



UniversitätsSpital
Zürich

BRAIN METASTASES 2018

**INTEGRATIVE APPROACH TO PRECISION
PATHOLOGY**

OUTLINE

Background

Aims of pathological examination

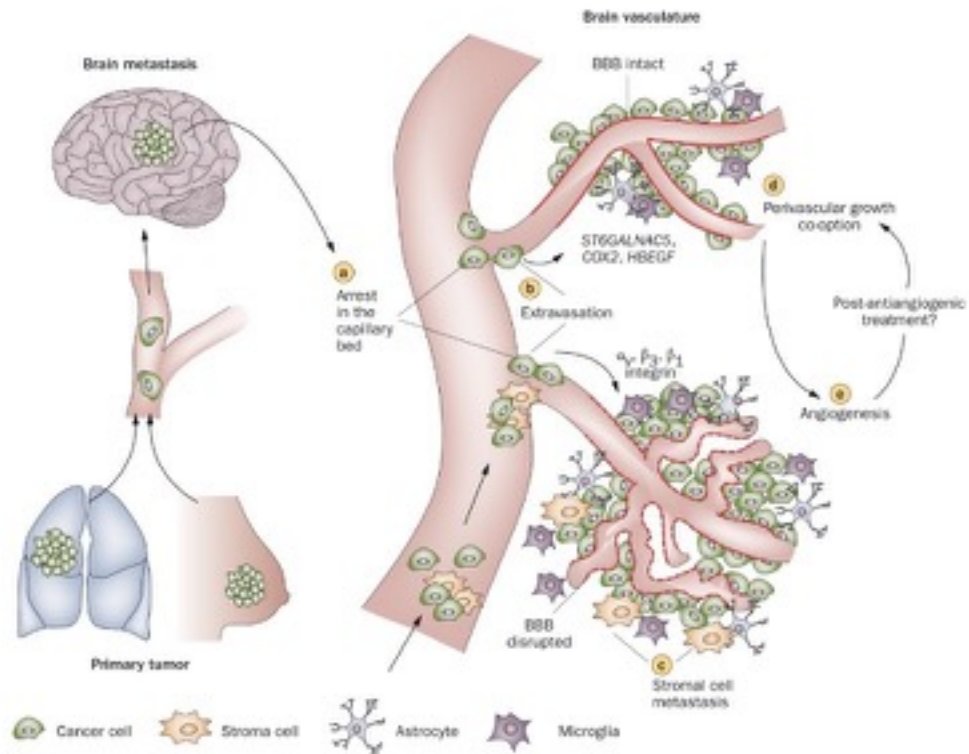
Microscopic approach

Molecular methods

Limitations

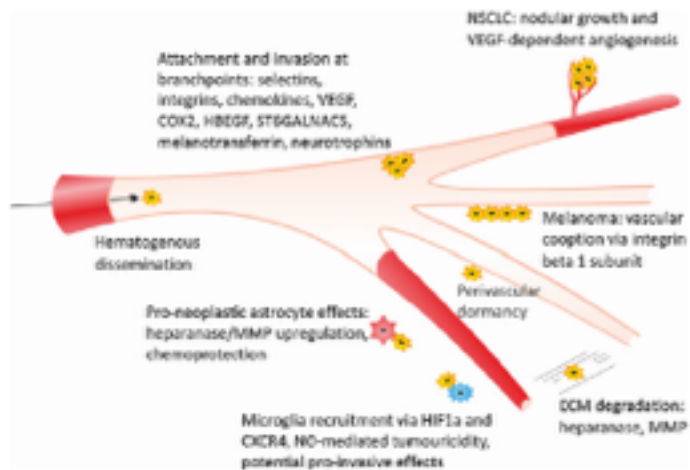
Outlook

THE PROCESS

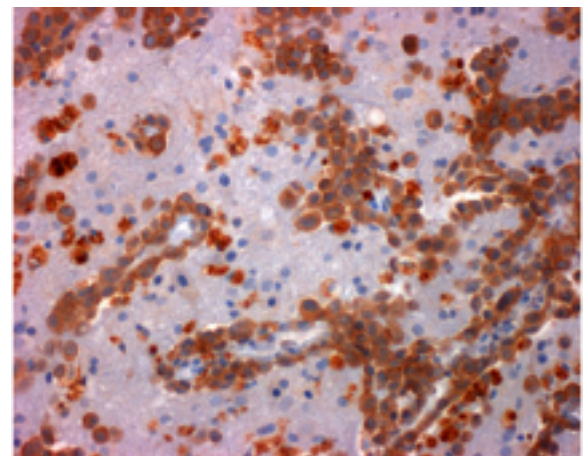
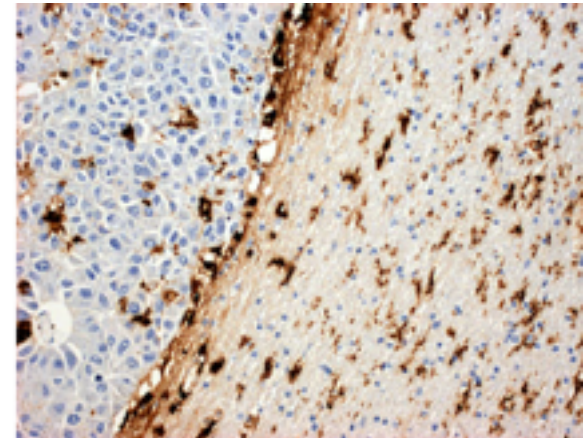


Eichler AF. Nat. Rev. Clin. Oncol. 2011

THE PROCESS

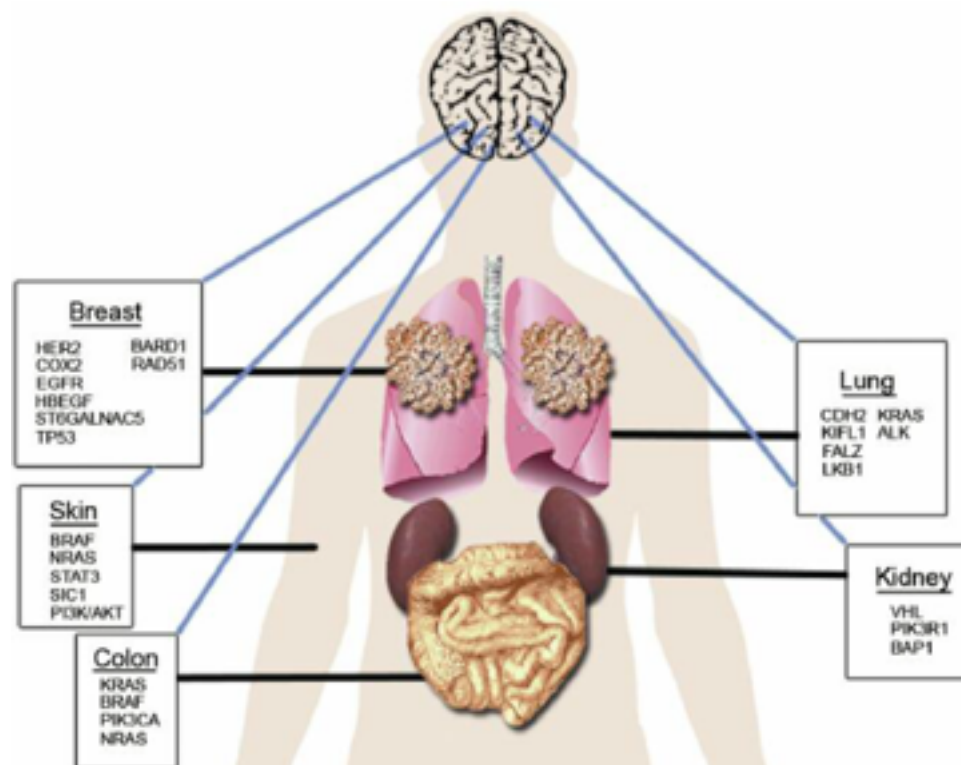


Preusser, 2018



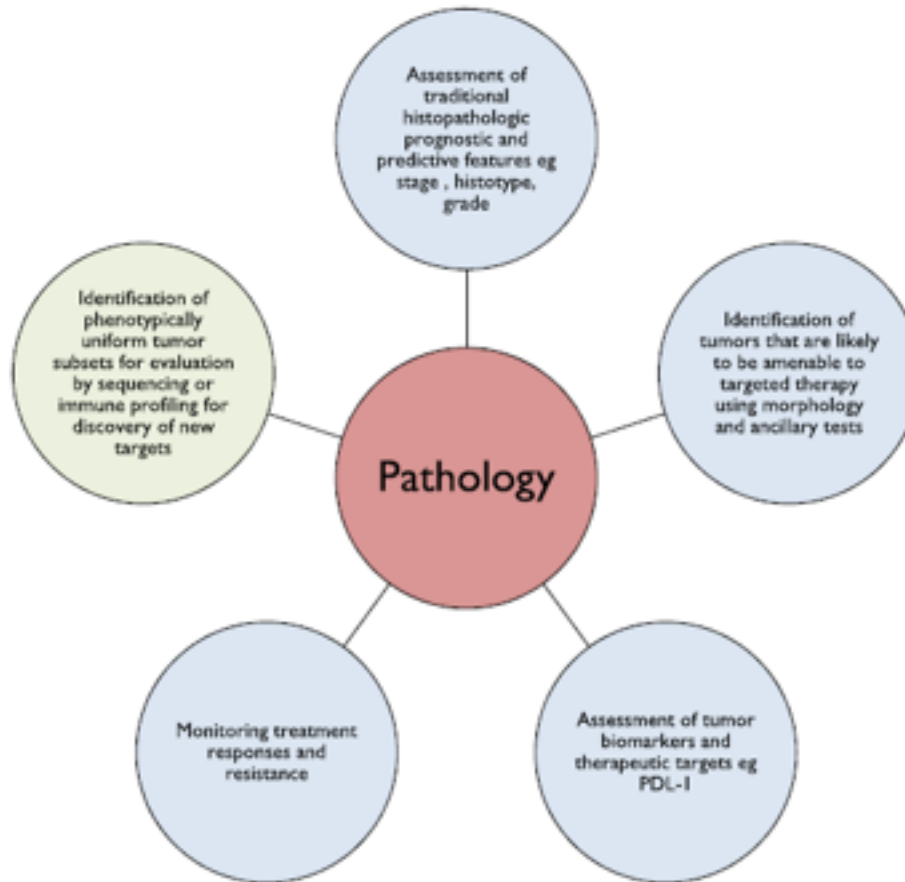
BRAFV600E (VE1)

MARKERS PREDICTIVE OF METASTASIS



Cooper, 2018

IS PATHOLOGIC DIAGNOSIS IMPORTANT?



THE USUAL SUSPECTS

Carcinoma/ neuroendocrine tumor

Adenocarcinoma

Poorly differentiated carcinoma

Squamous cell/ transitional cell carcinoma

Neuroendocrine tumor

Melanoma

Lymphoma

Extragonadal germ cell tumor

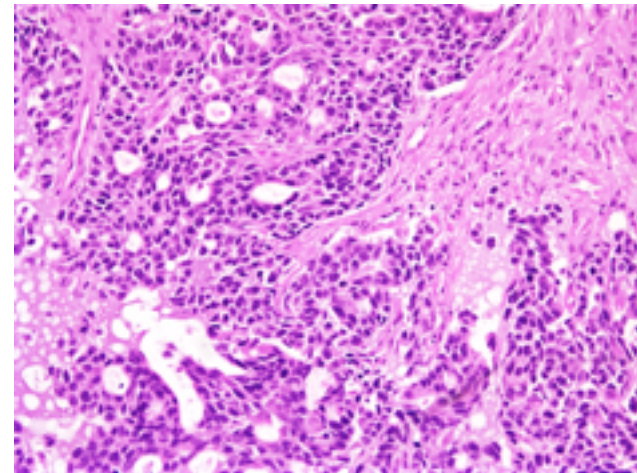
Sarcoma

Undifferentiated «cancer of unknown primary»

BASIC HISTOPATHOLOGY

Growth pattern

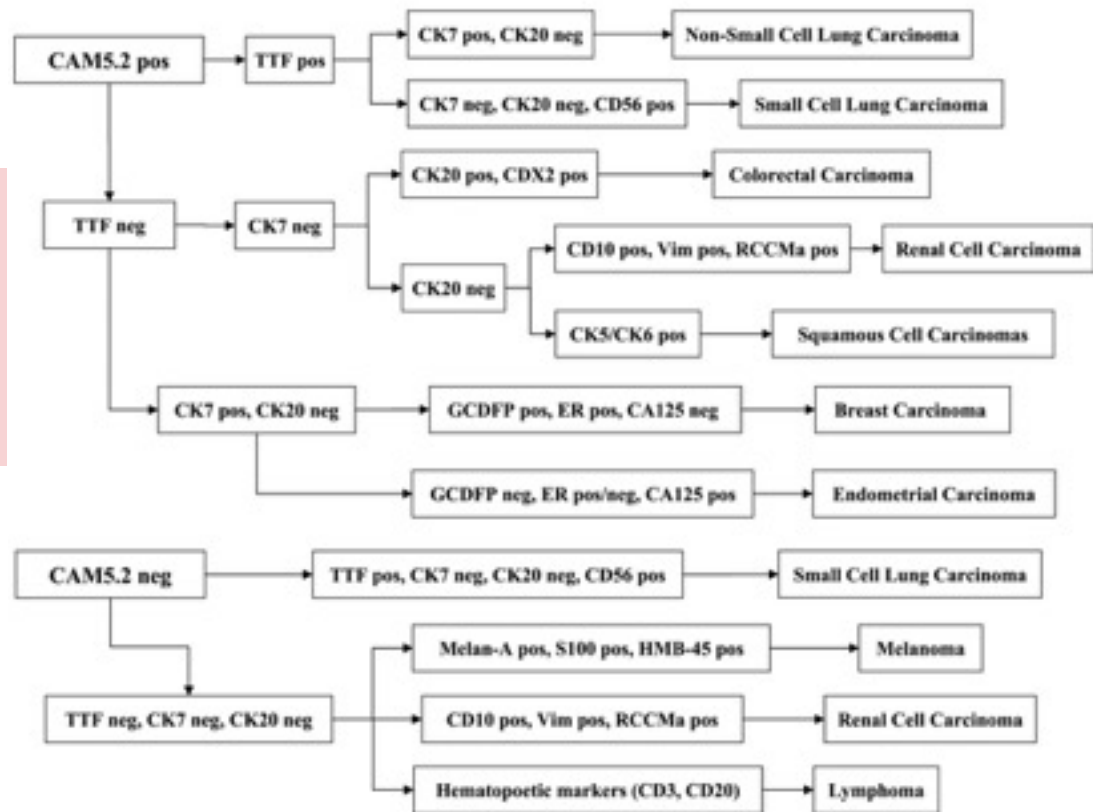
- **Epithelial/epithelioid:** carcinoma, “epithelioid” sarcoma, melanoma, glioma
- **Spindle cell:** sarcomatoid carcinoma, sarcoma, and melanoma
- **Small cell:** lymphoma, sarcoma, neuroendocrine tumour, «PNET», melanoma



IMMUNOHISTOCHEMISTRY

Advantages

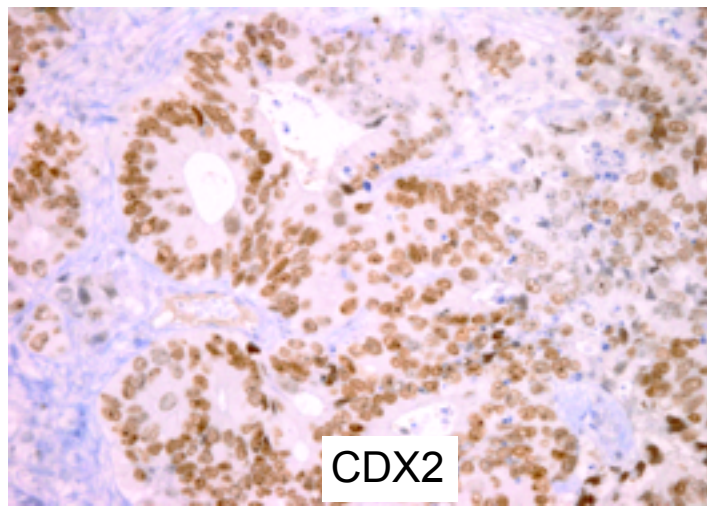
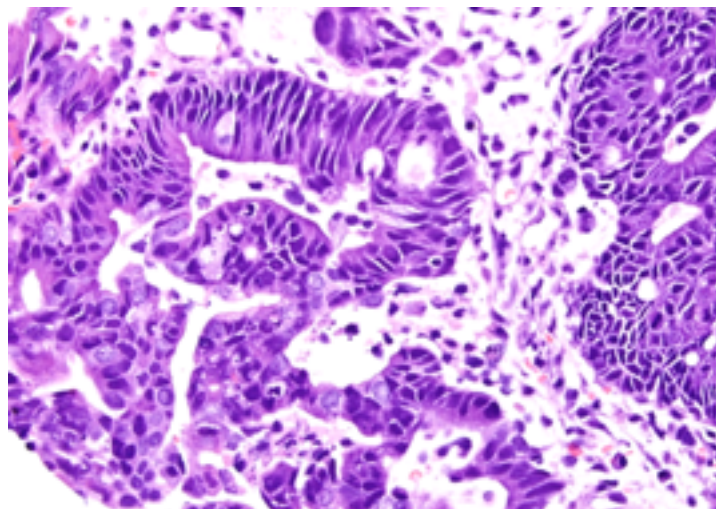
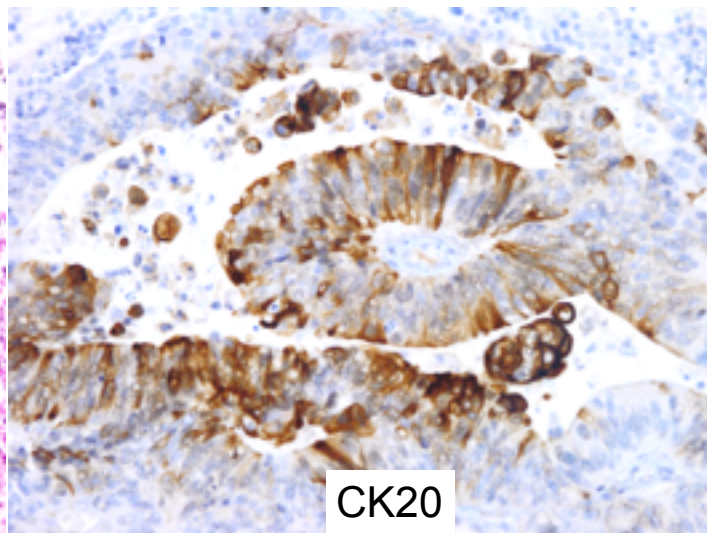
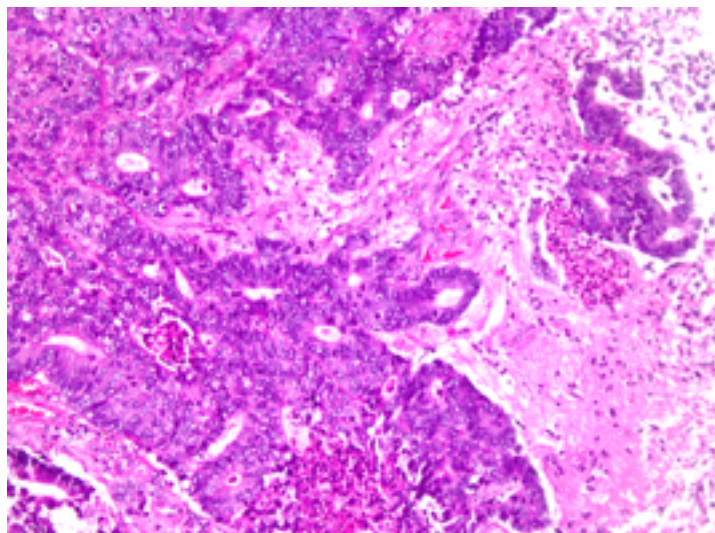
- Widely available
- Affordable
- In situ localization
- Rapid turnaround



Becher MW, J Neuropath Exp Neurol 2006;65, 935-944

TTF1(-), CK7(-), CK20(+), CX2(+)

→ Colon adenocarcinoma



IHC DETECTION OF MOLECULAR ALTERATIONS

Molecular alteration	Immunostain principle	Examples
Chromosomal translocation	Translocation (gene fusion)→positive staining	ALK translocation in lung Ca, NUT midline carcinoma
Gene mutation	Stabilization of protein	P53 mutations in many cancer types
	Mutation-specific Ab shows presence of altered protein (due to gene mutation)	BRAF V600E mutation in various cancers, EGFR L858R, exon 19 lung adenocarcinoma
Gene deletion/ loss of function	Inactivating mutation, deletion/ promoter hypermethylation of gene	Loss of INI-1 staining in sinonasal carcinoma, malignant rhabdoid tumors
Gene amplification	Increased gene copy number→overexpression	HER2 amplification in breast & gastric Ca

LIMITATIONS

Subjective

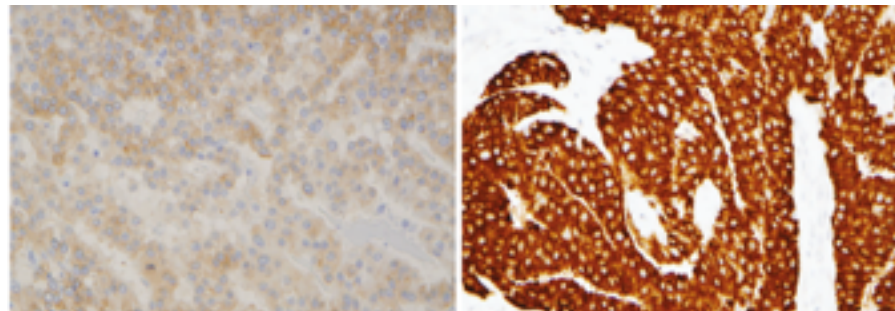
Immunoreactivity inhomogenous, may be lost, eg,

CK- staining loss in poorly differentiated carcinoma

Aberrant antibody expression, eg,

**Melanoma metastases may show aberrant
cytokeratin expression**

False positive/negative results



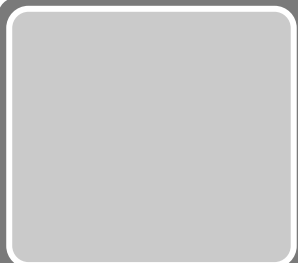
MOLECULAR APPROACHES



Genetics

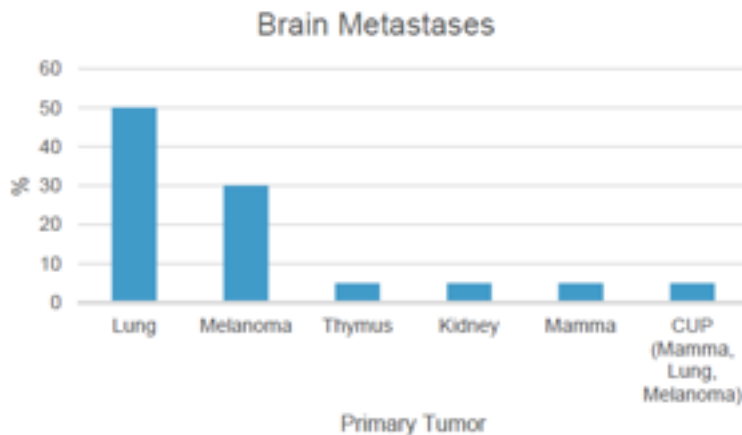


Epigenetics

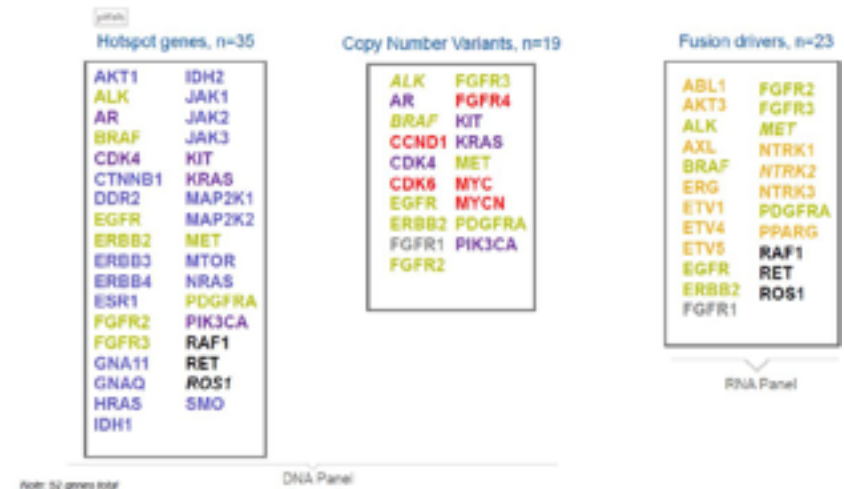


Proteomics

MOLECULAR DIAGNOSTICS - NGS



Oncomine Focus Assay – Gene List



Requires 10ng

Genetic alterations detected in 80% cases

(*EGFR*, *KRAS*, *NRAS*, *BRAF*, *MET*, *ALK*, *KIT*, *PIK3CA*, *MYC*)

MOLECULAR APPROACHES - NGS

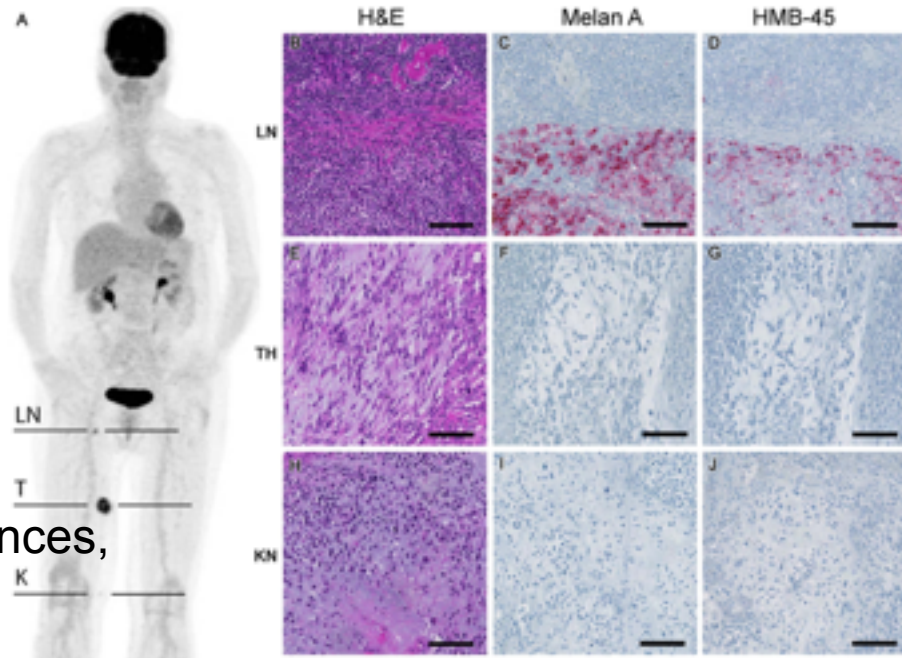
Verheem Aethir
<https://doi.org/10.1007/s00438-018-2570-5>

BRIEF REPORT

New observations in tumor cell plasticity: mutational profiling in a case of metastatic melanoma with biphasic sarcomatoid transdifferentiation

Niels J. Rupp¹ · Markus Rechsteiner² · Sandra N. Freiburger¹ · Daniela Lenggenhager¹ · Marijana Urošević² · Irene A. Burger³ · Elisabeth J. Rushing⁴ · Daniela Mihic-Probst¹

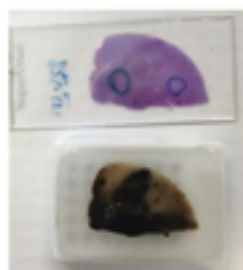
Morphological differences do not necessarily indicate genetic differences, and vice versa



NGS showed concordant NRAS p.Q61R mutation in all three tumors

FAST-TRACK

Idylla™ Mutation Test



Punch Biopsy
/ Sections



Available Tests

CE-IVD labelled:

- *BRAF/NRAS* test for colorectal cancer

Validated for diagnostics at our institute:

- *BRAF/NRAS* test for melanoma
- *EGFR* test for lung cancer
- *KRAS* test for colorectal cancer

TEST RESULT REPORT

For in-house use only. Not for distribution outside the institution.



Sample ID	6200744722.18	TTP Version	2.1
Sample type	FFPE tissue	Expiration date	05 Jan 2017
Cartridge ID	26760880		
Test type	KRAS		
Lot ID	00002075		
Instrument serial number	022071		
Instrument software version	20160204_0604		
Console software version	4.1.0.73		
Test request completed	15 Feb 2017 (13:30)		
Test started	15 Feb 2017 (13:30)		
Test ended	15 Feb 2017 (13:30)		
Test status	Released result, Automatic, 15 Feb 2017 (13:30)		
Operator	Administrative		

Test Result (1) In Vitro Diagnostic Medical Devices. For use in diagnostic procedures.

Idylla™ KRAS Mutation Test

STATUS	MUTATION DETECTED IN KRAS CODON 12
Mutation	G13A
Protein	p.G13A
Nucleotide Change	c.35G>A

Sample Criteria

- Tumor cell content should be $\geq 10\%$

Limit of Detection

- for most mutations $\leq 1\%$ - $\leq 5\%$

SAME MUTATION, DIFFERENT IMPACT

Tumor type	BRAF V600E mutation	Clinical use
Colorectal adenoCa	X	Prognosis, lack of response to EGFR monoclonal Ab; sporadic origin in MSI tumors, ?benefit in NEC rectal Ca
Melanoma	X	Response to BRAF inhibitors
Papillary thyroid	x	More aggressive, response to BRAF inhibitors



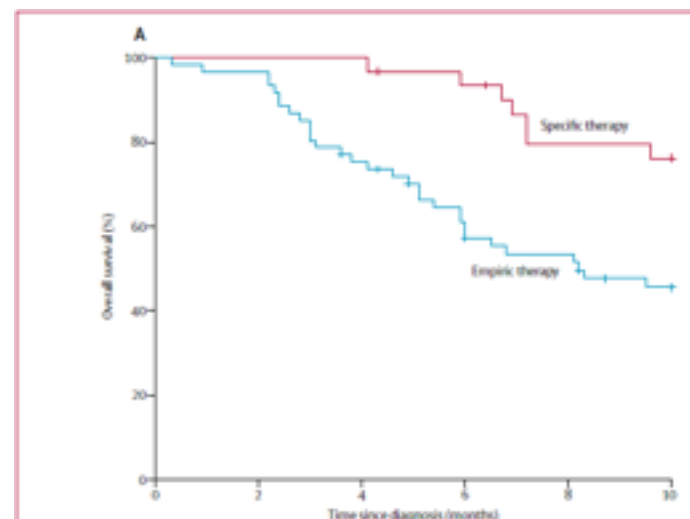
Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis

Sebastian Moran, Anna Martínez-Cardús, Sergi Sayols, Eva Musulén, Carme Balañá, Anna Estival-Gonzalez, Cátia Moutinho, Holger Heyn,

**EPICUP classifier
based on microarray
DNA signatures**

**Training set 2,700
known primaries +
mets plus validation
set of 7,691**

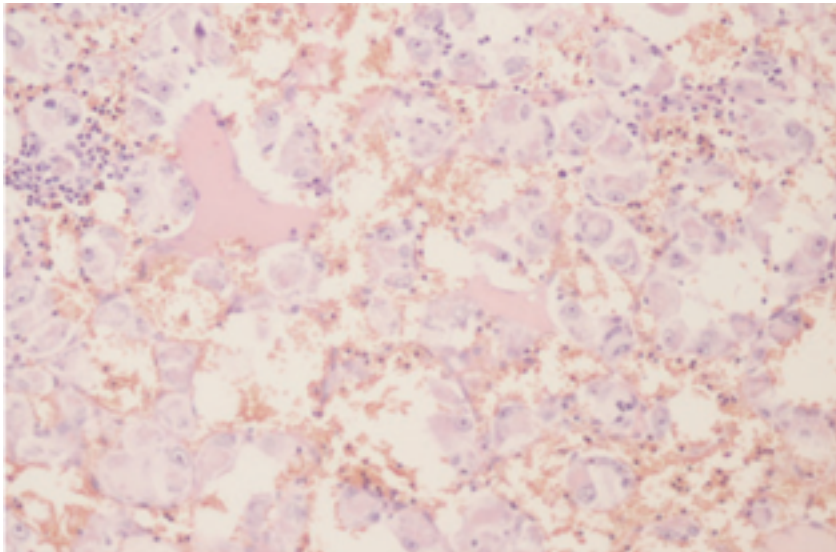
**Specificity 99.6%,
sensitivity 97.7%**



MESENCHYMAL TUMORS

ONCOLOGY LETTERS 12: 956-958, 2016

Alveolar soft part sarcoma associated with lung and brain metastases: A case report



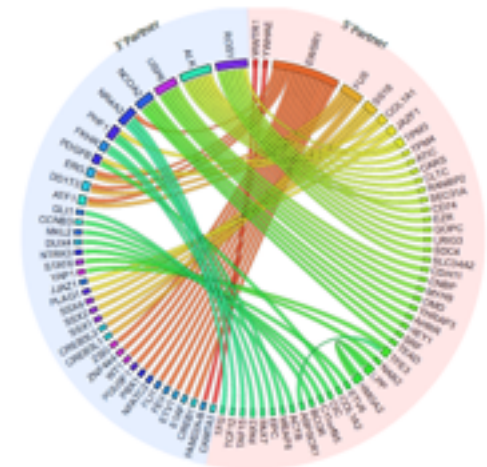
Wang 2016

Assay Targets

Includes the following genes and their fusion partners:

ALK	FUS	NTRK3	TCF12
CAMTA1	GLI1	PDGFB	TFE3
CCNB3	HMGA2	PLAG1	TFG
CIC	JAZF1	ROS1	USP6
EPC1	MEAF6	SS18	YWHAE
EWSR1	MKL2	STAT6	
FOXO1	NCOA2	TAF15	

Sarcoma Fusion Map





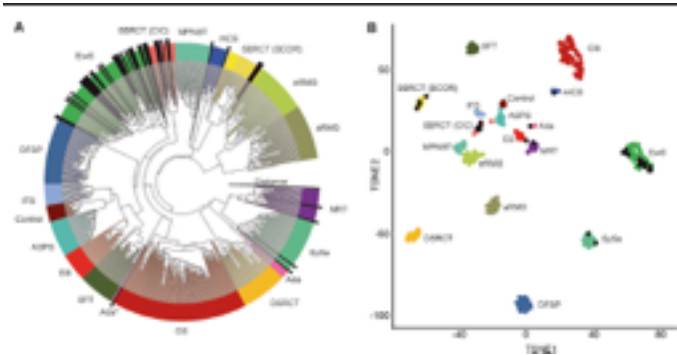
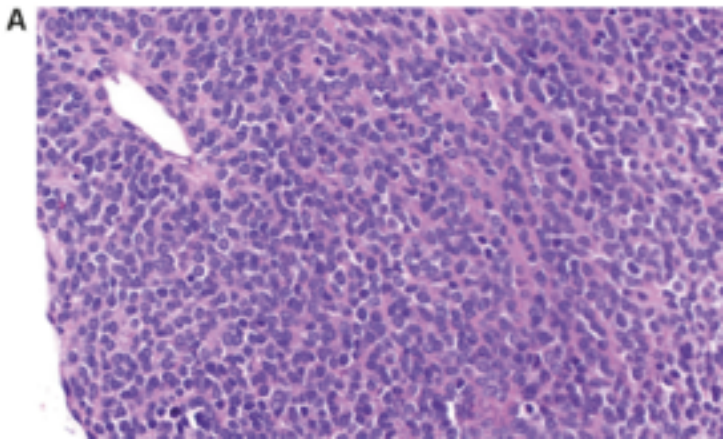
Array-based DNA-methylation profiling in sarcomas with small blue round cell histology provides valuable diagnostic information

Christian Koelsche^{1,2,3} · Wolfgang Hartmann⁴ · Daniel Schimpf^{1,2,3} · Damian Stichel^{1,3} · Susanne Jabar^{5,6,7} ·

Ewing sarcoma, CD99, fusions EWSR-FLI1 & EWSR-ERG ~90%

Ewing-like mimics show CIC-DUX4, BCOR-CCNB3 fusions

Assignment 30 NOS tumors to methylation groups of reference sarcoma sets



LIMITATIONS

Molecular panels lack full coverage

Results vary according to quality/quantity of tumor cells

Intratumoral mutational heterogeneity

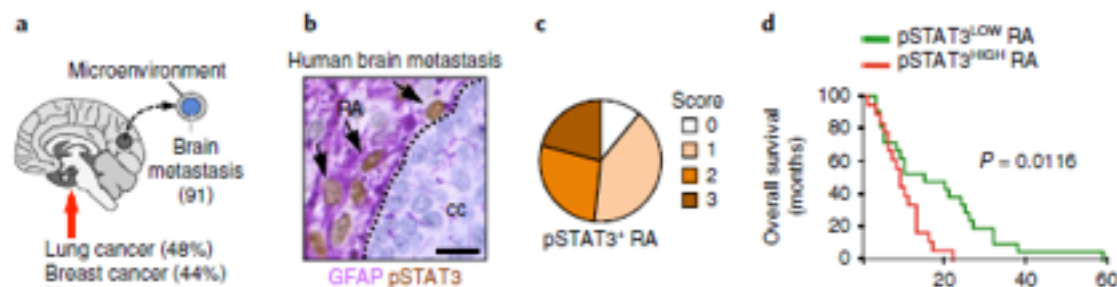
Mutational process is dynamic, discordance between primary and metastasis, which varies according to alteration

Tumors of different origin may share molecular profile

Lack in situ visualization

STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis

Neibla Priego¹, Lucía Zhu¹, Cátia Monteiro¹, Manon Mulders¹, David Wasilewski^{1,18}, Wendy Bindeman¹,



SUMMARY/OUTLOOK

Routine histological and immunohistochemical methods continue to play a pivotal role in diagnosis, prognosis, and predicting response to therapy

Molecular analyses of tissues and body fluids are complementary to standard histological approaches

Rapidly advancing «omics» technologies will likely provide new options for precision pathological diagnosis

MERCI