Invited Review

Iminosugars: A host-targeted approach to combat *Flaviviridae* infections

Lisa Evans DeWald [a], Chloe Starr [a], Terry Butters [b], Anthony Treston [a], Kelly L. Warfield [a,*]

[a] *Emergent BioSolutions, Gaithersburg, MD, 20879, USA*
[b] *Oxford Glycobiology Institute, Oxford, OX1 3QU, UK*

A B S T R A C T

N-linked glycosylation is the most common form of protein glycosylation and is required for the proper folding, trafficking, and/or receptor binding of some host and viral proteins. As viruses lack their own glycosylation machinery, they are dependent on the host's machinery for these processes. Certain iminosugars are known to interfere with the N-linked glycosylation pathway by targeting and inhibiting α-glucosidases I and II in the endoplasmic reticulum (ER). Perturbing ER α-glucosidase function can prevent these enzymes from removing terminal glucose residues on N-linked glycans, interrupting the interaction between viral glycoproteins and host chaperone proteins that is necessary for proper folding of the viral protein. Iminosugars have demonstrated broad-spectrum antiviral activity *in vitro* and *in vivo* against multiple viruses. This review discusses the broad activity of iminosugars against *Flaviviridae*. Iminosugars have shown favorable activity against multiple members of the *Flaviviridae* family *in vitro* and in murine models of disease, although the activity and mechanism of inhibition can be virus-specfic. While iminosugars are not currently approved for the treatment of viral infections, their potential use as future host-targeted antiviral (HTAV) therapies continues to be investigated.

1. Introduction

Host factors required for the replication of multiple viruses provide for a good target for the development of broad-spectrum antiviral drugs. Recent advances in the development of novel antiviral therapies have taken advantage of similarities in viral replication strategies by identifying and targeting host-cell pathways required for viral replication that are common to *Flaviviridae* or other viruses. One such pathway is the host N-linked glycosylation pathway, which has been shown to be required for the replication of multiple enveloped viruses (Watanabe et al., 2019). The aim of this review is to highlight the anti-*Flaviviridae* activity of iminosugars, a promising class of host-targeted antivirals (HTAVs) that can interfere with the host's N-linked glycosylation pathway by inhibiting α-glucosidases in the endoplasmic reticulum (ER).

2. N-linked glycosylation

Protein glycosylation is an essential post-translational modification required for the proper folding and function of glycoproteins and plays a key role in many cellular processes, including protein-protein and cell-cell interactions. N-linked glycosylation, the most common form of protein glycosylation, is catalyzed at the luminal face of the ER. The oligosaccharyltransferase complex (OST) co-translationally attaches the preformed N-linked glycan core, $Glc_3Man_9GlcNAc_2$, to an asparagine residue within the sequence asparagine-X-serine/threonine (N-X-S/T) of the target peptide, where "X" represents any amino acid residue except proline. Several factors can contribute to the glycosylation efficiency of N-X-S/T motifs, including the ability of the OST complex to access the sequon as well as the sequence content (Kasturi et al., 1997; Martinez-Duncker et al., 2014). Glycosylation efficiency is typically higher when the target sequence contains a threonine rather than a serine (Kasturi et al., 1997).

Processing of the attached N-linked oligosaccharide begins with sequential cleavage of terminal glucose residues by the ER α-glucosidases I and II. The distal glucose residue (α1,2-glucose) is cleaved by α-glucosidase I to generate $Glc_2Man_9GlcNAc_2$ (Kornfeld and Kornfeld, 1985), which can then associate with the ER membrane-bound lectin, malectin, or be further processed by α-glucosidase II (Schallus et al., 2008). The ER α-glucosidase II enzyme is responsible for consecutively cleaving the second and third glucose residues to generate $Man_9GlcNAc_2$ (Kornfeld and Kornfeld, 1985). However, prior to cleavage of the third glucose residue, the chaperone proteins, calnexin and calreticulin, can bind to the monoglucosidated glycan and assist with folding and proper

disulfide bond formation through interaction with a protein disulfide isomerase (Hebert et al., 1995; Molinari and Helenius, 1999). Once the third glucose residue is cleaved, proteins that are not properly folded can be transiently reglycosylated by UDP glucotransferase 1 to prevent further processing and return to the calnexin/calreticulin cycle. Proteins may progress through a series of reglycosylation, refolding, and processing steps until properly folded and sent to the Golgi apparatus for further processing. If proper folding is not achieved, the ER enzyme mannosidase I may remove the mannose residues from the oligosaccharide to prevent reglycosylation and reprocessing through the calnexin cycle, and the protein may be targeted for degradation by the ER-associated degradation pathway (Avezov et al., 2008).

The ER glycosylation machinery is not only required for host protein glycosylation, it is also critical for the lifecycle of diverse viruses. N-linked glycosylation is a necessary component for the replication of many viruses as it is required for the proper folding, trafficking, and/or receptor binding of some viral proteins (Watanabe et al., 2019; Yap et al., 2017). Because viruses do not possess their own glycosylation machinery, they rely on host-cell machinery for these processes. Viral dependency on this pathway was demonstrated in a case study of two patients with MOGS-CDG, a rare congenital disorder caused by absent expression of α-glucosidase I and a lack of N-linked glycan processing in the ER (Sadat et al., 2014). Although these patients had hypogammaglobulinemia and other complications, they also had no documented history of viral illness. These patients had no immune recognition against childhood vaccinations consisting of live virus vaccines directed against measles, mumps, rubella, and varicella viruses, which contain glycosylated envelopes, but had normal antibody responses when vaccinated with nonreplicating infectious agents presenting proteins, polysaccharides, and other immunogens, including the diphtheria-tetanus-acellular pertussis, hepatitis B, 23-valent pneumococcal polysaccharide, and conjugated *Haemophilus influenzae* type B vaccines. Additionally, cells isolated from these patients had increased resistance to infection by the glycosylation-dependent human immunodeficiency virus (HIV) and influenza A virus *in vitro,* but not to the nonenveloped viruses, adenovirus or poliovirus 1, confirming this pathway has an essential role in the replication of certain viruses.

3. *Flaviviridae* replication and the role of the host glycosylation pathway

3.1. *Genomic organization and replication strategies employed by Flaviviridae*

Although there are distinct differences in the mode of transmission, tissue tropism, and course of infection between different members of the *Flaviviridae* family (Supplemental Table 1), all viruses in this family have similar replication cycles and genomic organization. The *Flaviviridae* RNA genome ranges from 9,000 to 13,000 bases containing one open reading frame that encodes for a single polyprotein (Simmonds et al., 2017). After the virus is internalized through receptor-mediated endocytosis, the viral RNA genome is released into the cytoplasm where it is translated into the polyprotein, which is processed by viral and host-cell proteases to produce ~3–4 structural and ~6–8 non-structural (NS) proteins, depending on the virus. RNA replication occurs in membrane-associated viral replication organelles that are derived from the ER (Neufeldt et al., 2018; Paul and Bartenschlager, 2015). Immature viral particles are assembled on the surface of the ER and are transported through the trans-Golgi network where they are further processed to produce mature virus particles that are subsequently released from the cell by exocytosis.

Structural proteins are derived from the amino terminus end of the polyprotein to allow the virus to maximize production of these proteins, while the NS proteins are derived from the carboxyl-terminal end. NS proteins are involved with proteolysis, viral assembly, regulation of host immunity, and RNA replication, and include sequences that are characteristic of an RNA-dependent RNA polymerase, serine protease, and RNA helicase (Brinton, 2002; Fernandez-Garcia et al., 2009; Simmonds et al., 2017; Zhang et al., 2017). Pestiviruses, pegiviruses, and hepaciviruses also contain an additional P7/P13 protein that is believed to be important for viral maturation that is not expressed by members of the *flavivirus* genus. Structural proteins, which are required to form progeny virus particles, include two or three membrane-associated glycoproteins that differ slightly between genera. Hepaciviruses, flaviviruses, and pestiviruses also contain a structural basic capsid protein (C) which assembles to form the nucleocapsid that encases the viral genetic material.

The membrane-associated glycoproteins contain a variable number of potential N-linked glycosylation sites depending on the virus and the protein. During viral replication, N-linked glycans are added to the target N-X-S/T motifs in viral glycoproteins and processed by the host ER glycan processing and protein folding machinery.

3.2. *Flavivirus replication and protein glycosylation*

Flavivirus structural proteins include the C protein, precursor membrane glycoprotein (prM), and envelope (E) protein, although only membrane-associated E proteins are exposed on the surface of mature particles. The E protein is glycosylated and co-translationally processed in the ER lumen where it heterodimerizes with prM, which can function as a chaperone to stabilize E proteins (Idris et al., 2016; Zhang et al., 2003) and ensure proper protein folding (Li et al., 2008; Lorenz et al., 2002; Yap et al., 2017; Yu et al., 2009). The prM-E heterodimers further oligomerize (Konishi and Mason, 1993; Zhang et al., 2003) and assemble into immature, noninfectious virions containing nucleocapsid protein complexed with viral RNA (Lorenz et al., 2002, 2003; Mackenzie and Westaway, 2001). The immature virions are translocated to the Golgi complex where the low pH environment promotes reorganization of the prM-E heterodimers, allowing for the host protease, furin, to cleave prM to the mature membrane protein (M) (Fernandez-Garcia et al., 2009; Li et al., 2008; Stadler et al., 1997; Yu et al., 2008a). This results in the formation of head-to-tail E homodimers and the generation of mature infectious particles that are eventually released from the cell by exocytosis (Kuhn et al., 2002; Mukhopadhyay et al., 2003; Stiasny and Heinz, 2006). The dengue virus (DENV) prM/M protein contains an N-linked glycosylation site at N69 and may contain other sites at additional locations (N7, N31, N52) (Courageot et al., 2000). The ability of prM to act as a chaperone for E protein is thought to be dependent on glycosylation of prM (Courageot et al., 2000; Idris et al., 2016). Removal of the N-linked glycosylation site from the prM protein of Japanese encephalitis virus (JEV) and certain West Nile virus (WNV) strains can significantly decrease the amount of newly formed virus particles secreted from mammalian cells (Hanna et al., 2005; Kim et al., 2008; Zai et al., 2013).

Several potential N-linked glycosylation motifs have also been identified in the flavivirus E glycoprotein (Dai et al., 2016; Gavel and von Heijne, 1990; Hahn et al., 1987; Ishak et al., 2001; Johnson et al., 1994; Mukhopadhyay et al., 2003; Zhang et al., 2003). The DENV E protein is glycosylated at two asparagines, N67 and N153 (Chambers et al., 1990; Hacker et al., 2009; Johnson et al., 1994). N153 is a conserved glycosylation site in most *Flaviviridae* (present as N154 in some viruses), whereas N67 is unique to DENV. Glycosylation of N67 is thought to mediate the interaction between the virus and the host-cell receptor DC-SIGN and is required for assembly and egress of DENV (Bryant et al., 2007; Mondotte et al., 2007; Pokidysheva et al., 2006).

The E protein of most flaviviruses, aside from DENV, contains only the single, conserved N153/154 glycosylation site. N-linked glycosylation of the flavivirus E protein has been associated with pH sensitivity, neurovirulence, infectivity, and virus secretion (Beasley et al., 2005; Bryant et al., 2007; Guirakhoo et al., 1993; Hanna et al., 2005; Kawano et al., 1993; Lee et al., 1997, 2010; Mondotte et al., 2007; Mossenta et al., 2017; Vorndam et al., 1993). Deletion of the N153/154

glycosylation site results in decreased secretion of DENV (Lee et al., 2010), tick-borne encephalitis virus (TBEV) (Goto et al., 2005; Yoshii et al., 2013), ZIKV (Mossenta et al., 2017), and WNV (Hanna et al., 2005) from mammalian cells and attenuation of Zika virus (ZIKV) in mouse models of disease (Fontes-Garfias et al., 2017). Despite the importance of this glycosylation site for many flaviviruses, not all flavivirus E proteins are N-glycosylated. For WNV, there is a correlation between viruses that contain the E protein glycosylation site and those that are responsible for many of the major outbreaks of human disease (Hanna et al., 2005).

The flavivirus genome also encodes seven NS proteins: NS1, NS2A/B, NS3, NS4A, 2K, NS4B, and NS5 (Apte-Sengupta et al., 2014; Lopez-Denman and Mackenzie, 2017; Yu et al., 2008b; Zhang et al., 2017). NS1 contains two potential N-glycosylation sites (N130 and N207) that are conserved in flaviviruses (Flamand et al., 1999; Putnak et al., 1988; Winkler et al., 1989), in addition to less conserved sites (e.g. N175 in WNV) (Akey et al., 2014). NS1 glycosylation is necessary for proper replication and virulence of DENV, although the mechanism of how NS1 glycosylation is involved in these processes is not well understood (Muylaert et al., 1996; Somnuke et al., 2011).

3.3. HCV replication and protein glycosylation

Hepatitis C virus (HCV) structural proteins include the C protein and two envelope proteins, E1 and E2, which are dependent on each other for proper protein folding (Brazzoli et al., 2005; Cocquerel et al., 2001). E1 and E2 contain 9–11 and 4–5 highly conserved glycosylation sites, respectively, depending on the viral genotype (Goffard and Dubuisson, 2003). Following glycosylation, E1 and E2 can form noncovalently-bound heterodimers that interact with the ER chaperone protein, calnexin, to assist with productive folding of the proteins (Beljelarskaya et al., 2016; Chapel et al., 2006; Choukhi et al., 1998; Op De Beeck et al., 2001). Alternatively, the glycoproteins can interact with calreticulin and form disulfide bridges, resulting in the production of misfolded protein aggregates (Beljelarskaya et al., 2016; Chapel et al., 2006; Choukhi et al., 1998; Dubuisson, 2000; Op De Beeck et al., 2001) Formation of E1-E2 heterodimers is important for viral fusion, entry, and secretion (Lindenbach and Rice, 2013). Mutation studies demonstrated that specific glycosylation sites in the HCV envelope proteins are important for the correct folding and heterodimerization of E1 and E2, including N1 and N4 in E1, and N1, N8 and N10 in E2 (Freedman et al., 2016; Goffard et al., 2005; Helle et al., 2010). Glycosylation of several sites in E2 is also believed to contribute to immune evasion (Helle et al., 2010).

3.4. Evidence of Flaviviridae dependency on host ER α-glucosidases

Considering the important role N-linked glycosylation can have in the folding and function of *Flaviviridae* glycoproteins, it is likely that impeding this pathway could lead to detrimental effects on viral replication. Consistent with this concept, the ER α-glucosidases have been confirmed as antiviral targets for multiple viruses in the *Flaviviridae* family, including HCV, DENV, yellow fever virus (YFV), and (ZIKV). Downregulation of either α-glucosidase enzyme in Huh7.5 cells by shRNA resulted in a 5-fold reduction in the production of HCV particles without impacting cell growth or viability (Qu et al., 2011). In a separate study, CRISPR/Cas9 technology was used to generate Huh7.5 cell lines lacking expression of α-glucosidase I or α-glucosidase II, and the impact on viral replication and particle secretion was measured following infection with DENV, YFV, or ZIKV (Ma et al., 2018a). A significant reduction in intracellular viral RNA and virus secretion (~10–1, 000-fold) was observed for DENV and YFV in α-glucosidase I and II knockout Huh7.5 cells compared to control parental cells. Expression of α-glucosidase II was not required for the replication of ZIKV; however, loss of α-glucosidase 1 expression reduced ZIKV secretion by greater than 10-fold. These studies provide evidence that the ER α-glucosidases

have a functional role in the replication of several virulent *Flaviviridae* pathogens.
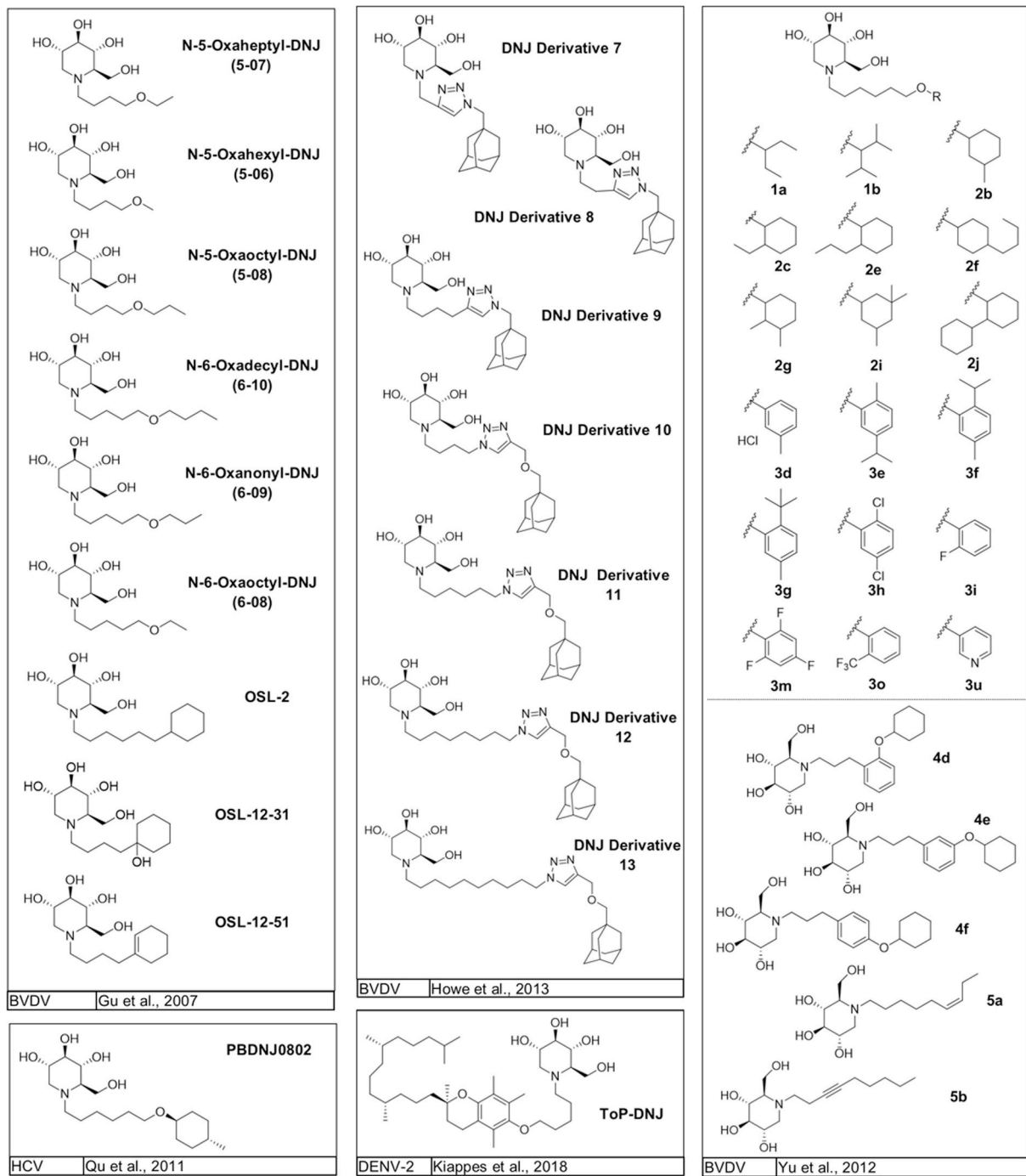
4. Iminosugars

The required involvement of the host's ER glycosylation machinery for replication of diverse viruses makes it a key target for broad-spectrum HTAV therapies. Iminosugars are sugar mimetics that can function as competitive inhibitors of a variety of glycosidase and glycosyltransferase enzymes that interact with sugar substrates. Certain iminosugars are known to inhibit the ER glycosylation pathway by targeting and inhibiting α-glucosidase I and α-glucosidase II and comprise the largest class of α-glucosidase inhibitors currently in development (Simone et al., 2018). Perturbing ER α-glucosidase function can prevent the enzymes from removing terminal glucose residues on N-linked glycans, thereby inhibiting the interaction with the calnexin/calreticulin chaperone proteins and proper folding of some viral glycoproteins. This can ultimately lead to the activation of the cellular unfolded protein response, which can detect the misfolded viral proteins and send them for degradation, resulting in decreased production of virions. Alternatively, it is possible that some misfolded viral glycoproteins may escape detection and incorporate into viral particles, greatly reducing their infectivity (Fischer et al., 1996).

The naturally occurring compounds 1-deoxynojirimycin (DNJ) and castanospermine (CAST) were some of the first iminosugars identified to have antiviral properties. Medicinal chemistry strategies to design derivatives of these iminosugars have led to the development of compounds with increased activity and decreased associated cytotoxicity. Numerous analogs and derivatives of DNJ and other iminosugars have been evaluated for antiviral activity against a diverse range of enveloped viruses, including members of the *Flaviviridae* family (Figs. 1–5) and Ebola, Marburg, influenza A and B, herpes simplex, hepatitis B, Sindbis, Semliki Forest, chikungunya, measles, Newcastle disease, severe acute respiratory syndrome, Lassa, Junín, Rift Valley fever, vesicular stomatitis, and human immunodeficiency viruses (Chang et al., 2013a; Miller et al., 2018).

Although iminosugars are not currently approved for use as antiviral therapies, they have a good safety profile and a long history of use in

Castanospermine (Cast)

In Vitro	
BVDV	Ouzounov et al., 2002; Whitby et al., 2004
DENV-1	Courageout et al., 2000; Whitby et al., 2005
DENV-2	Whitby et al., 2005; Gu et al., 2007; Lad et al., 2013; Sayce et al., 2016
DENV-3	Whitby et al., 2005
DENV-4	Whitby et al., 2005
JEV	Lad et al., 2013
WNV	Whitby et al., 2005; Gu et al., 2007
YFV	Whitby et al., 2005
In Vivo	
DENV-2	Whitby et al., 2005; Watanabe et al., 2012
WNV	Whitby et al., 2005

Celgosivir (BuCast)

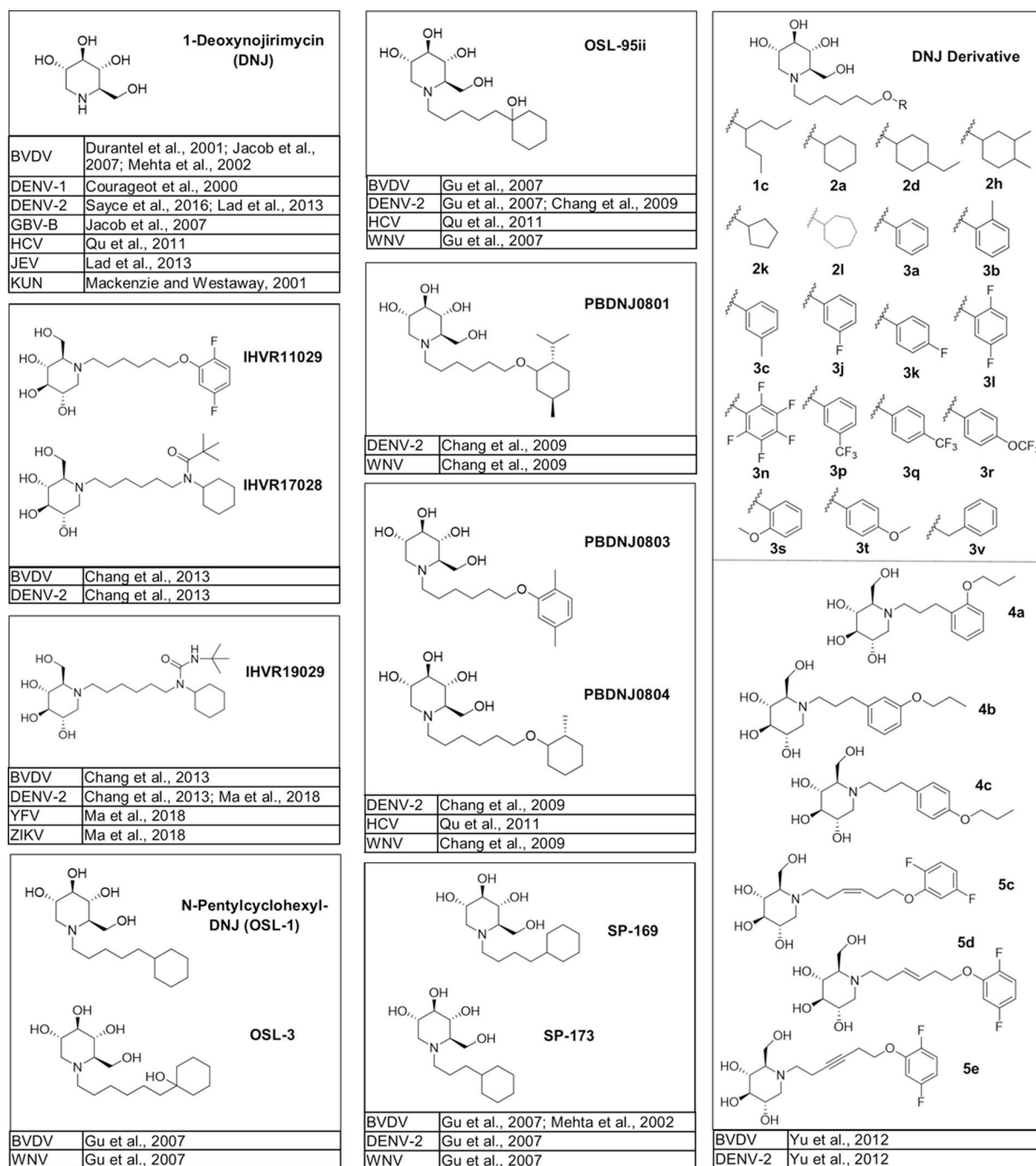| *In Vitro*	
BVDV	Whitby et al., 2004
DENV-1	Rathore et al., 2011; Watanabe et al., 2016
DENV-2	Rathore et al., 2011; Watanabe et al., 2016; Sayce et al., 2016
DENV-3	Rathore et al., 2011
DENV-4	Rathore et al., 2011
In Vivo	
DENV-1	Watanabe et al., 2016
DENV-2	Rathore et al., 2011; Watanabe et al., 2012; Watanabe et al., 2016; Sayce et al., 2016
Clinical	
DENV	NCT02569827; NCT01619969
HCV	NCT00292084; NCT00217139; NCT00157534

Fig. 1. Bicyclic iminosugars evaluated for activity against members of the *Flaviviridae* family. Structures for bicyclic iminosugars that have been tested for activity *in vitro* and *in vivo* against members of the *Flaviviridae* family, both of which have advanced to in clinical trials as potential HTAVs to combat *Flaviviridae* infections (Watanabe et al., 2012).

Fig. 2. DNJ derivatives evaluated *in vitro* for activity against a single member of the *Flaviviridae* family. Structures for monocyclic glucose-mimicking iminosugars that have been tested for activity *in vitro* against a single virus in the *Flaviviridae* family (Kiappes et al., 2018).

humans. Several iminosugars are approved by the Food and Drug Administration (FDA) for other indications which require long-term treatment. The N-alkylated DNJ derivative, N-butyl-deoxynojirimycin (*N*B-DNJ/Miglustat/Zavesca®), is a glucosylceramide synthase enzyme inhibitor approved for use to treat adult patients with type 1 Gaucher's disease, a lysosomal storage disorder that results from impaired break down of glucocerebroside due to decreased production of the enzyme, glucocerebrosidase. Migalastat (Galafold™) is approved for the treatment of another type of genetic lysosomal storage disorder, Fabry disease, which is caused by increased levels of globotriaosylceramide due to a deficiency in the production of the enzyme, alpha-galactosidase A. An FDA-approved DNJ derivative, N-hydroxyethyl-1-deoxynojirimycin

(BAY m 1099/Miglitol/Glyset®), is used to help control type 2 diabetes by targeting an α-glucosidase in the small intestines to prevent the breakdown of large carbohydrates into glucose. Acarbose (PRECOSE®) is similarly approved in the United States and other countries for the treatment of diabetes, as is voglibose (Voglib) in Japan. Several of these medications have been used in human patients for over 25 years and are considered safe treatment options for the intended indications. The clinical long-term use of iminosugars to treat these chronic diseases demonstrates the safety of this class of compounds and supports the further development of iminosugars for other indications, such as the treatment or prevention of certain viral infections. Although treatment of viral infections would require systemic delivery, for many viruses it

Fig. 3. DNJ derivatives evaluated *in vitro* for activity against multiple members of the *Flaviviridae* family. Structures for monocyclic glucose-mimicking iminosugars that have been tested for activity *in vitro* against two or more members of the *Flaviviridae* family (Schul et al., 2007).

would consist of a much shorter duration of treatment than for chronic indications, which is expected to limit the impact on host physiology. One of the practical challenges that needs to be considered when developing novel iminosugars for antiviral indications is the interaction between iminosugars and intestinal glucosidases, which is associated with undesired gastrointestinal side-effects. This can potentially be overcome by designing selective ER α-glucosidase inhibitors with reduced activity against the gastrointestinal enzymes and ensuring good bioavailability to minimize inhibition of intestinal glucosidases. It should be pointed out that gastrointestinal distress caused by long-term *N*B-DNJ (Zavesca®) use can be mitigated by changing dietary habits, such as reducing carbohydrate load with oral administration of the drug.

5. *In vitro* efficacy and mechanism of anti-*Flaviviridae* activity

A substantial amount of research has been performed in the past 20 years evaluating the activity of iminosugars against members of the *Flaviviridae* family (Figs. 1–5). Data from published studies assessing the antiviral effect of iminosugars against *Flaviviridae* in cell culture are summarized in Table 1 (bicyclic iminosugars), Table 2 (monocyclic glucose-mimicking iminosugars), and Table 3 (galactose-mimicking and other iminosugars). The details for each study are provided in Supplemental Table 2.

Fig. 4. DNJ derivatives evaluated *in vitro* and *in vivo* for activity against members of the *Flaviviridae* family. Structures for monocyclic glucose-mimicking iminosugars that have been tested for activity against *Flaviviridae in vitro* and in animal models of disease or that have advanced to clinical development for *Flaviviridae* infections.

Fig. 5. DGJ and other non-glucose-mimicking iminosugars evaluated *in vitro* or *in vivo* for activity against members of the *Flaviviridae* family. Structures for iminosugars containing headgroups that mimic galactose (DGJ and DGJ-derivatives) or mannose (DMJ) that have been tested for activity against *Flaviviridae in vitro* or in animal models of disease.

Table 1

In vitro activity of bicyclic iminosugars against members of the *Flaviviridae* family. References are provided in Fig. 1 and details for each study are included in Supplemental Table 2.

Bicyclic Iminosugars						
Compound(s)	Virus					Summary of Results
	BVDV	DENV	JEV	WNV	YFV	
Castanospermine (CAST)	X	X	X	X	X	**DENV (1–4)** in multiple cell types: Reduced production of infective particles. $IC_{50} = 1–85.7$ μm. Resulted in altered prM glycosylation (**DENV-2** in BHK-21 cells) and impaired formation of prME heterodimers (**DENV-1** in Neuro 2a cells). **WNV** in BHK-21 cells: minimal to no inhibitory effect at up to 500 μM **YFV** in BHK-21 cells: 57% reduction at 50 μM **JEV** in PS cells: ≤10-fold reduction in virus titer **BVDV** in MDBK cells: $IC_{50} = 5–367$ μM; additive activity when used in combination with *N*B-DNJ
Celgosivir (BuCast)	X	X				**DENV-1** and **-2** in multiple cell types: $EC_{50} = 0.06–51$ μM. Blocked transport of NS1 and E proteins from ER to Golgi and resulted in defect in NS1 glycosylation in BHK-21 cells **DENV-3** and **-4** in BHK-21 cells: $EC_{50} = 0.31–0.68$ μM. **BVDV**: Additive or synergistic activity in MDBK cells when used in combination with ribavirin or IFN-α.

Abbreviations: BVDV, bovine viral diarrhea virus; DENV, dengue virus; EC_{50}, half maximal effective concentration; ER, endoplasmic reticulum; IC_{50}, half maximal inhibitory concentration; IFN-α, interferon alpha; JEV, Japanese encephalitis virus; MDBK, Madin-Darby bovine kidney; NS1, non-structural protein 1; PS, porcine stable; prM: precursor membrane protein; prME: precursor membrane envelope protein complex; WNV, West Nile virus; YFV, yellow fever virus.

5.1. Flaviviruses

The antiviral activity of iminosugars has been evaluated *in vitro* against multiple flaviviruses that pose a risk to human health, including all four DENV serotypes, with a strong focus on DENV-2 (Miller et al., 2018), as well as JEV (Wu et al., 2002), YFV (Ma et al., 2018a; Whitby et al., 2005), ZIKV (Ma et al., 2018a), WNV (Chang et al., 2009; Gu et al., 2007; Whitby et al., 2005), and the WNV variant strain, Kunjin virus (KUNV) (Mackenzie and Westaway, 2001). A large number of iminosugars tested exhibited moderate to strong activity against flaviviruses *in vitro*, with IC_{50}s ranging from 40 nM to 20 μM, although the level of inhibition was dependent on the molecule, cell type, and virus. The variable response of flaviviruses was demonstrated when the antiviral activity of celgosivir, the butylated pro-drug of the bicyclic iminosugar, CAST, was measured against all four DENV serotypes in multiple cell lines. Celgosivir inhibited DENV 1–4 in BHK-12 cells, with half maximal inhibitory concentrations (IC_{50}s) in the nanomolar range (Rathore et al., 2011; Watanabe et al., 2016). However, when efficacy was evaluated against DENV-1 and DENV-2 in Vero, Huh-7, and THP-1 cells, the IC_{50} varied, ranging from 0.75 to 51 μM depending on the serotype, MOI, and cell type (Watanabe et al., 2016).

The variable response of flaviviruses to iminosugar treatment was also recognized when the antiviral activity of CAST was evaluated against multiple viruses in parallel, including DENV, WNV, and YFV, in BHK-21 cells (Whitby et al., 2005). CAST treatment demonstrated inhibition against all four DENV serotypes, with >95% reduction in the number of infected cells at 50 μM, as measured by immunostaining and flow cytometry, but resulted in minimal to no inhibitory effect against WNV at concentrations up to 500 μM. Decreased inhibition of YFV was also observed compared to DENV, although there was still a 93% reduction at 500 μM relative to untreated cells. Plaque assays of cell supernatants confirmed this differential inhibitory response to treatment. Cells exposed to 50 μM of CAST had a 910-fold reduction in the production of infectious DENV particles, but only a 1.7- and 6.8-fold reduction was observed for WNV and YFV, respectively (Whitby et al., 2005). Despite the lack of antiviral activity of the bicyclic iminosugar CAST against WNV, several monocyclic iminosugars demonstrated strong anti-WNV activity in BHK-21 cells with dose-dependent inhibition and IC_{50}s ranging between 1 and 10 μM (Chang et al., 2009; Gu et al., 2007), although most were still less efficacious than DENV (Chang et al., 2009).

The difference in sensitivity to iminosugar treatment between DENV and other flaviviruses was further demonstrated in HEK293 cells treated with IHVR-19029. The IC_{50} was 12.5 times higher for YFV than DENV, and 17.6 times higher for ZIKV than DENV (Ma et al., 2018a).

Iminosugar efficacy has also been assessed against JEV in two published studies, both demonstrating a similar trend in antiviral activity when compared to DENV (Wu et al., 2002; Lad et al., 2013). The DNJ derivative, *N*N-DNJ, which is known to have more potent antiviral activity than DNJ, inhibited the production of infectious JEV and DENV-2 in BHK-12 cells, however, the reduction was much greater at lower concentrations for DENV-2 compared to JEV. Other iminosugars (CAST, DNJ, and DMJ) had minimal to no impact on JEV replication, reducing viral RNA or infectious particle secretion by only ≤10-fold, but inhibited DENV secretion by up to 10^6 plaque forming units (pfu). Similarly, *N*N-DNJ (100 μM) also reduced the secretion of infectious KUNV particles by more than 10^3 pfu in Vero cells despite DNJ having no inhibitory effect when tested at the same concentration (Mackenzie and Westaway, 2001). It is possible that differences in the number and/or location of glycosylation sites within viral proteins may contribute to the range in antiviral activity of iminosugars observed against different flaviviruses (Miller et al., 2018).

Depending on the structure, iminosugars are capable of differentially inhibiting glycolipid or glycoprotein processing enzymes. The ability of some iminosugars to inhibit glycolipid processing was originally thought to contribute to the anti-DENV activity of this class of compounds (Chatel-Chaix and Bartenschlager, 2014; Sayce et al., 2016; Welsch et al., 2009; Wichit et al., 2011). To better understand the mechanism iminosugars use to inhibit DENV, Sayce et al., evaluated the enzymatic targets of diverse bicyclic glucose- (CAST and celgosivir), monocyclic glucose- (DNJ, *N*B-DNJ, and *N*N-DNJ) and galactose-mimicking (deoxygalactonojirimycin (DGJ), *N*B-DGJ, and *N*N-DGJ) iminosugars, and correlated the target with the anti-DENV activity in primary human monocyte derived macrophages (MDMs) (Sayce et al., 2016). To identify the enzymatic target, they performed both *in vitro* glucosidase and glycosidase enzyme inhibition assays as well as measured the generation of glycosphingolipids (GSLs) or free oligosaccharides (FOS). Generation of triglucosylated or monoglucosylated FOS is used as a marker for ER α-glucosidase I and II inhibition, respectively, whereas the generation of the glycosphingolipid monosialodihexosylganglioside (GM3) is used as an indicator to measure glycolipid processing (Alonzi et al., 2008; Neville et al., 2004; Platt et al., 1994a, 1994b; Warfield et al., 2016b). Sayce et al., demonstrated that monocyclic and bicyclic glucose-mimicking iminosugars inhibit glycoprotein processing, but only monocyclic compounds also inhibit glycolipid processing enzymes. Galactose-mimicking iminosugars inhibited glycolipid processing but not glycoprotein processing. Interestingly, the level of glucosyltransferase enzymatic inhibition correlated with the length of the alkyl tail, despite whether the iminosugar mimicked glucose or galactose, with longer tails having stronger inhibition

Table 2

In vitro activity of DNJ and DNJ derivatives against members of the *Flaviviridae* family. References are provided in Figs. 2-4 and details for each study are included in Supplemental Table 2.

Monocyclic Glucose-mimicking Iminosugars										
Compound(s)	Virus									Summary of Results
	BVDV	DENV	HCV	GBV-B	JEV	KUN	WNV	YFV	ZIKV	
2-THO-DNJ (UV-12)		X								**DENV-2** in Vero cells: $IC_{50} = 27.71$ μM, $CC_{50} > 500$ μM
CM-9-78		X	X							**DENV-2** in BHK-21 or A549 cells: $EC_{50} = 1.5–6.75$ μM, $CC_{50} > 40$ μM **HCV** chimera in Huh7.5 cells: $EC_{50} = 52.5$ μM, $CC_{50} > 500$ μM; shift in electrophoresis mobility for E2 and E2-p7 proteins
CM-10-18 (DNJ Derivative 7)	X	X	X							All viruses, multiple cell lines: $EC_{50} = 1.1–27.2$ μM, $CC_{50} > 100$ μM. Synergistic antiviral activity against **DENV-2** in A549 cells when combined with ribavirin.
1-Deoxynojirimycin (DNJ)	X	X	X	X	X	X				Low activity against most viruses with reported EC_{50} and EC_{90} values typically >100 μM. Low cytotoxicity (CC_{50}s reported as >500 to >10,000 μM, depending on cell type and highest concentration tested). **DENV-1** in Neuro 2a cells: E protein misfolding and impaired formation of prME heterodimers Greater effect on **DENV-2** secretion (6 log pfu/mL reduction) at 100 μm/mL compared to **JEV** (~0.6–1 log pfu/mL reduction) in PS cells No inhibitory effect observed against **KUN** in Vero cells at 100 μM
IHVR11029; IHVR17028	X	X								**BVDV** in MDBK cells and **DENV-2** in BHK-21 cells: $EC_{50} = 0.3–1.3$ μM, $CC_{50} > 500$ μM
IHVR19029	X	X						X	X	Various cell types: $EC_{50} = 0.25–1.7$ μM (**BVDV**, **DENV-2**) and 21.5–30 μM (**YFV**, **ZIKV**), $CC_{50} > 100–500$ μM. Synergistic antiviral effect against **YFV** when used in combination with T-705.
MON-DNJ (UV-4/UV-4B)		X								**DENV 1–4** in Vero cells: $IC_{50} = 2.1–86.49$ μM, $CC_{50} > 500-1,000$ μM **DENV-2** in MDMs: $IC_{50} = 3.09$ μM, $CC_{50} = 3,150$ μM
N-5-Oxaheptyl-DNJ (5–07); N-5-Oxahexyl-DNJ (5–06); N-5-Oxaoctyl-DNJ (5–08); N-6-Oxadecyl-DNJ (6–10); N-6-Oxanonyl-DNJ (6–09); N-6-Oxaocytyl-DNJ (6–08)	X									MDBK cells: 5–07 and 5–06, no antiviral activity (up to 100 μM); 5–08 and 6–10, minimal antiviral activity (up to 100 μM); 6–09 and 6–08, moderate antiviral activity (up to 100 μM) but not improved compared to *N*N-DNJ. Series not advanced. $CC_{50} > 300$ μM for all compounds.
N-7-Oxadecyl-DNJ (UV-3)	X	X								**BVDV** in MDBK cells: $IC_{50} = 17–80$ μM, $CC_{50} > 5,000$ μM, reduced secretion of viral RNA in MDBK cells. **DENV-2** in Vero cells: $IC_{50} = 41$ μM, $CC_{50} = >500$ μM
NAP DNJ (UV-5)		X								Vero cells: $IC_{50} = 2$ μM, $CC_{50} = 350$ μM; MDMs: $IC_{50} = 0.04$ μM, $CC_{50} = 300$ μM
NB-DNJ (UV-1)	X	X	X							**BVDV** in MDBK cells: Large range in IC_{50}s (2.5 to >200 μm), depending on MOI and assay readout. Infecting with lower MOI resulted in lower IC_{50} across assays; Dose dependent reduction in secretion of infectious virus particles that correlated with misfolding of viral glycoprotein E2; Synergistic when used in combination with IFN-α2b; Treatment of persistently infected MDBK cells in combination with IFN and ribavirin (containing 10 μM NB-DNJ) was sufficient to permanently eliminate virus after 9 passages following drug withdrawal. **HCV** chimera/replicon system: Dose dependent decrease in production of infectious particles in Huh7-Lunet and Huh7.5 cells; Reduced electrophoretic mobility of E1 and E2 glycoproteins. In Sf9 cells, E1 and E1 glycoproteins accumulated in ER, had reduced mobility shift, and interaction with calnexin was disrupted. **DENV-2**: $IC_{50} = 6–10.6$ μM, $CC_{50} > 100$ μM (MDMs); $IC_{50} = 162$ μM, $CC_{50} > 500$ μM (Vero cells)
NN-DNJ (N-nonyl-DNJ/UV-2/DNJ Derivative 6)	X	X	X		X	X	X			**BVDV** in MBDK cells: Range in IC50s depending on assay and publication, with most ranging between 3 and 115 μM, CC_{50}s mostly > 100 μM **DENV-2** in multiple cell lines: $IC_{50} = 1–9$ μM, $CC_{50} = 20–317$ μM; interfered with prM, E, and NS1 protein

Table 2 (*continued*)

Monocyclic Glucose-mimicking Iminosugars										
Compound(s)	Virus									Summary of Results
	BVDV	DENV	HCV	GBV-B	JEV	KUN	WNV	YFV	ZIKV	
										interaction with calnexin in BHK-21 cells. **HCV** in Huh7/Huh7.5/Huh7-Lunet cells: Multiple genotypes evaluated. Dose dependent decrease in production of infectious particles. Reduced electrophoretic mobility shift of E1 and E2 glycoproteins (JFH1 replicon system); inhibition of p7 function for most genotypes tested except for JFH-1/452 (genotype 3a) in which a lower inhibition of infectious particle secretion (only ~40% reduction at 50 μm) and minimal reduction in p7 function were observed. In Sf9 cells using HCV VLPs, E1 and E2 glycoproteins accumulated in ER and had reduced mobility shifts; VLPs produced had reduced binding to huh7 cells. HCV JFH-1 replicon: Additive effect on reduction of secreted infectious virus when used in combination with Rimantadine. **JEV** in BHK-21 cells: Dose dependent inhibition (2–3 log reduction at 100 μM), CC_{50} = 150 μM; interfered with transient interaction between viral proteins (prM, E, and NS1) and calnexin. **KUN** in Vero cells: secreted infectious virus titer reduced by 3.5 log at 100 μM **WNV** in BHK-21 cells: IC_{50} < 4 μM, CC_{50} = 20–100 μM
OSL-1 (N-Pentylcyclohexyl-DNJ); OSL-3	X						X			**BVDV** in MDBK cells: IC_{50} < 20 μM, CC_{50} = ~200 μM **WNV** in BHK-21 cells: IC_{50} = 4–10 μM, dose dependent inhibition.
OSL-2	X									MDBK cells: IC_{50} > 70 μM, CC_{50} > 300 μM
OSL-12-31; OSL-12-51	X									MDBK cells: IC_{50} = ~70 μM (OSL-12-31) or >70 μM (OSL-1-51), CC_{50} > 300 μM
OSL-95ii	X	X	X				X			All viruses, various cell lines: IC_{50} = 2–28 μM (**WNV** <4 μM), CC_{50} > 40–500 μM HCV JC1 chimera in Huh7.5 cells: shift in electrophoresis mobility for E2 and E2-p7 proteins
PBDNJ0801		X					X			BHK-21 cells: EC_{50} = 0.1 μM (**DENV-2**) or 4.75 μM (**WNV**), CC_{50} = 70–80 μM
PBDNJ0802			X							Huh7.5 cells: EC_{50} = 3.5 μM, CC_{50} > 500 μM
PBDNJ0803; PBDNJ0804		X	X				X			BHK-21 cells: EC_{50} = 0.07–0.1 μM (**DENV-2**) and 1.5–3.5 μM (**WNV**), CC_{50} = 65–85 μM HCV chimera in Huh7.5 cells: EC_{50} = 1.7–5.4 μM, CC_{50} > 200 μM, electrophoretic mobility shift and degradation of E2 and E2-p7 proteins (PBDNJ0804)
SP-169; SP-173	X	X					X			**BVDV** in MDBK cells, **DENV-2/WNV** in BHK-21 cells: IC_{50} = 3–5 μM, CC_{50} > 100 μM
ToP-DNJ		X								**DENV-2**: No inhibition observed in Huh7.5 cells (up to 50 μM), IC_{50} = 12.7 μM in MDMs
DNJ Derivatives 7 - 13	X									Reduction in viral RNA and infectious virus secretion measured in MDBK cells. Derivative 7: no antiviral activity at up to 50 μM Derivatives 8 and 9: No reduction at 10 μM, 25–75% reduction at 50 μM, CC_{50} > 250 μM Derivatives 10 and 11: ~50% reduction at 10 μM, CC_{50} ≥ 200 μM Derivatives 12 and 13: ≥75% reduction at 10 μM, CC_{50} = 29–51 μM
DNJ Derivatives: 1a, 1b; 2b, 2c, 2e, 2f, 2g, 2i, 2j; 3d, 3e, 3f, 3g, 3h, 3i, 3m, 3o, 3u; 4d, 4e, 4f; 5a, 5b	X									Single cycle virus yield reduction plaque assay using MDBK cells: Derivative 1 compounds: EC_{50} = 3–7.5 μM, CC_{50} ≥ 450 μM Derivative 2 compounds: EC_{50} = 0.3–1.8 μM, CC_{50} ≥ 150–500 μM Derivative 3 compounds: EC_{50} = 0.4–5.0 μM, CC_{50} ≥ 62–500 μM Derivative 4 compounds: EC_{50} = 3.5–24 μM, CC_{50} = 295–420 μM Derivative 5 compounds: EC_{50} = 11 μM (5a) and >100 μM (5b), CC_{50} > 500 μM

Table 2 (*continued*)

Monocyclic Glucose-mimicking Iminosugars										
Compound(s)	**Virus**									Summary of Results
	BVDV	DENV	HCV	GBV-B	JEV	KUN	WNV	YFV	ZIKV	
DNJ Derivatives: 1c, 2a, 2d, 2h, 2k, 2l, 3a, 3b, 3c, 3j, 3k, 3l, 3n, 3p, 3q, 3r, 3s, 3t, 3v, 4a, 4b, 4c, 5d	X	X								**BVDV** in MDBK cells and **DENV-2** in BHK-21 cells: Activity of individual compounds comparable against DENV and BVDV. Derivative 1 compounds: $EC_{50} = 0.3–0.6$ μM, $CC_{50} \geq 480$ μM. Derivative 2, 3, and 4 compounds: $EC_{50} = 0.3–4$ μM, $CC_{50} \geq 400$ μM. Derivative 5 compounds: $EC_{50} = 0.9–47$ μM, $CC_{50} > 500$ μM
SP-116; SP-150; SP-156; SP-159; SP-164; SP-165; SP-166; SP-168; SP-187	X									Virus yield reduction using MDBK cells: SP-165, SP-166, SP-187: $IC_{50} = 3–12$ μM, $CC_{50} \geq 400$ μM. SP-116, SP-150, SP-156, SP-159, SP-168: $IC_{50} = 24–40$ μM, $CC_{50} \geq 500$ μM. SP-164: $IC_{50} = 150$ μM, $CC_{50} > 1,000$ μM

Abbreviations: BVDV, bovine viral diarrhea virus; CC_{50}, 50% cytotoxic concentration; DENV, dengue virus; EC_{50}, half maximal effective concentration; ER, endoplasmic reticulum; GBV-B, GB virus B; HCV, hepatitis C virus; IC_{50}, half maximal inhibitory concentration; IFN, interferon; JEV, Japanese encephalitis virus; KUN, Kunjin virus; MDBK, Madin-Darby bovine kidney; MDM, monocyte-derived macrophages; MOI, multiplicity of infection; PS, porcine stable; pfu, plaque-forming unit; prM: precursor membrane protein; prME: precursor membrane-envelope protein complex; VLP, virus-like particle; WNV, West Nile virus; YFV, yellow fever virus; ZIKV, zika virus.

Table 3

In vitro activity of iminosugars containing headgroups that mimic galactose (DGJ and DGJ-derivatives) or mannose (DMJ) tested for activity against members of the *Flaviviridae* family. References are provided in Fig. 5 and details for each study are included in Supplemental Table 2.

Galactose-mimicking and other iminosugars					
Compound(s)	Virus				Summary of Results
	BVDV	DENV	HCV	JEV	
DGJ (Deoxygalactonojirimycin)	X				No antiviral activity, $CC_{50} > 10,000$ μM
DGJ Derivative 6	X				Minimal to no antiviral activity, $CC_{50} > 250$ μM
MON-6d-DGJ		X			**DENV-2** in MDMs: no inhibitory effect up to 100 μM.
N7-DGJ (N7-oxanonyl-6deoxy-DGJ)	X				MDBK cells: $IC_{50} = 2.5–125$ μM depending on MOI (MOI 0.1 = 2.5 μM; MOI 1.0 = 125 μm), $CC_{50} > 4,000$ μM. No viral rebound after 22 passages in persistently infected MDBK cells when used in combination with IFN and ribavirin
NB-DGJ	X	X			No antiviral activity observed against **BVDV** in MDBK cells or **DENV-2** in MDMs
NN-6deoxy-DGJ	X				MDBK cells: $IC_{50} = 2–25$ μM, $CC_{50} = 187.5$ μM
NN-DGJ	X	X	X		**BVDV** in MDBK cells: $IC_{50} = 12.5–25$ μM, $CC_{50} = 237.5$ μM. **DENV-2** in MDMs, no antiviral activity (up to 31.6 μM). **HCV** in multiple genotypes and cell types: results varied, mostly ~moderate inhibition of secreted infectious virus. No change in the electrophoretic mobility of E1 or E2 in Huh7 or S*f*9 cells
DMJ (1-Deoxymannojirimycin)	X	X		X	Virus secretion: **BVDV** in MDBK cells, minimal to no inhibition (up to 15,000 μM); **DENV-2** in PS cells, reduced by ~6 log pfu/mL (at 200 μM/mL); **JEV** in PS cells, reduced by 0.6–1.0 log pfu/mL (at 200 μM/mL)

Abbreviations: BVDV, bovine viral diarrhea virus; CC_{50}, 50% cytotoxic concentration; DENV, dengue virus; EC_{50}, half maximal effective concentration; HCV, hepatitis C virus; IC_{50}, half maximal inhibitory concentration; IFN, interferon; JEV, Japanese encephalitis virus; MDBK, Madin-Darby bovine kidney; MDM, monocyte-derived macrophages; MOI, multiplicity of infection; PS, porcine stable; pfu, plaque-forming unit.

(Butters et al., 2000; Caputo et al., 2016; Mellor et al., 2002, 2003; Sayce et al., 2016). Consistent with the enzymatic data, the glucose-mimicking iminosugars celgosivir and *N*B-DNJ generated FOS (Sayce et al., 2016; Warfield et al., 2016b). In contrast, DGJ derivatives reduced GM3 levels but did not generate triglucosylated or monoglucosylated FOS. Only the glucose-mimicking iminosugars that inhibited enzymes required for either glycoprotein processing, or both glycoprotein and glycolipid processing, were efficacious against DENV-2, whereas iminosugars with a galactose-like configuration had no measurable effect on the virus.

In a similar study, the glucose-mimicking iminosugar, UV-4B (MON-DNJ), inhibited the enzymatic activity of both α-glucosidase I and II, but when an isomer of UV-4B designed to have a galactose-like configuration was tested, α-glucosidase I and II inhibition was lost (Warfield et al., 2016b). MDMs treated with UV-4B containing either a glucose- or galactose-like configuration had reduced levels of GM3; however, only UV-4B with a glucose-like configuration generated FOS and was active against DENV, while the galactose-mimicking isomer lacking α-glucosidase inhibition was not efficacious against the virus.

Because iminosugars have the potential to alter the levels and/or folding of viral glycoproteins, treatment can ultimately result in decreased viral production or reduced infectivity of progeny viruses. *In vitro* studies have been performed to evaluate the effects of DNJ, *N*N-DNJ, CAST, and celgosivir on DENV or JEV glycoproteins, and to determine whether misfolded viral proteins are degraded or incorporated into newly released virus particles with decreased infectivity (Courageot et al., 2000; Rathore et al., 2011; Sayce et al., 2016; Warfield et al., 2016b; Wu et al., 2002). These studies demonstrated that treatment with iminosugars can alter glycan processing of the virus prM, E,

and NS1 glycoproteins (Courageot et al., 2000; Rathore et al., 2011), prevent their interaction with calnexin (Wu et al., 2002), reduce proper folding of prM and E (Courageot et al., 2000), inhibit the formation and stability of prM-E heterodimer complexes (Courageot et al., 2000), prevent the transport of E and NS1 from the ER to the Golgi (Rathore et al., 2011), and reduce secretion of both E and NS1 from cells (Rathore et al., 2011). The fact that iminosugars can block the transport and secretion of some flavivirus glycoproteins suggests that inhibiting ER α-glucosidase function likely results in decreased formation and secretion of new viruses rather than the formation of less infectious particles. Two separate studies have confirmed that this is the main mechanism employed by UV-4B, *N*B-DNJ, and celgosivir to inhibit DENV (Sayce et al., 2016; Warfield et al., 2016b). In these studies, the authors performed plaque assays to measure the amount of infectious virus secreted from iminosugar treated MDMs and compared that to quantitative polymerase chain reaction results measuring the total amount of viral RNA secreted from the cells. Data from both studies were consistent and found that there was a direct correlation between the decrease in secreted infectious virus titer and total virus production, providing evidence that the viral glycoproteins are likely misfolded and targeted for degradation rather than partially misfolded and incorporated into newly released particles with decreased infectivity.

5.2. HCV and BVDV

Prior to the development of *in vitro* culture systems for HCV, the closely related virus, bovine viral diarrhea virus (BVDV), was commonly used as a surrogate model. Cytopathic and noncytopathic BVDV culture systems have been used to evaluate the antiviral activity and mechanism of action of iminosugars in Madin-Darby bovine kidney (MDBK) cells (Branza-Nichita et al., 2001; Chang et al., 2013b; Chapman et al., 2005; Durantel et al., 2001, 2004; Gu et al., 2007; Howe et al., 2013; Jacob et al., 2007; Jordan et al., 2002; Mehta et al., 2002; Ouzounov et al., 2002; Whitby et al., 2004; Woodhouse et al., 2008; Yu et al., 2012; Zitzmann et al., 1999). Several DNJ derivatives were shown to inhibit BVDV by preventing the secretion of viral particles and viral RNA (Durantel et al., 2001; Jordan et al., 2002; Zitzmann et al., 1999). Similar to subsequent observations for flaviviruses, the antiviral effect elicited by the long-alkyl-chain DNJ derivative, *N*N-DNJ, was stronger than that of the short-alkyl-chain derivative, *N*B-DNJ. Treatment with DNJ derivatives was also shown to result in the assembly and release of low levels of viral particles with decreased infectivity (Durantel et al., 2001). The production of noninfectious particles was less pronounced when cells were treated with *N*B-DNJ compared to *N*N-DNJ, which may account for the stronger anti-BVDV activity associated with the long-alkyl-chain derivatives (Durantel et al., 2001).

To further evaluate the impact of the alkyl-chain length, as well as other potential inhibitory mechanisms of iminosugars, Durantel, et al., evaluated and compared the anti-BVDV activity of long- and short-alkyl-chain glucose-derived DNJ derivatives to that of long-alkyl-chain galactose-derived iminosugar derivatives of DGJ, which do not inhibit ER α-glucosidases (Durantel et al., 2001). Both DGJ and DNJ derivatives inhibited BDBV in cell culture, unlike what was later observed for flaviviruses, in which only DNJ derivatives demonstrated antiviral activity. However, only the long-alkyl-chain DGJ derivative and not the short-alkyl-chain derivative was efficacious against BVDV. This effect was solely the consequence of the production of noninfectious virions, rather than a reduction in viral secretion. Deletion of the viral membrane-spanning protein p7 has a similar impact on BVDV replication *in vitro*, also resulting in the production of viral particles with decreased infectivity without inhibiting viral secretion in media (Harada et al., 2000). P7 was also shown to be essential for the production of infectious HCV virus (Jones et al., 2007; Sakai et al., 2003; Steinmann et al., 2007). The long-alkyl-chain iminosugar derivatives, *N*N-DNJ, *N*7-DGJ, and *N*N-DGJ, were found to inhibit HCV p7 ion channel activity in a dose-dependent manner (Pavlovic et al., 2003). Interestingly,

DENV-2 and JEV, which do not encode for the p7/p13 protein, are not inhibited by DGJ derivatives (Wu et al., 2002).

While many of the initial studies used BVDV as a surrogate for HCV, the use of HCV virus-like particles (VLPs) and the later developed *in vitro* HCV infectivity systems helped to confirm the anti-HCV activity of iminosugars against multiple genotypes (Fig. 1 and Table 2). Exposure to *N*N-DNJ inhibited the release of HCV particles in a dose-dependent manner in Huh7 cells transfected with JFH-1 or chimeric derivatives containing structural proteins of other genotypes, including 1a (H77), 1b (CON-1), 2a (JS, CJ-1), and 3a (452), but was less active against the 3a (452) chimera (Griffin et al., 2008). To evaluate the potential cause for decreased activity against the 3a (452) chimera, the impact of iminosugar treatment on p7 function was evaluated by measuring the ability of p7 to release a fluorescent dye from liposomes. *N*N-DNJ had minimal to no impact on 3a (452) p7 function; however, *N*N-DNJ reduced the activity of p7 from all other HCV isolates tested, correlating with the *in vitro* viral inhibition data (Griffin et al., 2008). Alignment of the p7 sequences from different HCV isolates identified a polymorphism (F25A) in the 3a HCV genotype (Foster et al., 2011). When a mutant chimeric JFH-1 virus containing the p7 F25A polymorphism was exposed to *N*N-DNJ or *N*N-DGJ treatment, minimal to no effect was observed on virus secretion, whereas secretion of wild-type JFH-1 virus was largely inhibited following exposure to the iminosugars.

Data from mechanistic studies show that iminosugars have similar effects on HCV and the surrogate BVDV culture systems. Iminosugars prevent the release of BVDV and HCV viral RNA and infectious virus with minimal to no impact on viral RNA synthesis (Durantel et al., 2001; Jordan et al., 2002; Qu et al., 2011; Zitzmann et al., 1999). Treatment with iminosugars also alters the interaction between BVDV or HCV glycoproteins and ER chaperone proteins (Branza-Nichita et al., 2001; Chapel et al., 2006; Choukhi et al., 1998; Durantel et al., 2007), and results in an electrophoretic mobility shift caused by deficient glycan processing, which is accompanied by the production of misfolded glycoproteins and/or decreased formation of E1-E2 heterodimers (Branza-Nichita et al., 2001; Chapel et al., 2006; Qu et al., 2011).

The antiviral effect of *N*B-DNJ was attributed to inefficient glucose trimming of BVDV envelope glycoproteins by the ER α-glucosidases, inhibiting the association of the envelope proteins with calnexin and resulting in the production of misfolded viral envelope glycoproteins and decreased E1-E2 heterodimer formation (Branza-Nichita et al., 2001). The HCV glycoproteins E1 and the E2 precursor, E2-NS2, were found to interact with multiple ER chaperones, although only calnexin interacted primarily with monomers or noncovalent E1-E2 complexes, which are formed prior to the final folding stage (Choukhi et al., 1998). By using HCV VLPs, it was shown that iminosugars inhibit N-linked glycan processing to interrupt this interaction, resulting in the production of VLPs containing unprocessed N-linked glycans and misfolded proteins that have a decreased binding affinity to hepatoma cells (Chapel et al., 2006).

In addition to having a direct effect on viral glycoproteins, it is possible iminosugars may also regulate infectivity by altering the expression or perturbing the function of host glycoproteins that are required for viral entry and replication. Although changes to host glycoproteins have been implicated as a contributor to the antiviral activity of iminosugars against the severe acute respiratory syndrome coronavirus (SARS-CoV), the impact of potential alterations to host-protein glycosylation on *Flaviviridae* entry or replication has not been evaluated (Zhao et al., 2015). Miller et al., 2012 recently reported that iminosugars may alter the function and expression of some host glycoproteins involved in DENV replication, although the possible effect these changes may have on DENV infection is not yet understood (Miller et al., 2019).

6. *In vivo* efficacy of iminosugars against *Flaviviridae*

Despite the extensive *in vitro* efficacy studies that have been

performed evaluating the antiviral activity of iminosugars against multiple members of the *Flaviviridae* family, *in vivo* studies have been primarily limited to murine models of DENV infection, particularly DENV-2 (for a more in-depth review, refer to Miller et al., 2018). This has been due, in part, to a lack of suitable small animal models available for HCV and BVDV (Billerbeck et al., 2013), and because of the stronger *in vitro* antiviral activity observed against DENV compared to other flaviviruses. Animal models of JEV and WNV disease are the only *Flaviviridae* infection models, other than DENV, that have been used in published studies to evaluate the antiviral activity of iminosugars (Whitby et al., 2005; Wu et al., 2002). In fact, the first *in vivo* model system used to evaluate the efficacy of an iminosugar against a *Flaviviridae* family member was the murine model of JEV disease (Wu et al., 2002). A survival benefit was observed in comparison to control treated animals when female ICR mice were administered *N*N-DNJ (200 mg/kg, oral administration, once daily) beginning one day before simultaneous intraperitoneal and intracranial challenge with JEV. Considering that *N*N-DNJ was notably more efficacious against DENV-2 than JEV (RP9 strain) *in vitro*, the observation that *N*N-DNJ protected mice against a lethal JEV infection *in vivo* supported the continued examination of iminosugars against other flaviviruses.

The potential *in vivo* anti-flavivirus activity of iminosugars was next examined against WNV and DENV-2 (Whitby et al., 2005). C57BL/6 mice were infected with 10^2 pfu of WNV (3000.0259 strain) by footpad injection and A/J mice were infected intracranially with 10^5 pfu of mouse-neuroadapted DENV-2 (NGC strain). Mice were treated intraperitoneally with up to 250 mg/kg CAST or vehicle once daily beginning on the day of infection (Whitby et al., 2005). While CAST treatment resulted in a significant survival benefit against DENV-2 compared to control treated mice, it had no measurable effect on mortality when mice were infected with WNV. This *in vivo* response correlated with observations *in vitro*, where CAST had minimal to no inhibitory effect against WNV at concentrations of up to 500 μM. However, unlike CAST, which is a bicyclic iminosugar, several monocyclic iminosugars, such as *N*B-DNJ (Table 1), have proven to be efficacious *in vitro* against WNV but have not been tested *in vivo* (Chang et al., 2009; Gu et al., 2007). Despite the lack of activity of CAST against WNV in the murine model, it is possible treatment with monocyclic iminosugars would result in a survival benefit based on the results of *in vitro* studies. These monocyclic compounds may elicit their anti-WNV activity through a separate or additional mechanism, such as through their ability to also inhibit the glycolipid biosynthesis pathway, although alteration of glycosphingolipid activity was shown to not be the mechanism of anti-DENV activity for the iminosugar, UV-4 (Sayce et al., 2016; Warfield et al., 2016b).

Two iminosugars, UV-4B and celgosivir, successfully protected mice against a lethal challenge with DENV-2 when treatment was delayed and initiated up to 48 h post-challenge. (Perry et al., 2013; Rathore et al., 2011; Warfield et al., 2016b). Both of these molecules have advanced to clinical trials, with UV-4B being evaluated in early phase safety and pharmacokinetic studies, and celgosivir in adults with confirmed dengue fever (discussed below) (Low et al., 2014; Spurgers et al., unpublished data). Several other iminosugars have demonstrated efficacy in murine models of DENV-2 disease, including antibody dependent enhancement (ADE) models. ADE is an increase in the infectivity of a virus due to preexisting, poorly neutralizing antibodies that can facilitate entry of the virus into host cells and exacerbate disease severity. The highest known risk of severe dengue disease is in patients previously infected with a different DENV serotype who have intermediate levels of pre-existing anti-DENV antibodies (Katzelnick et al., 2017). Murine models of ADE have been used to better represent severe DENV infections in humans. Passive administration of anti-DENV antibodies has been shown to be sufficient to enhance DENV disease in mice using both mouse-adapted and clinical DENV isolates (Balsitis et al., 2010). Clinical features of lethal DENV-ADE disease models include thrombocytopenia, vascular leakage, elevated serum cytokine levels, and increased systemic viral burden in serum and tissue phagocytes, all hallmarks of severe dengue disease in humans (Balsitis et al., 2010).

Celgosivir is the only iminosugar for which data has been published evaluating the *in vivo* efficacy against a DENV serotype other than DENV-2 (Watanabe et al., 2016). AG129 mice were infected intravenously with 7×10^7 pfu DENV-1 (2402 strain) after being exposed to the 4G2 anti-E antibody to induce ADE, and mice were treated orally with vehicle or celgosivir (10 mg/kg or 50 mg/kg) twice daily, starting at the time of infection. Although a survival benefit was not observed for animals in the low-dose group (10 mg/kg), all mice in the high-dose group (50 mg/kg) survived infection.

7. Anti-*Flaviviridae* activity of iminosugar drug combinations

Drug combination therapies are often considered for the treatment of viral infections. Combination treatment may minimize the potential for resistant strains to develop against an individual drug, as well as maximize compound efficacy if a synergistic benefit is observed with the combination treatment. Iminosugars were first evaluated in clinical trials for an antiviral indication as part of a combination therapy with zidovudine (AZT) for the treatment of HIV infections, although these studies did not proceed further than Phase 2 (Fischl et al., 1994). Prior to the recent development and approval of highly efficacious direct-acting antivirals against HCV (discussed in more detail below), the standard therapy for patients consisted of a less effective combination of interferon (IFN) and ribavirin, which could be associated with multiple, and sometimes serious, side effects (Das and Pandya, 2018; Elloumi et al., 2007; Ferenci et al., 2005; Manns et al., 2006). In pursuit of improving the treatment regimen, the potential benefit of incorporating iminosugars into this therapeutic modality was evaluated. *In vitro* drug synergy studies were performed using BVDV culture systems to evaluate the antiviral efficacy of iminosugars when used in combination with IFN, ribavirin, or a second iminosugar (Jacob et al., 2007; Ouzounov et al., 2002; Whitby et al., 2004; Woodhouse et al., 2008). When BVDV-infected MDBK cells were treated with a combination of two iminosugars, CAST and *N*B-DNJ, the observed antiviral activity was additive with no statistically significant synergistic or antagonistic antiviral effect, which would be expected considering CAST and *N*B-DNJ have the same cellular target (Ouzounov et al., 2002). However, when *N*B-DNJ was combined with IFN-α2b under the same conditions, treatment resulted in synergistic antiviral activity against BVDV. In a separate study, synergistic antiviral activity was also observed when BVDV-infected MDBK cells were treated with celgosivir plus INF-α or celgosivir plus ribavirin in two or three out of four experiments, respectively (Whitby et al., 2004).

To extend observations beyond dual-drug treatment combinations, the antiviral activity of triple-drug combinations, consisting of IFN, ribavirin, and an iminosugar, was evaluated in MDBK cells persistently infected with a noncytopathic strain of BVDV (Woodhouse et al., 2008). Cells were passaged in the presence of IFN and ribavirin, with or without the addition of one of three iminosugars (*N*7-DGJ, *N*B-DNJ, or *N*N-DNJ). Treatment was sustained over several passages and the impact on long-term viral clearance was measured following removal of one or all drugs. Cells treated with the dual-drug combination of IFN and ribavirin maintained viral clearance only if treatment was continued; removal of the drugs resulted in a loss of viral clearance over time. However, with the addition of an iminosugar to the treatment regimen, viral clearance was sustained, even after removal of the drugs. *N*B-DNJ had the greatest impact on maintaining viral clearance. Treatment of cells for nine weeks with the three-drug cocktail containing 10 μM NB-DNJ was sufficient to permanently eliminate the virus.

The antiviral activity of iminosugar drug combinations was also evaluated against members of the *Flavivirus* genera. Synergistic antiviral activity was observed when HEK293 cells were infected with the vaccine strain of YFV (17D) and treated with the iminosugar, IHVR-19029, and the viral RNA polymerase inhibitor, favipiravir (T-705). While the

antiviral efficacy of this drug combination was not evaluated in an *in vivo* flavivirus disease model, T-705 was found to potentiate the antiviral activity of IHVR-19029 in a murine model of Ebola virus disease (Ma et al., 2018a). Ribavirin was also found to have a synergistic antiviral effect *in vitro* against DENV-2 when combined with the iminosugar CM-10-18 (Chang et al., 2011). In this study, the enhanced activity of ribavirin by CM-10-18 was confirmed using a murine model of DENV-2 infection. These studies support the potential use of iminosugars in drug combination therapies for the treatment of flavivirus infections.

8. Broad-spectrum antiviral activity of iminosugars

Considering that many viruses exploit the host's N-linked glycosylation machinery for the morphogenesis of progeny virus particles, it is not surprising that the antiviral activity of some iminosugars expands far beyond members of the *Flaviviridae* family. Several iminosugars have proven to be efficacious in small animal models of disease for influenza A, influenza B, herpes simplex, Ebola, and Marburg viruses (Bridges et al., 1995; Chang et al., 2013b; Stavale et al., 2015; Warfield et al., 2015, 2016a; Zhang et al., 2013), as well as against many diverse DNA and RNA viruses using cell culture systems (refer to table 20.3 from Miller et al., 2018 and Table 2 from Chang et al., 2013a,b). Included in this group of viruses are the coronaviruses, SARS-CoV and the recently identified SARS-CoV-2, responsible for the ongoing global pandemic (Wu et al., 2004; Zhao et al., 2015; Rajasekharan et al., 2020). Iminosugars have been shown to decrease the production of infectious virions *in vitro* as well as alter the N-linked glycan structure of the SARS-CoV and SARS-CoV-2 cellular receptor, angiotensin I-converting enzyme 2 (ACE2), impairing entry of SARS-CoV and human coronavirus NL63 (HCoV-NL63) spike glycoprotein-pseudotyped lentiviral particles through a post-receptor binding mechanism (Zhao et al., 2015).

Similar to what has been observed for *Flaviviridae*, the antiviral activity of iminosugars against other viruses varies with the compound, virus, and cell type. The mechanism of antiviral activity can also vary depending on the compound and the virus. Influenza virus and HIV are both good examples of the differential effects that can be observed following treatment with iminosugars. Extensive research has been performed evaluating the effect of iminosugars against influenza virus, which is discussed further in a separate review (Plummer et al., 2015). Interestingly, iminosugars tend to have low potency against influenza virus *in vitro*, yet treatment provides partial or full protection against lethal infections in murine models of disease (Warfield et al., 2015). Studies evaluating the mechanism of anti-HIV activity of iminosugars found that treating infected cells with iminosugars can alter the glycosylation of the viral gp120 glycoprotein (Fischer et al., 1995, 1996). This can impair the ability of new HIV particles to enter cells following binding to CD4 rather than reduce the production of secreted progeny viruses, as is observed for flaviviruses. Despite the differences in the level and mechanism of antiviral activity of iminosugars, the broad antiviral potential of this class of compounds supports further research into the potential use of these molecules for the treatment of diverse viral infections.

9. High barrier to development of *in vivo* drug resistance to iminosugars

One of the many challenges in the antiviral drug development field is understanding how to design compounds that will remain efficacious despite the high genetic variability of most viruses, which leads to rapid generation of antiviral drug resistance. For example, influenza virus resistance to oseltamivir (Tamiflu®) has been noted after a single mutation, and recent publications discussed resistance to baloxavir marboxil (XOFLUXA™), which was approved in the U.S. in October, 2018 (Bloom et al., 2010; Hauge et al., 2009; Meijer et al., 2009; Omoto et al., 2018; Takashita et al., 2019a, 2019b). Current strategies proposed for overcoming this challenge include the development of drug combination

therapies that contain drugs targeting multiple viral proteins and HTAVs, both of which are less likely to generate resistant strains compared to direct-acting antivirals targeting a single viral protein. For HTAVs, this is due to the detrimental effect resistant mutations would likely have on viral replication if the virus was critically dependent on the host factor targeted by the drug.

In order to determine whether DENV has the potential to mutate and develop resistance to iminosugars, Plummer et al., performed an *in vivo* passaging study in mice (Plummer et al., 2015). DENV (DENV-2 strain S221) was passaged in STAT1$^{-/-}$/2$^{-/-}$ mice treated orally with either UV-4B or vehicle (water) for 4 serial passages; samples were pooled by group after each passage and evaluated to determine whether the virus could mutate and develop resistant strains. Only 13 nonsynonymous mutations were identified in pooled samples from UV-4B treated mice, 12 of which were also identified in virus isolated from vehicle-treated control animals. Viruses isolated after each passage were still susceptible to treatment with UV-4B. Considering that DENV is transmitted from invertebrate (mosquito) to vertebrate hosts rather than from vertebrate to vertebrate (e.g., mouse to mouse), and to address the possible impact pooling the samples may have had on diluting mouse-specific responses, the authors also identified mutations in viruses isolated from individual mice after a single passage in the presence or absence of UV-4B. After only a single passage *in vivo*, 19 nonsynonymous mutations were identified in DENV glycosylated proteins (M, E, and NS1) that were specific to UV-4B treatment. Despite the increased number of nonsynonymous mutations identified, these viruses were still susceptible to treatment with UV-4B providing no evidence that viral escape mutants were generated after single or multiple cycles of proliferation in the presence of the iminosugar *in vivo*. A high barrier to the development of drug resistant DENV viral strains was also observed *in vitro* when DENV was passaged up to 30 times in cells in the presence of UV-4B (Warfield et al., unpublished data).

The potential for UV-4B to induce the development of drug resistant viral strains was also evaluated in a murine model of influenza A virus (Warfield et al., 2019). Similar to what was observed in the DENV model, when mouse-adapted influenza A virus was passaged in the presence or absence of UV-4B for five successive passages, the virus maintained sensitivity to UV-4B treatment in mice. Mice infected with recombinant viruses containing individual or combinations of nonsynonymous mutations identified in the passaged virus were also still sensitive to UV-4B treatment, further confirming the high genetic barrier to the generation of drug resistant strains following exposure to an iminosugar antiviral.

10. Clinical trials: iminosugars as antivirals

Three candidate iminosugars have advanced to clinical trials to evaluate safety and efficacy against viral infections: celgosivir (HCV, DENV, and HIV), *N*B-DNJ (HIV), and UV-4B (DENV). HCV was the first of the two *Flaviviridae* family members to be included in a clinical trial assessing the therapeutic potential of an iminosugar (Table 4). Prior to the approval of current direct-acting antiviral therapies for HCV infections, several Phase 2 clinical trials were completed to evaluate the safety and efficacy of celgosivir against chronic HCV genotype 1 infections when administered as a monotherapy (NCT00157534) or in combination with PEGylated IFN-α2b or PEGylated IFN-α2b plus ribavirin (NCT00332176 and NCT00217139). In the initial Phase 2a randomized, dose-ranging trial, 43 patients with chronic HCV infections who were either IFN-intolerant or had never been treated with IFN were enrolled to receive celgosivir treatment (200 or 400 mg once daily or 200 mg twice daily) for 12 weeks. Viral load reduction was only observed for 2 of the 35 patients who completed the full treatment course (Durantel, 2009; Yoshida et al., 2006). Although well tolerated, celgosivir treatment only provided a measurable therapeutic benefit when used as part of a drug combination in other trials. Clinical trials were conducted to evaluate the safety and efficacy of celgosivir plus

Table 4
Clinical stage development of iminosugars for the treatment of *Flaviviridae* infections.

Compound	Indication/Virus	Study Details	Study Endpoint/Readouts	Results	References
Celgosivir	HCV (genotype 1)	Phase 2a randomized, dose-ranging trial; 43 adult participants, ages 18–65 with chronic HCV infections who were IFN-intolerant or never treated with IFN; 200 or 400 mg once daily or 200 mg twice daily for 12 weeks	Safety and efficacy (viral load reduction)	Viral load reduction observed for 2 of the 35 patients who completed the full treatment course. Well tolerated but no measurable therapeutic benefit.	NCT00157534; Durantel 2009; Yoshida et al. (2006)
	DENV	Phase 1 randomized, double-blind, placebo-controlled study; 50 adult participants, ages 21–60 with uncomplicated DENV fever (fever ≥38 °C for less than 48 h); 24 participants received celgosivir (400 mg loading dose, 200 mg maintenance dose every 12 h for a total of 9 doses) and 26 participants received placebo control	Safety, pharmacokinetics, and efficacy (primary: viral load reduction and fever reduction; secondary: NS1 and NS1 clearance)	Celgosivir was well tolerated by patients; no measurable reduction in viral load or fever burden.	NCT01619969; Low et al. (2014)
		Phase 2 double-blind, placebo-controlled study; Adult participants ages 21–65 with acute febrile illness with fever >37.5 °C for less than 48 h; celgosivir arm: 150 mg every 6 h for a total of 20 doses	Safety and efficacy (primary: viral load reduction, AUC; secondary: fever clearance time, duration of illness, time to NSI clearance)	NA/not yet recruiting	NCT02569827
Celgosivir ± standard care (PEGylated IFN-α2b + Ribavirin)	HCV (genotype 1)	Phase 2 randomized, active controlled study; 50 adult participants, ages 18–65 with chronic HCV infections who were not previously treated with IFN; 400 mg or 600 mg celgosivir qd plus standard care, or standard care alone, for 12 weeks	Safety, tolerability, pharmacokinetics, efficacy (viral load reduction)	NA	NCT00332176
Celgosivir + PEGylated IFN-α2b +/− Ribavirin	HCV (genotype 1)	Phase 2 randomized, active controlled study; 60 participants ages 18–65 with chronic HCV infections who did not respond or only partially responded to prior treatment with PEGylated IFN-α2b; Study arms not indicated, 12-week treatment	Safety and efficacy (viral load reduction)	Non-responders treated with the triple drug combination had an increased average early viral response (42%) when compared to patients that received the standard treatment of PEGylated IFN-α2b and ribavirin (10%), and a greater reduction in viral load (−1.6310) compared to the control group receiving the standard treatment (−0.92 log10).	NCT00217139
	HCV (genotype 1)	Phase 2 (extension of clinical trial NCT00217139); allow patients to switch and/or continue celgosivir (plus IFN or IFN and ribavirin) treatment for an additional 36 weeks	Safety and efficacy (viral load reduction)	N/A	NCT00292084
UV-4B	DENV	Phase 1 randomized, double-blind, placebo-controlled, single-ascending dose study; 64 participants, healthy subjects received placebo control or up to 1,000 mg UV-4B (single dose)	Safety, tolerability, and pharmacokinetics	No serious adverse events.	NCT02061358; Spurgers et al., unpublished data
		Phase 1 randomized, double-blind, placebo-controlled, multiple ascending dose study; healthy adult subjects, ages 18–45; placebo or UV-4B TID treatment (ascending doses) for 7 days	Safety, tolerability, and pharmacokinetics	Terminated early before DENV-infected patients were dosed; product development ceased for business reasons.	NCT02696291

Abbreviations: BID, twice daily; DENV, dengue virus; h, hours; HCV, hepatitis C virus; IFN, interferon; NA, not available; NS1, non-structural protein 1; qd, once daily; RT-PCR, reverse transcription polymerase chain reaction; TID, three times daily; WNV, West Nile virus.

either PEGylated IFN-α2b or PEGylated IFN-α2b and ribavirin in patients not previously treated with IFN (NCT00332176) or patients who did not respond or only partially responded to prior treatment with PEGylated IFN-α2b (NCT00217139). Non-responders treated with the triple drug combination had an increased average early viral response (42%) when compared to patients that received the standard treatment of PEGylated IFN-α2b and ribavirin (10%), and a greater reduction in viral load (−1.63 $\log_{10}$) compared to the control group receiving the standard treatment (−0.92 $\log_{10}$) (NCT00217139) (Durantel, 2009). A follow-up trial was conducted (NCT00292084) as an extension of the original clinical trial (NCT00217139) to allow patients from the original trial an opportunity to switch treatments and receive therapy for an additional 36 weeks, although results from this study are not yet reported.

More recently, celgosivir entered a proof of concept, Phase 1b double-blind, randomized clinical trial (NCT01619969) for the treatment of DENV infections in adult patients with uncomplicated dengue fever (as defined by patients having a fever of at least 38 °C for less than 48 h) (Low et al., 2014). Fifty patients were enrolled in the study, with 24 assigned to the celgosivir group and 26 to the placebo group. Patients

receiving celgosivir treatment were administered an initial loading dose of 400 mg, followed by a maintenance dose of 200 mg every 12 h. Treatment was continued until patients received a total of nine doses. While celgosivir was well tolerated by patients in the test group, there was no measurable reduction in viral load or fever burden. It is possible that use of a subtherapeutic dose may have contributed to these results. A follow-up Phase 2 clinical trial (NCT02569827) was recently approved to evaluate a higher-dose regimen of 150 mg, administered every 6 h for a total of 20 doses, but is not yet recruiting. A potent derivative of *N*B-DNJ, UV-4B, also advanced to Phase 1 clinical trials as a prelude to trials in DENV infections. No serious adverse events were reported in a Phase 1 single-ascending dose study at doses up to 1,000 mg (NCT02569827). However, the subsequent Phase 1 multiple-ascending dose trial (NCT02696291) was terminated early before DENV-infected patients were dosed.

The iminosugars *N*B-DNJ and celgosivir also entered clinical trials as a combination therapy with zidovudine for HIV infections (celgosivir: NCT00002151, NCT00002150; NB-DNJ: NCT00001993, NCT00002079, NCT00000791), but neither advanced past Phase 2 (Fischl et al., 1994). These studies demonstrated that both *N*B-DNJ and celgosivir are well tolerated by patients, although minor but reversible osmotic diarrhea was observed due to inhibition of α-glucosidases in the gut.

11. Other *Flaviviridae* ER-associated host dependency factors

Several gain- or loss-of-function genetic screens have been performed to identify host factors required for flavivirus or HCV replication in various cell lines (Lin et al., 2017; Ma et al., 2015; Marceau et al., 2016; Petrova et al., 2019; Savidis et al., 2016; Zhang et al., 2016). While considerable overlap was observed for flavivirus host dependency factors between screens, there was substantial diversity between genes required for DENV and HCV replication, with DENV being largely affected by the loss of protein expressing genes that comprise different ER-associated protein complexes, while viral receptors, metabolic enzymes, and RNA binding proteins, were among the genes that had the most significant impact on HCV replication.

Several screens identified members of the OST complex, which is an essential component in the N-linked glycosylation pathway, as being essential for flavivirus propagation. The catalytic subunit of the OST complex consists of one of two paralogous forms of STT3, STT3A and STT3B, which have partial functional redundancy (Shrimal et al., 2015). STT3A/B associates with at least six other subunits, including Ribophorin I (RPN1), ribophorin II (RPN2), DDOST (OST48), DAD1, OST4, and MAGT1 (IAP) or Tusc3 (N33), to make up different forms of the complex (Shrimal et al., 2015). Viral dependency on OST was confirmed for multiple flaviviruses, including DENV 1–4, WNV, ZIKV, JEV and YFV, although dependency varied between virus, cell-type, and subunit (Lin et al., 2017; Marceau et al., 2016; Savidis et al., 2016; Zhang et al., 2016). STT3A and STT3B expression were shown to be required for DENV propagation, while STT3A, but not STT3B, is required for ZIKV, JEV, WNV, and YFV replication in 293T and/or HAP1 cells. Despite the involvement of the OST complex in the N-linked glycosylation pathway, the N-glycosylation catalytic activity of STT3A/B was not required for DENV replication (Lin et al., 2017; Marceau et al., 2016). Expression of a STT3A or STT3B mutant lacking catalytic activity was able to overcome DENV inhibition observed in STT3A or STT3B knockout cell lines, respectively, suggesting the mechanism is at least partially independent of viral protein glycosylation. Instead, the OST complex was found to interact with viral NS proteins of the RNA replication complex, demonstrating that OST plays a structural role though a physical interaction with a viral protein that is required for DENV RNA replication. Although, because STT3A and STT3B may have partially redundant functions, a possible impact on N-glycosylation of viral glycoproteins cannot be eliminated as any substantial change on viral protein glycosylation would be observed at a later stage in the viral replication cycle

than the primary mechanism observed.

The anti-flavivirus potential of perturbing OST function was confirmed using the OST inhibitor, NG-1, which was shown to have broad anti-flavivirus activity *in vitro*, reducing viral RNA production of DENV, ZIKV, WNV and YFV (Lopez-Sambrooks et al., 2016; Puschnik et al., 2017). The mechanism of anti-flavivirus activity of NG-1 was also shown to be independent of the catalytic N-glycosylation function of the OST complex; however, it is possible that higher concentrations could alter viral protein glycosylation (Puschnik et al., 2017).

In addition to the OST complex, these genome-wide screens identified multiple other host protein candidates important for flavivirus replication that could be targeted in the future development of novel HTAVs. Specifically, several other ER-associated proteins were identified in loss-of-function screens as flavivirus host dependency factors, including members of the translocon-associated protein (TRAP) complex, ERAD pathway, ER-associated signal peptidase complex (SPCS1), and the ER membrane complex (EMC) (Le Sommer et al., 2012; Marceau et al., 2016; Savidis et al., 2016; Zhang et al., 2016). The TRAP complex, along with Sec61 and OST complexes, form the translocon where the cotranslational insertion of proteins occurs in the ER, and is required for proper expression of ER-targeted glycoproteins (Braunger et al., 2018; Pfeffer et al., 2016). The SPCS complex cleaves signal peptides on various membrane-associated and secreted proteins. SPC1 was demonstrated to affect posttranslational processing of the JEV NS2B protein rather than viral entry, RNA replication, or translation (Ma et al., 2018b). The EMC complex may associate with the OST complex and is involved in the processing and maturation of transmembrane proteins (Volkmar et al., 2019; Zakaria et al., 2018). EMC proteins were found to have a role in regulating the levels of host AXL or heparin sulfate protein expression on the cell surface, which is important for the early phase of replication for DENV and ZIKV (Jemielity et al., 2013; Meertens et al., 2012; Richard et al., 2015; Savidis et al., 2016). Infectivity assays using Huh-7 cells lacking expression of the EMC component, EMC4, confirmed the requirement of this host protein for DENV-2, DENV-4, YFV, and ZIKV replication, although WNV replication was unchanged in EMC4 knockout cells (Barrows et al., 2019). Similarly, the EMC2 subunit was also found to be dispensable for WNV propagation, although replication appeared to be slightly delayed (Ma et al., 2015).

12. Other clinical therapeutics for the treatment of flavivirus infections

While numerous vaccines and other biologics have advanced to clinical development for the prevention or treatment of flavivirus infections, only a few small molecules have reached this stage of development, with most targeting DENV (Table 5). As of August 2019, all drugs that have been evaluated in the clinic for DENV (safety and/or efficacy studies) were previously approved for other indications, except for three experimental drugs: the iminosugars UV-4B and celgosivir (discussed above), and balapiravir. Balapiravir is a prodrug of a cytidine analog that functions by inhibiting the viral polymerase protein and was originally developed for the treatment of chronic HCV infections. Balapiravir demonstrated promising results against HCV in a Phase 1b study (Roberts et al., 2008); however, clinical development was terminated following dose-related adverse events in patients in a Phase 2 clinical trial (NCT00517439) investigating extended combination therapy with pegylated IFN and ribavirin (Nelson et al., 2012; Roberts et al., 2008). The positive safety profile observed during the short course treatment in the Phase 1b trial led to balapiravir being investigated for the treatment of DENV infections, which would require only acute treatment unlike chronic HCV infections (Roberts et al., 2008). Although well-tolerated, balapiravir did not measurably reduce viremia, NS1 antigenemia, or fever clearance time. Failure to meet the efficacy endpoint is thought to be due, in part, to a decrease in the conversion of the prodrug to the active triphosphate form in infected patients (Chen et al., 2014).

Table 5
Clinical stage development of small molecules for the treatment of flavivirus infections or symptoms associated with disease caused by flavivirus infections.

Compound	Indication/Virus	Study Details	Study Endpoint/Readouts	Results	References
Approved Drugs					
Chloroquine	DENV	Randomized, double-blind, placebo-controlled study; Participants included patients over 14 years of age hospitalized in Ho Chi Minh City, Vietnam, with suspected uncomplicated DENV infections, including 257 confirmed cases; Participants received 600 mg chloroquine (day 1–2) and 200 mg on day 3, or placebo control	Safety and efficacy (negative RT-PCR detection of viral RNA in plasma and clearance of NS1 from blood).	Trend of lower incidence of hemorrhagic fever with chloroquine treatment; no reduction in duration of NS1 antigenaemia or DENV viremia; treatment was associated with more adverse events (e.g. vomiting) compared to the placebo-treated control group, with one case of hematemesis, with no reduction in the duration of NS1 antigenaemia or DENV viremia.	ISRCTN38002730; Tricou et al. (2010)
Ivermectin	DENV	Phase 2/3, randomized, double-blind, placebo-controlled study; 360 participants, children and adult patients ages 15 and older with DENV infection (fever >38 °C for ≤ 72 h); 200–400 µg/kg ivermectin or placebo once daily for 2 or 3 days	Safety and efficacy (primary: time to resolution of viremia; secondary: time to clearance of NS1 antigen, time until fever resolved)	Preliminary data suggests that there is no difference in viremia with ivermectin treatment. Treatment may result in a reduction in body temperature and NS1 serum levels with administration of a high dose.	NCT02045069; Low et al. (2017)
	DENV	Phase 2 non-randomized, open label study; Pediatric participants, ages 1–15, with DENV infections (acute fever within last 72 h); 400 µg or 600 µg ivermectin per 1 kg body weight once daily for three days	Primary: pharmacokinetics, Secondary: pharmacodynamics, safety (occurrences of adverse events), viremia and NS1 antigenemia clearance	NA; recruiting, current status unknown	NCT03432442
Ketotifen	Vascular leakage during DENV fever	Phase 4 randomized, double-blind, placebo-controlled study; Adult participants ages 21–60 with DENV fever (≥ 37.5 °C for ≤ 72 h), excluding patients with severe dengue; 2 mg Ketotifen or placebo BID for a total of 10 doses	Efficacy (primary: fluid accumulation in the pleural cavity; secondary: multiple secondary outcome measures)	NA; recruitment status unknown	NCT02673840
Lovastatin	DENV	Phase 2, randomized, double-blind, placebo-controlled study; Adult Vietnamese participants with acute DENV infections; 40 mg or 80 mg lovastatin or placebo control once daily for up to 5 days	Safety and tolerability of lovastatin, including rate of adverse events	Well-tolerated; No benefit observed, although not powered to statistically measure efficacy and selected dose may have been subtherapeutic.	ISRCTN03147572; Whitehorn et al. (2016)
Oseltamivir	Thrombocyt-openia and plasma leakage in DENV infections	Phase 2 randomized, placebo-controlled, double-blinded study; Participants ages 16 and above with DENV infections (fever ≤ 6 days, admitted to hospital); 75 mg oseltamivir phosphate BID or placebo for a maximum of 5 days	Safety and effects of treatment on plasma leakage and thrombocytopenia (time to platelet recovery)	NA; ongoing, no longer recruiting	ISRCTN35227717
Prednisolone	Prevention of DENV associated complications without prolonging viral clearance	Randomized, placebo-controlled, partially blinded study; Participants included Vietnamese children and young adults, ages 6–20, with suspected DENV infection (fever ≤ 72 h); Low or high dose prednisolone (doses based on body weight ranges) or placebo daily for 3 days	Safety (clinical and virological parameters)	Adverse effects, including prolongation of viremia, were not observed in patients following prednisolone treatment. No benefit to disease progression observed with prednisolone treatment compared to the placebo treated control group, although the study was not powered to statistically assess such effects.	ISRCTN39575233; Tam et al. (2012)
Novel Inhibitors					
AVI-4020	WNV neuroinvasive disease	Phase 1 exploratory study; Adult participants ages 18 to 75 with presumptive acute neuroinvasive WNV disease	Safety, tolerability, pharmacokinetics, and potential effectiveness on neurological status (measured by NIH Stroke Scale and Galsgow Coma Score)	Study terminated due to limited number of patients meeting eligibility criteria.	NCT00091845
Balapiravir	DENV	Phase 1, randomized, double-blind, placebo-controlled study; 64 adult male participants, ages 18–65, with confirmed DENV	Safety. tolerability, pharmacokinetics, and efficacy (viral load reduction, body temperature, quality of life)	Well-tolerated; no measurable reduction in viremia, NS1 antigenemia, or fever clearance time.	NCT01096576; Nguyen et al. (2013)

Table 5 (*continued*)

Compound	Indication/Virus	Study Details	Study Endpoint/Readouts	Results	References
		infections and symptom onset of < 48 h; 1,500 mg or 3,000 mg balapiravir, or placebo, BID for 5 days			
Galidesivir	YFV	Phase 1, randomized, double-blind, placebo-controlled study; 2 part study with 66 adult participants, ages 18 and older, hospitalized in Brazil with first onset of symptoms occurring within previous 7 days; part 1 is a dose-ranging study (3 doses), IV treatment every 12 h for 7 days, optimized dose level will be selected for part 2	Safety, tolerability, pharmacokinetics, and efficacy (viral load reduction, improvement in signs and symptoms and clinical manifestations)	NA; recruiting	NCT03891420

Abbreviations: BID, twice daily; DENV, dengue virus; h, hours; HCV, hepatitis C virus; IFN, interferon; NA, not available; NS1, non-structural protein 1; RT-PCR, reverse transcription polymerase chain reaction; TID, three times daily; WNV, West Nile virus; YFV, yellow fever virus.

The FDA-approved compounds chloroquine, ivermectin, predniso-lone, and lovastatin have also been evaluated in clinical trials for the treatment of DENV infections or for their ability to relieve associated symptoms without exacerbating infectivity. These compounds were considered to be promising candidates with the potential to be fast-tracked for approval because of their known safety profiles. Unfortunately, in a majority of these clinical studies, treatment did not provide a measurable benefit or was associated with adverse events.

Unlike for flaviviruses, significant advancement has been made for HCV antiviral drug development in the last five to ten years, with recent approval of multiple direct-acting antivirals. These combination therapies, which are discussed more thoroughly in recent reviews (Asselah et al., 2018; Das and Pandya, 2018; Vermehren et al., 2018), have increased safety, tolerability, and efficacy, and require shorter treatment regimens compared to the previous standard of care consisting of IFN and ribavirin.

13. Considerations for future research

It is nearly impossible to halt trends that promote the geographical expansion of some flaviviruses, such as climate change, global travel, and rapidly growing urbanization (Boldescu et al., 2017). Considering the paramount international health problems that are associated with some flavivirus infections, the lack of available therapeutics to treat most diseases caused by these infections, and the continued risk for the development of drug resistant strains, further development of novel antivirals for the treatment of flavivirus infections will continue to remain a priority. The dependency of *Flaviviridae* and other viruses on the host for N-linked glycosylation and glycoprotein production provides an opportunity for a host-targeted approach to broad-spectrum antiviral drug development.

While significant advances have been made in HCV antiviral drug development, most available therapies are not suitable for use in children, and there are a small percentage of adults (<5%) that do not respond to treatment. Drug development for HCV infections is still advancing with multiple other direct-acting antivirals being developed and tested in preclinical and clinical studies. The possible emergence of drug resistant strains will continue to have to be monitored, particularly because nearly all of the approved therapies and investigational agents directly target viral proteins. While the use of drug combinations can minimize the risk of resistant strains from developing, the inclusion of a host-targeted antiviral, such as an iminosugar, in a combination cocktail could further reduce the potential for viruses to mutate and no longer respond to the approved treatment.

One of the practical challenges that needs to be considered when developing novel iminosugars as HTAVs is that, because both the virus and the host depend on ER glucosidases for glycan processing, the development of potent compounds could potentially have a negative impact on the host. Regulatory agency concerns around this issue have likely slowed development of HTAV therapies compared to direct-acting antivirals. However, good therapeutic window and high tolerability have been observed for some iminosugars in animal infection models, suggesting that viral proteins may be more susceptible to enzyme inhibition than host proteins. It is likely viral proteins are being synthesized at an exponentially higher rate compared to host proteins during the proliferative phase of an acute viral infection, which may provide a window between drug levels required to impact the virus and levels that might result in significant impact on the host. Experimentally, the unique sensitivity of M-protein to glucosidase inhibition in hepatitis B virus infected cells, demonstrated by Block et al., supports the idea that a therapeutic window can be found (Block and Jordan, 2001; Simsek et al., 2005). However, replication rates and viral burden can vary both in infected individuals and for different viruses, which may require some 'tailoring' of drug dosage or schedule. Demonstrating an acceptable therapeutic window for HTAVs remains the next hurdle for approval of HTAV drug candidates by international regulatory agencies.

Further research investigating virus lifecycles and viral/host interactions could eventually lead to the identification of novel host targets for the development of HTAV to combat flavivirus infections. As with many other viruses, a combination of antivirals may be necessary to maximize the response and, as drugs become available, research providing insight into their potential synergy when used in combination with other antivirals is warranted. With continued efforts, the future provides hope for available treatment options to combat flavivirus infections caused by both known viruses and those yet to be identified.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2020.104881.

References

Akey, D.L., Brown, W.C., Dutta, S., Konwerski, J., Jose, J., Jurkiw, T.J., DelProposto, J., Ogata, C.M., Skiniotis, G., Kuhn, R.J., Smith, J.L., 2014. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. Science 343, 881–885.

Alonzi, D.S., Neville, D.C., Lachmann, R.H., Dwek, R.A., Butters, T.D., 2008. Glucosylated free oligosaccharides are biomarkers of endoplasmic-reticulum alpha-glucosidase inhibition. Biochem. J. 409, 571–580.

Apte-Sengupta, S., Sirohi, D., Kuhn, R.J., 2014. Coupling of replication and assembly in flaviviruses. Curr. Opin. Virol. 9, 134–142.

Asselah, T., Marcellin, P., Schinazi, R.F., 2018. Treatment of hepatitis C virus infection with direct-acting antiviral agents: 100% cure? Liver Int. 38 (Suppl. 1), 7–13.

Avezov, E., Frenkel, Z., Ehrlich, M., Herscovics, A., Lederkremer, G.Z., 2008. Endoplasmic reticulum (ER) mannosidase I is compartmentalized and required for N-glycan trimming to Man5-6GlcNAc2 in glycoprotein ER-associated degradation. Mol. Biol. Cell 19, 216–225.

Balsitis, S.J., Williams, K.L., Lachica, R., Flores, D., Kyle, J.L., Mehlhop, E., Johnson, S., Diamond, M.S., Beatty, P.R., Harris, E., 2010. Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. PLoS Pathog. 6, e1000790.

Barrows, N.J., Anglero-Rodriguez, Y., Kim, B., Jamison, S.F., Le Sommer, C., McGee, C. E., Pearson, J.L., Dimopoulos, G., Ascano, M., Bradrick, S.S., Garcia-Blanco, M.A., 2019. Dual roles for the ER membrane protein complex in flavivirus infection: viral entry and protein biogenesis. Sci. Rep. 9, 9711.

Beasley, D.W., Whiteman, M.C., Zhang, S., Huang, C.Y., Schneider, B.S., Smith, D.R., Gromowski, G.D., Higgs, S., Kinney, R.M., Barrett, A.D., 2005. Envelope protein glycosylation status influences mouse neuroinvasion phenotype of genetic lineage 1 West Nile virus strains. J. Virol. 79, 8339–8347.

Beljelarskaya, S.N., Orlova, O.V., Drutsa, V.L., Orlov, V.A., Timohova, A.V., Koroleva, N. N., Popenko, V.I., Ivanov, A.V., Spirin, P.V., Prassolov, V.S., Rubtsov, P.M., Kochetkov, S.N., 2016. Hepatitis C virus: the role of N-glycosylation sites of viral genotype 1b proteins for formation of viral particles in insect and mammalian cells. Biochem. Biophys. Rep. 7, 98–105.

Billerbeck, E., de Jong, Y., Dorner, M., de la Fuente, C., Ploss, A., 2013. Animal models for hepatitis C. Curr. Top. Microbiol. Immunol. 369, 49–86.

Block, T.M., Jordan, R., 2001. Iminosugars as possible broad spectrum anti hepatitis virus agents: the glucovirs and alkovirs. Antivir. Chem. Chemother. 12, 317–325.

Bloom, J.D., Gong, L.I., Baltimore, D., 2010. Permissive secondary mutations enable the evolution of influenza oseltamivir resistance. Science 328, 1272–1275.

Boldescu, V., Behnam, M.A.M., Vasilakis, N., Klein, C.D., 2017. Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond. Nat. Rev. Drug Discov. 16, 565–586.

Branza-Nichita, N., Durantel, D., Carrouee-Durantel, S., Dwek, R.A., Zitzmann, N., 2001. Antiviral effect of N-butyldeoxynojirimycin against bovine viral diarrhea virus correlates with misfolding of E2 envelope proteins and impairment of their association into E1-E2 heterodimers. J. Virol. 75, 3527–3536.

Braunger, K., Pfeffer, S., Shrimal, S., Gilmore, R., Berninghausen, O., Mandon, E.C., Becker, T., Forster, F., Beckmann, R., 2018. Structural basis for coupling protein transport and N-glycosylation at the mammalian endoplasmic reticulum. Science 360, 215–219.

Brazzoli, M., Helenius, A., Foung, S.K., Houghton, M., Abrignani, S., Merola, M., 2005. Folding and dimerization of hepatitis C virus E1 and E2 glycoproteins in stably transfected CHO cells. Virology 332, 438–453.

Bridges, C.G., Ahmed, S.P., Kang, M.S., Nash, R.J., Porter, E.A., Tyms, A.S., 1995. The effect of oral treatment with 6-O-butanoyl castanospermine (MDL 28,574) in the murine zosteriform model of HSV-1 infection. Glycobiology 5, 249–253.

Brinton, M.A., 2002. The molecular biology of West Nile Virus: a new invader of the western hemisphere. Annu. Rev. Microbiol. 56, 371–402.

Bryant, J.E., Calvert, A.E., Mesesan, K., Crabtree, M.B., Volpe, K.E., Silengo, S., Kinney, R.M., Huang, C.Y., Miller, B.R., Roehrig, J.T., 2007. Glycosylation of the dengue 2 virus E protein at N67 is critical for virus growth in vitro but not for growth in intrathoracically inoculated Aedes aegypti mosquitoes. Virology 366, 415–423.

Butters, T.D, van den Broek, L.A.G.M, Fleet, G.W.J, Wormald, M.R., Dwek, R.A., Platt, F. M., 2000. Molecular requirements of imino sugars for the selective control of N-linked glycosylation and glycosphingolipid biosynthesis. Tetrahedron: Asymmetry 11, 113–124.

Caputo, A.T., Alonzi, D.S., Marti, L., Reca, I.B., Kiappes, J.L., Struwe, W.B., Cross, A., Basu, S., Lowe, E.D., Darlot, B., Santino, A., Roversi, P., Zitzmann, N., 2016. Structures of mammalian ER alpha-glucosidase II capture the binding modes of broad-spectrum iminosugar antivirals. Proc. Natl. Acad. Sci. U. S. A. 113, E4630–E4638.

Chambers, T.J., Hahn, C.S., Galler, R., Rice, C.M., 1990. Flavivirus genome organization, expression, and replication. Annu. Rev. Microbiol. 44, 649–688.

Chang, J., Wang, L., Ma, D., Qu, X., Guo, H., Xu, X., Mason, P.M., Bourne, N., Moriarty, R., Gu, B., Guo, J.T., Block, T.M., 2009. Novel imino sugar derivatives demonstrate potent antiviral activity against flaviviruses. Antimicrob. Agents Chemother. 53, 1501–1508.

Chang, J., Schul, W., Butters, T.D., Yip, A., Liu, B., Goh, A., Lakshminarayana, S.B., Alonzi, D., Reinkensmeier, G., Pan, X., Qu, X., Weidner, J.M., Wang, L., Yu, W., Borune, N., Kinch, M.A., Rayahin, J.E., Moriarty, R., Xu, X., Shi, P.Y., Guo, J.T., Block, T.M., 2011. Combination of alpha-glucosidase inhibitor and ribavirin for the treatment of dengue virus infection in vitro and in vivo. Antivir. Res. 89, 26–34.

Chang, J., Guo, J.T., Du, Y., Block, T., 2013a. Imino sugar glucosidase inhibitors as broadly active anti-filovirus agents. Emerg. Microb. Infect. 2, e77.

Chang, J., Warren, T.K., Zhao, X., Gill, T., Guo, F., Wang, L., Comunale, M.A., Du, Y., Alonzi, D.S., Yu, W., Ye, H., Liu, F., Guo, J.T., Mehta, A., Cuconati, A., Butters, T.D., Bavari, S., Xu, X., Block, T.M., 2013b. Small molecule inhibitors of ER alpha-glucosidases are active against multiple hemorrhagic fever viruses. Antivir. Res. 98, 432–440.

Chapel, C., Garcia, C., Roingeard, P., Zitzmann, N., Dubuisson, J., Dwek, R.A., Trepo, C., Zoulim, F., Durantel, D., 2006. Antiviral effect of alpha-glucosidase inhibitors on viral morphogenesis and binding properties of hepatitis C virus-like particles. J. Gen. Virol. 87, 861–871.

Chapman, T.M., Davies, I.G., Gu, B., Block, T.M., Scopes, D.I., Hay, P.A., Courtney, S.M., McNeill, L.A., Schofield, C.J., Davis, B.G., 2005. Glyco- and peptidomimetics from three-component Joullie-Ugi coupling show selective antiviral activity. J. Am. Chem. Soc. 127, 506–507.

Chatel-Chaix, L., Bartenschlager, R., 2014. Dengue virus- and hepatitis C virus-induced replication and assembly compartments: the enemy inside–caught in the web. J. Virol. 88, 5907–5911.

Chen, Y.L., Abdul Ghafar, N., Karuna, R., Fu, Y., Lim, S.P., Schul, W., Gu, F., Herve, M., Yokohama, F., Wang, G., Cerny, D., Fink, K., Blasco, F., Shi, P.Y., 2014. Activation of peripheral blood mononuclear cells by dengue virus infection depotentiates balapiravir. J. Virol. 88, 1740–1747.

Choukhi, A., Ung, S., Wychowski, C., Dubuisson, J., 1998. Involvement of endoplasmic reticulum chaperones in the folding of hepatitis C virus glycoproteins. J. Virol. 72, 3851–3858.

Cocquerel, L., Meunier, J.C., Op de Beeck, A., Bonte, D., Wychowski, C., Dubuisson, J., 2001. Coexpression of hepatitis C virus envelope proteins E1 and E2 in cis improves the stability of membrane insertion of E2. J. Gen. Virol. 82, 1629–1635.

Courageot, M.P., Frenkiel, M.P., Dos Santos, C.D., Deubel, V., Despres, P., 2000. Alpha-glucosidase inhibitors reduce dengue virus production by affecting the initial steps of virion morphogenesis in the endoplasmic reticulum. J. Virol. 74, 564–572.

Dai, L., Song, J., Lu, X., Deng, Y.Q., Musyoki, A.M., Cheng, H., Zhang, Y., Yuan, Y., Song, H., Haywood, J., Xiao, H., Yan, J., Shi, Y., Qin, C.F., Qi, J., Gao, G.F., 2016. Structures of the zika virus envelope protein and its complex with a flavivirus broadly protective antibody. Cell Host Microbe 19, 696–704.

Das, D., Pandya, M., 2018. Recent advancement of direct-acting antiviral agents (DAAs) in hepatitis C therapy. Mini Rev. Med. Chem. 18, 584–596.

Dubuisson, J., 2000. Folding, assembly and subcellular localization of hepatitis C virus glycoproteins. Curr. Top. Microbiol. Immunol. 242, 135–148.

Durantel, D., 2009. Celgosivir, an alpha-glucosidase I inhibitor for the potential treatment of HCV infection. Curr. Opin. Invest. Drugs 10, 860–870.

Durantel, D., Branza-Nichita, N., Carrouee-Durantel, S., Butters, T.D., Dwek, R.A., Zitzmann, N., 2001. Study of the mechanism of antiviral action of iminosugar derivatives against bovine viral diarrhea virus. J. Virol. 75, 8987–8998.

Durantel, D., Carrouee-Durantel, S., Branza-Nichita, N., Dwek, R.A., Zitzmann, N., 2004. Effects of interferon, ribavirin, and iminosugar derivatives on cells persistently infected with noncytopathic bovine viral diarrhea virus. Antimicrob. Agents Chemother. 48, 497–504.

Durantel, D., Alotte, C., Zoulim, F., 2007. Glucosidase inhibitors as antiviral agents for hepatitis B and C. Curr. Opin. Invest. Drugs 8, 125–129.

Elloumi, H., Houissa, F., Hadj, N.B., Gargouri, D., Romani, M., Kharrat, J., Ghorbel, A., 2007. Sudden hearing loss associated with peginterferon and ribavirin combination therapy during hepatitis C treatment. World J. Gastroenterol. 13, 5411–5412.

Ferenci, P., Fried, M.W., Shiffman, M.L., Smith, C.I., Marinos, G., Goncales Jr., F.L., Haussinger, D., Diago, M., Carosi, G., Dhumeaux, D., Craxi, A., Chaneac, M., Reddy, K.R., 2005. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J. Hepatol. 43, 425–433.

Fernandez-Garcia, M.D., Mazzon, M., Jacobs, M., Amara, A., 2009. Pathogenesis of flavivirus infections: using and abusing the host cell. Cell Host Microbe 5, 318–328.

Fischer, P.B., Collin, M., Karlsson, G.B., James, W., Butters, T.D., Davis, S.J., Gordon, S., Dwek, R.A., Platt, F.M., 1995. The alpha-glucosidase inhibitor N-butyldeoxynojirimycin inhibits human immunodeficiency virus entry at the level of post-CD4 binding. J. Virol. 69, 5791–5797.

Fischer, P.B., Karlsson, G.B., Dwek, R.A., Platt, F.M., 1996. N-butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with impaired gp120 shedding and gp41 exposure. J. Virol. 70, 7153–7160.

Fischl, M.A., Resnick, L., Coombs, R., Kremer, A.B., Pottage Jr., J.C., Fass, R.J., Fife, K.H., Powderly, W.G., Collier, A.C., Aspinall, R.L., et al., 1994. The safety and efficacy of combination N-butyl-deoxynojirimycin (SC-48334) and zidovudine in patients with HIV-1 infection and 200-500 CD4 cells/mm3. J. Acquir. Immune Defic. Syndr. 7, 139–147.

Flamand, M., Megret, F., Mathieu, M., Lepault, J., Rey, F.A., Deubel, V., 1999. Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble hexamer in a glycosylation-dependent fashion. J. Virol. 73, 6104–6110.

Fontes-Garfias, C.R., Shan, C., Luo, H., Muruato, A.E., Medeiros, D.B.A., Mays, E., Xie, X., Zou, J., Roundy, C.M., Wakamiya, M., Rossi, S.L., Wang, T., Weaver, S.C., Shi, P.Y., 2017. Functional analysis of glycosylation of zika virus envelope protein. Cell Rep. 21, 1180–1190.

Foster, T.L., Verow, M., Wozniak, A.L., Bentham, M.J., Thompson, J., Atkins, E., Weinman, S.A., Fishwick, C., Foster, R., Harris, M., Griffin, S., 2011. Resistance mutations define specific antiviral effects for inhibitors of the hepatitis C virus p7 ion channel. Hepatology 54, 79–90.

Freedman, H., Logan, M.R., Law, J.L., Houghton, M., 2016. Structure and function of the hepatitis C virus envelope glycoproteins E1 and E2: antiviral and vaccine targets. ACS Infect. Dis. 2, 749–762.

Gavel, Y., von Heijne, G., 1990. Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/Ser acceptor sites: implications for protein engineering. Protein Eng. 3, 433–442.

Goffard, A., Dubuisson, J., 2003. Glycosylation of hepatitis C virus envelope proteins. Biochimie 85, 295–301.

Goffard, A., Callens, N., Bartosch, B., Wychowski, C., Cosset, F.L., Montpellier, C., Dubuisson, J., 2005. Role of N-linked glycans in the functions of hepatitis C virus envelope glycoproteins. J. Virol. 79, 8400–8409.

Goto, A., Yoshii, K., Obara, M., Ueki, T., Mizutani, T., Kariwa, H., Takashima, I., 2005. Role of the N-linked glycans of the prM and E envelope proteins in tick-borne encephalitis virus particle secretion. Vaccine 23, 3043–3052.

Griffin, S., Stgelais, C., Owsianka, A.M., Patel, A.H., Rowlands, D., Harris, M., 2008. Genotype-dependent sensitivity of hepatitis C virus to inhibitors of the p7 ion channel. Hepatology 48, 1779–1790.

Gu, B., Mason, P., Wang, L., Norton, P., Bourne, N., Moriarty, R., Mehta, A., Despande, M., Shah, R., Block, T., 2007. Antiviral profiles of novel iminocyclitol compounds against bovine viral diarrhea virus, West Nile virus, dengue virus and hepatitis B virus. Antivir. Chem. Chemother. 18, 49–59.

Guirakhoo, F., Hunt, A.R., Lewis, J.G., Roehrig, J.T., 1993. Selection and partial characterization of dengue 2 virus mutants that induce fusion at elevated pH. Virology 194, 219–223.

Hacker, K., White, L., de Silva, A.M., 2009. N-linked glycans on dengue viruses grown in mammalian and insect cells. J. Gen. Virol. 90, 2097–2106.

Hahn, C.S., Dalrymple, J.M., Strauss, J.H., Rice, C.M., 1987. Comparison of the virulent Asibi strain of yellow fever virus with the 17D vaccine strain derived from it. Proc. Natl. Acad. Sci. U. S. A. 84, 2019–2023.

Hanna, S.L., Pierson, T.C., Sanchez, M.D., Ahmed, A.A., Murtadha, M.M., Doms, R.W., 2005. N-linked glycosylation of west nile virus envelope proteins influences particle assembly and infectivity. J. Virol. 79, 13262–13274.

Harada, T., Tautz, N., Thiel, H.J., 2000. E2-p7 region of the bovine viral diarrhea virus polyprotein: processing and functional studies. J. Virol. 74, 9498–9506.

Hauge, S.H., Dudman, S., Borgen, K., Lackenby, A., Hungnes, O., 2009. Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. Emerg. Infect. Dis. 15, 155–162.

Hebert, D.N., Foellmer, B., Helenius, A., 1995. Glucose trimming and reglucosylation determine glycoprotein association with calnexin in the endoplasmic reticulum. Cell 81, 425–433.

Helle, F., Vieyres, G., Elkrief, L., Popescu, C.I., Wychowski, C., Descamps, V., Castelain, S., Roingeard, P., Duverlie, G., Dubuisson, J., 2010. Role of N-linked glycans in the functions of hepatitis C virus envelope proteins incorporated into infectious virions. J. Virol. 84, 11905–11915.

Howe, J.D., Smith, N., Lee, M.J., Ardes-Guisot, N., Vauzeilles, B., Desire, J., Baron, A., Bleriot, Y., Sollogoub, M., Alonzi, D.S., Butters, T.D., 2013. Novel imino sugar alpha-glucosidase inhibitors as antiviral compounds. Bioorg. Med. Chem. 21, 4831–4838.

Idris, F., Muharram, S.H., Diah, S., 2016. Glycosylation of dengue virus glycoproteins and their interactions with carbohydrate receptors: possible targets for antiviral therapy. Arch. Virol. 161, 1751–1760.

Ishak, H., Takegami, T., Kamimura, K., Funada, H., 2001. Comparative sequences of two type 1 dengue virus strains possessing different growth characteristics in vitro. Microbiol. Immunol. 45, 327–331.

Jacob, J.R., Mansfield, K., You, J.E., Tennant, B.C., Kim, Y.H., 2007. Natural iminosugar derivatives of 1-deoxynojirimycin inhibit glycosylation of hepatitis viral envelope proteins. J. Microbiol. 45, 431–440.

Jemielity, S., Wang, J.J., Chan, Y.K., Ahmed, A.A., Li, W., Monahan, S., Bu, X., Farzan, M., Freeman, G.J., Umetsu, D.T., Dekruyff, R.H., Choe, H., 2013. TIM-family proteins promote infection of multiple enveloped viruses through virion-associated phosphatidylserine. PLoS Pathog. 9, e1003232.

Johnson, A.J., Guirakhoo, F., Roehrig, J.T., 1994. The envelope glycoproteins of dengue 1 and dengue 2 viruses grown in mosquito cells differ in their utilization of potential glycosylation sites. Virology 203, 241–249.

Jones, C.T., Murray, C.L., Eastman, D.K., Tassello, J., Rice, C.M., 2007. Hepatitis C virus p7 and NS2 proteins are essential for production of infectious virus. J. Virol. 81, 8374–8383.

Jordan, R., Nikolaeva, O.V., Wang, L., Conyers, B., Mehta, A., Dwek, R.A., Block, T.M., 2002. Inhibition of host ER glucosidase activity prevents Golgi processing of virion-associated bovine viral diarrhea virus E2 glycoproteins and reduces infectivity of secreted virions. Virology 295, 10–19.

Kasturi, L., Chen, H., Shakin-Eshleman, S.H., 1997. Regulation of N-linked core glycosylation: use of a site-directed mutagenesis approach to identify Asn-Xaa-Ser/Thr sequons that are poor oligosaccharide acceptors. Biochem. J. 323 (Pt 2), 415–419.

Katzelnick, L.C., Gresh, L., Halloran, M.E., Mercado, J.C., Kuan, G., Gordon, A., Balmaseda, A., Harris, E., 2017. Antibody-dependent enhancement of severe dengue disease in humans. Science 358, 929–932.

Kawano, H., Rostapshov, V., Rosen, L., Lai, C.J., 1993. Genetic determinants of dengue type 4 virus neurovirulence for mice. J. Virol. 67, 6567–6575.

Kiappes, J.L., Hill, M.L., Alonzi, D.S., Miller, J.L., Iwaki, R., Sayce, A.C., Caputo, A.T., Kato, A., Zitzmann, N., 2018. ToP-DNJ, a selective inhibitor of endoplasmic reticulum alpha-glucosidase II exhibiting antiflaviviral activity. ACS Chem. Biol. 13, 60–65.

Kim, J.M., Yun, S.I., Song, B.H., Hahn, Y.S., Lee, C.H., Oh, H.W., Lee, Y.M., 2008. A single N-linked glycosylation site in the Japanese encephalitis virus prM protein is critical for cell type-specific prM protein biogenesis, virus particle release, and pathogenicity in mice. J. Virol. 82, 7846–7862.

Konishi, E., Mason, P.W., 1993. Proper maturation of the Japanese encephalitis virus envelope glycoprotein requires cosynthesis with the premembrane protein. J. Virol. 67, 1672–1675.

Kornfeld, R., Kornfeld, S., 1985. Assembly of asparagine-linked oligosaccharides. Annu. Rev. Biochem. 54, 631–664.

Kuhn, R.J., Zhang, W., Rossmann, M.G., Pletnev, S.V., Corver, J., Lenches, E., Jones, C.T., Mukhopadhyay, S., Chipman, P.R., Strauss, E.G., Baker, T.S., Strauss, J.H., 2002. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. Cell 108, 717–725.

Lad, V.J., Shende, V.R., Gupta, A.K., 2013. Effect of catanospermine, 1-deoxynojirimycin or 1-deoxymannojirimycin on biological and functional activities of Japanese encephalitis virus in porcine stable kidney cells. Microbiol. Res. 4 (e3), 12–15.

Le Sommer, C., Barrows, N.J., Bradrick, S.S., Pearson, J.L., Garcia-Blanco, M.A., 2012. G protein-coupled receptor kinase 2 promotes flaviviridae entry and replication. PLoS Neglected Trop. Dis. 6, e1820.

Lee, E., Weir, R.C., Dalgarno, L., 1997. Changes in the dengue virus major envelope protein on passaging and their localization on the three-dimensional structure of the protein. Virology 232, 281–290.

Lee, E., Leang, S.K., Davidson, A., Lobigs, M., 2010. Both E protein glycans adversely affect dengue virus infectivity but are beneficial for virion release. J. Virol. 84, 5171–5180.

Li, L., Lok, S.M., Yu, I.M., Zhang, Y., Kuhn, R.J., Chen, J., Rossmann, M.G., 2008. The flavivirus precursor membrane-envelope protein complex: structure and maturation. Science 319, 1830–1834.

Lin, D.L., Cherepanova, N.A., Bozzacco, L., MacDonald, M.R., Gilmore, R., Tai, A.W., 2017. Dengue virus hijacks a noncanonical oxidoreductase function of a cellular oligosaccharyltransferase complex. mBio 8.

Lindenbach, B.D., Rice, C.M., 2013. The ins and outs of hepatitis C virus entry and assembly. Nat. Rev. Microbiol. 11, 688–700.

Lopez-Denman, A.J., Mackenzie, J.M., 2017. The IMPORTance of the nucleus during flavivirus replication. Viruses 9.

Lopez-Sambrooks, C., Shrimal, S., Khodier, C., Flaherty, D.P., Rinis, N., Charest, J.C., Gao, N., Zhao, P., Wells, L., Lewis, T.A., Lehrman, M.A., Gilmore, R., Golden, J.E., Contessa, J.N., 2016. Oligosaccharyltransferase inhibition induces senescence in RTK-driven tumor cells. Nat. Chem. Biol. 12, 1023–1030.

Lorenz, I.C., Allison, S.L., Heinz, F.X., Helenius, A., 2002. Folding and dimerization of tick-borne encephalitis virus envelope proteins prM and E in the endoplasmic reticulum. J. Virol. 76, 5480–5491.

Lorenz, I.C., Kartenbeck, J., Mezzacasa, A., Allison, S.L., Heinz, F.X., Helenius, A., 2003. Intracellular assembly and secretion of recombinant subviral particles from tick-borne encephalitis virus. J. Virol. 77, 4370–4382.

Low, J.G., Sung, C., Wijaya, L., Wei, Y., Rathore, A.P.S., Watanabe, S., Tan, B.H., Toh, L., Chua, L.T., Hou, Y., Chow, A., Howe, S., Chan, W.K., Tan, K.H., Chung, J.S., Cherng, B.P., Lye, D.C., Tambayah, P.A., Ng, L.C., Connolly, J., Hibberd, M.L., Leo, Y.S., Cheung, Y.B., Ooi, E.E., Vasudevan, S.G., 2014. Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. Lancet Infect. Dis. 14, 706–715.

Low, J.G., Ooi, E.E., Vasudevan, S.G., 2017. Current status of dengue therapeutics research and development. J. Infect. Dis. 215, S96–S102.

Ma, H., Dang, Y., Wu, Y., Jia, G., Anaya, E., Zhang, J., Abraham, S., Choi, J.G., Shi, G., Qi, L., Manjunath, N., Wu, H., 2015. A CRISPR-based screen identifies genes essential for west-nile-virus-induced cell death. Cell Rep. 12, 673–683.

Ma, J., Zhang, X., Soloveva, V., Warren, T., Guo, F., Wu, S., Lu, H., Guo, J., Su, Q., Shen, H., Solon, E., Comunale, M.A., Mehta, A., Guo, J.T., Bavari, S., Du, Y., Block, T.M., Chang, J., 2018a. Enhancing the antiviral potency of ER alpha-glucosidase inhibitor IHVR-19029 against hemorrhagic fever viruses in vitro and in vivo. Antivir. Res. 150, 112–122.

Ma, L., Li, F., Zhang, J.W., Li, W., Zhao, D.M., Wang, H., Hua, R.H., Bu, Z.G., 2018b. Host factor SPCS1 regulates the replication of Japanese encephalitis virus through interactions with transmembrane domains of NS2B. J. Virol. 92 (12) e00197-18.

Mackenzie, J.M., Westaway, E.G., 2001. Assembly and maturation of the flavivirus Kunjin virus appear to occur in the rough endoplasmic reticulum and along the secretory pathway, respectively. J. Virol. 75, 10787–10799.

Manns, M.P., Wedemeyer, H., Cornberg, M., 2006. Treating viral hepatitis C: efficacy, side effects, and complications. Gut 55, 1350–1359.

Marceau, C.D., Puschnik, A.S., Majzoub, K., Ooi, Y.S., Brewer, S.M., Fuchs, G., Swaminathan, K., Mata, M.A., Elias, J.E., Sarnow, P., Carette, J.E., 2016. Genetic dissection of Flaviviridae host factors through genome-scale CRISPR screens. Nature 535, 159.

Martinez-Duncker, I., Diaz-Jimenez, D.F., Mora-Montes, H.M., 2014. Comparative analysis of protein glycosylation pathways in humans and the fungal pathogen Candida albicans. Internet J. Microbiol. 2014, 267497.

Meertens, L., Carnec, X., Lecoin, M.P., Ramdasi, R., Guivel-Benhassine, F., Lew, E., Lemke, G., Schwartz, O., Amara, A., 2012. The TIM and TAM families of phosphatidylserine receptors mediate dengue virus entry. Cell Host Microbe 12, 544–557.

Mehta, A., Ouzounov, S., Jordan, R., Simsek, E., Lu, X., Moriarty, R.M., Jacob, G., Dwek, R.A., Block, T.M., 2002. Imino sugars that are less toxic but more potent as antivirals, in vitro, compared with N-n-nonyl DNJ. Antivir. Chem. Chemother. 13, 299–304.

Meijer, A., Lackenby, A., Hungnes, O., Lina, B., van-der-Werf, S., Schweiger, B., Opp, M., Paget, J., van-de-Kassteele, J., Hay, A., Zambon, M., 2009. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007-08 season. Emerg. Infect. Dis. 15, 552–560. European Influenza Surveillance, S.

Mellor, H.R., Nolan, J., Pickering, L., Wormald, M.R., Platt, F.M., Dwek, R.A., Fleet, G.W., Butters, T.D., 2002. Preparation, biochemical characterization and biological properties of radiolabelled N-alkylated deoxynojirimycins. Biochem. J. 366, 225–233.

Mellor, H.R., Platt, F.M., Dwek, R.A., Butters, T.D., 2003. Membrane disruption and cytotoxicity of hydrophobic N-alkylated imino sugars is independent of the inhibition of protein and lipid glycosylation. Biochem. J. 374, 307–314.

Miller, J.L., Lachica, R., Sayce, A.C., Williams, J.P., Bapat, M., Dwek, R.A., Beatty, P.R., Harris, E., Zitzmann, N., 2012. Liposome-mediated delivery of iminosugars enhances efficacy against dengue virus in vivo. Antimicrob. Agents Chemother. 56, 6379–6386.

Miller, J.L., Tyrrell, B.E., Zitzmann, N., 2018. Mechanisms of antiviral activity of iminosugars against dengue virus. Adv. Exp. Med. Biol. 1062, 277–301.

Miller, J.L., Hill, M.L., Brun, J., Pountain, A., Sayce, A.C., Zitzmann, N., 2019. Iminosugars counteract the downregulation of the interferon gamma receptor by dengue virus. Antivir. Res. 170, 104551.

Molinari, M., Helenius, A., 1999. Glycoproteins form mixed disulphides with oxidoreductases during folding in living cells. Nature 402, 90–93.

Mondotte, J.A., Lozach, P.Y., Amara, A., Gamarnik, A.V., 2007. Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. J. Virol. 81, 7136–7148.

Mossenta, M., Marchese, S., Poggianella, M., Slon Campos, J.L., Burrone, O.R., 2017. Role of N-glycosylation on Zika virus E protein secretion, viral assembly and infectivity. Biochem. Biophys. Res. Commun. 492, 579–586.

Mukhopadhyay, S., Kim, B.S., Chipman, P.R., Rossmann, M.G., Kuhn, R.J., 2003. Structure of West Nile virus. Science 302, 248.

Muylaert, I.R., Chambers, T.J., Galler, R., Rice, C.M., 1996. Mutagenesis of the N-linked glycosylation sites of the yellow fever virus NS1 protein: effects on virus replication and mouse neurovirulence. Virology 222, 159–168.

Nelson, D.R., Zeuzem, S., Andreone, P., Ferenci, P., Herring, R., Jensen, D.M., Marcellin, P., Pockros, P.J., Rodriguez-Torres, M., Rossaro, L., Rustgi, V.K., Sepe, T., Sulkowski, M., Thomason, I.R., Yoshida, E.M., Chan, A., Hill, G., 2012. Balapiravir plus peginterferon alfa-2a (40KD)/ribavirin in a randomized trial of hepatitis C genotype 1 patients. Ann. Hepatol. 11, 15–31.

Neufeldt, C.J., Cortese, M., Acosta, E.G., Bartenschlager, R., 2018. Rewiring cellular networks by members of the Flaviviridae family. Nat. Rev. Microbiol. 16, 125–142.

Neville, D.C., Coquard, V., Priestman, D.A., te Vruchte, D.J., Sillence, D.J., Dwek, R.A., Platt, F.M., Butters, T.D., 2004. Analysis of fluorescently labeled glycosphingolipid-derived oligosaccharides following ceramide glycanase digestion and anthranilic acid labeling. Anal. Biochem. 331, 275–282.

Nguyen, N.M., Tran, C.N., Phung, L.K., Duong, K.T., Huynh Hle, A., Farrar, J., Nguyen, Q.T., Tran, H.T., Nguyen, C.V., Merson, L., Hoang, L.T., Hibberd, M.L., Aw, P.P., Wilm, A., Nagarajan, N., Nguyen, D.T., Pham, M.P., Nguyen, T.T., Javanbakht, H., Klumpp, K., Hammond, J., Petric, R., Wolbers, M., Nguyen, C.T., Simmons, C.P., 2013. A randomized, double-blind placebo controlled trial of balapiravir, a polymerase inhibitor, in adult dengue patients. J. Infect. Dis. 207, 1442–1450.

Omoto, S., Speranzini, V., Hashimoto, T., Noshi, T., Yamaguchi, H., Kawai, M., Kawaguchi, K., Uehara, T., Shishido, T., Naito, A., Cusack, S., 2018. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci. Rep. 8, 9633.

Op De Beeck, A., Cocquerel, L., Dubuisson, J., 2001. Biogenesis of hepatitis C virus envelope glycoproteins. J. Gen. Virol. 82, 2589–2595.

Ouzounov, S., Mehta, A., Dwek, R.A., Block, T.M., Jordan, R., 2002. The combination of interferon alpha-2b and n-butyl deoxynojirimycin has a greater than additive antiviral effect upon production of infectious bovine viral diarrhea virus (BVDV) in vitro: implications for hepatitis C virus (HCV) therapy. Antivir. Res. 55, 425–435.

Paul, D., Bartenschlager, R., 2015. Flaviviridae replication organelles: oh, what a tangled web we weave. Annu. Rev. Virol. 2, 289–310.

Pavlovic, D., Neville, D.C., Argaud, O., Blumberg, B., Dwek, R.A., Fischer, W.B., Zitzmann, N., 2003. The hepatitis C virus p7 protein forms an ion channel that is inhibited by long-alkyl-chain iminosugar derivatives. Proc. Natl. Acad. Sci. U. S. A. 100, 6104–6108.

Perry, S.T., Buck, M.D., Plummer, E.M., Penmasta, R.A., Batra, H., Stavale, E.J., Warfield, K.L., Dwek, R.A., Butters, T.D., Alonzi, D.S., Lada, S.M., King, K., Klose, B., Ramstedt, U., Shresta, S., 2013. An iminosugar with potent inhibition of dengue virus infection in vivo. Antivir. Res. 98, 35–43.

Petrova, E., Gracias, S., Beauclair, G., Tangy, F., Jouvenet, N., 2019. Uncovering flavivirus host dependency factors through a genome-wide gain-of-function screen. Viruses 11 (1), 68.

Pfeffer, S., Dudek, J., Zimmermann, R., Forster, F., 2016. Organization of the native ribosome-translocon complex at the mammalian endoplasmic reticulum membrane. Biochim. Biophys. Acta 1860, 2122–2129.

Platt, F.M., Neises, G.R., Dwek, R.A., Butters, T.D., 1994a. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. J. Biol. Chem. 269, 8362–8365.

Platt, F.M., Neises, G.R., Karlsson, G.B., Dwek, R.A., Butters, T.D., 1994b. N-butyldeoxygalactonojirimycin inhibits glycolipid biosynthesis but does not affect N-linked oligosaccharide processing. J. Biol. Chem. 269, 27108–27114.

Plummer, E., Buck, M.D., Sanchez, M., Greenbaum, J.A., Turner, J., Grewal, R., Klose, B., Sampath, A., Warfield, K.L., Peters, B., Ramstedt, U., Shresta, S., 2015. Dengue virus evolution under a host-targeted antiviral. J. Virol. 89, 5592–5601.

Pokidysheva, E., Zhang, Y., Battisti, A.J., Bator-Kelly, C.M., Chipman, P.R., Xiao, C., Gregorio, G.G., Hendrickson, W.A., Kuhn, R.J., Rossmann, M.G., 2006. Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. Cell 124, 485–493.

Puschnik, A.S., Marceau, C.D., Ooi, Y.S., Majzoub, K., Rinis, N., Contessa, J.N., Carette, J.E., 2017. A small-molecule oligosaccharyltransferase inhibitor with pan-flaviviral activity. Cell Rep. 21, 3032–3039.

Putnak, J.R., Charles, P.C., Padmanabhan, R., Irie, K., Hoke, C.H., Burke, D.S., 1988. Functional and antigenic domains of the dengue-2 virus nonstructural glycoprotein NS-1. Virology 163, 93–103.

Qu, X., Pan, X., Weidner, J., Yu, W., Alonzi, D., Xu, X., Butters, T., Block, T., Guo, J.T., Chang, J., 2011. Inhibitors of endoplasmic reticulum alpha-glucosidases potently suppress hepatitis C virus virion assembly and release. Antimicrob. Agents Chemother. 55, 1036–1044.

Rajasekharan, S., Bonotto, R.M., Kazungu, Y., Alves, L.N., Poggianella, M., Orellana, P.M., Skoko, N., Polez, S., Marcello, A., 2020. Repurposing of miglustat to inhibit the coronavirus severe acquired respiratory syndrome SARS-CoV-2. https://doi.org/10.1101/2020.05.18.101691.

Rathore, A.P., Paradkar, P.N., Watanabe, S., Tan, K.H., Sung, C., Connolly, J.E., Low, J., Ooi, E.E., Vasudevan, S.G., 2011. Celgosivir treatment misfolds dengue virus NS1 protein, induces cellular pro-survival genes and protects against lethal challenge mouse model. Antivir. Res. 92, 453–460.

Richard, A.S., Zhang, A., Park, S.J., Farzan, M., Zong, M., Choe, H., 2015. Virion-associated phosphatidylethanolamine promotes TIM1-mediated infection by Ebola, dengue, and West Nile viruses. Proc. Natl. Acad. Sci. U. S. A. 112, 14682–14687.

Roberts, S.K., Cooksley, G., Dore, G.J., Robson, R., Shaw, D., Berns, H., Hill, G., Klumpp, K., Najera, I., Washington, C., 2008. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. Hepatology 48, 398–406.

Sadat, M.A., Moir, S., Chun, T.W., Lusso, P., Kaplan, G., Wolfe, L., Memoli, M.J., He, M., Vega, H., Kim, L.J.Y., Huang, Y., Hussein, N., Nievas, E., Mitchell, R., Garofalo, M., Louie, A., Ireland, D.C., Grunes, C., Cimbro, R., Patel, V., Holzapfel, G., Salahuddin, D., Bristol, T., Adams, D., Marciano, B.E., Hegde, M., Li, Y., Calvo, K.R., Stoddard, J., Justement, J.S., Jacques, J., Priel, D.A.L., Murray, D., Sun, P., Kuhns, D.B., Boerkoel, C.F., Chiorini, J.A., Di Pasquale, G., Verthelyi, D., Rosenzweig, S.D., 2014. Glycosylation, hypogammaglobulinemia, and resistance to viral infections. N. Engl. J. Med. 370, 1615–1625.

Sakai, A., Claire, M.S., Faulk, K., Govindarajan, S., Emerson, S.U., Purcell, R.H., Bukh, J., 2003. The p7 polypeptide of hepatitis C virus is critical for infectivity and contains functionally important genotype-specific sequences. Proc. Natl. Acad. Sci. U. S. A. 100, 11646–11651.

Savidis, G., McDougall, W.M., Meraner, P., Perreira, J.M., Portmann, J.M., Trincucci, G., John, S.P., Aker, A.M., Renzette, N., Robbins, D.R., Guo, Z., Green, S., Kowalik, T.F., Brass, A.L., 2016. Identification of zika virus and dengue virus dependency factors using functional genomics. Cell Rep. 16, 232–246.

Sayce, A.C., Alonzi, D.S., Killingbeck, S.S., Tyrrell, B.E., Hill, M.L., Caputo, A.T., Iwaki, R., Kinami, K., Ide, D., Kiappes, J.L., Beatty, P.R., Kato, A., Harris, E., Dwek, R.A., Miller, J.L., Zitzmann, N., 2016. Iminosugars inhibit dengue virus production via inhibition of ER alpha-glucosidases–not glycolipid processing enzymes. PLoS Neglected Trop. Dis. 10, e0004524.

Schallus, T., Jaeckh, C., Feher, K., Palma, A.S., Liu, Y., Simpson, J.C., Mackeen, M., Stier, G., Gibson, T.J., Feizi, T., Pieler, T., Muhle-Goll, C., 2008. Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. Mol. Biol. Cell 19, 3404–3414.

Schul, W., Liu, W., Xu, H.Y., Flamand, M., Vasudevan, S.G., 2007. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. J. Infect. Dis. 195, 665–674.

Shrimal, S., Cherepanova, N.A., Gilmore, R., 2015. Cotranslational and posttranslocational N-glycosylation of proteins in the endoplasmic reticulum. Semin. Cell Dev. Biol. 41, 71–78.

Simmonds, P., Becher, P., Bukh, J., Gould, E.A., Meyers, G., Monath, T., Muerhoff, S., Pletnev, A., Rico-Hesse, R., Smith, D.B., Stapleton, J.T., Ictv Report, C., 2017. ICTV virus taxonomy profile: flaviviridae. J. Gen. Virol. 98, 2–3.

Simone, M.I., Mares, L.J., Eveleens, C.A., McCluskey, A., Pappin, B.B., Kiefel, M.J., Houston, T.A., 2018. Back to (non-)Basics: an update on neutral and charge-balanced glycosidase inhibitors. Mini Rev. Med. Chem. 18, 812–827.

Simsek, E., Mehta, A., Zhou, T., Dwek, R.A., Block, T., 2005. Hepatitis B virus large and middle glycoproteins are degraded by a proteasome pathway in glucosidase-inhibited cells but not in cells with functional glucosidase enzyme. J. Virol. 79, 12914–12920.

Somnuke, P., Hauhart, R.E., Atkinson, J.P., Diamond, M.S., Avirutnan, P., 2011. N-linked glycosylation of dengue virus NS1 protein modulates secretion, cell-surface expression, hexamer stability, and interactions with human complement. Virology 413, 253–264.

Stadler, K., Allison, S.L., Schalich, J., Heinz, F.X., 1997. Proteolytic activation of tick-borne encephalitis virus by furin. J. Virol. 71, 8475–8481.

Stavale, E.J., Vu, H., Sampath, A., Ramstedt, U., Warfield, K.L., 2015. In vivo therapeutic protection against influenza A (H1N1) oseltamivir-sensitive and resistant viruses by the iminosugar UV-4. PLoS One 10, e0121662.

Steinmann, E., Penin, F., Kallis, S., Patel, A.H., Bartenschlager, R., Pietschmann, T., 2007. Hepatitis C virus p7 protein is crucial for assembly and release of infectious virions. PLoS Pathog. 3, e103.

Stiasny, K., Heinz, F.X., 2006. Flavivirus membrane fusion. J. Gen. Virol. 87, 2755–2766.

Takashita, E., Kawakami, C., Morita, H., Ogawa, R., Fujisaki, S., Shirakura, M., Miura, H., Nakamura, K., Kishida, N., Kuwahara, T., Mitamura, K., Abe, T., Ichikawa, M., Yamazaki, M., Watanabe, S., Odagiri, T., On Behalf Of The Influenza Virus Surveillance Group Of, J., 2019a. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. Euro Surveill. 24 (3), 1800698.

Takashita, E., Kawakami, C., Ogawa, R., Morita, H., Fujisaki, S., Shirakura, M., Miura, H., Nakamura, K., Kishida, N., Kuwahara, T., Ota, A., Togashi, H., Saito, A., Mitamura, K., Abe, T., Ichikawa, M., Yamazaki, M., Watanabe, S., Odagiri, T., 2019b. Influenza A(H3N2) virus exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019. Euro Surveill. 24 (12), 1900170.

Tam, D.T., Ngoc, T.V., Tien, N.T., Kieu, N.T., Thuy, T.T., Thanh, L.T., Tam, C.T., Truong, N.T., Dung, N.T., Qui, P.T., Hien, T.T., Farrar, J.J., Simmons, C.P., Wolbers, M., Wills, B.A., 2012. Effects of short-course oral corticosteroid therapy in early dengue infection in Vietnamese patients: a randomized, placebo-controlled trial. Clin. Infect. Dis. 55, 1216–1224.

Tricou, V., Minh, N.N., Van, T.P., Lee, S.J., Farrar, J., Wills, B., Tran, H.T., Simmons, C.P., 2010. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. PLoS Neglected Trop. Dis. 4, e785.

Vermehren, J., Park, J.S., Jacobson, I.M., Zeuzem, S., 2018. Challenges and perspectives of direct antivirals for the treatment of hepatitis C virus infection. J. Hepatol. 69 (5), 1178–1187.

Volkmar, N., Thezenas, M.L., Louie, S.M., Juszkiewicz, S., Nomura, D.K., Hegde, R.S., Kessler, B.M., Christianson, J.C., 2019. The ER membrane protein complex promotes biogenesis of sterol-related enzymes maintaining cholesterol homeostasis. J. Cell Sci. 132.

Vorndam, V., Mathews, J.H., Barrett, A.D., Roehrig, J.T., Trent, D.W., 1993. Molecular and biological characterization of a non-glycosylated isolate of St Louis encephalitis virus. J. Gen. Virol. 74 (Pt 12), 2653–2660.

Warfield, K.L., Plummer, E., Alonzi, D.S., Wolfe, G.W., Sampath, A., Nguyen, T., Butters, T.D., Enterlein, S.G., Stavale, E.J., Shresta, S., Ramstedt, U., 2015. A novel iminosugar UV-12 with activity against the diverse viruses influenza and dengue (novel iminosugar antiviral for influenza and dengue). Viruses 7, 2404–2427.

Warfield, K.L., Barnard, D.L., Enterlein, S.G., Smee, D.F., Khaliq, M., Sampath, A., Callahan, M.V., Ramstedt, U., Day, C.W., 2016a. The iminosugar UV-4 is a broad inhibitor of influenza A and B viruses ex vivo and in mice. Viruses 8, 71.

Warfield, K.L., Plummer, E.M., Sayce, A.C., Alonzi, D.S., Tang, W., Tyrrell, B.E., Hill, M. L., Caputo, A.T., Killingbeck, S.S., Beatty, P.R., Harris, E., Iwaki, R., Kinami, K., Ide, D., Kiappes, J.L., Kato, A., Buck, M.D., King, K., Eddy, W., Khaliq, M., Sampath, A., Treston, A.M., Dwek, R.A., Enterlein, S.G., Miller, J.L., Zitzmann, N., Ramstedt, U., Shresta, S., 2016b. Inhibition of endoplasmic reticulum glucosidases is required for in vitro and in vivo dengue antiviral activity by the iminosugar UV-4. Antivir. Res. 129, 93–98.

Warfield, K.L., Schaaf, K.R., DeWald, L.E., Spurgers, K.B., Wang, W., Stavale, E., Mendenhall, M., Shilts, M.H., Stockwell, T.B., Barnard, D.L., Ramstedt, U., Das, S.R., 2019. Lack of selective resistance of influenza A virus in presence of host-targeted antiviral, UV-4B. Sci. Rep. 9, 7484.

Watanabe, S., Rathore, A.P., Sung, C., Lu, F., Khoo, Y.M., Connolly, J., Low, J., Ooi, E.E., Lee, H.S., Vasudevan, S.G., 2012. Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial. Antivir. Res. 96, 32–35.

Watanabe, S., Chan, K.W., Dow, G., Ooi, E.E., Low, J.G., Vasudevan, S.G., 2016. Optimizing celgosivir therapy in mouse models of dengue virus infection of serotypes 1 and 2: the search for a window for potential therapeutic efficacy. Antivir. Res. 127, 10–19.

Watanabe, Y., Bowden, T.A., Wilson, I.A., Crispin, M., 2019. Exploitation of glycosylation in enveloped virus pathobiology. Biochim. Biophys. Acta Gen. Subj. 1863, 1480–1497.

Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C.K., Walther, P., Fuller, S.D., Antony, C., Krijnse-Locker, J., Bartenschlager, R., 2009. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. Cell Host Microbe 5, 365–375.

Whitby, K., Taylor, D., Patel, D., Ahmed, P., Tyms, A.S., 2004. Action of celgosivir (6 O-butanoyl castanospermine) against the pestivirus BVDV: implications for the treatment of hepatitis C. Antivir. Chem. Chemother. 15, 141–151.

Whitby, K., Pierson, T.C., Geiss, B., Lane, K., Engle, M., Zhou, Y., Doms, R.W., Diamond, M.S., 2005. Castanospermine, a potent inhibitor of dengue virus infection in vitro and in vivo. J. Virol. 79, 8698–8706.

Whitehorn, J., Nguyen, C.V.V., Khanh, L.P., Kien, D.T.H., Quyen, N.T.H., Tran, N.T.T., Hang, N.T., Truong, N.T., Hue Tai, L.T., Cam Huong, N.T., Nhon, V.T., Van Tram, T., Farrar, J., Wolbers, M., Simmons, C.P., Wills, B., 2016. Lovastatin for the treatment of adult patients with dengue: a randomized, double-blind, placebo-controlled trial. Clin. Infect. Dis. 62, 468–476.

Wichit, S., Jittmittraphap, A., Hidari, K.I., Thaisomboonsuk, B., Petmitr, S., Ubol, S., Aoki, C., Itonori, S., Morita, K., Suzuki, T., Suzuki, Y., Jampangern, W., 2011. Dengue virus type 2 recognizes the carbohydrate moiety of neutral glycosphingolipids in mammalian and mosquito cells. Microbiol. Immunol. 55, 135–140.

Winkler, G., Maxwell, S.E., Ruemmler, C., Stollar, V., 1989. Newly synthesized dengue-2 virus nonstructural protein NS1 is a soluble protein but becomes partially hydrophobic and membrane-associated after dimerization. Virology 171, 302–305.

Woodhouse, S.D., Smith, C., Michelet, M., Branza-Nichita, N., Hussey, M., Dwek, R.A., Zitzmann, N., 2008. Iminosugars in combination with interferon and ribavirin permanently eradicate noncytopathic bovine viral diarrhea virus from persistently infected cells. Antimicrob. Agents Chemother. 52, 1820–1828.

Wu, S.F., Lee, C.J., Liao, C.L., Dwek, R.A., Zitzmann, N., Lin, Y.L., 2002. Antiviral effects of an iminosugar derivative on flavivirus infections. J. Virol. 76, 3596–3604.

Wu, C.Y., Jan, J.T., Ma, S.H., Kuo, C.J., Juan, H.F., Cheng, Y.S., Hsu, H.H., Huang, H.C., Wu, D., Brik, A., Liang, F.S., Liu, R.S., Fang, J.M., Chen, S.T., Liang, P.H., Wong, C. H., 2004. Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc. Natl. Acad. Sci. U. S. A. 101, 10012–10017.

Yap, S.S.L., Nguyen-Khuong, T., Rudd, P.M., Alonso, S., 2017. Dengue virus glycosylation: what do we know? Front. Microbiol. 8, 1415.

Yoshida, E., Kunimoto, D., Lee, S.S., 2006. Results of a phase II dose ranging study of orally administered celgosivir as monotherapy in chronic hepatitis C genotype-1 patients. Gastroenterology 130, A784.

Yoshii, K., Yanagihara, N., Ishizuka, M., Sakai, M., Kariwa, H., 2013. N-linked glycan in tick-borne encephalitis virus envelope protein affects viral secretion in mammalian cells, but not in tick cells. J. Gen. Virol. 94, 2249–2258.

Yu, I.M., Zhang, W., Holdaway, H.A., Li, L., Kostyuchenko, V.A., Chipman, P.R., Kuhn, R. J., Rossmann, M.G., Chen, J., 2008a. Structure of the immature dengue virus at low pH primes proteolytic maturation. Science 319, 1834–1837.

Yu, L., Nomaguchi, M., Padmanabhan, R., Markoff, L., 2008b. Specific requirements for elements of the 5' and 3' terminal regions in flavivirus RNA synthesis and viral replication. Virology 374, 170–185.

Yu, I.M., Holdaway, H.A., Chipman, P.R., Kuhn, R.J., Rossmann, M.G., Chen, J., 2009. Association of the pr peptides with dengue virus at acidic pH blocks membrane fusion. J. Virol. 83, 12101–12107.

Yu, W., Gill, T., Wang, L., Du, Y., Ye, H., Qu, X., Guo, J.T., Cuconati, A., Zhao, K., Block, T.M., Xu, X., Chang, J., 2012. Design, synthesis, and biological evaluation of N-alkylated deoxynojirimycin (DNJ) derivatives for the treatment of dengue virus infection. J. Med. Chem. 55, 6061–6075.

Zai, J., Mei, L., Wang, C., Cao, S., Fu, Z.F., Chen, H., Song, Y., 2013. N-glycosylation of the premembrane protein of Japanese encephalitis virus is critical for folding of the envelope protein and assembly of virus-like particles. Acta Virol. 57, 27–33.

Zakaria, M.K., Carletti, T., Marcello, A., 2018. Cellular targets for the treatment of flavivirus infections. Front. Cell Infect. Microbiol. 8, 398.

Zhang, W., Chipman, P.R., Corver, J., Johnson, P.R., Zhang, Y., Mukhopadhyay, S., Baker, T.S., Strauss, J.H., Rossmann, M.G., Kuhn, R.J., 2003. Visualization of membrane protein domains by cryo-electron microscopy of dengue virus. Nat. Struct. Biol. 10, 907–912.

Zhang, G.B., Tian, L.Q., Li, Y.M., Liao, Y.F., Li, J., Bing, F.H., 2013. Protective effect of homonojirimycin from Commelina communis (dayflower) on influenza virus infection in mice. Phytomedicine 20, 964–968.

Zhang, R., Miner, J.J., Gorman, M.J., Rausch, K., Ramage, H., White, J.P., Zuiani, A., Zhang, P., Fernandez, E., Zhang, Q., Dowd, K.A., Pierson, T.C., Cherry, S., Diamond, M.S., 2016. A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. Nature 535, 164–168.

Zhang, X., Jia, R., Shen, H., Wang, M., Yin, Z., Cheng, A., 2017. Structures and functions of the envelope glycoprotein in flavivirus infections. Viruses 9.

Zhao, X., Guo, F., Comunale, M.A., Mehta, A., Sehgal, M., Jain, P., Cuconati, A., Lin, H., Block, T.M., Chang, J., Guo, J.T., 2015. Inhibition of endoplasmic reticulum-resident glucosidases impairs severe acute respiratory syndrome coronavirus and human coronavirus NL63 spike protein-mediated entry by altering the glycan processing of angiotensin I-converting enzyme 2. Antimicrob. Agents Chemother. 59, 206–216.

Zitzmann, N., Mehta, A.S., Carrouee, S., Butters, T.D., Platt, F.M., McCauley, J., Blumberg, B.S., Dwek, R.A., Block, T.M., 1999. Imino sugars inhibit the formation and secretion of bovine viral diarrhea virus, a pestivirus model of hepatitis C virus: implications for the development of broad spectrum anti-hepatitis virus agents. Proc. Natl. Acad. Sci. U. S. A. 96, 11878–11882.