RAF Dimerizes in Complex with RAS During MAPK Pathway Activation

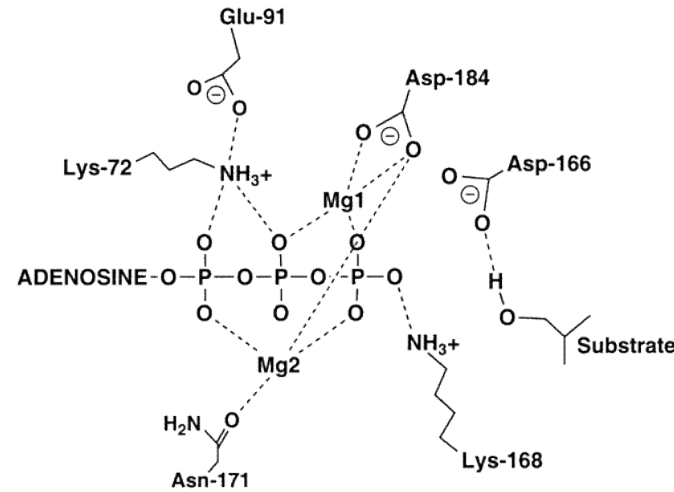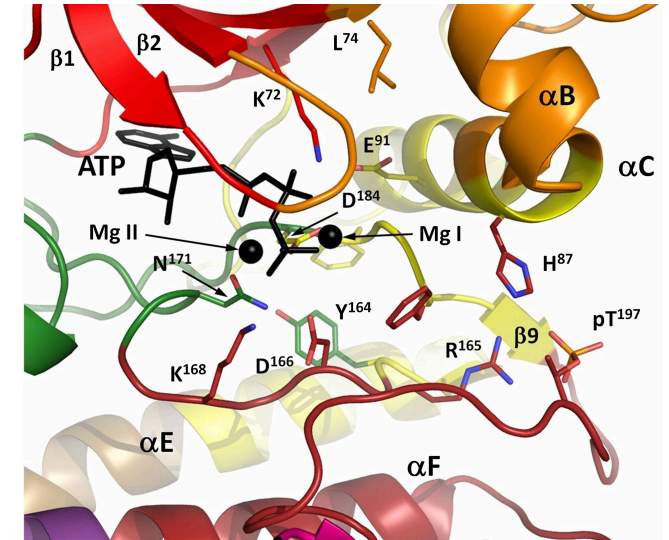## Kinase Cascade from RAS
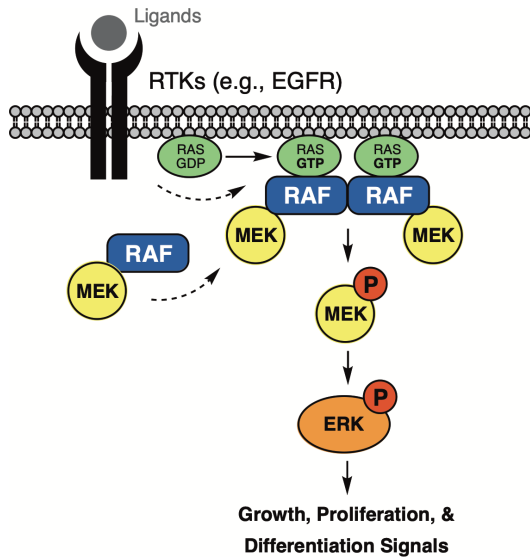
Charges Stabilize Mechanism

Constellation of Charged Residues

RAF Biology Deconstructed
In normal tissues, RAF dimerizes to set αC-Helix for activity- also in Class 2 & 3 BRAF alterations.
The V600 mutations activate monomeric BRAF: Approved BRAFi only work for V600 mutations.
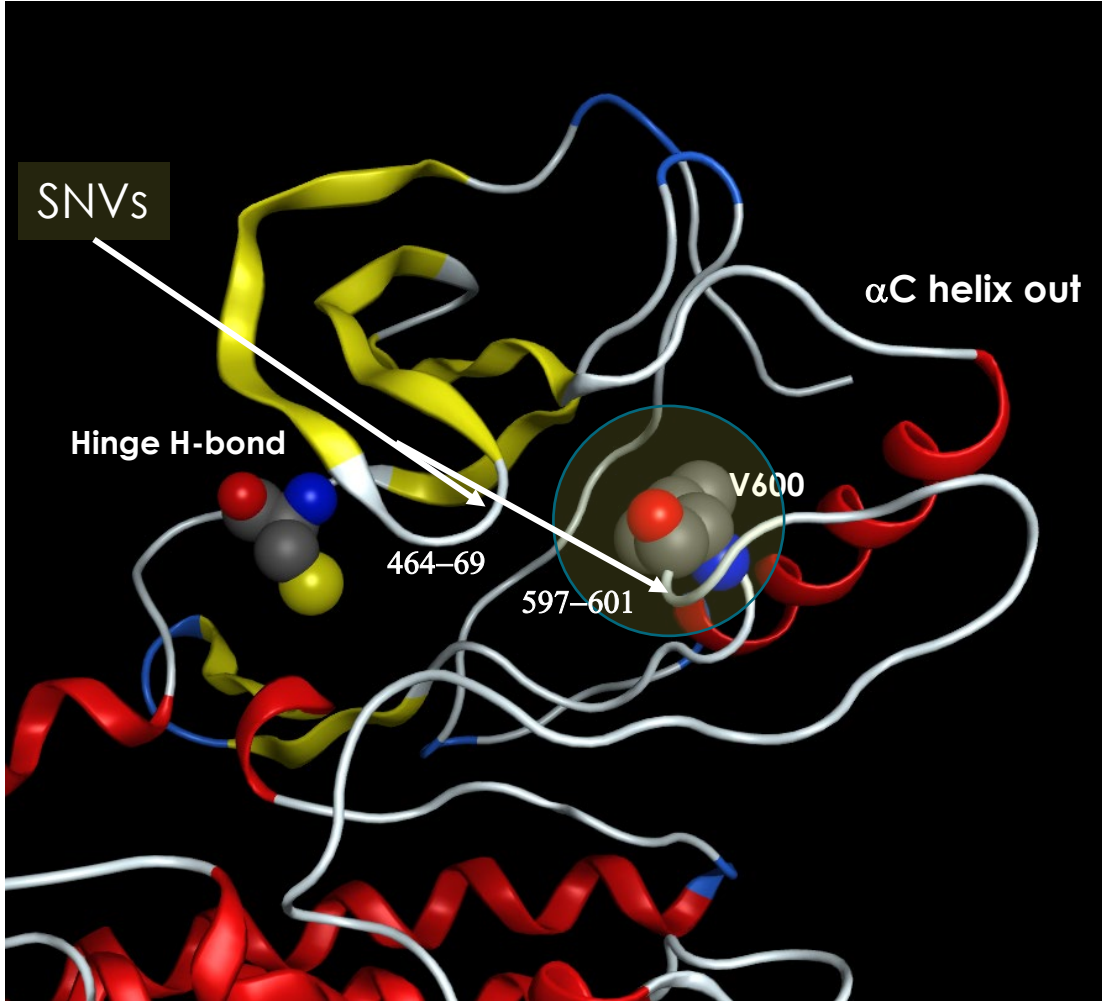
RAF Inhibitor Pharmacology Deconstructed
For RAF dimers, binding to one kinase ATP site (1st protomer) can activate the 2nd protomer kinase
For Class 2 & 3 BRAF alterations, successful dimer inhibitors must bind and inhibit both protomers equally

Adapted from: J. Adams, Chemical Reviews, 2001

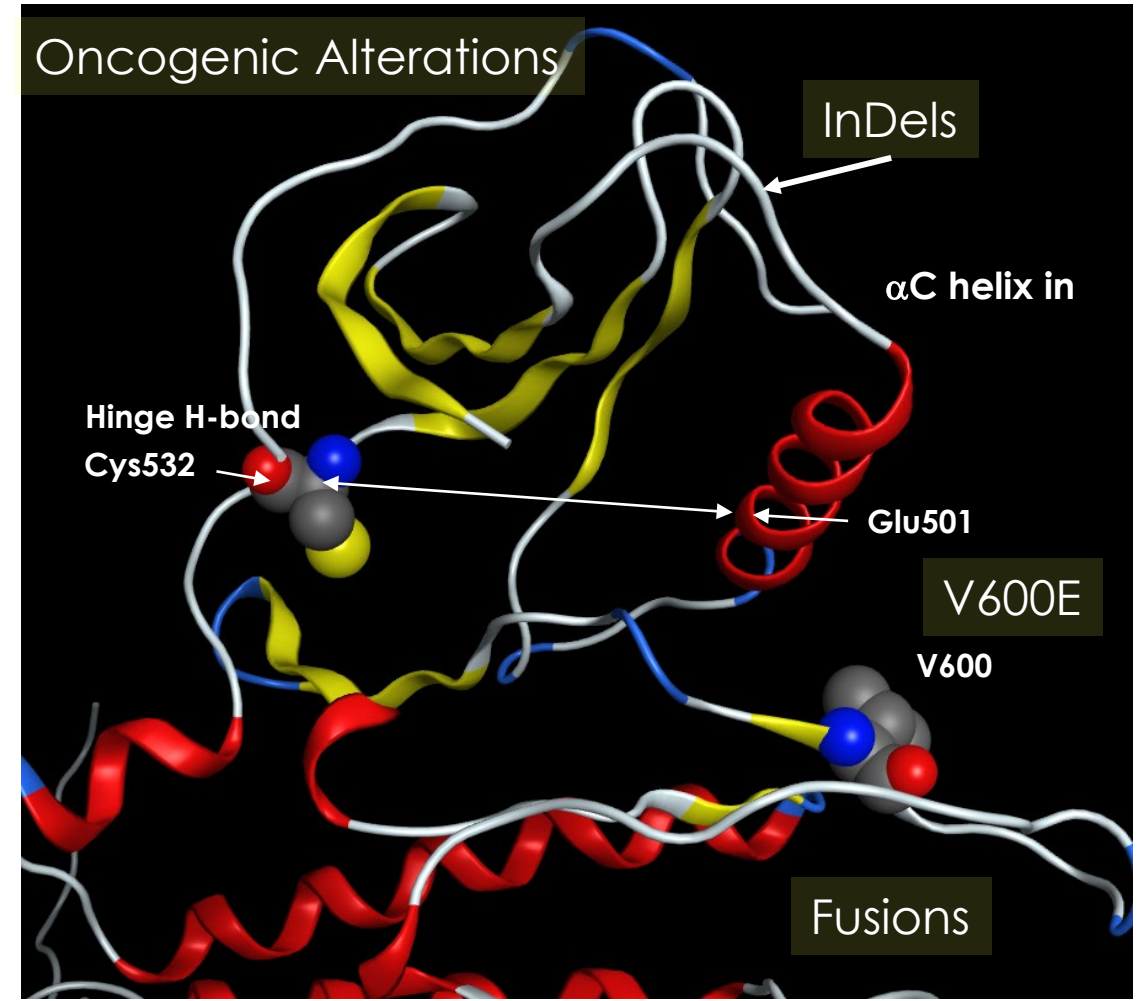# Structure of Active and Inactive BRAF by Cryo-EM
Phosphorylation, Dimerization, & αC-Helix Conformational Changes

Inactive BRAF

Active BRAF

Inactive BRAF panel labels: SNVs, Hinge H-bond, αC helix out, V600, 464–69, 597–601

Active BRAF panel labels: Oncogenic Alterations, InDels, αC helix in, Hinge H-bond, Cys532, Glu501, V600E, V600, Fusions
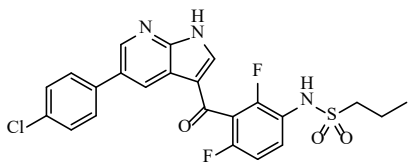
22.5 Å ⬅ α-Carbon Distance Cys532 to Glu501 ➡ 19.6 Å

Approved RAF Inhibitors Are Limited by Paradoxical Activation

RAS **RAS**

BRAF^{Mut} **B/CRAF**

Approved RAF inhibitors:
Vemurafenib
Dabrafenib
Encorafenib

MEK

ERK

Growth Signaling

Paradoxical activation due to drug altered BRAF asymmetric dimerization or CRAF in heterodimer

2nd site remains unbound at relevant exposures, and is conformationally activated

Need molecule that can <u>potently</u> inhibit both kinase active sites

1St Gen BRAF inhibitors can cause squamous cell carcinoma in skin cells due to paradoxical activation, and are combined with MEK inhibitor
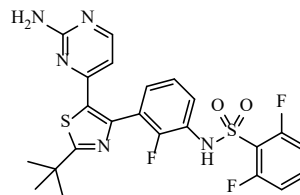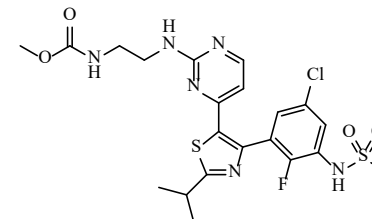
The RAF Inhibitor Landscape

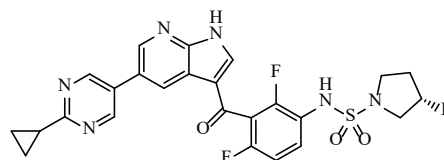## Gen 1: V600E BRAFi, paradoxical dimer activation
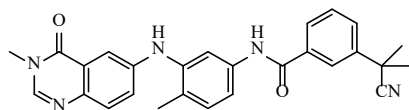

vemurafenib

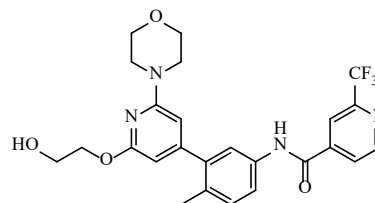dabrafenib

encorafenib

Dimer Buster

PLX8394

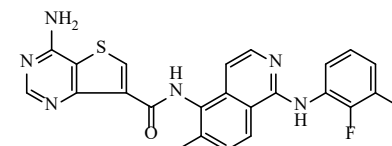## Gen 2: Pan-RAF Dimer Inhibitors

AZ-628

(2016) Cancer Cell **30**: 1-14
2016 X-Ray 4RZW

Naporafenib

(2020) J. Med. Chem., **63**, 2013–2027
2019 X-Ray 6NOP

Belvarafenib

(2021) Nature **594**: 418-423
2020 X-Ray 6XFP

The Asymmetric BRAF Dimer- High & Low Affinity Protomer Conformations

The high affinity, first protomer binding event sets second protomer in an active conformation
The low affinity site is not bound up to 10uM concentrations in cell culture, but was co-crystallized
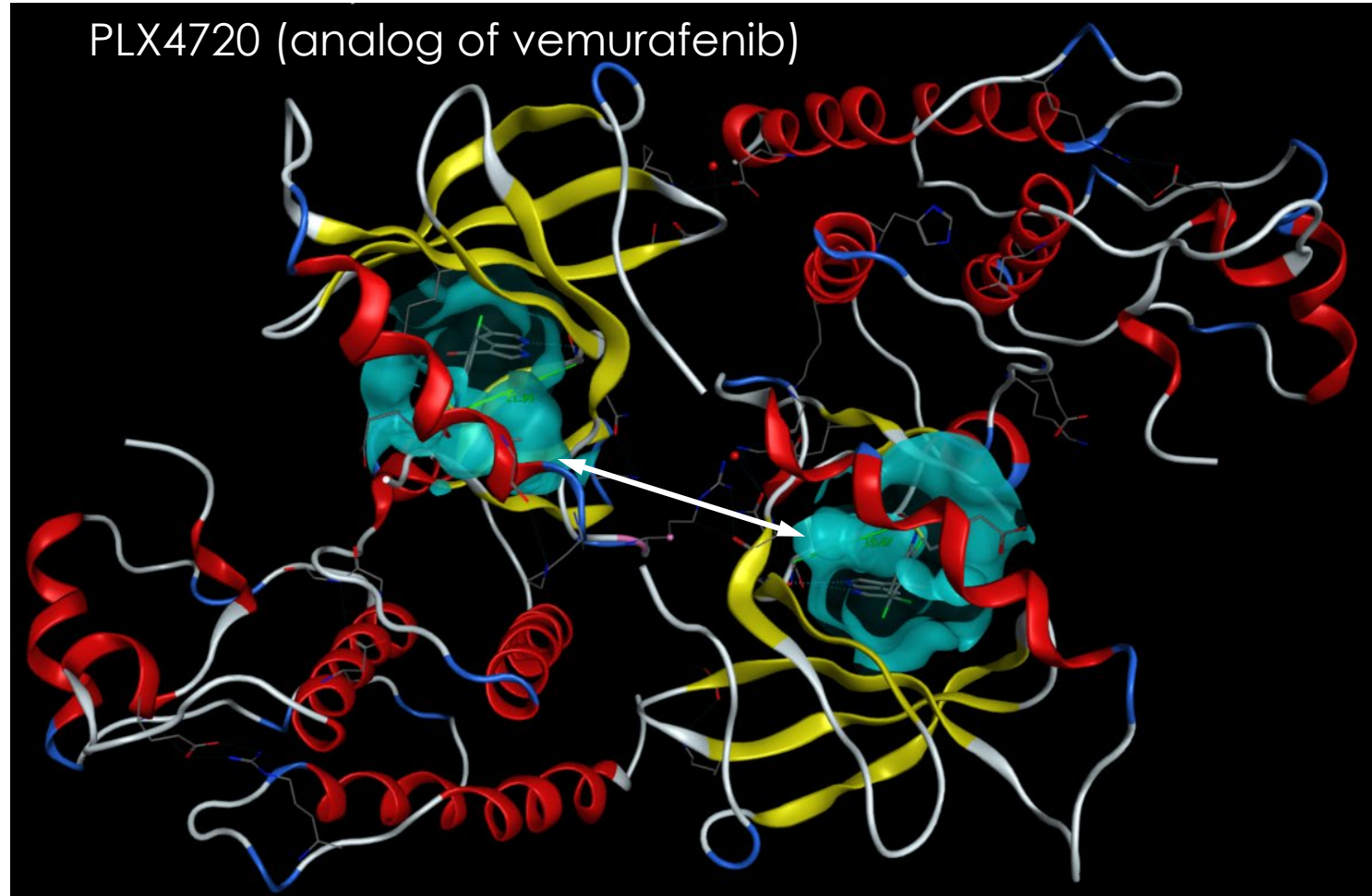
PLX4720 (analog of vemurafenib)

NCI-H358, wtRAF & mKRAS

BJ, wtRAF

Tsai et. al., PNAS, 105, (2008) pg 3041
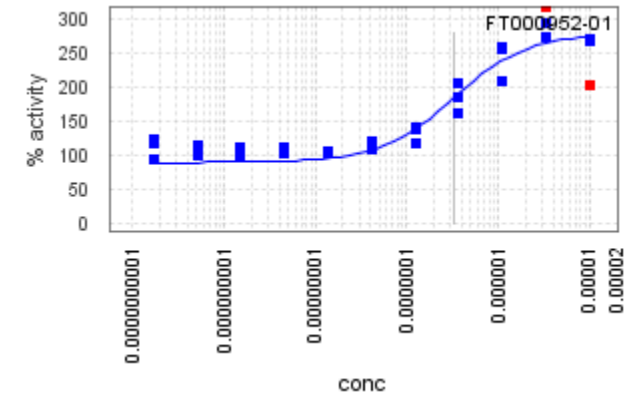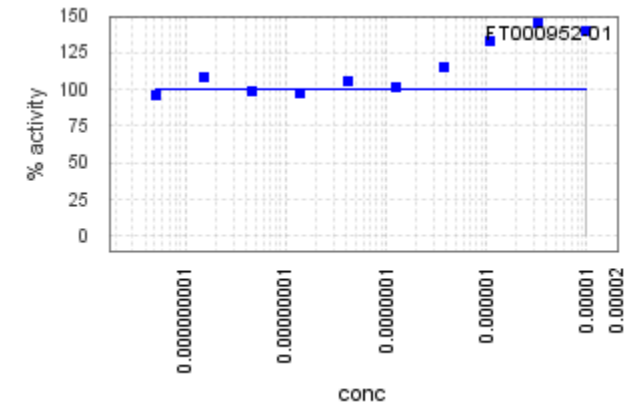
Zoom in of Back Pocket RAF Asymmetry with PLX4720:
Single Crystal Structure with Clear High and Low Affinity Modes in Unit Cell

Potent Inhibition by PLX4720…
Buried lipophilic n-Pr into deep hydrophobic pocket, pushing out αC-Helix
Sulfonamide makes 3 good H-bond
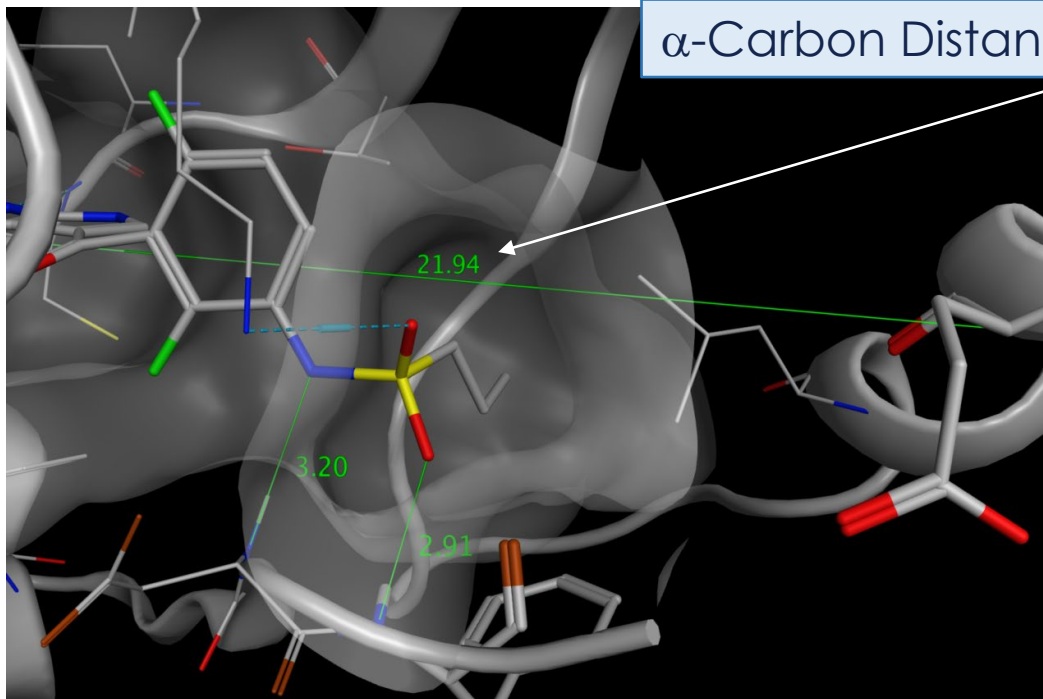
<u>Weak</u> Inhibition by PLX4720…
Lipophilic n-Pr into points out to solvent with αC-Helix in active position
Sulfonamide buries one oxygen as unmet H-bond acceptor

α-Carbon Distance Cys532 to Glu501
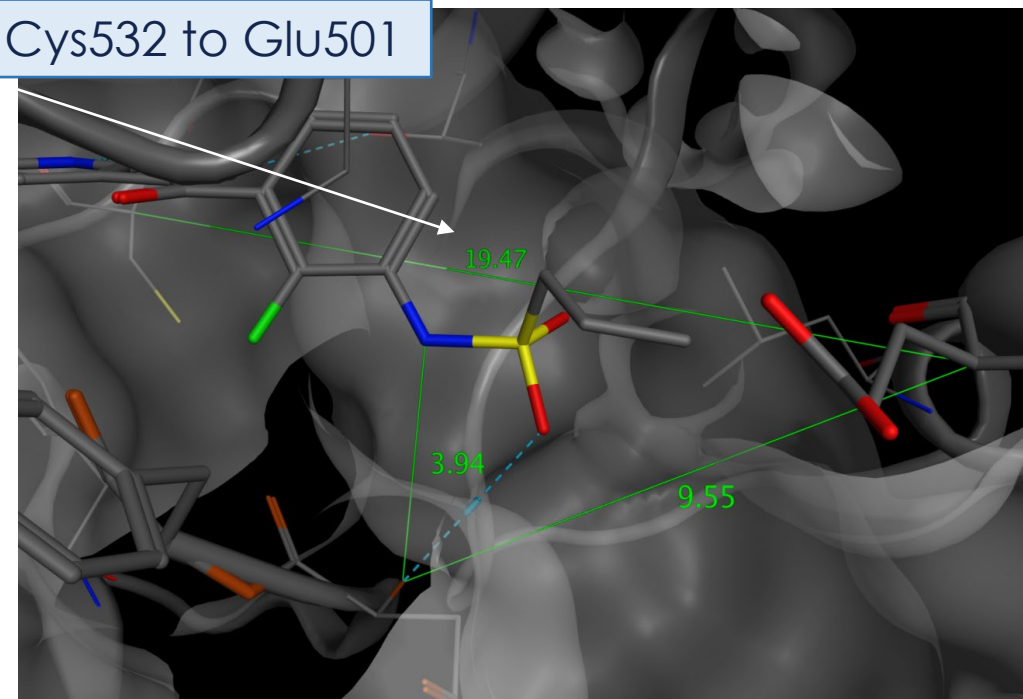
This is the RAF form to target for oncogenic alterations that activate dimers

Confirmed: Vemurafenib Bound to Single Promoter of Asymmetric BRAF Dimer

Bollag, G. et. al., Nature, 467, (2010) pg 596
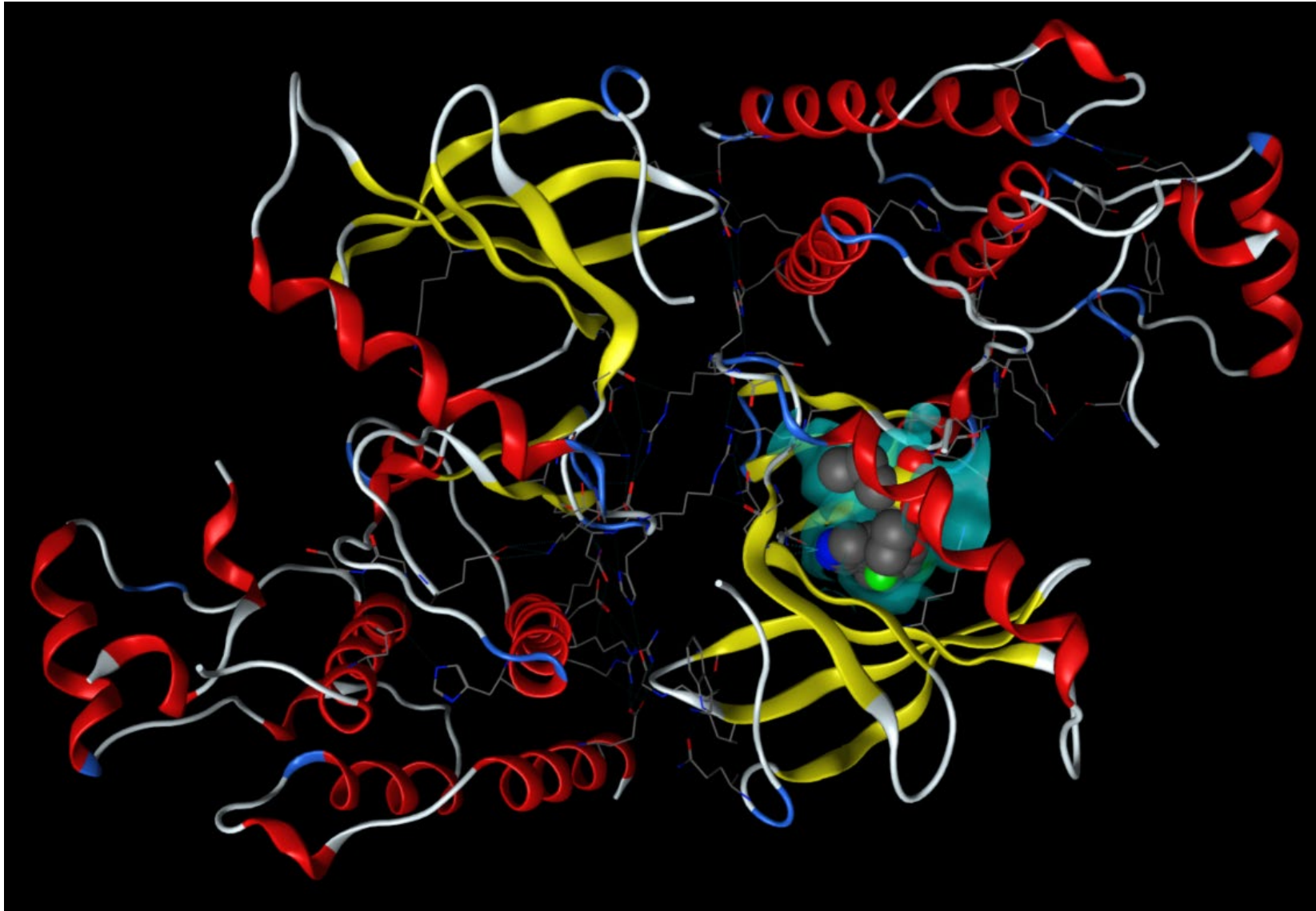
The RAF Opportunity

Targeting RAF Mutant-Driven and Dependent Cancers With No Approved Targeted Therapies

Approved Class I BRAF inhibitors: Vemurafenib, Dabrafenib, and Encorafenib

Patients with Class II / III BRAF alterations represent an unmet need

Kinnate's approach with exarafenib targets dimer signaling while minimizing MAPK paradoxical activation in wild-type signaling

Our Focus

Wildtype | Class I | Class II | Class III

Growth Signaling
(through MEK/ERK Activation)

**+ RAF Dependent Cancers
(e.g., RAS-mediated)**
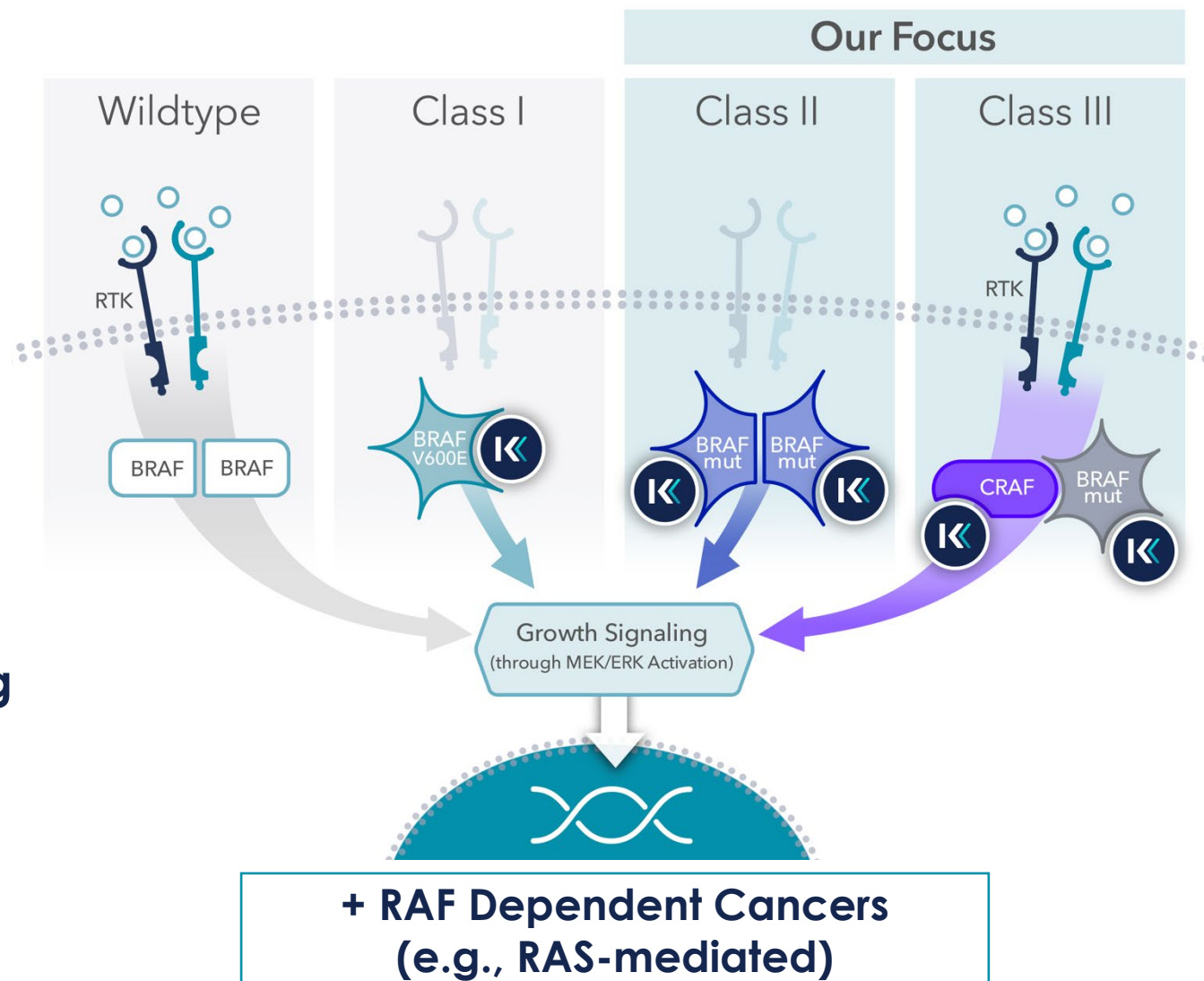
Goal: Target DFG-out Back Pocket Binder with great Pharmaceutical Properties

Reminder-
Fill "DFG-out" back-pocket for potent affinity to **both** protomers
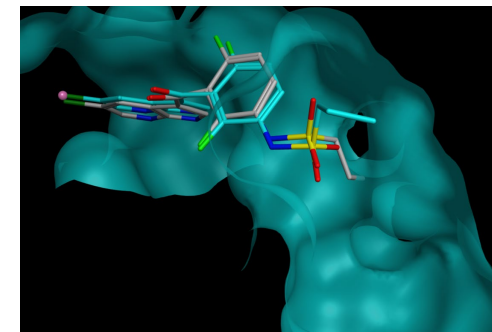
AZ-628
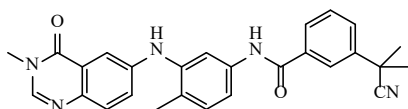
cLogP = 3.9
pKa = 2.0
f-sp^3 = 0.15
Rot. Bonds = 5

Naporafenib
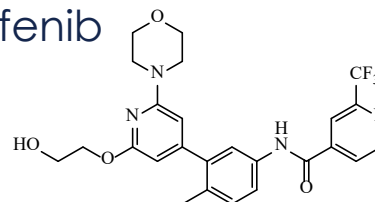
cLogP = 3.4
pKa = 3.7
f-sp^3 = 0.32
Rot. Bonds = 7
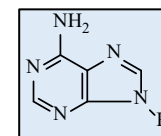
Adenine of ATP
Focus on morpholine
for **selectivity**

Belvarafenib

cLogP = 5.3
pKa = 5.4
f-sp^3 = 0.04
Rot. Bonds = 4

2016 X-Ray 4RZW
18.7 Å Cys to Glu αC
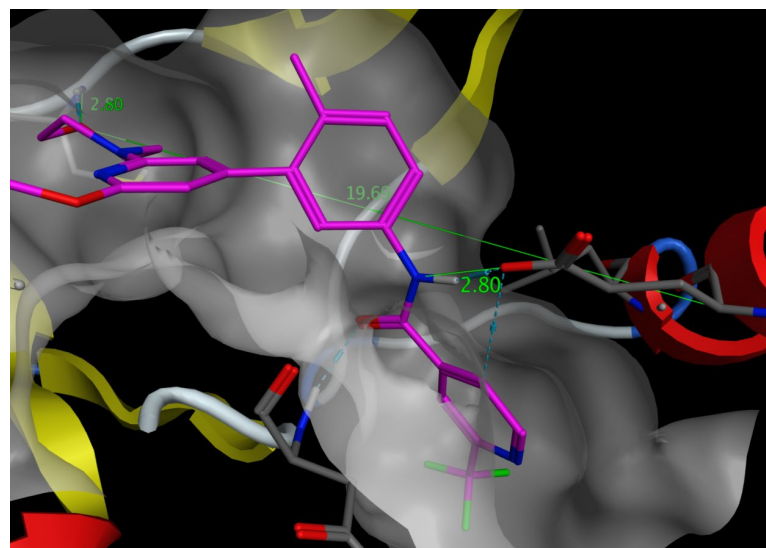
J. Med. Chem. 2020, 63, 2013–2027, 2019 X-Ray 6NOP
19.7 Å Cys to Glu αC
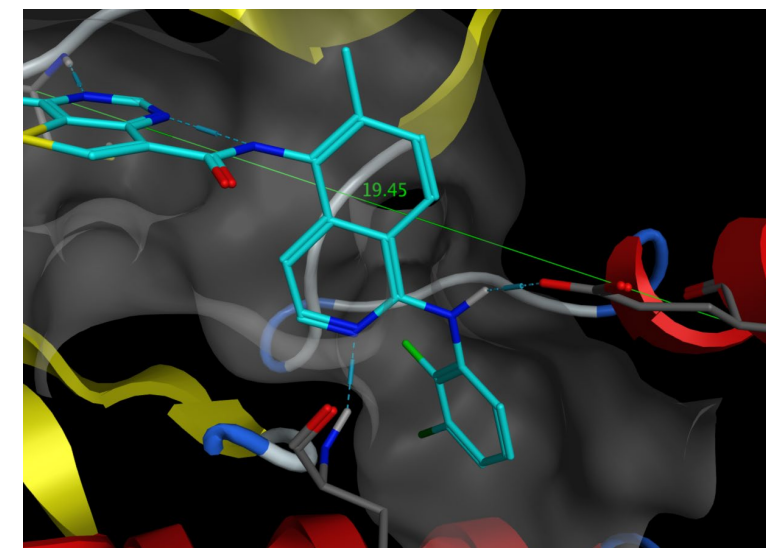
6XFP crystal structure 6/2020
18.8 Å Cys to Glu αC

10

There are Significant Liabilities in 2nd Gen Pan-RAF Inhibitor
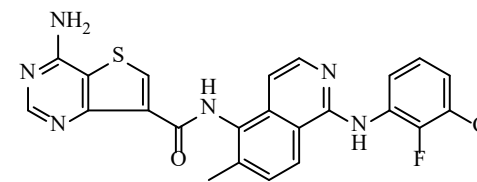
cLogP	= 3.4	5.3
pKa	= 3.7	5.4
f-sp^3	= 0.32	0.04
Rot. Bonds	= 7	4

exarafenib

cLogP	= 2.7
pKa	= 5.3
f-sp^3	= 0.54
Rot. Bonds	= 8

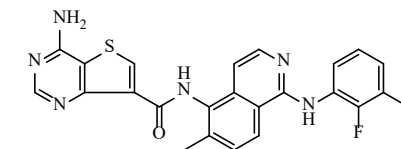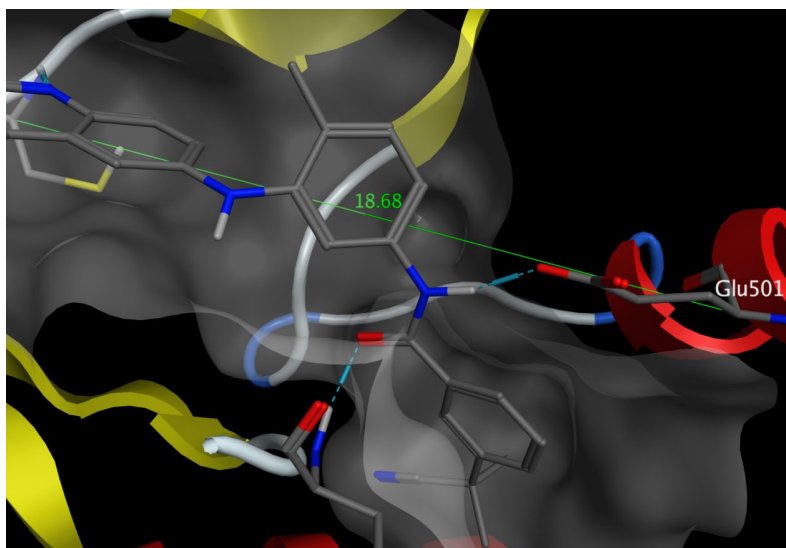*pERK Inhibition EC50 (nM)

	Erasca Naporafenib	Hanmi / Genentech Belvarafenib
Class II / III Cell Potency (nM)*	5 to 32 nM	4 to 42 nM
Human Plasma Free Fraction (%)	<1	<1
Aqueous Solubility (uM):		
pH = 2	50	266
pH = 4.5	7	0.4
pH = 7.4	6	0.1

> Improve aqueous solubility, lower unbound clearance to increase free drug exposure for **greater target coverage** in the clinical setting

1. Increase basic
2. Reduce planarity/rigidity
3. Decrease lipophilicity
4. Modify anilide => proteolysis to an aniline toxicophore
5. Avoid aromatic halogens, a structural class associated with skin toxicity, which could be additive to MAPKi mechanism-based toxicity

Four Critical Factors to a Successful Pan-RAF Inhibitor

Exarafenib is Designed to Achieve Inhibition of Class II and Class III BRAF-driven Cancers
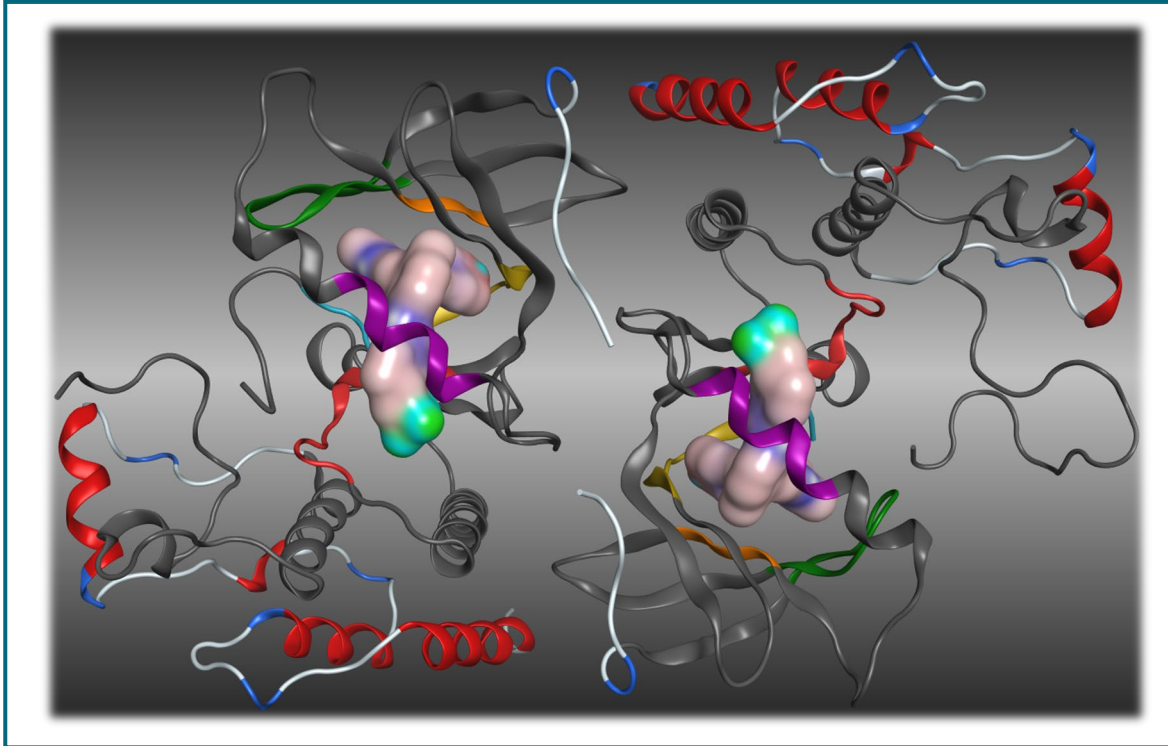
1 Selective drug — Limited off-target activity

2 Broad alteration coverage — Ability to cover heterogenous nature of BRAF Class II & Class III alterations

3 Equipotent inhibition — Equally inhibits across both RAF kinases in the dimer, Narrows concentration levels at which paradoxical activation occurs

4 Superior pharmaceutical properties — Robust PK with high free fraction to enable more drug available to bind to target

Urea Saturated Heterocycles Simultaneously Satisfy Multiple Design Strategies
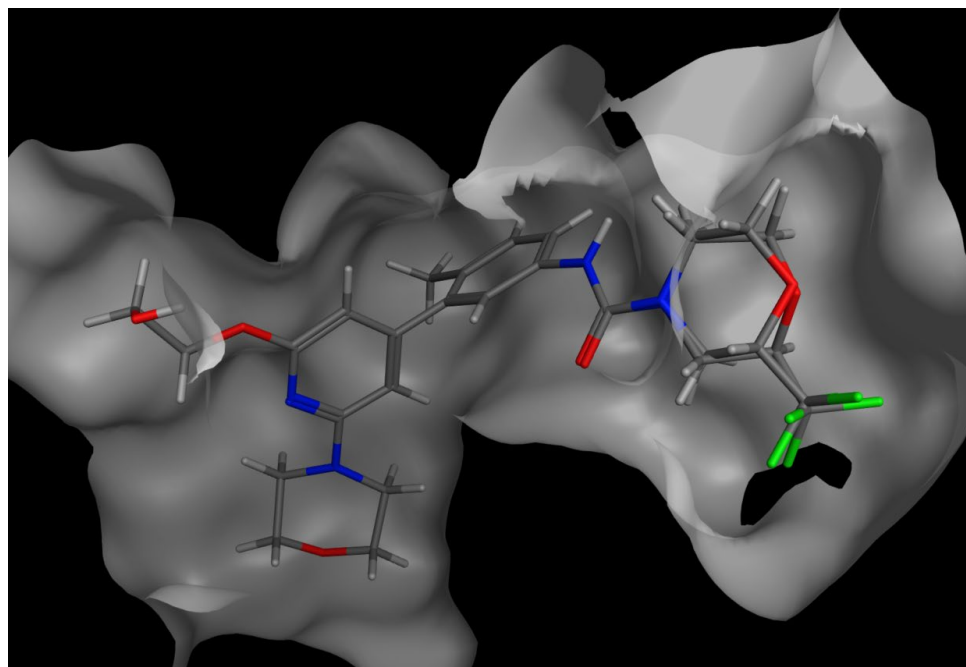
Selectivity Targeted by Focusing on Morpholine Hinge Binder and Tight Fit of Back Pocket

Only Subtle Binding Differences Between Epimers: CF_3 Occupy the Same Space

1. Increase basicity
✓ 2. Reduce planarity/rigidity
✓ 3. Decrease lipophilicity
✓ 4. Modify anilide => proteolysis to an aniline toxicophore
✓ 5. Avoid aromatic halogens, a structural class associated with skin toxicity (additive to MAPKi mechanism-based toxicity)

Saturated heterocyclic ureas: stable, less rigid, more soluble
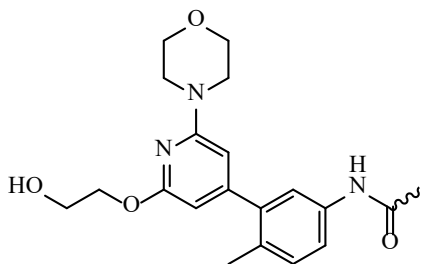
✔ 1. Reduce planarity/rigidity
✔ 2. Decrease lipophilicity
✔ 3. Modify anilide => proteolysis to an aniline toxicophore
✔ 4. Avoid aromatic halogens, a structural class associated with skin toxicity (additive to MAPKi mechanism-based toxicity)

| Compound	Back Pocket	cLogP	sp³ Ratio	Solubility pH 7.4	Hep Stable %R	Class 2 H2405	Class 3 WM3629	Class 1 A-375	BRAF Enz
Naporafenib		3.4	0.32	6	83	4.0	3.6	169	2.6
1784		2.2	0.50	62	93	15	12	850	2
1790		2.2	0.50	56	54	15	8	700	6
1604		2.8	0.50	17	9		12		17
1743		2.3	0.50	83	35	14	7	1600	

$IC_{50}s$ in nM

Pyrrolidine Urea Fits Contour with Trifluoroethyl Filling Deep into Pocket

Vs.

| 0.53 | ← | SP3 Ratio | → | 0.32 |
| +1 (at least) | ← | Rotatable Bonds | | |
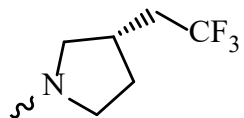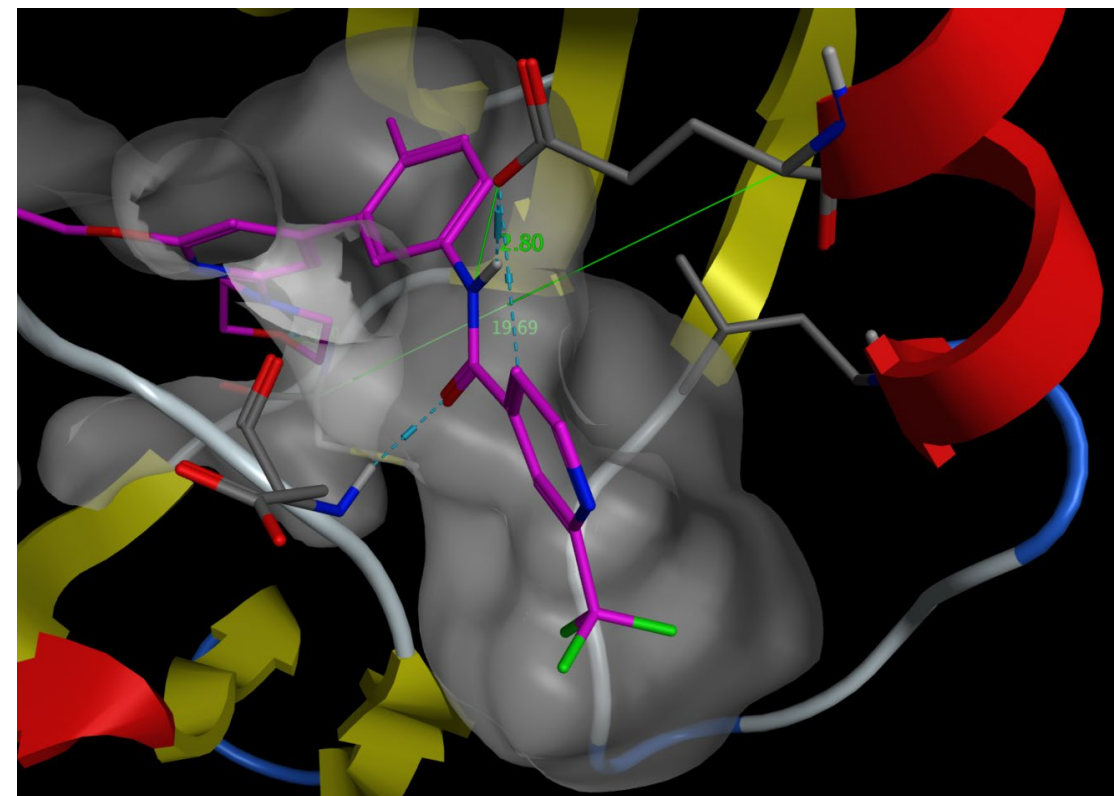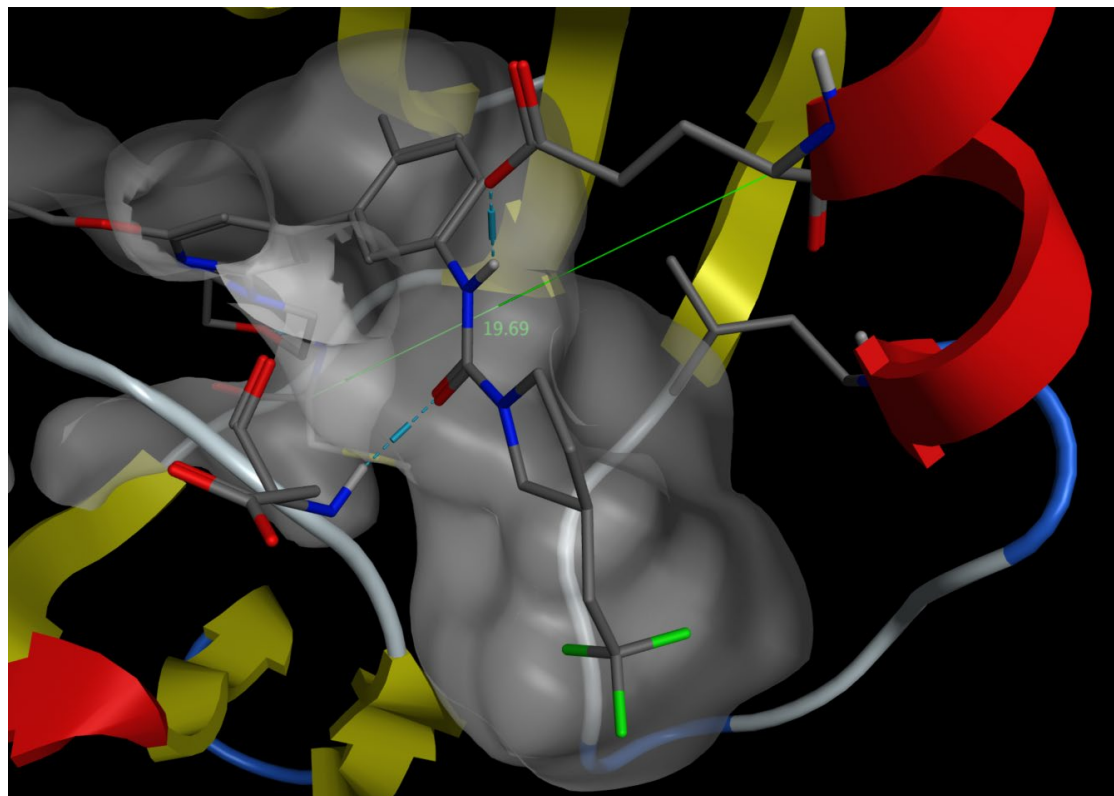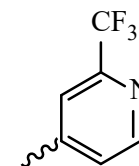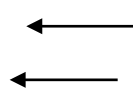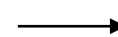
5-membered heterocyclic ureas with exocyclic extension
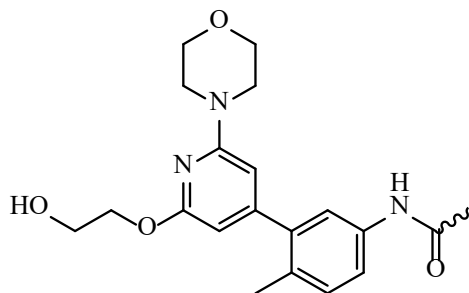
Compound	Back Pocket	cLogP	sp³ Ratio	Solubility pH 7.4, 4.5	Hep Stable %R	Class 2 H2405	Class 3 WM3629	Class 1 A-375	BRAF
Naporafenib		3.4	0.32	6, 7	83	4.0	3.6	169	2.6
1996		2.7	0.50	57	46	7	7	730	5.3
1965		2.6	0.52	43, 35	56	15	4	238	1.9

IC_{50}s in nM

Solvent Exposed Side of Adenine Pocket: Branching to Disrupt Crystal Packing, Increase Solubility, Modify Potency/Stability
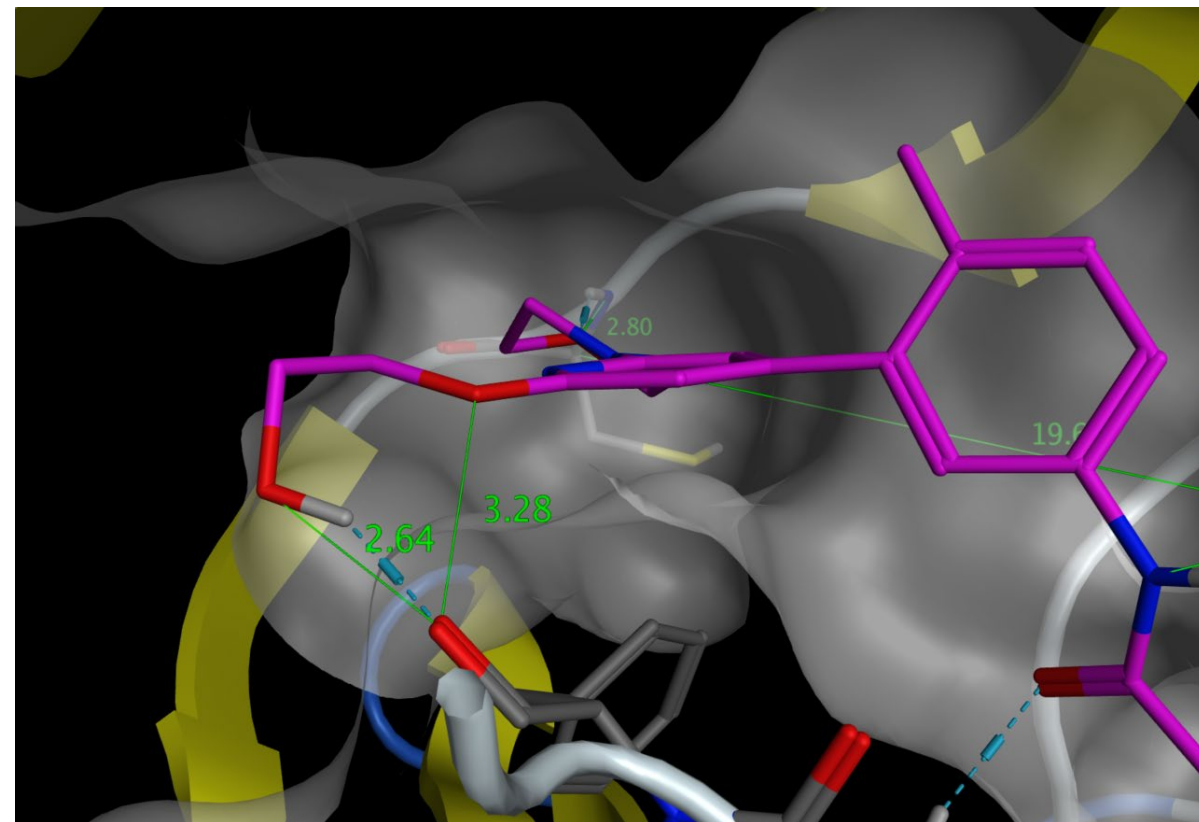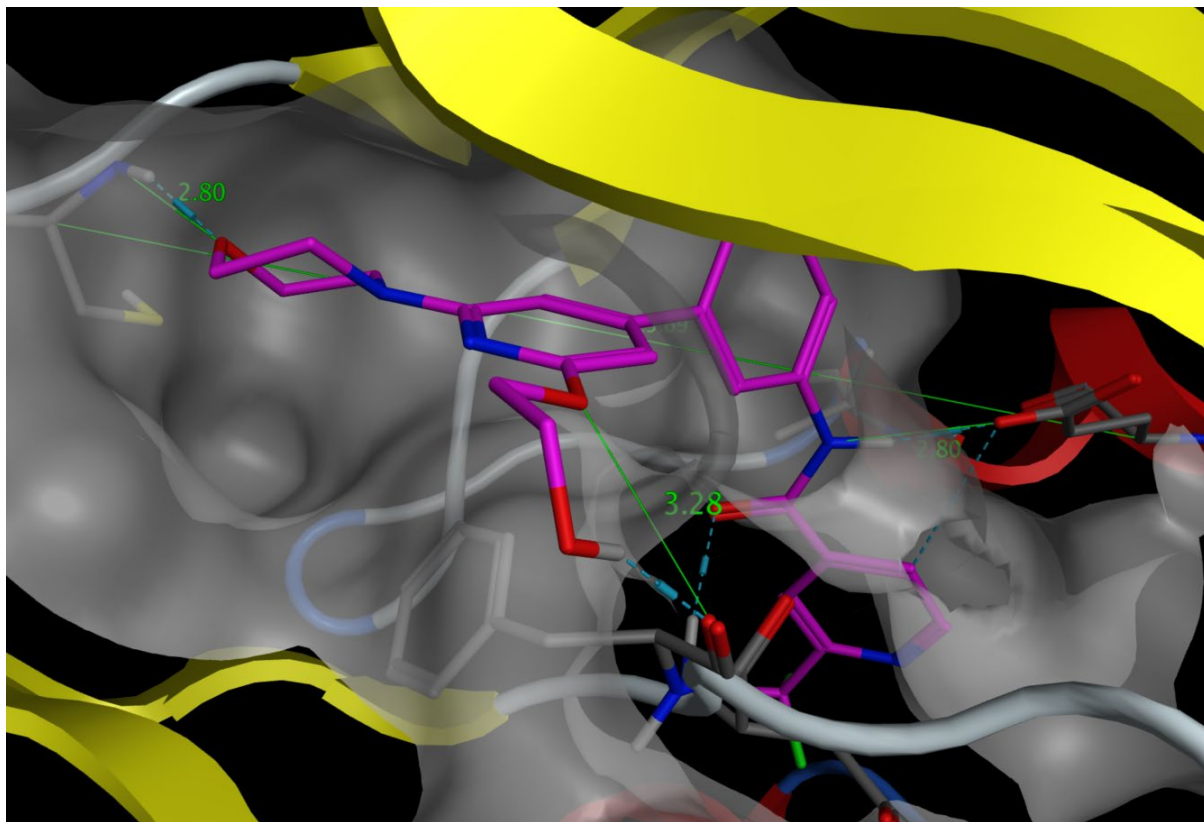
Branching to disrupt crystal packing
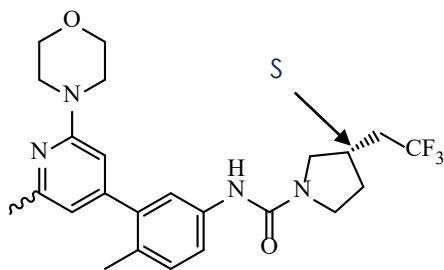
methyl adds lipophilicity, but branch minimize negative impact on solubility

Compound	Solvent Exposed	cLogP	sp³ Ratio	pKa	Solubility pH 7.4, 4.5	Hep Stable %R	Class 2 H2405	Class 3 WM3629	Class 1 A-375	BRAF
1966 - S		2.6	0.52	3.8	39, 35	56	10	9	238	
2280 - S		2.9	0.54	4.0	23, 26	23	12	14	94	5.4
2297 - S		3.3	0.56	4.2	15	60	10	8	48	5.4

IC_{50}s in nM

α-Branching brings in greater Class 1 potency
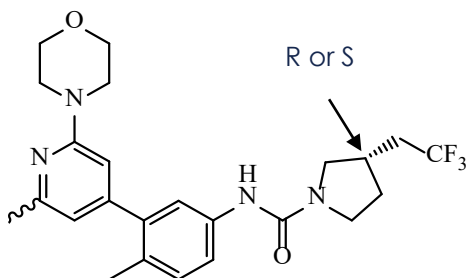
Compound	Solvent Exposed	cLogP	sp³ Ratio	pKa	Solubility pH 7.4, 4.5	Hep Stable %R	Class 2 H2405	Class 3 WM3629	Class 1 A-375	BRAF
1966 - S	HO~O~	2.7	0.52	3.8	39, 35	56	10	9	238	
2464- S	HO~O~	2.9	0.54	3.8	17	38	6	15	42	5.6
2465 - R	HO~O~	2.9	0.54	3.8	17		8.7	8.5	146	5.0
2480 - S	HO~O~	2.9	0.54	3.8	19	45	15	40	302	7.9
2481 - R	HO~O~	2.9	0.54	3.8			51	18	1550	9.2

IC_{50}s in nM

Although greatly improved solubility, desired greater solubility for oral absorption at acidic pHs

Target increased basicity with pKa > 4.5

Modify Linker Atom to Nitrogen

➢ Increases electron richness of pyridine core: pKa increase from 3.8 to 5.3

➢ Makes direct H-bond to backbone carbonyl oxygen

➢ Reduces logD
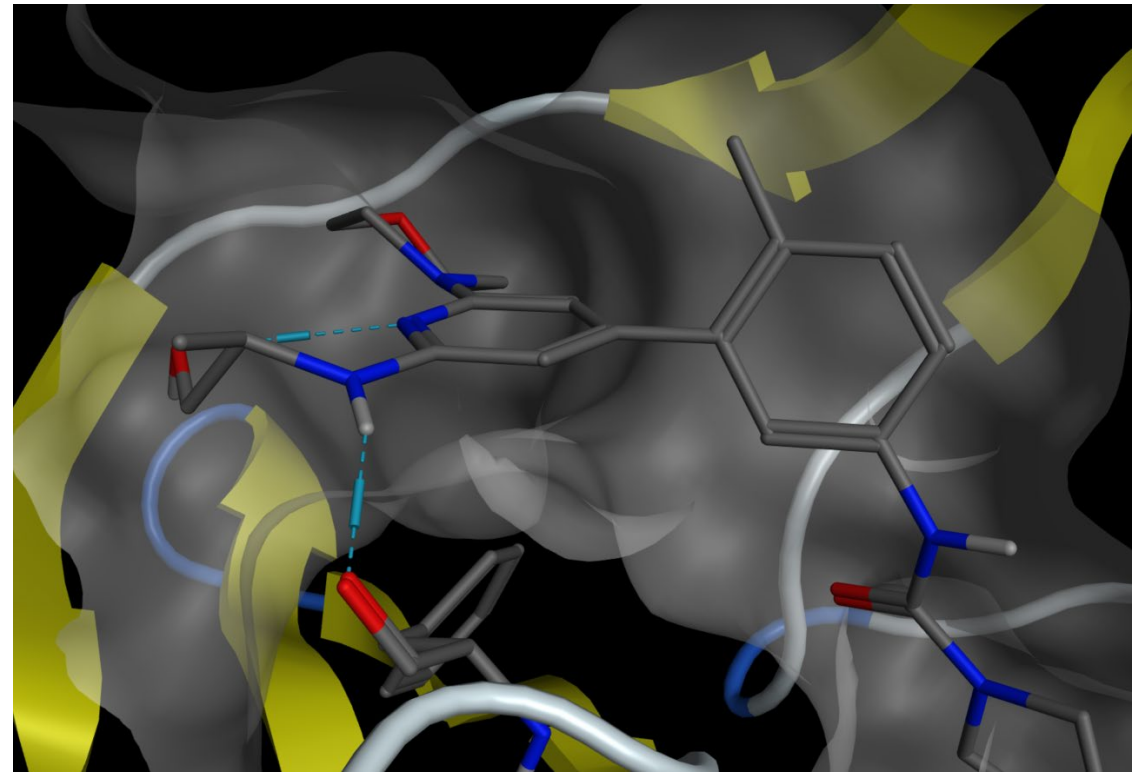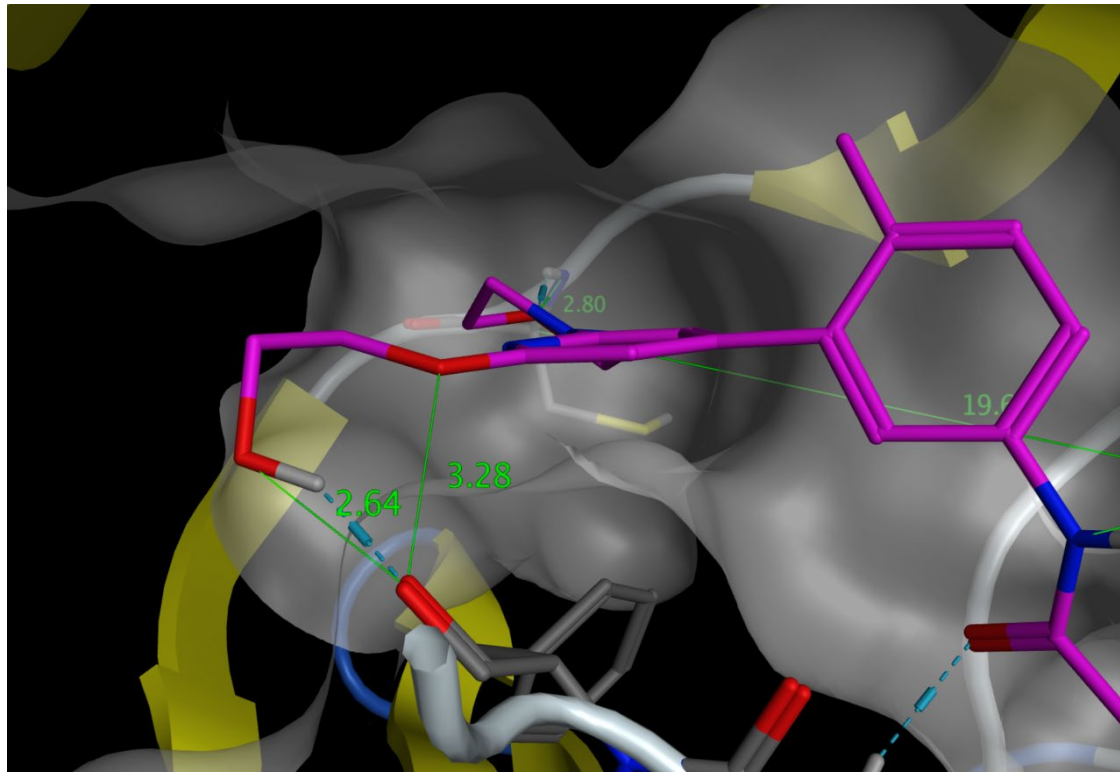
N-Linker for Higher pKa, Shift of pH Solubility Curve - Greater PK Consistency
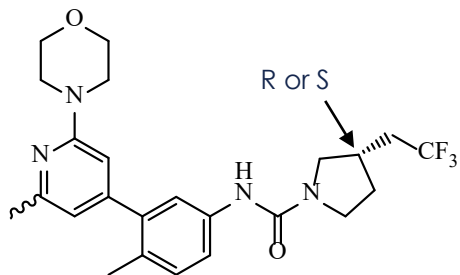
Exarafenib

Compound	Solvent Exposed	cLogP	sp³ Ratio	pKa	Solubility pH 7.4, 4.5	Hep Stable %R	Class 2 H2405	Class 3 WM3629	Class 1 A-375	BRAF
2464- S		2.9	0.54	3.8	17	38	6	15	42	5.6
2786 - S		3.1	0.54	5.3	39, 233	78	8.0	18	174	8.5
2787 - S		3.1	0.54	5.3	29, 196	85	7.9	8.1	62	5.7

$IC_{50}s$ in nM

Good PK across preclinical species

Clp ml/min/Kg	%F	T 1/2	Dose
Human Projections ⇨ 6	60	2 to 3 h	200 to 300 mg BID

Superior Pharmaceutical Properties for Exarafenib May Enhance *In Vivo* Target Exposure
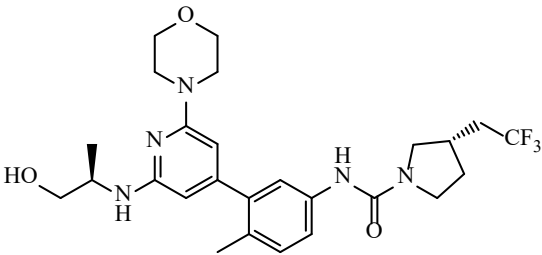
	Erasca Naporafenib	Hanmi / Genentech Belvarafenib	**Kinnate Exarafenib**
Class II / III Cell Potency (nM)*	**5 to 32 nM**	**4 to 42 nM**	**9 to 51 nM**
Human Plasma Free Fraction (%)	<1	<1	7
Aqueous Solubility (uM): **pH = 2** **pH = 4.5** **pH = 7.4**	50 7 6	266 0.4 0.1	312 196 29

Relevant physiological pH

cLogP	= 3.4	5.3	2.7
pKa	= 3.7	5.4	5.3
sp^3	= 0.32	0.04	0.54
Rot. Bonds	= 7	4	8

> Improved aqueous solubility, low unbound clearance, and increased free drug exposure all enhance the likelihood that exarafenib may achieve **greater target coverage** in the clinical setting

Greater than 7-fold Less Total Plasma Concentration Needed for Similar Target Coverage

*pERK Inhibition EC50 (nM)

Exarafenib Offers Differentiated Selectivity Versus Other Pan-RAF Approaches

Kinase Inhibition
- > 90%
- > 75%
- > 50%

DDR1

RAF family

Non-RAF Kinase Inhibition

Kinnate Exarafenib

Erasca Naporafenib

Hanmi / Genentech Belvarafenib
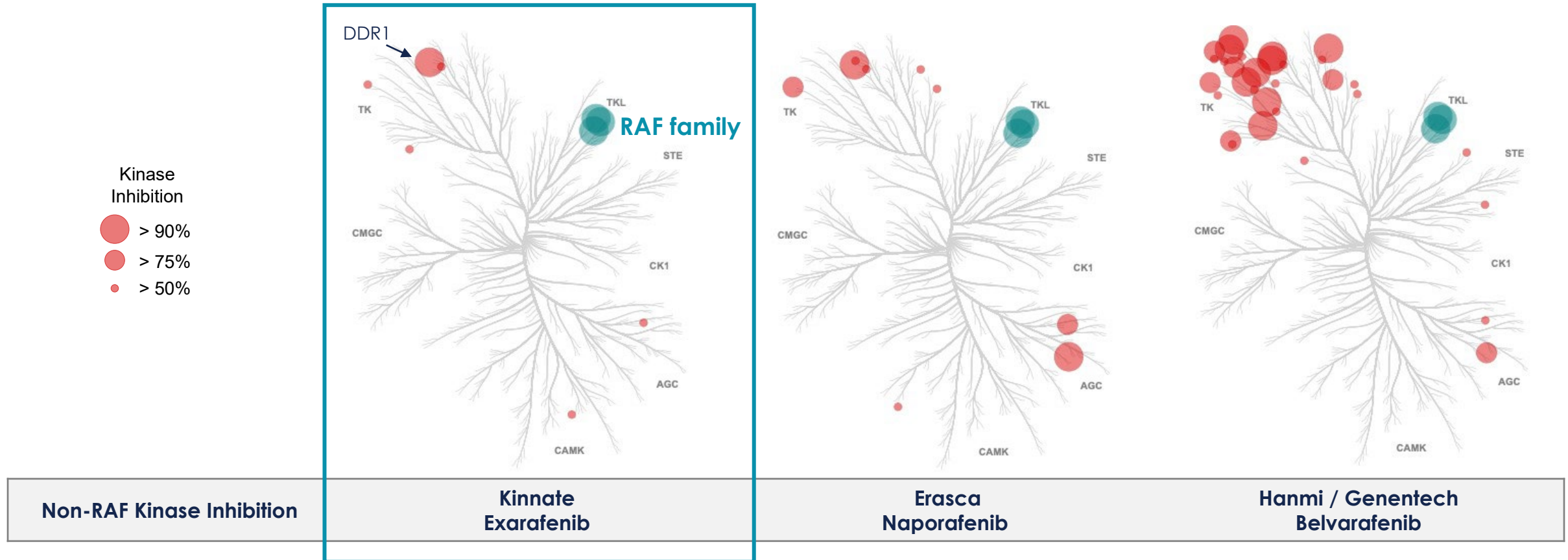
- Kinome profiling at 1 µM across > 600 kinases at Reaction Biology (including wild type, atypical, mutant)
- Only wild type kinases pictured in kinome trees

Dimer Inhibition Demonstrated Across Several Cell Lines
While Maintaining Selectivity Against Non-BRAF Mutated Cells

| BRAF Status	Tumor Cell Line	Lineage	MAPK Pathway Alteration(s)	pERK Inhibition EC50 (nM)			
				Pfizer MEKi Binimetinib	Erasca Naporafenib	Hanmi / Genentech Belvarafenib	**Exarafenib**
Class I	A-375	Melanoma	BRAF^{V600E}	7	171	67	**62**
	Colo800	Melanoma	BRAF^{V600E}	6	242	108	**112**
Class II	BxPC-3	Pancreatic	BRAF$^{indel(VTAPTP)}$	3	32	42	**51**
	OV-90	Ovarian	BRAF$^{indel(NVTAP)}$	4	24	22	**26**
	NCI-H2405	NSCLC	BRAF$^{indel(LNVTAP)}$	6	5	8	**10**
Class III	WM3629	Melanoma	BRAF^{D594G}, NRAS^{G12D}	5	6	4	**9**
	CAL-12T	NSCLC	BRAF^{G466V}	3	19	41	**18**
Wild Type (WT)	NCI-H358	NSCLC	BRAF^{WT}, KRAS^{G12C}	1	153	303	351
	CHL-1	Melanoma	BRAF^{WT}, NRAS^{WT}	5	291	443	580
	BJ	Normal fibroblast	Wild type	31	4686	2923	7963

- MEK inhibitors do not differentiate against WT

- Naporafenib & Belvarafenib have similar profile in cells, but suffer from sub-optimal properties & exposure *in vivo*

Exarafenib Antitumor Activity in Class II and Class III BRAF Models of NSCLC

Class II BRAF NSCLC CDX
NCI-H2405 (BRAF$^{L485\_P490delinsY}$)
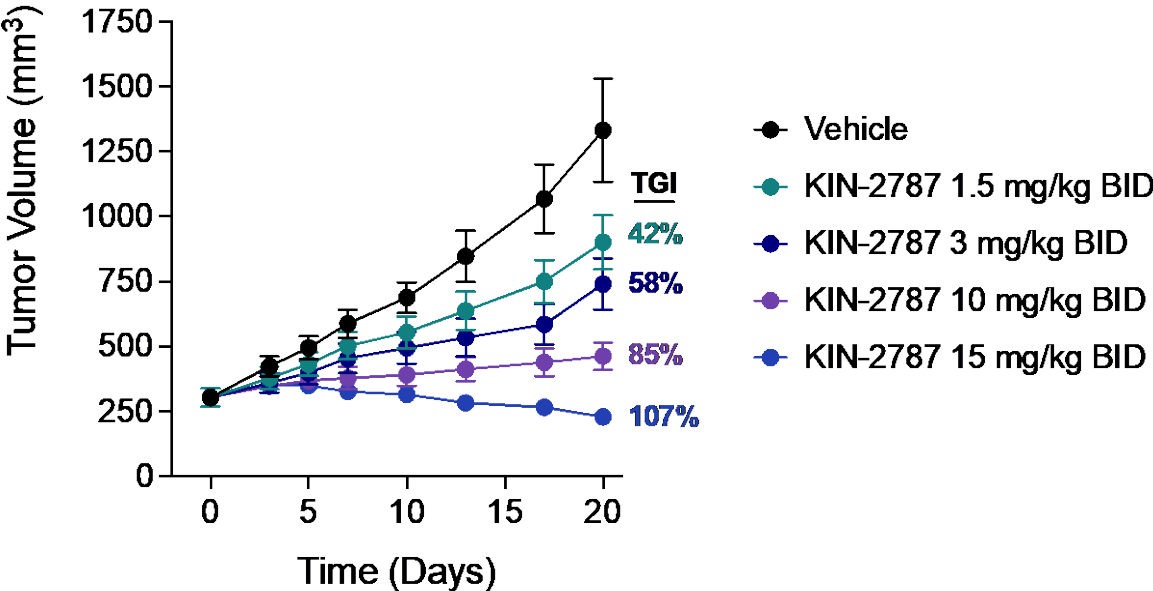
Class III BRAF NSCLC PDX
BRAF^{G466V}; NRAS^{Q61R}
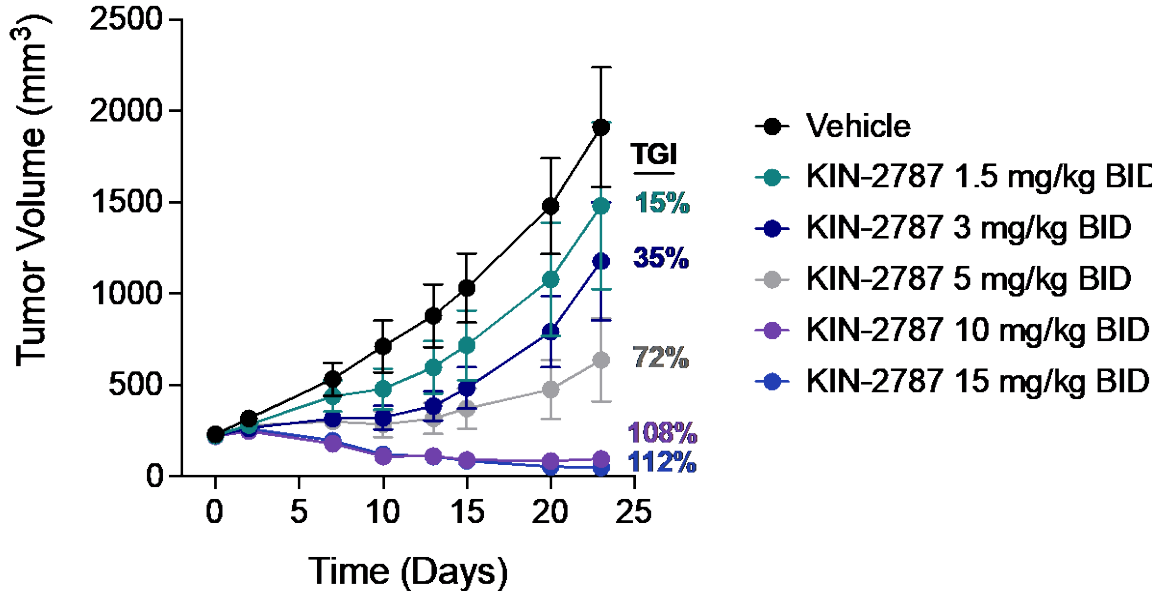
**Exarafenib (KIN-2787) demonstrates dose-dependent activity against Class II and Class III BRAF mutant
cell line- and patient-derived xenograft models of NSCLC**

Manabe, T. et. al., IASLC, Feb 2022 Targeted Therapies of Lung Cancer

Four Critical Factors to a Successful Pan-RAF Inhibitor

The Design of Exarafenib Achieves These Objectives

1 Selective drug — Limited off-target activity

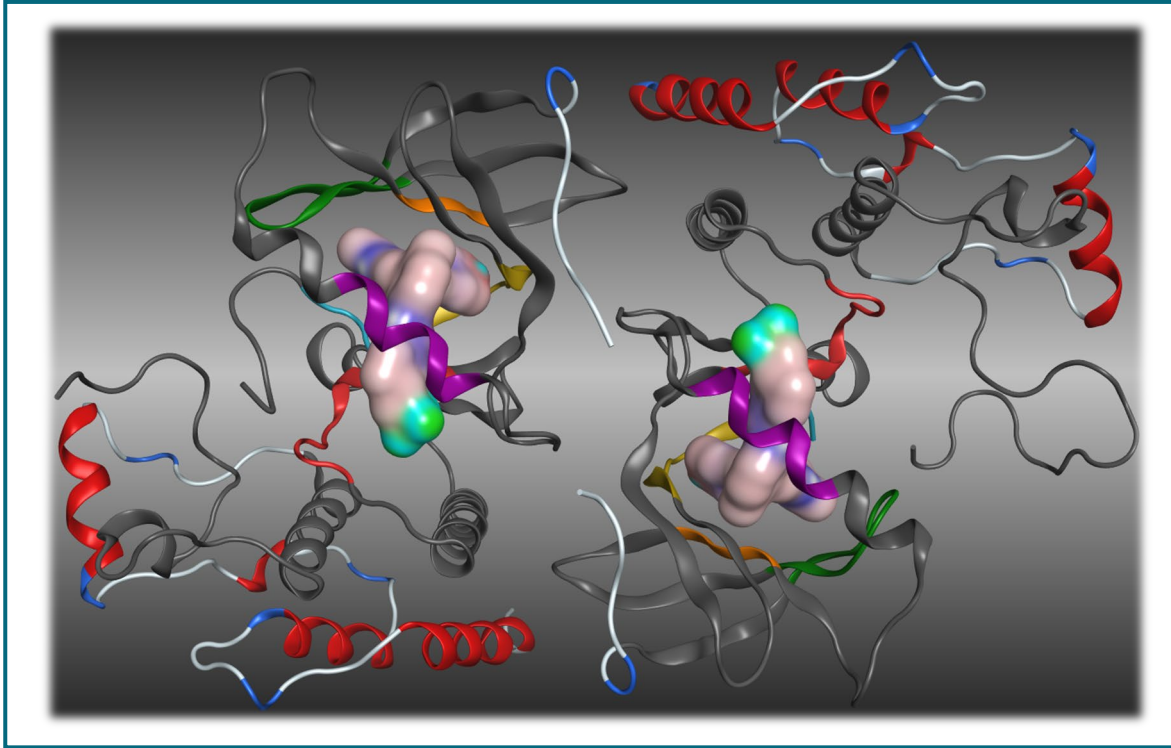**2 Broad alteration coverage** — Ability to cover heterogenous nature of BRAF Class II & Class III alterations

3 Equipotent inhibition — Equally inhibits across both RAF kinases in the dimer, Narrows concentration levels at which paradoxical activation occurs

4 Superior pharmaceutical properties — Robust PK to stay above paradoxical activation zone, High free fraction to enable more drug available to bind to target

Exarafenib Development Plan: Ongoing Phase 1 Trial

Active at More Than 30 Sites Globally

A1. Dose Escalation (Monotherapy)
Up to ~40 participants (enrolling)

BRAF Class I, II or III alterations (all tumor types) & NRAS^{Mut} Melanoma → Dose escalation to MTD / RP2D →

Began dosing in H2 2021
Dose escalation data in H1 2023

B. Dose Expansion
Total of ~75 participants across 3 cohorts

Cohort 1 (25 pts)
Melanoma BRAF Class II & Class III

Cohort 2 (25 pts)
NSCLC BRAF Class II & Class III

Cohort 3 (25 pts)
Other Solid Tumors BRAF Class II & Class III

- Enriched for patients with tumors and alterations of interest based on observed activity; Dosed at RP2D

- Potential to explore NRAS^{mut} Melanoma monotherapy and Class I refractory in dose expansion, dependent on dose escalation data

A2. Dose Escalation (Combination)
Up to ~36 participants (enrolling)

Exarafenib + Binimetinib
Incl. NRAS^{Mut} Melanoma

Initiated in Q2 2022
Initial data expected H1 2023

Study Status*

- **MTD not yet determined**
- **Achieved meaningful exposures that were dose proportional & exceeded the predicted efficacious thresholds based on preclinical models**
- **Encouraging initial clinical responses observed thus far**
- **Potential future cohort DL7, 500 mg bid**

- Phase 1 trial objectives: Evaluate safety, PK & PD; establish MTD/RP2D; assess preliminary anti-tumor activity (NCT 04913285)
- Population: Adults with advanced and unresectable or metastatic solid tumors
- Part A1: Participants that are BRAF Class I alteration-positive would be pre-treated by an approved Class I BRAF inhibitor, where indicated
- MTD, maximum tolerated dose; RP2D, recommended phase 2 dose; PK, pharmacokinetics; PD, pharmacodynamics
*As of October 11, 2022

Dedicated to Memory of Kinnate Founder Steve Kaldor

1962 - 2022

Acknowledgement of Exarafenib Discovery Team

Toufike Kanouni PTL, Steve Kaldor, Eric Murphy, Eric Martin, Lee Arnold, Morgan Boren, Jason Cox, Elizabeth Gardiner, Rob Kania, Ping Jiang, Om Makwana, Nichol Miller, Sanjeev Thohan, Noel Timple, John Tyhonas, Angie Vassar, Tim Wang, Richard Williams, Scott Womble

Thank You
Questions?

Our mission

is to inspire hope

for those battling cancer by
expanding on the promise

of targeted therapies.