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Committee for Medicinal Products for Human Use (CHMP)

CHMP 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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List of abbreviations

ADME	Absorption, distribution, metabolism, elimination
AE	Adverse event
AUC	Area under curve
BID	Twice daily
BIW	Twice weekly
CDC	Centre for Disease Cotnrol
CMV	Cytomegalovirus
CRRT	Continuous renal replacement therapy
DIC	Disseminated intravascular coagulation
Dpi	Days post infection
EBOV	Ebolavirus
EC50	50% effective concentration
EMA	European Medicines Agency
EVD	Ebolavirus disease
FDA	Food and Drug Administration
i.m.	Intramuscular
i.v.	Intravenous
MARV	Marburg Virus
MTD	Maximal tolerated dose
mtRNA	Mitochondrial RNA
NHP	Non-human primate
NP	Nucleoprotein
PFU	Plaque forming units
PK	Pharmacokinetics
PMO	Phosphorodiamidate morpholino oligomer
QD	Once daily
RESTV	Reston Ebolavirus
SiRNA	Silencing RNA
SUDV	Sudan ebolavirus
VP	Viral protein

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.

In August 2014, the World Health Organization (WHO) declared a public health emergency of international concern in the contexst of the worst Ebola virus disease outbreak in history affecting mainly Western Africa. As of January 2016, 28 602 confirmed, probable, and suspected cases have been reported in Guinea, Liberia, and Sierra Leone, with 11 301 deaths since the onset of the Ebola outbreak. The majority of these cases and deaths were reported between August and December 2014, after which case incidence began to decline as a result of the rapid scale-up of treatment, isolation, and safe burial capacity in the three countries. The WHO declared that human-to-human transmission of Ebola virus ended in Sierra Leone on 7 November 2015, in Guinea on 29 December 2015 and was declared to have ended in Liberia on 14 January 2016 (WHO Ebola Situation Report, 20 January 2016). Currently, sporadic cases likely related to virus flare-up in survivor have occurred and may occur again. Indeed, today, there are over 10,000 survivors of Ebola virus disease. A number of medical problems have been reported in survivors, including mental health issues. Ebola virus may persist in some body fluids, including semen.

No medicinal products have yet been approved for the treatment of Ebola virus disease (EVD) in the European Union (EU). However, the European Medicines Agency (EMA) has been made aware of several therapeutic candidates in early stages of development with putative action against EVD and in September 2014, given the dynamics of the outbreak and considering the number of repatriations of infected individuals to the EU, 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, 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 EVD in accordance with Article 5(3) of Regulation (EC) No. 726/2004.

In December 2014, EMA published an interim assessment report on experimental medicines under development for treating patients infected with Ebola[1]. This first interim report included information on seven experimental medicines intended for the treatment of people infected with the Ebola virus:

- BCX4430
- Brincidofovir
- Favipiravir
- TKM-100802/130803 (TKM-Ebola)
- AVI-7537
- ZMapp
- Anti-Ebola $F(ab')_2$

[1] The European Medicines Agency (EMA). Interim assessment report. Review under Article 5(3) of Regulation (EC) no 726/2004. London: EMA, http://www.ema.europa.eu/docs/en_GB/document_library/Report/2014/12/WC500179062.pdf (2015, accessed on 28 January 2016)

Following this initial review, further data became available for the above mentioned products as well as on other treamtments under development for the treatment of Ebola. It was agreed that products for which no direct antiviral activity against the Ebola virus exist should not be included in the review. Evidence of such direct activity could be established for instance by *in vitro* or *in vivo* studies.

Based on the information provided by the sponsors of these treatments, the CHMP considered that two additional products should be included in the review:

- GS-5734
- EBOTAb

Although it is acknowledged that other medicinal products may be currently under development for the treatment of EVD, this report only reflects the data made available for the above listed products.

During its meeting in January 2016, and in light of the current epdiemiological situation, the CHMP decided that the scientific review under Article 5(3) of Regulation (EC) No 726/2004 should be concluded.

2. Scientific Discussion

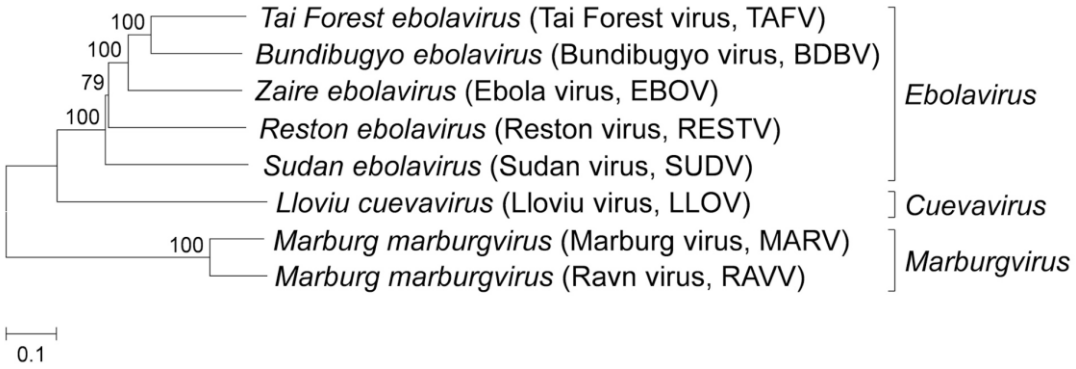
2.1. Introduction

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 2014 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 2014)



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, Rambaout 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 has been summarised in a number of recent publications (Schieffelin et al. 2014, Qin E. et al 2015, Zhang X. et al 2015 and Fitzpatrick et al. 2015).

The presumed incubation period is estimated between 2- 21 days. As compared with other febrile patients admitted without EBOV infection, EBOV patients show 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 (Lanini et al. 2015, Zhang X. et al. 2015, Fitzpatrick et al. 2015).

The average EVD case fatality rate over the course of the outbreak has resulted to be approximately 50%. However, fluctuation of fatality rate over time has been reported at different Ebola treatment centres (see figure 3).

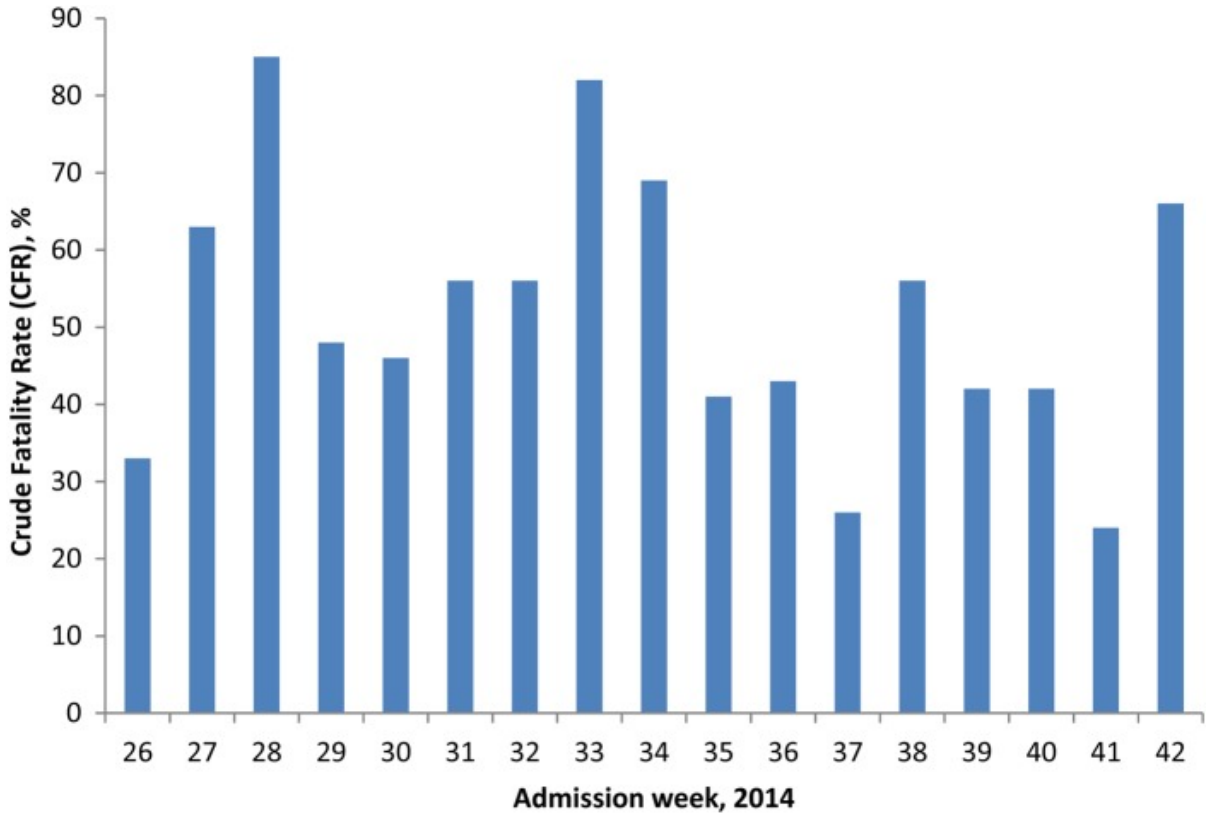


Figure 3 - Fluctuation of crude fatality rate by week of admission (26–42), Kailahun Ebola Case Management Centre, Sierra Leone, 2014 (from Fitzpatrick et al 2015)

It has become clear that continuous viral shedding in semen may occur for very long periods in patients surviving EBOV infection (possibly longer than the 3 months stated in Guidance documents), with a risk for sexual transmission (Mate et al, 2015). It is also clear that an unknown proportion of survivors may experience late complications, such as uveitis, that may occur many weeks after the clearance of viremi (Varkey et al, 2015). These issues require further investigation, and maybe also become subject to future clinical studies with investigational treatments for EVD (outside the acute disease).

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.

Use of a mouse model susceptible to wild type EBOV infection subsequent to engraftment of a human immune system has also been reported (see below).

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 10^5 plaque forming units (pfu) given subcutaneously and ~3 days with a lower dose of 10^3 pfu given intramuscularly). Similarly, time to death depends on the infectious dose, with death generally occurring 6-7 days after an intramuscular challenge with 10^3 pfu, and 9-12 days with a low dose of 10 pfu, which is still generally lethal. Disease course and time to death appears to be slightly faster in cynomolgus macaques than in rhesus macaques. An important factor in the experimental conditions to be controlled is the actual virus used for the challenge as indeed it was shown that several passages of the seed stock impacted on the virulence of the strain used and the course of disease in NHPs (Geisbert T., 2015).

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.

2.2. Antiviral treatments against Ebola virus

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

- Four nucleos(t)ide polymerase inhibitors: **BCX4430**, **brincidofovir**, **favipiravir** and **GS-5734**;
- Two oligonucleotide based products: **TKM-100802** and **AVI-7537**;
- A cocktail of monoclonal antibodies: **ZMapp**;
- Platforms describing the plans for the production of polyclonal immunoglobulins derived from immunised horses: **anti-Ebola F(ab')$_2$ fragments**.
- A purified polyclonal ovine antibody raised against soluble recombinant EBOV-GP ectodomain: **EBOTAb**

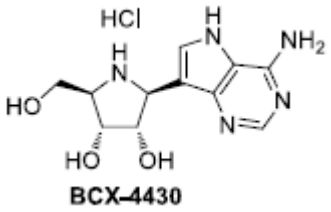
No discussion on efficacy in rodents is presented in cases where NHP studies have been performed.

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 4). The active substance is soluble in water.

Figure 4 Structure of BCX4430

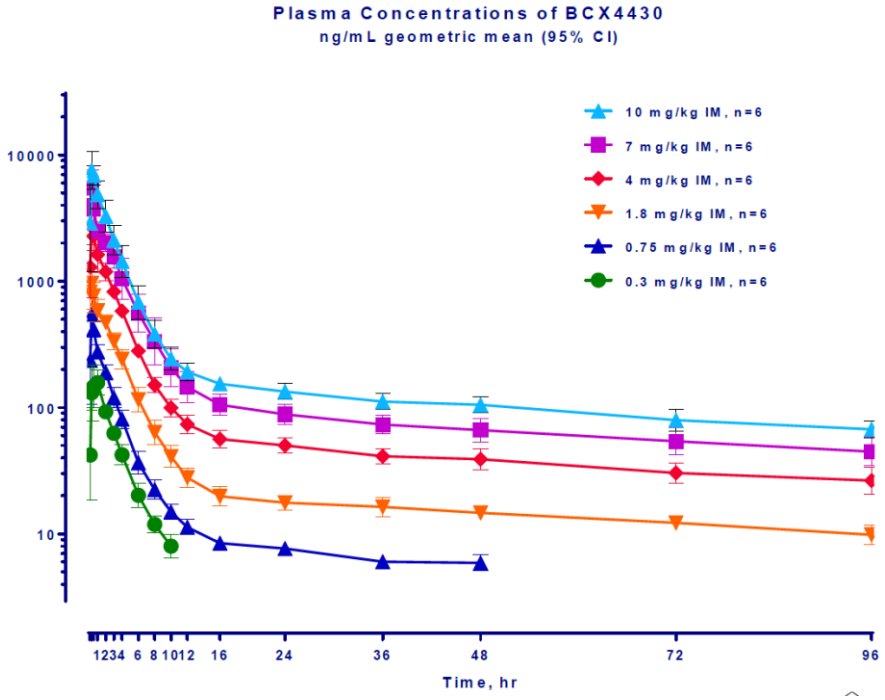


The active substance is metabolised into BCX6870 (5'-triphosphate metabolite). The triphosphate undergoes pyrophosphate cleavage, producing the monophosphate that is incorporated into nascent viral RNA strands. The monophosphate would be expected to cause premature termination of transcription and replication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

Preliminary pharmacokinetic data from the single ascending dose part of the first-in-man study of BCX4430 administered intramuscularly has been provided and are depicted below.



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. Very limited data from studies in Rhesus macaque challenged with EBOV indicate survival and antiviral effects of doses of BCX4430 16 mg/kg and 25 mg/kg BID compared to control animals. The number of animals studied, the route of administration and time point for initiation of BCX4430 treatment as well as information regarding the EBOV-strain, inoculum and route of administration of EBOV was not provided by the sponsor.

2.2.1.4. Safety aspects

Preliminary safety data from the single ascending dose part of the first-in-man study of BCX4430 administered intramuscularly has been provided. Single doses of BCX4430 were reported to be generally safe and well tolerated with no seroius adverse events. The most frequently reported adverse event was injection site pain (13/32 subjects), consistent with intramuscular injections Injection site pain was ameliorated by co-administration of lidocaine. Transient elevation of Creatine kinase (CK) and Aspartate aminotransferase (AST), which according to the company, is consistent with IM injections. There was no indication of liver, renal or cardiac toxicity.

The multiple ascending dose (MAD) part of the study will evaluate the safety, tolerability, and pharmacokinetics of 7 days of daily dosing with BCX4430 IM versus daily placebo. Three Cohorts are planned at doses of 2.5, 5 and 10 mg/kg.

Preclinical studies performed hitherto in NHP and rats suggest possible liver and renal toxicities, lymphoid depletion, and testicular effects at higher doses. Injection site toxicity has been reported in both species. The potential effects of BCX4430 and its 5-triphosphate metabolite, BCX6870, on mitochondrial function were evaluated. BCX6870 can be inefficiently utilized by mtRNA polymerase in the absence of adenosine triphosphate (ATP), in contrast BCX6870 up to 100 µM did not inhibit mtRNA polymerase activity and incorporation of adenosine monophosphate (AMP) into mitochondrial RNA transcript in the presence of 5 µM of un-labelled ATP. BCX4430 at concentrations of up to 50 µM did not exhibit mitochondrial toxicity in cultured cells. No mitochondrial toxicity was seen when cultured cells were forced to rely on oxidative metabolism with galactose.

2.2.1.5. Other relevant information

The company has not presented any proposed dose for the treatment of EBOV infection. No information is given on the current supply of BCX4430.

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. Phase 2 studies have also been performed to treat adenovirus infection in such patients.

2.2.2.1. Quality aspects

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

2.2.2.2. Efficacy and safety aspects

EBOV is a negative single-stranded RNA-virus, with an RNA-polymerase acting within the replication cycle. Despite BCV generally targeting DNA virus, where DNA-polymerase differs from that found in an RNA virus, initial *in vitro* experiments indicated that BCV had substantial antiviral activity to EBOV, with EC50 values in the same range as those seen for dsDNA-viruses, such as CMV, that have been treated successfully with brincidofovir in humans or in animal models.

Since substantial safety data had already been generated with BCV for the treatment of CMV-infection, the drug was therefore considered a potential candidate for clinical trials during the rapidly increasing 2014 epidemic of Ebola-infection. There was also compassionate use of brincidofovir in patients with EBOV infection (see below).

Planned clinical trials were never initiated, as preliminary data from studies in mice became available. These did not show any indication of activity in the mouse EBOV model. Both normal mice and mice with a humanized immune system were used, and there was no difference in survival (all animals died, except one virally infected animal that received placebo) or clinical symptoms score between animals receiving BCV or placebo. No data on viral loads in treated versus untreated animals have been made available. Furthermore, no PK data was provided from these studies and thus no information on the BCV plasma levels in mice and any potential correlation to doses achieved in humans was possible.

The dose and regimen of BCV used in these studies were based on data primarily from studies with mousepox virus (Ectromelia, a DNA virus), where it is known that the active antiviral is CDV-PP, which has a half-life in peripheral blood mononuclear cells of approximately 6 days.

As stated above, a limited number of patients with EBOV infection have been treated with BCV as compassionate use. Data from such patients have been submitted, but are not considered sufficient to evaluate the antiviral or clinical activity of BCV; they are too few, non-comparative and several patients also received other experimental agents.

The contrasting results obtained *in vitro* and *in vivo* (mice) have been followed up by further extensive *in vitro* works performed by the CDC in collaboration with the NIH, US (published by McMullan et al, Antiviral Research 2015, article in press).

In these further studies it was found that cidofovir without the lipid HDP component indeed lacked EBOV activity. The HDP component per se showed weak activity, but still much lower than that of BCV. The same pattern was shown for the enantiomer of cidofovir which cannot be phosphorylated, as well as for tenofovir; these analogues per se showed no activity to EBOV, but when linked to the HDP component the EC50 values were similar to that of BCV. In summary, it was concluded that the anti-EBOV activity of BCV was not meditated by phosphorylated cidofovir, but required the HDP component.

When choosing the dose for the EBOV-studies in mice it was presumed that the mechanism of action was the same for EBOV as for the other (DNA-) viruses previously studied (phosphorylated cidofovir inhibiting the viral polymerase). Now this has been shown not to be the case. Since pharmacokinetics, half-life included, differ substantially between the prodrug BCV and phosphorylated cidofovir (which

has a relatively long, intracellular half-life), this may explain the absence of any relevant effects in animals. On the basis of these data, further studies (in animals and humans) if considered, would have mandated higher or more frequent dosing. Such dosing of BCV, however, would in turn lack support in a safety database from previous human studies. Of note, diarrhoea was considered a dose-limiting side effect of BCV in the CMV development program.

On January 30, 2015, the company announced that it has decided to suspend current and future development of BCV for the treatment of Ebola virus disease.

2.2.2.3. Other relevant information

Due to the sponsor´s decision and available scientific evidence, brincidofovir is no longer considered a candidate for the treatment of Ebola virus infection.

2.2.3. Favipiravir

Favipiravir (6-fluoro-3-hydroxypyrazine-2-carboxamide; T-705) is a nucloside analogue that is phosphorylated within cells, resulting in a tri-phosphate analogues (T-705RTP), the active metabolite. As with other nucleoside analogues, studies suggest that favipiravir interferes with viral RNA replication.

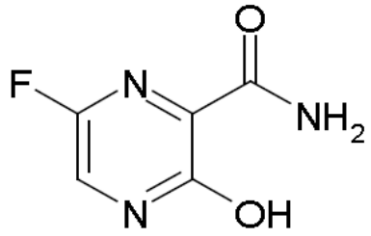
Favipiravir has been approved in Japan since March 2014 for an outbreak of novel or re-emerging influenza virus infections and its use is limited to cases in which other anti-influenza virus agents are either ineffective or insufficiently effective. The approved posology is 1,600 mg orally twice daily 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, delivered as 200 mg tablets. The quality has been assessed and found acceptable at this stage of development in several EU Member States, without any critical issues identified.

Figure 5 Structure of favipiravir



2.2.3.2. Pharmacology (kinetics)

Favipiravir is rapidly absorbed, with a median t_{max} 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.

Within the scope of this review, no pharmacokinetic data have been presented from NHPs or humans with EBOV infection for this orally administered 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 been studied in two NHP studies, with EBOV-k challenge. Doses tested were 250-400 mg/gl loading dose, followed by 150-300 mg/kh maintenance dose. All but one animal (first study) given favipiravir in these studies died; time to death was longer than for control animals and viral load was lower.

One clinical trial was performed in EBOV patients (JIKI study, 4 Ebola treatment centers in Guinea). The study was single armed, where survival rates (primary endpoint: mortality at day 14) were compared to those obtained in historical controls during the preceeding 3 months at one of the centers (same treatment and care, favipiravir excluded).

Adults were given 2,400 mg at hour (H) 0, 2,400 mg at H8 and 1,200 mg at H16, then 1,200 mg twice a day for 9 days. Children were dosed according to weight (supported by PK-modeling).

Body weight (kg)	Doses on Day-0 (mg)			Daily doses from Day-0 to Day-9 (mg)		
	H0	H8	H16	H0	H8	H12 if treatment BID H16 if treatment TID
10 - 15	600	600	200	200	200	200
16 – 21	800	800	400	400	-	400
22 – 35	1200	1200	600	600	-	600
36 – 45	1600	1600	800	800	-	800
46 – 55	2000	2000	1000	1000	-	1000
> 55	2400	2400	1200	1200	-	1200

An interim analysis was presented in February 2015, concerning 69 paients above age 14 (here called adults), and 11 children of age <11 (Sissoko et al, oral presentation of the CROI 2015). The majority of the adult patients had been ill for >72 hours (74%).

As can be seen below, there was no statistically significant impact on mortality in the overall adult cohort, compared to the historical controls.

Table 1: outcomes by viral load at baseline

	JIKI				3-months Pre-trial				
	Included		Dead		Admitted		Dead		*P*
	n	(column %)	n	(row %)	n	(column %)	n	(row %)	
Any Ct value*	69	(100%)	33	(48%)	478	(100%)	272	(57%)	0.16
Ct <20	28	(42%)	26	(93%)	232	(48%)	197	(85%)	0.39
Ct ≥20	39	(58%)	6	(15%)	246	(52%)	75	(30%)	0.052
20 ≤ Ct < 25	24	(36%)	6	(25%)	154	(32%)	57	(37%)	
Ct ≥ 25	15	(22%)	0	(0%)	92	(20%)	18	(20%)	

* Including 2 missing *Ct* values at baseline

In the cohort, outcomes correlated to baseline viral load, where a cut equivalent to 10^8 copies/ml was quite predictive of survival. This level of viremia corresponds to a CT-value of ≥20, defined as number of cycles of RNA replication to detect a positive signal (i.e. inversely correlated to viral load). Among the adults with a baseline viral load higher than 10^8 copies/ml (28/69, 42%) the mortality was 100% despite favipiravir therapy. In contrast, the mortaility rate in those with a lower baseline viral load was 2/41 (7%); this was lower than seen in historical controls with the same baseline viral load strata (mortaility rate 75/246, 30%). It is unclear whether this subgroup analysis was pre-planned.

Outcomes for the children were not presented; the proportion with a high baseline viral load, however, was high (>10^8 copies/ml in 7/10 with such data).

2.2.3.4. Safety

The main 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 (loading doses up to 3600 mg day 1, 800-1800 mg day 2-5).

Specific safety data from the JIKI trail have not been presented.

2.2.3.5. Other relevant information

The company has not presented any future plans with regards to favipiravir for the treatment of EBOV infection.

2.2.4. GS-5734

GS-5734 is a novel nucleotide prodrug formulated for i.v. administration under development for the treatment of EVD.

The mechanism of EBOV inhibition by GS-5734 has not been studied in detail to date. GS-5734 is believed to induce the inhibition of EBOV RNA synthesis and has been designed to deliver the monophosphate of parent nucleoside analogue GS-441524 into cells. Inside cells, the GS-441524 monophosphate undergoes conversion to pharmacologically active nucleoside triphosphate form GS-443902. Metabolism of GS-5734 and/or the diastereomeric mixture GS-466547 to triphosphate GS-443902 has been demonstrated in multiple cell types relevant for EBOV replication.

While there is no direct biochemical evidence for the inhibition of EBOV RNA-dependent RNA polymerase by GS-443902, the compound acts as an analogue of adenosine triphosphate (ATP) and selectively inhibits respiratory syncytial virus (RSV) RNA polymerase. RSV and EBOV share similar mechanism of RNA transcription/replication and exhibit a relatively high degree of homology within the active sites of their respective RNA polymerases, suggesting that GS-443902 is likely to inhibit EBOV RNA polymerase as the primary mechanism of action of GS-5734.

A phase I study is currently ongoing (single ascending dose study to evaluate safety, tolerability and PK of GS-5734 in healthy subjects) and three more Phase I studies are planned (a multiple ascending dose study, a renal impairment study and a safety and PK-study in subjects with prior EVD).

2.2.4.1. Quality aspects

GS-5734 is a single diastereomer monophosphoramidate prodrug of the pharmacologically active triphosphate, GS-443902.

The structure of the drug substance has not been presented to EMA by the manufacturer. It is stated to be a solid substance with the molecular formula $C_{27}H_{35}N_6O_8P$. Its solubility in water at pH 2 is 0.75 mg/ml and in pH 7 0.04 mg/ml.

GS-5734 is supplied as a sterile aqueous based concentrated solution containing 5 mg/ml GS-5734. It is stated that is should be diluted prior to IV administration. It is filled in glass vials and should be stored frozen (-25°C to -10°C). After thawing, vials can be stored up to 24 hours at room temperature prior to dilution and administration.

Based on the very limited quality information provided, no conclusions on the quality of the drug product could be made.

2.2.4.2. Pharmacology (kinetics)

Preliminary data from the first-in-man study indicate that following IV administration, GS-5734 exhibits a concentration-time profile generally consistent with that observed in nonclinical species with a short half-life of the prodrug GS-5734 ($t1/2\alpha$ = 5 min; $t1/2\beta$ = 60 min) and a long terminal elimination half-life of the terminal nucleoside metabolite GS-441524 ($t1/2 \geq 27$ hr).

GS-5734 and metabolite pharmacokinetics appear to have generally dose-proportional exposure over the dose range studied to date.

2.2.4.3. Efficacy aspects

The *in vitro* EC50s of GS-5734 were 0.06-0.14 µM against a genetically modified reporter EBOV strain in HMVEC cells and Huh-7 cells and against EBOV-k in HeLa cells and human macrophages.

GS-5734 has been studied in two NHP studies with EBOV challenge (EBOV strain, size of inoculum and route of administration not provided). The first study was performed with i.m administration. There were indications of some efficacy; this was limited, however, which was considered possibly due to slow and variable systemic uptake from the i.m administration site. Therefore, an IV formulation of GS-5734 was developed and tested in EBOV-infected rhesus monkeys to examine the effect of both the time to treatment initiation and the dose of GS-5734. Doses used and numbers treated are shown below. Treatment initiation was at fixed time point (preceding symptomatic EBOV and unguided by detectable viremia

Table 2. Design of *in vivo* efficacy study with GS-5734 administered IV

Phase[a]	Group, Number of Animals (N)	Treatment	GS-5734 Treatment Initiation	Treatment Schedule[b] (IV, once-daily)
A	Group 1, N = 3	Vehicle[c]	NA	Day 0-13: Vehicle
	Group 2, N = 6	GS-5734 3 mg/kg	Day 0	Day 0-11: 3 mg/kg GS-5734 Day 12-13: Vehicle
	Group 3, N = 6	GS-5734 3 mg/kg	Day 2	Day 0-1: Vehicle Day 2-13: 3 mg/kg GS-5734
B	Group 4, N = 3	Vehicle	NA	Day 2-14: Vehicle
	Group 5, N = 6	GS-5734 10/3 mg/kg	Day 2	Day 2: 10 mg/kg GS-5734 Day 3-13: 3 mg/kg GS-5734 Day 14: Vehicle
	Group 6, N = 6	GS-5734 10/3 mg/kg	Day 3	Day 2:Vehicle Day 3: 10 mg/kg GS-5734 Day 4-14: 3 mg/kg GS-5734
	Group 7, N = 6	GS-5734 10 mg/kg	Day 3	Day 2:Vehicle Day 3-14: 10 mg/kg GS-5734

NA = not applicable
a Phase A study arms were conducted prior to Phase B study arms. The doses and regimens evaluated in Phase B were adapted in response to results from Phase A.
b Additional vehicle treatments were administered to all GS-5734-treated animals to maintain experimental blinding.
c Vehicle (12% SBECD in water) or GS-5734 was administered once-daily by IV bolus injection.

All vehicle-treated animals succumbed to the infection or were euthanized due to their poor health status by Day 9, with a median survival time of 7.5 days. In contrast, improvements in survival until Day 28 were observed in all GS-5734-treated groups (table below).

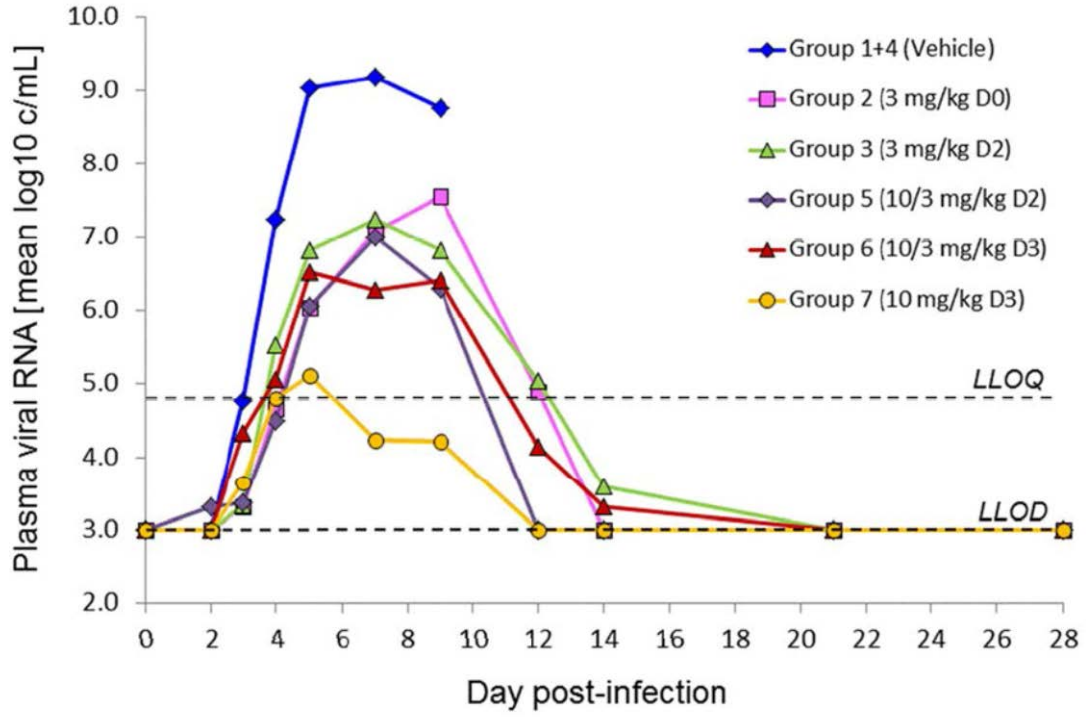
Table 3. Survival rate of EBOV-infected rhesus monkeys treated with once-daily IV administration of GS-5734

	Group 1+4 Vehicle Day 0	Group 2 GS-5734 3 mg/kg Day 0	Group 3 GS-5734 3 mg/kg Day 2	Group 5 GS-5734 10/3 mg/kg Day 2	Group 6 GS-5734 10/3 mg/kg Day 3	Group 7 GS-5734 10 mg/kg Day 3
Survival Day 28, N (%)	0 (0%)	2 (33%)	4 (67%)	2 (33%)	6 (100%)	6 (100%)
P-value versus Vehicle group	—	0.23	0.007	0.28	< 0.001	< 0.001
Median days of survival (95% CI)	7.5 (6.0 – 9.0)	10.0 (7.0 – NA)	NA	9.0 (8.0 – NA)	NA	NA

CI = confidence interval; NA = Not Applicable
Animals were infected on Day 0 and the GS-5734 treatment started on Day 0, 2, or 3 as indicated in the table above.
P-values are from Logrank test, using Dunnett-Hsu adjustment for multiple comparisons testing survival rates at Day 28.

IV administration of GS-5734 reduced the plasma vRNA levels across all groups of treated animals (figure below). Despite differential survival rate, treatment Groups 2, 3, 5, and 6 showed similar plasma viremia suppression with a mean vRNA reduction on Day 5 ranging from –2.2 to -3.0 $\log_{10}$ copies/mL compared to the vehicle-treated Group (Group 1 + 4).

Figure 6. Effects of GS-5734 administered IV on the mean plasma vRNA levels



LLOQ = lower limit of quantification (8×10^4 vRNA copies/mL); LLOD = lower limit of detection (10^3 vRNA copies/mL)

Group 7 (10 mg/kg, beginning Day 3), exhibited the most pronounced suppression of vRNA (difference vs vehicle at day 5 being -3.9 $\log_{10}$).

There werre no data available to demonstrate anti-EBOV efficacy in humans.

The sponsor has provided two case narratives of patients treated with GS-5734, a neonatal female patient born with EVD with persistent viremia and concern for Ebola CNS disease despite 4 ZMapp 150 mg infusions and a 39-year old female patient who had previously recovered from an acute phase of EVD and was readmitted to the hospital with a late complication possibly caused by reactivation of the Ebola virus. The neonatal patient was treated with a 12-day course of GS-5734 10 mg IV daily. The 39 year old patient was treated with GS-5734 for 14 days (150 mg for 2 days followed by 225 mg for 12 days). Both patients recovered and did not show treatment related adverse effects or toxicities.

2.2.4.4. Safety aspects

Only preliminary data are available from healthy subjects from the ongoing Phase I study and only two patients have received GS-5734 for the treatment of EBOV. One of these patients had late mainly neurological complications pertaining to the CNS. The data is yet too sparse to draw any conclusions of the safety profile in humans.

In preclinical studies the kidney was identified as the target organ for toxicity. Even though it cannot be excluded that GS-5734 may affect mitochondrial functions there seem to be some margin to the lowest concentration where negative effects on mitochondria occurred provided that the assumptions made regarding the projected clinically efficacious dose are correct.

The expected safety profile of GS-5734 in EBOV infected patients is unknown.

2.2.4.5. Other relevant information

The sponsor has not provided a proposal for dosing in humans for the treatment of EVD. It is however anticipated that GS-5734 will be administered for up to 14 days.

The 150 mg once daily dose, selected for further study in humans, is based on toxicology studies in cynomolgus monkey and in the rat and safety and pharmacokinetic results of the single-ascending dose study in healthy subjects indicating that GS-5734 doses of 150 and 225 mg are safe and well tolerated.

A 10 mg/kg dose of GS-5734 administered intravenously was efficacious in EBOV-infected rhesus monkeys providing protection against the lethal effects of EVD. The pharmacokinetics of a single 10 mg/kg IV dose to rhesus monkeys and a single dose of 150 mg to humans demonstrated similar systemic concentration-time exposure profiles. Therefore, the sponsor considers that a dose of 150 mg will provide drug exposures that may be efficacious in the treatment of EVD.

The sponsor states that GS-5734 is available in quantities to support the planned Phase 1 studies.

2.2.5. TKM-Ebola

2.2.5.1. Quality aspects

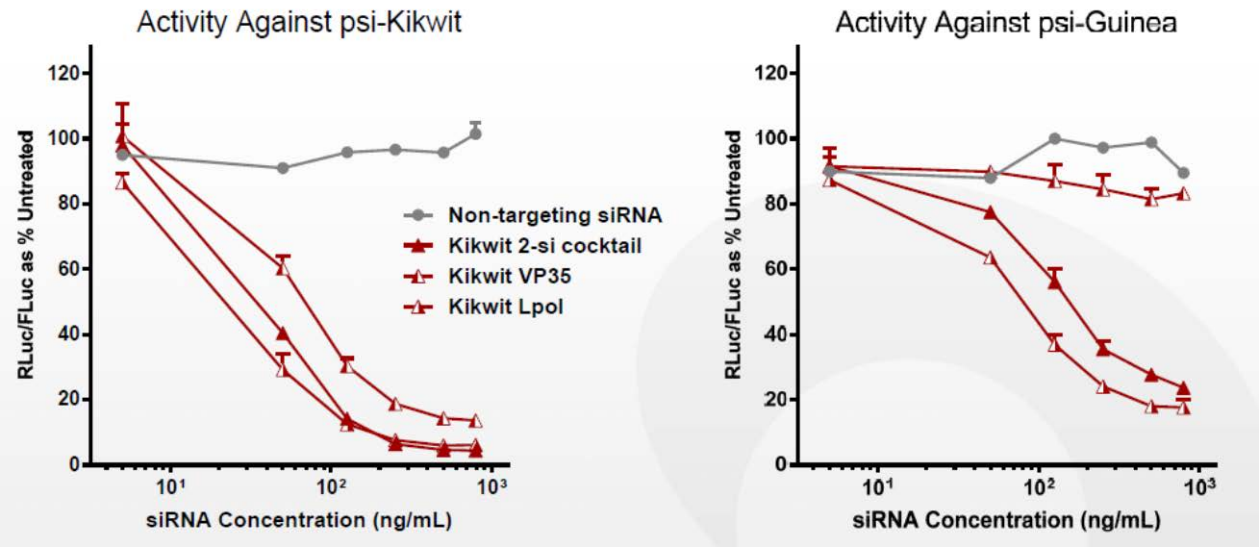
TKM-Ebola is a lipid nanoparticle (LPN) (60-120 nm) formulation containing two synthetic small interfering RNAs (siEbola-2 drug substance) designed to selectively reduce the expression of EBOV L polymerase and VP35 and thereby repress EBOV replication and pathogenesis in the infected cell.

The initially studied formulation, TKM-100802, was not optimised for use against EBOV-guinea (EBOV-g), the strain responsible for the present outbreak. The company therefore developed a new medicinal product with a modified drug substance (with changes in the oligonucleotides sequences) optimised to the 2014 isolate (TKM-130803).

2.2.5.2. Efficacy and safety 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, with the VP35 component demonstrating virtually no activity.



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 core NHP study TKM-100802 0.5 mg/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.

In humans, TKM-100802 was initially evaluated in a single ascending dose study in healthy volunteers. 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 cytokine release syndrome or infusion reaction and concern for potential prolonged hypotension or other adverse cardiac events led the U.S. Food and Drug Administration (FDA) to place further clinical development on a full clinical hold, later changed to a partial clinical hold to allow TKM-100802 to be used to treat individuals with confirmed or suspected Ebola virus infection.

The Rapid Assessment of Potential Interventions & Drugs for Ebola (RAPIDE) trial was an open-label, single arm trial conducted in West Africa to investigate the clinical efficacy of the new formulation TKM-130803, adapted to the EBOV strain responsible for the current outbreak. The primary endpoint was the mortality rate at day 14 after entry into the trial, which was compared to historical controls. TKM-130803, 0.3 mg/kg, was administered as a 2-hour intravenous infusion (IV) once daily for seven days. The trial was stopped due to futility.

Arbutus Biopharma (formerly Tekmira Pharmaceuticals) subsequently decided to suspend further clinical development of TKM-Ebola.

2.2.5.3. Other relevant information

TKM-Ebola in not available for use and is no longer being developed by Arbutus/Tekmira. Due to the sponsors decision to suspend further clinical development of TKM-Ebola the drug is no longer considered a candidate for the treatment of Ebola virus infection.

2.2.6. AVI-7537

2.2.6.1. 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. The nucleotide sequence of this synthetic oligomer targets the membrane associated VP24 gene transcript of the Ebola virus. The target sequence associated with the viral gene transcript was selected in part due to its apparent conserved nature, suggesting that this portion of the gene is highly resistant to mutation. 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.

2.2.6.2. Efficacy and safety aspects

In NHPs a dose of 40 mg/kg i.v. (treatment 1 hour after a lethal viral challenge) showed survival effects compared to control animals. Data on antiviral effects were not submitted.

The safety of AVI-7537 has been evaluated in one clinical single ascending dose study as part of the compound AVI-6002. AVI-6002 was generally well tolerated.

In general, with regard to current clinical and preclinical experience with oligonucleotide-based therapeutics, kidney toxicity may be considered as a class effect. In preclinical experiments, as expected AVI-7537 did show accumulation in the kidney and to a lesser extent in the liver.

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.

2.2.6.3. Other relevant information

At this time, no further research is planned by the sponsor for the treatment of EVD..

2.2.7. ZMapp

2.2.7.1. 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 could be made.

2.2.7.2. Pharmacokinetics

There are no human pharmacokinetic data available.

2.2.7.3. 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.

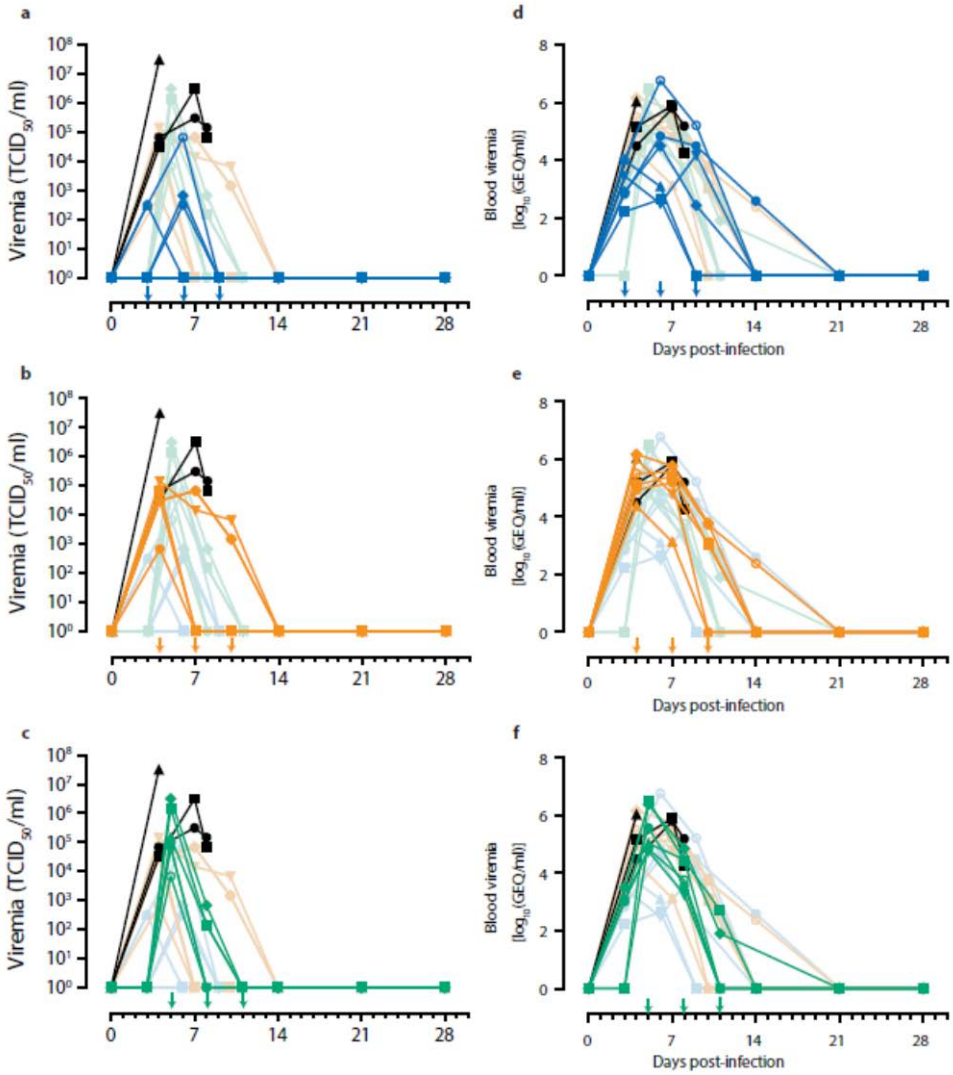
Table 4 Clinical findings of EBOV-infected NHPs from 1 to 28 dpi

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 7 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 7 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, TCID50 of Group D (blue); b, TCID50 of Group E (orange); c, TCID50 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).



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 nine patients with EBOV infection treated with ZMapp. Treatment was commenced between 5 and 16 days after the onset of symptoms. Two of the nine 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. Two patients 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. Another patient that also survived received only one dose. All patients received supplementary care, two patients also received convalescent plasma, and one of them also received brincidofovir and TKM-Ebola.

Six out of nine 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.

2.2.7.4. Safety aspects

In anticipation of potential infusion reactions, several of the patients discussed above were reported to have been pre-medicated with an antihistamine (diphenhydramine, promethazine, or chlorphenamine) prior to receiving each dose of ZMapp. Patients were treated with one to three doses of 42.5 mg/kg to 50 mg/kg administered as an intravenous infusion at three day intervals.

In eight patients, infusion-related reactions were observed after the first dose. 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.

2.2.7.5. Other relevant information

On 9 October 2015, orphan designation was granted by the European Commission for ZMapp for the treatment for Ebola virus disease.

A PK study in 16 healthy volunteers was planned for initiation in November 2015, but has been postponed until mid 2016.

In a phase 2 study sponsored by the US National Institute of Allergy and Infectious Diseases (NIAID) in West Africa and the United States (NCT02363322) has been conducted in patients that were randomized to best supportive care or the same in combination with three doses of ZMapp three days apart. No data from the study were provided to the CHMP.

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.

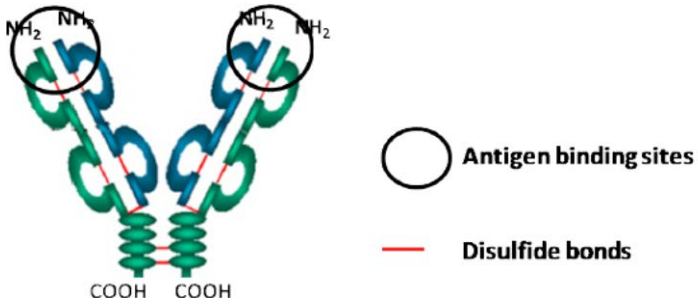
At this stage, the data are insufficient to establish any dose regimen for the treatment of EBOV infection.

At time of adoption of this report, the latest available information received from the sponsor is that ZMapp is only available for use in clinical trials.

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

Anti-Ebola F(ab')$_2$ are specific polyclonal anti-Ebola immunoglobulin F(ab')$_2$ fragments of equine origin (Figure 8).

Figure 8 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 company is developing a specific polyclonal immunoglobulin (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.

2.2.8.1. 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 only limited 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.

The selected antigen for the immunisation of horses to trigger a humoral immune response capable of neutralizing EBOV *in vivo* is an Ebola Glycoprotein (GP) based antigen. A description of the major steps of the manufacturing process has geen provided. Data presented by Fab'entech shows that hyperimmunisation of horses with Ebola Zaïre glycoprotein has generated equine plasma containing specific anti-Ebola antibodies. The 2 months post-immunization hyperimmune plasma has been used to manufacture equine IgG immunoglobulin and F(ab')$_2$ fragments.

The anti-Ebola specific activity of the equine hyperimmune plasma and the F(ab')2 fragments was semi-quantified via a direct ELISA assay developed at Fab'entech. Although no detailed information about the assay is provided, the data submitted shows significant titers of anti-Ebola virus specific activity in plasma and purified F(ab')2 fragments. Also results from studies using an *in vitro* neutralization assay in Vero E6 cells are provided. The details about the neutralizing assay are limited. Neutralization titres from plasma sampled two and three months post-immunization was however provided demonstrating higher titres after 3 months versus 2 months. No neutralization was seen in

pre immune plasma samples. Additional studies have been carried out with different Ebola virus strains in order to assess cross neutralization of the product.

2.2.8.2. Pharmacokinetics

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

2.2.8.3. 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.

2.2.8.4. 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.

2.2.9. EBOTAb

2.2.9.1. Quality aspects

EBOTAb is a purified polyclonal antibody of ovine origin raised against soluble recombinant EBOV-GP ectodomain. This is a stable recombinant glycoprotein identical to the Zaire glycoprotein but lacking the transmembrane domain.

EBOTAb is provided as a liquid solution.

The manufacturing process which has been briefly described is based on the experience with intact ovine IgG-based antivenom (EchiTAbG). The resulting product, EBOTAb, is manufactured by a process similar to that for EchiTAbG and about 10% of the resulting purified IgG is directed specifically against the glycoprotein. No step for purification of the specific immunoglobulin is in place and there is no information about the possibility of further purification of the specific immunoglobulin.

The immunogen for the primary immunization of sheep is a recombinant filovirus glycoprotein identical to the Zaire glycoprotein but lacking the transmembrane domain. The glycoprotein is expressed in a human cell line (HEK 293T cells) and includes an additional hexa-histidine tag. Additional information and characterization of the immunogen would be needed for a proper quality assessment.

Sera from sheep immunised with recombinant EBOV glycoprotein were assessed for binding titres and affinity. Results showed strong antibody responses at week 6 which increased at subsequent bleeding dates.

The ovine antisera neutralised the Zaire strain of the virus as assessed by an *in vitro* cytotoxicity assay. EBOTAb was prepared from pools of antisera and comprised intact immunoglobulins at a concentration of approximately 50 g/L.

Information about adventitious agents safety evaluation has not been provided and therefore no conclusion of the virus or TSE safety could be drawn.

2.2.9.2. Pharmacokinetics

No pharmacokinetic data have been provided

4.2.8.3 Efficacy aspects

An initial NHP study has been reported. *Macacae Fascicularis* (N=3 placebo, N=4 per treatment group) were challenged with 1000 pfu EBOV Gabon 2001 strain. Treatment with EBOTAb 309 mg (70-100 mg/kg) was initiated 24, 48 and 72 hours post challenge, in three respective treatment groups. The dosing schedule was as follows:

Actual Dosing Schedule:

Day:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hours:	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336
Group 1: Controls	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group 2: 24 hr	C	T	T	T	-	T	T	-	T	-	T	-	T	-	-
Group 3: 48 hr	C	-	T	T	-	T	T	T	-	T	-	T	-	T	-
Group 4: 72 hr	C	-	-	T	-	T	T	T	T	-	T	-	T	-	T

C Challenge
T Treat with 6 ml EBOTAb 51.5 mg/ml
- No treatment
Red highlighted text indicates dosing schedule adjustments made during study

All placebo treated animals died. Survival in the three groups, starting at 24, 48 and 72 hours post challenge, was 75%, 50% and 25% respectively. No pharmacokinetic data or data on antiviral effects have been submitted.

4.2.8.4 Safety aspects

There are no preclinical or clinical safety data available. Potential concerns are immediate or delayed hypersensitivity reactions, including systemic effects like anaphylaxis or serum sickness.

3. Overall summary

The last two years have seen the largest recorded outbreak of EVD. This epidemic has prompted intensified drug development in the field, including the first formal clinical trials in patients with EVD. The CHMP has reviewed available data for drug candidates presumed to have direct acting antiviral properties. The agents reviewed include four nucleos(t)ide analogues, two RNA-silencing oligonucleotides, one cocktail of three monoclonal antibodies and two platforms for the creation of

polyclonal sera. Within the context of this procedure, the CHMP has considered quality, preclinical and clinical data submitted by companies.

The assumption that these candidate medicinal products may potentially have clinically relevant anti-EBOV activity relied 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 (mainly in cynomolgus and rhesus macaques).

NHP models are generally considered the most appropriate animal model for the selection of drug candidates for the treatment of human EVD.

As mentioned above, despite the considerable difficulties surrounding the conduct of clinical trials in this condition, a few studies of the treatment of EVD have been performed. Interim results from a pilot study with the nucleoside analogue favipiravir conducted in Guinea were provided. The primary endpoint was mortality at day 14 after presentation, compared to historical controls. This data did not deliver clear evidence of clinical efficacy. Further, a study of the oligonucleoside TKM-Ebola, performed in West Africa was terminated early based on futility, and thus also failed to show efficacy compared to historical controls. While the reported studies are relatively small and not designed as prospective RCTs, it is notable that the efficacy noted *in vitro* and estimated via some of the preclinical models of potential agents were not translated into a measurable clinical benefit.

A randomized controlled trial comparing ZMapp, a cocktail of three monoclonal antibodies targeting the EBOV glycoprotein, with best supportive care has also been conducted in Liberia, but no results have been made available for CHMP assessment. No clinical efficacy data are available for the two platforms for the production of polyclonal sera with have been reviewed (Anti-Ebola $F(ab')_{2,}$ EBOTAb. For the other drug candidates reviewed (nucleos(t)ides BCX-4430, GS-5734 and brincidofovir; antisense oligonucleotide AVI-7534), no data from clinical trials in patients with EVD are available. It is also noted that a recently published trial failed to show an impact of convalescent plasma therapy on mortality, compared to historical controls (van Griensven et al, 2016).

In the beginning of the epidemic, many patients treated in the US or the EU received the nucleotide analogue brincidofovir under compassionate use. This was based on *in vitro* data indicating an anti-EBOV effect, bridging via animal models showing efficacy in different DNA-virus infections, and not least the fact that the drug was readily available. Subsequently, brincidofovir failed to show any efficacy in rodent EBOV models, and data have shown that the *in vitro* findings were not due to inhibition of the EBOV RNA polymerase, as preliminarily supposed.

Following the developments discussed above, the sponsors of TKM-Ebola and brincidofovir have announced that development for the treatment of EVD has been terminated. Development of AVI-7537 has also been discontinued.

At the time of writing, the outbreak is apparently over with the exception of flare-ups of cases from survivors. Outcomes from clinical trials available for CHMP assessment did not provide proof of concept of the efficacy of antiviral treatment in patients presenting with symptomatic EBOV infection. It is noted that the NHP experiments, as conducted in some of the pertinent cases, may be considered to mimic a post-exposure prophylaxis rather than a treatment situation.

Notwithstanding the absence of evidence of the clinical efficacy of antiviral therapy for EVD in the reviewed datasets, and the current epidemiological situation, drug development in the field remains important, as future outbreaks of EVD or other related diseases cannot be considered unlikely.

However, development pathways for those drugs still in development may need to be reconsidered in light of the epidemiological situation, and of the evidence that have been generated during the recent outbreak.

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