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REVIEW ARTICLE

# 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 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 EBOV 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, epidemic, GP, mAbs, monoclonal antibodies, therapeutic

## History

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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 (Leroy et al., 2005; WHO, 1978). Yet, no vaccine or specific treatment against Ebola infection is commercially available.

The EBOV is an enveloped, nonsegmented, 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 & Geisbert, 2011). In brief, experimental evidence suggests that fruit bats are its main natural reservoir (Leroy et al., 2004, 2005). Human outbreaks have been associated with previous occurrences of nonhuman primate (NHP) outbreaks. The patient-zero cases have been mainly hunters, infected when manipulating dead NHPs (gorillas and 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; Saéz et al., 2014). Indeed, the Guinean 2-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; Saéz et al., 2014). 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., 2015; Pandey et al., 2014; Richards et al., 2015).

The genome of the EBOV was elucidated in 1993 (Sanchez et al., 1993). The EBOV 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 (Carroll et al., 2013; Feldmann & Geisbert, 2011). 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

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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 nonsynonymous, 173 synonymous and 133 noncoding) between the 2014 EBOV and all previously published EBOV sequences. With all these new sequences included, the EBOV 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 (Brady, 2014; Enserink, 2014; Leroy et al., 2014). As of November 1 2015, there are more than 28 635 documented cases of the infection (including more than 11 300 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 UK and one in Italy (WHO, 2015).

### Current therapeutic strategies to treat Ebola

The clinical manifestations of EVD have been reviewed elsewhere (Bah et al., 2015; Feldmann & Geisbert, 2011). 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 & 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 World Health Organization (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 & Feldmann, 2014). Regarding therapeutic approaches against EBOV, the main strategies tested in animal models include the use of phosphorodiamidate morpholino oligomers (Iversen et al., 2012; Warren et al., 2015; Warfield et al., 2006), 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.

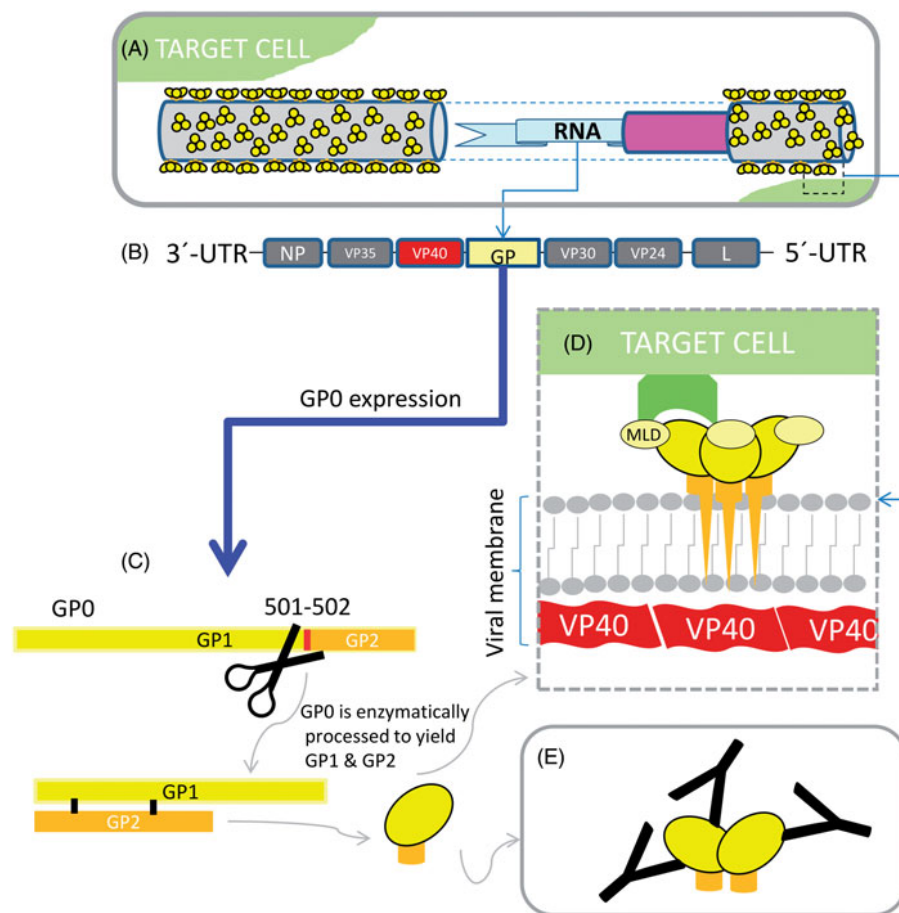
Amongst the experimental therapies that have been proposed and tested against EVD, passive immunization using full-length mAbs is arguably the most promising strategy. Several anti-Ebola mAb have been identified and studied by a number of research groups (Lee et al., 2008; Marceau et al., 2014; Qiu et al., 2011; Shedlock et al., 2010; Takada et al., 2003; Wilson et al., 2000) 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, 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 (WHO, 2014). 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 (Goodman, 2014; Hampton, 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. (2014). 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., 2014). ZMapp™ targets different epitopes of the EBOV GP. Before the current outbreak, this mAb cocktail had not been used in humans. Its predecessors had been tested only in murine and non-human primate (NHP) 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 & Roehrig, 2001), influenza infection (DiLillo et al., 2014; Vanderlinden & Naesens, 2014), SARS (Sui et al., 2004), among others. Several reports on antibodies with a high affinity for EBOV proteins are available (Beccquart et al., 2014; Lee et al., 2008; Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013). Most of these

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 magenta). (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-translational 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 (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 MLD, a highly glycosylated domain in GP1 (indicated in lighter shade of yellow), covers the receptor binding domain of GP. (E) Secreted GP is believed 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.



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 (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 & Saphire, 2009;

Mehedi et al., 2011; Figure 1). The sGP may act as a distractor to the host immune system (de La Vega et al., 2015; Misasi & 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 (Figure 1C), 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 (Malashkevich et al., 1999; White & Shornberg, 2012).

The most accurate information on the three-dimensional (3D) structure of transmembrane GP has been derived from a small number of well-executed studies (Beniac et al., 2012; Lee et al., 2008; 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 & Saphire, 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



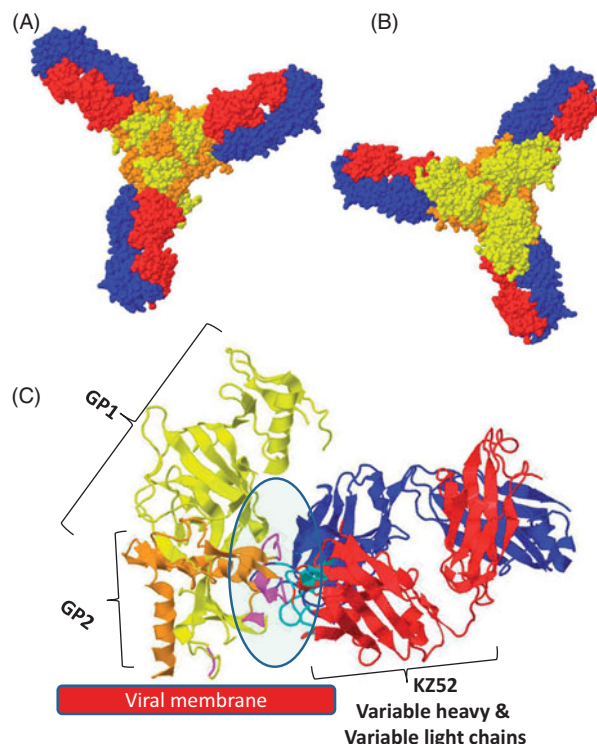


Figure 2. 3D view of the complexity of three mAbs binding to GP-EBOV, as resolved using X-ray crystallography (Lee et al., 2008; Lee & Saphire, 2009): (A) bottom view (as seen from the viral surface). The GP1 subunit is colored in yellow; GP2 is colored in orange. In the case of mAb KZ52, only the FAB region is presented (variable light chains in blue; variable heavy chains in red). (B) Top view (the mucin-like domain is not presented). (C) Zoom of the conformational epitope for KZ52 in GP1-GP2. The specific residues involved in the interaction between GP and mAb KZ52 are indicated in magenta and cyan, respectively.

VP40 (Beniac et al., 2012) and covered by a lipid bilayer originating from the cells of the host upon viral budding (Figure 1A and D).

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 (Lennemann et al., 2014), spans from residues 313 to 501 in GP (Figure 3A and B). Several neutralizing antibodies, including two comprised in MB-003 (Olinger et al., 2012), are directed against the MLD (Tran et al., 2014; Figure 4). 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  $\beta$ 1 integrin, on the surfaces of infected cells (Francica et al., 2009; Lennemann et al., 2014; Reynard et al., 2009).

The glycan cap is the other highly glycosylated region within GP1 as it contains six 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 GP Receptor Binding Domain (RBD) residues required for binding. EBOV entry into murine macrophages still occurred independently in 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 RBD 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 (Lee & Saphire, 2009; White & Shornberg, 2012). *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 & Geisbert, 2011; Gehring et al., 2014). Infection is then distributed through the lymphatic and vascular system to other tissues (Feldmann & Geisbert, 2011; Martinez et al., 2015). 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) (Martinez et al., 2015). 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., 2002; Simmons et al., 2003). There is a dispute on the role of folate receptors as facilitators of EBOV entry (Chan et al., 2001; Simmons et al., 2003).

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 5). 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

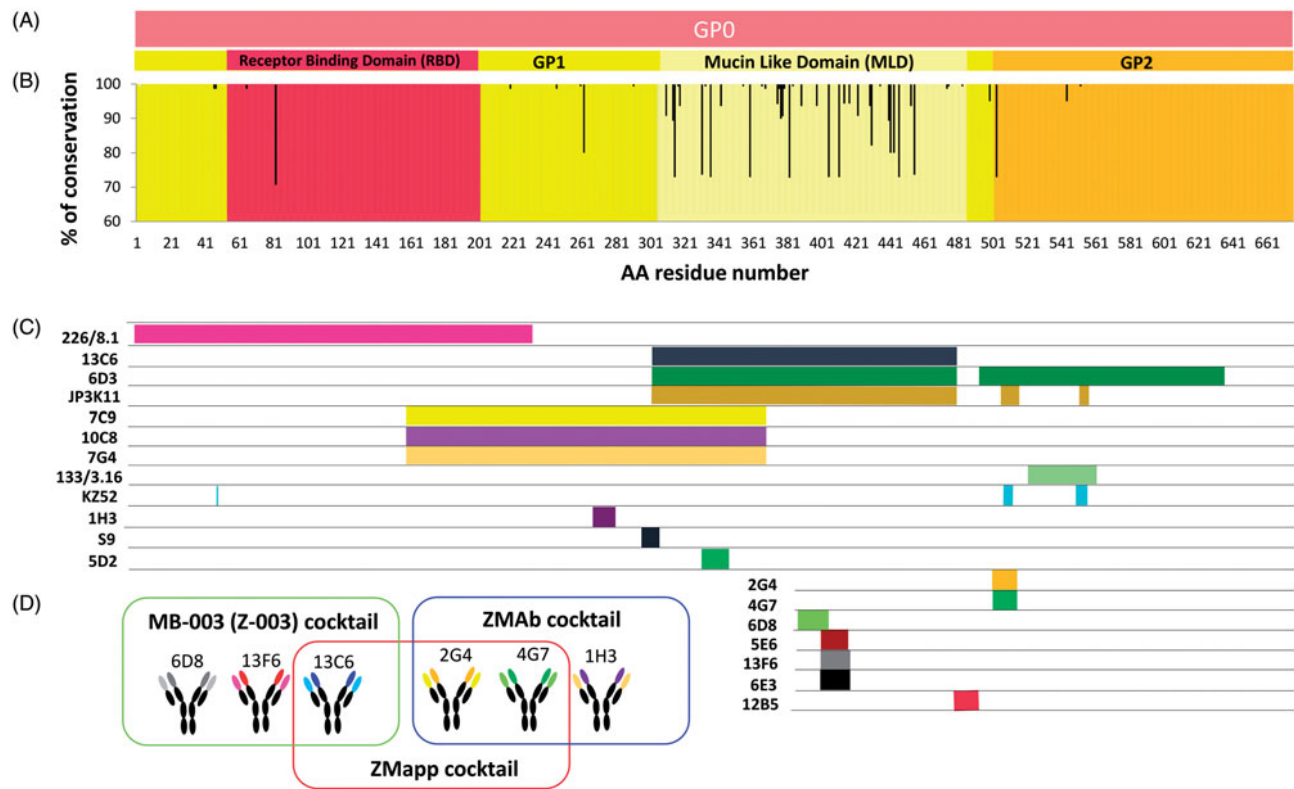


Figure 3. Different anti-GP mAbs bind to different epitopes in EBOV-GP. (A) The different subregions on GP1 (yellow section) and GP2 (orange section) are represented. The RBD in GP1 is indicated in red; the MLD in GP1 is indicated in light yellow. (B) The percentage of genetic conservation among Zaire 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) ZMapp™ is composed of mAbs included in predecessor formulations (MB-003 and ZMAb, produced by Mapp Biopharmaceutical Inc., San Diego, CA, and Defyrus Inc., Toronto, Canada and Defyrus Inc., Toronto, Canada, respectively).

domain, facilitating further membrane fusion between the virus and the cell (at the endosome). This proteolytic cleavage of the glycan cap appears to be required for infection (Chandran et al., 2005; Misasi et al., 2012) and the endosomal cholesterol transporter Niemann-Pick C1 (NPC1) is believed to be an important intracellular receptor (Carette et al., 2011; Côté et al., 2011; White & Shornberg, 2012). Recently, Sakurai et al. (2015) demonstrated that the endosomal calcium channels, called two-pore channels, 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; Figure 4).

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 NHPs was published in 2012 (Dye et al., 2012; Olinger et al., 2012), after 15 years of research and multiple failed attempts to prove the therapeutic potential of anti-EBOV antibodies (Qiu & Kobinger, 2014). Dye et al. (2012) used polyclonal antibodies

that were directly recovered and concentrated from NHPs that survived EBOV infection to treat NHPs that were lethally challenged with EBOV. The polyclonal mix provided full protection to the animals even when the first dose was administered 48 h 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 & Meyers, 2015; Kreil, 2015). The first successful case of the protective use of an anti-GP (EBOV) mAb cocktail in NHPs 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 NHPs, and there is very limited but promising therapeutic evidence in humans (Qiu & 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 (Tables 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 EBOVs, antibody binding to GPs is very

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 Shedlock et al., 2010); (B) Bottom and (C) top views 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 dimeric versions of sGP (the secreted form of GP) and (G) ssGP.

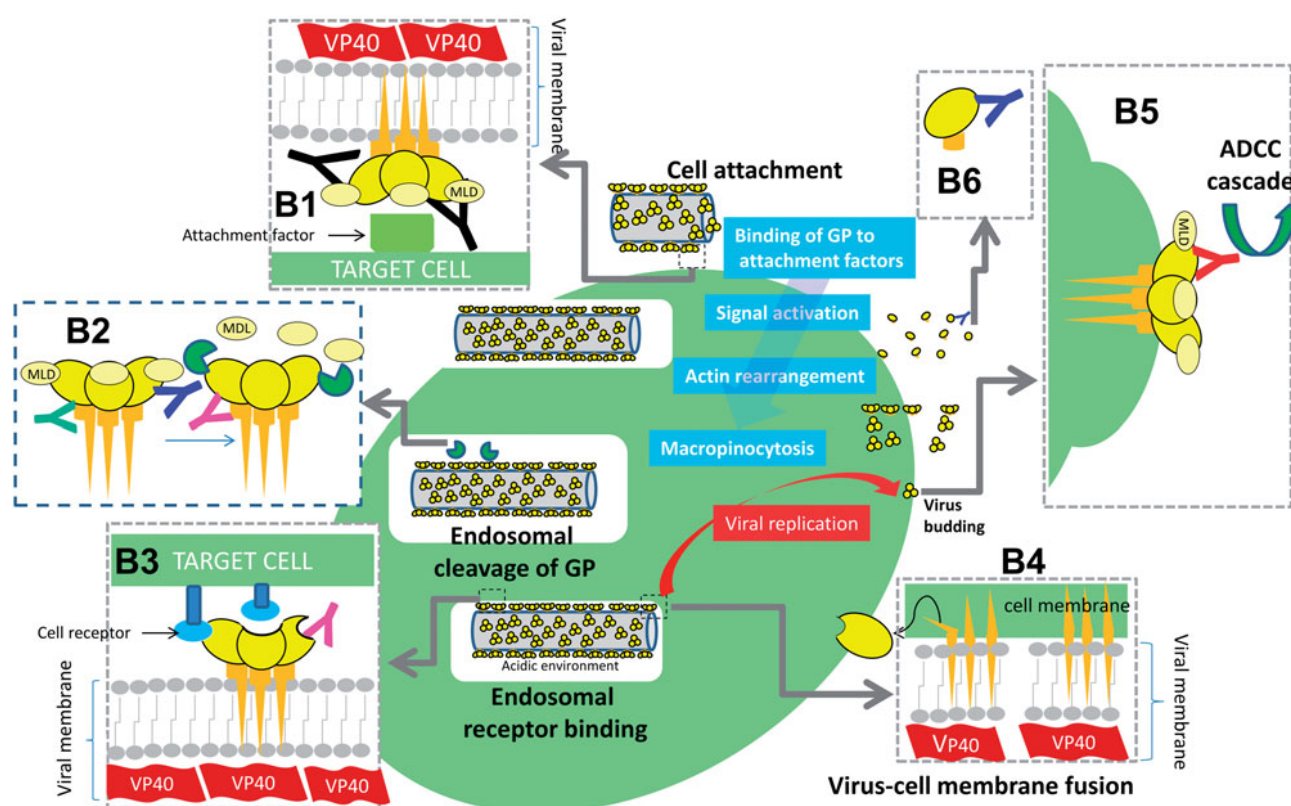
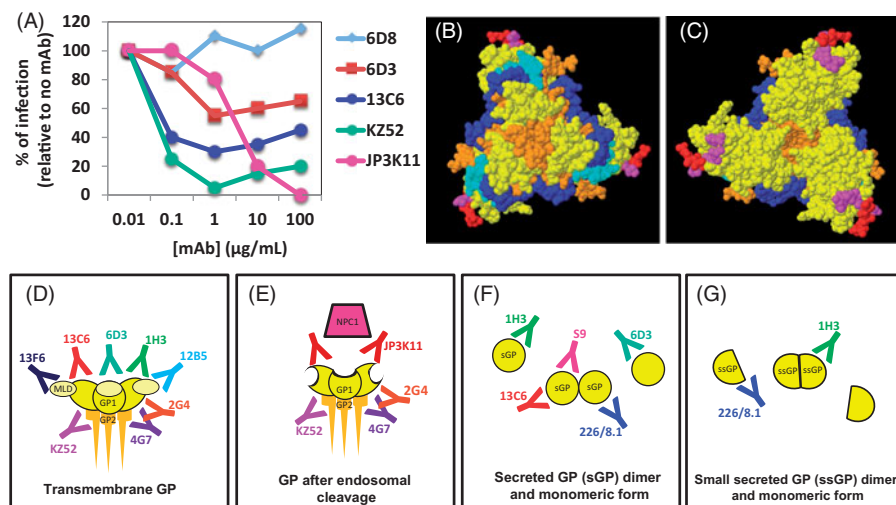


Figure 5. Known anti-GP mAbs interfere with key GP functions at different stages of the progression of EBOV infection. (A) Upon interaction with the host cell through attachment factors (not precisely receptors), a complex series of biochemical signals are triggered, eventually leading to EBOV entry 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. NPC1) 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.

specific (Kamata et al., 2014). However, some of the mAbs in Tables 1–3 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 and 4 illustrates, in a graphical way, the GP regions targeted by each of these mAbs. This information is very



Table 1. More than 15 anti-GP (EBOV) full-length mAbs have been described in the literature.

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	Z: GP1, GP2	100	Conformational	Lee et al. (2008)
	505–513				
	549–556				
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	Z, S, IC: GP1, sGP	93.6	Conformational	Wilson et al., (2000)
	494–635				
6D3	302–479	Z, S, IC: GP1, GP2	93.6	Conformational	Wilson et al. (2000)
	494–635				
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	Z: Cleaved GP1	90	Conformational	Shedlock et al. (2010)
	505–514 549–556				
S9	293–307	Z: GP1, sGP	100	Linear	Marceau et al. (2014)

Notes: The level of conservation among different Zaire EBOV variants varies from 76% to 100%. Among them, examples of linear and conformational epitopes can be found. Z, Zaire; S, Sudan; IC, Ivory Coast.

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%). However, mAb 226/8.1 targets an epitope (Ponomarenko et al., 2014; Takada et al., 2003) (Tables 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 1–3. 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 (Figure 3D) used in three different cocktails (named MB-003, ZMAb and ZMapp™) that have proven to be protective against EBOV lethal challenges in NHPs (Olinger et al., 2012; Pettitt et al., 2013; Qiu et al., 2013, 2014). 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). The KZ52-conformational epitope contains residues of GP1 and GP2 (Figure 2C) (Lee et al., 2008; Lee & Saphire, 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 NHPs (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*) and in Chinese hamster ovary (CHO) cells. 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 nonmammalian (lacking core fucose) glycosylation pattern. In a pilot study on NHP 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 EBOV challenge and were treated with three doses of 50 mg/kg of MB-003 several hours after they presented Ebola symptoms (4–5 after exposure). Three of seven

Table 2. Amino acid sequence of GP epitopes targeted by different anti-Ebola mAbs.

mAb	Aminoacidic sequence
1H3	SNTTGKLIWKVNPEI
2G4	REAI VNAQPKCNPNL
4G7	REAI VNAQPKCNPNL
5D2	DPG T N T T I E D H K I M A
5E6	ATQV E Q H H R R I D N D S
7C9	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPOAKKDFSSHPLREPVNATEDPSSGGYYSTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLQLNETIYISGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A
7G4	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPOAKKDFSSHPLREPVNATEDPSSGGYYSTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLQLNETIYISGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A
10C8	GAFFLYDRLASTVIYRGTTFAEGVVAFLILPOAKKDFSSHPLREPVNATEDPSSGGYYSTIRYQATGFGTNETE YLFEVDNLTYVQLESRFTPQFLQLNETIYISGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEEL SFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A
KZ52	L V V N A Q P K C N P H N Q D G L I C
13F6	ATQV E Q H H R R I D N D S T A
6D8	H N T P V Y K L D I S E A T Q V E
12B5	G K L G L I T I N T I A G V A G L I
6E3	ATQV E Q H H R R I D N D S T A
13C6	RSEELSFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A T I S T S P Q P P T T K P G P D N S T H N T P V Y K L D I S E A T Q V E Q H H R R I D N D S T A S D T P P A T T A A G P P K A E N T N T S K G T D L L D P A T T I S P Q N H S E T A G N N N T H Q D T G E E S A S S G K L T G G R R A R R E A I V N A Q P K C N P N L H Y W T T Q D E G A A I G L A W I P Y F G P A A E G I Y T E G L M H N Q D G L I C G L R Q L A N E T T Q A L Q L F L R A T T E L R T F S I L N R K A I D F L L Q R W G G T C H I L G P D C C I E P H D W T K N I T D K I D Q I I H D F V D K T L
6D3	RSEELSFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A T I S T S P Q P P T T K P G P D N S T H N T P V Y K L D I S E A T Q V E Q H H R R I D N D S T A S D T P P A T T A A G P P K A E N T N T S K G T D L L D P A T T I S P Q N H S E T A G N N N T H Q D T G E E S A S S G K L T G G R R A R R E A I V N A Q P K C N P N L H Y W T T Q D E G A A I G L A W I P Y F G P A A E G I Y T E G L M H N Q D G L I C G L R Q L A N E T T Q A L Q L F L R A T T E L R T F S I L N R K A I D F L L Q R W G G T C H I L G P D C C I E P H D W T K N I T D K I D Q I I H D F V D K T L
133/3.16	Q D E G A A I G L A W I P Y F G P A A E G I Y T E G L M H N Q D G L I C G L R Q
226/8.1	M G V T G I L Q L P R D R F K R T S F F L W V I I L F Q R T F S I P L G V I H N S T L Q V S E V D K L Y C R D K L S T N Q L R S V G L N L E G N G V A T D V P S A T K R W G F R S G V P P K V V N Y E A G E W A E N C Y N L E I K K P D G S E C L P A A P D G I R G F P C R Y V V H K V S G T G R C A G D F A F H K E G A F L Y D R L A S T V I Y R G T T F A E G V V A F L I L P O A K K D F S S H P L R E P V N A T E D P S S G Y Y S T I R Y Q A T G F G T N E T E Y
JP3K11	RSEELSFTAVSNRAKNISGQSPARTSSDPG T N T T I E D H K I M A S E N S S A M V Q V H S Q G R E A A V S H L T T L A T I S T S P Q P P T T K P G P D N S T H N T P V Y K L D I S E A T Q V E Q H H R R I D N D S T A S D T P P A T T A A G P P K A E N T N T S K G T D L L D P A T T I S P Q N H S E T A G N N N T H Q D T G E E S A S S G K L V N A Q P K C N P N H N Q D G L I C
S9	T K K N L T R K I R S E E L S C

Notes: The conserved amino acids among different Zaire EBOV variants are marked in black; amino acid positions with differences among Zaire EBOV variants (1976–2014) are indicated in gray.

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- $\alpha$ ) 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 NHPs (Qiu et al., 2014). In a first round of experiments, the authors tested a single dose of individual mAbs or their combinations. 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 3, 4 or 5 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 (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 (WHO, 2014). 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, 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

Table 3. Subtype (IgGx), nature (neutralizing or non-neutralizing), and animal model used to asses therapeutic efficacy of different anti-Ebola mAbs.

mAb	Subtype	Neutralizing/non-neutralizing	Animal models used	References
1H3	IgG2a	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	Qiu et al. (2012a, 2012b, 2013, Audet et al. 2014)
2G4	IgG2b	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	Qiu et al. (2012a, 2012b, 2013, Audet et al. 2014)
4G7	IgG2a	Neutralizing	Mice, guinea pigs, cynomolgus, rhesus macaques	Qiu et al. (2012a, 2012b, 2013, Audet et al. 2014)
5D2	IgG2a	Non-neutralizing (38%)	Mice, guinea pigs	Qiu et al. (2012b)
5E6	IgG2a	Non-neutralizing	Mice, guinea pigs	Qiu et al. (2012b)
7C9	IgG2a	Non-neutralizing	Mice, guinea pigs	Qiu et al. (2012b)
7G4	IgG1	Non-neutralizing	Mice, guinea pigs	Qiu et al. (2012b)
10C8	IgG2a	Non-neutralizing	Mice, guinea pigs	Qiu et al. (2012b)
KZ52	IgG1	Neutralizing	Mice, guinea pigs, rhesus macaques	Lee et al. (2008) and 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/neutralizing (40%)	Mice	Wilson et al. (2000), Shedlock et al. (2010)
133/3.16	IgG1	Neutralizing	Mice	Takada et al. (2003, 2007)
226/8.1	IgG1	Neutralizing	Mice	Takada et al. (2003, 2007)
JP3K11	IgG1	Neutralizing	–	Shedlock et al. (2010)
S9		Neutralizing	Mice, guinea pigs	Marceau et al. (2014)

Note: Relevant references are included.

animal model does not necessarily imply that it provides protection in another animal model.

Nakayama & Saijo (2013) have comprehensively reviewed the different animal models used to study EBOV infection, mainly rodents and NHPs, and have summarized their strengths and weaknesses. NHP models, 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 (Geisbert et al., 2015; Nakayama & Saijo, 2013) 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 have 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 3C and 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 and (d) 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 NHPs (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 2–4 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

Table 4. Amino acid sequence of the variable heavy (VH) and light (VL) chains of the antibodies including in mAb cocktails proven to be protective in NHPs in EBOV lethal challenge experiments.

mAb	Heavy chain	Light chain	Reference
13C6	MGRLTSSFLLLIVPAYVLXQLTLKESG PGILKPSQTLSTLCSLGSFSLSTSGVGV GWFQPSGKGLEWALIWWDKYY NPSLKSQSLSKDFSRNQVFLKISNVDI ADTATYYCARRDPFGYDNAMGYWG QGSTVTVSSAKTTAPPVYPLVPGSL	MGIKMKSQTQAFVFAFLWLSGVDGDI VMTQSQKFMSTSVGDRVSLTCKASQN VGTAVAWYQQKPGQSPKLLIYSASNR YTGVPDRFTGSGSGTDFTLTISNMQSE DLADYFCQQYSSYPLTFGAGTKLELR RADAAPTVSIFPPS	Hart et al. (2005)
2G4	GGGLMQPGGSMKLSVASGFTFSNY WMNWVRQSPKGLEWVAEIRLKSNN YATHYAESVKGRFTISRDDSKRSVYL QMNTLRAEDTGIIYCTRGNGNYRAM DYWGQGSTVTVSSAKTTTPPS	ASLSVSVGETVSITCRASENIYSSLA YQQKQKQSPQLLVYSATILADGVPSR FSGSGSGTQYSLKINSLSQSEDFGTYYC QHFVGTPYTFGGGKLEIKRAD	Hart et al. (2005)
4G7	GPELMPGASVKISCKASGSSFTGFSM NWWKQNGKSLWIGNIDTYGGTTY NQKFKGKATLTVDKSSSTAYMQLKSL TSEDSAVYYCARSAYYGSTFAYWGQ GTLVTVSAAKTTPAS	ASLSASVGETVTITCRASENIYSYLA YQQKQKQSPQLLVYNKTLIEGVPSR FSGSGSGTQFSLKINSLPEDFGSYFCQ HHFGTPTFTGSGTELEIKRAD	Hart et al. (2005)
1H3	GAELVKPGASVKLSCTASGFNIKDTYI HWVKQPEQGLEWIGRIDPANGNTK YDPKFQKATITADTSSNTAYLQLSGL TSEDYAVYYCARESRISTMLTGYFDY WGQGTTLTVSSAKTTAPAS	AIMSASPGKVTMTCSASSVSVMY YQQKQKQSPQLLVYDTSNLSAGVPVRF SGSGSGTSYSLTISRMEAEADATYYCQ QWSSYPYTFGGGKLEIKRAD	Jones et al. (2013)
6D8	MDFGLIFFIVALLKGVQCDVKLLESGG GLVQPGGSLKLSCAASGDFSRYSWMS WVRQAPGKGLEWIGEPDSTINYTP SLKDKFIISRDNAKNTLYLQMSKVRSE DTALYYCTRQGYGNYWGQGTTLIV SSAKTTAPPVYPLVPGSL	MKLPVRLVLMFWIPASSSDVLLTQIP LSLPVSLGDQASISCRSSQSVHSNGNT YLEWYLQKPGQSPKLLIYKASNRFSG VPDRFSGSGSGTDFTLKINRVEAEDLG VYYCLQGSHPSTFGGGKLEIKRAD AAPTVSIFPPSSKLG	Jones et al. (2013)
13F6	MELGLSWIFLVLTGKGVKCEVQVVE GGGLVKPGGSLKLSCAASGFAFSSYD MSWVRQTPEKRLWVAYISRGGGYT YYPDTVKGRFTISRDNKNTLYLQMS SLKSEDTAMYYCSRHIYYGSSHYAM DYWGQGSTVTVSSAKTTAPPVYPLAPGSL	MAWIXLIFFVLHCSGFSQVLVTQSSS ASFSLGASAKLTCTLSRQHSYTIIEWY QQQPLKPPRYVMELKKDGSHTGDGI PDRFSGSSSGADRYLSISNIQPEDEAIY CGVGDTIKEQFVYVFGGGTKVTVLGQ PKSTPTLTVPFSSSEELKENKATLVCLI SNFSPSGVTVAWKANGTPTQGVDT NPTKEGKFMASFLHLSQDQWRSHN SFTCQVTHEGDTVEKSLSPAEC	Jones et al. (2013)
10C8	GAELVRSGASVKLSCTSSGFNIKDYFL HWVKQRPEQGLEWIGWIDPENGDT YAPKFQDKATMTADTSSNTAYLHLSS LTSEDYGVYYCNADGNYGKNYWGQ GTTLTVSSAKTTAPAS	LSPVSLGDQASISCRSSQSLVHSNGN TFLHWYLQKPGQSPKLLIYRVSNRFSG VPDRFSGSGSGTDFTLKISRVEAEDLG VYFCSQSTHVPYTFGGGKLEIKRAD	Jones et al. (2013)
7C9	GAELVKPGASVKLSCTASGFNIKDTY MHVVKERPDKGLEWIGRIDPANGNT KCDSRFQKATITADTSSNTAYLQLSS LTSEDYAVYYCARRIFGKGFDFWGQ GTTLTVSSAKTTAPAS	SSLVVSAGEKVTMSCKSSQSLFNSGDQ KNYLAWYQQKPGQPPKLLIYGASTRE SGVPDRFTGSGSGTDFTLTISVQAED LAVYYCQNDQFYPTFGDGTCLDLKRAD	Jones et al. (2013)
5E6	GGGLVKPGGSLKLSCAASGSAFSRYD MSWVRQTPEKRLWVAYISRGGGFIY YPDTVKGRFTISRDNKNTLYLQMSS LKSDDTAMYYCARHVVYGGSSPLYAM DYWGQGSTVTVSSAKTTAPAS	SASFSLGASAKLTCTLSQHSFTTIEW YQQQPLKPPKYVMELKKDGSHTGD GIPDRFSGSSSGADRYLSISNIQPEDEAI YICVGVDITINEQFVYVFGGGTKVTVLG	Jones et al. (2013)
5D2	GPGLVRPSQLSLTCTVTGYSITSDYA WNWIRQFPGNKLEWLGYYTNTGSTGF NPSLKSRISTRDTSKNQFFLQLISVTE DTATYHCARGLAYWGQGTTLVTSAA KTTPAS	LTLSVTIGQPASISCKSSQSLDSDGKT YLNWLLQRPQSPKRLIYLVSKLDSG VTDRTGSGSGTDFTLKISRVEAEDLG VYYCWQGTHTSPFTFGSGTKLEIKRAD	Jones et al. (2013)
KZ52	EVQLLESGGGLVKPGGSLRLSCAASG FTLINYRXNWVRQAPGKLEWVSSIS SSSYIHYADSVKGRFTISRDNKNTLYLQMS SLYLQXNSLRAEDTAVYYCVRE GPRATGYXADVDFDIWGQGT XVTVSSASTKGPSVFPLAP SSKSTSGGTAAALGLV KDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYS LSSVTVPSSSLGTQ TYICNVNHKPSN TKVDKKVEPK	ELVXTQSPDSLAVSLGERATINCKSSQSV LYSSNNKSYLAWYQQK GQPPKLLIYWASTRESGVPDRFSGSGSGT DFTLTISLQAEDVAVY YCYQYYSAPLTFGGGKVEIKRTVAA PSVFIFFPSDEQLKSGTASVVCLLNPF YPREKAVQWKVDNALQSGNSQESVTE QDSKDSYSLSSLTLSKADYEKHKVYACE VTHQGLRSPVTKSFNR	Lee et al. (2008)

Note: The VH and VL sequence of mAb KZ52, protective in guinea pigs but not in human-primates are also included.



immune system to mount a sufficiently efficient immune response.

High anti-GP titers appear to be correlated with EBOV infection survival. However, 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, 4 and 5).

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<sup>+</sup> animals were capable of mounting a certain level of protection. Results suggested that protection in mice was mainly mediated by B cells and CD4<sup>+</sup> T cells. A high correlation between GP-specific total immunoglobulin G levels and survival was found in both vaccinated guinea pigs and NHPs. 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.

Figures 3 and 4 illustrate 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 (Figures 3 and 4B–C) in different GP forms (Figure 4D–G). Since some epitopes are not accessible to mAb binding in some GP forms (i.e. MLD in transmembrane GP protects the epitopes located nearby the RBD), 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 & Shornberg, 2012). 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 4A). 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  $\approx 1 \mu\text{g/mL}$ . JP3K11 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: JP3K11 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). However, 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<sup>+</sup> referred in Wilson et al., 2000) recognize linear nonglycosylated 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 electron microscopy, attempted to precisely identify the binding sites of each conformational antibody contained in MB-003, ZMAb and ZMapp<sup>TM</sup> cocktails. Their results confirmed that mAb 13C6, constituent of MB-003 and ZMapp<sup>TM</sup>, 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 MAbs 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 threefold 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 6). 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. However, CHO-derived MB-003 formulations still protected NHPs 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 (Sabalza et al., 2014). 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, 2015). However, mAb doses required for each patient might be high. Based on the doses that have proven to be effective in preclinical studies in NHPs (i.e.  $50 \text{ mg kg}^{-1} \text{ mAb}^{-1}$ ; 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 5000 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 6–8 weeks old are co-transfected with constructs containing the genetic information for the synchronous production of both heavy and light chains of each antibody (Castilho et al., 2011; Giritch et al., 2006). 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; Sabalza et al., 2014). 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  $2 \text{ g mAb L}^{-1}$  (after purification) can be produced after 2 weeks of fed-batch culture in a standard  $10\text{--}13 \text{ m}^3$  stirred-tank bioreactor using CHO cells, the warhorse for the production of glycosylated biopharmaceuticals (Garza-García et al., 2014; Li et al., 2010). Producing 100 kg of mAbs would require processing 50 000 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  $10 \text{ m}^3$ -CHO cell culture is approximately US\$10 M. 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  $10\text{--}13 \text{ m}^3$  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 10 March 2015, 252 kg of mAbs would be required. That is approximately 3.0% of the yearly worldwide installed capacity for mAb production (Ecker & Ransohoff, 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 as 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) offer 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 6).

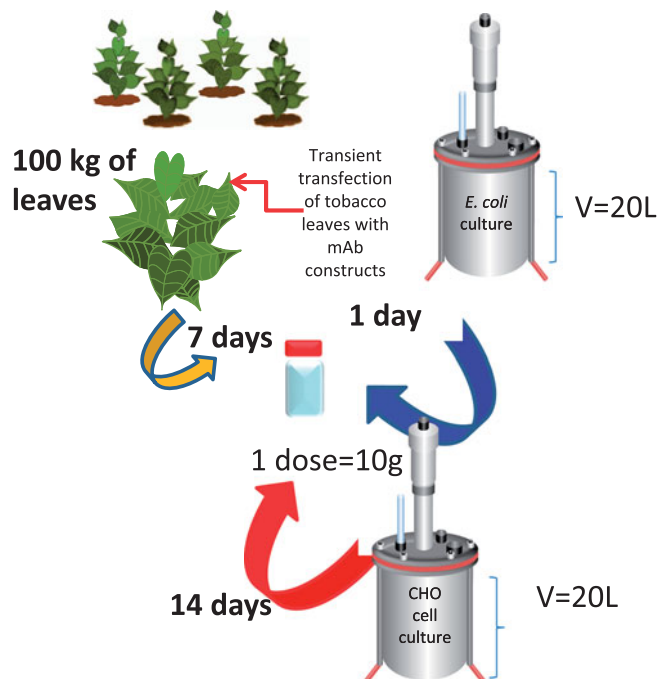


Figure 6. Comparison of different platforms to produce anti-EBOV immune therapeutics. While transient expression in tobacco leaves is not an easily scalable solution, and CHO cell culture exhibits limitations in capacity and cost, other alternatives such as the production of mAb fragments in bacterial cultures could be a cost-effective alternative to face EBOV epidemics.

### The road ahead

MAb-based anti-EBOV therapies must be further investigated to assure their safety and effectiveness at large-scale clinical interventions. The first formal clinical trial for ZMapp™, the first ever conducted for a mAb anti-Ebola therapy, was announced on February 2015 to occur in Liberia and USA (NIH, 2015), and results have not made available yet.

Even preclinical data in NHPs 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 NHPs 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 be 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.

### Declaration of interest

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