Molecular Pathology in XXIst Century

M. Dietel

Institute of Pathology
(Rudolf-Virchow-Haus)
Humboldt University, Berlin

e-mail: manfred.dietel@charite.de

Today's Challenges in Anatomic and Molecular Pathology

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.

This is the prerequisite for precision medicine.

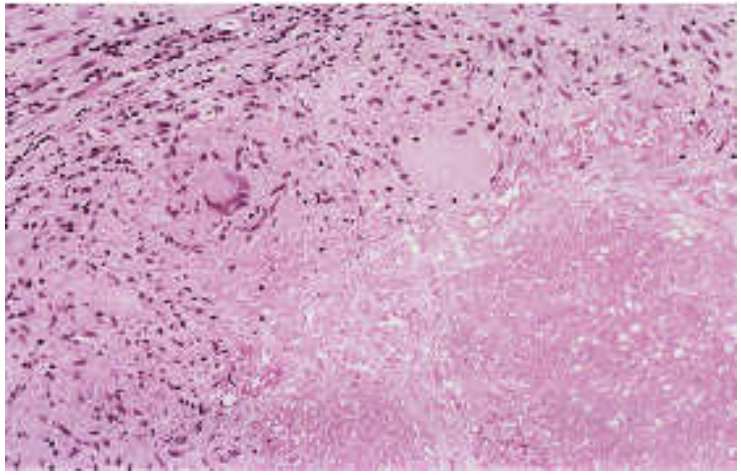
But it to be emphasized that the technical results have to be interpreted by an experienced tumor board including pathologists. Only then the diagnostic, prognostic and predictive information can be interpreted adequately to assign the optimal treatment to individual patients.

Challenges in Anatomic and Molecular Pathology

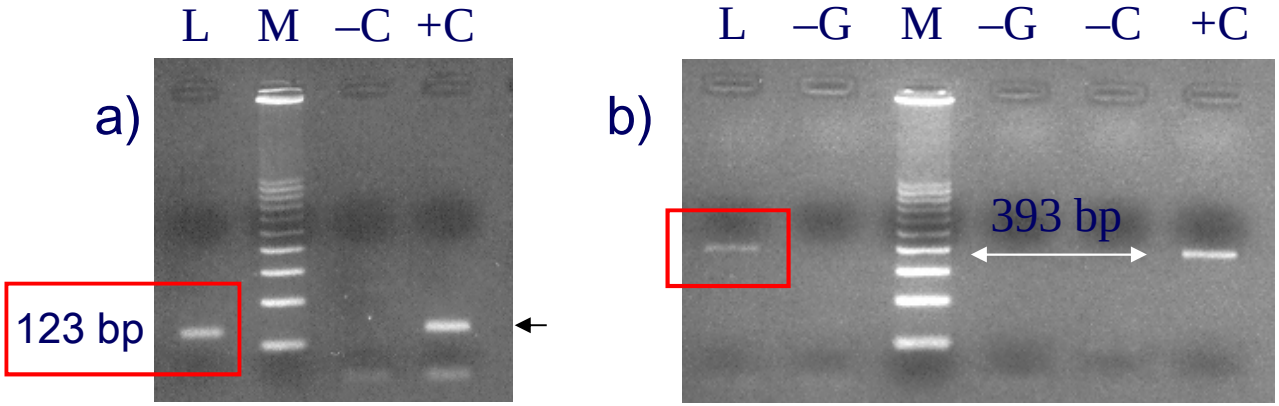
New approaches in tissue-based diagnostic

- 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)



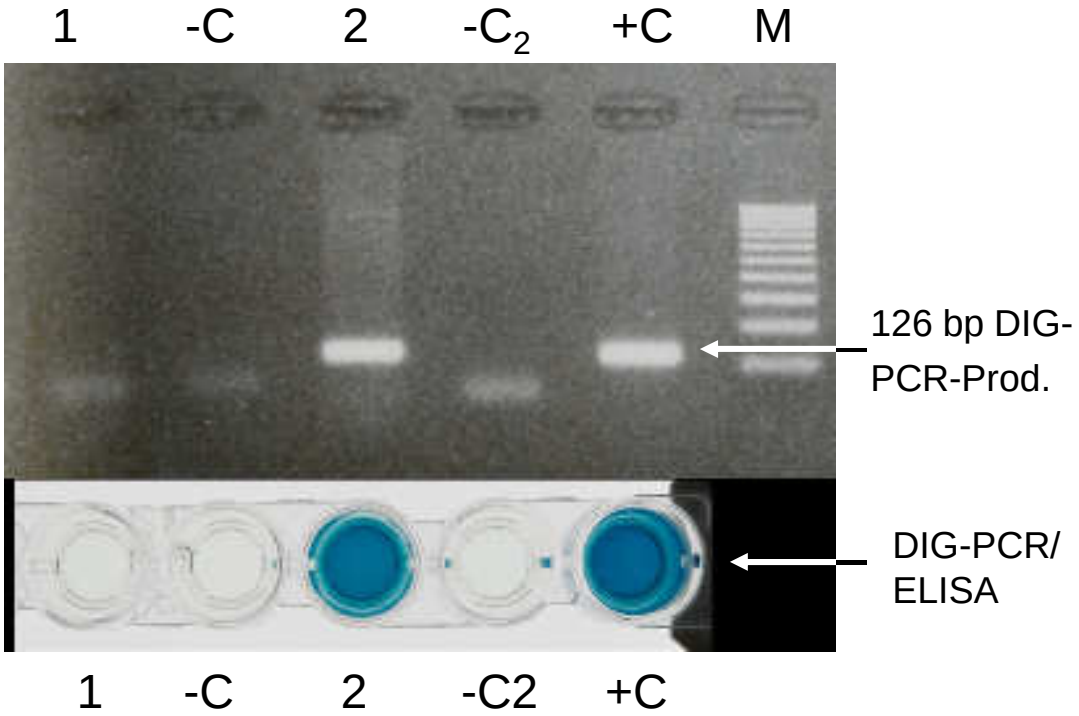
(a, b) detection of mycobacterial DNA (*M. tuberculosis*), Gel electrophoresis of the PCR products

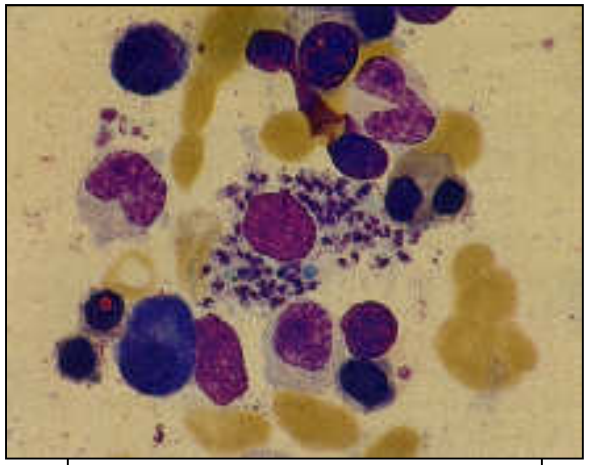
a) *Mycobacterium tuberculosis* complex-PCR,
b) Detection of the *M. tuberculosis* mtp 40 Gen,
the specificity of all products were verified by hybridisation

formalin fixed paraffin embedded tissue

Leishmaniosis*-Detection by DIG-PCR/ELISA

3,3% Agarosegel-Elektrophoresis





Leishmaniosis (bone marrow)

1 tisue-DNA w/o detection, **2** tissue-DNA with proof of *L. donovani*;
-C/-C$_2$ PCR negative control; **+C** PCR positive control; M DNA-ladder 100 bp

* Flagellate protozoe, type of hemoflagellates

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

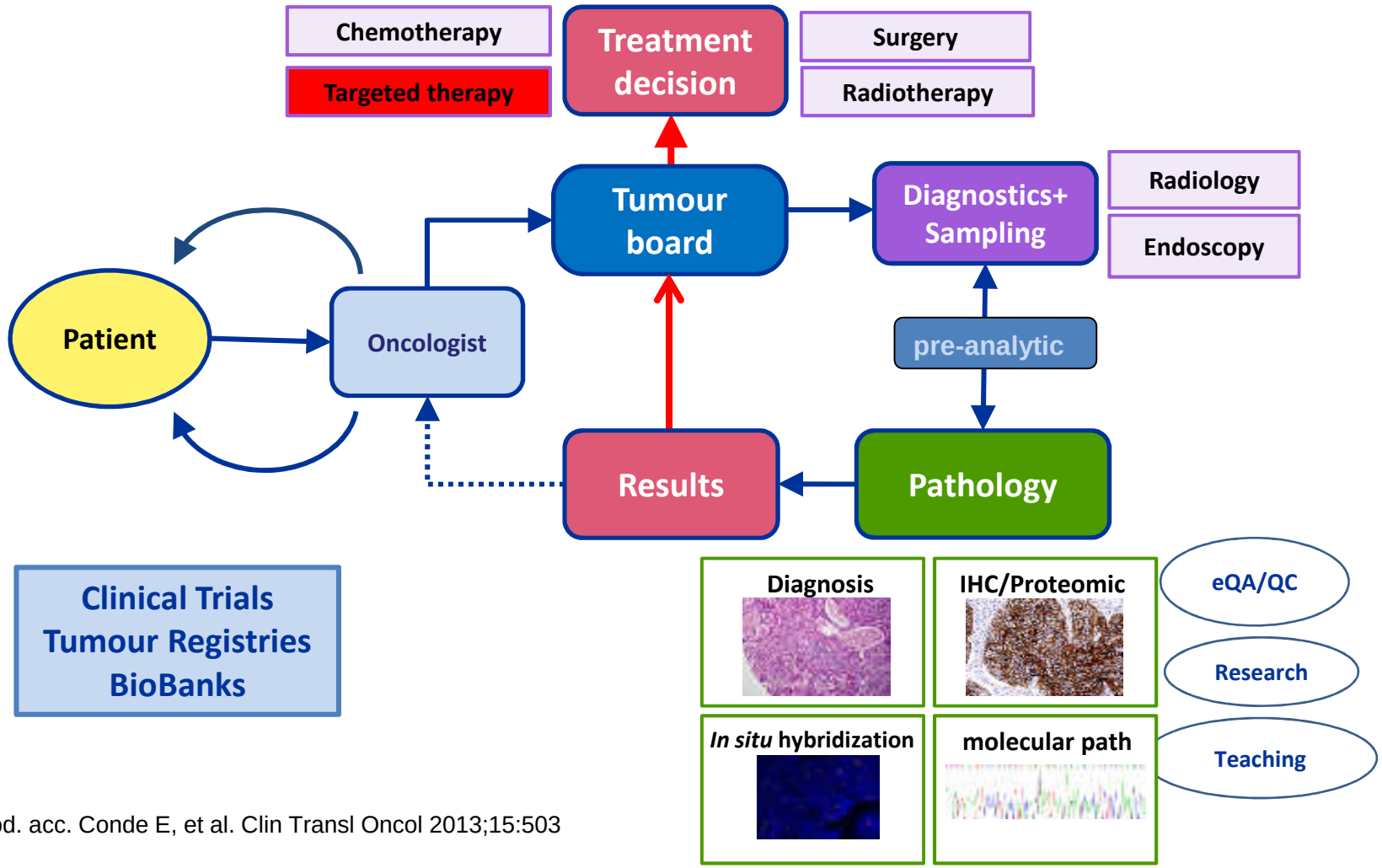
- Amoeba (Entamoeba histolytica)
- Fungi PCR/typing
- Leishmania
- Mycoplasma (consensus/pneumoniae)
- Pneumocystis carinii (P. jirovecii)
- Toxoplasma gondii

Challenges in Anatomic and Molecular Pathology

New approaches in tissue-based diagnostic

- pathology of infectious diseases
- tumor-pathology

Multidisciplinary cooperation enables personalised oncology



Mod. acc. Conde E, et al. Clin Transl Oncol 2013;15:503

Predictive tissue-based biomarkers for targeted therapies

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

- T
- 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.

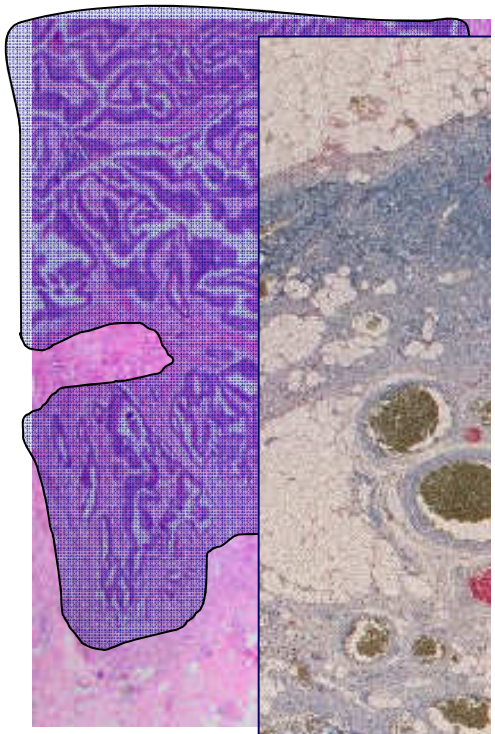
All these substances have been developed on the basis of histologically characterised human tissue.

This underlines the importance of biobanks.

*Strongly suggested by FDA's Drug-Diagnostic Co-Development Initiative

CHARITÉ

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.

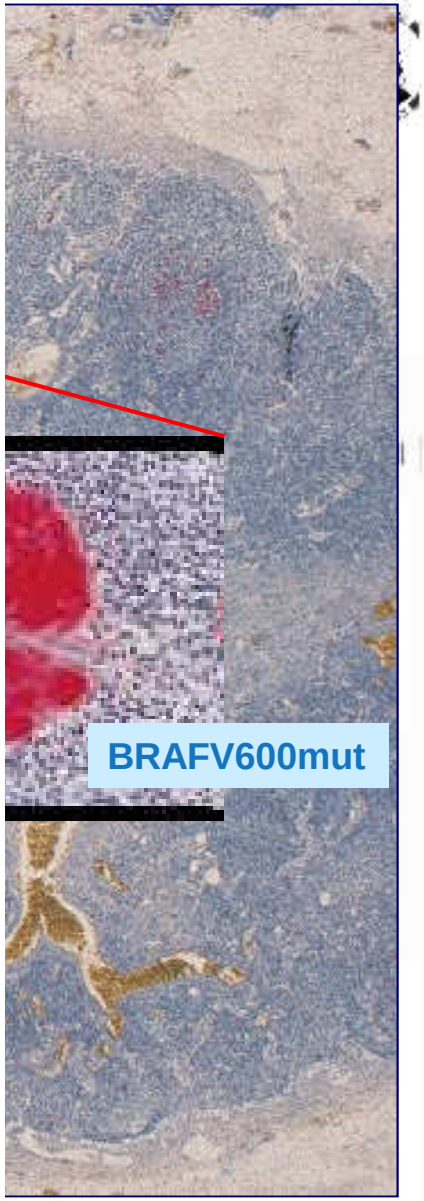
Leitung des Ringversuches:
Prof. Dr. med. P. Schirmacher, Prof. Dr. med. M. Dietel,
Dr. R. Penzel, Dr. Chr. Schewe

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 / 30881970, Mail: bvd@pathologie.de

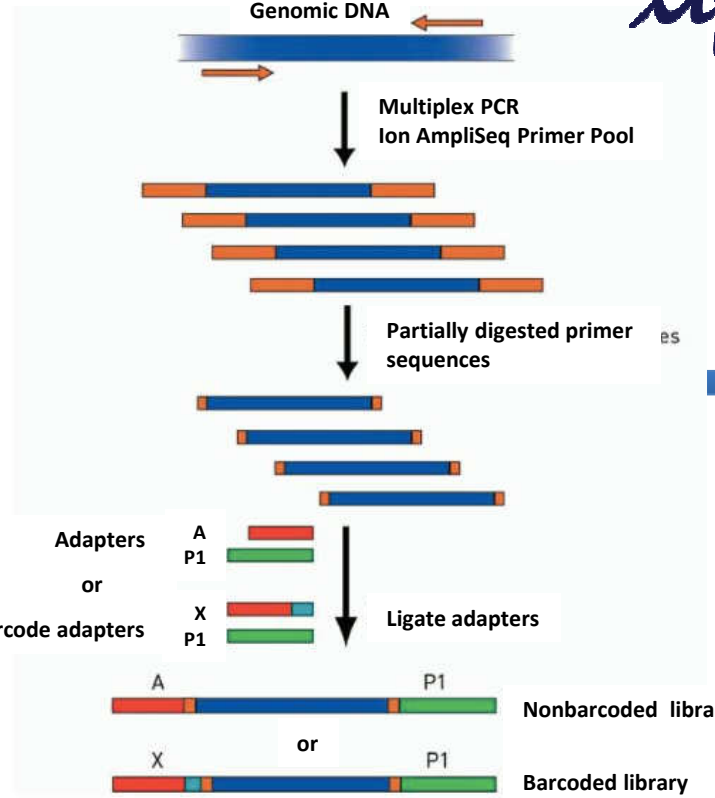


BRAFV600mut

Institut für Pathol

CHARITÉ

Integrating Next Generation Sequencing in Diagnostic Pathology



life technologies

IonTorrent PGM

Ion AmpliSeq™ Cancer Panel targets 46 critical genes

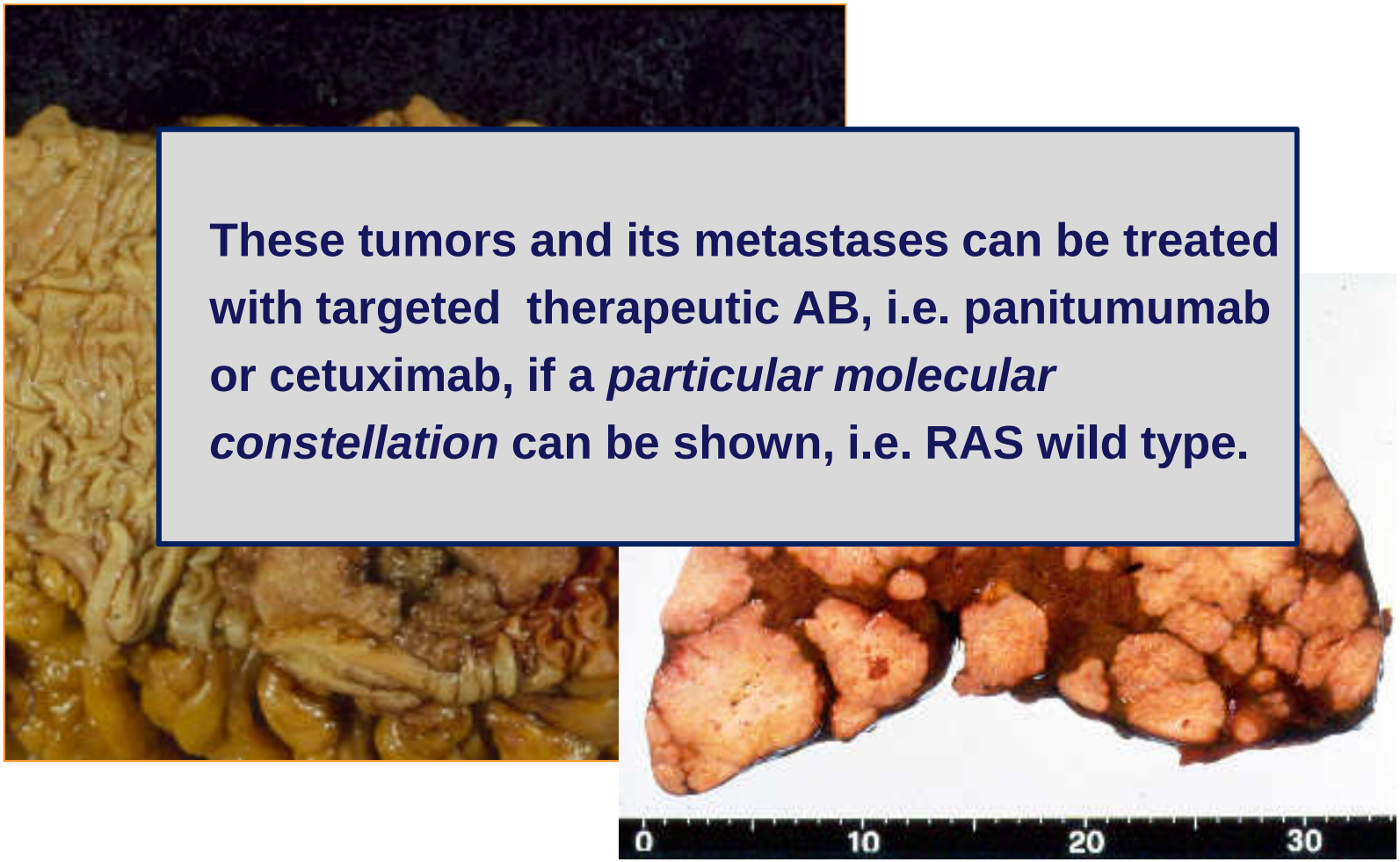
ABL1	ERBB4	KDR	PTPN11
AKT1	FBXW7	KIT	RB1
ALK	FGFR1	KRAS	RET
APC	FGFR2	MET	SMAD4
ATM	FGFR3	MLH1	SMARCB1
BRAF	FLT3	MPL	SMO
CDH1	GNAS	NOTCH1	SRC
CDKN2A	HNF1A	NPM1	STK11
CSF1R	HRAS	NRAS	TP53
CTNNB1	IDH1	PDGFRA	VHL
EGFR	JAK2	PIK3CA	
ERBB2	JAK3	PTEN	

CHARITÉ

Tumor Entities Important in Predictive Molecular Pathology

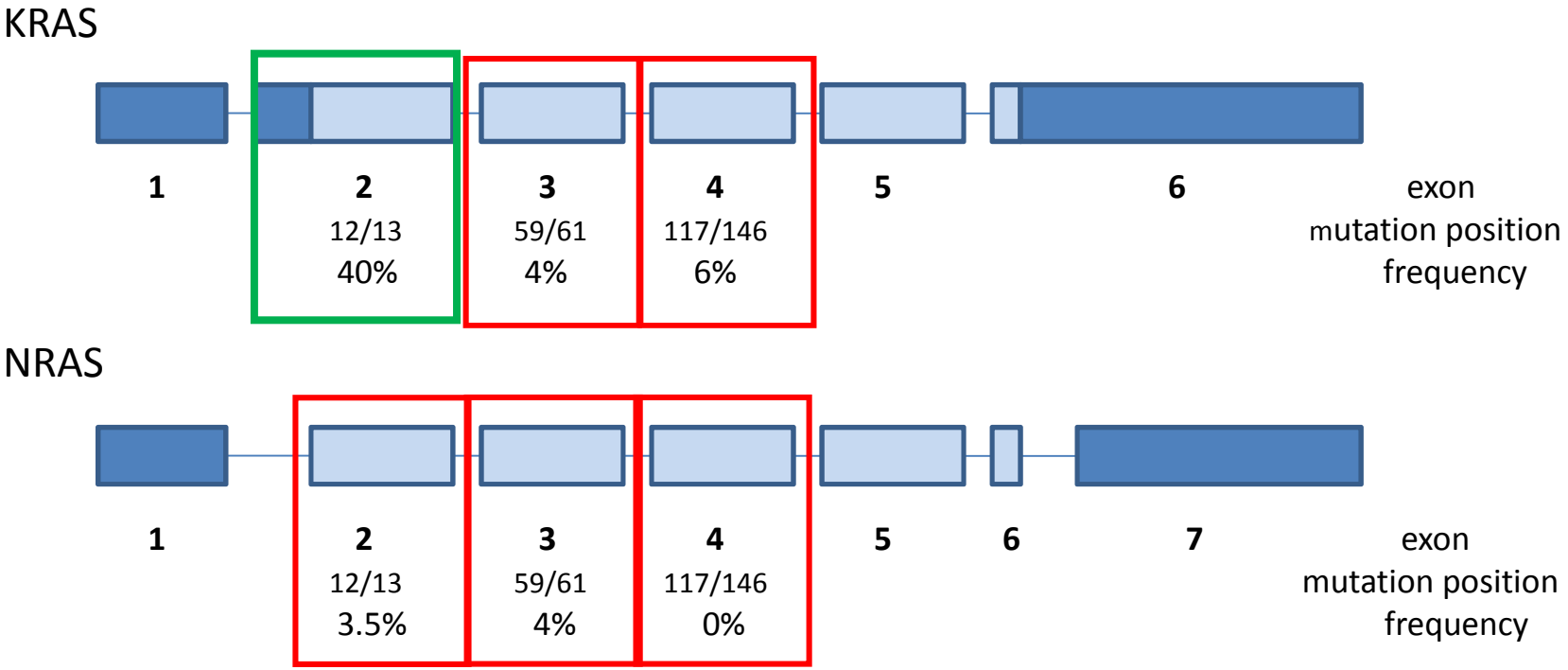
- Colon cancer
- Malignant melanoma
- Rare tumors
- Ovarian cancer
- NSCLC
- Breast cancer
- Check-point inhibitors

Invasive colorectal cancer with liver metastases



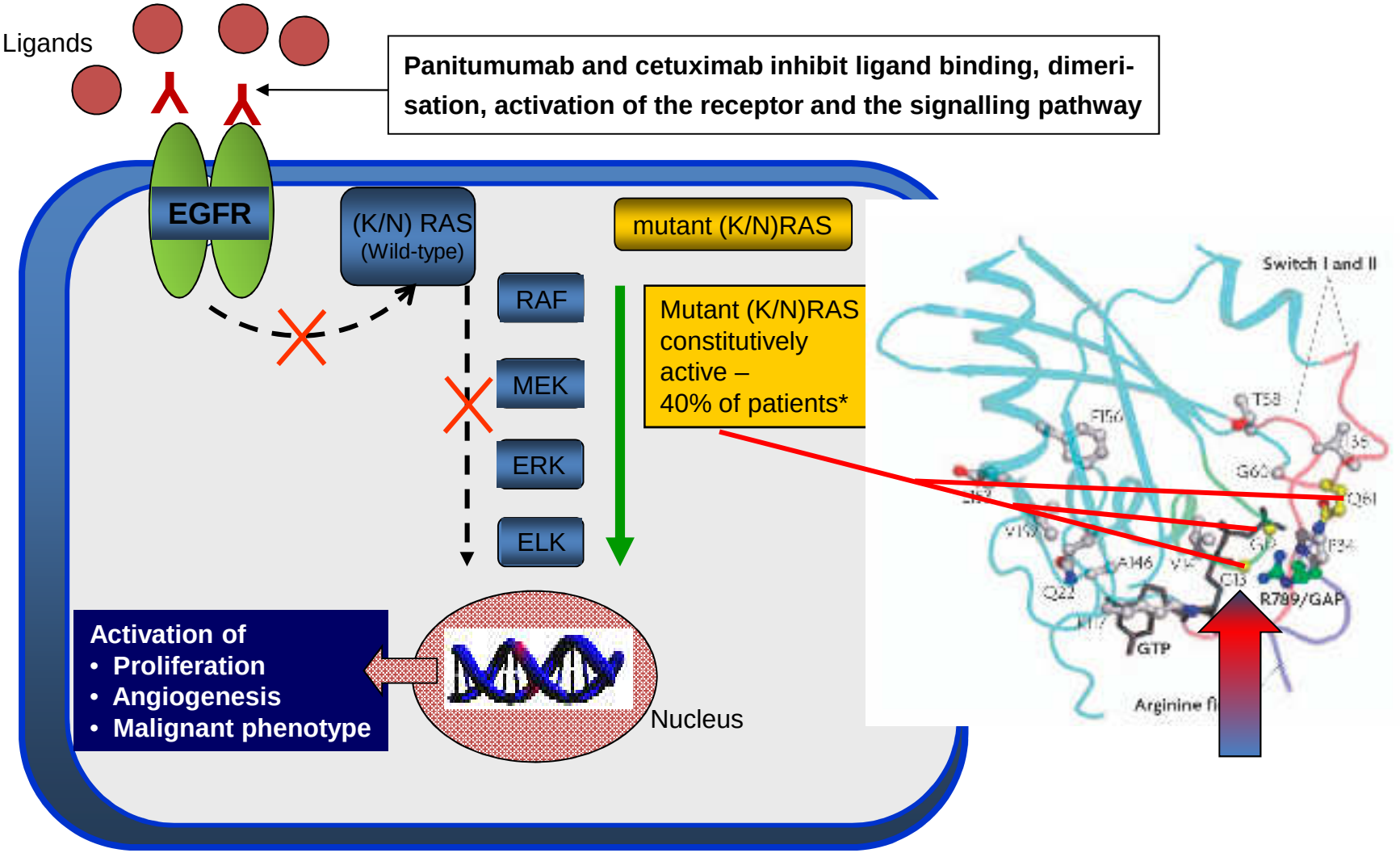
These tumors and its metastases can be treated with targeted therapeutic AB, i.e. panitumumab or cetuximab, if a *particular molecular constellation* can be shown, i.e. RAS wild type.

Mutations in KRAS and NRAS genes in colorectal cancer



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!

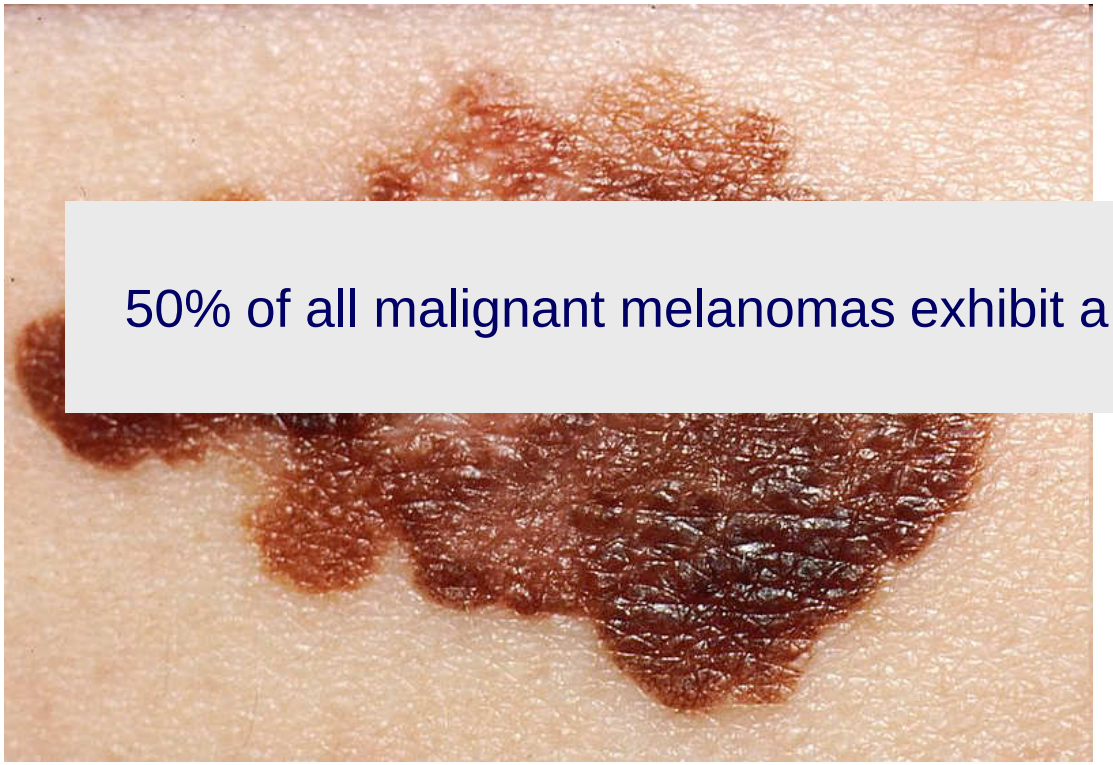
RAS-MAPK signalling pathway



Schubbert S *et al*. Nat Rev Cancer 2007;7:295-308;

*Friday BB, Adjei AA. Biochim. Biophys. Acta. 2005; 1756:127-144.

Malignant Melanoma



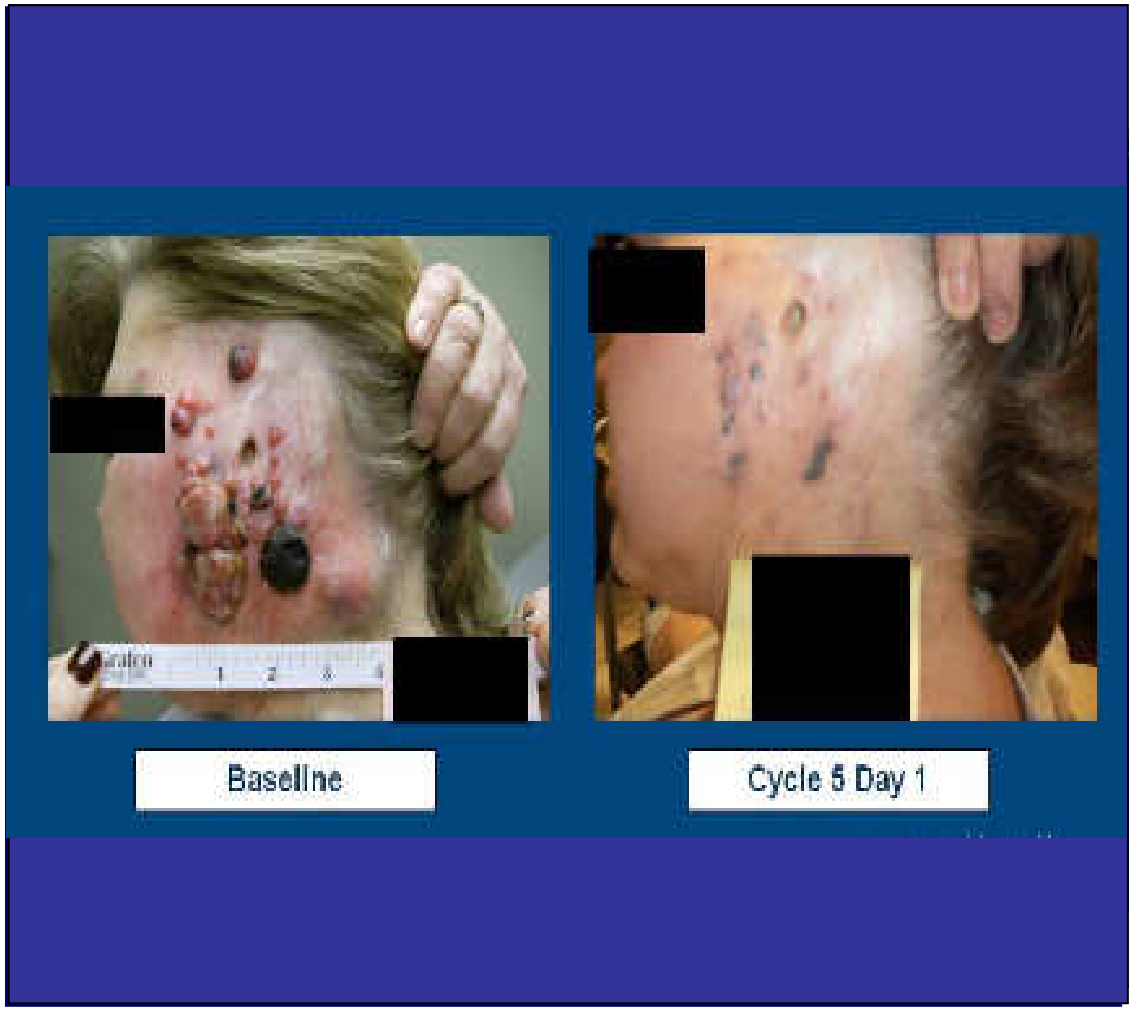
50% of all malignant melanomas exhibit a BRAF-Mutation

*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

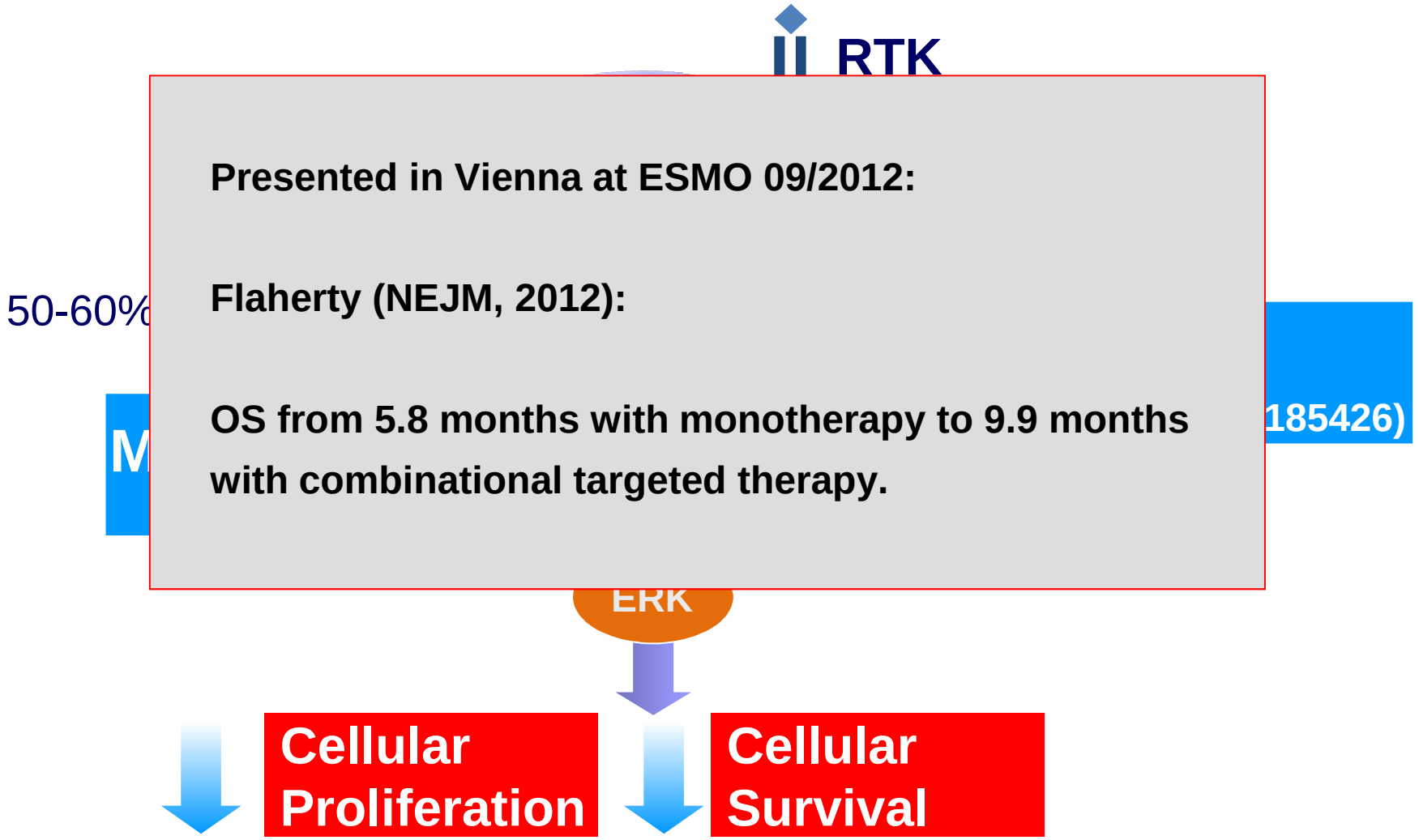
Vemurafenib inhibits V600 mutated BRAF kinase

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

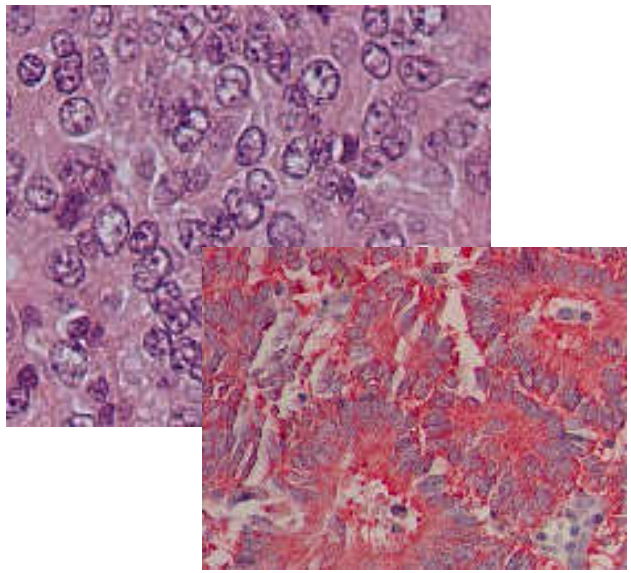
Next generation sequencing and rare tumor entities -

an issue of up-coming importance !

Up-coming Molecular Diagnostic

histological diagnosis

metastasized neuro-endocrine carcinoma, grade 3



standard sequential molecular diagnostics

KRAS
BRAF
EGFR exons 18,19, 21
cKIT
usw.

no mutations

NGS based molecular diagnostics

IonAmpliseq* Cancer Panel in 46 gene (total 604 loci).

other relevant mutations

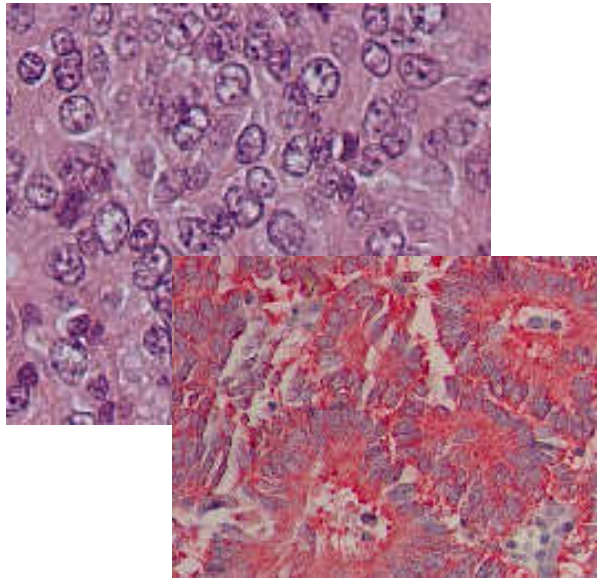
????

***Ion Torrent**

Up-coming Molecular Diagnostic

histological diagnosis

metastasized neuro-endocrine carcinoma, grade 3



standard sequential molecular diagnostics

KRAS
BRAF
EGFR exons 18,19, 21
cKIT
usw.

NGS based molecular diagnostics

IonAmpliseq* Cancer Panel in 46 gene (total 604 loci).

ABL
APC
ALK
KRAS
BRAF

Iressa ⇒ **EGFR mut exon 20**
ERBB2

FGFR-inhibitor ⇒ **FGFR2 mut**
FGFR3
cKIT

sorafinib/sufitinib ⇒ **KDL mut**

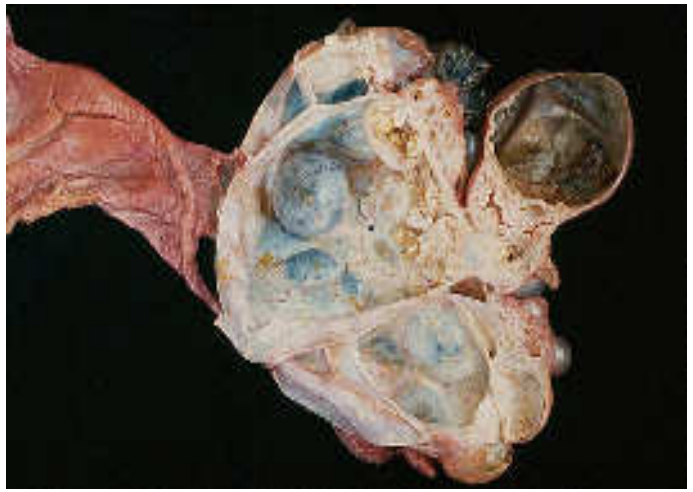
604 further loci…….

Next Generation Pathology of Ovarian Cancer

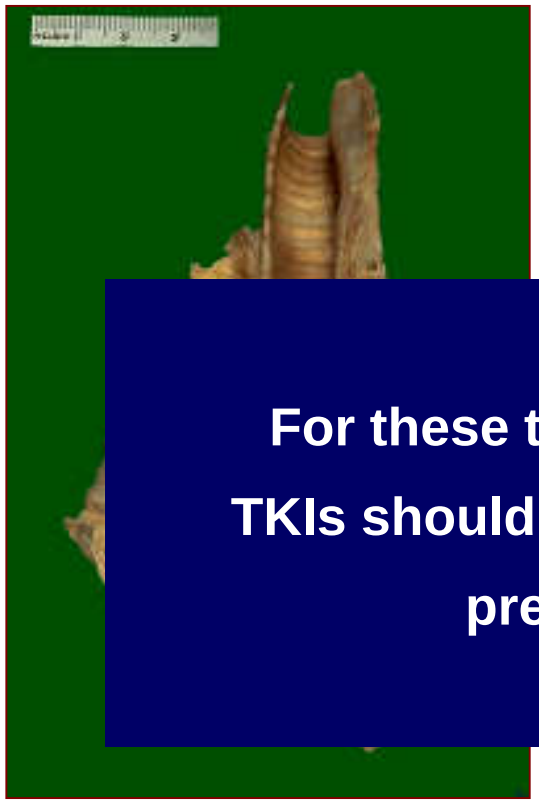
Up-coming challenge in companion diagnostic of ovarian cancer:

Routine BRCA-testing as prerequisite for treatments with the PARP inhibitor Olaparib and in near future TNBC.

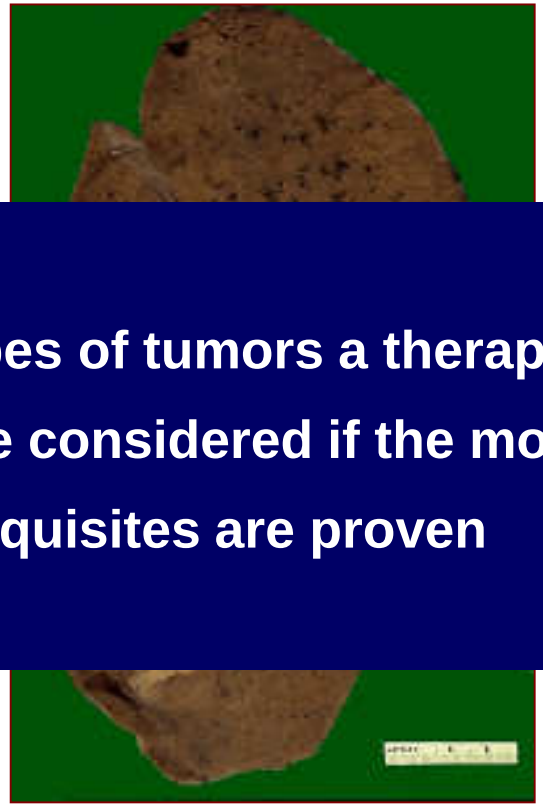
This can be done only by NGS.



NSCLC - Macroscopy

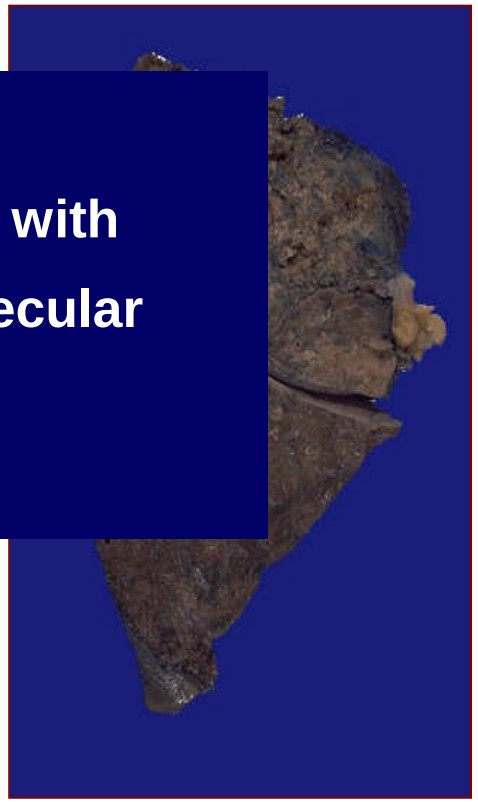


central
squamous cell carcinoma



peripheral
adenocarcinoma

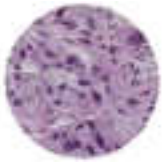
adeno carcinoma
broncho-alveolar type



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]

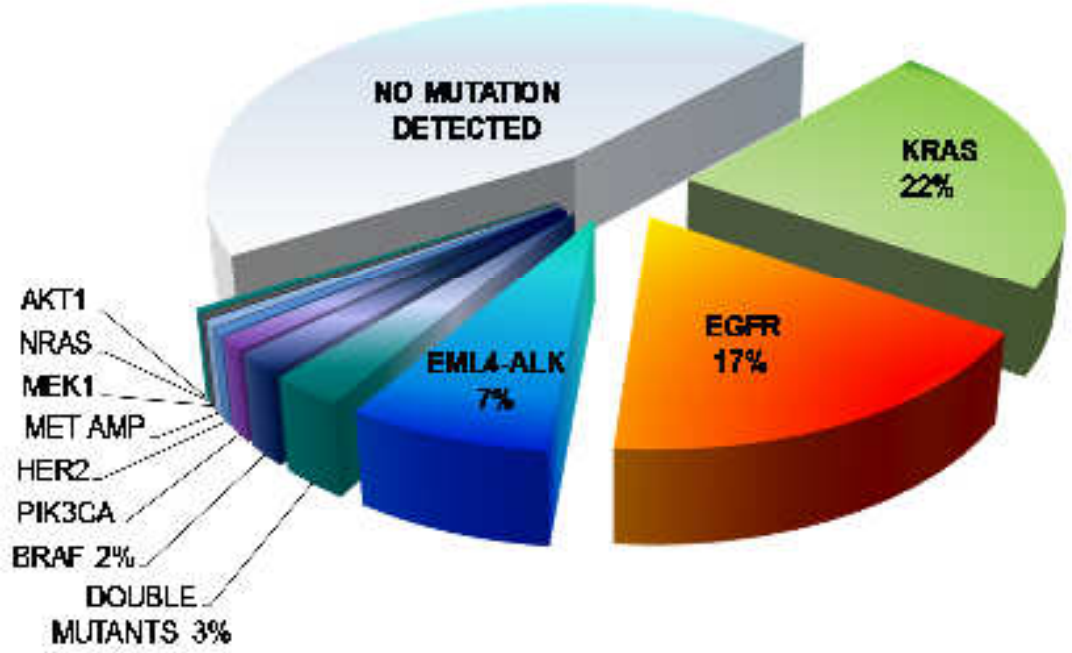


Adenocarcinoma

Squamous-cell carcinoma

Large cell carcinoma

2012
Targeting oncogenic drivers



Actionable driver mutations identified in 54% of lung adenocarcinoma tumours

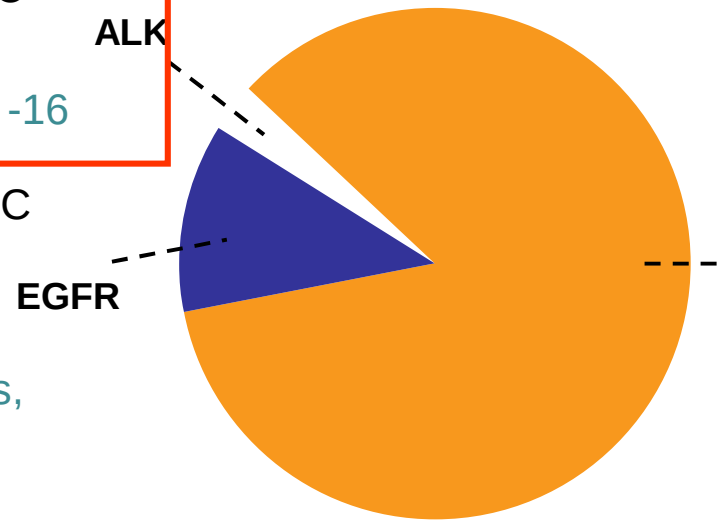
LCMC, Lung Cancer Mutation Consortium

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

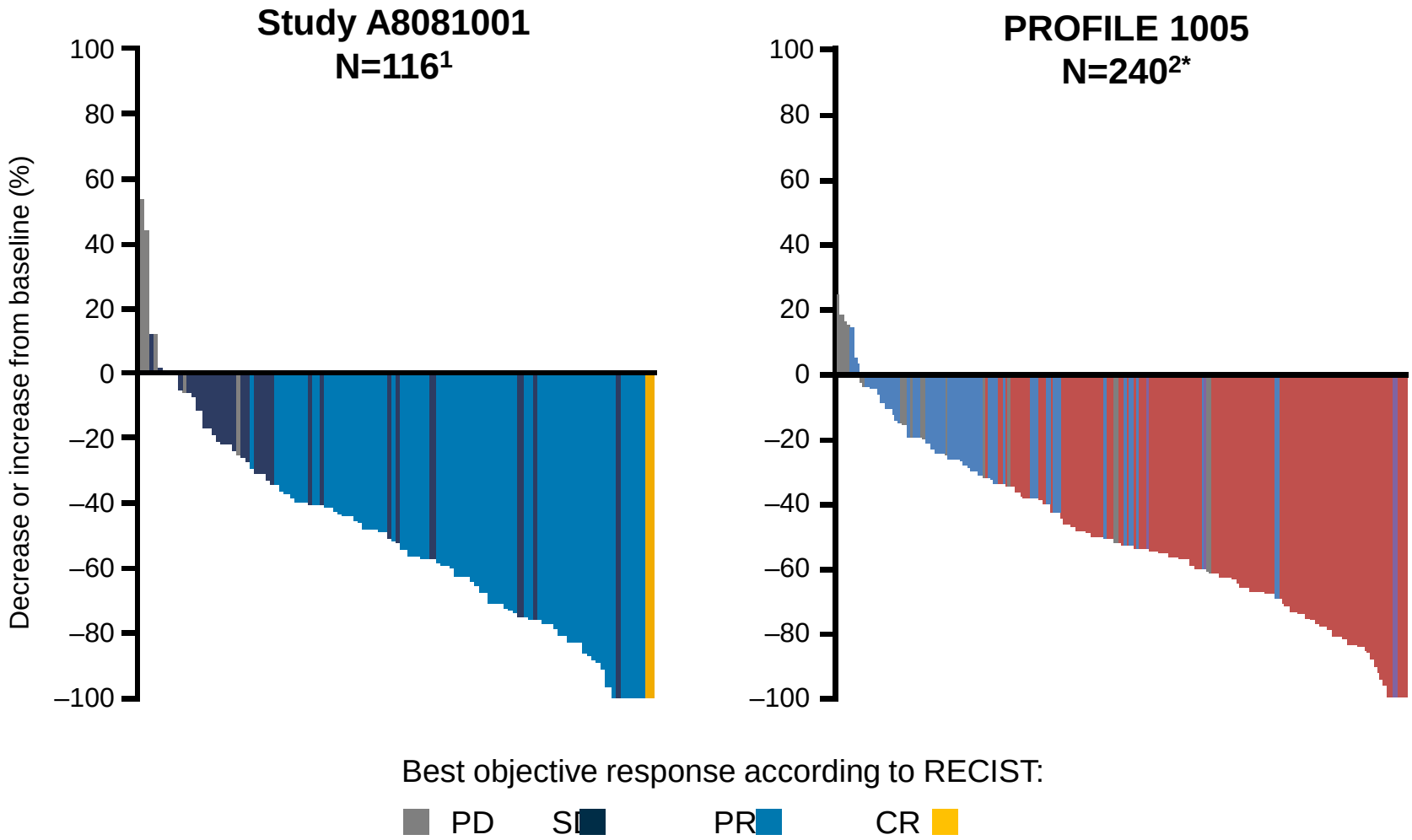


Chemotherapy in unselected patients[2]
RR 20%, OS <12 mths

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

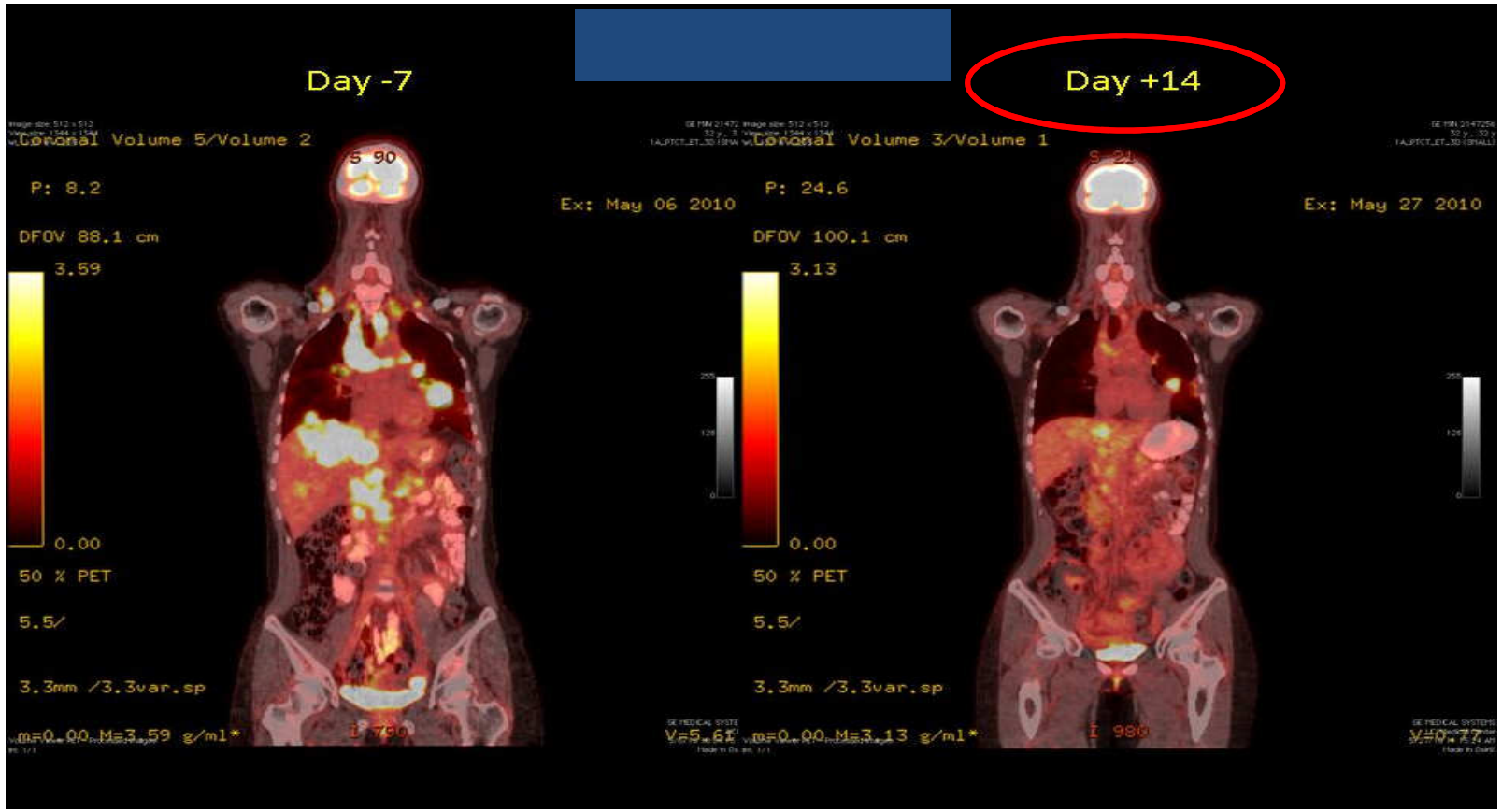
Tumour responses to crizotinib by patient



1. Camidge DR, et al. Lancet Oncol 2012;10:1011−9;
2. Kim DW, et al. Presented at ASCO 2012; Abstract 7533

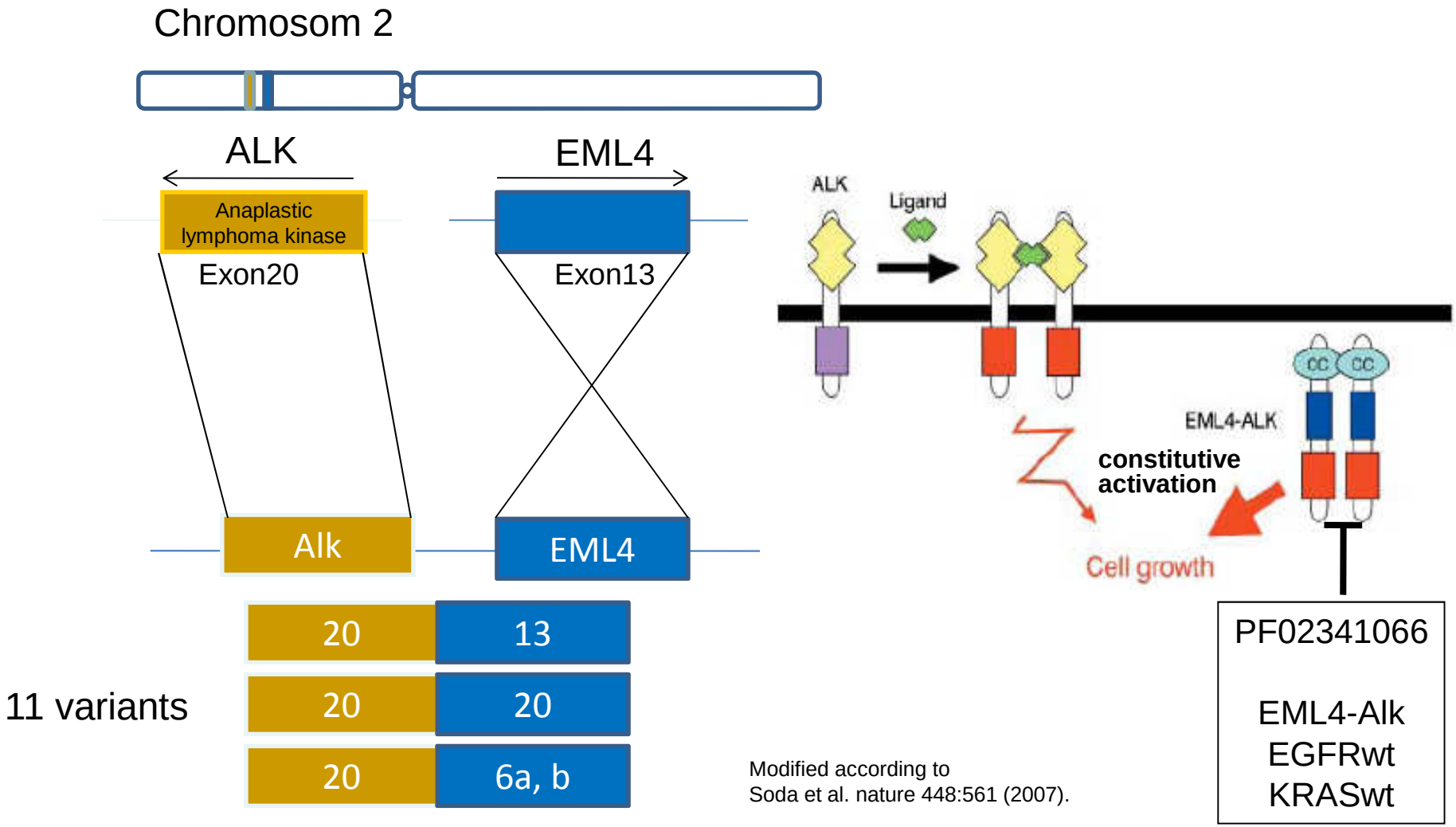
*Mature population, excluding those with early death, indeterminate response and non-measurable disease

Rapid Responses Seen In Some Patients

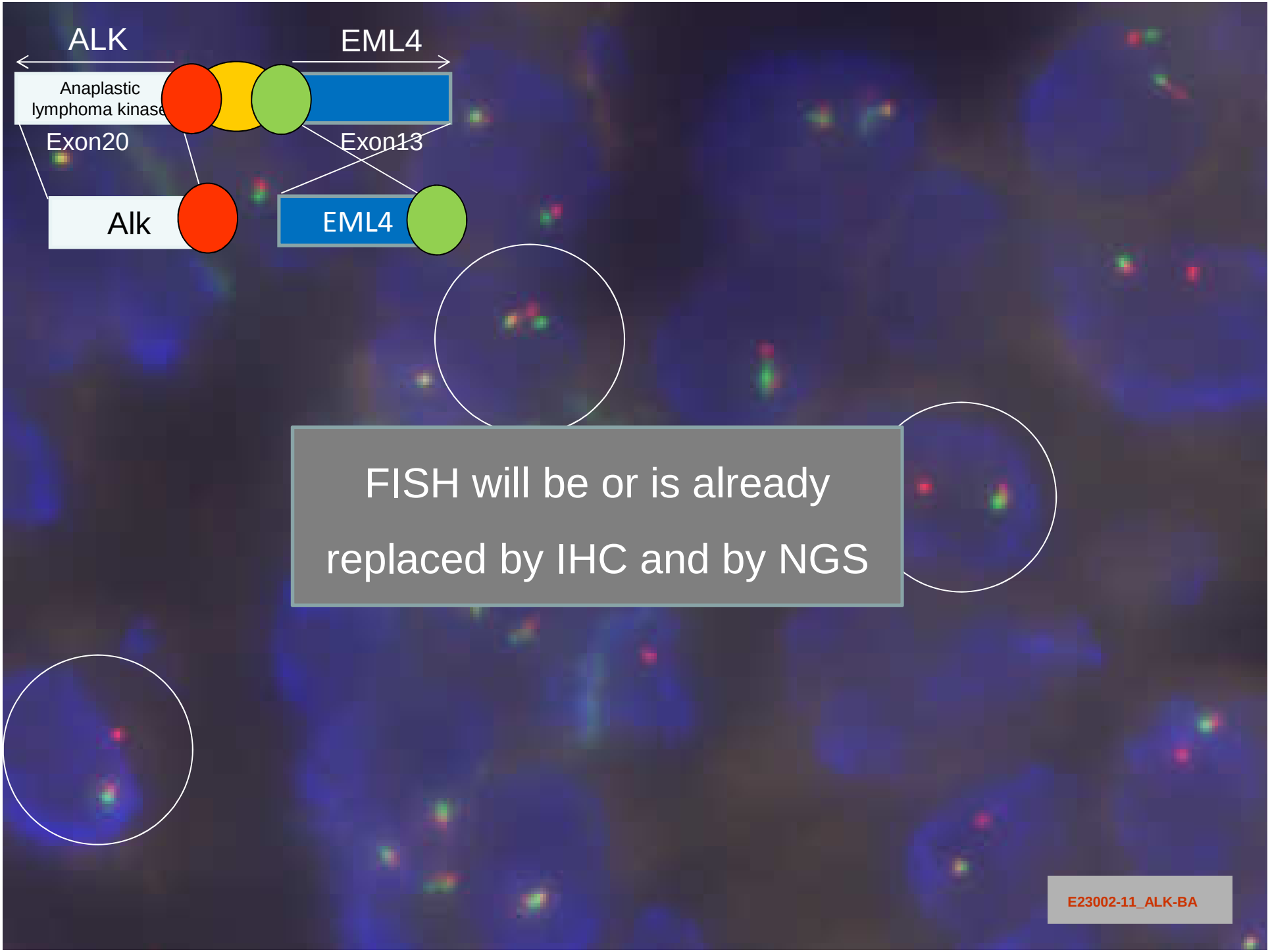


Ou et al. J Thoracic Oncol 2010;5:2044–2046 Camidge RD et al.: ASCO 2011

EML4-ALK Fusion in NSCLC



Modified according to
Soda et al. nature 448:561 (2007).


ALK
EML4
Anaplastic
lymphoma kinase
Exon20
Exon13
Alk
EML4
FISH will be or is already
replaced by IHC and by NGS
E23002-11_ALK-BA

The next step: How to fight resistance

Almost all tumors become resistant to targeting drugs.

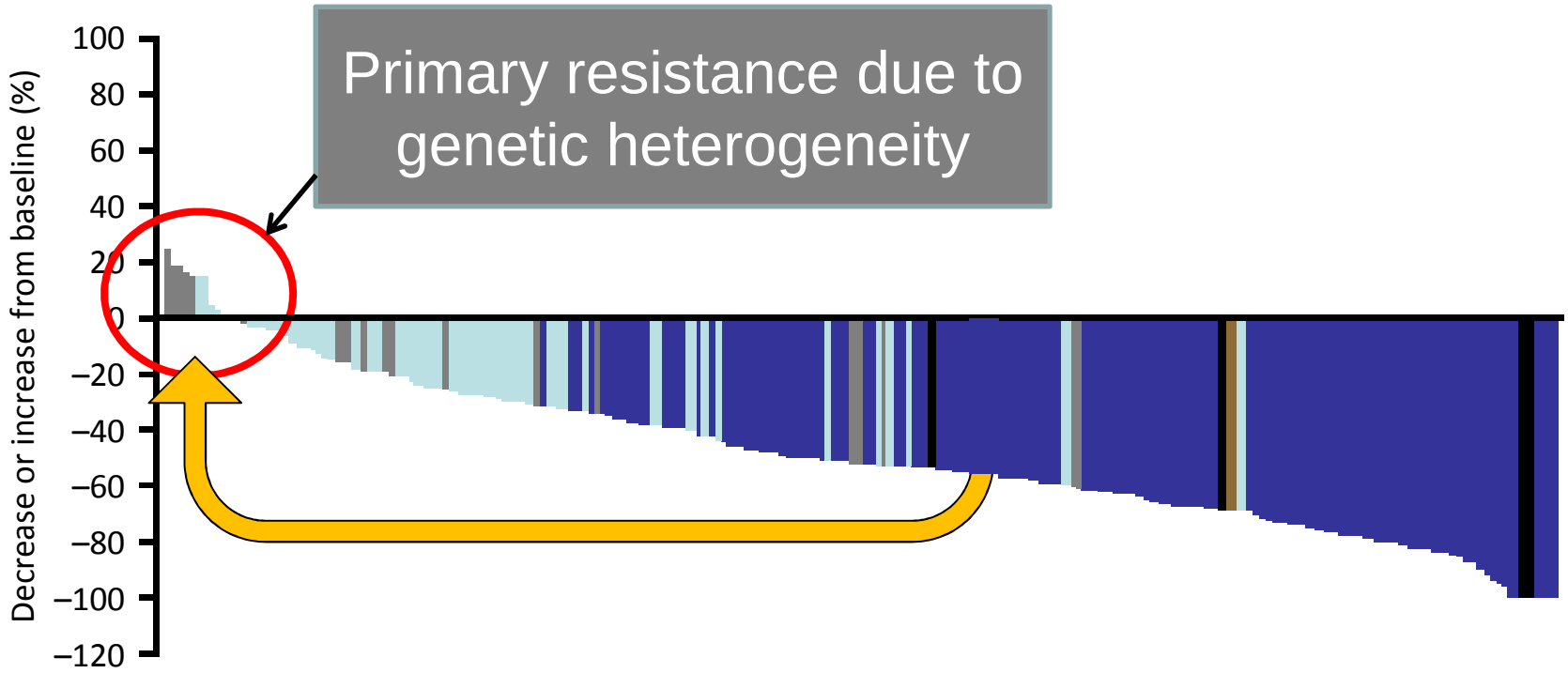
Novel approaches that have already proven successful include the development of second-generation and third-generation inhibitors and the combination of some of these inhibitors with antibodies directed against the same target or other targets (check points).

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**

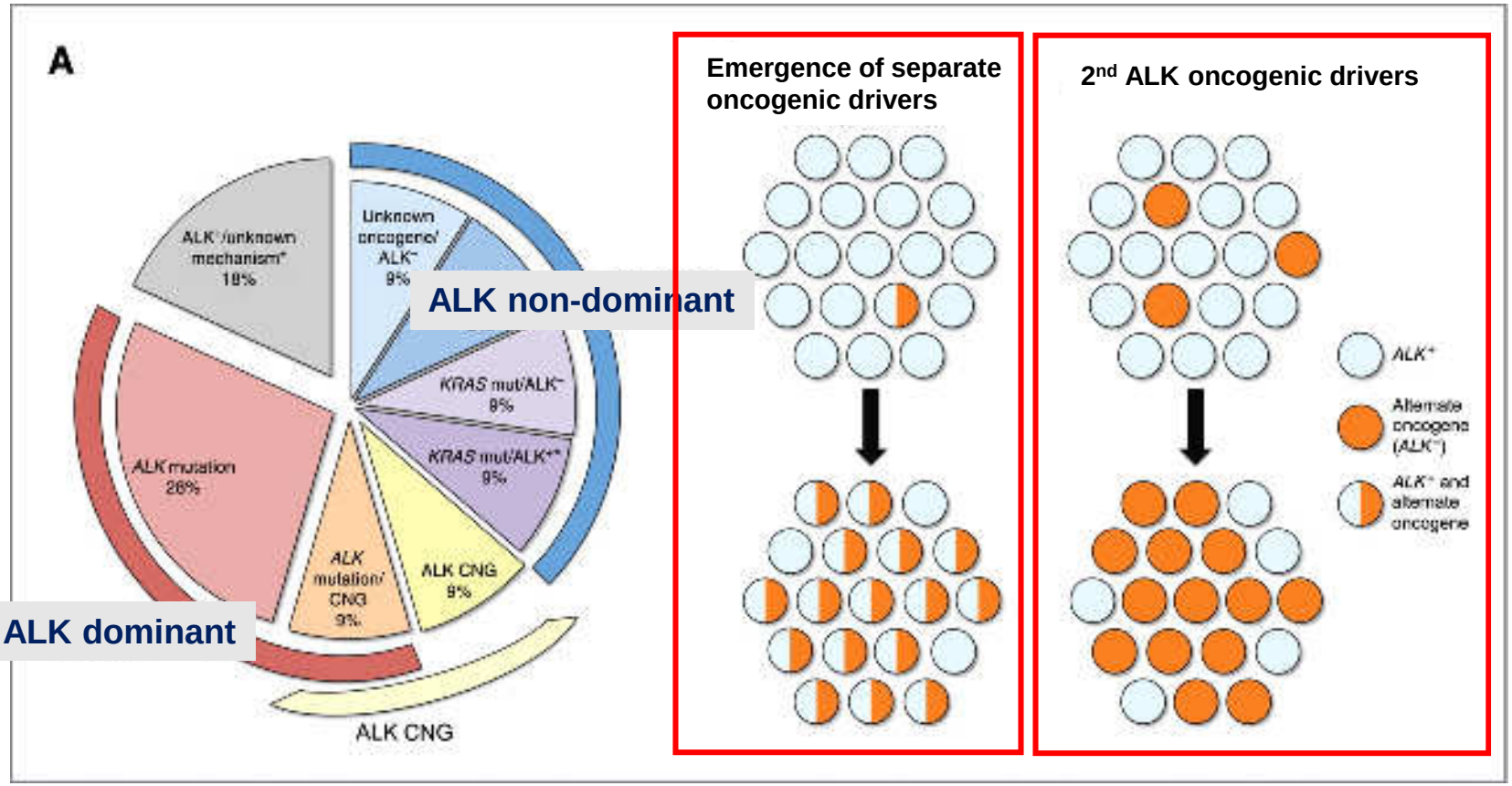
Majority of ALK+ tumors respond to Crizotinib – some show primary resistance



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**

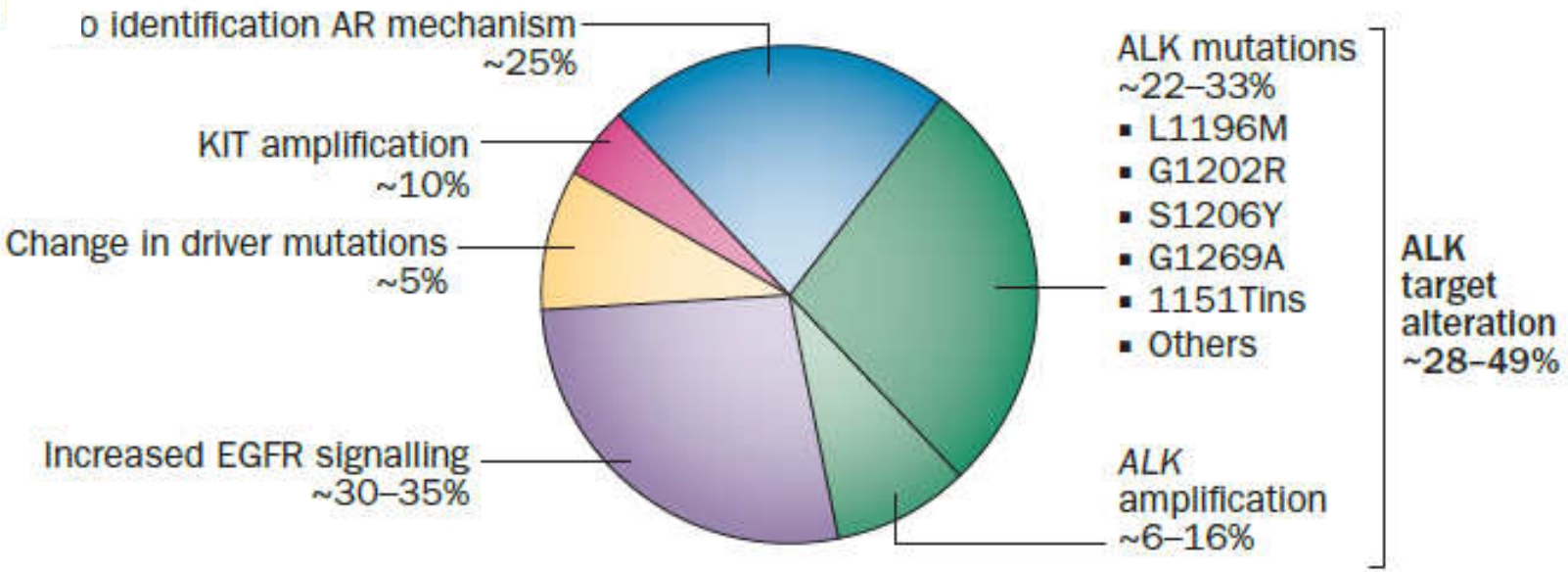
Crizotinib resistance mechanisms in patients with ALK+ NSCLC and models for potential mechanisms of alternate oncogene acquisition



Robert C. Doebele et al. Clin Cancer Res 2012;18:1472-1482

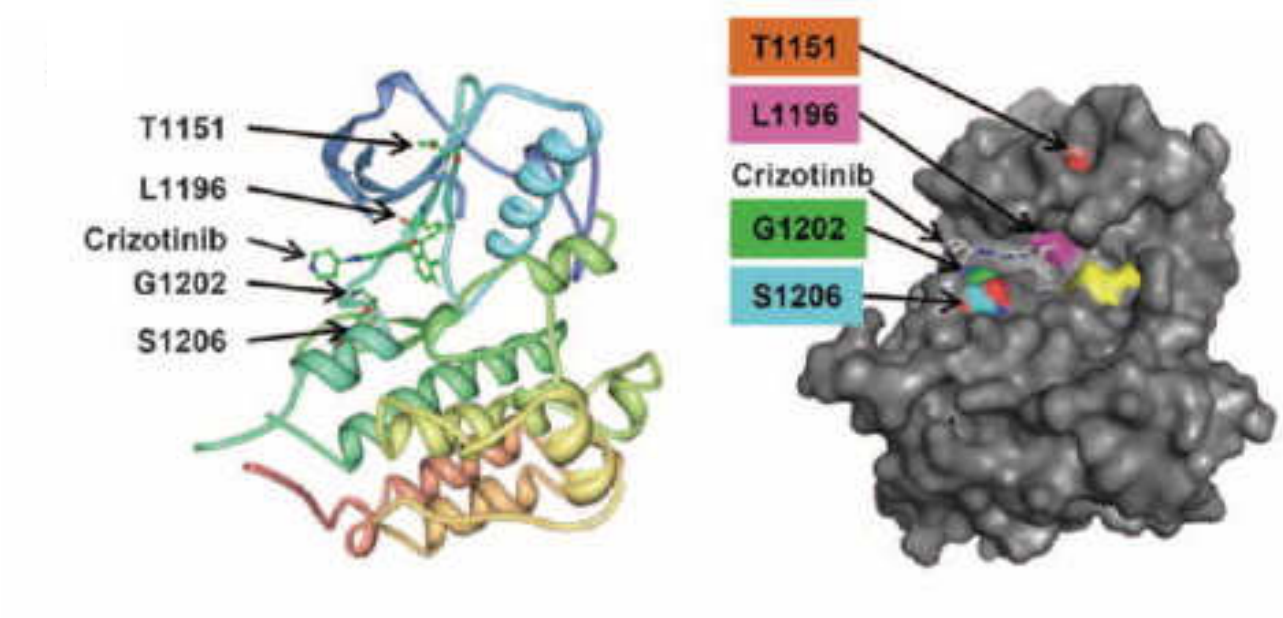
Clinical Cancer Research
AACR

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

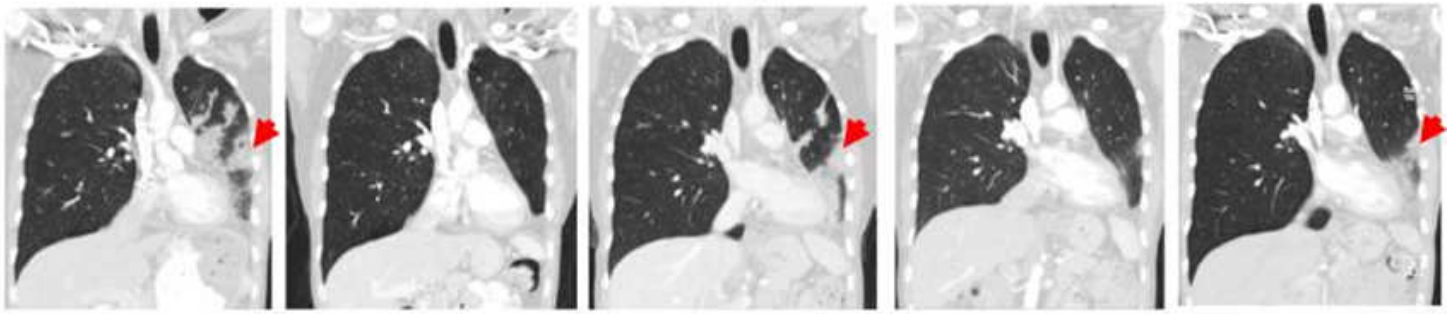
For example:

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



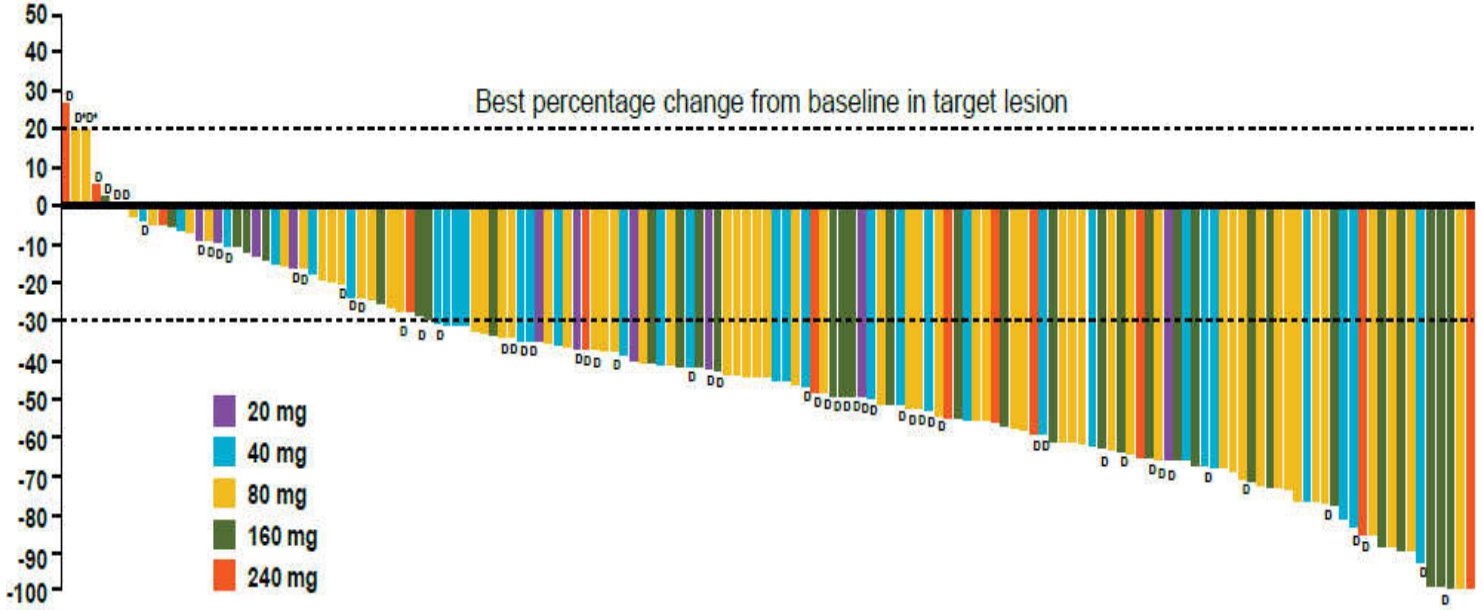
Baseline | After 8 weeks of crizotinib | After 34 months of crizotinib | After 12 weeks of Ceritinib | After 15 months of Ceritinib

EML4-ALK sequence: **ALK mut** S1206Y G1202R

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)

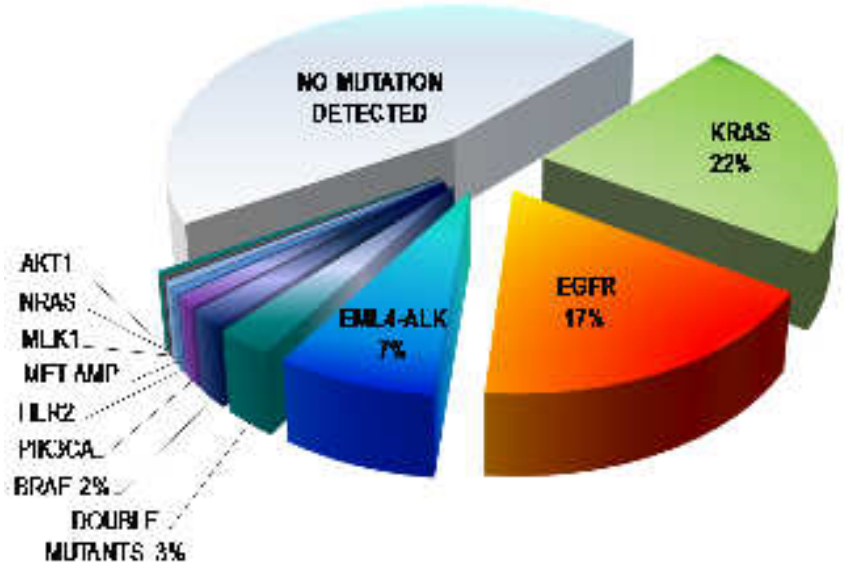
Presented by Pasi A Jänne at the 2016 European Lung Cancer Conference. Ann Oncol 2016; 26(Suppl 1): i80, LBA3.

2nd Generation ALK-Inhibitors

Other ALK-inhibitors in development include:

ceritinib and alectinib

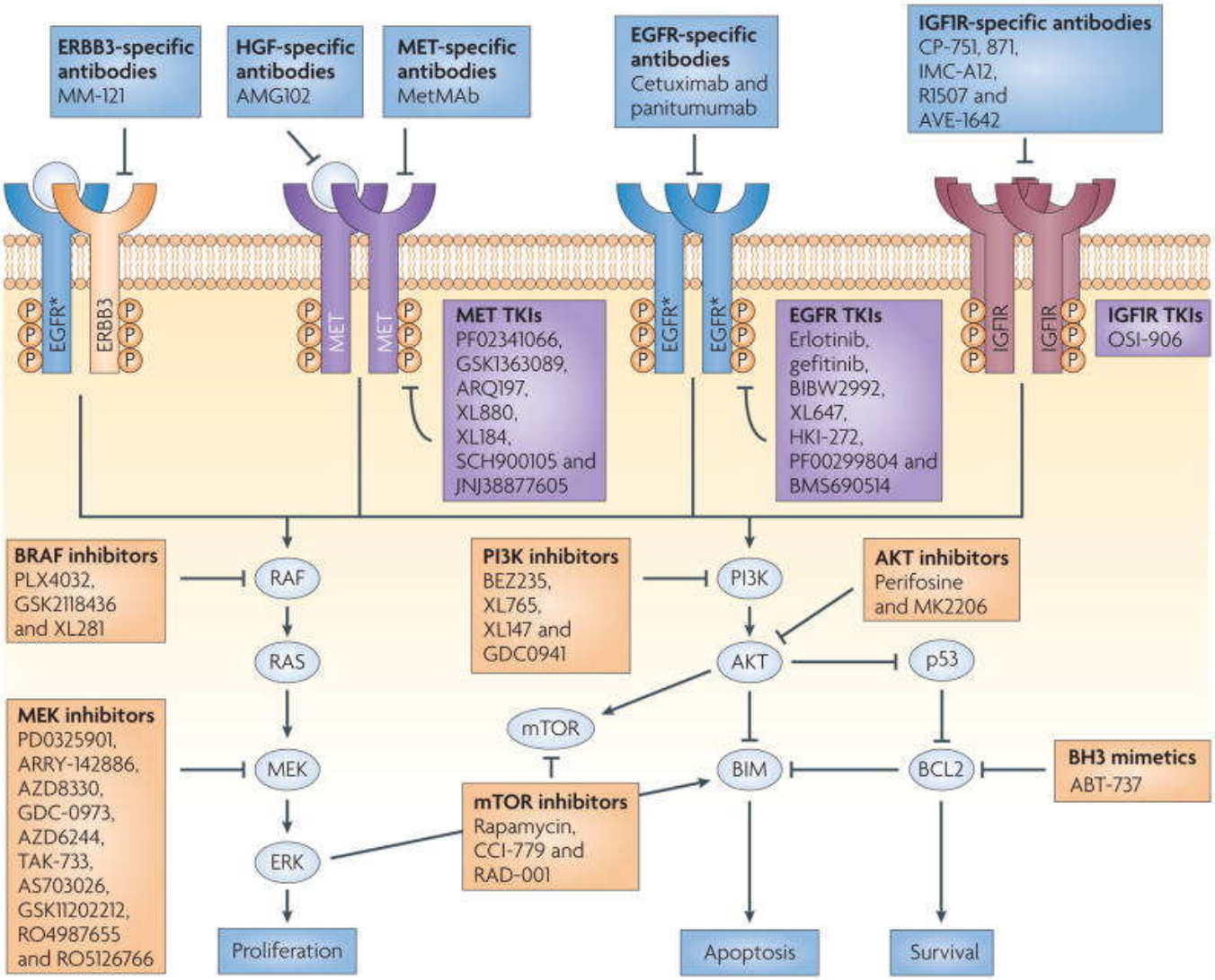
Irreversibly binding, pan-HER inhibitors in clinical development include:
dacomitinib (Phase 3) and HM781-36B (Phase 1; solid tumours)



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

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

Multi-pathway Inhibition as Strategy to treat TKI-resistant NSCLC



Pao et al., Nat Rev Cancer 2011

Next Steps in Molecular Pathology – Multigene Assays in Breast Cancer

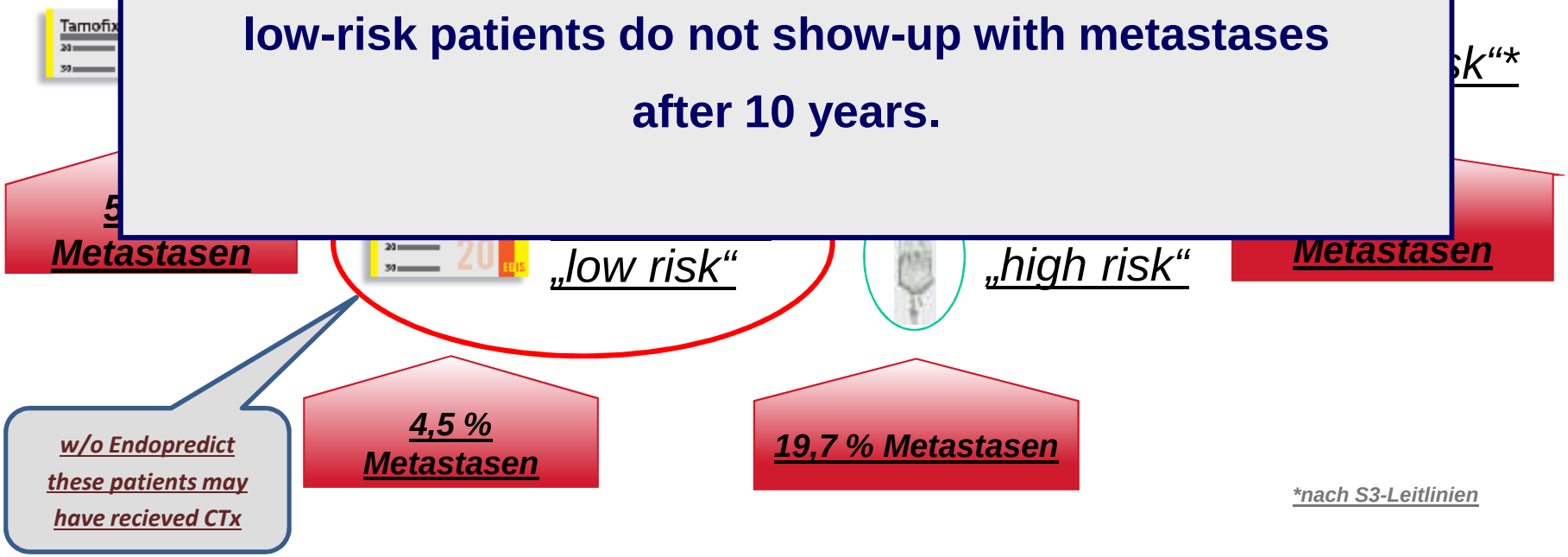
- **Multi-gene analyses, predictive molecular pathology and response to chemotherapy in breast cancer**
- **The development of new multi-gene assays (2nd generation) aimed to answer the following clinical question**

„Which patient with ER+ and Her2 neg. breast carcinoma will show a good prognosis when treated by endocrine therapy only?“

Stratification by EndoPredict$_{clin}$ ®

1702 Patientinnen in ABCSG 6 & 8

Following the EPclin-based predictive data 96% of the low-risk patients do not show-up with metastases after 10 years.



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

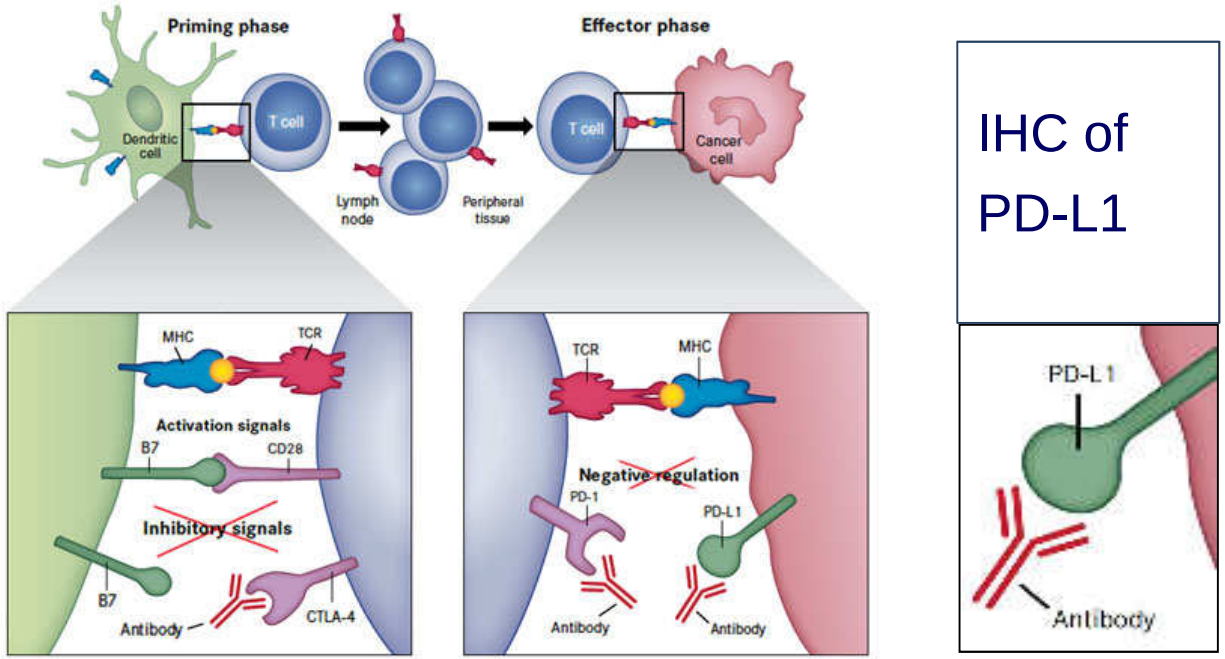
Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

Edward B. Garon, M.D., Naiyer A. Rizvi, M.D., Rina Hui, M.B., B.S., Natasha Leighl, M.D., Ani S. Balmanoukian, M.D., Joseph Paul Eder, M.D., Amita Patnaik, M.D., Charu Aggarwal, M.D., Matthew Gubens, M.D., Leora Horn, M.D., Enric Carcereny, M.D., Myung-Ju Ahn, M.D., Enriqueta Felip, M.D., Jong-Seok Lee, M.D., Matthew D. Hellmann, M.D., Omid Hamid, M.D., Jonathan W. Goldman, M.D., Jean-Charles Soria, M.D., Marisa Dolled-Filhart, Ph.D., Ruth Z. Rutledge, M.B.A., Jin Zhang, Ph.D., Jared K. Lunceford, Ph.D., Reshma Rangwala, M.D., Gregory M. Lubiniecki, M.D., Charlotte Roach, B.S., Kenneth Emancipator, M.D., and Leena Gandhi, M.D., for the KEYNOTE-001 Investigators*

CHARITÉ

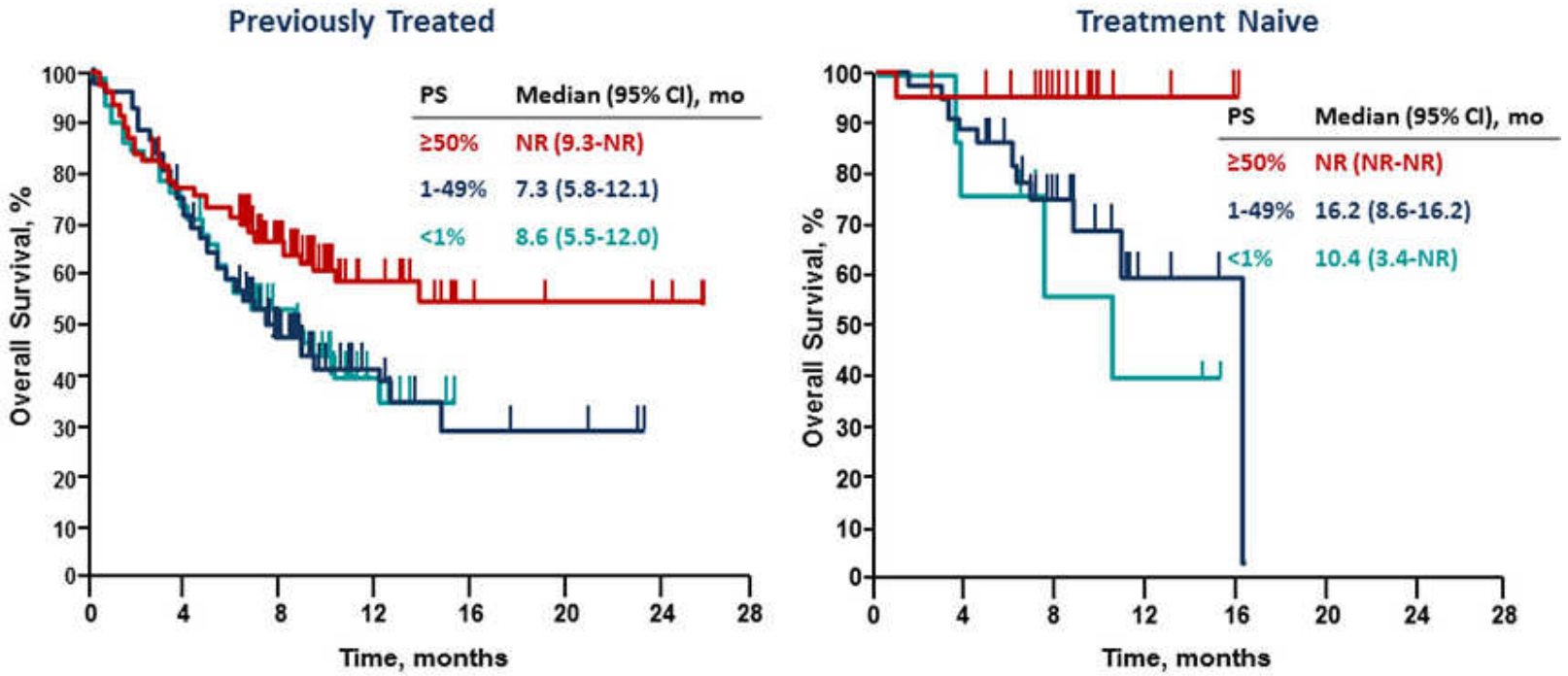
Immuntherapy of Cancer

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



n at risk

99	74	45	18	5	4	3	0
127	89	43	12	5	4	0	0
68	49	30	6	0	0	0	0

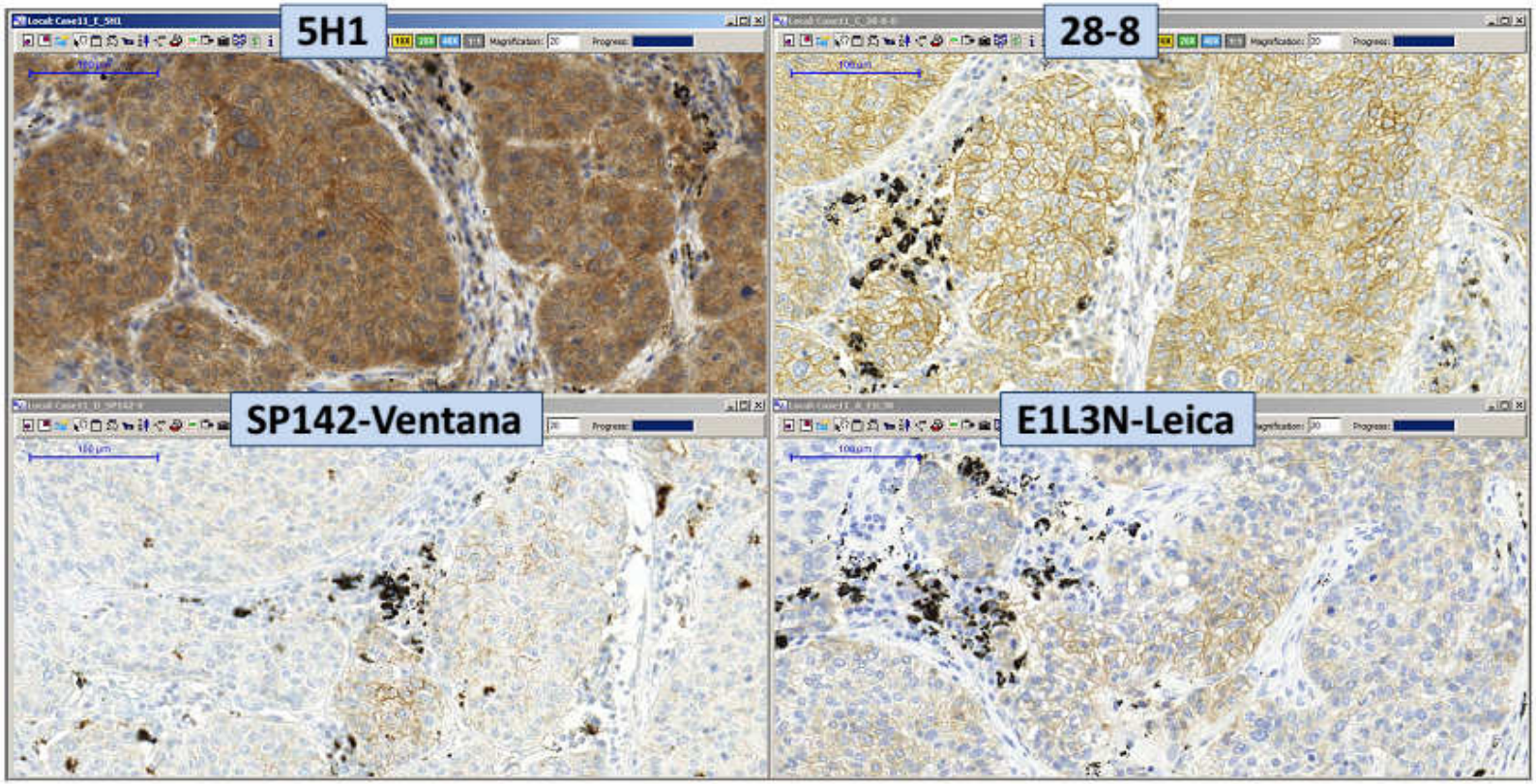
n at risk

20	18	11	4	0	0	0	0
34	30	15	3	1	0	0	0
8	6	3	2	0	0	0	0

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

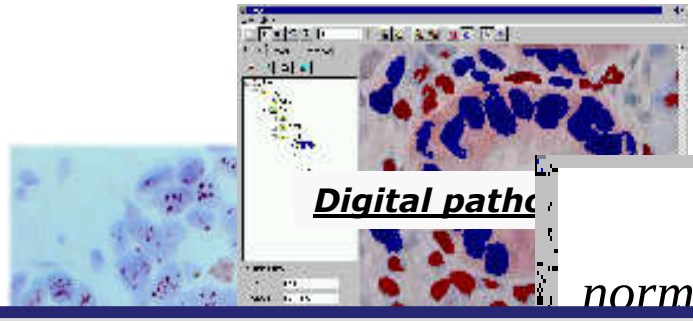
Garon_AACR 2015_19Apr15

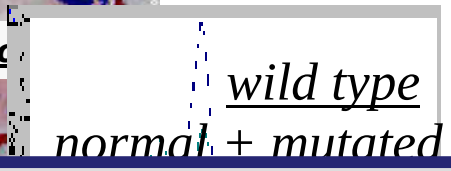
Different Staining Pattern of PD-L1 due to Applied AB



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%

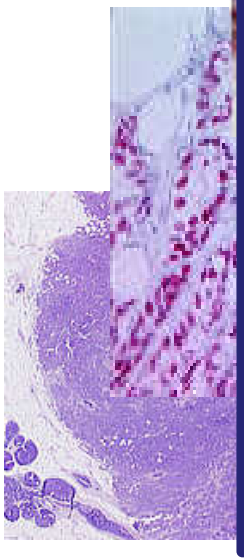
Clinical data tissue

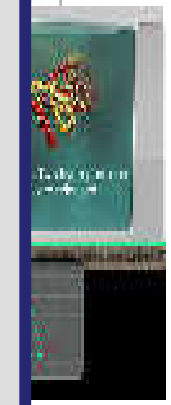




All test are done on for-malin fixed paraffin embedded tissue

Personalized medicine is based on a "combined morphological-molecular pathology report" including classical morphology (HE/IHC/(F)ISH) and diverse molecular analyses – to do this in a fast and reliable manner will be the future challenge of pathology





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.


Institut für Pathologie,
Rudolf-Virchow-Haus, Charité
Humboldt-Universität zu Berlin
Alexander Ufer
CHARITÉ