**Supplementary Table S1.** Relative IC50’s of ALK+ and ALK- ALCL cell lines to the ALK tyrosine kinase inhibitors crizotinib and ceritinib

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Crizotinib | Ceritinib |
|  |  | IC50 | S.E.M. | IC50 | S.E.M. |
| ALK-Positive | Karpas-299 | 129.8 | 1.96 | 54.79 | 4.95 |
| SUP-M2 | 67.76 | 3.71 | 15.57 | 1.06 |
| DHL-1 | 128.9 | 5.92 | 67.94 | 4.35 |
| ALK-Negative | MAC 2A | 850.78 | 69.05 | 1015.83 | 47.54 |

**Supplementary Table S2.** *ALK* Kinase-Domain Sequencing Results

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Comparison** | **Subclone-Specific Mutation** | **Mutation Identification Method\*** | **Known ALK Inhibitor Resistance** | **Notes** | **Supplementary Reference** |
| **MuTect** | **Seurat** | **Sanger** |
| Karpas-299 vs. K299-CR1000 | *None* | ✓ | ✓ | ✓ | *-* |  | *-* |
| Karpas-299 vs. K299-CRS | *None* | ✓ | ✓ | ✓ | *-* |  | *-* |
| Karpas-299 vs. K299-CR1000-2 | *None* |  |  | ✓ | *-* |  | *-* |
| SUP-M2 vs. SUP-CR500 | G1272R | ✓ |  |  | NR | NR | *-* |
| L1291Q | ✓ |  |  | NR | NR | *-* |
| I1171S |  |  | ✓ | CrizotinibR CeritnibS | Modifies the structure of the kinase-inhibitor complex; Identified in vitro (for both EML4-ALK and ALK+ ALCL), as well as in vivo for EML4-ALK | (1-4) |
| SUP-M2 vs. SUP-CRS |  I1171S |  |  | ✓ | CrizotinibR CeritnibS | Modifies the structure of the kinase-inhibitor complex; Identified in vitro (for both EML4-ALK and ALK+ ALCL), as well as in vivo for EML4-ALK | (1-4) |
| SU-DHL-1 vs. DHL1-CR500 | R1192P | ✓ | ✓ | ✓ | NR | Identified in neuroblastoma (unknown function) | (5) |
| SU-DHL-1 vs. DHL1-CRS | D1232G | ✓ |  |  | NR |  | - |
| Karpas-299 vs. K299-LR150 | *None* | ✓ | ✓ | ✓ | *-* |  | *-* |
| Karpas-299 vs. K299-LRS | D1372G | ✓ |  |  | NR |  | *-* |
| SUP-M2 vs. SUP-LR150 | F1174L | ✓ | ✓ | ✓ | CrizotinibR CeritnibR | Neuroblastoma (confers ligand-independence to full-length ALK); Identified in vitro as well as in EML4-ALK patient samples | (2-4, 6) |
| S1186I | ✓ |  |  | NR |  | - |
| SUP-M2 vs. SUP-LRS | S1206T | ✓ |  |  | CrizotinibR CeritnibS | Occurs at the solvent front leading to a bulkier and charged side chain that is incompatible with crizotinib or ceritinib binding due to steric hindrance; Identified in vitro as well as in EML4-ALK patient samples | (2, 4, 7) |
| I1233T | ✓ |  |  | NR | I233 forms the conserved hydrophobic patch that anchors the catalytic loop to establish correct positioning with respect to the DFG motif and ATP. | (8) |
| F1271Y | ✓ |  |  | NR | F1271 makes direct contact with F1174, making up the DFG motif, which is important for ATP binding. | (9) |

NR: Not previously reported

\* Mutations validated by more than one method carry greater confidence.

R = resistant

S = sensitive