# SUPPLEMENTARY DATA

**Supplementary Tables**

**Supplementary Table S1:** BLU-667 selectivity for RET over selected kinases

|  |  |  |
| --- | --- | --- |
| **Kinase** | **BLU-667 IC50 (nM)** | **Fold-change over RET IC50\*** |
| **RET** | 0.12 | 1 |
| **DDR1** | 1.9 | 16 |
| **JAK1** | 2.4 | 20 |
| **TRKC** | 7.1 | 59 |
| **FLT3** | 15 | 125 |
| **JAK2** | 19 | 158 |
| **TRKA** | 58 | 483 |
| **PDGFRb** | 76 | 633 |
| **LIMK1** | 120 | 1000 |
| **FGFR1** | 136 | 1133 |
| **c-SRC** | 139 | 1158 |
| **MLK2/MAP3K10** | 154 | 1283 |
| **PEAK1** | 159 | 1325 |
| **FGFR3** | 204 | 1700 |
| **MLK3/MAP3K11** | 227 | 1892 |
| **ROS/ROS1** | 245 | 2042 |
| **c-KIT** | 245 | 2042 |
| **YES/YES1** | 258 | 2150 |
| **FLT4/VEGFR3** | 355 | 2958 |
| **JAK3** | 389 | 3242 |
| **TYK2** | 394 | 3283 |
| **BMX/ETK** | 690 | 5750 |

\*Fold-change calculated by dividing the IC50 of BLU-667 for each kinase by the IC50 of BLU-667 for RET

# Supplementary Table S2: Inhibition of proliferation in *RET*-driven cell lines

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | **IC50,** **nM** | | | |
| **Cell line** | **RET alteration** | **Alteration type** | **Cancer type** | **BLU-667** | **Cabozantinib** | **Vandetanib** | **RXDX-105** |
| LC2/ad | CCDC6-RET | Fusion | NSCLC | **3.7** | 329 | 85 | 33 |
| TT | C634W | Missense (extracellular) | MTC | **15.4** | 554 | 554 | 107 |
| MZ-CRC-1 | M918T | Missense  (kinase domain) | MTC | **4.2** | 63 | 17 | 25 |
| TPC-1 | CCDC6-RET | Fusion | PTC | **10.9** | 338 | 207 | 240 |
| Ba/F3 | KIF5B-RET | Fusion | Engineered | **16.5** | 341 | 793 | 196 |
| Ba/F3 | KIF5B-RET V804M | Fusion/missense mutation | Engineered | **15.3** | 3023 | 9228 | 4116 |
| Ba/F3 | KIF5B-RET V804L | Fusion/missense mutation | Engineered | **4.6** | 5582 | 8360 | 2940 |
| Ba/F3 | KIF5B-RET V804E | Fusion/missense mutation | Engineered | **21.5** | >10,000 | 8723 | 5488 |

**Supplementary Table S3. Multi-kinase inhibitor mutagenesis screens**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Concentration over IC50** | **Number of clones** | | | | | | |
| **V804E** | **V804L** | **V804M** | **Y806C** | **Y806H** | **Y806N** | **D898Y** |
| **Regorafenib** | 16× | 1 | 2 | 4 | 0 | 0 | 0 | 0 |
| 32× | 5 | 7 | 11 | 0 | 0 | 0 | 0 |
| 64× | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | | | | | | | |  |
| **Vandetanib** | 16× | 0 | 3 | 5 | 0 | 0 | 0 | 0 |
| 32× | 10 | 21 | 12 | 6 | 1 | 2 | 0 |
| 64× | 5 | 6 | 11 | 1 | 1 | 0 | 0 |
|  | | | | | | | |  |
| **Ponatinib** | 16× | 22 | 16 | 20 | 0 | 0 | 0 | 0 |
| 32× | 25 | 8 | 1 | 0 | 0 | 0 | 0 |
| 64× | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
|  | | | | | | | |  |
| **Cabozantinib** | 16× | 14 | 2 | 10 | 5 | 0 | 1 | 1 |
| 32× | 26 | 0 | 14 | 0 | 0 | 0 | 0 |
| 64× | 16 | 0 | 0 | 0 | 0 | 0 | 0 |

**Supplementary Figures**

**Supplementary Figure S1. Relative change in body weight of mice treated with BLU-667 or cabozantinib**

Body weight of mice treated with BLU-667 remained stable or increased in mice harboring KIF5B-RET (A) and KIF5B-RET V804L (B) Ba/F3 allografts. Mice treated with cabozantinib did not reach the end of the study due to significant toxicity manifested by severe weakness. BLU-667 was well tolerated throughout the *in vivo* studies. Data are the mean + SEM. BID, twice-daily; QD, once-daily; SEM, standard error of the mean.



**Supplementary Figure S2. BLU-667 inhibits growth of CCDC6-RET and CCDC6-RET V804M CRC PDX tumors**

(A-D) Antitumor activity of BLU-667 and cabozantinib and waterfall plots showing the best response as a percent change in tumor volume taken on the last day of treatment in *CCDC6-RET*-driven CRC PDX tumors (A, B) and *CCDC6-RET V804M*-driven CRC PDX tumors (C, D). Nine mice were used per treatment group. Data are the mean + SEM. \*\**P*<0.01, \*\*\**P*<0.001, one-way ANOVA with a Dunnett’s (CCDC6-RET) or a Games-Howell (CCDC6-RET V804M) multiple comparisons test. BID, twice daily; QD, once daily; SEM, standard error of the mean.



**Supplementary Figure S3. RET pathway inhibition from KIF5B-RET V804L Ba/F3 allograft tumors treated with BLU-667**

Phosphorylation of RET and SHC are reduced in tumor lysates harvested from mice bearing Ba/F3 KIF5B-RET V804L allografts that were treated with BLU-667 at the indicated doses. No effect is evident on RET pathway components in tumor lysates from mice treated with cabozantinib at 60 mg/kg QD. BID, twice-daily; QD, once-daily.



**Supplementary Figure S4. Circulating levels of soluble VEGFR-2 in mice baring KIF5B-RET NSCLC PDX tumors.**

Circulating soluble VEGFR-2 levels from KIF5B-RET NSCLC PDX-bearing mice treated with BLU-667 or cabozantinib at the indicated times after administration of the last dose. Data are the mean + SD. \**P*<0.001, one-way ANOVA with a Dunnet’s multiple comparisons test. BID, twice daily; QD, once daily; SD, standard deviation.



**Supplementary Figure S5. Rapid decrease of RET M918T ctDNA and *DUSP6/SPRY4* expression in an MTC patient treated with BLU-667.**

ctDNA analysis of RET M918T levels in plasma from an MTC patient reveals rapid drop in circulating M918T mutant allele fraction during early treatment cycles. Pre- and post-treatment tumor biopsy reveals 93% decrease in *DUSP6* and 86% decrease in *SPRY4* mRNA expression after 28 days of treatment with BLU-667. MAF, mutant allele fraction.

