EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 November 2014
EMA/756544/2014 Corr. 1

Interim assessment report

Review under Article 5(3) of Regulation (EC) No 726/2004

Medicinal products under development for the treatment of Ebola

Procedure no: EMEA/H/A-5(3)/1410

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. Background information on the procedure

Ebola virus, a member of the *Filoviridae* family together with Marburg virus, is one of the most virulent and deadly pathogens currently known, causing an acute febrile illness with severe diarrhoea and vomiting and sometimes haemorrhagic manifestations, and leading to multi-organ failure and death in a substantial proportion of affected symptomatic individuals.

The current outbreak of Ebola virus disease (EVD) is the largest to date in terms of number of worldwide cases with 15,351 infected persons as at 21 November 2014, including 5,459 deaths[1], representing a case-fatality rate of 36%. Suspected cases of EVD have been reported in six countries (Guinea, Liberia, Mali, Sierra Leone, Spain, and the United States of America) and previously in two other countries (Nigeria and Senegal) as at 18 November 2014.

Data from the affected countries show that the current outbreak has so far occurred in 2-3 waves, with the latest wave, the most intense, triggering an exponential increase in the number of cases in recent weeks, underlining the urgent need for safe and effective treatments.

No medicinal products have yet been approved for the treatment of EVD in the European Union (EU). However, the European Medicines Agency (EMA) was made aware of several therapeutic candidates in early stages of development with putative action against EVD.

Given the dynamics of the outbreak, it was considered necessary to have an overview of all quality, non-clinical, and clinical data available from these experimental treatments in order to support decisions on potential emergency use for individual patients.

Consequently, in view of the above and in accordance with Article 5(3) of Regulation (EC) No. 726/2004, on 23 September 2014, the EMA requested the Committee for Medicinal Products for Human Use (CHMP) to review the currently available quality, preclinical and clinical data for experimental treatments against Ebola.

This review covers only medicinal products currently under development for the treatment of Ebola. It was agreed that products under development for which no direct antiviral activity against the Ebola virus exist should not be included in the ongoing review. Evidence of such direct activity could be established for instance by in vitro or *in vivo* studies.

Data were provided by seven different companies whose products fulfil the above-mentioned criteria. The therapeutic agents considered by the CHMP in this review were the following:

- BCX4430
- Brincidofovir
- Favipiravir
- TKM-100802
- AVI-7537
- ZMapp

[1] http://apps.who.int/iris/bitstream/10665/144117/1/roadmapsitrep_21Nov2014_eng.pdf?ua=1

- Anti-Ebola F(ab')$_2$

2. Scientific Discussion

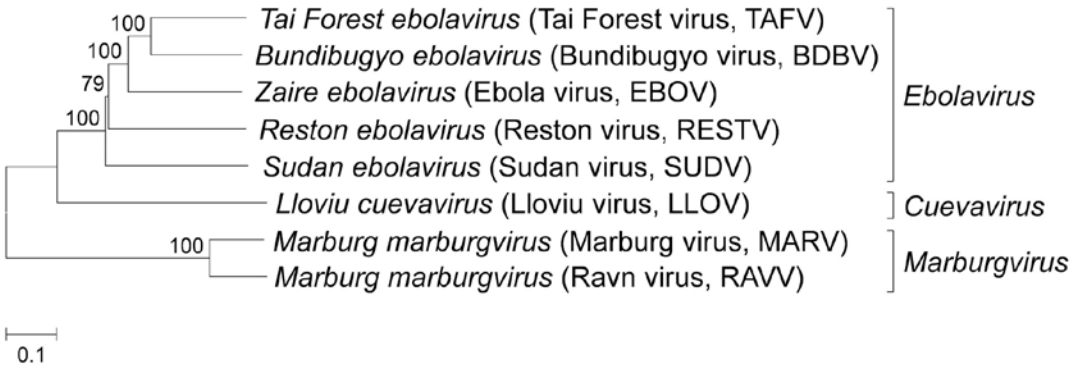
2.1. Introduction

This document provides a short introduction to the virus, the disease and the animal models relevant for studying potential therapies, followed by a summary of the available data for each of the above mentioned products. Unless otherwise stated, the introductory information on the virus and the disease has been obtained from recently published reviews (such as Ansari 2014, Nakayama 2013 and Feldman 2014).

2.1.1. The virus

Figure 1 shows a brief outline of the taxonomy of filoviruses. Each of the filoviruses shown in the figure is known to have caused human disease, except Reston virus (RESTV) which hitherto has only been shown to be pathogenic in non-human primates (NHPs).

Figure 1 Filovirus phylogenetics by nucleotide sequence (From Nakayama 2013)



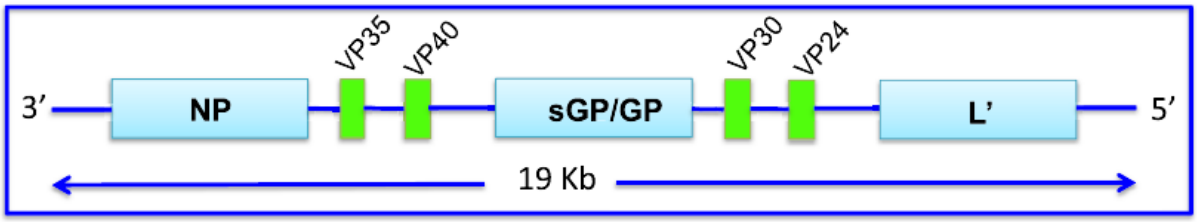
The Ebola virus was first recognised in 1976 in the Democratic Republic of Congo (Congo-Kinshasa), formerly Zaire, and was initially proposed as a new strain of Marburg virus. However, it was subsequently shown to be a new species, initially named *Zaire ebolavirus* and later called Ebola virus (EBOV). The initial strain from 1976, a reference strain ever since, was named EBOV Mayinga (EBOV-m). Another reference strain was isolated in the 1995 outbreak in Congo-Kinshasa and called EBOV-kikwit (EBOV-k). The present outbreak started in southern Guinea in December 2013, close to the borders of Sierra Leone and Liberia. Phylogenetic analysis indicates that the agent causing this outbreak, EBOV-Guinea (EBOV-g), belongs to a lineage of the EBOV-Zaire virus (Dudas, Rambaut 2014).

EBOV is a lipid enveloped, heavily glycosylated, non-segmented negative strand RNA virus (Ansari 2014). As part of viral replication the original RNA-strand is converted to a positive sense RNA (mRNA) prior to translation by the means of an RNA-dependent polymerase.

The EBOV genome contains seven genes encoding for a nucleoprotein (NP), glycoprotein (GP), the mentioned RNA polymerase (L) and four structural proteins (VP24, VP30, VP35 and VP40) (Figure 2). GP, which is also expressed in a soluble form (sGP) is responsible for host receptor binding and fusion with the cell membrane. sGP represent the majority of GP

transcripts. Other structural genes have inhibitory effects on both innate and adaptive immune responses.

Figure 2 Organisation of the EBOLA virus genome (From Ansari 2014)



NP, nucleoprotein; VP24, 30, 35 and 40 are structural proteins; sGP/GP, soluble & membrane forms of the glycoprotein, L', RNA dependent RNA polymerase

Based on human autopsy studies, EBOV is primarily seen in mononuclear phagocytic cells (dendritic cells, monocytes, and macrophages), endothelial cells and hepatic sinusoidal cells. The virus and its pathological effects have also been shown in adrenocortical cells, which may contribute to shock development typical for EBOV infection. Hence, the virus can gain entry to a wide range of cells; specific receptors or mechanisms for cell entry have not been defined.

In addition to the above, a prominent feature of filovirus pathophysiology in humans is an extensive bystander death of uninfected lymphocytes due to apoptosis and severe coagulopathy.

Viral loads in the infected patient reach high levels during the course of the disease. Viral titres have been reported to be associated with outcome (death or survival) at least in the rural setting; in an outbreak of Sudan ebola virus (SUDV) in Uganda in 2000 and 2001, viral loads typically seen in fatal cases where around 10^8 to 10^9 copies/ml; in surviving patients these tended to be around 2 $\log_{10}$ copies lower (Towner 2004).

Despite the high reported case fatality rate, there are data indicative of milder or non-symptomatic infections with subsequent longstanding humoral and cellular immunity. In a large serological survey performed in rural parts of Gabon (>4,000 persons) an EBOV-specific immunity was seen in around 15% of the population (Becquart et al. 2010).

2.1.2. Ebola virus disease

The clinical picture was recently summarised in a case series of 106 patients (86 with a complete follow-up) with confirmed EBOV infection admitted to Kenema Government Hospital in Sierra Leone within the present outbreak (Schieffelin et al. 2014). This hospital has a dedicated branch for the treatment of haemorrhagic fevers. Supportive treatment with intravenous fluids, broad spectrum antibiotics and antimalarial drugs were available.

The presumed incubation period was 6-12 days. Symptoms at the time of admission are shown in Figure 3 for both fatal and non-fatal cases. As compared with other febrile patients admitted without EBOV infection, EBOV patients showed significant elevations in blood urea nitrogen, creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase. As was the case with the SUDV outbreak described by Towner 2004, viral load has been predictive of outcome in the present outbreak (Figure 4).

Average time from patient reported onset of symptoms to death (from hypovolemic shock or multi-organ failure) was 10 days. The case fatality rate was 74%. Patients who survived were discharged after a mean duration of illness of 21 days.

Figure 3 Signs and Symptoms in Patients with Fatal and Nonfatal EVD (from Schieffelin et al 2014)

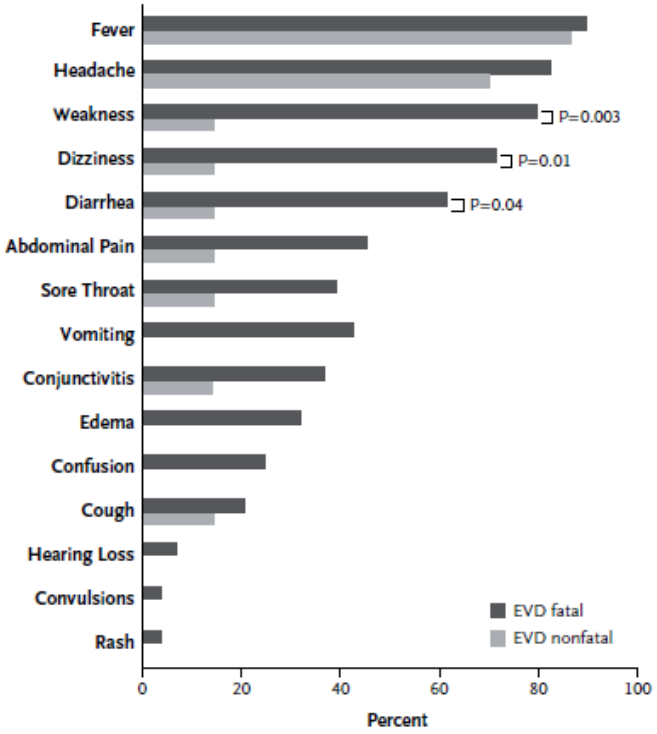
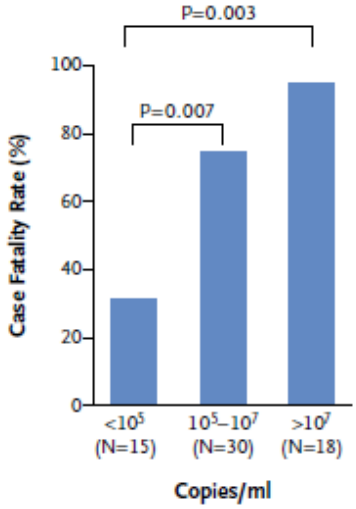


Figure 4 Case Fatality Rates among Patients with Ebola Virus Disease (EVD) (from Schieffelin et al 2014)



2.1.3. Animal models for the study of EBOV infection

Models for the study of EBOV infection have been developed in rodents (mice, guinea pigs and hamsters) and in non-human primates (NHPs).

In mice models, serial passage of wild-type EBOV is needed to yield lethal virulence, and effects differ by type of challenge (intra-peritoneal versus subcutaneous inoculation). Mouse-adapted EBOV has mutations in both coding and non-coding regions, leading to amino acid changes in VP35, VP24, NP and L viral proteins. Similar to humans and NHPs, viral loads are high during the course of the disease, and extensive pathological effects are seen in the

liver, spleen and other organs. However, fever is not seen and coagulopathy is also lacking in moribund mice.

Guinea pig models resemble mice models in some respects; EBOV needs adaption to yield lethal disease and the adapted virus shows substitutions leading to amino acid changes in NP, L and VP24. In contrast to what is seen in mice models, however, coagulopathy (falling platelets and fibrin deposits) does occur in Guinea pig models.

Of the rodent models studied, Syrian golden hamsters challenged with mouse-adapted EBOV have reactions most similar to those seen with wild-type EBOV infections in NHPs and humans (including target cells and coagulopathy).

So, although rodent models are useful in studying EBOV, the pathophysiology differs in the different models, and all the models require the use of an adapted virus.

In contrast, challenge with wild-type EBOV in NHPs (mainly cynomolgus and rhesus macaques) generally (though not universally) yields a lethal infection which closely resembles that seen in humans, with regards to pathophysiology and clinical features, including coagulopathy with disseminated intravascular coagulation (DIC), haemorrhagic shock and organ failure. As in humans, extensive bystander death of lymphocytes is seen in NHPs (not the case in rodents). Time to viraemia in NHPs depends on the infectious dose (~24 hrs with a high dose of 105 plaque forming units (pfu) given subcutaneously and ~3 days with a lower dose of 103 pfu given intramuscularly). Similarly, time to death depends on the infectious dose, with death generally occurring 6-7 days after an intramuscular challenge with 103 pfu, and 9-12 days with a low dose of 10 pfu, which is still generally lethal.

In the majority of NHP studies performed with agents discussed in section 2.2 of this report, the monkeys have been challenged with the virus at an intramuscular dose of 10^3 pfu or slightly lower. This is of interest when considering results obtained with the test agents given at different times in relation to the viral challenge. For example, test agent may be given 1-2 days post-viral challenge, when viraemia is still generally at a low (non-quantifiable) level, or delayed until 72 hours post challenge, which is the time point for quantifiable viraemia. In humans with EBOV infection it has been shown that viral loads may reach high levels (> 10^7 copies/ml) as early as one day after onset of symptoms with peak values (up to 10^{10} copies/ml) being seen 2-4 days later (Towner 2004).

2.2. Antiviral treatments against Ebola virus

This report provides an overview of all quality, non-clinical and clinical data made available for:

- Three nucleos(t)ide polymerase inhibitors: **BCX4430**, **brincidofovir**, and **favipiravir**;
- Two oligonucleotide based products: **TKM-100802** and **AVI-7537**;
- A cocktail of monoclonal antibodies: **ZMapp**;
- A platform describing the plans for the production of polyclonal immunoglobulins derived from immunised horses: **anti-Ebola F(ab')$_2$ fragments**.

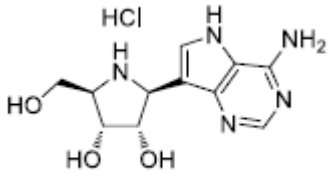
No discussion on efficacy in rodents is presented in cases where NHP studies have been performed.

2.2.1. BCX4430

2.2.1.1. Quality aspects

BCX4430 is a nucleoside analogue acting as an inhibitor of RNA polymerase. The active substance is chiral and isolated as the pure stereoisomer (Figure 5). The active substance is soluble in water.

Figure 5 Structure of BCX4430



BCX–4430

The active substance is metabolised into BCX6870 (5'-triphosphate metabolite). The triphosphate undergoes pyrophosphate cleavage, producing the monophosphate that is incorporated into nascent viral RNA strands. The monophosphate would be expected to cause premature termination of transcription and replication of viral RNA.

As the relevant information on the drug substance or drug product has not been made available to the EMA from the drug product manufacturer, an assessment of the quality of the product cannot be made.

2.2.1.2. Pharmacology (kinetics)

There are currently no clinical pharmacokinetic data available for BCX4430.

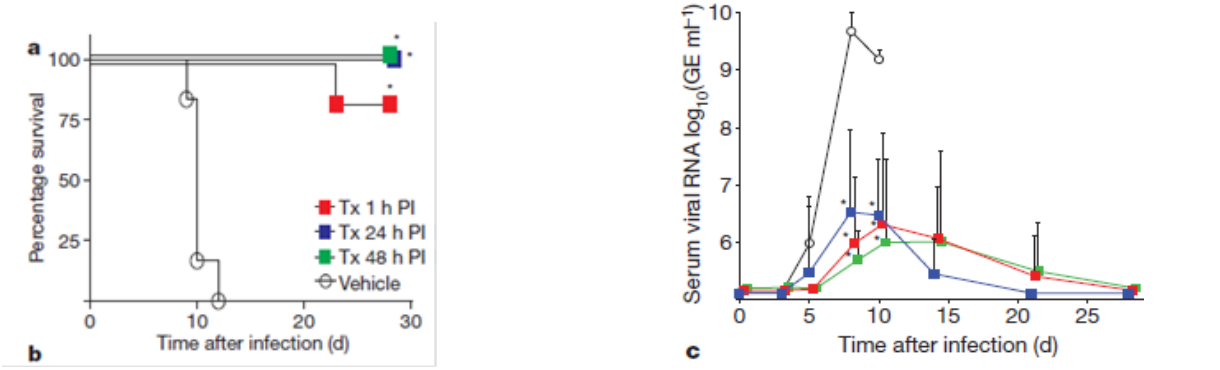
2.2.1.3. Efficacy aspects

The *in vitro* 50% effective concentration (EC50) value for BCX4430 against EBOV-k was shown to be 12 µM in a study with human macrophages pre-treated with BCX4430 for 18 hours before infection (Warren et al 2014).

Effects on survival have been shown in rodent models of EBOV infection. Studies are ongoing in NHPs challenged with EBOV.

The EC50 values for BCX4430 in EBOV and Marburg virus are similar (12 µM versus 5 µM). In a study of Marburg virus in NHPs (Warren et al. 2014), BCX4430 dosed at 15 mg/kg was administered intramuscularly twice daily beginning 1-48 h after infection and continuing for 14 days. 17 out of 18 NHPs given BCX4430 survived compared with none of those given the control vehicle (Figure 6, left). Effects on viral loads were also significant in the BCX4430-treated animals (Figure 6, right). Treatment was instituted prior to quantifiable viraemia.

Figure 6 Post-exposure protection of MARV-infected cynomolgus macaques by BCX4430.



Kaplan-Meier survival curves

Serum viral RNA load. GE, genomic equivalent

2.2.1.4. Safety aspects

There are currently no clinical safety data available with BCX4430.

Preclinical studies have been performed hitherto in NHP and rats. The potential effects of BCX4430 and its 5-triphosphate metabolite, BCX6870, on mitochondrial function were evaluated. BCX4430 at concentrations of up to 50 µM did not exhibit mitochondrial toxicity in cultured cells.

2.2.1.5. Other relevant information

The company has not presented any proposed dose for the treatment of EBOV infection. According to the company, current supply is limited and the medicinal product is mainly available for a planned phase 1 trial.

2.2.2. Brincidofovir

Brincidofovir is in late-stage development for the treatment and prophylaxis of cytomegalovirus (CMV) infection in haematopoietic stem cell transplant patients and there are data on a fairly large number of these patients in the present safety database. Phase 2 studies have also been performed to treat adenovirus infection in these patients.

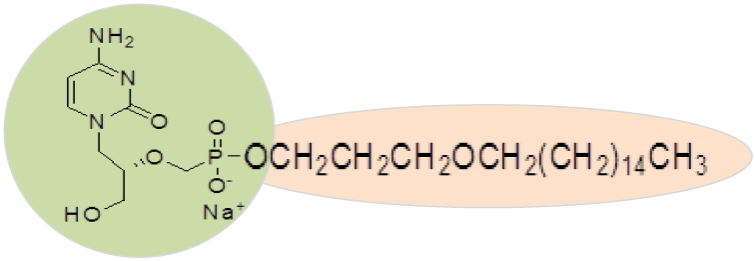
2.2.2.1. Quality aspects

Drug substance

Brincidofovir (CMX001; BCV) is an orally-available lipid conjugate of the nucleotide analogue cidofovir (CDV) that provides for intracellular delivery of the active antiviral cidofovir-diphosphate (CDV-PP). It should not be understood as an oral prodrug for CDV.

Brincidofovir is a white to off-white solid and has a solubility of 0.013 mg/ml (deionised water). It contains a stereogenic centre carbon and is produced as a pure S-enantiomer (Figure 7).

Figure 7 Structure of brincidofovir



The stability data provided support the proposed retest period of 12 months.

Drug product

Brincidofovir is supplied for oral administration as a 100 mg tablet and 10 g/ml oral suspension.

These formulations have been developed using common compendial excipients.

According to stability information on batches of the finished product (100 mg tablets), the shelf-lives of 36 months and 12 months for the tablets stored in bottles and blisters, respectively, are considered acceptable at this stage of development.

2.2.2.2. Pharmacology (kinetics)

Brincidofovir may be administered either as tablets (100 mg) or as a suspension (10 mg/ml). Due to drug-related gastro-intestinal symptoms of brincidofovir, it is recommended to administer brincidofovir with a meal. No data were provided on the bioavailability of brincidofovir in patients with symptomatic EBOV infection.

Brincidofovir readily crosses cell membranes, and the lipid phosphate ester linkage is cleaved in the cytoplasm and subsequently phosphorylated to produce CDV-PP by the consecutive actions of pyrimidine nucleoside monophosphate kinase and one of several intracellular kinases (with pyruvate kinase considered the most efficient). Major cytochrome P450 (CYP) isoenzymes do not seem to contribute to the elimination of brincidofovir.

Unlike CDV (which is associated with nephrotoxicity) brincidofovir is not a substrate of the renal uptake transporter OAT1. In addition, when brincidofovir is administered, the plasma exposure to CDV is relatively low: $AUC_{0\text{-}24h}$ of CDV contributed to ~5% of the brincidofovir derived radioactivity $AUC_{0\text{-}24h}$, in the human ADME study (of note, due to its longer t½, the contribution of CDV would probably be greater if comparing AUC_{inf}). In the subjects with renal impairment receiving brincidofovir, the exposure to CDV was increased, but the CDV C_{max} values remained <10% of that reported with CDV.

At the 1 mg/kg dose level, the plasma $t_{1/2}$ for brincidofovir was 27 hours and the $t_{1/2}$ for the CDV metabolite was 65 hours. Brincidofovir is extensively metabolised. The company states, based on limited data, that brincidofovir is not removed by dialysis.

2.2.2.3. Efficacy aspects

Data on the antiviral activity against EBOV are only available from *in vitro* studies. Although brincidofovir was originally developed for the treatment of various infections from DNA viruses, it was screened for *in vitro* activity against EBOV at the University of Texas and

confirmatory studies were conducted at the Centers for Disease Control and Prevention (CDC) in Atlanta. EBOV Mayinga (EBOV-m) was used in these experiments.

The activity differs by time of pre-incubation (i.e. time between the addition of the medicinal product until cell lines are infected) and to some extent incubation. EC50 values in the order of 0.01-0.1 µM were seen with 48/24 hours pre-incubation and higher values (0.5-5 µM) with a pre-incubation of 2 hours.

In general, this *in vitro* activity is similar to that seen for other viruses that are lethal in rodent models, such as poxviruses, where brincidofovir led to high survival rates. This is the basis for the potential anti-EBOV efficacy in humans. The company's dosing proposal is also based on such bridging data (Table 1).

Table 1 In vitro activity of brincidofovir (CMX001) and CDV on various virus

Viral family	Virus	EC50	
		CMX001	CDV
Adenovirus	AdV type 7	0.02	1.3
Herpesviruses	CMV	0.001	0.4
	EBV	0.03	65.5
	HHV type 6A	0.003	2.7
	HHV type 8	0.02	2.6
	HSV type 1	0.01	3.0
	HSV type 2	0.02	6.5
	VZV	0.0004	0.5
Papillomaviruses	HPV type 11	17	716
Polyomaviruses	BKV	0.13	115
	JCV	0.045	> 0.1
Poxviruses	Vaccinia virus	0.8	46
	Variola Virus (Small pox)	0.1	27
Retroviruses	HIV, strain IIIB	0.03	88.5

So far no efficacy data from rodents or NHP have been presented.

It is notable that following brincidofovir dosing, the relative exposure to CDV (which is highly nephrotoxic) is considerably higher in several species, particularly in monkeys, compared to what is seen in humans (where the AUC of CDV is 5-10% of that seen for brincidofovir).

2.2.2.4. Safety aspects

As is the case with CDV, preclinical data for brincidofovir indicate carcinogenicity and reproductive toxicity.

Over 900 healthy subjects or patients infected with various double-stranded DNA-viruses have received brincidofovir. A few patients with EBOV infection have been treated with

brincidofovir. However, no clinical safety data from such patients have been available for review.

Doses up to 200 mg once weekly or 100 mg twice weekly were generally well tolerated. The dose-limiting toxicity of brincidofovir is gastrointestinal. Diarrhoea, and, less frequently, nausea, vomiting, anorexia, loss of appetite, hypoalbuminemia and abdominal pain, have been observed in subjects receiving brincidofovir, particularly at doses of 200 mg twice weekly for more than 2 to 4 weeks.

Increases in serum aminotransferase levels, particularly alanine transaminase, but also aspartate aminotransferase, have been reported. These increases were dose-proportional in terms of frequency and intensity but serum levels generally returned to baseline after discontinuation of dosing. Severe hepatobiliary adverse events have been reported in a few virally infected patients receiving brincidofovir therapy.

Although brincidofovir is a lipid conjugate of CDV, no nephrotoxicity, myelotoxicity or ocular toxicity has been noted, presumably due to differences in tissue distribution. However, due to the potential for CDV to accumulate during dosing with brincidofovir, dosing with brincidofovir is not recommended in patients with end-stage renal disease (creatinine clearance <15 ml/min) unless a patient is undergoing some form of dialysis, which removes CDV. No data on brincidofovir use in patients on continuous renal replacement therapy (CRRT) has been reported.

It is notable that the assumption of clinically relevant anti-EBOV activity of brincidofovir is based on the bridging argument that EC50 values for EBOV are similar to the values for other viruses where clinical activity has been shown in animal models or humans.

While CDV and brincidofovir are both prodrugs of cidofovir-PP, their EC50 values are not similar (see Table 1). Brincidofovir and CDV exhibit different tissue distribution *in vivo*, which is why the high nephrotoxicity of cidofovir is not seen with brincidofovir. Thus, the available data do not provide a rationale for the use of CDV in patients with EBOV infection. In this context, it should be noted that adequate hydration is a prerequisite to reduce the nephrotoxicity of CDV, and that fluid loss is one of the clinical hallmarks of EBOV infection.

2.2.2.5. Other relevant information

The company proposes a loading dose of 200 mg (or 4 mg/kg) orally, followed by 100 mg (or 2 mg/kg) twice weekly for a total of 14 days (5 doses in total). Apart from the loading dose, which is based on preclinical double-stranded DNA virus disease models, the company's proposed dose is similar to the dose used in human CMV studies. An unconfirmed assumption would be that bioavailability in EBOV-infected patients does not greatly differ from bioavailability in patients with other conditions.

At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

The company declared that treatment is available for the current clinical trial use.

2.2.3. Favipiravir

Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide; T-705) is a nucleic acid analogue that is ribosylated and phosphorylated within cells forming T-705 ribosylmonophosphate (T-705RMP) which can be further phosphorylated to the di- and tri-phosphate analogues (T-

705RDP and T-705RTP respectively). T-705RTP (T705M6) is the active metabolite. As with other nucleoside analogues, studies suggest that favipiravir interferes with viral RNA replication.

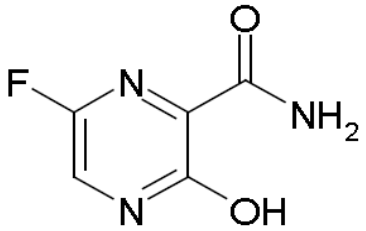
Favipiravir has been approved in Japan since March 2014 for an outbreak of novel or re-emerging influenza virus infections and its use is limited to cases in which other anti-influenza virus agents are either ineffective or insufficiently effective. The approved posology is 1,600 mg orally twice daily for 1 day followed by 600 mg twice daily for 4 days.

Favipiravir is currently being tested for treating uncomplicated influenza infection at a differential dosing regimen (taking into account differential pharmacokinetic parameters between Japanese and non-Japanese subjects): 1800 mg twice daily at day 1 then 800 mg twice daily from day 2 to 5.

2.2.3.1. Quality aspects

The drug substance is a small chemical molecule (shown in Figure 8) delivered in the form of 200 mg tablets. The quality has been assessed and found acceptable at this stage of development in several EU Member States and in other regions for use in a phase 3 clinical trial. No critical issues have been identified concerning the quality of this product at this stage of development.

Figure 8 Structure of favipiravir



2.2.3.2. Pharmacology (kinetics)

Favipiravir is rapidly absorbed with a median t_{max} of 1 hour (following a single dose) and of 2 hours (following multiple doses). The oral bioavailability of favipiravir in patients with symptomatic EBOV infection is unknown. The compound has a terminal $t_{½}$ of 2 to 4 hours. The main elimination pathway of favipiravir is likely via metabolism. *In vitro* data have indicated that CYP isoenzymes are not involved, and that the major metabolite (M1) may be formed by aldehyde oxidase. A glucuronide metabolite has also been detected in plasma and urine.

Following single doses of 30 mg to 1,200 mg, favipiravir exposure increases in an almost dose-proportional manner. However, non-linear pharmacokinetics have been suggested in studies with multiple doses of favipiravir: the t½ of the compound increases to some extent, favipiravir plasma levels accumulate in some studies, and the relative metabolite-to-parent ratio of metabolite M1 has been shown to be reduced (from 43% at day 1 to 6% at day 5). The underlying cause of the non-linearity is suggested to be the saturation of aldehyde oxydase. There are no data on alternative metabolic pathways of favipiravir.

In studies completed in Japan, patients had approximately 50% higher blood levels than patients in studies completed in the US. It is not known if differences in diet and/or ethnic background contributed to the observed differences.

No pharmacokinetic data have been presented from NHPs or humans with EBOV infection for this orally administrered agent.

2.2.3.3. Efficacy aspects

The *in vitro* EC50 of favipiravir for EBOV-m and EBOV-k has been estimated to be 10.5 to 63 µg/ml (67 µM - 402 µM) (Oestereich et al. 2014 and Smither et al. 2014). In comparison, the EC50 for influenza A and B are estimated to be 0.045 to 3.8 µg/ml.

Favipiravir has also been studied in NHP studies. Final results are not yet available.

2.2.3.4. Safety aspects

Over 2,500 healthy subjects or patients infected with influenza virus have received at least one dose of favipiravir. Notably, the recommended and studied doses for the treatment of influenza virus are lower than those currently being planned for EBOV. Clinical safety data are not yet available for the dose regimen proposed against EBOV disease. A few Ebola-virus-infected patients have been treated with favipiravir and data from these patients are scarce. The adverse events of favipiravir seen during the development of the product for influenza include mild to moderate diarrhoea, abdominal pain, headache and asymptomatic elevations of blood uric acid.

2.2.3.5. Other relevant information

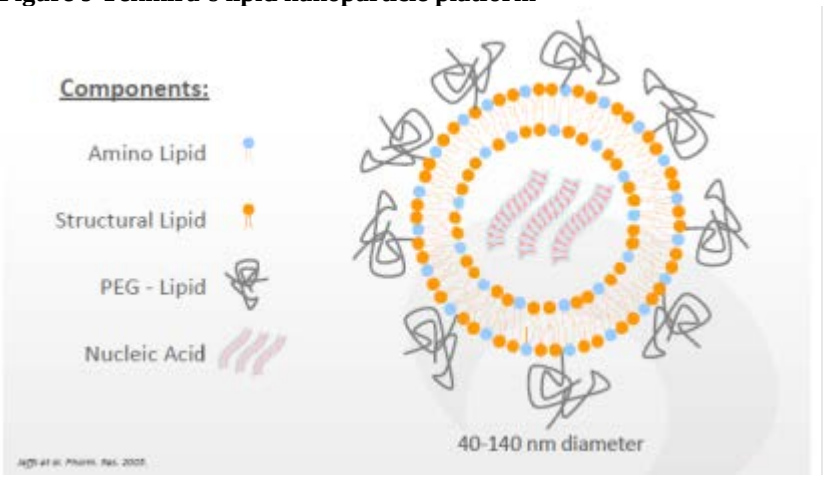
At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

2.2.4. TKM-100802

2.2.4.1. Quality aspects

TKM-100802 is a lipid nanoparticle (LPN) (60-120 nm) formulation containing two synthetic small interfering RNAs (siEbola-2 drug substance) designed to selectively reduce the expression of EBOV L polymerase and VP35 and thereby repress EBOV replication and pathogenesis in the infected cell.

Figure 9 Tekmira's lipid nanoparticle platform[2]



[2] Contrary to the drawing representing the drug formulation, the particle is in reality not a bilayer liposome but a true and dense nanoparticle.

The present product is not optimised for use against EBOV-guinea (EBOV-g), the strain responsible for the present outbreak. The company expects to release a new medicinal product with a modified drug substance (with changes in the oligonucleotides sequences) optimised to the 2014 isolate from early December 2014. Considering the documentation provided on the product's manufacture, the introduction of an updated target sequence adapted to the 2014 isolate of the two active substances is not expected to negatively influence the quality of the finished product.

The drug product is a sterile formulation of the siEbola-2 drug substance with lyoprotectants and lipids of which two are novel excipients. It is presented as a lyophilised product containing 5 mg of the drug substance in a glass vial. The storage temperature is 5 ± 3°C. The product is reconstituted to 1 mg/ml with water for injection prior to use.

2.2.4.2. Pharmacology (kinetics)

The siRNA containing LPNs are expected to enter the cells via endocytosis, where pH changes trigger the release of the two siRNAs. It is suggested that the lipid excipients are catabolised via endogenous cellular pathways and that the siRNA components probably undergo nuclease-mediated metabolism.

In a single-ascending intravenous dose study in healthy volunteers (TKM-EBOV-002), four dose levels from 0.075 to 0.5 mg/kg of TKM-100802 were investigated (each cohort contained 3 subjects that received TKM-100802). C_{max} appeared to increase in a greater than dose-proportional manner over the dose range, whereas the increase in the AUC was approximately proportional with dose. However, the inter-individual variability was substantial, precluding any firm conclusions. The plasma concentration-time profiles and the pharmacokinetic parameters of siLpol-2 and siVP35-2 were highly correlated, suggesting similar retention properties within the LPN.

Since there are no data for TKM-100802 in humans following multiple dosing, preliminary simulations based on the single-dose data (and assuming linear kinetics) have been performed. These simulations suggest that a dose of 0.24 mg/kg/day for 7 days results in C_{max} values that are similar to those observed for the single-dose MTD (i.e. 0.3 mg/kg) in humans. Based on the similarity in plasma exposure between humans and monkeys, it is suggested that a dose level of at least 0.2 mg/kg/day may be required to meet exposure targets derived from efficacious dose levels in the monkey infection study.

2.2.4.3. Efficacy aspects

The nucleotide sequences for siLpol-2 and siVP35-2 were selected through *in vitro* screening studies that confirmed the antiviral activity and mechanism of action of these siRNAs (EC50 ranging from <0.003 nM to 0.04 nM at 48h post infection in HepG2 cells using wild-type and engineered virus systems). The oligonucleotide sequences of siLpol-2 and siVP35-2 possess 100% complementarity to EBOV Lpol and VP35 mRNAs.

Sequence data indicate that the 2014 West African emergent strains of EBOV-g differ from the TKM-100802 siRNA target sites (EBOV-k strain) by one nucleotide in the case of siL-pol-2, and two nucleotides in the case of siVP35-2. All publically available viral sequences (3 Guinea and 99 Sierra Leone) from the current outbreak share these 3 single nucleotide polymorphisms (SNPs) in the targeted regions. When assessed in a plasmid-based virus-free system, both components had lower activity against the EBOV-g strain.

TKM-100802 has been studied against EBOV-k in three different NHP studies. No studies with EBOV-g were provided by the company in the context of this review.

In the first study two different doses (0.2 and 0.5 mg/kg) were given intravenously once daily and within 1.5 hours of viral challenge. With the lower dose, viraemia similar to that seen in untreated animals was detected in all monkeys from day 5 and onwards (survival: 4 out of 6). Viraemia was seen also with the higher dose, but at lower levels (survival: 6 out of 6).

The second study assessed the impact of different dosing regimens. Of the six animals receiving a daily treatment regimen of 0.5 mg/kg TKM-100802, all survived to day 41 (the scheduled termination time).

In the third study TKM-100802 0.5mg/kg was given 1 to 4 days after viral challenge. Animals randomised to saline succumbed to infection beginning on day 6 and all animals died by day 9. Five out of six animals (83.3%) treated with TKM-100802 24 hours post infection and three out of six treated 48 hours post infection survived. When TKM-100802 was given 72 hours after infection, four out of six (66.7%) animals survived. On the other hand, when TKM-100802 was given 96 hours after infection, zero out of six animals survived. Thus, survival benefits were seen when the agent was given up to 3 days post challenge, but not when given 4 days post challenge. In the latter case, there was also no apparent effect on viraemia.

2.2.4.4. Safety aspects

siRNA can induce sequence-specific off-target effects by two mechanisms, referred to as either “siRNA-like” or “miRNA-like” mechanisms. A bioinformatic analysis indicated that siEbola-2 is highly selective for EBOV Lpol and VP35 mRNA targets and that no contiguous complementarity of more than 15 nucleotides exists between siEbola-2 and any human mRNAs, indicating a low risk of off-target siRNA-like effects.

The clinical safety of TKM-100802 has been evaluated in a single ascending dose study in 19 healthy volunteers (14 treated with TKM-100802 and 5 with placebo). Four dose levels from 0.075 to 0.5 mg/kg of TKM-100802 were tested. The maximum tolerated dose for TKM-100802 in healthy human volunteers was considered to be 0.3 mg/kg. However, findings in the study of adverse events led the U.S. Food and Drug Administration (FDA) to place further clinical development on a full clinical hold in July 2014.

In August 2014, the FDA changed the full clinical hold to a partial clinical hold to allow TKM-100802 to be used to treat individuals with confirmed or suspected Ebola virus infection. TKM-100802 has been administered to patients during the current Ebola outbreak. At this time, no safety data have been provided to the EMA for these patients.

2.2.4.5. Other relevant information

The company’s recommended dose for TKM-100802 is 0.3 mg/kg daily, administered intravenously over approximately 60 minutes. This is based on and limited by the safety findings in healthy volunteers.

At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

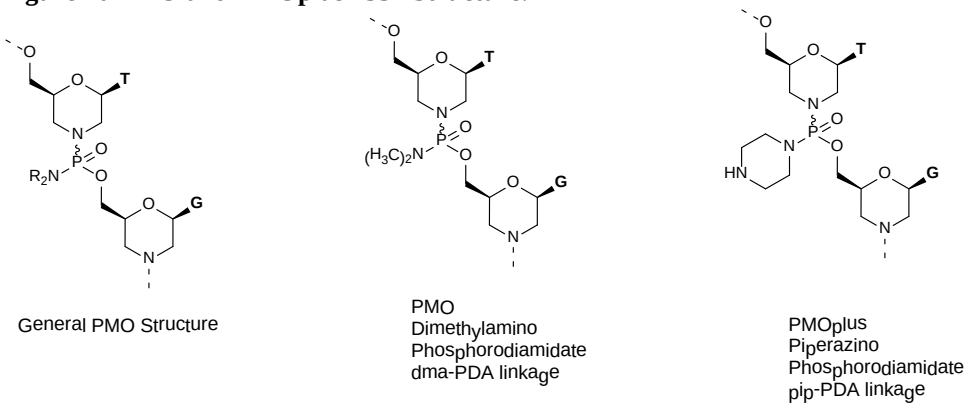
Current supplies of TKM-100802 directed against the EBOV-k strain are limited. The company expects to release a new medicinal product with a modified drug substance optimised to Guinea genome from early December 2014.

2.2.5. AVI-7537

2.2.5.1. Quality aspects

AVI-7537 is an antisense oligonucleotide aiming at repressing virus replication. The active substance is a 19-mer phosphorodiamidate morpholino oligomer (PMO) with up to five positive charges (PMOplus) replacing certain phosphorodiamidate neutral linkages (Figure 10). The company has evaluated the sequence of the Ebola strain circulating in the current outbreak and there do not appear to be any mismatched bases in the VP24 region that AVI-7537 targets.

Figure 10 PMO and PMOplus 7537 Structure.



The morpholino ring is a 6-member ring as opposed to the natural 5-member ribose ring of RNA and DNA. When the morpholino subunits are linked to each other, the functional group formed by the intersubunit linkage is neutral (centre image Figure 10; format of Sarepta PMO basic subunit). The inclusion of a dimethylamino or piperazinyl phosphorodiamidate linkages into a PMO drug imparts a net positive charge at physiological pH, hence the term PMOplus. The synthetic nature of the PMO, containing morpholino rings connected by phosphorodiamidate linkages renders the compound metabolically stable and resistant to DNAse and RNAse cleavage. Information on the sequence of AVI-7537 is shown in Table 2.

Table 2 AVI-7537 Sequence

Drug Substance	Target	Target Description	Sequence*	Length
AVI-7537	VP24	Membrane Associated Viral Protein 24	GCC +ATG GT+T TT+T TC+T C+AG G	19
* + indicates the position of the positively charged linkages between the subunits.				
A= Adenine; C = Cytosine, G = Guanine; T = Thymine				

2.2.5.2. Pharmacokinetics

AVI-7537 was administered to healthy volunteers (4 subjects receiving AVI-7537 in each cohort) at six dose levels ranging from 0.005 mg/kg to 4.5 mg/kg. AVI-7537 C_{max} and AUC

parameters seemed to increase in a dose-proportional manner. As for TKM-100802 there was a late second peak followed by a slow decline, but the clinically more meaningful t½ was estimated to 2-4 h.

At the four higher dose levels the median value of clearance was 127 ml/h/kg. The contribution of the renal clearance to the total clearance increased with dose, from 1.7% (CV 151%; dose 0.05 mg/kg) to 44% (CV 15%; dose 4.5 mg/kg) of total elimination. Other pathways contributing to the elimination of AVI-7537 are uncertain.

2.2.5.3. Efficacy aspects

AVI-7537 targets the VP24 gene. Initially in its development, the product was part of a compound known as AVI-6002 which contained (in a 1:1 ratio) AVI-7537 together with another compound targeting VP35 (AVI-7539). Data subsequently showed that efficacy seen in NHPs derived from AVI-7537 alone. Data on survival in studies with AVI-6002 are shown in Table 3. Administration of drug was shortly after viral challenge in all studies. Data on antiviral effects were not submitted.

Table 3 Survival in Rhesus Monkeys Infected with EBOV-k, treated with AVI-6002 or Control

Group	Dose (mg/kg)	Route	Regimen	Survival (n/N)	Days until death
Experiment 6					
Control	0	SC + IP	Day 0 - Day 14	0/1	D10
AVI-6002	30-42			3/4	D11
Control	0	SC + IP	Day 0 - Day 14	0/1	D7
AVI-6002	30	SC + IP		2/4	D15, D16
Experiment 7					
Vehicle control	0	IV	Day 0 - Day 14	0/1	D8
Scramble control[a]	40			0/4	D7, D8, D8, D8
AVI-6002	4			0/5	D7, D8, D8, D9, D16
AVI-6002	16			1/5	D7, D8, D9, D13
AVI-6002	28			3/5	D9, D9
AVI-6002	40			3/5	D10, D11

a) Scramble control is a PMO*plus* compound with a sequence that does not bind to Ebola virus mRNA

2.2.5.4. Safety aspects

The safety of AVI-7537 has thus far been evaluated in one clinical single ascending dose study as part of the compound AVI-6002. Six dose levels from 0.01 to 9 mg/kg of AVI-6002 were tested (corresponding to 0.005-4.5 mg/kg of AIV-7537). Four healthy subjects received AVI-6002 and one received placebo in each dose-cohort. AVI-6002 was generally well tolerated.

There are some supportive safety data on the dose intended for treatment of EVD from the multiple ascending dose study with AVI-7288 (a compound related to AVI-7537 for the treatment of Marburg virus disease). Five dose levels from 1 to 16 mg/kg/day for 14 days were tested. Six healthy subjects received AVI-7288 and two received placebo in each dose-cohort. AVI-7288 was generally well tolerated. One patient in the 12 mg/kg group was discontinued at day 6 due to a >3 fold increase in the urine albumin to creatinine ratio. This is an observation correlating to the known renal toxicity of oligomers found in preclinical studies.

In general, with regard to current clinical and preclinical experience with oligonucleotide-based therapeutics, kidney toxicity may be considered as a class effect. Further to this, no information has been provided on potential binding of the oligonucleotide to sequences in the human genome. Binding to human RNA/DNA could have two consequences: 1) binding to RNA will likely result in inhibition of gene expression, the consequences of which depend on the function of the particular gene and 2) there could be effects on the formation of triple helices when the oligonucleotide binds to the duplex DNA, potentially resulting in site-directed mutagenesis.

2.2.5.5. Other relevant information

At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

2.2.6. Z-Mapp

2.2.6.1. Quality aspects

ZMapp is composed of three mouse/human chimeric IgG1 monoclonal antibodies: c13C6, c2G4 and c4G7, directed against three distinct epitopes in EBOV glycoprotein. Each of the three mouse/human chimeric mAbs comprises one ZMapp drug substance. The three individual mAb substances are then combined in equal mass ratio in the ZMapp medicinal product to be administered intravenously. The formulated ZMapp medicinal product is stored at -20°C.

The active substances are produced in a plant (*N. benthamiana*) using a transient *Agrobacterium* expression system. The plant has been genetically modified to produce a N-glycosylation pattern similar to that of mammalian cells.

The purification steps applied to the plant extracts are in line with what is expected from a recombinant antibody. Regarding the quality of ZMapp, little information has been provided concerning the manufacture and control and stability of active substances and the medicinal product. The company has indicated that further information would be made available in the near future. However, at present, based on the limited quality information provided, no conclusions on its suitability for human use can be made.

2.2.6.2. Pharmacokinetics

Seven patients have been treated with ZMapp. There are no pharmacokinetic data available on these patients.

2.2.6.3. Efficacy aspects

The three monoclonal antibodies comprising ZMapp were selected from a panel of six different monoclonal antibodies which were part of prior mAb “cocktails” MB-003 and Zmab. The combination considered to be most effective was identified through studies with various combinations performed in guinea pigs and NHPs. In the initial studies, the mAbs were given 24 hours post infection. Subsequently combinations of different mAbs were administrated 3 days post infection in an attempt to extend the treatment window and presumably make the model more predictive of a human treatment situation.

Following these studies, the combination of mAb c13C6 from MB003 and c2G4 and c4G7 from Zmab were selected for ZMapp. In what may be considered the core NHP experiment for ZMapp, rhesus macaques were assigned to either one of three treatment groups of six or to a control group of three animals. All animals in the treatment groups received three doses of ZMapp (50mg/kg per dose) spaced 3 days apart (Qiu et al. 2014).

After a lethal intramuscular challenge with 1,000 x TCID50 (or 628 pfu) of EBOV-k, the animals were treated with ZMapp at 3, 6 and 9 days post infection (dpi) in Group D; 4, 7, and 10 dpi in Group E; or 5, 8 and 11 dpi in Group F. The control animals (Group G) were given a mAb 4E10 (anti-HIV) as an IgG isotype control (n=1) or PBS (n=2) in place of ZMapp starting at 4 dpi.

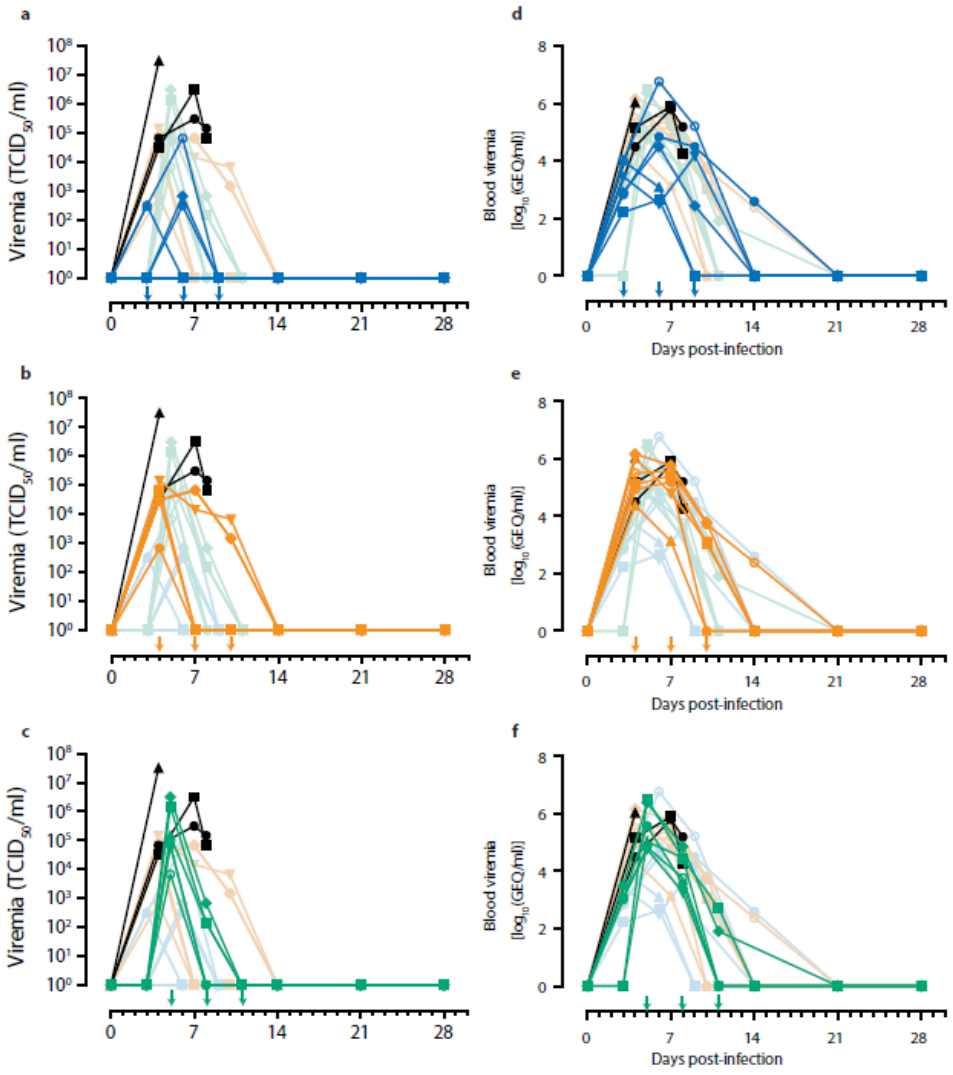
Table 4 Clinical findings of EBOV-infected NHPs from 1 to 28 dpi

Animal ID	Treatment group	Clinical findings Body temperature	Rash	White blood cells	Platelets	Biochemistry	Outcome
D1	50 mg kg^{-1} ZMapp, 3 dpi	Fever (3, 6, 14, 21 dpi)		Leukocytosis (3, 6, 21 dpi)	Thrombocytopenia (3, 6, 9, 14, 21 dpi)	ALB↓ (14, 21 dpi), ALP↓ (9, 14, 21, 28 dpi), AMY↓ (9 dpi), GLOB↑ (21, 28 dpi)	Survived
D2	50 mg kg^{-1} ZMapp, 3 dpi			Leukocytopenia (21, 28 dpi)	Thrombocytopenia (28 dpi)	PHOS↓ (9 dpi)	Survived
D3	50 mg kg^{-1} ZMapp, 3 dpi	Fever (3 dpi)		Leukocytosis (3, 14 dpi)	Thrombocytopenia (3, 21, 28 dpi)	ALT↓ (6 dpi)	Survived
D4	50 mg kg^{-1} ZMapp, 3 dpi			Leukocytopenia (14 dpi)	Thrombocytopenia (14, 21 dpi)	ALT↓ (9 dpi), CRE↑ (14 dpi)	Survived
D5	50 mg kg^{-1} ZMapp, 3 dpi	Fever (3 dpi)		Leukocytopenia (21, 28 dpi)	Thrombocytopenia (6, 9 dpi)	ALB↓ (9 dpi), BUN↓ (3, 6, 14, 21, 28 dpi)	Survived
D6	50 mg kg^{-1} ZMapp, 3 dpi				Thrombocytopenia (6 dpi)		Survived
E1	50 mg kg^{-1} ZMapp, 4 dpi				Thrombocytopenia (4, 7, 21 dpi)	AMY↓↓ (4, 21 dpi), AMY↓ (7, 10, 14 dpi), CRE↓ (21, 28 dpi)	Survived
E2	50 mg kg^{-1} ZMapp, 4 dpi	Fever (4 dpi)		Leukocytosis (4, 10 dpi)	Thrombocytopenia (4, 7, 10, 21 dpi)	ALT ↓↓ (4 dpi), GLU↑ (4 dpi)	Survived
E3	50 mg kg^{-1} ZMapp, 4 dpi	Fever (4 dpi)		Leukocytosis (4, 10 dpi)	Thrombocytopenia (7, 10, 14 dpi)	CRE↓ (14 dpi)	Survived
E4	50 mg kg^{-1} ZMapp, 4 dpi		Severe rash (5, 6, 7, 8 dpi), Mild rash (9 dpi)	Leukocytosis (10, 14, 21, 28 dpi)	Thrombocytopenia (4, 7, 10, 14 dpi)	ALP↑ (7, 10, 14 dpi), ALT ↑↑↑ (7 dpi), ALT ↑↑ (10 dpi), AMY↓ (4, 7, 10 dpi), TBIL↑↑↑ (7 dpi), TBIL↑ (10, 14 dpi), PHOS↓ (7, 10 dpi), K^+↓ (4 dpi)	Survived
E5	50 mg kg^{-1} ZMapp, 4 dpi	Fever (7 dpi)		Leukocytosis (4 dpi)	Thrombocytopenia (4, 7, 10, 14 dpi)	ALT↑ (7 dpi), AMY↓ (4, 7 dpi), PHOS↓ (10 dpi)	Survived
E6	50 mg kg^{-1} ZMapp, 4 dpi	Fever (4 dpi)	Mild rash (7, 8, 9 dpi)	Leukocytosis (4, 10, 14 dpi)	Thrombocytopenia (4, 7, 10, 14 dpi)	ALP↑ (7, 10 dpi), ALT ↑↑↑ (7, 10, 14 dpi), AMY↓ (7, 10 dpi), TBIL↑↑ (7 dpi), TBIL↑↑↑ (10 dpi), TBIL↑ (14 dpi), PHOS↓ (7 dpi), GLOB↑ (21 dpi)	Survived
F1	50 mg kg^{-1} ZMapp, 5 dpi			Leukocytosis (11 dpi)	Thrombocytopenia (3, 5, 8, 11 dpi)	AMY↓ (5 dpi), PHOS↓ (11 dpi), CRE↓ (28 dpi)	Survived
F2	50 mg kg^{-1} ZMapp, 5 dpi	Fever (3, 5 dpi)	Mild rash (8 dpi)	Leukocytosis (3, 5, 11 dpi)	Thrombocytopenia (3, 5, 8, 11, 14, 21 dpi)	PHOS↓ (11 dpi), CRE↓↓ (11 dpi)	Survived
F3	50 mg kg^{-1} ZMapp, 5 dpi			Leukocytopenia (8 dpi), Leukocytosis (3 dpi)	Thrombocytopenia (5, 8, 11, 21 dpi)	ALT↑ (8 dpi), CRE↓↓ (14 dpi)	Survived
F4	50 mg kg^{-1} ZMapp, 5 dpi	Fever (3, 5 dpi)		Leukocytopenia (8 dpi)	Thrombocytopenia (5, 8, 11, 28 dpi)	PHOS↓ (8 dpi)	Survived
F5	50 mg kg^{-1} ZMapp, 5 dpi	Fever (3 dpi)		Leukocytosis (3, 11, 14 dpi)	Thrombocytopenia (5, 8, 11 dpi)	PHOS↓ (5, 8 dpi), CRE↓ (8, 11, 21, 28 dpi)	Survived
F6	50 mg kg^{-1} ZMapp, 5 dpi	Fever (3 dpi)		Leukocytopenia (8, 21, 28 dpi)	Thrombocytopenia (8, 11, 21 dpi)	PHOS↓ (5, 8, 11 dpi), GLU↑ (5 dpi)	Survived
G1	PBS, 4 dpi		Severe rash (4 dpi)	Leukocytopenia (4 dpi)	Thrombocytopenia (4 dpi)	AMY↓ (4 dpi)	Died, 4 dpi
G2	Control mAb, 4 dpi		Severe rash (8 dpi)	Leukocytopenia (7, 8 dpi)	Thrombocytopenia (4, 7, 8 dpi)	ALP↑ (8 dpi), ALT↑ (7 dpi), ALT ↑↑↑ (8 dpi), CRE↑ (8 dpi)	Died, 8 dpi
G3	PBS, 4 dpi	Fever (4, 8 dpi)	Severe rash (8 dpi)	Leukocytopenia (7, 8 dpi)	Thrombocytopenia (4, 7, 8 dpi)	ALP↑ (8 dpi), ALT↑ (7, 8 dpi), AMY↓ (7 dpi), AMY ↓↓ (8 dpi), TBIL↑ (8 dpi), PHOS↓ (7 dpi)	Died, 8 dpi

Hypothermia was defined as below 35 °C. Fever was defined as >1.0 °C higher than baseline. Mild rash was defined as focal areas of petechiae covering <10% of the skin, moderate rash was defined as areas of petechiae covering 10 to 40% of the skin, and severe rash was defined as areas of petechiae and/or ecchymosis covering >40% of the skin. Leukocytopenia and thrombocytopenia were defined as a >30% decrease in the numbers of white blood cells and platelets, respectively. Leukocytosis and thrombocytosis were defined as a twofold or greater increase in numbers of white blood cells and platelets above baseline, where white blood cell count > 11×10^3. ↑, two- to threefold increase; ↑↑, four- to fivefold increase; ↑↑↑, greater than fivefold increase; ↓, two- to threefold decrease; ↓↓, four- to fivefold decrease; ↓↓↓, greater than fivefold decrease. ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; TBIL, total bilirubin; BUN, blood urea nitrogen; PHOS, phosphate; CRE, creatinine; GLU, glucose; K^+, potassium; GLOB, globulin.

It is notable that ZMapp conferred 100% survival when given 5 days post infection and to NHPs displaying considerable clinical and laboratory abnormalities due to EBOV infection (Table 4). Five days post infection was the latest time-point at which ZMapp was administered. Figure 11 below illustrates levels of viraemia over time in the NHPs. Note that the matrix for the polymerase chain reaction (PCR) in this study was blood rather than serum or plasma.

Figure 11 Viraemia for each ZMapp-treated group (From Qiu et al. 2014)
Arrows indicate treatment days. Faded symbols/lines are the other two treatment groups, for comparison.
Control group (Group G) is shown in black on all three panels. a, $TCID_{50}$ of Group D (blue); b, $TCID_{50}$ of Group E (orange); c, $TCID_{50}$ of Group F (green). d, Viraemia byRT–qPCR of Group D (blue); e, Viraemia by RT–qPCR of Group E (orange); f, Viraemia by RT–qPCR of Group F (green).



Extended Data Figure 2 | Viraemia for each ZMapp-treated group. Arrows indicate treatment days. Faded symbols/lines are the other two treatment groups, for comparison. Control group (Group G) is shown in black on all three panels. **a**, $TCID_{50}$ of Group D (blue); **b**, $TCID_{50}$ of Group E (orange); **c**, $TCID_{50}$ of Group F (green). **d**, Viraemia by RT–qPCR of Group D (blue); **e**, viraemia by RT–qPCR of Group E (orange); **f**, viraemia by RT–qPCR of Group F (green).

As stated above, EBOV-g is the virus responsible for the present West African outbreak. *In vitro* assays were carried out to compare the binding affinity of c13C6, c2G4 and c4G7 to sucrose purified EBOV-g and EBOV-k. As measured by ELISA assay, the ZMapp components showed slightly better binding kinetics for EBOV-g than for EBOV-k. Additionally, the neutralising activity of individual mAbs was evaluated in the absence of complement for c2G4 and c4G7, and in the presence of complement for c13C6, as they have previously been

shown to neutralise EBOV only under these conditions. The results supported the ELISA binding data, with comparable neutralising activities seen between the two viruses.

The documentation submitted by the company contains descriptions of seven patients with EBOV infection treated with ZMapp. Treatment was commenced between 6 and 16 days after the onset of symptoms. Two of the seven patients received the recommended full three-dose course (50 mg/kg for each dose). Three additional patients received three doses, but with a reduced dose of approximately 42.5 mg/kg. One patient died prior to receiving the second dose. One patient received only 2 doses, since he became asymptomatic and his blood EBOV PCR was negative following his second dose. All patients received supplementary care, and one patient also received convalescent plasma.

Five out of seven patients survived. Preliminary virological data indicate that administration of ZMapp may have decreased the viral load in several patients; however, in the absence of a control group, this cannot presently be ascertained.

2.2.6.4. Safety aspects

No clinical studies have been performed yet with ZMapp and no preclinical safety data have been provided by the company.

In anticipation of potential infusion reactions, all seven patients discussed above were pre-medicated with an antihistamine (diphenhydramine, promethazine, or chlorphenamine) prior to receiving each dose of Zmapp. Patients were treated with 42.5 mg/kg to 50 mg/kg administered as an intravenous infusion at three day intervals.

In all patients, infusion-related reactions were observed after the first dose (fever in four patients; generalised seizure in one patient; hypotension, shortness of breath, agitation, confusion and restlessness in one patient; tachycardia and macular rash in one patient; flushing in one patient and itchy palms in one patient). These reactions were alleviated by slowing down the infusion. In the patient that experienced a seizure, the infusion was halted and resumed after the seizure resolved. Fever was treated with antipyretics. The consecutive administrations were generally well tolerated, however one patient experienced hypotension, chest pain, difficulty breathing, fever and rigors. The patient's condition stabilised after the infusion was halted and the infusion of two litres of saline.

2.2.6.5. Other relevant information

There are no human pharmacokinetic data. The company proposes a dose of 50 mg/kg given every third day. This is the same dose and dose regimen as used in NHP studies (Qiu et al. 2014). The original rationale for this particular regimen has not been fully clarified, nor has a discussion on presumed similarities between human versus NHP pharmacokinetics and exposure been provided.

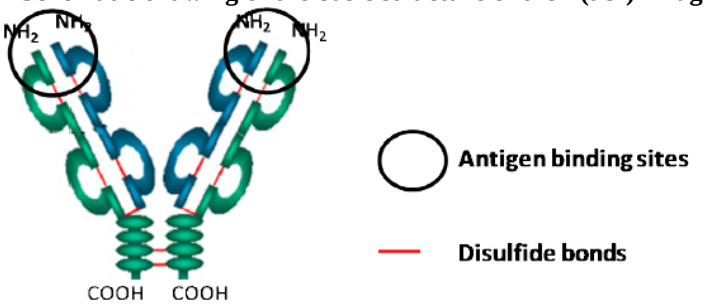
At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

At present there is no ZMapp available, and the ZMapp being produced in the upcoming half year is for use in clinical trials.

2.2.7. Anti-Ebola F(ab')$_2$

Anti-Ebola F(ab')$_2$ are specific polyclonal anti-Ebola immunoglobulin F(ab')$_2$ fragments of equine origin (Figure 12).

Figure 12 Schematic drawing of the basic structure of the F(ab')2 fragment



The drug product is planned to be supplied as a sterile solution in glass vials stored at +5 °C. The route of administration has not been stated.

The specific product against Ebola has not yet been manufactured. The company plans to develop the specific polyclonal immunoglobulins (anti-Ebola F(ab')$_2$ fragments of equine origin) targeting the Ebola virus. The product development is based on quality and safety data from an already established platform used for similar products from the same company, i.e. specific polyclonal immunoglobulin F(ab')$_2$ fragments directed against emerging and unaddressed diseases.

2.2.7.1. Quality aspects

The proposed manufacturing process and its control, both for the active substance and medicinal product, specifications, test methods, shelf-life and stability studies are based on the experience gained with similar products since no batches of anti-Ebola F(ab')$_2$ fragments are available yet. A specific potency assay is also under development. Information on viral safety and the capacity of the manufacturing process to remove/inactivate viruses has also been presented and is based on the equivalent specific inactivation steps from similar products.

Although no specific data are available for this product, the experience in the manufacture and purification of F(ab')$_2$ fragments from horse serum of equivalent products is of relevance in the development of the product. However, no scientific review has been presented supporting the use of the proposed glycoprotein-based antigen for immunisation of horses to trigger a humoral immune response capable of neutralizing EBOV *in vivo*. Neither is it clear from the documentation if this kind of antigen is or has been used in the production of the company's other products. Another critical aspect to be developed concerns a specific potency assay that should be appropriately validated and shown to correlate with neutralising activity *in vivo*. No additional viral validation studies are considered necessary at this stage if the manufacturing process is maintained as described, i.e. equivalent to other similar products already marketed.

2.2.7.2. Pharmacokinetics

There are currently no clinical pharmacokinetic data available for the anti-ebola $F(ab')_2$ fragments.

2.2.7.3. Efficacy aspects

Passive transfer of polyclonal, EBOV-specific antibodies has been evaluated in NHP models of EBOV with inconsistent results. The company cites experiences reported by Jahrling et al. 1996, with IgG from hyper-immunised horses and from Dye at al. 2012, using concentrated, polyclonal IgG antibody from NHPs that survived experimental challenge with EBOV as evidence that post-exposure antibody treatments can protect NHPs infected with EBOV. It is notable that this rationale presupposes that the Fc-part of the antibody is of no importance for the efficacy of neutralising antibodies against EBOV.

2.2.7.4. Safety aspects

There are currently no clinical or preclinical data with anti-ebola $F(ab')_2$ fragments as the specific product has not yet been manufactured.

Based on what is known from other available equine immunoglobulin $F(ab')_2$ fragment products, possible adverse reactions that could be anticipated with anti-ebola $F(ab')_2$ fragments are immediate or delayed hypersensitivity reactions varying from mild local reactions to serious systemic effects like anaphylaxis or serum sickness.

3. Overall summary

The present standard of care for EBOV infection is supportive care, including fluid replacement and support of failing organ systems. The high rate of mortality has prompted the compassionate use of candidate medicinal products alone or in combination.

The assumption that these candidate medicinal products may potentially have clinically relevant activity relies on:

- *In vitro* antiviral studies (which may be supported by *in vitro*/*in vivo* bridging);
- Rodent studies (in mice, guinea pigs and hamsters);
- NHP studies (in cynomolgus and rhesus macaques).

NHP models are generally considered as the most appropriate animal model for the selection of drug candidates for the treatment of human EBOV infection. However, no therapeutic intervention has been appropriately tested in EBOV patients to confirm the predictive value of this model.

Within the context of this procedure, the CHMP has considered quality, preclinical and clinical data submitted by companies on experimental therapies where there is *in vitro* or *in vivo* evidence of antiviral activity against EBOV. Seven experimental compounds were considered.

Key data of relevance for potential effect in EBOV infection, available up to October 2014, are provided in the below table.

Agent (company) class or type of product	Activity *in vitro* / EC50 (strain) (cell lines)	*In vivo* efficacy against EBOV infection and data on human exposure	
		NHPs	**Humans**
BCX4430 (BioCryst) Polymerase inhibitor, adenosine analogue	12 µM for EBOV-k, 3 µM for SUDV 4 µM for MARV, of relevance for NHP study (18 hrs pre-incubation human macrophages)	Cynomolgus macaques (MARV) No available data on EBOV. In a study, MARV 15 mg/kg BID IM was given 1 hr prior to and 24-48 hrs post infection. The total survival rate was 17 out of 18 exposed animals (0 out of 6 in the control group).	No efficacy or safety data in humans
Brincidofovir (Chimerix) Polymerase inhibitor (cytidine analogue)	Depending on time of pre-incubation, and incubation; 0.02-5 µM for EBOV-m (2-48 hrs pre-incubation, Vero, HUVEC, Huh7)	*In vivo* data not available for EBOV (rodents/NHPs or humans). The company describes a bridging argument that EC50 values for EBOV are similar to the values for other viruses where clinical activity has been shown in animal models.	Safety in humans available from large studies for the treatment of CMV and adenovirus in transplanted patients. A few EBOV infected patients have received the medicinal product; no data were submitted
Favipiravir (Fujifilm Corporation/Toyam) Polymerase inhibitor (purine analogue)	Depending on strain and cell line, 67 µM for EBOV-m (1h pre-incubation Vero)	Ongoing	Safety in humans generated in large influenza studies (loading doses up to 3600 mg on day 1, 800-1800 mg on day 2-5). A few Ebola-virus-infected patients have been treated with favipiravir
TKM-100802 (Tekmira) Interfering RNAs targeting L polymerase and VP35, delivered as lipid nanoparticle.	0.04 nM at 48 hrs post infection, EBOV-m (HepG2)	3 studies performed in Rhesus macaques (EBOV-k) *Dosing starting 1h-1.5 hrs post challenge:* 0.5 mg/kg/d iv was tested in 2 studies and yielded 100% survival (n=12). Lower survival with dose	TKM-100802 was tested in 14 healthy volunteers (single doses from 0.075 to 0.5 mg/kg). A grade 3 cytokine release syndrome was

		0.2 mg/kg/d (66%) or 0.5 mg/kg on alternate days (50%). *Dosing (0.5 mg/kg/day iv) starting 24, 48, or 72 post challenge:* survival rates of 5 out of 6, 5 out of 6 and 4 out of 6 animals, respectively. *When delaying therapy for 96 hrs:* 0 out of 6 animals survived (Survival rate in control group: 0 out of 6).	seen in 1 out of 2 subjects treated with the highest dose. (Formulation on partial hold by FDA, which, however, has permitted the use in patients with suspected or confirmed EBOV infection).
AVI-7537 (Sarepta) Antisense oligonucleotide targeting VP24	Not available	NHP studies performed. Most data generated with a previous compound (AVI-6002, a 1:1 mix of AVI-7537 and AVI-7539. AVI-7539 later shown not to be active). Survival was enhanced in Rhesus macaques exposed to EBOV-k	PK and tolerability in healthy volunteers (n=30) given AVI-6002 up to 9 mg/kg (i.e. AVI-7537 0.005-4.5 mg/kg) No patients reported to have received this product.
ZMapp (Leafbio Inc.) 3 monoclonal antibodies targeting the EBOV glycoprotein		Several NHP studies. In core study, 3 doses of 50 mg/kg iv (every third day) were given to Rhesus macaques starting 3, 4 and 5 days post infection with EBOV-k. 6 out of 6 animals survived in all 3 cases. 0 out of 3 control animals survived.	7 patients received the product. Infusion reactions were noted.
Anti-Ebola F(ab')$_2$ (Fab'entech) Hyperimmune horse sera	Not available. At present the discussion concerns a production platform for polyclonal antibodies.		

For four of these compounds (Zmapp, TKM-100802, favipiravir and AVI-7537) there are ongoing or finalised studies in NHP models, primarily performed with the EBOV-kikwit strain. For BCX-4430 there are data from rodent models of EBOV. For brincidofovir, the assumption of potential anti-EBOV activity is based on a similar *in vitro* EC50 as for double-stranded DNA viruses where there are *in vivo* data in animals and/or humans. The hyper-immune horse serum concept concerns a platform for the development of polyclonal anti-Ebola F(ab')$_2$ fragments through immunisation with EBOV glycoprotein; the assumption of

potential efficacy is based on passive transfer experiments of other polyclonal sera and that the Fc fragment is not important for efficacy.

In principle medicinal products with anti-EBOV effects could be used as post-exposure prophylaxis or as treatment of symptomatic infection. Of particular interest in relation to potential human use is the efficacy of delayed onset of therapy after viral challenge in the NHP model. In this context, it is notable that data are available only for ZMapp and TKM-100802. For AVI-7537 (as part of the compound AVI-6002), data are only available from the NHP model when the product is given on the same day as the viral challenge. At that time, viraemia is generally not detected in the animals, and it is presumed that the model under such circumstances would be more representative of human post-exposure prophylaxis than of actual treatment of an established symptomatic infection in humans.

For TKM-100802 it has been shown that RNA sequence differences between the guinea and kikwit varieties impacts *in vitro* activity in such a way that the efficacy shown against EBOV-kikwit in the NHP model cannot be presumed to be representative of EBOV-guinea, the virus responsible for the present outbreak.

It is notable that none of the compounds alone appears to provide full inhibition of detectable viral replication, as would be the case with combination antiviral regimens against some other viral diseases. No data on combination therapy are available and therefore the potential benefit of such strategies remains unknown.

Two of the reviewed compounds are proposed to be given orally. There are however no data on the bioavailability of oral antiviral agents in EBOV infection, which is characterised by profuse diarrhoeas and vomiting.

Safety in patients with EBOV infection has not been characterised. It is recognised that patients with EBOV infection may be differently susceptible to identified adverse reactions such as gastrointestinal side effects, nephrotoxicity or infusion reactions with hypotension. Some candidate medicinal products have potential for or have demonstrated side effects which may be less well tolerated in patients with EBOV infection than in healthy volunteers, and may potentially influence the outcome negatively. Moreover, pharmacokinetics may be altered in the disease state.

4. Overall conclusion

Based on the available data for products included in the review, the CHMP concluded that:

- The amount of preclinical and human safety data available are highly variable, ranging from no human data to relatively large amounts of data on the treatment of other viral diseases;
- While some of these products have been given to a limited number of patients with EBOV infection on a compassionate-use basis, available clinical data are not sufficient for an evaluation of efficacy, safety, and pharmacokinetics in the target population at this stage;
- The appropriate dosing of all the products reviewed is uncertain.

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