

Impact of AS1411 on Mechanistic Target of Rapamycin Complexes 1 and 2 (mTORC1/2) Activity

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Introduction and Background: AS1411 is a cancer-selective oligonucleotide that binds nucleolin, a multifunctional protein that drives both tumorigenesis and malignancy. Although many aspects of AS1411's activity are understood, the exact molecular mechanism is unknown. A link between AS1411 and mTOR was first discovered during its Phase II trial, when a patient with an mTOR-driven metastatic renal cancer had a durable complete response to AS1411 treatment. Other researchers have shown that AS1411 blocks nucleolin-mediated stabilization of mTOR mRNA in neurons in a subcellular localization-dependent manner, but whether this function exists in cancer is unknown. This led us to hypothesize that **AS1411 modulates mTOR signaling by a nucleolin-mediated mechanism.**

Specific Aims: We aim to determine the following: (1) if AS1411 affects mTORC1/2 activity, mTOR protein, or mRNA levels, and (2) if the biological effects of AS1411 depend on inhibition of mTORC1/2 signaling.

Methods: Effects on total mTOR protein, its post-translational modifications (PTMs), and downstream pathways were explored with time-course and dose-response western blots (WBs) in A549 non-small cell lung cancer cells and CHP-134 neuroblastoma cells, which respond to AS1411 with GI50 values of 1 and 4 μ M, respectively. Results were compared to C-rich oligo (CRO) and ATP-dependent mTOR inhibitor (Torin1) as negative and positive controls. WBs were probed for mTOR, p-mTOR s2448/s2481, pS6, pAKTs473 and GAPDH.

Results: In both cell lines, AS1411 inhibited mTORC1 and mTORC2 activity (i.e. pS6 and pAKTs473 levels) in a dose- and time- dependent manner. Interestingly, although Torin1 was quicker to inhibit mTORC1, it did not inhibit mTORC2 activity in any time point, whereas AS1411 treatment did. As expected, AS1411 does not appear to alter mTOR levels or its PTMs in whole cell extracts.

Conclusions and Future Directions: Our research has demonstrated that AS1411 can inhibit mTORC1/2 activity, although the mechanism of this effect is still unknown at present. We are currently assessing subcellular fractions for mTOR protein and mRNA to determine if AS1411's effect is specific to a particular cellular location. Understanding AS1411's mechanism of action and the pathways involved may elucidate additional roles for nucleolin in cancer biology and identify new regulatory aspects of mTOR signaling. Moreover, our studies may disclose clinical biomarkers to identify patients who would most benefit from AS1411 treatment.