It Takes a Matured mAb to Treat Ebolavirus Infection

Ami Patel[1] and David B. Weiner[1,*]
[1]Vaccine & Immunotherapy Center, The Wistar Institute, Philadelphia, PA, USA
*Correspondence: dweiner@wistar.org
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Pan-Ebolavirus immunotherapy would provide a rapid-response treatment during Ebola virus outbreaks. In this issue of *Cell Host & Microbe*, Wec et al. (2019) and Bornholdt et al. (2019) optimize the MBP134 mAb cocktail through antibody affinity maturation, improving its protective efficacy against three Ebolaviruses: EBOV, SUDV, and BDBV.

Ebolaviruses belong to the Filovirus family and cause severe illness characterized by a sudden onset fever, fatigue, loss of appetite, and diarrhea as well as inducing a hemorrhagic syndrome recognized as Ebola virus disease (EVD) (Baseler et al., 2017). The first EVD outbreak was reported in 1976. Since then members of the *Ebolavirus* genus have been responsible for several outbreaks as well as the devastating 2013–2016 West Africa EVD epidemic and the current severe outbreak in the Democratic Republic of Congo (DRC). Five *Ebolavirus* species infect humans: Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV), Tai Forest virus (TAFV), and Reston virus (RESTV). All are symptomatic in humans except for RESTV, which is non-pathogenic in people. The current EBOV-EVD outbreak in the Democratic Republic of Congo in North Kivu (2018) is associated with a 65% case fatality rate (WHO, 2018). The prevention and treatment of advanced EVD has been extremely challenging; however, recent advances in experimental vaccines and immunotherapies are providing some hope for potential ways to control this emerging pathogen. Compellingly, efforts in anti-Ebolavirus monoclonal antibody (mAb) discovery have led to the development of promising immunotherapies to treat EVD caused by EBOV infection. As the frequency of EVD outbreaks increases, there is an urgent public health need for preventative and therapeutic countermeasures against multiple *Ebolavirus* species that share overlapping geographic distributions.

Passive delivery of serum antibodies to treat infectious diseases was first used to treat infections more than 100 years ago (reviewed in Casadevall et al., 2004). Treatment by injection of pathogen-specific immunoglobulins (Igs) or intravenous Ig (IVIG) derived from animal and human sources has been an important approach for prevention or treatment of several bacterial (diphtheria, botulism, tetanus) and viral (cytomegalovirus, hepatitis A, measles, rabies, vaccinia, varicella) infections. In contrast for Ebola virus infection, no clear benefit was observed when convalescent patient sera were administered during several EVD outbreaks or when studied in non-human primate animal models (Mire et al., 2016). Studies with single monoclonal antibodies (mAbs) were also initially stymied by their lack of efficacy in larger animal models. Despite these challenges, the potential protective capacity of antibodies against EVD is supported by evidence from protection studies with prophylactic anti-EBOV vaccines (Marzi et al., 2013).

The Ebolavirus surface glycoprotein (GP) is the major antigenic target located on the virus surface. GP can be further divided into several regions with potential epitope targets for protective mAbs—the glycan cap, chalice base, fusion loop, membrane proximal external region, heptad repeat region, and mucin-like domain—and their role in mAb-mediated protection is becoming clearer. Several groups have performed elegant studies isolating mouse and monkey antibodies from GP-vaccinated animals and fully human antibodies from EVD survivors from the EBOV-Kikwit 1995 and BDBV-2007 outbreaks and the EBOV-West Africa 2014 epidemic. The important ZMapp cocktail consists of three human/mouse chimeric mAbs (2G4, 4G7, 13c6) that were originally isolated from vaccinated mice. ZMapp is the most advanced clinical candidate representing the first EVD mAb cocktail to be evaluated during the 2013–2016 West Africa epidemic. It was determined to be safe and well tolerated during the West Africa 2014 outbreak and was associated with more favorable recovery in people, although the studies did not reach statistical significance (Davey et al., 2016). Other advanced candidates are the single mAb114 isolated from an EBOV-Kikwit 1995 survivor (Corti et al., 2016) and REGN-EB3, a cocktail of three mAbs isolated from transgenic mice immunized with an anti-GP DNA vaccine and boosted with protein (Sivapalasingam et al., 2018). ZMapp, mAb114, and REGN-EB3 are currently approved for compassionate use for EVD treatment in the DRC North Kivu 2018 outbreak.

One highlight of these studies and others is the ability to discover potent mAbs using a range of isolation techniques, including hybridoma (2G4, 4G7, 13c6, REGN-EB3) and EBV immortalized B cells (mAb114). Building on these studies, newer studies have taken advantage of antigen-specific single B cell sorting technology and high-throughput screening methods including cell-based and yeast displays. Together, these approaches are helping to identify new and potentially more potent anti-GP mAbs (Figure 1). However, the identification of cross-reactive antibodies against all the three most pathogenic *Ebolaviruses* (EBOV, SUDV, and BDBV) has been challenging due to distant homology between the viruses.

Wec et al. (2019) determined that a previously identified mAb from an EBOV-EVD survivor also shared specificities for

BDBV and weakly for SUDV due to differences in the GP binding site. Following natural infection, affinity maturation is utilized by the immune system to improve antibody specificity and affinity for antigen while also ensuring low reactivity to self-antigens. *Saccharomyces cerevisiae* (yeast) systems can be utilized to display different antibody variable heavy (VH) and variable light (VL) genes, enabling rapid screening and down-selection of potential mAb candidates. In this issue of *Cell Host & Microbe*, Wec et al. (2019) utilized a yeast display system to rapidly interrogate different complementary determining region (CDR) variants to mature the affinity of the anti-EBOV and anti-BDBV antibody for improved binding and potency against SUDV. This candidate was combined with a second mAb to develop the MBP-134 cocktail with optimized effector functions, demonstrating protection mice and guinea pigs against all three viruses. The companion paper by Bornholdt et al. (2019) demonstrates that the MBP-134 cocktail is highly protective in ferrets and non-human primates against EBOV, BDBV, and SUDV.

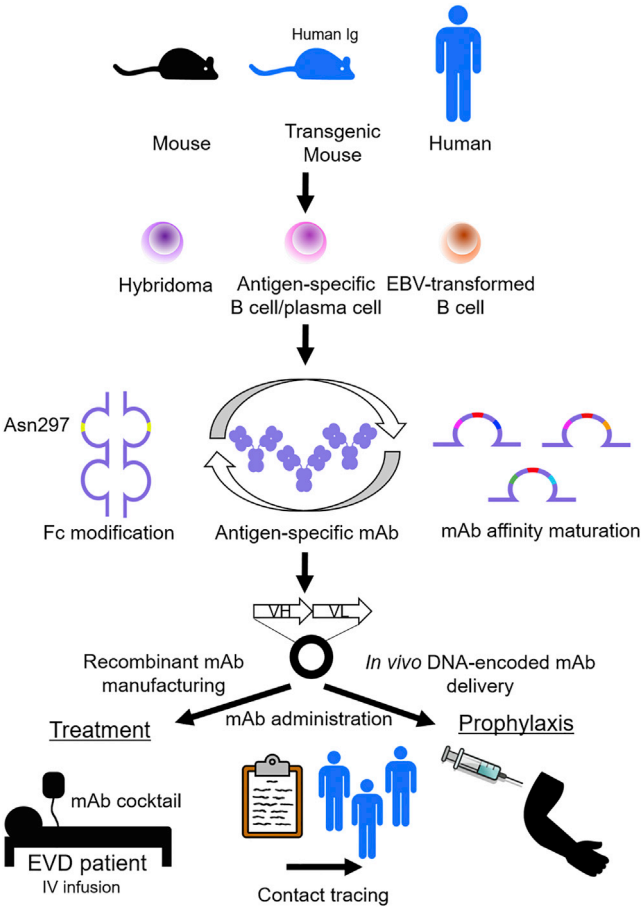

Figure 1. Anti-*Ebolavirus* Immunotherapy
Ebola virus GP-specific mAbs can be isolated from mice, human-Ig transgenic mice, or humans using several methodologies including hybridoma, EBV transformation, and single-cell sorting technologies. Affinity maturation and Fc modifications may be utilized to improve mAb potency. The mAb sequences variable heavy chain (VH) and light chain (VL) sequences can then be inserted into plasmid DNA for recombinant mAb production for therapeutic delivery against EVD or potentially direct *in vivo* delivery for prophylactic delivery.

An important obstacle for EVD mAb therapy is the need for high doses infusions. In the ongoing compassionate use trials in the DRC, ZMapp is being delivered as three doses of 50 mg/kg (over 4 hr) and mAb114 is a single 50 mg/kg dose (over 30–60 min) (http://clinicaltrials.gov, NCT03719586). In the most recent REGN-EB3 trial, a single dose of 150 mg/kg recombinant mAb demonstrated superior protection in macaques and was the most promising for delivery in humans (Sivapalasingam et al., 2018). In this issue, Bornholdt et al. (2019) demonstrate that the MBP134 cocktail is protective at a single, lower dose of 25 mg/kg. The findings show uniform protection when MBP134 is administered 5 days post infection with EBOV, BDBV, or SUDV, demonstrating the expanded breadth of this cocktail against multiple filoviruses. Further improvements in formulation and delivery routes might additionally improve dosing. Fc region modifications to increase antibody half-life may improve mAb persistence *in vivo*, possibly further contributing to lower injection doses.

Given the unpredictable nature of EVD outbreaks, the feasibility and logistics for mAb field delivery during a prolonged outbreak should be an important part of the conversation. Advances in alternative manufacturing technologies, such as using optimized cell lines or plants, will be highly valuable for production and distribution. New technologies such as synthetic DNA-encoded anti-Ebolavirus mAbs (Patel et al., 2018), as well as other vector approaches, are important new tools for further study promising simpler delivery of potential life-saving mAbs/cocktails such as ZMapp, mAb114, REGN-EB3, and MBP134. Such approaches may enable post-exposure prophylaxis during contact tracing, where serum levels required for protection are likely not needed to be as high as during advanced EVD. The studies by Wec et al. (2019) and Bornholdt et al. (2019) contribute to and advance the next generation of *pan-Ebolavirus* immunotherapies, likely allowing administration to a wider population base supporting protection against a more diverse Ebolavirus species outbreaks representing an important step forward for field delivery.

DECLARATION OF INTERESTS

A.P. declares no competing interests. D.B.W. has received grant funding, participates in industry collaborations, has received speaking honoraria, and has received fees for consulting, including serving on scientific review committees and board services. Remuneration received by D.B.W. includes direct payments or stock or stock options, and in the interest of disclosure, he notes potential conflicts associated with this work with Inovio and possibly others. In addition, he has a patent DNA vaccine delivery pending to Inovio.

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