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Repurposed Therapeutic Agents Targeting the Ebola Virus: A Systematic Review

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ABSTRACT

Background: The Ebola virus has been responsible for numerous outbreaks since the 1970s, with the most recent outbreak taking place between 2014 and 2016 and causing an international public health emergency. Ebola virus disease (EVD) has a high mortality rate and no approved targeted treatment exists to date. A number of established drugs are being considered as potential therapeutic agents for the treatment of EVD.

Objective: We aimed to identify potential drug repositioning candidates and to assess the scientific evidence available on their efficacy.

Methods: We conducted a systematic literature search in MEDLINE, Embase, and other relevant trial registry platforms for studies published between January 1976 and January 2017. We included drug screening, preclinical studies, and clinical studies on repurposed drugs for the treatment of EVD. The risk of bias for animal studies and nonrandomized clinical studies was assessed. The quality of reporting for case series and case reports was evaluated. Finally, we selected drugs approved by established regulatory authorities, which have positive in vitro study outcomes and at least one additional animal or clinical trial.

Results: We identified 3301 publications, of which 37 studies fulfilled our inclusion criteria. Studies were highly heterogeneous in terms of study type, methodology, and intervention. The risk of bias was high for 13 out of 14 animal studies. We selected 11 drugs with potential anti-EVD therapeutic effects and summarized their evidence.

Conclusions: Several established drugs may have therapeutic effects on EVD, but the quality and quantity of current scientific evidence is lacking. This review highlights the need for well-designed and conducted preclinical and clinical research to establish the efficacy of potential repurposed drugs against EVD.

Introduction

Since its discovery in 1976, the Ebola virus has been the pathogen responsible for an increasing number of epidemics.[1] The most widespread epidemic took place in western Africa between December 2013 and June 2016, and resulted in a total of 28,652 reported cases and 11,325 reported deaths as of April 2016.[2] The World Health Organization declared the recent epidemic a public health emergency of international concern and called for intensified efforts to develop therapeutic agents targeting the Ebola virus.[3] Although the large-scale epidemic may have ended, the emergence of sporadic new cases continues to pose a risk for future outbreaks.[4]

Ebola virus disease (EVD) is often considered a disease of poverty because it takes place in the form of sudden outbreaks amongst poor populations and under limited resources.[5] As with many other diseases of poverty, research and drug development for EVD have been neglected for many years because it is commercially unattractive for drug developers to invest significant

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resources. For example, a study from 2002 revealed that in the period between 1975 and 1999, only 13 out of 1393 new, approved drugs were specifically indicated for tropical diseases, accounting for $< 1\%$ of all new approved drugs.[6]

Nonetheless, the recent EVD outbreak led to accelerated de novo drug development efforts for EVD.[7] These efforts were promoted by an expedited approval process by regulators such as the US Food and Drug Administration (FDA) and the European Medicines Agency.[8,9] Yet, after more than 2 years since the start of the epidemic, results of many experimental drugs are considered either questionable or negative, with only 1 potential vaccine being considered a true breakthrough.[10] To date, none of the experimental drugs has been fully approved for the treatment or prevention of EVD.

Due to the urgent need for an effective and accessible EVD treatment, there were additional efforts to study approved and established drugs as potential anti-EVD therapeutic agents, a concept known as drug repurposing or drug repositioning.[11] This concept may have significant advantages in the case of EVD, which overcomes the limitations of experimental drug development. First, repurposed drugs usually have well-known safety and pharmacokinetic profiles, which leads to shorter development cycles and lower costs.[12] In addition, these drugs may often tap into an already established manufacturing and distribution network, which shortens production and delivery times in cases of rapidly spreading epidemics. Finally, depending on which repurposed drugs are being identified, they may already be marketed as generics, which is a clear advantage in countries with resource-poor health care systems.

A variety of literature reviews have been published on potential therapeutic targets for EVD, some of which also include an overview of possible candidates for drug repurposing.[13-18] However, no systematic review dedicated to repurposed therapeutic agents targeting EVD exists to date. Herein, we present a systematic review with the aim of identifying potential drug repurposing candidates and assessing the scientific evidence available on their efficacy.

Methods and Design

Protocol and registration

We undertook a systematic review based on an a priori protocol that was registered with PROSPERO (CRD42015024349) and published in a peer-reviewed journal.[19] This systematic review was reported according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) statement.[20]

Eligibility criteria

Study designs

Study designs included drug library screening studies yielding at least 1 approved therapeutic agent (including high throughput screening studies or virtual, in silico drug screens); preclinical trials (including in vitro trials and studies on animal models); clinical trials (including randomized controlled trials, controlled clinical trials, prospective and retrospective comparative cohort studies, and caseã control studies); and cross-sectional studies, case series, and case reports.

Intervention

Potential repurposed drugs selected for further assessment must fulfill the following criteria: drugs that are already approved by at least 1 regulatory authority US Food and Drug Administration (FDA), European Medicines Agency (EMA), Japan Pharmaceuticals and Medical Devices Agency (PMDA), and drugs with positive in vitro study outcomes and at least 1 additional animal or clinical trial.

Study population, timing, and setting

There were no restrictions on the type of participants in preclinical or clinical trials. In addition, there were no restrictions on the type of setting. We included studies published from 1976, the year of discovery of EVD.

Comparators

There were no restrictions on the type of comparator.

Outcomes

The primary outcomes included mortality, sequelae of the infection, and serious adverse events. Secondary outcomes include adverse events. Outcomes were collected as reported. We extracted outcomes in all data forms (eg, dichotomous and continuous) as reported in the included studies.

Languages

We included articles reported in the English, German, French, and Spanish languages.

Publication status

We included articles published in scientific journals as well as unpublished ones.

Information sources

Literature search strategies were developed using medical subject headings and text words related to EVD. We performed a systematic literature search in MEDLINE, Embase, and Cochrane Central Register of Controlled Trials.

The search was carried out on January 2, 2017, for studies published between January 1, 1976, and the date the searches were run.

To identify ongoing and unpublished studies, we searched the World Health Organization International Clinical Trials Registry Platform, ClinicalTrials.gov, and European Union Clinical Trials Register. In addition, we searched the reference lists of selected studies as well as the Websites of regulatory authorities (FDA and European Medicines Agency).

Search strategy

We developed a search strategy with the help of an information specialist (**Supplementary file 1**). The database records yielded by all search strategies were exported into EndNote Version X7.5, Clarivate Analytics, USA and duplicates were manually removed. The results of our database searches and records identified from other sources were documented and depicted in a PRISMA flow diagram.

Study selection

Before formal screening, a preliminary study screen was used by 2 authors (HS and OE) to carry out a pilot screening of 50 randomly chosen studies from the search results spreadsheet. Following the pilot screening, both authors independently screened the titles and abstracts yielded by the search against the inclusion criteria. In addition, they screened the reference lists of all selected articles. Studies selected at title and abstract levels were further screened for eligibility by assessing the full text of the article. We retrieved additional information from study authors

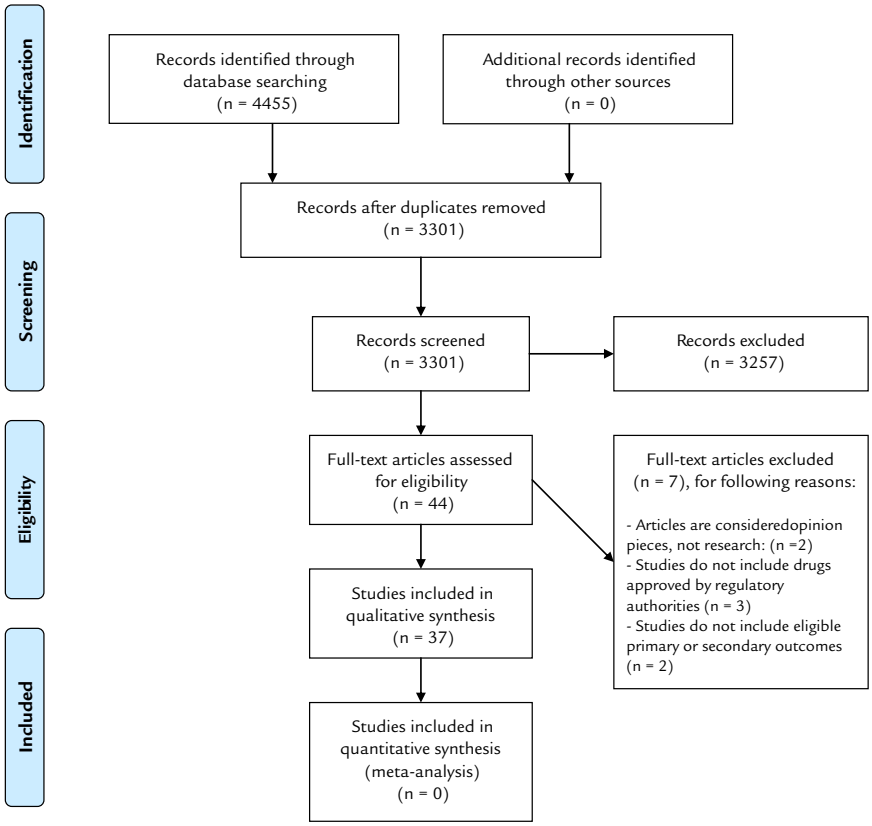

Figure 1. Preferred Reporting Items for Systematic reviews and Meta-analyses flow diagram of the study search and selection.

where necessary to resolve questions about eligibility. Opinion of a third reviewer (SKL) was sought to arrive at a consensus in case of disagreement on a study for inclusion. We documented the reasons for excluding trials at the full-text screening level. Neither of the review authors was blinded to the journal titles or to the study authors or institutions. We reported the results of the study selection process and reasons for exclusion at the full-text screening level using a PRISMA flow diagram.

Data extraction and data items

A preliminary data extraction spreadsheet was used to conduct a pilot test carried out by both authors using 5 randomly selected articles. The data extraction sheet was refined accordingly after the pilot studies. The refined spread sheet was then used by both authors to independently extract data items from all included screening, preclinical and clinical studies. Opinion of a third reviewer (SKL) was sought to arrive at a consensus in case of disagreement. Study authors were contacted for further clarification if necessary. If a selected study included items from more than 1 study type, we extracted all eligible items available. Qualitative data reported in studies was excluded from the review and thus was not extracted. However, if an included study drew conclusions based on qualitative data, we reported those conclusions separately in the characteristics of study table.

Selection of potential drugs

Following study selection and data extraction, 2 authors (HS and OE) independently selected drugs with potentially promising results based on the aforementioned drug selection criteria. The opinion of a third reviewer (SKL) was sought to arrive to a consensus in case of disagreement on a drug for inclusion.

Risk of bias in individual studies

Two authors (OE and HS) independently assessed the risk of bias of included studies. The risk of bias of in vitro studies and screen studies were not assessed due to the nonexistence of an appropriate risk of bias tool for such studies. Internal validity of preclinical studies (animal studies) was assessed using the Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool.[21] Per the instructions outlined in the Cochrane handbook for systematic reviews of interventions, the Newcastle Ottawa scale was used to assess risk of bias of nonrandomized studies. The National Heart, Lung, and Blood Instituteãs quality assessment tool for case series was used to assess the quality of reporting of case reports and case series, due to the lack of a suitable risk of bias tool for such studies.[22] Overall risk of bias was judged as low risk, unclear risk, and high risk, whereas the overall quality of reporting was judged as good, fair, or poor after comparing the individual assessments (by OE and HS).

Statistical analysis

We initially intended to conduct a quantitative analysis of primary and secondary outcomes in accordance with our protocol for systematic review.[19] However, due to the lack of an adequate number of sufficiently homogenous studies, we synthesized the study results narratively.

Results

Study selection

Our search results are presented in a PRISMA flow diagram (**Figure 1**). Our search yielded 4455 records, of which 3301

remained after the removal of duplicates. Following screening at title and abstract level, 44 studies were still included. After reviewing the full text of these studies against our inclusion criteria, 37 studies were selected. No additional studies were identified following reference searches. No additional or ongoing studies were selected from International Clinical Trials Registry Platform, ClinicalTrials.gov, European Union Clinical Trials Register, or the Websites of regulatory authorities.

Study characteristics and results

A total of 37 selected studies were classified based on principal study type into drug screening, preclinical studies, or clinical studies.[23-53] This resulted in 11 drug screening,[23-33] 17 preclinical studies,[34-50] and 9 clinical studies.[51-59]

Drug screening studies

We summarized the main characteristics of selected drug screening studies and listed all positively testing drugs with confirmed regulatory approval (**supplementary file 2**). These studies were a heterogeneous group that included 8 high-throughput screens and 3 virtual drug screens. Six of these studies introduced new drug screening methodologies and carried out pilot screens. In addition, different studies used varying methods to detect positively testing drugs. The most commonly applied technique was the measurement of luciferase activity in infected cells, a technique used in 3 studies.[23,28,31] Other applied techniques included measurement of the inhibition of enhanced green fluorescent protein expression and inhibition of beta-lactamase activity. In addition to the conduction of drug library screens, 3 studies included additional preclinical trials (in vitro and in vivo) on selected drug candidates.[24,25,27] Relevant items from these trials were extracted and analyzed together with the results from other preclinical studies.

Preclinical studies

We summarized the main characteristics of preclinical studies examining the effects of potential drug candidates on EVD (**Table I**). In addition to the data from the 17 preclinical studies selected, we also included relevant data from 3 drug screening studies,[24,25,27] which included in vitro and in vivo trials. In vitro trials were carried out on varying cell lines. The half maximal inhibitory concentration or the half maximal effective concentration (EC_{50}) of tested drugs was extracted. If unavailable, we reported the half maximal inhibitory concentration or EC_{50} values from the World Health Organization categorization and prioritization table.[60] A total of 14 studies included experiments on animals.[26,27,29,39,41-50] Three of these trials were carried out on monkey species,[39,44,50] such as the rhesus macaques, whilst the remaining 11 studies where carried on small animal models, such as mice, hamsters, or guinea pigs.

Clinical studies

We summarized the main characteristics of the 9 clinical studies selected (**Table II**). These include 3 case reports,[52-54] 1 case series,[55] and 5 nonrandomized cohort studies.[51,56-59] Convalescent blood products were included in the treatment regimen in a total of 6 studies.[52-55,57,59] Finally, the only end point extracted was the mortality rate because it was the only adequately documented end point shared by all studies.

Risk of bias

From a total of 36 selected studies, 14 animal studies,[26,27,29,39,41-50] and 9 clinical studies[51-59] were assessed for risk of bias within studies (**Supplementary file 3**). All of the animal studies were found to have a high risk of bias except 1 study with an unclear risk of bias. The high risk of bias in the animal studies was mainly due to selection bias, performance bias, and detection bias. The animal studies failed to mitigate selection bias because the test animals were selected without a proper randomization process, and thus the samples obtained were not representative of the population intended to be analyzed. The animal studies also failed to mitigate performance bias and detection bias by not blinding the investigators.

The clinical studies were single case reports or case series (4 studies)[52-55] and nonrandomized cohort studies (5 studies).[51,56-59] Of the 4 case studies, 2 studies[52,53] were judged to have poor reporting quality, 1 study[55] was judged to have fair reporting quality, and the last study[54] had good reporting quality. All the nonrandomized cohort studies[51,56-59] were found to have a low risk of bias.

Synthesis of results

Due to the significant variation of selected study types, designs, participants, and reported outcome measures, we chose to carry out a narrative synthesis of results rather than a meta-analysis. Based on our eligibility criteria, we selected a total of 11 potential therapeutic agents for the treatment of EVD for further analysis. We summarized the data extracted on each potential drug (**Table III**).

Discussion

Summary of evidence

The subsequent section discusses the evidence available on 11 selected drugs with potential therapeutic effects on EVD. It also takes additional factors into consideration, such as the proposed mechanism of action, history of antiviral activity, safety, and applicability in the setting of an EVD epidemic. Details of potential repurposed drugs for the treatment of EVD are shown in Table III.

Chloroquine

Chloroquine is a readily available, well-tolerated antimalarial agent with a long history of use in the treatment and prophylaxis of malaria. It also has well documented antiviral effects at various stages of a viral life cycle, including anti-HIV-1 activity[61] as well as inhibitory effects on the severe acute respiratory virus syndrome coronavirus.[62] In addition, studies show systemic anti-inflammatory effects of chloroquine by downregulating the production of proinflammatory factors such as interferon (IFN)-ŏ and tumor necrosis factor-ŏ.[63]

The potential activity of chloroquine on the Ebola virus may be achieved by inhibiting virus entry into host cells. This is believed to take place by inhibiting various factors such as vesicle sorting and endosome-membrane fusion, as well as increasing endosomal pH.[27] Chloroquine was shown to successfully inhibit the Ebola virus in vitro in different studies and with various cell lines.[27,36,39,46] Animal studies, on the other hand, revealed mixed results. In 2013, Madrid et al[27] carried out an animal trial in which 8 out of 10 mice survived Ebola infection when treated with chloroquine twice daily at 90 mg/kg IP. In another study, intraperitoneal treatment with chloroquine at the same dose showed no significant protection and high toxicity in a mouse and hamster model.[46] In 2015, Madrid et al[48] were able to reproduce positive results upon repeat testing of chloroquine on Ebola-infected mice under identical conditions (90 mg/kg IP twice daily). Chloroquine administered orally at 40 mg/kg once daily showed no increase in survival.[48] Our search did not yield any clinical trials for chloroquine.

Table I
Characteristics of preclinical studies.

Author Year Country	Study design and candidates tested	Cell lines studied (in vitro)	Ebola virus strain studied	Animal model characteristics	Relevant results
Subramanian et al[34] 2008 United States	In vitro tests of varying doses of albumin-IFN-α or albumin-IFN-β on Ebola virus replication	Vero cells	Zaire 76	Not applicable	Tests showed marked inhibition of viral replication even with low concentrations of albumin-IFN-α and albumin-IFN-β
Salata et al[35] 2015 Italy/ Sweden	In vitro tests of amiodarone and its metabolite MDEA on the inhibition of the Ebola virus	Vero cells	pVR-1012-ZEBOVGP Plasmid	Not applicable	Study reveals inhibitory effects of amiodarone and its metabolite MDEA on Ebola virus infection
Long et al[36] 2015 United Kingdom	In vitro tests of chloroquine, bafilomycin A1, omeprazole, and esomeprazole on the inhibition of Ebola pseudovirus	HEK-293T cells, A549 cells	EBOV-B GP (FJ217161) Plasmid	Not applicable	Chloroquine, omeprazole, and esomeprazole showed inhibitory effects on pseudotyped Ebola virus
Hensley et al[37] 2015 United States	In vitro tests of lamivudine on different cell lines and different Ebola prototypes, using toremiphene as a positive control	Vero E6, HEP G2 cells, human monocyte-derived macrophages	Homo sapiens-tc/COD/1995/ Kikwit (EBOV/Kik)	Not applicable	No direct inhibition of Ebola RNA polymerase or replication through lamivudine
Gehring et al[38] 2014 Germany	In vitro tests of amiodarone, haloperidol, verapamil, dronedarone, and other established drugs on the Ebola virus	Vero E6	Mayinga strain of Ebola virus	Not applicable	Amiodarone, dronedarone, and verapamil were shown to inhibit filoviral cell entry
Dowall et al[39] 2015 United Kingdom	In vitro and animal studies of chloroquine on Ebola-infected cells/animals	MRC-5 cells	Strain ME718, 1976/ Yambuku-Ecran	12 guinea pigs; 6 received 33.75 mg/kg chloroquine BID and 6 were control	Positive in vitro results but negative animal study outcome (significantly poorer outcome for treated animals compared with nontreated ones)
McCarthy et al[40] 2016 Canada	In vitro tests of IFN-α, IFN-ß, lamivudine, favipiravir, and other drugs as monotherapies or in combination in inhibiting viral replication	293-T-cells	trVLP related to Zaire Ebola virus	Not applicable	IFN-α and IFN-ß monotherapy inhibited viral replication in vitro. Authors also identified drug combinations with positive in vitro inhibition
Smith et al[41] 2013 United States	Animal studies of human recombinant IFN-β, and an experimental IFN-α- antibody	Not applicable	Ebola virus-Z strain	Small number of rhesus macaques divided to 4 groups (2 Ebola virus test groups, 1 MARV test group, 1 control group)	Recombinant IFN-β significantly prolonged survival of rhesus macaques infected with a lethal dose of Ebola virus, but no change to mortality
Smither et al[42] 2014 United Kingdom	In vitro and animal studies on the efficacy of favipiravir in the treatment of Ebola virus in vitro and murine model	Vero C1008 cells	EBOV E718 and EBOV Kikwit	3 groups of 6 IFN α/β receptor deficient female mice	Positive in vitro inhibition and 100% protection against aerosol Ebola infection in mice
Oestereich et al[43] 2014 Germany	In vitro and in vivo testing of the efficacy of favipiravir against Ebola virus	Vero E6	Zaire EBOV Mayinga 1976 strain	Groups of 5-10 mice underwent nasal inoculation with Ebola virus	Positive in vitro inhibition and100% protection of all infected mice
Herbert et al[44] 2015 United States	Relevant part of study tests efficacy of imipramine and other experimental compounds in vitro and in vivo	Murine peritoneal macrophages, human macrophages	Recombinant EBOV and EBOV GP/ rVSV	3 groups of wild-type mice: Experimental treatment group, imipramine group, and a control group	In vitro, imipramine significantly inhibited Ebola virus replication. In vivo, no significant protection or delay of death from imipramine treatment (at 20 mg/kg IP daily or every other day)
Jahrling et al[45] 1999 United States	Relevant part of the efficacy study investigates treatment of cynomolgus monkeys infected with Ebola virus with recombinant IFN-α2b	Not applicable	Zaire EBOV	4 cynomolgus monkeys in test group (receiving high dose IFN α2b) and 2 in control group	A slight delay of death and delay of development of viremia was noticed for the test group. None of the animals survived
Falzarano et al[46] 2015 United States	In vitro and in vivo testing of the activity of chloroquine against Ebola virus	Vero E6	Mouse-adapted Ebola virus	BALB/c mice or Syrian hamsters divided to 4 groups: Treatment, vehicle, mock infected, and combination treatment with chloroquine, doxycycline, and azithromycin	Chloroquine inhibited EBOV replication in vitro, but only at cytotoxic doses. It showed no efficacy and high toxicity for chloroquine in mice or hamsters. No combination-treated or vehicle treated animal survived

Rhein et al[47] 2015 United States	In vitro testing of IFN-γ on infected macrophage cells followed by in vivo testing on infected mice	Murine peritoneal macrophages	Ebola virus GP/rVSV	IFN-γ treated vs untreated BALB/c mice with lethal dose of MA-EBOV	In vitro, IFN-γ inhibited Ebola virus infection of macrophages. In vivo, IFN-γ administered 24 h before or after infection significantly reduced mortality, morbidity, and serum viral titers of lethally challenged mice compared with control
Johansen et al[24] 2013 United States	In vitro tests and animal studies of a subgroup of drugs from the screening study carried out	Vero E6, human HepG2 cells, HUVECs	eGFP-EBOV (in vitro), EBOV/Kik, EBOV/May, Sudan EBOV	Female C57BL/6 mice were challenged with Ebola virus and were treated after 1 h with drug or vehicle for 6 sessions	Clomiphene and toremiphene were identified to have inhibitory effects in vitro and protective effects in murine models
Johansen et al[25] 2015 United States	In vitro and in vivo testing of 30 subselected approved drugs that tested positively in a high throughput screen	Vero E6, human HepG2 cells	eGFP-EBOV (in vitro), and ma-EBOV (in vivo	Female C57BL/6 mice were challenged with Ebola virus and were treated after 1 h with drug or vehicle for 10 d	25 out of 30 subselected approved drugs inhibited Ebola virus-VLP entry in vitro by more than 90%. Only clomipramine, sertraline, bepridil, toremiphene, and clomiphene showed survival benefit
Madrid et al[27] 2013 United States	In vitro tests and in vivo, murine Ebola virus infection model testing of prochlorperazine, chloroquine, and other established drugs	Vero cells	Zaire Ebola virus	C57BL/6 and Balb/c mice were given test drug once daily PO for 7 d from day of virus inoculation	Chloroquine disrupted entry and replication of 2 or more viruses (including Ebola virus) in vitro and protected mice against Ebola virus challenge
Madrid et al[48] 2016 United States	In vitro tests and in vivo efficacy testing on selected drug candidates (azithromycin, amodiaquine, chloroquine, amiodarone, clomiphene, prochlorperazine, benztropine, and chlorotetracycline)	Vero cells	Zaire Ebola virus	Each drug was tested in 10 Balb/c female mice, administered once or twice daily for 7 d starting on day of infection, via oral or intraperitoneal route	Azithromycin (100 mg/kg, twice daily IP), chloroquine (90 mg/kg, twice daily IP), and amiodarone (90 mg/kg, twice daily IP) showed significant increases in survival in murine model. Significant efficacy was only reproducible with chloroquine. Studies of azithromycin and chloroquine in a guinea pig model revealed no improved survival
Cong et al[49] 2016 United States	In vitro and animal studies evaluating the activity of lamivudine and zidovudine against the Ebola virus	Vero E6, Huh 7, HeLa, Hep G2, 293T cells, and primary MDMs	EBOV/Kik and GPA-EBOV/ May	Treatment with lamivudine 20 mg/kg PO once daily (n = 6), or water (n = 7) as mock control. Treatment was started on Day 3 before intraperitoneal challenge and continued to Day 9 postchallenge	Lamivudine and zidovudine had no in vitro detectable antiviral activity against Ebola virus/Kik in any cell line infected. In addition, lamivudine was not protective against Ebola virus in a guinea pig model (6 out of 6 treated guinea pigs died, compared with 6 out of 7 animals in the control group)
Mire et al[50] 2016 Canada	Animal tests of convalescent ZEBOV und SEBOV sera on rhesus monkeys	Not applicable	ZEBOV-Makona	After onset of viremia, 4 monkeys were treated with ZEBOV-Makona convalescent macaque sera, 3 monkeys were treated in parallel with SEBOV convalescent macaque sera, and 2 monkeys were controls	8 out 9 monkeys died because of the Ebola virus infection. Results show no protection by the treatment with convalescent serum after onset of viremia

eGFP = enhanced green fluorescent protein (eGFP); GP/rVSV = glycoprotein/recombinant vesicular stomatitis virus; HUVECs = human umbilical vein endothelial cells; IFN = interferon; MA-EBOV = mouse-adapted Ebola virus; MARV, Marburg-Virus; MDEA = mono-N-desethylamiodarone; MDMs = monocyte-derived macrophages; SEBOV = Sudan Ebola virus; trVLP = Transcription- and replication-competent virus-like particle; VLP = Virus-like particle; ZEBOV = Zaire Ebola virus.

Table II
Characteristics of clinical studies.

Author Year Country	Study design and comments of interest	Population size Gender	Drug tested, dosage	Treatment duration	Comparator	Mortality
Sissoko et al[51] 2016 France/ Guinea	Multicenter, nonrandomized, historically controlled JIKI Phase II trial conducted on EVD patients in Guinea	126 included, 111 analyzed, results of 99 adults published 64% female	Favipiravir, Day 0: 6000 mg; from Day 1 1200 mg BID	9 d from Day 0	No direct control group, comparator is the 3-mo pretrial mortality rate at the same centers	Mortality was 20% for baseline cycle threshold $\geq$ 20 (95% CI, 11.6% to 32.4%) compared with 30% in pretrial population. Mortality was 91% (95% CI, 78.8% to 91.1%) for cycle threshold $<$ 20, compared with 85% in pretrial population
Bai et al[58] 2016 Sierra Leona/ China	Retrospective case series for the treatment of patients with favipiravir compared with control group	39 patients in treatment group, 85 control group	Favipiravir 800 BID on Day 1, then 600 mg BID on Day 2, then at least 5 d of standard therapy	3-11 d or until discharge	85 patients receiving standard care	Higher survival rate for treatment group compared with control (56.4% vs 35.3%; $P = 0.027$). All treated patients had medium to high viral load
Borobia et al[52] 2015 Spain	Case report on a single case of Ebola infection in Spain treated with a combination of convalescent plasma and favipiravir	1 patient	Convalescent plasma, favipiravir (loading dose of 50 mg/kg BID, maintenance dose of 25 mg/kg TID)	11 d from Day 9	None	Patient survived
Nicholson-Roberts et al[53] 2015 United Kingdom/ Sierra Leone	Case report on management of a severe case of EVD with supportive care and convalescent whole blood	1 male patient	Convalescent whole blood (500 mL over 2 d)	2 d	None	Patient survived
Florescu et al[54] 2015 United States	Case report on the management of a case of EVD with supportive care, convalescent plasma, and an experimental drug brincidofovir	1 male patient	200 mg brincidofovir PO on Day 6, followed by 100 mg doses on Day 9, 13, and 16. On Day 8, 3 U convalescent plasma were given	Convalescent plasma: 1 d	None	Patient survived
Mupapa et al[55] 1999 Democratic Republic of Congo	Observational study on 8 patients with confirmed EVD receiving treatment with convalescent blood transfusions	8 female patients	Convalescent blood, variable quantity	Variable duration	No direct control, overall case fatality rate of the Ebola epidemic in Kikwit	2 out 9 patients died (12.5% Mortality compared with 80% overall case fatality rate)
Gignoux et al[56] 2016 Liberia/ France	Retrospective analysis of relative risk of mortality for treatment with 2 different antimalarial drugs, using adjusted and unadjusted regression models	381 in total, divided into 4 groups	Artesunate-amodiaquine (dose according to age)	3-d course	Group receiving artemether-lumefantrine	50.7% (36 out of 71) mortality rate for artesunate-amodiaquine group compared to 64.4% (125 out of 194) for the artemether-lumefantrine group
Van Griensven et al[57] 2016 Guinea/ Belgium	Nonrandomized, comparative trial including 84 patients treated with up to 500 mL convalescent plasma with Unknown levels of neutralizing antibodies	84 patients in treatment group 57% female	2 U ABO-compatible convalescent plasma, 200ã 250 mL each	Transfusion on day of diagnosis or up to 2 d later	418 patients in control group treated during previous 5 mo	31% mortality in the treatment group and 38% for control (not significant). The difference was reduced after adjustment for age and cycle-threshold value (adjusted risk difference, -3 percentage points; 95% CI, -13 to 8). No serious adverse reactions associated with the use of convalescent plasma were observed
Sahr et al[59] 2016 Sierra Leone	Nonrandomized, controlled case series including 69 EVD patients to assess treatment of convalescent whole blood	69 in total, 44 in treatment group, 25 in control group	1 U ABO-compatible convalescent whole blood (450 mL)	Transfusion within 24 h of admission	25 patients who chose not to receive convalescent blood treatment	27.9% mortality rate in the treatment group compared with 44% for control group (not significant). Significant drop in viral load 24 h after transfusion

EVD = Ebola virus disease; JIKI = efficacy of favipiravir against Ebola.

Table III
Characteristics of potential therapeutic agents targeting Ebola virus.

Drug name	Drug type Current application	Preclinical or clinical evidence on Ebola virus treatment	Proposed mechanism of action
Toremiphene	SSRI Approved for breast cancer treatment	In vitro: More than 90% inhibition (EC_{50} = 0.57 ö¥M) Mice: Intraperitoneal 50% survival of EBOV challenge (P = 0.0441)	Late-stage virus entry inhibition, likely involving the triggering of viral fusion
Amodiaquine	Approved antimalarial drug	In vitro: Positive inhibition in drug screens, (EC_{50} = 2 ö¥M) Mice: No increased survival at 60 mg/kg, twice daily for 7 d Humans: Retrospective analysis showed significantly lower mortality (50.7%) for amodiaquine compared with lumefantrine (64.4%)	Similar to chloroquine, Inhibition expected at the stage of viral entry into host cells, potentially through interfering with factors mediating virus cellular entry
Chloroquine	Approved antimalarial	In vitro: Positive inhibition, (EC_{50} = 16 ö¥M) Guinea pigs: In 1 study, no increased survival or time to death at a range of doses. In a second study, chloroquine group showed poorer results than control group. Mice: Mixed results; in one study, no protection and high toxicity of mice at 90 mg/kg IP. In another study: protection at 90 mg/kg PO was reproduced once upon repeat testing	Inhibition of Ebola virus entry into host cells, potentially through interfering with factors mediating virus cellular entry, such as endosome pH, vesicle sorting, and endosome-membrane fusion initiation
Amiodarone	Antiarrhythmic agent approved for treatment of cardiac arrhythmias	In vitro: Inhibition of Ebola virus (EC_{50} = 1.4â 7.6 ö¥M) Mice: No survival benefit at 60 mg/kg; 0%â 40% at 90 mg/kg dose Humans: Compassionate use on 65 patients in Sierra Leone with a mortality of 40%, compared with 50% for treatment unit population, unknown statistical significance	Indirect inhibition by inducing cell structure changes, resembling an NPC-like phenotype. Direct inhibition of virus entry at the stage of viral fusion
Clomiphene	SERM, approved for female fertility treatment	In vitro: More than 90% inhibition (EC_{50} = 2.2 ö¥M) Mice: Intraperitoneal, 90% survival of EBOV challenge (P < 0.0001). In a second study, no survival benefit shown Humans: Used in combination treatment (together with irbesartan and atorvastatin) for some patients	Late stage virus entry inhibition, likely involving the triggering of fusion
Sertraline	SSRI approved for treatment of depression	In vitro: More than 90% inhibition (EC_{50} = 1.15 ö¥M) Mice: 7 out of 10 survival of treatment group (P = 0.0019)	Inhibition likely at steps late in the viral entry pathway, close to NPC1-dependent viral fusion. Studies indicate broad filovirus inhibition
Bepridil	Calcium channel blocker approved for treatment of angina pectoris	In vitro: More than 90% inhibition (IC_{50} = 4.54 ö¥M) Mice: 10 out of 10 survival of treatment group (P < 0.0001)	Inhibition likely at steps late in the viral entry pathway, close to NPC1-dependent viral fusion. Studies indicate broad filovirus inhibition
Favipiravir	Antiviral drug approved in Japan for treatment of Influenza virus infection	In vitro: Inhibition of Ebola virus (IC_{50} = 64 ö¥M) Mice: 100% protection at 300 mg/kg/d in 2 independent studies Humans: Results of efficacy trial in Guinea indicate no improved survival for patients with cycle threshold > 20. For patients with a cycle threshold < 20, results suggest higher survival for treatment group. A second retrospective study in Sierra Leone showed significant improvement in survival for patients with medium to high viral load	Favipiravir is an oral nucleotide analog. It is converted by host enzymes into its active metabolite, which inhibits the viral RNA polymerase. After incorporation into viral RNA, it causes lethal mutagenesis
Interferons ǒ, ǒ2b, ö, and ǒ	Immune modulators approved for treatment of hepatitis C and autoimmune disorders	In vitro: IFN-ǒ inhibited Ebola virus infection of macrophages, albumin-IFN-ǒ, albumin-IFN-ö inhibited Ebola virus replication Mice: IFN-ǒ administered 24 h before or after infection significantly reduced mortality of lethally-challenged mice and reduced morbidity Monkeys: IFN- ǒ2b treated monkeys showed slight delay of death and delay of development of viremia, none survived. In a different study, IFN-ö prolonged survival but did not change mortality	Activity involves enhancing host defenses (eg, by activating macrophages into a M1-phenotype, which is antiviral and proinflammatory)
Convalescent blood	Donation and use of human blood products is approved and applied worldwide. Indications include anemia	Monkeys: No improved survival for rhesus macaques treated with convalescent serum from macaque convalescent sera Humans: In 2 nonrandomized comparative/controlled studies, treatment of patients with up to 500 mL convalescent plasma or whole blood did not significantly improve survival Other studies include 1 case report (1 patient, survived) and 1 observational study (9 patients; 87.5% survival). An additional case report describes use of convalescent serum as well as favipiravir	Inhibition via EBOV neutralizing antibodies present in convalescent plasma
Azithromycin	Antibiotic approved for treatment of many bacterial infections	In vitro: inhibition of Ebola virus (EC_{50} = 2.79) Mice: Treatment with 100 mg/kg azithromycin IP twice daily resulted in 10%-60% overall survival. 0% survival of orally treated mice	Remains to be determined

EBOV = Ebola virus; EC_{50} = half maximal effective concentration; IC_{50} = half maximal inhibitory concentration; IFN = interferon; NPC1 = Niemann-Pick disease, type C1 membrane protein; SERM = selective estrogen receptor modulators; SSRI = selective serotonin reuptake inhibitor.

The mixed results provided by animal studies may be explained by the range of doses tested. With an EC_{50} of 16 ö¥M, higher doses may be necessary to produce consistently positive results. This may lead to poor outcome due to an increase in drug related toxicity. Furthermore, chloroquine may be more effective if given prophylactically due to its activity during the early stages of a viral cycle. Hence, additional animal studies for dose finding are recommendable before clinical trials.

Amodiaquine

Amodiaquine is an antimalarial agent structurally related to chloroquine and widely used in Africa. In 1 in vitro study,[27] amodiaquine was shown to inhibit the Ebola virus as well the Marburg virus more potently than chloroquine, as demonstrated with lower EC_{50} values. However, in the only animal study yielded by our search, mice treated with 60 mg/kg IP amodiaquine twice daily for 7 days showed no survival benefit.[46]

In a retrospective observational study of patients with EVD, a switch from an antimalarial containing lumefantrine to amodiaquine was associated with a significant decrease in case fatality rates at Mûdecins Sans Frontiûres-led EVD treatment units.[56] It remains unknown whether the improved survival rate was due to the antiviral activity of amodiaquine or possible toxicity of lumefantrine. Another limitation of this study was unmeasured patient characteristics (ie, confounding variables) that could have influenced the mortality rate.

Toremiphene and clomiphene

Toremiphene and clomiphene are well-established selective estrogen reuptake modulators approved for the treatment of breast cancer and infertility, respectively. Both drugs were able to inhibit Ebola virus entry in vitro by more than 90%. This was likely the result of late-stage entry inhibition that affects the triggering of fusion.[27]

In a mouse model, 9 out of 10 animals treated with 60 mg/kg IP clomiphene survived a lethal dose of Ebola virus. For the toremiphene treatment group, 5 out of 10 mice treated with the drug 60 mg/kg survived. Two control groups showed 0% survival rates.[27] In another animal study, mice treated with 60 mg/kg IP clomiphene twice daily showed no survival benefit compared with a control group.[48] Additional animal trials at different doses are essential to confirm the survival benefit association with these drugs.

There are certain concerns that limit the practicality of use of toremiphene and clomiphene as EVD therapeutic agents. Higher doses than the standard clinical range may be necessary to achieve a therapeutic effect on EVD, which would increase the risk of serious side effects. These include ocular adverse effects for clomiphene and serious electrolyte derangements for toremiphene. These drugs may therefore be better suited as candidates for combination treatments.

IFNs

IFNs are signaling proteins that are produced and secreted by host cells in response to pathogens or tumor cells. They are currently approved for the treatment of hepatitis C[64] as well as certain autoimmune disorders like multiple sclerosis.[65] During recent viral outbreaks, IFNs have been suggested as a potential treatment for emerging viral infections, such as the severe acute respiratory syndrome coronavirus.[66] The antiviral activity of IFNs appears to occur via the induction of IFNs-induced transmembrane proteins, which inhibit a broad range of viruses at different stages of the virus life cycle, including viral entry and fusion.[67,68]

The potential use of IFNs in the treatment of EVD has been assessed by several studies since the 1990s.[34,41,45,47] In vitro studies have shown potent inhibition of EVD across multiple cell types. In 1 animal study, IFN-ő was shown to protect mice challenged with a lethal dose of Ebola virus.[45] Two additional studies involving nonhuman primates treated with IFN-ö or IFN-ŏ2b revealed an increase in survival time but no change in mortality.[41,45]

Despite a potential increase in the availability of IFNs as therapeutic agents,[69] there are several concerns on the practicality of its use during an EVD outbreak. Certain side effects of IFN such as fever and myalgia may be difficult to control in an EVD treatment unit. In addition, it is important to rule out comorbidity with malaria before an interferon treatment, which may be difficult to achieve and may further delay treatment.

Convalescent blood products

The transfusion of blood products is an established treatment method for a variety of noninfectious conditions. In the case of infectious diseases, there are several reports on the off-label use of convalescent blood products.[70,71] We chose to include convalescent blood products in our assessment due to the similar properties shared with repurposed therapeutics. These include their presumed availability in the setting of an EVD epidemic, positive reports on tolerability, and the absence of safety-related events in 2 nonrandomized studies.[57,59] Nonetheless, it is important to note that transfusion-transmitted infections remain a significant problem in Africa, and it may be difficult to distinguish between transfusion-related complications and EVD progression.[72,73]

The use of whole blood as a form of passive immunotherapy for the treatment of EVD was reported during the EVD outbreak in the Democratic Republic of Congo in 1995.[55] In the observational study, 7 out of 8 patients who received convalescent blood transfusion survived, in contrast to an 80% mortality rate during that outbreak. However, all 7 survivors received convalescent blood between Day 7 and Day 15 after the onset of symptoms. In a separate analysis, it was shown that the high rate of survival was due to the treatment of patients later in the course of disease, when they have a higher probability of survival.[74]

During the recent West Africa EVD outbreak, the World Health Organization approved the use of blood or plasma transfusions from convalescent patients.[75] Our search yielded 3 single case reports of foreign patients who contracted EVD during their stay in western Africa and survived following transfusion therapy.[52-54] An additional potential therapeutic agent was administered in 2 cases.[52,54] The largest study on the treatment of EVD with convalescent blood enrolled 102 confirmed EVD patients in a Mûdecins Sans Frontiûres-led treatment unit in Guinea, of whom 84 were included in the primary analysis.[57] The study showed no significant improvement in survival for patients receiving up to 500 mL convalescent plasma with unknown levels of neutralizing antibodies, even after adjusting for age and cycle-threshold value. A recently published study from Sierra Leone[59] similarly did not show a significant improvement in survival, but revealed a significant drop in viral load after 24 hours of treatment with convalescent whole blood. Furthermore, results from a recent animal study by Mire et al[50] did not show protection by the treatment of rhesus monkeys with convalescent sera. It remains unknown whether convalescent blood products with known titers of anti-Ebola virus antibodies, alternative administration regimens, or a subselection of patients would yield different results.

Amiodarone, sertraline, and bepridil

Amiodarone is a widely available, commonly used multi-ion channel blocker, approved for the treatment of atrial fibrillation and ventricular tachycardic arrhythmias. It has been identified as a potent inhibitor of the Ebola virus in various endothelial and epithelial cell lines at concentrations that are commonly reached

in humans treated with this drug.[35,38] Its mechanism of action appears to rely on the induction of a Niemann-Pick C-like phenotype that inhibits late endosomal filovirus entry.[76]

In the only small animal study yielded by our search, treatment with 90 mg/kg amiodarone IP twice daily significantly improved the survival of Ebola virus-challenged mice. A repeat trial under identical conditions was unable to reproduce the significant improvement in survival rates.[48] Furthermore, amiodarone was reportedly used in Sierra Leone to treat EVD patients in 1 treatment unit on a compassionate basis, but the potential effects and their statistical significance could not be determined.[77]

Other therapeutic agents that appear to inhibit filovirus entry in a similar fashion to amiodarone are sertraline and bepridil. Sertraline is a selective serotonin reuptake inhibitor used in the treatment of depression. Bepridil is a calcium channel blocker once used to treat angina pectoris. Both drugs were found to effectively inhibit Ebola virus in vitro by more than 90%.[25] In a murine model, 7 out of 10 mice treated with 10 mg/kg sertraline twice daily survived, compared with 100% mortality in the control group. In the same study, 10 out of 10 mice treated with 12 mg/kg bepridil survived.[25]

Favipiravir

Favipiravir is a relatively new viral RNA polymerase inhibitor, approved in 2014 by Japan Pharmaceuticals and Medical Devices Agency for the treatment of influenza A virus infections. It is considered to have broad-spectrum antiviral activity against RNA viruses and has demonstrated activity against hemorrhagic fever-causing viruses, such as the arenaviruses and bunyaviruses.[78,79] To unfold its antiviral activity, favipiravir is initially converted to its active metabolite, which acts as an RNA polymerase inhibitor, mainly via direct competition with guanosine-5'-triphosphate (GTP). In addition, favipiravir causes lethal mutagenesis after being incorporated into the viral RNA.[80]

Studies on the Ebola virus involving favipiravir have shown promising outcomes in vitro and in small animal trials.[40,42,43] In 2 animal studies with different mice models and treatment regimens, treatment with favipiravir was associated with a 100% survival rate.[42,43] However, the outcome of a Phase II clinical trial was not as clear.[51] In the nonrandomized, cohort study, patients were stratified based on their baseline cycle threshold value, reflecting their viral load. Only patients with moderate to high viremia (cycle threshold value $\geq$ 20) showed a tendency for a lower mortality rate compared with historical records, although the difference was not statistically significant.[51] In a single-center retrospective study by Bai et al,[58] a series of 39 treated patients with medium to high viral load showed a significantly higher survival rate compared with the control group. Additional randomized, multicenter trials are necessary to confirm efficacy in target groups and to eliminate confounding variables.

Azithromycin

Azithromycin is a well-established antibiotic, commonly prescribed for the treatment of various bacterial infections. In addition, it has been proposed to have some anti-viral activity in the treatment of upper respiratory tract viral infections.[81] These effects are believed to be the result of the amplification of the systemic antiviral response mediated by the IFN pathway. It is not known to have a direct antiviral inhibitory effect.

Azithromycin was among several drugs investigated for anti-Ebola virus activity in vitro and in small animal models.[48] It has shown potent in vitro inhibition of the Ebola virus. Small animal studies generated mixed results. In a murine model, treatment with 100 mg/kg azithromycin twice daily was initially associated with a 60% survival rate, compared with 20% for the control group ($P = 0.02$). Repeat testing under identical conditions did not reproduce statistically significant results. In addition, a different treatment regimen with 210 mg/kg oral azithromycin PO once daily was associated with a 0% survival rate. An efficacy screening using different doses of azithromycin on guinea pigs similarly did not yield positive outcomes.[48]

Limitations

This systematic review was carried out on the basis of a previously published protocol to comprehensively search and select relevant studies and to identify potential repurposed drugs for EVD. However, limitations that are either inherent to the methodology applied in this review or to the studies included still exist. First, despite a search strategy that was designed to be highly sensitive, we cannot be certain that all studies on this topic have been captured. This is due to the pressing nature of an acute EVD epidemic, which resulted in a rapidly evolving research scene, with many studies currently underway.[82] Second, this review was synthesized narratively, which may increase the risk of bias if 1 study is given more weight compared with others.[83] Efforts to avoid such bias in this review include systematic selection of articles, extraction of data, and identification of potential drugs by 2 authors independently. Multireviewer extraction has been previously shown to decrease the risk of error compared with single-reviewer extraction.[84]

There are several limitations inherent to the studies included in this review. First, a clear majority of included studies were categorized as screening, in vitro, or animal studies. Results from these studies cannot be used to reliably predict a positive response in humans.[85,86] In our review, only 3 studies were carried out on nonhuman primates, which are considered the gold standard for filovirus models because they resemble the human clinical manifestation of filoviral disease.[87] The remaining 11 animal studies were carried out using adapted small animal models, such as mice, hamsters, and guinea pigs, which are not naturally susceptible to the Ebola virus. In these studies, it was necessary to use a genetically adapted virus that has undergone numerous mutations to achieve virulence and lethality. The genetic variation of an adapted Ebola virus together with differences in disease development compared with infected humans limit our ability to extrapolate results from these studies. Furthermore, our risk of bias assessment revealed a high risk of bias for 13 out of 14 animal studies. This limitation appears to be a shared concern amongst other fields of drug development.[88]

Our review included a total of 9 clinical studies that did not have a high level of internal validity or a high level of evidence. Based on the Oxford 2011 levels of evidence,[89] 4 out of 9 studies are case reports or case series with a low level of evidence,[52-55] and 5 trials are nonrandomized studies with a midrange level of evidence.[51,56-59] This lack of high-level evidence may have several reasons. First, until the recent EVD epidemic, this condition has been neglected and no major clinical research attempts were carried out. Second, clinical research during an outbreak faces many ethical and practical obstacles that influence study design, the number of participants, and settings.[90,91]

Conclusions

This systematic review offers a comprehensive overview on the current state of the art with regard to drug repurposing for EVD. It addresses the different stages of repurposed drug development for EVD, from screening chemical libraries to clinical trials. Authors of the review identified 11 therapeutic agents with potentially

promising therapeutic influence on EVD. At this stage, none of these therapeutic agents can be recommended for the treatment of EVD. This review highlights the need for well-designed and well-conducted preclinical and clinical research to establish the efficacy of these drugs in the proposed indication. It may be a useful aid for researchers to identify gaps in the evidence on the various drugs presented.

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Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.curtheres.2017.01.007.

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