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REVIEW ARTICLE

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Norovirus antivirals: Where are we now?

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Abstract

Human noroviruses inflict a significant health burden on society and are responsible for approximately 699 million infections and over 200 000 estimated deaths worldwide each year. Yet despite significant research efforts, approved vaccines or antivirals to combat this pathogen are still lacking. Safe and effective antivirals are not available, particularly for chronically infected immunocompromised individuals, and for prophylactic applications to protect high-risk and vulnerable populations in outbreak settings. Since the discovery of human norovirus in 1972, the lack of a cell culture system has hindered biological research and antiviral studies for many years. Recent breakthroughs in culturing human norovirus have been encouraging, however, further development and optimization of these novel methodologies are required to facilitate more robust replication levels, that will enable reliable serological and replication studies, as well as advances in antiviral development. In the last few years, considerable progress has been made toward the development of norovirus antivirals, inviting an updated review. This review focuses on potential therapeutics that have been reported since 2010, which were examined across at least two model systems used for studying human norovirus or its enzymes. In addition, we have placed emphasis on antiviral compounds with a defined chemical structure. We include a comprehensive outline of direct-acting antivirals and offer a discussion of host-modulating compounds, a rapidly expanding and promising area of antiviral research.

Natalie E. Netzler and Daniel Enosi Tuipulotu contributed equally to this review.

KEYWORDS
antivirals, direct-acting antivirals, host-targeting drugs, norovirus, therapy

1 | INTRODUCTION

1.1 | Norovirus and disease manifestation

Human noroviruses are a predominant etiological agent of acute gastroenteritis worldwide, estimated to cause over one-fifth of all gastroenteritis cases globally [1] and approximately 699 million infections per annum.[2] Norovirus is responsible for over 200 000 estimated deaths each year,[3] with the largest proportion occurring in children from lower income nations.[3]

Norovirus-associated disease is usually self-limiting, and symptoms such as diarrhea, vomiting, nausea, low-grade fever, and abdominal cramps, usually resolve within two to four days in healthy adults.[4] Human norovirus affects people of all ages and demographics worldwide,[5] however, increased infection rates occur within high-risk groups such as neonates, the elderly and the immunocompromised. Immunocompromised and transplant recipients would benefit from antivirals, as norovirus infection can persist for years in some cases [6,7] and norovirus infection the transplant setting is a major health concern.

Transmission of human norovirus primarily occurs from person-to-person and also through contaminated food and water.[8] Because norovirus is highly contagious with a low infectious dose (estimated to range between 18 and 1000 viral particles),[9] outbreaks are particularly common in semi-enclosed and closed settings such as nursing homes, hospitals, restaurants, cruise ships, social catered events, child-care centers, within the military,[10] and navy.[11,12] Prolonged shedding of norovirus post infection could also contribute to norovirus spread, and has been reported as a mechanism for nosocomial transmission.[13] To reduce norovirus transmission, prophylactic antivirals to prevent large-scale outbreaks are essential to protect high-risk groups.

1.2 | Social, health, and economic burden

In 2010 the World Health Organization's "*Global estimates of the burden of foodborne disease*" found that norovirus was the most common cause of death from foodborne gastroenteritis[14] and was also responsible for 70 000 deaths in children under five years of age.[3] These statistics were associated with lower income nations, where basic sanitation and wastewater infrastructure is often lacking; contributing to high levels of viral transmission.

This social burden is reflected by a substantial economic impact, with norovirus infection estimated to incur over USD $4.2 billion in direct annual health costs and $60.3 billion in societal costs worldwide, from factors such as forced business closures, hospitalizations, and days off work.[3] Additionally, norovirus infections have been recognized as the fourth greatest cause of disability-adjusted life years across the globe.[14] These social and economic impacts justify the intensive research undertaken to identify effective antiviral and vaccine solutions for human norovirus.

1.3 | Virus genome and classification

Human norovirus is classified within the *Caliciviridae*, a diverse family of positive-sense RNA viruses, currently divided into five accepted genera including; *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, and *Nebovirus*,[15] with several additional genera proposed.[16–19] The *Norovirus* genus is further classified into seven genogroups (GI-GVII), with each genogroup containing numerous genotypes, based on capsid and polymerase protein coding sequence diversity.[20] Recombinant viruses with different polymerase and capsid genotypes are common[21] and many viruses detected today are recombinant in nature.[22]

Genogroup I (GI), GII, and GIV noroviruses can infect humans, with GII noroviruses responsible for approximately 80% to 90% of norovirus infections worldwide.[23] In particular, the genogroup II, genotype 4 (GII.4) strains are recognized as causing the majority (~70%) of GII norovirus global cases and outbreaks[24–26] and have historically been responsible for six reported pandemics. In temporal order they included; 1995 (US 95-96 variant), 2002 (Farmington Hills 2002 variant), 2004 (Hunter 2004), 2006 (Den Haag 2006b), 2009 (New Orleans, 2009), and 2012 (Sydney 2012), respectively.[27] While GII.4 noroviruses persist as the dominant strain in circulation worldwide, a number of viruses from other genotypes have emerged in recent years. For example, a sudden increase and high prevalence of the GII.17 strain in Asian countries occurred between 2014 and 2015,[28–30] although the same high prevalence of this strain was not reflected in other parts of the world, with lower levels detected in Australasia, Europe, and North America compared to the Asian outbreaks during that same period.[31–33]

The human norovirus positive-sense, single-stranded RNA genome is 7.5 to 7.7 kb (Figure 1) and encapsidated within a nonenveloped, icosahedral 27 to 35 nm virion. The genome has three open reading frames (ORFs). ORF1 encodes a polyprotein that is posttranslationally cleaved into seven nonstructural proteins (p48 [NS1/2], NTPase [NS3], p22 [NS4], VPg (NS5], a viral protease [Pro, 3C-like, NS6], and a viral RNA-dependent RNA polymerase [RdRp, NS7]), by the virus-encoded 3C-like cysteine protease (3CLpro) (Figure 1) (reviewed in Atmar[34]; Karst[35]; Karst et al[36]). ORF2 and ORF3 encode the proteins VP1 and VP2, respectively; VP1 is the major capsid protein and VP2 is the minor capsid protein, likely involved in capsid assembly and genome encapsidation.[37] The VP1 protein structure comprises the shell (S) and protruding (P) domains; the S domain encloses the viral RNA, while the antigenically variable P domain forms the outer surface of VP1, and is also involved in cell attachment.[38,39] The VP1 protein can be expressed in baculovirus which then self-assembles into virus-like particles (VLPs). These VLPs are antigenically and structurally indistinguishable to virions produced by the complete virus.[40]

1.4 | Models for studying norovirus infection

Despite the clinical significance of norovirus infection, antiviral studies have been hindered, because until recently, human norovirus could not be successfully propagated in cell culture. Recent breakthroughs have enabled human norovirus to be cultured in B cells[41] and intestinal enteroids,[42] which represent milestones in the field of norovirus biology. However, the modest replication levels generated by these new systems (≤3.5 log increase in B cells[41,43]

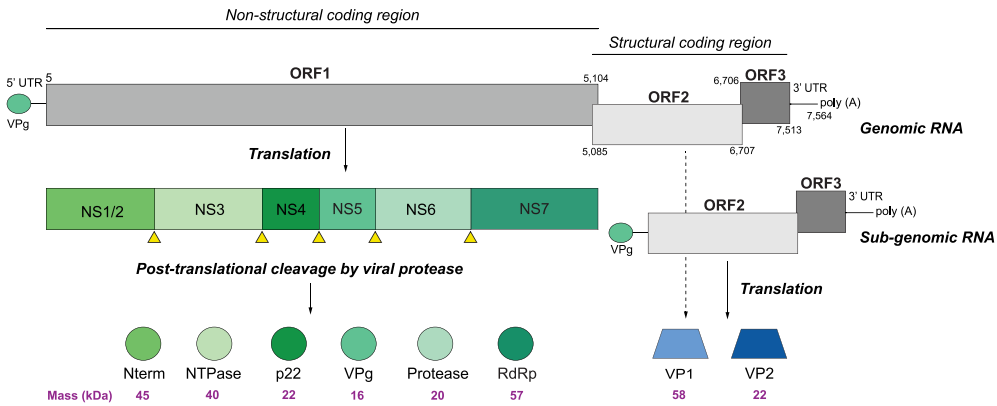


FIGURE 1 Schematic of the human norovirus genome. The norovirus genome is a positive-sense, single-stranded RNA genome comprising three ORFs that encode the nonstructural proteins: p48/N-terminal (NS1/2), NTPase (NS3), p22 (NS4), VPg (NS5), protease (NS6), and RNA polymerase (NS7); and the structural proteins: VP1 and VP2. The numbers at the edges of each domain indicate nucleotide positions. Genome illustration is based on the norovirus GII.4 Sydney 2012 sequence (GenBank accession number JX459908). ORF, open reading frames [Color figure can be viewed at wileyonlinelibrary.com]

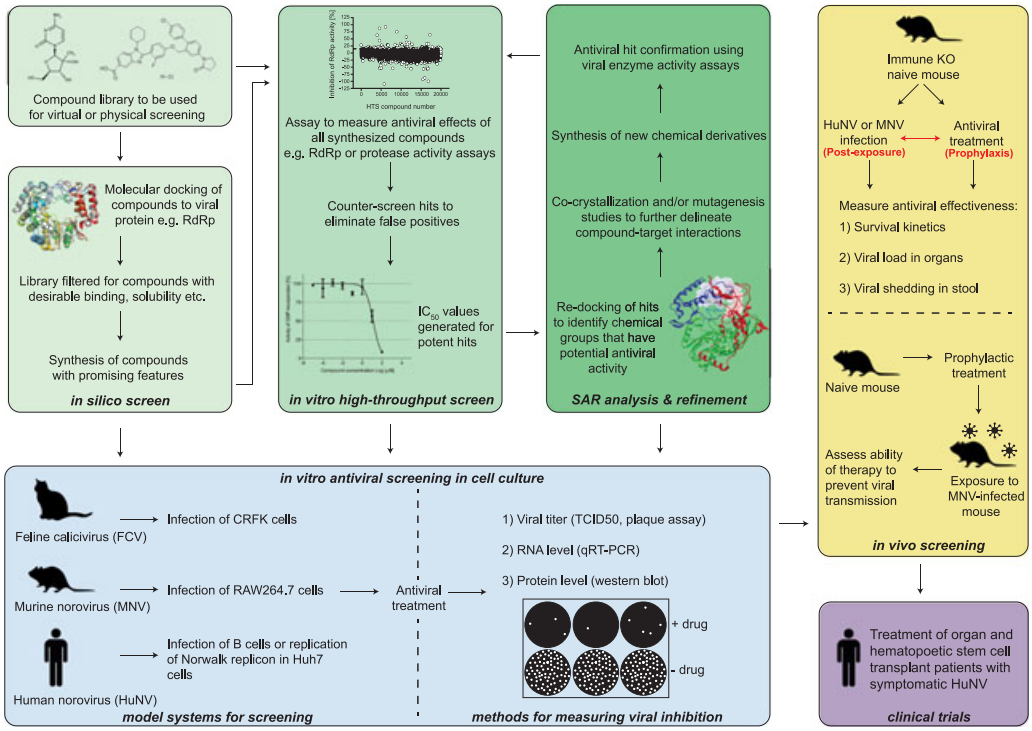


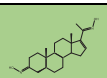
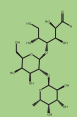
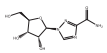
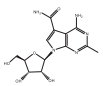
FIGURE 2 Current methods for the identification and characterization of norovirus antivirals. A flow chart depicting the methods and tools available for assessing the effectiveness of norovirus antivirals. Panels in green involve a combination of in silico and in vitro methods. Panels in blue and yellow represent in vitro and in vivo methods, respectively. The purple panel represents clinical testing in human patients. CRFK, Crandell Rees feline kidney; IC_{50}, half maximal inhibitory concentration; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RdRp: RNA-dependent RNA polymerase; SAR, structure-activity relationship; TCID50, tissue culture infective dose [Color figure can be viewed at wileyonlinelibrary.com]

and ≤3.8 log increase in enteroids[42]) means that they require optimization before widespread use for antiviral screening and development.

The GI.1 (Norwalk virus) norovirus replicon system has been used to assess antiviral candidates against the human virus in lieu of a viral culture system (Figure 2). The Norwalk replicon consists of an intact ORF1, ORF3, and genomic 3′ end, however, ORF2 is disrupted by a neomycin gene, engineered into the VP1-encoding region. As such, while the subgenomic promoter is preserved, the expression of an intact VP1 is disrupted. Self-replicating and stably expressed in Huh-7 cells or BHK-21 cell lines,[44] the Norwalk replicon has proven itself as a useful tool to screen potential antiviral compounds (Table 1). However, replication levels of the Norwalk replicon are relatively low (approximately 1×10^3 Norwalk RNA copies per cell[44]), when compared to other replicon systems such as those for hepatitis C virus (HCV). The HCV replicons typically yield more than 1×10^4 copies per cell (AA Eltahla, 2018 personal communication), and have been used to identify many successful antiviral candidates. Moreover, they are amenable to high-throughput antiviral screening (reviewed in Horscroft et al[45]), which has directly led to many of the direct-acting antivirals (DAAs) used to treat HCV infections today (reviewed in Asselah et al[46]).

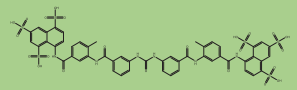
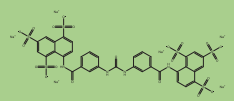
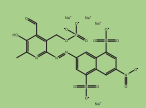
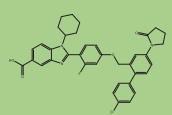
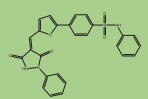
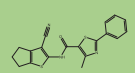
Other models have also been used to study antiviral efficacy against norovirus (Figure 2). For example, researchers have used in vitro enzyme activity assays, such as viral polymerase assays[47] and protease assays[48] (Figure 2) to screen compounds for antiviral activity in a high-throughput format.[49] In addition, X-ray crystallography and in silico modeling can be used to examine ligand and viral protein interactions, to further elucidate antiviral mechanisms.[50]

TABLE 1 Antiviral compounds within this review with known structures that have been examined in more than one system

Target	Index [#]	Compound	Mw	Chemical Structure	Structural studies		Protein assay		Replicon	Infectious culture			Animal model		Clinical trials	Reference(s)
					MNV	HuNV	MNV	HuNV	HuNV	FCV	MNV	HuNV	MNV	HuNV		
Attachment	2.1.1	^ZINC04041115	344.5			+		+								[85]
	2.1.2	^2'-fucosyllactose	488.4			+		+								[86-87]
	2.1.3	Citrate	189.1			+		+								[88]
	2.1.4	mAb D8	~150 kDa	NA				+						+		[89-91]
	2.1.5	^Nb-85	~15 kDa	NA		+		+								[92-93]
Polymerase (NA)	2.2.1	2'-C-methylcytidine	257.2				+	+	+	+	+	+	+	+		[67, 109-112, 189]
	2.2.2	Favipiravir	157.1				+	+	+	+	+		+			[118-119, 189]
	2.2.3	Ribavirin	244.2		+				+		+					[111, 114, 121-122]
	2.2.4	CMX521	323.3		+				+		+				+	[128]

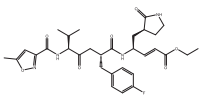
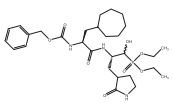
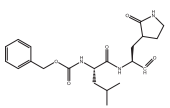
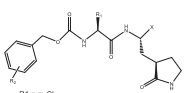
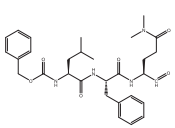
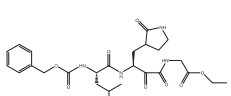
(Continues)

TABLE 1 (Continued)

Target	Index [#]	Compound	Mw	Chemical Structure	Structural studies		Protein assay		Replicon	Infectious culture			Animal model		Clinical trials	Reference(s)
					MNV	HuNV	MNV	HuNV	HuNV	FCV	MNV	HuNV	MNV	HuNV		
Polymerase (NNI)	**2.2.5**	Suramin	1297.3			+	+	+			+					[130, 135]
	2.2.6	NF023	1162.8		+		+	+								[130]
	2.2.7	NAF2	286.2			+		+								[131, 133]
	2.2.8	PPNDS	694.3		+	+	+	+			+					[132-133]
	2.2.9	JTK-109	638.1			+	+	+			+					[132]
	2.2.10	^Compound 54	485.5		+	+	+	+			+					[50]
	2.2.11	^NIC02	365.5					+	+		+					[47]

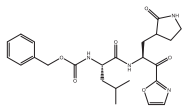
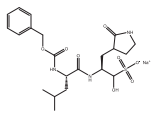
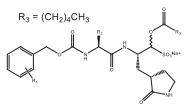
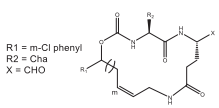
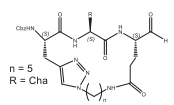
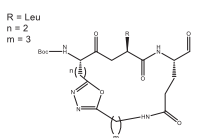
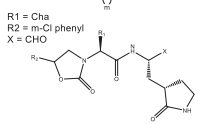
(Continues)

TABLE 1 (Continued)

Target	Index [#]	Compound	Mw	Chemical Structure	Structural studies		Protein assay		Replicon	Infectious culture			Animal model		Clinical trials	Reference(s)
					MNV	HuNV	MNV	HuNV	HuNV	FCV	MNV	HuNV	MNV	HuNV		
Protease	**2.3.1**	Rupintrivir	598.6					+	+	+	+					[48, 138-139]
	2.3.2	^Compound 7d	581.6					+	+							[141]
	2.3.3	^Compound 4	403.4					+	+		+					[142]
	2.3.4	^Compound 16	500.1	 R1 = m-Cl R2 = Cha X = CHO		+		+	+		+		+			[48, 144]
	2.3.5	^syc-10	552.6			+		+								[145]
	2.3.6	^Compound 6d	532.5					+	+							[48, 141, 146]

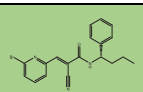
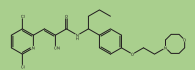
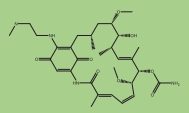
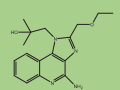
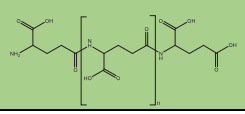
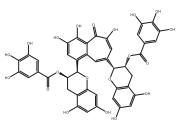
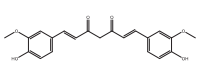
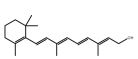
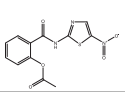
(Continues)

TABLE 1 (Continued)

Target	Index [#]	Compound	Mw	Chemical Structure	Structural studies		Protein assay		Replicon	Infectious culture			Animal model		Clinical trials	Reference(s)
					MNV	HuNV	MNV	HuNV	HuNV	FCV	MNV	HuNV	MNV	HuNV		
Protease	**2.3.7**	^Compound 8a	470.5					+	+							[146]
	2.3.8	^GC376	507.5			+		+	+	+	+					[48, 147-148]
	2.3.9	^Compound 11	656.2					+	+							[149]
	2.3.10	^Compound 24	518.2			+		+	+		+					[151, 154]
	2.3.11	^Compound 8	624.3			+		+	+							[153]
	2.3.12	^Compound 17	523.3			+		+	+		+					[150]
	2.3.13	^Compound 9	502.2			+		+	+							[152]

(Continues)

TABLE 1 (Continued)

Target	Index [#]	Compound	Mw	Chemical Structure	Structural studies		Protein assay		Replicon	Infectious culture			Animal model		Clinical trials	Reference(s)
					MNV	HuNV	MNV	HuNV	HuNV	FCV	MNV	HuNV	MNV	HuNV		
Host	**2.4.1**	WP1130	384.2						+		+		+			[156-158]
	2.4.2	^Compound 9	503.4						+		+					[156]
	2.4.3	17-DMAG	653.1				+				+		+			[161]
	2.4.4	IFN-lambda	20 kDa	NA							+		+			[174-176]
	2.4.5	^R-848	314.3						+		+					[179]
	2.4.6	γ-PGA	~2,000 kDa						+		+		+			[180]
Unknown	**2.5.1**	^Theaflavin digallate	868.7							+	+					[183-186]
	2.5.2	Curcumin	368.3						+		+					[187]
	2.5.3	Vitamin A	286.4								+		+			[188]
	2.5.4	Nitazoxanide	307.2						+	+	+				+	[189-191, 194-200]

Abbreviation: FCV, feline calicivirus; HuNV, human norovirus; mAb, monoclonal antibody; MNV, murine norovirus; Mw, molecular weight (g/mol); Nb: nanobody; ^, denotes a representative from a series of compounds that displays the highest level of potency.

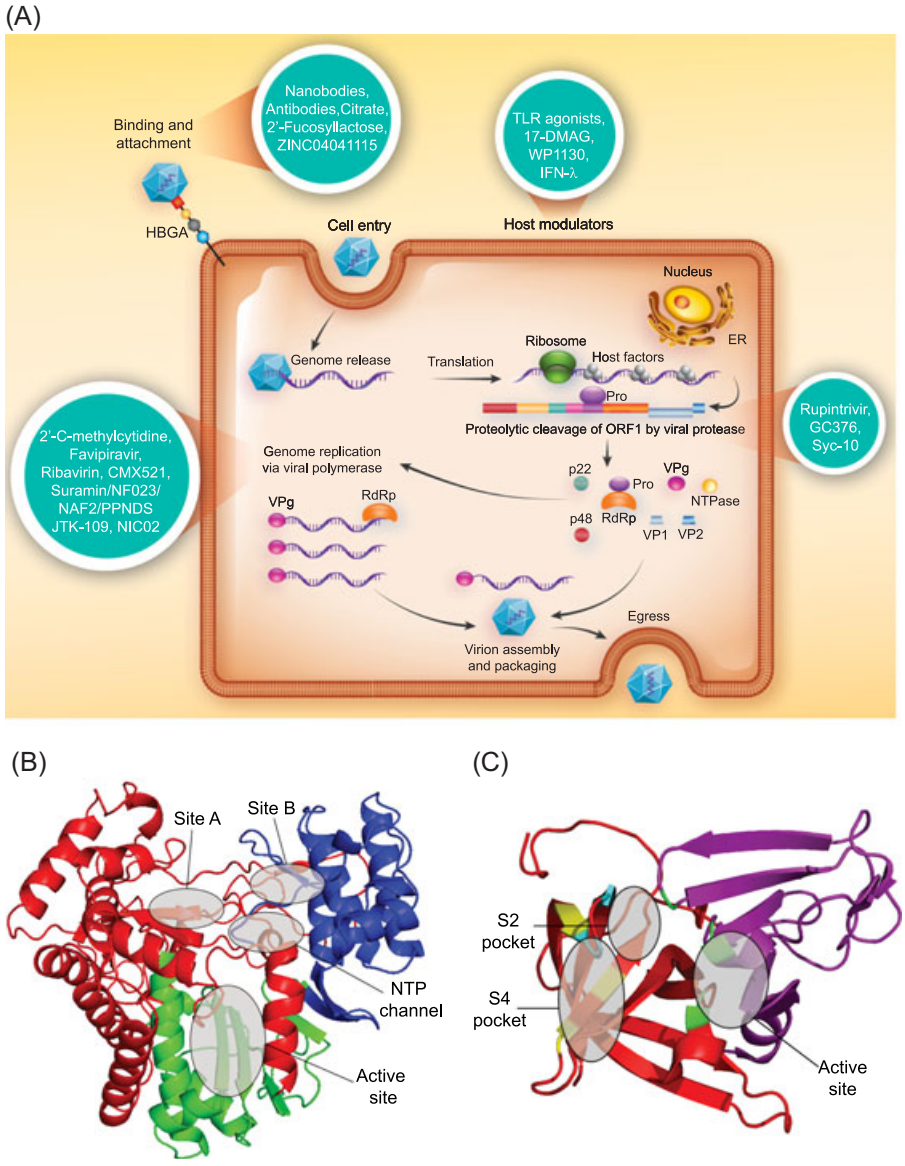


FIGURE 3 The replication cycle and antiviral targets for human norovirus. (A), A schematic of the complete norovirus replication cycle is presented and antivirals that have been developed against norovirus are depicted in turquoise circles. The listed compounds represent only those antivirals which have a known target and a more extensive list of antivirals has been described in Table 1. (B), A ribbon diagram of the human norovirus GII.4 Sydney 2012 RdRp with fingers (red), thumb (blue), and palm (green) domains color-coded. The NNI binding sites (Site A, Site B, and the NTP channel) and the NA binding site (active site and Motif C) are labeled. (C), A ribbon diagram of the GI.1 Norwalk virus protease (2FYQ) with N- and C-terminal chains colored in magenta and red, respectively. Residues of the catalytic triad are colored in green. Residues for the S2 (cyan) and S4 (yellow) pockets[145] are shown as representative binding sites for protease inhibitors. NTP, nucleoside triphosphate [Color figure can be viewed at wileyonlinelibrary.com]

Surrogate viruses from within the *Caliciviridae* family have also been exploited to screen for inhibitory activity of antiviral candidates across several calicivirus genera (Figure 2). These surrogates include murine norovirus (MNV; *Norovirus)*, feline calicivirus (FCV; *Vesivirus*), porcine sapovirus (*Sapovirus*), rabbit hemorrhagic disease virus (RHDV; *Lagovirus*), and Tulane virus, from the proposed genus *Recovirus*.[51] MNV, in particular, has been used as the predominant human norovirus surrogate as it is classified within the same genus and can be robustly propagated in cell culture,[52,53] making it amenable to viral disinfection and sterilization studies.[54–56] Additional features that make MNV desirable for antiviral screening include its ability to be manipulated through reverse genetics, whilst in vivo studies in mice of many genetic backgrounds are straightforward.[57,58]

Various animal models have also been used for human norovirus challenge studies and include: nonhuman primates such as chimpanzees, macaques, marmosets, and tamarins.[59–61] Additionally, gnotobiotic or miniature pigs and gnotobiotic calves[62–66] have also been used, as well as knockout and humanized mice for norovirus infection studies.[67–69] However, no animal model has been deemed entirely suitable, due to obvious differences in clinical disease, gut physiology, microbiomes, naivety to norovirus infection compared to human populations, and the low genetic diversity of laboratory test animals.[36]

1.5 | Viral replication cycle and antiviral targets

Every stage of the human norovirus replication cycle represents a unique target for antiviral development (Figure 3). However, the development of antiviral therapies that target viral replication requires a detailed understanding of norovirus biology and viral gene functionality, much of which is still to be elucidated. An overview of the human norovirus lifecycle and the specific antiviral targets are outlined in Figure 3. The stages of the replication cycle for antiviral targeting include: host cell attachment, internalization, genome release, viral genome replication mediated by the viral RdRp, translation of the genomic and subgenomic templates using the VPg and host cell machinery, viral protease cleavage of the viral polyprotein to yield mature viral proteins, followed by assembly, packaging, and cell egress (reviewed in Thorne and Goodfellow[70]).

1.6 | A review of norovirus antiviral therapies

While a number of other norovirus antiviral reports have been published in the last three years,[71–75] there has been significant recent progress in the field, which now warrants an updated review. Herein we include a comprehensive overview of peer-reviewed studies since 2010, including antiviral candidates examined in at least two different systems (e.g. viral enzyme inhibition assays and MNV cell culture). It should be noted that plant or food extracts that have antinorovirus effects have been omitted from this study when the active antiviral compound is unknown, with this review focusing on compounds that have a defined structure (as outlined in Table 1). We include studies using the recently developed human norovirus cell culture system in B cells.[41] We also discuss recently identified broad-spectrum antivirals with antinorovirus activity and provide a more comprehensive overview of host-modulating compounds, which is a recent, novel and exciting area within norovirus antiviral research. Table 1 lists the individual antiviral compounds, or in the case where several very similar derivatives have been studied, it lists the most potent compound of the group as a representative derivative, with relevant citations. Each compound is indexed for clarity throughout the manuscript and in Table 1.

2 | DRUGS WITH KNOWN TARGETS

2.1 | Attachment and entry

Cell attachment and entry are features of the viral replication cycle that have been extensively investigated as antiviral targets for human immunodeficiency virus (HIV), dengue virus (DENV), and HCV,[76] amongst others. To

design compounds capable of targeting viral entry, knowledge of the viral entry receptor/s is usually required. Recently, CD300lf was identified as the entry receptor for MNV, however, the counterpart receptor for human norovirus has yet to be discovered.[77,78] As such, there is an absence of antivirals that target norovirus entry and a bias toward compounds that prevent norovirus cellular attachment.

The most widely studied attachment targets are the histo-blood group antigens (HBGAs). HBGAs are complex carbohydrates that are presented abundantly on the surface of mucosal epithelia of the gastrointestinal tract. They interact with the surface P2 domain of the VP1 capsid protein[79,80] and are thought to aid viral attachment, and perhaps even entry.[81–83] HBGAs are determined by host cell genetics (reviewed in Tan and Jiang[82]) and play an important role in terms of both virus and host interactions, and for noroviruses to initiate infection.[81,84]

In one study, in silico screening was performed on a drug library (>2 million compounds) to identify molecules that interact strongly with HBGAs and prevent norovirus attachment.[85] Potential hits ($n = 160$) were confirmed in vitro with enzyme-linked immunosorbent assay (ELISA)-based human norovirus VP1/HBGA blocking assays, which revealed that compounds with a cyclopenta-a-dimethyl phenanthrene base structure ($n = 4$) display potent inhibition ($IC_{50} < 10\,\mu M$) of HBGA-norovirus binding.[85] The most potent compound in this series was ZINC04041115 [2.1.1] with an approximate EC_{50} of 2.5 μM.[85] Studies by others using ELISA-based VLP-binding assays have shown that the human milk oligosaccharides (HMOs) 2′-fucosyllactose (2′FL) [2.1.2] and 3′-fucosyllactose (3′ FL) inhibit the binding of GII.10 norovirus VLPs to HBGAs at low millimolar concentrations (5.5-30.2 mM).[86] 2′FL was later shown to also inhibit binding of GII.17 and GI.1 VLPs to HBGAs with IC_{50} values ranging between 13 to 20 mM and 38 to 50 mM, respectively.[87]

In a similar fashion, citric acid [2.1.3] has been shown to bind norovirus GII.10 VLPs causing an altered morphology that prevents VLP-HBGA binding.[88] In addition, X-ray crystallography has revealed that these HMOs interact directly with the P domain of the capsid, thus providing a clear mechanism for the binding inhibition observed.[86–88] Despite these advances, attachment inhibitors have only been tested against VLPs from less common genotypes and thus their effectiveness against a broader spectrum of norovirus strains needs to be demonstrated.

Passive immunotherapy with monoclonal antibodies (mAbs) or nanobodies (Nbs) is an antiviral strategy to prevent norovirus attachment and entry, which would be particularly useful for immunocompromised patients. Therapeutic mAbs [2.1.4] generated from human and chimpanzee B cells have been described in several studies and were shown to block carbohydrate binding by norovirus VLPs.[89–91] However, only mAb D8 was shown to provide protection against Norwalk virus infection in a chimpanzee model[89] (Table 1). Moreover, mAbs generated thus far have limited cross-genotypic activity, which makes them a less than optimal antiviral against the antigenically diverse norovirus.

More recently, Nbs have been shown to have broadly neutralizing activity against VLPs of important norovirus genotypes via interaction with the capsid P domain.[92,93] Nanobodies are recombinant, single-domain, camelid heavy-chain antibody fragments of ~15 kDa which can bind with high affinity to a specific antigen (reviewed in Muyldermans[94]; Harmsen and De Haard[95]). The best-described norovirus Nb is Nb-85 [2.1.5] which has a broad binding capability against GII.1, GII.2, GII.4, GII.12, GII.17, and G1.11 VLPs, preventing attachment to HBGAs.[92,93] The cross-genotypic activity displayed by Nbs illustrates that these molecules have the potential to overcome the narrow antigenic spectrum typically displayed by conventional mAbs. However, despite these findings, mAb and Nb studies have been based mostly on VLP-binding and structural analysis of that binding (Table 1) and thus the effects of such compounds against norovirus in cell culture or in vivo need to be explored further before continued development toward clinical application.

2.2 | Polymerase inhibitors

Of the human norovirus antiviral targets, the RdRp is one with numerous preclinical candidates identified that can inhibit its activity (Table 1). Critical for viral replication, the RdRp is a highly attractive antiviral target, as it largely

lacks host homologs minimizing the chance of off-target adverse effects.[96] The human norovirus polymerase forms the canonical RdRp structure resembling a closed right hand, with fingers, palm, and thumb domains[97] (Figure 3B), likely acting as a homodimer in it active state.[97–99]

The RdRp-targeting antivirals are divided into two major classes; the nucleoside analogs (NAs) and the non-nucleoside inhibitors (NNIs). NAs and/or NNIs have been successful in the treatment of HCV, HIV, herpesvirus and hepatitis B virus (HBV) infections (reviewed in Eltahla[100]; Zhang and Wang[102]; Fung et al[103]; Usachet al[104]), and candidates from both classes have been assessed for antiviral activity against norovirus within this review (outlined below).

2.2.1 | Nucleoside analogs

NAs inhibit RNA synthesis through mimicry of incoming nucleoside triphosphates (NTPs), which upon incorporation subsequently cause chain termination,[105] or less commonly, increase mutations during viral genome transcription that results in lethal mutagenesis (also known as error catastrophe).[106] Since NAs bind in the highly conserved RdRp active site, they generally demonstrate broad-spectrum antiviral activity compared to NNIs. Therefore, NAs developed against other RNA viruses have been examined for repurposing as human norovirus therapeutics. In terms of norovirus, 2′-C-methylcytidine (2CMC) [2.2.1] is the most intensely studied and was initially developed as an antiviral therapy against HCV. 2CMC is a chain terminating cytidine analog with broad-spectrum in vitro activity against other flaviviruses including DENV, yellow fever virus, and West Nile virus.[107] However, the development of the oral 2CMC prodrug, Valopicitabine, was halted for use against HCV following reports of undesirable gastrointestinal side effects.[108]

Despite 2CMC being discontinued for clinical development, it is still widely reported as a potential norovirus antiviral. In one study, MNV plaque formation and RNA synthesis were inhibited by 2CMC, with EC_{50} values of 2.0 μM and 1.6 μM, respectively.[109] 2CMC was also found to reduce RNA synthesis of the Norwalk replicon in a dose-dependent fashion, with an EC_{50} of 18 μM.[110] Another study reported a similar 2CMC potency for MNV RNA level reduction (6.9 μM), but potency against the Norwalk replicon was found to be 14-fold higher (1.3 μM).[111] This difference was proposed to be due to either varying methodology between studies, or differences in drug purity.[110] In the human norovirus BJAB cell culture system, 2CMC inhibited human norovirus replication with an EC_{50} of 0.3 μM.[67] Despite the variation in 2CMC potency observed between these in vitro systems, collectively these studies illustrate that the polymerase is an excellent antiviral target for cross-genogroup inhibition of norovirus replication.

The NA 2CMC has also shown promise as a potential norovirus antiviral in mouse model studies. Knockout mice infected with MNV and treated with 2CMC were protected from mortality, diarrhea, and had reduced norovirus genome titers in tissues and stool (1.0-1.5 $\log_{10}$ reduction), compared to mock-treated animals.[67,110] Additionally, MNV-infected mice treated with a high dose of 2CMC (100 mg/kg/day for 5-7 days) demonstrated reduced transmission to uninfected sentinel mice caged together, and offered prophylactic protection for up to 18 days.[112]

In an attempt to improve the safety and efficacy of 2CMC therapy, several derivatives have been examined for antinorovirus activity, for example, 2′-*F*-2′-*C*-methylcytidine (2FCMC). One study evaluated the inhibitory activity of 2CMC, 2FCMC, β-D-N(4)-hydroxycytidine (NHC) and the HBV/HIV NA lamivudine against the replication of MNV and the Norwalk replicon.[111] EC_{50} values for 2CMC and 2FCMC in MNV cell culture were 6.9 and 12.7 μM, respectively,[111] while NHC and lamivudine had no inhibitory effect on replicon RNA synthesis. Indeed, both 2CMC and 2FCMC demonstrated dose- and time-dependent inhibition of Norwalk replicon RNA levels, although 2CMC (EC_{50} 1.3 μM) was found to be more than 2-fold more potent than 2FCMC (EC_{50} 3.2 μM).[111] While the search continues for a safer derivative of 2CMC for the treatment of norovirus infection, no effective alternative has been identified thus far.

Favipiravir (T705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) [2.2.2] is another broad-spectrum NA that has been assessed as a potential antiviral against human norovirus. T705 is a purine analog,[113] which was shown to

induce lethal mutagenesis against MNV,[114] consistent with results from other viruses.[115] T705 is approved for treatment of influenza in Japan[116] and also inhibits replication of several viruses using in vitro and mouse models, including flaviviruses, arenaviruses, hantaviruses,[113] and Ebola virus.[117] T705 has poor antiviral activity against MNV replication in cell culture, inhibiting virus-induced cytopathic effects with an EC_{50} of 250 μM, and RNA synthesis with an EC_{50} of 124 μM,[118] with a therapeutic index of just 4.3.[118] The human norovirus replicon has also been used to examine the antiviral efficacy of T705, which exhibited a modest EC_{50} of 21 μM and a CC_{50} of more than 100 μM.[119] Despite clinical approval for influenza, T705 displays a level of potency that is likely too low to be pursued further as a norovirus therapeutic.

Another broad-spectrum NA examined against norovirus is ribavirin (RBV; 1-α-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) [2.2.3]. RBV has broad-spectrum antiviral activity against both DNA and RNA viruses and has been used clinically to treat HCV, hepatitis E virus, Lassa fever, and respiratory syncytial virus, amongst others (reviewed in Snell[120]). A guanoside analog, RBV was first shown to inhibit the human norovirus replicon and MNV replication in 2007, with an EC_{50} of around 40 μM for Norwalk replicon and an 82% reduction of replicon genome levels at 100 μM compared to mock-treated controls.[121] As expected for an NA, the binding site of RBV was shown to be the polymerase active site by cocrystallization studies in the MNV RdRp.[122] However, RBV poorly inhibits MNV (EC_{50} of 63.5 μM) as well as the Norwalk replicon (EC_{50} of 40 μM).[111]

RBV exerts its inhibitory action early against MNV, with effects detectable at 8 hpi (MOI 0.01), suggesting chain termination as a possible mode of NA action.[114] However, the sequencing of MNV populations after four serial passages within the RAW264.7 cell line and in the presence of 200 μM RBV showed a three-fold increase in mutations per nucleotide compared to untreated populations.[114] These observations are consistent with reports of RBV having multiple modes of action, including mutagenesis, direct inhibition of RNA polymerases, unbalancing intracellular NTP pools, and interference of 5′ cap structures for capped viruses.[120,123–127] Despite this, the modest inhibition of RBV against MNV and the Norwalk replicon, coupled with the numerous adverse effects reported with RBV treatment, suggests that this antiviral is not desirable as an antinorovirus agent.

CMX521 [2.2.4] is a novel NA discovered through an HTS, and according to the manufacturer's press releases, has potent and pan-genotypic activity against norovirus.[128] CMX521 is reportedly in the recruitment stage of phase I clinical trials to evaluate the safety, tolerability, and pharmacokinetics in less than or equal to 50 healthy adults. No peer-reviewed publications were available when writing this review, with results projected to be released later in 2018.[128]

2.2.2 | Non-nucleoside inhibitors

NNIs generally exhibit narrow-spectrum antiviral activity and bind allosterically to block conformational rearrangements of the viral polymerase required to form an active replication complex.[129] Currently, there are three known NNI binding sites on the norovirus RdRp. One binding pocket is within the NTP access path located between the fingers and thumb domains,[130] the second pocket is termed Site A, a positively charged NTP traversal channel with flexible amino acid side chains,[131] and the third pocket is Site B, a highly conserved allosteric binding pocket present across the *Caliciviridae* and located within the thumb region[131,132] (Figure 3B).

Several therapeutics have been repurposed as NNIs against human norovirus. One example includes the sleeping sickness medication suramin [2.2.5], which is a large, symmetric polyanionic naphthylurea, with a molecular mass of 1297.29 g/mol. Suramin was found to potently inhibit in vitro activity of both the human and MNV polymerases[130] with IC_{50} values of 24.6 and 70.0 nM, respectively. Similarly, the smaller suramin derivative NF023 [2.2.6] inhibited human and mouse norovirus RdRp activities with IC_{50} values of 71.5 and 200 nM, respectively.[130] Cocrystallization studies of these compounds with the human norovirus polymerase identified the suramin and NF023 binding pocket within the NTP pathway, between the fingers and thumb domains.[130]

Other suramin derivatives that inhibit in vitro norovirus RdRp transcription are naphthalene disulfonate (NAF2; 286.27 g/mol) [2.2.7] and pyridoxal-5′-phosphate-6-(2′-naphthylazo-6′-nitro-4′,8′-disulfonate) tetrasodium salt

(PPNDS; 694.36 g/mol) [2.2.8].[131,132] NAF2 and PPNDS inhibited the human norovirus RdRp in an enzyme assay with IC_{50} values of 14 and 0.45 μM, respectively, and PPNDS also inhibited the MNV RdRp with an IC_{50} of 0.88 μM.[131,133] Cocrystallization studies revealed that NAF2 binds into both Site A and Site B, while PPNDS binds solely in Site B.[131] Further X-ray synchrotron crystallization studies by the same group confirmed that PPNDS also binds allosterically within Site B of the MNV RdRp by mimicking two stacking RNA bases.[133]

Despite the promising potency of suramin and its derivatives, including PPNDS, these compounds demonstrate limited bioavailability and exhibit poor cell permeability,[134] greatly reducing their antiviral efficacy in viral culture.[132,135,136] While suramin has been shown to inhibit MNV replication in cell culture (EC_{50} 0.3 μM), the large structure and low cell permeability meant delivery required a liposome system to enter RAW264.7 cells.[135] Similarly, PPNDS was found to be much less potent in MNV cell culture, demonstrating only 20.5% inhibition of MNV replication in a plaque assay at 10 μM, compared to 98.0% inhibition at the same concentration in the RdRp assays.[132] Moreover, PPNDS has been eliminated from other enzyme inhibition studies due to nonspecific, off-target effects,[137] and is therefore likely to be an unsuitable drug candidate for further antinorovirus development.

Repurposing antivirals developed against other viruses can sometimes reveal drug candidates with broad-spectrum activities for further development. A recent study evaluated the antiviral activity of six HCV NNIs against the human norovirus RdRp[132] including; Filibuvir, JTK-109, Lomibuvir, Nesbuvir, Setrobuvir, and Tegobuvir using a fluorescent polymerase assay. All HCV NNIs had an IC_{50} of more than 100 μM except JTK-109. JTK-109 [2.2.9], was shown to have broad-spectrum activity across several genera of the *Caliciviridae*, inhibiting the human norovirus polymerase with an IC_{50} of 4.3 μM MNV replication in cell culture with an EC_{50} of 6.1 μM, and against calicivirus RdRps in an in vitro activity assay with EC_{50} values ranging from 0.1 to 2.3 μM.[132] In the same study, PPNDS inhibited the human and mouse norovirus RdRps by 1.4 and 2.3 μM, respectively,[132] which is less potent than previously reported above,[133] with the variation likely due to different methodologies. Norovirus RdRp mutant studies, docking studies and combinational antagonism with PPNDS suggested the binding site of JTK-109 is within the highly conserved Site B pocket.[132]

To further exploit the broad-spectrum RdRp binding pocket Site B,[132] a high-throughput in silico screen of approximately 300 000 commercially available compounds was performed to identify ligands that bind to Site B of the human norovirus and MNV RdRps.[50] Sixty-two compounds were selected from the screen and assessed for inhibition of the human norovirus RdRp, revealing a hit (compound **11**) with an IC_{50} of 5.0 μM. Synthesis of derivatives of this compound produced a novel candidate (compound **54** [2.2.10]) with IC_{50} values of 5.6 μM (human norovirus RdRp) and 12.1 μM (MNV RdRp). Further in silico molecular modeling and biological antagonism studies with PPNDS suggested the binding site of compound **54** was indeed within the targeted broad-spectrum Site B pocket, however, it was relatively ineffective against MNV in cell culture, with an EC_{50} of more than 50 μM.[50]

These issues of cell permeability, specificity, compound toxicity, and potency could all be addressed by extensive medicinal chemistry to improve the "druggability" of these developmental NNIs.

Several potential norovirus NNIs have also been identified by HTS of compound libraries using viral enzyme activity assays. A case in point is the HTS of almost 20 000 compounds for RdRp inhibition using a fluorescent activity assay by Eltahla et al.[47] Four lead compounds were identified (NIC02 [2.2.11], NIC04, NIC10, and NIC12) that had IC_{50} values against the human norovirus polymerase ranging from 5.0 to 9.8 μM, and inhibited both the Norwalk replicon and MNV replication in cell culture with EC_{50} values between 30.1 to >100 μM and 4.8 to 38.1 μM, respectively.[47] These NNIs represent scaffolds with the potential to be structurally modified for increased potency.

While a growing body of research has been invested in identifying antivirals that target the norovirus polymerase, to date all candidates are still in early preclinical development and the search for a compound continues in earnest.

2.3 | Protease inhibitors

Much like the RdRp, the norovirus protease (NS6; Figure 3C) represents a desirable antiviral target since it plays an essential role in viral replication, through cleavage of the NS polyprotein, which is necessary for the production of

viral progeny. Norovirus protease inhibitors (PIs) have been tested over a wide range of model antiviral systems (Figure 2) and represent the class of calicivirus antivirals with the most number of compounds (Table 1). Rupintrivir [2.3.1] was one of the first PIs described for norovirus, and although this compound was originally developed for human rhinovirus, Kim et al[48] demonstrated broad-spectrum antiviral activity of Rupintrivir against other picornaviruses, coronaviruses, and caliciviruses, including; MNV, FCV, and the Norwalk replicon. Subsequent studies have supported these initial observations and showed that Rupintrivir inhibited Norwalk replicon with EC_{50} values of 0.3 and 1.3 μM.[138,139] However, in some cases PI potency does not translate in vivo, for example, Rupintrivir demonstrates poor pharmacokinetic properties and limited bioavailability,[140] and is, therefore, not ideal as an antinorovirus therapy.

The remaining norovirus PIs described in the literature were mostly developed by one group of researchers and are broadly divided into transition state (TS) inhibitors and TS mimics (reviewed in Galasiti et al[72]). The TS mimics include α-hydroxyphosphonates (e.g. compound 7d [2.3.2][141]) which effectively inhibit the Norwalk replicon at low micromolar concentrations.[141] Although potent, TS mimics are far less studied than TS inhibitors and represent a small proportion of the compounds described in Table 1.

The first TS inhibitors designed included a series of peptidyl aldehydes that incorporated a glutamine surrogate in their structure,[142] and take advantage of the preference for the norovirus protease to cleave at glutamine-glycine peptide sites.[143] Within this original series of peptidyl aldehydes ($n = 10$), compounds **4** [2.3.3] and **5** displayed inhibition of MNV infection (EC_{50} of 5.5 and 20.3 μM, respectively) and the Norwalk replicon (EC_{50} of 2.1 and 7.8 μM, respectively).[142] Several variants of the these primary hits were developed in later studies and included: dipeptidyl aldehydes (e.g. compound 16 [2.3.4]),[48,144] tripeptidyl aldehydes (e.g. syc10 [2.3.5]),[145] peptidyl α-ketoamides (e.g. compound 6d [2.3.6]),[48,141,146] α-ketoheterocycles (e.g. compound 8a [2.3.7],[146] bisulfite adducts (e.g. GC376, [2.3.8]),[48,147,148] and ester or carbamate prodrugs of bilsulfite adducts (e.g. compound 11 [2.3.9]).[149] Each of these variants have displayed an equal or a greater level of antiviral activity when compared to the original peptidyl aldehyde inhibitors, with the most potent of these variants displaying low nanomolar potency against MNV (in vitro and in vivo) and the Norwalk replicon [2.3.4].[144]

Another subclass of TS inhibitors includes macrocyclic compounds[150–153] which are structurally modified versions of the aforementioned peptide-based TS inhibitors. These macrocyclic compounds were originally designed to improve the membrane permeability and oral bioavailability of TS inhibitors to increase their suitability for the clinical application.[153] Several studies have investigated the inhibitory effects of these macrocyclic TS inhibitors (EC_{50} range: 1.5->20 μM) such as compound 24 [2.3.10]),[151,154] as well as triazole-based (EC_{50} range, 3.8-88.3 μM) (e.g. compound 8 [2.3.11]),[153] oxadiazole-based (EC_{50} range, 2.5-51.2 μM; e.g. compound 17 [2.3.12]),[150] and oxazolidinone-based (EC_{50} range, 6.7-17.5 μM; e.g. compound 9 [2.3.13])[152] variants against the Norwalk replicon. Although none of the macrocyclic TS inhibitors have reached the potency achieved by the peptide-based TS inhibitors, several macrocyclic variants display broad-spectrum antiviral activity against members of the picornavirus-like supercluster similar to that of Rupintrivir.[154] The effectiveness of PIs across multiple genera and norovirus genogroups illustrates that the protease is an excellent antiviral candidate for the treatment of norovirus infections.

2.4 | Host-factor drugs

A pitfall of using some DAAs is the emergence of resistance mutations which can undermine their effectiveness, although combinational therapy is a proven option in HIV and HCV therapy to circumvent this. In comparison to DAAs, antivirals that target the host generally have a higher barrier to resistance than some classes of DAAs, particularly PIs and NNIs which are known to have a low barrier to resistance (reviewed in Kaufmann et al[155]). Thus host-targeted therapies represent an important antiviral class to be considered for the treatment of norovirus infections. Host-factor drugs can target individual cellular components that directly interact with the virus or aid viral replication. Alternatively, host-factor drugs may influence multiple cellular components that culminate in antiviral defenses.

2.4.1 | Protein targets

A recently explored class of host-targeted norovirus antivirals include deubiquitinase (DUB) inhibitors.[156–158] DUB inhibitors are a class of enzymes involved in regulation of the ubiquitin-proteasome system which is commonly exploited by viruses for replication (reviewed in Luo[159]). WP1130 [2.4.1] is an example of a small synthetic DUB inhibitor (molecular weight of 384.2 g/mol) that was shown to effectively inhibit MNV and norovirus replication through induction of the unfolded protein response.[158] However, this initial study revealed that inhibition of MNV in mice was limited to the small intestine due to poor bioavailability of WP1130.[158] To address the poor bioavailability, libraries of WP1130 variants were developed and tested for improved antiviral efficacy.[156,157] Out of 59 derivatives, compound **9** [2.4.2] was found to be the most potent antiviral.[156] Treatment at 2.5 μM with this derivative resulted in an 84.7% reduction in replication of the Norwalk replicon and a 2.5-log reduction in MNV titer in infected cells; more than double the potency of the parental compound.[156]

Another host protein that has been identified as an antiviral target is heat-shock protein 90 (Hsp90), a molecular chaperone involved in the maturation of proteins responsible for multiple biological processes (reviewed in Li et al[160]). Vashist et al[161] initially showed using siRNA knockdown and overexpression studies that Hsp90 interacts with the 5′ and 3′ termini the MNV genome and plays a key role in MNV replication.[161] The authors then showed that abolishment of Hsp90 activity using 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG [2.4.3]) potently inhibited MNV replication in vitro (IC_{50} of 60 nM and CC_{50} of 4.5 μM) and reduced MNV titer (~ 1-log) in an in vivo mouse model.[161]

Despite potential cytotoxicity from some host-targeted compounds (reviewed in Engel et al[162]), generally, therapeutics that target specific host proteins result in effective antiviral activity with minimal off-target effects, which are desirable features for norovirus therapeutics.

2.4.2 | Immunomodulators

Immunomodulators are an excellent therapeutic option for viral infections due to their ability to induce a powerful host response against intracellular parasites. The best example of immunomodulators are interferons (IFNs) and for over a decade, studies have shown that type I and II IFNs, as well as their receptors, provide protection against murine and human norovirus infections.[44,52,53,163–168] However, the role of type III IFNs (IFN-λ), in norovirus infection and their potential as norovirus antivirals has only recently been explored.[169] IFN-λ [2.4.4] binds to a heterodimeric receptor comprised of IFN-λ receptor 1 (IFNLR1) and interleukin-10R2 (IL-10R2), which induces the expression of many of the same genes induced by type I and II IFNs.[170–173] However, unlike type I and II IFN, a single dose of IFN-λ (1 μg) has been shown to both prevent and clear persistent MNV infection in mice.[174] IFN-λ was shown to target nonhematopoietic cells[174] and intestinal epithelial cells which express high levels of IFNLR1.[175] Using a MNV transmission model, endogenous IFN-λ expression (induced using a transfected plasmid) was shown to block MNV transmission from mice infected with the acute CW3 MNV strain.[176] IFN-λ treatment also prevented intestinal CW3 replication, inflammation, and antibody responses in mice,[176] which illustrates that it has an integral role in the prevention of norovirus transmission. Taken together, the antiviral activity displayed by IFN-λ in these studies reveal that it has potential as a therapeutic to not only cure infections but also prevent norovirus spread if given prophylactically.

More recently the group of immunomodulators known as Toll-like receptor (TLR) agonists have been explored as norovirus antivirals. These compounds have been used for many years as vaccine adjuvants, but have also been shown to inhibit the replication of RNA viruses HIV and HBV.[177,178] TLR agonists induce the innate immune response and stimulate IFN production, which is known to have antiviral activities against MNV and human norovirus in vitro.[44,52,53,163–168] Several TLR7 agonists were shown to display potent inhibition of MNV infection, including R-848 [2.4.5] (23.5 nM), GS-9620 (0.59 μM), Gardiquimod (0.13 μM) and R-837 (1.5 μM), with therapeutic indices more than 30 for each compound.[179] Furthermore, conditioned media generated by stimulation of THP-1

cells with 10 μM of R-848 reduced replication of the Norwalk replicon by 50%.[179] More recently the TLR4 agonist, poly-γ-glutamic acid (γ-PGA) [2.4.6] was explored as an antiviral and when tested against MNV in vitro displayed an EC_{90} value < 100 nM.[180] In addition, a 50 mg/kg dosage of γ-PGA to mice before and following MNV infection resulted in ≥47% reduction in viral genomes.[180] Given that the TLR4 agonist monophospholipid A is approved for use as an adjuvant for HPV and HBV vaccines (reviewed in Dowling and Mansell[181]) and the TLR7 agonist R-837 is already approved for the treatment of HPV-associated genital warts,[182] repurposing TLR4 and TLR7 agonists could hugely expedite their use for norovirus clinical treatment.

2.5 | Compounds with unknown targets

Several compounds have demonstrated antiviral activity where the mechanism of inhibition is unknown. These include flavonoid-type molecules, such as theaflavin digallate [2.5.1], myricetin and epigallocatechin-3-gallate, among many others that display broad-spectrum medicinal properties, although they display limited inhibition of norovirus infection in vitro.[183–186] The mechanism of action of flavonoids against norovirus is thought to be a result of anti-inflammatory pathway activation. Similarly, phytochemicals such as curcumin [2.5.2] are a class of compounds that display inhibition of norovirus in vitro,[187] however, the mechanism of inhibition is poorly understood. Vitamin A [2.5.3] has been shown to inhibit MNV in vitro, albeit poorly with less than 50% inhibition at 50 U/mL and ~50% inhibition of the Norwalk replicon at 100 U/mL. Vitamin A-induced changes in the microflora are thought to be the mechanism responsible for these antiviral effects.[188] Lastly, nitazoxanide (NTZ) [2.5.4] (covered in section 3) has shown promise as an antiviral therapy, but the defined mechanism of activity against norovirus has yet to be determined. Most recently NTZ was shown to potently inhibit FCV replication in cell culture with an EC_{50} of 0.6 μM,[189] and the GI norovirus replicon at a clinically relevant concentration (5 μg/mL),[190] which was later shown to result in a broad antiviral response.[191] Despite these observations, the latter study also showed that NTZ was ineffective against MNV suggesting that further antiviral investigations are warranted.[189]

3 | DRUGS IN CLINICAL DEVELOPMENT

To date, no norovirus antiviral or vaccine is approved for medical use, and the only norovirus antiviral candidate to complete clinical trials is NTZ. This compound was originally developed in the 1970s, and is currently an FDA-approved therapy for treating *Giardia* and *Crytosporidium* infections.[192] NTZ has demonstrated broad-spectrum antimicrobial activity against a range of bacterial, protozoan, and viral infections, including inhibition of the Norwalk replicon (EC_{50} 1.6 μM) (reviewed in Rossignol[193]).

In phase II randomized double-blind trial, NTZ therapy was administered to 25 of 50 patients (≥ 12 years) that tested positive for rotavirus or norovirus infection. Treatment resulted in a significant reduction in the duration of gastroenteritis symptoms (norovirus, $P = 0.0295$ and rotavirus, $P = 0.0052$) from 2.5 to 1.5 days when compared to the placebo.[194]

Additional anecdotal studies have supported the efficacy of NTZ treatment for norovirus. NTZ successfully treated one immunosuppressed transplant patient infected with norovirus that had experienced 10 consecutive days of gastroenteritis symptoms.[195] Four days after commencing NTZ treatment, a complete resolution of symptoms was recorded without a reduction in immunosuppressive drugs.[195] NTZ treatment was also shown to resolve diarrheal symptoms and clear norovirus in stool samples from a pediatric patient with chronic norovirus, following kidney transplantation.[196]

Although the above-mentioned studies suggest that NTZ is a promising therapy for the treatment of norovirus infections, there is an equal amount of evidence revealing that NTZ is ineffective against norovirus

infections.[197–200] Despite this contrary evidence, NTZ represents the only therapeutic option currently available apart from RBV, immunoglobulins and supportive care to patients with persistent infections.

4 | CONCLUSIONS

Human norovirus is a pervasive pathogen that creates a significant social and economic impact and causes hundreds of thousands of deaths each year. Despite intensive research for safe and effective norovirus antivirals, none have yet been clinically approved, and the majority of candidates are still in the early stages of preclinical development. This review outlines recent therapeutic candidate studies to provide an updated overview of the current human norovirus antiviral development pipeline.

Optimization of human norovirus culture in enteroid and B cell systems offers the promise that in time, more robust and reliable culture methods will be developed allowing greater replication levels. These improved systems may enhance antiviral studies to provide a stronger platform for effective norovirus antiviral development.

The landscape of antiviral development for norovirus is changing. The rapidly developing field of host immunomodulatory therapies is opening the door to potential treatments for many viral infections, with promising results already published for norovirus. Moreover, recent discoveries have reported highly conserved binding pockets on critical viral enzymes, such as the norovirus polymerase, which could allow for the further development of broad-spectrum antivirals.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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