Discovery of (10R)-7-Amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3-h][2,5,11]-benzoxadiazacyclotetradecine-3-carbonitrile (PF-06463922), a Macrocyclic Inhibitor of Anaplastic Lymphoma Kinase (ALK) and c-Ros Oncogene 1 (ROS1) with Preclinical Brain Exposure and Broad Spectrum Potency against ALK-Resistant Mutations

T.W. Johnson *et. al. J. Med. Chem.* **2014**, 57, 4720.

And

PF-06463922, an ALK/ROS1 Inhibitor, Overcomes Resistance to First and Second Generation ALK Inhibitors in Preclinical Models

H.Y. Zou *et. al. Cancer Cell* **2015**, 28, 70.

Evan Carder
Wipf Group Current Literature
February 11, 2017

Phase I. Discovery of Lead Candidates

Lead Optimization Objectives

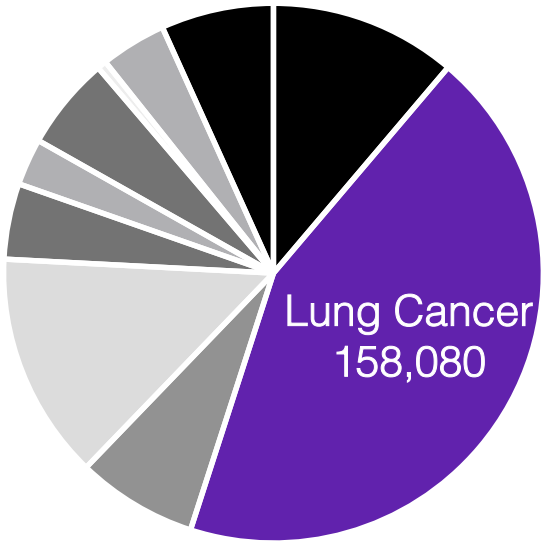
- Biochemical and cellular potency
- Selectivity profile
- Physiochemical properties
- Pharmocokinetic properties
- In-vitro toxicity
- Chemical stability
- Synthetic tractability
- Patentability

Preclinical Objectives

- Mechanism of action
- Pharmacokinetic profile
- Pharmacodynamic profile
- Safety and Toxicity profile
- Investigational New Drug (IND) application

Cancer: Estimated New Cases and Deaths in 2016

Type of Cancer	Estimated New Cases	Estimated Deaths
1. Breast Cancer	246,660	40,450
2. Lung Cancer	224,390	158,080
3. Prostate Cancer	180,890	26,120
4. Colon and Rectum Cancer	134,490	49,190
5. Bladder Cancer	76,960	16,390
6. Melanoma of the Skin	76,380	10,130
7. Non-Hodgkin Lymphoma	72,580	20,150
8. Thyroid Cancer	64,300	1,980
9. Kidney and Renal Pelvis Cancer	62,700	14,240
10. Leukemia	60, 140	24,400

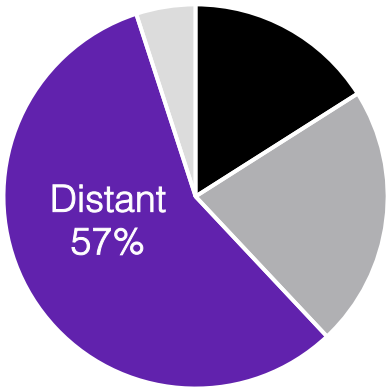


Estimated Deaths in 2016

National Cancer Institute Surveillance, Epidemiology, and End Results Program (seer.cancer.gov)

NCI: Lung Cancer Statistics

Percent of Cases by Stage



Localized (16%)

Confined to primary site

Regional (22%)

Spread to regional lymph nodes

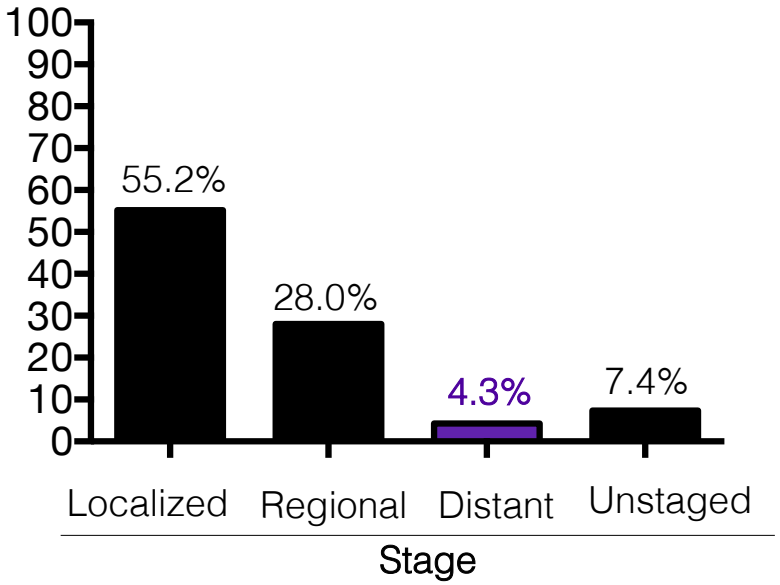
Distant (57%)

Cancer has metastasized

Unknown (5%)

Unstaged

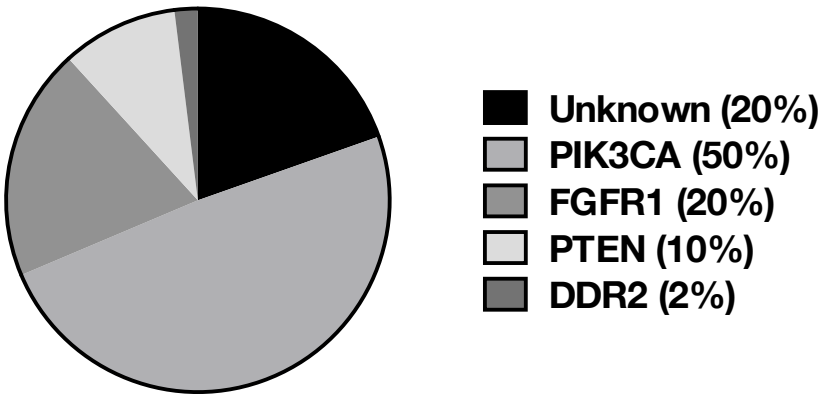
5-Year Relative Survival



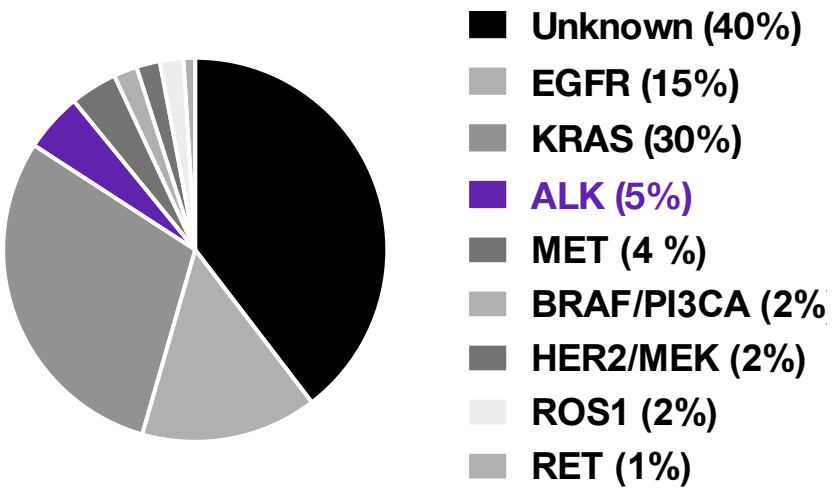
National Cancer Institute Surveillance, Epidemiology, and End Results Program (seer.cancer.gov)

Molecular Subtypes of Non-Small Cell Lung Carcinoma (NSCLC)

Squamous Cell Carcinoma

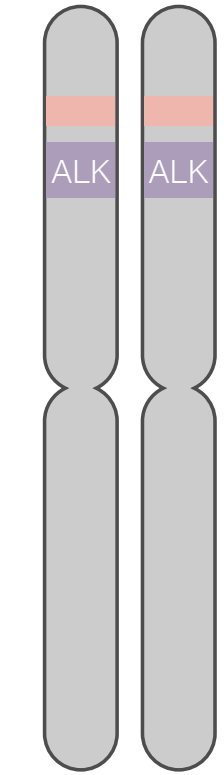


Adenocarcinoma



Transl. Lung Cancer Res. **2015**, 4, 36.

Tumorgenesis: ALK-Specific Genomic Alterations



Chromosome 2
Wild Type

Genomic
Alteration

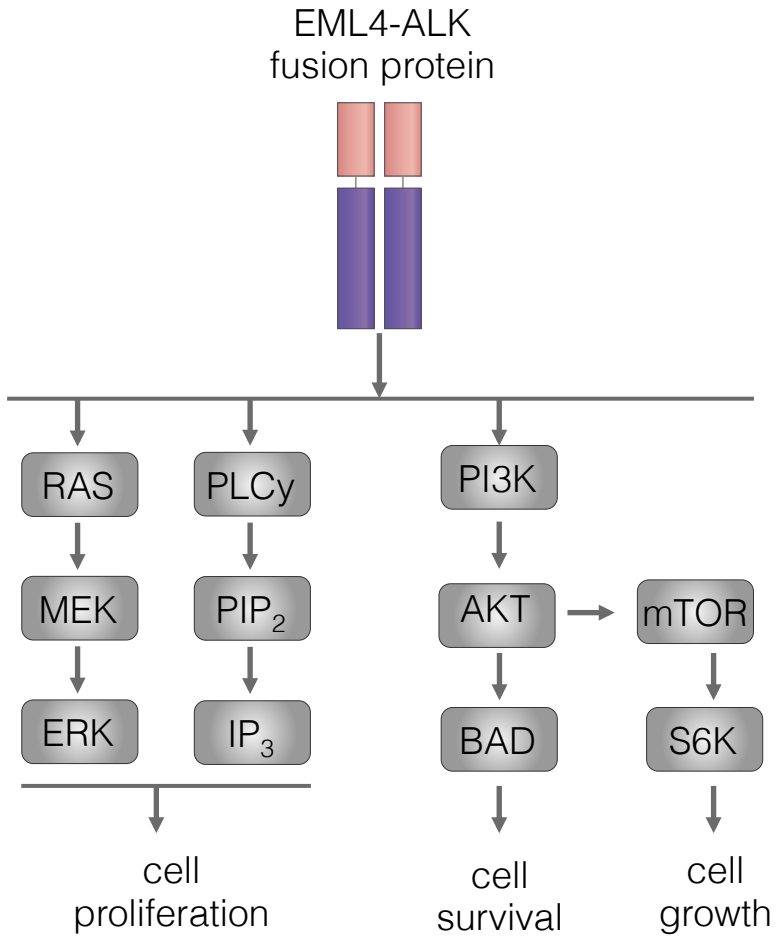
ALK Point Mutations

ALK Amplification

ALK Translocation

Anaplastic Lymphoma Kinase (ALK)

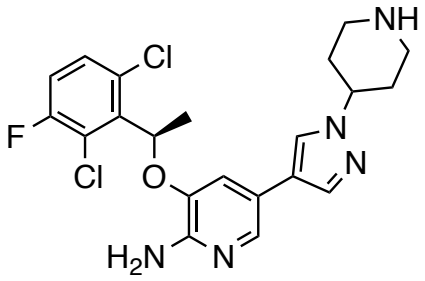
- Receptor tyrosine kinase (RTK)
- Primarily expressed in brain tissue
- Important for the development and function of the nervous system
- Fusion protein leads to truncated ALK resulting in a constitutively active cytoplasmic tyrosine kinase
- Constitutively active ALK can enhance cell growth, proliferation, survival, and motility.



Nat. Rev. Cancer **2013**, 13, 685.

Selected ALK Inhibitors for the Treatment of ALK+ Metastatic NSCLC

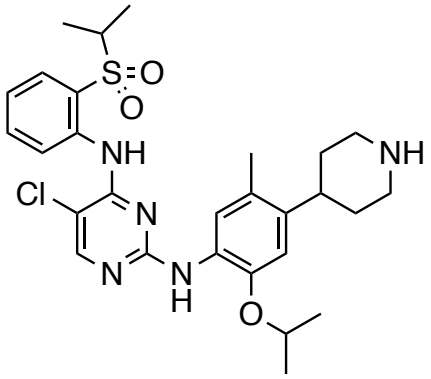
1st Generation



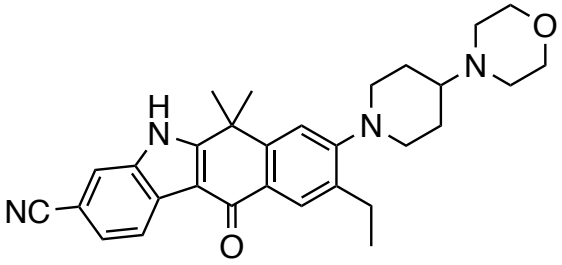
Crizotinib (Pfizer)

MET, ALK, ROS1 kinase inhibitor
Type I ATP competitive
FDA approval in 2011
Response rate: 60%
Progression free survival: 10 months
Subject to resistance mechanisms
Poor brain penetration

2nd Generation

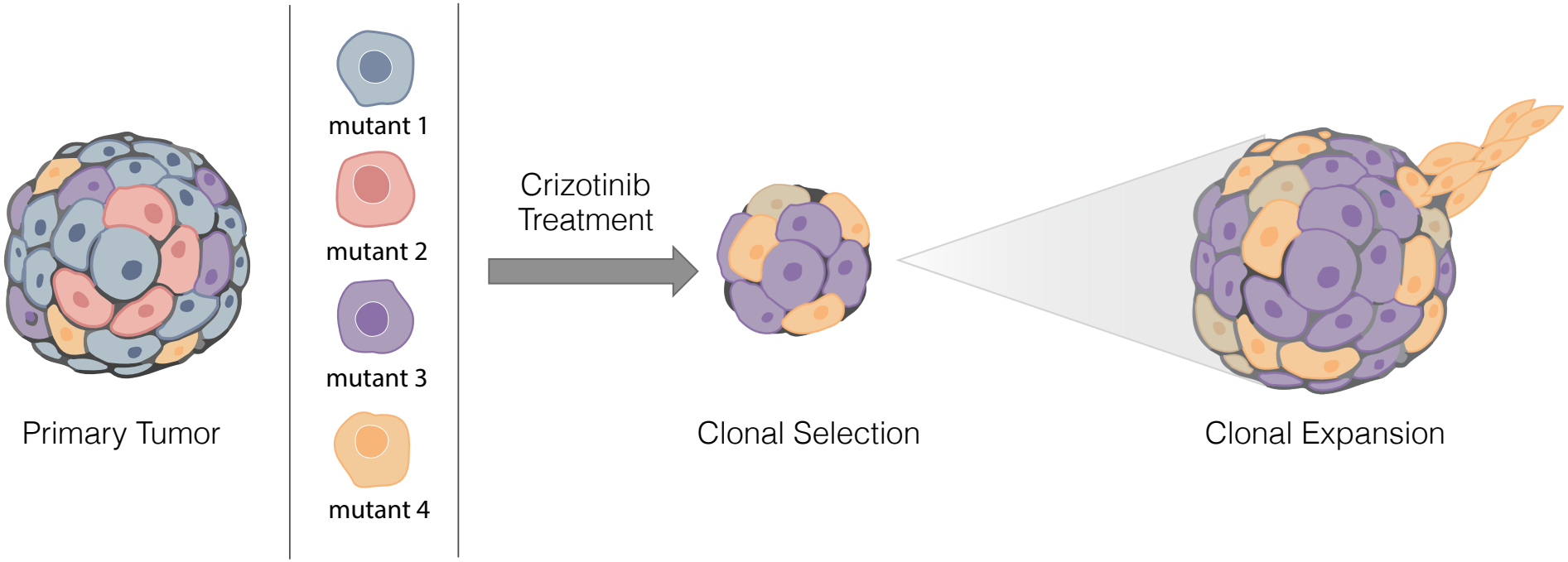


Ceritinib (Novartis)
FDA approval 2014



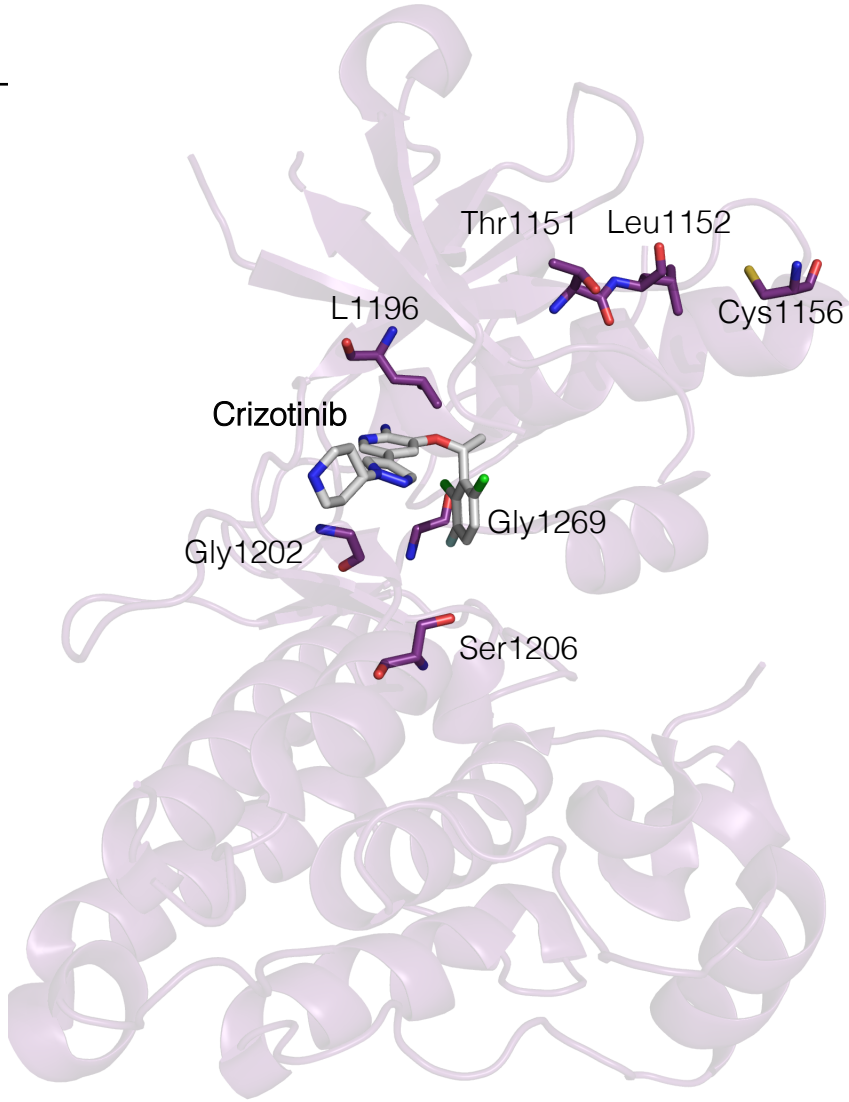
Alectinib (Roche)
FDA approval 2015

Therapeutic Resistance and Cancer Recurrence



Crizotinib Resistance: Clinical Mutants of ALK

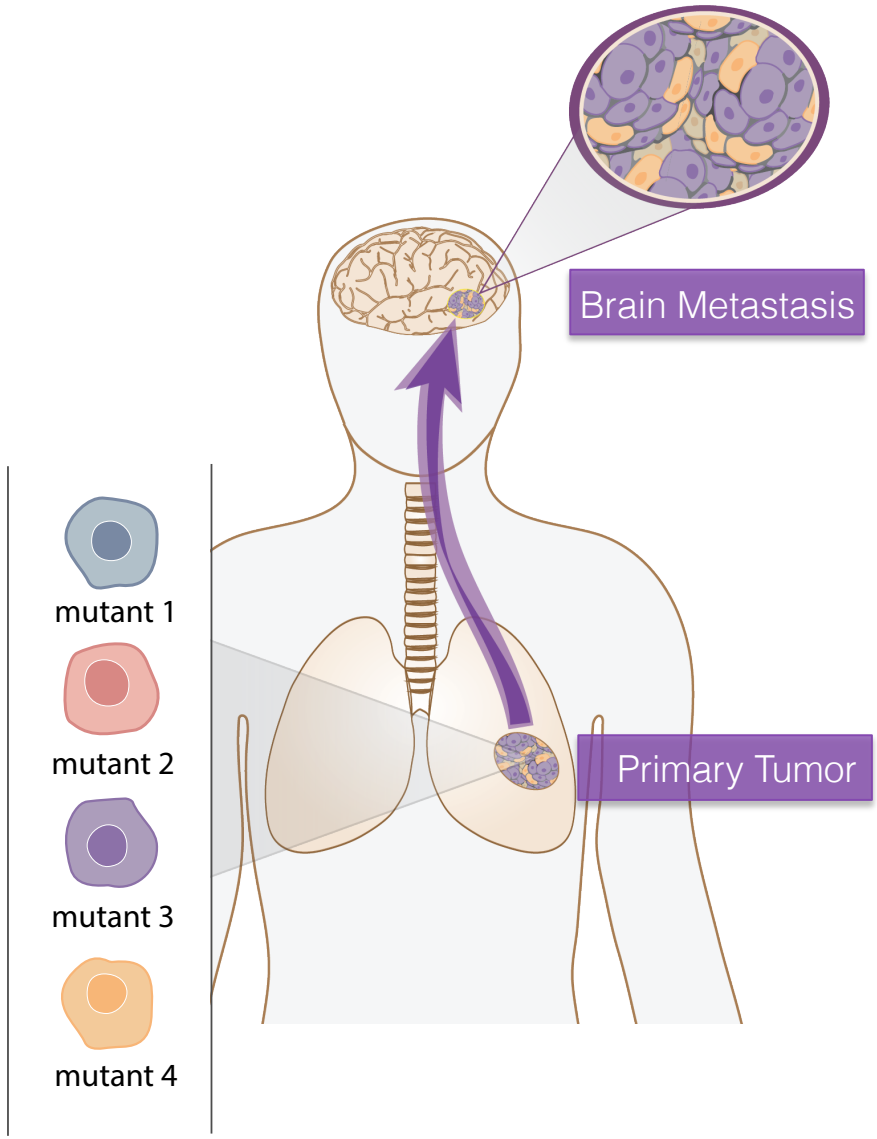
ALK Mutation	Biological Function	Crizotinib pALK IC_{50} (nM)
-	-	80
L1196M	Gatekeeper residue	843
G1269A	ATP-binding pocket	605
G1202R	Solvent front, steric hindrance	1148
S1206Y	Solvent front	626
1151Tins	Increased ATP affinity for ALK	3039
C1156Y	Loop N-terminal of alpha C; increased kinase activity	478
L1152R	Loop N-terminal of alpha C	1026
F1147L	Decreased stability of ALK-crizotinib complex	165



Program Objectives

Development of a novel ALK therapeutic

- Biochemical and cellular potency
- Kinase selectivity
- Orally bioavailable
- ADMET properties
- Overcomes resistant mutations
- CNS penetrable

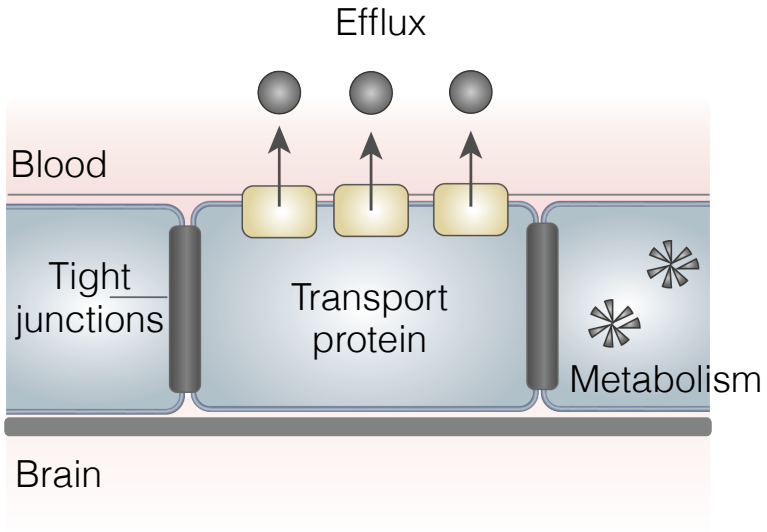


In-Vitro Parameters Evaluated

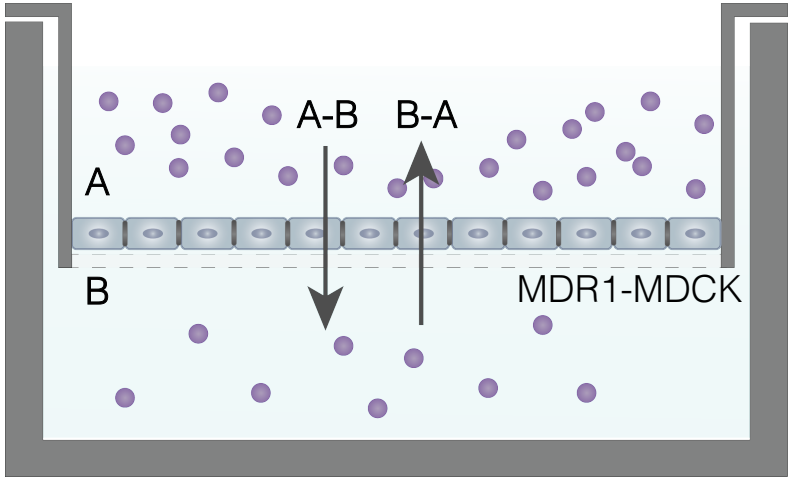
ALK K_i (nM)	pALK cell IC_{50} (nM)	log D^a	LipE^b	HLM Cl^c	MDR BA/AB (ratio)^d
ALK-L1196M K_i (nM)	pALK-L1196M cell IC_{50} (nM)				

Parameter	Function	Objective
Inhibitor dissociation constant (K_i)	Inhibitor binding affinity	K_i < 20 nM
Half maximal inhibitory concentration (IC_{50})	Functional Potency	Biochemical IC_{50} < 25 nM Cellular IC_{50} < 200 nM
Distribution coefficient (LogD)	Lipophilicity at a specific pH	LogD 2-3
Lipophilic efficiency (LIPE)	Estimates druglikeness	LiPE > 6
Human liver microsome clearance	Clearance rate due to oxidative metabolism	HLM Cl < 10 mL/min/Kg
Multidrug resistance efflux ratio (MDR BA/AB)	P-gp protein transporter efflux	MDR BA/AB < 2.5

Blood Brain Barrier

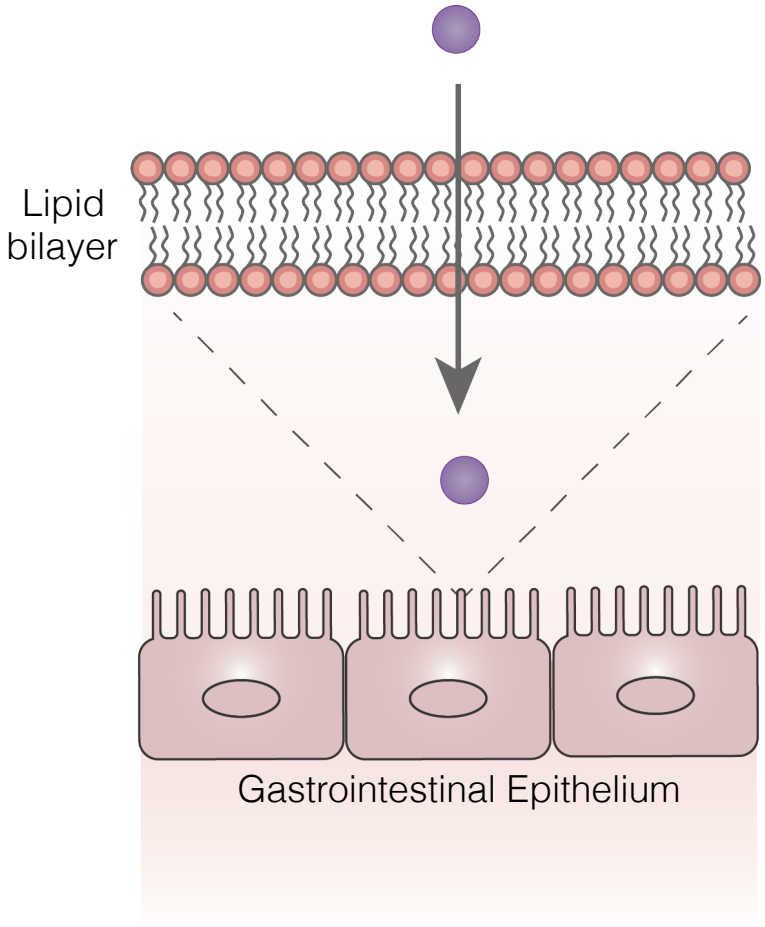


In-Vitro Blood Brain Barrier Assay



LC-MS quantitation → MDR ratio = $\frac{[B\text{-}A]}{[A\text{-}B]}$

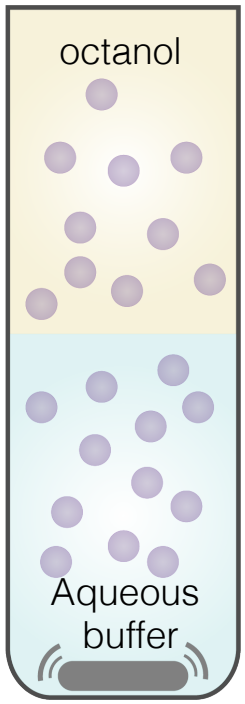
Cell Permeability



Shake-Flask Assay

$$LogD_{7.4} = \frac{[A]_{octanol}}{[A]_{Aqueous}}$$

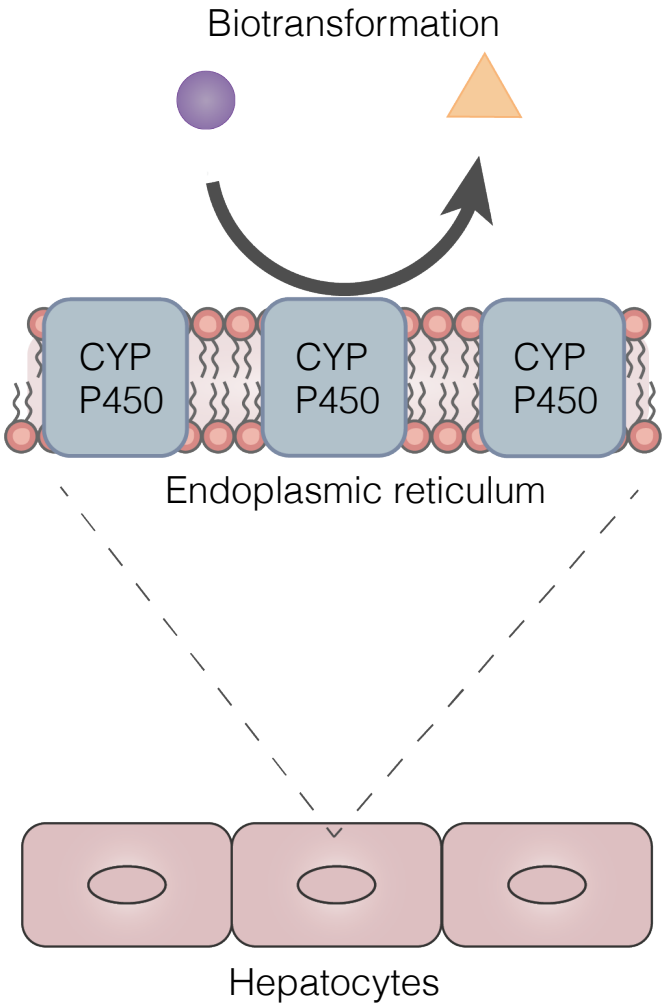
LC-MS quantitation



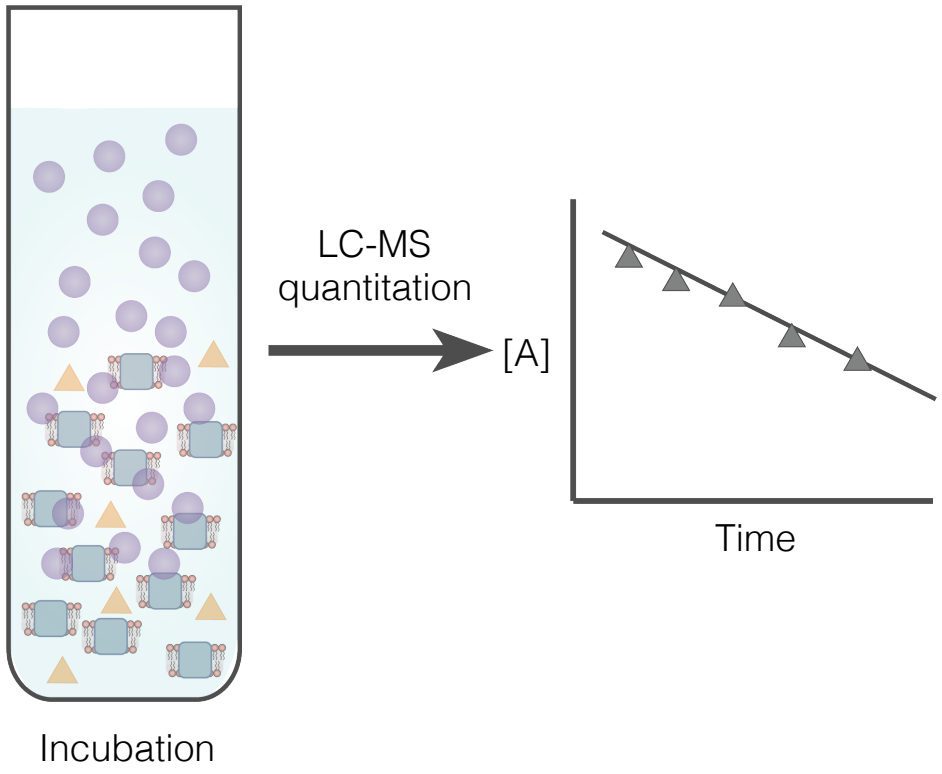
Shake-flask

$LogD_{7.4}$	$[A]_{octanol} : [A]_{aqueous}$
1	10:1
2	100:1
3	1,000:1

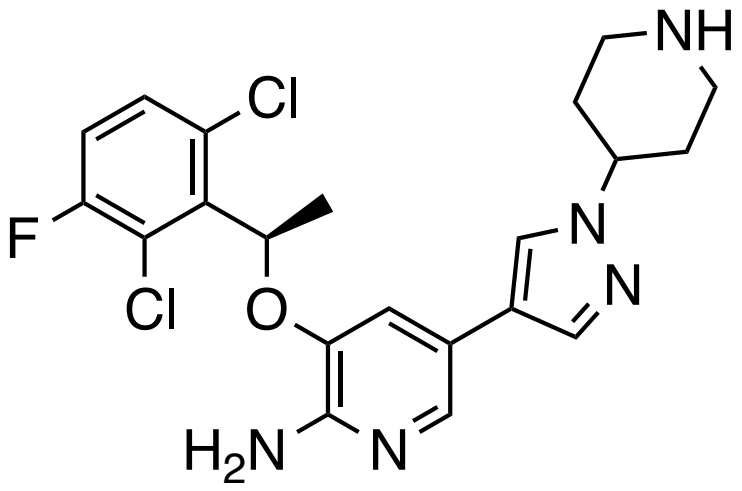
Liver Detoxification



Human Liver Microsome Assay

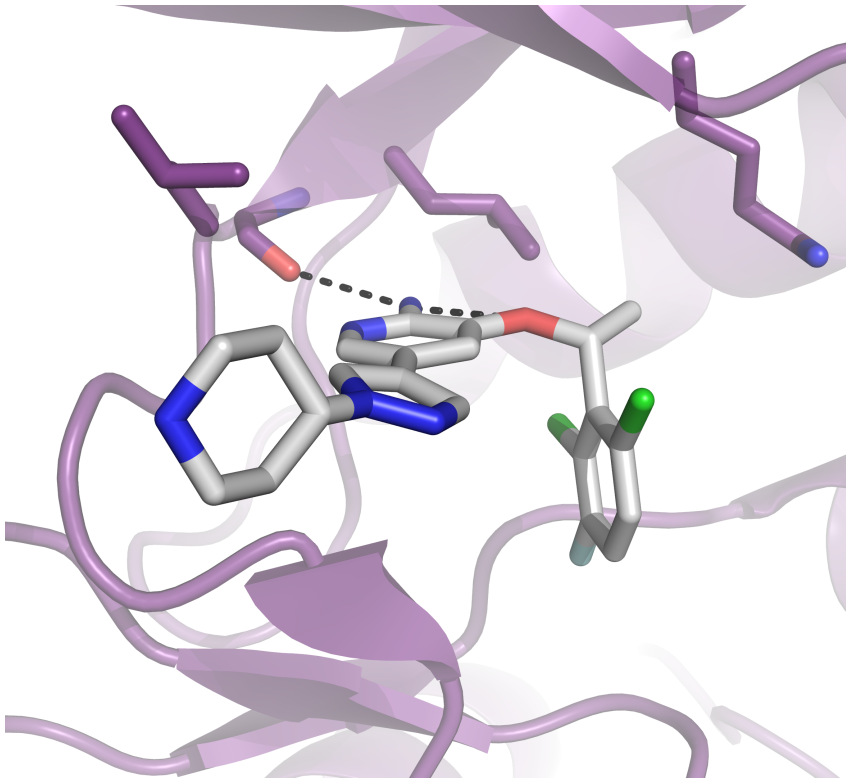


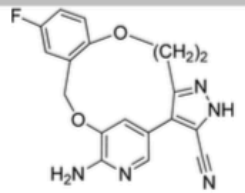
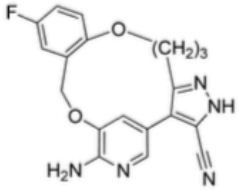
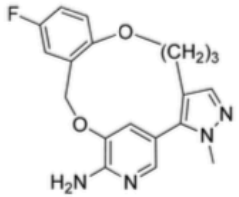
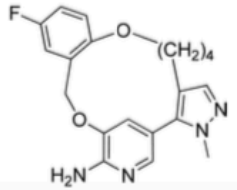
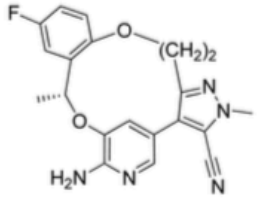
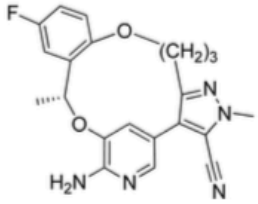
Structure-Based Drug Design (SBDD)

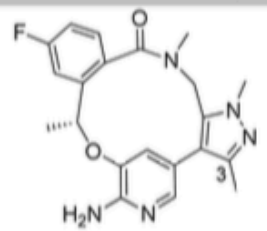
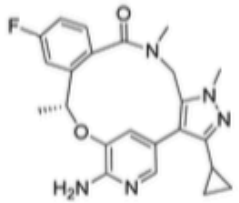
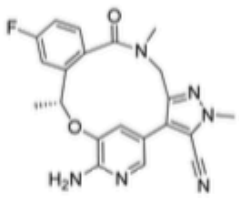
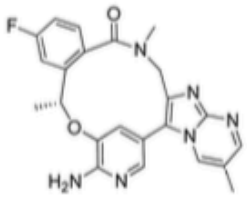
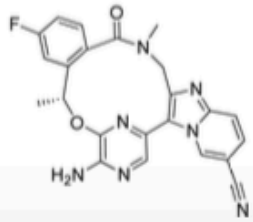


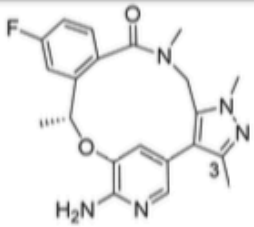
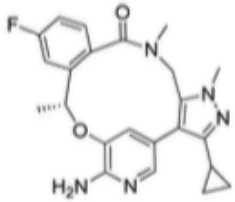
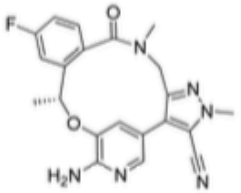
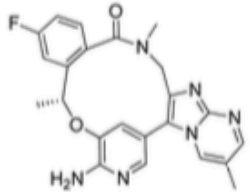
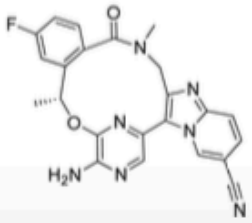
Crizotinib

Crizotinib bound to ALK

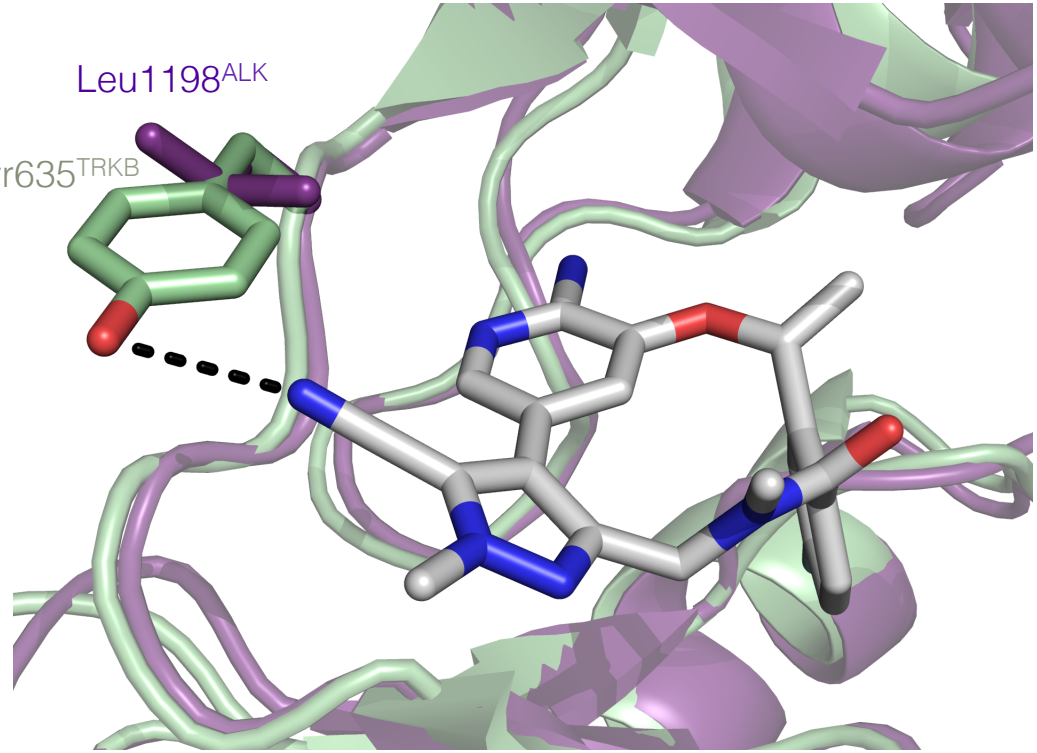


compd. no.	structure	ALK K_i (nM) ALK-L1196M K_i (nM)	pALK cell IC_{50} (nM) pALK-L1196M cell IC_{50} (nM)	log D^a	$LipE^b$
7a		0.36 1.6	22 101	3.0	4.0
7b		0.22 <0.1	15 112	3.6	3.4
7c		3.8 29	86 654	3.4	2.8
7d		5 36	524 3655	3.1	2.3
7e		<0.1 0.57	1.0 20	3.3	4.4
7f		<0.1 0.62	0.9 21	3.8	3.9

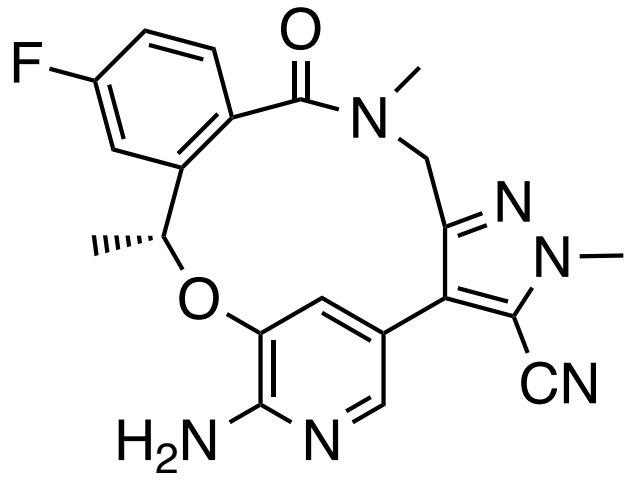
compd. no.	structure	ALK-L1196M K_i (nM)	pALK-L1196M cell IC_{50} (nM)	log D^a	HLM Cl[b]	MDR BA/AB (ratio)[c]
8a		0.29	14	2.2	8.6	28.3/8.1 (4.2)
8j		<0.1	5.8	2.9	14.6	16.3/8.0 (2.0)
8k (PF-06463922)		0.70	21	2.3	<8	28.0/19.3 (1.5)[d]
8l		2.0	365	1.7	<8	32.4/1.74 (20.2)
8m		0.56	45	2.4	<8	22.1/3.8 (5.8)

compd. no.	structure	TrkB K_i (nM) (selectivity)[e]
8a		0.5 (1.7x)
8j		0.4 (2.0x)
8k (PF-06463922)		23 (38x)
8l		77 (39x)
8m		65 (93x)

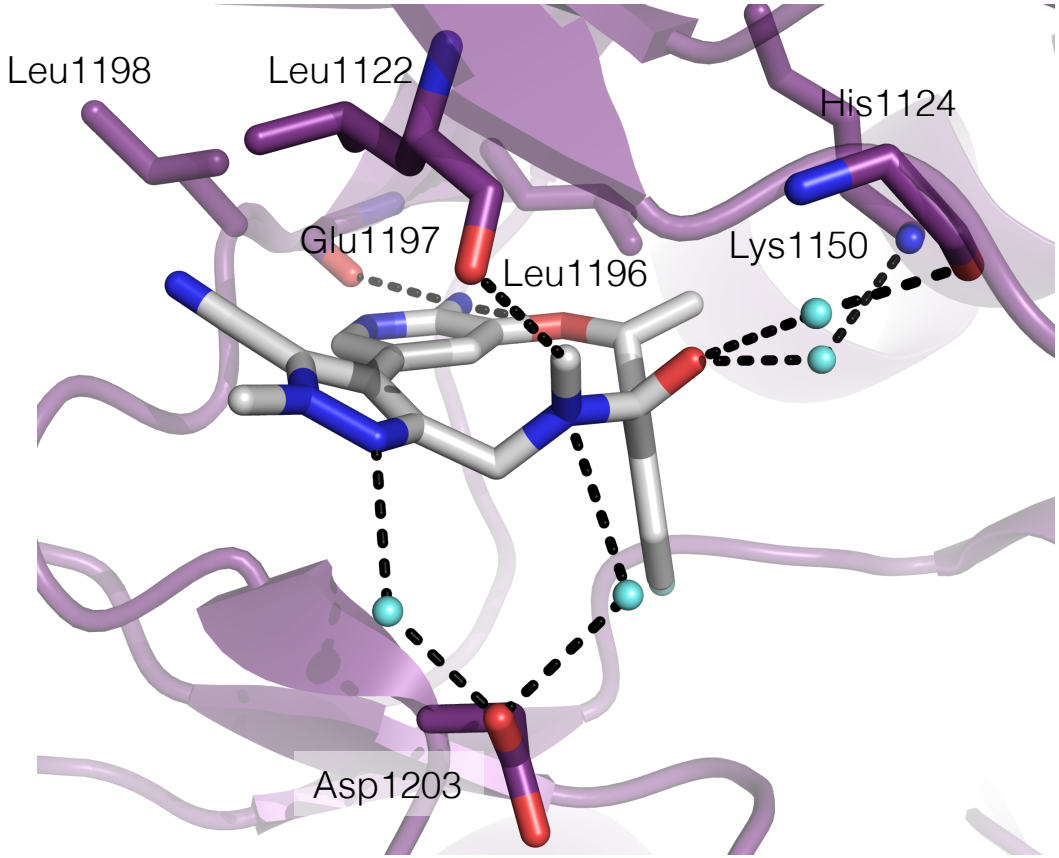
Overlay of ALK and TRKB Kinase



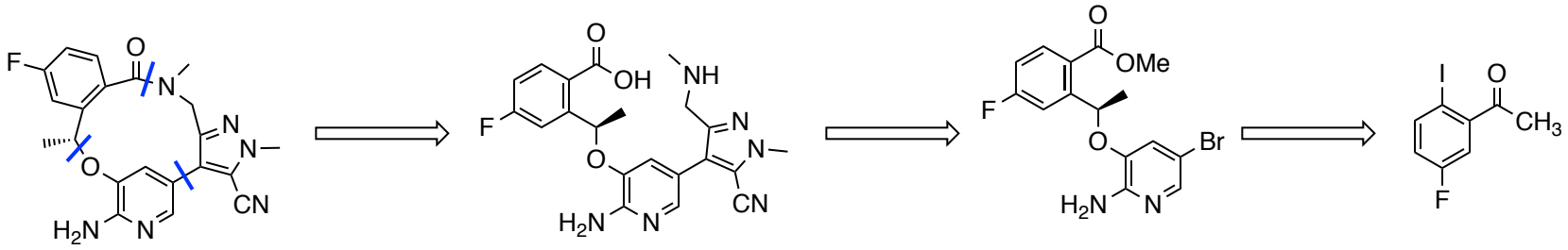
PF-06463922-ALK Kinase Co-Crystal Structure



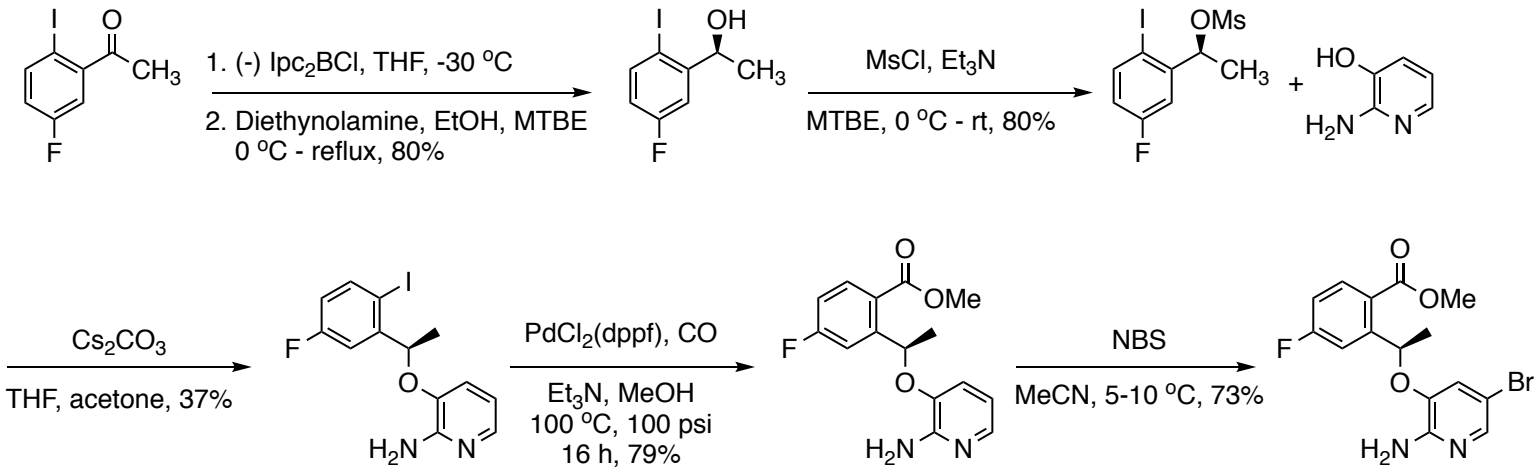
PF-06463922



PF-06463922 Synthesis



PF-06463922 Synthesis



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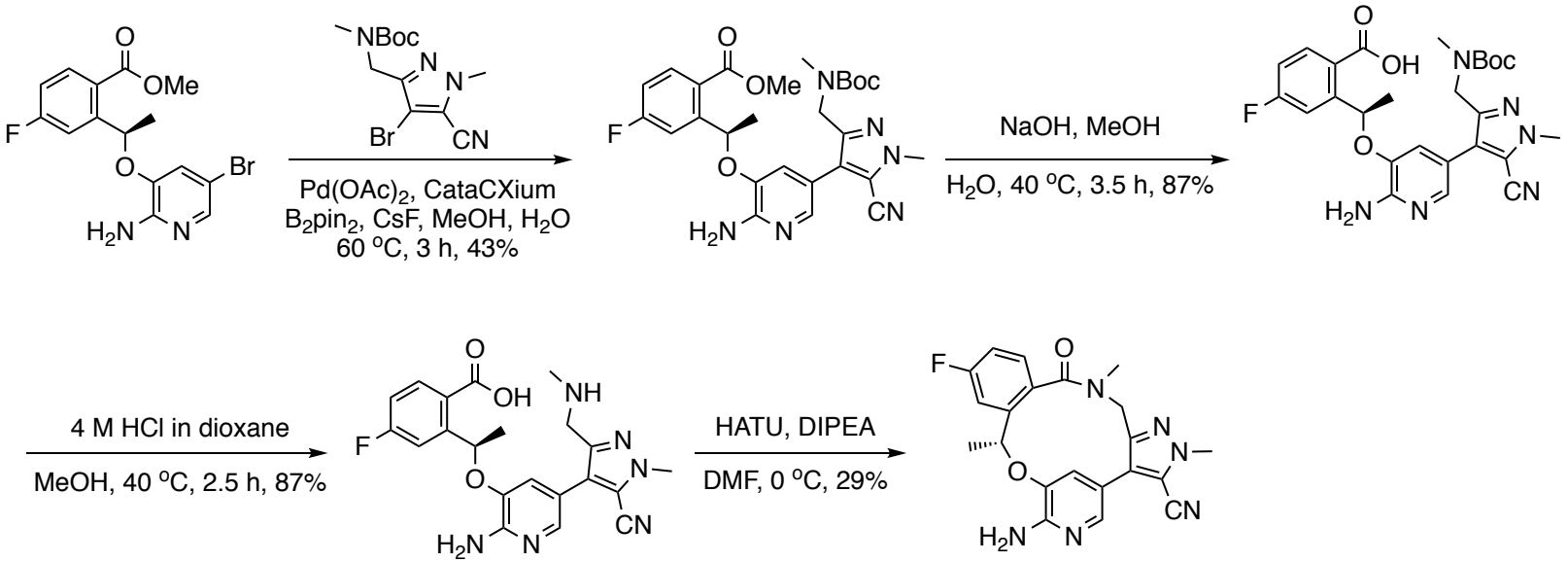
PF-06463922 Synthesis



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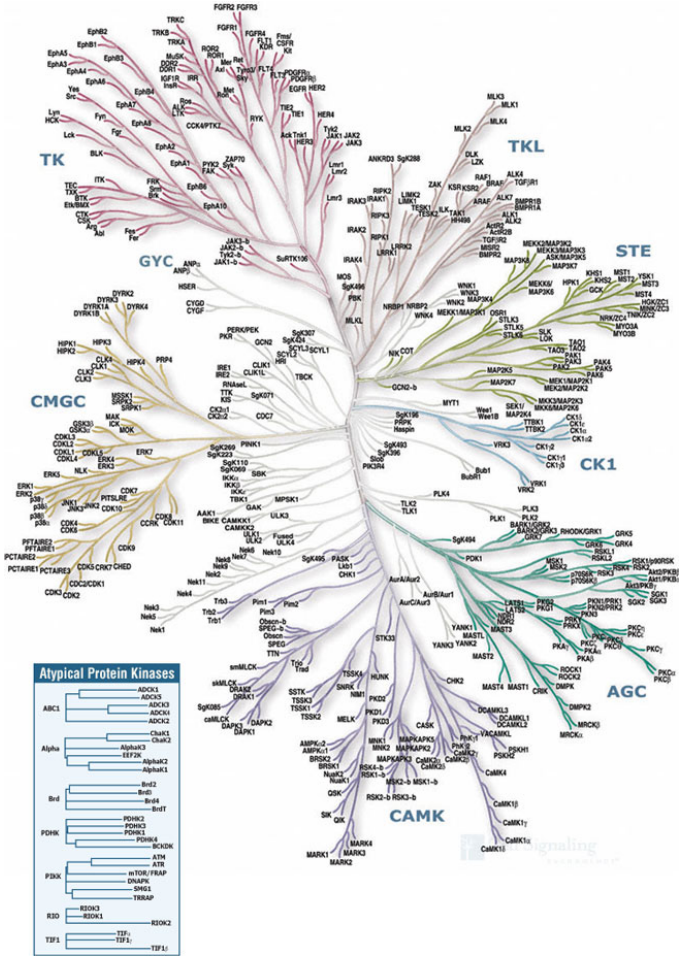
PF-06463922 Kinase Selectivity

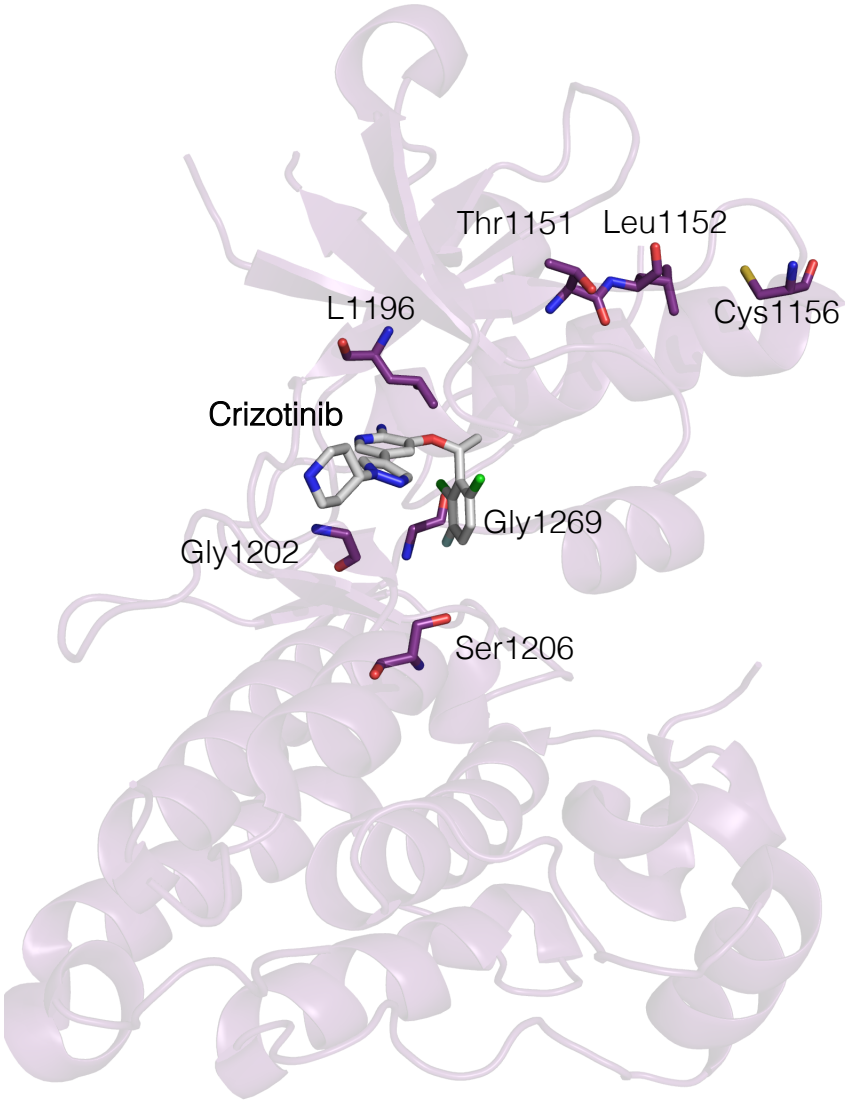


Enzyme-Based Selectivity of PF-064639

Kinase	% Inhibition 1 μM	IC50[a] (Ki[b]) nM	Fold shift (ALK-L1196M)
ROS1	102	<0.005[b]	<0.03
LTK (TYK1)	111	2.7	3.9
FER	104	3.3	4.7
FES (FPS)	97	6.0	8.6
PTK2B (FAK2)	101	14	20
TNK2 (ACK)	99	17	24
PTK2 (FAK)	99	17	24
NTRK2 (TRKB)	103	23	33
NTRK1 (TRKA)	87	24	34
NTRK3 (TRKC)	95	46	66

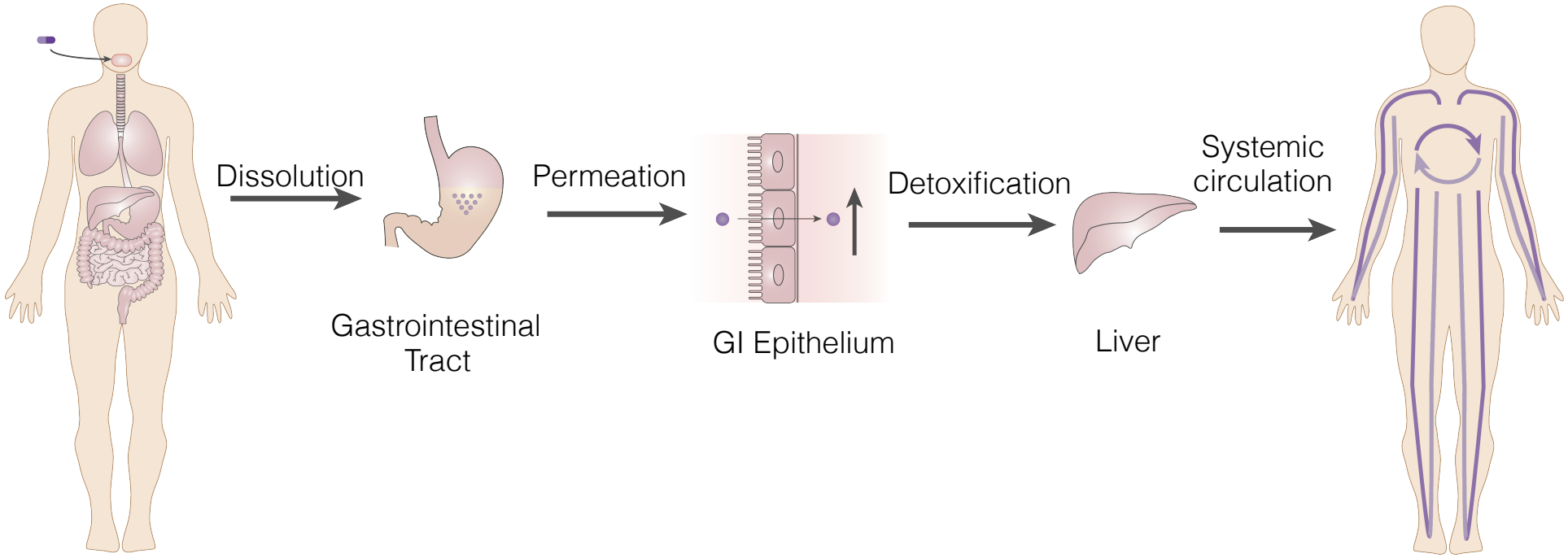
PF-06463922 Efficacy Against Clinical Mutants of ALK

ALK Mutation	Crizotinib pALK IC_{50} (nM)	PF-06463922 pALK IC_{50} (nM)
-	80	1.3
L1196M	843	21
G1269A	605	1.6
G1202R	1148	77
S1206Y	626	4.2
1151Tins	3039	38
C1156Y	478	1.6
L1152R	1026	9.0
F1147L	165	0.2



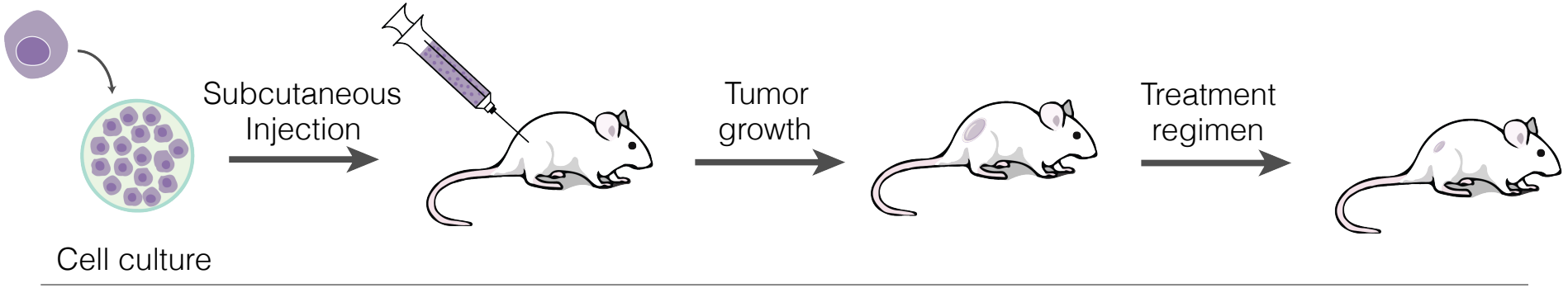
Oral Bioavailability (F)

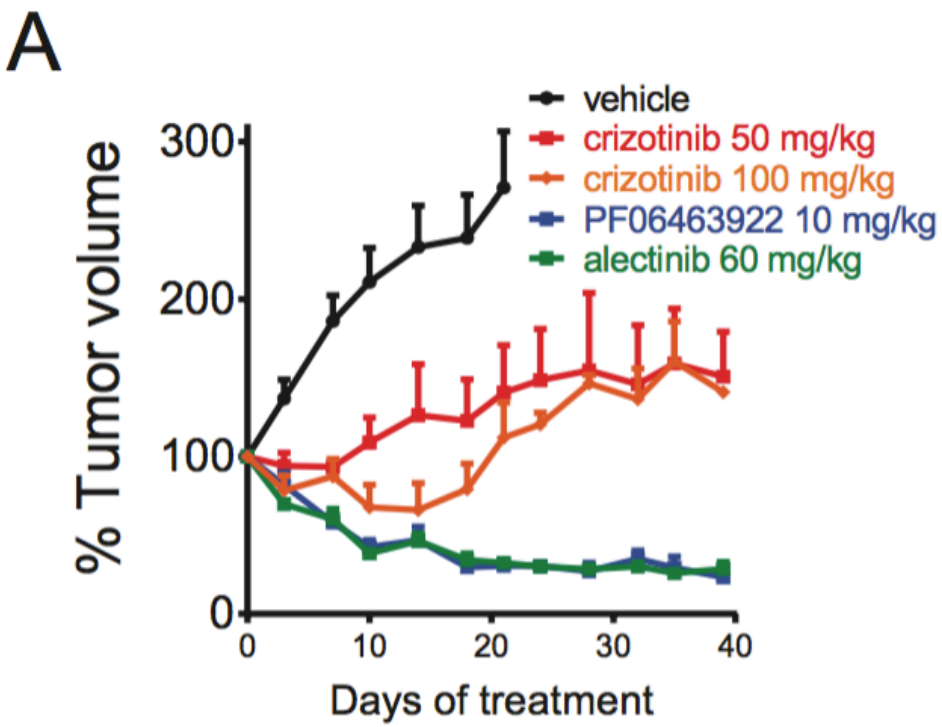
The fraction or percentage of orally administered drug that enters systemic circularion



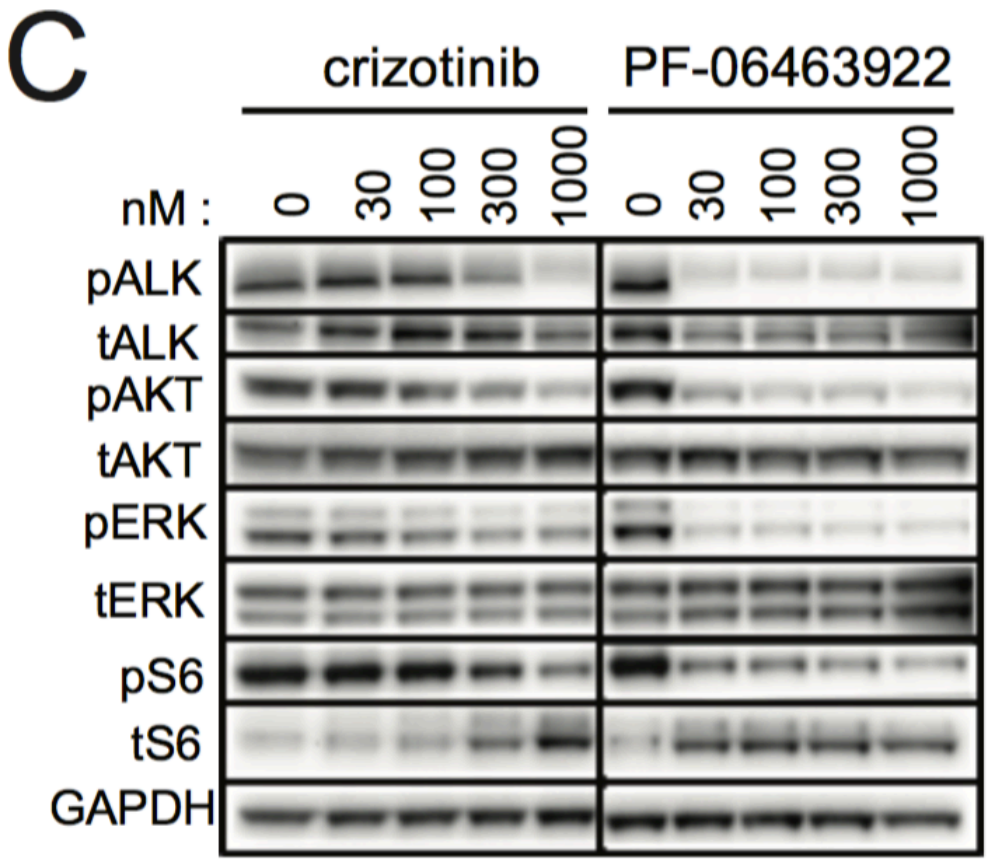
route[a,b]	F (%)
iv	
po	100

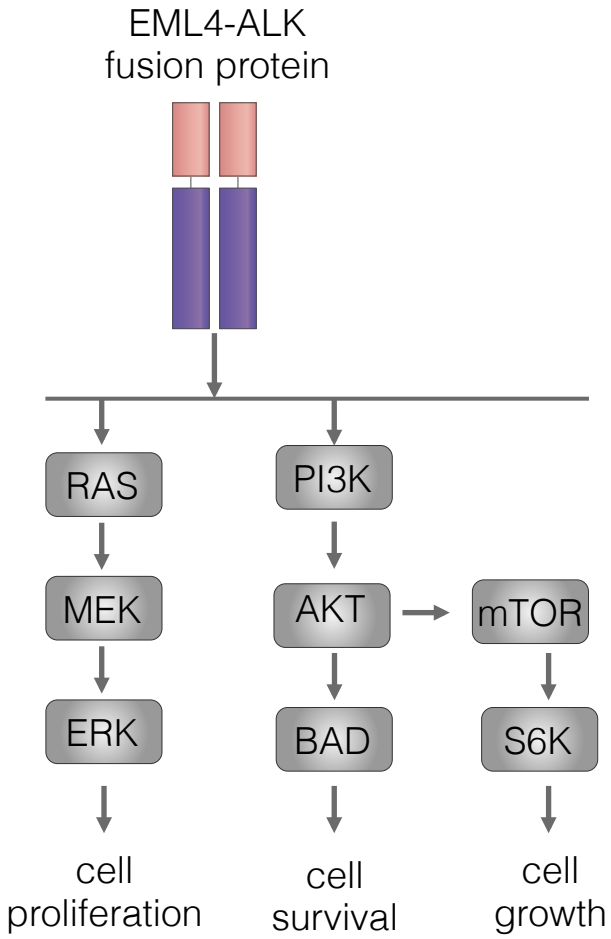
Tumor Xenograft Study



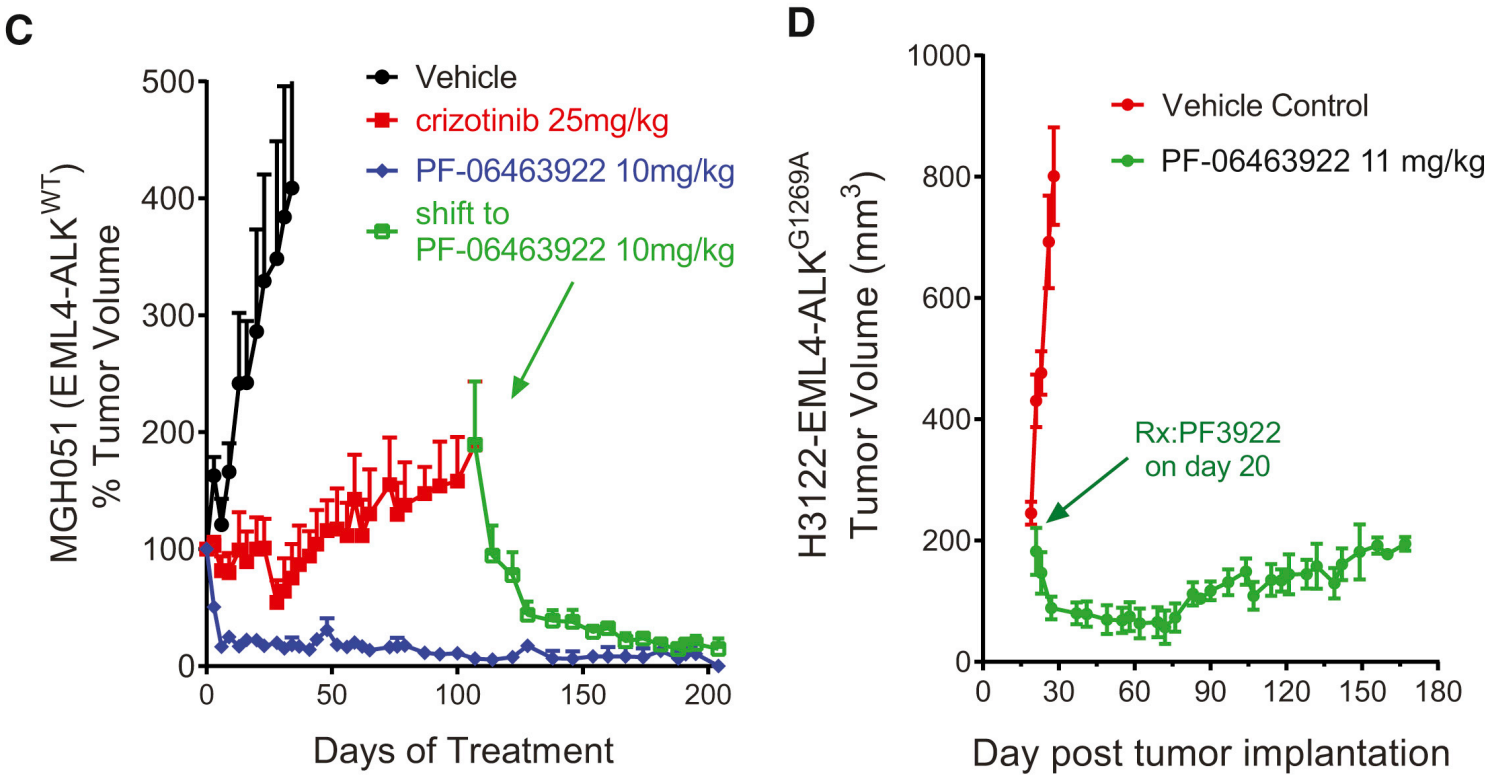


Pharmacodynamic Analysis

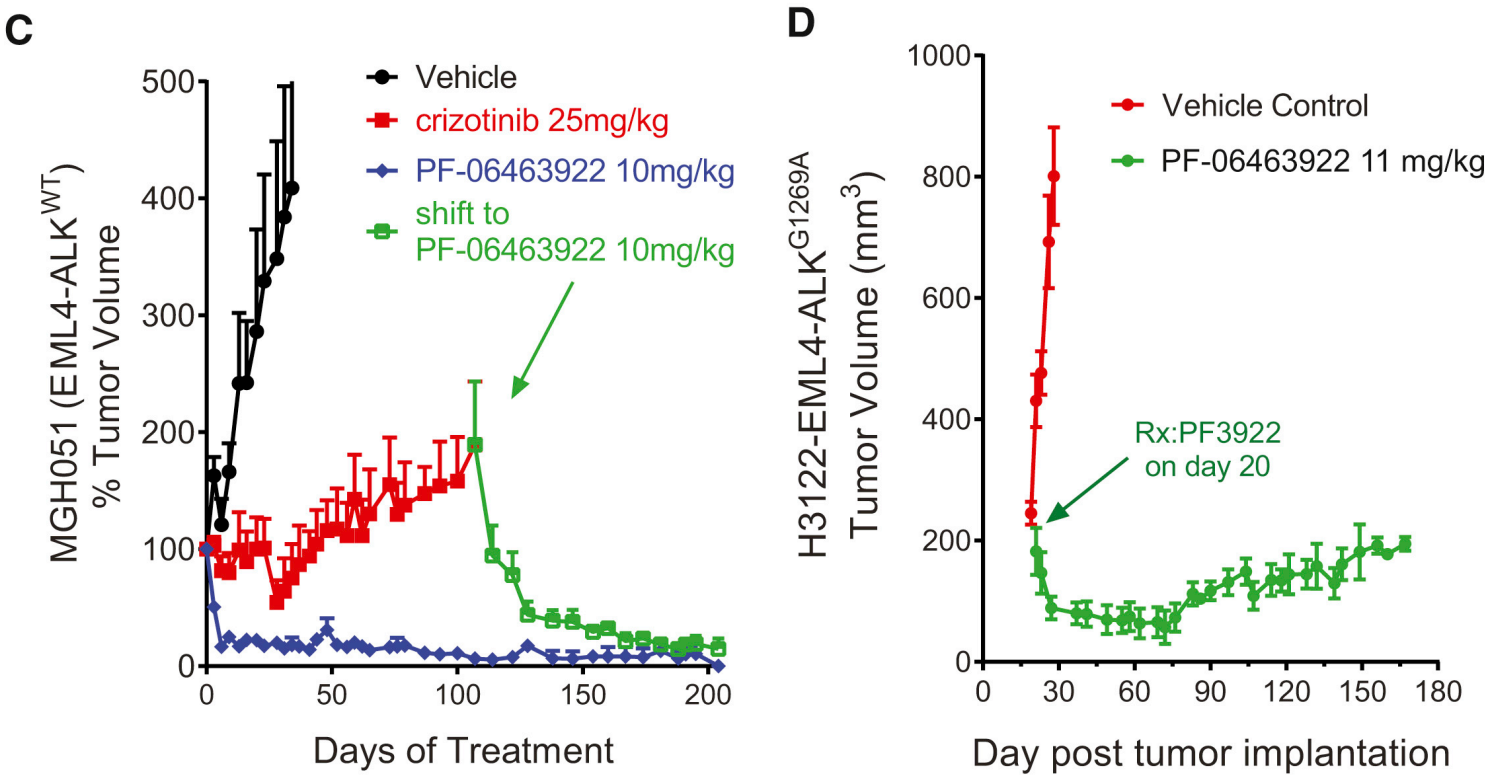




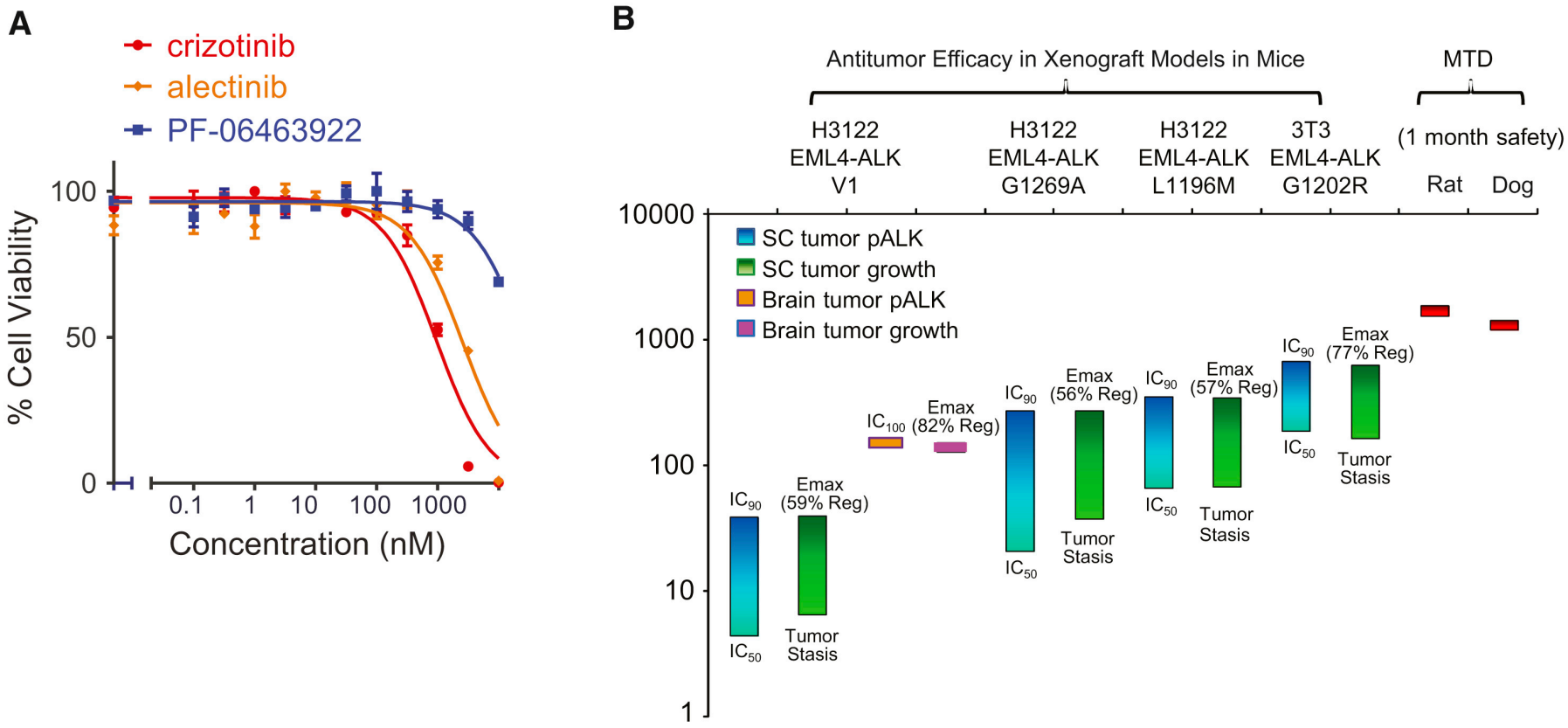
Preclinical Pharmacology Profile



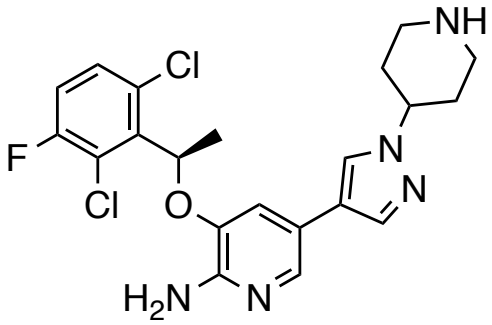
Preclinical Pharmacology Profile

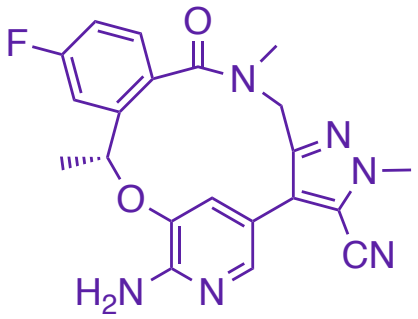


Preclinical Safety



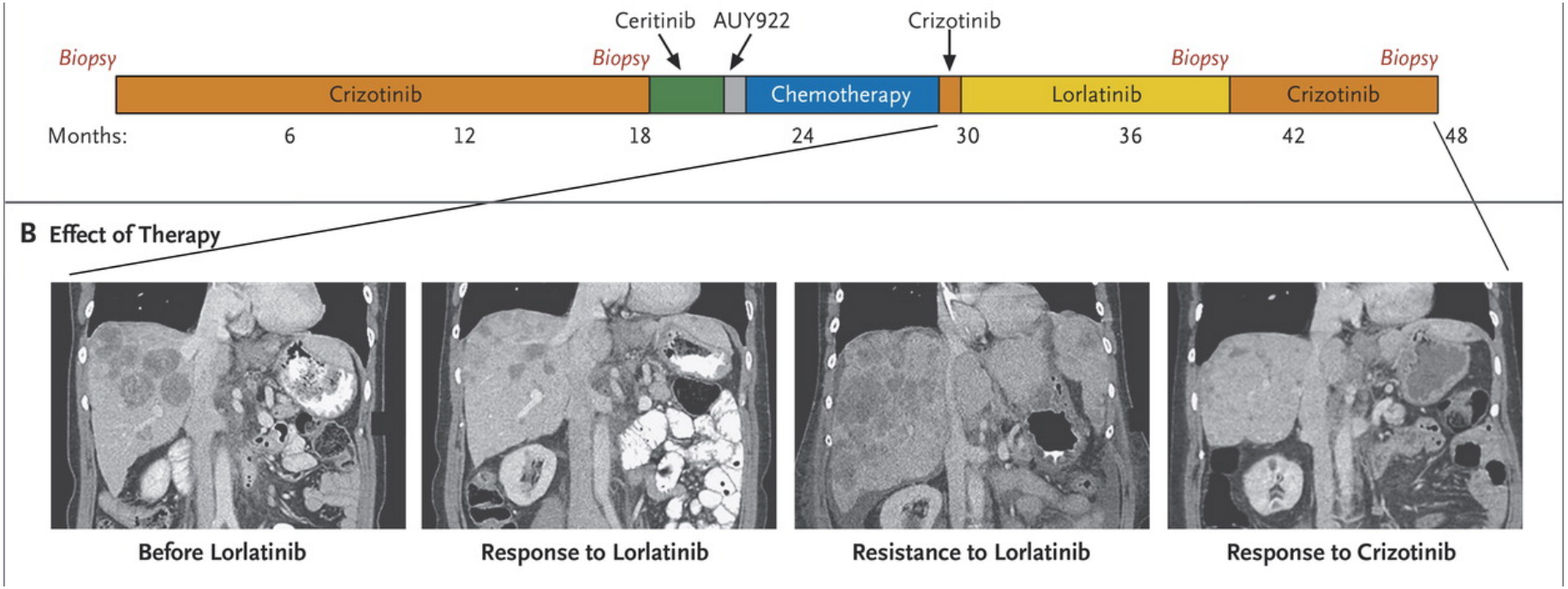
Summary and Conclusions





	Crizotinib		PF-06463922
ALK cell IC_{50}	80 nM	62x	1.3 nM
ALK-L1196M cell IC_{50}	843 nM	40x	21 nM
MDR BA/AB	45	30x	1.5
CNS free AUC (uM h), rat	-		3.47

Clinical Trials



ALK L1198F + C1156Y mutant is resistant to Lorlatinib (PF-06463922)

- Essentially all patients develop resistance to targeted therapies
- Sequential or combination treatment provides clinical benefit
- Tumor heterogeneity is evident and presents a significant therapeutic challenge

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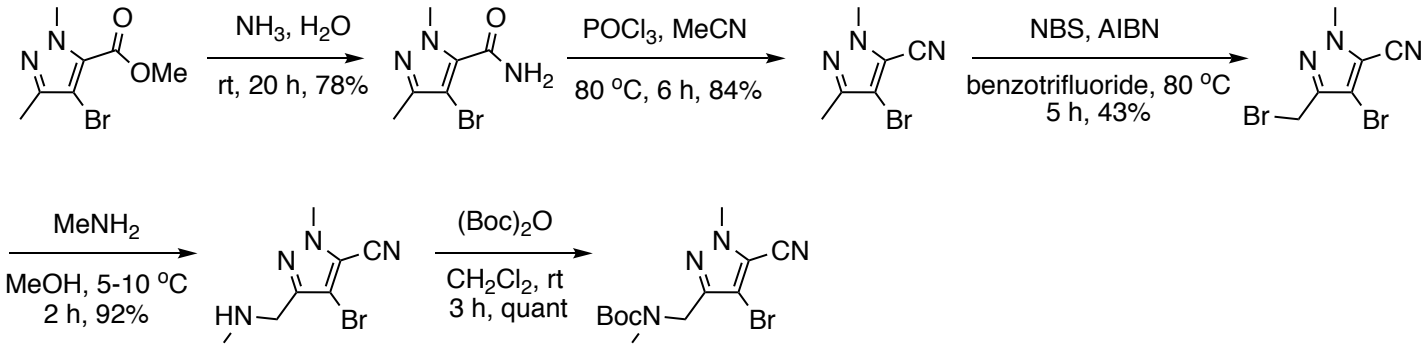
Macrocycle Synthesis



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