Forodesine has anticancer immunomodulatory properties through the adenosine pathway

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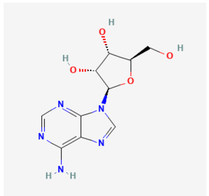
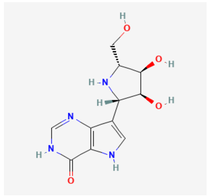
Introduction



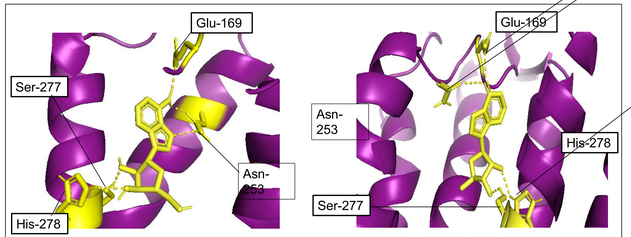
Adenosine



Forodesine

Background. Adenosine is overproduced in the tumor microenvironment, where it restricts protective anticancer immune responses. Previous work from our lab showed that adenosine-mediated activation of the Adenosine A2A Receptor (A2AR) causes suppression of CD8 T-cells effector functions in tumor and infection models (1,2). Importantly, our group showed that adenosine analogs could restore T-cell responses through blocking A2AR in tumor and infection models (1,2). Nucleoside analogs are a class of drugs which resemble the structure of adenosine and other nucleosides. Given the similarities between the analogs and adenosine, we **hypothesize** that these compounds may have immunomodulatory properties through interaction with A2AR. **Methods.** To address this hypothesis, we used a computational drug design approach (1) to model the interaction of adenosine analogs with A2AR, functional assays in A2AR-expressing cell lines and primary T-cells, mouse models of immunosuppression and cancer models

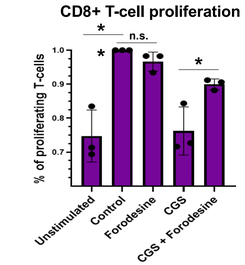
Molecular Docking of forodesine predicts binding to A2AR

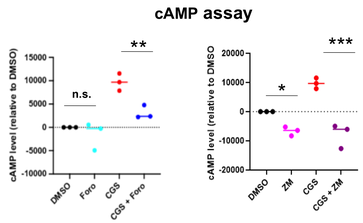


	ΔG	Interacting Residues
Adenosine	**-7.3**	**Asp-169, Asn-253, Ser-277, His-278**
Forodesine	**-7.3**	**Asp-169, Asn-253, Ser-277, His-278**

We screened nucleoside analogs for their binding to A2AR using molecular docking – Pymol and AutodockVina software (1): we calculated the affinity for binding to A2AR (ΔG) and found the residues of interaction between A2AR and the analogs. The nucleoside analog, forodesine, was among the best scoring analogs, showing a similar ΔG and interacting residues as adenosine, suggesting that it likely binds to A2AR (3).

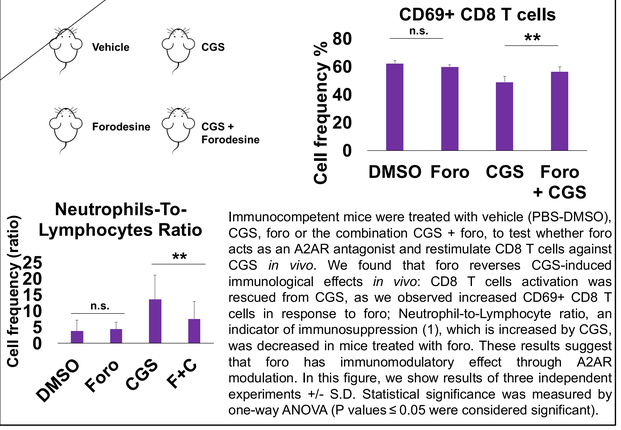
Forodesine antagonizes A2AR activation





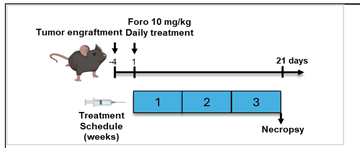
To investigate whether forodesine (foro) has immunomodulatory properties through A2AR, we measured the proliferation of CD8 T-cells treated with forodesine or the A2AR agonist CGS-21680 (CGS) or the combination of the two drugs. To do this, we isolated splenic CD8 T-cells from C57BL/6 mice and stained cells with CFSE (Carboxyfluorescein Succinimidyl Ester) dye to measure proliferation using flow cytometry. We found that the adenosine agonist CGS suppresses the proliferation of CD8 T-cells whereas foro rescues the proliferation from CGS. To confirm the capacity of foro to antagonize A2AR signaling, we also measured cyclic AMP (adenosine monophosphate) (cAMP), the direct second mediator of A2AR, in an A2AR-expressing cells line (A2AR CHO cells) treated with foro, CGS or the A2AR antagonist ZM-241385 (ZM). We found that foro, like ZM, antagonizes the production of cAMP in response to CGS. These results suggest that foro can counteract CGS binding to A2AR, suggesting that foro binds to and has immunomodulatory properties through A2AR. In this figure, we show results of three independent experiments +/- standard deviation. Statistical significance was measured by one-way ANOVA (P values ≤ 0.05 were considered significant).

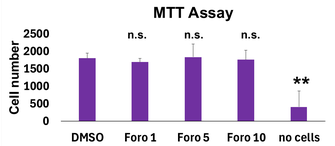
Forodesine has immunomodulatory properties through A2AR

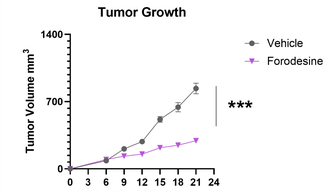


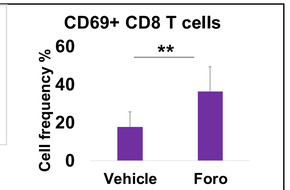
Immunocompetent mice were treated with vehicle (PBS-DMSO), CGS, foro or the combination CGS + foro, to test whether foro acts as an A2AR antagonist and restimulate CD8 T cells against CGS *in vivo*. We found that foro reverses CGS-induced immunological effects *in vivo*: CD8 T cells activation was rescued from CGS, as we observed increased CD69+ CD8 T cells in response to foro; Neutrophil-to-Lymphocyte ratio, an indicator of immunosuppression (1), which is increased by CGS, was decreased in mice treated with foro. These results suggest that foro has immunomodulatory effect through A2AR modulation. In this figure, we show results of three independent experiments +/- S.D. Statistical significance was measured by one-way ANOVA (P values ≤ 0.05 were considered significant).

Forodesine stimulates anticancer T cell responses









We tested foro in a highly immunosuppressive Triple-Negative Breast Cancer (TNBC) model, C0321 (2), to determine whether the drug immunomodulatory properties result in stimulation of anticancer T cell responses. First, we did an MTT assay and found that foro does not directly kill C0321 TNBC cells. In this study, 10^6 C0321 cells were engrafted in the mammary fat pad of female immunocompetent FVB mice. Foro or vehicle (PBS-DMSO) was administered via IP, daily, for 21 days from the formation of palpable tumors. We observed that foro significantly reduces tumor growth and stimulates CD8 T cells activation in tumors, suggesting that the immunomodulatory properties of foro translate into antitumor effects. Statistical significance was measured by one-way ANOVA (P values ≤ 0.05 were considered significant).

Conclusions

We found that selected nucleoside analogs, like forodesine, act as A2AR antagonists, a function which confers immunomodulatory properties. Importantly, we found that forodesine reverses several immunological effects which are associated with tumor-induced immunosuppression and reduced anticancer immune responses. Our findings suggest that nucleoside analogs have previously unrecognized immunomodulatory properties which could be exploited for drug design of novel immunotherapeutic drugs.

Future work will focus on characterizing the immunomodulatory effects of nucleoside analogs and development of novel compounds leveraging our previous findings.

References

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