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REVIEW

Patent landscape of novel technologies for combating category-A Arenavirus infections

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ABSTRACT

Introduction: Arenavirus are unique category-A pathogens that are also classified as Orphan diseases. Very few options exist currently for treating Viral Hemorrhagic Fever (VHF) caused by viruses belonging to the Arenaviridae family [1]. The current review provides detailed patent landscape and a description of selected technologies developed for combating category-A *Arenavirus*. Currently, Arenavirus infections are epidemic [2] but could cause widespread pandemics due to ease of dissemination and lack of immunity against these viruses.

Areas covered: The key strings for selected Arenavirus VHF were run separately in MCPaIRS®, PatSeer, and Questel database. The search was limited to Title, Abstract and Claim fields; one member per patent family was considered for analysis.

Expert opinion: Synthetic molecules dominate the patent landscape, while natural products have not been extensively claimed for the treatment of Arenavirus infection. The broad-spectrum activity has been highly desired for Arenavirus treatment, but few reports have experimentally tested it. With each year, a constant increase in number of patents published is seen, while the maximum number of applications was filed in 2017. The research in VHF is driven by public funds; the maximum numbers of patents were filed by publicly funded organizations.

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Arenavirus; MCPaiRS®; Questel; ribavirin; NIAID; WHO; VHF

1. Introduction

Viral infections are a major challenge to the survival of mankind [1]. Viruses are constantly evolving and seem to be catching up with the progress in new technologies for combating infections. With several strains discovered newly and as some old strains evolve to challenge human health, there is increasing a need for effective therapies and diagnosis [2].

Viral infection can be pandemic, epidemic, or sporadic depending on the occurrences [3]. Viral Hemorrhagic Fever (VHF) are multisystem syndrome characterized by hemorrhage, infections are caused by viruses belonging to Arenaviridae, Bunyaviridae, Filoviridae, and Flaviridae family [4]. Currently, VHF's are epidemic with a good probability of becoming widespread pandemic [5]. With globalization and a more connected world, chance encounter of these viruses have increased. A case in this regard is the scare caused by the disappearance of a vial of Guanarito virus from the Galveston National Laboratory [6]. *Arenavirus* in wrong hands could threaten world peace.

National Institute of Allergy and Infectious Diseases (NIAID) annually publishes a list of emerging infectious pathogens based on the threat posed to public health; the list includes three categories from A to C [7]. The focus of the current work is *Arenaviruses*, which are declared as Category-A pathogens by NIAID. With Category-A pathogens posing high risk to public health, easily transmitted among humans and animals, high mortality rates, delayed diagnosis and reduced availability of therapies [7]. The category-A *Arenaviruses* include *Lymphocytic Choriomeningitis Mammaarena Virus (LCMV), Junin Virus, Machupo virus, Guanarito mamma Arenavirus, Lassa mamma arenavirus. Arenaviruses* are mostly epidemic and commonly occur in isolated parts of the Americas and some parts of Africa [8], which is reflected in the naming of diseases and these regions are also remote and lack resources. LCMV infection is mild, in most of the cases (70%) it is asymptomatic, while all other Arenavirus infections have 15-20% mortality rate [7]. The general clinical manifestation of Arenaviruses infection is onset of fever over 2–3 days, followed by febrile illness and the virus has incubation time of about 2 weeks [1].

The current work provides a detailed patent landscape analysis for each of the above diseases and a description of selected technologies developed for combating these infections. These diseases are categorized as rare diseases by WHO, which in coordination with national public health agencies continuously monitors outbreak, establishes treatment standards and emergency response protocols [9].

Arenaviruses have evolved a three pronged strategy to infect its host including–ease of dissemination (vector borne/aerosol mediated), rapid systemic infection coupled with low immunogenicity and altered capillary permeability of blood [1]. The general morphology of Arenavirus is spherical with an envelope containing lipid molecules, glyco-proteins (GP) and embedded ribosomes. The genome is made up of one pair of single stranded, negative sense RNA with hairpin structure [4]. The Viral genome can be divided into two segments Large (L)

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Article highlights

- Arenavirus infections are category-A pathogens causing sporadic infections, which are also designated as Orphan diseases.
- Currently available treatments are supportive care and Ribavirin.
- VHF are epidemic, with a good probability of becoming widespread pandemic.
- VHF have 15–20% mortality rate, with the exception of LCMV. LCMV infections are mild and asymptomatic in 70% of the cases.
- Synthetic molecules dominate the patent landscape, while natural products have not been extensively claimed for the treatment of Arenavirus infection.
- A constant increase in number of patents published is seen, while the maximum number of applications was filed in 2017.
- The research in VHF is driven by public funds; maximum numbers of patents were filed by publicly funded organizations.

and small (S) interconnected by an intergenic region, wherein the S segment encodes a nucleocapsid protein(NP), and envelop glycoprotein. The L segment of viral RNA encodes a zinc binding protein and an RNA polymerase. Nucleo protein antigens are the most conserved among the Arenaviridae family [10]. Glycoprotein is composed of two units, GP1 and GP2 which enable the virus binding to cell surface receptor and entry into cells [10].

Arenavirus multiplication occurs in the cytoplasm of infected cell, initially the L-segment is translated into nucleoprotein (NP) and the accumulation of NP signals the replication of viral RNA. The templates thus formed serves in translation of viral proteins [4]. The viral release is through budding, during which the viral components assemble at the cytoplasmic membrane. The budding of viral progeny is assisted by Z protein [7].

Working with *Arenaviruses* requires all experiments to be carried out at the highest containment, i.e. Biosafety Level-4. Very few options exist currently for treating *Arenaviruses* infection, while several are in developmental stage [11]. The most common treatment is supportive care with fluid replacement [12]. Some *Arenaviruses* strains have 100% morbidity in children while it varies in adults [1]. Currently, the requirement is for early diagnosis, reduction in hospitalization days, awareness among physicians and the local population. Arenavirus infections are seasonal and chance infection is dependent on exposure to the vector or aerosol particles. These rodent-transmitted infections occur majorly during harvest season among agricultural laborers and field workers [12]. Each of the Arenaviruses has its specific rodent reservoir; viral particles are known to propagate in vectors without showing any symptoms [13]. Arenavirus infections are unique category-A pathogens that are also classified as Orphan or Rare diseases in many countries including the USA and European Union. *Orphanet* is a network funded by INSERM and European commission that collects and disseminates information on orphan diseases with the aim of improving patient's quality of life and management of rare diseases [7]. The ORPHA number is a numerical Identifier given by ORPHAnet to Arenavirus infection and is indicated in Table 1. Orphan status eases the regulatory hurdles and provides tax benefits for drug development to orphan diseases, also expenditure on rare disease research can be claimed as tax exemptions [8]. On the regulation front, US-FDA & EMA has formulated animal rule to fast track development of orphan drugs in which drugs with established safety profile can be used for treatment without clinical trials [6]. Currently, investment by private players on Arenavirus therapy is low due to its reduced prevalence in developed countries.

Most of Arenavirus cases are zoonotic infections wherein, the carrier rodents shed the viral particles in saliva, urine, and feces. Infection in humans is through accidental contact or through inhalation of aerosol particles dispersed by the rodent carrier [13]. Due to ease of transmission, both vertical and horizontal transfer of virus has been reported, while human to human transmissions are rare [1]. Arenaviruses infection is associated with nonspecific clinical manifestation and hence difficult to diagnose, often leading to wrong diagnosis [7]. Virus isolation and in-vitro culture in Vero E6 cell line has been the gold standard test for VHF diagnosis. Currently, polymerase chain reaction (PCR) based diagnostics are available and are finding increased usage [14]. Delay in diagnosis leads to increased risk of horizontal transmission among humans. Usage of personnel protection equipment and standard containment procedures can reduce the chances of secondary infections and nosocomial infections to near zero [1].

Ribavirin is a broad spectrum antiviral used for the treatment of Arenavirus infection; it works by inhibiting IMP dehydrogenase and leading to inhibition of viral replication through depletion of GTP [15]. Common adverse reactions reported for intravenous administration of ribavirin include anemia and thrombocytosis [16]. Sulfated polysaccharides [17], nucleoside analogues [8] and phenothiazines [1] have also been reported to be effective in inhibiting Arenavirus in-vitro. Convalescent serum administered to infected patients has shown limited effectiveness in reducing viremia in patients [3].

The key strings (supplementary material-1) were run individually in Questel, PatSeer, and MCPaIRS® databases with search fields limited to Title, Abstract, and Claims. One member per patent family was considered for analysis. The resulting documents were de-duplicated followed by manual categorization and grouping into new chemical entities, vaccines, gene therapy, and diagnostic groups based on the focus

Table 1. Table indicating Arenavirus associated mammalian carrier and corresponding orphan disease ID.

Virus	Disease	ORPHA Number	Vector
Lassa virus	Lassa Fever	99,824	*Mastomys natalensis*
LCMV	Lymphocytic Chriomeningitis	-	*Mus musculus*
Junin Virus	Argentine Hemorrhagic Fever	319,223	*Calomys musculinus,*
Machupo virus	Bolivian Hemorrhagic Fever	319,229	*Calomys cf. callosus*
Guanarito mammarena virus	Venezuelan Hemorrhagic Fever	319,234	*Zygodontomys brevicauda*

of independent claims. Patents related only to the production method of viral particles or vaccines and personnel protection methods/equipments were not considered for analysis. The patents were further grouped based on their patent family legal status, to identify active patents. Patents published in last 25 years were included in the analysis, with 1 September 2019 as cutoff.

A brief description of VHF's followed by active patents describing treatment or diagnosis is provided in the following sections.

2. Argentine hemorraghic fever (AHF)

AHF is caused by Junin virus, cases of Junin virus infection are commonly reported during harvest season in Argentina which lasts from February to May [18]. Clinical manifestation includes shock, hypotension, petechiae, ecchymoses. Clinical findings include leukopenia, thrombocytopenia, and proteinuria, bleeding, and neurologic signs [12]. With the availability of only ribavirin established for treatment, search for new antiviral compounds has gained momentum. Some of the active patents relating to AHF infections are as follows:

Host recognition of infectious particles is the first step in generating immunity in humans, the host recognition is mediated by pattern recognition receptors like STING and NOD2. STING receptors are sensitive to double-stranded DNA and cyclic di-nucleotides, upon activation STING receptor signals interferon production. WO2019161171A1 [19] discloses cyclic-di-nucelotides (CDN) which are STING agonists activating the host innate immunity against viral infection. The patent also encompasses a polymeric nanoparticle formulation containing Compound-1 with a higher circulating half-life. CDN exert their pharmacologic action by inducing expression of STING receptor. Compound-1 was tested for inhibition of Junin virus in plaque assay, reduction in virus yield was observed upon treatment with 2,2 fluoro-cyclic adenothymidine(Compound-1). 10022375 USDB2 [20] discloses Immucillins as inhibitors of viral RNA polymerase. The compound-1 has 90% clearance (plasma concentration) within 6 h of oral administration in mice. In neutral red uptake assay, the compound showed EC_{50} of 16 µg/ml and IC_{50} of 240 µg/ml when tested in Vero cells infected with Junin virus (Candid1 strain). Tests have shown that the Immucillins have broad spectrum antiviral activity.

Bergamottin is naturally occurring furanocoumarin, found in grapefruit and pomelo. It is known as an antioxidant in Chinese medicine. CN109864988A [21] describes the use of Bergamottin as an antiviral inhibitor, with an IC_{50} of 3.3 µM, inhibiting Junin virus in-vitro. Bergamottin acts by interfering with the entry of viral particles by binding to the glycoprotein on the viral surface. 9879003 USDB2 [22] discloses compounds that exert therapeutic action by covalently modifying cysteine residues of the host target proteins, particularly cysteine residues at the active site of enzyme molecules. The advantage of this approach is that such compounds have broad spectrum antiviral activity. QL-XII-47 a tricyclic quinoline derivative compound, was tested against Junin virus for antiviral activity, and was seen to inhibit the cytopathic effects during infection. 20150297677 USDA1 [23] discloses antibodies against human T-cell Immunoglobulin and Mucin(TIM)-domain containing proteins including its four subtypes for inhibiting virus entry into the host cell. The antibodies bind to TIM preventing interaction of host cells with the virus envelope. Mouse monoclonal antibody against TIM1 inhibited Junin virus entry into Huh7 cells by 70% at a concentration of 50 nM.

Iminosugars such as de-oxyno-jirimycin(DNJ) derivatives interfere with the morphogenesis of Junin virus, 20100222384 USDA1 [24] describes the synthesis of DNJ derivatives. During screening for Junin virus inhibition by DNJ derivatives, NAP-DNJ (structure included in supplementary material-3) showed excellent inhibition of the virus with EC50 of 10 µM and reduction of viral yield was also noticed.

CN110204613A [25] discloses a polyclonal antibody against Junin virus GP1. The antigen was expressed in HEK293 cells by recombinant transfer of viral GP protein, the viral protein was purified and injected into horse. The polyclonal antibody obtained from horse serum and purified by chromatography for use in the treatment of humans, it has broad spectrum activity against new world Arenavirus. The antibody has EC_{50} of 13.88 µg when tested in Junin virus infected VeroE6 cells.

RU2525938C1 [26] discloses a set of fluorescent labeled oligonucleotide primers for real time detection of Junin viral RNA by PCR, with a minimum detection limit of 100 genomic equivalent copies.

3. Bolivian hemorrhagic fever (BHF)

BHF is endemic to Bolivia, the number of reported cases had been declining, probably due to better rodent control. A spike in reported cases is seen since 2006, with the latest reported outbreak in 2019 [27]. Limited resources have been allocated for BHF by local health authorities, which are overburdened with other diseases. Agricultural workers are the highest risk group due to proximity with the carrier rodent [3]. The clinical manifestations are very similar to AHF, intravenous Ribavirin has shown effectiveness against BHF. Horizontal transfer of Machupo virus among humans has been reported.

CN105929170B [28] discloses a rapid, simple, and sensitive indirect immune fluorescence detection of IgG antibody against the Machupo virus. IgG antibody specific to virus glycoprotein is detected using a secondary antibody that is fluorescently labeled. The method was tested in HEK 293 cells infected with Machupo virus; is sensitive, easy to use and exhibits good specificity.

RU2525937C1 [26] discloses fluorescence labeled primers for detection of Machupo virus in body fluids. The primer binds to S-segment of the viral genome and can be used for real-time detection of virus. Sensitivity of the test was 100 genomic equivalents/reaction.

4. Venezuelan hemorrhagic fever (VeHF)

VeHF is endemic to central Venezuela first reported in 1989, several sporadic cases have been reported since then [12]. Clinical manifestations include acute febrile illness, weakness, headache, mylagia, vomiting with diarrhea. VeHF is seasonal with nearly half of the reported cases are in the months of

December and January coinciding with the agricultural activity, with the majority of identified victims being agriculturists [12].

The document 9498470 USDB2 [29] discloses Benzimidazole compounds synthesized for improved potencies, solubility. Based on structure-activity relationship, 25 Benzimidazole compounds were synthesized and subsequently screened for antiviral activity against Guanarito virus. Of the 25 screened only five compounds showed inhibition, except for one molecule (compound-12) all the molecules had an EC_{50} of <1 μM.

5. LCMV fever

LCMV is the only Arenavirus with reported global footprint; this is partly due to its carrier *Mus musculus*, the common mice [30]. LCMV also has the least mortality among Arenaviruses, most of the cases are asymptomatic or mild febrile illness is reported. Clinical manifestations of disease include low glucose in CSF, meningitis and encephalitis. Most of the reported cases of LCMV infection have been traced to rodent pets or chance encounter of the carrier mouse. Infection in gestational period can cause still birth, microcephaly, or chorioretinitis [13].

WO2019010422A1 [31] discloses programmable antiviral therapy that targets viral RNA replication using type VI CRISPR nuclease system. Plasmid carrying guide RNAs targeted to different parts of the LCMV genome was transfected to HEK293 cells using Lipofectamine-2000. This was followed by LCMV infection to HEK-293 cells and the viral particles were measured after 24 h of infection by qualitative RT-PCR. By modifying the guide RNA, the system can be multiplexed and can be used for targeting different viral strains without inducing any resistance. The CRISPR system shows reduction in viral load and guide RNA targeted to L-segment of the LCMV showed a very high reduction in viral inhibition. 9238042 USDB2 [32] discloses antisense oligomers for the treatment of LCMV infection. The oligomers were tested in mouse model (FVB strain). The oligomer PPMO IL17RC-SD12 when administered intra-peritoneal into LCMV infected FVB mouse, increased the survival of mouse by 66%. Along with significant reduction in viral load, the treated animals showed increase in liver and kidney function. PPMO IL17RC-SD12 is antisense, synthetic nucleotide modulating immune dysfunction mediated by IL-17/IL-23 signaling pathway. PPMO IL17RC-SD12 targets interleukin expression by binding to target RNA forming double stranded RNA complexes which are cleared by ribonucleases. The nucleotide is modified with phosphorodiamidate linkages and morpholino bases. 20180086749 USDA1 [33] discloses synthesis of compound ML-416; it has broad spectrum antiviral activity, low cytotoxicity (CC50 = 70 μM). ML-416 is very effective against LCMV, it acts by interfering with pyrimidine synthesis. Three times reduction in viral load was seen upon administering ML-416 in Vero E6 cells.

Bepecin is a pentadecapeptide which is nontoxic, nonteratogenic and non-genotoxic. A salt form of bepecin with enhanced gastric stability has been presented in the invention. EP2968442B1 [34] discloses Bepecin as antiviral against LCMV. Bepecin has broad spectrum antiviral activity against LCMV. In mouse models, bepecin (0.020 mg/kg body weight of animal) either delayed the LCMV infection symptoms or prevented death of the animal. Bepecin was more effective in preventing mortality when administered 2 h prior to viral infection, while the survival was reduced to 20 days post infection when bepecin was given after infection. 9943585 USDB2 [35] discloses a method for attenuation of LCMV virus by deleting IGR linking the two segments (L and S) of the viral genome. The attenuated virus is administered as vaccine to generate immunity against the wild type LCMV. IGR functions as transcription stop signal and is involved in genome organization, deletion of which leads to the formation of replication defective virus like particles.

CN105601750B [36] discloses a recombinant C-peptide fusion protein to induce immunity against LCMV infection. The fusion protein consists of human C-peptide linked to S-segment of the LCMV (167–224 amino acids) through a linker peptide (2–10 amino acids). The C-peptide acts as a guide is resistant to proteases and prevents elimination. The fusion protein was tested in Mouse models, about 0.25 mg of the fusion protein was injected intra-peritoneally and the immunization was repeated twice with a gap of 3 weeks. Production of antibodies was evaluated by indirect ELISA. 7553932 USDB1 [37] discloses treatment of viral infection by targeting host proteins. It is disclosed that upon injecting (intra parental) IL-10 R neutralizing antibody to BALB/c mouse at different times, viral titer was reduced followed by reversal of lymphopenia. Inhibition of virus is mediated by CD8 T cells.

WO2018106712A1 [38] discloses a human Monoclonal Antibody (hMAb) against glycoprotein of Lasa virus. The hMAb is seen to have cross reactivity with glycoprotein from LCMV, since the GP from Arenavirus is highly conserved. The hMab was found to neutralize all four serotypes of LCMV strains. The antibody can be used for treatment or diagnostics. 8883429 USDB2 [39] discloses a sensitive ELISA-based method for detection of acute, chronic or persistent LCMV infection in human serum samples. The method is based on detection of Nucleo Protein (NP) specific antibodies present in serum using horseradish peroxidase conjugated monoclonal antibody.

CN102399904B [40] discloses primers for detection of mouse LCMV by real-time fluorescence quantitative PCR, the method is simple, fast, and sensitive. For specificity and accuracy, the primers were designed to target the conserved sequences from the S-segment of the viral genome.

6. Lassa fever

Lassa fever was first reported in 1969 during a nosocomial outbreak and is the largest cause of febrile illness in West Africa [5]. Clinical diagnosis of Lassa fever includes fever, dysuria, pharyngitis, conjunctivitis, vomiting, diarrhea, facial edema, bleeding. About 50% of neonates infected with Lassa virus have permanent hearing loss, high mortality rates are observed in patients with lower immunity and nosocomial infections [11]. Reported cases range from 100,000 to 300,000 annually with 16% mortality rate [5]. The incubation period of Lassa fever varies from 1 week to 3 weeks. 20170114060 USDA1 [41] discloses broad spectrum host oriented antiviral drugs for treatment of Lassa fever. The

compounds inhibit ORAI-1 receptor that regulates calcium entry, which is essential for budding of new viral particles formed. Typical compounds include 2-aminoethoxydiphenyl borate (2-APB), Synta66. 10383852 USDB2 [42] discloses a method of treating viral infection through depletion of Guanosine nucleoside/nucleotides. Depletion can be achieved by administration of compounds like mycophenolic acid (MPA) through diet. MPA exhibits broad spectrum antiviral activity, it was tested for protecting Vero E6 cells against Lassa fever virus infection. MPA inhibited the virus with EC_{50} of 0.79 µM.

CN106511343B [43] discloses the use of lacidipine for antiviral treatment. Lacidipine is a synthetic dihydro pyrrole pyridine molecule and a potent calcium channel blocker, currently used for treating hypertension. Lacidipine was tested for inhibition of Lassa virus in Vero E6 cells, EC_{50} was found to be 2.6 µM. Lacidipine interferes with the GP mediated viral entry into the Vero cells. 8148428 USDB2 [44] discloses a high-throughput screen consisting of 400,000 small molecules against Lassa virus glycoprotein. Ten compounds were found to have EC_{50} below 100 nM in plaque reduction assay. Among the tested compounds, compound 408,306 was the most potent and selective to Lassa virus with EC_{50} of 20 nM. Compound-408,306 is a hydrazide compound that inhibits the entry of viral particle into the cell. 20190203211 USDA1 [45] discloses REV-ERBα as a target for inhibition of viral entry in cells. REV-ERBα regulates cellular metabolism and immunity, also functions as a major component of the feedback loop in transcriptional regulation of the circadian clock. Pseudo particles expressing viral envelope glycoprotein from Lassa virus and reporter genes were inoculated to Huh-7 cells, pretreated with GSK4112 or SR9009 at different concentrations. Inhibition was observed at sub-optimal concentrations and broad spectrum inhibition was also seen. In further experiments, siRNA directed against REV-ERBα also inhibited viral entry into Huh-7 cells. 8455455 USDB1 [46] discloses siRNA-lipid complex called Stable Nucleic Acid Lipid Particle (SNALP) for inhibition of Lassa virus. The siRNA has been chemically modified to obtain low immunogenic profile. siRNA were designed to target various sections of the Lassa virus genome particularly the Nucleoprotein and the RNA polymerase. siRNA in combination with lipoFectamine-2000 were added to HepG2 expressing LASV proteins., The siRNA targeting the Nucleoprotein showed inhibition at lower concentration (KD_{50}). 8999925 USDB2 [47] discloses peptides for the treatment of Lassa virus infection. The synthetic peptides were tested against the recombinant Vesicular stomatitis virus (rVSV) expressing Lassa virus glycoprotein. The peptides were tested for plaque reduction in Vero cells, peptides showed sequence specificity and complete inhibition of plaque formation at 20 µM. For rVSV, peptide-1 showed IC_{50} of 63.8 µM. The possible mechanism of viral inhibition is through change in bilayer rigidity of the viral particle and interference in viral entry.

WO2019145739A1 [48] discloses plasmid vectors as vaccine against Lassa virus nucleoprotein. The plasmid, contain expression cassettes coding Modified Vaccinia virus Ankara (MVA), Lassa Nucleoprotein, reporter gene-GFP and promoters, was injected intramuscularly into female Dunkin-Hartley guinea-pigs. Weight gain, antibody response and clinical symptoms of Lassa virus infection were monitored throughout the study. After 38 days post vaccination with plasmid the animals were challenged with Lassa virus, no clinical symptoms were observed in vaccinated groups. Whereas unimmunized animals met humane end points, weight loss, and high body temperature were also recorded. 10342861 USDB2 [49] discloses codon optimized live attenuated vaccines (LAV) which produce robust humoral and cellular immunity with a single immunization. CD optimized LAV has low virulence, the strongest immunogenicity, and high efficacy.

WO2019/018501A1 [50] discloses a live-attenuated vaccine based on MVA(GEO-LM01) vector for immunizing against Lassa fever. The method encompasses a recombinant MVA expressing Lassa virus like particles (VLP) to elicit robust immunity mediated by T-cell after a single dose. The vector produces broadly neutralizing antibodies specific to all known strains of Lassa virus. The vector consists of mutated glycoprotein (R207 C, E329P, and G360 C) which upon translation produces GPC and Z proteins that self assembles to form VLP to serve as immunogens. The vector was evaluated in animal trials consisting of CBA/J mice. The immunized mice were challenged 14 days after vaccination by intracranial injection of 1000 plaque forming units (pfu) of rMVA, and the animals were monitored for weight change, mortality and morbidity. It was observed that all the immunized animals survived, whereas the non immunized control group died after 8 days.

EP2731628B1 [51] discloses a method of delivery for DNA vaccines into live animals by electroporation. The electroporation device is Minimally invasive Intradermal Device (MID), with three sets of electrodes arranged triangularly. The electrodes are equally spaced with a gap of 3 mm on one side and 5 mm on the other sides. The device was validated for transfection of guinea pigs and non-human primates. Using MID, 100 µg of DNA vaccine encoding codon optimized optimized-Lassa virus Glycoprotein precursor complex under CMV promoter control was transfected in guinea pigs and compared with appropriate controls. Four weeks after vaccination the animals were challenged with 1000pfu of wild type Lassa virus, injected subcutaneously. 83% of the vaccinated animals survived, will all the animals in control group succumbed to infection.

WO2018/115525A1 [52] discloses mRNA-based vaccines against Lassa virus. Polymer-lipoid nanoparticles were used for the delivery of mRNA. Lipid nanoparticle (LNP) containing mRNA coding Lassa virus GPC was used to vaccinate female BALB/c mice, the resulting antibody titers were measured by ELISA. The vaccine composition was further tested in a phase-1 clinical trial, in which healthy human volunteers were administered intradermally mRNA vaccine for two times. Blood sera were collected from volunteers and assayed for virus neutralizing antibodies.

CN105296507A [53] discloses Lassa fever virus like particles. The nucleic acids coding various Lassa fever proteins were cloned into Baculovirus vector and expressed in insect cell line. The virus like particles thus formed were isolated and purified and administered to healthy BALB/c mice, the antibody titers thus formed were measured by ELISpot assay. Antibody neutralization activity was measured using

immunized mice serum, VLP were found to be more immunogenic if administered along with adjuvant. 10357562 USDB2 [54] discloses monoclonal antibody against glycoprotein of Lassa virus. Human origin Mab is IgG class, was tested in guinea pigs. LASV Josiah challenged guinea pigs were administered via intra-peritoneal route with two monoclonal antibodies MAb GP19.7E and MAb GP10.4B at 30 mg/Kg and 15 mg/Kg, respectively. The animals were monitored for diseases symptoms, control animals showed typical signs of Lassa virus infection and succumbed by day16 post-infection; whereas Mab treated animals did not show any symptoms of Lassa virus infection. The antibodies provided complete protection and prolonged the survival. The antibodies were isolated from peripheral blood mononuclear cells (PBMC) obtained from convalescence blood.

CN105548539B [55] discloses an indirect immuno-fluorescence method for detection of anti-Lassa virus IgG antibody. Lassa virus immunized rabbit serum was used as positive control and sera samples from humans not infected with Lassa virus were used as negative control. Fluorescent labeled secondary antibody was used to detect IgG.

CN109338016A [56] discloses a rapid detection kit for Lassa virus diagnosis based on fluorescence PCR. The kit also includes positive and negative controls for fast and easy diagnosis. The primers are designed to be specific and sensitive to Lassa virus.

CN109055617A [57] discloses a RT-LAMP method, with high sensitivity and specificity, for the detection of Lassa virus. Primers were directed to bind viral conserved NP gene, five sets of primers were designed. The isothermal amplification was carried at 63°C, the reaction was monitored using calcein, a metal ion indicator. Lassa virus positive samples give a green color due to the interaction of Magnesium ions and calcein. 10251904 USDB2 [58] discloses compounds that inhibit Arenavirus polymerases, the compounds are modified nucleosides. The details of synthesis have been included in the patent, about 32 compounds were synthesized and screened for inhibition of viral polymerase and antiviral activity in-vitro. Of the tested compounds, compound-9 and compound-32 showed inhibition of Lassa virus. The effect of compound-9 and 32 was tested in HeLa cells. Compound-9 and Compound-32 were effective at 2 μM and 1.65 μM EC_{50} respectively. The compound-9 is also marketed by Gilead Sciences as *Remdesivir* for the treatment of Ebola HF. *Remdesivir* is designated as orphan drug and has been approved for use in EU and USA [59].

7. Patents with broad-spectrum antiviral activity

Due to evolutionary similarity among different Arenavirus, drugs show cross-reactivity in inhibition of Arenaviruses. Broad-spectrum neutralizing abilities are economically favorable due to sporadic and low prevalence of these infections. Several patents claim broad-spectrum activity, patents which demonstrate broad-spectrum neutralizing abilities are;

WO2019173602A1 [60] discloses halogen containing nucleotides for treatment of Junin virus and LCMV. The halogen containing nucleotides are targeted for inhibiting viral RNA polymerase. Several molecules have been synthesized by combinatorial approach, of which two compounds 4′-Fluorouridine (EIDD-02749) and Cyclo-sal 4′-Fluorouridine (EIDD-02838) have been tested against Junin virus. The complete drug profile of EIDD-02749 has been studied and shown to have EC_{50} of 1.86 μM when tested in Vero E6 cell line. EIDD-02749 was tested for cytotoxicity (CC_{50} 380 μM), stability (20 min in human plasma) and excellent tolerability in mice. EIDD-02838 was effective with 0.23 μM EC_{50} and 12-min plasma stability in humans. EIDD-02749 and EIDD-02838 are broad spectrum antiviral effective against several strains of Arenaviridae. 4′-Fluorouridine (EIDD-02749) was effective in inhibiting LCMV and has EC_{50} 7.22 μM when tested in Vero E6 cell line.

Phosphodiesterase type-IV (PDE4) is involved in degrading cAMP in immune cells, thus regulating the release of inflammatory cytokines. 8461177 USDB2 [61] discloses the antiviral activity of Benzimidazole compounds and derivatives against Arenaviruses. Compound-1 shows antiviral activity against Junin virus, which acts as inhibitor of PDE4. The compound-1 has been tested and compared with Ribavirin for protection of Guinea pigs challenged with Junin virus. When administered orally the compound-1 improved the survival of animals by 30% as compared to 100% survival with Ribavirin. These molecules are hydrophobic and have poor aqueous solubility. Compound-1 has EC_{50} less than 0.05 μM against Machupo virus glycoprotein. The compound-1 has broad-spectrum antiviral activity, has been tested against Lassa virus, VSV, Junin, and Guanarito. Compound-1 has EC_{50} less than 0.13 nM against Lassa virus glycoprotein.

In CN109745319A [62] Isavuconazole and its prodrug Isaconazole sulfate are reported as antiviral for treating Junin virus and Lassa virus infection. Isavuconazole is approved by USFDA for the treatment of invasive Aspergillosis. Isavuconazole's antiviral activity has been reported for the first time, in HEK293 cells infected by the Junin virus, drug has an IC_{50} of 0.6 μM. The antiviral mechanism of Isavuconazole is by inhibition of glycoprotein mediated viral entry into the host cell.

Transferrin receptor is involved in the internalization of viral particles during infection. The patent 9439973 USDB2 [63] discloses RNA aptamer loaded on to lipid nanoparticles (300 nm size). The aptamer specifically binds to the human transferrin receptor without affecting its binding to the human cell surface. The aptamer contains 2′-Fluoro modified nucleotides and effectively inhibits Junin virus internalization. The aptamer was tested for protection of U2OS cell line infected with recombinant VSV containing GFP tag and Junin virus Glycoprotein. At a concentration of 1 μM the aptamer showed significant inhibition of Junin virus. The aptamer was tested for protection of U2OS cell line infected with recombinant VSV containing GFP tag and Machupo virus Glycoprotein. At a concentration of 1 μM, the aptamer showed significant inhibition of Machupo virus.

8. Conclusion

Viruses belonging to the Arenaviridae family present a unique threat; they are cause of serious epidemic VHF with a high possibility of giving rise to pandemic. The outbreaks are

continuously monitored by public health agencies where these VHF's are endemic [7]. Significant resources are invested to develop diagnostics and therapy, yet an effective treatment is still elusive. The Arenaviruses are categorized as category-A pathogens by NIAID for the past 10 years [7], to encourage private investment, and several of the VHF's are designated as Orphan diseases which provide tax exemptions.

The complete patent landscape is represented in the supplementary material-2. Two strategies commonly adopted in fighting Arenaviruses are targeting the host cell proteins and attenuation of viral entry. Of these, the former approach of targeting host cell proteins is advantageous as it leads to lesser chances of resistance and can provide broad spectrum neutralizing abilities. Host cell targeting is based on targeting either downstream effectors genes like interferon signaling or immune-related genes and receptors.

Viral Glycoprotein and RNA polymerase are primary targets for viral inhibition, with several antagonists designed against these proteins. Ribavirin has been highly effective in the treatment of some Arenavirus strains, but effectiveness reduces in some strains [15]. Several cases of drug re-purposing are evident from the patent landscape; one particular case is of *Remdesivir*. Viral nucleoprotein is conserved among several strains of Arenaviridae, but not targeted extensively could be excellent candidate for broad spectrum antibodies.

Broad-spectrum neutralizing antibodies and small molecules are desired because the Arenaviruses present similar clinical manifestations that are difficult to diagnose. Several technologies are present in patent literature which shows promising broad-spectrum activity.

Vaccines can be administered prophylactically and can be advantageous in places where these diseases are regular in occurrences. Antibodies including monoclonal, heavy chain fragments and polyclonal have been developed and tested in animal models but yet to be proven in a clinical setting. Attenuated Arenaviruses providing single shot [16] immunity has been patented but concerns regarding safety are yet to be clinically tested. Modified or replication deficient recombinant Adenovirus and MVA carrying Arenavirus glycoproteins have shown promising results in animal trials.

Nucleic acid-based therapies include RNA aptamers and targeted CRISPR-nuclease based system. CRISPR–nuclease system enables to target one particular genetic element of Arenaviruses, this recombinant technology is very promising. The guide RNA stability during transfection has also been addressed by chemical modification. Vectors coding for viral proteins regulated by suitable promoter elements have been tested as vaccines and antisense-based viral gene silencing methods have been developed. Most nucleic acid-based therapies are tested in cell culture or animals, due to ethical consideration none have progressed to the clinical testing.

Most Arenaviruses share clinical manifestations which often lead to miss diagnosis by physician; several VHF cases are commonly diagnosed as dengue fever [14]. Currently, real-time based PCR followed by culturing the virus in Vero E6 is used for confirming the type of VHF. Virus has an incubation period of 2 weeks, early diagnosis is required to prevent nosocomical infections. Drugs like Ribavirin are effective when given early in the incubation phase when the viremia load is less [8]. The problem in early diagnosis is partly due to the remote location of these endemic diseases and the lack of resources and awareness. Isothermal PCR-based LAMP has been reported, which is suitable for remote diagnosis without the requirement of sophistication. LAMP method of diagnosis is cost-effective, easy to implement and interpret, and provides high sensitivity due to usage of multiple primers.

A one-to-one comparison of technologies could be limiting due to variation in the testing procedures and the models used for infection. Various animal models such as Guinea pigs, BALB mice, non-human primate models like *Rhesus macaques*, Cynomologus monkey have been employed for testing [30]. For cell culture studies, VeroE6 cells are commonly used to determine plaque reduction in presence of the test agent. Apart from Vero cells, Huh-7 and HEK-293 are also employed.

The cost associated with testing new VHF therapeutics is high due to the requirement of BSL-4 facilities, which are few. For preliminary testing, recombinant adenoviruses or MVA or VSV carrying Arenavirus targets are expressed and testing carried at BSL-2 facilities.

9. Expert opinion

Viral RNA polymerase has been frequently targeted using modified nucleotides and inhibitors. Synthetic molecules dominate the patent landscape, while natural products have not been extensively studied for the treatment of Arenavirus infection.

Viral entry blocking has been a common approach for antibodies. Since viral nucleoprotein is conserved throughout Arenaviridae family, it can be targeted to develop antibodies with broadspectrum neutralizing capabilities.

Further, there is lack of established animal infection models. All experiments need to be conducted in BSL-4 facility, which adds to the cost of development [64]. To overcome the requirement of BSL-4 during preliminary screening, required Arenavirus genes are cloned into vectors such as eukaryotic plasmids, MVA and Adenoviral vectors, which can be handled in BSL-2. Though cost-effective the method may be susceptible to false positives hits [65].

Broad-spectrum activity has been highly desired for Arenavirus treatment, but few reports have experimentally tested it. A one-to-one comparison of technologies could be limiting due to variation in the testing procedures and the models used for infection.

Year on year filing trend (supplementary data-2) indicates a constant increase in number of patents published with each year, with maximum number of applications filed in 2017. Since 2015 an exponential increase in patent filling is observed, which follows out-break of Ebola hemorrhagic fever in 2014. The outbreak has brought the focus on developing therapies for other VHF.

The research in VHF is driven by public funds, maximum patents where filed by publicly funded organizations, among which BROAD institute had the highest number of applications assigned. The BROAD institute hosts Drug Repurposing

Lab and is leader in CRISPR-based gene silencing. A significant number of patents are held by national departments such as the US Army, the US Department of Health & Human Services. Among the private players, Kineta, Inc. leads the number of patents filed. Kineta, Inc., is a clinical stage biotechnology company that has developed a small molecule against Lassa virus. The molecule blocks entry of the virus into cells, is currently under Phase-1 human safety studies. Kineta Inc, hosts a Biodefense program funded by NIH and Wellcome Trust for developing broad-spectrum antivirals against Arenaviruses. The other top private players are Agenovir and Aurigene Discovery Technologies Ltd, with five patents each.

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Declaration of interest

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