Unraveling signal escape: Vertical inhibition

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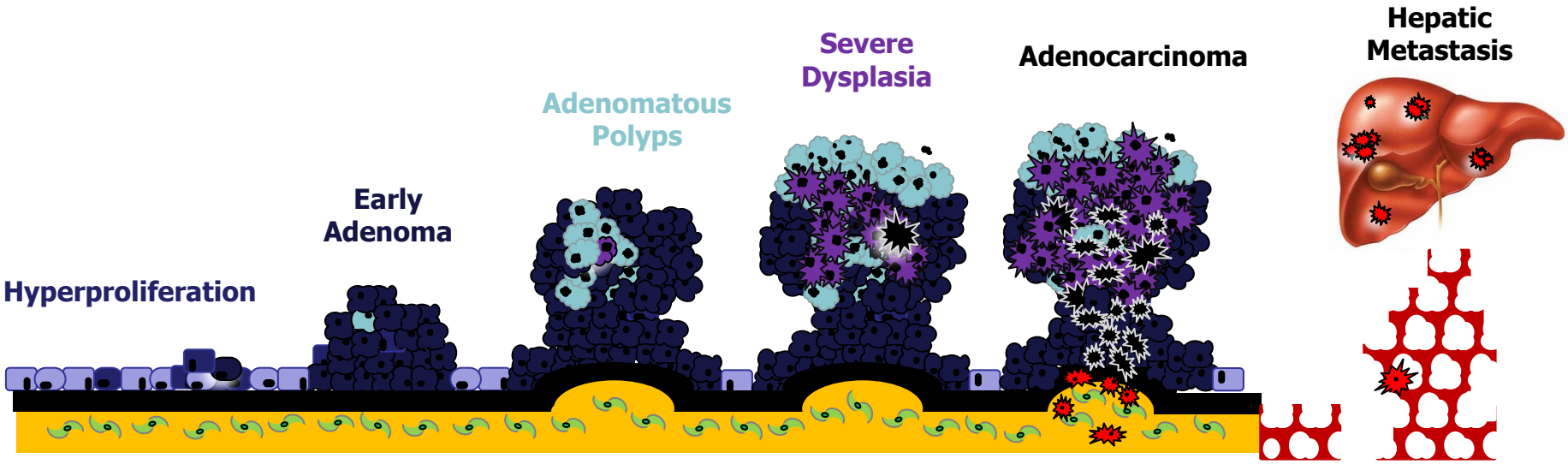
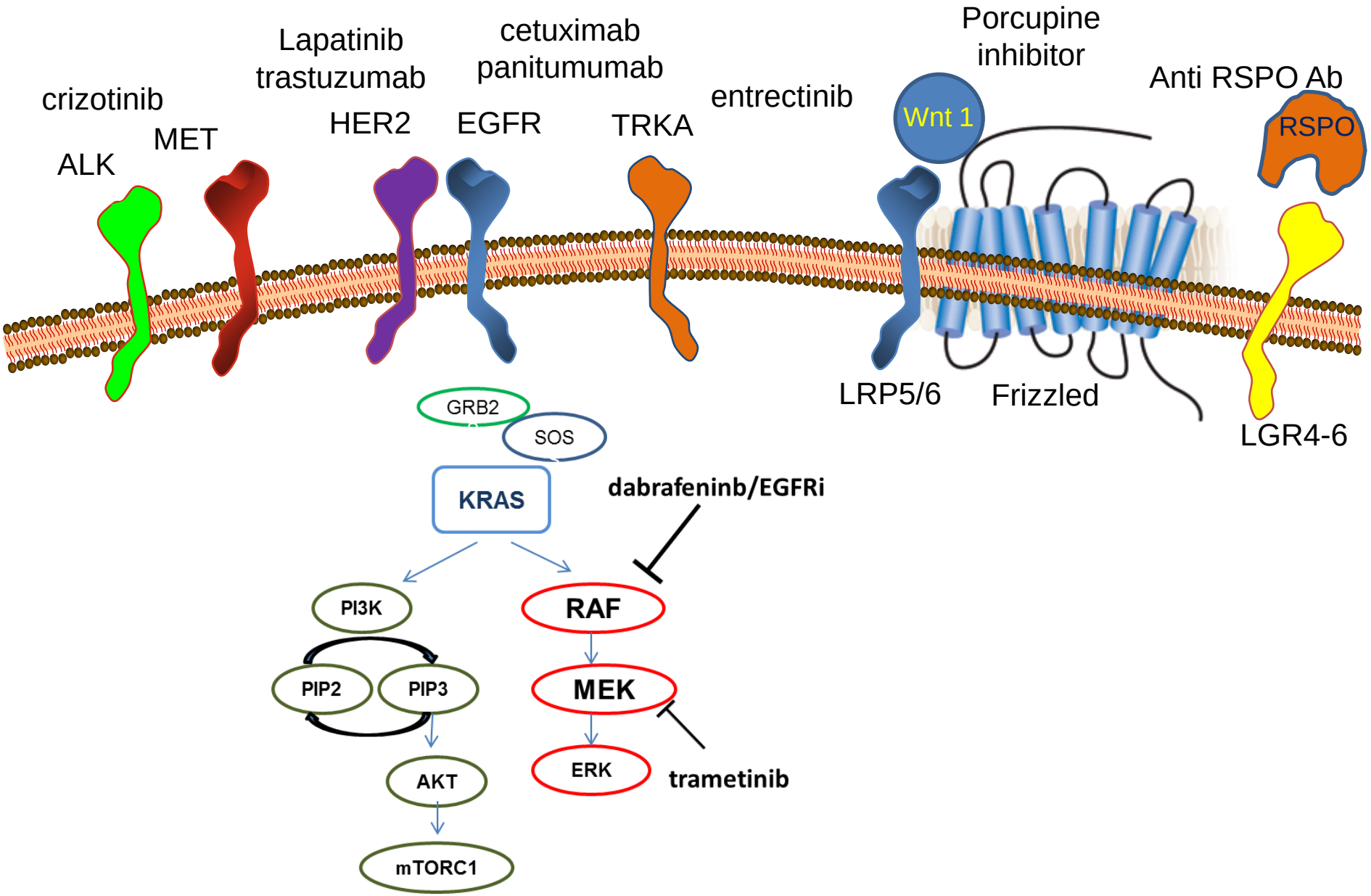
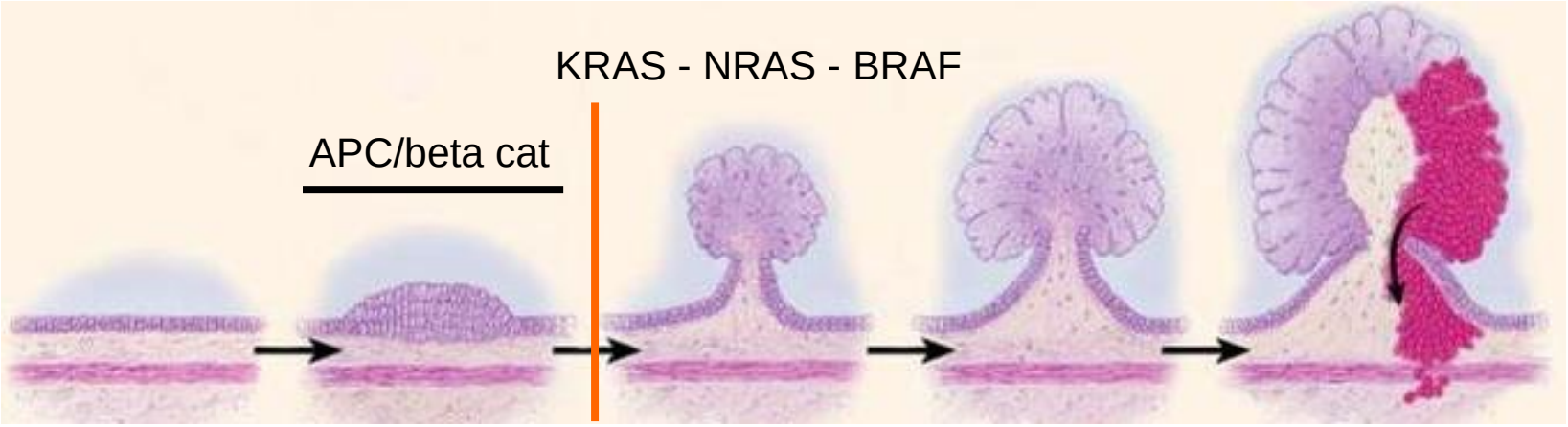
- Oncogenic pathways in colorectal cancer
- Drug resistance and signaling escape
- Lesion-specific therapeutic responses
- Tumor evolution as a therapeutic target

Colorectal cancer

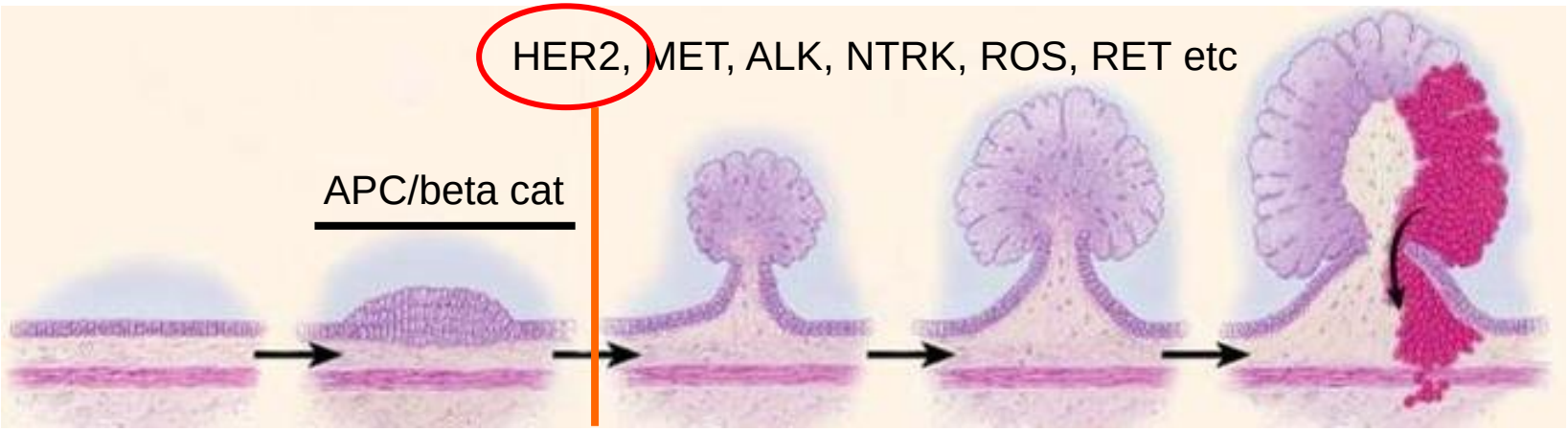


Actionable targets in CRC

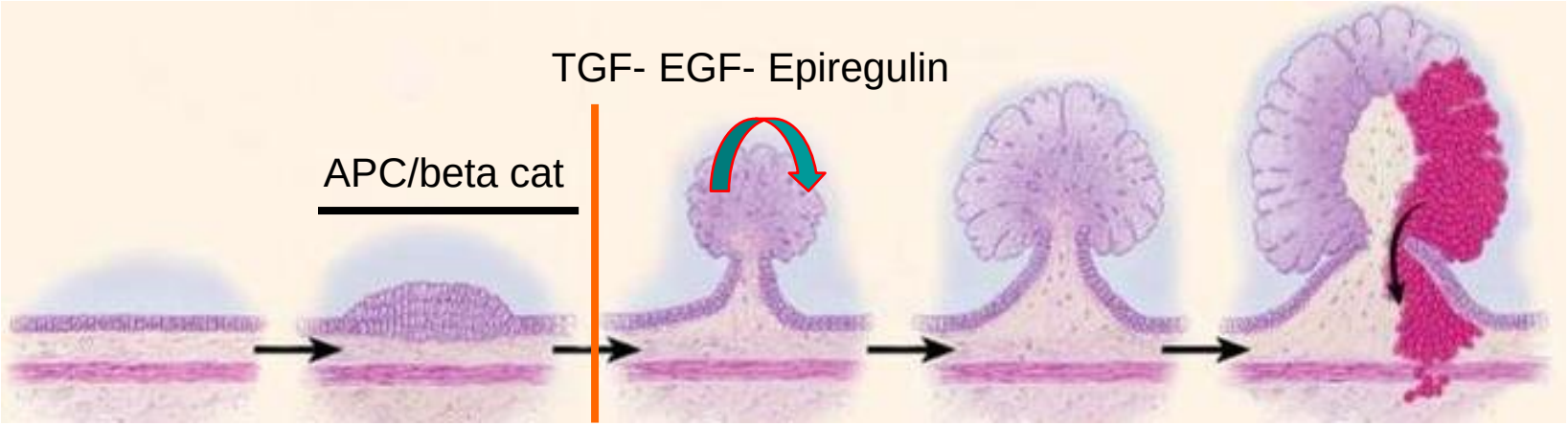



KRAS - NRAS - BRAF
APC/beta cat

50 %

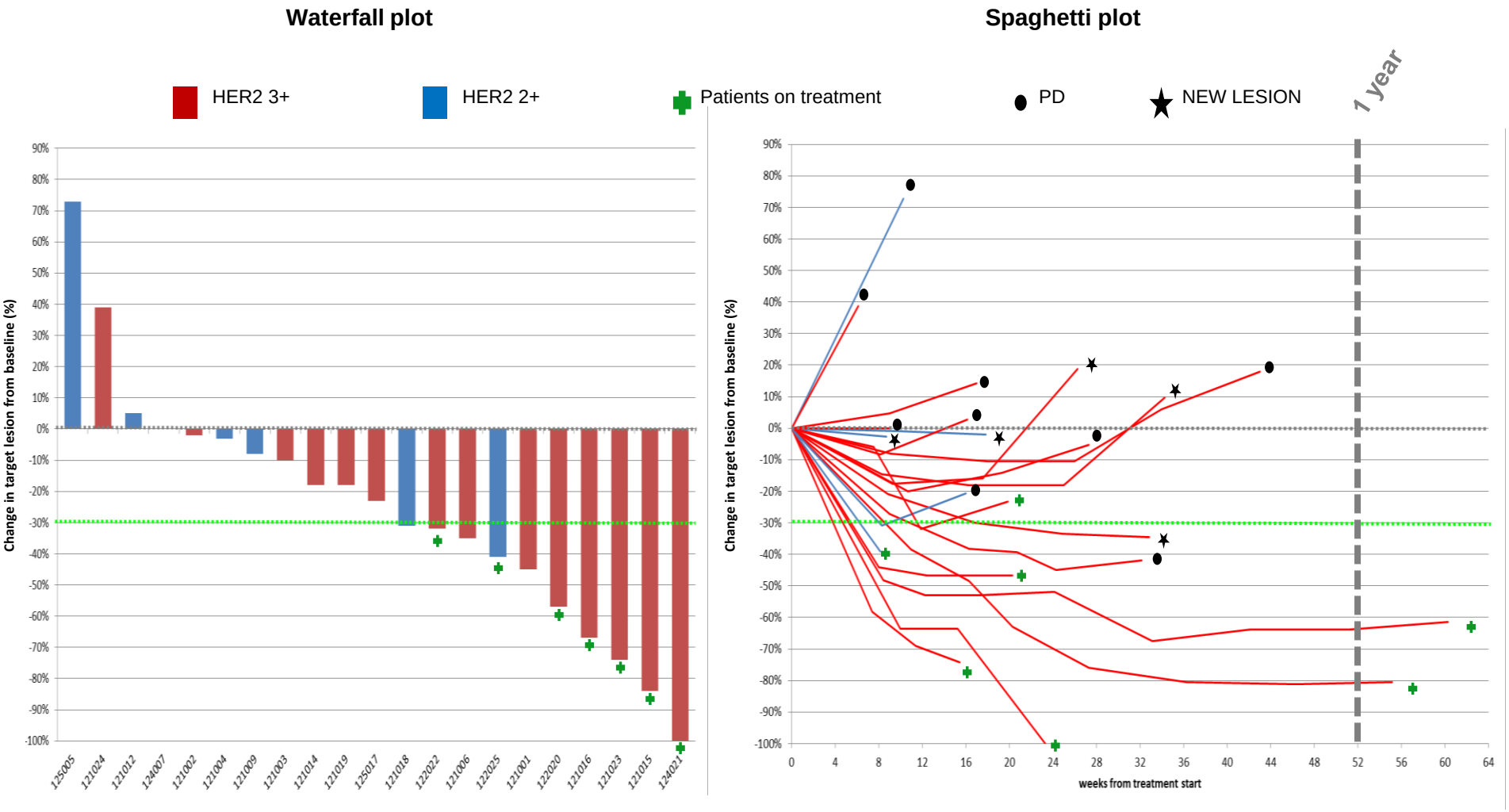

HER2, MET, ALK, NTRK, ROS, RET etc
APC/beta cat

20-30 %

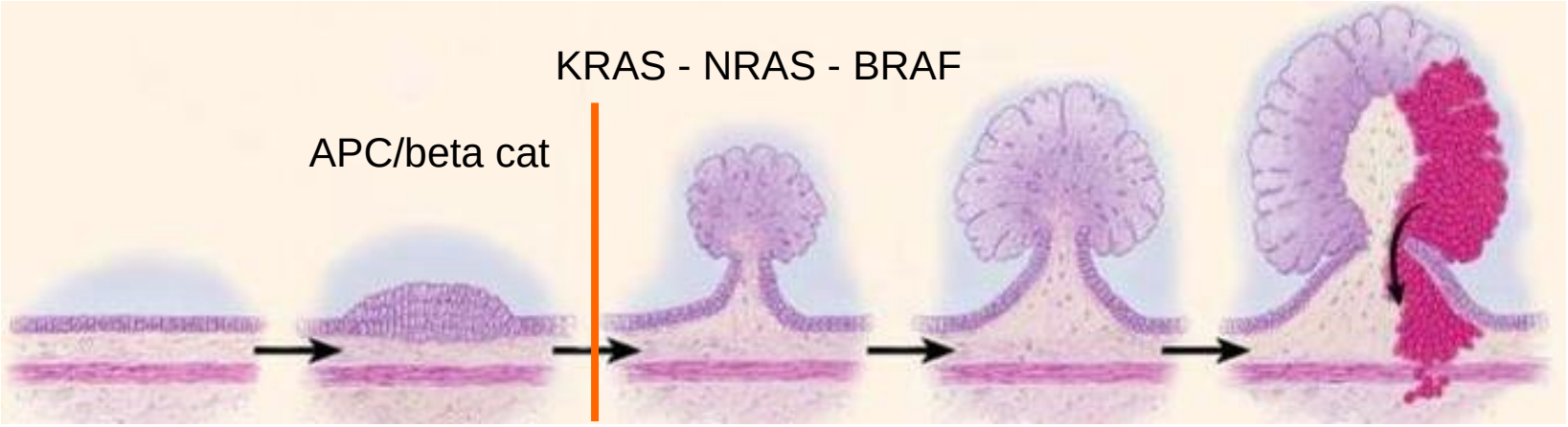

TGF- EGF- Epiregulin
APC/beta cat

≈15 %

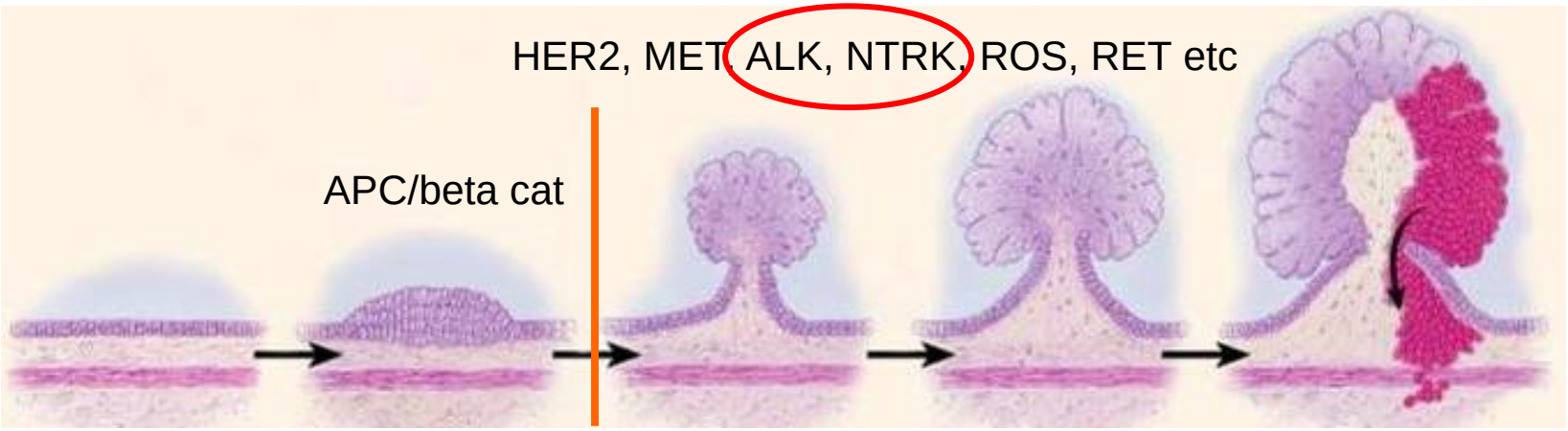
Response and resistance to HER2 blockade



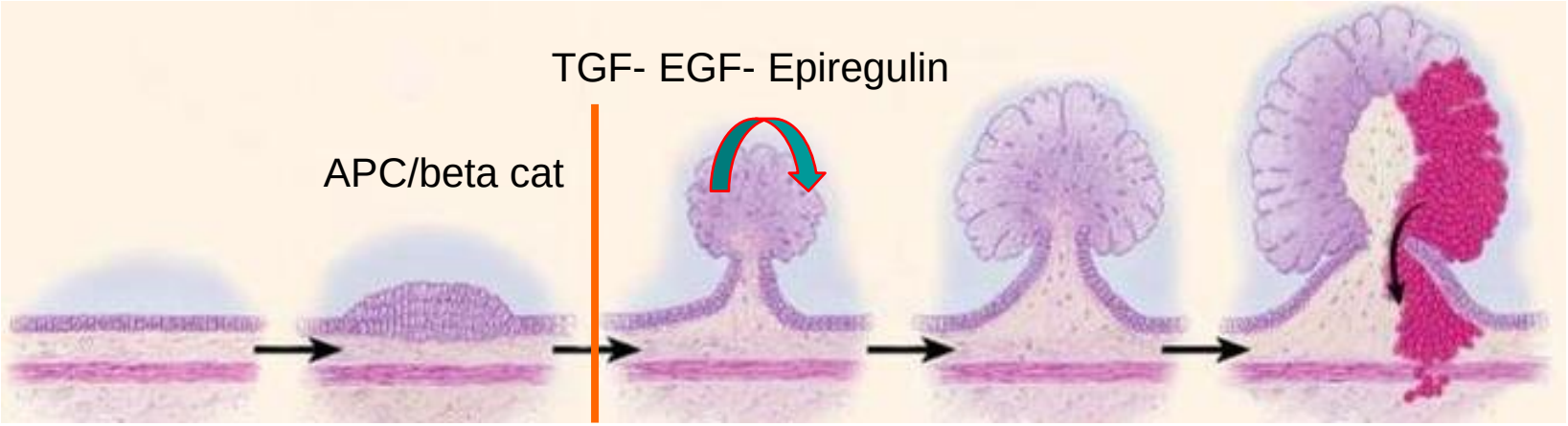
Sartore-Bianchi et al., In press


KRAS - NRAS - BRAF
APC/beta cat

50 %

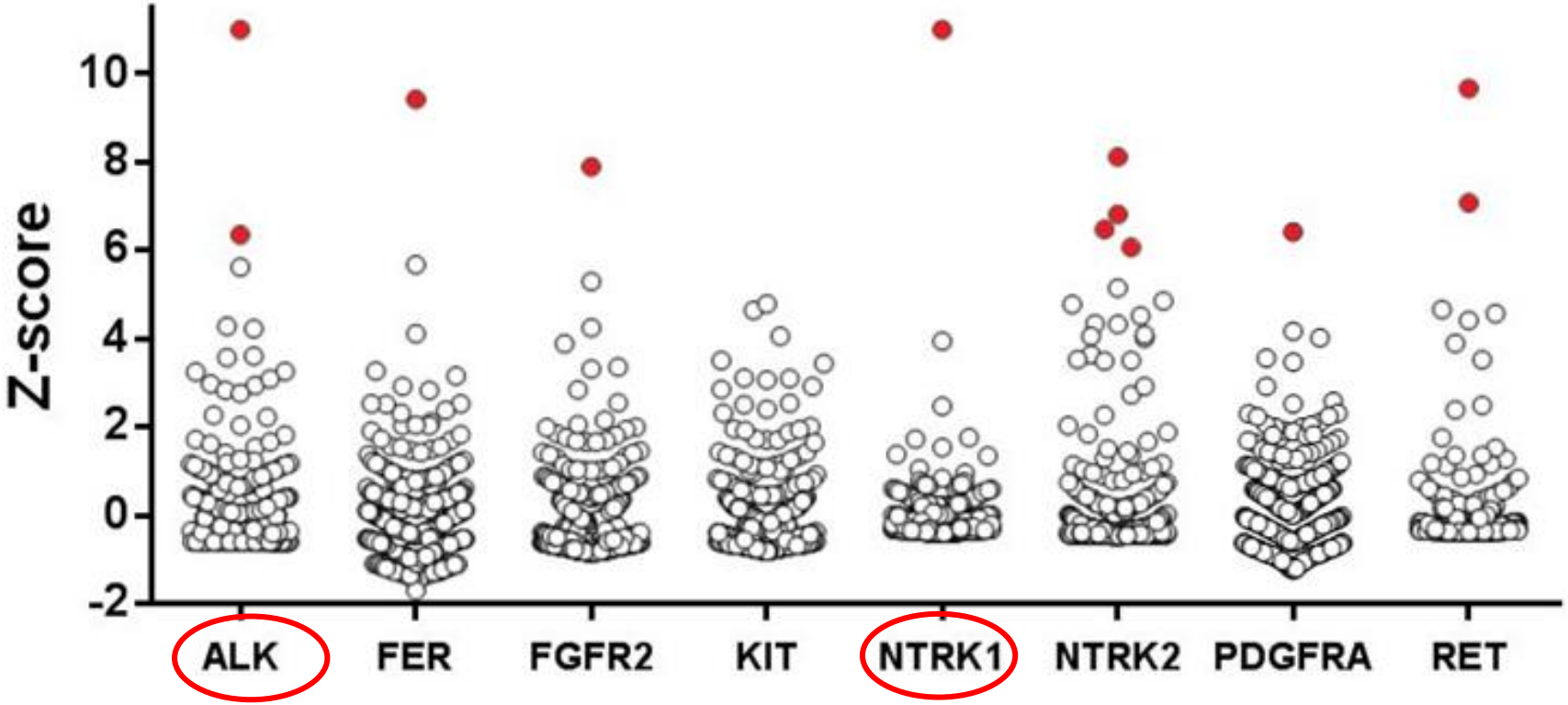

HER2, MET, ALK, NTRK, ROS, RET etc
APC/beta cat

20-30 %


TGF- EGF- Epiregulin
APC/beta cat

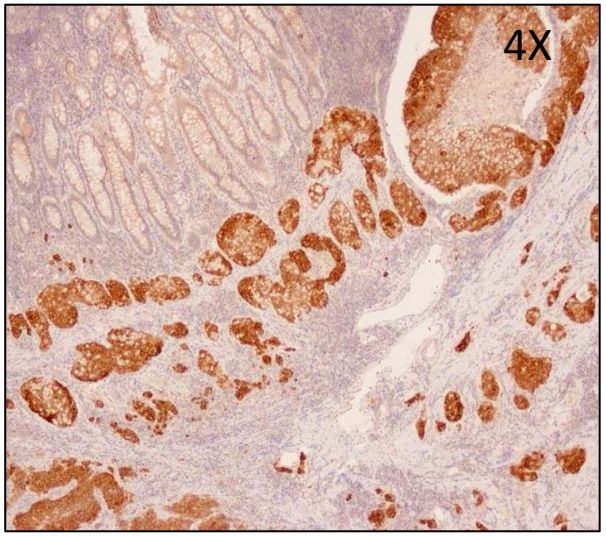
≈15 %

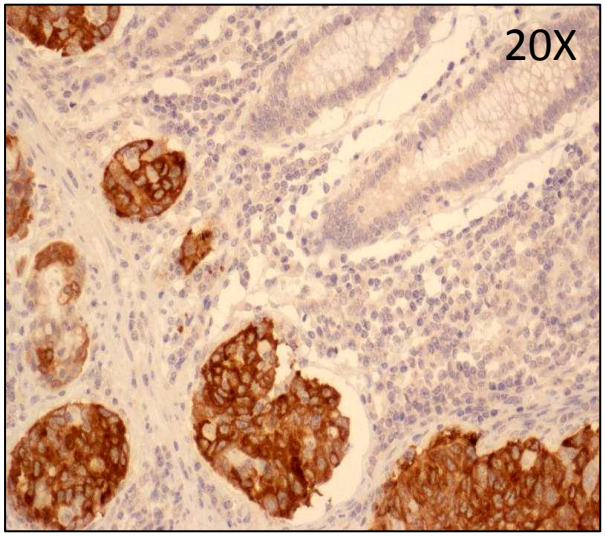
TRKA and ALK are actionable target in CRC

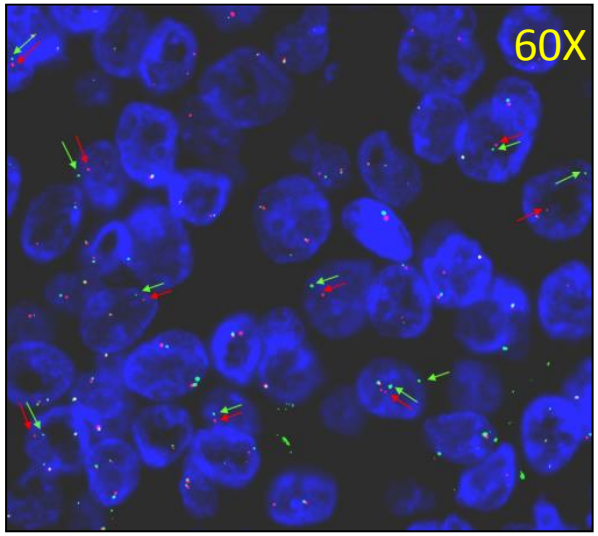


Medico et al., Nat Comm 2015

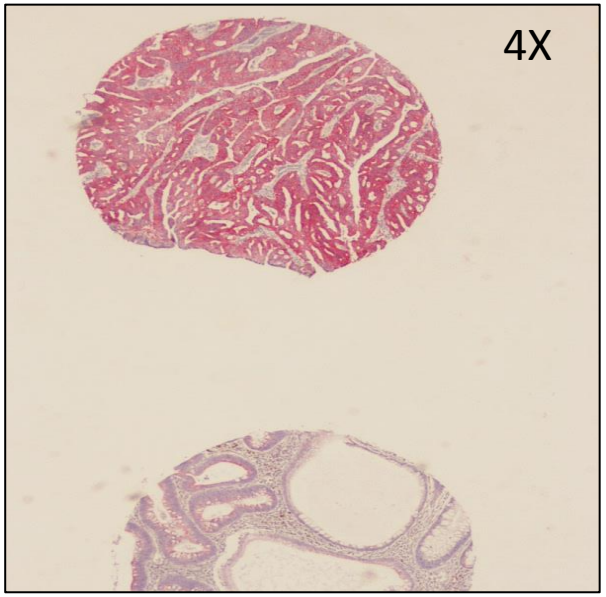
CRC patient: ALK translocated

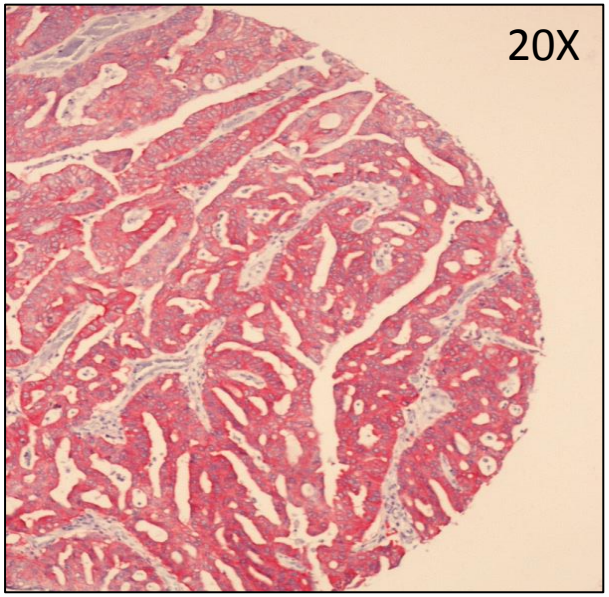


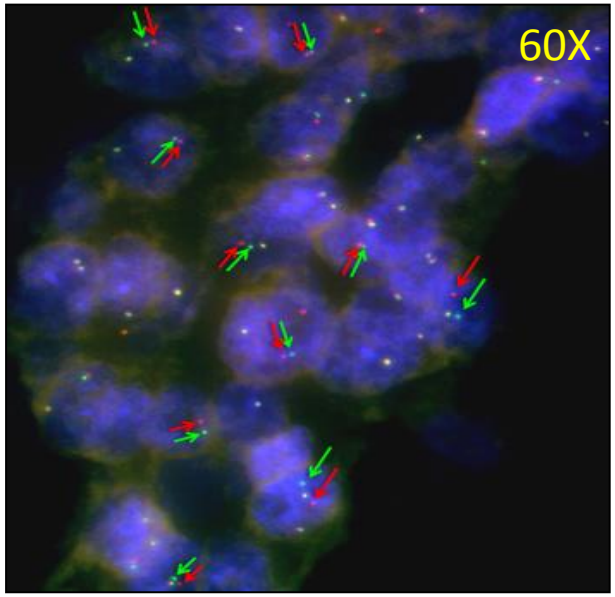




CRC patient: NTRK1 translocated

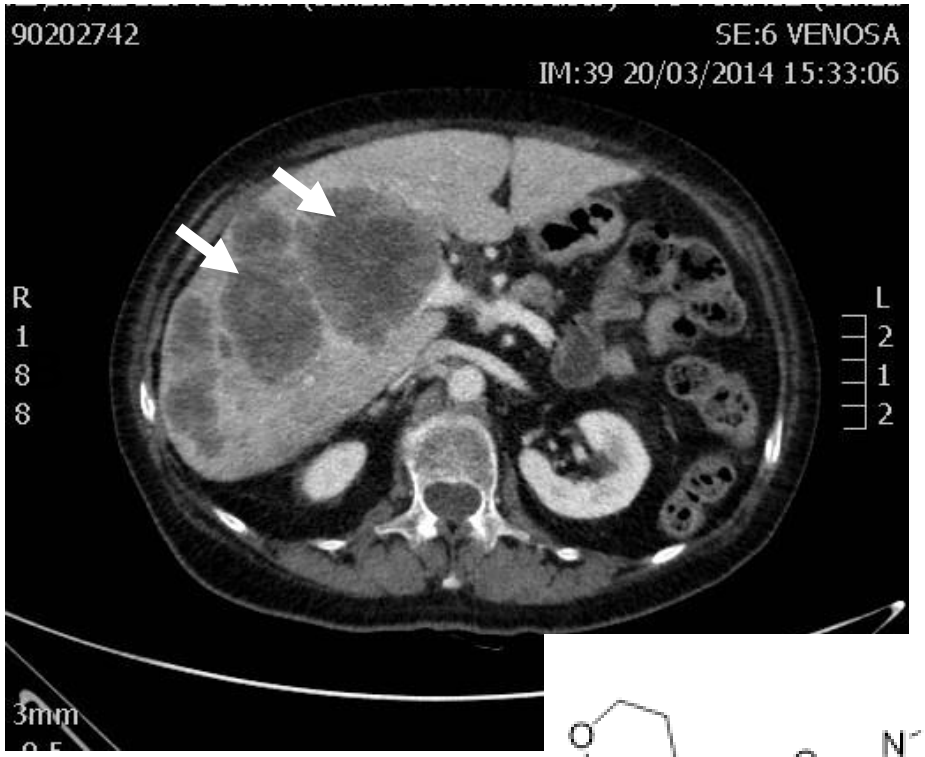


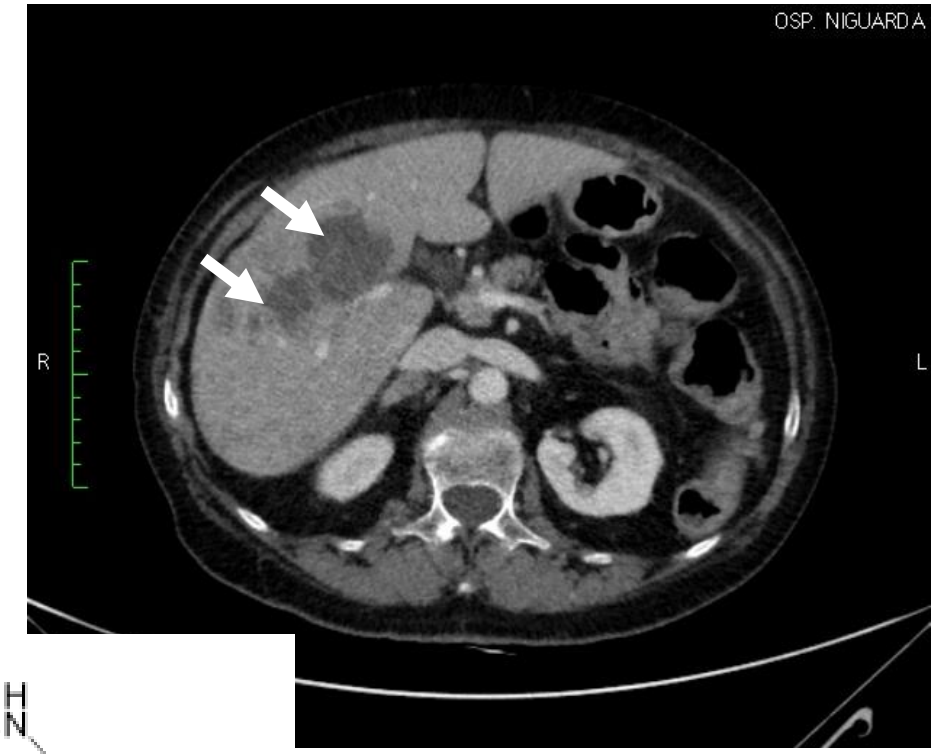


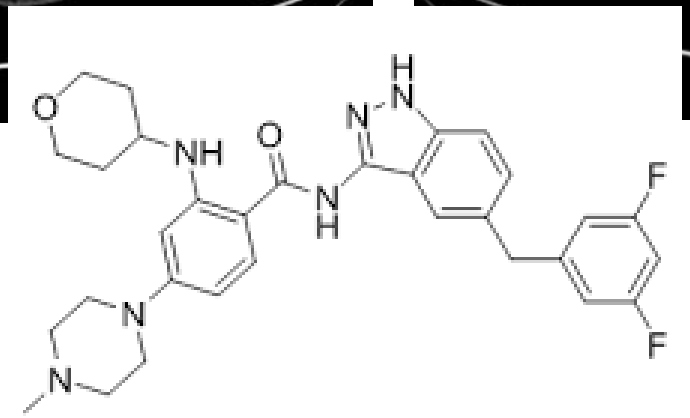


Medico et al., Nature Commun 2015

Targeting NTRK1 in CRC with entrectinib

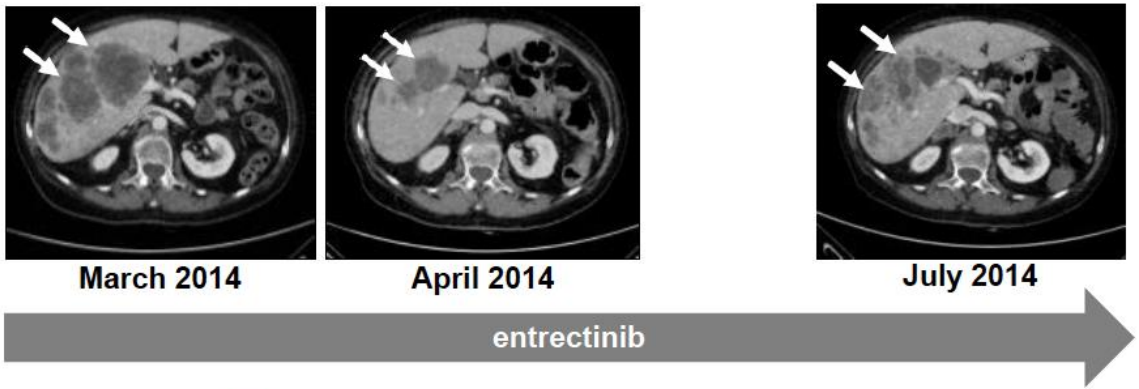


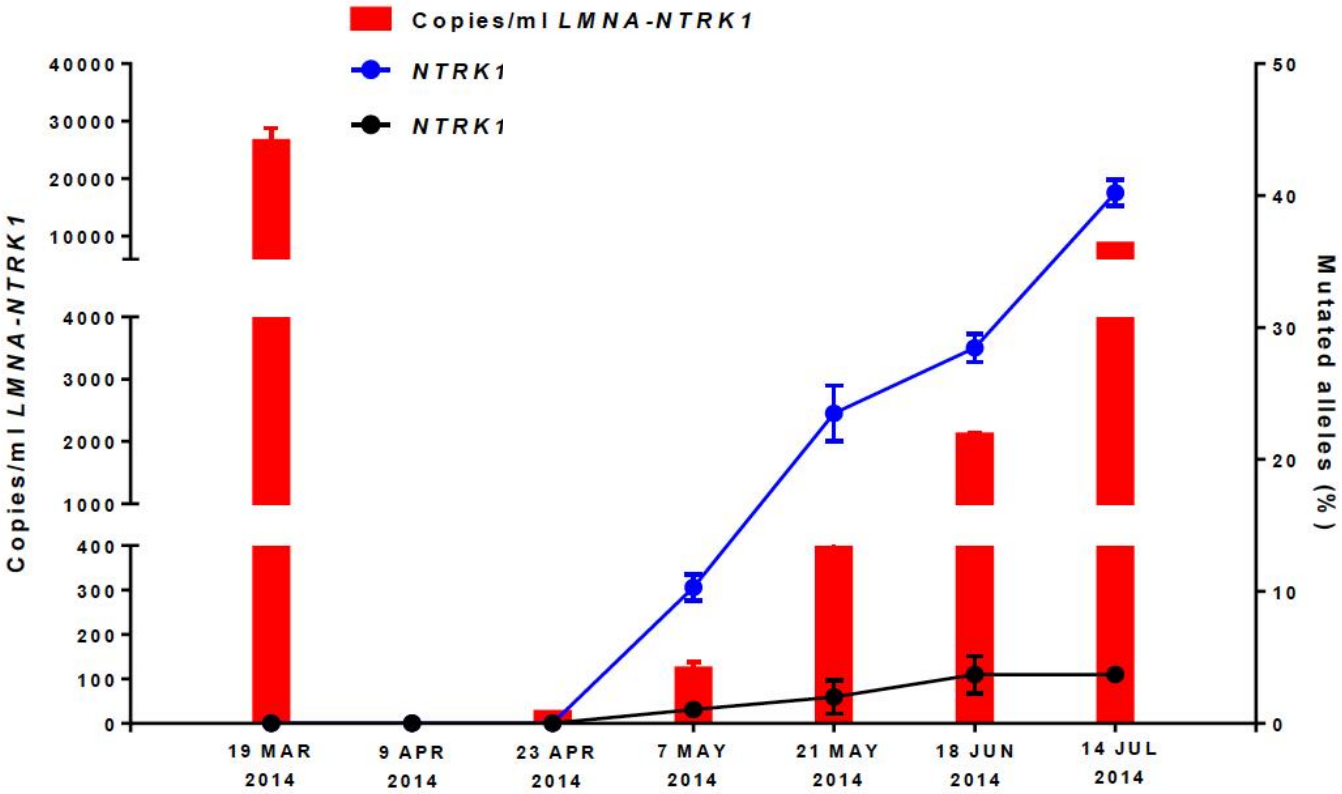




Sartore-Bianchi et al., JNCI 2016

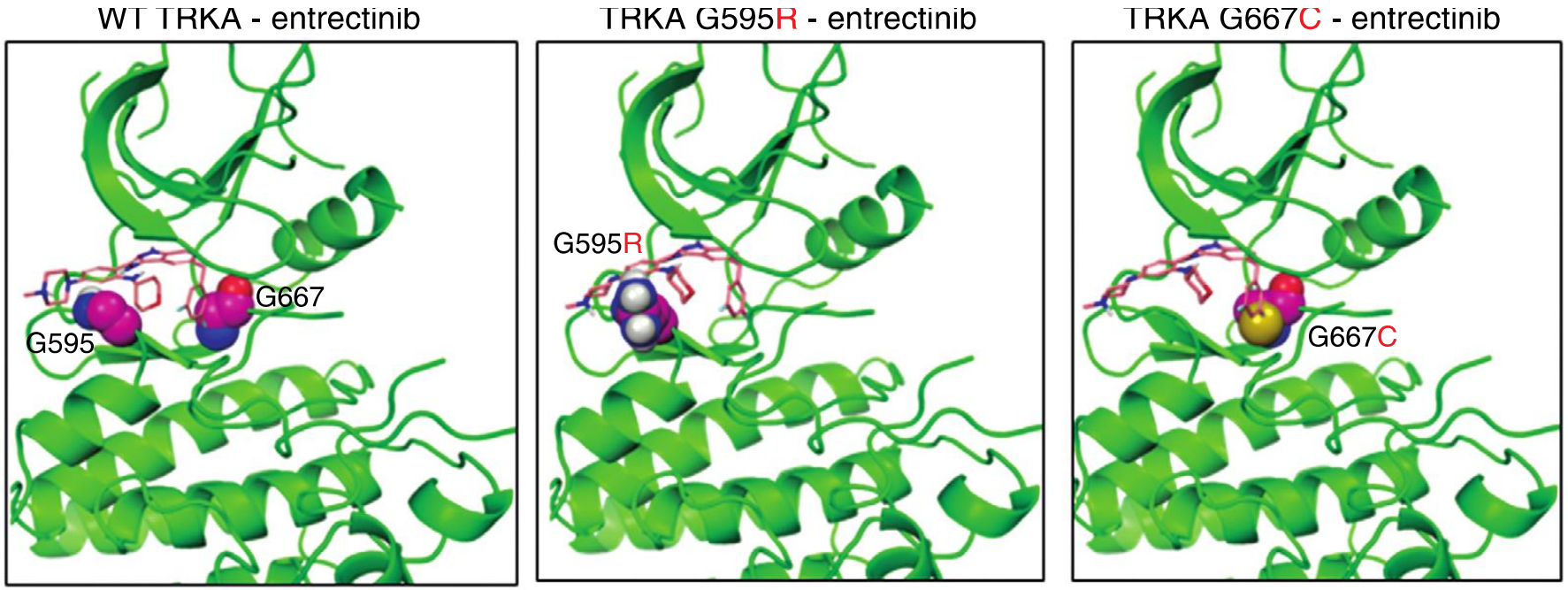
NTRK1 inhibition is short lived



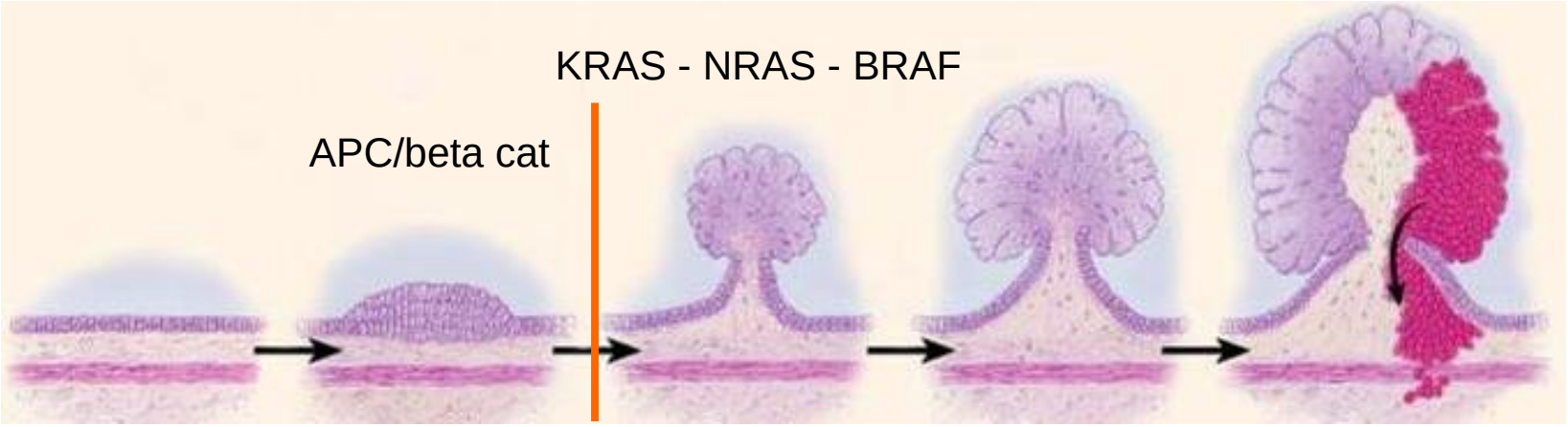


Russo et al., Cancer Discovery 2015

TRKA resistance mutations



Gene	Codon	Mutation at resistance	Tissue of detection	Associated with resistance to
NTRK1	595	p.G595R	Colon	Entrectinib
ALK	1202	p.G1202R	Lung	Crizotinib/ceritinib
ROS1	2032	p.G2032R	Lung	Crizotinib
EGFR	796	p.G796A/R	Lung	Erlotinib/gefitinib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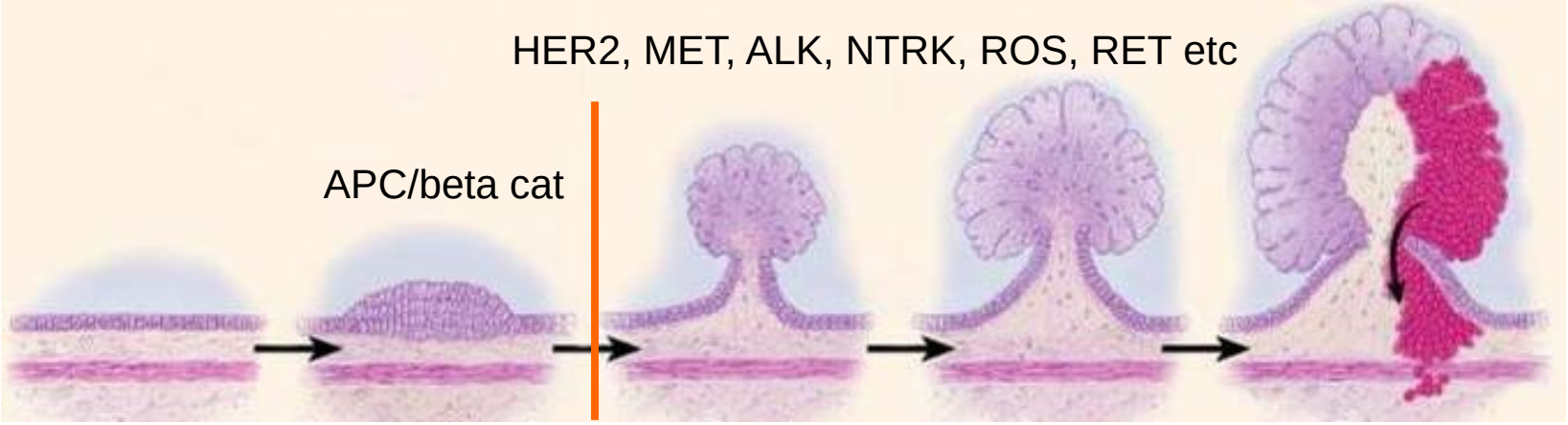
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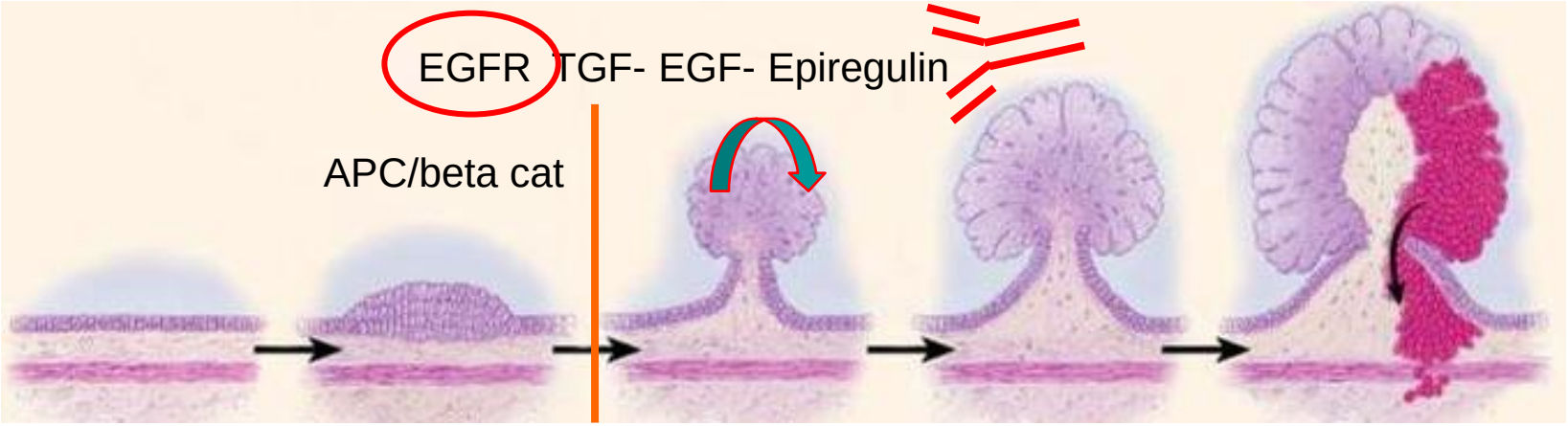
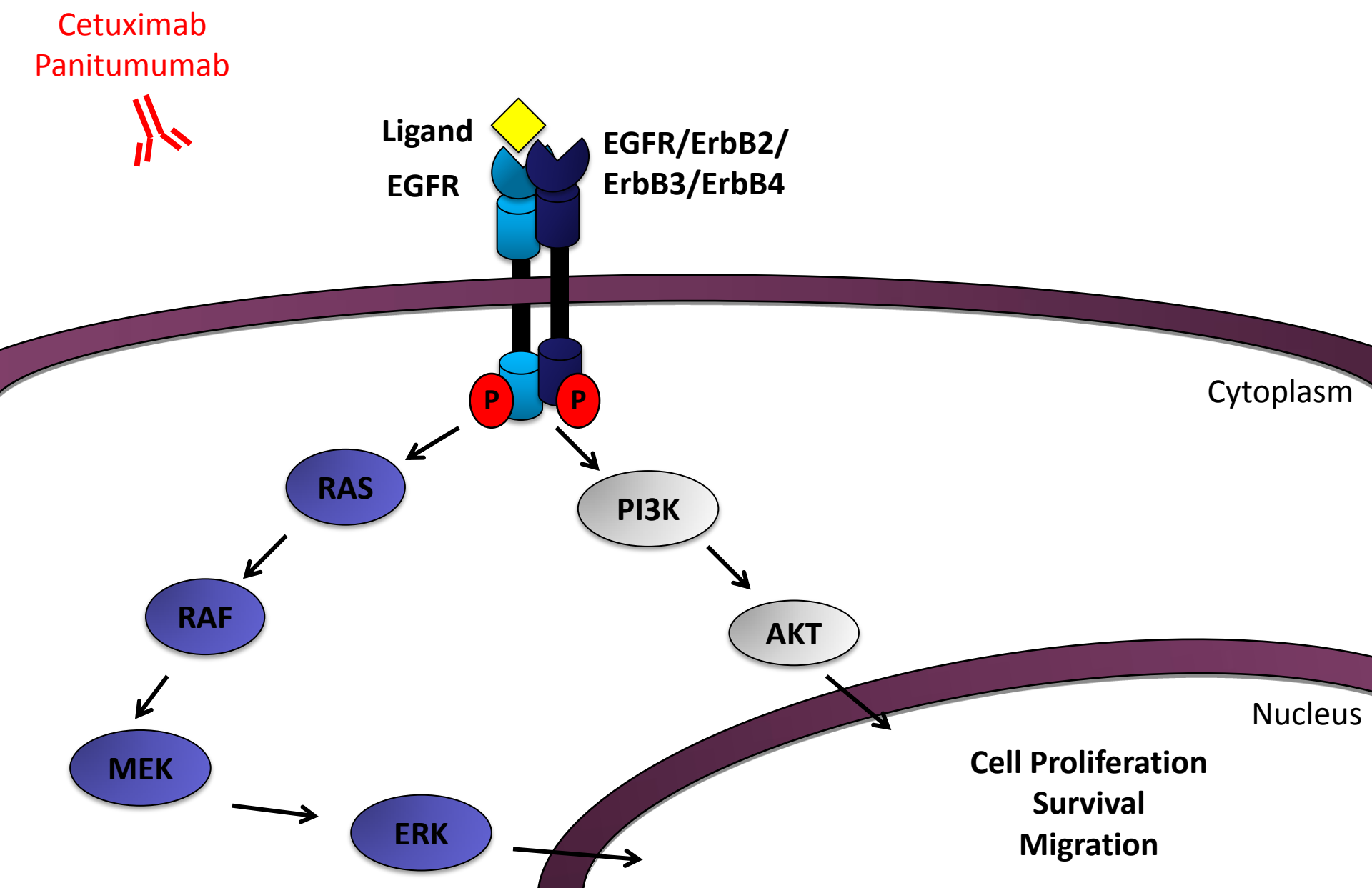
APC/beta cat

20-30 %

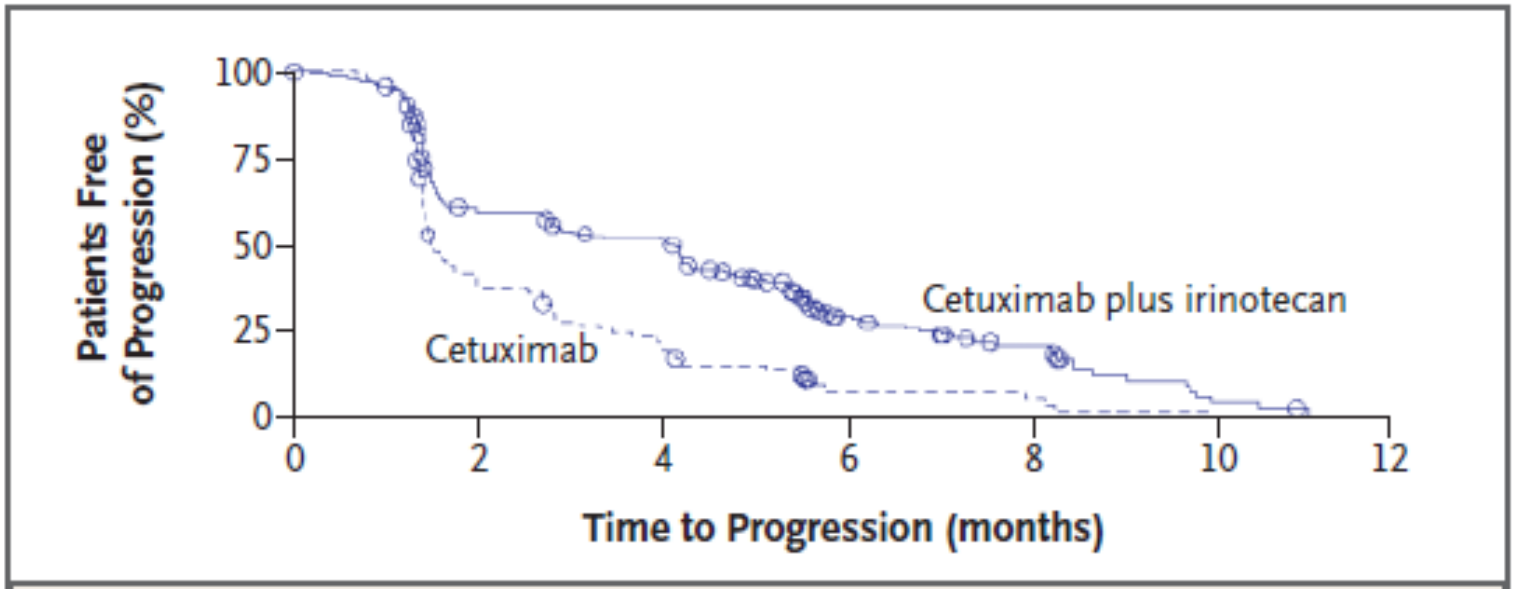

EGFR
TGF- EGF- Epiregulin
APC/beta cat

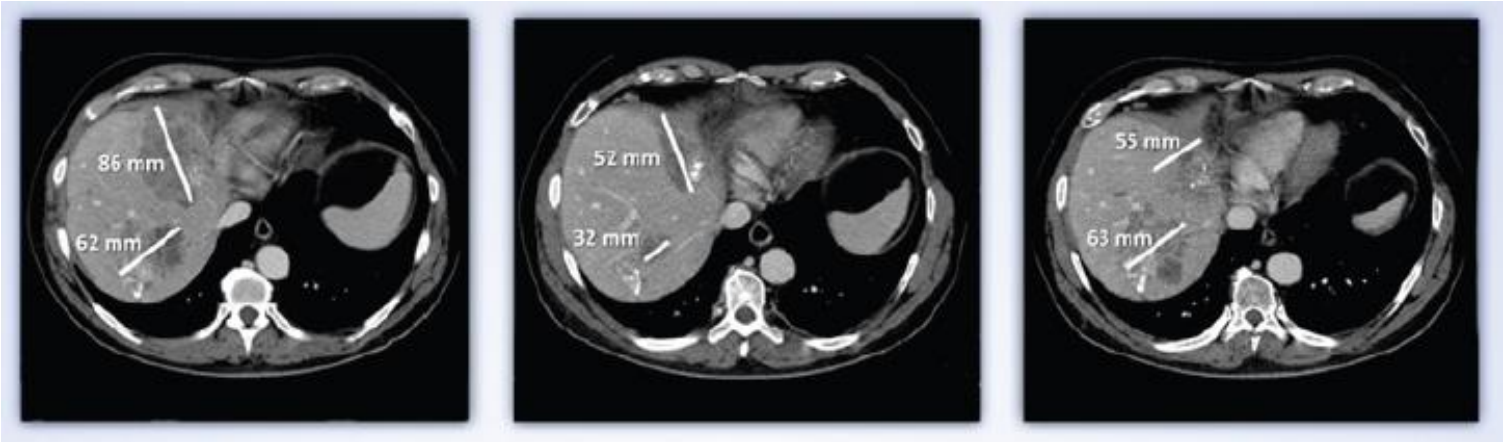
≈15 %

Anti EGFR therapy and colorectal cancer



Response to EGFR blockade is short lived





Cunningham D *et al.*, NEJM. 2004

Mechanisms of resistance in cells and xenos

Pre-Treatment												Post-Treatment											
Parental cell Line	KRAS exon 2	KRAS exon 3	KRAS exon 4	NRAS exon 2	NRAS exon 3	NRAS exon 4	BRAF exon 15	PIK3CA exon 9	PIK3CA exon 20	EGFR exon 12	MEK1 K57N	Resistant cell Line	KRAS exon 2	KRAS exon 3	KRAS exon 4	NRAS exon 2	NRAS exon 3	NRAS exon 4	BRAF exon 15	PIK3CA exon 9	PIK3CA exon 20	EGFR exon 12	MAP2K1 K57N
CCK81												CCK81 R1 Cetux										■	
												CCK81 R2 Cetux	■										
												CCK81 R3 Cetux	■										
DiFi												DiFi R1 Cetux											
												DiFi R2 Cetux											
HCA46												HCA46 R1 Cetux											
												HCA46 R2 Cetux	■										
												HCA46 R3 Cetux											■
LIM1215												LIM1215 R1 Cetux	■		■	■						■	
												LIM1215 R2 Cetux	■										
												LIM1215 R3 Cetux			■								
												LIM1215 R4 Cetux	■										
												LIM1215 R5 Cetux										■	
NCIH508								■				NCIH508 R1 Cetux								■			
								■				NCIH508 R2 Cetux								■			
OXCO2												OXCO2 R1 Cetux	■						■				
												OXCO2 R2 Cetux				■						■	
												OXCO2 R3 Cetux	■									■	

Misale et al., Nature 2012

Arena et al., Science Translational Medicine *in press*

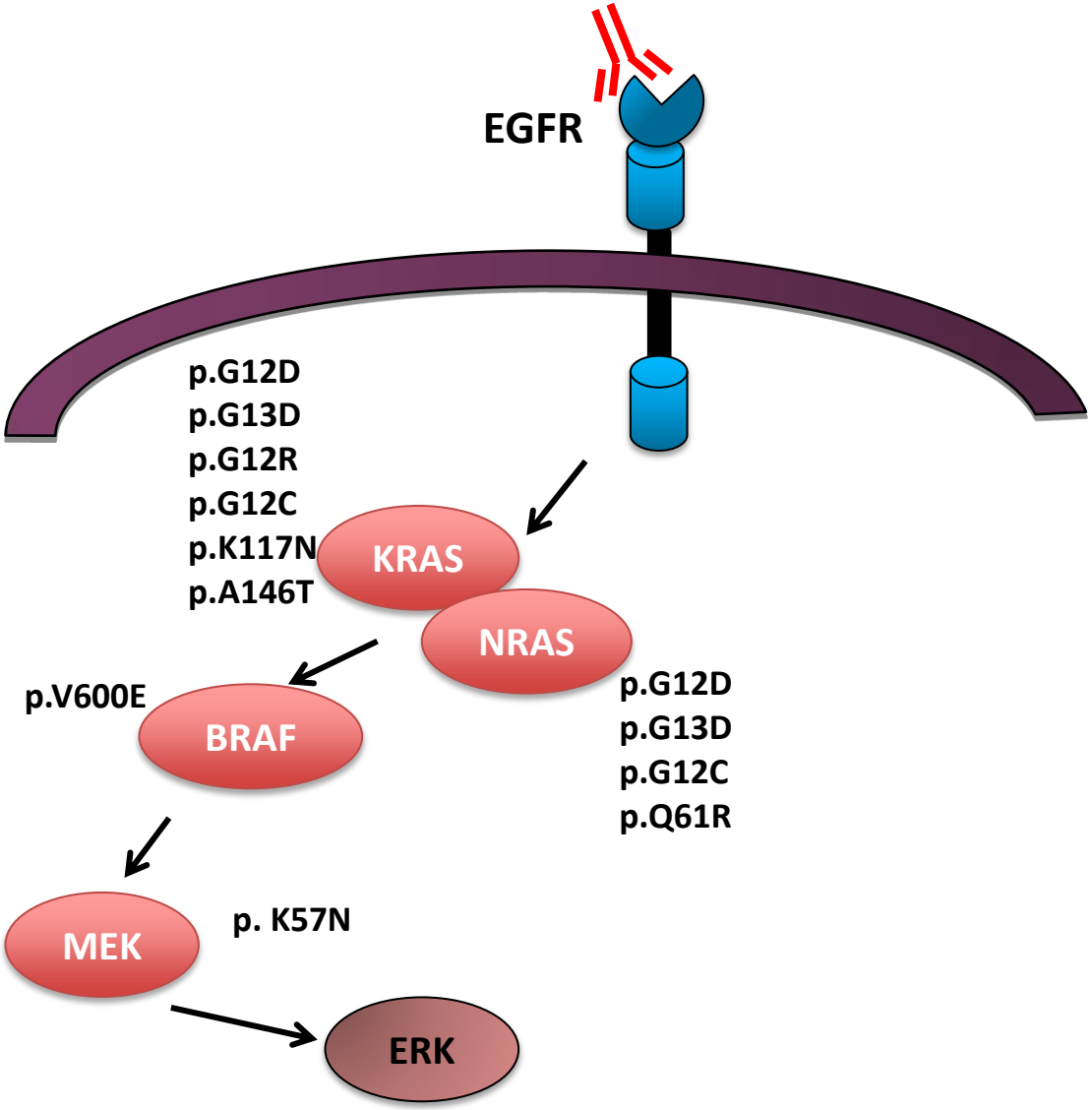
Mechanisms of resistance in patients

	Pre-Treatment										Post-Treatment									
#Patients	KRAS exon 2	KRAS exon 3	KRAS exon 4	NRAS exon 2	NRAS exon 3	NRAS exon 4	BRAF exon 15	PIK3CA exon 9	PIK3CA exon 20	EGFR exon 12	KRAS exon 2	KRAS exon 3	KRAS exon 4	NRAS exon 2	NRAS exon 3	NRAS exon 4	BRAF exon 15	PIK3CA exon 9	PIK3CA exon 20	EGFR exon 12
4												■	■						■	
5																	■	■		
8														■			■			
11															■					
13											■									
15															■					
16																■				
17																				■
18											■									
20											■									
21											■									
23																				■
26								■				■						■		
27																				■
31																				■
33														■						
34													■		■					
35								■	■					■	■			■	■	■
36																	■			
37															■					

Misale et al., STM 2014

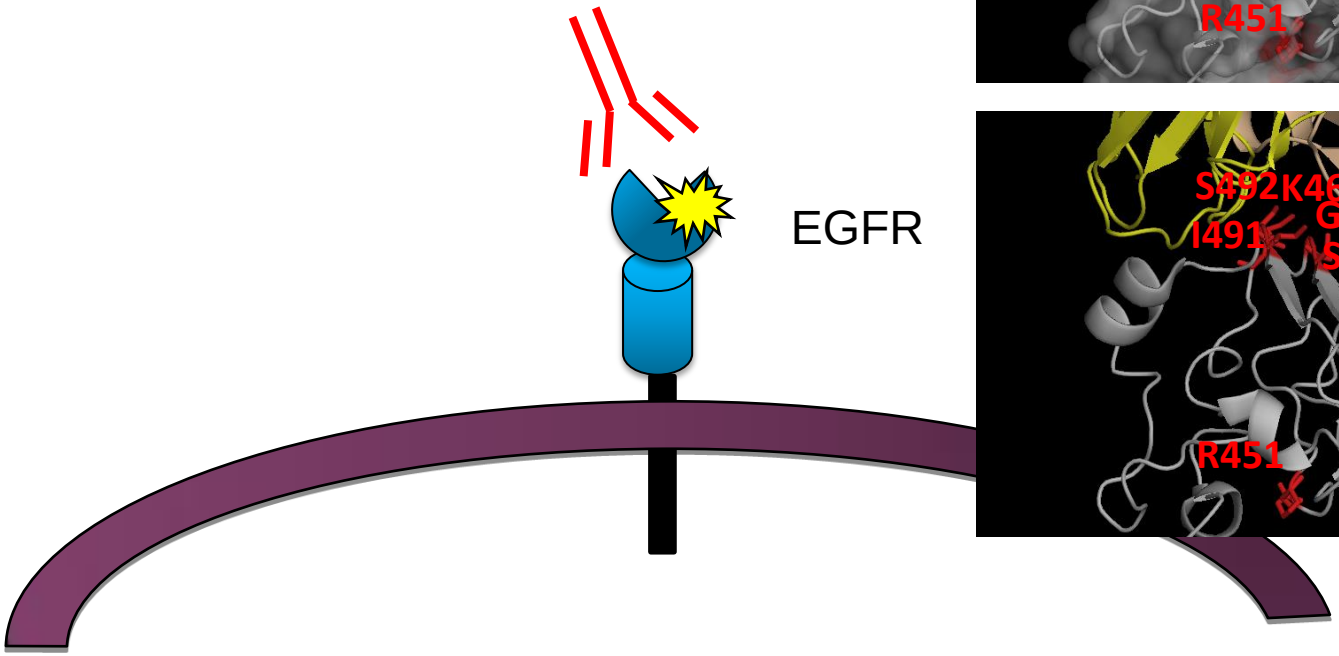
Siravegna et al., Nat Med 2015

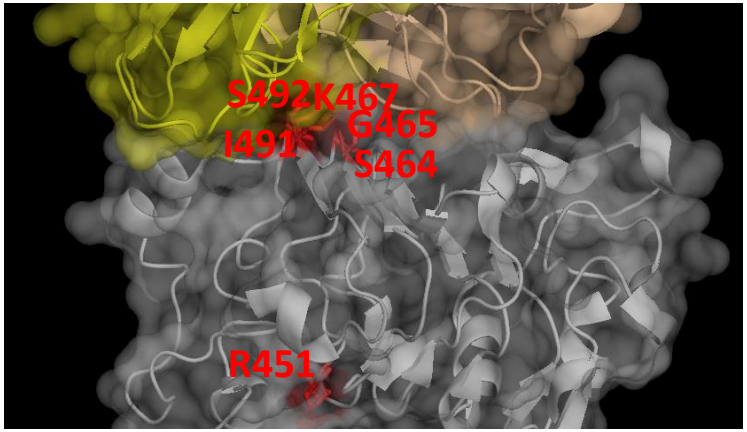
Mechanism (1)
Downstream signaling reactivation



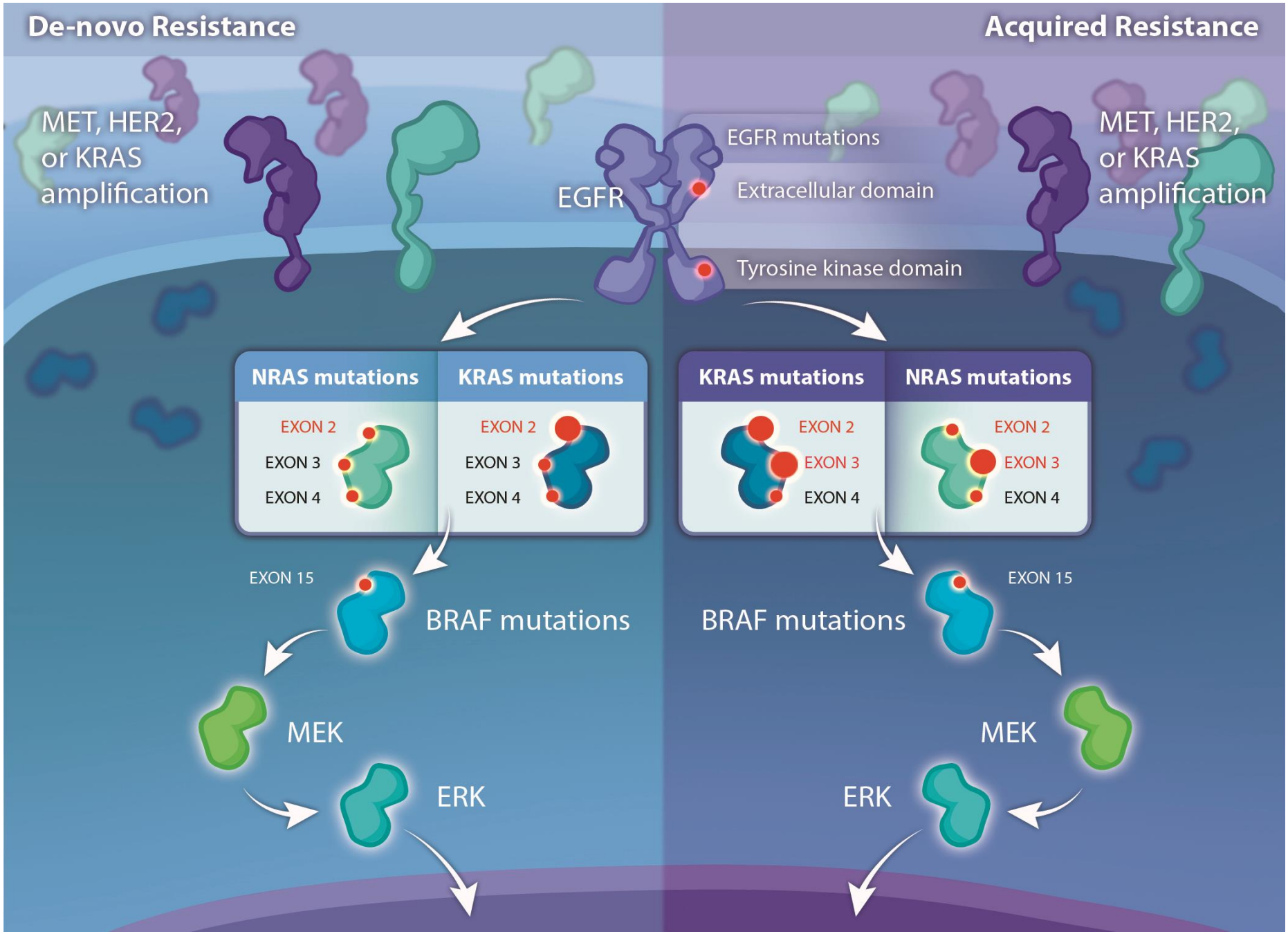
Mechanism (2)
Mutations of the target: EGFR ECD

EGFR S492R
EGFR R451C
EGFR S464L
EGFR G465R
EGFR K467T
EGFR I491M



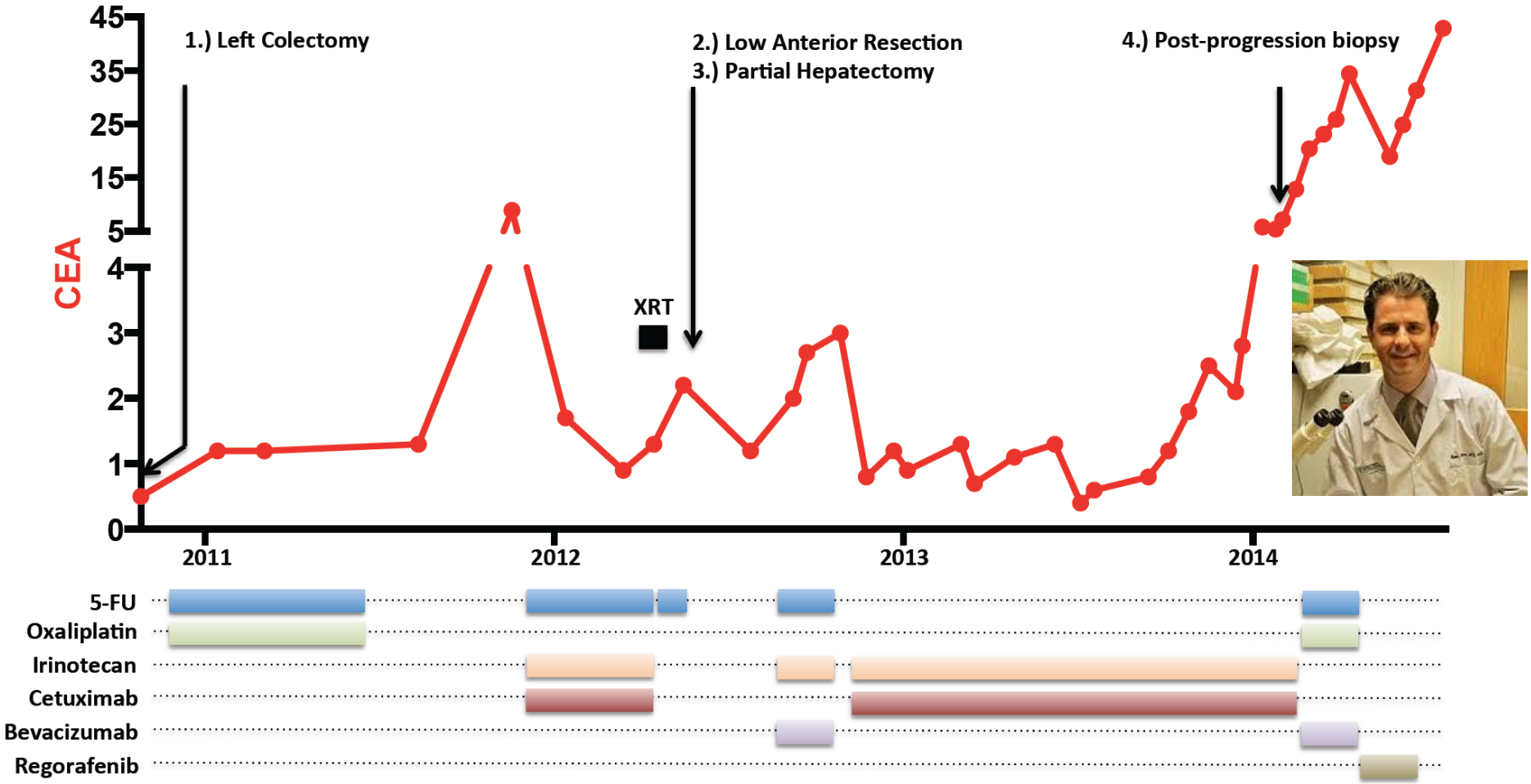


Arena et al., *CCR* 2015


De-novo Resistance
Acquired Resistance
MET, HER2, or KRAS amplification
EGFR
EGFR mutations
Extracellular domain
Tyrosine kinase domain
MET, HER2, or KRAS amplification
NRAS mutations
KRAS mutations
EXON 2
EXON 3
EXON 4
EXON 2
EXON 3
EXON 4
KRAS mutations
NRAS mutations
EXON 2
EXON 3
EXON 4
EXON 2
EXON 3
EXON 4
EXON 15
BRAF mutations
BRAF mutations
EXON 15
MEK
MEK
ERK
ERK

- Oncogenic pathways in colorectal cancer
- Drug resistance and signaling escape
- Lesion-specific therapeutic responses
- Tumor evolution as a therapeutic target

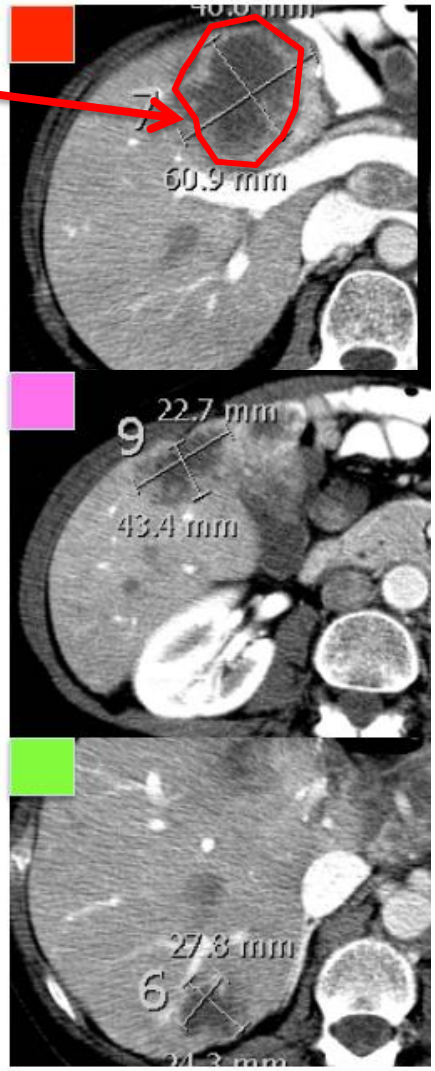
Resistance to EGFR blockade drives lesion-specific responses in colorectal cancer



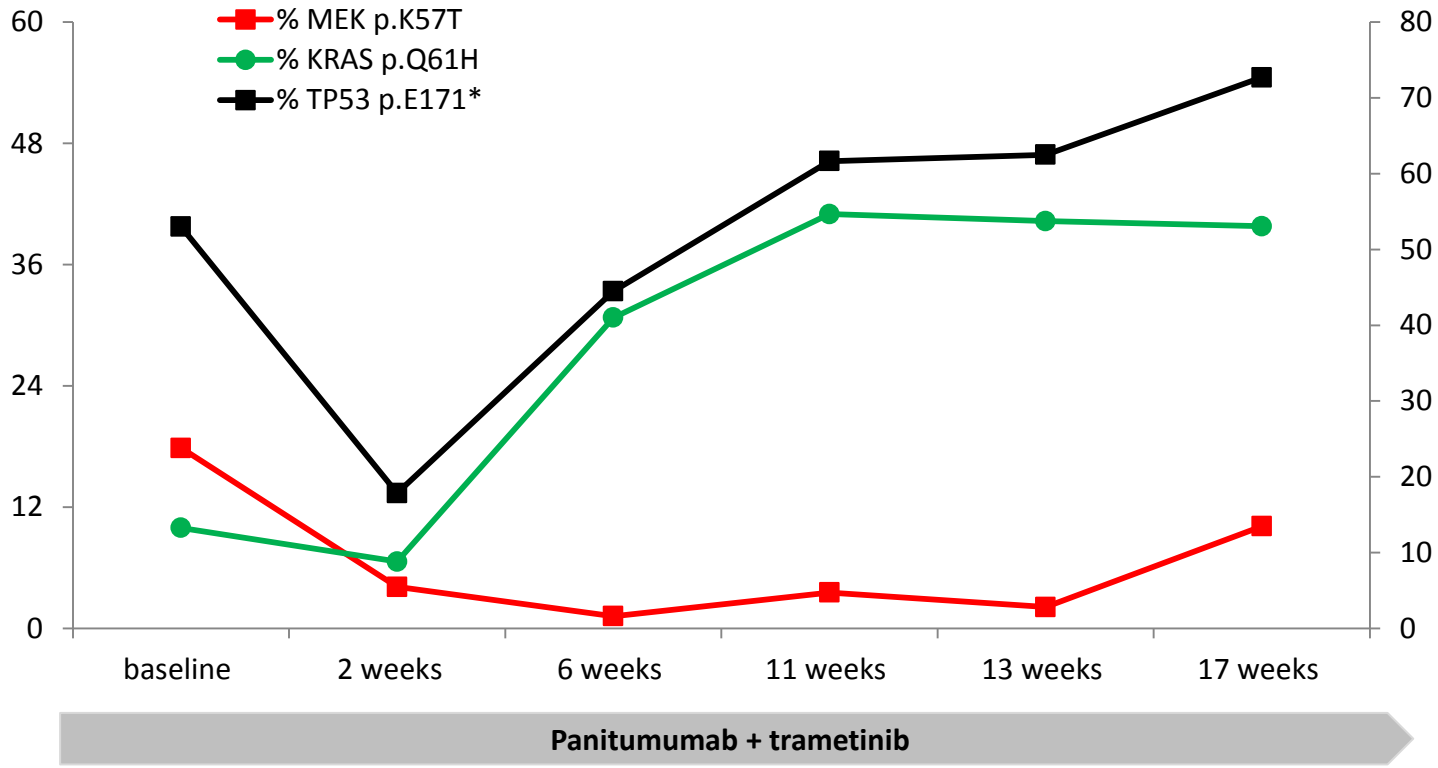
MAP2K1 K57T emerges during to EGFR blockade

TP53 p.E171*
MAP2K1 p.K57T

TISSUE SAMPLES	TP53	RAS/RAF	MAP2K1
Primary Tumor, 2011	p.E171*	WT	WT
Liver metastasis (pre-cetux), 2012	p.E171*	WT	WT
Post-cetux progression liver biopsy, 2014	p.E171*	WT	p.K57T

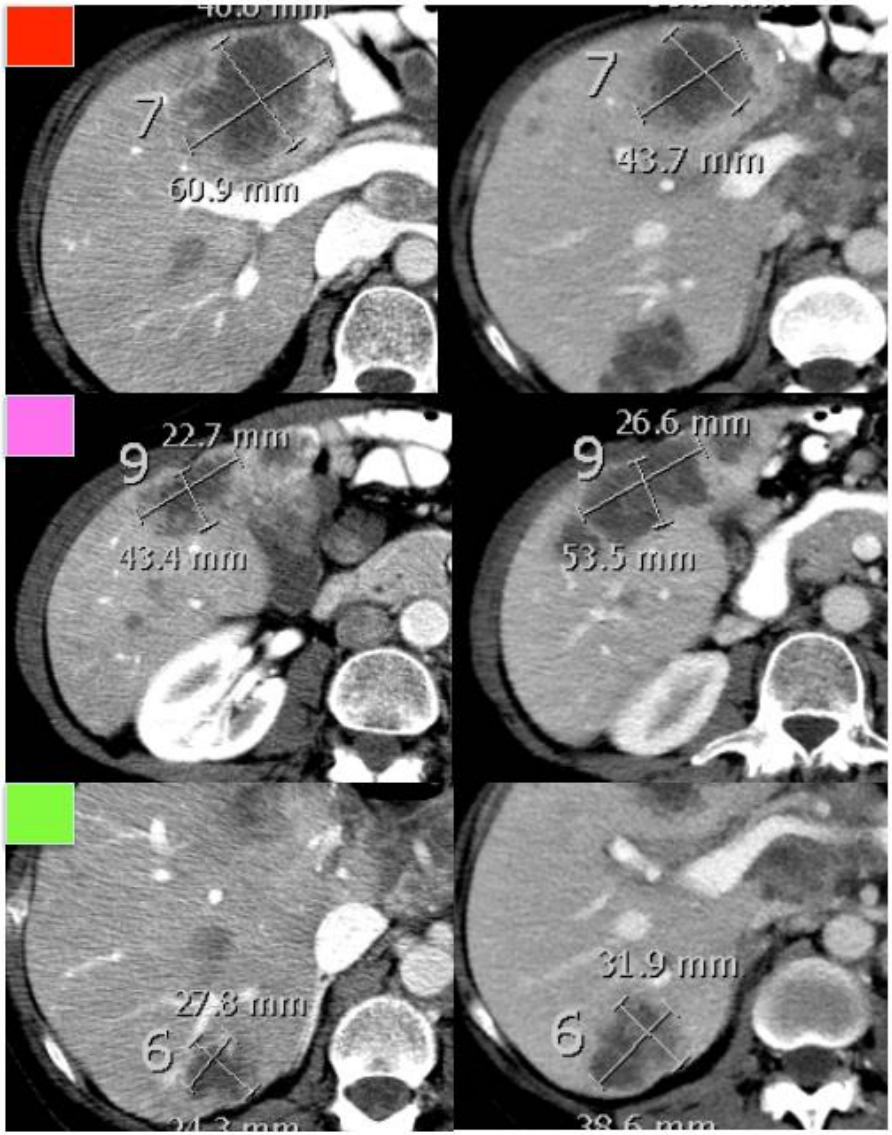


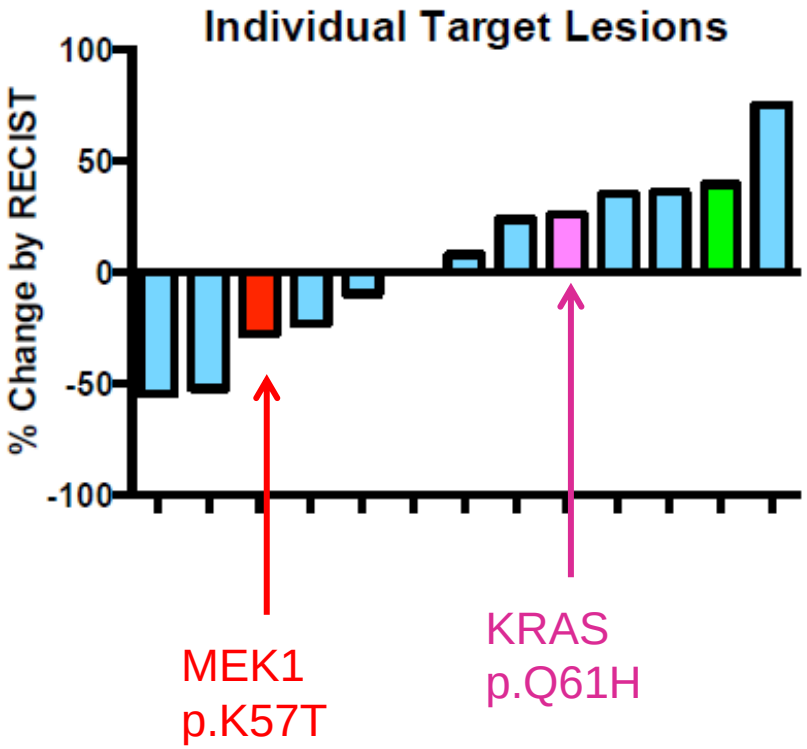
Clonal evolution and lesion specific response in cfDNA



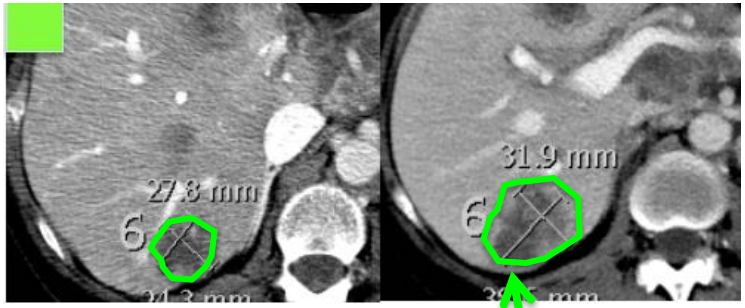
Cosmic	Gene	Description	coord	Nchange	AAchange	var_effect	% mutant reads
207	**KRAS**	Kirsten rat sarcoma viral oncogene homolog	chr12:25380275	c.A183C	p.Q61H	nonsynonymous	7,00935
29	**TP53**	tumor protein p53	chr17:7578419	c.G511T	p.E171*	stopgain	52,9787
8	**MAP2K1**	mitogen-activated protein kinase kinase 1	chr15:66727454	c.A170C	p.K57T	nonsynonymous	13,9896

Clonal evolution and lesion-specific responses



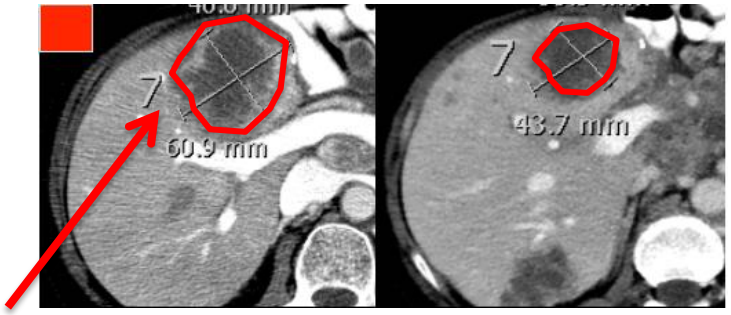


Panitumumab + trametinib (5 months)

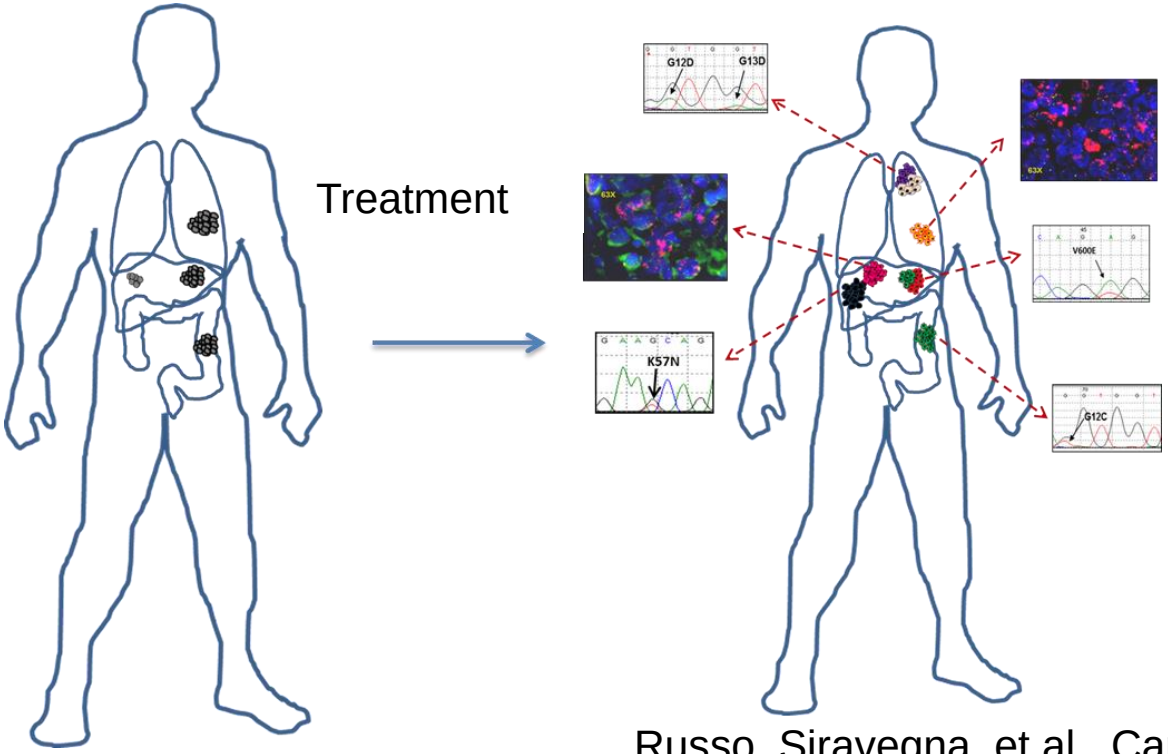


TP53 p.E171*
KRAS p.Q61H

Panitumumab + trametinib (5 months)

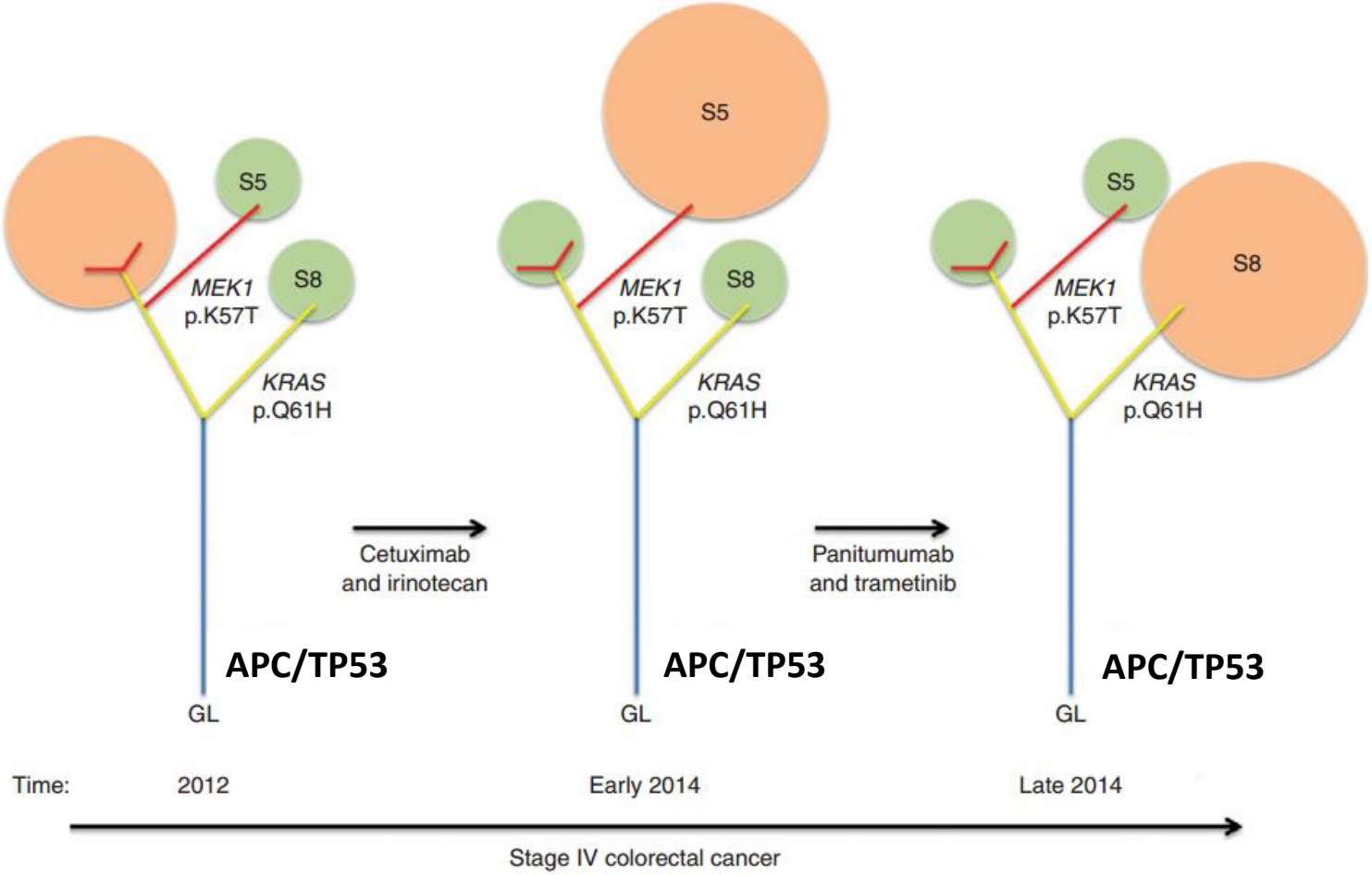


TP53 p.E171*
MAP2K1 p.K57T



Russo, Siravegna et al., Cancer Discovery 2016

Pruning Cancer's Evolutionary Tree with Lesion-Directed Therapy



Modified by Hiley and Swanton, Canc Discov in the spotlight 2016

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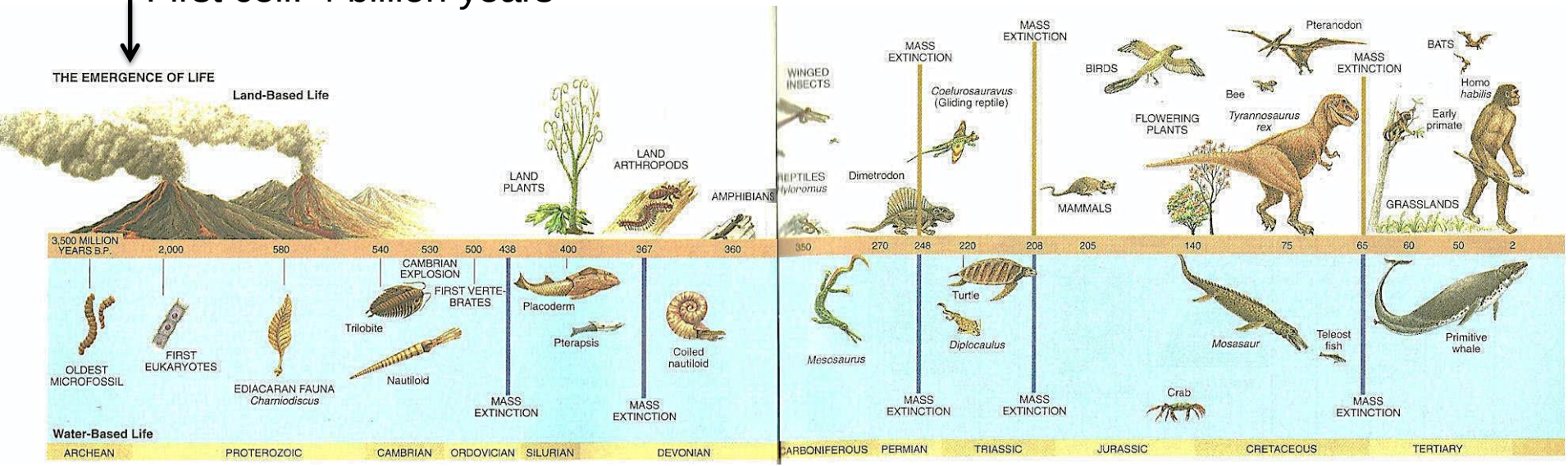
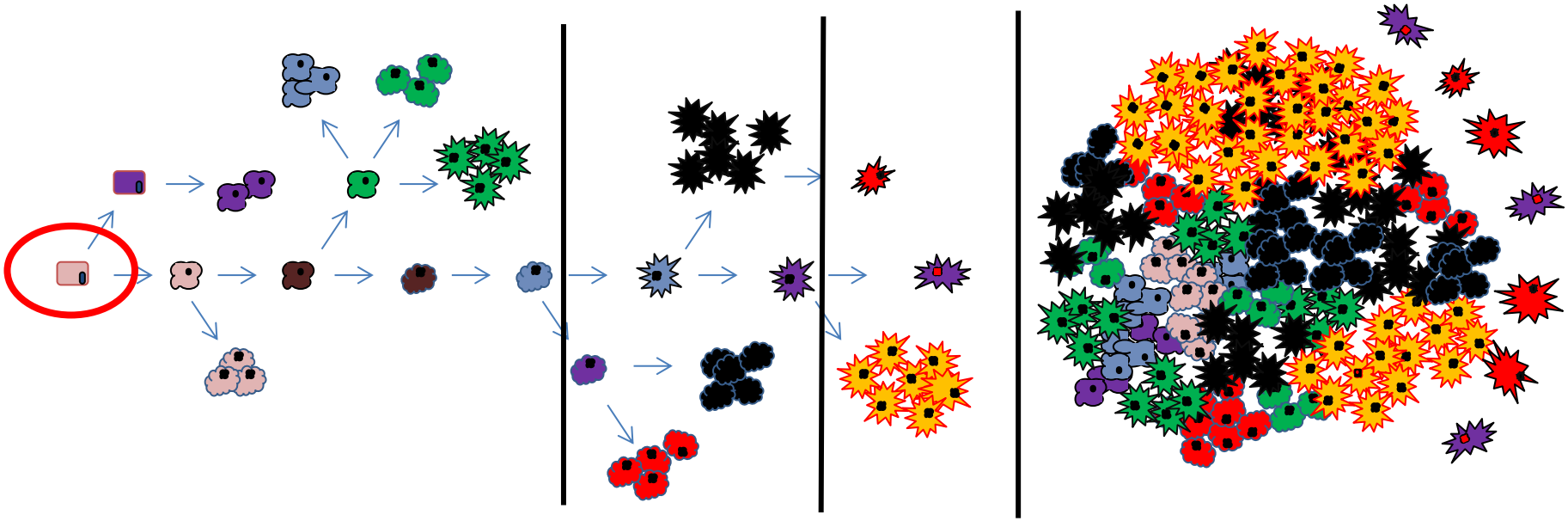
Cancer evolution as a therapeutic target

First cell: 4 billion years





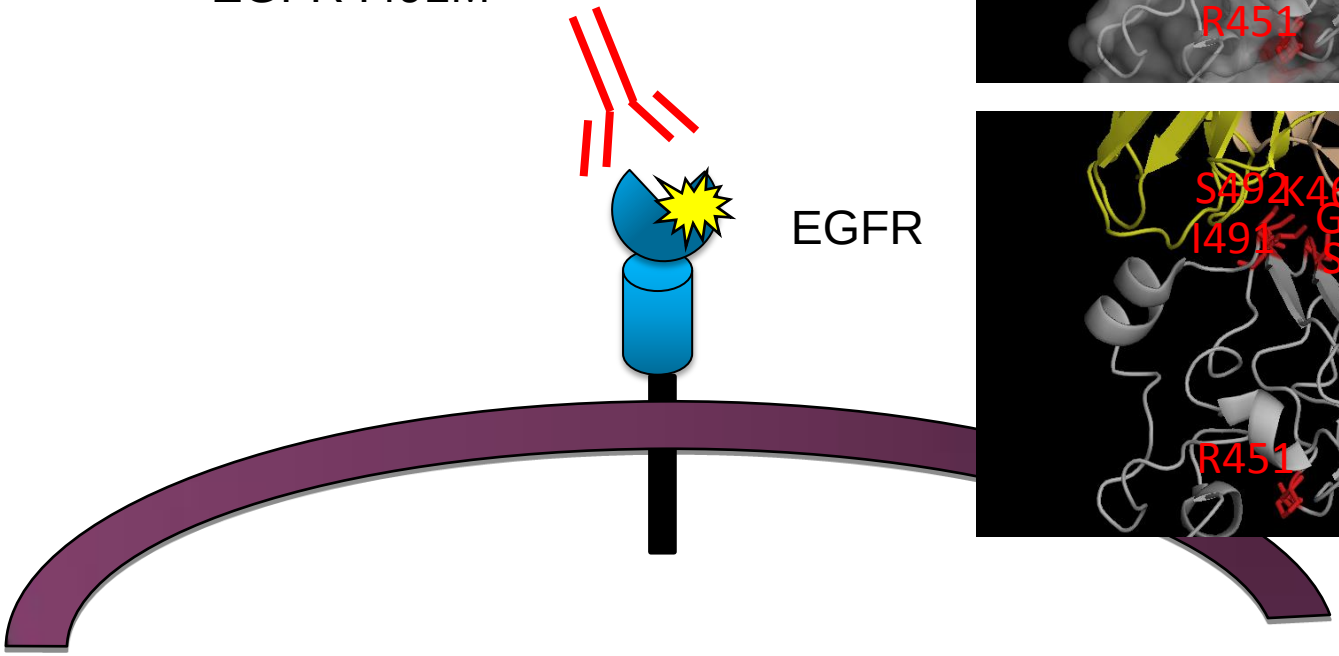
What are we up against ?

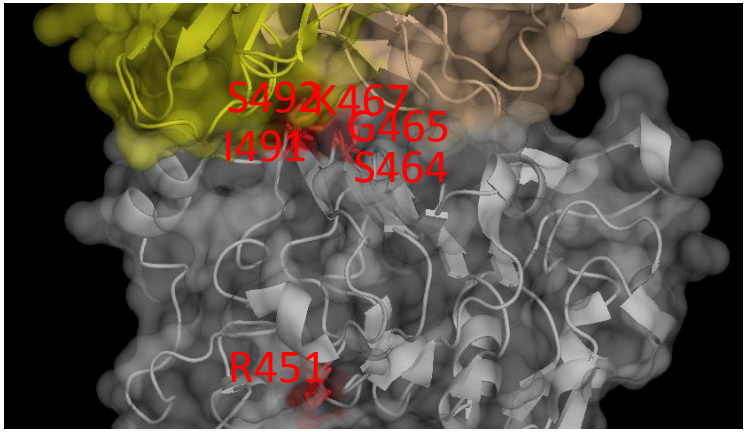
-1-

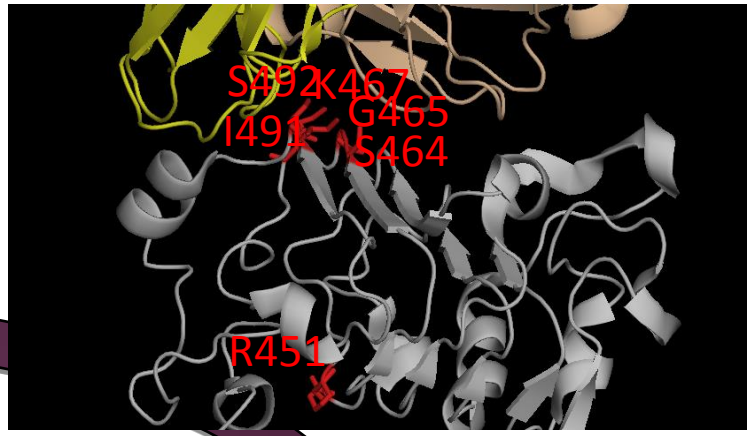
Therapies that adapt to tumor evolution

Targeting EGFR extracellular domain mutations

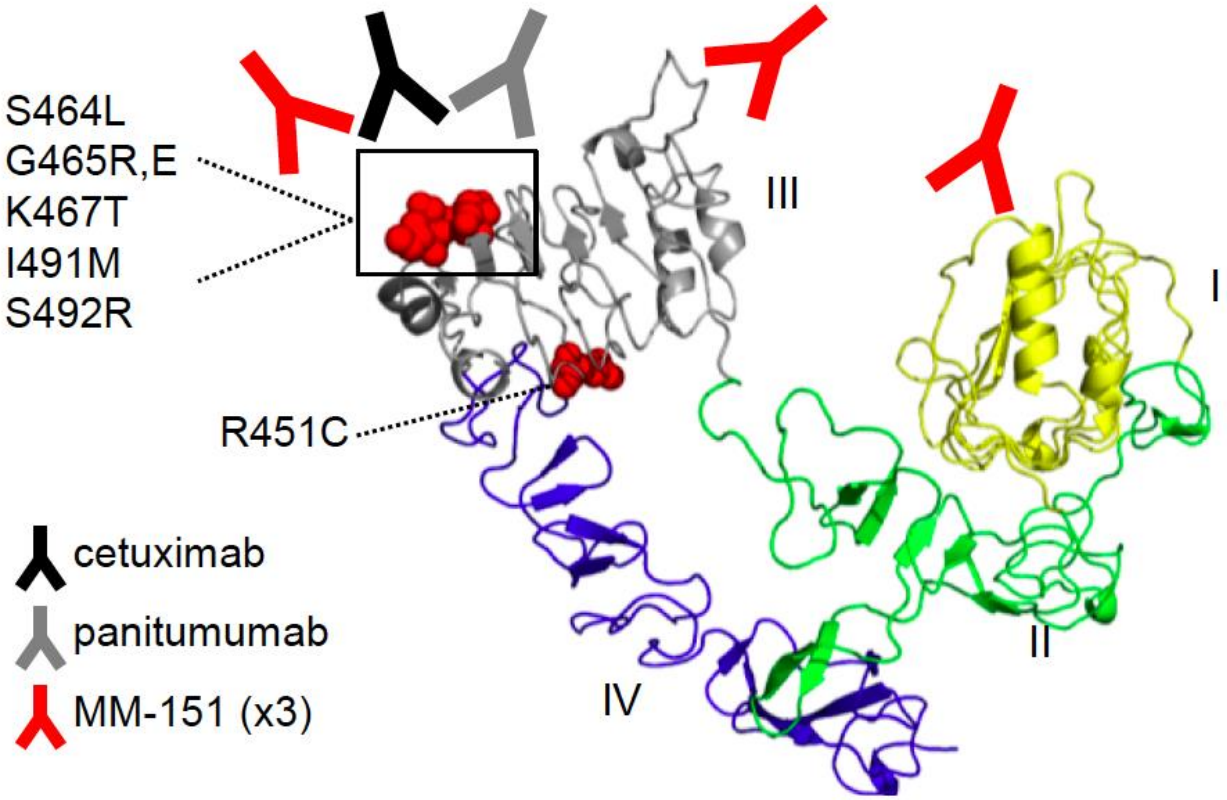
EGFR S492R
EGFR R451C
EGFR S464L
EGFR G465R
EGFR K467T
EGFR I491M





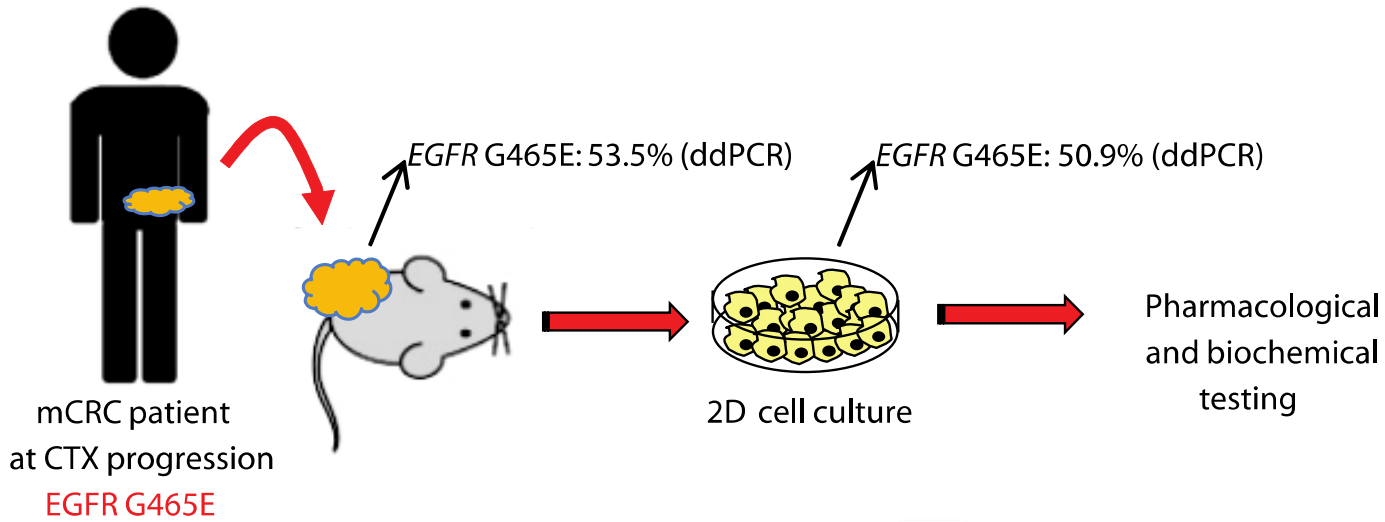


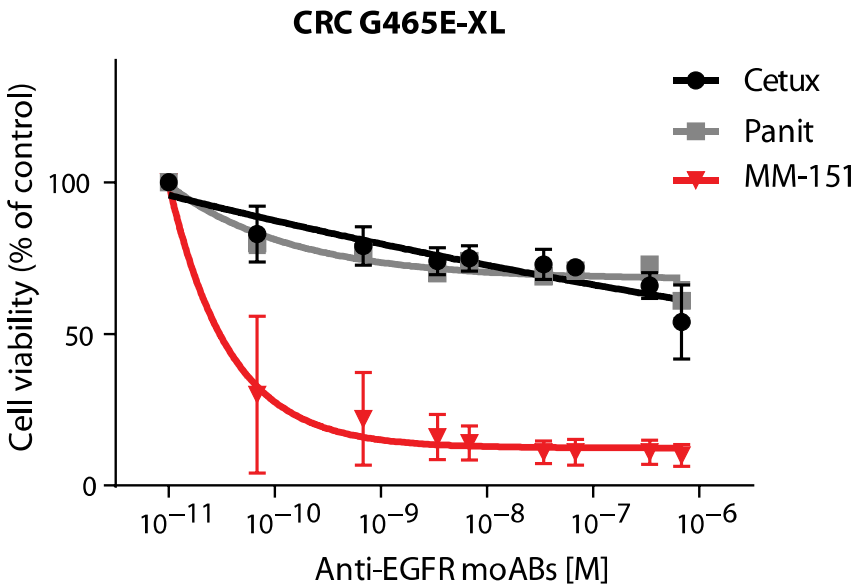
MM-151 targets EGFR ECD mutations

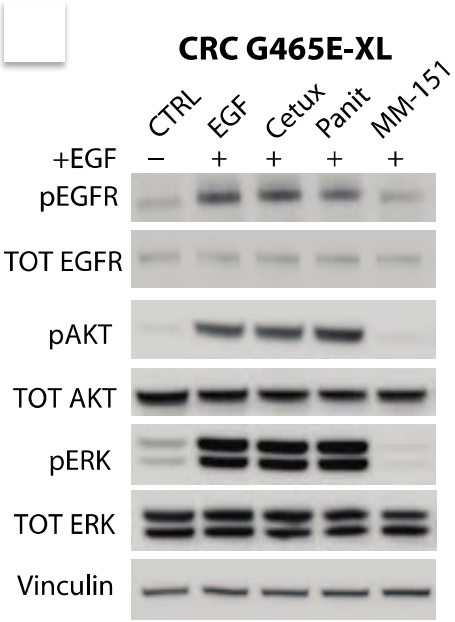


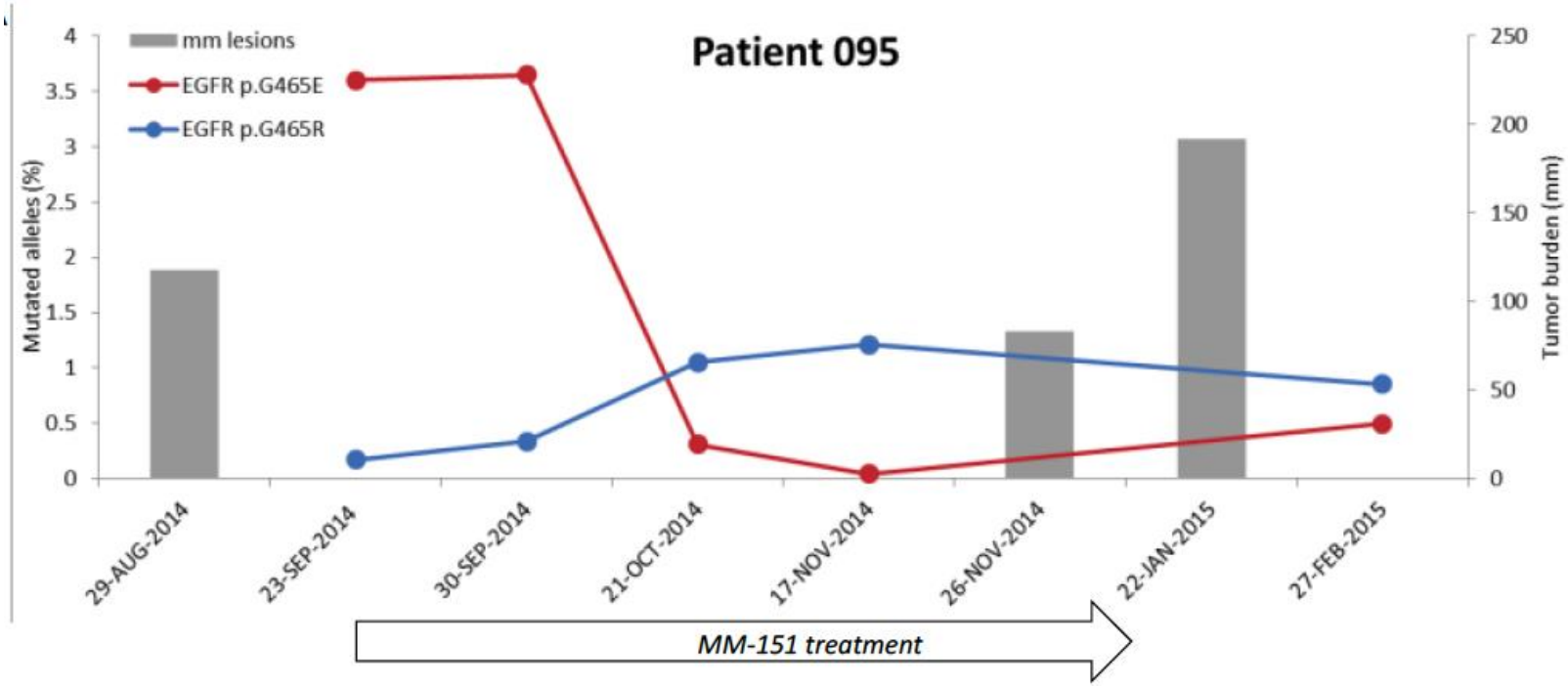
Arena et al., Science Translational Medicine *in press*

MM151 on patient's avatar with ECD mutations

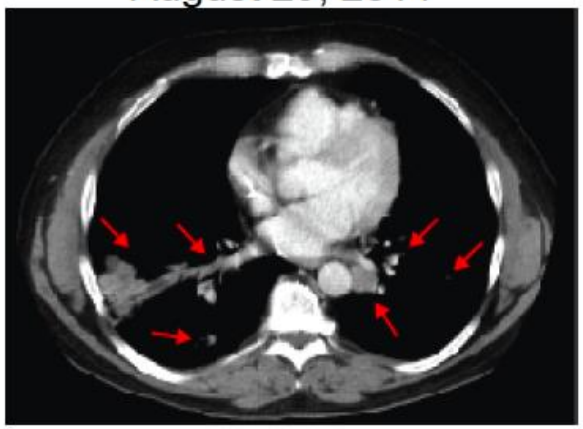




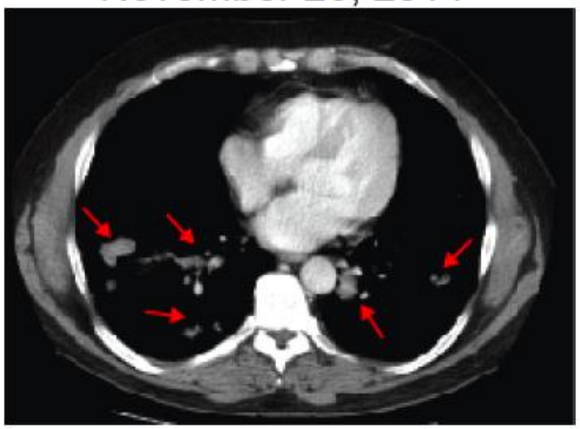




Baseline
August 29, 2014



First Assessment
November 26, 2014

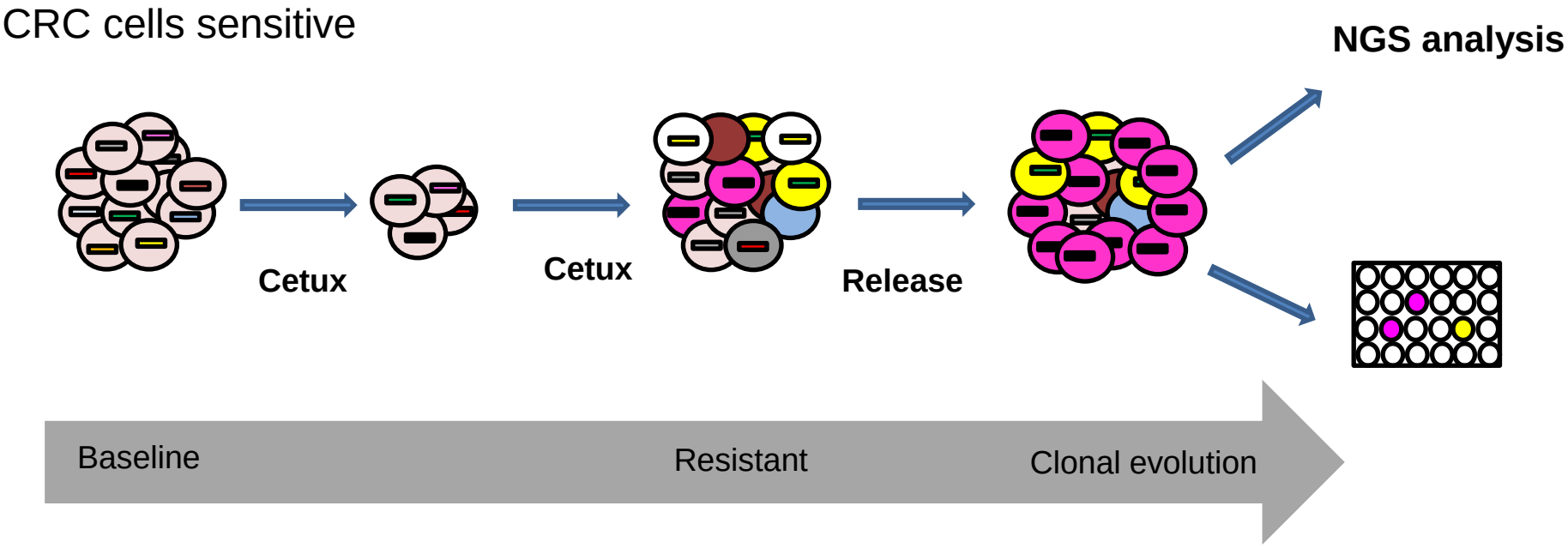


Arena et al., Science Translational Medicine *in press*

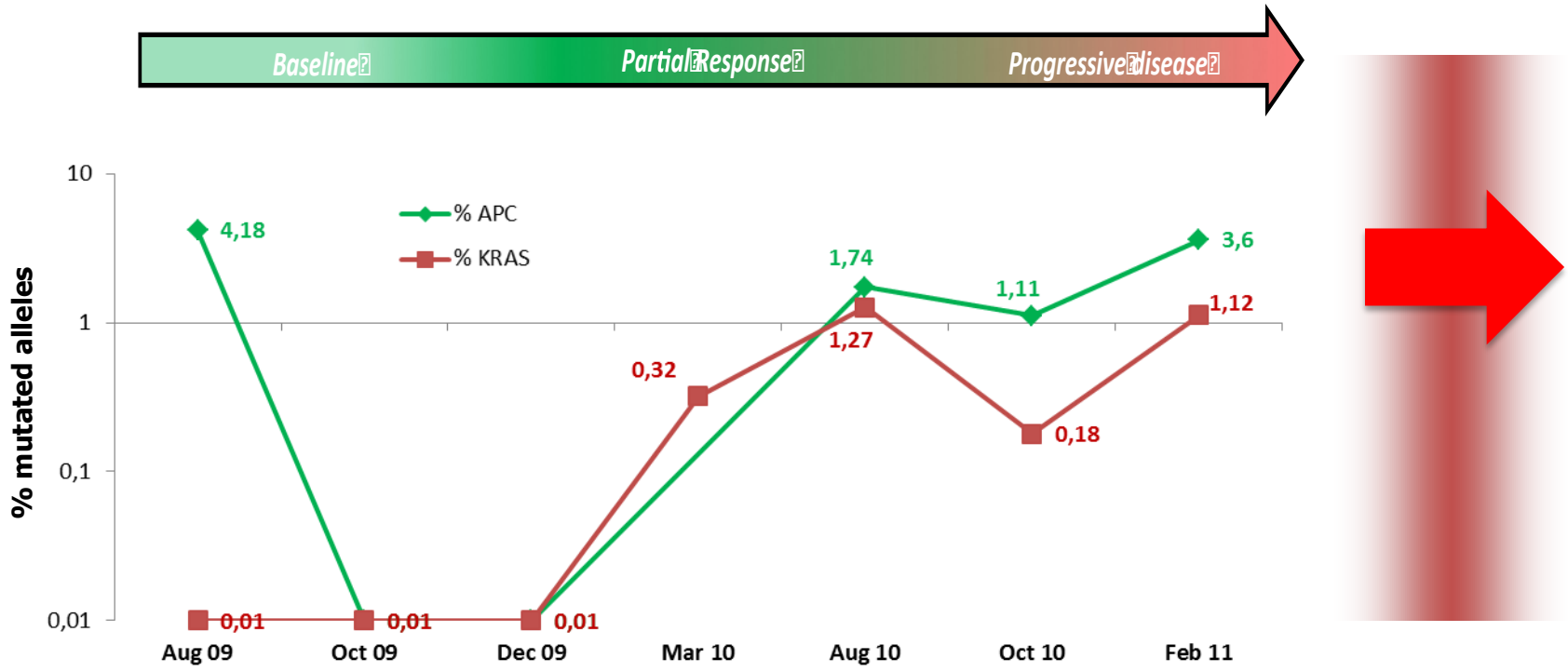
-2-

Fighting evolution with evolution

Modeling clonal evolution and drug resistance

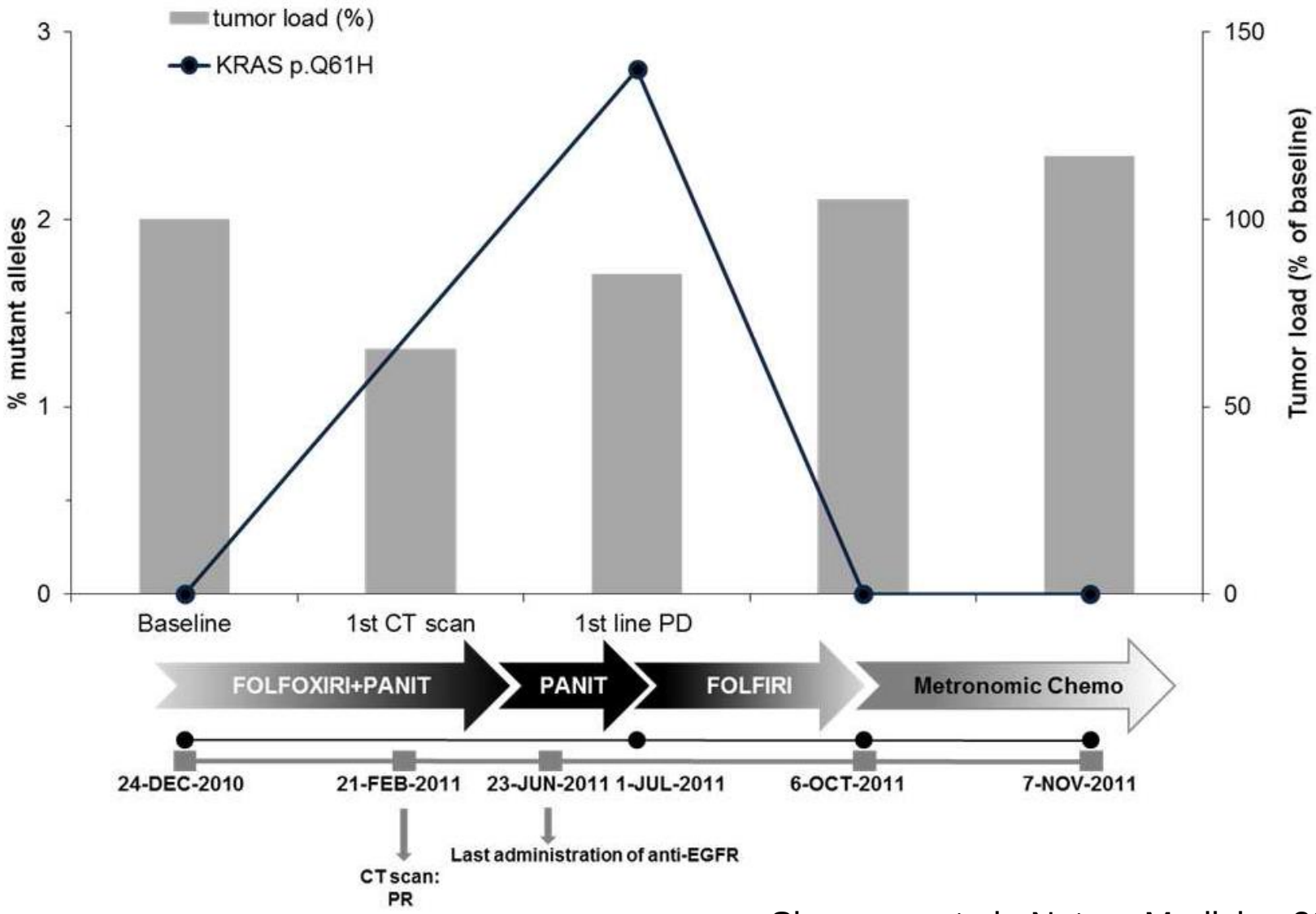


Clonal evolution and resistance to EGFR blockade



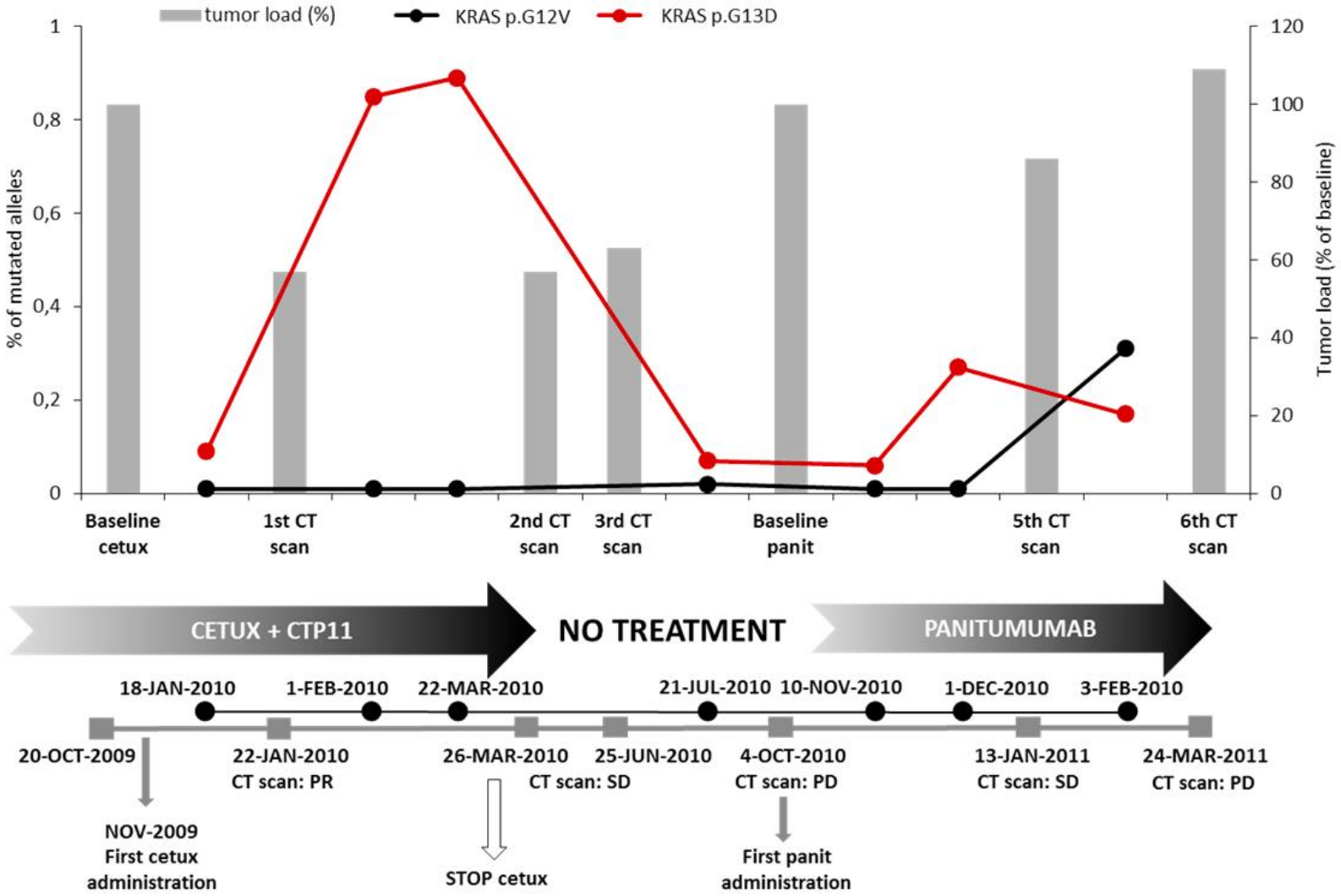
What happens to resistant clones upon progression?

KRAS clones decline upon withdrawal of EGFR antibodies



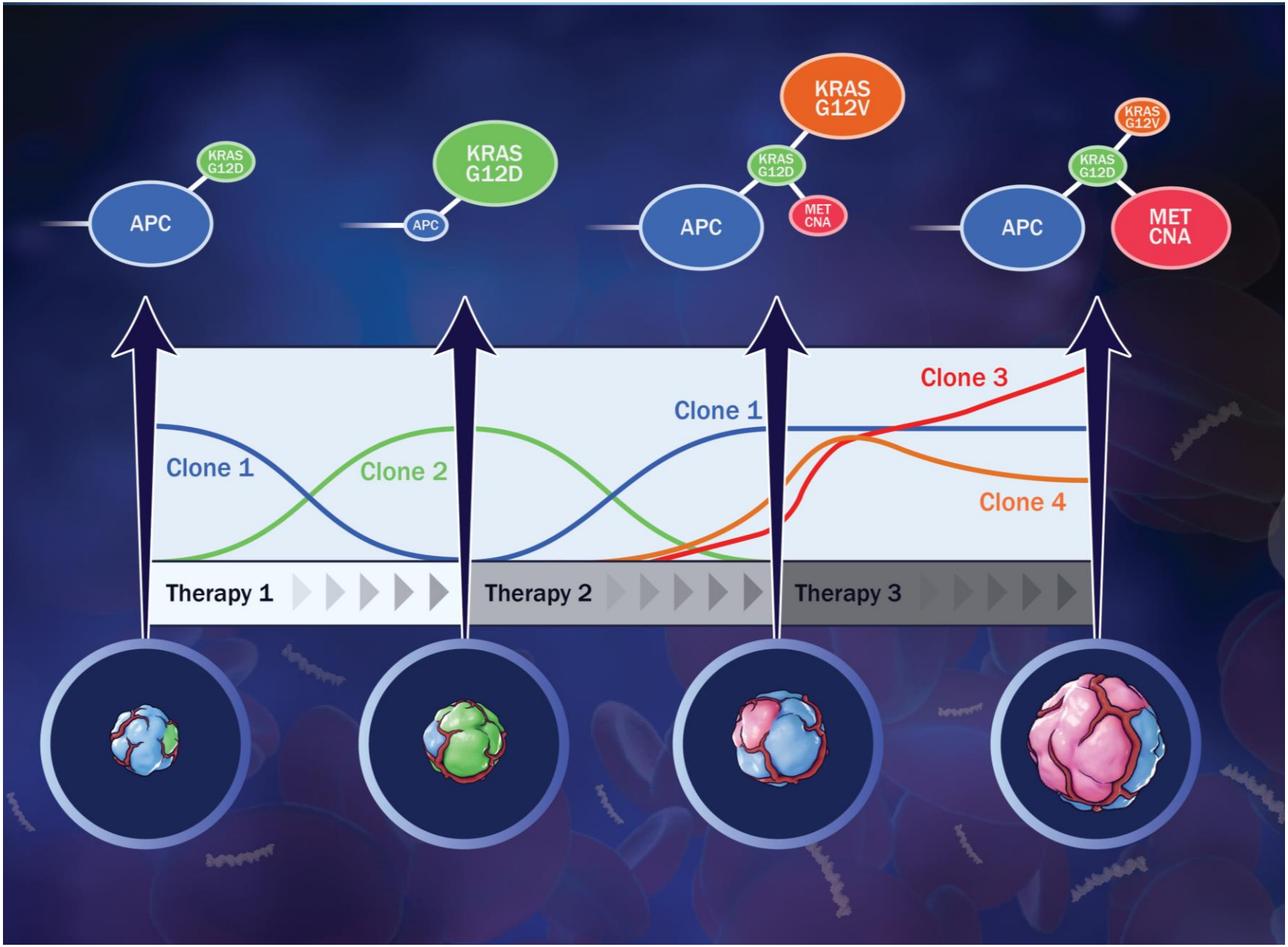
Siravegna et al., Nature Medicine 2015

When KRAS clone decline in blood, re-challenging with anti-EGFR antibodies can be clinically effective



Siravegna et al., Nature Medicine 2015

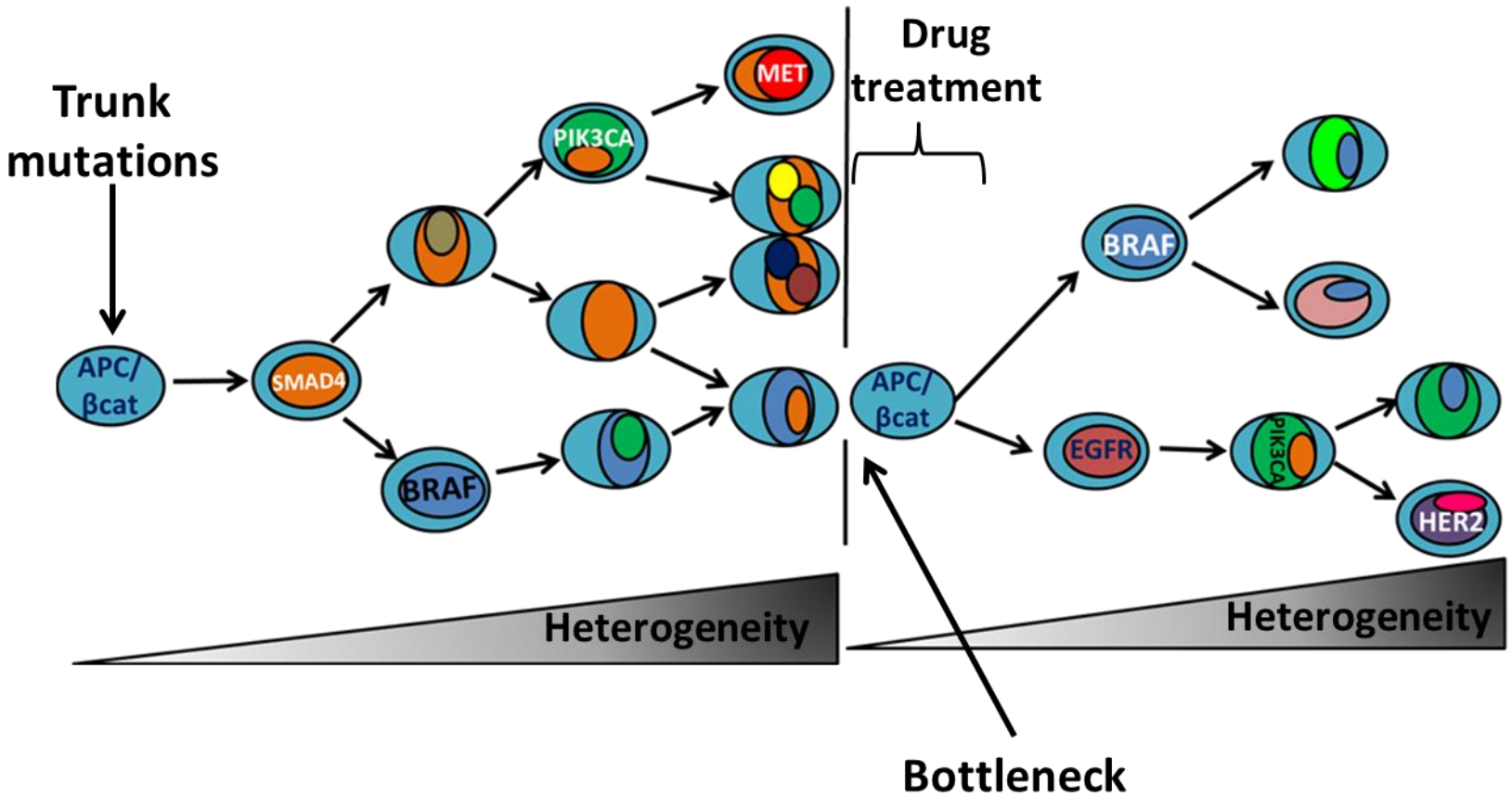
Tumor evolution in the blood of patients



-3-

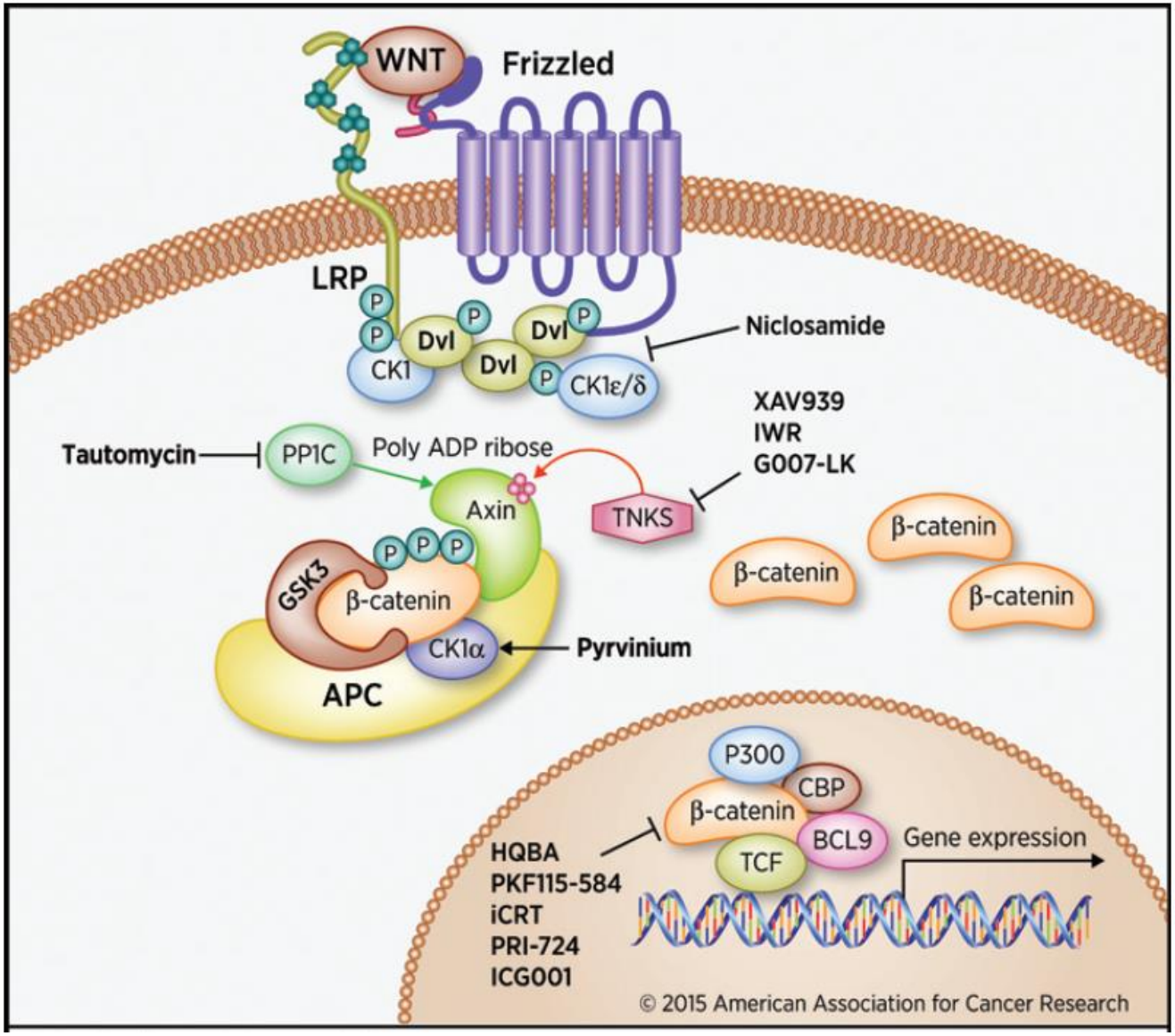
Targeting evolution from the trunk

Can we bypass heterogenity from the trunk?

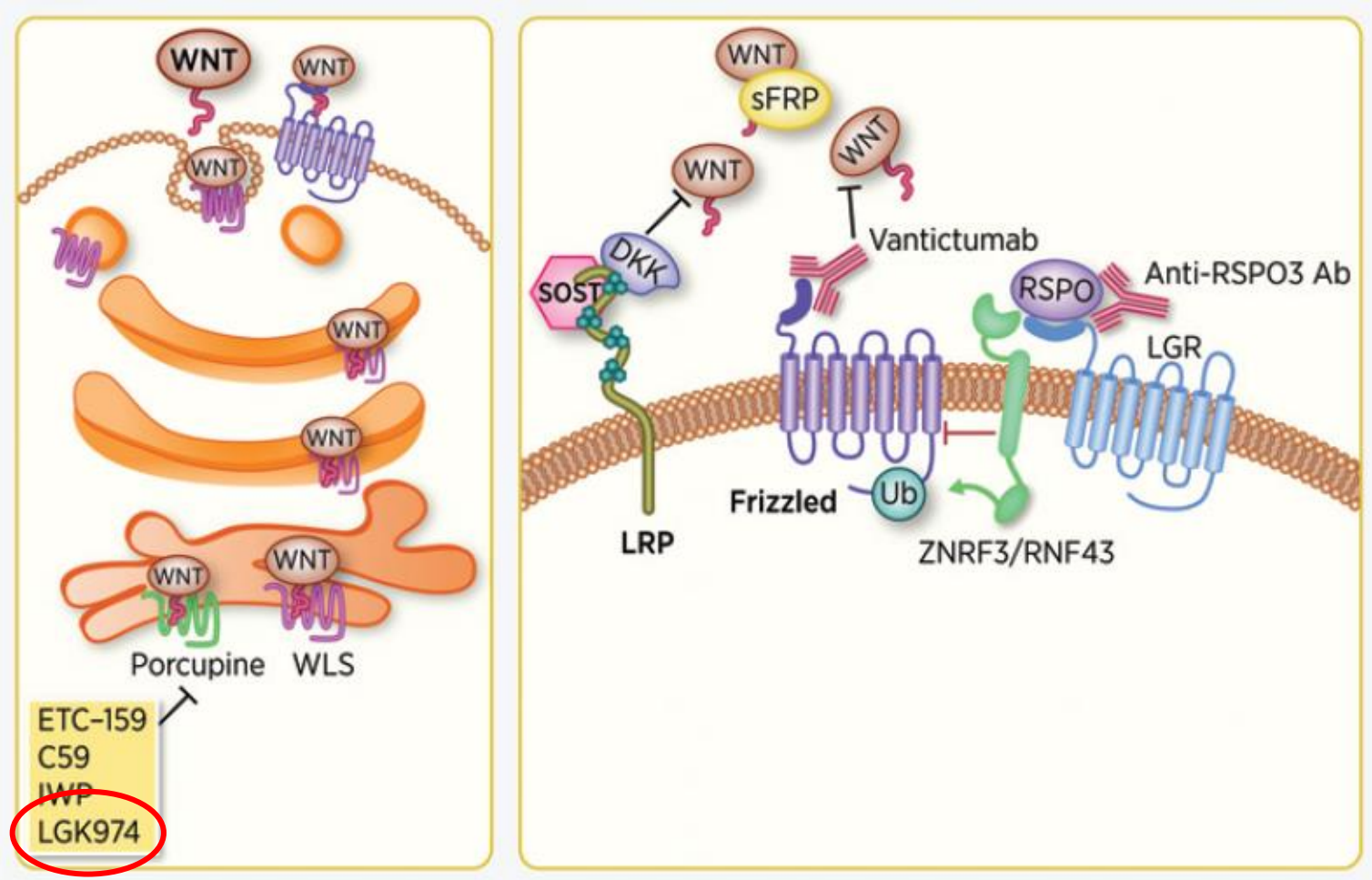


Do CRC cells remain addicted to «trunk-mutations» after developping multiple resistance mechanisms ?

Targeting the trunk of colorectal cancer evolution



Inhibition of βcat/APC Pathway



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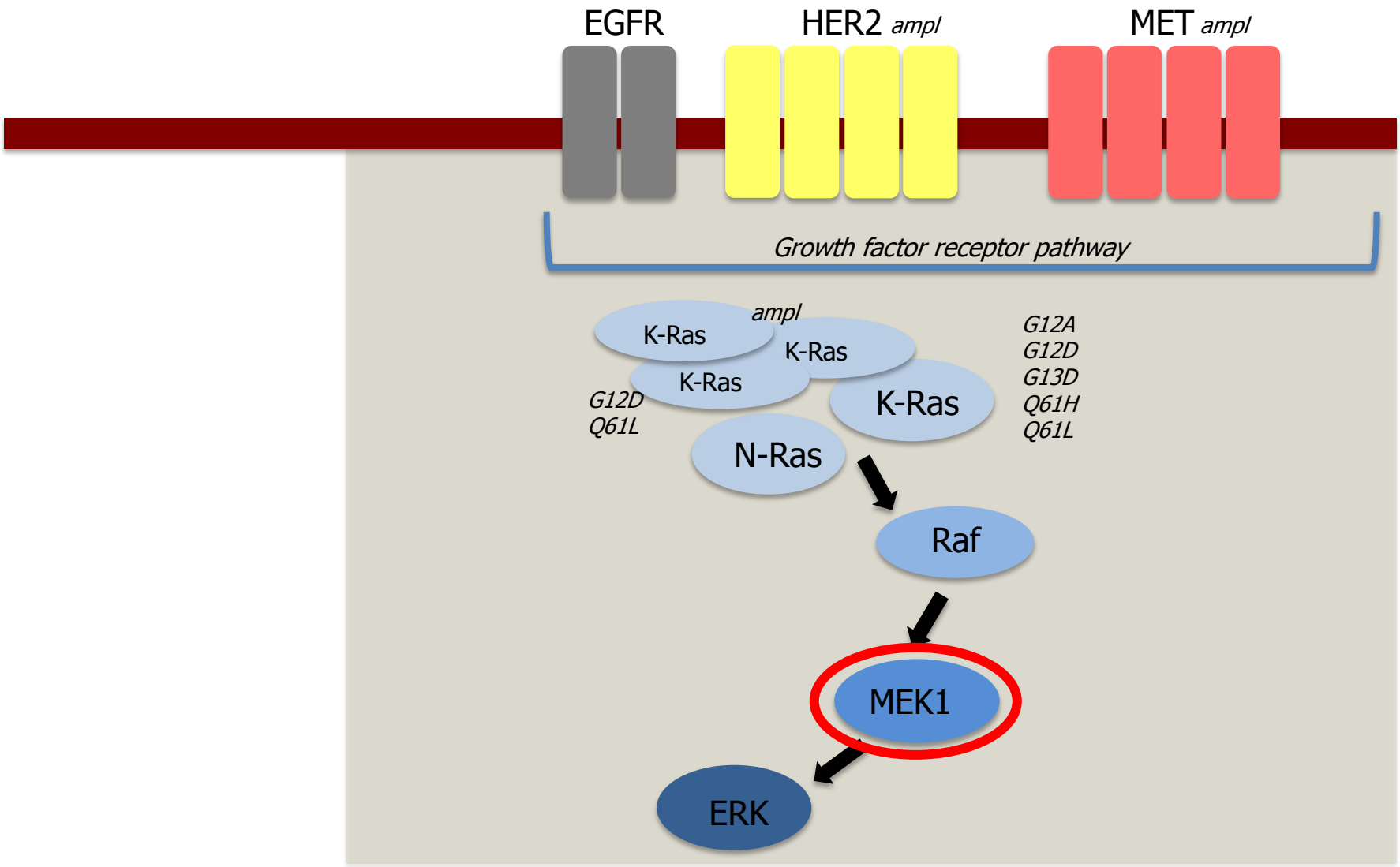
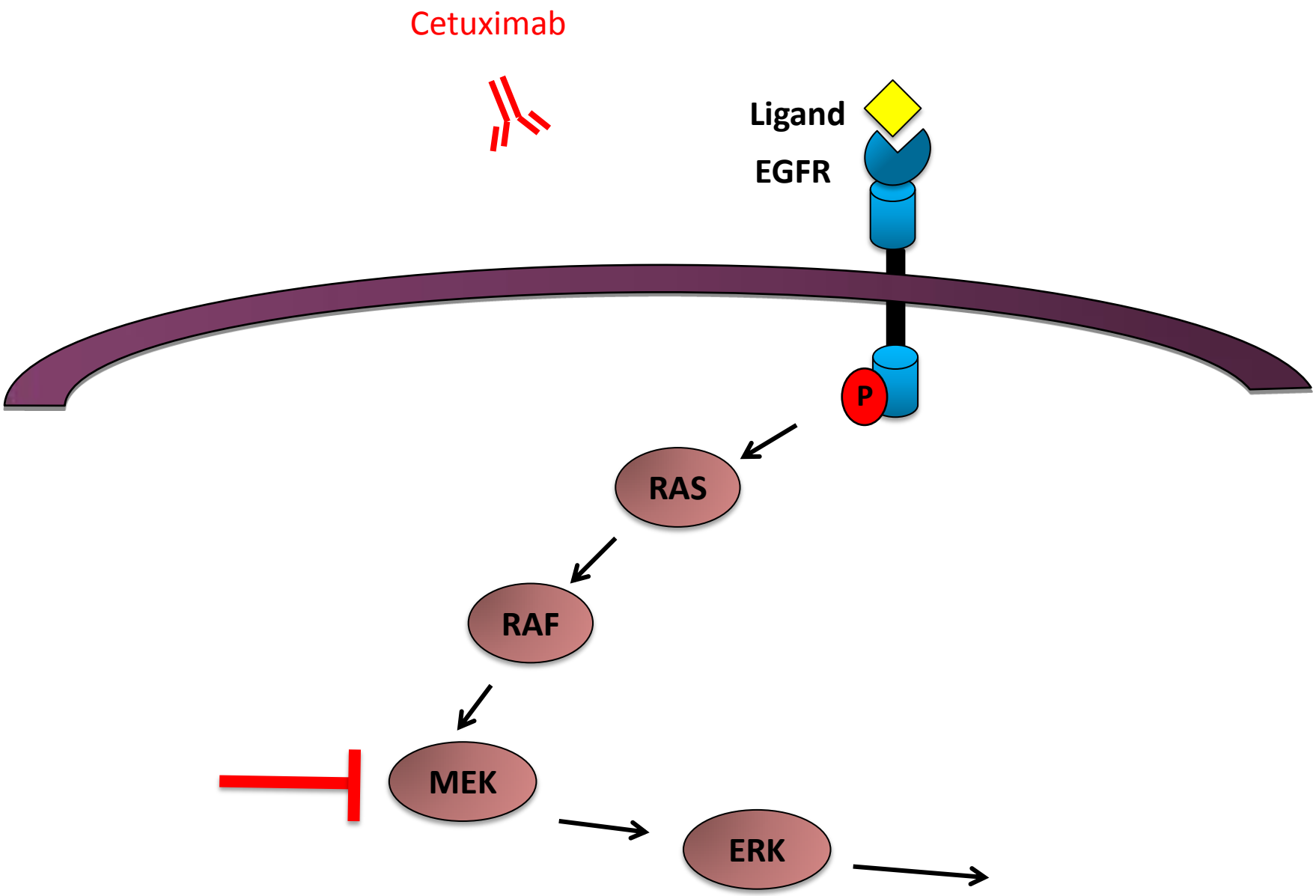
-4-

Preventing evolution: Vertical inhibition

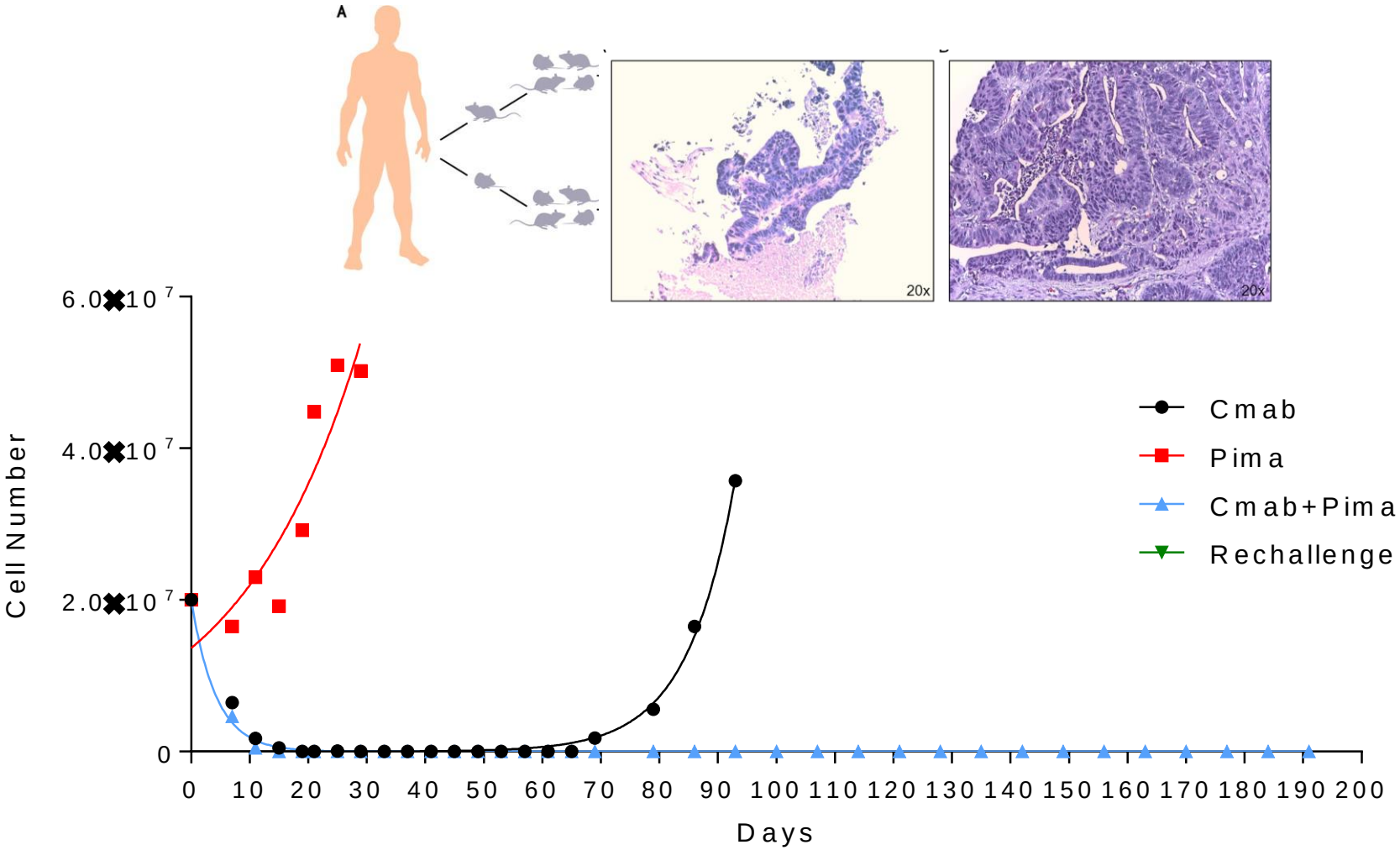
Drug resistance and biochemical convergence



Vertical targeting the EGFR-MEK pathway to delay resistance

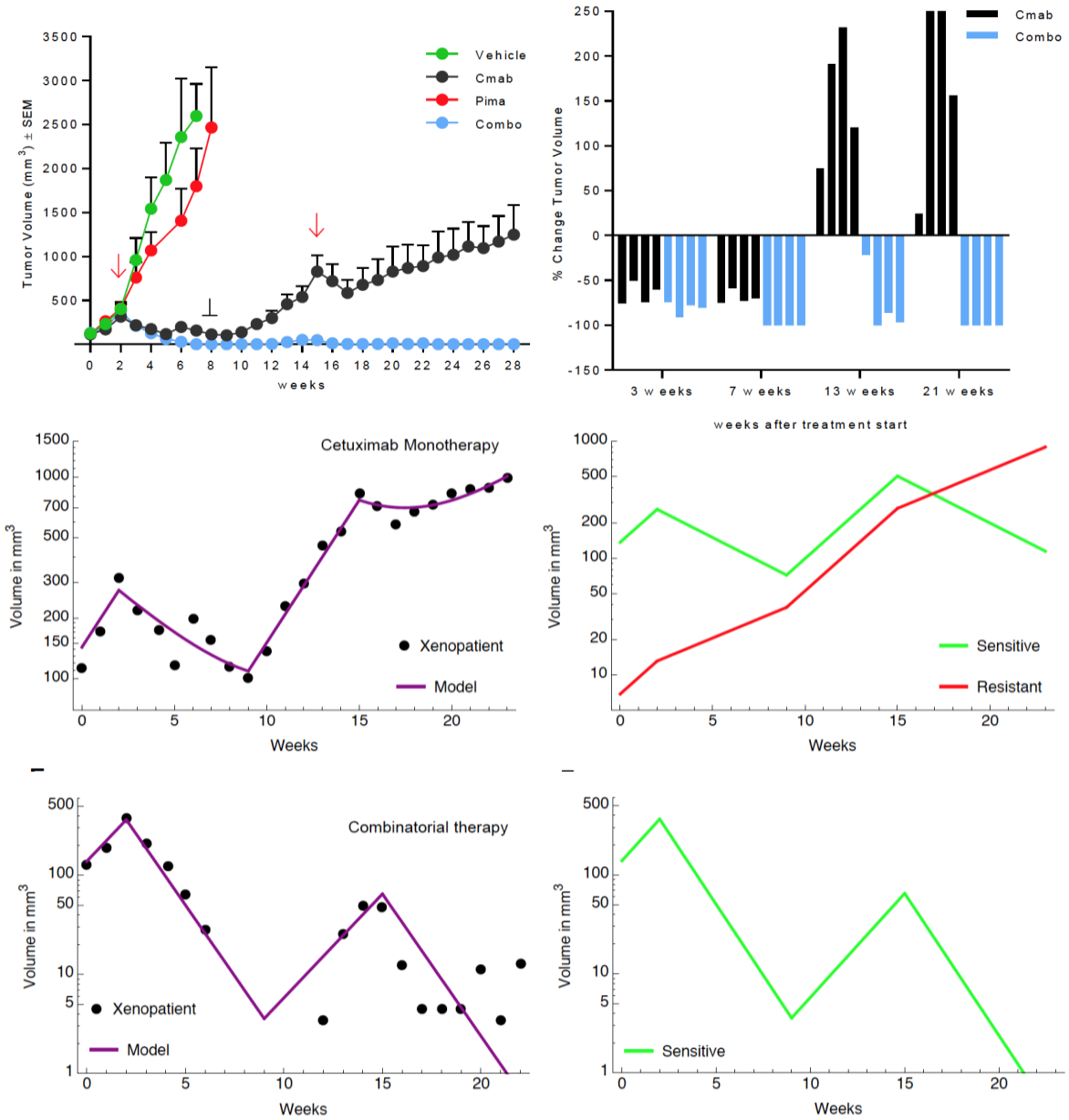


EGFR-MEK combo prevents the onset of resistance



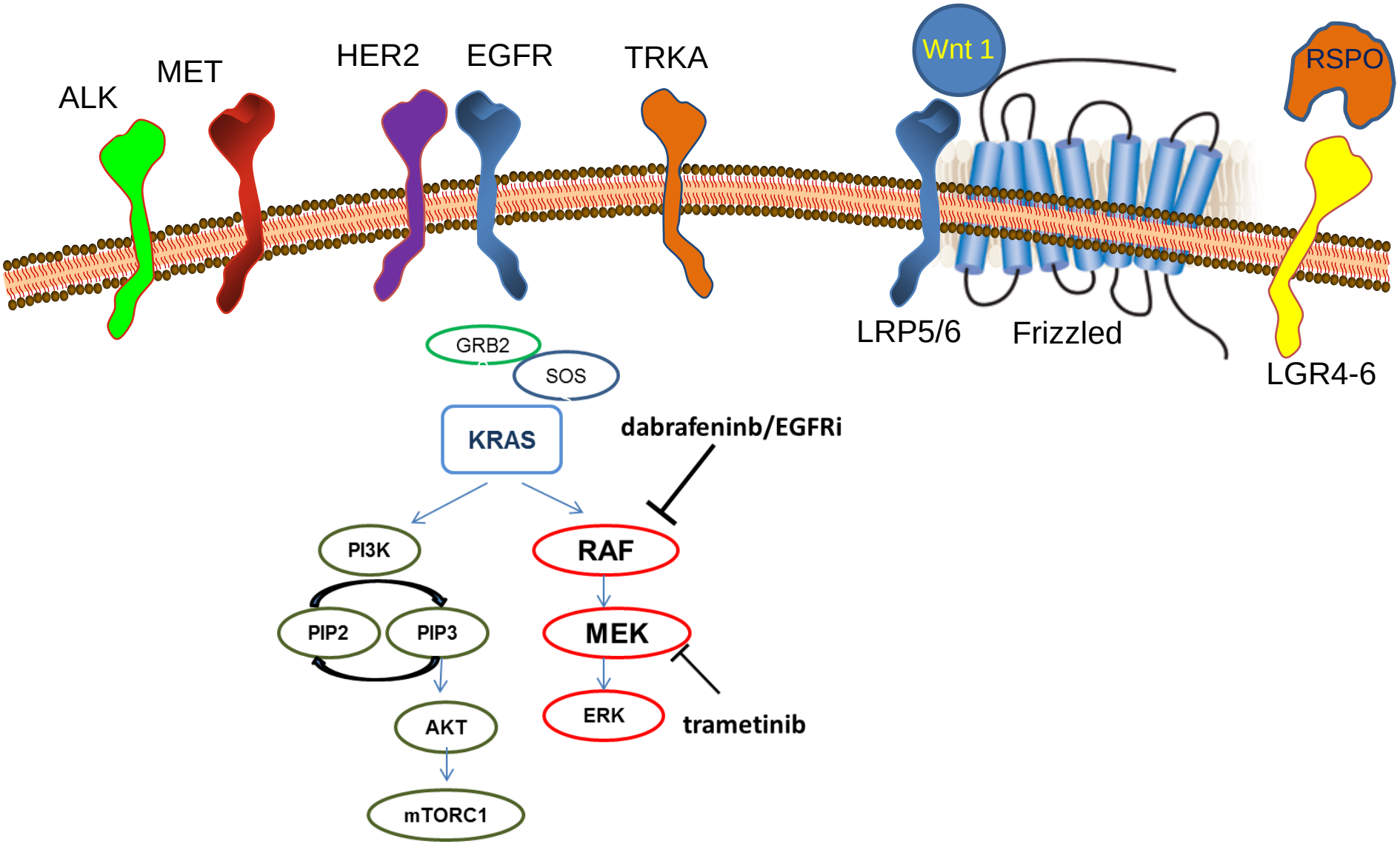
Misale et al., *Nature Comm* 2015

EGFR-MEK blockade and resistance in a patient's avatar



Bozic and Nowak

Drug resistance and clonal evolution in colorectal cancer



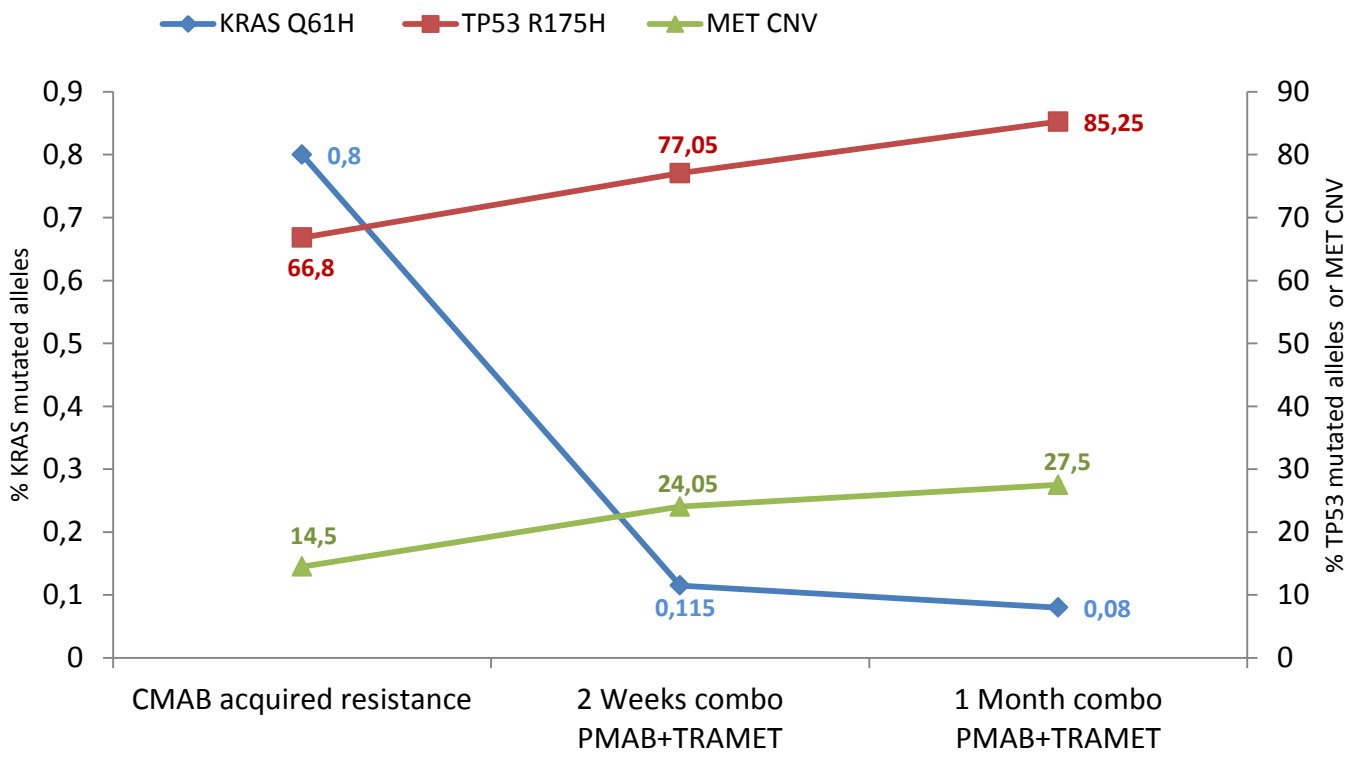
Josep Tabernero
Gary Li
Ryan Corcoran

Salvatore Siena
Andrea Sartore Bianchi
Emanuele Valtorta
Silvia Marsoni

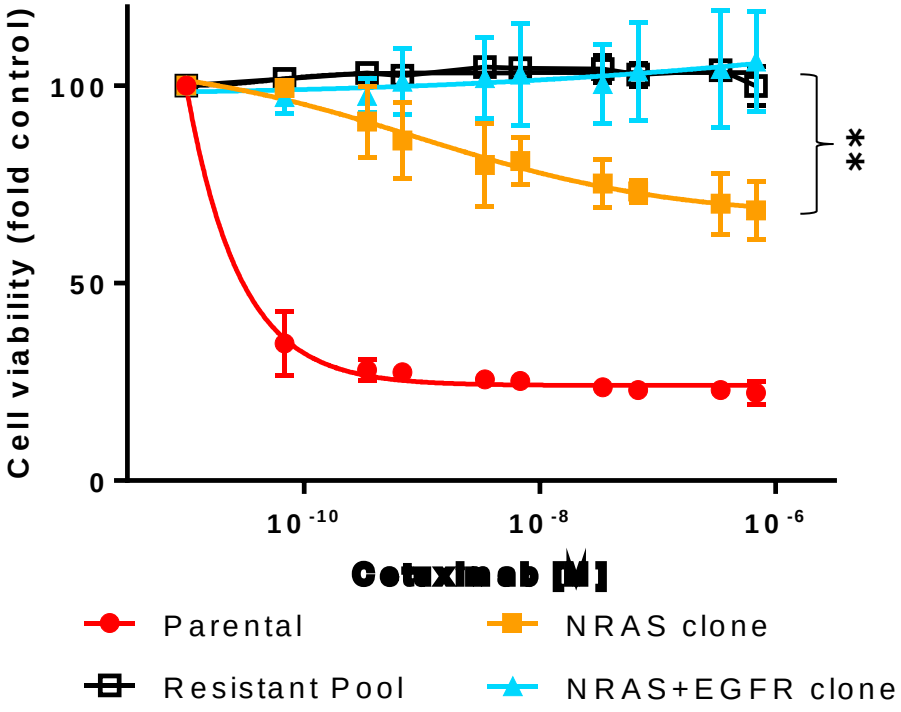
Federica Di Nicolantonio
Livio Trusolino
Andrea Bertotti
Enzo Medico

Clara Montagut
Beatriz Bellosillo
Rachel Nering
Fotios Loupakis

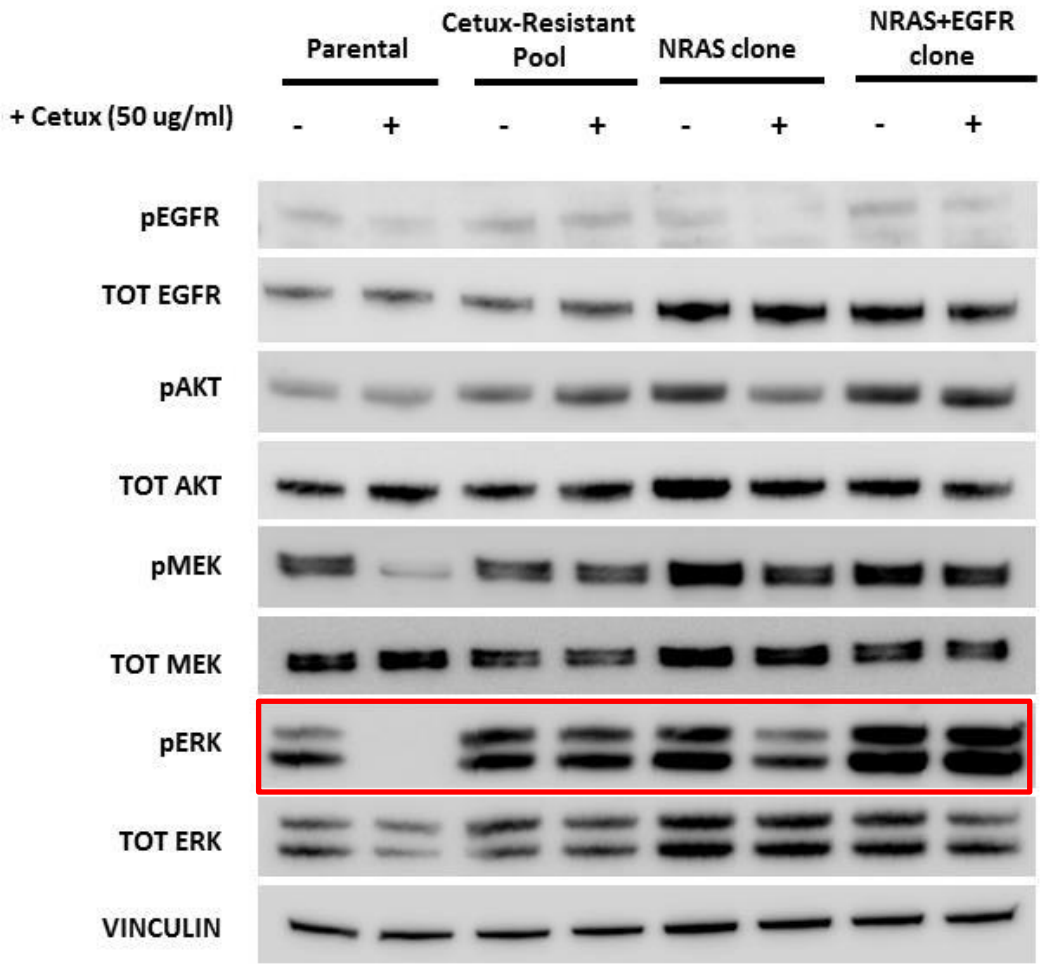
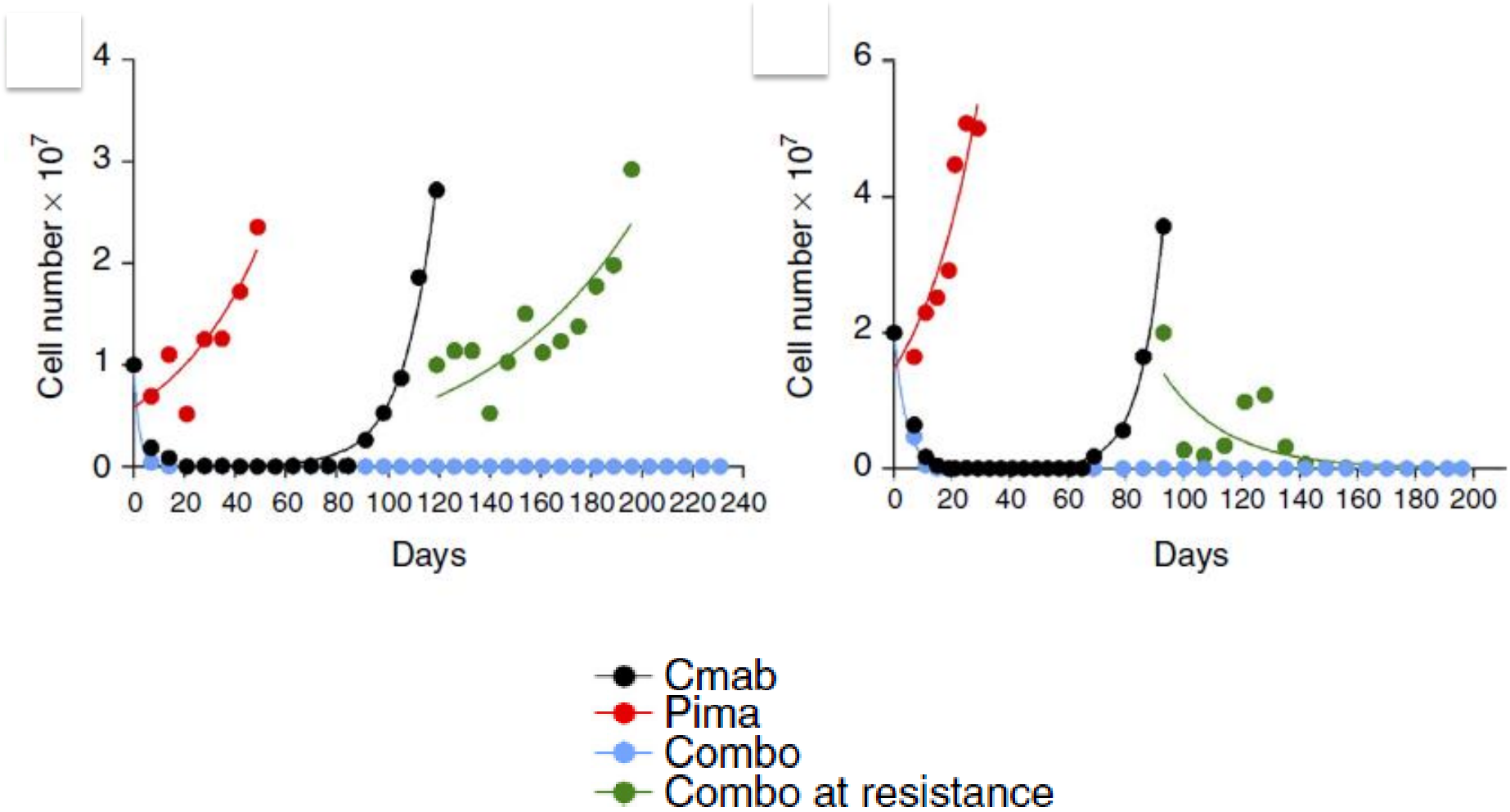
MGH-RC019


KRAS Q61H
TP53 R175H
MET CNV
% KRAS mutated alleles
% TP53 mutated alleles or MET CNV
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
90
80
70
60
50
40
30
20
10
0
0,8
77,05
85,25
66,8
24,05
27,5
14,5
0,115
0,08
CMAB acquired resistance
2 Weeks combo PMAB+TRAMET
1 Month combo PMAB+TRAMET

Clonal fitness: sensitivity to cetuximab



Clonal Fitness: Effects on MAPK Signaling



Concomitant blockade of EGFR and MEK halts the emergence of resistance in CRC cell lines