



Journal Club

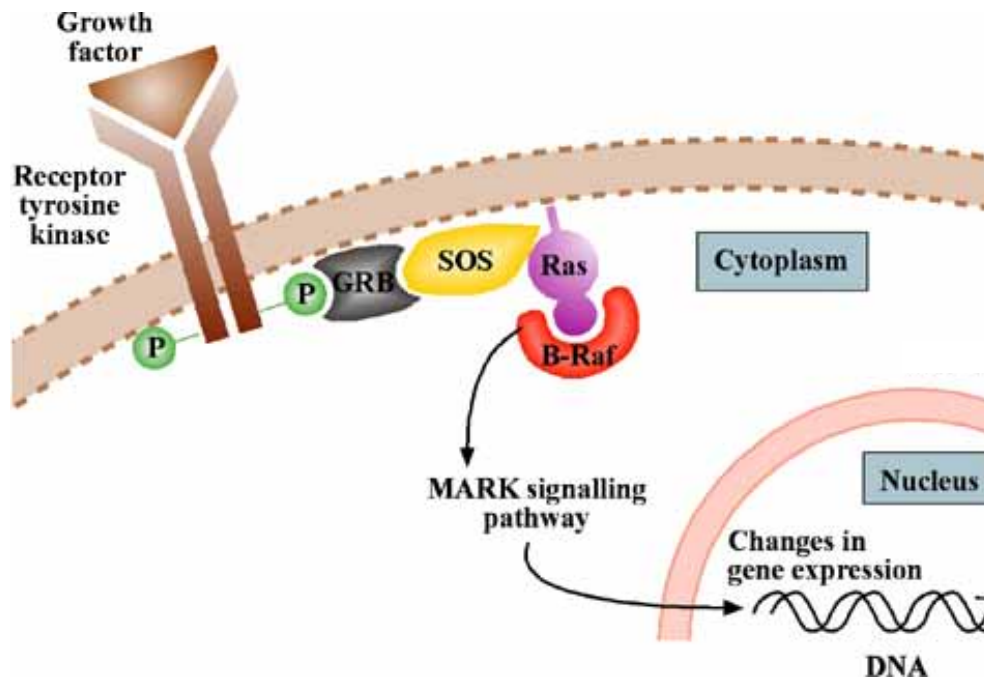
Medecine-Science

Session II: Innovations in Melanoma Treatment

David PUYRAIMOND-ZEMMOUR, TCEM₁ Oncology Paris VI
Eimad SHOTAR, TCEM₁ Radiology Paris V
CRI, 10/20/2010

Introduction

- The Ras-Raf-MEK-ERK signaling pathway has been implicated in human oncogenesis





Introduction

- The Ras-Raf-MEK-ERK signaling pathway has been implicated in human oncogenesis
- Mutations in BRAF have been found in 8% of human cancers
 - 50% of melanomas
 - 30-70% of thyroid cancers
 - 10% of colorectal cancers



Introduction

- The Ras-Raf-MEK-ERK signalling pathway has been implicated in human oncogenesis
- Mutations in BRAF have been found in 8% of human cancers
- Metastatic melanoma is an aggressive disease for which there are few effective therapies
 - The 2 therapies approved by the FDA are high dose Il-2 and decarbazine: response rates 10-20%
 - Neither improve overall survival
 - The median survival among patients treated with decarbazine <8 months

Discovery of selective inhibitors of oncogenic B-Raf kinase

PNAS

Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity

James Tsai*, John T. Lee[†], Weiru Wang*, Jiazhong Zhang*, Hanna Cho*, Shumeye Mamo*, Ryan Bremer*, Sam Gillette*, Jun Kong[†], Nikolas K. Haass[†], Katrin Sproesser[†], Ling Li[†], Keiran S. M. Smalley[†], Daniel Fong*, Yong-Liang Zhu*, Adhirai Marimuthu*, Hoa Nguyen*, Billy Lam*, Jennifer Liu*, Ivana Cheung*, Julie Rice*, Yoshihisa Suzuki*, Catherine Luu*, Calvin Settachatgul*, Rafe Shellooe*, John Cantwell*, Sung-Hou Kim[‡], Joseph Schlessinger^{§¶}, Kam Y. J. Zhang*, Brian L. West*, Ben Powell*, Gaston Habets*, Chao Zhang*, Prabha N. Ibrahim*, Peter Hirth*, Dean R. Artis*, Meenhard Herlyn^{¶¶}, and Gideon Bollag*[¶]

*Plexikon, Inc., 91 Bolivar Drive, Berkeley, CA 94710; [†]Department of Molecular and Cellular Oncogenesis, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104; [‡]220 Calvin Laboratory, University of California, Berkeley, CA 94720; and [§]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520

LETTER

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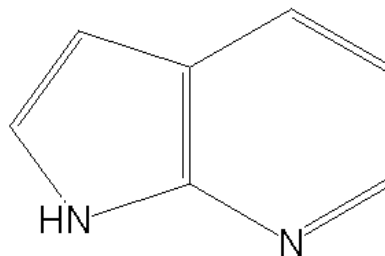
Clinical efficacy of a RAF inhibitor needs broad target blockade in *BRAF*-mutant melanoma

Gideon Bollag¹, Peter Hirth¹, James Tsai¹, Jiazhong Zhang¹, Prabha N. Ibrahim¹, Hanna Cho¹, Wayne Spevak¹, Chao Zhang¹, Ying Zhang¹, Gaston Habets¹, Elizabeth A. Burton¹, Bernice Wong¹, Garson Tsang¹, Brian L. West¹, Ben Powell¹, Rafe Shellooe¹, Adhirai Marimuthu¹, Hoa Nguyen¹, Kam Y. J. Zhang¹, Dean R. Artis¹, Joseph Schlessinger², Fei Su³, Brian Higgins³, Raman Iyer³, Kurt D'Andrea⁴, Astrid Koehler³, Michael Stumm³, Paul S. Lin¹, Richard J. Lee³, Joseph Grippo³, Igor Puzanov⁵, Kevin B. Kim⁶, Antoni Ribas⁷, Grant A. McArthur⁸, Jeffrey A. Sosman⁵, Paul B. Chapman⁹, Keith T. Flaherty^{4†}, Xiaowei Xu⁴, Katherine L. Nathanson⁴ & Keith Nolop¹

Discovery of selective inhibitors of oncogenic B-Raf kinase

I Scaffold and structure based Discovery of the inhibitors

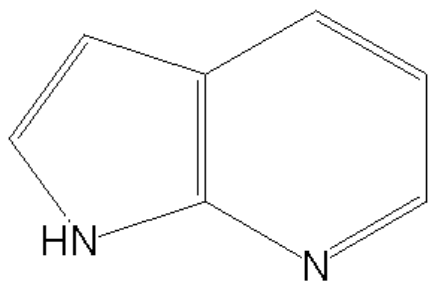
- 20 000 compounds (150-350 daltons) were screened at concentration of 200 μ M for multiple kinase affinity
- 238 compounds inhibited the activity of of Pim-1, p38 and CSK by at least 30%
- More than 100 structures showing compound bound to kinase were solved by cocrystallography
- The structure of 7-azaindole binding to ATP-binding site was solved



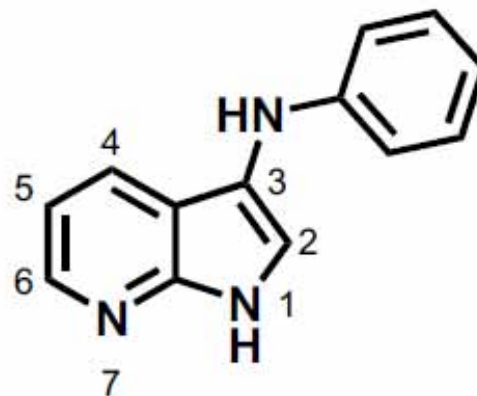
Discovery of selective inhibitors of oncogenic B-Raf kinase

I Scaffold and structure based Discovery of the inhibitors

- 7-azaindole has $IC_{50} > 200\mu M$ for Pim-1
- Mono-substituted 7-azaindoles with increasing affinity were synthesized



7-azaindole
 $IC_{50} > 200\mu M$

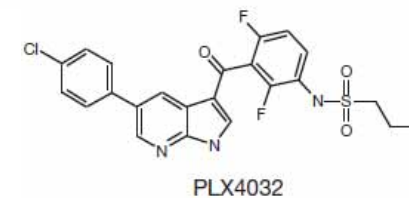
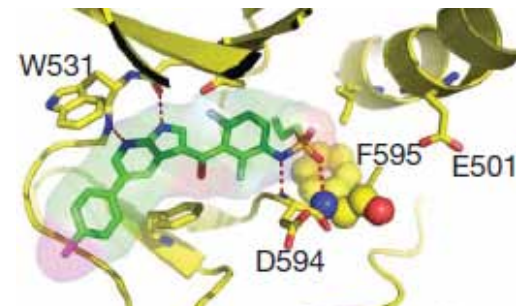
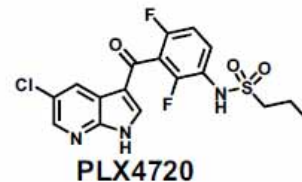
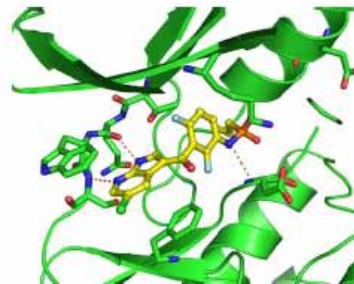
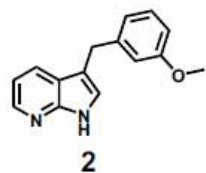
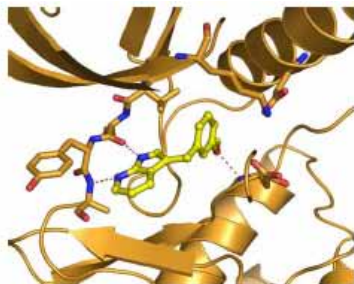
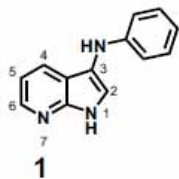
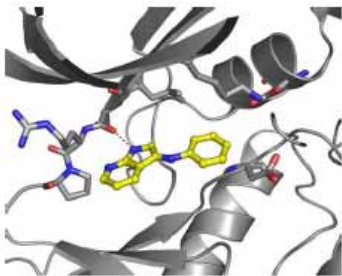


3-aminophenyl analogue
 $IC_{50} = 100\mu M$ for Pim-1

Discovery of selective inhibitors of oncogenic B-Raf kinase

I Scaffold and structure based Discovery of the inhibitors

- 7-azaindole has $IC_{50} > 200\mu M$ for Pim-1
- Mono-substituted 7-azaindoles with increasing affinity were synthesized
- Mono and di-substituted 7-azaindoles were screened against B-raf



Discovery of selective inhibitors of oncogenic B-Raf kinase

II Biochemical characterisation of the novel inhibitors

PLX4720

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

Kinases with IC₅₀s > 5,000 nM: ABL1, AKT1, AKT2, AKT3, ALK, BTK, CAMK2A, CDK2/CYCLIN A, CHK1, CHK2, CK1 ϵ , CLK1, EGFR, EPHA2, EPHB4, ERK2, FER, FLT3, GRK2, GSK3 β , HCK, IGF1R, IKK β , IRAK4, JAK3, JNK1, JNK2, JNK3, KIT, MAPKAPK2, MEK1, MER, MET, MKK6, MLK1, MST1, NEK2, P38 α , P70S6K, PAK3, PDGFR α , PDK1, PIM1, PKA, PKC μ , PKC θ , PLK1, PYK2, ROCK1, SYK, TAO1, TIE2, TRKA, TSSK1, ZIPK. We are unable to reliably measure wild-type c-Raf-1 activity. However, by using an activated form of c-Raf-1 with Y340D and Y341D mutations, an IC₅₀ of 6.7 nM is determined. This suggests that PLX4720 binds with high affinity to the "active" form of both B-Raf and c-Raf-1.

PLX4032

Assay	IC ₅₀ nM*
B-RAF-V600E	31
C-RAF	48
B-RAF	100
SRMS	18
ACK1	19
MAP4K5 (KHS1)	51
FGR	63
LCK	183
BRK	213
NEK11	317
BLK	547
LYNB	599
YES1	604
WNK3	877
MNK2	1717
FRK (PTK5)	1884
CSK	2339
SRC	2389

Discovery of selective inhibitors of oncogenic B-Raf kinase

Therapeutics, Targets, and Chemical Biology

Cancer
Research

RG7204 (PLX4032), a Selective BRAF^{V600E} Inhibitor, Displays Potent Antitumor Activity in Preclinical Melanoma Models

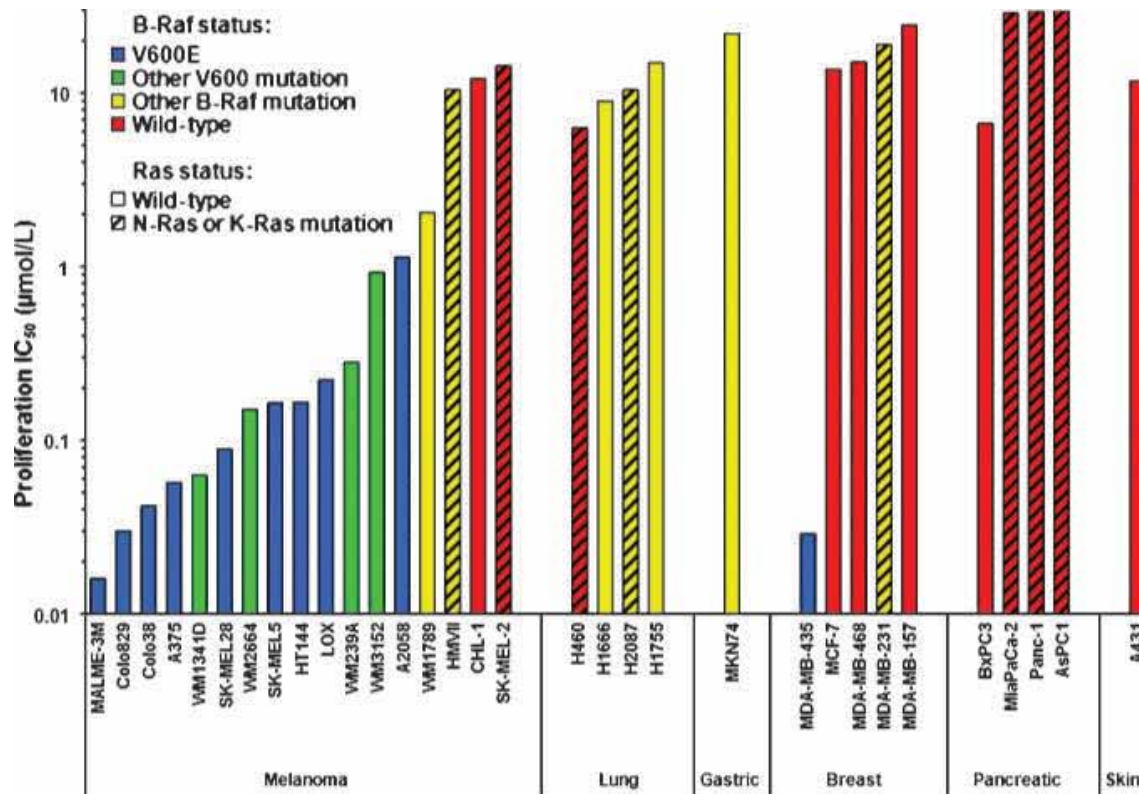
Hong Yang¹, Brian Higgins¹, Kenneth Kolinsky¹, Kathryn Packman¹, Zenaida Go², Raman Iyer², Stanley Kolis³, Sylvia Zhao³, Richard Lee⁴, Joseph F. Grippo⁴, Kathleen Schostack⁴, Mary Ellen Simcox¹, David Heimbrook¹, Gideon Bollag⁵, and Fei Su¹

Discovery of selective inhibitors of oncogenic B-Raf kinase

III Cellular selectivity of B-Raf inhibitors

Discovery of selective inhibitors of oncogenic B-Raf kinase

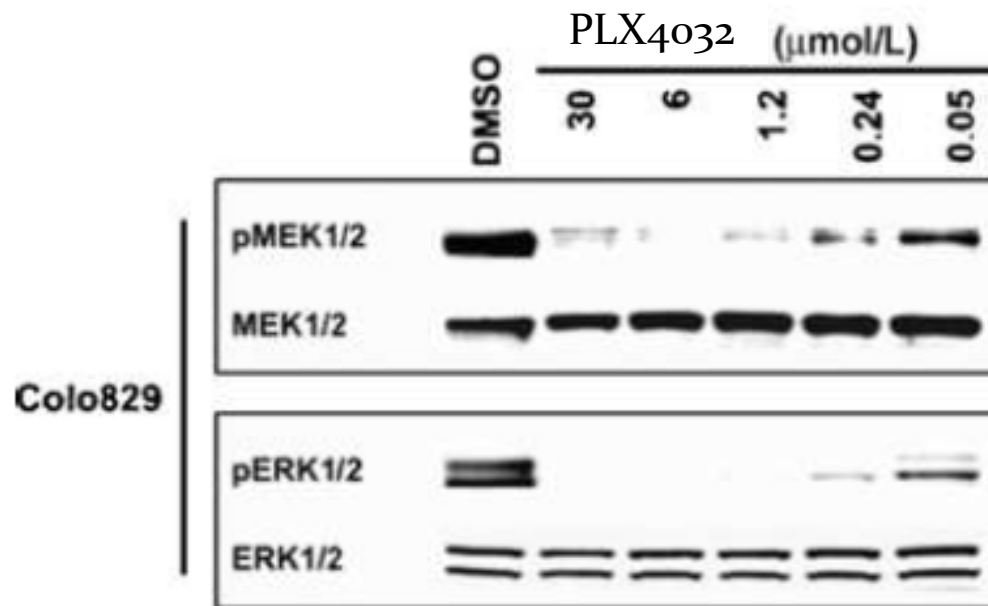
III Cellular selectivity of B-Raf inhibitors



- Cell line mutation status and PLX4032 affect:
 - Cellular proliferation

Discovery of selective inhibitors of oncogenic B-Raf kinase

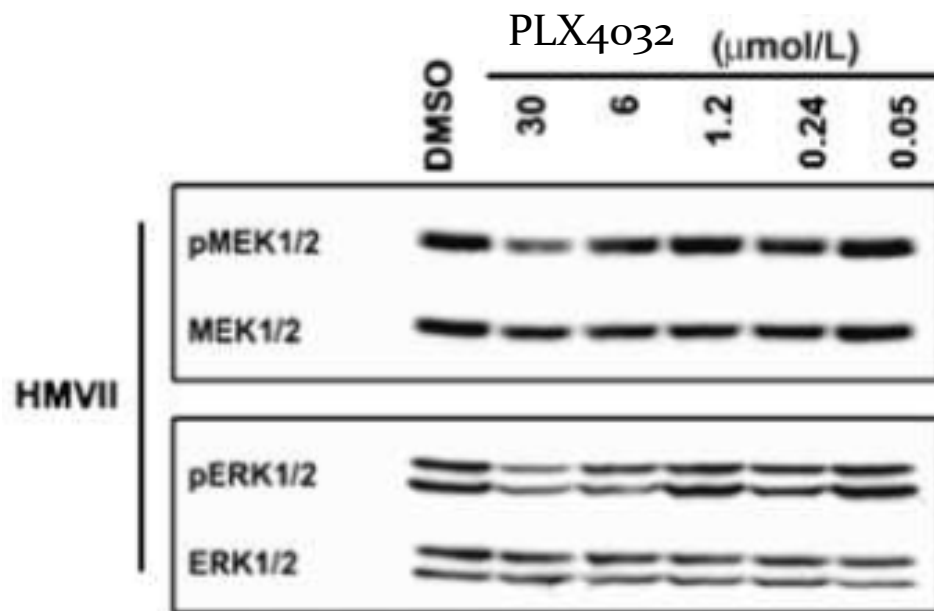
III Cellular selectivity of B-Raf inhibitors



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 - MEK and ERK phosphorylation

Discovery of selective inhibitors of oncogenic B-Raf kinase

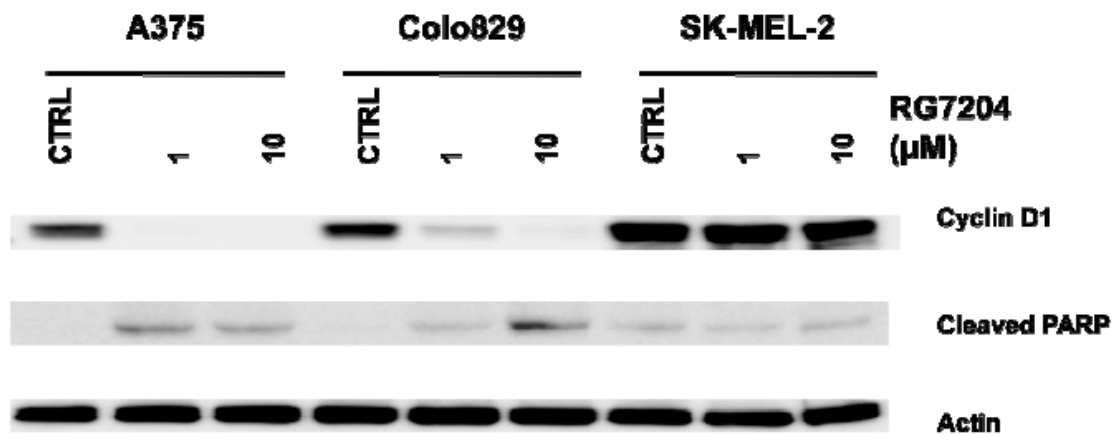
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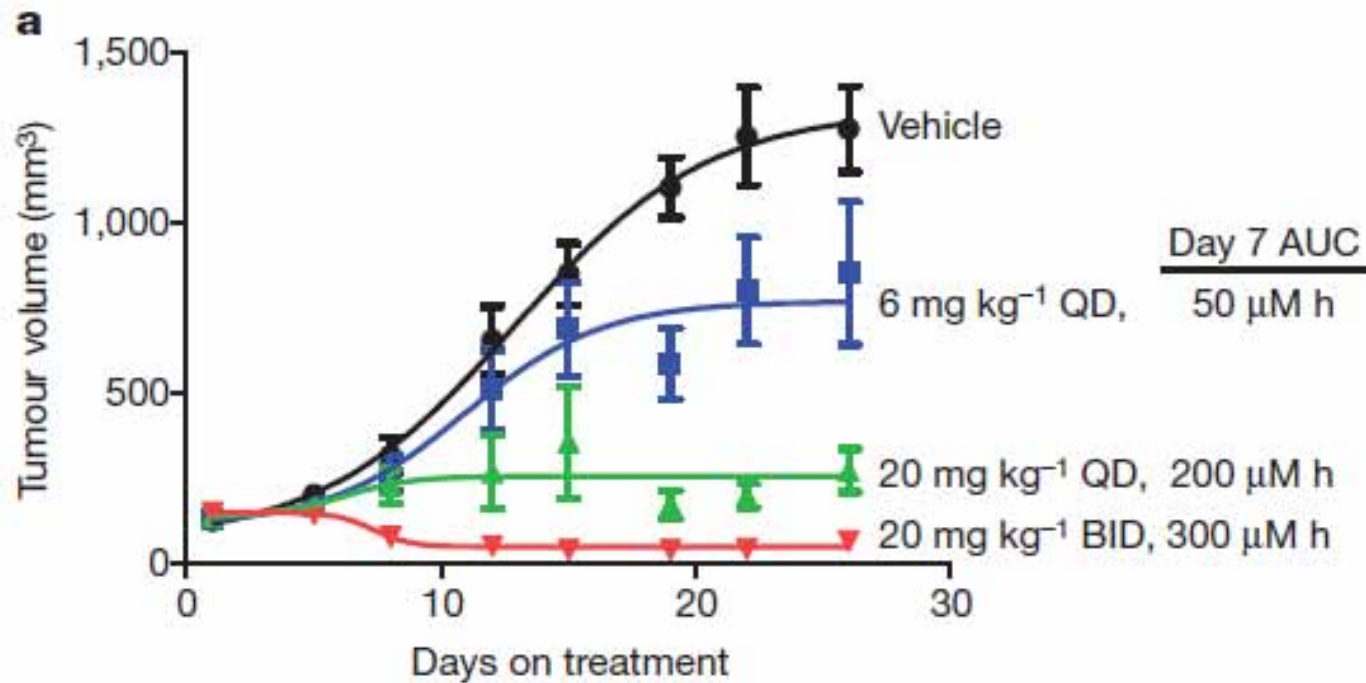
III Cellular selectivity of B-Raf inhibitors



- Cell line mutation status and PLX4032 affect:
 - Cellular proliferation
 - MEK and ERK phosphorylation
 - Cell cycle and apoptosis

Discovery of selective inhibitors of oncogenic B-Raf kinase

IV PLX4032 affects melanoma xenograft tumor growth





Discovery of selective inhibitors of oncogenic B-Raf kinase

- Scaffold-based drug design approach and Crystallography-guided optimization allowed discovery of BRAF (V600E) inhibitors
 - PLX4720: a highly selective inhibitor
 - PLX4032: a partially selective inhibitor
- Both inhibitors show activity in cellular assays
 - ERK phosphorylation
 - Cell proliferation
- In BRAF (V600E)-mutant xenograft studies, both inhibitors demonstrated dose dependant inhibition of tumor growth

Discovery of selective inhibitors of oncogenic B-Raf kinase

- Due to their pharmacokinetics in rodents, PLX4032 and PLX4720 were prioritized over a panel of related compounds
- PLX4032 was chosen over PLX4720 because its pharmacokinetic properties scaled more favorably in beagle dogs and cynomolgus monkeys



- What does this mean ?????????? ;-/



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Inhibition of Mutated, Activated BRAF in Metastatic Melanoma

Keith T. Flaherty, M.D., Igor Puzanov, M.D., Kevin B. Kim, M.D., Antoni Ribas, M.D.,
Grant A. McArthur, M.B., B.S., Ph.D., Jeffrey A. Sosman, M.D., Peter J. O'Dwyer, M.D., Richard J. Lee, M.D., Ph.D.,
Joseph F. Grippo, Ph.D., Keith Nolop, M.D., and Paul B. Chapman, M.D.



I Primary goals

- To define the safety and pharmacokinetic characteristics of treatment with continuous, twice daily administration of PLX4032
- To determine the maximum dose that could be administered until Adverse effects prevented further increases (recommended phase II dose)
- To determine the objective response rate, the duration of response and the rate of progression among patients who had melanoma tumors with the V600E BRAF mutation and who were given the recommended phase 2 dose of PLX4032



II Methods

i Study design

- Dose escalation phase
 - The drug was formulated as a highly bioavailable microprecipitated bulk powder
 - Patients enrolled initially received the MBP formulation at dose of 80mgx2/day
 - Doses were not escalated unless patients received min 4 weeks of a given dose with dose-limiting side effects observed in $<1/3$
 - The recommended phase2 dose was defined as the highest dose at which no more than $1/6$ patients had dose-limiting side effects



II Methods

i Study design

- Dose escalation phase
- Extension phase
 - Using recommended phase 2 dose
 - A cohort with V600E BRAF melanoma was treated



II Methods

ii Patients

- Eligibility criteria
 - Written informed consent
 - Age 18 or more
 - Solid tumor confirmed histologically, refractory to standard therapy or for which standard therapy did not exist
 - Eastern Cooperative Oncology Group performance status score of 0 or 1
 - Life expectancy of 3 months or longer
 - Absence of known progression or unstable brain metastases
 - Adequate hepatic, hematologic and renal function



II Methods

ii Patients

- Eligibility criteria
 - In the dose escalation phase
 - Trial open to patients with any type of tumor
 - In the extension cohort
 - V600E BRAF mutation harboring melanoma patients



II Methods

iii Study Assessment

- Safety evaluations

- at baseline, day 8, day 15, day 29 and every 4 weeks thereafter
- Physical examination
- ECG
- Blood count, chemical testing and urinalysis
- Adverse events were graded according to the Common Terminology Criteria for Adverse Events (version 3.0)
- Protocol was amended to include dermatologic evaluation and thoracic CT scans



II Methods

iii Study Assessment

- Safety evaluations
- Response evaluation
 - CT studies were performed at 8-week intervals
 - Response Evaluation Criteria in Solid Tumors (RECIST)
 - **Complete response:** disappearance of all target lesions
 - **Partial response:** decrease by at least 30% in the sum of the largest diameter of each target lesion (relative to baseline)
 - **Progressive disease:** increase by at least 20%...
 - **Stable disease:** everything else



II Methods

iii Study Assessment

- Safety evaluations
- Response evaluation
- Pharmacokinetic assessments
 - On days 1 and 15 then every 4 weeks
 - HPLC with detection by mass spectroscopy



II Methods

iii Study Assessment

- Safety evaluations
- Response evaluation
- Pharmacokinetic assessments
- Further evaluation
 - ^{18}F -fluorodeoxyglucose-positron-emission tomography (FDG-PET) at baseline, day 15 and every 4 weeks
 - Selected patients underwent biopsy at baseline and day 15 (cutaneous or superficial lymph-node lesions)



II Methods

iv Statistical analysis

- 32 patients
- 95% confidence ($\alpha=0.05$), with 80% power ($\beta=0.2$)
- An observed rate of 40% would be consistent with a true response rate of more than 10%

III Results

i Patients

Table 1. Baseline Characteristics of the Patients, According to Study Cohort.*

Characteristic	Dose-Escalation Cohort (N = 55)	Extension Cohort (N = 32)
Age — yr		
Median	63	52
Range	23–89	23–83
Sex — no. (%)		
Male	34 (62)	19 (59)
Female	21 (38)	13 (41)
Tumor type — no. (%)		
Melanoma	49 (89)	32 (100)
Thyroid	3 (5)	0
Other	3 (5)	0
Extent of metastatic melanoma — no. (%) [†]		
Stage M1a	7 (14)	6 (19)
Stage M1b	6 (12)	2 (6)
Stage M1c	36 (73)	24 (75)
LDH >ULN — no. (%)		13 (41)
ECOG performance status score — no. (%)		
0	28 (51)	15 (47)
1	27 (49)	17 (53)
No. of previous chemotherapy regimens — no. (%) [‡]		
0	5 (10)	7 (22)
1	16 (33)	9 (28)
2	5 (10)	4 (12)
≥3	23 (47)	12 (38)

III Results

ii Adverse events

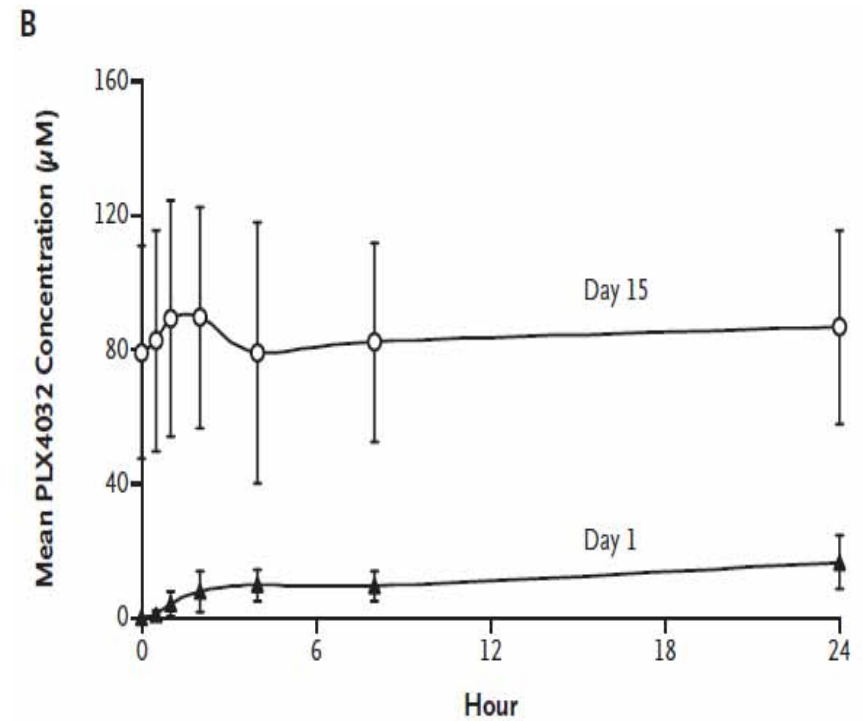
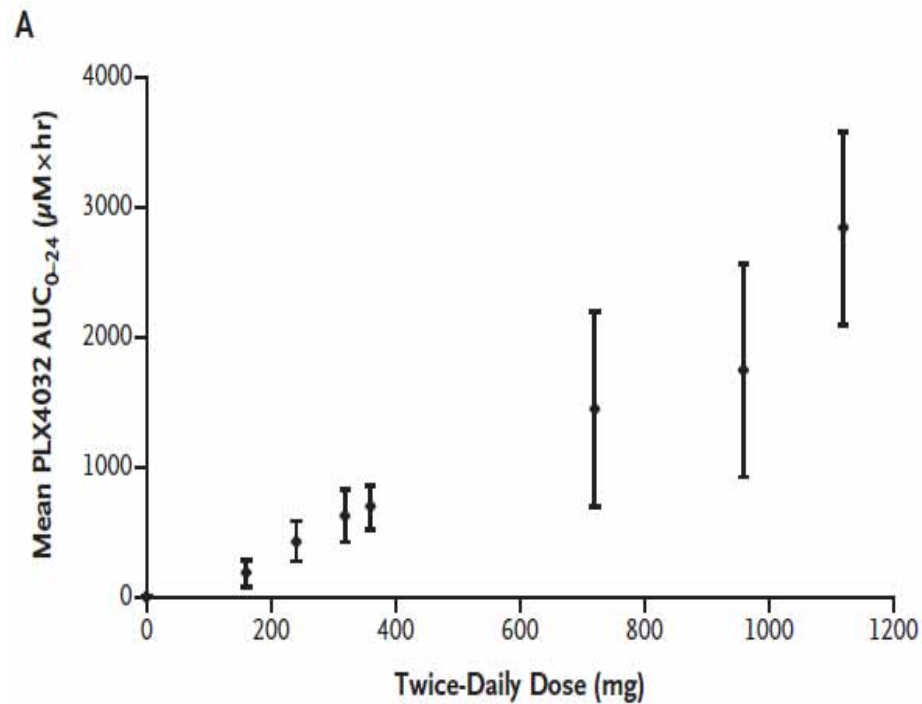
- 720mg x2/day no dose limiting effects were noted
- At 1120mg x2/day 4/6 patients had dose limiting side effects
- 960mg x2/day was tolerated by the first 6 patients.
Recommended phase 2 dose

Table 2. Drug-Related Adverse Events of Grade 2 or Higher Reported in More Than 5% of the 87 Study Patients, According to the Dose of PLX4032 Given Twice Daily.*

Event	<240 mg (N=30)	240 mg (N=4)	320 or 360 mg (N=8)	720 mg (N=7)	960 mg (N=32)	1120 mg (N=6)	Total (N=87)
Arthralgia							
Grade 2	0	1 (25)	2 (25)	0	10 (31)	1 (17)	14 (16)
Grade 3	0	0	0	0	1 (3)	1 (17)	2 (2)
Rash							
Grade 2	1 (3)	0	0	1 (14)	7 (22)	1 (17)	10 (12)
Grade 3	0	0	0	0	1 (3)	3 (50)	4 (3)
Squamous-cell carcinoma, keratoacanthoma type							
Grade 2	0	0	0	0	0	0	0
Grade 3	1 (3)	2 (50)	3 (38)	0	10 (31)	2 (33)	18 (21)
Nausea							
Grade 2	1 (3)	0	1 (11)	1 (14)	4 (12)	1 (17)	8 (9)
Grade 3	0	0	0	0	1 (3)	0	1 (1)
Fatigue							
Grade 2	0	0	0	0	2 (6)	1 (17)	3 (3)
Grade 3	0	0	0	0	2 (6)	2 (33)	4 (5)
Photosensitivity reaction							
Grade 2	0	0	0	1 (14)	4 (12)	1 (17)	6 (7)
Grade 3	0	0	0	0	1 (3)	0	1 (1)
Palmar-plantar dysesthesia							
Grade 2	0	0	0	0	2 (6)	1 (17)	3 (3)
Grade 3	0	0	0	0	2 (6)	0	2 (2)
Pruritus							
Grade 2	0	0	0	0	4 (12)	0	4 (5)
Grade 3	0	0	0	0	0	1 (17)	1 (1)
Lymphopenia							
Grade 2	0	0	2 (25)	0	2 (6)	0	4 (5)
Grade 3	0	0	0	0	0	1 (17)	1 (1)

III Results

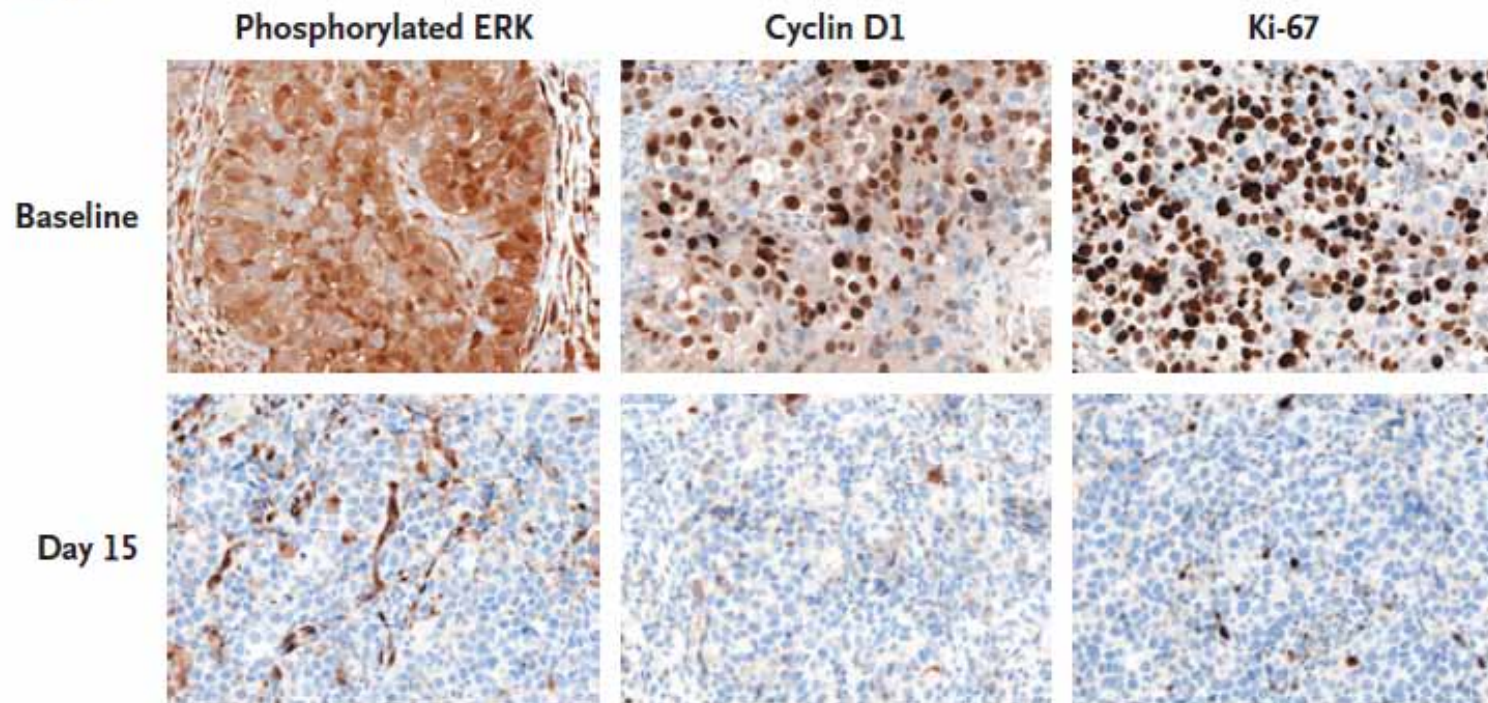
iii Pharmacokinetics



III Results

iv Pharmacodynamics

Tumor Biopsy

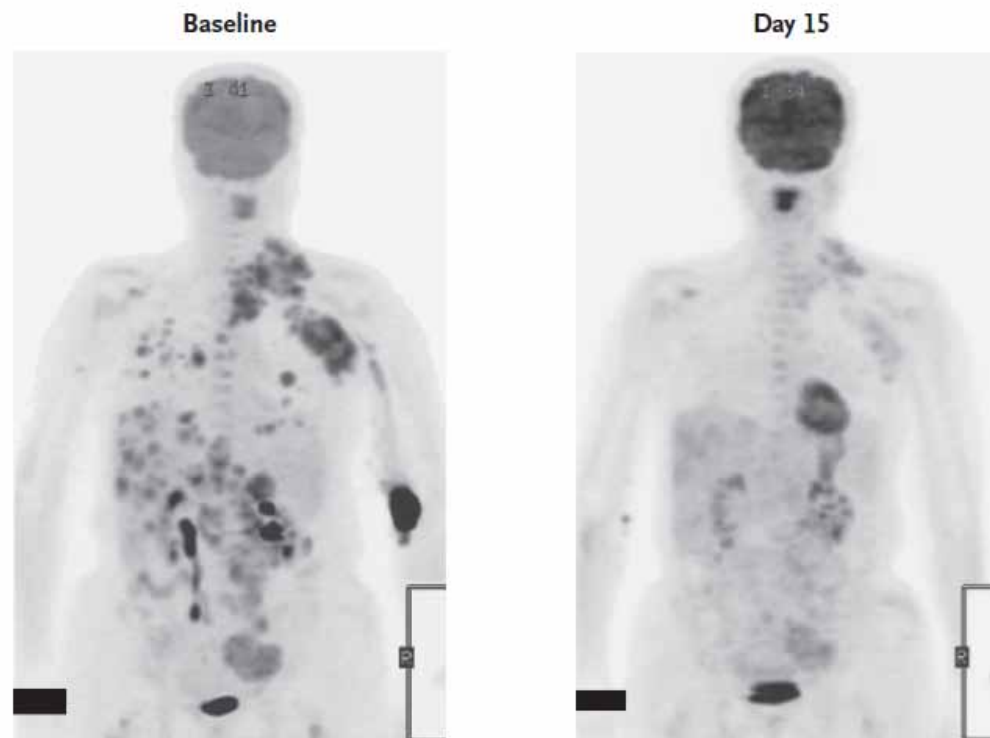


- PLX4032 reduces levels phosphorylated ERK, cycline D1 and Ki-67 in tumor biopsies

III Results

iv Pharmacodynamics

FDG-PET

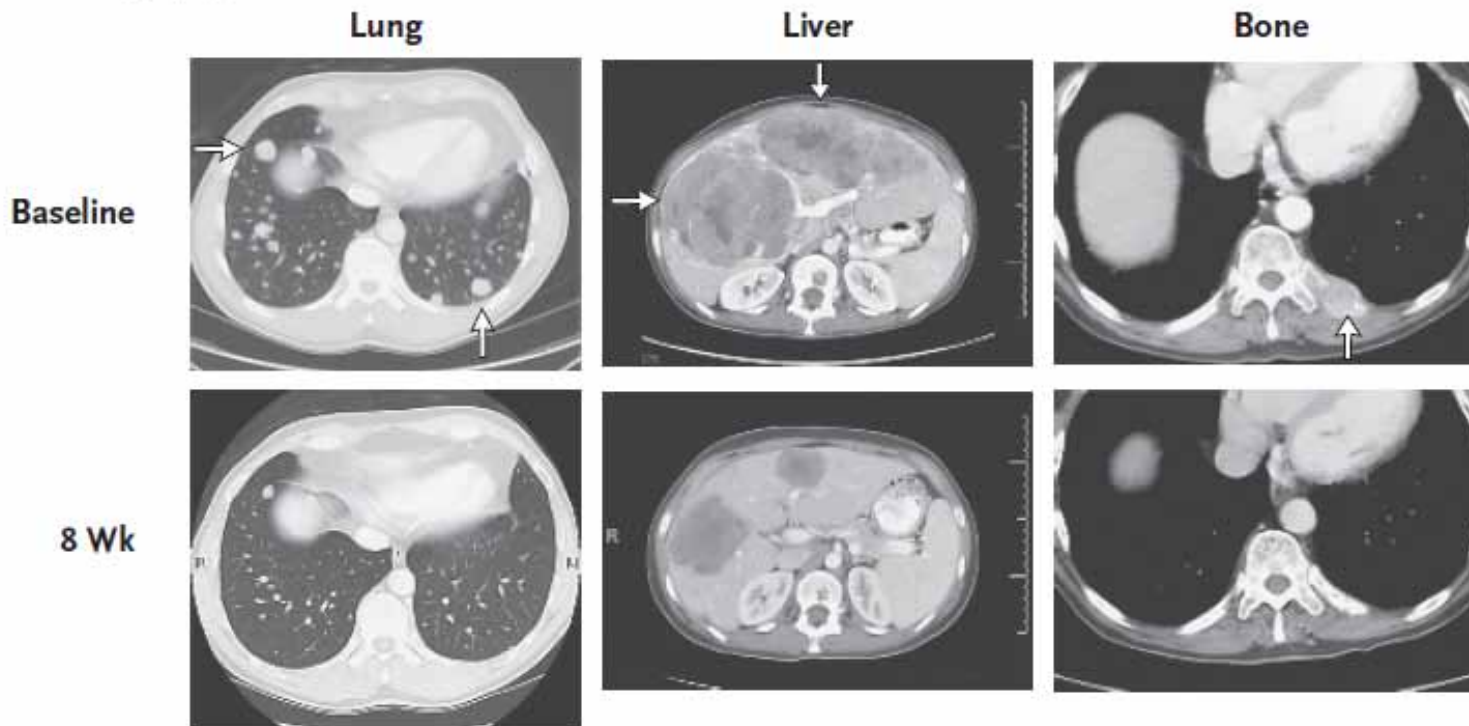


- PLX4032 reduces FDG tumor uptake measured by FDG-PET

III Results

iv Pharmacodynamics

Computed Tomography

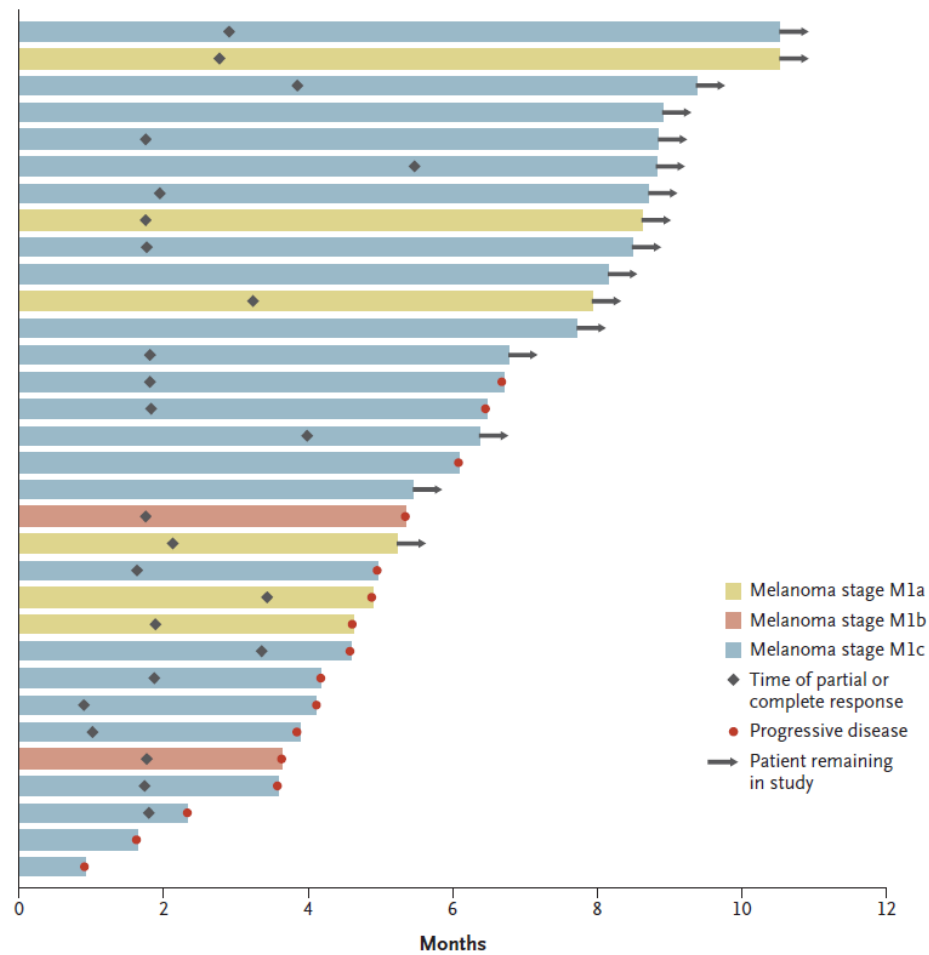


- PLX4032 leads to metastatic lesion regression in some patients

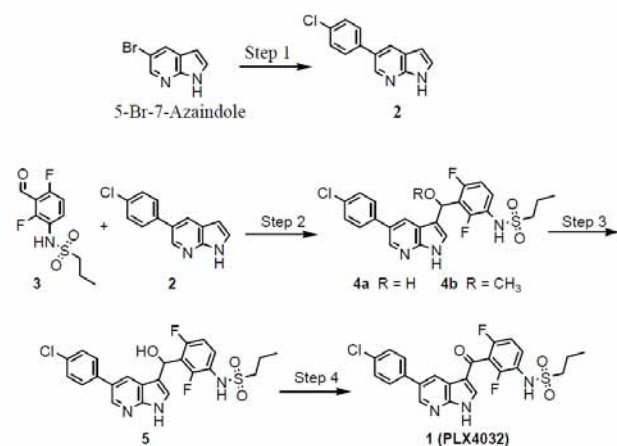
III Results

v Tumor response during extension phase

Response over Time

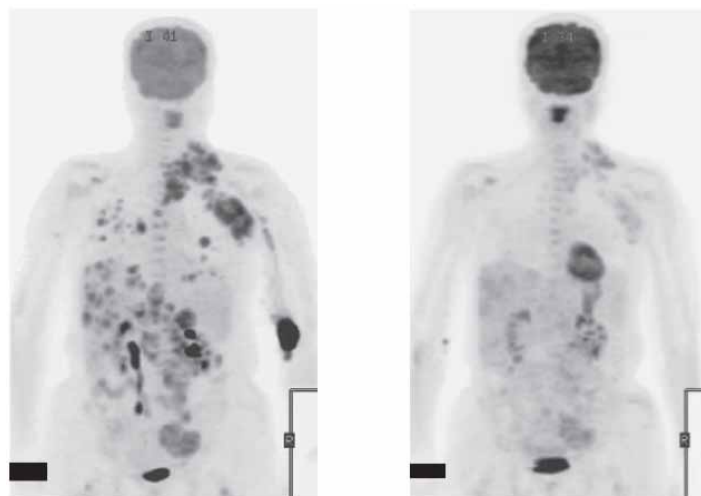


CONCLUSION



From Scaffold and structure based
Discovery of the inhibitors...

To preliminary clinical
evaluation





PERSPECTIVES

- Phase II and III trials are underway...