BRAIN METASTASES PHENOTYPE HETEROGENEITY

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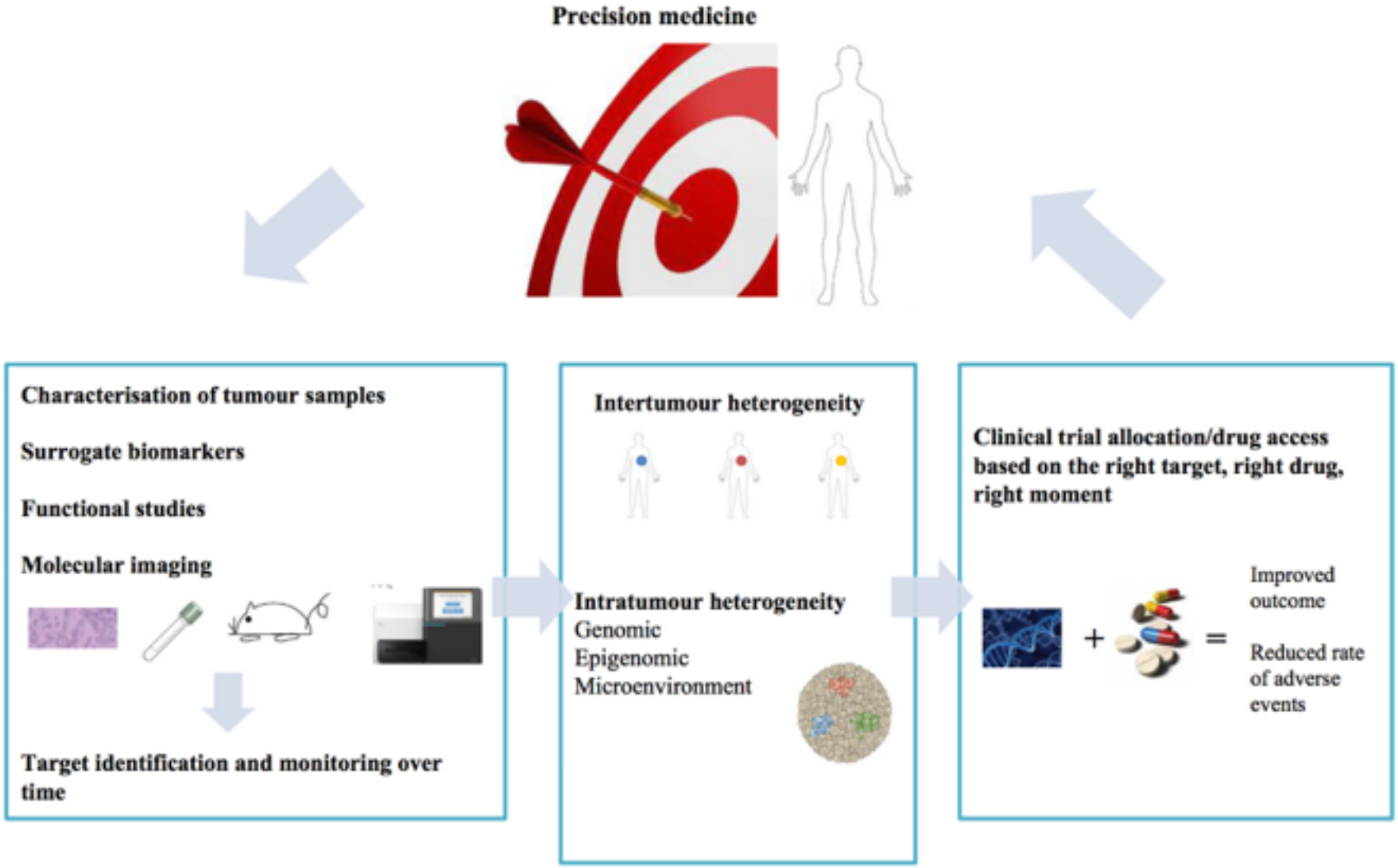
Disclosure

- Research funding: GSK, Roche
- Speaker: AstraZeneca, Roche
- Advisory roles: Amgen, AstraZeneca, BMS, Boehringer, Lilly, Pfizer, Roche

Precision medicine

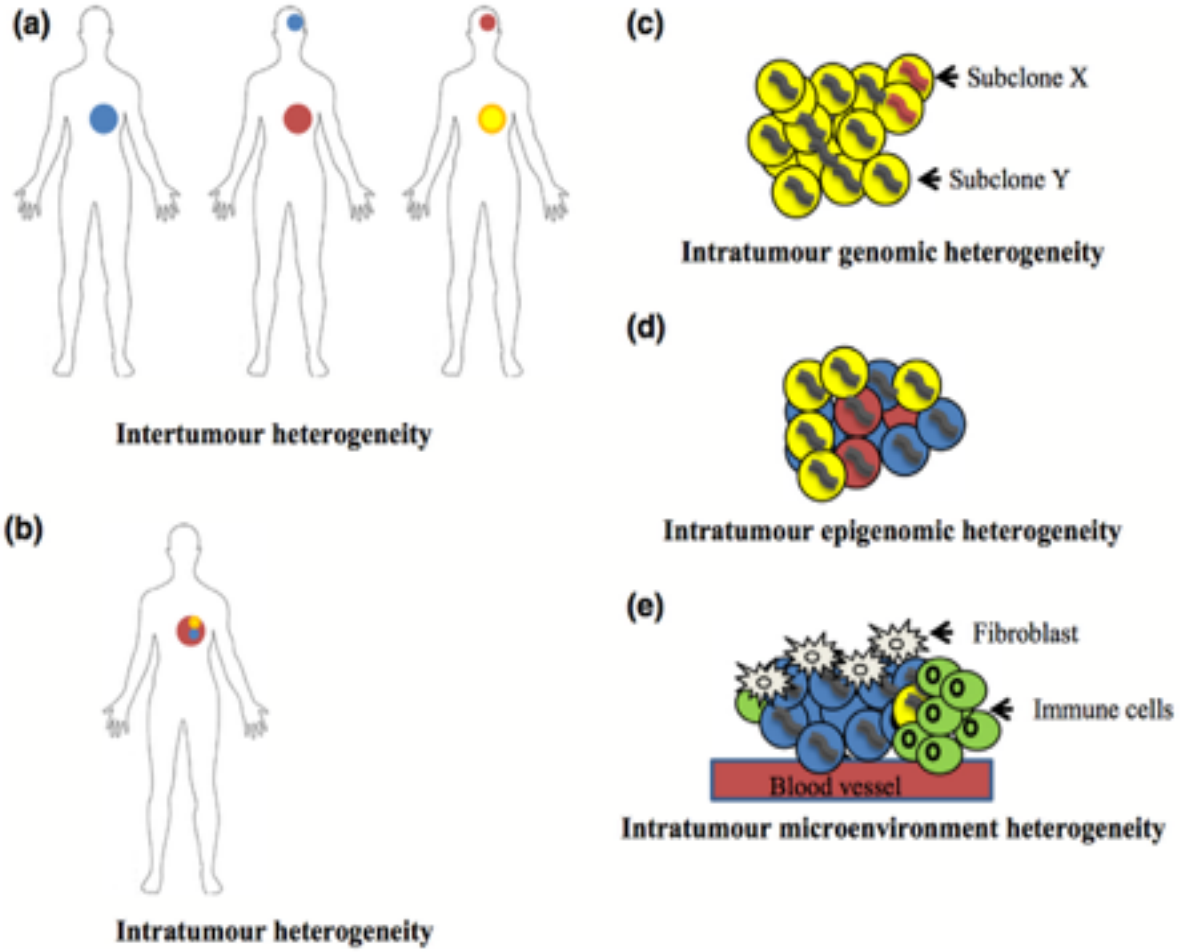


Seoane, J Intern Med. 2014

Barriers for precision medicine

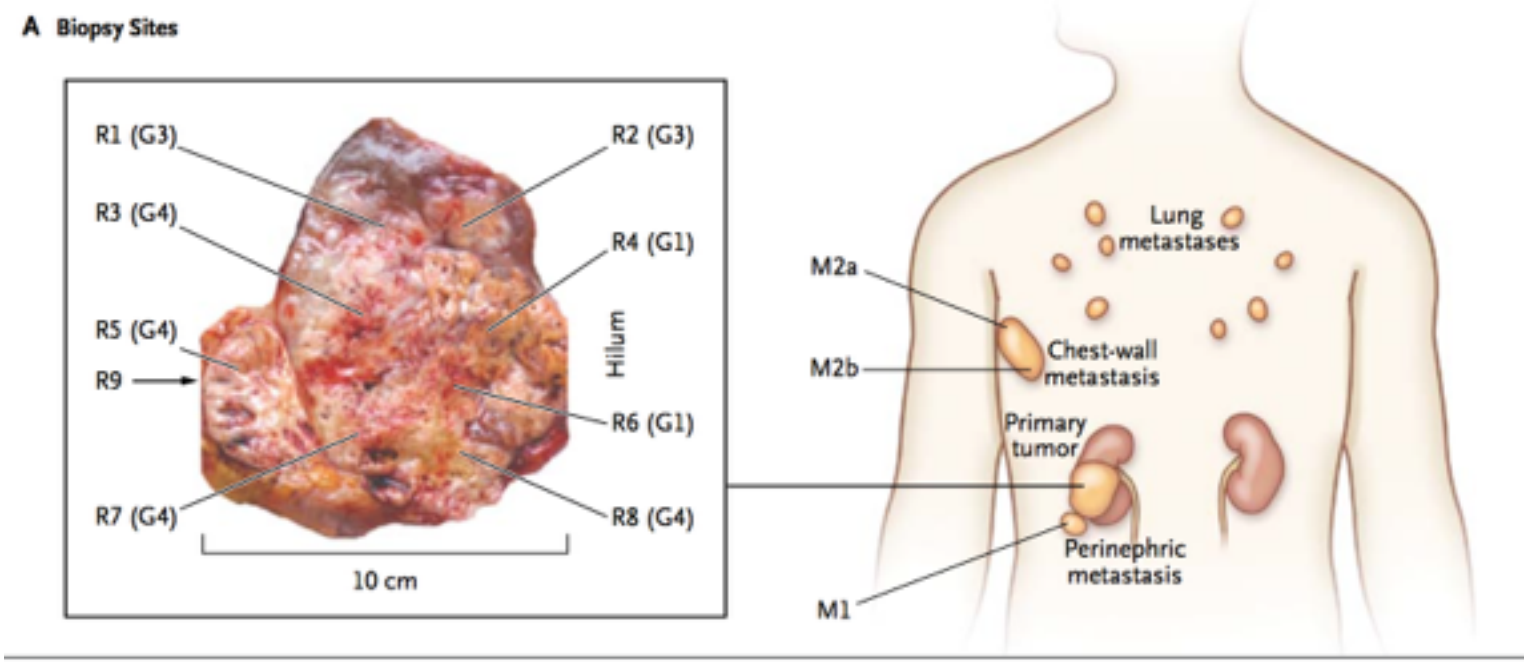
- Tumor heterogeneity
- Complexity of identifying and validating predictive molecular biomarkers
- Technical limitations of molecular tests
- Slow progres in unraveling the biology of some types of cancer and resistance mechanisms
- High costs of targeted agents
- Reimbursement and regulatory issues

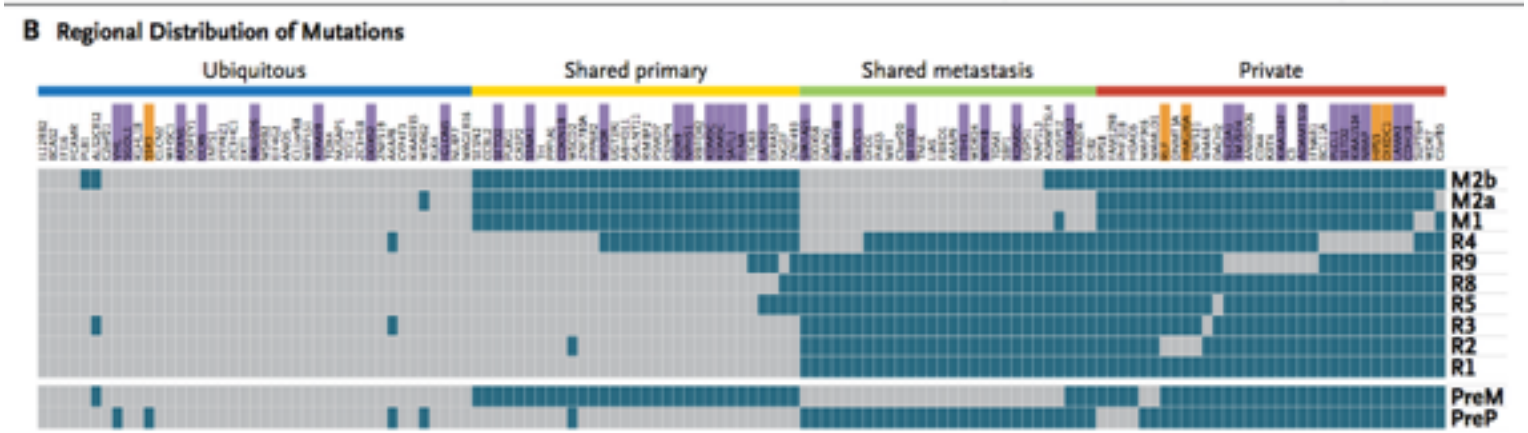
Patterns of tumor heterogeneity



Seoane, Journal Intern Med 2014

Intratumor heterogeneity and branched evolution





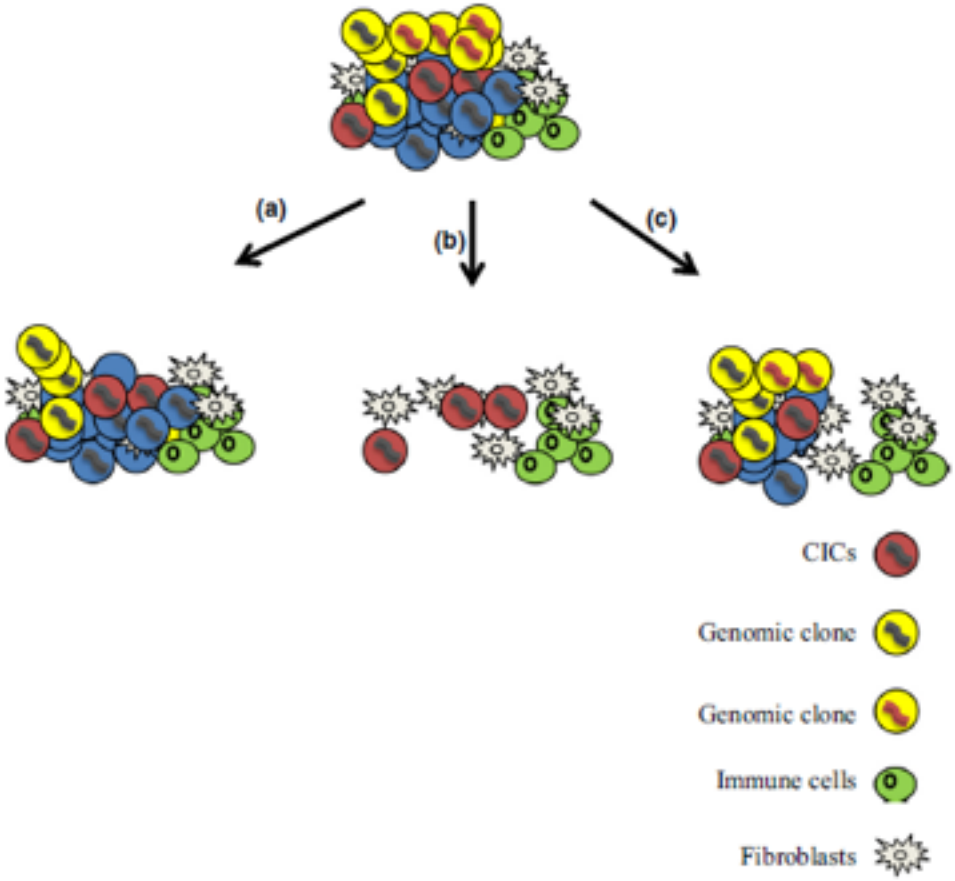
Gerlinger, NEJM 2012

Functional heterogeneity and treatment impact on clone selection (Darwinian model)

a) Genomic selection (treatment affects a cellular clone with specific alteration)

b) Epigenomic selection (treatment affects a cellular compartment with specific epigenetic status)

c) Micrenvironment selection (treatment affects cells present in a specific stromal niche)



Seoane, Journal Intern Med 2014

Types of tumor heterogeneity

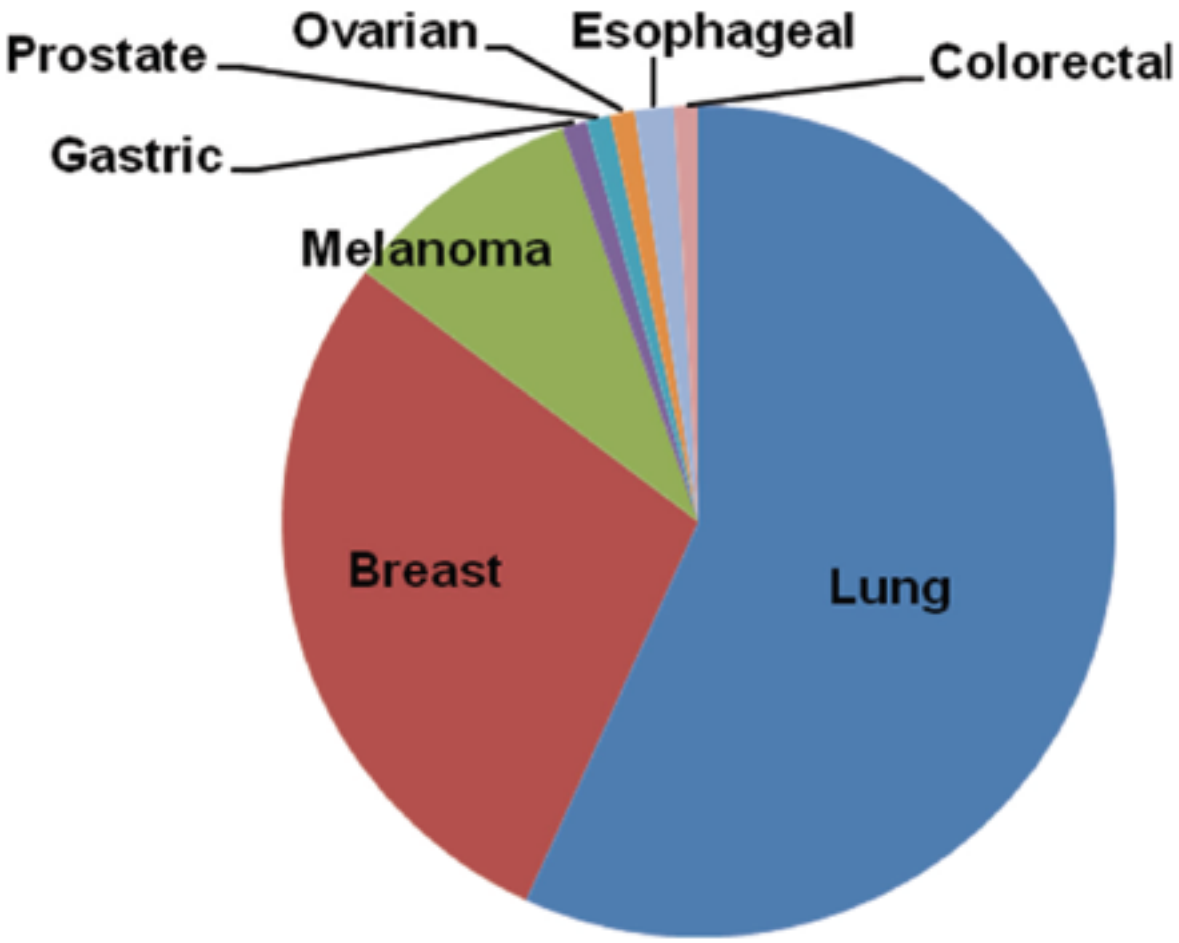
- Spatial
- Temporal

Tumor heterogeneity across various malignancies

Malignancy	Description	Heterogeneity
NSCLC	*EGFR*	Intratumor
Melanoma	*BRAF*^{V600E}	Intra- and intertumor
Triple negative breast cancer	*TP53*, *PIK3CA*, *PTEN*	Intratumor
Renal cancer	Branched evolutionary growth through multiregion exome sequencing	Intra- and intertumor
Ovarian cancer	Key driver gene alterations (e.g., *PIK3CA*, *CTNNB1*, *NF1*)	Intratumor

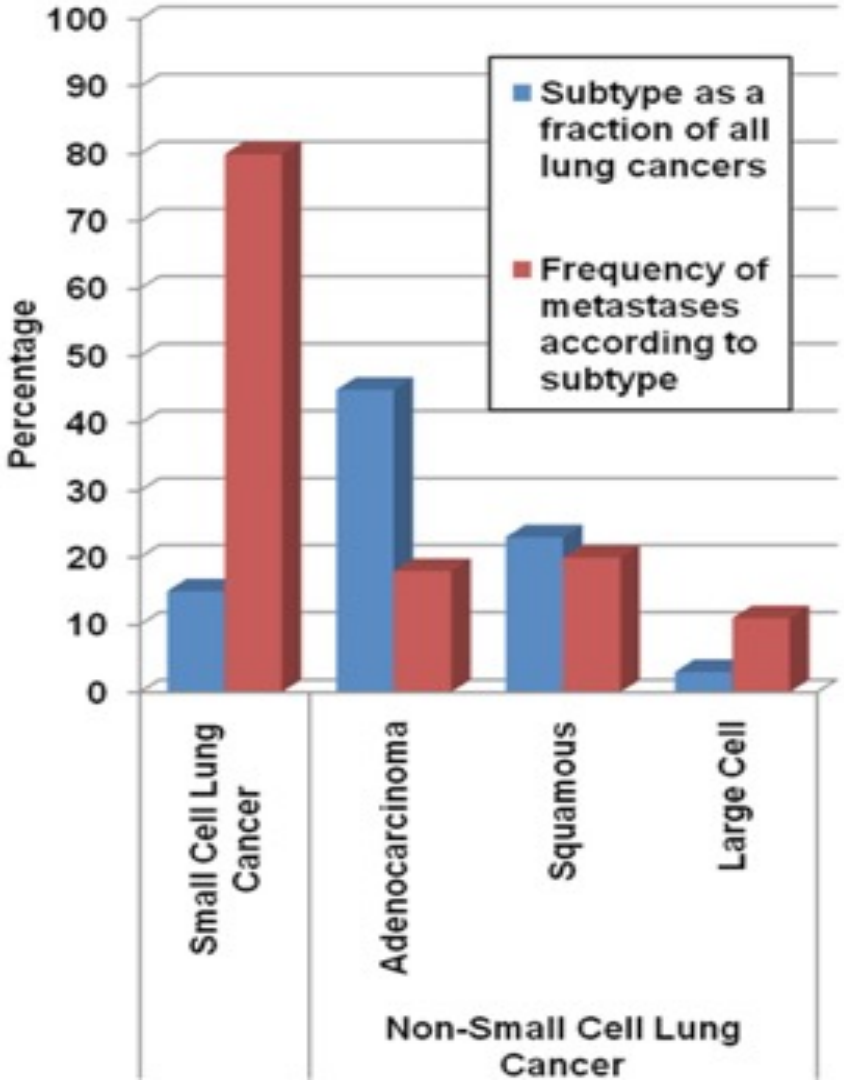
Tanigami, Cancer Sci 2008; Yancovitz, PLoS ONE 2012; Shah, Nature 2012; Nike-Zainal, Cell 2012; Gerlinger, N Engl J Med 2012; Bashashati, J Pathol 2013

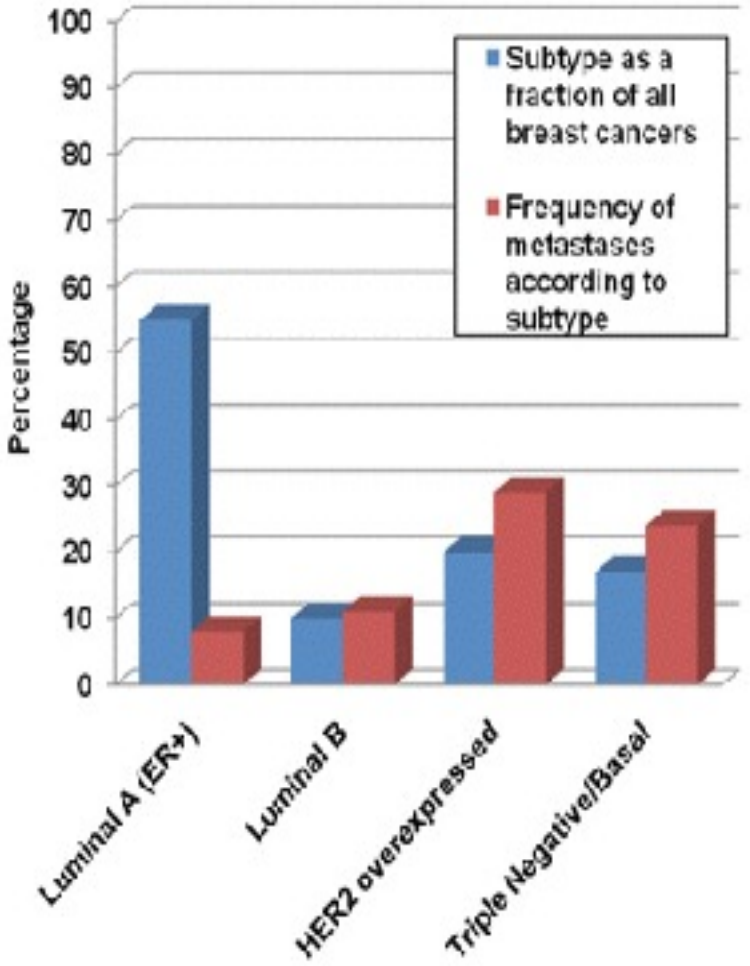
Breakdown of brain metastates according to originating cancer



Bollig-Fischer, OA Molecular Oncology 2013

Frequency of brain metastases according to lung and breast cancer subtypes





Bollig-Fischer, OA Molecular Oncology 2013

Current brain metastases management

- Neurosurgery or radiosurgery possible in a minority of patients
- Whole brain irradiation assosciated with considerable toxicity and QoL deterioration
- Chemotherapy ineffective due to limited blood-brain barrier penetration
- Molecular therapies critically under-utilized
- Paucity of prospective clinical studies allowing or addressing patients with brain metastases
- Dire need for more effective therapies

Difficulties in clinical research on brain metastases heterogeneity

- Brain metastases are not commonly biopsied or resected and the availability of clinical samples is limited
- Potential selection bias (prevalence of small tumors)
- Variability of tumor cell-intrinsic mechanisms contributing to growth of brain metastases
- Most studies based on archival formalin-fixed material, primary tumor datasets with brain relapse data
  or experimental models
- Impact of specific brain microenvironment
- Confounding effect of prior therapies

Potentially actionable alterations in BM

Gene	No. cases	Alteration	Breast–BM	Lung–BM	Mel–BM	Oes–BM	Drugs	Reference
KRAS	8	Amp		Q755, Q822			MEK/PI3K/mTORi[c]	
		p.G12V		Q631 *(68.32)*, Q782 *(79.59)*			MEKi[a], PI3K/mTORi[b]	[110,111]
		p.G12C		Q757 *(62.83)*				
		p.G13C		Q747 *(89.01)*				
		p.G13D		Q756 *(91.36)*				
		p.Q61H		Q739 *(67.15)*				
PIK3CA	8	Amp	Q851	Q630, Q634, Q637, Q822			PI3K/AKT/mTORi[a]	[112]
		p.H1047R	Q678 *(25.64)*, Q751 *(64.29)*,					
PTEN	2	CN-loss	Q639, Q772*				PI3K/AKT/mTORi[b]	[116]
AKT1	1	Amp		Q756			AKT/mTORi[c]	
AKT3	1	Amp	Q851				AKT/mTORi[c]	
AURKA	1	Amp		Q755			CDK/AURKAi[c]	
BRCA1	1	CN-loss	Q639*				PARPi[a]	[117]
CCND1	1	Amp				Q734#	CDKi[c]	
CDK4	1	Amp		Q782			CDKi[c]	
HRAS	1	Amp	Q678				PI3K/RAF/MEKi[c]	
KIT	1	*p*.L572P			Q746 *(99.39)*		KITi eg imatinib[c]	
MEK1	1	Amp		Q737			PI3K/RAF/MEKi[c]	

Potential drug targets in 31/36 cases (variuos malignancies), including 25 with clinical evidence supporting a genotype–drug efficacy relationship

Saunus J Pathol 2015

NON-SMALL CELL LUNG CANCER

EGFR activating mutations in paired primary and metastatic NSCLC (67 EGFR TKI-naive patients)

- Loss of *EGFR* mutation: 9/18 (50%)
- Gain of *EGFR* mutation: 17/26 (65%)
- Overall discordance rate: 18/67 (27%)
- 12 BM: 1 loss (8%), 7 gains (58%), 4 concordant (33%)

Gow, Ann Oncol 2009

EGFR mutations in paired primary tumors and brain metastases

Author	N	*EGFR* activating mutations in BM	Concordance in matched pairs
Matsumoto	19	63%	6/8
Han	5	60%	4/5
Wojas-Krawczyk	143	6.3%	32/32
Munfus-McCray	10	40%	Highly concordant

Matsumoto, Int J Cancer 2006; Han, Clin Lung Cancer 2011; Wojas-Krawczyk, Clin Exp Metastasis 2013; Munfus-McCray, Hum Pathol 2011

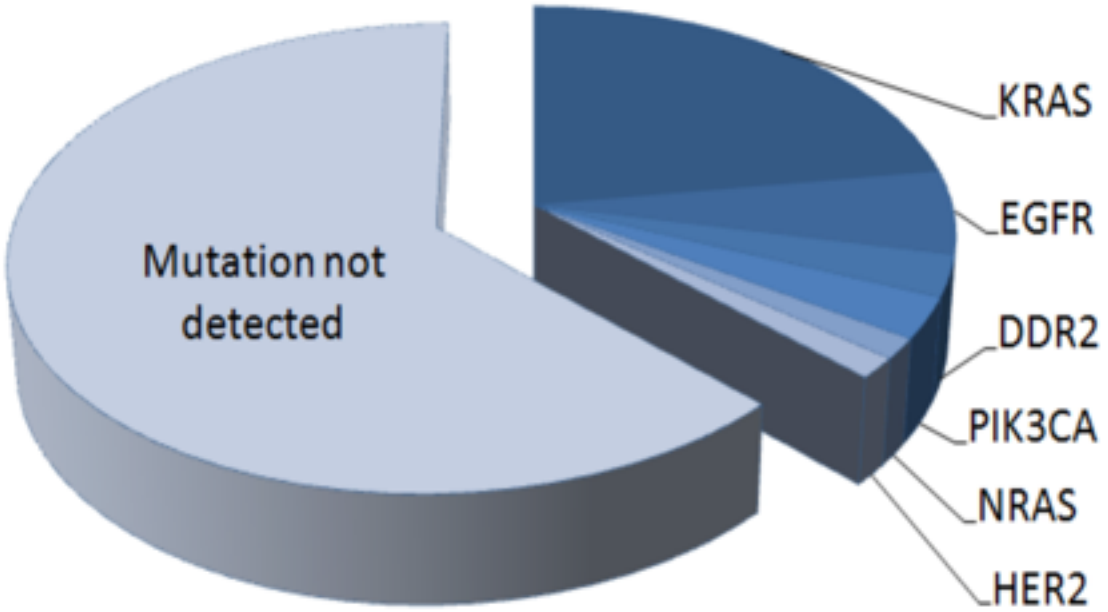
Efficacy of first-generation EGFR inhibitors in NSCLC brain metastases

Study	Treatment	Selection	Phase	*N*	RR (%)
Unselected patients					
Ceresoli et al. (51)	Gefitinib	European	II	41	27
Wu et al. (52)	Gefitinib	East Asian, adenocarcinoma	II	40	32
Selected patients					
Hotta et al. (53)	Gefitinib	East Asian	II	57	43
Porta et al. (54)	Erlotinib	*EGFR* mutation	II	69	82
Kim et al. (55)	Gefitinib or erlotinib	*EGFR* mutation, East Asian, adenocarcinoma	II	23	70
Li et al. (41)	Gefitinib	*EGFR* mutation, East Asian	II	110	89
Wu et al. (57)	Erlotinib	East Asian, *EGFR* mutation, and/or adenocarcinoma	II	48	56
Kim et al. (60)	Gefitinib or erlotinib	East Asian, never-smoker, adenocarcinoma	II	23	74

Hanjani et al. Clin Cancer Res 2012

Driver mutations present in 36% (52/145) of NSCLC brain metastases

Gene	Mutations incidence
KRAS	34 (23%)
EGFR	9 (6%)
DDR2	3 (2%)
PIK3CA	3 (2%)
NRAS	2 (1.5%)
HER2	1 (1%)
BRAF	not detected



Nicoś, WCLC 2015; P3.04-033

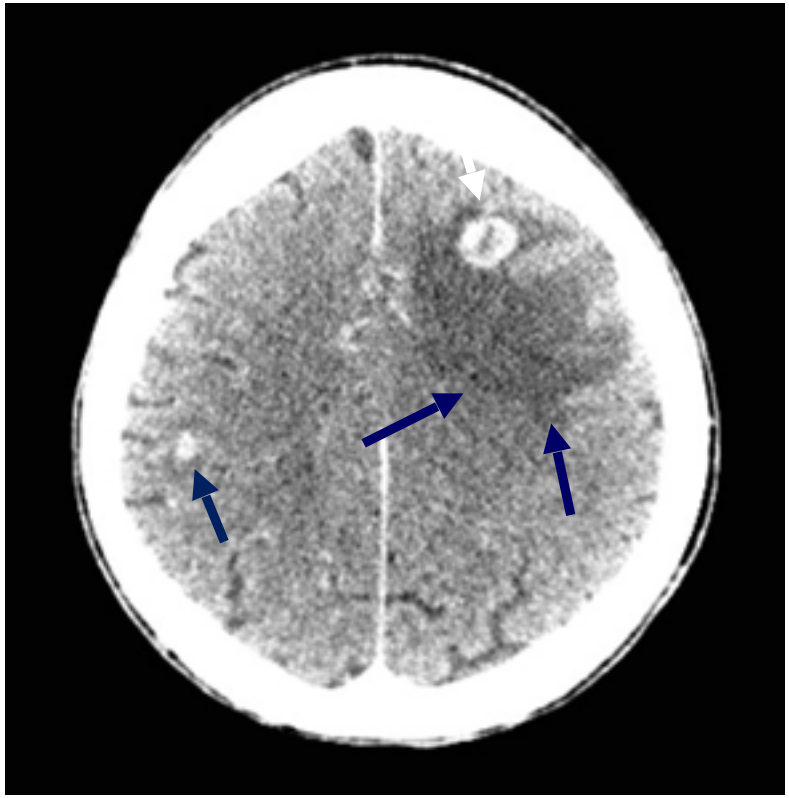
ALK/EML4 alterations in brain metastases

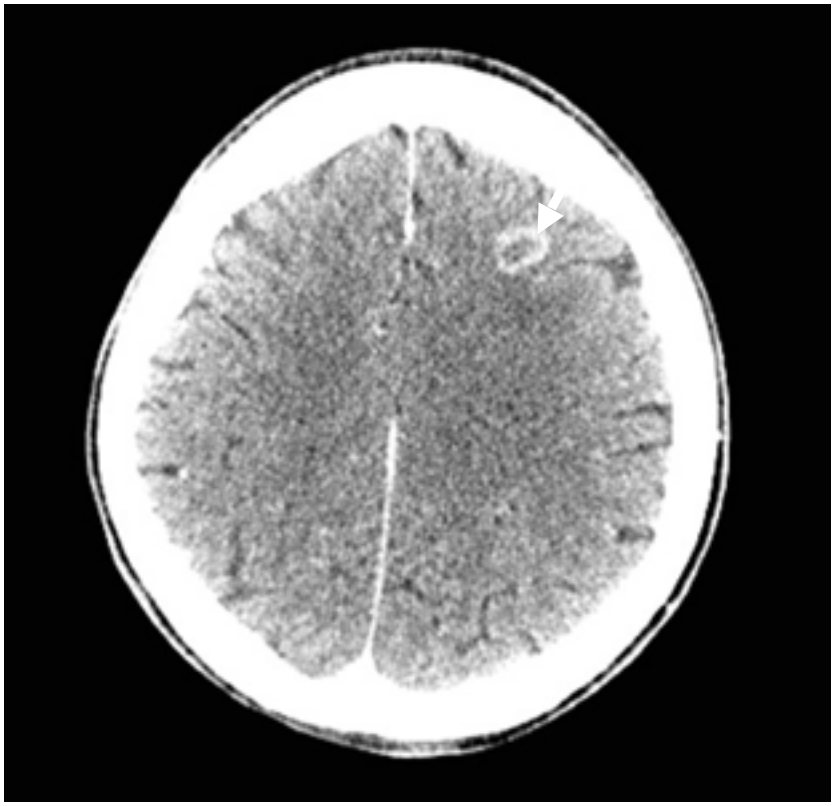
Tumor type	FISH *ALK* translocation	FISH *ALK* amplification	IHC ALK+	FISH *EML* translocation not involving *ALK*
Adenocarcinoma	4/151 (2.6%)	16/151 (10.6%)	4/179 (2.2%)	3/151 (2%)
Squamous cell cancer	0/5	2/5	0/18 (0%)	0/5
Adenosquamous cancer	1/9	0/9	1/13 (7.7%)	1/9
Large cell carcinoma	0/7	1/7	0/8	1/7
Neuroendocrine large cell carcinoma	0/3	0/3	0/3	0/3

Assay	Matched pairs (N)	Primary tumor	Brain metastases
FISH *ALK-EML* translocation	16	2	2
FISH *ALK* amplification	14	0	2
IHC ALK	29	2	2

Preusser, Lung Cancer 2013

Response to crizotinib in brain metastases in a patient with ALK rearrangement

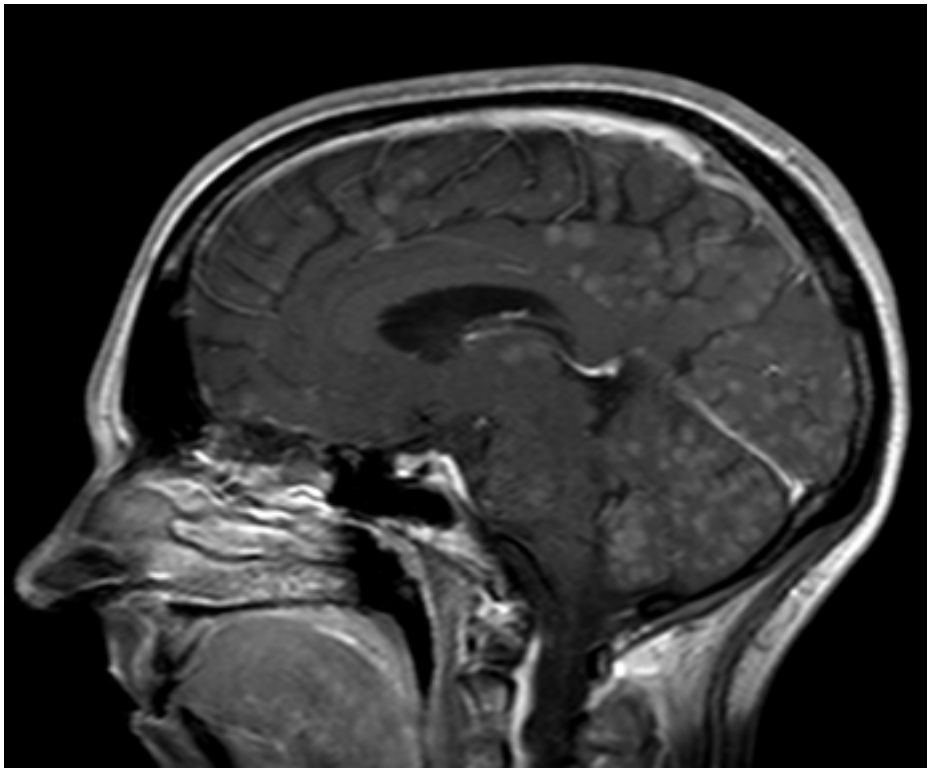




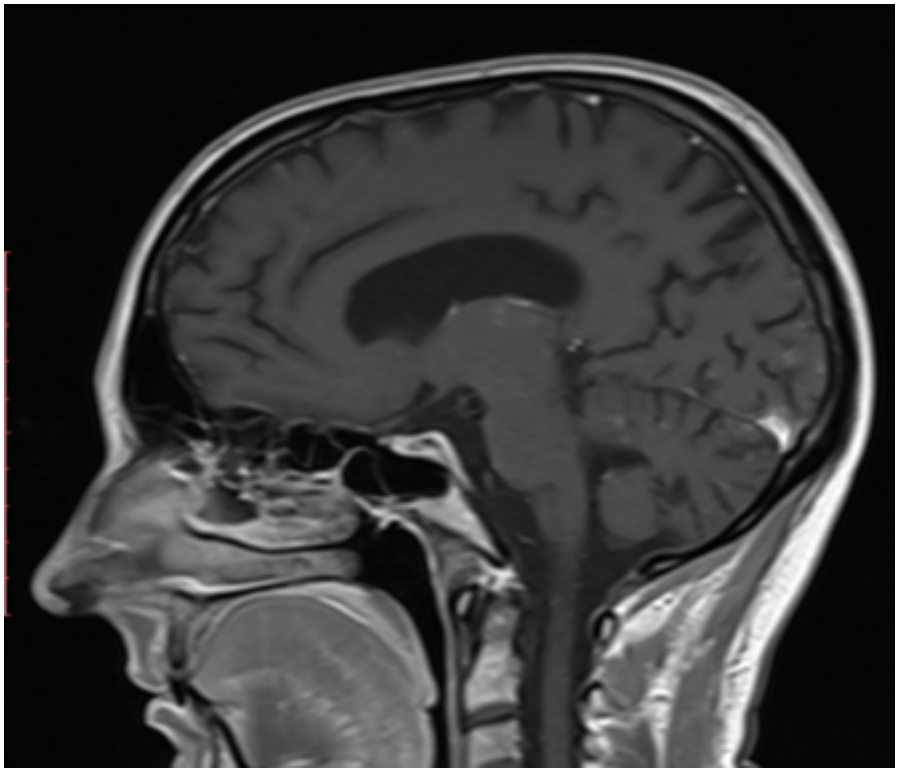
Crizotinib penetration to CSF <1%

Medical University of Gdańsk series; Courtesy Dr Dziadziuszko

Miliary brain metastases in a patient with *ROS1* rearrangement



After 6 months on crizotinib

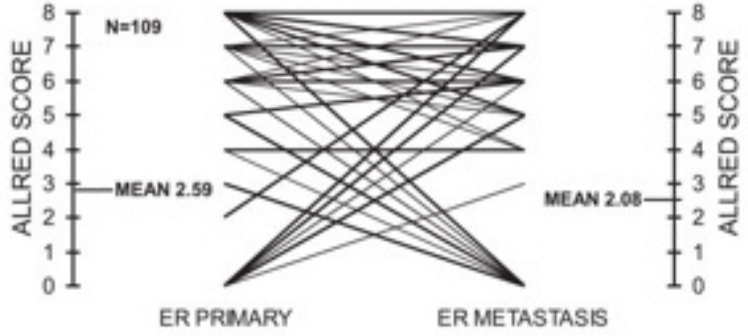


After WBRT and another 4 months on crizotinib

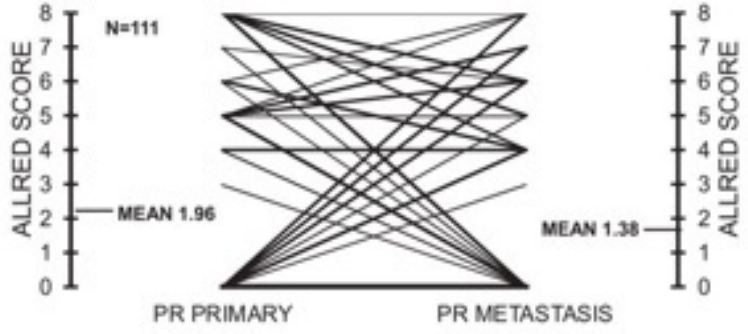
Dziadziuszko et al, J Thorac Oncol 2015

BREAST CANCER

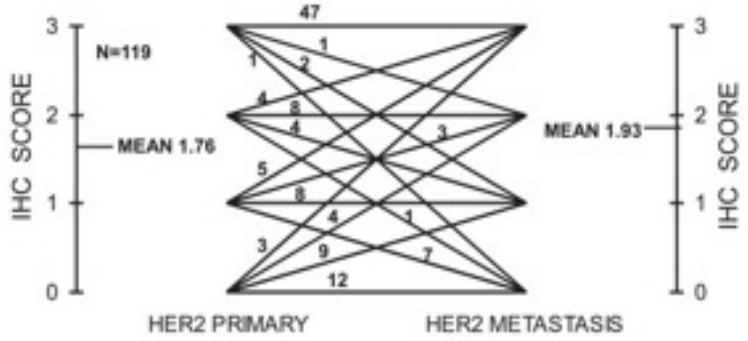
ER, PR and HER2 phenotypes in 120 paired primary tumors and brain metastases



ERα conversion rate: 29%;
43% loss, 19% gain (P=0.005)
Impact of hormonal therapy (P=0.021



PR conversion rate: 29%;
56% loss, 14% gain (P < 0.001)
Impact of hormonal therapy (P=0.001



HER2 conversion rate: 14%;
12% loss, 14% gain (P < 0.60)
No impact of trastuzumab

Duchnowska, Breast Cancer Res 2012

IHC vs. FISH analysis in paired primary tumors and BM

Immunohistochemistry

Case No.	Primary	Metastasis a	Metastasis b	Metastasis c
5	0 (0.0)	2+ (2.4)	2+ (2.6)	. . .
6	0 (0.0)	0 (1.2)	0 (0.7)	0 (0.0)
8	2+ (1.9)	0 (0.2)	0 (0.0)	0 (0.0)
12	0 (0.0)	2+ (1.7)	2+ (1.2)	3+ (3.1)
13	1+ (0.6)	2+ (1.9)	2+ (2.0)	. . .
17	3+ (4.0)	3+ (2.8)	. . .	. . .
21	1+ (0.4)	2+ (2.0)	. . .	. . .
26	3+ (4.0)	3+ (5.3)	. . .	. . .
27	0 (0.0)	0 (0.0)	. . .	. . .
29	2+ (1.2)	0 (0.0)	. . .	. . .
30	2+ (2.2)	1+ (0.7)	. . .	. . .
31	0 (0.0)	0 (0.0)	. . .	. . .

8/12 discrepant

FISH

Case No.	Primary	Metastasis a	Metastasis b	Metastasis c
5	2.3	2.5	2.4	. . .
12	1.2	1.2	1.2	1.2
13	1.2	1.4	. . .	. . .
17	4.1	5.7	. . .	. . .
21	1.0	1.2	. . .	. . .
26	10.1	10.1	. . .	. . .
27	1.2	1.1	. . .	. . .
29	1.1	1.2	. . .	. . .
30	2.6	2.2	. . .	. . .
31	1.3	1.5	. . .	. . .

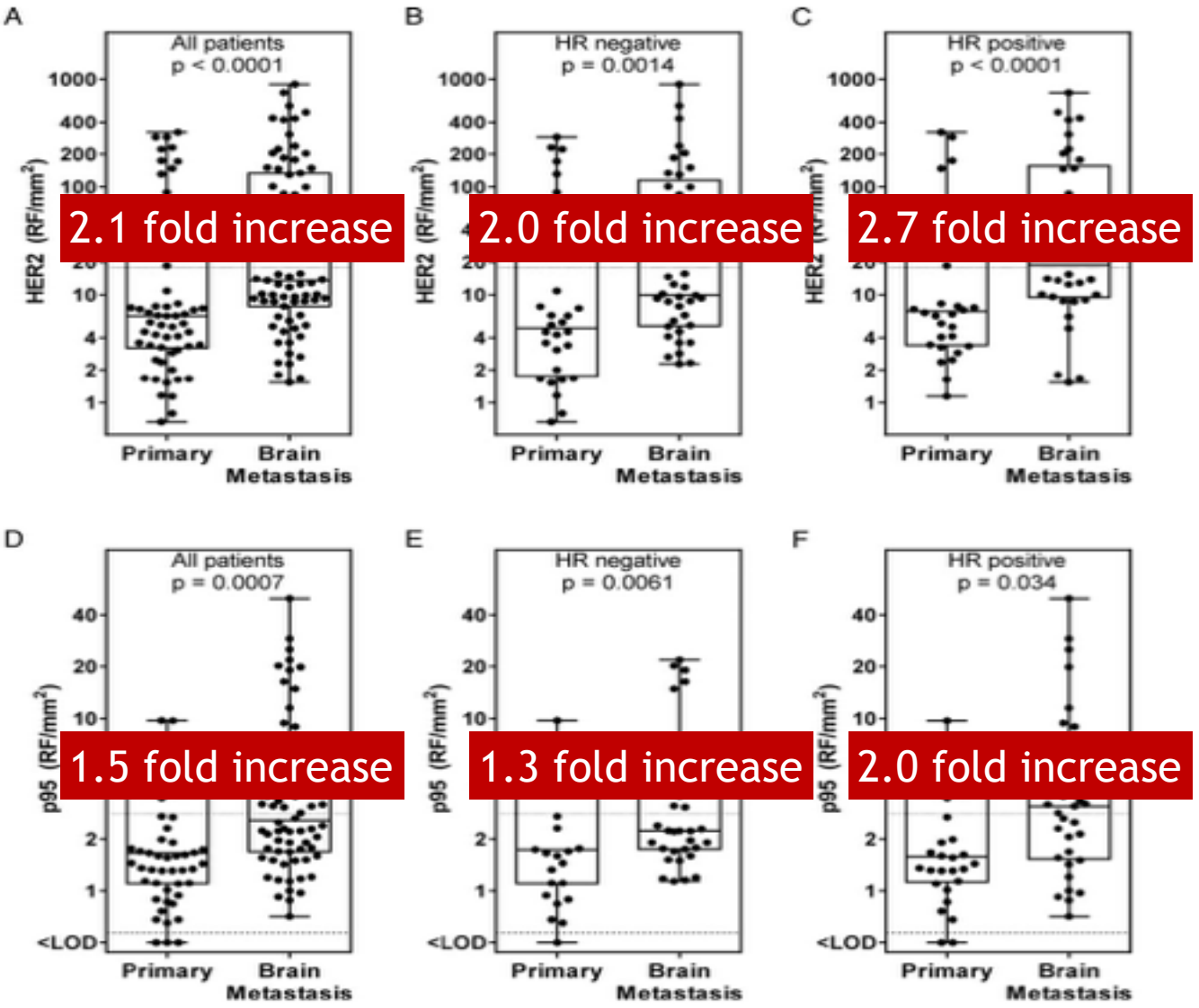
None discrepant

Lear-Kaul, Arch Pathol Lab Med 2003

Quantitative HER2 and p95HER2 levels in 75 paired primary tumors and brain metastases

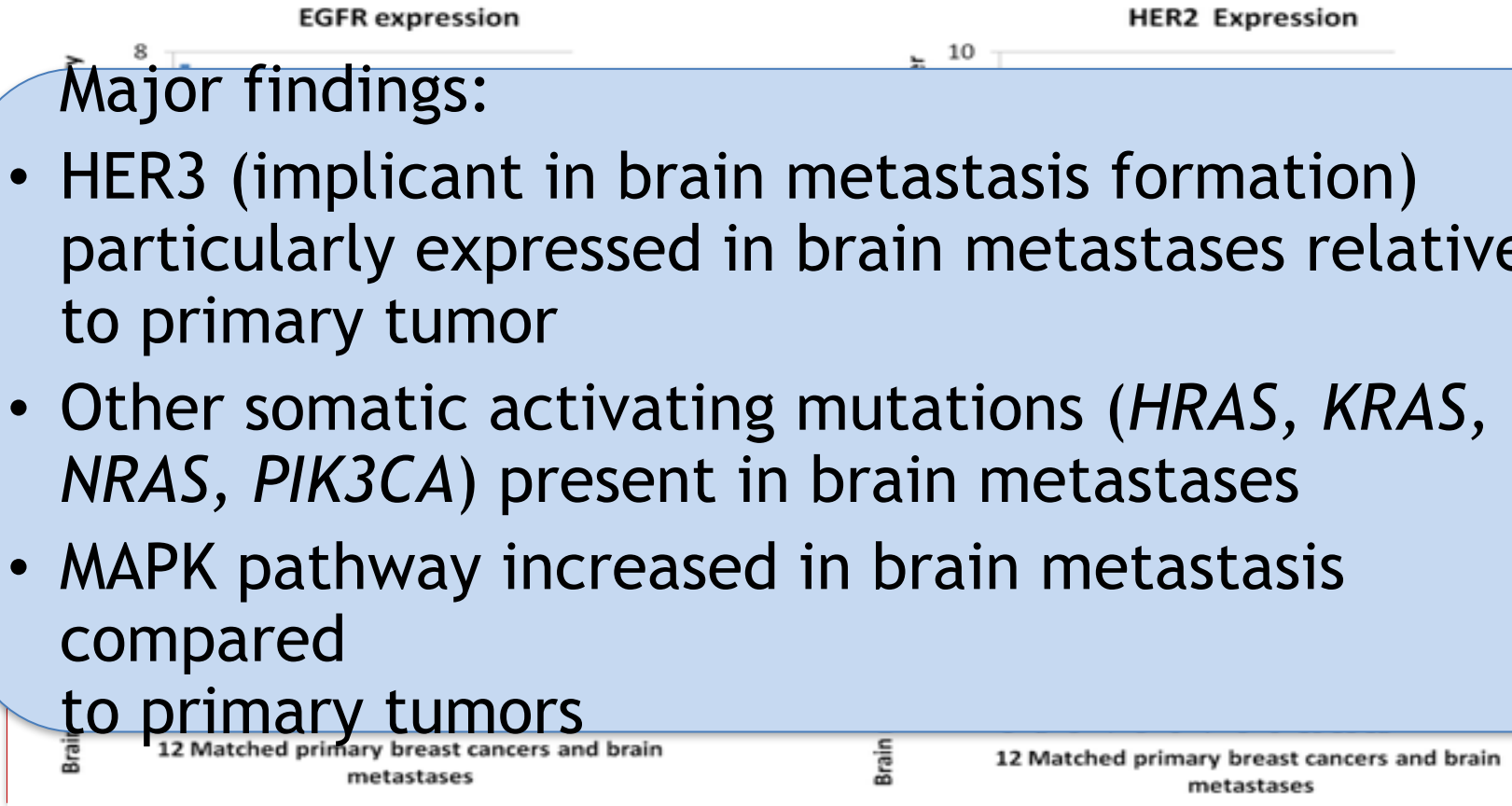
HER2

p95



Duchnowska, Neuro-Oncol 2015

RT-PCR and DASL for *HER* family genes in 12 paired primary tumors and brain metastases



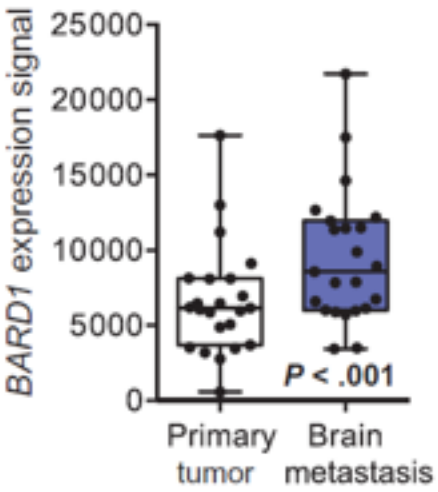
Major findings:

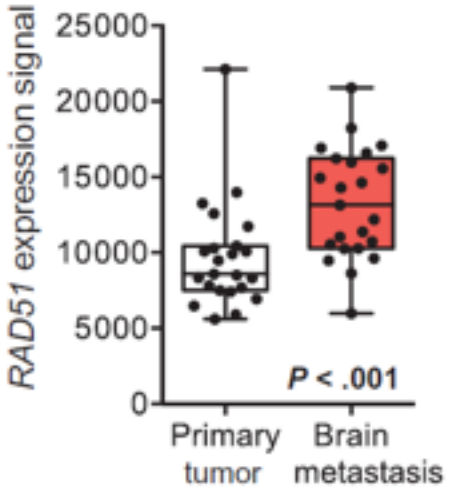
- HER3 (implicant in brain metastasis formation) particularly expressed in brain metastases relative to primary tumor
- Other somatic activating mutations (*HRAS*, *KRAS*, *NRAS*, *PIK3CA*) present in brain metastases
- MAPK pathway increased in brain metastasis compared to primary tumors

Summary of RT-PCR and DASL assay for 12 matched samples – Positive values indicate increased fold change in metastases and negative values increased fold change in primary cancers

Da Silva, Breast Cancer Res 2010

Expression of the DNA repair genes: *BARD1* and *RAD51* in 23 paired primary tumors and BM

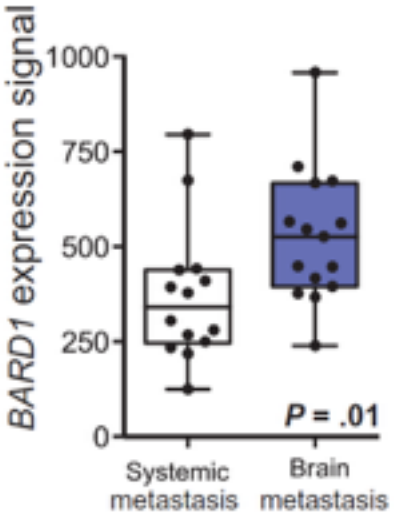


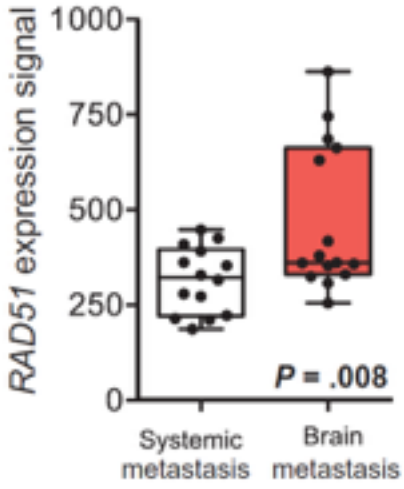


BM vs. primary tumor

BARD: 1.74 fold; p<.001

RAD51: 1.46 fold; p<.001





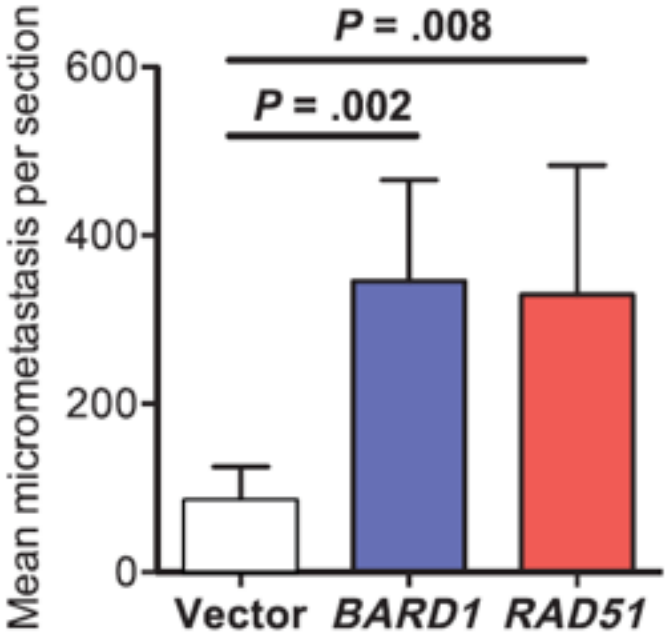
BM vs. other metastatic sites

BARD: 1.49 fold; p=.01

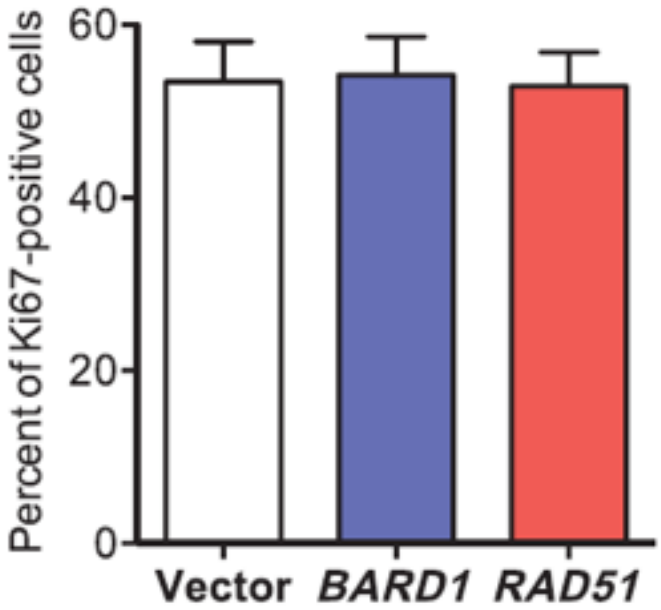
RAD51: 1.44 fold; p=.008

Woditschka, JNCI 2014

Effect of *BARD1* and *RAD51* overexpression in MDA-MB-231-BR cells on brain vs lung metastases development (mouse model)



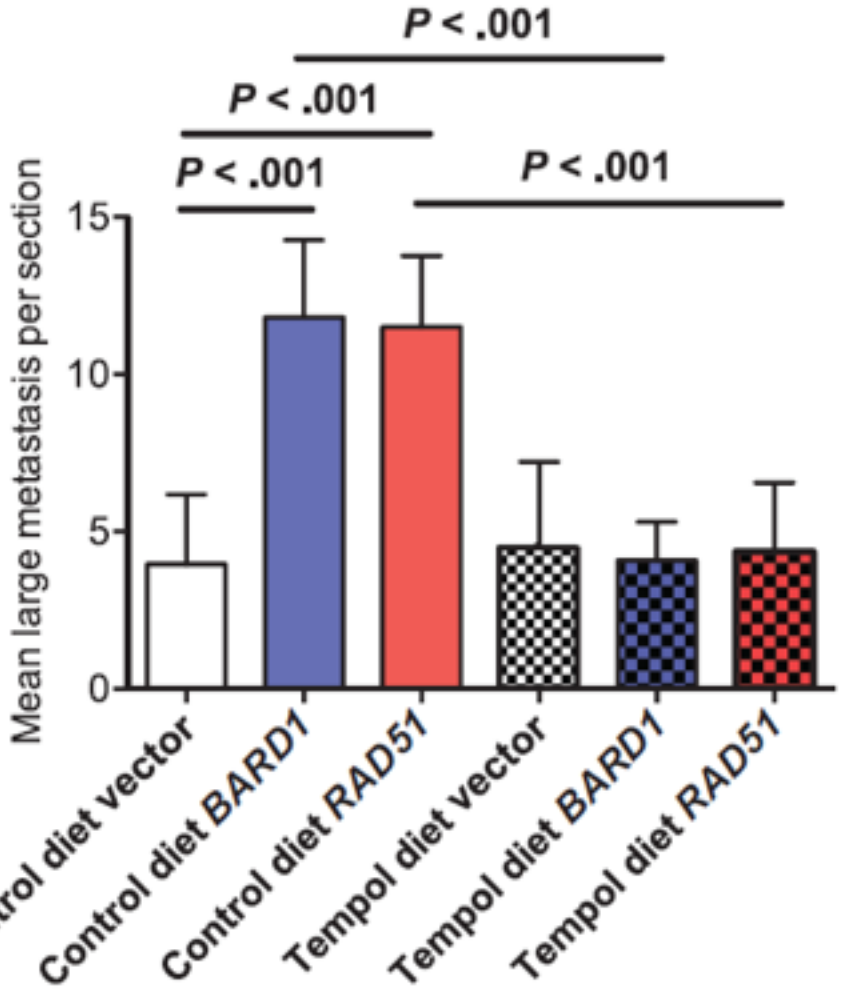
Brain metastases



Lung metastases

Woditschka, JNCI 2014

Supression of brain metastases (induced by *BARD1* and *RAD51* overexpression) by tempol, a brain-permeable oxygen radical scavenger (mouse model)

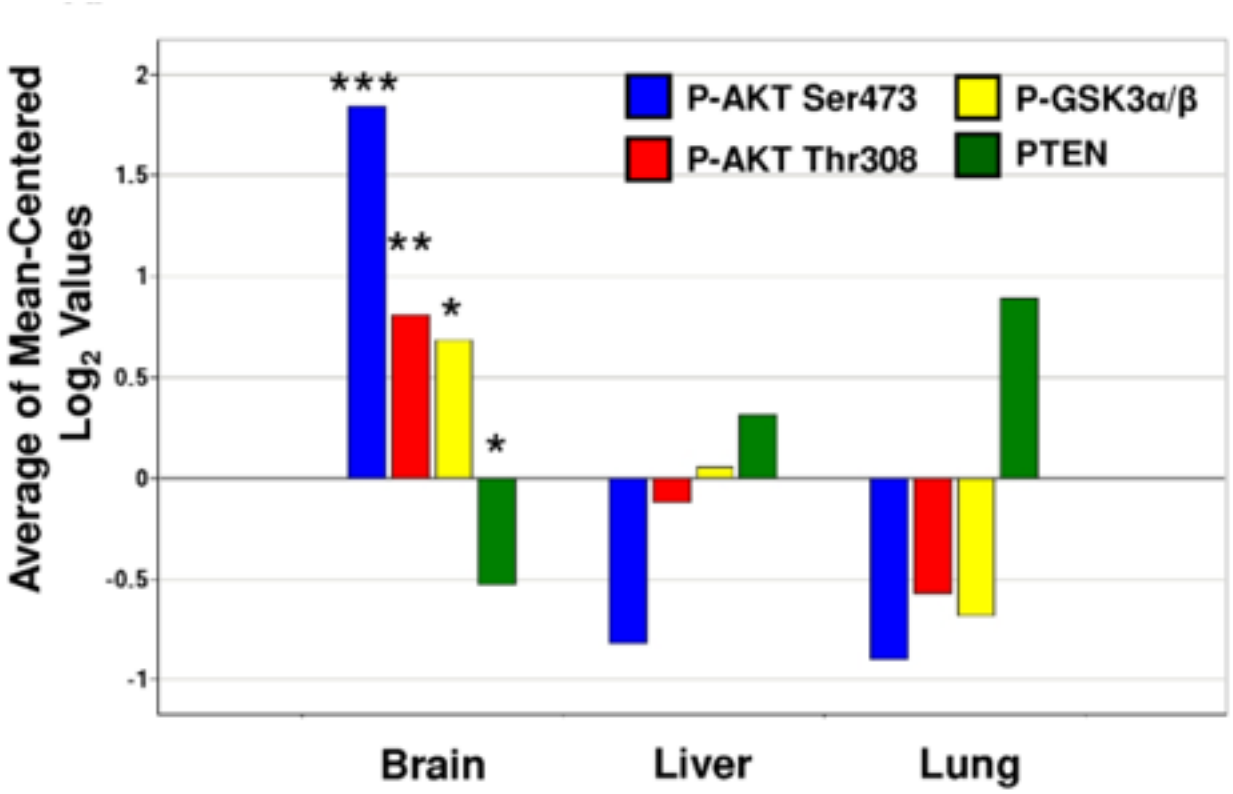


Woditschka, JNCI 2014

MELANOMA

AKT activation by metastatic site

Distant metastases (10 brain, 5 liver, and 5 lung)



P-AKT-Ser473 in BM
- vs. liver (*P*=.002)
- vs. lung (*P*=0.008)

P-AKT-Thr308 in BM
- vs. liver (*P*=.04)
- vs. lung (*P*=.01)

P-GSK3α/β in BM
- vs. lung (*P*=.003)

PTEN in BM
- vs. lung (*P*=.05)

Davies, Clin Cancer Res 2009

Consistency between *BRAF/NRAS* mutation status in primary and secondary melanomas

Site	N	Consistency
Lymh nodes	84	93%
Visceral	25	96%
Brain	20	80%
Skin	36	75%

Colombino, J Clin Oncol 2012

COLON CANCER

Prevalence of *BRAF*, *KRAS*, *NRAS* and *PIK3CA* mutations in colorectal cancer metastases

Mutation	Liver	Lung	Brain	P
BRAF	3.1%	0%	6.5%	0.184
KRAS	32%	62%	57%	0.003
NRAS	7.7%	6.0%	4.3%	0.917
PIK3C	7.7%	20%	24%	0.044

High concordance of *KRAS* mutations between primary cancer and corresponding brain metastases

Tie, Clin Cancer Res 2011

Summary

- Tumor heterogeneity poses a challenge for cancer treatment managing
- Current strategies based on single tumor-biopsy samples may hinder personalized-medicine approaches
- Sampling bias may impact treatment selection
- Comprehensive assessment of tumor partraits may facilitate individual treatment decisions
- Assessment and quantification of inter- and intratumor heterogeneity remains logistic and clinical challenge

Let's join efforts!