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Uses and Challenges of Antiviral Polyclonal and Monoclonal Antibody Therapies

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Abstract: Viral diseases represent a major public health concern and an ever-present risk for developing into a future pandemic. Antiviral antibody therapeutics, either alone or in combination with other therapies, have emerged as valuable preventative and treatment options, including during a global emergency. Here we will discuss polyclonal and monoclonal antiviral antibody therapies, focusing on the unique biochemical and physiological properties that make them well suited as therapeutic agents. We will describe the methods of antibody characterization and potency assessment throughout development, highlighting similarities and differences between polyclonal and monoclonal products as appropriate. In addition, we will consider the benefits and challenges of antiviral antibodies when used in combination with other antibodies or other types of antiviral therapeutics. Lastly, we will discuss novel approaches to the characterization and development of antiviral antibodies and identify areas that would benefit from additional research.

Keywords: antiviral therapy; antiviral antibodies; antibody combination therapy; antibody potency; potency assays

Introduction

Infectious diseases are a major global health burden with eight major diseases (HIV/AIDS, malaria, measles, hepatitis, dengue fever, rabies, tuberculosis and yellow fever) exacting a toll estimated at more than 156 million life years lost in 2016 alone[1]. The coronavirus disease 2019 (COVID-19) pandemic has further exacerbated the cost in human life and long-term health outcomes. Emerging and re-emerging viral diseases, such as Ebola, Zika, Hantavirus, Lassa fever, Hendra, highly pathogenic avian influenza, and others, continue to pose a risk not only for local/regional outbreaks but for becoming the next pandemic. The availability of safe and effective prophylaxis and treatment options for these and other infectious diseases is a top public health priority. Antibody therapeutics have long been used in communicable disease settings, for example post-exposure prophylaxis for rabies or hepatitis B with respective hyper- or specific- immune globulin (IG), or the use of monoclonal antibody (mAb) therapies for the prevention of respiratory syncytial virus (RSV) infection. Recent approvals of mAb therapies for human immunodeficiency virus type-1 (HIV-1) and Ebola virus (EBOV), as well as the rapid development and emergency use authorization of several mAbs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) for prophylaxis and treatment of COVID-19, further highlight the potential of these molecules, either alone or in combination with other therapies, to make a significant impact on public health. In this review, we will discuss the biochemical and physiologic characteristics that render antibody molecules desirable therapeutics, preclinical assays that can be used to assess potency, and the benefits and challenges of antibody combination therapies, as well as highlighting areas in need of additional research.

Antibodies as therapeutics

With very few exceptions, antibody therapeutics approved to date are isotype G immunoglobulins (IgG). IgGs are protein macromolecules secreted in the blood of most vertebrates[2] by differentiated plasma B cells that have a high affinity and specificity for their respective antigen. The IgG molecules can then be purified from human or animal plasma to produce polyclonal immune globulin products. These types of products, such as diphtheria antitoxin[3], represent some of the first products to be licensed in the United States. In over a century of development, polyclonal products have undergone tremendous advances in the manufacturing process and characterization of safety and efficacy attributes. In the last few decades, antibody therapeutic development has shifted toward the development of monoclonal antibodies - IgG molecules that are produced *in vitro* after mature B cells have been isolated, immortalized, and cultured.

The structural and functional features of IgG antibodies render them well suited for use as therapeutics. Structurally, the molecule can be thought of as modular, with two identical heavy chains (HC) and two identical light chains (LC). The IgG HC is comprised of four domains, a variable (V) domain and three constant (CH1, CH2, and CH3) domains with a hinge region between the CH1 and CH2 domains (Figure 1a). The LC is comprised of two domains, a variable (V) domain and a constant (CL) domain. The fragment antigen binding (Fab) region in each chain contains both V and constant (CH1 or CL) domains, with the former housing the complementarity determining regions (CDR) responsible for epitope recognition and antibody specificity. When properly folded, the CDRs of the HC and LC come together to form the antigen-binding pocket. The fragment crystallizable (Fc) region, comprised of the HC CH2 and CH3 domains, is responsible for downstream processes (Fc effector functions) that result in immune activation and the ultimate destruction of the antigen. There are four different IgG isotypes (IgG1, IgG2, IgG3 and IgG4), with polymorphic variants for each isotype[4]. In addition, IgG antibodies have a single N-glycan in the constant region. These biochemical properties (i.e., sequence and glycan structures) play an important role in physicochemical (i.e., stability, shelf-life), pharmacokinetic, and pharmacodynamic properties of the antibody therapeutic, and thus should be well characterized during development.

The use of IgG products as prophylactic and therapeutic modalities for viral diseases is predicated on their ability to bind to one or more antigens on the surface of viral particles and/or infected cells via the antigen-binding pockets. They can neutralize the ability of viruses to enter cells by blocking attachment or fusion, inactivate virus particles, or trigger the killing of infected cells through Fc-mediated effector functions (Figure 1b)[5]. For the latter, the antigen-antibody complex is recognized by effector molecules, such as the C1q component of complement or Fc gamma receptors (FcγRs) present on the surface of effector cells, giving rise to immune signaling cascades that culminate with the clearance of viruses and/or infected cells. In some cases, Fc effector functions have been shown to enhance the antiviral activity of specific antibodies[5-9].

On the other hand, antibody-dependent enhancement (ADE) of viral infection or disease can also occur, as has been documented in humans for dengue virus[10]. ADE can arise after natural infection, vaccination, or passive transfer of antibody therapies. It is widely thought that ADE occurs when antibodies of insufficient avidity or concentration are unable to neutralize the virus, but can facilitate the uptake of the virus-antibody complex by FcγR-bearing cells such as monocytes, dendritic cells, or macrophages[11], resulting in increased viral production, enhanced immune activation (e.g., cytokine production), and more severe disease[12]. In addition to flaviviruses[13, 14], ADE has been observed for mAbs against influenza virus, HIV-1, and EBOV in cell culture, but not typically when tested in animal models or in clinical trials, with a few exceptions[11, 13]. The risk of ADE can be reduced by engineering substitutions into the Fc region that disrupt FcγR binding, although these substitutions may also disrupt Fc effector functions that could contribute to clinical efficacy[15, 16]. Thus, when selecting antibodies best suited for use as an antiviral product, it is critical to optimize binding both to the antigen and FcγRs. For mAbs, IgG isotypes can be selected, Fc glycosylation patterns can be modified, or Fc regions can be engineered with substitutions that enhance or diminish select Fc effector functions. Although ADE in cell culture and animal studies has been observed with antiviral specific polyclonal immune globulins (IG)[17], clinical ADE has not been reported for any FDA-approved specific IG products.

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During pharmaceutical development, mAb domains often undergo extensive biochemical engineering to optimize the properties of the antibody. For example, the CDRs can be grafted onto the framework regions of V domains from other mAbs and still retain their antigen binding properties in the context of a known protein fold. The Fc region can also be modified to alter pharmacokinetic properties and effector functions. On the other hand, although not subjected to Fc engineering, depending on the antigen or donor population, specific antiviral polyclonal IGs can be "enriched" for a particular isotype[18], subclass, or glycosylation signature, leading to different Fc effector functions compared to other polyclonal IG products. For example, IgG1 and IgG4 are the most prevalent subclasses following measles infection or vaccination, with significant differences in titers in infected versus vaccinated individuals[19]. In addition, anti-SARS-CoV-2 antibodies from convalescent donors can have distinct glycosylation patterns depending on disease severity[20]. We will discuss some of the methods currently used to design, produce, and characterize antibody products, highlighting the differences between polyclonal and monoclonal antibody therapies.

a) b)

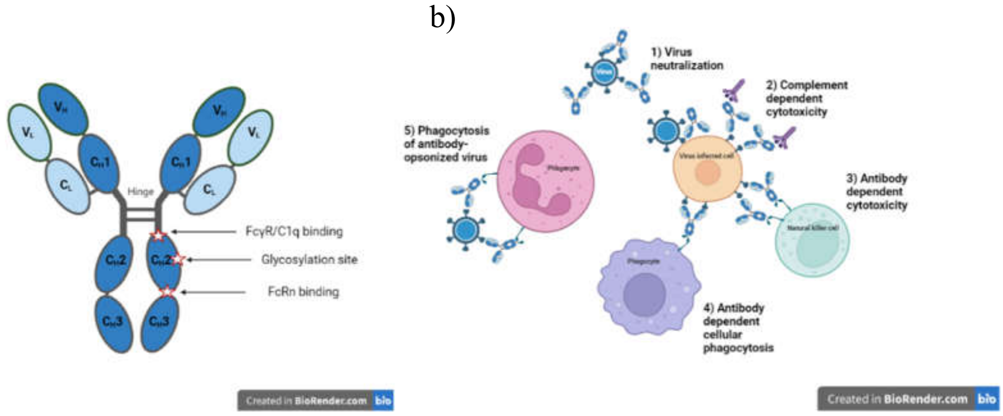

Figure 1. Structural and functional features of isotype G immunoglobulins (IgG). 1a. Structural features of IgG antibodies. The IgG macromolecule is a tetramer of two identical heavy (H) and light (L) chains depicted in dark and light blue, respectively, each containing variable (VH, VL) and constant (CH, CL) regions, as shown. The glycosylation site and the locations responsible for receptor and complement binding, are marked. These regions can be engineered to modulate downstream properties of IgG products. 1b. Antiviral functions of IgG antibodies. Antiviral pharmacologic properties of antibody therapies include 1) neutralization of viral entry to its cell target 2) complement and 3) antibody-mediated cytotoxicity of infected cells, 4) phagocytosis of infected cells and 5) clearing of opsonized virus by phagocytosis.

Production and Characterization of Antibody Therapies

Specific Polyclonal Antibody Therapies

Specific polyclonal immune globulins (SpIG) are purified from pooled animal or human plasma. The first products were developed in 1898 and were comprised of little more than serum from horses vaccinated with live viruses, bacterial toxins, or snake venom. In 1903, diphtheria antitoxin made from vaccinated horses became the first licensed product in the United States. Research during World War II stimulated a major breakthrough in purification of IGs and other proteins from human plasma. IG purification methods are based on sequential alcohol precipitations, each with specific conditions of pH, ionic strength, temperature, protein concentration, and alcohol concentration[21, 22].

For some products, purely chromatographic methods or caprylate precipitation methods have partially or completely supplanted alcohol precipitation. These changes are often driven by the need to increase yield of IgG (thus increasing availability)[23]. Nevertheless, alcohol-based fractionation remains as the backbone of early steps in production of IG products and is often combined with subsequent caprylate or polyethylene glycol precipitations. Modern IG products are further purified using column chromatography to remove unwanted plasma proteins. In addition, a minimum of two orthogonal, robust, dedicated viral clearance steps are performed, which often include solvent-detergent treatment and nanofiltration, as well as other virucidal (caprylate, heat treatment, low pH) and partitioning (chromatography, precipitations, depth filtration) steps. All viral clearance steps must be validated and found to be robust using scaled-down models of the manufacturing process and actual manufacturing intermediates spiked with virus as starting material. It should be emphasized that modern IG purification is highly complex with multiple steps, each of which must be controlled to result in a safe and intact product. Every manufacturing method is unique with respect to purification details and methodology (such as mixing speeds, equipment used, precipitation times, buffer types and concentrations, centrifugation vs. precipitation), and equipment. Thus, each product is also unique with respect to levels and types of plasma protein impurities and IG stability.

Specific polyclonal IG is used as the overarching term for all polyclonal preparations that are enriched for certain antiviral, antibacterial, or antitoxin antibodies. Antibody enrichment for human antibodies is achieved by either immunizing donors, or screening and selecting high-titer plasma from routine donations (as for Cytogam[24]) or convalescent donors (as for early versions of SARS-CoV-2 IG investigational products[25, 26]). "Hyperimmune" polyclonal antibodies are derived from animal or human donors who have been immunized intentionally for the purpose of obtaining high titer plasma (e.g., rabies, anthrax, and tetanus immune globulins). Nevertheless, convalescent plasma is often inaccurately referred to as "hyperimmune," even though donors were not immunized. Under FDA-approved plasma center collection protocols, and after investigational safety studies are completed, hyperimmune plasma can be collected from consenting immunized donors.

For purposes of final product testing, a validated bioassay demonstrating neutralization in cell culture or in animals, is ideally performed for SpIG products. In special cases, adequate cell culture or animal models are not available at the time of licensure. In this situation, a binding assay has usually been selected and validated for product release contingent on discussions with FDA. Likewise, national or international IgG standards may be lacking. In these instances, an internal IgG standard is developed by the manufacturer.

Table 1. FDA-approved polyclonal antibodies for the prevention or treatment of viral diseases.

Target	Trade Name(s)	Donors	Indications	Used With
Rabies[27-29]	HyperRAB, Imogam, KedRab	Vaccinated	Post-exposure prophylaxis	Rabies vaccine (required, see prescribing information for rabies IG)
Varicella[30]	VARIZIG	Donations selected from high-titer donors after natural infection*	Post-exposure prophylaxis in patients at risk for severe infection	Concomitant use of acyclovir reported to occur in clinical practice[31]
Vaccinia[32]	Vaccinia Immune Globulin (Human)	Vaccinated	Treatment of severe complications after smallpox vaccination	Investigational antiviral drugs and/or cidofovir[33, 34]

Cytomegalovirus (CMV)[24]	CytoGam	Donations selected from source plasma	Prevention of CMV disease in patients receiving organ transplants from CMV donors	Ganciclovir recommended in prescribing information; other drugs recommended in practice guidelines[35]
Hepatitis A (HAV)[36]	GamaSTAN	Regular donors	Pre- and Post-exposure prophylaxis	None
Hepatitis B (HBVIG/IGIV)[37-39]	HyperHEP B, Nabi-HB	Vaccinated	Post-exposure prophylaxis	None
	HepaGam-B	Vaccinated	Post-exposure prophylaxis Prevention of HBV recurrence in HBsAg+ liver transplant recipients	Concomitant treatment with other drugs recommended in practice guidelines[40]
Measles[36,†]	GamaSTAN	Regular donors	Prevention or attenuation of measles in susceptible individuals	None
Rubella[28]	GamaSTAN	Regular donors	To modify rubella in exposed pregnant women who will not be undergoing a therapeutic abortion	None

*GamaSTAN may be used only if VariZIG is not available[36]. †In patients receiving IG products to correct antibody deficiencies, doses of intravenous IG (IVIG) that should prevent measles infections for travelers to measles-endemic areas are suggested[41].

Antiviral SpIG products licensed in the United States are shown in Table 1. A number of SpIGs (human, bovine, or equine plasma-derived) are under investigation for other viral infections, including SARS-CoV-2 (at least 12 studies in clinicaltrials.gov, e.g., including an International

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Network for Strategic Initiatives in Global HIV Trials study, NCT04910269) and influenza (NCT04850898). SpIGs from animal sources are produced by hyperimmunizing donor animals. Advantages of large animal donors (horses, sheep, or cattle) include the ability to immunize more frequently (which increases the yield and avidity of specific antibodies), to use experimental vaccinations, and to safely collect larger volumes of plasma. A major disadvantage includes potential allergic reactions in patients due to animal proteins, including the active ingredient. Animal-derived antibodies are often treated with pepsin or trypsin, to remove the Fc portion and reduce immunogenicity. These fragments lack effector functions that could be important for antibody activity, depending upon the virus. An interesting strategy has been developed using transchromosomic cattle that produce full-length human IgG antibodies. The cattle are knocked out for bovine antibody heavy and lambda light chains but contain an artificial chromosome encoding the respective human IgG chains. Chimeric antibodies consisting of human IgG heavy chains and bovine kappa light chains are removed during manufacturing[42], thus the resulting IG product manufactured from these bovines contain only human IgGs, thus lowering the risk of immunogenicity. These transchromosomic bovines have been successfully hyperimmunized[43].

Treatment timing and dosing for SpIG.

Treatment timing relative to infection depends on demonstrable efficacy of the product for pre- or post-exposure prophylaxis. Pre- and post-exposure prophylaxis can be effective (if adequately dosed) largely because viral burdens are relatively low. Even if an infection has been initiated, post-exposure prophylaxis attenuates disease severity of measles, HAV, and varicella zoster[30, 36]. When vaccines are given concomitantly with specific IG, such as for rabies, passive immunization provides a defensive "bridge" that acts immediately to neutralize the virus until vaccine responses arise. It is important that the dose of rabies IG (RIG) is not so high that it suppresses the vaccine response. In such contexts, both a minimum and maximum potency should be defined to assure optimal function of both RIG and the vaccine. Pharmacokinetic studies performed in healthy immunocompetent human subjects are used to define the dose of SpIG that is needed to avoid suppression of vaccine responses yet still be able to provide protection until vaccine responses are sufficiently developed.

Treatment of symptomatic viral disease with SpIG is much more challenging and often ineffective. In these cases, the viral burden may exceed the capacity of the IG, viruses may be relatively inaccessible within infected cells, and cellular immune responses may also be suppressed by the virus[44]. Notable lack of efficacy by specific IG for treatment of symptomatic infections such as rabies, influenza, HAV, HBV, measles, and varicella have been observed. The time windows for effective post-exposure prophylaxis of each infection have been established based on such failures. Treatment with CMVIG and HBVIG(IV) can prevent symptomatic disease in transplanted patients but are not curative. Vaccinia Immune Globulin is used to treat severe complications (eczema vaccinatum and progressive vaccinia) caused by live vaccinia virus vaccine (ACAM2000), which is used to prevent smallpox. Recently licensed replication-deficient vaccinia virus (Jynneos) generates an immune response but is thought to be incapable of causing eczema vaccinatum or progressive vaccinia. Both vaccines are indicated for prevention of smallpox. Jynneos is also licensed for prevention of monkeypox[45].

Monoclonal Antibodies

To date, the FDA has approved four mAb therapies to prevent or treat viral diseases (Table 2): palivizumab for prevention of RSV in preterm infants and infants with other specific conditions, ibalizumab for treatment of HIV-1 in patients failing their current anti-retroviral regimen, and two products for treatment of Ebola virus disease caused by *Zaire ebolavirus*. One of these products, Inmazeb, consists of three mAbs that target non-overlapping epitopes on EBOV glycoprotein and represents the first co-formulated mAb cocktail approved by the FDA[46].

Multiple mAbs are currently in advanced stages of clinical development or have been approved in other countries. Nirsevimab, a half-life extended mAb that targets the RSV fusion (F) protein [47], was recently approved by the European Medicines Agency for the prevention of RSV lower

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respiratory tract disease in neonates and infants during their first RSV season. In addition, three mAb products targeting the rabies virus glycoprotein have been approved in other countries: two in India (Rabishield, a single mAb, and TwinRab, a cocktail of two mAbs[48]), and one in China (ormutivimab[49]).

Several mAbs and mAb combinations that target the SARS-CoV-2 spike protein were rapidly developed after the onset of the COVID-19 pandemic and received emergency use authorization (EUA) from the FDA for the pre-exposure prophylaxis, post-exposure prophylaxis, and/or treatment of COVID-19. Although highly effective against early SARS-CoV-2 variants, these products are not currently authorized in the United States due to the emergence and widespread circulation of variants that are resistant to neutralization by these mAbs in cell culture[50-56]. However, if future variants emerge that are susceptible to these products, their authorization status may change. Refer to the FDA website for updated information on the status of EUAs for mAbs and other COVID-19 therapeutics[57].

In addition to the approved and previously authorized mAbs and those directed against SARS-CoV-2, many other mAbs have been or are under development against existing and emerging diseases (see [58-61] for some examples).

Table 2. FDA-approved monoclonal antibodies for the prevention or treatment of viral diseases.

Non-proprietary name	Trade name	Target	Indication
palivizumab	Synagis	RSV F protein	for the prevention of serious lower respiratory tract disease caused by RSV in pediatric patients (specific conditions and age limitations)
ibalizumab	Trogarzo	CD4 (post attachment HIV-1 inhibitor)	in combination with other antiretroviral(s), for the treatment of HIV-1 infection in heavily treatment-experienced adults with multidrug resistant HIV-1 infection failing their current antiretroviral regimen
atoltivimab, maftivimab, odesivimab-ebgn	Inmazeb	Ebola virus glycoprotein	for the treatment of infection caused by *Zaire ebolavirus* in adult and pediatric patients, including neonates born to a mother who is RT-PCR positive for *Zaire ebolavirus* infection
ansuvimab-zykl	Ebanga	Ebola virus glycoprotein	for the treatment of infection caused by *Zaire ebolavirus* in adult and pediatric patients, including neonates born to a mother who is RT-PCR positive for

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	Zaire ebolavirus infection

Historically, therapeutic mAbs were derived from immunized mice or rats and engineered as chimeric or humanized mAbs to reduce the immunogenicity due the "foreignness" of rodent mAbs in humans. Currently, most mAbs are of human origin, derived from "humanized mice" that express human germline V(D)J region genes, or from phage display libraries generated from human donor lymphocytes. However, many antiviral mAbs are isolated directly from previously infected patients (see[58, 62-64]). Regardless of the source, many considerations inform the selection and engineering of candidate mAbs.

Fc engineering approaches: Most mAbs developed for viral diseases are selected first for their ability to neutralize virus entry. However, Fc effector functions play a major role in the immune system's response to infectious diseases[8]. For mAbs, the contribution of Fc effector functions to disease protection has been demonstrated for several viruses including Ebola virus[65], HIV-1[6,66, 67], influenza[68], SARS-COV-2[69], and Rift Valley fever virus[70]. However, ADE of infection or disease is a possible negative consequence of FcγR binding[71-73]. Therefore, depending on what is known about specific viral diseases, different approaches are used to engineer the Fc region of mAbs to either enhance or diminish FcγR binding. Amino acid residues have been identified in the IgG Fc region that contact the complement component C1q, FcγRs, or the neonatal Fc receptor (FcRn), which is responsible for the long half-life of IgG (reviewed in[74, 75]). Substitutions can be engineered at these residues to alter Fc effector functions or extend the half-life of a mAb, which allows less frequent dosing[76].

In addition to Fc engineering, there is a better understanding of specific Fc glycan structures and their association with different effector functions, e.g., afucosylated mAbs have better antibody dependent cellular cytotoxicity (ADCC) compared to highly fucosylated antibodies, and galactosylation is associated with complement dependent cytotoxicity (CDC) and can influence ADCC activity[77] . Therefore, cell lines have been engineered to produce mAbs with up to 100% afucosylation to enhance ADCC activity[74, 78]. The understanding of the relationship between antibody glycan structures and Fc effector functions is ongoing and additional strategies may be developed to further engineer mAb glycan structures. For example, the effect of galactosylation on ADCC activity may depend on the specific linkage of the galactose monosaccharide[79]. Fc effector functions can be reduced by introducing substitutions at the glycosylation site (N297) in the CH2 domain to prevent the addition of a glycan[80, 81], thus providing another glycoengineering approach for antiviral mAbs.

Other approaches for the development of mAbs: Three of the four approved monoclonal antiviral products are single mAbs, but the anti-Ebola virus mAb cocktail of atoltivimab, maftivimab, odesivimab-ebgn was the first fixed dose co-formulated mAb combination product approved by the FDA. Many other mAbs to treat viral diseases and for other indications are used in combination, but only a few to date are co-formulated[82]. The advantage of antibody cocktails over a single mAb is that they might be less susceptible to escape, depending on the different targeted epitopes. As seen for the anti-SARS-CoV-2 mAb combinations previously authorized for the prophylaxis or treatment of COVID-19, they all target the SARS-CoV-2 receptor binding domain of the SARS-CoV-2 spike protein but have little neutralization activity against current variants. MAbs that target regions outside the receptor binding domain could neutralize virus or mediate Fc effector functions and might be less susceptible to escape. For example, a recent report demonstrated that mAbs targeting the conserved fusion peptide region adjacent to the S2′ cleavage site of the spike protein are broadly neutralizing against betacoronaviruses[83].

Advantages and Disadvantages of Polyclonal and Monoclonal Antibodies

There are advantages and disadvantages when selecting a product for treatment or prophylaxis of viral diseases, some of which are summarized in Table 3. For approved products, the choice is often based on which products are available for a specific viral disease. For example, currently only SpIG products are approved in the United States to treat rabies, CMV, HBV, varicella or vaccinia, whereas only mAb therapies are approved to prevent or treat RSV, HIV-1, and EBOV disease. There are other considerations that also play a role in development or deployment of antibody therapies in

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an infectious disease setting. Although resistance to polyclonal antibodies has been reported[84], polyclonal antiviral products are less likely to result in treatment-emergent resistance, or the formation of anti-drug antibodies, whereas both issues are a larger concern for mAb products. On the other hand, given the relative ease of engineering, development, and production of mAbs, they are well suited for rapid development, especially in an emerging infectious disease setting. Both types of products can have drawbacks that include the potential to interfere with the immune response to the vaccine or natural infection, as well as specific diagnostics, and the potential to result in enhanced infection or disease, as already described. Despite these limitations, the benefit-to-risk ratio for these approved products is favorable, as demonstrated in clinical trials and by routine clinical use in viral disease settings.

Table 3. Advantages and disadvantages of specific polyclonal and monoclonal antibodies for the prophylaxis or treatment of viral diseases.

	Specific Polyclonal Antibodies	Monoclonal Antibodies
Advantages	• React with multiple target epitopes on the same or different viral proteins • Contain more than one type of neutralizing antibody • Less susceptible to resistance • Potential for higher avidity • Mix of IgG isotypes provides expanded potential for Fc effector functions	• Highly specific for a single well-defined epitope • Can be engineered in V domain for affinity, avidity, specificity, and/or the Fc region to enhance or abrogate Fc effector functions or extend half-life. • Can combine multiple mAbs targeting different epitopes or proteins • Can be manufactured and released using platform strategies, which allows rapid entry into clinical trials • Batch-to-batch consistency
Disadvantages	• Large potentially variable pool of donors who must be screened for pathogen safety • Potential for antibody dependent enhancement of infection/disease • May dampen immune responses after vaccination with live viruses (e.g., measles, mumps, etc.). • Can interfere with diagnostic assays • Presence of low amounts of other plasma proteins, which could contribute to adverse events	• Individual mAbs susceptible to resistance • Potential for antibody dependent enhancement of infection/disease • May dampen immune responses after vaccination • Can interfere with diagnostic assays • Can induce the formation of anti-drug antibodies

Evaluation of Antiviral Activity

Prior to being evaluated in clinical studies as antiviral therapies, biological activity and potential mechanisms of action of antibodies are investigated in preclinical studies performed in model systems. In practice, assays to assess antibody activity usually fall into three broad categories: biochemical (e.g., binding) assays, cell culture assays, and animal models. Early during the pharmaceutical development of the antibody therapies, these assays are performed as part of candidate selection and then to characterize the antibody product that is being developed. Multiple such assays can be performed with the goal of understanding different aspects of antibody antiviral activity. Examples include antibody binding affinity, epitope characterization, neutralization activity, and assays to characterize Fc effector functions. Some of these assays will be developed as quality control potency assays to ensure lot-to-lot consistency and stability of the product. Federal regulations define potency as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data… to effect a given result." (21 CFR. 600.3(s)). Thus, for antiviral antibody therapies, potency assays provide a quantitative measure of the antibody activity linked to its primary mechanism of action. Fit-for-purpose potency assays are often performed prior to initiation of Phase 1 clinical studies, and full validation is completed by the time of a biologics license application (BLA) submission. FDA guidance describing current thinking on the development and validation of such studies for mAbs is available in draft form at the time of writing this article[85]. Some points to consider when designing preclinical studies to evaluate antiviral activity and assess potency are discussed below.

Types of Potency Assays

SpIGs and mAbs may exert their antiviral effects via one or more potential mechanisms: virus neutralization, ADCC, opsonization and phagocytosis, complement lysis and/or complement dependent cytotoxicity [86-88]. There are just a few examples of antibodies potentially acting at virus post-entry steps[87, 88] but their role in the overall antiviral humoral immune response is still to be established. If SpIGs or mAbs under clinical development have multiple mechanisms of action, multiple assays are developed and implemented for quality control. In general, the selected potency methods should reflect the product's proposed mechanism as closely as possible. Potency is usually evaluated by a comparison to an appropriately qualified reference standard and is expressed as a percentage of the reference material value. For SpIGs, international or national standards are often used, e.g., for anti-rabies, anti-hepatitis B virus, or anti-measles IGs. The potency is then expressed in international units or alternative units, as appropriate. As for all quality control release methods, key assays for demonstrating the antiviral mechanism(s) of action should be shown to be suitable for their intended purposes during development and validated by the time of an application for approval. Ideally, potency assays which adequately reflect the proposed mechanism(s) of action should be qualified and implemented before pivotal clinical trials[85].

Antibody-antigen binding is a necessary step for both virus neutralization and Fc effector functions. Therefore, binding assays such as an enzyme-linked immunosorbent assay (ELISA) or a surface plasmon resonance (SPR) assay are a logical approach to evaluate drug potency. There is significant experience with these types of assays, and they may be easier to qualify and validate compared to cell-based methods used to assess the antiviral activity of therapeutic antibodies. In general, potency binding assays are developed and used during early stages of product development. However, direct binding assays may not provide a comprehensive assessment of the product's mechanism of action. For antibodies targeting virus-cellular receptor(s) interactions, inhibitory binding assays (ELISA or SPR) may better reflect their mechanism of action, but even these assays may not fully represent the antibody-mediated suppression of the complex virus-cell fusion process. Furthermore, broadly neutralizing antibodies may target complex, conformation-dependent, non-linear epitopes which can be challenging to reproduce in a binding assay.

In comparison to binding assays, cell-based methods can provide a more comprehensive assessment of antibody-mediated antiviral activity, either via virus neutralization and/or Fc effector functions[85].

There is already significant expertise with the development and validation of cell-based ADCC potency assays for a variety of mAbs for the treatment of different neoplasms[89] and the qualification/validation of methods to evaluate the ADCC activity of antiviral antibodies follow the same general principles. However, challenges remain regarding the selection and qualification of relevant target and effector cells employed in these assays (discussed later in the manuscript).

Virus neutralization assays can employ authentic (wt) viruses, replication-competent pseudotyped virions, or cell-fusion capable, but replication- incompetent pseudotyped virus-like particles (VLPs). Pseudotyped viruses and VLPs are considered safer alternative methods for

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studying a growing number of viruses which pose enormous health and socioeconomic risks because of their high pathogenicity (including Ebola, Sudan, Marburg, Hendra and Nipah viruses, SARS, and MERS) or their capacity to cause a widespread pandemic (HIV-1, SARS-CoV-2, certain influenza virus A subtypes). Furthermore, highly pathogenic viruses require biosafety level-3 (BSL-3) or BSL-4 facilities, which have high costs and limited availability, impeding the successful development of new therapeutic modalities.

Pseudotyped viruses, also referred to as chimeric viruses[90], are typically generated by replacing the gene(s) expressing the surface glycoprotein(s) of a virus with low pathogenicity (e.g., vesicular stomatitis virus) with the gene(s) encoding the envelope (Env) glycoprotein(s) of a BSL-3 or a BSL-4 pathogen (e.g., EBOV), thus creating a replication-competent virus that can be used in a BSL-2 environment[90-95]. However, in the case of HIV-1, replication competent, infectious pseudotyped viruses were created within the same species by replacing the original envelope gene with the one from a different HIV-1 strain, usually for the purpose of studying virus tropism and/or neutralization susceptibility[96-101]. In general, *in vitro* infection with either wt or pseudotyped replication competent viruses involves a self-spreading infection among the target cells unless the time of the assay is shortened by design. Reporter genes are often inserted into the genome of pseudotyped viruses to assess the level of infection[90, 94, 95, 101-103] as an alternative to measuring viral proteins, nucleic acids, or cytopathic effects[91, 98, 100, 104]. Target cells, stably transfected to express a reporter gene(s) when infected can also be used to quantify virus infection and assess the activity of antivirals, including neutralizing antibodies[101, 105-109].

In addition to pseudotyped viruses, fusion-competent, but replication-incompetent VLPs can be used to assess virus fusion and entry. VLPs are produced by co-transfecting producer cells (usually 293T cells) with a plasmid(s) encoding the desired virus surface glycoprotein(s) and a plasmid (or plasmids(s)) encoding viral proteins necessary for VLP production. In general, VLP contain either an incomplete or no viral genome which renders them capable of just a single round of virus entry followed by a partial or no virus replication. Currently, VLPs have been successfully generated for both enveloped and non-enveloped viruses [102, 110-120]. For the generation of enveloped VLP, retroviral (HIV-1 or murine leukemia virus derived) or rhabdoviral (VSV) based packaging vector systems are commonly used[110, 119], although other vectors are also described[101, 108, 121]. As with the pseudotyped viruses, to facilitate the assessment of VLP cell fusion and entry, VLP are often engineered to include a reporter gene encoding an enzyme or a fluorescent protein (luciferase, alkaline phosphatase, β-galactosidase, green fluorescent protein), where expression reflects the level of infection[97, 99, 102, 103, 113, 114, 122].

Alternatively, VLPs can be used to infect stably transfected cell lines containing a reporter gene under the control of a viral regulatory protein[123-125]. The TZM-bl cell line, stably transfected with the luciferase and β-galactosidase genes under the control of the HIV-1 long terminal repeat promoter, activated by the HIV-1 tat protein, is probably the best-known example of this approach[95, 106, 109, 124, 126].

A more elaborate VLP system, based on EBOV minigenomes that encode a reporter gene, has been designed to study almost all aspects of the EBOV life cycle in a BSL-2 environment [112, 127]. This system may potentially be used for the development and/or screening of anti-EBOV antibodies, but its applicability for this purpose remains to be demonstrated.

It should be noted that for reporter gene encoding virus particles (wt/pseudotyped viruses or VLPs), the reporter gene expression depends not only on the virion-cell fusion, but also on post-entry events leading to the synthesis of the encoded protein. To study solely the viral cell entry process, replication competent virions or VLPs have been designed to incorporate an enzyme or a fluorescent protein that is expressed in producer cells. This is achieved by utilizing vectors that encode chimeric molecules, consisting of the "reporter protein" fused to a viral protein, which directs the entire molecule into the budding virions[112, 128-133]. The assays that employ "reporter protein" containing virions can be a valuable tool to study virus entry inhibitors, but currently there are limited data, compared to the reporter gene-based methods, regarding their use for the assessment of virus neutralizing antibodies[132].

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A broad range of enveloped viruses belonging to different families and including human pathogens, can induce cell-cell fusion between infected cells and neighboring non-infected cells[134]. This phenomenon served as the basis for the development of assays measuring the level of fusion between virus surface glycoprotein-expressing cells (effector cells) and cells expressing the relevant virus receptor(s) (target cells) via quantitation of giant, multinuclear cells (syncytia) formation, fluorescent dye transfer, or reporter gene expression. The virus envelope-mediated cell-cell fusion assays are a useful tool for assessing virus-cell fusion inhibitors, including neutralizing antibodies, as a rapid surrogate for the virus entry methods. Also, like the pseudotyped and VLP systems, cell-cell fusion assays allow studying in BSL-2 environment of viruses that are otherwise restricted to a higher level of biocontainment (BSL-3 or BSL-4)[107, 135-138].

The variety of methods which can be used to evaluate the effects of neutralizing antibodies raise the issue of how these methods compare to each other regarding their sensitivity and ability to predict a correlation between the *in vitro* and *in vivo* results. To address this issue, efforts have been made to apply a standardized approach among different labs for the assessment of antibody-mediated virus neutralization[124, 139, 140].

In general, multiple *in vitro* binding and cell-based methods are used for antibody characterization during product development, with the goal of defining the antibody's mechanism(s) of action and the critical quality attributes potentially affecting its anti-viral activities, as both the neutralization and Fc -mediated effects of the antibody are being investigated. Assays employing wt infectious viruses are likely to remain an important part of product characterization and serve as a basis for comparison with the alternative virus neutralization methods. However, as mentioned earlier, biosafety concerns may limit the use of authentic BSL3-3 and BSL4-4 pathogens. The use of pseudotyped viruses and/or VLP can offer less restrictive biosafety requirements and may facilitate antibody characterization in several additional ways: **a.** pseudotyped viruses/VLP can be more readily manipulated allowing faster assessment of potential mutations in the virus surface glycoproteins **b.** the level of entry may be easier to quantify, and **c.** panels of pseudotyped VLP, representing a wide range of virus strains that are generated using the same packaging system, can be created and tested for their neutralization susceptibility[126, 141-143]. Ideally, the pseudotyped viruses or VLP should closely resemble the corresponding authentic viruses, but certain differences may exist regarding shape, glycosylation, and density of the envelope glycoproteins due to the packaging system and/or producer cells used. The incubation times, readout methods and target cells may also be different[140, 144]. However, similar neutralization sensitivity was demonstrated when replication competent HIV-1 and HIV-1 Env pseudotyped VLP (generated using an HIV-1 derived packaging system) were both produced in 293T cell line and tested on the same target cells[99, 106]. Also, similar neutralization profiles were observed for spike protein pseudotyped VLP and authentic SARS-CoV-2 in Vero E6 target cells[90].

Usually, one or more of the characterization methods are adapted to become a potency assay(s) for the purpose of drug substance and drug product release and stability testing. Development and implementation of an adequate potency assay(s) is a critical quality control measure to ensure that each lot is consistently produced with the potency necessary to achieve clinical efficacy and that such potency is maintained over the shelf life of the product. Data regarding the validation of certain commonly used pseudotyped virus/VLP assays have already been published[101, 109]. It should be emphasized that adequate qualification of the assays' critical reagents is an integral part of the validation process. Detailed information regarding the generation, quantitation, and stability of the virus/VLP stocks should be provided. Determination of the ratio of functional (infectious, or fusion-capable) to non-functional virus/virus-like particles may also be important for qualification of the virus stocks [140]. Finally, control measures should be in place to ensure consistency in the performance of the target cells in the virus neutralization assays or both the target and effector cells in the virus envelope mediated cell-cell fusion assays [85].

Cells for Potency Assays

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For most viruses, there are a wide variety of cell lines that can be used to assess the potency of polyclonal and monoclonal antibodies with antiviral activity. For example, for SARS-CoV-2 alone, cell lines that have been used to assess antibody neutralization activity include Vero/Vero E6 (African green monkey kidney), 293/293T (human embryonic kidney), HeLa (human cervical carcinoma), Huh7 (human hepatocellular carcinoma), Calu-3 (human lung carcinoma), HT1080 (human fibrosarcoma), U2OS (human osteosarcoma), and HOS (human osteosarcoma) cells, often engineered to stably express human angiotensin-converting enzyme 2 (ACE2, the SARS-CoV-2 receptor) and/or transmembrane serine protease 2 (TMPRSS2, a protease involved in SARS-CoV-2 entry)[50, 52, 55, 145-150].

When selecting an appropriate cell line for the evaluation of antibodies, a large number of factors can be considered: physiologic relevance, activity to be measured (e.g., neutralization, ADCC, etc.), desired assay throughput and readout (e.g., plaque formation), feasibility of using primary cells in lieu of immortalized cell lines, expression of host factors, tissue and species origins, and potential for amino acid polymorphisms in the receptor or coreceptor that might affect activity. For example, if an animal cell line will be used, species-specific differences in receptor or coreceptor expression levels and/or amino acid sequences may affect antibody activity. Likewise, if immortalized cell lines will be used, the results may not accurately reflect those obtained with primary cells. For example, several research groups have reported that the neutralization activity of some anti-SARS-CoV-2 mAbs is affected by ACE2 expression levels, leading to variable potency (in terms of both the half-maximal effective concentration [EC_{50}] values and maximal percent inhibition) in different cell lines[145, 151-153]. If primary cells will be used, it may be beneficial to test cells from multiple donors (ideally of different sexes and ethnicities) to assess variability in potency. For viruses that infect multiple cell types (e.g., herpesviruses), either due to expression of the same receptor on multiple cell types or the use of multiple receptors, antiviral activity can be assessed in distinct cell types.

In addition to neutralization assays, cell type is an important factor to consider for other types of antibody assays as well, including assessments of Fc effector functions (e.g., ADCC, ADCP, CDC), ADE, and other types of studies. Characterization of the effector function is a consideration for both monoclonal and polyclonal antibodies. In general, these assays have not been well standardized, and it is often unclear which cell types and Fc effector functions are most likely to be relevant for clinical efficacy. To further complicate matters, Fc effector function assays often involve at least two cell types: target cells expressing the antigen and immune effector cells that respond to the IgG-bound antigen. In most cases, target cells consist of a cell line (e.g., CHO, Jurkat, or 293T) that has been transiently transfected or engineered to constitutively or inducibly express viral antigen(s), such as envelope protein. For example, Fc effector functions were assessed for the FDA-approved anti-EBOV mAbs (Inmazeb and Ebanga) using target cell lines with inducible expression of the EBOV glycoprotein[154, 155]. Alternatively, virus or viral-like particles may be used as targets in some assays (e.g., for ADCP). For effector cells, common cell types that have been used include Jurkat (immortalized human T) or NK-92 (immortalized human NK-like) cells engineered to stably express specific FcγRs and primary human cell types, such as NK cells, monocytes, monocyte-derived macrophages, or peripheral blood mononuclear cells. Many manufacturers have developed reporter cell lines (often Jurkat-based) to quantify FcγR activation as a surrogate for ADCC, and some research has been performed to compare these assays to classical ADCC assays[156, 157]. Fc effector function assays can be performed with cells that express different FcγRs and FcγR variants, e.g., the FcγRIIIa F158 and V158 variants, which have distinct binding affinities for IgG1 and IgG3[158].

The selection of cell lines for assessment of ADE is also an important issue, particularly for viruses in which ADE is known to be a significant issue (e.g., Dengue and Zika viruses). For these viruses, ADE has been assessed using K562 (FcγRIIa+ human erythroleukemia), Raji (human B lymphoblastoid), U937 (human myeloid leukemia), THP-1 (human monocytic leukemia), and primary monocytes or macrophages[159-162]. In many cases, mAbs are engineered to have Fc substitutions that are expected to enhance, diminish, or abrogate Fc effector functions and/or ADE. In these cases, cell culture studies can be performed to verify that the substitutions have the intended effects. It may be beneficial to test multiple versions of a mAb in parallel to identify one with optimal properties, i.e., versions with an unmodified Fc region, different Fc substitutions, or distinct Fc

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glycosylation patterns. Lastly, cell lines for other types of studies should also be carefully selected, such as for studies of antibody resistance mechanisms, cell-cell transmission, and cell-cell fusion or syncytium formation.

Resistance

As part of development, it is critical to characterize the resistance mechanisms and pathways of antiviral antibodies in preclinical (and later clinical) studies. There are two different mechanisms by which viral resistance can develop. Naturally occurring resistance arises as the virus naturally evolves, as strains with increased fitness (e.g., due to enhanced infectivity, replication, or immune escape) become dominant in the human population. This type of resistance is unrelated to treatment but may lead to treatment failure or non-response. In contrast, treatment-emergent resistance happens in response to the specific antibody treatment, with treatment providing the selective pressure for the emergence of the resistant variant. The potential for antiviral antibody therapies to be affected by either form of resistance should be assessed in preclinical studies. These studies are often highly valuable for informing the dose and dosing interval, optimization of treatment regimens (e.g., mAb monotherapy vs. mAb combinations vs. mAbs+other antivirals), interpretation of clinical resistance data, identification of patients infected with susceptible viral variants, likelihood of cross-resistance with other mAbs (important for rescue/salvage therapy), and genomic surveillance efforts. Approaches for resistance characterization vary widely depending on the virus, antibody, and antibody target. For example, approaches for SARS-CoV-2 have included testing the effects of single amino acid substitutions in the spike protein (S) on antibody binding in biochemical assays, screening large libraries of S proteins with substitutions using a yeast display system, determining the effects of S substitutions on antibody neutralization in cell culture using pseudotyped VLPs, and performing resistance selection in cell culture and animal models with replication-competent chimeric (i.e., VSV-spike) and authentic viruses[147, 163-166]. For mAbs that target host proteins, resistance could potentially arise from genetic polymorphisms that alter the expression or sequence of the host protein, rather than through changes in the virus. In these cases, differences in host genetics (e.g., single-nucleotide polymorphisms) can be assessed by bioinformatics and, if necessary, biochemical or cell culture studies.

Some studies performed to assess resistance, particularly those involving the selection or characterization of replication-competent authentic or recombinant viruses, can raise significant ethics and biosecurity concerns. These studies are conducted only under appropriate biocontainment and in strict accordance with all applicable institutional, local, regional, and national guidelines and regulations. In some cases, it may be possible to adequately characterize antibody resistance using biochemical or cell culture assays that involve only the viral antigen or a replication-defective virus. In other cases, it may be possible to select for antibody resistance using a replication-competent virus that poses less risk, such as a replication-competent chimeric virus, a related animal virus that expresses the same epitope but cannot infect human cells, or a deliberately attenuated version of a human virus. One caveat of these approaches is that the substitutions observed in these viruses may not accurately predict the resistance substitutions observed with authentic virus. When an authentic virus must be used due to unavailability of other approaches or inadequate resistance information from such approaches, risk can be mitigated by using an authentic virus that is susceptible to current vaccines and antivirals and to which vaccine-induced or natural immunity is already widespread.

Animal Studies

Animal studies can be a powerful tool for assessing the safety and efficacy of antiviral antibody therapies prior to commencing clinical trials. To be useful, an animal model should recapitulate as closely as possible the critical aspects of human disease, including susceptibility to the pathogen, route of infection, viral tropism, severity of outcomes, pathophysiology of systemic and end organ disease as applicable, and host responses. Although non-human primates, being evolutionarily closest to humans, are most likely to fulfill these criteria, many other mammalian species have been successfully used as models of viral disease, including for assessing antibody therapies. Examples include mice for West Nile virus, ferrets for influenza, cotton rats for RSV, and hamsters for hantavirus and SARS-CoV-2[167].

The role of animal studies in the development of antiviral therapies can be illustrated by the recent COVID-19 pandemic. For SARS-CoV-2, initial cell culture studies focused on identifying pathways underlying viral entry, tropism, molecular pathways of disease processes, and mechanisms for neutralization in cell lines and organ-like systems, with the ultimate goal being the discovery of effective preventive and therapeutic strategies, including antibody therapies. Although critical for identifying and measuring neutralization activity of antiviral antibodies, cell culture studies such as these cannot account for *in vivo* activity or their distribution in the mucosal or lung tissue - often the point of entry and viral replication for SARS-CoV-2. Studies in animal models, including hamsters, transgenic mice, ferrets, and non-human primates validated the findings from cell culture studies and demonstrated the potential for efficacy, including of anti-SARS-CoV-2 antibodies[168-175]. Similar studies were used to support EUA packages for mAbs that received such authorizations (https://www.fda.gov/drugs/coronavirus-covid-19-drugs/cder-scientific-review-documents-supporting-emergency-use-authorizations-drug-and-biological). These studies demonstrated that monoclonal and polyclonal antibodies have the potential to protect against disease when used as prophylaxis and to improve outcomes when used as a therapeutic, thus providing preliminary data to support their investigation in clinical trials. They also demonstrated that ADE was not observed.

A critical question for advancing any therapeutic modality to clinical trials is related to the starting dose, which should be both safe and potentially efficacious. Although the definite proof of safe and effective dose will come from well-designed and adequately controlled clinical trials, a scientific, data driven rationale should inform the dose(s) chosen to advance to the clinic. Data supporting safety are obtained from nonclinical toxicology, safety pharmacology and other pertinent studies, as outlined in the appropriate FDA guidance documents[176]. Deriving a potentially efficacious dose, on the other hand, is not as standardized, and multiple approaches can be applied based on the availability of cell culture and animal models, new or existing pharmacokinetic (PK) and pharmacodynamic (PD) data in both animals and humans, physiologically based pharmacokinetic (PBPK) models, and biomarkers that correlate with efficacy. To continue with the example of the recent pandemic, the data submitted by sponsors in EUA packages sheds light on some of the methods used[57]. For all these antibodies, a necessary component and often the first step in efficacious dose estimating was identification of the antibody concentration providing 90% of maximal effect (EC_{90} value), then target clinical exposures that exceed this level, taking into account antibody distribution to the respiratory tract. Animal data can assist in deriving such concentrations, even through direct measurements or through methods to relate serum to tissue concentrations such as antibody biodistribution coefficient or allometric scaling[177]. It should be noted that anatomical and physiological characteristics differ, depending on the species and the degree of phylogenetic similarity with humans. Thus, biodistribution of the antibody to the site of action (such as lung or gastro-intestinal tract) may be quite different. This may also be further influenced by variability in disease presentation and pathological processes, for example.

Other considerations that influence the translatability of efficacy data from animal studies include differences in expression patterns of Fc receptors on effector cells and the affinity of the human antibodies to the animal orthologs[178, 179]. In addition, Fc modifications intended to alter FcRn or FcγR binding may not have the same effects in animals and humans. For example, M252Y/S254T/T256E, referred to as "YTE" variant, has a half-life four times longer than unmodified IgG1 in humans but a rapid clearance in rodents[57, 180]. To overcome these limitations, transgenic and humanized mouse models that incorporate human FcγR genes have been developed[181, 182].

In certain infectious disease settings, specifically when clinical trials are not feasible or ethical, adequate and well-controlled animal studies can be used to provide substantial evidence for efficacy through a pathway known as approval under the Animal Rule. Detailed advice on considerations when developing a therapy under this pathway are described in the pertinent FDA guidance[183]. The guidance outlines many clinical and nonclinical aspects of such programs, including considerations for choosing the animal models, such as the challenge agent, susceptibility to disease, mechanisms of virulence, pathophysiology and its comparison with human disease (natural history studies), trigger for intervention, mechanism of action for the treatment, dose, and the necessary studies that can be used to derive a dose and dosing regimen in humans (such as PK and/or PD studies in animals and humans). It should be noted that seeking regulatory approval under the Animal Rule is not a way to

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circumvent performing clinical studies or to simplify the approval process for a therapeutic. Safety and PK studies in humans are still needed, whereas performing studies to adequately characterize at least two animal models, often undertaken under high containment conditions in BSL-4 facilities, adds significant complexity to this process. This is reflected by the number of approvals: a total of sixteen drugs are approved under this rule but none of them are antiviral antibodies, although there are four antibodies targeting bacterial infections or toxins (please refer to the FDA database for up-to-date information, see FDA webpage[184]).

Combining Antiviral Antibodies and Other Therapies

In the following sections we will discuss combination therapies containing antiviral antibodies, highlighting potential benefits and challenges. We will also provide examples from clinical practice and scientific literature of the use of antiviral antibodies with other drugs or biologics. The intent is not to endorse any specific combinations, but to give a panoramic view of the potential for treatments that, if proven safe and effective, can help combat viral diseases and make an impact on public health.

Combinations of Specific Polyclonal Antibodies with Vaccines or Drugs

The combination of SpIG with vaccines for pre- or post-exposure prophylaxis has been longstanding, dating back to historical use of live vaccinia virus for smallpox vaccination in combination with Vaccinia Immune Globulin, to prevent complications in patients with risk factors for serious adverse events such as eczema vaccinatum[185]. In contrast, vaccine-SpIG combinations for rabies[186], HAV[187], and HBV[37-39] are used to prevent viral spread until host vaccine responses are fully developed. In these settings, SpIG is effective if given early enough after exposure, when viral burdens are low and before an effective vaccine response can develop. This "window of opportunity" to neutralize virus with SpIG so as to prevent viral spread is defined for passive immune therapies for rabies (0-7 days)[27-29], HAV (0-2 weeks)[36], measles (0-6 days)[36], HBV (0-14 days; 0-12 hours for infant born to infected mother; as soon as possible to recipient of HBV+ blood)[38, 39], and varicella zoster (0-4 days)[30].

Several principles are common to vaccine-SpIG combination treatments. First, the vaccine and SpIG if given at the same time, should always be administered at different anatomic sites (separate limbs). Secondly, live vaccines should be withheld for 3-6 months after SpIG because other antibodies contained in the IG products may interfere with effectiveness.

Combinations of Specific Polyclonal Antibodies with Drugs

The combination of SpIG and drugs is most often used in transplant settings, where the goal is suppression of viral activation and clinical disease, and prevention of organ damage. The combination of SpIG and an antiviral drug provides orthogonal methods for viral control or clearance. For example, CMVIGIV is used often in combination with ganciclovir or similar drugs, to prevent cytomegalovirus disease associated with transplantation of kidney, lung, liver, pancreas or heart[24]. Acyclovir and related drugs are reportedly used with varicella IG sometimes, based on clinical judgement, and there have not been clinical trials directly comparing VARIZIG alone or with the addition of acyclovir[32]. Hepatitis B virus (HBV) Immune Globulin (Hepagam B only) is licensed as a monotherapy to prevent recurrence of HBV in HBV-infected liver transplant recipients[39]. However, clinical guidelines recommend use of HBIG + nucleoside analogs for liver transplant patients at higher risk for endogenous HBV recurrence, or to prevent transmission of HBV from a transplanted organ (infected donor)[40]. Several nucleoside analogs are now licensed for treatment of HBV. Finally, Vaccinia Immune Globulin (VIG) has been used to treat complications of smallpox vaccination. In immunocompromised SCID mouse models of progressive vaccinia, frequent administration of VIG staves off clinical signs of disease and death. However, when treatment stops, the mice succumb to viral infection several months later[188]. Progressive vaccinia that occurs in immunocompromised patients after vaccinia exposure may be slowed or halted by VIG although complete resolution of infection is thought to coincide with improved function of the immune system

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based on anecdotal reports[189]. The ability of VIG + cidofovir to eliminate vaccinia in a proportion of SCID mice suggests that antibody + drug treatment could be advantageous for severely immunocompromised patients[188]. Generally, treatment of eczema vaccinatum and progressive vaccinia are combined with investigational antivirals and/or cidofovir [33, 34]. Controlled human studies of adjunct drug treatments are not currently feasible due to rarity of severe vaccinia infections.

Combinations of Monoclonal Antibodies

The first mAb combination approved for a viral disease, Inmazeb, consists of three mAbs that have non-overlapping epitopes and can simultaneously bind to EBOV glycoprotein: atoltivimab, maftivimab, and odesivimab. All three mAbs are human IgG1 antibodies without substitutions in the Fc regions. In nonclinical assays, these mAbs had distinct mechanisms of action: atoltivimab neutralized virus and mediated Fc effector functions, including FcγRIIIa activation (used as a surrogate of ADCC) and ADCP. Maftivimab neutralized virus but did not activate FcγRIIIa or ADCP. Lastly, odesivimab did not neutralize virus but activated FcγRIIIa and ADCP[155]. In addition, as noted above, several mAb combinations were previously authorized by the FDA for the pre-exposure prophylaxis, post-exposure prophylaxis, or treatment of COVID-19: casirivimab+imdevimab (REGEN-COV), bamlanivimab+etesevimab, and cilgavimab+tixagevimab (Evusheld). Casirivimab and imdevimab are human IgG1 antibodies with unmodified Fc regions that target non-overlapping spike epitopes, neutralize virus, and activate ADCC and ADCP. Bamlanivimab and etesevimab are human IgG1 antibodies with unmodified (bamlanivimab) or LALA-modified (etesevimab) Fc regions that target partially overlapping spike epitopes, neutralize virus, and activate FcγRIIIa (bamlanivimab only[190, 191]). Cilgavimab and tixagevimab are human IgG1 antibodies with YTE-TM-modified Fc regions to extend antibody half-life, reduce Fc effector functions, and minimize the risk of ADE[191, 192]. They target non-overlapping spike epitopes, neutralize virus, and did not mediate Fc effector functions in cell culture. In addition, mAb combinations are currently under development for many other viruses, including CMV, HIV-1, influenza virus, and rabies virus[48, 193-196]. For general regulatory advice on the codevelopment of mAbs, we encourage readers to refer to the appropriate guidance document, which applies only to drugs and biologics regulated by CDER[197].

Potential Benefits of Monoclonal Antibody Combinations

Synergy. The activity of mAb combinations in cell culture is often additive, i.e., similar to what would be predicted based on the potency of the individual mAbs and the molar ratio at which they are combined. However, in some cases, mAb combinations may be antagonistic (discussed below) or synergistic in terms of neutralization activity and/or Fc effector functions. For example, several mAb combinations with synergistic neutralization activity have been identified for HIV-1 and SARS-CoV-1[198-201]. Relative to individual mAbs, synergistic interactions between mAbs could lead to enhanced antiviral activity or comparable activity at lower concentrations, potentially leading to reduced or less frequent dosing and fewer adverse reactions in patients. In some cases, mAb combinations might also have synergistic effects on viral replication or disease in patients. However, in clinical trials of mAb combinations for viral diseases, individual mAbs are often not tested due to concerns about resistance, such that it is not possible to determine whether the combinations are synergistic in humans. Thus, these types of studies are often best performed in animal models.

Greater Breadth of Activity. As individual mAbs are highly specific for a single epitope, their activity can be significantly affected by naturally occurring amino acid polymorphisms in or near the epitope. Thus, mAb combinations are often designed to target non-overlapping epitopes on the same viral protein, leading to broader activity against different virus types, genotypes, subtypes, and/or variants than individual mAbs. For example, the anti-rabies virus mAbs R172 and R173, which target non-overlapping sites on the viral glycoprotein, are being developed in combination to ensure sufficient breadth of activity against viral variants circulating in North America[193]. However, mAb combinations have also been developed that target different viral proteins or overlapping epitopes on the same viral protein. For example, the anti-CMV mAbs LJP538 and LJP539 target different viral proteins, while the anti-SARS-CoV-2 mAbs bamlanivimab and etesevimab target partially overlapping epitopes on the same protein[191, 202]. In addition to the number of mAbs, epitope conservation is an important factor to consider. In principle, a single mAb that targets a highly conserved epitope could have broader activity than a combination of several mAbs that target poorly conserved epitopes.

Reduced Likelihood of Resistance. Relative to individual mAbs, combinations of mAbs are generally less susceptible to the development of treatment-emergent resistance, as resistance would likely require amino acid changes in or near both epitopes. Thus, resistance may develop less frequently or with delayed kinetics relative to individual mAbs. For example, in several studies of anti-SARS-CoV-2 mAbs, resistance was readily selected in cell culture with single mAbs but not combinations of mAbs targeting non-overlapping spike epitopes[163, 166, 203]. In a clinical trial, SARS-CoV-2 resistance emerged less frequently with a mAb combo (bamlanivimab+etesevimab) than with one of the mAbs alone (bamlanivimab), although it should be noted that these mAbs target partially overlapping epitopes and an etesevimab-only control arm was not included[204]. Likewise, HIV-1 resistance to mAbs is thought to be less likely to develop clinically with mAb combinations as opposed to single mAbs[205]. Note that this potential benefit of mAb combinations may only apply when both mAbs are active against the viral variant at the time of treatment initiation.

Multiple Mechanisms of Action. Like individual antibodies, mAbs in combinations may have multiple mechanisms of action, including neutralization and Fc effector functions. However, in contrast to individual antibodies, mAbs in combinations can be designed to specialize in different functions. This is perhaps best illustrated by Inmazeb, which, as described above, contains three mAbs: one with only neutralization activity, one with only Fc effector function activity, and one with both activities in cell culture[155]. However, the relative contribution of each antibody and each mechanism of action to clinical efficacy is often unclear (see below).

Potential Challenges of Monoclonal Antibody Combinations

Resistance. In principle, mAb combinations are expected to have broader activity and to be less susceptible to resistance than individual mAbs. However, these issues represent significant challenges for mAb combinations as well. For example, most anti-SARS-CoV-2 mAb combinations were found to have significantly reduced activity in cell culture against the SARS-CoV-2 Omicron BA.1 variant, which emerged in November 2021. According to a recent review, casirivimab+imdevimab (REGEN-COV), bamlanivimab+etesevimab, and cilgavimab+tixagevimab (Evusheld) had 840-, 740-, or 75-fold reduced neutralization activity (based on geometric mean EC_{50} values), respectively, against BA.1 in cell culture[206]. As another example, in a recent Phase 1 clinical trial of a combination of three anti-gp120 mAbs for the treatment of HIV-1, participants had transient decreases in viral load, followed by the rebound of viruses with partial or complete resistance to two of the mAbs in cell culture[207]. The authors hypothesized that at least four broadly neutralizing mAbs may need to be combined for HIV-1 treatment to provide broad activity and prevent resistance.

Uncertain Contribution of Each mAb to Clinical Efficacy. In mAb combinations for viral diseases, it is often unclear to what extent each mAb contributes to clinical efficacy. The FDA generally requests sponsors who are developing combinations of two or more investigational drugs to demonstrate that the combination is more effective than each single drug alone[197]. However, nonclinical data demonstrating that the combination is superior to the single drugs in some aspect (e.g., better activity or less resistance) can be considered sufficient when the combination is intended to treat a serious disease or condition and there is a strong rationale for the use of the combination (e.g., prevention of resistance). In the cases of FDA-approved and previously authorized mAb combinations for viral diseases, individual mAbs were not tested in the trials that evaluated clinical efficacy. Thus, there is significant uncertainty about the extent to which each mAb contributes to clinical efficacy, and whether the mAb combination would retain efficacy against viral variants resistant to one of the mAbs in cell culture.

Uncertain Contribution of Neutralization vs. Fc Effector Functions to Clinical Efficacy. As for individual antibodies, mAbs in combinations often have multiple mechanisms of action that include neutralization and Fc effector functions, unless the Fc regions have been modified to disrupt effector functions. Thus, it is often unclear to what extent neutralization and Fc effector functions contribute to clinical efficacy. Likewise, it is often unclear if a mAb combination found to lack neutralization activity against a particular viral variant in cell culture could at least partially retain clinical efficacy through Fc effector functions. To further complicate matters, cell culture assays to assess neutralization and Fc effector functions are poorly standardized, and it usually unclear which Fc effector functions or assays are the most relevant to clinical efficacy. Such challenges have frequently arisen with anti-SARS-CoV-2 mAbs, due to the continued emergence of novel variants with varying degrees of susceptibility to neutralization by mAbs and mAb combinations. These issues can be addressed in animal models by comparing different versions of mAbs (e.g., unmodified Fc vs. modified Fc with disrupted or enhanced FcγR binding), as has been done for SARS-CoV-2[208-211]. However, the extent to which animal models can predict clinical efficacy remains unclear, as animals have differences in FcγRs and IgG-FcγR interactions compared to humans.

Other Challenges. In addition to the issues described above, other challenges for the development of mAb combinations for viral diseases include the potential for antagonism between mAbs, particularly those with partially overlapping epitopes, low distribution of mAbs to some sites of interest (e.g., 6.5-15% for lung epithelial lining fluid)[212-214], lack of standardization of nonclinical assays and reagents, leading to highly variable results across assays, potential diminishment of mAb activity by soluble antigen (or subviral or virus-like particles), potential for cross-resistance with other mAbs that have the same target, potential interference with diagnostic assays, potential attenuation of the immune response after vaccination or infection, potential for the development of anti-drug antibodies, and uncertainty about the optimal dose and, in the case of repeated administration, dosing schedule.

In addition to challenges related to clinical outcomes, there are some chemistry, manufacturing, and controls (CMC) challenges related to co-formulated mAbs. These include methods that can identify all mAbs in the drug product and that they are present at a consistent ratio in each lot; and understanding the nature of aggregates due to co-formulation and high concentrations. Multiple potency assays may be needed if the mAbs have different mechanisms of action, for example neutralization or Fc-mediated effector functions. Another challenge from both the CMC and clinical standpoints is when drug product is diluted in an IV solution and large volumes are infused. The contribution of potential endotoxin from both the drug product and the diluent should be considered. The compendial limit for infusion solutions is not more than 0.5 endotoxin units (EU) per mL[215]. Depending on the volume of diluted drug product to be delivered, the endotoxin release criteria for drug product may need to be adjusted to comply with the endotoxin limits for patients of less than 5.0 EU/kg/hour.

Combinations of Monoclonal Antibodies with Other Types of Antivirals

In addition to mAb combinations, there are many examples of mAbs and mAb cocktails being used in combination with other types of antivirals for viral diseases (Table 4). These mAbs target either a viral glycoprotein or a host receptor or co-receptor. The antivirals include approved drugs (e.g., oseltamivir), approved drugs being studied against a different virus (e.g., remdesivir), and investigational drugs (e.g., VIR-2218, a small-interfering RNA [siRNA]). These antivirals belong to many different classes, including nucleos(t)ide analog reverse transcriptase inhibitors, RNA-dependent RNA polymerase inhibitors, protease inhibitors, capsid inhibitors, neuraminidase inhibitors, fusion inhibitors, coreceptor antagonists, therapeutic vaccines, latency-reversing agents, and immunomodulators. In most cases, the mAbs and antivirals target different viral proteins (or a viral protein and a host protein). However, there are also cases of mAbs being combined with antivirals that target the same protein, such as the combination of leronlimab and maraviroc, which both target the HIV-1 coreceptor CCR5[216].

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Table 4. Examples of Combinations of mAbs and Other Types of Antivirals.

Virus	mAb(s) (Target)	Antiviral(s)	Stage	References
HBV/HDV	VIR-3434 (HBsAg)	VIR-2218±NrtI±pegIFN	phase 2	NCT04856085*, also see[217, 218]
HCV	various (HCV receptors)†	Various†	preclinical	[219]
HIV-1	teropavimab+zinlirvimab (gp120)	various‡	phase 1-2	NCT04811040* Also see[220, 221]
HIV-1	leronlimab (CCR5)	approved antiretrovirals	phase 2-3	[216, 222]
HIV-1	ibalizumab (CD4)	approved antiretrovirals	approved	[223]
IAV	various (HA) §	oseltamivir	phase 2	[194]
IAV	CR9114+F3A19 (HA)	favipiravir	preclinical	[224]
MARV	MR186-YTE (GP)	remdesivir	preclinical	[225]
SUDV	ADI-15878+ADI-23774 (GP)	remdesivir	preclinical	[226]

*Clinicaltrials.gov study number †The mAbs tested were OM-7D3-B3 (anti-CLDN1 mAb), NK-8H5-E3 (anti-SR-BI mAb), and QV-6A8-F2C4 (anti-CD81 mAb). The antivirals tested were HCV NS3/4A protease inhibitors (simeprevir, danoprevir, boceprevir, telaprevir), NS5A inhibitors (daclatasvir), and NS5B nucleotide analog polymerase inhibitors (sofosbuvir). ‡The therapeutics being tested in combination with these mAbs in clinical trials include FDA-approved antiretrovirals (e.g., the HIV-1 capsid inhibitor lenacapavir) and investigational drugs, such as peptide fusion inhibitors, therapeutic vaccines, latency-reversing agents, and immunomodulators (e.g., pegylated interferon). §The mAbs tested in combination with oseltamivir in clinical trials include CT-P27 (a combination of two mAbs), MEDI8852, MHAA4549A, and VIS410. Abbreviations: GP, glycoprotein; HA, hemagglutinin; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV-1, human immunodeficiency virus type-1; IAV, influenza A virus; mAb, monoclonal antibody; MARV, Marburg virus; NrtI, nucleos(t)ide analog reverse transcriptase inhibitor; pegIFN, pegylated interferon-α; SUDV, Sudan virus.

As is the case for mAb cocktails, the major benefits of combinations of mAbs and other antiviral drugs include the potential for synergy, broader activity against different viral variants, and reduced likelihood of resistance. Many of the combinations listed in Table 4 were reported to have synergistic effects on viral replication or disease progression in cell culture and/or animal models. For example, several mAbs targeting HCV receptors were found to exhibit synergistic activity against HCV when combined with approved drugs for HCV in cell culture and human liver-chimeric mice[219]. Likewise, several mAbs targeting HA were found to exhibit synergistic activity against IAV when combined with oseltamivir, an FDA-approved influenza neuraminidase inhibitor, in mice and ferrets[227-230]. While little clinical data are available for most of these combinations, the combination of VIR-3434, a mAb that targets HBsAg, and VIR-2218, an siRNA that targets HBV gene expression, was found to have enhanced antiviral activity (in terms of serum HBsAg reductions) than either drug alone in a phase 2 trial[217]. In other studies, combinations of mAbs targeting the MARV or SUDV glycoproteins and remdesivir were found to extend the therapeutic window of antiviral therapy in MARV- or SUDV-infected rhesus macaques[225, 226]. The authors hypothesized that such combinations might also extend the therapeutic window in humans. Other potential benefits of these combinations include enhanced antiviral activity across different tissues (due to different drug distribution profiles), broader antiviral activity, shorter treatment schedules, reduced doses, and lower risk of adverse events (due to reduced doses or dosing durations). For some viral diseases, these combinations may not have major benefits relative to the individual drugs for the general patient population but could still prove useful for specific sub-populations, such as patients who are immunocompromised or transplant recipients, cannot tolerate, do not respond, or have contraindications to standard-of-care therapies, have drug-resistant virus, or have severe disease.

Challenges for the development of combinations of mAbs and other antiviral drugs can include insufficient breadth of antiviral activity, the development of resistance, and the uncertain contribution of each drug to clinical efficacy, particularly in cases where the individual drugs cannot be tested alone (e.g., due to concerns about resistance). For example, although mAbs and other antiviral drugs usually target different proteins, resistance may still arise because the exposure of one drug in a particular tissue is low or because the patient is infected with a viral variant that is already resistant to one of the drugs at the time of treatment initiation. In other cases, these combinations may fail to improve antiviral activity or clinical efficacy relative to the individual drugs but lead to higher rates of adverse events. Combinations that include mAbs or antiviral drugs that target host proteins may result in toxicities or have variable activity across patients due to differences in host genetics. Given that mAbs and other antiviral drugs will usually have different dosage forms and half-lives, it may also be difficult to determine the optimal dosing regimen, or the regimen might be complex, leading to problems with patient adherence and increasing the chance of drug resistance.

Future Directions and Conclusions

As the applications of antiviral antibody therapies expand, there are several areas that can benefit from further development. The international standardization of assays and reagents (e.g., viruses and cell lines) for measuring antibody activity could help address the variability in potency often observed for the same antibody in different assays or laboratories (e.g., 10-100-fold range in EC_{50} values for anti-SARS-CoV-2 mAbs)[231]. In addition, more work is needed to better understand the role of Fc effector functions in viral diseases using cell culture, animal models, and clinical studies. Furthermore, preclinical assays are being developed that are more physiologically relevant, especially potency assays that capture multiple functions of the antibody. For example, organ-on-a chip and microphysiological systems can incorporate multiple cell types including immune cells, simulate blood flow and organ perfusion, and provide data that serves as a bridge between standard cell culture assays and clinical studies. Although still early in development and not commonly used in regulatory applications, such technologies are expected to become increasingly powerful and more widely used. These systems can also help address ethical concerns and societal pressures to replace, reduce, and refine animal research, and they have the potential to provide information that is more predictive of clinical efficacy.

Another area with unharnessed potential, especially for SpIG therapies, is the selection of specific glycosylation signatures to modulate downstream immune responses[232]. These strategies have been proposed for use in the setting of autoimmune disease, but they could potentially be applied to viral diseases as well. When combined with other novel technologies, such as the production of recombinant IG preparations[233], such methods have the potential to result in antiviral antibody preparations with improved properties.

Production of SpIG from convalescent plasma in a pandemic setting remains time-consuming and challenging. Convalescent plasma is often the earliest available antibody-containing treatment that could be effective for prevention of severe disease. Advances in technologies that can be used to rapidly and inexpensively select donations containing high titers of neutralizing antibodies (from among thousands of donations) are needed both for direct use of convalescent plasma and manufacturing of SpIG. Biosensor-based methods that reliably measure neutralizing potency in plasma donations and products, and that can be used in a low biocontainment (BSL-2) setting are promising. In addition to improved donor screening, technical advances in manufacturing that would maximize the yield of SpIG during a pandemic could include affinity matrices or changes in manufacturing steps to allow virus-specific IgM to copurify with IgG.

For mAbs, large volumes of product are usually infused intravenously for several hours. The length of infusion time may depend on the amount of mAb needed per body weight and whether a patient is experiencing infusion-related reactions typical of mAbs. One strategy to reduce the time of infusion is to co-formulate a high concentration of the mAb with recombinant human hyaluronidase[234]. The recombinant human hyaluronidase degrades hyaluronic acid in the extracellular matrix, facilitating rapid delivery of large volume subcutaneous injections and bioavailability of the product. This has already been accomplished with several mAbs for oncology, including the combination of rituximab, trastuzumab, daratumumab or trastuzumab and pertuzumab with recombinant human hyaluronidase[235]. This approach is being studied with an anti-HIV mAb (clinicaltrials.gov #NCT03538626). These formulations provide more convenient dosing for patients.

Other developments for anti-viral mAbs include bispecific antibodies, single domain antibodies derived from camelids, and other scaffold proteins such as DARPIns and Adnectins that are engineered in the loop regions between more structured regions of the core domain to mimic antibody CDRs[236-239]. Some of these technologies may be able to target epitopes that are difficult for traditional antibodies to recognize. Furthermore, these novel constructs may be more cost effective to manufacture than mAb cocktails and lower doses may be as effective as higher doses of a mAb cocktail. However, clinical studies are needed to determine efficacy and safety and to see if there are issues, such as immunogenicity, related to these novel products.

Whether alone or in combination, antiviral antibody therapies can provide important prophylaxis and treatment options to help relieve the burden of viral diseases. This space is rapidly evolving, and, as more experience is gained through successful clinical applications, the products of the future have the potential to overcome many of the challenges we describe, while continuing to fulfill the promise of safety and effectiveness.

References

1. Armitage C. The high burden of infectious disease. *Nature*. 2021;598(S9)
2. Parra D, Takizawa F, Sunyer JO. Evolution of B cell immunity. *Annu Rev Anim Biosci*. Jan 2013;1:65-97. doi:10.1146/annurev-animal-031412-103651
3. *Science and the Regulation of Biological Products*. Center for Biologics Evaluation and Research; 2002. https://www.fda.gov/about-fda/histories-product-regulation/science-and-regulation-biological-products
4. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol*. 2014;5:520. doi:10.3389/fimmu.2014.00520
5. Gunn BM, Yu WH, Karim MM, et al. A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus. *Cell Host Microbe*. Aug 8 2018;24(2):221-233 e5. doi:10.1016/j.chom.2018.07.009
6. Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch JV. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. *Cell*. Sep 11 2014;158(6):1243-1253. doi:10.1016/j.cell.2014.08.023
7. DiLillo DJ, Palese P, Wilson PC, Ravetch JV. Broadly neutralizing anti-influenza antibodies require Fc receptor engagement for in vivo protection. *J Clin Invest*. Feb 2016;126(2):605-10. doi:10.1172/JCI84428
8. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol*. Jan 2018;18(1):46-61. doi:10.1038/nri.2017.106
9. Phelps M, Balazs AB. Contribution to HIV Prevention and Treatment by Antibody-Mediated Effector Function and Advances in Broadly Neutralizing Antibody Delivery by Vectored Immunoprophylaxis. *Front Immunol*. 2021;12:734304. doi:10.3389/fimmu.2021.734304
10. Halstead SB. Dengue Antibody-Dependent Enhancement: Knowns and Unknowns. *Microbiol Spectr*. Dec 2014;2(6)doi:10.1128/microbiolspec.AID-0022-2014
11. Bournazos S, Gupta A, Ravetch JV. The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol*. Oct 2020;20(10):633-643. doi:10.1038/s41577-020-00410-0
12. Screaton G, Mongkolsapaya J, Yacoub S, Roberts C. New insights into the immunopathology and control of dengue virus infection. *Nat Rev Immunol*. Dec 2015;15(12):745-59. doi:10.1038/nri3916
13. Brown JA, Singh G, Acklin JA, et al. Dengue Virus Immunity Increases Zika Virus-Induced Damage during Pregnancy. *Immunity*. Mar 19 2019;50(3):751-762.e5. doi:10.1016/j.immuni.2019.01.005
14. Martín-Acebes MA, Saiz JC, Jiménez de Oya N. Antibody-Dependent Enhancement and Zika: Real Threat or Phantom Menace? *Front Cell Infect Microbiol*. 2018;8:44. doi:10.3389/fcimb.2018.00044
15. Kotaki T, Kurosu T, Grinyo-Escuer A, et al. An affinity-matured human monoclonal antibody targeting fusion loop epitope of dengue virus with in vivo therapeutic potency. *Sci Rep*. Jun 21 2021;11(1):12987. doi:10.1038/s41598-021-92403-9

16. Lu J, Chen L, Du P, et al. A human monoclonal antibody to neutralize all four serotypes of dengue virus derived from patients at the convalescent phase of infection. *Virology*. Nov 2022;576:74-82. doi:10.1016/j.virol.2022.09.007
17. Pinto AK, Hassert M, Han X, et al. The Ability of Zika virus Intravenous Immunoglobulin to Protect From or Enhance Zika Virus Disease. *Front Immunol*. 2021;12:717425. doi:10.3389/fimmu.2021.717425
18. Wilson CS, Hoopes EM, Falk AC, Moore DJ. A human IgM enriched immunoglobulin preparation, Pentaglobin, reverses autoimmune diabetes without immune suppression in NOD mice. *Sci Rep*. Jul 11 2022;12(1):11731. doi:10.1038/s41598-022-15676-8
19. Isa MB, Martinez LC, Ferreyra LJ, et al. Measles virus-specific IgG4 antibody titer as a serologic marker of post-vaccinal immune response. *Viral Immunol*. Summer 2006;19(2):335-9. doi:10.1089/vim.2006.19.335
20. Siekman SL, Pongracz T, Wang W, et al. The IgG glycome of SARS-CoV-2 infected individuals reflects disease course and severity. *Front Immunol*. 2022;13:993354. doi:10.3389/fimmu.2022.993354
21. Cohn EJ, Strong LE, Hughes WL, et al. Preparation and properties of serum and plasma proteins; a system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. *J Am Chem Soc*. March 1 1946;68(3):459-75. doi:10.1021/ja01207a034
22. Oncley JL, Melin M, Richert DA, Cameron JW, Gross PM. The separation of the antibodies, isoagglutinins, prothrombin, plasminogen and beta1-lipoprotein into subfractions of human plasma. *J Am Chem Soc*. Feb 1949;71(2):541-50. doi:10.1021/ja01170a048
23. Lebing W, Remington KM, Schreiner C, Paul HI. Properties of a new intravenous immunoglobulin (IGIV-C, 10%) produced by virus inactivation with caprylate and column chromatography. *Vox Sang*. Apr 2003;84(3):193-201. doi:10.1046/j.1423-0410.2003.00285.x
24. CytoGam Prescribing Information. https://www.accessdata.fda.gov/spl/data/2a40733c-106b-41cf-94f0-f10a03180ac8/2a40733c-106b-41cf-94f0-f10a03180ac8.xml
25. Vandeberg P, Cruz M, Diez JM, et al. Production of anti-SARS-CoV-2 hyperimmune globulin from convalescent plasma. *Transfusion*. Jun 2021;61(6):1705-1709. doi:10.1111/trf.16378
26. Burnouf T, Gathof B, Bloch EM, et al. Production and Quality Assurance of Human Polyclonal Hyperimmune Immunoglobulins Against SARS-CoV-2. *Transfus Med Rev*. Jul 2022;36(3):125-132. doi:10.1016/j.tmrv.2022.06.001
27. HyperRAB prescribing information. https://www.accessdata.fda.gov/spl/data/f993778d-01fb-4670-af67-a0e08d6b258b/f993778d-01fb-4670-af67-a0e08d6b258b.xml
28. Imogam prescribing information. https://www.accessdata.fda.gov/spl/data/8026005f-7587-47fe-bb78-ec6247a3434b/8026005f-7587-47fe-bb78-ec6247a3434b.xml
29. Kedrab prescribing information. https://www.accessdata.fda.gov/spl/data/5e5c130a-693b-47f9-b44a-3d8f9cde3f98/5e5c130a-693b-47f9-b44a-3d8f9cde3f98.xml
30. VariZIG prescribing information. https://www.accessdata.fda.gov/spl/data/272379b7-f0e7-4560-8d79-3fd0024c3010/272379b7-f0e7-4560-8d79-3fd0024c3010.xml
31. Levin MJ, Duchon JM, Swamy GK, Gershon AA. Varicella zoster immune globulin (VARIZIG) administration up to 10 days after varicella exposure in pregnant women, immunocompromised participants, and infants: Varicella outcomes and safety results from a large, open-label, expanded-access program. *PLoS One*. 2019;14(7):e0217749. doi:10.1371/journal.pone.0217749
32. Vaccinia Immune Globulin prescribing information. https://www.fda.gov/media/78174/download
33. Centers for Disease C, Prevention. Household transmission of vaccinia virus from contact with a military smallpox vaccinee--Illinois and Indiana, 2007. *MMWR Morb Mortal Wkly Rep*. May 18 2007;56(19):478-81.
34. Centers for Disease C, Prevention. Progressive vaccinia in a military smallpox vaccinee - United States, 2009. *MMWR Morb Mortal Wkly Rep*. May 22 2009;58(19):532-6.
35. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant*. Sep 2019;33(9):e13512. doi:10.1111/ctr.13512
36. GamaSTAN prescribing information. https://www.accessdata.fda.gov/spl/data/38a323af-7c25-42d1-9c29-532ef61999b8/38a323af-7c25-42d1-9c29-532ef61999b8.xml
37. HyperHEP B prescribing information. https://www.accessdata.fda.gov/spl/data/391b2218-8a15-4e5e-8717-aa49efcc2210/391b2218-8a15-4e5e-8717-aa49efcc2210.xml
38. Nabi-HB prescribing information. https://www.accessdata.fda.gov/spl/data/ee1560c0-18e1-b617-e053-2a95a90aa1af/ee1560c0-18e1-b617-e053-2a95a90aa1af.xml
39. HepaGAM B prescribing information. https://www.accessdata.fda.gov/spl/data/56525de0-f47d-11eb-85b4-0800200c9a66/56525de0-f47d-11eb-85b4-0800200c9a66.xml
40. Te H, Doucette K. Viral hepatitis: Guidelines by the American Society of Transplantation Infectious Disease Community of Practice. *Clin Transplant*. Sep 2019;33(9):e13514. doi:10.1111/ctr.13514
41. FDA. Letter to Immune Globulin (Human) Licensed Manufacturers: Option to Lower Lot Release Specification for Required Measles Antibody Potency Testing. https://www.fda.gov/media/118428/download

42. Gardner CL, Sun C, Luke T, et al. Antibody Preparations from Human Transchromosomic Cows Exhibit Prophylactic and Therapeutic Efficacy against Venezuelan Equine Encephalitis Virus. *J Virol*. Jul 15 2017;91(14)doi:10.1128/JVI.00226-17
43. Saied AA, Nascimento MSL, do Nascimento Rangel AH, et al. Transchromosomic bovines-derived broadly neutralizing antibodies as potent biotherapeutics to counter important emerging viral pathogens with a special focus on SARS-CoV-2, MERS-CoV, Ebola, Zika, HIV-1, and influenza A virus. *J Med Virol*. Oct 2022;94(10):4599-4610. doi:10.1002/jmv.27907
44. Stauft CB, Tegenge M, Khurana S, et al. Pharmacokinetics and Efficacy of Human Hyperimmune Intravenous Immunoglobulin Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Adult Syrian Hamsters. *Clin Infect Dis*. Aug 24 2022;75(1):e459-e465. doi:10.1093/cid/ciab854
45. Rao AK, Petersen BW, Whitehill F, et al. Use of JYNNEOS (Smallpox and Monkeypox Vaccine, Live, Nonreplicating) for Preexposure Vaccination of Persons at Risk for Occupational Exposure to Orthopoxviruses: Recommendations of the Advisory Committee on Immunization Practices - United States, 2022. *MMWR Morb Mortal Wkly Rep*. Jun 3 2022;71(22):734-742. doi:10.15585/mmwr.mm7122e1
46. Tshiani Mbaya O, Mukumbayi P, Mulangu S. Review: Insights on Current FDA-Approved Monoclonal Antibodies Against Ebola Virus Infection. *Front Immunol*. 2021;12:721328. doi:10.3389/fimmu.2021.721328
47. Hammitt LL, Dagan R, Yuan Y, et al. Nirsevimab for Prevention of RSV in Healthy Late-Preterm and Term Infants. *N Engl J Med*. Mar 3 2022;386(9):837-846. doi:10.1056/NEJMoa2110275
48. de Melo GD, Hellert J, Gupta R, Corti D, Bourhy H. Monoclonal antibodies against rabies: current uses in prophylaxis and in therapy. *Curr Opin Virol*. Apr 2022;53:101204. doi:10.1016/j.coviro.2022.101204
49. Kaplon H, Crescioli S, Chenoweth A, Visweswaraiah J, Reichert JM. Antibodies to watch in 2023. *MAbs*. Jan-Dec 2023;15(1):2153410. doi:10.1080/19420862.2022.2153410
50. Cao Y, Wang J, Jian F, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature*. Feb 2022;602(7898):657-663. doi:10.1038/s41586-021-04385-3
51. Dejnirattisai W, Huo J, Zhou D, et al. SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell*. Feb 3 2022;185(3):467-484 e15. doi:10.1016/j.cell.2021.12.046
52. Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature*. Feb 2022;602(7898):671-675. doi:10.1038/s41586-021-04389-z
53. Sheward DJ, Kim C, Fischbach J, et al. Omicron sublineage BA.2.75.2 exhibits extensive escape from neutralising antibodies. *Lancet Infect Dis*. Nov 2022;22(11):1538-1540. doi:10.1016/S1473-3099(22)00663-6
54. Group AC--TfIwC-S. Tixagevimab-cilgavimab for treatment of patients hospitalised with COVID-19: a randomised, double-blind, phase 3 trial. *Lancet Respir Med*. Oct 2022;10(10):972-984. doi:10.1016/S2213-2600(22)00215-6
55. Imai M, Ito M, Kiso M, et al. Efficacy of Antiviral Agents against Omicron Subvariants BQ.1.1 and XBB. *N Engl J Med*. Jan 5 2023;388(1):89-91. doi:10.1056/NEJMc2214302
56. Wang Q, Iketani S, Li Z, et al. Alarming antibody evasion properties of rising SARS-CoV-2 BQ and XBB subvariants. *Cell*. Dec 14 2022;doi:10.1016/j.cell.2022.12.018
57. Coronavirus Disease 2019 (COVID-19) EUA Information. Accessed 02/17/2023, https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs
58. Corti D, Lanzavecchia A. Efficient Methods To Isolate Human Monoclonal Antibodies from Memory B Cells and Plasma Cells. *Microbiol Spectr*. Oct 2014;2(5)doi:10.1128/microbiolspec.AID-0018-2014
59. Dibo M, Battocchio EC, Dos Santos Souza LM, et al. Antibody Therapy for the Control of Viral Diseases: An Update. *Curr Pharm Biotechnol*. 2019;20(13):1108-1121. doi:10.2174/1389201020666190809112704
60. Hastie KM, Cross RW, Harkins SS, et al. Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell*. Aug 8 2019;178(4):1004-1015 e14. doi:10.1016/j.cell.2019.07.020
61. Li H, Buck T, Zandonatti M, et al. A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med*. Oct 26 2022;14(668):eabq0991. doi:10.1126/scitranslmed.abq0991
62. Corti D, Misasi J, Mulangu S, et al. Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. *Science*. Mar 18 2016;351(6279):1339-42. doi:10.1126/science.aad5224
63. Karuna ST, Corey L. Broadly Neutralizing Antibodies for HIV Prevention. *Annu Rev Med*. Jan 27 2020;71:329-346. doi:10.1146/annurev-med-110118-045506
64. Wrammert J, Smith K, Miller J, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature*. May 29 2008;453(7195):667-71. doi:10.1038/nature06890
65. !!! INVALID CITATION !!! 5;
66. Lewis GK. Role of Fc-mediated antibody function in protective immunity against HIV-1. *Immunology*. May 2014;142(1):46-57. doi:10.1111/imm.12232
67. Asokan M, Dias J, Liu C, et al. Fc-mediated effector function contributes to the in vivo antiviral effect of an HIV neutralizing antibody. *Proc Natl Acad Sci U S A*. Aug 4 2020;117(31):18754-18763. doi:10.1073/pnas.2008236117

68. Vanderven HA, Kent SJ. The protective potential of Fc-mediated antibody functions against influenza virus and other viral pathogens. *Immunol Cell Biol*. Apr 2020;98(4):253-263. doi:10.1111/imcb.12312
69. Zhang A, Stacey HD, D'Agostino MR, Tugg Y, Marzok A, Miller MS. Beyond neutralization: Fc-dependent antibody effector functions in SARS-CoV-2 infection. *Nat Rev Immunol*. Dec 19 2022:1-16. doi:10.1038/s41577-022-00813-1
70. Cartwright HN, Barbeau DJ, McElroy AK. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere*. Oct 27 2021;6(5):e0055621. doi:10.1128/mSphere.00556-21
71. Taylor A, Foo SS, Bruzzone R, Dinh LV, King NJ, Mahalingam S. Fc receptors in antibody-dependent enhancement of viral infections. *Immunol Rev*. Nov 2015;268(1):340-64. doi:10.1111/imr.12367
72. Smatti MK, Al Thani AA, Yassine HM. Viral-Induced Enhanced Disease Illness. *Front Microbiol*. 2018;9:2991. doi:10.3389/fmicb.2018.02991
73. doi:10.1016/b978-0-12-809468-6.00033-4
74. Almagro JC, Daniels-Wells TR, Perez-Tapia SM, Penichet ML. Progress and Challenges in the Design and Clinical Development of Antibodies for Cancer Therapy. *Front Immunol*. 2017;8:1751. doi:10.3389/fimmu.2017.01751
75. Liu R, Oldham RJ, Teal E, Beers SA, Cragg MS. Fc-Engineering for Modulated Effector Functions-Improving Antibodies for Cancer Treatment. *Antibodies (Basel)*. Nov 17 2020;9(4)doi:10.3390/antib9040064
76. Ko S, Jo M, Jung ST. Recent Achievements and Challenges in Prolonging the Serum Half-Lives of Therapeutic IgG Antibodies Through Fc Engineering. *BioDrugs*. Mar 2021;35(2):147-157. doi:10.1007/s40259-021-00471-0
77. Reusch D, Tejada ML. Fc glycans of therapeutic antibodies as critical quality attributes. *Glycobiology*. Dec 2015;25(12):1325-34. doi:10.1093/glycob/cwv065
78. Golay J, Andrea AE, Cattaneo I. Role of Fc Core Fucosylation in the Effector Function of IgG1 Antibodies. *Front Immunol*. 2022;13:929895. doi:10.3389/fimmu.2022.929895
79. Hatfield G, Tepliakova L, Gingras G, et al. Specific location of galactosylation in an afucosylated antiviral monoclonal antibody affects its FcgammaRIIIA binding affinity. *Front Immunol*. 2022;13:972168. doi:10.3389/fimmu.2022.972168
80. Tao MH, Morrison SL. Studies of aglycosylated chimeric mouse-human IgG. Role of carbohydrate in the structure and effector functions mediated by the human IgG constant region. *J Immunol*. Oct 15 1989;143(8):2595-601.
81. Bolt S, Routledge E, Lloyd I, et al. The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains in vitro immunosuppressive properties. *Eur J Immunol*. Feb 1993;23(2):403-11. doi:10.1002/eji.1830230216
82. Liu D, Shameem M. Antiviral monoclonal antibody cocktails as a modern weapon in combating pandemics. *Ther Deliv*. Feb 2022;13(2):67-69. doi:10.4155/tde-2021-0079
83. Dacon C, Tucker C, Peng L, et al. Broadly neutralizing antibodies target the coronavirus fusion peptide. *Science*. Aug 12 2022;377(6607):728-735. doi:10.1126/science.abq3773
84. Tarafdar S, Virata ML, Yan H, et al. Multiple epitopes of hepatitis B virus surface antigen targeted by human plasma-derived immunoglobulins coincide with clinically observed escape mutations. *J Med Virol*. Feb 2022;94(2):649-658. doi:10.1002/jmv.27278
85. Center for Drug Evaluation and Research OoPQ. Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens, Guidance for Industry (Draft). https://www.fda.gov/media/165746/download
86. Huber M, Trkola A. Humoral immunity to HIV-1: neutralization and beyond. *J Intern Med*. Jul 2007;262(1):5-25. doi:10.1111/j.1365-2796.2007.01819.x
87. Klasse PJ. Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives. *Adv Biol*. 2014;2014doi:10.1155/2014/157895
88. Reading SA, Dimmock NJ. Neutralization of animal virus infectivity by antibody. *Arch Virol*. 2007;152(6):1047-59. doi:10.1007/s00705-006-0923-8
89. Jiang XR, Song A, Bergelson S, et al. Advances in the assessment and control of the effector functions of therapeutic antibodies. *Nat Rev Drug Discov*. Feb 2011;10(2):101-11. doi:10.1038/nrd3365
90. Schmidt F, Weisblum Y, Muecksch F, et al. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. *J Exp Med*. Nov 2 2020;217(11)doi:10.1084/jem.20201181
91. Clapham PR. Vesicular stomatitis virus pseudotypes of retroviruses. *Methods Mol Biol*. 1992;8:95-102. doi:10.1385/0-89603-191-8:95
92. Kim Y, Zheng X, Eschke K, et al. MCMV-based vaccine vectors expressing full-length viral proteins provide long-term humoral immune protection upon a single-shot vaccination. *Cell Mol Immunol*. Feb 2022;19(2):234-244. doi:10.1038/s41423-021-00814-5

93. Racine T, Kobinger GP, Arts EJ. Development of an HIV vaccine using a vesicular stomatitis virus vector expressing designer HIV-1 envelope glycoproteins to enhance humoral responses. *AIDS Res Ther*. Sep 12 2017;14(1):55. doi:10.1186/s12981-017-0179-2
94. Takada A, Feldmann H, Stroeher U, et al. Identification of protective epitopes on ebola virus glycoprotein at the single amino acid level by using recombinant vesicular stomatitis viruses. *J Virol*. Jan 2003;77(2):1069-74. doi:10.1128/jvi.77.2.1069-1074.2003
95. Takada A, Robison C, Goto H, et al. A system for functional analysis of Ebola virus glycoprotein. *Proc Natl Acad Sci U S A*. Dec 23 1997;94(26):14764-9. doi:10.1073/pnas.94.26.14764
96. Bannert N, Farzan M, Friend DS, et al. Human Mast cell progenitors can be infected by macrophagetropic human immunodeficiency virus type 1 and retain virus with maturation in vitro. *J Virol*. Nov 2001;75(22):10808-14. doi:10.1128/JVI.75.22.10808-10814.2001
97. Connor RI, Chen BK, Choe S, Landau NR. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology*. Feb 1 1995;206(2):935-44. doi:10.1006/viro.1995.1016
98. Freed EO, Englund G, Martin MA. Role of the basic domain of human immunodeficiency virus type 1 matrix in macrophage infection. *J Virol*. Jun 1995;69(6):3949-54. doi:10.1128/JVI.69.6.3949-3954.1995
99. Louder MK, Sambor A, Chertova E, et al. HIV-1 envelope pseudotyped viral vectors and infectious molecular clones expressing the same envelope glycoprotein have a similar neutralization phenotype, but culture in peripheral blood mononuclear cells is associated with decreased neutralization sensitivity. *Virology*. Sep 1 2005;339(2):226-38. doi:10.1016/j.virol.2005.06.003
100. Lundquist CA, Zhou J, Aiken C. Nef stimulates human immunodeficiency virus type 1 replication in primary T cells by enhancing virion-associated gp120 levels: coreceptor-dependent requirement for Nef in viral replication. *J Virol*. Jun 2004;78(12):6287-96. doi:10.1128/JVI.78.12.6287-6296.2004
101. Sarzotti-Kelsoe M, Daniell X, Todd CA, et al. Optimization and validation of a neutralizing antibody assay for HIV-1 in A3R5 cells. *J Immunol Methods*. Jul 2014;409:147-60. doi:10.1016/j.jim.2014.02.013
102. Matsuura Y, Tani H, Suzuki K, et al. Characterization of pseudotype VSV possessing HCV envelope proteins. *Virology*. Aug 1 2001;286(2):263-75. doi:10.1006/viro.2001.0971
103. Renelt S, Schult-Dietrich P, Baldauf HM, et al. HIV-1 Infection of Long-Lived Hematopoietic Precursors In Vitro and In Vivo. *Cells*. Sep 23 2022;11(19)doi:10.3390/cells11192968
104. Riepler L, Rossler A, Falch A, et al. Comparison of Four SARS-CoV-2 Neutralization Assays. *Vaccines (Basel)*. Dec 28 2020;9(1)doi:10.3390/vaccines9010013
105. Chikere K, Webb NE, Chou T, et al. Distinct HIV-1 entry phenotypes are associated with transmission, subtype specificity, and resistance to broadly neutralizing antibodies. *Retrovirology*. Jun 23 2014;11:48. doi:10.1186/1742-4690-11-48
106. Mann AM, Rusert P, Berlinger L, Kuster H, Gunthard HF, Trkola A. HIV sensitivity to neutralization is determined by target and virus producer cell properties. *AIDS*. Aug 24 2009;23(13):1659-67. doi:10.1097/QAD.0b013e32832e9408
107. Miyamoto F, Kawaji K, Oishi S, Fujii N, Kaku M, Kodama EN. Anti-HIV-1 activity determined by beta-galactosidase activity in the multinuclear activation of an indicator assay is comparable with that by a conventional focus counting method. *Antivir Chem Chemother*. Apr 2015;24(2):77-82. doi:10.1177/2040206615614164
108. Spenlehauer C, Gordon CA, Trkola A, Moore JP. A luciferase-reporter gene-expressing T-cell line facilitates neutralization and drug-sensitivity assays that use either R5 or X4 strains of human immunodeficiency virus type 1. *Virology*. Feb 15 2001;280(2):292-300. doi:10.1006/viro.2000.0780
109. Sarzotti-Kelsoe M, Bailer RT, Turk E, et al. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. *J Immunol Methods*. Jul 2014;409:131-46. doi:10.1016/j.jim.2013.11.022
110. Bentley EM, Mather ST, Temperton NJ. The use of pseudotypes to study viruses, virus sero-epidemiology and vaccination. *Vaccine*. Jun 12 2015;33(26):2955-62. doi:10.1016/j.vaccine.2015.04.071
111. Comas-Garcia M, Colunga-Saucedo M, Rosales-Mendoza S. The Role of Virus-Like Particles in Medical Biotechnology. *Mol Pharm*. Dec 7 2020;17(12):4407-4420. doi:10.1021/acs.molpharmaceut.0c00828
112. Du R, Cui Q, Caffrey M, Rong L. Ebola Virus Entry Inhibitors. *Adv Exp Med Biol*. 2022;1366:155-170. doi:10.1007/978-981-16-8702-0_10
113. Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J Virol Methods*. Jan 2012;179(1):226-32. doi:10.1016/j.jviromet.2011.11.003
114. Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J Virol Methods*. Sep 2009;160(1-2):7-13. doi:10.1016/j.jviromet.2009.04.037
115. Khetawat D, Broder CC. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J*. Nov 12 2010;7:312. doi:10.1186/1743-422X-7-312

116. Nooraei S, Bahrulolum H, Hoseini ZS, et al. Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *J Nanobiotechnology*. Feb 25 2021;19(1):59. doi:10.1186/s12951-021-00806-7
117. Rudometova NB, Shcherbakov DN, Rudometov AP, Ilyichev AA, Karpenko LI. Model systems of human immunodef iciency virus (HIV-1) for in vitro eff icacy assessment of candidate vaccines and drugs against HIV-1. *Vavilovskii Zhurnal Genet Selektsii*. Mar 2022;26(2):214-221. doi:10.18699/VJGB-22-26
118. Steeds K, Hall Y, Slack GS, et al. Pseudotyping of VSV with Ebola virus glycoprotein is superior to HIV-1 for the assessment of neutralising antibodies. *Sci Rep*. Aug 31 2020;10(1):14289. doi:10.1038/s41598-020-71225-1
119. Steffen I, Simmons G. Pseudotyping Viral Vectors With Emerging Virus Envelope Proteins. *Curr Gene Ther*. 2016;16(1):47-55. doi:10.2174/1566523216666160119093948
120. Wang B, Meng XJ. Structural and molecular biology of hepatitis E virus. *Comput Struct Biotechnol J*. 2021;19:1907-1916. doi:10.1016/j.csbj.2021.03.038
121. Ryu W-S. Virus Vectors. *Molecular Virology of Human Pathogenic Viruses*. 1st ed. Elsevier Inc.; 2017:263-275:chap 19.
122. Gasmi M, Glynn J, Jin MJ, Jolly DJ, Yee JK, Chen ST. Requirements for efficient production and transduction of human immunodeficiency virus type 1-based vectors. *J Virol*. Mar 1999;73(3):1828-34. doi:10.1128/JVI.73.3.1828-1834.1999
123. Salmon P, Trono D. Lentiviral vectors for the gene therapy of lympho-hematological disorders. *Curr Top Microbiol Immunol*. 2002;261:211-27. doi:10.1007/978-3-642-56114-6_11
124. Todd CA, Greene KM, Yu X, et al. Development and implementation of an international proficiency testing program for a neutralizing antibody assay for HIV-1 in TZM-bl cells. *J Immunol Methods*. Jan 31 2012;375(1-2):57-67. doi:10.1016/j.jim.2011.09.007
125. Wei X, Decker JM, Liu H, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. *Antimicrob Agents Chemother*. Jun 2002;46(6):1896-905. doi:10.1128/AAC.46.6.1896-1905.2002
126. Seaman MS, Janes H, Hawkins N, et al. Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies. *J Virol*. Feb 2010;84(3):1439-52. doi:10.1128/JVI.02108-09
127. Hoenen T. Minigenome Systems for Filoviruses. *Methods Mol Biol*. 2018;1604:237-245. doi:10.1007/978-1-4939-6981-4_18
128. Cavrois M, De Noronha C, Greene WC. A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. *Nat Biotechnol*. Nov 2002;20(11):1151-4. doi:10.1038/nbt745
129. Li J, Bentsman G, Potash MJ, Volsky DJ. Human immunodeficiency virus type 1 efficiently binds to human fetal astrocytes and induces neuroinflammatory responses independent of infection. *BMC Neurosci*. May 12 2007;8:31. doi:10.1186/1471-2202-8-31
130. Saeed MF, Kolokoltsov AA, Davey RA. Novel, rapid assay for measuring entry of diverse enveloped viruses, including HIV and rabies. *J Virol Methods*. Aug 2006;135(2):143-50. doi:10.1016/j.jviromet.2006.02.011
131. Tobiume M, Lineberger JE, Lundquist CA, Miller MD, Aiken C. Nef does not affect the efficiency of human immunodeficiency virus type 1 fusion with target cells. *J Virol*. Oct 2003;77(19):10645-50. doi:10.1128/jvi.77.19.10645-10650.2003
132. Tscherne DM, Manicassamy B, Garcia-Sastre A. An enzymatic virus-like particle assay for sensitive detection of virus entry. *J Virol Methods*. Feb 2010;163(2):336-43. doi:10.1016/j.jviromet.2009.10.020
133. Wyma DJ, Jiang J, Shi J, et al. Coupling of human immunodeficiency virus type 1 fusion to virion maturation: a novel role of the gp41 cytoplasmic tail. *J Virol*. Apr 2004;78(7):3429-35. doi:10.1128/jvi.78.7.3429-3435.2004
134. Leroy H, Han M, Woottum M, et al. Virus-Mediated Cell-Cell Fusion. *Int J Mol Sci*. Dec 17 2020;21(24)doi:10.3390/ijms21249644
135. Bossart KN, Broder CC. Viral glycoprotein-mediated cell fusion assays using vaccinia virus vectors. *Methods Mol Biol*. 2004;269:309-32. doi:10.1385/1-59259-789-0:309
136. Moulard M, Phogat SK, Shu Y, et al. Broadly cross-reactive HIV-1-neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes. *Proc Natl Acad Sci U S A*. May 14 2002;99(10):6913-8. doi:10.1073/pnas.102562599
137. Saw WT, Matsuda Z, Eisenberg RJ, Cohen GH, Atanasiu D. Using a split luciferase assay (SLA) to measure the kinetics of cell-cell fusion mediated by herpes simplex virus glycoproteins. *Methods*. Nov 15 2015;90:68-75. doi:10.1016/j.ymeth.2015.05.021
138. Wang L, Zhao J, Nguyen LNT, et al. Blockade of SARS-CoV-2 spike protein-mediated cell-cell fusion using COVID-19 convalescent plasma. *Sci Rep*. Mar 10 2021;11(1):5558. doi:10.1038/s41598-021-84840-3
139. Oguntuyo KY, Stevens CS, Hung CT, et al. Quantifying Absolute Neutralization Titers against SARS-CoV-2 by a Standardized Virus Neutralization Assay Allows for Cross-Cohort Comparisons of COVID-19 Sera. *mBio*. Feb 16 2021;12(1)doi:10.1128/mBio.02492-20

140. Schendel SL, Saphire EO. Assay Standardization for Neutralizing Antibody. Why Different Labs Can Get Different Results and A Path Forward. In "Therapeutic Neutralizing Monoclonal Antibodies: Report of a Summit sponsored by Operation Warp Speed and the National Institutes of Health". August 20, 2020:52-66.
141. Li Y, O'Dell S, Walker LM, et al. Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. *J Virol*. Sep 2011;85(17):8954-67. doi:10.1128/JVI.00754-11
142. Lorenzi JCC, Mendoza P, Cohen YZ, et al. Neutralizing Activity of Broadly Neutralizing anti-HIV-1 Antibodies against Primary African Isolates. *J Virol*. Mar 1 2021;95(5)doi:10.1128/JVI.01909-20
143. Sanders DA. No false start for novel pseudotyped vectors. *Curr Opin Biotechnol*. Oct 2002;13(5):437-42. doi:10.1016/s0958-1669(02)00374-9
144. Li Q, Liu Q, Huang W, Li X, Wang Y. Current status on the development of pseudoviruses for enveloped viruses. *Rev Med Virol*. Jan 2018;28(1)doi:10.1002/rmv.1963
145. Chen RE, Zhang X, Case JB, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nat Med*. Apr 2021;27(4):717-726. doi:10.1038/s41591-021-01294-w
146. Cho A, Muecksch F, Schaefer-Babajew D, et al. Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. *Nature*. Dec 2021;600(7889):517-522. doi:10.1038/s41586-021-04060-7
147. Dong J, Zost SJ, Greaney AJ, et al. Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol*. Oct 2021;6(10):1233-1244. doi:10.1038/s41564-021-00972-2
148. Lusvarghi S, Pollett SD, Neerukonda SN, et al. SARS-CoV-2 BA.1 variant is neutralized by vaccine booster-elicited serum but evades most convalescent serum and therapeutic antibodies. *Sci Transl Med*. May 18 2022;14(645):eabn8543. doi:10.1126/scitranslmed.abn8543
149. Shi R, Shan C, Duan X, et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature*. Aug 2020;584(7819):120-124. doi:10.1038/s41586-020-2381-y
150. Yamasoba D, Kimura I, Nasser H, et al. Virological characteristics of the SARS-CoV-2 Omicron BA.2 spike. *Cell*. Jun 9 2022;185(12):2103-2115 e19. doi:10.1016/j.cell.2022.04.035
151. Farrell AG, Dadonaite B, Greaney AJ, et al. Receptor-Binding Domain (RBD) Antibodies Contribute More to SARS-CoV-2 Neutralization When Target Cells Express High Levels of ACE2. *Viruses*. Sep 16 2022;14(9)doi:10.3390/v14092061
152. Lempp FA, Soriaga LB, Montiel-Ruiz M, et al. Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies. *Nature*. Oct 2021;598(7880):342-347. doi:10.1038/s41586-021-03925-1
153. VanBlargan LA, Errico JM, Halfmann PJ, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nat Med*. Mar 2022;28(3):490-495. doi:10.1038/s41591-021-01678-y
154. FDA. Integrated Review Application Number 761172. Accessed 2/17/2023, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/761172Orig1s000IntegratedR.pdf
155. FDA. Multi-Discipline Review, Application Number 761169. Accessed 2/17/2023, https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/761169Orig1s000MultidisciplineR.pdf
156. Hsieh YT, Aggarwal P, Cirelli D, Gu L, Surowy T, Mozier NM. Characterization of FcγRIIIA effector cells used in in vitro ADCC bioassay: Comparison of primary NK cells with engineered NK-92 and Jurkat T cells. *J Immunol Methods*. Feb 2017;441:56-66. doi:10.1016/j.jim.2016.12.002
157. Parekh BS, Berger E, Sibley S, et al. Development and validation of an antibody-dependent cell-mediated cytotoxicity-reporter gene assay. *MAbs*. May-Jun 2012;4(3):310-8. doi:10.4161/mabs.19873
158. de Taeye SW, Rispens T, Vidarsson G. The Ligands for Human IgG and Their Effector Functions. *Antibodies (Basel)*. Apr 25 2019;8(2)doi:10.3390/antib8020030
159. Goncalvez AP, Engle RE, St Claire M, Purcell RH, Lai CJ. Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention. *Proc Natl Acad Sci U S A*. May 29 2007;104(22):9422-7. doi:10.1073/pnas.0703498104
160. Huang X, Yue Y, Li D, et al. Antibody-dependent enhancement of dengue virus infection inhibits RLR-mediated Type-I IFN-independent signalling through upregulation of cellular autophagy. *Sci Rep*. Feb 29 2016;6:22303. doi:10.1038/srep22303
161. Littaua R, Kurane I, Ennis FA. Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. *J Immunol*. Apr 15 1990;144(8):3183-6.
162. Stettler K, Beltramello M, Espinosa DA, et al. Specificity, cross-reactivity, and function of antibodies elicited by Zika virus infection. *Science*. Aug 19 2016;353(6301):823-6. doi:10.1126/science.aaf8505
163. Baum A, Fulton BO, Wloga E, et al. Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science*. Aug 21 2020;369(6506):1014-1018. doi:10.1126/science.abd0831
164. Copin R, Baum A, Wloga E, et al. The monoclonal antibody combination REGEN-COV protects against SARS-CoV-2 mutational escape in preclinical and human studies. *Cell*. Jul 22 2021;184(15):3949-3961 e11. doi:10.1016/j.cell.2021.06.002

165. Starr TN, Greaney AJ, Addetia A, et al. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. *Science*. Feb 19 2021;371(6531):850-854. doi:10.1126/science.abf9302
166. Weisblum Y, Schmidt F, Zhang F, et al. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *Elife*. Oct 28 2020;9doi:10.7554/eLife.61312
167. Ruiz SI, Zumbrun EE, Nalca A. Animal Models of Human Viral Diseases. *Animal Models for the Study of Human Disease*. 2017:853-901.
168. Beddingfield BJ, Maness NJ, Fears AC, et al. Effective Prophylaxis of COVID-19 in Rhesus Macaques Using a Combination of Two Parenterally-Administered SARS-CoV-2 Neutralizing Antibodies. *Front Cell Infect Microbiol*. 2021;11:753444. doi:10.3389/fcimb.2021.753444
169. Haagmans BL, Noack D, Okba NMA, et al. SARS-CoV-2 Neutralizing Human Antibodies Protect Against Lower Respiratory Tract Disease in a Hamster Model. *J Infect Dis*. Jun 15 2021;223(12):2020-2028. doi:10.1093/infdis/jiab289
170. Jha A, Barker D, Lew J, et al. Efficacy of COVID-HIGIV in animal models of SARS-CoV-2 infection. *Sci Rep*. Oct 10 2022;12(1):16956. doi:10.1038/s41598-022-21223-2
171. Kim C, Ryu DK, Lee J, et al. A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein. *Nat Commun*. Jan 12 2021;12(1):288. doi:10.1038/s41467-020-20602-5
172. Maisonnasse P, Aldon Y, Marc A, et al. COVA1-18 neutralizing antibody protects against SARS-CoV-2 in three preclinical models. *Nat Commun*. Oct 20 2021;12(1):6097. doi:10.1038/s41467-021-26354-0
173. Winkler ES, Gilchuk P, Yu J, et al. Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. *Cell*. Apr 1 2021;184(7):1804-1820 e16. doi:10.1016/j.cell.2021.02.026
174. Yadav PD, Mendiratta SK, Mohandas S, et al. ZRC3308 Monoclonal Antibody Cocktail Shows Protective Efficacy in Syrian Hamsters against SARS-CoV-2 Infection. *Viruses*. Dec 3 2021;13(12)doi:10.3390/v13122424
175. Zost SJ, Gilchuk P, Case JB, et al. Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature*. Aug 2020;584(7821):443-449. doi:10.1038/s41586-020-2548-6
176. FDA. S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, Guidance for Industry. Accessed 2/17/2023, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s6r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals
177. Mahmood I, Tegenge MA. Prediction of tissue concentrations of monoclonal antibodies in mice from plasma concentrations. *Regul Toxicol Pharmacol*. Aug 2018;97:57-62. doi:10.1016/j.yrtph.2018.06.004
178. Keeler SP, Fox JM. Requirement of Fc-Fc Gamma Receptor Interaction for Antibody-Based Protection against Emerging Virus Infections. *Viruses*. May 31 2021;13(6)doi:10.3390/v13061037
179. Schmaljohn AL, Orlandi C, Lewis GK. Deciphering Fc-mediated Antiviral Antibody Functions in Animal Models. *Front Immunol*. 2019;10:1602. doi:10.3389/fimmu.2019.01602
180. Robbie GJ, Criste R, Dall'acqua WF, et al. A novel investigational Fc-modified humanized monoclonal antibody, motavizumab-YTE, has an extended half-life in healthy adults. *Antimicrob Agents Chemother*. Dec 2013;57(12):6147-53. doi:10.1128/aac.01285-13
181. Smith P, DiLillo DJ, Bournazos S, Li F, Ravetch JV. Mouse model recapitulating human Fcγ receptor structural and functional diversity. *Proc Natl Acad Sci U S A*. Apr 17 2012;109(16):6181-6. doi:10.1073/pnas.1203954109
182. Proetzel G, Roopenian DC. Humanized FcRn mouse models for evaluating pharmacokinetics of human IgG antibodies. *Methods*. Jan 1 2014;65(1):148-53. doi:10.1016/j.ymeth.2013.07.005
183. FDA. Product Development Under the Animal Rule, Guidance for Industry. Accessed 2/17/2023, https://www.fda.gov/media/88625/download
184. FDA. Animal Rule Approvals. Accessed 2/17/2023, https://www.fda.gov/drugs/nda-and-bla-approvals/animal-rule-approvals
185. Sharp JC, Fletcher WB. Experience of anti-vaccinia immunoglobulin in the United Kingdom. *Lancet*. Mar 24 1973;1(7804):656-9. doi:10.1016/s0140-6736(73)92215-0
186. Bahmanyar M, Fayaz A, Nour-Salehi S, Mohammadi M, Koprowski H. Successful protection of humans exposed to rabies infection. Postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum. *JAMA*. Dec 13 1976;236(24):2751-4.
187. Nelson NP, Weng MK, Hofmeister MG, et al. Prevention of Hepatitis A Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices, 2020. *MMWR Recomm Rep*. Jul 3 2020;69(5):1-38. doi:10.15585/mmwr.rr6905a1
188. Fisher RW, Reed JL, Snoy PJ, et al. Postexposure prevention of progressive vaccinia in SCID mice treated with vaccinia immune globulin. *Clin Vaccine Immunol*. Jan 2011;18(1):67-74. doi:10.1128/CVI.00280-10
189. Bray M, Wright ME. Progressive vaccinia. *Clin Infect Dis*. Mar 15 2003;36(6):766-74. doi:10.1086/374244
190. FDA. REGEN-COV (casirivimab and imdevimab). 2/17/2023. https://www.fda.gov/media/145611/download
191. FDA. Bamlanivimab and etesevimab. Accessed 2/17/2023, https://www.fda.gov/media/145802/download

192. FDA. EVUSHELD™ (tixagevimab co-packaged with cilgavimab). Accessed 2/17/2023, https://www.fda.gov/media/154701/download
193. Ejemel M, Smith TG, Greenberg L, et al. A cocktail of human monoclonal antibodies broadly neutralizes North American rabies virus variants as a promising candidate for rabies post-exposure prophylaxis. *Sci Rep*. Jun 7 2022;12(1):9403. doi:10.1038/s41598-022-13527-0
194. Koszalka P, Subbarao K, Baz M. Preclinical and clinical developments for combination treatment of influenza. *PLoS Pathog*. May 2022;18(5):e1010481. doi:10.1371/journal.ppat.1010481
195. Maertens J, Logan AC, Jang J, et al. Phase 2 Study of Anti-Human Cytomegalovirus Monoclonal Antibodies for Prophylaxis in Hematopoietic Cell Transplantation. *Antimicrob Agents Chemother*. Mar 24 2020;64(4)doi:10.1128/AAC.02467-19
196. Mahomed S, Garrett N, Baxter C, Abdool Karim Q, Abdool Karim SS. Clinical Trials of Broadly Neutralizing Monoclonal Antibodies for Human Immunodeficiency Virus Prevention: A Review. *J Infect Dis*. Feb 13 2021;223(3):370-380. doi:10.1093/infdis/jiaa377
197. FDA. Codevelopment of Two or More New Investigational Drugs for Use in Combination, Guidance for Industry. Accessed 2/17/2023, https://www.fda.gov/media/80100/download
198. Li A, Katinger H, Posner MR, et al. Synergistic neutralization of simian-human immunodeficiency virus SHIV-vpu+ by triple and quadruple combinations of human monoclonal antibodies and high-titer anti-human immunodeficiency virus type 1 immunoglobulins. *J Virol*. Apr 1998;72(4):3235-40. doi:10.1128/jvi.72.4.3235-3240.1998
199. Miglietta R, Pastori C, Venuti A, Ochsenbauer C, Lopalco L. Synergy in monoclonal antibody neutralization of HIV-1 pseudoviruses and infectious molecular clones. *J Transl Med*. Dec 13 2014;12:346. doi:10.1186/s12967-014-0346-3
200. ter Meulen J, van den Brink EN, Poon LL, et al. Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. *PLoS Med*. Jul 2006;3(7):e237. doi:10.1371/journal.pmed.0030237
201. Zhong L, Haynes L, Struble EB, Tamin A, Virata-Theimer ML, Zhang P. Antibody-mediated synergy and interference in the neutralization of SARS-CoV at an epitope cluster on the spike protein. *Biochem Biophys Res Commun*. Dec 18 2009;390(3):1056-60. doi:10.1016/j.bbrc.2009.10.115
202. Patel HD, Nikitin P, Gesner T, et al. In Vitro Characterization of Human Cytomegalovirus-Targeting Therapeutic Monoclonal Antibodies LJP538 and LJP539. *Antimicrob Agents Chemother*. Aug 2016;60(8):4961-71. doi:10.1128/AAC.00382-16
203. Copin R, Baum A, Wloga E, et al. The monoclonal antibody combination REGEN-COV protects against SARS-CoV-2 mutational escape in preclinical and human studies. *Cell*. Jul 22 2021;184(15):3949-3961.e11. doi:10.1016/j.cell.2021.06.002
204. Gottlieb RL, Nirula A, Chen P, et al. Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. *Jama*. Feb 16 2021;325(7):632-644. doi:10.1001/jama.2021.0202
205. Spencer DA, Shapiro MB, Haigwood NL, Hessell AJ. Advancing HIV Broadly Neutralizing Antibodies: From Discovery to the Clinic. *Front Public Health*. 2021;9:690017. doi:10.3389/fpubh.2021.690017
206. Cox M, Peacock TP, Harvey WT, et al. SARS-CoV-2 variant evasion of monoclonal antibodies based on in vitro studies. *Nat Rev Microbiol*. Feb 2023;21(2):112-124. doi:10.1038/s41579-022-00809-7
207. Julg B, Stephenson KE, Wagh K, et al. Safety and antiviral activity of triple combination broadly neutralizing monoclonal antibody therapy against HIV-1: a phase 1 clinical trial. *Nat Med*. Jun 2022;28(6):1288-1296. doi:10.1038/s41591-022-01815-1
208. Chan CEZ, Seah SGK, Chye H, et al. The Fc-mediated effector functions of a potent SARS-CoV-2 neutralizing antibody, SC31, isolated from an early convalescent COVID-19 patient, are essential for the optimal therapeutic efficacy of the antibody. *PLoS One*. 2021;16(6):e0253487. doi:10.1371/journal.pone.0253487
209. Schäfer A, Muecksch F, Lorenzi JCC, et al. Antibody potency, effector function, and combinations in protection and therapy for SARS-CoV-2 infection in vivo. *J Exp Med*. Mar 1 2021;218(3)doi:10.1084/jem.20201993
210. Winkler ES, Gilchuk P, Yu J, et al. Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. *Cell*. Apr 1 2021;184(7):1804-1820.e16. doi:10.1016/j.cell.2021.02.026
211. Yamin R, Jones AT, Hoffmann HH, et al. Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2 efficacy. *Nature*. Nov 2021;599(7885):465-470. doi:10.1038/s41586-021-04017-w
212. Chigutsa E, Jordie E, Riggs M, et al. A Quantitative Modeling and Simulation Framework to Support Candidate and Dose Selection of Anti-SARS-CoV-2 Monoclonal Antibodies to Advance Bamlanivimab Into a First-in-Human Clinical Trial. *Clin Pharmacol Ther*. Mar 2022;111(3):595-604. doi:10.1002/cpt.2459

213. Chigutsa E, O'Brien L, Ferguson-Sells L, Long A, Chien J. Population Pharmacokinetics and Pharmacodynamics of the Neutralizing Antibodies Bamlanivimab and Etesevimab in Patients With Mild to Moderate COVID-19 Infection. *Clin Pharmacol Ther*. Nov 2021;110(5):1302-1310. doi:10.1002/cpt.2420
214. Magyarics Z, Leslie F, Bartko J, et al. Randomized, Double-Blind, Placebo-Controlled, Single-Ascending-Dose Study of the Penetration of a Monoclonal Antibody Combination (ASN100) Targeting Staphylococcus aureus Cytotoxins in the Lung Epithelial Lining Fluid of Healthy Volunteers. *Antimicrob Agents Chemother*. Aug 2019;63(8)doi:10.1128/aac.00350-19
215. General Chapter: USP. General Tests and Assays, Biological Tests and Assays, <85> Bacterial Endotoxins Test. *USP–NF Rockville, MD: USP; DOI: https://doiorg/1031003/USPNF_M98830_02_01*.
216. Murga JD, Franti M, Pevear DC, Maddon PJ, Olson WC. Potent antiviral synergy between monoclonal antibody and small-molecule CCR5 inhibitors of human immunodeficiency virus type 1. *Antimicrob Agents Chemother*. Oct 2006;50(10):3289-96. doi:10.1128/AAC.00699-06
217. Vir Biothechnology, Inc. Press Release, 11/6/2022. Accessed 2/17/2023, https://investors.vir.bio/news-releases/news-release-details/vir-biotechnology-presents-new-data-evaluating-potential-vir-0
218. Vir Biothechnology, Inc. Press Release, 06/25/22. Accessed 2/22/2022, https://investors.vir.bio/news-releases/news-release-details/vir-biotechnology-announces-new-clinical-data-its-broad
219. Xiao F, Fofana I, Thumann C, et al. Synergy of entry inhibitors with direct-acting antivirals uncovers novel combinations for prevention and treatment of hepatitis C. *Gut*. Mar 2015;64(3):483-94. doi:10.1136/gutjnl-2013-306155
220. Caskey M, Klein F, Lorenzi JC, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature*. Jun 25 2015;522(7557):487-91. doi:10.1038/nature14411
221. Mouquet H, Scharf L, Euler Z, et al. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc Natl Acad Sci U S A*. Nov 20 2012;109(47):E3268-77. doi:10.1073/pnas.1217207109
222. Drug Database: Leronlimab. Accessed 2/17/2023, https://clinicalinfo.hiv.gov/en/drugs/leronlimab/patient
223. TROGARZO® (ibalizumab-uiyk). Accessed 2/17/2023, https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761065s013lbl.pdf
224. Kiso M, Yamayoshi S, Kawaoka Y. Triple combination therapy of favipiravir plus two monoclonal antibodies eradicates influenza virus from nude mice. *Commun Biol*. May 7 2020;3(1):219. doi:10.1038/s42003-020-0952-y
225. Cross RW, Bornholdt ZA, Prasad AN, et al. Combination therapy protects macaques against advanced Marburg virus disease. *Nat Commun*. Mar 25 2021;12(1):1891. doi:10.1038/s41467-021-22132-0
226. Cross RW, Bornholdt ZA, Prasad AN, et al. Combination therapy with remdesivir and monoclonal antibodies protects nonhuman primates against advanced Sudan virus disease. *JCI Insight*. May 23 2022;7(10)doi:10.1172/jci.insight.159090
227. Nakamura G, Chai N, Park S, et al. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza A antibodies. *Cell Host Microbe*. Jul 17 2013;14(1):93-103. doi:10.1016/j.chom.2013.06.004
228. Paules CI, Lakdawala S, McAuliffe JM, et al. The Hemagglutinin A Stem Antibody MEDI8852 Prevents and Controls Disease and Limits Transmission of Pandemic Influenza Viruses. *J Infect Dis*. Aug 1 2017;216(3):356-365. doi:10.1093/infdis/jix292
229. Tharakaraman K, Subramanian V, Viswanathan K, et al. A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proc Natl Acad Sci U S A*. Sep 1 2015;112(35):10890-5. doi:10.1073/pnas.1502374112
230. Yi KS, Choi JA, Kim P, et al. Broader neutralization of CT-P27 against influenza A subtypes by combining two human monoclonal antibodies. *PLoS One*. 2020;15(7):e0236172. doi:10.1371/journal.pone.0236172
231. Cox M, Peacock TP, Harvey WT, et al. SARS-CoV-2 variant evasion of monoclonal antibodies based on in vitro studies. *Nat Rev Microbiol*. Feb 2023;21(2):112-124. doi:10.1038/s41579-022-00809-7
232. Washburn N, Schwab I, Ortiz D, et al. Controlled tetra-Fc sialylation of IVIg results in a drug candidate with consistent enhanced anti-inflammatory activity. *Proc Natl Acad Sci U S A*. Mar 17 2015;112(11):E1297-306. doi:10.1073/pnas.1422481112
233. Keating SM, Mizrahi RA, Adams MS, et al. Generation of recombinant hyperimmune globulins from diverse B-cell repertoires. *Nat Biotechnol*. Aug 2021;39(8):989-999. doi:10.1038/s41587-021-00894-8
234. Frost GI. Recombinant human hyaluronidase (rHuPH20): an enabling platform for subcutaneous drug and fluid administration. *Expert Opin Drug Deliv*. Jul 2007;4(4):427-40. doi:10.1517/17425247.4.4.427
235. Pitiot A, Heuze-Vourc'h N, Secher T. Alternative Routes of Administration for Therapeutic Antibodies-State of the Art. *Antibodies (Basel)*. Aug 26 2022;11(3)doi:10.3390/antib11030056
236. Sroga P, Safronetz D, Stein DR. Nanobodies: a new approach for the diagnosis and treatment of viral infectious diseases. *Future Virology*. 2020;15(3):195-205. doi:10.2217/fvl-2019-0167
237. Nyakatura EK, Soare AY, Lai JR. Bispecific antibodies for viral immunotherapy. *Hum Vaccin Immunother*. Apr 3 2017;13(4):836-842. doi:10.1080/21645515.2016.1251536

 doi:10.20944/preprints202304.0506.v1

238. Walser M, Mayor J, Rothenberger S. Designed Ankyrin Repeat Proteins: A New Class of Viral Entry Inhibitors. *Viruses*. Oct 12 2022;14(10)doi:10.3390/v14102242
239. Wensel D, Sun Y, Davis J, et al. A Novel gp41-Binding Adnectin with Potent Anti-HIV Activity Is Highly Synergistic when Linked to a CD4-Binding Adnectin. *J Virol*. Jul 15 2018;92(14)doi:10.1128/JVI.00421-18