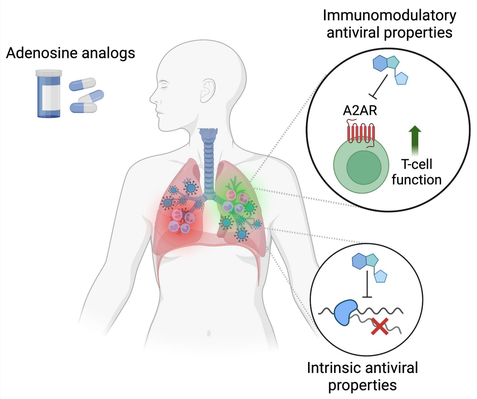
Adenosine analogs have immunomodulatory antiviral properties through the Adenosine A2A Receptor pathway

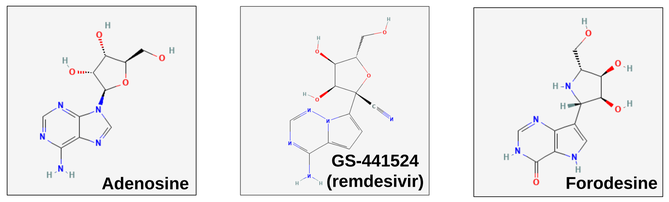
Brionna King[1], Zhi Huang[1], Peter Hewins[2], Thomasina Cook[2], Oygul Mirzalieva[3], Kristina Larter[1], Dorota Wyczechowska[4], Judy S. Crabtree[1,5], Jone Garai[4], Li Li[4], Jovanny Zabaleta[4], Mallory T. Barbier[4], Luis Del Valle[4,6], Kellie A. Jurado[2], Lucio Miele[1] and Giulia Monticone[1]

[1]Department of Genetics, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA, [2]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [3]Department of Biochemistry and Molecular Biology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA, [4]Department of Interdisciplinary Oncology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA, [5]Precision Medicine Program, Louisiana State University Health Sciences Center, New Orleans, LA, USA, [6]Department of Pathology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, USA

Introduction



The COVID-19 pandemic has unveiled an urgent need for new antivirals to control emerging infectious diseases and potential future pandemics. Current efforts to design antivirals have been focusing on approaches that directly interfere with pathogens. However, given the sometime drastic impairment of the immune system in viral diseases, antiviral therapies alone do not always guarantee a full recovery. In the past decade, immunotherapy has revolutionized oncology, but its application to infectious diseases remains largely unexplored. Adenosine analogs are antiviral drugs which resemble the structure of adenosine (1), a metabolite that can suppress CD8+ T-cell responses through activation of the Adenosine A2A Receptor (A2AR) (2).



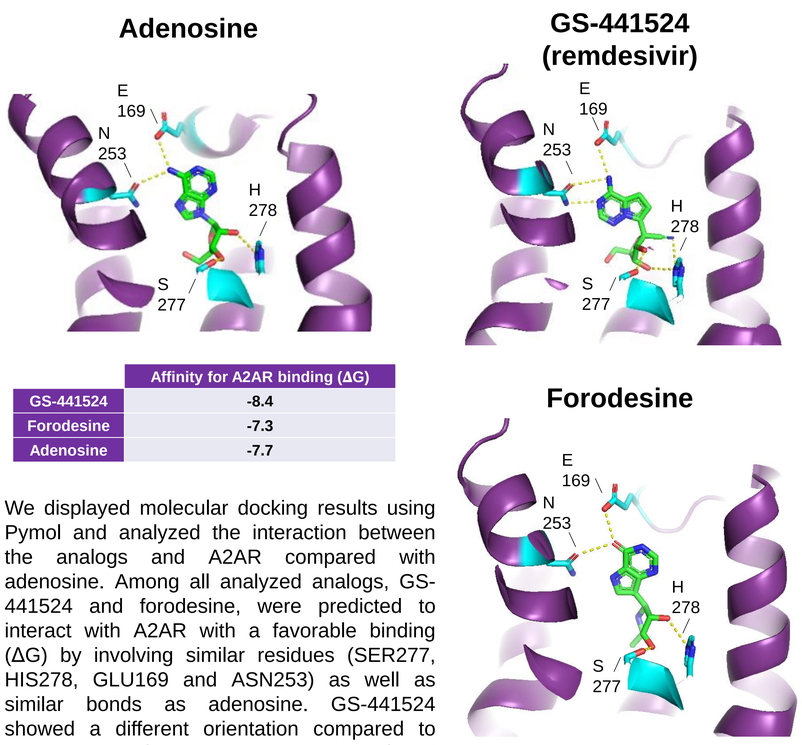
Adenosine is overproduced in some infectious diseases, like COVID-19, where it restricts protective antiviral immune responses. Adenosine analogs have a known intrinsic antiviral effect through blockade of viral replication (1). However, given the similarities between adenosine analogs and adenosine, we **hypothesize** that these compounds may have immunomodulatory properties, distinct from their intrinsic antiviral effect, through interaction with A2AR. Therefore, adenosine analogs may be endowed with dual – immunomodulatory and intrinsic - antiviral functions. To address this hypothesis, we used a computational drug design approach to model the interaction of adenosine analogs with A2AR (3), and functional assays in primary T-cells, mouse models of immunosuppression and SARS-CoV-2 infection models to test the immunomodulatory properties of adenosine analogs and their antiviral function.

Screening of adenosine analogs

Adenosine Analog	ΔG	Interacting residues
GS-441524 (remdesivir metabolite)	**-8.4**	**Glu-169, Asn-253, Ser-277, His-278**
Forodesine	**-7.3**	**Glu-169, Asn-253, Ser-277, His-278**
Riboprine	-8.2	His-278, Ser-277
8-chloroadenosine	-7.2	His-278
Maribavir	-7.2	Tyr-9
Vidarabine	-7.0	Ser-277
Aristeromycin	-7.0	Glu-169, Asn-253, Ser-277
Decoyinine	-6.4	Ser-277
Galidesivir	-7.4	Ser-277, Thr-88
Adenosine	**-7.7**	**Glu-169, Asn-253, Ser-277, His-278**

We screened adenosine analogs, known or predicted to inhibit the replication of SARS-CoV-2 and used in clinical settings (1), for biding to A2AR. As shown in the table, using molecular docking (AutodockVina software), we calculated the affinity (ΔG) for binding to A2AR and the residues of interaction between A2AR and the analogs. GS-441524 and forodesine showed the lowest ΔG and similar interacting residues as adenosine, suggesting they likely bind to A2AR.

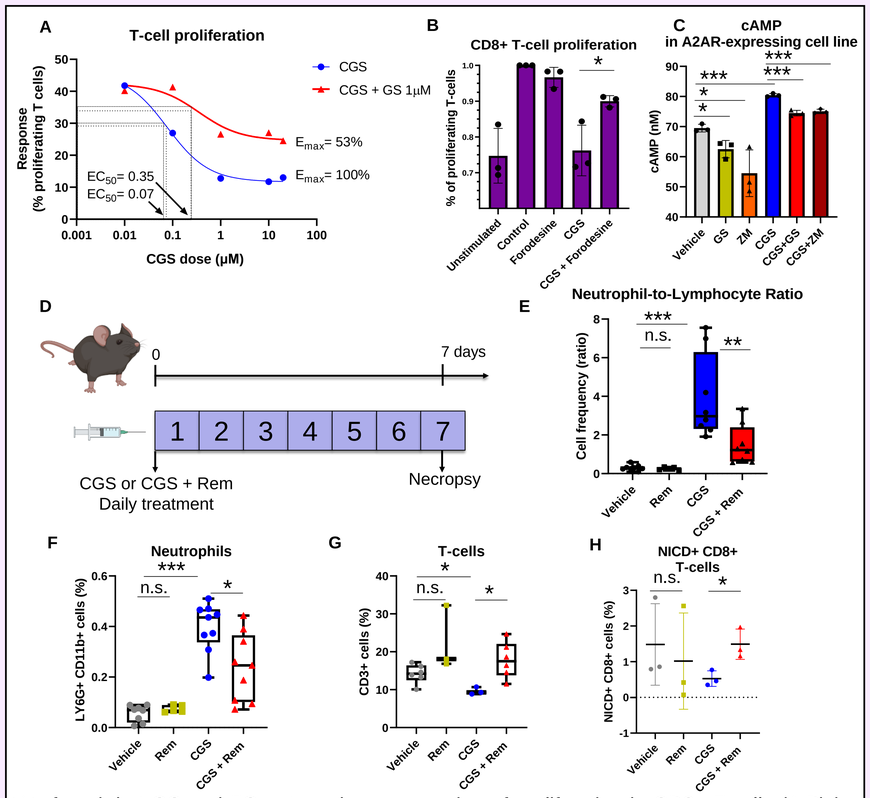
Molecular docking of adenosine analogs predicts binding to A2AR



	Affinity for A2AR binding (ΔG)
GS-441524	-8.4
Forodesine	-7.3
Adenosine	-7.7

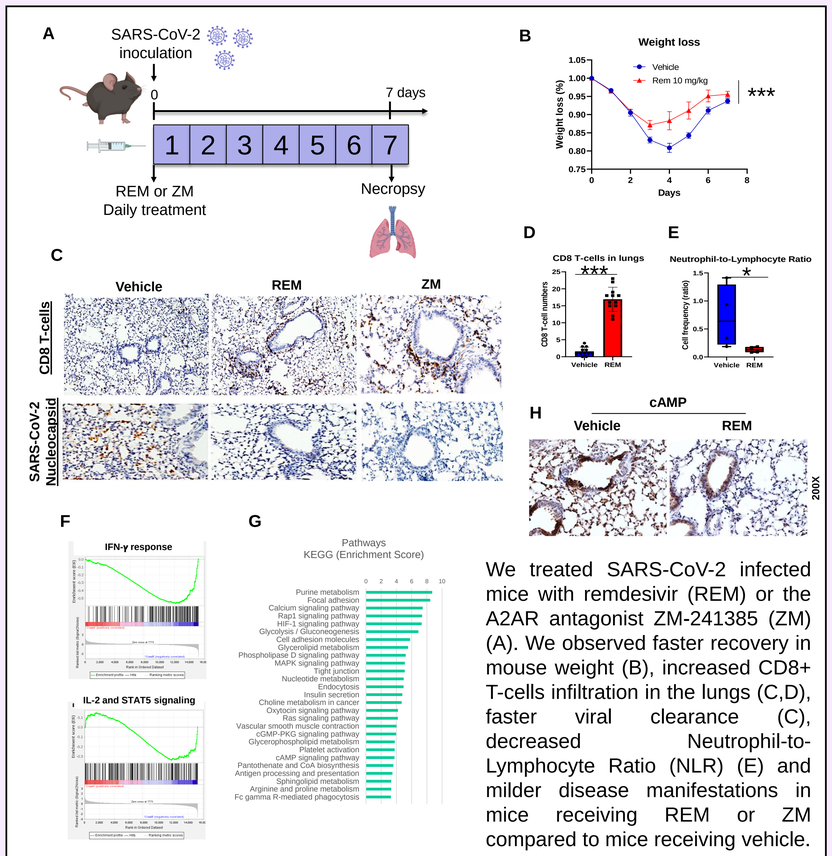
We displayed molecular docking results using Pymol and analyzed the interaction between the analogs and A2AR compared with adenosine. Among all analyzed analogs, GS-441524 and forodesine, were predicted to interact with A2AR with a favorable binding (ΔG) by involving similar residues (SER277, HIS278, GLU169 and ASN253) as well as similar bonds as adenosine. GS-441524 showed a different orientation compared to adenosine while forodesine had an almost identical orientation as adenosine.

Adenosine analogs antagonize A2AR activation and its immunological effects



We found that GS and FO antagonize suppression of proliferation in CD8+ T-cells (A,B) by the A2AR agonist CGS-21680 (CGS) as well as the production of cyclic AMP (cAMP), the direct secondary mediator of A2AR, in an A2AR-expressing cell line (C), similarly to the A2AR antagonist ZM-241385 (ZM). We also found that remdesivir (REM) treatment strongly reverses CGS-induced immunological effects *in vivo* in non-infected immunocompetent mice (D), including reducing the Neutrophil-to-Lymphocyte Ratio (NLR) (E) and the number of circulating neutrophils (F), while increasing splenic T-cells (G,H).

Adenosine analogs promote antiviral immune responses in SARS-CoV-2 infected mice



We treated SARS-CoV-2 infected mice with remdesivir (REM) or the A2AR antagonist ZM-241385 (ZM) (A). We observed faster recovery in mouse weight (B), increased CD8+ T-cells infiltration in the lungs (C,D), faster viral clearance (C), decreased Neutrophil-to-Lymphocyte Ratio (NLR) (E) and milder disease manifestations in mice receiving REM or ZM compared to mice receiving vehicle.

Consistently, we found transcriptional upregulation of genes involved in adaptive immune responses and A2AR antagonism, including IFN-γ response and IL-2 signaling (G), significant changes in genes of pathways (KEGG analysis) associated with A2AR, including cAMP signaling pathway, purine metabolism and HIF-1 signaling pathway (G) in the lungs of mice treated with REM. Finally, we found decreased cAMP expression, the second mediator of A2AR, in correspondence to CD8+ T-cell infiltration in the lungs of REM treated mice (H).

Conclusions

We found that selected adenosine analogs act as A2AR antagonists, a function which confers immunomodulatory properties and is distinct from their intrinsic antiviral activity. Importantly, we found that adenosine analogs modulate several immunological factors which are critical in the context of viral diseases that impair the immune system, like COVID-19: treatment with the analogs results in rapid viral clearance and recovery in SARS-CoV-2 infected mice by boosting CD8+ T-cell effector function and other immune components through A2AR antagonism.

Our findings support a new rationale for the design of next-generation antiviral therapeutics with dual – immunomodulatory and intrinsic - antiviral properties. These compounds could represent game-changing therapies against emerging viral diseases and future pandemics.

References

1. Rabie AM, Abdalla M. A Series of Adenosine Analogs as the First Efficacious Anti-SARS-CoV-2 Drugs against the B.1.1.529.4 Lineage: A Preclinical Repurposing Research Study. ChemistrySelect. 2022 Dec 13;7(46):e202201912. doi: 10.1002/slct.202201912. Epub 2022 Dec 8. PMID: 36718467; PMCID: PMC9877610.
2. Monticone G, Huang Z, Csibi F, Leit S, Ciccone D, Champhekar AS, Austin JE, Ucar DA, Hossain F, Ibba SV, Boulares AH, Carpino N, Xu K, Majumder S, Osborne BA, Loh C, Miele L. Targeting the Cbl-b-Notch1 axis as a novel immunotherapeutic strategy to boost CD8+ T-cell responses. Front Immunol. 2022 Aug 26;13:987298. doi: 10.3389/fimmu.2022.987298. PMID: 36090975; PMCID: PMC9459147.
3. Monticone G, Huang Z, Hewins P, Cook T, Mirzalieva O, King B, Larter K, Miller-Ensminger T, Sanchez-Pino MD, Foster TP, Nichols OV, Ramsay AJ, Majumder S, Wyczechowska D, Tauzier D, Gravois E, Crabtree JS, Garai J, Li L, Zabaleta J, Barbier MT, Del Valle L, Jurado KA, Miele L. Novel immunomodulatory properties of adenosine analogs promote their antiviral activity against SARS-CoV-2. EMBO Rep. 2024 Aug;25(8):3547-3573. doi: 10.1038/s44319-024-00189-4. Epub 2024 Jul 15. PMID: 39009832; PMCID: PMC11315900.

Contacts: bking9@lsuhsc.edu, gmonti@lsuhsc.edu