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Anti-Ebola therapies based on monoclonal antibodies: Current state and challenges ahead

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Abstract

The 2014 Ebola outbreak, the largest recorded, took us largely unprepared, with no available vaccine or specific treatment. In this context, the World Health Organization (WHO) declared that the humanitarian use of experimental therapies against Ebola Virus (EBOV) is ethical. In particular, an experimental treatment consisting of a cocktail of three monoclonal antibodies (mAbs) produced in tobacco plants and specifically directed to the Ebola virus glycoprotein (GP) was tested in humans, apparently with good results. Several mAbs with high affinity to the GP have been described. This review discusses our current knowledge on this topic. Particular emphasis is devoted to those mAbs that have been assayed in animal models or humans as possible therapies against Ebola. Engineering aspects and challenges for the production of anti-Ebola mAbs are also briefly discussed; current platforms for the design and production of full-length mAbs are cumbersome and costly.

Keywords

Ebola; mAbs; monoclonal antibodies; therapeutic; epidemic; GP

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The Ebola virus in brief: Epidemiology, and genetic variability

The Ebola virus (more formally called EBOV, formerly known as *Zaire ebolavirus*) is one of the most aggressive and feared pathogens known to humans. Its first documented outbreak occurred in 1976 (WHO, 1978; Leroy et al., 2005). Yet no vaccine or specific treatment against Ebola infection is commercially available.

The Ebola virus is an enveloped, non-segmented, RNA virus. EBOV, together with the Marburg virus, belongs to the Filoviridae family. The ecology and epidemiology aspects of Ebola Virus Disease (EVD) have been reviewed elsewhere (Feldmann and Geisbert, 2011). In brief, experimental evidence suggests that fruit bats are its main natural reservoir (Leroy et al., 2005; Leroy et al., 2004). Human outbreaks have been associated with previous occurrences of nonhuman primate outbreaks. The patient-zero cases have been mainly hunters, infected when manipulating dead nonhuman primates (gorillas, chimpanzees) or duikers. Other zero patients include subjects that accidentally came in contact with bats (i.e., workers in bat-infested cotton factories or mines) (Chiappelli et al., 2015; Saenz et al., 2015). Indeed, the Guinean two-year old kid, believed to be the patient zero of the current West Africa outbreak, most probably became infected while playing in a hollow tree infested by insectivorous bats (Baize et al., 2014; Saenz et al., 2015). Subsequent dissemination often occurred by direct contact amongst individuals living together (Leroy et al., 2004), through patient care or through ritual burial practices (Chiappelli et al., 2014; Pandei et al., 2014; Richards et al., 2015).

The genome of the Ebola virus was elucidated in 1993 (Sanchez et al., 1993). The Ebola virus has been diversified into five different species: Zaire, Sudan, Ivory Coast, Reston, and Bundibugyo ebolavirus. All species originated in Africa, with the exception of Reston, which was discovered in Reston Virginia, from a macaque imported from the Philippines (Feldmann and Geisbert, 2011; Caroll et al., 2013). The Zaire species, the protagonist of the current outbreak, is the most virulent. The genetic differences among species are relatively high; a 35% genetic divergence among all species has been reported, based on sequences available up to 2011 (Grard et al., 2011). Until 2013, only 22 complete genome sequences for the EBOV Zaire species were available in international repositories. Most of these were collected during the outbreaks of 1976, 1990, and 2007–2008 in the Democratic Republic of Congo. Gire et al. (2014) have studied 99 EBOV genome sequences from 78 confirmed EVD patients during the current outbreak, providing new and valuable information on the genetic identity of the Zaire EBOV. The authors found 341 fixed substitutions (35 non-synonymous, 173 synonymous, and 133 noncoding) between the 2014 EBOV and all previously published EBOV sequences. With all these new sequences included, the Ebolavirus resource database at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/genome/viruses/variation/ebola) has 149 complete genome sequences of the Zaire EBOV available. Considering these sequences, the EBOV genome variability increases from 35% to 40%–45%. It should be noted that the advances achieved so far in the understanding of the infection mechanisms and in the design of an experimental vaccine and therapies against EBOV are based on the genome information available before the current outbreak. The implications of new genomic variations might be important for Ebola diagnostics and therapy.

The recent Ebola outbreak that began in West Africa in December, 2013, made evident that we are unprepared to effectively control this disease (Leroy et al., 2014; Enserink, 2014; Brad, 2014). As of July 12, 2015, there are more than 27670 documented cases of the infection (including more than 11250 deaths) in six different African countries: Guinea, Liberia, Nigeria, Mali, Sierra Leone and Senegal (WHO, 2015). Additionally, there have been eight cases outside West Africa, five in the USA, one in Spain, one in the United Kingdom and one in Italy (WHO, 2015).

Current therapeutic strategies to treat Ebola

The clinical manifestations of EVD have been reviewed elsewhere (Feldmann and Geisbert, 2011; Bah et al., 2015). As the virus reproduces and spreads in the body, it interferes with blood clotting and disrupts electrolyte balance. Based on the current treatment for EVD, which consists of supportive care, patients are frequently dehydrated and need intravenous or oral fluids with solutions that contain electrolytes (Bah et al., 2015; Lyon et al., 2014; Zhong et al., 2014). Maintaining oxygen levels and modulating coagulation (Feldmann and Geisbert, 2011; Geisbert et al., 2003) are important parts of the treatment scheme for Ebola patients (Bah et al., 2015; Lyon et al., 2014; Zhong et al., 2014). Such interventions can help sustain some patients and allow them to recover. An adequate level of support care might improve survival significantly (Bah et al., 2015; Lyon et al., 2014), but even in such conditions patients can progress toward multiorgan failure, shock, and death. Based on WHO reports, the overall fatality rate for the current Ebola outbreak is 48–49% (WHO, 2015) with a span that goes from 0 to 90% as a strong function of the quality of the supportive care received (Lyon et al., 2014).

As stated before, no commercial vaccines or specific therapies are currently available to combat Ebola. Several experimental vaccines and drugs have been tested in animal models with promising results, and some of them are currently in clinical trials (Kuehn, 2015). A comprehensive analysis of the state of the art in vaccine development strategies against EBOV can be found elsewhere (Marzi et al., 2014). Regarding therapeutic approaches against EBOV, the main strategies tested in animal models include the use of phosphorodiamidate morpholino oligomers (PMOs) (Warfield et al., 2006; Iversen et al., 2012; Warren et al., 2015) small interference RNA molecules (siRNA) (Geisbert et al., 2010; Thi et al., 2014), small-molecule antiviral drugs (Oestereich et al., 2014; Smither et al., 2014; Warren et al., 2014), and full-length mAbs.

Amongst the experimental therapies that have been proposed and tested against EVD, passive immunization using full-length mAbs is arguably the most promising strategy. Several anti-Ebola mAb have been identified and studied by a number of research groups (Wilson et al., 2000; Takada et al., 2003; Lee et al., 2008; Shedlock et al., 2010; Qiu et al., 2011; Marceau et al., 2014) and several mAb cocktails have been developed by different research groups and companies (Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013; Qiu et al., 2014), mainly based on knowledge derived from the study of viral species or strains isolated between 1976 and 1995.

Exceptional humanitarian use of Anti-Ebola mAb-based therapies

In the context of the largest Ebola outbreak ever registered, the WHO has declared that the use of experimental drugs for the humanitarian treatment of Ebola patients is ethical. An experimental treatment (ZMapp™, from Mapp Biopharmaceutical, Inc., San Diego, CA) consisting of a cocktail of three mAbs, produced in tobacco leaves, has been administered to several patients (Hampton, 2014; Goodman, 2014; Qiu et al., 2014) under this humanitarian exception. The evolution of two of the three patients first treated with ZMapp™, two American health workers infected in Liberia and treated at the Emory Hospital, in Atlanta USA, was recently documented by Lyon et al. (2015). Both patients improved their conditions shortly after receiving a first dose of ZMapp™. Since this improvement occurred in the context of aggressive rehydration, electrolyte balancing, and other care measures, the significance of the effect of the mAb cocktail cannot be conclusively established from this application (Lyon et al., 2015). ZMapp™ targets different epitopes of the Ebola virus GP. Before the current outbreak, this mAb cocktail had not been used in humans. Its predecessors had been tested only in murine and nonhuman primate models (Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013).

Interfering with viral attachment or (viral entry) into mammalian cells is a common therapeutic approach to fight envelope viruses (Wisskirchen et al., 2014). The therapeutic use of full-length antibodies against viral infection has been proposed to obstruct viral entry in the context of West Nile (Oliphant et al., 2005), herpes simplex (Highlander et al., 1988), dengue (Crill et al., 2001), influenza infection (Vanderlinden and Naesens, 2014; DiLillo et al., 2014), and SARS (Sui et al., 2004), among others. Several reports on antibodies with a high affinity for EBOV proteins are available (Lee et al., 2008, Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013, Becquart et al., 2014). Most of these studies refer to antibodies that specifically bind to different epitopes of the GP. Indeed, all mAbs proposed to be used as anti-Ebola therapeutics have the virus transmembrane GP as their target. This is logical as transmembrane GP is key to initiate virus attachment and fusion to host membranes (Nanbo et al., 2010; Sakurai et al., 2015; see Figure 1).

GP: The therapeutic target of anti-Ebola mAb-based therapies

GP is its only surface capsid protein, i.e., a transmembrane protein with spiked protrusions on the surface of the virus (see Figure 1) and plays a key role in many important EBOV functions, including the interaction with host cell receptors to activate viral attachment and/or entry. GP is also the most antigenic of the EBOV proteins. For instance, serum from EVD survivors collected a few days after the end of symptoms react mainly with GP peptides (Becquart et al., 2014). Currently, GP is believed to be required, but it is not sufficient for *in vivo* virulence (Groseth, et al., 2012). Here we provide a brief summary of the current knowledge on the structure and functions of GP, particularly those relevant to the design and/or efficacy of anti-GP mAb therapies.

Several glycoproteins (forms of GP) originate from the GP-encoding RNA sequence: a transmembrane form of GP (normally referred to in literature simply as GP), secreted GP (sGP), and a smaller version of sGP (named small sGP or ssGP) are among the most relevant

(Lee et al., 2009; Mehedi et al., 2011; Figure 1). The sGP may act as a distractor to the host immune system (de la Vega et al., 2014; Misasi and Sullivan, 2014; Mohan et al., 2012). It is highly present as a dimer in solution in the serum of infected patients, and serves as a binding target for some anti-GP antibodies produced by the host, perhaps effectively diminishing the number of antibodies available for virus neutralization (Mohan et al., 2012; Ramanan et al., 2011). The transmembrane GP of EBOV is a protein containing a high number of both N-linked and O-linked carbohydrates (Takada et al., 1997). Mature transmembrane GP is a trimer of GP1-GP2 subunits linked by disulfide bonds. Each of these subunits is generated by the proteolytic cleavage of GP0, a precursor polypeptide, during virus assembly. GP1, the membrane-distal subunit, is responsible of viral adhesion to host cells and regulates GP2, the transmembrane subunit, which participates in membrane fusion (White and Schornberg, 2015; Malashkevich et al., 1999).

The most accurate information on the three-dimensional (3D) structure of transmembrane GP has been derived from a small number of well-executed studies (Lee et al., 2008; Beniac et al., 2012; Tran et al., 2014). The structure of the trimeric GP ectodomain (Figure 2) has been more graphically referred as a “chalice,” consisting of a base, a head, and a glycan cap (Lee et al., 2008; Lee et al., 2009). The base projects a transmembrane anchor of 22 residues (651–672 in GP) that attaches GP2 to the viral membrane (Malashkevich et al., 1999), which is structurally composed of protein VP40 (Beniac et al., 2012) and covered by a lipid bilayer originating from the cells of the host upon viral budding (Figure 1(A), (B)).

Within the GP1 subunit, three regions have been frequently referred to in the literature as key to the binding and immune-evasion functions of EBOV: the glycan cap, the mucin-like domain (MLD), and the receptor-binding domain (RBD). The glycan cap and the MLD are highly glycosylated GP1 regions. The MLD, containing both N- and O-linked glycans (Lennenmann et al., 2014), spans from residues 313 to 501 in GP (Figure 3). Several neutralizing antibodies, including two comprised in MB-003 (Olinger et al., 2012), are directed against the MLD (Tran et al., 2014). Recently, Tran *et al.* (2014) used cryoelectron tomography of EBOV virus-like particles to show the exact 3D location of MLD with respect to the rest of the GP molecule. Functions attributed to MLD include: enhancing viral attachment to target cell surfaces (Marzi et al., 2007; Matsuno et al., 2010), protecting conserved regions of GP from antibody recognition, and sterically masking important immune regulatory molecules, such as MHC1 (major histocompatibility complex 1) or β1 integrin, on the surfaces of infected cells (Lennemann et al., 2014; Francica et al., 2009; Reynard et al., 2009).

The glycan cap is the other highly glycosylated region within GP1 as it contains 6 N-linked glycosylation sites. The hyper-glycosylated character of GP1 has a major role in this steric immune shielding/masking. In an elegant set of site-directed mutagenesis experiments by Lennemann *et al.* (2014), the N-linked glycan sites on EBOV GP1 (a total of 15) were systematically disrupted to better understand their role in GP function. The loss of EBOV GP1 glycosylation sites enhanced pseudo-virion infection in Vero cells. Results also indicated that the glycan cap/MLD domains mask the GP1 RBD residues required for binding. EBOV entry into murine macrophages still occurred independently on the presence of GP1 N-glycans, suggesting that N-glycan interactions are not required for entry, (at least)

into this cell type, one of the main primary EBOV targets. Also, the removal of all non-MLD GP1 N-glycans enhanced antibody sensitivity. All together, these observations suggest that N-linked glycans on the EBOV GP1 core protect GP from antibody neutralization despite the effect that these glycans might have diminishing infection efficiency (Lennemann et al., 2014).

The role of the glycan cap as a protector of the receptor-binding domain is well understood. However, there is still incomplete knowledge on the mechanisms of EBOV-host cell fusion and subsequent viral entry. A good summary of the current knowledge in this particular area has been recently provided by Gehring et al., (2014). Possibly a number of regions in the GP glycan cap interact with cell receptors to mediate/trigger the fusion of the viral and host membranes (White and Schornberg, 2015; Lee et al., 2009). *In vivo* GP appears to interact with and infect a wide variety of cells in different tissues. Monocytes, macrophages, and dendritic cells are considered EBOV primary targets (Feldmann and Geisbert, 2011; Gehring et al., 2014). Infection is then distributed through the lymphatic and vascular system to other tissues (Feldmann and Geisbert, 2011; Martines et al., 2014). Affected cells in these tissues include: alveolar macrophages, endothelial cells, fibroblasts, and other interstitial cells (in the lung); Kupffer cells and hepatocytes (in the liver); epidermal dendritic cells, endothelial cells, connective tissue fibroblasts, epithelium cells of the sweat and sebaceous glands (in the skin); cells of the mononuclear phagocytic system, dendritic cells, and fibroblasts (in the spleen and lymph nodes); mononuclear cells within the lamina propria (in the mucosa of the GI tract); endothelial cells (in renal tissue); and monocytes, interstitial cells, and endothelium cells (in testes) (Martines et al., 2014). Several molecules have been suggested as GP1 binding receptors/attachment factors (Gehring et al., 2014) in different cell types, including T-cell Ig and mucin domain 1 (TIM-1) (Kondratowicz et al., 2011) and C-Type Lectins (i.e., L-SIGN and DC-SIGN) in dendritic cells (Alvarez et al., 2000; Simmons et al., 2003). There is a dispute on the role of folate receptors as facilitators of EBOV entry (Simmons et al., 2003; Chan et al., 2001).

Experimental evidence indicates that cell binding triggers a chain of biochemical signals that lead to viral entry into the cell through macropinocytosis (Nanbo et al., 2010; Figure 4). Endosomal proteolysis of the GP, apparently mediated by low pH-dependent cysteine proteases (i.e., Cathepsin B and L), removes the glycan cap and exposes the receptor binding domain, facilitating further membrane fusion between the virus and the cell (at the endosome). This proteolitic cleavage of the glycan cap appears to be required for infection (Misasi et al., 2012; Chandran et al., 2005) and the endosomal cholesterol transporter Niemann–Pick C1 (NPC1) is believed to be an important intracellular receptor (White and Schornberg, 2015; Carette et al., 2011; Côté et al., 2011). Recently, Sakurai et al. (2015) demonstrated that the endosomal calcium channels, called two-pore channels (TPCs), are required for EBOV entry into host cells. The binding of mAbs to GP interferes at least partially with the virus's first interaction with host cells, its later entry and infection at the endosome (Figure 5). Precisely, the main rationale for the use of a mAb cocktail (instead of a single mAb) is to extend the breath of protection by binding to multiple GP regions and to more efficiently mitigate immune escape (Both et al., 2013; Ter Meulen et al., 2006).

Anti-GP mAb-based therapies: From animal models to a potential clinical use

The first set of experiments that documented a successful use of passive immunization for the EBOV infection in nonhuman primates was published in 2012 (Olinger et al., 2012; Dye et al., 2012), after 15 years of research and multiple failed attempts to prove the therapeutic potential of anti-EBOV antibodies (Qiu and Kobinger, 2014). Dye *et al.* (2012) used polyclonal antibodies that were directly recovered and concentrated from nonhuman primates (NHP) that survived EBOV infection to treat NHP that were lethally challenged with EBOV. The polyclonal mix provided full protection to the animals even when the first dose was administered 48 hours after the EBOV challenge. During the current outbreak, treatment with plasma or whole blood from convalescent patients has been used. In particular, the massive implementation of EVD treatment using convalescent plasma has been evaluated as a cost-effective countermeasure against EBOV (Gutfraind and Meyers, 2015; Kreil, 2015). The first successful case of the protective use of an anti-GP (EBOV) mAb cocktail in nonhuman primates, that were lethally challenged with EBOV, was reported by Olinger *et al.* (2012).

Currently, mAb-based therapies have proven to be the most efficient strategy to reverse the progression of a lethal EBOV challenge in nonhuman primates, and there is very limited but promising therapeutic evidence in humans (Qiu and Kobinger, 2014). Nearly 20 anti-GP full-length mAbs have been described in the recent literature, including 1H3, 2G4, 4G7, 5D2, 5E6, 7C9, 7G4, 10C8, KZ52, 13F6, 6D8, 12B5(14G7), 13C6, 6D3, 133/3.16, 226/8.1, 6E3, JP3K11, and S9. At least four different groups have led research efforts on the development of these anti-Ebola therapeutic candidates (see Table 1–3). All of these mAbs target different epitopes (some linear, but most of them conformational) of the GP Zaire EBOV protein.

While antibody cross-reactivity has been reported among VP40s and NPs of Ebola viruses, antibody binding to GPs is very specific (Kamata et al., 2014). However, some of the mAbs in Tables 1 to 3 also recognize GPs from other EBOV species. For example, mAbs 13C6 and 6D3 bind to epitopes that are highly conserved among Zaire, Sudan, and Ivory Coast EBOVs. Remarkably, a recent paper by Flyak et al. (2015) reported that several anti-GP Marbug mAbs also bind EBOV GP. Figure 3 illustrates, in a graphical way, the GP regions targeted by each of these mAbs. This information is very relevant from the perspective of drug design or drug prescription/personalization. Note that even among the Zaire EBOV strains, some mAbs may not recognize the same epitope with equal affinity in different genetic variants. For instance, the GP region between AA 305 and 510 comprehends the most genetic variations in the Zaire EBOV strains. Therefore, therapeutic mAbs that would target epitopes within this region might not bind with the same GP from different patients or geographic locations. An extreme case for Zaire EBOV is mAb 6E3, which binds to an epitope located in a region with relatively low conservation (76%). On the other hand, mAb 226/8.1 targets an epitope (Takada et al., 2003; Ponomarenko et al., 2014) (Table 1 and 2), which is highly conserved in Zaire EBOV (Figure 3). Next, we review a body of published research that documents the potential therapeutic use of the most promising anti-GP EBOV

mAbs among the 20 referred to in Tables 1 to 3. In particular, we refer to experimental evidence on the use of one mAb, named KZ52 (Lee et al., 2008), (see Figure 2), and six mAbs used in three different cocktails (named MB-003, ZMAb, and ZMapp™) that have proven to be protective against EBOV lethal challenges in nonhuman primates (Qiu et al., 2014; Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013). The genetic sequences of mAb KZ52, and all mAbs contained in MB-003, ZMAb, and ZMapp™, have been disclosed (Table 4).

MAb KZ52 is one of the first and best described neutralizing EBOV mAbs. Lee *et al.* (2008) analyzed the 3D structure of the GP bound to antibody KZ52, originally isolated from a 1995 Kikwit outbreak human survivor (Figure 2). The authors used X-ray crystallography to resolve, at the interaction site, the structural details between residues at the surface of GPs and the variable regions (Table 4) of KZ52. The antibody recognizes a relatively small (~20 GP residues), glycan-unprotected region of the protein neighboring the viral membrane surface (see Figure 2(A), (B), (E)). The KZ52-conformational epitope contains residues of GP1 and GP2 (Figure 2(C)) (Lee et al., 2008, 2009). The KZ52 mAb proved to be protective against lethal EBOV challenge in guinea pigs when administered before and immediately after infection (Parren et al., 2002). In these experiments, the medium to high levels of viremia in survivor animals suggested that other mechanisms (i.e., tagging, to activate the elimination of infected cells) besides virus neutralization were responsible for protection. Interestingly, KZ52 was unable to provide protection against lethal challenge in nonhuman primates (Oswald et al., 2007) even at doses of 50 mg/kg, which have been shown to be sufficient in other disease/mAb cases.

Olinger *et al.*(2012) reported the expression of three anti-GP virus mAbs (named c13C6, h-13F6, and c6D8) in whole plant (*Nicotiana benthamiana*) cells and in Chinese hamster ovary (CHO). The authors tested these three mAbs (MB-003, a predecessor of ZMapp™) and found them to be protective against lethal Ebola challenge in rhesus macaques when administered 1 h postinfection. The tobacco version of MB-003 was approximately three times more effective than its CHO analog in murine models, presumably due to their non-mammalian (lacking core fucose) glycosylation pattern. In a pilot study on primate models, three 16 mg/kg doses of each tobacco-derived mAb protected three out of three Ebola-infected macaques when MB-003 was administered within the first 48 h after virus exposure. In a more refined experiment (Pettitt et al., 2013), a group of macaques received a lethal Ebola virus challenge and were treated with three doses of 50 mg/kg of MB-003 several hours after they presented Ebola symptoms (four to five days after exposure). Three out of seven macaques survived the challenge. A combination therapy with ZMAb—another cocktail composed of the murine mAbs 1H3, 2G4, and 4G7—and Ad-IFN (adenovirus-vectored interferon-α) was 100% protective in rhesus macaques when administered three days after positive Ebola diagnosis (Qiu et al., 2013).

In a recent report, the different mAbs contained in MB-003 and ZMAb were tested in lethal challenge EBOV experiments in guinea pigs and nonhuman primates (Qiu et al., 2014). In a first round of experiments, the authors tested a single dose of individual mAbs or combinations of them. In these experiments, no individual mAb was able to provide protection levels above 33% of survival; c13C6 proved to be the most effective mAb in

single-dose experiments. In contrast, some mAb cocktails provided between 50% and 67% protection levels in guinea pigs. In a second round of lethal challenge experiments, this time using a scheme of three doses of mAb cocktails, some mAb combinations provided full protection. Promisingly, the administration of the mAb therapy started three, four, or five days after the lethal challenge, when viremia, as measured by RT-PCR, was high. The mAb cocktail that rendered the best results was a combination of the mouse/human chimeric mAbs c13C6 (from MB-003), c2G4 (from ZMAb), and the murine mAb m4G7 (from ZMAb). This mAb formulation (see Figure 3D) was selected by Mapp Biopharmaceuticals, Inc. to be further tested under the brand name of ZMapp™ (produced by Mapp Biopharmaceuticals, Inc.), which was recently approved by WHO for humanitarian use on Ebola patients. Clearly, mAb therapy using the ZMapp™ cocktail has proven to be helpful as an emergency resource to treat Ebola patients, although the evidence that exists at this point is insufficient to anticipate high proficiency in large-scale clinical intervention (Goodman et al., 2014).

The limited availability of robust and reliable in vitro assays/platforms that are capable of predicting the therapeutic value of an anti-EBOV mAb in animal models is an important challenge in anti-EBOV mAb research and development; the ability of a monoclonal antibody to neutralize EBOV in cell culture assays does not necessarily mean that this mAb will be protective in animal models. Moreover, the characteristics and conditions that make an antibody protective against EBOV in animal models and/or in humans are not fully understood. Furthermore, the protective ability of a specific mAb in one animal model does not necessarily imply that it provides protection in another animal model.

Nacayama and Saijo (2013) have comprehensively reviewed the different animal models used to study EBOV infection, mainly rodents and non-human primates (NHP), and have summarized their strengths and weaknesses. NHP, particularly rhesus and cynomolgus macaques, better mimic EBOV infection in humans and more closely reproduce the symptoms of the disease than do rodents. However, the use of NHP presents researchers with more significant practical and ethical hurdles (Nacayama and Saijo, 2013; Geisbert et al., 2015) than does the use of rodents. Different rodent models have been developed to do EBOV research, including some knockout variants (Brannan et al., 2015). The practicality of using rodents in the laboratory and the possibility of using genetically modified animals has made them an attractive model with which to study the protective effect of anti-EBOV therapies and vaccines. However, the EBOV has to be adapted to cause lethal infection in rodents, which represents a highly restrictive situation given the high risk presented by handling a BSL-4 pathogen, such as EBOV. Moreover, some widely used rodents, such as mice and guinea pigs, do not exhibit some of the distinctive symptoms of EBOV in primates, such as fever and rash, which further suggests dissimilarities between the anti-EBOV immune response in rodents and primates. In general, anti-EBOV protection in rodents is not a conclusive indicator of protection in humans. Probably the best example of this is the case of mAb KZ52, which is neutralizing and protective in rodents but not in NHPs. In the particular case of anti-EBOV mAb therapies, NHP studies appear to be a mandatory step in predicting efficacy in humans. However, no clear correlation can be established between therapeutic effectiveness in NHPs and humans due to the limited amount of clinical data in humans.

Anti-Ebola mAb-based therapies: Incomplete knowledge on mechanisms of action

As previously stated, the use of anti-GP mAb cocktails is mainly intended to interfere with the functions of EBOV-GP by binding GP at different epitopes (Figure 4). In brief, anti-EBOV mAbs are believed to directly interfere with EBOV infection at four different stages: (a) during host cell attachment, (b) during endosomal protease cleavage, (c) at endosomal NPC1 receptor binding, (d) and during virus-host cell membrane fusion (Figure 5). However, our knowledge on the mechanisms by which the anti-EBOV mAb therapy may work remains incomplete. A summary of the current knowledge on the effects and mechanisms of action of mAb-based therapies follows.

In experiments where MB-003 was administered to nonhuman primates (Olinger et al., 2012; Pettitt et al., 2013), the number of viral gene copies in surviving monkeys, as measured by RT-PCR, was significantly reduced by two to four orders of magnitude after the first two dosages, suggesting that the therapy effectively interferes with viremia progression. In addition, in these experiments, the titer of specific anti-GP IgGs was much higher in survivors. A plausible interpretation of this observation is that mAb therapy retards the infection progress long enough to allow the host immune system to mount a sufficiently efficient immune response.

High anti-GP titers appear to be correlated with EBOV infection survival. On the other hand, a considerable body of experimental evidence indicates that EBOV has multiple ways to evade and interfere with the host immune response. Some (but not all) of these interference strategies are mediated by transmembrane or sGPs (see Figures 1 and 4).

Wong *et al.* (2012) evaluated the correlation between immune responses and survival in rodents lethally challenged or vaccinated with EBOV using knockout mice with an impaired ability to generate normal B and/or T cell responses. In particular, vaccinated animals with impaired B cell response were unable to survive the challenge, while their wild type counterpart did. Impaired CD4+ animals were capable of mounting a certain level of protection. Results suggested that protection in mice was mainly mediated by B cells and CD4+ T cells. A high correlation between GP-specific total immunoglobulin G (IgG) levels and survival was found in both vaccinated guinea pigs and nonhuman primates. Although the mechanisms for protection after vaccination are not necessarily the same as those for infection intervention, these results clearly suggest that GP plays a relevant role in the infection propagation.

Figure 3 and 4 illustrates the GP regions where each of the mAbs listed in Tables 1 and 2 bind to GP. This information might be useful in the design of mAb cocktail therapies. Note that mAbs may inhibit different GP roles by binding to different epitopes (Figure 3,4B–C)) in different GP forms (Figure 4D–G). Since some epitopes are not accessible to mAb binding in some GP forms (i.e. MDL in transmembrane GP protects the epitopes located nearby the receptor binding domain), some mAbs can only bind the cleaved forms of GP. As stated before, anti-EBOV directly interfere with the progression of EBOV infection at different stages. During host cell attachment, mAbs with binding affinity for the exposed

epitopes of transmembrane GP (Figure 4B–D) might sterically impede GP binding to different cell attachment factors (Figure 5A). Other mAbs might obstruct the enzymatic cleavage conducted by proteases at the endosomes (Figure 5B) or block the binding of the cleaved forms of GP (Figure 4E) to cell receptors also present at endosomes, (i.e. NPC1) hence obstructing the activation of the process of endosomal virus-cell fusion (see White and Schornberg, 2015). Moreover, GP also undergoes a GP1-GP2 endosomal cleavage to release GP1 and trigger the complex structural rearrangement that mediates virus-cell membrane fusion. Experimental evidence suggests that some mAbs may bind simultaneously to GP1 and GP2 regions obstructing cleavage or rearrangement (Dias et al., 2011).

The recognition of different epitopes at different stages of viral infection results in differences in observable binding affinities and neutralization kinetics of each mAb. Shedlock et al. (2010) published the most comprehensive comparative study of anti-GP binding affinities published so far (see Figure 4A). In experiments designed to measure the extent of infection inhibition by using five different anti-GP EBOV mAbs (6D3, 13C6, KZ52, 6D8, and JP3K11), the authors exposed endothelial cell cultures to GP-pseudoviral particles in the presence of different concentrations of each mAb. The observed inhibition profiles differed significantly from mAb to mAb (Figure 4(A)). The most drastic infection inhibition was obtained when KZ52 was used, closely followed by 13C6. Interestingly, in both cases, results suggested maximum inhibition at an *in vitro* concentration of ≈1 μg/mL. JP3K1 was also capable of interfering with infection efficiently in this model, but at much higher concentrations. The authors also report and discuss other important differences in binding/neutralizing behavior among these mAbs. Both KZ52 and JP3K11 bind to conformational epitopes that contain residues in GP1 and GP2. However, the mechanisms by which these two mAbs inhibit infection could be different: JP3K1 can bind to both trimeric GP and cleaved GP. Some controversy remains on the ability of KZ52 to bind effectively to cleaved GP1. Shedlock et al. (2010) found that KZ52 is unable to bind GP-pseudoviral particles (not actual EBOV particles or the isolated GP molecule), which were treated with Cathepsin to cleave GP. Other reports have demonstrated that KZ52 is capable of binding to the purified cleaved GP trimmer (Bale et al., 2011; Hood et al., 2010). It is believed that KZ52 binds cleaved GP restraining conformational changes required for membrane fusion (Dias et al., 2011). On the other hand, 13C6 and 6D3 recognized epitopes in GP and sGP, exhibiting a greater affinity for sGP than for transmembrane GP, probably due to the increased exposure of the corresponding epitope on sGP compared to GP (Shedlock et al., 2010; Murin et al., 2014). The 6D8, a mAb that recognizes a linear epitope in MLD, was unable to interfere with infection and did not exhibit affinity for sGP or cleaved GP (GP loses the MLD during cleavage) (Shedlock et al., 2010). Some other mAbs that also target the MLD have been proven to be protective in animal models. In particular, mAbs 13F6 and 12B5 (12B5 is most probably equivalent to mAb 14G7, referred in Wilson et al., 2000) recognize linear non-glycosylated epitopes of the MLD (Olal et al., 2012. The precise binding site for these two mAbs (13F6 and 12B5) has been described in detail (Olal et al., 2012). In a recent paper, Murin et al. (2014), using single particle EM, attempted to precisely identify the binding sites of each conformational antibody contained in MB-003, ZMAb and ZMapp™ cocktails. Their results confirmed that mAb 13C6, constituent of MB-003 and ZMapp™, binds perpendicularly to the expected plane of the membrane,

straight down onto the surface of the GP, in the region of the glycan cap. Similarly, 1H3 binds the glycan cap of GP partially interfering with 13C6. The authors also showed that MAb 13F6 and 12B5 bind the MLD without interfering with each other. MAbs 2G4, 4G7, and 16F6 simultaneously target epitopes at the base of GP. The epitopes of c4G7 and c2G4 overlap extensively. These antibodies differ mainly in their angle of approach to the overlapping binding sites. While c4G7 most likely simultaneously binds GP1 and GP2, c2G4 appears to bind almost exclusively to GP2. mAb 4G7 binds slightly lower on GP, encompassing some of the GP1 base, similar to KZ52. The footprints of both c2G4 and c4G7 identified by Murin et al. (2014), as well as the footprint of KZ52 determined crystallographically (Lee et al., 2008), all include residue Q508 of GP2. A point mutation at Q508 abolishes the binding of c2G4 and c4G7 (Qiu et al., 2013), and also abolishes binding of KZ52 (Murin et al., 2014).

Some experimental evidence suggests that glycosylation (and differences in glycosylation patterns) might play a role in the design and selection of the recombinant platform for the production of anti-Ebola mAbs (Zeitlin et al., 2011). There was a three-fold difference in effectiveness between MB-003 formulations produced in tobacco versus those expressed in CHO cell lines. This suggests that the differences in glycosylation (e.g., the absence of fucose) attached at the constant region portion of the full-length antibody might play a role on the observed therapeutic effect. The absence of core fucose on full-length antibodies increases the binding of mAb to FcγRIII, a well-characterized cell receptor in macrophages (Guilliams et al., 2014). This results in a significant enhancement in antibody-dependent cell-mediated cytotoxicity (ADCC) activity, as compared with a fucosylated antibody, such as those produced by most CHO cell lines (Figure 5E). In conclusion, this observation suggests that full-length antibodies without fucose are better infected-cell markers than full-length antibodies with fucose, but it does not indicate the relative importance of ADCC activation versus simple virus entry interference. On the other hand, CHO-derived MB-003 formulations still protected nonhuman primates from lethal Ebola challenge when administered at a higher dose, suggesting that ADCC stimulation is not an absolute requirement for protection (Olinger et al., 2012).

In summary, there are two main mechanisms that appear to be involved in the therapeutic effect of anti-GP mAbs: interference with viral functions (namely attachment, cleavage or entry) and tagging for immune system attack. We do not know the relative importance of these two effects; no peer-reviewed published research is yet available on this particular topic.

Bottlenecks in anti-Ebola mAb production: Scaling up, cost, and development time

Anti-Ebola mAb therapy appears to be a promising resource to combat EVD. One severe problem remains: the production of mAbs is a complex process from a biopharmaceutical engineering perspective, and currently available production platforms are not sufficiently effective to respond quickly in an emergency. The current Ebola outbreak is unprecedented in terms of the number of people infected, the rapidness of progression, and the broadness of

geographical extent (WHO, 2014b). On the other hand, mAb doses required for each patient might be high. Based on the doses that have proven to be effective in preclinical studies in nonhuman primates (i.e., 50 mg kg^{-1} mAb^{-1} (Olinger et al., 2012)), a simple extrapolation to humans (average weight of 70 kg) suggests that approximately 10.0 g of mAbs would be required to treat each Ebola patient.

In a scenario in which 5,000 people require ZMapp™ treatment per month (as it has happened in October, 2014), 50,000 g will be required monthly. So far, ZMapp™ has been produced in tobacco plants by a high-yield transient expression strategy in which tobacco leaves from plants that are six to eight weeks old are co-transfected with constructs containing the genetic information for the synchronous production of both heavy and light chains of each antibody (Giritch et al., 2006; Castilho et al., 2011). This strategy is labor intensive but definitely useful for producing sufficient amounts of each mAb rapidly for pilot studies. However, to produce 50 kg of mAbs, approximately 150 tons of leaves would have to be transfected. The separation processes required to recover mAbs from tobacco biomass is not straightforward (Fulton et al., 2015) and has yet to be optimized and scale-up to be suitable for handling tons of biomass. Stable expression in mature tobacco plants is another option that is both less labor intensive and more scalable, but developing a tobacco plant with stable expression requires an investment of several months (Hood et al., 2002; Ma et al., 2003). A more practical option is expression in mammalian cells. Therapeutic mAbs are commercially produced by recombinant technology in suspended mammalian cell cultures in stirred tanks (Li et al., 2010). In an optimized commercial process, at least 2 g mAb L^{-1} (after purification) can be produced after two weeks of fed-batch culture in a standard 10–13 m^3 stirred-tank bioreactor using CHO cells, the warhorse for the production of glycosylated biopharmaceuticals (Li et al., 2010; Garza-García et al., 2014). Producing 100 kg of mAbs would require processing 50000 L of culture media monthly. This proposition is feasible, but the high complexity and cost of mammalian cell culture are serious drawbacks of this technological alternative; the operational cost for a single 10 m^3-CHO cell culture is approximately US$10M. Most importantly, to construct and isolate a stable, high-producing, CHO cell clone would demand no less than 90 days of development work; the optimization and scale-up of a CHO cell culture to a 10 m^3 bioreactor would demand three or four additional months (Li et al., 2010). Even assuming that an optimized process is in place to produce mAb cocktails in CHO cells, this technological path could still be compromised due to the relatively low productivity of this production platform and the high dose required to treat an Ebola patient. Illustratively, to treat 24,000 patients, approximately the total number of confirmed cases reported up to March 10st 2014, 252 kg of mAbs would be required. That is approximately 3.0% of the yearly worldwide installed capacity for mAb production (Ecker et al., 2014). The use of properly engineered mAb fragments, instead of full-length mAbs, is a therapeutic alternative that has yet to be investigated for Ebola. The use of antibody fragments to neutralize viruses or prevent virus infection is not novel; proof of concept experiments have shown their potential applications in the context of different viral infections such us HIV (Lülf et al., 2014), Influenza A H1N5 (Bal et al., 2015), SARS (Sui et al., 2004), HPV (Culp et al., 2007), and West Nile virus (Gould et al., 2005). A recent contribution by Rodríguez-Martínez et al. (2015) offers proof-of-principle of the application

of three anti-EBOV mAb fragments, containing the variable regions the mAbs KZ52, 13C6, and 13F6, in immunological assays to specifically detect recombinant GP.

The production of mAb fragments provides with several important technological (and possibly even therapeutic) advantages. MAb fragments have been suggested before to interfere with other viral infections. It can be easily and massively produced in simpler bioreactors at 1/50 of the cost required to produce full-length mAbs from CHO cells (Figure 5).

The road ahead

MAb-based anti-EBOV therapies must be further investigated to assure their safety and effectiveness at large-scale clinical interventions. No mAb-based cocktail has yet been the subject of a formal clinical trial yet. The ZMapp™ cocktail might enter into clinical trials in the USA this year.

Even preclinical data in non-human primates is still limited; only a handful of mAbs have been tested in NHPs, and only three mAbs, as a cocktail, have been tested in humans. Ideally, the set of mAbs tested in animal models should be expanded. More research is needed to fully understand the mechanisms by which different mAbs (and mAb cocktails) interfere with the progression of EVD in NHP and other more widely available animal models. A rational extrapolation of preclinical data to humans can only be accomplished when the first sets of clinical data becomes available.

Recently, the Viral Hemorrhagic Fever Immunotherapeutic Consortium (VIC), a worldwide research consortium headed by Erika Saphire (known as VIC), initiated a massive screening of anti-GP antibodies from laboratories across the globe in order to identify the best anti-EBOV therapeutic candidates for further preclinical testing. A deeper understanding of the mechanisms by which different mAbs interfere with EBOV infection will provide elements for a more rational design of anti-EBOV mAb cocktails. Relevant details of the process of EBOV entry and infection propagation are still unknown. For example, the importance of the MLD cleavage in exposing the RBD for further interaction of the virus with inner and outer cell surfaces needs to the clarified. In addition, the relative importance of mAb interference with relevant GP functions versus mAb tagging to induce ADCC activity has not yet been investigated. Finally, there are technological issues to be resolved to make feasible the massive production of anti-EBOV mAbs. Currently available platforms have yet to be adapted to produce sufficient mAb quantities fast enough to respond to epidemic outbreaks.

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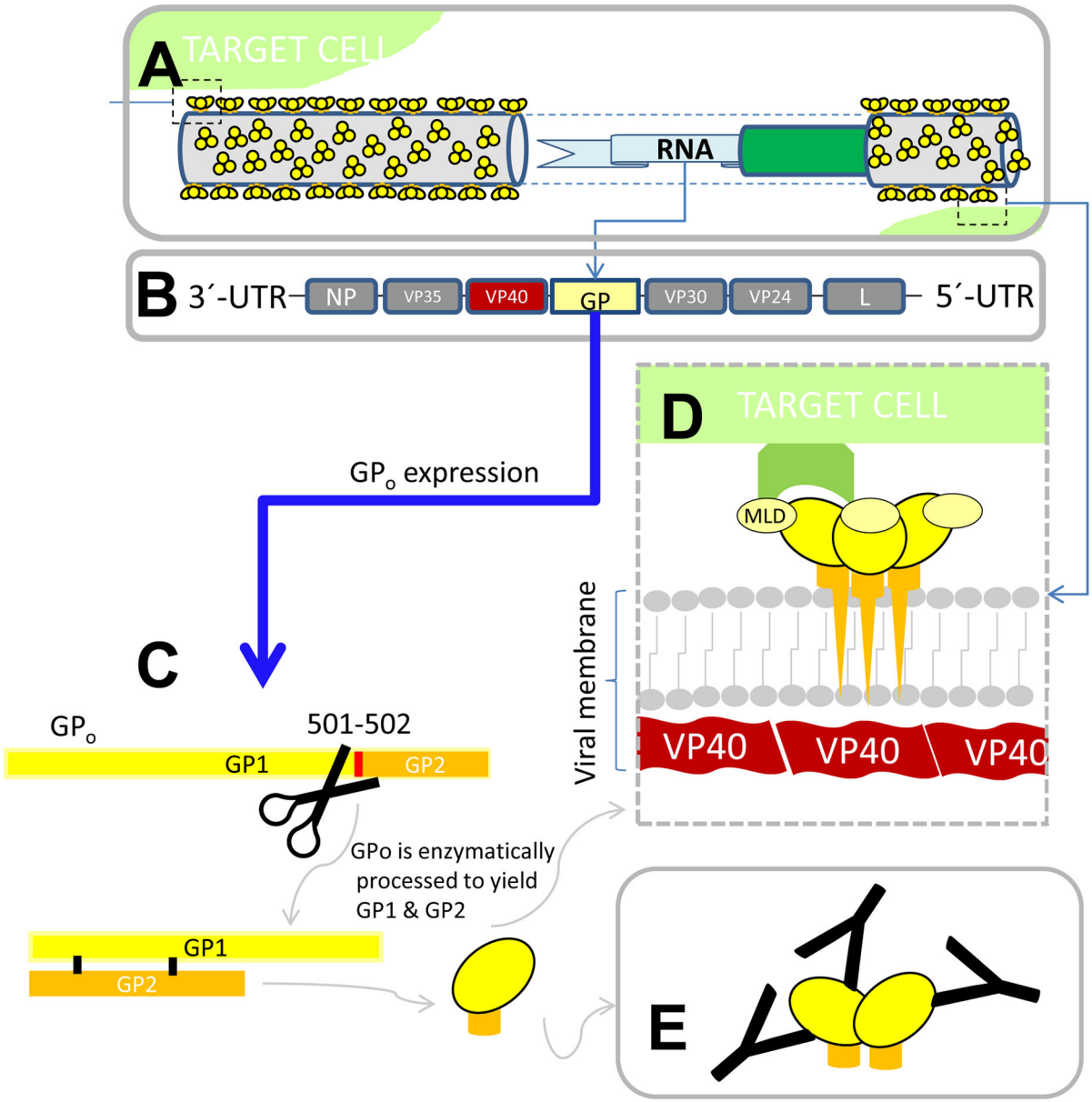

Figure 1.

The GP of EBOV. (A) The cylindrical EBOV capsid contains genetic information encoded in single strand, negative sense RNA enclosed in an inner structure composed of NP (indicated in green). (B) GP is expressed as a 676-residue protein (GP0) from the fourth of a total of seven RNA protein genes present in EBOV. (C) GP0 undergoes a post-transductional furin cleavage at the 501–502 site to render two GP units (GP1 and GP2) that bridge together through a disulfide bond to generate GP monomers. Different GP forms originate through transcriptional editing, mainly secreted dimers (sGP), small secreted monomers (small ssGP), and transmembrane GP. (D) Transmembrane GP is a trimer that is anchored in a bilipid layer and structurally supported by VP40. Each of the monomers conforming to the trimer contains a chalice-shaped domain (GP1; indicated in yellow) and a basal/

transmembrane domain (GP2; indicated as an orange stalk). The mucin like domain (MLD), a highly glycosylayed domain in GP1 (indicated in lighter shade of yellow), covers the receptor binding domain of GP. (E) Secreted GP (sGP) is believe to act as a distractor of the host immune system, serving also as a target for neutralizing antibodies, diminishing the number of mAb units effectively available for viral entry interference.

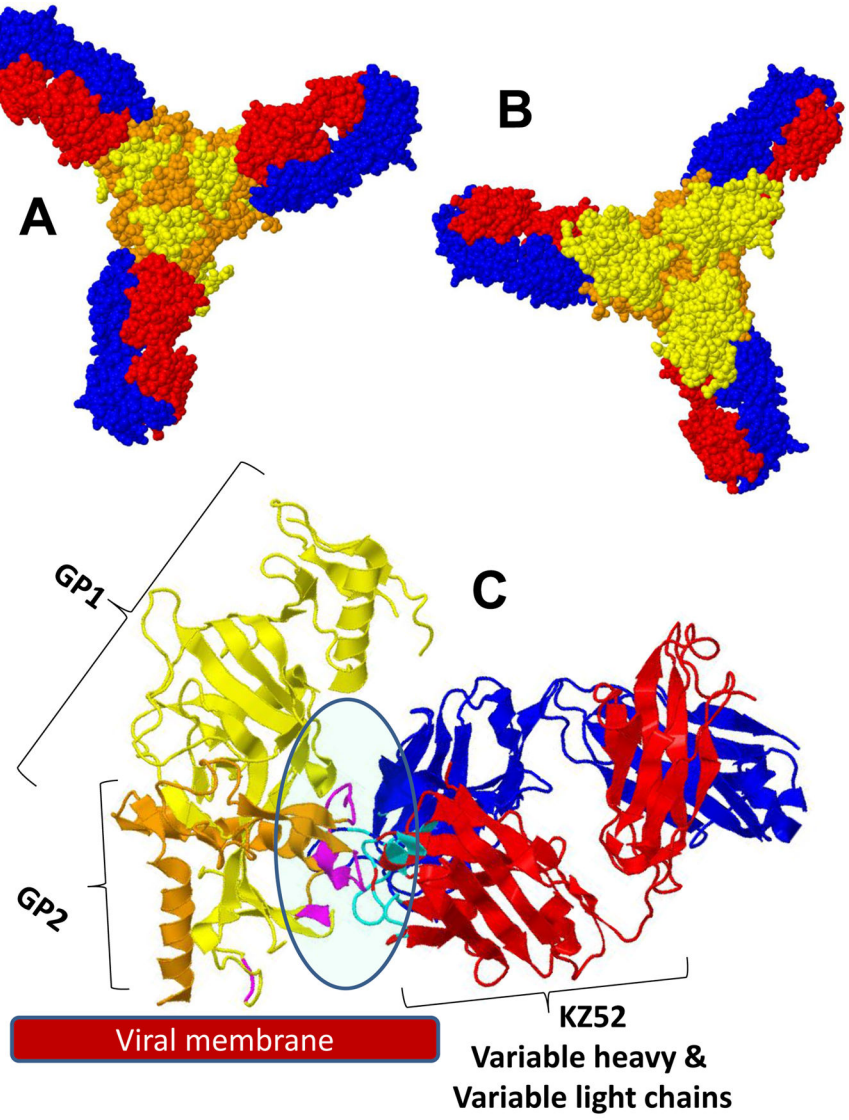

Figure 2.

3-D view of the complexity of three monoclonal antibodies binding to GP-EBOV, as resolved using X-ray crystallography (Lee et al., 2008; Lee et al., 2009): (A) bottom view (as seen from the viral surface). The GP1 subunit is colored in yellow; GP2 is colored in orange. In the case of mAb KZ52, only the FAB region is presented (variable light chains in blue; variable heavy chains in red). (B) Top view (the mucin-like domain is not presented).

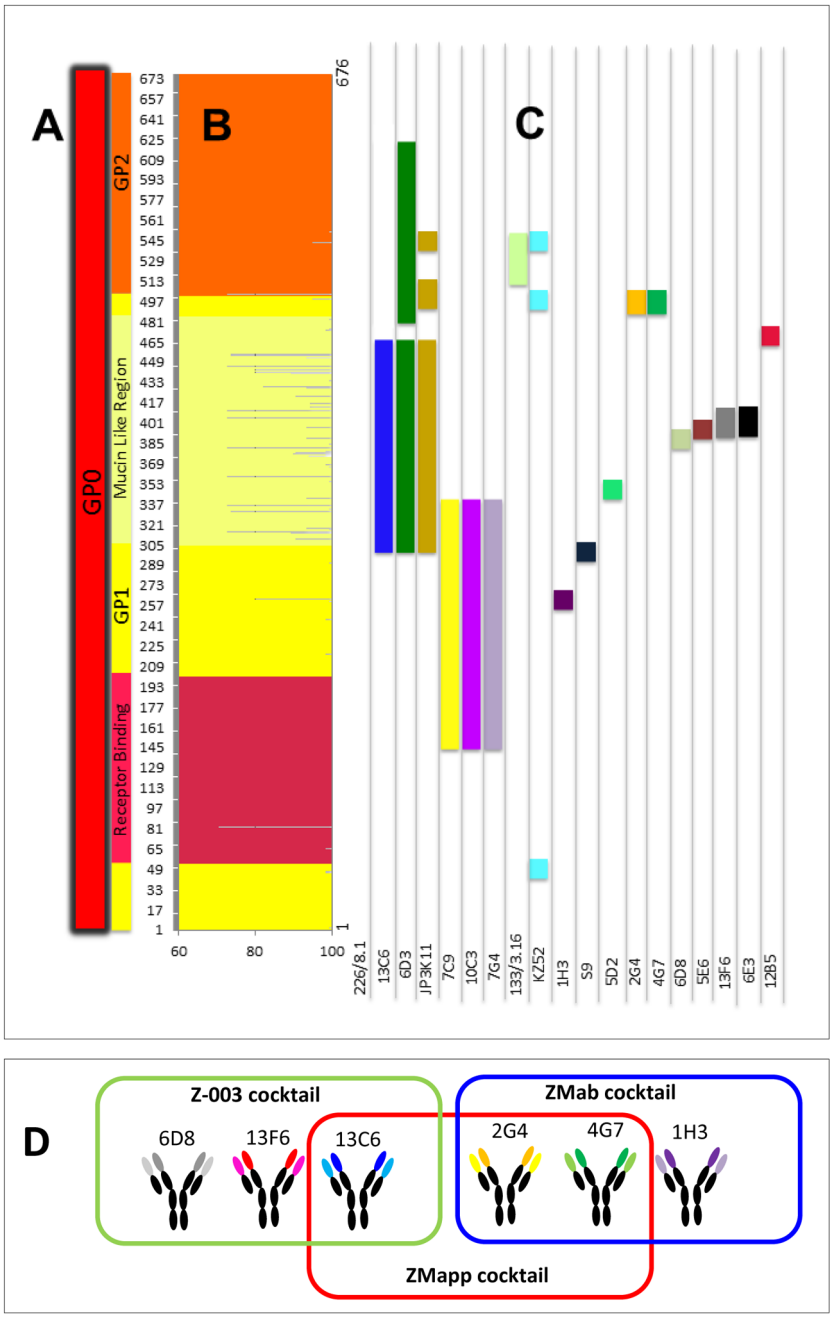

Figure 3.

Different anti-GP mAbs bind to different epitopes in EBOV-GP. (A) The different subregions on GP1 (yellow section) and GP2 (orange section) are represented. The receptor-binding domain (RBD) in GP1 is indicated in red; the mucin-like domain (MLD) in GP1 is indicated in light yellow. (B) The percentage of genetic conservation among Zaire EBOV variants ranges from 100% to 76%. The MLD is the least-conserved domain in GP1. The RBD in the GP1 is a well-conserved region. (C) Mapping of the epitopes of 19 anti-GP mAbs reported in the literature. Different epitopes are coded with different colors (one epitope per column). In most cases, the epitope location was derived from studies in which

truncated GP proteins were exposed to mAb binding. Therefore, all the residues within each color segment do not necessarily interact effectively with the corresponding binding mAb. (D) Zoom of the conformational epitope for KZ52 in GP1-GP2. (F) ZMapp™ is composed of mAbs included in predecessor formulations (MB-003 and ZMAb, produced by Mapp Biopharmaceutical, Inc, and Dephyrus Inc., respectively)

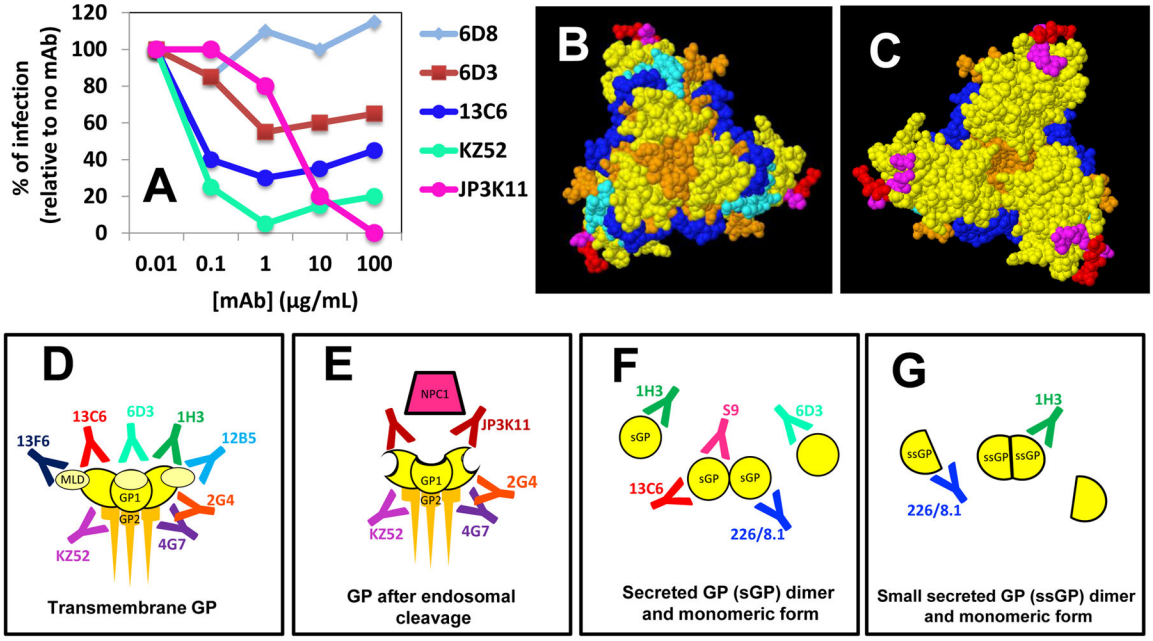

Figure 4.

Different mAbs bind to different GP epitopes and interfere at different functional levels. (A) A plot of the percentages of infection inhibition (in vitro) at different mAb concentrations for five different anti-GP mAbs (data modified from Sheldock et al. 2010); (B) Bottom and (C) top view of the chalice of the GP trimer. The epitopes for selected anti-GP mAbs have been indicated with different colors: S9 (red); 1H3 (magenta); JP3K11 (light blue); 133/3.16 (blue). (D) Some mAbs bind to transmembrane GP (E), and/or the enzymatically cleaved form of GP; (F) and/or the monomeric or dimmer versions of sGP (the secreted form of GP) and (G) ssGP.

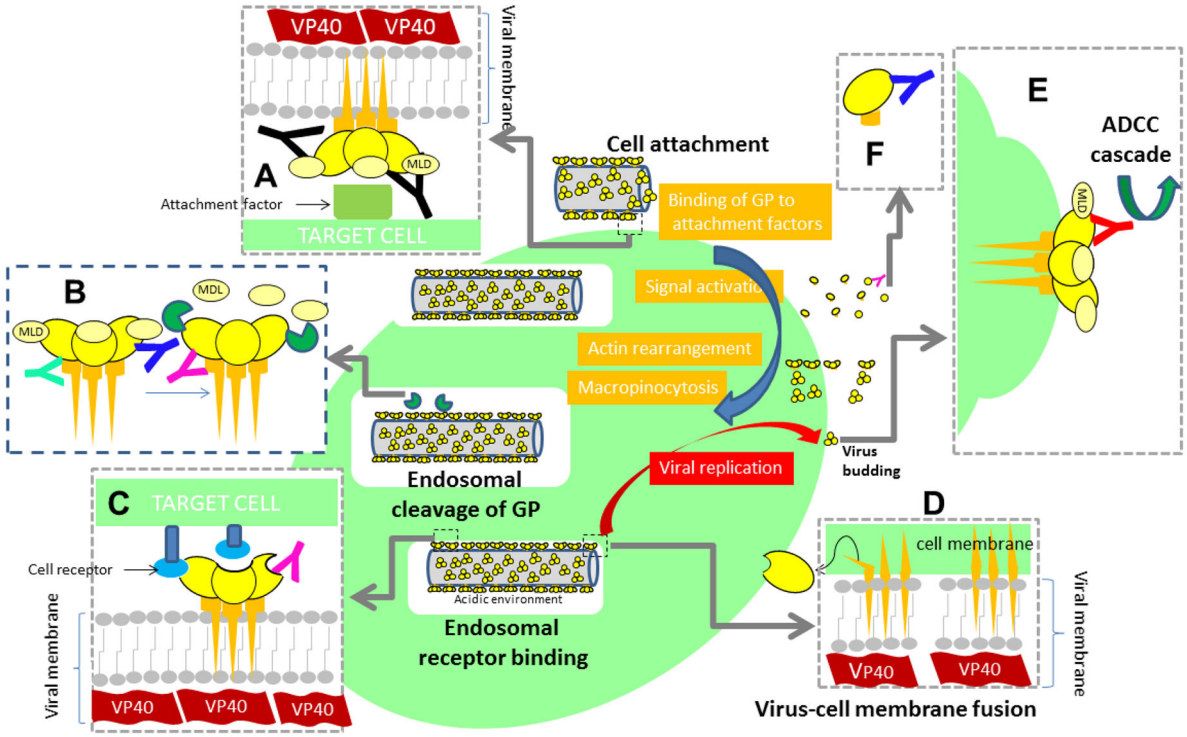

Figure 5.

Known anti-GP mAbs interfere with key GP functions at different stages of the progression of EBOV infection. (A) Upon interaction with the host cell through attachment factors (not precisely receptors), a complex series of biochemical signals are triggered, eventually leading to EBOV entry through macropinocytosis and endosome formation. Several mAbs are known to interfere with virus-cell attachment (black mAb). (B) Transmembrane GP is cleaved by proteases (dark green symbols) within endosomes. This enzymatic cleavage removes the MLD region (indicated in a lighter shade of yellow) and the glycan cap exposing the RBD at GP1. Several mAbs bind cleaved forms of GP and, by doing so, interfere with GP binding to cell receptors (pink antibody) and (C) further enabling the interaction of viral GP with cell receptors (blue ovals) through the RBD. The interaction of cleaved GP with cell receptors (v.gr. NCP1) triggers virus-cell membrane fusion. After GP binding to receptors, (D) GP is further cleaved, and a significant portion of GP1 is lost, the remaining GP1-GP2 peptide undergoes a geometrical rearrangement to initiate fusion. Some antibodies simultaneously bind epitopes at G1 and G2 interfering with the series of structural arrangements required for virus-cell membrane fusion. (E) Anti-GP mAbs may also bind to the GP molecules exposed at the surface of infected cells, marking them for further host immune response and attacking through mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC). (F) Binding to sGP conceivably decreases the number of mAb units available to interfere with transmembrane or cleaved GP.

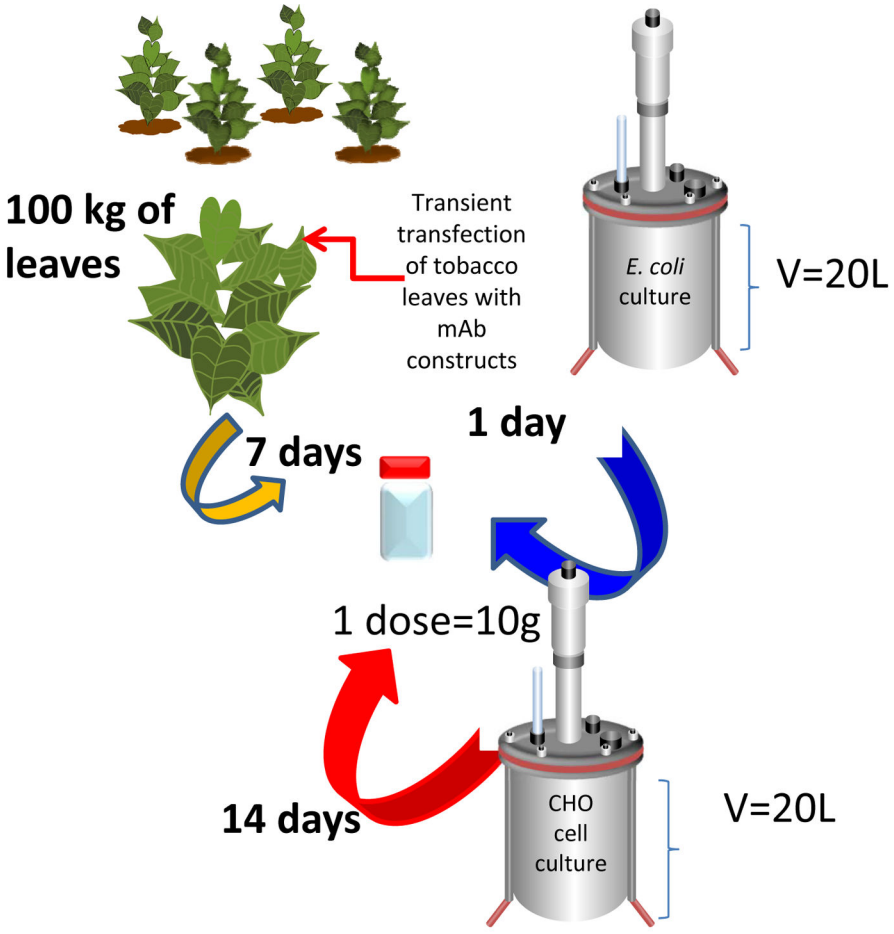

Figure 6.

Comparison of different platforms to produce anti-EBOV immune therapeutics. While transient expression in tobacco leaves is not an easily scalable solution, and CHO cell culture exhibits limitations in capacity and cost, other alternatives such as the production of mAb fragments in bacterial cultures could be a cost-effective alternative to face EBOV epidemics.

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Table 1

More than 15 anti-GP (EBOV) full-length mAbs have been described in the literature. The level of conservation among different Zaire EBOV strains varies from 76% to 100%. Among them, examples of linear and conformational epitopes can be found. Z(Zaire), S(Sudan), IC(Ivory Coast).

mAb	Target AA sequence	Specific strain: GP region	Percentage of conservation	Epitope type (linear or conformational)	Reference
1H3	267–281	Z: GP1, sGP	100%	Conformational	(Qiu et al., 2011)
2G4	501–515	Z: GP1, GP2	93.3%	Conformational	(Qiu et al., 2011)
4G7	501–515	Z: GP1, GP2	93.3%	Linear	(Qiu et al., 2011)
5D2	329–343	Z: GP1	73.3%	Linear	(Qiu et al., 2011)
5E6	401–415	Z: GP1	80%	Linear	(Qiu et al., 2011)
7C9	157–369	Z: GP1	91.5%	Conformational	(Qiu et al., 2011)
7G4	157–369	Z: GP1	91.5%	Conformational	(Qiu et al., 2011)
10C8	157–369	Z: GP1	91.5%	Conformational	(Qiu et al., 2011)
KZ52	51–52 505–513 549–556	Z: GP1, GP2	100%	Conformational	(Lee et al., 2008)
13F6	401–417	Z: GP1	76.4%	Linear	(Wilson et al., 2000)
6D8	389–405	Z: GP1	82.4%	Linear	(Wilson et al., 2000)
12B5	477–493	Z: GP1	94.1%	Linear	(Wilson et al., 2000)
6E3	401–417	Z: GP1	76.4%	Linear	(Wilson et al., 2000)
13C6	302–479 494–635	Z, S, IC: GP1, sGP	93.6%	Conformational	(Wilson et al., 2000)
6D3	302–479 494–635	Z, S, IC: GP1, GP2	93.6%	Conformational	(Wilson et al., 2000)
133/3.16	521–560	Z: GP1, GP2	97.5%	Conformational	(Takada et al., 2003)
226/8.1	1–232	Z: GP1, sGP, ssGP	97.4%	Conformational	(Takada et al., 2003)
JP3K11	302–479 505–514 549–556	Z: Cleaved GP1	90%	Conformational	(Shedlock et al., 2010)
S9	293–307	Z: GP1, sGP	100%	Linear	(Marceau et al., 2014)

Table 2

Amino acid sequence of GP epitopes targeted by different anti-Ebola mAbs. The conserved amino acids among different Zaire EBOV strains are marked in black; amino acid positions with differences among Zaire EBOV strains are indicated in gray.

mAb	Aminoacidic sequence
1H3	SNTTGKLIWKVNPEI
2G4	REAIVNAQPKCNPNL
4G7	REAIVNAQPKCNPNL
5D2	DPGTNTTTEDHKIMA
5E6	ATQVEQHHRRTDNDS
7C9	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLA
7G4	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLA
10C8	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLA
KZ52	LVVNAQPKCNPHNQDGLIC
13F6	ATQVEQHHRRTDNDSTA
6D8	HNTPVYKLDISEATQVE
12B5	GKLGLITNTIAGVAGLI
6E3	ATQVEQHHRRTDNDSTA
13C6	RSEELSFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQ PPTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDTPPATTAAGPPKAENTNTSKGTDLLDPATTT SPQNHSETAGNNNTHHQDTGEESASSGKLTGGRRARREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFG PAAEGIYTEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIE PHDWTKNITDKIDQIIHDFVDKTL
6D3	RSEELSFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQ PPTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDTPPATTAAGPPKAENTNTSKGTDLLDPATTT SPQNHSETAGNNNTHHQDTGEESASSGKLTGGRRARREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFG PAAEGIYTEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIE PHDWTKNITDKIDQIIHDFVDKTL
133/3.16	QDEGAAIGLAWIPYFGPAAEGIYTEGLMHNQDGLICGLRQ
226/8.1	MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSEVDKLVCRDKLSSTNQLRSVGLNLEGNGV ATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPC AGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQA TGFGTNETEY
JP3K11	RSEELSFTAVSNRAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQ PPTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDTPPATTAAGPPKAENTNTSKGTDLLDPATTT SPQNHSETAGNNNTHHQDTGEESASSGKLVNAQPKCNPNHNQDGLIC
S9	TKKNLTRKIRSEELSC

Table 3

Subtype (IgGx), nature (neutralizing or non-neutralizing), and animal model used to asses therapeutic efficacy of different anti-Ebola mAbs. Relevant references are included.

mAb	Subtype	Neutralizing/non-neutralizing	Animal models used	References
1H3	IgG2a	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	(Qiu et al., 2012;Qiu et al., 2013)
2G4	IgG2b	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	(Qiu et al., 2012;Qiu et al., 2013)
4G7	IgG2a	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	(Qiu et al., 2012;Qiu et al., 2013)
5D2	IgG2a	Non-neutralizing (38%)	Mice, guinea pigs	(Qiu et al., 2012)
5E6	IgG2a	Non-neutralizing	Mice, guinea pigs	(Qiu et al., 2012)
7C9	IgG2a	Non-neutralizing	Mice, guinea pigs	(Qiu et al., 2012)
7G4	IgG1	Non-neutralizing	Mice, guinea pigs	(Qiu et al., 2012)
10C8	IgG2a	Non-neutralizing	Mice, guinea pigs	(Qiu et al., 2012)
KZ52	IgG1	Neutralizing	Mice, guinea pigs, rhesus macaques	(Lee et al., 2008; Parren et al., 2002;)
13F6	IgG2a	Non-neutralizing	Mice, rhesus macaques	(Wilson et al., 2000; Olinger et al., 2012; Zeitlin et al., 2011))
6D8	IgG2a	Non-neutralizing/neutralizing with complement/neutralizing	Mice, rhesus macaques	(Wilson et al., 2000; Shedlock et al., 2010; Olinger et al., 2012; Pettitt et al., 2013)
12B5	IgG1	Non-neutralizing	Mice	(Wilson et al., 2000)
6E3	IgG1	Non-neutralizing	Mice	(Wilson et al., 2000)
13C6	IgG2a	Non-neutralizing/neutralizing with complement/neutralizing 65%	Mice, rhesus macaques	(Wilson et al., 2000; Shedlock et al., 2010; Olinger et al., 2012)
6D3	IgG2a	Non-neutralizing/neutralizing40%	Mice	(Wilson et al., 2000; Shedlock et al., 2010)
133/3.16	IgG1	Neutralizing	Mice	(Takada et al., 2003)
226/8.1	IgG1	Neutralizing	Mice	(Takada et al., 2003)
JP3K11	IgG1	Neutralizing	-----	(Shedlock et al., 2010)
S9		Neutralizing	Mice, guinea pigs	(Marceau et al., 2014)

Table 4

Genetic sequence of the variable heavy (VH) and light (VL) chains of the antibodies including in mAb cocktails proven to be protective in non-human primates in EBOV lethal challenge experiments. The **VH and VL** sequence of mAb KZ52, protective in guinea pigs but not in human-primates are also included.

mAb	Chain Heavy	Chain Light	Ref
13C6	MGRLTSSFLLLIVPAYVLXQLTLKESGPGI LKPSQTLSLTCSLSGFSLSTSGVGVGWFR QPSGKGLEWLALIWWDDDKYYNPSLKS QLSISKDFSRNQVFLKISNVDIADTATYYC ARRDPFGYDNAMGYWGQGTSVTVSSAK TTAPPVYPLVPGSL	MGIKMKSQTQAFVFAFLWLSGVDGDIV MTQSQKFMSTSVGDRVSLTCKASQNVG TAVAWYQQKPGQSPKLLIYSASNRYTGV PDRFTGSGSGTDFTLTISNMQSEDLADYF CQQYSSYPLTFGAGTKLELRRADAAPTV SIFPPS	Hart et al., 2005
2G4	GGGLMQPGGSMKLSCVASGFTFSNYWM NWVRQSPEKGLEWVAEIRLKSNNYATH YAESVKGRFTISRDDSKRSVYLQMNTLR AEDTGIYYCTRGNGNYRAMDYWGQGTS VTVSSAKTTPPS	ASLSVSVGETVSITCRASENIYSSLAWYQ QKQGKSPQLLVYSATILADGVPSRFSGSG SGTQYSLKINSLQSEDFGTYYCQHFWGT PYTFGGGTKLEIKRAD	Hart et al., 2005
4G7	GPELEMPGASVKISCKASGSSFTGFSMNW VKQSNGKSLEWIGNIDTYYGGTTYNQKF KGKATLTVDKSSSTAYMQLKSLTSEDSA VYYCARSAYYGSTFAYWGQGTLVTVSA AKTTAPS	ASLSASVGETVTITCRASENIYSYLAWYQ QKQGKSPQLLVYNAKTLIEGVPSRFSGS GSGTQFSLKINSLQPEDFGSYFCQHHFGT PFTFGSGTELEIKRAD	Hart et al., 2005
1H3	GAELVKPGASVKLSCTASGFNIKDTYIHW VKQGPEQGLEWIGRIDPANGNTKYDPKF QGKATITADTSSNTAYLQLSGLTSEDTAV YYCARESRISTMLTTGYFDYWGQGTTLT VSSAKTTAPS	AIMSASPGEKVTMTCSASSSVSYMYWY QQKPGSSPRLLIYDTSNLASGVPVRFSGS GSGTSYSLTISRMEAEDAATYYCQQWSS YPYTFGGGTKLEIKRAD	Jones et al., 2013
6D8	MDFGLIFFIVALLKGVQCDVKLLESGGGL VQPGGSLKLSCAASGFDFSRYWMSWVR QAPGKGLEWIGEINPDSSTINYTPSLKDKF IISRDNAKNTLYLQMSKVRSEDTALYYCT RQGYGYNYWGQGTTLIVSSAKTTAPPVY PLVPGSL	MKLPVRLLVLMFWIPASSSDVLLTQIPLS LPVSLGDQASISCRSSQSIVHSNGNTYLE WYLQKPGQSPKLLIYKASNRFSGVPDRF SGSGSGTDFTLKINRVEAEDLGVYYCLQ GSHVPSTFGGGTKLEIKRADAAPTVSIFP PSSKLG	Jones et al., 2013
13F6	MELGLSWIFLVLTLKGVKCEVQVVESGG GLVKPGGSLKLSCAASGFAFSSYDMSWV RQTPEKRLEWVAYISRGGGYTYYPDTVK GRFTISRDNAKNTLYLQMSSLKSEDTAM YYCSRHIYYGSSHYYAMDYWGQGTSVT VSSAKTTAPPVYPLAPGSL	MAWIXLIFFVLHCSGSFSQLVLTQSSSAS FSLGASAKLTCTLSRQHSTYTIEWYQQQ PLKPPRYVMELKKDGSHSTGDGIPDRFS GSSSGADRYLSISNIQPEDEAIYICGVGDT IKEQFVYVFGGGTKVTVLGQPKSTPTLT VFPPSSEELKENKATLVCLISNFSPSGVTV AWKANGTPITQGVDTSNPTKEGNKFMA SSFLHLTSDQWRSHNSFTCQVTHEGDTV EKSLSPAECL	Jones et al., 2013
10C8	GAELVRSGASVKLSCTSSGFNIKDYFLHW VKQRPEQGLEWIGWIDPENGDTEYAPKF QDKATMTADTSSNTAYLHLSSLTSEDTG VYYCNADGNYGKNYWGQGTTLTVSSAK TTAPS	LSLPVSLGDQASISCRSSQSLVHSNGNTF LHWYLQKPGQSPKLLIYRVSNRFSGVPD RFSGSGSGTDFTLKISRVEAEDLGVYFCS QSTHVPPYTFGGGTKLEIKRAD	Jones et al., 2013

mAb	Chain Heavy	Chain Light	Ref
7C9	GAELVKPGASVKLSCTASGFNIKDTYMH WVKERPDKGLEWIGRIDPANGNTKCDSR FQGKATITADTSSNTAYLQLSSLTSEDTA VYYCARRIYFGKGFDFWGQGTTLTVSSA KTTAPS	SSLSVSAGEKVTMSCKSSQSLFNSGDQK NYLAWYQQKPGQPPKLLIYGASTRESGV PDRFTGSGSGTDFTLTISSVQAEDLAVYY CQNDQFYPPTFGDGTKLDLKRAD	Jones et al., 2013
5E6	GGGLVKPGGSLKLSCAASGSAFSRYDMS WVRQTPEKRLEWVAYISRGGGFIYYPDT VKGRFTISRDNAKNTLYLQMSSLKSDDT AMYYCARHVYYGSSPLYAMDYWGQGT SVTVSSAKTTAPS	SASFSLGASAKLTCTLSSQHSTFTIEWYQ QQPLKPPKYVMELKKDGSHSTGDGIPDR FSGSSSGADRYLSISNIQPEDEAIYICGVG DTINEQFVYVFGGGTKVTVLG	Jones et al., 2013
5D2	GPGLVRPSQSLSLTCTVTGYSITSDYAWN WIRQFPGNKLEWLGYITNTGSTGFNPSLK SRISITRDTSKNQFFLQLISVTTEDTATYH CARGLAYWGQGTLVTVSAAKTTAPS	LTLSVTIGQPASISCKSSQSLLDSDGKTYL NWLLQRPGQSPKRLIYLVSKLDSGVTDR FTGSGSGTDFTLKISRVEAEDLGVYYCW QGTHSPFTFGSGTKLEIKRAD	Jones et al., 2013
KZ52	EVQLLESGGGLVKPGGSLRLSCAASGFTL INYRXNWVRQAPGKGLEWVSSISSSSSYI HYADSVKGRFTISRDNAENSLYLQXNSL RAEDTAVYYCVREGPRATGYSXADVFDI WGQGTXVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSL GTQTYICNVNHKPSNTKVDKKVEPK	ELVXTQSPDSLAVSLGERATINCKSSQSV LYSSNNKSYLAWYQQKPGQPPKLLIYW ASTRESGVPDRFSGSGSGTDFTLTISSLQA EDVAVYYCQQYYSAPLTFGGGTKVEIKR TVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLRSPVTKSFNR	Lee et al., 2008