REVIEW

Perspectives towards antiviral drug discovery against Ebola virus

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Ebola virus disease (EVD), caused by Ebola viruses, resulted in more than 11 500 deaths according to a recent 2018 WHO report. With mortality rates up to 90%, it is nowadays one of the most deadly infectious diseases. However, no Food and Drug Administration-approved Ebola drugs or vaccines are available yet with the mainstay of therapy being supportive care. The high fatality rate and absence of effective treatment or vaccination make Ebola virus a category-A biothreat pathogen. Fortunately, a series of investigational countermeasures have been developed to control and prevent this global threat. This review summarizes the recent therapeutic advances and ongoing research progress from research and development to clinical trials in the development of small-molecule antiviral drugs, small-interference RNA molecules, phosphorodiamidate morpholino oligomers, full-length monoclonal antibodies, and vaccines. Moreover, difficulties are highlighted in the search for effective countermeasures against EVD with additional focus on the interplay between available in silico prediction methods and their evidenced potential in antiviral drug discovery.

KEYWORDS
advancement, antiviral, discovery, drugs, Ebola virus, in silico methods, therapeutic

1 | INTRODUCTION

The Filoviridae family consists of three genera, namely *Marburgvirus*, *Cuevavirus*, and *Ebolavirus* (EBOV).[1] The *Ebolavirus* includes five virus species included *Sudan ebolavirus*, *Tai Forest ebolavirus*, *Bundibugyo ebolavirus*, *Reston ebolavirus*, and *Zaire ebolavirus*.[2,3]

High mortality rates associated with Ebola virus disease (EVD) outbreaks in humans illustrate their extreme vulnerability as host for filoviruses. Bodily fluids of an infected animal or individual serve as a mode of transmission, through exposure to cuts, wounds, and mucous membranes, direct or accidental injection,[4-7] and recently suspected cases of sexual transmission.[8,9] During disease prognosis, macrophages, monocytes, and dendritic cells are generally infected first, which later progressed to major cellular targets.[10-13] The action of viral proteins (VP24 and VP35) causes suppression of type-I interferon resulting in dysregulation of the immune response and

activation of T-cells as result of EBOV infection,[14,15] with various disease manifestations.[16-19]

Among clinical signs, initial nonspecific symptoms arise (malaise, fever, and gastrointestinal infection[19,20] followed by the state of shock, severe uveitis, vision loss,[7,21] organ failure, and ultimately death.[22,23] Disease progression towards the EBOV disease accounts for 90% mortality rate in humans.[24-27] Due to hypovolemic shock and multiorgan damage, death typically occurs between 6 and 16 days after the chronic symptoms of hemorrhagic illness.[28]

EVD outbreaks tend to rely on supportive care measures with fluid and electrolyte replacement. During the 2014-2016 outbreak (which resulted in 28 616 infections and 11 310 deaths), a coordinated and collaborative global effort with some stakeholders and agencies have been carried out resulting in some vaccine candidates and therapeutics capable of protecting high-risk populations with unprecedented efficiency. In 2018, the Ministry of Heath of Democratic Republic of Congo (DRC) reported two consecutive

EVD outbreaks, of which the first on 8 May (May-June 2018 Equateur province DRC outbreak) and the second on 1 August (August-present, 2018, Kivu province DRC outbreak). The first outbreak resulted in 54 EVD cases (38 confirmed and 16 probable) and 33 deaths (overall case-fatality ratio of 61%) as of 24 July, 2018 (declared the end of this outbreak),[29] while the latter is ongoing, which includes 238 cases (203 confirmed and 35 probable) and 155 deaths resulting in a case-fatality rate of 65.1% (as of 21 October, 2018).[30] Preclinical efforts toward specific EBOV countermeasures have been enduring for years with first treatment and vaccine clinical trials conducted during the 2013 to 2016 EBOV outbreak in West-Africa.[31-37] During the DRC outbreaks, the countermeasures were evaluated in West-Africa EVD outbreak and demonstrated their clinical efficacy. In addition, a small number of studies (case reports) exists for the experi countermeasures has been enduring mental use of anti-Ebola antiviral and vaccine leads.[38-41] EBOV vaccine development has already been covered in previously published reviews.[34,42-45] In brief, the recent update includes the development of replication-competent rVSV-ZEBOV (rVSV) used in May-June 2018 Equateur DRC outbreak in a ring vaccination trial (Phase III; NCT03161366), which proved to be well tolerated with improved efficacy and safety.[35,36] Currently, rVSV is being investigated along with other investigational therapeutics in the ongoing Kivu DRC outbreak (Phase II; NCT03719586). A second update includes Ad26-ZEBOV/MVA-BN-Filo prime-boost vaccine evidenced as safe and well tolerated.[46,47] Both rVSV and Ad26-ZEBOV/MVA-BN-Filo prime-boost vaccine have been evaluated in Phases I, II, and III clinical trials. Other nonreplicating vaccines are virus-vector-based evidenced to be highly effective in non-human primates (NHPs).[48-53] The current study is focused on the therapeutic advancement towards EVD and the challenges encountered.

1.1 | Biological targets of EBOV

Identification of suitable biological drug targets is the primary step towards therapeutic development.[54,55] Validated through a combination of genetic,[56] biochemical,[57,58] structural, and computational strategies,[54,55] a variety of drug targets have been identified in both host and pathogen.[59]

Encased in a lipid envelope, filoviruses are filamentous in shape.[60] Linear, nonsegmented, negative-sense single-stranded RNA encodes a 19-kb genome containing the genetic information for seven structural proteins and considered as potential drug targets, namely transcription activator VP30, polymerase cofactor VP35, matrix proteins VP40 and VP24, nucleoprotein (NP), glycoprotein (GP), and RNA-dependent RNA polymerase (L).[61,62] The structure and function of these proteins aided in deciphering the molecular mechanisms of filovirus lifecycle and have been explanatorily reviewed in a series of three reviews by Martin et al., describing aspects of filovirus entry,[63] replication cycle,[64] assembly, and budding.[65] Briefly, GP, a heterodimeric complex of $GP_{1,2}$ surface protein, orchestrates viral entry into the host cell and participates in virus egress.[66] Due to the major role of $GP_{1,2}$ in viral entry, numerous approaches targeting the entry

process have been explored to block EBOV replication at an early stage, namely immune-based therapies,[67-70] peptide-based antiviral molecules, a broad range of small molecules, reviewed by Rhein and Maury,[71] and more specific entry inhibitors targeting the fusion events characterized by the GP_2/NPC1 (Niemann-Pick C1) interaction.[72,73]

After entry by macropinocytosis, replication and transcription cycles involve the releasing of viral nucleocapsids into the host cell cytoplasm, resulting in the synthesis of new viral proteins and genomes. The whole process of assembly and budding is coordinated by NP, VP24, and VP40, and enhanced by GP.[74] NP binds to the viral RNA and creates RNP complex with polymerase L and viral proteins VP24, VP30, and VP35.[75] VP30 and NP play an important role in the RNA-binding activity.[75] The multifunctional protein, VP35, suppresses host-innate immunity and has been found to be involved in transcription/replication processes together with nucleocapsid assembly.[76,77] The interactions between these proteins (ie, NP-NP and NP-VP35) are essential to regulate the formation of the EBOV replication complex for efficient transcription/replication. Blocking this nucleocapsid formation by protein-protein interaction inhibitors can lead to potential inhibition of EBOV. for example, VP35-derived peptide (first identified NP ligand) that specifically binds to NP, blocks NP oligomerization and causes the release of RNA from NP-RNA complex.[78,79] Other studies identified 18β-glycyrrhetinic acid and licochalcone-A[80] to potentially disrupt the association of NP-RNA complexes, aptamers,[81] pyrrolidinone compounds,[82] and recently MCCB4 (ene-thiazolidinedione group-containing compound),[83] that specifically lead to specific VP35-NP interaction. VP24 functions as nucleocapsid maturation factor[84] and transcription/replication modulator.[85] Additionally, it has been identified as a target for protein-protein interaction inhibitors, namely for a macrocyclic peptide inhibitor VP24-KPNA5 (karyopherin α5) which specifically disrupt VP24-KPNA interaction.[86] VP40 is a matrix protein which coordinates with VP24 toward viral assembly, budding,[87] and regulation of viral transcription.[88] All structural/functional features and recent advances for these crucial regulatory proteins, essential in viral messenger RNA (mRNA) synthesis and genome replication, have been critically reviewed[64] and therefore constitute key targets for designing EBOV-specific drugs.

1.2 | Current experimental therapeutics against EVD

Key strategies recognized to combat EBOV include: (i) directly targeting the virus, (ii) modulating the host factors or immune response, and (iii) disease management. Critically targeting the viral lifecycle is among the most popular strategies for EBOV therapeutics.[61,63,89-91] This is done either by targeting the initial binding and/or entry of the virus into the host cells or the later viral replication and packaging. EBOV antiviral compounds mainly encompass small molecules, antisense therapies, and immunotherapeutic drugs.

Current treatment of EVD is centered upon modulating coagulation and maintaining oxygen levels in Ebola patients.[13,92-94] Because of these therapeutic interventions, sustenance and recovery have been reported. However, significant improvement might be observed as a result of an adequate level of support care.[27,92,93,95,96] To date, no commercial vaccines or specific therapies are available for EBOV. However, various studies have been reported that explain the comprehensive development of vaccines,[11,42,43,61] small-molecule inhibitors,[90,91,97-103] and repurposed Food and Drug Administration (FDA) approved drugs against Ebola.[90,100,104-110] Table 1 provides an overview of anti-EBOV compounds and their level of efficacy, reported in either IC_{50} or EC_{50}, in vitro assays against EBOV, clinical status, and observational studies.

1.3 | Small molecules: direct EBOV inhibitors

Development of potent small molecules directed against the RNA-dependent RNA polymerase L required for viral replication is the most promising therapeutics. The three most potent nucleotide viral polymerase inhibitors, GS-5734, BCX4430, and favipiravir (T-705), have demonstrated in vitro and animal efficacy in EBOV-infected mice and NHPs. They have reported antiviral effects due to intracellular conversion to their corresponding nucleoside triphosphate for incorporation into the viral genome and inhibition of viral polymerase.

By the end of the West-Africa EBOV outbreak, GS-5734 clearly indicated 100% protection of rhesus monkeys following lethal EBOV challenge[113] and an improved highly potent in vitro efficacy against Mayinga and Makona strains as compared to favipiravir.[112] Moreover, GS-5734 has been recently administered for the first time to a newborn baby.[127] BCX4430 has been tested against for its broad-spectrum inhibition of various viruses including, arenaviruses, bunyaviruses, coronaviruses, paramyxoviruses, and flaviviruses.[89,128,129] Additionally, animal survival efficacy accounted for 90% to 100% in mice[128] and 67% to 100% in rhesus monkeys using increased doses of BCX4430.[111] Favipiravir (T-705) remained a potential anti-EBOV candidate during the West-Africa outbreak[94,130] and has reported up to 100% survival rate in EBOV-infected mice even with the lowest oral dose of 37.5 mg/kg once daily. In comparison, NHP resulted in 17% to 50% survival rate.[94,115]

Among various FDA screens, multiple compounds including amodiaquine, diphenylpyraline, ketotifen, diphenoxylate were reported to inhibit EBOV replication, while others including, verapamil, dronedarone, sertraline, toremifene, chloroquine, teicoplanin, and amiodarone were considered EBOV entry inhibitors.[90,100,104,110] Very recently, tilorone ($EC_{50} = 0.23\,\mu M$) was reported to be the most potent small-molecule inhibitor with a single-daily dose of 25 and 50 mg/kg intraperitoneal and proved efficacious in protecting 90% of mice from a lethal EBOV challenge.[116] Some of these inhibitors are shown in Figure 1. Other studies include the identification of coumarin-based antihistamine-like molecules,[131] benzoquinoline compounds (SW456 compound reported to be the most potent compound in the infectious EBOV assay, $IC_{50} = 0.5\,\mu M$),[132] ellagic acid ($IC_{50} = 1.4\,\mu$ M),[133] and vindesine ($IC_{50} = 0.34\,\mu M$),[134] that proved to have potential EBOV inhibition.

1.4 | Immunotherapeutics and other treatments

Direct antivirals preventing viral entry incorporate large numbers of immune-therapeutics under development.[64,98,135] EVD treatment has recently been linked with the modulation of the immune system. Cytokines and chemokines play an immunomodulatory role during EBOV infection promote viral clearance by an enhanced immune response. However, an overwhelming inflammatory cytokine release can cause an undesirable effect. Therefore, numerous research groups have currently identified and studied a variety of anti-Ebola monoclonal antibodies (mAbs), leading to the development of several commercialized mAb cocktails as recently reviewed.[136]

EBOV GP constitutes a prime target for therapeutic antibodies. ZMAb, a cocktail of three mouse mAbs (1H3, 2G4, and 4G7), resulted in 50% to 100% protection,[67,69] while MB-003, a cocktail of three mouse-human chimeric mAbs (13C6, 13F6, and 6D8), demonstrated 67% protection after EBOV (Kikwit strain) infection in rhesus monkeys.[68] To identify the most protective combination, ZMapp was introduced as a combination of three EBOV-GP mAbs (13C6, 2G4, and 4G7) and demonstrated 100% protection for rhesus monkeys.[70] The functional mechanism of binding of ZMapp has been described for its neutralizing activity targeting the GP base and glycan cap.[137] Further studies reported the further optimization of ZMapp against NHPs by using two chimeric mAbs (13C6 and 2G4), designated as MIL77, which protected all EBOV (Makona strain) infected rhesus monkeys.[138]

Very recently, adeno-associated virus-mediated mAb 5D2 or 7C9 delivers 100% protection against mouse-adapted EBOV infection, whereas neutralizing mAb 2G4 revealed 83% protection.[139] Also, a coformulated cocktails REGN3470-3471-3479 (three human 3-mAb cocktails REGN3470, REGN3471, and REGN3479 in 1:1:1 ratio) is currently being evaluated and proved safe and well tolerated with no observed immunogenicity after a randomized, first-in-human Phase I clinical trial (NCT002777151).[37]

Other therapeutics include the nucleic acid-based inhibitors with phosphorodiamidate morpholino oligomers (PMOs) and small-interference RNAs (siRNAs), involved in promoting degradation of mRNA transcripts and constitute two essential classes of antisense therapies.[102,140,141] Both compounds target vital proteins involved in EBOV transcription/translation processes VP24, VP35, and viral polymerase L. The development underlying the modification of PMOs and siRNA has been elaborated[142] and recently reviewed.[143] PMO combination, as in AVI-6002 (AVI-7537 and AVI-7539), has shown significant efficacy and safety in NHP[126] when directed against both VP35/VP24 (63% protection) and VP24 alone (75% protection)[126] without further development. TKM-100803, a lipid siRNA nanoparticle product that targets viral RNA polymerase L, VP35, and VP24 demonstrated no survival advantage after Phase II single-arm trial[122] without further development of TKM-EBOV modifications.

TABLE 1 Ebola virus (EBOV) drug candidates, their efficacies (EC$_{50}$ or IC$_{50}$) and clinical status

Compound/drug	EBOV target and description	EBOV clinical trial phase	Results/status	Ebola preclinical data	
				In vitro efficacy data	**Animal efficacy data**
BCX4430	A novel adenosine nucleotide analogue that inhibits RNA polymerase L activity by incorporating into new viral RNA chains and cause chain termination	Phase I (NCT02319772)	Phase I complete; generally safe and well tolerated up to 10 mg/kg daily for 7 d	EC$_{50}$: 11.8 μM EC$_{90}$: 25.4 μM (IM Kikwit) EC$_{50}$:3.4 μM EC$_{90}$: 10.5 μM (Boniface)[89]	Mice: 90% survival at 150 mg/kg BID PO; 100% survival at 150 mg/kg BID IM; NHP: 100% survival at 25 mg/kg BID; days 0-14; 100% survival rate at 100 mg/kg loading dose BID; days 2-3; 67% survival at maintenance dose of 25 mg/kg BID till days 3-17[111]
GS-5734	a novel monophosphoramidate prodrug of adenosine analogues, it selectively inhibits EBOV replication by targeting its RNA-dependent RNA polymerase and converts it into active triphosphate nucleotides in efficient cells	Phase II (NCT02818582)	Phase I complete; Phase II, given intravenously in survivors with viral perseverance in their semen	EC$_{50}$ replication: 0.021-0.066 μM EC$_{90}$: 0.053-0.203 μM (Mayinga) EC$_{50}$: 0.014 μM EC$_{90}$: 0.045 μM (Makona) VTR: EC$_{50}$: 0.003 μM Reporter Assay EC$_{90}$: 0.013 μM (Makona)[112]	NHP: 100% protection at 10 mg/kg IV days 3-15 (extensively summarized in Warren et al[113]
Favipiravir (T-705; avian)	a pyrazine derivative that was discovered during a screen of compounds against influenza virus A/PR/8/34 (H1N1); it modified intracellularly to purine derivative and inhibits RNA polymerase L with significant efficacy	Phase III (NCT02329054 and NCT02363322)	Limited efficacy in patients with lower to moderate levels of virus (C$_t$ > 20); T-705 was well tolerated[114]	EC$_{50}$: 44.2 μg/mL[114]	Mice: 300 mg/kg qD PO; day 1; 90% survival; 150, 75, and 37.5 mg/kg qD PO; days 0 -14; 100% survival 300, 150, 75, and 37.5 mg/kg BID PO; days 0-14; 100% survival (While mice gained weight) 8, 1.6, 0.325 mg/kg PO qD; survival rate 90%, 10%, and 0%, respectively[115] NHP: 400/200 qD PO; days 0-10; 17% survival 250/150 mg/kg PO BID; days 0-14; and 125/75 mg/kg PO BID; days 0-

(Continues)

TABLE 1 (Continued)

Compound/drug	EBOV target and description	EBOV clinical trial phase	Results/status	Ebola preclinical data	
				In vitro efficacy data	Animal efficacy data
					14; All NHPs succumbed between days 11-15 and days 8-11[115] 150 and 180 mg/kg BID days 1-21 (with 1-d loading dose of 250 mg/kg); ~50% survival
Amiodarone	Cationic amphiphilic drug (CAD) amiodarone is a widely used antiarrhythmic drug which inhibits EBOV infection (in vitro) at an early stage of viral replication	Phase II (NCT02307591)	Terminated: unknown statistically significant	IC_{50} entry: 5.6 µM; IC_{50} entry (lentivirus): 2.2 µM; IC_{50} replication: 0.4 µM[90]	Mice: No survival rate of 60 mg/kg. 0% to 40% survival at 90 mg/kg.[101] NPH: N/A
Amodiaquine	CAD[103]	Preclinical	Showed significantly lower mortality (50.7%) for amodiaquine compared with lumefantrine (64.4%)	EC_{50} entry: 2.6 µM; EC_{50} replication: 34 µM	Mice: No increased survival at 60 mg/kg BID IP; days 0-7.[101] NPH: N/A
Chloroquine	CAD	N/A	Well tolerated	EC_{50} entry: 4.7 µM; EC_{50} replication: 16 µM[116]	Mice: Mixed results across several dose/studies; IP and PO with 0% to 80% survival rate. Hamsters: No efficacy at 50 mg/kg IP in combination with doxycycline (2.5 mg/kg) and azithromycin (50 mg/kg). Guinea pigs: no protection up to 100 mg/kg.
Hydroxychloroquine	CAD	N/A	N/A	EC_{50} replication: 22 µM; EC_{50} entry: 9.5 µM[117]	N/A
Clomiphene	CAD; entry inhibitor	N/A	Used in combination treatment together with irbesartan and atorvastatin for some patients. Well tolerated at prescribed doses	EC_{50} replication: 11 µM; EC_{50} entry:1.3 µM[116]	Mice: 10% survival at 60 mg/kg IP BID.[116] NPH: N/A
Toremifene	CAD; entry inhibitor	N/A	N/A	EC_{50}: 1.73 µM (Kikwit); EC_{50}:0.973 µM (Mayinga)[100]; EC_{50}:1.10 µM (Makona)[118]	Mice: 50% survival at 60 mg/kg IP qD on days 0, 1, 3, 5, 7, 9.[100] NPH: N/A

(Continues)

TABLE 1 (Continued)

Compound/drug	EBOV target and description	EBOV clinical trial phase	Results/status	Ebola preclinical data	
				In vitro efficacy data	Animal efficacy data
Amiodarone	CAD; entry inhibitor	Phase II (NCT02307591)	Terminated early; reduction in case-fatality rate; not statistically significant	IC_{50} entry: 5.6 μM IC_{50} entry (lentivirus): 2.2 μM IC_{50} replication: 0.4 μM[90]	Mice: 90 mg/kg; 0-40% survival.[101] NPH: N/A
Azithromycin	CAD	Registered as NCT02380625 (not yet open to recruitment)	Well tolerated in critically ill patients	EC_{50} replication: 5.1 μM[101] EC_{50} VLP entry: 2.79 μM EC_{90} VLP entry: 15.8 μM[119]	Mice: 10%-60% survival at 100 mg/kg BID IP. 0% survival by PO route. Guinea pigs: no efficacy.[101]
Sertraline	CAD; entry inhibitor	N/A	Well tolerated in healthy adults and children	IC_{50}: 3.13 μM (Vero) IC_{50}: 1.44 μM (HepG2)	Mice: 70% survival at 10 mg/kg PO qD. NPH: N/A
Bepridil	Glycoprotein[120]	N/A	N/A	IC_{50}: 5.08 μM (Vero) IC_{50}: 3.21 μM (HepG2) (Mayinga)[120]	Mice: 100% survival at 12 mg/kg NPH: N/A
Brincidofovir (BCV, CMX001)	Monophosphoramidate prodrug of an adenosine analogue selectively inhibits viral RNA (L) polymerase	Phase II (NCT02271347)	Clinical trial halted due to low enrollment And withdrawn by the company for further development as EBOV therapeutic.[31]	EC_{50}: 0.88 μM (Makona) EC_{50}: 0.66-0.79 μM (kikwit, Huh7)[121]	No preclinical efficacy reported so far[31]
TKM-100802/TKM-130803	Small interfering RNA-lipid nanoparticle product that targets viral RNA polymerase L, VP35, and VP24	TKM-100802: Phase I (NCT02041715) TKM-130803: Phase II (PACTR201501000997429)	TKM-100802: Terminated TKM-130803: Terminated; failure to achieve a survival probability and did not demonstrate efficacy[122]	EC_{50}: 50 ng/mL (Makona) EC_{50}: 50-100 ng/mL (Kikwit) EC_{50}: 100-250 ng/mL (Makona) EC_{50}: 1-50 ng/mL (Makona)[123,124]	NHP: 100% survival against Kikwit (0.5 mg/kg qD IV) and Makona; (0.5 mg/kg qD IV) after viral challenge[123]

(Continues)

TABLE 1 (Continued)

Compound/drug	EBOV target and description	EBOV clinical trial phase	Results/status	Ebola preclinical data	
				In vitro efficacy data	Animal efficacy data
ZMapp	A cocktail of three human chimeric neutralizing mAbs (c13C6, c2G4, and 4G7) selected from MB-003 and ZMab antibody cocktails. Selectively targets the viral glycoprotein	Phase II (NCT02363322)	Suspended: due to no conclusion on efficacy available	EC$_{50}$: <0.1-1 μg/mL (Gueckedou)	NHP: 100% survival at 50 mg/kg, 3 doses with 3-d interval IV[70]
Convalescent whole blood/plasma (Ebola-Tx)	ABO-compatible plasma from a separate convalescent donor, Targets whole virus/glycoprotein	Phase II/III (NCT02342171)	Completed; No adverse reactions associated and found no significant improvement in efficacy in survival[125]	N/A	NHP: No efficacy observed with whole blood; efficacy of concentrated IgG from survivors
AVI-7537	VP24	Phase I (NCT01593072)	Withdrawn before enrollment; further development has been suspended due to funding constraints	EC$_{50}$: 0.585 μM	NHP: 75% survival at 40 mg/kg qD IV.[126]
Tilorone hydrochloride (tilorone)	N/A	N/A	N/A	EC$_{50}$: 0.23 μM[116]	Mice: 25 and 50 mg/kg qD IP DO-8; proved efficacious in protecting 90% of mice from a lethal EBOV challenge[116]

Abbreviations: BID, twice daily; IM, intramuscular injection; IP, intraperitoneal injection; IV, intravenous injection; mAb, monoclonal antibody; NHP, non-human primate; PO, oral administration; qD, once daily; siRNA, small interfering RNA; VTR, herpesvirus telomerase RNA.
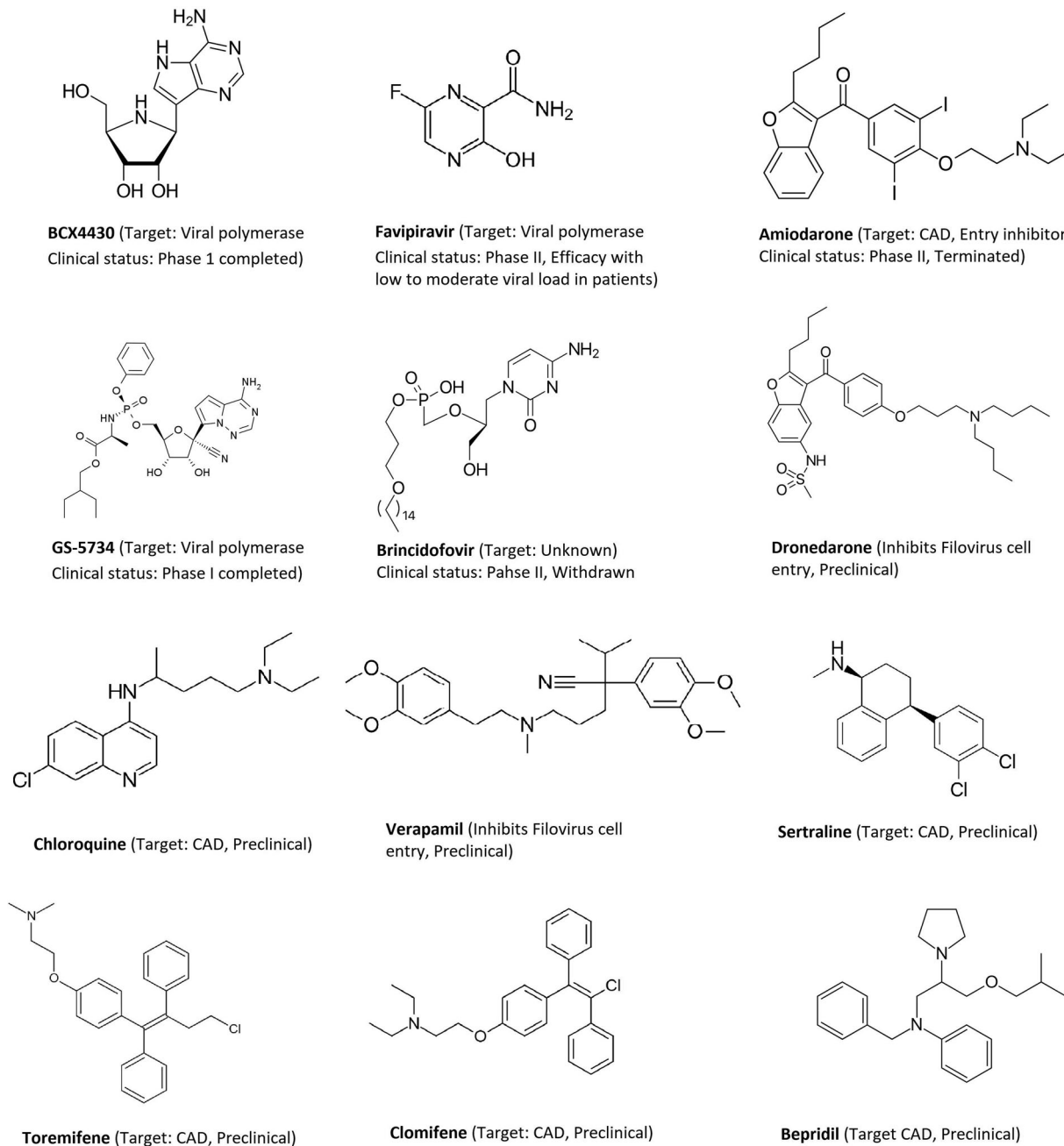
FIGURE 1 Few drug candidates to treat Ebola virus disease

Additionally, managing EVD includes treatment of clinical manifestations like hemorrhage or coagulation abnormalities.[24,92] Electrolyte balance gets disturbed as soon as the virus starts to reproduce and spreads across the body. Patients require fluid intake (intravenous or oral) rich in electrolytes, to treat dehydration and to restore electrolyte balance.[21,92,93,144] Moreover, EBOV infection results in disturbed blood clotting. For that purpose, anticoagulants like recombinant nematode anticoagulant protein c2 (rNAPc2) and recombinant human-activated protein C (rhAPC)[145] known to affect coagulation pathways have been investigated and resulted in reduced morbidity and fatality.[146]

1.5 | 2014-2016 EBOV outbreak—an overview

Regardless of no effective treatment against EVD, potential drug candidates indicated promising results in animal models after the West-Africa 2014-2016 outbreak.[31,64,81,89,90,94,99,100,113,126,128,130,147,148] Safety and efficacy concerns arose due to the limited duration of the outbreak. For this reason, EBOV therapeutics in advanced development had only been evaluated in the initial two phases of the clinical trials.[31,32,149] Bafilomycin, chlorpromazine, cytochalasin B, mannose-binding lectin, and ZMapp were reported to inhibit viral entry,[70,100,150-152] while other mAbs and cocktails are under development and most are now in preclinical stages.[138,139,153-156] Because of

successful preclinical NHPs data,[70,138] ZMapp was recently used on four patients evacuated from West-Africa[157] and successfully treated two patients repatriated to the United States.[93] In 2015, a randomized controlled trial (Prevail II; NCT02363322) of 72 patients demonstrated 22% deaths (8 of 36 patients), who were treated with ZMapp compared with the group of patients who received standard care alone (37% deaths; 13 of 35 patients).

During the outbreak, two prominent vaccines, rVSVΔG-ZEBOV-GP (rVSV) and cAd3-EBO, were tested in clinical trials and proved to be efficacious.[35,36,158,159] rVSV is currently in Phase II (NCT02876328 and NCT02788227) and is being tested in the United States and Africa. Against viral polymerase, several drugs have been reported and tested.[81,99,128] BCX4430 and GS-5734 have successfully crossed Phase I clinical trials (Table 1). In male EBOV patients, GS-5734 is currently being tested for the reduction of viral load in semen.[89,113] Very recently, the antiviral activity of drugs was discovered, and selected molecular probes have been identified for EBOV infection. Clomiphene and toremifene, selective estrogen receptor modulators (SERMs), have been identified as a result of this in vitro screening.[100] SERMs block Ebola entry by causing a reduction in the accumulation of endolysosomal calcium and the concentration of cellular sphingosine[160] and have been approved by the FDA for treating EBOV infections.

Although the 2014-2016 outbreak highlighted several therapeutic compounds[161] that successfully crossed Phase I clinical trials, some of them indicated safety concerns.[34,44,117,162] The clinical trials conducted during the outbreak lacked proper controls and statistical power due to the severity and urgency of the disease.[163] TKM-130803 and amiodarone reached Phase II of clinical trials in Sierra Leone, which were later terminated due to lack of demonstrating efficacy and statistical power.[122,163] FDA halted TKM-100802 because of the release of cytokines triggered by the action of the siRNA causing flu-like symptoms in treated individuals.[164,165] Likewise, brincidofovir, an oral bioactive molecule, failed to cross the Phase II clinical trials for efficacy, safety, and tolerability, resulting in discontinuation.[31] Another study, the JIKI trial, was carried out in 2014 to test the efficacy of favipiravir. The current favipiravir data suggests its efficacy for low to moderate viral loads.[32] This trial which was conducted in four Ebola treatment centers in Guinea and relied on the use of historical controls. Because of the ambiguity of the results, JIKI was criticized for its design.[32] Another clinical trial of nonrandomized Ebola-Tx, carried out in Guinea evaluating convalescent plasma, failed to demonstrate an improved survival rate.[125,166] Similarly, amodiaquine showed good invitro efficacy in inhibiting EBOV activity[104] but showed liver-related toxic effects during artesunate-amodiaquine combinatory treatment.[167]

1.6 | 2018 EBOV outbreak (May-June 2018, August-present)—an update

Two consecutive outbreaks are reported in the following year. On 8 May 2018, the Government of the Democratic Republic of Congo (DRC) reported an EVD outbreak (Zaire EBOV strain) in the north-west of the country. According to the last updated situation report of Equateur DRC outbreak (24 July 2018), a total of 54 EVD cases (38 laboratories confirmed and 16 probable) were reported since the beginning of this outbreak on 4 April 2018 till 2 June. Of these 54 cases, (29 from Iboko, 21 from Bikoro and 4 from Wangata health zones), 33 died resulting in a case-fatality ratio of 61%.[29] This is the ninth outbreak in DRC in the last four decades (since 1976), with the last outbreak dated in May 2017 (8 cases with 4 deaths). Due to improved efficacy and safety of rVSV (evaluated in more than 10 000 individuals),[35,36] the test treatment was administered during the May-June 2018 DRC outbreak, currently being investigated in a ring vaccination trial (Phase III; NCT03161366). After the declaration of the end of Equateur DRC outbreak, another EVD outbreak was reported in Kivu and Ituri provinces of DRC on 1st August 2018. As of 21st October 2018, a total of 238 cases (203 laboratories confirmed and 35 probable) have been reported, including 155 deaths, resulting in a case-fatality ratio of 65.1%.[30] To date, the 10th ongoing outbreak in DRC has raised serious concerns due to the spread in surrounding regions. In this outbreak, investigational therapeutics including ZMapp, remdesivir(GS-5734), and mAB114 (a monoclonal antibody used for the very first time to treat infected individuals) are being investigated together with rVSV (Phase II; NCT03719586).

1.7 | In silico methods for anti-EBOV drug discovery

In silico methods in drug discovery hold great potential and may prove beneficial at any stage in the preclinical development of drug candidates.[168] Especially, areas like target validation, the design of compound libraries,[169,170] hit identification,[171-173] hit-to-lead optimization,[174] and preclinical candidate identification can essentially benefit from exponentially increasing in silico tools, with unprecedented accuracy. A report from Bayer HealthCare[175] illustrates the significance of integrating computational drug design in pharmaceutical companies. This report states that computer-aided design methods (CADD) have aided in approximately half of the 20 new chemical entities currently being tested in Phase I clinical trials. Figure 2 highlights the interconnected stages and different phases in the drug discovery process mapped with EBOV updates.

1.8 | Homology modeling

Protein modeling plays a significant role in the drug discovery process. The goal of homology modeling is structure prediction from a known sequence with accuracy comparable to experimentally resolved structures.[176-178] Restrictions linked with this technique are the presence of inserts and loop sequences, which cannot be accurately predicted in the absence of a three-dimensional (3D) crystal structure.[179] The gap between known protein sequences and identified protein structures is significantly growing. Given an enormous amount of data through a vast array of DNA sequencing
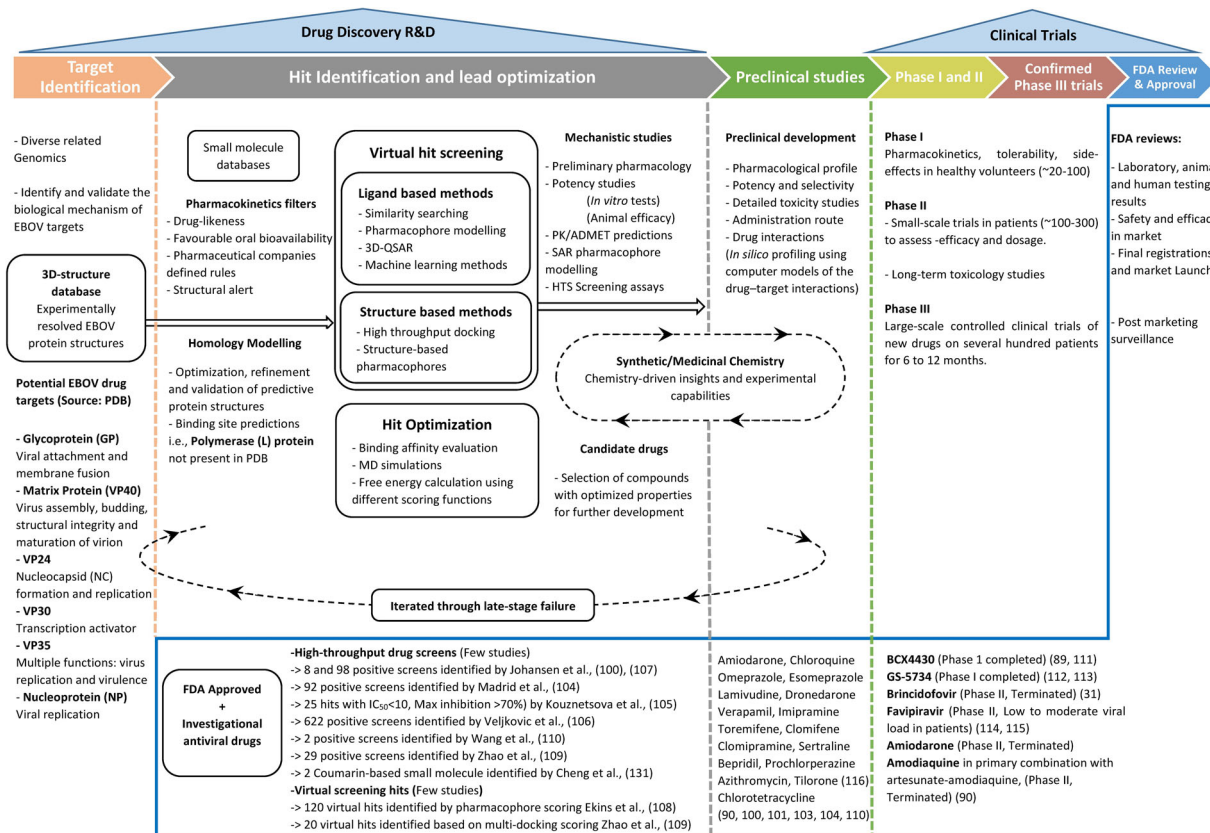
FIGURE 2 Stepwise drug discovery process from target identification, hit-to-lead optimization, and clinical trials along with the progress in the development of small-molecule inhibitors against EBOV. ADMET, absorption, distribution, metabolism, excretion, and toxicity; HTS, high through-put screen; PDB, protein data bank; PK, pharmacokinetics; QSAR, quantitative structure-activity relationship; R&D, research and development; SAR, structure-activity relationship

techniques available, experimental structure identification techniques require attention.[180] Computational techniques are actively exploited in the pharmaceutical industry for the prediction of 3D protein models.[124,168] To expand the scope of computational methods and to improve model accuracy, efforts are being made continuously. These approaches help to predict the tertiary structure of a protein through its amino acid sequence to combat this issue.[176] Depending on the available information, these methods can be characterized as either de novo or homology modeling. Template-based modeling also referred to as homology modeling or comparative modeling, is the most trusted method for model design.[176] Similar folding properties of the members of a protein family with the core structure unaffected by modifications in the sequence are fundamental criteria governing homology modeling.[181] Models are generated given target-protein sequences and X-ray, cryo-EM, or NMR-determined structures. Even with a low sequence similarity (~20%), accurate models can be obtained using homology modeling.[182-185] For this, a template structure is initially selected to identify similar experimentally determined structures, after which template-target sequence alignment is performed. The 3D model is then energetically refined to optimize model quality. The refinement of the model includes optimizing bond lengths and angles and removing clashes in geometry. If required, additional structural

modifications can be applied, until a relevant and accurate model is obtained. However, the refinement of the model often does not meet the desired level of accuracy.[186] A number of potential in silico studies have been documented over last few years which include homology modeling of unresolved EBOV polymerase[187,188] and docking-based virtual screening of compounds that have potential to bind with important residues lining the binding pocket of EBOV VP40, VP24, VP30, and VP35.[82,108,189-194]

1.9 | Molecular dynamics simulation to elucidate ligand-protein interactions

Although optimal ligand-receptor interactions can be predicted through molecular docking,[195] not all key interactions between the ligand and the active site of the receptor will be accurately depicted. Hence, molecular docking followed by molecular dynamics (MD) simulations of the obtained complexes can help in understanding interaction modes. Rastelli et al[196] reported sulfonamide derivatives to bind effectively within the active site of aldose reductase. Contrary to these predictions, the experiments demonstrated lower activity and binding potential of these compounds, therefore, negating the prediction made. Later, the in silico refinements of these compounds using MD revealed the interruption of key

interactions between sulphonamide ligands and the receptor due to an additional water molecule. The migration of this water molecule from outside explained the reduced activity of these compounds when tested experimentally.[196] In another study by Cavalli et al,[197] MD simulations were used as a platform to discern several different docked complexes of propidium and human acetylcholinesterase and most stable structures identified which correlated with the experimentally verified binding modes.

Interestingly, MD simulation assisted in the discovery and development of antiviral drugs.[198-200] For the first time, a combination of MD refinements of postdocking complexes and ensemble-based molecular docking has helped to reveal a unique symmetrical binding mode of daclatasvir with hepatitis C virus (HCV) NS5A protein. This drug is currently in Phase III clinical trials and is being tested for different HCV genotypes.[201] Moreover, through MD simulations, identification of a trench adjacent to the active site of HIV-1 integrase has been made possible.[202] The role of the trench in ligand binding later became evident when a site-directed mutagenesis study was carried out. These findings helped in the design of potent HIV-1 integrase inhibitors with enhanced antiviral activity. A 3D structure model of major coreceptor of HIV-1, CCR5, has been constructed through the use of MD simulations.[203] Furthermore, the development of antiviral drugs against influenza virus (IFV) has also benefitted from MD simulations. Through the use of this method, a universal cavity (150-cavity) adjacent to the binding site of the natural substrate has been reported with neuraminidase (NA) proteins of human 2009 pandemic H1N1, avian H5N1, and human H2N2 strains.[204]

Recently, MD simulations have been used to study the molecular behavior of ZIKV NS3-helicase, both in the presence and absence of single-stranded RNA, and the potential implications for NS3-helicase activity/inhibition.[205] Recent studies have reported notable examples of MD-driven drug discovery.[199] These studies prove the usefulness of MD simulation in understanding molecular interactions and the mechanism of drug binding,[206,207] especially against the drug-resistant viruses.[206,208,209]

1.10 | Hit-to-lead optimization

Hit-to-lead optimization is the most essential phase to closely examine the chemical scaffold concerning its absorption, distribution, metabolism, and excretion (ADME) challenges in the drug discovery process. In silico ligand-profiling[210,211] benefited from the boost of repurposing drugs[91,104,108,194] and the notion of designing drugs with controlled selectivity profiles.[212] This approach is aimed at: (i) The utilization of phenotypic screening hits to predict potential targets and their mechanism of action, (ii) identifying off-targets potentially responsible for adverse reactions and side effects, and (iii) careful analysis of ADMET (absorption, distribution, metabolism, excretion, and toxicity) parameters to propose potential hits.

Another useful in silico method, particularly beneficial for lead optimization, is quantitative structure-property relationship (QSPR) modeling which is useful for the identification of key structural features responsible for interacting with the target protein. For many ADME endpoints measured in the pharmaceutical industry, QSPR models have prospectively shown their ability to extract knowledge from a wide variety of chemical scaffolds proving their utility as predictive models.[213] QSPR models, based on machine learning techniques, are desirable to achieve the optimal potency and ADME properties. To reduce the risk of failure in trials, a useful QSAR/QSPR model is necessary to accurately predict the activity of a compound for each drug discovery project. However, these models do not provide adequate information about the modifications that should be made to the tested compound in the next cycle of drug design. To address this issue, the matched molecular pair analysis technique is another promising approach. This method assesses the mean effect of different substituents on various ADME parameters, such as: (i) permeability,[214] (ii) solubility,[215] (iii) clearance,[214] and (iv) cytochrome P450 inhibition.[147] The design of a new scaffold that interacts with the desired pharmacological target can be benefitted based on these findings. Molecular substitutions that are closely linked with the molecular properties can guide the design of such scaffolds. Several studies are reporting the use of quantitative structure-activity relationship modeling in lead optimization.[216-219]

Computational drug discovery has proven to accelerate the challenging process of designing and optimizing new drug candidates. Hierarchical virtual screening of ligand-based and structure-based methods delineated their validity in finding potential hits, even in the early phase of drug discovery. Because of the increased efficiency on Ebola hit-to-lead optimization, an interplay between the several stages of in silico drug design has been depicted in Figure 3. Because of the rapid development of faster architectures and comprehensive algorithms for high-level computations, the impact of computational structure-based drug design on antiviral drug discovery and lead optimization will have a more profound impact in future years. Not only hit identification but also elucidation of its biological target has provided information for use in drug discovery research. In this perspective, Perilla et al,[220] have described the physical properties of the HIV-1 capsid protein using the all-atom MD simulations. Andoh et al,[221] performed the all-atom MD calculation study of entire poliovirus and found rapid equilibrium exchange of water molecules across the capsid, finally concluded the capsid to function as a semipermeable membrane. The next study by Le et al,[222] less restrictive to a single target, studied drug interactions of Tamiflu and Relenza to multiple evolutionary correlated proteins. More specifically swine influenza A/H1N1, Spanish H1N1, and avian H5N1 flu N1 NAs were investigated using MD techniques for possible drug resistance mechanisms, in combination with electrostatic analysis. The research group created a molecular model of the swine influenza A/H1N1 type-I NA based on the avian H5N1 type-I NA, after which all three NAs were simulated as apo-conformation and compared with its bound state with oseltamivir (Tamiflu) or zanamivir (Relenza). When compared with each other the simulations identified conserved and unique drug-protein interactions across all three proteins mediated by hydrogen bonds. This elucidation of key molecular interactions was used to predict mutations that could lead to drug resistance.
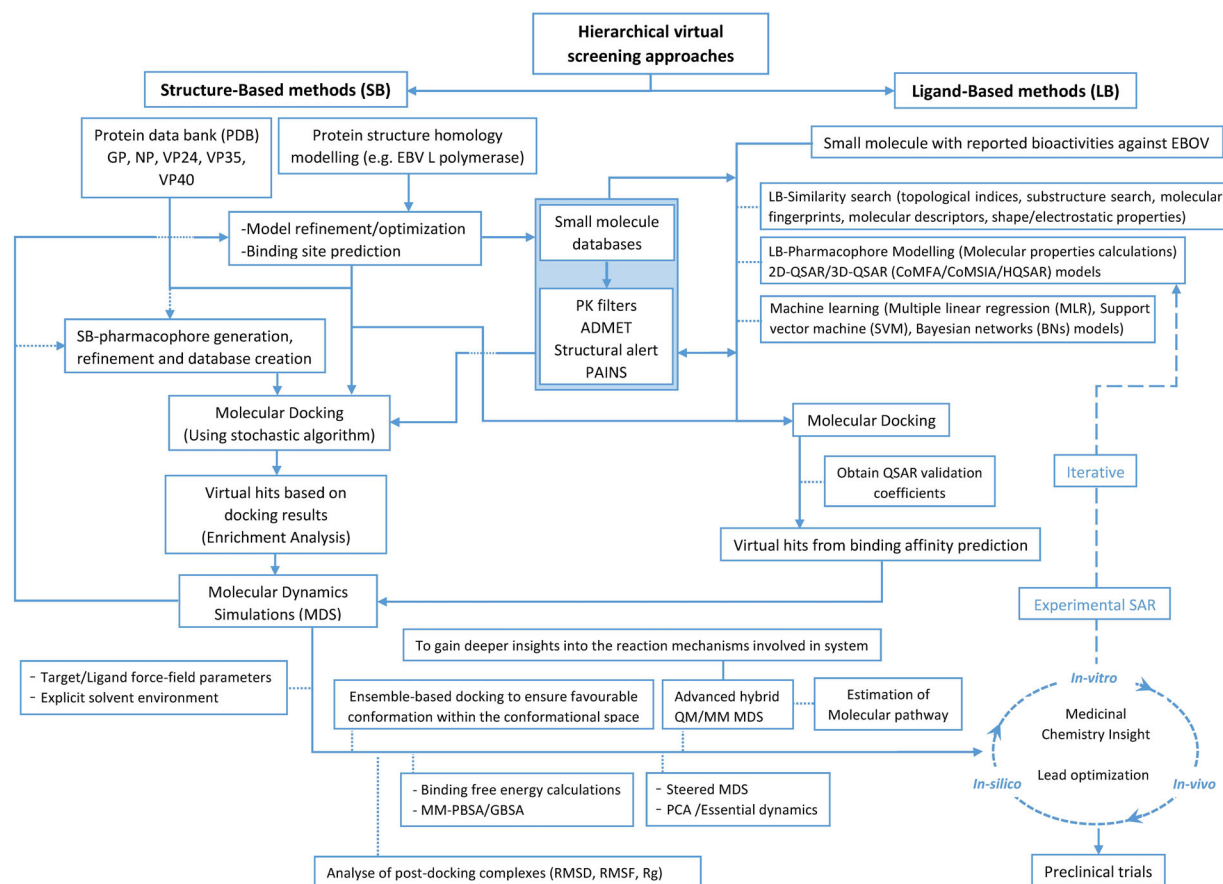
FIGURE 3 Detailed comprehensive workflow toward efficient anti-EBOV drug discovery. ADMET, absorption, distribution, metabolism, excretion, and toxicity; CoMFA, comparative molecular field analysis; CoMSIA, comparative molecular similarity indices analysis; GBSA, generalized born surface area; MM, molecular mechanics; PAINS, filters for removal of pan assay interference compounds; PBSA, Poisson-Boltzmann surface area; PCA, principal component analysis; PDB, protein data bank; QM; quantum mechanics; QSAR, quantitative structure-activity relationship; Rg, radius gyration; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation

1.11 | Some advances in MD simulations

More advanced nudge elastic band or catalytic MD techniques identify reaction or conformational transition paths.[223] Advanced hybrid QM/MM MD simulations have been proven extremely beneficial to gain more profound insights into the reaction mechanisms involved in the investigated biosystems. To more specifically asses which amino acids are interacting, per-residue energy decoupling has the potential to identify key interactions with the target. Many drug candidates are known to bind to less populated structures within the target's conformational space. In this view, ensemble-based docking describes a method in which the ligand is docked to multiple conformational forms a biomolecular target instead of only one. Based on the hypothesis of the influence of induced fit in enzymes, the normal-mode analysis could prove to be helpful in the elucidation of the collective motions of protein domains that underlie their conformational changes upon binding a ligand.[224] In other research, slow motions have been extracted from MD trajectories by using principal component analysis and MD simulation clustering.[225,226] The same technique might be incorporated in the elucidation of interacting amino acids of the target with the ligand of

interest, as described using per-residue energy decoupling. Steered MD simulations applying predefined degrees of freedom can be used after identifying the catalytic important domain movements of the target and the relation with ligand binding. The refinement quality of postdocking complexes is generally assessed by plotting the root-mean square deviation and root-mean square fluctuation of obtained trajectories. For comparison purposes between MD-refined complex systems, binding-free energy calculations using the molecular mechanics Poisson-Boltzmann surface area/generalized born surface area can be incorporated in the workflow. Like these, numerous recent studies have been performed with the aid of MD simulations in search of direct antivirals[205,218,226-233] and investigating drug resistance mechanisms.[226,227,234-237]

Furthermore, computational power has increased exponentially over the past 30 years with the sequential development of more powerful supercomputer units (high-performance computing). With the assumption of a continuation of Moore's law, which is reasonable given the latest advancements in computing power, a one–million-fold increase in processing power is expected. However, until quantum computing becomes a reality, a maximum level of processing power is expected due to limitations in computational resources. Specialized

supercomputers designed especially for all-atom MD simulations have been developed that show to the ability to reach millisecond timescales that represent interesting biological processes.[238,239] Efforts being made in quantum computing offer an exciting outlook.[240] To date, actual quantum computers are still in their initial stage of development with quantum computational operations executed only experimentally on a very small number of quantum bits. With these timewise developments, it might become possible to simulate large biological systems and determine, computationally, the 3D folding of proteins starting from their amino acid sequence.[241] In drug discovery processes the applicability is very promising, especially in target identification and interaction analysis.

2 | PROPOSED CHALLENGES

The success rates for drug discovery pipeline involving target identification, screening, hit-to-lead optimization, and preclinical candidate selection are within the range of 69% to 85%.[242] Failure of a discovery project accounts for several reasons; notably, (i) unclear underlying mechanism of target protein, (ii) lack of leads, (iii) poor potency, (iv) lack of efficacy, (v) inappropriate drug-like properties, and (vi) unexpected high animal-toxicity levels. However, the success rates of the clinical development of drugs vary distinctly. An experimental drug in Phase I clinical trials has approximately 10% chance to reach the market.[242] Inadequate efficacy of drugs accounts for two-thirds of recent Phase III clinical trial failures, half of the trials for Phase II trials and approximately 16% for Phase I trials.[242] Most drugs fail during clinical trials even though all experimental drugs enter human clinical trials based on extensive preclinical data indicating the efficacy in vivo. Moreover, experiments with Ebola strains require a BSL4 safety level, which narrows the possibilities and slows the progress in anti-EBOV drug research. The complexities of human biology, amplified by the limitations of target-based drug discovery approach[243] poses a significant challenge.

The magnitude of the recent EBOV outbreak, coupled with drug discovery and development challenges has necessitated the need to explore broad-spectrum alternative strategies. Despite no current countermeasures, experimental drug and vaccine development is progressing with some in the earliest stages of product development. Repurposing drugs provide an alternative way to accelerate the process of drug design and discovery. Investigation of FDA-approved compounds in new directions opens avenues for devising a strategy against challenging diseases, in particular, EVD. Drugs gain FDA approval after rigorously being screened through a set of criteria including pharmacokinetic and pharmacodynamics, dosage, toxicity, safety, and efficacy. Repurposed drugs bypass Phase I of clinical trials accelerating the drug discovery process and additionally eliminating the logistic considerations like manufacturing and distribution. However, when repurposing such drugs, any unfavorable data gained from these clinical trials may not serve any purpose against the approved drug, it might cut down on the development timeline.

Furthermore, studying the mechanism of action of such repurposed drugs may be difficult. Therefore, experiments specifically designed to identify the mechanism of action of repurposed compounds may provide insights into the EBOV lifecycle, and can help devise new trials against EVD pandemic. Additionally, drug resistance can also be a major clinical problem for the treatment of EBOV-infected individuals, as only a small number of mutations can drastically change the biological properties of RNA viruses,[244] HIV virus,[245] and influenza viruses.[246]

The statistical power of preclinical studies is crucial for the efficiency of clinical studies, preventing the unnecessary testing of a large number of compounds.[247] A large proportion of drugs proceeding into clinical trials never showed animal efficacy which leads to a large number of useless therapeutics.[248] Moreover, the statistical power of clinical studies poses an additional challenge to enroll a sufficient number of participants in a clinical trial to demonstrate a statistically significant study.[249] EBOV-infected animal models need to be reliable enough to reflect the patient's situation. Furthermore, the overall aspects of experimental procedures and efficacy should be estimated to a higher level.[250]

Additionally, pharmacokinetics properties particularly plasma half-life, are representative of drug efficacy. With the use of animal models, the importance of interspecies translation of half-life becomes more evident. In the light of recent clinical trials, although many treatment options for EVD have been proposed, there exists no FDA-approved drug yet. The genetics and immunological profile vary from one population to another. This poses a further challenge for evaluating drug safety profile in different populations. Identifying viable hits through in silico hit identification and screening is almost achievable for any target, however, hit-to-lead optimization remains cumbersome. A reasonable argument is that computational methods that accurately predict binding constants for chemically diverse compounds and large datasets, still need to be optimized. Second, ADMET properties are difficult to predict for large datasets because it is impossible to simplify them to a single molecular event. Incontrovertibly, it is the ADMET properties that cause the failure of most drug candidates. Increased attention has been paid to the pharmacokinetic properties during lead optimization. As a result, poor pharmacokinetic properties, once a major issue, today account for only 10% of clinical failures, mostly in Phase I.[251] With the joint efforts by regulatory institutions, meta-analytic analysis with controlled experimental protocols and performance can yield safe and unprecedented predictive results for human clinical trials.

3 | CONCLUDING REMARKS

After the largest, most devastating Ebola outbreak (2014-2016), efforts toward EVD treatment have gained vital importance. This outbreak has highlighted an urgent need to develop an efficacious treatment that can be used to curtail future outbreaks. As a result of clinical research, numerous countermeasures have been developed including vaccines (rVSV-ZEBOV and Ad26-ZEBOV/MVA-BN-Filo

prime-boost vaccine), nucleoside and nucleotide analogues (BCX4430, favipiravir, and GS-5734), plasma transfusions (Ebola-Tx), immunotherapeutics (Zmapp and MIL77), nucleic acid-based drugs, and repurposed drugs. The scientific community has to overcome multiple challenges to ensure a licensed efficacious drug for future outbreaks. In this regard, it is required to widen the prospects in the development of therapeutic agents with broad-spectrum activity against filoviruses like Marburg virus, Sudan virus, or other viral pathogens. However, the drug discovery and development pipeline lead to only a small number of compounds that enter clinical trials, thus making it not just a challenging but also a time-consuming process. Until the next outbreak, drug development efforts rely on efficacy characterization in animal models of EVD. The EBOV outbreaks have also reconfirmed the significance of the immunological basis of vaccine protection to the scientific community. This will not only help to assist the progression of vaccine candidates in development, but also vaccine efficacy can be assessed for potential outbreaks of genetically diverse strains in the coming episodes.

Within the entire process from drug discovery to authorization, a great potential can be attributed to in silico methods of drug discovery and may prove beneficial at any stage in the preclinical development of drug candidates. In silico drug discovery methods have already changed the perception of drug design and development. Methods in computational chemistry, particularly MD simulations and QSPR, will significantly impact the trajectory of the drug discovery process in the pharmaceutical industry. With an increased understanding of human biology, clinical trials are expected to gain success. MD simulations, in this case, will make useful contributions in understanding the underlying molecular processes and biological functions. Through the application of QSPR modeling, improvising the ability to design better molecules is an achievable goal. With the presence of an enormous amount of data, computational approaches are the most sought after methods to answer biological problems. With advancement in understanding the mechanism and mode of action of EBOV, future in silico work will have an essential role in the development of drug candidates against the devastating EVD.

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