Pralsetinib (BLU-667) demonstrates robust activity in *RET*-fusion-driven intracranial tumor models

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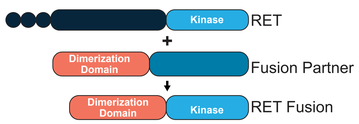
RET kinase is oncogenic in diverse cancer subtypes

Oncogenic alterations in *RET*, a tyrosine kinase receptor, cause ligand-independent kinase activation across a wide range of cancers, driving tumor formation and growth.[1] *RET* fusions are implicated as the underlying cause of disease in ~1–2% of patients with NSCLC.[2,3] Patients with NSCLC have an estimated 5-year survival rate of 18%.[4] Brain metastases, which portend a particularly poor prognosis, occur in about 40% of these patients.[5]

RET Fusions



RET Mutations

Frequency of Oncogenic *RET* Alterations in Solid Tumors

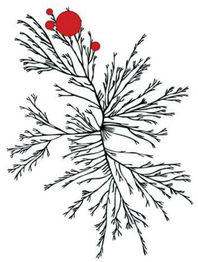
Indication	*RET* Alteration	Frequency
NSCLC[2,3]	Fusions	~1–2%
Advanced MTC[6]	Activating mutations	~90%
PTC[7]	Fusions	~20%
Colon, breast, other tumor types[8,9]	Fusions	<1%

Pralsetinib selectively inhibits oncogenic RET fusions and gatekeeper mutants

Variant	Biochemical IC_{50} (nM)
RET wild-type	0.4
RET V804L	0.3
RET V804M	0.4
RET M918T	0.4
CCDC6-RET	0.4

RET Cell Lines	Cellular IC_{50} (nM)
Ba/F3-KIF5b-RET	10.1
Ba/F3-KIF5b-RET V804L	8.1
Ba/F3-KIF5b-RET V804M	14.1
Ba/F3-KIF5b-RET V804E	8.1
LC2/ad (CCDC6-RET)	3.7
TPC-1 (CCDC6-RET)	10.9
MZ-CRC (RET M918T)	4.2
TT (RET C634W)	15.4

Kinome Selectivity for RET



Kinome illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).[a]

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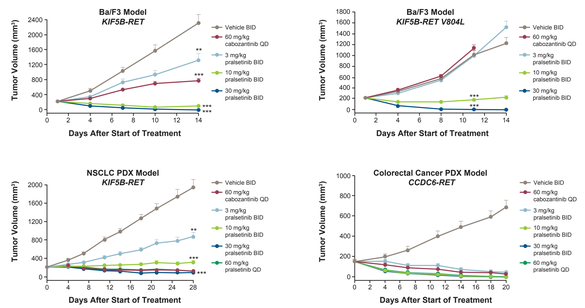
Pralsetinib was crafted to selectively target oncogenic *RET* fusions and activating mutations. Pralsetinib displays subnanomolar biochemical inhibitory activity across activated RET kinase fusions and mutations and low nanomolar anti-proliferative activity against *RET*-fusion or mutant-driven cell lines. When screened against a panel of human kinases, pralsetinib inhibited RET (large dot) most potently. Those kinases inhibited by pralsetinib within 50x RET IC_{50} are shown with medium dot and within 100X RET IC_{50} shown with small dot.

Pralsetinib demonstrated strong anti-tumor activity across *RET*-fusion in vivo models

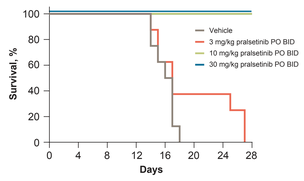


**P<0.01; ***P<0.001.

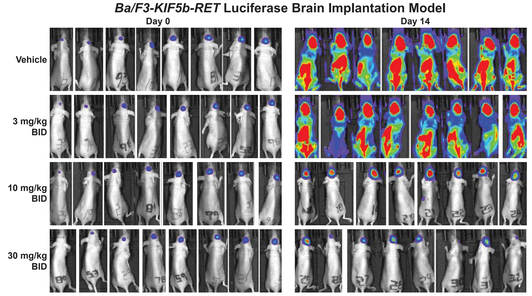
Oral administration of pralsetinib demonstrated dose-dependent anti-tumor activity in *RET*-fusion-driven models, including a Ba/F3 allograft model expressing either a *KIF5B-RET* fusion or a *KIF5B-RET*(*V804L*) gatekeeper mutant. Cabozantinib was administered at the MTD in mice (60 mg/kg QD) and showed anti-tumor activity similar to pralsetinib in models with a WT *RET*-kinase domain, but not in models driven by gatekeeper mutant *RET*. Pralsetinib demonstrated anti-tumor activity in an NSCLC *KIF5B-RET* fusion-driven model, as well as a *CCDC6-RET* fusion-positive colorectal cancer PDX. Pralsetinib was well tolerated in all models at all doses tested.

Pralsetinib showed significant anti-tumor activity in *KIF5b-RET*-driven intracranial tumors

BA/F3-*KIF5b-RET* Brain Implantation Model



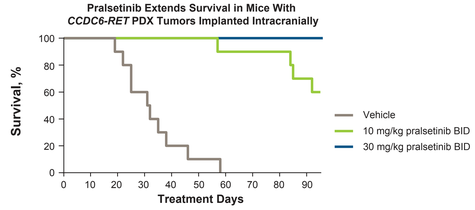
***Ba/F3-KIF5b-RET* Luciferase Brain Implantation Model**

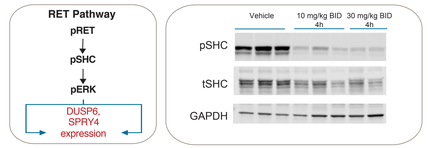


Ba/F3-*KIF5B-RET* cells engineered to express luciferase were injected intracranially into mice. Image analysis confirmed intracranial tumor growth and luciferase detection. Imaging occurred periodically throughout the experiment to measure disease. The health of vehicle-treated mice declined starting at day 14, with all animals requiring euthanasia by day 18. Treatment with 3 mg/kg pralsetinib BID demonstrated a moderate survival advantage, while mice treated with 10 or 30 mg/kg BID pralsetinib survived through 28 days (3 mg/kg BID, *P*<0.05; 10 or 30 mg/kg BID, both *P*<0.001).

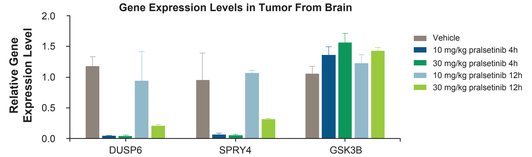
Pralsetinib demonstrated anti-tumor activity and strong target engagement in an intracranial *CCDC6-RET*-driven tumor model

Pralsetinib Extends Survival in Mice With *CCDC6-RET* PDX Tumors Implanted Intracranially





Gene Expression Levels in Tumor From Brain

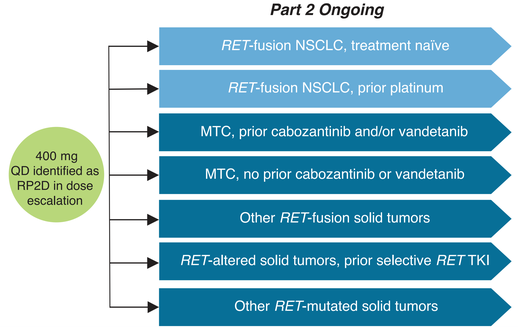


Anti-tumor activity: Each mouse was intracranially injected with *CCDC6-RET* PDX cells. After tumor inoculation, body weight was measured twice per week. The median survival for vehicle-treated mice was 31.5 days. Six of 10 mice treated with 10 mg/kg pralsetinib BID survived through 95 days; all 10 mice treated with 30 mg/kg pralsetinib BID survived through 95 days (both *P*<0.001).

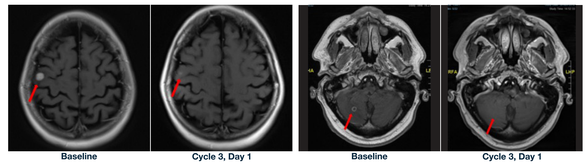
Intracranial tumor target engagement: Each mouse was intracranially injected with *CCDC6-RET* PDX cells. Enrollment was performed on a rolling basis, with body weight used as an early marker for tumor progression. Therapy was initiated immediately upon enrollment. Enrollment continued until there were ≥4 animals per group. Immunoblot demonstrated high levels of pSHC inhibition after dosing with 10 or 30 mg/kg pralsetinib. Human *DUSP6*/*SPRY4* transcripts decreased >90% with 10 or 30 mg/kg pralsetinib at 4h and indicated full pathway inhibition at doses demonstrating anti-tumor activity. Negative control, human GSK3B expression was not inhibited with pralsetinib treatment.

Pralsetinib is being evaluated in a clinical trial for patients with RET-altered tumors

Part 2 Ongoing



Pralsetinib was active against intracranial metastases in the clinical setting[10]



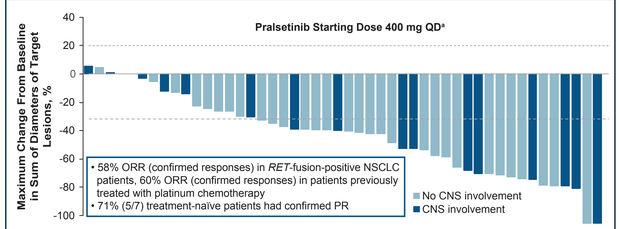
- 52-year-old woman, *RET*-fusion-positive NSCLC, prior platinum and checkpoint inhibitor
- Near-complete resolution of previously untreated target brain metastasis after 2 months of pralsetinib 400 mg QD
- Continues to receive treatment with ongoing confirmed PR (70% shrinkage) at 10+ months (data cut-off 16 Aug 19)

Images courtesy of Dr. Stephen Liu, Georgetown University, Washington, DC

- 59-year-old man, *RET*-fusion-positive NSCLC, prior platinum and checkpoint inhibitor
- Complete resolution of previously untreated nontarget brain metastasis after 2 months of pralsetinib 400 mg QD
- Continues to receive treatment with ongoing confirmed CR at 10+ months (data cut-off 16 Aug 19)

Images courtesy of Dr. P Cassier Centre, Leon Berard, Lyon, France

Pralsetinib demonstrated anti-tumor activity in patients with CNS involvement[10]



*Patients enrolled by 14 Nov 18, with a data cut-off of 28 Apr 19. The response-evaluable population included patients with measurable disease at baseline and ≥1 evaluable post-treatment disease assessment and excluded four patients who previously received >1 cycle of a selective RET inhibitor. All responses are confirmed on 2 consecutive assessments as per RECIST 1.1.

Adverse Events (Pralsetinib starting dose 400 mg)	Treatment-Emergent AE		Treatment-Related AE	
	All (≥15% overall)	Grade ≥3	All	Grade ≥3
Constipation	30%	2%	17%	2%
Neutropenia[a]	26%	13%	26%	13%
AST increased	24%	5%	20%	2%
Fatigue	21%	3%	13%	3%
Hypertension	20%	13%	13%	10%
Anemia	18%	7%	11%	4%
Diarrhea	18%	2%	9%	-
Pyrexia	18%	-	2%	-
ALT increased	17%	3%	13%	2%
Cough	17%		3%	-
Dry mouth	17%	-	12%	-

Additional grade ≥3 treatment-related AEs (≥2%): increased CPK (3%), leukopenia[a] (3%).
Across the entire study (n=276), the rate of discontinuation due to treatment-related toxicity was 4%.
[a]Combined term including decreased neutrophils and neutropenia. [b]Combined term including leukopenia and white blood cell count decreased.

CONCLUSIONS

- Pralsetinib has broad anti-tumor activity in intracranial tumor models regardless of *RET*-fusion partner
- Pralsetinib showed broad, durable anti-tumor activity in patients with *RET*-fusion NSCLC, both systemically and in the brain
- ARROW clinical trial enrollment continues in treatment naïve *RET*-fusion-positive NSCLC (NCT03037385)

Acknowledgments

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Abbreviations

AE, adverse event; *ALT*, alanine aminotransferase; AST, aspartate aminotransferase, BID, twice daily; CI, confidence interval; CPK, creatine phosphokinase; CR, complete response; CRC, colorectal cancer; DCR, disease control rate (best response of SD or better); h, hour; IC_{50}, half maximal inhibitory concentration; MTC, medullary thyroid cancer; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; ORR, overall response rate; PD, progressive disease; PDX, patient-derived xenograft; PO, orally; PR, partial response; PTC, papillary thyroid cancer; QD, once daily; RP2D, recommended phase 2 dose; SD, stable disease; TKI, tyrosine kinase inhibitor; WT, wild-type.

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