Pediatric Preclinical Testing Program (PPTP): Evaluation of the MEK1/2 Inhibitor AZD6244 against Juvenile Pilocytic Astrocytoma (JPA) Xenografts

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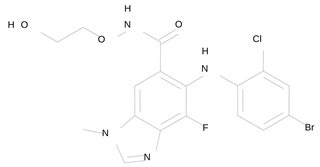
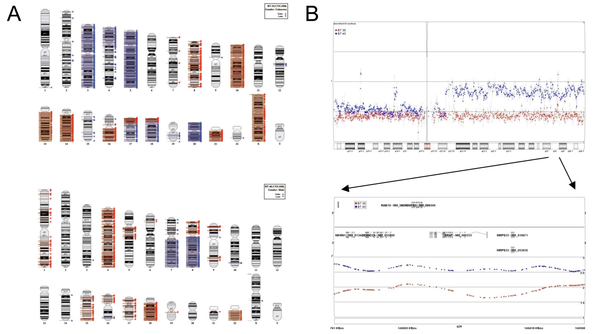
Background

- AZD6244 (ARRY-142886) is a potent, highly specific small molecule inhibitor of MEK1/2 that is not competitive with ATP.

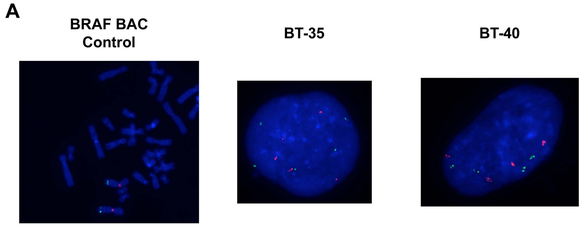


- In Stage 1 testing of AZD6244 against the PPTP in vitro panel:
  - Growth inhibition was observed in a minority of the 23 PPTP cell lines.
  - Kasumi-1, a cell line with an activating KIT mutation, was the most responsive cell line and the only cell line with a clear cytotoxic response to AZD6244.
- In Stage 1 testing of AZD6244 against the PPTP *in vivo* tumor panels, the following was observed:
  - AZD6244 induced significant differences in EFS distribution in 10 of 37 (27%) solid tumor models and 0 of 6 acute lymphoblastic leukemia (ALL) models,
  - No objective responses were observed.
- Recent reports describing molecular characterization of juvenile pilocytic astrocytomas (JPAs) has shown tandem duplication producing a novel fusion gene (KIAA1549-BRAF) that lacks the BRAF regulatory domain and leads to constitutive activation of BRAF.
- Activating point mutations in BRAF are less frequent in JPA, accounting for only 5 percent of cases.
- AZD6244 was evaluated against two JPA xenografts, BT-35 and BT-40, that are used for secondary testing by the PPTP.

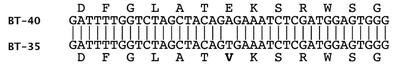
Characterization of BT-35 and BT-40



Copy number analysis of BT-35 and BT-40 xenografts (*Affymetrix SNP6.0 arrays*) .
- Panel A. Ideograms of xenograft genomes. Genomic segments of at least 100 kb with copy gain (blue triangles) or loss (red triangles) for BT-35 (top) and BT-40 (bottom).
- Panel B. Copy number estimates for BT-35 (red squares) and BT-40 (blue squares) on chromosome 7 (top) and Hidden Markov model copy number states for the BRAF locus at 7q34 (bottom). BT-35 and BT-40 showed no evidence for focal gain in the region of the BRAF gene, while BT-40 demonstrated gain of the entire long arm of chromosome 7.

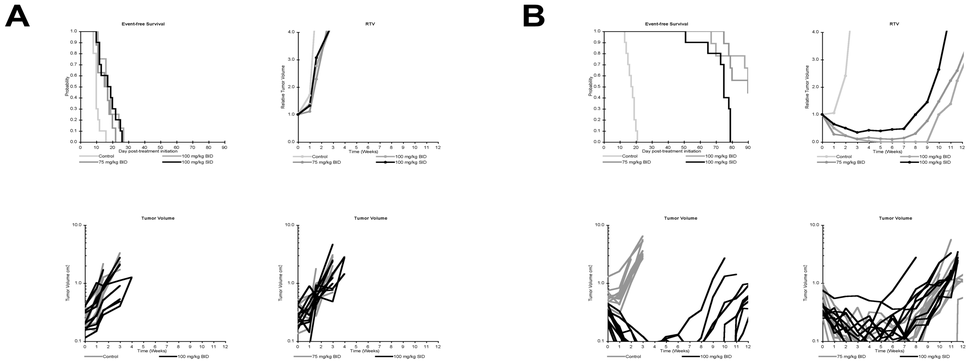


Fluorescence in situ hybridization (FISH) using Chr 7 centromeric probes (red) and BRAF probes (Green) show no evidence for BRAF duplication in BT-35 and BT-40.

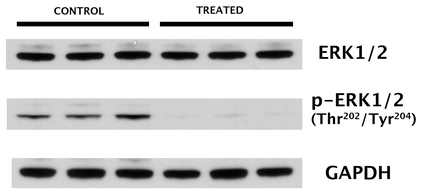


Sequence analysis for BRAF in BT-35 and BT-40 xenografts shows wildtype BRAF for BT-35 and the V600E mutation for BT-40.

AZD6244 in Vivo Activity against JPA Xenografts



- Kaplan-Meier curves for EFS, median relative tumor volume graphs, and individual tumor volume graphs are shown for BT-35 (A) and BT-40 (B). AZD6244 was dissolved in 0.5% hydroxypropyl methyl cellulose, 0.1% Polysorbate 80 and administered p.o.
- For individual growth curve plots: left panel Control (gray), AZD6244 100 mg/kg BID x 5 SID x2/week (black); right panels AZD6244 75 mg/kg BID x5 SID x 2/week (gray) and 100 mg/kg SID x 7 (black).
- AZD6244 had no activity against BT-35 (wildtype BRAF), but induced complete regressions against BT-40 (V600E BRAF).
- Twice-daily dose regimens (100 mg/kg or 75 mg/kg) were more effective than a daily dose regimen (100 mg/kg).



Western blot analysis of OS-33 xenografts treated with either vehicle or AZD6244 at 100mg/kg BID for 5 days. Tumors were harvested 1 hour after the first dose on day 5. AZD6244 at this dose completely inhibited ERK1/2 phosphorylation.

AZD6244 was provided for testing by AstraZeneca.
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CONCLUSIONS

- AZD6244 was highly active against the BT-40 JPA xenograft that harbors constitutively activated BRAF as a result of a V600E mutation.
- AZD6244 was inactive against BT-35, which has wildtype BRAF, just as it was against most of the PPTP Stage 1 xenograft panels.
- AZD6244 blocks MAPK pathway signaling in solid tumor xenografts at the 100 mg/kg dose that is effective against BT-40.
- The BT-40 xenograft model may be valuable for developing rational combinations of molecularly-targeted agents against JPAs with BRAF activation.
- The complete regressions induced by AZD6244 against a BRAF-mutant pilocytic astrocytoma xenograft are a strong activity signal pointing to the potential utility of MEK inhibition for this tumor type.
- Further dose-response testing will provide insight into whether tumor regressions for BT-40 can occur at doses that produce drug exposures achievable in the clinical setting.