Introduction to Molecular Pathology

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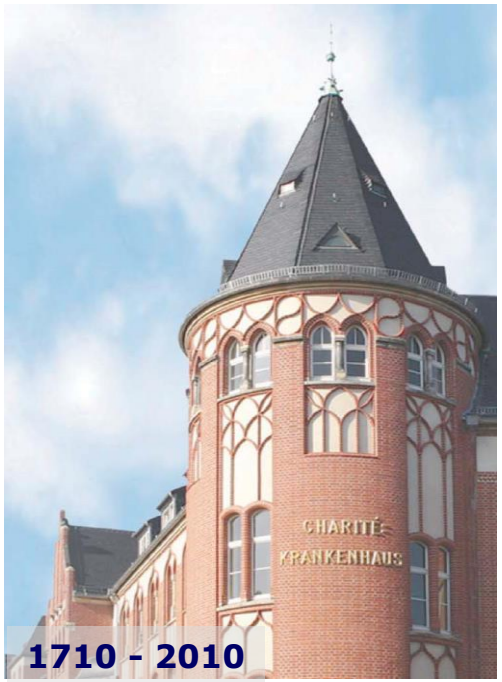

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Todays Challenges in Anatomic Pathology

The main goal of diagnostic pathology is to

extract from the patient's tissue

as many information as possible

by applying classical, immunological and molecular techniques.

But it should not be forgotten that the methodological results have to be interpreted by an experienced pathologist who is the one to bring together the diagnostic, prognostic and predictive information and to assign them to the disease of the individual patient.

Predictive Molecular Pathology and Personalized Medicine

A prerequisite of personalized medicine is the capability to predict pre-therapeutically the response of individual tumors to certain (targeted) drug.

For this prediction one needs reliable and reproducible biomarker and predictive assays.

This is the current challenge of predictive molecular pathology.

Prediction is difficult, especially about the future

Niels Bohr, 1885-1962

2010

Thinking back to infectious diseases and seeing the current development in cancer – HER2, KRAS, BRAF, EGFR, c-MET etc. - we shouldn't be too pessimistic.

The predictive power of tissue based analyses is underestimated.

Predictive tissue-based biomarkers for targeted therapies

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

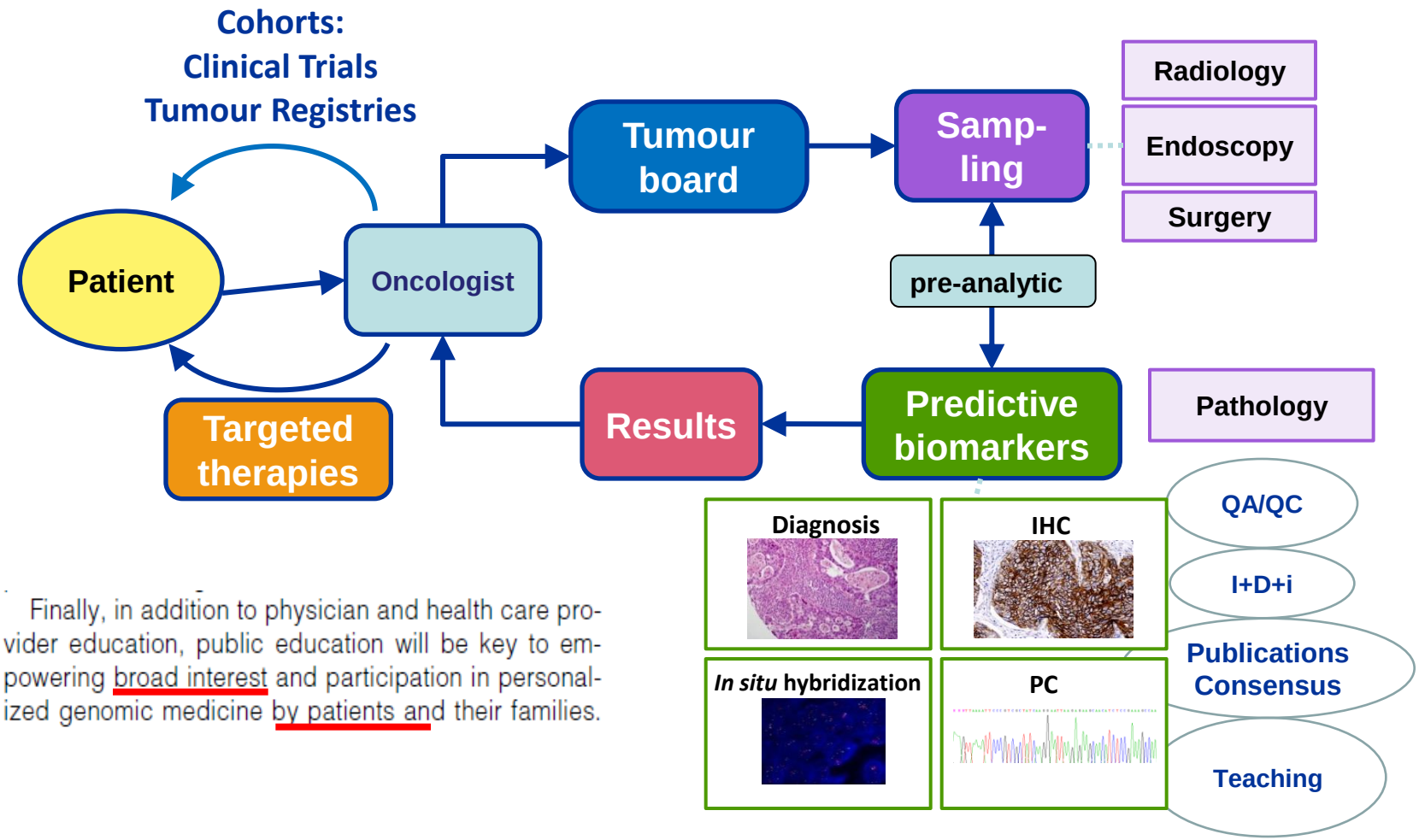
- T
- C
- F
- C
- E
- C
- N
- L
- V
- I
- I
- F
- C
- T

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

Multidisciplinary cooperation enables personalised oncology

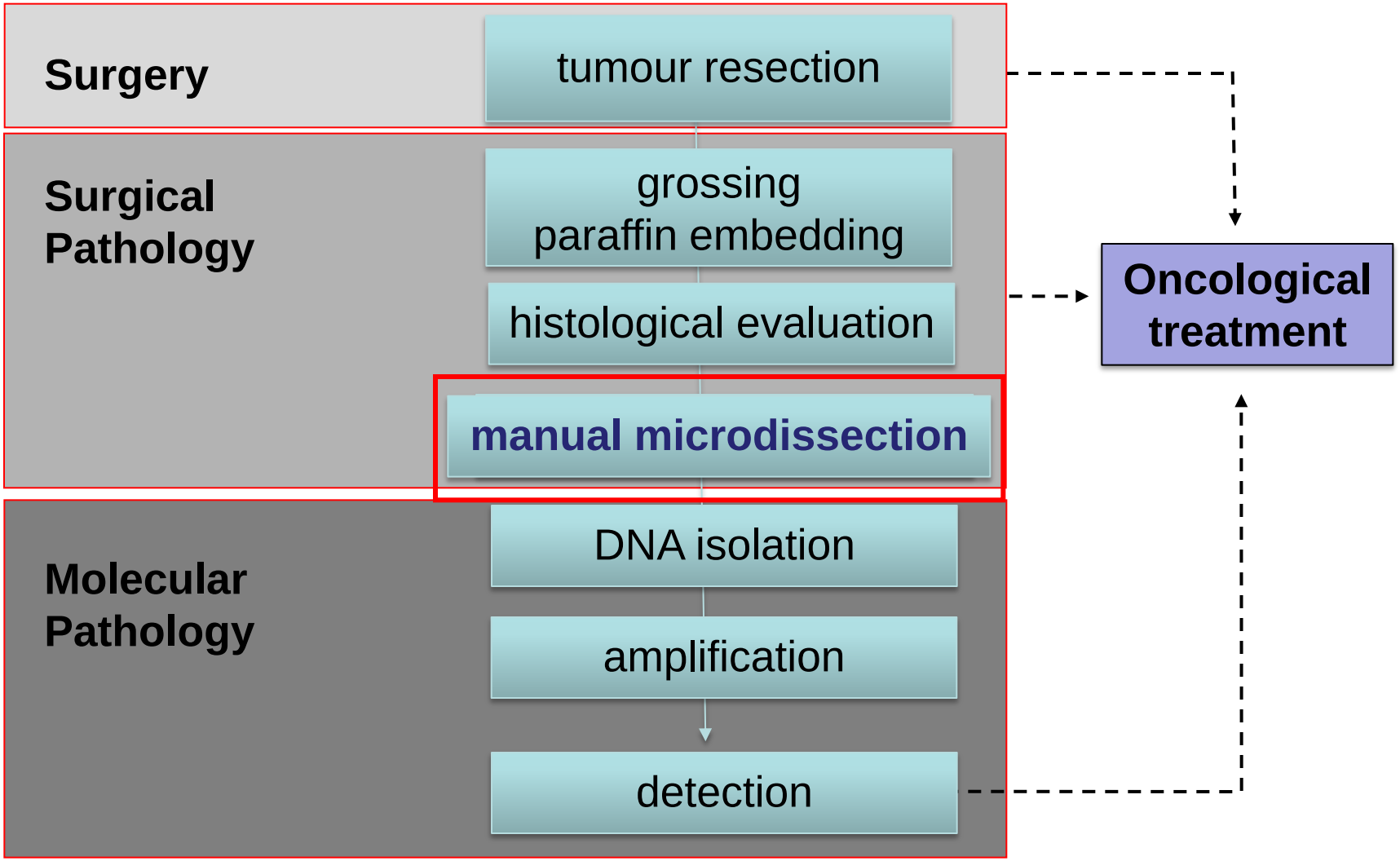


Finally, in addition to physician and health care provider education, public education will be key to empowering broad interest and participation in personalized genomic medicine by patients and their families.

Conde E, et al. Clin Transl Oncol 2013;15:503–8; AMP Whole Genome Analysis WG. J Mol Diagn 2011;13:249–51

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

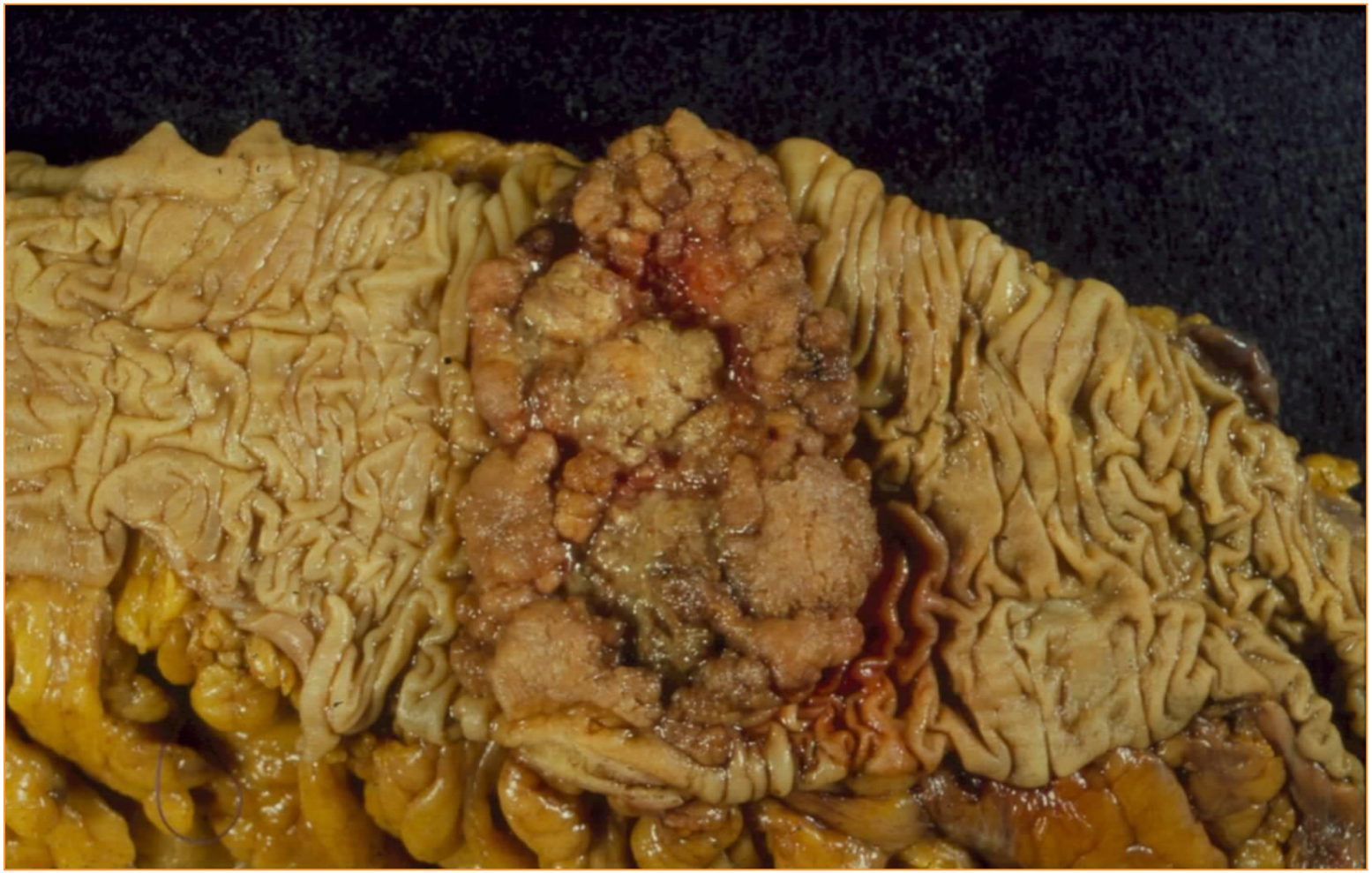
Recommendations for sample preparation and molecular analysis



Tumor Entities Important in Predictive Molecular Pathology

- Colon cancer
- NSCLC
- Malignant melanoma
- Breast cancer
- Upcoming challenges

Invasive colorectal cancer



Drug-Diagnostic-Co-Development Initiative

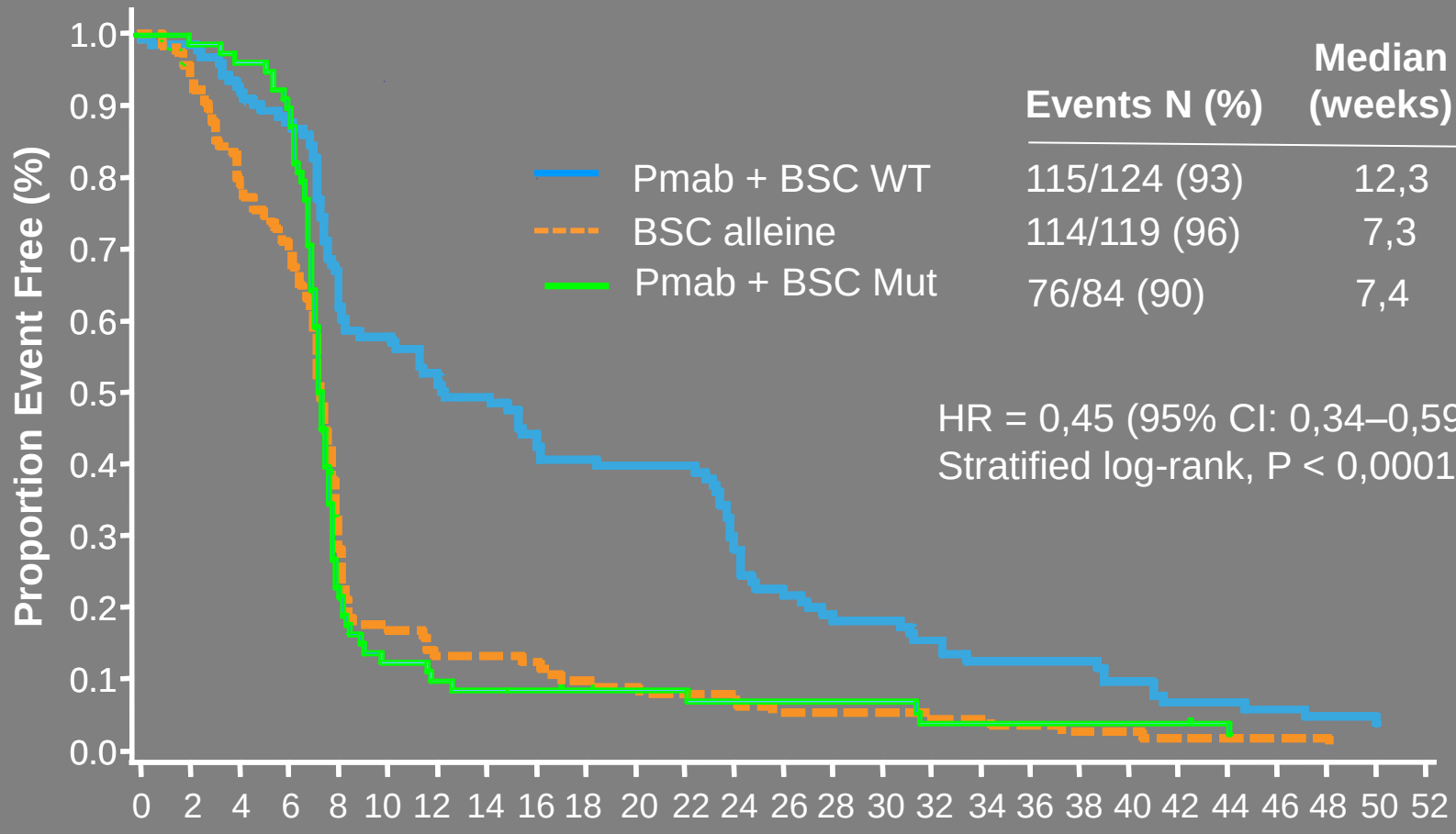
EMA/FDA asked for a pre-therapeutic eligibility test.

Example: therapeutic anti-EGFR antibodies,

e.g. **panitumumab**

cetuximab

Met. Colon-Ca: Wild-type/mut *KRAS*/BSC



	Events N (%)	Median (weeks)
Pmab + BSC WT	115/124 (93)	12,3
BSC alleine	114/119 (96)	7,3
Pmab + BSC Mut	76/84 (90)	7,4

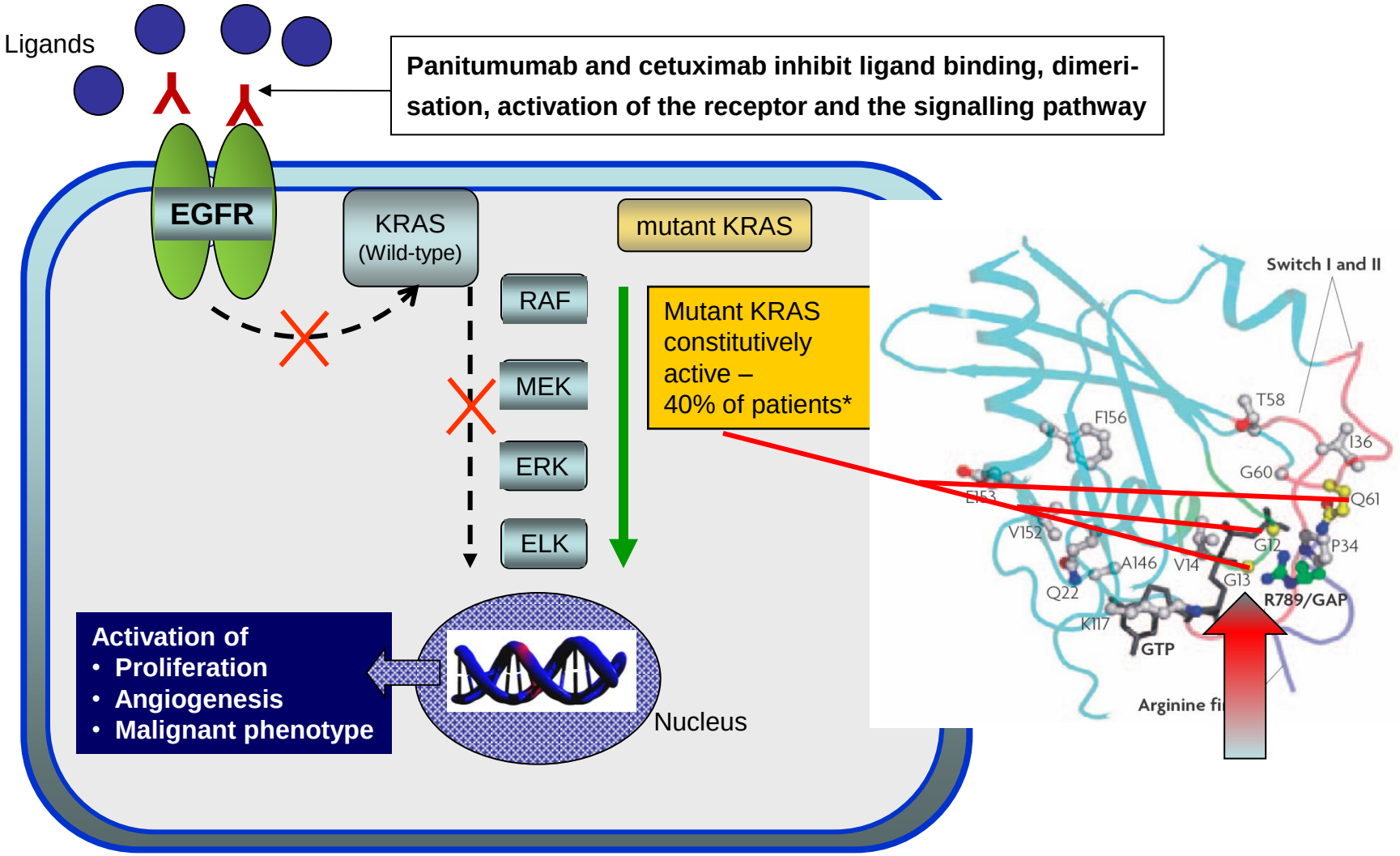
HR = 0,45 (95% CI: 0,34–0,59)
Stratified log-rank, P < 0,0001

Patients at Risk

Pmab + BSC	124	119	112	106	80	69	63	58	50	45	44	44	33	25	21	20	17	13	13	13	10	7	7	6	5	5
BSC alleine	119	109	91	81	38	20	15	15	14	11	10	9	9	6	6	6	6	5	4	3	3	2	2	2	2	1

Amado R, et al. *ESMO* 2007;a0007; *JCO*, 26 (2008 1626-1634

KRAS-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.

Relevance of different types of

(K)RAS and BRAF mutations

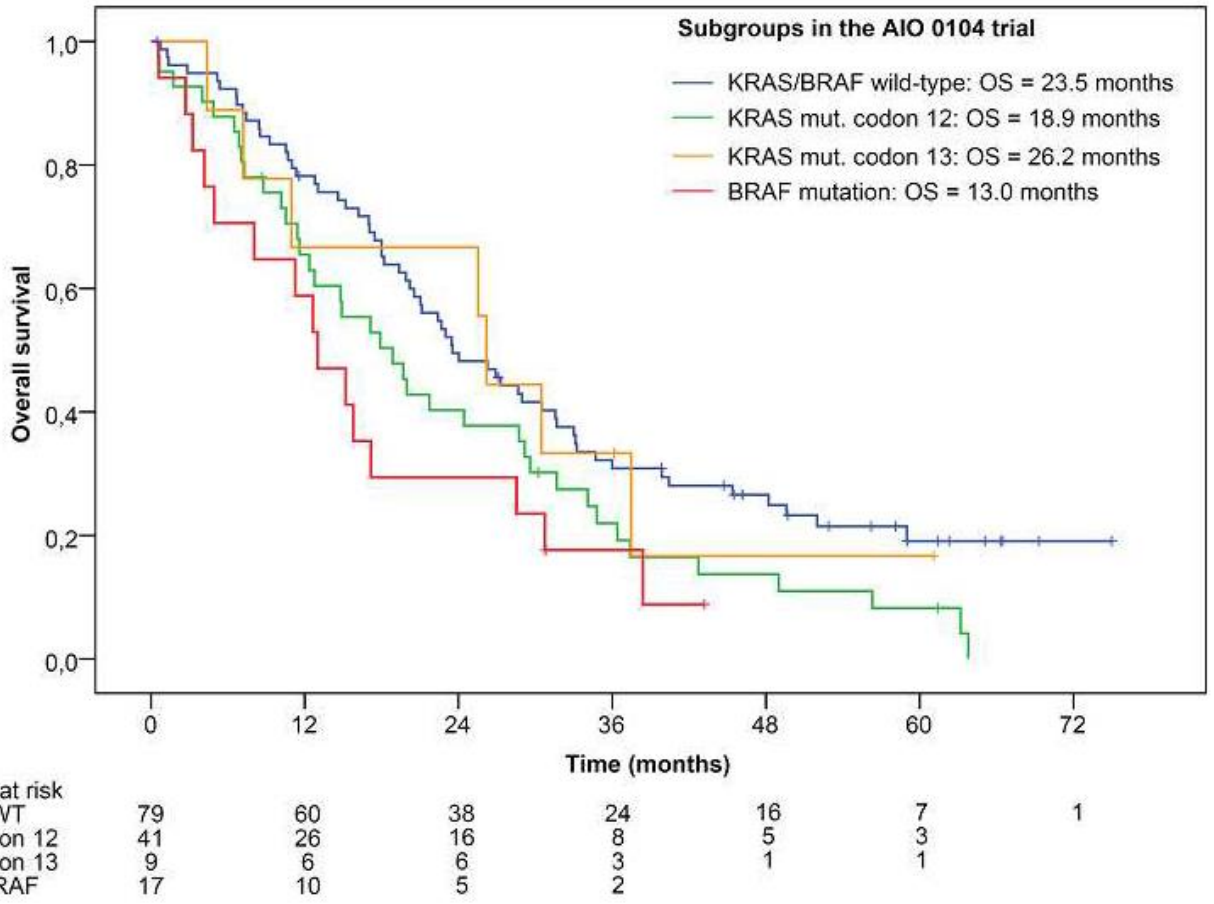
Treatment efficacy - according to mut status

Parameter	*KRAS/ BRAF* wild-type	*KRAS* mutation codon 12	*KRAS* mutation codon 13	*BRAF* mutation	p-values
All patients					
Number of patients	79 (54%)	41 (28%)	9 (6%)	17 (12%)	
ORR (%)-(95% CI)	59 (47-71)	47 (32-63)	66 (35-88)	57 (33-79)	0.61
DCR (%)-(95% CI)	92 (83-97)	97 (82-98)	100 (70-100)	79 (52-93)	0.22
Response not assessable	15	5	0	3	
Median PFS (ms)	8	5.8	9.9	4.2	0.058
95% CI	6.6-9.3	4.4-7.1	7.9-12.0	1.4-7.0	
Median OS (ms)	23.5	18.9	26.2	13.0	0.032
95% CI	17.7-29.4	12.6-25.1	24.3-28.1	7.7-18.3	

Table Legend: ORR: overall response rate; DCR: disease control rate; CI:confidence interval; PFS: progression-free survival; OS: overall survival. Percentages based on non-missing data. P-values ORR and DCR: chi-square-test, p-values PFS and OS: log rank.

Modest, DP et al.: Int J Cancer 2011, accepted preprint

Overall survival in the AIO KRK 0104 –trial

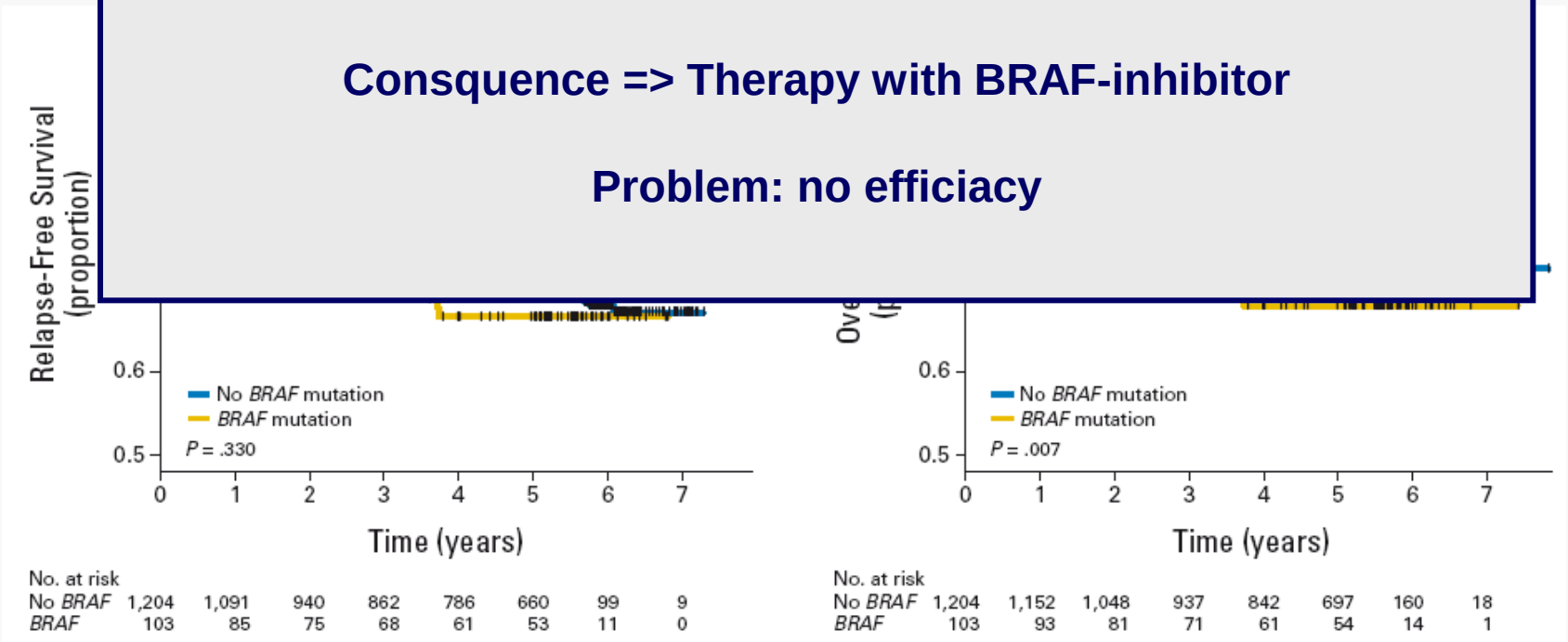


Prognostic role of BRAF in stage II and III resected colon cancer

Results of the translational study on the PETACC-3, EORTC 40 993,
Sakk 60-00 Trial, N= 1307; BRAF-mutated= 103 (7,9 %)

Consquence => Therapy with BRAF-inhibitor

Problem: no efficiacy



Roth A.D. et al. (2009) J Clin Oncol 28:466-474

LETTER

doi:10.1038/nature10868

Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR

Anirudh Pra[illegible]n[2],
Roderick L.

To investigate the potency of combinational targeted therapy and the companion diagnostic is the great challenge of the next couple of years.

We repo[illegible]nergy with BR[illegible]t inhibitio[illegible]r erlotinib is strong[illegible]ally, we find tha[illegible]n supports[illegible]lls express low levels of EGFR and are therefore not subject to this feedback activation. Consistent with this, we find that ectopic expression of EGFR in melanoma cells is sufficient to cause resistance to PLX4032. Our data suggest that BRAF(V600E) mutant colon cancers (approximately 8–10% of all colon cancers2,3,5), for which there are currently no targeted treatment options available, might benefit from **combination therapy consisting of BRAF and EGFR inhibitors**.

Treatment efficacy - according to mut status

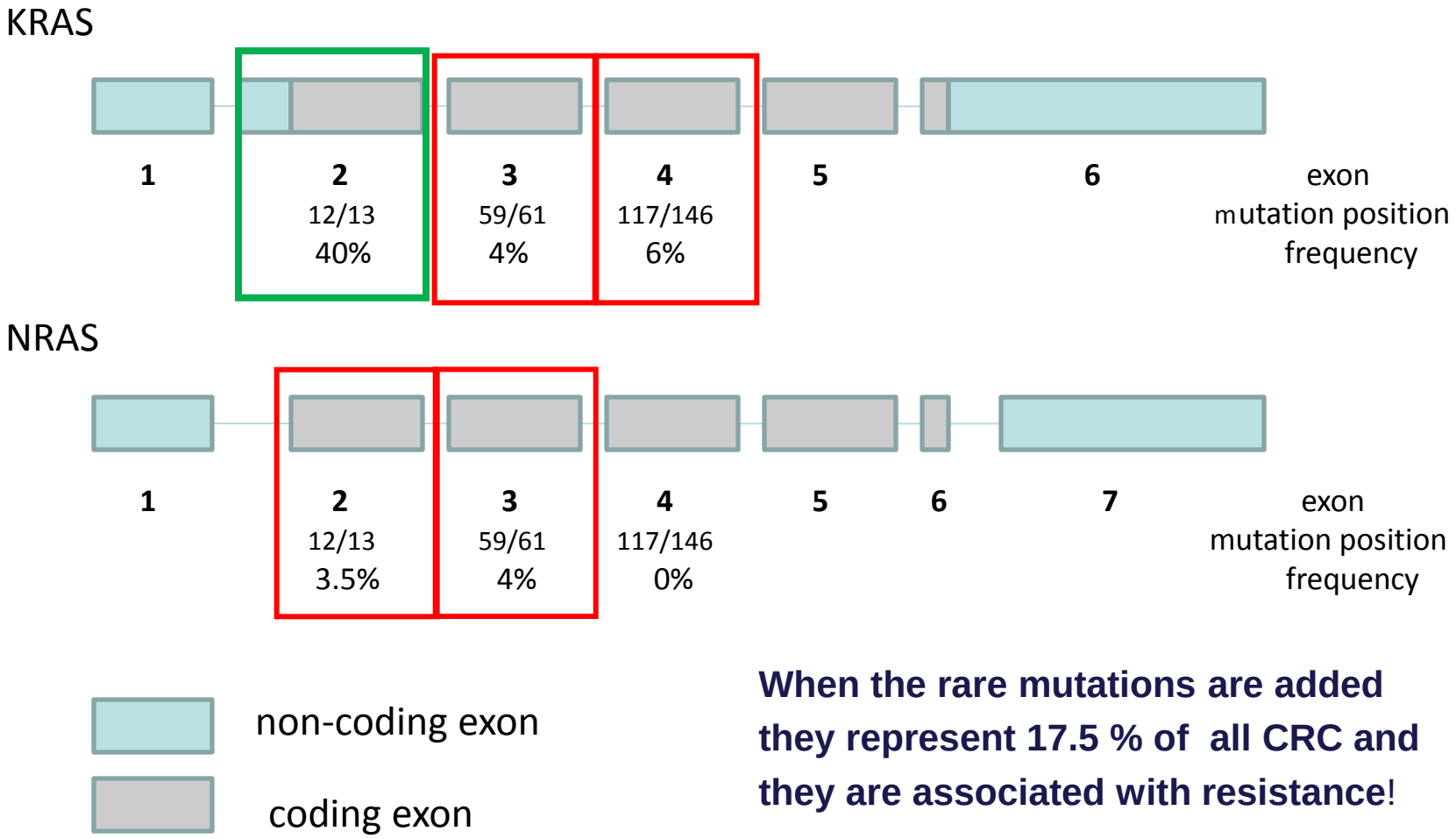
In CRC ca. 55% are KRAS wild type.

Out of these only ca. 50% (i.e. 25% of all CRC) respond to EGFR antibodies.

Why?

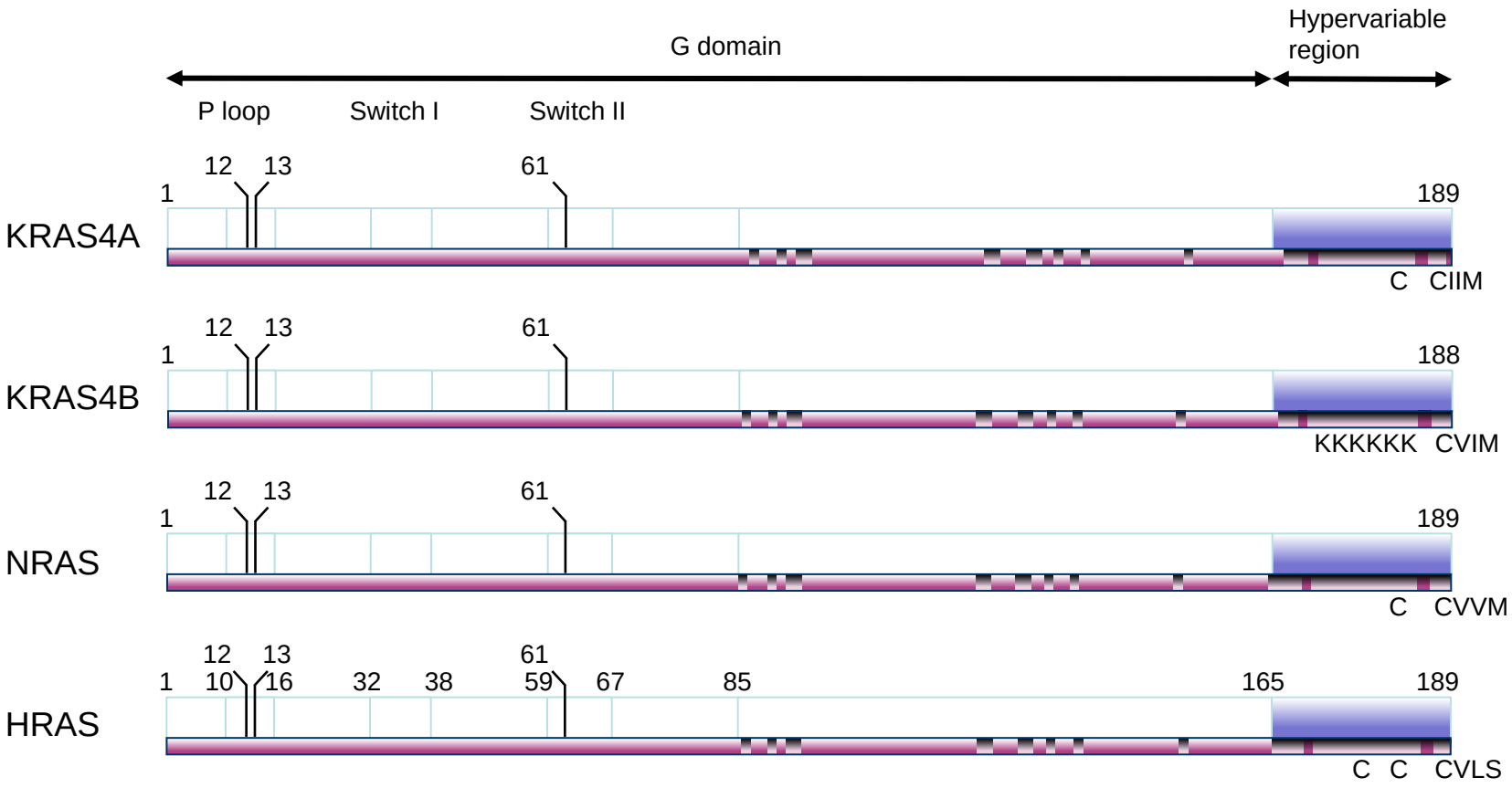
How can they be detected or stratified?

Mutations in KRAS and NRAS genes in colorectal cancer



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

Three Cellular *RAS* Genes Encode Four Highly Homologous 21 kD Proteins

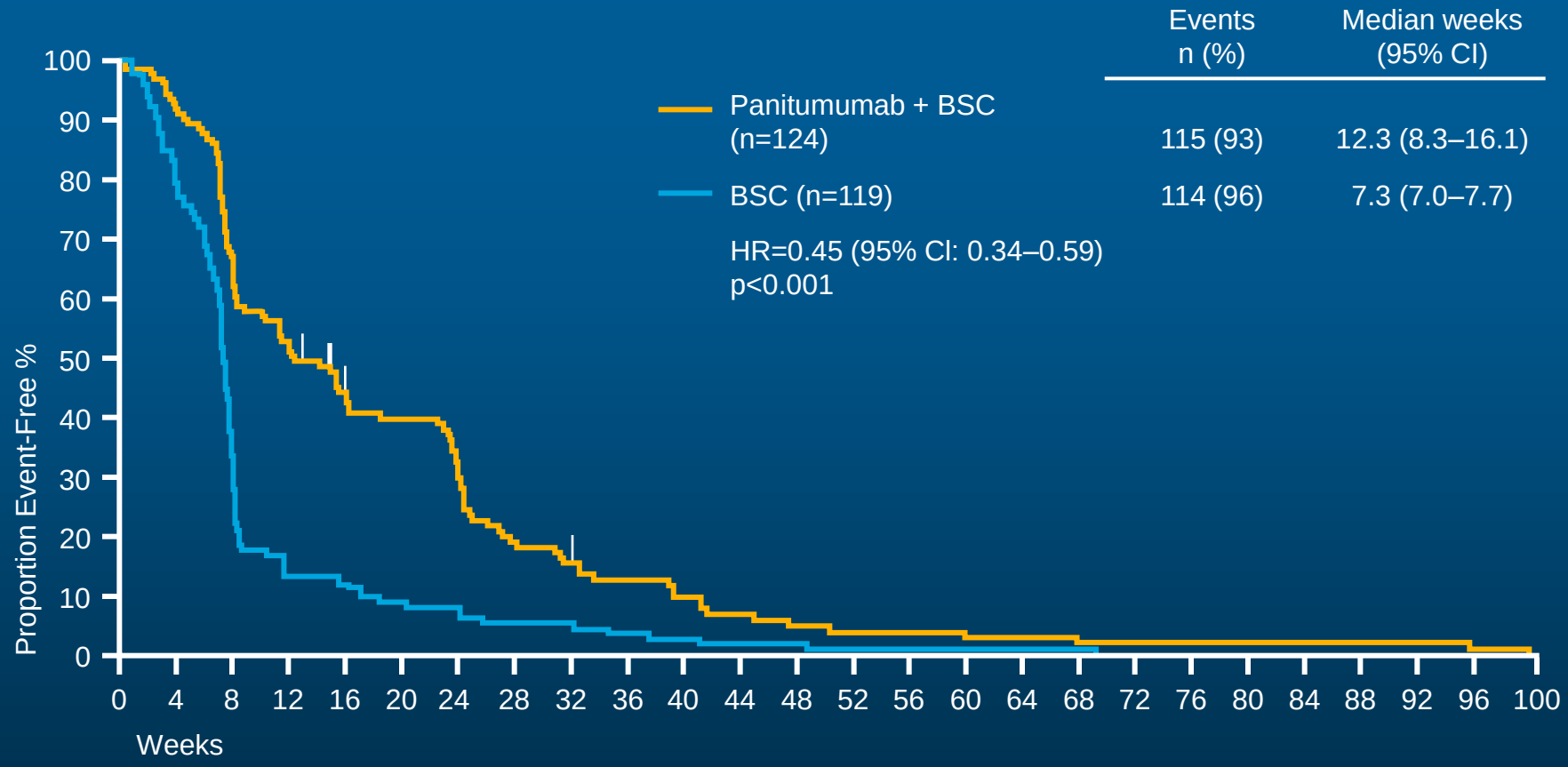


Adapted from Schubbert S, et al. Nat Rev Cancer 2007; 7:295-308.

Boxes at the bottom of each isoform representation show the conserved residues in magenta and the variable residues in pink.

20020408 Trial *RAS* (Exon 4) Analysis

PFS in Patients with WT *KRAS* Exon 2 mCRC

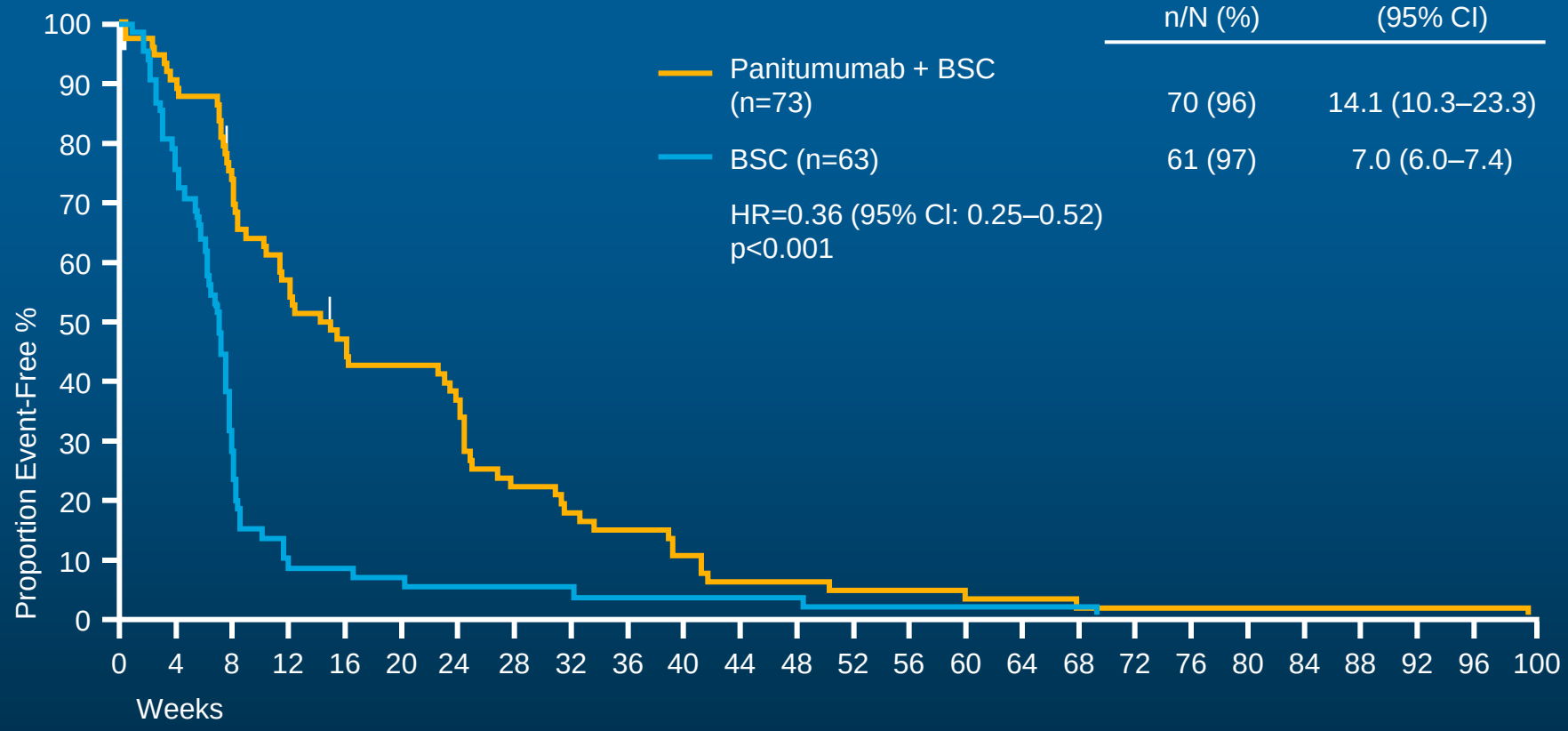


	Events n (%)	Median weeks (95% CI)
Panitumumab + BSC (n=124)	115 (93)	12.3 (8.3–16.1)
BSC (n=119)	114 (96)	7.3 (7.0–7.7)

Patterson SD, et al. J Clin Oncol 31, 2013 (suppl; abstr 3617); 1. Amado RG, et al. J Clin Oncol 2008; 26:1626-34.

20020408 Trial *RAS* (Exon 4) Analysis

PFS in Patients with WT *RAS** mCRC



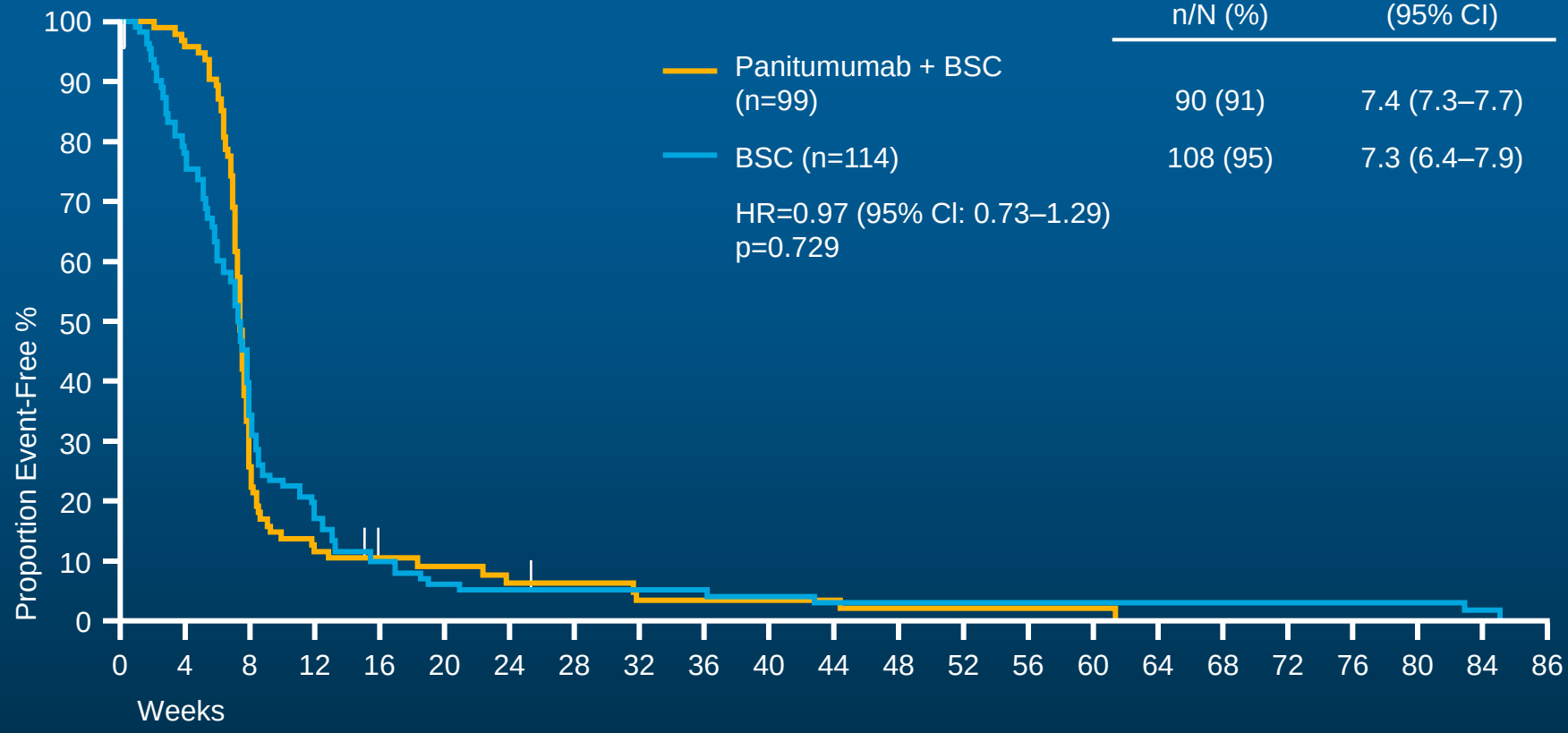
	Events n/N (%)	Median weeks (95% CI)
Panitumumab + BSC (n=73)	70 (96)	14.1 (10.3–23.3)
BSC (n=63)	61 (97)	7.0 (6.0–7.4)

*WT KRAS and NRAS exons 2, 3, and 4

Patterson SD, et al. J Clin Oncol 31, 2013 (suppl; abstr 3617).

20020408 Trial *RAS* (Exon 4) Analysis

PFS in Patients with MT *RAS** Exon mCRC

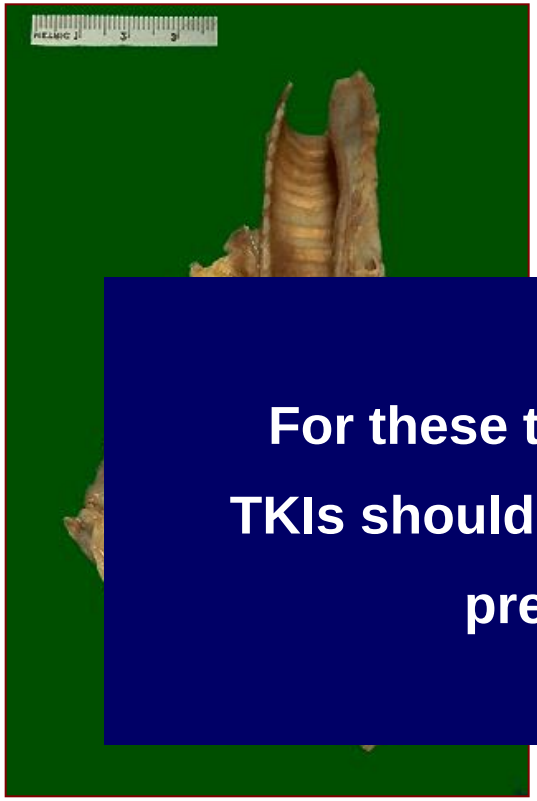


	Events n/N (%)	Median weeks (95% CI)
Panitumumab + BSC (n=99)	90 (91)	7.4 (7.3–7.7)
BSC (n=114)	108 (95)	7.3 (6.4–7.9)

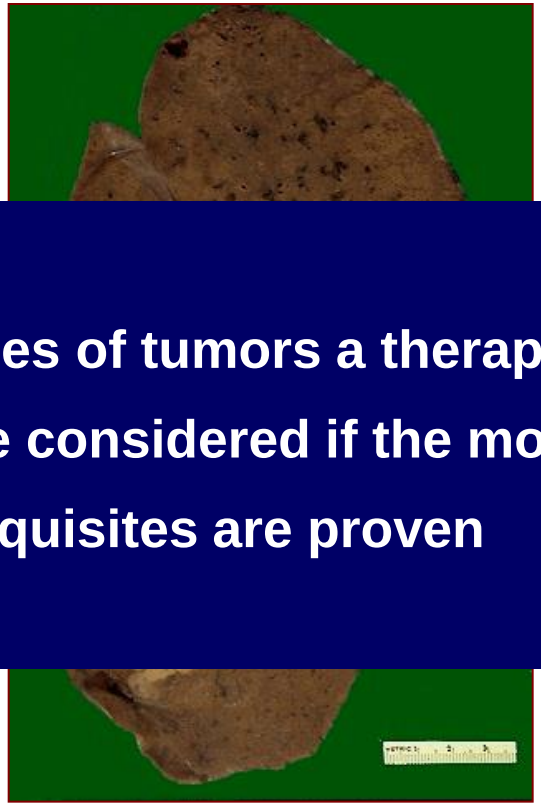
*MT in any KRAS and NRAS exons 2, 3, and 4

Patterson SD, et al. J Clin Oncol 31, 2013 (suppl; abstr 3617).

NSCLC - Macroscopy

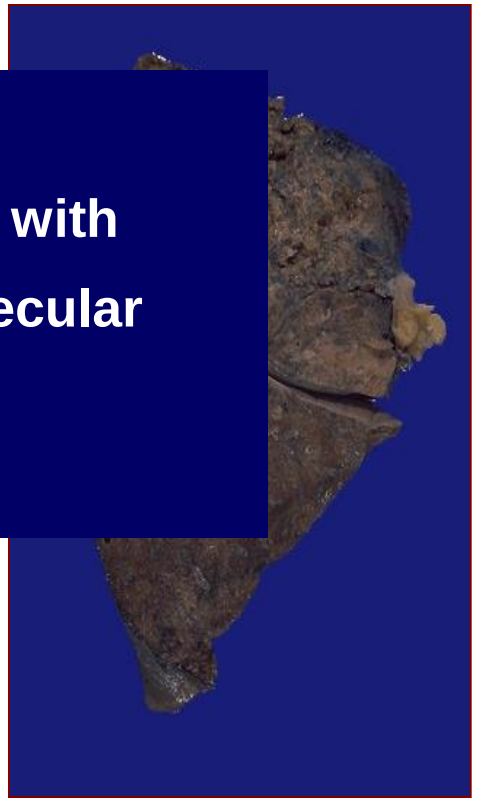


central
squamous cell carcinoma



peripheral
adenocarcinoma

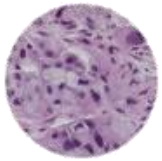
adeno carcinoma
broncho-alveolar type



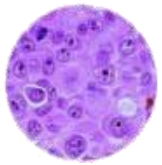
For these types of tumors a therapy with TKIs should be considered if the molecular prerequisites are proven

NSCLC: Past and Current Landscape

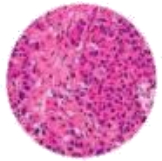
1999
Histology-driven selection[1]



Adenocarcinoma

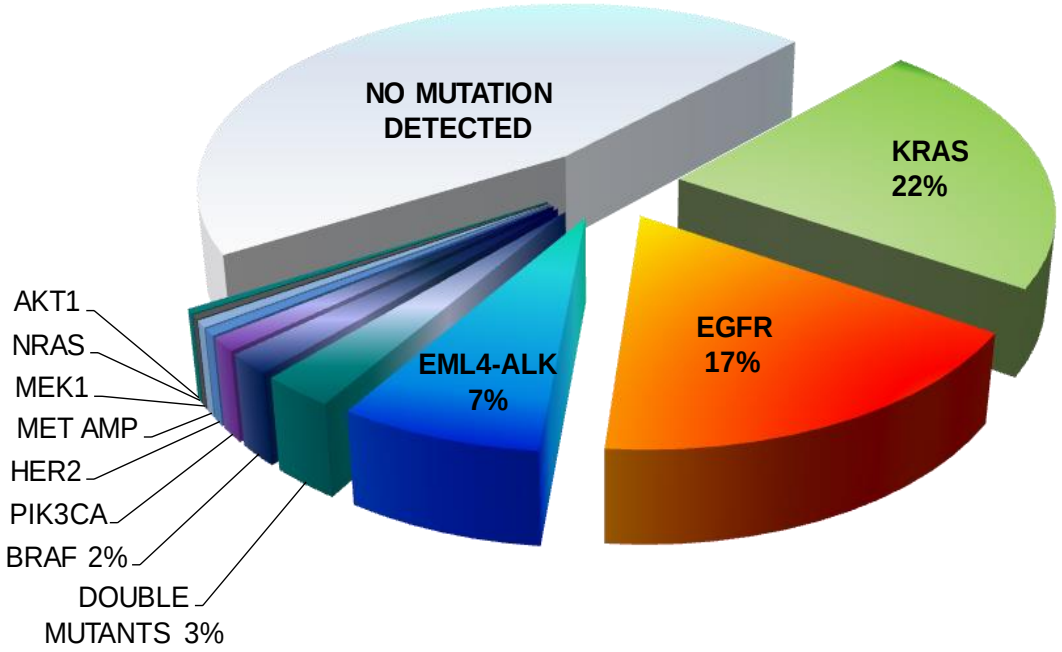


Squamous-cell carcinoma



Large cell carcinoma

2012
Targeting oncogenic drivers

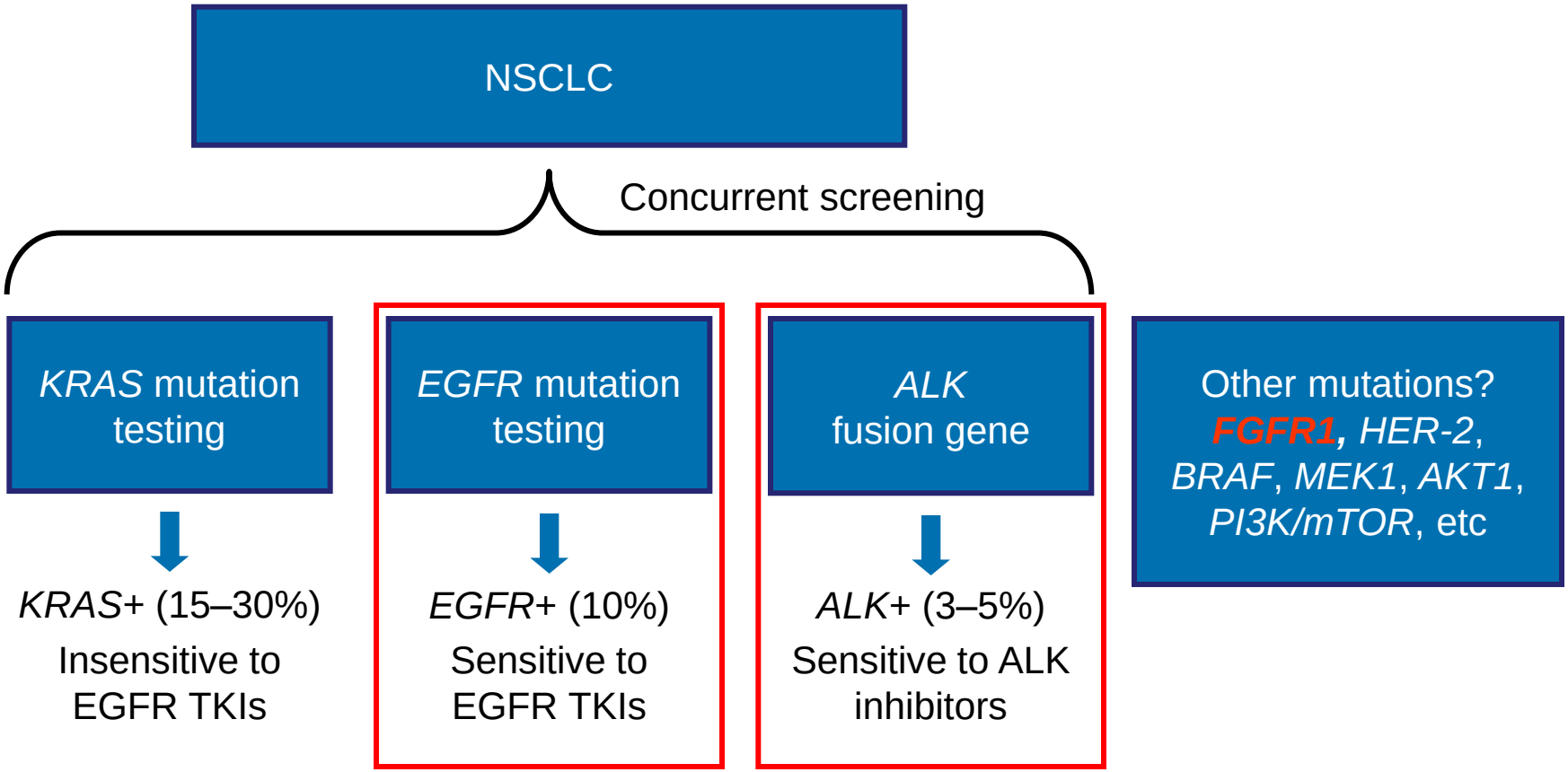


Actionable driver mutations identified in 54% of lung adenocarcinoma tumours

LCMC, Lung Cancer Mutation Consortium

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

Molecular Screening in NSCLC



MEK1, mitogen activated protein kinase kinase 1;
AKT1, v-akt murine thymoma viral oncogene homolog 1; mTOR, mammalian target of rapamycin;
TKI, tyrosine kinase inhibitor.

Modified from Horn L, Pao W. *J Clin Oncol.* 2009;26:4232–35.
Shaw AT, et al. *J Clin Oncol.* 2009;27:4247–53

Targeted Therapy in NSCLC

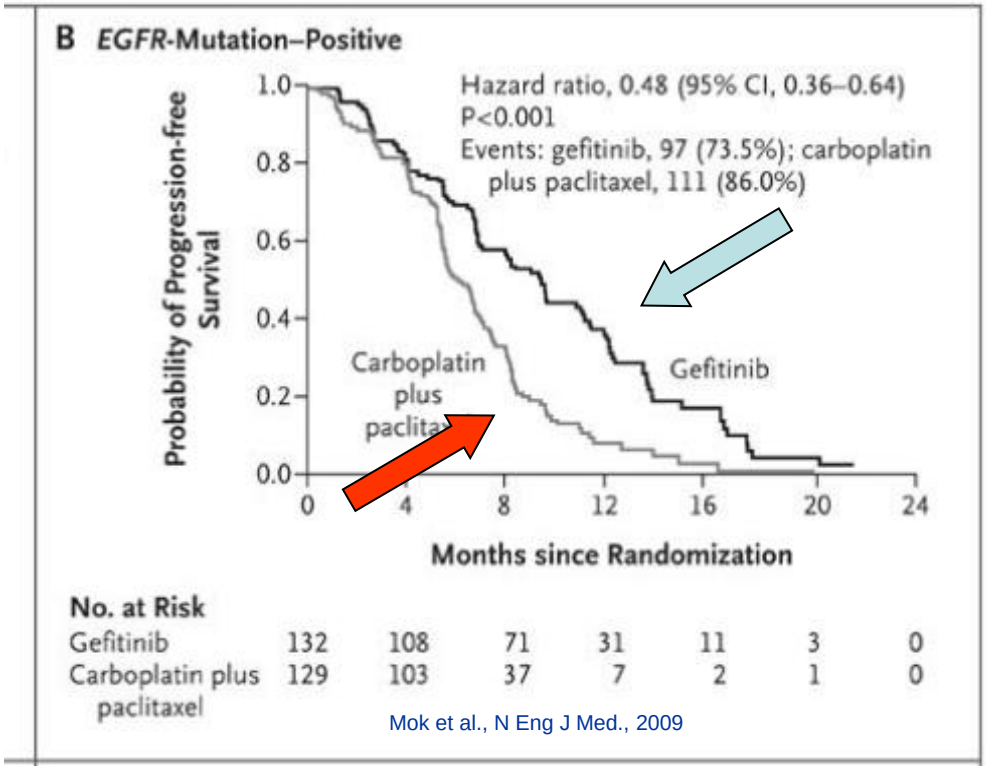
EMA/FDA: kinase inhibitors can be approved only in combination with a diagnostic eligibility test.

Example:

- therapeutic anti-EGFR
- kinase inhibitors

Gefitinib

(Iressa, Astra Zeneca)

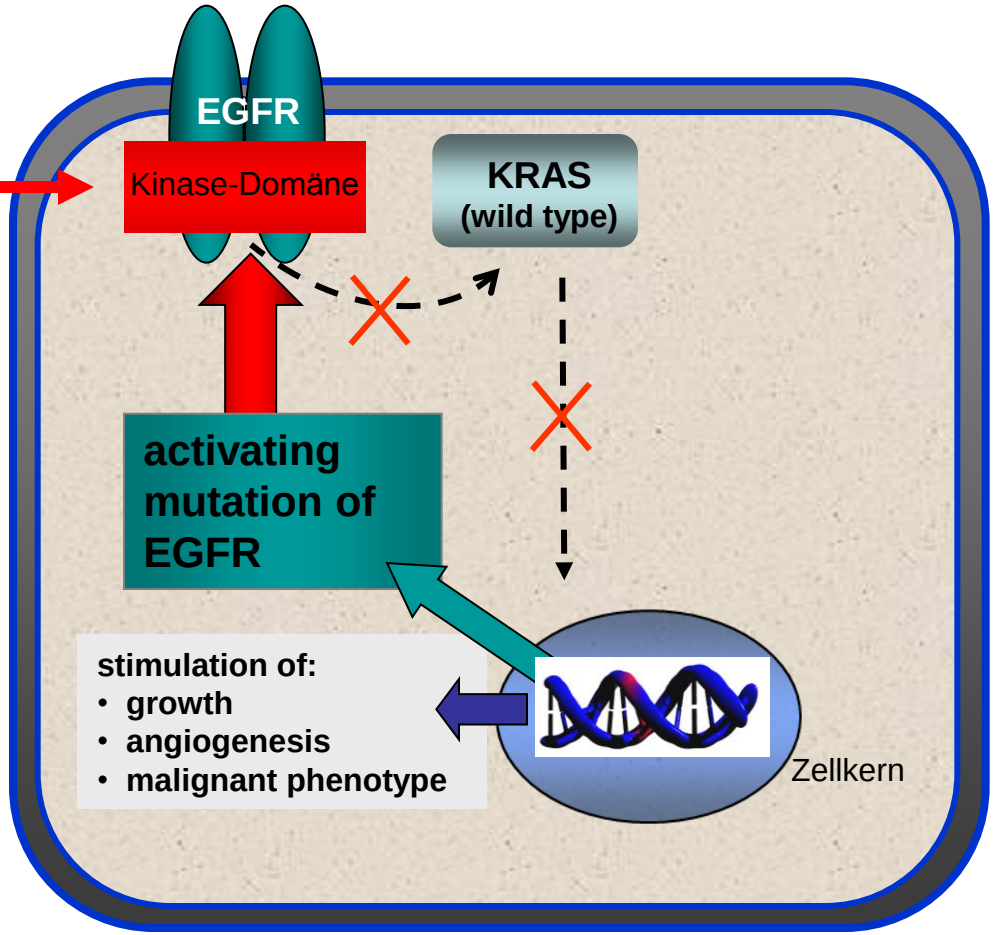


Mok et al., N Eng J Med., 2009

EGFR-mutations and EGFR tyrosine-kinase-inhibitors

EGFR-mutations
- **NSCLC (10-15%)**

tyrosine-kinase-inhibitors interfere with activated receptors and the corresponding pathway



Summary

577 cases* included,

174 cases no sufficient PCR product for exon 20

35 cases have less than 30% tumor.

=> 368 specimen sequenced.

Based on the experience of >3000 cases it is strongly recommended to test all 4 exons

Pro				
56				
Exon				
Exon 21	14 cases	3,8	**25,0**	40-45
Exon 18	6 cases	1,6	**10,7**	5
Exon 20	4 cases	1,1	**7,2**	<1

The results correspond with those from the other institutes of the German panel institutes of the German Soc. of Path.

*Sharma SV et al. Nature Reviews Cancer 2007; 7: 169-181

Targeted Therapy in NSCLC

EMA (FDA): Therapeutic kinase inhibitors have been approved only in combination with a diagnostic eligibility test.

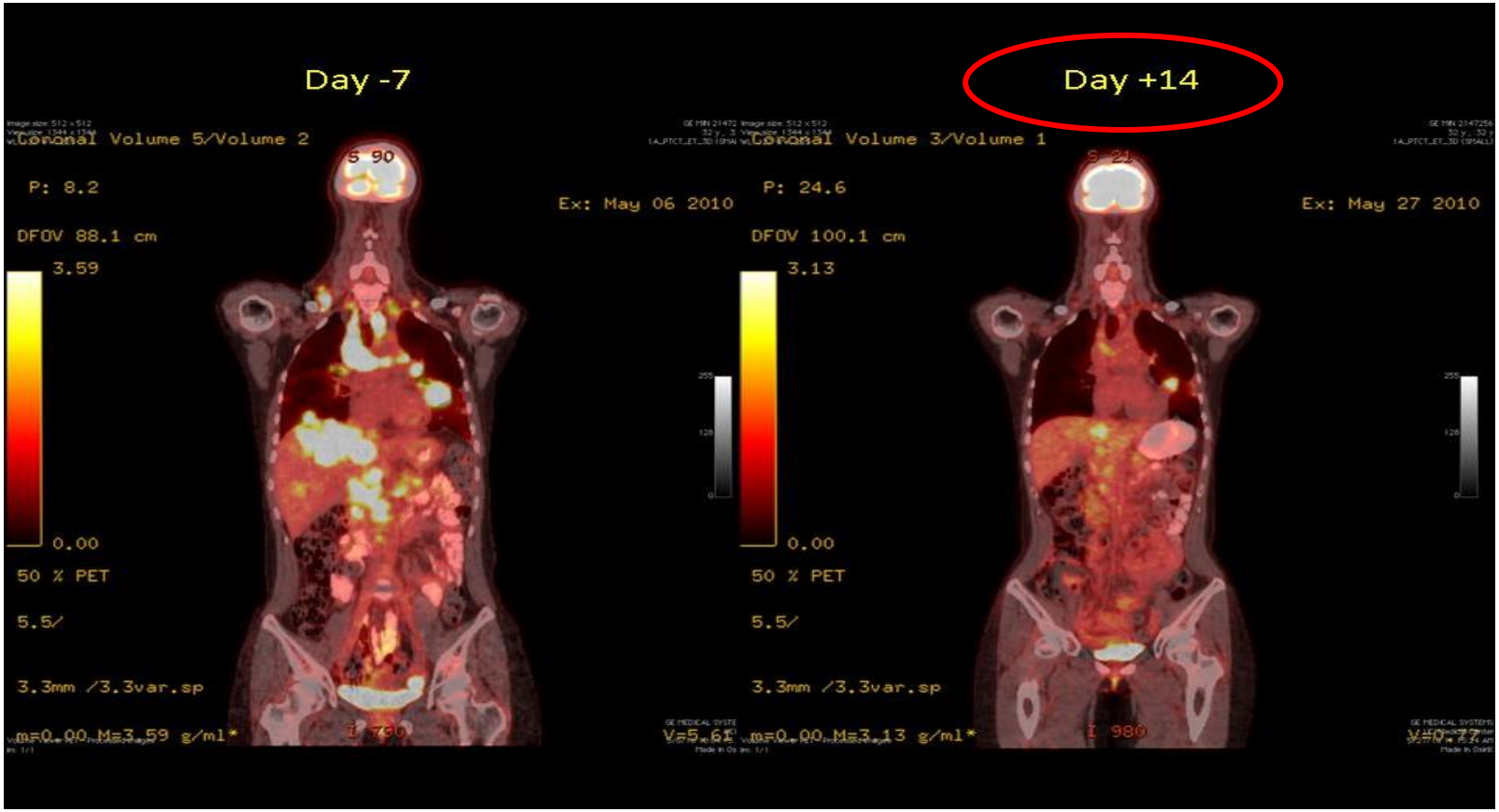
Examples:

Xalkori – mutEML4-Alk (Crizotinib, Pfizer)

Gefitinib – mutEGFR (Iressa, Astra Zeneca)

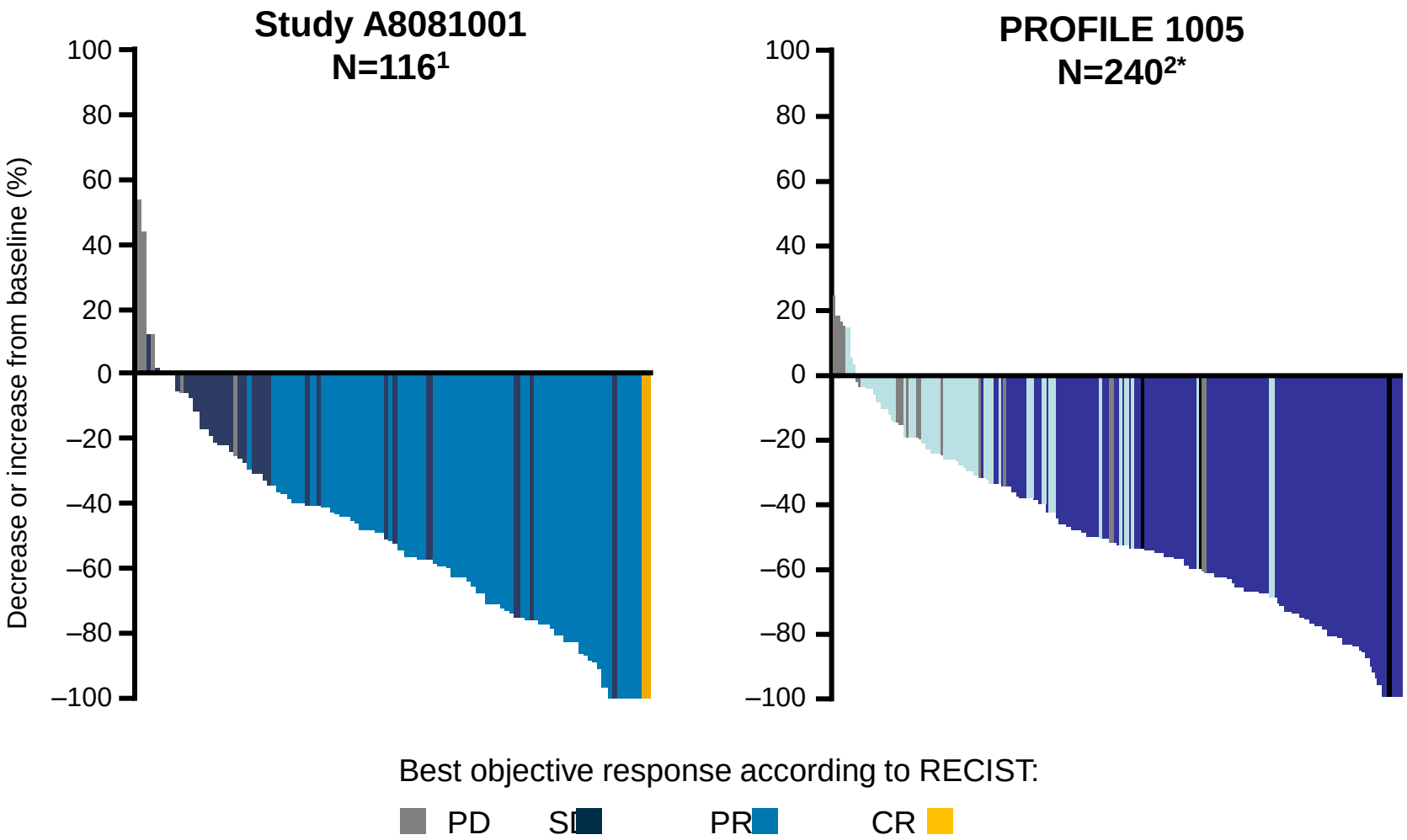
Erlotinib – mutEGFR (Tarceva, Roche)

Rapid Responses Seen In Some Patients



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

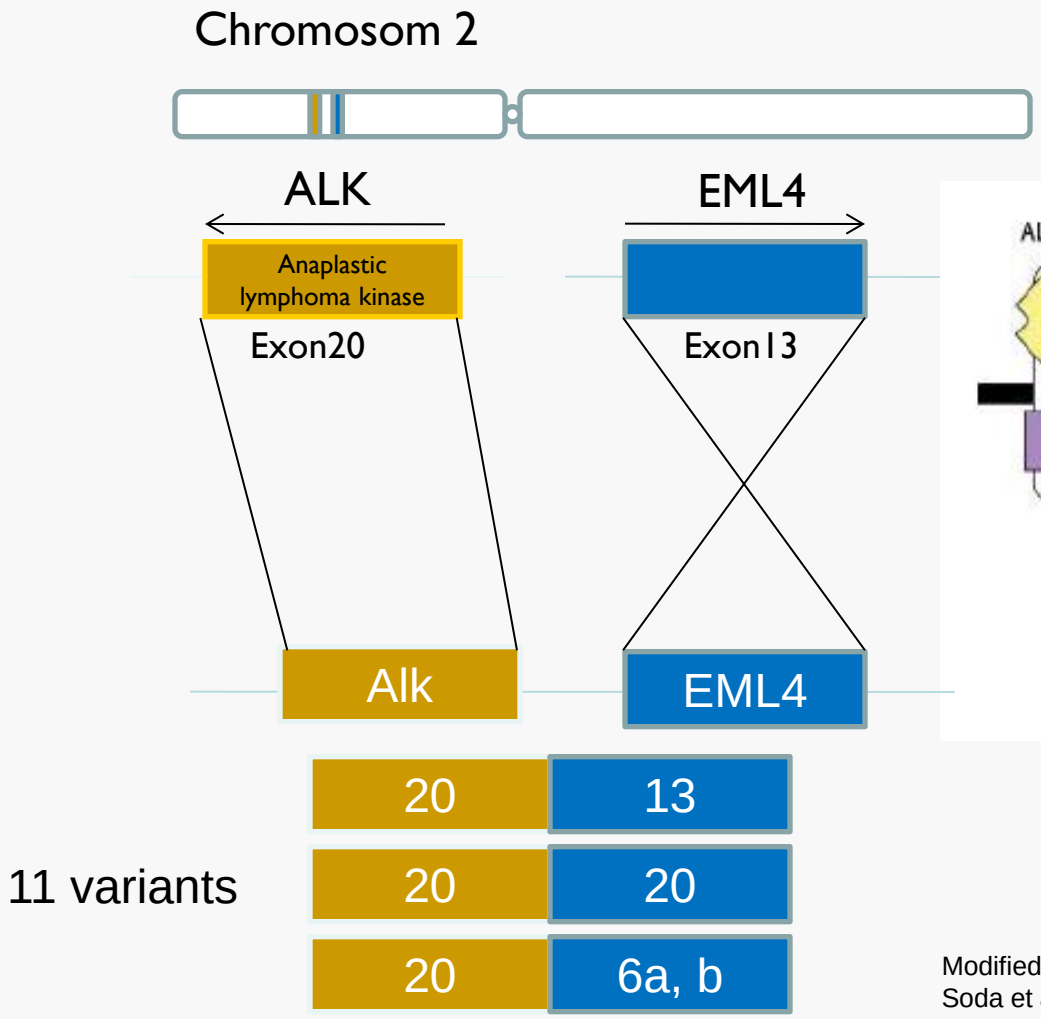
Tumour responses to crizotinib by patient



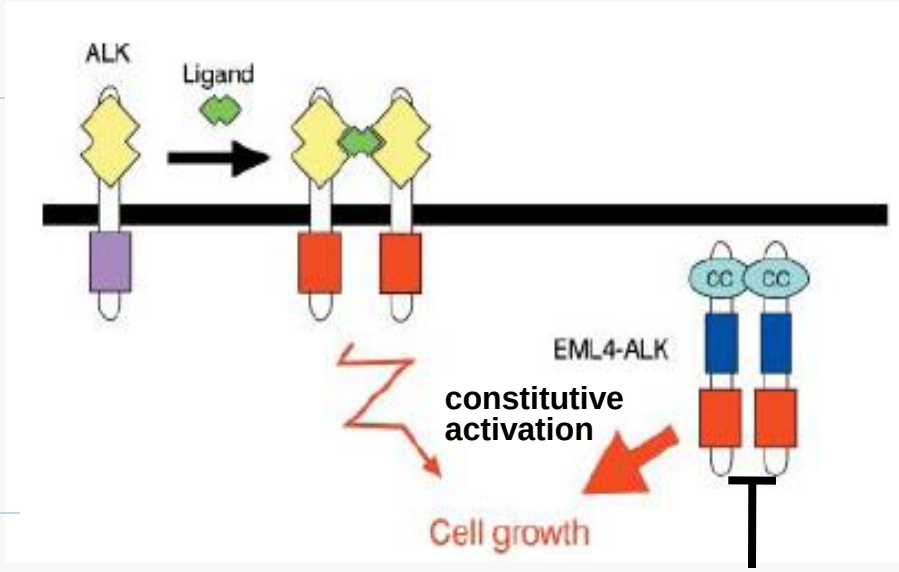
1. Camidge DR, et al. Lancet Oncol 2012;10:1011−9;
2. Kim DW, et al. Presented at ASCO 2012; Abstract 7533

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

EML4-ALK Fusion in NSCLC



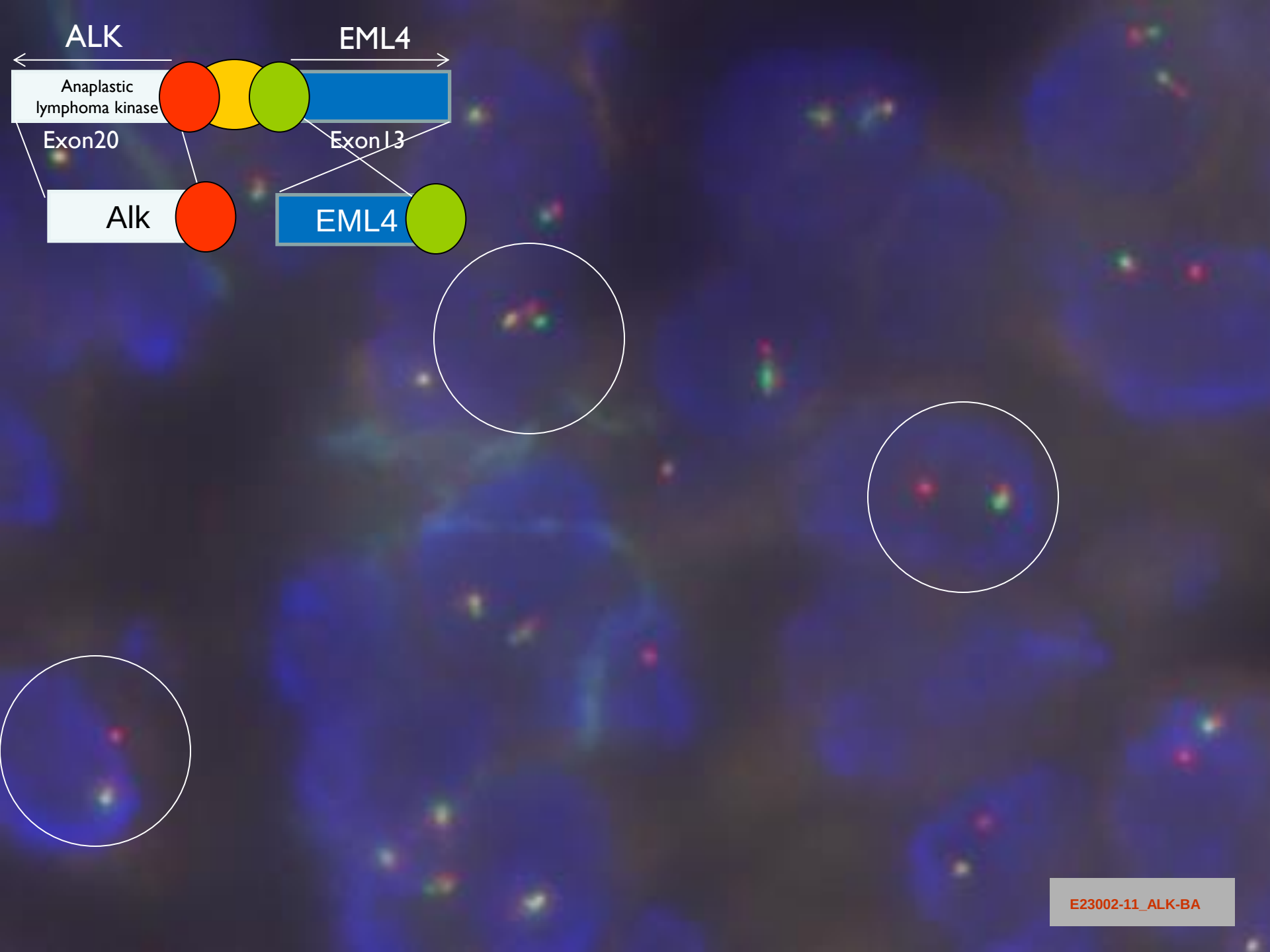
11 variants



PF02341066

EML4-Alk
EGFRwt
KRASwt

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

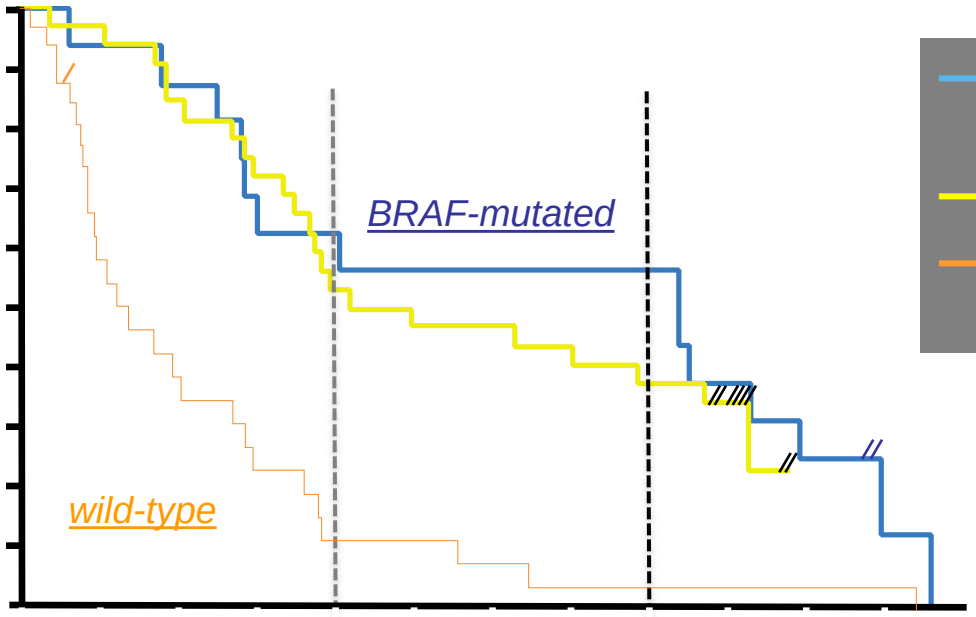

ALK
EML4
Anaplastic
lymphoma kinase
Exon20
Exon13
Alk
EML4
E23002-11_ALK-BA

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 phase I overall survival: Updated KM estimates (08. 2011)



- V600E dose escalation (n=16)
- Extension (n=32)
- WT or sub-therapeutic exposure (n=33)

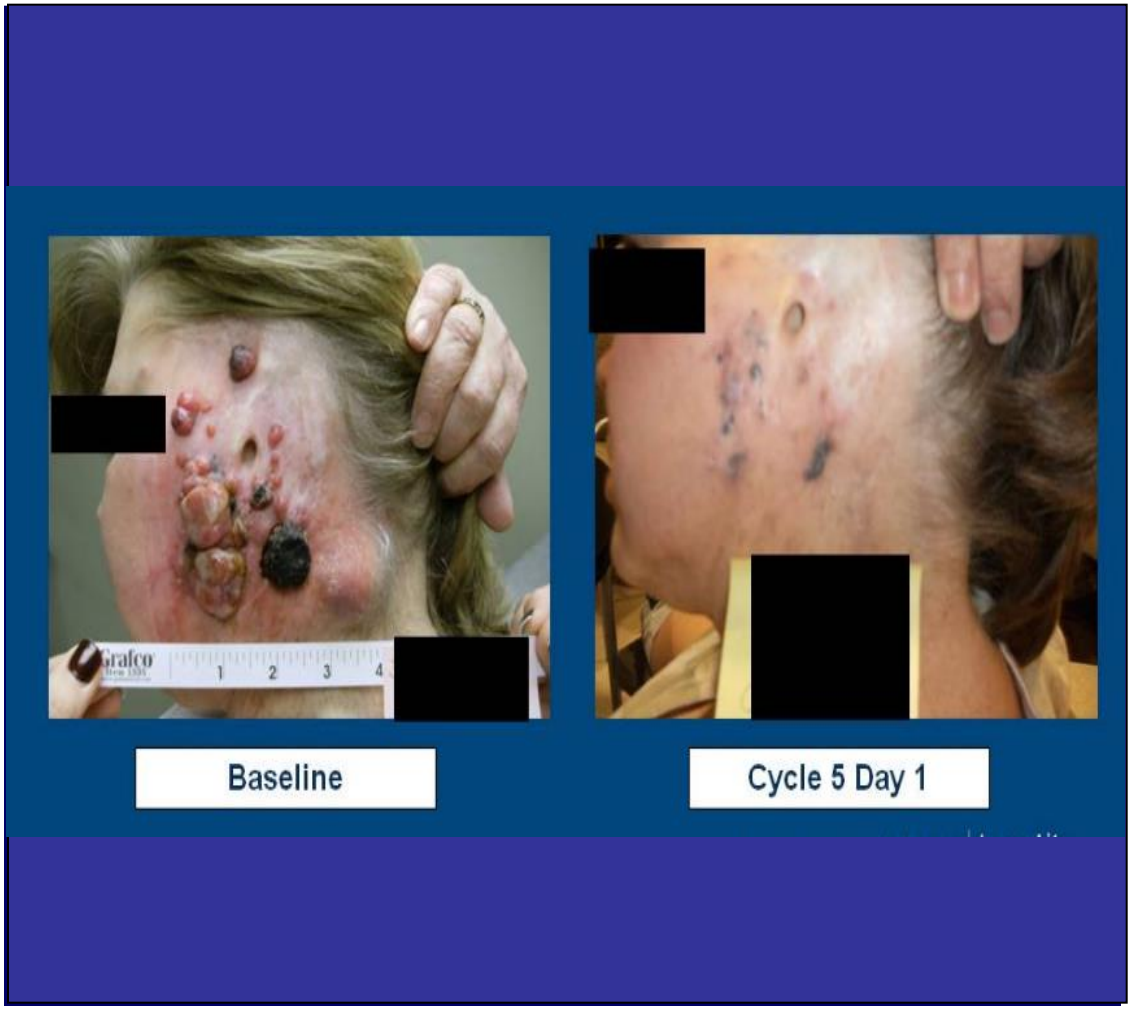
	Median OS (month)
Dose escalation	25.2
Extension	13.8
WT or sub-therapeutic.	4.18

Extension cohort landmark
Estimated survival: 1 year = 50%, 2 years = 38%

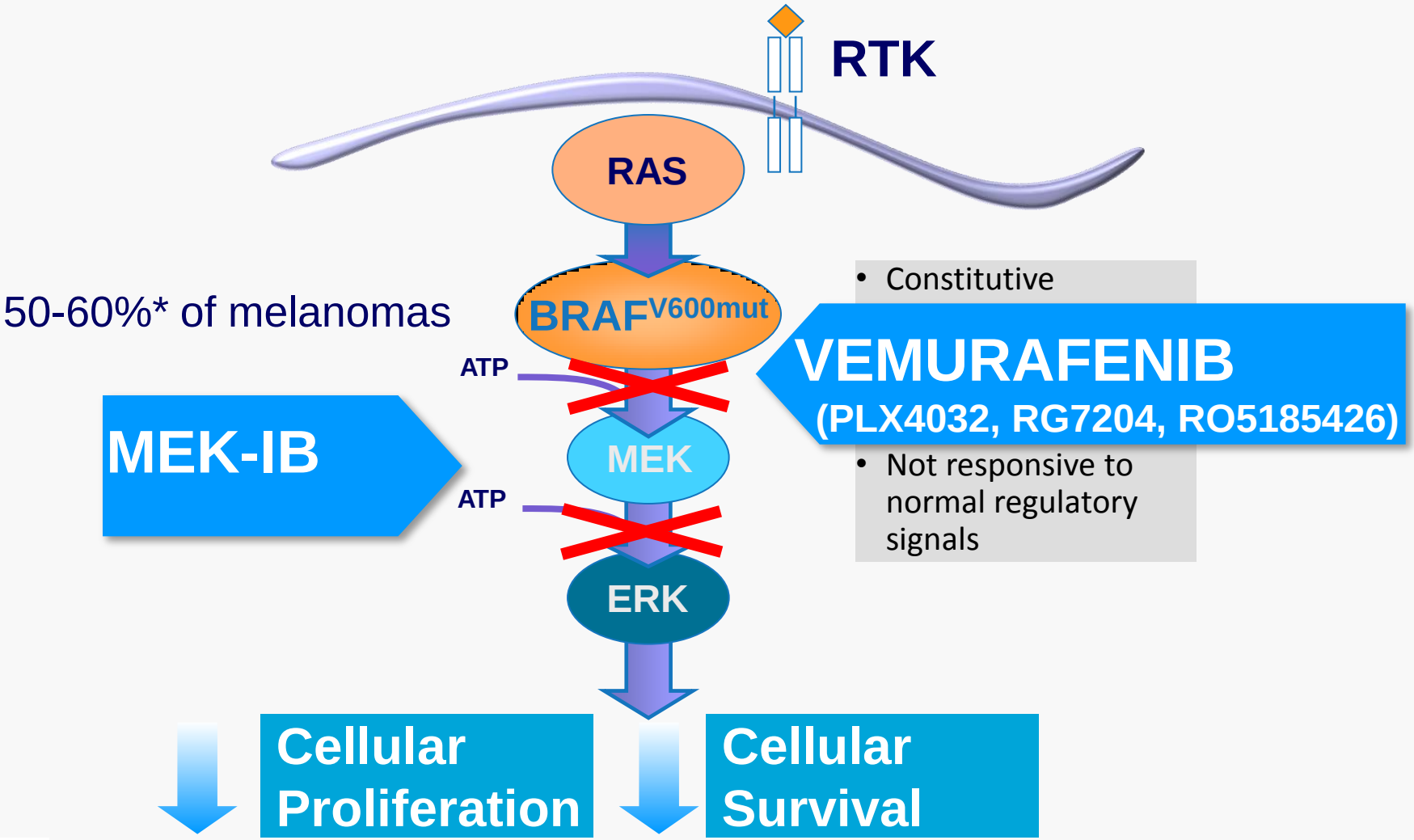
Vemurafenib inhibits V600 mutated BRAF kinase

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



*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

Rationale for Combination of BRAFi (GSK436) + MEKi (GSK212) in BRAF Mutant Tumors

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.

Goals of Combination

1. **Synergy in combination**
2. **Prevent/overcome potential monotherapy resistance**
3. **Potentially decrease incidence of BRAFi-induced hyper-proliferative skin lesions**

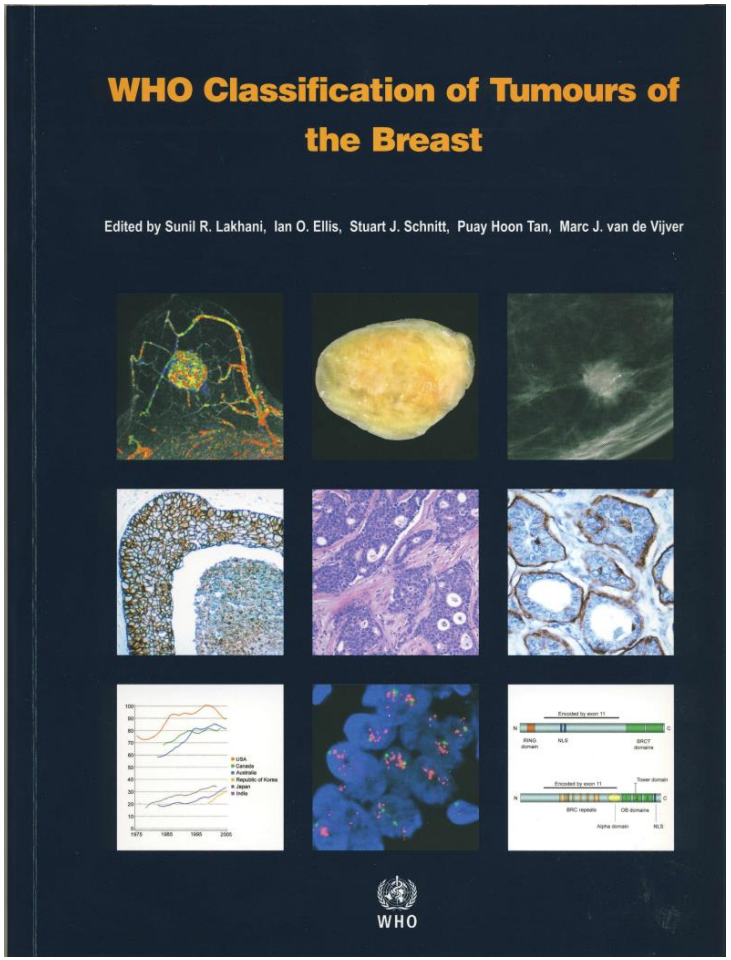
[1] Data presented at ASCO 2010

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?“

Gene expression profile-defined signatures (multigene assays) predicting clinical outcome

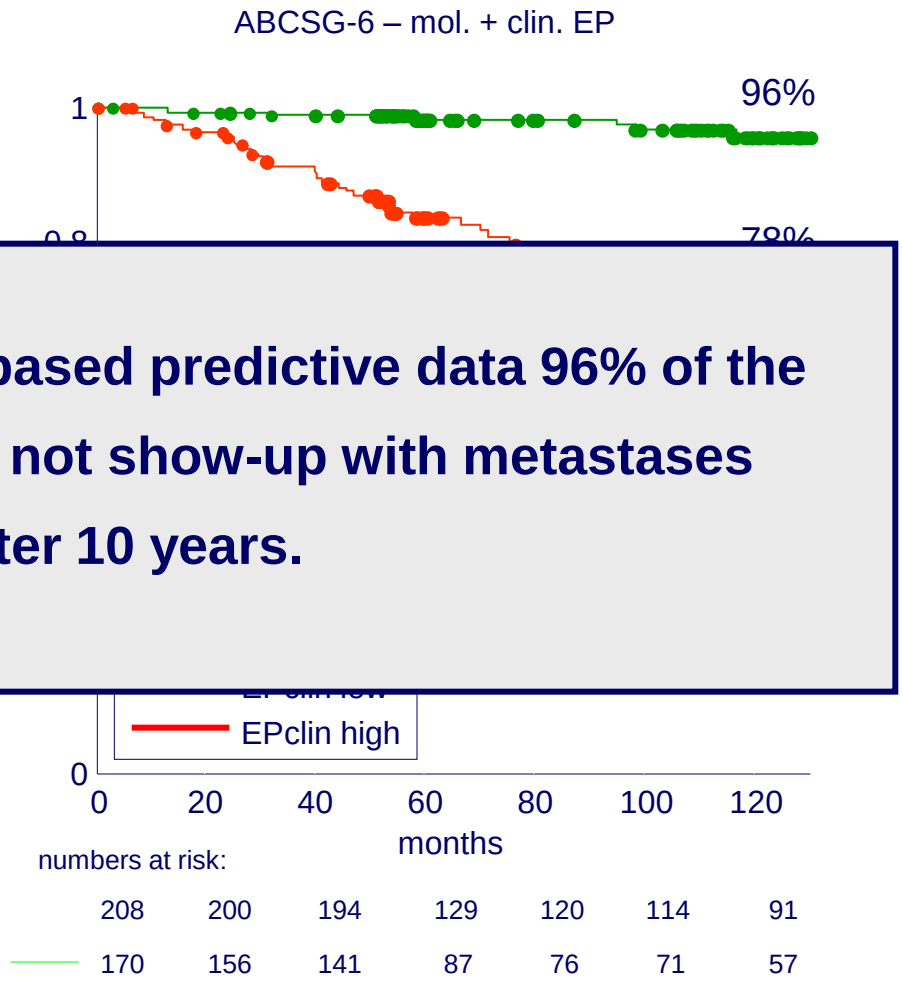


The supervised approach of classifying tumors has identified prognostic signatures.

Probably the most promising and clinically useful area for the application of genetic analysis is the prediction of response to treatment, including chemotherapy, hormonal therapy, and radiation.

The prognostic/predictive gene-expression profiles can be used in clinical practice.

EPclin: Validation



Martin
Raimu

A new
ER-pos
indepe
clinica

Clin Ca

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

Stratification by EndoPredict$_{clin}$ ®

1702 Patientinnen in ABCSG 6 & 8

nach S3-Leitlinien

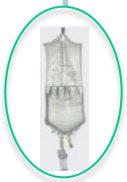
*248 Pat. „low risk“**

*1.371 Pat. „intermed. risk“**

*83 Pat. „high risk“**

5,3 % Metastasen

***840** EPclin „low risk“*



***531** EPclin „high risk“*

44,5 % Metastasen

w/o Endopredict these patients may have recieved CTx

4,5 % Metastasen

19,7 % Metastasen

**nach S3-Leitlinien*

MEDICAL UNIVERSITY OF VIENNA

ABCSG
AUSTRIAN BREAST & COLORECTAL CANCER STUDY GROUP

The EndoPredict Score identifies late distant metastases in ER+/HER2- breast cancer patients

Peter Dubsky, Jan C. Brase, Karin Fisch, Raimund Jakesz, Christian F. Singer, Richard Greil, Otto Dietze, Karsten E. Weber, Christoph Petry, Ralf Kronenwett, Margaretha Rudas, Michael Knauer, Michael Gnant and Martin Filipits

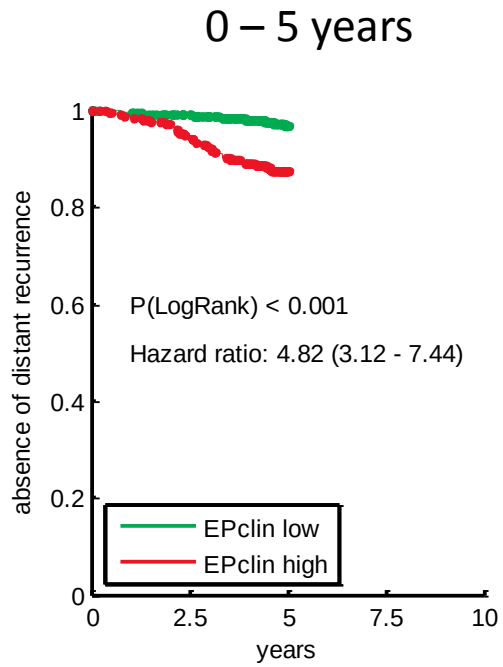
for the

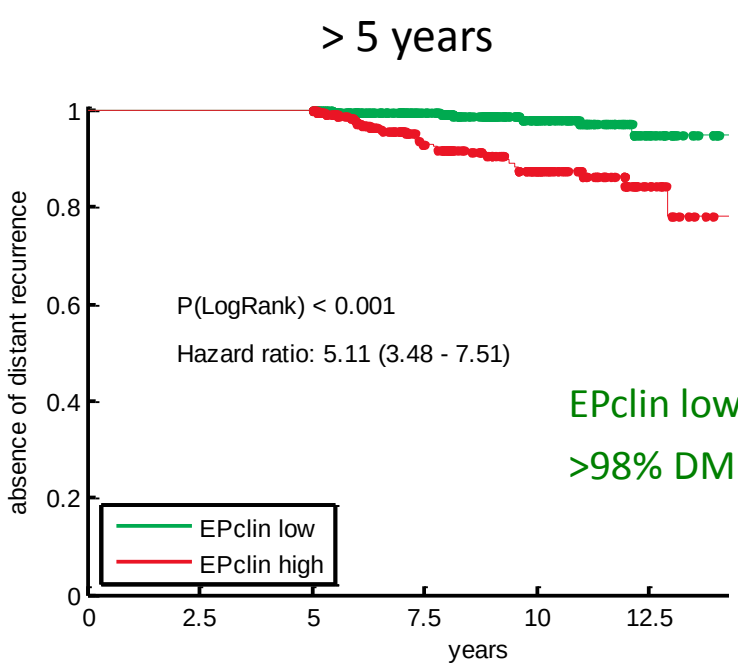
Austrian Breast and Colorectal Cancer Study Group

bgz BRUST GESUNDHEITS ZENTRUM
MedUni Wien | AKH Wien

COMPREHENSIVE CANCER CENTER VIENNA

EndoPredict: Zeigt frühe *und* späte Metastasen an





EPclin low: 64% with
>98% DMFS @> 5 years

numbers at risk:

1066	1029	682	
636	572	373	

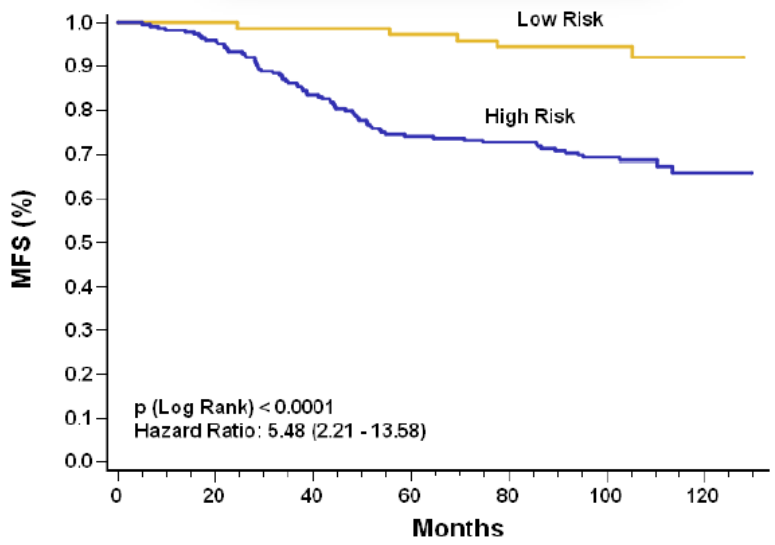
numbers at risk:

642	298	150	32
356	173	101	21

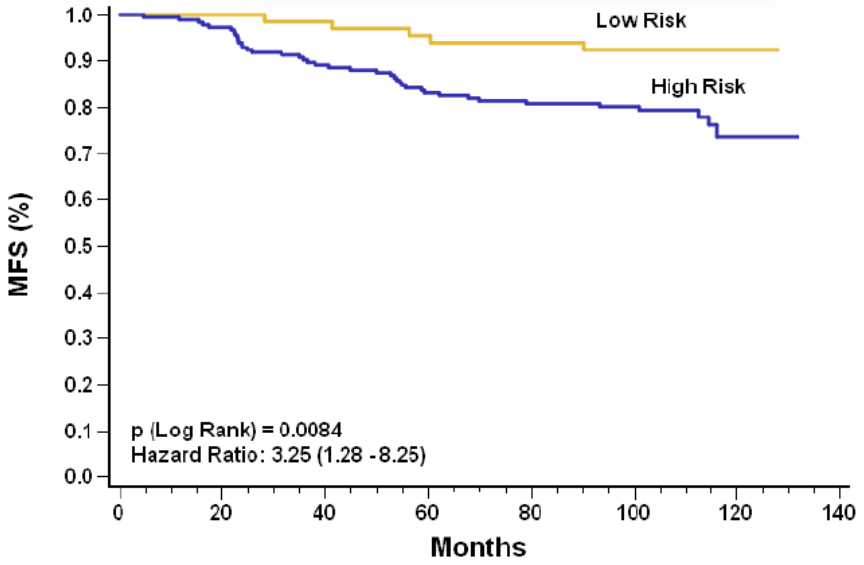
Dubsky et al., SABCS 2012

GEICAM 9906-Studie: EP ist prognostisch in prä- und postmenopausalen Patientinnen

a) **premenopausal**

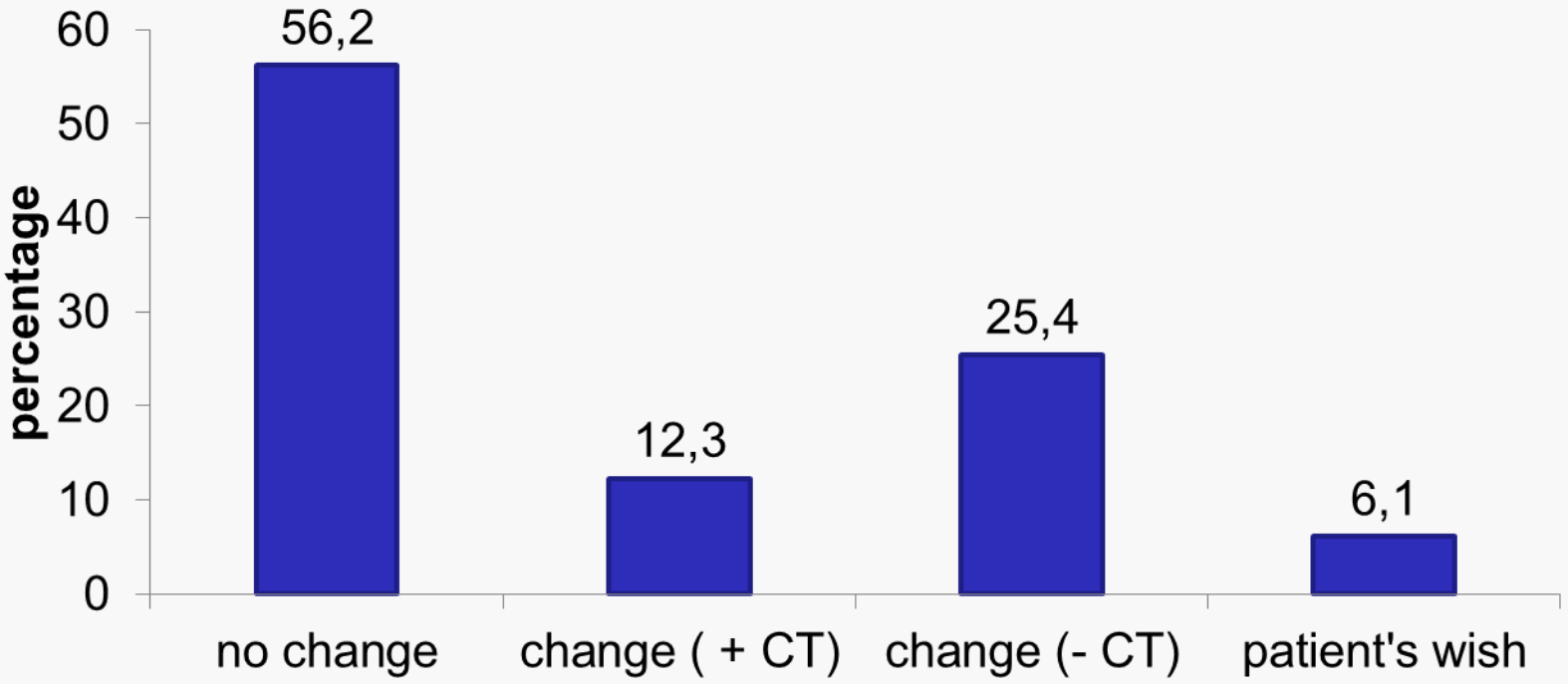


b) **postmenopausal**



Martin et al., SABCS 2012

How often therapy is changing due to test results?



- Endopredict tests during 1st y at Charité n=167
- Results based on 130 patients, retrospektiv evaluated

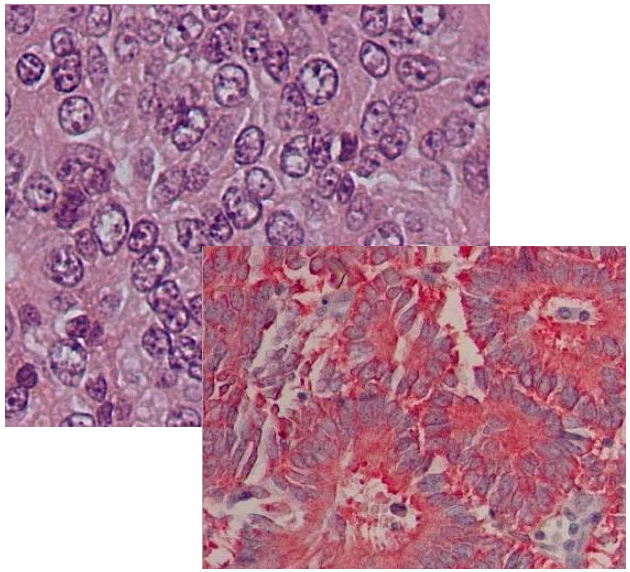
Berit Müller, 2012

A look into the future, exemplified by a current case

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

parallel molecular diagnostics

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

other relevant mutations

????

***Ion Torrent**

Proprietary Semiconductor Sequencing Technology

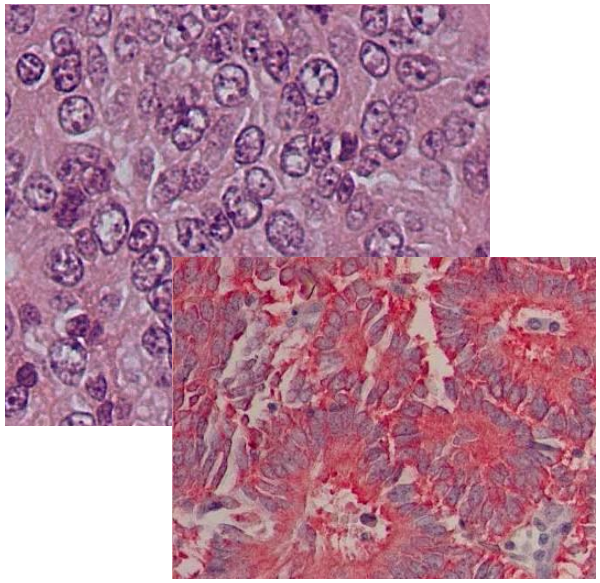
Ion Torrent®, the chip is the mashine

If a nucleotide, for example a C, is added to a DNA template and is then incorporated into a strand of DNA, a hydrogen ion will be released. The charge from that ion will change the pH of the solution, which can be detected by our proprietary ion sensor.

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.

parallel 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…….

Clinical data tissue

All test are done on ixed d

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