

Progress in Anti-Mammarenavirus Drug Development

Subjects: Virology

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Mammarenaviruses are prevalent pathogens distributed worldwide, and several strains cause severe cases of human infections with high morbidity and significant mortality. Currently, there is no FDA-approved antiviral drugs and vaccines against mammarenavirus and the potential treatment option is limited to an off-label use of ribavirin that shows only partial protective effect and associates with side effects. For the past few decades, extensive research has reported potential anti-mammarenaviral drugs and their mechanisms of action in host as well as vaccine candidates. This review describes current knowledge about mammarenavirus virology, progress of antiviral drug development, and technical strategies of drug screening.

Keywords: mammarenavirus ; antiviral drug ; drug repurposing ; high-throughput screening

1. Introduction

Members of the family *Arenaviridae* are classified into four genera based on phylogenetic analysis of their RNA-directed RNA polymerase (L protein) and nucleoprotein (NP) sequences: *Antennavirus*, *Hartmanivirus*, *Mammarenavirus*, and *Reptarenavirus* [1]. Antennaviruses (2 species) were discovered in actinopterygian fish by next-generation sequencing, and no biological isolate has been reported yet. Hartmaniviruses (4 species) and reptarenaviruses (5 species) infect captive snakes, and some of them have been associated with boid inclusion body disease (BIBD). Mammarenaviruses (39 species) infect mainly rodents, and the infection is generally asymptomatic. Current knowledge about the biology of snake and fish arenaviruses is very limited, and their zoonotic potential unknown. In contrast, some mammarenaviruses have been found to infect and cause disease in humans.

Mammarenaviruses are enveloped viruses with a bi-segmented single-stranded negative-sense RNA genome [1]. Mammarenaviruses cause chronic infections of their rodent natural reservoirs across the world, but some of them have zoonotic potential. Human infections occur through mucosal exposure to aerosols or by direct contact of abraded skin with infectious materials [2]. Based on their antigenic properties, mammarenaviruses have been classified into two distinct groups, Old world (OW) mammarenaviruses, aka Lassa-lymphocytic choriomeningitis virus serocomplex, including viruses present in Africa and the worldwide distributed lymphocytic choriomeningitis virus (LCMV), and the New World (NW) mammarenaviruses, aka "Tacaribe serocomplex", including viruses indigenous to the Americas [3]. Both OW and NW mammarenaviruses include several species members that can cause severe hemorrhagic fever (HF) diseases in humans that are associated with high morbidity and significant mortality; these viruses include Lassa (LASV), Junin (JUNV), Machupo (MACV), Guanarito (GTOV), Sabia (SABV), Chapare (CHPV), and Lujo (LUJV) [4]. Concerns posed by human pathogenic mammarenaviruses are exacerbated by the overall lack of FDA-licensed vaccines and current anti-mammarenavirus therapy being limited to off-label use of ribavirin that is only partially effective, has a narrow therapeutic window, and can be associated with side effects [5]. The only mammarenavirus vaccine tested in humans is the live-attenuated Candid#1 strain of JUNV that has been shown to be safe and provide effective protection against Argentine HF (AHF) disease caused by JUNV [6][7]. Accordingly, Candid#1 is approved in Argentina for use in populations at high risk of JUNV infection [8].

2. Drugs Targeting Different Steps of Mammarenavirus Life Cycle

2.1. Cell Entry

Completion of mammarenavirus cell entry requires a GP2-mediated fusion event between viral and cellular membranes, a process triggered by the acidic environment of the late endosome. Therefore, targeting the GP2-mediated fusion event is an attractive strategy to inhibit mammarenavirus infection. Accordingly, a high-throughput screen (HTS) of a small molecule library identified a series of molecules (ST-193, ST-294, and ST-336) that target GP2 and inhibit viral entry [9][10]. ST-193 has been shown to confer significant protection against LASV infection in a guinea pig model [11], and the optimized chemical analog of ST-193 compound, LHF-535, was shown to be a potent broad-spectrum inhibitor of HF

mammarenaviruses via targeting the GP2-mediated fusion step of virus cell entry [12]. Another GP2-mediated fusion inhibitor, F3406, was shown to inhibit LCMV multiplication [13]. AVP-p, a peptide derived from the Pichinde virus (PICV) GP2 subunit, was found to bind the prefusion state of the GP complex, which arrests the GP2-mediated fusion event [14]. Arbidol (umifenovir) was developed as an inhibitor of influenza virus infection, but it has been shown to exhibit broad-spectrum antiviral activity against different viruses by interfering with different steps, including cell entry of the virus life cycle [15]. Arbidol inhibits both LASV and Ebola virus (EBOV) GP-mediated fusion events required to complete the virus cell entry process [16][17]. Screening of a natural product library identified tangeretin as a cell entry inhibitor of seven different HF-causing viruses [18].

Several compounds have been shown to inhibit mammarenavirus cell entry via off-target activities distinct from their intended therapeutic effects. The clotrimazole-derivative TRAM-34 is an ion channel blocker that antagonizes the calcium-activated potassium channel KCa3.1. TRAM-34 specifically inhibited mammarenavirus GP2-mediated fusion, and this anti-mammarenaviral effect was independent of its channel blocking activity [19]. Losmapimod, an p38 MAPK inhibitor, was developed as a therapeutic drug for chronic obstructive pulmonary disease (COPD) and has been reported to inhibit LASV entry by blocking the pH-dependent GP2-mediated fusion without requiring inhibition of p38 MAPK [20]. NH125, a selective eEF-2 kinase inhibitor, inhibited cell entry of recombinant VSVs expressing envelope glycoproteins of avian influenza virus, EBOV, and LASV due to the compound lysosomotropic properties and independently of eEF-2 kinase inhibition [21]. ZCL278 was identified as an inhibitor of Cdc42, a small GTPase that regulates actin polymerization [22]. Subsequently, ZCL278 was shown to exhibit broad-spectrum anti-viral activity, including against mammarenaviruses, by redistributing viral particles from endosomal to lysosomal membranes, and this antiviral activity was not dependent on the downregulation of Cdc42 activity [23]. The antifungal drug isavuconazole, which was approved by the FDA as an orphan drug for aspergillosis, mucormycosis, and candidiasis, was shown to inhibit viral fusion, targeting the SSP-GP2 interface of LASV [24]. The adamantyl diphenyl piperazine 3.3 was developed to target the lysosome-associated membrane protein 1 (LAMP1) by competing with cholesterol, preventing the interaction between LAMP1 and LASV GP [25].

2.2. Viral Genome Replication

The nucleoside analog ribavirin has been shown to inhibit mammarenavirus replication in cell culture systems, as well as to have clinical benefits when used to treat LF cases at early times after the onset of clinical symptoms [26][27]. Several different mechanisms of action have been proposed to account for the antiviral activity of ribavirin, including up-regulation of interferon responses [28], GTP pool depletion by inhibiting IMP dehydrogenase (IMPDH), and direct inhibition of viral RNA-directed RNA polymerase (RdRp) activity [29]. Due to its broad-spectrum antiviral activity against multiple RNA and DNA viruses, most likely different mechanisms of action, and combination of them, contribute to the antiviral activity of ribavirin. Notably, a recent study has shown that the main mechanism of the antiviral activity of ribavirin against LASV, is by protecting LASV-infected cells from death [30].

Favipiravir was initially developed as an antiviral drug targeting the influenza virus polymerase and subsequently shown to exhibit a broad-spectrum activity against different RNA viruses, including mammarenaviruses, bunyaviruses, flaviviruses, alphaviruses, picornaviruses, and noroviruses [31][32]. BCX4430 is another broad-spectrum inhibitor of many types of RNA viruses, including mammarenaviruses, which interferes with the activity of the viral RdRp by acting as an RNA chain terminator [33].

Peptide-conjugated phosphorodiamidate morpholino oligomers (PPMO), designed to target conserved regions within mammarenavirus genome RNA, were effective against multiple mammarenaviruses, including JUNV, LCMV, TCRV, and PICV [34]. The inhibitors of DEDDh family of 3'-5' exonucleases aurintricarboxylic acid (ATA) and pontacyl violet 6R (PV6R) have been reported to inhibit the 3'-5' exonuclease activity of LASV NP [35], which in addition to its role counteracting the host cell IFN-I response, has been shown to play a critical role in viral fitness in IFN-I deficient cells [36]. As metal-chelating pharmacophores, diketo acids, polyphenols, and *N*-hydroxyisoquinoline-1,3-diones were able to inhibit the endonuclease activity of arenavirus L protein, which resulted in inhibition of the cap-snatching mechanism used by mammarenavirus polymerases to initiate transcription of viral mRNAs [37]. A carboxamide-derivatized disulfide, NSC4492, was reported to show antiviral activity against JUNV and TCRV [38] and this compound was shown to impair viral RNA synthesis of JUNV via targeting the replication complex [39]. KP-146 was shown to have dual roles in its antiviral activity against LCMV, not only interfering with vRNP activity responsible for directing LCMV genome replication and gene transcription but also inhibiting Z protein-mediated budding process [36].

2.3. Processing of GPC

The mammarenavirus GPC precursor is co-translationally cleaved by cellular signal peptidases to generate a 58 amino acid-long stable signal peptide (SSP) and the immature GP1/GP2 precursor [40]. Subsequent processing of GP1/GP2 by

the cellular subtilisin kexin isozyme-1 (SKI-1)/site-1 protease (S1P) into GP1 and GP2 is required for the production of infectious progeny [41][42]. GP1, GP2, and the SSP form the mature trimeric GP spike complex [40]. Decanoyl-RRLL-chloromethylketone (dec-RRLL-CMK) was developed as a SKI-1/S1P inhibitor based on the cleavage recognition site present within LASV GPC [43] and was shown to exert a potent antiviral activity against LCMV as well as additive antiviral drug activity in combination with ribavirin [44]. PF-429242, a small molecule inhibitor of S1P, was shown to interfere with the proteolytic processing of GPC, which correlated with the compound's ability of inhibiting multiplication of LCMV and LASV in cultured cells [45]. In S1P-deficient cells, wild-type LCMV consistently underwent extinction without emergence of S1P-independent escape variants [44]. Moreover, PF-429242 efficiently and rapidly cleared persistent virus from infected cells, and interruption of drug treatment did not result in re-emergence of infection, indicating that PF-429242 treatment resulted in virus extinction [46]. These findings indicate a high genetic barrier for the emergence of viral variants capable of using an alternative host cellular protease for the processing of GPC, thus making S1P a very attractive target for the development of antiviral drugs against mammarenaviruses.

2.4. Virion Assembly and Cell Egress

Assembly and cell release of infectious mammarenavirus progeny involves Z-L, Z-NP, and Z-GP interactions to facilitate the co-localization of all viral proteins for the assembly of mature infectious particles [47]. Functional studies have shown that the matrix Z protein plays a key role in mammarenavirus budding, a process mediated by the interaction of Z late (L) domain motifs, PTAP and PPPY, with components of the cellular ESCRT complexes [48]. Z-mediated budding also requires myristoylation of the Z protein at a glycine (G) in position 2 to target Z to the plasma membrane, the location of arenavirus budding [49]. Accordingly, treatment with 2-hydroxymyristic acid, an inhibitor of the N-myristoyltransferase (NMT), impaired Z budding activity and production of mammarenavirus infectious progeny [49]. Valproic acid (VPA), a short-chain fatty acid, used in anti-epileptic therapy [50], was shown to inhibit Z-mediated budding of LCMV, likely due to VPA-mediated alteration of the lipid composition of cellular membranes, which is critical in virus budding [51]. Compound 0013, identified as a potent inhibitor of the interaction between the PTAP L domain and Tsg101, a member of the host cell ESCRT complex proteins, was shown to inhibit viral budding by blocking the Z-Tsg101 interaction [52]. The ubiquitin ligase Nedd4 E3 is also a component of the ESCRT complex [53]. A small molecule termed compound1 was identified as an inhibitor of Z-Nedd4 interaction, resulting in inhibition of viral budding [54]. BEZ-235, a phosphatidylinositol 3-kinase (PI3K) inhibitor, was shown to inhibit Z protein-mediated budding of LCMV and LASV by a mechanism of action yet to be determined [55]. In addition to driving virion assembly and release, Z proteins have been reported to modulate various host mechanisms such as repression of translation by binding and counteracting eIF4E [56] and suppression of host innate immune responses [57], suggesting potential host targets for developing antiviral drugs. Recent Z interactome study has identified human proteins that interact with arenavirus Z and validated potential host targets for antiviral therapeutics, including ADP ribosylation factor 1 (ARF1), ATP synthase, H transporting mitochondrial F1 complex beta polypeptide (ATP5B), ATPase H transporting lysosomal 38-kDa V0 subunit d1 (ATP6V0D1), inosine monophosphate dehydrogenase 2 (IMPDH2), peroxiredoxin 3 (PRDX3), and Ras-related protein Rab5c [58].

2.5. Monoclonal Antibodies

Monoclonal antibody-based therapies represent an attractive strategy to treat infections by highly pathogenic mammarenaviruses. A monoclonal antibody specific for human transferrin receptor 1 (hTfR1), the receptor used by pathogenic NW mammarenaviruses, inhibits viral entry of several NW mammarenaviruses, including JUNV, GTOV, CHAV, SABV, and MACV [59]. In addition, monoclonal antibodies targeting mammarenavirus GP have been reported to have potent neutralizing activity against MACV and LASV that correlated with inhibition of virus multiplication [60][61]. A recent study documented the isolation of human monoclonal antibodies from LF survivors and characterized their epitope and neutralization profiles, showing that 80% of the monoclonal antibodies with neutralizing activity targeted complex epitopes involving LASV GP1 and GP2 subunits [62]. Importantly, this study identified several human monoclonal antibodies with neutralizing activities against members of the main four lineages of LASV, and some of them showed cross-reactivity to LCMV, LUJO, and MACV. This finding has provided insights to develop therapeutic strategies based on the use of broadly reactive monoclonal antibodies.

2.6. Targeting Host Factors

As strict parasites, viruses rely on many host cell factors to complete their life cycles. Therefore, there is increasing interest in targeting host cell factors required for virus multiplication as an antiviral drug strategy. Direct-acting antivirals (DAAs) that target specific viral gene products and functions are likely to be well tolerated by the infected host cell. Still, they are limited by the common problem in antiviral therapy posed by the emergence of drug-resistant variants. In contrast, the emergence of viral variants resistant to host-targeting antivirals (HTAs) is usually significantly reduced or entirely absent, but HTAs can be associated with significant side effects. However, side effects associated with the use of

HTAs might be manageable in the case of acute infections, such as HF disease caused by arenaviruses, where the duration of the treatment would be rather short.

Dihydroorotate dehydrogenase (DHODH) small molecule inhibitors A3 and A77172 interfere with de novo pyrimidine biosynthesis and exhibit potent antiviral activity against LCMV and JUNV [63][64]. Likewise, de novo purine biosynthesis is a potential cellular target for the development of HTAs. Inhibition of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the purine biosynthesis pathway, was shown to be associated with a broad-spectrum antiviral activity against RNA viruses including JUNV [65][66]. S-adenosylhomocysteine hydrolase (SAHH) is an important cellular enzyme for regulating viral mRNA capped structures, and inhibition of SAHH activity by 3-deazaneplanocin was associated with potent antiviral activity against TCRV and PICV [67]. ATPase Na⁺/K⁺ transporting subunit alpha 1 (ATP1A1) and prohibitin (PHB) were identified as mammarenavirus NP-interacting host cell proteins and act as pro-viral factors that promote mammarenavirus multiplication. Accordingly, the ATP1A1 inhibitors, ouabain and bufalin, as well as the PHB inhibitor, rocaglamide A, exhibit potent antiviral activity against LCMV and LASV infection [68].

Anti-mammarenaviral drugs discussed in this section have been summarized in **Table 1**.

Table 1. Drugs with anti-mammarenaviral activity.

Target	Drug Name	Mechanism
Viral entry	ST-193	- GP2 targeting compounds
	ST-294	
	ST-336	
	LHF-535	
	F3406	- Inhibition of pH-dependent membrane fusion
	AVP-p	
	arbidol	
	tangeretin	
	TRAM-34	- Calcium-activated potassium channel blocker
		- Inhibition of pH-dependent membrane fusion
	losmapimod	- p38 MAPK inhibitor
		- Inhibition of pH-dependent membrane fusion in p38 MAPK down regulation-independent manner
	NH125	- eEF-2 kinase inhibitor
		- GP-mediated fusion inhibition due to lysosomotropic properties
	ZCL278	- Cdc42 inhibitor
		- Fusion inhibition by redistributing viral particles from endosomal to lysosomal membranes
	isavuconazole	- Antifungal drug for aspergillosis
		- Inhibition of pH-dependent membrane fusion by targeting the SSP-GP2 interface
	dec-RRLL-CMK	- Blocking the proteolytic processing of GPC by inhibition of host protease S1P
	PF-429242	
	adamantyl diphenyl piperazine 3.3	- Blocking the interaction between host LAMP1 and viral GP

Target	Drug Name	Mechanism
Viral genome replication	ribavirin	<ul style="list-style-type: none"> - Upregulation of host IFN responses - Cellular GTP depletion - Viral RdRp inhibition
	favipiravir BCX4430	- Viral RdRp inhibition
	PPMO	- Interfering with viral RNA synthesis and translation
	ATA PV6R	- Inhibition of NP exonuclease activity
	diketo acids polyphenols N-hydroxyisoquinoline-1,3-diones	- Inhibition of L endonuclease activity
	NSC4492 KP-146	- Targeting the vRNP to impair viral RNA synthesis
	A3	- DHODH inhibitor
	A771726	- Inhibition of de novo pyrimidine biosynthesis
	acridone	- IMPDH inhibitor
	bredinin	- Inhibition of de novo purine biosynthesis
	3-deazaneplanocin	- Inhibition of SAHH activity which is important in viral mRNA capped structure
	ouabain	- ATP1A1 inhibitor
	bufalin	- Preventing interaction with viral NP
	rocaglamide	<ul style="list-style-type: none"> - PHB inhibitor - Preventing interaction with viral NP

Target	Drug Name	Mechanism
Virion assembly and budding	2-hydroxymyristic acid	- Inhibition of NMT which mediates myristoylation of Z protein
		- Blocking Z-mediated budding
	valproic acid	- Altering lipid composition of cellular membranes which is critical in virus budding
	compound0013	- Tsg101 inhibitor
		- Blocking interaction between Tsg101 and viral Z protein that is required for virion egress
	compound1	- Inhibiting the interaction between Nedd4 and Z protein, blocking viral budding
	BEZ-235	- PI3K inhibitor
		- Inhibition of Z-mediated budding via unknown mechanism
	KP-146	- Dual roles in viral genome replication and budding

3. High-Throughput Screening for Discovery of Anti-Mammarenaviral Drugs

The development of mammarenavirus reverse genetics (RG) systems has provided investigators with a novel and powerful approach for the investigation of the cis-acting sequences and trans-acting factors that control arenavirus replication, gene expression, and budding, as well as the rescue of infectious mammarenaviruses from cloned cDNAs. These advances in mammarenavirus molecular genetics have facilitated the generation of recombinant mammarenaviruses expressing reporter genes of interest that have enabled the development of cell-based assays and HTS strategies to identify novel anti-mammarenaviral drugs and assess how they target each of the different steps of the virus life cycle [69]. Different strategies, including the development of tri-segmented mammarenaviruses [69] and the use of the self-cleaving P2A linker to facilitate expression of a reporter gene and NP from the same bi-cistronic NP mRNA [13], have been used to generate these recombinant mammarenaviruses.

However, the use of recombinant infectious HF mammarenaviruses expressing reporter genes for antiviral drug screening campaigns or the investigation of drug mechanism of action would face the complications imposed by the requirement of BSL4 biocontainment. Hence, the advantage of the implementation of RG approaches to develop non-infectious cell-based assays recreating each of the key steps of the virus life cycle and that is amenable to HTS, which can be used without the need of a high-level biocontainment facility. Thus, a number of different platforms have been developed to screen for compounds capable of inhibiting cell entry mediated by GPs of different HF mammarenaviruses, using pseudotype viruses [70][71][72]. Likewise, the biosynthetic processes of replication and transcription of HF mammarenavirus genomes can be mimicked using cell-based minireplicon, or minigenome (MG) systems [73]. These cell-based MG systems are based on the intracellular reconstitution of a functional vRNP directing expression of a reporter gene whose expression level serves as a surrogate of the vRNP activity. For this, cells are transfected with plasmids expressing the viral trans-acting factors L and NP, together with a plasmid that allows for intracellular synthesis of an RNA containing the open reading frame of a reporter gene under the control of the cis-acting regulatory sequences of the S or L genome RNA [74]. In addition to these transient transfection-based MG systems, cell lines have been generated to constitutively express a functional, non-infectious vRNP of LCMV or LASV [75]. This overcomes some technical complications related to transient transfection in the context of HTS. Thus, cell lines expressing LCMV and LASV functional vRNPs were successfully used to screen different compound libraries, resulting in the identification of a number of hits that were confirmed to exhibit antiviral activity against infectious LCMV and LASV [75].

Generation of infectious virus-like particle (iVLP) systems containing functional virus MGs allows for modeling of not only viral genome replication but also cell entry and budding [76]. A chimeric protein consisting of a secretion deficient form of *Gaussia* luciferase (GLuc) fused to the C-terminus of LASV Z protein was successfully used to develop a cell-based assay to quantify Z-mediated budding activity. This assay has features compatible with its use in HTS [77], as levels of GLuc in the tissue culture supernatant serve as an accurate surrogate of Z budding activity.

4. Drug Repurposing Strategy

The Discovery and development of novel drugs require significant investments and resources and an average processing time for market authorization of 10 to 17 years [78]. The rapid development of antiviral therapeutics is important to combat emerging viruses. Finding novel applications of clinically approved drugs can accelerate the drug development process and significantly reduce risks during clinical trials assessing the new drug application. Accordingly, repurposing existing drugs is considered an attractive strategy to combat emerging viral infections [79]. This has been illustrated by efforts to combat the current COVID-19 pandemic, where screening of libraries of already approved drugs resulted in the rapid identification of anti-SARS-CoV-2 drug candidates that were very rapidly advanced to clinical trials [80]. Among the listed compounds in **Table 1**, ribavirin, arbidol and favipiravir are currently being tested in COVID-19 patients in clinical trials [80].

Screening of a library of FDA-approved drugs using VSV pseudotyped with LASV GP identified a number of inhibitors of LASV GP-mediated cell entry [71][81]. Likewise, screening of the Repurposing, Focused Rescue, and Accelerated Medchem (ReFRAME) library identified several potent anti-mammarenaviral compounds [82]. Importantly, selected hits initially identified based on their anti-LCMV activity, which were confirmed to show potent antiviral activity against the HF causing mammarenaviruses LASV and JUNV. These compounds exerted their antiviral activity via targeting host cellular factors, including enzymes required for pyrimidine and purine biosynthesis, regulators of apoptosis, and the mitochondrial electron transport complex III [82]. Recently, this ReFRAME library was used to screen for antiviral drugs against SARS-CoV-2, and the existing pharmacological and safety data on the identified hits will facilitate their rapid testing in the clinic [83]. In addition to HTS formats to rapidly identify novel targets and antiviral drug candidates, function-focus based assays have also been successfully used to identify compounds that could be repurposed as antiviral drugs. For example, screening of a collection of kinase inhibitors identified several cellular kinases that were involved in LASV GP-mediated viral entry, including protein kinase C, phosphoinositide 3-kinase, and human hepatocyte growth factor receptor (HGFR), which is a receptor tyrosine kinase [84].

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