# Response and resistance to BRAF inhibitors in melanoma

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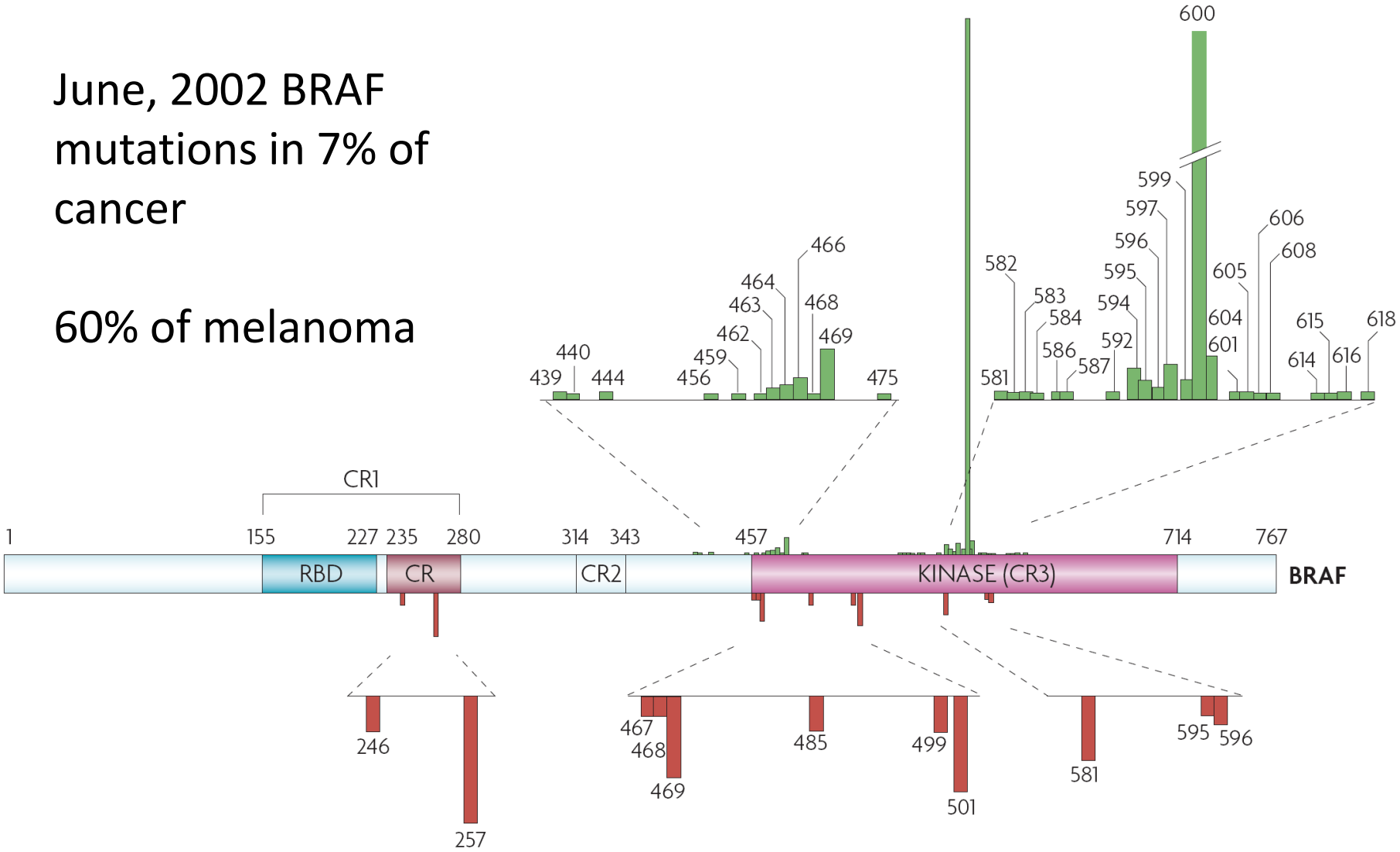
# Disclosures

- Roche/Genentech: consultant
- GlaxoSmithKline: consultant

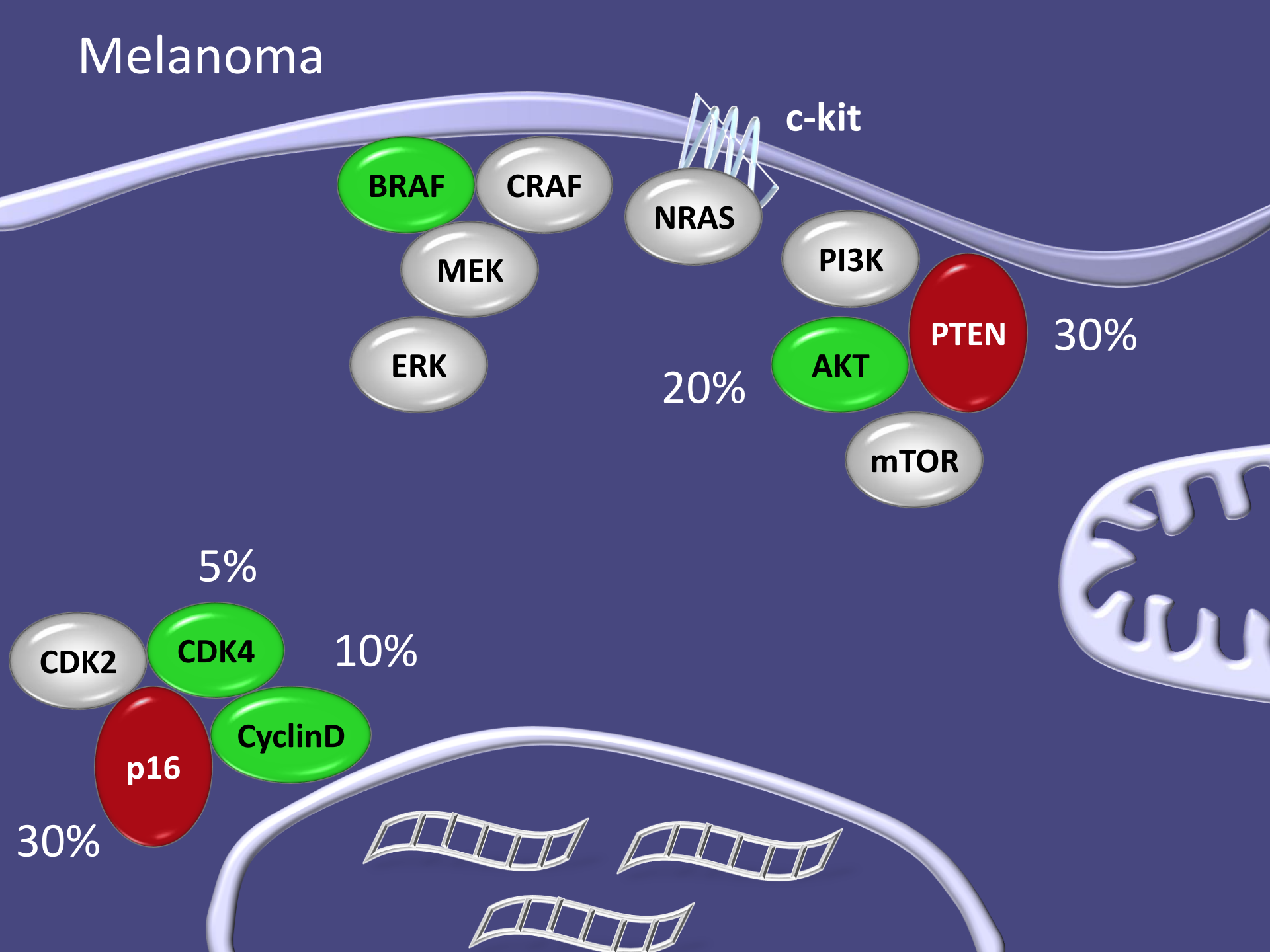
# BRAF mutations in human cancer

June, 2002 BRAF mutations in 7% of cancer

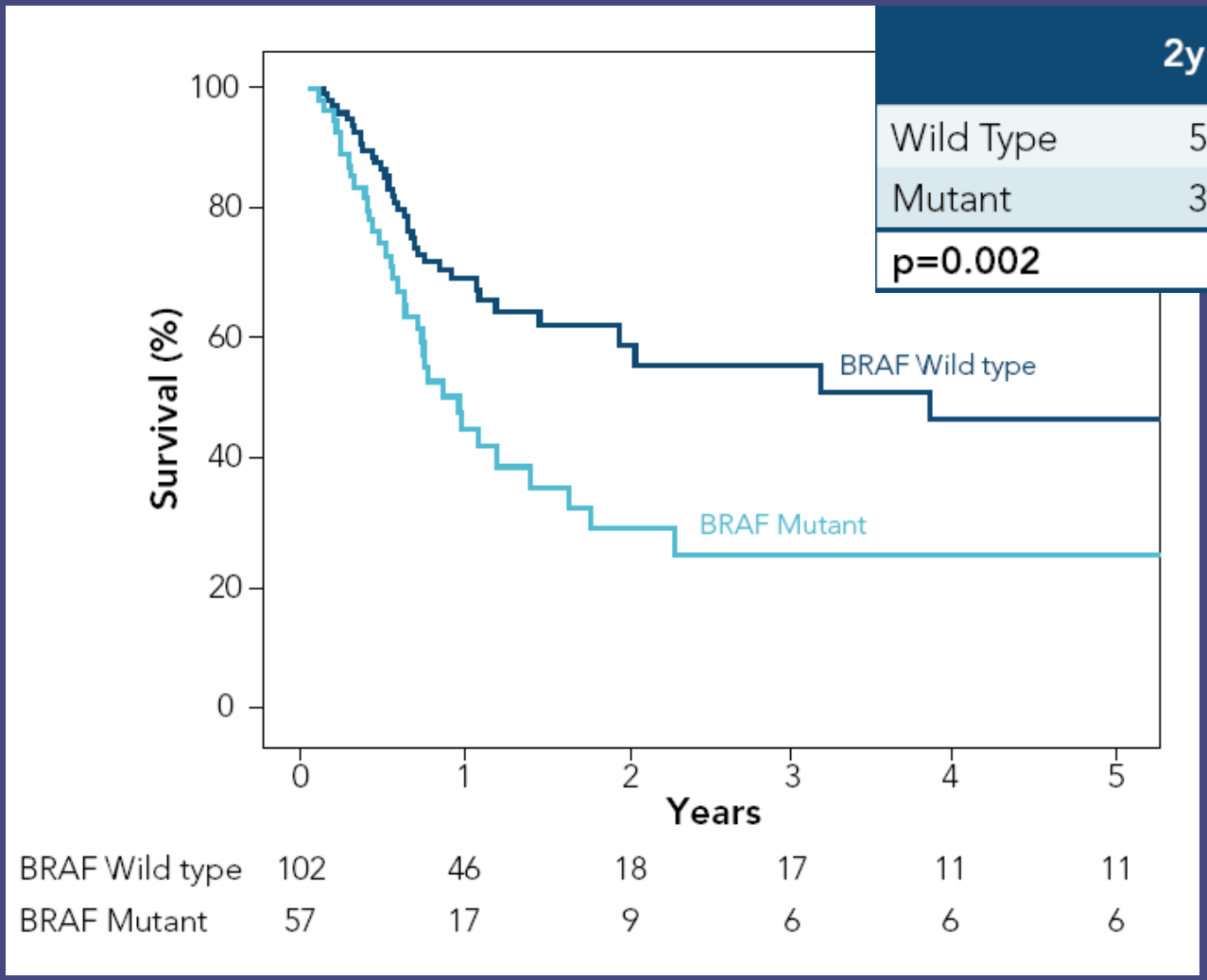
60% of melanoma




Bollag G. Nat Rev Cancer 2007 Apr;7(4):295

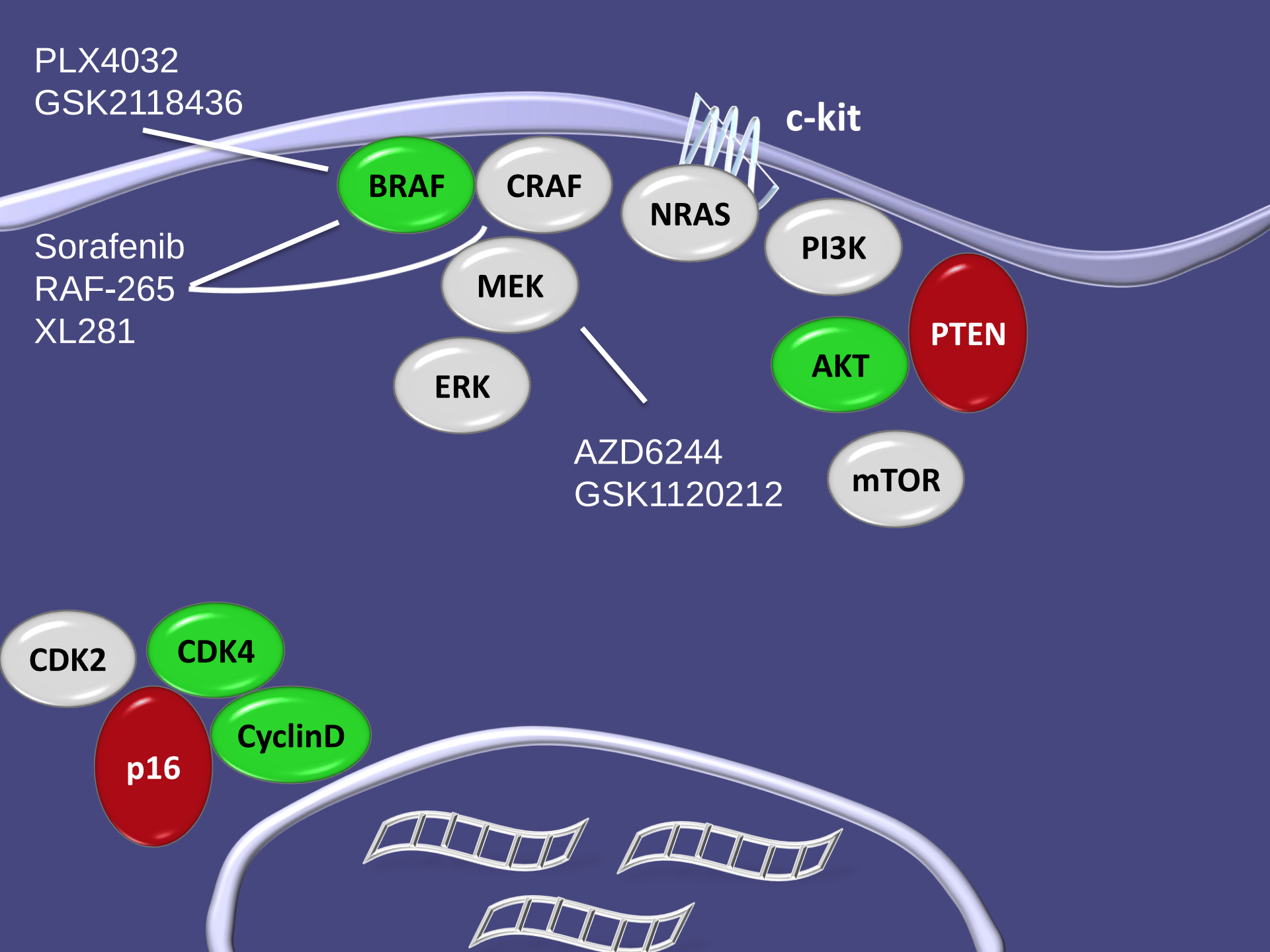

Melanoma
c-kit
BRAF
CRAF
NRAS
MEK
ERK
PI3K
PTEN
AKT
mTOR
20%
30%
5%
CDK2
CDK4
10%
CyclinD
p16
30%

# BRAF mutation as a poor prognostic factor

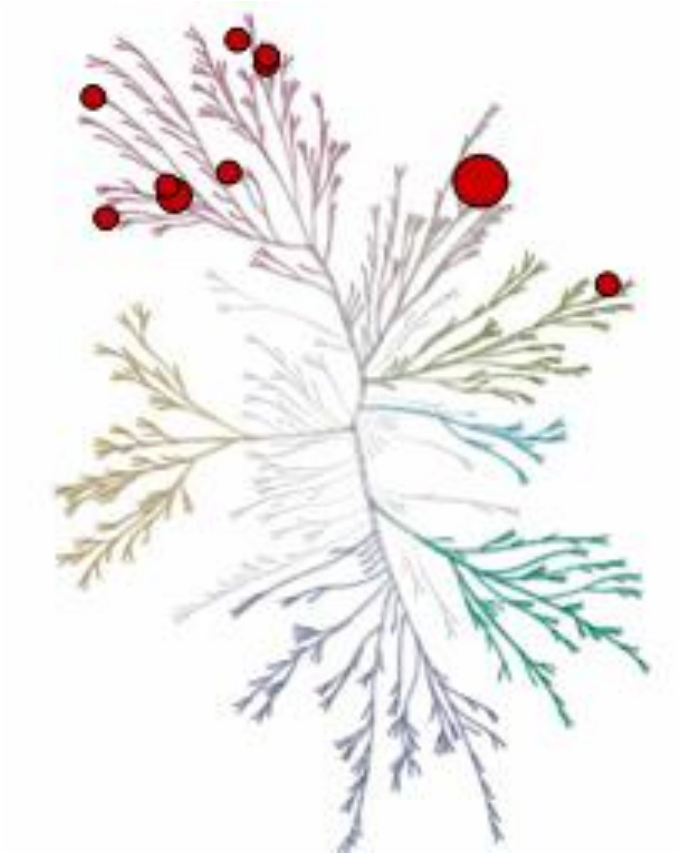



| | 2yr OS | 5yr OS | Median Survival |
|---|---|---|---|
| Wild Type | 59% | 47% | 46 mo. |
| Mutant | 30% | 25% | 11 mo. |
| p=0.002 | | | |

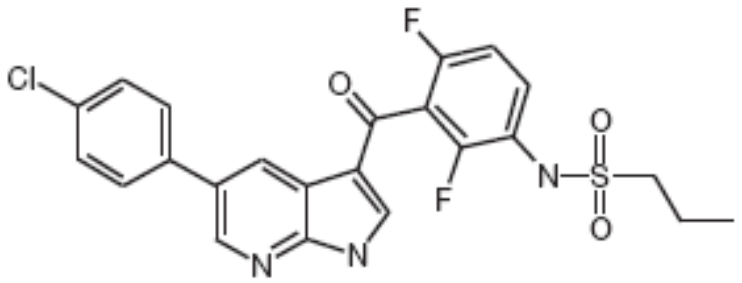
Long G et al.
ASCO 2010


PLX4032
GSK2118436
c-kit
BRAF
CRAF
NRAS
PI3K
Sorafenib
RAF-265
XL281
MEK
PTEN
AKT
ERK
AZD6244
GSK1120212
mTOR
CDK2
CDK4
CyclinD
p16

# PLX4032 selectivity



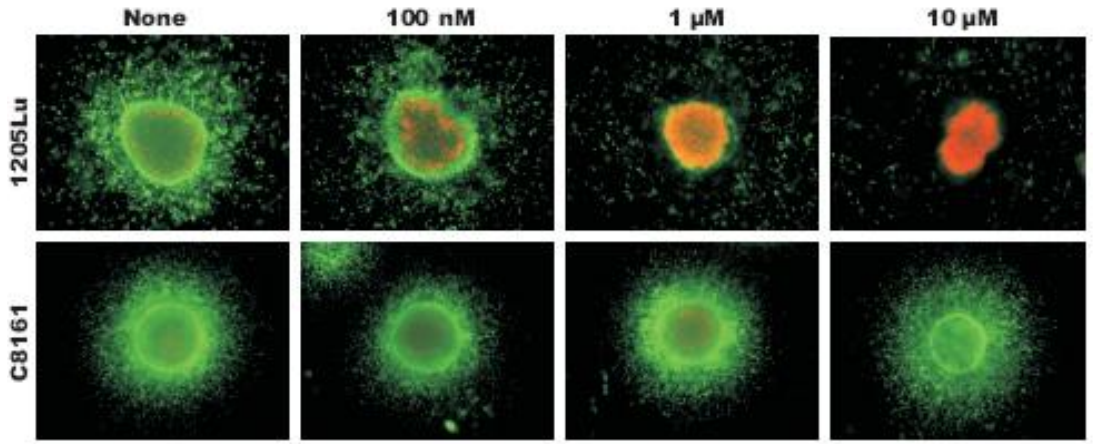
PLX4032



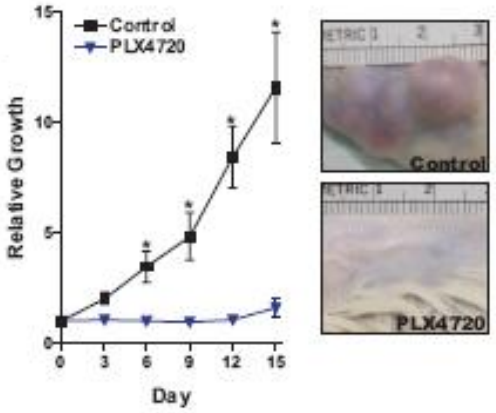

PLX4032

# PLX4720/4032: selective BRAF inhibitors are selective for BRAF mutant melanoma in vitro & in vivo

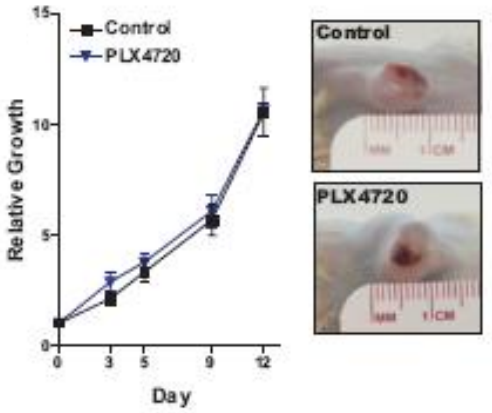
| Assay | $IC_{50}$, nM |
|---|---|
| B-Raf V600E | 13 |
| B-Raf | 160 |
| BRK | 130 |
| FRK | 1,300 |
| CSK | 1,500 |
| SRC | 1,700 |
| FAK | 1,700 |
| FGFR | 1,900 |
| KDR | 2,300 |
| HGK | 2,800 |
| CSF1R | 3,300 |
| AURORA A | 3,400 |




**BRAF mutant**




**BRAF wild-type**



# GSK2118436 (selective RAF inhibitor)

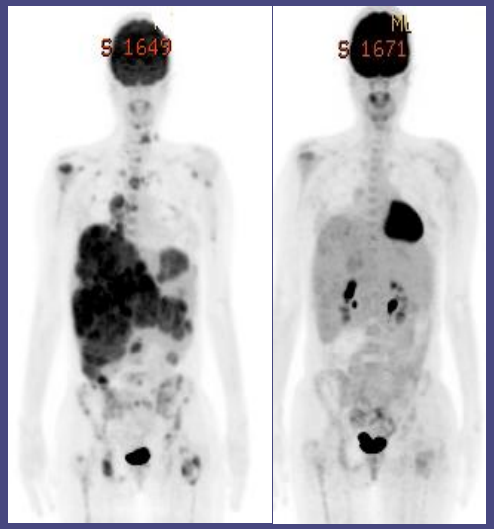
| ENZYME | STATUS | IC50 (nM) |
|---|---|---|
| B-RAF | V600E | 0.6 |
| B-RAF | V600K | 0.5 |
| B-RAF | V600D | 1.9 |
| B-RAF | WT | 12 |
| C-RAF | WT | 5 |

- ATP-competitive; reversible inhibitor
- Selective against 270 kinase panel
  - 10 of 270: IC50 10-100nM
  - 260 of 270: IC50 from 100nM to >10,000nM
- Selective against BRAF mutant cell lines
  - >1000-fold selectivity for mutant/wt BRAF

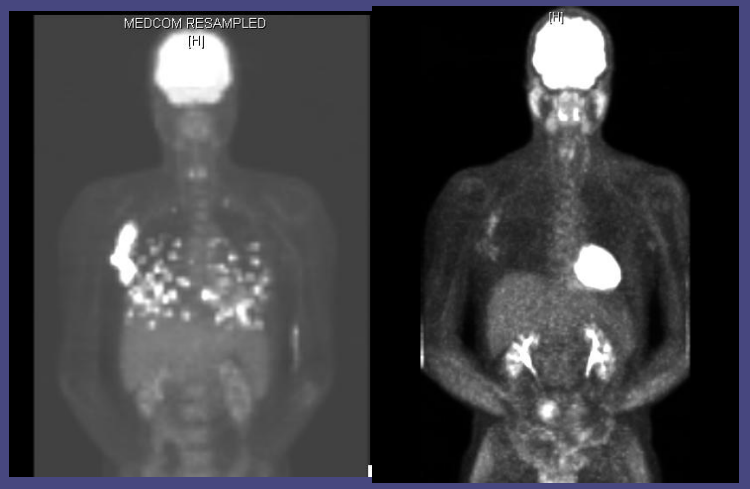
Kefford R et al. ASCO 2010

# PLX4032

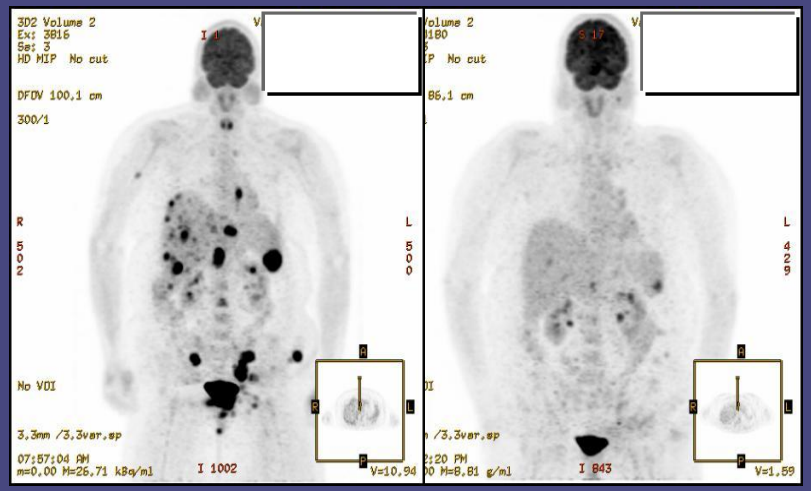
# PET Scans at Baseline and Day 15 after PLX4032



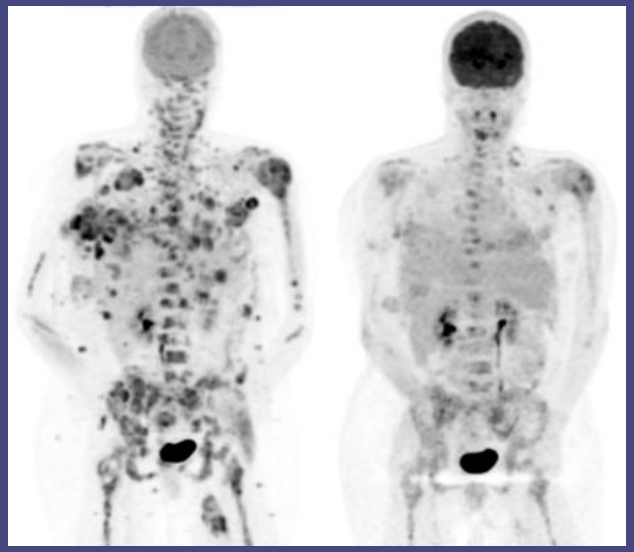

#69 MDA




#63 MSKCC

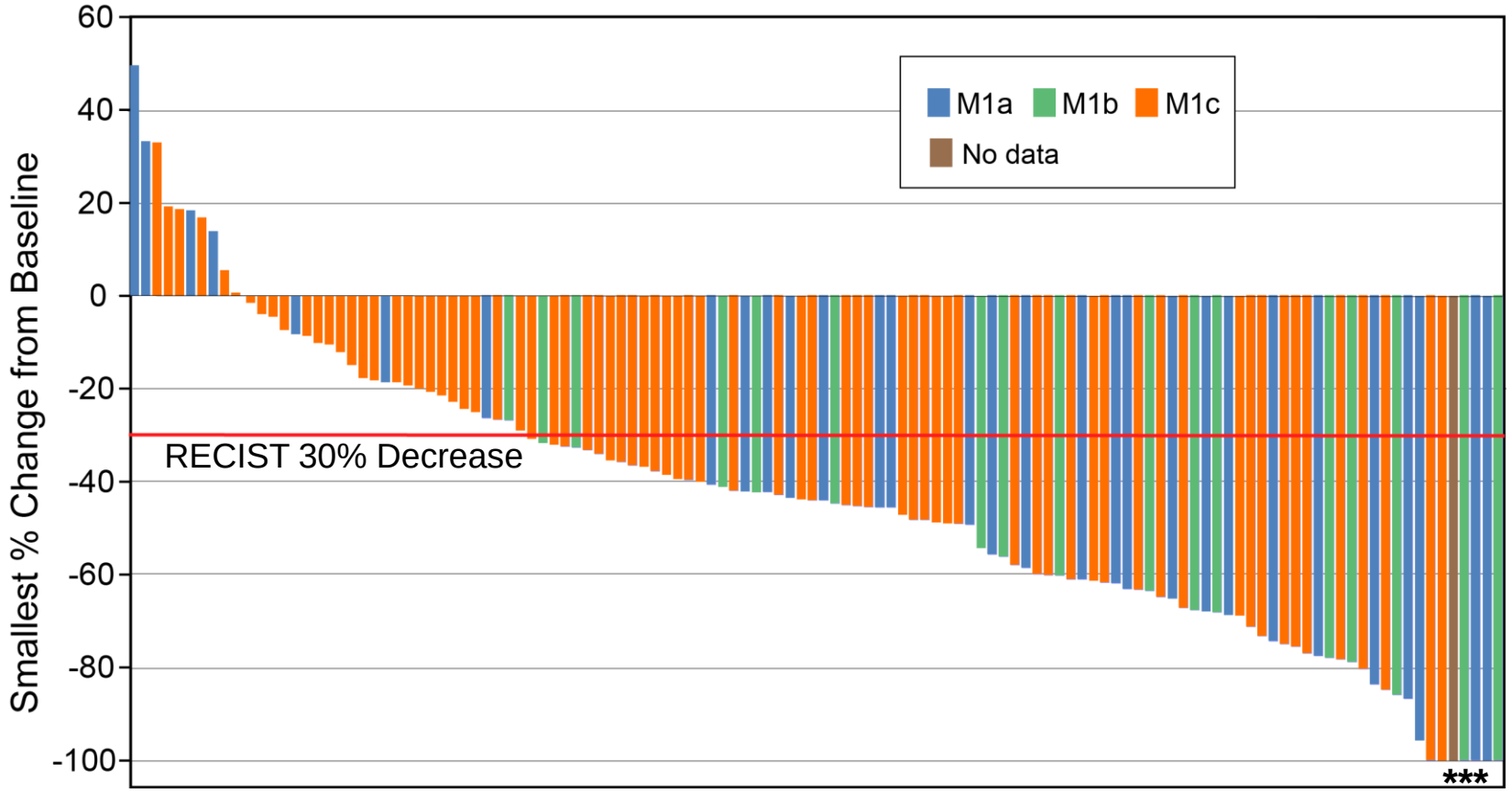



#56 Vanderbilt



#59 Peter MacCallum

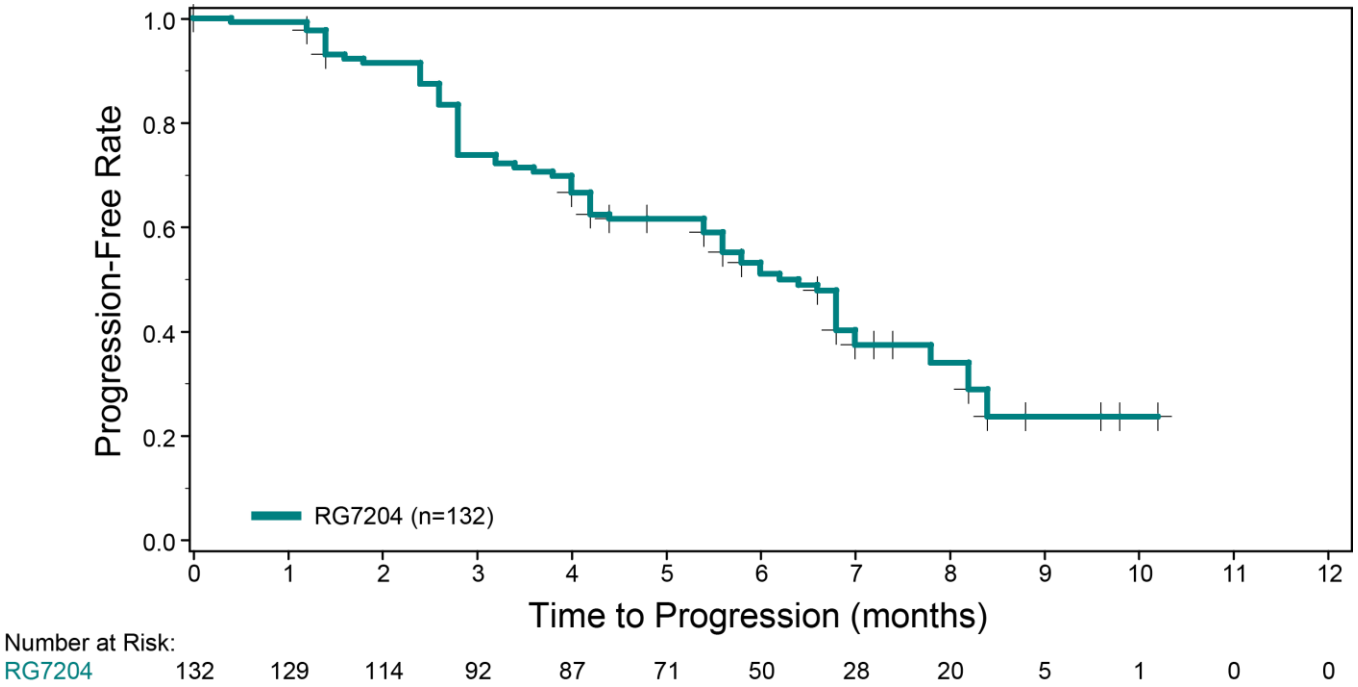
# Tumor Regression (Target Lesions) Occurred in Majority of Patients (IRC)




*** 7 patients had 100% tumor shrinkage, **3 of which had confirmed CR;**
1 patient had unconfirmed CR and 3 patients had non-target lesions present

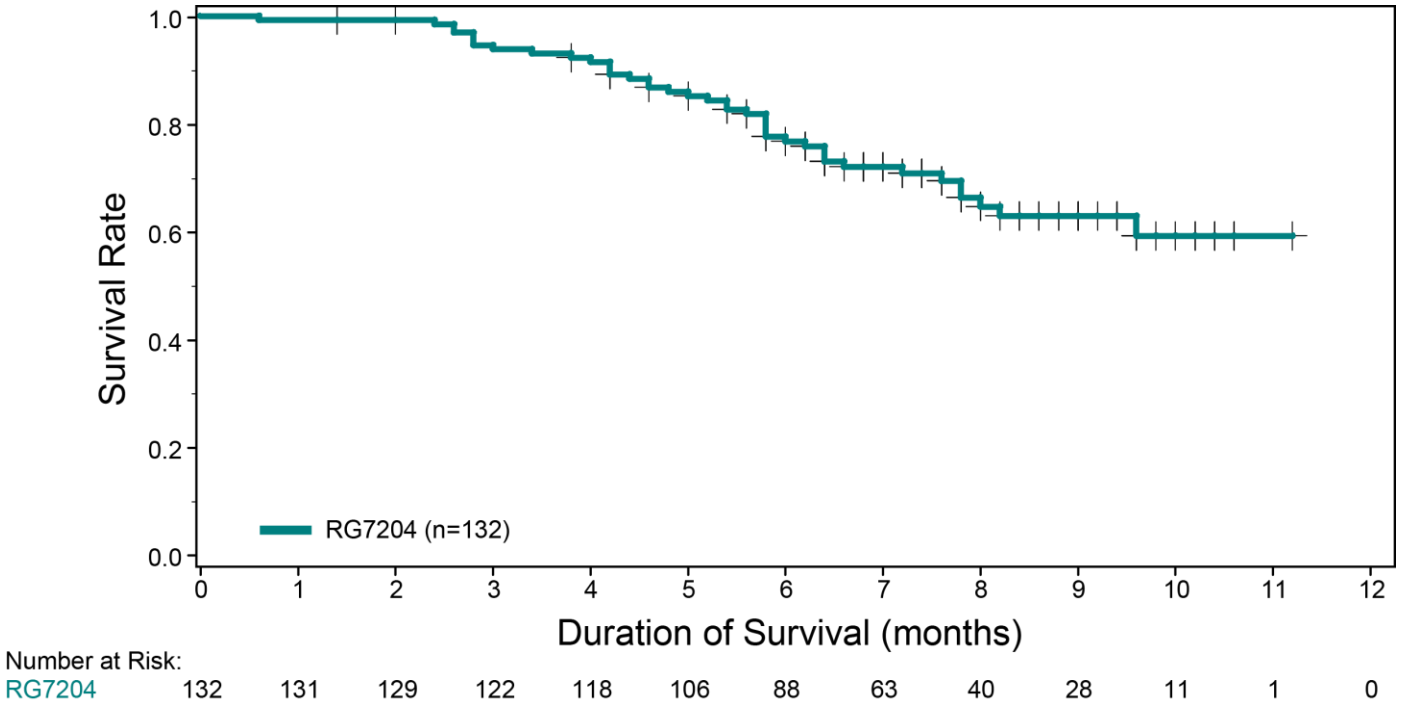
- 122 patients had baseline and ≥ 1post-baseline scan with measurable disease

# PFS (IRC)




| | n=132 |
|---|---|
| PD or death, n (%) | 78 (59.1) |
| Progression-free | 54 (40.9) |
| Median PFS, mo (95% CI) | 6.2 (5.6–6.8) |
| 6 mo PFS rate (95% CI) | 0.51 (0.42–0.60) |

# Overall Survival



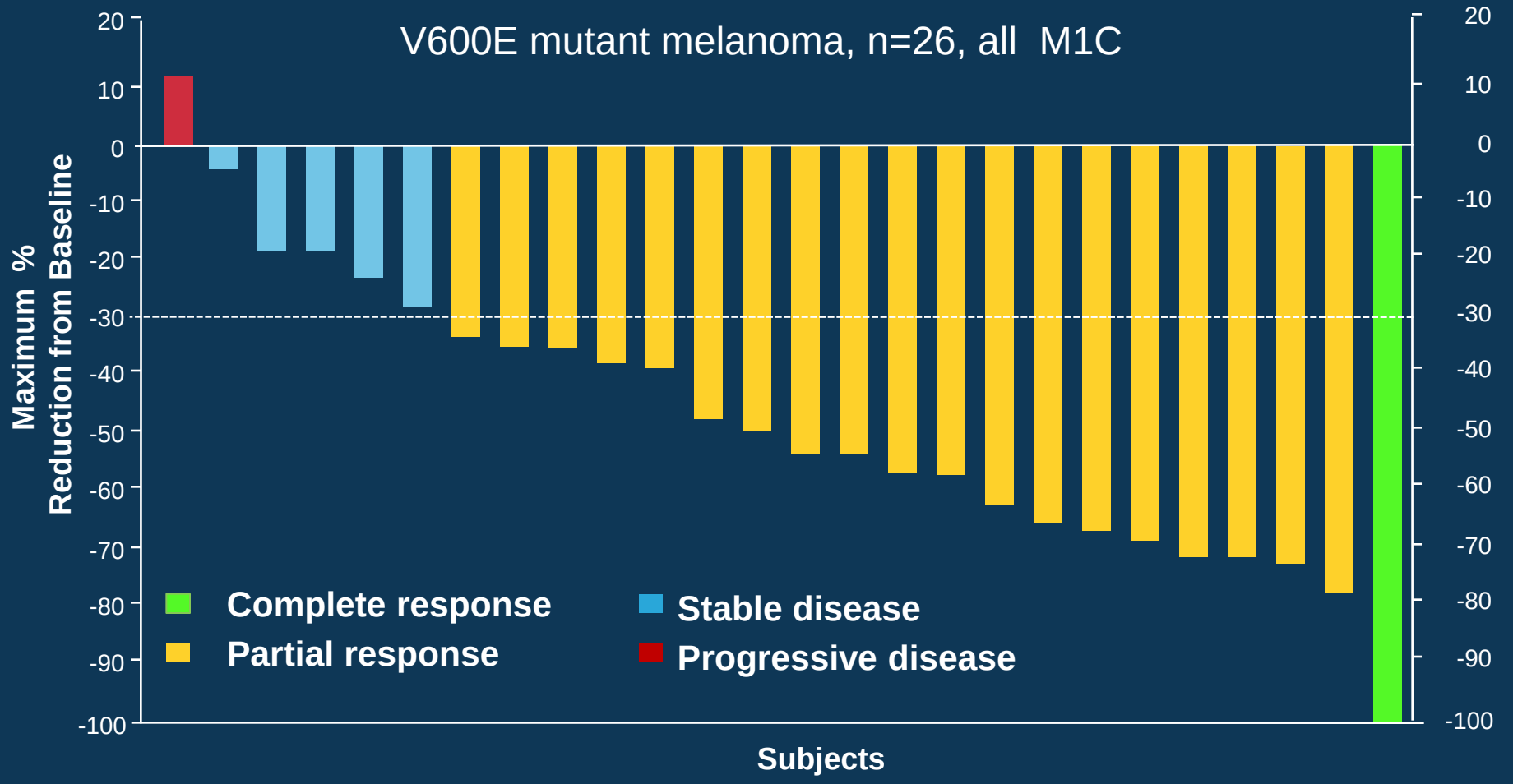

| | n=132 |
|---|---|
| **Alive (i.e., censored), n (%)** | **91 (69)** |
| **Death, n (%)** | **41 (31)** |
| **Median OS, mo (95% CI)** | **NR** |

NR=not reached

# GSK2118436

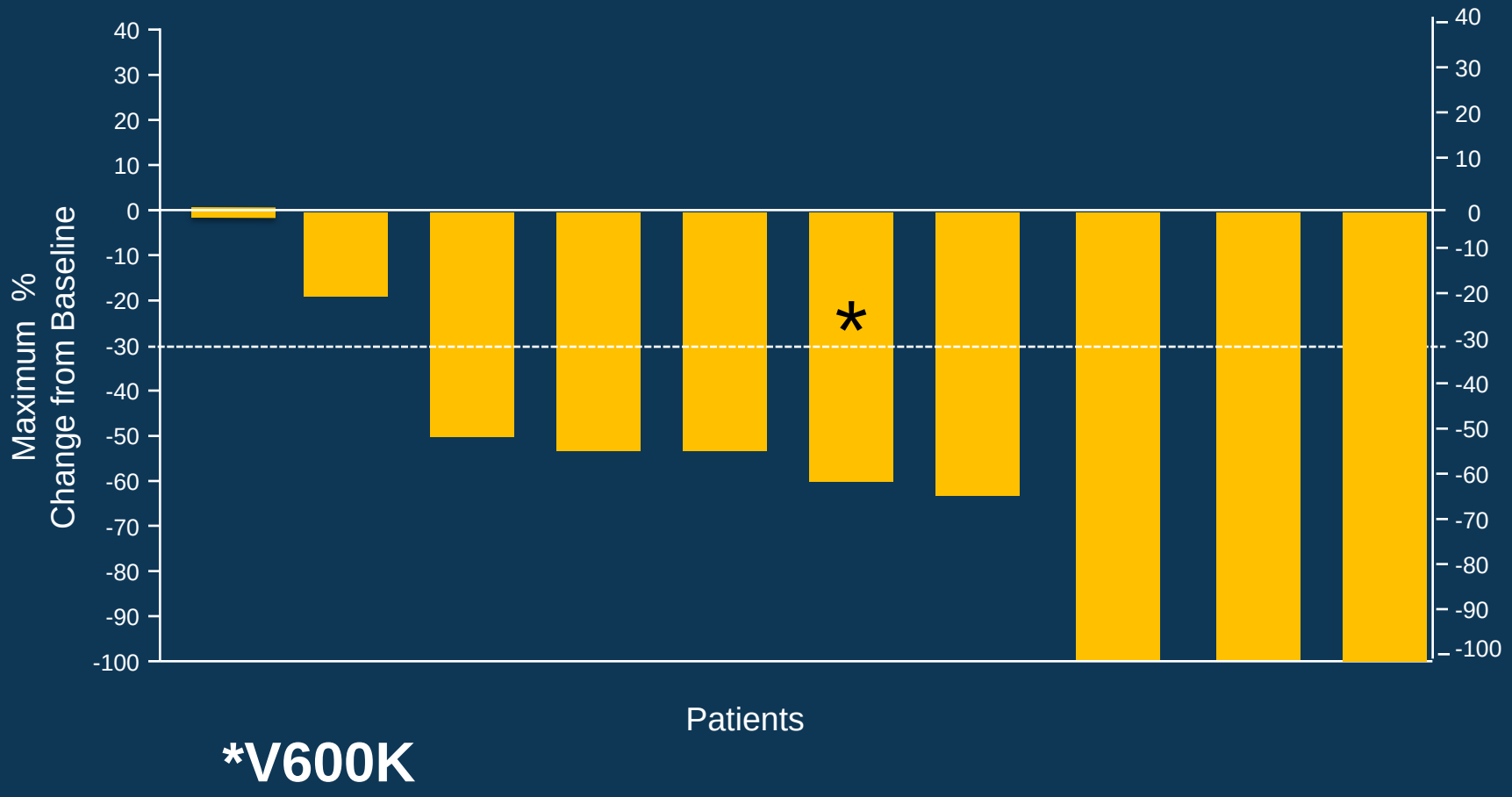
# GSK2118436: phase II expansion at 150 mg BID

## Overall Response Rate 77% (20/26)




Kefford R et al. SMR 2010

# Response in Brain Lesions with GSK2118436 (n=10)




***V600K**

Long, G. ESMO Oct 2010

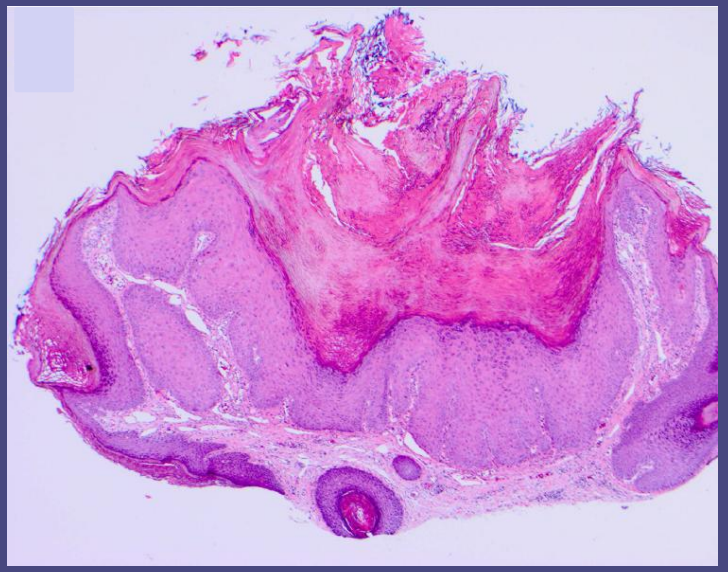
# Toxicity

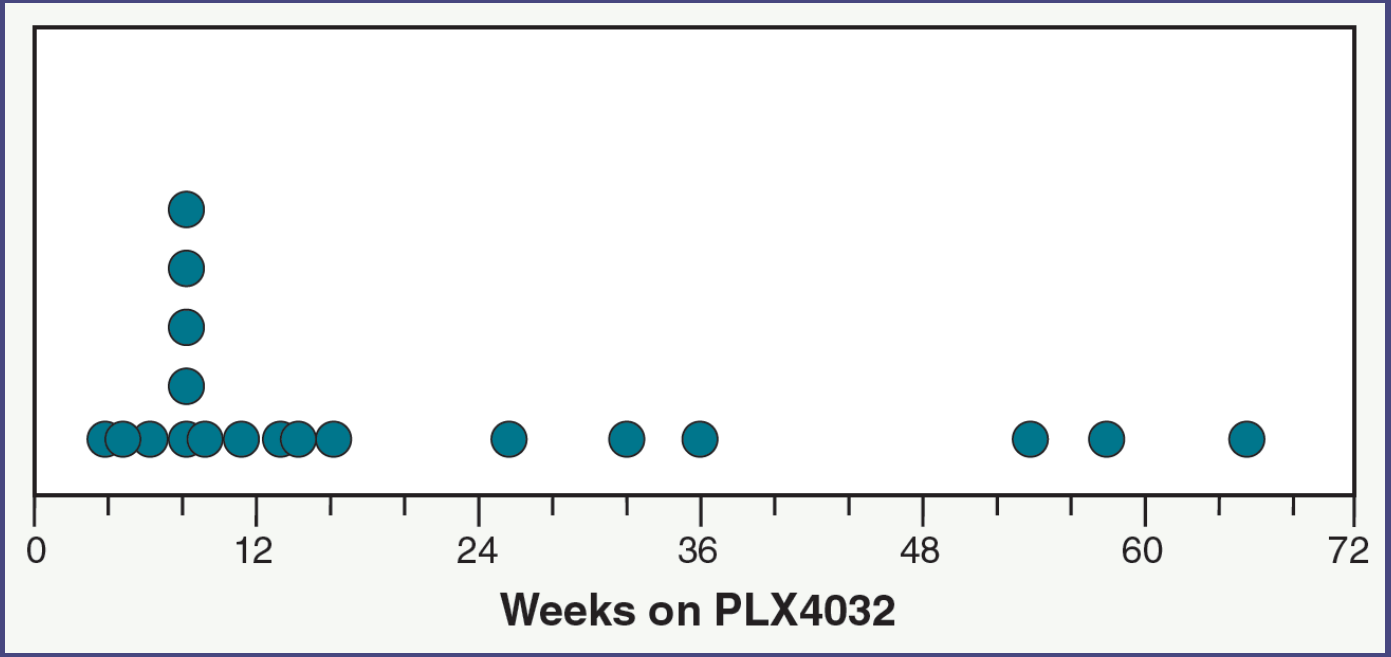
# Most common treatment-related toxicities

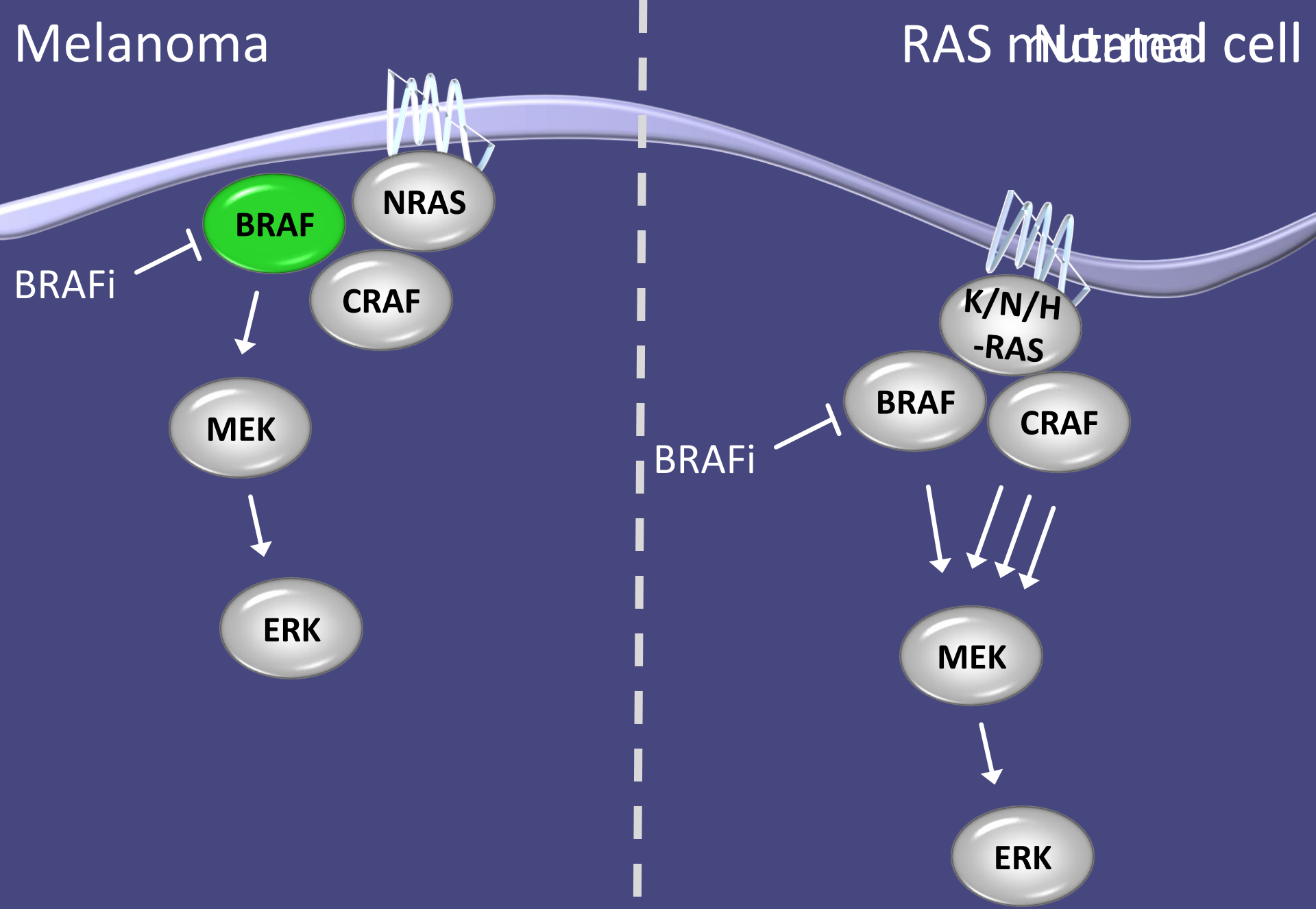
| PLX4032 Adverse Event | % of patients with toxicity |
|---|---|
| Rash | 68 % |
| Arthralgia | 48 % |
| Photosensitivity | 42 % |
| Fatigue | 32 % |
| Cutaneous squamous cell carcinoma (keratoacanthoma) | 23 % / 7 % |

| GSK2118436 Adverse Event | % of patients with toxicity |
|---|---|
| Pyrexia | 43 % |
| Rash | 30 % |
| Headache | 26 % |

# Keratoacanthoma

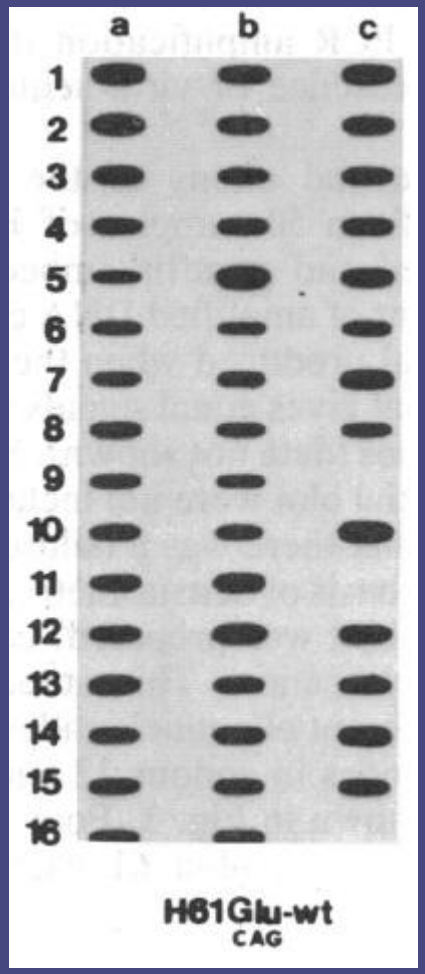




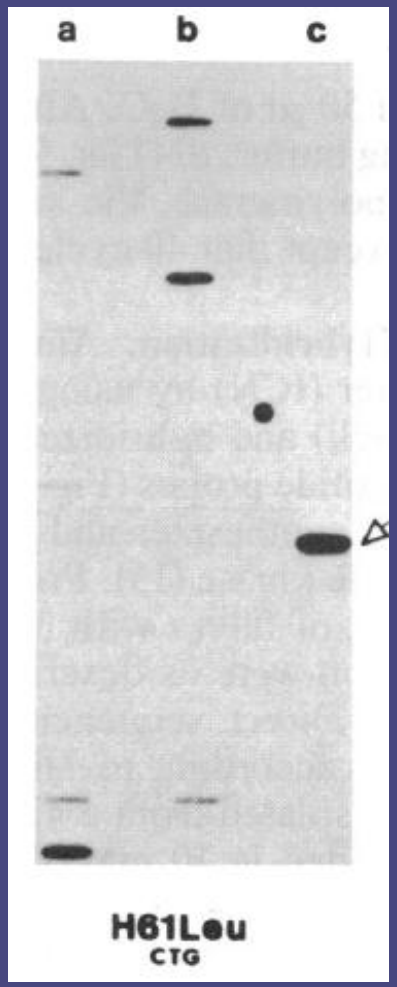



Heidorn et al. Cell 2010; Poulikakos et al. Nature 2010; Hatzivassiliou et al. Nature 2010

# HRAS mutations in KAs and SCCs







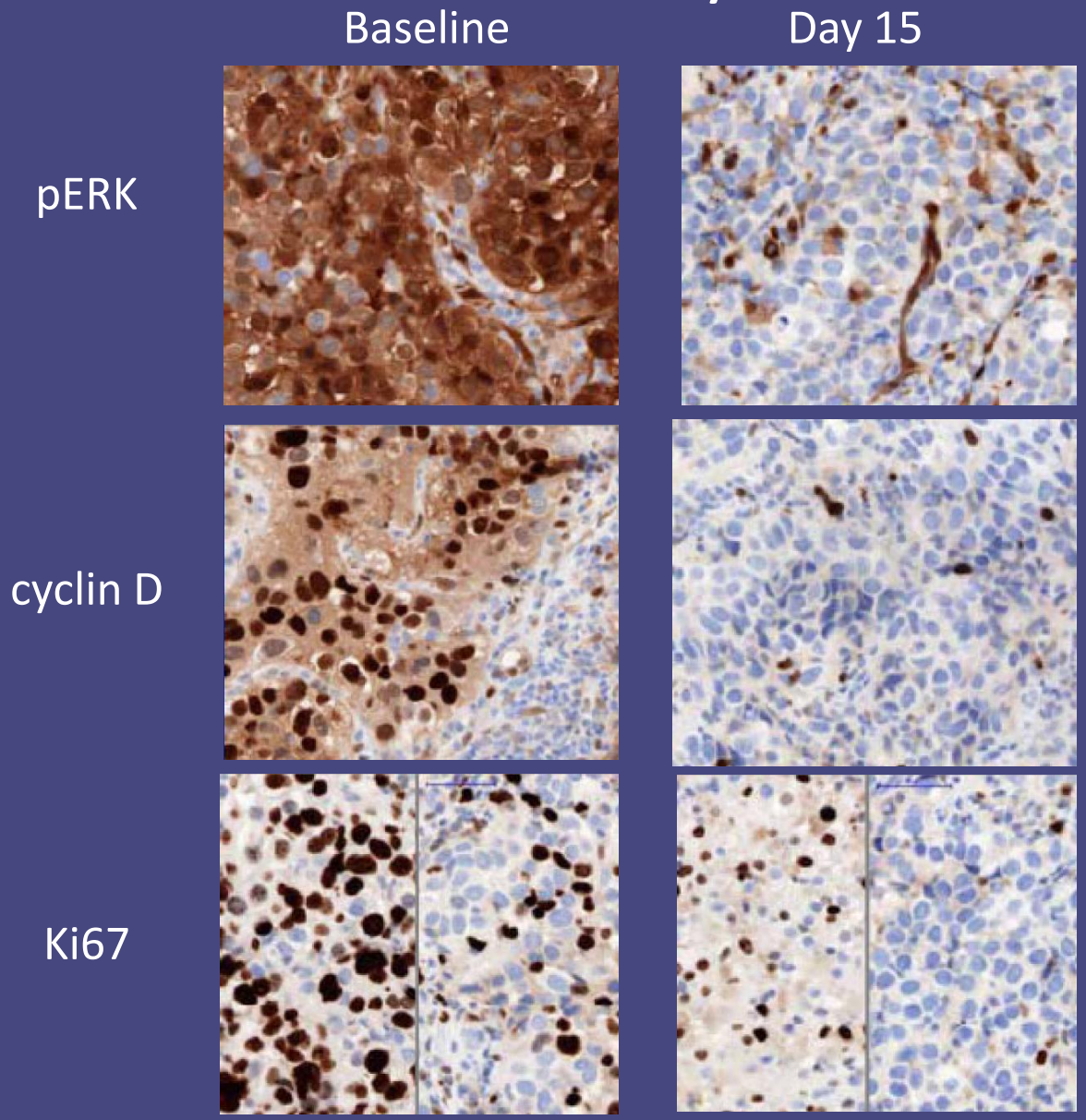

16 mutations among 56 KAs

6 mutations among 50 SCCs

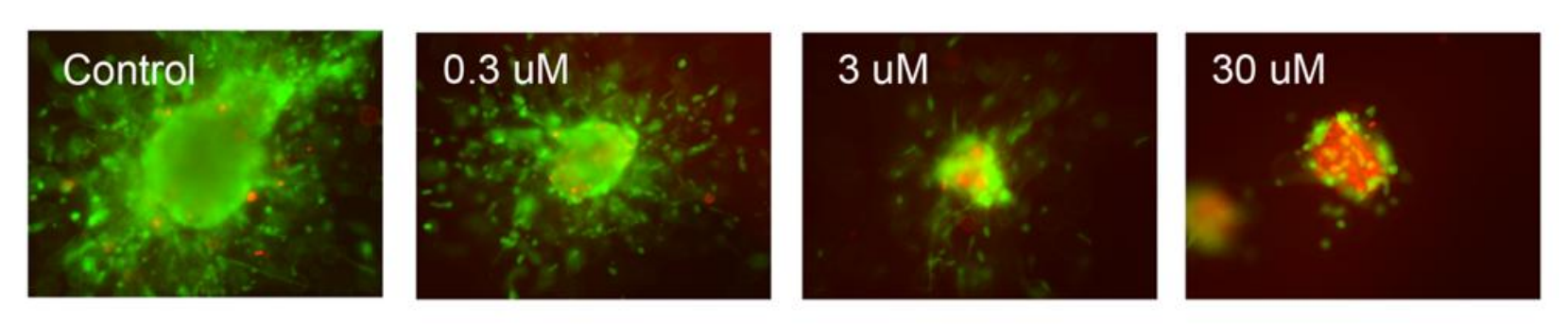
Corominas M et al. PNAS 1989;86:6372

# Mechanisms of resistance: primary

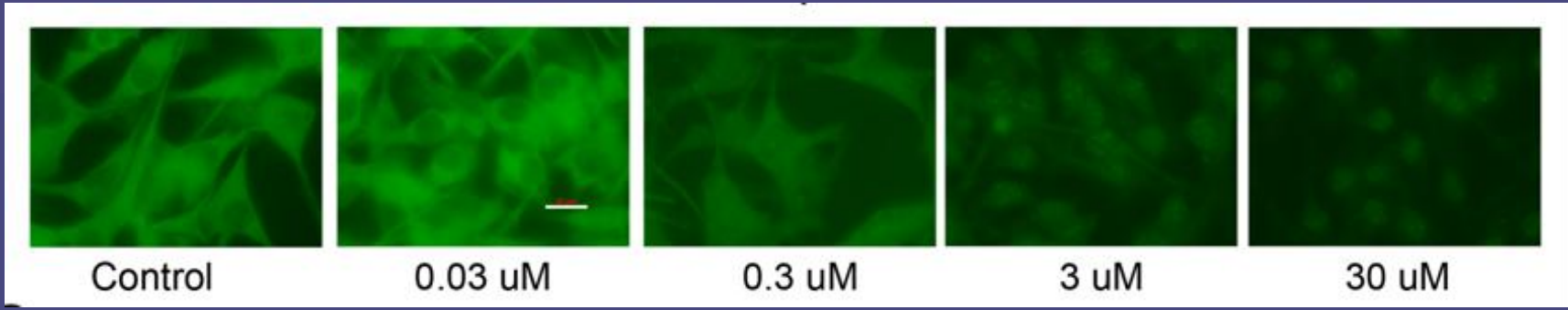
# Changes in MAP kinase signaling and markers of cell cycle



# Residual MAP kinase activity

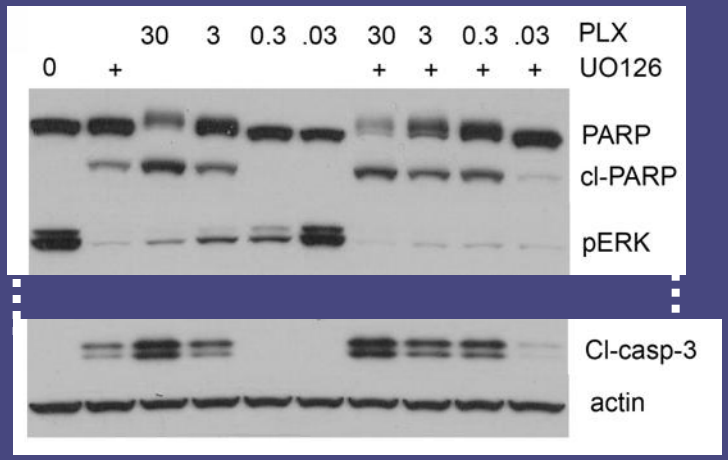



Dose dependent induction of apoptosis (PLX4720)

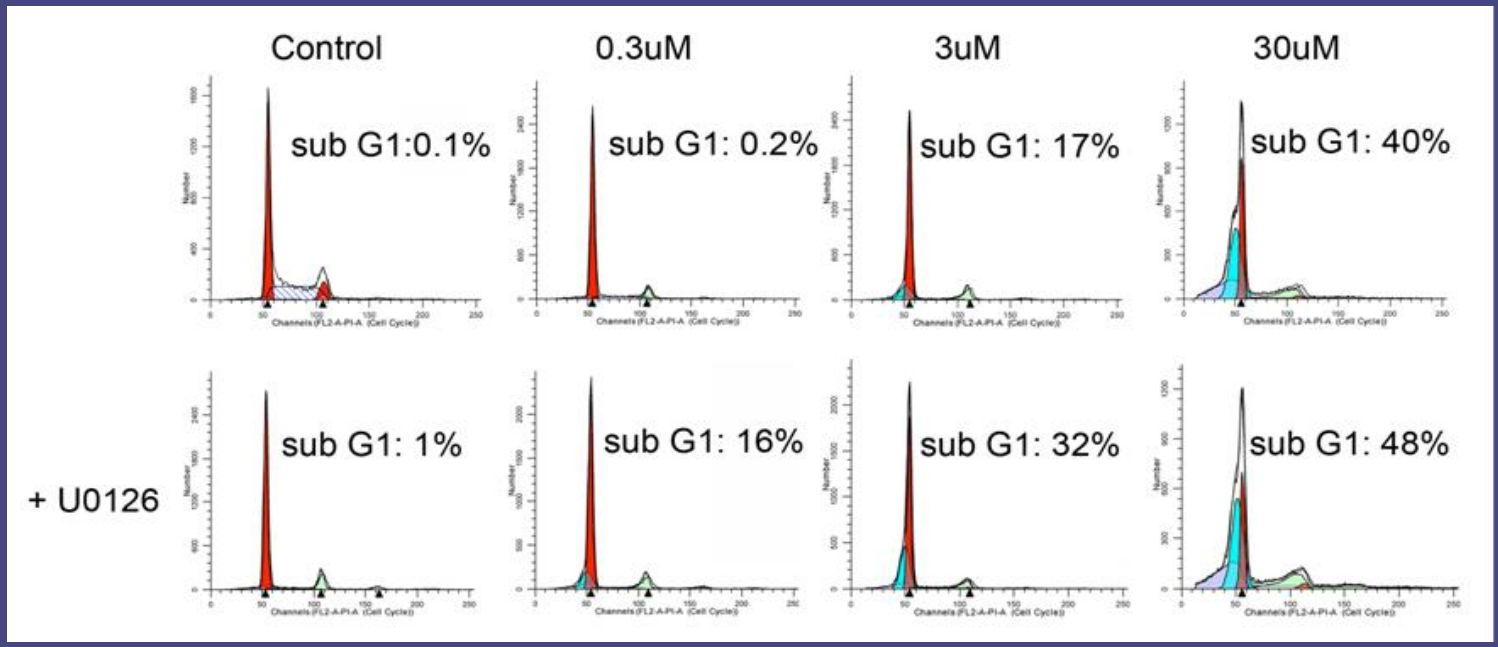



Residual nuclear pERK even at high concentrations

# Combined BRAF & MEK inhibition



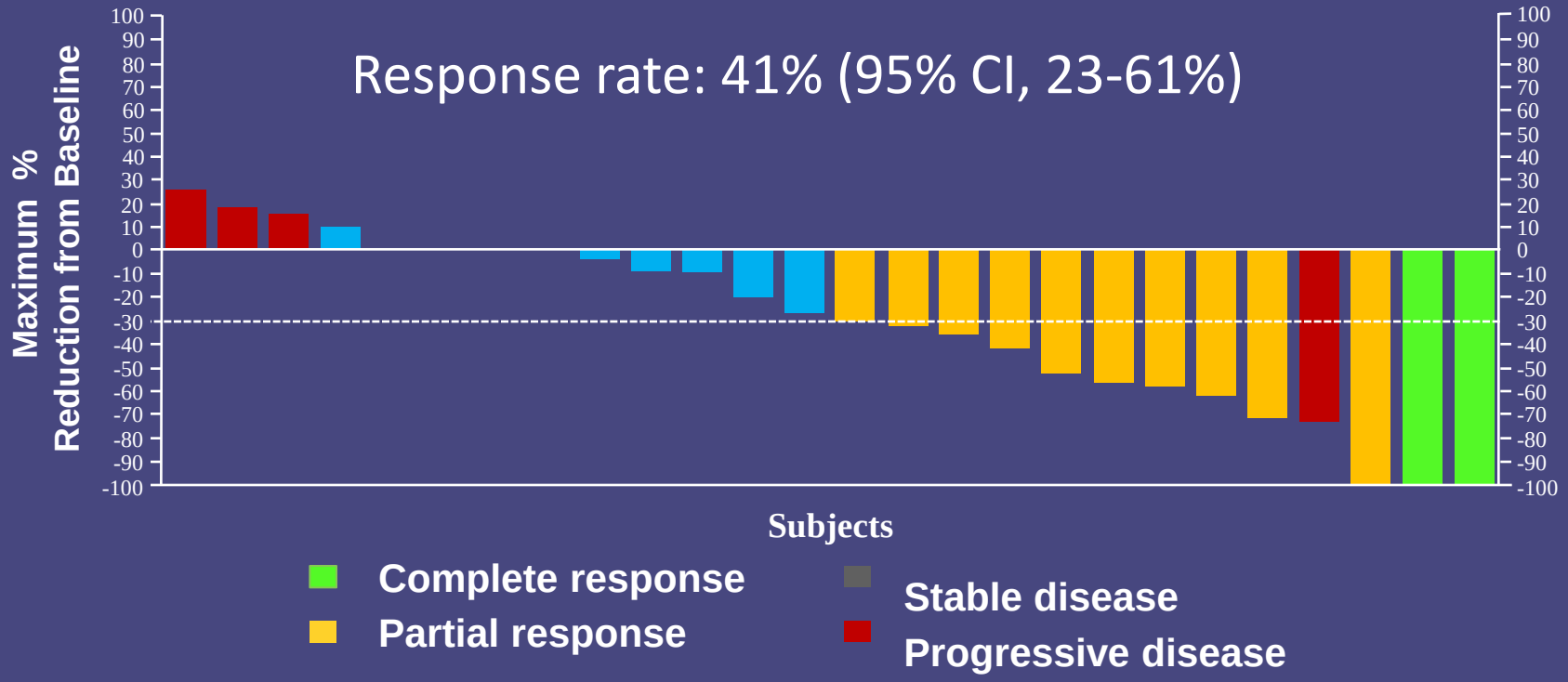

Increased PARP & caspase 3 cleavage at lower concentrations of PLX4720



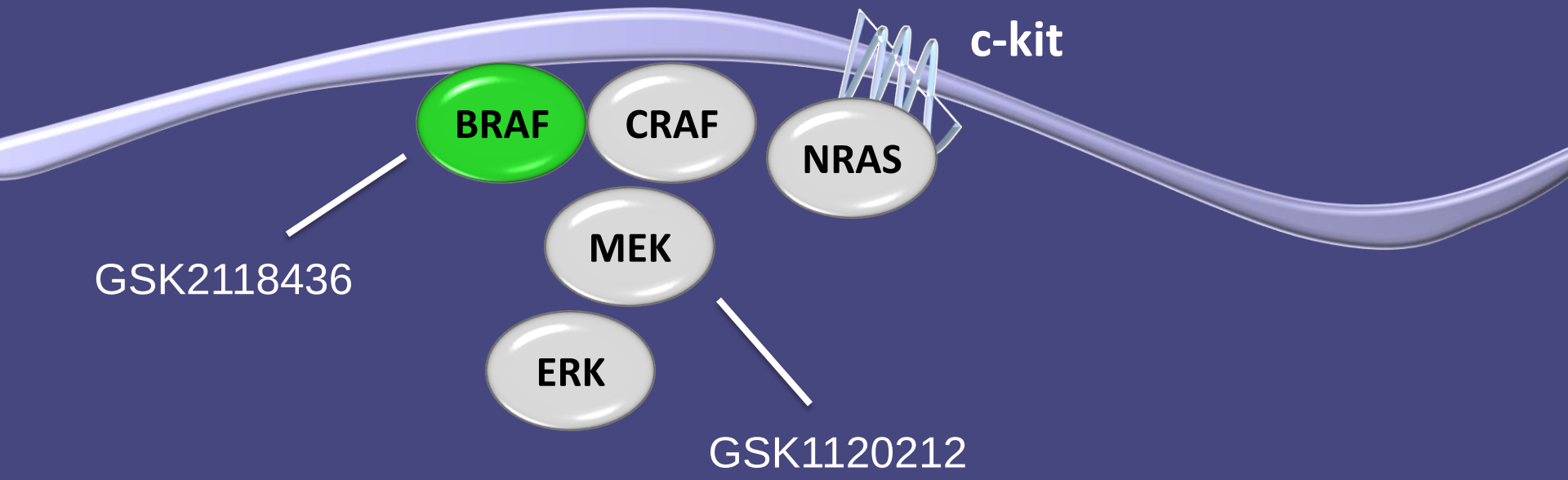

More apoptosis at lower concentrations of PLX4720

# GSK1120212 (MEK inhibitor) in BRAF mutant melanoma (n=29)

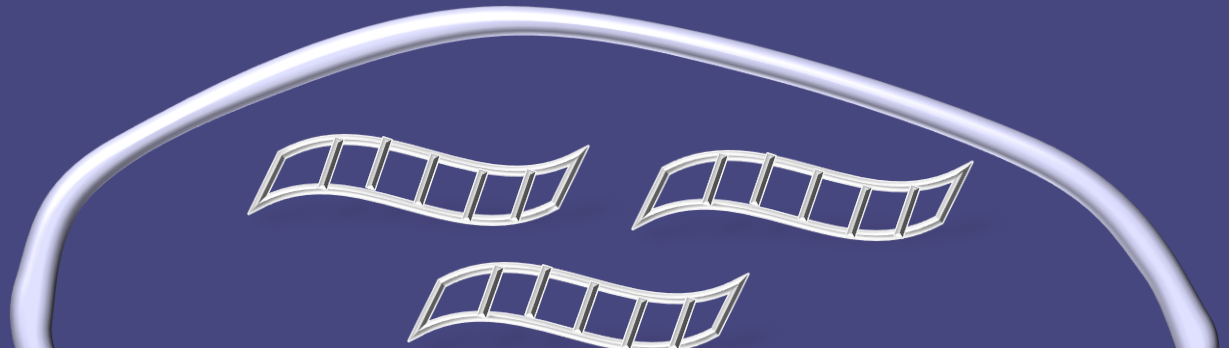
- 2 CR and 10 PR
- ~ 90% M1c;  48% history of brain metastases
- No prior treatment with a BRAF inhibitor




**Scans unavailable for 2 patients with clinical PD and 1 WD**




An Dose-Escalation, Phase IB/II Study to Investigate the Safety, Pharmacokinetics, Pharmacodynamics and Clinical Activity of the BRAF Inhibitor GSK2118436 in Combination with the MEK Inhibitor GSK1120212 in Subjects with BRAF Mutant Metastatic Melanoma

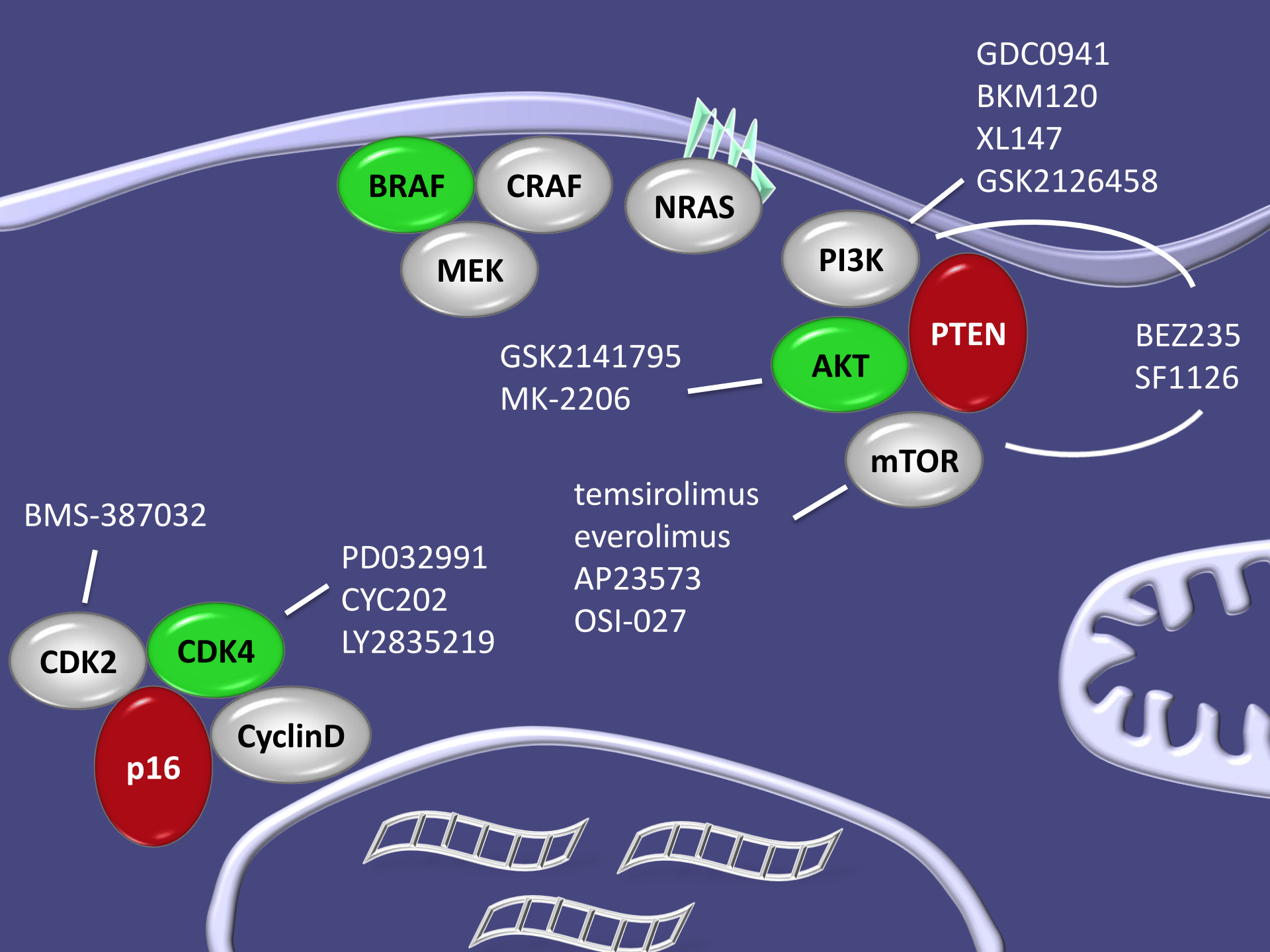


# Next generation BRAF inhibitors:

Increased potency and/or selectivity for BRAF

pan-RAF inhibitors with increased potency against CRAF

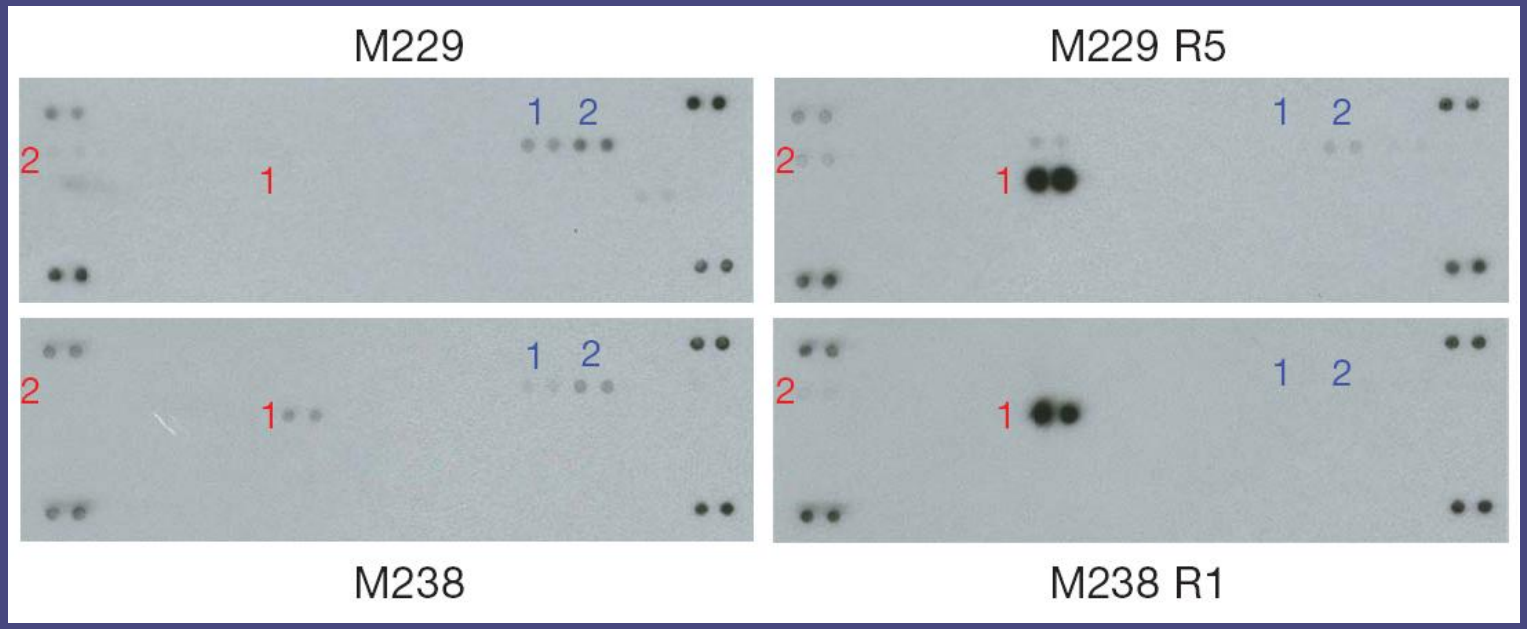
Dimerization blockers


GDC0941
BKM120
XL147
GSK2126458
BRAF
CRAF
NRAS
MEK
PI3K
PTEN
AKT
GSK2141795
MK-2206
BEZ235
SF1126
mTOR
temsirolimus
everolimus
AP23573
OSI-027
BMS-387032
PD032991
CYC202
LY2835219
CDK2
CDK4
CyclinD
p16

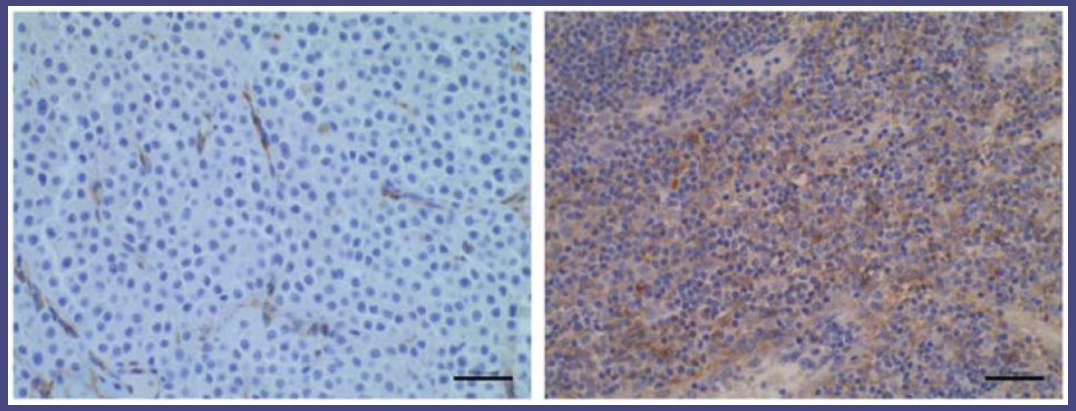
# Mechanisms of resistance: secondary

No secondary BRAF mutations

# PDGFRβ expression in resistant cell lines

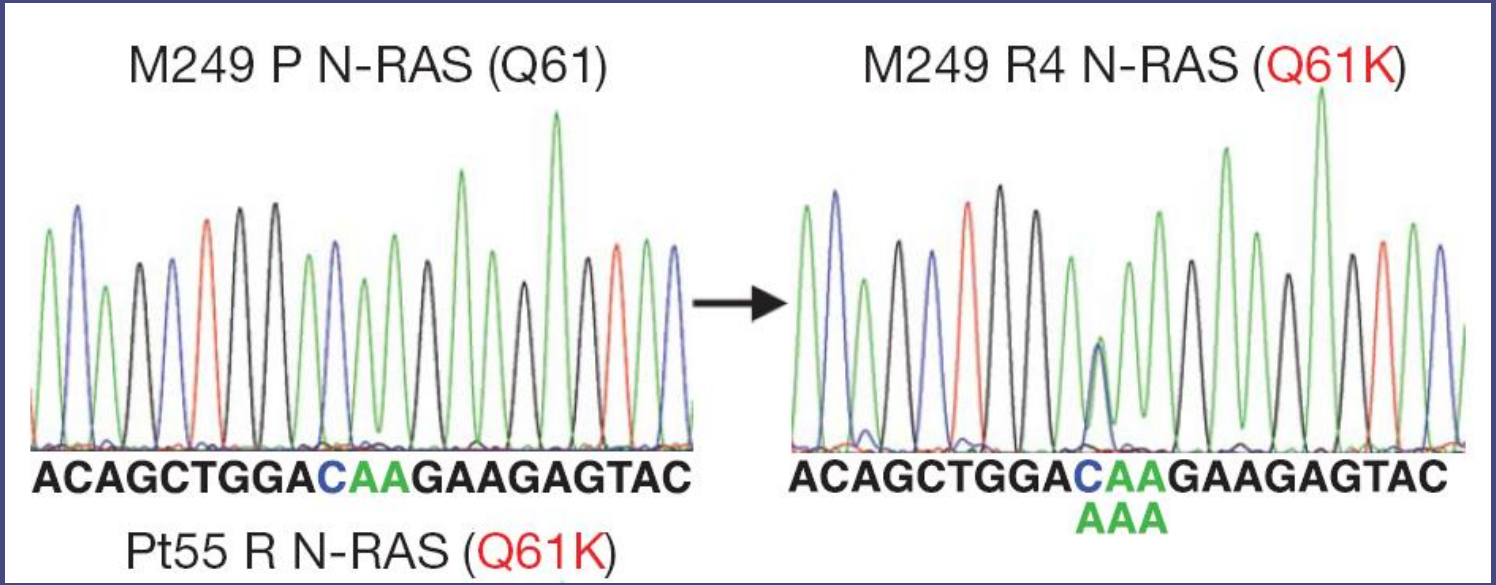


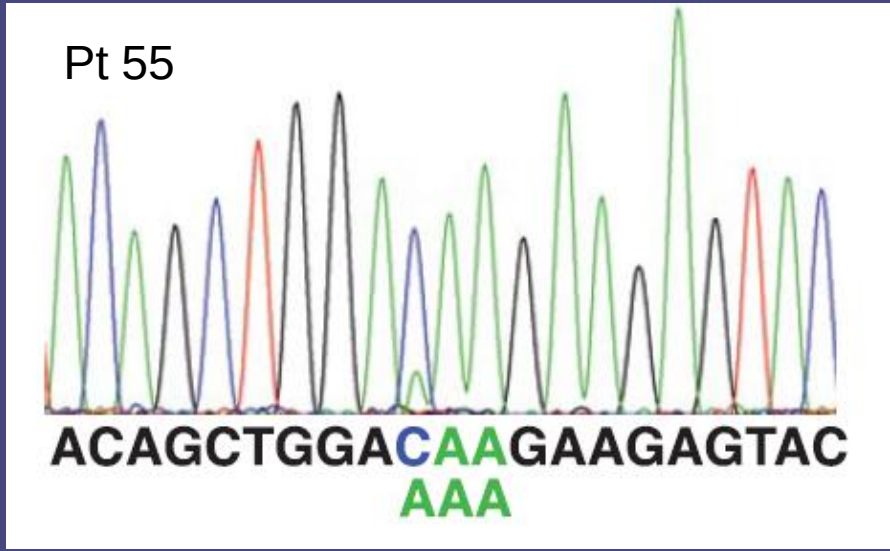

and patients



Nazarian et al. Nature 2010

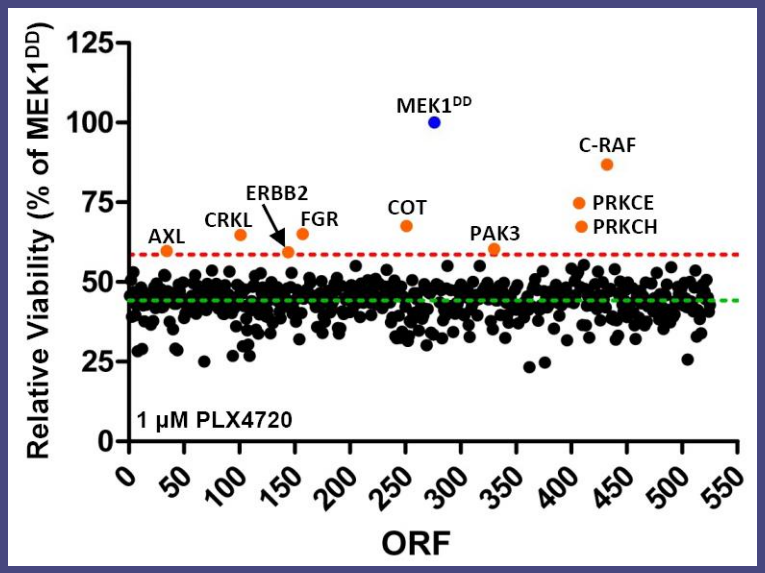
# Emergence of an NRAS mutation in vitro & in vivo



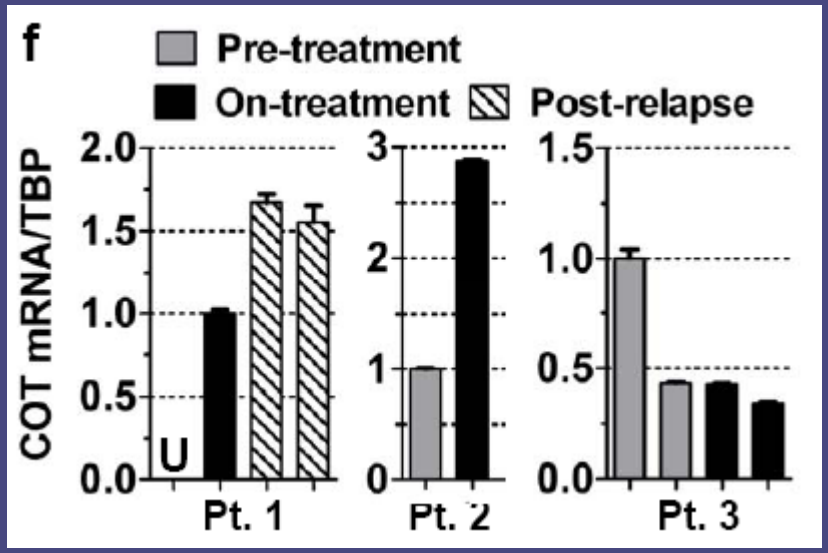




Nazarian et al.
Nature 2010

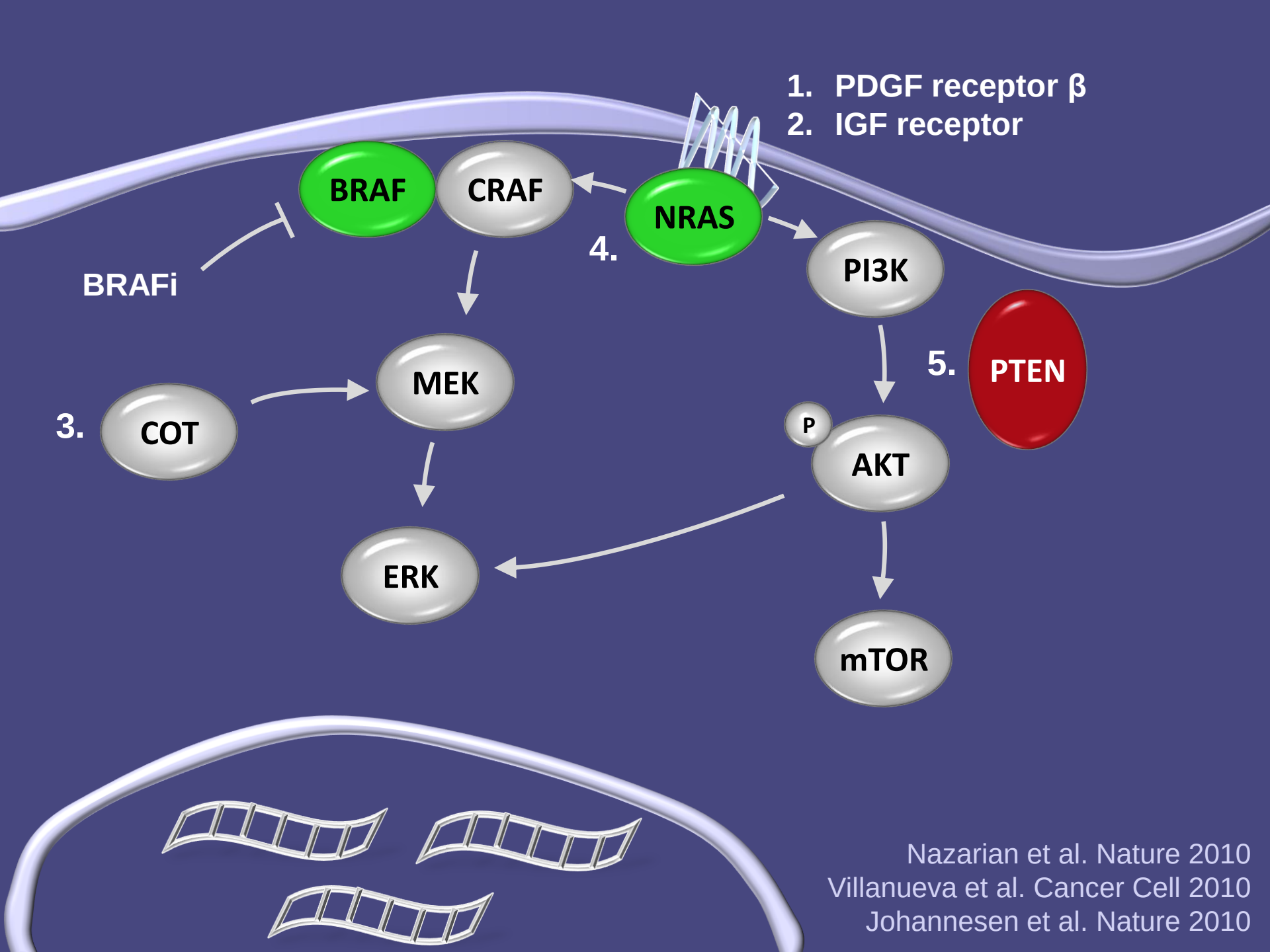
# Forced expression of kinome reveals COT (TPL2/MAP3K8 conferring resistance




| Secondary Screen | | | | | | | |
|---|---|---|---|---|---|---|---|
| A375 | | | | SKMEL28 | | | |
| Gene | $GI_{50}$ (µM) | Fold Change $GI_{50}$ | Rank | Gene | $GI_{50}$ (µM) | Fold Change $GI_{50}$ | Rank |
| *MAP3K8*/COT | >100.0 | 598 | 1 | *MAP3K8*/COT | >100.0 | ~100 | 1 |
| *RAF1* /C-RAF | ≥100.0 | 598 | 2 | *RAF1* /C-RAF | ≥10.0 | ≥10 | 2 |
| *CRKL* | >10.0 | 59.8 | 3 | *CRKL* | 9.7 | 9.7 | 3 |
| *FGR* | >10.0 | 59.8 | 4 | *FGR* | 5 | 5 | 4 |
| *PRKCE* | 4.41 | 26.4 | 5 | *PRKCH* | 2.26 | 2.26 | 5 |
| *PRKCH* | 4.14 | 24.7 | 6 | *PRKCE* | 1.91 | 1.91 | 6 |
| *ERBB2* | 1.33 | 7.95 | 7 | *AXL* | 1.18 | 1.18 | 7 |
| *AXL* | 1 | 5.98 | 8 | *ERBB2* | 1 | 1 | 8 |
| *PAK3* | 0.4934 | 2.95 | 9 | *PAK3* | 0.9041 | 0.9041 | 9 |



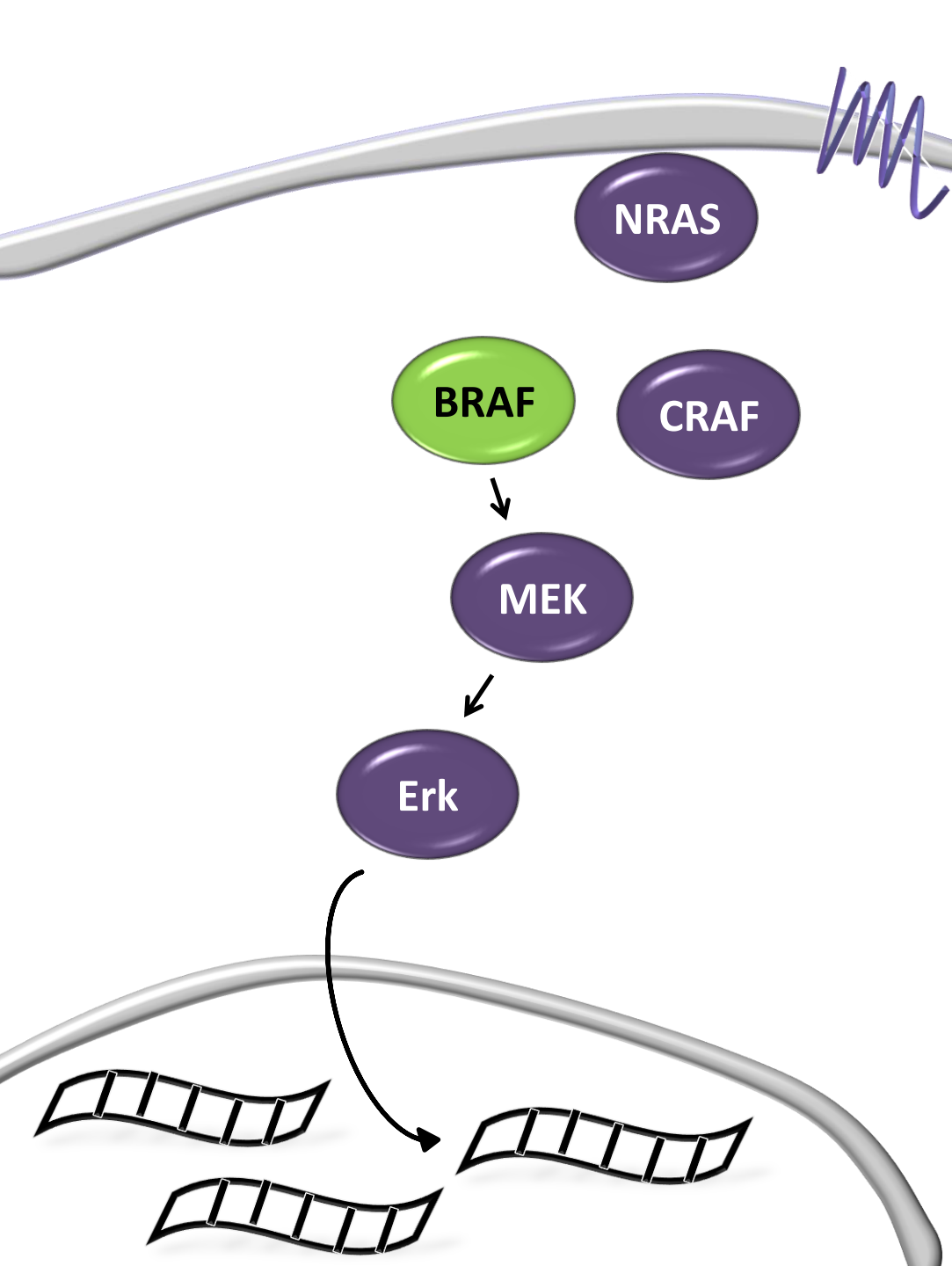

Johannesen et al.
Nature 2010




Nazarian et al. Nature 2010
Villanueva et al. Cancer Cell 2010
Johannesen et al. Nature 2010

# BRAF inhibition: future directions

- Establish mechanism(s) of primary & secondary resistance
- Combinations with inhibitors of concomitantly activated oncogenic pathways
- Development of agents/regimens that intercept mechanisms of resistance: sequential therapy
- Combinations with immunotherapy




Upregulated genes:
Cyclin D
p27
MYC

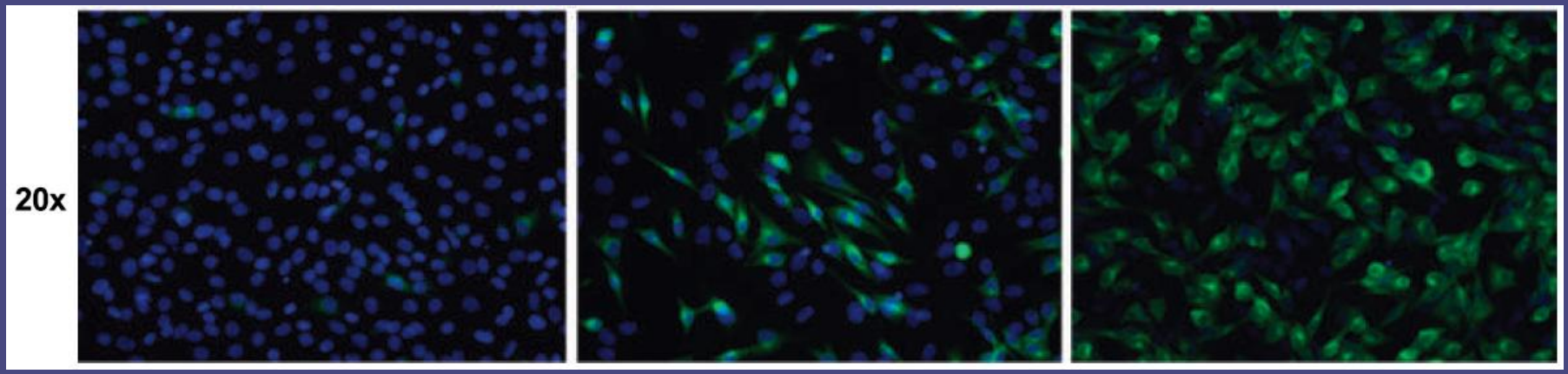
Downregulated genes:
MITF
BIM

Packer LM et al. PCMR 2009 Dec;22(6):785

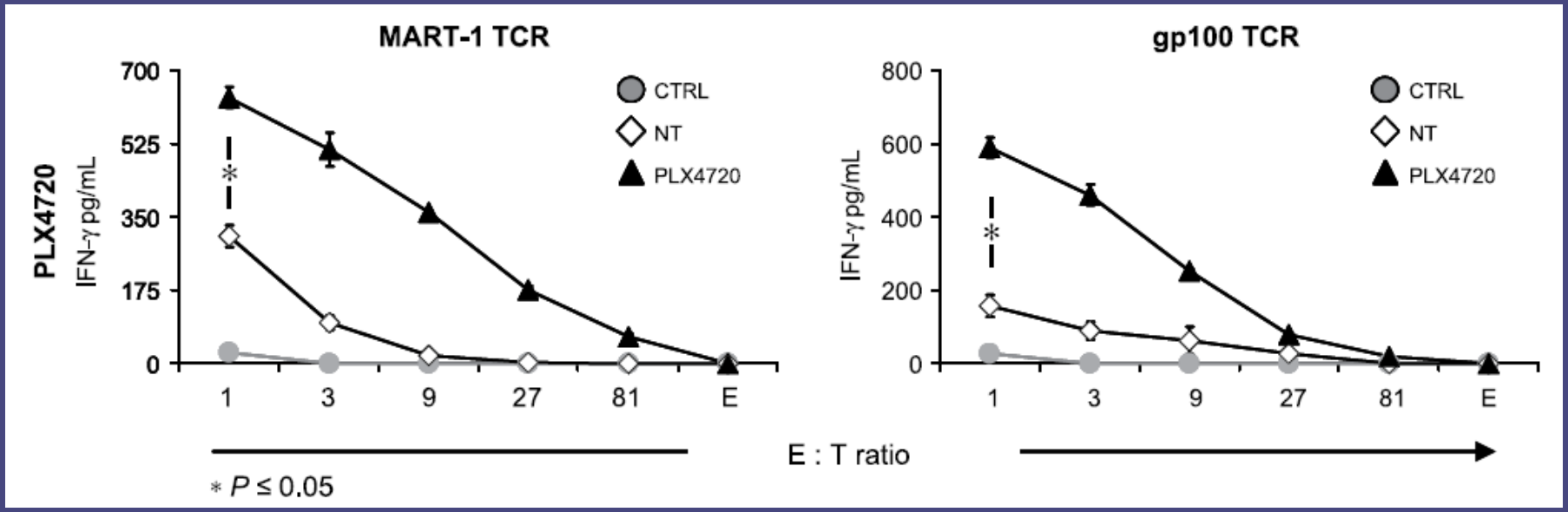
# Effect of BRAF inhibition of antigen expression & T cell recognition

Boni et al. Cancer Res 2010

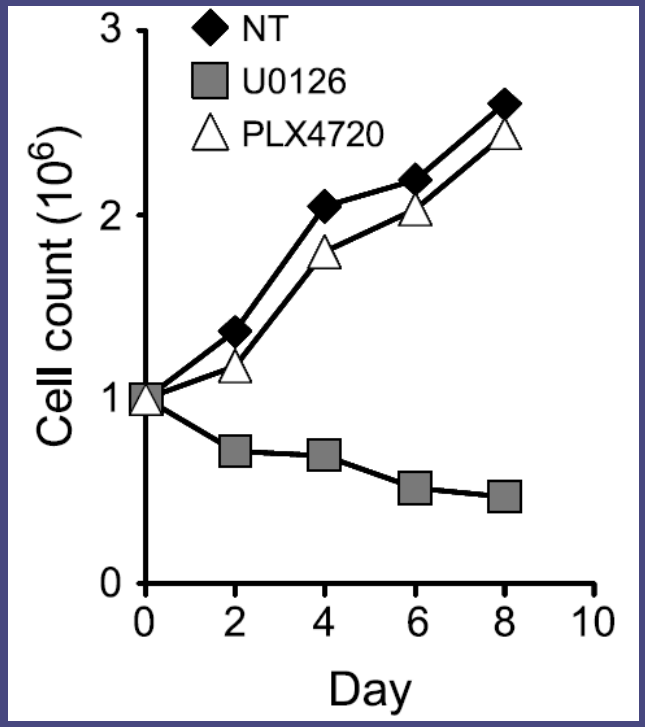
MART-1 expression in UACC903

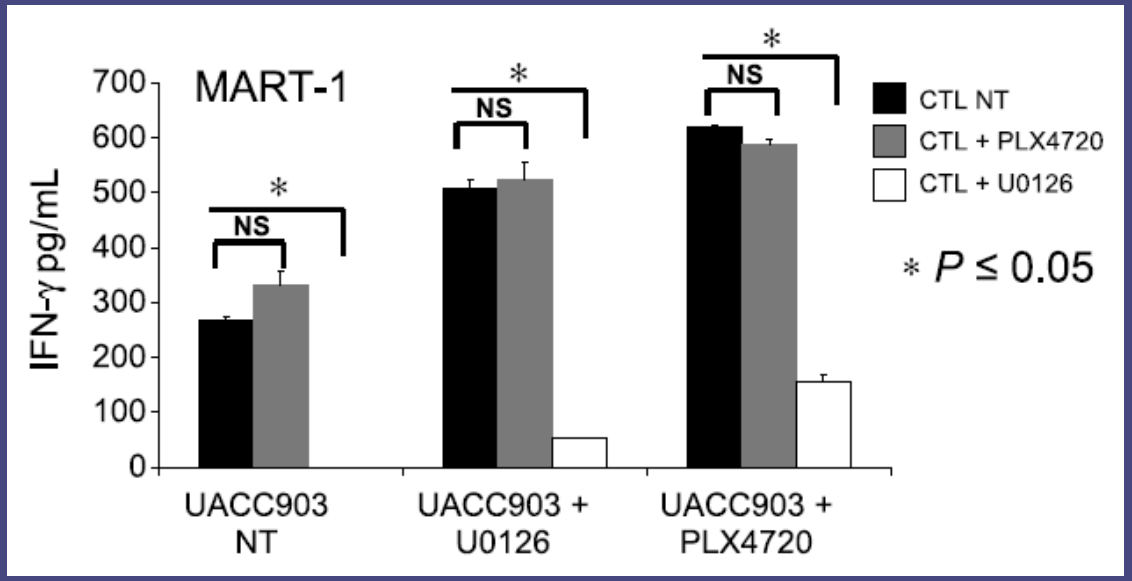



Control PD0325901 PLX4720



# MEK inhibitor, but not BRAF inhibitor, impair lymphocyte viability




Boni et al. Cancer Res 2010