To conclude, Sugiura et al.[4] have elegantly demonstrated the therapeutic potential for disrupting the CD80–PD-L1 duplex to liberate PD-L1 and enable PD-L1–PD-1-induced T cell anergy. This method of eliciting PD-1 signal propagation could represent a new and impactful strategy in the treatment of autoimmunity. ❐

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Competing interests

D.A.A.V. declares competing financial interests and has submitted patents covering LAG3 that are licensed or pending and is entitled to a share in net income generated from licensing of these patent rights for commercial development. D.A.A.V.: cofounder and stock holder of Novasenta, Potenza, Tizona, Trishula; stock holder of Oncorus, Werewolf, Apeximmune; patents licensed and royalties for Astellas, BMS, Novasenta; scientific advisory board member of Tizona, Werewolf, F-Star, Bicara, Apeximmune; consultant for Astellas, BMS, Almirall, Incyte, G1 Therapeutics; research funding from BMS, Astellas and Novasenta.

Check for updates

COVID-19

Bispecific antiviral neutralizing antibodies are twice as nice

Increasingly, human monoclonal antibodies have been deployed against COVID-19, but combinations are typically needed for recognition of diverse viral variants. Bispecific antibodies could make the task of manufacturing and delivering combinations more efficient.

James E. Crowe Jr

Human monoclonal antibodies (mAbs) are being used in the clinic to prevent or treat SARS-CoV-2 infection, and experience thus far suggests that combinations of antibodies are potentially able to sustain broad recognition of viral variants as they arise, but developing, testing and approving mAb combination products is complex. One approach to reducing the logistical challenges of combination products is the development of bispecific antibodies (bsAbs) (Fig. 1). In this issue of *Nature Immunology*, Li et al. report the development and testing of an engineered bispecific human mAb against SARS-CoV-2[1].

Antibodies (initially in the form of horse immune serum) have been used for the prevention or treatment of infectious diseases since the nineteenth century. Now there are more than 100 licensed human mAbs in clinical use[2], but therapeutic application of these antibodies has been focused almost exclusively on cancer and autoimmune diseases, with only 7 mAb products licensed for infectious diseases. The COVID-19 pandemic really has been the first scenario in which human mAbs have been used at scale against an infection across a broad population, and many lessons have been learned about how to use these potent and specific tools. Four antibody drugs have obtained emergency use authorization (from the US Food and Drug Administration) or conditional marketing authorization (from the European Medicines Agency) for mAbs against COVID-19 so far[3], but challenges have arisen.

Perhaps the most important of these challenges is the evolution of SARS-CoV-2 variants of concern that threaten to escape the activity of human mAbs (and vaccine-induced immunity too). The use of a single mAb to prevent or treat infection with an RNA virus is usually feasible if the mAb selected recognizes a highly conserved functional domain on the viral protein, and the size of the outbreak is small. However, monotherapy runs some risk of failure, as the RNA-dependent RNA polymerases used to replicate genomes in RNA viruses are error-prone, which leads to the potential for the generation and selection of mAb escape variants. At the scale of the current pandemic, SARS-CoV-2 has had millions of 'shots on goal' to generate variants, so even the mathematically improbable ensemble of more than 30 mutations that occurred in the Omicron variant was perhaps statistically inevitable. Remarkably, some of the broadly reactive mAbs deployed medically in the population retained activity against diverse variants of concern

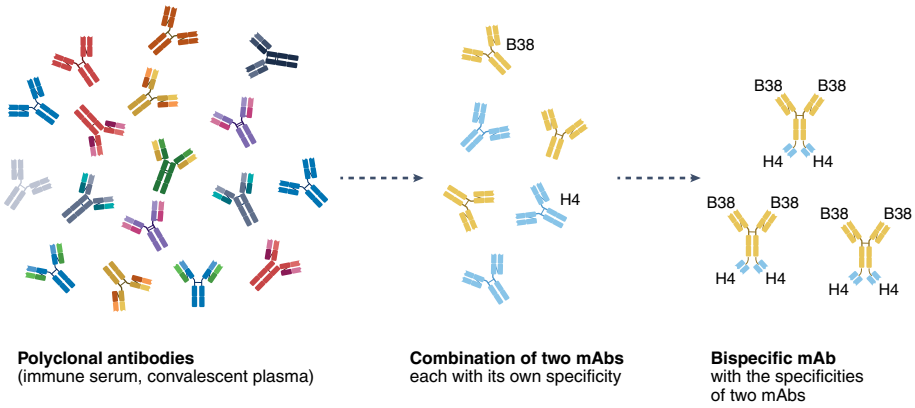

Fig. 1 | Simplification of complexity in antibody preparations over time. a, Immune serum and convalescent plasma contain not only antiviral antibodies but also antibodies of unrelated specificity. **b**, Combinations of mAbs are frequently used in development programs for antiviral antibodies to inhibit RNA viruses. **c**, A bispecific mAb (shown as an IgG–ScFv bsAb here) can incorporate the specificities of two mAbs in a single engineered immunoglobulin molecule. Created with BioRender.com.

through to the Delta variant until Omicron emerged, and the therapeutic mAb sotrovimab (which targets the SARS-COV-2 spike protein) and the prophylactic combination of the mAbs tixagevimab and cilgavimab (which also target the spike protein) even retain substantial activity against the Omicron variant[4].

So, one of the lessons learned during the current pandemic is that combinations of antibodies may be useful in reducing or preventing the emergence of escape variants. Another benefit of some mAb combinations is that the functional activity of the combination might exceed the combined activity of the constituent mAbs, a property called 'synergy' that stems from cooperative effects during simultaneous binding of two mAbs directed against different antigenic sites on a viral protein[5]. Through rational selection processes, rare synergistic combinations can be identified, and their mechanisms of action can be identified with molecular and structural studies.

Challenges arise for antibody developers and regulators when antibody combinations are developed. The cost and complexity of a combination treatment is higher than that of a monotherapy. Should the antibodies be formulated in a single vial (allowing a single injection) or in separate vials (simplifying formulation problems, but requiring two injections)? Should regulators require manufacturers to perform clinical trials of each antibody alone and then also in combination (more than tripling cost, time to clinic and other resource commitments)? On the one hand, the *primum non nocere* principle ('first do no harm') stemming from the Hippocratic oath in medicine advises caution. On the other hand, patients die when life-saving medicines, such as potent neutralizing antibodies against COVID-19, are delayed.

One interesting engineering approach that might alleviate some of these difficulties is the technology of bsAbs, which are antibodies with binding sites directed against two different epitopes or antigens. There are now more than 100 formats in which bsAbs can be constructed genetically in the research laboratory, and three bsAbs have been approved by the US Food and Drug Administration for clinical application. BsAbs have been developed to treat infection with viruses such as HIV[6]. In fact, trispecific antibodies against HIV have been developed[7], and several trispecific antibodies or antibodies with higher-order complexity (multispecific antibodies) are being tested in clinical trials.

Li et al. explored two bsAb formats, dual variable domain–immunoglobulin and immunoglobulin G–single-chain variable fragment (IgG–ScFv), to combine the variable regions of the previously identified experimental mAbs B38 and H4 that block binding of the spike protein to its receptor in humans, ACE2[1] (Fig. 1). One of the constructs, bsAb15 (an IgG–ScFv), displayed affinity for binding to the receptor-binding domain of SARS-CoV-2 spike protein and neutralization potency against SARS-CoV-2 similar to that of the parental mAbs. This bsAb showed efficacy in mice expressing human ACE2 and in a rhesus macaque SARS-CoV-2-infection model of both prophylaxis and treatment. Notably, the investigators were able to identify escape mutant viruses in vitro that were selected with individual mAbs but could not detect escape with the bsAb, and the bsAb did neutralize the escape mutant viruses isolated from each of the monotherapy selections. These studies show that bsAbs can be developed to have some of the advantageous features of combinations of individual mAbs. Ultimately, this study serves principally as a proof of concept for combinations assembled as a bispecific molecule. It should be noted that the construct has some limitations in that it can be escaped, as the naturally occurring Beta (B.1.351) variant exhibited resistance to bsAb15. The Gamma variant might also be resistant, given it has K417T and E484K substitutions critical for escape.

In summary, human mAbs have been of remarkable benefit for some patients with COVID-19, and their use is likely to spread to other infectious targets. Lessons learned during this pandemic inform best principles for using mAbs against infectious diseases. Combining mAbs is an approach that is here to stay for many RNA viruses, and bsAbs may be one technical approach to reducing the complexity of manufacture and development. ❐

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Competing interests

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