Gewebebasiertes Biobanking für die translationale prädiktive Forschung

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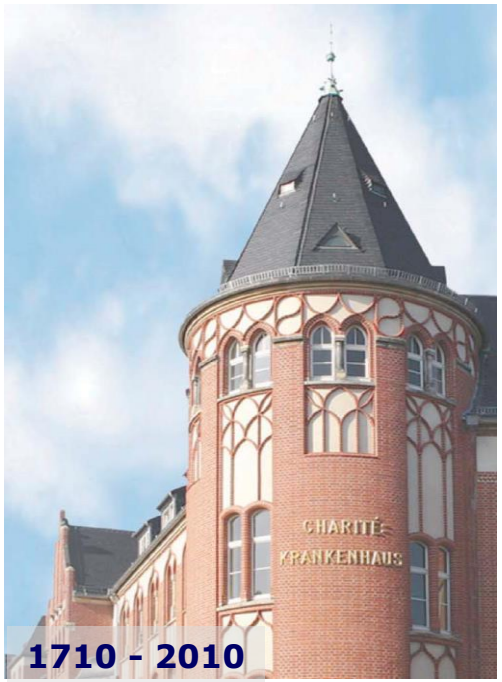

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Today's Challenges in Precision Medicine

The goal of diagnostic pathology was to provide a correct diagnosis, but today the task extended greatly to

extract from the patient's tissue as many information as possible

by applying in parallel classical, immunological (proteomic) and molecular techniques.

The capability to predict pre-therapeutically the response of infections or individual tumors to certain (targeted) drug(s) is based on reliable and reproducible biomarker and predictive assays, which can be developed only on the basis of systematically structured biobanks.

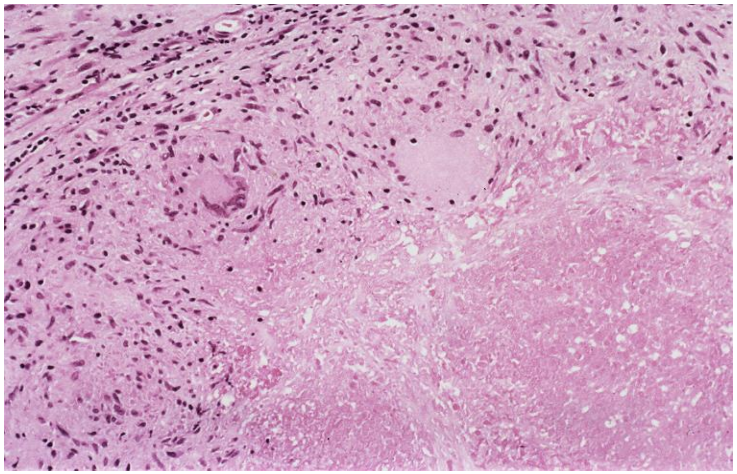
This is the prerequisite for precision medicine.

Challenges in Anatomic and Molecular Pathology

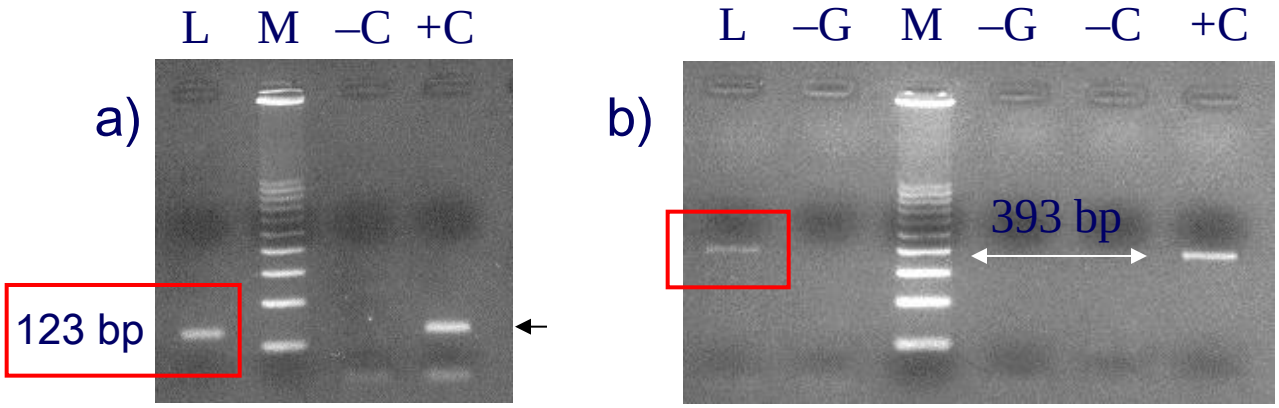
New approaches in tissue-based diagnostic

- pathology of infectious diseases
- tumor-pathology

Tissue-based diagnostic of tuberculosis



Necrotizing granuloma with epitheloid histiocytes and Langerhans-type giant cells often without detection of acid-fast bacilli in the Ziehl-Neelsen stain
(H&E x 100)



(a, b) detection of mycobacterial DNA (*M. tuberculosis*), Gel electrophoresis of the PCR products

a) *Mycobacterium tuberculosis* complex-PCR,
b) Detection of the *M. tuberculosis* mtp 40 Gen,
the specificity of all products were verified by hybridisation

formalin fixed paraffin embedded tissue

MICROORGANISM

Molecular Pathology

Our institute performs a broad panel of molecular diagnostic tests based on FFPE specimens:

PCR based detection of microorganism

Viruses

- Adenovirus
- Cytomegalovirus (CMV)
- Enterovirus
- Epstein-Barr virus (EBV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Human herpes simplex virus (HSV-1, -2)
- Human herpesvirus 6 (HHV-6)
- Human herpesvirus 8 (HHV-8)
- Human papillomavirus (HPV), detection and typing
- Parvovirus B19
- Polyomavirus (BKV/JCV)
- Varicella zoster virus (VZV)

Bacteria

- Bartonella (henselae/quintana)
- Borrelia burgdorferi (Lyme disease)
- Chlamydia trachomatis
- Helicobacter pylori
- Listeria
- Mycobacteria consensus (MOTT)
- Mycobacteria tuberculosis complex (Tbc)
- Pseudomonas aeruginosa
- Stenotrophomonas maltophilia
- Treponema pallidum
- Tropheryma whipplei
- Yersinia

Other pathogens

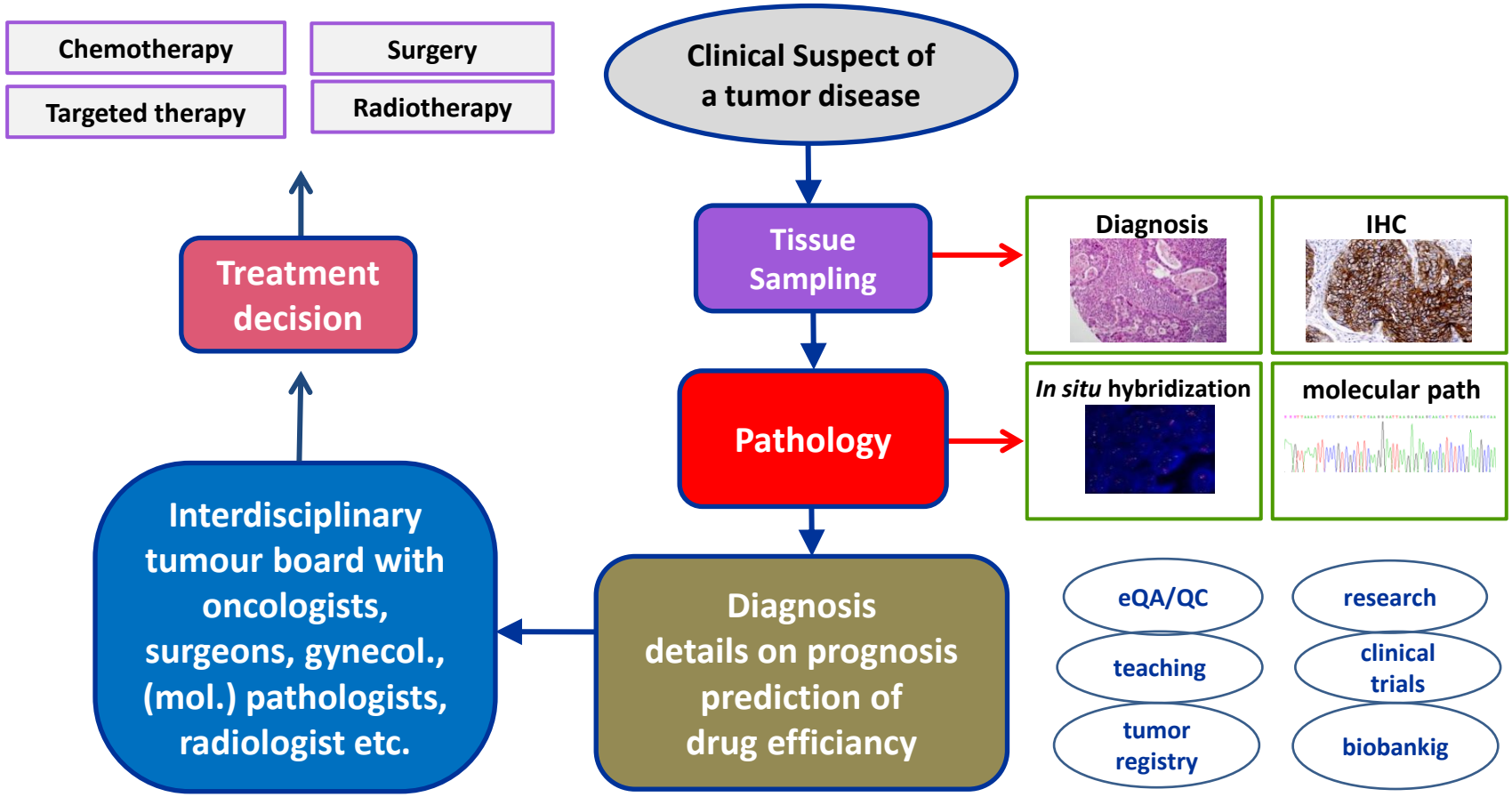
- Amoeba (Entamoeba histolytica)
- Fungi PCR/typing
- Leishmania
- Mycoplasma (consensus/pneumoniae)
- Pneumocystis carinii (P. jirovecii)
- Toxoplasma gondii

Challenges in Anatomic and Molecular Pathology

New approaches in tissue-based diagnostic

- pathology of infectious diseases
- tumor-pathology

Multidisciplinary Cooperation Enables Precision Oncology



Predictive tissue-based biomarkers for targeted therapies

FDA / EMA-approved drugs associated with companion diagnostic / eligibility tests* (selection)

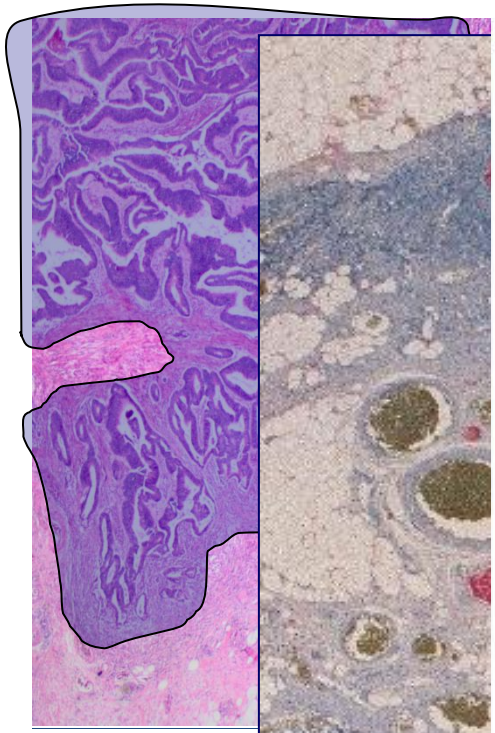
- Trastuzumab/Pertuzumab→ metast. breast cancer, overexpression/amplification of ***HER-2***
- L
- T
- C
- P
- N
- C
- B
- C
- V
- I
- I
- P
- C
- Check point inhibitors → various tumor entities, **PD1/PDL-1 overexpression**

Already now, in 35% of all tumors a predictive molecular test is appropriate. Notably, prediction of tumour response is exclusively tissue-based.

All these substances have been developed on the basis of histologically characterised human tissue.

This underlines the importance of biobanks.

What is one of the irreplaceable role of anatomic pathology in the procedure of molecular biomarker analysis?



Qualitätssicherungs-Initiative Pathologie

QuIP

Ringversuche Immunhistochemie
und Molekularpathologie

Teilnahmezertifikat

4. Ringversuch EGFR-Mutationsbestimmung beim NSCLC.

2013

Prof. Dr. med. Manfred Dietel
Charité - Universitätsmedizin Berlin
Institut für Pathologie
Charitéplatz 1
10117 Berlin

hat am Ringversuch ‚EGFR-Mutationstestung beim NSCLC' mit Erfolg teilgenommen.

Leitung des Ringversuches:
Prof. Dr. med. P. Schirmacher, Prof. Dr. med. M. Dietel,
Dr. R. Penzel, Dr. Chr. Schewe

Prof. Dr. med. P. Schirmacher
Deutsche Gesellschaft für Pathologie e. V.

Prof. Dr. med. W. Schlake
Bundesverband Deutscher Pathologen e. V

Bestandteil dieser Teilnahmebescheinigung ist die getrennt gefasste, inhaltliche Beurteilung der Untersuchung.

Träger der Ringversuche Immunhistochemie und Molekularpathologie QuIP
Deutsche Gesellschaft für Pathologie e.V., Berlin, Tel: 030 / 25760727, Mail: geschaeftsstelle@dgp-berlin.de
Bundesverband Deutscher Pathologen e.V., Berlin, Tel: 030 / 3088197-0, Mail: bvd@pathologie.de

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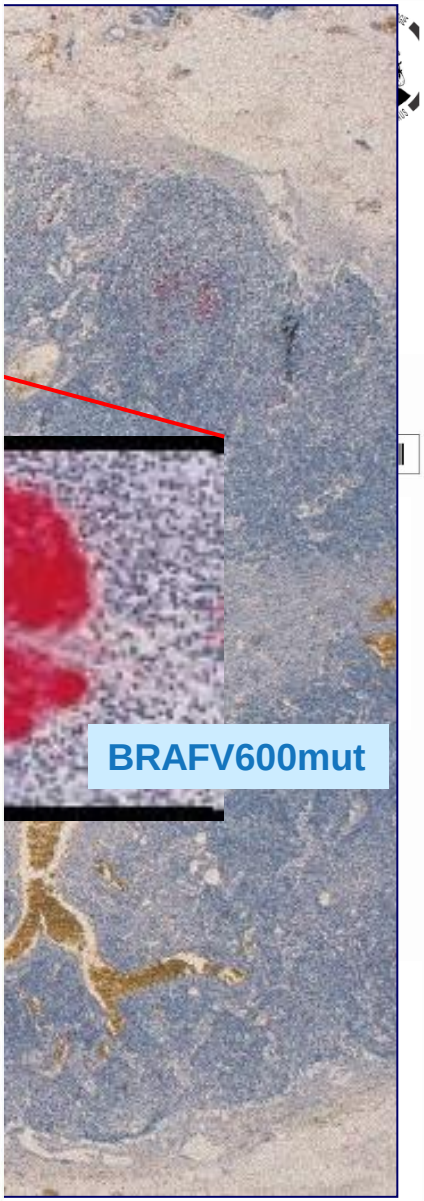


BRAFV600mut

CHARITÉ

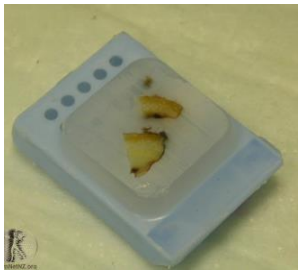
Technical Prerequisites

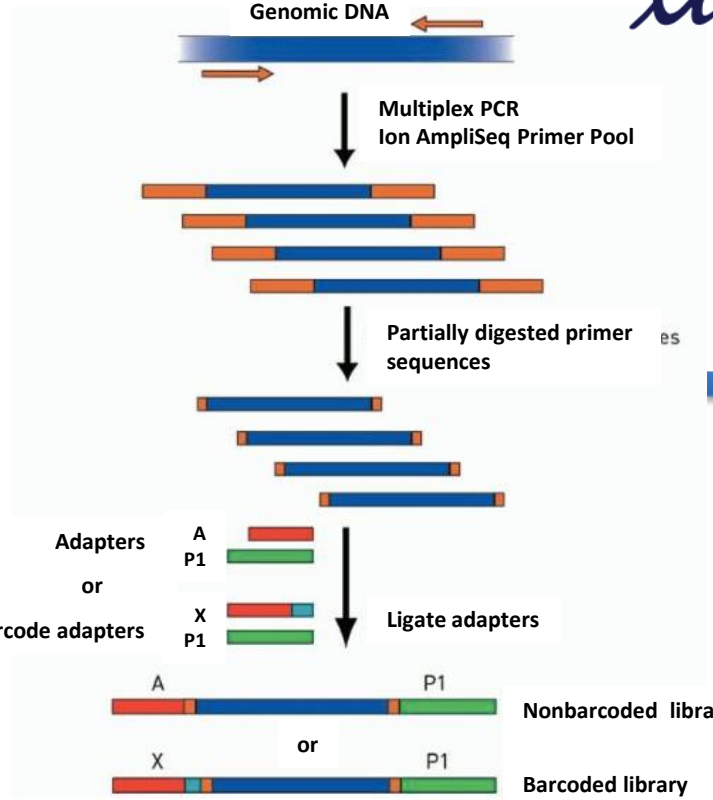
- Adequately fixed tissue/cells in buffered formalin
- Adequately processed FFPE tissue
- Robust DNA/RNA extraction methods
- Techniques/instruments will are able to process relatively short DNA sequences (150 - 200 bp)
- Reliable and reproducible bio-informatics

All these data can be provided by a well organized and well annotated FFPE-tissue based biobank.

Whole exome or genome analyses will be possible within a couple of years.

Integrating Next Generation Sequencing in Diagnostic Pathology





life technologies™

IonTorrent PGM

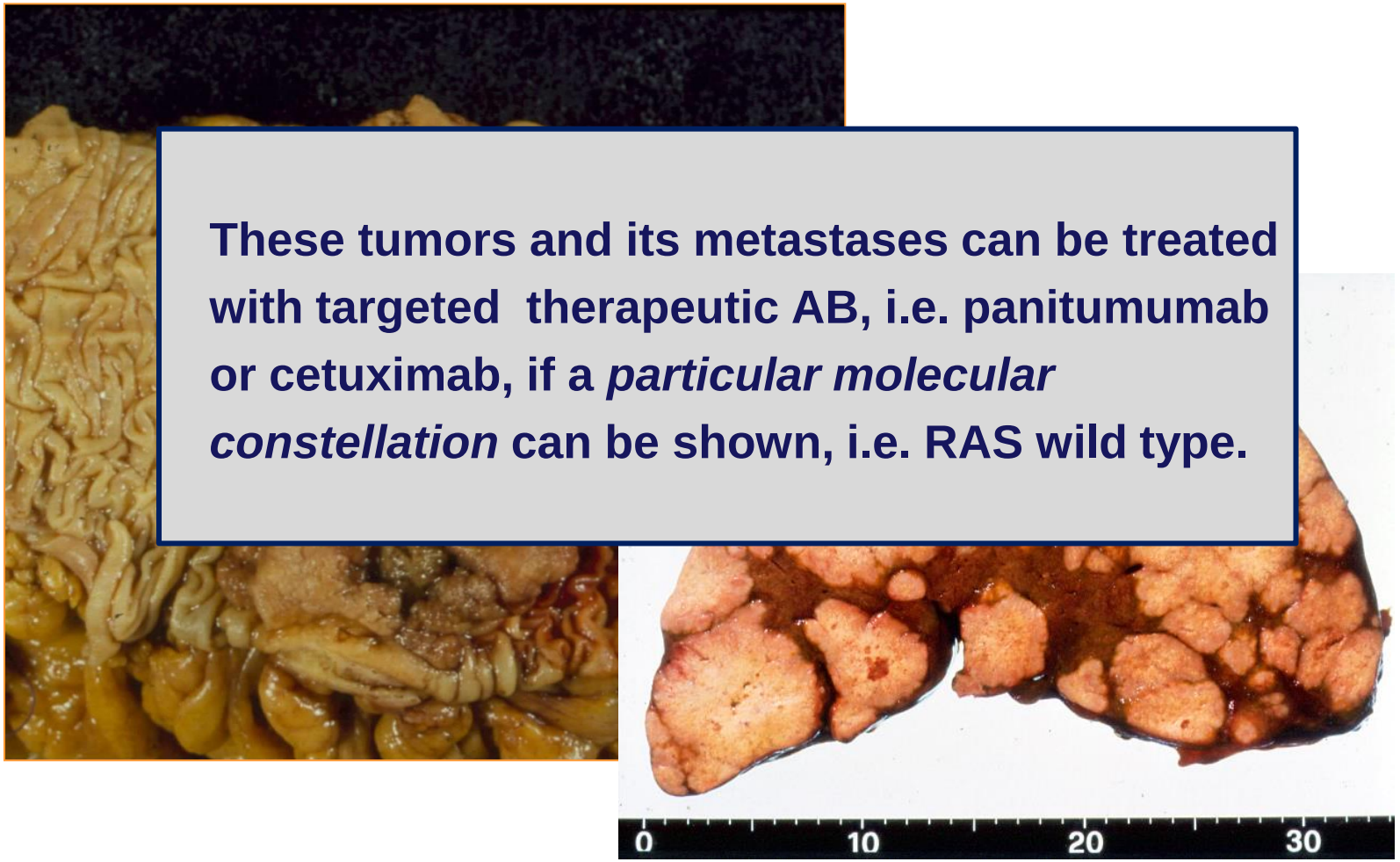
Ion AmpliSeq™ Cancer Panel targets 46 critical genes

ABL1	*ERBB4*	*KDR*	*PTPN11*
AKT1	*FBXW7*	*KIT*	*RB1*
ALK	*FGFR1*	*KRAS*	*RET*
APC	*FGFR2*	*MET*	*SMAD4*
ATM	*FGFR3*	*MLH1*	*SMARCB1*
BRAF	*FLT3*	*MPL*	*SMO*
CDH1	*GNAS*	*NOTCH1*	*SRC*
CDKN2A	*HNF1A*	*NPM1*	*STK11*
CSF1R	*HRAS*	*NRAS*	*TP53*
CTNNB1	*IDH1*	*PDGFRA*	*VHL*
EGFR	*JAK2*	*PIK3CA*	
ERBB2	*JAK3*	*PTEN*	

Tumor Entities Important in Predictive Molecular Pathology

- Colon cancer
- Malignant melanoma
- Ovarian cancer
- NSCLC
- Check-point inhibitors

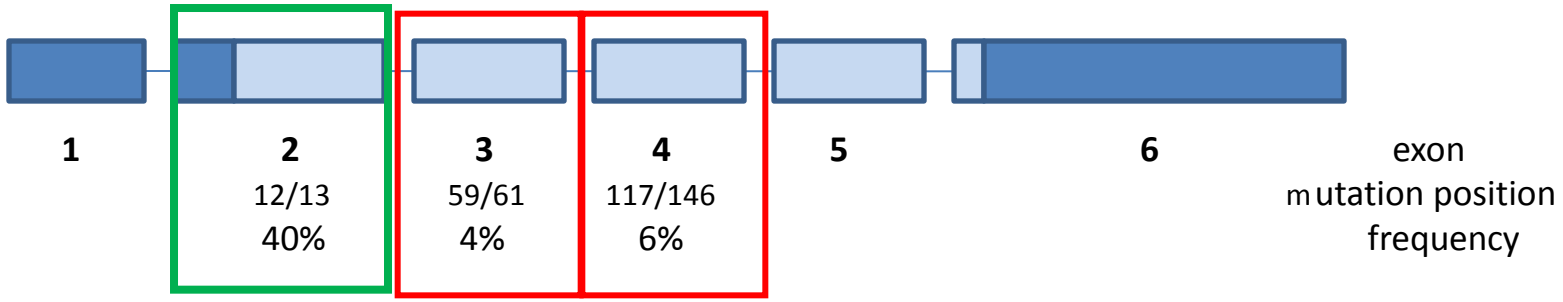
Invasive colorectal cancer with liver metastases



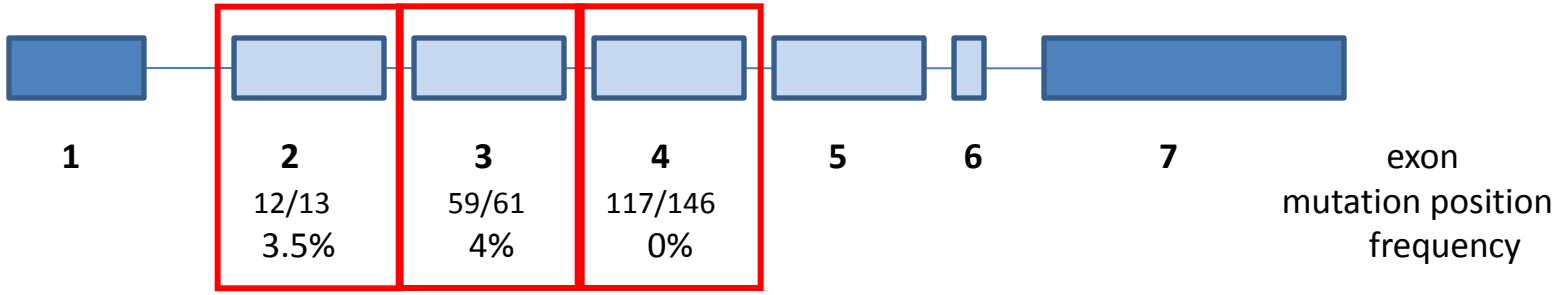
These tumors and its metastases can be treated with targeted therapeutic AB, i.e. panitumumab or cetuximab, if a *particular molecular constellation* can be shown, i.e. RAS wild type.

Mutations in KRAS and NRAS genes in colorectal cancer

KRAS



NRAS



non-coding exon

coding exon

When the rare mutations are added they represent 17.5 % of all WT-CRC and 10% of all mCRC and they are associated with resistance!

Malignant Melanoma

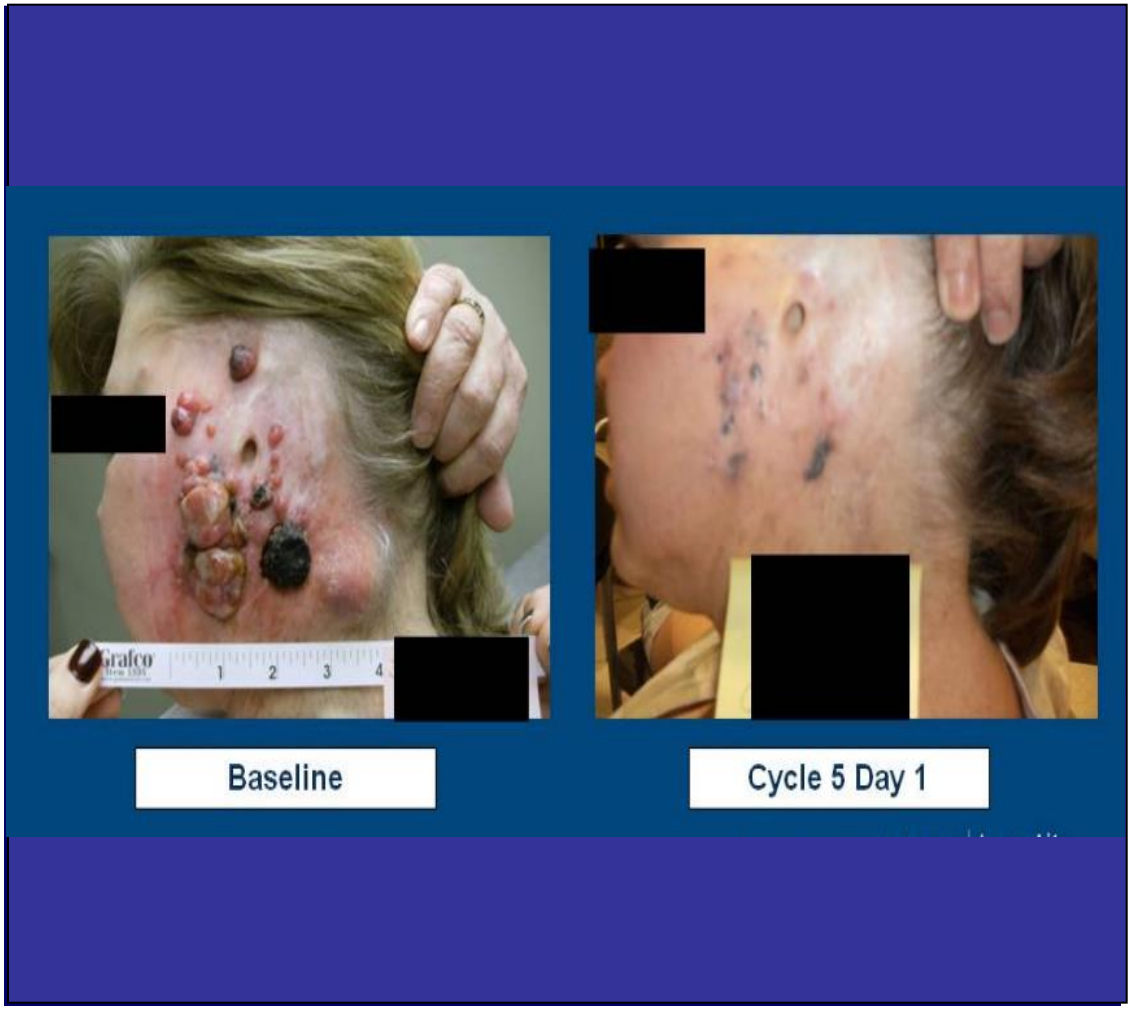
50% of all malignant melanomas exhibit a BRAF-Mutation

*Total V600 mutation rate for BRIM-3 (cobas® 4800 BRAF V600 Mutation Test); 9.9% of the cobas-positive cases subjected to retrospective Sanger sequencing had V600K mutations

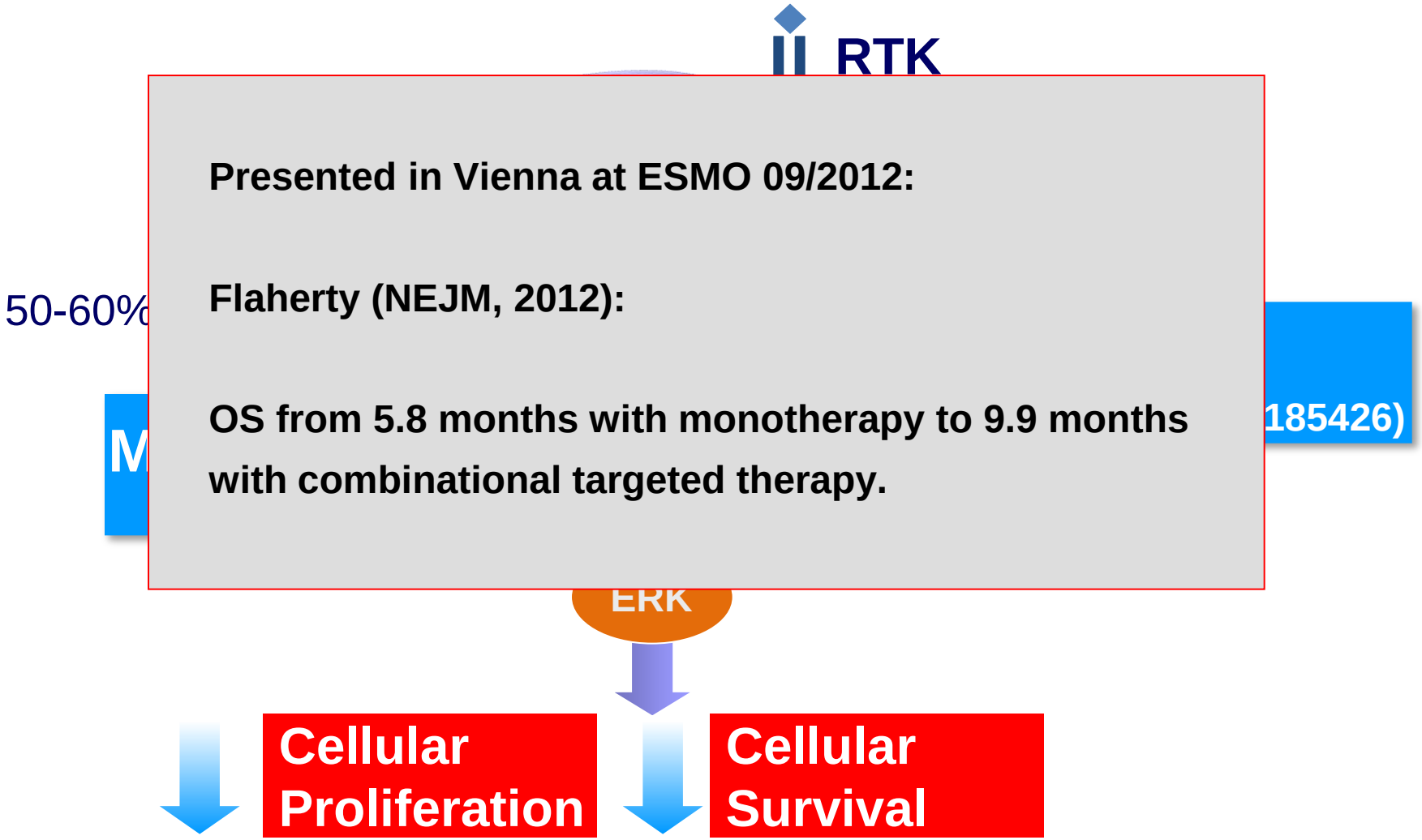
Vemurafenib inhibits V600 mutated BRAF kinase

Response to BRAF-inhibitors is given only if a BRAF mutation is present

This has to be tested prior to the therapy.



Vemurafenib inhibits V600 mutated BRAF kinase



Presented in Vienna at ESMO 09/2012:

Flaherty (NEJM, 2012):

OS from 5.8 months with monotherapy to 9.9 months with combinational targeted therapy.

*Total V600 mutation rate for BRIM-3 (cobas® 4800 BRAF V600 Mutation Test); 9.9% of the cobas-positive cases subjected to retrospective Sanger sequencing had V600K mutations

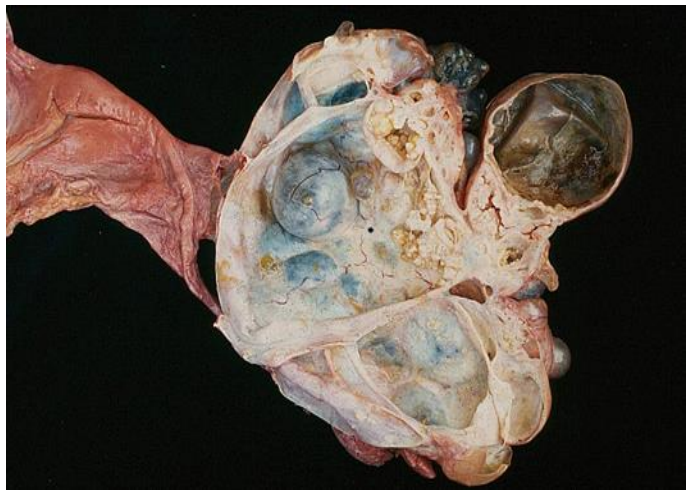
Next generation sequencing and rare tumor entities -

an issue of up-coming importance !

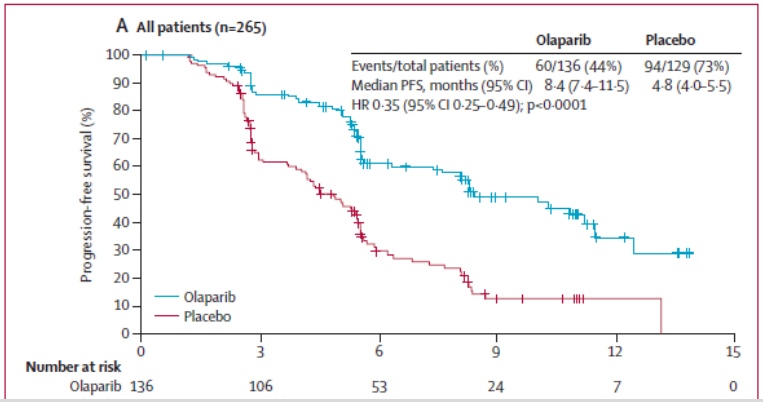
Next Generation Pathology of Ovarian Cancer

Up-coming challenge in companion diagnostic of ovarian cancer:

Routine BRCA-testing as prerequisite for treatments with the PARP inhibitor Olaparib.



n *Lancet Oncol* 2014



all pts

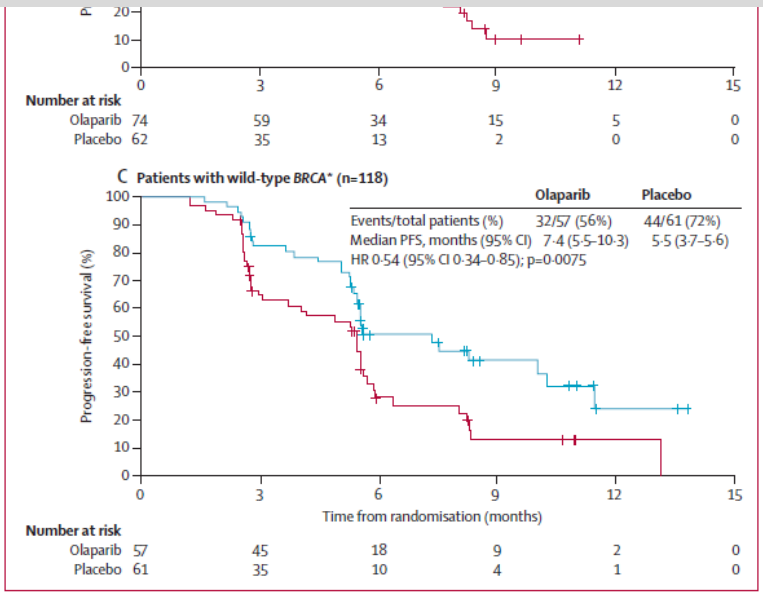
med. PFS 8.4 vs 4.8 months

HR 0.35 (0.25-0.49)

P = 0.0001

EMA-Zulassung: Olaparib für das platin-sensitive Rezidiv des high-grade serösen,

BRCA-mutierten Ovarial-, Tuben-, Peritonealkarzinoms



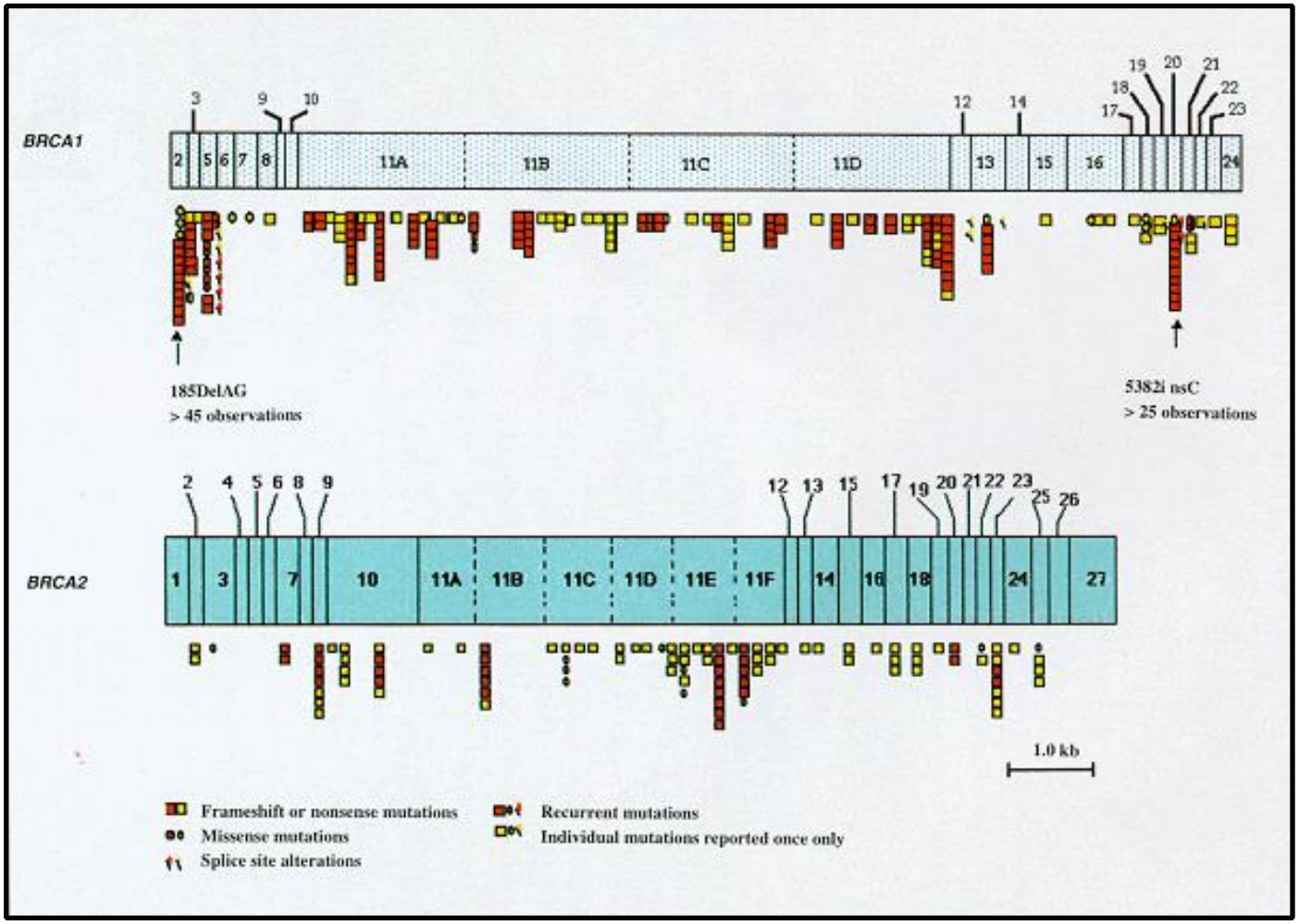
BRCA wt

med. PFS 7.4 vs 5.5 months

HR 0.54 (0.34-0.85)

P = 0.0075

Broad Distribution of BRCA-Mutations



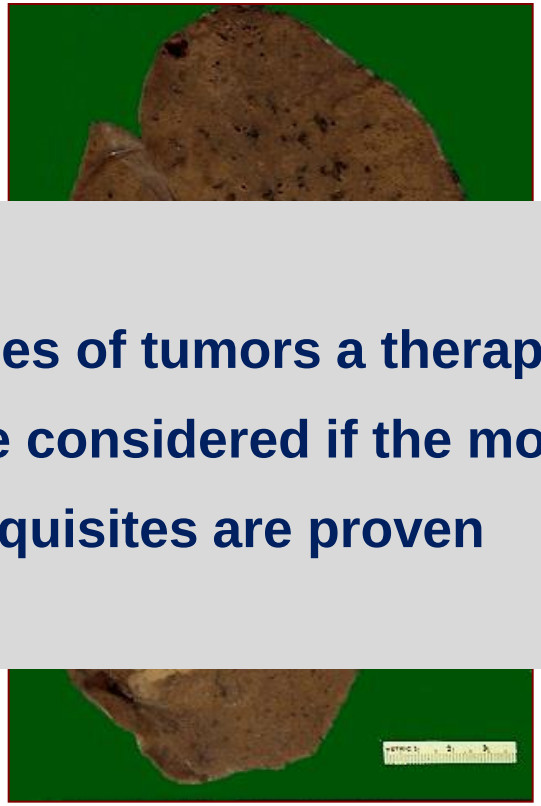
Next Generation Pathology of Ovarian Cancer

BRCA muation analyses can be done only by NGS.

The next entity will be the triple negative breast cancer (TNBC) near future.

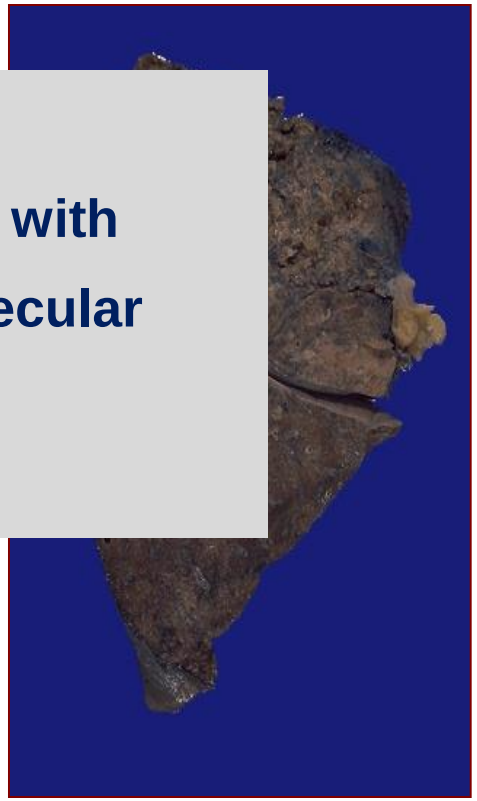
NSCLC - Macroscopy

central
squamous cell carcinoma



peripheral
adenocarcinoma

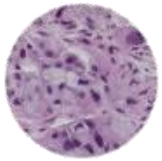
adeno carcinoma
broncho-alveolar type



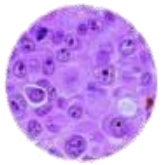
For these types of tumors a therapy with TKIs should be considered if the molecular prerequisites are proven

NSCLC: Past and Current Landscape

1999
Histology-driven selection[1]



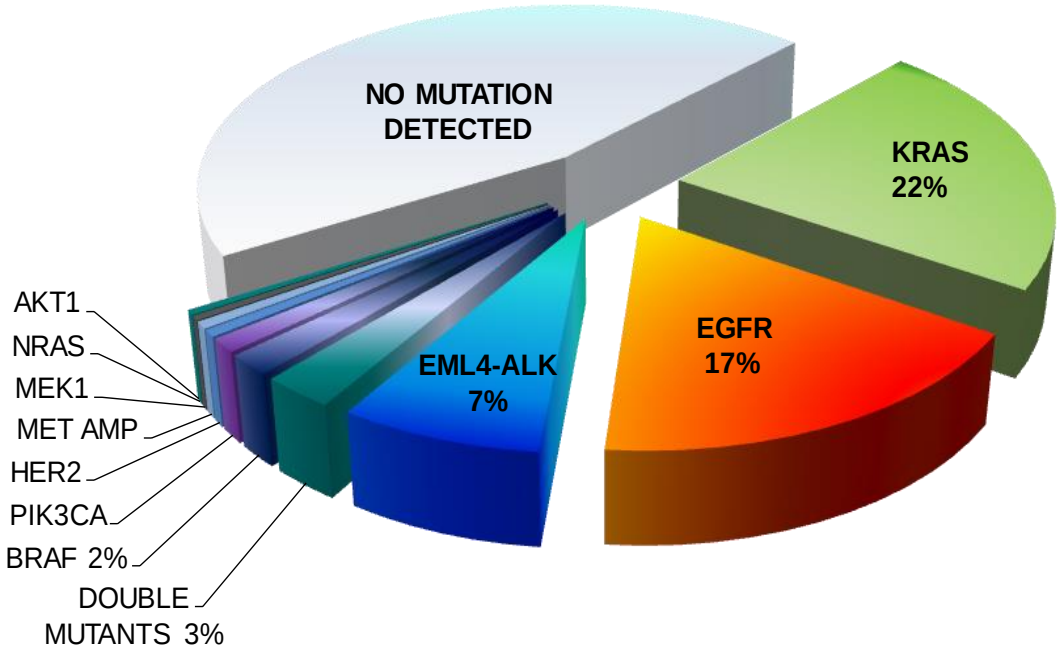
Adenocarcinoma



Squamous-cell carcinoma

Large cell carcinoma

2012
Targeting oncogenic drivers



Actionable driver mutations identified in 54% of lung adenocarcinoma tumours

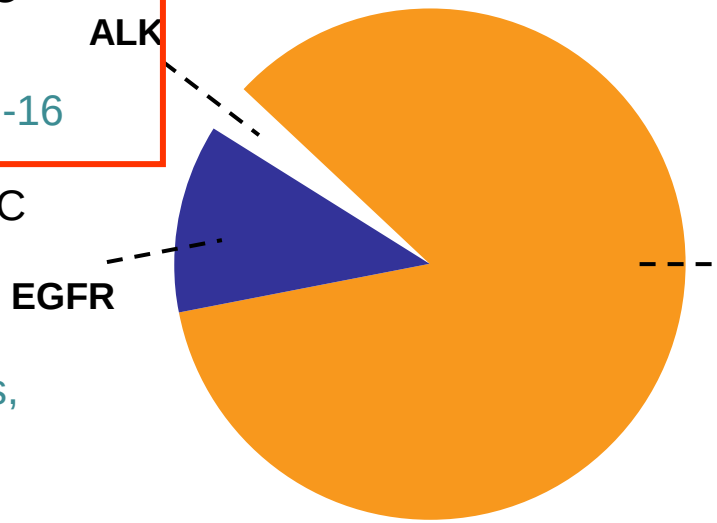
LCMC, Lung Cancer Mutation Consortium

Kris MG, et al. Presented at ASCO 2011; Abstract CRA7506

Currently, Two Approved Personalised Treatment Options: Substantial Benefit for ~15 – 20 % of Patients

Crizotinib in ALK-positive NSCLC (US, EU filed)[1]
RR 60%, PFS 8 months, OS 14 -16

EGFR-TKIs in EGFR-mut NSCLC
Gefitinib, Erlotinib (US, EU)
(Afatinib filed in EU)[3–5]
RR 60–80%, PFS 10–13 months,
OS 19–30 months

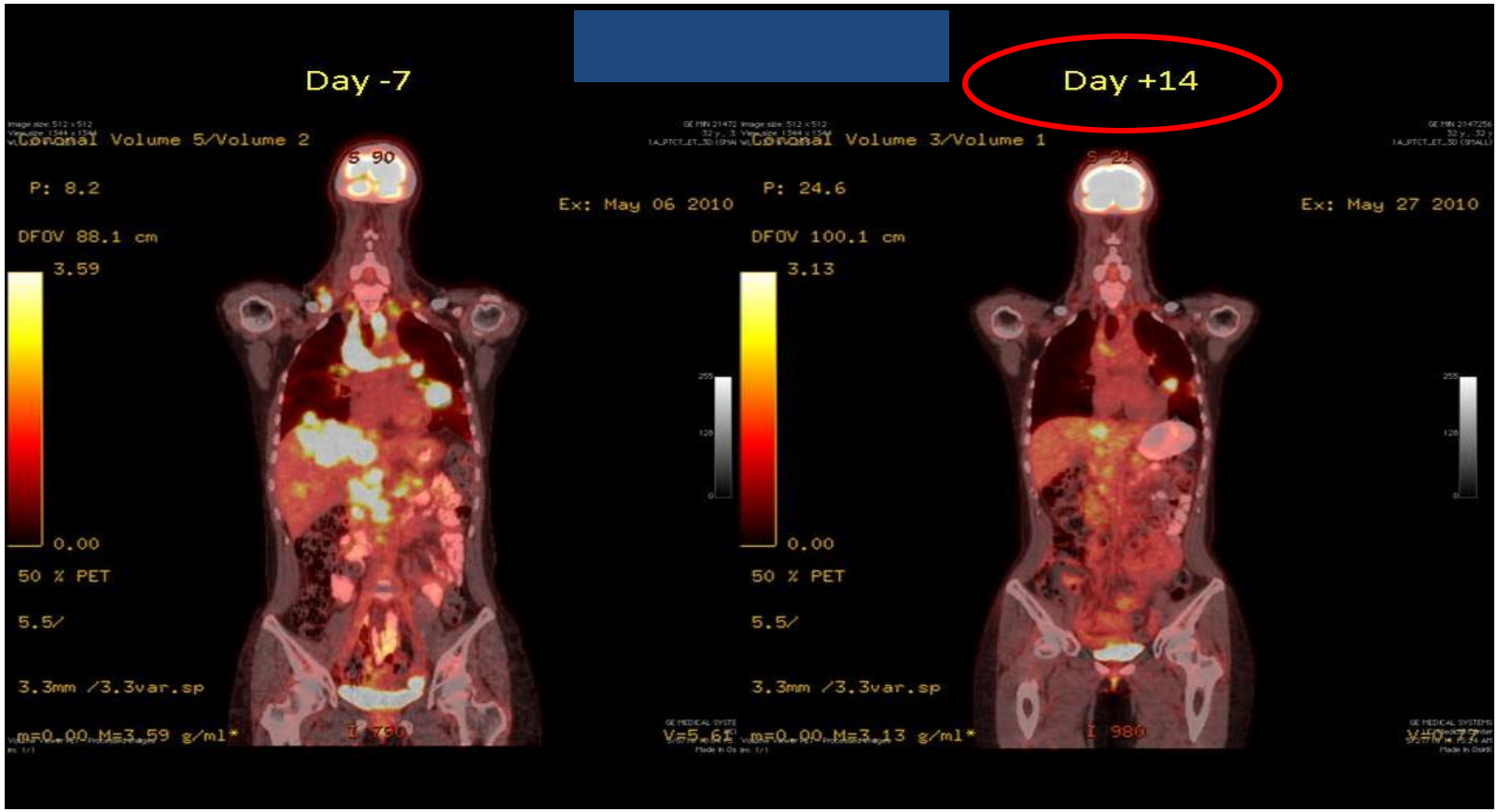


Chemotherapy in unselected patients[2]
RR 20%, OS <12 mths

Dacomitinib (PF-00299804; Pfizer Inc.) is an investigational compound not currently licensed for use in any market; Crizotinib (PF-02341066; Pfizer Inc.) is not yet approved in member states of the European Union. Crizotinib is currently licensed for use in Argentina, Canada, Israel, India, Japan, South Korea, Macau, Mexico, Switzerland, and the USA.

1. Kim D-W, et al. Presented at ASCO 2012; Abstract 7533
2. Schiller JH, et al. N Engl J Med 2002; 346:92–8
3. Maemondo M, et al. N Engl Med 2010;362: 2380-8
4. Rosell R, et al. Lancet Oncol 2012;13: 239–46
5. Yang C-H, et al. Presented at ASCO 2012; Abstract LBA7500

Rapid Responses Seen In Some Patients



Ou et al. J Thoracic Oncol 2010;5:2044–2046 Camidge RD et al.: ASCO 2011

The next step: How to fight resistance

Almost all tumors become resistant to targeting drugs.

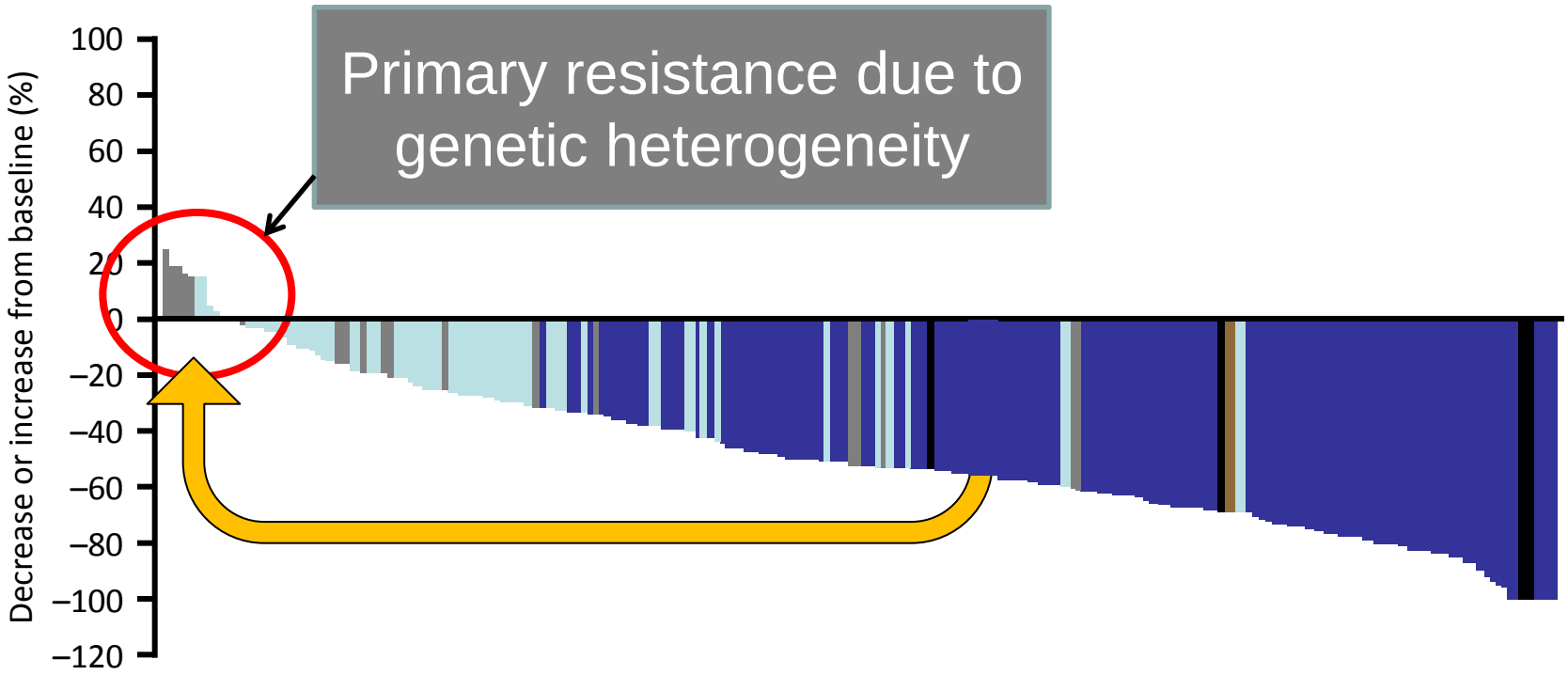
Novel approaches that have already proven successful include the development of second-generation and third-generation inhibitors and the combination of some of these inhibitors with antibodies directed against the same target or other targets (check points).

Consequently, clinical studies assessing combinations of drugs targeting both the original and the bypass pathways (after resistance) are now being explored in this setting.

Resistance to ALK Inhibitors

- **Primary resistance, e.g. to crizotinib, alectinib or ceritinib**
- **Acquired resistance,**
  - **ALK dominant □ reinstituting ALK signalling in the presence of the inhibitor.**
    - **2ndary ALK mutation(s) with steric hindrance of ALK inhibitors**
    - **Copy number gain**
  - **ALK non-dominant □ activation of bypass tracks**
    - **New non-ALK mutations: EGFR, KRAS, KIT, IGF-1R, EMT**

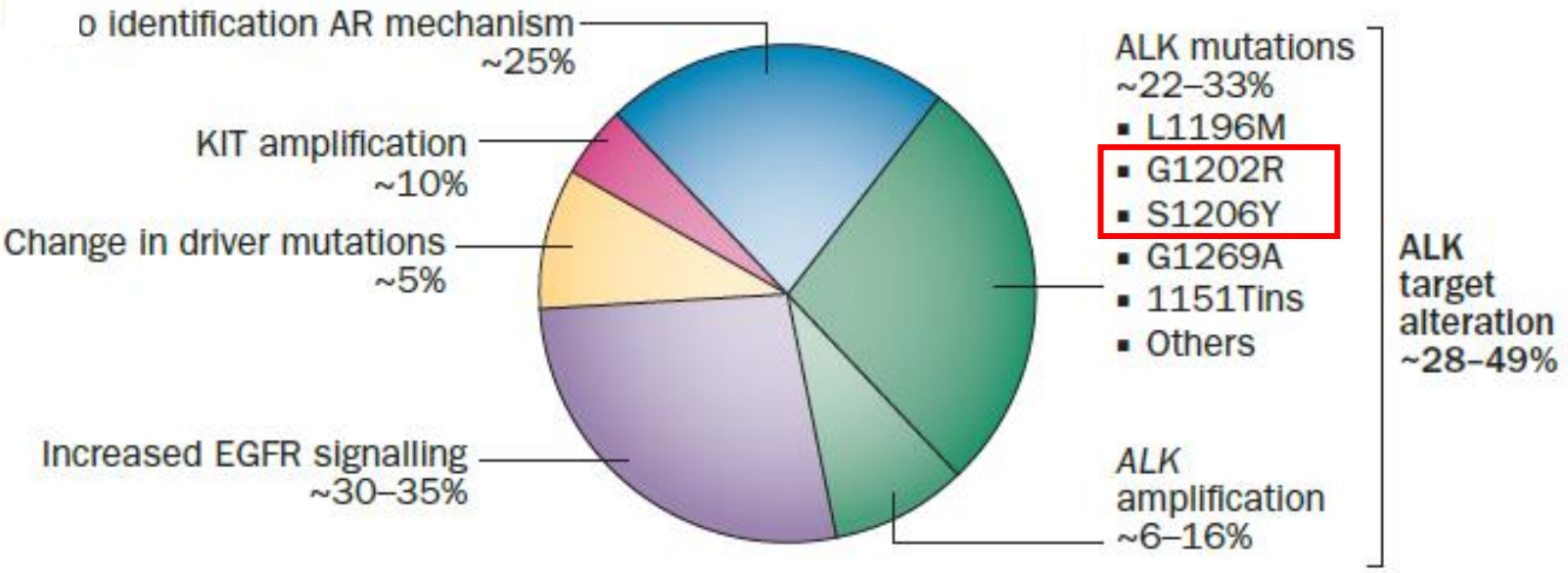
Majority of ALK+ tumors respond to Crizotinib – some show primary resistance



Resistance to ALK Inhibitors

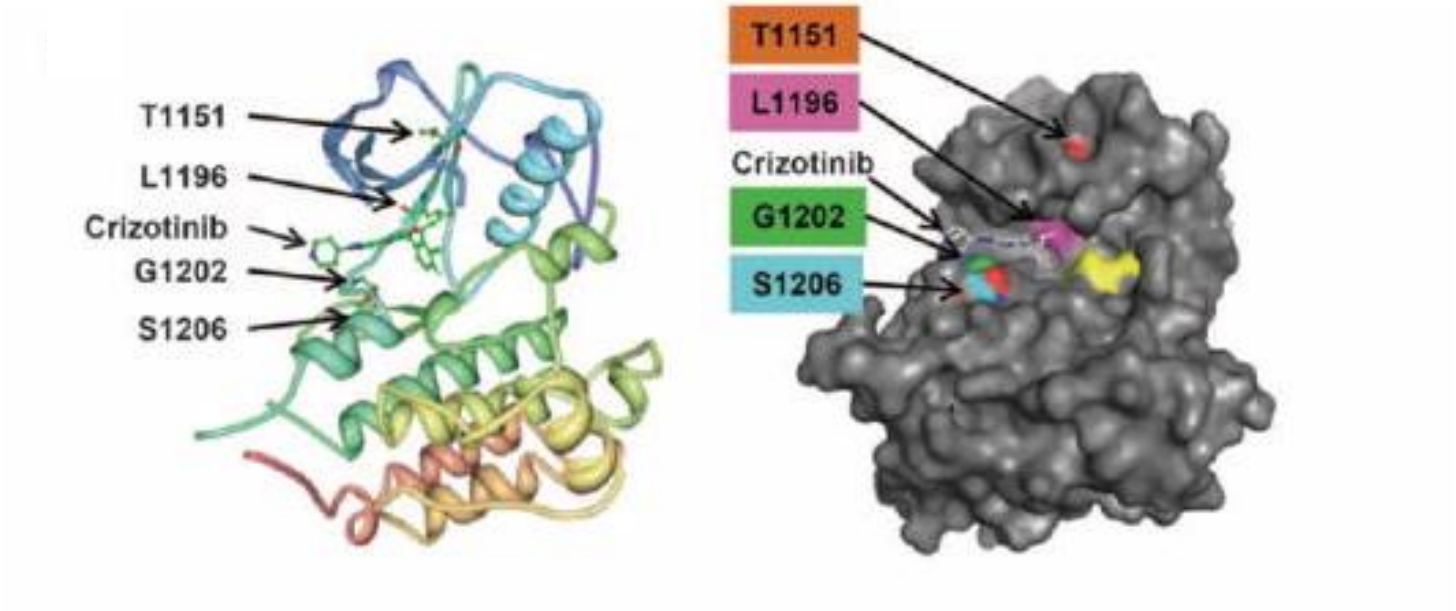
- **Primary resistance,** **e.g. to crizotinib, alectinib or ceritinib**
- **Acquired resistance** (after treatment)
  - **ALK dominant**
    - **2ndary ALK mutation(s) with steric hindrance of ALK inhibitors**
    - **Copy number gain**
  - **ALK non-dominant**
    - **New non-ALK mutations: EGFR, KRAS, KIT, IGF-1R, EMT**

Mechanisms of acquired resistance in ALK-rearranged NSCLC resistant to crizotinib



R.Katayama et al. Sci Transl Med. 2012 Feb 8;4(120):120ra17.

ALK gene amplification and multiple *ALK* resistance mutations in cancers with acquired crizotinib resistance



One Step Forward: New Drugs to Fight Resistance

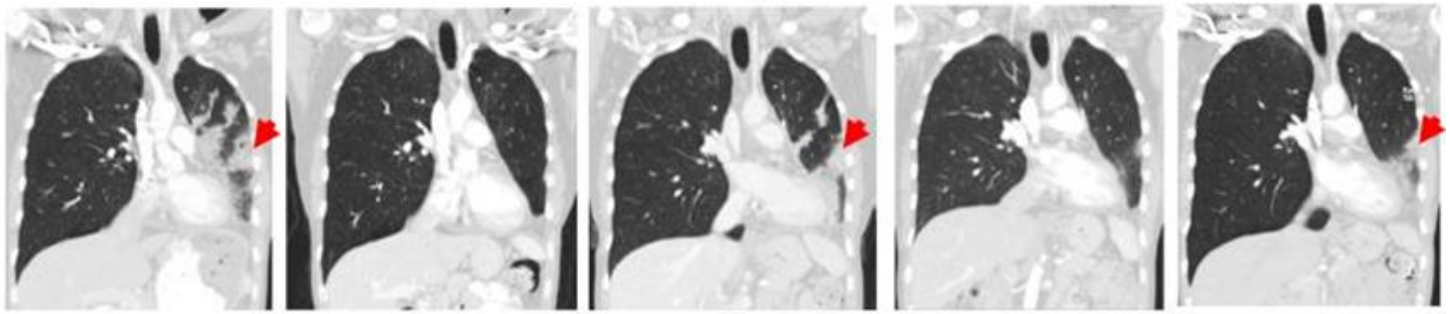
For example:

Crizotinib resistant NCSLC showed sensitivity to ceritinib,

but became resistant again only many months later

Patient Id	EML4-ALK sequence at Crizotinib Resistance	EML4-ALK sequence at Ceritinib Resistance
MGH011	S1206Y	G1202R
MGH015	WT	WT
MGH023	WT	F1174C
MGH034	WT	WT
MGH049	WT	WT
MGH051	WT	G1202R
MGH057	N/A	WT
MGH061	WT	WT
JFCR013	N/A	WT
JFCR021	G1269A (right lung)	F1174V (left lung) and G1202R (right lung)

MGH011 Lung CT scan

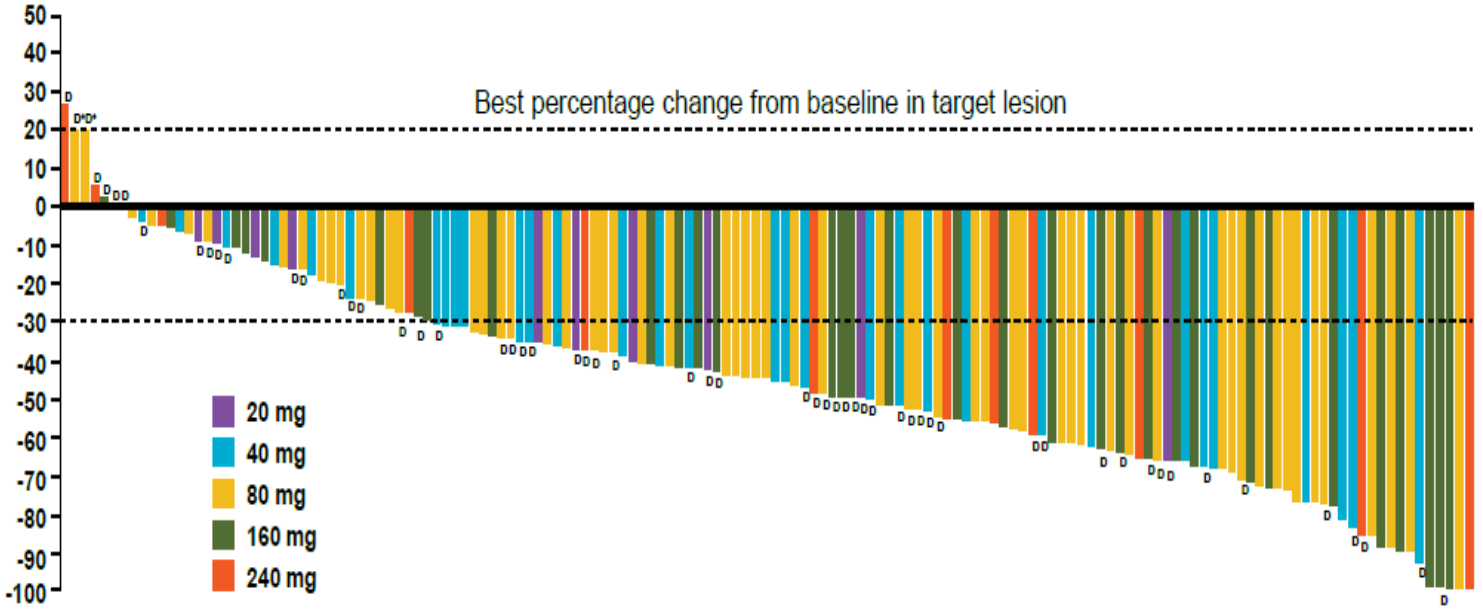


Baseline	After 8 weeks of crizotinib	After 34 months of crizotinib	After 12 weeks of Ceritinib	After 15 months of Ceritinib
EML4-ALK sequence:	**ALK mut**	**S1206Y**		**G1202R**

EGFR: AZD9291 □ 66% ORR in T790M positive patients*

Medical Affairs

*as assessed by central tumor tissue testing



DCR (CR+PR+SD) in patients with centrally tested T790M positive tumours was 90% (141 / 157; 95% CI 84, 94)

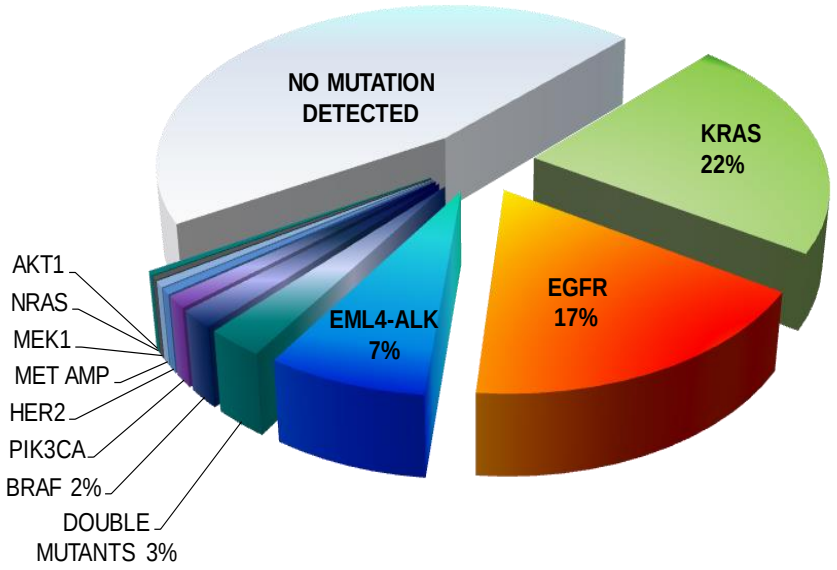
	20 mg	40 mg	80 mg	160 mg	240 mg	Total
N (157)	10	32	61	41	13	157
ORR (95% CI)	50% (19, 81)	59% (41, 76)	66% (52, 77)	51% (35, 67)	54% (25, 81)	59% (51, 66)

Presented by Pasi A Jänne at the 2015 European Lung Cancer Conference. Ann Oncol 2015; 26(Suppl 1): i60, LBA3.

2nd Generation ALK-Inhibitors

Another ALK-inhibitor is in development, alectinib

Irreversibly binding, pan-HER inhibitors in clinical development include:
dacomitinib (Phase 3) and HM781-36B (Phase 1; solid tumours)



Dacomitinib (PF-00299804; Pfizer Inc.) is an investigational compound not currently licensed for use in any market

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Up-coming Proteomic Diagnostics for Check-point Inhibitors

The NEW ENGLAND JOURNAL of MEDICINE

Garon EB, ASCO 2015
Keynote-001 Phase Ib
NSCLC: 15% Adeno, 80% Platte
DAKO **22C3**

ORIGINAL ARTICLE

Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

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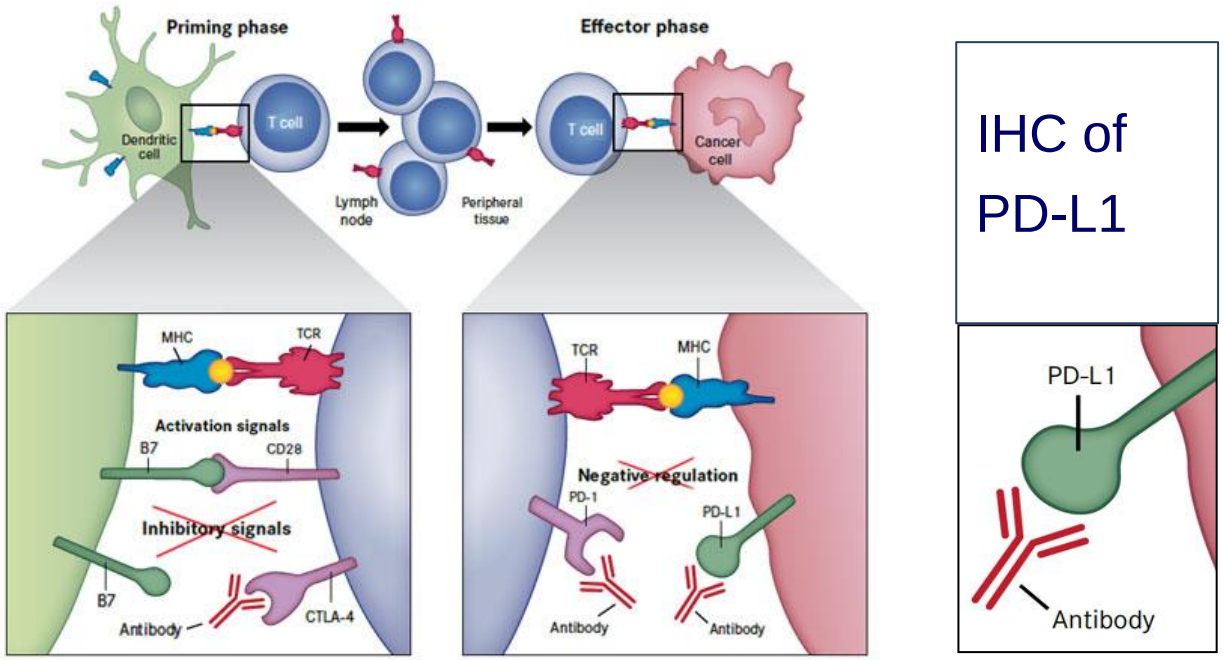
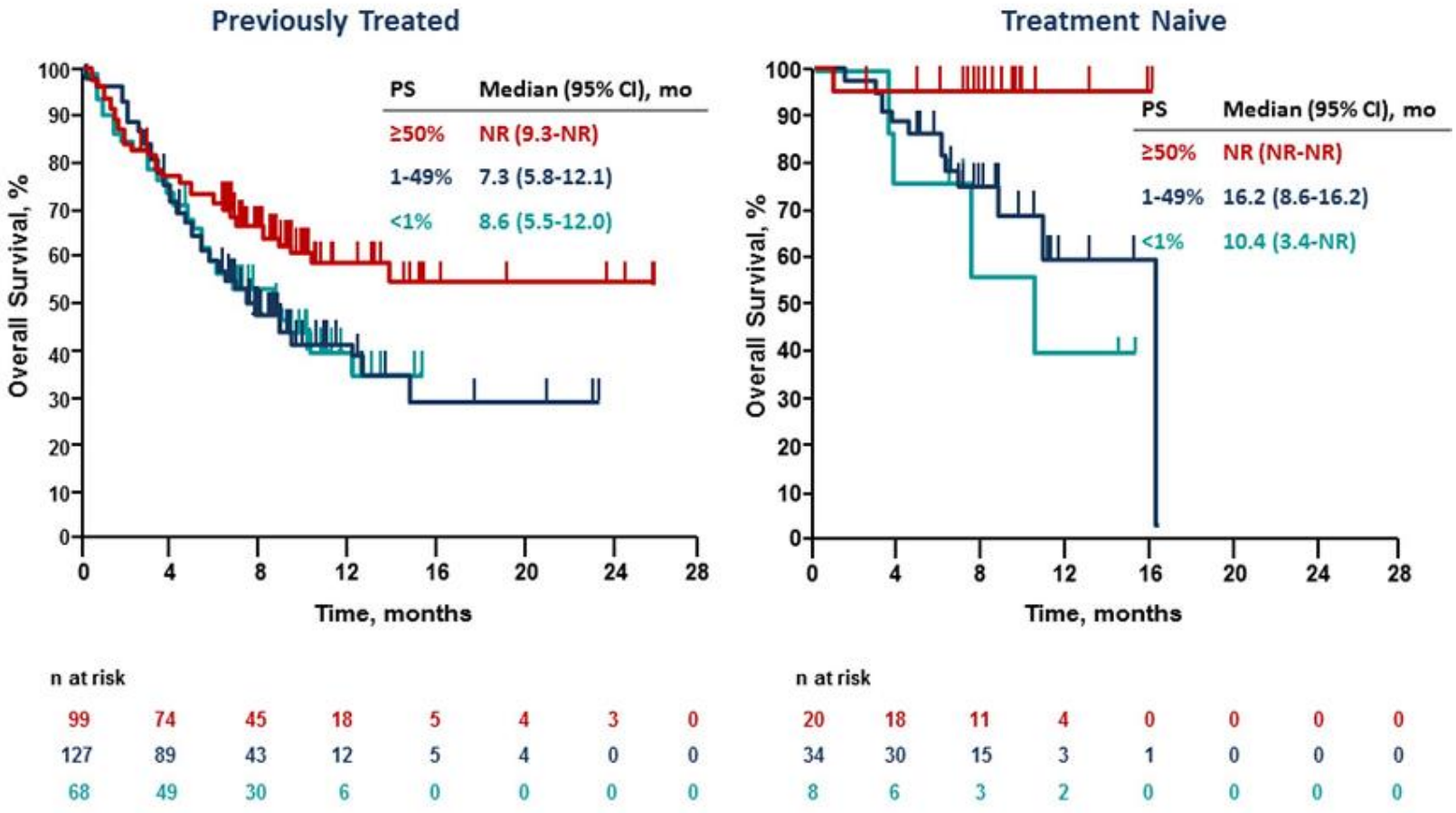
Immuntherapy of Cancer

Stimulation of the immunsystem by blocking immun-suppressive receptor protein interactions => PD-1/PD-L1



The Role of Anti-PD-L1 Immunotherapy in Cancer – OncLive - published online

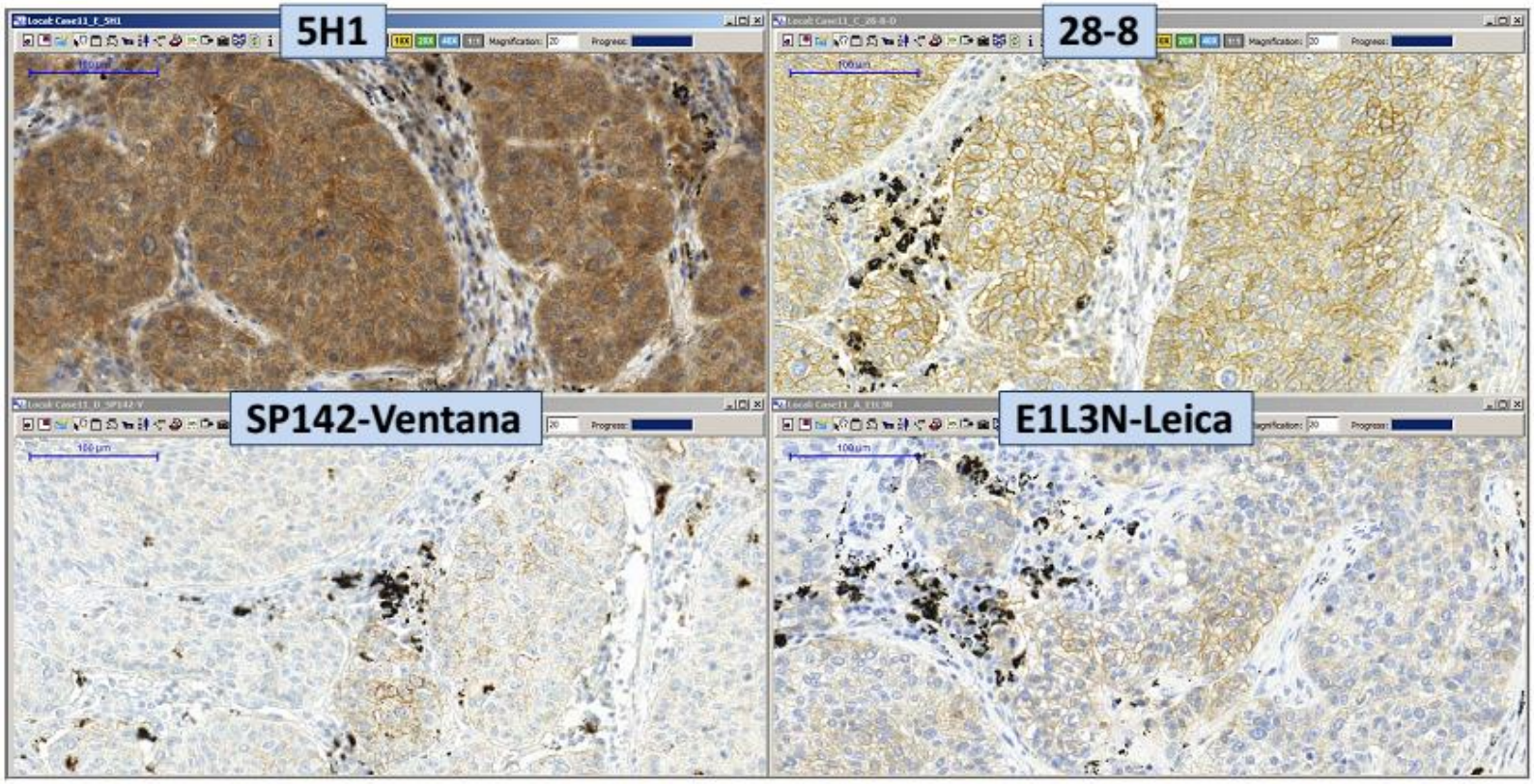
OS by IHC Determined PD-L1 Expression, Evaluable Patients by Prior Treatment



OS was assessed in all patients whose samples were stained within 6 months of cutting.
Analysis cut-off date: August 29, 2014.

Garon_AACR 2015_19Apr15

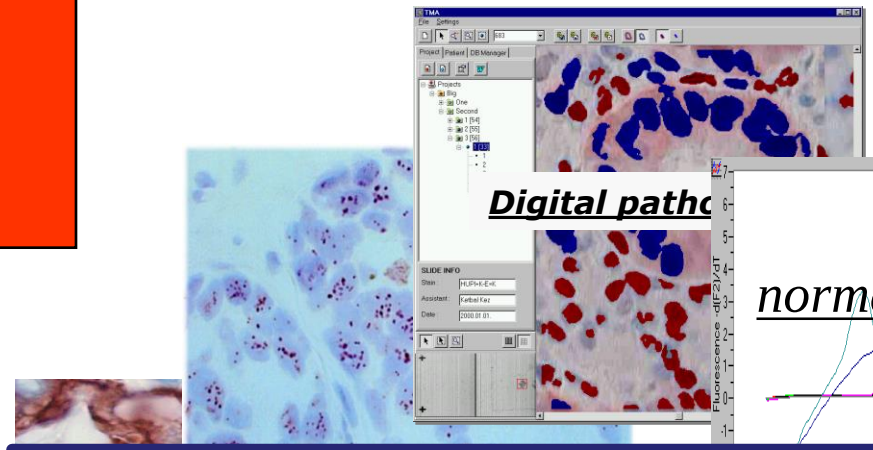
Different Staining Pattern of PD-L1 due to Applied AB

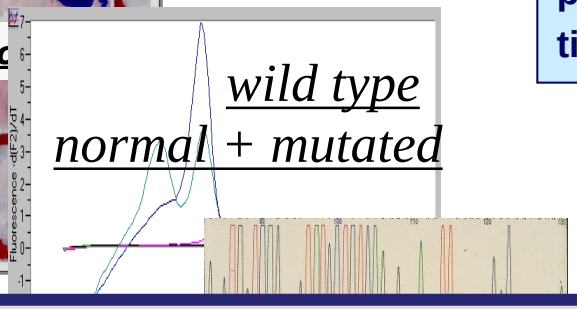


Case 11	Pathologists									Modus	Agreement
	P1	P2	P3	P4	P5	P6	P7	P8	P9		
Tumor, E1L3N	4	2	3	4	4	5	4	4	6	4	56%
Tumor, SP142	5	4	3	4	5	5	5	4	5	5; 4	56%
ImmuneCells, E1L3N	1	0	0	1	1	1	0	0	0	0	56%
ImmuneCells, SP142	1	1	1	1	1	1	0	1	1	1	89%

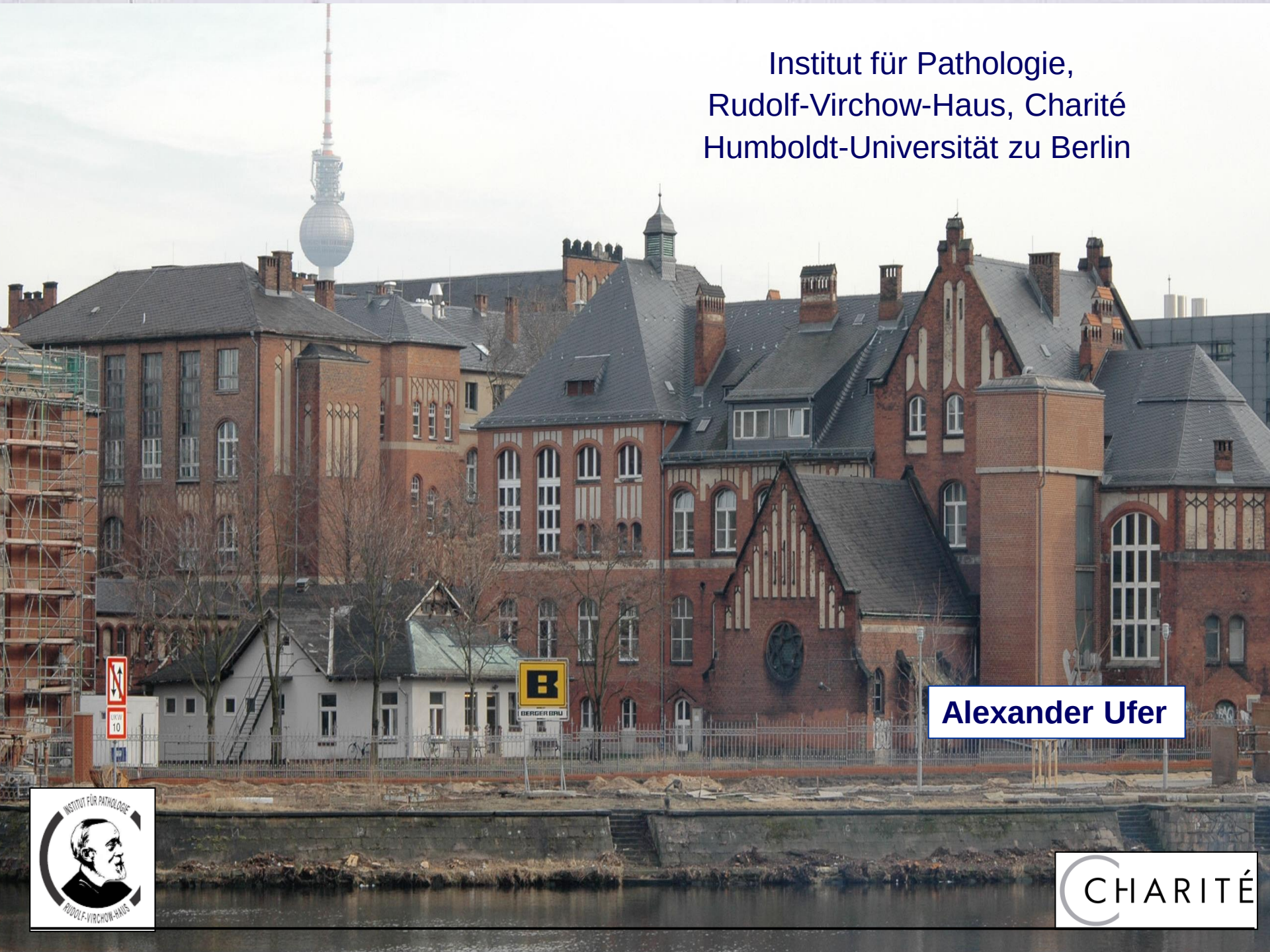
Clinical data tissue

All test are done on formalin fixed paraffin embedded tissue





New developments in precision medicine are based on a combined morphological-molecular pathology approach, which is only possible in relation to well annotated biobanks.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Next Generation Pathology

It has to be emphasized that next generation molecular pathology requires

- **next generation hospitals with**
- **next generation oncologists and**
- **next generation pathologists.**

To achieve these goals here in China we are on the way to set up a joint venture on molecular pathology.