^{3,4}, Michael F. Berger^{5,9}, Javier Cortes^{1,3}, Jorge S. Reis-Filho² & Joan Seoane^{1,3,6}



Indications for liquid biopsies in patients with brain metastases?

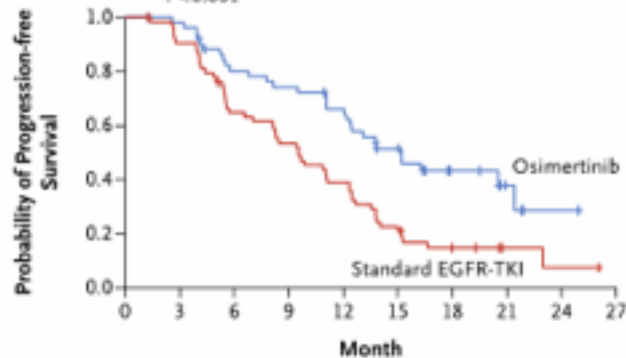
Indications for liquid biopsies (in patients with brain metastases)

Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer

J.-C. Soria, Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewaskulyong, K.H. Lee, A. Dechaphunkul, F. Imamura, N. Nogami, T. Kurata, I. Okamoto, C. Zhou, B.-C. Cho, Y. Cheng, E.K. Cho, P.J. Voon, D. Planchard, W.-C. Su, J.E. Gray, S.-M. Lee, R. Hodge, M. Marotti, Y. Rukazenzov, and S.S. Ramalingam, for the FLAURA Investigators^a

B Progression-free Survival in Patients with CNS Metastases

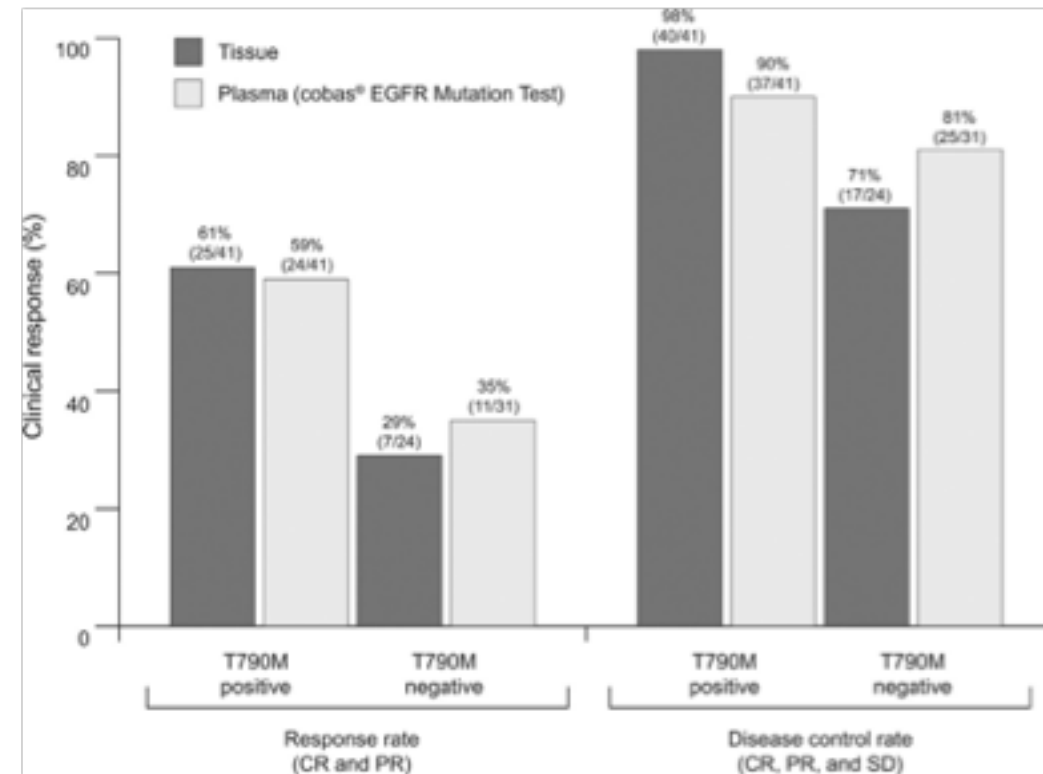
| | No. of Patients | Median Progression-free Survival (95% CI) mo |
|---|-----------------|--|
| Osimertinib | 53 | 15.2 (12.1–21.4) |
| Standard EGFR-TKI | 63 | 9.6 (7.0–12.4) |
| Hazard ratio for disease progression or death, 0.47 (95% CI, 0.30–0.74) | | |
| P<0.001 | | |



| No. at Risk | 53 | 51 | 40 | 37 | 32 | 22 | 9 | 4 | 1 | 0 |
|-------------------|----|----|----|----|----|----|---|---|---|---|
| Osimertinib | 53 | 51 | 40 | 37 | 32 | 22 | 9 | 4 | 1 | 0 |
| Standard EGFR-TKI | 63 | 57 | 40 | 33 | 24 | 13 | 6 | 2 | 1 | 0 |

EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291

Kenneth S. Thress^{a,*}, Roz Brant^b, T. Hedley Carr^c, Simon Dearden^d, Suzanne Jenkins^e, Helen Brown^d, Tracey Hammett^f, Mireille Cantarini^g, J. Carl Barrett^a



Indications for liquid biopsies (in patients with brain metastases)

➤ Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study

Thomas Bachelot, Gilles Romieu, Mario Campone, Véronique Diéras, Claire Cropet, Florence Dolenc, Marta Jimenez, Emilie Le Rhun, Jean-Yves Pierga, Anthony Gonçalves, Marianne Leheurteur, Julien Domont, Maya Gutierrez, Hervé Curié, Jean-Marc Ferrero, Catherine Labbe-Devilliers

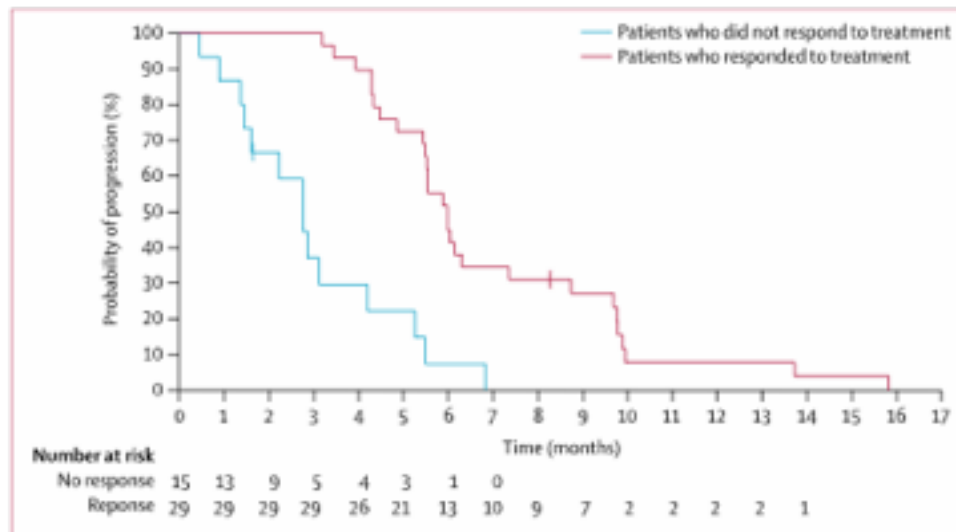
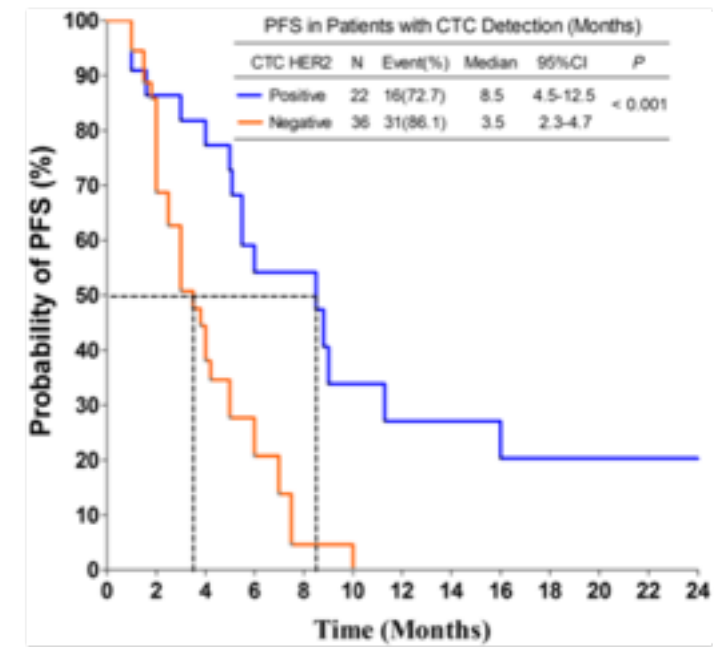


Figure 2: Time to progression, by CNS response (volumetric analysis; N=44)

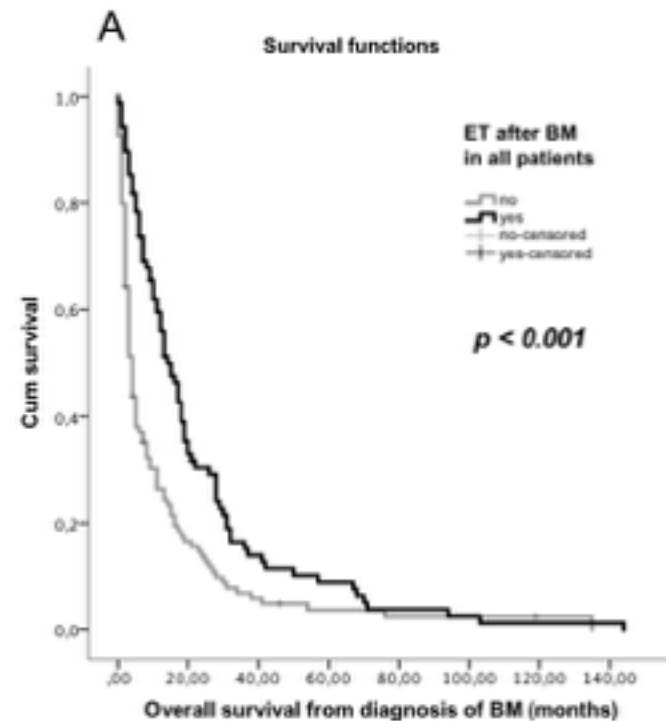
Real-time HER2 status detected on circulating tumor cells predicts different outcomes of anti-HER2 therapy in histologically HER2-positive metastatic breast cancer patients

Shaohua Zhang^{1†}, Lei Li^{1†}, Tao Wang¹, Li Bian¹, Haixu Hu², Chunhong Xu², Bing Liu², Yi Liu^{2*}, Massimo Cristofanilli^{3*} and Zefei Jiang^{1*}



Indications for liquid biopsies (in patients with brain metastases)

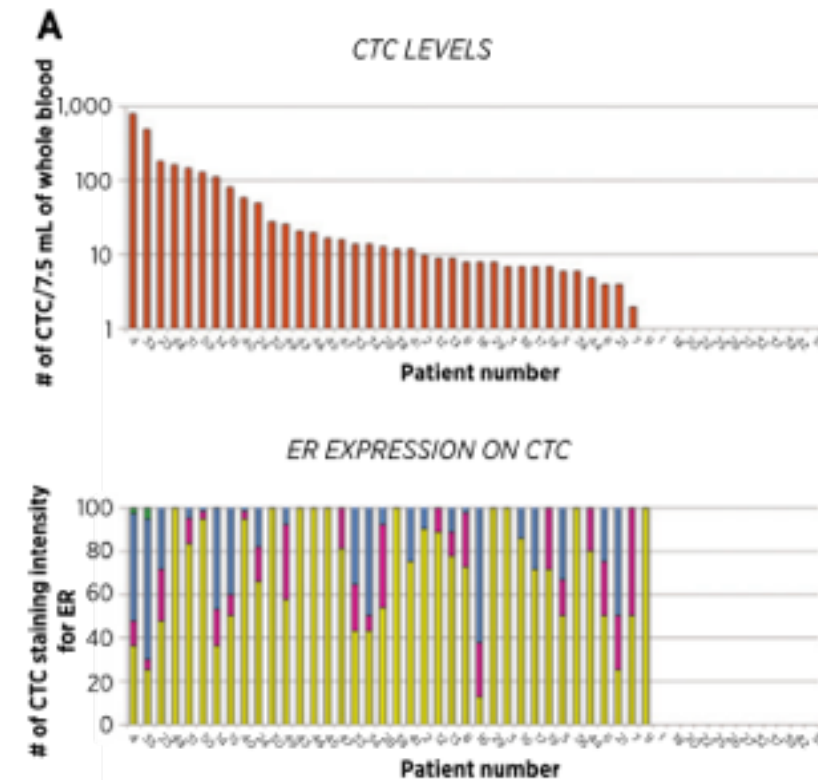
Continued endocrine therapy is associated with improved survival in patients with breast cancer brain metastases



Bergen E, Berghoff AS et al Clin Can Res 2018

Development of Circulating Tumor Cell-Endocrine Therapy Index in Patients with Hormone Receptor-Positive Breast Cancer

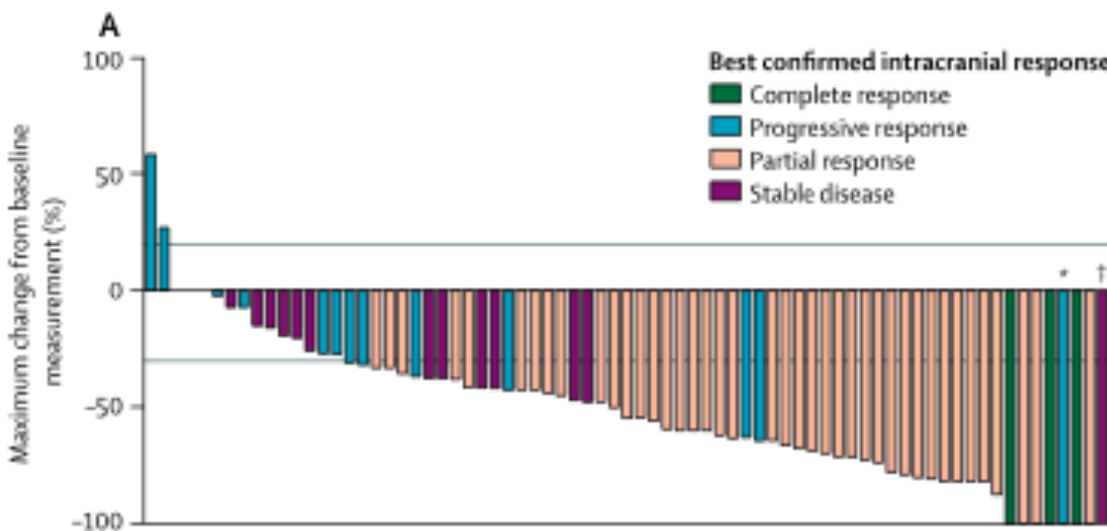
Costanza Paoletti¹, Maria C. Muñoz¹, Dafydd G. Thomas¹, Kent A. Griffith², Kelley M. Kidwell², Nahomi Tokudome¹, Martha E. Brown¹, Kimberly Aung¹, M. Craig Miller³, Dorothy L. Blossom¹, Anne F. Schott¹, N. Lynn Henry¹, James M. Rae¹, Mark C. Connelly³, David A. Chianese³, and Daniel F. Hayes¹



Indications for liquid biopsies (in patients with brain metastases)

Dabrafenib plus trametinib in patients with BRAF^{V600}-mutant melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial

Michael A Davies*, Philippe Saiag*, Caroline Robert, Jean-Jacques Grob, Keith T Flaherty, Ana Arance, Vanna Chiarion-Sileni, Luc Thomas, Thierry Lesimple, Laurent Mortier, Stergios J Maschos, David Hogg, Iván Márquez-Rodas, Michele Del Vecchio, Céleste Lebbé, Nicolas Meyer, Ying Zhang, Yingjie Huang, Bijoyesh Mookerjee, Georgina V Long



Mutation analysis of *BRAF* and *KIT* in circulating melanoma cells at the single cell level

K Sakaizawa¹, Y Goto¹, Y Kuniwa¹, A Uchiyama¹, K Harada², S Shimada², T Saida¹, S Ferrone³, M Takata¹, H Uhara¹ and R Okuyama^{4,1}
¹Department of Dermatology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan; ²Department of Dermatology, University of Yamaguchi School of Medicine, 1-1-1 Shimokawato, Chuo 409-3898, Japan; ³Departments of Surgery, Immunology and Pathology, University of Pittsburgh Cancer Institute, 5117 Center Avenue Suite 2.26d, Pittsburgh, PA 15213, USA

Table 2 Clinical characteristics of the 11 melanoma patients

| No. | Sex | Age (years) | Histopathological type | AJCC stage | Metastatic sites | No. of CTC in 5 ml of PB |
|-----|-----|-------------|------------------------|------------|--|--------------------------|
| 1 | M | 54 | Mucosal | IV | Lymph nodes, lung, brain, spinal cord | 2–5 |
| 2 | F | 78 | Mucosal | IV | Lymph nodes, lung, liver | 2 |
| 3 | M | 67 | Mucosal | BC | Lymph nodes | 5 |
| 4 | F | 68 | Mucosal | IV | Lymph nodes, lung | 1–5 |
| 5 | F | 64 | Non-CSD | IV | Lymph nodes, lung, liver, skin | 1–20 |
| 6 | M | 61 | Non-CSD | IV | Lymph nodes, lung, brain, skin | 2–7 |
| 7 | F | 62 | Non-CSD | IV | Lymph nodes, lung, peritoneum | 1–3 |
| 8 | M | 73 | Non-CSD | IV | Lymph nodes, digestive tract, pleura, abdominal cavity, skin | 1 |
| 9 | M | 69 | Unknown | IV | Lymph nodes, liver, digestive tract, bone, adrenal, skin | 8 |
| 10 | F | 62 | Acral | BC | Lymph nodes | 6 |
| 11 | M | 84 | Acral | IV | Lymph nodes, lung, brain | 7 |

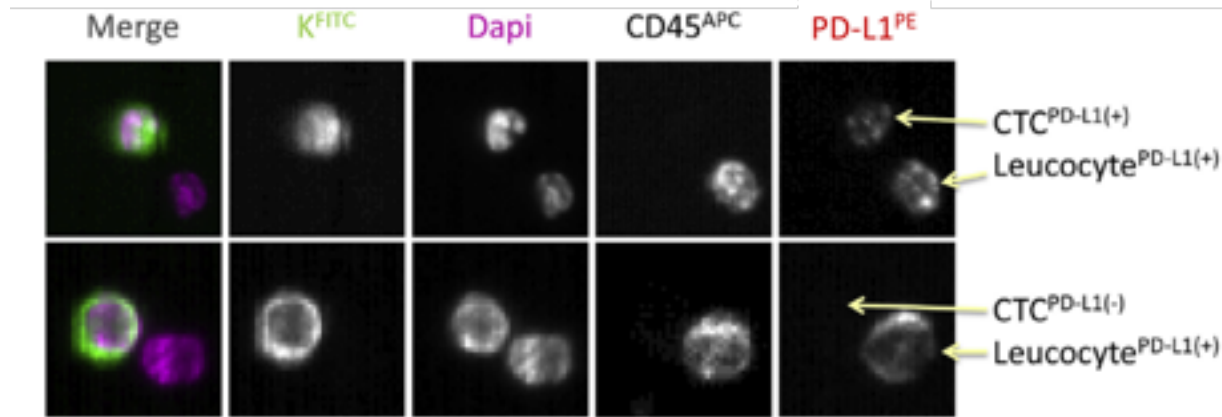
Abbreviations: CTC = circulating tumour cells; non-CSD = melanoma on non-chronic sun-damaged skin; PB = peripheral blood.

Table 3 Genotypes of *BRAF* and *KIT* in melanoma patients

| BRAF (exon 15) | | | | | KIT (exons 11, 13, and 17) | | | |
|----------------|-----------|------------|-------------------------------------|---------------------|----------------------------|------------|------------------------|---------------------|
| Tissue | | Single CTC | | | Tissue | | Single CTC | |
| No. | Primary | Metastasis | No. of cells sequenced | Success rate of PCR | Primary | Metastasis | No. of cells sequenced | Success rate of PCR |
| 1 | Wild type | Wild type | Wild type (1) | 1/1 | D820Y | Wild type | UR | 0/3 |
| 2 | Wild type | NA | Wild type (1) | 1/1 | Wild type | NA | UR | 0/1 |
| 3 | Wild type | NA | Wild type (1) | 1/5 | Wild type | NA | NE | NE |
| 4 | V600E | UR | V600E (1) | 1/1 | Wild type | UR | V560G (1) | 1/2 |
| 5 | V600E | V600E | V600E (1), V600K (1), wild type (1) | 3/3 | Wild type | Wild type | Wild type (1) | 1/3 |
| 6 | V600E | Wild type | V600E (3) | 3/3 | Wild type | Wild type | NE | NE |
| 7 | Wild type | Wild type | Wild type (1) | 1/2 | UR | Wild type | NE | NE |
| 8 | Wild type | Wild type | Wild type (1) | 1/1 | Wild type | Wild type | NE | NE |
| 9 | NA | V600E | Wild type (2) | 2/4 | NA | Wild type | NE | NE |
| 10 | Wild type | Wild type | NE | NE | N822Y | Wild type | Wild type (1) | 1/6 |
| 11 | Wild type | Wild type | NE | NE | Wild type | N822Y | Wild type (1) | 1/2 |

Abbreviations: CTC = circulating tumour cells; NA = tissues not available; NE = not examined; UR = sequence unreadable due to PCR failure.

Liquid biomarkers for the response to immune checkpoint inhibitors

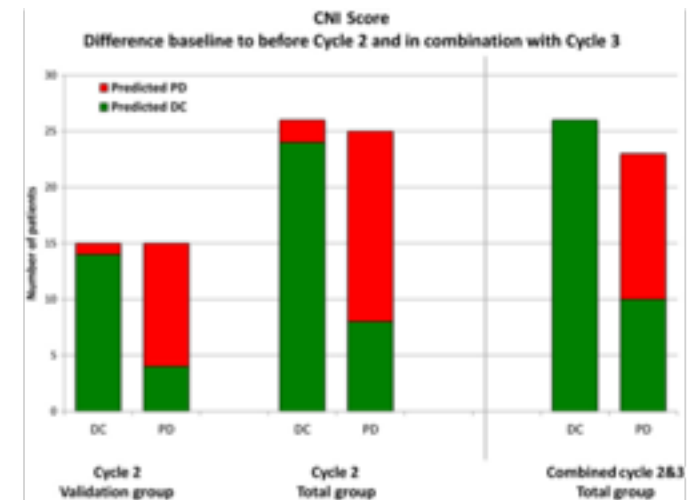
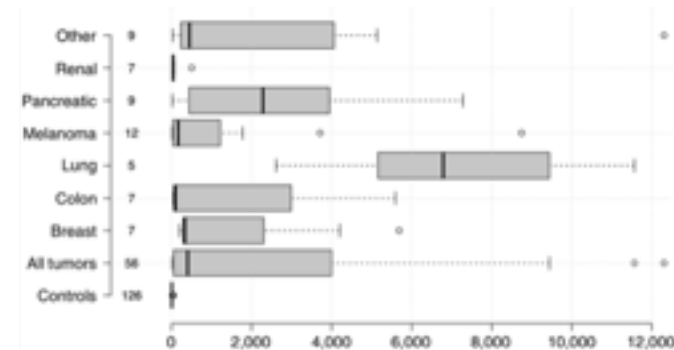


PD-L1 expression found on CTCs in 11/16 patients

Mazel *et al.* (2015) *Mol Onc*

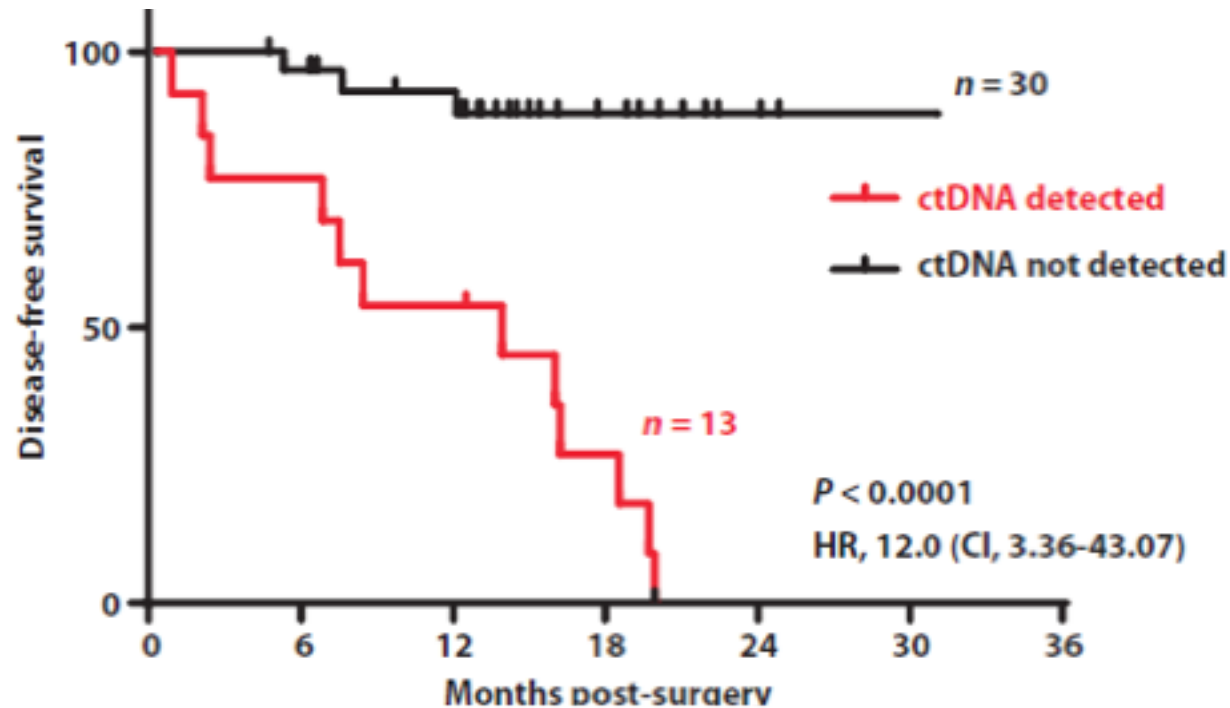
Tumor Cell-Free DNA Copy Number Instability Predicts Therapeutic Response to Immunotherapy

Glen J. Weiss¹, Julia Beck², Donald P. Braun³, Kristen Bornemann-Kolatzki², Heather Barilla¹, Rhiannon Cubello¹, Walter Quan Jr¹, Ashish Sangal¹, Vivek Khemka¹, Jordan Waypa¹, William M. Mitchell⁴, Howard Urnovitz², and Ekkehard Schütz²

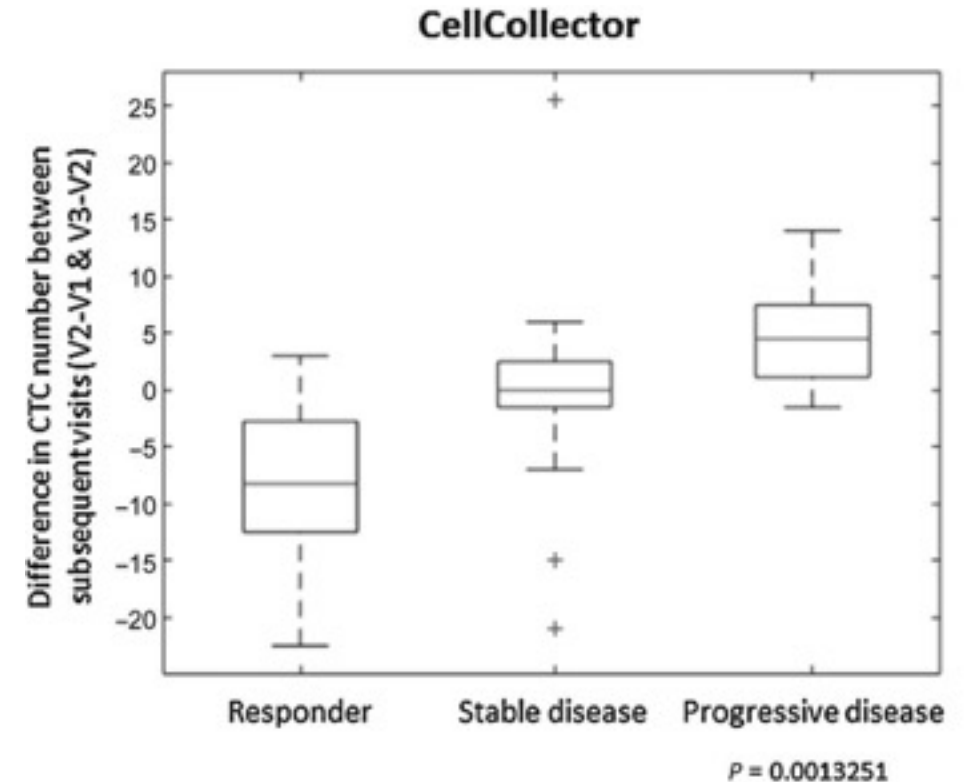


Disease monitoring using liquid biomarkers/ circulating tumor cells

Breast cancer



Lung cancer

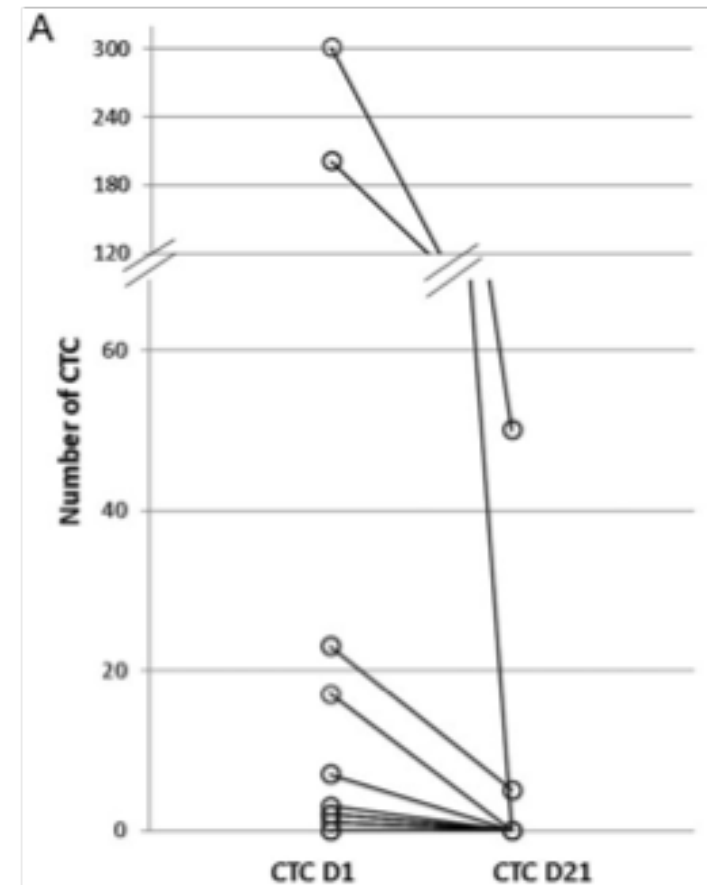
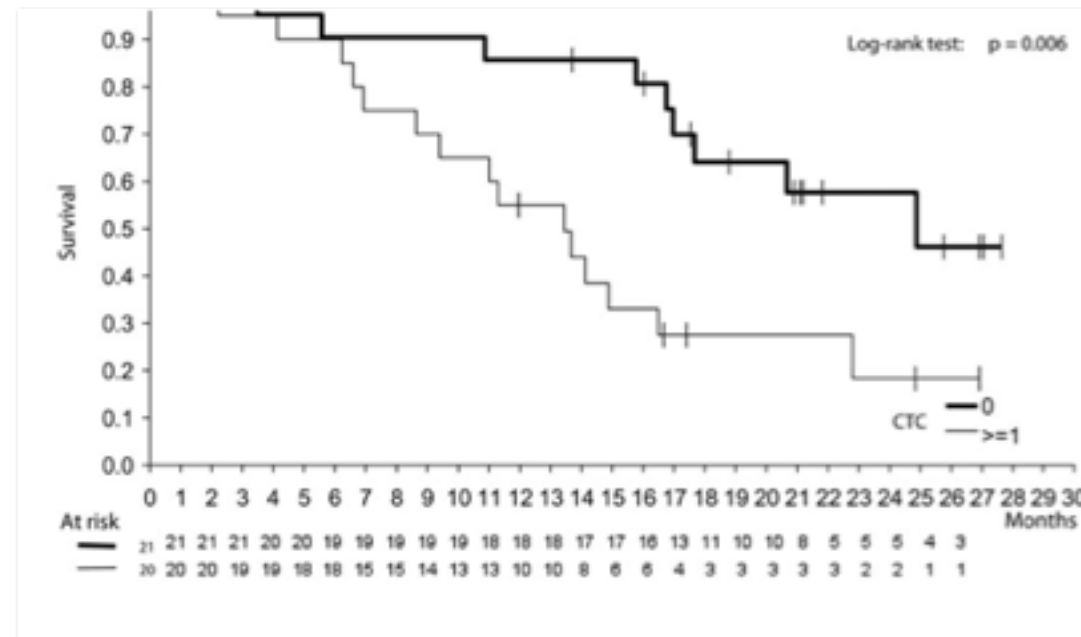


Gorges *et al.* (2016) *Clin Cancer Res*
Garcia-Murillas *et al.* (2015) *Sci Transl Med*

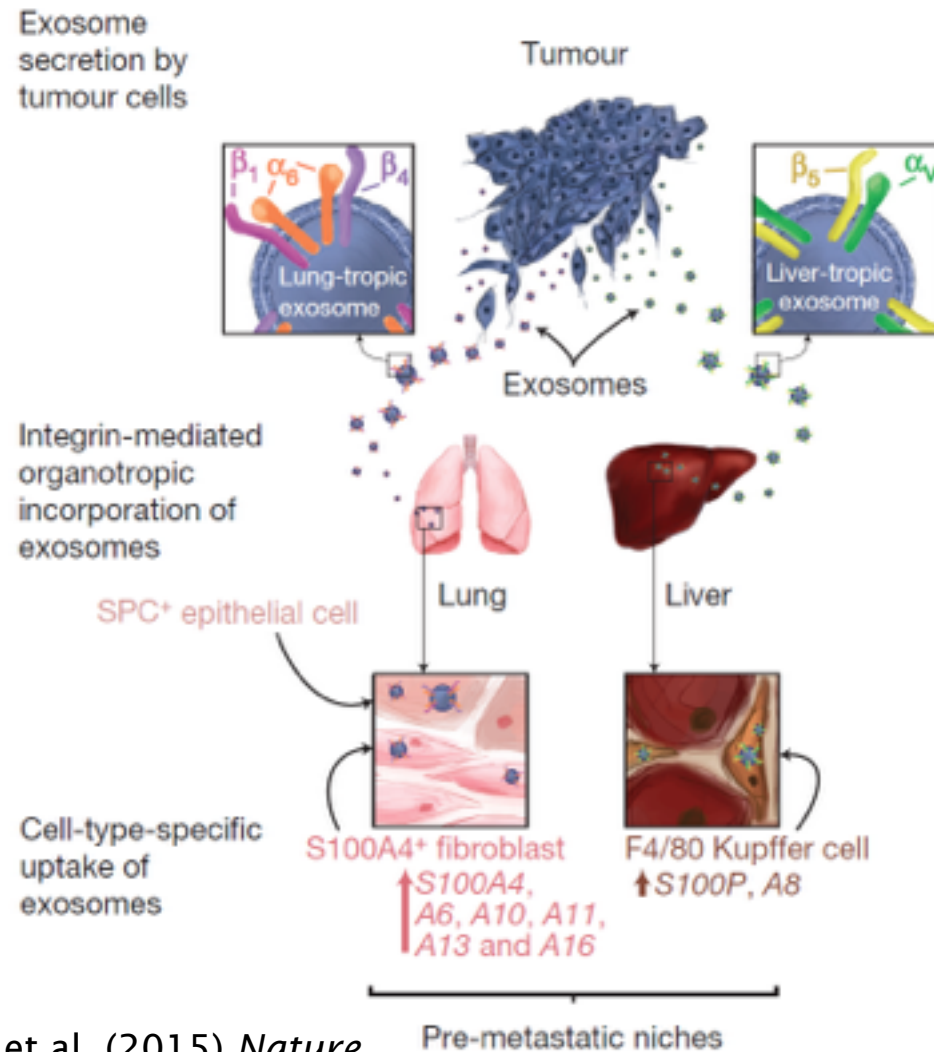
Disease monitoring using liquid biomarkers/ circulating tumour cells

Circulating tumor cells and brain metastasis outcome in patients with HER2-positive breast cancer: the LANDSCAPE trial

J.-Y. Pierga^{1*}, F.-C. Bidard¹, C. Cropet², P. Tresca¹, F. Dalenc³, G. Romieu⁴, M. Campone⁵, C. Mahier Ait-Oukhatar⁶, E. Le Rhun⁷, A. Gonçalves⁸, M. Leheutteur⁹, J. Dômont¹⁰, M. Gutierrez¹¹, H. Curé¹², J.-M. Ferrero¹³, C. Labbe-Devilliers⁵ & T. Bachelot¹⁴

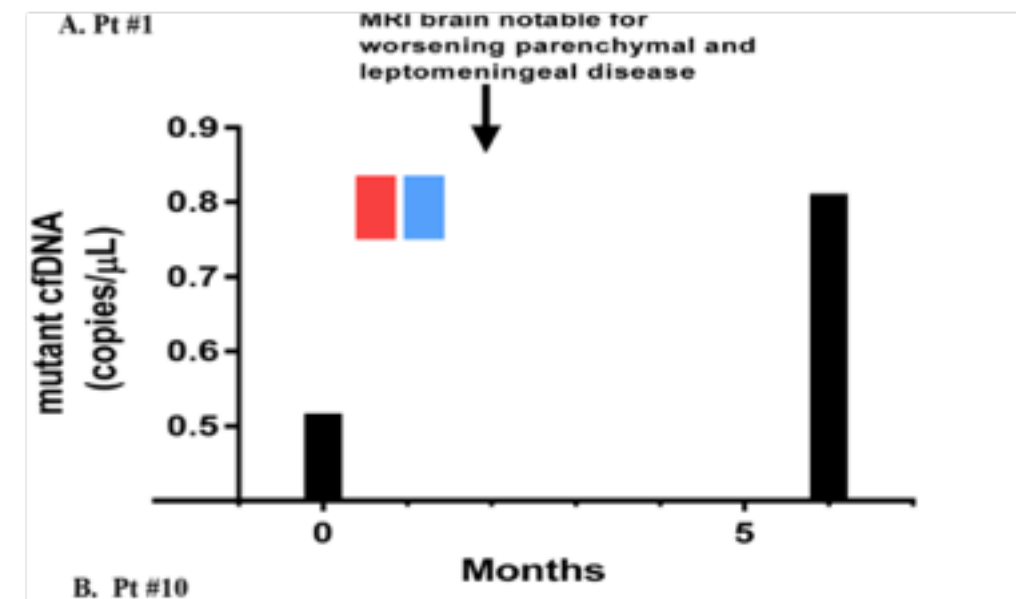


Outlook: Liquid biomarkers to predict clinical course



Quantification of tumor-derived cell free DNA(cfDNA) by digital PCR (DigPCR) in cerebrospinal fluid of patients with BRAF^{V600} mutated malignancies

Parisa Momtaz¹, Elena Pentsova¹, Omar Abdel-Wahab¹, Eli Diamond¹, David Hyman¹, Taha Merghoub¹, Daoqi You¹, Billel Gasmi¹, Agnes Viale¹, Paul B. Chapman^{1,2}



Hoshino et al. (2015) *Nature*

Summary

- High clinical potential but many open questions!
- Liquid biopsies facilitate information on
 - Genetic alterations - targeted therapies and immune modulating therapies
 - Prognosis
 - Disease monitoring
- Outlook: Prediction of metastasis development?
- Need for
 - Paired tissue/liquid collections: tumour heterogeneity? CSF vs. Blood?
 - Optimize analysis

Thank you for your attention!

Anna.Berghoff@meduniwien.ac.at

Analytical performance and clinical utility

CellSearch System (FDA-cleared)



Keratin-PE
positive



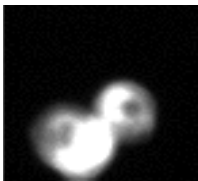
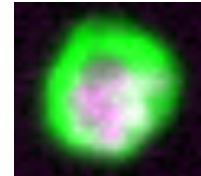
DAPI
positive



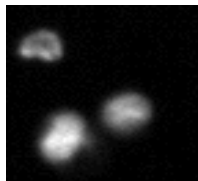
CD45-APC
negative



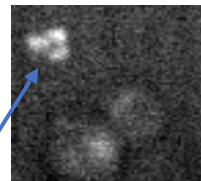
Tumor cell



Cytoplasm

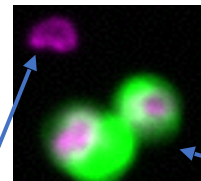


Nucleus



CD45+
membrane

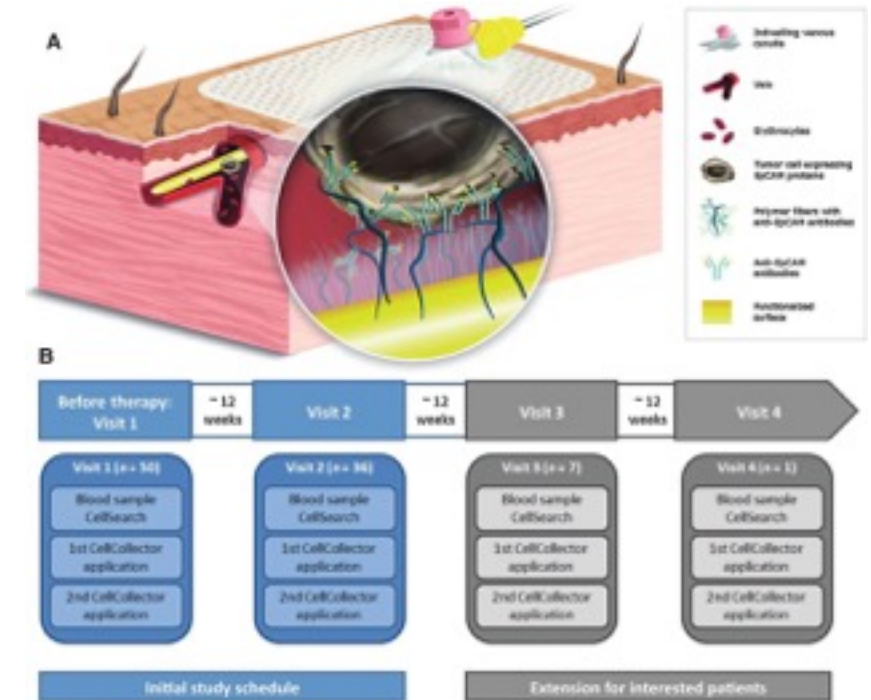
Cell membrane



Leukocyte

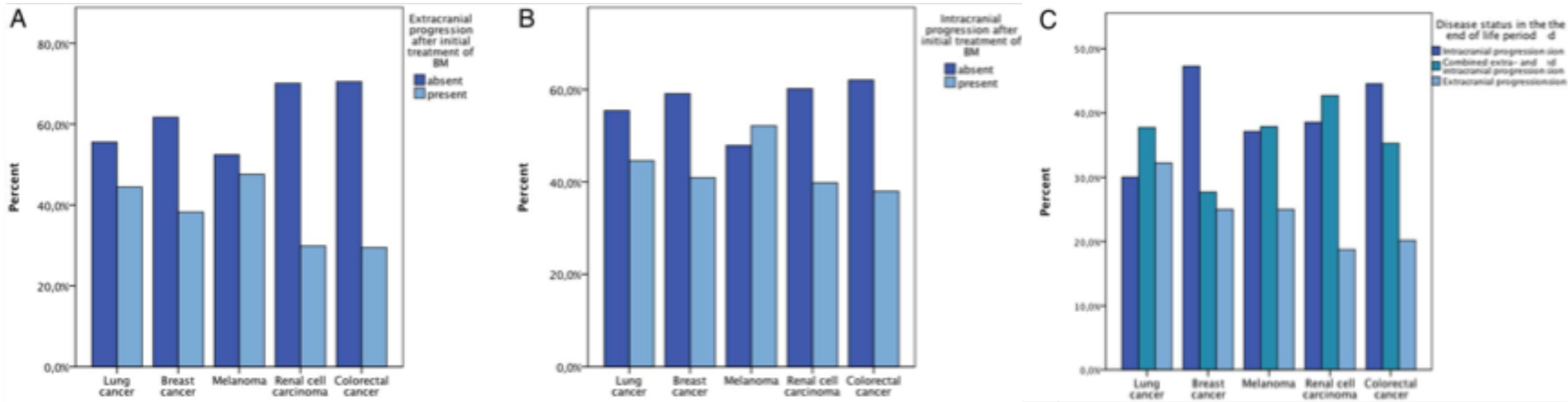
Tumor cells

Composite



Gorges *et al.* (2016) *Clin Cancer Res*

The clinical challenge of treating brain metastases – the intra- and extracranial compartment



Berghoff et al, ESMO Open (2016)

Liquid prognostic biomarkers in patients with brain metastases

Combining standard clinical blood values for improving survival prediction in patients with newly diagnosed brain metastases—development and validation of the LabBM score

Anna S. Berghoff, Fabian Wolpert, Tim Holland-Letz, Romina Koller, Georg Widhalm, Brigitte Gatterbauer, Karin Dieckmann, Peter Birner, Rupert Bartsch, Christoph C. Zielinski, Michael Weller, and Matthias Preusser

| | 0 points | 0.5 point | 1.0 point |
|----------------|------------|-----------|-----------|
| Platelet count | NR OR >ULN | <LLN | |
| LDH | NR | | >ULN |

