The A2AR antagonist AZD4635 prevents adenosine-mediated immunosuppression of CD103+ dendritic cells

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Abstract LB-192

Introduction

Adenosine signaling is a normal physiologic process preventing autoimmunity, that is co-opted by tumors as an immune escape mechanism. AZD4635 (HTL-1071) is a selective oral $A_{2A}R$ antagonist, currently in clinical trials as a single agent and in combination with durvalumab (anti-PD-L1 Ab) in patients with solid malignancies. The role of antigen presentation by dendritic cells (DC), in particular CD103+ DCs, is critical to drive anti-tumor immunity, and is impaired under conditions of high extracellular adenosine. Previously we demonstrated enhanced T cell function contributing to tumor efficacy in the syngeneic model MC38 with AZD4635 alone and in combination with anti-PD-L1. Using mouse OVA models, we now demonstrate that AZD4635 monotherapy, and when combined with anti-PD-L1, could significantly prevent adenosine (NECA)-mediated immunosuppression in mouse CD103+ cross-presenting DC, leading to improved OVA-specific T cell function, and contributing to tumor efficacy. AZD4635 also blocked NECA- induced immuno-suppression in the human HLA-A2+ Melan-A model system, resulting in antigen-specific priming of naïve CD8+ T cells. Thus, we demonstrate that AZD4635's MOA includes restoration of DC function, augmenting the elicitation of antigen-specific T cell responses.

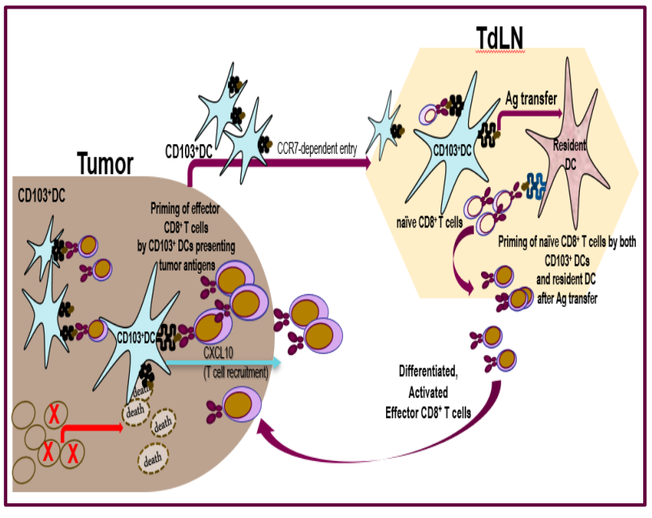

Figure 1. CD103+ DC Antigen Cross-presentation to T cells: CD103+ DC traffic tumor antigen from tumors into TdLN where they directly prime naïve T cells or transfer these antigens to other tissue-resident DC subsets to elicit CD8+ T cell cross-priming and activation.

Methods

OVA Antigen Presentation *in vitro*: AZD4635 (3μM) was tested for effects in reversing DC immunosuppression induced by 5μM 5'-N-ethylcarboxamidoadenosine (NECA), a stable adenosine analog, in mouse CD103+ DC cultures [1]. Antigen presentation of bound K^b-SIINFEKL complexes was measured by flow cytometry with antibody clone # 25-D1.16.

Human Melan-A Tumor Antigen-specific Assays: AZD4635 (3 μM) was tested *in vitro* in reversing NECA (5 μM)-induced DC dysfunction of Mo-DC to prime Melan-A antigen-specific (ELAGIGILTV) T cells from autologous naïve HLA-A2+ CD8+ T cells [2].

***Ex-vivo* CD103+ DC Antigen Presentation:** CD103+ DC were flow sorted from TdLN of mice bearing MC38-OVA tumors from AZD4635 tumor efficacy studies. Sorted cells were co-cultured with OT-I CD8+ T cells with proliferation measured 4 days later.

***In vivo* Tumor Efficacy Studies:** Treatments of MC38-OVA tumor-bearing mice started once tumors reached ~100mm3. Mice (n=10) were treated for 14 days: Vehicle, AZD4635 50 mg/kg BID, anti-PD-L1 10 mg/kg IP 2x/wk.

Results

AZD4635 Rescues Adenosine-mediated Inhibition of CD103+ DC Generation

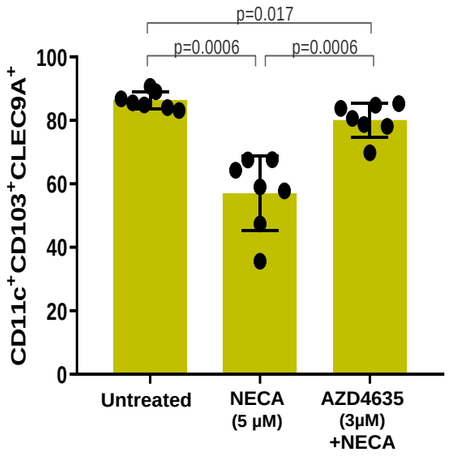

Figure 2. NECA significantly reduced the generation of CD103+ DC in mouse BM cultures with FLT3L + GM-CSF. AZD4635 treatment restored CD103+ DC differentiation to nearly normal levels.

Results

AZD4635 Reduces Adenosine-mediated Suppression of Antigen Presentation

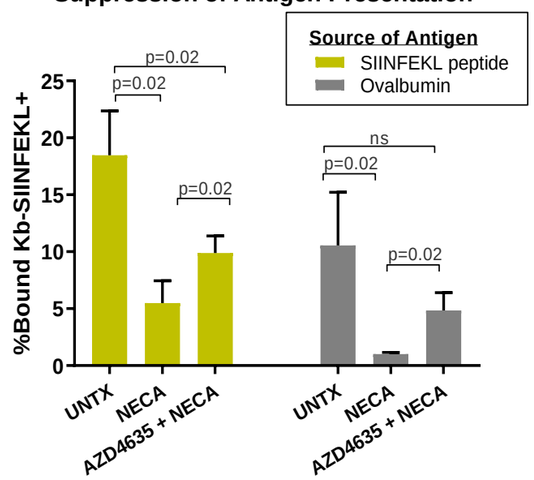

Figure 3. Quantification of K^b-bound SIINFEKL peptide complexes (antigen presentation) on CD103+ DC.

AZD4635 Improves Antigen T cell Responses by Correcting Adenosine Defects in DC

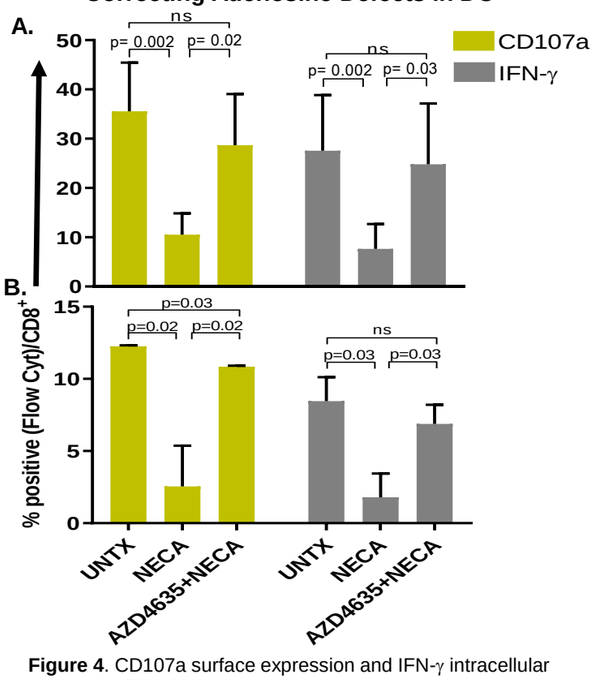

Figure 4. CD107a surface expression and IFN-γ intracellular staining of OT-I CD8+ T cells following overnight co-culture with CD103+ DC incubated with **A.** SIINFEKL peptide, **B.** Killed MC38-Ova cells

AZD4635 Enhances CD103+ DC Cross-priming and combines with anti-PD-L1 Contributing to Tumor Efficacy

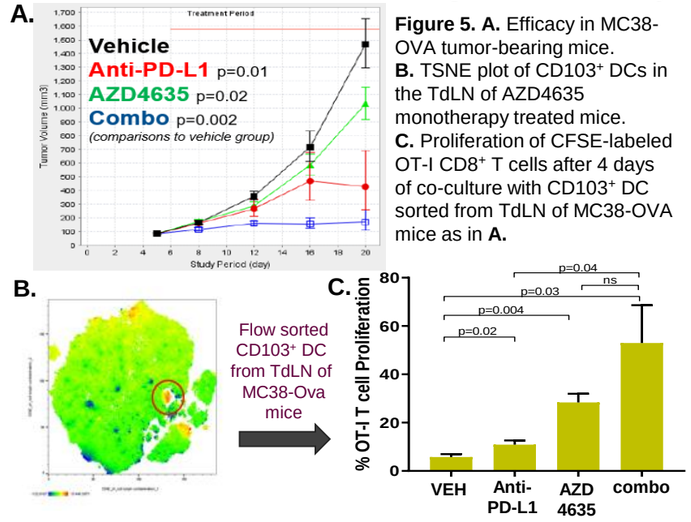

Figure 5. A. Efficacy in MC38-OVA tumor-bearing mice. **B.** TSNE plot of CD103+ DCs in the TdLN of AZD4635 monotherapy treated mice. **C.** Proliferation of CFSE-labeled OT-I CD8+ T cells after 4 days of co-culture with CD103+ DC sorted from TdLN of MC38-OVA mice as in **A.**

AZD4635 Reverses Tolerogenic Human DCs and Promotes T cell Priming to Tumor-associated Antigens *in vitro*

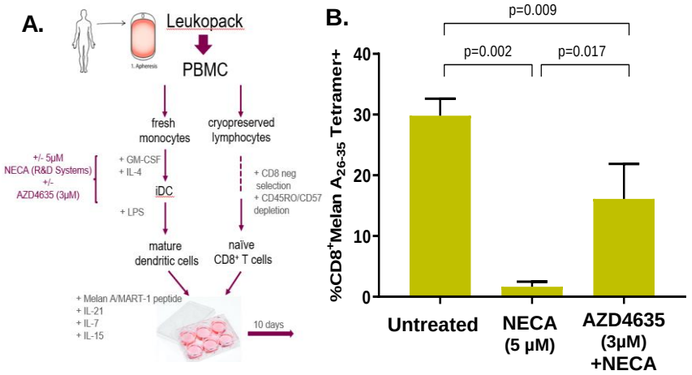

Figure 6. **A**. Experimental design of DC and Melan-A tetramer+ T cells generation. **B**. Flow cytometry detection of Melan-A- specific T cells from 3 HLA-A2+ healthy donors using the protocol shown in **A.**

Conclusions

- Adenosine receptor signaling antagonism by AZD4635 improved differentiation and antigen presentation by DCs, including CD103+ crosspresenting/crosspriming DCs, leading to better priming, expansion and function of antigen-specific T cells.
- AZD4635's MOA includes restoration of DC function, supporting its anti-tumor activity.

References

1. Mayer, C.T. *et al*., *Blood*, 2014, 124:3081-3091.
2. Wölfl, M and Greenberg, P.D., *Nature Protocols*, 2014, (9):4: 950-966.

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