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**Voigt, S., Hofmann, J., Edelmann, A., Sauerbrei, A., Köhl, J.-S.**  
**Brincidofovir clearance of acyclovir-resistant herpes simplex virus-1 and adenovirus infection after stem cell transplantation**  
**(2016) Transplant Infectious Disease, 18 (5), pp. 791-794.**

TID-16-C-035.R2, Received 25 February 2016, revised 25 May 2016, 24 June 2016, accepted for  
publication 25 July 2016

Case report

## **Brincidofovir clearance of acyclovir-resistant herpes simplex virus-1 and adenovirus infection after stem cell transplantation**

S. Voigt, J. Hofmann, A. Edelmann, A. Sauerbrei, J.-S. Kühl

**S. Voigt<sup>1,2,\*</sup>, J. Hofmann<sup>3,\*</sup>, A. Edelmann<sup>3</sup>, A. Sauerbrei<sup>4</sup>, J.-S. Kühl<sup>2</sup>**

<sup>1</sup>Department of Infectious Diseases, Robert Koch Institute, Berlin, Germany

<sup>2</sup>Department of Pediatric Oncology/Hematology/SCT, Charité-Universitätsmedizin  
Berlin, Germany

<sup>3</sup>Institute of Medical Virology, Helmut-Ruska-Haus, Charité-Universitätsmedizin Berlin,  
and Labor Berlin Charité-Vivantes GmbH, Germany

<sup>4</sup>Institute of Virology and Antiviral Therapy, German Consulting Laboratory for HSV  
and VZV, Jena University Hospital, Jena, Germany

\*These authors contributed equally.

Running title: Voigt et al: Viral infections and clearance with brincidofovir

Correspondence to:

Sebastian Voigt, MD

Department of Infectious Diseases, Robert Koch Institute,

Nordufer 20,

1  
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3 13353 Berlin, Germany  
4

5  
6 Tel: +49 30 18 754 2271  
7

8  
9 Fax: +49 30 18 10 754 2271  
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11  
12 Email: [sebastian.voigt@charite.de](mailto:sebastian.voigt@charite.de)  
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15 **Abstract:**  
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18 Infections with adenovirus (AdV) and herpesviruses can result in considerable morbidity  
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20 and mortality in pediatric hematopoietic stem cell transplant (SCT) recipients. Herpes  
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22 simplex virus (HSV) reactivations are usually prevented by acyclovir (ACV) prophylaxis,  
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24 whereas cidofovir (CDV) has been used off indication to manage AdV infections. We  
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26 report a child with myelodysplastic syndrome undergoing multiple SCT, who  
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28 experienced HSV-1 disease including severe mucositis and herpetic whitlow, as well as  
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30 high viral load AdV DNAemia. Both ACV and CDV were ineffective; however, viral  
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32 loads were decreased with brincidofovir, resulting in viral clearance. A subsequent  
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34 Epstein–Barr virus disease with relevant meningoencephalitis responded to rituximab.  
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41 Key words: adenovirus; Epstein–Barr virus; herpes simplex virus; brincidofovir;  
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43 hematopoietic stem cell transplantation  
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6 In healthy individuals, herpesvirus infections are usually controlled by the immune  
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8 system, whereas they can lead to devastating conditions in immunosuppressed stem cell  
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10 transplant (SCT) recipients. In these patients, herpes simplex virus type 1 (HSV-1)  
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12 reactivations occur frequently without acyclovir (ACV) prophylaxis and occur as early as  
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14 the pre-engraftment phase (1). Under ACV prophylaxis, HSV-1 reactivations in pediatric  
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16 SCT recipients are rare and mainly caused by resistance-associated mutations in the viral  
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18 thymidine kinase gene (2). If present, these mutations usually define cross-resistance to  
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20 brivudine and the structurally similar nucleoside analogs penciclovir and its prodrug  
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22 famciclovir (3). In addition, long-term ACV prophylaxis has been associated with a risk  
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24 of developing ACV-resistant virus (4). The pyrophosphate analog foscarnet can be  
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26 alternatively used for treatment, if no resistance-associated mutation is present in the viral  
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28 DNA polymerase. B lymphocytes involved in lymphoproliferative disease occurring after  
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30 SCT are targeted with rituximab.  
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37 Moreover, adenovirus (AdV) infections have been reported to contribute  
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39 significantly to morbidity and mortality in pediatric SCT patients, especially during  
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41 episodes of high level AdV DNAemia (5). Apart from a desirable but often challenging  
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43 reduction of immunosuppression, cidofovir (CDV) has been proven useful for AdV  
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45 DNAemia treatment but its use is frequently limited by nephro- and myelotoxicity (6).  
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47 Brincidofovir (BCV) is an orally available analog of CDV containing a lipid side chain  
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49 facilitating the transport of the molecule into the cell (7). Several groups have reported an  
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51 inhibitory effect for double-stranded DNA viruses including AdV (8–12) and safety data  
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53 in patients are published (7, 13). Analysis of a recent phase 3 trial to evaluate BCV  
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3 prophylaxis against cytomegalovirus demonstrated gastrointestinal adverse events and  
4 acute graft-versus-host disease was suspected and treated (14).  
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8 Here, we report a child with myelodysplastic syndrome (MDS) undergoing  
9 multiple SCT who experienced HSV-1 disease including severe mucositis and herpetic  
10 whitlow as well as high viral load AdV DNAemia. Both ACV and CDV were ineffective;  
11 however, viral loads were decreased with BCV, resulting in viral clearance.  
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### 20 **Case report**

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22 A 5-year-old girl was diagnosed with MDS (FAB RC) and received a bone marrow graft  
23 from a 9/10 matched unrelated donor following conditioning according to the EWOG-  
24 MDS protocol (15). Both recipient and donor were initially HSV-1, Epstein–Barr virus  
25 (EBV), and AdV immunoglobulin-G seropositive. The day after SCT, the girl received  
26 standard ACV prophylaxis for prevention of HSV and varicella zoster virus infections  
27 and was continued until day 233 before foscarnet treatment commenced.  
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36 At 136 days after transplantation, the girl developed a secondary graft failure (see  
37 Fig. 1). She received a second reduced-intensity conditioning including fludarabine,  
38 alemtuzumab, cyclophosphamide, and melphalan with a subsequent, partially T-cell-  
39 depleted, peripheral blood SCT from the initial donor. On day 215 after initial SCT, she  
40 rejected again and received a third SCT from a new, also 9/10 identical matched-  
41 unrelated bone marrow donor after conditioning with fludarabine, thiotepa, 4 Gy total  
42 body irradiation, and anti-thymocyte globulin.  
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53 Despite ACV prophylaxis, HSV-1 reactivated and DNA became detectable in  
54 blood, cheek swab, as well as mouth washes after the second SCT. Phenotyping by  
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3 plaque-reduction assay revealed an ACV-resistant strain, and subsequent genotyping  
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5 confirmed the presence of a C336Y resistance-associated mutation in the viral thymidine  
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7 kinase gene (2). The girl presented with severe stomatitis and pharyngitis before herpetic  
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9 whitlow developed on day 221 after the first SCT (Figs. 1 and 2A). Almost  
10  
11 simultaneously, EBV DNAemia occurred and was treated with foscarnet (Fig. 1).  
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15 AdV DNA positivity in stool started 178 days and AdV DNAemia (species C)  
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17 began 237 days after the first SCT, when second graft rejection occurred. CDV was  
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19 started but AdV in plasma increased to  $>10^6$  copies/mL within 3 weeks, while HSV-1  
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21 DNAemia remained positive. Therefore, BCV was obtained by emergency use  
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23 authorization and substituted for CDV and ACV. AdV and HSV-1 DNA in plasma  
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25 became undetectable shortly after starting treatment. Herpetic whitlow resolved 75 days  
26  
27 after BCV introduction (Figs. 1 and 2B).  
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32 An EBV rebound on day 286 with beginning lymphoproliferative disease  
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34 developed into meningoencephalitis with neurologic deficits and was eventually treated  
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36 with foscarnet and a single dose of rituximab.  
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## 41 **Discussion**

42  
43 ACV has long been the mainstay in therapy for HSV diseases and is frequently used to  
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45 prevent viral reactivation in the post-SCT period (9, 16). Although it is unusual, our  
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47 patient reactivated HSV-1 under ACV prophylaxis and developed a fulminant herpetic  
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49 whitlow. Hematologically, the situation was aggravated by recurrent episodes of graft  
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51 rejection and constant lymphopenia, necessitating re-conditioning. This combination of  
52  
53 graft rejections and constant lymphopenia, necessitating re-conditioning. This combination of  
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55 graft rejections and multiple viral reactivations, including an ACV-resistant HSV-1 strain  
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3 and high viral load AdV DNAemia, led to a life-threatening condition. AdV treatment  
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5 with CDV failed and required another approach with BCV, which has been shown to  
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7 have *in vitro* activity against HSV-1 and AdV (17, 18).  
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10 In our patient, BCV controlled both HSV-1 and AdV DNAemia within a short  
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12 period of time without adverse side effects, especially no impairment of hematopoiesis  
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14 early after SCT. Still, a second EBV DNAemia occurred under BCV administration.  
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16 Whether this reflects an inferior efficacy of BCV on EBV or simply the patient's  
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18 reestablished lymphopoiesis remains unclear. However, the relevant EBV  
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20 meningoencephalitis may indicate a poor penetration of BCV into the brain.  
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24 BCV is still being tested in clinical trials, and it is still too soon to provide a  
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26 recommendation as to whether it might be useful in hematopoietic SCT recipients with  
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28 viral infections (19). However, the need for novel compounds is obvious, and a beneficial  
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30 effect of BCV in another pediatric HSCT recipient has been reported (20). Given the lack  
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32 of available broad-spectrum antiviral agents and the frequent occurrence of multiple viral  
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34 infections after SCT, BCV might prove a good option for these complicated patients.  
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#### 41 **Acknowledgements:**

42  
43 *Conflict of interest:* The authors declare that there is no conflict of interest.  
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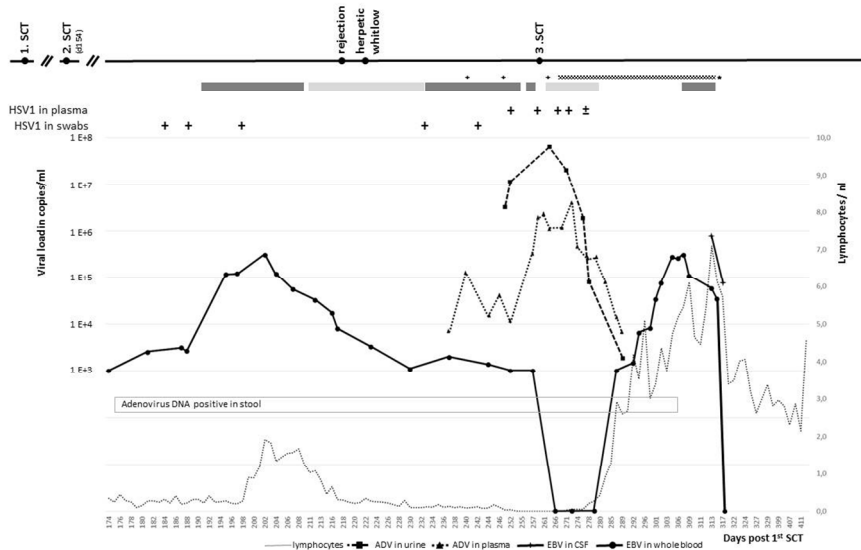
## Figure legends

**Fig. 1.** Time course of virus reactivations and treatment. Viral loads of Epstein–Barr virus (EBV) measured in whole blood and cerebrospinal fluid, as well as of adenovirus (ADV) in ethylenediamine tetraacetic acid (EDTA) plasma and urine are shown in relation to the days after the first stem cell transplant (SCT). Polymerase chain reaction (PCR) followed standard protocols with a limit of detection of about 1000 copies/mL. Continued shedding of ADV in stool specimens was found between days 178 and 314 post SCT. Qualitative herpes simplex virus (HSV)-1 PCR was performed in EDTA plasma and oral fluids/swabs (days 184, 189 and 198), cheek swab (day 238), and digit swab (day 231). Resistance analyses, performed in the samples from days 189 (wild-type/mutant mixture) and 198 (mutant only), revealed the C336Y substitution in the viral thymidine kinase.

Antiviral treatment (start and stop dates and days of treatment, respectively, are given in brackets): ✦ cidofovir (5 mg/kg/week; days 241, 248, 262); ✦ rituximab (375 mg/m<sup>2</sup> day 309); ◻ acyclovir (3 x 10 mg/kg/day or 3 x 15 mg/kg/day; days 212–233; 260-270); ◼ foscarnet (2 x 60 mg/kg/day; days 191–211; 234–259; 307–325); ◼ brincidofovir (2 mg/kg/biweekly; days 267–306). The top line indicates SCT dates, rejection events, and first appearance of herpetic whitlow.

**Fig. 2.** (A) Herpetic whitlow before brincidofovir administration. (B) Herpetic whitlow after brincidofovir administration.

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Figure 2

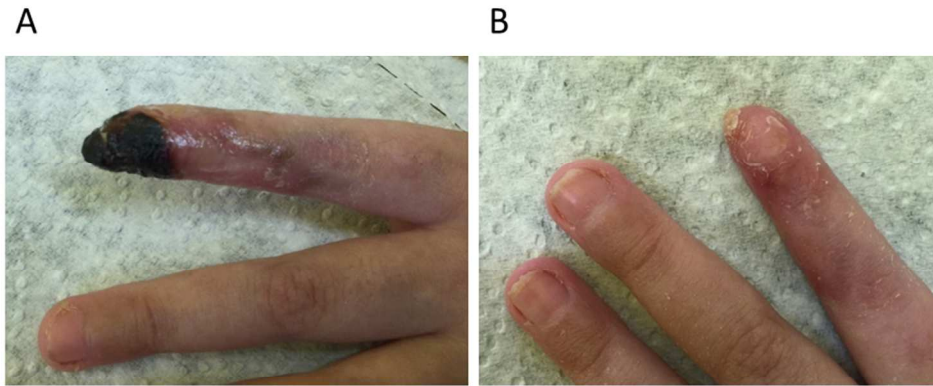


Figure 2

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