The Potential Role of ATN-161 as an Integrin α5β1 Inhibitor in Preventing Common Viral Infections

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Introduction

- Integrins are transmembrane proteins which mediate many cellular signaling processes such as immunological function, extracellular matrix composition, cell adhesion, and apoptosis [1].
- RGD-Integrins, or integrins which bind peptides containing the sequence Arginine-Glycine-Aspartate, have been shown to be implicated in the adhesion or internalization of many viruses [1].
- α5β1 integrins, which are of the RGD-integrin class, play a role in the pathogenesis of the human adenovirus type 2 [2, 3], Epstein-Barr Virus (EBV) [4], Human Immunodeficiency Virus (HIV-1) [5], Foot-and-Mouth Disease Virus (FMDV) [6], Ebola Virus [7], Porcine Hemagglutinating Encephalomyelitis Virus (PHEV) [8, 9], and also the Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) [10, 11].
- ATN-161 is a pentapeptide that acts a noncompetitive inhibitor of the α5β1 integrin complex and was shown to be an effective anti-tumor agent in a 2006 phase I clinical trial [12].
- ATN-161 is capable of blocking integrin-dependent signaling, making it an attractive potential therapeutic for the prevention and treatment of α5β1 - mediated viral infections.

Research Question

Could ATN-161, an integrin α5β1 inhibitor, act as an effective antiviral therapeutic?

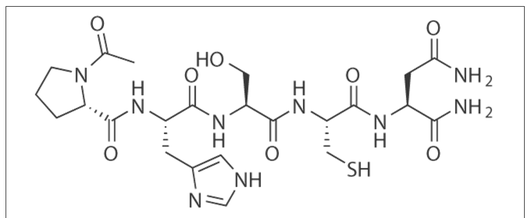

Figure 1: Structure of ATN-161

Methods

- A total of 14 papers were selected from PubMed and Google Scholar using the search terms “ATN-161”, “ATN-161 virus”, and “integrin a5b1 virus”.
- Inclusion criteria were studies which examined ATN-161 itself or other integrin α5β1 inhibitors and their effect on viral infection. Reviews as well as studies analyzing integrin complexes besides α5β1 (e.g., αVβ3) were excluded.

References

[1] Stewart, Phoebe L and Glen R Nemerow. “Cell Integrins: Commonly Used Receptors for Diverse Viral Pathogens.” Trends in microbiology, vol. 15, no. 11, 2007, pp. 500-507. **[2]** Summerford, Candace et al. “Avβ5 Integrin: A Co-Receptor for Adeno-Associated Virus Type 2 Infection.” Nature medicine, vol. 5, no. 1, 1999, pp. 78-82. **[3]** Asokan, Aravind, et al. “7. Adeno-Associated Virus Type 2 (AAV2) Contains an Integrin alpha5beta1 Recognition Sequence Essential for Viral Cell Entry.” Molecular Therapy, 13, 2006: S3. **[4]** Tugizov, Sharof M et al. “Epstein-Barr Virus Infection of Polarized Tongue and Nasopharyngeal Epithelial Cells.” Nature medicine, vol. 9, no. 3, 2003, pp. 307-314. **[5]** Monini, Paolo et al. “Hiv-1 Tat Promotes Integrin-Mediated Hiv Transmission to Dendritic Cells by Binding Env Spikes and Competes Neutralization by Anti-Hiv Antibodies.” PLoS One, vol. 7, no. 11, 2012, p. e48781. **[6]** Jackson, Terry et al. “Foot-and-Mouth Disease Virus Is a Ligand for the High-Affinity Binding Conformation of Integrin Aβ1: Influence of the Leucine Residue within the Rgdl Motif on Selectivity of Integrin Binding.” Microbiology, vol. 81, no. 5, 2000, pp. 1383-1391. **[7]** Schornberg, Kathryn L et al. “Aβ1-Integrin Controls Ebolavirus Entry by Regulating Endosomal Cathepsins.” Proceedings of the National Academy of Sciences, vol. 106, no. 19, 2009, pp. 8003-8008. **[8]** Lv, Xiaoling, et al. “ATN-161 reduces virus proliferation in PHEV-infected mice by inhibiting the integrin α5β1-FAK signaling pathway.” Veterinary microbiology 233 (2019): 147-153. **[9]** Lv, Xiaoling, et al. “Porcine hemagglutinating encephalomyelitis virus activation of the integrin α5β1-FAK-cofilin pathway causes cytoskeletal rearrangement to promote its invasion of N2a cells.” Journal of virology 93.5 (2019): e01736-18. **[10]** Beddingfield, Brandon J et al. “The Integrin Binding Peptide, Atn-161, as a Novel Therapy for Sars-Cov-2 Infection.” Basic to Translational Science, vol. 6, no. 1, 2021, pp. 1-8. **[11]** Amruta, Narayanappa, et al. “In-vivo Protection from SARS-CoV-2 infection by ATN-161 in k18-hACE2 transgenic mice.” bioRxiv (2021). **[12]** Cianfrocca, M. E., et al. “Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH 2), a beta integrin antagonist, in patients with solid tumours.” British journal of cancer 94.11 (2006): 1621-1626. **[13]** del Portillo, Francisco Garcıa, Jose Antonio Bengoechea, and Junkal Garmendia. “Host cell kinases, a5 and b1 integrins, and Rac1 signalling on the microtubule cytoskeleton are important for non-typable Haemophilus influenzae invasion of respiratory epithelial cells.” Microbiology 158 (2012): 2384-2398. **[14]** Hatley, Richard JD et al. “An Av-Rgd Integrin Inhibitor Toolbox: Drug Discovery Insight, Challenges and Opportunities.” Angewandte Chemie International Edition, vol. 57, no. 13, 2018, pp. 3298-3321.

Table 1: Role of integrin α5β1 with regards to different viruses.

Virus	Integrin	Role of Integrin α5β1	Model	Reference
Human adenovirus type 2	α5β1	Cell attachment, Cell entry	Embryonic kidney cells	2, 3
Epstein-Barr virus (EBV)	α5β1	Cell entry	Tongue cells, oropharyngeal epithelial cells	4
Human immunodeficiency virus 1 (HIV-1)	α5β1	Cell attachment	Dendritic cells	5
Foot and Mouth Disease virus (FMDV)	α5β1	Cell attachment	α5β1 coated plate	6
Ebola virus	α5β1	Cell entry	Chinese hamster ovary (CHO) cells	7
Porcine hemagglutinating encephalomyelitis virus (PHEV)	α5β1	Cell entry	Mouse neuroblastoma cells	8, 9
SARS-CoV-2	α5β1	Cell attachment, Cell entry	Pulmonary epithelial cells, α5β1 coated plate, African green monkeys (*Chlorocebus atheiops*) kidney cells, Mouse lung tissue	10, 11

Discussion

ATN-161 on PHEV:

- Integrin α5β1 expression and protein are increased upon PHEV infection.
- Treatment with ATN-161 lowered integrin α5β1 expression and protein levels.
- ATN-161 reduced viral expression in PHEV-infected mice.

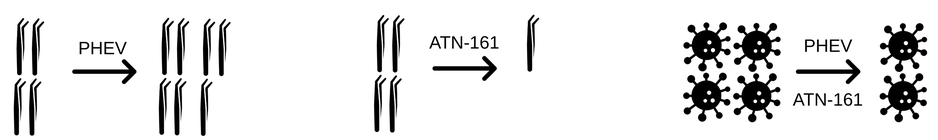

Figure 2: Results of ATN-161 treatment in PHEV. Integrin α5β1 expression levels in PHEV-infected mice brain tissue, after ATN-161 treatment, and viral expression in PHEV-infected mice treated with ATN-161.

ATN-161 on SARS-CoV-2:

- Integrin α5β1 expression is increased upon SARS-CoV-2 infection.
- Treatment with ATN-161 inhibits SARS-CoV-2-induced integrin α5β1 expression in mice lungs
- ATN-161 reduces viral load in mice responders

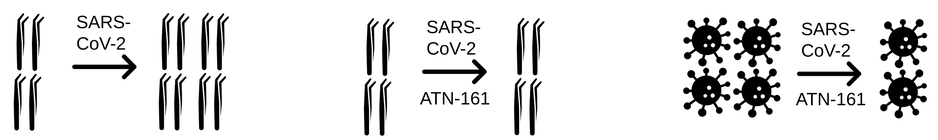

Figure 3: Results of ATN-161 treatment in SARS-CoV-2. Integrin α5β1 expression levels in infected mice, treated mice, and viral expression in SARS-CoV-2 infected mice treated with ATN-161.

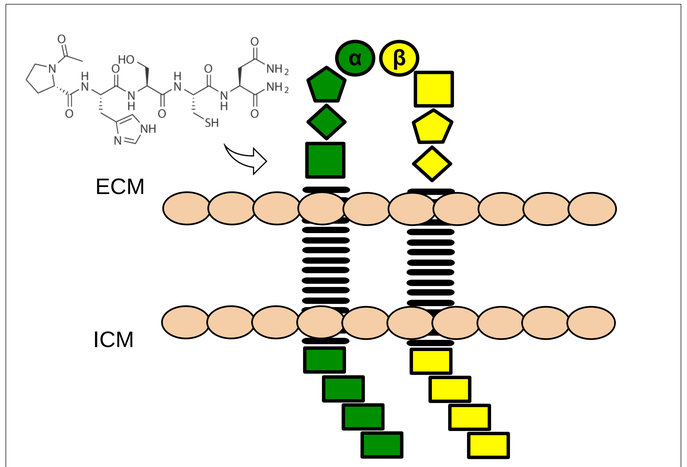

Figure 4: Integrin Structure. ATN-161 binds the N-terminus of the α5 region.

Findings

- ATN-161 has been shown to be a well-tolerated anti-tumorigenic acting in a U-shaped dose response in a Phase I clinical trial in renal cell carcinomas and gliomas [12].
- ATN-161 is capable of acting as a noncompetitive inhibitor of integrin α5β1 with a strong potential to prevent and minimize viral infection within the *Coronaviridae* family [9, 11].
- ATN-161 appears to decrease integrin α5 and/or β1 subunits, therefore directly targeting viral entry and/or attachment [9, 11].
- Interestingly, many viruses, including the PHEV and SARS-CoV-2, upregulate their expression of alpha α5 and/or β1 integrins, allowing for targeted antiviral therapy [9, 11].
- PHEV-infected and SARS-CoV-2 infected mice each showed decreased viral load and histological improvement when treated with ATN-161 [8, 9, 10, 11].
- Treatment of ATN-161 may represent a potential therapeutic approach against SARS-CoV-2.

Discussion

- RGD-integrins play a role in the mechanism of pathogenesis of both viruses and bacteria [1-11, 13].
- Viral attachment and/or entry is mediated by integrin α5β1 in several viruses including the Human Adenovirus 2, Epstein-Barr Virus, Human Immunodeficiency Virus 1 Tat protein, Foot-and-Mouth Disease Virus, Ebola Virus, Porcine Hemagglutinating Encephalomyelitis Virus, and SARS-CoV-2.
- To our best knowledge, viral infections will lead to changes in the expression of integrin α5 and/or integrin β1 within their host cells, making ATN-161 an even more appealing therapeutic target [8, 9, 11].
- Overall, ATN-161 has the potential to clinically minimize and even prevent α5β1 - mediated infection processes, including SARS-CoV-2.
- Although integrins serves a diverse array of functions, integrin-targeted medications have been FDA approved. For example, natalizumab is an integrin α4β1 antagonist alleviating multiple sclerosis and Crohn’s disease symptoms and tirofiban is an αIIbβ3 integrin antagonist for treating acute coronary syndrome [14].
- Given that ATN-161 has already undergone clinical trials and has an established safety profile, future in-vivo experiments may rapidly promote ATN-161 to clinical trials for the prevention and treatment of α5β1 - mediated viruses.