**Supplementary Tables 1-4**

Table S1. *In Vitro* Kinase IC50 Values (nM) for Native and Mutant Recombinant KIT

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| KIT genotype | KIT exon | Ponatinib | Imatinib | Sunitinib | Regorafenib |
| Native | - | 6 | 640 | 110 | 533 |
| V559D | 11 | 0.8 | 36 | 139 | 57 |
| V559D/ V654A | 11/ 13 | 38 | >3000 | 38 | >3000 |
| V559D/ T670I | 11/ 14 | 2 | >3000 | 124 | 353 |
| V560G | 11 | 0.3 | 38 | 154 | 27 |
| V654A | 13 | 160 | 2590 | 20 | 1020 |
| T670I | 14 | 3 | >3000 | 17 | 66 |
| D816H | 17 | 11 | 2470 | 119 | 736 |
| D820E | 17 | 4.4 | 169 | 117 | 194 |
| A829P | 18 | 1.0 | 197 | 137 | 147 |

Table S2. Summary of Ba/F3 KIT Cell Lines Generated in this Study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| KIT Mutant Abbreviation | Primary Mutation | | Secondary Mutation | |
| Location | Genotype | Location | Genotype |
| Native | - | - | - | - |
| Ex9 | Exon 9 (ECD) | Ins (502AY) | - | - |
| Ex9/ V654A | Exon 13 (ATP pocket) | V654A |
| Ex9/ D816H | Exon 17 (A-loop) | D816H |
| Δ550-557 | Exon 11 (JM) | Del 550\_557 | - | - |
| Δ551-554 | Exon 11 (JM) | Del 551\_554 | - | - |
| Δ557-558 | Exon 11 (JM) | Del 557\_558 | - | - |
| Δ557-558/ V654A | Exon 13 (ATP pocket) | V654A |
| Δ557-558/ N655K | Exon 13 (ATP pocket) | N655K |
| Δ557-558/ T670I | Exon 14 (gatekeeper) | T670I |
| Δ557-558/ D816G | Exon 17 (A-loop) | D816G |
| Δ557-558/ D816H | Exon 17 (A-loop) | D816H |
| Δ557-558/ D820A | Exon 17 (A-loop) | D820A |
| Δ557-558/ D820G | Exon 17 (A-loop) | D820G |
| Δ557-558/ N822K | Exon 17 (A-loop) | N822K |
| Δ557-558/ A829P | Exon 18 (A-loop) | A829P |
| K558NP | Exon 11 (JM) | Del/Ins (K558NP) | - | - |
| V560D | Exon 11 (JM) | V560D | - | - |
| V560D/ V654A | Exon 13 (ATP pocket) | V654A |
| V560D/ D816H | Exon 17 (A-loop) | D816H |

Extracellular domain (ECD), juxtamembrane domain (JM), ATP binding pocket (ATP pocket) and activation loop (A-loop).

Table S3. Summary of IC50 Viability Values in Ba/F3 KIT Cells

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Ponatinib | Imatinib | Sunitinib | Regorafenib |
| Parental | 2,128 ± 159 | >10,000 | 8,951 ± 413 | >10,000 |
| Native | 37 ± 5 | 145 ± 37 | 8 ± 4 | 128 ± 28 |
| Ex9 | 56 ± 5 | 143 ± 10 | 5 ± 1 | 138 ± 63 |
| Ex9/ V654A | 564 ± 11 | 2621 ± 154 | 18 ± 3 | 814 ± 66 |
| Ex9/ D816H | 188 ± 20 | 1584 ± 434 | 373 ± 136 | 560 ± 62 |
| Δ550-557 | 3 ± 1 | 13 ± 3 | 5 ± 1 | 22 ± 3 |
| Δ551-554 | 15 ± 1 | 90 ± 5 | 42 ± 1 | 141 ± 10 |
| Δ557-558 | 3 ± 0 | 27 ± 8 | 7 ± 2 | 30 ± 14 |
| Δ557-558/ V654A | 60 ± 16 | 545 ± 56 | 10 ± 0 | 350 ± 67 |
| Δ557-558/ N655K | 15 ± 1 | 65 ± 3 | 34 ± 1 | 88 ± 5 |
| Δ557-558/ T670I | 12 ± 2 | >10,000 | 12 ± 2 | 132 ± 25 |
| Δ557-558/ D816G | 5 ± 1 | 180 ± 43 | 234 ± 51 | 88 ± 12 |
| Δ557-558/ D816H | 13 ± 2 | 292 ± 51 | 204 ± 65 | 170 ± 21 |
| Δ557-558/ D820A | 8 ± 2 | 369 ± 76 | 339 ± 82 | 134 ± 29 |
| Δ557-558/ D820G | 3 ± 1 | 233 ± 37 | 196 ± 34 | 79 ± 11 |
| Δ557-558/ N822K | 3± 0 | 483 ± 24 | 225 ± 19 | 131 ± 9 |
| Δ557-558/ A829P | 2 ± 0 | 179 ± 27 | 319 ± 88 | 33 ± 6 |
| K558NP | 1 ± 0 | 2 ± 1 | 2 ± 0 | 4 ± 1 |
| V560D | 2 ± 0 | 3 ± 0.2 | 2 ± 0.2 | 3 ± 0 |
| V560D/ V654A | 101 ± 30 | 461 ± 106 | 8 ± 1 | 465 ± 96 |
| V560D/ D816H | 58 ± 7 | 407 ± 69 | 285 ± 54 | 447 ± 12 |

Values shown are the mean and standard deviation calculated from three independent experiments.

Table S4. Summary of KIT Driven Ba/F3 *In Vivo* Studies

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Model | Total growth inhibition (%) | | | |
| Ponatinib | | Imatinib | Sunitinib |
|  | 10 mg/kg | 30 mg/kg | 300 mg/kg | 80 mg/kg |
| Δ557-558 | -8 | -89 | -90 | N.T. |
| Δ557-558/ V654A | N.T. | 65 | No effect | -100 |
| Δ557-558/ T670I | N.T. | -100 | 31 | -100 |
| Δ557-558/ D816H | 66 | -24 | 43 | N.T. |

Total growth inhibition = [(treated final volume – treated starting volume) / (vehicle final volume – vehicle starting volume)] x100. Negative values indicate tumor regression. Not tested (N.T.)