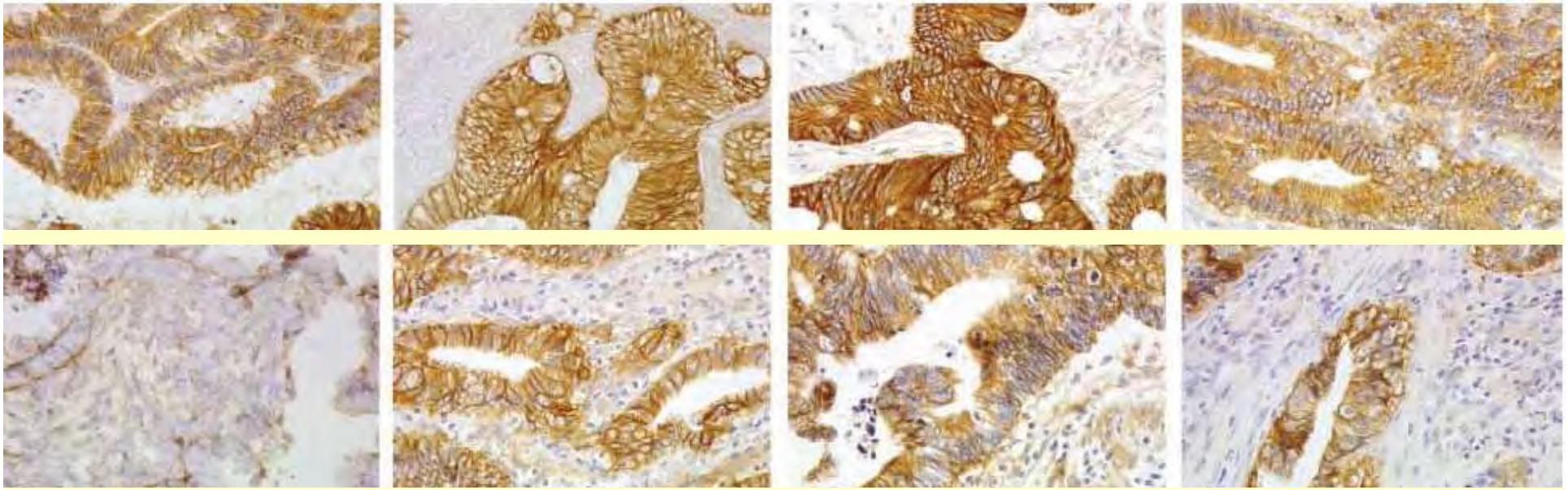
Testování KRAS u kolorektálního karcinomu: současný stav

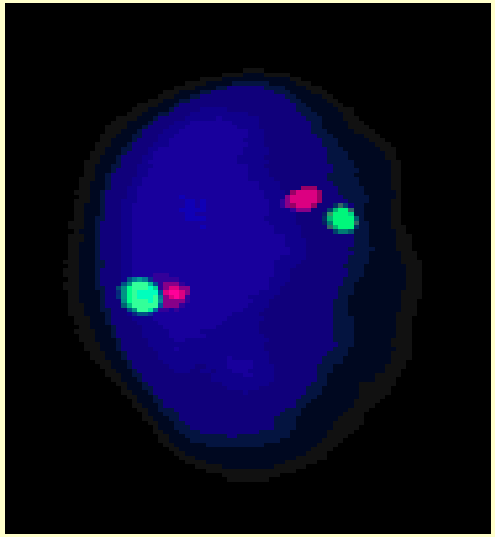
Ryška A, Vošmiková H, Nenutil R, Hajdúch M, Povýšil C, Šíma R, Matějčková M, Uvírová M

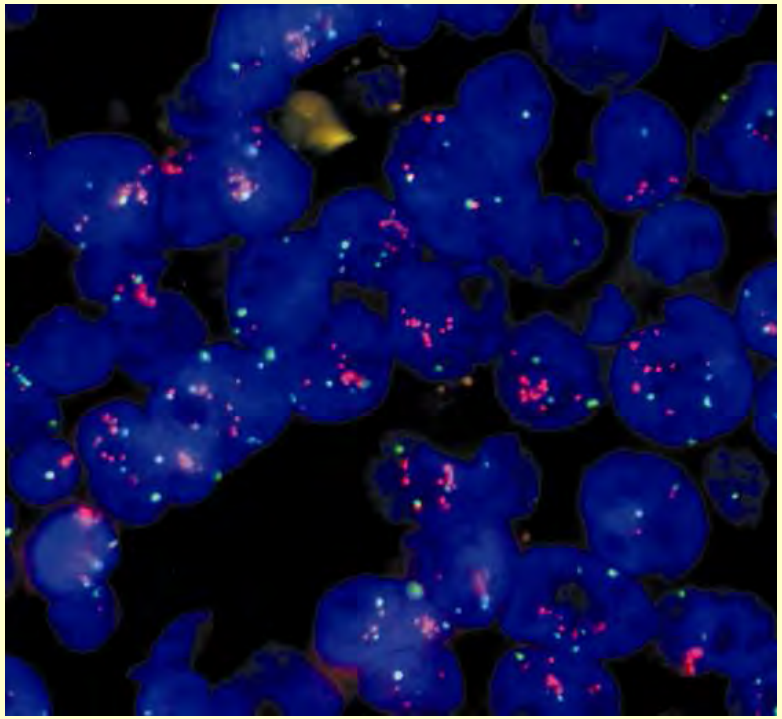
Hradec Králové, Brno, Praha, Olomouc, Plzeň, Ostrava

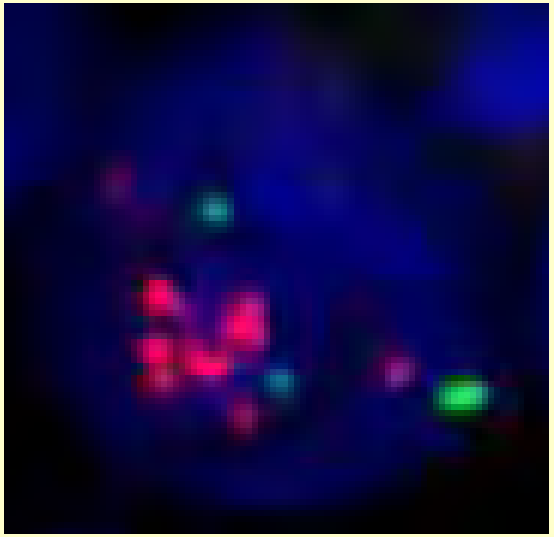
Fingerlandův ústav patologie LF UK a FN
Hradec Králové

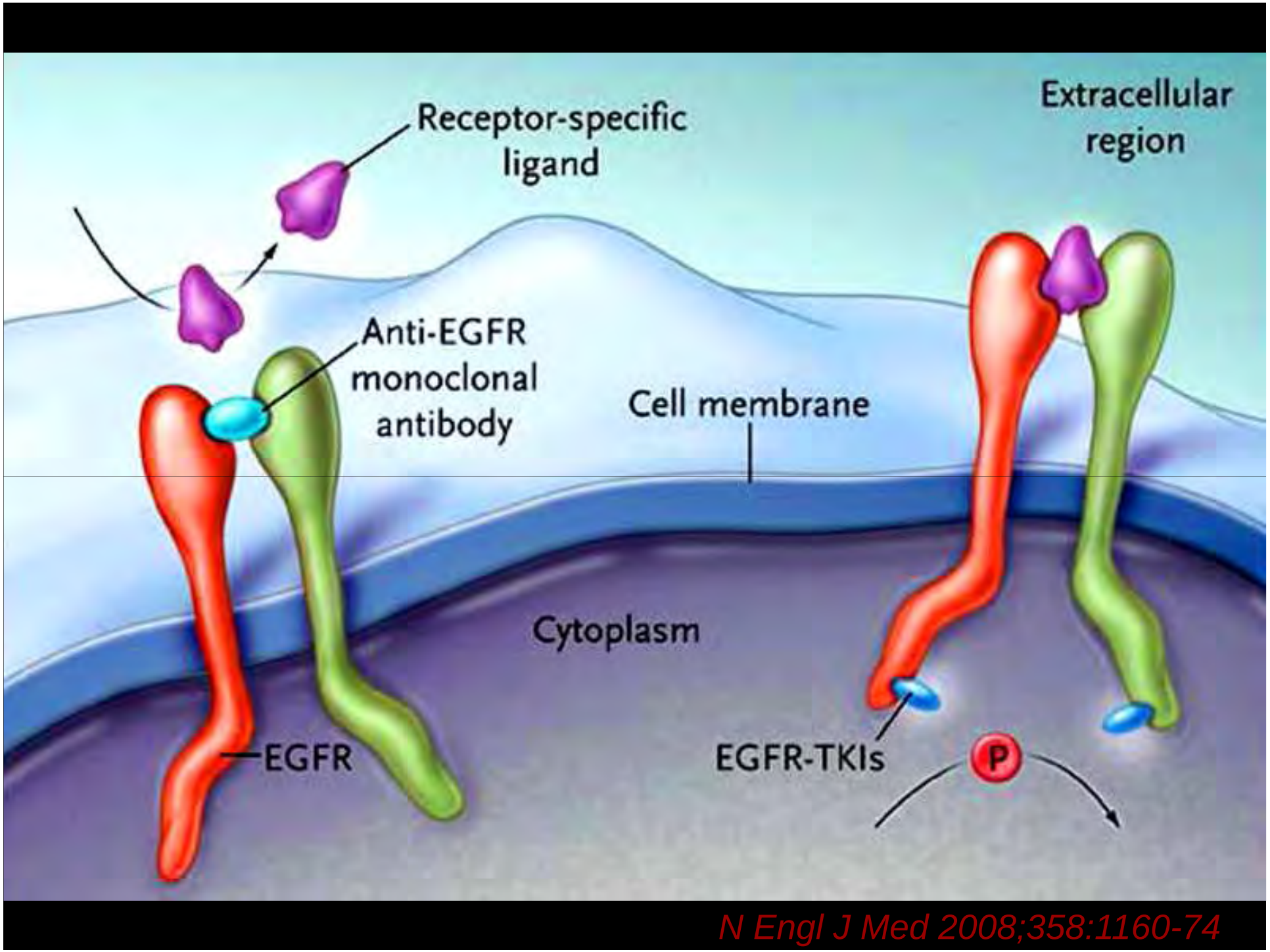

12
YOU'RE DOING IT WRONG



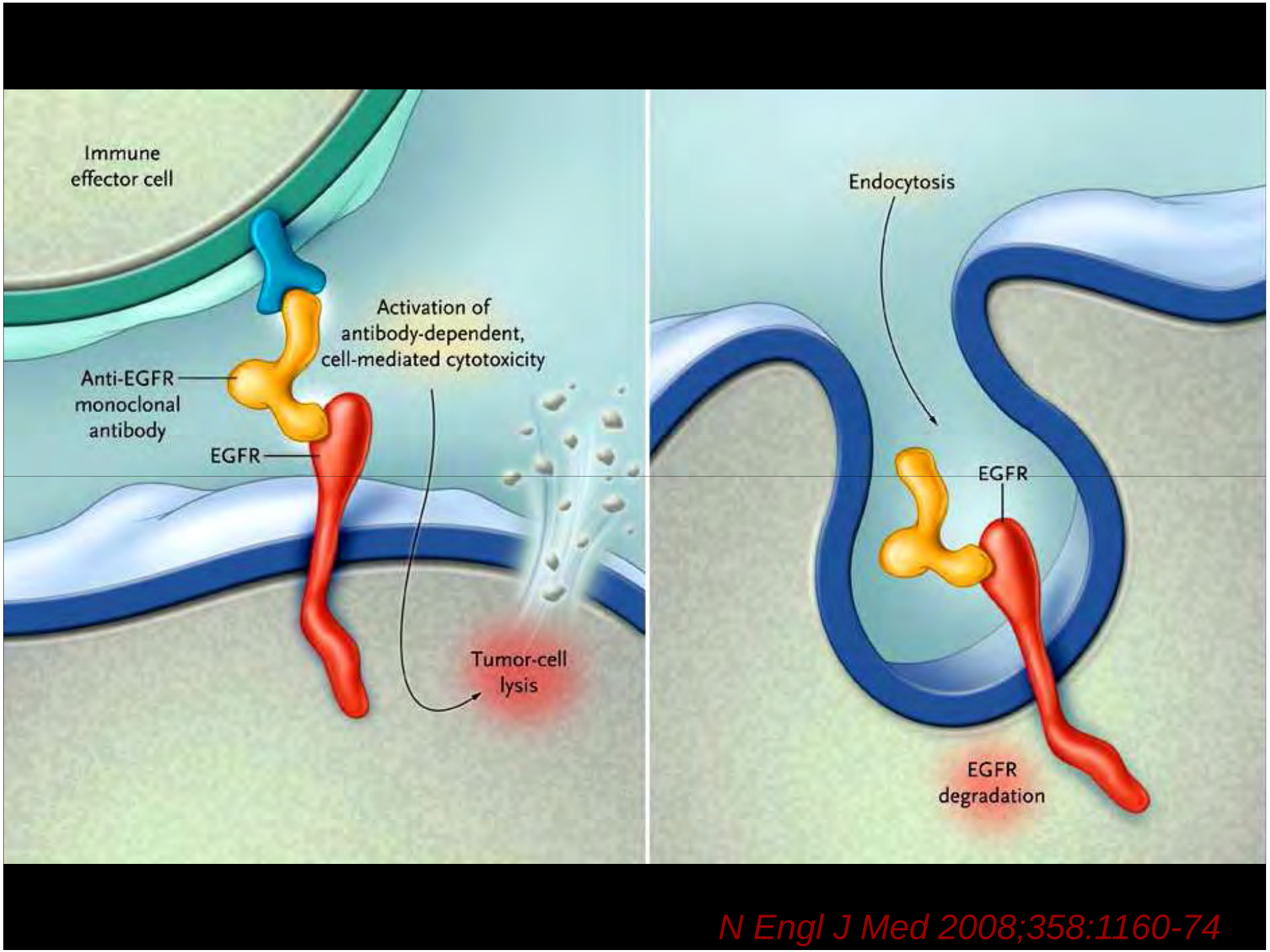






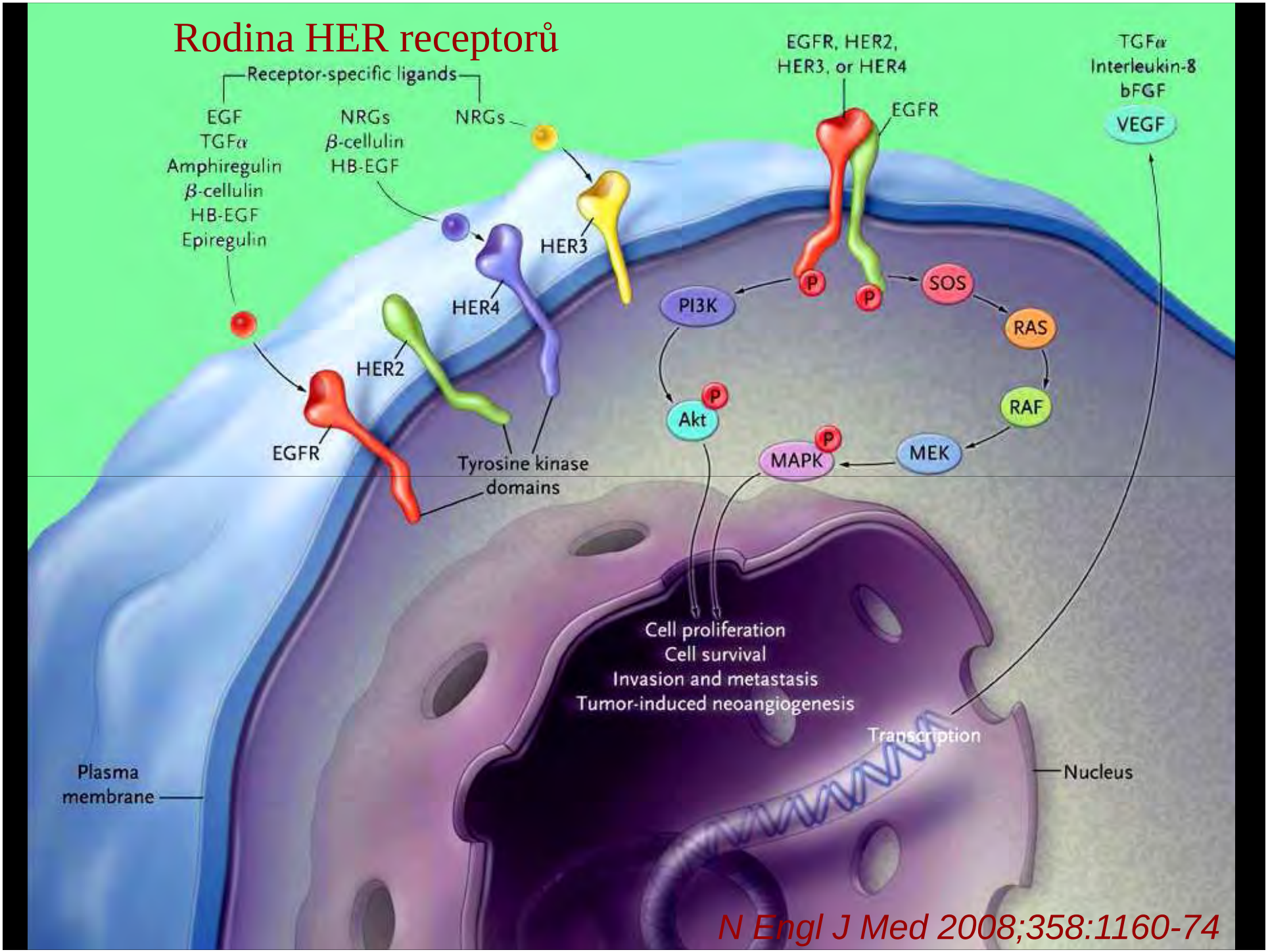


N Engl J Med 2008;358:1160-74

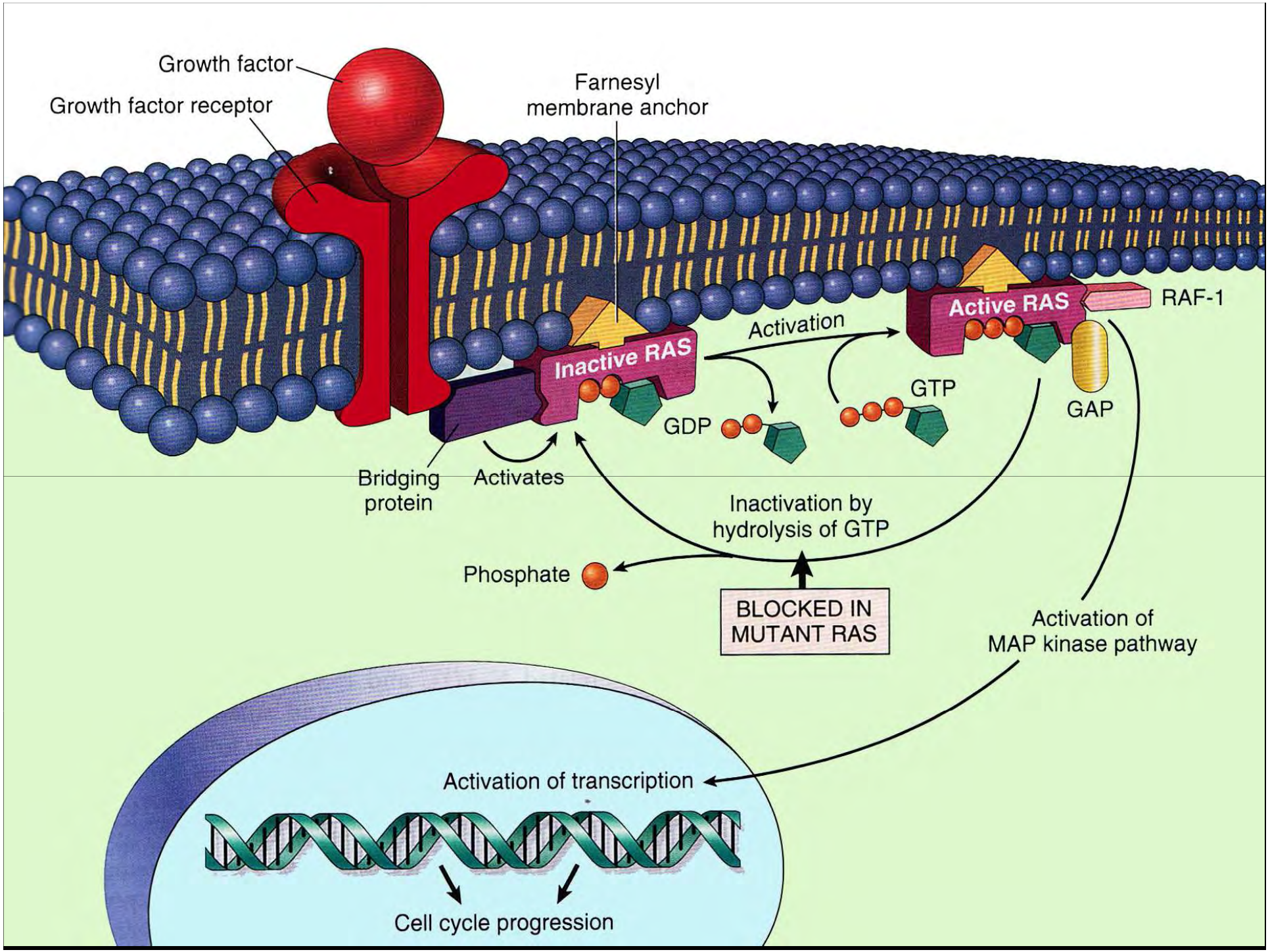


N Engl J Med 2008;358:1160-74

Rodina HER receptorů



N Engl J Med 2008;358:1160-74

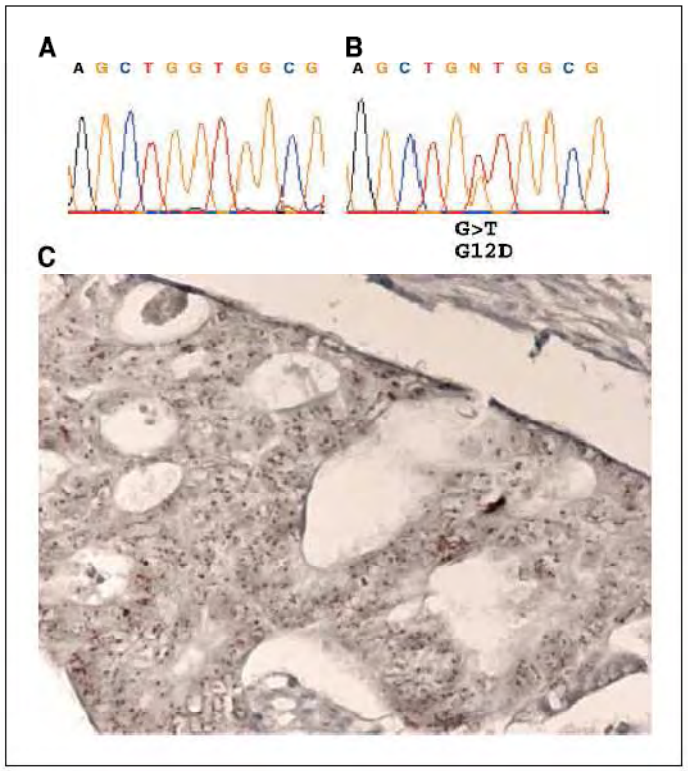

Growth factor
Growth factor receptor
Farnesyl membrane anchor
Inactive RAS
Activation
Active RAS
RAF-1
GTP
GDP
GAP
Bridging protein
Activates
Inactivation by hydrolysis of GTP
Phosphate
BLOCKED IN MUTANT RAS
Activation of MAP kinase pathway
Activation of transcription
Cell cycle progression

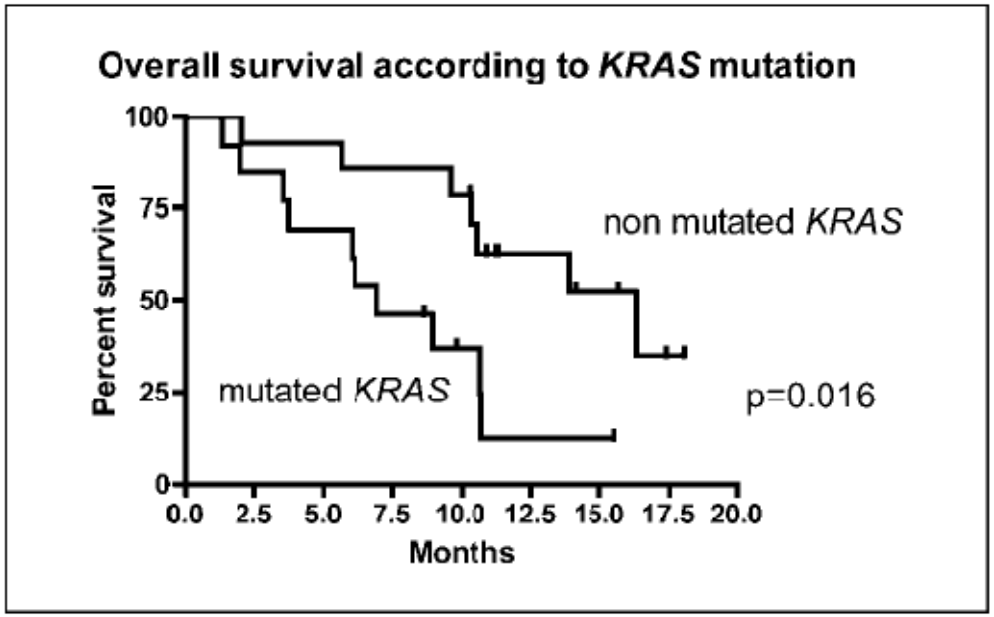
Priority Report

KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer

Astrid Lièvre,[1,3] Jean-Baptiste Bachet,[3] Delphine Le Corre,[1] Valérie Boige,[4] Bruno Landi,[2] Jean-François Emile,[3] Jean-François Côté,[1,2] Gorana Tomasic,[4] Christophe Penna,[3] Michel Ducreux,[4] Philippe Rougier,[3] Frédérique Penault-Llorca,[5] and Pierre Laurent-Puig[1,2]

Cancer Res 2006; 66: (8). April 15, 2006





K-ras mutace u 0/11 s pozitivní odpovědí na léčbu vs. 13/19 bez odpovědi p=0,0003

original article

Annals of Oncology 19: 508–515, 2008
doi:10.1093/annonc/mdm496
Published online 12 November 2007

KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab

W. De Roock[1], H. Piessevaux[2], J. De Schutter[1], M. Janssens[3], G. De Hertogh[4], N. Personeni[5], B. Biesmans[1], J.-L. Van Laethem[6], M. Peeters[7], Y. Humblet[8], E. Van Cutsem[5] & S. Tejpar[1,5*]

Results: OR was seen in 27 of 66 KRAS wild-type (WT) patients versus 0 of 42 in KRAS mutants. Median OS was significantly better in KRAS WT versus mutants (43.0 versus 27.3 weeks; $P = 0.020$). Decrease in tumor sizes was significantly larger at all time points in WT patients. KRAS WT patients with an initial relative decrease of tumor size >9.66% at week 6 had a significantly better median OS compared with all other patients (74.9 versus 30.6 weeks; $P = 0.0000025$). Within KRAS WT patients OS was significantly better in patients with an initial decrease compared with those without [median OS: 74.9 versus 30.6 weeks ($P = 0.00000012$)].

VOLUME 26 · NUMBER 10 · APRIL 1 2008

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Wild-Type *KRAS* Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer

Rafael G. Amado, Michael Wolf, Marc Peeters, Eric Van Cutsem, Salvatore Siena, Daniel J. Freeman, Todd Juan, Robert Sikorski, Sid Suggs, Robert Radinsky, Scott D. Patterson, and David D. Chang

Results

KRAS status was ascertained in 427 (92%) of 463 patients (208 panitumumab, 219 BSC). *KRAS* mutations were found in 43% of patients. The treatment effect on PFS in the wild-type (WT) *KRAS* group (hazard ratio [HR], 0.45; 95% CI: 0.34 to 0.59) was significantly greater ($P < .0001$) than in the mutant group (HR, 0.99; 95% CI, 0.73 to 1.36). Median PFS in the WT *KRAS* group was 12.3 weeks for panitumumab and 7.3 weeks for BSC. Response rates to panitumumab were 17% and 0%, for the WT and mutant groups, respectively. WT *KRAS* patients had longer overall survival (HR, 0.67; 95% CI, 0.55 to 0.82; treatment arms combined). Consistent with longer exposure, more grade III treatment-related toxicities occurred in the WT *KRAS* group. No significant differences in toxicity were observed between the WT *KRAS* group and the overall population.

Published Ahead of Print on February 2, 2009 as 10.1200/JCO.2009.21.9170
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2009.21.9170

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

Provisional Clinical Opinion

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.

Virchows Arch (2008) 453:417–431
DOI 10.1007/s00428-008-0665-y

REVIEW AND PERSPECTIVE

KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program

J. H. J. M. van Krieken · A. Jung · T. Kirchner · F. Carneiro · R. Seruca · F. T. Bosman · P. Quirke · J. F. Fléjou · T. Plato Hansen · G. de Hertogh · P. Jares · C. Langner · G. Hoefler · M. Ligtenberg · D. Tiniakos · S. Tejpar · G. Bevilacqua · A. Ensari

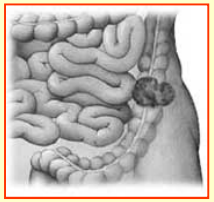
Zásadní role "primárního" patologa !

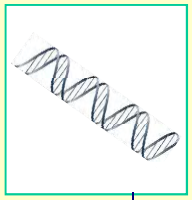
- záchyt případu, primární diagnóza
- výběr tkáně vhodné pro vyšetření
- zajištění vyšetření
- zahrnutí výsledku do dokumentace

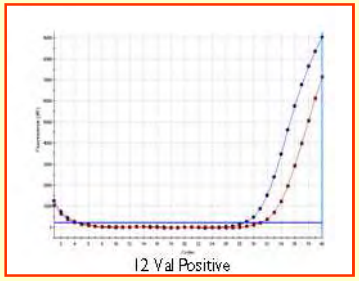
Vyšetřování mutací *k-ras* u mCRC v ČR

Vyšetření mutací *k-ras* – provedení

DNA je extrahována z parafínových bločků, příp. z čerstvé tkáně





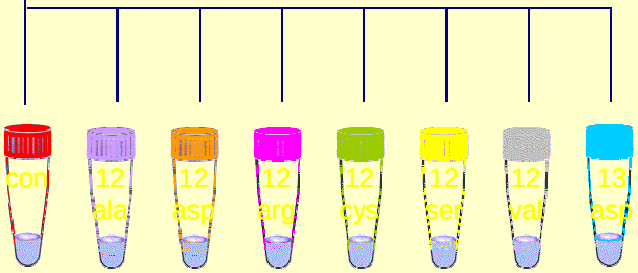


Analýza srovnáním mutantní DNA s normální

PCR reakce

RT PCR analýza

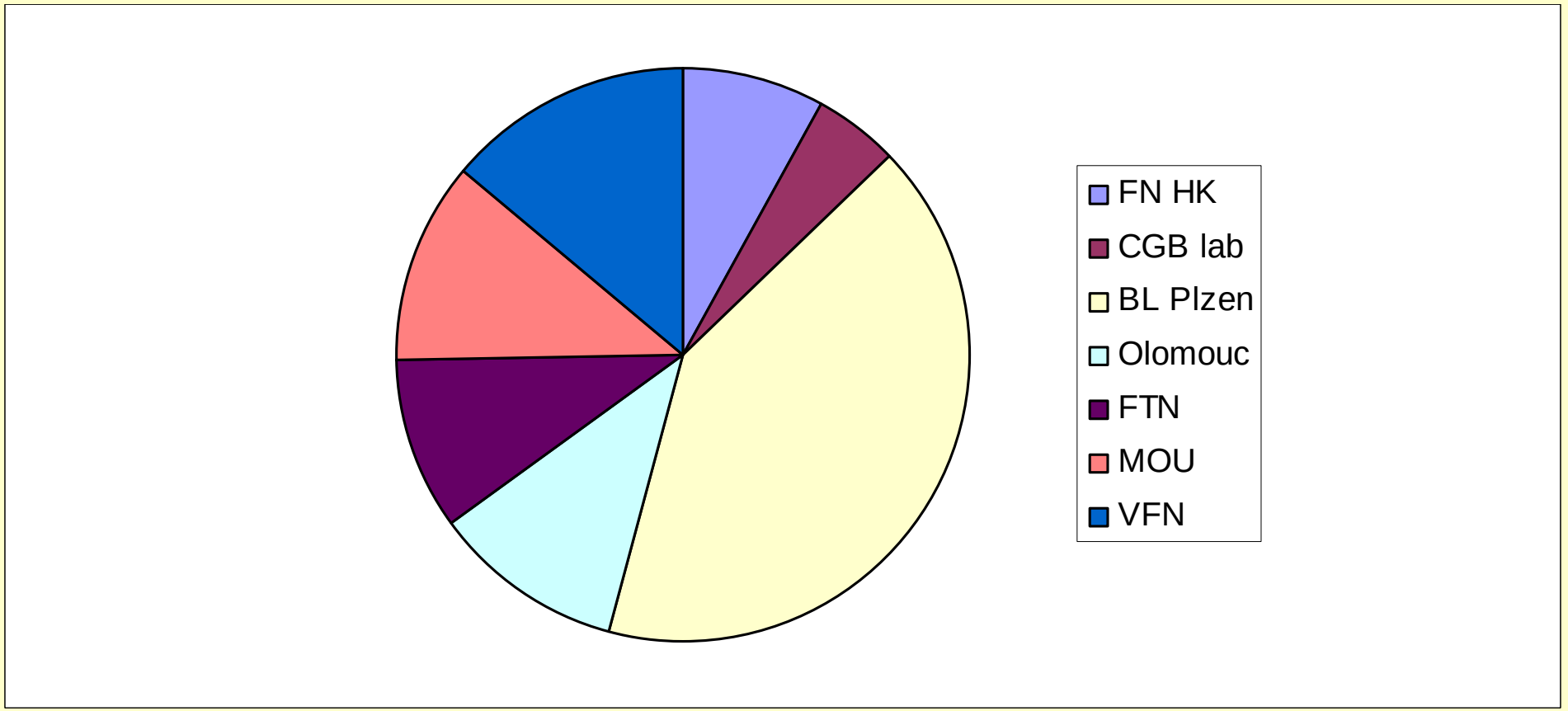
- detekce cca 1% mutované DNA
- 7 nejčastějších somatických mutací v kodonech 12 a 13 exonu 1



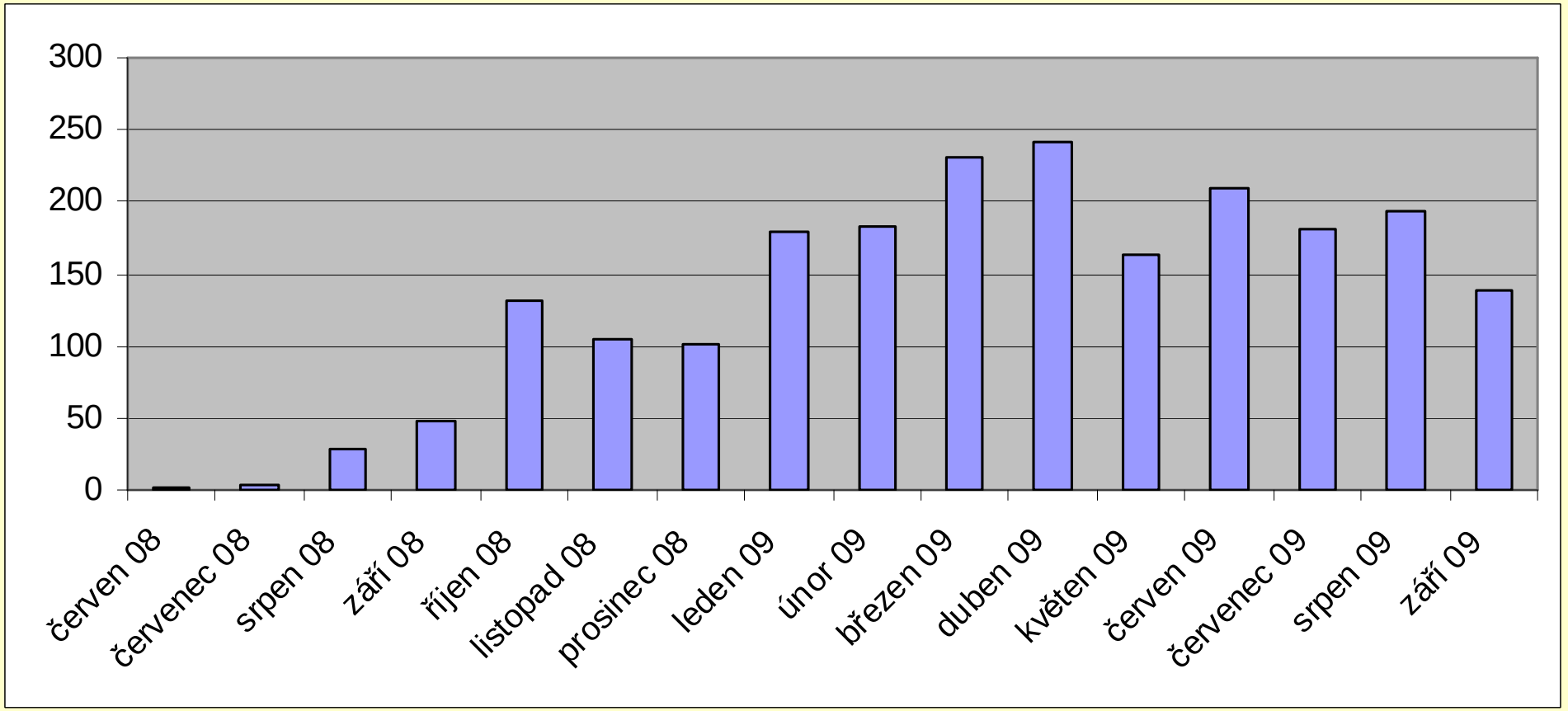
WT x mut

Vyšetřování mutací *k-ras* u mCRC v ČR

červen 2008 - září 2009 (n=2117)

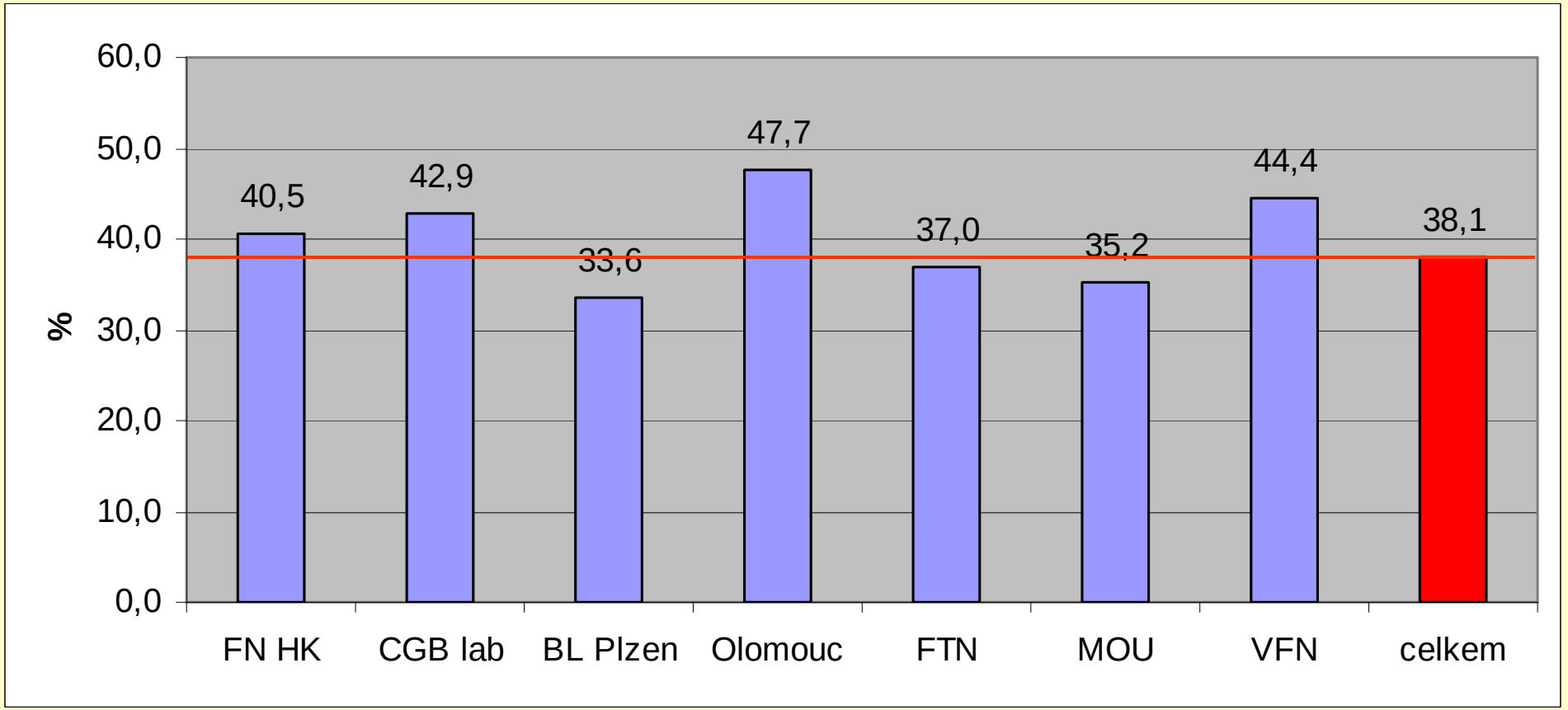


Vyšetřování mutací *k-ras* u mCRC v ČR
(data k 20. 9. 2009)



Předpokládaný počet vyšetření za r. 2009 = 2500

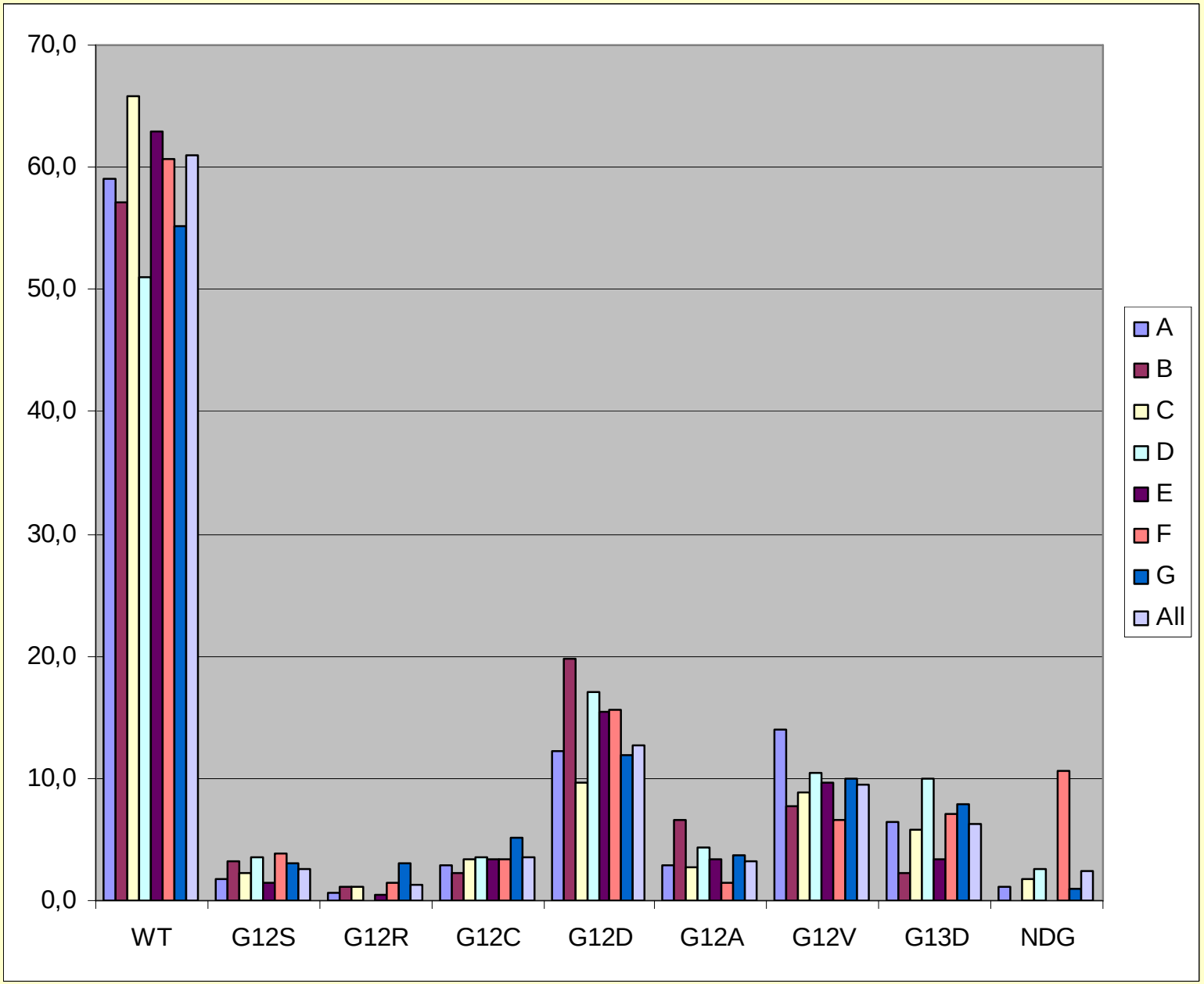
Zastoupení mutací *k-ras* u mCRC v ČR



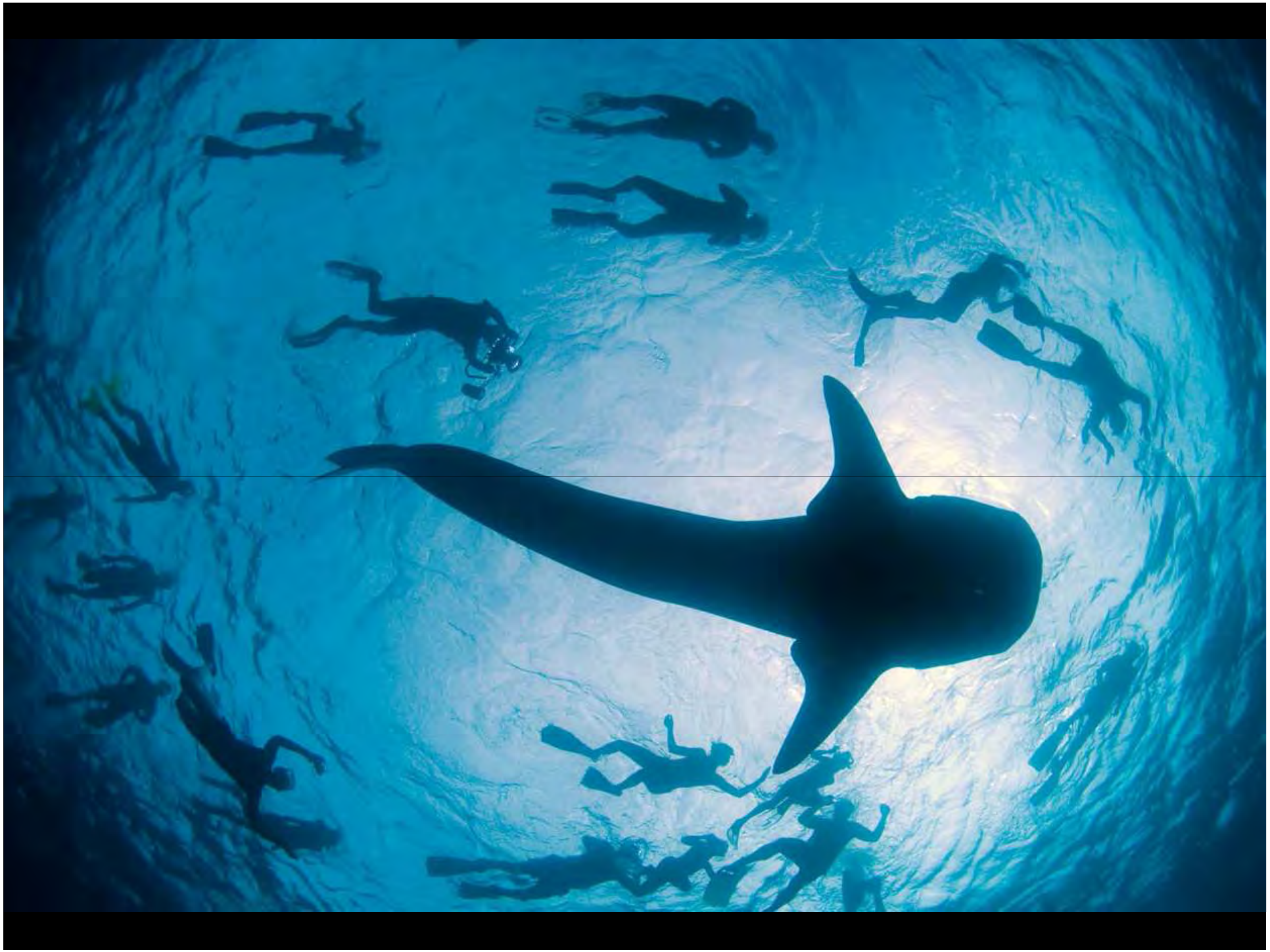
Zastupení jednotlivých mutací *k-ras* u mCRC

	A	B	C	D	E	F	G	All
WT	59,1	57,1	65,9	51,1	63,0	60,7	55,1	**60,9**
G12S	1,8	3,3	2,3	3,5	1,4	3,8	3,1	**2,6**
G12R	0,6	1,1	1,2	0,0	0,5	1,4	3,1	**1,2**
G12C	2,9	2,2	3,4	3,5	3,4	3,3	5,1	**3,5**
G12D	12,3	19,8	9,6	17,0	15,4	15,6	12,0	**12,6**
G12A	2,9	6,6	2,7	4,4	3,4	1,4	3,8	**3,1**
G12V	14,0	7,7	8,9	10,5	9,6	6,6	9,9	**9,4**
G13D	6,4	2,2	5,8	10,0	3,4	7,1	7,9	**6,3**
NDG	1,2	0,0	1,7	2,6	0,0	10,6	1,0	**2,4**
Případy	**173**	**91**	**879**	**235**	**208**	**236**	**295**	**2117**

Zastupení jednotlivých mutací *k-ras* u mCRC

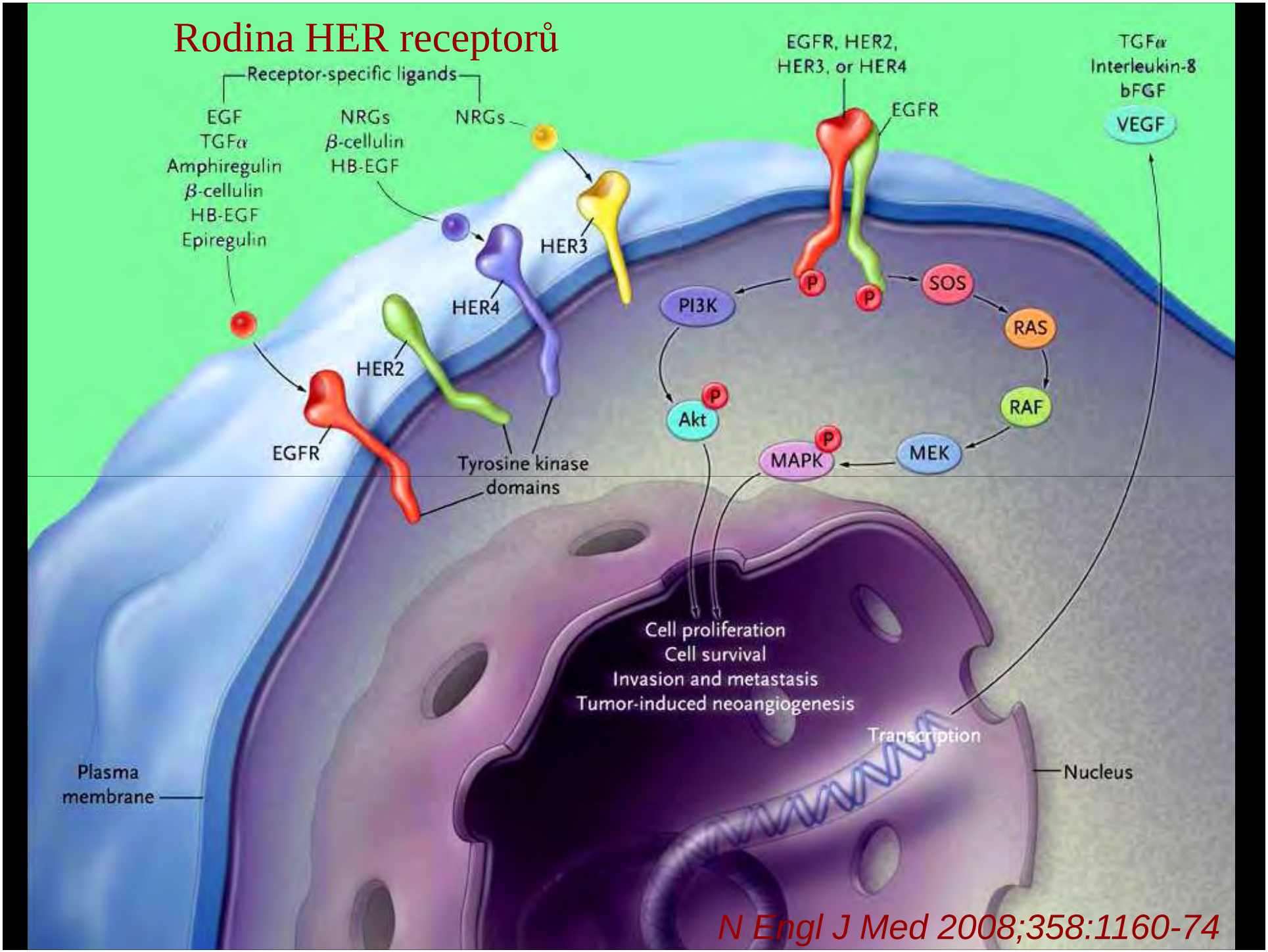



One *Gene* to rule them all,
One *Gene* to find them, One *Gene* to bring them all and in the darkness bind them.




The largest maze in the world, at Reignac-sur-Indre, Indre-et-Loire Department, France (N 47°13'-E 0
http://www.yannarthusbertrand

Rodina HER receptorů

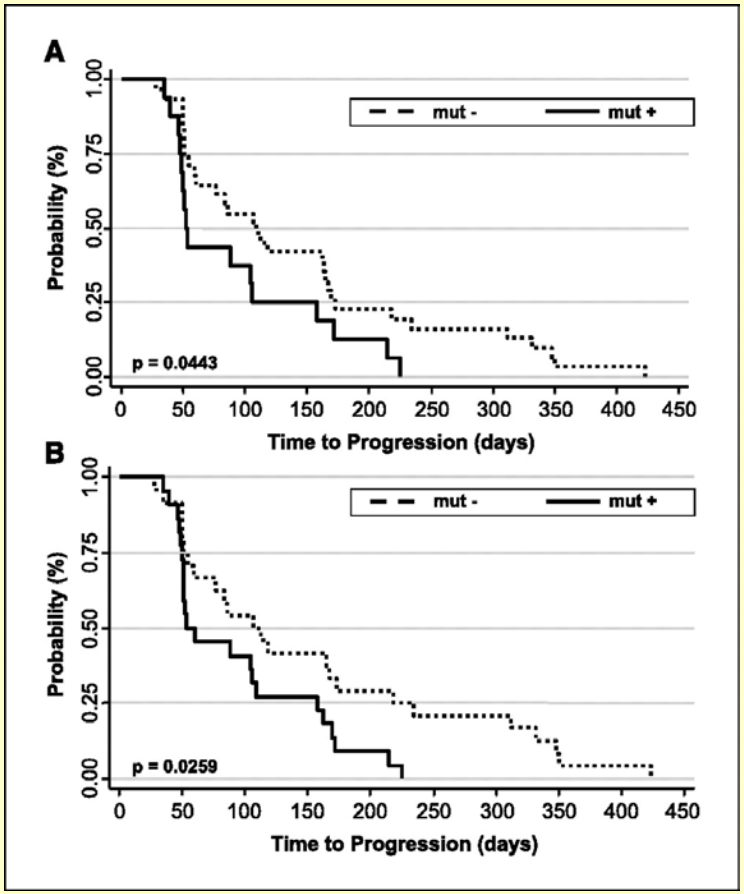


N Engl J Med 2008;358:1160-74

Oncogenic Activation of the RAS/RAF Signaling Pathway Impairs the Response of Metastatic Colorectal Cancers to Anti–Epidermal Growth Factor Receptor Antibody Therapies

Silvia Benvenuti,[1] Andrea Sartore-Bianchi,[2] Federica Di Nicolantonio,[1] Carlo Zanon,[1] Mauro Moroni,[2] Silvio Veronese,[2] Salvatore Siena,[2] and Alberto Bardelli[1,3]

Cancer Res 2007; 67: (6). March 15, 2007



$P = 0.017$). These results suggest that patients carrying either *K-RAS* or *BRAF* mutations are less likely to achieve partial response to therapy with anti-EGFR mAbs. Our data also indicate that signaling pathways rather than single genes should be the focus of genetic studies aimed at dissecting the molecular basis of targeted therapies.

British Journal of Cancer (2009) 100, 1087 – 1094

www.bjcancer.com

Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may be clinically relevant

F Molinari[1,4], V Martin[1,4], P Saletti[2], S De Dosso[2], A Spitale[3], A Camponovo[1], A Bordoni[3], S Crippa[1], L Mazzucchelli[1] and M Frattini[*,1]

[1]Institute of Pathology, Locarno, Switzerland; [2]Oncology Institute of Southern Switzerland, Ospedale San Giovanni, Bellinzona, Switzerland; [3]Ticino Cancer Registry, Locarno, Switzerland

- 38 mCRC
- 12 - antiEGFR terapie
- srovnání primárního tu a meta

- EGFR (IHC + FISH)
- kras (sekvenování)
- BRAF (sekvenování)
- PTEN (IHC)

	EGFR IHC			*EGFR* FISH			*K-Ras*			*BRAF*			PTEN IHC			
Case no.	T	LN	M	T	LN	M	T	LN	M	T	LN	M	T	LN	M	Clinical response
1	+	+	–	P	A	A	WT	WT	WT	WT	WT	WT	+	+	–	–
2	+	NA	–	P	NA	D	G12A	NA	G12A	WT	NA	WT	–	NA	–	–
3	+	+	–	P	NV	P	WT	WT	G12C	WT	WT	WT	+	+	–	–
4	+	NA	–	A	NA	P	G12D	NA	G12D	WT	NA	WT	–	NA	–	–
5	+	+	–	P	P	P	G12A	G12A	G12A	WT	WT	WT	–	–	–	–
6	+	+	–	D	D	P	WT	WT	WT	WT	WT	WT	–	–	–	–
7	+	NA	–	P	NA	P	G12C	NA	WT	WT	NA	WT	–	NA	–	–
8	+	NA	–	NV	NA	NV	WT	NA	WT	NV	NA	NV	+	NA	–	–
9	I	I	I	P	P	P	G13D	G13D	G13D	WT	WT	WT	I	I	I	–
10	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	–	–
11	+	+	–	A	A	P	WT	WT	WT	WT	WT	WT	+	+	–	
12	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	–	–
13	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	–	–
14	+	NA	–	P	NA	P	WT	NA	WT	WT	NA	WT	+	NA	–	–
15	+	NA	–	D	NA	D	WT	NA	WT	WT	NA	WT	+	NA	–	–
16	+	NA	–	P	NA	P	G12D	NA	G12D	WT	NA	WT	+	NA	–	–
17	+	NA	–	P	NA	P	G13D	NA	WT	WT	NA	WT	+	NA	–	–
18	+	NA	–	P	NA	P	G13D	NA	G13D	WT	NA	WT	–	NA	–	–
19	+	NA	–	L	NA	L	WT	NA	WT	WT	NA	WT	+	NA	–	–
20	+	+	–	P	P	P	G12V	G12V	G12V	WT	WT	WT	+	+	–	–
21	+	NA	–	D	NA	P	WT	NA	WT	WT	NA	WT	+	NA	–	–
22	+	+	–	P	P	D	WT	WT	WT	WT	WT	WT	+	+	–	–
23	+	+	–	D	D	P	G12A	G12A	G12A	WT	WT	WT	+	+	–	–
24	+	NA	–	NV	NA	NV	NV	NA	NV	NV	NA	NV	+	NA	–	–
25	+	+	–	D	D	D	WT	WT	WT	V600E	V600E	V600E	+	+	–	NR
26	+	+	–	D	D	D	G12D	G12D	G12D	WT	WT	WT	+	+	–	–
27	+	+	–	D	P	P	G12A	G12A	G12A	WT	WT	WT	–	–	–	NR
28	+	+	–	P	NV	A	G12A	G12A	G12A	WT	WT	WT	+	–	–	NR
29	+	+	–	D	D	P	G12D	G12D	G12D	WT	WT	WT	+	+	–	NR
30	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	–	PR
31	+	+	–	P	P	P	WT	WT	WT	V600E	V600E	V600E	+	–	–	NR
32	+	NA	–	D	NA	D	WT	NA	WT	WT	NA	WT	+	NA	–	NR
33	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	–	PR
34	I	NA	I	P	NA	P	G12S	NA	G12S	WT	NA	WT	I	NA	I	NR
35	+	NA	–	P	NA	P	G12A	NA	G12A	WT	NA	WT	+	NA	–	NR
36	+	NA	–	P	NA	P	WT	NA	WT	WT	NA	WT	+	NA	–	NR
37	+	NA	–	D	NA	P	WT	NA	WT	WT	NA	WT	+	NA	–	–
38	+	NA	–	A	NA	A	WT	NA	WT	WT	NA	WT	–	NA	–	NR

EGFR IHC			*EGFR* FISH			*K-Ras*			*BRAF*			PTEN IHC			
T	LN	M	T	LN	M	T	LN	M	T	LN	M	T	LN	M	Clinical resp
+	+	+	D	D	D	WT	WT	WT	V600E	V600E	V600E	+	+	+	NR
+	+	+	D	D	D	G12D	G12D	G12D	WT	WT	WT	+	+	+	–
+	+	+	D	P	P	G12A	G12A	G12A	WT	WT	WT	–	–	–	NR
+	+	+	P	NV	A	G12A	G12A	G12A	WT	WT	WT	+	–	–	NR
+	+	+	D	D	P	G12D	G12D	G12D	WT	WT	WT	+	+	+	NR
+	NA	+	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	+	PR
+	+	+	P	P	P	WT	WT	WT	V600E	V600E	V600E	+	–	–	NR
+	NA	+	D	NA	D	WT	NA	WT	WT	NA	WT	+	NA	+	NR
+	NA	+	A	NA	A	WT	NA	WT	WT	NA	WT	+	NA	+	PR
+	NA	+	P	NA	P	G12S	NA	G12S	WT	NA	WT	+	NA	+	NR
+	NA	+	P	NA	P	G12A	NA	G12A	WT	NA	WT	+	NA	+	NR
+	NA	+	P	NA	P	WT	NA	WT	WT	NA	WT	+	NA	–	NR
+	NA	+	D	NA	P	WT	NA	WT	WT	NA	WT	+	NA	+	–
+	NA	+	A	NA	A	WT	NA	WT	WT	NA	WT	–	NA	–	NR

Published Ahead of Print on November 2, 2009 as 10.1200/JCO.2008.21.6796
The latest version is at http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2008.21.6796

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

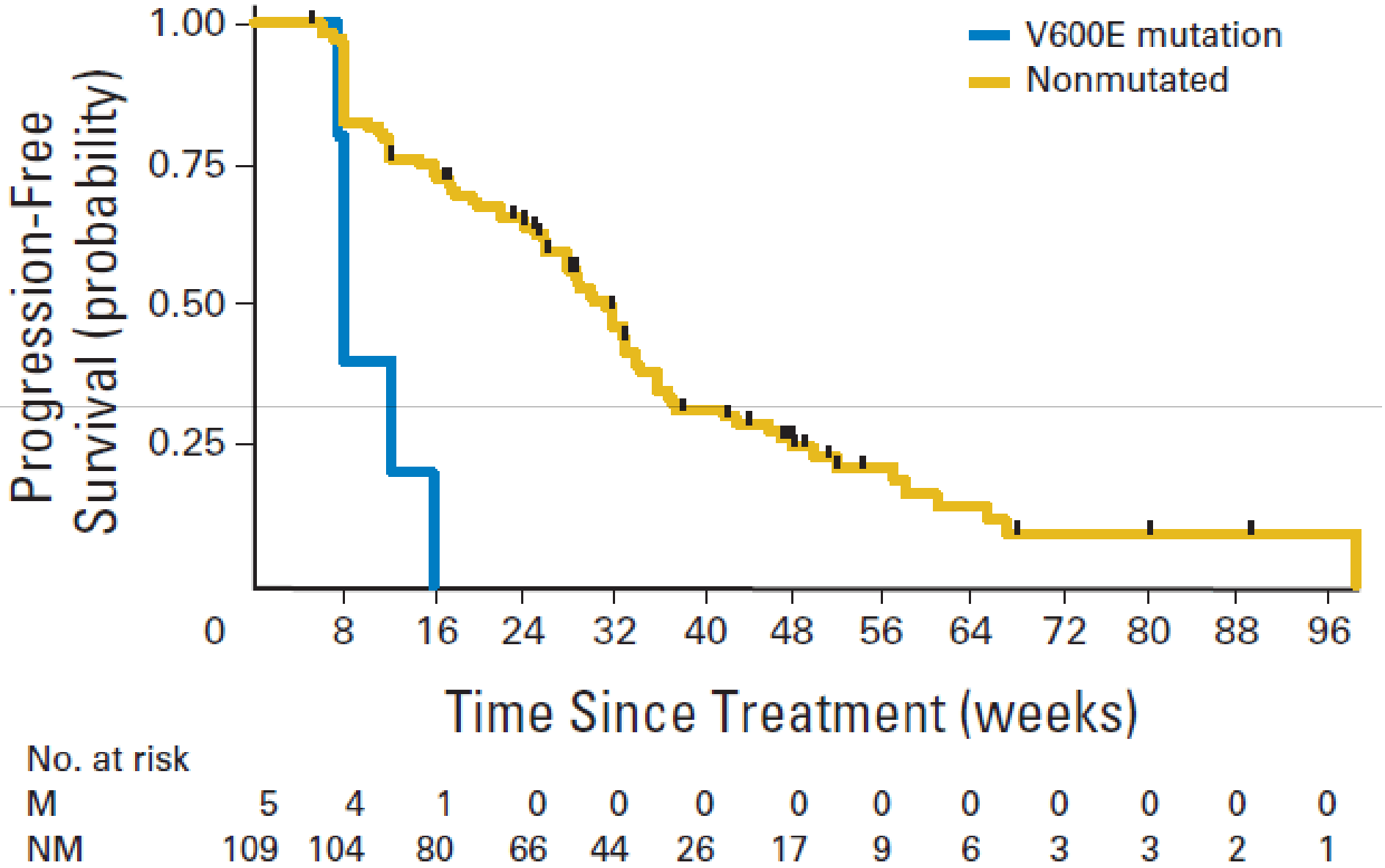
Analysis of *PTEN*, *BRAF*, and *EGFR* Status in Determining Benefit From Cetuximab Therapy in Wild-Type *KRAS* Metastatic Colon Cancer

Pierre Laurent-Puig, Anne Cayre, Gilles Manceau, Emmanuel Buc, Jean-Baptiste Bachet, Thierry Lecomte, Philippe Rougier, Astrid Lievre, Bruno Landi, Valérie Boige, Michel Ducreux, Marc Ychou, Fréderic Bibeau, Olivier Bouché, Julia Reid, Steven Stone, and Frédérique Penault-Llorca

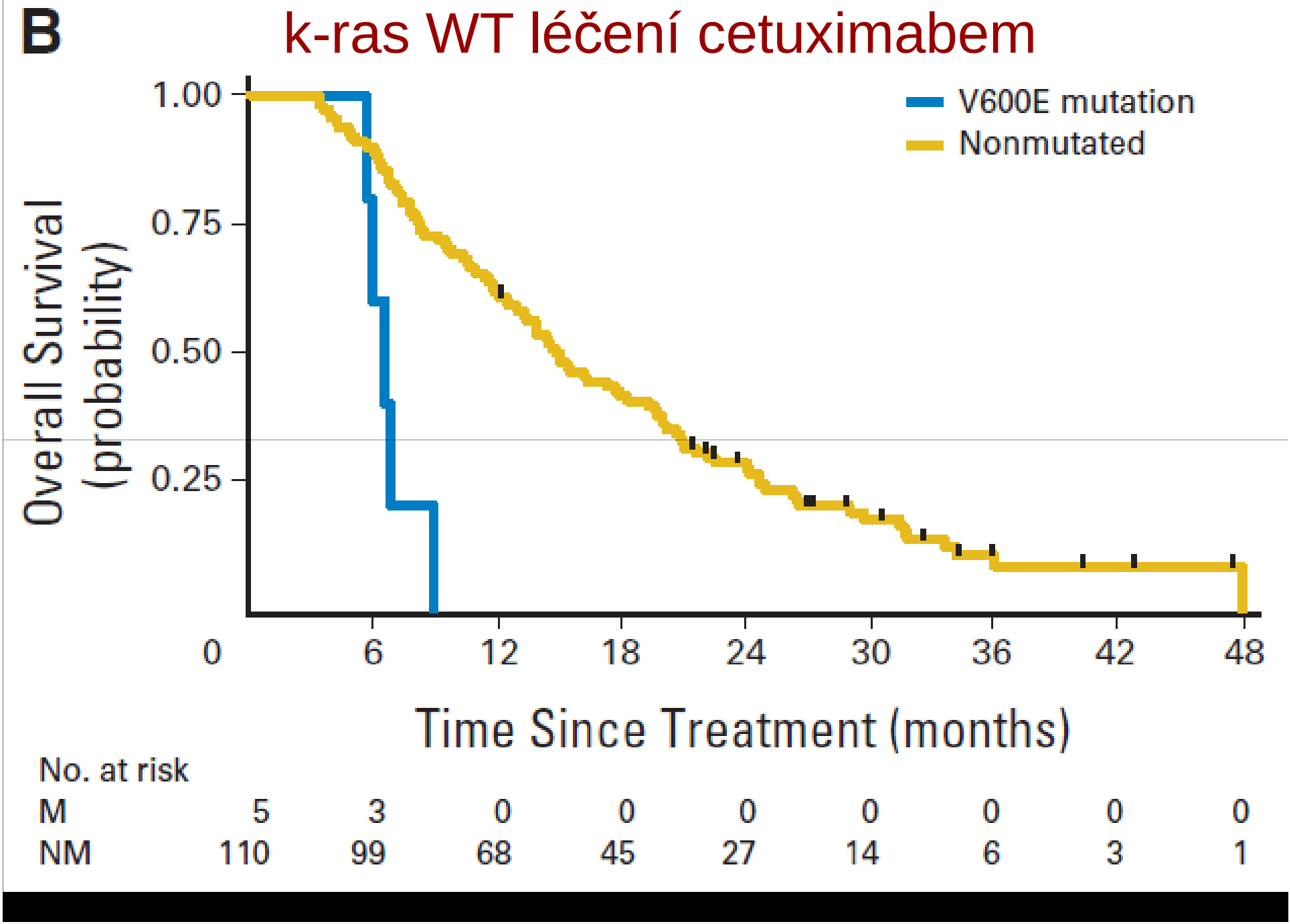
See accompanying editorial doi: 10.1200/JCO.2009.24.8013 and article doi: 10.1200/JCO.2009.22.4295

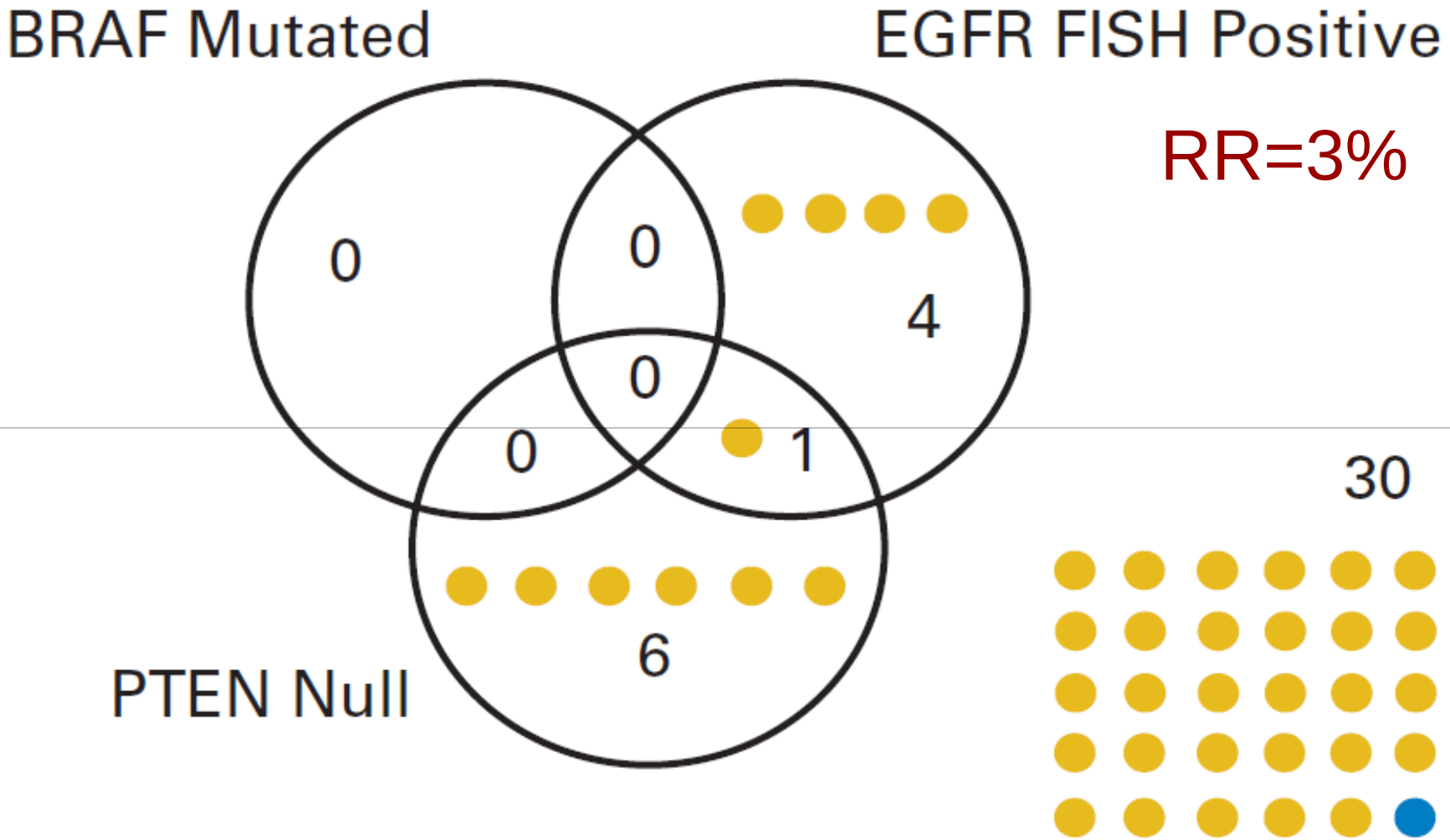
- retrospektivní studie
- 173 pacientů s mCRC
- k-ras mutace u 31,3% (n=53)
- k-ras WT léčení cetuximabem (n=116)
- detekce mutace BRAF (V600E)
- detekce amplifikace/polysomie EGFR (ISH)
- detekce ztráty exprese PTEN (IHC)

k-ras WT léčení cetuximabem

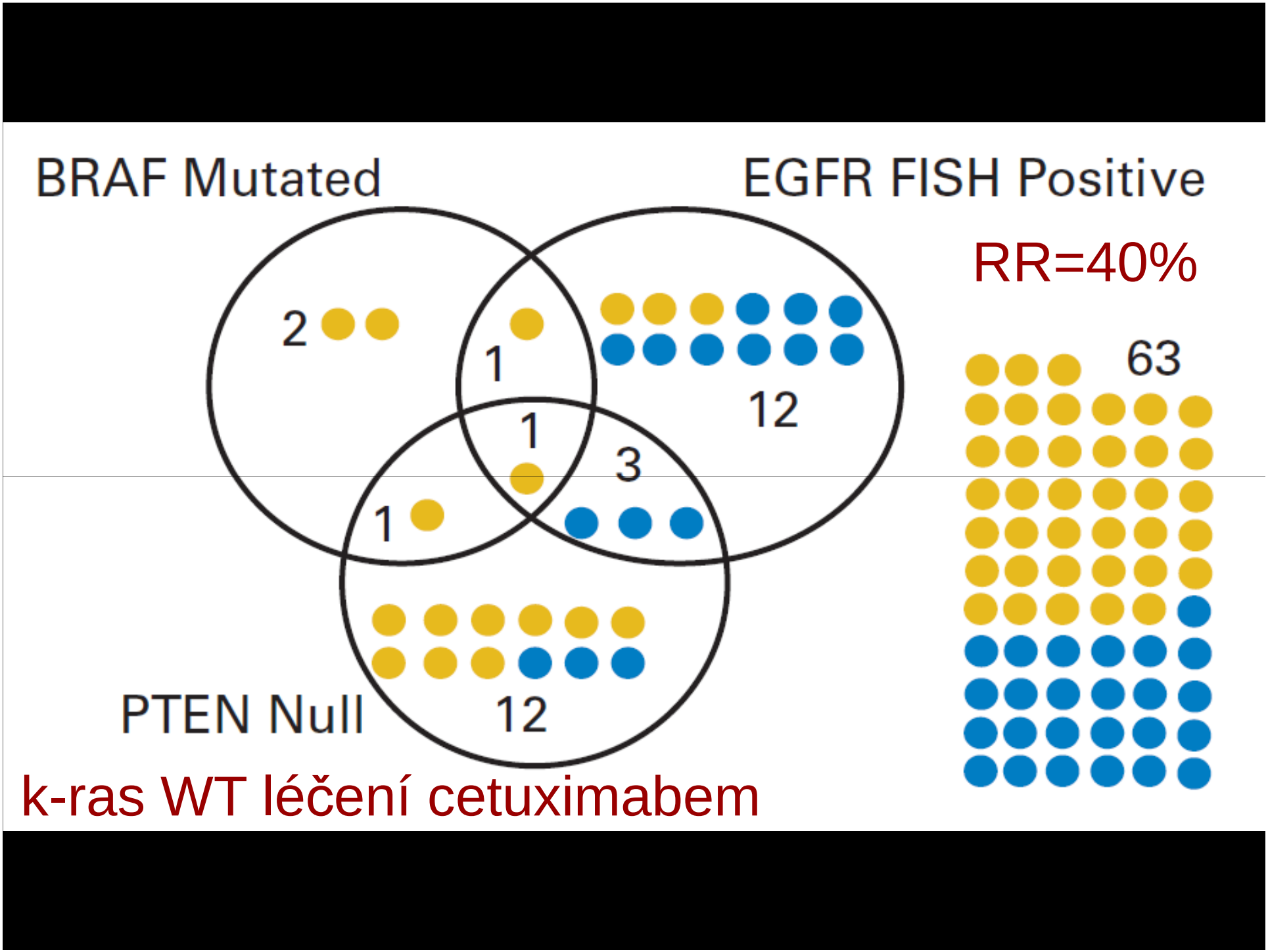


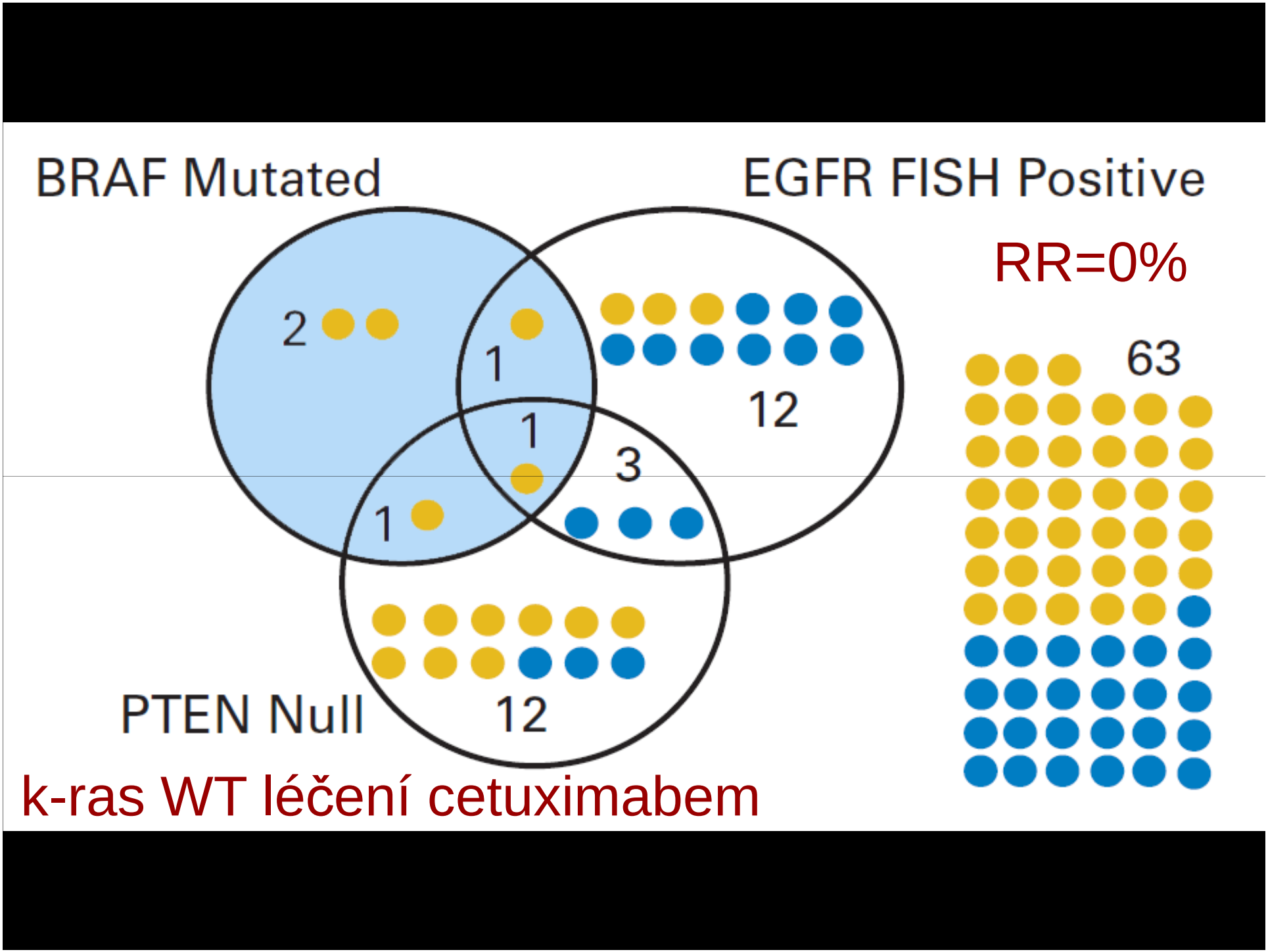
k-ras WT léčení cetuximabem

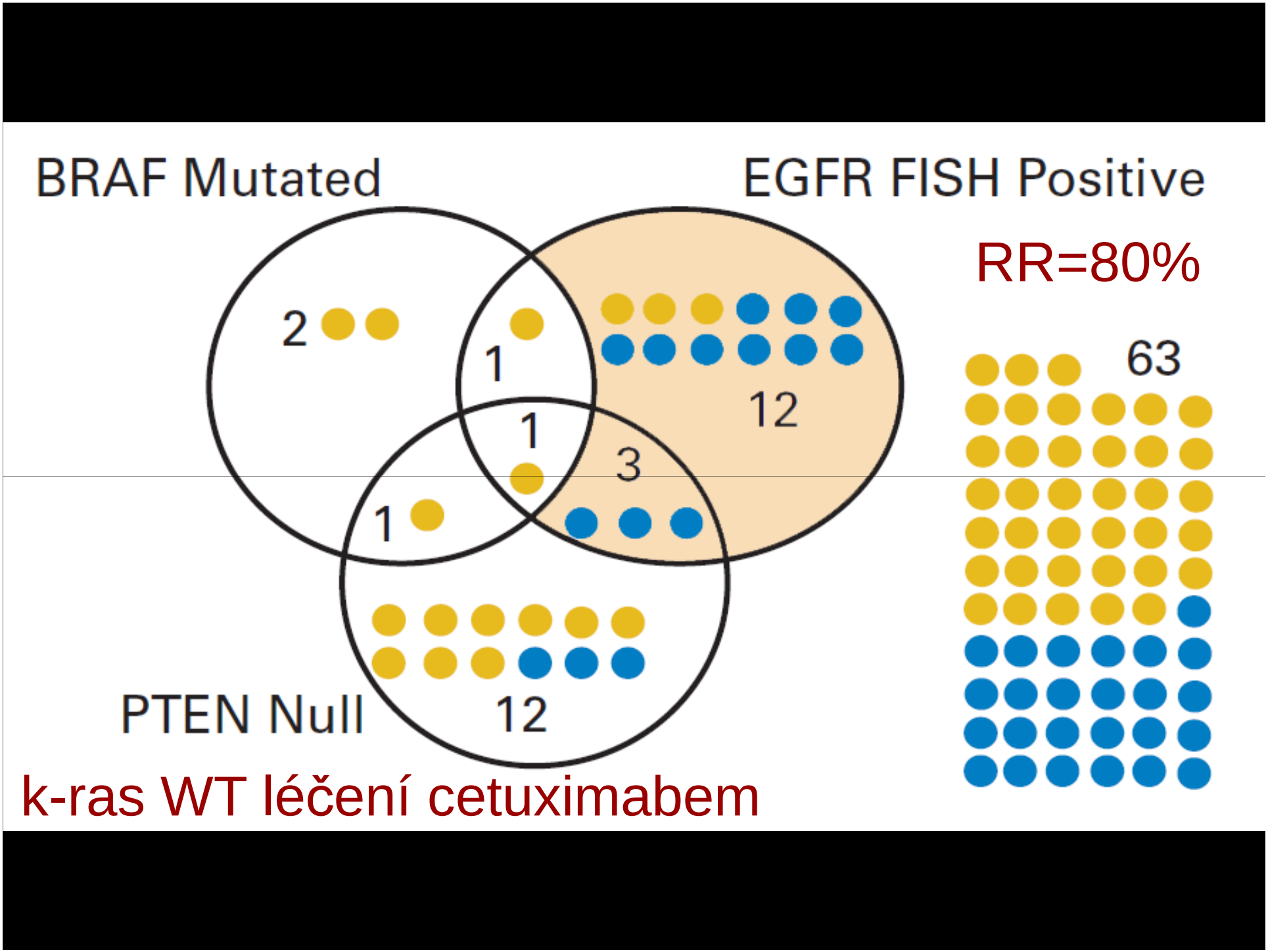


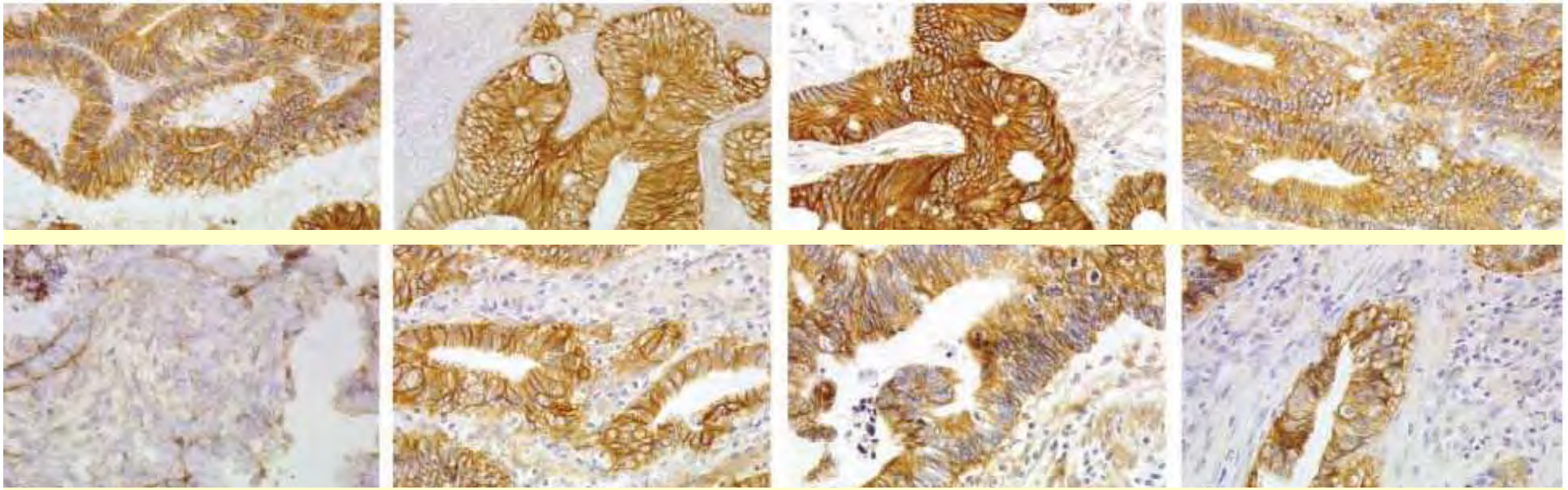


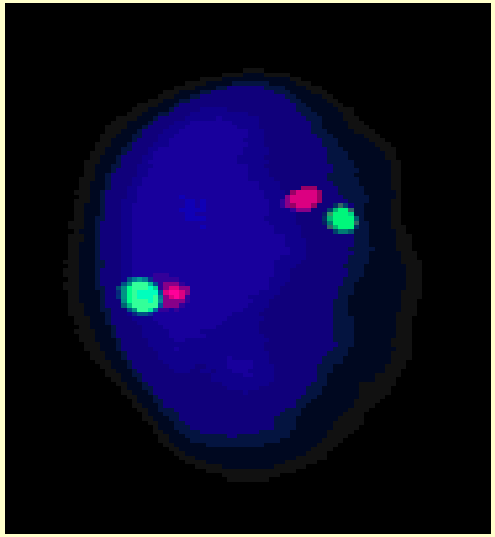
k-ras mutovaní léčení cetuximabem

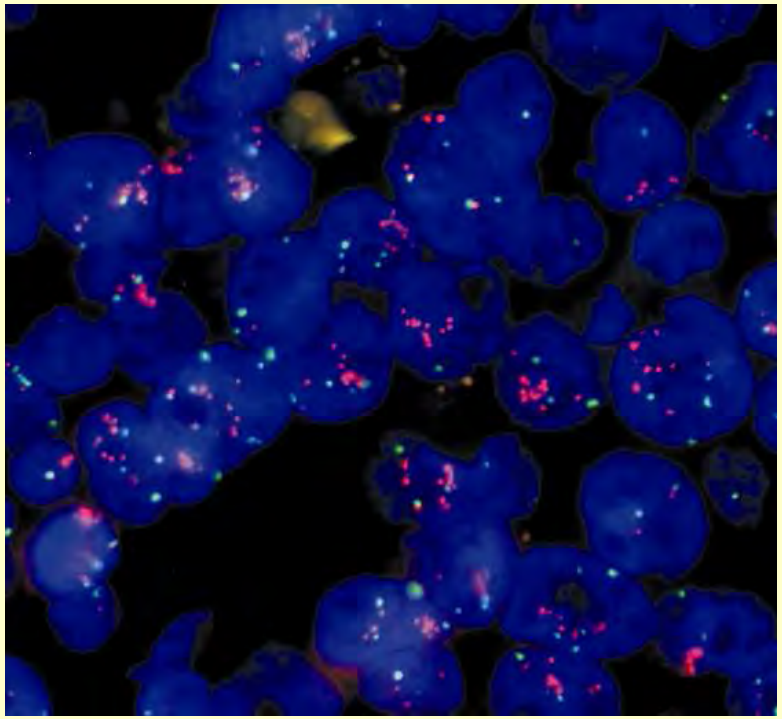

BRAF Mutated
EGFR FISH Positive
RR=40%
2
1
12
1
3
1
63
PTEN Null
12
k-ras WT léčení cetuximabem

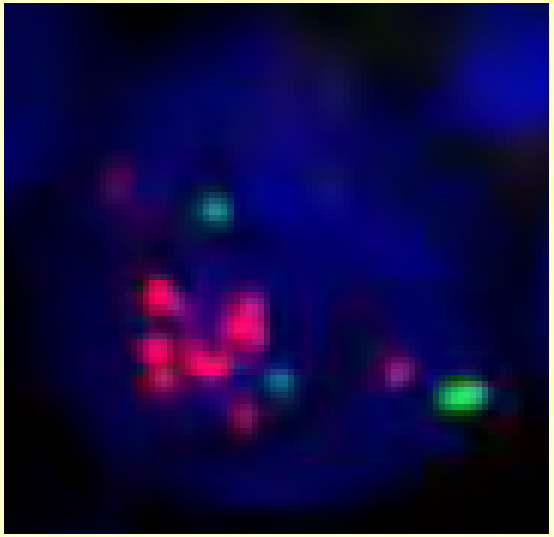

BRAF Mutated
EGFR FISH Positive
RR=0%
2
1
12
1
3
1
63
PTEN Null
12
k-ras WT léčení cetuximabem


BRAF Mutated
EGFR FISH Positive
RR=80%
2
1
12
1
3
1
63
PTEN Null
12
k-ras WT léčení cetuximabem









Annals of Surgical Oncology 15(2):649–654
DOI: 10.1245/s10434-007-9667-2

Cetuximab Shows Activity in Colorectal Cancer Patients With Tumors for Which FISH Analysis Does Not Detect an Increase in *EGFR* Gene Copy Number

Antoine Italiano, MD,[1,2] Philippe Follana, MD,[1] François-Xavier Caroli, MD,[3] Jean-Luc Badetti, MD,[4] Daniel Benchimol, MD,[3] Georges Garnier, MD,[5] Jean Gugenheim, MD,[3] Juliette Haudebourg, MD,[6] Frédérique Keslair, MS,[2] Gérard Lesbats, MD,[7] Gérard Lledo, MD,[8] Jean-Francois Roussel, MD,[9] Florence Pedeutour, PharmD, PhD,[2] and Eric François, MD[1]

Results: By IHC (n = 47), 39 patients (83%) had EGFR-positive tumors. *EGFR* gene copy gain was detected in 8 (19.5%) of 41 tumors. Neither EGFR expression assessed by IHC nor *EGFR* gene copy gain assessed by FISH were statistically significantly correlated with objective response rate, disease control rate, progression-free survival, and overall survival. Of the 33 patients whose tumors were FISH negative, 8 patients (24.2%) had a partial response, and 10 (30.3%) had stable disease.

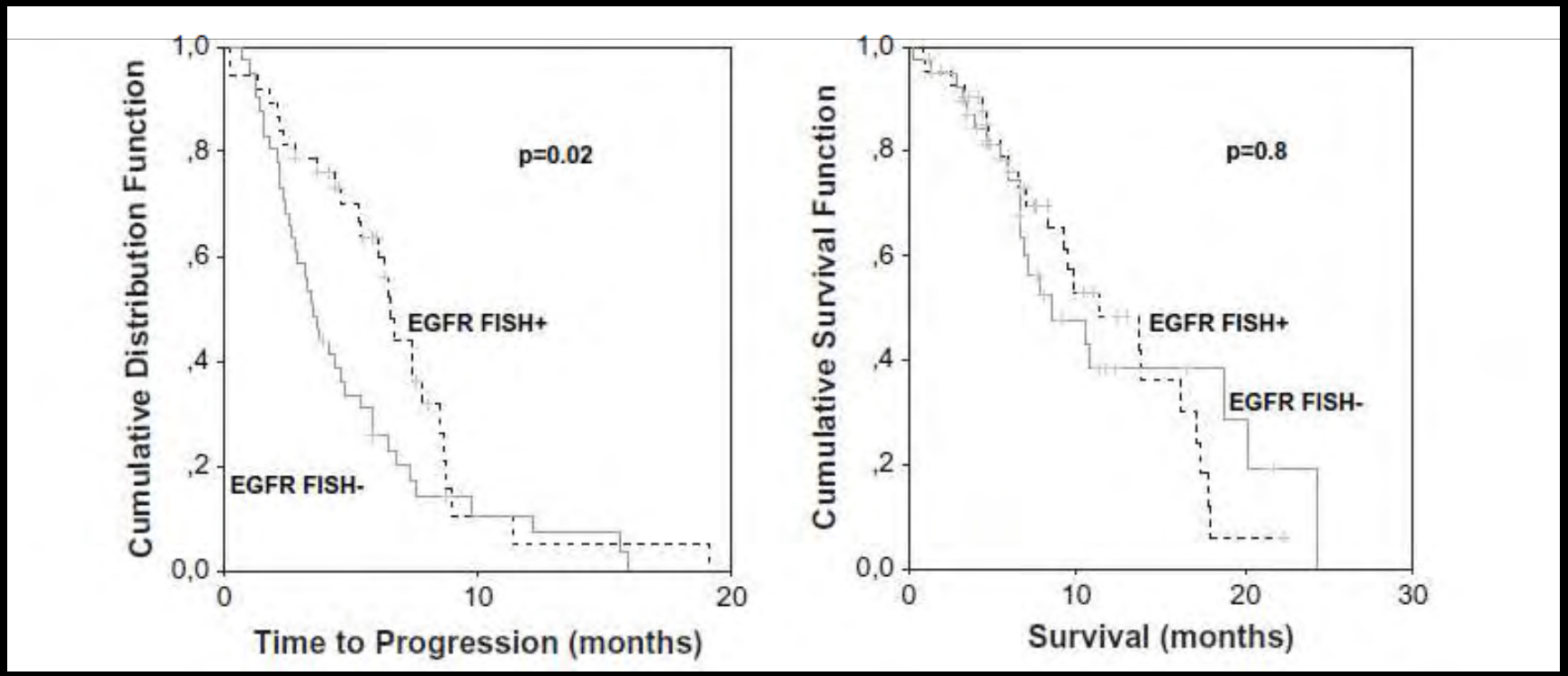
Conclusions: *EGFR* FISH analysis does not seem to be a sufficiently robust test for selecting candidate CRC patients for cetuximab therapy.

original article

Annals of Oncology 19: 717–723, 2008
doi:10.1093/annonc/mdm492
Published online 31 December 2007

EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients

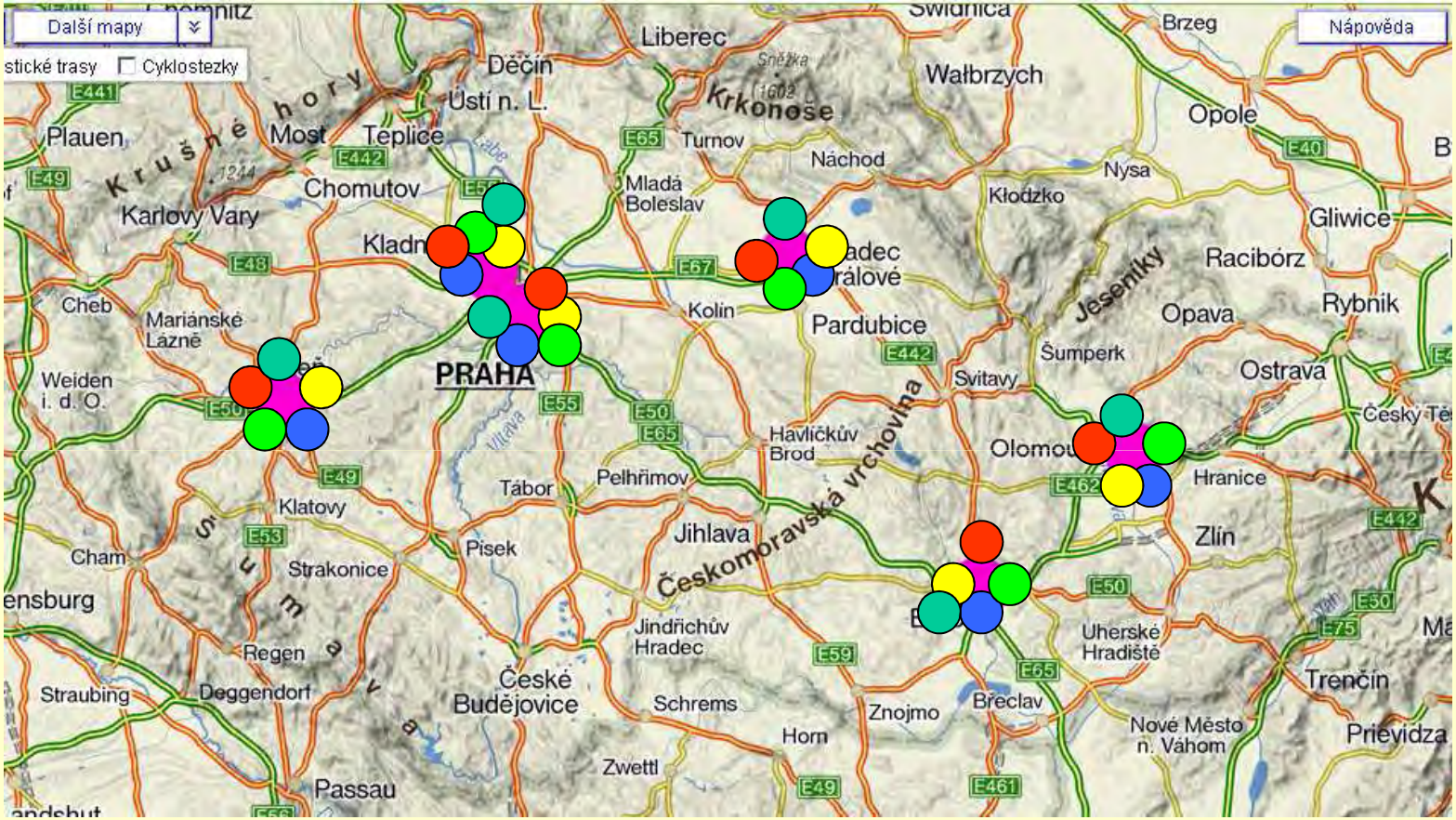
F. Cappuzzo[1*], G. Finocchiaro[1,2], E. Rossi[3], P.A. Jänne[4], C. Carnaghi[5], C. Calandri[6], K. Bencardino[7], C. Ligorio[1], F. Ciardiello[7], T. Pressiani[1], A. Destro[1,8], M. Roncalli[1,8], L. Crino[9], W. A. Franklin[2], A. Santoro[1] & M. Varella-Garcia[1]



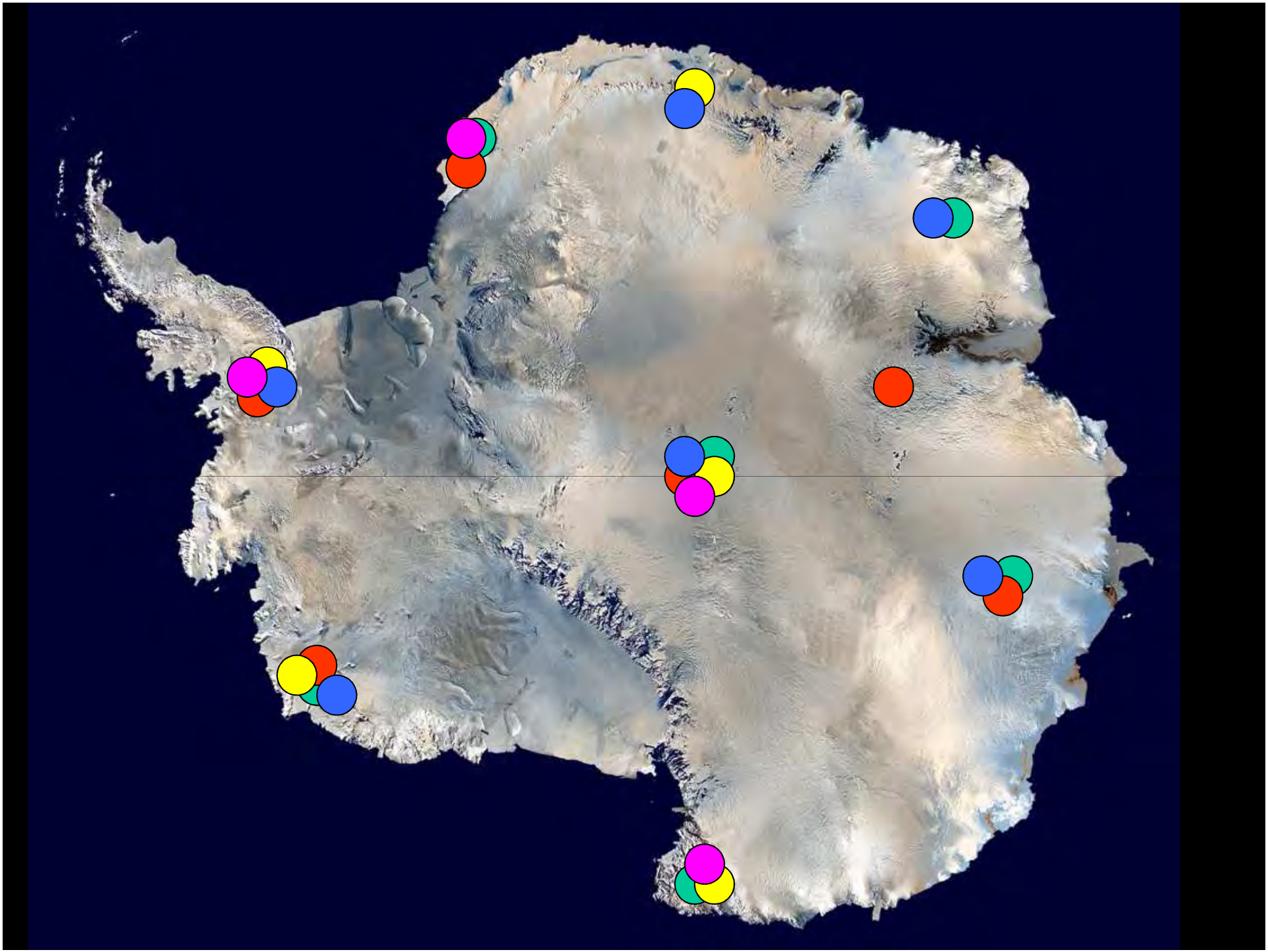
Závěry pro praxi

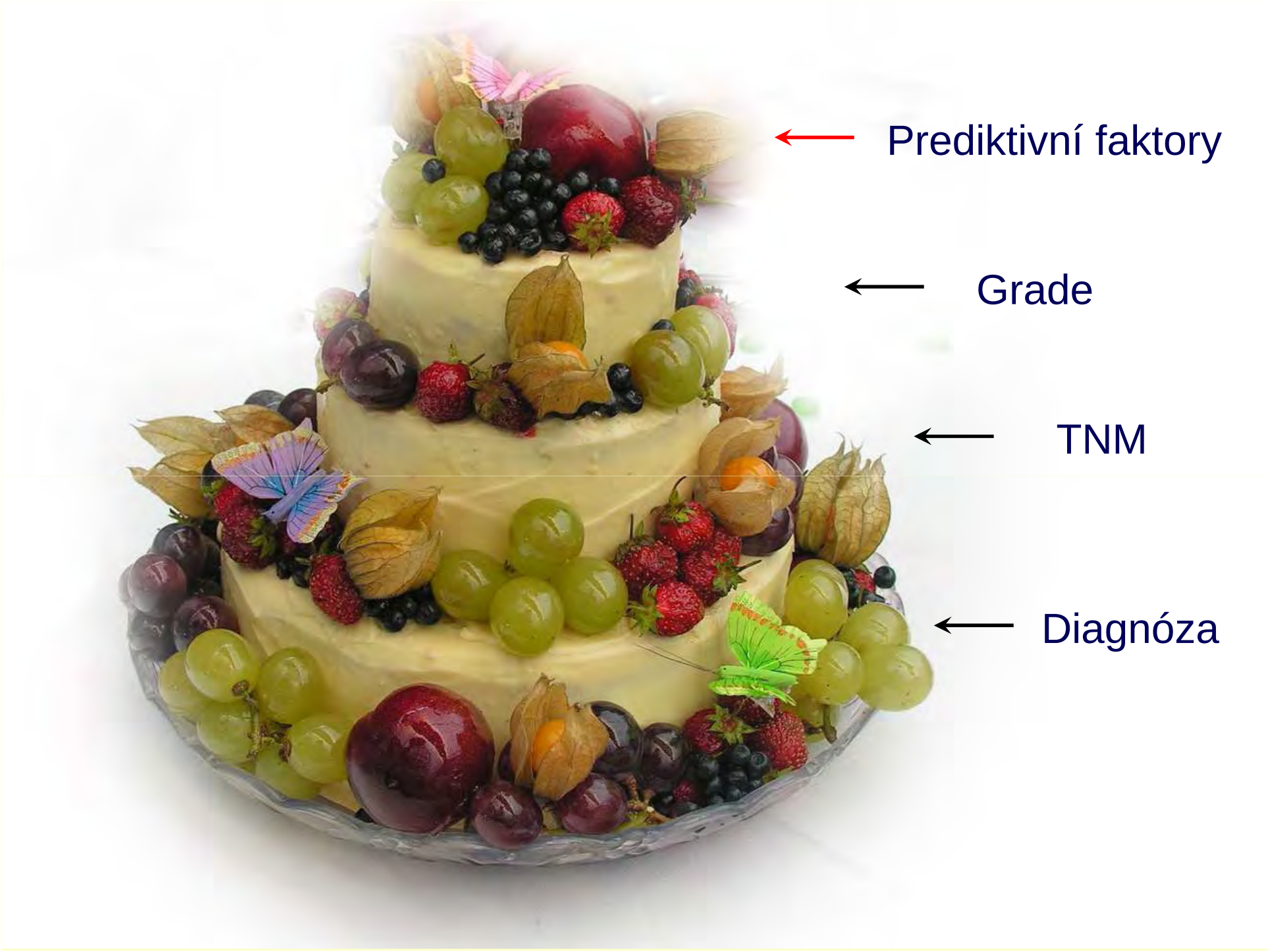
- Mechanismy účinku molekulární léčby jsou komplexní
- Funkční signální dráhy
- Vzájemné interakce - síť
- Heterogenita nádorové populace, diskrepance mezi prim. tu a meta
- IHC detekce EGFR je obsolétní x stále vyžadována
- K-ras WT – nejdůležitější, nikoli ale jediný molekulární prediktor odpovědi

Co nás čeká v nejbližší budoucnosti?



- HER2
- EGFR
- K-ras
- EGFRmut
- BRAF
- EGFR-FISH




Prediktivní faktory
Grade
TNM
Diagnóza