Discovery of BLU-667 for *RET*-driven cancers

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AACR
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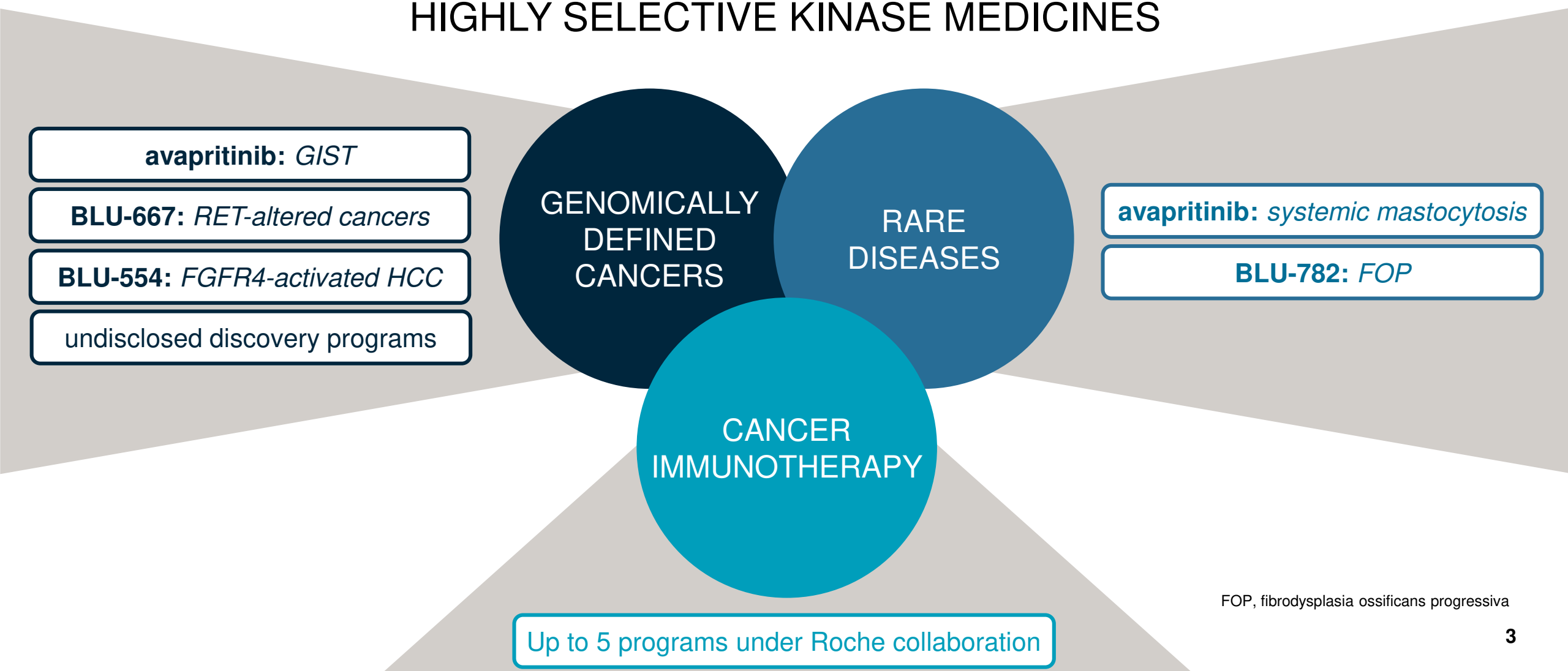
Disclosures

- **I am an employee and shareholder of Blueprint Medicines**
- **BLU-667 is an investigational therapy discovered and currently in development by Blueprint Medicines**

A robust and diverse portfolio focused on kinase inhibitor medicines

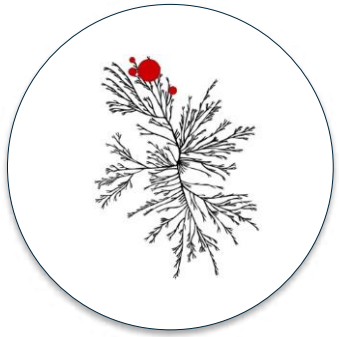
HIGHLY SELECTIVE KINASE MEDICINES



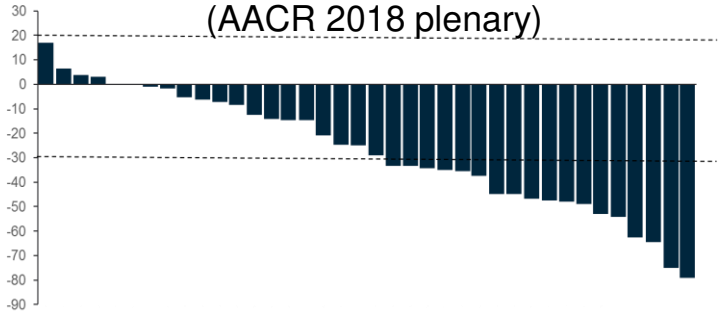
FOP, fibrodysplasia ossificans progressiva

Each clinical-stage TKI has achieved rapid proof-of-concept

BLU-667

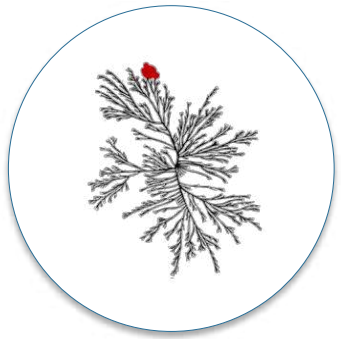


RET-altered Solid Tumors
(AACR 2018 plenary)

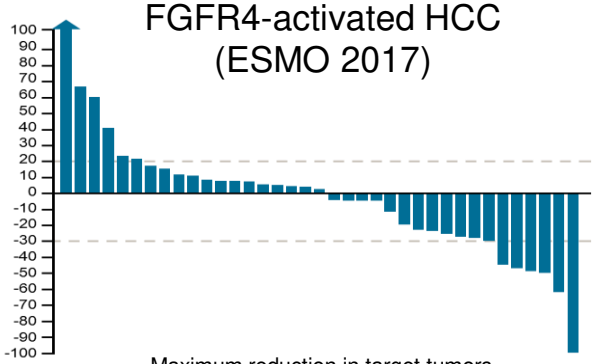


Maximum reduction in target tumors

BLU-554

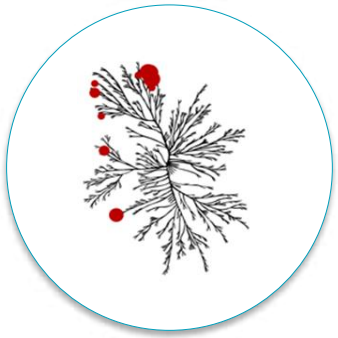


FGFR4-activated HCC
(ESMO 2017)

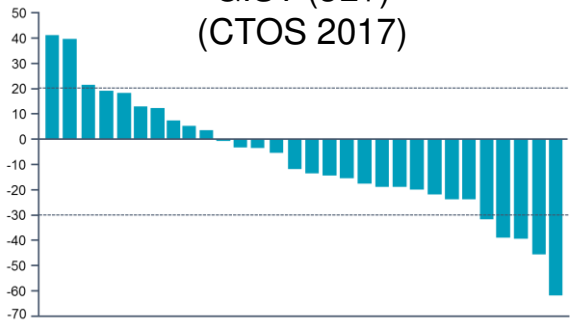


Maximum reduction in target tumors

avapritinib (formerly BLU-285)

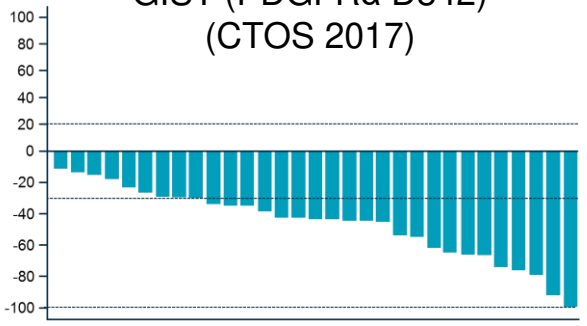


GIST (3L+)
(CTOS 2017)



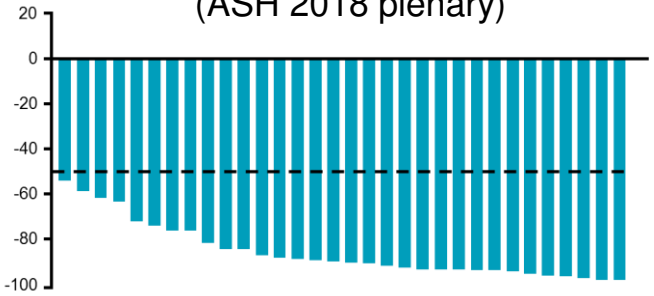
Maximum reduction in target tumors

GIST (PDGFRα D842)
(CTOS 2017)



Maximum reduction in target tumors

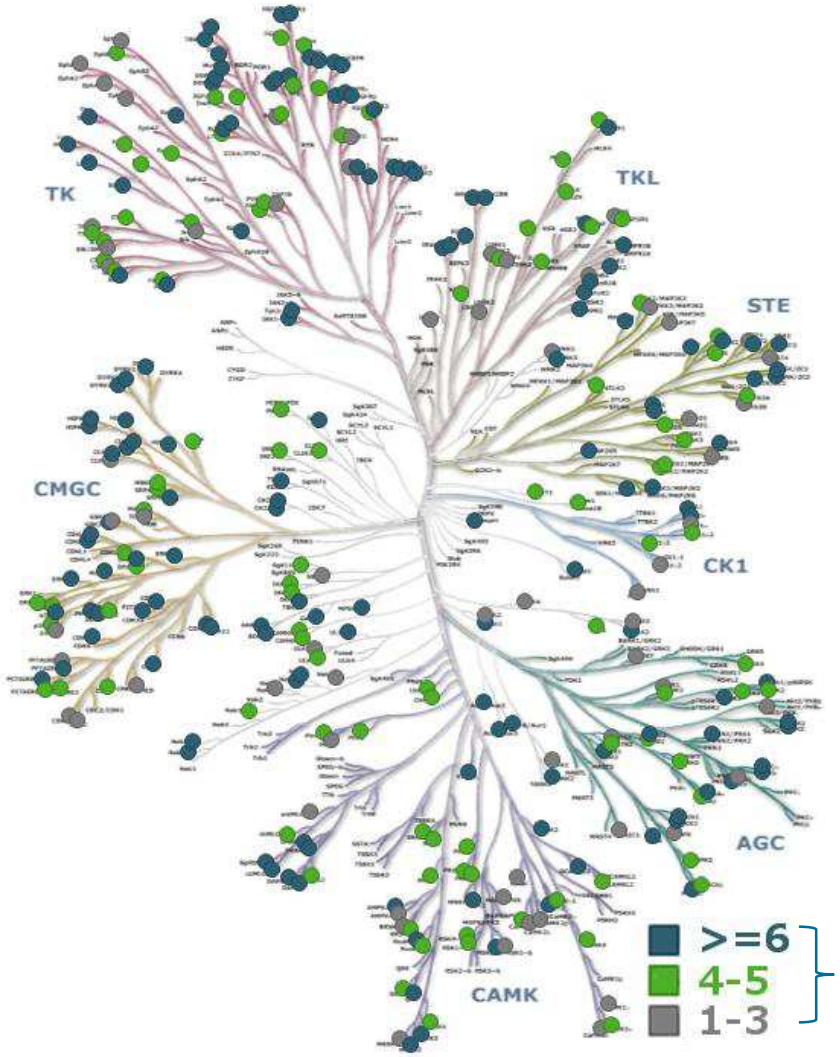
Advanced Systemic Mastocytosis
(ASH 2018 plenary)



Maximum reduction in serum tryptase

Avapritinib GIST data presented at November 2017 CTOS Annual Meeting. Data cutoff: October 11, 2017; Avapritinib systemic mastocytosis data presented at December 2017 ASH Annual Meeting. Data cutoff: October 4, 2017; BLU-554 data presented at September 2017 ESMO Congress. Data cutoff: August 18, 2017; BLU-667 data presented at April 2018 AACR Annual Meeting. Data cutoff: April 6, 2018. Kinome illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com) (CSTI). The foregoing website is maintained by CSTI, and Blueprint Medicines is not responsible for its content; GIST, gastrointestinal stromal tumors; HCC, hepatocellular carcinoma; 3L+, third-line or later treatment.
TKI = Tyrosine kinase inhibitor

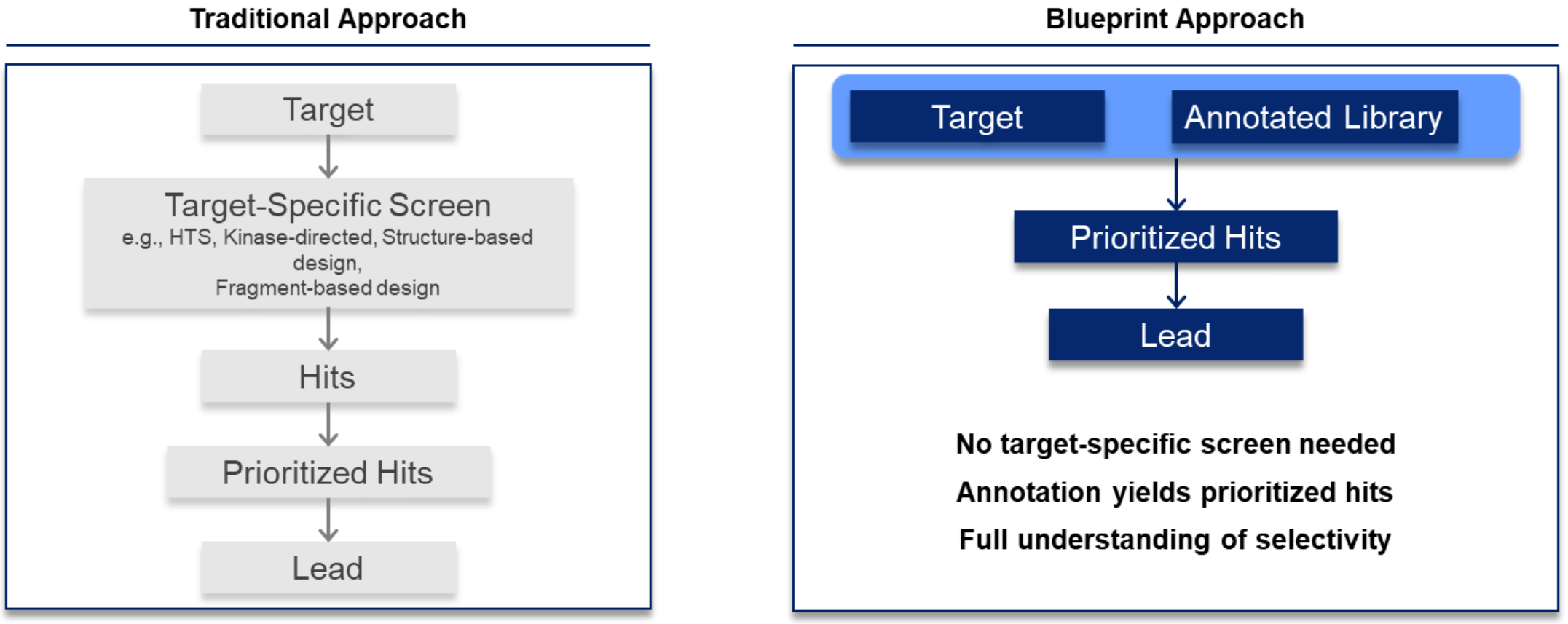
Broad coverage of the kinome with highly diverse collection



- 10,000+ carefully crafted and tested molecules from over 100 scaffolds
- Broad and deep coverage of kinome
  - >85% coverage - 1 scaffold
  - ~70% coverage - 3 scaffolds
  - ~45% coverage - 6 scaffolds
- High quality, differentiated med chem starting points
- Library compounds pre-screened against human wildtype kinases and several disease associated mutants

Kinome illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com) The foregoing website is maintained by CSTI, and Blueprint Medicines is not responsible for its content

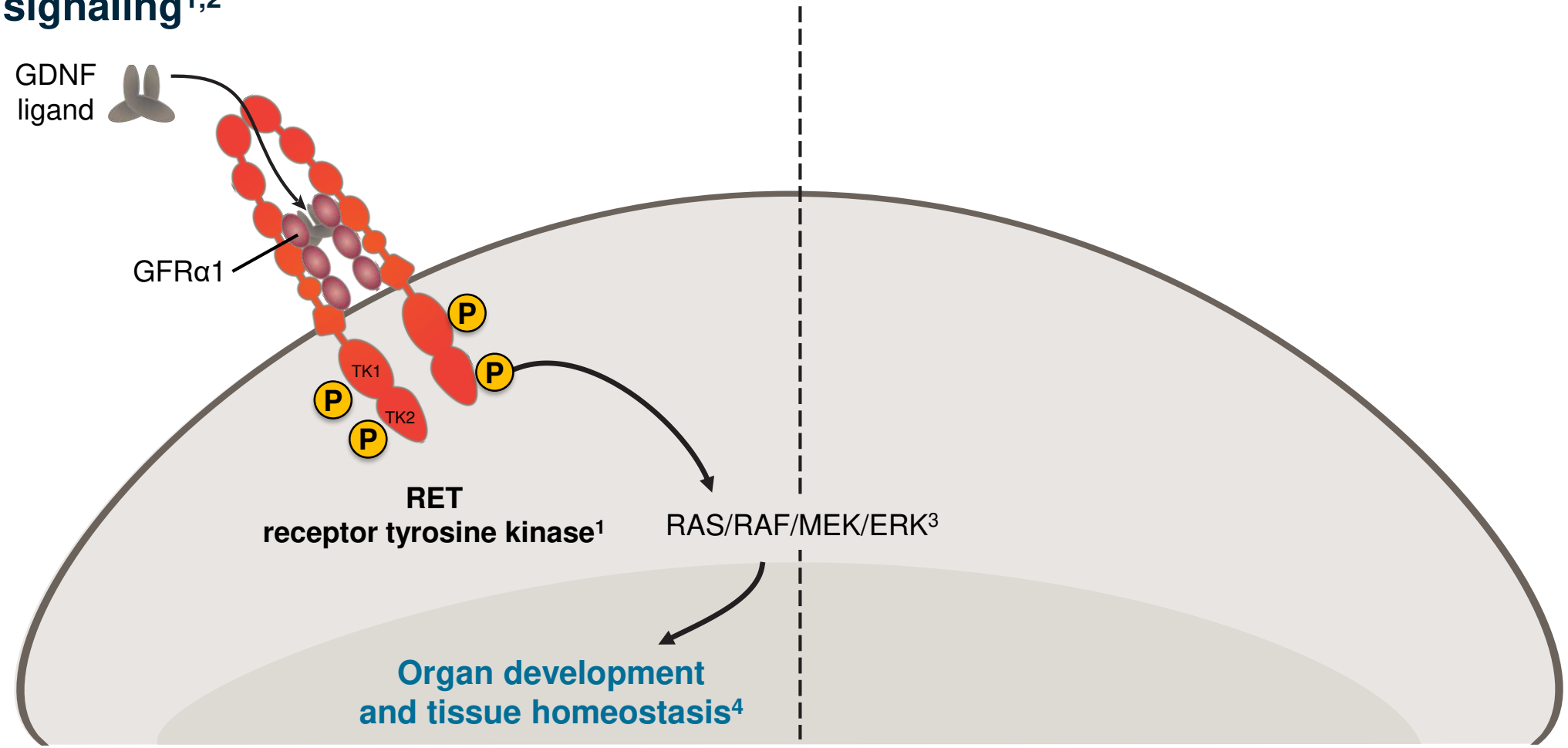
The fully annotated library accelerates high quality hit identification



- **Rapid program progression through accelerated hit identification, efficient prioritization, and informed optimization**

RET is an RTK required for normal development[1]

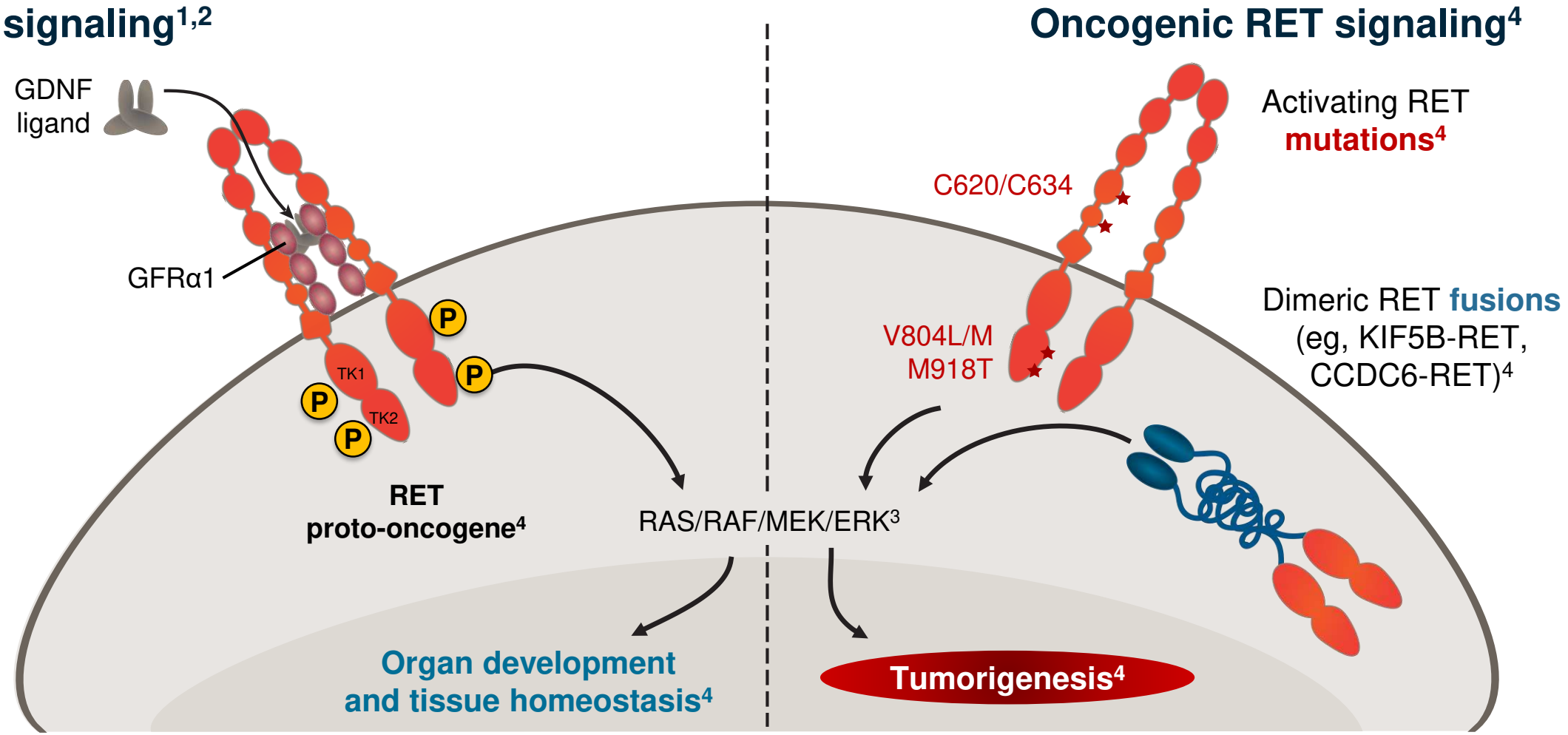
Normal RET signaling[1,2]



ERK, extracellular signal-regulated kinase; GDNF, glial cell line-derived neurotrophic factor; GFR, GDNF family receptor; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; P, phosphorylation; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; RET, rearranged during transfection; RTK, receptor tyrosine kinase; TK, tyrosine kinase.

1. Mulligan LM. *Nat Rev Cancer*. 2014;14(3):173-186. 2. Pützer BM et al. In: Diamanti-Kandarakis E, ed. *Contemporary Aspects of Endocrinology*. IntechOpen; 2011. https://www.intechopen.com/books/contemporary-aspects-of-endocrinology/molecular-diagnostics-in-treatment-of-medullary-thyroid-carcinoma. Accessed August 23, 2018. 3. Pratilas CA et al. *Proc Natl Acad Sci U S A*. 2009;106(11):4519-4524. 4. Drilon A et al. *Nat Rev Clin Oncol*. 2018;15(3):151-167.

Alterations in RET structure and function can lead to tumorigenesis[1]

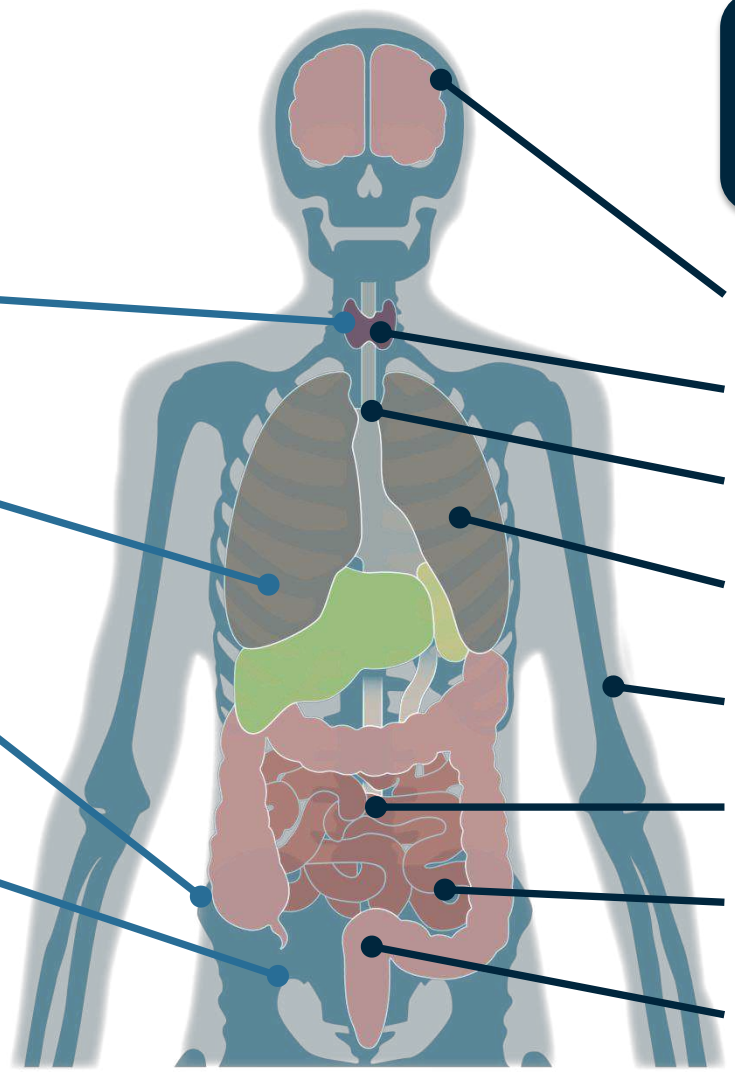


1. Mulligan LM. *Nat Rev Cancer*. 2014;14(3):173-186. 2. Pützer BM et al. In: Diamanti-Kandarakis E, ed. *Contemporary Aspects of Endocrinology*. IntechOpen; 2011. https://www.intechopen.com/books/contemporary-aspects-of-endocrinology/molecular-diagnostics-in-treatment-of-medullary-thyroid-carcinoma. Accessed August 23, 2018. 3. Pratilas CA et al. *Proc Natl Acad Sci U S A*. 2009;106(11):4519-4524. 4. Drilon A et al. *Nat Rev Clin Oncol*. 2018;15(3):151-167.

RET alteration occurs in a wide range of tumor type[1,2]

RET fusions
- NSCLC (1-2%)[1]
- PTC (10%)[1,2]

RET mutations
- MTC (60%)[1]



PTC (10-20%)[1,2,3]

NSCLC (1-2%)[1]

CMML[4]

Ovarian epithelial carcinoma (1.9%)[2]

Meningioma (5.6%)[2]

MTC (60%)[1]

Esophageal adenocarcinoma (1.4%)[2]

Breast carcinoma (0.2%)[2]

Melanoma (0.7%) and basal cell carcinoma (12.5%)[2]

Gastric adenocarcinoma (0.7%)[2]

Ureter urothelial carcinoma (16.7%)

Colorectal adenocarcinoma (0.7%)[2]

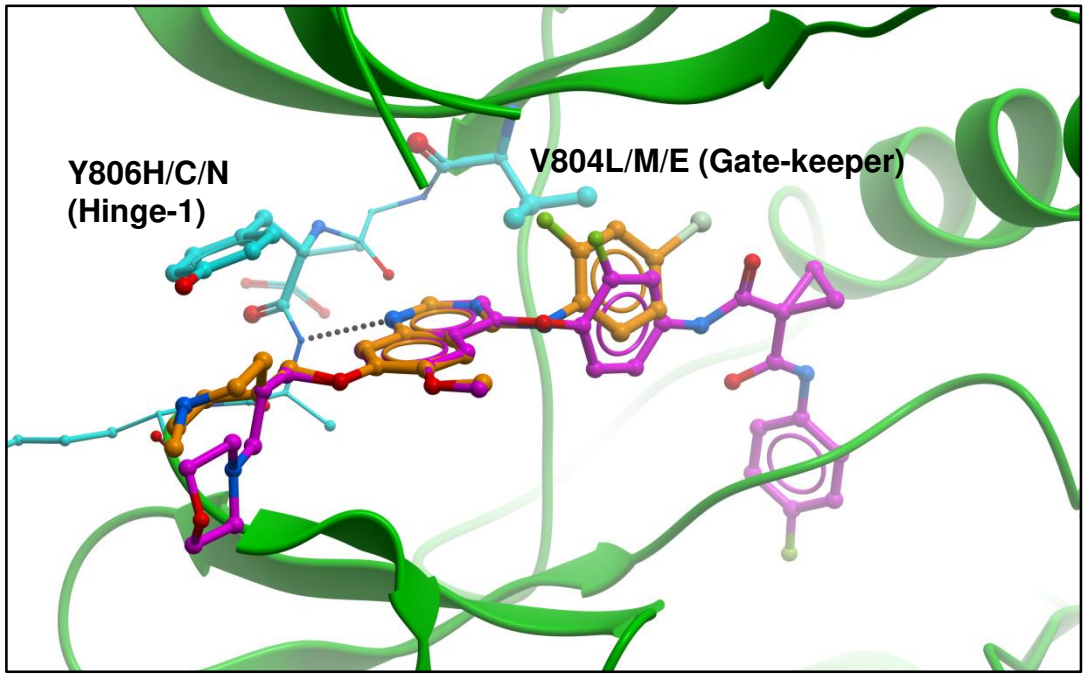
MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer; PTC, papillary thyroid cancer.

1. Drilon A et al. *Nat Rev Clin Oncol*. 2018;15(3):151-167. 2. Kato S et al. *Clin Cancer Res*. 2017;23(8):1988-1997.
3. Prescott JD et al. *Cancer*. 2015; 121(13):2137-2146. 4. Ballerini P et al. *Leukemia*. 2012;26(11):2384-2389.

Patients with *RET*-altered cancers have not yet achieved the promise of precision therapy

Ideal RET inhibitor profile:

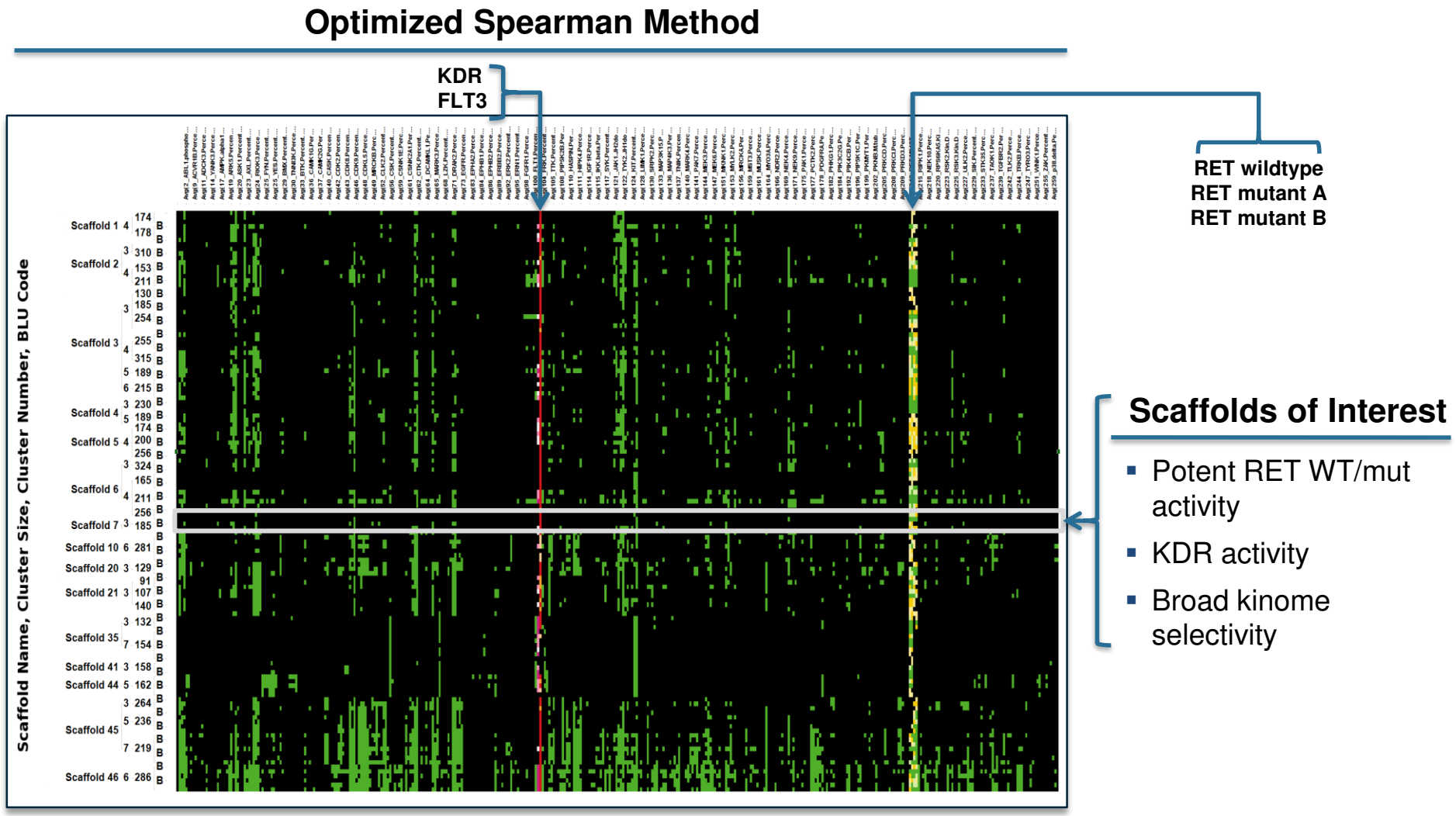
1. Potently inhibit RET wild-type fusions (NSCLC & other cancers)
2. Potently inhibit oncogenic RET mutants (thyroid cancer)
3. Spare VEGFR2 in a kinome-selective manner
4. *Prevent on-target resistance mutations*



RET + Vandetanib Crystal Structure

In vitro resistance screens have confirmed that multi-kinase inhibitors are vulnerable to RET mutations at V804(M/L/E) or Y806(H/C/N)

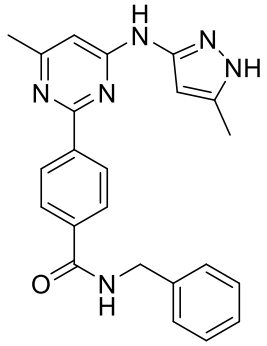
Activity-based clustering to identify hits from Blueprint library



Scaffolds of Interest

- Potent RET WT/mut activity
- KDR activity
- Broad kinome selectivity

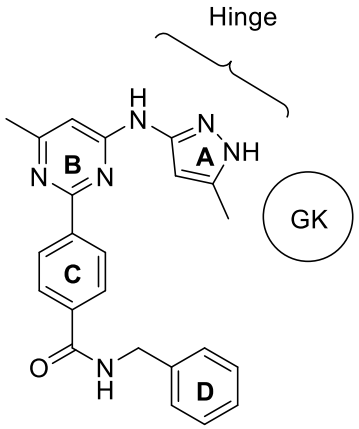
Blueprint library delivers multiple gatekeeper-agnostic RET inhibitor scaffolds

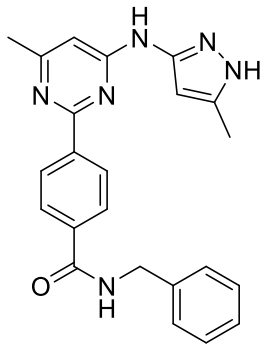


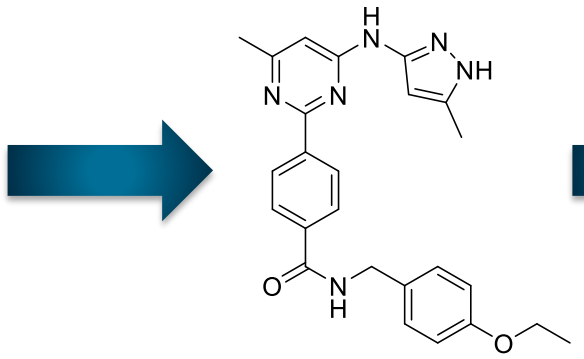
	Scaffold 1	Scaffold 2	Scaffold 3	Scaffold 4	Scaffold 5
RET WT IC_{50} (nM)	56	13	9	7	85
RET V804L IC_{50} (nM)	30	17	12	5	52
pRET Cell IC_{50} (nM)	*3300*	*765*	*1500*	*1725*	
KDR/RET	*26x*	*10x*	*56x*	*28x*	*9x*
S(10) @ 3 µM*	*0.089*	*0.071*	*0.041*	*0.046*	*0.054*
Papp / efflux	*16 / 3*	*7.5 / 6*			*22 / 1*
HLM / RLM ER**	*0.39 / 0.53*	*0.51 / 0.19*	*0.60 / 0.53*	*0.83 / 0.87*	*0.55 / 0.53*
Solubility (µM)	*13*	*96*	*1*	*5*	*6*

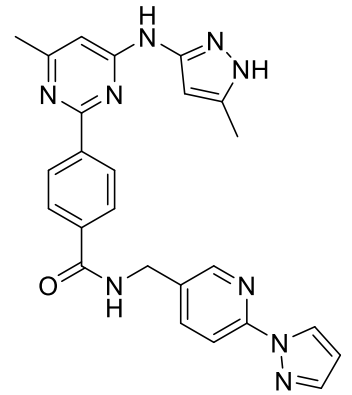
*number of kinases inhibited at <10 POC divided by total number of human wt kinases
**human / rat liver microsome in vitro extraction ratio

Progression of benzyl amide SAR leads to initial potency breakthrough







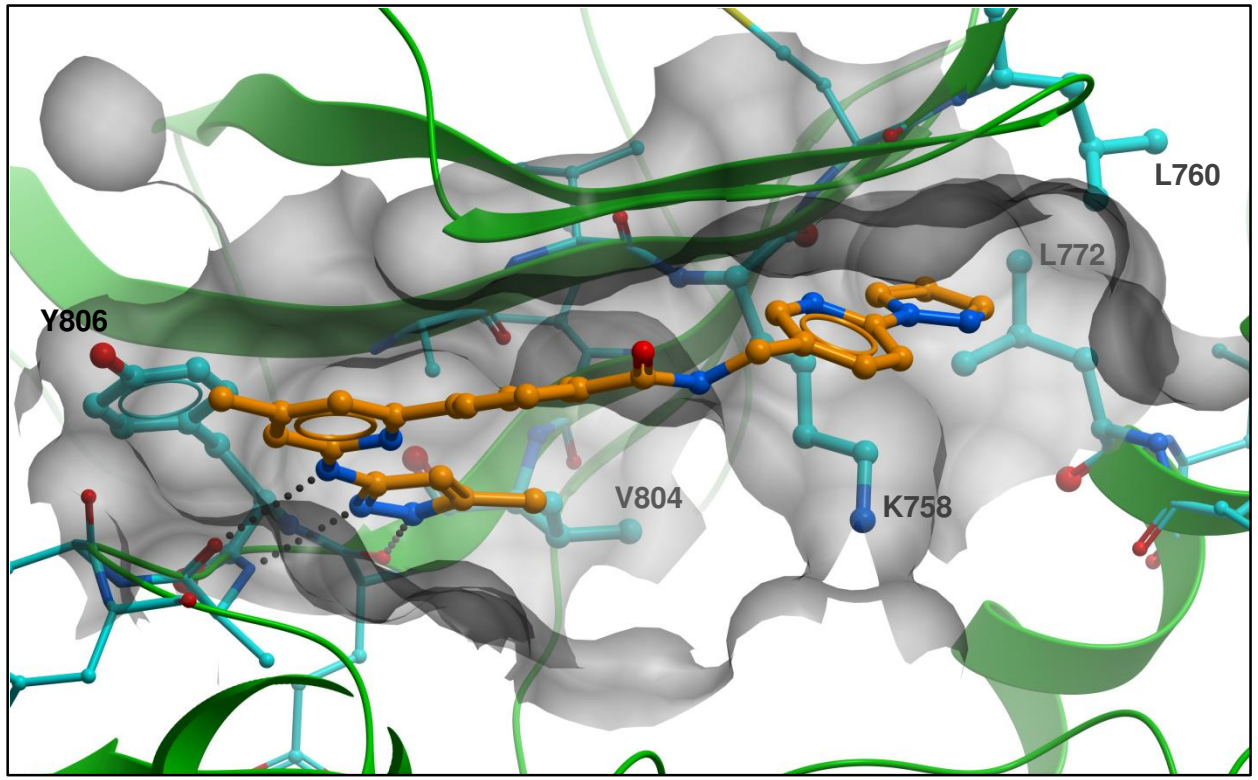


Compound	1	2	3
RET WT IC_{50} (nM)	56	10	2.1
pRET Cell IC_{50} (nM)	*3300*	*409*	*29*
KDR/RET	*26x*	*70x*	*48x*
Papp / efflux	*16 / 3*	*21 / 2*	*3.0 / 17.4*
HLM / RLM ER	*0.39 / 0.53*	*0.54 / 0.49*	*0.00 / 0.28*
Solubility (µM)	*13*	*48*	*9*

GK = Gatekeeper

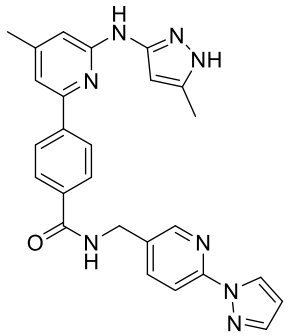
SAR = Structure activity relationship

X-Ray crystal structure of Compound 4 (B-ring pyridine analog)



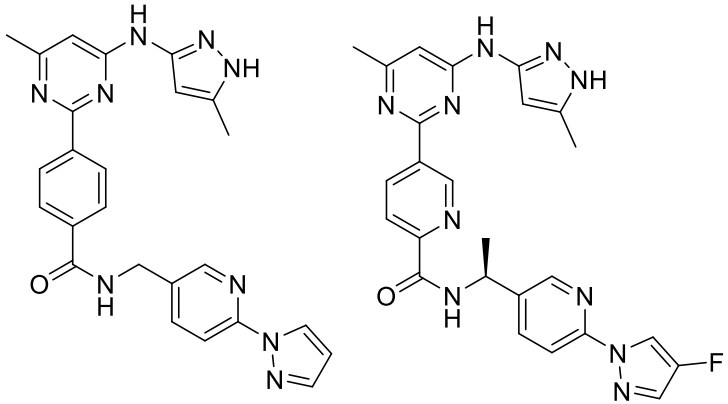
Key features of scaffold

- Methylaminopyrazole hinge binder avoids gatekeeper pocket
- Aminopyrazole makes triplet H-bond interaction with kinase hinge
- Arylamide linker provides scaffolding to access pocket beyond catalytic Lys (K758); no specific protein interactions
- Terminal pyrazole accesses post-Lys pocket



Compound	4
RET WT IC_{50} (nM)	1.8

Further SAR development leads to advanced compound



Compound	3	5
RET WT IC_{50} (nM)	2.1	1.6
pRET Cell IC_{50} (nM)	*29*	*58*
KDR/RET	*48x*	*49x*
Papp / efflux	*3.0 / 17.4*	*11 / 1.3*
HLM / RLM ER	*0.00 / 0.28*	*0.35 / 0.27*
Solubility (µM)	*9*	*16*
Mouse $t_{1/2}$ @ 15 mg/kg PO (h)	*2*	*7*

BID = twice daily dosing

fu = free fraction

Compound 5:

- First project compound to show full tumor growth inhibition in mouse RET tumor model
- Confirmed IC_{90} required for tumor regression
- Advanced to human dose projection – 6 g BID

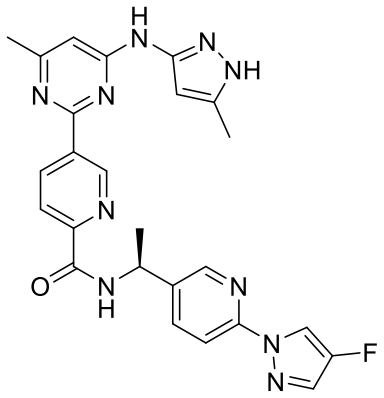
To lower dose projection, need to improve:

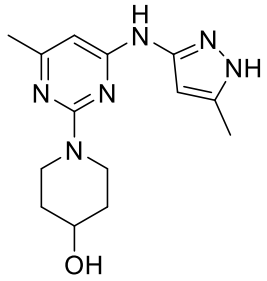
- Potency
- Higher species pharmacokinetics
- Intrinsic clearance (issue masked by high HLM binding)

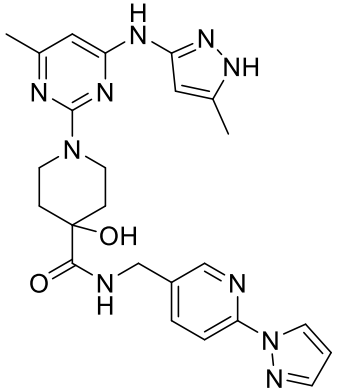
Compound	5
HLM fu	*0.09*
cLogD	*3.5*
measured LogD	*5.0*

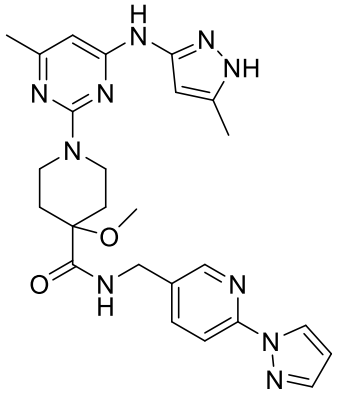
Replacement of the aryl linker leads to potent alternate series

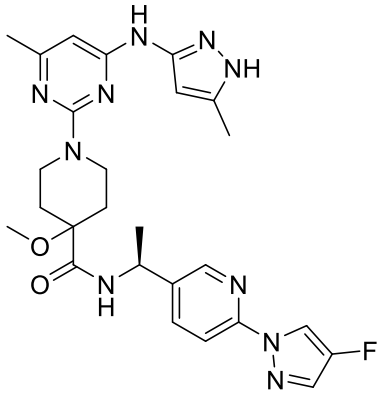
- Aryl linker replaced with saturated linker to improve physical properties
- Increased 3-dimensionality in linker leads to dramatic improvement in potency and solubility





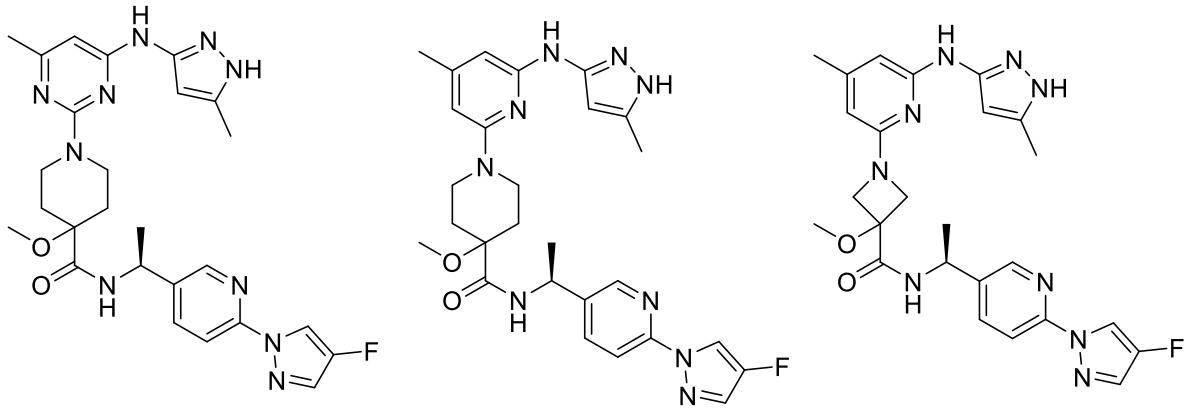






Compound	5	6	7	8	9
RET WT IC_{50} (nM)	1.6	402	4.9	4.0	0.5
pRET Cell IC_{50} (nM)	*58*		*1660*	*58*	*3.0*
KDR/RET	*49x*		*29x*	*34x*	*67x*
Papp / efflux	*11 / 1.3*		*0.4 / 56*	*6 / 9*	*8 / 5*
HLM / RLM ER	*0.35 / 0.27*		*0.34 / 0.27*	*0.53 / 0.69*	*0.65 / 0.46*
Solubility (µM)	*16*		*88*	*>100*	*62*

Advanced N-Linked compounds plagued by high unbound clearance and short half-life



Compound	9	10	11
RET WT IC_{50} (nM)	0.5	0.6	0.9
pRET Cell IC_{50} (nM)	*3.0*	*2.4*	*10*
KDR/RET	*67x*	*176x*	*411x*
HLM / RLM ER	*0.65 / 0.46*	*0.24 / 0.26*	*0.46 / 0.28*
Rat IV Cl (mL/min/kg)	*29*	*15*	*23*
Rat IV Clu (mL/min/kg)	*916*	*9109*	*2431*
Rat $t_{1/2}$ (h)	*1.2*	*1.2*	*0.9*

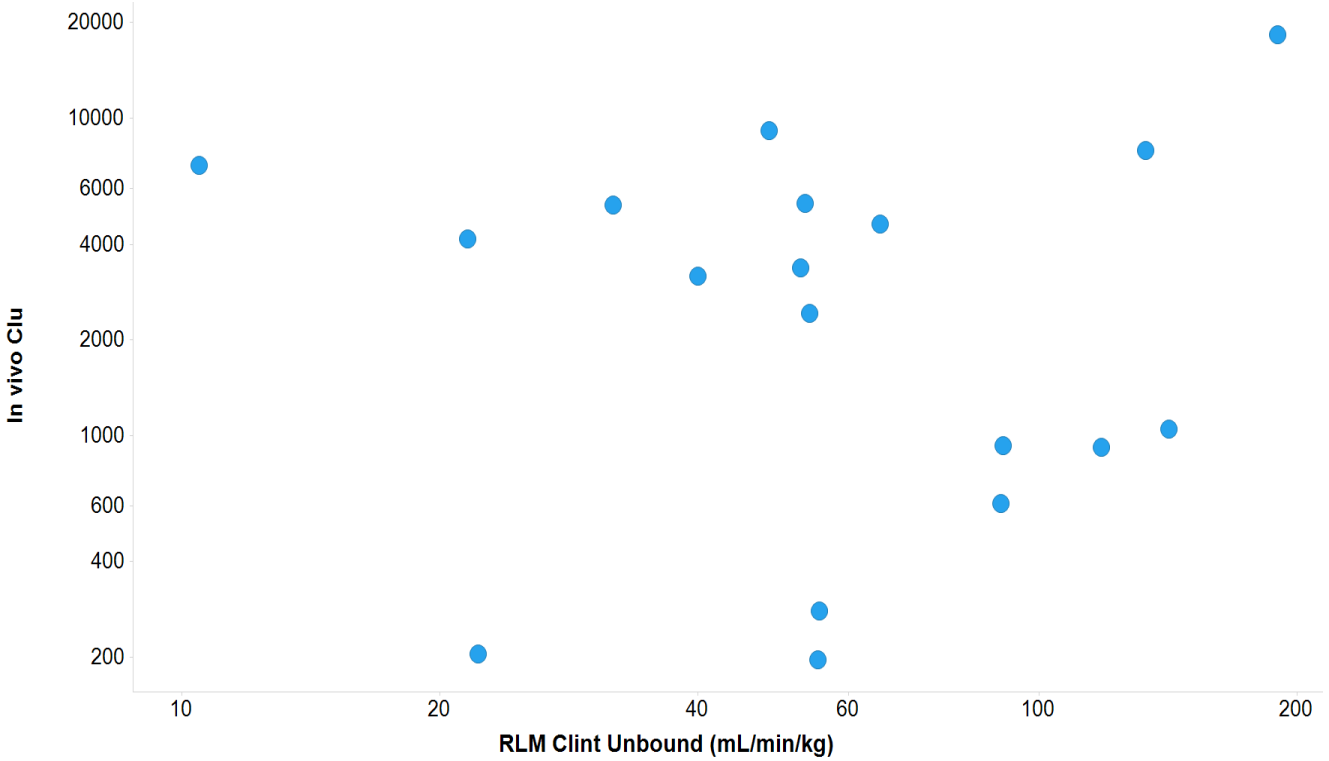
- N-linked series addressed only the potency aspect of an improved dose projection
- Still need to improve pharmacokinetic profile

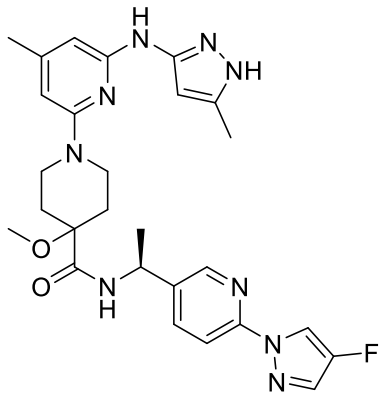
Cl = Clearance

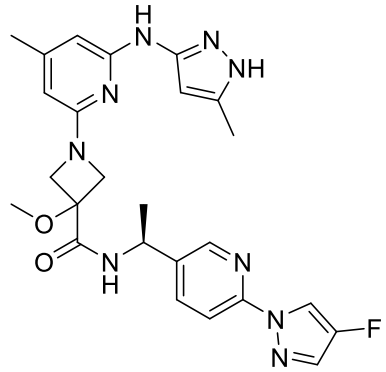
Clu = Unbound clearance

No IVIVC or effect of ABT pretreatment on PK of N-linked series

No in vitro – in vivo correlation (IVIVC):





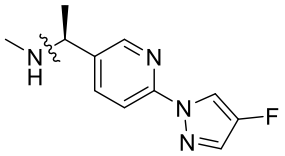


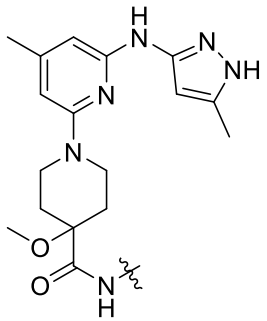
Compound	10	10 + 50 mg/kg ABT	11	11 + 50 mg/kg ABT
Rat IV Cl (mL/min/kg)	*15*	*15*	*22*	*19*
Rat $t_{1/2}$ (h)	*0.7*	*0.8*	*0.9*	*1.2*

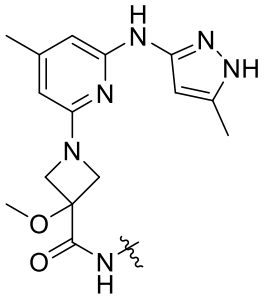
- Oxidative metabolism not a driver of clearance
- Needed alternative hypothesis to improve Cl / dose projection

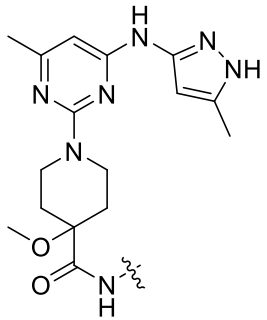
ABT = 1-Aminobenzotriazole

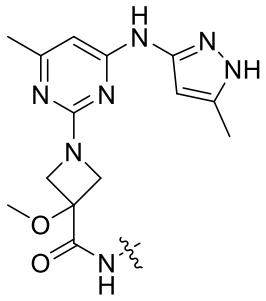
Trend observed in ring electronics and unbound clearance leads to C-linked designs

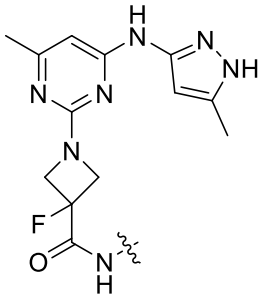








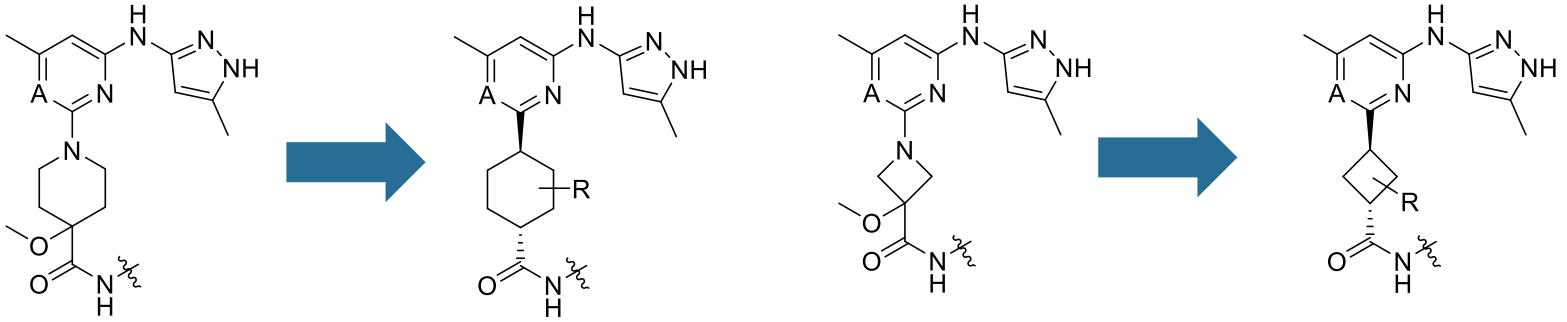




Compound	10	11	9	13	14
Rat IV Clu (mL/min/kg)	*9109*	*2431*	*916*	*608*	*279*

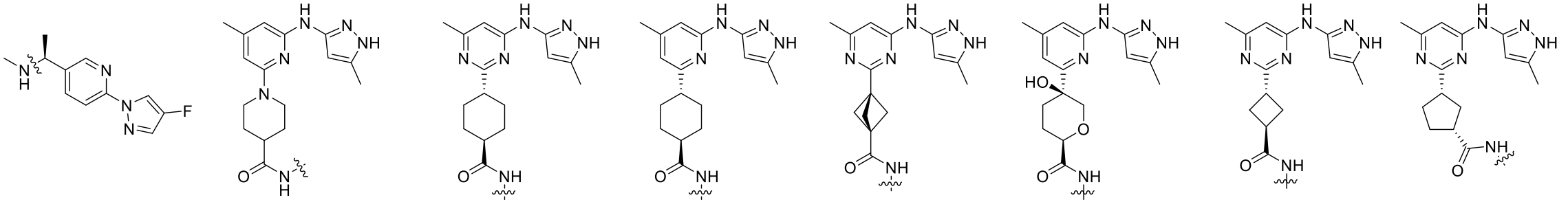
Hypothesis: Decreasing pKa of B ring leads to dramatic improvements in Clu

Design: sp^3 carbon linked analogs will decrease electron density of B ring and improve Clu



Broad exploration of carbon linkers shows improved unbound clearance and half-lives

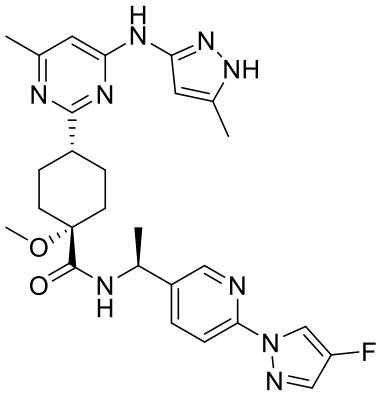
- Synthesized and profiled a wide array of C-linked compounds to pick best linkers for further development

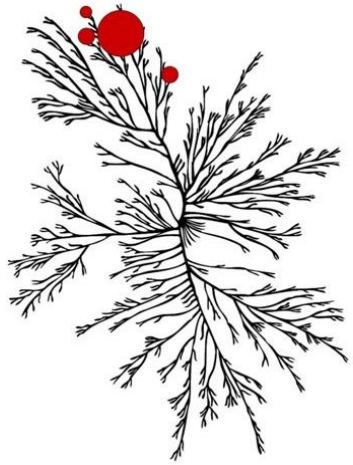


Compound	15	16	17	18	19	20	21
RET WT IC_{50} (nM)	1.7	0.8	5.5	2.0	1.0	0.3	0.4
pRET Cell IC_{50} (nM)	*90*	35	232	319	*15*	*14*	*8.9*
Rat IV Cl (mL/min/kg)	*11*	*17*	*3.4*	*24*	*2.7*	*26*	*14*
Rat IV Clu (mL/min/kg)	*8461*	*420*	1848	383	*1353*	*515*	*465*
Rat $t_{1/2}$ (h)	*1.1*	*3.8*	3.9	3.1	*4.4*	*1.2*	*1.8*

- Trans cyclohexyl linker gives excellent balance of potency, unbound clearance, and half-life

Advancement of trans cyclohexyl series leads to discovery of BLU-667





Enzymatic IC_{50} (nM)	
RET WT	0.4
RET CCD6	0.4
RET M918T	0.4
RET V804L	0.3
RET V804M	0.4
RET V804E	0.7
RET Y806H	1.0
KDR/RET	80x

In vivo potency (nM)	
RET IC_{50}, u	1.1
RET IC_{90}, u	6.9

In vitro Stability	
HLM ER	0.14
RLM ER	0.10
DLM ER	0.21
MkLM ER	0.48

Physicochemical Properties	
MW	533
LogD (pH 7.4)	3.0
TPSA	127
FaSSIF (µM)	48
Caco-2 (efflux ratio)	21 (1.0)

Cellular IC_{50} (nM)	
RET WT IC_{50} (nM)	4.0

Pharmacokinetic Profile (IV Dosing)

	Cl (mL/min/kg)	Clu (mL/min/kg)	Vdss (L/kg)	$t_{1/2}$ (h)
Rat	14	710	3.3	3.8
Dog	2.0	235	0.49	3.5
Monkey	6.5	131	1.7	3.7

PO Dosing

%F
>100
>100
100

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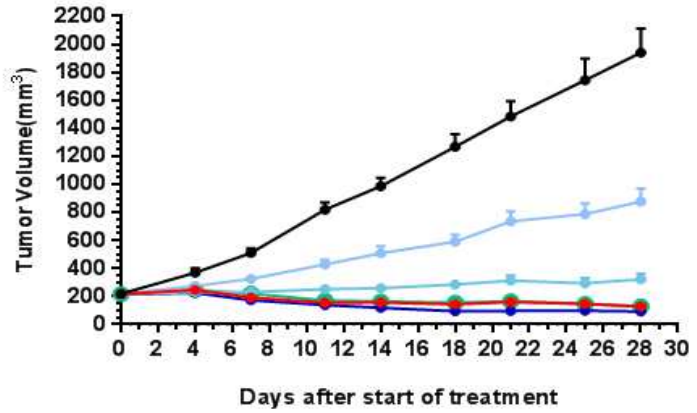
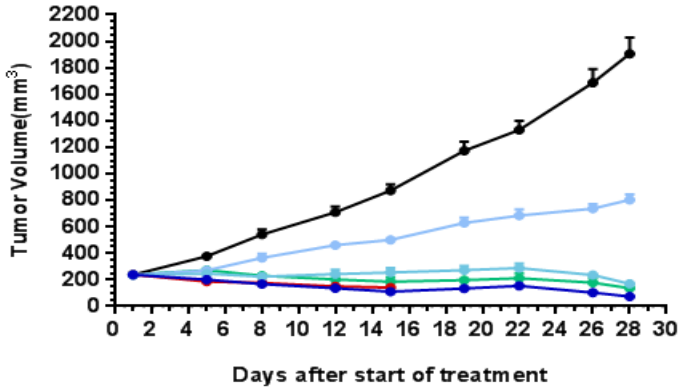
Targeted RET inhibition induces regression in RET-altered in vivo tumor models

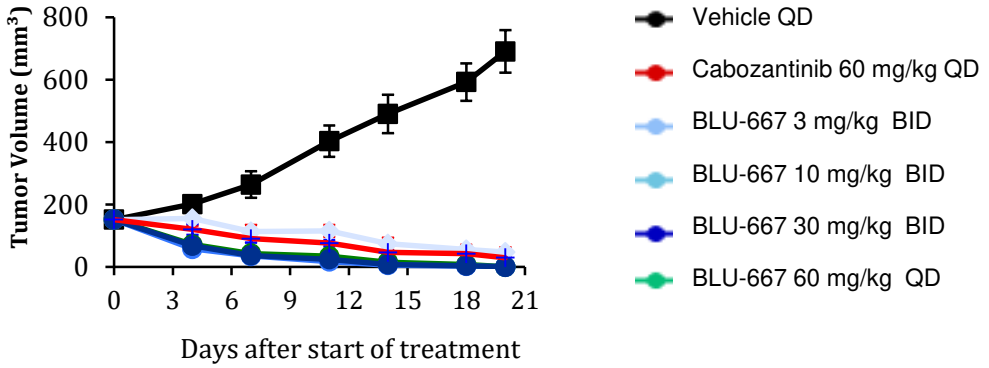
Lung Adenocarcinoma PDX
KIF5B-RET Fusion



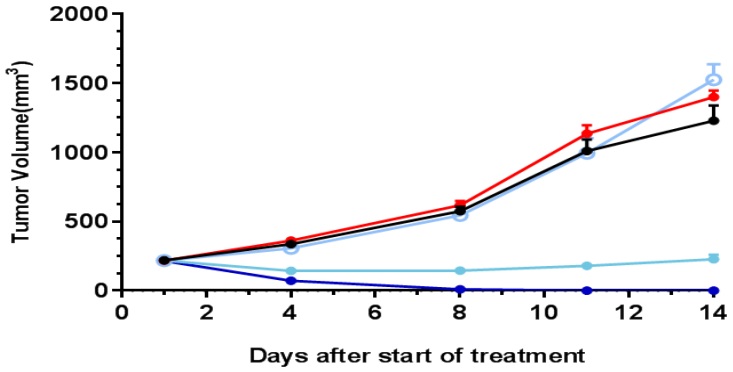
Medullary Thyroid Cancer Xenograft
Mutant (***RET C634W***)



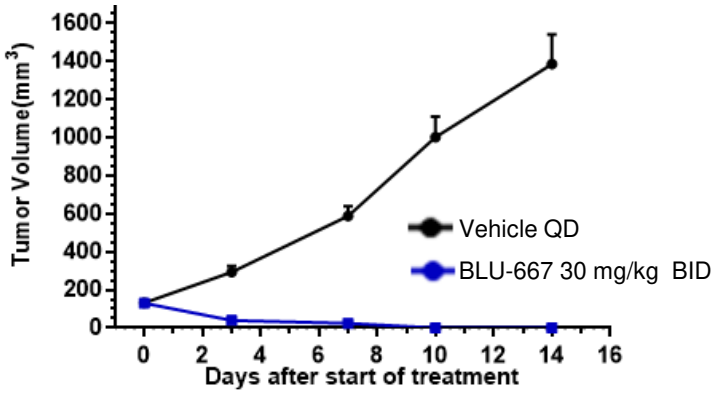
Colorectal Cancer PDX
CCDC6-RET Fusion



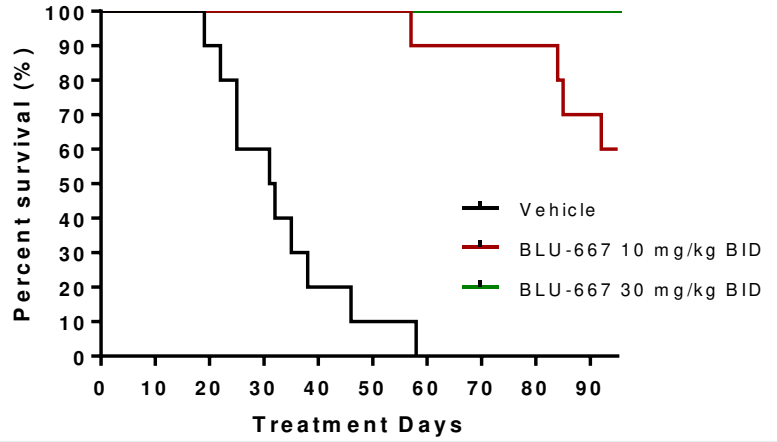
Ba/F3 ***KIF5B-RET(V804L)***



Ba/F3 ***KIF5B-RET(V804E)***



Intracranial *CCDC6-RET* CRC

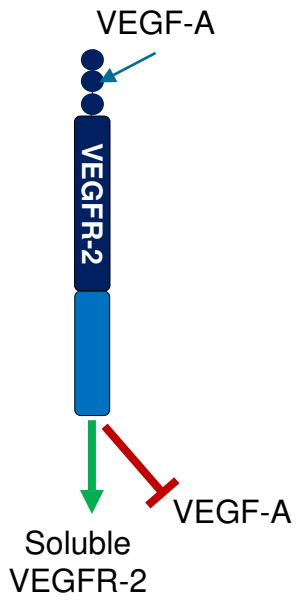


1. Subbiah V et al. *Cancer Discovery* 2018.

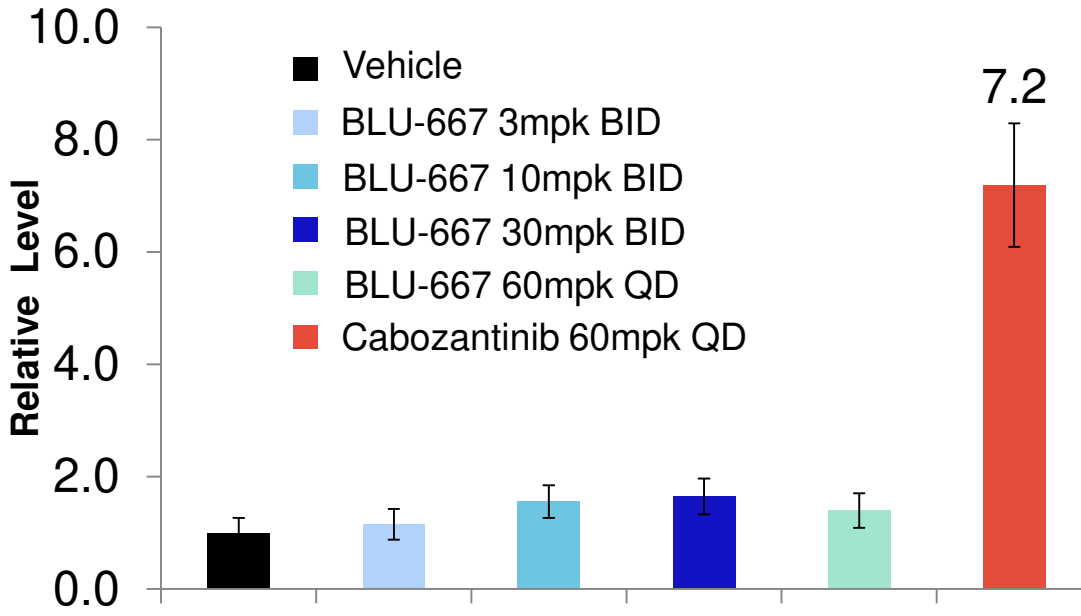
Active doses of BLU-667 do not functionally impact VEGFR-2 in PDX models

Drug	VEGF-A	sVEGFR-2
Cabozantinib	↑	↓
Vandetanib	↑	↓
Sunitinib	↑	↓
Axitinib	↑	↓
Sorafenib	↑	↓
Telatinib	↑	↓
Brivanib	↑	↓
Motesanib	↑	↓
Cediranib	↑	↓

VEGFR-2/KDR Signaling



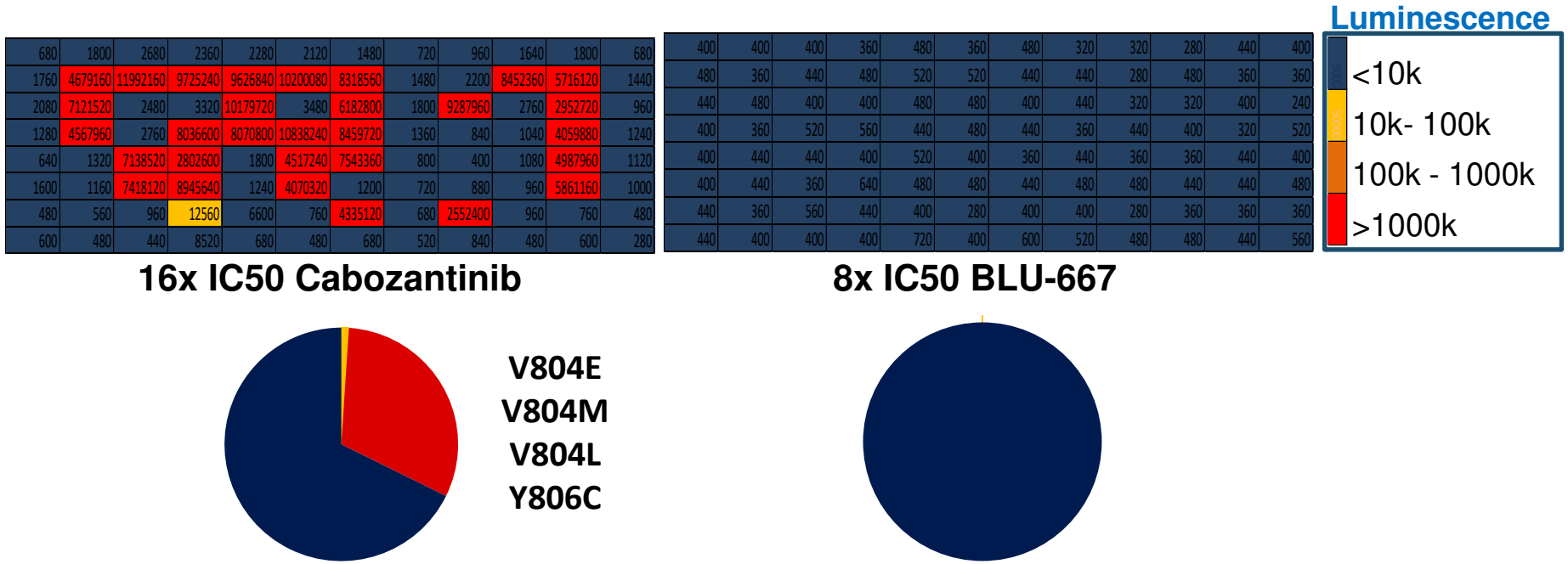
Plasma VEGF-A Levels Following Treatment of *KIF5B-RET* Tumor-bearing Mice with BLU-667 or Cabozantinib



1. Subbiah V et al. *Cancer Discovery* 2018.

BLU-667 prevents RET resistance mutants

Ba/F3 *KIF5B-RET* → **ENU** → **8x - 64x IC50** → **Cell Number**
(WT) (mutagen) 2-3 weeks (ATP; Luminescence)



By suppressing resistance mutants that confer resistance to MKIs, BLU-667 has the potential to overcome and prevent emergence of clinical resistance

Conclusions

- Blueprint Medicines Library provided multiple starting scaffolds with activity against RET wt and predicted resistance mutations
- Cell potency was improved ~1000x while retaining broad activity against resistance mutants and KDR sparing profile
- DMPK optimization faced with poor IVIVC was overcome by identifying a trend in electronic properties and unbound clearance
- BLU-667 is active in WT, gatekeeper mutant, and intracranial preclinical tumor models at doses that spare in vivo KDR activity
- Potently inhibits RET wild-type fusions (NSCLC & other cancers) and oncogenic mutations (MTC)
    - High preliminary response rates and durable activity in phase 1 dose escalation
    - BLU-667 has been generally well tolerated with most AEs being Grade 1/2

Program outlook and anticipated milestones

- BLU-667 phase 1 dose expansion is open and enrolling globally
- Plan to initiate a Phase 3 trial in first-line RET-fusion NSCLC in the second half of 2019
- Plan to initiate a Phase 2 combination trial of BLU-667 and osimertinib in treatment-resistant, EGFR-mutant NSCLC harboring an acquired RET alteration in the second half of 2019
- Plan to submit an NDA to the FDA for second-line RET-fusion NSCLC and second-line RET-mutant MTC in the first half of 2020

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- Investigators, and research coordinators
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