Preclinical pharmacodynamics and antitumor activity of AZD4635, a novel adenosine 2A receptor inhibitor that reverses adenosine mediated T cell suppression

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Abstract

Accumulation of extracellular adenosine within the microenvironment is a strategy exploited by tumors to escape immune surveillance. Adenosine signaling through the high affinity adenosine 2A receptor (A2AR) on immune cells elicits a range of immunosuppressive effects which can promote tumor growth and limit the efficacy of immune checkpoint inhibitors. Here we describe the preclinical pharmacology of AZD4635 (HTL-1071), an oral A2AR antagonist which binds to human A2AR with a K_i of 1.7 nM. In ex vivo T cell assays, AZD4635 reversed adenosine mediated suppression and restored IFNγ secretion in cells incubated with 5’-N-ethylcarboxamidoadenosine (NECA), a stable analog of adenosine. The therapeutic benefit of A2AR blockade was evaluated in the MC38 syngeneic mouse tumor model. Inhibition of A2AR signaling led to a reduction in tumor growth alone and in combination with anti-PD-L1 Ab. AZD4635 treated tumors had increased expression of genes associated with immune activation and increased expression of co-stimulatory molecules on antigen presenting cells (APCs). AZD4635 is currently in a Phase 1 clinical trial as a single agent and in combination with durvalumab (anti-PD-L1 Ab) in patients with solid malignancies (NCT02740985).

Introduction

• Accumulation of extracellular adenosine within the microenvironment is a strategy exploited by tumors to escape immune surveillance.
• Adenosine signaling through the high affinity adenosine 2A receptor (A2AR) on immune cells elicits a range of immunosuppressive effects
• Blockade of the A2AR receptor can reverse adenosine mediated immune suppression to enhance anti-tumor immunity

Results

AZD4635 (HTL-1071), is a potent A2AR antagonist. AZD4635 binds to human A2AR with a K_i of 1.7 nM and with >30-fold selectivity over other adenosine receptors as measured by radioligand receptor occupancy assay.

Adenosine levels are high and spatially heterogeneous in mouse syngeneic tumors. Accumulation of intratumoral adenosine in syngeneic tumors, measured by desorption electrospray ionisation - mass spectrometry (DESI-MS), demonstrated that adenosine levels are high and spatially heterogeneous. Scale bar: arbitrary abundance threshold at 50%.

AZD4635 enhances the anti-tumor activity of anti-PD-L1 Ab in established MC38 syngeneic tumors. The therapeutic benefit of A2AR blockade was evaluated in the established MC38 syngeneic colorectal cancer model. Inhibition of A2AR signaling by AZD4635 led to a reduction in tumor growth alone and in combination with anti-PD-L1 (error bars represent SEM, ***p<0.001).

AZD4635 is active against human and mouse A2AR. CHO cells stably expressing human or mouse A2AR were incubated with adenosine in the presence of AZD4635. AZD4635 is capable of inhibiting adenosine mediated cAMP accumulation in both human and mouse A2AR expressing cells (error bars represent SD).

AZD4635 reverses adenosine mediated T cell suppression. AZD4635 (10 µM) restores IFNγ secretion in murine T cells incubated with NECA, a stable analog of adenosine in an ex vivo IFNγ ELISA. (Error bars represent SD, **p<0.01, ***p<0.001)

AZD4635 increases expression of genes associated with immune activation. MC38 tumors were treated for 14 days with AZD4635 and changes in surface protein expression were assessed by flow cytometry. AZD4635 increased expression of co-stimulatory markers (CD86) and markers of antigen presentation (MHCII and a co-stimulatory signal) on antigen presenting cells (APCs).

AZD4635 is currently in a Phase 1 clinical trial as a single agent and in combination with durvalumab (anti-PD-L1 Ab) in patients with solid malignancies (NCT02740985).

Conclusions

• AZD4635 (HTL-1071) is an oral A2AR antagonist which binds to human A2AR with a K_i of 1.7 nM and reverses adenosine mediated T cell suppression
• Treatment with AZD4635 leads to a reduction in tumor growth alone and in combination with anti-PD-L1 Ab. Increased expression of genes associated with immune activation and increased expression of co-stimulatory markers on APCs
• AZD4635 is currently in a Phase 1 clinical trial as a single agent and in combination with durvalumab (anti-PD-L1 Ab) in patients with solid malignancies (NCT02740985).