PLX8394

BRAF-MAPK Paradox Breaker

CRUK Combinations Alliance

Plexxikon Inc.

March 23, 2016

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Plexxikon's Development Pipeline

Compound	Target	Indication	Stage of Development
PLX4032 (vemurafenib)	BRAF	Adjuvant Melanoma	Ph3
PLX3397 (pexidartinib)	FMS	PVNS (TGCT)	Ph3
PLX3397 + RT + TMZ	FMS, KIT	Adjuvant GBM	Ph2
PLX3397 + paclitaxel	FMS, KIT	Advanced Ovarian Cancer	Ph2
PLX3397 + pembro	FMS	Melanoma, Solid Tumors	Ph2
PLX3397	KIT	KIT-mutant Melanoma	Ph2
PLX3397	FMS	Alzheimer's/Imaging	Ph1
PLX7486	TRK, FMS	Pain, Oncology	Ph1
PLX9486	KIT-mutant	GIST, KIT-mutant tumors	Ph1
PLX8394	BRAF	BRAF-mutant tumors	Ph1
PLX51107 (2016)	BRD4	Leukemia	Pre-IND
PLX73086 (AC708) (2016)	FMS	TGCT, non-oncology	Pre-IND

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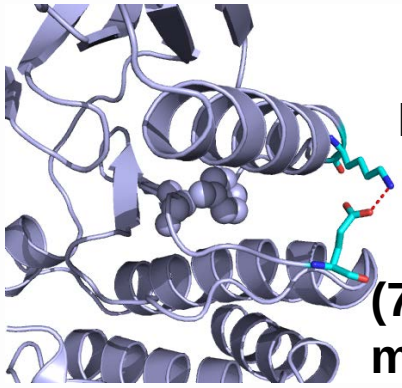
Clinically Actionable BRAF Mutations in Melanoma

Oncogenic BRAF mutation at V600 promotes RAS-independent MAPK signaling in melanoma (*6.27.2002*)

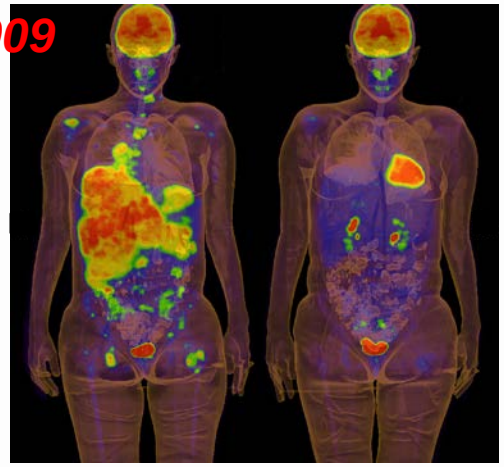


K507

E600

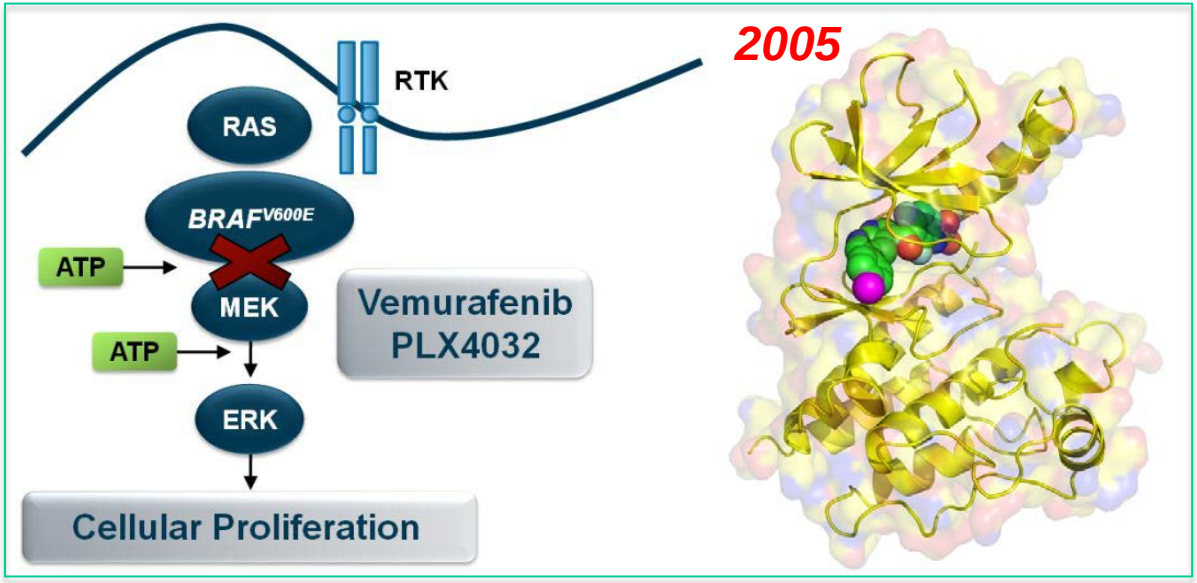
(700 fold more active)

2009



FDA approval

8.17.2011



2005

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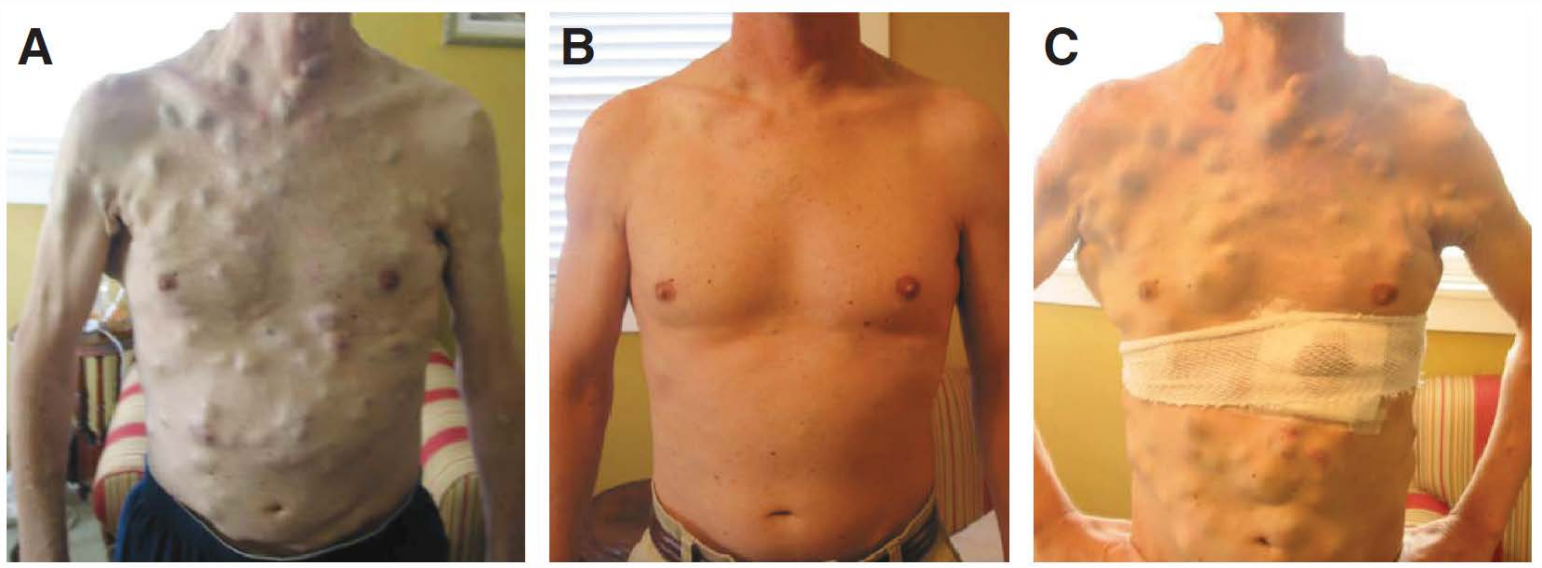
PLX8394 – Rationale for Collaboration with Combinations Alliance

- Due to its paradox-breaking properties, PLX8394 may be the most combinable BRAFi inhibitor in clinical development
- Plexxikon is developing PLX8394 as a single agent in niche indications, based on vemurafenib efficacy but desiring improved tolerability
- Proposed combinations for the Combinations Alliance
  - MEK inhibitors
  - Immunotherapies
    - Anti-PD1
    - Anti-PDL1
    - Anti-CTLA4
    - Other immune checkpoint inhibitors
  - EGFR inhibitors
  - Epigenetic modulators (HDACi, BRDi)

BRAF-mutant Glioblastoma Responding to Vemurafenib

Vemurafenib – Response and Resistance

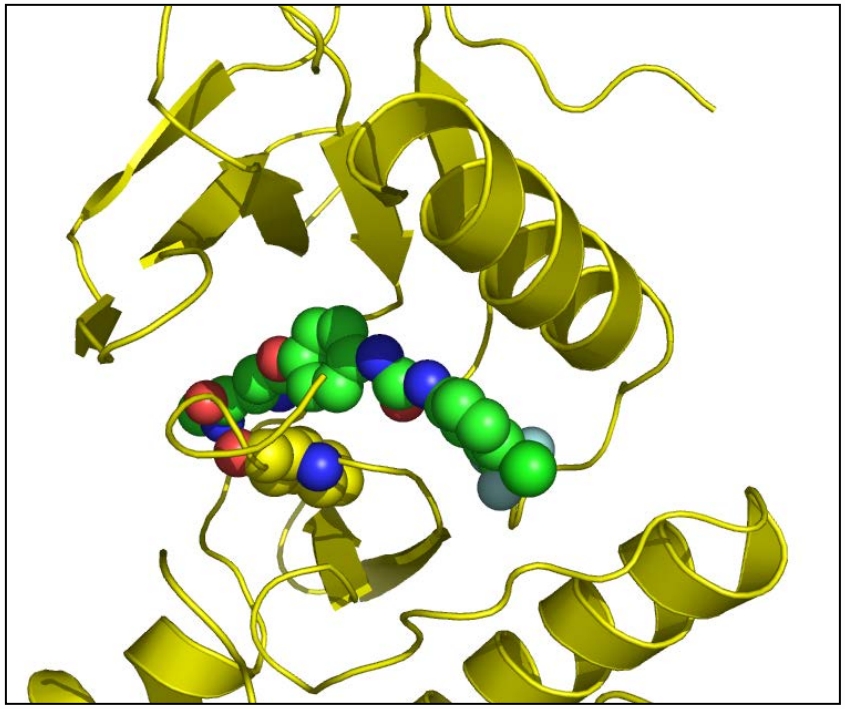


Reactivation of MAPK pathway through acquired MEK1^{C121S} mutation

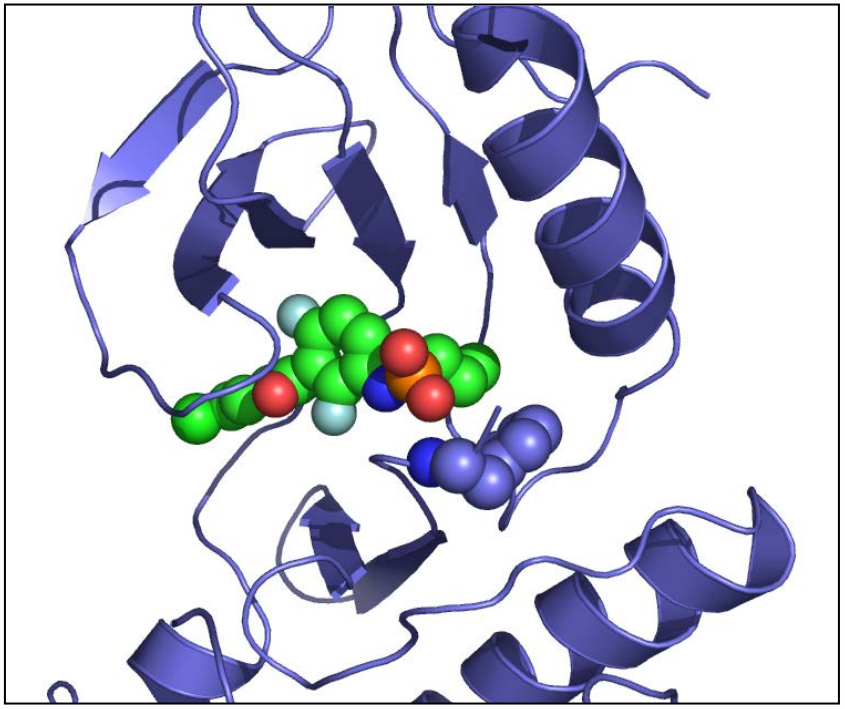
Wagle, Garraway, et al. J Clin Oncol. 2011

Correlation between Clinical Response and Mode of Inhibition

Sorafenib binds preferably the "DFG-out" state of Raf



Vemurafenib binds preferably the "DFG-in" state of Raf



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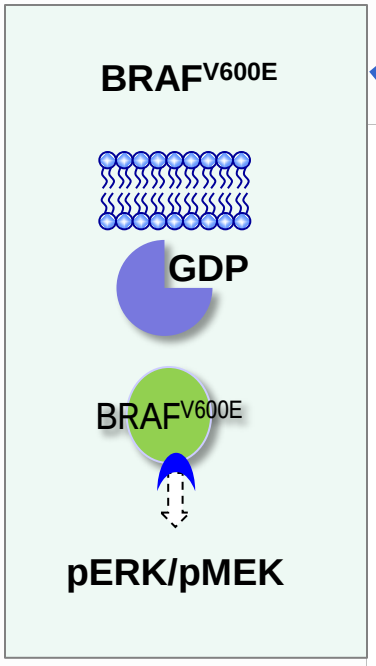
Paradoxical Activation of MAPK Pathway by BRAF Inhibitors in RAS-activated Cells

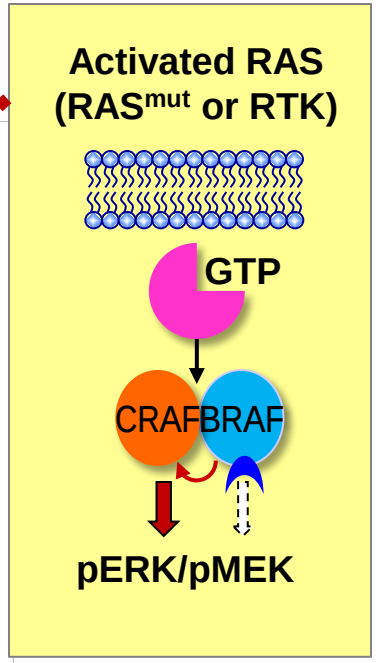
Phospho-Selectivity

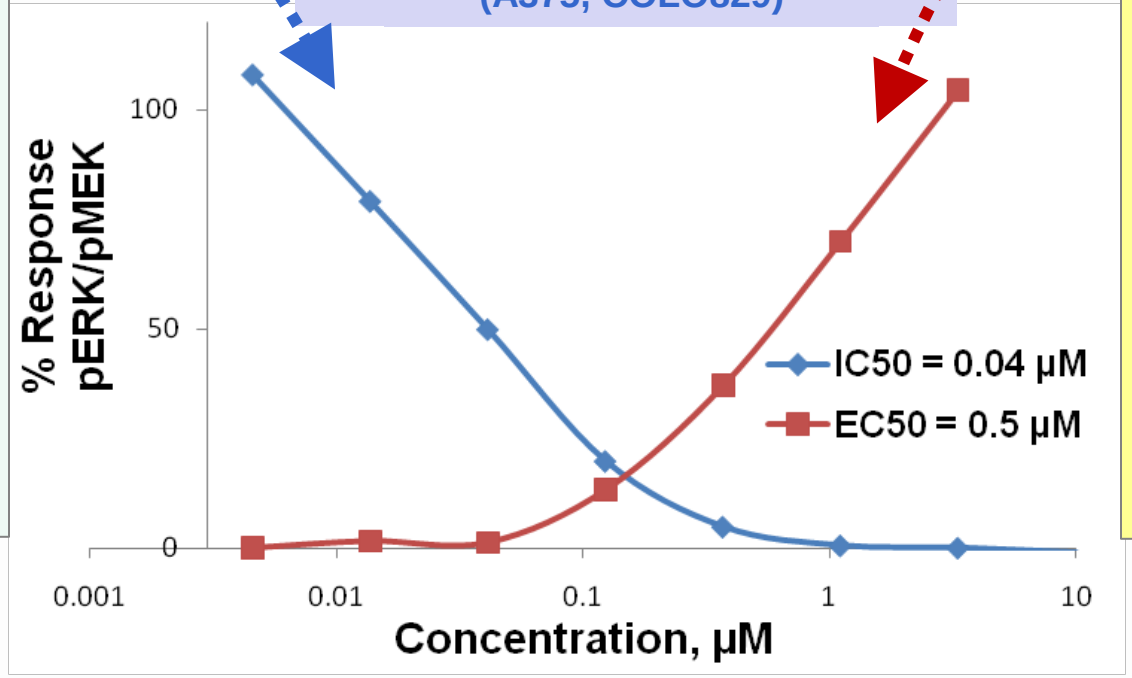
$$= \frac{\text{EC50 for RAS pathway activation (IPC-298, B9, HCT116)}}{\text{IC50 for BRAF pathway inhibition (A375, COLO829)}}$$

Inhibition



Activation





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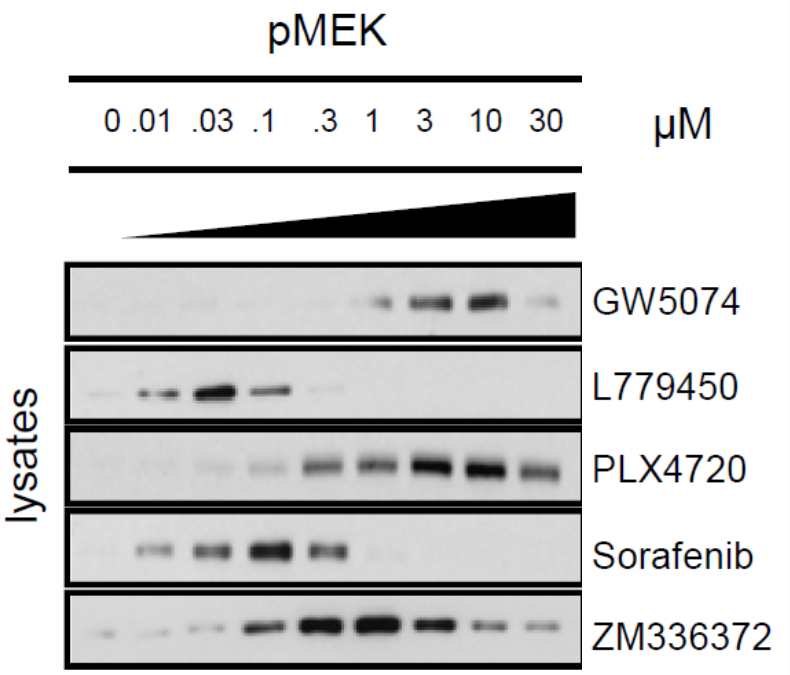
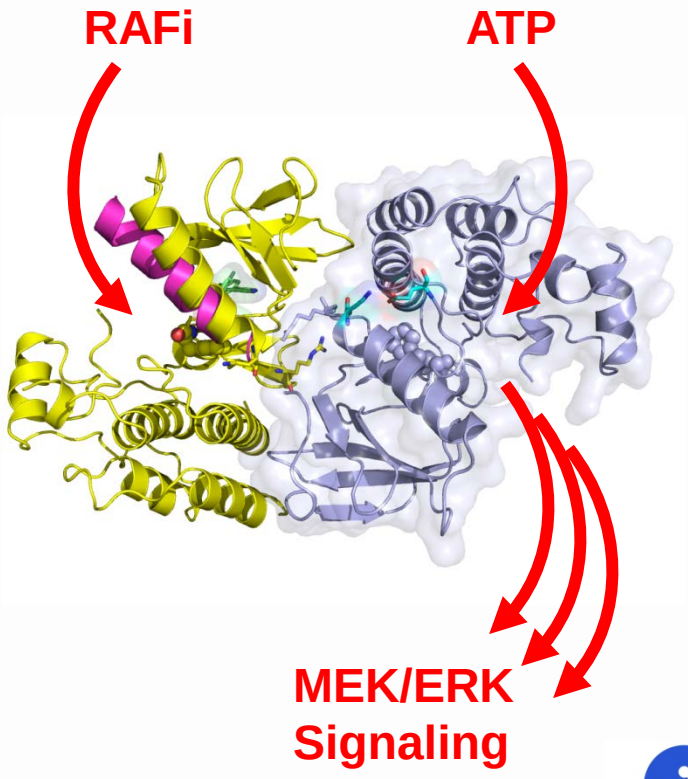
Studies on the RAF Inhibitor Paradox

5 different RAF inhibitors all activate the MEK/ERK Pathway

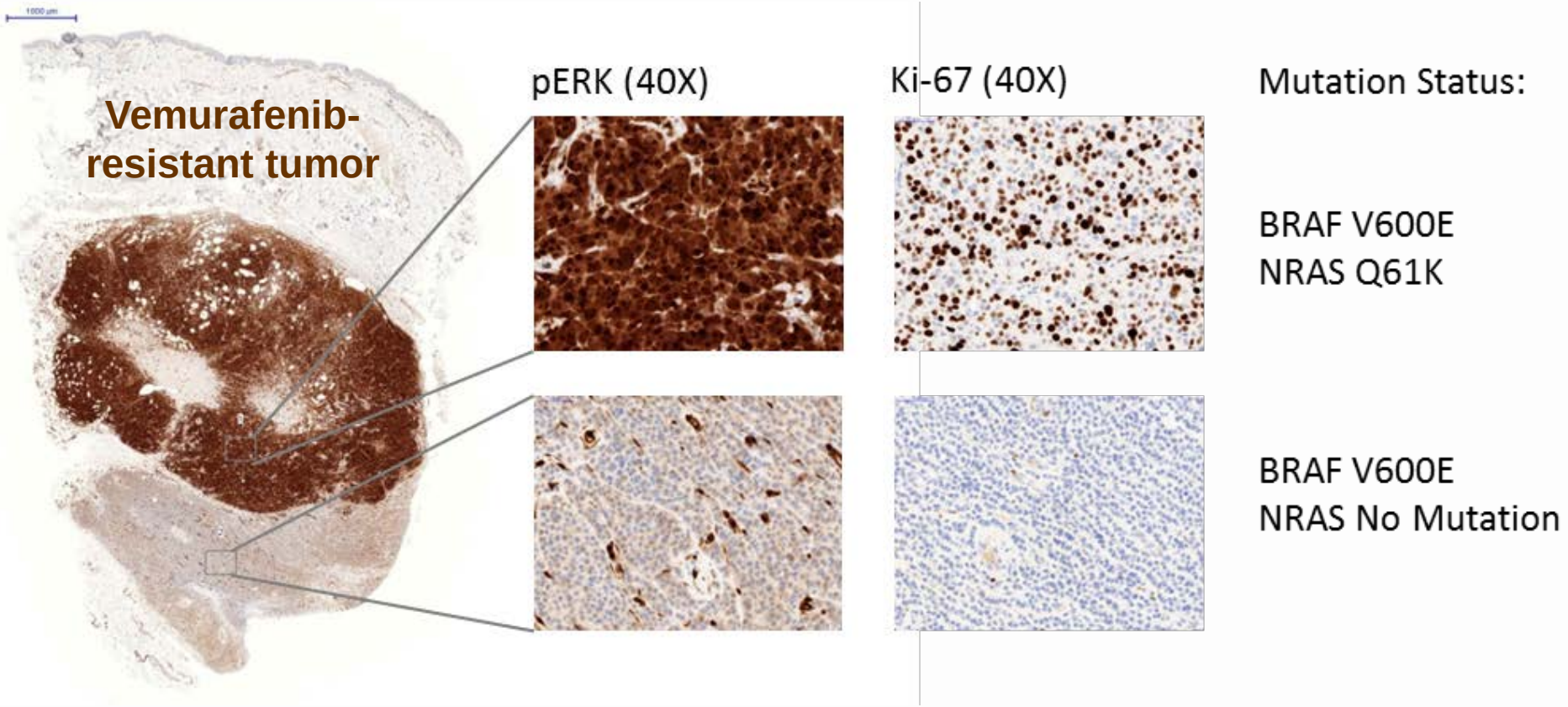
Cell 2010
Nature 2010
Nature 2010
PCMR 2010
Oncogene 2010
Neoplasia 2010
PNAS 2010





Poulikakos et al. Nature 2010

Paired Biopsy Data – Resistant Tumors Usually Re-Activate the MAP Kinase Pathway



Trunzer et al. J Clin Oncol. 2013

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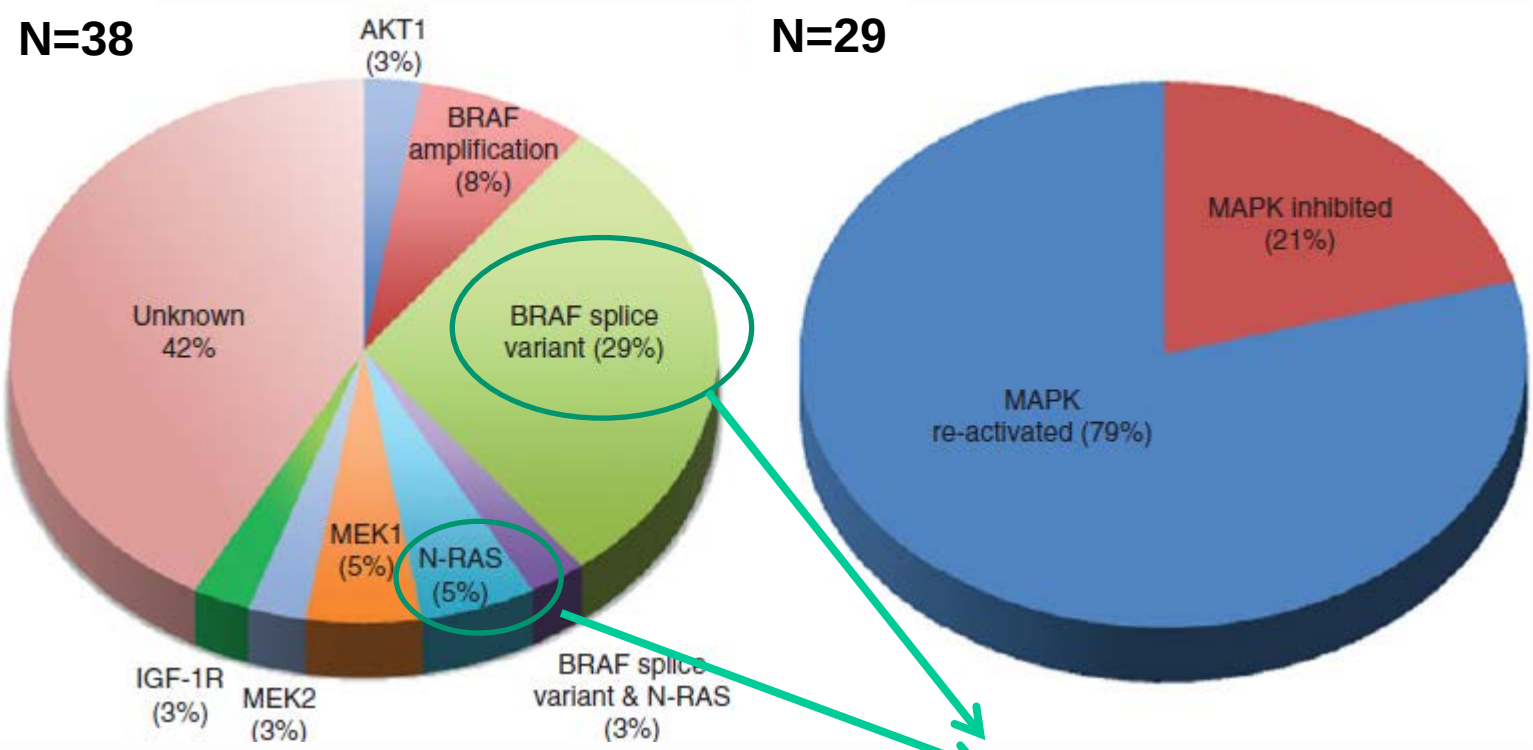
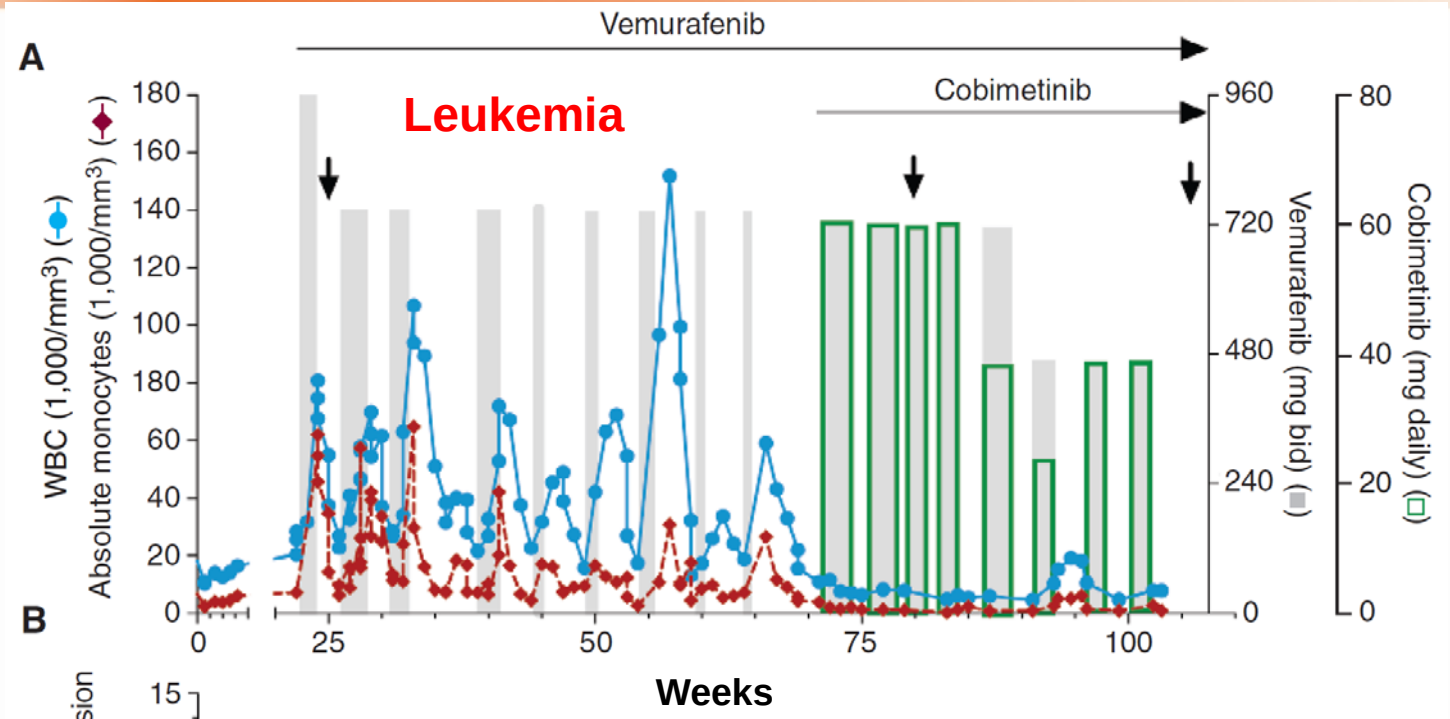
BRAF Inhibitor Resistance Mechanisms



- Aberrant splicing of BRAF
- Elevated expression of CRAF, COT1, mutant BRAF
- Activating mutations in NRAS, MEK1/2, AKT1 or BRAF
- Lost of PTEN (activation of PI3K), Lost of NF1 (activation of RAS)
- Activation of RTKs (PDGFRβ, IGF-1R, EGFR)
- Microenvironment (e.g. stromal derived HGF)

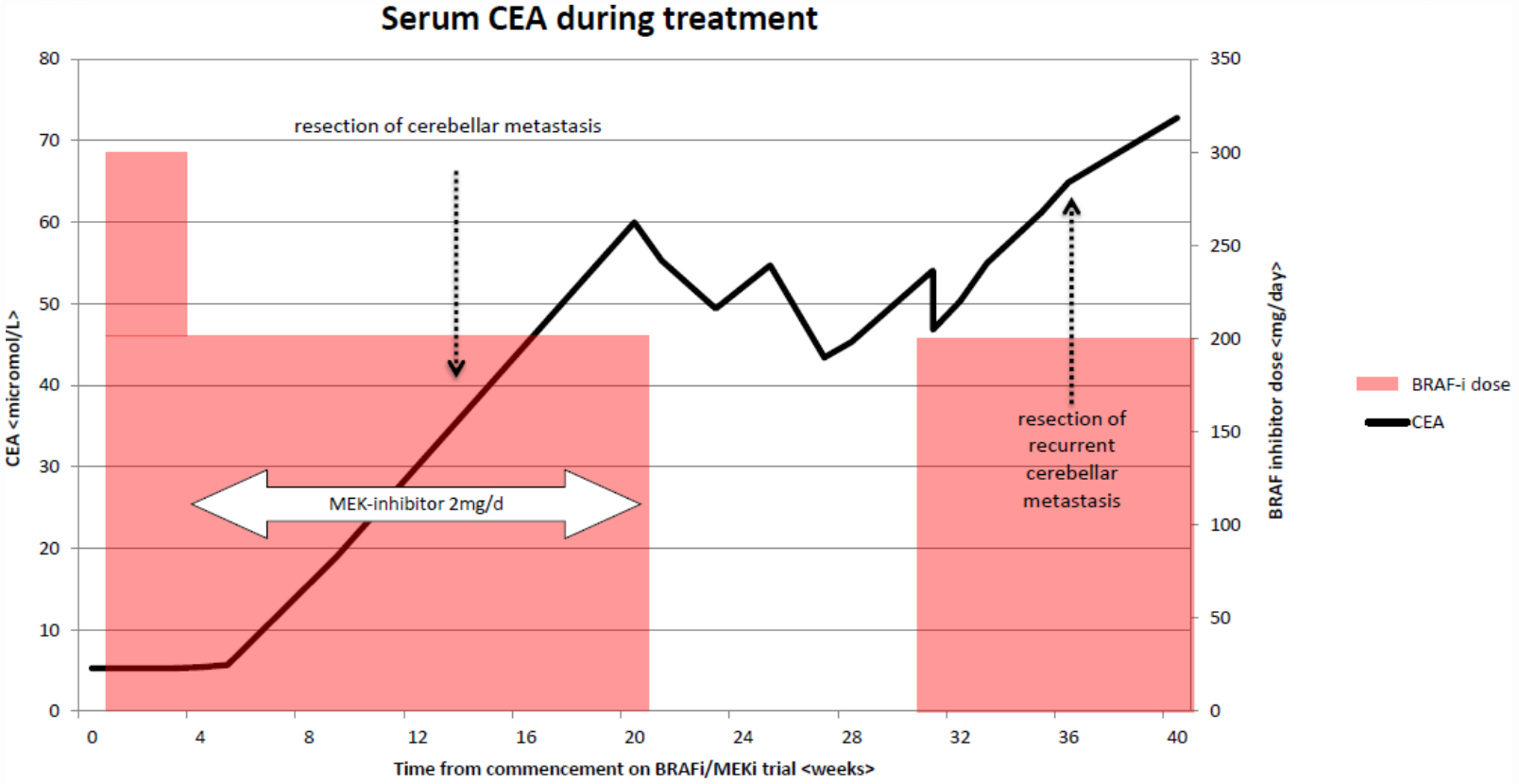
Rizos et al., Clin Cancer Res 2014

Progression of RAS-Mutant Leukemia during RAF Inhibitor Treatment



Callahan et al. NEJM 2012
Abdel-Wahab et al. Cancer Discovery 2014

Progression of RAS-Mutant Metastatic CRC during RAF/MEK Inhibitor Treatment

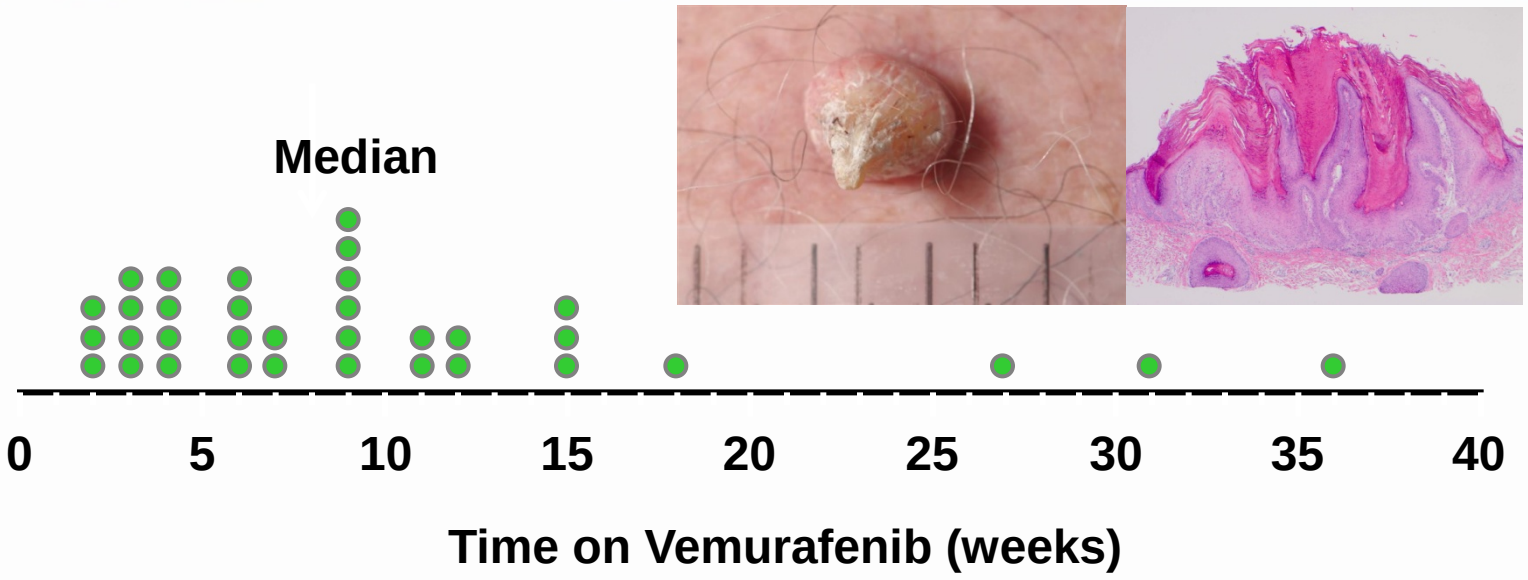


Paradoxical activation can be seen in other premalignant lesions even with RAFi-MEKi combined treatment.

Andrews, et al., Cebon et al, JCO 2013

Rapid cSCC emergence on RAF inhibitors

Majority of tumors have *RAS* mutations



- **Median time to first incidence 8 weeks (range 2–36)**
- **Each dot represents one patient:**

  # weeks to development of first cuSCC/KA lesion
- **21/35 (60%) of samples have *RAS* mutations**

Su et al. NEJM 2012

PLX8394: Next Generation BRAF Inhibitor
Paradox Breaker (PB)

- Opportunity:

  Avoiding paradoxical MAPK pathway activation

- Hypothesis:

  PLX8394 as a ‘paradox breaker’ might

  – Improve response of some V600 tumors

  – Delay resistance (1st gen compounds enable pathway re-ignition)

  – Reduce toxicities such as skin lesions

  – Combine better with Immunotherapy

- Status:

  Currently in clinical development

LETTER

doi:10.1038/nature14982

RAF inhibitors that evade paradoxical MAPK pathway activation

Chao Zhang[1], Wayne Spevak[1], Ying Zhang[1], Elizabeth A. Burton[1], Yan Ma[1], Gaston Habets[1], Jiazhong Zhang[1], Jack Lin[1], Todd Ewing[1], Bernice Matusow[1], Garson Tsang[1], Adhirai Marimuthu[1], Hanna Cho[1], Guoxian Wu[1], Weiru Wang[1], Daniel Fong[1], Hoa Nguyen[1], Songyuan Shi[1], Patrick Womack[1], Marika Nespi[1], Rafe Shellooe[1], Heidi Carias[1], Ben Powell[1], Emily Light[1], Laura Sanftner[1], Jason Walters[1], James Tsai[1], Brian L. West[1], Gary Visor[1], Hamid Rezaei[1], Paul S. Lin[1], Keith Nolop[1], Prabha N. Ibrahim[1], Peter Hirth[1] & Gideon Bollag[1]

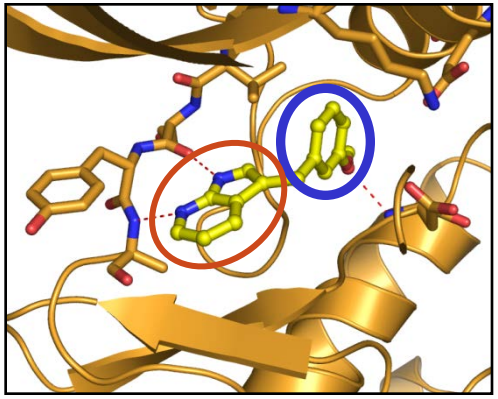
Oncogenic activation of BRAF fuels cancer growth by constitutively promoting RAS-independent mitogen-activated protein kinase (MAPK) pathway signalling[1]. Accordingly, RAF inhibitors have brought substantially improved personalized treatment of metastatic melanoma[2–5]. However, these targeted agents have also revealed an unexpected consequence: stimulated growth of certain cancers[6–9]. Structurally diverse ATP-competitive RAF inhibitors can either inhibit or paradoxically activate the MAPK pathway, depending

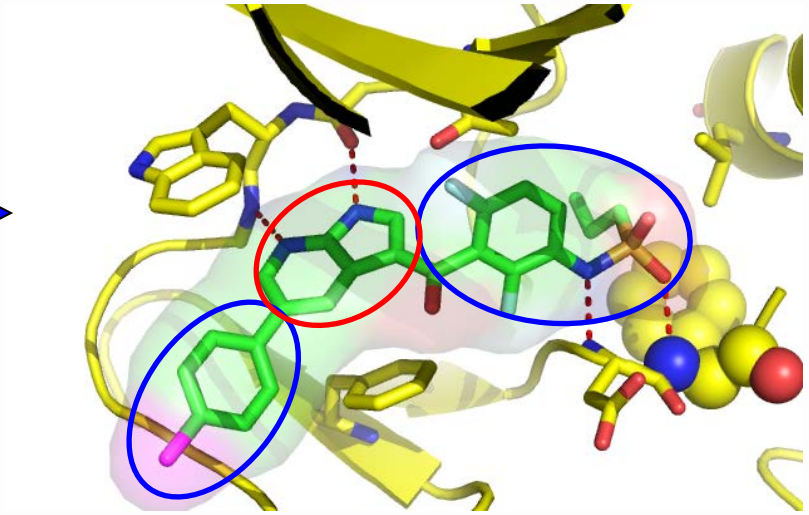
the MAPK pathway in cells bearing oncogenic RAS or elevated upstream receptor signalling[10–12]. This paradox can promote cellular proliferation and manifest clinically with progression of cutaneous squamous cell carcinomas (cuSCC) and keratoacanthomas, sometimes within weeks of therapy initiation[6,15]. These paradox-induced skin tumours have an uncharacteristically high incidence of RAS mutations[6,16], raising the concern that the same mechanism might accelerate progression of other RAS-driven cancers. Recent case reports of increased incidence of prim-

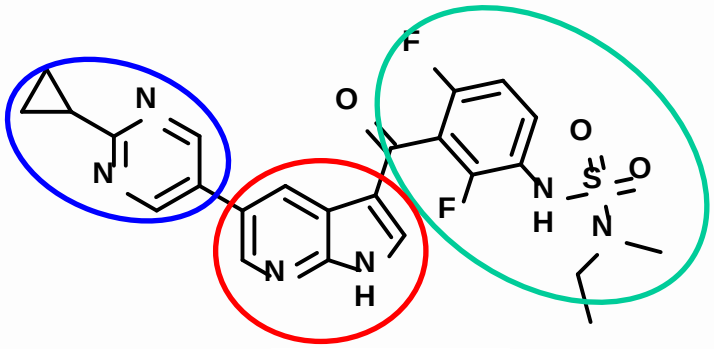
Zhang et al., *Nature* **526**, 523-586 (22 October 2015).

From Hit to Development Candidate

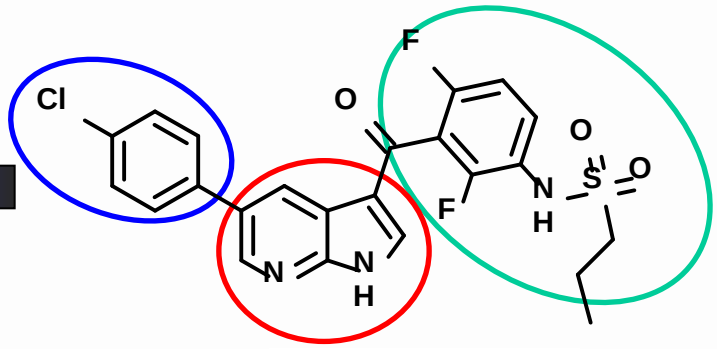
Discovery of selective RAF kinase inhibitors







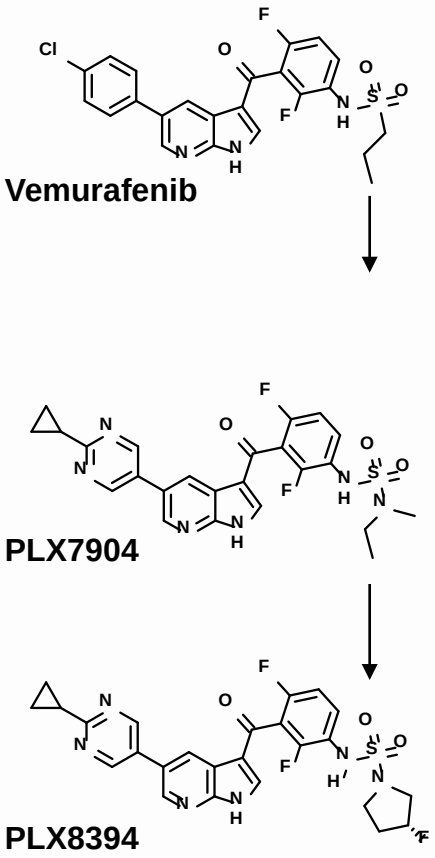
PLX8394

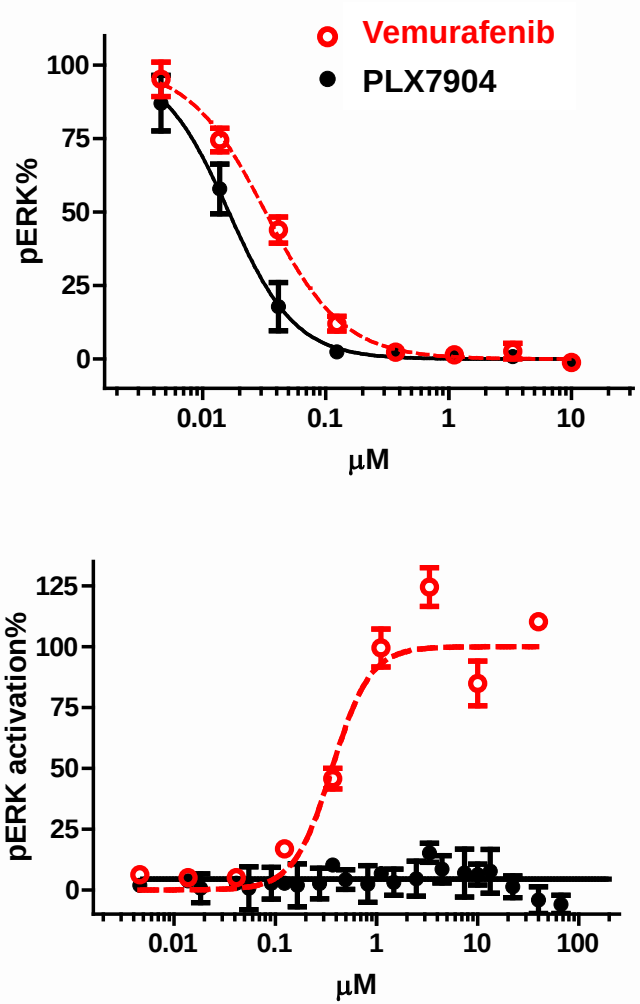


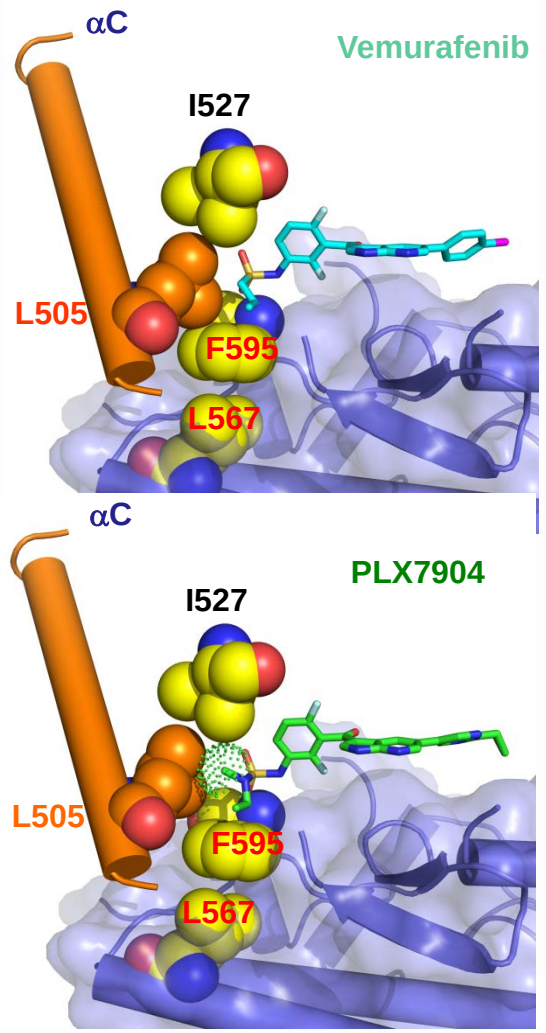
Vemurafenib

Nature 2010, 2015

Next-Gen BRAF Inhibitors Overcome Paradoxical MAPK Pathway Activation







Zhang et al., Nature 526, 583 (2015)

Allosteric Switch?

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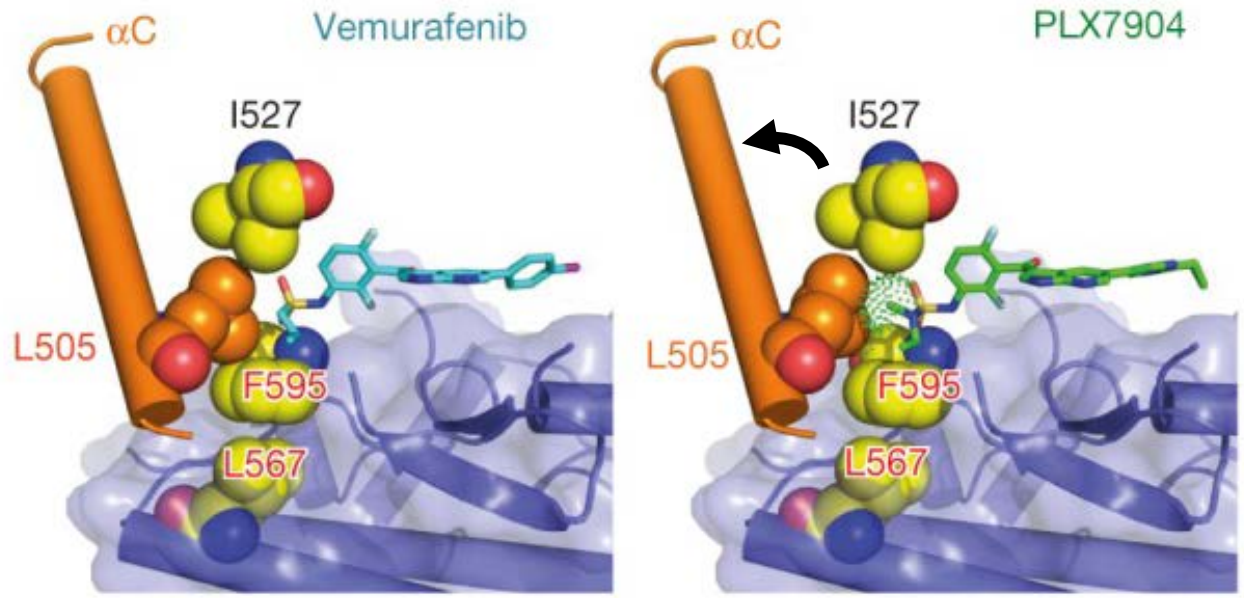
1st - generation BRAFi induce BRAF-CRAF Heterodimers, PLX8394 does not



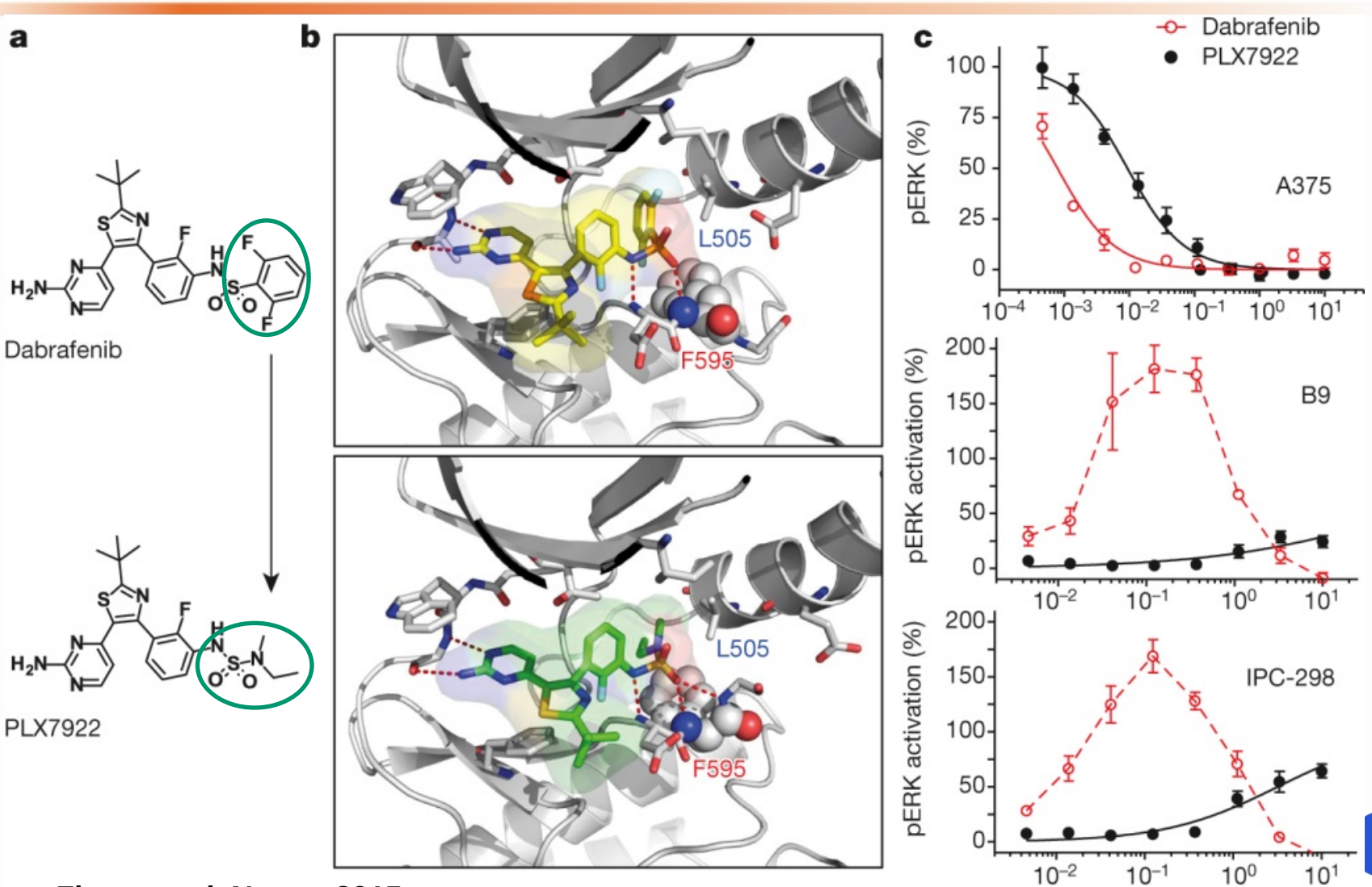
Dimerization
In B9 Cells

Vemurafenib

PLX8394

Zhang et al. Nature 2015

‘Paradox Breaking’ property can be transferred to another chemical series



Zhang et al. Nature 2015

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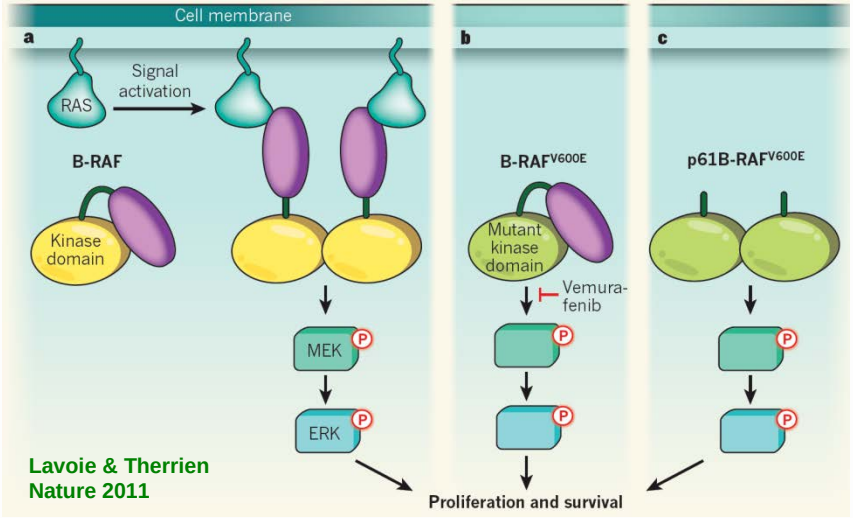
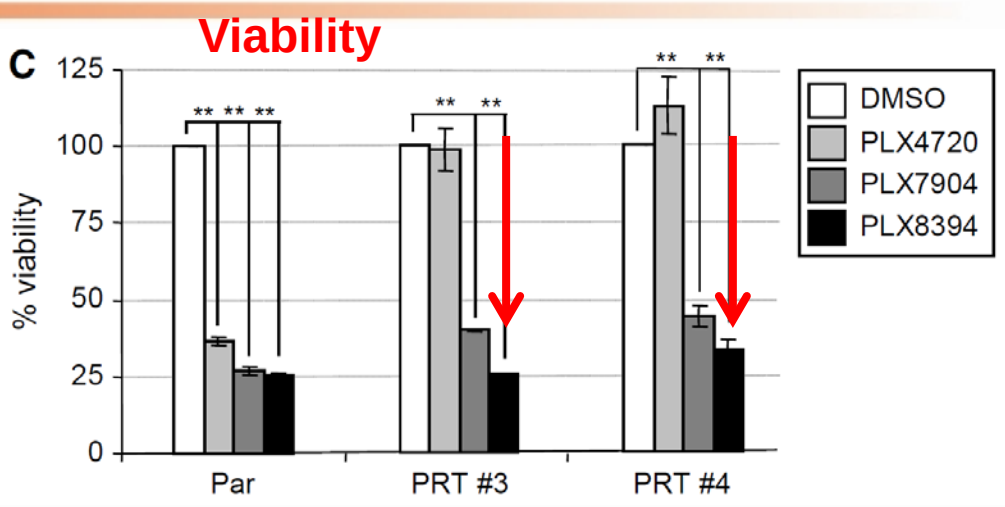
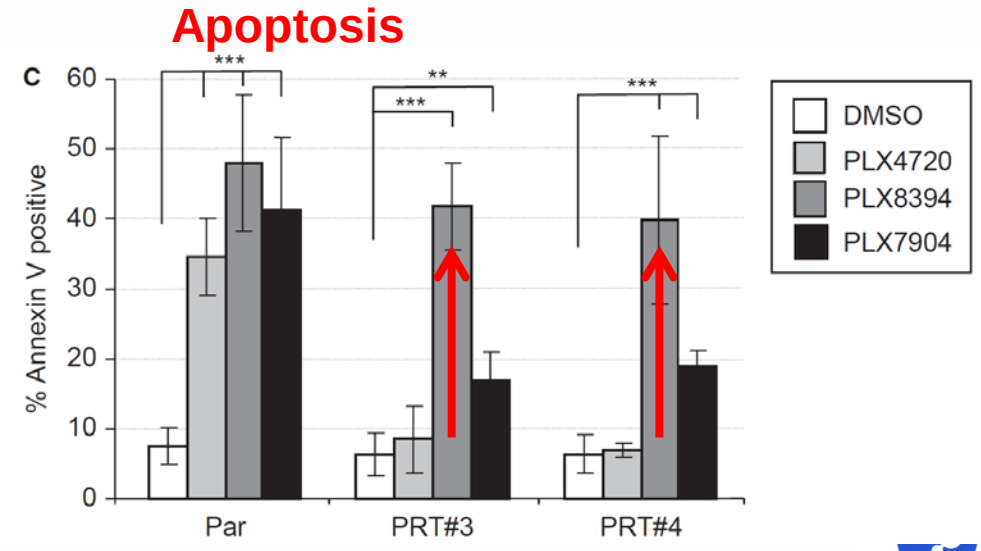
PLX8394 Is Active Against Vemurafenib-Resistant Cells







From **Poulikakos, Rosen, Solit, Nature 2011**

Resistance to BRAF^{V600E} melanoma

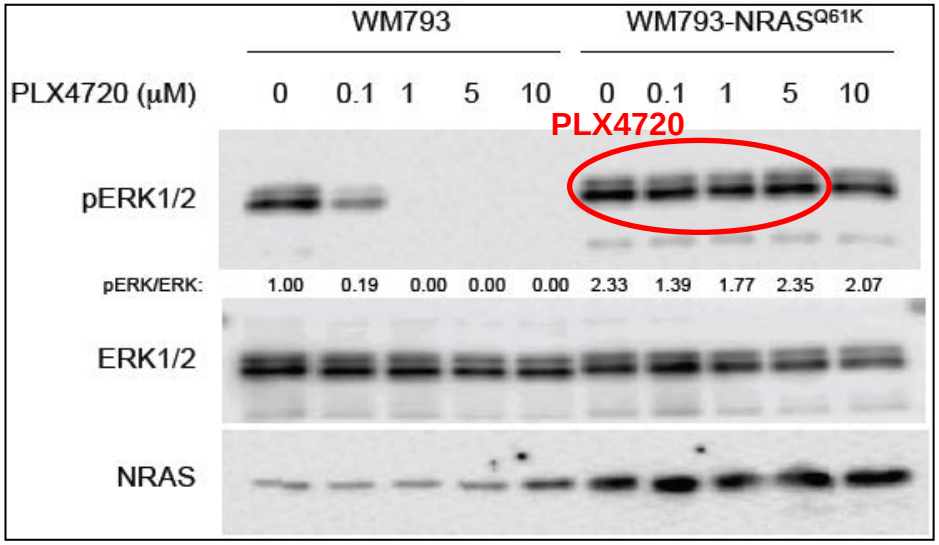
In vitro and in human patients

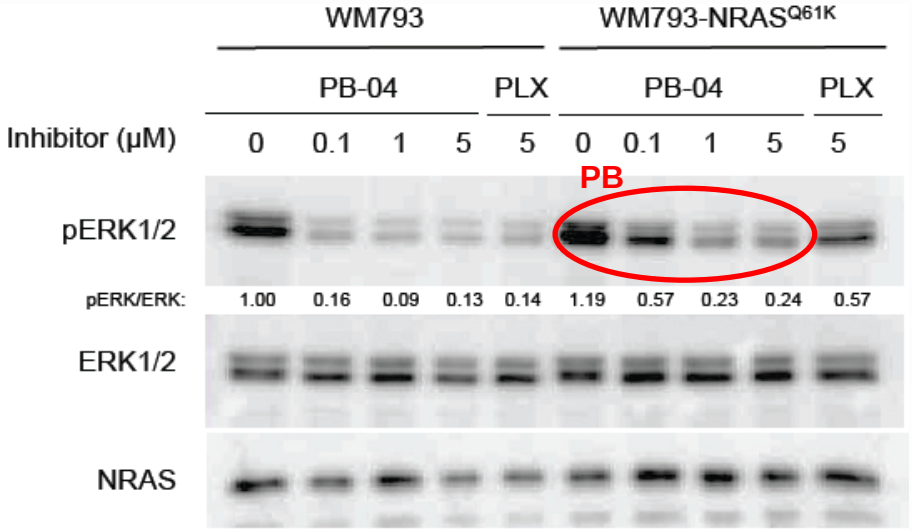
Mediated by alternatively spliced BRAF^{V600E}

Constitutive dimers are resistant to Vemurafenib

Basile, Le, Hartsough & Aplin. ***Pigment Cell Melanoma Research 2014***

PLX8394 inhibits ERK activation in BRAF^{mut}/NRAS^{mut} co-expressing melanoma cells

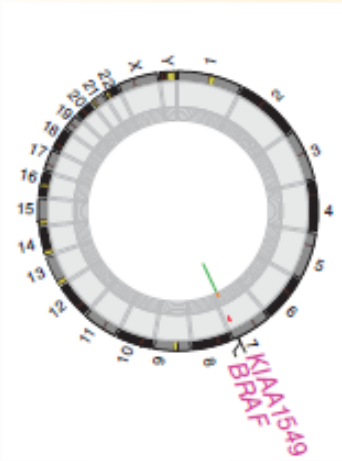


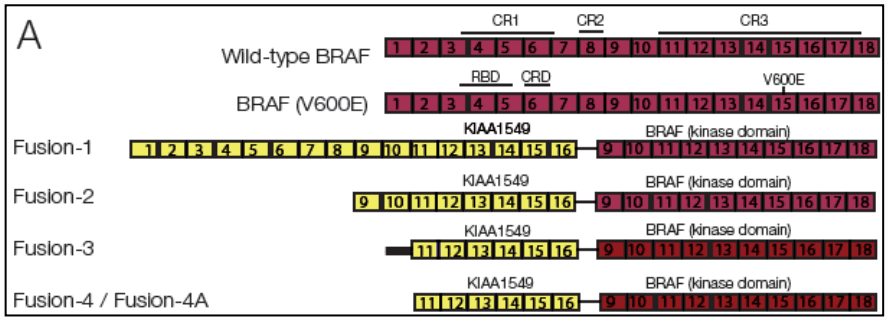


Re-activation of the ERK signaling pathway and development of acquired resistance are sometimes mediated by acquired mutations in NRAS (or selection of a small population of cells co-expressing mutant BRAF and NRAS)

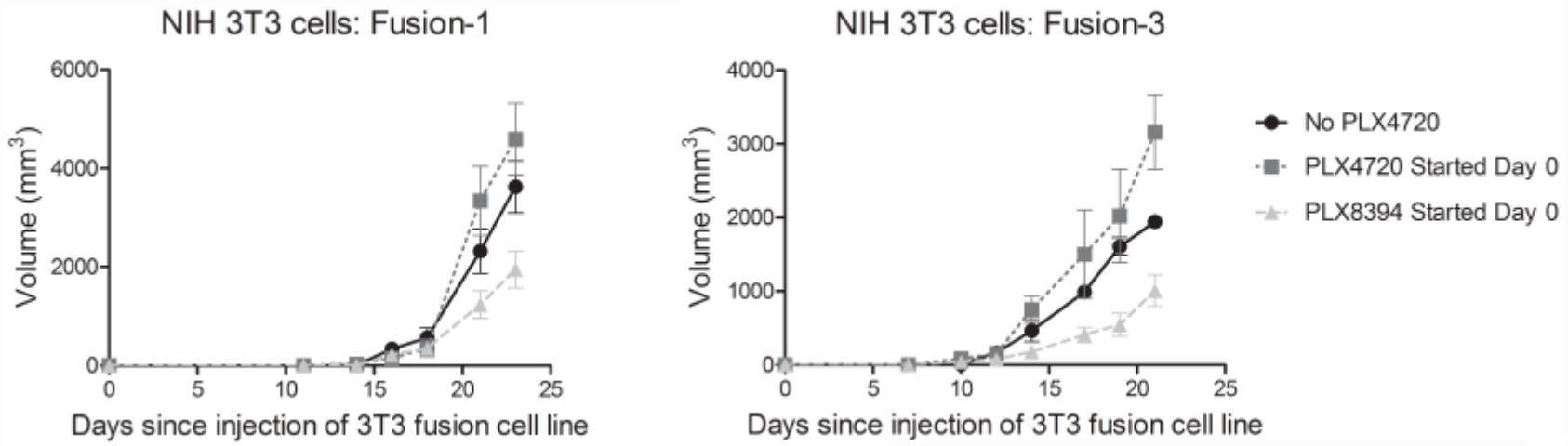
Le, Blomain, Rodeck & Aplin. ***Pigment Cell Melanoma Research 2013***

Effective against BRAF Kinase Fusions in vivo





>60% of pediatric astrocytoma caused by BRAF-fusion; paradoxical activation & BRAFi resistance



Adam Resnick et al., CHOP

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Frequency of BRAF mutations

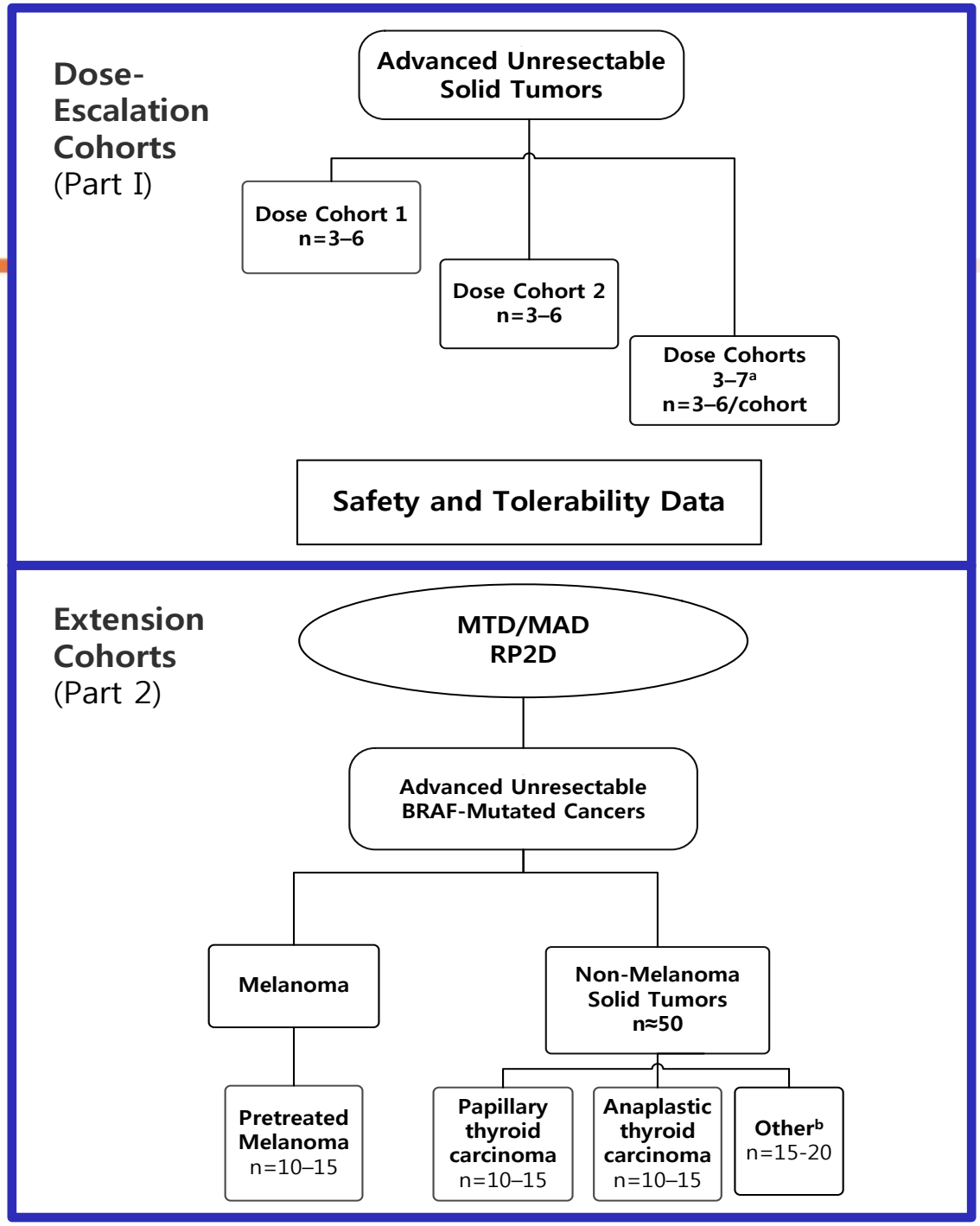
Multiple Potential Indications

~100% →	• Hairy Cell Leukemia, Papillary Craniopharyngiomas
30-80%	• Melanoma • Papillary and Anaplastic Thyroid Cancer • Langerhans Cell Histiocytosis, Erdheim-Chester Disease • Serous Ovarian Cancer • Astrocytoma/Glioblastoma
8-30%	• Cholangiocarcinoma • Colorectal Cancer
< 8%	• Non Small Cell Lung Cancer • Bladder Cancer • Prostate Cancer • Multiple Myeloma • Breast Cancer • GIST, Gastric Cancer, Barrett's Esophageal Cancer • Head and Neck Cancers

PLX120-03: PLX8394 Phase I Trial
STUDY DESIGN

- Part 1: “3+3” Dose escalation phase (n=up to 42)
  - Patients with advanced solid tumors refractory to standard therapy or no standard therapy exists or considered appropriate by the investigators
- Part 2: Extension cohort phase (n=65) at RP2D
  - Metastatic Melanoma
  - Papillary thyroid carcinoma (PTC)
  - Anaplastic thyroid carcinoma (ATC)
  - NSCLC, colorectal carcinoma (CRC) and other BRAF mutated malignancies

Study Design



[a] Potential, depending on the safety and tolerability data.

[b] For example, colorectal cancer, non-small-cell lung cancer, cholangiocarcinoma, histiocytosis (e.g., LCH, ECD).

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