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In vitro comparison of currently available and investigational antiviral agents against pathogenic human double-stranded DNA viruses: A systematic literature review

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ABSTRACT

Background: Double-stranded (ds) DNA virus infections often occur concomitantly in immunocompromised patients. We performed a systematic search of published in vitro activity for nine approved and investigational antivirals to understand the spectrum of in vitro activity against dsDNA viruses.

Methods: A literature search was performed (PubMed and the WoS Core Collection) using keywords related to: 1) targeted approved/developmental antivirals (acyclovir, artesunate, brincidofovir, cidofovir, cyclopropavir (filociclovir), foscarnet, ganciclovir, letermovir, and maribavir); 2) pathogenic dsDNA viruses; 3) in vitro activity. We summarized data from 210 publications.

Results: Activity against ≤3 viruses was documented for maribavir (cytomegalovirus, Epstein-Barr virus), and letermovir, while activity against > 3 viruses was shown for ganciclovir, cidofovir, acyclovir, foscarnet, cyclopropavir, artesunate, and brincidofovir. The EC_{50} values of brincidofovir were the lowest, ranging from 0.001 to 0.27 μM, for all viruses except papillomaviruses. The next most potent agents included cidofovir, ganciclovir, foscarnet, and acyclovir with EC_{50} values between 0.1 μM and > 10 μM for cytomegalovirus, herpes simplex virus, and adenovirus.

Conclusion: Most of the identified antivirals had in vitro activity against more than one dsDNA virus. Brincidofovir and cidofovir have broad-spectrum activity, and brincidofovir has the lowest EC_{50} values. These findings could assist clinical practice and developmental research.

1. Introduction

Patients undergoing solid organ transplantation or allogeneic hematopoetic cell transplant (allo-HCT) are susceptible to viral infection, including reactivation of latent double-stranded DNA (dsDNA) viruses, due to immunosuppression required to avoid the rejection of the allograft and/or to prevent graft-versus-host disease. Allo-HCT patients are particularly at risk in the immediate post-transplant period before immune reconstitution, complicating clinical management and causing significant morbidity and mortality (Hiwarkar et al., 2018; Lion, 2014; Park et al., 2015). An added complication of dsDNA viral reactivations is that they can occur concomitantly in allo-HCT recipients (Ariza-Heredia et al., 2014; Hill et al., 2017; Huang et al., 2017). The most frequently detected dsDNA virus infections in plasma in the first 180 days after allo-HCT include cytomegalovirus (CMV) (44–65%) followed by BK virus (BKV) (54%), human herpesvirus-6 (HHV-6) (46–61%), adenovirus (AdV) (7–10%), and Epstein-Barr virus (EBV) (9–16%) (Hill et al., 2017; Huang et al., 2017). Detection of multiple viruses is common, with ≥2 viruses detected in 33–62% of allo-HCT recipients. The overall burden of dsDNA viruses is associated with increased mortality, indicating the unmet need for treatment options that provide coverage against multiple viruses in this patient population (Hill et al., 2017; Huang et al., 2017). Similarly, solid organ transplant recipients are at an increased risk of morbidity and mortality due to dsDNA infections (Beam and Razonable, 2012; Florescu et al., 2013; Leboeuf et al., 2017; Loginov et al., 2006; Pape et al., 2016; Razonable and Hayden, 2013). CMV is again one of the most common pathogens (Beam and Razonable, 2012; Razonable and Hayden, 2013), while BKV

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is particularly problematic after kidney transplantation (Leboeuf et al., 2017; Pape et al., 2016).

Although there are several approved antivirals with activity against one or more dsDNA viruses and others in development, their spectrum of antiviral activity and potency vary due to different mechanisms of action. As new agents are introduced into the armamentarium, understanding the gaps in coverage is important to ensure optimal use and/or novel surveillance strategies. Because most antivirals are usually approved for use against only one particular virus, labelled indications may not adequately describe the full spectrum of coverage for a particular agent, and although data on antiviral activity in vitro are available in the scientific literature, it is scattered across multiple publications and hence is not easily accessible to clinicians managing patients with these viral infections.

In order to ascertain the full spectrum of in vitro antiviral coverage against the dsDNA viruses, we undertook a systematic literature review to consolidate all available data into a single source, reporting antiviral activities of nine available or investigational therapeutic agents against major pathogenic human dsDNA viruses. This effort was supported by Chimerix Inc. who assisted by supporting a professional medical writer to undertake the literature search and assist in the drafting of the manuscript under direction of the authors.

2. Methods

2.1. Data sources

Data on in vitro antiviral activity were retrieved from articles published in English and obtained from searches of MEDLINE (US National Library of Medicine, Bethesda, MD, USA) and the Web of Science (WoS) Core Collection (Clarivate Analytics, Philadelphia, PA, USA). All searches were performed with no limit on date of publication.

2.2. Search terms

Three searches were performed to identify publications related to: 1) the dsDNA viruses; 2) the antivirals of interest (Table 1); and 3) in vitro activity based on half-maximal effective concentration (EC_{50}) data. The EC_{50} is the concentration at which inhibition of viral growth is 50% of the maximum response within the specified exposure time. The antivirals of interest include approved agents and new therapies in development. Publications on the following nine approved/developmental antivirals were identified: acyclovir, artesunate, brincidofovir, cidofovir, cyclopropavir or filociclovir, foscarnet, ganciclovir, letermovir, and maribavir. The list of keywords used in each Boolean search is detailed in Table 2. Searches were current as of October 2018.

2.3. Screening and selection

Titles and abstracts of the resulting reference list were screened; single case reports, data from non-human dsDNA viruses, and publications not focusing on at least one of the nine antivirals of interest were excluded.

2.4. Data extraction

For each antiviral compound, EC_{50} values reported in abstracts, main text, figures, or tables of selected publications were extracted. Data extraction was performed by one individual and independently verified by a second individual. A hierarchical approach to data extraction was employed whereby plaque reduction assay data, which is generally considered the 'gold standard' assay, was categorized as the top tier of evidence and was included if available. If this was not available, data from DNA-based assays (including but not limited to quantitative polymerase chain reaction [qPCR]), reporter gene assays, or immunoassays were included. Activity against HPV is presented as CC_{50} (the concentration resulting in 50% cytotoxicity) instead of an EC_{50} since cytotoxicity was used as the readout for the assay. Where values were reported in μg/ml in the original publication, these were converted to μM using molecular weights of 225.21 for acyclovir, 255.23 for ganciclovir, 279.187 for cidofovir, and 126.005 for foscarnet.

For the purposes of categorizing the in vitro activity data, an EC_{50} value of 10 μM was selected as a suitable threshold for determining the relative strength of the in vitro activity. For presentation purposes, data were grouped by herpesvirus family members and non-herpesviruses.

3. Results

The systematic review identified 3429 references (after the exclusion of 142 duplicate records). During the detailed review, 3129 references were excluded because a) they were single case reports, b) they detailed studies on non-human dsDNA viruses, c) they did not contain data on one of the antivirals of interest, d) they were excluded based on our hierarchical data extraction, or e) a combination of these factors (Fig. 1). The final list of references comprised 210 articles, found in 85 journals with publication dates from 1980 until 2018 (Fig. 1). In vitro activity data against the dsDNA viruses were extracted and compiled into two separate tables: human herpesviruses (Table 3) and other dsDNA viruses (Table 4). Individual EC_{50} values were plotted on scatter plots to visualize the range in activity for the individual antivirals (Fig. 2). Using a cut-off EC_{50} value of 10 μM, the antiviral activity of each compound against the dsDNA viruses included in the literature search was summarized and arranged

Table 1
List of antivirals with approved indications or in development.

Antiviral	Status	Indications	Reference
Acyclovir	Approved (US/EU)	Treatment and prophylaxis of mucocutaneous, ocular, and systemic HSV infections	ZOVIRAX® Prescribing Information
Ganciclovir	Approved (US/EU)	Treatment of CMV retinitis in immunocompromised patients. Prevention of CMV disease in adult transplant recipients.	CYTOVENE®—IV Prescribing Information
Foscarnet	Approved (US/EU)	Treatment of CMV retinitis in patients with AIDS and treatment of refractory HSV infections in immunocompromised patients	FOSCAVIR® Prescribing Information
Cidofovir	Approved (US/EU)	CMV retinitis	VISTIDE® Prescribing Information
Brincidofovir	In development	In development for AdV, smallpox	https://clinicaltrials.gov/ct2/show/NCT03339401
Letermovir	Approved (US/EU)	Prevention of CMV reactivation in CMV seropositive adult HCT recipients	PREVYMIS™ Prescribing Information
Maribavir	In development	In development for CMV	https://clinicaltrials.gov/ct2/show/NCT02931539
Cyclopropavir (filociclovir)	In development	In development for CMV	https://clinicaltrials.gov/ct2/show/NCT02454699
Artesunate	In development	Approved for treatment of malaria. In development for CMV.	(Dondorp et al., 2005, 2010; Sharma et al., 2014a; Wolf et al., 2011)

AdV, adenovirus; AIDS, acquired immune deficiency syndrome; CMV, cytomegalovirus; HCT, hematopoietic cell transplant; HSV, herpes simplex virus.

Table 2
Literature search terms.

Search	Keywords
1	Cidofovir OR brincidofovir OR CMX001 OR CMX-001 OR HDP-CDV OR hexadecyloxypropyl cidofovir OR acyclovir OR ganciclovir OR foscarnet OR maribavir OR letermovir OR 1263w94 OR AIC246 OR cyclopropavir OR filociclovir OR CMV423 OR methylenecyclopropane OR artesunate OR benzimidazole
2	Adenovirus OR herpes virus OR herpesvirus OR cytomegalovirus OR Epstein-Barr virus OR varicella zoster OR BK virus OR JC virus OR papillomavirus OR variola OR vaccinia OR dsDNA virus
3	IC50 OR EC50 OR inhibitory concentration OR inhibition OR inhibit OR inhibitors OR susceptibility OR susceptibilities OR in vitro efficacy
4	1 AND 2 AND 3

based on spectrum of activity from broadest spectrum to narrowest in the following order: brincidofovir > cidofovir > ganciclovir > artesunate > acyclovir > cyclopropavir > maribavir > letermovir > foscarnet (Fig. 2).

3.1. In vitro activity against the human herpesviruses

All nine antiviral compounds included in our search were reported to have in vitro activity against one or more herpesviruses (Table 3 and Fig. 3). Acyclovir, ganciclovir, foscarnet, cyclopropavir, cidofovir, and brincidofovir displayed activity against > 3 herpesviruses.

In particular, acyclovir had reported EC_{50} values for HSV-1 ranging from 0.11 to 18.6 μM, while values for HSV-2 ranged from 0.34 to 23 μM (Appendix A (Field et al., 2013; Leary et al., 2002; Sudo et al., 1994)). There was a single report of an EC_{50} value for acyclovir of 0.0025 μM against HSV-1 using a macrophage cell line (Brand et al., 2001). For ganciclovir, the lowest EC_{50} values were those reported for CMV (range: 0.04–37.2 μM), HSV-1 (range: 0.2–0.86 μM), HSV-2 (0.016–2.5 μM), and VZV (range: 0.52–1.3 μM) (Appendix B (Andrei et al., 2005; Andrei et al., 2000; Andrei et al., 1995; Hartline et al., 2005b; Hobden et al., 2011; Smee et al., 1983; Zhou et al., 2009)). For foscarnet, in vitro activity was reported for all herpesviruses, though EC_{50} values ranged widely with values for CMV ranging from 27.8 μM to 300 μM (Appendix C (Piret et al., 2016; Tatarowicz et al., 1991)). The lowest EC_{50} values for cidofovir were for HHV-8 (0.05–9.2 μM), CMV (0.26–9.5 μM), and VZV (0.5 μM) (Appendix D (Drew et al., 2006; Hartline et al., 2005b; Kern et al., 2005; Medveczky et al., 1997; Williams-Aziz et al., 2005)). For brincidofovir, potent activity was reported against all herpesviruses, with the lowest EC_{50} values for VZV (0.0004 μM) and the highest for HSV-1 (0.06 μM) (Appendix E (Hostetler, 2009; Lanier et al., 2010; Williams-Aziz et al., 2005)). Letermovir had potent activity only against CMV (0.0051 μM), with EC_{50} values > 10 μM reported for all other herpesviruses except HHV-8, for which no data were available (Appendix F (Marschall et al., 2012)). For maribavir, the lowest reported EC_{50} values were for EBV (0.15–1.1 μM), while the highest values were for HHV-6 (> 133 μM). Values for CMV ranged between 0.31 and 19.4 μM (Appendix G (Chou et al., 2007; Williams et al., 2003; Zacny et al., 1999)). Cyclopropavir had potent activity for CMV (0.36–1.9 μM), HHV-6 (1–7.8 μM), and HHV-8 (6.5 μM) with less potent activity reported for EBV (45 μM), HSV-1

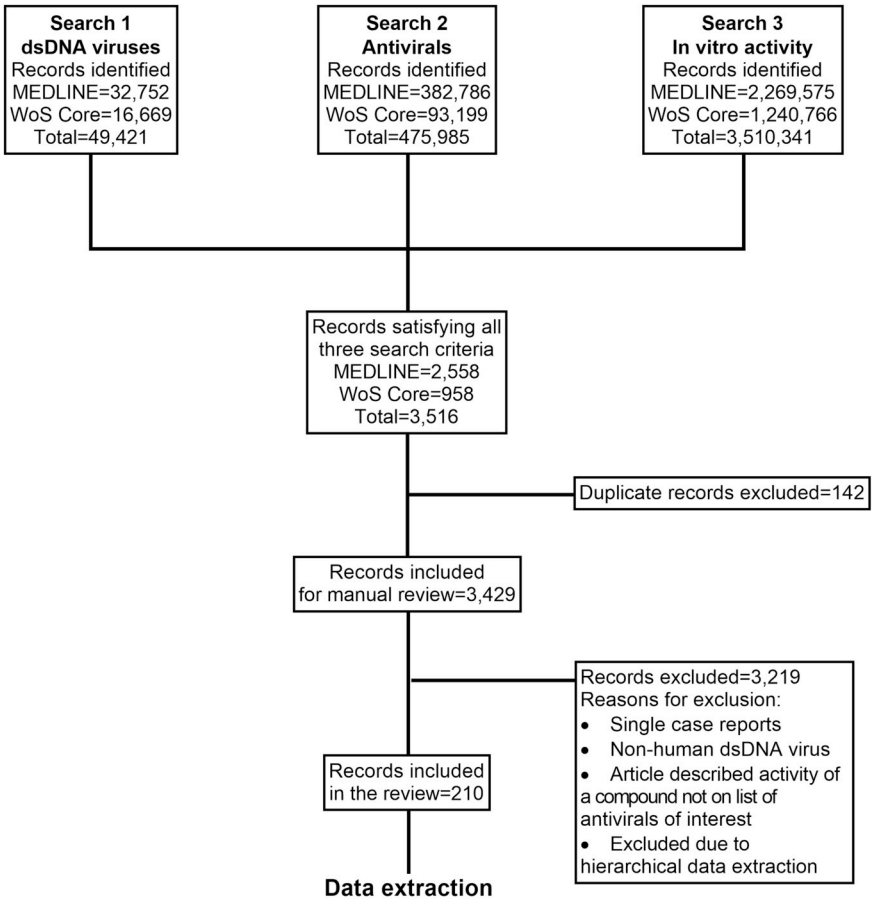

Fig. 1. Literature search summary. Footnote: dsDNA, double-stranded DNA.

Table 3
Antiviral activity based on EC_{50} values against human herpesviruses.

Virus (strain)	EC_{50} (μM)								
	Acyclovir	Ganciclovir	Foscarnet	Cidofovir	Brincidofovir	Letermovir	Maribavir	Cyclopropavir	Artesunate
	N = 72	N = 3	N = 22	N = 6	N = 8	N = 1	–	N = 2	–
HSV-1	**0.0025–10**	**0.2–0.86**	**5.8– > 793**	**0.7–5.7**	**0.009–0.06**	**> 10**	ND	**> 380–420**	ND
HSV-1 (KOS)	**0.11–2.9**		**50.3–253**						
HSV-1 (McIntyre)	**1.91**								
HSV-1 (McKrae)	**3.2**								
HSV-1 (SC16)	**0.15–18.6**		**26– > 793**						
	N = 36	N = 2	N = 12	N = 4	N = 8	N = 1	–	N = 1	–
HSV-2	**3.5–23**	0.016–2.5	**50.6–278**	**6.5–9.1**	**0.009–0.027**	**> 10**	ND	> 380	ND
HSV-2 (G)	**0.34–4.4**		**78.2**	**5.3**	**0.01–0.029**				
HSV-2 (SB5)	**0.75–23**		**174– > 793**						
HSV-2 (M)	**1.63–4.4**								
	N = 35	N = 3	N = 8	N = 1	N = 1	N = 1	–	N = 1	–
VZV			**28.3–130**				ND	> 380	ND
VZV (Oka)	**7**	**0.52–1.3**	**39.8–67**			> 10			
VZV (Ellen)	**3.6–16.4**			**0.5**	**0.0004**				
VZV (Kawaguchi)	**1.18–4.1**								
	N = 18	N = 8	N = 2	N = 8	N = 3	N = 1	N = 1	N = 1	N = 2
EBV	0.3– > 10	1.17–5.0	45.3–156	1.04– > 170	0.02–0.04	> 10	0.15–1.1	45	1.5–7.21
	N = 22	N = 55	N = 17	N = 19	N = 7	N = 1	N = 7	N = 8	N = 1
CMV (AD169)	**3.8–150**	**0.04–6.7**	**27.8–300**	**0.6–1.1**	**0.001**	**0.0051**	**0.54–19.4**	**0.36–1.4**	**3.7**
CMV (Coffman)	**5.5**	**5.5–15.3**		**1.9**	**0.001**			**1.9**	
CMV (Davis)	**3.4**	**0.6–5.9**		**0.5**	**0.001**			**1**	
CMV (Toledo)	**37.2**	**8.2–37.2**		**3.8–9.5**	**0.03**			**1.3**	
CMV (Towne)	**3.2–79**	**0.45–13.3**	**39–185**	**0.26–0.5**	**0.001**		**0.31**	**0.91**	
	N = 20	N = 22	N = 19	N = 4	N = 2	N = 1	N = 3	N = 2	N = 1
HHV-6			5.8–31			> 10			
HHV-6A	10–180	2.6–31.9	5.8–60	**5.7**–11.7	0.003		**> 125– > 133**	1	3.8
HHV-6B	119–185	5.2–68.6	0.7–98	1.4–1.6	0.007		**> 106**	6–7.8	
	N = 6	N = 6	N = 5	N = 5	N = 1	–	–	N = 1	–
HHV-8	31–138	0.4–23	6.5– > 449	0.05–9.2	0.02	ND	ND	6.5	ND
Source references	Appendix A	Appendix B	Appendix C	Appendix D	Appendix E	Appendix F	Appendix G	Appendix H	Appendix I

N = number of articles.
Bold text denotes data derived from plaque reduction assays. Non-bold text is derived from other assays, including DNA-based methods (qPCR and others), reporter gene assays, or immunofluorescence assays. CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; ND, no data; VZV, varicella zoster virus.

Table 4
Antiviral activity based on EC_{50} values against human non-herpesvirus dsDNA viruses.

Viral Family	Virus	EC_{50} (μM)								
		Acyclovir	Ganciclovir	Foscarnet	Cidofovir	Brincidofovir	Letermovir	Maribavir	Cyclopropavir	Artesunate
		N = 4	N = 3	–	N = 2	N = 2	N = 1	–	–	–
Adenovirus	AdV-2	> 500– > 1000	**5.4**				> 10	ND	ND	ND
	AdV-3				**2**	**0.01**				
	AdV-5				**0.5–6.2**	**< 0.009**				
	AdV-7	> 100			**1.3**	**0.02**				
	AdV-8				**1**	**0.03**				
	AdV-19		**7.2**							
	AdV-22		**4.5**							
	AdV-31				**1.4**	**0.28**				
		–	–	–	N = 1	N = 1	–	–	–	–
Papillomaviruses	HPV type 11*	ND	ND	ND	200	17	ND	ND	ND	ND
		–	–	–	N = 2	N = 4	–	–	–	N = 1
Polyomaviruses	BKV	ND	ND	ND	115	0.13–0.27	ND	ND	ND	4.2
		–	–	–	N = 1	N = 4	–	–	–	
	JCV	ND	ND	ND	> 0.1	0.006–0.1	ND	ND	ND	2.9
		N = 1	N = 1	–		N = 9	–	–	–	–
Poxviruses	VACV	**> 144**	**> 392**	ND	7.68–62	0.2–1.2	ND	ND	ND	ND
		–	–	–		N = 5	–	–	–	–
	VARV	ND	ND	ND	1.37–28.45	0.05–0.21	ND	ND	ND	ND
Source references		Appendix A	Appendix B	Appendix C	Appendix D	Appendix E	Appendix F	Appendix G	Appendix H	Appendix I

N = number of articles.
Bold text denotes data derived from plaque reduction assays. Non-bold text is derived from other assays, including DNA-based methods (qPCR and others), reporter gene assays, or immunofluorescence assays. AdV, adenovirus; BKV, BK virus; HPV, human papilloma virus; JCV, JC virus; ND, no data; VACV, vaccinia virus; VARV, variola virus. *The HPV assay is based on cytotoxicity against HPV-transformed cell lines, therefore values reported are CC_{50} rather than EC_{50} values.

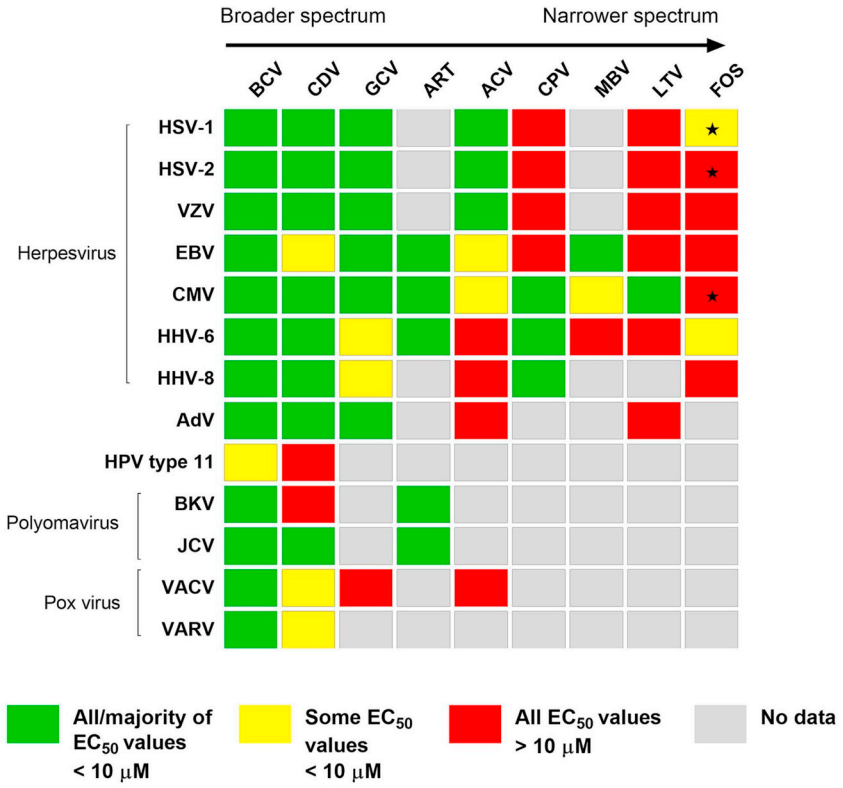

Fig. 2. Heat map of antivirals ordered by spectrum of activity (EC_{50} < 10 μM). Antivirals were ordered according to their spectrum of activity using an EC_{50} cut-off value of 10 μM. Footnote: Data for HHV-6A and HHV-6B are combined in the category HHV-6. See Table 3 for separate data for HHV-6A and HHV-6B. *Approved for treatment of CMV retinitis in patients with AIDS and treatment of refractory HSV infections in immunocompromised patients. **Antivirals:** ACV, acyclovir; ART, artesunate; BCV, brincidofovir, CDV, cidofovir; CPV, cyclopropavir; FOS, foscarnet; GCV, ganciclovir; LTV, letermovir; MBV, maribavir. **Viruses**: AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein Barr virus; HSV-1, herpes simplex virus-1; HSV-2, herpes simplex virus-2; HHV-6, human herpesvirus-6; HHV-8, human herpesvirus-8; HPV, human papilloma virus; JCV, JC virus; VACV, vaccinia virus; VZV, varicella zoster virus; VARV, variola virus. Note: The HPV assay is based on cytotoxicity against HPV-transformed cell lines, therefore values reported are CC_{50} rather than EC_{50} values. Source references are listed in the appendices.

(> 380–420 μM), HSV-2 (> 380 μM), and VZV (> 380 μM) (Appendix H (Gentry et al., 2013; Kern et al., 2005; Prichard and Whitley, 2014)). Reported EC_{50} values for artesunate were relatively low, with data available for CMV (3.7 μM), EBV (1.5–7.21 μM), and HHV-6A (3.8 μM), but no data were available for HHV-8, HSV-1, HSV-2, and VZV (Appendix I (Kaptein et al., 2006; Marschall et al., 2012; Milbradt et al., 2009)).

3.2. *In vitro activity against the non-herpesviruses*

For non-herpesviruses, in vitro data were relatively sparse in comparison with the findings for herpesviruses (Table 4 and Fig. 3). In vitro activity data were available for acyclovir against AdV (EC_{50} > 100 to > 1000 μM) and vaccinia virus (VACV) (EC_{50} > 144 μM) (Appendix A (Kern et al., 2002; Naesens et al., 2005; Wildner et al., 2003)). Similarly, ganciclovir had reported activity against AdV and VACV, with the most potent activity against AdV (5.4–7.2 μM) and less potent activity against VACV (EC_{50} > 392 μM) (Appendix B (Kern et al., 2002; Taylor et al., 1988)). For cidofovir, activity was reported against all the non-herpesviruses, with the lowest EC_{50} values for AdV (0.5–6.2 μM) (Appendix D (Gordon et al., 1996; Hartline et al., 2005a)). Similarly, brincidofovir had reported activity against all the non-herpesviruses, with EC_{50} values in the sub-μM range for all except HPV, with the lowest values for AdV (< 0.009–0.28 μM). Brincidofovir also had potent activity against BKV (0.13–0.27 μM), JCV (0.006–0.1 μM), VACV (0.2–1.2 μM), and variola virus (VARV) (0.05–0.21 μM) (Appendix E (Gosert et al., 2011; Hartline et al., 2005a; Hostetler, 2009; Jiang et al., 2010; Kern et al., 2002; Lanier et al., 2010; Olson et al., 2014)). There was a single report of letermovir activity against AdV (> 10 μM) (Appendix F (Marschall et al., 2012)), and reports of activity of artesunate against JCV (2.9 μM) and BKV (4.2 μM) (Appendix I (Sharma et al., 2014a; Sharma et al., 2014b)). There were no reports of activity against the non-herpesviruses for foscarnet, maribavir, and cyclopropavir.

4. Discussion

An understanding of the spectrum of antiviral activity is important for researchers and clinicians to aid decision-making, but in vitro antiviral activity data are currently scattered over hundreds of publications. Furthermore, although certain approved antivirals have activity against multiple dsDNA viruses, approved indications are often more narrow such that drug labels do not provide the necessary information. To consolidate available data into a useful reference source, we undertook a systematic literature search of published reports of in vitro activity against the major pathogenic dsDNA viruses for nine developmental or approved antivirals.

Our analyses highlight major differences in the breadth of antiviral activity for different agents, and these findings are important to understand for clinical application. Few of the antivirals in our search provide potent broad-spectrum activity against the full range of dsDNA viruses. Using an EC_{50} threshold of 10 μM, brincidofovir, cidofovir, ganciclovir, cyclopropavir, artesunate, and acyclovir had activity against three or more dsDNA viruses, with brincidofovir having activity against all the dsDNA virus species. At a more stringent threshold of 1 μM, brincidofovir, cidofovir, ganciclovir, and acyclovir had activity against three or more dsDNA viruses and brincidofovir had activity against all except HPV. Brincidofovir and cidofovir had the broadest spectrum of antiviral activity, with brincidofovir demonstrating higher in vitro potency (100–1000-fold greater) than cidofovir (Gosert et al., 2011; Hartline et al., 2005a; Hostetler, 2009; Jiang et al., 2010; Kern et al., 2002; Lanier et al., 2010; Olson et al., 2014; Williams-Aziz et al., 2005), with EC_{50} values in the sub-nM to μM range (0.0004–1.2 μM). Other antivirals either had a narrow spectrum of activity, limited data available, or had EC_{50} values ranging up to > 100 μM. However, it is important to note that in vitro activity does not necessarily translate into in vivo efficacy, as many factors play a role in determining clinical efficacy, such as pharmacokinetics, pharmacodynamics, and tolerability. A high EC_{50} may be overcome by dosing, as is the case for foscarnet—low in vitro activity is compensated by a high clinical dose

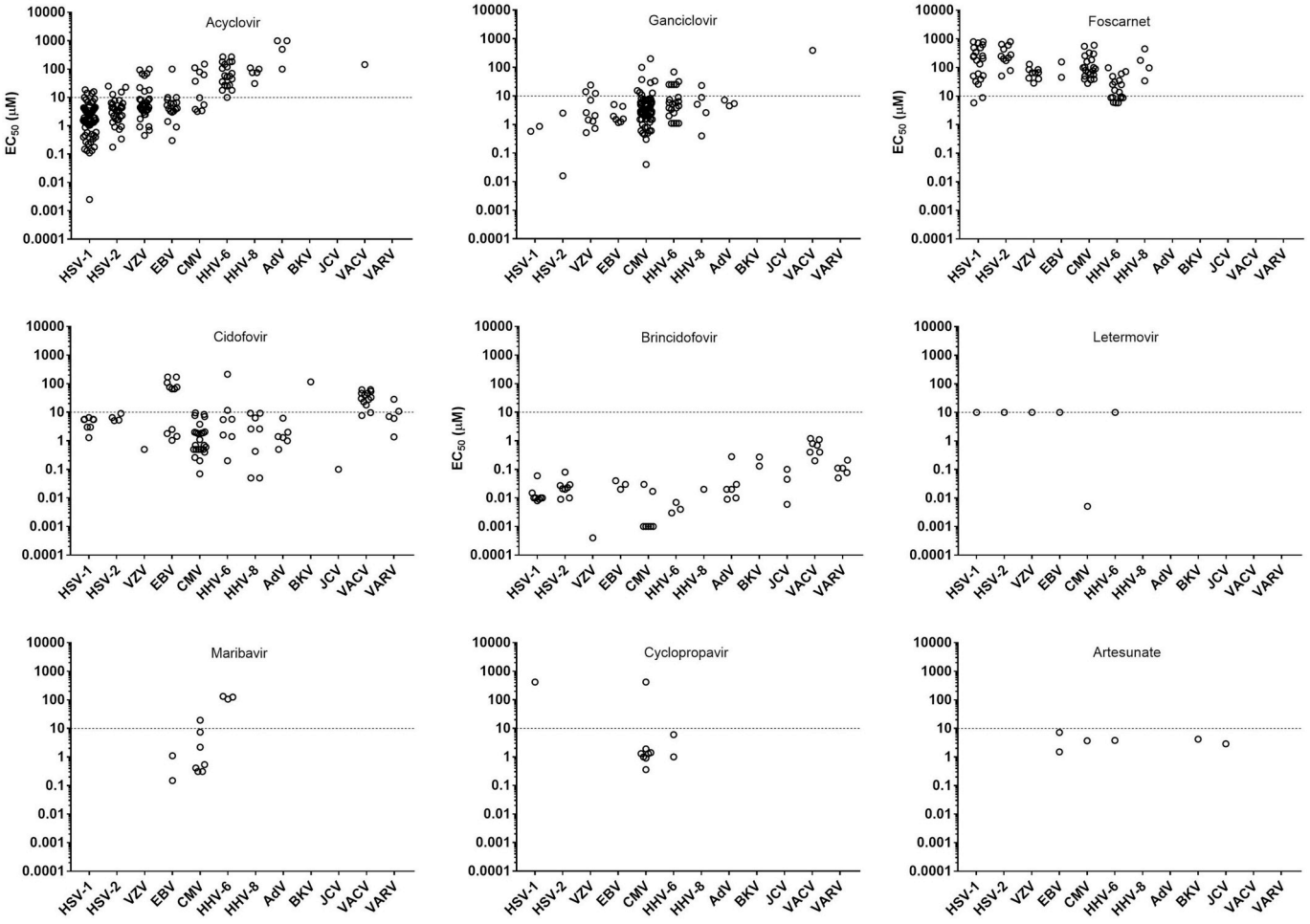

Fig. 3. Scatter plot of antiviral activity vs. dsDNA viruses. All data points are plotted as open circles; some data points overlap. Data reported for CMV, HSV-1, and HSV-2 are derived from plaque reduction assays for all reported antivirals, as were data for VZV using acyclovir, cidofovir, brincidofovir, letermovir, and foscarnet. Data for EBV and HHV-8 were derived from methods that include reporter gene assays and DNA-based methods (including qPCR), as were data for HHV-6 using brincidofovir and for HSV-2 using ganciclovir. AdV, adenovirus; BKV, BK virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV, human herpesvirus; HSV, herpes simplex virus; JCV, JC virus; qPCR, quantitative polymerase chain reaction; VACV, vaccinia virus; VARV, variola virus; VZV, varicella zoster virus. Footnote: Data for HHV-6A and HHV-6B are combined in the category HHV-6. See Table 3 for separate data for HHV-6A and HHV-6B.

(90 mg/kg IV BID), which leads to clear clinical efficacy for the treatment of CMV or HSV. There may also be differences in absorption or distribution into different body compartments such as the central nervous system. Metabolism and excretion or elimination are also important characteristics to consider. Nevertheless, in vitro activity is a good indication of the likely activity of any compound in vivo; a lack of in vitro activity, or a high EC_{50} (eg, > 10 μM) value is likely to translate into suboptimal efficacy in vivo.

Acyclovir was approved in 1982, and together with its prodrug valacyclovir remains the mainstay of therapy for HSV and VZV infections. Prophylaxis with acyclovir is standard practice for HSV or VZV seropositive transplant recipients, which has significantly decreased the incidence of disease caused by these viruses (Klysik et al., 2018 [Epub ahead of print]). Ganciclovir (and its prodrug valganciclovir) and foscarnet (despite its high EC_{50} against CMV in vitro) have been the primary agents used to treat or prevent CMV infections; because both drugs have activity against the other human herpesviruses, they provide coverage for HSV, VZV, and HHV-6 when they are employed for CMV prevention or preemptive therapy (Meesing and Razonable, 2018). This is not the case for some of the newer agents for treatment or prevention of CMV (Table 1). Letermovir has been recently licensed for prevention of CMV in allogeneic HCT recipients (Merck, 2017). It has a novel mechanism of action that prevents CMV egress. Because its molecular target is specific to CMV, it is not active against the other dsDNA viruses (Bowman et al., 2017; Verghese and Schleiss, 2013). Maribavir has activity against CMV and EBV and is currently being developed to treat CMV infection in allo-HCT patients and those with resistant/refractory infections (NCT02931539), but like letermovir, does not provide coverage for all the other prevalent human herpesviruses. Cyclopropavir is a developmental drug under investigation for the treatment of CMV infections. It is currently in early phase investigation as an oral formulation (NCT02454699; NCT01433835). Brincidofovir is currently in phase II trials for the treatment of adenovirus infection (NCT03339401), and is also in development for the treatment of smallpox.

The lack of coverage for HSV or VZV means that acyclovir is required to be co-administered to prevent disease due to these viruses in patients treated with letermovir, maribavir, or cyclopropavir, although a recent study suggests that brincidofovir may be worthy of further study for prophylaxis and/or pre-emptive treatment of HSV and VZV in allo-HCT recipients (Lee et al., 2018). It is also possible that HHV-6 related diseases could become more common if ganciclovir and foscarnet are replaced by CMV-specific agents without HHV-6 activity. Although there are no approved treatments for HHV-6 and HHV-8, ganciclovir, foscarnet and cidofovir, and the investigational agent brincidofovir, have activity against these viruses (Table 3 and Fig. 3) (Coen et al., 2014; Prichard and Whitley, 2014). Both ganciclovir and foscarnet have been investigated as prophylaxis or preemptive therapy

for HHV-6 infection in high-risk allo-HCT recipients; however, these studies have not shown an improvement in outcomes (Ishiyama et al., 2011, 2012; Ogata et al., 2008, 2013, 2018). The authors of this study speculated that this may be due to failure of these drugs to penetrate the cerebrospinal fluid to therapeutically relevant levels. On the other hand, the lipid conjugate brincidofovir may penetrate the cerebrospinal fluid more efficiently (Tippin et al., 2016) which potentially complements its very low EC_{50} in vitro (Table 3, Fig. 3).

There are no approved treatments for diseases caused by AdV, BKV, or JCV in the US or EU. Most of these infections are generally managed clinically with cidofovir, despite known kidney and hematologic toxicity (Gilead, 2010). Artesunate is an intriguing agent, which has been licensed to treat malaria, but has also been investigated to treat CMV (with mixed results) and been reported to have activity against HHV-6, BKV, and JCV (Sharma et al., 2014a, 2014b). There are no licensed antiviral options for therapeutic treatment of HPV, though topical cidofovir has been used for patients with severe HPV infections (Stier et al., 2013).

A major limitation of our review is that there is no consensus methodology for assessing antiviral activity across different dsDNA viruses. Consequently, EC_{50} values can vary greatly between publications. Although the plaque reduction assay is considered the 'gold standard' for assessing antiviral activity, it is not applicable for all viruses and it can be employed with a variety of modifications that may affect the results obtained. For example, the characteristics of the cell line such as the passage number or growth characteristics can influence the findings, as can the passage number of the virus used in the assay, and/or whether a clinical isolate or a laboratory strain was used. Other factors such as the method of preparation or isolation of the virus can also influence assay results (Tille, 2013). There are also differences in how individual laboratories score and characterize plaques based on plaque size. Despite these differences, the plaque reduction assay remains the most accepted assay for assessing in vitro antiviral activity, and our data extraction strategy was designed to identify and report these data where available. Where plaque reduction assay data were not available, data from other assay techniques such as reporter gene assays, DNA-based techniques such as qPCR, or immunofluorescence assays were reported. In these assays, factors such as multiplicity of infection (infectious virus units/cell) or growth phase of the cells could have an impact on the assay results. Nevertheless, by including data from these assays where plaque reduction assay data are not available, our systematic review presents a comprehensive overview of the available literature. An additional limitation is that the compounds used in the publications included in our literature search came from a variety of sources and may have different levels of purity and activity.

The absence of standardized methodology for assessing antiviral activity has been addressed by two recent articles that describe the development of a robust in vitro testing platform using an automated 384-well format. The readout was based either on qPCR or cytopathic effect; these methods were used to assess the activity of a panel of antivirals against polyomaviruses, herpesviruses, orthopoxviruses, and adenovirus (Hartline et al., 2018; Keith et al., 2018). Their investigation demonstrated consistent and robust results that were in accordance with data from previously published work, including the activity data described in our systematic review. It should be pointed out that data presented in this review is generated by several investigators using different assay methods, including data generated from plaque reduction assays. This raises the possibility of improvements in the capacity to assess antiviral activity of existing agents in the future or the identification of new agents with potential antiviral activity.

Despite the limitations inherent in comparing data across many publications with a range of antiviral assays, this systematic review represents the first comprehensive resource gathering the most current information on the overall in vitro antiviral activity of available and developmental antivirals against major pathogenic dsDNA viruses. We found that the spectrum of antiviral activity ranged from broadest to narrowest, based on a cut-off value of 10 μM, as follows: brincidofovir > cidofovir > ganciclovir > artesunate > acyclovir > cyclopropavir > maribavir > letermovir > foscarnet (Fig. 3). While in vitro values may not necessarily translate into clinical efficacy, we believe that this review is a useful resource for clinicians and researchers seeking a consolidated description of in vitro antiviral activity against the dsDNA viruses, particularly those managing immunocompromised patients who are at the greatest risk of disease from these viruses.

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Glossary

AdV	adenovirus
allo-HCT	allogeneic hematopoietic cell transplant
BKV	BK polyomavirus
CMV	human cytomegalovirus
ds	double-stranded
EBV	Epstein-Barr virus
HHV	human herpesvirus
HIV	human immunodeficiency virus
HPV	human papillomavirus
HSV	herpes simplex virus
JCV	JC polyomavirus
qPCR	quantitative polymerase chain reaction
VACV	vaccinia virus
VARV	variola virus
VZV	varicella zoster virus

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.01.008.

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