M1069 as Dual A_{2A}/ A_{2B} Adenosine Receptor Antagonist Counteracts Immune-Suppressive Mechanisms of Adenosine and Reduces Tumor Growth In Vivo

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CONCLUSIONS

- M1069 is a potent and selective, dual A_{2A}/A_{2B} adenosine receptor antagonist, which is expected to counteract immune-suppressive mechanisms in the presence of high concentrations of adenosine and enhance the antitumor activity of chemotherapies.
- In adenosine rich settings, M1069 demonstrates potent (superior compared to A_{2A}-selective antagonist):
  - Suppression of VEGF production from murine and human myeloid cells;
  - M1069 reverses differentiation of murine bone-marrow-derived dendritic cells (BMDCs) in vitro toward the cells with stronger antigen-presenting anti-tumorigenic properties;
  - Antitumor activity in murine $CD73^{hi}$ 4T1 mammary tumor model as monotherapy or in combination with chemotherapies.

INTRODUCTION

M1069 is a selective dual antagonist of A_{2A} and A_{2B} adenosine receptors that is being developed to counteract the immunosuppressive effects of adenosine signaling and stimulate antitumor immune responses in patients with advanced malignancies. While the A_{2A} adenosine receptor was considered the major contributor to adenosine-mediated tumor immunosuppression, A_{2B} has recently emerged as another potential therapeutic target. M1069 has demonstrated preclinical antitumor activity superior to A_{2A}-selective antagonists in adenosine-rich settings in vitro and in vivo, both as a monotherapy and in combination with chemotherapeutic agents cisplatin and paclitaxel in the 4T1 tumor model. The preclinical data are consistent with a primary mechanism of action of M1069 that involves the reversal of immune suppression via dual antagonism of pro-tumorigenic A_{2A} and A_{2B} adenosine receptors in adenosine rich TME, where A_{2B} acts complementary or compensatory to A_{2A}. M1069 is currently being evaluated as monotherapy in a Phase I, first-in-human clinical trial in patients with advanced solid tumors (NCT05198349).

STRUCTURE and BINDING

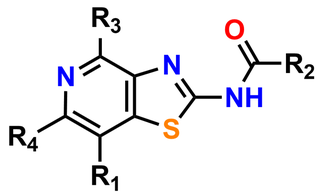


Figure 1. General Structural Class of M1069 Drug Substance

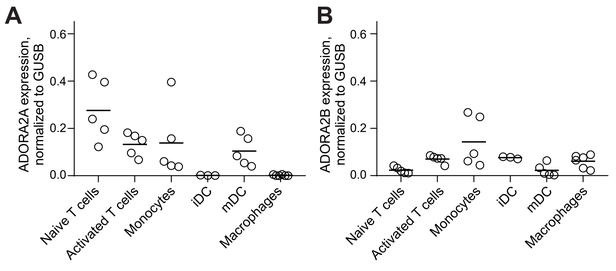
Table 1. Potency and Selectivity of M1069 on Human Adenosine Receptors

Parameter	Human A_{2A}	Human A_{2B}
Potency (cAMP, IC_{50}) in A_{2A}- or A_{2B} - over expressing HEK293 cells	0.130 nM	9.03 nM
Potency in human T cells (cAMP, IC_{50})	0.22 nM	
Potency in human whole blood at 10 µM NECA (pCREB, IC_{50})	119.5 nM	
Selectivity vs A_1	> 10,000-fold	153-fold
Selectivity vs A_3	> 10,000-fold	> 1,000-fold

cAMP: cyclic adenosine monophosphate; NECA: 5′-N-ethylcarboxamide adenosine; pCREB: phosphorylated cAMP response element binding protein

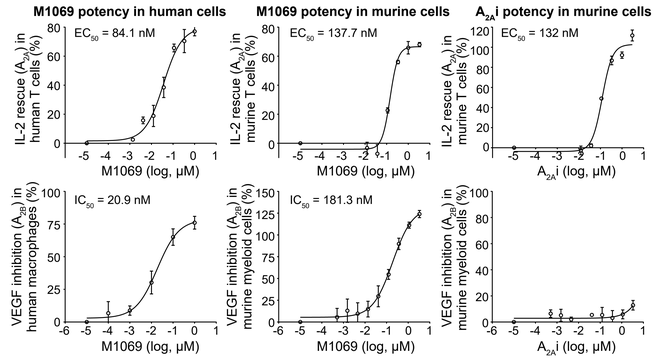
RESULTS

Figure 2. A_{2A} and A_{2B} Adenosine Receptor Expression on Primary Human Immune Cells Depends on Their Activation Status



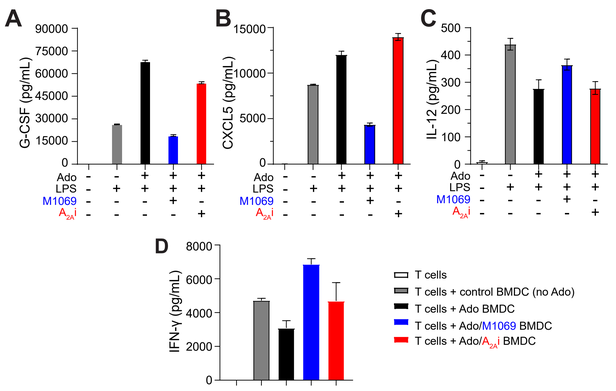
mRNA expression levels of (**A**) A_{2A} and (**B**) A_{2B} in various subsets of immune cells (naïve or activated $CD3^+$ T cells, $CD14^+$ monocytes, $CD14^+$ monocyte-derived macrophages, $CD14^+$ monocyte-derived immature DCs (iDCs) or maturated (mDCs) by subsequent incubation of iDCs with LPS. Adenosine receptor copy numbers were normalized to GUSB copy numbers. Data from 3 (iDCs) and 5 (all other immune cell subsets) donors.

Figure 3. Rescue of IL-2 Secretion from T Cells (A_{2A}) and Inhibition of VEGF Production from Myeloid Cells (A_{2B}) by M1069 or Reference A_{2A} Selective Antagonist (A_{2A}i) at 10 µM NECA



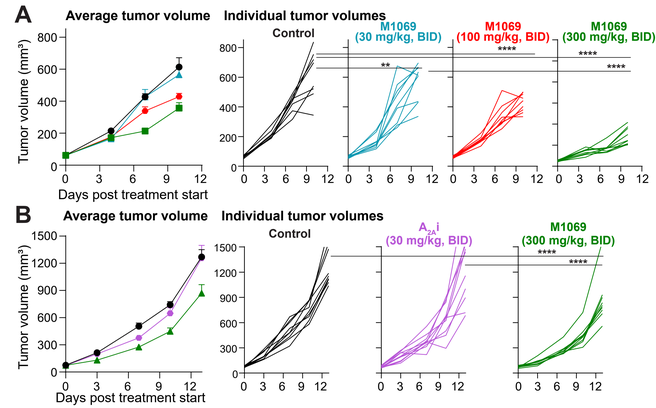
M1069 rescues IL-2 secretion (A_{2A}) by both human and murine T cells and decreases VEGF production (A_{2B}) from human and murine myeloid cells in the presence of 10 µM NECA in vitro, while A_{2A}i rescues IL-2 production, but does not decrease VEGF production, in murine immune cells. Rescue of IL-2 secretion or inhibition of VEGF was assessed by ELISA in cell culture supernatants collected after 48 or 24 hours of treatment, respectively.

Figure 4. M1069 Reverses Differentiation of Murine BMDCs Toward the Cells with Anti-Tumorigenic Properties in the Presence of High Adenosine (10 µM)



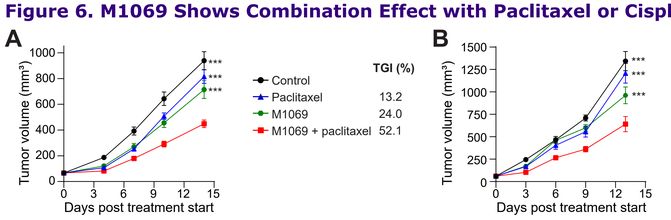
BMDCs were differentiated from murine bone marrow cells with or without a mix of adenosine and adenosine deaminase inhibitor EHNA (each at 10 µM final concentration) and M1069 or A_{2A}i, throughout the differentiation protocol. (**A-C**) Cytokine secretion from BMDCs in cell supernatants collected after the last 24 hour maturation of BMDCs with LPS. (**D**) IFN-γ production induced by murine T cells after 3-days of coculture with adenosine-differentiated murine BMDCs treated with either M1069 or A_{2A}i in allogenic one-way MLR assay.

Figure 5. M1069 Tumor Growth Inhibition is Superior to an A_{2A} Selective Antagonist (A_{2A}i) in the Murine $CD73^{hi}$ 4T1 Tumor Model



Female BALB/c mice were inoculated orthotopically with $CD73^{hi}$ 4T1 mammary tumor cells and once the average tumor volume reached ~60-80 mm^3, mice were randomized into the groups and treated BID with (**A**) vehicle or different doses of M1069 throughout the study or (**B**) with vehicle, A_{2A}i or M1069. The doses for M1069 and A_{2A}i were adjusted based on the PK/PD (pCREB inhibiton) results. Average or individual tumor volumes with SEM are presented. Asterisks denote a significant difference between the groups.

Figure 6. M1069 Shows Combination Effect with Paclitaxel or Cisplatin in the $CD73^{hi}$ Murine 4T1 Mammary Tumor Model



BALB/c mice were inoculated orthotopically with CD73hi 4T1 tumor cells and once the average tumor volume reached ~60-80 mm^3, mice were randomized into the groups and treated with vehicle, M1069 alone, or in combination with either (A) Paclitaxel (45 mg/kg, Day 0), (B) Cisplatin (5 mg/kg, Day 0, 7). Average tumor volumes with SEM are presented. Asterisks denote a significant difference relative to combination therapy.

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