The TRK Inhibitor Entrectinib Enhances the Efficacy of Temozolomide and Irinotecan in a Xenograft Model of Neuroblastoma

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Statistical Analysis: Linear mixed effects model was used to test the rate of tumor volume over time between groups.

Introduction

Neuroblastoma:
• The most common extracranial solid tumor in children, comprising 8–10% of all childhood cancers.
• Accounts for almost 15% of cancer deaths in children.
• Demonstrates incredible clinical heterogeneity from spontaneous regression to aggressive disease with progression despite intense multimodality therapy.
• Evidence suggests that the differences in behavior may be associated with differential expression of the Trk family of receptor tyrosine kinases.
• Trk expression in neuroblastoma has been associated with MYCN amplification and poor prognosis.

Entrectinib:
• A novel, orally available, selective tyrosine kinase inhibitor of the Trk (A/B/C), Ret, and NTRK1/2/3 family.
• Currently in two Phase I/II clinical trials (STARTRK-1 and ALKA-372-001).
• Good oral bioavailability with no dose limiting toxicity.
• Currently in two Phase III clinical trials (STARTRK-1 and ALKA-372-001).
• Low expression of TrkB has been associated with poor outcome in neuroblastoma.

Materials and Methods

• Compounds: Entrectinib (Ignyta, San Diego) was dissolved in DMSO for in vitro studies. For in vivo studies, it was reconstituted in 0.5% MethoCell solution containing 1% Tween 60 at final dosing volume of 10mg/kg. Tamoxifen (Teva, 20mg capsule) and Irinotecan (hexareps, 20mg/ml) were diluted in saline for i.v. dosing.
• Cell lines: In vitro studies were carried out on Sh-SY5Y and NLF cells stably transfected with TRKB (A/B/C), Ret, and NTRK1/2/3.
• In vivo experiments: For in vivo studies, a xenograft model was used to evaluate the efficacy of single agent or combination therapy in vivo. The SY5Y TrkB xenografts were orthotopically implanted in NOD/SCID mice and animals were treated with the following regimens:
  - Entrectinib alone at 60mg/kg, BID x 7days/week, PO; Temozolomide (7.5 mg/kg)/Irinotecan (0.63 mg/kg); QD x 5 days/week, PO.
  - Entrectinib + T17 (7 days and 5 days respectively) or vehicle alone.

Results

Figure 1: Effect of Entrectinib on TrkB inhibition in vitro

A. Western blot showing increased inhibition of phospho-Trk in treated with increasing concentrations of Entrectinib in:
• Sh-SY5Y cells transfected with TrkB
• NLF cells transfected with TrkB

B. TrkB expression in:
• Sh-SY5Y cells transfected with TrkB
• NLF cells transfected with TrkB

RNA isolated from the cells were run on NGS platform for TrkB expression in kinase domain

Figure 2: Effect of Entrectinib on SY5Y-TrkB neuroblastoma xenografts as a single agent

Nununu mice xenografted with the SY5Y-TrkB cells were given Entrectinib at 60mg/kg, QD x 7days/week, PO; Control group received vehicle alone. Animals were taken out of the study when tumor volume reached 2 cm3.

A. Average tumor growth
B. Survival graph
C. Western blots comparing tumors from control group to tumors harvested at 1hr, 4hr and 6hr post dosing with Entrectinib.

Figure 3: Growth Assay (SRB) showing increased growth inhibition with increasing concentrations of Entrectinib used in combination with Temozolomide in vitro compared to single agents or Temozolomide + Irinotecan treatment

Figure 4: Effect of Entrectinib in combination with Temozolomide and Irinotecan on SY5Y-TrkB neuroblastoma xenografts

Nununu mice xenografted with the SY5Y-TrkB cells were given either Entrectinib alone at 60mg/kg, BID x 7days/week, PO; Temozolomide (7.5 mg/kg)/Irinotecan (0.63 mg/kg); QD x 5 days/week, PO; Entrectinib + T17 (7 days and 5 days respectively) or vehicle alone. Animals were taken out of the study when tumor volume reached 3 cm3.

A. Average tumor growth
B. Survival graph

Conclusions

• Entrectinib shows inhibition of Trk phosphorylation at nanomolar levels in both the TrkB transfected cells lines tested in this study
• Cell growth inhibition depends on the amount of Trk expressed in the cells
• Sh-SY5Y-TrkB cells with more TrkB expression show a distinct dose dependent cell growth inhibition compared to NLF clones that express lesser TrkB
• Single agent treatment of xenograft models shows a pronounced reduction in tumor growth rate and a longer and more significant survival time for the animals
• Combination of Entrectinib with chemotherapeutic agents (Temozolomide + Irinotecan) shows increased cell growth inhibition in vitro compared to single agents or Temozolomide + Irinotecan treatment
• Combination of Entrectinib with Temozolomide and Irinotecan results in significant delay in tumor growth and a significant EFS when compared to single agent alone or Temozolomide + Irinotecan group
• Our data supports the incorporation of Entrectinib in clinical trials of neuroblastomas and other tumors relying on the Trk pathway for tumorigenesis

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