ATL313, A Potent, and Selective A2A Agonist as a Novel Drug Candidate for the Treatment of Multiple Myeloma

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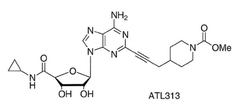
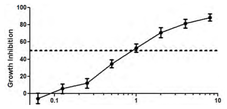
Abstract

Adenosine A2A receptor agonists, in combination with standard of care Multiple Myeloma (MM) therapeutics, demonstrate potent and highly synergistic anti-proliferative effects in B-cell malignancies. We have previously reported that A2A agonists show significant anti-tumor synergy with established MM drugs in several anti-tumor models including: tumor cell lines, in vivo xenograft models and in primary human MM tumor cells ex vivo. While these initial studies robustly demonstrate the proof of concept for this unexpected synergistic interaction, they were conducted with research compounds at doses and routes of administration resulting in exposure above therapeutically relevant levels. Here we describe the preclinical evaluation of ATL313, a potent and highly selective adenosine A2A receptor agonist that synergizes with established anti-MM agents resulting in enhanced efficacy in pre-clinical models of MM. ATL313 shows binding affinity for the human A2A receptor in the low single digit nM range and shows at least 80-fold selectivity for A2A compared to other adenosine receptor subtypes. As with other agonists examined, the activity of ATL313 is dependent on expression of the A2A receptor in the target cell and demonstrates single agent inhibition of proliferation in MM cell lines with an IC50 of 0.5-1 nM in the MM.1S cell line. ATL313 potently synergizes with glucocorticoids (dexamethasone and prednisolone), bortezomib, lenalidomide, melphalan and doxorubicin as well as emerging drug classes including HDAC inhibitors and HSP90 inhibitors. Substantial increases in inhibition of proliferation and cell killing and 2 to 100 fold potency shifts are observed with ATL313 combinations in MM cell lines including those both sensitive and resistant to current MM agents. In MM.1S cells, addition of 0.5 nM ATL313 to 100 nM Dexamethasone results in 95% inhibition of proliferation as compared to approximately 45% and 70% inhibition with either ATL313 or dexamethasone alone respectively. This combination results in a 30 fold shift in the dexamethasone IC50 and a combination index of 0.1 indicating high levels of synergy. Furthermore, the combination results in nearly complete cell killing as compared to reductions in cell number of 10% and 70% by ATL313 or dexamethasone respectively. Importantly, we have observed that A2A agonists are effective in combination in cells resistant to dexamethasone. Evaluation of A2A agonist combinations in a panel of 83 cell lines including solid tumor types and hematological malignancies demonstrates that synergy is highly selective for B-cell malignancies with little to no activity in solid tumor cell lines. We have now translated these in vitro results with ATL313 to a mouse xenograft model where the ATL313 is delivered using continuous infusion osmotic pumps. Doses of 10, 33 and 100 ng/kg/min were evaluated (in addition to QD subcutaneous bolus dosing at 0.1 mg/kg) and demonstrate synergistic anti-proliferative effects with no significant body weight loss. Mice bearing subcutaneous MM.1S tumors show a 43% regression in tumor volume after treatment with the combination of dexamethasone (1 mg/kg, s.c QD) and ATL313 (100 ng/kg/min) for 24 days as compared to a 500% or 700% increase after treatment with either ATL313 or dexamethasone, respectively. Furthermore, treatment with ATL313 in combination with dexamethasone confers a statistically significant survival advantage as compared to either agent alone. In summary, we report the preclinical evaluation of ATL313, a potent and highly druggable A2A agonist as a novel, selective and synergistic drug candidate for the treatment of MM. Our preclinical data provides compelling evidence in support of the further development of ATL313 for use in multi-drug combination therapy of MM.

A Potent, Selective Adenosine A2AR Agonist

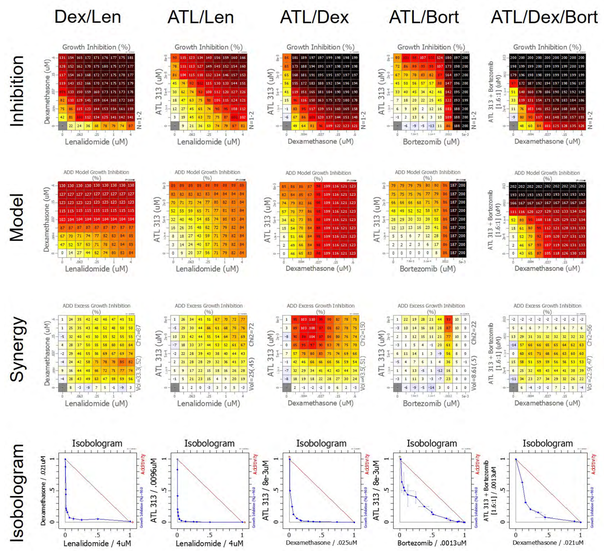


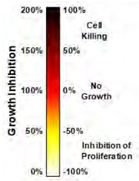


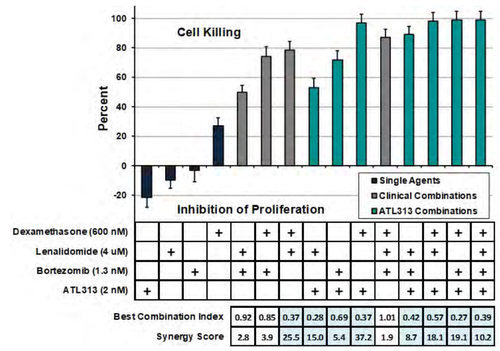
ATL313

- Interacts with the human A2A receptor with a binding potency of 1.9 nM and a functional potency of 0.9 nM and is approximately 30-fold selective for human A2A vs. A1 and 85-90-fold selective for human A2A vs. A2B or A3 receptors
- Inhibits growth of MM.1S cells with an IC50 of 1 nM
- Demonstrates favorable, drug-like properties in multiple tests including: genotoxicity, protein binding, metabolic stability and inhibition of receptors/enzymes, CYP450 and HERG

ATL313 Potently Synergizes with Myeloma Drugs in Pair-wise and Higher Order Combinations

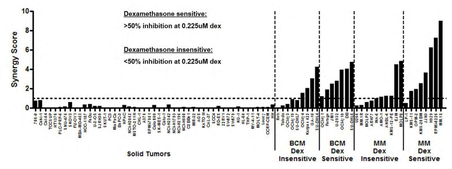


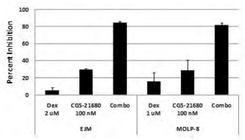




- ATL313 synergizes with lenalidomide, dexamethasone and bortezomib in 2-, 3- and 4-way combinations
- ATL313 synergies are equivalent to or greater than those with approved MM drugs
- ATL313 synergizes are evident at clinically relevant concentrations
- Synergies containing dexamethasone result in the greatest level of cell killing

A2A Agonist Synergies are Broadly Active, Highly Selective and Overcome Drug Resistance

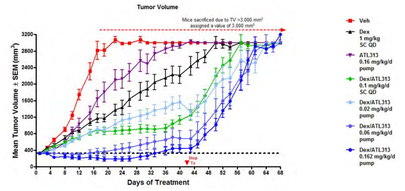


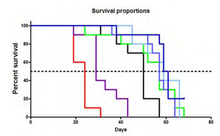


- A2A agonist synergies are highly selective for B-cell malignancies vs. solid tumors or normal cells
- A2A agonists synergize with dexamethasone and melphalan (not shown) in dexamethasone sensitive and resistant cell lines

In vitro study methods: Cell lines were seeded in 384 or 1536 well plates and treated with single agents and combinations in a dose matrix format. To assess synergy, each test point is compared to the expected combination effects assuming Loewe dose-additivity, derived from the single agent response curves. The synergy score sums up the excess inhibition over the model with weights to account for drug dilution factors, and favor synergy at high inhibition levels. Growth inhibition is calculated using treated and untreated values from a 72hr timepoint untreated values from a time 0 timepoint. Inhibition of proliferation is calculated from treated and untreated values from the 72 hr timepoint. Animal study methods: Mice were inoculated in both flanks with 1.5x107 cells in in matrigel. Once tumors were established animals were randomized, Alzet pumps were implanted subcutaneously in appropriate animal groups and QD SC dosing commenced. Tumor volume = (a2 x b/2) where 'a' was the smallest diameter and 'b' was the largest diameter. Animals were bearing tumors of 3,000 mm3 or greater were sacrificed. Growth curves were fit to the tumor volume versus time data for the treatment period by least squared nonlinear regression. Growth rate parameters were compared using F-tests. Survival data was analyzed using a Kaplan-Meier Survival analysis. For all statistical analyses, P values less than 0.05 were considered significant.

ATL313 Synergizes with Dexamethasone in vivo





Significance vs. Dexamethasone Alone		
Group	Tumor Growth	Survival
Dex + ATL313 SC	<0.0001	0.0528
Dex + ATL313 20 ng/kg/d pump	0.4352	0.066
Dex + ATL313 60 g/kg/d pump	0.1589	0.071
Dex + ATL313 160 ng/kg/d pump	0.0341	.0003

- ATL313 has only modest anti-tumor activity as a single agent
- ATL313 significantly enhances the anti-tumor effect and survival benefit of dexamethasone
- Continuous exposure to low levels of ATL313 is sufficient for efficacy

Summary

- ATL313 is a potent, selective and drug-like adenosine A2A receptor agonist
- ATL313 demonstrates high synergy in 2-, 3- and 4-way combinations with lenalidomide, dexamethasone and bortezomib
- A2A agonist combinations are broadly active in dexamethasone sensitive and resistant cell lines and are highly selective for tumor vs. normal cells
- ATL313 combination effects translate into xenograft models of multiple myeloma at therapeutically relevant doses
- These results provide compelling evidence in support of the further development of ATL313 for use in multi-drug combination therapy of MM

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