Ebola Virus Infection: a review on the pharmacokinetic and pharmacodynamic properties of drugs considered for testing in human efficacy trials

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Title

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Abstract

The 2014-2015 outbreak of Ebola virus disease (EVD) is the largest epidemic to date in terms of number of cases, of death and affected areas. In October 2015, no antiviral agents had proven an antiviral efficacy in patients. However in September 2014 WHO inventoried and regularly updated since then a list of potential drug candidates with demonstrated antiviral efficacy *in vitro* or in animal models. This includes agents belonging to various therapeutic classes, namely direct antiviral agents (favipiravir and BCX4430), combination of antibodies (ZMapp), type I interferons, RNA interference-based drugs (TKM-Ebola and AVI-7537) and anticoagulant drug (rNAPc2).

Here, we review the pharmacokinetic and pharmacodynamic information that are presently available on these drugs, using data obtained in healthy volunteers for pharmacokinetics and data obtained in human clinical trials or animal models for pharmacodynamics. Future studies evaluating these drugs in clinical trials will be critical to confirm their efficacy in humans, propose appropriate doses and evaluate the possibility of treatment combinations.

Keypoints

In response to the 2014-2015 outbreak in West Africa, WHO prioritized a list of drug candidates developed or repurposed for Ebola virus infection treatment.

Here we reported available information on pharmacokinetics and pharmacodynamics of the drugs which can be considered for clinical development or have already been tested in clinical trials in July 2015, according to WHO.

As most information was gathered from healthy volunteer and non-human primate studies, assessment of these drugs in Ebola virus infected patients will require further investigation.

1.   Introduction

1.1.   Epidemiology

Ebola virus (EBOV) was first discovered in 1976 when an outbreak of Ebola hemorrhagic fever occurred in central Africa and caused 280 deaths out of 318 confirmed cases [1]. Since then, 24 outbreaks have occurred in several African countries. The 2014-2015 outbreak initiated in Guinea before spreading to Sierra Leone, Liberia and other surrounding countries is the most severe and deadly outbreak so far with 28331 reported cases and 11310 reported deaths up to September 20[th]2015 [2], corresponding to an overall fatality rate of 40%. Depending on viral strain and available medical care, larger fatality rates up to 90% in some settings have been previously reported [3].

1.2.   Ebola virus

Genus *Ebolavirus* belongs the *Filoviridae* family, order *Mononegavirales.* It includes four EBOV species highly pathogenic in humans: *Zaire ebolavirus* (responsible for the majority of cases reported until now), *Sudan ebolavirus*, *Bundibugyo ebolavirus* and *Taï Forest ebolavirus* (formerly Cote d'Ivoire *ebolavirus*) [4,5]. EBOV is a lipid enveloped, heavily glycosylated, non-segmented negative strand RNA virus (Figure 1) [6,7]. Phylogenetic analysis indicates that the agent causing the recent outbreak in Western Africa, EBOV-Guinea, with isolated reference strains EBOV-Makona and EBOV-Gueckedou, belongs to an evolutionary lineage within the species *Zaire ebolavirus*[8].

1.3.   Natural history of the disease

EBOV is transmitted between humans by mucosae contact with infected fluid [9]. Previous studies based on seroprevalence analysis in various African populations [10] have shown that *filovirus* infections can commonly be associated with asymptomatic or mild infections and that EBOV genome could be detected in the blood of asymptomatic seroconverters exposed to documented EBOV symptomatic patients [11]. After an incubation period of 6-12 days, symptomatic patients enter an acute phase of infection during which they become highly contagious [6].First symptom onset associates fever, asthenia, myalgia and progressive gastrointestinal syndrome, including diarrhea and vomiting. This can lead to intravascular volume depletion, electrolyte perturbations, hypoperfusion,multi-organ failure including severe renal impairment and finally shock [6,12]. Lately, disseminated intravascular coagulation and blood leakage, consequence of massive cytokines release and viral replication in endothelial cells, may lead to hemorrhage syndrome, mostly represented by gastro-intestinal bleeding. However, in the current outbreak, only less than 20% of patients present bleeding [13]. In the case series of Sierra Leone, average time from reported onset of symptoms to death was 10 days, and survivingpatients were discharged after a mean illness duration of 21 days [12].

1.4. Medical care

1.4.1. Supportive care

In the absence of an approved specific treatment, current medical care primarily relies on intensive supportive care [13], in particular intravenous fluids and electrolytes solution, oral rehydration to maintain intravascular volume. Sepsis management and blood transfusion can also be considered. Treatment of other concomitant disease such as malaria is recommended along with empiric antibiotics for enteric pathogens especially at the gastrointestinal phase of the illness[13,14].

1.4.2. Convalescent plasma

The use of convalescent plasma was among the first therapeutic approaches. These plasmas, collected in patient who recovered from EBOV infection, are expected to contain polyclonal immunoglobulins targeting EBOV proteins [15]. However, the kinetics of appearance of immunoglobulins to EBOV, and more importantly that of sero-neutralizing antibodies are poorly characterized. They seem to be slower than in classical acute viral infections, probably because of the deepfunctional immunodeficiency observed during the disease. In fact albeit clinical trials have attempted to assess the efficacy of convalescent plasma, no conclusive evidence has been reported yet [15].

1.4.3. Current approaches for specific treatment

In order to accelerate and rationalize the evaluation of these putative agents, WHO issued in 2014 and has frequently updated since then a document for *Categorization and prioritization of drugs for consideration for testing or use in patients infected with Ebola*[16]. Here we review the pharmacokinetic and pharmacodynamic properties reported for the drugs categorized in class A and B in the 3 July 2015 document, which are already or can be considered for clinical trial. These drugs are antivirals (favipiravir, BCX4430), immunotherapy based on monoclonal antibodies (ZMapp), or on immunomodulation (type-I interferons (IFN)) and antisense therapy such as small interfering RNAs (TKM-Ebola) or oligonucleotides (AVI-7537). Other intervention based on drugs approved for other diseases have been proposed, but will not be developed here as there is a lack of information on their efficacy in EBOV disease.

In the following session, we report, for each drug candidate, chemical structure or composition, mechanism of action (Table 1 and Figure 2), pharmacokinetic characteristics in human or alternatively in animals (Table 2), available data on safety, *in vitro* EC50 assessment (Table 3), efficacy in non-human primates (NHP) studies (Table 4 and Figure 3) if available or alternatively in rodent. Case reports and clinical trials are described to support efficacy in EBOV infected patients.

2. Drug candidates

2.1. Favipiravir

Favipiravir (T-705) is a broad spectrum antiviral developed by Toyama Chemical Co Ltd. It has been approved in Japan and is now in phase III of clinical development in USA for the treatment of complicated or resistant flu [17]. Favipiravir is a purine nucleic acid analogue which is ribosylated and phosphorylated intracellularly into its active form, T-705RTP. This active metabolite then interferes with viral replication, probably by inhibiting the RNA-dependent RNA polymerase [18]. It was also found to increase the mutation rate of virus as observed with the influenza virus [19].

2.1.1. Pharmacokinetics& Safety

The pharmacokinetics of favipiravir was firstly characterized in Japanese healthy volunteers in several dose escalating trials with doses ranging from 30 to 2400 mg for single administration and from 800 to 1200 mg daily for repeated administration. After a single oral dose, favipiravir concentration increases to Cmax within 2 hours and then decreases rapidly with an elimination rate corresponding to a short half-life of 2-5.5h [Toyama in house documentation]. Both Tmax and half-life increase after multiple doses. Favipiravir is eliminated via metabolism, mainly by aldehyde oxidase, leading to the inactive metabolite T705M1, and marginally by xanthine oxidase. Most metabolites are excreted under hydroxylated forms via kidney. The fraction of metabolites excreted in the urine increases over time to reach 80-100% after 7 days. Favipiravir exhibits a dose- and time-dependent pharmacokinetics which is possibly due to saturation and/or auto-inhibition of the main enzymatic pathway, as favipiravir was shown to inhibit aldehyde oxidase *in vitro* [20]. During the clinical development of favipiravir in USA, a lower plasma concentration of approximately 50% has been observed in American patients as compared to Japanese patients.

The most frequent adverse events of favipiravir reported during the development for influenza treatment include mild to moderate diarrhea, asymptomatic increase of blood uric acid and transaminases and decrease of neutrophil count [20].

2.1.2. Efficacy

Favipiravir was shown to have a high activity against EBOV *in vitro*. It effectively blocks the production of infectious virus with an EC50 of 10 μg/mL in an *in vitro* experiment using Vero E6 cells and wild-type Zaire EBOV Mayinga 1976 strain [21]. A higher EC50 value of about 31 – 63 μg/mL was reported in another study, using Vero C1008 cells and EBOV E718/ EBOV Kikwit strains [22].

Preclinical data in murine models also demonstrated a strong efficacy of favipiravir against EBOV. In one study, A129 IFNα/β receptor$^{-/-}$ knockout mice were challenged by aerosol inoculation of 1000 focus-forming units (FFU) of wild-type EBOV E718 and then left untreated (N=12) or treated with

150 mg/kg BID one hour post-challenge (N=6)[22]. All mice starting treatmentat day 6 survived whereas all untreated mice died within 8 days post-challenge. In another study, C57BL/6 IFNα/β receptor$^{-/-}$ knockout mice were challenged by intranasal inoculation of 1000 FFU of Zaire 1976 EBOV and then left untreated (N=10) or treated with 150 mg/kg BID starting from day 6 (N=5) or day 8 (N=5) post-challenge. All mice receiving treatment at day 6 survived, while untreated mice and those receiving treatment at day 8 died within 10 days after infection [21]. The strong antiviral effect of favipiravir, with an average effectiveness in blocking viral production of 99.6% at steady-state was confirmed in a pharmacokinetic-viral kinetic model developed to characterize the data of the second study [23]. However the analysis revealed that time was needed to achieve this steady state, with an anti-viral effectiveness of only 49.9% and 94.6% at day 1 and 2, suggesting that favipiravir, in order to be fully effective, needs to be administered early. Studies in NHP models are ongoing but data are not yet available.

In fall 2014, at the peak of the epidemics, favipiravir was the only drug meeting the three following criteria: strong antiviral effect in animal model, good safety profile and large stocks readily available. This prompted the decision to evaluate favipiravir in a non-comparative proof-of-concept trial, in which all patients received favipiravir along with standardized care (JIKI trial) [24]. Using a modelling approach based on the pharmacokinetic data obtained in Japanese and preclinical results, a ten-day treatment with a loading dose of 6000 mg on day 1 and a maintenance dose of 2400 mg/day was used for adults [25]. These doses are larger than what is approved in Japan for complicated influenza (3200 mg on day 1, followed by 1200 mg for 4 days [20]). For children, doses were calculated related to body weight [26]. Between December 2014 and April 2015, 126 patients were included, with a mortality rate of 52.6% (excluding patients receiving also convalescent plasma, 95% confidence interval [43.1%-61.9%]), compared to 55% in the pretrial period [24]. The baseline viral load was a critical predictor of survival with a mortality rate of 20% (95% confidence interval [11.6%-32.4%]) in patients with less than 7.7 $\log_{10}$ copies/mL compared to 91% (95% confidence interval [78.8%-96.4%]) in adults with more than 7.7 $\log_{10}$ copies/mL. In patients with less than 7.7 $\log_{10}$ copies/mL, the pretrial mortality was larger and equal to 30.5 %, suggesting that an effect of favipiravir merits further study in this population. Although the absence of comparator group and the reduced number of included patients did not allow for a formal safety assessment, no signal of toxicity was reported in the JIKI trial [24].

2.2. BCX4430

BCX4430 is a broad spectrum antiviral developed by BioCryst Pharmaceuticals, originally intended to target hepatitis C virus, but subsequently developed for treatment of filovirus infections such as EBOV [27]. BCX4430 isan adenosine analogue, which is metabolized into triphosphate active form,

BCX4430-TP. This active metabolite reduces the production of viral RNA by inhibiting the RNA polymerase activity via inducing premature termination of RNA chain synthesis [27]. The drug nucleotide has high selectivity for viral RNA polymerase. No evidence was found for the incorporation of BCX4430 nucleotide into human DNA and RNA [27].

2.2.1. *Pharmacokinetics& Safety*

The pharmacokinetics of BCX4430 has been only evaluated in animal models, with dosesranging from 2 to 50 mg/kg. In rodents and cynomolgus macaques, BCX4430 concentration decreases rapidly in the plasma with a half-life of 5-10 min [27]. However, the half-life of its principal active metabolite, BCX4430-TP, in the liver in rats was substantially longer (6.2 h). High bioavailability and rapid absorption via intramuscular route was observed in animal models [27]. *In vitro* experiments showed that BCX4430 exhibited no mutagenicity, produced no detectable chromosomal aberrations in human lymphocyte. A phase I study to evaluate the safety, tolerability and pharmacokinetics of BCX4430 is ongoing [16].

2.2.2. *Efficacy*

BCX4430 exhibited a strong *in vitro* antiviral effect against EBOV with an EC50 of 3.13 µg/mL using HeLa cells and EBOV Kikwit strain [27]. The efficacy of BCX4430 against EBOV infection has been evaluated in two different NHP models [28–30]. In one study, infected cynomolgus macaques were given various doses (from 3.4 to 16 mg/kg BID) 48 hours post-challenge. The results of this study showed that BCX4430 significantly prolonged the survival time but did not improve survival rate even at the highest dose tested [28]. In another study, infected rhesus macaque monkeys were given high intramuscular doses of BCX4430 (16 mg/kg BID or 25 mg/kg BID) 30-120 minutes after virus challenge for 14 days[29,30]. At the end of the follow-up period all of six NHP receiving 25 mg/kg survived compared to four of six in the group receiving 16 mg/kg and none in the control group (N=3, all dead within 9 days). The mean peak viral load (at day 8 in all animals) was 3 $\log_{10}$ copies/mL lower in treated NHP compared to untreated NHP (6 vs 9 $\log_{10}$copies/mL, respectively) [29,30].

2.3. ZMapp

ZMapp, developed by Mapp Biopharmaceutical, is a combination of three humanized monoclonal antibodies (c13C6, c2G4 and c4G7 in equal proportion) targeting the EBOV glycoprotein [31]. ZMapp components are produced by bioengineering in *Nicotianabenthamiana*, a plant able to express pharmaceutical proteins. These antibodies were demonstrated to have large neutralizing activity *in vitro* [31], suggesting ability to link with strong affinity to viral particles, inhibiting their fusion with the target cells and enhancing their clearance. Besides, monoclonal antibodies were also thought to accelerate the elimination of infected cells expressing viral glycoprotein, through antibody-dependent cellular cytotoxicity mechanism or complement [32,33].

Another similar cocktail of three monoclonal antibodies addressing the same binding domain sequence as ZMapp, known as MIL-77, is produced by MabWorksusing mammalian Chinese Hamster Ovary (CHO) cells to obtain larger yield. Since no proof of equivalence of MIL-77 and Zmapp has been provided, WHO recommended to complete ZMapp therapeutic evaluation before considering MIL-77 [16].

2.3.1. *Pharmacokinetics& Safety*

A phase I clinical trial to assess the pharmacokinetics and safety of ZMapp is ongoing in healthy volunteers with a unique dose level of 50 mg/kg and results are planned to be released in 2016 [34]. Preliminary information on drug's safety can be obtained from seven infected repatriated patients receiving the drug as compassionate therapy. The common side effects reported during immunoglobulin infusion were fever, hypotension, tachycardia, rash, polypnea[35], which were handled using preventive antihistamine treatment and acetaminophen co-medication. One patient experienced generalized seizures, which disappeared after a temporary interruption of treatment.

2.3.2. *Efficacy*

The efficacy of monoclonal antibodies cocktails, such as MB003 and ZMab,in preventing and treating EBOV disease in rodent and NHP has been proved in several studies [36–39], with survival rates of 50-100% and 43% in rhesus macaques treated with monoclonal antibodies cocktails started at 1 day and 5 days after the challenge, respectively[36–38].

ZMapp combination was obtained by selecting the most efficient antibodies in the MB003 and ZMabcocktails[31]. The *in vitro* EC50 of the three monoclonal antibodies in ZMapp were reported between 0.1 and 1 µg/mL using Ebola-Guinea strain in veroE6 cells culture. ZMapp was then evaluated in a NHP study where 21 rhesus macaques infected with 628 pfu of Kikwik Ebola virus by IM route were left untreated (N=3) or treated with three doses of 50 mg/kg given at three-day interval. The treatment was initiated at 3, 4 or 5 days post-challenge (N=6 in each group). All the treated animals survived whereas all in the control group died within 8 days after infection. In monkeys whose treatment started on day 5 after the challenge, EBOV disease symptoms were reversed by day 7 and viral load reached the limit of quantitation by day 9 after treatment initiation.

ZMapp clinical use was restricted due to its limited supply. European Medicines Agency reported that five of seven patients who received the drug as a compassionate use at day 6 to 16 after the onset of symptom, in combination with intensive supportive care, survived [35]. Yet no imputability can be assessed from these single case observations, receiving different dosing and sometime other investigational treatments. An adaptive randomized clinical trial is ongoing in West Africa, promoted

by NIAID [40] to evaluate the efficacy of ZMapp with other potential candidate treatments as comparators, with a fixed dose of 50 mg/kg administered every 3 days.

2.4. Interferons

Interferons α and β belong to the class of type-I IFN, a family of cytokines with antiviral, antiproliferative andimmunoregulatory properties [41,42]. These cytokines are the major effectors of the innate immune response to viral infection, through host cell genes regulation. They hamper intracellular viral replication by several mechanisms, including viral mRNA degradation, inhibition of viral transcription and translation and interference with the release of viral particles. Besides, they enhance infected cells clearance by activating apoptosis mechanism and recruiting cytotoxic cells [43]. As EBOV infection is associated with a strong alteration of host immune response, started by the downregulation of type-I IFN [44,45] and massive lymphocyte apoptosis [46], IFN supplementation may help control the infection and the associated unregulated inflammatory syndrome. Several recombinant IFNs with chemical structures close to the natural type I IFNs have been commercialized (IFNα-2a, IFNα-2b, IFNβ-1a, IFNβ-1b).

2.4.1. Pharmacokinetics& Safety

Usual dose per injection range is 3 to 36 MIU three times a week for INFα and about 30-44 µg weekly for IFNβ, respectively, depending on the indication and administration route. The recombinant type-I IFNs are poorly absorbed from the gastrointestinal tract and therefore have to be given parentally [47,48]. Following an IV bolus administration, IFN concentration decreases rapidly with a terminal half-life of 4-16h for IFNα and 1-2h for IFNβ [47]. By subcutaneous route, IFN has a good bioavailability (>80%) and is rapidly absorbed, with peak serum concentrations observed after 1-8 hours and 3-15 hours for IFNα and IFNβ, respectively [47]. The terminal half-life of IFNβ is prolonged in a subcutaneous administration [49].

The type-I IFNs share a similar safety profile. The most frequently encountered side effects include influenza-like symptoms (myalgia, asthenia, fevers, fatigue and headache), neuropsychiatric consequences (depression, irritability, memory impairment), myelosuppression (neutropenia and thrombocytopenia), dermatological troubles, and the development or exacerbation of autoimmune disease, in particular thyroiditis [41,50]. These side effects were reported for long duration treatment, and may have lesser impact in short treatments for acute infection.

2.4.2. Efficacy

The antiviral activity of type-I IFN has been proved *in vitro* in VeroE6 cells, using an engineered EBOV (Zaire 76) expressing green fluorescent protein with an EC50 of <0.4 ng/mL for IFNβ and 2 ng/mL for IFNα [51].

The efficacy of IFNs monotherapy in treating EBOV infection has been evaluated in two NHP studies. The results showed that IFN given in monotherapy as post-exposure therapy had no effect on survival rates but appeared to prolong the survival time from 6 days in control group (N=2) to 7.5 days in cynomolgus monkeys receiving IFNα-2b (N=4) and from 8.3 days in control group (N=26, experiment and historical controls) to 13.8 days in monkeys treated with IFNβ (N=5) [52,53]. Peak of viral load appeared later, at day 7 post-challenge, in monkeys receiving IFNα-2b (N=4) [52] in comparison with non-treated monkeys (peak at day 5 post-challenge, N=2). In a separate study including two species of NHP infected by 1000 pfu IM of EBOV Kikwit, administration of IFNα in combination with ZMab at day 3 or 4 after the challenge improved the survival rates up to 75% in cynomolgus macaques (N=4) and 100% in rhesus macaques (N=4), compared to a survival rate of 50% in ZMab monotherapy (N=4) [39,54].

WHO mentioned an ongoing clinical trial of IFN in Guinea (not yet registered on clinicaltrial.gov at the end of September 2015) including patient with early onset of symptoms [16].

2.5. TKM-Ebola

TKM-Ebola, developed by Arbutus biopharma (formerly known as Tekmira), belongs to a new therapeutic class based on RNA interference technology. This drug is composed of two small interfering RNA, siLpol-2 and siVP35-2, whose sequences are complementary to those of EBOV viral polymerase and VP35 genes, respectively. As siRNA are very unstable, they are encapsulated and protected in lipid nanoparticles coated with polyethylene glycol molecules [35,55]. The two siRNA in TKM-Ebola silence the corresponding viral genes by inhibiting mRNA translation and enhancing host cell mediated viral mRNA destruction [56].

The initial formulation of TKM-Ebola, TKM 100-802 siEbola-2, was 100% sequence complementary to the corresponding genes of the EBOV Kikwit strain. However, these siRNA have several mismatches when compared to the gene sequences of the EBOV Guinea (Makona) strain. To address the potential loss of efficacy, Tekmira developed a new formulation TKM 100-802 siEbola-3 specifically targeting the Guinea strain[55], the major strain responsible of the outbreak in West Africa.

2.5.1. Pharmacokinetics& Safety

The pharmacokinetics of TKM-Ebola was characterized in healthy volunteers in a single escalating dose phase I clinical trial [57] with doses ranging from 0.075 to 0.5 mg/kg. The two siRNA, siLpol-2 and siVP35-2, were shown to have comparable plasma concentration time profiles, suggesting the drug PK is mostly ruled by the distribution and metabolism of lipid nanoparticles and this finding can be extrapolated to other siRNAs sequences with the same vectorization[35]. Preliminary data obtained

from 24 patients suggest a greater than dose-proportional increase in Cmax and an approximately dose-proportional increase in the AUC.

Most of the reported adverse events, fever, rigors, dizziness, chest tightness and raise heart rate can be related to transient inflammatory response, started during the first 6 hours of perfusion and disappeared within 24 hours post-infusion[16,58]. Furthermore, one severe cytokine release syndrome was diagnosed when treated with the highest dose (0.5 mg/kg). Thus, the maximal dose was limited at 0.3 mg/kg daily for the future studies.

2.5.2. *Efficacy*

The efficacy of the two components of TKM-Ebola was demonstrated *in vitro* using both Kikwit and Guinea strains on HepG2 cells, with EC50 reported between 50 and 250 ng/mL [55]. A mixture of these two siRNA and another targeting VP24 gene (2 mg/kg), was administered to two groups of rhesus macaques infected by 1000 pfu of Kikwit EBOV at 30 minutes after infection, followed by three doses given at two-day interval (N=3) or six doses given at one-day interval (N=4) for 6 days. All monkeys receiving the daily treatment survived compared to two out of three who received two-day interval treatment[59]. The two most effective siRNA, siLpol-2 and siVP35-2, were selected among this cocktail to constitute TKM 100-802 siEbola-2. In a second study, three rhesus monkeys infected with EBOV Makona strain (1000 pfu) via intramuscular route were given daily doses of 0.5 mg/kg of TKM 100-802 siEbola-3 by infusion at day 4 post-infection, when viremia and clinical symptoms had well been established [55]. All the three monkeys survived up to day 28 while all the two untreated monkeys died on day 8 and 9. Median peak viral load was also strongly reduced (1-4 $\log_{10}$ copies/mL) in the treatment group compared to the control group [55].

TKM-Ebola has been used in USA in two adult patients as compassionate treatment in combination with extensive supportive care and convalescent plasma [58]. The two patients survived despite of severe disease-related clinical and biological alteration. A phase II single arm clinical trial was conducted in Sierra Leone to evaluate the efficacy of TKM-Ebola in patients. In July 2015, Tekmira announced that a predefined statistical endpoint was reached in an intermediate analysis, indicating the trial would be discontinued due to low probability to demonstrate an overall therapeutic benefit [60].

2.6. AVI-7537

AVI-7537, developed by Sarepta Therapeutics, is a small RNA-like oligomer, with linkage to a 6-member ring, instead of the natural 5-member ribose ring of RNA and DNA [61]. This structure, called PMOplus, renders the RNA-like oligomer metabolically stable and resistant to DNAse and RNAse cleavage. The inclusion of five positive charges in AVI-7537 enhance drug's stability and its binding to the negatively charge RNA [62]. Having the same principle as other antisense therapies,

AVI-7537 targets the specific sequences of the VP24 gene of EBOV and interferes with the mRNA translation of this protein, therefore, affecting viral replication. Initially in its development, the product was part of a compound known as AVI-6002 which contained (in a 1:1 ratio) AVI-7537 and another oligomer targeting VP35 (AVI-7539) [62,63].

2.6.1. Pharmacokinetics& Safety

The pharmacokinetics and safety of AVI-7537 was assessed in a Phase I single-ascending dose study, with doses ranging from 0.005 to 4.5 mg/kg [61]. The mean Cmax and AUC values of AVI-7537 approximatively follow a dose-proportional pharmacokinetics. The half-life was about 2-5 h. Urinary excretion of intact drug accounted for no more than 44.0% of the total elimination at the highest dose. Other pathways contributing to the elimination of AVI-7537 are uncertain. The AVI-7537 renal clearance was not measurable for lower doses (≤0.05 mg/kg) and increased linearly with dose. This is likely to be due to low affinity between the PMO*plus* agent and plasma proteins, resulting in a greater filtered fraction in the kidney and the increased steady state volume of distribution observed at higher doses, which is about 400 mL/kg, compared to 100-200 ml/kg in low doses (≤0.05 mg/kg).

AVI-7537 was safe and well tolerated across the doses studied. Adverse effects associated to treatment, including gastrointestinal and nervous systems disorders, occurred in 50% of patients received AVI-6002, but were dose independent.

2.6.2. Efficacy

AVI-7537 was shown to effectively inhibit viral mRNA translation in a cell-free *in vitro* translation system using rabbit reticulocyte lysate with an EC50 of 585 nM[62].

In vivo efficacy of AVI-7537 was evaluated in several NHP studies using rhesus macaques challenged with 1000 pfu of EBOV Kikwit strain by intramuscular injection. In two proof-of-concept studies, five out of eight rhesus monkeys treated with 40 mg/kg of AVI-6002, starting at 30-60 minutes after the challenge survived whereas the untreated monkey died within 7 days [63]. A dose-escalating experiment was conducted subsequently, in which rhesus monkeys were treated 30-60 minutes after the challenge with either 4 mg/kg (N=5), 16 mg/kg (N=5), 28 mg/kg (N=5) or 40 mg/kg (N=5) of AVI-6002 or with a scramble control (PMOplus formulation which does not target the EBOV gene sequences) or placebo (N=4 and N=1, respectively) [62,63]. All monkeys in the control and scramble control groups died by day 8 after infection. A dose-dependent survival was observed in this study, with 0%, 20%, 60% and 60% survival in the groups receiving 4 mg/kg, 16 mg/kg, 28 mg/kg and 40 mg/kg, respectively[62,63]. In the last study, rhesus monkeys were given intravenously 40 mg/kg of either AVI-6002 (N=8), AVI-7537 (N=8), AVI-7539 (N=8) or saline solution (N=6) at 30-60 minutes after the challenge then once daily for 14 days [64]. The survival rates were 62.5%, 75%, 0% and 0%,

respectively, indicating that AVI-7537 alone was sufficient to confer protection from EBOV infection [64]. The peak viral loads following AVI-7537 and AVI-6002 treatments showed no significant difference but they were significantly lower than those of AVI-7539 and control groups [64].

The clinical development of AVI-7527 (AVI-6002) was pending due to funding issues. Based on the body surface, the dose of 28-30 mg/kg needed to achieve 50% survival in the NHPs was estimated to translate to 9 mg/kg of AVI-6002 or 4.5 mg/kg of AVI-7537 [61].

2.7. rNAPc2

The Recombinant Nematode Anticoagulant Protein c2 (rNAPc2), originally cloned from a parasitic nematode, *Ancylostomacaninum*(dog hookworm) [65] is a potent, long-acting anticoagulant developed by ARCA Pharma. It was shown to have no intrinsic antiviral action *in vitro* for a concentration range of 0.045–100 µg/mL. This protein, bound to the circulating coagulation Factor X, acts as an inhibitor of the complex Factor VIIa/Tissue Factor [65]. This complex physiologically enables the extrinsic pathway of the coagulation, and is widely implied in the unregulated disseminated intravascular coagulation process leading to hemorrhagic symptoms in patients infected by EBOV [66]. Therefore, rNAPc2 was though to limit the coagulopathy and associated complications (renal failure, hemorrhage, multiple organ failure) [67].

2.7.1. Pharmacokinetics& Safety

The pharmacokinetics of rNAPc2 was assessed in humans following subcutaneous or intravenous administration in three phase I clinical studies using healthy volunteers with the doses ranging from 0.3 µg/kg to 7.5 µg/kg [67,68]. rNAPc2 was shown to have a linear pharmacokinetics within the studied dose range [67,68]. As a result of a high affinity between rNAPc2 and plasma clotting factor X, rNAPc2 has a prolonged elimination half-life ($t_{1/2}$) of more than 50 hours and is distributed predominantly in the plasma compartment, leading to a small distribution volume[67,68]. The fact that rNAPc2 is closely bound to clotting Factor X in blood circulation, has similar half-life and is not detected in the urine suggests that the complex rNAPc2/Factor X may be cleared via the same elimination route of the unbound Factor X in the liver[68]. The accumulated data obtained in more than 700 patients from several phase I and II clinical studies suggest that rNAPc2 is safe and well tolerated following subcutaneous doses up to 10 µg/kg or IV dose up to 7.5 µg/kg in healthy volunteers [67–71]. Bleeding was the major side effect [69–71], but was related to invasive procedure (surgery and catheterization) or co administration with platelet aggregation inhibitors. This adverse effect can be monitored and, if occurs, can be reversed with recombinant Factor VIIa.

2.7.2. Efficacy

The efficacy of rNAPc2 has been evaluated in a NHP model using rhesus macaques challenged by 1000 pfu of Zaire 95 Ebola virus [72]. The drug was administered at the dose of 30 µg/kg daily by subcutaneous route at 10 minutes (N=6) or 24 hours (N=3) after the viral challenge, respectively. Three of the nine treated monkeys survived, whereas all the three monkeys in the control group died. The mean survival time of dead animals was significantly longer in treated monkeys (11.7 vs 8.3 days).

3.   Conclusion

The 2014-2015 outbreak has accelerated the development of various molecules for the treatment of EBOV disease. In this paper, we reviewed available pharmacokinetics/pharmacodynamics information of the most advanced therapeutic agents whose effectiveness against EBOV infection has been evaluated*in vivo* in clinical studies or in animal models.

The pharmacokinetics information reported in this review was collected only in healthy volunteers. However, EBOV disease causes dramatic alteration of vital function [6] in particular renal impairment, hepatic necrosis, blood leakage, coagulopathy, multiple organ failure. These systemic syndromes, together with therapeutic interventions such as dialysis and large volume electrolyte infusions may drastically modify drug plasma concentration [73,74]. Therefore, and in spite of the difficulties due to the absence of analytical devices on the field and to the transfer of infectious samples to BSL4 facilities, it will remain particularly important to collect frequent measurement of drug concentration in infected individuals to fully characterize the drug's pharmacokinetics in the context of EBOV infection.

For most drugs, NHP model is used to assess the *in vivo* efficacy before clinical development. However, important limitations of this model need to be kept in mind. Firstly, the infection route is systematically via intramuscular injection while it is not the common infection route in human [9]. Secondly, the inoculum (usually 1000 pfu), set to correspond to the maximal amount of virus introduced by a needle stick accident [75], is probably much larger than in most of human infections. Partlybecause of these two differences, the evolution of clinical symptoms and death in NHP models is much more rapid than in humans. In particular there is no asymptomatic infection cases,no or only short incubation period and all untreated animals succumb within 10 days, to compare with an incubation period of 2 to 21 days and a mortality rate of 40% to 90% in humans [3]. As a consequence of the short natural history of the NHP infection and of the technical constraints that limit the number of experiments, all experiments published relied on early treatment compared to what can be done in the clinical setting [6]. In addition, given the small number of animals reported in NHP

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studies, subtle differences in the experimental conditions, such as the challenge used, the supportive care provided to treated animals, the decision process to euthanize animals or the genetic differences across NHP species, can be sufficient to substantially modify the outcome of different studies. Therefore, the comparison of different NHP experiments should be done with caution, especially when they are not yet published in a peer-reviewed journal.

This review did not pretend to be exhaustive and we made the choice to present only drugs categorized in class A and B by WHO. A number of agents that have shown anti-EBOV activity *in vitro* or *in vivo* in animals were not presented in this review. Among them, we can cite brincidofovir, a broad spectrum antiviral developed by Chimerix. Its demonstrated *in vitro* efficacy against EBOV and approval for other viral infection (CMV) supported its evaluation in a clinical trial [76]. However, due to insufficient enrollment, the study was stopped and the development of brincidofovir for EBOV infection was discontinued by Chimerix. Recently, encouraging results of an antiviral developed by Gilead, GS-5734, have been reported as late breaker abstract for the annual conference of the Infectious Diseases Society of America held in October 2015 [77]. GS-5734 is a prodrug of adenine nucleotide analogue, which undergoes fast conversion to a long half-life triphosphate metabolite (>10h). GS-5734 inhibits EBOV (Kikwit and Makona strains) with a high *in vitro* efficacy (EC50 of 0.01 to 0.2 μM). Intravenous administration with a dose of 10 mg/kg initiated on day 3 led to 100% survival and a 5 $\log_{10}$ copies/mL reduction of viral load in treated monkeys compared to the placebo group [77]. First administration in patient was allowed in October 2015 for compassionate care [78]. Several new compounds or drugs approved for other indications have also been identified to have activity against EBOV *in vitro* with different mechanisms of action such as preventing viral entry [79–84] or interfering with viral replication by targeting host factors [85–87] and may warrant future *in vivo* evaluation. Likewise future developments will probably involve combination therapy with drugs having different mechanisms of action, as done for other viral infections such as HIV or HCV. For instance, the combination of ZMab and IFNα was shown to improve the survival rates in monkeys compared to ZMab monotherapy [39] and a drug trial evaluating the combination of favipiravir and ZMapp is also planned.

In severe acute infection, as many patients may already develop high viremia and be in critical conditions when treatment starts, it is crucial to rapidly achieve high level of drug exposure. Consequently, clinical development plans of these drugs should consider the need for loading doses to reach the target exposure as quickly as possible in order to maximize clinical benefits.

Modeling and simulation of pharmacokinetic data obtained could be of critical importance to support the search for an optimal dosing regimen, in particular in sanitary crisis where the need of therapeutic response may shorten the usual drug evaluation. Further, and following what has been done in other

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viral infections, such as influenza or hepatitis C virus[88,89], a better anticipation of the effect of drugs on the outcome could be obtained by developing mechanistic model of viremia. However, the use of this approach is still limited by the lack of data on the viral kinetics and other markers which may be related to treatment outcome.

Lastly, we focused here on the effect of drugs during acute infection. However some case reports have shown the presence of EBOV in semen as well as in ocular aqueous humor three months and nine weeks after the clearance of viremia, respectively[90,91]. These findings, if confirmed, suggest that antiviral therapy using drugs with high permeability to immune privileged organs may also be needed in some patients long after the disappearance of EBOV-related symptoms.

Overall vaccines remain the best way to prevent and rapidly control future outbreaks [92]. A number of vaccine candidates are currently under development, including inactivated virus, virus-like particles, DNA vaccines and recombinant viral vector-based vaccines [93]. One of the most advanced is rVSV-ZEBOV, a vaccine developed by Merck, showing promising results in an intermediate analysis of a phase III trial [94].

In summary a large number of molecules are currently tested in animals and in clinical trials. These drugs, used alone or in combination, hold the promise that significant breakthrough may be done in a near future. However for that purpose a lot of information will need to be collected to better understand the effect of these drugs on the course of the disease and optimize the search for a cure.

Compliance with Ethical Standards

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Table 1.

Drug	Chemical structure (or source)	Molecularweight	Target	Assay technique	References
Favipiravir	6-fluoro-3-hydroxy-2-pyrazinecarboxamide Purine base analogue	157.1	Viral polymerase	High Performance Liquid Chromatography (HPLC) with UV detection	[18,20]
BCX-4430	[(2S,3S,4R,5R)-2-(4-amino-5H-pyrrolo[3,2-d]pyrimidin-7-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol] Adenosine analogue	265.3	Viral polymerase	Protein-precipitation and high-performance liquid chromatography using tandem mass spectrometric detection (LC-MS/MS)	[27]
ZMapp	Association of 3 human–mouse chimaeric monoclonal antibodies (c13C6, 2G4,c4G7)	_	Viral glycoprotein	ELISA assay	[31,32]
IFNα and β	Protein, single chain of 165/166 amino-acids	17000-27000	Activator of antiviral intracellular, innate and adaptive immune response	ELISA immunometric assay	[42]
TKM-100802	Two siRNA encapsulated in lipid nanoparticles siEBOV-2: siVP35-2: GCAACTCATTGGACATCAT siLpol-2: GTACGAAGCTGTATATAAA siEBOV-3: siVP35-3: GCAATTCATTGGACATTAT siLpol-3: GTACGAAGCTGTACATAAA	_	L polymerase and viral protein 35 mRNAs	_	[55]
AVI-7537	RNA-like oligomer with 5 PMO*plus*linkages Sequence : 5' GCC +ATG GT+T TT+T TC+T C+AG G 3'	6826	Viral protein 24 mRNA	Capillary gel electrophoresis and fluorescent probe hybridization assay.	[61]
rNAPc2	Protein, single chain of 85 amino-acids	9732	Anticoagulant, inhibitor of FVIIa/Tissue factor complex	ELISA immunometric assay	[65,68]

Table 2.

Drug	Route	Time	D	C_{max}	AUC	t_{max}	$t_{1/2}$	CL/F	V_d/F	Reference
Favipiravir	oral	First day	400 mg	16.59 mg/mL	39.41 mg.h/L	[0.25-0.75] h	1.6 h	10.15L/h	23.4 L	Toyama documentation
	oral	First day	1600 mg	59.43 mg/mL	397.79 mg.h/L	[0.5-1.5] h	4.6 h	4.02 L/h	26.7 L	Toyama documentation
	oral	First day	2400 mg	92.17 mg/mL	1297.56 mg.h/L	[0.75-3] h	4.5 h	1.85 L/h	12.0 L	Toyama documentation
	oral	Steady state	400 mg BID	30.56 mg/L	193.69 mg.h/mL	[0.5-2] h	4.5 h	2.07 L/h	13.4 L	Toyama documentation
	oral	Steady state	600 mg BID	61.50 mg/L	470.53 mg.h/mL	[0.5-1.5] h	5.8 h	1.28 L/h	10.7 L	Toyama documentation
IFNα	IV	First day	36 MIU	_	_	_	[3.7-8.5] h	[0.13-0.22] L/h/kg	[0.22-0.75] L/kg	[49]
	IV	First day	5 MIU	188.2 IU/mL	208 IU.h/mL	0.5 h	1.7 h	24.04 L/h	23.6 L	[48]
	IM	First day	5 MIU	47.6 IU/mL	518.7 IU.h/mL	6.7 h	2.2 h	9.64 L/h	30.6 L	[48]
IFNβ	IV/IM/ subcutaneous	First day	[5-10] MIU/m2	_	_	[3-12] h	[2-7] h	_	_	[49]
	IM	First day	12 MIU	25.9 IU/mL	657 IU.h/mL	12.6 h	_	18.26 L/h	_	[50]
	IM	Steady state	12 MIU every two weeks	23.9 IU/mL	634 IU.h/mL	15.3 h	_	18.93 L/h	_	[50]
AVI-7537	IV	First day	1.5 mg/kg	6460 ng/mL	10100 ng.h/mL	0.5 h	2.8 h	152 mL/h/kg	406 mL/kg	[61]
	IV	First day	3.0 mg/kg	20900 ng/mL	27000 ng.h/mL	0.5 h	4.6 h	114 mL/h/kg	334 mL/kg	[61]
	IV	First day	4.5 mg/kg	24100 ng/mL	35300 ng.h/mL	0.5 h	4.0 h	126 mL/h/kg	453 mL/kg	[61]
rNAPc2	subcutaneous	First day	0.7 µg/kg	17.2 ng/mL	505 ng.h/mL	7 h	52.0 h	0.7 mL/h/kg	48 mL/kg	[68]
	subcutaneous	First day	3.5 µg/kg	80.3 ng/mL	2471 ng.h/mL	7 h	44.2 h	0.8 mL/h/kg	46 mL/kg	[68]
	subcutaneous	First day	5 µg/kg	108.8 ng/mL	3379 ng.h/mL	9 h	49.6 h	0.7 mL/h/kg	51 mL/kg	[68]
	subcutaneous	Steady state	1.5 µg/kg every 2 days	66.8 ng/mL	2441 ng.h/mL	8 h	78.9 h	0.622 mL/h/kg	70.9 mL/kg	[68]
	subcutaneous	Steady state	3 µg/kg every 2 days	116 ng/mL	4351 ng.h/mL	7 h	70.8 h	0.702 mL/h/kg	72.8 mL/kg	[68]
	subcutaneous	Steady state	5 µg/kg every 2 days	213 ng/mL	8491 ng.h/mL	12 h	71.9 h	0.591 mL/h/kg	61.5 mL/kg	[68]

Table 3.

Drug	EC50	Viral strain	Cells	Measurement method	Reference
Favipiravir (T705)	10 μg/mL	Mayinga 1976	Vero E6	reduction of viral titer (immuno-assay)	[21]
	31-63 μg/mL	Kikwit 1995/E718	Vero E6	percentage EBOV plaque reduction (cytopathiceffect)	[22]
BCX-4430	11.8 μmol/L	Kikwit 1995	HeLa	inhibition of viral replication	[27]
ZMapp	1 μg/mL (13C6)	Gueckedou 2014	Vero E6	percentage EBOV plaque reduction (cytopathic effect)	[31]
	< 0.1 μg/mL (2G4)	Gueckedou 2014	Vero E6	percentage EBOV plaque reduction (cytopathic effect)	[31]
	0.1 μg/mL (4G7)	Gueckedou 2014	Vero E6	percentage EBOV plaque reduction (cytopathic effect)	[31]
albumin-INFα	23.3 pmol/L	Engineered EBOV expressing GFP	Vero E6	reduction of viral titer (fluorescence measurement)	[51]
albumin-INFβ	< 4.7 pmol/L	Engineered EBOV expressing GFP	Vero E6	reduction of viral titer (fluorescence measurement)	[51]
TKM-100802siEbola3	50 ng/mL 50-100 ng/mL	Makona 2014 Kikwit 1995	HepG2	inhibition of viral mRNA production by high-content imaging assays	[55]
TKM-100802 siEbola2	100-250 ng/mL 1-50 ng/mL	Makona 2014 Kikwit 1995	HepG2	inhibition of viral mRNA production by high-content imaging assays	[55]
AVI-7537	585 nmol/L	_	_	Inhibition of viral mRNA translation	[62]
rNAPc2	> 100 μg/mL	Kikwit 1995	Vero E6	percentage EBOV plaque reduction (cytopathic effect)	[72]

Table 4.

Drug	Macaque species	Number of monkeys/ group	Ebola strain	Dosing and route	Treatment initiation related to viral challenge	Overall survival in experimental group	Median survival in experimental group (days)	Median survival in control group (days)	Reference
favipiravir	_	_	_	_	_	_	_	_	_
BCX-4430	Rhesus	6	_	16 mg/kg BID IM	0.5 to 4 h	66.7%	_	8	[29]
	Rhesus	6	_	25 mg/kg BID IM	0.5 to 4 h	100.0%	_	8	[29]
	Cynomolgus	6	_	16 mg/kg BID IM	2 days	0.0%	12	7	[28]
ZMapp	Rhesus	18	Kikwit 1995	50 mg/kg, 3 doses with 3 days interval IV	3, 4, 5 days	100.0%	> 25	8	[31]
INFα	Cynomolgus	4	Kikwit 1995	20 MU/kg daily IM	18 h	0.0%	7.5	6	[52]
INFβ	Rhesus	5	Kikwit 1995	10.5 µg/kg every two days subcutaneous injections	1 and 18 h	0.0%	10	8	[53]
	Rhesus	5	Kikwit 1995	35 µg/kg daily subcutaneous injections	1 h	0.0%	9	8	[53]
TKM-100802 siEbola-2	Rhesus	6	Kikwit 1995	0.2 mg/kg once daily IV	1.5 h	66.0%	_	_	[35]
	Rhesus	6	Kikwit 1995	0.5 mg/kg once daily IV	1.5 h	100.0%	_	_	[35]
	Rhesus	6	Kikwit 1995	0.5 mg/kg once daily IV	1 days	83.3%	_	_	[35]
	Rhesus	6	Kikwit 1995	0.5 mg/kg once daily IV	2 days	50.0%	_	_	[35]
	Rhesus	6	Kikwit 1995	0.5 mg/kg once daily IV	3 days	66.7%	_	_	[35]
	Rhesus	6	Kikwit 1995	0.5 mg/kg once daily IV	4 days	0.0%	_	_	[35]
TKM-100802 siEbola-3	Rhesus	3	Makona 2014	0.5 mg/kg once daily IV	3 dpi	100.0%	> 25	9	[55]
AVI-7537	Rhesus	13	Kikwit 1995	20 mg/kg once daily IV (in combination with AVI-7539 at 1:1 ratio)	1 h	61.5%	> 25	7	[63]
	Rhesus	8	Kikwit 1995	20 mg/kg once daily IV (in combination with	1 h	62.5%	> 25	8	[64]

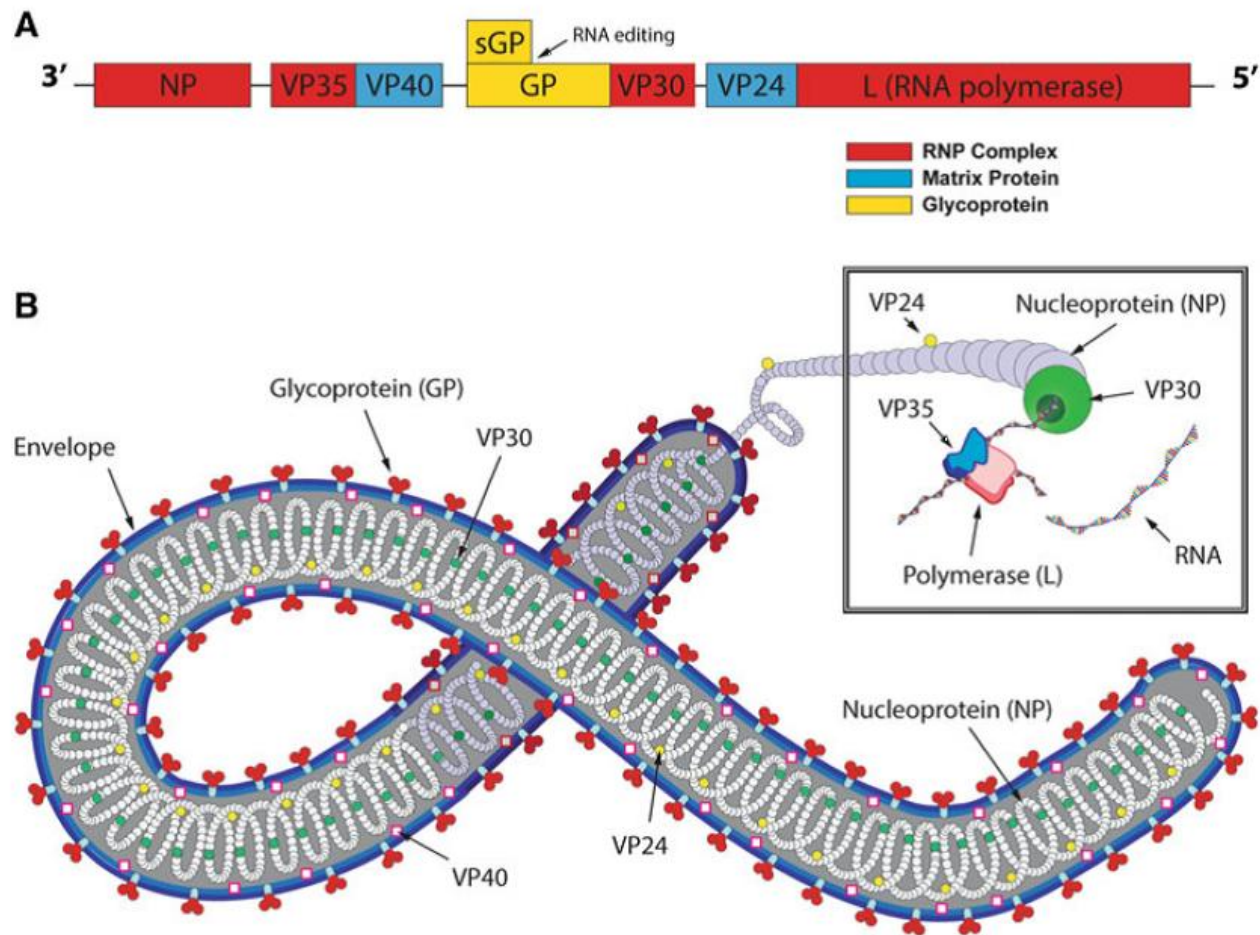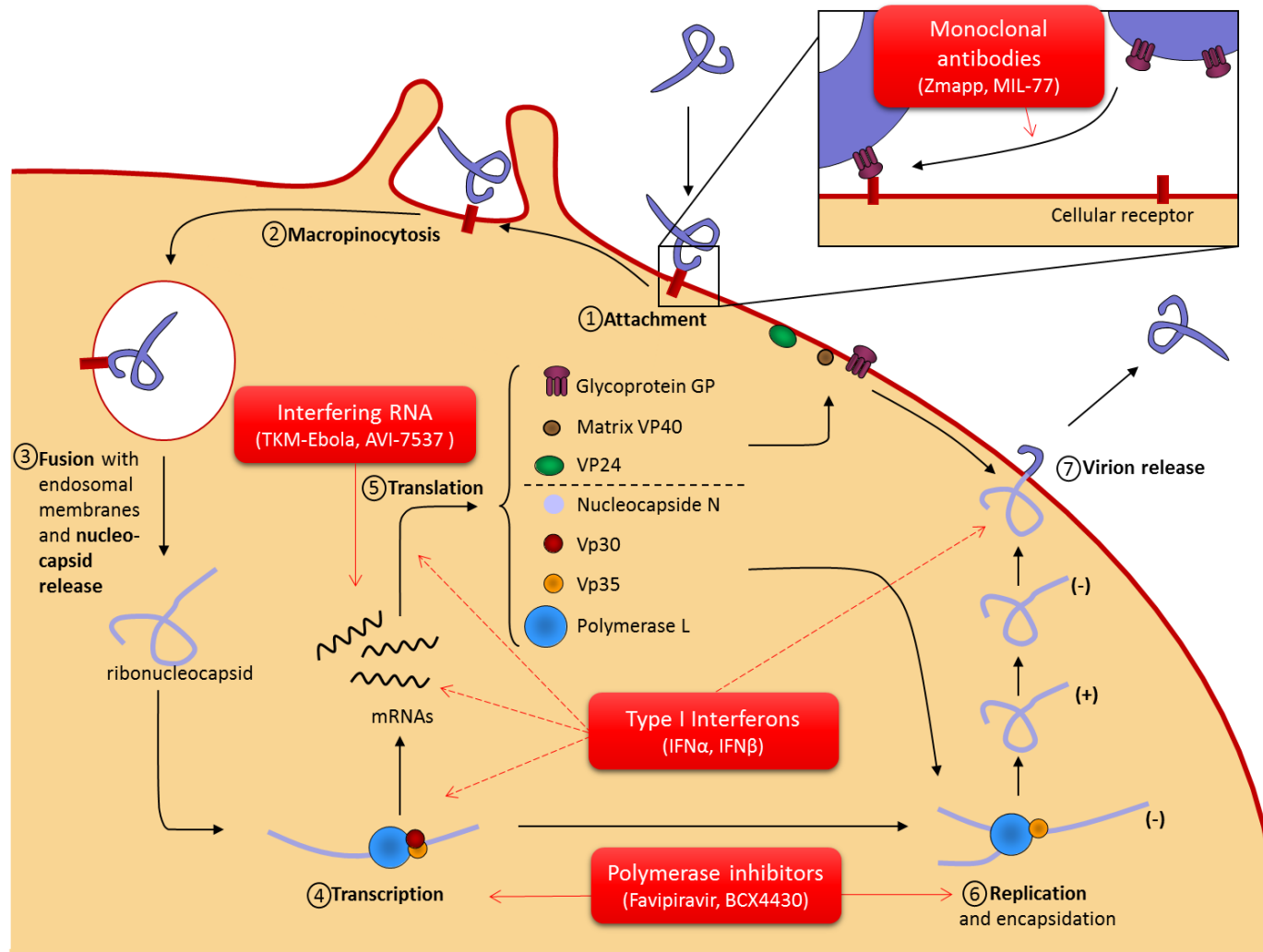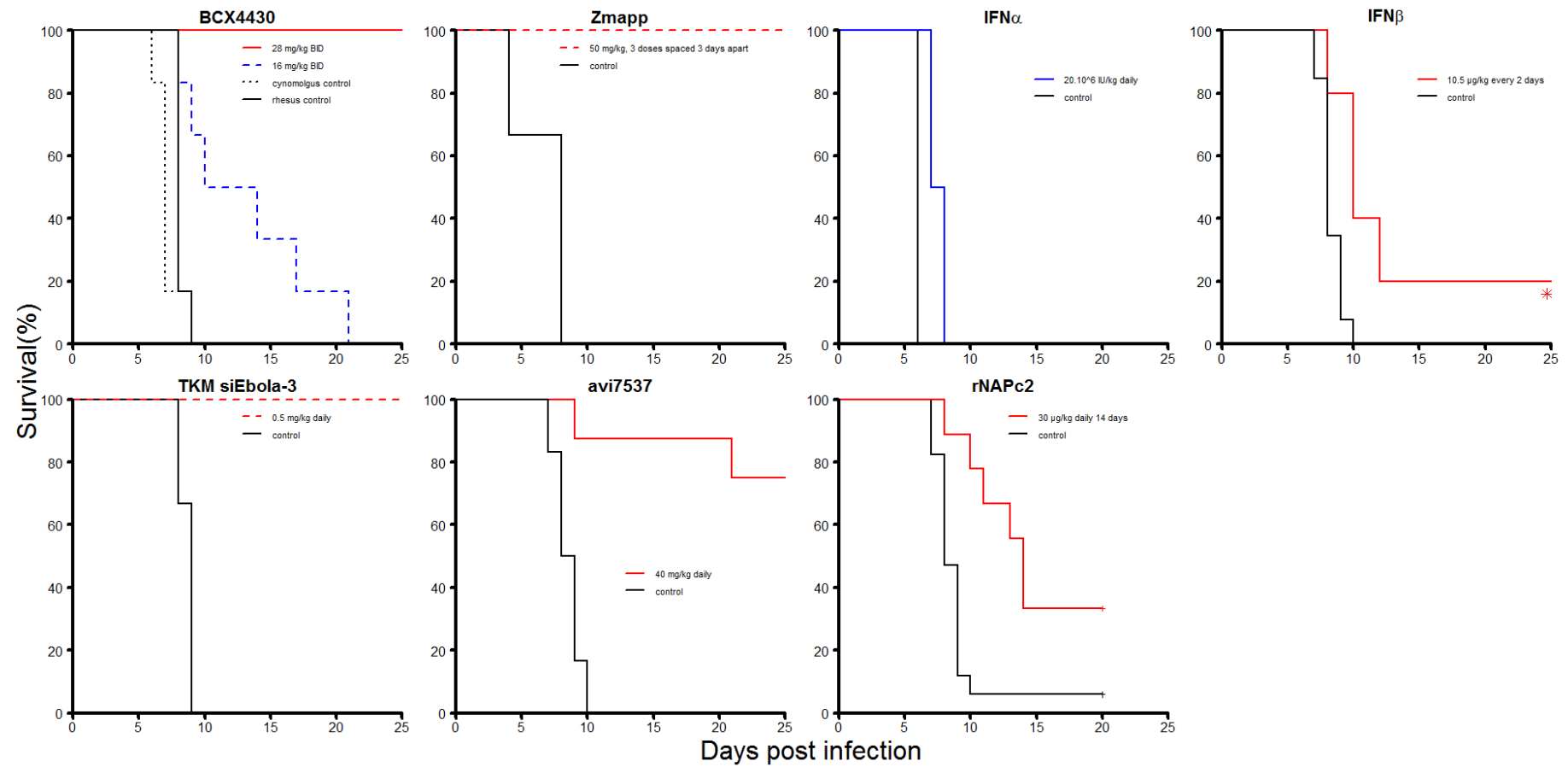|			AVI-7539 at 1:1 ratio)					
	Rhesus	8	Kikwit 1995	40 mg/kg once daily IV	1 h	75.0%	> 25	8	[64]
rNAPc2	Rhesus	9	Kikwit 1995	30 µg/kg daily subcutaneous injections	10 min or 24 h	33.3%	14	8	[72]

Figure1.

Figure2.

Figure3.

Tables and Figures captions:

Table 1. Chemical structure, molecular weight, target and assay technique of the Ebola drugs candidate*s*

Table 2. Pharmacokinetic parameters of Ebola drugs candidates obtained in healthy volunteers and calculated by non-compartmental analysis. Data were not available for BCX4430, ZMapp and TKM-Ebola. Ranges represent minimum and maximal reported value of the parameter.

Table 3. *In vitro* experiment conditions and efficacy (EC50) of the Ebola drug candidates

Table 4. Summary of survival rates obtained in nonhuman primate experiments with different Ebola drug candidates. Viral challenge was performed injecting 1000 pfu of the mentioned viral strain by intramuscular route, excepted for ZMapp study, where inoculum was 628 pfu and BCX4430 studies, where this data is not available. Survival rate in control group was 0% for the different reported studies, excepted for study [**72**], where 1 of 17 NHP survived.

Figure 1. Structure of Ebola virus. EBOV is an enveloped virus presenting a single-stranded RNA genome of nearly 19000 nucleotides, encoding seven proteins: structural nucleoprotein (NP), polymerase cofactor (VP 35), VP 40, transcription activator (VP30), VP24, RNA-dependent RNA polymerase (L) and Glycoprotein (GP). GP, also expressed in a soluble form (sGP), is responsible for host receptor binding and fusion with the cell membrane. Reproduced from Choi and Croyle. Biodrugs 2013[7].

Figure 2. Ebola viral lifecycle and targets of different therapeutic classes. Steps of virus life cycle: (1) attachment, (2) fusion with endosomal membranes, (3) nucleocapsid release, (4) mRNA transcription, (5) viral protein translation, (6) genome replication and (7) viral assembly and release. Polymerase inhibitors hamper replication and transcription processes (4)(6), directly targeting the viral polymerase L. Monoclonal antibodies (ZMapp, MIL-77) binds to viral glycoprotein and therefore inhibit viral attachment (1) but also increase virions and infected cells clearance (not represented). Interfering RNAs inhibit the viral mRNA translation process (5), and enhance viral mRNA degradation. Type I interferons have pleiotropic indirect effects through host cell genes regulation, leading to viral mRNA degradation, inhibition of viral transcription (4) and translation (5), interference with the release of viral particles(7), facilitation apoptosis of infected cells and enhancement of innate and adaptive immune response (not represented). Modified from Yazdanpanah et al, Intensive Care Medicine 2015 [13].

Figure 3. Survival of NHP infected by EBOV and treated with highest doses of candidates drugs. Data from rhesus macaques and cynomolgus macaques are in red and blue, respectively. Colored solid line stands for post exposure prophylaxis experiments (treatment initiation within 24h post challenge), colored dashed line for curative treatment (treatment initiation after 24h post challenge and black line for untreated control) + marks the end of study following. Survival plots were drawn from data reported in [28,29,31,52,53,55,64,72]using the dose where the best survival rate was observed.