Mechanisms of Resistance to BRAF/MEK Inhibitors How to overcome them

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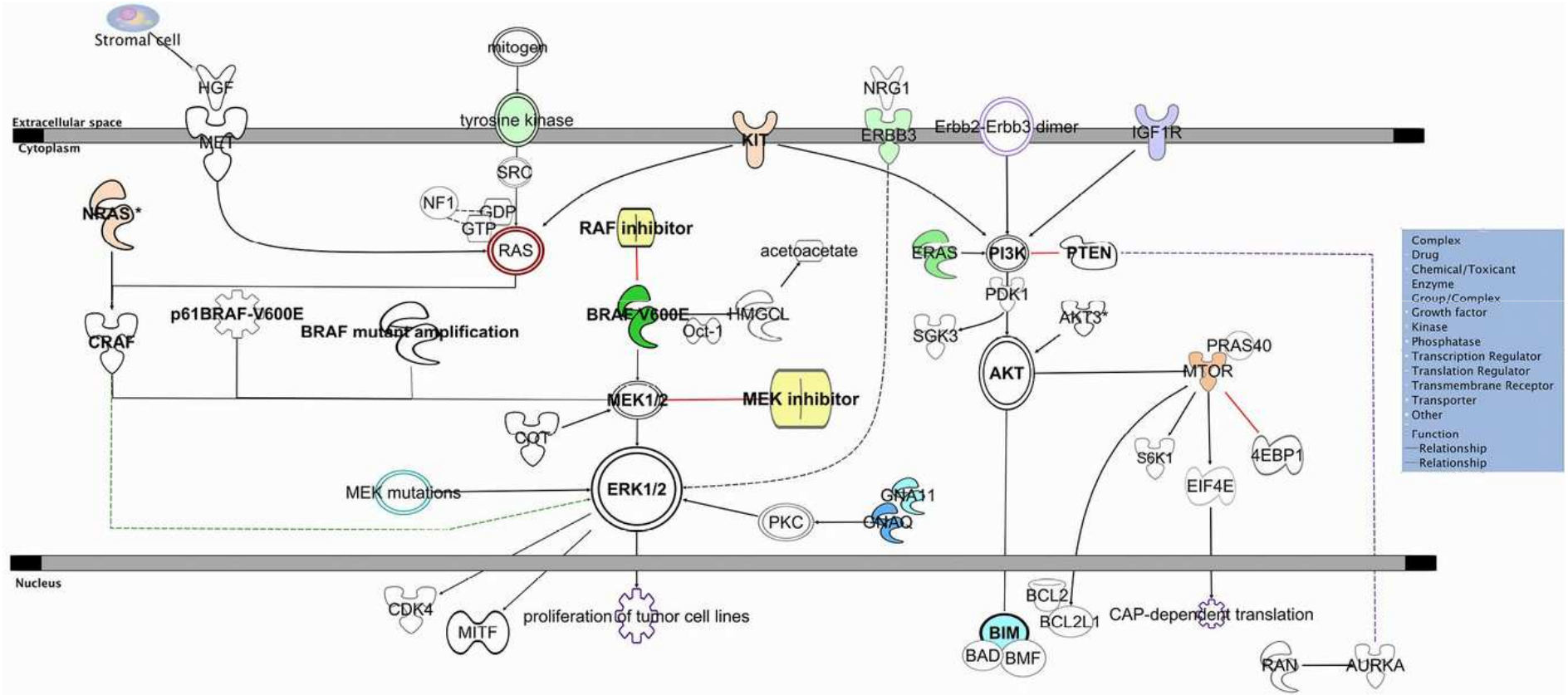
June 2016
GEM

Summary

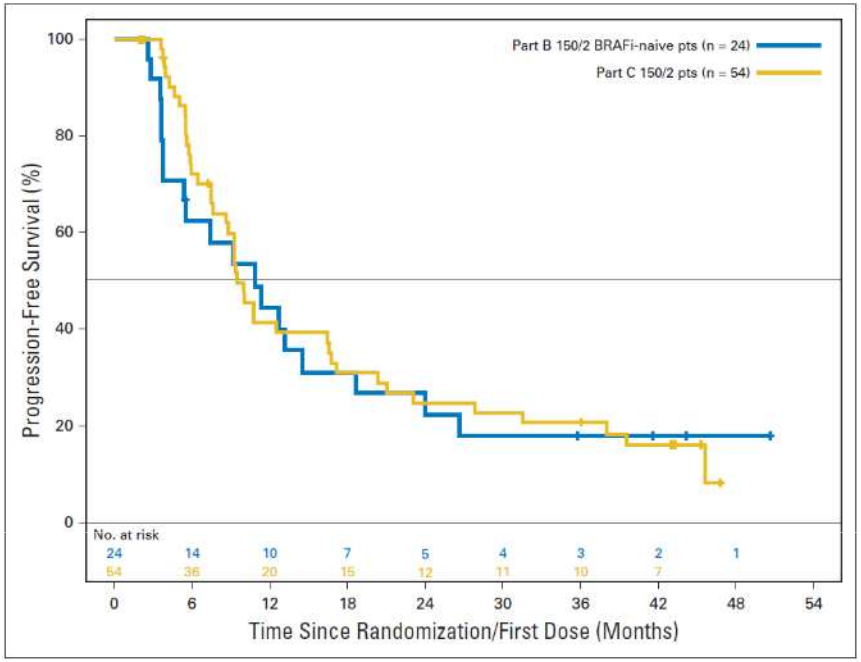
1. Pathway
2. Different responders
    1. Long responders (15-20%): CR----------**BRAFi+MEKi**
    2. Refractary patiens (5-10%): Intrinsic resistance----**Immunotherapy, novel drugs**
    3. Resistance (80%): adaptative and acquired
        - 3.1. Heterogeneity: ----------**Targeted drugs at progression: cfDNA,**
          Novel combinations 1st line
          Novel drugs
        - 3.2. Adaptative resistance----------**On/off schedule**

Main pathways in BRAF mutant melanoma



Karachaliou et al. ATM 2016

1. Long term survival Dabra + Trame



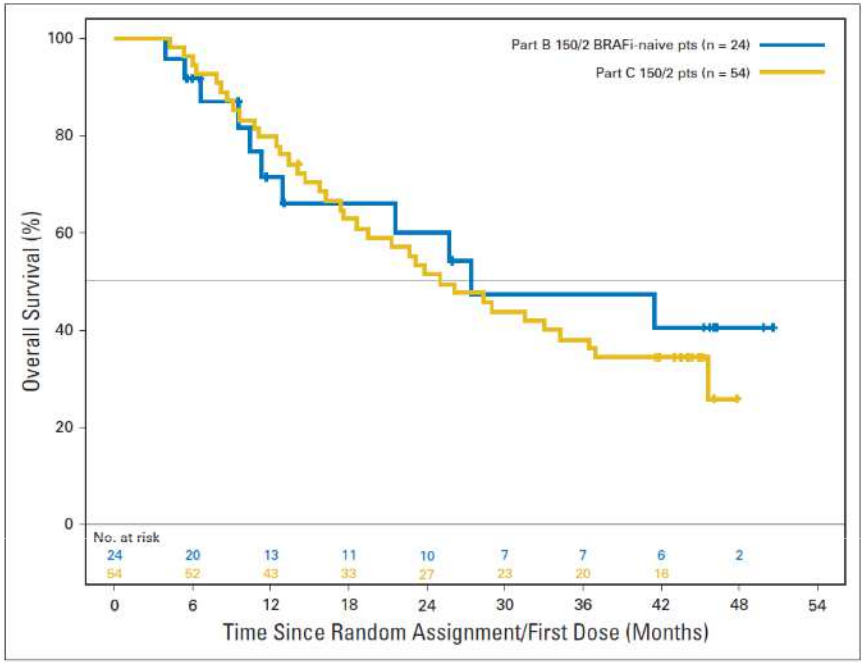


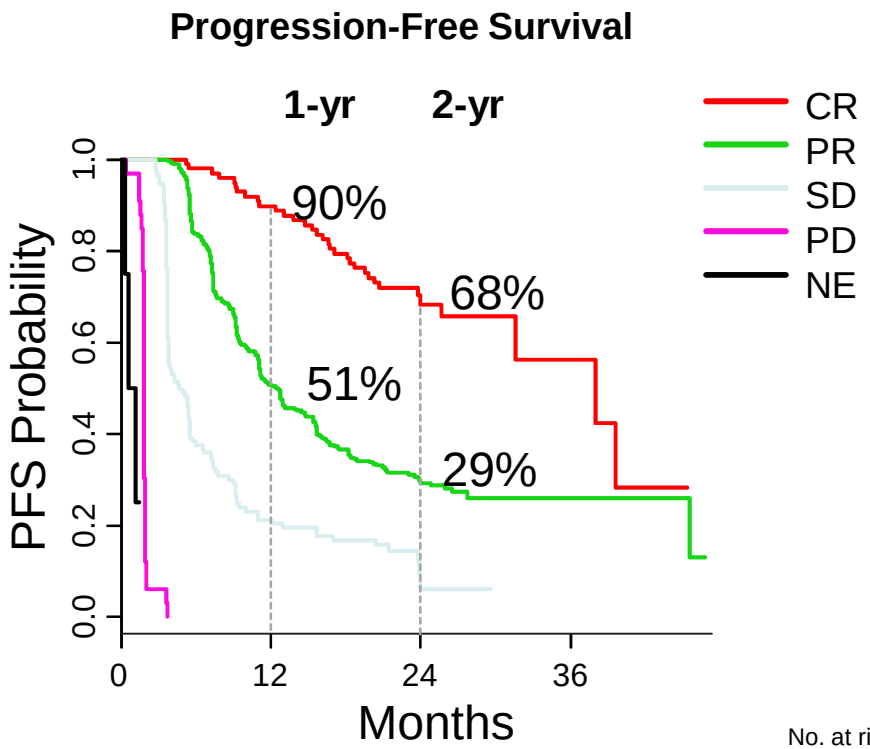
Table 2. Baseline Characteristics, Best Response, and OS in Patients Treated With a Combination of Dabrafenib 150 mg Twice Daily and Trametinib 2 mg Once Daily: Part C (n = 54)

Factor	No.	HR	Median OS, Months	1-Year OS, %	2-Year OS, %	3-Year OS, %
RECIST best response						
Stable disease	13		21.3 (8.6 to not reached)	69 (37.3 to 87.2)	35 (10.9 to 60.2)	35 (10.9 to 60.2)
Partial response	33	0.98 (0.44 to 2.19)	23.1 (16.2 to 34.3)	79 (60.6 to 89.3)	48 (30.8 to 64.1)	33 (18.2 to 49.3)
Complete response	8	0.38 (0.12 to 1.25)	— (29.0 to not reached)	100	88 (38.7 to 98.1)	63 (22.9 to 86.1)

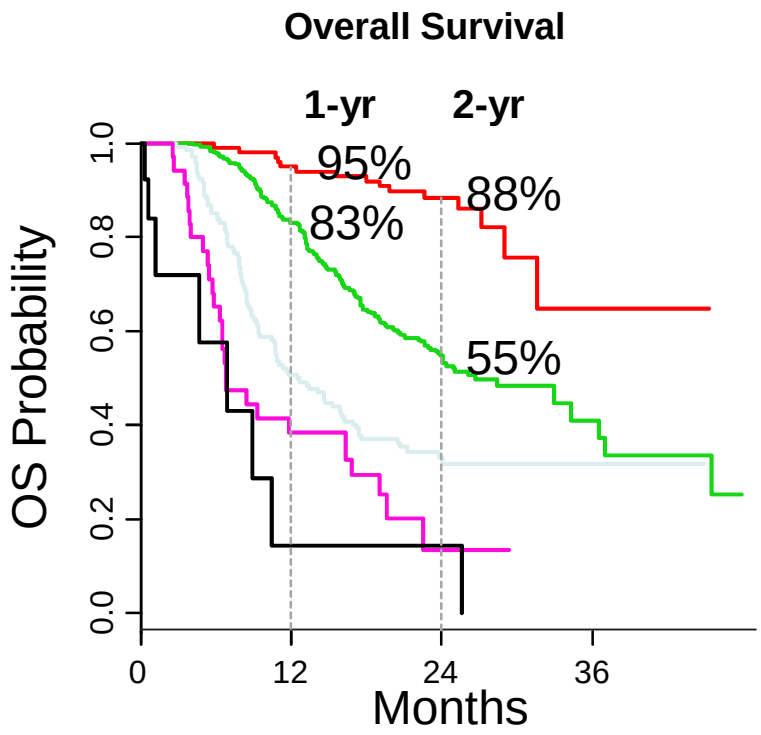
Abbreviations: ECOG, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; RECIST, Response Evaluation Criteria in Solid Tumors; ULN, upper limit of normal.

Long et al, JCO. Jan, 2016. DOI: 10.1200/JCO.2015.62.9345

PFS and OS by Best Response

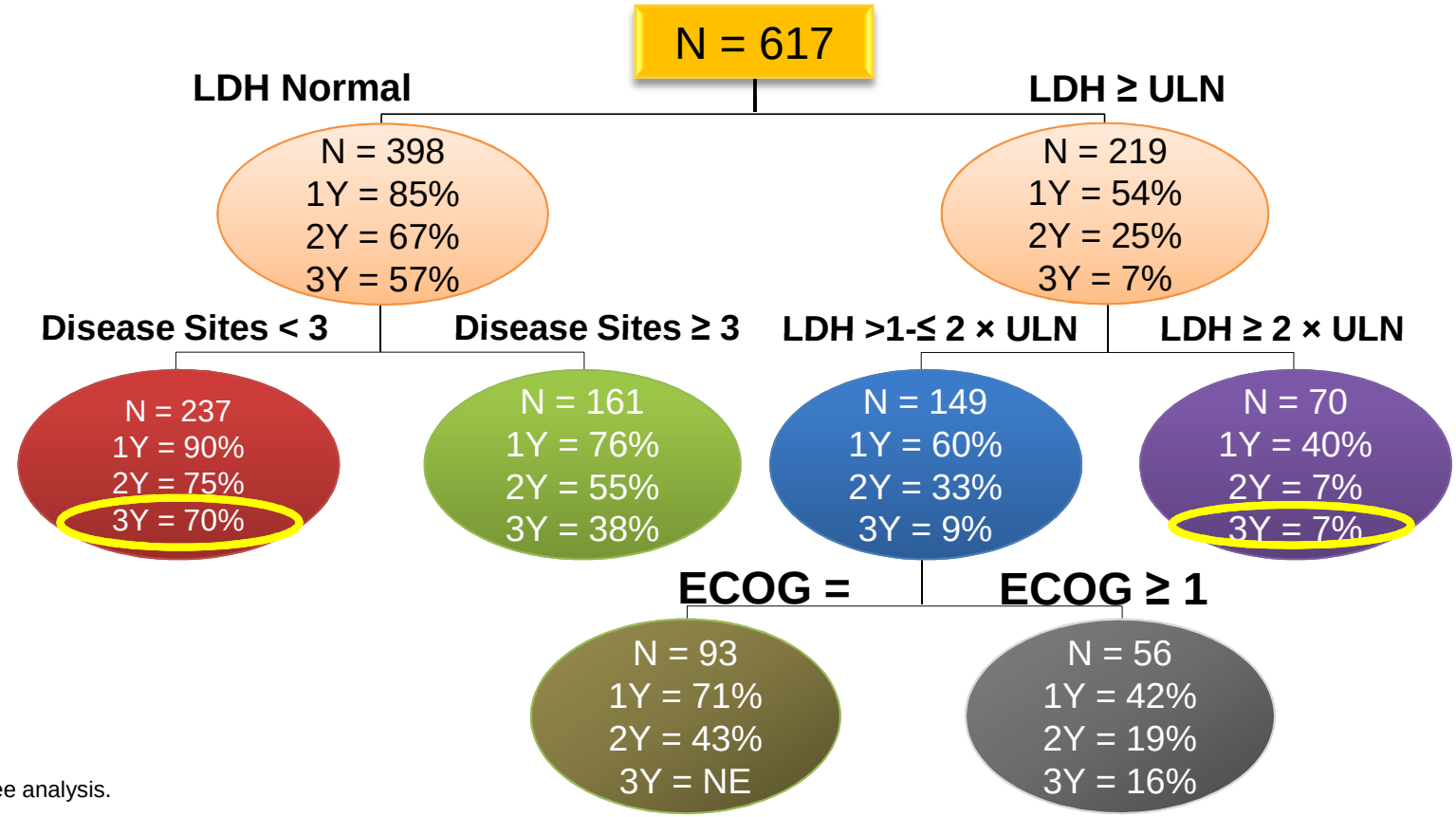


No. at risk				
Complete Response (CR)	100	87	33	5
Partial Response (PR)	316	151	50	5
Stable Disease (SD)	150	24	2	0
Progressive Disease (PD)	35	0	0	0
Not Evaluable (NE)	16	0	0	0



No. at risk				
Complete Response	100	94	52	5
Partial Response	316	255	107	11
Stable Disease	150	68	24	4
Progressive Disease	35	13	2	0
Not Evaluable	16	1	1	0

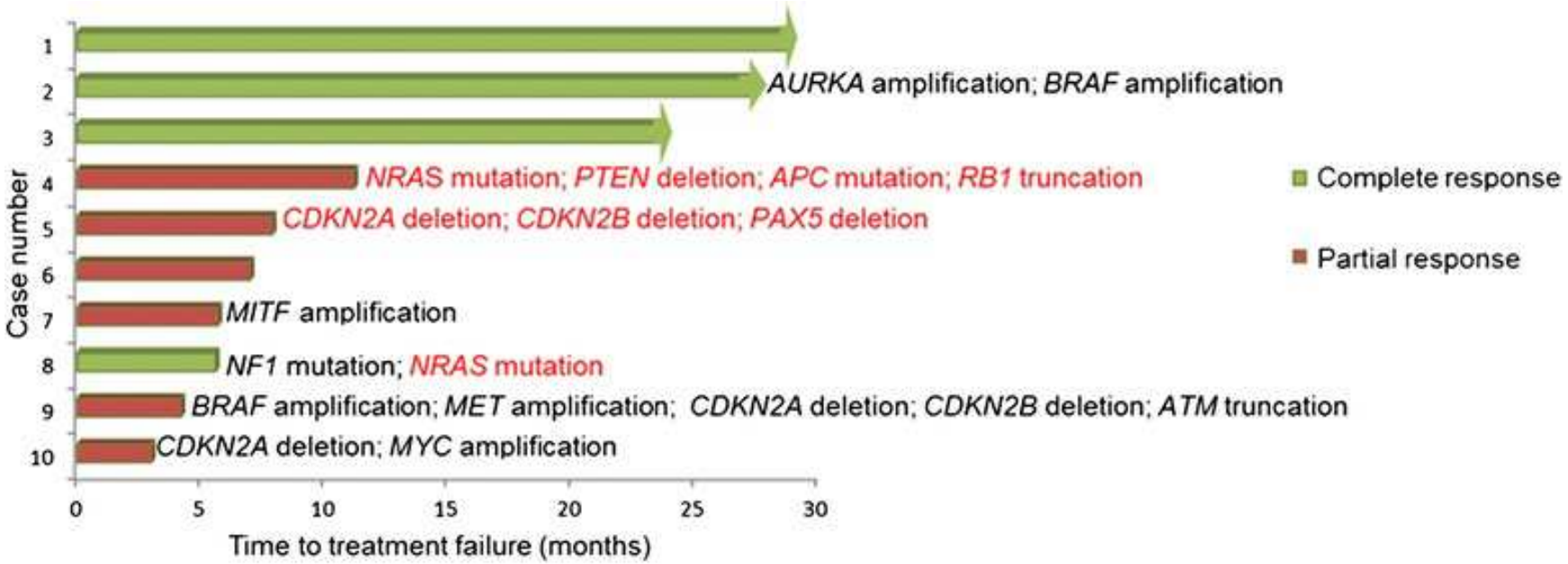
Five Baseline Factors Influenced OS



[a] Regression tree analysis.

NE, not estimable.

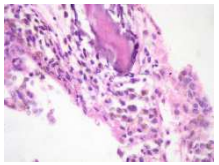
Long survivors with BRAFi: only BRAF mutations on WES

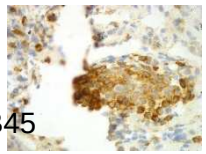


Wheler. BMC Cancer 2015

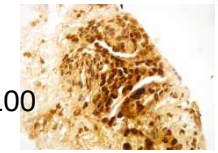
Bone Biopsy

H-E

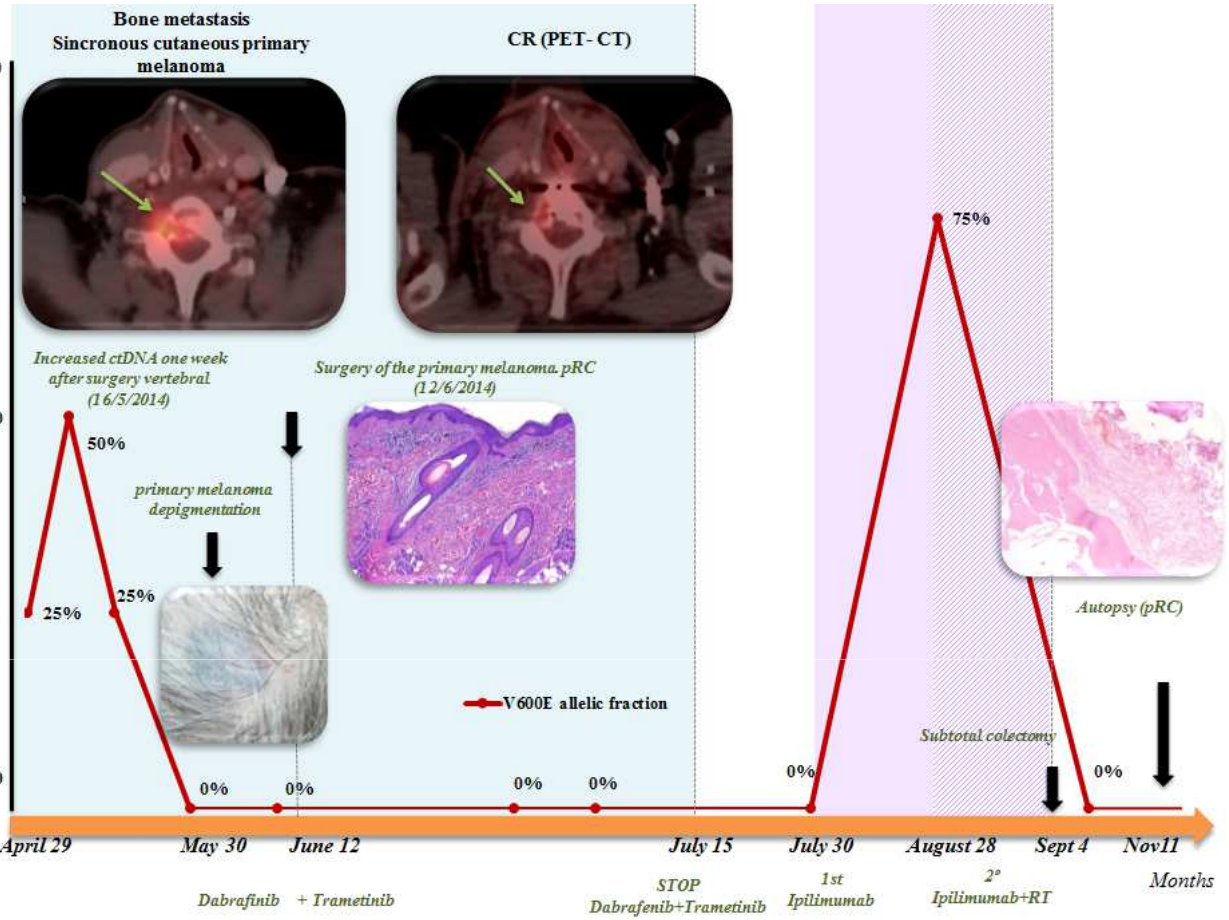


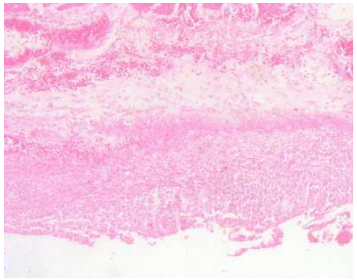


HMB45



S-100





Autopsy: colitis perforation

Targ Oncol
DOI 10.1007/s11523-015-0410-9

THERAPY IN PRACTICE

Ongoing Response in BRAF V600E-Mutant Melanoma After Cessation of Intermittent Vemurafenib Therapy: A Case Report

Andrew J. Dooley[2] · Avinash Gupta[1] · Mark R. Middleton[1]

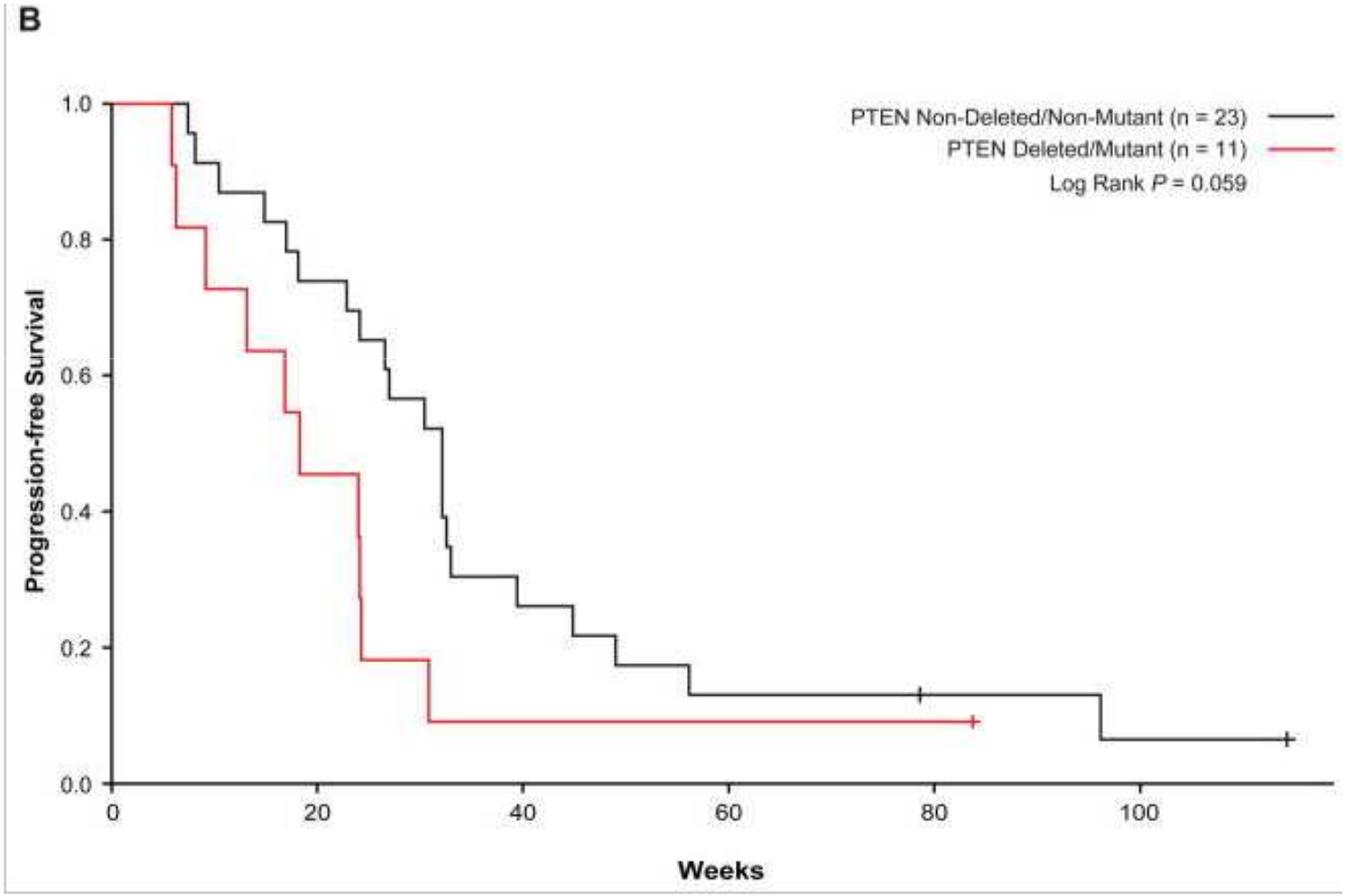
Dooley et al. Targ Oncol 2016

Gonzalez-Cao, BoadaA et al. Oncotarget In press

2. BRAFi Resistant Mechanisms: intrinsic resistance

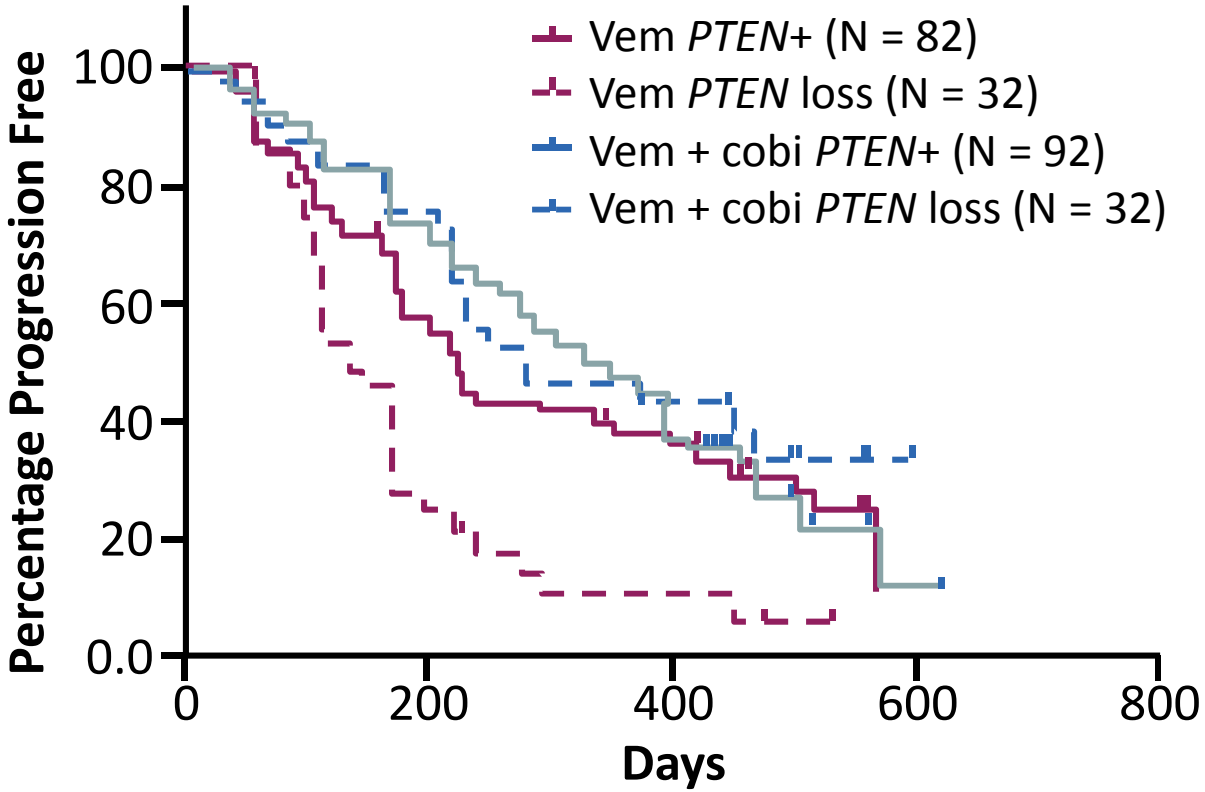
	%	Primary/acquir	Treatment
NRAS mut	15	P/A	Mek+simvastatin, Meki+ CDK4i, MEKi+EGFRi, MEKi+abt263, smvastatin+flavopiridol, simvastatin+CDK4i, MEKi+PI3Ki PLX7904, **ERKi** **MEKi+nefinavir**
BRAF FUSION	2	P	MEKi
MEK mut (NO P162S)	15	A/P	**ERKI** (SCH7729 MERK)
NF	**2**	A/P	MEKi+MTORi, CRAFi+BRAFi, panRAFi (AZ628), **ERKi**
COT sobre	**?**	A/P	-
RAC1	**3/14**	P	PAKi
MELP124	**10/134**	P	MEKi, ERKi
EGFR, pdgfr	**6/16**	A/P	Dasatinib, AKTi+EGFRi, (¡?), braf+pi3ki Holidays, HSP90i
HGF	**?**	P	BRAFi+METi, BRAFi+AKTi
PTEN loss, or RB inact	**10-30**	P/A	BRAFi+everolimu BRAFi+PI3Ki
ERB2, ERB3	**?**	A/P	BRAFi+Lapa ¿?
MED12	**?**	Mediator A	BRAFi+TGFBi (YR-290)
BCL2A1-MITF	**?**	A(P	R to ERKi, BRAFi, MEKi; S BRAFi+bcla2i, obatoclax; Antiretroviral drugs

PTEN deletion/mutant pretreatment in BRAFi



Hartsough J Invest Dermatol 2013, Shi CCR 2013

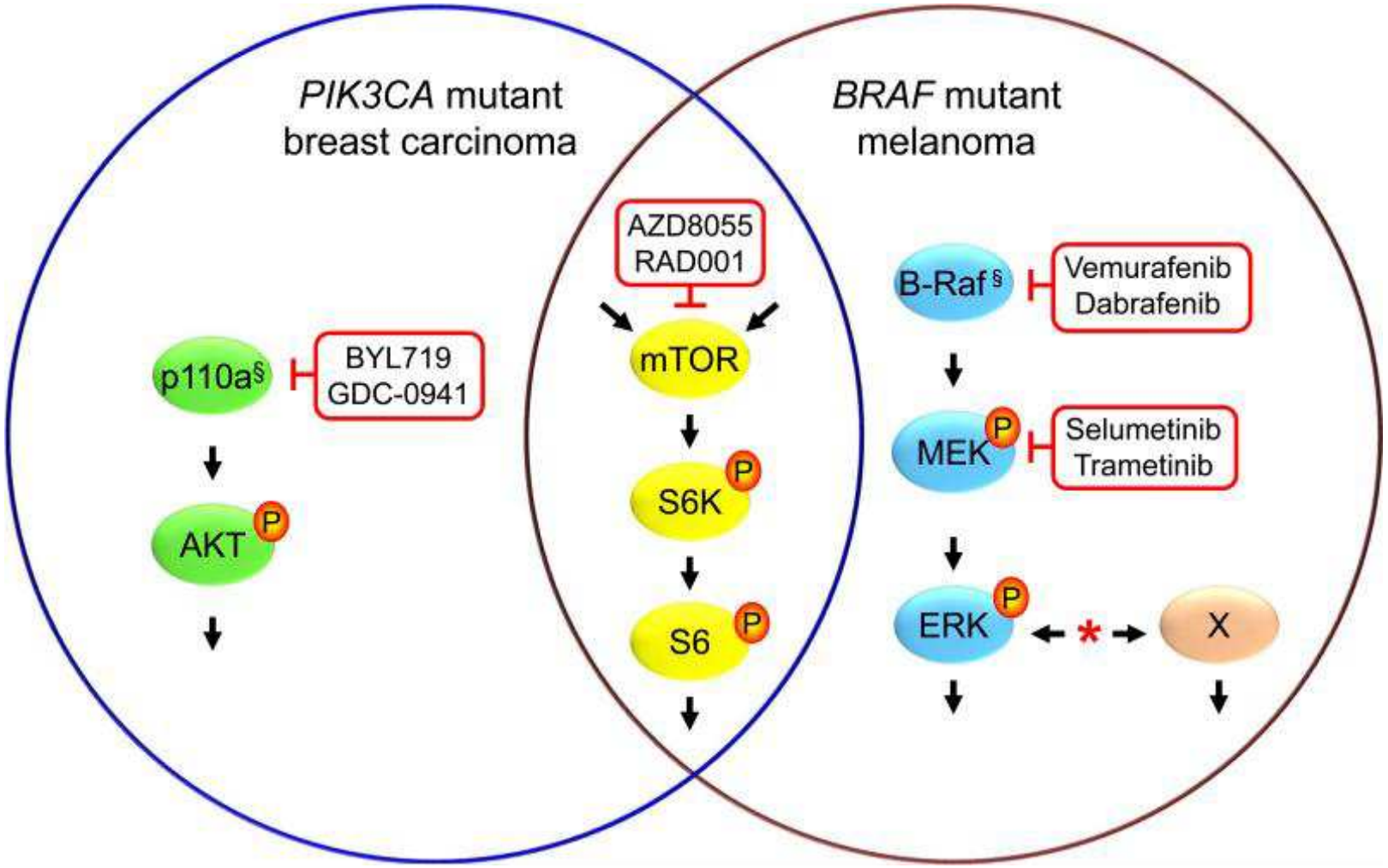
Addition of Cobimetinib to Vemurafenib Overcomes the Negative Impact of *PTEN* Loss on PFS



PTEN+: H score ≥50
PTEN loss: H score <50

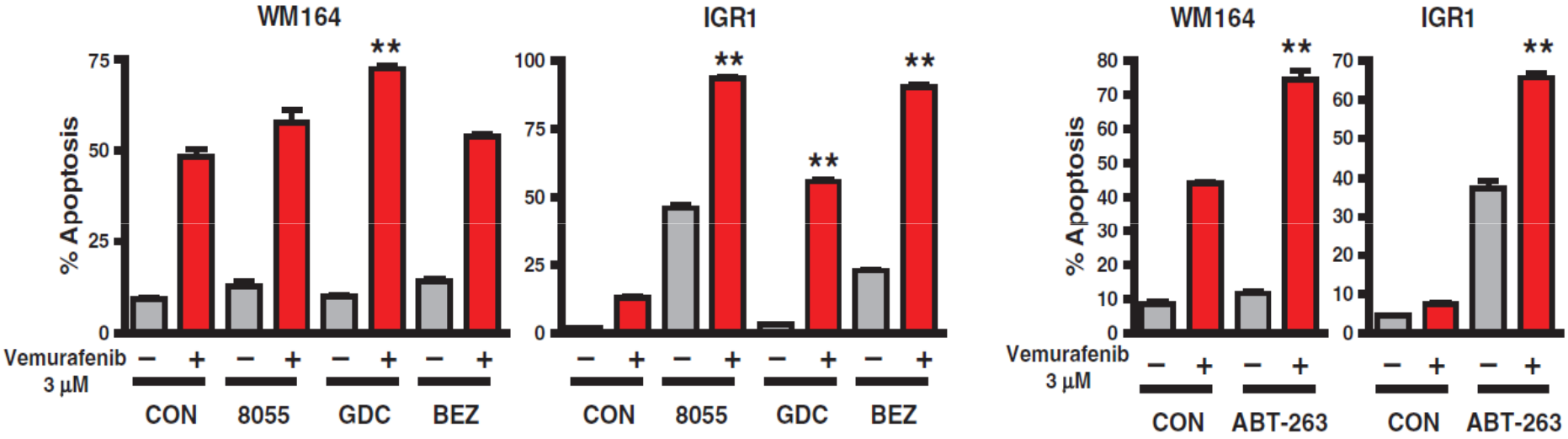
HR 0.36 (95% CI, 0.19-0.65)
For PTEN loss Vem + Cobi vs. Vem

McArthur. ECCO 2015



Gentry. Cancer Cell 2013

TORC1 suppression increases the apoptotic response in BRAF-mutant melanomas



AZD8055 (mTORC inhibitor), GDC0941 (pan-PI3K inhibitor), BEZ235 (dual PI3K-TORC inhibitor), ABT-263 (BH3 mimetic)

P-S6 measurement can effectively identify tumors with ERK-independent resistance. Combinations of RAF inhibitor with a TORC inhibitor, a PI3K inhibitor, or a BH3 mimetic, may be effective.

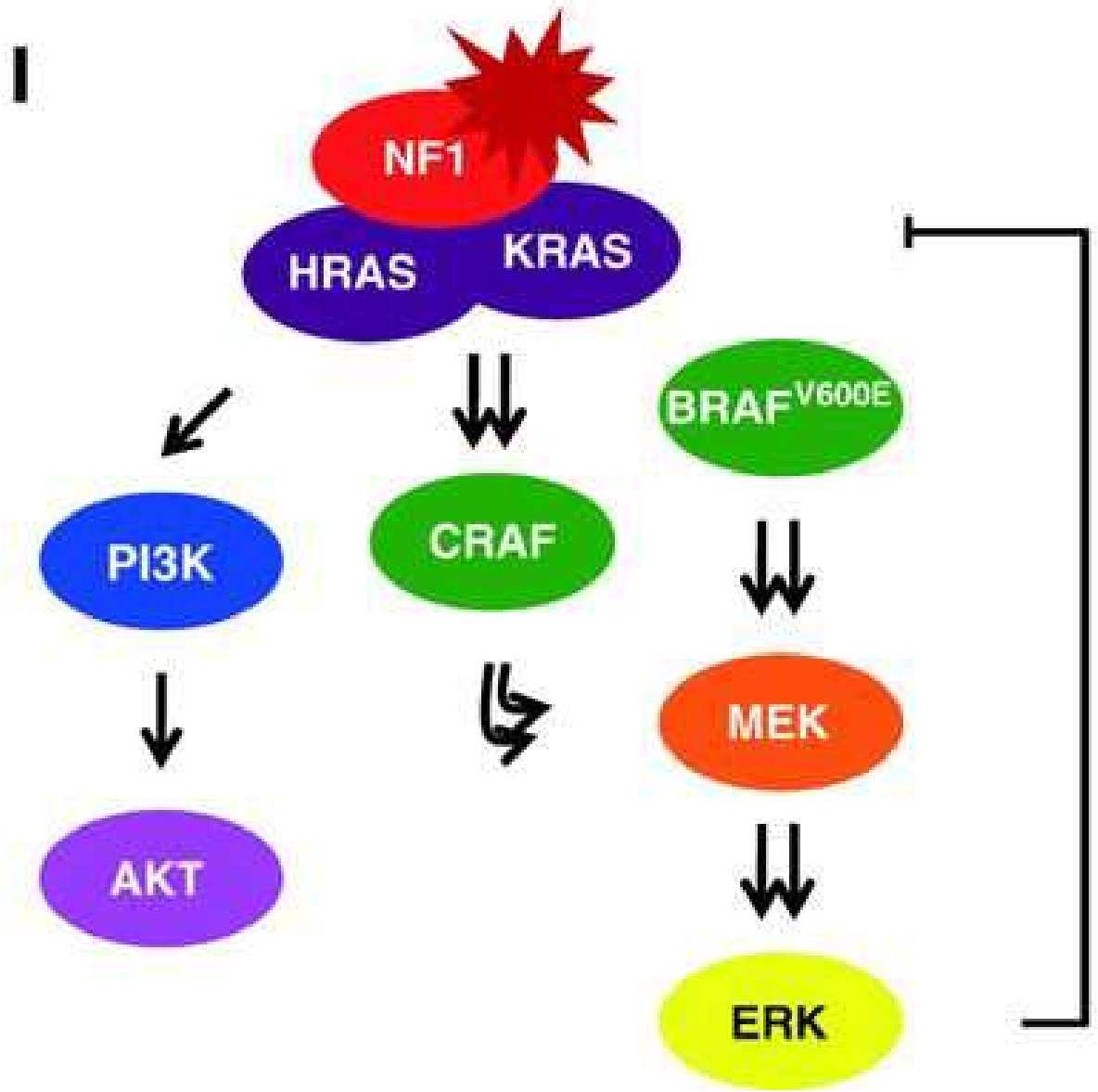
Back up information

Corcoran et al. STM 2013

Clinical trials using mTOR inhibition

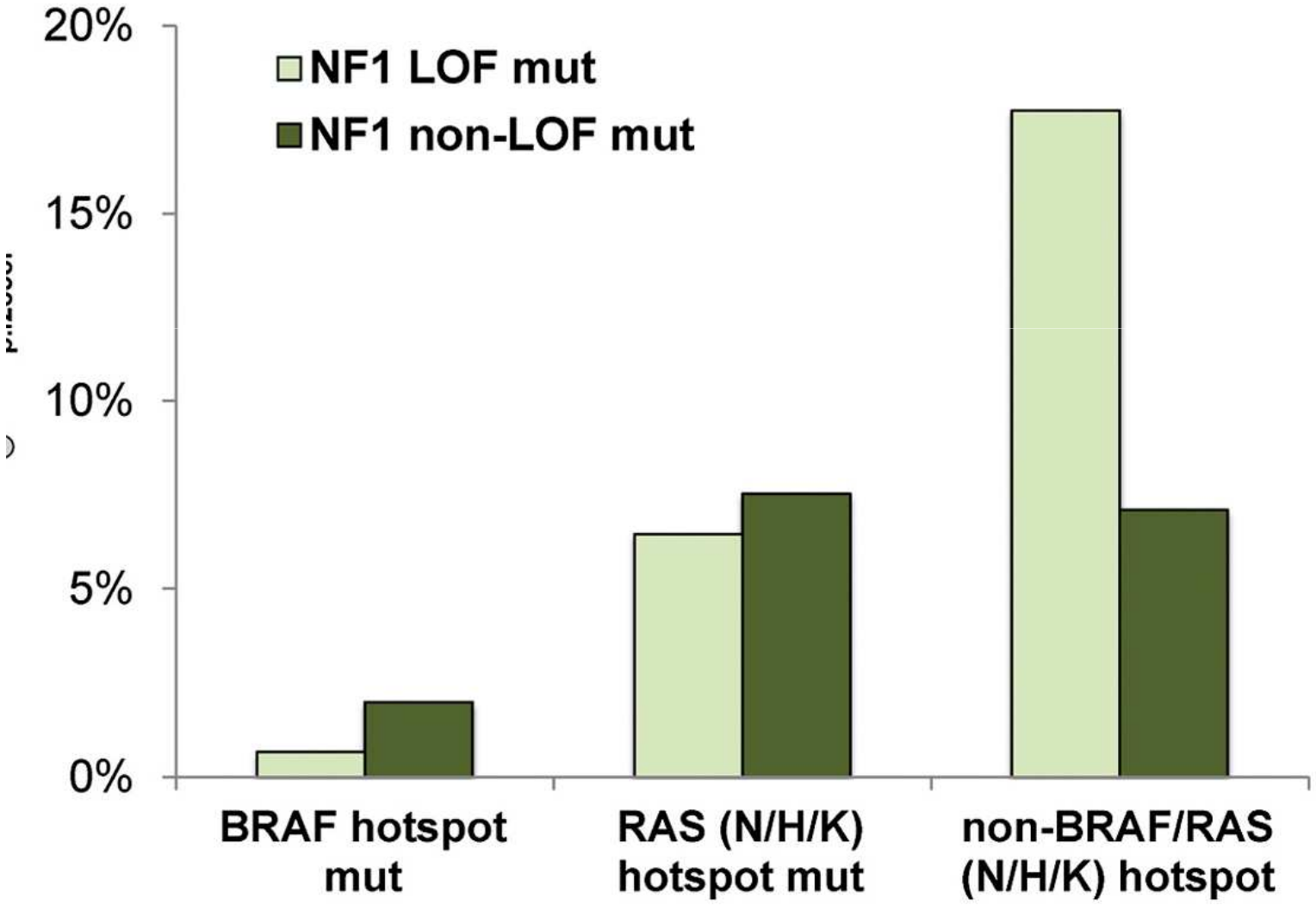
Drug	Mec	Combination	Status	Nº
BEZ235	DUAL pi3k/torc	MEKi	Completed	
everolimus	mTOR	several	Completed without published results	
AZD8055	mTOR		Completed	
ABT-263 (navitoclax)	BH3 mimetic	combination	Ongoing	NCT0189585

NF1 critically regulates KRAS and HRAS in melanomas



Maertens O et al. Cancer Discovery 2013;3:338-349

NF1 was mutated in 38.7% of non *BRAF/NRAS* melanomas (29/75) and in ~70% of non *BRAF/NRAS* samples with a UV-signature

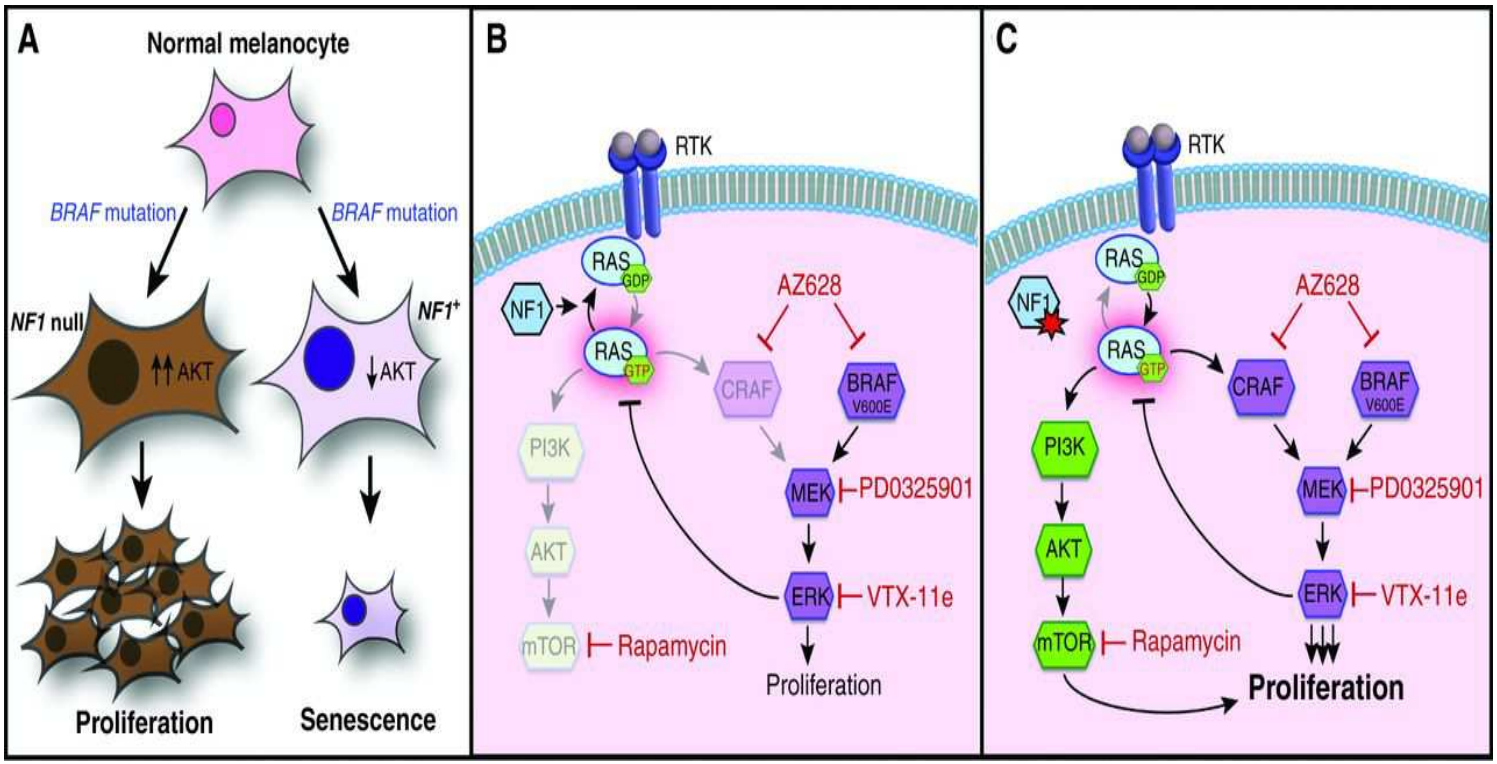


CGAN. Cell 2015

NF1 loss

16/121 melanomas harbored a NF1 missense or nonsense mutation
But clinically resistance has been only demonstrated in **one patient** that had it in the initial byopsy and had a short PFS

Sensitive to pan-RAF inhibitor AZ628, the ERK inhibitor VTX11e,
and the combination of a MEK + mTOR inhibitor (PD0325901 and rapamycin)

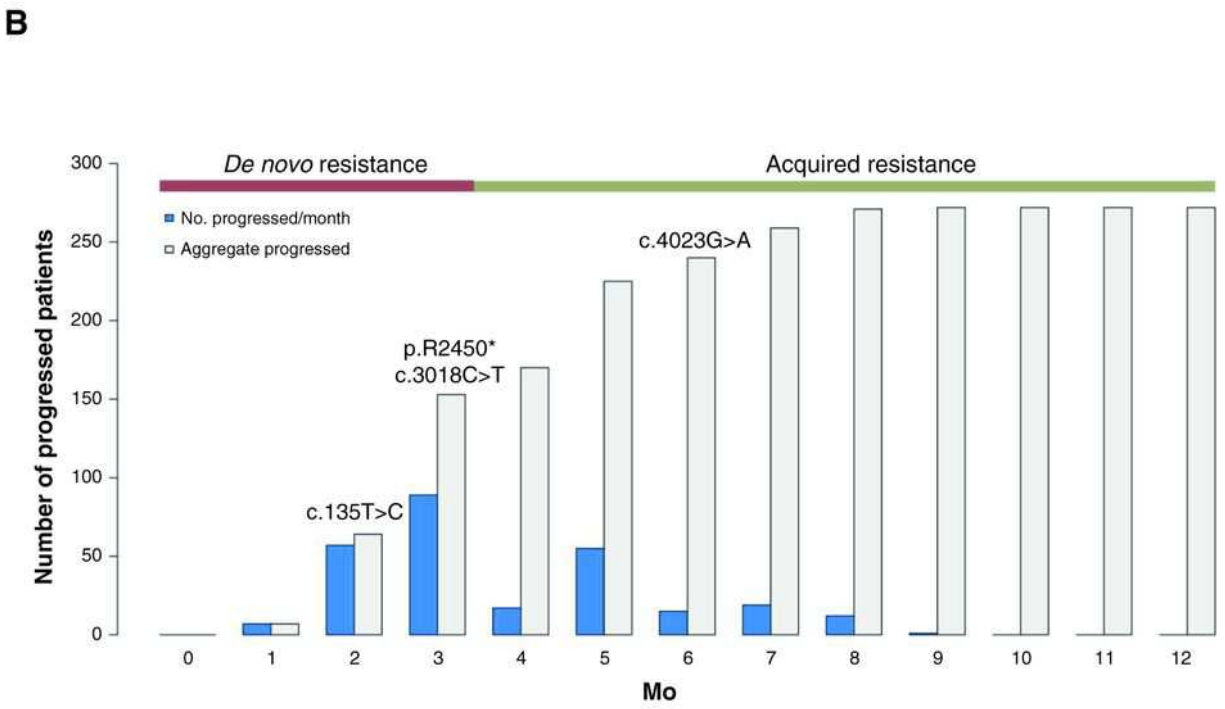


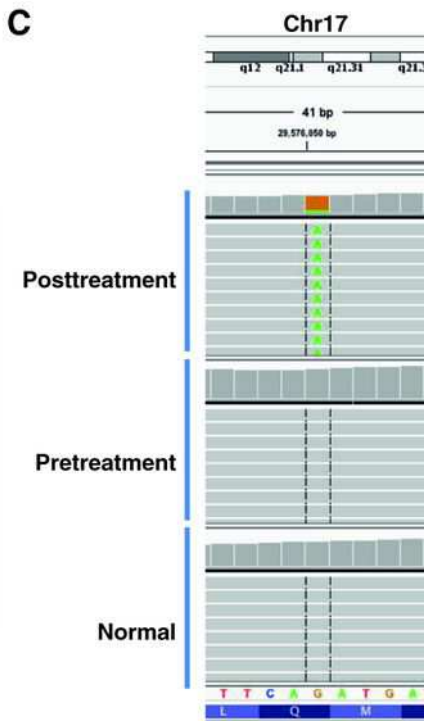
Gibney G T , Cancer Discovery 2013; Maertena, Cancer Discov 2013

Whole-exome sequencing identifies NF1 mutations in tumors of melanoma patients exhibiting resistance to vemurafenib

A

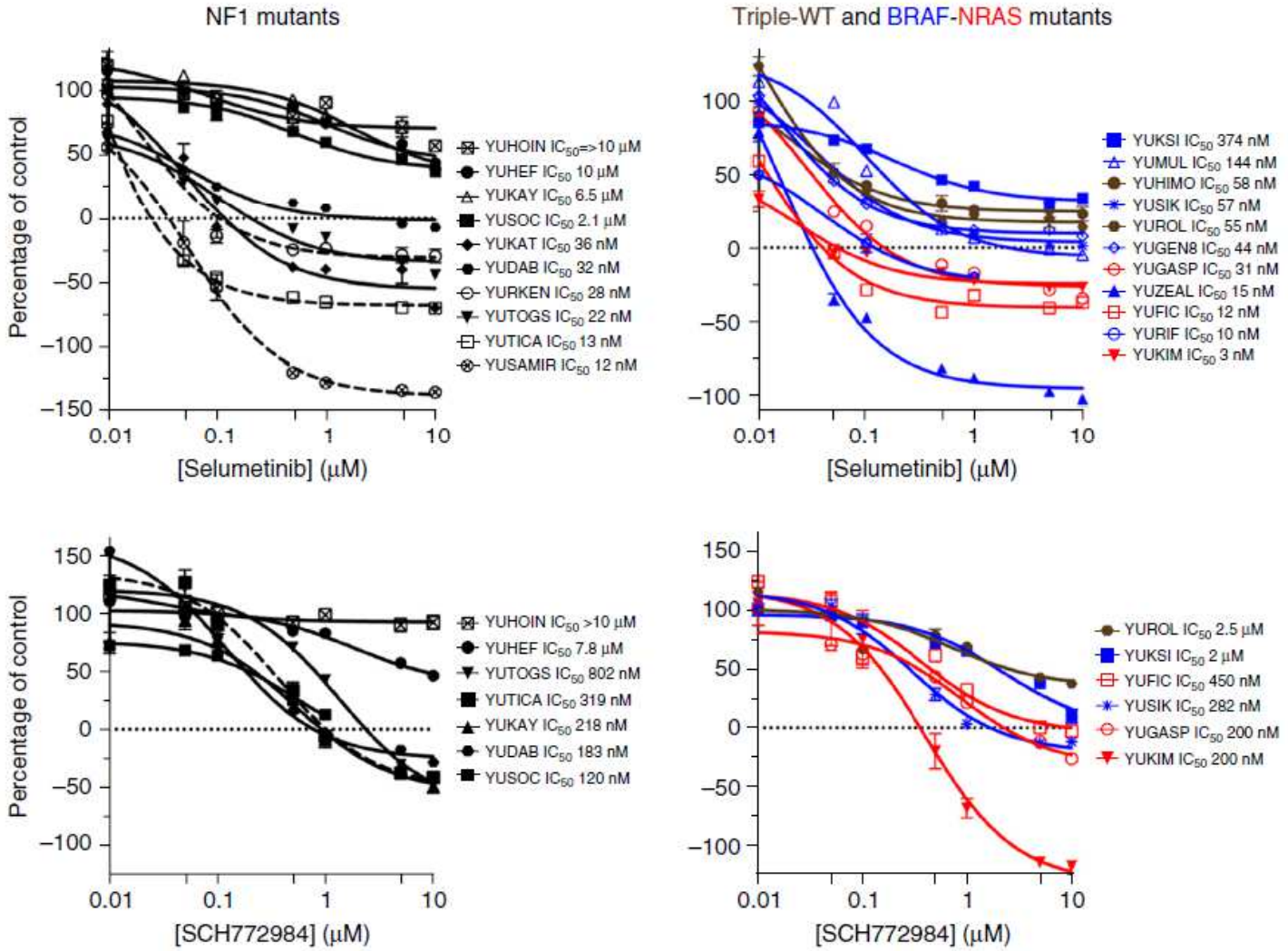
Patient	PFS (mo)	Resistance	cDNA	Protein	Candidate splice motif	Splice motif sequence	Site broken?
15	1.5	*De novo*	c.135C>T	p.N45N	Enhancer	ATCAAT	Yes
45	5	Acquired	c.4023G>A	p.Q1341Q	Splice site	AACCTCCTTCAGAT	Yes
46	2.5	*De novo*	c.7248C>T	p.R2450*	N/A	N/A	N/A
50	2	*De novo*	c.3018C>T	p.V1006V	Enhancer	ATGGTC	Yes





Whittaker S R et al. Cancer Discovery 2013;3:350-362

NF1 melanoma: growth responses to selumetinib and ERKi SCH772984

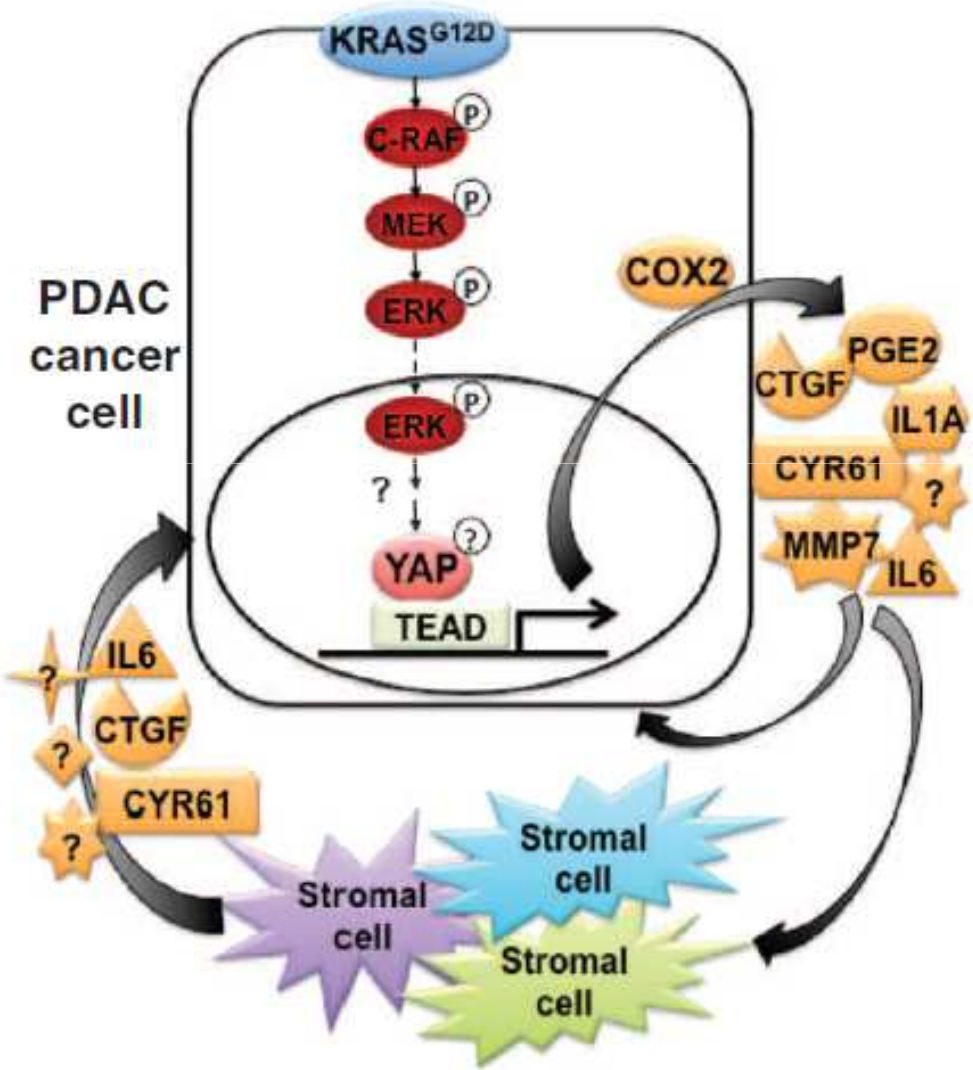


Krauthammer. Nat Genetics 2015

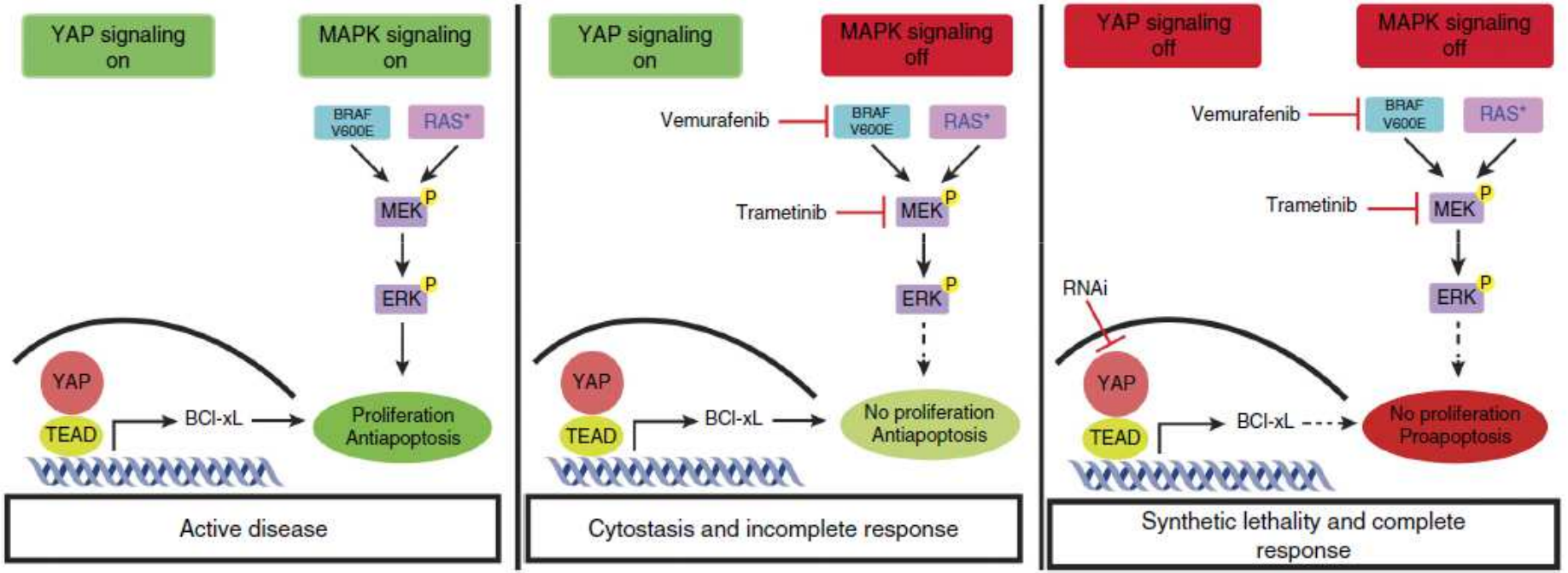
Clinical trials

Drug	Mec	Combination	Status	Nº
LXH254 (Nov)	PanRAF	-	Ongoing	NCT0260781
MLN2480	PanRAF	-	Ongoing	NCT02327169 NCT01425008 (melanoma)
AZD628	PanRAF	-	preclinical	
VTX11e	ERKi	-	preclinical	
everolimus	mTOR	several	Completed without published results	

Stroma

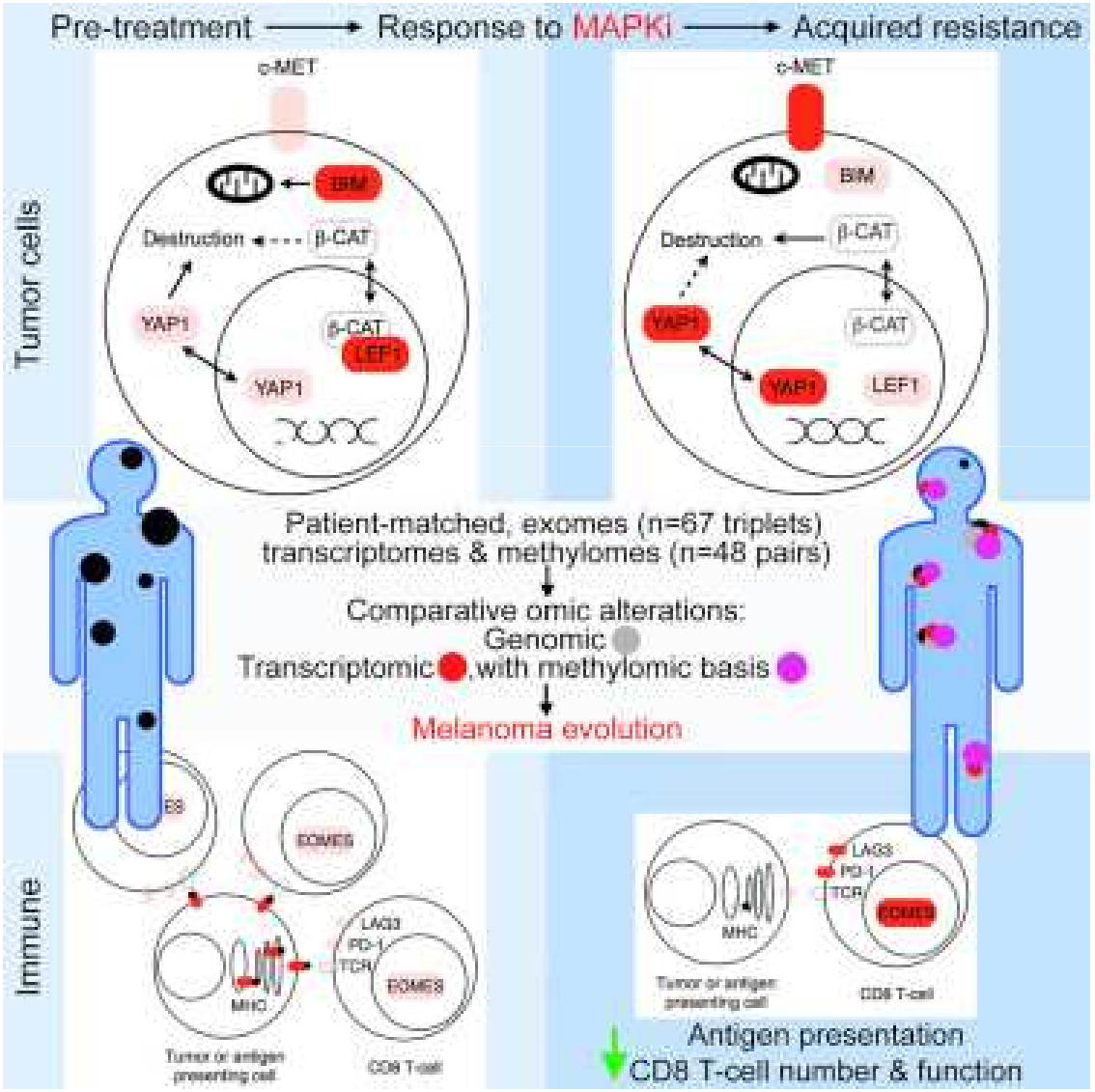


Zhang et al. Sci Signal 2014

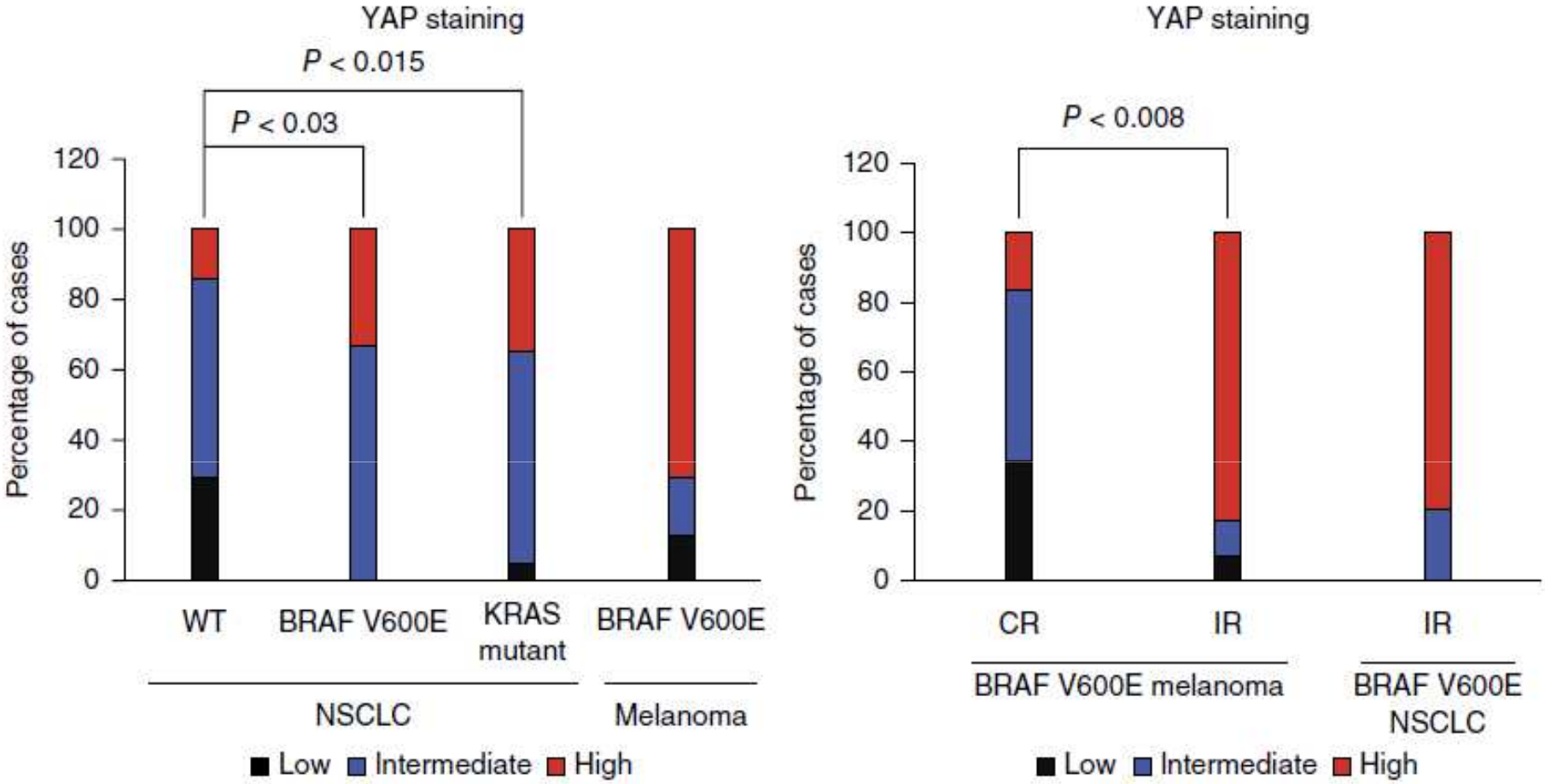


Lin et al. Nat Genet 2015

β-catenin-LEF1 and YAP1 signaling antagonistically co-regulate the tumor cell-intrinsic, apoptotic threshold of melanoma to MAPKi.



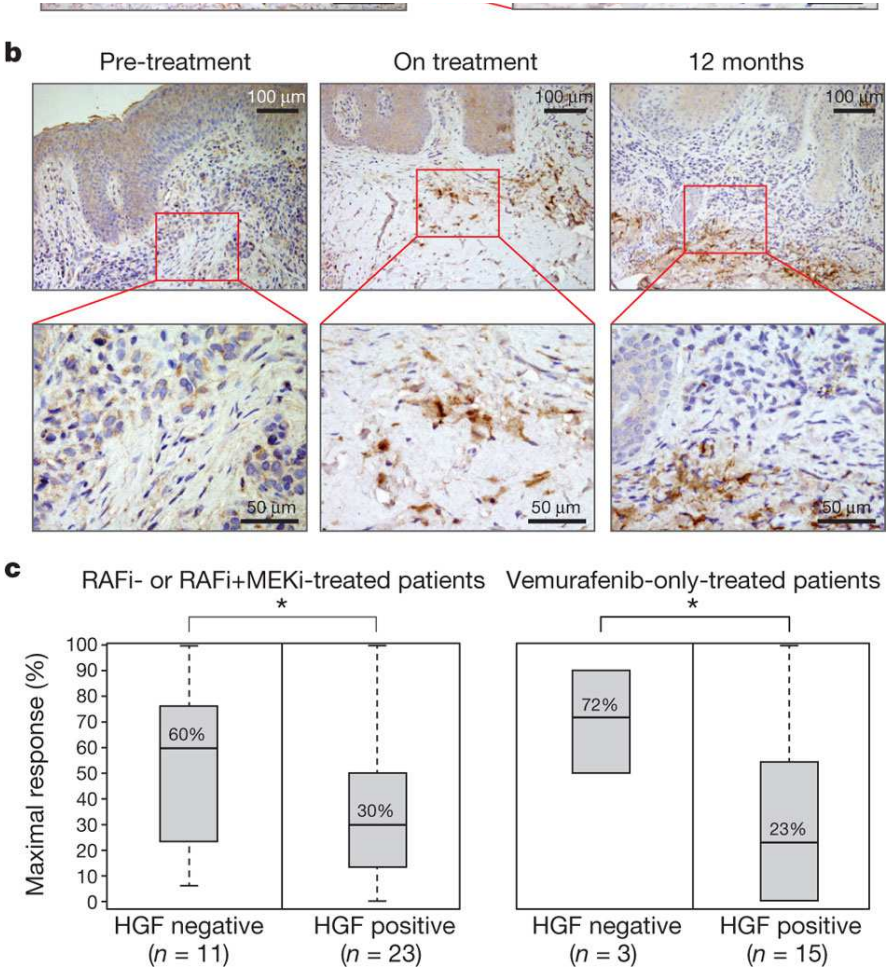
Hugo Cell 2015



Lin et al. Nat Genet 2015

HGF from stromal cells: poor response

- Stromal cell lines secrete HGF in co-culture with BRAFmut cell lines
- Innate resistance
- BRAFi+METi

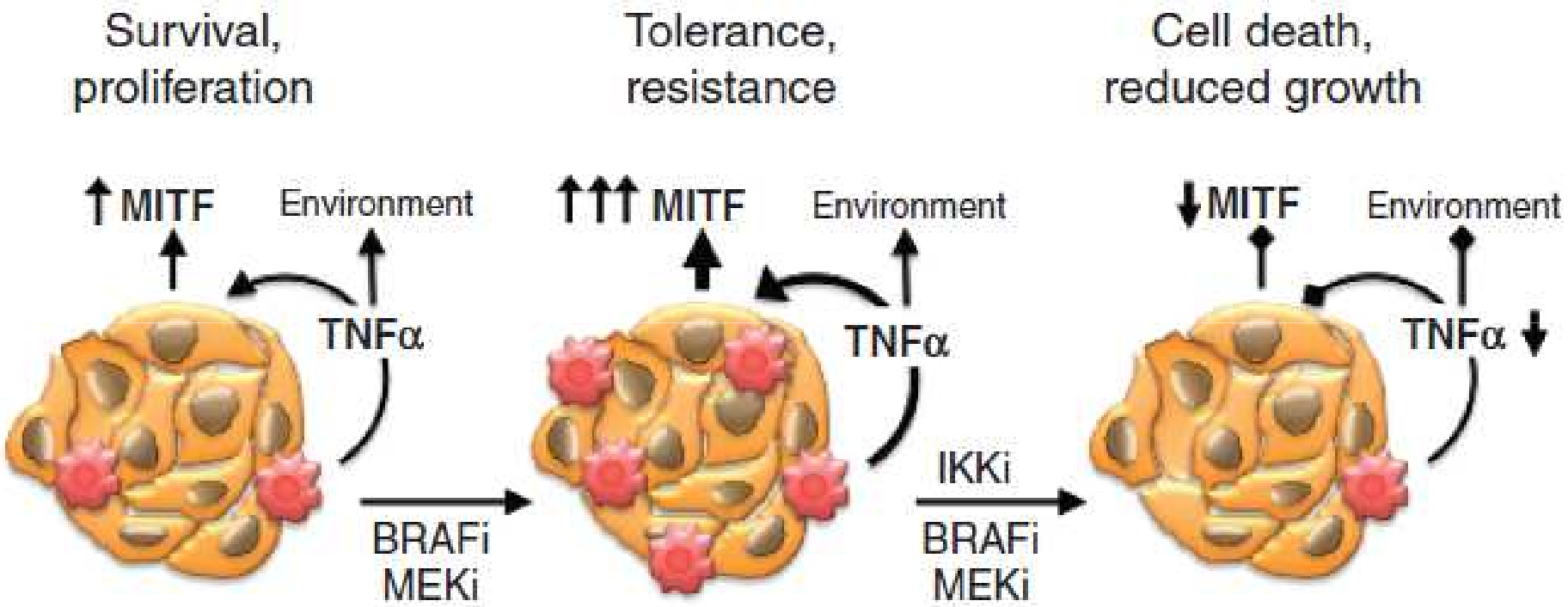


Straussman Nature 2012

Clinical trials

Drug	Mec	Combination	Status	Nº
INC280 (Nov)	cMETi	BRAFi/MEKi	Ongoing	Logic2

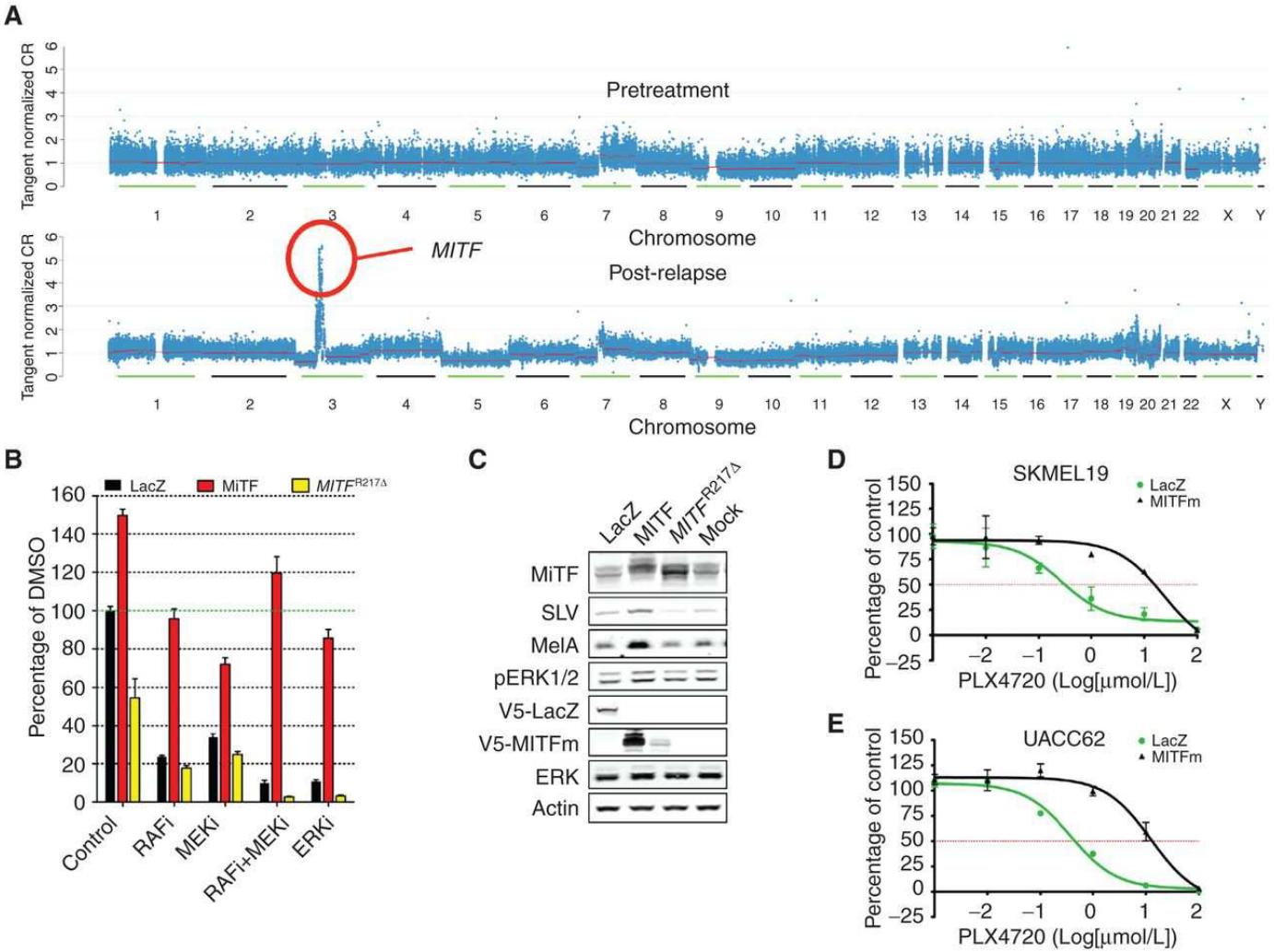
Macrophage derived TNF: MITF upregulation



MITF target BCL2A1 has been shown to antagonize BRAF inhibition

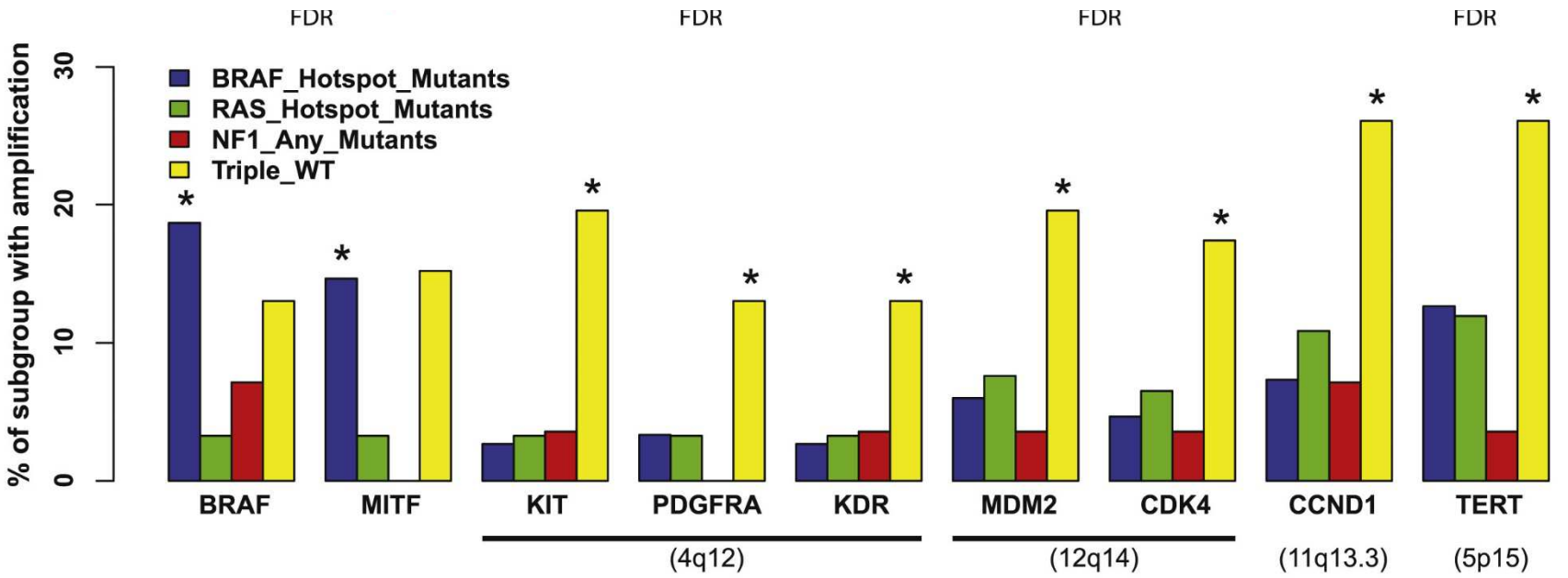
Smith et al. Cancer Discov 2014

Acquired MITF amplification: resistance to BRAFi, MEKi and ERKi



Van Allen E M et al. Cancer Discovery 2014;4:94-109

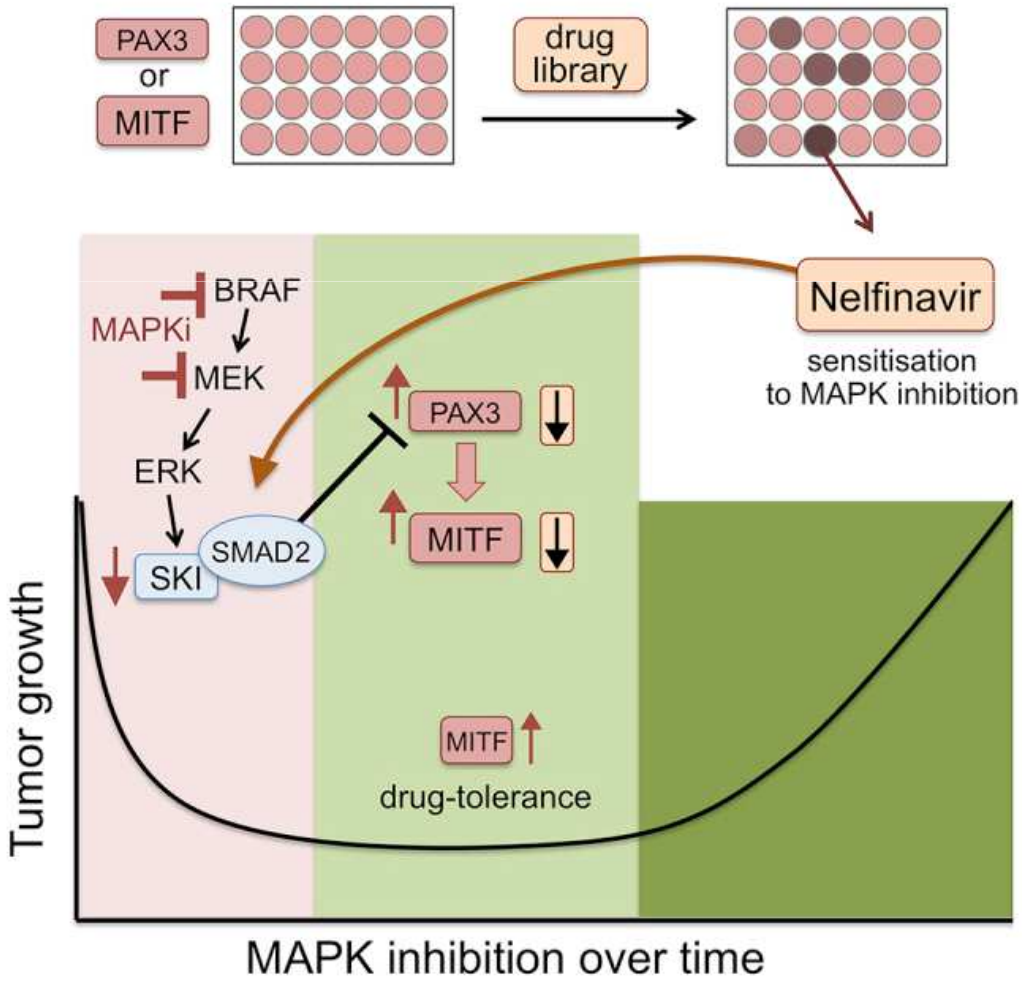
Amplifications



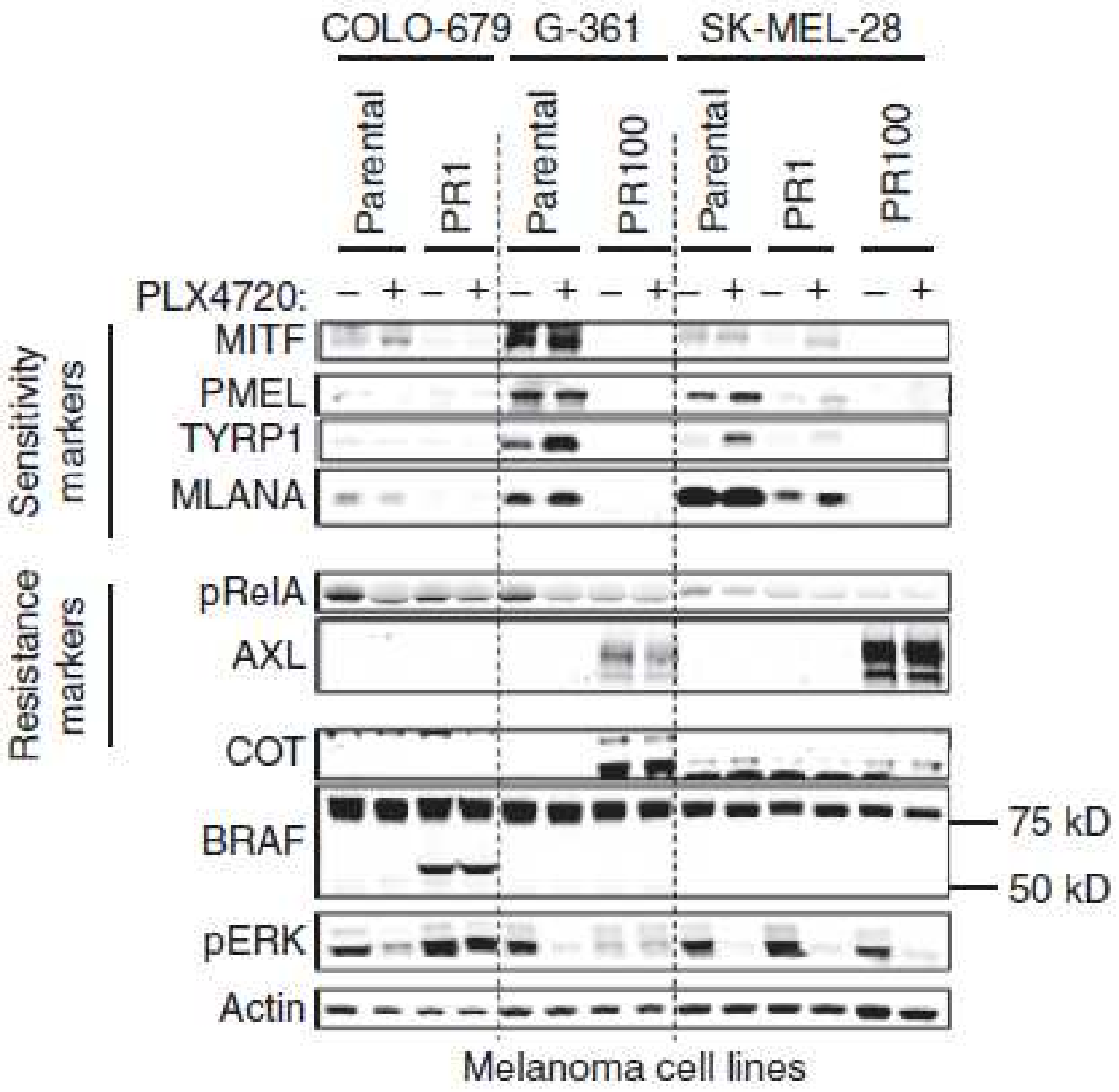
CGAN. Cell 2015

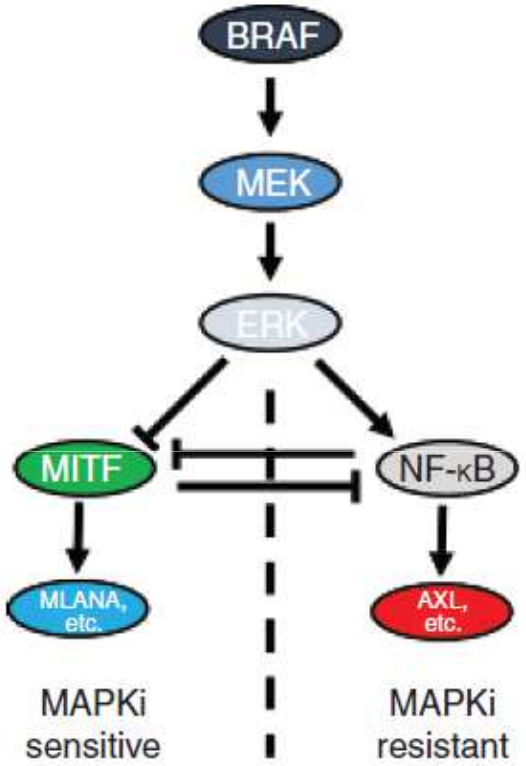
Drug repositioning identifies nelfinavir mesylate as a suppressor of MITF expression

PAX3-mediated
overexpression of MITF as a reversible
resistance mechanism to MAPK-pathway



Smith . Cancer Cell 2016





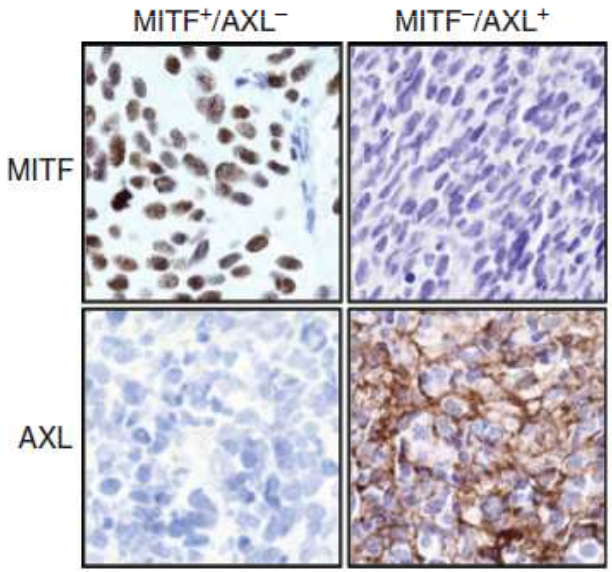
Intrinsically resistant cells showed diminished MITF expression and MITF transcriptional activity (as measured by levels of MITF target genes *TYRP1* , *MLANA* , and *PMEL* ; 4/4 lines) and increase of AXL expression (2/4 lines). *Intrinsic resistance is not simply related to AXL expression.*

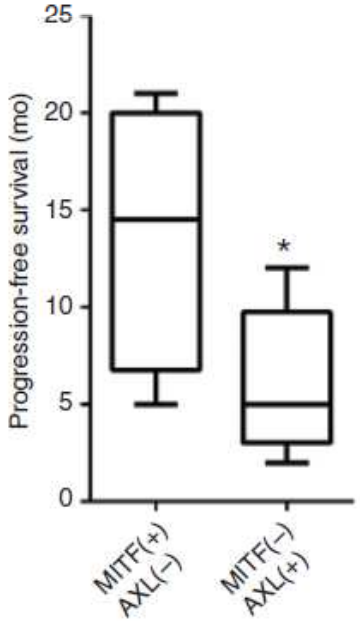
Konieczkowski et al. Cancer Discov 2014

Low MITF/AXL ratio predict early resistance to ERK inhibition

- MITF low/AXL high is common in BRAF and NRAS melanomas
- MITF low in naive melanoma predict high resistance
- Also Mitf low in some melanomas with adquired resistance:
  - they must be treated with **AXLi combined with MAPKi**

Mullerl et al. Nat Commun 2014





Konieczkowski et al. Cancer Discov 2014

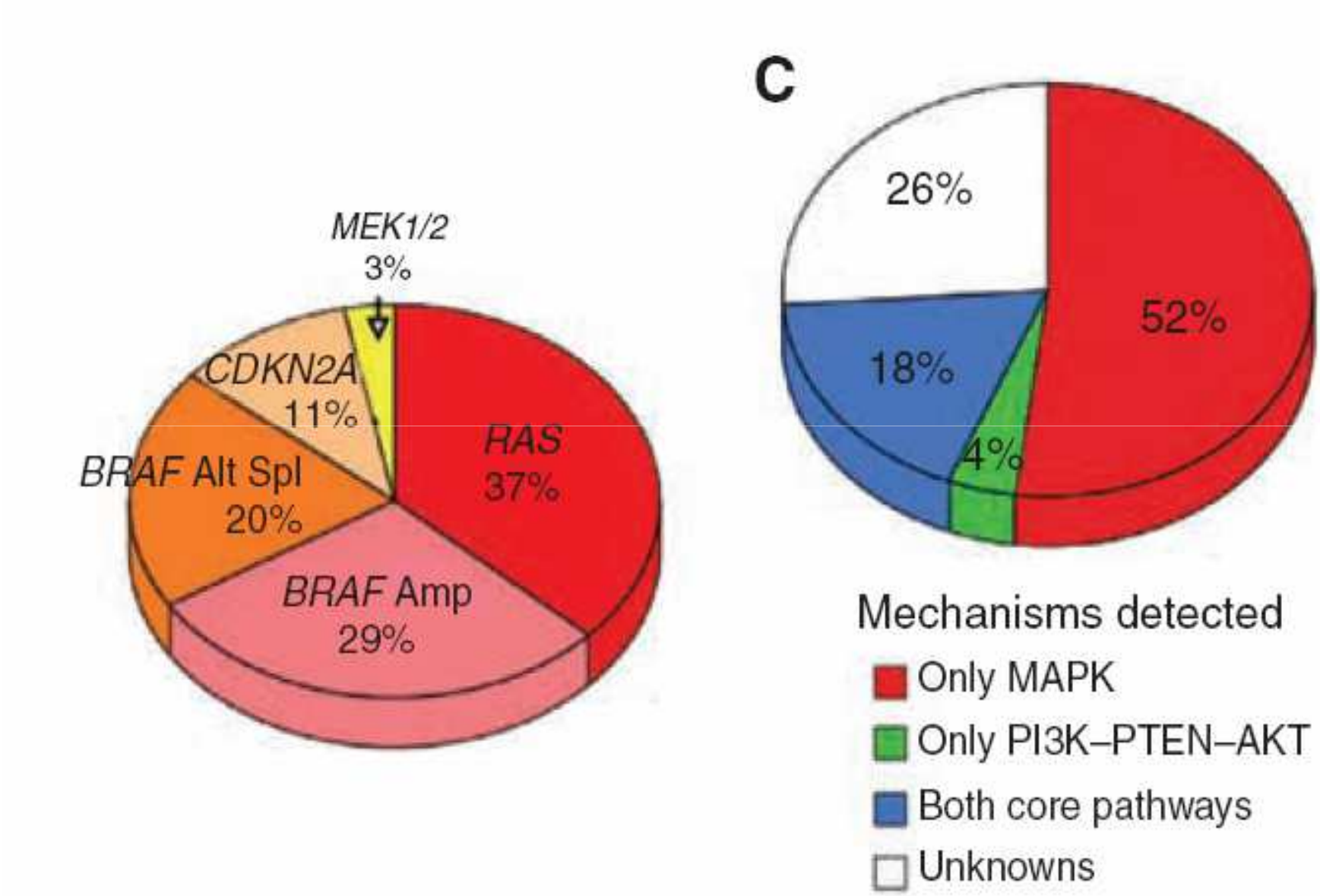
Clinical trials

Drug	Mec	Combination	Status	Nº
BMS345541	IKKi	-	preclinical	
cabozantinib	AXLi	-	No trials in cutaneous melanoma	
Foretinib (GSK1363089)	AXL/METi		Completed without results	
BMS777607	AXLi		preclinical	
Bosutinib (SKI606)	AXLi		preclinical	
MGCD265 MGCD516	AXLi		preclinical	

Clinical trials

Drug	Mec	Combination	Status	Nº
Nelfinavir	PI3Ki/Radisens	RT/TMZ	Ongoing GBM/NSCLC	

3. Acquired resistance

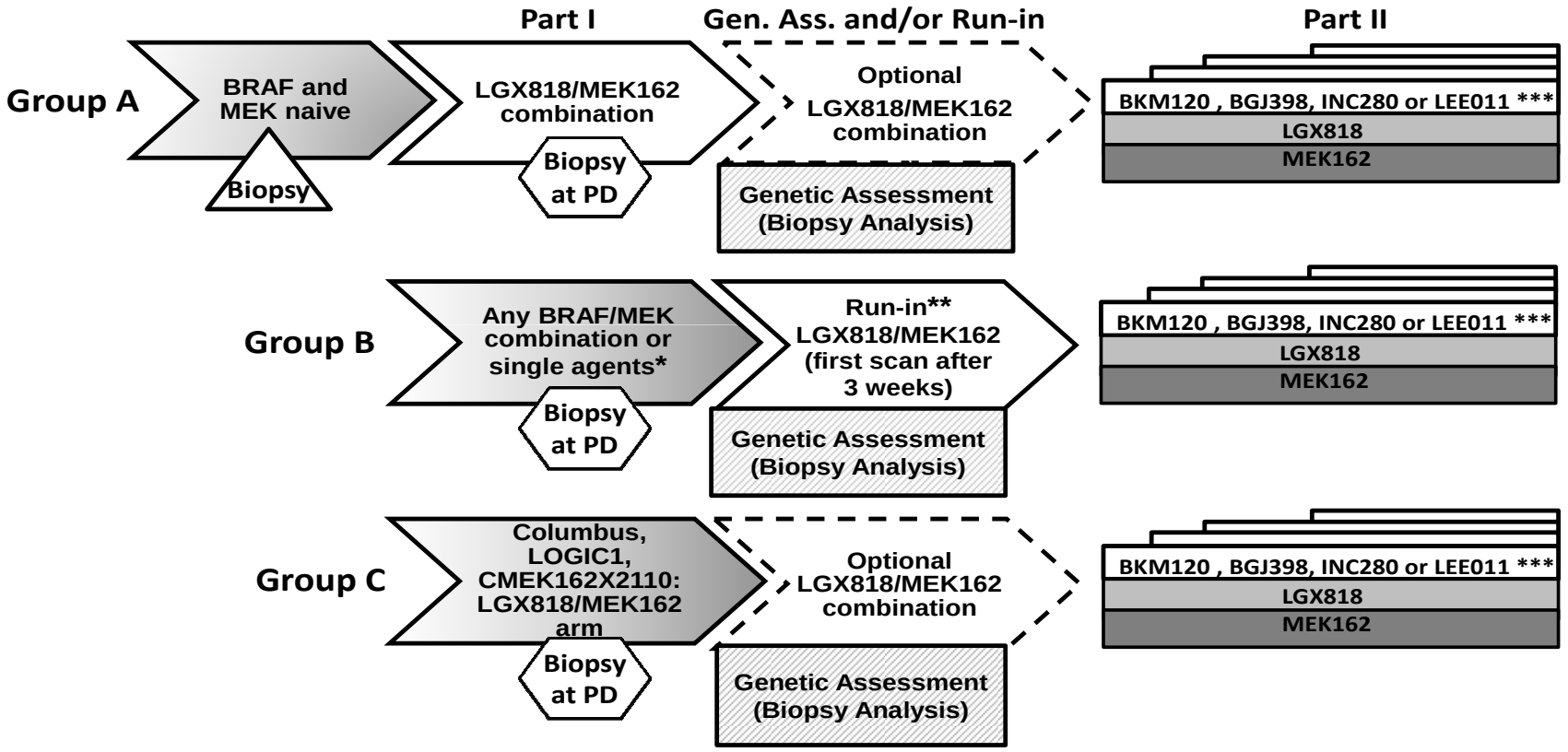


Shi H. Cancer Discovery 2013 Nov

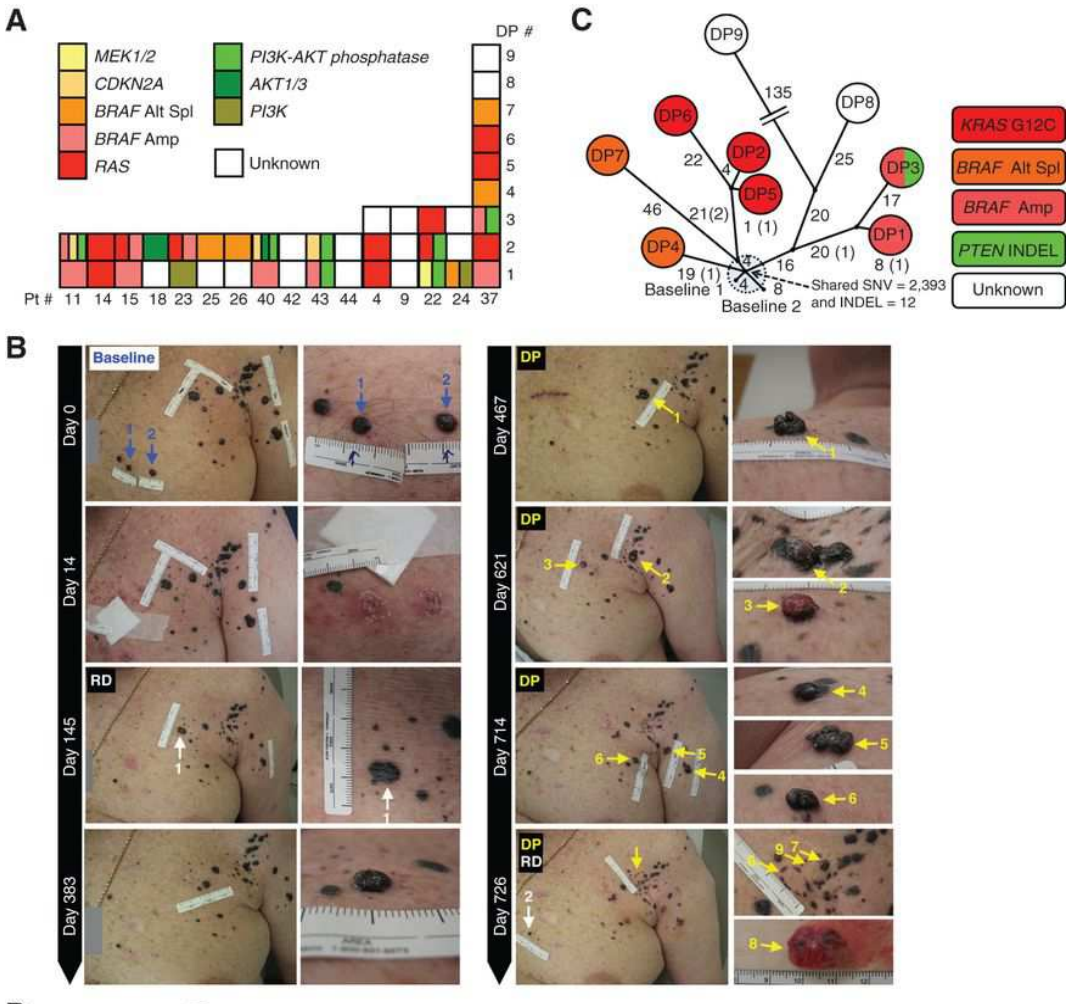
3. Acquired resistance

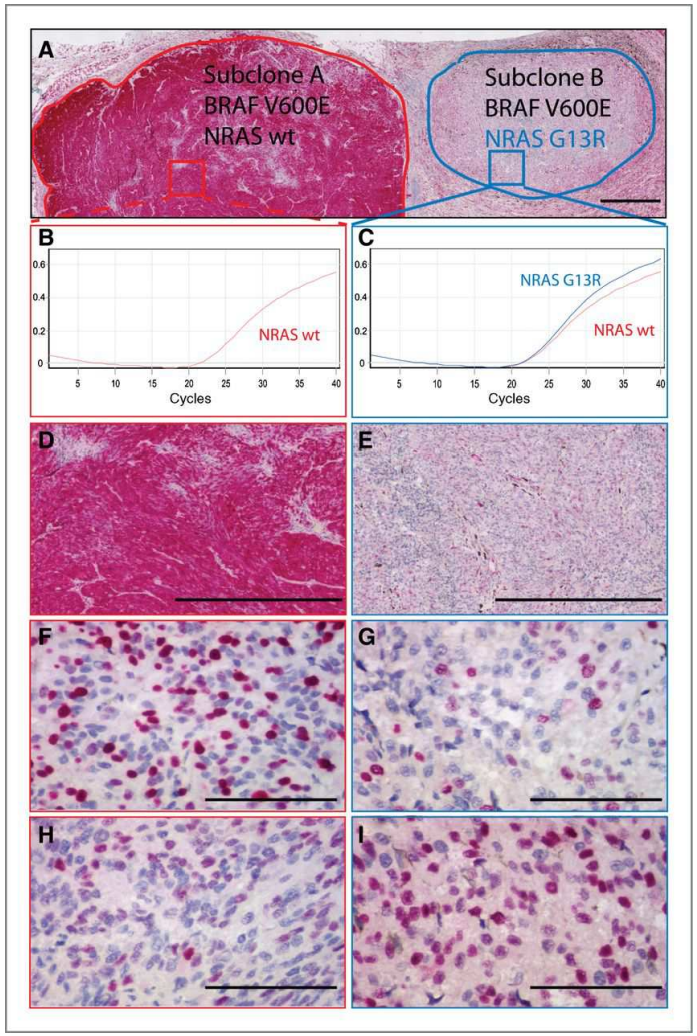
	%	Primary/acquir	Treatment
NRAS mut	15	P/A	Mek+simvastatin, Meki+ CDK4i, MEKi+EGFRi, MEKi+abt263, smvastatin+flavopiridol, simvastatin+CDK4i, MEKi+PI3Ki PLX7904, ERKi
Braf ampl	15	A	BRAFi intemitente , Dosis de braf mayor, ERKi
Splicing	20	A	ERKi+ BRAFi, drug holidays, higher doses of BRAFi or MEKi, PLX7004
MEK mut (NO P162S)	15	A/P	ERKI (SCH7729 MERK)
NF	**2**	A/P	MEKi+MTORi, CRAFi+BRAFi, panRAFi (AZ628), ERKi
COT sobre	**?**	A/P	-
IGFR,	**4/11**	A	MEKi+IGFRi, BRAFi+IGFRi, MEKi+ AKTi or mtori or pi3ki, HSP90i
EGFR, pdgfr	**6/16**	A/P	Dasatinib, AKTi+EGFRi, (¡?), braf+pi3ki Holidays, HSP90i
FGFR3	**?**	A	MEK+FGFRi
PTEN loss, or RB inact	**10-30**	P/A	BRAFi+everolimu BRAFi+PI3Ki
ERB2, ERB3	**?**	A/P	BRAFi+Lapa ¿?
MED12	**?**	Mediator A	BRAFi+TGFBi (YR-290)
BCL2A1-MITF	**?**	A(P	R to ERKi, BRAFi, MEKi; S BRAFi+bcla2i, obatoclax

LGX818, MEK162, BKM120, BGJ398, INC280, LEE011 (LOGIC 2)



Heterogenity intratumoral/intrapatient





Shi H et al. Cancer Discovery 2014; Wilmott 2012; Van Allen 2014; Romano 2014; Turajlic 2014

BRAF/MEK acquired resistance: how to overcome it?

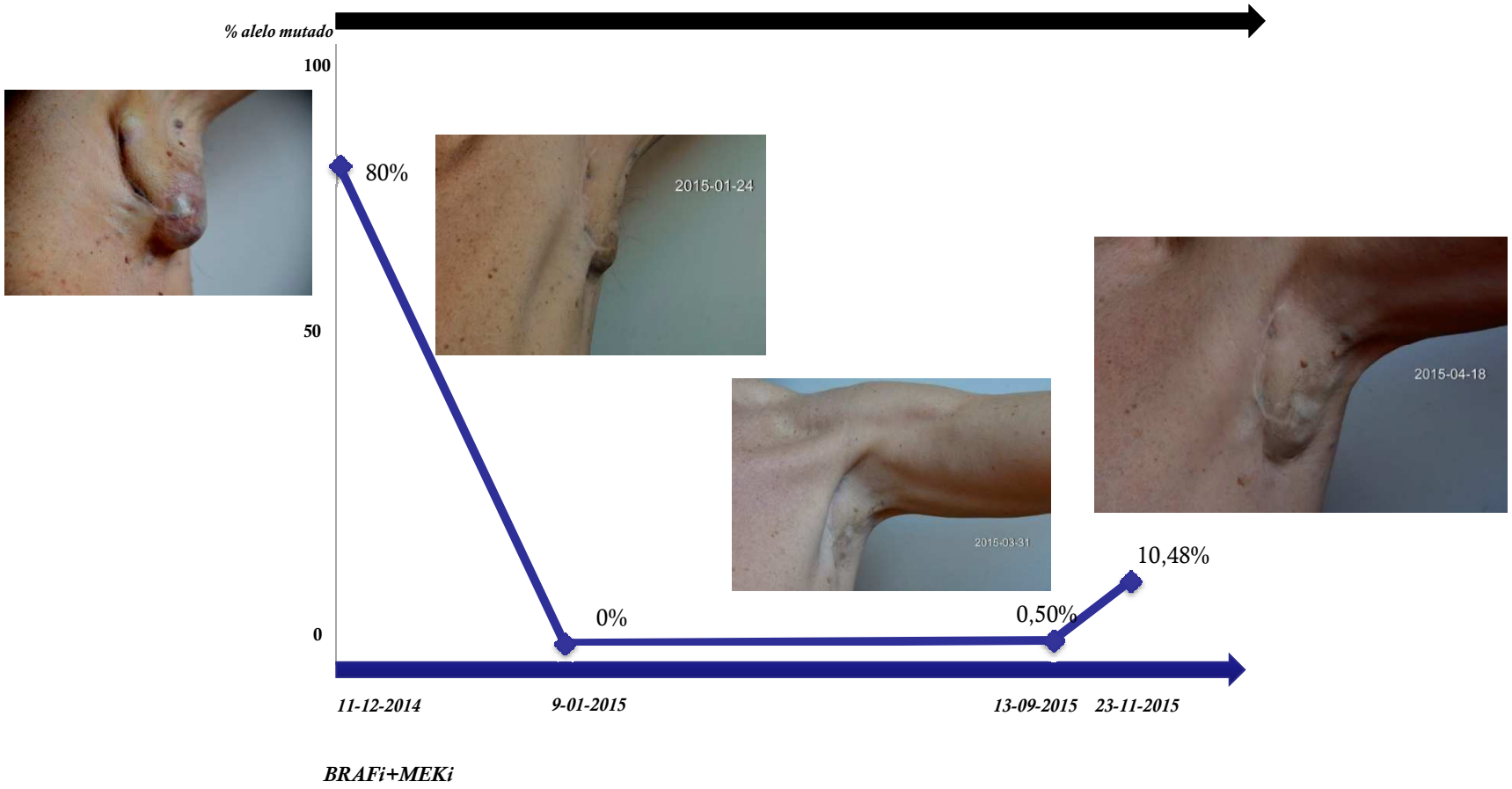
3.1. Identification of the most relevant mechanism of resistance: cfDNA analysis

3.2. Triple combinations in first line setting: trying to improve PFS

3.3. Deloyed adaptative resistance: on/of Schedule, other combinations?

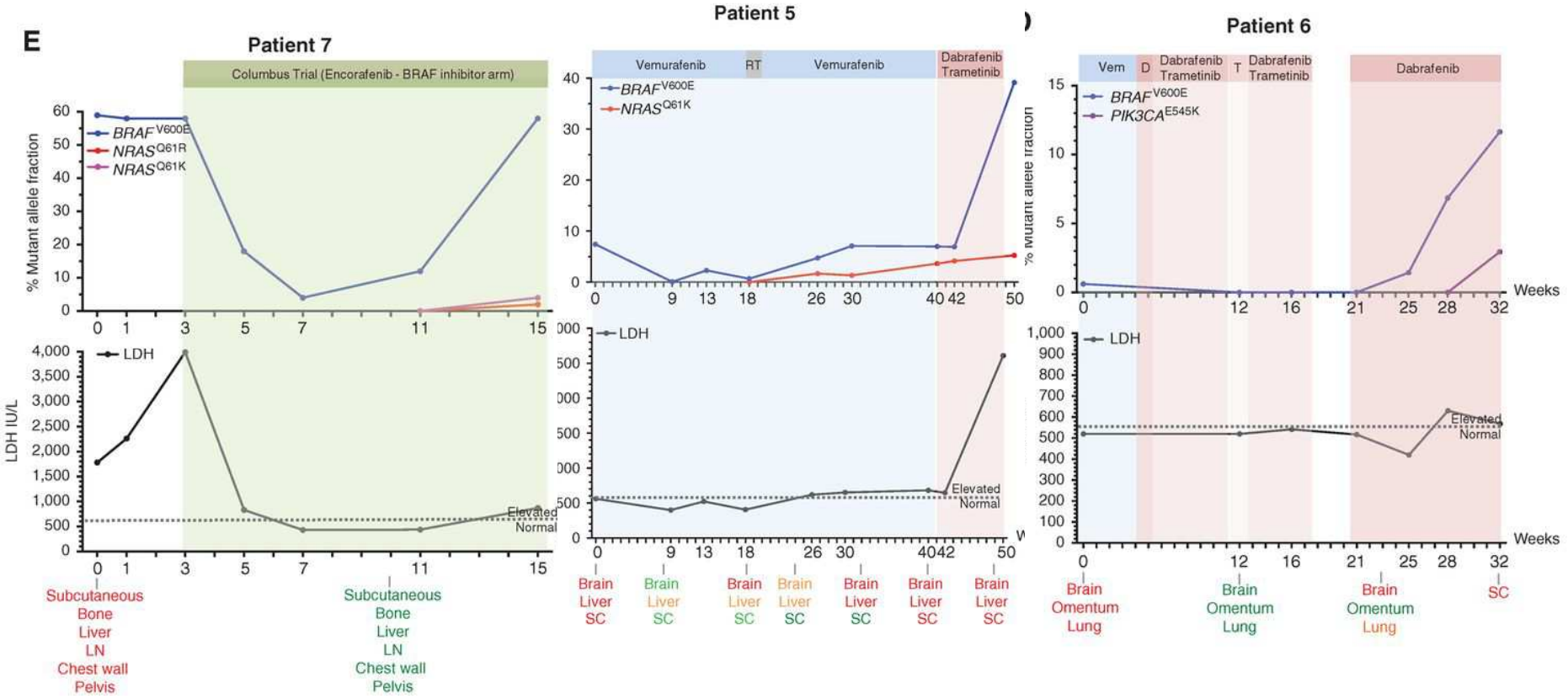
3.4. Using at progression drugs with a broader spectrum of inhibition

3.1 cfDNA as a tool for selecting the targeted drug



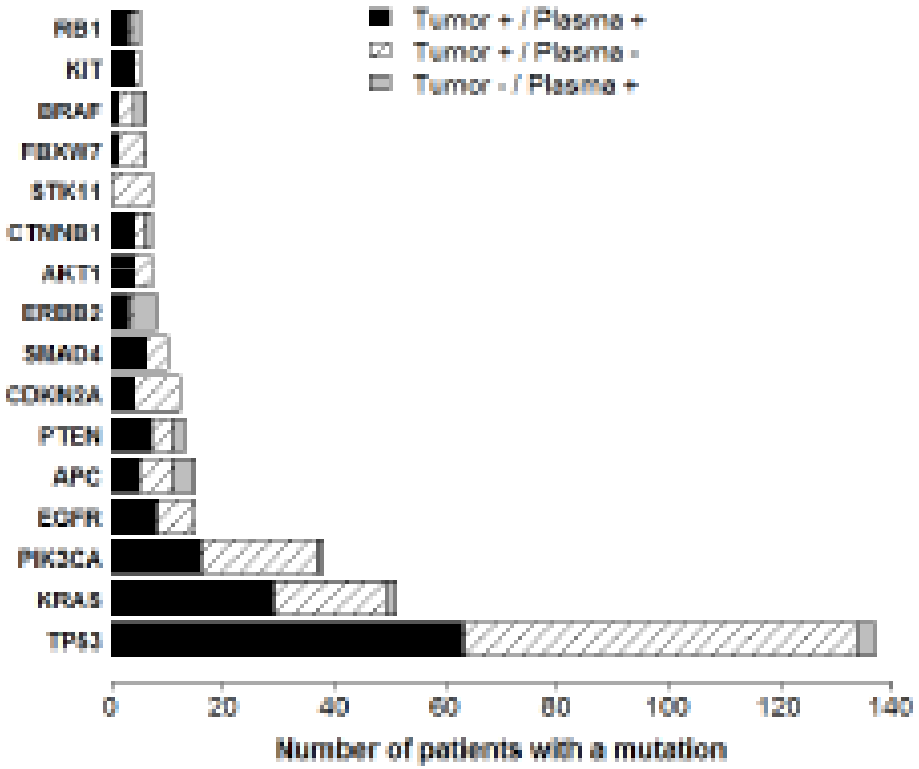
Gonzalez-Cao 2015

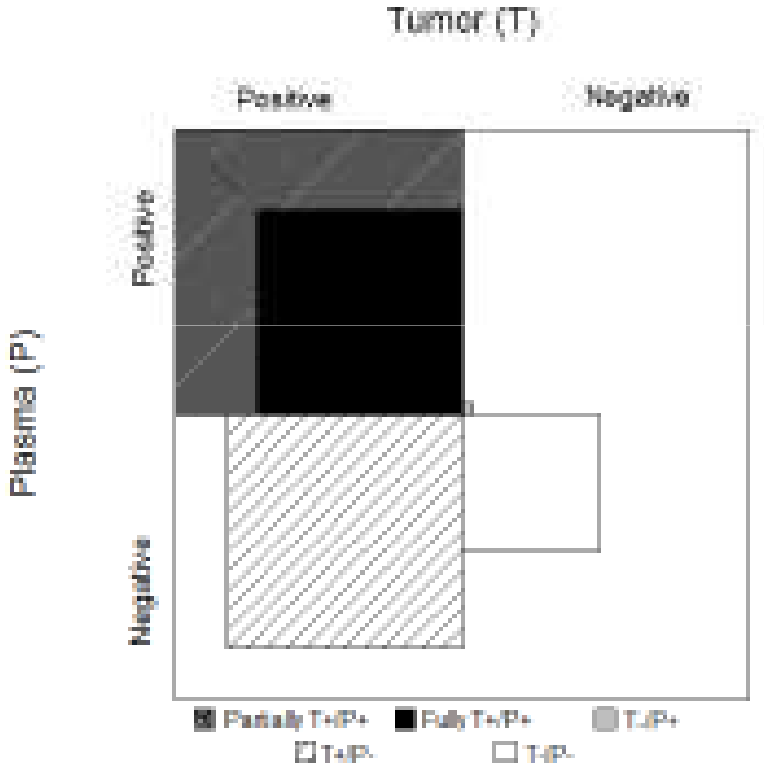
ctDNA can be used to monitor patient response



Maria Romina Girotti et al. Cancer Discov 2016;6:286

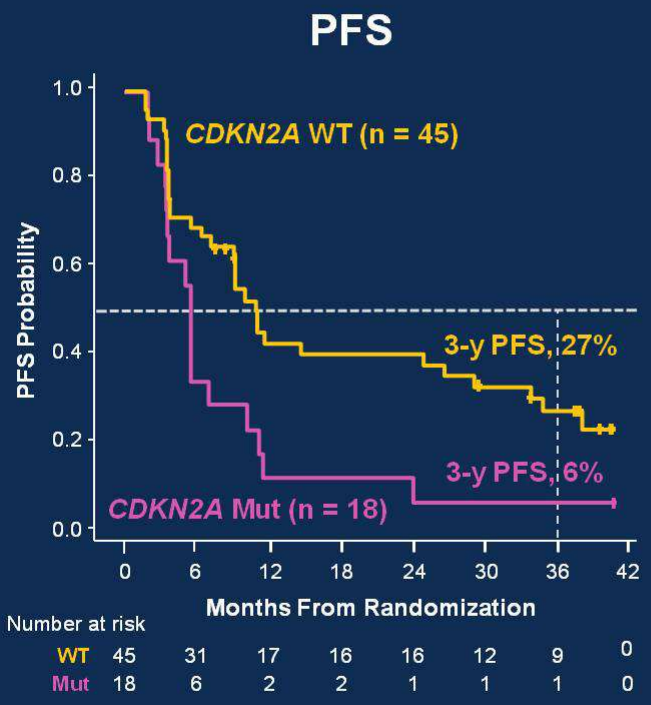
cfDNA next-generation sequencing in MOSCATO trial

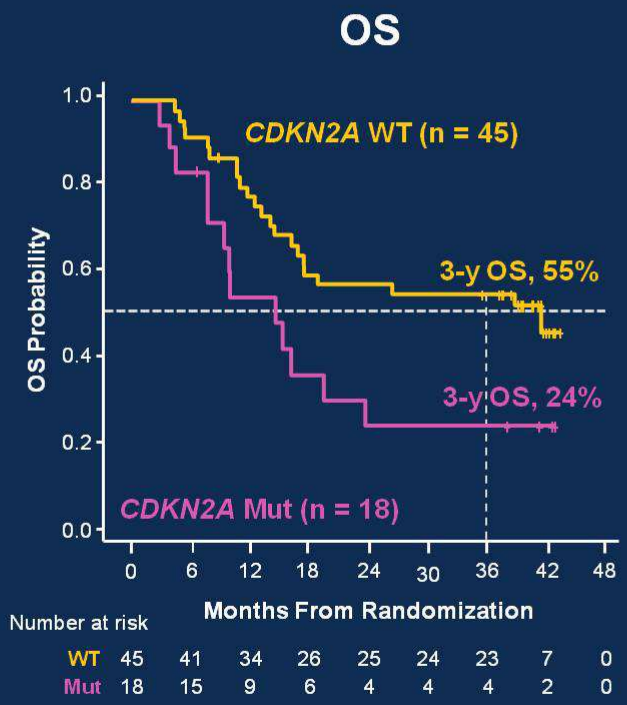




Jovelet. Clin Cancer Res 2016

COMBI-d: *CDKN2A* Loss in the Dabrafenib + Trametinib Arm

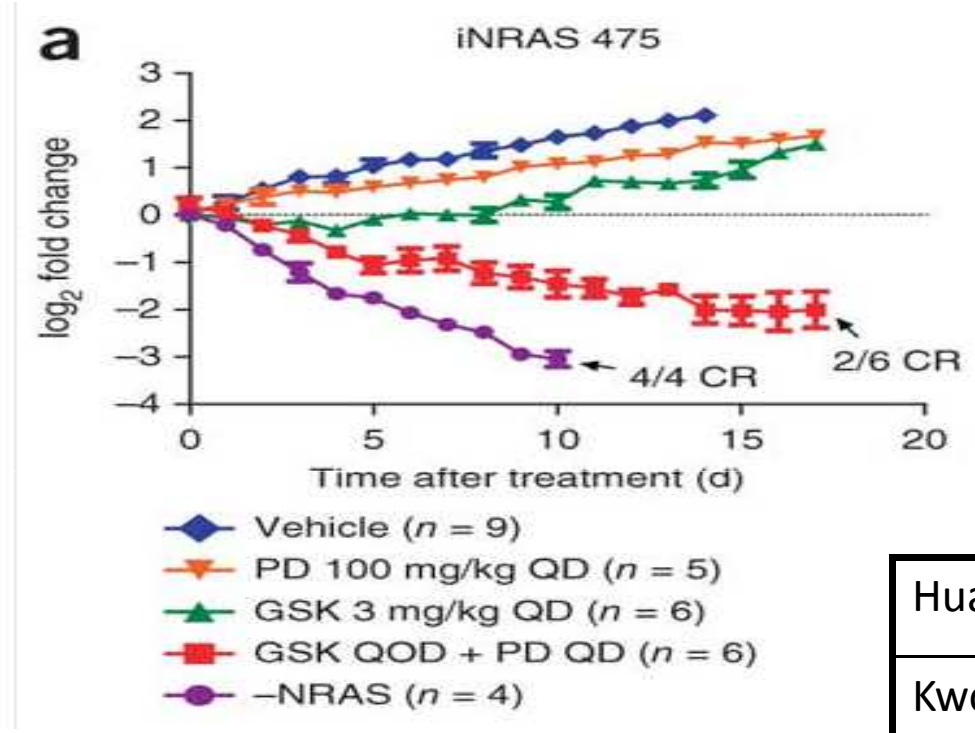




- *CDKN2A* mutation and deletion were significantly associated with poorer OS ($P = 0.027$[a]) and PFS ($P < 0.001$[a])
- Preclinical data suggest that combination with CDK4/6 inhibitors could be a beneficial strategy

[a] Cox proportional hazards *P* value; +, censored.

NRAS treatment:
PD-0332991(CDk4/6i)+GSK 1120212(MEKi)



CR in 33% of mice

Kwong. Nature 2012

Huang[1]	**Simvast+Falvop/MEKi**
Kwong[2]	**MEKi+CDK4i**
Corcoran[3]	**MEKi+abt-263 (bcl-xli)**
Posch[4]	**MEKi+PI3Ki**
Greger[5]	**MEKi+BRAFi+PI3Ki**
Means-Powell[6]	**METi+Sorafenib**

1.Can Discovery 2013
2.Nature 2012
3.Cell 2013
4.PNAS 2013
5.Mol Cancer T 2012
6. ASCO 2012

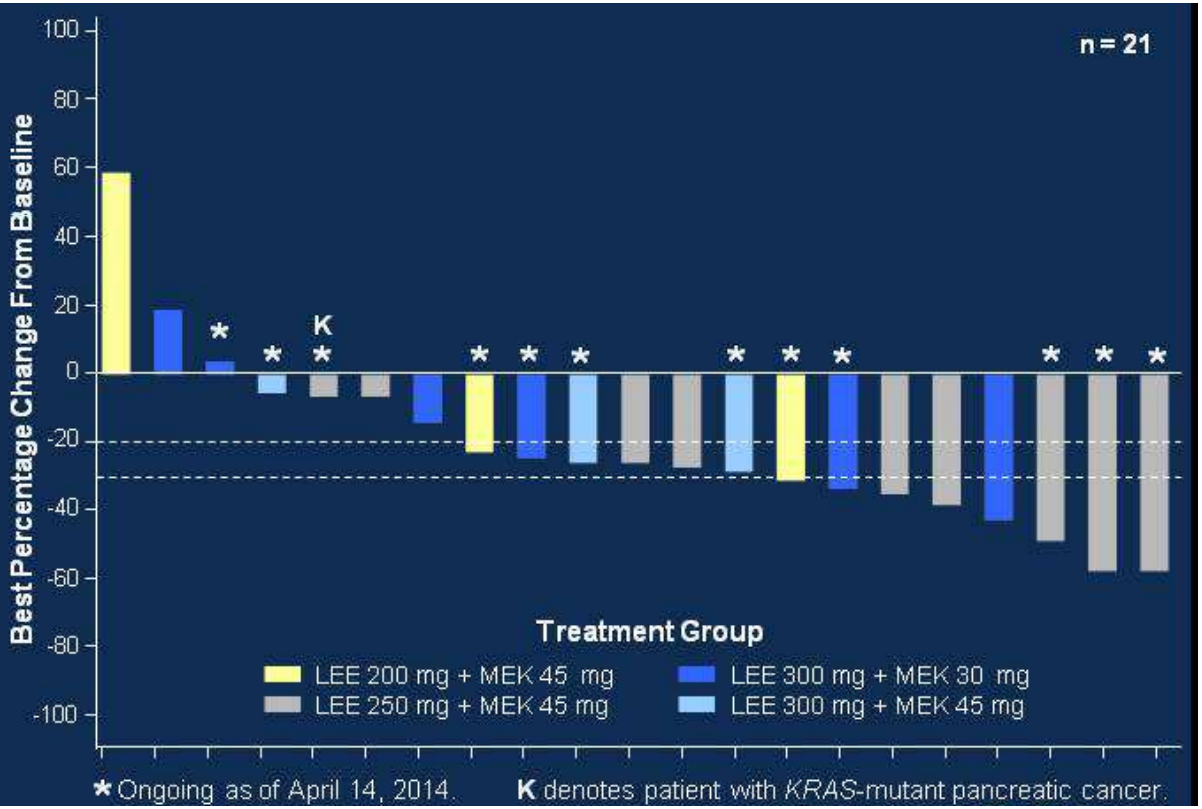
Nazarian. Nature 2010; McCarthur 2011; Ribas, JCO 2013; Van Allen, 2014

A phase 1b/2 study of LEE011 in combination with binimetinib (MEK162) in patients with *NRAS*-mutant melanoma

CDK4/6i (LEE011)+MEKi (MEK162)

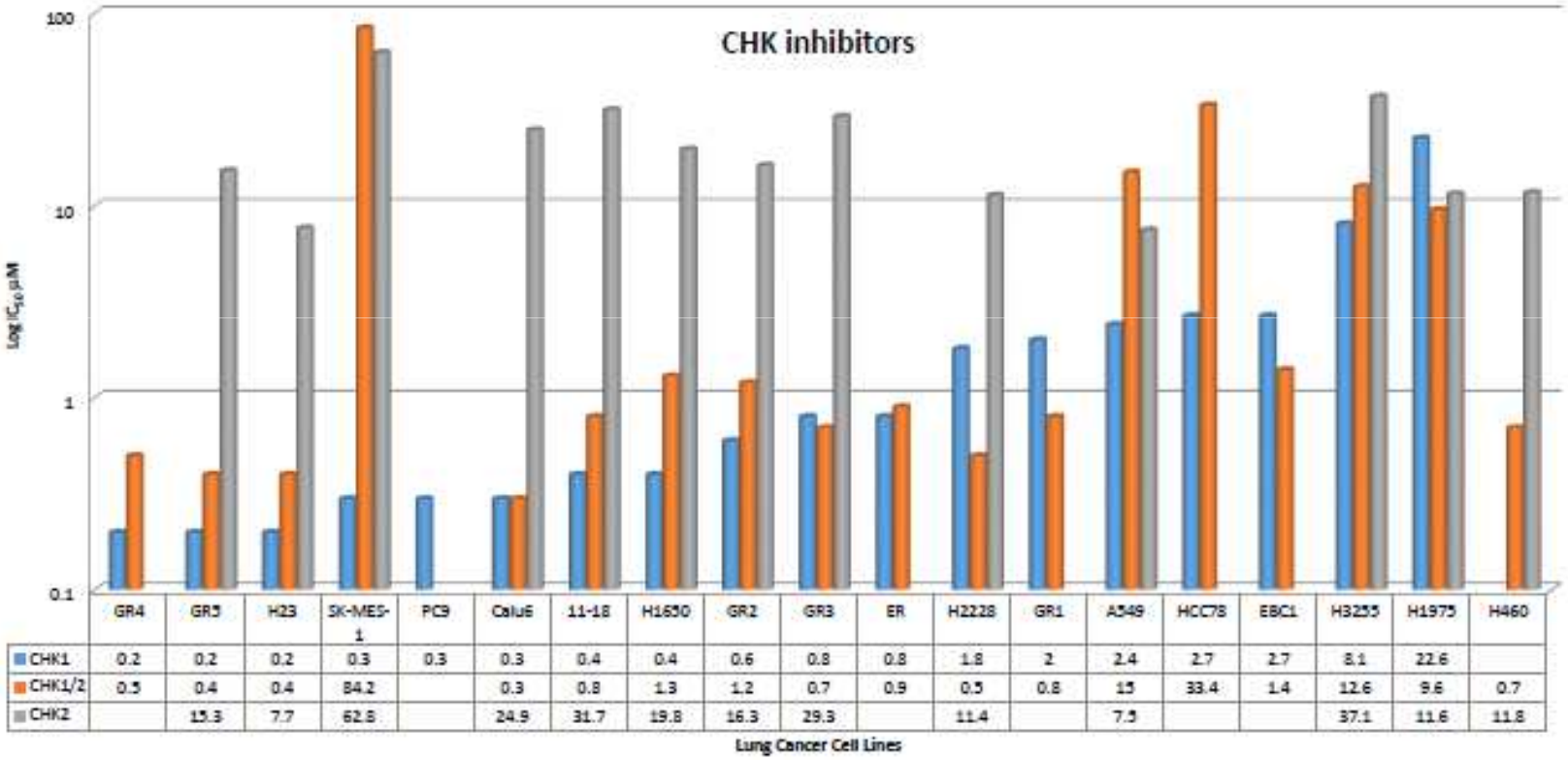
OR 82% (N 22)

Response, n (%)	LEE011 + Binimetinib (N = 22)
Complete response (CR)	0
Partial response (PR) Confirmed PR Unconfirmed PR	7 (32) 3 (14) 4 (18)
Stable disease (SD)	11 (50)[a]
Progressive disease	3 (14)
Unable to evaluate	1 (4)



Sosman. ASCO 2014, abs 9009

CHK1 inhibitors in RAS mutant cell lines



	GR4	GR5	H23	SK-MES-1	PC9	Calu6	11-18	H1650	GR2	GR3	ER	H2228	GR1	A549	HCC78	EBC1	H3255	H1975	H460
CHK1	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.6	0.8	0.8	1.8	2	2.4	2.7	2.7	8.1	22.6	
CHK1/2	0.5	0.4	0.4	84.2		0.3	0.8	1.3	1.2	0.7	0.9	0.5	0.8	15	33.4	1.4	12.6	9.6	0.7
CHK2		15.3	7.7	62.8		24.9	31.7	19.8	16.3	29.3		11.4		7.5			37.1	11.6	11.8

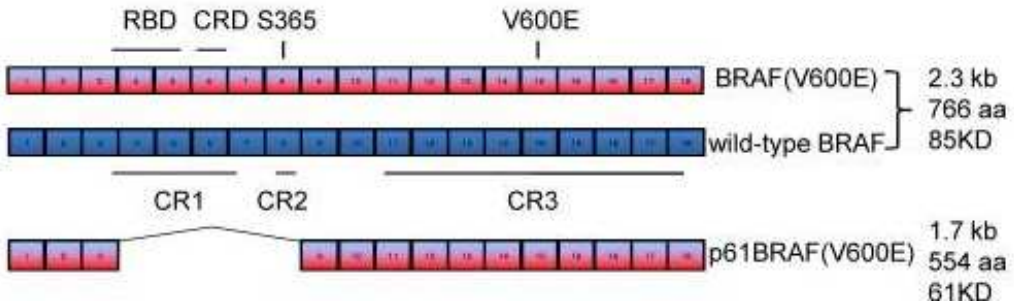
Morales D, Rosell R et al. ASCO 2016

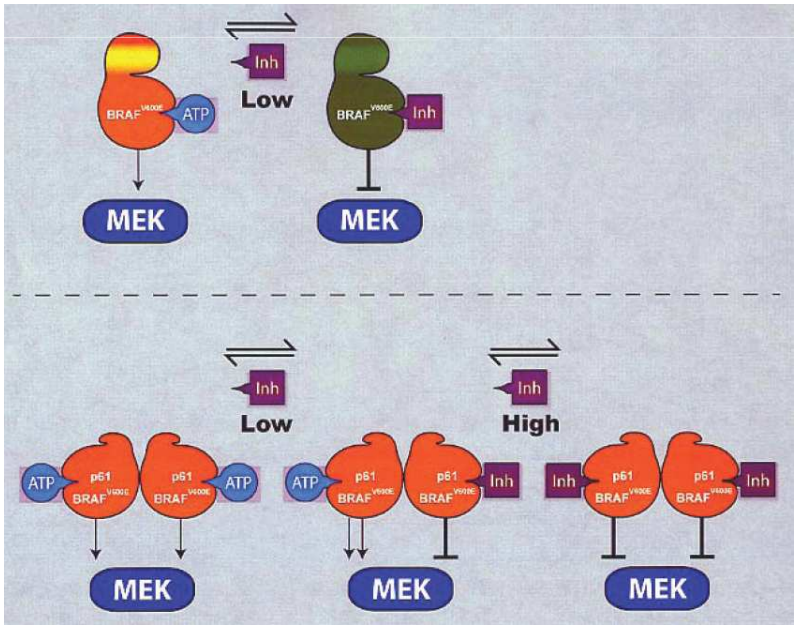
Clinical trials

Drug	Mec	Combination	Status	Nº
LEE011	CDK4/6	BRAF/MEKi	Ongoing	2011-005875-17 LOGIC-2
Abemaciclib (LY2835219)	CDK4/6	single	Ongoing Phase II	NCT02308020
SAR245409	PI3Ki/mTORi	pimasertib	Completed 2011	NCT01390818
BKM120	PI3Ki	BRAFi	Unknown 2013	NCT01512251

Splicing forms p61BRAFV600

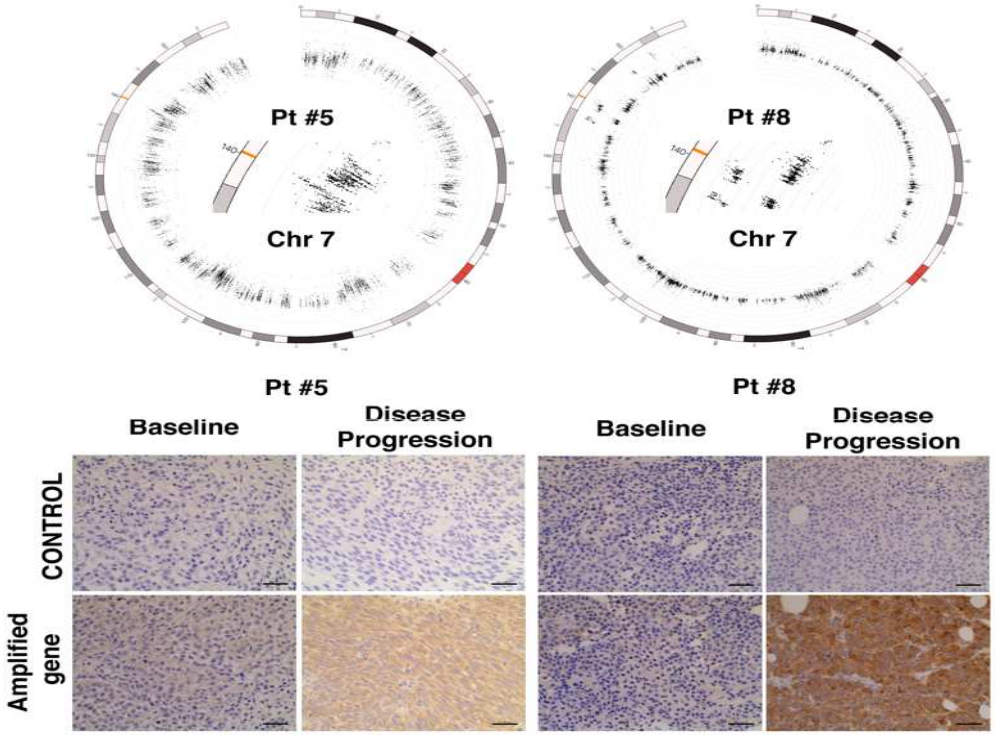
- **6/19** patients: 61kd variant form of BRAF(V600E) that lacks exons 4-8, a region
- It could be sensible to higher dose or combination, unless it is also observed in pts with combined treatment (Hartsoughet al , 2014)





Poulikakos P. Nature 2011

Whole exome sequencing identified *B-RAF*^{V600E} amplification



4/20 patients

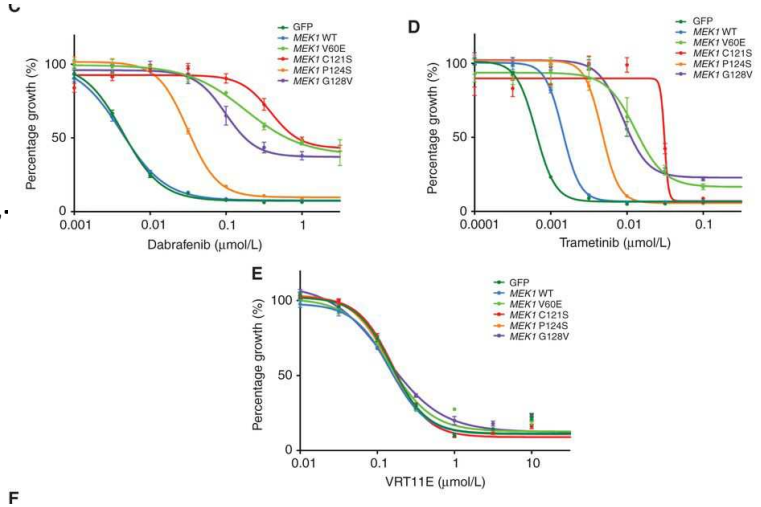
In vitro testing: growth inhibition could be achieved with higher dose of BRAFi

Shi. Nature 2012, Thakur 2013

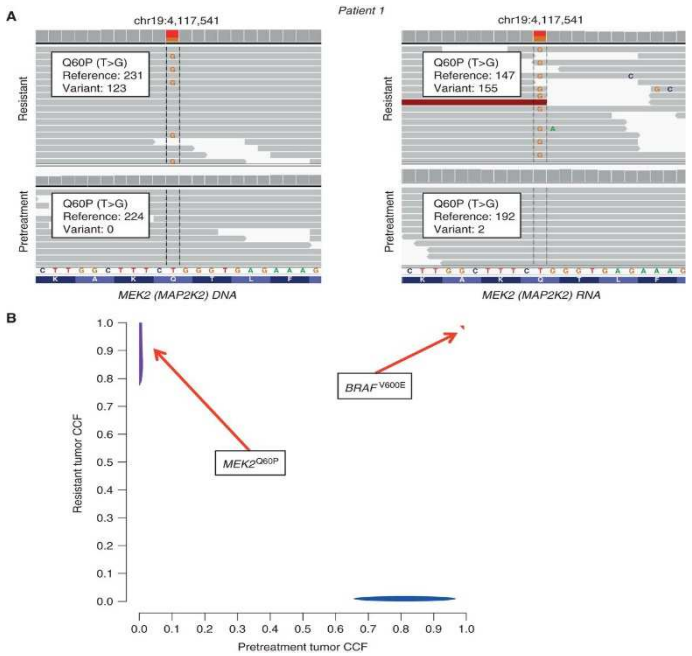
MEK1/2 mutations

- *MEK1*^{P124} mutations co-occur at low frequency (~ with *BRAF*^{V600} mutations. Pre therapy MEK1 codon 124 **6/87**; OR 2/6 (Sosman 2012)
- MEK1Q56P (1 pts to BRAFi)(Trunzer, 2013).
- MEK1P162S (1/5 pts): no gives resistance
- *MEK1* mutation at progression **3/20** cases: sensituve t combination
- MEK2 Q60P (1/5 pts): resit combination but sensible to ERKi (Wagle, 2014)
- MEK2 C125S (R to BRAFi and MEki);
- MEK2 V35M and L46F and N126D

(also resistance to BRAFi or MEKi, but no so intense as C125S (Van Allen 2014)



Van Allen et al. Cancer Discovery 2014



Wagle N et al. Cancer Discovery 2014

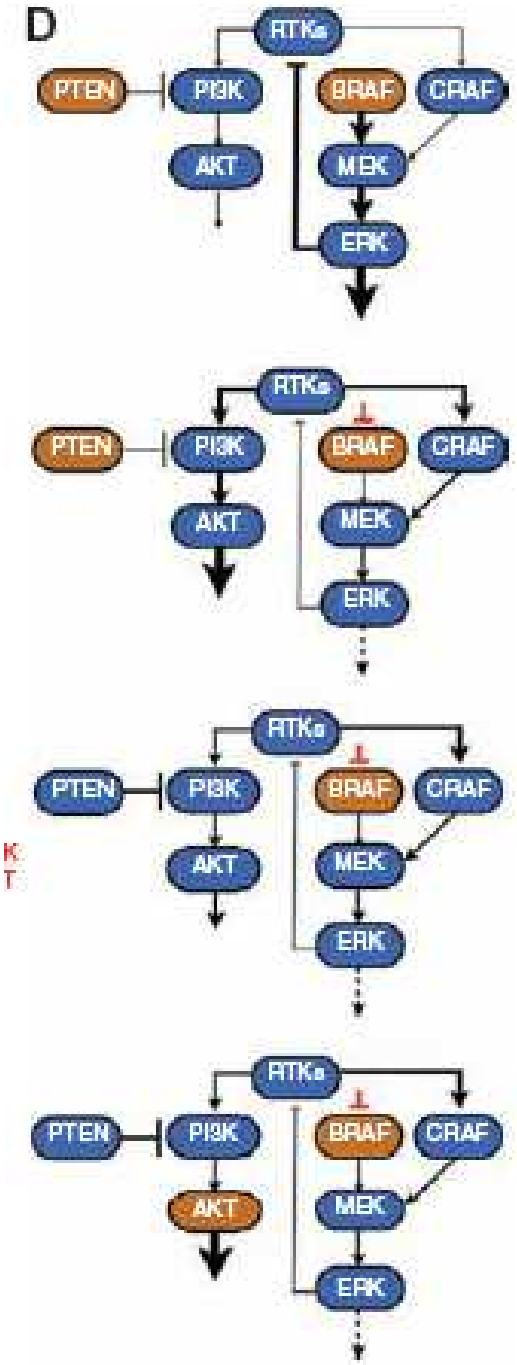
AKT1 mut (Q79K)

Acquired resistance to BRAFi in PTEN wild type

Adaptative resistance: early rebound of AKT pathway in wild PTEN null or PTEN wild but AKT mutant

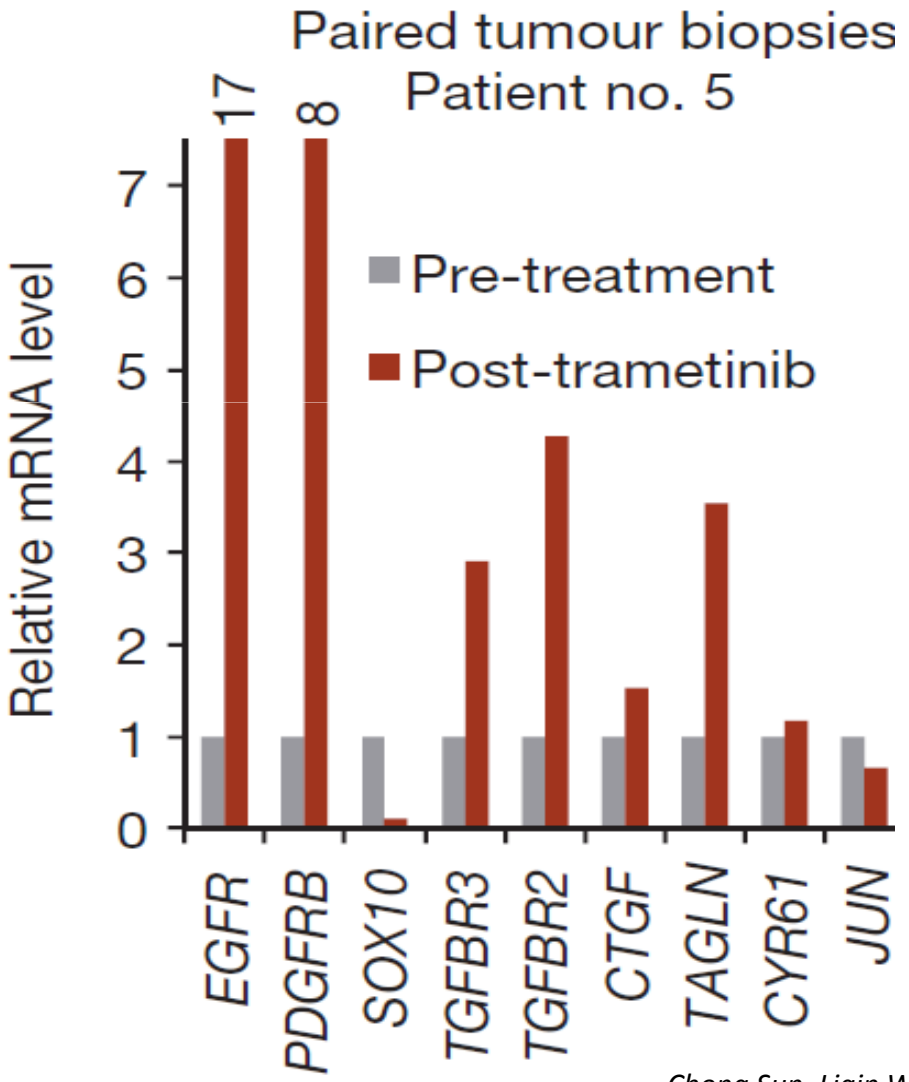
Early rebounf of PDGFRB (not EGFR) and downregulation of MYC (adaptative AKT signalling)

In melanomas without early adaptative AKT upregulation is more frequent late resistance by AKT than in melanomas with early AKT rebound



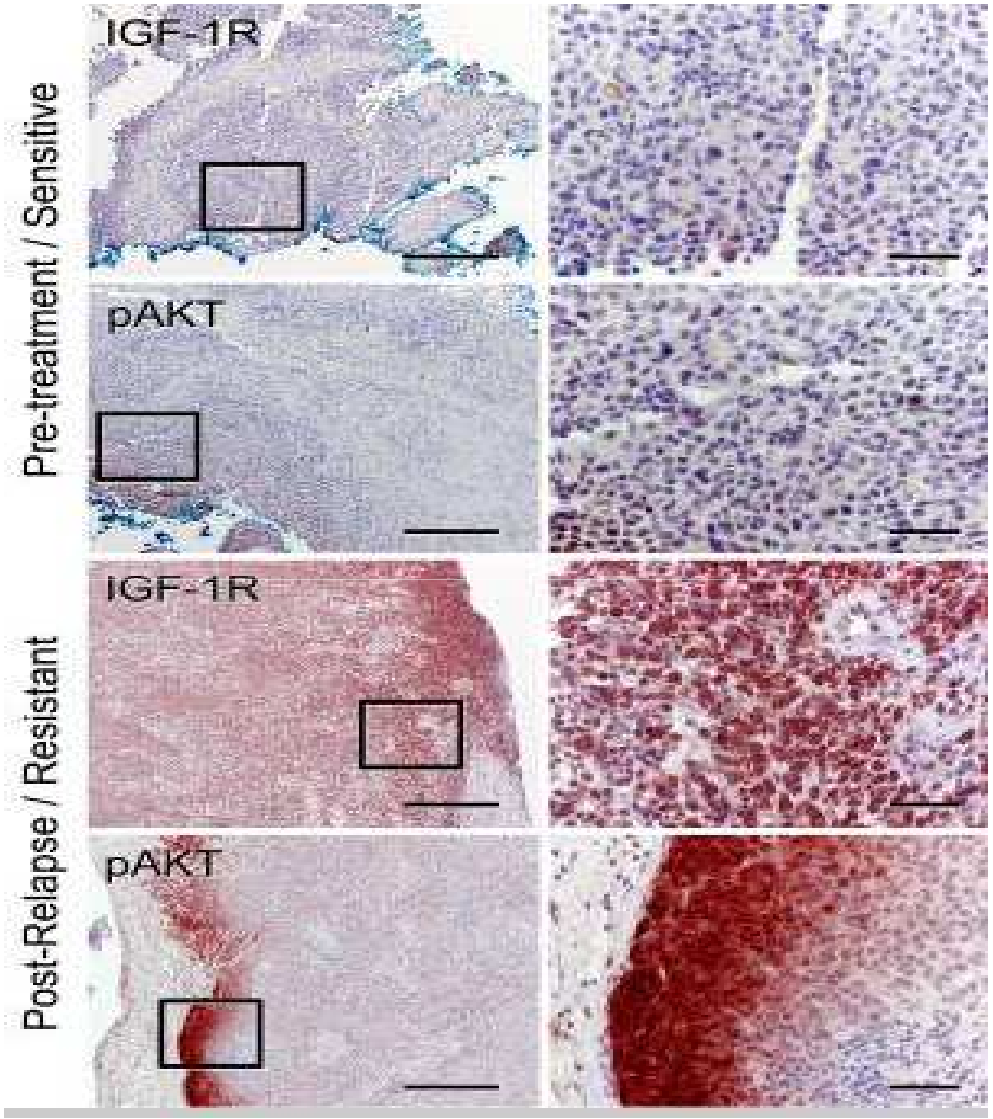
Shi H, Ribas et al C Diiscovery 2014:4: 69

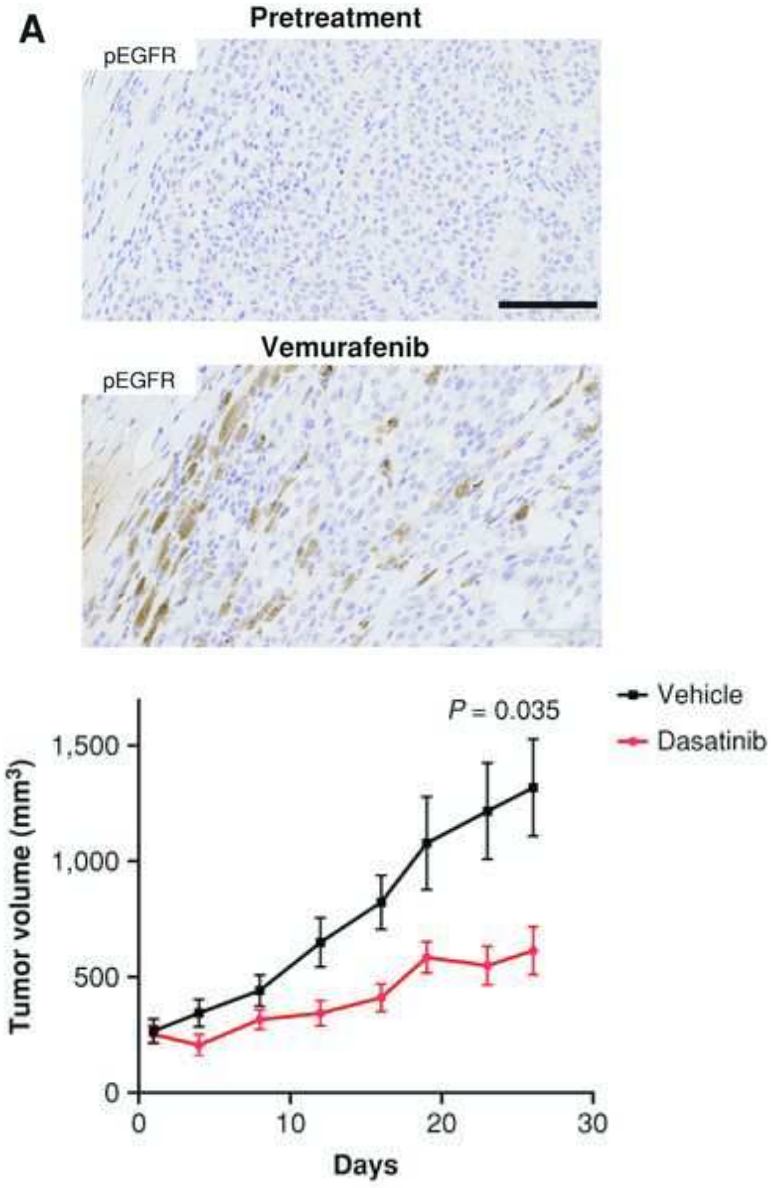
SOX10 TGFB signaling increases EGFR and PDGFRB expression



Chong Sun, Liqin Wang et Sidong Huang et al Nature. 2014

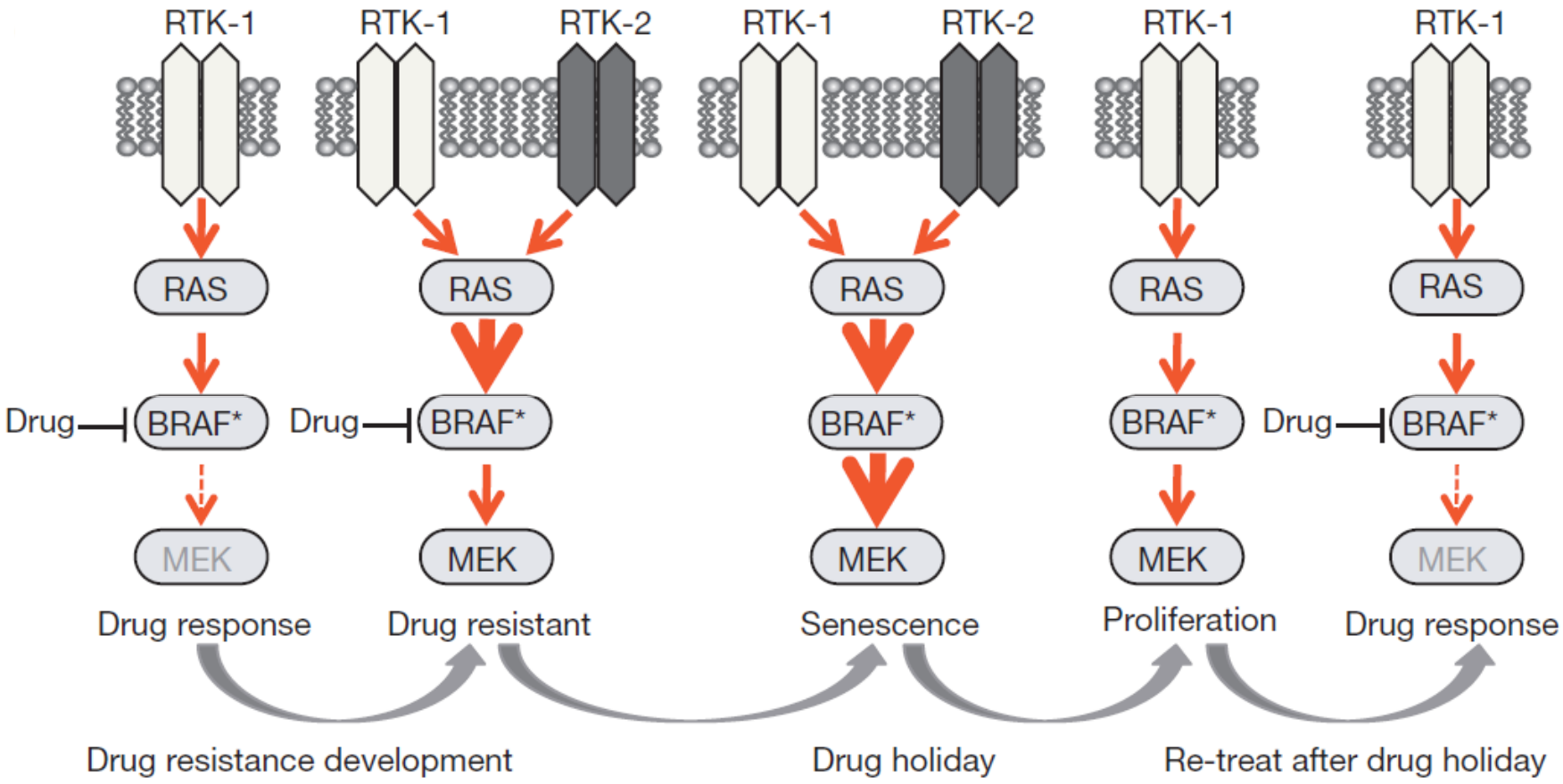
IGF-1R, EGFR, erb2, erb3, PDGF





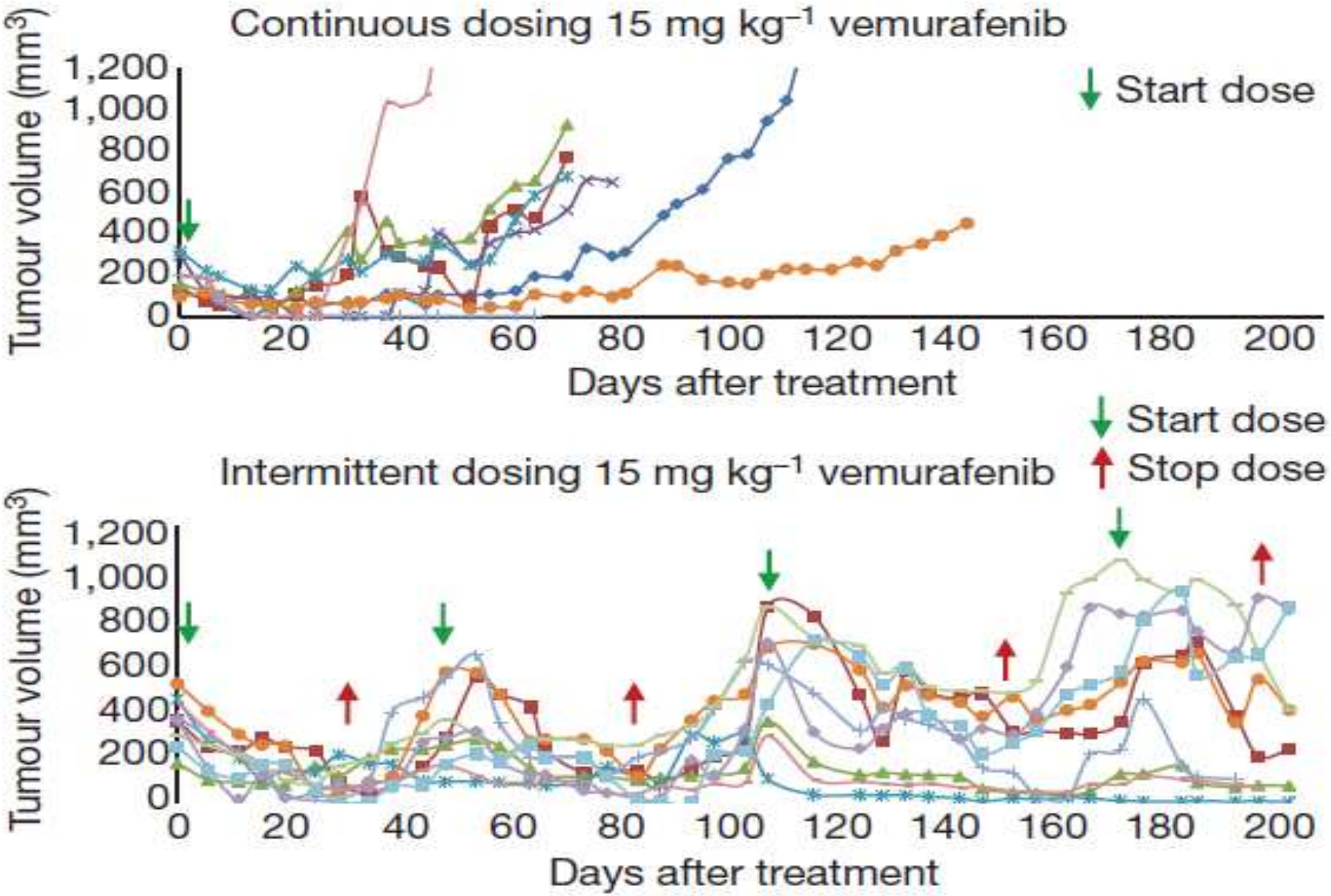
Villanueva. Cancer Cell 2010
Gopal, CR 2010

3.3. BRAF/MEK adaptative resistance: on/off schedule



Back up information

Chong Sun, Liqin Wang et Sidong Huang et al Nature. 2014

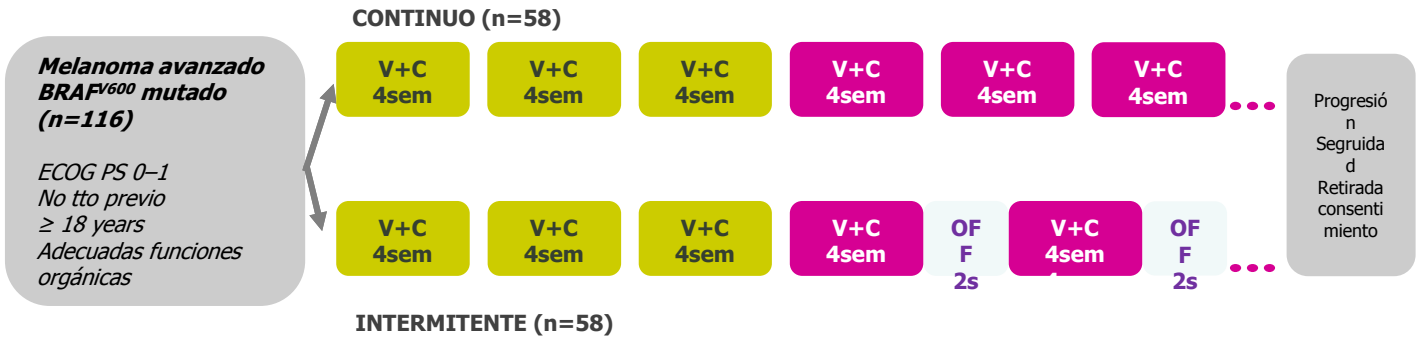


Discontinuous dosing strategy attenuates continued dependency on BRAF (V600E)-MEK-ERK signaling in resistant tumors , akin to reintroducing EGFR TKIs in EGFR mutant NSCLCs following chemotherapy *(Sequist et al. Science Trans Med 2011) (Chmielecki 2011)*

Das Thakur et al. Nature 2013

Intermittent Vemurafenib (iBRAF) + Cobimetinib (iMEK): GEM-01-15

GEM: Grupo Español de Melanoma



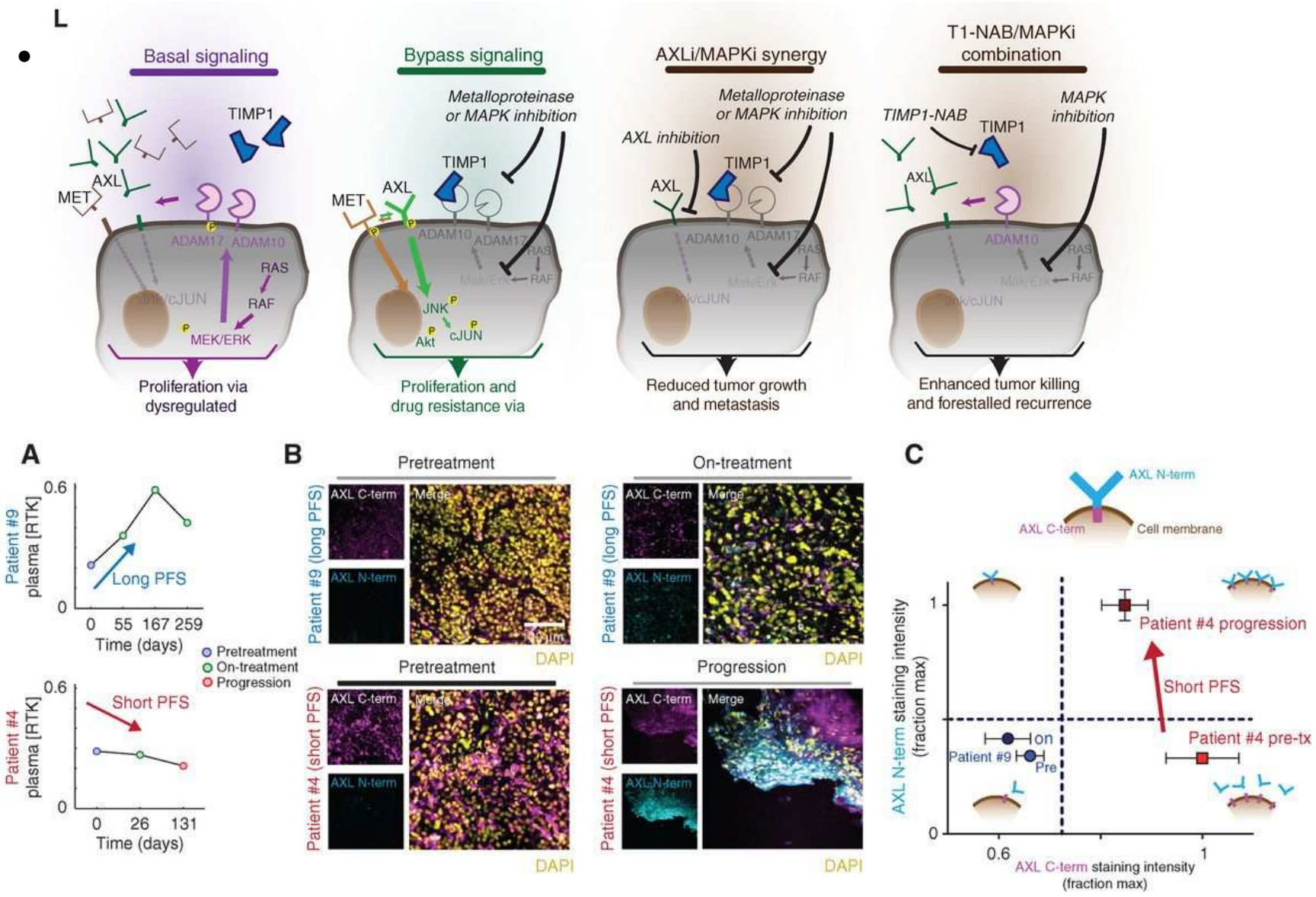
Objetivo principal:
- SLP

Objetivos secundarios
- Tasa de respuesta
- SLP 1 año, SLP 2 años
- SG, SG 1 año, SG 2 años
- Seguridad
- cfDNA BRAF V600 subestudio biomarcadores

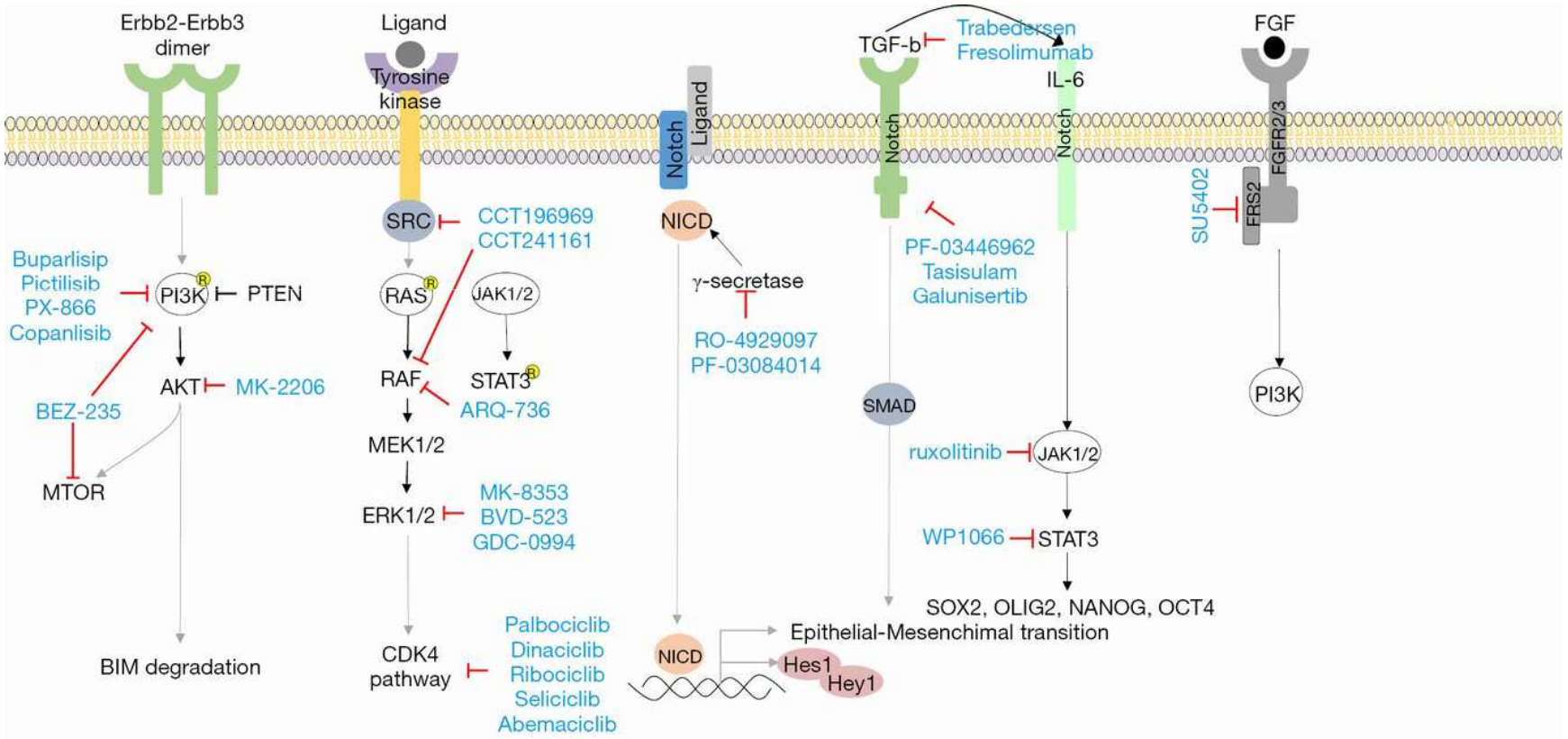
Vemurafenib (960mg BID oral 1-28d) (ROCHE)
Cobimetinib (60mg QD oral 1-21d) (ROCHE)

Justificación
- Optimizar esquema de tratamiento V-C
- Retrasar resistencias
- Reducir perfil de seguridad
- Reducir coste tratamiento/mes

Reduced Proteolytic Shedding of Receptor Tyrosine Kinases Is a Post-Translational Mechanism of Kinase Inhibitor Resistance



Miles A. Miller et al. Cancer Discov 2016;6:382-399



Gonzalez Cao M et al. ATM 2015

Conclusions

- Resistance to BRAF inhibitors is mediated by different mechanisms.
  - Secondary NRAS mutations
  - Upregulation of RTKs (PDGFRβ, IGF1R, AXL)
  - BRAF truncations or amplification
- Adaptative resistance
  - On-off scheudle
- Heterogeneous
  - Target key signaling node: ERKi, pan RAFi
  - Methods for looking at the most prevalent resistant mechanism: cfDNA