

Review Article

Antivirals that target the host IMP α / β 1-virus interface

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Although transport into the nucleus mediated by the importin (IMP) α / β 1-heterodimer is central to viral infection, small molecule inhibitors of IMP α / β 1-dependent nuclear import have only been described and shown to have antiviral activity in the last decade. Their robust antiviral activity is due to the strong reliance of many different viruses, including RNA viruses such as human immunodeficiency virus-1 (HIV-1), dengue (DENV), and Zika (ZIKV), on the IMP α / β 1-virus interface. High-throughput compound screens have identified many agents that specifically target this interface. Of these, agents targeting IMP α / β 1 directly include the FDA-approved macrocyclic lactone ivermectin, which has documented broad-spectrum activity against a whole range of viruses, including HIV-1, DENV1–4, ZIKV, West Nile virus (WNV), Venezuelan equine encephalitis virus, chikungunya, and most recently, SARS-CoV-2 (COVID-19). Ivermectin has thus far been tested in Phase III human clinical trials for DENV, while there are currently close to 80 trials in progress worldwide for SARS-CoV-2; preliminary results for randomised clinical trials (RCTs) as well as observational/retrospective studies are consistent with ivermectin affording clinical benefit. Agents that target the viral component of the IMP α / β 1-virus interface include N-(4-hydroxyphenyl) retinamide (4-HPR), which specifically targets DENV/ZIKV/WNV non-structural protein 5 (NS5). 4-HPR has been shown to be a potent inhibitor of infection by DENV1–4, including in an antibody-dependent enhanced animal challenge model, as well as ZIKV, with Phase II clinical challenge trials planned. The results from rigorous RCTs will help determine the therapeutic potential of the IMP α / β 1-virus interface as a target for antiviral development.

Introduction

Compartmentalisation is a key feature of the eukaryotic cell, with transport between the different compartments essential for cellular function [1]. The double membrane of the nuclear envelope encircles and thereby defines the nuclear compartment, which sequesters the cell's genetic material, as well as the processes of RNA transcription and subsequent RNA maturation processes such as mRNA splicing. Protein translation occurs in the cytoplasmic compartment, so that specific signal-dependent transport into and out of the nucleus is necessary for the cell to function; i.e. mRNA needs to be exported to the cytoplasm for translation to occur, while all components of the protein machinery that mediate and/or regulate key nuclear processes such as transcription [2–4] and mRNA splicing/processing need to be imported into the nucleus.

The transport of proteins >45 kDa into and out of the nucleus is mediated by members of the importin (IMP) superfamily, of which there are multiple distinct α and β forms [5,6]. The best-characterised pathway by which host proteins enter the nucleus is that mediated by the IMP α / β 1 heterodimeric transport complex [7]. Host proteins dependent on IMP α / β 1 for nuclear import include those central to the antiviral response, such as members of the signal transducer and activators of transcription (STATs) and nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) transcription factor families.

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Many viruses exploit the host cell nuclear trafficking machinery, and in particular, the pathway mediated by the host IMP α / β 1 heterodimer, in order to enhance their ability to replicate or to subvert the host immune response [3]. Nuclear transport of particular viral proteins has been shown to be critically important for infection by flaviviruses (e.g. dengue virus — DENV or Zika virus — ZIKV; non-structural protein 5 — NS5), lentiviruses (e.g. human immunodeficiency virus — HIV-1; integrase — IN), alphaviruses (e.g. Venezuelan equine encephalitis virus — VEEV; capsid protein — CP), alphainfluenza viruses (Influenza A; nucleoprotein — NP), lyssaviruses (e.g. P1 and P3 forms of the Rabies virus — RV; phospho — P — protein), orthopneumoviruses (e.g. respiratory syncytial virus — RSV; matrix — M — protein), and others [3,4,8–13]. Over the last decade or so, studies using high-throughput screening (HTS) approaches targeting the host–virus interface (i.e. IMP α / β 1 recognition of viral proteins) have identified small molecular inhibitors [14–17], which target either the host IMP α / β 1 heterodimer, or the specific viral protein [18]. Many these are exciting prospects as antiviral drugs.

The host cell nuclear import pathway

To gain access to the nucleus, proteins generally require nuclear localisation signals (NLSs), which are recognised by either the IMP α subunit within the IMP α / β 1 heterodimer, or by one of the IMP β s directly [5,6,2,5]. The IMP: cargo complex is then translocated through the nuclear pore complex (NPC). Once within the nucleus, binding of Ran-GTP to IMP β dissociates the complex, freeing the protein cargo to perform its nuclear function [19,20]. This process is illustrated in Figure 1. As hinted at above, there are multiple distinct IMP α s; these can be grouped into three subfamilies, but all are highly homologous, and function in the same way within the IMP α / β 1 heterodimer to mediate nuclear import through a shared domain structure of ten α -helical armadillo (ARM) repeats involved in NLS recognition, and a flexible N-terminal importin- β -binding (IBB) domain (see Figure 2) [21]. X-ray crystallographic evidence indicates that NLS-binding is most commonly mediated by IMP α ARM repeats 2–4 (‘major NLS-binding site’) [22], with IMP α ARM repeats 6–8 (‘minor NLS-binding site’) [23] used to a lesser extent, as well as in combination with the major site in the case of certain NLSs. The IBB domain itself contains an NLS-like sequence, so that when the IMP α IBB domain is not bound to IMP β 1, this sequence is bound in the NLS-binding pocket [24], essentially ‘autoinhibiting’ IMP α to prevent binding to NLS-containing cargoes in the absence of IMP β 1 [20,21]. The distinct IMP α s are believed to have certain differences in NLS-binding specificity, as well as tissue distribution [3,21,25,26], potentially having more specialised roles in transporting particular import cargoes and not others [2]. STAT1, for example, has been reported to interact with IMP α 5 and not IMP α 1 [27], while the Ran nucleotide exchange factor RCC1 appears to be transported into the nucleus only by IMP α 3/ α 4 [28]. Consistent with this, certain viral proteins have been identified as being recognised only by specific IMP α s; these include Hendra and Nipah virus W protein [25,29] and avian virus Influenza A nucleoprotein (NP) which preferentially bind IMP α 3, whereas mammalian virus Influenza A NP binds IMP α 7 [30].

Viral proteins subvert the host nuclear import machinery

The host cell nuclear transport can be exploited by viral proteins that target nuclear processes to antagonise the host antiviral response. These include DENV NS5, which accesses the nucleus in order to impact many host nuclear processes (Figure 1); this leads to a range of outcomes such as delay of production of interleukin 8 (IL-8) that favour a host cell environment conducive to viral replication [8,31], effects on host cell spliceosome complexes to reduce the efficiency of pre-mRNA splicing of genes important for the antiviral response [32], and inhibition of recruitment of Paf1/RNA polymerase II complex component PAF1C to suppress interferon (IFN)-stimulated genes [33]. Other nuclear localising viral proteins that antagonise the host antiviral response, include RV P3-protein, in part through binding to STAT-1 and promyelocytic leukaemia tumour suppressor protein to impact IFN signalling [34], and RSV M, which modulates infected host cell transcriptional outcomes [3,35].

In the case of other proteins such as HIV-1 IN and influenza A NP, nuclear access is critical to the viral life cycle [4]. Dependent on IMP α / β 1 [36], for example, nuclear import of HIV-1 IN/the HIV-1 preintegration complex (PIC) is essential to enable the DNA form of the HIV-1 genome to be integrated into the host DNA to enable productive infection [4,9,36,37]. VEEV CP, in contrast, appears to bind to the host IMP α / β 1 heterodimer, as well as the IMP β -homologue nuclear export protein EXP1, to form a tetrameric complex that accumulates at the nuclear pore and blocks nuclear import of host proteins, thereby reducing the host IFN- α / β response [38,39]. In the case of SARS-CoV-1, the causative agent of severe acute respiratory syndrome (SARS),

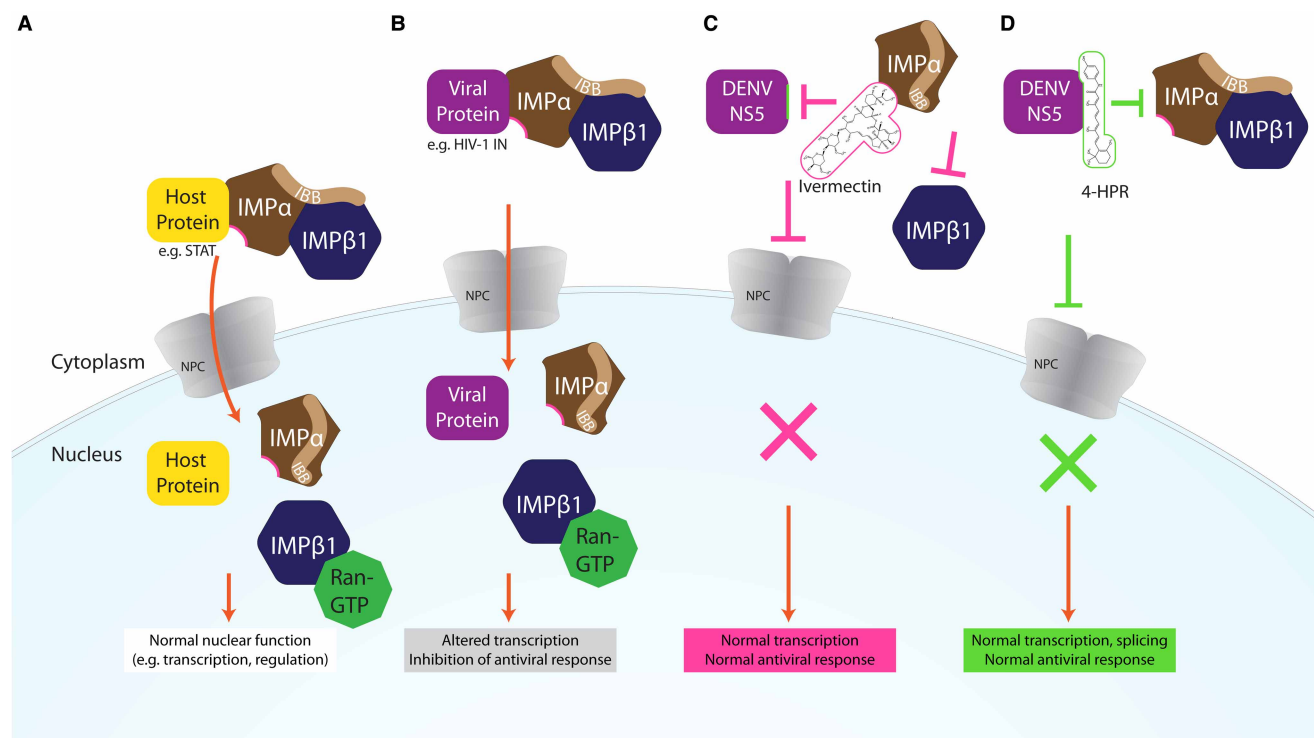


Figure 1. Schematic representation of the host IMP α / β 1-dependent nuclear import pathway (A), showing how it is co-opted by viral proteins in viral infection (B), and how small molecule antivirals can impact the pathway (C,D).

(A) Host proteins (e.g. transcription factor STAT-2) contain nuclear localisation signals which are recognised by IMP α (brown), after the autoinhibitory IMP β -binding (IBB) domain of IMP α binds IMP β 1, forming the IMP α / β heterodimer. This complex is then translocated across the nuclear pore complex (NPC), and the cargo is released after the binding of Ran-GTP to IMP β 1 dissociates the complex. The cargo can then carry out its normal nuclear function, such as transcriptional regulation of the antiviral response. (B) During viral infection, specific NLS-containing viral proteins (e.g. HIV-1 IN, purple) are imported into the nucleus by the same IMP α / β 1 dependent mechanism, where they can interfere with normal cellular functions, such as altering transcription to antagonise the antiviral response in order to maximise the rate of virus production. (C) Ivermectin (pink) binds IMP α , dissociating the IMP α / β 1 heterodimer and preventing binding to its viral (as well as host) protein target(s), thereby preventing its nuclear import and downstream transcriptional effects. (D) 4-HPR (green) specifically binds viral protein DENV2 NS5, preventing binding of the IMP α / β 1 and nuclear import, and associated downstream effects on transcription and splicing.

open reading frame (ORF) 6 has been shown to target host IMP α , and sequester it at the rough ER/Golgi, to prevent its key role in mediating STAT-1 nuclear import, thus mollifying the host cell antiviral response [40].

The critical importance of the IMP α / β 1-virus axis to viral infection has been formally demonstrated for many viruses, including DENV, where mutagenesis of the NS5 NLS nuclear targeting through impaired IMP α / β 1 recognition results in an attenuated virus and markedly reduced infectious virus production [8]. In addition, several structurally distinct small molecule inhibitors that inhibit NS5 nuclear import (see below), also reduce infectious virus production, underlining the critical importance of nuclear targeting of NS5 in DENV infection. Similar results have been obtained for HIV, where inhibiting the nuclear import of HIV-1 IN protein dramatically reduces HIV-1 infection [41,42], and for VEEV, where mutations within the CP NLS region result in attenuated nuclear accumulation, and are correlated with a non-pathogenic phenotype [39]. Finally, that nuclear trafficking of RV P-protein is critical to infection is implied by the observation that an attenuated non-lethal chicken embryo (CE) cell-adapted strain (Ni-CE) of the highly pathogenic RV Nishigahara (Ni) strain, harbours mutations that impact nucleocytoplasmic distribution [12].

Inhibitors targeting IMP α / β 1: ivermectin

HTS using chemical compound libraries and recombinant proteins has been used to identify many small molecule inhibitors targeting host IMP α / β (see Table 2). Ivermectin (22,23-dihydroavermectin B), the best-studied of these, is a macrocyclic lactone produced by the bacterium *Streptomyces avermitilis*, which was discovered in

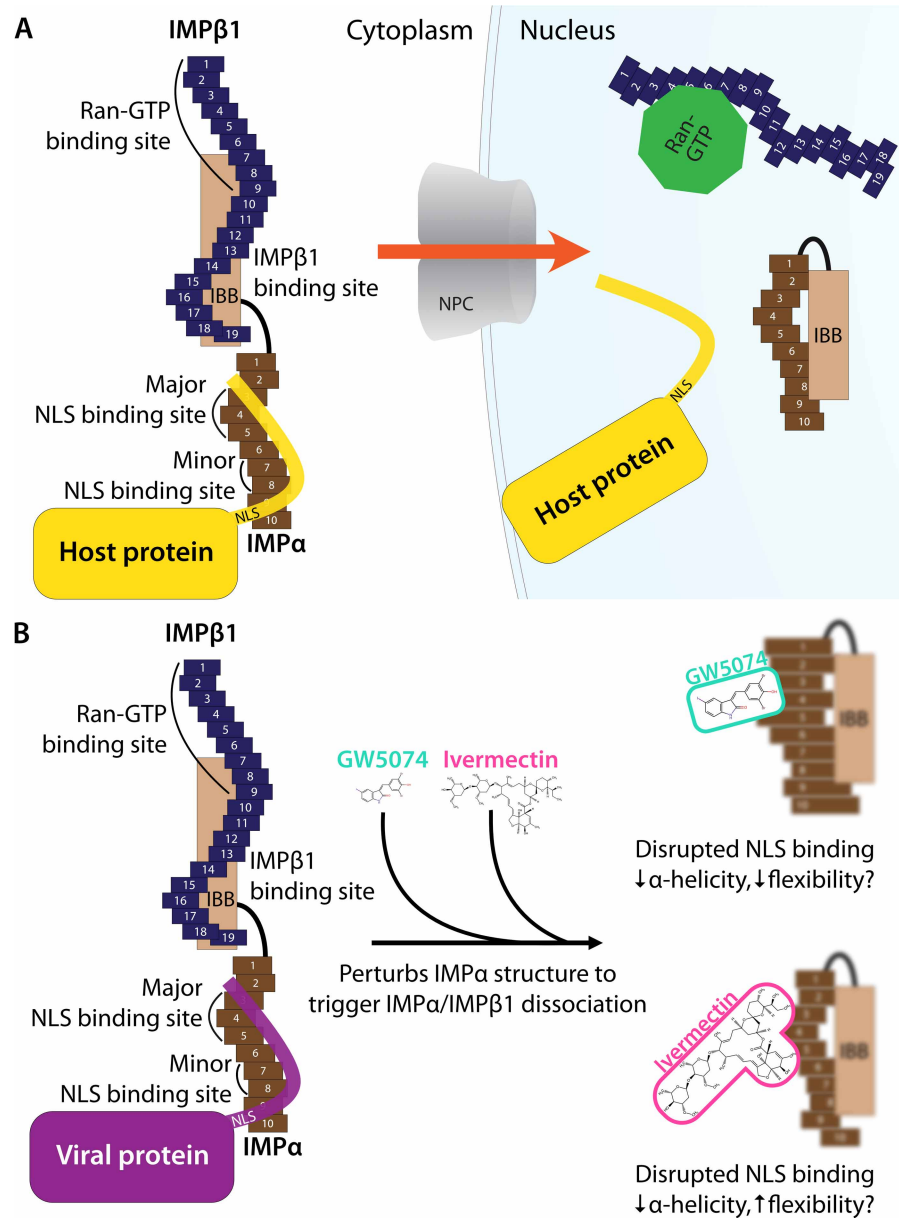


Figure 2. Schematic representation of IMP α and IMP β 1 structural/functional domains; impact of inhibitors.

(A) Schematic representation of the IMP α / β 1 complex based on the crystal structures of human IMP α and IMP β 1. IMP α comprises 10 armadillo (ARM) repeats and an IMP β -binding (IBB) domain. IMP α ARM repeats 2–4 form the major cargo NLS binding site, while ARM 7–8 form the minor NLS binding site. IMP β 1 comprises 19 HEAT repeats, where repeats 7–19 bind the IMP α IBB domain, and Ran-GTP binds to the N-terminus/repeat 8. In the cytoplasm, IMP β 1 (blue) binds IMP α (brown) via the IBB domain, exposing the major NLS binding site for cargo protein (yellow) binding. After transport into the nucleus across the NPC, Ran-GTP binds to IMP β 1, altering its conformation and displacing and releasing the IBB domain, which then binds the NLS binding sites in an autoinhibitory manner, preventing NLS binding. (B) Ivermectin and GW5074 bind directly to IMP α to perturb its structural integrity as confirmed by biophysical measurements including circular dichroism, thermostability analysis, and analytical ultracentrifugation [44,47], to reduce IMP α -helicity/impact flexibility, and thereby prevent IMP α binding to NLS-containing proteins or IMP β 1.

1975, and approved for use in humans as an antiparasitic agent 10 years later. It is US FDA-approved for treating a range of ectoparasitic and endoparasitic infestations, with an excellent safety profile and millions of people treated globally for onchocerciasis (river blindness) and lymphatic filariasis [43]. Ivermectin is one of

many agents listed on the WHO List of Essential Medicines, with the work identifying it/establishing its properties recognised by the Nobel Prize for Physiology or Medicine in 2015.

The first indication that ivermectin might have a role impacting viral protein nuclear import, and thereby potential as an antiviral, was afforded in 2011 in a chemical compound library HTS to identify inhibitors of binding of IMP α / β 1 to HIV-1 IN [14]. A nested counterscreening strategy confirmed ivermectin to be an agent likely directly interacting with IMP α / β 1 rather than IN to inhibit IMP α / β 1:IN binding (IC₅₀ of \sim 5 μ M) [14]. Consistent with this, ivermectin was found to inhibit nuclear accumulation of IN and the well-characterised IMP α / β 1-recognised nuclear import cargo SV40 large tumour antigen (T-Ag), but not of cargoes that are transported through IMP-dependent pathways that do not rely on IMP α , such as the IMP β 1-recognised telomere repeat factor-1 [14]. Ivermectin has since been shown to inhibit the binding of IMP α / β to a range of viral proteins, including DENV NS5 (see Figure 1), as well as host cell proteins (see Table 1).

Recent work has confirmed that the mechanism of action of ivermectin in this context is through direct binding to IMP α , resulting in structural changes that decrease α -helicity and likely impact flexibility, as implied by circular dichroism (CD)/thermostability (TSA) measurements (see Figure 2) [44]. These changes prevent interaction with NLS-containing viral/host protein cargoes, as well as IMP β 1; ivermectin can also dissociate preformed IMP α / β heterodimer, which is the form IMP α needs to be in to mediate nuclear import [44]. Thus, ivermectin prevents recognition of nuclear import cargoes by IMP α and the IMP α / β 1 (see Figure 1C), significantly reducing their nuclear accumulation and thereby diminishing suppression of the host antiviral response in infection. Inhibition of nuclear accumulation by ivermectin has been demonstrated for many different viral proteins [14,18] as well as host proteins such as NF- κ B p65 [45] in transfected and infected cell systems (see Table 1). Ivermectin's ability to inhibit the binding of IMP α to the viral proteins has also been shown in a cellular context using the technique of biomolecular fluorescence complementation [32].

The IMP α -dependent host-targeted mode of action of ivermectin explains the wide range of viruses for which ivermectin has demonstrated antiviral effects both *in vitro* and *in vivo* [18]. *In vitro* antiviral properties have been demonstrated for a wide range of viruses, including Betacoronavirus SARS-CoV-2 (COVID19), the lentivirus HIV-1, alphainfluenzavirus (influenza A), flaviviruses (DENV, WNV, ZIKV, yellow fever virus — YFV), alphaviruses (CHIKV, Sindbis, Semliki forest virus, VEEV), the Hendra henipavirus, mastadenoviruses (human adenovirus — HAdV — B and C), the BK betapolyomavirus and PSV varicellovirus (Table 1). In the case of DENV and ZIKV, ivermectin antiviral activity has been confirmed in an *ex vivo* human disease-relevant model of peripheral mononuclear blood monocytes (PBMCs) [15,44]. It is noteworthy that ivermectin was also independently identified by HTS for antiviral agents able to inhibit CHIKV replication using a luciferase reporter-carrying virus [46].

Other inhibitors targeting IMP α / β 1

Compound library HTS has enabled the identification of small molecule IMP α -targeting inhibitors other than ivermectin (see Table 1). GW5074 was identified as an inhibitor of IMP α / β 1-DENV NS5 binding [15,47]. Recent efforts to characterise the properties of GW5074 have revealed that, in analogous fashion to ivermectin, it binds to IMP α , eliciting changes in α -helicity/flexibility implicated by CD/TSA measurements (see Figure 2), that lead to dissociation of the IMP α / β 1 heterodimer/inhibition of NS5 nuclear import [47]. This is likely the basis of GW5074's ability to limit infectious virus production of DENV, ZIKV, and WNV [47]. Another host-targeted inhibitor, gossypol (GSP) discovered in the same HTS that identified ivermectin (see above) [14] has been shown to inhibit interaction of IMP α / β 1:Hendra virus (HENV) V protein [48]. GSP has also been shown to have antiviral effects against WNV [49].

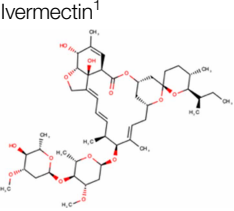
In silico structure-based-drug-design HTS has also been successfully applied to identify nuclear transport inhibitors [17]. *In silico* screening of 1.5 million compounds for the ability to dock into the NLS-binding site of IMP α and thereby mimic binding of the VEEV CP NLS to IMP α identified a range of different small molecules that were subsequently confirmed experimentally as being able to inhibit binding of IMP α / β 1 to VEEV CP. Several of the lead compounds, including 1111864 (see Table 1) were confirmed to inhibit CP nuclear accumulation, as well as VEEV replication in infected cells [17].

Nuclear import inhibitors targeting specific viral proteins

In addition to the host-targeted inhibitors discussed above, several inhibitors have been identified that specifically target the virus side of the IMP α / β 1-virus axis to prevent viral protein nuclear import (see Table 2). The steroid progesterone/glucocorticoid receptor antagonist mifepristone was the first such inhibitor to be identified in HTS,

Table 1. *In vitro* properties of IMP α inhibitors with antiviral effects

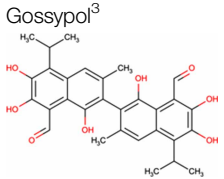
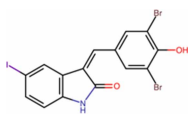
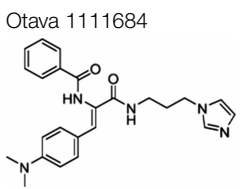
Part 1 of 2

Compound	Documented action in nuclear import (IC ₅₀)	Antiviral against	Effective concentration (assay)/fold reduction (assay)
 <p>Ivermectin¹</p>	<p>Inhibits interaction <i>in vitro</i> of IMPα with HIV IN [14], DENV2 NS5 (1 μM) [42,44], T-ag [15], Hendra V (15 μM) [48], IMPβ1 (7 μM) [44]</p> <p>Inhibits interaction of IMPα with T-ag and NS5 in a cell context as visualised by quantitative BiFc [44]</p> <p>Inhibits ColP from cell lysates of IMPα with T-ag, Adenovirus E1A [81]</p> <p>Inhibits nuclear accumulation in transfected cells of IMPα/β1- but not β1-recognised viral proteins such as T-ag [16,42], DENV2 NS5 [42], VEEV CP [16], PRV UL42 [60], HIV-1 IN [14,42], hCMV UL44 [42], Influenza A vRNPs [11] as well as host cargoes (e.g. [8,42,46])</p> <p>Reduces nuclear localisation in infected cells of DENV1-4 NS5 [82], VEEV CP [51] and adenovirus E1A [81]</p>	<p>Betacoronavirus SARS-CoV-2</p>	<p>EC₅₀ = 2.2/2.8 μM (qPCR/ released/ cell-associated virus) [59]</p> <p>5 μM > 5000-fold [59]</p>
		<p>HIV-1 (VSV-G-pseudotyped NL4-3.Luc.R-E-HIV)</p>	<p>50 μM > 2-fold (luciferase) [42]</p>
		<p>Influenza VLPs (avian influenza A/MxA escape mutants)</p>	<p>10 μM total inhibition (luciferase) [11]</p>
		<p>Flaviviruses YFV (17D)</p>	<p>EC₅₀ = 5/0.5 nM (CPE/qPCR) [83]</p> <p>3 μM > 50000-fold (PFU) [46]</p>
		<p>DENV1 (EDEN1)</p>	<p>EC₅₀ = 2.3/3.0 μM (CFI, 2 hosts) [82]</p>
		<p>DENV2 (NGC)</p>	<p>EC₅₀ = 0.7 μM (qPCR) [83]</p> <p>EC₅₀ = 3.3/0.4 μM (PFU, 2 hosts²) [44]</p> <p>EC₅₀ = 0.4/0.6 μM (pfu/qPCR) [44]</p> <p>EC₅₀ = 2.1/1.7 μM (CFI, 2 hosts) [82]</p> <p>50 μM total inhibition (PFU) [42]</p>
		<p>DENV2 (EDEN2)</p>	<p>EC₅₀ = 1.7 μM (CFI) [82]</p> <p>EC₅₀ = 1.9 μM (CFI) [82]</p>
		<p>DENV3 (EDEN3)</p>	<p>EC₅₀ = 4 μM (qPCR) [83]</p>
		<p>DENV4 (EDEN4)</p>	<p>EC₅₀ = 1/0.5 μM (PFU/qPCR) [44]</p>
		<p>WNV (NY99)</p>	<p>EC₅₀ = 1.6/1.3 μM (PFU, 2 hosts²) [44]</p>
		<p>WNV (MRM61C)</p>	<p>EC₅₀ = 1.9/0.6 μM (luciferase, 2 hosts) [46]</p> <p>3 μM > 5000-fold (PFU) [46]</p>
		<p>ZIKV (Asian/Cook Islands/2014)</p>	<p>3 μM > 200-fold (PFU) [46]</p>
		<p>Alphaviruses Chikungunya virus (CHIKV-Rluc)</p>	<p>1 μM c. 20-fold (PFU) [51]</p>
		<p>Sindbis (HR)</p>	<p>est. EC₅₀ = 2 μM (TCID/luciferase) [48]</p>
		<p>Semliki forest virus VEEV (TC83)</p>	<p>EC₅₀ = c. 2.5 μM; 10 μM 20-fold (qPCR) [81]</p>
<p>Hendra (Australia/ Horse/1994)</p>	<p>10 μM c. 8-fold (qPCR) [81]</p>		
<p>Adenovirus HAdV-C5</p>	<p>Est. EC₅₀ 1.5 μM (PFU/CPE/qPCR) [84]</p>		
<p>HAdV-B3</p>	<p>Est. EC₅₀ c. 0.8 μM 1000-fold [60]</p>		
<p>BK polyomavirus (BKPyV)</p>			
<p>Pseudorabies</p>			

Continued

Table 1. *In vitro* properties of IMP α inhibitors with antiviral effects

Part 2 of 2

Compound	Documented action in nuclear import (IC ₅₀)	Antiviral against	Effective concentration (assay)/fold reduction (assay)
 <p>Gossypol³</p>	Inhibits interaction <i>in vitro</i> of IMP α with Hendra Virus V (10 μ M) [48] Inhibits nuclear accumulation in WNV infected cells of NS5 [49]	WNV (MRM61C) Hendra (Australia/ Horse/1994)	10 μ M 100-fold (PFU) [49] 10 μ M 6-fold (TCID/luciferase) [48]
 <p>GW5074</p>	Inhibits interaction <i>in vitro</i> of IMP α with DENV2 NS5 (5 μ M) [47], Hendra (V 15 μ M) [48], IMP β 1 (10 μ M) [47] Inhibits nuclear accumulation in DENV2 infected cells of NS5 [47]	Flaviviruses DENV2 NGC ZIKV (Asian/Cook Islands/2014) WNV (MRM61C)	EC ₅₀ = 0.8/1.4 (PFU/PCR) [47] EC ₅₀ = 0.3/0.5 (PFU/PCR) [47] EC ₅₀ = 5.2/4.8 (PFU/PCR) [47]
 <p>Otava 1111684</p>	Inhibits interaction <i>in vitro</i> of IMP α with VEEV CP [17] Inhibits nuclear accumulation of VEEV CP-GFP in transfected cells [17] Reduces nuclear localisation in infected cells of VEEV CP [17] Reduces rate/extent of VEEV CP-GFP nuclear import (FRAP) [17]	Alphavirus VEEV (TC83)	EC ₅₀ = 9.9 μ M (lum) ⁴ [17] 10 μ M > 100-fold [17]

¹US Food and Drug Administration (FDA)-approved broad-spectrum antiparasitic agent, including against endoparasitic infestations (strongyloidiasis, onchocerciasis) and ectoparasites causing scabies, pediculosis and rosacea [66]; reported to inhibit helicase activity of DENV2/YFV/WNV NS3 in a FRET-based assay [83];

²Includes *ex vivo* model of human infection in PBMCs;

³Abandoned as human male contraceptive due to side effects (hypokalaemic paralysis, testicular damage) [85,86];

⁴Selectivity index of 3.7.

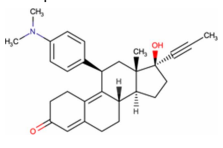
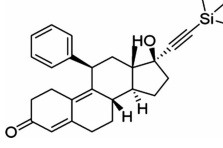
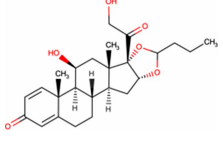
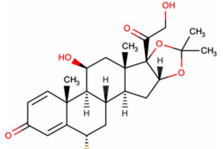
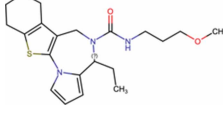
Abbreviations: ADE, antibody-dependent enhancement of infection; BiFc, bimolecular fluorescence complementation; DENV, Dengue virus; CFI, cell-based flavivirus infection assay (immunostaining for virus); CoIP, co-immunoprecipitation; CP, capsid protein; CPE, cytopathogenic effect (host cell); CHIKV, Chikungunya virus; EC₅₀, half-maximal effective concentration; Est., estimated; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; HAdV, human adenovirus; hCMV, human cytomegalovirus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IBB, importin β -binding domain; IC₅₀, half-maximal inhibitory concentration; IN, integrase; lum, luminescence assay; NS5, non-structural protein 5; PCR, polymerase chain reaction; PFU, plaque forming units (infectious virus); PRV, pseudorabies virus; RdRp, RNA-dependent RNA polymerase; SV40, simian virus 40; T-ag, SV40 large T-antigen; TCID, tissue culture infectious dose (estimation of viral load based on CPE); VEEV, Venezuelan equine encephalitis virus; VLP, virus-like particle; vRNP, viral ribonucleoprotein complex; VSV-G, vesicular stomatitis virus glycoprotein; WNV, West Nile virus; YFV, Yellow Fever virus; ZIKV, Zika virus.

through its ability to inhibit the interaction of recombinant IMP α / β 1 with HIV-1 IN [14]. Nested counterscreening was used to establish that its action was specific to IMP α / β 1: IN and not due to direct effects on IMP α / β 1 [14]), with more recent NMR studies confirming its direct binding to the IN core domain, with effects within the vicinity of key residues within the IN NLS [37]. Importantly, mifepristone was able to inhibit nuclear import of IN in an *in vitro* reconstituted nuclear import assay [37]. *In vitro* results had previously indicated strong antiviral activity against HIV-1 [50], with activity also documented against VEEV and HAdV [51–53]; since neither of these viruses possess an IN-homologue, the precise mechanism by which mifepristone impacts nuclear accumulation of CP in VEEV-infected cells [51] and of the HAdV genome in HAdV-infected cells [53] is unclear. A limitation on the clinical utility of mifepristone is the fact that it is abortifacient; medicinal chemistry and SAR analysis were able to be used to generate novel mifepristone analogues that lacked detectable progesterone receptor antagonism, but retained the ability both to inhibit VEEV CP nuclear accumulation and to limit VEEV infection [52], underlining how medicinal chemistry/SAR analysis can produce an inhibitor with improved properties.

The glucocorticoid budesonide was identified in the same HTS campaign as mifepristone (IC₅₀ of c. 1 μ M), subsequent characterisation using NMR confirming that budesonide also binds to the HIV-1 IN NLS region

Table 2. In vitro properties of viral protein cargo-targeted inhibitors with antiviral effects

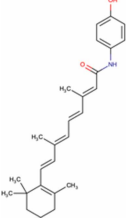
Part 1 of 2

Compound	Documented action (IC50)	Antiviral against	Effective concentration (assay, host)/fold reduction in virus production (assay, host cell line)
<p>Mifepristone¹</p> 	<p>Inhibits interaction <i>in vitro</i> of HIV IN with Impα/β (27 μM) [14] Binds to HIV IN core domain but not Impα/β (HSQC NMR) [37] 50 μM inhibits GFP-HIV-1 IN nuclear accumulation in transfected cells [14] 5 μM reduces HIV-1 IN nuclear accumulation > 5-fold in <i>in vitro</i> reconstituted nuclear transport assay [37] 5 μM reduces VEEV CP nuclear accumulation in infected cells [51] Reduces rate/extent of VEEV CP-GFP nuclear import (FRAP) [52] 50 μM reduces HAdV genome nuclear import (cellular fractionation/qPCR) [53]</p>	<p>HIV-1 VEEV Human adenovirus HAdV5</p>	<p>10 μM > 8-fold (EGFP reporter virus, CEMx174) [50] 10 μM >20-fold (ELISA, PBMC) [50] 200 μM > 3-fold (luciferase, HeLa) [42] EC50 = 19.9 μM (PFU, Vero) [52] 10 μM > 10-fold (PFU, U87MG) [51] 10 μM > 5-fold (PFU, Vero) [51] 10 μM > 15-fold (PFU, Vero) [52] EC50 = 2 μM (PFU) [53]</p>
<p>Mifepristone analogue 50²</p> 	<p>50 μM inhibits nuclear import of transfected GFP-CP (FRAP) Reduces recovery of VEEV CP-GFP nuclear fluorescence after FRAP [52] 50 μM inhibits nuclear accumulation of CP in infected cells [52]</p>	<p>VEEV</p>	<p>EC50 = 7.2 μM (luciferase, Vero) [52] 10 μM > 7-fold (PFU, Vero) [52]</p>
<p>Budesonide³</p> 	<p>Inhibits interaction <i>in vitro</i> of HIV IN with Impα/β (1.2 μM) [37] Binds to HIV IN core domain but not Impα/β (HSQC NMR) [37] 5 μM reduces HIV-1 IN nuclear accumulation > 2-fold in <i>in vitro</i> reconstituted nuclear transport assay [37] 100 μM inhibits HIV PIC nuclear import [37]</p>	<p>HIV-1</p>	<p>EC50 = 79.4 μM (luciferase, MT-2, single cycle) [37] EC50 = 49.4 μM (luciferase, TZMbl) [37]</p>
<p>Flunisolide⁴</p> 	<p>Inhibits interaction <i>in vitro</i> of HIV IN with Impα/β (1.4 μM) [37] Binds to HIV IN core domain but not Impα/β (HSQC NMR) [37] 250 μM inhibits HIV PIC nuclear import [37]</p>	<p>HIV-1</p>	<p>EC50 = 77.5 μM (luciferase, MT-2, single cycle) [37] EC50 = 105 μM (luciferase, TZMbl) [37]</p>
<p>G281-1564</p> 	<p>Inhibits interaction <i>in vitro</i> of VEEV CP and Impα/β (12 μM) [16] 50 μM inhibits extent and rate of GFP-CP nuclear accumulation in transfected cells (1.8-fold, 1.4-fold, respectively) [16]</p>	<p>VEEV</p>	<p>EC50 = 10.8 μM (luciferase, Vero)⁵ [16] 50 μM > 10-fold (PFU, Vero) [16]</p>

Continued

Table 2. In vitro properties of viral protein cargo-targeted inhibitors with antiviral effects

Part 2 of 2

Compound	Documented action (IC50)	Antiviral against	Effective concentration (assay, host)/fold reduction in virus production (assay, host cell line)
N-(4-hydroxyphenyl) retinamide/fenretinide/4-HPR ⁶ 	Inhibits interaction <i>in vitro</i> of DENV1 NS5:Imp α / β (2.3 μ M) [82]; DENV2 NS5:Imp α / β (0.8 μ M) [15]; DENV2 NS5:Imp α Δ IBB (1.1 μ M) [15]; ZIKV NS5:Imp α / β (1.1 μ M) [87] Inhibition of DENV2 NS5 nuclear accumulation in infected cells [15,47,77] Destabilises DENV 3 NS5 RdRp (thermostability assay) [44,47] 15 μ M inhibits nuclear accumulation of influenza vRNPs (IF) [11]	Flaviviruses	EC50 = 2.6 μ M (PFU, Huh-7) [15] EC50 (ADE ⁷) = 0.8 μ M (PFU, THP-1) [15] EC50 (ADE ⁷) = 0.8 μ M (PFU, PBMC) [15] EC50 = 2.1 μ M (PFU, Huh-7) [15] EC90 = 2.0 μ M (PFU, Vero) [77] EC50 = 1.4 μ M (PFU, Huh-7) [15] EC50 = 2.1 μ M (PFU, Huh-7) [15] EC50 = 2.6 μ M (PFU, Vero) [87] EC90 = 1 μ M (PFU, multiple hosts) [78] 10 μ M > 1000-fold (PFU, Vero) [78] 10 μ M > 7-fold (PFU, Vero) [15] 10 μ M > 100-fold (PFU, Vero) [77] 10 μ M > 10-fold (PFU, Vero) [49] 3 μ M > 100-fold (BHK-21, PFU) [46] EC50 = 0.5/3.1 μ M (PFU, multiple hosts) [46] 10 μ M > 100-fold (PFU, Vero) [77] 10 μ M > 4-fold inhibition (luciferase) [11]
		DENV1 (EDEN1)	
		DENV2 (EDEN2)	
		DENV2 (NGC)	
		DENV3 (EDEN3)	
		DENV4 (EDEN4)	
		ZIKV (Asian/Cook Islands/2014)	
		ZIKV (Asian/PF13-251013-1)	
		ZIKV (African/MR-766)	
		WNV (Kunjin)	
		YFV	
		Alphavirus CHIKV	
Modoc			
Influenza VLPs ⁸ (avian influenza A/MxA escape mutants)			

¹Approved for human use in medical termination of pregnancy, hyperglycaemia treatment in Cushing's syndrome patients;

²Analogue of mifepristone ((8S,13S,14S,17S)-17-hydroxy-13-methyl-11-phenyl-17-((trimethylsilyl) ethynyl)-1,2,6,7,8,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one) lacking progesterone receptor antagonism [52];

³Approved for asthma, chronic obstructive pulmonary disease, allergic rhinitis and nasal polyps, inflammatory bowel diseases;

⁴Approved for the treatment of asthma, allergic rhinitis;

⁵Selectivity index > 9.3;

⁶Progressed to clinical trials (incl. Phase III) for indications including breast, bladder and paediatric cancers, macular degeneration; other actions include increased phosphorylation of eIF2 α , promoting an antiviral state not related to long-chain ceramide biosynthesis, PERK pathway or ATF-4 [15,55,77];

⁷ADE model uses subneutralising concentrations of anti-E protein antibody prior to infection;

⁸Mechanism of action against influenza to be determined.

Abbreviations (see Table 1): ADE, antibody-dependent enhancement of infection; DENV, Dengue virus; CFI, cell-based flavivirus infection assay (immunostaining for virus); CoIP, co-immunoprecipitation; CP, capsid protein; CPE, cytopathogenic effect (host cell); CHIKV, Chikungunya virus; EC50, half-maximal effective concentration; Est., estimated; FRAP, fluorescence recovery after photobleaching; FRET, fluorescence resonance energy transfer; GFP, green fluorescent protein; HAAdV, human adenovirus; hCMV, human cytomegalovirus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSQ NMR, heteronuclear single quantum coherence nuclear magnetic resonance; IBB, importin β -binding domain; IC50, half-maximal inhibitory concentration; IF, immunofluorescence; IN, integrase; lum, luminescence assay; NS5, non-structural protein 5; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PIC, preintegration complex; PFU, plaque forming units (infectious virus); PRV, pseudorabies virus; RdRp, RNA-dependent RNA polymerase; SV40, Simian virus 40; T-ag, SV40 large T-antigen; TCID, tissue culture infectious dose (estimation of viral load based on CPE); VEEV, Venezuelan equine encephalitis virus; VLP, virus-like particle; vRNP, viral ribonucleoprotein complex; VSV-G, vesicular stomatitis virus glycoprotein; WNV, West Nile virus; YFV, Yellow Fever virus; ZIKV, Zika virus.

[37]. SAR/NMR analysis of closely related molecules showed that flunisolide also bound IN, but causes greater structural perturbation than budesonide, consistent with flunisolide being a more potent inhibitor of IN nuclear accumulation *in vitro* [37]. Importantly, both flunisolide and budesonide could be confirmed to inhibit nuclear import of the HIV PIC, as inferred from quantitative polymerase chain reaction analysis of unintegrated nuclear viral DNA [25]. Both budesonide and flunisolide also showed robust antiviral activity towards HIV-1, including in a single round replication assay (see Table 2).

From HTS of a > 14 000 compound library for inhibitors of IMP α / β 1:VEEV CP binding, followed by a nested counterscreen, many chemical scaffolds were identified that retained potent inhibitory activity [16]. Subsequent *in silico*-informed SAR analysis focussed on several of these, with compound G281–1564 confirmed to be a specific inhibitor of IMP α / β 1:CP interaction. Experiments in transfected cells confirmed inhibition of CP but not SV-40T-Ag nuclear accumulation, with fluorescence recovery after photobleaching experiments demonstrating inhibitory effects on both the rate and extent of CP nuclear import. Importantly, G281–1564 was found to limit VEEV infection in cell culture (EC₅₀ of 10.8 μ M) [16]; subsequent work indicated that G281–1564 prevents delays in cell cycle progression, which in part may depend on CP nuclear localisation/IMP α / β 1:CP binding [54].

One of the most promising inhibitors targeting the virus side of the IMP α / β 1-virus axis is N-(4-hydroxyphenyl) retinamide (4-HPR, a.k.a. fenretinide), which was identified through HTS as a specific inhibitor of IMP α / β 1:DENV NS5 interaction (IC₅₀ of 0.8 μ M) [15]. 4-HPR was shown to reduce the nuclear accumulation of DENV NS5 in infected cells, as well as strongly inhibiting viral replication of all four circulating DENV strains (DENV1–4) with EC₅₀ values between ~1–2 μ M, including in an *ex vivo* model of severe antibody-dependent enhanced (ADE) human infection using PBMCs [15]. 4-HPR also limits infection by other flaviviruses, including ZIKV (EC₅₀ of 1–2 μ M), WNV, and YFV (see Table 2). SAR analysis together with the use of other inhibitors confirmed 4-HPR's specificity, and that its mechanism of antiviral action is though inhibiting NS5 nuclear import, rather than other effects (e.g. on ceramide metabolism) [15]. TSA assays confirmed that 4-HPR destabilises DENV2 NS5 RNA-dependent RNA polymerase (RdRp) domain, and not IMP α [44], highlighting its specificity for the viral component of the host–virus interface.

It is of interest that 4-HPR has also been identified in HTS (see above) as an inhibitor of CHIKV [46], and reported to limit infection by the arbovirus Modoc and influenza A viruses. Like CHIKV, Modoc, and influenza virus do not have a correlate of flavivirus NS5 (well-conserved in DENV1–4, ZIKV, YFV, WNV, Japanese Encephalitis Virus) that localises in the nucleus of the infected host cell to impact transcription/splicing, so that 4-HPR's antiviral action in this case may be through other cellular pathways (ceramide biosynthesis/unfolded protein response) [47,55].

Pre-clinical/clinical studies for IMP α targeting inhibitors

Host-directed agents that impact essential cellular activities like nuclear transport must be used with caution in a clinical setting. Of the small molecule inhibitor drugs targeted against the host IMP α / β 1 side of the IMP α / β 1: virus interface, ivermectin is the only one that has progressed from to human clinical trials for viral indications. Although it has an established safety profile in humans [43,56], and is FDA-approved for many parasitic infections [56–58], ivermectin targets host nuclear import function that is unquestionably important in the antiviral response, with titration of a large proportion of the IMP α repertoire of a cell/tissue/organ is likely to lead to toxicity. Where a host-directed agent like ivermectin can optimally be used to treat viral infection may well be in the initial stages of infection or potentially prophylactically by keeping the viral load low so that the body's immune system has an opportunity to mount a full antiviral response [44,59].

Pre-clinical studies in a lethal mouse PRV challenge study showed that ivermectin administration (0.2 mg/kg) 12 h post-infection rescued 50% of mice, while the same dose at the time of infection rescued 60% of mice [60]. Results from a human Phase III clinical trial for the treatment of patients presenting with fever and confirmed DENV diagnosis showed that daily administration (0.4 mg/kg for 3 days) was safe and effective at improving virus clearance, but the authors concluded that dosing regimen modification was required to achieve the clinical benefit, potentially due to the timing of administration of the doses [61].

The current SARS-CoV-2 pandemic has already seen >67 million infections and >1.5 million deaths (nearing 300 000 in the US alone [62]) worldwide [63]. SARS-CoV-2 is a respiratory virus, so it is noteworthy that pharmacokinetic modelling, based on both the levels of ivermectin achievable in human serum from standard 0.2 mg/kg dosing and robust measurement in large animal experiments, indicates that concentrations of ivermectin 10 times higher than the c. 2.5 μ M EC₅₀ indicated by *in vitro* experiments (Table 1) are likely

achievable in the human lung [64]. Modelling based on different assumptions predicts lower values, but highlights the long-term stability of ivermectin in the lung (>30 days) [65]. Currently, almost 80 clinical trials are in progress globally for the treatment or prevention of SARS-CoV-2 [66–68], including variations on dosing regimens, combination therapies (see also [69–71]) and prophylactic protocols. Preliminary results from prophylactic study NCT04422561 of asymptomatic family close-contacts of confirmed COVID-19 patients indicate that two doses of ivermectin 72 h apart (0.25 mg/kg) are efficacious, resulting in an 87% relative risk reduction in the development of symptoms (58.4% of the untreated group vs 7.4% of the ivermectin-treated group).

Results from retrospective/observational trials for ivermectin [72–75] are also consistent with clinical benefit in the context of SARS-CoV-2 infection. Mymensingh Medical College Hospital (Bangladesh) reported that none of 115 subjects receiving a single 12 mg dose of ivermectin developed pneumonia/cardiovascular complications, compared with 9.8% (pneumonia) and 1.5% (ischemic stroke) in 133 control subjects [72]. Ivermectin-treated patients became SARS-CoV-2 negative faster (median 4 compared with 15 days), had shorter hospital stays (median 9 versus 15 days), and lower mortality (0.9 versus 6.8%). Importantly, significantly fewer ivermectin group patients developed respiratory distress (2.6 versus 15.8%), or required oxygen (9.6 versus 45.9%) or intensive care management (0.09 versus 8.3%). Comparable results were obtained in a 196 patient propensity-matched cohort study at Broward Health Medical Centre (Florida, U.S.A.) [73], with significantly lower mortality (13.3%) in subjects receiving ivermectin (0.2 mg/kg), compared with 24.5% mortality in those not receiving it, with more significant differences for patients with severe pulmonary involvement (mortality rates of 38.8 versus 80.7%). These early results are consistent with efficacy, but it is clear that only the results from large rigorous randomised clinical trials (RCTs) [66,67] will establish ivermectin's utility to treat or prevent SARS-CoV-2 infection.

Pre-clinical/clinical studies for viral protein targeting inhibitors

Of the small molecule inhibitors targeting the virus side of the IMP α / β 1:virus interface, only mifepristone has thus far been investigated clinically in any detail. Mifepristone was shown to reduce virus production and viral DNA copy number in HAdV-infected mice, concomitant with inhibition of HAdV genome nuclear import [53]. However, although a Phase I/II clinical trial of mifepristone (75–225 mg/day) in HIV patients indicated it was safe and well-tolerated, there was no significant effect on plasma HIV-1 RNA levels or CD4⁺ lymphocyte counts [76].

In the case of 4-HPR as a therapeutic for flavivirus infection, results are thus far restricted to mouse models. In AG129 mice, twice-daily dosage of 4-HPR at 180 mg/kg (resulting in ~15 μ M plasma concentration of 4-HPR), reduced viremia >50-fold in DENV2 infection [77]. In a lethal challenge model of ADE-DENV1 infection in AG129 mice, 20 mg/kg 4-HPR once-daily rescued 20% of mice, whilst twice-daily treatment rescued 70% of mice [15], concomitant with reduced inflammatory cytokine responses (tumour necrosis factor α , IL-6, -10, and -12) [15]. Efficacy was also seen in AG129 mice for ZIKV infection, where 4-HPR at 60 mg/kg twice-daily reduced viremia by ~10-fold [78]. Previous human Phase II/III trials for a variety of adult and paediatric solid tumour indications have demonstrated 4-HPR plasma trough concentrations exceeding 7 μ M (up to a peak of >50 μ M) are achievable with a tolerable safety profile [79,80], which compares favourably with EC50 values of 1–2 μ M for DENV in human PBMCs [15]. Based on these results as well as 4-HPR's established human safety and pharmacokinetic profiles in adults and children, human clinical trials are planned to investigate 4-HPR's exciting potential as a therapeutic and potentially prophylactic agent against DENV.

Future prospects

It is clear from the last decade that small molecules targeting the host IMP α / β 1:virus interface can be found that have potent antiviral activity, and exciting potential to treat human infection [66]. While host-targeted 'broad spectrum' inhibitors such as ivermectin can affect a range of viruses that share the need for the same nuclear import pathways as part of their lifecycle, potential toxicity must be considered, due to the essential nature of these import pathways for host cell function [4]. Virus-targeted inhibitors like 4-HPR, on the other hand, do not inhibit host protein nuclear import and hence are not toxic, but inevitably have a more restricted spectrum of antiviral activity as a result [66].

Conventional HTS (e.g. [14–16]) have proven effective at identifying promising lead compounds which are specific and effective at achievable concentrations, with *in silico* HTS emerging as a complementary approach to identify promising leads, and facilitate focussed library selection (e.g. [17,52]). Drugs such as ivermectin and 4-HPR are clearly exciting prospects, but the possibilities have by no means been yet exhausted in terms of

agents that target the host IMP α / β 1:virus axis, let alone other host IMP:virus interfaces. It is clear that further HTS and development of novel approaches to target these and other interfaces are likely to prove beneficial in the current landscape, in terms of identifying new possible agents to control existing and emerging viral pathogens.

Perspectives

- Nuclear import is essential to the infectious cycles of a range of viruses of medical significance. Inhibitors of nuclear import targeting either the host or virus side of the IMP α / β 1:virus interface have been identified in recent years, and shown to possess potent antiviral activity.
- Host-targeted inhibitor of nuclear import ivermectin is showing exciting early results as a treatment for COVID-19 infection. Virus-targeted inhibitor of nuclear import of flavivirus NS5, 4-HPR, appears to be an exciting prospect for future clinical trials.
- The process of nuclear import, and the IMP α / β 1:virus interface in particular, remains a viable target of interest for the future identification and development of antivirals to tackle existing and emerging viral disease.

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Competing Interests

D.A.J. is an inventor on a patent application for the use of ivermectin for coronavirus infection, and a patent on the use of 4-HPR in flavivirus infection. A.J.M. declares no competing interests.

Author Contributions

A.J.M. drafted the manuscript, D.A.J. provided overall direction, and helped revise the manuscript.

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Abbreviations

ADE, antibody-dependent enhanced; ARM, α -helical armadillo; CD, circular dichroism; CE, chicken embryo; DENV, dengue; HIV-1, human immunodeficiency virus-1; HTS, high-throughput screening; IBB, importin- β -binding; NLSs, nuclear localisation signals; NP, nucleoprotein; PBMCs, peripheral mononuclear blood monocytes; PIC, preintegration complex; RCTs, randomised clinical trials; SARS, severe acute respiratory syndrome; STATs, signal transducer and activators of transcription; TSA, thermostability; WNV, West Nile virus; ZIKV, Zika.

References

- 1 Aguzzi, A. and Altmeyer, M. (2016) Phase separation: linking cellular compartmentalization to disease. *Trends Cell Biol.* **26**, 547–558 <https://doi.org/10.1016/j.tcb.2016.03.004>
- 2 Poon, I.K.H. and Jans, D.A. (2005) Regulation of nuclear transport: central role in development and transformation? *Traffic (Copenhagen, Denmark)* **6**, 173–186 <https://doi.org/10.1111/j.1600-0854.2005.00268.x>
- 3 Fulcher, A.J. and Jans, D.A. (2011) Regulation of nucleocytoplasmic trafficking of viral proteins: an integral role in pathogenesis? *Biochim. Biophys. Acta* **1813**, 2176–2190 <https://doi.org/10.1016/j.bbamcr.2011.03.019>
- 4 Caly, L., Wagstaff, K.M. and Jans, D.A. (2012) Nuclear trafficking of proteins from RNA viruses: potential target for antivirals? *Antivir. Res.* **95**, 202–206 <https://doi.org/10.1016/j.antiviral.2012.06.008>

- 5 Fried, H. and Kutay, U. (2003) Nucleocytoplasmic transport: taking an inventory. *Cell Mol. Life Sci.* **60**, 1659–1688 <https://doi.org/10.1007/s00018-003-3070-3>
- 6 Terry, L.J., Shows, E.B. and Wenthe, S.R. (2007) Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science* **318**, 1412–1416 <https://doi.org/10.1126/science.1142204>
- 7 Smith, K.M., Antonio, V.D., Bellucci, L., Thomas, D.R., Caporuscio, F., Ciccarese, F. et al. (2018) Contribution of the residue at position 4 within classical nuclear localization signals to modulating interaction with importins and nuclear targeting. *Biochim. Biophys. Acta Mol. Cell Res.* **1865**, 1114–1129 <https://doi.org/10.1016/j.bbamcr.2018.05.006>
- 8 Pryor, M.J., Rawlinson, S.M., Butcher, R.E., Barton, C.L., Waterhouse, T.A., Vasudevan, S.G. et al. (2007) Nuclear localization of dengue virus nonstructural protein 5 through its importin α/β -recognized nuclear localization sequences is integral to viral infection. *Traffic (Copenhagen, Denmark)* **8**, 795–807 <https://doi.org/10.1111/j.1600-0854.2007.00579.x>
- 9 Piller, S., Caly, L. and Jans, D. (2003) Nuclear import of the pre-integration complex (PIC): the achilles heel of HIV? *Curr. Drug Targets* **4**, 409–429 <https://doi.org/10.2174/1389450033490984>
- 10 Rawlinson, S.M., Pryor, M.J., Wright, P.J. and Jans, D.A. (2006) Dengue virus RNA polymerase NS5: a potential therapeutic target? *Curr. Drug Targets* **7**, 1623–1638 <https://doi.org/10.2174/138945006779025383>
- 11 Götz, V., Magar, L., Dornfeld, D., Giese, S., Pohlmann, A., Höper, D. et al. (2016) Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Sci. Rep.* **6**, 23138–23115 <https://doi.org/10.1038/srep23138>
- 12 Ito, N., Moseley, G.W., Blondel, D., Shimizu, K., Rowe, C.L., Ito, Y. et al. (2010) Role of interferon antagonist activity of rabies virus phosphoprotein in viral pathogenicity. *J. Virol.* **84**, 6699–6710 <https://doi.org/10.1128/JVI.00011-10>
- 13 Wiltzer, L., Okada, K., Yamaoka, S., Larrous, F., Kuusisto, H.V., Sugiyama, M. et al. (2014) Interaction of rabies virus P-protein with STAT proteins is critical to lethal rabies disease. *J. Infect. Dis.* **209**, 1744–1753 <https://doi.org/10.1093/infdis/jit829>
- 14 Wagstaff, K.M., Rawlinson, S.M., Hearn, A.C. and Jans, D.A. (2011) An AlphaScreen®-based assay for high-throughput screening for specific inhibitors of nuclear import. *J. Biomol. Screen.* **16**, 192–200 <https://doi.org/10.1177/1087057110390360>
- 15 Fraser, J.E., Watanabe, S., Wang, C., Chan, W.K.K., Maher, B., Lopez-Denman, A. et al. (2014) A nuclear transport inhibitor that modulates the unfolded protein response and provides in vivo protection against lethal dengue virus infection. *J. Infect. Dis.* **210**, 1780–1791 <https://doi.org/10.1093/infdis/jiu319>
- 16 Thomas, D.R., Lundberg, L., Pinkham, C., Shechter, S., DeBono, A., Baell, J. et al. (2018) Identification of novel antivirals inhibiting recognition of Venezuelan equine encephalitis virus capsid protein by the importin α/β heterodimer through high-throughput screening. *Antivir. Res.* **151**, 8–19 <https://doi.org/10.1016/j.antiviral.2018.01.007>
- 17 Shechter, S., Thomas, D.R., Lundberg, L., Pinkham, C., Lin, S.-C., Wagstaff, K.M. et al. (2017) Novel inhibitors targeting Venezuelan equine encephalitis virus capsid protein identified using in silico structure-based drug design. *Sci. Rep.* **7**, 17705 <https://doi.org/10.1038/s41598-017-17672-9>
- 18 Jans, D.A., Martin, A.J. and Wagstaff, K.M. (2019) Inhibitors of nuclear transport. *Curr. Opin. Cell Biol.* **58**, 50–60 <https://doi.org/10.1016/j.ceb.2019.01.001>
- 19 Pemberton, L.F. and Paschal, B.M. (2005) Mechanisms of receptor-mediated nuclear import and nuclear export. *Traffic (Copenhagen, Denmark)* **6**, 187–198 <https://doi.org/10.1111/j.1600-0854.2005.00270.x>
- 20 Stewart, M. (2007) Molecular mechanism of the nuclear protein import cycle. *Nat. Rev. Mol. Cell Biol.* **8**, 195–208 <https://doi.org/10.1038/nrm2114>
- 21 Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T. and Adam, S.A. (2004) Importin α : a multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**, 505–514 <https://doi.org/10.1016/j.tcb.2004.07.016>
- 22 Fontes, M.R.M., Teh, T. and Kobe, B. (2000) Structural basis of recognition of monopartite and bipartite nuclear localization sequences by mammalian importin- α . *J. Mol. Biol.* **297**, 1183–1194 <https://doi.org/10.1006/jmbi.2000.3642>
- 23 Chen, M.-H., Ben-Efraim, I., Mitrousis, G., Walker-Kopp, N., Sims, P.J. and Cingolani, G. (2005) Phospholipid scramblase 1 contains a nonclassical nuclear localization signal with unique binding site in importin α . *J. Biol. Chem.* **280**, 10599–10606 <https://doi.org/10.1074/jbc.M413194200>
- 24 Kobe, B. (1999) Autoinhibition by an internal nuclear localization signal revealed by the crystal structure of mammalian importin α . *Nat. Struct. Biol.* **6**, 388–397 <https://doi.org/10.1038/7625>
- 25 Smith, K.M., Tsimbalyuk, S., Edwards, M.R., Cross, E.M., Batra, J., Costa, T. et al. (2018) Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses. *Nat. Commun.* **9**, 3703 <https://doi.org/10.1038/s41467-018-05928-5>
- 26 Holt, J.E., Ly-Huynh, J.D., Efthymiadis, A., Hime, G.R., Loveland, K.L. and Jans, D.A. (2007) Regulation of nuclear import during differentiation; the IMP α gene family and spermatogenesis. *Curr. Genom.* **8**, 323–334 <https://doi.org/10.2174/138920207782446151>
- 27 Sekimoto, T., Imamoto, N., Nakajima, K., Hirano, T. and Yoneda, Y. (1997) Extracellular signal-dependent nuclear import of Stat1 is mediated by nuclear pore-targeting complex formation with NPI-1, but not Rch1. *EMBO J.* **16**, 7067–7077 <https://doi.org/10.1093/emboj/16.23.7067>
- 28 Köhler, M., Speck, C., Christiansen, M., Bischoff, F.R., Prehn, S., Haller, H. et al. (1999) Evidence for distinct substrate specificities of importin α family members in nuclear protein import. *Mol. Cell Biol.* **19**, 7782–7791 <https://doi.org/10.1128/MCB.19.11.7782>
- 29 Ao, Z., Jayappa, K.D., Wang, B., Zheng, Y., Kung, S., Rassart, E. et al. (2010) Importin α 3 interacts with HIV-1 integrase and contributes to HIV-1 nuclear import and replication. *J. Virol.* **84**, 8650–8663 <https://doi.org/10.1128/JVI.00508-10>
- 30 Gabriel, G., Klingel, K., Otte, A., Thiele, S., Hudjetz, B., Arman-Kalcek, G. et al. (2011) Differential use of importin- α isoforms governs cell tropism and host adaptation of influenza virus. *Nat. Commun.* **2**, 156 <https://doi.org/10.1038/ncomms1158>
- 31 Rawlinson, S.M., Pryor, M.J., Wright, P.J. and Jans, D.A. (2009) CRM1-mediated nuclear export of dengue virus RNA polymerase NS5 modulates interleukin-8 induction and virus production. *J. Biol. Chem.* **284**, 15589–15597 <https://doi.org/10.1074/jbc.M808271200>
- 32 De Maio, F.A., Risso, G., Iglesias, N.G., Shah, P., Pozzi, B., Gebhard, L.G. et al. (2016) The dengue virus NS5 protein intrudes in the cellular spliceosome and modulates splicing. *PLoS Pathog.* **12**, e1005841 <https://doi.org/10.1371/journal.ppat.1005841>
- 33 Shah, P.S., Link, N., Jang, G.M., Sharp, P.P., Zhu, T., Swaney, D.L. et al. (2018) Comparative flavivirus-host protein interaction mapping reveals mechanisms of dengue and zika virus pathogenesis. *Cell* **175**, 1931–1945.e18 <https://doi.org/10.1016/j.cell.2018.11.028>
- 34 Chelbi-Alix, M.K., Vidy, A., Bougrini, J.E. and Blondel, D. (2006) Rabies viral mechanisms to escape the IFN system: the viral protein P interferes with IRF-3, Stat1, and PML nuclear bodies. *J. Interf. Cytokine Res.* **26**, 271–280 <https://doi.org/10.1089/jir.2006.26.271>

- 35 Hu, M., Bogoyevitch, M.A. and Jans, D.A. (2020) Impact of respiratory syncytial virus infection on host functions: implications for antiviral strategies. *Physiol. Rev.* **100**, 1527–1594 <https://doi.org/10.1152/physrev.00030.2019>
- 36 Anna C, H. and David A, J. (2006) HIV-1 integrase is capable of targeting DNA to the nucleus via an importin α/β -dependent mechanism. *Biochem. J.* **398**, 475–484 <https://doi.org/10.1042/BJ20060466>
- 37 Wagstaff, K.M., Headey, S., Telwatte, S., Tyssen, D., Hearn, A.C., Thomas, D.R. et al. (2018) Molecular dissection of an inhibitor targeting the HIV integrase dependent preintegration complex nuclear import. *Cell. Microbiol.* **59**, e12953 <https://doi.org/10.1111/cmi.12953>
- 38 Ryman, K.D. and Klimstra, W.B. (2008) Host responses to alphavirus infection. *Immunol. Rev.* **225**, 27–45 <https://doi.org/10.1111/j.1600-065X.2008.00670.x>
- 39 Atasheva, S., Fish, A., Fornerod, M. and Frolova, E.I. (2010) Venezuelan equine encephalitis virus capsid protein forms a tetrameric complex with CRM1 and importin α/β that obstructs nuclear pore complex function. *J. Virol.* **84**, 4158–4171 <https://doi.org/10.1128/JVI.02554-09>
- 40 Frieman, M., Yount, B., Heise, M., Kopecky-Bromberg, S.A., Palese, P. and Baric, R.S. (2007) Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. *J. Virol.* **81**, 9812–9824 <https://doi.org/10.1128/JVI.01012-07>
- 41 Bukrinsky, M.I., Sharova, N., McDonald, T.L., Pushkarskaya, T., Tarpley, W.G. and Stevenson, M. (1993) Association of integrase, matrix, and reverse transcriptase antigens of human immunodeficiency virus type 1 with viral nucleic acids following acute infection. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6125–6129 <https://doi.org/10.1073/pnas.90.13.6125>
- 42 Wagstaff, K.M., Sivakumaran, H., Heaton, S.M., Harrich, D. and Jans, D.A. (2012) Ivermectin is a specific inhibitor of importin α/β -mediated nuclear import able to inhibit replication of HIV-1 and dengue virus. *Biochem. J.* **443**, 851–856 <https://doi.org/10.1042/BJ20120150>
- 43 Laing, R., Gillan, V. and Devaney, E. (2017) Ivermectin – old drug, new tricks? *Trends Parasitol.* **33**, 463–472 <https://doi.org/10.1016/j.pt.2017.02.004>
- 44 Yang, S.N.Y., Atkinson, S.C., Wang, C., Lee, A., Bogoyevitch, M.A., Borg, N.A. et al. (2020) The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β heterodimer. *Antivir. Res.* **177**, 104760 <https://doi.org/10.1016/j.antiviral.2020.104760>
- 45 Ci, X., Li, H., Yu, Q., Zhang, X., Yu, L., Chen, N. et al. (2009) Avermectin exerts anti-inflammatory effect by downregulating the nuclear transcription factor kappa-B and mitogen-activated protein kinase activation pathway. *Fundam. Clin. Pharm.* **23**, 449–455 <https://doi.org/10.1111/j.1472-8206.2009.00684.x>
- 46 Varghese, F.S., Kaukinen, P., Gläsker, S., Bespalov, M., Hanski, L., Wennerberg, K. et al. (2016) Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. *Antivir. Res.* **126**, 117–124 <https://doi.org/10.1016/j.antiviral.2015.12.012>
- 47 Yang, S.N.Y., Atkinson, S.C., Fraser, J.E., Wang, C., Maher, B., Roman, N. et al. (2019) Novel flavivirus antiviral that targets the host nuclear transport importin α/β heterodimer. *Cells* **8**, 281 <https://doi.org/10.3390/cells8030281>
- 48 Atkinson, S.C., Audsley, M.D., Lieu, K.G., Marsh, G.A., Thomas, D.R., Heaton, S.M. et al. (2018) Recognition by host nuclear transport proteins drives disorder-to-order transition in Hendra virus V. *Sci. Rep.* **8**, 23 <https://doi.org/10.1038/s41598-017-18157-5>
- 49 Lopez-Denman, A.J., Russo, A., Wagstaff, K.M., White, P.A., Jans, D.A. and Mackenzie, J.M. (2018) Nucleocytoplasmic shuttling of the West Nile virus RNA-dependent RNA polymerase NS5 is critical to infection. *Cell. Microbiol.* **20**, e12848 <https://doi.org/10.1111/cmi.12848>
- 50 Schafer, E.A., Venkatachari, N.J. and Ayyavoo, V. (2006) Antiviral effects of mifepristone on human immunodeficiency virus type-1 (HIV-1): targeting Vpr and its cellular partner, the glucocorticoid receptor (GR). *Antivir. Res.* **72**, 224–232 <https://doi.org/10.1016/j.antiviral.2006.06.008>
- 51 Lundberg, L., Pinkham, C., Baer, A., Amaya, M., Narayanan, A., Wagstaff, K.M. et al. (2013) Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan equine encephalitis virus replication. *Antivir. Res.* **100**, 662–672 <https://doi.org/10.1016/j.antiviral.2013.10.004>
- 52 DeBono, A., Thomas, D.R., Lundberg, L., Pinkham, C., Cao, Y., Graham, J.D. et al. (2019) Novel RU486 (mifepristone) analogues with increased activity against Venezuelan equine encephalitis virus but reduced progesterone receptor antagonistic activity. *Sci. Rep.* **9**, 2634 <https://doi.org/10.1038/s41598-019-38671-y>
- 53 Marrugal-Lorenzo, J.A., Serna-Gallego, A., González-González, L., Buñuales, M., Poutou, J., Pachón, J. et al. (2018) Inhibition of adenovirus infection by mifepristone. *Antivir. Res.* **159**, 77–83 <https://doi.org/10.1016/j.antiviral.2018.09.011>
- 54 Lundberg, L., Fontenot, J., Lin, S.-C., Pinkham, C., Carey, B.D., Campbell, C.E. et al. (2018) Venezuelan equine encephalitis virus capsid implicated in infection-induced cell cycle delay in vitro. *Front. Microbiol.* **9**, 3126 <https://doi.org/10.3389/fmicb.2018.03126>
- 55 Fraser, J.E., Wang, C., Chan, W.K.K., Vasudevan, S.G. and Jans, D.A. (2016) Novel dengue virus inhibitor 4-HPR activates ATF4 independent of protein kinase R-like endoplasmic reticulum kinase and elevates levels of eIF2 α phosphorylation in virus infected cells. *Antivir. Res.* **130**, 1–6 <https://doi.org/10.1016/j.antiviral.2016.03.006>
- 56 Canga, A.G., Prieto, A.M.S., Liébana, M.J.D., Martínez, N.F., Vega, M.S. and Vieitez, J.J.G. (2008) The pharmacokinetics and interactions of ivermectin in humans—a mini-review. *AAPS J.* **10**, 42–46 <https://doi.org/10.1208/s12248-007-9000-9>
- 57 Crump, A. and Imura, S. (2011) Ivermectin, ‘Wonder drug’ from Japan: the human use perspective. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **87**, 13–28 <https://doi.org/10.2183/pjab.87.13>
- 58 World Health Organization. World Health Organization Model List of Essential Medicines 2019. Available from: <https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf>.
- 59 Caly, L., Druce, J.D., Catton, M.G., Jans, D.A. and Wagstaff, K.M. (2020) The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antivir. Res.* **178**, 104787 <https://doi.org/10.1016/j.antiviral.2020.104787>
- 60 Lv, C., Liu, W., Wang, B., Dang, R., Qiu, L., Ren, J. et al. (2018) Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus in vitro and vivo. *Antivir. Res.* **159**, 55–62 <https://doi.org/10.1016/j.antiviral.2018.09.010>
- 61 Yamasmith, E., Saleh-arong, F.A., Avirutnan, P., Angkasekwinai, N., Mairiang, D., Wongsawat, E. et al. Efficacy and Safety of Ivermectin against Dengue Infection: A Phase III, Randomized, Double-blind, Placebo-controlled Trial. The 34th Annual Meeting The Royal College of Physicians of Thailand. 2018
- 62 Centres for Disease Control and Prevention. CDC COVID Data Tracker 2020. Available from: https://covid.cdc.gov/covid-data-tracker/#cases_totalcases
- 63 Johns Hopkins University Coronavirus Resource Centre. COVID-19 Dashboard 2020. Available from: <https://coronavirus.jhu.edu/map.html>
- 64 Arshad, U., Pertinez, H., Box, H., Tatham, L., Rajoli, R.K.R., Curley, P. et al. (2020) Prioritization of anti-SARS-Cov-2 drug repurposing opportunities based on plasma and target site concentrations derived from their established human pharmacokinetics. *Clin. Pharmacol. Ther.* **108**, 775–790 <https://doi.org/10.1002/cpt.1909>

- 65 Schmith, V.D., Zhou, J. and Lohmer, L.R.L. (2020) The approved dose of ivermectin alone is not the ideal dose for the treatment of COVID-19. *Clin. Pharmacol. Ther.* **108**, 762–765 <https://doi.org/10.1002/cpt.1889>
- 66 Jans, D.A. and Wagstaff, K.M. (2020) Ivermectin as a broad-spectrum host-directed antiviral: the real deal? *Cells* **9**, 2100 <https://doi.org/10.3390/cells9092100>
- 67 Jans, D.A. and Wagstaff, K.M. (2020) The broad spectrum host-directed agent ivermectin as an antiviral for SARS-CoV-2? *Biochem. Biophys. Res. Commun.* In press <https://doi.org/10.1016/j.bbrc.2020.10.042>
- 68 Jans, D.A. and Wagstaff, K.M. Ivermectin as Broad-Spectrum Host-Directed Antiviral 2020. Available from: <https://encyclopedia.pub/2895>
- 69 Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y. et al. (2020) Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* **395**, 1569–1578 [https://doi.org/10.1016/S0140-6736\(20\)31022-9](https://doi.org/10.1016/S0140-6736(20)31022-9)
- 70 Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G. et al. (2020) A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *N. Engl. J. Med.* **382**, 1787–1799 <https://doi.org/10.1056/NEJMoa2001282>
- 71 Héctor, C., Roberto, H., Psaltis, A. and Veronica, C. (2020) Study of the efficacy and safety of topical ivermectin + iota-carrageenan in the prophylaxis against COVID-19 in health personnel. *J. Biomed. Res. Clin. Investig.* **2** <https://doi.org/10.31546/2633-8653.1007>
- 72 Khan, M.S.I., Khan, M.S.I., Debnath, C.R., Nath, P.N., Mahtab, M.A., Nabeka, H. et al. (2020) Ivermectin treatment may improve the prognosis of patients with COVID-19. *Archivos De Bronconeumología* **56**, 828–830 <https://doi.org/10.1016/j.arbres.2020.08.007>
- 73 Rajter, J.C., Sherman, M.S., Fattah, N., Vogel, F., Sacks, J. and Rajter, J.-J. (2020) Use of ivermectin is associated with lower mortality in hospitalized patients with COVID-19 (ICON study). *Chest* In press. <https://doi.org/10.1016/j.chest.2020.10.009>
- 74 Alam, M.T., Murshed, R., Bhiyuan, E., Saber, S., Alam, R.F. and Robin, R.C. (2020) A case series of 100 COVID-19 positive patients treated with combination of ivermectin and doxycycline. *J. Bangladesh Coll. Physicians Surg.* **13**, 10–15 <https://doi.org/10.3329/jbcps.v38i0.47512>
- 75 Rahman, M.A., Iqbal, S.A., Islam, M.A., Niaz, M.K., Hussain, T. and Siddiquee, T.H. (2020) Comparison of viral clearance between ivermectin with doxycycline and hydroxychloroquine with azithromycin in COVID-19 patients. *J. Bangladesh Coll. Physicians Surg.* **13**, 5–9 <https://doi.org/10.3329/jbcps.v38i0.47514>
- 76 Para, M.F., Schouten, J., Rosenkranz, S.L., Yu, S., Weiner, D., Tebas, P. et al. (2010) Phase I/II trial of the anti-HIV activity of mifepristone in HIV-infected subjects ACTG 5200. *J. Acquir. Immune Defic. Syndr.* **53**, 491–495 <https://doi.org/10.1097/QAI.0b013e3181d142cb>
- 77 Carocci, M., Hinshaw, S.M., Rodgers, M.A., Villareal, V.A., Burri, D.J., Pilankatta, R. et al. (2015) The bioactive lipid 4-hydroxyphenyl retinamide inhibits flavivirus replication. *Antimicrob. Agents Chemother.* **59**, 85–95 <https://doi.org/10.1128/AAC.04177-14>
- 78 Pitts, J.D., Li, P.-C., de Wispelaere, M. and Yang, P.L. (2017) Antiviral activity of N-(4-hydroxyphenyl) retinamide (4-HPR) against Zika virus. *Antivir. Res.* **147**, 124–130 <https://doi.org/10.1016/j.antiviral.2017.10.014>
- 79 Garaventa, A., Luksch, R., Lo Piccolo, M.S., Cavadini, E., Montaldo, P.G., Pizzitola, M.R. et al. (2003) Phase I trial and pharmacokinetics of fenretinide in children with neuroblastoma. *Clin. Cancer Res.* **9**, 2032–2039 PMID: 12796365
- 80 Cooper, J.P., Reynolds, C.P., Cho, H. and Kang, M.H. (2017) Clinical development of fenretinide as an antineoplastic drug: pharmacology perspectives. *Exp. Biol. Med. (Maywood, N.J)* **242**, 1178–1184 <https://doi.org/10.1177/1535370217706952>
- 81 King, C.R., Tessier, T.M., Dodge, M.J., Weinberg, J.B. and Mymryk, J.S. (2020) Inhibition of human adenovirus replication by the importin α / β 1 nuclear import inhibitor ivermectin. *J. Virol* **94**, e00710–20 <https://doi.org/10.1128/jvi.00710-20>
- 82 Tay, M.Y.F., Fraser, J.E., Chan, W.K.K., Moreland, N.J., Rathore, A.P., Wang, C. et al. (2013) Nuclear localization of dengue virus (DENV) 1–4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor ivermectin. *Antivir. Res.* **99**, 301–306 <https://doi.org/10.1016/j.antiviral.2013.06.002>
- 83 Mastrangelo, E., Pezzullo, M., Burghgraeve, T.D., Kaptein, S., Pastorino, B., Dallmeier, K. et al. (2012) Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J. Antimicrob. Chemother.* **67**, 1884–1894 <https://doi.org/10.1093/jac/dks147>
- 84 Bennett, S.M., Zhao, L., Bosard, C. and Imperiale, M.J. (2015) Role of a nuclear localization signal on the minor capsid proteins VP2 and VP3 in BKPvY nuclear entry. *Virology* **474**, 110–116 <https://doi.org/10.1016/j.virol.2014.10.013>
- 85 Porat, O. (1990) Effects of gossypol on the motility of mammalian sperm. *Mol. Reprod. Dev.* **25**, 400–408 <https://doi.org/10.1002/mrd.1080250414>
- 86 Yu, Z.H. and Chan, H.C. (1998) Gossypol as a male antifertility agent – why studies should have been continued. *Int. J. Androl.* **21**, 2–7 <https://doi.org/10.1046/j.1365-2605.1998.00091.x>
- 87 Wang, C., Yang, S.N.Y., Smith, K., Forwood, J.K. and Jans, D.A. (2017) Nuclear import inhibitor N-(4-hydroxyphenyl) retinamide targets Zika virus (ZIKV) nonstructural protein 5 to inhibit ZIKV infection. *Biochem. Biophys. Res. Commun.* **493**, 1555–1559 <https://doi.org/10.1016/j.bbrc.2017.10.016>