**In vivo and Pharmacodynamic Profiling of the KSP Inhibitor ARRY-520 Supports Potent Activity in Hematological Cancers and Drug Resistant Tumors**


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**Introduction**

Anti-mitotic therapy has proven clinical benefit in the treatment of cancer: microtubule-targeted drugs, such as taxanes, vinca alkaloids, and epothilones, are used to treat a variety of cancers, including breast, ovarian, lung, and leukemia. One disadvantage of these drugs is the mechanism-based toxicity arising from disruption of microtubule dynamics in cellular processes not involved in proliferation. This lack of specificity leads to neurotoxicity, which can be dose-limiting with these agents.

Mitotic kinesins, a family of motor proteins involved in all phases of mitosis including chromosome and spindle pole body dynamics and microtubule depolymerization, provide an alternative target for anti-mitotic therapy. These proteins couple the energy of ATP hydrolysis to mechanochemical force resulting in movement of microtubules. Kinesin spindle protein (KSP, egCdc11) is a mitotic kinesin involved in the early stages of mitosis. KSP is responsible for centrosome separation, which is required for formation and maintenance of the bipolar spindle. Inhibition of KSP prevents the formation of a bipolar spindle, resulting in cells arrested in mitosis with an abnormal monopolar spindle. KSP inhibition is an attractive approach to the design of novel antimitotic drugs with a distinct mechanism compared to microtubule-targeted therapeutics.

KSP is expressed in non-dividing cells, and as a consequence KSP inhibitors are selective for proliferating cells. KSP inhibitors are highly mitotic-specific and act as promising anti-cancer agents. Because KSP inhibition is a novel mechanistic approach to anti-mitotic therapy, with potentially unique mechanisms of resistance, it is also possible that KSP inhibitors will show activity in tumors that are refractory to standard therapies.

**Materials and Methods**

**Cell Culture**

Growth conditions for the cells included appropriate growth medium and incubation with 5% CO2 at 37°C. Cells were passaged with standard methods at a density that did not exceed 70% confluence. Media was replaced every 48 hours. Cell viability was determined at the end of the assay, using the Promega CellTiter-Blue® assay, and the EC50 for inhibition of viability was determined.

**Drug Treatments**

Cells were treated with serial dilutions of ARRY-520. Activity of ARRY-520 was determined at 72 hours by cell viability assay (CellTiter-Blue®).

**IHC Analysis of Spindle Morphology**

IHC analysis of spindle morphology was performed by staining the paraffin embedded tumors. Spindle morphology was analyzed by staining tumor sections for tubulin, and apoptosis was analysed by TUNEL stain. Cell number was determined from Hoechst stained samples, and data reported as mean +/- SEM.

**Summary of in vitro and in vitro antitumor activity**

Activity vs. paclitaxel and ispinesib

<table>
<thead>
<tr>
<th>Compound</th>
<th>HT-29 (paclitaxel)</th>
<th>A2780 (paclitaxel)</th>
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<tbody>
<tr>
<td>ARRY-520</td>
<td>10 mg/kg</td>
<td>40 mg/kg</td>
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<tr>
<td>Ispinesib</td>
<td>3 mg/kg</td>
<td>6 mg/kg</td>
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</tbody>
</table>

**Summary**

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