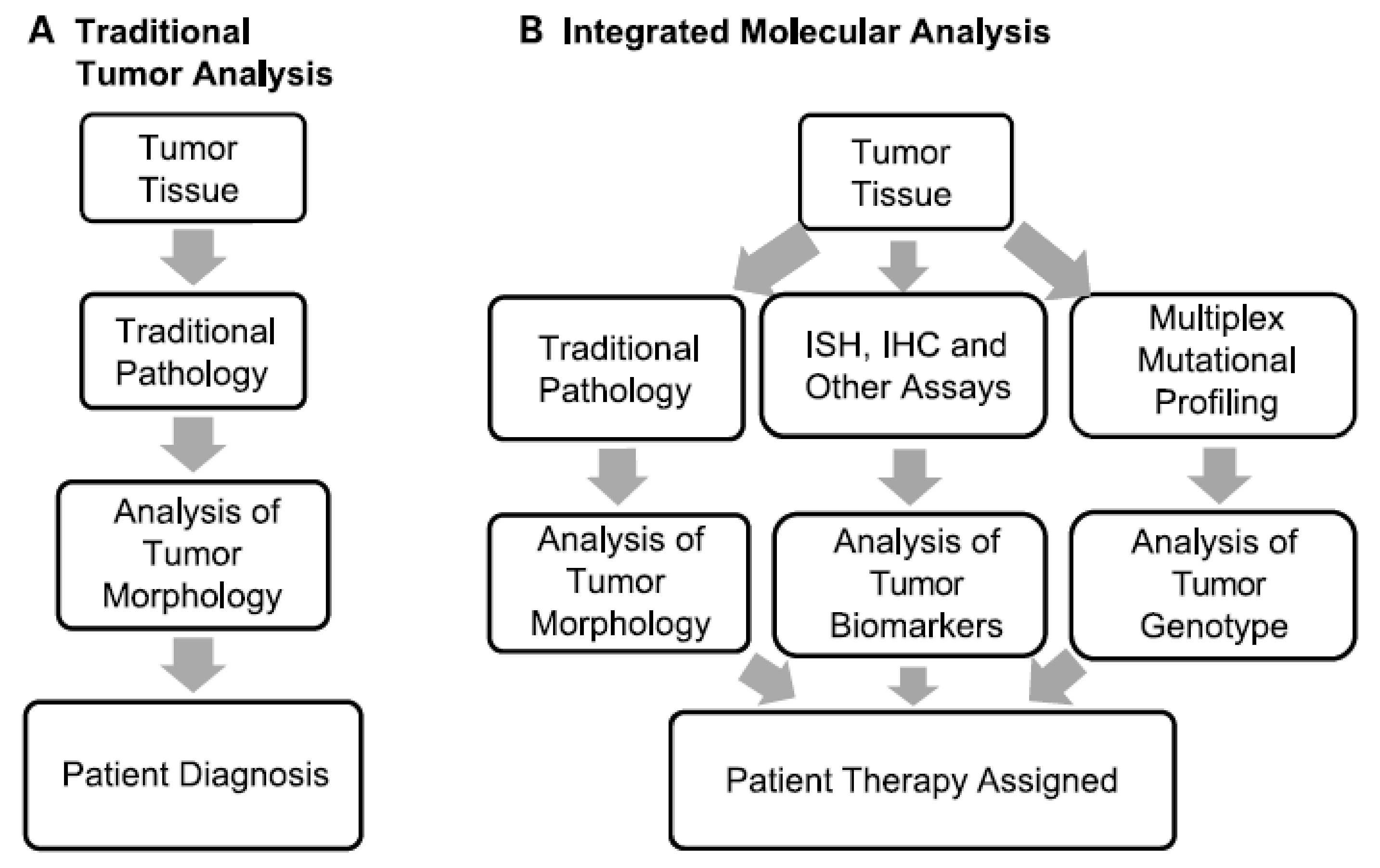
Il patologo e la terapia oncologica personalizzata

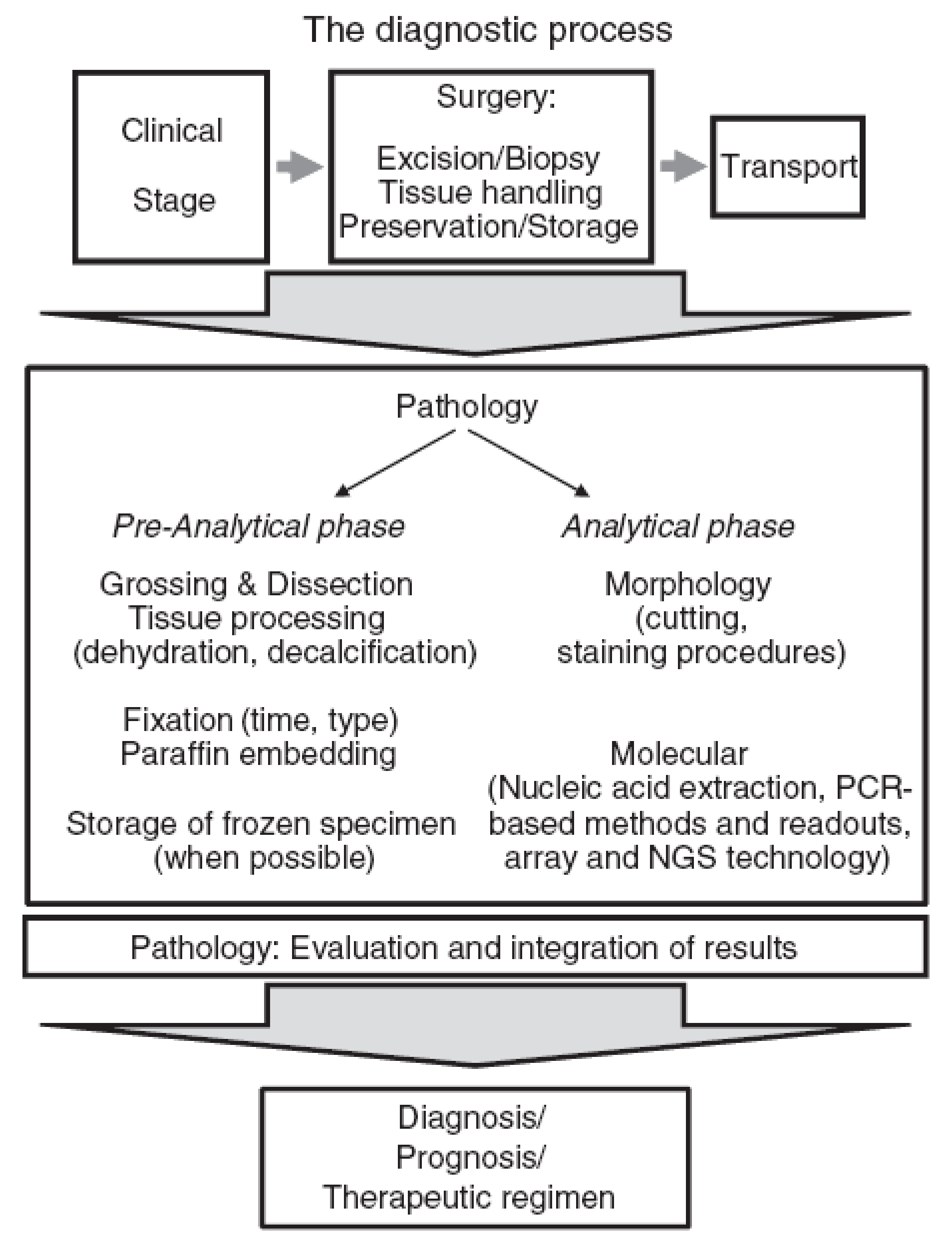
Prof. Giovanni Lanza
U.O. Anatomia Patologica

Qualità e innovazione nel dipartimento di diagnostica per immagini e medicina di laboratorio di Ferrara: IIa edizione

Cona, 25 maggio 2013

Integration of molecular pathology into cancer clinic





The diagnostic process

Clinical Stage

Surgery:

Excision/Biopsy
Tissue handling
Preservation/Storage

Transport

Pathology

Pre-Analytical phase

Grossing & Dissection
Tissue processing
(dehydration, decalcification)

Fixation (time, type)
Paraffin embedding

Storage of frozen specimen
(when possible)

Analytical phase

Morphology
(cutting,
staining procedures)

Molecular
(Nucleic acid extraction, PCR-based methods and readouts, array and NGS technology)

Pathology: Evaluation and integration of results

Diagnosis/
Prognosis/
Therapeutic regimen

Raccomandazioni per l'analisi dei riarrangiamenti del gene ALK nel carcinoma polmonare non a piccole cellule

A cura del Gruppo di Lavoro di AIOM e SIAPEC-IAP

AIOM: Andrea Ardizzoni, Lucio Crinò, Cesare Gridelli,
Nicola Normanno, Giorgio Scagliotti, Carmine Pinto *(Coordinatore)*

SIAPEC-IAP: Antonio Marchetti, Mauro Papotti, Giulio Rossi,
Massimo Barberis, Eugenio Maiorano, Gian Luigi Taddei,
Claudio Clemente *(Coordinatore)*

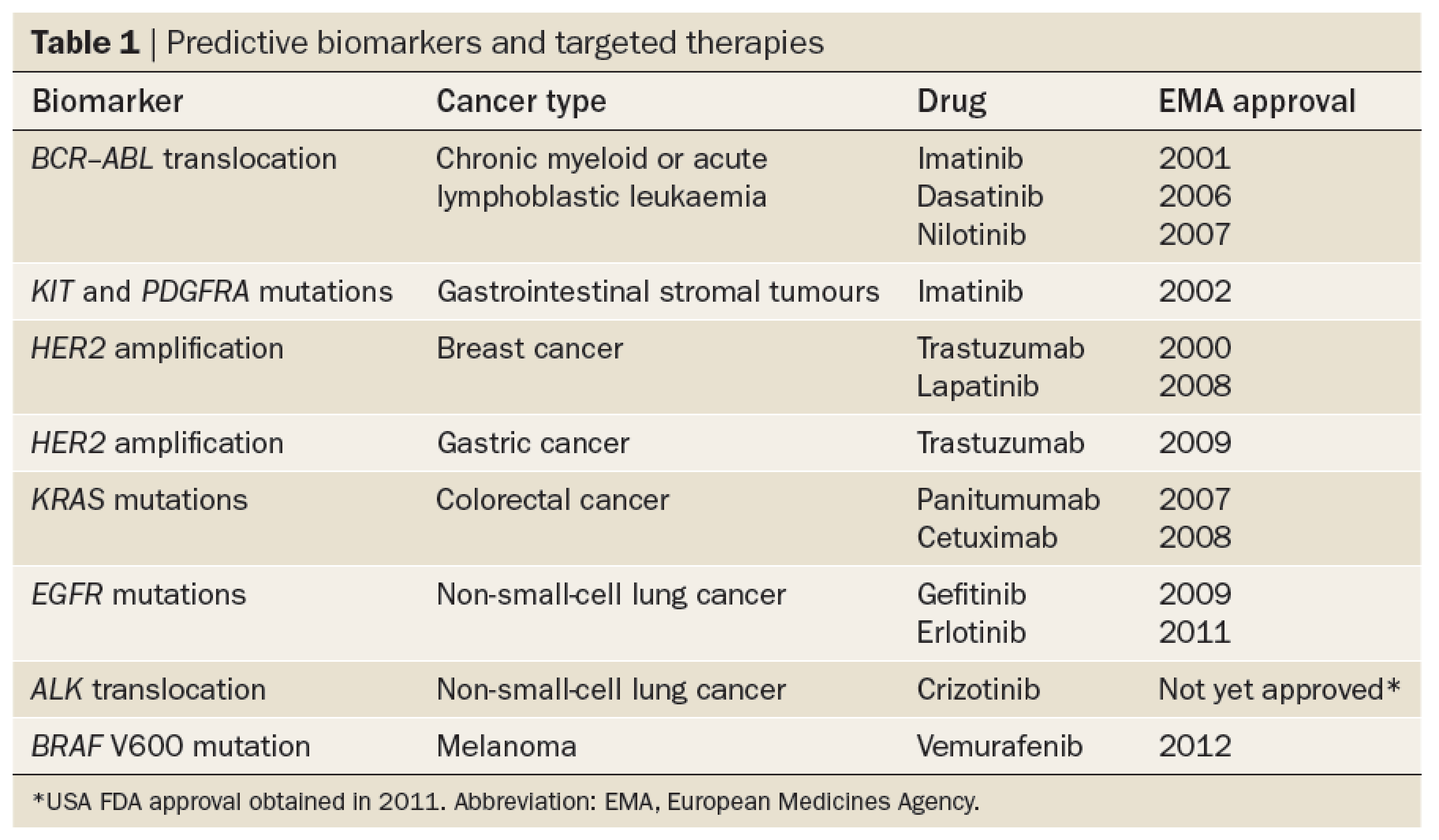


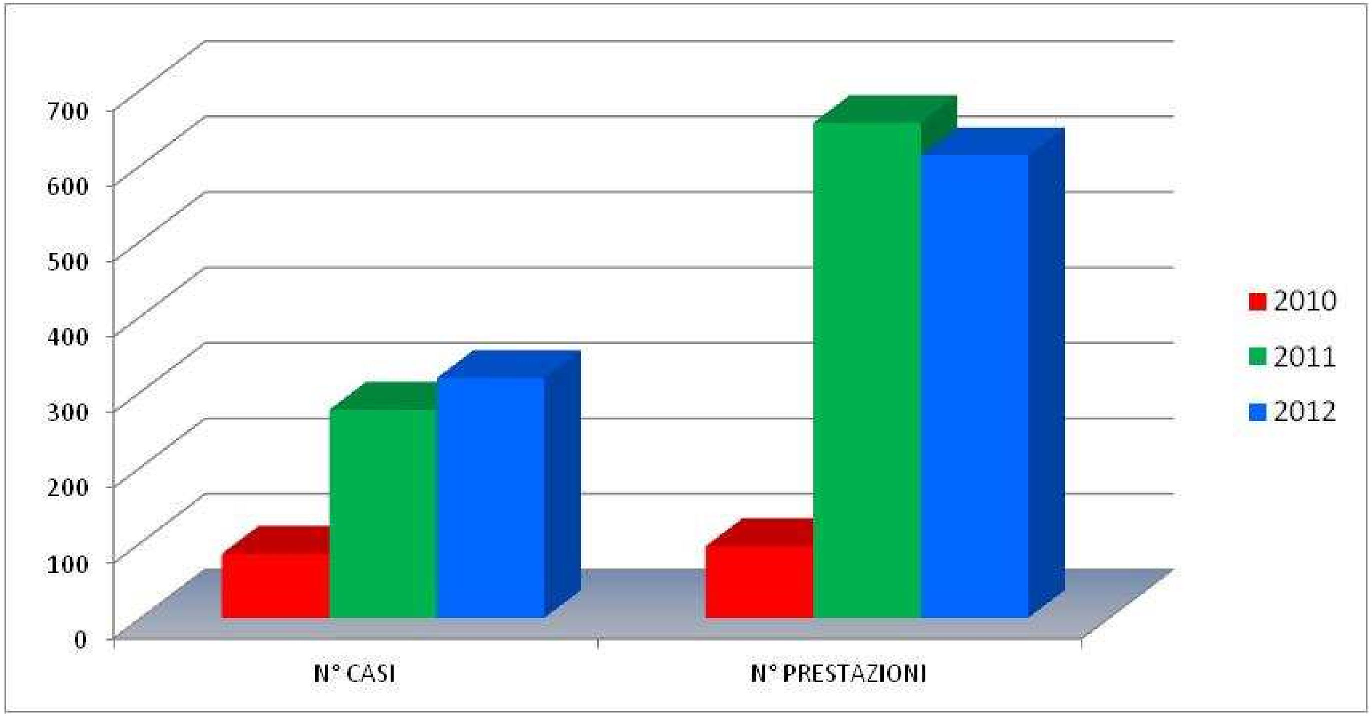
Table 1 | Predictive biomarkers and targeted therapies

Biomarker	Cancer type	Drug	EMA approval
BCR–ABL translocation	Chronic myeloid or acute lymphoblastic leukaemia	Imatinib Dasatinib Nilotinib	2001 2006 2007
KIT and *PDGFRA* mutations	Gastrointestinal stromal tumours	Imatinib	2002
HER2 amplification	Breast cancer	Trastuzumab Lapatinib	2000 2008
HER2 amplification	Gastric cancer	Trastuzumab	2009
KRAS mutations	Colorectal cancer	Panitumumab Cetuximab	2007 2008
EGFR mutations	Non-small-cell lung cancer	Gefitinib Erlotinib	2009 2011
ALK translocation	Non-small-cell lung cancer	Crizotinib	Not yet approved*
BRAF V600 mutation	Melanoma	Vemurafenib	2012

*USA FDA approval obtained in 2011. Abbreviation: EMA, European Medicines Agency.

DIAGNOSTICA BIOTECNOLOGICA: ANALISI DI SEGMENTI DI DNA MEDIANTE SEQUENZIAMENTO

	2010	2011	2012
N°CASI	85	276	318
N°PRESTAZIONI	95	656	613
RICAVI €	14.815,25	102.303,20	95.597,35

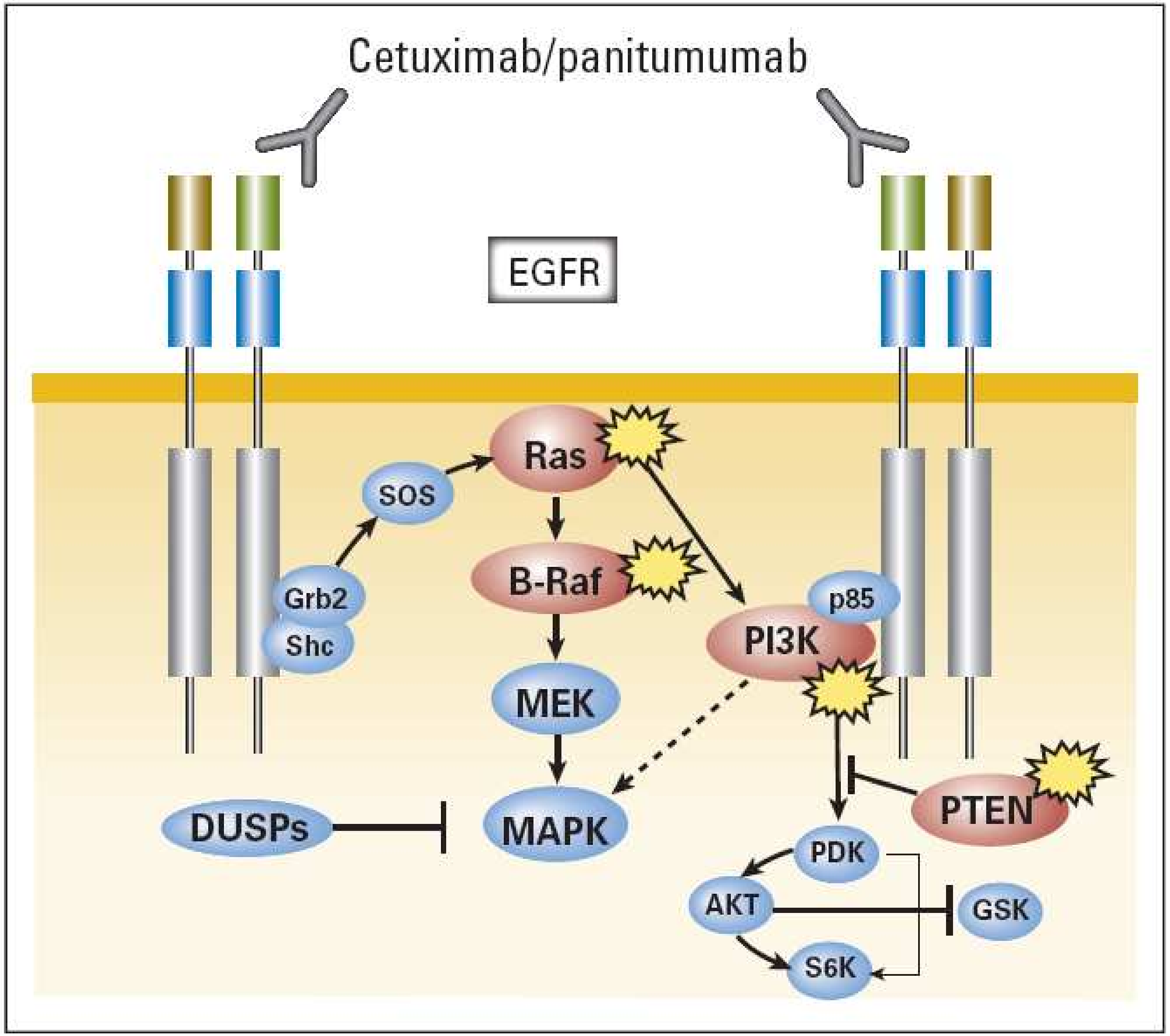


Carcinoma del colon-retto

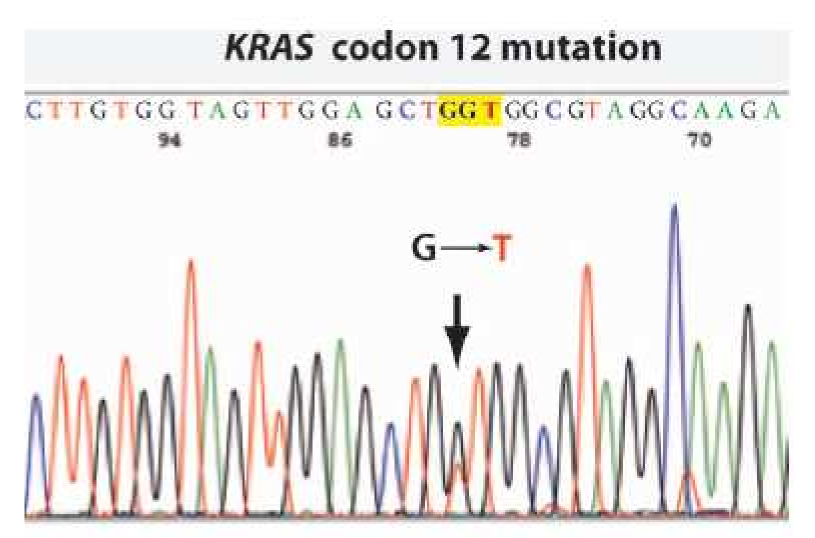
- **Mutazione di KRAS**
- Mutazione di BRAF
- Mutazione di PIK3CA
- Instabilità dei microsatelliti (MSI)
- Espressione proteine del mismatch repair
- Metilazione promoter MLH1
- Espressione di MGMT
- Metilazione promoter MGMT

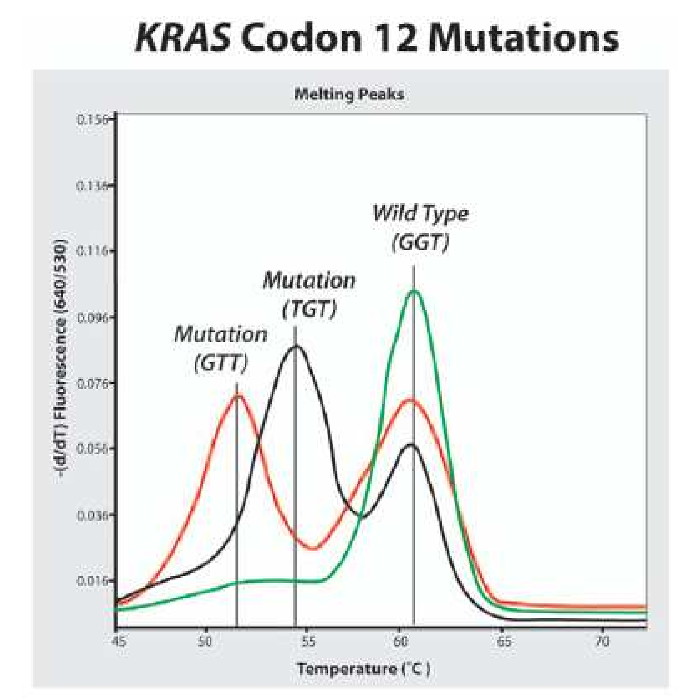
KRAS oncogene

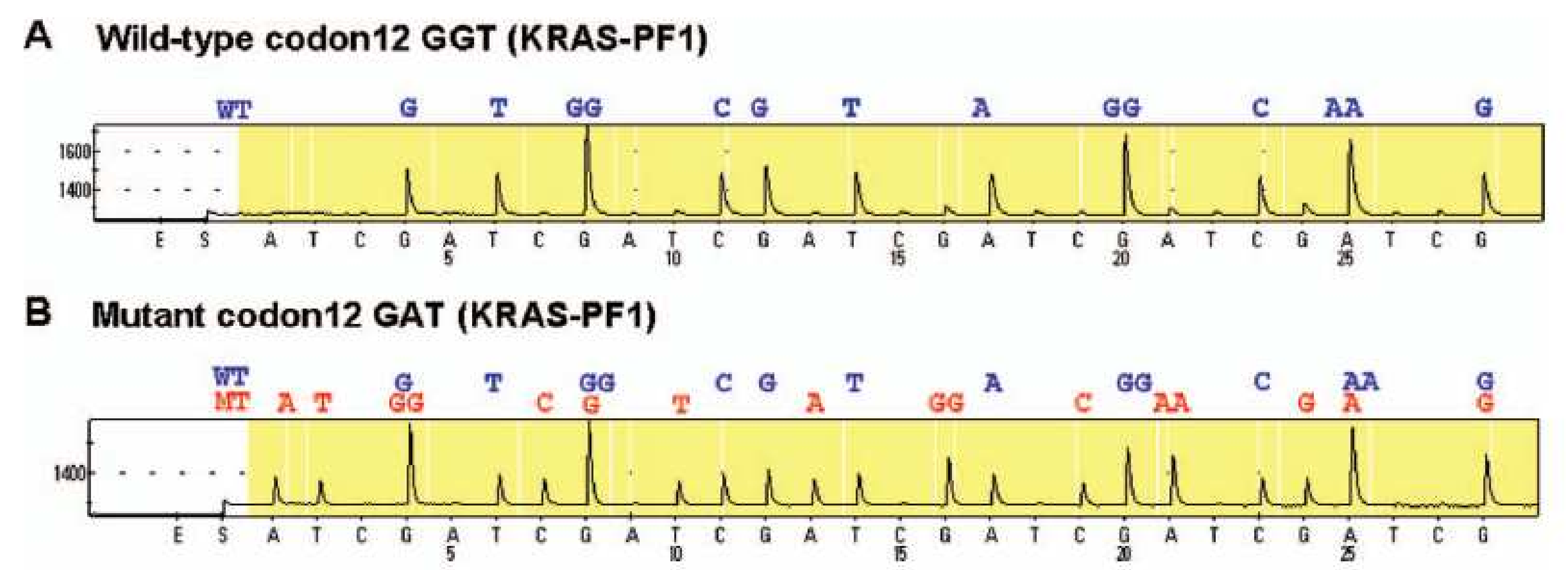
- Mutated in approximately 35% to 45% of CRCs
- Somatic missense mutations that lead to single amino acid substitutions
- The most frequent alterations are detected in codons 12 (~82% of all reported mutations) and 13 (~17%) in exon 2. Mutations in other positions (such as codon 61) account for a minor proportion (1-4%) of cases
- *KRAS* mutation is an early event in colorectal carcinogenesis


Cetuximab/panitumumab
EGFR
Ras
SOS
B-Raf
Grb2
Shc
p85
PI3K
MEK
MAPK
DUSPs
PTEN
PDK
AKT
GSK
S6K

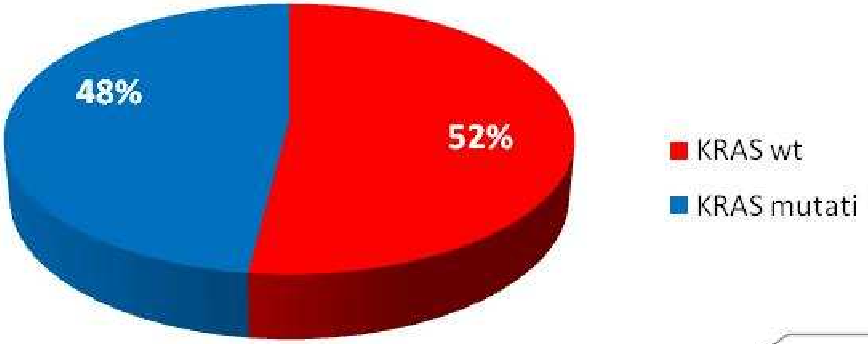
KRAS Mutation Analysis Methods

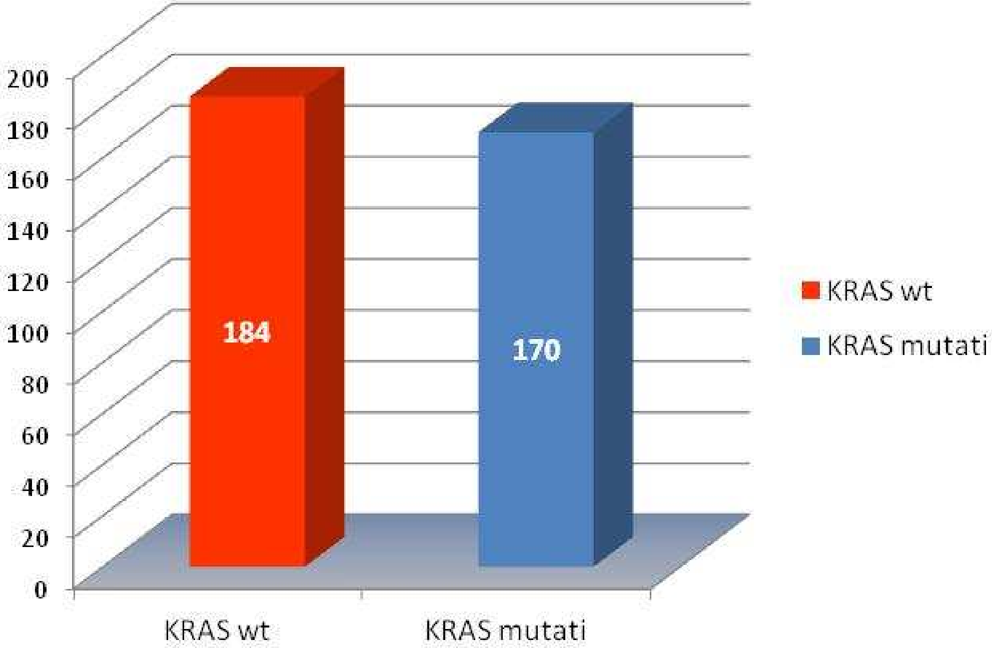




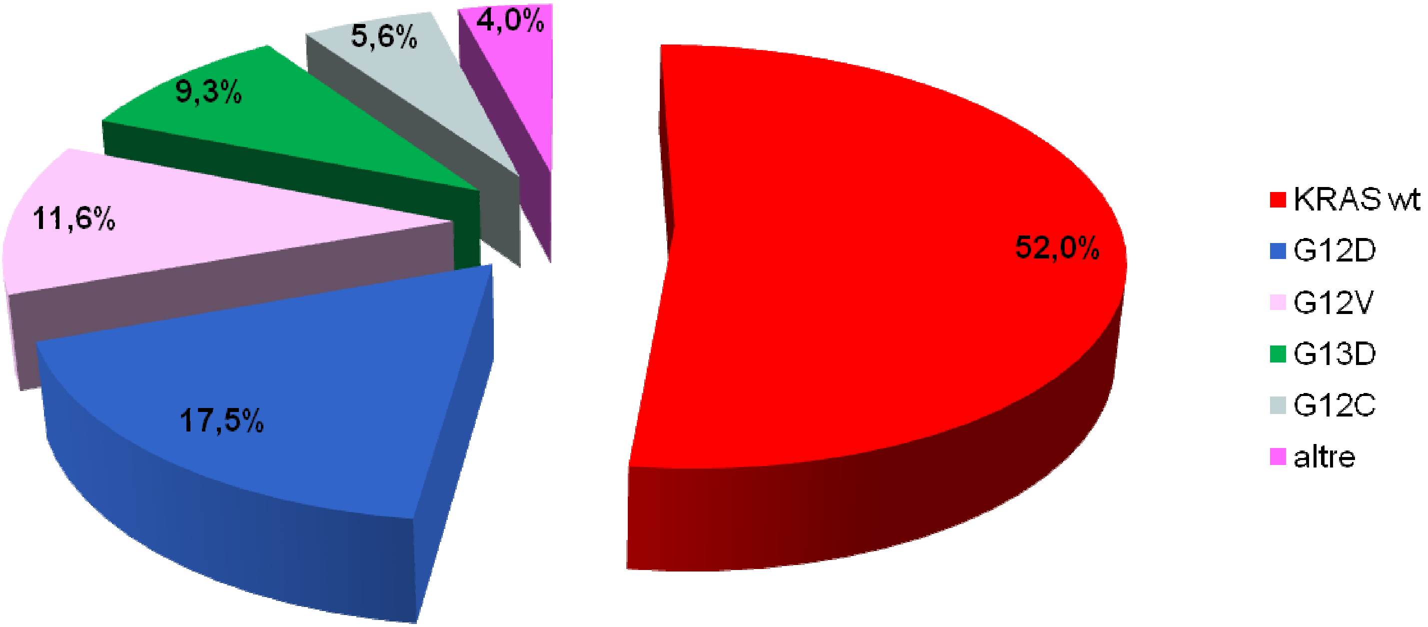


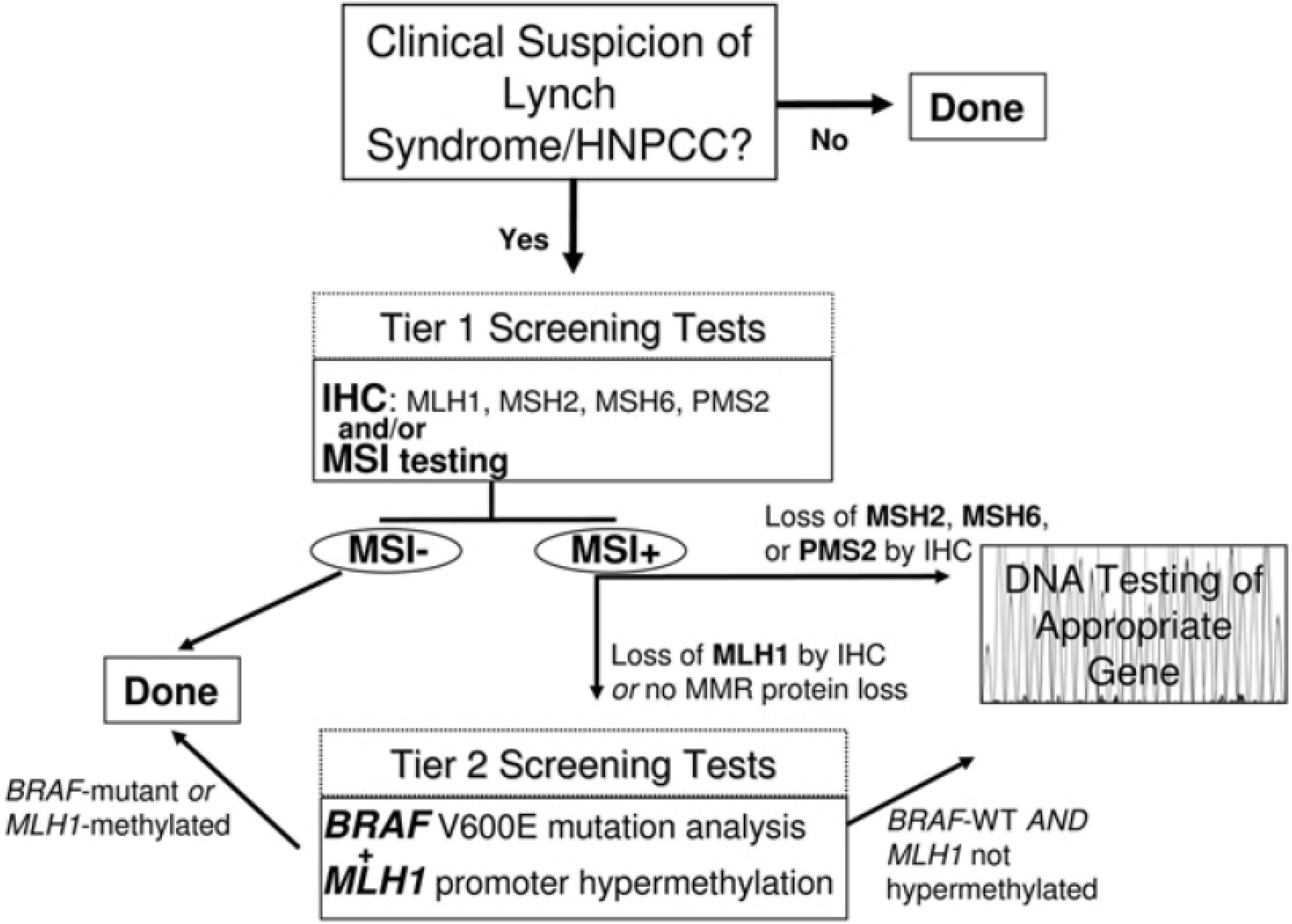
CARCINOMA DEL COLON-RETTO
Analisi molecolare mutazione KRAS 2009-2013 (354 casi)





CARCINOMA DEL COLON-RETTO
Analisi biomolecolare mutazione KRAS 2009-2013 (354 casi)





Clinical Suspicion of Lynch Syndrome/HNPCC?

No

Done

Yes

Tier 1 Screening Tests

IHC: MLH1, MSH2, MSH6, PMS2
and/or
MSI testing

MSI-

MSI+

Done

Loss of **MSH2**, **MSH6**, or **PMS2** by IHC

DNA Testing of Appropriate Gene

Loss of **MLH1** by IHC *or* no MMR protein loss

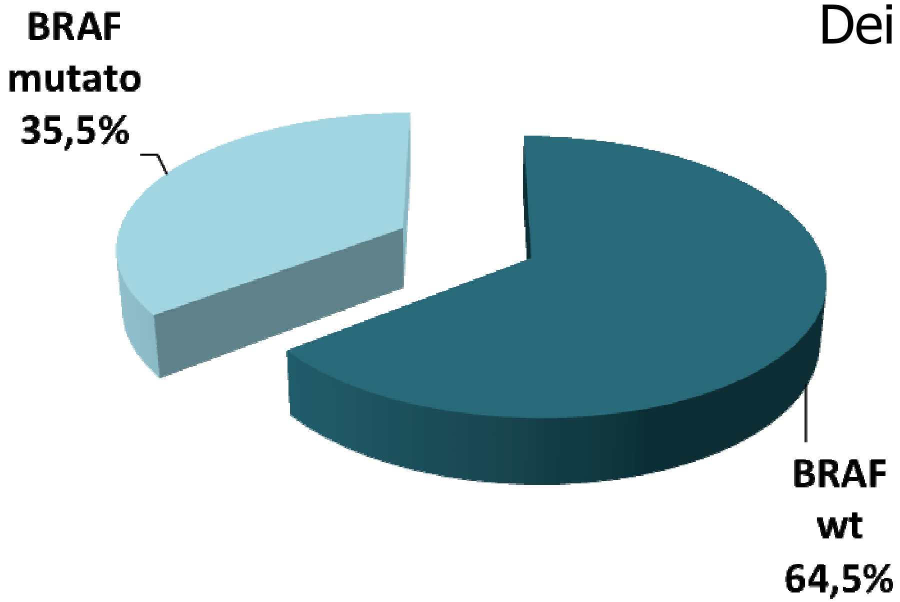
Tier 2 Screening Tests

BRAF V600E mutation analysis
\+
MLH1 promoter hypermethylation

BRAF-mutant *or* *MLH1*-methylated

BRAF-WT *AND* *MLH1* not hypermethylated

Analisi mutazionale di BRAF

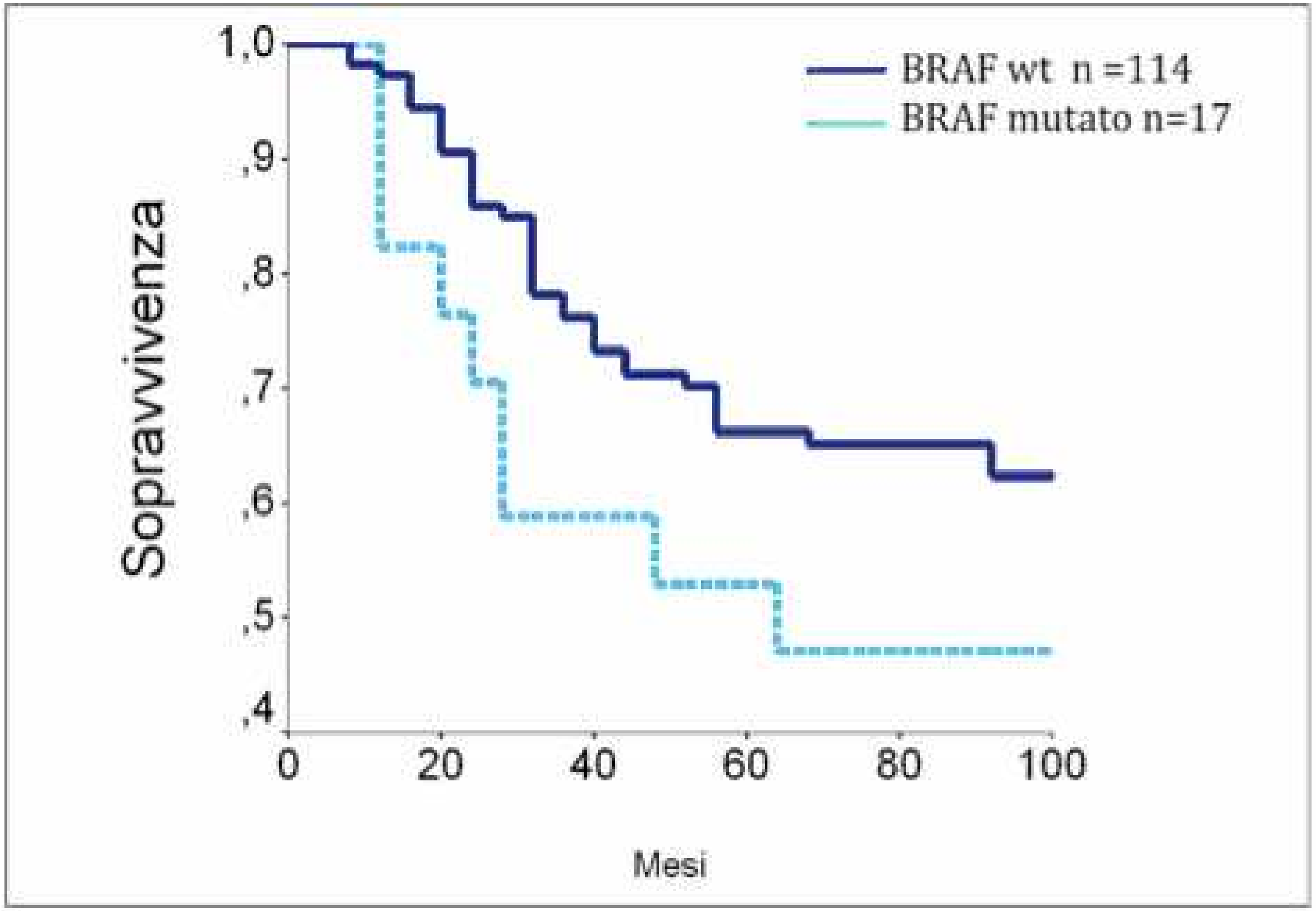


Dei 699 carcinomi colorettali analizzati:

- **248 (35,5%)** con mutazione V600E
- **451 (64,5%)** wild type

BRAF	**MMR-p**	**MMR-d**
V600E	40 (11,8%)	208 (57,8%)
Wild type	299 (88,2%)	152 (42,2%)

BRAF status e sopravvivenza



131 pazienti con tumore MMR-p

Sopravvivenza tumore specifica a 5 anni:

- **66% BRAF wild type**
- **47% BRAF mutato**

P = 0,08

Carcinoma polmonare

- Mutazione di EGFR
- Mutazione di KRAS
- ALK
- Mutazione di BRAF

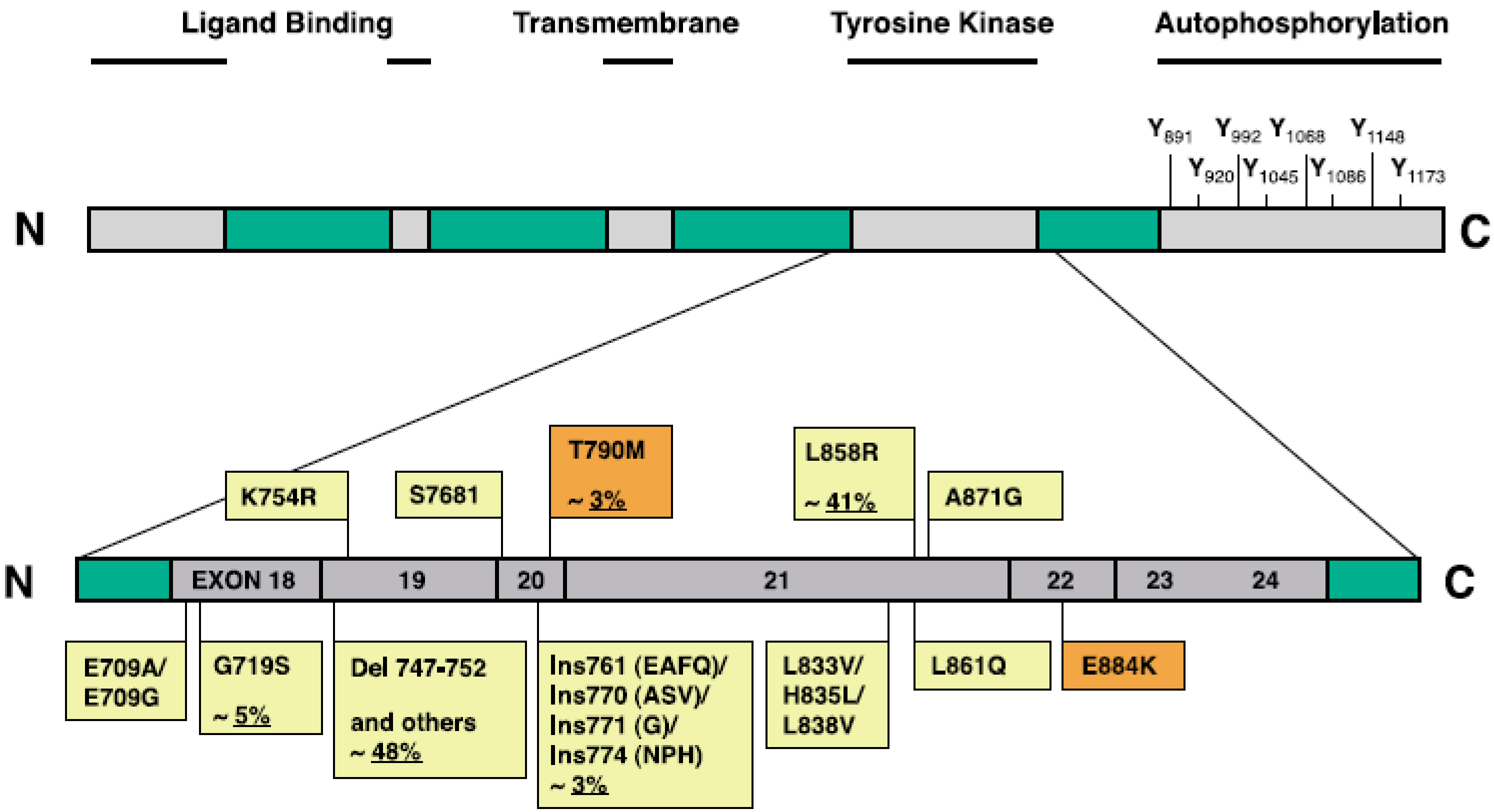


Figure 1 EGFR kinase domain mutations in NSCLC. Structural organization of EGFR and localization of clinically relevant alterations in the kinase domain (yellow boxes). Frequencies are indicated and mutations that may confer resistance to gefitinib (T790M) or erlotinib (T790M, E884K) are highlighted (orange boxes).

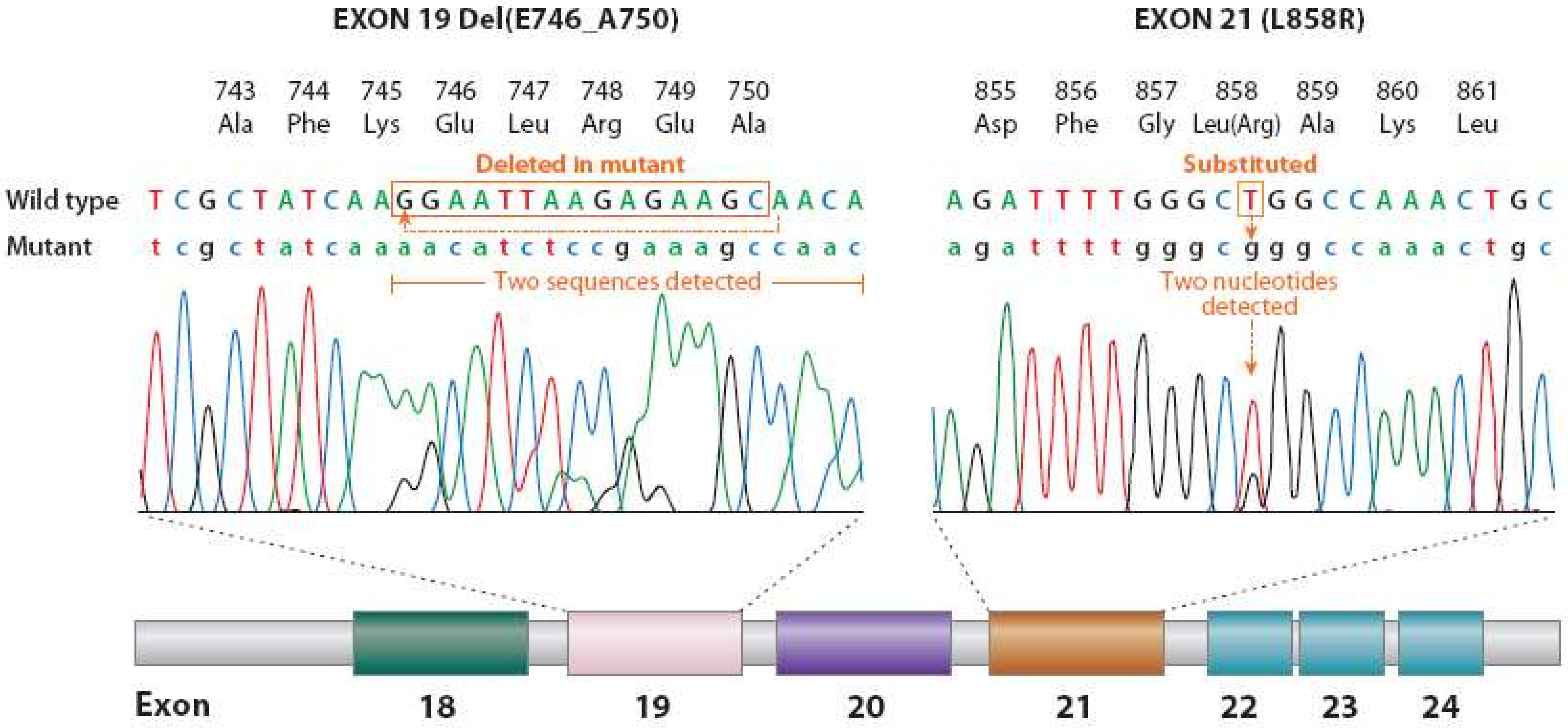
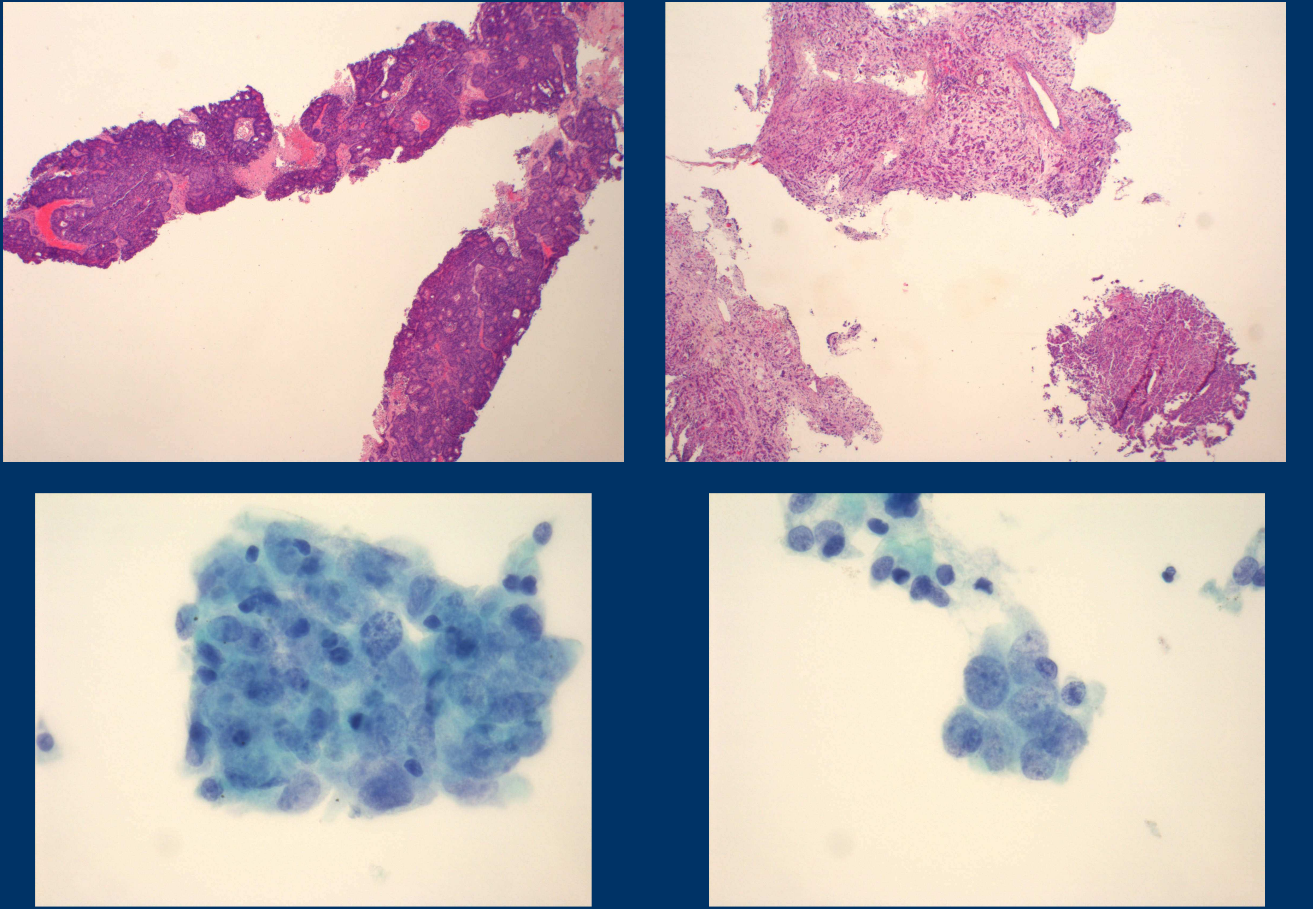
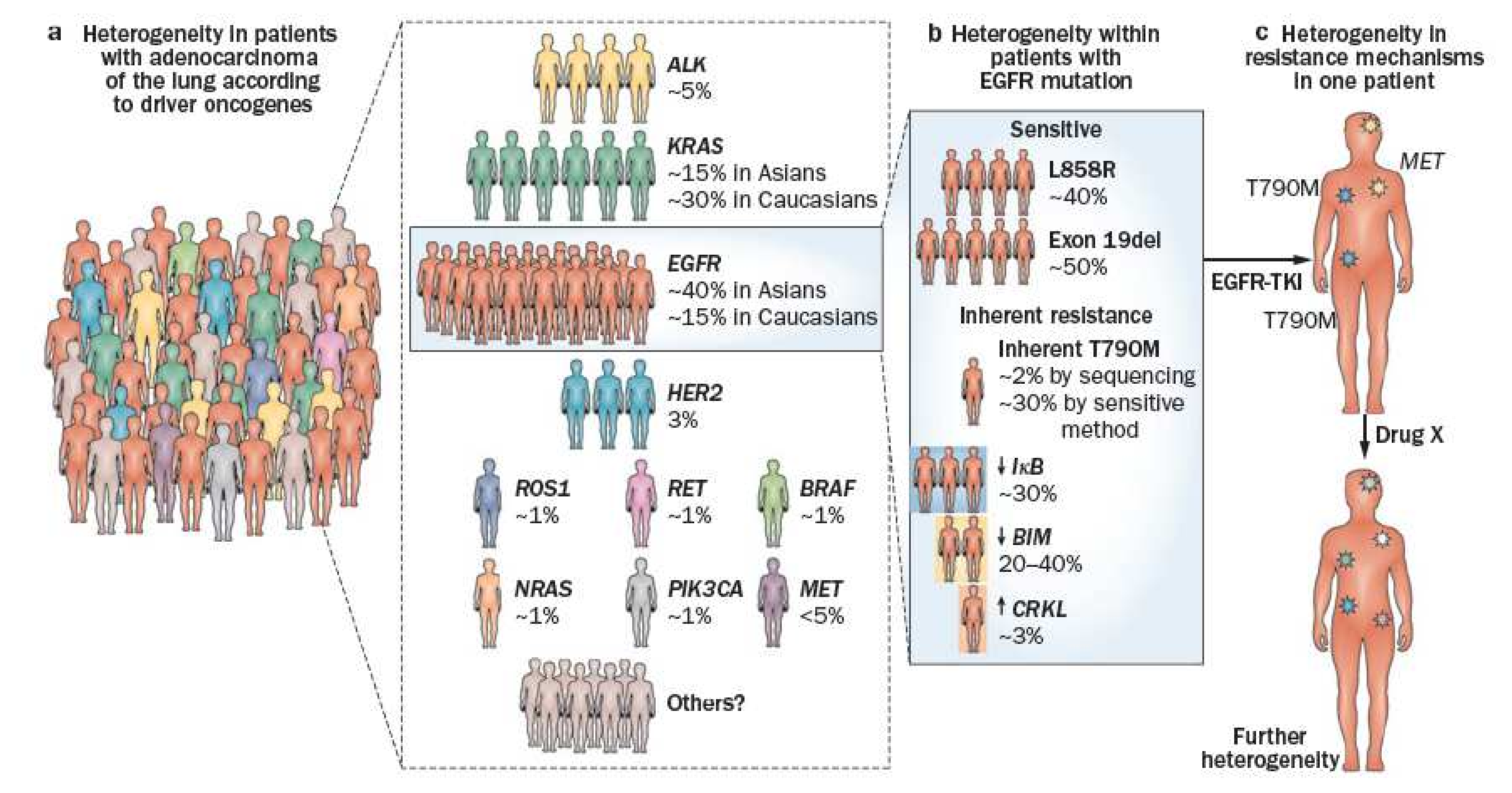


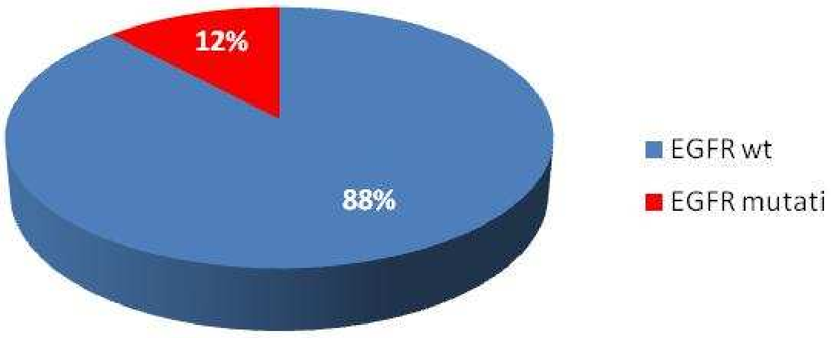
Figure 2

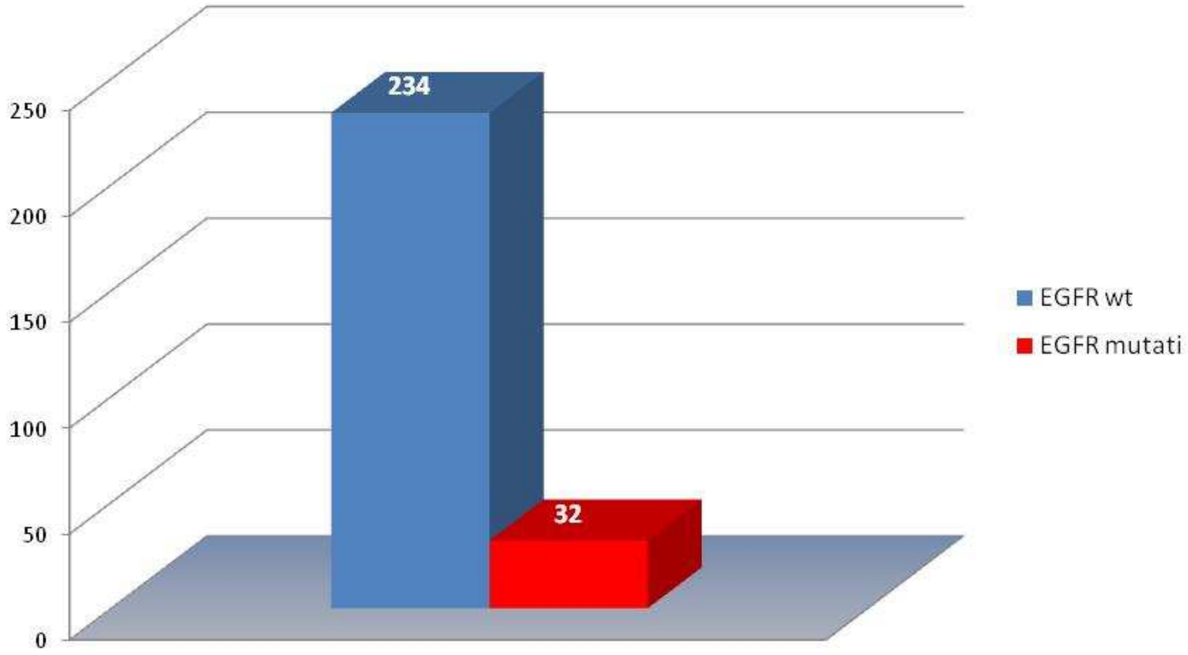
Amino acid and nucleotide sequence changes in exon 19 deletion and exon 21 L858R mutations involving the tyrosine kinase domain of epidermal growth factor receptor.



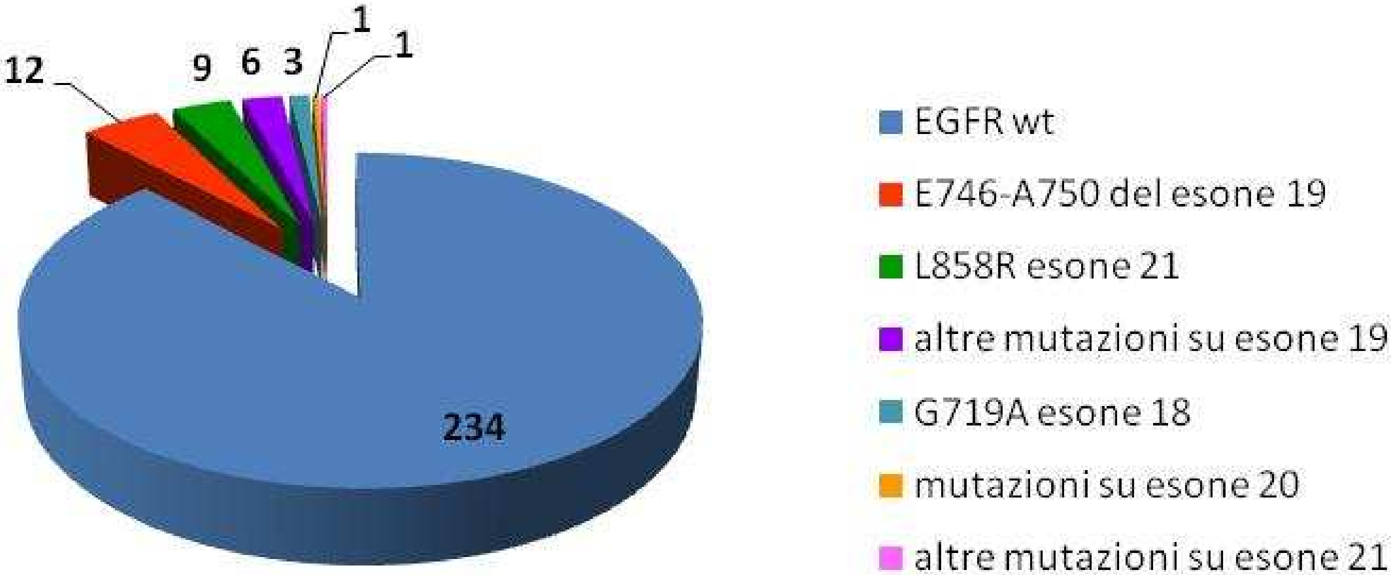

a Heterogeneity in patients with adenocarcinoma of the lung according to driver oncogenes
ALK
~5%
KRAS
~15% in Asians
~30% in Caucasians
EGFR
~40% in Asians
~15% in Caucasians
HER2
3%
ROS1
~1%
RET
~1%
BRAF
~1%
NRAS
~1%
PIK3CA
~1%
MET
<5%
Others?
b Heterogeneity within patients with EGFR mutation
Sensitive
L858R
~40%
Exon 19del
~50%
Inherent resistance
Inherent T790M
~2% by sequencing
~30% by sensitive method
↓ IκB
~30%
↓ BIM
20–40%
↑ CRKL
~3%
c Heterogeneity in resistance mechanisms in one patient
MET
T790M
T790M
EGFR-TKI
Drug X
Further heterogeneity

ADENOCARCINOMA DEL POLMONE
Analisi biomolecolare mutazione EGFR (266 casi: 200 istologici, 66 citologici)

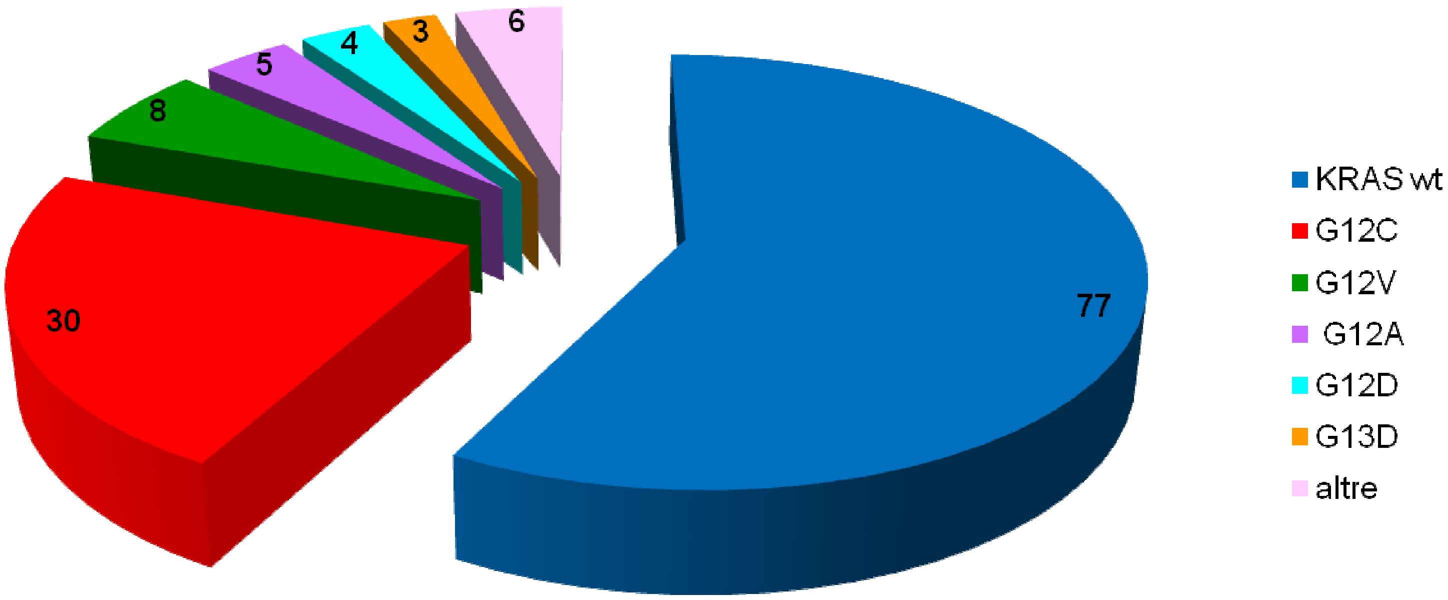




ADENOCARCINOMA DEL POLMONE
Analisi biomolecolare mutazione EGFR (266 casi: 200 istologici, 66 citologici)



ADENOCARCINOMA DEL POLMONE
Analisi biomolecolare mutazione KRAS (133 casi)

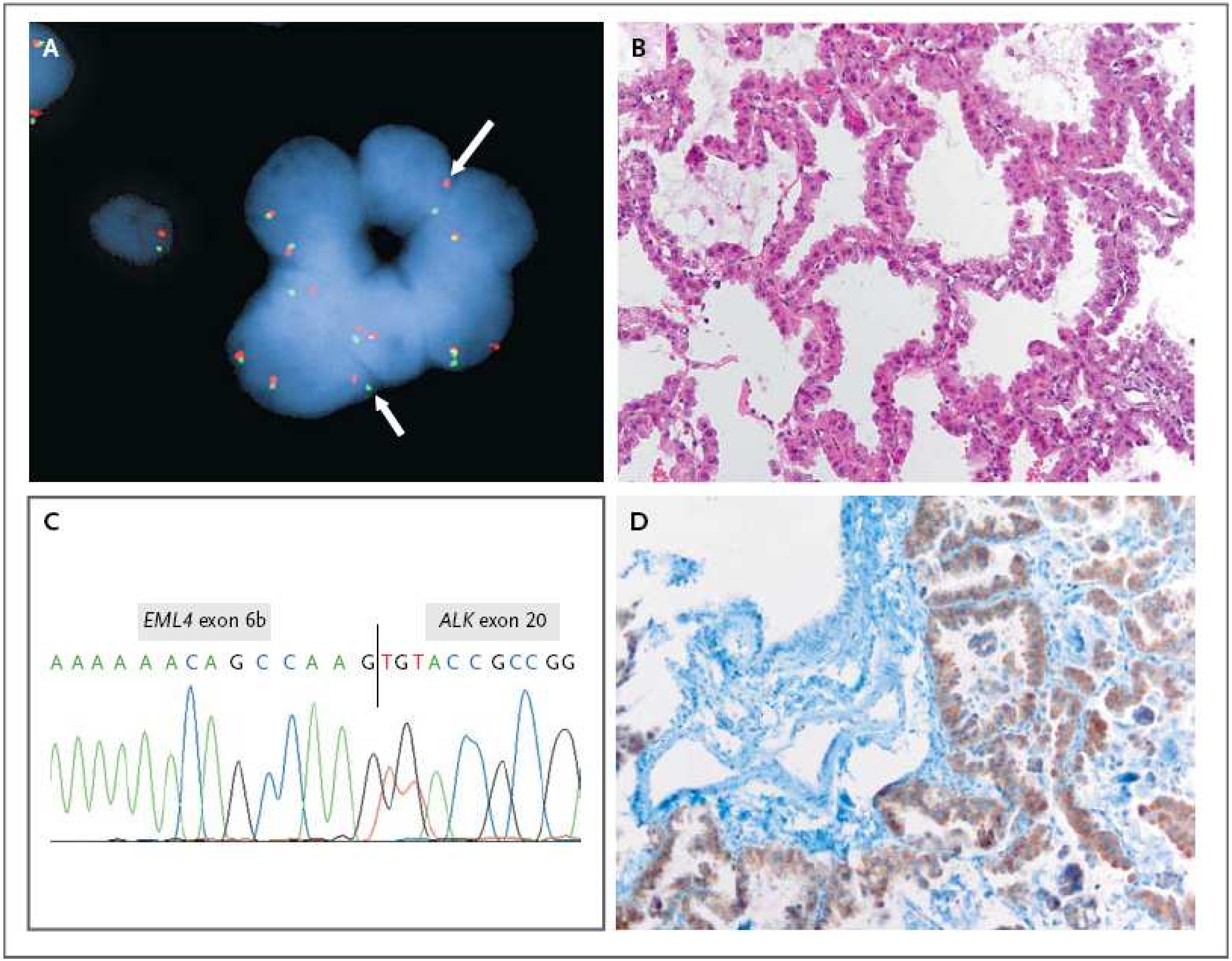


The NEW ENGLAND
JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 28, 2010 VOL. 363 NO. 18

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Eunice L. Kwak, M.D., Ph.D., Yung-Jue Bang, M.D., Ph.D., D. Ross Camidge, M.D., Ph.D.,
Alice T. Shaw, M.D., Ph.D., Benjamin Solomon, M.B., B.S., Ph.D., Robert G. Maki, M.D., Ph.D.,
Sai-Hong I. Ou, M.D., Ph.D., Bruce J. Dezube, M.D., Pasi A. Jänne, M.D., Ph.D., Daniel B. Costa, M.D., Ph.D.,
Marileila Varella-Garcia, Ph.D., Woo-Ho Kim, M.D., Thomas J. Lynch, M.D., Panos Fidias, M.D.,
Hannah Stubbs, M.S., Jeffrey A. Engelman, M.D., Ph.D., Lecia V. Sequist, M.D., M.P.H., WeiWei Tan, Ph.D.,
Leena Gandhi, M.D., Ph.D., Mari Mino-Kenudson, M.D., Greg C. Wei, Ph.D., S. Martin Shreeve, M.D., Ph.D.,
Mark J. Ratain, M.D., Jeffrey Settleman, Ph.D., James G. Christensen, Ph.D., Daniel A. Haber, M.D., Ph.D.,
Keith Wilner, Ph.D., Ravi Salgia, M.D., Ph.D., Geoffrey I. Shapiro, M.D., Ph.D., Jeffrey W. Clark, M.D.,
and A. John Iafrate, M.D., Ph.D.


A
B
C
EML4 exon 6b
ALK exon 20
A A A A A A C A G C C A A G T G T A C C G C C G G
D

Imaging, Diagnosis, Prognosis

Clinical
Cancer
Research

A Novel, Highly Sensitive Antibody Allows for the Routine Detection of *ALK*-Rearranged Lung Adenocarcinomas by Standard Immunohistochemistry

Mari Mino-Kenudson[1], Lucian R. Chirieac[2], Kenny Law[2], Jason L. Hornick[2], Neal Lindeman[2], Eugene J. Mark[1], David W. Cohen[3], Bruce E. Johnson[4], Pasi A. Jänne[4], A. John Iafrate[1], and Scott J. Rodig[2]

Abstract

Purpose: Approximately 5% of lung adenocarcinomas harbor an *EML4-ALK* gene fusion and define a unique tumor group that may be responsive to targeted therapy. However *ALK*-rearranged lung adenocarcinomas are difficult to detect by either standard fluorescence *in situ* hybridization or immunohistochemistry (IHC) assays. In the present study, we used novel antibodies to compare ALK protein expression in genetically defined lung cancers and anaplastic large cell lymphomas.

Experimental Design: We analyzed 174 tumors with one standard and two novel monoclonal antibodies recognizing the ALK protein. Immunostained tissue sections were assessed for the level of tumor-specific ALK expression by objective quantitative image analysis and independently by three pathologists.

Results: ALK protein is invariably and exclusively expressed in *ALK*-rearranged lung adenocarcinomas but at much lower levels than in the prototypic *ALK*-rearranged tumor, anaplastic large cell lymphoma, and as a result, is often not detected by conventional IHC. We further validate a novel IHC that shows excellent sensitivity and specificity (100% and 99%, respectively) for the detection of *ALK*-rearranged lung adenocarcinomas in biopsy specimens, with excellent interobserver agreement between pathologists (κ statistic, 0.94).

Conclusions: Low levels of ALK protein expression is a characteristic feature of *ALK*-rearranged lung adenocarcinomas. However, a novel, highly sensitive IHC assay reliably detects lung adenocarcinomas with *ALK* rearrangements and obviates the need for fluorescence *in situ* hybridization analysis for the majority of cases, and therefore could be routinely applicable in clinical practice to detect lung cancers that may be responsive to ALK inhibitors. *Clin Cancer Res; 16(5); 1561–71. ©2010 AACR.*

The Journal of Molecular Diagnostics, Vol. 15, No. 3, May 2013

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the Journal of Molecular Diagnostics

jmd.amjpathol.org

ALK Status Testing in Non–Small Cell Lung Carcinoma

Correlation Between Ultrasensitive IHC and FISH

Eugen C. Minca,* Bryce P. Portier,* Zhen Wang,* Christopher Lanigan,* Carol F. Farver,* Yan Feng,† Patrick C. Ma,†
Valeria A. Arrossi,* Nathan A. Pennell,† and Raymond R. Tubbs*

From the Departments of Molecular and Anatomic Pathology, Pathology and Laboratory Medicine Institute, and the Departments of Translational Hematology and Oncology Research and Solid Tumor Oncology,† Tausig Cancer Institute Cleveland Clinic Foundation, Cleveland, Ohio*

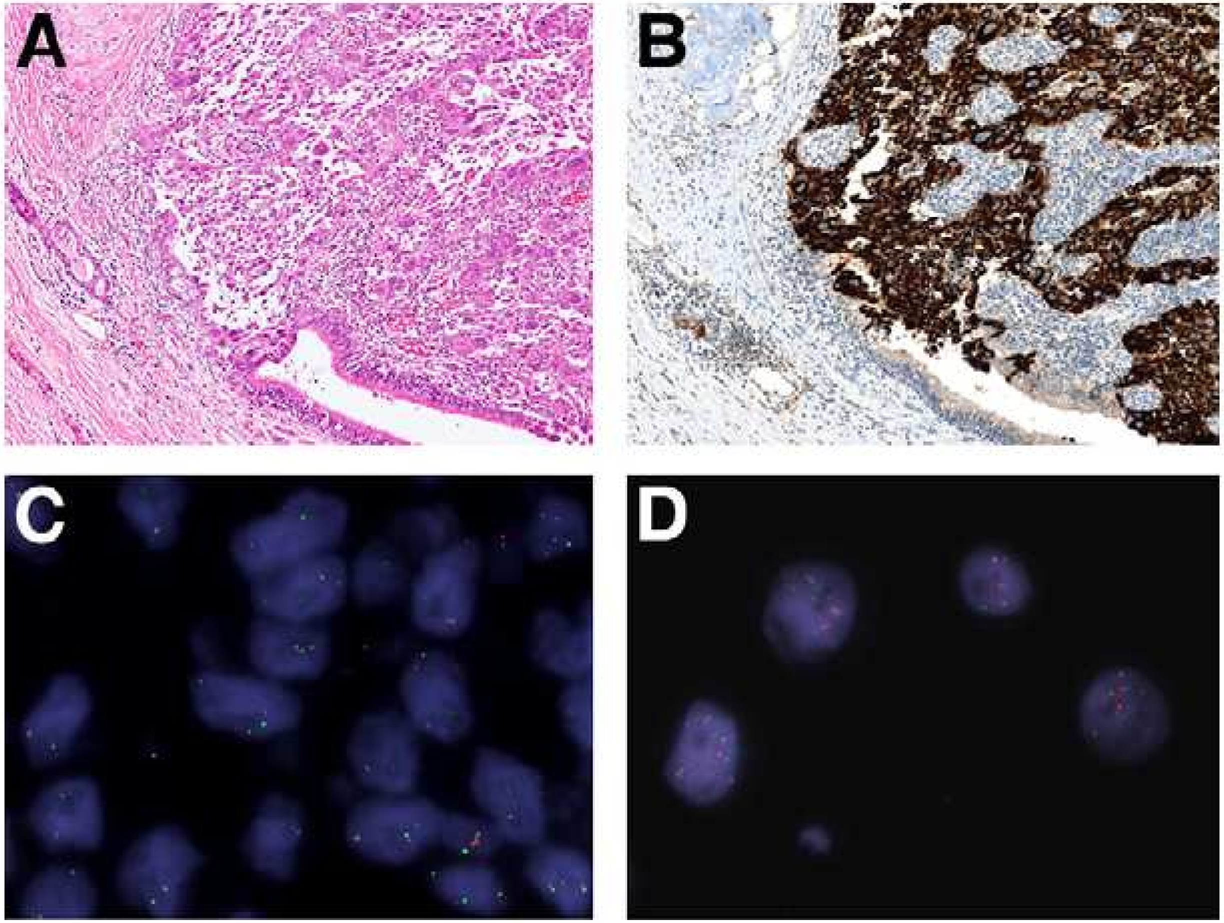


Figure 1 IHC for ALK expression. Representative images of NSCLC (**A**) positive for ALK expression by D5F3-IHC (**B**) and for *ALK* rearrangements by FFPE-FISH (**C**) and ThinPrep-FISH (**D**). Original magnification: ×100 (**A** and **B**); ×1000 (**C** and **D**).

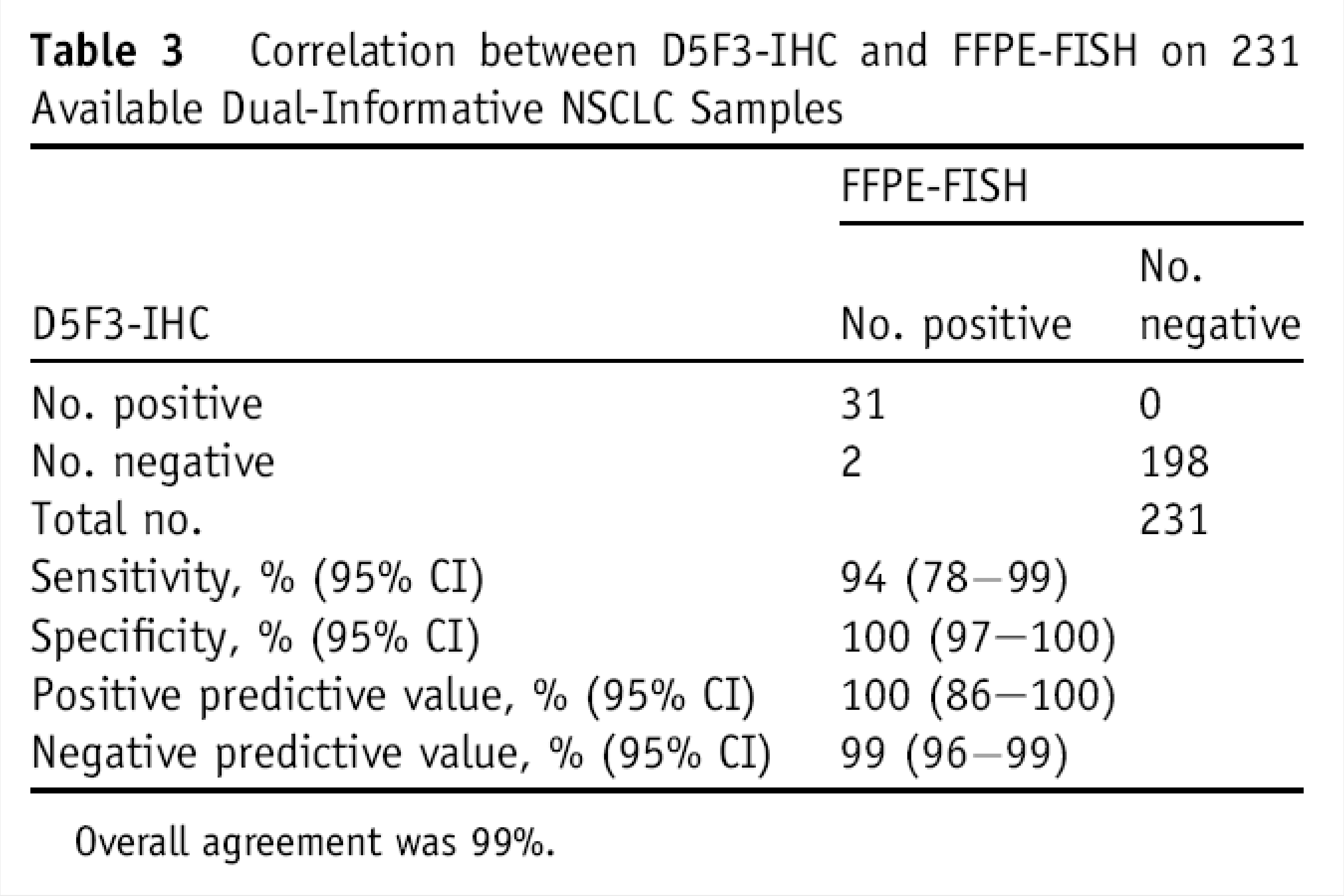
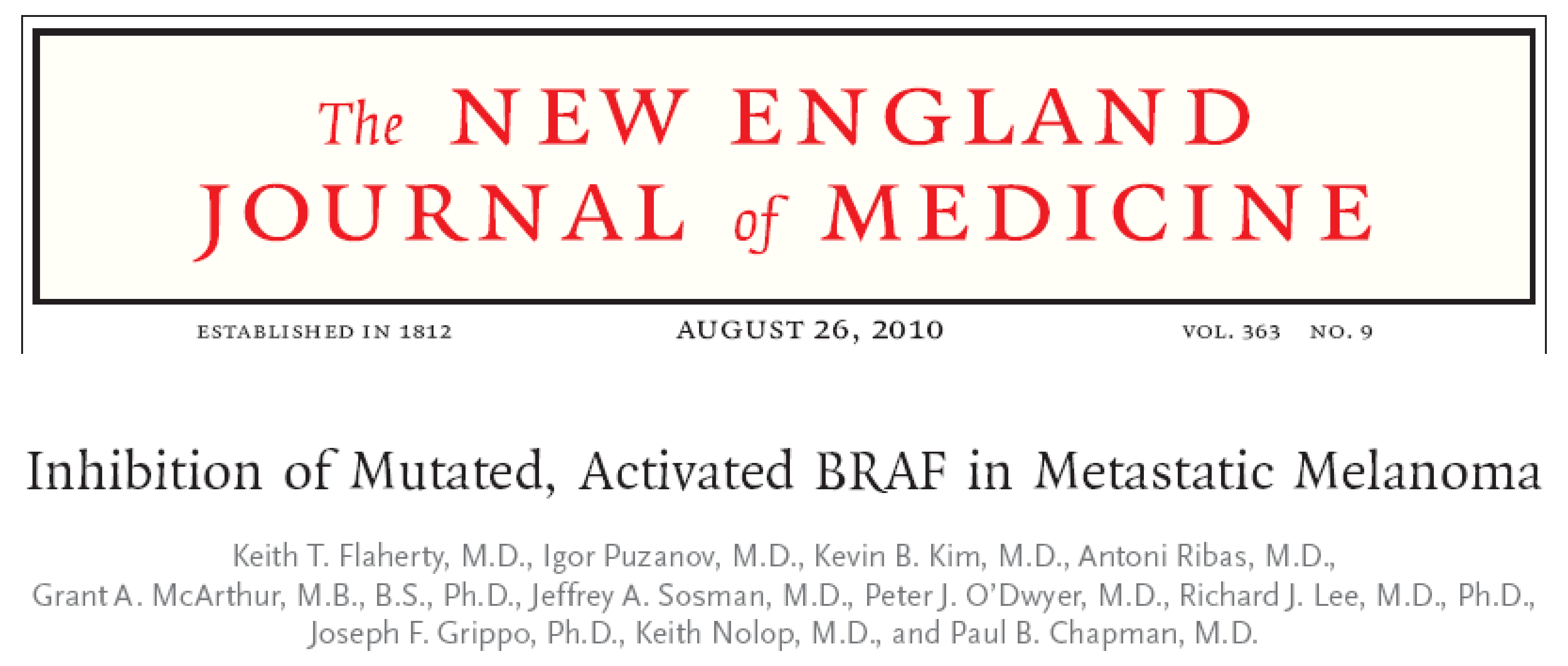


Table 3 Correlation between D5F3-IHC and FFPE-FISH on 231 Available Dual-Informative NSCLC Samples

	FFPE-FISH	
D5F3-IHC	No. positive	No. negative
No. positive	31	0
No. negative	2	198
Total no.		231
Sensitivity, % (95% CI)	94 (78–99)	
Specificity, % (95% CI)	100 (97–100)	
Positive predictive value, % (95% CI)	100 (86–100)	
Negative predictive value, % (95% CI)	99 (96–99)	

Overall agreement was 99%.



The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 26, 2010 VOL. 363 NO. 9

Inhibition of Mutated, Activated BRAF in Metastatic Melanoma

Keith T. Flaherty, M.D., Igor Puzanov, M.D., Kevin B. Kim, M.D., Antoni Ribas, M.D.,
Grant A. McArthur, M.B., B.S., Ph.D., Jeffrey A. Sosman, M.D., Peter J. O'Dwyer, M.D., Richard J. Lee, M.D., Ph.D.,
Joseph F. Grippo, Ph.D., Keith Nolop, M.D., and Paul B. Chapman, M.D.

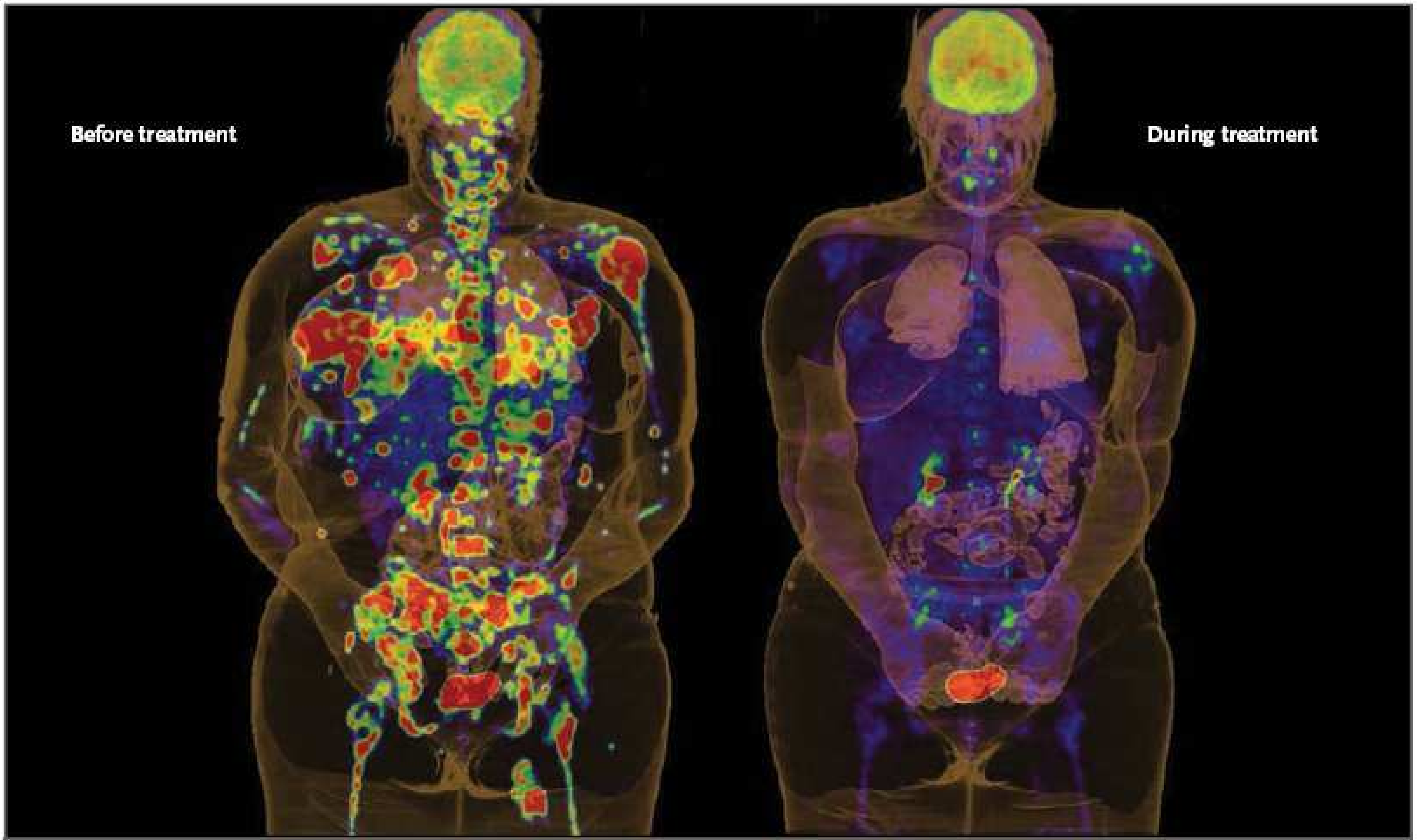


Figure 2. Targeting Treatment to a Specific Variant in the Melanoma Gene.

Shown are three-dimensional representations of glucose metabolism in ^{18}F-fluorodeoxyglucose (FDG)–PET scans obtained at baseline and 2 weeks after the initiation of treatment in a patient with melanoma carrying the V600E *BRAF* mutation. The patient was treated with the BRAF inhibitor PLX4032. Hypermetabolism of injected radioactive glucose is indicated by the red, green, and yellow signals and is a feature of dividing cancer cells, as well as being a normal feature of brain and bladder metabolism or excretion. (Images courtesy of Grant McArthur, Jason Callahan, and Rod Hicks of the Peter MacCallum Cancer Centre.)

Dabrafenib in *BRAF*-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial

Lancet 2012

Axel Hauschild, Jean-Jacques Grob, Lev V Demidov, Thomas Jouary, Ralf Gutzmer, Michael Millward, Piotr Rutkowski, Christian U Blank, Wilson H Miller Jr, Eckhart Kaempgen, Salvador Martín-Algarra, Boguslawa Karaszewska, Cornelia Mauch, Vanna Chiarion-Sileni, Anne-Marie Martin, Suzanne Swann, Patricia Haney, Beloo Mirakhur, Mary E Guckert, Vicki Goodman, Paul B Chapman

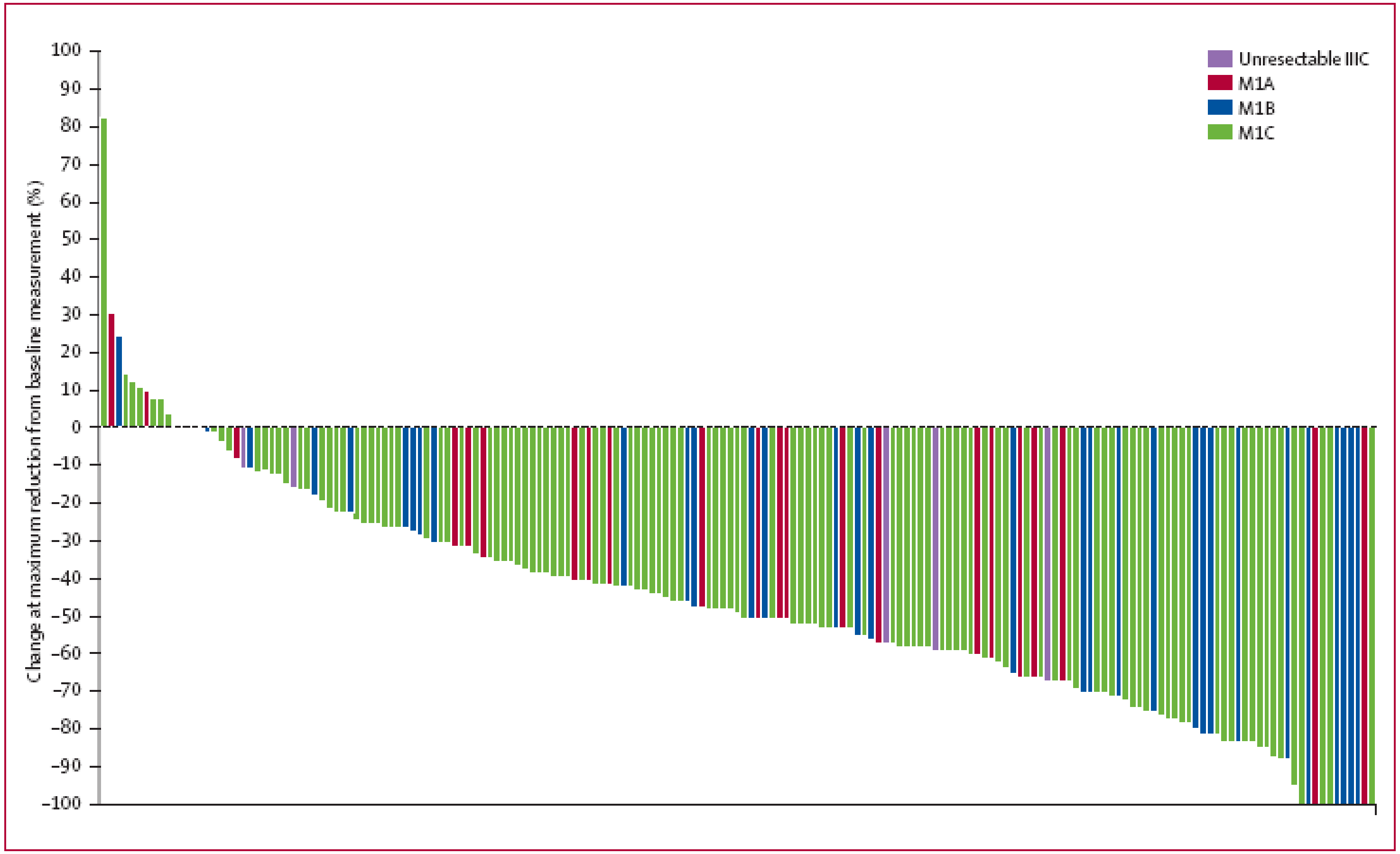
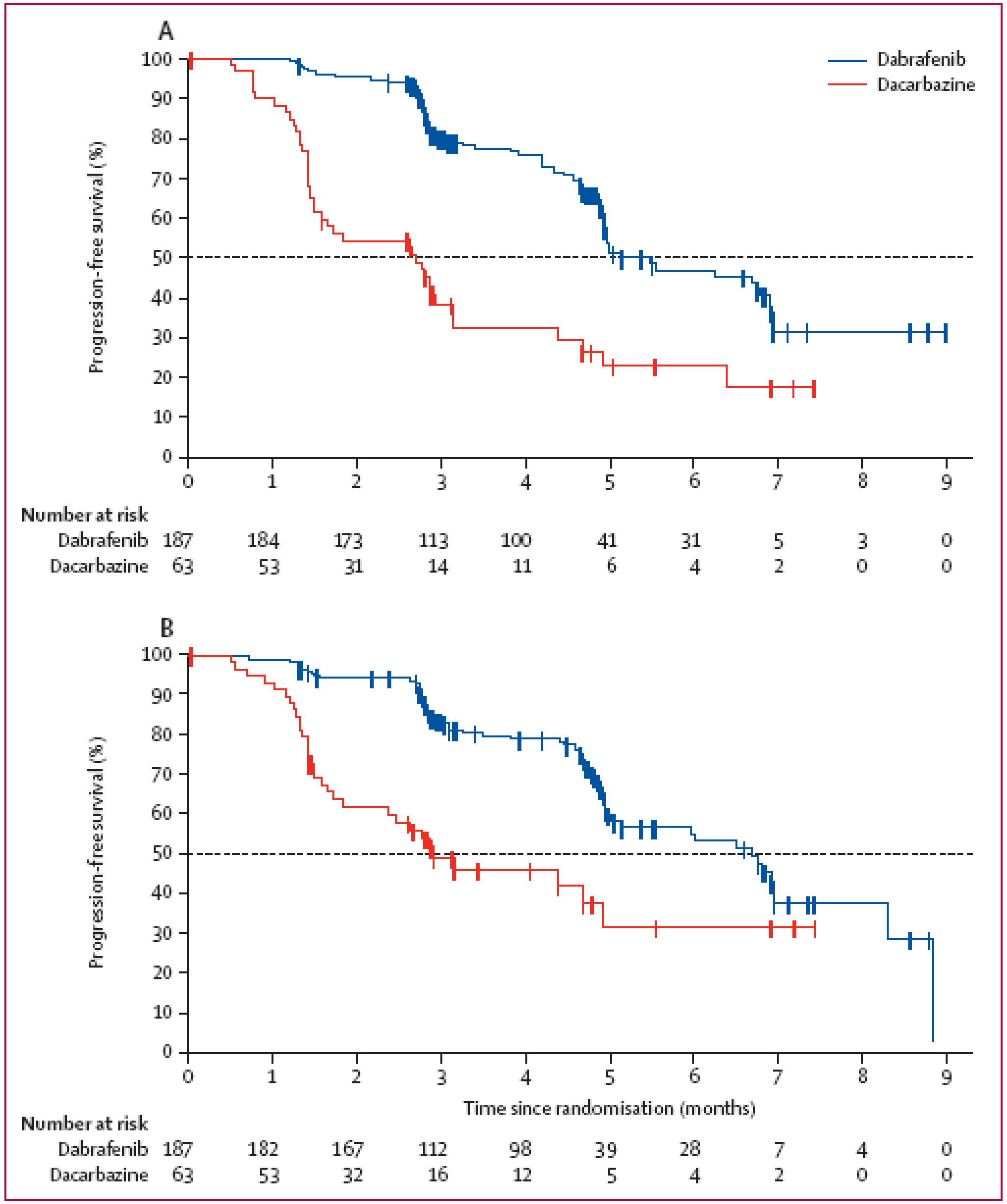


Figure 3: **Maximum tumour percent change from baseline in patients treated with dabrafenib**
Maximum reduction from baseline measurement is shown for patients in the dabrafenib group (n=187). Individual patients are indicated by each bar, with colour indicating the baseline disease stage.



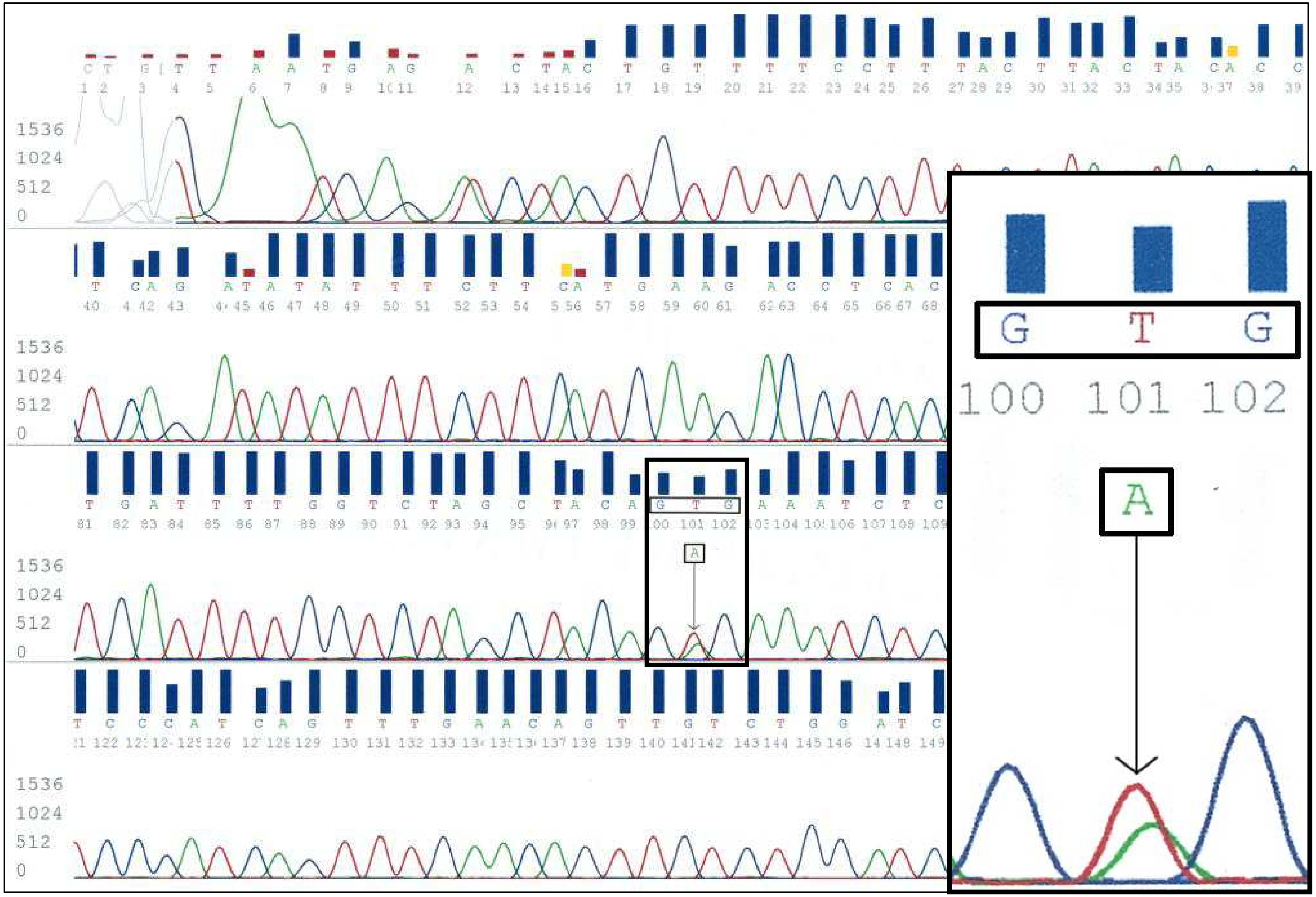
Number at risk

	0	1	2	3	4	5	6	7	8	9
Dabrafenib	187	184	173	113	100	41	31	5	3	0
Dacarbazine	63	53	31	14	11	6	4	2	0	0

Number at risk

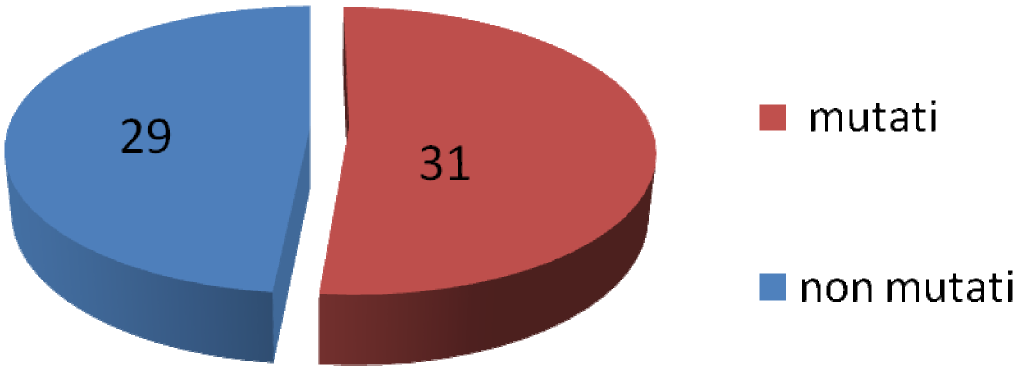
	0	1	2	3	4	5	6	7	8	9
Dabrafenib	187	182	167	112	98	39	28	7	4	0
Dacarbazine	63	53	32	16	12	5	4	2	0	0

Mutazione V600E

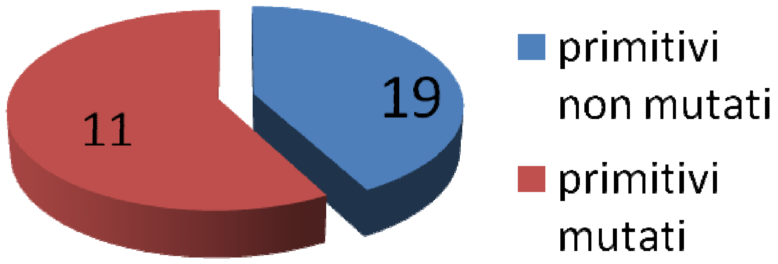


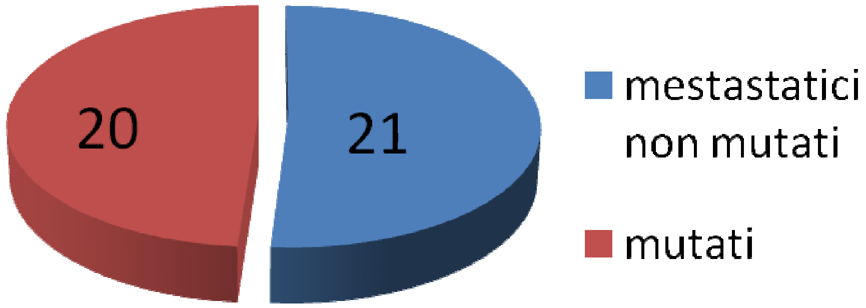
MELANOMA MALIGNO
Analisi molecolare della mutazione di BRAF

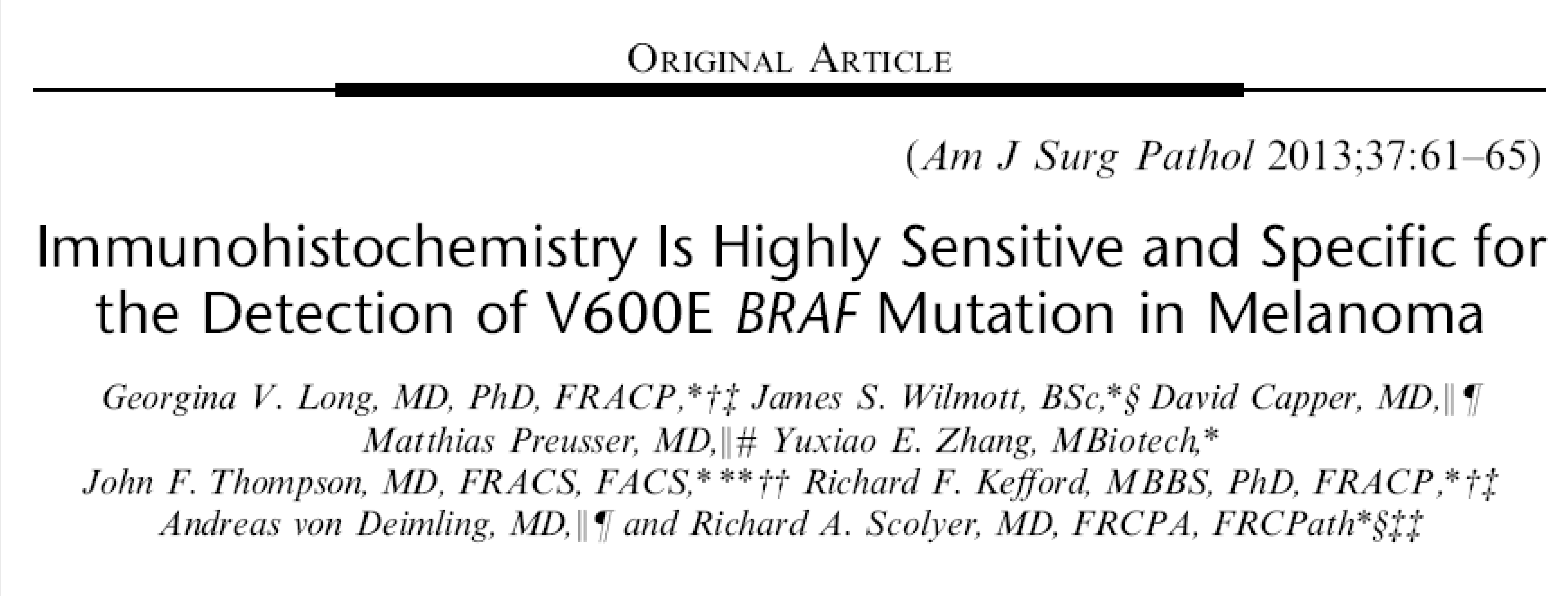
Mutazioni attivanti **BRAF** sono state riscontrate in 31/60 **(51,7%)** casi esaminati



In particolare mutazione di **BRAF** è stata riscontrata
nel **57,8%** dei **campioni di tumore primitivo** (11/19)
nel **48,8% dei campioni metastatici** (20/41)







ORIGINAL ARTICLE

(*Am J Surg Pathol* 2013;37:61–65)

Immunohistochemistry Is Highly Sensitive and Specific for the Detection of V600E *BRAF* Mutation in Melanoma

Georgina V. Long, MD, PhD, FRACP,[*†‡] *James S. Wilmott, BSc,*[*§] *David Capper, MD,*[∥¶]
Matthias Preusser, MD,[∥#] *Yuxiao E. Zhang, MBiotech,*[*]
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Andreas von Deimling, MD,[∥¶] *and Richard A. Scolyer, MD, FRCPA, FRCPath*[*§‡‡]

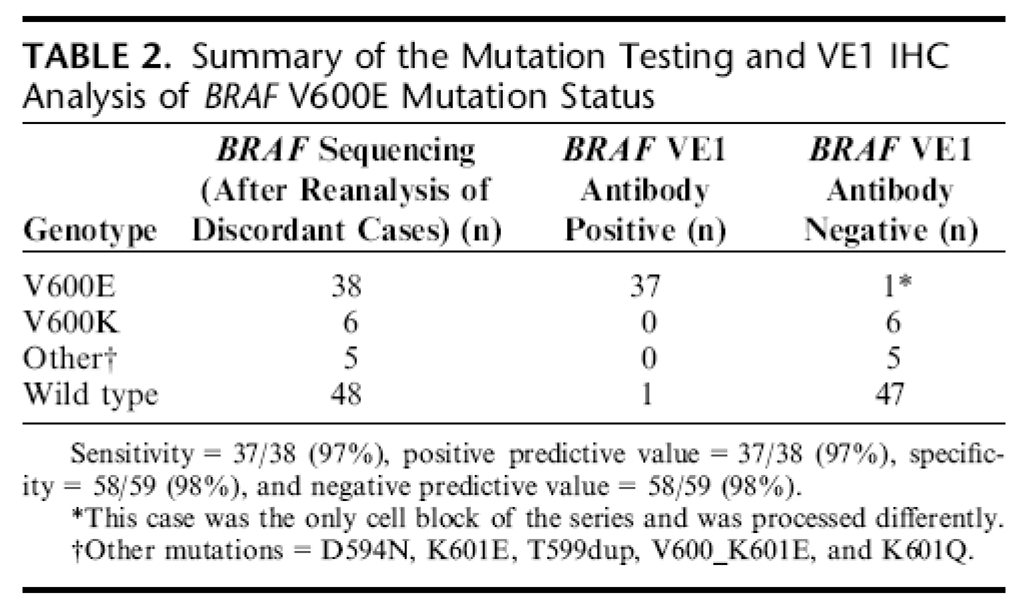

TABLE 2. Summary of the Mutation Testing and VE1 IHC Analysis of *BRAF* V600E Mutation Status

Genotype	***BRAF*** **Sequencing (After Reanalysis of Discordant Cases) (n)**	***BRAF*** **VE1 Antibody Positive (n)**	***BRAF*** **VE1 Antibody Negative (n)**
V600E	38	37	1[*]
V600K	6	0	6
Other[†]	5	0	5
Wild type	48	1	47

Sensitivity = 37/38 (97%), positive predictive value = 37/38 (97%), specificity = 58/59 (98%), and negative predictive value = 58/59 (98%).

[*] This case was the only cell block of the series and was processed differently.

[†] Other mutations = D594N, K601E, T599dup, V600_K601E, and K601Q.

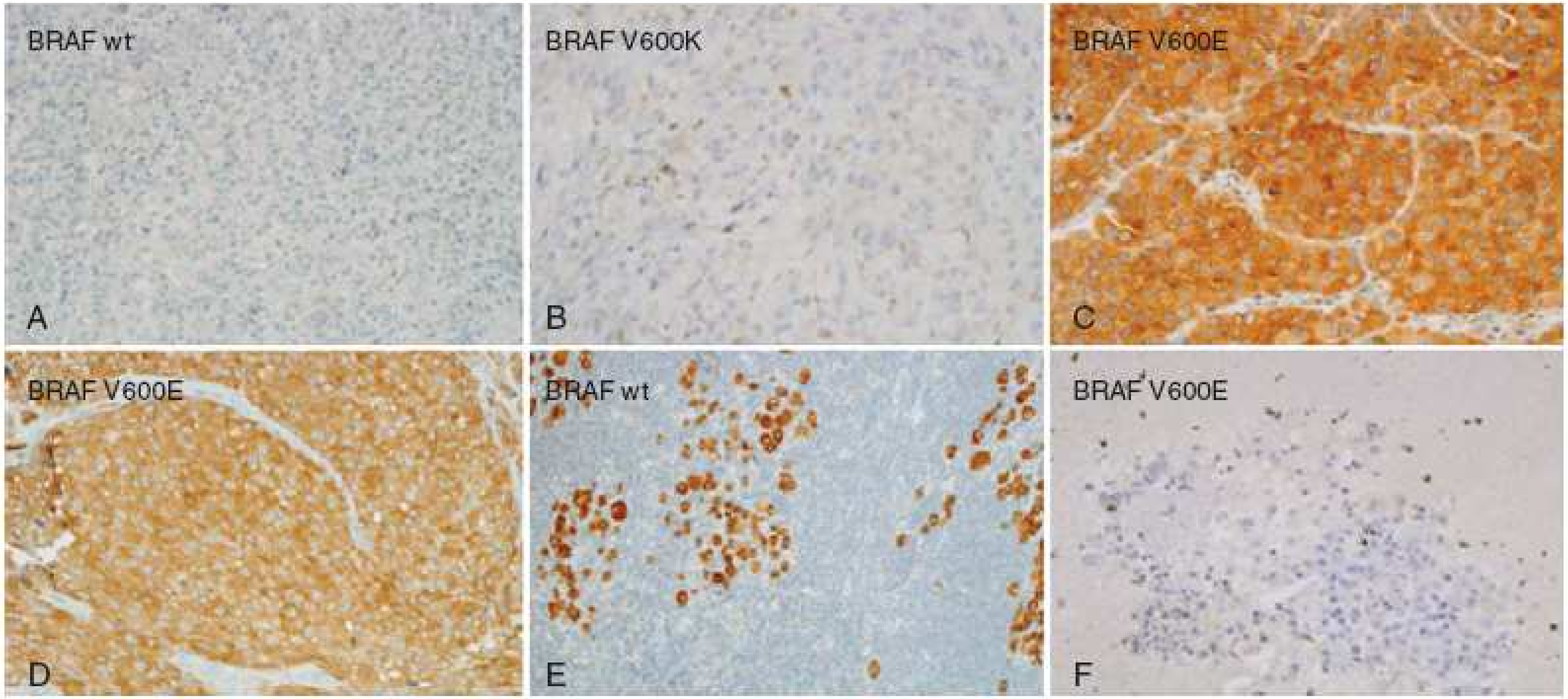

FIGURE 1. IHC staining with the VE1 antibody visualized using the chromogen diaminobenzene (brown staining) in metastatic melanoma. A, *BRAF* wild-type (wt) melanoma, which is negative for VE1. B, *BRAF* V600K-mutated melanoma, which is negative for VE1 (brown melanin pigment is present in a few melanoma cells). C, *BRAF* V600E-mutated melanoma strongly positive for VE1. D, Discordant case that was strongly VE1 positive and *BRAF* wt on original mutation testing. Upon retesting, a *BRAF* V600E mutation was detected. E, Lymph node containing scattered single and small clusters of strongly VE1-positive melanoma cells in a background of numerous lymphocytes. This case was *BRAF* wt on original and repeat mutation testing. F, Discordant fine-needle biopsy case showing negative VE1 staining in the cell block preparation. Mutation testing detected a *BRAF* V600E mutation. The cell block was prepared and fixed using techniques that differed from those used for all other cases (which utilized FFPE tissues).

Long 2013

Raccomandazioni per la determinazione dello stato di HER2 nel carcinoma gastrico

A cura del gruppo di lavoro AIOM-SIAPEC-IAP

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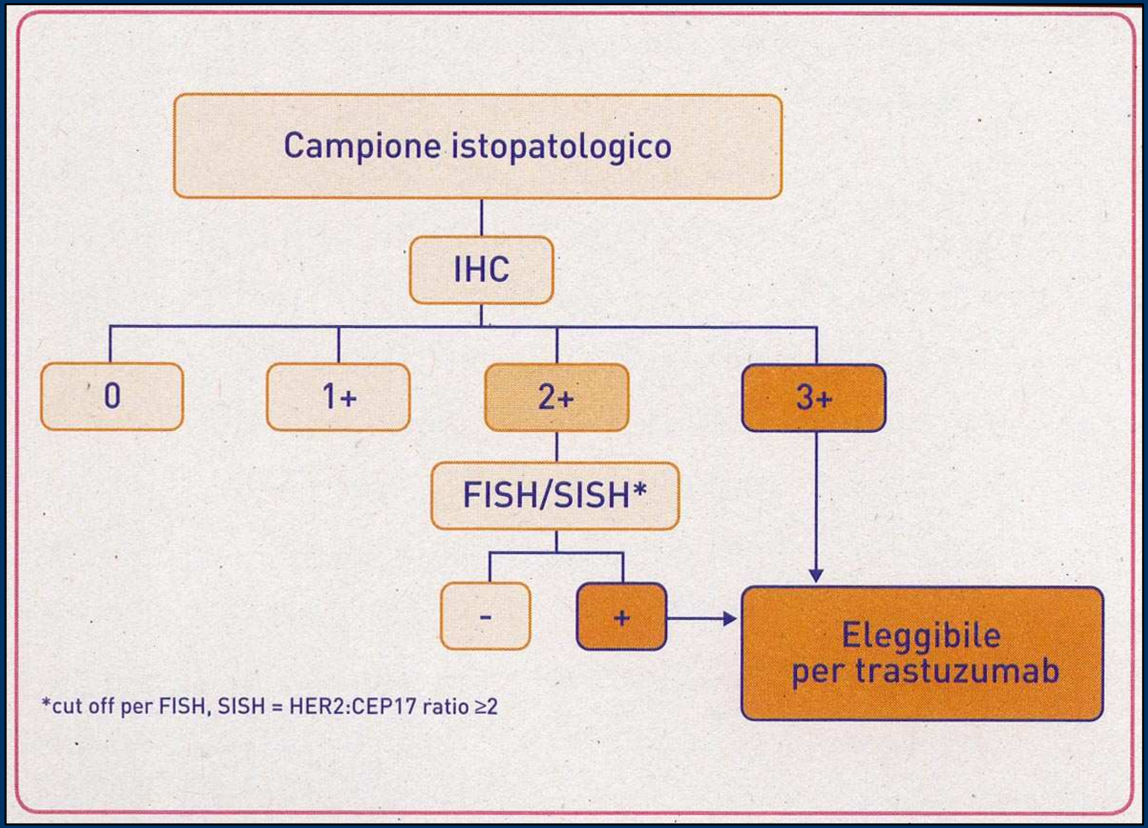


Campione istopatologico

IHC

0

1+

2+

3+

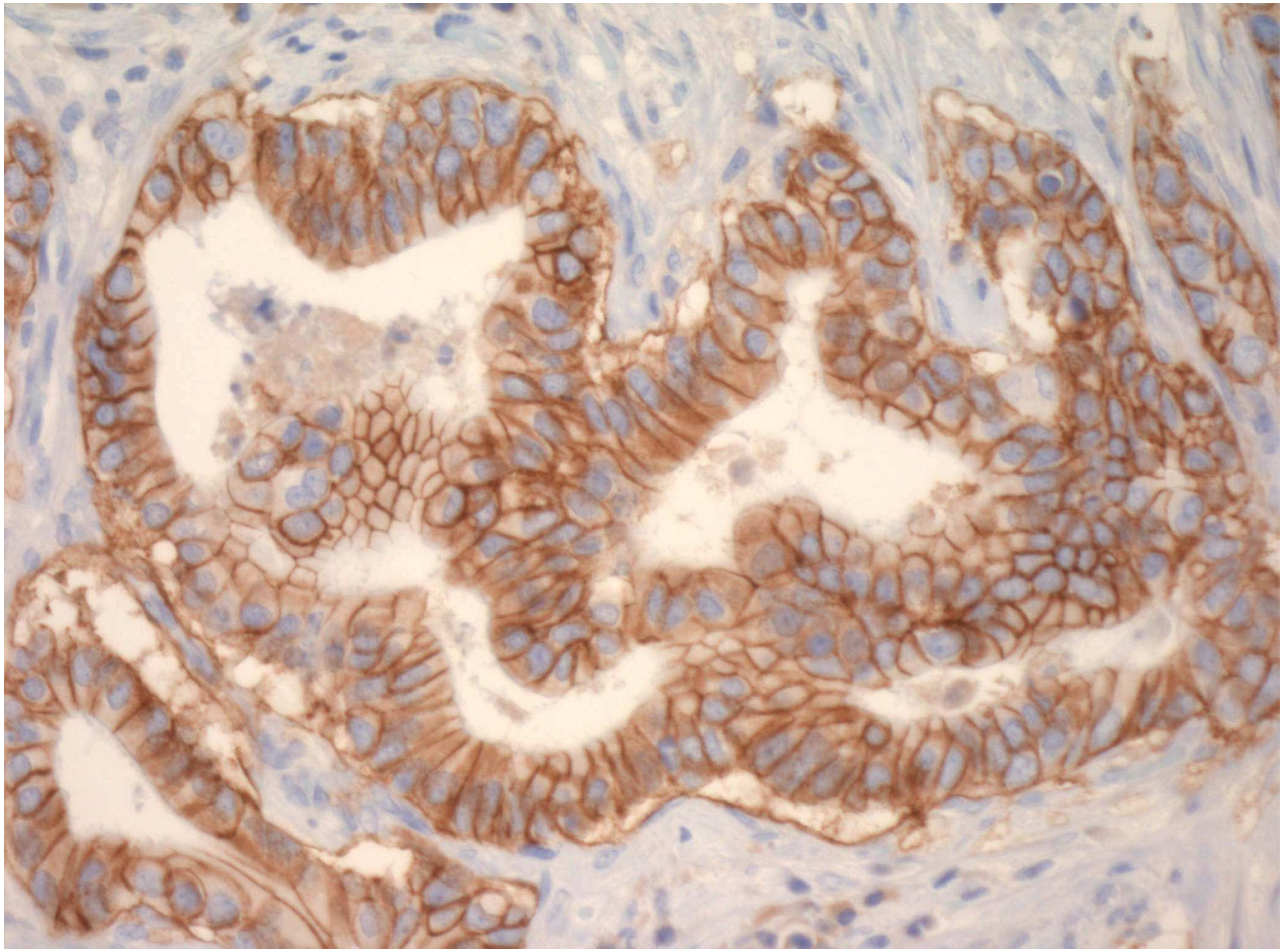
FISH/SISH*

-

+

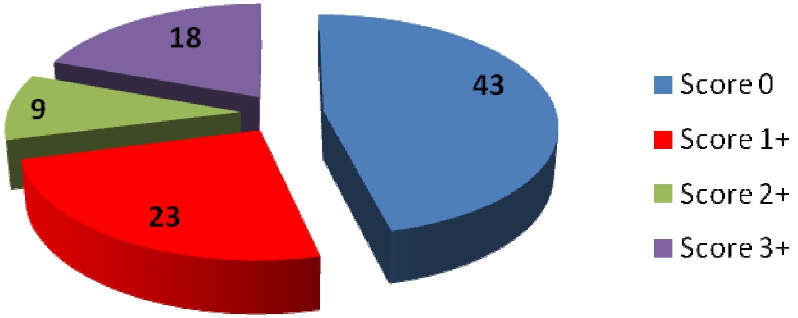
Eleggibile per trastuzumab

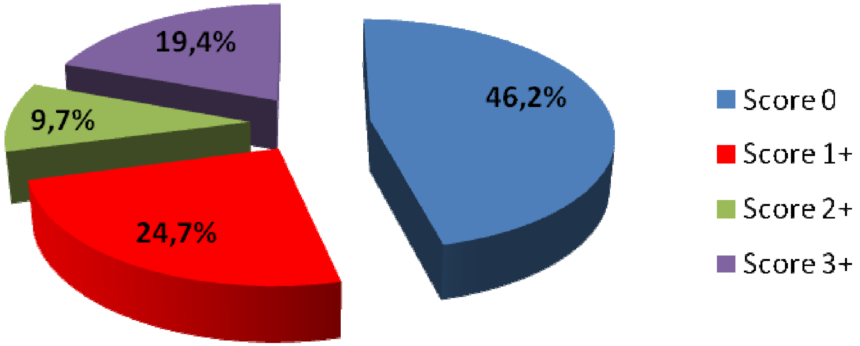
*cut off per FISH, SISH = HER2:CEP17 ratio ≥2



Adenocarcinoma gastrico con iperespressione di HER2 (score 3+)

Adenocarcinoma gastrico
Analisi immunoistochimica espressione HER2 2010-2013: 93 casi





SISH score 2+: 2 amplificati
7 non amplificati

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