The Anti-Tumor Efficacy of Immunogenic Chemotherapy is Enhanced by the Dual $A_{2a}R/A_{2b}R$ Antagonist Etrumadenant, Relieving the Necessity for an Extended Chemotherapy Regimen

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Abstract 801

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INTRODUCTION

- High levels of extracellular adenosine generated in the tumor microenvironment (TME) engage A_{2a} and A_{2b} adenosine receptors on immune cells, resulting in immunosuppression (Figure 1).
- Chemotherapy releases adenosine triphosphate (ATP) into the tumor microenvironment, where it is rapidly converted into adenosine, primarily by the ectoenzymes CD39 and CD73.
- Etrumadenant (etruma) is a selective, small-molecule, dual $A_{2a}R/A_{2b}R$ antagonist. It was specifically designed to potently block adenosine-induced immunosuppression in the TME. We have previously shown that etrumadenant blocks the immunosuppressive effects of adenosine in immune cells and enhances anti-tumor immune responses in mouse syngeneic tumors.
- Herein, we describe the capacity for etruma to drive enhanced tumor control and immune activity in mouse tumor models using immunogenic chemotherapeutic agents with different chemotherapy doses or with reduced dosing regimens.
- Etruma is currently being studied in several Ph2 studies in combination with chemotherapy or other immune-enhancing regimens: NCT04381832, NCT04660812, NCT04262856.

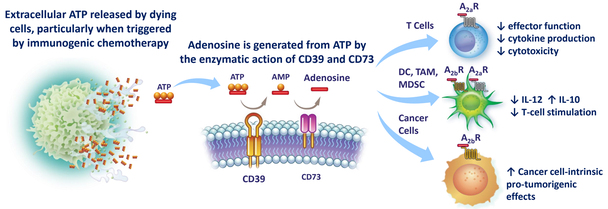

Figure 1. Diagram of adenosine production from ATP released into the TME by immunogenic chemotherapy. Hydrolysis of ATP by the ectoenzymes CD39 and CD73 produces adenosine, which exerts immunosuppressive effects by binding to adenosine receptors expressed on immune cells and may promote cell growth via-$A_{2b}R$-mediated signaling on cancer cells.

METHODS

- **Isolated immune cell experiments:** $CD8^+$ T cells and dendritic cells (DC) were isolated from healthy human blood by negative selection. DC were matured with LPS/IFN-γ for 24 hours in the presence of NECA (synthetic adenosine receptor agonist) +/- antagonists.
- **CREB phosphorylation assay:** CREB phosphorylation (pCREB) in $CD8^+$ T cells was determined by phospho-flow cytometry using mouse whole blood stimulated with NECA (5 μM) for 20 min at 37°C *ex* vivo. The dose of etruma used in anti-tumor efficacy studies was selected based upon full inhibition of adenosine receptor-mediated pCREB between dose intervals (100 mg/kg, BID)
- **Mouse *in vivo* studies:** C57BL/6 female mice (6-8 weeks old) were injected subcutaneously on the right flank with B16F10 or AT3-OVA cells. 4T1 cells were implanted in the mammary fat pad of 6-8 week old female Balb/c mice. Etruma was administered at 100 mg/kg BID or 200 mg/kg QD at the initiation of the chemotherapy regimen.

Table 1: Potency and Selectivity of Adenosine Receptor Antagonists *

Potency (nM)	Etrumadenant (etruma)	$A_{2a}R$ antagonist[a]	$A_{2a}R$ antagonist[b]
$A_{2a}R$	1.4	1.5	0.2
$A_{2b}R$	2.0	123	141

[a] Proprietary $A_{2a}R$-selective adenosine receptor antagonist
[b] Compound 35 from patent application WO2018178338 (iTeos Therapeutics)
* Potency data generated by measuring cAMP levels in CHO cells stably expressing $A_{2a}R$ or $A_{2b}R$ following stimulation with NECA

RESULTS

Adenosine Signaling Drives Suppression Through $A_{2a}R$ and $A_{2b}R$ in Immune Cells Which is Reversed by Etrumadenant

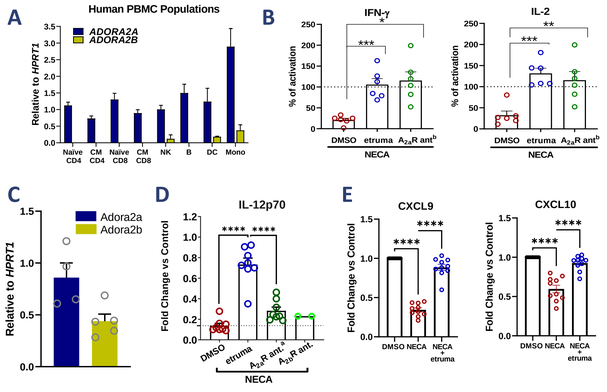

Figure 2. **(A)** Adenosine receptor gene expression of sorted human immune cells from healthy donors. **(B)** IFN-γ and IL-2 production from primary human $CD8^+$ T cells activated for 72 h in the presence of NECA (5 μM) and adenosine receptor antagonists (1 μM). **These results demonstrate that 1 μM of the adenosine receptor antagonists used in this experiment are capable of fully suppressing adenosine-mediated suppression driven by 5 μM NECA. (C)** Real-time PCR for adenosine receptors ADORA2A and ADORA2B expression in DCs enriched from healthy human blood shows near equal expression of each receptor. **(D)** IL-12p70 and **(E)** CXCL9 and CXCL10 suppression by NECA (5 μM) in DCs was rescued by etrumadenant (1 μM) after 24 hours in culture whereas blockade of $A_{2a}R$ or $A_{2b}R$ alone was less effective. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. $A_{2b}R$ antagonist: GS-6201 purchased from TOCRIS. See Table 1 for details on $A_{2a}R$ ant a and b.

$A_{2b}R$ is Highly Expressed on Cancer Cell Lines and Drives Gene Expression Changes Which are Blocked by Etrumadenant

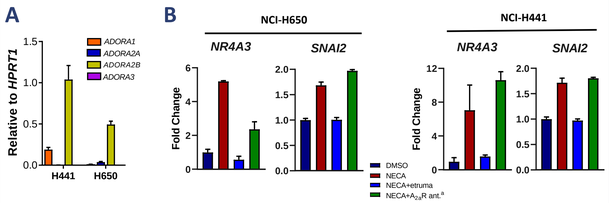

Figure 3. **(A)** Real-time PCR for adenosine receptor expression from NSCLC cell lines showing high expression of $A_{2b}R$ and substantially lower levels of expression for all other adenosine receptors. **(B)** Real-time PCR from NSCLC cell lines that were stimulated with NECA (5 μM) in the presence of dual vs $A_{2a}R$-selective antagonists (1 μM, see table 1 for details) showing that etruma is capable of blocking NECA-stimulated gene expression changes driven by $A_{2b}R$.

Etrumadenant Inhibits Peripheral CREB Phosphorylation and Suppresses B16F10 Tumor Growth

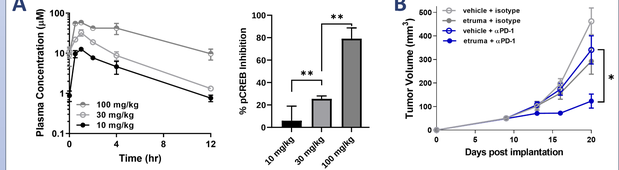

Figure 4. **(A)** Pharmacokinetic profile of etrumadenant administered orally to C57Bl/6 mice (left panel). NECA was utilized to stimulate pCREB on T cells in whole blood from mice 12 hours after etrumadenant dosing (right panel). **(B)** Mice bearing B16F10 tumors were treated with α-PD-1 antibodies (Q3Dx4) beginning at a tumor volume of 50 mm^3 concurrently with etrumadenant (100 mg/kg PO, BID) or vehicle control. *p<0.05, **p<0.01.

Etrumadenant Combines with Platinum-Based Chemotherapy to Enhance AT3-OVA Tumor Control and Immune Infiltration

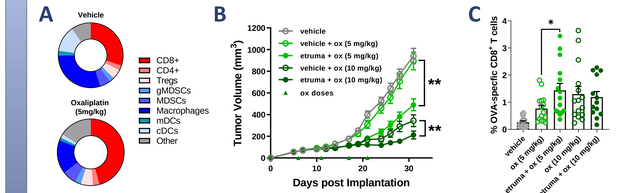

Figure 5. **(A)** Profiling of tumor-infiltrating immune cells in the AT3-OVA tumor in vehicle vs oxaliplatin-treated tumors. **(B)** Tumor volume from AT3-OVA model. Dosing started at 50 mm^3, etrumadenant (100 mg/kg, PO, BID) and oxaliplatin dosing Q4Dx4, as indicated. **(C)** Flow cytometric analysis of OVA-specific tumor infiltrating $CD8^+$ T cells displayed as % of total CD45+ cells. *p<0.05, **p<0.01.

Etrumadenant in Combination with Doxorubicin Reduces 4T1 Syngeneic Tumor Growth and Lung Metastases

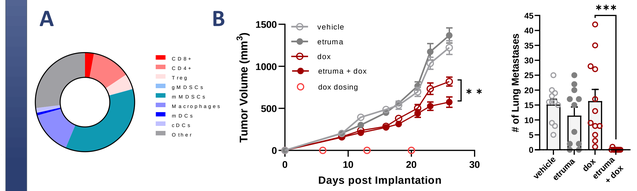

Figure 6. **(A)** Profiling of tumor-infiltrating immune cell shows that myeloid cells comprise a large portion of the immune cell infiltrate in 4T1 tumors. **(B)** Tumor volume from 4T1 model. Dosing started at 50 mm^3, etrumadenant (100 mg/kg, PO, BID) and doxorubicin (6 mg/kg) dosing as indicated. Number of lung metastases were quantified at the end of the study (right panel). **p<0.01, ***p<0.001.

Etrumadenant Combines with Doxorubicin Chemotherapy to Enhance AT3-OVA Tumor Control and Immune Infiltration

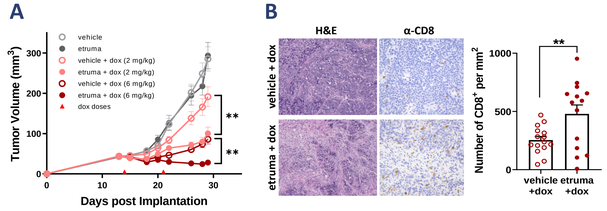

Figure 7. **(A)** Tumor volume from AT3-OVA model. Dosing started at 50 mm^3 with etrumadenant (100 mg/kg, PO, BID) and doxorubicin (administered Q4Dx2) initiated on the same day. Tumor growth was significantly reduced in the etruma + dox group at both doses compared to vehicle + dox. **(B)** Tumors from the 6 mg/kg doxorubicin treated group were taken for histologic analysis. H&E staining (images on the left) showed an enhanced stromal compartment in the etruma + dox group versus dox alone, suggesting an enhanced immune infiltrate. $CD8^+$ cells were identified by chromogenic immunohistochemistry (images on the right) and quantified (graph) demonstrating that the combination of etruma and dox led to a significant enhancement of infiltrating $CD8^+$ cells. **p<0.01.

Etrumadenant in Combination with Doxorubicin Provides Similar Tumor Control as an Extended Course of Chemotherapy

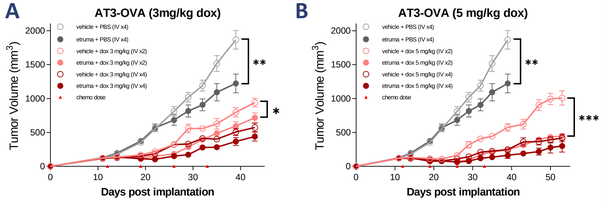

Figure 8. **(A)** AT3-OVA tumors were administered doxorubicin (3 mg/kg) as a 2 or 4 dose regimen in combination with etrumadenant (200 mg/kg, PO, QD) starting at 100 mm^3, as indicated. A significant reduction in tumor volume was observed with etrumadenant as a monotherapy and a small but significant reduction was observed in the etruma + dox group vs vehicle + dox in the abbreviated chemotherapy group. **(B)** A substantially larger reduction in tumor growth was observed in the etruma + dox group vs vehicle + dox in the abbreviated chemotherapy group at the higher (5 mg/kg) dose of doxorubicin. These data suggest that the greatest therapeutic benefit of doxorubicin, when combined with etruma, may result from the initial, priming, doses of chemotherapy. . *p<0.05, **p<0.01, ***p<0.001.

CONCLUSIONS

- These studies demonstrate the ability of etrumadenant to block adenosine-driven immunosuppression and to combine with chemotherapeutic agents in preclinical models.
- Furthermore, our studies suggest that combination of etrumadenant with a truncated course of immunogenic chemotherapy (either fewer cycles or a reduced dose level) may be sufficient to enhance immune activation and yield similar anti-tumor effects as a full course of chemotherapy.