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

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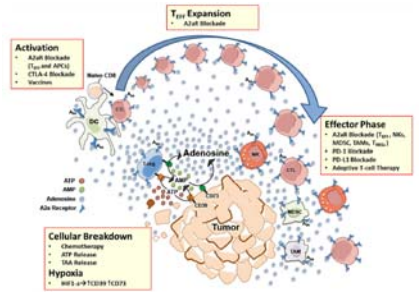
Abstract

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 ($A_{2A}R$) 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 $A_{2A}R$ antagonist which binds to human $A_{2A}R$ 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 $A_{2A}R$ blockade was evaluated in the MC38 syngeneic mouse tumor model. Inhibition of $A_{2A}R$ 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 ($A_{2A}R$) on immune cells elicits a range of immunosuppressive effects
- Blockade of the A_{2A} receptor can reverse adenosine mediated immune suppression to enhance anti-tumor immunity



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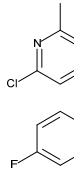
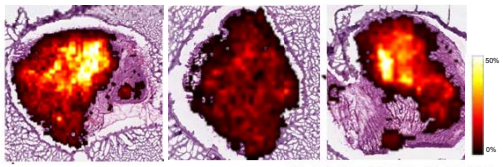
Adenosine elicits a range of immune suppressive effects within the tumor microenvironment

Results

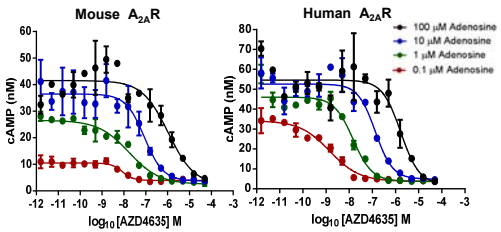


Adenosine Receptor	Binding Affinity (nM)	
	Adenosine	AZD4635
A_1R	100	160
$A_{2A}R$	310	1.7
$A_{2B}R$	15,000	64
A_3R	290	>10,000

AZD4635 (HTL-1071), is a potent $A_{2A}R$ antagonist. AZD4635 binds to human $A_{2A}R$ with a Ki of 1.7 nM and with > 30-fold selectivity over other adenosine receptors as measured by radioligand receptor occupancy assay

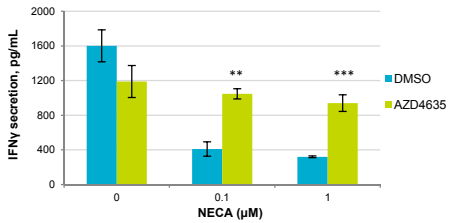


Adenosine levels are high and spatially heterogeneous in mouse syngeneic tumors. Accumulation of intratumoral adenosine in syngenic tumors, measured by desorption electrospray ionisation - mass spectroscopy (DESI-MS), demonstrated that adenosine levels are high and spatially heterogeneous. Scale bar: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

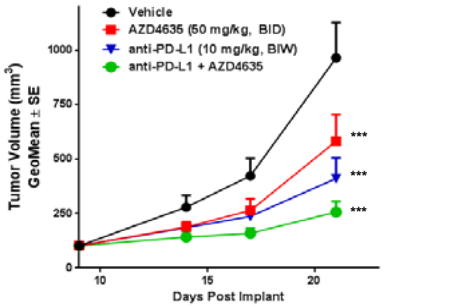


Adenosine Concentration (µM)	AZD4635 IC_{50} (nM)	
	Mouse $A_{2A}R$	Human $A_{2A}R$
10	96.7 ± 12.8	142.9 ± 11.7
1	19.6 ± 3.7	10.0 ± 5.6
0.1	9.7 ± 1.7	0.79 ± 0.67

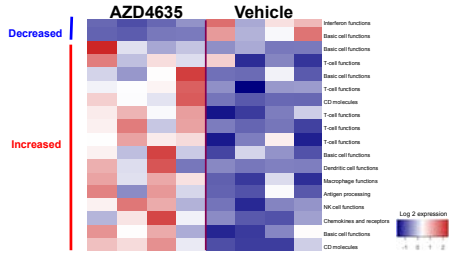
AZD4635 is active against human and mouse $A_{2A}R$. CHO cells stably expressing human or mouse $A_{2A}R$ were incubated with adenosine in the presence of AZD4635. AZD4635 is capable of inhibiting adenosine mediated cAMP accumulation in both human and mouse A2aR expressing cells (error bars represent SD)



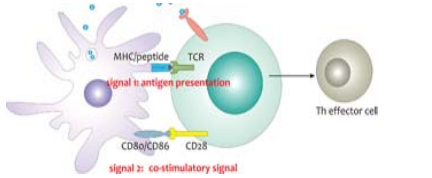
AZD4635 reverses adenosine mediated T cell suppression. AZD4635 (10 µM) restores IFNγ secretion in murine T cells incubated with NECA, a stable analog of adenosine in an *ex vivo* IFNγ ELISA, (Error bars represent SD, **p<0.01, *** p<0.001)



AZD4635 enhances the anti-tumor activity of anti-PD-L1 Ab in established MC38 syngeneic tumors. The therapeutic benefit of $A_{2A}R$ blockade was evaluated in the established MC38 syngeneic colorectal cancer model. Inhibition of $A_{2A}R$ 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)

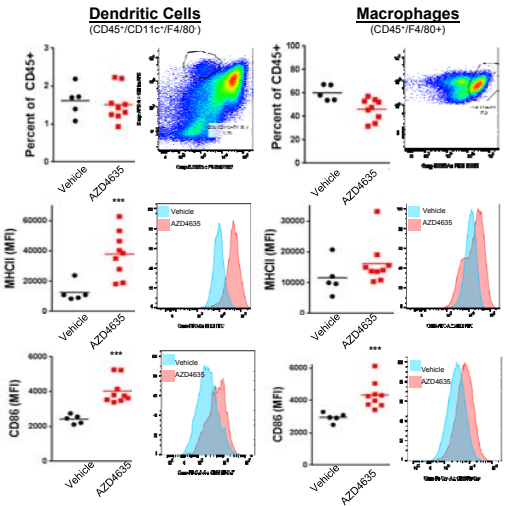


AZD4635 increases expression of genes associated with immune activation. MC38 tumors were treated for 14 days with AZD4635 and gene expression changes were evaluated by the NanoString Mouse PanCancer Immune Profiling Panel. Unsupervised clustering of gene expression changes revealed upregulation of antigen processing and T-cell function. Genes filtered for significance (p≤0.05) and magnitude (fold change>2)



De Koker S. *et al. (2011) Chem. Soc. Rev.* 40, 320-339

Efficient antigen presentation by APCs requires expression of MHCII and a co-stimulatory signal



AZD4635 increases expression of co-stimulatory markers on APCs. 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) on dendritic cells and macrophages, but did not increase total population frequencies in MC38 tumors (*** p<0.001)

Conclusions

- AZD4635 (HTL-1071) is an oral $A_{2A}R$ antagonist which binds to human $A_{2A}R$ 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)

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