BLU-667 is a Potent and Highly Selective RET Inhibitor Being Developed for *RET*-Driven Cancers

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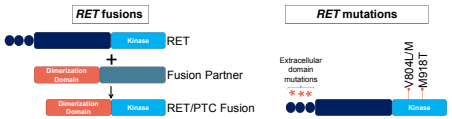
Poster B151

RET Kinase is Oncogenic in Diverse Cancer Subtypes

Rearranged during **t**ransfection (RET) is a highly conserved receptor tyrosine kinase that transduces signaling from the GDNF-family of neurotrophic factors. Activating *RET* mutations are observed in sporadic medullary thyroid cancer (MTC) and multiple endocrine neoplasia type 2 (MEN2). In other tumor types, chromosomal translocations oncogenically activate RET by fusing the kinase domain to a dimerization domain of another gene, creating RET fusion proteins with constitutive kinase activity. Although *RET* kinase fusions were initially observed in papillary thyroid cancer (PTC), recent genomic analyses of diverse tumor types identified *RET* fusions in non-small cell lung cancer (NSCLC), colon, breast, ovary, salivary carcinomas, chronic myelomonocytic leukemia (CMML) and inflammatory myofibroblastic tumors (IMFTs).



- **1-2% of NSCLC patients**
- 10% of PTC patients
- <1% of patients with colon, ovary, breast, or hematological cancer
- **60% of MTC patients harbor oncogenic *RET* mutations**
- M918T is the most prevalent *RET* mutation
- Germline V804L or V804M mutations are associated with familial MTC

Frequency of Oncogenic *RET* Alterations in Solid Tumors

Indication	*RET* Alteration	Frequency
NSCLC	Fusions	1-2%
MTC	Activating Mutations	60%
PTC	Fusions	10%
Colon, breast, other tumor types	Fusions	<1%

RET-Altered Cancer Patients Have High Medical Need

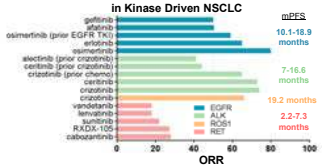
Multikinase inhibitors (mKIs) currently being tested in *RET*-altered NSCLC patients were not designed to selectively target RET and exhibit:

- **Broad kinome activity, often with potent inhibition of VEGFR-2**
- **Off-target related dose limiting toxicities that hamper their ability to fully inhibit RET**
- **Dramatically lower ORR and duration of response in NSCLC compared to selective kinase inhibitors targeting other kinase drivers**

mKIs Tested in *RET*-Altered NSCLC Patients

Multi-Kinase Inhibitor	Original Target(s)
Vandetanib	VEGFR-2 / EGFR
Lenvatinib	VEGFR1-2 / FGFR1-3 PDGFRα / KIT
Sunitinib	VEGFR-2 / pan-RTK
RXDX-105	RAF / RET
Cabozantinib	VEGFR-2 / MET

ORR and mPFS for Kinase Inhibitors in Kinase Driven NSCLC



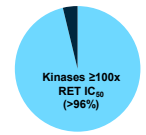
BLU-667 is Designed to Transform Treatment of *RET*-Altered Cancers

- **Crafted to target oncogenic RET fusions and activating mutations**
- **Highly active against activating and resistance mutations at the gatekeeper (V804) residue**
- **Kinome selectivity allowing full in vivo RET inhibition at well tolerated doses**

BLU-667 is a sub-nanomolar RET inhibitor with ~90 fold selectivity over VEGFR-2

Compound	Biochemical IC_{50} (nM)						IC_{50} Ratio (VEGFR-2 / RET)
	WT RET	RET V804L	RET V804M	RET M918T	CCDC6-RET	VEGFR-2	
BLU-667	**0.4**	**0.3**	**0.4**	**0.4**	**0.4**	**35**	**88x**
Cabozantinib	11	45	162	8	34	2	0.2x
Vandetanib	4	3597	726	7	20	4	1x
RXDX-105	3	188	102	4	7	17	6x

Kinases Inhibited by BLU-667



BLU-667 selectivity was assessed in a biochemical screen of a broad panel of 371 kinases.

BLU-667 is a highly potent RET inhibitor that exhibits ≥ 100X selectivity for RET over 96% of kinases tested

BLU-667 is a Potent and Selective Inhibitor of Oncogenic RET Fusions and Mutations

BLU-667 Potently Inhibits RET Autophosphorylation

Compound	pRET IC_{50} (nM)	pRET IC_{50} Ratio (vs. BLU-667)
BLU-667	**5.0**	**1x**
Cabozantinib	61.9	12x less potent
Vandetanib	833.1	167x less potent
RXDX-105	128.6	26x less potent

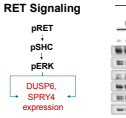
BLU-667 Inhibits *KIF5B-RET* Driven Proliferation and is Active on Gatekeeper (V804) Mutants

Compound	IC_{50} (nM)	IC_{50} Relative to WT		
	WT	V804L	V804M	V804E
BLU-667	**16.5**	**1.3x**	**0.3x**	**1.3x**
Cabozantinib	341	9x	16x	36x
Vandetanib	793	12x	11x	11x
RXDX-105	195.8	21x	15x	28x

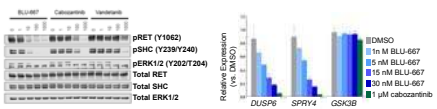
BLU-667 is More Potent than mKIs on *RET* Fusions Identified in NSCLC Patients

Cell Line	RET Alteration	Cancer Subtype	BLU-667 IC_{50} (nM)	Proliferation IC_{50} Relative to BLU-667		
				Cabozantinib	Vandetanib	RXDX-105
Ba/F3 LC2/ad	*KIF5B-RET*	Engineered	16.5	21x	48x	12x
	CCDC6-RET	NSCLC	3.7	89x	23x	9x

RET Signaling



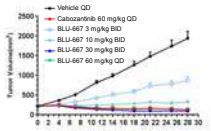
LC2/ad (*CCDC6-RET* NSCLC Cell Line)



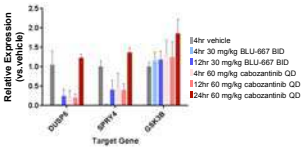
BLU-667 potently inhibited *RET* fusion-driven cell proliferation, including cell lines harboring a *KIF5B-RET* fusion, the most prevalent *RET* fusion identified in *RET*-rearranged NSCLC patients. Inhibition of cell proliferation was associated with potent downregulation of RET autophosphorylation and downstream signaling. Consistent with these observations, the transcriptional targets of the MAPK pathway DUSP6 and SPRY4 were downregulated by BLU-667 in a dose-dependent manner. Expression of GSK3B, a component of the parallel PI3K/AKT pathway, was not substantially impacted, confirming DUSP6 and SPRY4 as RET pathway biomarkers.

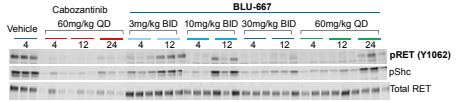
BLU-667 Robustly Suppresses Oncogenic KIF5B-RET Signaling and Tumor Growth Without Functionally Impacting VEGFR-2

***KIF5B-RET* NSCLC PDX**



BLU-667 Inhibits RET Pathway Target Genes In Vivo





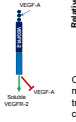
BLU-667 demonstrated dose dependent anti-tumor activity in a *KIF5B-RET*-driven NSCLC PDX. Modulation of KIF5B-RET activity in tumor lysates was confirmed by decreased pRET and pShc levels. DUSP6 and SPRY4 were also effectively downregulated by RET inhibition in vivo.

Active Doses of BLU-667 Do Not Functionally Impact VEGFR-2 In Vivo

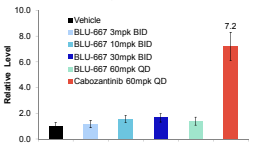
Increased VEGF-A and decreased soluble VEGFR-2 (sVEGFR-2) are class effects of VEGFR-2 inhibitors:

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



Circulating VEGF-A and sVEGFR-2 (not shown) were measured in the plasma of treated mice at the end of treatment. Cabozantinib, but not BLU-667, increased circulating VEGF-A and decreased sVEGFR-2 (not shown).

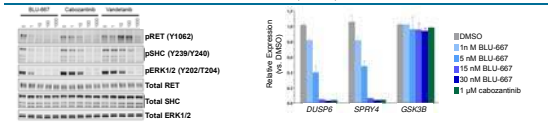
BLU-667, but not cabozantinib, inhibited tumor growth without biomarker evidence of VEGFR-2 inhibition

BLU-667 Inhibits RET Signaling and Cell Proliferation of Thyroid Cancer Cells with Activating *RET* Alterations

BLU-667 is More Potent than mKIs at Inhibiting *RET*-Driven Thyroid Cancer Cell Proliferation

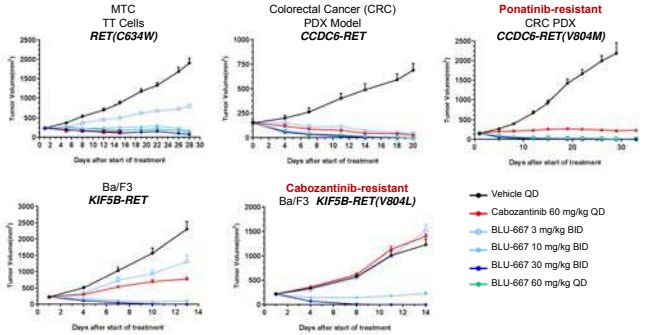
Cell Line	*RET* Alteration	Cancer Subtype	IC_{50} (nM)	Proliferation IC_{50} Relative to BLU-667		
			BLU-667	Cabozantinib	Vandetanib	RXDX-105
TT	*RET (C634W)*	MTC	15.4	36x	36x	7x
MZ-CRC-1	*RET (M918T)*	MTC	4.2	15x	4x	6x
TPC-1	*CCDC6-RET*	PTC	10.9	31x	19x	22x

MZ-CRC-1 Cells *RET(M918T)*



BLU-667 potently inhibited the proliferation of MTC and PTC cell lines harboring oncogenic *RET* alterations. Downregulation of RET pathway signaling was confirmed by examining pRET, pShc, and pERK1/2 levels. DUSP6 and SPRY4 were potently suppressed by BLU-667, confirming these as lineage independent biomarkers of oncogenic RET signaling.

BLU-667 has Broad Anti-tumor Activity on Diverse *RET*-Driven Cancers Including Tumors Harboring Gatekeeper (GK) Mutations



Oral administration of BLU-667 demonstrated dose dependent anti-tumor activity in *RET*-driven models including an MTC cell line xenograft driven by a *RET (C634W)* mutation, a *CCDC6-RET* fusion positive colorectal cancer PDX, as well as a GK mutant *CCDC6-RET(V804M)* model. BLU-667 was also active in Ba/F3 allograft models expressing either a *KIF5B-RET* fusion or a *KIF5B-RET(V804L)* GK mutant. Cabozantinib was administered at the MTD in mice (60 mg/kg QD) and showed antitumor activity similar to BLU-667 in models with a WT *RET* kinase domain, but not in models driven by GK mutant *RET*. Moreover, cabozantinib-treated animals suffered a rapid decline in health after 14 days of dosing in the TT and Ba/F3 in vivo studies. BLU-667 was well-tolerated in all models at all doses tested.

BLU-667 Phase 1 Study Initiated and First Patient Enrolled In March 2017

Part 1: Dose escalation *Enrolling*	Part 2: Dose expansion *Planned*
Escalation	NSCLC with RET fusion, prior TKI that inhibits RET
MD Anderson	NSCLC with RET fusion, no prior TKI that inhibits RET
Mass General Hospital	Medullary thyroid cancer
OHSU	RET-altered solid tumors other than NSCLC and MTC
UC Irvine	
U. Pennsylvania	
Duke	

GLOBAL EXPANSION ONGOING

NCT03037385 KEY OBJECTIVES: Identify MTD or RP2D and characterize initial anti-tumor activity

Conclusions

- **BLU-667 potently and selectively inhibits oncogenic RET fusions and activating mutations in preclinical models:**
  - **Broad, robust, anti-tumor activity against multiple *RET*-driven solid tumor types**
  - **Highly active against V804 gatekeeper substitutions that are mKI resistant**
  - **Spares VEGFR-2 at doses that completely inhibit RET**
  - **DUSP6 and SPRY4 have been identified as robust PD biomarkers of RET activity**
- **BLU-667 has entered Phase 1 clinical testing in patients with *RET*-altered NSCLC, thyroid cancer and other solid tumors**

BLU-667 has the potential to be a transformative medicine for patients with *RET*-driven malignancies