DPAGT1 inhibitor as general therapy for viral infections

Anviron
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This is a speculative assessment of how some viruses may respond from therapeutic disruption of cellular mechanisms that are known to be upregulated by these virus families. Research in this area is proposed but not yet complete. ANV221 and other candidates discussed herein have not been approved for use in humans.

Executive Summary

The Anviron team is developing a family of small molecule drug candidates that are designed to inhibit dysregulated cellular processes that are known to be upregulated in various solid tumors. While we intend to continue to advance these candidates to treat oncological applications, there are other attributes of our candidates that may benefit non-oncology applications and we welcome investment support for this purpose. For example, some viral infections have been shown to upregulate the same dysregulated cellular processes that our candidates target, viruses from the coronavirus and flavivirus (i.e. Zika, West Nile) families in particular.

A general therapy against viral infection would be beneficial to the evolving Covid-19 pandemic as well as future pandemics from these virus families. In addition, our candidates have known antibiotic properties, another important area worth exploring.

Viruses, protein folding and the unfolded protein response

Viruses reproduce by inserting genetic instructions into an infected cell, co-opting the cell's reproductive machinery to produce more copies of the virus. These newly created viruses exit the cell and repeat the process with neighboring cells. Successful viruses are also able to evade immune responses and either thwart or reprogram cellular reproductive checkpoint mechanisms. Two important checkpoints relevant to this paper are endoplasmic reticulum stress response and unfolded protein responses.

Virion production requires a sustained manufacturing and folding of proteins. Viral infections (including coronavirus) place a heavy demand on the protein folding machinery of the host endoplasmic reticulum (ER), a major site of protein synthesis, folding, modification and sorting in the eukaryotic cells[i]. To survive ER stress, host cells mount an unfolded protein response (UPR) to decrease ER protein load and enhance protein-folding capacity.

It is also known that viruses directly elicit the UPR to enhance their replication[ii], the UPR has been shown to be amongst the most upregulated pathway in SARS-Cov[iii] and it has been shown that pharmacological inhibition of the UPR, specifically the IRE1α and ATF6α branches, reduced the replication of both MHV and SARS-CoV-2 coronavirus virion release by ~1,000-fold[iv].

Paradoxically, pharmacological activation of all three UPR branches by toxic antibiotic Tunicamycin has been shown to also be an effective therapeutic strategy for treating SARS-Cov and other viral infections[v] via it's targeting of an early step in protein folding.

Activation and inhibition of the UPR seem to be in conflict as therapeutic strategies. Here we examine this dichotomy, attempt to reconcile the science, and introduce our proof-of-concept discovery as a general therapy to treat viral infections including SARS-CoV.

Inhibiting UPR as a therapeutic strategy

First we look at inhibiting UPR as an therapeutic strategy. The mammalian UPR encompasses three signaling branches, namely inositol-requiring enzyme-1α (IRE1α), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6α (ATF6α).[vi] The ATF6α branch has been shown to remove the accumulated protein load, preventing any further addition to the stress, so that normal function of the ER can be restored. It's a logical extension to surmise that by inhibiting ATF6α, the ER remains stressed and viral production of proteins is inefficient, resulting in a decrease in virion reproduction which was confirmed experimentally. [iv]

The IRE1α branch of UPR has dual functions as both a kinase and an endonuclease (Hetz et al., 2011). As an endonuclease, IRE1 cleaves 26bp from X-box binding protein 1 (XBP1) which removes a premature stop codon. This allows XBP1 mRNA to encode a full length XBP1. XBP1 coordinates with ATF6 to regulate chaperones and other proteins involved in folding (ERAD; Lee et al., 2003; Adachi et al., 2008)[vii]. XBP1 also regulates and promotes expansion of the ER (Sriburi et al., 2004). Pharmacological inhibition of IRE1 branch of UPR will prevent XBP1 from coordinating with ATF6 to increase production of chaperone proteins and prevent the expansion of the ER during protein folding stress. Pharmacological inhibition of IRE1 and ATF6 concurrently will prevent the UPR from expanding or alleviating ER stress, homeostasis will be avoided and production of viral proteins will be significantly attenuated resulting in reduction of virion production. This supposition was confirmed experimentally where virion release was reduced by a factor of 1,000. [iv]

Activating UPR as a therapeutic strategy against viral infections:

Increasing evidence supports an intersection between the host UPR and inflammation, in particular the production of pro-inflammatory cytokines and type I IFN in response to viral immune (pattern recognition receptor) engagement. (Front. Microbiol., 16 May 2014 | https://doi.org/10.3389/fmicb.2014.00222)

Innate activation of an inflammatory response is an important, early acting defense mechanism from newly encountered viral dangers and activation of the UPR can be effective at early suppression of infection. It is equally important that the innate immune response be well regulated as the release of pro-inflammatory cytokines, especially interferon (IFN)-α and IFN-γ, is correlated with lethal SARS[viii]. One example of this was recently explored where researchers confirmed that people missing an important allele, fail to express a prenylated form of **2'-5'-oligoadenylate synthetase 1** (OAS1), an essential protein involved in the innate immune response to viral infection that is known to block SARS-Cov-2[ix] whereas the shorter, non-

prenylated OAS1 protein was ineffective. Inflammatory responses due to initiation of UPR or other mechanisms during SARS-CoV infection, in persons lacking the genetic machinery to produce effective responses, such as a prenylated form of OAS1, might contribute to a "robust but ineffective" innate immune response and could be one causative factor in the SARS-Cov-2 "cytokine storms" cited in lethal SARS. Furthermore, antiviral therapies to address genetic deficiencies such as prenylated OAS1, were found to be specific to SARS-CoV-2, with limited usefulness to address future viral threats.

Viral infection and N-glycosylation

Viral infection has been found to upregulate N-glycosylation to support the production and transport of viral proteins. N-glycosylation (in mammals) is the adding of glycan (sugars) to facilitate proper protein folding. Some effective viral therapies involve inhibition of the manufacturing of specific protein enzymes, for example in the treatment of human immunodeficiency (HIV) and hepatitis C (HCV) viruses as well as the prospective Sars-Cov from Pfizer that targets the SARS-CoV-2 main protease (M^{pro}). It is our supposition that a therapeutic that can safely attenuate the production of viral proteins in a family of viruses that require this upregulation, without triggering runaway inflammatory conditions in vulnerable immune systems would be an effective general therapy to counter viral infections including SARS-CoV.

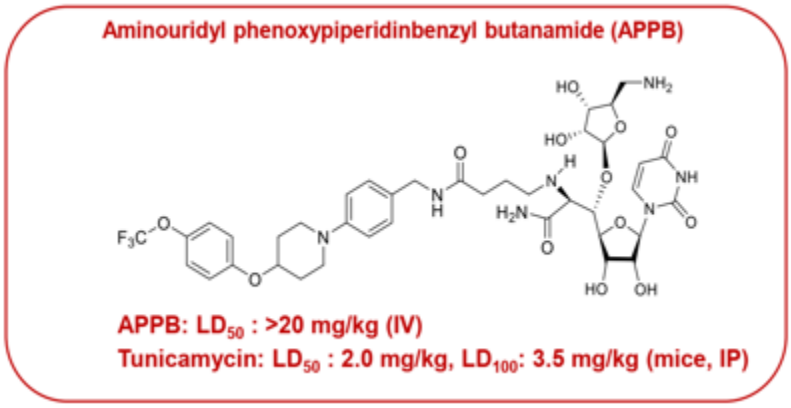
We propose that hindering N-glycosylations in infected cells could be an effective strategy to prevent assembly of the virion or key virion proteins during infections where this upregulation is important for the infection, such as SARS-CoV and other virus families. This general pharmacologic must possess several important features:

1. It must inhibit N-glycosylation in a manner that avoids the cascade of collective inflammatory signaling.
2. It must inhibit N-glycosylation of viral features that drive further infection.
3. It must inhibit N-glycosylation in a manner that prevents viruses from reprogramming the UPR to advance their purposes.
4. Inhibition of N-glycosylation must be specific to infected cells and demonstrate low toxicity to healthy cells.

Tunicamycin is an effective inhibitor of the enzyme encoded from the dolichyl-phosphate N-acetylglucosaminephosphotransferase 1 (DPAGT1) gene which catalyzes the first step of N-glycosylation, the primary protein folding mechanism in the ER.

SARS-Coronavirus (SARS-CoV) S protein are N-glycosylated at the several known glycosylation sites and constitute important processes in replication of SARS-CoV. Our referenced studies concluded that tunicamycin inhibits the formation of S glycoproteins in vitro. The antigenicity of SARS-CoV S proteins was significantly reduced by the treatment with tunicamycins, resulting in the production of spike-less non-infectious virions. While Tunicamycin meets our first three criteria, **it does not meet the final criteria.** Tunicamycin is not specific to infected cells, is toxic to healthy cells, and not suitable for use in humans. An alternate pharmacological inhibitor of N-glycosylation is needed that meets all criteria.

Anviron research team has developed a well-tolerated inhibitor of N-glycan protein biosynthesis (ANV221) that has been demonstrated to be as effective at inhibiting DPAGT1 enzyme as Tunicamycin in our animal studies.



Anviron APPB toxicity comparison with Tunicamycin

Anviron's DPAGT1 encoding inhibitors have been shown to be an effective oncological therapy in solid tumors that rely on upregulation of N-glycosylation to drive proliferative and metastatic behaviors. Our candidate has demonstrated effectiveness (in vivo) studies to selectively inhibit the first step in N-glycosylation in cells where glycosylation is upregulated. Our candidate was also shown to be well tolerated in vivo mouse models. We believe it would be viable and effective therapy in infections that upregulate this mechanism such as Coronavirus (i.e. SARS-CoV), Flaviviruses (i.e. West Nile, Zika) as well as other infectious conditions that rely on similar mechanisms.

Summary

Anviron's family of small molecule candidates are designed to inhibit dysregulated cellular process that are known to occur in various cancers. While we intend to pursue these promising aspects of our candidates, there are other properties of our molecules that can be impactful toward infectious diseases. Our team is seeking investment to extend our oncology research into areas that potentially align with national and global health priorities.

Additional References

"The growth of coronavirus in the presence of inhibitor tunicamycin resulted in the production of spikeless, non-infectious virions which were devoid of S protein… We concluded that tunicamycin inhibits E2, S, M glycoproteins of coronaviruses."

- **Inhibition of N-linked Glycosylation by Tunicamycin May Contribute to The Treatment of SARS-CoV-2**, Dawooda, Altobjeb, Microbial Pathogenesis, Volume 149, December 2020, 104586

"…viral yields (in Flaviviruses) and viral RNA were markedly reduced following Tunicamycin treatment."

- **Viral priming of cell intrinsic innate antiviral signaling by the unfolded protein response,** Carletti, Zakaria, et al, Nature Communications (2019), 10(1), 1-9. DOI:10.1038/s41467-019-11663-2

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*"Ultimately, the (MHV) virus alters the UPR, **preventing the induction of UPR**-responsive genes, which induces the blockage of protein synthesis in the host cell and favours the translation of viral proteins. This modified response would allow MHV to escape the innate defence cell signalling pathways during coronavirus replication."*

- **Manipulation of the unfolded protein response: A pharmacological strategy against coronavirus infection,** Echavarria-Consuegra, Cook, et al
PLoS Pathogens (2021), 17(6), e1009644. DOI:10.1371/journal.ppat.1009644

"Herpesviruses usurp cellular stress responses to promote viral replication and avoid immune surveillance…It is thought that the burst of herpesvirus glycoprotein synthesis during lytic replication causes ER stress, and that these viruses may have evolved mechanisms to manage UPR signaling to create an optimal niche for replication…highlight key evidence that herpesviruses hijack the UPR to aid infection."

- **Herpesviruses and the Unfolded Protein Response**, Johnston, McCormick, Viruses (2020), 12(1), 17.

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*"The ER stress response could be significantly induced in Cystic Fibrosis (CF) cells by pharmacological ER stress inducers Brefeldin A, **Tunicamycin,** and Thapsigargin… induction of the UPR pathway… reduced viral replication and shedding by up to two orders of magnitude."*

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