Monoclonal antibodies for the treatment of Ebola virus disease


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1. Introduction

1.1. Ebola virus disease: outbreak history

Ebola virus disease (EVD) was first recorded in 1976 when two outbreaks occurred, namely in (now South) Sudan, and in the Democratic Republic of Congo, formerly Zaire [1]. Since then, around 35 outbreaks have been reported mainly from Central Africa, with case fatality rates between 25% and 90% [2]. The latest one in West Africa was caused by Zaire ebolavirus and started in the Southeast of Guinea in December 2013. It rapidly spread to Liberia and Sierra Leone to become the largest recorded outbreak in history [3,4] and only tailed off slowly [5]. With more than 28,600 cases and more than 11,300 deaths (mortality rate 39.5%), it constituted a humanitarian disaster and public health emergency of international concern.

1.2. The Ebola viruses

EVD is caused by viruses of the Filoviridae family, which encompasses five antigenically distinct viruses; each named after the location of the outbreak in which they were first identified. These include Zaire ebolavirus, Sudan ebolavirus, Reston ebolavirus, Tai Forest ebolavirus (formerly known as Côte d’Ivoire ebolavirus), and Bundibugyo ebolavirus [8]. Zaire ebolavirus and Sudan ebolavirus most frequently cause disease in humans. Reston virus has not been linked to disease in humans.

The Ebola virus structure has been described in detail by Bharat et al. [9] and Beniac et al. [10] Messaoudi et al. [11] depict the Ebola virus ‘life cycle’ in detail. Ebola viruses (EBOV) produce nonstructural small soluble glycoproteins (ssGPs) and soluble glycoproteins (sGPs) [12]; the latter are secreted in large quantities from infected cells. The only EBOV transmembrane surface protein is another glycoprotein (GP), which forms trimeric spikes consisting of glycoprotein 1 (GP1) and glycoprotein 2 (GP2). This GP structure provides the target for vaccines and monoclonal antibodies (mAbs). Murin et al. [13] described GP structure and antibody binding sites in detail.

1.3. Clinical manifestations

After an incubation period of 2–21 days, EBOV can cause severe disease in humans, characterized by the sudden appearance of flu-like signs and symptoms including fever, chills, malaise, and myalgia; followed by gastrointestinal manifestations such as...
2. Overview of the market: prevention and treatment of Ebola virus disease

To date, there are no approved vaccines or treatments available for human use, and the current protocol for patients with suspected or confirmed EVD is quarantine, symptomatic management, and supportive care, including rehydration, restoration of electrolyte deficiencies, early treatment of secondary bacterial infections, empiric malaria treatment, and vital organ function support (blood transfusion, mechanical ventilation, and hemodialysis) in case of disease progression; mostly limited to settings which allow for maximal care [16]. However, during the latest outbreak in West Africa, significant progress has been made beyond these limited prevention and treatment options, despite the ethical, time-related, organizational, and technical obstacles that typically hamper the design and implementation of clinical trials in outbreak situations. To that end, the fast approval of several clinical vaccine trials was a quantum leap forward. Progress with the development of effective treatments has developed more slowly. However, several interventions prioritized by WHO [17] have reached phase II clinical trials, including favipiravir, TKM-130803, convalescent plasma, and a monoclonal antibody cocktail (ZMapp™, Mapp Biopharmaceutical, San Diego, CA, USA).

The focus of this review was on monoclonal antibodies and their combination considered as promising for the treatment of EVD in humans. In order to put their development into perspective, other interventions will be briefly discussed first.

2.1. Vaccines

The two most promising candidates are chimpanzee adenovirus 3 vector vaccine expressing Zaire ebolavirus GP (ChAd3-EBO-Z) and a recombinant vesicular stomatitis virus (rVSV-ZEBOV) vaccine. Both vaccines showed so far acceptable safety and tolerability profiles, and high titers of surface and neutralizing antibodies were induced after vaccination [18–21]. An interim analysis of the rVSV-ZEBOV phase II/III trial in Guinea demonstrated a promising 100% efficacy [22]. Phase II/III clinical trials for both vaccines are ongoing [23].

2.2. Favipiravir

Favipiravir, originally developed for the treatment of severe influenza, is a virus RNA polymerase inhibitor, which has shown to be effective in the treatment of EVD in mice. From a nonrandomized trial in Guinea, no conclusions could be drawn regarding efficacy [24].

2.3. TKM-100802/TKM-130803

TKM-100802 is a small RNA-interfering (siRNA) molecule directed against the gene products encoding for two viral proteins: L polymerase, involved in transcription and replication of EBOV, and Viral Protein-35, involved in suppression of the host immune response [25]. Survival rate was 100% in a limited number of NHPs treated [26]. TKM-100802 is currently on partial clinical hold by the Food and Drug Administration (FDA) due to concerns about the occurrence of a cytokine release syndrome, a proinflammatory reaction mediated by activated immune cells, following administration of the drug [25]. TKM-130803 is a new formulation of TKM-100802 in which the siRNA has been adapted by three nucleotide substitutions to enhance specificity to the EBOV that caused the latest outbreak. A nonrandomized, single-arm, phase II trial conducted in 2015 in Sierra Leone failed to show improved survival compared to historical survival rates [25].

diarrhea, nausea, and vomiting [14]. Disease progression is rapid, and clinical signs such as ecchymosis, petechial bleedings, and maculopapular rash can be observed. Laboratory findings include leukopenia, thrombocytopenia, and elevated serum amiotransferase concentrations. In addition, signs and symptoms of respiratory and central nervous system infection such as chest pain, dry cough, behavioral disorders, and seizures can be present. The terminal disease phase leads to either death or a frequently prolonged convalescence period. Death by shock and multiorgan failure typically occurs within 6–10 days after onset of disease [14].

The high fatality rate of EVD, in combination with the absence of treatment and vaccination options, constitutes an important public health threat, illustrated by the classification of Ebola virus as category A pathogens of Bioterrorism by the Centers for Disease Control and prevention (CDC) [15].
2.4. Convalescent plasma (polyclonal antibody therapy)

The rationale behind treatment with convalescent plasma is that EVD survivors have developed a protective immune response against the virus through generation of neutralizing antibodies and/or polyclonal Ebola-specific IgG that may or may not neutralize the virus but still may exhibit other protective mechanisms. Administration of these antibodies by transfusion could support virus clearance. Furthermore, the administration of these antibodies likely prevents virus infection/replication/dissemination within the patient, rather than virus clearance.

An important prerequisite to prevent infection with blood-borne diseases is that safety of virus-inactivated convalescent plasma transfusions is guaranteed, which can be challenging in EVD endemic areas [27]. Clinical trials were initiated in Guinea, Sierra Leone, and Liberia; without severe adverse reactions reported to date. While no efficacy data are available yet, experience from nonrandomized studies suggests safety, acceptability, and feasibility of convalescent plasma as EVD treatment [28]. Of note, in their concise overview on antibody therapeutics for EVD authored by the major leaders in the field of monoclonal antibody cocktail development [29], Zeitlin et al. present remarkable original data of an EVD patient treated sequentially with polyclonal antibodies in the form of convalescent plasma and then subsequently with monoclonal antibodies (ZMAb: see below); with the administration of the latter resulting in a dramatic increase in anti-Ebola IgG levels and a pronounced reduction of Ebola viral load, suggesting a synergistic effect.

2.5. Monoclonal antibodies

As comprehensively reviewed recently by Gonzalez-Gonzalez et al. [30]; up to date, around 20 monoclonal antibodies have been identified and characterized, of which some were found promising to progress to testing in nonhuman primate models. This included KZ52, isolated from a human survivor and successful in protecting mice and guinea pigs from lethal infection, but failing to protect NHPs when administered as single antibody [13].

The monoclonal antibodies, which emerged from testing in NHPs as promising for treatment of humans are subject to this review and are discussed in detail below. They include the single antibody MAb114; the MAb cocktails MIL-77E (two MAbs combined) and MB-003, ZMAb and ZMapp™ (three antibodies combined, respectively; Figure 1).


3.1. Introduction of the compounds

MAb114 is a human monoclonal antibody, recently isolated from a survivor of the 1995 EVD outbreak in the Democratic Republic of Congo [31]. MB-003 and ZMAb are monoclonal antibody cocktails isolated from immunized mice. MB-003 contains three mouse–human mAbs: 13C6, 6D8, and 13F6, which are IgG antibodies consisting of mouse variable regions chimerized with human constant regions and produced in tobacco plants (Nicotiana benthamiana). The mAbs were obtained from mice injected with Venezuelan equine encephalitis virus replicons encoding Ebola virus GP [32]. ZMAb contains three mAbs: 2G4, 4G7, and 1H3, which are murine immunoglobulins G (IgG) obtained from mice immunized with vesicular stomatitis virus (VSV), with the VSV GP gene replaced with one encoding Ebola virus GP [33,34]. ZMapp™ is a cocktail of three chimeric mouse–human mAbs produced in tobacco plants. Capitalizing on the two predecessor cocktails (Figure 1), it contains the mAbs 13C6, 2G4, 4G7 [35]. MIL77E is a chimeric antibody cocktail containing two mAbs (13C6 and 2G4 from ZMapp™) and is produced in engineered Chinese hamster ovary cells [36].

3.2. Chemistry

All mAbs are IgG antibodies (Figure 1): large molecules, composed of four polypeptide chains, comprising two identical light chains and two identical heavy chains, forming a flexible Y-shaped structure. The two heavy chains are linked with each other by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond. Each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these sequences corresponds to a discrete, compactly folded protein domain region. The light chain of the IgG molecule is made up of two domains, whereas the heavy chain contains four [37].
Murin et al. [13] describe the specific structure of EVD-protective antibodies in detail and discuss them in relation to the specificities of the GP target; González-González et al. summarize the up-to-date knowledge on the structure and mode of action of mAbs as well as the structure of the targeted Ebola virus GP [30].

3.3. Pharmacodynamics

Monoclonal antibodies bind GP, the only surface protein of Ebola virus, which plays a key role in virus attachment and fusion to host membranes. The individual mAbs each binds to one of three distinct regions; the GP base (where GP1 and GP2 interface), a glycan cap, or a mucin-like domain (Figure 2). MAb binding to the GP base (4G7 and 2G4) are neutralizing in vitro, whereas antibodies binding the glycan caps (mAb114, 1H3, and 13C6) or the mucin-like domains (13F6 and 6D8) appear non-neutralizing in vitro [13], with the exception of mAb114 [31]. 13C6 and 6D8 do neutralize in presence of complement [32]. During Ebola virus entry, mucin-like domains and glycan caps as well as their binding mAbs are removed before receptor engagement and therefore appear non-neutralizing; whereas the GP base with its binding mAbs remains intact before entry. MB-003 does not contain mAbs binding the GP base, but only mAbs binding domains that are cleaved off GP before viral infusion. ZMAb contains one mAb against the glycan cap and two against the GP base. Two mAbs (1H3 and 13C6) also bind sGP, the soluble version of GP, which is secreted from infected cells during EVD. It has been suggested that sGP acts as a decoy for antibodies, and therefore, mAbs which bind sGP may be less effective [13].

3.4. Pharmacokinetics and metabolism

To date, the half-life of monoclonal antibodies remains unknown, as pharmacokinetics and metabolism have not been studied in detail.

3.5. Clinical efficacy

To date, clinical trials on mAb114, MB-003, ZMAb, or MIL-77E have not been conducted. A phase I and phase I/II clinical trial on ZMapp™ are registered (Box 1). Available data are based on in vivo studies and studies in NHPs, except for ZMab and ZMapp™: four case reports are available describing compassionate use in humans with EVD. Table 1 provides a synopsis of existing in vivo studies in NHPs and humans. Table 2 summarizes all published efficacy study results for the different mAbs in NHPs. Table 3 provides an overview on biochemical studies and reviews to date.

3.5.1. mAb114

mAb114 has in vitro activity against recent and previous outbreak variants of Ebola and monotherapy with mAb114 fully protected three rhesus macaques when given as late as 5 days after a challenge with a lethal dose of EBOV [31]. This study suggests that a single antibody has therapeutic potential. However, although all three rhesus macaques survived EVD with only mAb114, the authors also saw that animals given mAb114 alone at 1–3 dpi had high viremia and showed clinical signs, whereas a combination of mAb114 and mAb100 prevented viremia and clinical signs, and thus, it is currently still preferable to administer a mAb cocktail, and

<table>
<thead>
<tr>
<th>Box 1. Drug summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug/molecule name</td>
</tr>
<tr>
<td>Phase</td>
</tr>
<tr>
<td>Indication</td>
</tr>
<tr>
<td>Pharmacology description/mechanism of action</td>
</tr>
<tr>
<td>Route of administration</td>
</tr>
<tr>
<td>Chemical structure</td>
</tr>
<tr>
<td>Pivotal trial</td>
</tr>
</tbody>
</table>

**Figure 2.** Viral entry and the effect of neutralizing antibodies.
(a) Ebola expresses trimers of Glycoprotein 1 (GP1) and Glycoprotein 2 (GP2) on its surface. GP1 is covered by a glycan cap and mucin like domain (MLD), both highly glycosylated regions, which cover conserved sites of receptor binding sites. After binding to a target cell receptor, Ebola virus particles the cell through macroinocytosis (II) or clathrin mediated endocytosis. They are then taken up in early endosomes (IV) and fuse with the endosome membrane to release viral RNA to allow for transcription, translation of viral proteins and viral replication (not shown in this figure, the lifecycle of Ebola is reviewed in more detail in Murin et al. [31]). An infected cell releases both Ebola virus particles and glycoprotein dimers and monomers, which are thought to act as decoy targets for neutralizing antibodies, thereby diminishing the number of antibodies available for viral entry interference.

(b) Neutralizing antibodies prevent viral entry into the target cell. Different antibodies bind to different sites of the glycoprotein. 2G4 and 4G7, which are part of ZMab and ZMapp, bind to the GP base (II), 13C6, which is part of MB-003 and ZMapp, and 1H3, which is part of ZMAb, bind to the glycan cap (II), and 6D8 and 13F6, which are part of MB-003, bind to the mucin like domain (III). Glycoprotein structure and antibody binding are described in more detail in Murin et al. [13].
<table>
<thead>
<tr>
<th>Antibody treatment</th>
<th>Title</th>
<th>Author</th>
<th>Population</th>
<th>Objective</th>
<th>Conclusion</th>
<th>Year/ reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAb114</td>
<td>Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody</td>
<td>Corti et al.</td>
<td>Rhesus macaques</td>
<td>Isolate mAbs from human EVD survivors and identify those who show protection as a single or dual-combination agent</td>
<td>Monotherapy with mAb114 shows 100% survival in NHPs when administrated as late as 5 days after challenge with EBOV</td>
<td>2016 [31]</td>
</tr>
<tr>
<td>MB-003</td>
<td>Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques</td>
<td>Olinger et al.</td>
<td>Rhesus macaques</td>
<td>Efficacy of RAMP MB-003 vs. CHO MB-003, given 1, 24, and 48 h p.i.</td>
<td>75% and 83% survival; MB-003 administration 1 h p.i. derived from CHO and RAMP, respectively 67% survival 24 and 48 h p.i. with RAMP MB-003</td>
<td>2012 [38]</td>
</tr>
<tr>
<td></td>
<td>Therapeutic interventions of Ebola virus infection on rhesus macaques with the MB-003 monoclonal antibody cocktail</td>
<td>Pettitt et al.</td>
<td>Rhesus macaques</td>
<td>Efficacy of MB-003 in NHP administered after the onset symptoms</td>
<td>43% survival with MB-003 administration after fever + viremia</td>
<td>2013 [39]</td>
</tr>
<tr>
<td>ZMAb</td>
<td>Successful treatment of Ebola virus-infected cynomolgus macaques with monoclonal antibodies mAbs and Ad- vectored IFN-α therapy rescue Ebola-infected NHP when administered after the detection of viremia and symptoms</td>
<td>Qiu et al.</td>
<td>Cynomolgus macaques</td>
<td>Evaluate survival and immune response in EBOV-infected NHP treated with ZMAb 100% (4/4) and 50% (2/4) survival with ZMAb 25 mg/kg administration i.v. at D3,6,9 beginning at 24 h and 48 h p.i., respectively</td>
<td>ZMAb + Ad-IFN simultaneously 75–100% survival. ZMAb following Ad-IFN 50% survival</td>
<td>2012 [40]</td>
</tr>
<tr>
<td></td>
<td>Sustained protection against Ebola virus infection following treatment of infected NHP with ZMAb</td>
<td>Qiu et al.</td>
<td>Cynomolgus macaques</td>
<td>Evaluate whether NHP EVD survivors treated with ZMAb are still protected at subsequent exposure 100% and 67% survival when rechallenged 10 and 13 weeks after initial exposure, respectively</td>
<td></td>
<td>2013 [42]</td>
</tr>
<tr>
<td>ZMapp™</td>
<td>Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp</td>
<td>Qiu et al.</td>
<td>Rhesus macaques</td>
<td>Identify the optimized antibody combination from MB-003 and ZMAb components and determine the therapeutic window The optimized combination ZMapp™ (13C6, 2G4, 4G7) showed 100% survival in NHP initiated up to 5 days p.i.</td>
<td></td>
<td>2014 [35]</td>
</tr>
<tr>
<td>MIL-77E</td>
<td>Two-mAb cocktail protects macaques against the EBOV Makona variant</td>
<td>Qiu et al.</td>
<td>Rhesus macaques</td>
<td>Confirm whether CHO-produced MIL-77E shows the same efficacy as plant-produced ZMapp™ and evaluate the impact of removing mAb 4G7 MIL-77E shows a comparable efficacy with ZMapp™ even when used as a 2 mAb cocktail. However, treatment was initiated at day 3 compared to day 5 for Zmapp™</td>
<td></td>
<td>2016 [36]</td>
</tr>
<tr>
<td><strong>Case reports/series in humans</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>ZMAb</td>
<td>Ebola virus disease complicated with interstitial pneumonia</td>
<td>Petrosillo et al.</td>
<td>1 human</td>
<td>Describe the course of EVD under optimized conditions ZMAb administering was associated with a sharp decay of plasma viral loads (twice) Rapid recovery, virus half-life decrease, but in the context of aggressive supportive care measures Improvement of both patients was observed shortly after receiving ZMapp™; however, this occurred in the context of receiving other care as well</td>
<td></td>
<td>2015 [43]</td>
</tr>
<tr>
<td></td>
<td>Clinical features and viral kinetics in a rapidly cured patient with EVD</td>
<td>Schibler et al.</td>
<td>1 human</td>
<td>Describe clinical, biological, and virological FU of EVD</td>
<td></td>
<td>2015 [44]</td>
</tr>
<tr>
<td>ZMapp™</td>
<td>Clinical care of two patients with Ebola virus disease in the United States</td>
<td>Lyon et al.</td>
<td>2 humans</td>
<td>Report the clinical course of EVD in two health-care workers optimally treated in Emory University Hospital</td>
<td></td>
<td>2014 [45]</td>
</tr>
</tbody>
</table>

RAMP: rapid antibody manufacturing platform; CHO: Chinese hamster ovary; p.i.: postinfection; NHP: nonhuman primate; EBOV: Ebola virus; mAbs: monoclonal antibodies; Ad-IFN: adenovirus-vectored interferon-α; EVD: Ebola virus disease; FU: follow-up; mAb: monoclonal antibody.
Table 2. MB-003, ZMAb, ZMapp™, MIL-77E, and mAb114 efficacy in NHPs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Challenge virus</th>
<th>Challenge dose</th>
<th>NHP model (all macaques)</th>
<th>Time of first administration</th>
<th>Dose</th>
<th>Number of doses (days of treatment)</th>
<th>Survival</th>
<th>Clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAb114</strong></td>
<td>EBOV</td>
<td><strong>Lethal dose</strong></td>
<td>Rhesus</td>
<td>24 h</td>
<td>50 mg/kg</td>
<td>2 (1, 2, 3)</td>
<td>3/3 (100%)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>120 h</td>
<td>50 mg/kg</td>
<td>3 (5, 6, 7)</td>
<td>3/3 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>MB-003</strong></td>
<td>EBOV</td>
<td>690 PFU</td>
<td>Rhesus</td>
<td>24 h</td>
<td>50 mg/kg</td>
<td>4 (1, 5, 8, 10)</td>
<td>2/3 (67%)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>50 mg/kg</td>
<td>4 (2, 6, 8, 10)</td>
<td>2/3 (67%)</td>
<td></td>
</tr>
<tr>
<td><strong>ZMAb (±Ad-IFN)</strong></td>
<td>EBOV</td>
<td>1067 PFU</td>
<td>Rhesus</td>
<td>103–120 h</td>
<td>50 mg/kg</td>
<td>3 (every 72 h)</td>
<td>3/7 (43%)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48 h</td>
<td>25 mg/kg</td>
<td>3 (2, 5, 8)</td>
<td>2/4 (50%)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1000 PFU</td>
<td>Cynomolgus</td>
<td>72 h</td>
<td>50 mg/kg + 1x10⁹ PFU Ad-IFN</td>
<td>3 (3, 6, 9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2512 PFU</td>
<td>Rhesus</td>
<td>72 h</td>
<td>50 mg/kg + 1x10⁹ PFU Ad-IFN</td>
<td>3 (3, 6, 9)</td>
</tr>
<tr>
<td><strong>ZMapp™</strong></td>
<td>EBOV</td>
<td>1000 PFU</td>
<td>Cynomolgus</td>
<td>24 h (Ad5-IFN) and 96 h (ZMAb)</td>
<td>50 mg/kg</td>
<td>3 (every 72 h)</td>
<td>2/4 (50%)</td>
<td>Yes¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72 h</td>
<td>50 mg/kg</td>
<td>3 (3, 6, 9)</td>
<td>6/6 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>628 PFU</td>
<td>Rhesus</td>
<td>72 h</td>
<td>50 mg/kg</td>
<td>3 (3, 6, 9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96 h</td>
<td>50 mg/kg</td>
<td>3 (4, 7, 10)</td>
<td>6/6 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>120 h</td>
<td>50 mg/kg</td>
<td>3 (5, 8, 11)</td>
<td>6/6 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>MIL-77E</strong></td>
<td>EBOV</td>
<td>1000 PFU</td>
<td>Rhesus</td>
<td>72 h</td>
<td>50 mg/kg</td>
<td>3 (3, 6, 9)</td>
<td>3/3 (100%)</td>
<td>No</td>
</tr>
</tbody>
</table>


* Data as provided by Wong et al. [54]

** Not defined in article.

¹ NCT02363322
follow-up studies, to the best of our knowledge, have not been conducted to date.

### 3.5.2. MB-003

Olinger et al. challenged eight rhesus macaques with a lethal dose of EBOV (690 plaque-forming units [PFU]). Initiation of treatment with MB-003 (50 mg/kg) started 24 and 48 h after virus challenge, with three animals in each treatment group. Two controls were treated with phosphate-buffered saline (PBS) or a control mAb. Animals received three additional doses (at day 5, 8, and 10 for the 24 h group and day 6, 8, and 10 for the 48 h group). In both groups, two animals survived with low viremia and showed no signs of illness, whereas both controls died on day 7. Hence, survival in this small group of subjects was 67% in the 24 h and the 48 h treatment groups, respectively, versus zero in controls [38].

To study the use as a therapeutic option rather than a postexposure prophylaxis, the same study group investigated whether MB-003 was effective after the onset of positive viremia (through reverse transcription polymerase chain reaction [RT-PCR]) and elevated temperature. They challenged nine rhesus macaques with an EBOV dose of 1067 PFU. Three of seven (43%) animals survived following treatment after symptom onset, in contrast to none of the two controls [39].

### 3.5.3. ZMAb

Qiu et al. studied the efficacy of ZMAb administrated 24 and 48 h after challenging cynomolgus macaques with a lethal dose of EBOV (1000 PFU). The four animals in each group were treated intravenously with three doses of ZMAb (25 mg/kg), each dose three days apart. One control received PBS. All four animals (100%) in the 24 h group survived; two of four animals (50%) in the 48 h group survived, whereas the one control did not (0%) [40]. The same study group tried to extend the treatment window of ZMAb by adding adenovirus-vectorized interferon-α (Ad-IFN) to the treatment. In the first experiment, four cynomolgus macaques received three doses of ZMAb (50 mg/kg) intravenously at a 3-day interval, beginning 3 days after a lethal dose of 1000 PFU of EBOV. The animals were supplemented with Ad-IFN (1 × 10⁹ PFU/kg) at receipt of the first dose of ZMAb. Three of the four animals survived the challenge with mild signs of disease; one had to be euthanized after a prolonged illness period. The second experiment focused on extending the treatment window. First, Ad-IFN was administered one day after EBOV challenge. Then, the ZMAb regime was started after onset of viremia and fever at day 4 in all four animals; two of them survived infection. In a third experiment, four rhesus macaques were treated with the same regime as the cynomolgus macaques in the first experiment (Ad-IFN and ZMAb initiated at day 3). In all four animals, viremia and fever higher than 40°C were detected before treatment. All four treated animals survived, whereas two controls (treated with PBS or control mouse IgG) succumbed to infection [41]. In another study, Qiu et al. performed an experiment with macaques surviving a previous EBOV challenge by receiving ZMAb or ZMAb and Ad-IFN. The animals were rechallenged 10 or 13 weeks after the initial challenge, respectively, to evaluate whether the immunity developed following the first infection was protective without further intervention. Survival rates were 100% and 67%, respectively [42]. Two cases of compassionate use of ZMAb in humans with EVD have been described. Petrosillo et al. describe the case of a physician with EVD who was evacuated to Italy for medical help. He was treated with convalescent plasma, favipiravir, and ZMAb. Having received two doses of ZMAb, a sharp and sustained decline of plasma viral load was seen, with an increase of IgM and IgG after the start of viremia decline [43]. Schibler et al. reported a case of EVD in a medical doctor who was airlifted to Switzerland for medical care. The patient...
received favipiravir and two doses of ZMAb (50 mg/kg). A temporal relation was seen between the ZMAb infusion combined with favipiravir and an accelerated decay of viral load [44]. Both patients compassionately treated with ZMAb survived. Since both patients received additional treatment, the significance of the effect of the mAb cocktail cannot be conclusively established.

To the best of our knowledge, four additional EVD patients were treated compassionately with ZMAb, of which all survived [33].

### 3.5.4. ZMapp™

ZMapp™ is the mAb cocktail, which evolved from MB-003 and ZMAb. It has shown a 100% efficacy in NHPs when administered up to 5 days after lethal challenge with EBOV. Multiple NHPs presented with severe stage of disease, indicated by the elevated liver enzymes, mucosal hemorrhages, and rash, at the time of administration [35]. A randomized, phase I/II safety and efficacy study is currently ongoing in Guinea, Sierra Leone, and the United States. No data on efficacy are available yet [17]. Several patients have been compassionately treated with ZMapp™. Lyon et al. describe two American health-care workers infected with EBOV who were transported to the USA and treated at Emory University in Atlanta. Both patients’ conditions improved shortly after a first dose of ZMapp™. Since this improvement occurred in the context of aggressive rehydration, electrolyte balancing, and others supportive care measures, the significance of the effect of the mAb cocktail cannot be conclusively established [45].

### 3.5.5. MIL77E

MIL77E, containing 13C6 and 2G4, showed full protection in NHPs when administered 3 days after challenge with a lethal dose of EBOV [36]. No further studies have yet been conducted with this combination of monoclonal antibodies. Of note, 13C6:2G4 was given in a 1:2 ratio, respectively. For all other antibody cocktails mentioned previously (i.e. MB-003, ZMAb, ZMapp), the ratio was 1:1:1.

### 3.6. Safety and tolerability

In the four primary NHP studies, there was no mentioning of adverse effects observed [39–42]. In the case report by Schibler et al., urticaria were observed on day 17 (ZMAb was given on days 5 and 8) likely due to a food allergy, although a delayed reaction to infusion of ZMAb cannot be excluded [44]. In the case report by Petrosillo et al., no adverse reactions were seen [43]. In the case series by Lyon et al., two patients were treated with ZMapp™, without adverse reactions [45].

### 3.7. Development of cross-reactive antibodies

Most of the currently investigated antibody cocktails are Ebola virus specific, and cross-neutralizing activity against Ebola viruses has not been demonstrated [46]. Ebola virus is responsible for the majority of filovirus hemorrhagic fever outbreaks, including the 2014 outbreak in West Africa. However, other members of the Filoviridae have also caused human epidemics, including seven outbreaks of Sudan virus, two outbreaks of Bundibugyo virus [47], and 12 outbreaks of Marburg virus [48]. Therefore, broadly protective treatment options are needed. Multiple mAbs have been generated, that elicit cross-reactivity in vitro and efficacy in mouse models for different Ebola virus species [49,50] including Marburg virus [51–53]. Furuyama et al. generated mAb 6D6, which was found to efficiently neutralize the infectivity of vesicular stomatitis virus (VSV) pseudo-typed with GPs of all known Ebola viruses (EBOV, SUDV, TAFV, BDBV, and RESTV), but not Marburg virus (MARV). In a mouse model of EBOV infection, 6D6 was 100% effective when given a single dose 24 h after challenge. A second mouse model of SUDV infection showed severe weight loss in untreated mice. Treatment with 6D6 24 h after infection delayed the onset of the disease and significantly reduced the weight loss [51]. Keck et al. created a set of pan-ebolavirus and pan-filovirus mAbs derived from cynomolgus macaques immunized repeatedly with a mixture of engineered GPs and virus-like particles (VLPs) for three different filovirus species (EBOV, SUDV, and MARV). The most protective mAb, FVM04 effectively neutralized EBOV and SUDV in vitro and showed weak binding to MARV. In a mouse model of EBOV infection, FVM04 was fully protective when used in two doses (2 h and 3 days postchallenge). When given a single dose 3 days postchallenge, survival rate was 40% [53].

### 3.8. MAbs production techniques and capacity

Anti-Ebolavirus mAb production is highly complex, and the currently available production facilities would not suffice to facilitate a quick large-scale outbreak response. González-González et al. [30] thoroughly examined the currently available and potential future techniques and highlight that firstly, MAb production requires for successful treatment attempts may amount to as much as 10 g/patient; and that within a massive outbreak situation, MAb needs may rapidly run up to many kilograms. Pointing out that with the current technique to produce MAb from transfected tobacco plants; in order to produce, as an example, 50 kg of MAb to allow for the treatment of maybe 5000 individuals, the authors calculate that 150 tons of tobacco leaves would have to be transfected. This is a dimension exceeding current facilities by far; apart from the fact that the MAb recovery process from tobacco biomass poses its own formidable technical challenges [30].

An alternative to full-length MAb production to be explored in the future could be a stable expression in mature tobacco plants, which would, even if technically feasible, require a considerable financial ad hoc effort and time to establish (which is precisely what is lacking in outbreak situations) or expression in mammalian cells, which appears theoretically feasible but would by far exceed the current global production capacity. A future option to be still thoroughly researched could be engineered mAb fragments in place of full-length MAb L, which could make it probably more easily into mass production [30].
4. Conclusions

Two promising vaccines are underway; however, mass immunization in the near future is unlikely, considering the costs and the amount of people at risk of getting EVD. Therefore, development of postexposure prophylaxis options or treatment for EVD is important. Monoclonal antibodies are currently the most promising experimental postexposure and treatment options against EVD, as evidenced by consistently higher rates of survival in lethally challenged NHPs [30,54].

Monoclonal antibodies of particular interest are mAb114 and ZMapp™ which both showed to be 100% protective in NHPs when given as late as 5 days after exposure [31,35]. All EVD patients compassionately treated with ZMAb or ZMapp™ survived, and a clear decrease in viral load following infusion was seen. However, considering the multiple additionally applied and different supportive treatments, it remains unclear whether this effect is due to the antibodies alone [43–45]. The only antibody treatment currently undergoing clinical trial is ZMapp™.

5. Expert opinion

Prevention and prophylaxis strategies, including early case detection, rapid diagnostic testing, and – hopefully soon – production and mass administration of vaccines will be key to prevent or at least curb future Ebola outbreaks. However, it goes without saying that the development of specific treatment options must not be neglected. As a consequence of the unprecedented large West African outbreak, research in this area has remarkably intensified recently.

Evidence from small-scale animal studies and some limited evidence from individual cases support the view that both suitable single mAbs and mAb cocktails are effective for the treatment of human EBOV infections following exposure; thus justifying further preclinical development and testing in clinical trials.

The ultimate goals in this field are a comprehensive understanding of why, how, and to what extent monoclonal antibodies play an essential role in overcoming disease; the identification of the most potent full-length mAbs, and ideally engineering highly efficient fragments thereof; the recognition of (situation-specific) optimal compositions of mAb cocktails; on an operational level, the development of a framework enabling the accomplishment of clinical studies in the challenging conditions of an ongoing outbreak; and the facilitation of large-scale production to enable timely mass deployment once needed.

Recent research by a small number of research groups leading the field has identified a multitude of mAbs effective in animal studies including nonhuman primates to facilitate survival of Ebola virus challenge; moreover, anecdotal evidence of individually treated patients has triggered the further clinical development of at least one full-length mAb cocktail. Over the past couple of years, insight into the viral GP target of the various mAbs as well as into their composition and role within the virus-host interaction has massively grown, as well that techniques to accelerate and refine the further developments of mAbs for the treatment of EVD have been developed.

While impressive progress has been made, knowledge on viral entry into host cells and the various mechanisms of Ebola virus to evade an efficient host immune response, as well as knowledge gaps on how mAb therapy precisely works is still incomplete.

Over the coming years, next-generation full-length antibodies and designed fragments from the most suitable candidates will be identified and progress to testing in NHPs, as well as that the understanding of the immunological complexity of non-neutralizing and neutralizing protective antibodies will grow further.

Challenges to move on with the development of Ebola therapies will remain multitude, however, including the potentially very high doses needed to yield clinical efficacy; epitope mutations which might reduce efficacy and require a constant evolution of (possibly outbreak-specific) mAb mixtures; as well as that monoclonal antibodies work best early after exposure, before the development of clinical disease, which poses a conceptual challenge and a common problem with antiviral therapy.

Adequately designed phase I studies are needed to pave the way for eventual, sufficiently powered phase II and III trials. This is a major obstacle, in view of the fact that the disease manifests itself in outbreaks, with associated ethical and logistical difficulties in timely conducting randomized controlled clinical trials. As well, anti-Ebolavirus mAb production is highly complex, and the currently available production facilities are not sufficient to facilitate a quick large-scale emergency response.

To that end, the probably biggest overall challenge today may be to develop innovative and ethically acceptable ways of facilitating appropriately designed ‘ad hoc’ clinical trials to be initiated swiftly (and seen through) in outbreaks-to-come, and to stimulate investment into adequate production techniques and facilities for rapid upscaling beforehand.

While more research data are pending, and in the light of high EVD mortality, an interim advice based on the clinical experience to date is that treatment of patients with mAbs is sensible, provided those could be made available timely in the quantities required. For the time being, however, there remains a clear discrepancy between the theoretical potential of MAb for large-scale EVD treatment and today’s experimental treatment options for very few individual study patients.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


• Very concise overview on mAb cocktail development against EVD by one of the groups leading the field.


• This paper elucidates the mechanism of action of monoclonal antibody cocktails.


• Demonstration that ZMapp emerging from MB-003 and ZMab cocktails resulted in a 100% survival rate in a limited number of EVD-infected macaques when treated within 5 days postchallenge.


• MIL-77E fully protects NHPs when administered 3 days postchallenge with a lethal EBOV dose.


• 60% of NHPs versus no controls survived EBOV infection with MB-003 administered up to 48 h postinfection.


• EBOV-challenged NHPs survived when given MB-003 after onset of disease.


• The authors demonstrate that EBOV-infected NHPs may develop strong specific immune responses and survive follow-up administration of ZMAB and interferon after disease onset.


Of NHPs which survived EBOV disease when challenged and administered ZMAb; 100% and 60%, respectively, survived when rechallenged at week 10 or 13, respectively.


