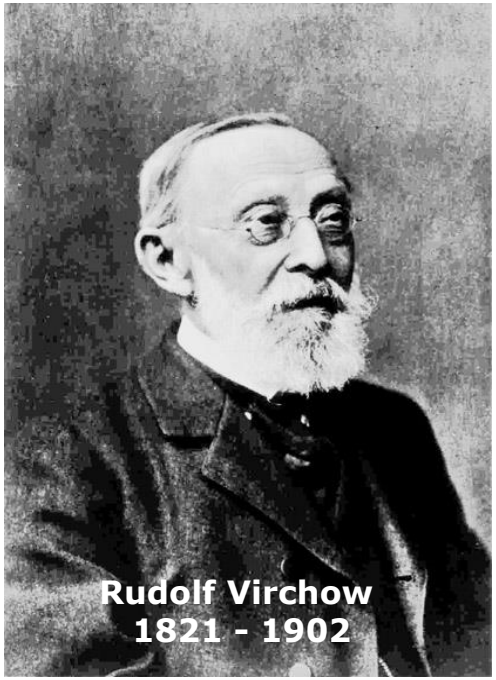
Workshop in Diagnostic Immunohistochemistry

Lung Cancer: IHC and Molecular Classification



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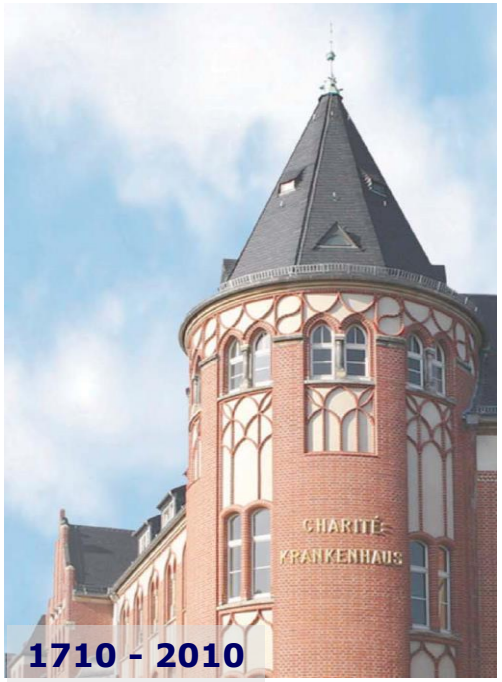

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Lung Cancer

In the workflow of LC materials technicians play fundamental role!

One key point is tissue handling!

Lung Cancer

Molecular assays to be done in NSCLC

Essential: EGFR, EML4-Alk, ROS1,

Addiitonal

BRAF, MET, PDGFR, MSI, KRAS, and others → next generation sequencing

<u>All assays should be controlled continuously in repeated ring trials (round Robin tests)</u>

Tissue work-up and Quality Control

All assays should be controlled continuously in repeated ring trials (round Robin tests).

Just one example: ALK

Tissue Work-up in the Histo-Lab

Comprehensive diagnosis of lung cancer requires:

- 1 or 2 4µm slides for H&E / PAS
- 4 to 6 4µm slides for IHC (syn, chrom A, CK5/6, CK7/8/18, p63, ERCC1, TTF1, ALK [IHC/FISH] ….controls)
- 2 to 3 10µm slides for EGFR-mut testing or NGS
- ? for controls and other assays.

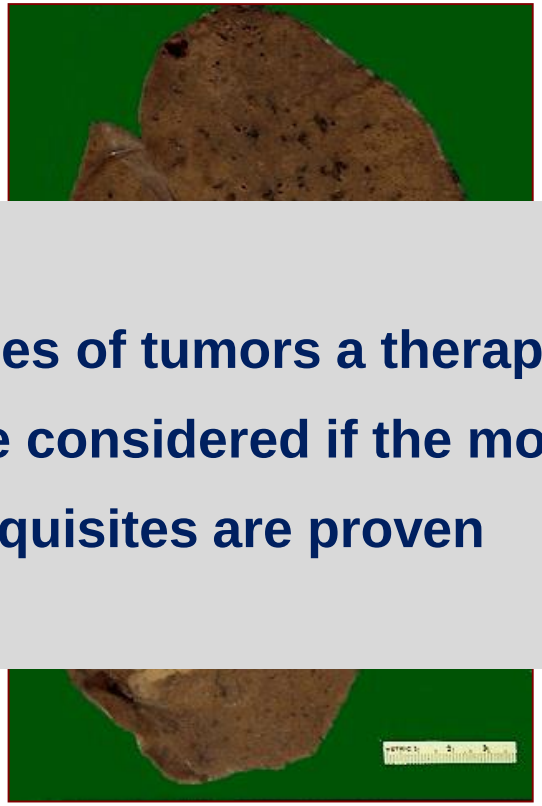
For the work-up of (small) biopsies this means in general:

- Very careful handling of the tissue
- Neatly embedding with all biopsies in on a similar level
- Cutting as precise as possible to enable all assays necessary
- Spare slides in advance to avoid re-cutting.

Thunnissen E, et al. Virchows Arch 2012;

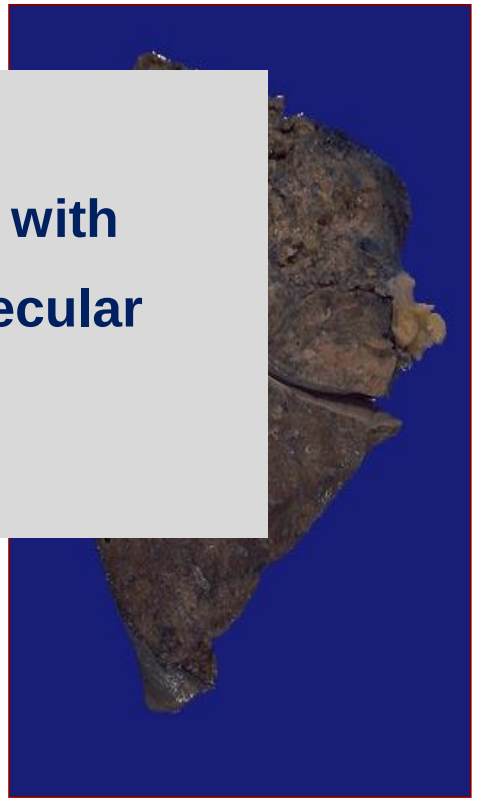
NSCLC - Macroscopy

central
squamous cell carcinoma



peripheral
adenocarcinoma

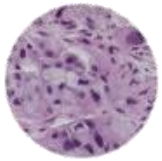
adeno carcinoma
broncho-alveolar type



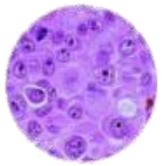
For these types of tumors a therapy with TKIs should be considered if the molecular prerequisites are proven

NSCLC: Past and Current Landscape

1999
Histology-driven selection[1]



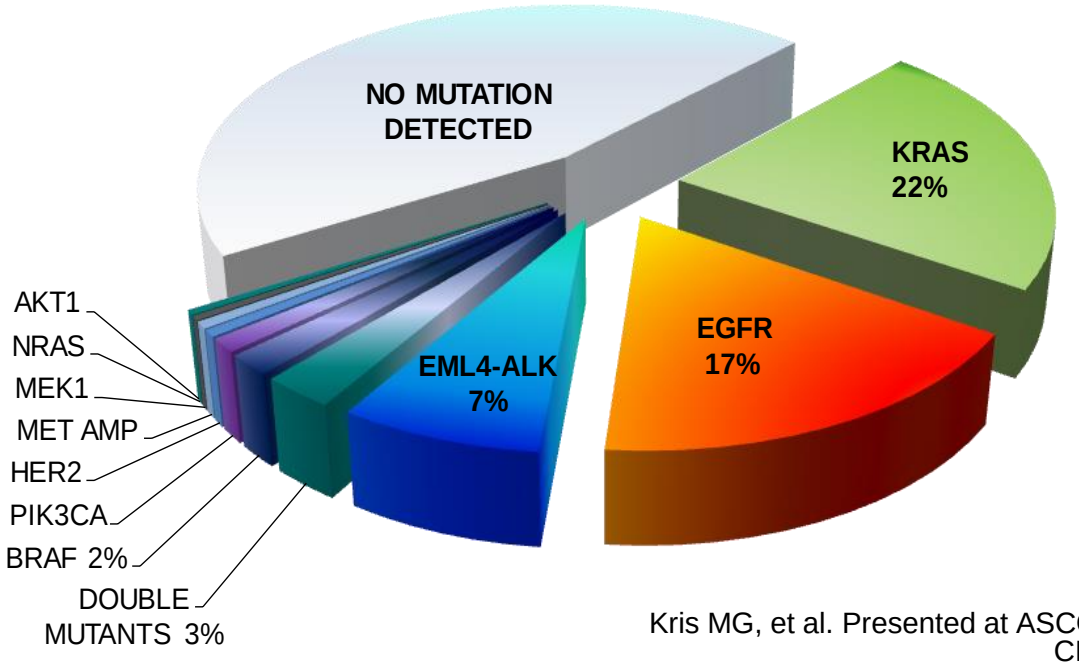
Adenocarcinoma



Squamous-cell carcinoma

Large cell carcinoma

2012
Targeting oncogenic drivers



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

Actionable driver mutations identified in 54% of lung adenocarcinoma tumors

Lung Cancer

Molecular assays to be done in NSCLC

Essential: EGFR, EML4-Alk, ROS1,

Addiitonal

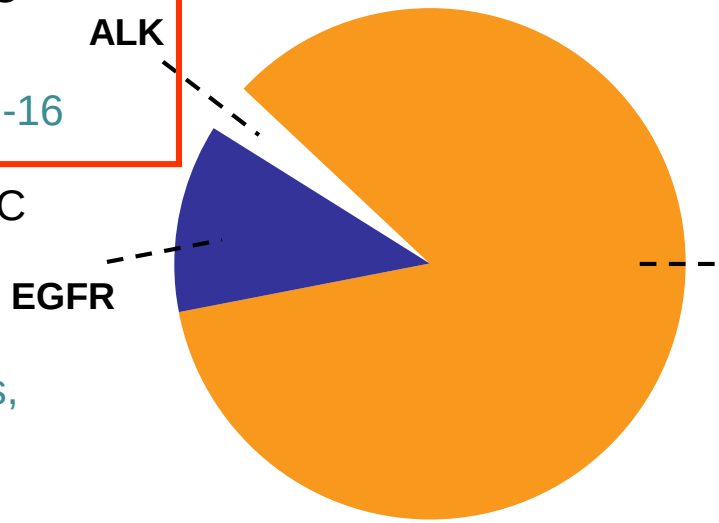
BRAF, MET, PDGFR, MSI, KRAS, and others → next generation sequencing

<u>All assays should be controlled continuously in repeated ring trials (round Robin tests)</u>

Currently, Two Approved Personalised Treatment Options:

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



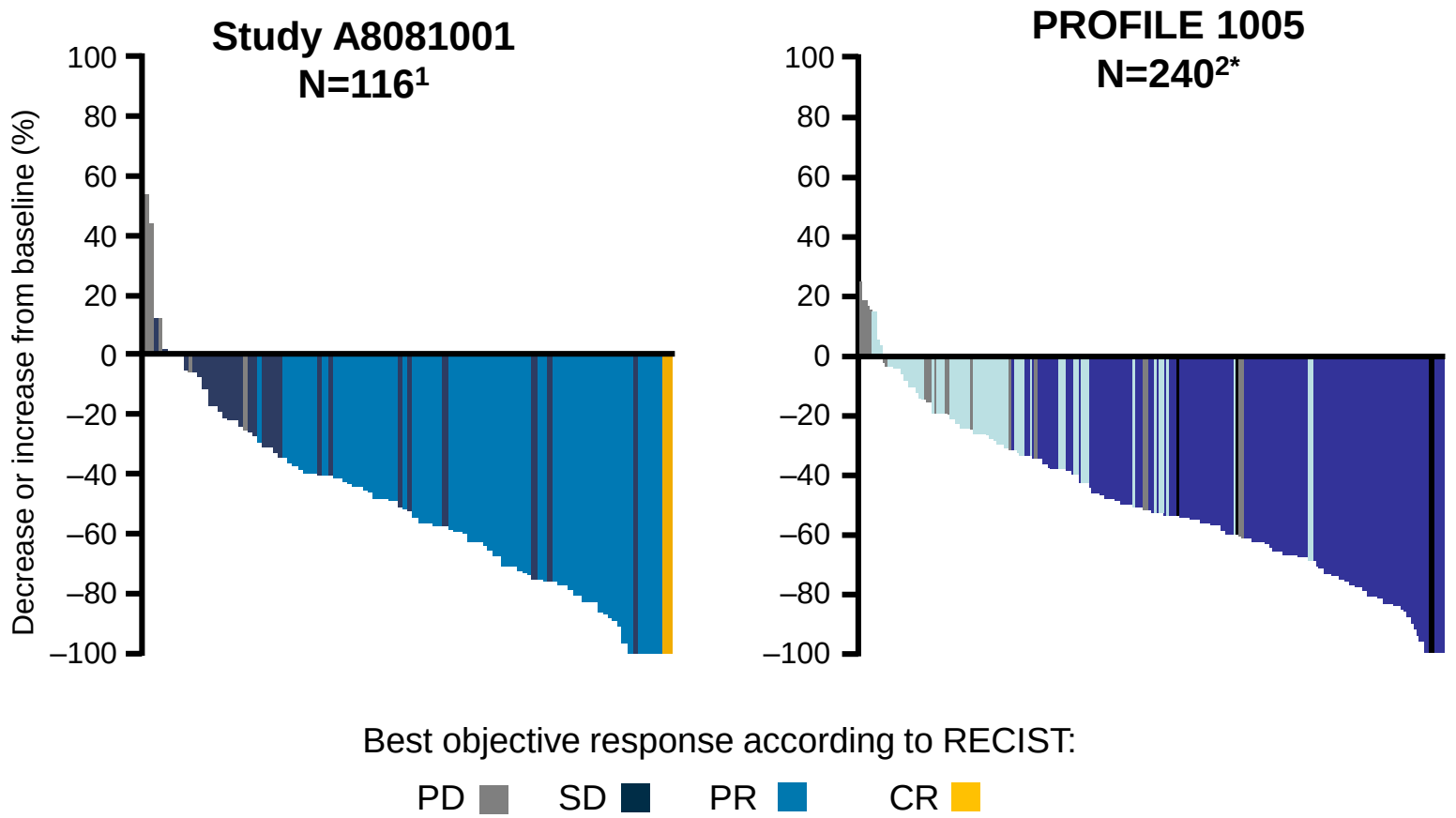
Chemotherapy in
unselected patients[2]
RR 20%, OS <12 mths

Substantial Benefit for ~15 – 20 % of Patients

•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

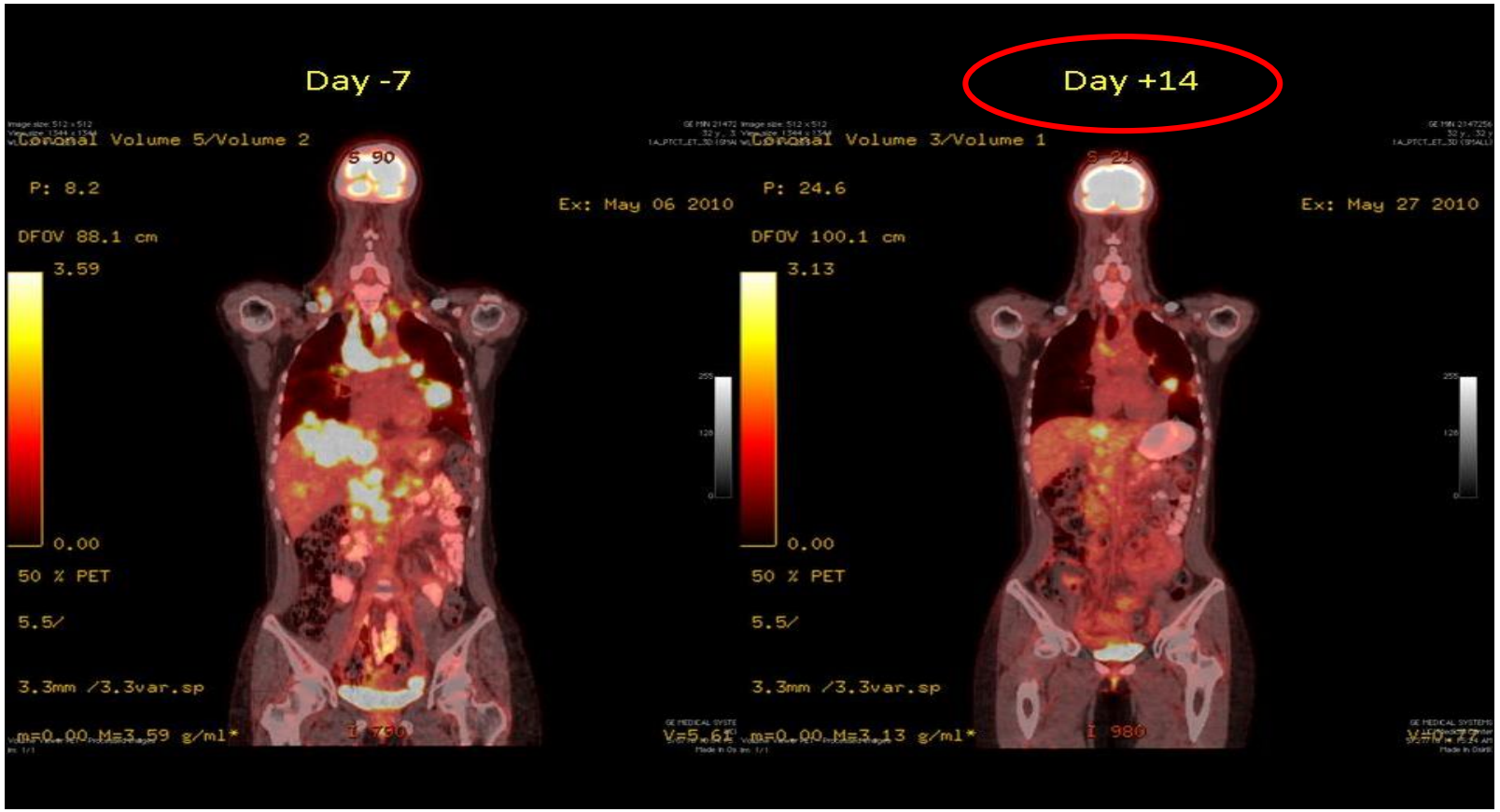
Tumour Response to Crizotinib by Alk+ Patients



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

•*Mature population, excluding those with early deaath, 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

The next step: How to fight resistance

Almost all tumors become resistant to targeting drugs.

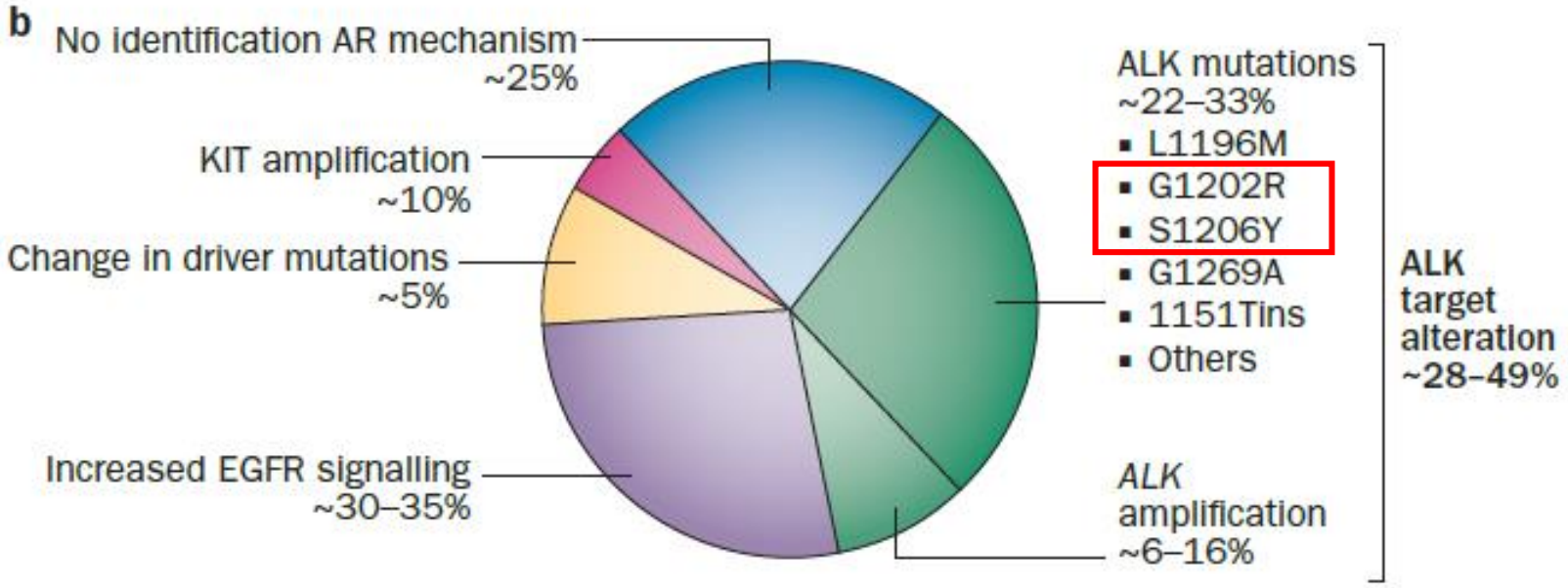
Novel approaches that have already proven successful include the development of second-generation and third-generation inhibitors and the combination of some of these inhibitors with antibodies directed against the same target or other targets (check points).

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 EGFR Inhibitors

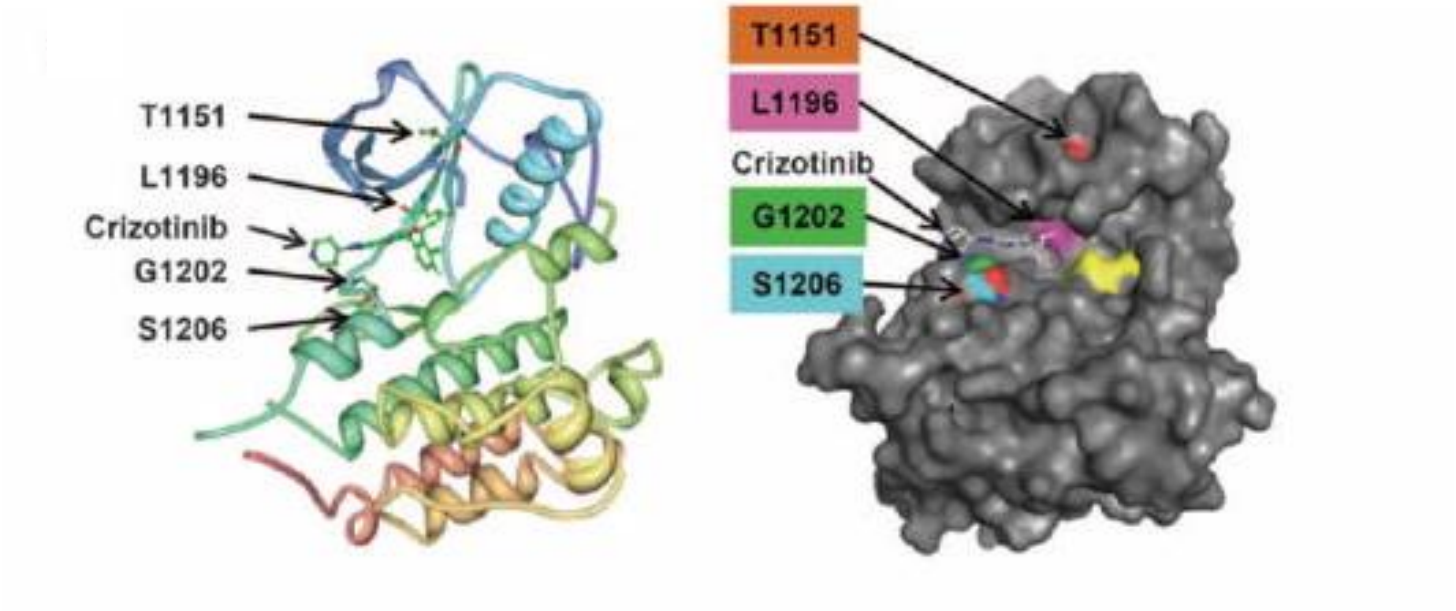
- **Primary resistance, e.g. to erlotinib or gefitinib**
- **Acquired resistance** (after treatment)
  - **EGFR dominant**
    - **2ndary EGFR mutation(s) with steric hindrance of 1st gen. EGFR inhibitors**
    - **EGFR amplification or point mutation**
  - **EGFR non-dominant**
    - **New non-EGFR mutations: Met, HER2, PIK3CA, BRAF, other**

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

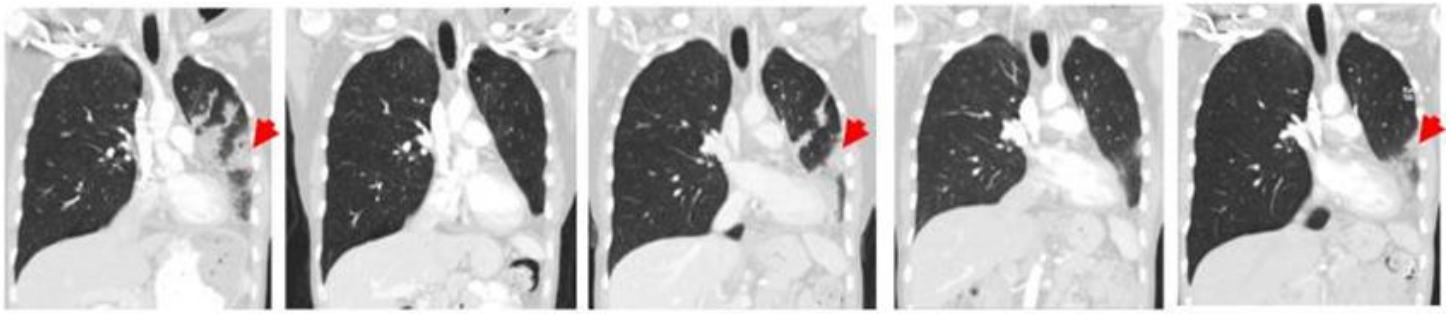
For example:

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

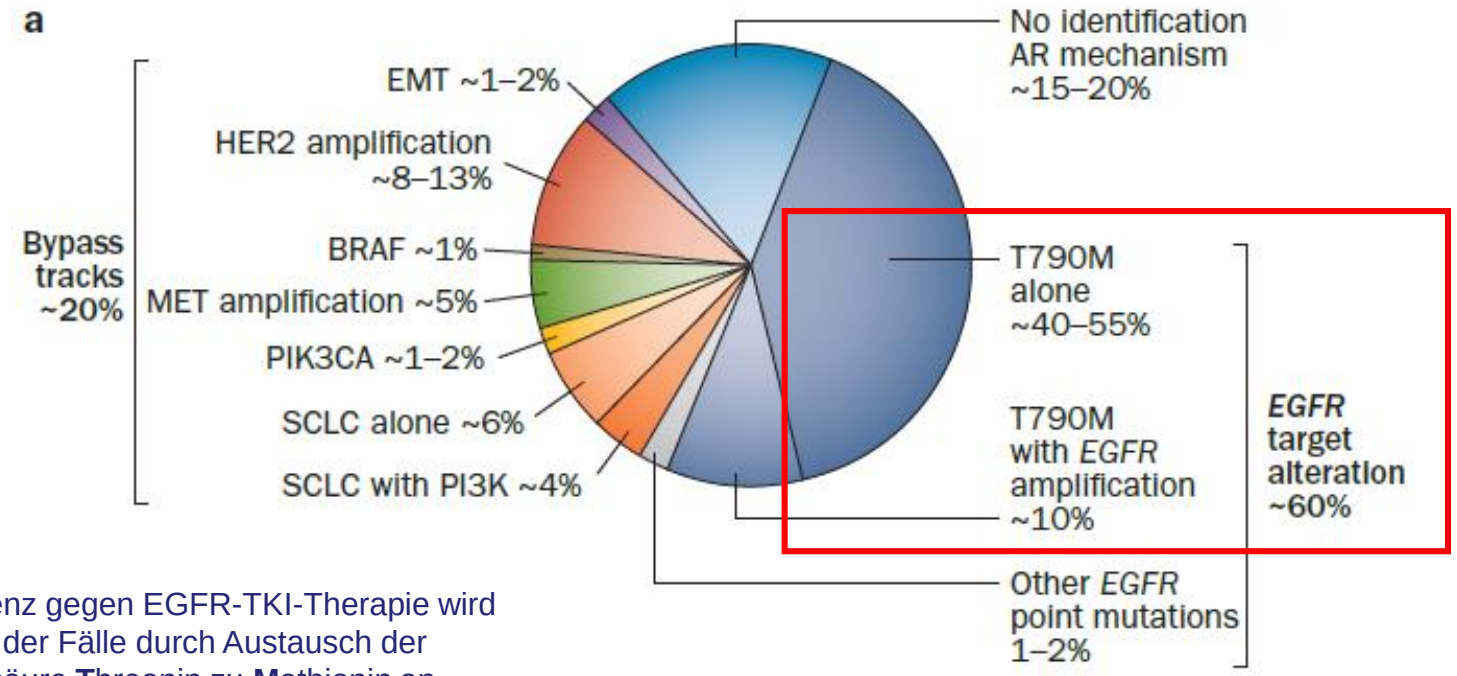


Baseline	After 8 weeks of crizotinib	After 34 months of crizotinib	After 12 weeks of Ceritinib	After 15 months of Ceritinib
EML4-ALK sequence: **ALK mut**		**S1206Y**		**G1202R**

Mechanisms of Acquired Resistance in EGFR-mutant NSCLC Resistant to First Generation EGFR TKIs Erlotinib/Gefitinib

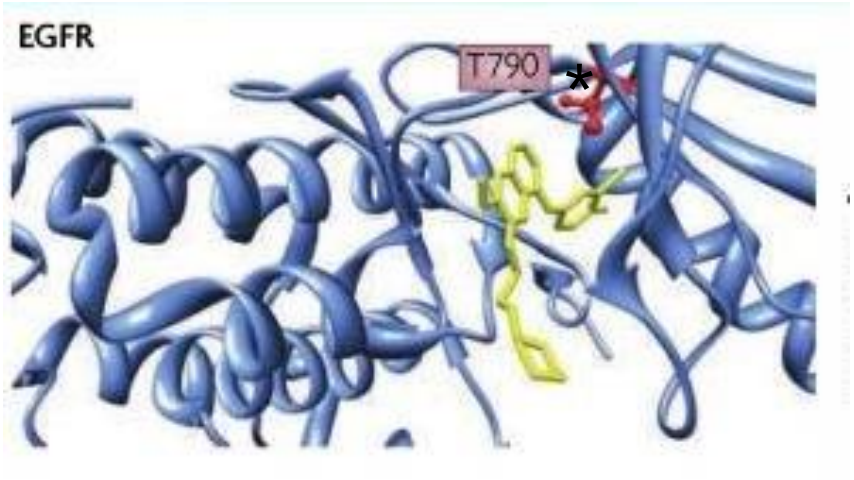
EGFR non-dominant

EGFR dominant



Resistenz gegen EGFR-TKI-Therapie wird in 50% der Fälle durch Austausch der Aminosäure **T**hreonin zu **M**ethionin an Position **790** des EGFR-Proteins vermittelt (**T790M**)

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

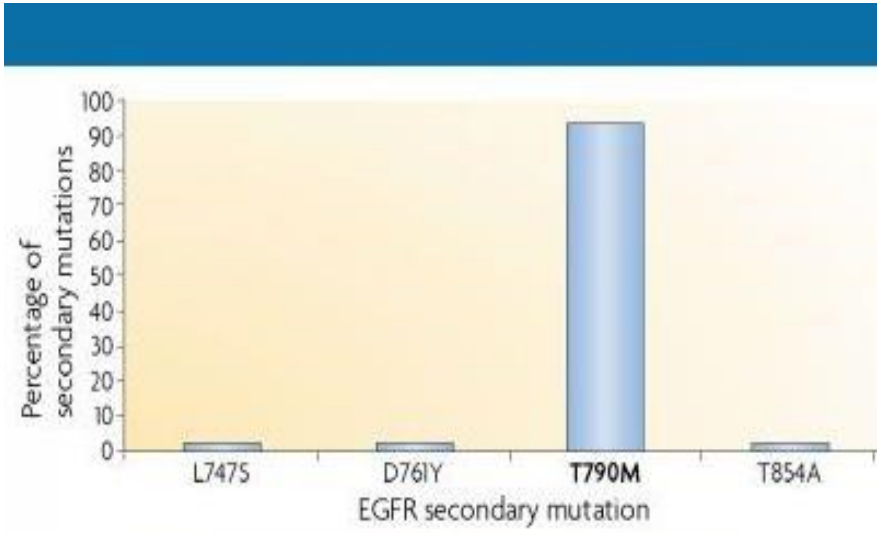


Gefitinib (yellow)

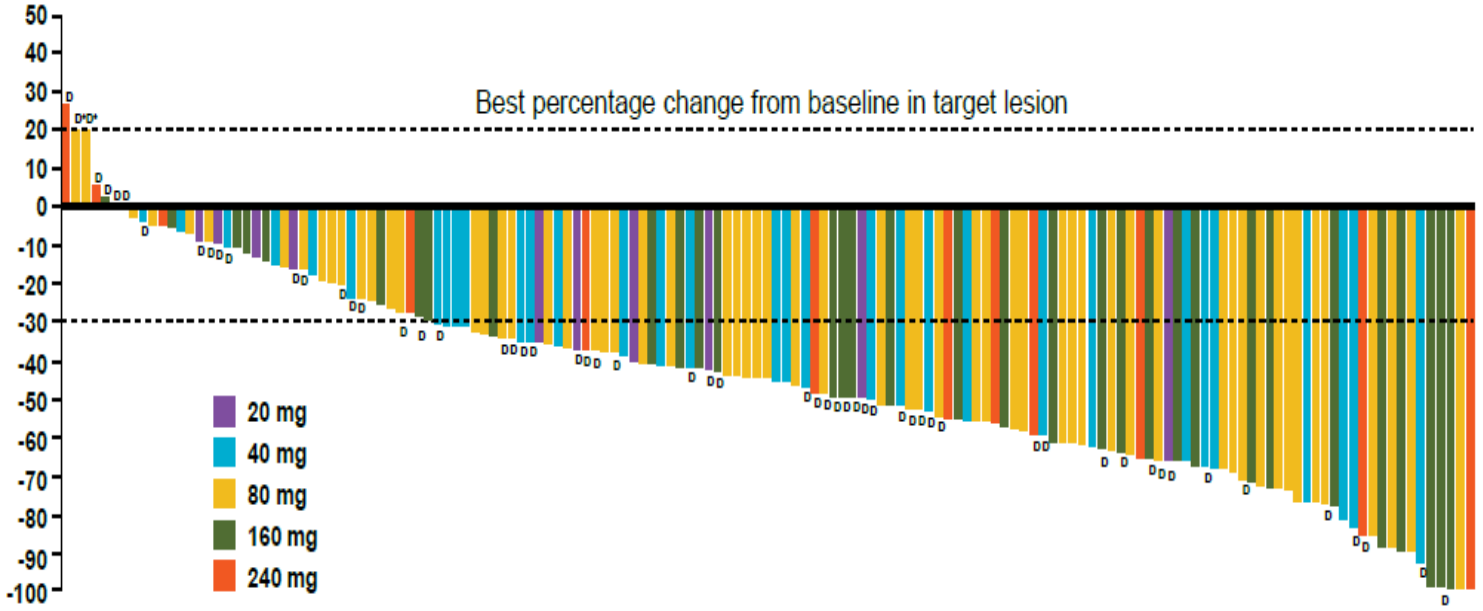
Second-site mutation frequency following development of acquired resistance to TKI therapy

T790M → threonine to methionine

*AZ9291 binding site



AZD9291 (Tagrisso) – 66% ORR in T790M positive patients*



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

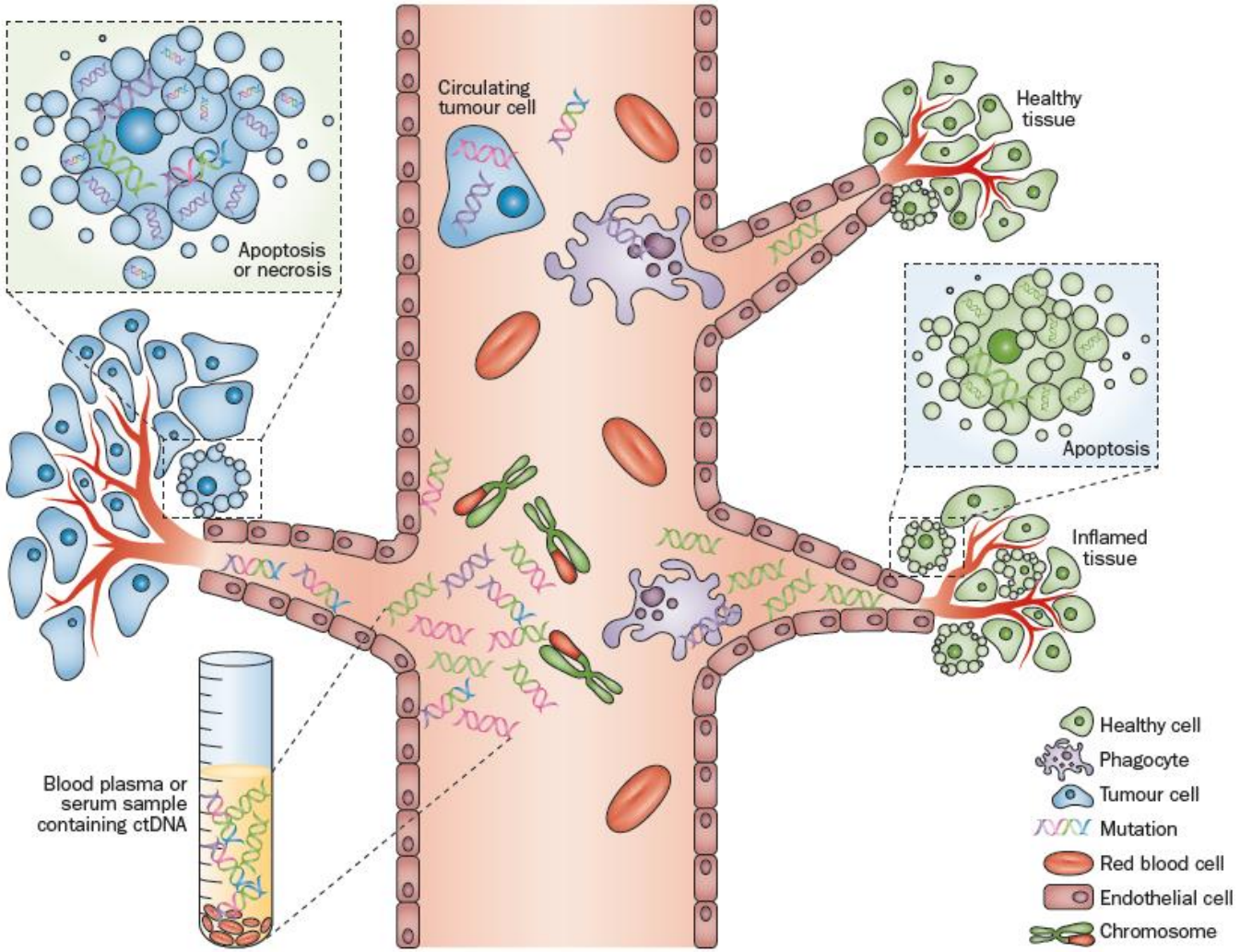
	20 mg	40 mg	80 mg	160 mg	240 mg	Total
N (157)	10	32	61	41	13	157
ORR (95% CI)	50% (19, 81)	59% (41, 76)	66% (52, 77)	51% (35, 67)	54% (25, 81)	59% (51, 66)

*as assessed by central tumor tissue testing

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

cfDNA und Liquid-Biopsie

tumor tissue



normal tissue

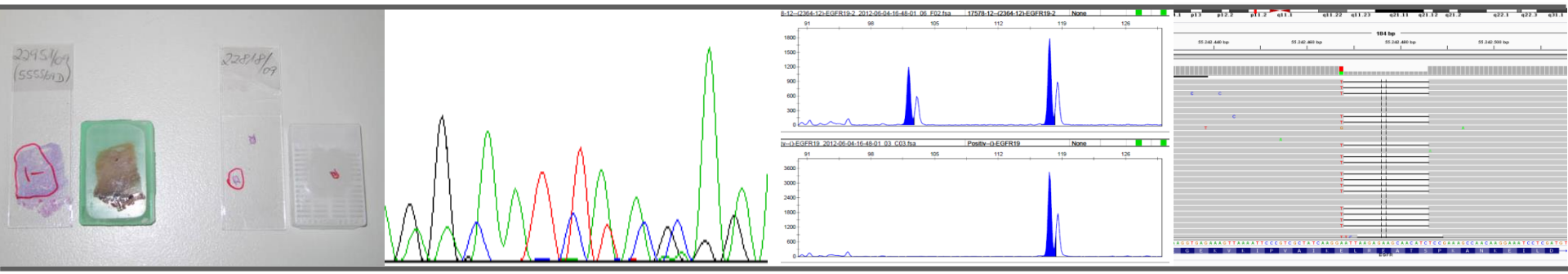
cfDNA, circulating free DNA; ctDNA, circulating tumor DNA.

Crowley, Nat Rev Clin Oncol 10, 472 (2013)

UNIKLINIK KÖLN

QuiP Qualitätssicherungs-Initiative Pathologie GmbH

CIO Centrum für Integrierte Onkologie Köln Bonn



Ring Trial EGFR T790M
Tissue and Blood

26.01.2016

Sabine Merkelbach-Bruse

Institut für Pathologie – Charité Berlin

CHARITÉ

Quality in Pathology

Qualitätssicherungs-
Initiative Pathologie GmbH

The German Society of Pathology and the Association of German Pathologists founded the QuiP initiative to perform nationwide quality control (ring trials) on IHC and molecular analyses

A prerequisite is always a well-organised biobank.

Qualitätssicherungs-Initiative Pathologie

QuIP

Ringversuche Immunhistochemie
und Molekularpathologie

Teilnahmezertifikat
1.Ringversuch EGFR-Mutationsbestimmung beim NSCLC

2009

Institut für Pathologie
Charité-Universitätsmedizin Berlin
(Direktor: Prof. Dr. M. Dietel)
hat am Ringversuch, EGFR-Mutationstestung beim NSCLC´ mit Erfolg teilgenommen.

Leiter des Ringversuchs:

Prof. Dr. P. Schirmacher,
Prof. Dr. M. Dietel
Prof. Dr. I. Petersen

Hannover/Regensburg , den 30.09.2009

Prof. Dr. med. Hans H. Kreipe
Gesellschaft für Pathologie e. V.

Prof. Dr. med. Ferdinand Hofstädter
Bundesverband Deutscher Pathologen e.V.

Träger der Ringversuche Immunhistochemie und Molekularpathologie QuIP
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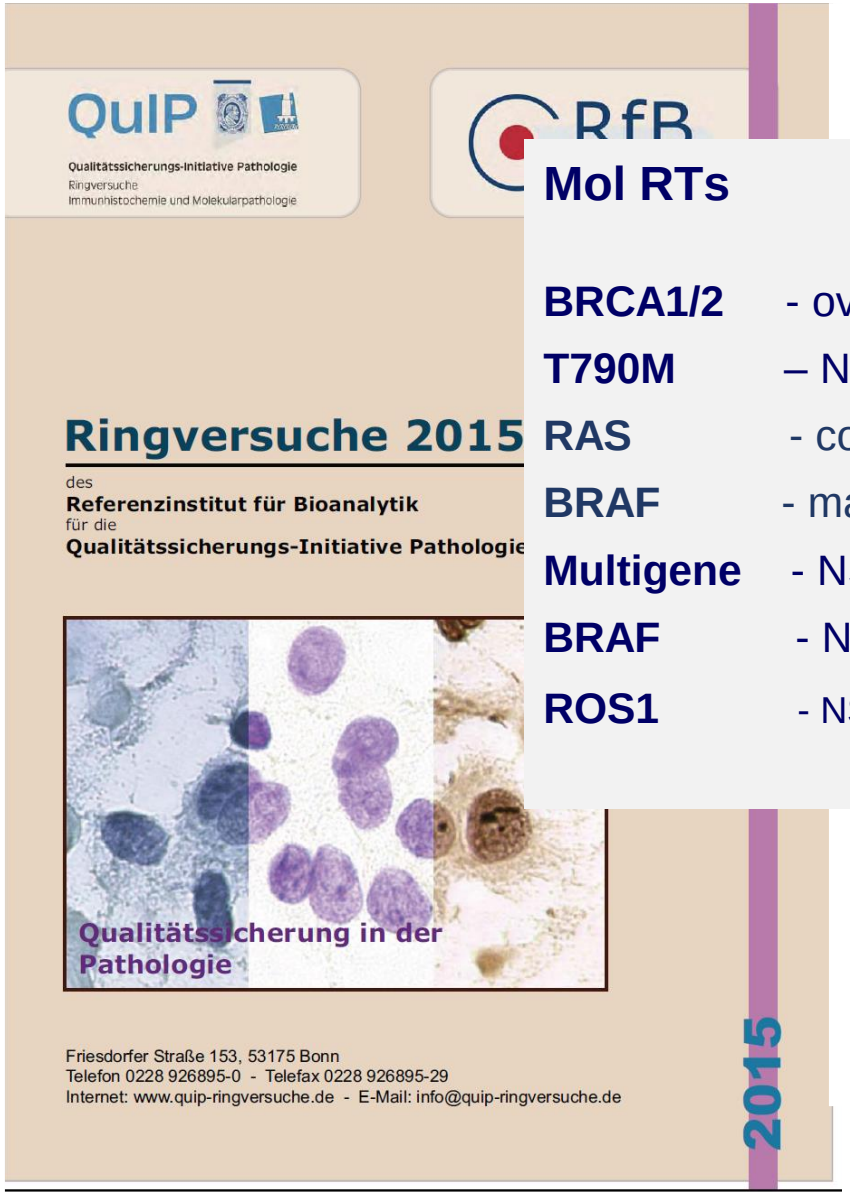
Bundesverband Deutscher Pathologen e.V., Berlin, Tel: 030 / 3088197-0, Mail: bv@pathologie.de

QuiP, Quality in Pathology

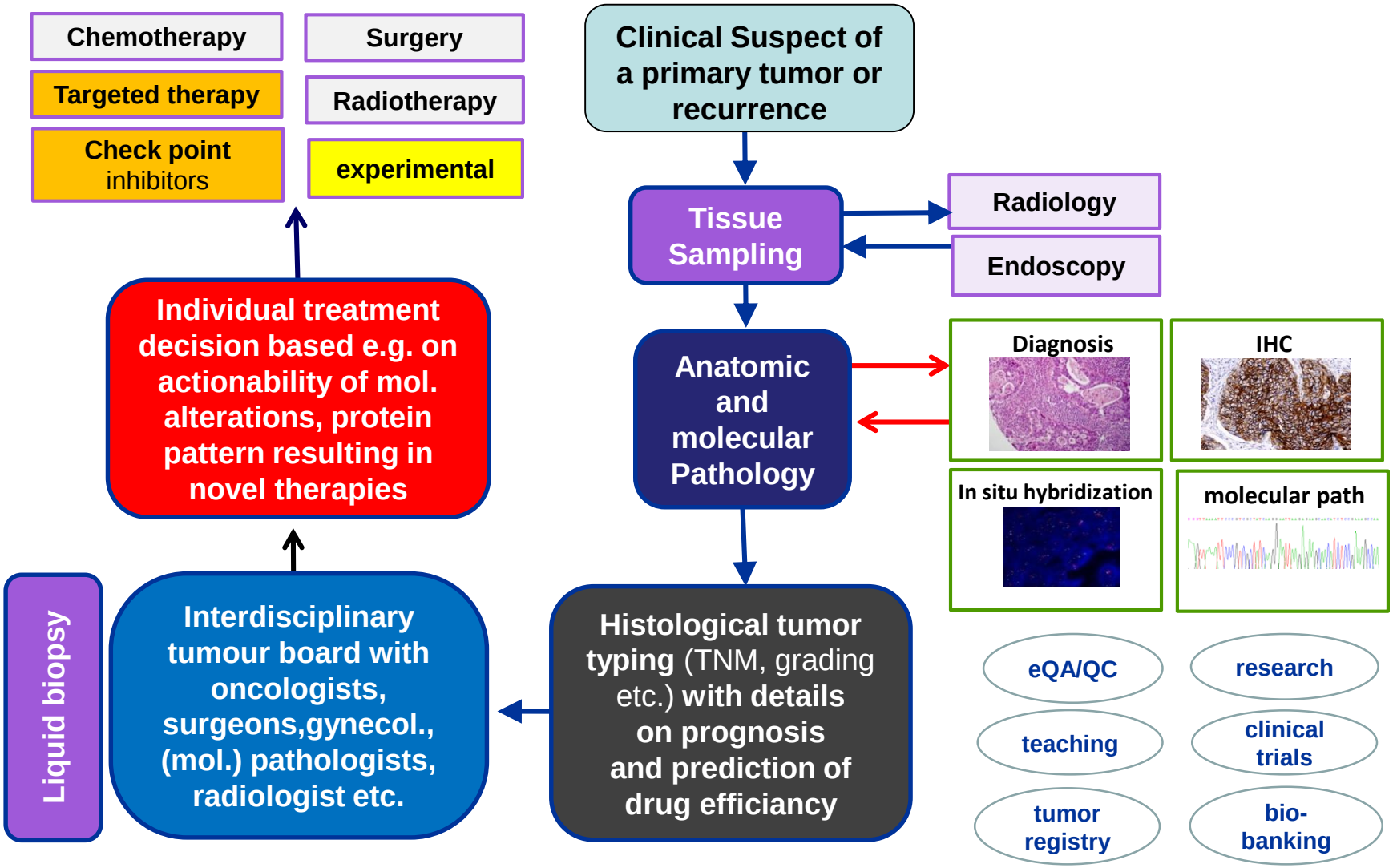


Inhaltsverzeichnis

Mol RTs

BRCA1/2	- ovarian carcinoma
T790M	– NSCLC (tissue and blood)
RAS	- colon
BRAF	- malignant melanoma
Multigene	- NSCLC – planned for 1st. quarter of 2017
BRAF	- NSCLC – 2nd qu. 2017
ROS1	- NSCLC – planned for 2nd half of 2017

Multidisciplinary Cooperation Enables Precision Oncology

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