ESMO
GOOD SCIENCE
BETTER MEDICINE
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European Society for Medical Oncology

PERSONALIZED
MEDICINE SYMPOSIUM

Targeting the HER/EGFR family in breast, lung and colorectal cancers

SIGNALLING PATHWAYS IN CANCER

Sitges, Barcelona, Spain 1-2 March 2013

GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

Pitfalls and challenges with tumor tissue specimens

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IIS-Fundacion Jimenez Diaz, Madrid
IMIM-Hospital del Mar, Barcelona

Sitges, March 1st 2013

No conflicts of interest to declare

Outline of the presentation

Topic #1. State of the art in predictive biomarkers to anti-HER therapies in lung, colorectal and breast cancer

Topic #2. Upcoming predictive biomarkers to anti-HER2 therapies

Topic #3. Laboratory policies: considerations about type of assay, sample selection, optimization in use and quality:

- Workflow with tumor samples
- Quality control
- Primary or metastatic tissue?
- Type of assay

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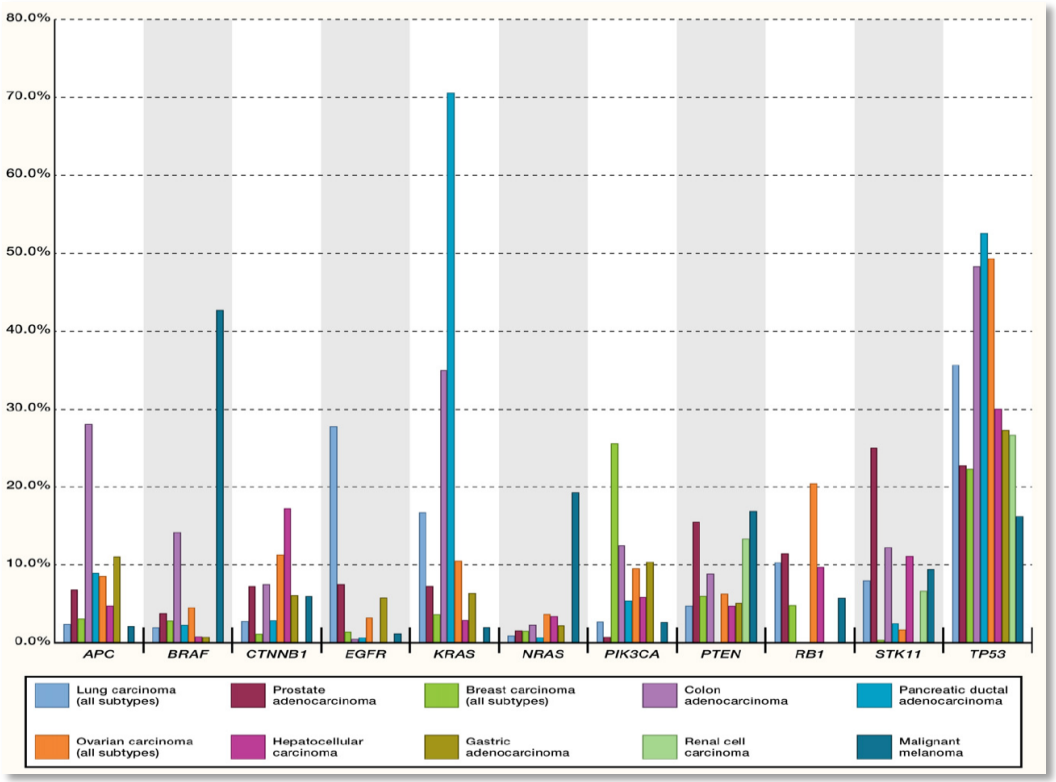
The genetic basis for cancer treatment decisions

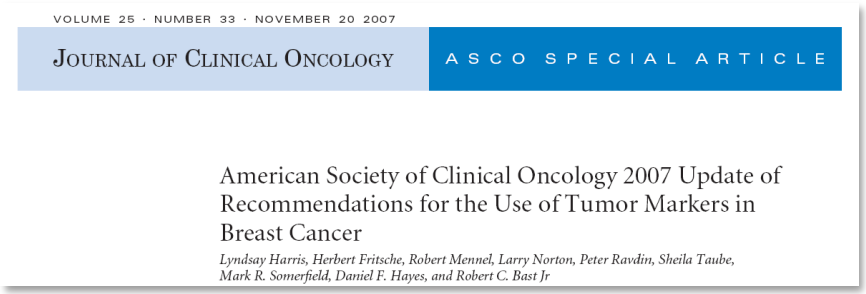


Genetic Marker	Application	Drug
BCR-ABL	Ph+ CML; Ph+ ALL	Imatinib, dasatinib, nilotinib
BCR-ABL/T315I	Resistance to anti-BCR-ABL agents	Imatinib, dasatinib, nilotinib
BRAF V600E	Metastatic melanoma	Vemurafenib
BRCA1/2	Metastatic ovarian cancer and breast cancer with BRCA 1/2 mutations	Olaparib, veliparib, iniparib
c-Kit	Kit (CD117)-positive malignant GIST	Imatinib
EGFR	Locally advanced, unresectable, or metastatic NSCLC	Erlotinib, gefitinib
EGFR T790M	Resistance to EGFR tyrosine kinase inhibitors in advanced NSCLC	Erlotinib, gefitinib
EML4-ALK	ALK kinase inhibitor for metastatic NSCLC with this fusion gene	Crizotinib
HER2 amplification	HER2-positive breast cancer or metastatic gastric or gastroesophageal junction adenocarcinoma	Trastuzumab
KRAS	Resistance to EGFR antibodies in metastatic colorectal cancer	Cetuximab, panitumumab
PML/RAR	Acute promyelocytic leukemia	ATRA, arsenic trioxide
TPMT	Deficiency is associated with increased risk of myelotoxicity	Mercaptopurine, azathioprine
UGT1A1	Homozygosity for UGT1A1*28 is associated with risk of toxicity	Irinotecan
DPD	Deficiency is associated with risk of severe toxicity	5-Fluorouracil

Bernards, R. Cell 2012
Dancey, JE. et al. Cell 2012

HER2 evaluation as a marker in breast cancer

1890 | 1960 | 1970 | 1980 | 1990 | 2000



HER2 gene cloned

HER2 over-expression linked to breast cancer progression

Trastuzumab approved by FDA for HER2+ advanced disease; IHC and FISH assays approved to test for HER2 status from patient samples

- HER2 expression and/or amplification should be evaluated in every primary invasive breast cancer, to guide selection of trastuzumab.
- Not recommended as prognostic in early breast cancer in absence of systemic therapy.
- Refer to "ASCO/CAP Clinical Practice Guidelines on HER2 Testing in Breast Cancer" regarding analysis of tissue HER2 status. *(Wolff A.C., et al. J Clin Oncol. 25:118-45, 2007)*
- Use IHC (expression) or FISH (amplification) tests to identify HER2 levels and identify benefit (or lack thereof) of trastuzumab therapy, in either adjuvant or metastatic settings.
- If considering chemotherapy for a patient with HER2 positive breast cancer who will not receive trastuzumab, strongly consider an anthracycline (if no contraindications).
- Use of HER2 not recommended to guide use of adjuvant taxane chemotherapy.
- Should not be used to withhold endocrine therapy for a patient with hormone-receptor positive breast cancer, nor should it be used to select one specific type of endocrine therapy over another.
- Measuring circulating extracellular domain of HER2 is not currently recommended for any clinical setting.

ASCO®

Highlights of the St Gallen International Expert Consensus on early breast cancer 2011

special article

Annals of Oncology 22: 1736–1747, 2011
doi:10.1093/annonc/mdr304
Published online 27 June 2011

Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011

A. Goldhirsch[1*], W. C. Wood[2], A. S. Coates[3], R. D. Gelber[4], B. Thürlimann[5], H.-J. Senn[6] & Panel members[†]

Intrinsic Subtype (1)	Clinico-pathologic definition	Notes	Type of therapy	Notes on therapy
Luminal A	**'Luminal A'** ER and/or PgR positive(76) HER2 negative (77) Ki-67 low (<14%)*	This cut-point for Ki-67 labelling index was established by comparison with PAM50 intrinsic subtyping (7). Local quality control of Ki-67 staining is important.	Endocrine therapy alone	Few require cytotoxics (e.g. high nodal status or other indicator of risk: see text).
Luminal B**	**'Luminal B (HER2 negative)'** ER and/or PgR positive HER2 negative Ki-67 high	Genes indicative of higher proliferation are markers of poor prognosis in multiple genetic assays (78). If reliable Ki-67 measurement is not available, some alternative assessment of tumor proliferation such as grade may be used to distinguish between 'Luminal A' and 'Luminal B (HER2 negative)'.	Endocrine ± cytotoxic therapy	Inclusion and type of cytotoxics may depend on level of endocrine receptor expression, perceived risk and patient preference.
	'Luminal B (HER2 positive)' ER and/or PgR positive Any Ki-67 HER2 over-expressed or amplified	Both endocrine and anti-HER2 therapy may be indicated.	Cytotoxics + anti-HER2 + endocrine therapy	No data are available to support the omission of cytotoxics in this group.
Erb-B2 overexpression	**'HER2 positive (non luminal)'** HER2 over-expressed or amplified ER and PgR absent		Cytotoxics + anti-HER2	Patients at very low risk (e.g. pT1a and node negative) may be observed without systemic adjuvant treatment.
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative	Approximately 80% overlap between 'triple negative' and intrinsic 'basal-like' subtype but 'triple negative' also includes some special histological types such as (typical) medullary and adenoid cystic carcinoma with low risks of distant recurrence. Staining for basal keratins (79) although shown to aid selection of true basal-like tumors, is considered insufficiently reproducible for general use.	Cytotoxics	
	Special histological types[*] A. Endocrine responsive		Endocrine therapy	
	B. Endocrine nonresponsive		Cytotoxics	Medullary and adenoid cystic carcinomas may not require any adjuvant cytotoxics (if node negative).

Molecular assays to select therapy in HER2 breast tumors

VOLUME 25 · NUMBER 1 · JANUARY 1 2007

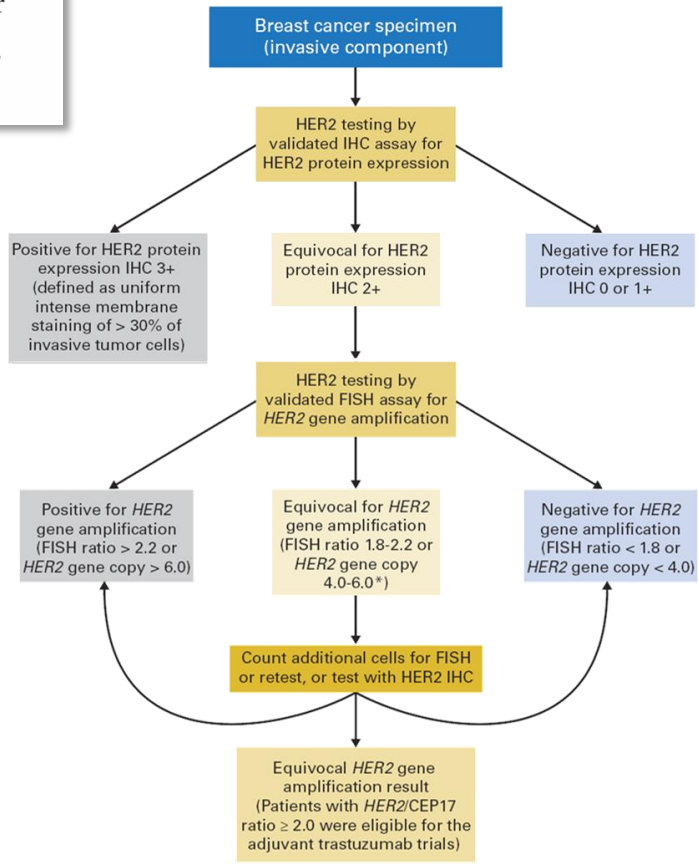
JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, and Daniel F. Hayes

Company Location	Name of test Status	Technology
Biogenex San Ramon, California	InSite HER2/neu CB11 FDA approved	Immunohistochemistry assay using a monoclonal antibody directed against the internal domain of HER2/neu available either in automated or manual formats
Dako Glostrup, Denmark	HER2 FISH pharmDx Kit FDA approved	FISH assay to determine HER2 gene amplification in formalin-fixed, paraffin-embedded breast cancer specimens. Gene amplification is determined from the ratio between the number of signals from the hybridization of the HER2 gene probe and the number of signals from the hybridization of the reference chromosome 17 probe (green signals)
Dako	HercepTest FDA approved	Semi-quantitative immunohistochemistry assay for determination of HER2 protein overexpression in breast cancer tissues routinely processed for histological evaluation
Genomic Health	Oncotype DX CLIA validated	RT PCR–based assay analyzes the expression of a panel of 21 genes, among them HER2. Oncotype DX predicts disease recurrence and assesses benefit from certain types of chemotherapy
Invitrogen Carlsbad, California	SPOT-Light HER2 CISH Kit FDA approved	Chromogenic *in situ* hybridization (CISH) using a DNA probe. Quantifiable results are visualized under a standard brightfield microscope.
Monogram Biosciences	HERmark Breast Cancer Assay CLIA-validated	Proximity-based assay, which provides direct quantitative measurements of HER2 total protein and HER2 homodimer levels
Siemens Healthcare Diagnostics Erlangen, Germany	HER2/neu ELISA FDA approved	Sandwich enzyme immunoassay using mouse monoclonal for capture and a different biotinylated mouse monoclonal antibody for the detection of human HER2/neu protein. Detection is by direct chemiluminescence. Protein is quantified by spectrophotometry
Ventana-Roche Tucson	Inform HER2 Silver in situ Hybridization Approved in Europe and elsewhere but not by FDA	Fully automated silver *in situ* hybridization assay for HER2 and chromosome 17 detection. Chromogenic signals are detected through the use of silver deposition technology. Results and morphological significance can be interpreted using conventional brightfield microscopy
Ventana-Roche	Pathway anti-HER2/neu (Clone CB11) FDA approved	Semiquantitative immunohistochemistry assay using a monoclonal antibody for the detection of c-erbB-2 (HER2) antigen using Ventana's family of automated instrument platforms
Vysis (Abbott)	PathVysion HER2 DNA Probe Kit FDA approved	Fluorescence *in situ* hybridization (FISH) assay to determine *HER2* amplification, using LSI HER2 probe, which spans *HER2*, and CEP 17 probe, which hybridizes to the alpha satellite DNA located at the centromere of chromosome



Predictive biomarkers to anti-EGFR therapies in mCRC

VOLUME 27 · NUMBER 12 · APRIL 20 2009

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Provisional Clinical Opinion: Testing for *KRAS* Gene Mutations in Patients With Metastatic Colorectal Carcinoma to Predict Response to Anti–Epidermal Growth Factor Receptor Monoclonal Antibody Therapy

Carmen J. Allegra, J. Milburn Jessup, Mark R. Somerfield, Stanley R. Hamilton, Elizabeth H. Hammond, Daniel F. Hayes, Pamela K. McAllister, Roscoe F. Morton, and Richard L. Schilsky

Based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment.

Predictive biomarkers to anti-EGFR therapies: KRAS mutations

Table 1. Randomized Clinical Trial Evidence on Relationship of KRAS Mutation Status to Efficacy of Anti-EGFR Monoclonal Antibodies in Patients With Metastatic Colorectal Cancer

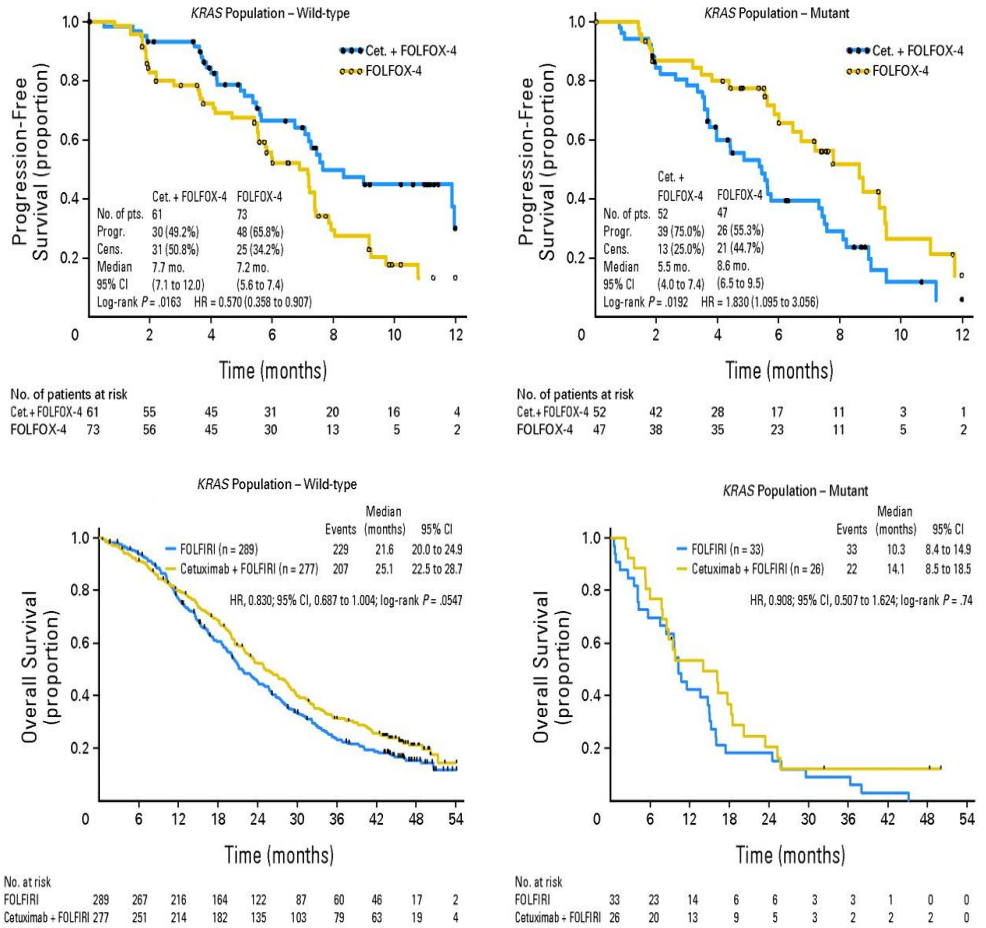
Study and Population	Treatments by Arm	Variable	*KRAS* WT		*KRAS* Mutated	
			Antibody Arm	Control Arm	Antibody Arm	Control Arm
van Cutsem et al, 2009[10]; CRYSTAL trial of first line therapy	FOLFIRI ± cetuximab	No. of patients	172	176	105	87
		Response rate, %	59.3	43.2	36.2	40.2
		95% CI	51.6 to 66.7	35.8 to 50.9	27.0 to 46.2	29.9 to 51.3
		P	.0025		.46	
		Median PFS, months	9.9	8.7	7.6	8.1
		HR	0.68		1.07	
		P	.017		.47	
Bokemeyer et al, 2008[9]; OPUS trial of first line therapy	FOLFOX ± cetuximab	No. of patients	61	73	52	47
		Response rate, %	60.7	37.0	32.7	48.9
		95% CI	47.3 to 72.9	26.0 to 49.1	20.3 to 47.1	34.1 to 63.9
		P	.011		.106	
		OR	2.54		0.51	
		95% CI	1.24 to 5.23		0.22 to 1.15	
		Median PFS, months	7.7	7.2	5.5	8.6
		HR	0.57		1.83	
		P	.016		.0192	
Punt et al, 2008[8]; CAIRO2 trial of first line therapy	(Capecitabine + oxaliplatin + bevacizumab) ± cetuximab	No. of patients	153	152	93	103
		Median PFS, months	10.5	10.7	8.6	12.5
		P	.10		.043	
		Median OS, months	22.2	23.0	19.1	24.9
		P	.49		.35	
Amado et al, 2008[1]; chemotherapy-refractory disease	Panitumumab *v* best supportive care	No. of patients	124	119	84	100
		Response rate, %	17	0	0	0
		Median PFS, weeks	12.3	7.3	7.4	7.3
		HR	0.45		0.99	
		95% CI	0.34 to 0.59		0.73 to 1.36	
Karapetis et al, 2008[6]; second- or subsequent-line therapy	Cetuximab *v* best supportive care	No. of patients	117	113	81	83
		Response rate, %	12.8	0	1.2	0
		Median PFS, months	3.7	1.9	1.8	1.8
		HR	0.40		0.99	
		95% CI	0.30 to 0.54		0.73 to 1.35	
		P	< .001		.96	
		Median OS, months	9.5	4.8	4.5	4.6
		P	.01 (for interaction, *KRAS* mutation status × treatment arm)			
		OS at 1 year, %	28.3	20.1	13.2	19.6
		HR (death)	0.55		0.98	
		95% CI	0.41 to 0.74		0.70 to 1.37	
		P	< .001		.89	

Table 2. Single-Arm Studies of Treatment of Metastatic CRC With Anti-EGFR Monoclonal Antibodies and *KRAS* Mutational Status

Study and Population	Treatments by Arm	Variable	*KRAS* WT	*KRAS* Mutated
Lievre et al, 2008[3]; second-line therapy	Cetuximab	No. of patients	65	24
		Response rate	40	0
		P	.001	
		PFS, weeks	31.4	10.1
		95% CI	19.4 to 36	8 to 16
		P	.0001	
		OS, months	14.3	10.1
		95% CI	9.4 to 20	5.1 to 13
		P	.026	
De Roock et al, 2008[4]	Cetuximab alone *v* with irinotecan	No. of patients	57	46
		Response rate	41	0
		P (cetuximab + irinotecan)	.000001	
		P (cetuximab alone)	.126	
		PFS cetuximab + irinotecan, weeks	34	12
		95% CI	28.5 to 40.0	5.4 to 18.7
		P	.016	
		PFS cetuximab, weeks	12	12
		95% CI	4.2 to 20.0	7.0 to 17.0
		P	.351	
		OS cetuximab + irinotecan, weeks	44.7	27.3
		95% CI	28.4 to 61.0	9.5 to 45.0
		P	.003	
		OS, weeks	27	25.3
		95% CI	8.9 to 45.1	0.0 to 70.0
		P	.330	
Khambata-Ford et al, 2007[7]	Cetuximab; second- or third-line treatment	No. of patients	50	30
		Response rate, %	10	0
Di Fiore et al, 2007[5]	Cetuximab plus chemotherapy	No. of patients	43	16
		Response rate, %	28	0
Benvenuti et al, 2007[2]	Panitumumab or cetuximab, or cetuximab plus chemotherapy	No. of patients	32	16
		Response rate, %	31	6

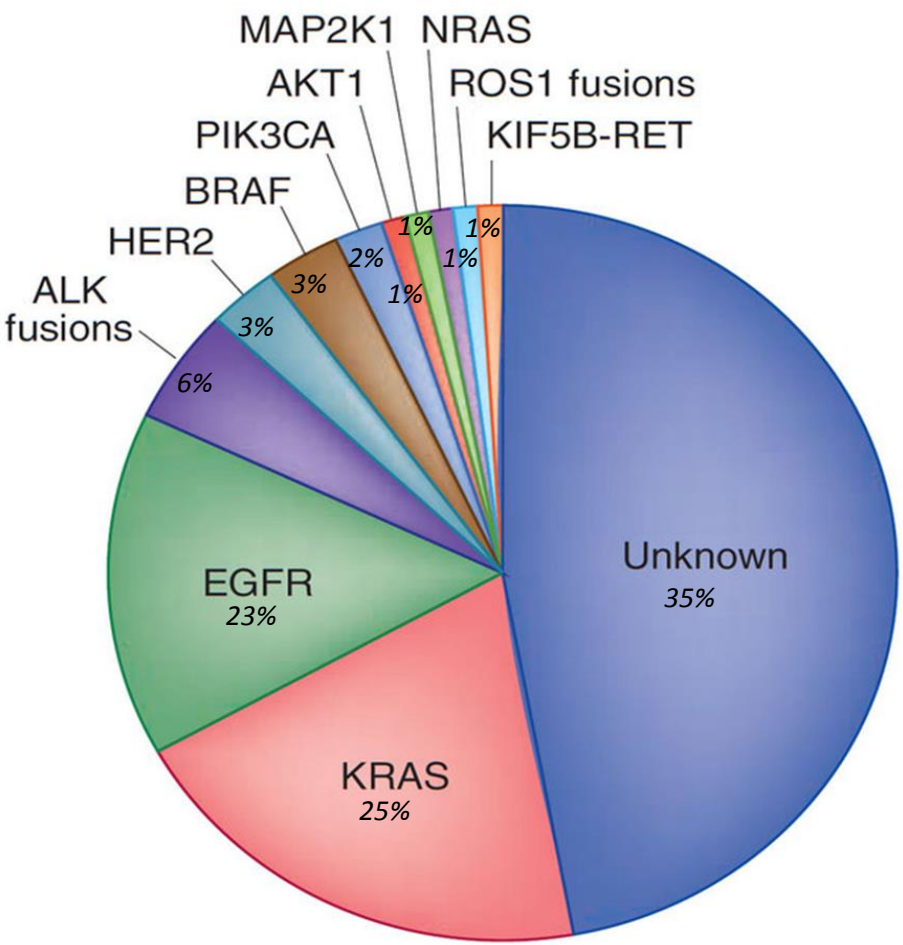
Allegra, CJ. et al.J Clin Oncol 2009

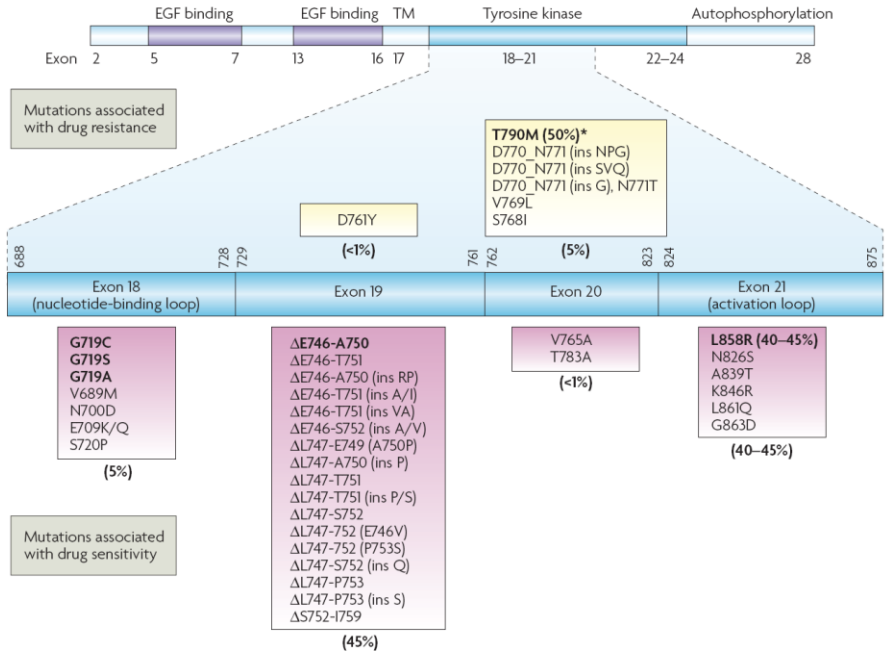
Cetuximab plus oxaliplatin, leucovorin, and fluorouracil (FOLFOX-4) or FOLFOX-4 alone
Cetuximab plus irinotecan, fluorouracil, and leucovorin (FOLFIRI) or FOLFIRI alone



Bokemeyer, C. et al. J Clin Oncol 2009
Van Cutsem, E. et al. J Clin Oncol 2011

Potential predictive biomarkers in NSCLC





Sequist, A et al. Ann Oncol 2011
Bergethon J et al. J Clin Oncol 2012

Predictive biomarkers to anti-EGFR therapies in NSCLC

VOLUME 29 · NUMBER 15 · MAY 20 2011

JOURNAL OF CLINICAL ONCOLOGY

ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Provisional Clinical Opinion: Epidermal Growth Factor Receptor (*EGFR*) Mutation Testing for Patients With Advanced Non–Small-Cell Lung Cancer Considering First-Line EGFR Tyrosine Kinase Inhibitor Therapy

Vicki Leigh Keedy, Sarah Temin, Mark R. Somerfield, Mary Beth Beasley, David H. Johnson, Lisa M. McShane, Daniel T. Milton, John R. Strawn, Heather A. Wakelee, and Giuseppe Giaccone

On the basis of the results of five phase III randomized controlled trials, patients with NSCLC who are being considered for first-line therapy with an EGFR TKI (patients who have not previously received chemotherapy or an EGFR TKI) should have their tumor tested for EGFR mutations to determine whether an EGFR TKI or chemotherapy is the appropriate first-line therapy.

clinical practice guidelines

Annals of Oncology 21 (Supplement 5): v116–v119, 2010
doi:10.1093/annonc/mdq189

Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up

G. D'Addario[1], M. Früh[2], M. Reck[3], P. Baumann[4], W. Kle
On behalf of the ESMO Guidelines Working Group*

Use of predictive markers for treatment

Activating EGFR mutations (Exons 19, 21) are predictive for response and progression-free survival to the tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib based on several trials. The incidence of EGFR mutations in a Caucasian population is ~10%. Higher rates are observed in never-smokers, in East-Asians, in patients with adenocarcinoma subtype and in women. Further prognostic and predictive molecular markers have been described but not prospectively validated.

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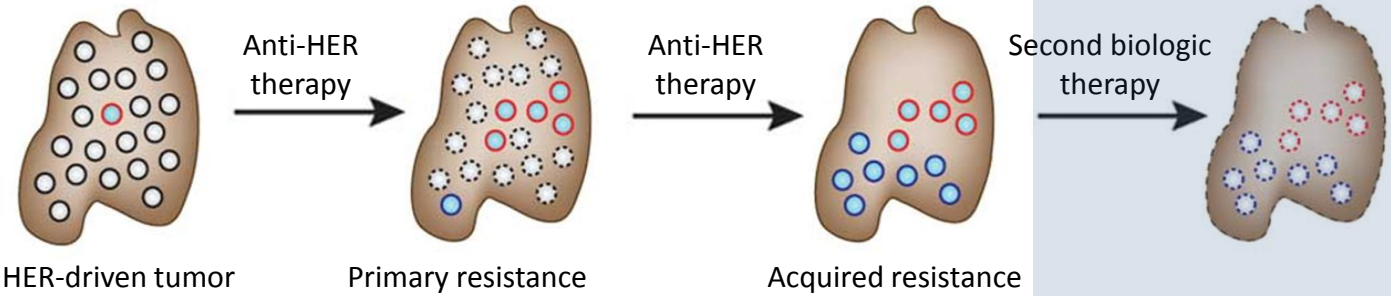
Resistance mechanisms to anti-HER therapies in cancer: primary and acquired resistance

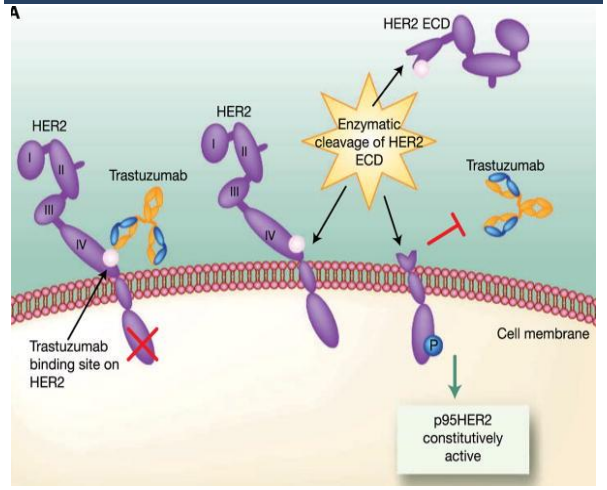


Biomarker: HER2 amplification (breast)
EGFR mutation (NSCLC)
EGFR-signaling, KRAS wt (CRC)

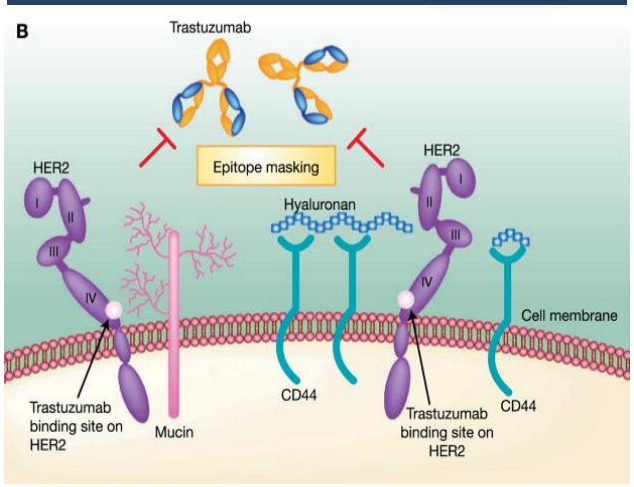
HER-driven cell
Primary resistant cell
Acquired resistant cell

Resistance mechanisms to anti-HER2 therapy in breast cancer

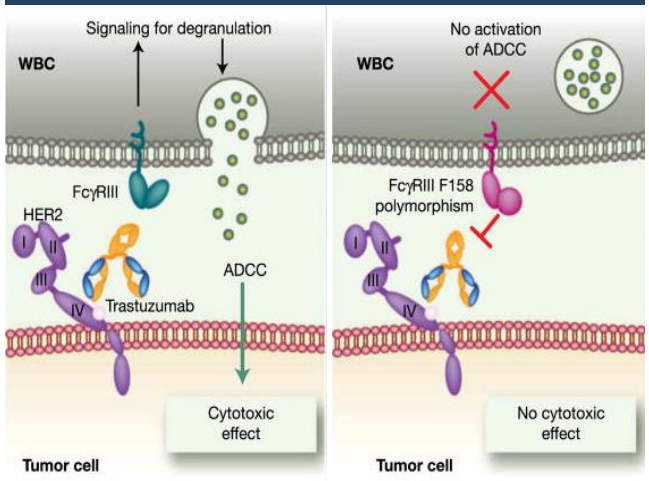
Constitutively active truncated HER2



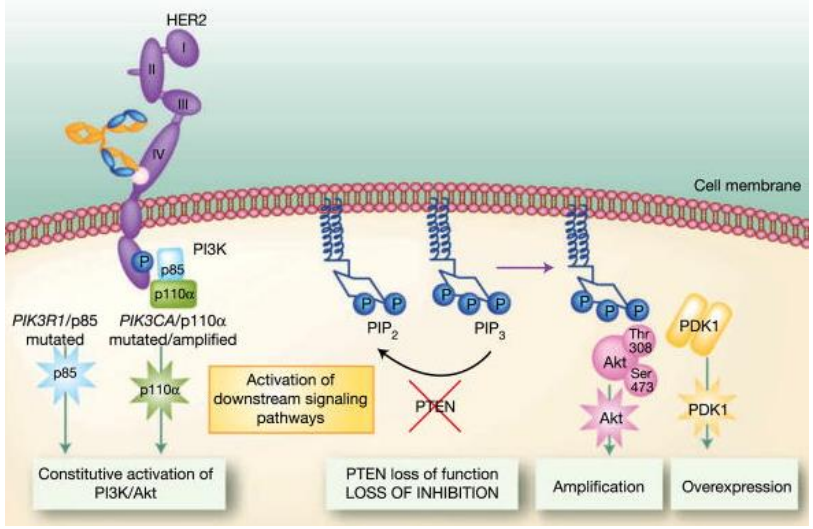
Epitope masking by MUC4 or CD44/ polymeric hyaluronan complex



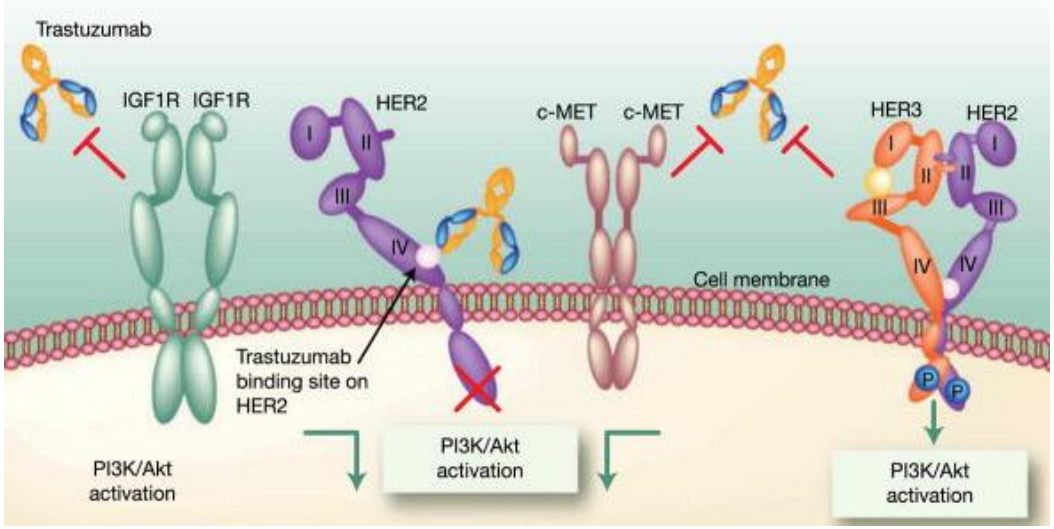
Failure to trigger immune-mediated mechanisms



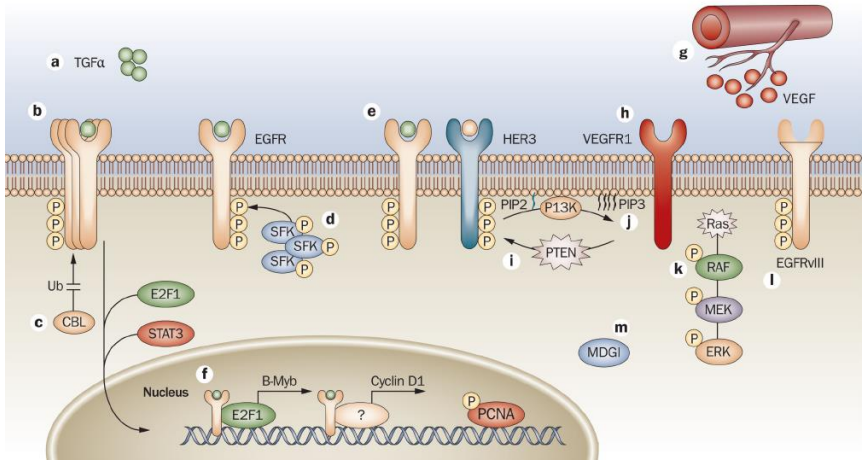
Upregulation of HER2 downstream signaling pathways



Signaling through an alternate receptor and/or pathway



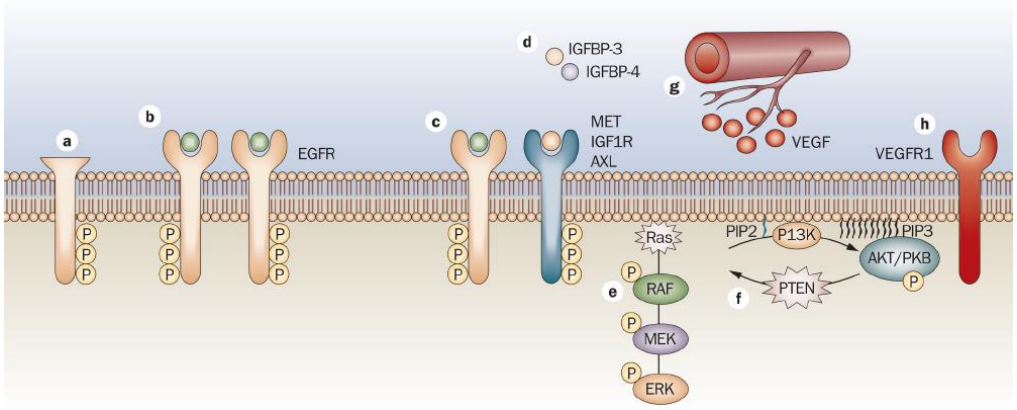
Predictive biomarkers to anti-EGFR antibodies

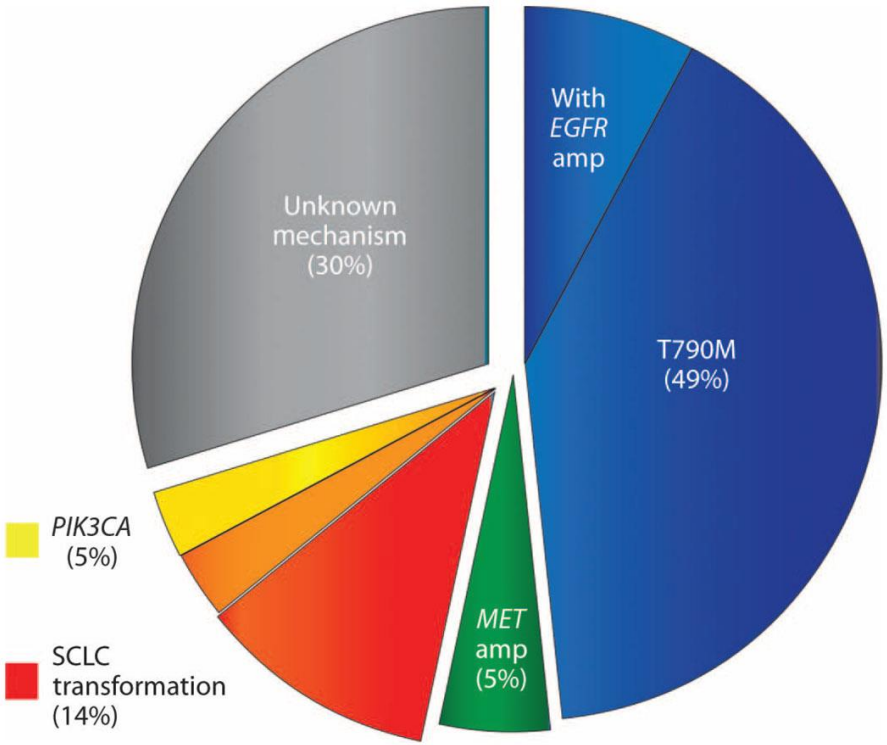


Biomarker	Prevalence	Detection method	Level of evidence	Regulatory status
KRAS mutation	40%	Direct sequencing, pyrosquencing, PCR	I (c12, 13), II (c61, 146)	Adopted by FDA, ASCO
BRAF mutation	10%	Direct sequencing, pyrosquencing, PCR	IIA	Under evaluation
PI3K mutation	5-10%	Direct sequencing, pyrosquencing, PCR	IIB	Under evaluation
P53 mutation	1-5%	Direct sequencing, pyrosquencing, PCR, IHC	IIB	Under evaluation
PTEN deletion	20%	IHC, direct sequencing, methylation-specific PCR	IIB	Under evaluation
EGFR CNV	40%	FISH, direct sequencing	III	Under evaluation
EGFR mutation	5%	Direct sequencing, PCR	III	Under evaluation
HER2 CNV	10%	FISH, direct sequencing	III	Under evaluation
HER2 mutation	1%	Direct sequencing, pyrosquencing, PCR	III	Under evaluation
MET CNV, mutation, expression	10%	IHC, FISH, direct sequencing,pyrosquencing, PCR	III	Under evaluation
Constitutive activation of EGFR effectors	50%		III	Under evaluation
Fcg receptor polymorphism	unknown	Genotyping	III	Under evaluation
Angiogenesis	unknown	Direct sequencing, PCR, IHC	III	Under evaluation

Wheeler, DL. et al. Nat Rev Clin Oncol 2010
Ross, JS. et al. Am J Clin Pathol 2011

Predictive biomarkers to anti-EGFR TKI

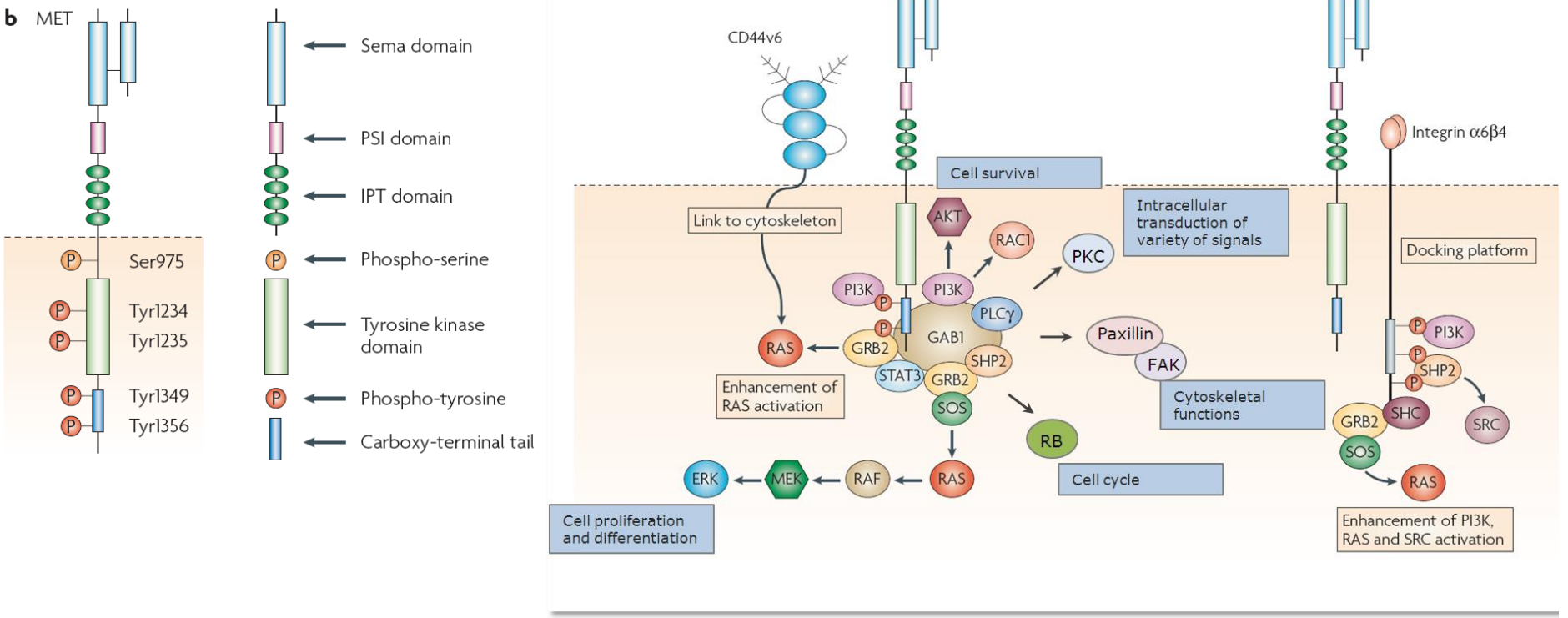




Wheeler, DL. et al. Nat Rev Clin Oncol 2010

Workman, P and Clarke, PA. Nat Rev Oncol 2011

MET pathway regulates EMT phenotype and induces EGFR-inhibition resistance



Gherardi, E. et al. Nat Rev Cancer 2012

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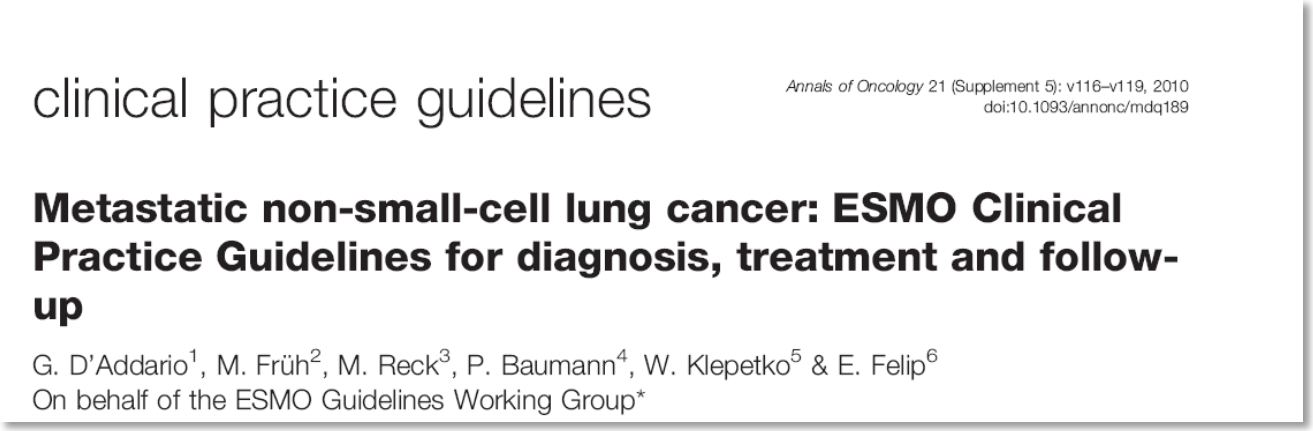
Topic #3. **Laboratory policies: considerations about type of assay, sample selection, optimization in use and quality:**

- **Workflow with tumor samples**
- **Quality control**
- **Primary or metastatic tissue?**
- **Type of assay**

Benchmarking of EGFR mutation diagnostics in NSCLC



clinical practice guidelines

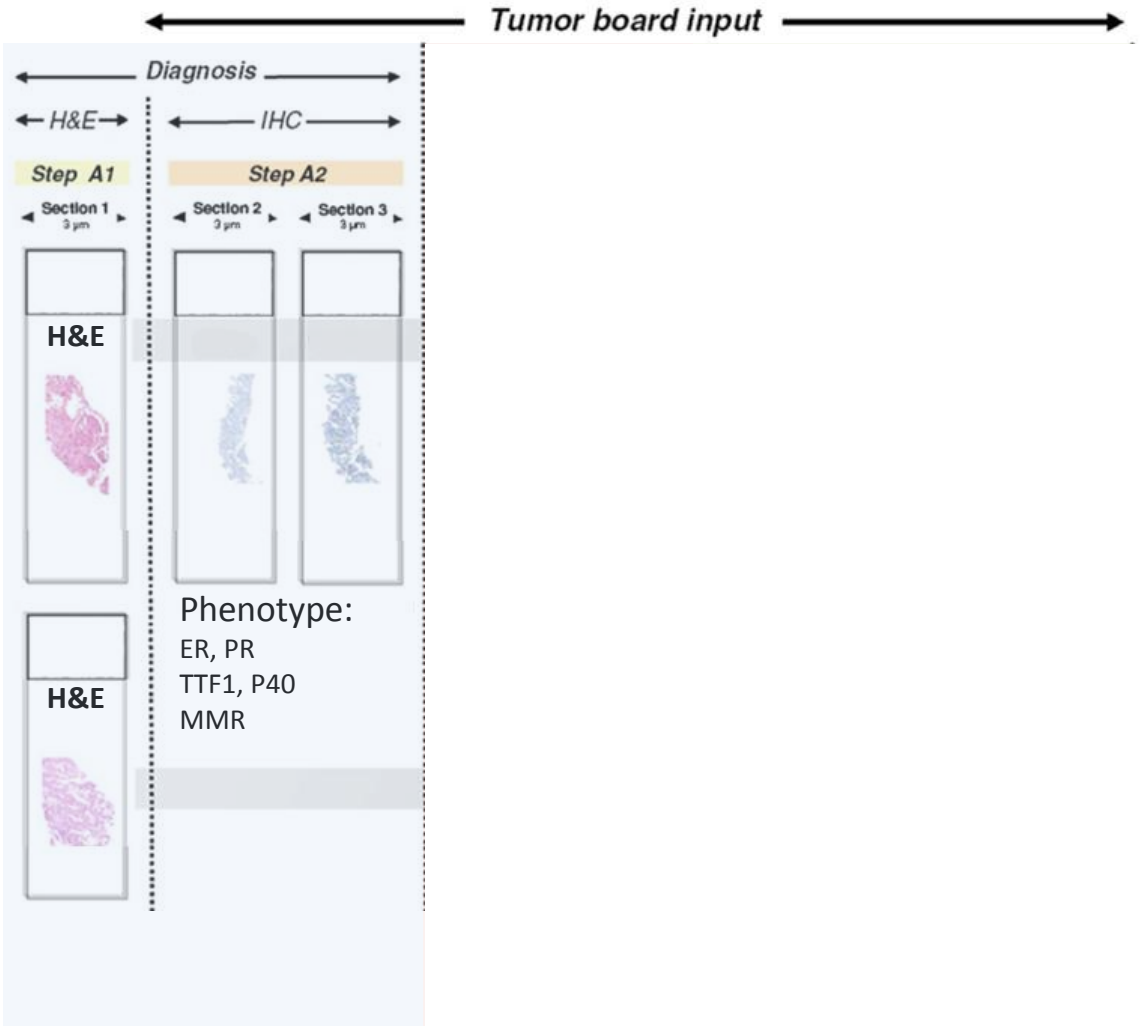
Annals of Oncology 21 (Supplement 5): v116–v119, 2010
doi:10.1093/annonc/mdq189

Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up

G. D'Addario[1], M. Früh[2], M. Reck[3], P. Baumann[4], W. Klepetko[5] & E. Felip[6]
On behalf of the ESMO Guidelines Working Group*

"If molecular testing is planned, appropriate biopsy methods should be utilized to obtain sufficient tissue for both pathological diagnosis and molecular analysis and the specimens should be handled appropriately."

Benchmarking of molecular diagnostics in cancer

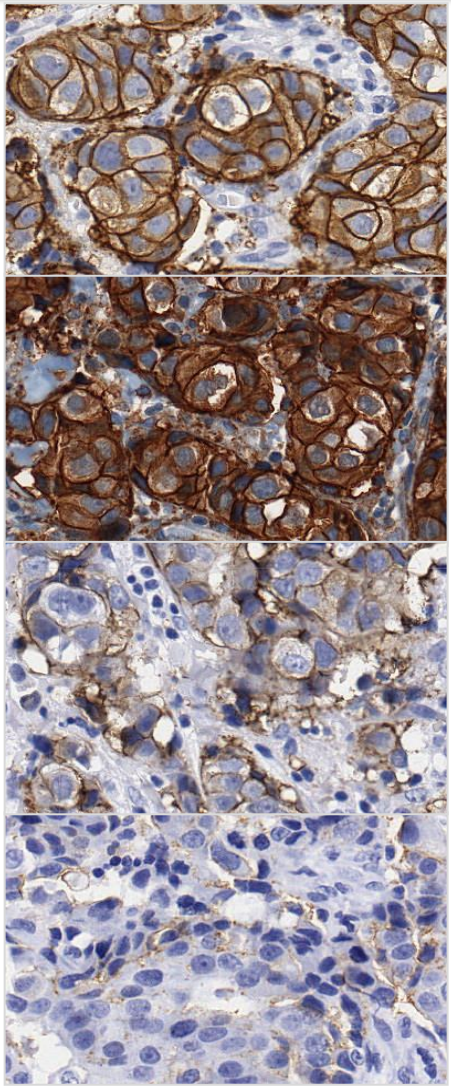


Effects of pre-analytical variables on the detection of biomarkers in FFPE tissue

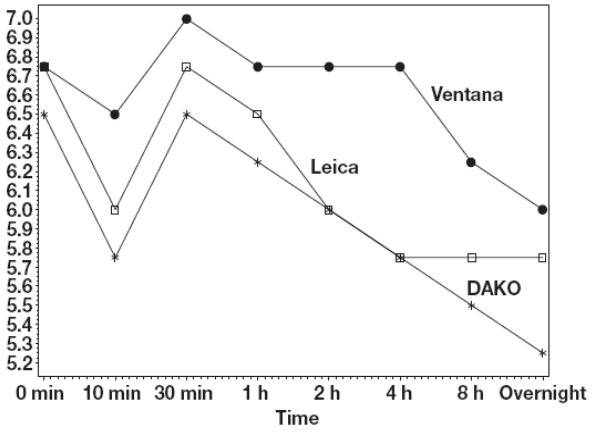
Preanalytic Variable	Analytic Effect	Antigen Dependent	Conflicting Reports	Source, y
Fixation delay (>12 h)	Alterations in the extent and intensity of immunostaining	Yes	No	Start et al,[5] 1992 von Wasielewski et al,[6] 2002 Hammond et al,[8] 2010
Fixative Concentration pH Buffer	Alterations in the extent and intensity of immunostaining, as well as nonspecific background staining	Yes	No	Pollard et al,[1] 1987 Williams et al,[2] 1997 von Wasielewski et al,[6] 2002 von Wasielewski et al,[11] 1998 Atkins et al,[12] 2004
Time in fixative	Alterations in the extent, distribution, and intensity of immunostaining	Yes	Yes	Pollard et al,[1] 1987 Williams et al,[2] 1997 von Wasielewski et al,[6] 2002 von Wasielewski et al,[11] 1998 Arber,[13] 2002 Goldstein et al,[14] 2003 Middleton et al,[15] 2009 Shi et al,[17] 2007 De Marzo et al,[18] 2002 Ibarra et al,[19] 2010
Dehydration Reagent Duration Temperature	Alterations in the extent and intensity of immunostaining	No	Yes	Pollard et al,[1] 1987 Williams et al,[2] 1997 Cerio and MacDonald,[29] 1986
Clearing Reagent Temperature	Alterations in the extent and intensity of immunostaining, as well as nonspecific background staining	No	Yes	Williams et al,[2] 1997 Cerio and MacDonald,[29] 1986
Paraffin embedding Temperature Duration	Alterations in the extent and intensity of immunostaining	No	Yes	Pollard et al,[1] 1987 Williams et al,[2] 1997 Cerio and MacDonald,[29] 1986
Section/slide adhesion Temperature and duration	Alterations in the intensity of immunostaining and nonspecific background staining	No	Yes	Pollard et al,[1] 1987 Williams et al,[2] 1997 Jones et al,[32] 2001
Storage of slide-mounted sections Temperature Duration	Alterations in the extent and intensity of immunostaining, as well as case status	Yes	Yes	Williams et al,[2] 1997 van den Broek and van de Vijver,[31] 2000 Bromley et al,[33] 1994 Shin et al,[35] 1997 Bertheau et al,[37] 1998 DiVito et al,[38] 2004 Jacobs et al,[39] 1996 Wester et al,[40] 2000 Fergenbaum et al,[41] 2004 Grabau et al,[42] 1998

Engel, KB and Moore, HM. Arch Pathol Lab Med, 2011

Delay to formalin fixation (cold ischemia) modifies the status of biomarkers



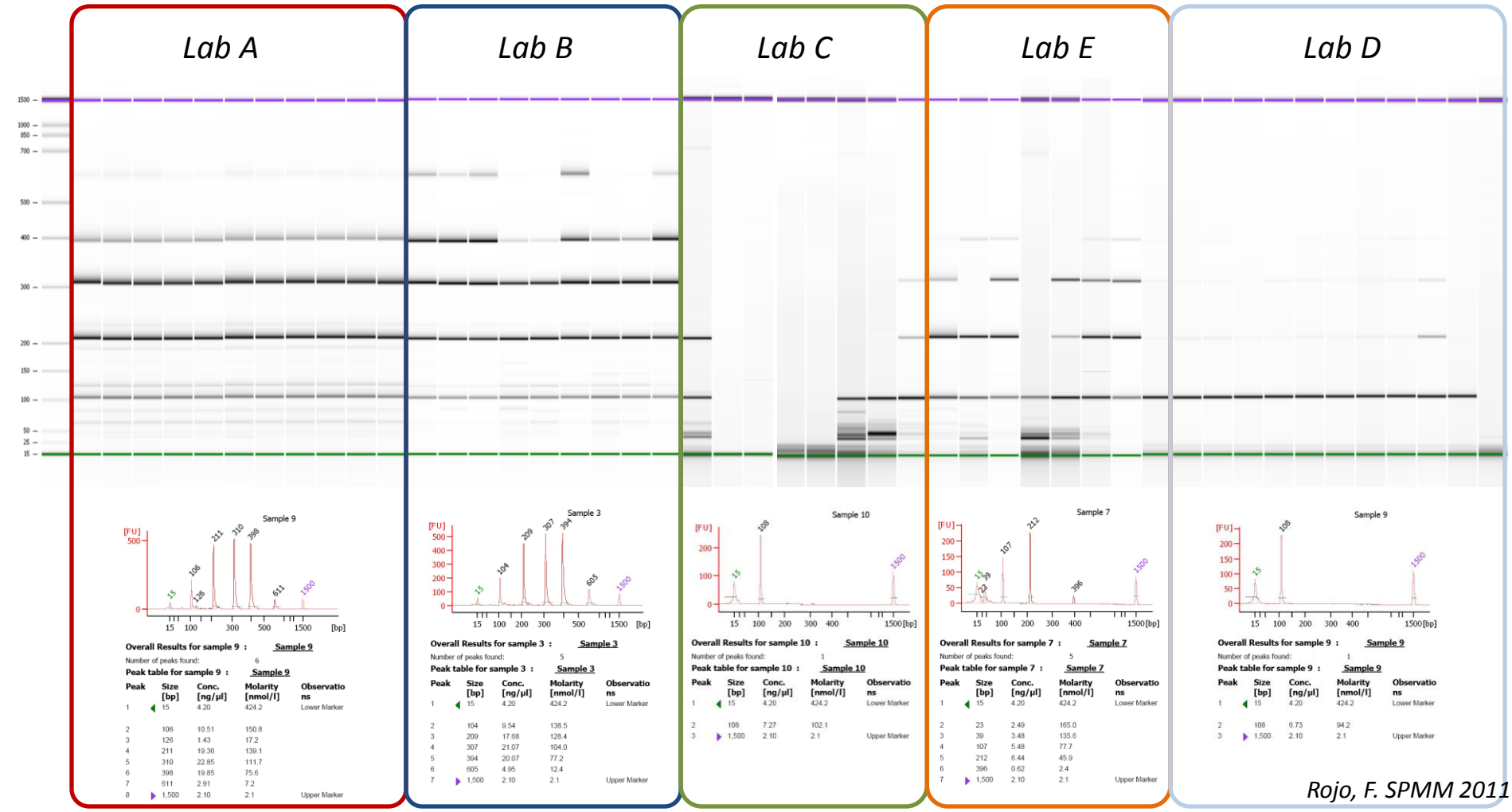
Rojo, F. SPMM 2011



	Reduction in staining	*Details*
HER2 protein	11/23 (48%)	Five cases: mild reduction at 48 h only Two cases: mild reduction at 24 and 48 h Two cases: mild reduction at 3, 4, 24, and 48 h One case: mild reduction at 4 h; significant reduction at 24 and 48 h One case: mild reduction at 3 and 4 h; significant reduction at 24 and 48 h

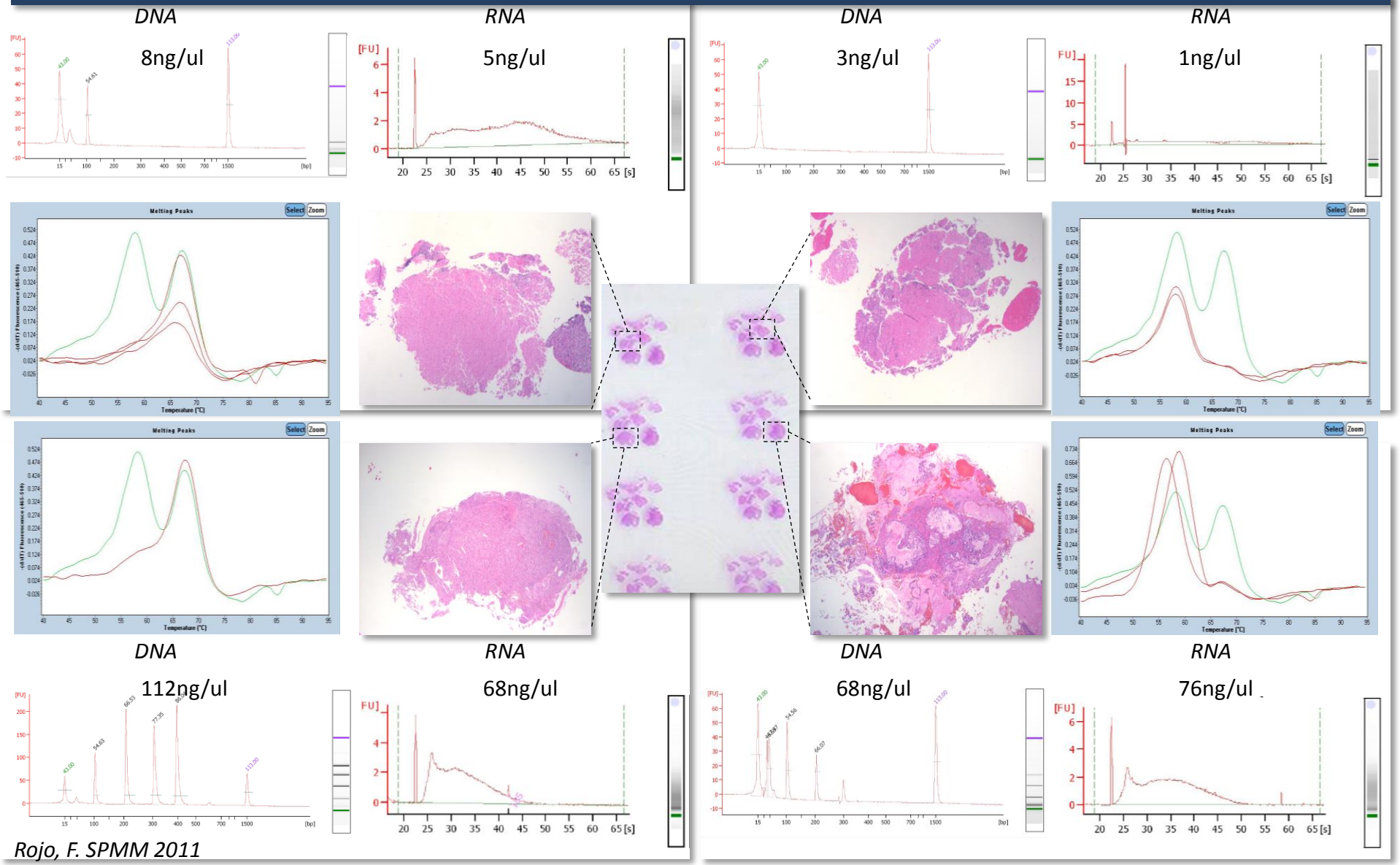
Khoury, T et al. Mod Pathol 2009
Yildiz-Aktas, IZ et al. Mod Pathol 2012

Preservation of DNA in FFPE archieved tissue samples: Standard quality control



Rojo, F. SPMM 2011

Importance of selection of tissue sample for molecular diagnosis



Rojo, F. SPMM 2011

Systematic bias in genomic classification due to non-neoplastic cell proportion in breast cancer

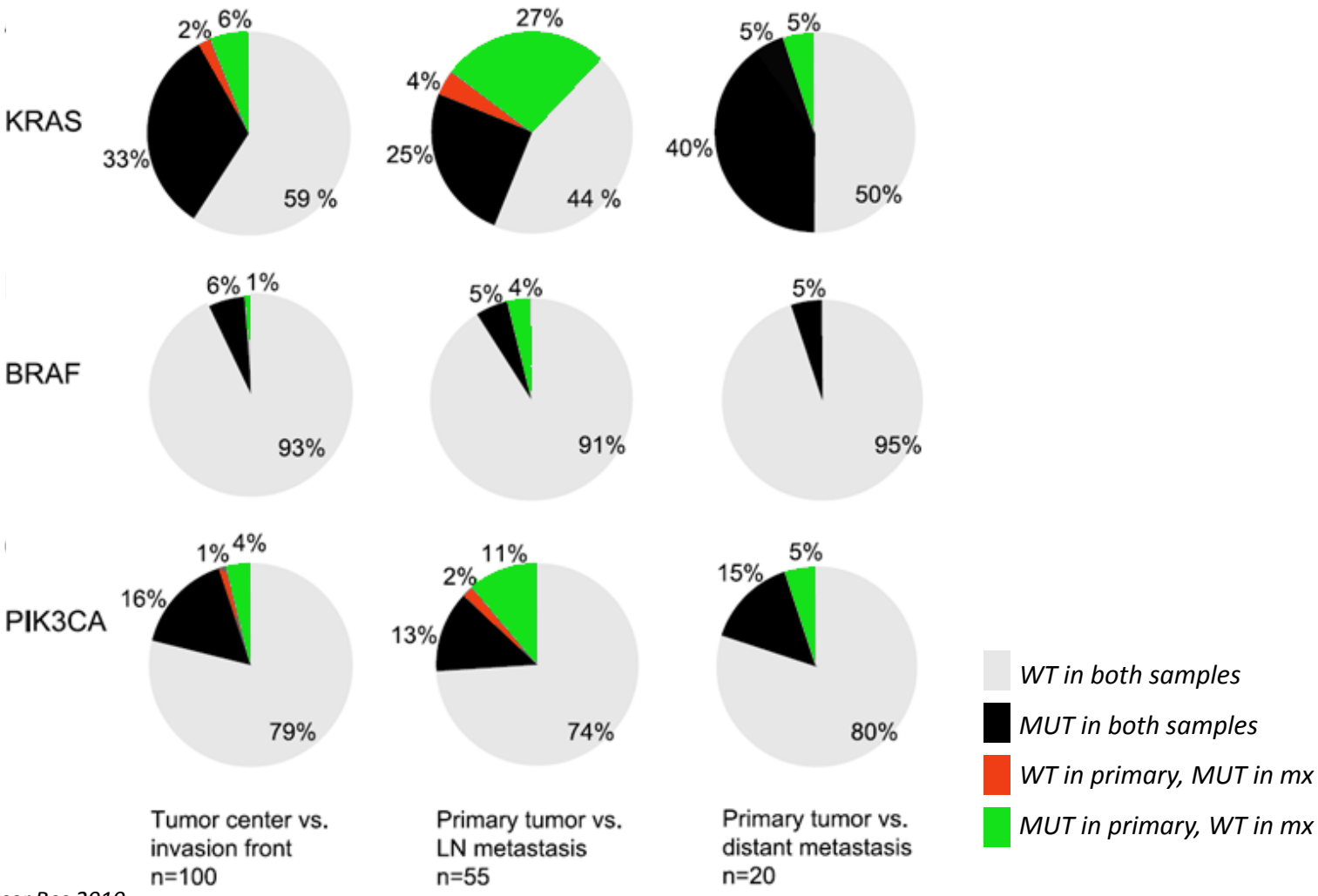
Elloumi, F. et al. BMC Med Genomics 2011

Loss of HER2 expression in metastatic sites of HER2+ primary breast cancer

Previous Study	Location	HER2-Positive Discordance Rate (%)	HER2-Negative Discordance Rate (%)
Masood et al[1]	Metastatic	8	0
Shimizu et al[2]	Metastatic	0	0
Simon et al[3]	Metastatic	6	2
Tanner et al[4]	Metastatic	0	0
Vincent-Salomon et al[23]	Primary	15	0
Salomon et al[5]	Metastatic	18	—
Xu et al[6]	Metastatic	0	—
Gancberg et al[7]	Metastatic	6	5
Taucher et al[8]	Primary	10	3
Burstein et al[19]	Primary	26	—
Regitnig et al[9]	Metastatic	0	15
Carlsson et al[10]	Metastatic	0	0
Zidan et al[11]	Metastatic	7	0
Gong et al[12]	Primary/Metastatic	12	0
Pectasides et al[13]	Metastatic	38	—
Hurley et al[20]	Primary	43	—
D'Andrea et al[14]	Metastatic	13	—
Harris et al[21]	Primary	11	—
Mittendorf et al[22]	Primary	32	—
Simmons et al[15]	Metastatic	0	9
Lower et al[16]	Metastatic	64	15
Wilking et al[17]	Metastatic	19	6
Thompson et al[18]	Metastatic	7	2

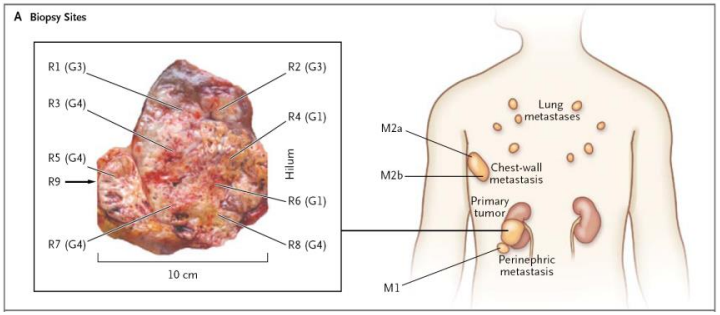
Niikura, N. et al. J Clin Oncol 2012

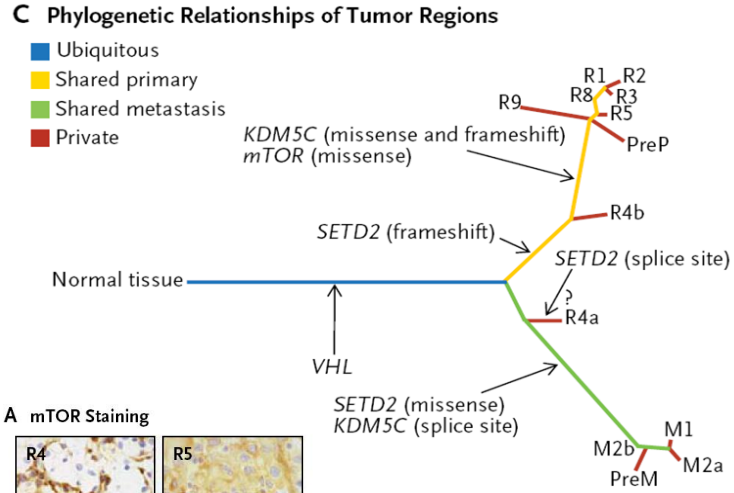
Heterogeneity of KRAS, BRAF and PIK3CA mutations in colorectal cancer

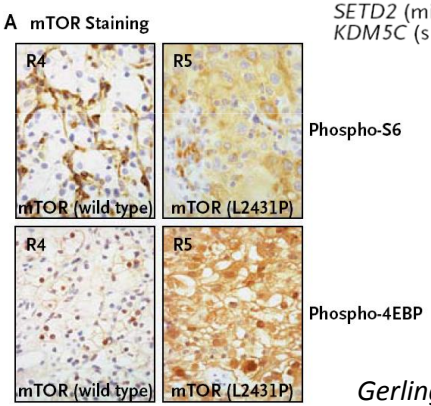


Baldus, SE. et al. Clin Cancer Res 2010

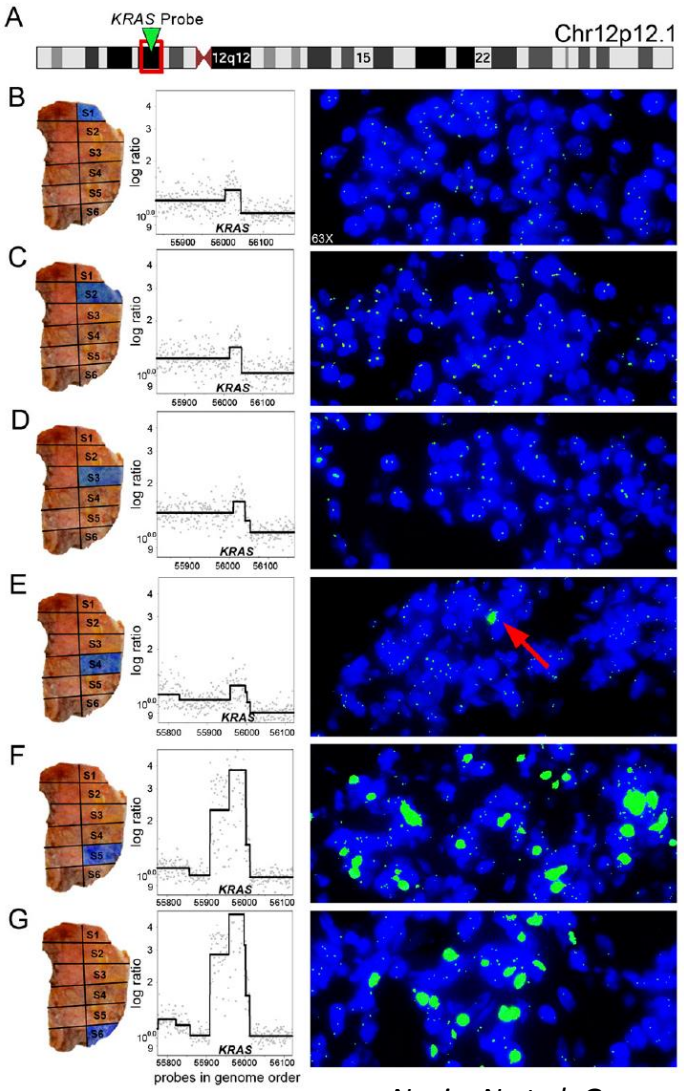
Intratumor heterogeneity: impact on biomarker detection





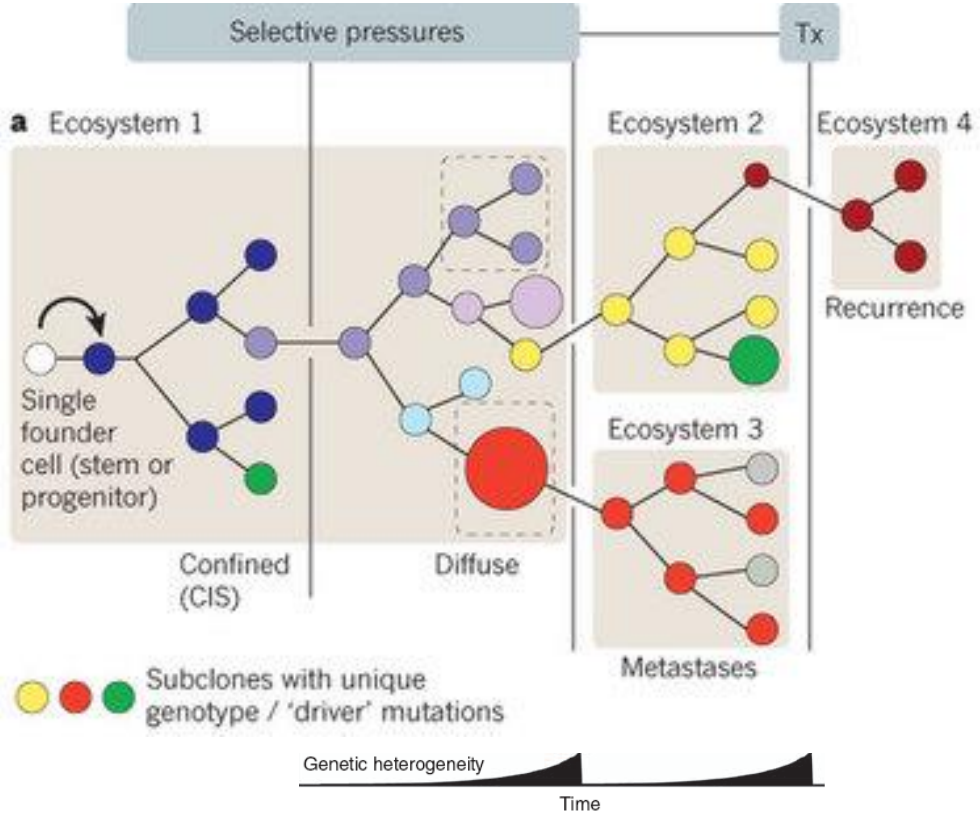


Gerlinger, M. et al. NEJM 2012



Navin, N et al. Genome Res 2010

Intratumor heterogeneity: impact on biomarker detection



Gerlinger, M and Swanton, C. BJC 2010

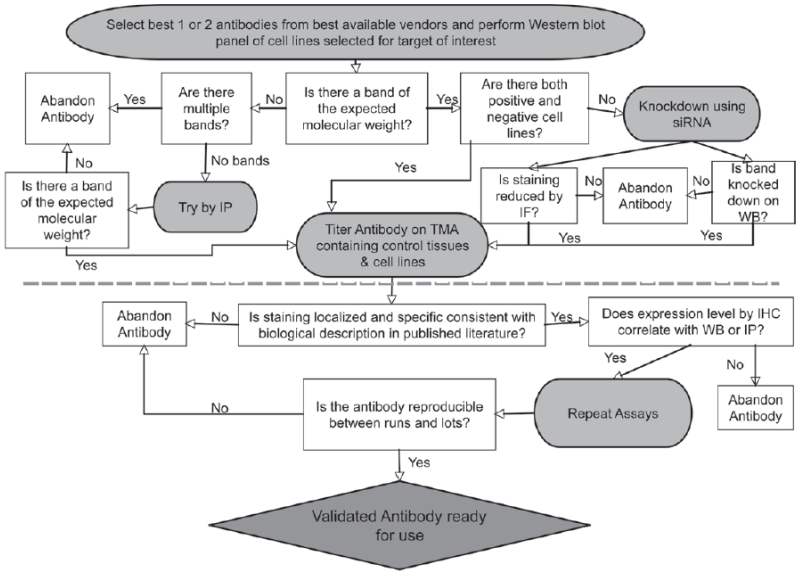
Limitations of single-biomarker (IHC) assays in cancer

Significant rates of discordance between local and central HER2 testing

Study	No. of cases retested	Method of retesting	Concordant cases, n (%)	Discordant cases, n (%)
NSABP B-31	104	IHC	85 (82)	19 (18)
		FISH	82 (79)	22 (21)
NCCTG 9831	119	IHC	88 (74)	31 (26)

Paik S, et al. J Natl Cancer Inst. 2002;94:852-854.
Roche PC, et al. J Natl Cancer Inst. 2002;94:855-857.

Approximately 20% of HER2 testing may be inaccurate



Method	Success rates
Western blot *only*	12/30 (40%)
IHC *only*	2/6 (33%)
Western blot/IHC	1/6 (17%)
Sandwich ELISA	1/30 (3%)
Western blot *and* sandwich ELISA <1 nM sensitivity	5/30 (17%)
Western blot *and* sandwich ELISA <100 pM sensitivity	1/30 (3%)
Western blot *and* sandwich ELISA <10 pM sensitivity	0/30 (0%)
Western blot *and* sandwich ELISA *and* IHC	1/6 (17%)
No performance in any application (i.e., complete failure)	11/30 (37%)

Bordeaux, J. et al. Biotechniques 2010

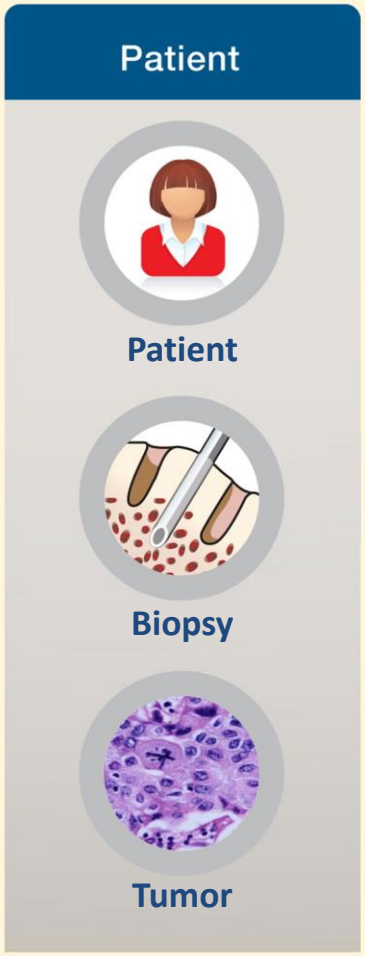
Brown, MC et al. Plos One 2011

Established technologies for KRAS mutation analysis

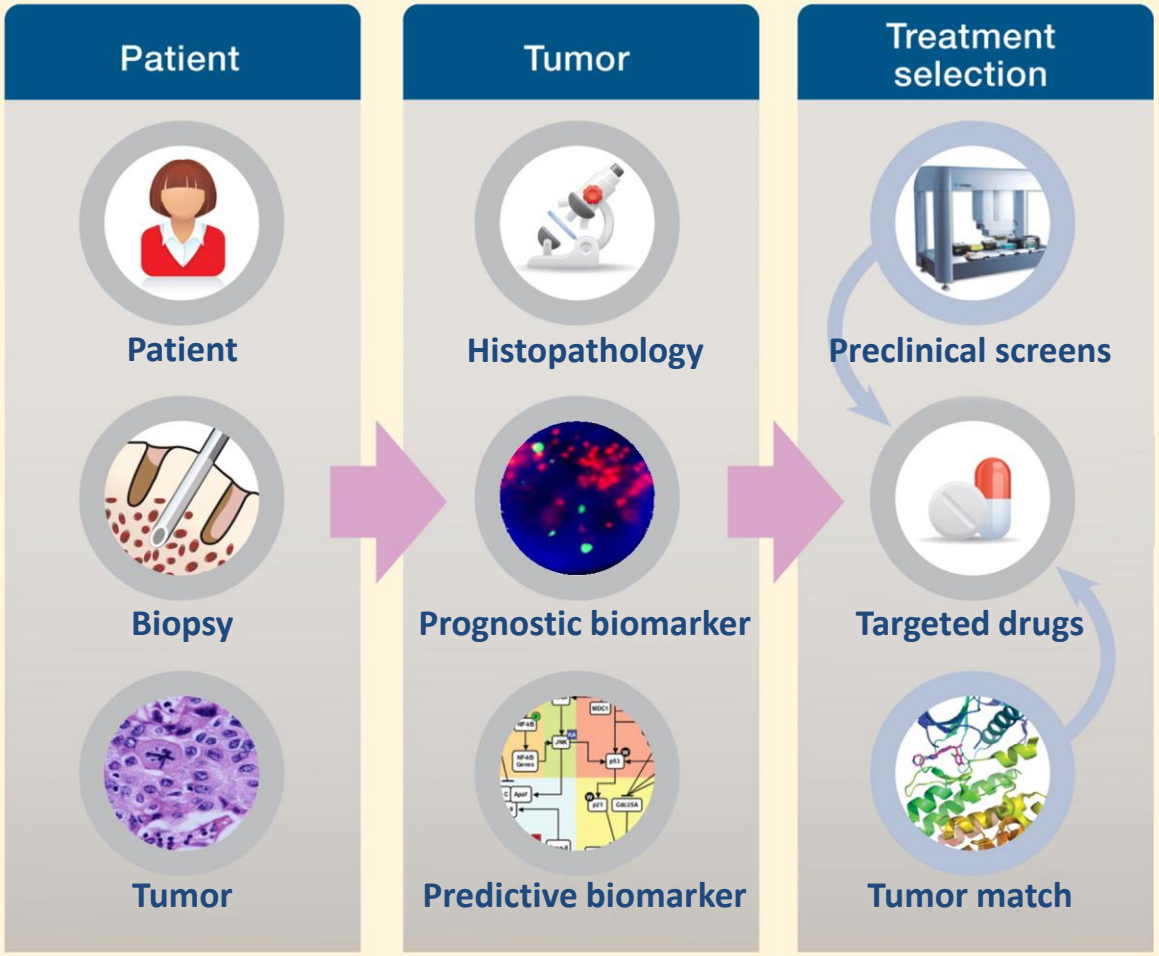
Method	Technology	Sensitivity, MT/WT%[a]	Time to Result	Pros	Cons
Direct sequencing					
Cycle sequencing	Sanger sequencing using dye-labeled dideoxynucleotide chain termination	15–25	4 d–2 wk (paraffin)	Gold standard Detects all mutations	Insensitive Labor intensive
Pyrosequencing	Measures pyrophosphate release during DNA extension	5–10	Fast	High-throughput Precise/reproducible Suitable for partially degraded samples	Expensive
PCR-based methods					
ARMS[a]	Mutation-specific PCR amplification	1	Rapid: <2 d (paraffin)	High sensitivity Rapid results	Detects single mutation per reaction Requires engineered primer/probe
TheraScreen[a]	Combination of ARMS, Scorpions[b] (allele-specific probe), and real-time PCR	1–5	Rapid: 2 d 2 h to process samples	Rapid results High sensitivity Commercially available	Detects only 7 common mutations Requires more tissue Very expensive
Allele-specific oligonucleotide hybridization					
Allele-specific probes	Probes hybridize to wild-type or mutant sequence impacting melting temperature	10	Rapid: <2 d (paraffin)	Rapid results	Low sensitivity
ViennaLab[b]	Hybridization of PCR products to array of allele-specific oligonucleotides	1	Rapid: 6 h to process samples	Detects 13 common mutations Less expensive than TheraScreen[b]	Complicated data interpretation

Ross, JS. Arch Pathol Lab Med 2012

Envisioning diagnostic medicine



Envisioning diagnostic medicine



Envisioning diagnostic medicine