Story of Anellovirus
Are they our foes or allies?

Prologue

It was about 30 years ago that I, as a graduate student attended a Keystone Symposium at Keystone, Colorado. The Keystone symposiums are typically small yet highly focused international meetings attracting top-notch scientists and their trainees (graduate students and postdocs) from all over the world to present their data. I was presenting a poster about phenotypic and functional characterization of HIV-1 specific $CD8^+$ T cells. Next to my poster was a poster about simian immunodeficiency virus (SIV) isolates from African green monkey (AGM). AGM is an Old World monkey belonging to a single species known as *Chlorocebus sabaeus*, and as its name suggests, their primary habitat is sub-Saharan Africa encompassing mixed forest-Sahara environment (*1*, *2*). I was a bit curious about my neighbor's poster but not really interested at the beginning. With reluctance, I took another glance at this poster and immediately noticed one striking observation; African green monkeys did get persistently infected by SIVagm with tremendous amount of viral load (on the average over 10^5 virions/ml of blood) but these monkeys did not develop acquire immunodeficiency syndrome (AIDS) and had no consequential death at all!! This was quite contrast to SIV infection in new world monkeys such as asian Macaques, which much like HIV-1 infection in human, establishes progressive infection that manifests in AIDS. Throughout the entire meeting, this really got me thinking, "how is it possible for any lentivirus to establish a commensal like relationship with its host or the relationship is not commensal but something else?"

During a little break in, otherwise the extremely busy, evening poster session (because alcohol was involved), I had a little discussion with my neighbor presenter about immunopathogenesis of progressive HIV-1 and SIV infections leading to AIDS. I fired the first question by asking him about his model of SIVagm; how the heck do AGMs survive the persistent SIVagm infection with exorbitant viral load? I still vividly remember what his simple answer was, "SIVagm is well adapted to the host (AGM) or vice versa. They are sort of like in a commensal relationship" He continued, "AGM simply does not elicit as strong immune responses, particularly cell mediate immune responses to SIVagm infection except during an acute infection." I later became aware that SIVagm infections in AGM lack a wide array of immune hallmarks of progressive HIV-1 and SIV infections in humans and New World Monkeys respectively (*3*–*5*). Still, he made yet another important comment. "However," he added, "if viral load increases another log-fold, AGM will start displaying clinical symptoms resembling human AIDS." Evidently, AGM is not completely free from lethal SIV progressive infection after all. It clearly has the upper limit of tolerance.

What sets the upper limit then? Mathematically, it is simply an intricate balance between effectiveness of the host immune system and an efficiency of virus production. If I have to guess, it would most likely be a degree to which the host immune system handles the virus. Oddly, AGM appears to have a fully functional immune system yet for some reasons suboptimal against SIVagm infection, allowing viral production to hit saturation. Interestingly, the saturation is set very early in the course of SIV infection. A peak of viral load in the acute phase pretty much remains the same throughout the chronic persistent phase without exhibiting any clinical sings of AIDS in AGM. According to the celebrated HIV viral set point theory (*6*) a level of viral load during acute infection determines potential clinical outcomes of the host. In short, the higher the viral load, quicker the development of AIDS and eventual death. While NWMs will progressively develop AIDS at this viral load, AGM will be doing just fine. In fact, AGM is not only saved from debilitating lethal SIV infection but also harbor massive amount of SIVagm for the rest of their entire lives. I have to admit it that this phenomenon can only be explained by acknowledging the commensal relationship between AGM and SIVagm or is it something else?

As a trained immunologist in an infectious disease field, I always thought that there was no such thing as a true commensal relationship between virus and its host, particularly for human. Virus needs a compatible host for replication, which in almost all the cases, result in sever illness or in an extreme case, death of the host. Some virus including pathogenic H5N1 Influenza virus and Severe acute respiratory syndrome *coronavirus* 2 (*SARS*-CoV-2) replicate significantly during acute infection and although infection is transient, without any clinical interventions the host will face catastrophic consequences including death. In contrast, other virus such as Herpes virus that can establish persistent chronic infection typically cause sever flu like symptoms during acute infection however, they cause only minor clinical complications during chronic persistent infection and likely the host will survive from such persistent infection unless his or her immune system is severely compromised. From an evolutionary standpoint, the persistent virus (I.e. Herpes virus) is thought better adapted to human than the non-persistent virus. However, even some persistent virus can be fairly pathogenic to human, particularly with the compromised immune system as mentioned. Still, do we have any knowledge of human persistent virus that is capable of massive virus replication and production but have absolutely no impact on health of the infected host?

Anellovirus Overview

Anellovirus family (Anelloviridae) is comprised of over 30 different genera and 100 species, establishes persistent infection in a wide variety of mammals including human, and is transmitted vertically and horizontally (*7*). Since it is beyond a scope of this article to cover the entire Anellovirus family, a discussion will be focused on the most well characterized member of Anelloviridae that is relevant to human virome, known as Transfusion Transmitted Virus, or Torque Teno virus (TTV) belonging to genus, Alphatorquevirus. Because of its mode of infection, Anellovirus is quite ubiquitous in human population; it is estimated that almost all humans on this planet are persistently infected by TTV (*8*), and TTV occupies the largest portion of total human virome (*9*). Importantly, TTV defies the most common conception of human virus, a pathogen that causes rapid and wide spread infection with unendurable human suffering and consequential death in some cases. In fact, persistent infection of TTV, irrespective of age, race, and sex appear to be nonpathogenic with no known clinical manifestations in human (*7*), raising a possibility that human may be a natural host of TTV. Surprisingly, the host immune system does appear to play a role in capping the viral load through perpetual immunosurveillence (*10*), though it is still debatable as to whether virus production is self-limiting or the immune controlled. Still, the recent accumulating evidence including the ones showing a significant increase in the viral load of the immunocompromized individuals in organ transplant recipients (*11*) and in progressive HIV-1 infection (*12*–*14*) seems to ratify the immune control of TTV over viral self-regulation governing viral load. However, as in the case of SIVagm infection in AGM, the relatively large amount of chronic viral load created in a day strongly suggests the imperfect or insufficient immune control to curve daily or steady-state viral production over time.

Torque Teno virus Genomic Features

Physical structure and genome organization of TTV is well investigated. The prototype TTV (Group 1a TA278) virion contains a genome that is the negative sense circular (~3.8kbp) DNA. There are five overlapping Open Reading Frames (ORFs), of which ORF1, at 2310bp, is the longest and suspected to encode the capsid protein (Figure 1). Overall, genomic sequence of TTV family are well conserved although there are islands of less conserved and highly variable regions (Figure. 5). The 770 residue ORF1 protein has remarkable sequence variability but all isolates have an arginine rich region near the N terminus (suspected genome interactions, and nuclear localization functions) (*15*, *16*) and three short hypervariable regions (HVR) towards the C terminus (*17*). In addition, there is a relatively conserved 110bp Non-Coding Region (NCR) which forms a hairpin like structure to aide replication of a circular genom (*18*). By convention, position 1 in the nucleotide sequence is 85bp upstream of a TATA Box located 29bp from the cap site of the lone viral transcript.

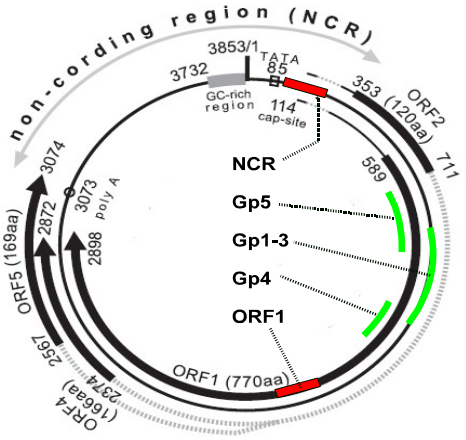

Figure 1. **Genome of TTV1 1a prototype α Anellovirus TA278**

Torque Teno virus Structural Features

TTVs (and all the other member of Anelloviridae) lack viral envelop(outer double layer lipid membrane), which turns out to be a common feature shared by the small DNA virus (*19*). Viral genome (DNA) is surrounded and protected by viral capsid proteins. Despite of the decade long technical impediment, the physical structure of the Anellovirus capsid, based on the Betatorquevirus, (also known as TTMV:Torque Teno MiniVirus) isolate, LY1 (*20*) finally has been resolved recently (*21*). According to the study, a capsid structure of TTMV, LY1 is T = 1 icosahedron (Fig.2A) ~ 30nm with a five-fold symmetry spike domain forming a crown like outer ring structure (Fig. 2B).

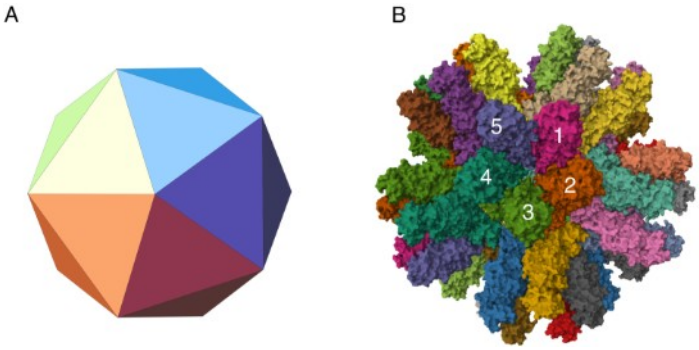

Figure 2. **Capsid Structure of Betatorquevirus, TTMV isolate, LY1 like particle assembly in vitro (A)** Structure of T=1 icosahedron with 5 fold symmetry. This image was generated with Vladimir Bulatov's Polyhedra Stellations Applet (https://bulatov.org/polyhedra/stellation_applet/) by Tomruen. **(B)** Cryo-EM structure of TTMV-LY1 anellovirus virus-like particle (PDB ID: 8CYG) published by Liou et. Al (Nat Commun 15: 7219-7219). C and N termini truncated TTMV-LY1 capsids were expressed in sf9 insect cell line and allowed to assemble in the particle that resembles the TTMV-Ly1 vision. Cryo-EM structure was uploaded to RCSB PDB (https://www.rcsb.org; H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, The Protein Data Bank (2000) Nucleic Acids Research 28: 235-242 https://doi.org/10.1093/nar/28.1.235). This particular image of 3D structure of the TTMV-Ly1 like particle was created by RCSB PDB Mol* (Sehnal, S. Bittrich, M. Deshpande, R. Svobodová, K. Berka, V. Bazgier, S. Velankar, S.K. Burley, J. Koča, A.S. Rose (2021) Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures (2021) Nucleic Acids Research 49:W431-W437 https://doi.org/10.1093/nar/gkab314).

Considering a level of genomic and proteomic conservation within Anellovaridae, it will be reasonable to assume that the viral capsid structure of TTVs (Alphatorquevirus) is nearly identical to the LY1 (Betatorquevirus) viral capsid structure. In fact, a predicted capsid structure from it's primary amino

acid (AA) sequence of a prototypical TTV1, Alphatorquevirus homin1 (XCH55654.1) by AlphaFold3 (*22*) gives a testament of the close structural resemblance between TTV1 and LY1 (Fig. 3).

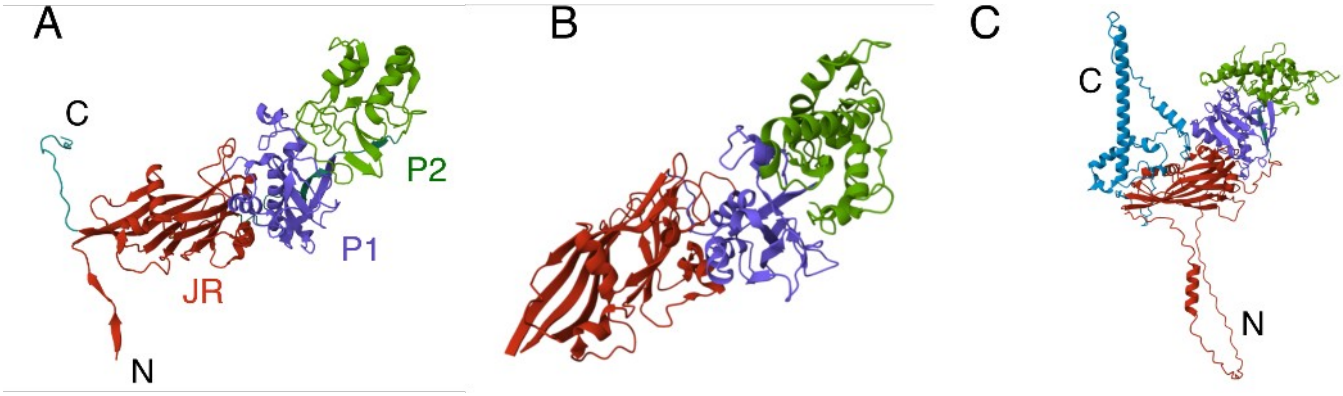

Figure 3. **Cryo-EM structure of TTMV-LY1, predicted Alphatorquevirus homin1 capsid proteins (protomer) (A)** TTMV Ly1 protomer with 3 distinct functional domains including jelly-roll, projection 1 (P1), and projection 2 (P2) domains. **(B)** A predicted structure of the Alphatorquevirus homin1 (XCH55654.1) capsid protomer without C and N terminal amino acid sequences, and **(C)** A predicted structure of the Alphatorquevirus homin1 (XCH55654.1) capsid protomer in the panel B with nascent C and N terminus, created by AlphaFold3.

A TTMV-Ly1 capsid protomer has the 5-fold symmetry with outermost P2 domain in the spike region forming a crown like structure pointing outward (Fig. 2B and 4). The P2 domain (Fig. 4A), according to Liou et.al. (*21*), plays a significant role in Anellovirus immune invasion (see discussion below).

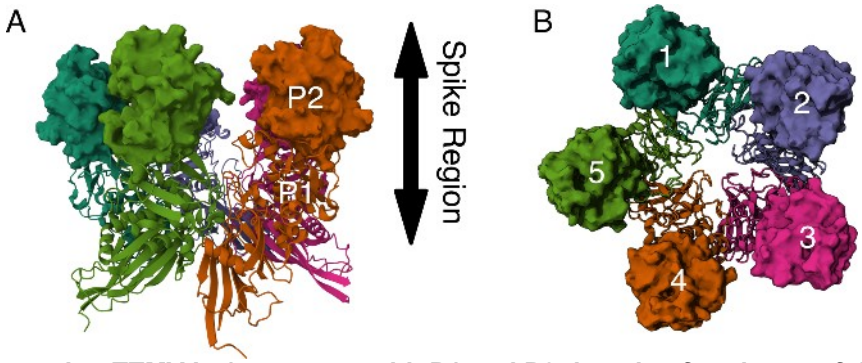

Figure 4. **Spike region TTMV-Ly1 protomer with P1 and P2 domains forming a 5-fold symmetry crown capsid structure (A)** The spike region consists of the inner P1 and outermost P2 domain. **(B)** A 5-fold symmetry crown structure of capsid protomers may play a role in virus-cell interaction. Also, apex of the P2 domain is facing the interstitial space and are therefore accessible to antibodies.

Notably, C and N terminus of TTMV-Ly1 protomers in this Cryo-EM study were truncated at the very end, leaving only small inner portion of C and N terminus sequences intact (Fig. 3A). Evidently, truncation of the C and N terminus does not interfere with proper protein folding, assembly of TTMV-Ly1 crown like structure, and the formation of Ly1 virion. This, however, does not mean both N an C terminus have some important biological functions. In fact, based on a 3D structure of the C terminus with its protruding section (Fig. 3C) hypothetically extending beyond the exterior of virion is implicated in virus-cell interaction (*21*). Unfortunately however, this and other purported functions of an extracellular arm of C terminus can not to be tested in this TTV1 viral assembly and remained to be seen at this point.

Torque Teno virus Immune Evasion Mechanism

During a life long chronic infection, TTV1 produces a myriad of viral particles a day. Under the presumed immune intervention, this however would not be possible unless TTVs possess some sort of an elaborate scheme(s) for immune evasion. Since other known persistent pathogens do have

some sort of the immune evasion mechanisms, it is reasonable to assume that TTVs should also have such immune evasion mechanism(s). Based on the structure of the TTMV-Ly1 protomer, Liou et.al. (*21*) proposed that the outer most spike region, the P2 domain with the hyper variable region (HVR) function as a decoy that fools the host immune system by masking highly conserved primary and secondary viral sequences in P1 and JR domains, thus diverting the immune response, particularly antibody mediated immunity (AMI) from such conserved regions of TTV capsid (Figure 5A and B). Any immune responses against the conserved region of virus is detrimental to the virus simply because such immune response could potentially wipe out an entire Annelovirus members from human population(a bit hyperbole consider what vaccine did to measles virus). Understandably, many persistent virus such as Herpes virus and Anellovirus have been selected through the course of virus-host evolution for having complex transformative immune evasion apparatus ensuing continuous viral production without harming the host (to a certain extent). To fully appreciate how the P2 domain of TTV1 is able to provide such an immune hack, it is necessary to take a close look at the immune evasion mechanism of TTV1.

Detailed examination of amino acid sequences of several TTV1 isolates does reveal such hyper variable P2 domains in ORF1 (Fig. 5A), as described in Liou et.al. (21). Moreover, the crown structure of the P2 spike domains appears to be one of the potential hotspots of antibody binding sites predicted by Discotope (protein structure based antibody epitope binding site prediction algorithm) (*23*) (Figure 5B and C). Liou et.al.'s claim seems to be well substantiated by these and other additional findings (not mentioned). However there is an interesting twist to this rather simple but somewhat admissible claim, which comes from a study by Venkataraman et.al. (*24*). Venkataraman et.al. found that among 156 individuals infected by variants of human TTVs, a majority of those TTV-specific antibodies were directed against epitopes within the C terminus of the capsid protomer (see Figure 2C and 3A in Venkataraman et.al (27)), which is in agreement with the Discotope prediction showing the C terminus as another hotspot for antibody binding (see Figure 5B and C). A key implication from this study is that the C terminus is immunodominant or immunologically more favorable over P2/HVR for antibody recognition. Again, this could be a serious issue for any TTVs if some of these antibodies recognizing and binding to the relatively more conserved C-terminus are capable of neutralizing virus infectivity, thus, potentially impeding virus production and lingering infection. Evidently, this is not the case since, as mentioned earlier, that TTV infection results in daily production of the enormous amount of the virus, almost exceeding any known persistent human virus, suggesting that none of these antibodies against C terminus are neutralizing.

Alternative speculation, as suggested by Venkataraman et.al.(26), is that C terminus of TTVs could be proteolytically cleaved by cellular proteases, sparing TTVs from extermination by AMI. Interestingly, these authors noted that Liou et.al. (*21*) originally proposed this process as yet another immune evasion mechanism. However, Liou et.al. (*21*) did not mention such proteolytic cleavage of C terminus playing a significant role in Anellovirus immune invasion. They just showed that C terminus deletion of their model system, LY1 Betatorquevirus did not impact an overall assembly of LY1 protomers into the fully mature LY1 capsid, and suggested that C terminus of the LY1 protomer through some post translational modifications or other protein modification could augment the formation of the wild type virus, and perhaps as a receptor for cell attachment and entry (*21*). Taken all together, I think that proteolytic cleavage of Anellovirus capsid C terminus as a decoy for immune evasion remains a bit far fetched. Most likely, it is just a part of the viral capsid that plays a critical role in viral assembly but not immune evasion.

Nevertheless, I have to admit that there is something eccentric about C-terminus of Anellovirus from a perspective of Anellovirus evolution. This elongated C terminus with a relatively and unusually long alpha helix which is believed to be protruding from the surface of virion is unique only to the members of Anellovridae, and it is indeed absent from its closest ancestral virus, Circovirus (*25*). Intriguingly, Circovirus unlike Anellovirus is pathogenic to its hosts (*26*) . For instance, the human Circovirus type

1 (HCirV-1) infection is strongly associated with hepatitis particularly among immunocompromised individuals (*27*).

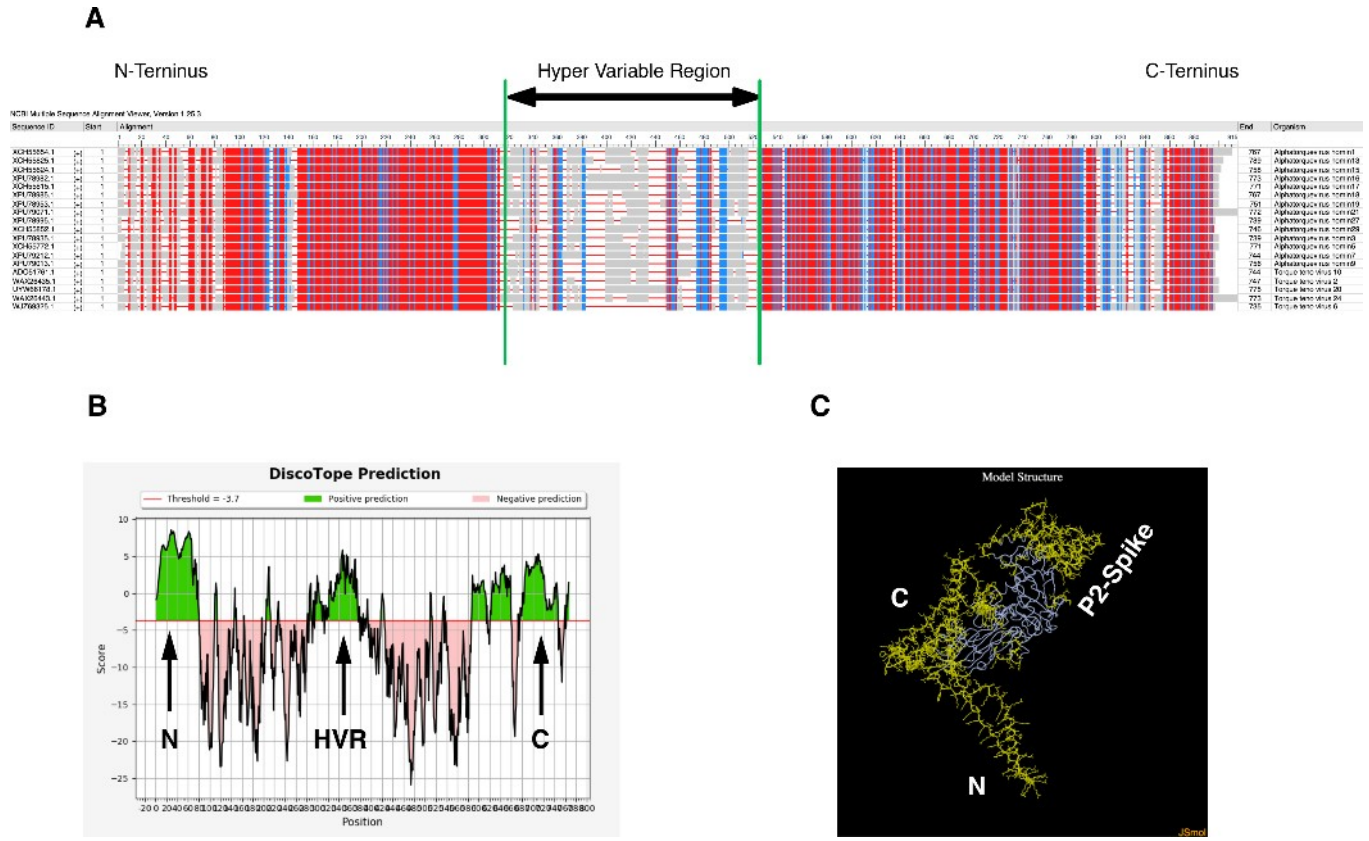

Figure 5. **Antibody accessible sites in the AlphaAnellovirus (TTV) Capsid Protomer** (**A)** Primary sequences of protomers from currently known19 distinct TTV species available in NCBI Virus data base (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/) were aligned by using MUSCLE (*28*) and resulting sequence conservation/variation is visualized by NCBI Multiple Alignment Viewer (*29*). Blue and red indicates the mismatches and matches respectively, red lines denotes sequence gaps, and grey highlights mismatches over the gap. As mentioned in the text, a large gap in the middle of the alignment is the region known as Hyper Variable Region (HVR) as well as N- and C- Terminus regions. **(B)** These regions are structurally permissive to antibody binding (green regions) as predicated by DiscoTope 2.0 (*30*) and **(C)** these corresponding regions (in yellow) occupy very much the exterior of the capsid protomer seen here in 3D.

Moreover, capsid protomers of Circovirus have Anellovirus equivalent JR domain but lack P1 and P2 domains as well as elongated C terminus found amongst Anellovirus. An obvious question here is whether having P1, P2 and elongated C terminus renders pathogenicity to Circovirus? This can be simply tested by swapping the P1, P2 domains and the C terminus from TTV and added them onto the JR domain of Circovirus to generate a chimeric TTV-Cicovirus protomer, complete with a C-JR-P1-P2 configuration. I have to admit though that whether such engineered Circovirus capsid protomers will successfully assemble infectious virions is an another matter, and from my experience, most likely it needs a lot of tinkering around. Sadly, nothing in Science is so easy and straightforward.

T Cell Responses to Torque Teno virus

Cleary, the host immunity is mounted against TTV1 as it should since TTVs are considered as "foreign" not "self". As such, the host immunity is trying to control persistent TTV1 infection through a TTV1-specific humoral immunity/AMI yet AMI alone is not sufficient to clear the virus. Fortunately, the humoral immunity is not the only type of immunity we possess. Another type of the immunity is known as a Cell Mediated Immunity (CMI) which is comprised predominantly by T cells ($CD4^+$ and $CD8^+$ T cells). $CD8^+$ T cells, ultimate immune effectors responsible for commencing the process of

intercellular pathogen clearance, play a major role in controlling in virus by simply performing a search and destroy mission to virus-infected cells. Downside of deploying virus-specific $CD8^+$ T cells is that potency of these cells must be tightly regulated, otherwise, they could pose serious damage to uninfected tissues surrounding the infected area and in worst case, the destruction can spread to the whole organ. This is why $CD8^+$ T cells undergoes multiple activation steps to ensure safety; first they get activated by specific cognate antigen(s) (in this case, TTV1 antigens), and second, they will proliferate, expand, and third, deliver rapid death to infected cells. A vast majority of activated virus-specific $CD8^+$ T cells undergo cell death soon after primary infection but a surviving fraction becomes a virus-specific memory $CD8^+$ T cells, which is a major driving force behind their incredible functional potency (*31*).

But then none really talks about TTV1-specific $CD8^+$ T cells, and in fact, there is a lack of or only a few studies of TTV1-specific $CD8^+$ T cells that I was able to find today. How come? If I would guess, this is likely due to a lack of definitive TTV1 antigens (epitopes, more precisely) of the TTV1-specific $CD8^+$ T cell discovered, because such antigen/epitope is a priori for studying any antigen-specific $CD8^+$ T cells. Unlike B cells, the $CD8^+$ T cell recognizes its cognate antigen in the form of a peptide with a string of average 9 amino acids derived from the antigenic protein, called “epitope” and such epitope must be presented by Major Histocompatiblity Complex class 1 (MHC class I) protein (for $CD8^+$ T cells). All I can say at this point is that not a whole lot of T cells biologist spend their effort and money into characterizing the TTV1 $CD8^+$ T cell eptitope, due to a lack of funding or something else. A sad fact is that none can do Science for “fun” any longer. It is a serious business; either publish or perish. It takes so much money, time and effort. Why would any T cell biologist waste their limited resourced to study a subject which has not funding/money.

Yet, I was hoping to find any published data on the class I TTV1 epitopes restricted (presented) by any human Class I protein called Human Leukocyte Antigen including the most common A*02(A*0201) allele in North America, through either google or PubMed searches but to no avail. Then I resorted on currently the largest compendium of all the published and unpublished (but characterized) MHC class I (and II) epitopes, Immune Epitope Database (IEDB) & Tools (https://www.iedb.org) (*32*) where I could search potential Anellovirus epitopes but once again I could not find any. It is sort of strange and at the same time disappointing not to find any studies of the T cell responses to Anellovirus, despite of having a tremendously large community of T cell biologists in the world. Perhaps, I have yet to discover some publications and evidently have slipped through my exhaustive searches but at this point I decided to go with another, a lot more entertaining (for some technophiles in audience) approach which is called “epitope prediction”. My rationale here is that rather than wasting time in arduous search, I will short cut the entire process by employing IEDB’s Class I epitope prediction tool to quickly access the presence of any potential TTV1 $CD8^+$ T cell epitopes with overall predicted scores and percentiles meeting expectations of T cell epitopes being processed, presented, and immunogenic.

The result of prediction is summarized in a following heatmap (Fig.6). This heatmap displays 6 categorical variables on x-axis and predicted epitopes on y-axis with normalized prediction scores in color. These categorical variables can be roughly grouped into two groups, “percentiles and scores”. Basically, the epitope with relatively low percentile (blue) and high scores (red) has higher probabilities of being the true genuine immunodominant epitope. I found 18 of such epitopes that could be considered to be the top HLA-A*0201 binder for having NetCpan_EL_Percentile values less than 1 by the IEDB prediction tool. Among them, 4 epitopes stand out with respectful protesome, tap, and processing scores. It should be mentioned that this is a result of the prediction, therefore must be taken with a grain of salt. There is no guarantee that the prediction works at all the time and although these candidate have greater chances of being real epitopes, they must be further tested and validated for T cell reactivity. Nonetheless, the latest IEDB MHC Class I epitope prediction tool is superb. In the real world test, this IEDB prediction tool will typically pick at least one of the real

authentic immunodominant epitope from its a top 10 candidate epitopes. At least one of these predicted epitopes will be the bona fide HLA-A*0201 restricted TTV1 $CD8^+$ T cell epitope expectantly.

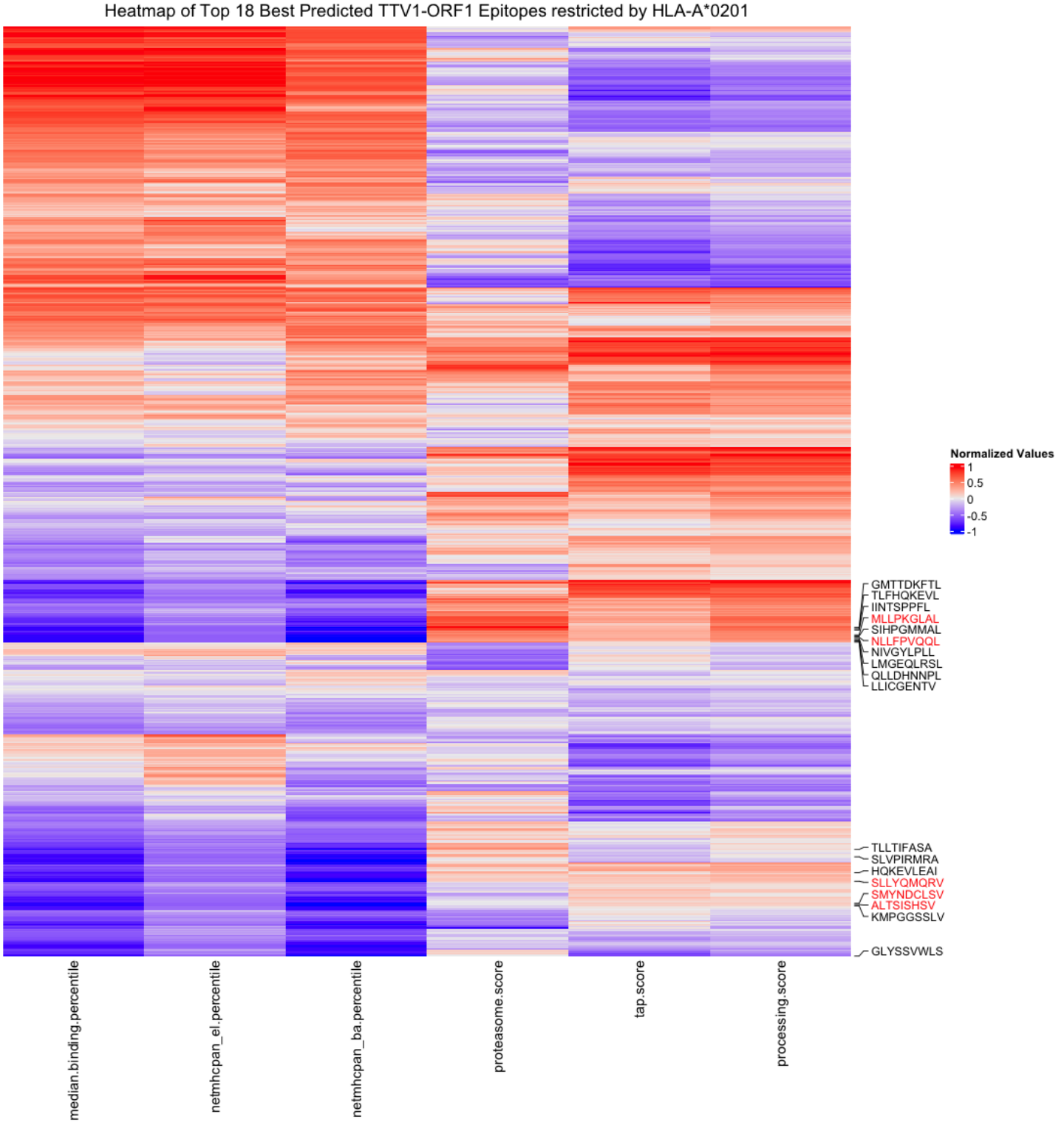

Figure 6. **Top 18 Best Predicted TTV1-ORF1 Epitopes restricted by HLA-A*0201** These top 18 TTV1-ORF1 MHC/HLA class I epitopes for $CD8^+$ T cells are chosen based on the percentiles (generated by NetMHCpan4.1EL (*33*)) being less than 1, as recommended by the IEDB MHC class I epitope prediction documentation. Red epitope sequences indicate the top 5 lowest percentile. Overall, IEDB MHC class I epitope prediction tool (https://nextgen-tools.iedb.org/pipeline?tool=tc1) yielded 759 distinct epitopes with varying degrees of prediction percentiles and scores. These scores and percentiles are normalized and plotted as heatmap to show relative values of scores and percentiles. Epitopes with lower percentiles (blue) and higher scores (red) are most likely the candidate for the actual epitope that is presented by HLA-A*0201 molecules and recognized by $CD8^+$ T cells

It is relatively simple (but laborious) to run an assay to test reactivities of human $CD8^+$ T cells specific for these 4 (or more) candidate epitopes or even scan an entire TTV1 capsid protein for the presence of human $CD8^+$ T cell epitopes by using a peptide library. Detecting such TTV1-specific human $CD8^+$ T cells, on the other hand, may not be as easy if the frequency of such $CD8^+$ T cells will be extremely low, potentially due to a massive viral production causing profound T cell exhaustion, a state in which $CD8^+$ T cells become dysfunctional and unresponsive to the specific viral epitope (in this case TTV1 epitopes) and doing so they lost capacities to produce key cytokines (soluble protein factors activates themselves and other immune cells in vicinity), and proliferate. Clonal expansion of antigen-specific $CD8^+$ T cells very much depends on these processes. Again, exceptionally high TTV1 viral load strongly suggests that TTV1-specific $CD8^+$ T cells are modestly or not all all effective in controlling persistent TTV1 infection. Perhaps masterful immune evasion by Anellovirus is real and it aids the virus establishing commensalism with human but how exactly does TTV1 evade $CD8^+$ T cells or CMI all together?

Torque Teno virus Immune evasion: Alternative Mechanism?

Just a quick recap. The presence of TTV1 anti-capsid antibodies is clear evidence of the fact that TTV1 can not escape from being detected and consequently targeted by the host immunity, however, TTV1-specific immune response mediated by AMI is apparently not be able to clear the infection due to the elaborateTTV1 evasion from AMI. As previously mentioned, a prevailing hypothesis is that HVR of the P2 domain is responsible for immune evasion from AMI by creating a perfect diversion (HVR) to the immune detection and clearance by AMI. This hypothesis makes a perfect sense if AMI predominantly targets HVR but that is not really the case. Moreover, cell mediated immunity (CMI) comprised of TTV1-specific T $CD4^+$ and $CD8^+$ T cells does not appear to be effective either, though data on CMI are too scarce to make any clear conclusions yet.

Still, these facts do not offer an acceptable answer as to how does TTV1 establish life-long persistent infection with such massive viral load? Contrary to Human Immunodeficiency Virus 1 (HIV-1), and Hepatitis B virus, both of which produce tremendous amount of virus by establishing long-term progressive infection with simultaneous active immune suppression, TTV1 does not establish progressive infection and does not appear to cause any sever immune dysfunctions with noticeable immunodeficiency at the global scale, which simply means that the host immunity overall is very much intact in individuals infected persistently withTTV1. It is almost as if the host immunity acknowledges the presence of virus and mount the immune response accordingly however, the response for some unknown reasons is extremely moderate at the best. This is probably because the immune system does not warrant that the infection is serious enough to elicit a much more robust immune responses, which is very reminiscent of the host immune **unresponsiveness (commensalism?)** to SIVagm infection in African Green Monkeys. If this really were the case for TTV1, much like SIVagm, another two log-fold increase in the TTV1 viral load would trigger robust immense immune responses and certainly would result in sever clinical complications associated with typical acute viral infection but again there are not enough data to genuinely support this idea, and we have no way of resolving whether or not a commensal relationship between human and TTV1 is real.

Perhaps, a key to understand TTV1 immune evasion and to ultimately prove commensalism between TTV1 and human is to shed a light on how the anti-TTV1 CMI is not elicited by persistent TTV1 infection even with tremendous viral load in the first place. In all likelihood, the TTV1 accomplishes its immune evasion by either active or passive suppression of the TTV1-specific CMI. Unfortunately, once again, it is nearly impossible to speculate this without further knowledge of CMI against Anellovirus, particularly Anellovirus-specific $CD8^+$ T cell. One of my takes on this (seriously wacky) is that TTV1 infection may be actively suppressing CMI by eliciting the regulatory T cell (Treg) response through the same mechanism that SARS-Cov-2 does. SARS-Cov-2 deploys a SARS-Cov-2 encoded NSP7 protein which possesses the epitope that activates a group of T cells that are known to suppress the CMI, known as regulatory T cells (Tregs), through which SARS-Cov-2 archives active

suppression of the anti-SARS-Cov-2 memory CMI (*34*). These Treg epitopes are collectively known as Tregitopes (*35*, *36*). Now the question is whether TTV1-ORF1 has such Tregitopes inscribed in its primary sequence. How can I determine this without actually running the experiment in the lab? Well, potentially, I could use some bioinformatic tools to determine if known Tregitopes and Tregitopes like epitopes could be aligned against the primary sequence of TTV1-ORF1. However, this unfortunately does not look promising (https://github.com/akhst7/AnelloVirus/blob/main/file4e9b4f0aafe3.pdf).. Alternatively, I could do the opposite, mapping the top most probably predicted HLA Class II epitopes 1 against the primary sequence of human IgG. It turns out that the original Tregitopes were discovered from the primary sequence of human IgG1 (*36*), and possibly yet undiscovered Tregitopes embedded in its primary sequence. However, this too sounds just as inauspicious as the first attempt. The last attempt was made to see if the Tregitope embedded in NSP protein of SRAS2-Cov-2 as mentioned above, and/or sequences similar to that could any way found in the primary sequence of the TTV1-ORF1 protein and this attempt seemingly is still pretty far fetched but looks a bit more promising. I found one such candidate Tregitope, **IVGYLPLLI** with border line criteria (See Figure 7). Clearly. this putative TTV1-ORF1 Tregitope, **IVGYLPLLI** must be further tested in the lab for its capacity to bind HLA ClassII molecule(s), to activate Tregs and to suppress CMI against TTV1.

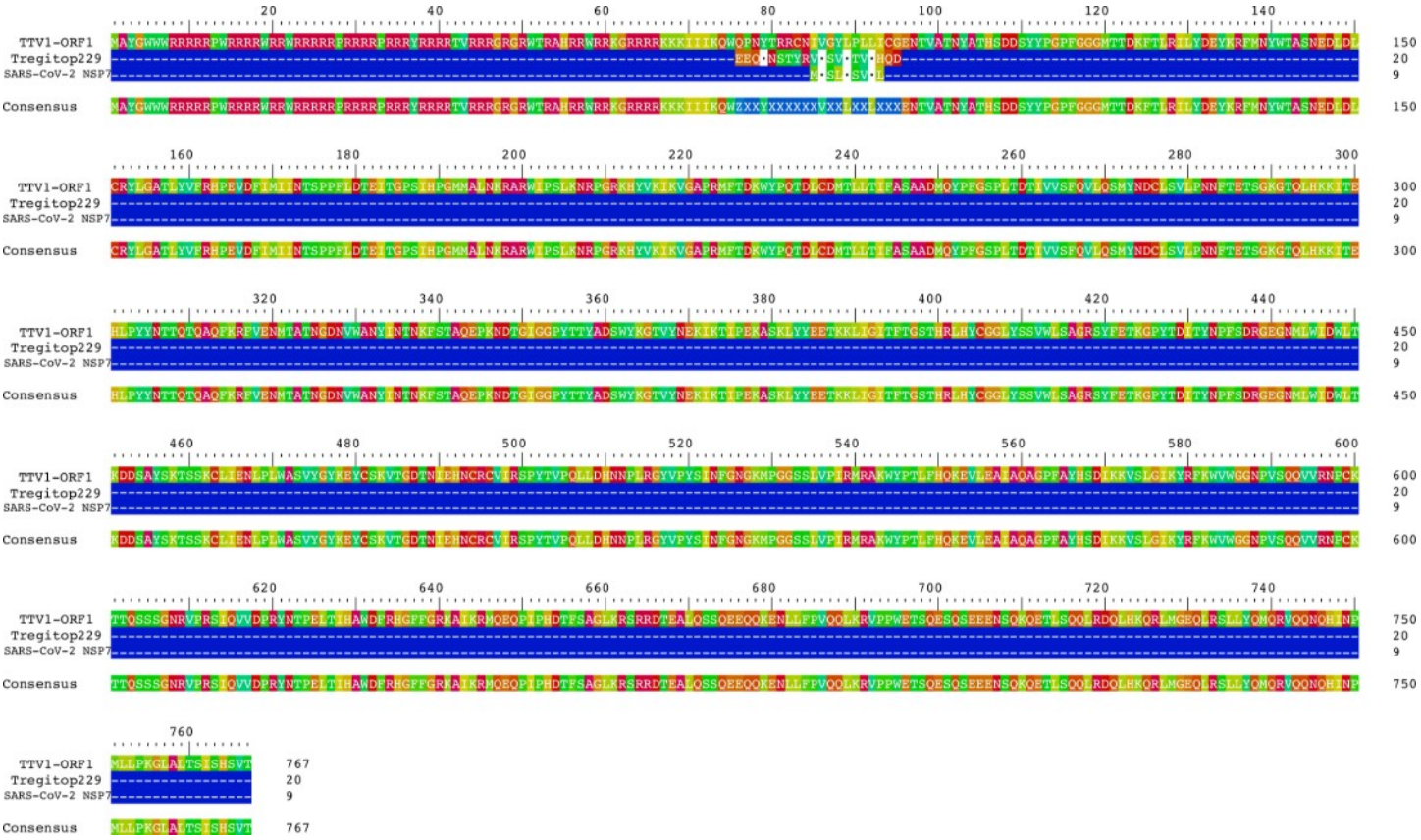

Figure 7 **Alignment of Human IgG Tregitopes 229 and SARS-CoV-2 NSP17 Tregitopes against TTV1-ORF1 (XCH55654.1)** Pairwise alignment of those previously characterized Tregitope 229 (*36*) and SARS-CoV-2 NSP17 Tregitope (EKMVSLLSVLLSM) (*34*) against TTV1-ORF1 (XCH55654.1) was made by using two R packages, DECIPHER (33) and Biostrings (*38*). The top sequence is the primary sequence of TTV1-ORF1 and following sequences are the peptide sequences of Tregitope 229 and SARS-CoV-2 NSP17 Tregitope. Numbers on the top of each rows display amino acid positions starting from 1st amino acid position in ORF1, and the bottom sequence denoted as consensus shows the amino acids in agreement between Tregitopes and TTV1-ORF1 primary sequences. Black dots represent matched amino acids between the primary sequence of Tregitopes and TTV1-ORF1. Notice the “x” in the consensus sequence which indicates that the amino acids in Tregitopes and TTV1-ORF are not in agreement. *AlignPair* function with a default parameter in DECIPHER was used to generate this alignment.

Conclusions

Despite recent advances in the physicochemical property of Anellovirus, many aspects, fundamental to the biology of Anellovirus still remain deep in abyss. The most intriguing and critical aspect of Anellovirus concerns the immune evasion mechanism which allows the virus to persist with enormous viral load in the absence of discernible immune mitigation and disease expression. As outlined above, the recent structural study (*39*) of the Anellovirus capsid protein shed a light to this matter and has proposed the HVR on the outermost P2 domain of the capsid protein as a viral decoy for Anellovirus immune evasion. This however, only explains for immune evasion from the antibody mediated immunity but not the cell (T cell) mediated immunity. Although I argued profusely above, my version of the alternative immune evasion mechanism remains at most fantasy. It should also be mentioned here that there is a whole array of other immune suppression mechanisms that TTV1 (or Anellovirus as a whole) can potentially deploy(*40*) Regardless, after all this, I have to concede the notion that Anellovirus could well be commensal to human. Much like a relationship between SIVagm in African Green Monkeys, through ages of viral evolution, the Anellovirus likely has become adapted to the host and hence, the host immune system simply decides to ignore its presence, allowing virus to establish the persistent infection with tremendous viral load simply because the virus is not pathogenic to human whatsoever. By all means, the story of Anellovirus does not end here, and for the academic interest as well as practical applications of Anellovirus, we need to advance our knowledge of Anellovirus immune evasion mechanism, particularly against T cell immunity.

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