23. VKD/VDGH – Führungskräfteseminar am 25. Februar 2016

Next Generation Sequencing – klinischer Nutzen heute und morgen
Reinhard Büttner, Uniklinik Köln

Reinhard Büttner
**Cologne Institute for Pathology-CIP**
CIO Köln Bonn
Reinhard.Buettner@uk-koeln.de
www.ngm-cancer.com

All patients with same diagnosis

Alternate therapy
non-responders and toxic responders

Standard therapy
Responders and Patients Not Predisposed to Toxicity

Understanding the Pathogenesis of Cancer
Driving a Rationale for IndividualisedTherapies
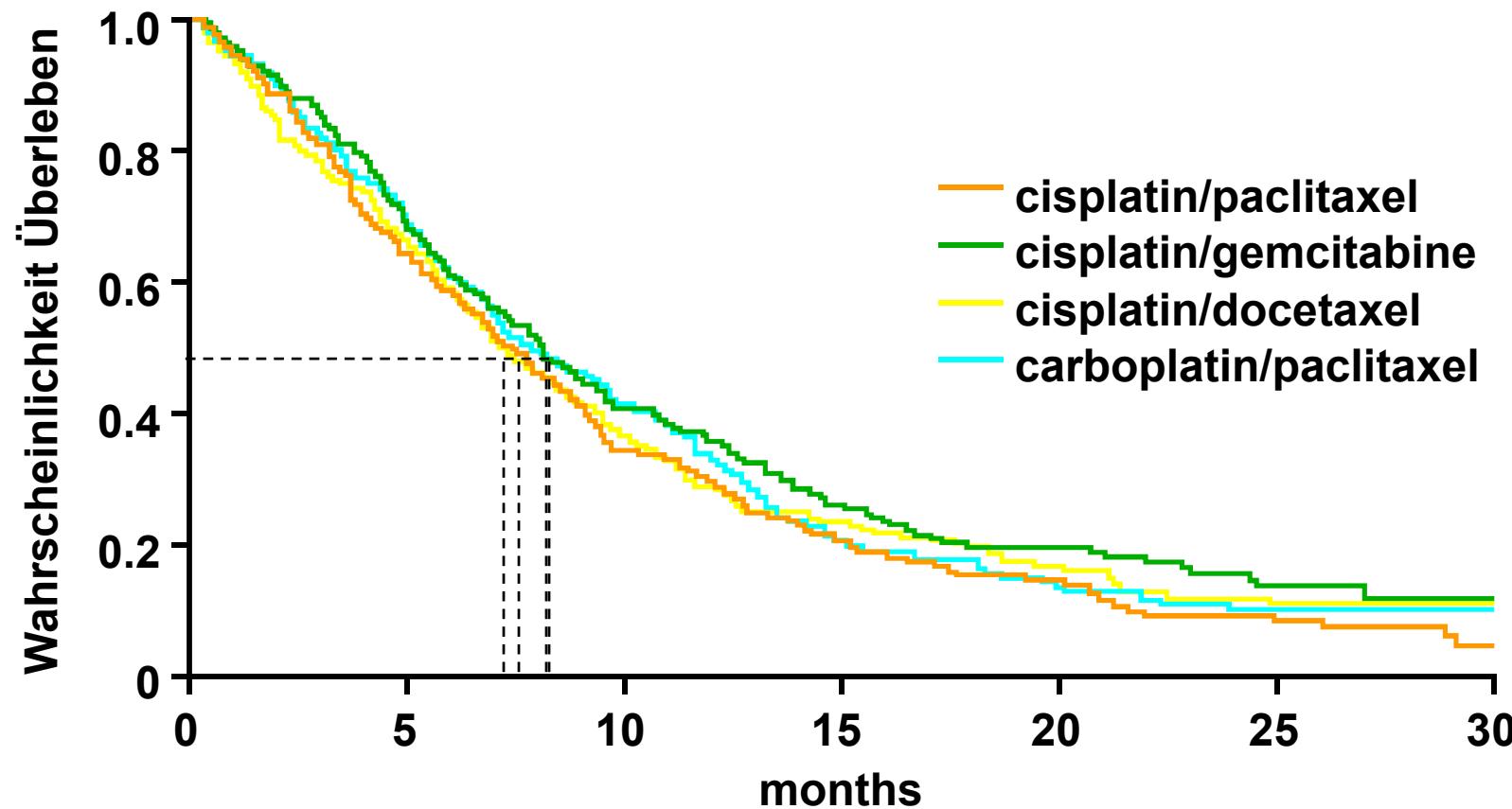
Hanahan & Weinberg Cell 2011

Lung Cancer serves as a Paradigm
The differences between chemotherapy regimens are negligible
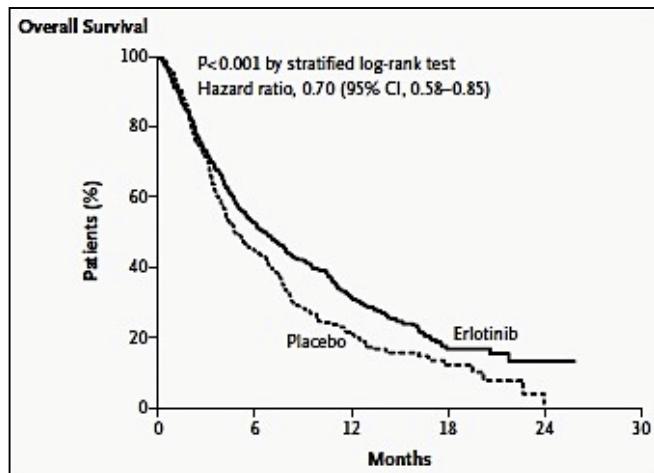
**E1504**

Schiller, et al. NEJM 2002

In unselected patients targeted drugs will add only marginal benefits (if at all)

EGFR-TKI mono
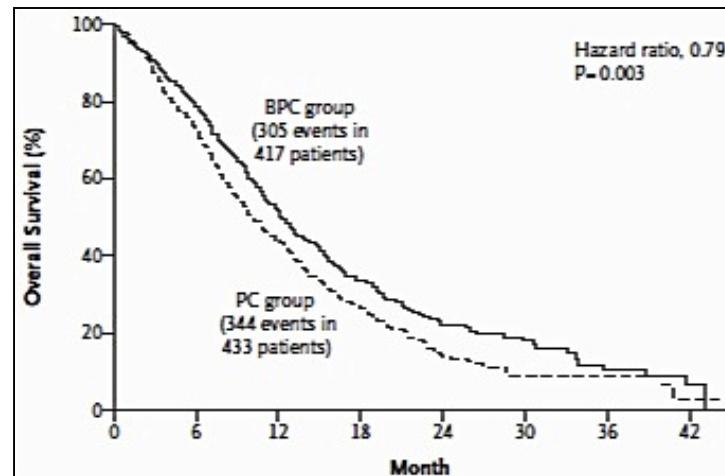
anti-VEGF mab + chemotherapy

Erlotinib vs. Plac.: SV + 2 m

Shepherd, 2005

**Bevacizumab + PC vs PC: SV + 2 m**
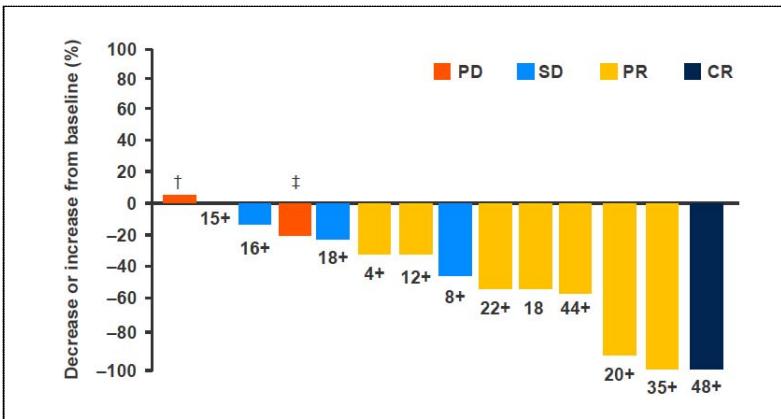
Sandler, 2006

Do we hit the right target in a specific patient ???
## Taregeted therapies vs Personalised Therapies
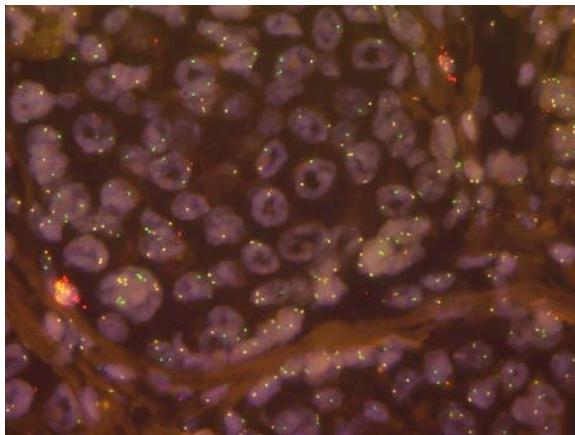
Rare Lesions and innovation NGM

www.lungcancergroup.de
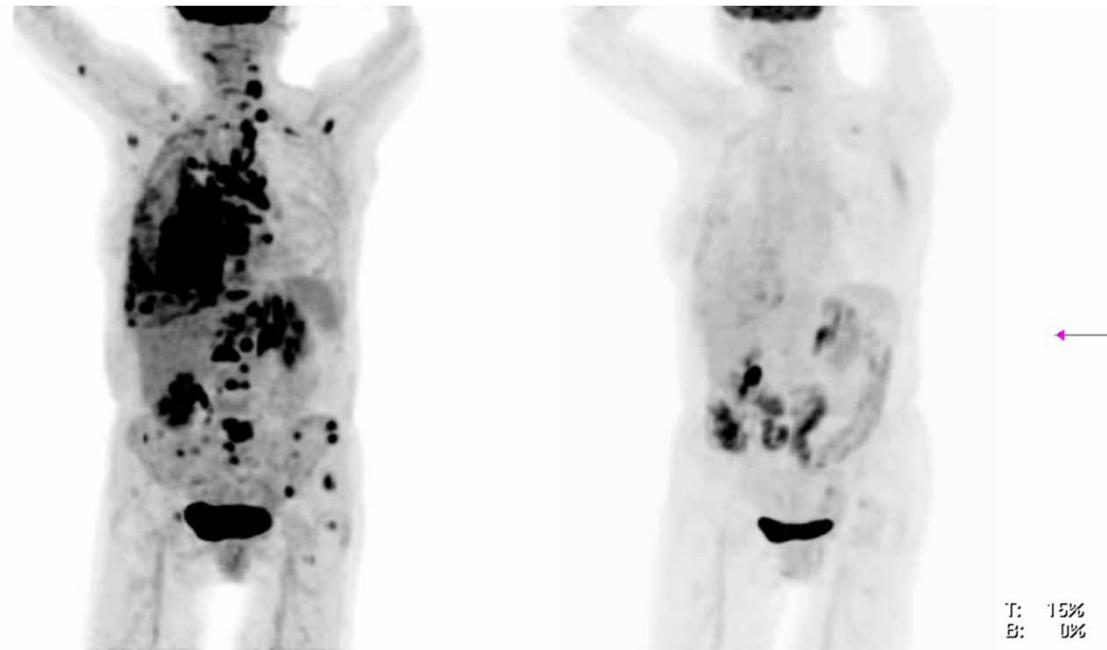
ASCO Juni 2013: Crizotinib wirksam bei ROS1+ Adenokarzinom

Shaw et al.,ASCO 2012 #7508
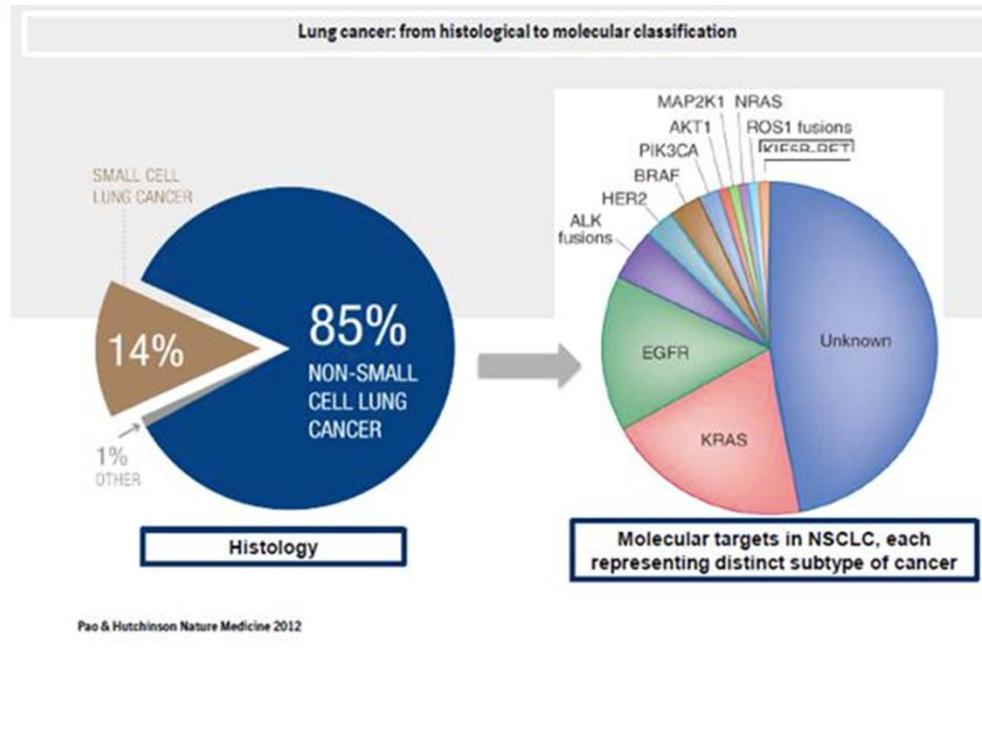
Aug. 2012: ROS1-FISH in NGM etabliert, Bos Lung Cancer 2012
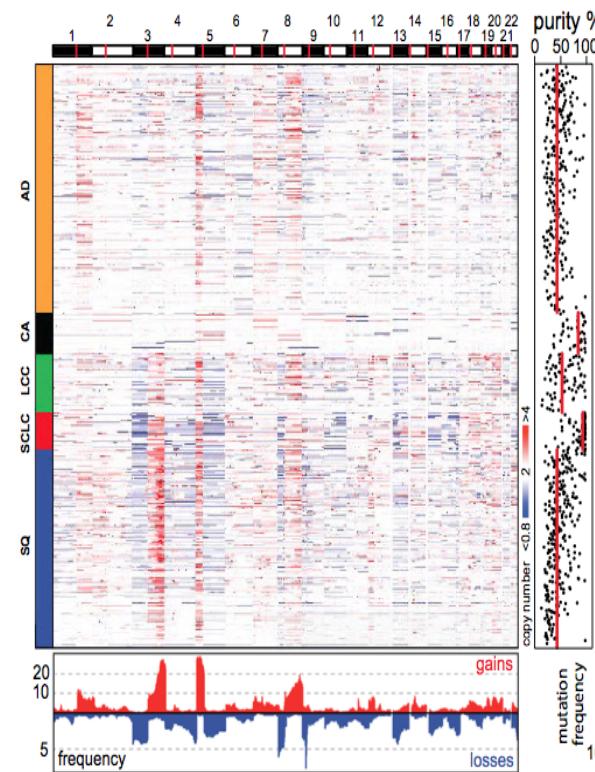
Sept. 2012: Crizotinib – Start bei ROS1+ Adenokarzinom-Patientin: Komplette Remission, anhaltend bis heute

Figure 1

Lung cancer: from histological to molecular classification

Molecular targets in NSCLC, each representing distinct subtype of cancer

Pao & Hutchinson Nature Medicine 2012
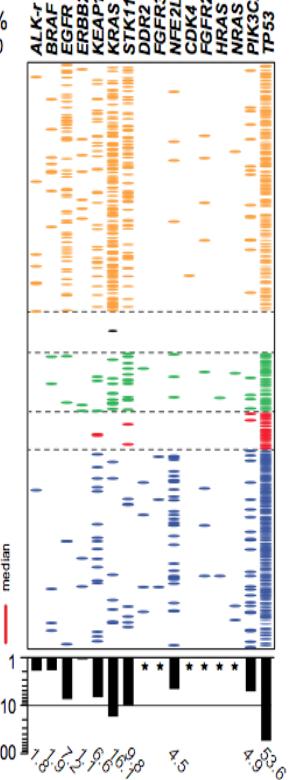
Seidel…Wolf, Buettner, Thomas Science Transl Med Oct30th, 2013

~5,000 Lung Cancer Genomes connected to clinical data (NGM-L)

Options for Personalised Therapies for NSCLC (2015)

Gene	Alteration	Frequency
EGFR	Mutation	10-15%
ALK	Rearrangement	4-5%
ROS	Rearrangement	1%
MET	Amplification	2-4%
BRAF	Mutation	1-3%
HER2	Amplification	2-4%
DDR2	Mutation	4%
RET	Rearrangement	1%
MEK1	Mutation	1%
FGFR1	Amplification	10%
KRAS, p53	Mutation	30-35% 50%
NRAS	Mutation	1%
PIK3CA	Mutation	1-3%
PTEN	Deletion	4%

→ **Standard 1st line**

→ **off label Crizotinib, 1st line**

→ **off label Crizotinib, Rez.**

→ **off label Dabrafenib, Rez.**

→ off label Lapatinib, Rez. ?

→ off label Dasatinib, Rez. ?

→ off label Cabozantinib o. Vandetanib, Rez.

→ **Clinical Studies**

- 🟩 drugs approved in NSCLC
- 🟨 drugs approved in NSCLC, but for other molecular subtype
- 🟦 drugs approved in other cancer
- ⬜ drugs in clinical development
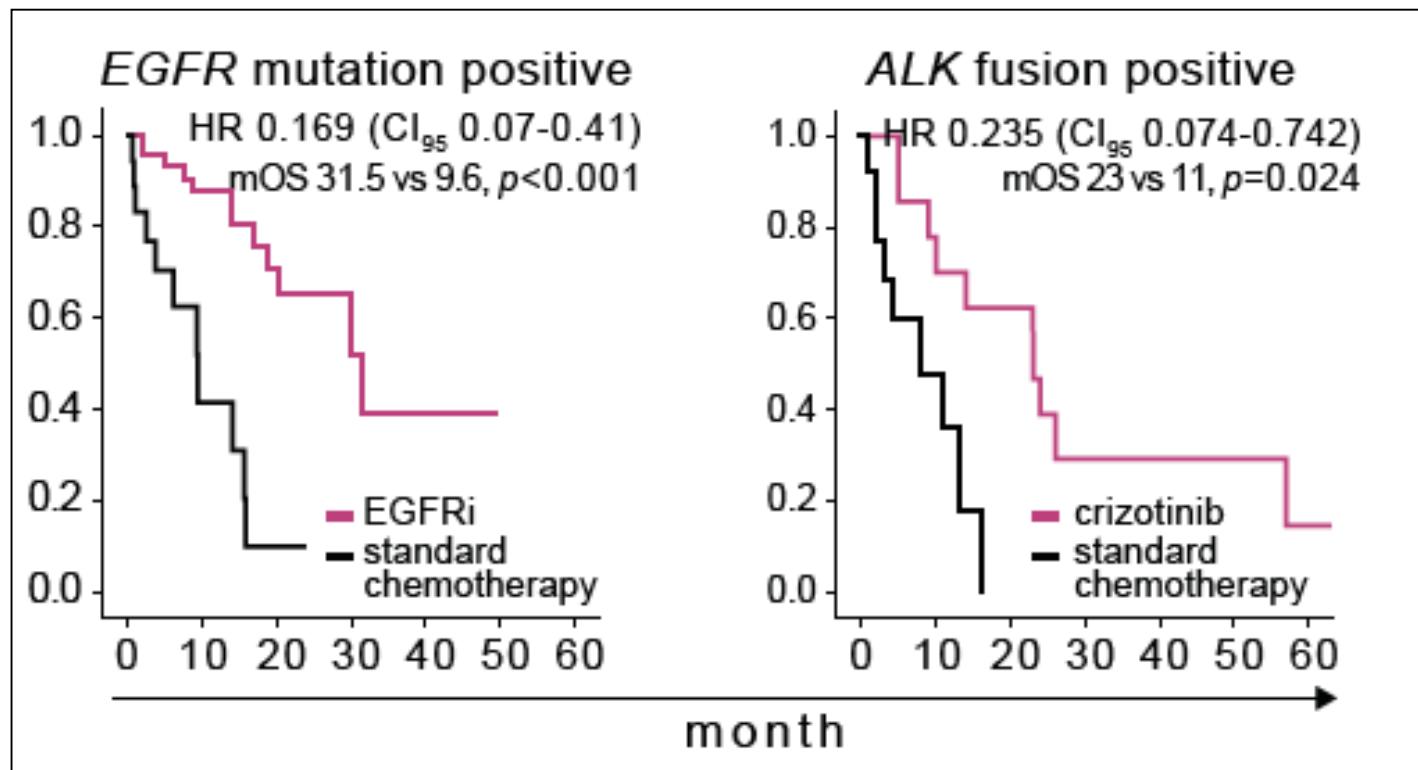
+ 3 additional therapies applying immune checkpoint antibodies CTLR-4, PD1, PD-L1
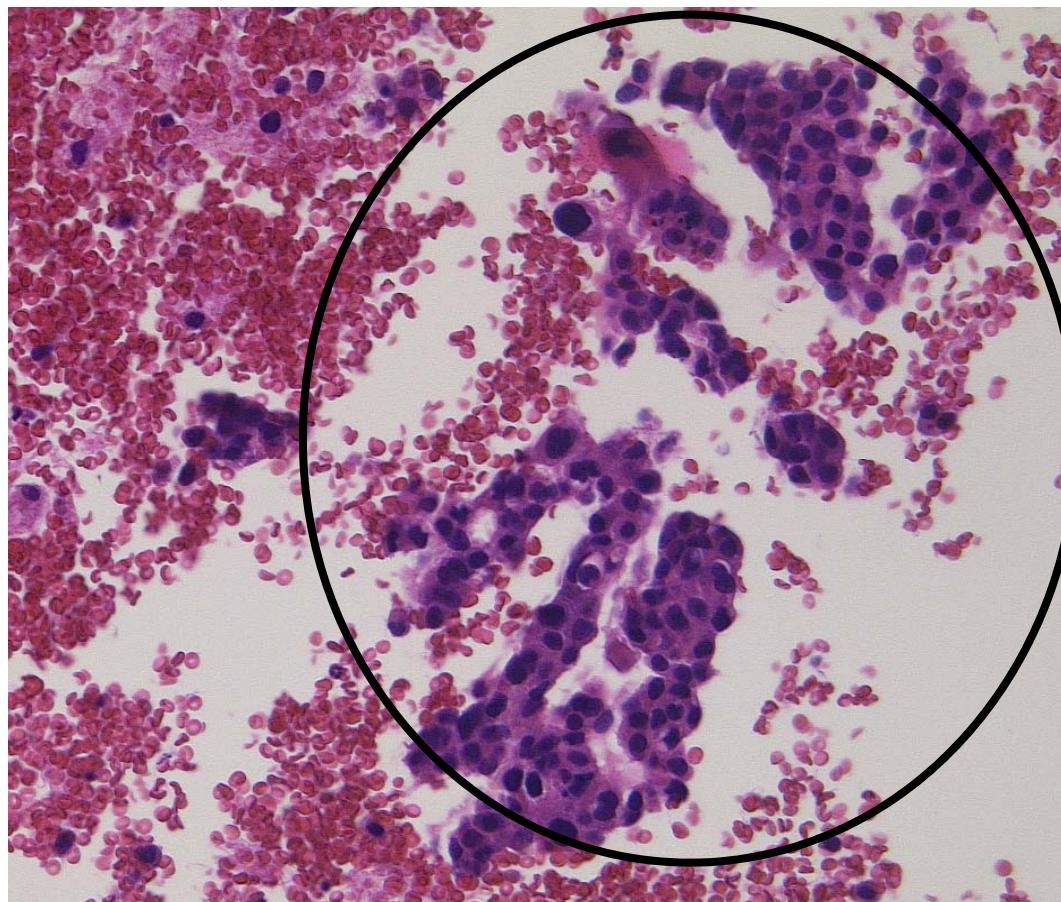
Improved survival of EGFR^{mut} und ALK^{transl} patients with personalised therapy as compared to chemotherapy

Subkkohorten des Network Genomic Medicine (NGM)

The Clinical Lung Cancer Genome Project and Network Genomic Medicine, Sci Transl Med 2013

Diagnostics

EBUS-TBNA, EBB, TBB, Cytoblock

1. Histology /Cytology

2. Immunhistochemistry

   PEC: p63, CK5
   AdCA: TTF1, CK7, NapsinA
   SCLC: CD56, panCKAE1/3
   LCC
   exclude lymphomas, metastases
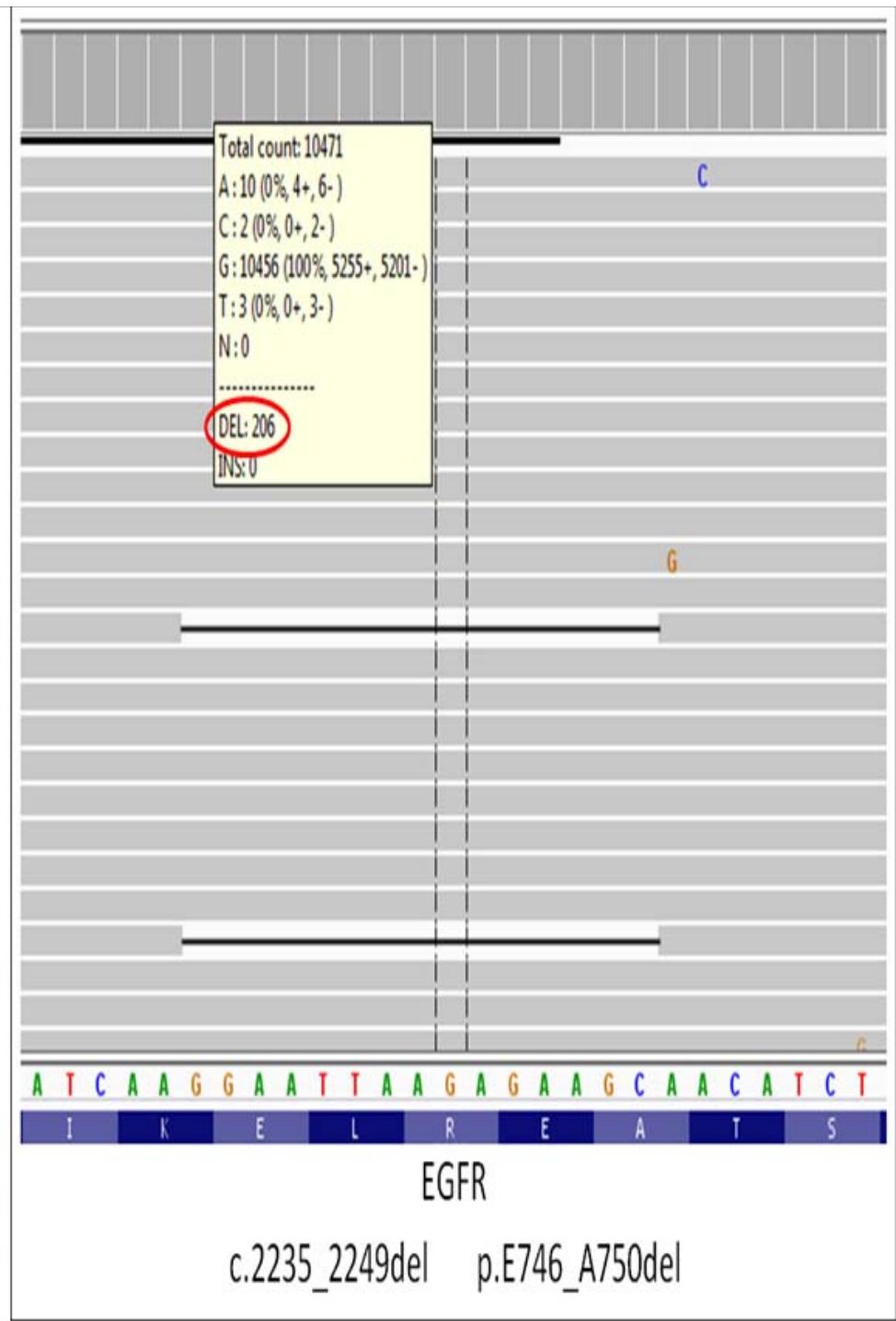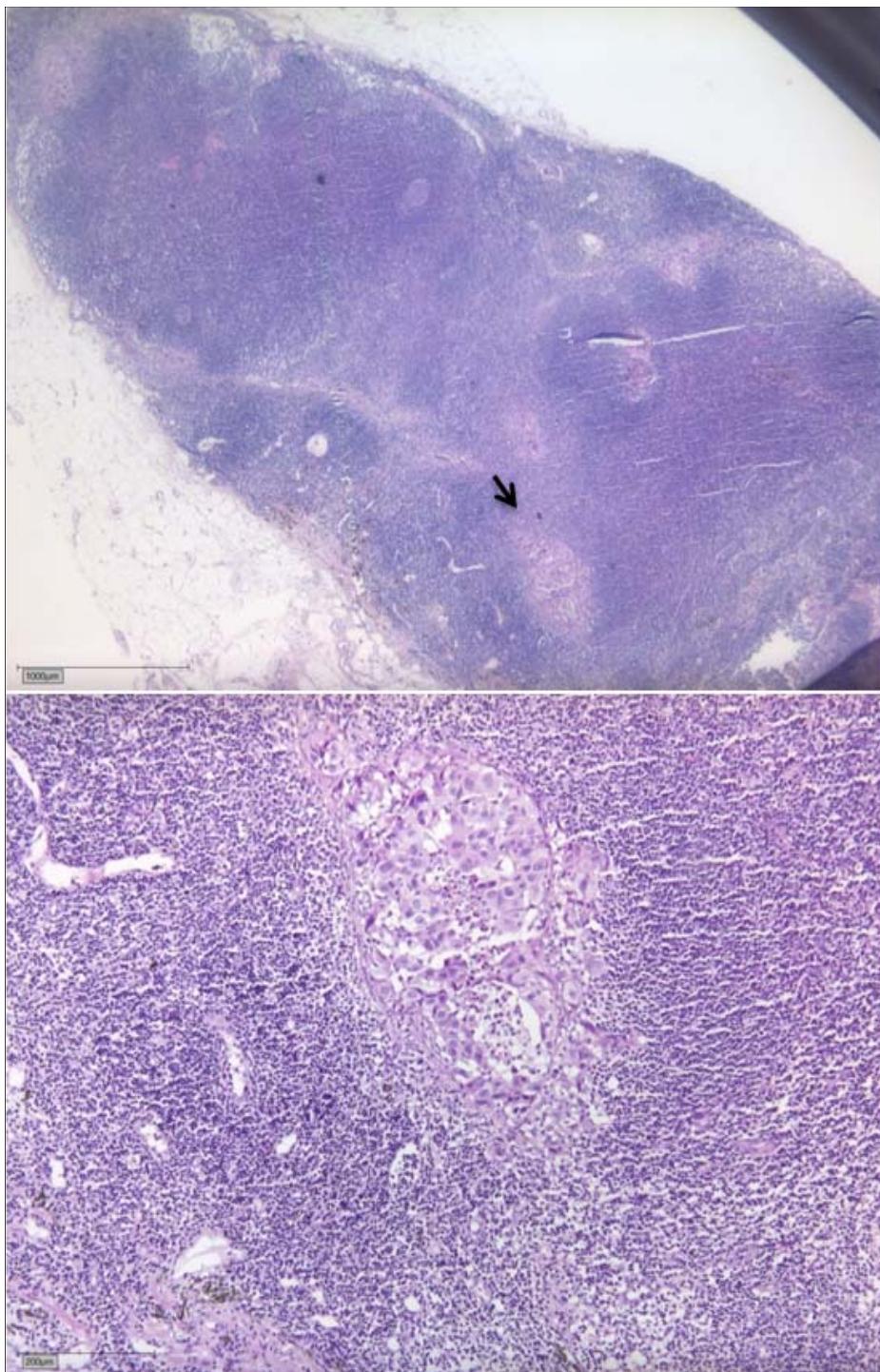
3. Single parameter molecular diagnostics (compagnion diagnostics
   ...... etc, etc....

4. Whole Genome Sequ   vs

   Multiplexing Informative Gene Sets

Total count: 10471
A : 10 (0%, 4+, 6-)
C : 2 (0%, 0+, 2-)
G : 10456 (100%, 5255+, 5201-)
T : 3 (0%, 0+, 3-)
N : 0
- - - - - - - - - - - - - - -
DEL: 206
INS: 0

C

G

A T C A A G G A A T T A A G A G A A G C A A C A T C T
I   K   E   L   R   E   A   T   S

EGFR

c.2235_2249del   p.E746_A750del

Sequenzielle Einzelanalysen
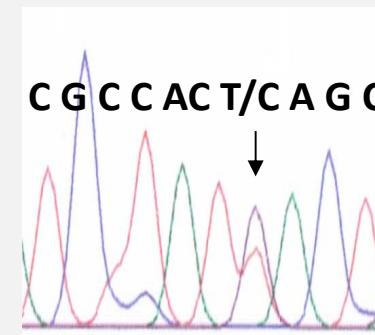
Pro Ziel Region werden

40ng FFPE DNA benötiget

DNA-Population aus FFPE

Amplifikation einer Zielregion z.B. Exon 2 KRAS

C G C C AC T/C A G C
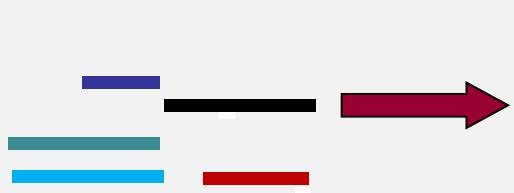
<u>**Sanger**</u> **Sequenzierung eines Amplikons**

Multiplex -Parallel Sequenzierung

Für gesamtes Panel werden

50ng FFPE DNA benötigt

DNA-Population aus FFPE

<u>**Simultane**</u> **Amplifikation von >300 Amplikons**

z.B. das gesamte Lungen Panel

TATCAAGGAATTAAGAGAAGCAACATCTCCG.

<u>**Parallel**</u> **Sequenzierung aller relevanten Amplikons**

König K,
JTO 2015

Datei Bearbeiten Ansicht Einfügen Format Datensätze Scripts Fenster Hilfe

51276 Datensätze | 52161 Gesamt (Unsortiert) | Alle zeigen Neuer Datensatz Datensatz löschen Suchen Sortieren

Layout: NGS Fall Anzeigen als: Seitenansichtsmodus

Mol_NR 3798
Barcode C13.28708
Kürzel C / 28708 / 2013
GBD

If_Untersuchung_ID 57185
LCGC-ID 7051
Untersuchung NGS_LUN3_#3

Ergebnis MolPatho
KRAS: c.35G>T p.G12V; TP53: c.359A>G p.K120R; TP53: c.215C>G p.P72R; TP53: c.542G>C p.R181P

NGS DATA SUMMARY
- 0 NGS bestellt
- 1 Ampliseq gestartet
- 2 Library ready
- 3 Data gesichtet
- 4 Validation bestellt
- 5 Validation ready
- 6 Befund

Diagnose LUN/M

MP Leistungserfassung

Comment_NGS

Coverage Statistic:

Minimum	31	Std.-Abw.	6.839,3
Maximum	36.069	Std.-Abw. i.V.	
Durchschn.	6.961,5	Durchschn.	1,0
Anzahl ges.	102	Maximum i.V.	
Anzahl uHu	1	Std.-Abw.	5,3

Gene	Chr	Pos1	Pos2	Cov				Type	SNM	c.	p.			Val%	c.	p.	Kommentar	Studie
KRAS	chr12	25380224	25380224	1072	3	0	0	silent	SNM	c.234T>C	p.F78F	IGV	+	11,325500	c.234T>C	p.F78F		
KRAS	chr12	25380260	25380260	44593	3	0	0	silent	SNM	c.198A>T	p.A66A	IGV	+	0,865827	c.198A>T	p.A66A		
KRAS	chr12	25398284	25398284	9297	3	0	0	missense	SNM	c.35G>T	p.G12V	IGV	-	26,551200	c.35G>T	p.G12V	Aktivierend	Einschluss in SORAVE (Everolimus + Sorafenib) Studie
MAP2K1	chr15	66727526	66727526	3558	3	0	0	missense	SNM	c.242T>G	p.V81G	IGV	+	0,859889	c.242T>G	p.V81G		
MAP2K1	chr15	66727529	66727529	3549	3	0	0	missense	SNM	c.245T>G	p.V82G	IGV	+	3,067950	c.245T>G	p.V82G		
MAP2K1	chr15	66727538	66727538	3520	3	0	0	missense	SNM	c.254T>G	p.V85G	IGV	+	1,738240	c.254T>G	p.V85G		
MAP2K1	chr15	66727562	66727562	3549	3	0	0	missense	SNM	c.278T>G	p.V93G	IGV	+	1,470590	c.278T>G	p.V93G		
TP53	chr17	7578236	7578236	612	3	0	0	missense	SNM	c.613T>A	p.Y205N	IGV	+	1,908960	c.613T>A	p.Y205N	nicht funktionelles Protein	
TP53	chr17	7578388	7578388	1954	3	0	0	missense	SNM	c.542G>C	p.R181P	IGV	-	25,736600	c.542G>C	p.R181P	nicht funktionelles Protein	
TP53	chr17	7579328	7579328	5297	3	0	0	missense	SNM	c.359A>G	p.K120R	IGV	-	14,271200	c.359A>G	p.K120R	nicht funktionelles Protein	
TP53	chr17	7579393	7579393	1301	3	0	0	silent	SNM	c.294T>C	p.P98P	IGV	+	1,244810	c.294T>C	p.P98P		
TP53	chr17	7579409	7579409	1698	3	0	0	missense	SNM	c.278T>C	p.L93P	IGV	+	1,218870	c.278T>C	p.L93P	neutral	Einschluss in P53/MDM2 Inhibitor Studie CGM097 prüfen
TP53	chr17	7579416	7579416	1696	3	0	0	missense	SNM	c.271T>C	p.W91R	IGV	+	1,379310	c.271T>C	p.W91R	neutral	Einschluss in P53/MDM2 Inhibitor Studie CGM097 prüfen

ID	If	Mol	Gene	Chr	Pos1	Pos2	Cov				Type	SNM	c.	p.		Val%	c.	p.	Kommentar	Studie	Datum
272739	57185-3-M3798		MAP2K1	chr15	66727526	66727526	3558	3	0	0	missense	SNM	c.242T>G	p.V81G	IGV	+	0,859889	c.242T>G	p.V81G			05.09.2013 09:46:48
272740	57185-3-M3798		MAP2K1	chr15	66727529	66727529	3549	3	0	0	missense	SNM	c.245T>G	p.V82G	IGV	+	3,067950	c.245T>G	p.V82G			05.09.2013 09:46:48
272741	57185-3-M3798		MAP2K1	chr15	66727538	66727538	3520	3	0	0	missense	SNM	c.254T>G	p.V85G	IGV	+	1,738240	c.254T>G	p.V85G			05.09.2013 09:46:48
272742	57185-3-M3798		MAP2K1	chr15	66727562	66727562	3549	3	0	0	missense	SNM	c.278T>G	p.V93G	IGV	+	1,470590	c.278T>G	p.V93G			05.09.2013 09:46:48
272743	57185-3-M3798		TP53	chr17	7578236	7578236	612	3	0	0	missense	SNM	c.613T>A	p.Y205N	IGV	+	1,908960	c.613T>A	p.Y205N	nicht funktionelles Protein		05.09.2013 09:46:48
272744	57185-3-M3798		TP53	chr17	7578388	7578388	1954	3	0	0	missense	SNM	c.542G>C	p.R181P	IGV	-	25,736600	c.542G>C	p.R181P	nicht funktionelles Protein		05.09.2013 09:46:48
272745	57185-3-M3798		TP53	chr17	7579328	7579328	5297	3	0	0	missense	SNM	c.359A>G	p.K120R	IGV	-	14,271200	c.359A>G	p.K120R	nicht funktionelles Protein		05.09.2013 09:46:48
272746	57185-3-M3798		TP53	chr17	7579393	7579393	1301	3	0	0	silent	SNM	c.294T>C	p.P98P	IGV	+	1,244810	c.294T>C	p.P98P			05.09.2013 09:46:48
272747	57185-3-M3798		TP53	chr17	7579409	7579409	1698	3	0	0	missense	SNM	c.278T>C	p.L93P	IGV	+	1,218870	c.278T>C	p.L93P	neutral	Einschluss in P53/MDM2 Inhibitor Studie CGM097 prüfen	05.09.2013 09:46:48
272748	57185-3-M3798		TP53	chr17	7579416	7579416	1696	3	0	0	missense	SNM	c.271T>C	p.W91R	IGV	+	1,379310	c.271T>C	p.W91R	neutral	Einschluss in P53/MDM2 Inhibitor Studie CGM097 prüfen	05.09.2013 09:46:48

100 Blättern

Hybrid Selection instead of Multiplex PCR:
- Fragmentation of DNA (Covaris)
- Ligation of Adapter and Barcodes

C. Hybridize biotinylated probes to targeted regions

D. Enrich using streptavidin beads

E. Elute from beads

Advantage:

fusions can be integrated

Disadvantage:

more sample input (10x)

more data output

Composition of *aClO* (= all cancers in one) panel *LTCGv3.0*

Gene	target	Gene	target	Gene	target
ABL1	exons	IDH1	exons	RHOA	Exon 2,3
ALK	breakpoints and exons	IDH2	exons	RICTOR	exons
APC	exons	IGF2R	exons	ROS1	breakpoints and exons
AR	exons	JAK2	exons	RPTOR	exons
ARAF	exons	KDR	exons	SMO	exons
ATM	exons	KEAP1	exons	STK11	exons
ATR	exons	KIF5B	breakpoint only	TGFBR2	exons
BCL6	exons	KIT	exons	TP53	exons
BRAF	breakpoints and exons	KNSTRN	Exon1	TSC1	exons
BRCA1	exons	KRAS	exons	TSC2	exons
BRCA2	exons	MAP2K1	Exon 2	VHL	exons
CCND1	exons	MDM2	exons		
CCNE1	exons	MET	whole gene		
CD74	breakpoints	MSH3	exons		
CDK4	exons	MTOR	exons		
CDK6	exons	MYC	exons		
CDKN2A	exons	MYCL1	exons		
CDKN2B	exons	MYCN	exons		
CTNNB1	exons	NF1	exons		
EGFR	whole gene	NF2	exons		
EML4	breakpoint	NFE2L2	exons		
ERBB2	exons	NOTCH 1	exons		
FGFR1	whole gene	NOTCH 2	exons		
FGFR2	breakpoints and exons	NOTCH 3	exons		
FGFR3	whole gene	NRAS	exons		
FLT1	exons	NRG1	breakpoint only		
FLT4	exons	NTRK1	breakpoints and exons		
GNA11	exons	OXA1L	Exon 1		
GNA13	exons	PDGFRa	breakpoints and exons		
GNAI2	exons	PDGFRb	breakpoints and exons		
GNAQ	exons	PIK3CA	exons		
GNAS	exons	PTCH1	exons		
GNAT2	exons	PTEN	exons		
GNG2	exons	RAC1	Exon2		
HDAC2	exons	RB1	exons		
HRAS	exons	RET	breakpoints and exons		

83 genes and gene regions

    Mutation analysis

    Rearrangement analysis

Size of *aClO*

(= all cancers in one) panel:

- **estimated total target region: 2.26 Mb**
- **suggested mean coverage: 200-400 x**

**S Merkelbach Bruse, M Odenthal
S Dümcke, R Büttner**

Size of CAIO (= cancer all in one) panel:

- estimation of total target region: 2.26 Mb
- suggested mean coverage: 200-400x
- minimal mean coverage: 150x

⇨ results in 48 samples per run using the „high output" mode of Illumina's NextSeq

⇨ cover: mutations

                  in-dels

                  amplifications, deletions

                  fusions

                  qMSI

                  (estimate mutational load)
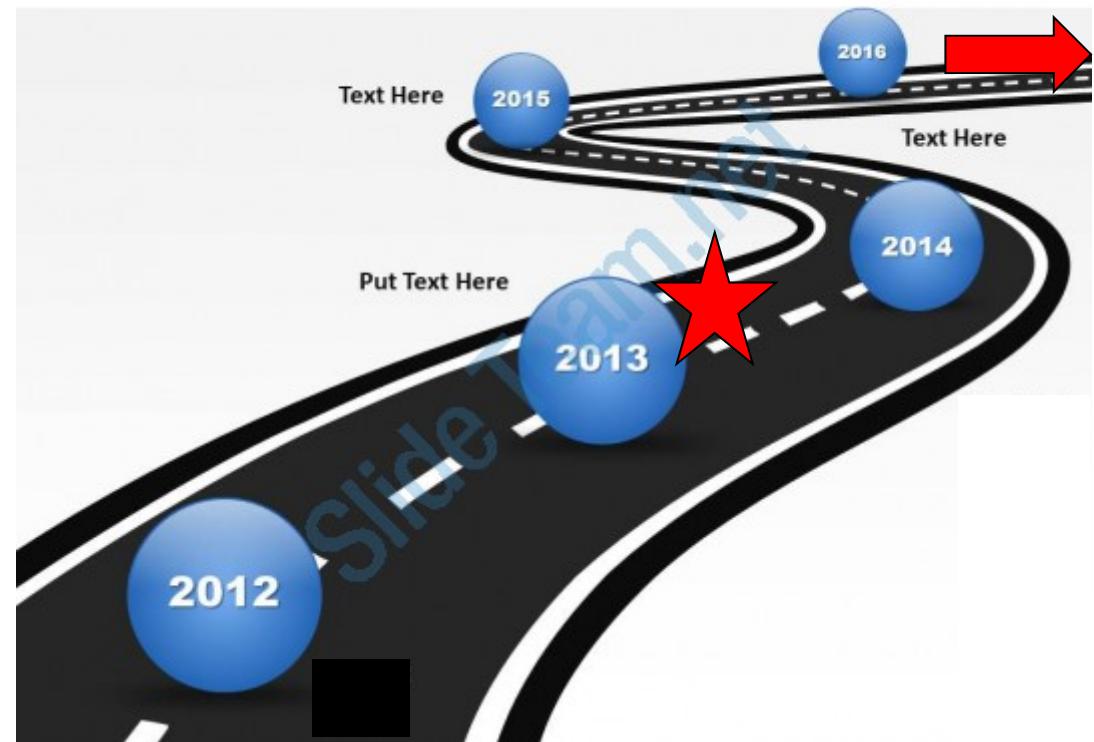
- *Genotyping for Personalised Oncology*

Is ist worth ?   Yes

    Is it durable ?   ???
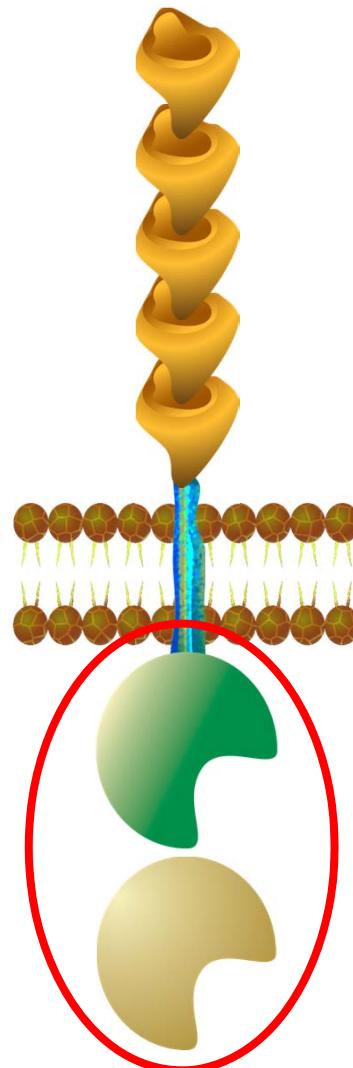
Tyrosinkinase Inhibitors (TKIs)
Mode of action
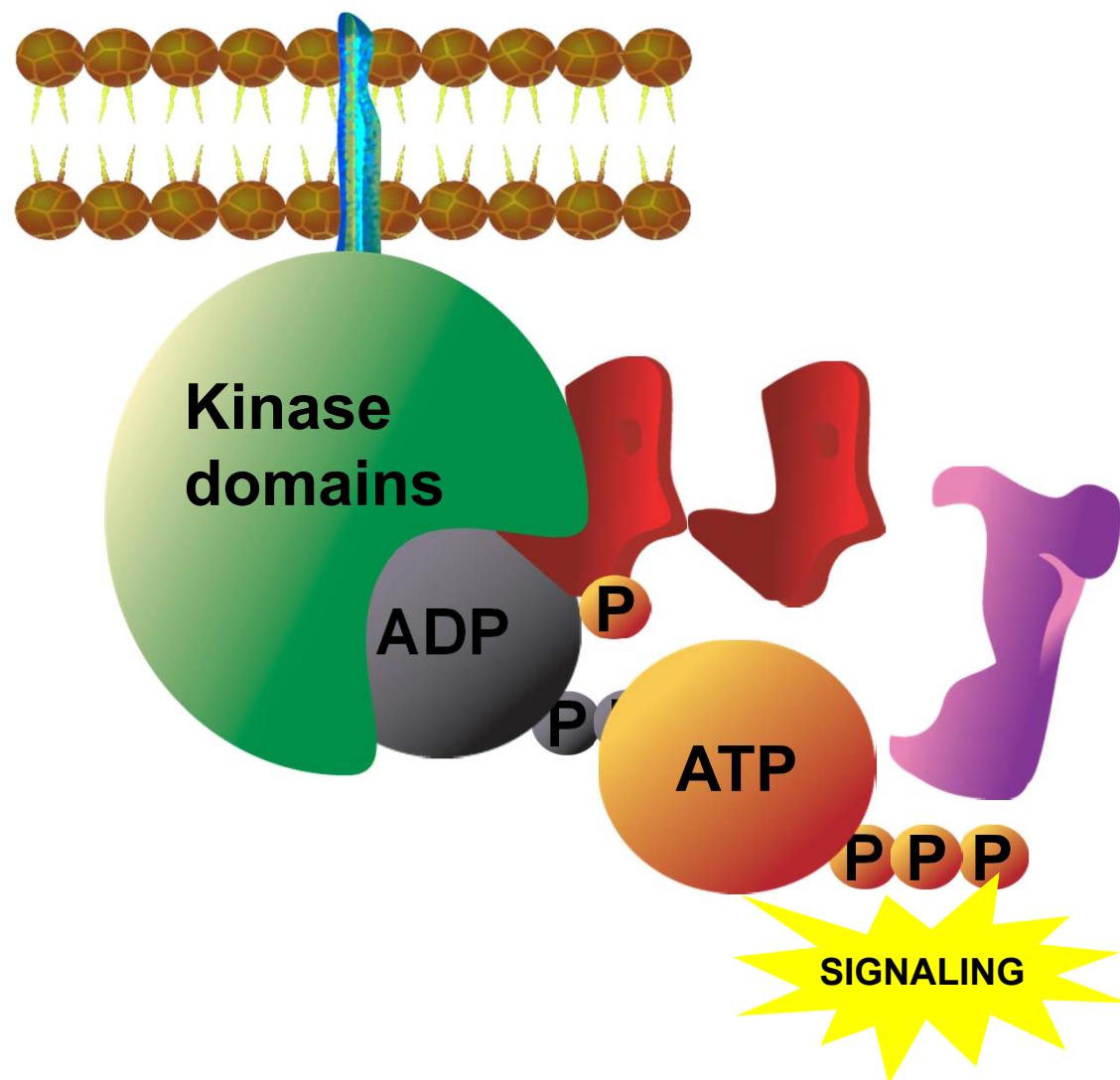
- **Structure of a tyrosine kinase (c-KIT)**

  − SCF binding site
  − 5 IgG domains
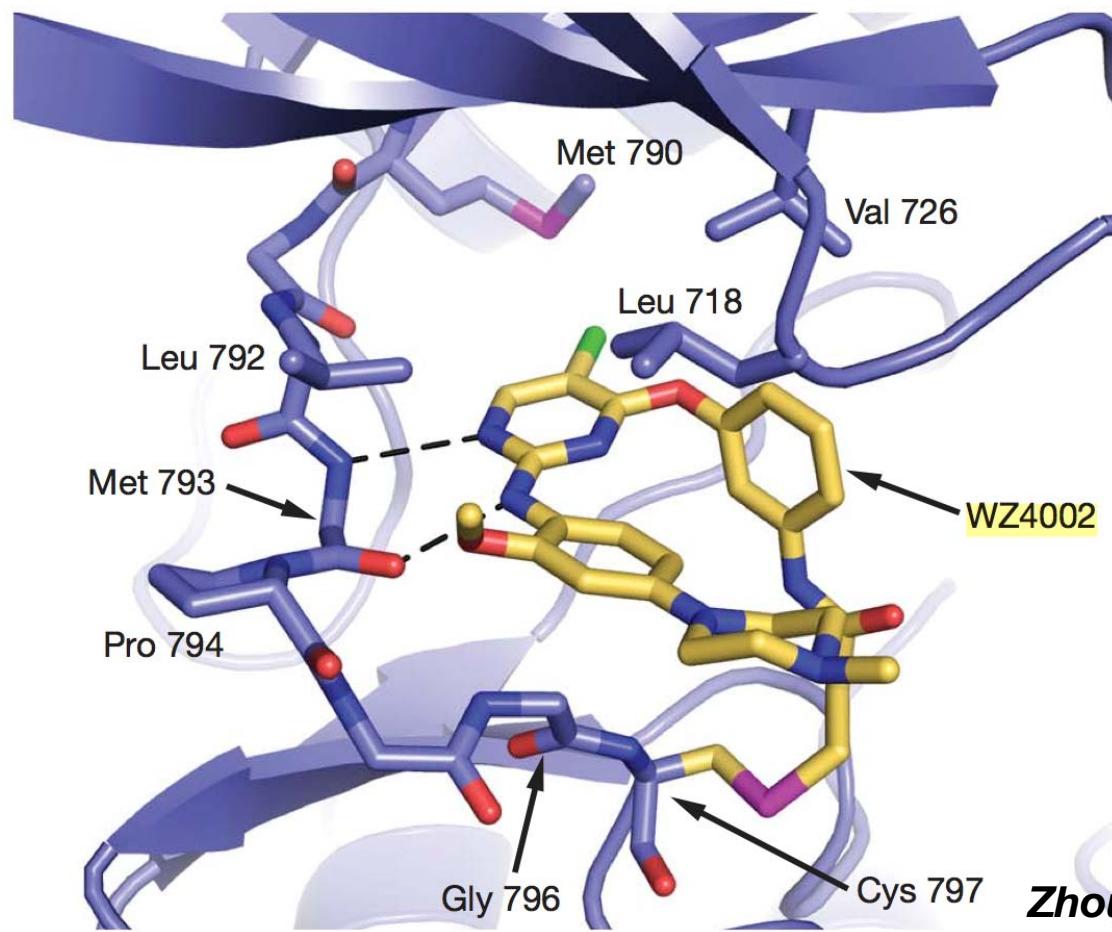
  2 tyrosine kinase domains

- **The KIT kinase domain activates a substrate protein, eg, PI3 kinase, by phosphorylation**

- **This activated substrate initiates a signaling cascade culminating in cell proliferation and survival**
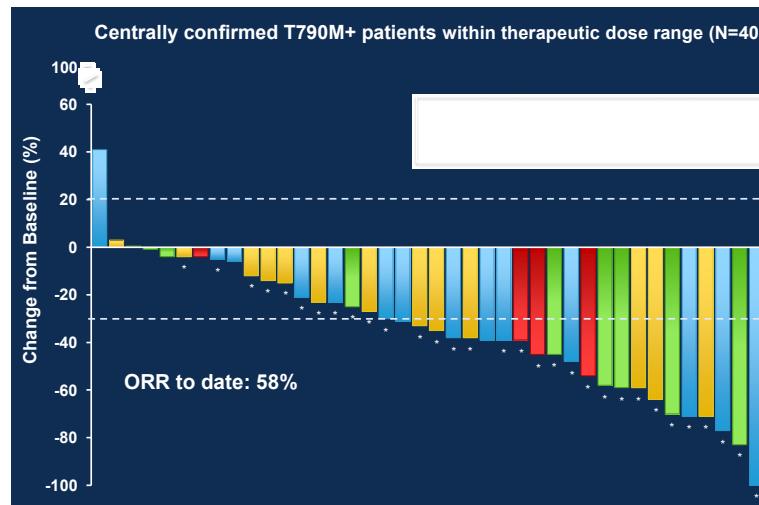
Overcoming resistance by structure-based compound design
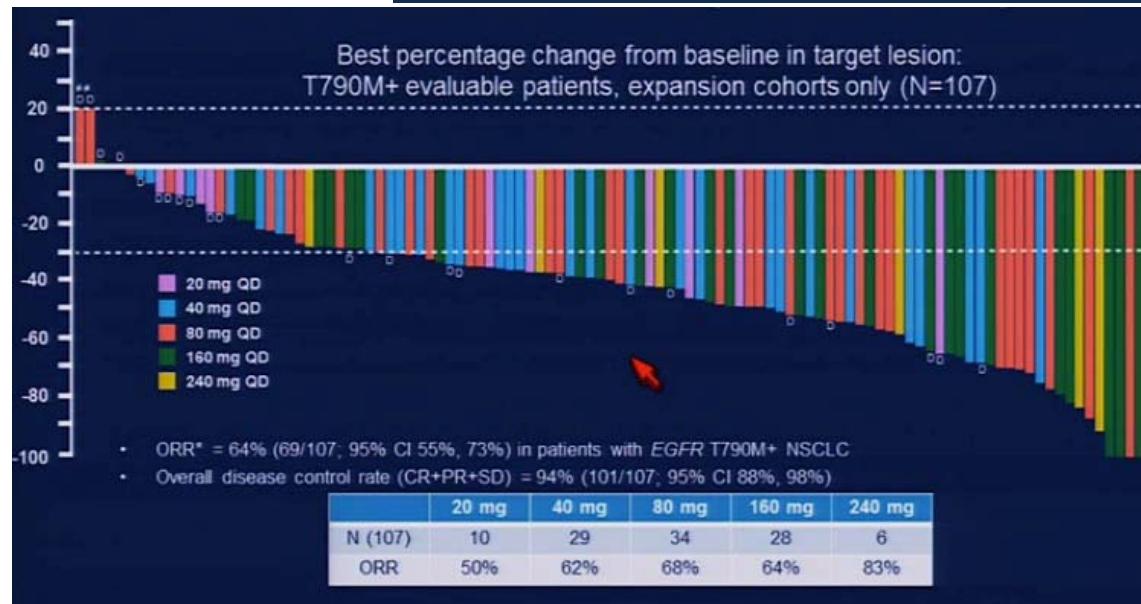
Zhou et al., Nature 2009
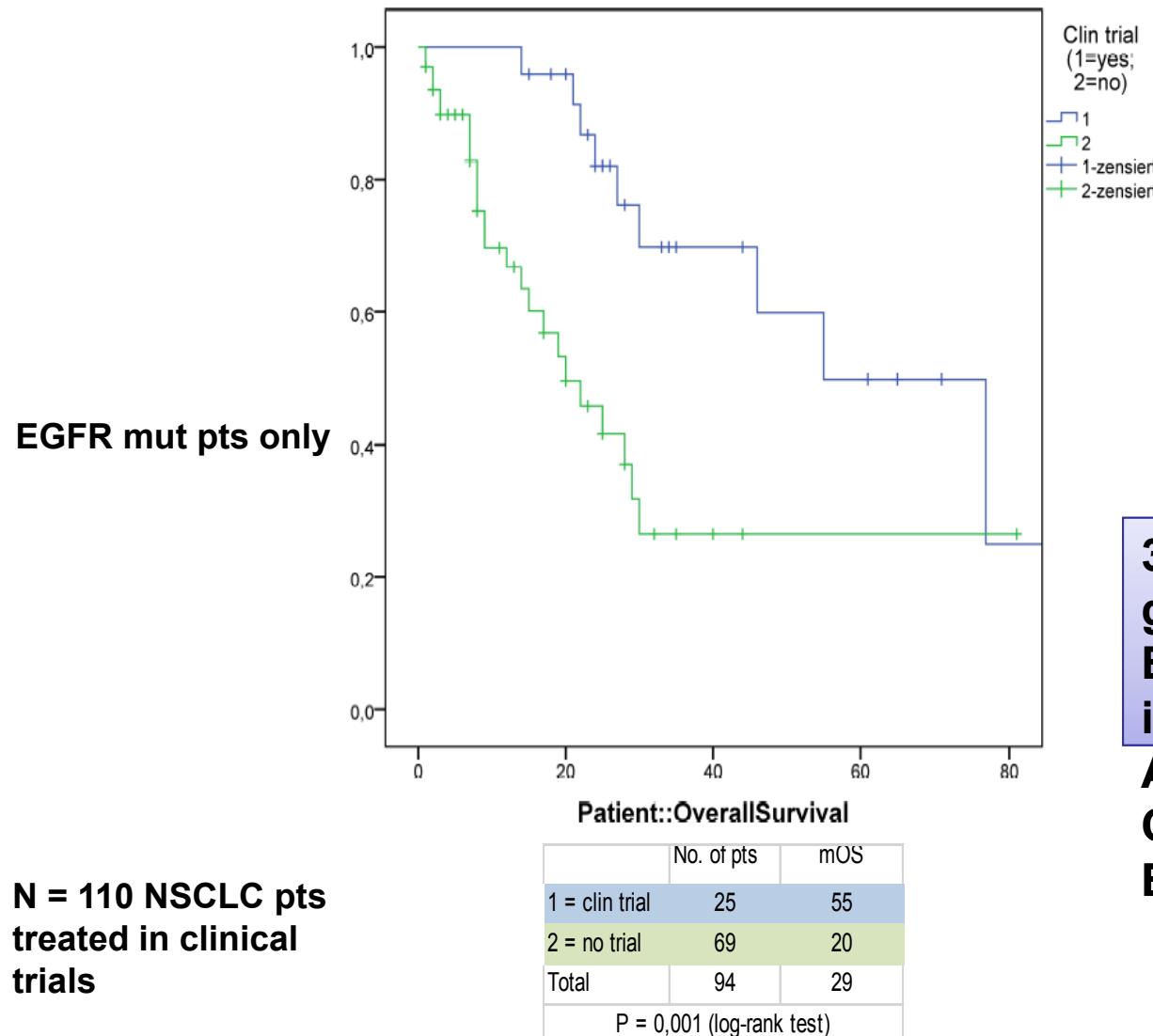
Clinical efficacy of next-gen EGFR inhibitors

CO-1686

Centrally confirmed T790M+ patients within therapeutic dose range (N=40)

Change from Baseline (%)

ORR to date: 58%

AZD9291

Best percentage change from baseline in target lesion:
T790M+ evaluable patients, expansion cohorts only (N=107)

- 20 mg QD
- 40 mg QD
- 80 mg QD
- 160 mg QD
- 240 mg QD

- ORR* = 64% (69/107; 95% CI 55%, 73%) in patients with *EGFR* T790M+ NSCLC
- Overall disease control rate (CR+PR+SD) = 94% (101/107; 95% CI 88%, 98%)

	20 mg	40 mg	80 mg	160 mg	240 mg
N (107)	10	29	34	28	6
ORR	50%	62%	68%	64%	83%

EGFR mut pts only

Patient::OverallSurvival

	No. of pts	mOS
1 = clin trial	25	55
2 = no trial	69	20
Total	94	29
P = 0,001 (log-rank test)		

N = 110 NSCLC pts treated in clinical trials

3rd generation EGFR inhibitors: AZD9291, CO-1686, EGF816
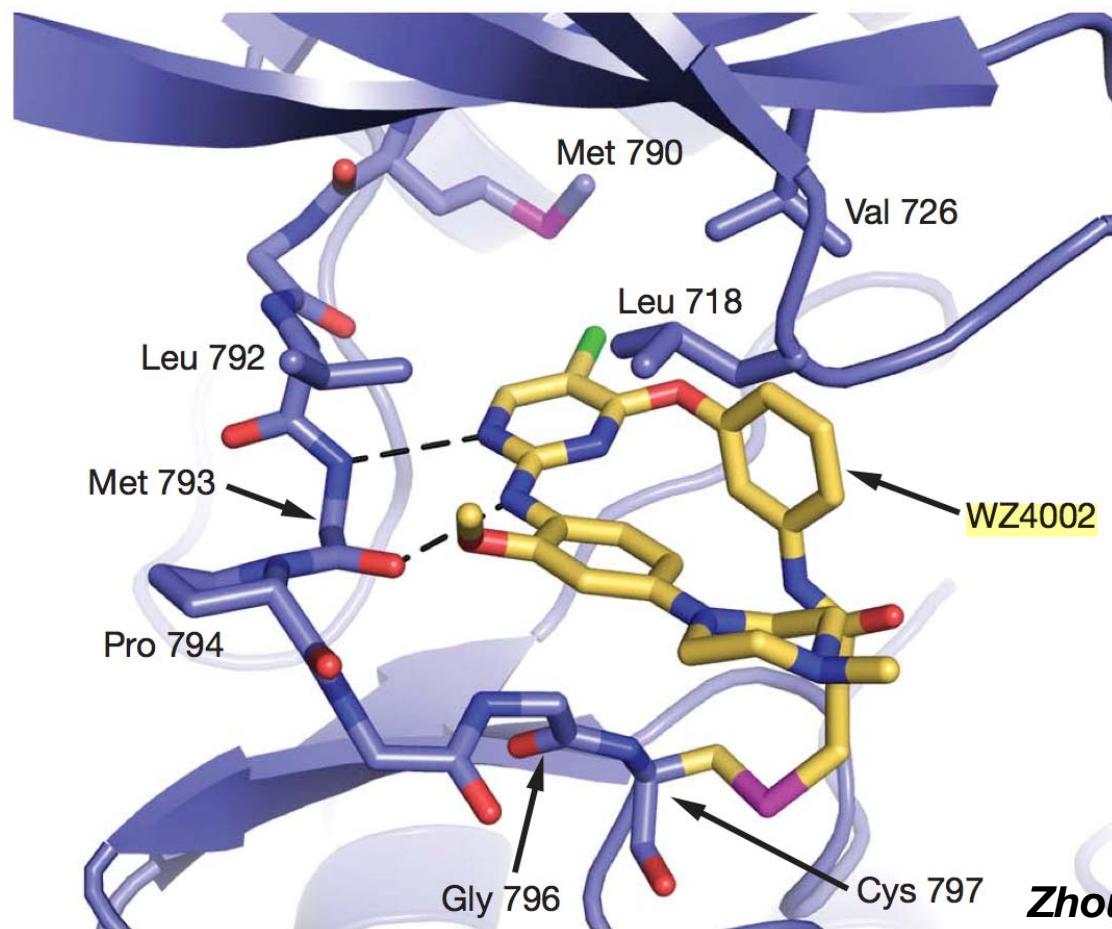
Surveillance of solid tumors by liquid biopsies

Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA.
Murtaza M, Nature. 2013 May 2;497(7447)

Overcoming resistance by structure-based compound design

Zhou et al., Nature 2009
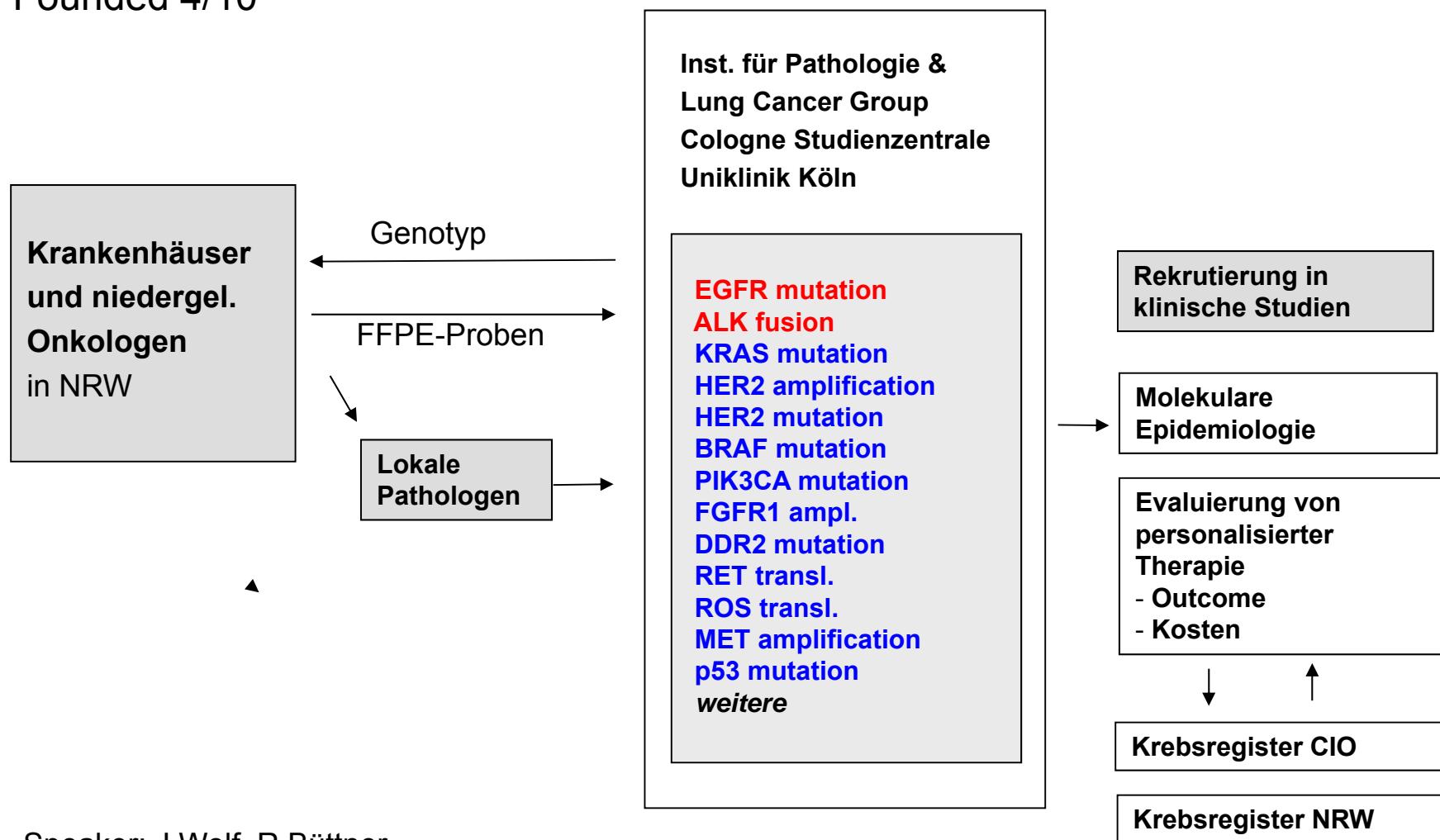
Network Genomic Medicine - NGM
Lung Cancer

Network Genomic Medicine Lung Cancer

**Inst. für Pathologie &
Lung Cancer Group
Cologne Studienzentrale**

**Krankenhäuser
und niedergel.
Onkologen**
in NRW

Gen

**Rekrutierung in
klinische Studien**

**Molekulare
Epidemiologie**

> **> 4,500 patients / 2014
> 10 % of all German stage IV
> Lung cancer patients**

**Evaluierung von
personalisierter
Therapie
- Outcome
- Kosten**

ROS transl.
MET amplification
p53 mutation
weitere

Krebsregister CIO

Krebsregister NRW
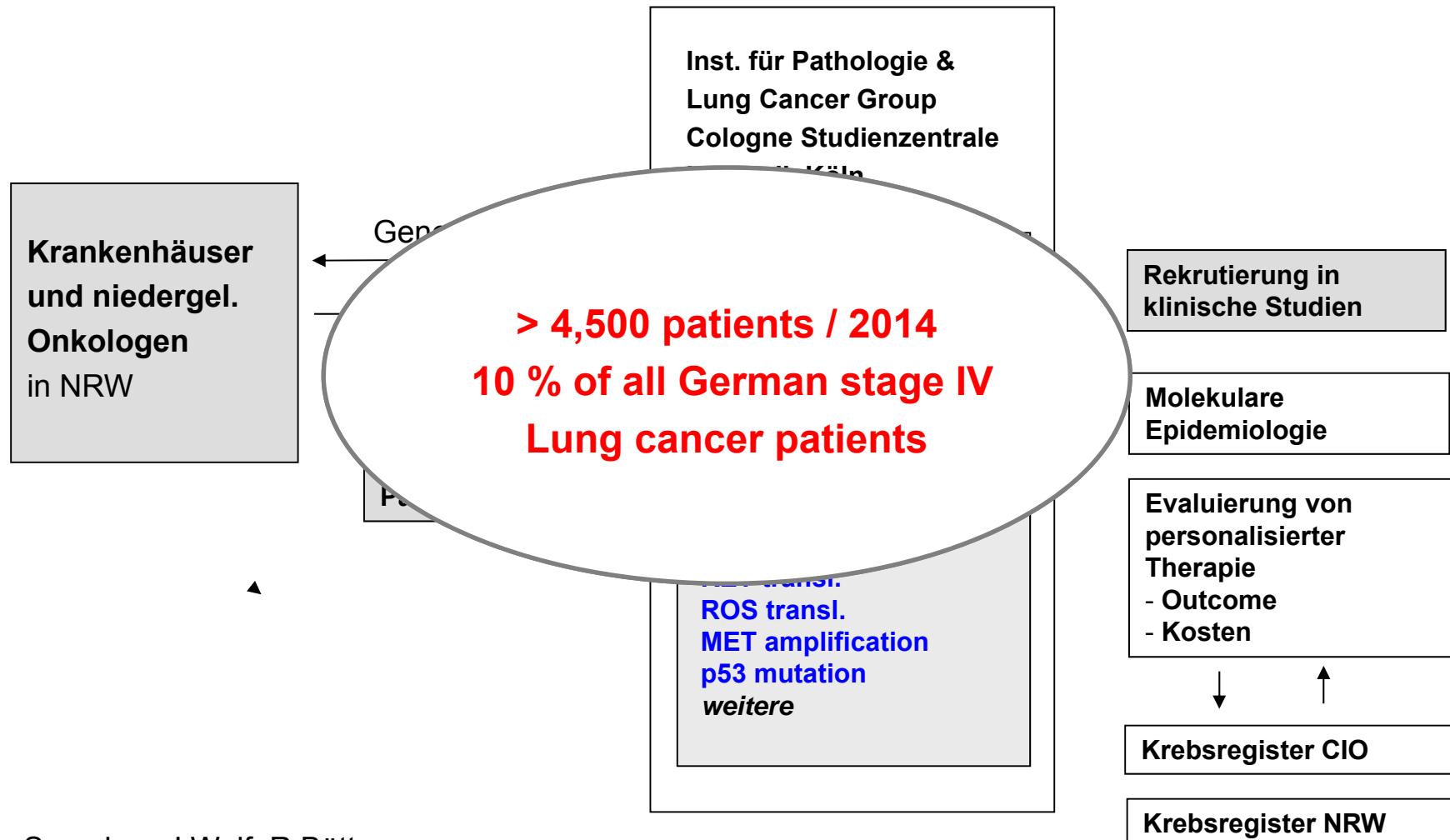
Sprecher: J.Wolf, R.Büttner
PI s: S.Michels, A. Scheel

Network
Genomic Medicine
Lung Cancer

Reimbursed by an Integrated Care Contract (IV)

> 225
NGM Members
In Germany
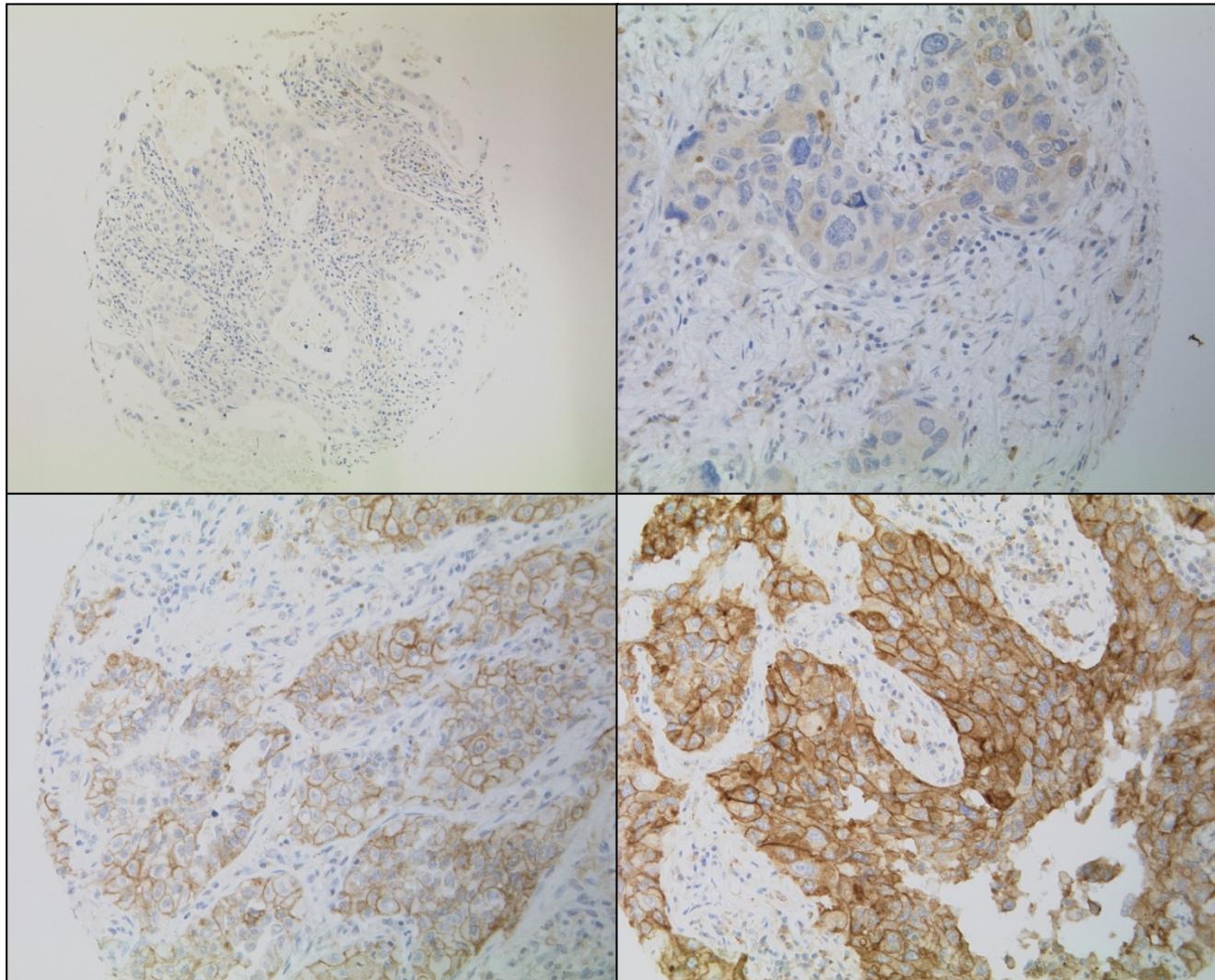
Studies Steering Committee

Networks provide a Win-Win situation
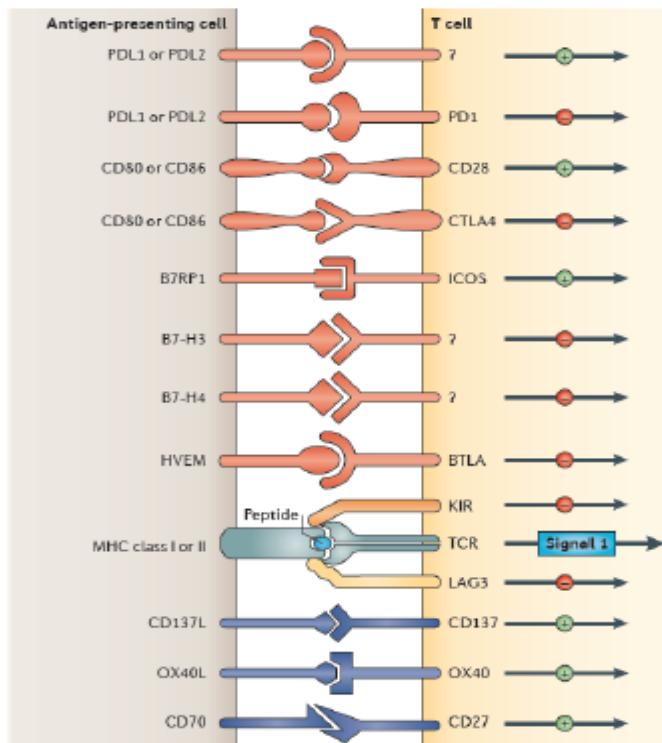
PD-L1 ICH, Schultheis et al, 2015
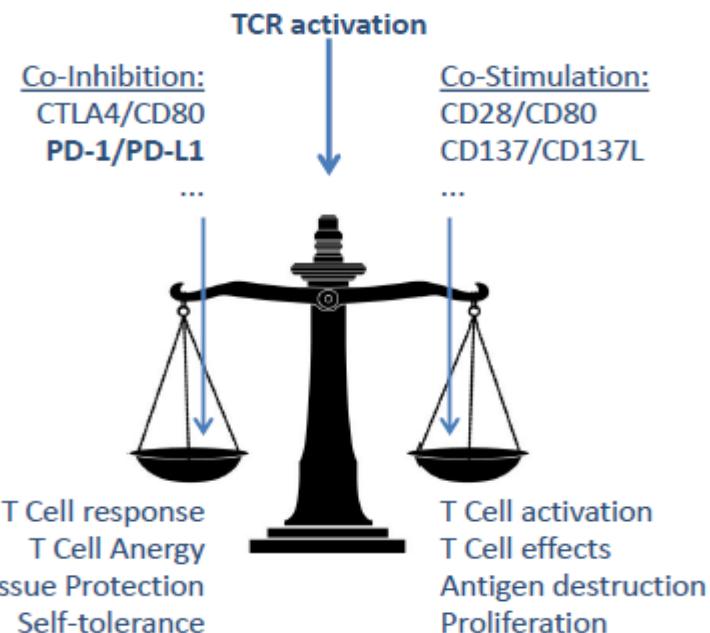
IHC: PD-L1, Klon 5H1.
Abbildungen: Dr. Anne Schultheis,
Pathologie Universitätsklinik Köln

# The role of PD-1 and PD-L1 pathway
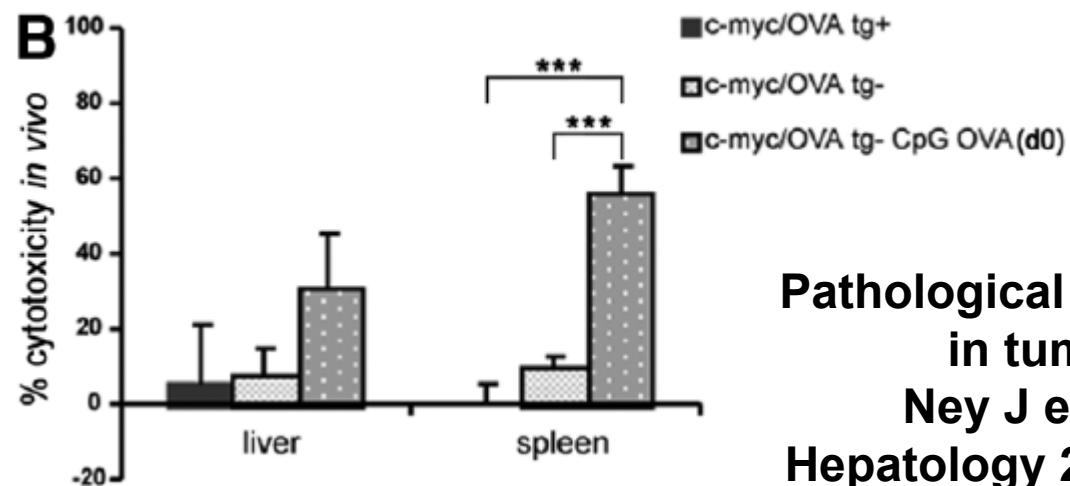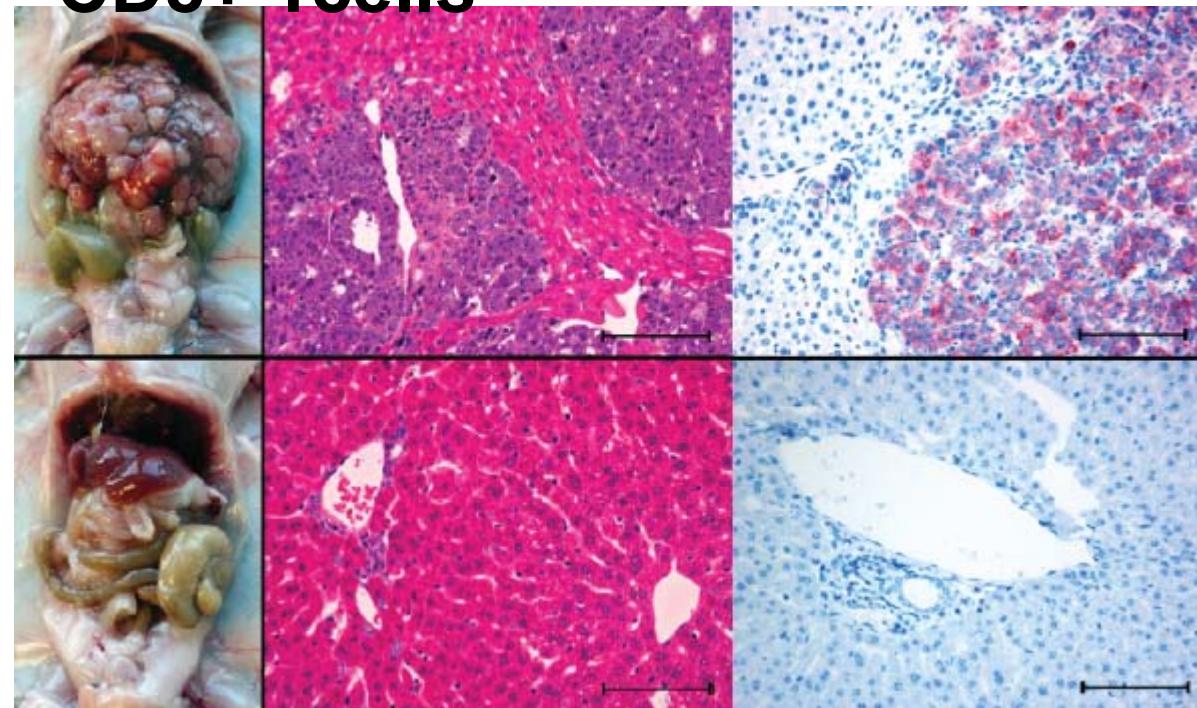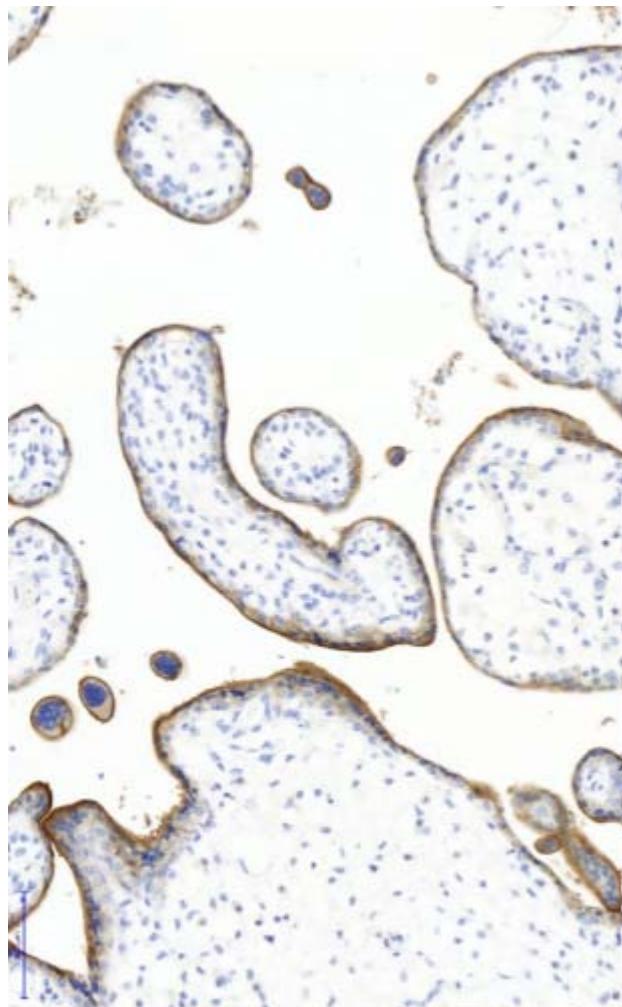
Pardoll. Nat Rev Cancer. 2012 Mar 22;12(4):252-64.
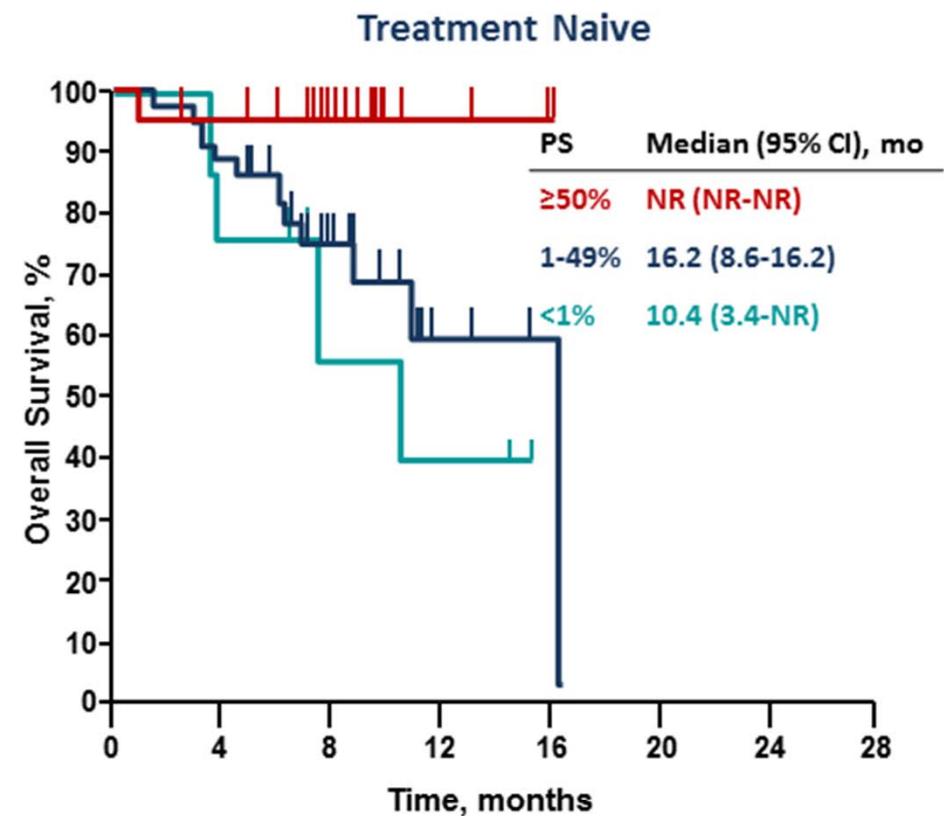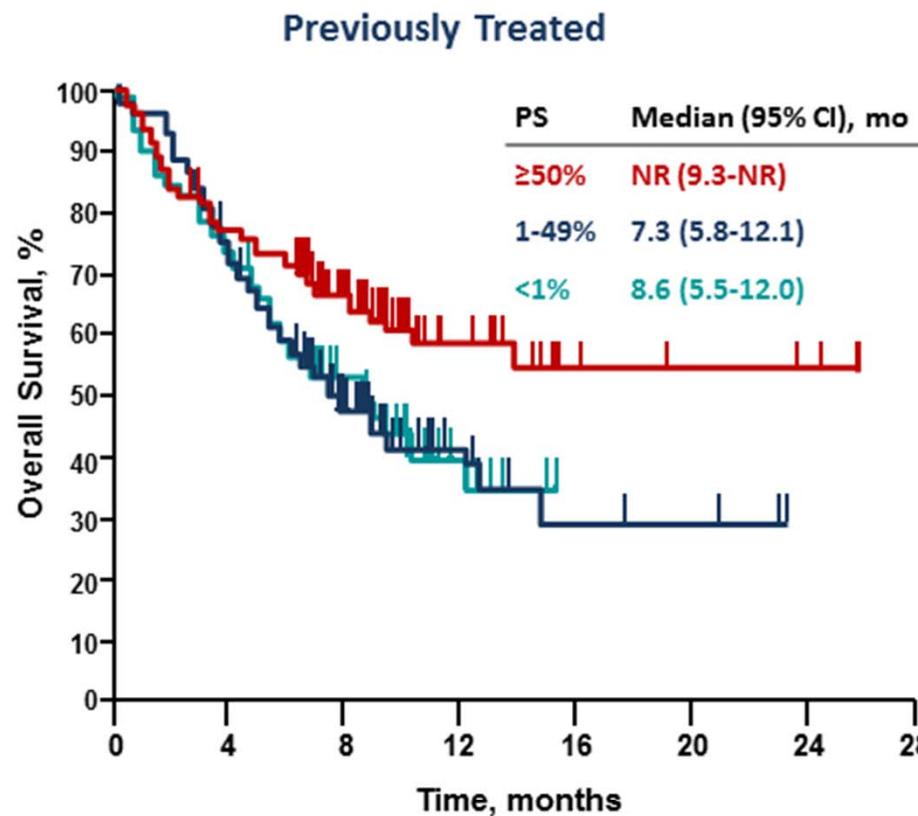
PD-L1 induces tolerance in antigen-specific CD8+ Tcells

Physiological role in placenta

Pathological role in tumors
Ney J et al., Hepatology 2009

OS by PD-L1 Expression, CTA-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

The challenge in PD-L1 testing: Currently four tests in development

	Merck	BMS	Roche	AZ
	KEYTRUDA pembrolizumab	Opdivo nivolumab	Atezolizumab MPDL3280a	Durvalumab MEDI-4736
Clone	22C3	28-8	SP142	SP263
Dxy	Dako	Dako	Ventana	Ventana
Cutoffs	TC: ≥1, ≥50	TC: ≥1, ≥5, ≥10	TC: ≥1, ≥10, ≥50 IC: ≥1, ≥5, ≥10	TC: ≥25, ≥90
Prospective	Yes	No	Yes	Yes
Inter Observer	95.6 (50%)	97.8 (1%) 98.5 (5%)	>90	96.7 (25%)
Inter Site	91.3 (50%)	90.2 (1%) 94.8 (5%)	–	–

PD-L1 IHC: Harmonisation urgently needed

German Society for Pathology initiative: Ring Trial for PD-L1 ICH test harmonization

Phase I: Interobserver Concordance in Scoring -NSCLC
- In Progress
- Projectmanagement: A. Scheel, Cologne

Phase II: Assay-Harmonization: Interobserver Concordance in Staining and Scoring NSCLC
- In Development
- Projectmanagement: Targos GmbH, Kassel

Status

RRT PD-L1: Round 1

Case	Pathologists									Modus	Agreement
	P1	P2	P3	P4	P5	P6	P7	P8	P9		
1	0	0	0	0	0	0	0	0	1	0	89%
2	6	6	5	5	6	6	6	6	6	6	78%
3	0	0	2	1	0	0	0	0	0	0	78%
4	0	0	0	0	0	0	0	0	0	0	100%
5	6	6	4	6	6	6	6	6	6	6	89%
6	2	1	2	1	3	3	2	3	2	2; 3	44%
7	0	0	0	0	0	0	0	0	1	0	89%
8	4	2	4	4	4	4	5	3	6	4	56%
9	5	4	5	5	5	5	5	6	6	5	67%
10	1	0	4	3	1	1	0	1	4	1; 4; 0	44%
11	4	2	3	4	4	5	4	4	6	4	56%
12	0	0	1	0	0	0	0	0	1	0	78%
13	0	1	0	0	0	0	0	0	0	0	89%
14	2	1	2	3	1	3	2	1	3	1; 2; 3	33%
15	5	5	5	4	5	6	5	5	6	5	67%

Concordance (7-step-score)

Mean (Light's κ) [95% CI]	0,43 [0,31 - 0,53]
Mean κw [95% CI]	0,75 [0,61 - 0,82]
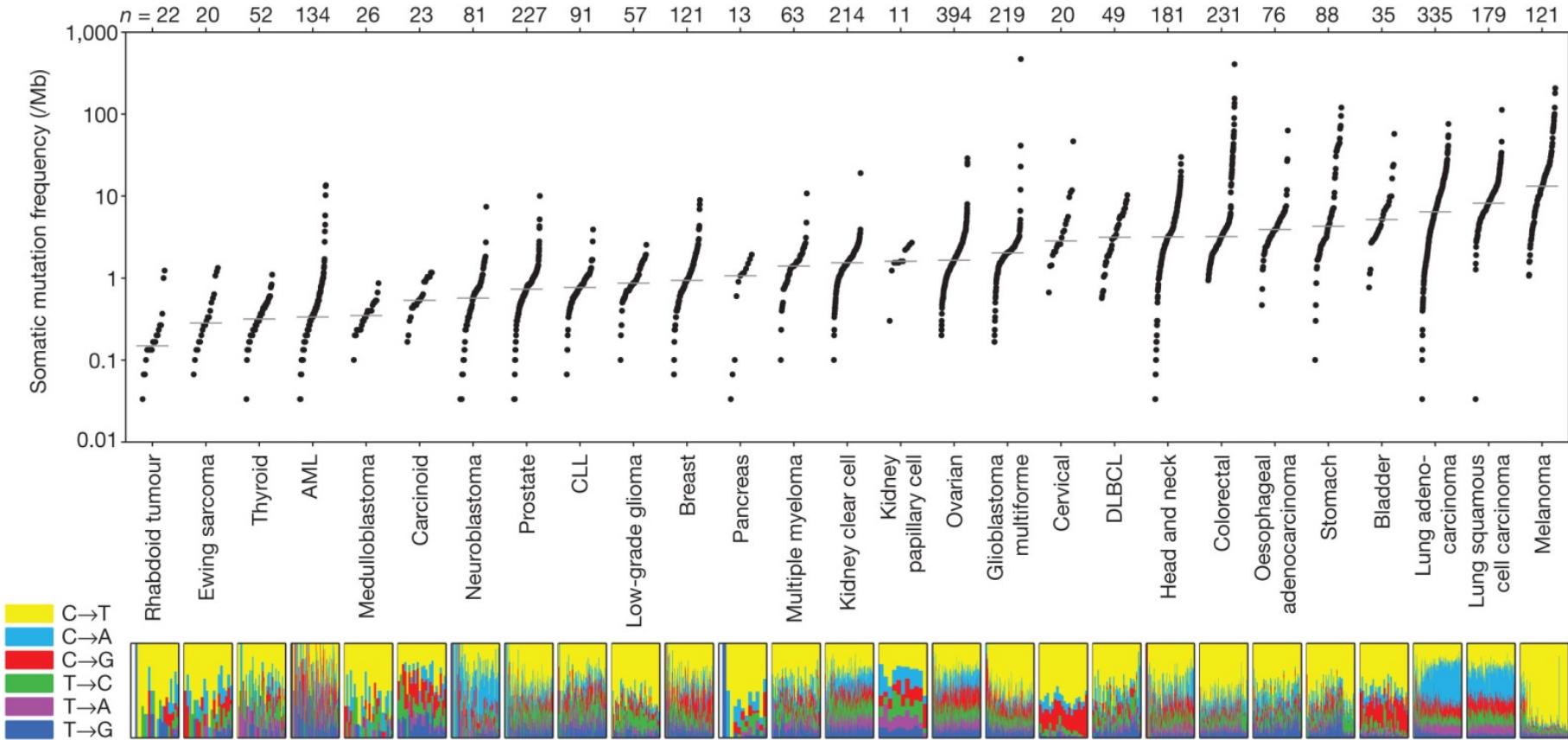
Concordance (2-step / ≥1%)

**Mean (Light's k) = 0,73 [0,60 - 0,88]**

Concordance (2-step / ≥50%)

Mean (Light's k) = 0,76 [0,58 - 0,92]

- **15 NSCLC-specimens stained centrally ('Case 1 - 15')**
- **Scoring by 9 German pathologists ('P1' - 'P9')**

'Working group PD-L1 IHC'
Manuscript in preparation

Somatic genetic alterations in cancer correlate with response to PD1 therapies

MS Lawrence et al. Nature, 1-5 (2013)

Van Allen EM[1], et al., Science. 2015 Sep 10.
**Genomic correlates of response to CTLA4 blockade in metastatic melanoma.
Overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit.**

**UNIKLINIK KÖLN**

Re-analyses of:
  Rizvi NA et al, Science 2015. 348(6230):124-8
  Van Allen EM et al, Science 2015. 350(6257):207-11
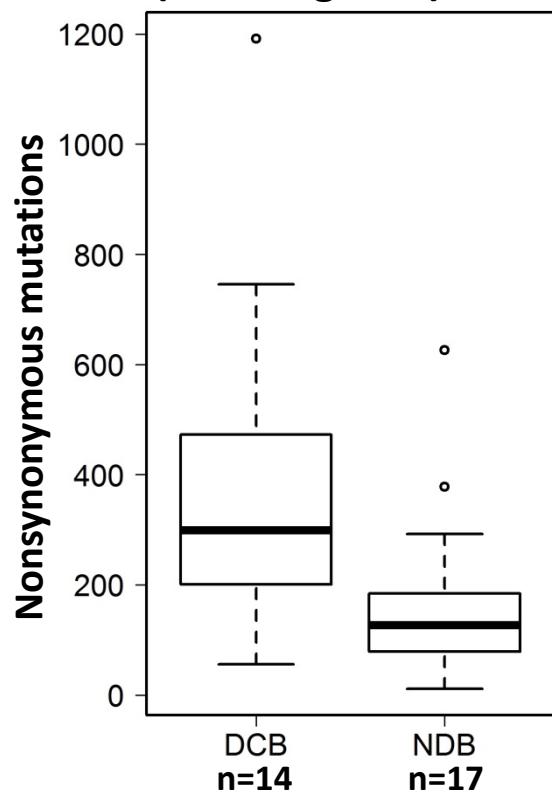
Simulation of 'CAIO panel'
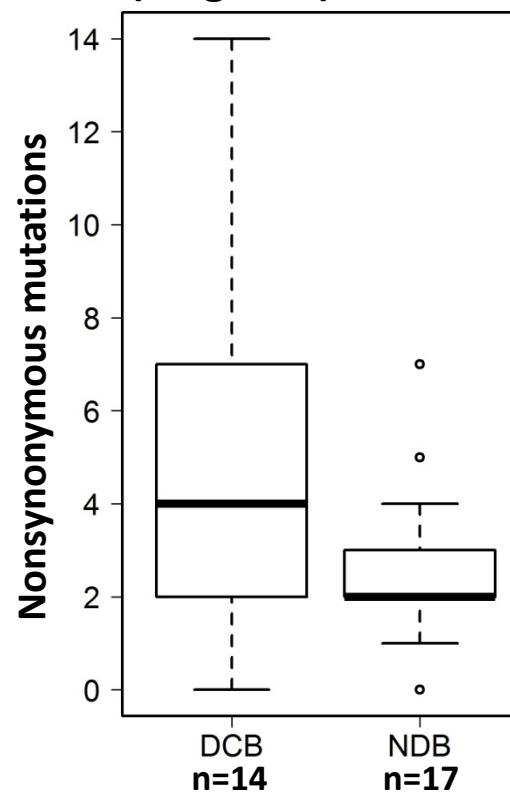
NSCLC - Nonsynonymous mutations
('Mutational load')

Published:
Whole exome seq
(20,500 genes)

Reanalysis:
**'CAIO panel' Cologne**
(93 genes)

Rizvi et al Science 2015: n=31 patients with NSCLC; Pembrolizumab (anti-PD-1). Number of nonsynonymous mutations determined by whole exome sequencing (published data) and 'CAIO panel' (reanalysis of same dataset).

DCB: Durable clinical benefit
**NDB: No durable benefit**

Data: Rizvi NA et al, Science 2015. 348(6230):124-8
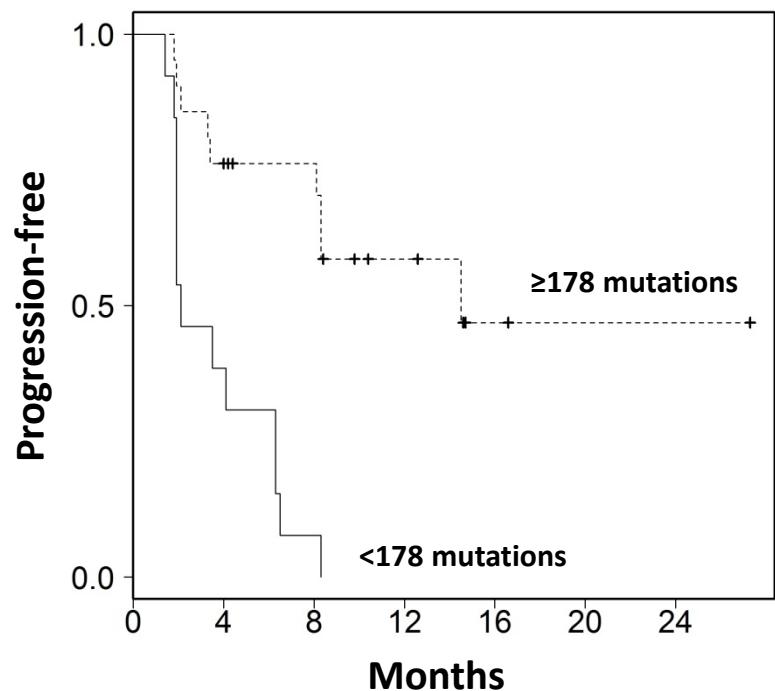
NSCLC - Progression-free survival
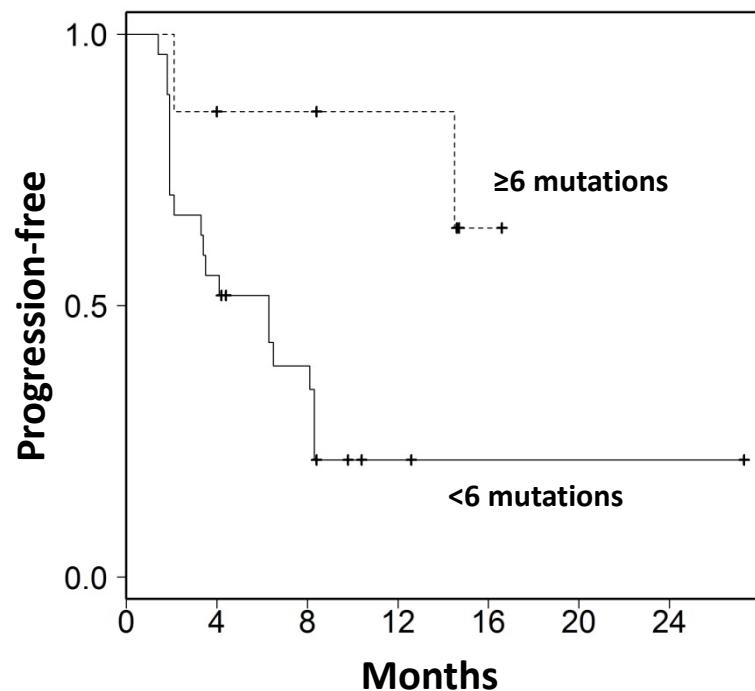
Published:
Whole exome seq (20,500 genes)
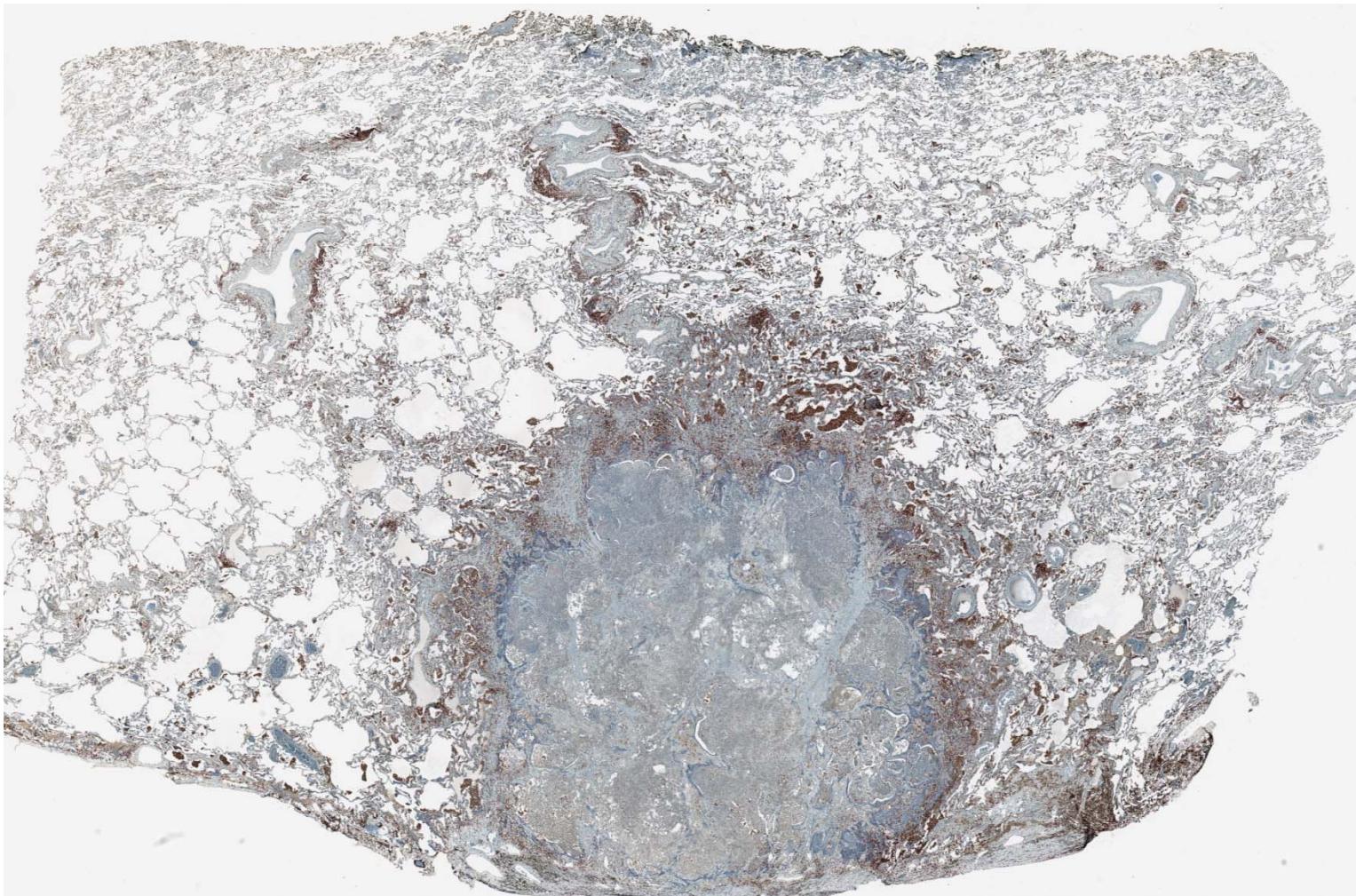
Reanalysis:
'CAIO panel' Cologne (93 genes)
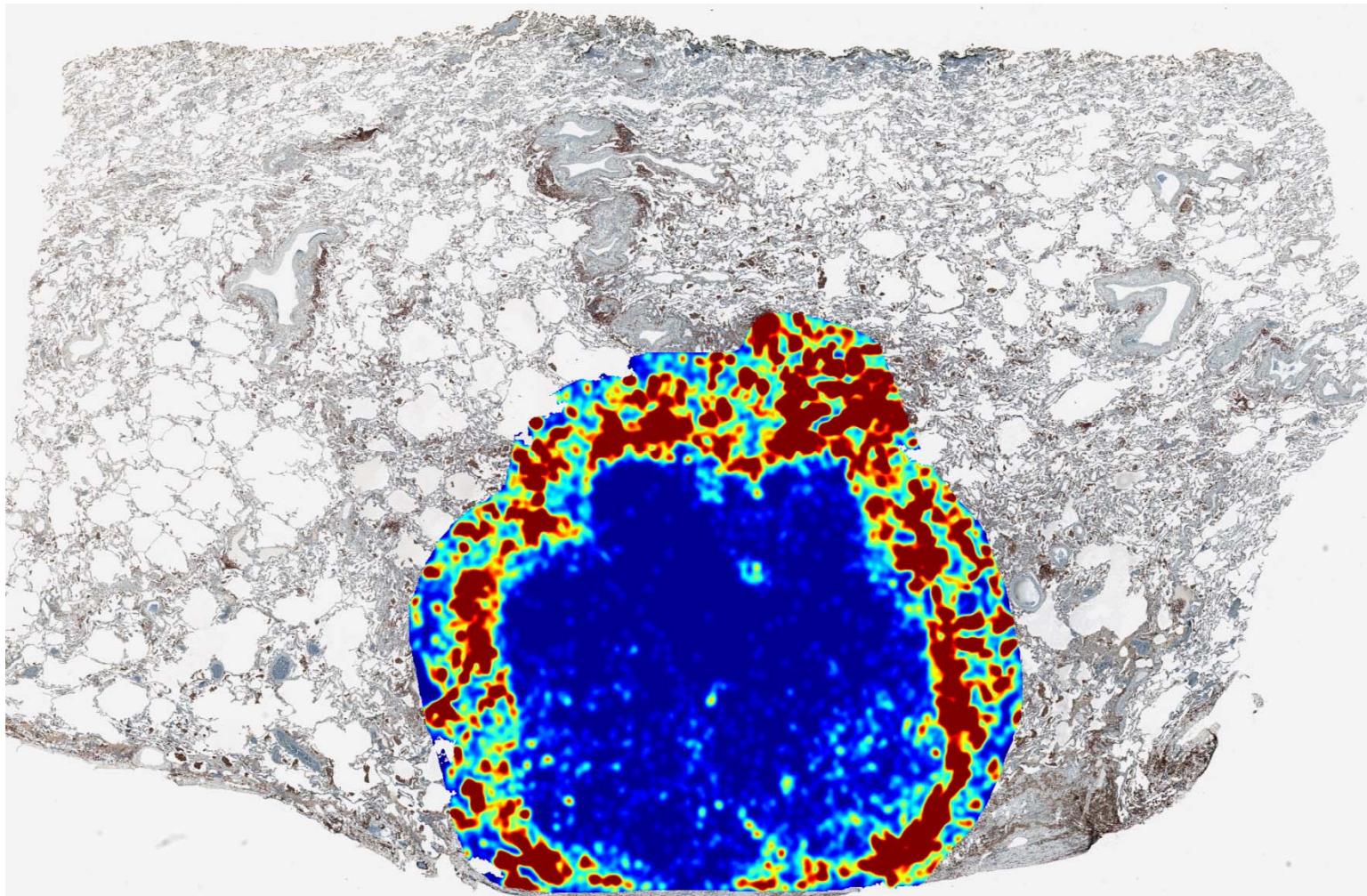
Data: Rizvi NA et al, Science 2015. 348(6230):124-8

Quantitative Pathology: CD4CD8 SqCC

CD3 (0-2000/mm^2): PD1

The concept of Personalised Medicine:

- In every single case determine the specific genomic vulnerability of a tumor

- Selectivity >> broad cytotoxicity

- Monitor genomic evolution under therapy (liquid biopsies)

- NGM networks and preclinical sciences (CCC)

- High-end integrative pathology

Thank you for attendance…

Andreas Scheel   **Institute of Pathology, Cologne**
Alexander Quaas
Margarethe Odenthal
Claudia Vollbrecht
Sabine Merkelbach-Bruse
Jana Fassunke
Michaela Ihle
Helen Künstlinger
Carina Heydt
Theresa Buhl
Ursula Rommerscheidt-Fuss
Alexandra Florin
Frank Ueckeroth
Michael Kloth
Michal R Schweiger

Peter Nürnberg   **Cologne Center for Genomics (Cologne)**
Janine Altmüller
Kerstin Becker
Christian Becker

Roman Thomas   **Institute of Genomics (Cologne)**
Martin Peifer

Thomas Henkel   **Targos (Kassel)**
Katrin Stamm
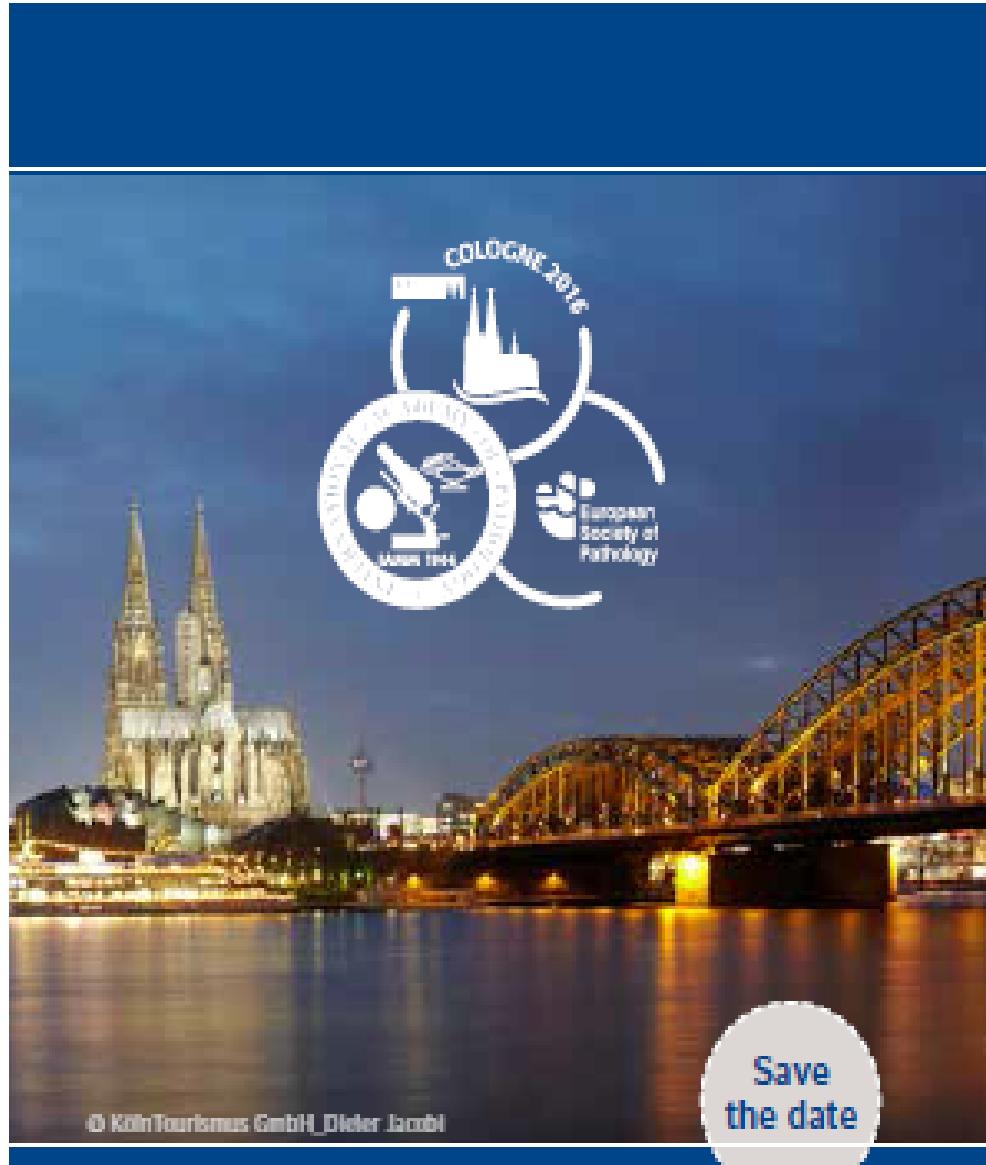
William J. Geese Bristol-Myers Squibb (Princeton, USA)
Lewis Strauss

Sven-Ernö Bikár   GENterprise GENOMICS (Mainz)

Jürgen Wolf

Center for Integrated Oncology Cologne/ Bonn
Lung Cancer Group Cologne

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European
Society of
Pathology

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the date

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and the

28th Congress of the European Society of Pathology

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Congress-Centrum Ost Kölnmesse, Cologne, Germany

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