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Status of antiviral therapeutics against rabies virus and related emerging lyssaviruses

Venice Du Pont[1], Richard K Plemper[1] and Matthias J Schnell[2]

Rabies virus (RABV) constitutes a major social and economic burden associated with 60 000 deaths annually worldwide. Although pre-exposure and post-exposure treatment options are available, they are efficacious only when initiated before the onset of clinical symptoms. Aggravating the problem, the current RABV vaccine does not cross-protect against the emerging zoonotic phylogroup II lyssaviruses. A requirement for an uninterrupted cold chain and high cost of the immunoglobulin component of rabies prophylaxis generate an unmet need for the development of RABV-specific antivirals. We discuss desirable anti-RABV drug profiles, past efforts to address the problem and inhibitor candidates identified, and examine how the rapidly expanding structural insight into RABV protein organization has illuminated novel druggable target candidates and paved the way to structure-aided drug optimization. Special emphasis is given to the viral RNA-dependent RNA polymerase complex as a promising target for direct-acting broad-spectrum RABV inhibitors.

Addresses
[1] Institute for Biomedical Sciences, Georgia State University, Atlanta, GA, United States
[2] Department of Microbiology and Immunology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, United States

Corresponding authors: Plemper, Richard K (rplemper@gsu.edu), Schnell, Matthias J (matthias.schnell@jefferson.edu)

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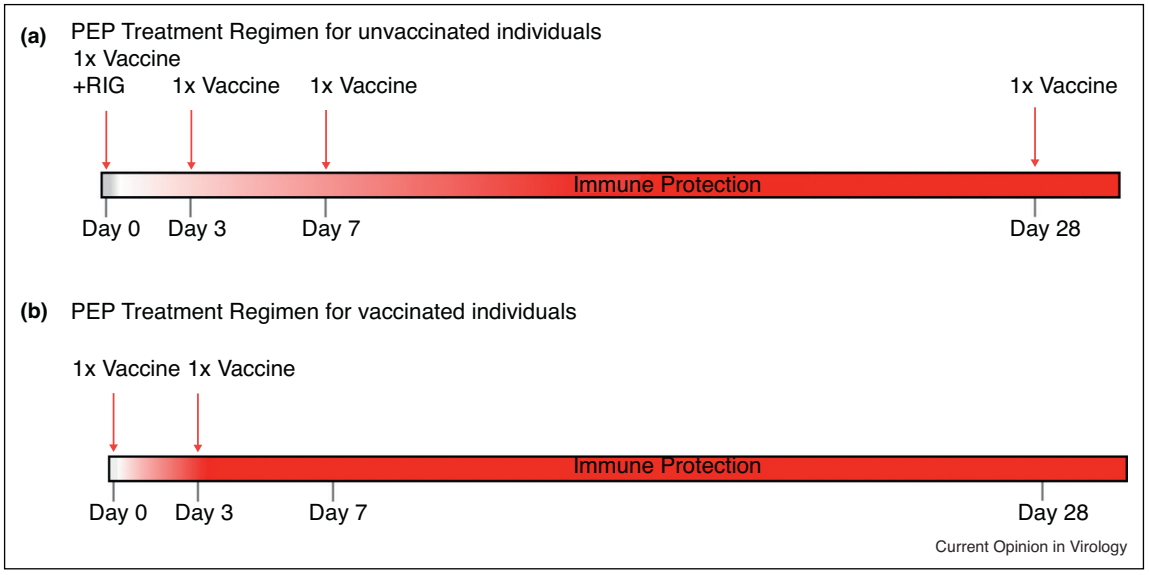
Introduction

The devastating signs and symptoms of rabies disease have been documented as far back as 2000 B.C. in the Eshnunna tablets of Mesopotamia [1]. Even now, in the second millennia A.D, rabies disease continues to be a social and economic hardship with approximately 60 000 deaths worldwide, nearly $8.6 billion in economic burden, and $1.5 billion spent on post-exposure prophylaxis treatment (PEP) alone [2]. The causative agents, lyssaviruses, within the *Rhabdoviridae* family, are characterized as zoonotic, neurotropic negative-sense non-segmented RNA viruses. Transmission of rabies virus (RABV) occurs typically through the transfer of infectious saliva from the percutaneous bite of a mammal, usually a dog [2]. Through axoplasmic transport, RABV enters the central nervous system (CNS) where it begins to replicate, causing severe neuronal dysfunction [3–5]. Rabies is vaccine-preventable as well as treatable early after infection. After the onset of clinical symptoms, however, almost all patients succumb to the infection, progressing toward coma and ultimately death [6•]. RABV's ability to effectively subvert the host immune system through evasion of TLR signaling, down-regulation of IFN signaling, and prevention of adaptive responses by maintaining lowered blood–brain barrier (BBB) permeability, and induction of T-cell apoptosis exemplifies why early intervention is critical [7,8,9•]. As depicted in Figure 1, treatment of rabies consists of rabies immune-globin (RIG) and four doses of the vaccine over a four-week period. PEP is recommended for previously vaccinated individuals as well, and consists of vaccine doses on days 0 and 3. A single PEP regimen costs at least $3000 in the United States [2]. This expense of rabies PEP is predominantly due to the high cost of producing human rabies immune-globin HRIG, a human plasma-based product, with a relatively short shelf life and need for extensive quality assurance [10]. A second contributor to the high treatment cost is the requirement of four doses of rabies vaccine, which typically costs $260 per dose in the USA and Europe. In Africa and Asia, where 95% of rabies-related deaths occur, PEP averages $40 and $49, respectively. This expense is often out of reach in areas with a daily family income of approximately $1–2. The number of people worldwide that receive rabies PEP as well as the crippling debt associated with it is estimated to reach a staggering 15 million annually [2]. Furthermore, the current vaccine is likely ineffective against emerging zoonotic lyssaviruses of phylogroup II such as Mokola (MOKV) and Lagos bat viruses [11–15]. The high cost of HRIG and the current vaccine, along with cold-chain requirements for both, present an urgent and unmet clinical need for the development of safe, cost-effective, efficacious, shelf-stable, and cross-protective antivirals against lyssavirus phylogroups associated with human rabies disease. Antiviral compounds could be used to replace the HRIG or other RIG component in current rabies PEP (Table 1).

Lyssavirus virion organization

Lyssaviruses contain RNA genomes of approximately 12 kb. The virion of lyssaviruses, as with the other family members of *Rhabdoviridae*, is characterized by a bullet-

Figure 1



Schematic diagram representing the current post-exposure prophylaxis treatment (PEP) schedule as recommended by the WHO, **(a)** for naïve individuals and **(b)** previously vaccinated individuals [2].

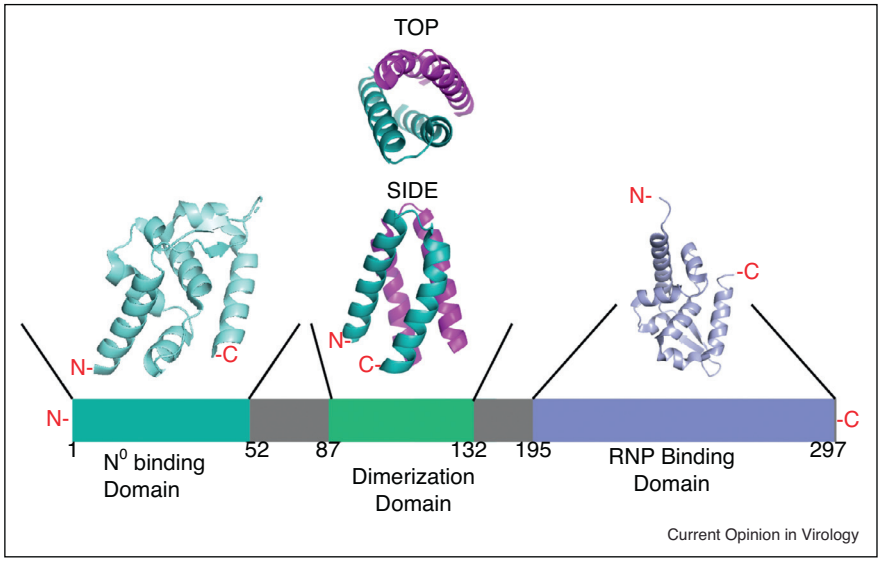
shape with a length of 180 nm, and average diameter of 70 nm. Lyssaviruses are enveloped by a lipid protein coat studded with the viral receptor glycoprotein (G). G is the predominant target for the host humoral immune response. The RABV G is a trimeric type-1 membrane protein, capable of major conformational rearrangements upon receptor binding and subsequent endocytotic internalization and acidification of the endosome [6•]. Nicotinic acetylcholine receptor (nAChR), the neuronal cell adhesion molecule (NCAM), and the p75 neurotrophin receptor (p75NTR) have all been implicated in serving as host receptors for RABV, and several other membrane components have been proposed to aid in viral entry as well [16]. Matrix protein (M) interacts with the cytoplasmic tails of G, lining the viral envelope [17,18]. Within the virion, the viral genome is encapsidated by nucleocapsid (N), resulting in a helical ribonucleoprotein complex (RNP). Encapsidation of the viral genome is a protective measure against RNAse digest and it reduces triggering of innate cellular immune response pathways. Recognition of N by the viral RNA-dependent RNA polymerase (vRdRp) is essential for viral replication [19]. N also plays a significant role in evading the innate immune response and enhancing viral pathogenicity through virion cell-to-cell spread [20]. The vRdRp is a hetero-oligomeric protein complex consisting of the large protein (L) and the phosphoprotein (P) [6•]. Of these, P, as depicted in Figure 2, is the noncatalytic cofactor of the vRdRp that mediates the interaction with RNP to position L for RNA synthesis. P also guides nascently folded RNA-free N (N^0) to newly synthesized genomic RNA during replication [19]. Up to five alternative start codons result in five N-terminally truncated P protein variants, which have been implicated as Type-1 IFN antagonists

Table 1

Host-directed anti-RABV compounds

Compound	Target	EC_{50} (uM)	CC_{50} (uM)	SI (CC_{50}/EC_{50})	Ref.
Catechin	Host Cell GAGs	36.50 ± 8.40	124.33 ± 33.53	3	[54,63•]
Quercetin	Host Cell GAGs	191.68 ± 24.25	670.02 ± 180.18	3	[54,63•]
3,4,5-Trimethoxybenzoic acid	Host Cell GAGs	2142.74 ± 266.37	>5042.41	2	[54,63•]
Trimethoxyacetophenone	Host Cell GAGs	1023.98 ± 64.62	3738.98 ± 1099.17	3	[54,63•]
3,4,5-Trimethoxybenzoic acid ethyl ester	Host Cell GAGs	822.23 ± 134.38	3204.20 ± 397.87	3	[54,63•]
Butyl gallate	Host Cell GAGs	109.79	113.23 ± 52.35	1	[54,63•]
PAV-866	ABCE1	~0.15–0.30	~2.5–10	~100	[55]
Sorafenib	Tyrosine Kinases	1.463	>160	109	[22•]
2-Piperidin-3-yl-benzothiazole analog	Nedd4	0.345	>1	>3	[126]
1-Acetyl-3-(2,2,2-trifluoroethyl)-urea analog	Nedd4	0.210	>1	>8	[126]

Figure 2



Schematic representation of the modular organization of the RABV phosphoprotein (P). The N^0 binding domain is teal, the dimerization domain is green, and the ribonucleoprotein binding domain (RNP) is periwinkle. The solved crystal structure for the N^0 binding domain is depicted in teal (PDB 3OA1). The solved crystal structure for the dimerization domain is depicted in green and pink with both top and side views (PDB 3L32). The solved structure for the RNP is depicted in periwinkle (PDB 1VYI) [19,127,135,137,138].

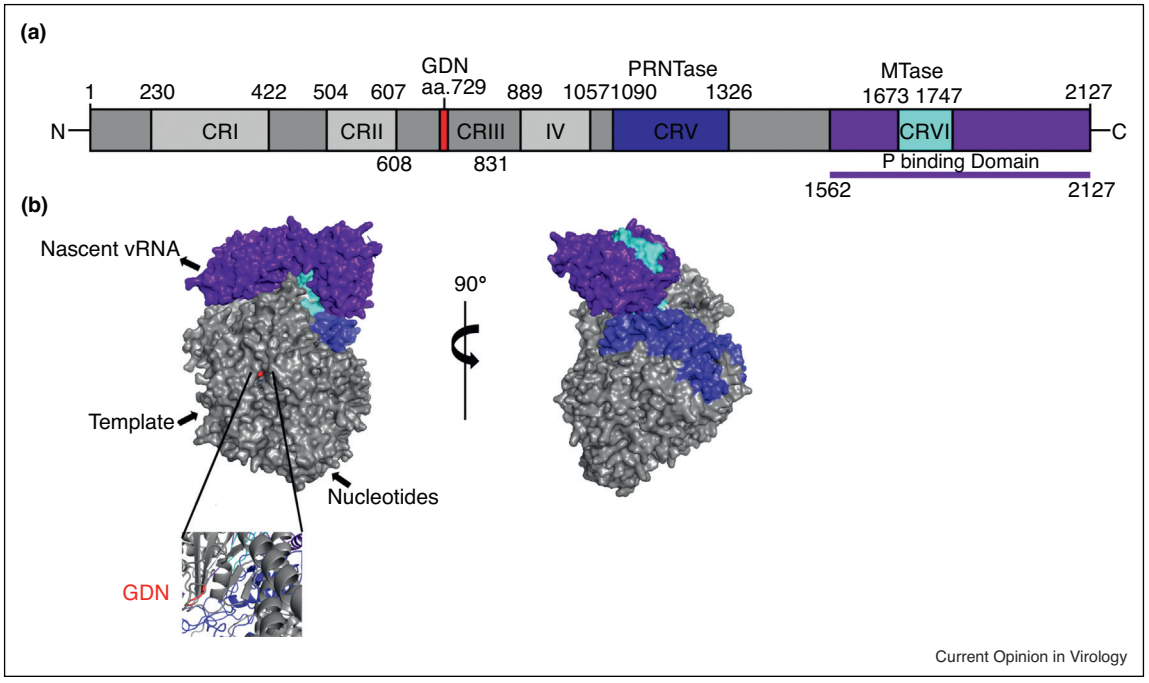
[21]. This is accomplished through direct interaction with STAT1 and STAT2 proteins as well as suppressing IRF-3 [22•,23,24]. L is approximately 250 kDa in mass and provides all enzymatic activity required for viral genome synthesis. Sequence homology among other members of *Mononegavirales* have revealed six highly conserved regions (CRs) within L, as shown in Figure 3a [25]. These CRs have been implicated in the different catalytic functions for productive replication. CRII and CRIII are required for phosphodiester bond formation, with III containing a GDN motif starting at residue 729 that is considered to form the catalytic center [26–28]. CRV is implicated in mediating viral mRNA capping through GDP polyribonucleotidyltransferase (PRNTase) activity [29–33]. CRVI contains a K-D-K-E motif that is characteristic for methyltransferase (MTase) activities [34–36].

Current treatment of RABV infection

The high cost and cold chain requirement of rabies biologics have revealed an urgent need for the development of alternative antiviral compounds. To date, there have been 14 documented survivors of symptomatic rabies disease, of whom all but one received vaccine and PEP. However, all of these survivors exhibit severe neurological sequelae [37]. The single documented survivor, who did not receive PEP nor vaccine, was treated with what is now termed 'The Milwaukee Protocol'. This treatment method involves induction of a therapeutic coma accompanied by ketamine and amantadine infusions [38]. This survivor had anti-RABV antibodies, suggesting that she was infected with an atypical RABV strain and that her immune system was responsible for the clearance, though the strain was never identified [39]. Also, this original Milwaukee protocol patient developed neurological side effects that never fully resolved [38]. Subsequent application of the Milwaukee protocol has resulted in 31 deaths and only 1–2 additional survivors, who both developed severe neuronal sequelae, casting considerable doubt on the overall efficacy of the approach [39,40].

Administered in conjunction with RIG and RABV vaccine, broad-spectrum antiviral therapeutics are used for the treatment of highly aggressive rabies cases. These include ribavirin, interferon-alpha (IFN-α), and ketamine/amantadine [41,42]. Ribavirin is a broad-spectrum guanosine nucleoside analog with unclear mechanism of action including inhibition of inosine monophosphate dehydrogenase (IMPDH) purine *de novo* synthesis, direct incorporation into nascent viral genomes causing lethal mutagenesis, and inhibition of mRNA capping [43–45]. Although efficacious *in vivo*, ribavirin has shown no activity against clinical rabies [41,46]. This is supported by a 15 year-long study, in which 16 RABV infected patients were treated with ribavirin, but no beneficial effect was observed [47]. Also, in two separate cases, one in Thailand, and one in the United States, ribavirin was administered, again demonstrating a lack of efficacy [46,48]. It is hypothesized that this disappointing performance of ribavirin is due to its interference with the Th1/Th2 immune response, thus hindering production of effective antibodies that are essential for RABV clearance [49].

Figure 3



(a) Schematic diagram depicting the domain organization of RABV large protein (L). The GDN polymerization motif is in red. The polyribonucleotidyltransferase (PRNTase) is in blue. The methyltransferase (MTase) is in cyan. The phosphoprotein (P) binding region is purple. Conserved regions (CR) of the non-segmented negative-sense RNA viruses are labelled CR I -VI. **(b)** Surface representation of the RABV L generated by homology modelling based on the coordinates reported for the closely related VSV L structure with the same color scheme as described by 3a. Below is a zoomed in ribbon representation of the GDN motif responsible for polymerase activity [25,33,36,128].

Inducing an antiviral state through triggering of innate immune response pathways, anti-RABV activity of IFN-α is ameliorated through counteraction by the lyssavirus phosphoprotein (P) [8,46]. Currently up to five truncated isotypes of P (P1-5) have been identified that play distinct roles in antagonizing the type-1 IFN response. P1 and P2 encode for a nuclear export signal and directly bind to phosphorylated STAT1, thus sequestering it to the cytoplasm [50,51]. P3 binds directly to microtubules to prevent STAT1 shuttling into the nucleus and it furthermore can interact directly with STAT1 to block DNA binding [52,53]. The inhibition of STAT1 also renders exogenous IFN-α ineffective for RABV therapy (Table 2).

Ketamine and amantadine are core components of the Milwaukee protocol anti-RABV approach. Both drugs are non-competitive NMDA receptor antagonists and have been shown to prevent uncoating and release of RABV particles. Ketamine requires prohibitively high concentrations to effectively block viral replication, however, which are not achievable in human therapy [38,40,42,54].

All current antiviral strategies to block RABV replication are thus compromised by substantial limitations, adding little to improve case fatality rates of symptomatic disease. This lack of an effective therapeutic option creates an urgent and currently unmet clinical need for the development of next-generation antivirals that can be used as an additional component of current combinatorial PEP and may improve management of established rabies cases.

Anti-RABV drug discovery

Most of the deaths attributed to RABV infection result from socio-economic barriers, reagent shortages, and inability to maintain an uninterrupted cold chain for the transport of biologics for PEP [11]. The discovery of novel antiviral compounds may offer a fresh avenue to address these concerns. Small-molecule antivirals in general have the advantages of cost-effective manufacture — addressing supply concerns — as well as high shelf-stability, enabling developing countries to stock-pile life-saving supplies [42]. Currently, there are no small-molecule inhibitors licensed for therapeutic use, despite several attempts at discovery [41,54–62]. Considering the previous antiviral activity of phenolic compounds against viruses such as HIV, herpes simplex virus and influenza virus, 24 representatives of this class were tested against RABV. According to visual scoring of viral cytopathic effects (CPE) after infection with the Pasteur virus

Table 2

Viral-directed anti-RABV compounds

Compound	Target	EC_{50} (uM)	CC_{50} (uM)	SI (CC_{50}/EC_{50})	Ref.
Ketamine	vRNA Synthesis	922.93 ± 68.48	3010.69 ± 171.26	3.3	[54,194]
Ribavirin	*de novo* purine synthesis	18.55	>200	>10	[57]
EICAR	*de novo* purine synthesis	0.90	>200	>200	[57]
EICNR	*de novo* purine synthesis	3.80	>200	>50	[57]
Favipiravir	vRdRp	32.4	>2500 mM	>1000	[60•,195•]

(PV) RABV strain, 50%-effective concentrations (EC_{50}) were in all cases >50 μM [54]. Although a specific mechanism of activity was not evaluated in this study, it was suggested that the antiviral activities of phenolic compounds may be attributed to their interaction with host cell group-specific antigens (GAGs), blocking viral entry [63•]. Another anti-RABV drug discovery campaign employed cell-free translation, using fluorescently tagged mRNA to screen the Prosetta compound library in search of hits that prevented nucleocapsid assembly. This exercise yielded a hit directed against the ABCE1 transporter that showed considerable cytotoxicity with 50% cytotoxic concentrations (CC_{50}) of 2.5–10 μM [55].

These early attempts for anti-RABV drug discovery did not yield viable hit candidates, but exposed some challenges to anti-RABV drug screening approach. A significant deterrent to automated anti-RABV drug discovery, for instance, is the BSL2/3 containment requirements imposed by replication-competent RABVs and mandatory rabies vaccination as well restriction to the laboratory of unvaccinated people. On the basis of the original reverse genetics system developed for the SAD-B19 RABV strain [64], however, minireplicon systems and single-cycle reporter RABV viruses were developed enabling the study of the RABV polymerase activity and in-cell replication in a BSL2 setting [65–73]. Analogous to the precedent set by successful screens employing single-cycle HIV, hepatitis C virus, and influenza virus reporter strains [74–76], the single-cycle-based approach in particular offers an exciting drug discovery perspective. Transient-transfection based minigenome drug screens have furthermore been attempted to identify, for instance, Ebola virus polymerase inhibitors [77–79]. Applied to the RABV problem, single-cycle reporter viruses and/or minireplicon systems may offer a viable solution to the biocontainment challenge to automated large-scale drug discovery.

Host-directed RABV inhibitors

The analysis of the host-RABV interactome has revealed cellular proteins that are essential for completion of the RABV life-cycle and may provide exploitable antiviral target opportunities [80•,81]. Targeting of host factors involved in viral replication and pathogenesis offers several advantages over direct-acting antivirals. The frequency of viral escape is typically reduced when host factors are targeted [62,82–84], due to the fact that the host genetic information is much more stable than that of error-prone RNA viruses and resistance mutations can never become fixed in circulating viral strains. Also, the potential for a broadened indication spectrum is heightened if the targeted host factor is highjacked by related viruses within the same family, preparing the path for targeting the emerging phylogroup II lyssaviruses [55].

However, host-directed antivirals are more prone to inducing severe adverse effects [85,86,87••]. For example, erlotinib, dasatinib, and ezetimibe are all broad-spectrum entry inhibitors of hepatitis C virus that have no documented escape mutations [88,89]. However, these drugs have revealed several adverse effects for lung, liver, and kidney functions, as well as cause rash and diarrhea in cancer patients, thus demonstrating the tightrope balance of targeting host proteins that are essential for cellular function [90–92]. Identifying pursuable host targets thus remains to be a challenging endeavor [93••,94]. RABV entry is mediated by clathrin-coated endocytosis which is dependent on actin cytoskeleton reorganization for internalization [95]. A closely related *Rhabdovirus*, vesicular stomatitis virus (VSV), employs a comparable entry strategy [96,97]. Dynamin is a critical component of multiple endocytotic pathways and plays a role in actin reassembly and organization [95,97]. Dynasore, a membrane-permeable inhibitor of dynamin GTPase activity, has been shown to inhibit the entry process for several viruses, including RABV and VSV [95,98]. A similar dynamin inhibitor, AMBA, has also shown antiviral effects against HSV, but the selectivity index ($SI = CC_{50}/EC_{50}$) was at a low <21. When tested *in vivo*, there was only a 50% survival rate of mice given a lethal RABV challenge, greatly compromising therapeutic potential against the RABV indication [99]. Since AMBA was a direct hit compound from a high-throughput screen, synthetic optimization to improve efficacy was suggested. However, greater affinity for dynamic targets must be anticipated to coincide with increased cytotoxicity, since dynamin is an essential cellular protein. Analysis of RABV entry into non-neuronal cells has revealed preference for cholesterol-rich microdomains [16,100–106]. Depletion of membrane cholesterol, however, did not affect viral entry, suggesting RABV may have another route of entry into these cell types [107].

RABV encephalitic pathogenesis is Raf/MEK/ERK kinase pathway-dependent [108]. Sorafenib is a small-molecule

tyrosine kinase inhibitor that curbs angiogenesis in cancer patients [109]. In an attempt of repurposing cancer drugs as antivirals, sorafenib has been highlighted as a broad-range antiviral against adenovirus, mumps virus, chikungunya virus, dengue virus, West Nile virus, Yellow fever virus, and enterovirus 71 [110–116]. When administered at non-cytotoxic levels, however, sorafenib given in combination with IFN-β reduced RABV load by less than one order of magnitude (74% inhibition) [22•]. Furthermore, this compound was associated with GI complications and other severe adverse effects in cancer patients, making it an unlikely antiviral candidate [117–123].

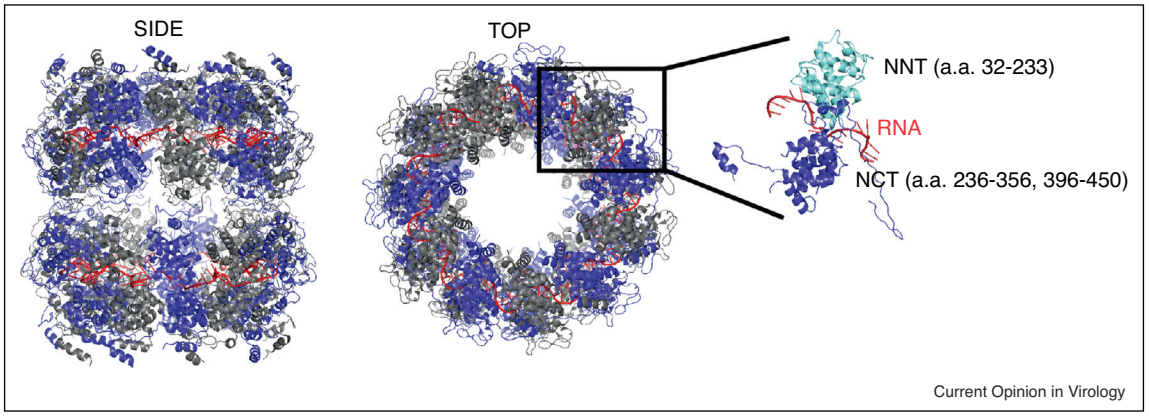
The neural precursor cell expressed developmentally downregulated protein 4 (Nedd4) is a highly conserved and universally expressed ubiquitin ligase that regulates steady state levels of many membrane proteins including ion channels and membrane receptors. The recently characterized WW domain of Nedd4 has been associated with a number of functions that facilitate protein-protein interactions [124]. This domain was shown to provide a binding site for M proteins of rhabdoviruses and filoviruses, promoting viral budding and egress [125]. A hit compound synthesized from an *in silico* screen based on the structural information of the WW domain showed some initial efficacy against RABV. Further synthetic optimization of the scaffold yielded two hit compounds that specifically targeted the Nedd4 WW domain, competitively inhibited viral M protein binding to the domain, and were only minimally cytotoxic [126]. Further *in vivo* evaluation is required, however, to determine whether the Nedd4 WW domain will persevere as a viable druggable target.

Direct-acting antivirals

Despite the anticipated strengths of host-directed antiviral approaches, unacceptable cytotoxicity often shifts focus towards direct-acting inhibitors that offer the potential for wider therapeutic windows [59]. A co-crystal structure of N with viral RNA was solved (Figure 4) that may be exploitable for drug discovery through structure-guided design [127]. The conserved nature of N and its critical interactions with multiple viral and cellular proteins make it overall an attractive druggable candidate [128–130]. Proof-of-concept comes from liposomally delivered siRNAs that were designed to address highly conserved N sequences across different RABV strains. RABV replication was significantly inhibited *in vitro* through this approach, validating druggability of RABV N. *in vivo*, however, the siRNAs protected at best 60% of lethally RABV challenged mice [61], which was attributed to inefficient delivery and uptake by the animals [59,61].

Conformational changes of N are likewise vital for its bioactivity. Phosphorylation at serine389, for instance, is considered to allow N to loosen its interaction with RNA, enabling access of the vRdRp to the encapsidated RNA template [131,132]. These dynamic changes in nucleoprotein structure may be targeted by allosteric inhibitors that trap N in specific conformations, preventing rearrangements required for polymerization [133]. In addition to targeting N directly, preventing the interaction of the viral P-L polymerase complex with N in the assembled RNP may be a viable antiviral strategy. Replication of the distantly related respiratory syncytial virus, for instance, is potently blocked by the small-molecule compound RSV604 that is considered to interfere with P-RNP binding [134]. Structural models of RNP and the C-terminal domain of P proteins of both RABV and MOKV revealed close structural homology and interaction mechanism similar to these employed by the related paramyxoviruses and pneumoviruses [99,135–138]. Through yeast-2-hybrid screening, several peptides were identified that bind directly to both RABV and MOKV P in highly conserved regions, inhibiting viral replication in minigenome assays.

Figure 4



Structural representation of the ribonucleoprotein complex (PDB 2GTT) with both side (left) and top (center) views. Individual nucleoprotein (N) protomers are depicted as alternating blue and grey with RNA as a red coil. The far right represents a single N protomer with the n-terminal residues (NNT) in cyan and the c-terminal residues (NCT) in blue [127,137].

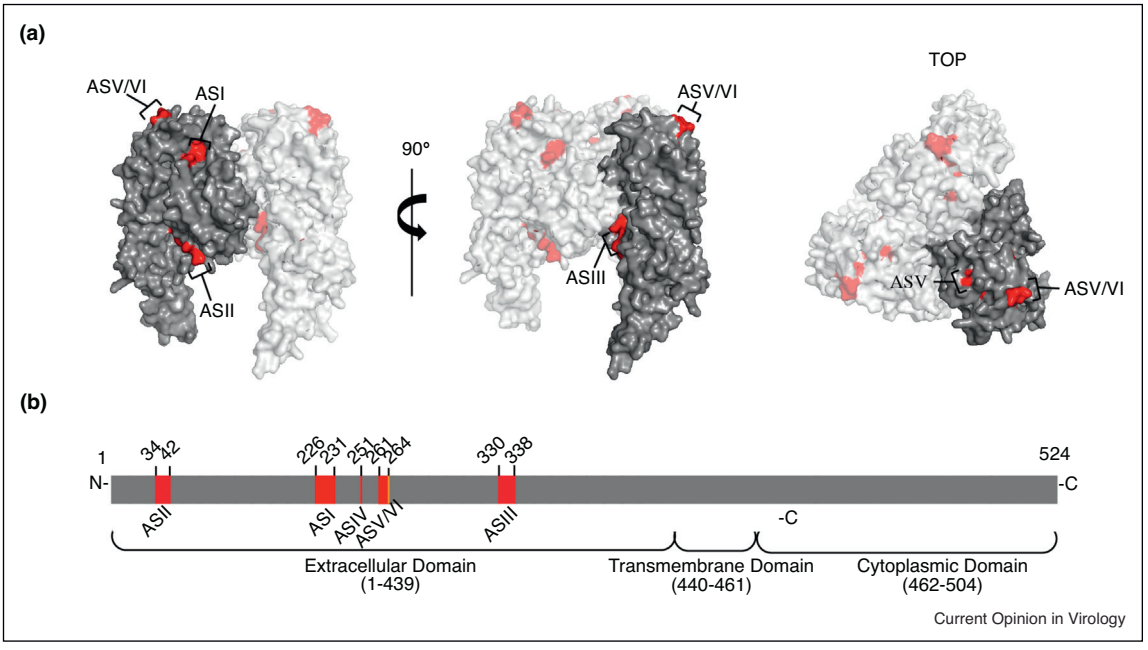
Mechanistically, inhibition by these peptides was due to disruption of RNP-P complex formation [139]. Although therapeutic peptides are often highly specific and show low toxicity, they are frequently proteolytically unstable, display poor membrane permeability, and are often immunogenic when repeat administration is required [140]. Replacement of P binding peptides with a small-molecule inhibitor that directly interacts with P should, therefore, be considered in search of a broad range therapeutic that is efficacious against both phylogroups, cost-effective, and shows superior stability.

M is of critical importance for viral particle formation, based on self-assembly upon interaction with RNP and the cytoplasmic domain of G [141]. M also induces mitochondria-mediated cellular apoptosis in neuronal cells to promote viral dissemination [142,143], and alters host cell protein biosynthesis through two mechanisms [144]: suppression of mRNA translation by interaction with Rae1 and block of mRNA export from the nucleus [145]; and shut-off of host gene expression by binding to, and/or modulating of, the phosphorylation site of host cell transcription factors [145]. The structures of both VSV and LABV M proteins have been solved [146], fueling the mechanistic appreciation of M assembly and opening a door towards structure-guided drug design against-specific M microdomains. Attractive antiviral targets include M domains involved in M homo-oligomerization, interaction with host proteins, or required for binding to the viral G protein [17,18,70,125,126,146–151].

Monoclonal antibodies (mAbs) targeting RABV G have proven antiviral efficacy [15,152–154]. Broad panels of antibodies against rabies G revealed primary clusters for antibody recognition in distinct regions of the G ectodomain [18,155–158]. As shown in Figure 5, these antigenic sites (AS) include ASI (a.a. 226–231), ASII (a.a. 34–42), ASIII (a.a. 330–338), ASIV (a.a. 251), ASV (a.a. 261–264), and ASVI (a.a. 264). Considerable efforts have been made to assemble antibody cocktails that target different AS' to prevent viral escape through antigenic drift and provide cross-protection based on across-strain conservation [14,42,159•,160•,161–164]. Despite the initial promise of the approach, antibody therapy does not address the cold-chain problematic or provide cross-protection against the emerging phylogroup II lyssavirus threat. Lifting the cold-chain limitation at least, small molecule fusion inhibitors targeting G should be considered nevertheless to block RABV entry [165]. These can be effective against viral targets, since resistance often coincides with a viral fitness penalty [166–169].

Harboring all catalytic centers of the viral polymerase complex, L has emerged as a leading drug target for many negative-sense RNA viruses because of its highly conserved nature and the density of candidate target sites

Figure 5



Schematic representation of the RABV glycoprotein (G) antigenic sites (AS).
(a) Side view (left) and 90° turn (center) and top view (right) of homology model of RABV G based on VSV G (PDB 2J6J). Residues of AS are highlighted in red for each view. **(b)** Linear schematic showing relative position and amino acid numbering for ASI-VI within the extracellular domain of G. [129,157,191,193].

including domains required for RNA synthesis, mRNA capping, or mRNA methylation [25,128,170–173,174••].

Favipiravir (T-705) is a broad-spectrum RNA virus inhibitor that is currently licensed in Japan for stock-piling against pandemic influenza viruses resistant to oseltamivir. T-705 has been shown to be effective against Ebola virus, also within order Mononegavirales and, therefore, was considered for use against RABV also [175–181]. When tested in mouse neuroblastoma neural 2a cells, T-705 indeed lowered RABV titers by three to four order of magnitude (EC_{50} of 32.4 μM against a circulating RABV strain and 44.3 μM against the vaccine strain). However, the survival rate of mice infected with RABV and treated with T-705 was only approximately 50% (five out of nine animals examined), and the surviving mice developed limb paralysis, indicating viral circulation within the nervous system [60•]. T-705 was also the least effective in a panel of drug combinations with IFN-α, suggesting that it is not an optimal antiviral agent against RABV [112].

Screening of several ribavirin analogs returned two compounds, EICAR and EICNR, that had superior antiviral potency in human neuroblastoma cells (EC_{50} 0.9 μM, and 3.8 μM, respectively), compared to the ribavirin EC_{50} of 18.6 μM [57]. However, testing of these compounds was limited to the RABV vaccine strain, necessitating further examination *in vivo* against clinically relevant pathogenic RABVs. If effective *in vivo*, these analogs may have the potential to replace ribavirin in current PEP.

Beyond ribavirin, ribonucleoside analogs present an exciting option for the discovery of broad-spectrum lyssavirus inhibitors. For instance, the ribonucleoside analogy N^4-hydroxycytidine reportedly blocks both seasonal and pathogenic influenza virus strains, respiratory syncytial virus, Ebola virus, chikungunya virus, and hepatitis B and C viruses [182•,183–188] with excellent pharmacological properties [182•]. If antiviral activity equally extends to RABV and related lyssaviruses, compounds like N^4-hydroxycytidine may present an example for a viable next-generation therapeutic option to address the rabies challenge.

Summary

The high cost of PEP for RABV and the lack of cross-protection against the emerging zoonotic lyssaviruses of phylogroup II have underscored the unmet demand of an updated treatment regimen. Ideal alternatives break the cold-chain requirement, be BBB permeable, RABV-specific, safe, and efficacious. Advances in molecular biology and a better understanding of RABV pathogenesis have led to new approaches to address the problem. Of all options, we consider the RABV polymerase complex to represent the most promising target for direct-acting antivirals due to the comparably low conservation of G across lyssaviruses [12,17,158,174••,189–192]. The heterologous polymerase complex offers druggable protein–protein interfaces, essential enzymatic centers, and opportunity for allosteric and competitive substrate-analog inhibitors. Crystal structures have been determined for the RABV N-RNA complex and the P-RNP [127,135,137] complex, providing an exciting starting point for future structure-guided drug design.

Acknowledgement

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- • of special interest
- •• of outstanding interest

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