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Research Article

Sting Pathway Activation by Orally Administered Attenuated dsRNA Vaccine Virus for Therapy of Viral Diseases

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Even after the Coronavirus disease 2019 (COVID-19) pandemic, the world's vaccine strategy is failing because vaccines are produced only after an epidemic is under way. This article argues that vaccination alone will not be sufficient to control COVID-19 or any other future pandemic (e.g., flu). Using non-pathogenic viruses to control unrelated ongoing infections could complement vaccination efforts. The attenuated dsRNA Infectious Bursal Disease Virus (IBDV), the drug candidate of the clinically validated orally administered viral superinfection therapy (SIT), is close to regulatory approval. IBDV signals the innate Stimulator of Interferon Genes (STING) pathway and has been proven to be safe and effective against five different families of viruses: hepatitis A, B, and C viruses (HAV, HBV, HCV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and herpes zoster viruses (HZV). Here, a blueprint for a registration strategy is proposed. Attenuated IBDV is a repurposed drug candidate as it has been used safely during 60 years of IBDV mass vaccination programs in poultry. IBDV can therefore be produced faster, cheaper, with less risk, and with higher success rates than traditional drug development. With SIT, a repeat of the US$12 trillion the world just spent on COVID-19 can be avoided.

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Introduction

The largest vaccination program in history has prevented 19.8 million out of a potential 31.4 million COVID-19 deaths in the first year of vaccination [1]. Despite such spectacular results, Dr. Seth Berkley, former CEO of Gavi, the Vaccine Alliance, warned the medical community that even after the COVID-19 pandemic, the world's vaccine strategy is failing because 'we keep relying on a market-based model that churns out millions of doses only after an epidemic is under way' [2]. According to Berkley, 'our best defense is having vaccines ready to use the moment disaster strikes.' This would be a daunting task because another influenza pandemic is inevitable.

Measles, polio, and smallpox vaccines provide long-lasting immunity because the causative viruses have low mutation rates. In contrast, SARS-CoV-2 viruses replicate quickly and mutate frequently that makes lasting vaccine immunity challenging [3]. SARS-CoV-2 is picking up about two single-letter mutations per month. The high circulation of the COVID-19 variants, the inequitable vaccine rollouts and inadequate control measures in some countries offer fertile ground for SARS-CoV-2 to take surprising evolutionary leaps. Circulation in animal reservoirs could also bring unexpected changes. The assumption that viruses evolve to become milder is a myth. The World Health Organization has warned that Disease X could result in 20 times more fatalities than COVID-19.

A compounding problem is that the frequency of zoonotic diseases is growing because people exert pressures on nature [4]. Animal industries in the United States pose serious risk of future pandemics, concluded a study by researchers at Harvard Law School and New York University. The study authors stated, 'In the wake of COVID-19, we no longer have to imagine what a large-scale infectious disease outbreak would look like in the United States. Still, COVID-19 fatality rates in the U.S. hovered just under 2% for most of the early pandemic. What if, instead of this coronavirus disease, it had been another such as SARS, a cousin to COVID-19, with a mortality rate of 14%, or MERS, a disease caused by another member of the viral family, that is 32% fatal?'

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The main objective of this article is to argue that vaccination alone will not be sufficient to control COVID-19 or any other future pandemic. Here we propose a blueprint for a doable and affordable post-infection plan "B" that would complement prophylactic vaccination efforts [5]. The viral superinfection therapy (SIT) is administered orally to patients with ongoing viral diseases. SIT is an intentional viral *coinfection* therapy during which a live attenuated bird vaccine virus, the infectious bursal disease virus (IBDV) is given orally to patients in order to activate the innate immune system as described below [6].

According to Virgin, we need to change our obsolete view of viruses [7]. The complex interactions between viruses and the host cannot be described by a 'monotheistic view' in which viruses are simply harmful pathogens. The virome is much more than a deadly threat to people or animals because host genes in combination with viral genes can contribute to host fitness. Healthy humans comprise 10^{13} cells, while they host about 10^{14} bacteria and 10^{15} viruses. This ecosystem is characterized by balanced coexistence (which developed over millions of years) rather than by a constant war. Viruses have by far the largest gene repertoire and sequence space available on earth. As a matter of fact, about half of the human genome consists of virus-related sequences, truncated viruses, or viral fossils. Even our innate antiviral defense system comes from viruses [8]. Intentional combinations of virus and host genes can therefore be therapeutically exploited. In such a way, counteracting the immune inhibitory capabilities of pathogenic viruses with apathogenic ones would make sense.

In the above context, the recent paper by Garcia et al [9] is relevant because they considered the activation of the Stimulator of Interferon Genes (STING) pathway by agonist drug candidates a promising strategy to inhibit multiple families of viruses. According to Rabie, the most attractive target for the therapy of viral diseases is RNA [10]. While the STING agonist drug candidates are still in preclinical development, exploiting a non-pathogenic dsRNA virus for the activation of the STING pathway has been clinically validated against four different families of viruses, as described below [5].

The non-pathogenic attenuated avian dsRNA vaccine virus, the Infectious Bursal Disease Virus (IBDV), has been proven to be clinically safe and effective for the post-exposure therapy of four different viral diseases

The translation of the idea of complementing host genes with an apathogenic virus is closest to regulatory approval in the so-called *viral superinfection therapy* (SIT), which is an intentional coinfection therapy for patients suffering from acute or chronic viral diseases [6]. During SIT, the orally administered repurposed avian apathogenic infectious bursal disease virus (IBDV) delivers its dsRNA cargo into host cells. Then, the viral dsRNA activates the native antiviral interferon (IFN) gene defense system of the host cells.

The innate immune system detects conserved pathogen-associated molecular patterns (PAMPs) of invading pathogens by germline-encoded pattern-recognition receptors (PRRs). The retinoic acid inducible gene-I (RIG-I)/melanoma differentiation associated gene 5 (MDA5)-mitochondrial antiviral-signaling protein (MAVS) axis and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) axis are the major sensing pathways for cytosolic RNA and DNA, respectively [11]. Emerging evidence indicates a crosstalk between the innate sensing of cytosolic DNA and RNA [12] (Fig.1.).

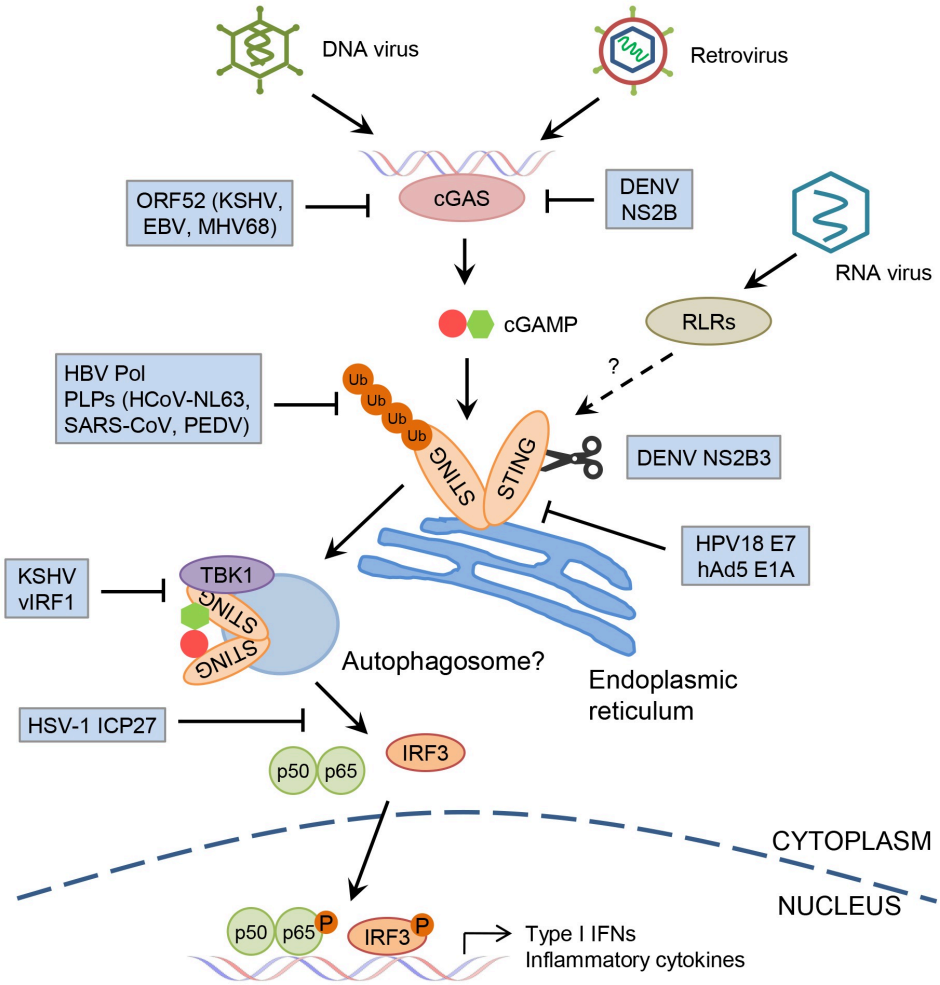

Figure 1. *The cGAS–STING pathway and its counteraction by viruses. (Reproduced from https://doi.org/10.1371/journal.ppat.1007148.g001).* Genomic DNA form DNA viruses or reverse transcription intermediates from retroviruses are recognized by cGAS, which catalyzes the production of cGAMP to bind and activate the ER-resident adaptor protein STING. STING then forms a complex with TBK1 and translocates from the ER to the perinuclear lysosomal compartments via an autophagy-like process. The STING–TBK1 complex subsequently activates transcription factors IRF3 and NF-κB to induce the production of type I IFNs and inflammatory cytokines to establish an antiviral state. Viruses have developed numerous strategies to antagonize the cGAS–STING pathway. Tegument protein ORF52 from gamma-herpesviruses inhibits cGAS binding to viral DNA, while nonstructural protein NS2B of DENV promotes cGAS degradation. Similarly, DENV NS2B3 protease cleaves STING and leads to its degradation. HBV polymerase and papain-like proteases of human coronaviruses prevent or remove the K63-linked Ub of STING. KSHV vIRF1 blocks the TBK1-mediated phosphorylation of STING, while HSV-1 ICP27 prevents the phosphorylation of IRF3 by TBK1. HPV18 E7 protein and hAd5 E1A protein bind to STING and inhibit its activation. cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; DENV, Dengue virus; ER, endoplasmic reticulum; HBV, Hepatitis B virus; hAd5, human adenovirus 5; HSV-1, herpes simplex virus 1; HPV18, human papillomavirus 18; ICP27, infected cell protein 27; IFN, interferon; IRF3, interferon regulatory factor 3; KSHV, Kaposi's sarcoma-associated herpesvirus; NF-κB, nuclear factor-κB; NS2B, nonstructural protein 2B; ORF52, open reading frame 52; P, phosphorylation; RLRs, RIG-I-like receptors; STING, stimulator of interferon genes; TBK1, TANK binding kinase 1; Ub, ubiquitination; vIRF1, viral interferon regulatory factor 1.

The cytosolic DNA sensing cGAS-STING-mediated signaling has a noncanonical role in the crosstalk of viral DNA and RNA sensing and controlling RNA viruses. Several RNA viruses, including vesicular stomatitis virus (VSV), Sendai

virus (SeV), dengue virus (DENV), West Nile virus (WNV), influenza A virus (IAV), Japanese encephalitis virus (JEV), and retroviruses, could be detected via STING signaling pathways [12][13]. A recent study by Garcia et al. screened innate immune agonists for antiviral activity and identified that cGAS-STING cytosolic DNA-sensing pathway stimulation contributes to broader inhibition of multiple families of RNA viruses, including Zika, West Nile, Chikungunya, and SARS-CoV-2 viruses [9]. Garcia et al considered the activation of the STING pathway by agonist drug candidates a promising strategy to inhibit multiple families of viruses. In this context it is important to note that even after a single dose, the IBDV-R903/78 drug candidate activated several genes in mice, which have been directly or indirectly associated with the STING pathway (Fig. 2. A, B). Consistent with this, multiple doses of IBDV were capable to inhibit the replication of DNA (HBV, HZV) and RNA (HCV, SARS-CoV-2) viruses in patients[6][14].

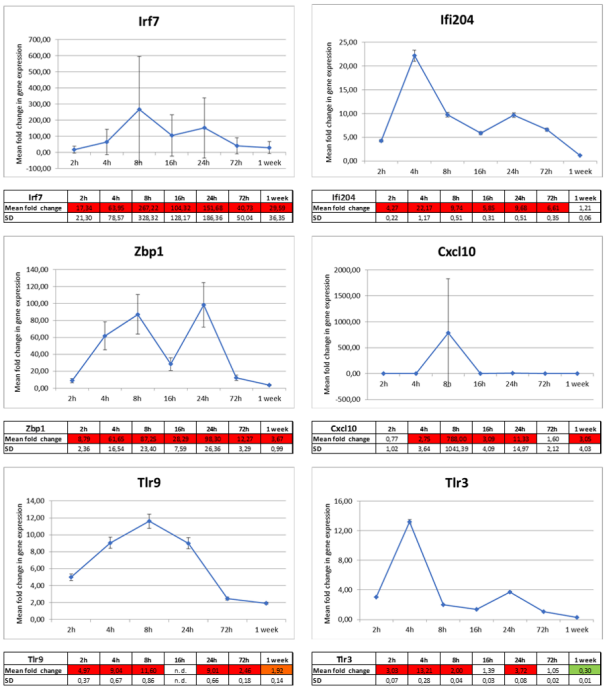

Figure 2A. *Expression levels of individual genes over time in the liver tissue after a single dose of intravenous IBDV in mice (for technical details see in [15]).*

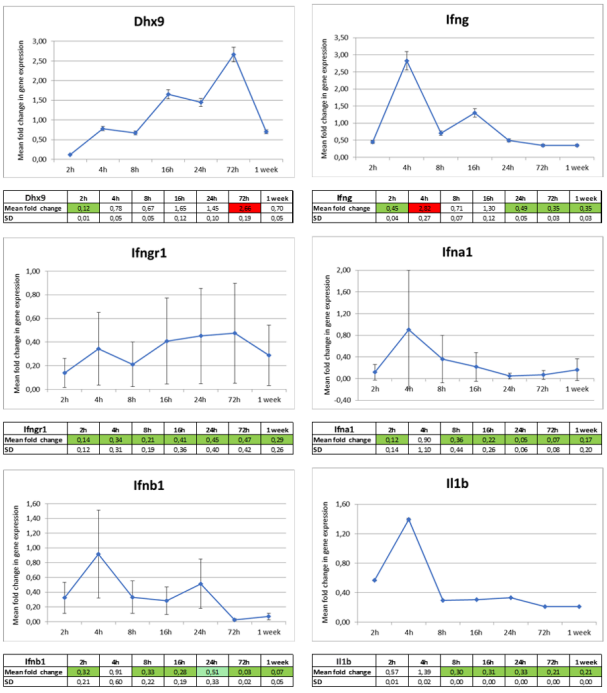

Figure 2B. *Expression levels of individual genes over time in the liver tissue after a single dose of intravenous IBDV in mice (for technical details see in [15]).*

The attenuated reversely engineered IBDV serotype R903/78 strongly induces IFN-β and IFN-λ (~30-fold and 5 to 10-fold, respectively) in the A549 human lung adenocarcinoma cell line, while IFN-γ is not induced (see Figure 1 in [16]). As IBDV did not lyse several mammalian cell lines (A549, HEK293, HepG2, U937, and THP1), it provides safety compared to lytic viruses [17].

In this way, inflammation and antiviral efficacy are separated, which opens up the therapeutic window. In chronic hepatitis B (CHB) patients, for example, such a particular cytokine induction profile makes it possible that IBDV maximizes antiviral efficacy with only minimal side effects. IBDV was used for 24 weeks in acute HBV/HCV patients. In severe decompensated hepatitis cases, IBDV was administered over a long period (about a year) for the maintenance of 'artificial viremia', while no serious side effects were observed [14]. Effective repeated oral IBDV administration is possible because a breakthrough infection can be achieved even in the presence of high levels of IBDV neutralizing antibodies (Fig. 3) [17]. IBDV treatment never induced an excessive release of pro-inflammatory cytokines, despite the fact that patients with decompensated liver disease had high-level viremia, which is a key driver of the cytokine storm [14].

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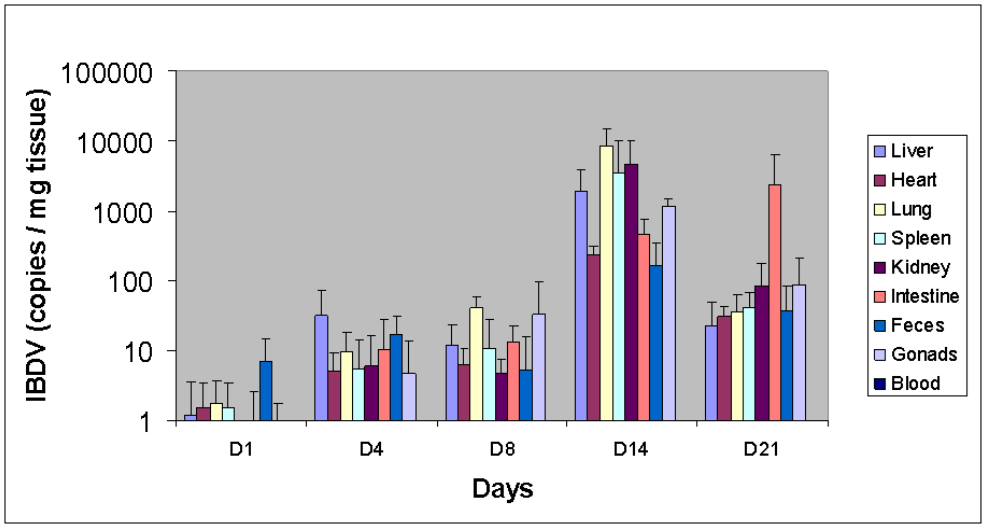

Figure 3. *Effective multiple oral administrations of R903/78 virus in mice in the presence of neutralizing antibodies.* Tissue distribution of R903/78 virus following multiple oral administrations in Balb/C mice were dosed with multiple oral (MO) delivery of $1.7x10^6$ IU of R903/78 virus at days 0, 3, 7, 13, and 20. Necropsy was performed on days 1, 4, 8, 14, and 21. Tissue RNA was quantified by quantitative real-time RT-PCR. The transduction efficiency is expressed as IBDV copy numbers per mg of tissue (Reproduced with permission from [17]).

IBDV has been proven to be safe and effective against the hepatitis A virus (HAV) in marmoset monkeys and in patients with hepatitis B virus, hepatitis C virus (HBV/HCV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and herpes zoster virus (HZV) infections [6]. Importantly, the feasibility of iatrogenic viral coinfection had been demonstrated by large controlled field trials involving more than 300 thousand people during three seasonal outbreaks of influenza and other associated acute respiratory infections with IFN-inducing live enteroviral vaccine strains in the former Soviet Union [18]. The immunostimulatory power of IBDV became obvious to the naked eye in a severe herpes zoster ophthalmicus infection of the first author of this paper [19] (Fig. 4).

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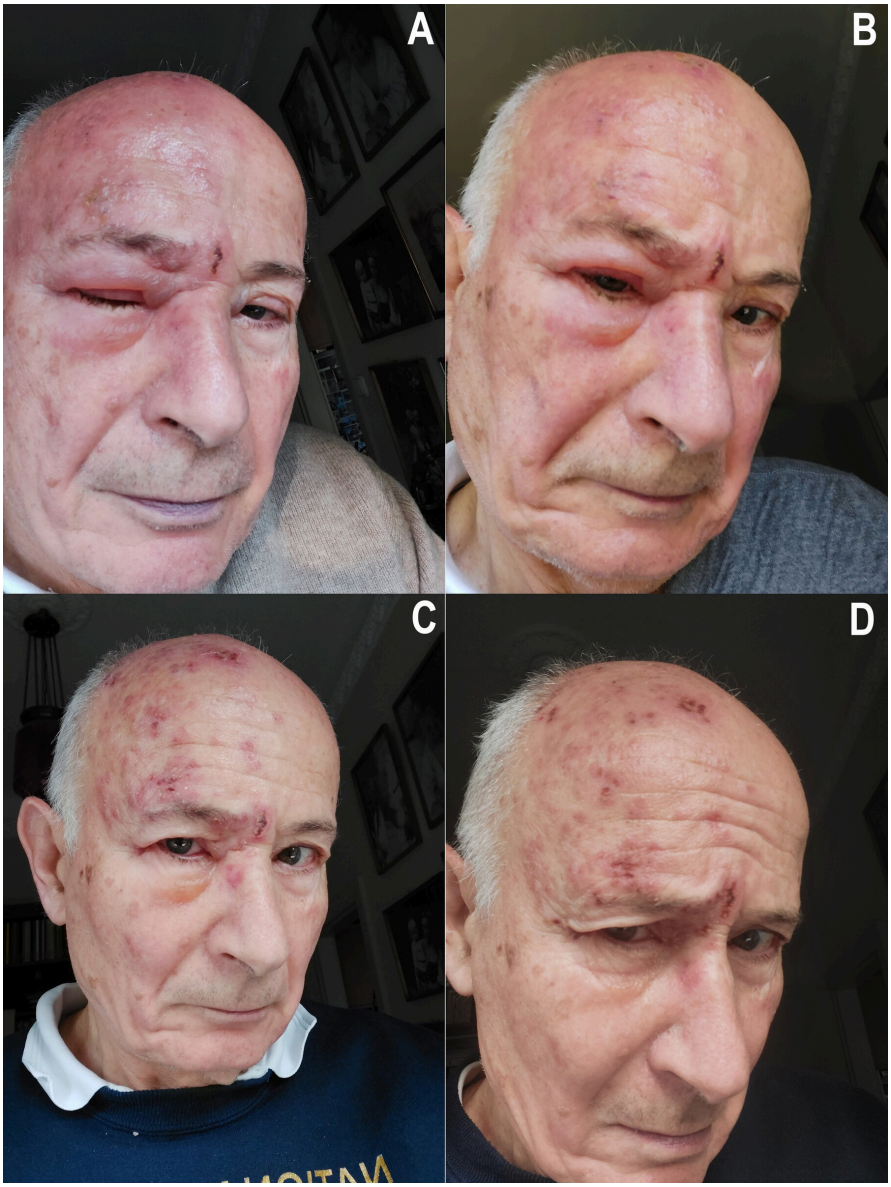

Figure 4. *The author's HZO with orbital edema at the peak of the disease and in recovery (A–D).* The selfie pictures were taken between October 9, 2021, and October 12, 2021. Consent for the publication of patient information was granted by Tibor Bakacs, M.D., Ph.D., D.Sc., as he was the patient and the treating physician in the autobiography. Reproduced from Bakacs, T. Healing of Severe Herpes Zoster Ophthalmicus Within a Few Days: An Autobiographical Case Report. Cureus. 2021, 13:e20303. doi:10.7759/cureus.20303 with permission from Cureus Inc. (see in 8. Site Content and User Submissions; https://www.cureus.com/terms; accessed on 24 January 2024).

Reverse genetic engineering made the IBDV-R903/78 drug candidate pharmaceutically safe, and it is simple to manufacture

Establishing a productive infection for a virus by jumping between donor and recipient host species depends on the relatedness of the two species. Birds and mammals, along with their viruses, diverged in evolution more than 200 million years ago. Therefore, thirteen mutations (a gigantic

jump) may be required for an avian influenza virus to cause a productive infection in humans. To pre-adapt to humans, the influenza virus requires the swine, which is an intermediate host [20] [21]. Fortunately, IBDV does not have such a natural genetic engineering laboratory. Consistent with this, no zoonosis cases have ever been reported over the past 60 years during IBDV mass vaccination programs in poultry. In order to prevent even a very low risk of zoonosis, which is a legitimate concern for the regulatory authorities, the IBDV-R903/78 drug candidate was created by reverse genetics technology. In this way, an easily testable homogeneous starting material was provided, which ensured batch-to-batch consistency without the need for repeated plaque purification [22].

The AGE1.CR.PIX cell line has been chosen for manufacturing the IBDV-R903/78 drug candidate. This cell line was created by the immortalization of the Muscovy embryonic duck retina cells. For pharmaceutically safe immortalization, a combination of E1 genes was chosen from human adenovirus type 5. This is a 'defined-risk approach' because the immortalizing genes are known and traceable [23]. The AGE1.CR.PIX cell line is banked under Good Medical Practice (GMP) conditions and is free of adventitious agents. Based on the Modified Vaccinia virus Ankara (MVA), this cell line was used for the production of clinical-grade, live-attenuated, vectored vaccines. AGE1.CR.PIX was selected because it is highly permissive for IBDV. Titers exceeded those of other cell lines by 100-1,000-fold. In this way, viral levels between 10^9 and 10^{10} infectious units (IU)/mL were reached. Since a typical clinical dose is 10^7 IU, 100,000 to 1 million doses can be produced in a 1 L fermentation volume. Host cell protein contamination was reduced by purification (below 10 ng/dose of host cell DNA). Please note that the oral or intranasal application of IBDV-R903/78 requires less stringent reductions of host cell DNA and protein. As IBDV-R903/78 is secreted into the cell culture supernatants, it does not require lysing of the cells. Downstream purification processes and expenses are therefore greatly reduced. The high virus yield combined with oral delivery allows for a simple formulation methodology.

A blueprint for a doable three-step approach for the registration of the IBDV-R903/78 drug candidate

Following a Phase I dose escalation safety study of the IBDV-R903/78 drug candidate in healthy adult volunteers, we propose a doable, three-step development strategy for the registration of the broad-spectrum post-infection IBDV antiviral superinfection therapy (SIT): 1) the safety and efficacy of the IBDV-R903/78 drug candidate will be tested as a short-term add-on therapy in herpes zoster patients; 2) IBDV-R903/78 will be tested as a primary long-term monotherapy in CHB patients; 3) IBDV-R903/78 will be tested in sequential combination with ultra-low doses of immune checkpoint inhibitors (ICIs) in those CHB patients who did not achieve a functional cure on IBDV monotherapy, as described briefly below.

1) Short-term standard of care (SoC) acyclovir (ACV) plus add-on IBDV-R903/78 drug candidate for the treatment of herpes zoster

A herpes zoster trial is proposed that will be modelled on the Immune Modulators for Treating COVID-19 (NIH ACTIV-1 IM) master protocol, which was designed to evaluate multiple investigational agents for the treatment of patients infected with SARS-CoV-2. Based on the autobiographical case report of a severe HZO infection of the first author of this article [19] , a hypothesis was generated predicting that bolstering the innate antiviral immunity of the HZ patients by an add-on immunostimulatory IBDV-R903/78 therapy would leverage the activity of the conventional SoC acyclovir (ACV) treatment. To this end, a small randomized phase I/II trial of IBDV-R903/78 in HZ patients was proposed [24]. Safety and tolerability of IBDV are assessed as primary endpoints, while immunogenicity and therapeutic efficacy are the exploratory endpoints. The healing periods of HZ symptoms will be compared during ACV monotherapy and ACV plus IBDV-R903/78 combination therapy. All patients will receive SoC ACV 800 mg five times daily. Half of the patients will also receive IBDV-R903/78 orally for 7 days as an add-on immunostimulatory therapy. Four doses (1.0 x 10^5, 1.0 x 10^6, 1.0 x 10^7, and 1.0 x 10^8 IU) will be orally administered daily for 7 days to reach the maximum tolerated dose (MTD). The starting dose will be 1 log below the autobiographical HZO study dose [19].

2) Long-term IBDV-R903/78 monotherapy for the functional cure of CHB patients

HBV is a manageable disease; therefore, ethical issues should be considered before giving non-registered therapies to HBV carriers. The most compelling argument for this is that nucleoside/nucleotide analogues (NAs) do not eliminate the risk of hepatocellular cancer (HCC) in CHB patients. In such a way, 80 million people may die from liver cancer [25].

During chronic HBV infection, the immune responses are weak in CHB patients, and HBV-specific T cells display the hallmarks of T cell exhaustion, which could be explained by the persistence of HBsAg. Functional cure of HBV infection can only be achieved when therapy targets the high viral burden and weak T cell response, respectively [25]. In other words, activation of both innate and adaptive immune responses is required for durable viral clearance. Such a goal can be achieved by mimicking the spontaneous resolution of HBV infection [15]. The safety and efficacy of long-term administration of the IBDV-R903/78 drug candidate should be tested in virally suppressed HBeAg-negative CHB patients. The IBDV-R903/78 virus containing 1×10^7 IU will be orally administered daily for 24 weeks. The primary objective is to determine the safety of the IBDV-R903/78 drug candidate in CHB patients. The secondary objectives are to determine the efficacy of the IBDV-R903/78 product in eliminating HBV infection in patients with CHB. To this end, the following parameters will be measured: (i) HbsAg, (ii)

HBV DNA, (iii) HBV RNA, (iv) HBV-specific CD8 T cells, and (v) serum transaminase levels.

3) Sequential ultra-low-dose ipilimumab plus nivolumab therapy for those CHB patients who did not achieve a functional cure during IBDV-R903/78 monotherapy

According to earlier experiences [14], long-term oral administration of IBDV alone was capable of eliminating HBV without lysing infected cells. Notwithstanding, functional HBV cure may not be achieved in all patients by IBDV monotherapy alone. The function of the exhausted HBV-specific T cells should be restored by an ultra-low-dose ipilimumab (0.3 mg/kg) plus nivolumab (0.5 mg/kg) therapy, which will be administered following the 24 weeks of IBDV monotherapy. HBeAg- patients were highly responsive to PD-1 blockade, demonstrating significantly improved IFN-γ+ and IL-2+ HBV-specific T cell responses, respectively [26]. Importantly, ultra-low doses of ICI drugs were proven to be safer than the established protocols in 131 unselected stage IV cancer patients without compromising efficacy [27]. To reinvigorate exhausted HBV-specific T cells, CHB patients will receive nivolumab and ipilimumab intravenously (Fig. 5).

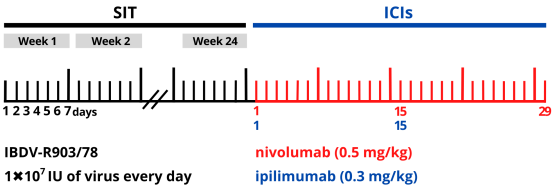

Figure 5. *The planned sequential combination of IBDV and ICI treatment courses for CHB patients.* The IBDV-R903/78 virus drug candidate (1×10^7 IU/dose/day) will be orally administered to HBeAg-negative CHB patients daily for 24 weeks. Then, patients will intravenously receive nivolumab (0.5 mg/kg) on days 1, 15, and 29 (in red) and ipilimumab (0.3 mg/kg) on days 1 and 15 (in blue). Reproduced from Sequential Combination of a Strong Interferon Inducer Viral Vector with Low Doses of Nivolumab Plus Ipilimumab Could Provide a Functional Cure in Chronic Hepatitis B Virus Infections: Technical Report Proposing a New Modality, Cureus 2022 Vol. 14 Issue 3 Pages e22750. Accession Number: 35371882. PMCID: PMC8970536. DOI: 10.7759/cureus.22750. https://www.ncbi.nlm.nih.gov/pubmed/35371882; with permission from Cureus Inc. (see in 8. Site Content and User Submissions; https://www.cureus.com/terms; accessed on 24 January 2024).

We predict that SIT in combination with ultra-low-dose nivolumab plus ipilimumab therapy will have a synergistic therapeutic effect on the innate and adaptive anti-HBV responses. In such a way, a functional cure in CHB patients can be achieved during a finite treatment course. Importantly, such ultra-low-dose ICI therapy will also be affordable in low-income countries where the burden of HBV infection is the highest [25]. Using a fraction of the nivolumab dose approved in the United States and Europe dramatically reduced the financial cost of immunotherapy, increasing access and improving patient outcomes in low- and middle-income countries [28].

Adjuvant IBDV-R903/78 therapy, specifically leveraging the activation of dsRNA sensors, would have the potential to contribute to existing cancer immunotherapies

'Cold tumors' are characterized by the lack of T cell infiltration due to impaired T cell priming and deficient T cell homing to tumor beds. Since antitumor responses depend on the infiltration of tumor-specific T cells, ICI drugs are not effective in cold tumors. In contrast, 'hot tumors' with significant T cell infiltration are associated with better ICI efficacy [29]. Oncolytic viruses (OVs) induce immunogenic cell death (ICD). The reason for this is that lysis of tumor cells results in a massive release of tumor-associated antigens (TAAs), pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs). Treatment-induced ICD by OVs could represent a promising strategy to reshape the 'cold' tumors into 'hot' ones by boosting the efficacy of ICI drugs [30]. Importantly, the STING pathway could also be activated by the orally administered dsRNA IBDV-R903/78 drug candidate without inducing ICD. In such a way, therapeutic benefits could be achieved without the safety issues associated with OVs (such as, for example, fever, seizure, cerebral edema, meningitis). This hypothesis could be tested in HBV-positive advanced hepatocellular cancer (HCC) patients. HCC is two diseases in one, both of which require intensive management: 1) the underlying liver dysfunction frequently caused by HBV infection; 2) the cancer itself. Here, we propose a two-pronged approach to treat both diseases, similar to that proposed above for CHB patients. First, the HBV-caused liver dysfunction should be targeted with orally dosed IBDV-R903/78. This will boost the immune system, changing the 'cold' tumors into immunologically receptive 'hot' ones. Then, the non-lytic viral therapy will be followed by an ultra-low-dose ICI drug therapy targeting the tumor itself while avoiding the well-known immune-related adverse events (irAEs) of standard ICI protocols.

Conclusions

IBDV is a repurposed drug candidate; it can therefore be produced faster, cheaper, less riskily, and with higher success rates than traditional drug development. Importantly, an expert team of US National Institutes of Health-sponsored ACTIV public-private partnership concluded that the IBDV drug candidate shows merit as a potential treatment for COVID-19. The German Paul Ehrlich Institute discussed the conditions for a Phase I COVID trial with the producer of the drug candidate, ProBioGen AG. IBDV is simple to manufacture, and it will be affordable even in

resource-limited countries. Spending many US$ trillions on the next pandemic could be avoided by completing the development of the clinically tested IBDV drug candidate. The comment of Dr. Richard Klausner, former director of the US National Cancer Institute, is worth recalling here: ‘As I go around the country, I talk about the tragedy of cancer to remind people that the tragedy is not our inability to prevent the inevitable or to do the impossible; the tragedy is when a person, a group, or a society fails to achieve the possible’ [31].

Statements and Declarations

Acknowledgements

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We'd like to thank all reviewers for their valuable feedback; most of their suggestions has been addressed in version 2.

Animal subjects

All mouse studies were done in accordance with national and international laws and regulations of animal experiments and were reviewed and approved by the Regional Animal Health Authorities, Csongrad County, Hungary, and by the Joint Local Ethics and Animal Welfare Committee of Avidin Ltd. in possession of ethical clearance entitled, "Investigation of the effect of non-pathogenic microbes and viruses." Issued protocol number XXIX./127/2013.

Conflict of interest statement

Tibor Bakacs declares stock/stock options from HepC Inc. and he is also a shareholder of HepC Inc. L.Z.F. and L.G.P. have nothing to declare.

References

1. ^*Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC: Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. The Lancet Infectious Diseases. 2022. 10.1016/S1473-3099(22)00320-6*
2. ^*Berkley S: Even after COVID, the world's vaccine strategy is failing. Nature. 2022, 612:377. 10.1038/d41586-022-04423-8*
3. ^*Callaway E: Beyond Omicron: what's next for COVID's viral evolution. Nature. 2021, 600:204-207. 10.1038/d41586-021-03619-8*
4. ^*Linder A, Jamieson D: Blind spots in biodefense. Science. 2023, 379:621-621. doi:10.1126/science.adg9237*
5. a, b*Bakacs T, Chumakov K, Safadi R, Kovesdi I: Editorial: Fighting fire with fire: Using non-pathogenic viruses to control unrelated infections. Front Immunol. 2022, 13:1046851. 10.3389/fimmu.2022.1046851*
6. a, b, c, d*Bakacs T, Volker S, Kovesdi I: An Orally Administered Nonpathogenic Attenuated Vaccine Virus Can Be Used to Control SARSCoV-2 Infection: A Complementary Plan B to COVID-19 Vaccination. Cureus. 2022, 14:e28467. 10.7759/cureus.28467*
7. ^*Virgin HW: The virome in mammalian physiology and disease. Cell. 2014, 157:142-150. 10.1016/j.cell.2014.02.032*
8. ^*Moelling K: Viruses: More Friends Than Foes. 2016. 10.1142/10230*
9. a, b*Garcia G, Jr., Irudayam JI, Jeyachandran AV, Dubey S, Chang C, Castillo Cario S, Price N, Arumugam S, Marquez AL, Shah A, Fanaei A, Chakravarty N, Joshi S, Sinha S, French SW, Parcells MS, Ramaiah A, Arumugaswami V: Innate immune pathway modulator screen identifies STING pathway activation as a strategy to inhibit multiple families of arbo and respiratory viruses. Cell Rep Med. 2023, 4:101024. 10.1016/j.xcrm.2023.101024*
10. ^*Rabie AM: RNA: The most attractive target in recent viral diseases. Chem Biol Drug Des. 2023:e14404. 10.1111/cbdd.14404*
11. ^*Wu J, Chen ZJ: Innate Immune Sensing and Signaling of Cytosolic Nucleic Acids. Annu Rev Immunol. 2014, 32:461-488. 10.1146/annurev-immunol-032713-120156*
12. a, b*Ni G, Ma Z, Damania B: cGAS and STING: At the intersection of DNA and RNA virus-sensing networks. PLoS Pathog. 2018, 14:e1007148. 10.1371/journal.ppat.1007148*
13. ^*Cai C, Tang Y-D, Xu G, Zheng C: The crosstalk between viral RNA- and DNA-sensing mechanisms. Cell Mol Life Sci. 2021, 78:7427-7434. 10.1007/s00018-021-04001-7*
14. a, b, c, d*Bakacs T, Safadi R, Kovesdi I: Post-infection viral superinfection technology could treat HBV and HCV patients with unmet needs. Hepatol Med Policy. 2018, 3:2. 10.1186/s41124-017-0028-x*
15. a, b, c*Bakacs T, Safadi R, Puskas LG, Feher LZ, Kovesdi I: Sequential Combination of a Strong Interferon Inducer Viral Vector With Low Doses of Nivolumab Plus Ipilimumab Could Provide Functional Cure in Chronic Hepatitis B Virus infections: Technical Report Proposing a New Modality. Cureus. 2022, 14:e22750. 10.7759/cureus.22750*
16. ^*Bakacs T, Sandig V, Slavin S, Gumrukcu S, Hardy WD, Renz W, Kovesdi I: A clinically validated, broadly active, oral viral superinfection therapy could mitigate symptoms in early-stage COVID-19 patients. Infect Disord Drug Targets. 2022. 10.2174/1871526522666220419130403*
17. a, b, c*Hornyak A, Lipinski KS, Bakonyi T, Forgach P, Horvath E, Farsang A, Hedley SJ, Palya V, Bakacs T, Kovesdi I: Effective multiple oral administration of reverse genetics engineered infectious bursal disease virus in mice in the presence of neutralizing antibodies. J Gene Med. 2015, 17:116-131. 10.1002/jgm.2830*
18. ^*Chumakov K, Benn CS, Aaby P, Kottilil S, Gallo R: Can existing live vaccines prevent COVID-19? Science. 2020, 368:1187-1188. 10.1126/science.abc4262*
19. a, b, c*Bakacs T: Healing of Severe Herpes Zoster Ophthalmicus Within a Few Days: An Autobiographical Case Report. Cureus. 2021, 13:e20303. 10.7759/cureus.20303*

20. ^Finkelstein DB, Mukatira S, Mehta PK, Obenauer JC, Su X, Webster RG, Naeve CW: Persistent host markers in pandemi c and H5N1 influenza viruses. J Virol. 2007, 81:10292-1029 9. JVI.00921-07 [pii];10.1128/JVI.00921-07 [doi]
21. ^Holmes EC: The comparative genomics of viral emergence. Proceedings of the National Academy of Sciences. 2010, 10 7:1742-1746. doi:10.1073/pnas.0906193106
22. ^Mundt E, Vakharia VN: Synthetic transcripts of double-str anded Birnavirus genome are infectious. Proc Natl Acad Sci U S A. 1996, 93:11131-11136.
23. ^Lewis AM, Jr., Krause P, Peden K: A defined-risks approac h to the regulatory assessment of the use of neoplastic cells as substrates for viral vaccine manufacture. Dev Biol (Base l). 2001, 106:513-535.
24. ^Bakacs T, Sandig V, Kovesdi I: Combination Therapy for th e Treatment of Shingles with an Immunostimulatory Vacci ne Virus and Acyclovir. Pharmaceuticals. 2023, 16:226.
25. a, b, cRevill PA, Chisari FV, Block JM, Dandri M, Gehring AJ, Guo H, Hu J, Kramvis A, Lampertico P, Janssen HLA, Levrero M, Li W, Liang TJ, Lim SG, Lu F, Penicaud MC, Tavis JE, Thi mme R, Members of the ICEHBVWG, Chairs I-HSG, Advisors I-HS, Zoulim F: A global scientific strategy to cure hepatitis B. Lancet Gastroenterol Hepatol. 2019, 4:545-558. 10.1016/ S2468-1253(19)30119-0
26. ^Chua C, Salimzadeh L, Ma AT, Adeyi OA, Seo H, Boukhaled GM, Mehrotra A, Patel A, Ferrando-Martinez S, Robbins S H, La D, Wong D, Janssen HLA, Brooks DG, Feld JJ, Gehring AJ: IL-2 produced by HBV-specific T cells as a biomarker of viral control and predictor of response to PD-1 therapy acro ss clinical phases of chronic hepatitis B. Hepatology Commu nications. 2023, 7:e0337. 10.1097/hc9.0000000000000337
27. ^Kleef R, Nagy R, Baierl A, Bacher V, Bojar H, McKee DL, Mo ss R, Thoennissen NH, Szasz M, Bakacs T: Low-dose ipilimu mab plus nivolumab combined with IL-2 and hyperthermi a in cancer patients with advanced disease: exploratory find ings of a case series of 131 stage IV cancers - a retrospective study of a single institution. Cancer Immunol Immunother. 2021, 70:1393-1403. 10.1007/s00262-020-02751-0
28. ^Mitchell AP, Goldstein DA: Cost Savings and Increased Acc ess With Ultra-Low-Dose Immunotherapy. J Clin Oncol. 20 23, 41:170-172. 10.1200/jco.22.01711
29. ^Liu YT, Sun ZJ: Turning cold tumors into hot tumors by im proving T-cell infiltration. Theranostics. 2021, 11:5365-538 6. 10.7150/thno.58390
30. ^Shi T, Song X, Wang Y, Liu F, Wei J: Combining Oncolytic Vi ruses With Cancer Immunotherapy: Establishing a New Gen eration of Cancer Treatment. Front Immunol. 2020, 11:683. 10.3389/fimmu.2020.00683
31. ^Klausner RD: Cancer, minorities & the medically underser ved. Cancer. 1998, 83:1703-1706. https://doi.org/10.1002/(S ICI)1097-0142(19981015)83:8+<1703::AID-CNCR7>3.0.CO; 2-Y

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