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Commentary
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Discovery and development of BVDU (brivudin) as a therapeutic for the treatment of herpes zoster

E. De Clercq*

Department of microbiology and immunology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat B-3000 Leuven, Belgium

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Dedicated to the memory of Prof. Jacques Gielen, devoted editor, eminent scientist and gentleman.

Abstract

This Commentary is dedicated to the memory of Dr. Jacques Gielen, the late Editor of Biochemical Pharmacology, whom I have known as both an author and reviewer for the Journal for about 25 years. This is, quite incidentally, about the time it took for bringing brivudin (BVDU) [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] from its original description as an antiviral agent to the market place (in a number of European countries, including Germany and Italy) for the treatment of herpes zoster in immunocompetent persons. BVDU is exquisitely active and selective against varicella-zoster virus (VZV) and herpes simplex virus type 1 (HSV-1). BVDU owes this high selectivity and activity profile to a specific phosphorylation by the virus-encoded thymidine kinase, followed by a potent interaction with the viral DNA polymerase. The (*E*)-5-(2-bromovinyl)-substituent can be considered as the hallmark for the activity of BVDU against VZV and HSV-1. Extensive clinical studies have indicated that BVDU as a single (oral) daily dose of 125 mg (for no more than 7 days) is effective in the treatment of herpes zoster, as regards both short-term (suppression of new lesion formation) and long-term effects (prevention of post-herpetic neuralgia). In this sense, BVDU is as efficient and/or convenient, if not more so, than the other drugs (acyclovir, valaciclovir, famciclovir) that have been licensed for the treatment of herpes zoster. There is one *caveat*; however, BVDU should not be given to patients under 5-fluorouracil therapy, as the degradation product of BVDU, namely (*E*)-5-(2-bromovinyl)uracil (BVU), may potentiate the toxicity of 5-fluorouracil, due to inhibition of dihydropyrimidine dehydrogenase, the enzyme involved in the catabolism of 5-fluorouracil.

Keywords: Brivudin; Herpes zoster; DNA polymerase

1. Introduction

BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine, brivudin] (Fig. 1) was originally synthesized in 1976 at the Chemistry Department of the University of Birmingham by P.J. Barr, A.S. Jones and R.T. Walker, as a potential radiation-sensitizing agent (assuming that it would be incorporated into DNA). Its potent and selective activity against herpes simplex virus type 1 (HSV-1) was first mentioned at the FEBS (Federation of European Biochemical Societies) Symposium on ''Antimetabolites in Biochemistry, Biology and Medicine'' (Prague, Czechoslovakia, 10–12 July 1978) [1] and the Fourth Symposium on the Chemistry of Nucleic Acid Components (Bechyne Castle, Czechoslovakia, 3–10 September 1978) [2]. When it was discovered, BVDU, and its closely related congener, IVDU [(*E*)-5-(2-iodovinyl)-2′-deoxyuridine] proved more potent and more selective in their activity against HSV-1 than all other anti-herpes compounds [3], and this has virtually remained the case, now 25 years later.

At the joint NATO Advanced Study Institute/FEBS Advanced Study Course held at Sogesta (near Urbino) in Italy (7–18 May 1979) on ''Nucleoside Analogues: Chemistry, Biology and Medical Applications'', P. Langen presented a long list of 5-substituted 2′-deoxyribopyrimidine nucleosides as anti-HSV-1 agents, the most active on the list being 5-(l-bromovinyl)-2′-deoxyuridine (Fig. 1), a compound obtained by the selective bromination and subsequent dehydrobromination of 5-ethyl-2′-deoxyuridine [4]. As it turned out later, the compound thus synthesized was not the 5-(l-bromovinyl)-but 5-(2-bromovinyl)-2′-deoxyuridine, and thus the superiority of BVDU over other anti-HSV-1 agents [5] was confirmed in a truly blinded fashion.

* Tel.: +32 16 337341; fax: +32 16 337340.
E-mail address: erik.declercq@rega.kuleuven.ac.be.

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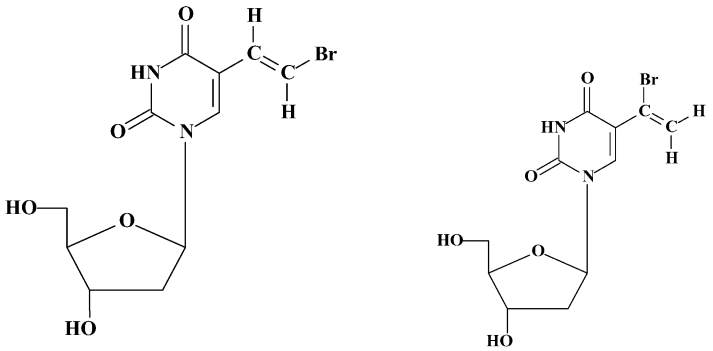

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Brivudin, Bromovinyldeoxyuridine (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU)

-(1-bromovinyl)-2'-deoxyuridine

Fig. 1. Brivudin [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU)] and 5-(l-bromovinyl)-2′-deoxyuridine.

The discovery of BVDU as a selective anti-herpesvirus agent came shortly after that of acyclovir [9-(2- hydroxyethoxymethyl)guanine] [6,7]. Shortly thereafter, the 2′-fluoro-2′-deoxyarabinofuranosylpyrimidine nucleosides, and particularly 2′-fluoro-5-iodoaracytosine (FIAC) were reported as potent and selective anti-herpesvirus agents [8,9]. For many years, these three compounds (acyclovir, BVDU and FIAC) would remain the ''gold standards'' or reference compounds for the development of new, and potentially more effective and/or selective antiviral agents [10].

From a (clinical) therapeutic viewpoint, the three compounds fared quite differently. Acyclovir became (worldwide) the drug of choice for the treatment of HSV-1, HSV-2 and varicella-zoster virus (VZV) infections (although it has now been replaced by its prodrug, valaciclovir, for the oral treatment of these infections). BVDU has been used for many years, albeit at a limited scale, for the topical treatment of herpetic keratitis, and for the oral treatment of VZV infections (in particular, shingles in immunocompromised patients). FIAC, and its uracil counterpart FIAU, once considered for the treatment of respectively cytomegalovirus (CMV) and hepatitis B virus (HBV) infections, are no longer pursued, and their commercial development towards therapeutic use for these (or other) virus infections has been stopped.

The antiviral potency and selectivity of BVDU, its activity spectrum, mechanism of action, structure–function relationship relative to that of other 5-substituted 2′-deoxyuridines, and clinical efficacy relative to that of acyclovir, FIAC and other anti-herpes agents have been reviewed repeatedly in the years that followed the initial discovery of BVDU as a selective inhibitor of HSV-1 [11–24]. From these initial studies BVDU not only emerged as a potent and selective inhibitor of HSV-1 [25], but also of VZV replication. In fact, in a comparative study of various anti-herpes drugs against VZV, the EC_{50} (50% effective concentration) of BVDU was 0.0024 μg/ml, as compared to 4.64 (g/ml for acyclovir, attesting to more than 10^3-fold superiority in potency of BVDU over acyclovir [26].

Within two years after its discovery, BVDU was introduced in the clinic, in Belgium, for the oral treatment of severe herpes zoster [27] and topical treatment of herpes simplex keratitis [28]. All patients, whether adults [29] or children [30], who were treated with oral BVDU for zoster or varicella, responded promptly to the treatment without any adverse side effects. In the treatment of herpetic keratitis, BVDU proved successful where other antiviral drugs including 5-iodo-2′-deoxyuridine (idoxuridine), 5-trifluoromethyl-2′-deoxyuridine (trifluridine), 9-β-D-arabinofuranosyladenine (vidarabine) and 9-(2-hydroxyethoxymethyl)-guanine (acyclovir) had failed [31]. Independently from the clinical studies in Belgium, BVDU has also been pursued in the former East Germany (DDR), for the treatment of herpetic keratitis [32] and VZV infections in patients with malignancies [33]. In these studies, as in ours, the compound proved clearly efficacious, without any adverse side effects.

BVDU (Brivudin) has originally been marketed under the name of Helpin® in the Deutsche Democratische Republik (DDR), and, later, in the whole of Germany, for the treatment of severe VZV and HSV-1 infections in immunosuppressed patients, where it was administered orally at 4× 125 mg per day for 5 days. Later BVDU has become available in Germany and some other European Countries under the trade name of Zostex®, Zerpex® or Brivirac®, for the treatment of herpes zoster in immunocompetent patients; it is given orally as a single dose of 125 mg daily for 7 days. This makes that in Europe there are four ''standard'' treatments available for varicella-zoster virus infections: brivudin (Zostex®, Zerpex®, Brivirac®), acyclovir (Zovirax®), valaciclovir (Valtrex®, Zelitrex®) and the diacetyl ester of 9-(4-hydroxy-3-hydroxymethyl-but-l-yl)-6-deoxyguanine (famciclovir) (Famvir®).

Table 1
Milestones in the development of BVDU as an antiviral drug

1979: First, full description of BVDU as a potent and selective anti-herpes agent, active against HSV-1 [3].
1980: Demonstration of activity of BVDU in the experimental treatment of HSV-1 keratitis in rabbits [34].
1980: First clinical use of BVDU in the treatment of patients with severe herpes zoster [27].
1980: Description of outstanding anti-HSV-1 activity of BVDU, as compared to several other anti-herpes drugs [25].
1981: Demonstration of in vivo efficacy of BVDU against simian varicella virus infection in monkeys, the only existing animal model reminiscent of VZV infections in humans [35].
1981: First clinical use of BVDU eye drops in the topical treatment of HSV-1 keratitis in humans [28].
1981: Elucidation of the target enzyme, the viral DNA polymerase, in the mechanism of action of BVDU[36].
1981: Recognition of the virus-encoded thymidine kinase (TK) as responsible for the activation of BVDU (and its close congener IVDU) and thus essential for BVDU and IVDU to exert their antiviral activity [37].
1981: First clinical use of (oral) BVDU in the treatment of herpes zoster opthalmicus [38].
1982: Description of the outstanding anti-VZV activity of BVDU, relative to that of other antiviral drugs [39].
1983: Demonstration of correlation between incorporation of BVDU into viral (HSV-1) DNA and its ensuing antiviral potency [40].
1984: Clinical study on the efficacy of BVDU in the treatment of severe herpes zoster in cancer patients [29].
1984: Remarkable pharmacological observation that BVDU can be regenerated from its degradation product BVU, as the consequence of the perfectly reversible reaction: BVDU + Thy o BVU + dThd [41].
1984: Experimental study demonstrating the efficacy of BVDU in the topical treatment of HSV-1 infection in hairless mice, an animal model for mucocutaneous HSV-1 infections, such as herpes labialis in humans [42].
1985: Clinical study on the efficacy of BVDU in the treatment of varicella and zoster in children with cancer [30].
1986: First demonstration of interference of BVU, the degradation product of BVDU, with the catabolism of 5-fiuorouracil, pointing to the potential of BVDU to elevate 5-fluorouracil levels, if BVDU is administered concomitantly with 5-fiuorouracil [43].
1986: Clinical trial again pointing to the efficacy of BVDU in the treatment of HSV and VZV infections in immunocompromised patients [44].
1987: First demonstration of potentiating effect of BVDU (via conversion to BVU) on the antitumor activity of 5-fiuorouracil in an experimental animal model [45].
1987: Demonstration that cytostatic activity of BVDU in tumor cells transfected by the HSV-1 (or HSV-2) thymidine kinase gene is mediated by an inhibitory effect at the thymidylate synthase level [46].
1988: Independent confirmation of clinical efficacy of BVDU in the treatment of herpes zoster in cancer patients [33].
1990: Independent confirmation of clinical efficacy of BVDU in the treatment of VZV infections in immunosuppressed children [47].
1991: Clinical study revealing efficacy of BVDU in (topical) treatment of herpetic keratitis resistant to other antiviral drugs such as idoxuridine, trifluridine and acyclovir [31].
1993: Differentiating mechanism of cytostatic action of BVDU in tumor cells transfected with the HSV-1 (or HSV-2) thymidine kinase gene, from that of other antiviral drugs such as ganciclovir [48].
1995: Double-blind clinical study demonstrating unequivocally that (oral) BVDU is equally (or even more) effective than acyclovir in the treatment of herpes zoster [49].

2. Milestones in the development of BVDU as an antiviral drug

In retrospect, the key events that led to the eventual development of BVDU as an antiviral drug for the treatment of HSV-1 and VZV infections have been summarized in Table 1.

3. Antiviral activity spectrum

The antiviral activity spectrum of BVDU is not restricted to HSV-1 and VZV but also encompasses several other herpesviruses such as suid herpesvirus type 1 (SHV-1), bovid herpesvirus type 1 (BHV-1), simian varicella virus (SVV), herpesvirus saimiri, and herpesvirus platyrrhinae (Fig. 2). Also, Epstein-Barr virus (EBV) is rather sensitive to BVDU, whereas HSV-2 and cytomegalovirus (CMV) are relatively resistant to the antiviral action of the compound [50]. Murine herpesvirus 68 (MHV-68), a murine gamma herpesvirus closely related to EBV, is also sensitive to BVDU, albeit to a lesser extent than EBV [51]. More sensitive are the bovine herpes mammillitis virus [52], the macropodid herpesvirus type 1 [53] and the macropodid herpesvirus type 2 [54]. The macropodid herpesviruses have been held responsible for the death of kangaroos and wallabies in European and North American zoos, and BVDU has been considered the drug of choice for experimental therapy of herpesvirus infections in captive macropodids [53,54]. Human CMV [which corresponds to human herpesvirus type 5 (HHV-5)], human herpesvirus type 6 (HHV-6) [55], human herpesvirus type 7 (HHV-7) [56] and human herpesvirus type 8 (HHV-8, or Kaposi's sarcoma-associated herpesvirus) [57] show little, if any, sensitivity to BVDU. The characteristic activity spectrum of BVDU (Fig. 2) thus explains why, from a human clinical viewpoint, the compound has been primarily pursued for the treatment of HSV-1 and VZV infections.

4. Mechanism of action

The exquisite potency of BVDU against HSV-1 and VZV, in comparison with the potency of other antiviral compounds, has been demonstrated with various clinical isolates of both HSV-1 [58] and VZV [59]. The mechanism of action of BVDU against HSV-1 and VZV (Fig. 3) depends on a specific phosphorylation by the virus-

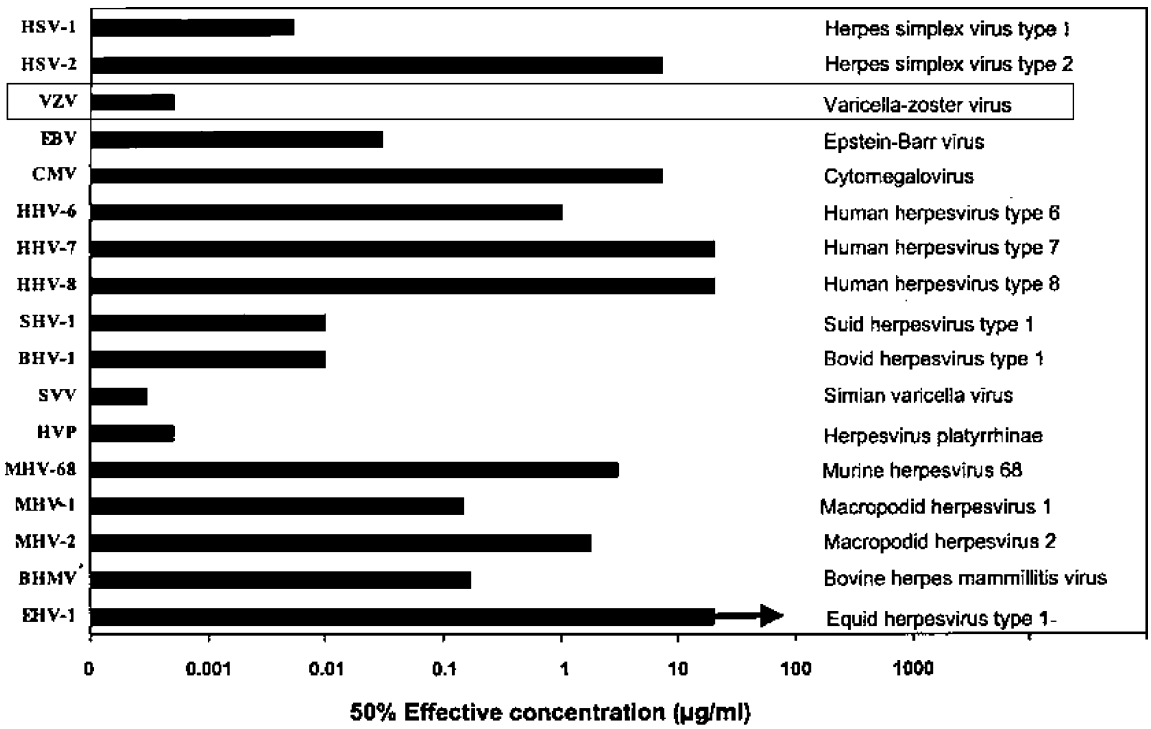

Fig. 2. Antiviral activity spectrum of BVDU.

encoded thymidine kinase (TK), the HSV-1 TK and VZV TK, which converts BVDU to its 5′-monophosphate (BVDU-MP) and 5′-diphosphate (BVDU-DP) [37]. Upon further phosphorylation by cellular kinase(s), i.e. nucleoside 5′-diphosphate (NDP) kinase, BVDU 5′-triphosphate (BVDU-TP) can then interact with the viral DNA polymerase, either as a competitive inhibitor with respect to the natural substrate (dTTP) [36], or as an alternative substrate, allowing the incorporation of BVDU-TP (as BVDU-MP) into the growing DNA chain (Fig. 3). This incorporation may affect both the stability and functioning of the DNA during the replication and transcription processes. In fact, a

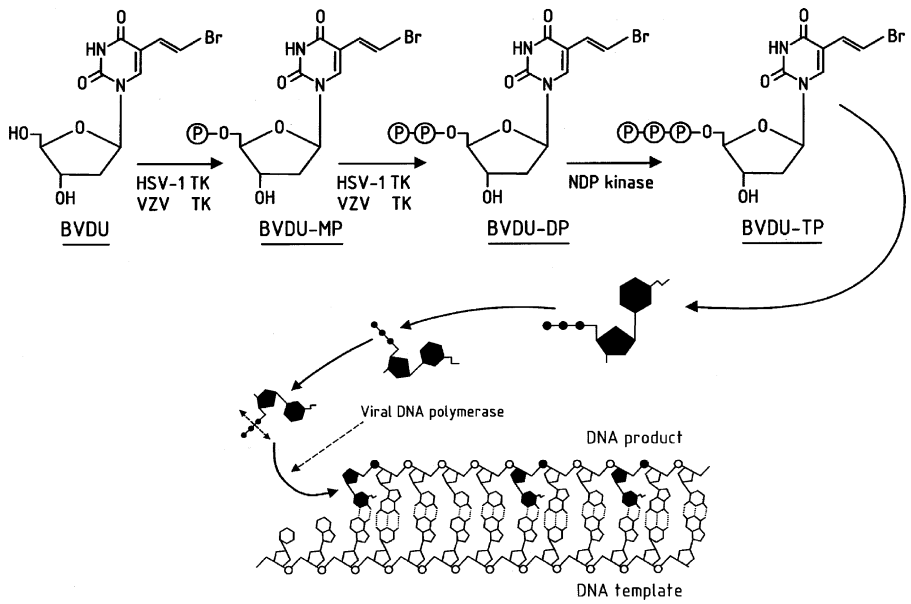

Fig. 3. Mechanism of action of BVDU. Following uptake by the (virus-infected) cells, BVDU is phosphorylated by the virus-encoded thymidine kinase (TK) to the 5′-monophosphate (BVDU-MP) and 5′-diphosphate (BVDU-DP), and further on to the 5′-triphosphate (BVDU-TP) by cellular kinases, i.e. nucleoside 5′-diphosphate (NDP) kinase. BVDU-TP can act as a competitive inhibitor/alternative substrate of the viral DNA polymerase, and as a substrate it can be incorporated internally (via internucleotide linkages) into the (growing) DNA chain.

close correlation has been found between the incorporation of BVDU into HSV-1 DNA, DNA integrity and viral infectivity [40,60].

A remarkable feature in the antiviral specificity of BVDU is that it is a highly potent inhibitor of HSV-1 but not HSV-2, so that it can be used as a marker for differentiating HSV-2 from HSV-1 strains [50]. The reason for the differential sensitivity of HSV-1 and HSV-2 towards BVDU resides in the fact that the HSV-2-encoded thymidine kinase, unlike its HSV-1 counterpart is unable to phosphorylate BVDU 5′-monophosphate to BVDU 5′-diphosphate [61]. This results in a substantial reduction in the supply of the active BVDU metabolite, BVDU-TP, in the HSV-2-infected cells [62], and, thus, reduced ability to interfere with viral DNA synthesis. BVDU-MP may interact as an alternate substrate [63] or inhibitor [64] of thymidylate synthase, but it is questionable that the interference of BVDU-MP with dTMP synthase contributes to the antiviral potency that is eventually achieved by BVDU.

Thus, the predominant determinant in the antiviral activity of BVDU is the virus-encoded thymidine kinase (TK), and the therewith-associated thymidylate kinase activity. The latter can apparently be regulated independently from the TK activity, as is the case for HSV-2 and also some HSV-1 isolates that have been more recently described [65]. One of these HSV-1 isolates had a single mutation (G → A at base position 502) that resulted in the substitution of threonine for alanine at amino acid position 168 in the viral TK: this led to a decreased dTMP kinase activity, concomitantly with a reduced sensitivity of the viral isolate towards BVDU.

5. Clinical efficacy

BVDU and its arabinofuranosyl counterpart BVaraU belong to the most potent inhibitors of VZV that have ever been described: BVDU inhibits VZV replication in cell culture at an EC_{50} of 0.001–0.003 (g/ml, and BVaraU at an even three-fold lower EC_{50} [26,59]. This exquisite potency has prompted the pursuit of both BVDU and BVaraU for the treatment of VZV infections in immunocompromised patients. The closest experimental model for VZV infections in humans is simian varicella virus (SVV) infection in monkeys, and BVDU was found effective in suppressing this disease when administered orally at either 15, 10 or 5, or even 1 mg/kg per day [35]. In a randomized double-blind trial, BVDU, when given orally at 7.5 mg/kg per day (that is 4× 125 mg-tablets per day), proved as (or even more) efficacious than acyclovir given intravenously at 30 mg/kg per day, in the treatment of herpes zoster in immunocompromised patients [49] (Fig. 4).

In another randomized double-blind clinical trial [66], BVaraU (sorivudine) administered orally at 40 mg daily was compared with acyclovir, given orally at 4 g (5× 800 mg) daily, both over a 10-day course, in the treatment of dermatomal herpes zoster in patients infected with human immunodeficiency virus (HIV): BVaraU effected a slightly faster cessation of new lesion formation (p = 0.07), a significantly accelerated cutaneous healing (crusting) (p = 0.02) and a similar resolution of zoster-associated pain (ZAP) (p = 0.22) when compared with acyclovir therapy. Thus, BVaraU could be considered as efficient a treatment for herpes zoster (in HIV-infected individuals) at a daily dose of 40 mg as acyclovir at a daily dose of 4 g.

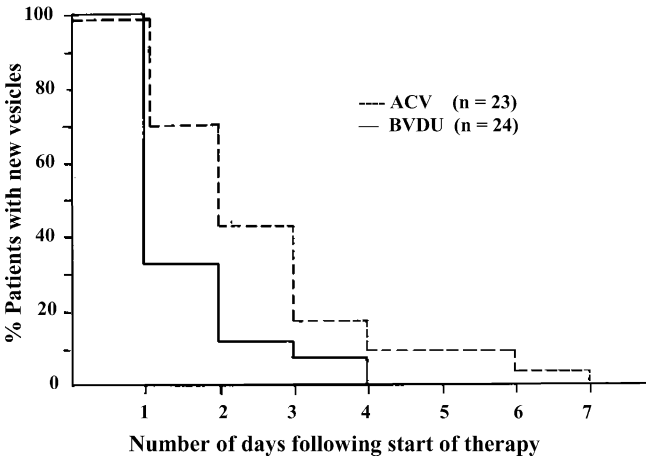

Fig. 4. Multicentered, double-blind, randomized study of oral brivudin vs. intravenous acyclovir in the treatment of severe herpes zoster in cancer patients [49]. BVDU was administered orally at a dose of 125 mg × 4 per day for 5 days. ACV was administered intravenously at a dose of 10 mg/kg × 3 per day for 5 days. BVDU group (24 patients) also received placebo (intravenously). ACV group (23 patients) also received placebo orally.

The efficacy of BVDU in the treatment of herpes zoster has been examined in several European clinical centers, with BVDU given at various dosage schedules (i.e., 50 or 125 mg twice daily; and 31.25, 62.5 or 125 mg once daily) in comparison with 4 g (5× 800 mg) daily for acyclovir, for a 7-day treatment period. The results of these multicentered studies with a large number of patients have been recently divulged at a number of international conferences.

In two randomized, double-blind, multicentered studies in immunocompetent patients, BVDU (brivudin, 125 mg, once daily) was compared with acyclovir (5× 800 mg, daily) and with famciclovir (3× 250 mg, daily), respectively, all treatments for 7 days, for their effects on acute herpes zoster as well as postherpetic neuralgia (zoster-associated pain). The results of these studies have been reported at several scientific meetings [67–76].

BVDU 125 mg once daily for 7 days was found to be superior to acyclovir 5× 800 mg daily in stopping viral replication in acute herpes zoster, as based on cessation of new lesion formation (or ‘‘time to last formation of new vesicles’’, the parameter which best reflects the end of virus replication), thus confirming in the clinical setting the far greater anti-VZV activity of BVDU (than of acyclovir) [73]. The incidence of potentially treatment-related adverse events was similar for BVDU (7.7%) and acyclovir (10.0%) [73].

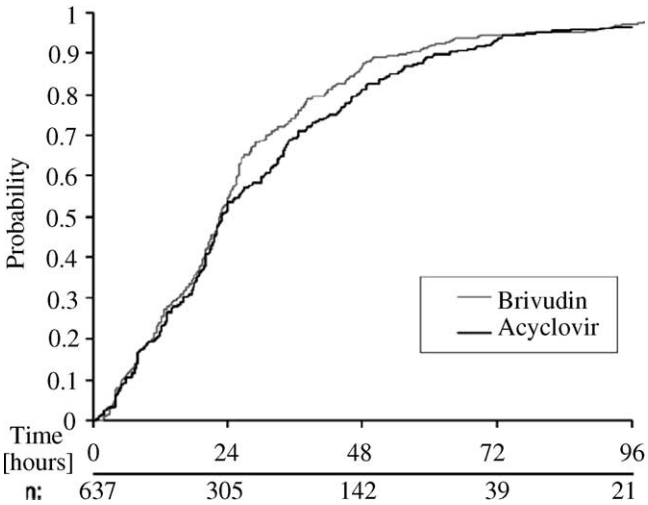

Fig. 5. Oral brivudin in comparison with acyclovir for improved therapy of herpes zoster in immunocompetent patients: results of a randomized, double-blind, multicentered study [73]. Intent-to-treat analysis of the primary endpoint "time from start of treatment to last vesicular eruption". Kaplan–Meier curves for herpes zoster patients treated with oral brivudin 125 mg once daily and for patients treated with acyclovir 800 mg 5× daily, both for 7 days. The letter *n* indicates the overall number of patients still showing vesicle formation at the respective time points.

BVDU 125 mg once daily was equivalent to famciclovir 3× 250 mg daily in reducing time to last occurrence of lesions; however, in patients with zoster ophthalmicus, cessation of vesicle formation occurred 31% faster with BVDU than with famciclovir [70].

Treatment with BVDU 125 mg once daily for 7 days resulted in a significantly lower incidence of postherpetic pain than standard treatment with acyclovir (5× 800 mg daily): 32.7% versus 43.5% ($p = 0.006$); mean duration of postherpetic neuralgia was similar with BVDU (173 days) and acyclovir (164 days) ($p = 0.270$) [73] (Fig. 5). Pain more than six months after study termination was reported by 12% of the BVDU patients as compared to 15.6% of the acyclovir patients [67,69]. As shown in Fig. 6, the monthly prevalence of postherpetic neuralgia was lower after BVDU than after acyclovir therapy [67], and thus, this

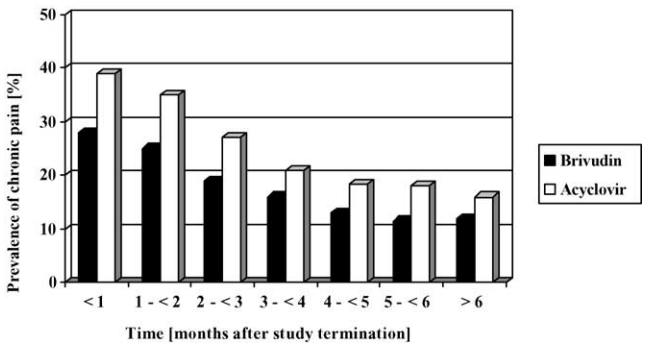

Fig. 6. Monthly prevalence of postherpetic pain after termination ($N = 545$) of randomized, double-blind study of oral BVDU (brivudin) at 125 mg once daily vs. acyclovir at 800 mg 5× daily for 7 days. Data taken from Wassilew et al. [67].

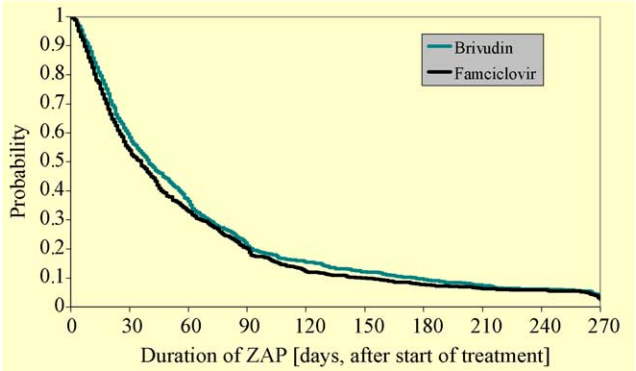

Fig. 7. Time to resolution of zoster-associated pain (per protocol population, $N = 1712$) in randomized, double-blind study of oral BVDU (brivudin) at 125 mg once daily vs. famciclovir at 250 mg 3× daily for 7 days. Data taken from Wassilew et al. [68].

study clearly showed that BVDU treatment during acute herpes zoster favorably affects the incidence of postherpetic neuralgia in immunocompetent elderly patients with herpes zoster [77].

BVDU 125 mg once daily for 7 days was as effective as famciclovir 250 mg three times daily for 7 days in reducing the prevalence of postherpetic neuralgia three months after start of treatment [68]. With respect to zoster-associated pain up to 9 months after the start of treatment, again equivalence of BVDU and famciclovir could be shown [68] (Fig. 7). In the "Per" protocol population ($N = 1712$), 21.1% of the brivudin patients and 20.2% of the famciclovir patients had zoster-associated pain (of any intensity) three months after the start of treatment [69].

In conclusion for the randomized, double-blind studies of BVDU versus acyclovir and famciclovir, these studies have confirmed important advantages of BVDU over currently available antiviral therapy [67–76]. These advantages include a reduced total daily dose (125 mg) and a reduced dosing frequency (only one daily drug intake), which is a great improvement particularly in the treatment of elderly patients who often require extensive co-medication [75].

As already mentioned [31], BVDU has been used for many years in the topical treatment (as 0.1% eyedrops) of herpetic keratitis, since it is efficacious against various manifestations of this disease (dendritic and geographic corneal ulcers, and stromal keratitits), also when clinically resistant to other antiviral drugs such as idoxuridine, fluridine, vidarabine, or acyclovir [31].

BVDU as a 5% cream in Beeler base (15 g of cetylalcohol, 1 g of cera alba, 10 g of propylene glycol, 2 g of sodium lauryl sulfate, and enough water to make 100 g) has been used, with success, in the topical treatment of recurrent herpes labialis. This use has been based on the protective activity seen with BVDU in the topical treatment of intracutaneous HSV infection in hairless (h/h) mice [42]. When an entirely blinded protocol was followed to assess the efficacy of BVDU in this HSV-1 model

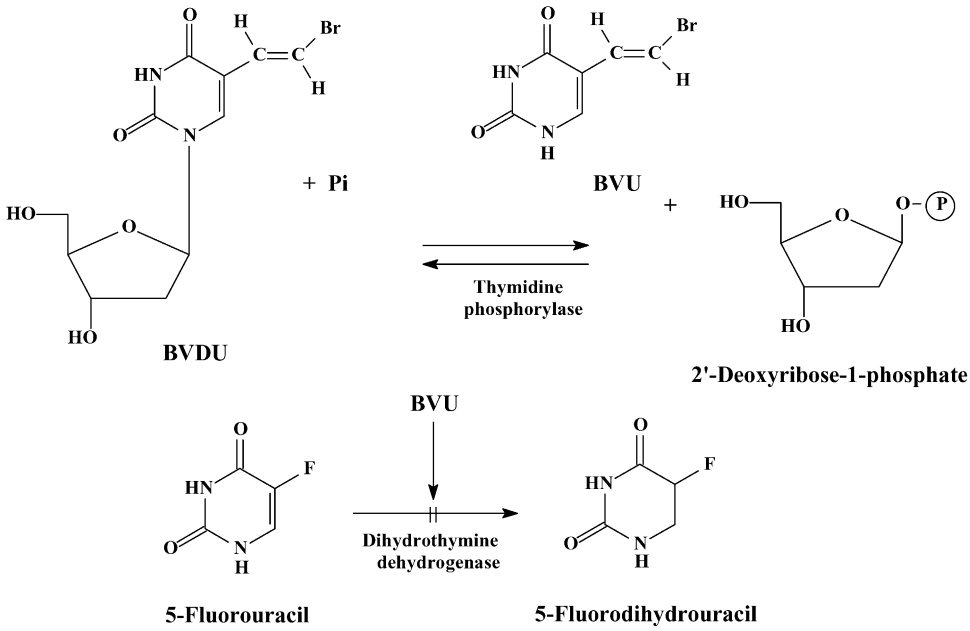

Fig. 8. Degradation of BVDU to BVU by thymidine phosphorylase and inhibition of the degradation of 5-fluorouracil by BVU.

infection [E. De Clercq, unpublished observations (1994)] BVDU, when formulated as an hydrogel cream at 5, 2 or 0.5%, completely suppressed all manifestations of the infection (i.e. skin lesions, paralysis of the hind legs, and mortality). At all three concentration levels, topical BVDU treatment resulted in a 100% survival rate at the 20th day post infection. In contrast, all the placebo-treated mice developed lesions within 4–7 days after the infection and succumbed within 7–14 days after the infection. These observations provide unequivocal evidence for the effectiveness of topical BVDU (at 5, 2 and 0.5%) in the treatment of intracutaneous HSV-1 infection [78].

6. Interaction with 5-fluorouracil

It has been known for more than 15 years [20] that BVDU is recognized as substrate by thymidine phosphorylase that converts BVDU to BVU [(*E*)-5-(2-bromovinyl)uracil] and 2-deoxyribose-1-phosphate (Fig. 8). The rapid metabolism of BVDU to BVU, which is as such inactive as an antiviral agent, obviously attenuates the antiviral potency of BVDU. However, the resulting BVU can be re-converted to BVDU, both in vitro and in vivo, through a pentosyl transfer reaction with any 5-substituted 2′-deoxyuridine, including 2′-deoxythymidine, as the pentosyl donor [20], thus restoring, at least in part, the antiviral potential of the compound.

BVU itself is a potent inhibitor of dihydropyrimidine dehydrogenase (DPD), the enzyme that is responsible for the first step in the catabolic pathway of pyrimidines. As DPD is also needed for the degradation of 5-fluorouracil, BVU protects 5-fluorouracil against breakdown and significantly increases its half-life. This marked increase in the half-life of 5-fluorouracil has also been demonstrated in cancer patients which had been given 5-fluorouracil (intravenously), concomitantly with BVDU (orally) [79]. The combination of BVDU with 5-fluorouracil results in a significant enhancement of the antitumor activity of 5-fluorouracil, as has been clearly shown in different tumor models in mice, i.e. adenocarcinoma 755 [80] and Lewis lung carcinoma [81]. In fact, the combination of BVDU with 5-fluorouracil significantly enhanced the life-span of mice bearing liver metastases of Lewis lung carcinoma [81].

While the combination of BVDU (or BVU) with 5-fluorouracil may be endowed with enhanced anti-tumor activity, one should also beware of increased toxicity associated with the elevated plasma levels of 5-fluorouracil [79]. The Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, reported that in 1993, fifteen deaths occurred in Japanese patients following the co-administration of BVaraU (sorivudine) with a 5-fluorouracil prodrug, and this within 40 days after sorivudine was approved by the Japanese government and began to be used clinically. Before death, all of the patients had severe symptoms of toxicity, such as diarrhea with bloody flux and marked decreases in white blood cell and platelet counts. Also, eight other Japanese patients that had received both drugs during this period had severe symptoms of gastrointestinal toxicity and myelotoxicity. Obviously, this severe toxicity could be attributed to the enhanced plasma and tissue levels of 5-fluorouracil, consequently to its retarded catabolism [82,83]; the culprit being BVU that was found to irreversibly inhibit DPD, as originally shown by Desgranges et al. [43], by covalent binding of a reduced form of BVU as a suicidal inactivator [84]. The formation of BVU, after oral administration of

BVaraU, could be due to the action of the thymidine phosphorylase(s) from enterobacteriaceae, i.e. *Klebsiella pneumoniae* [85], and/or the anaerobic *Bacteroides* species [86].

Even at the 40 mg once-daily oral dosage regimen for 10 consecutive days [66], BVaraU leads to a profound depression of DPD activity, and recovery of DPD activity occurrs only within four weeks of completion of BVaraU therapy [87]. This indicates that patients receiving sorivudine are not only at risk to develop potentially life-threatening toxicity from 5-fluorouracil (or any of its prodrugs) while receiving both drugs simultaneously but also for the next few weeks after the last dose of sorivudine [88]. More recent studies have addressed the mechanism-based inactivation of DPD by BVU [89]: in the presence of NADPH, the sulfhydryl group of Cys^{671} in the human DPD would interact with 5,6-dihydro-5-(2-bromoethyldenyl)uracil (BEDU), a putative allyl bromide type of reactive molecule, to form a sulfide bond with loss of hydrogen bromide [90].

Obviously, the concomitant use of BVDU and 5-fluorouracil (or a 5-fluorouracil derivative such as tegafur) should be prohibited, and for the reasons explained above, at least a 4-week interval should be respected before embarking upon a 5-fluorouracil-based therapy after the end of BVDU therapy. Yet, it should be kept in mind that BVDU and BVaraU, upon oral administration, do not have the same pharmacodynamics in BVU release. In the case of BVaraU, BVU is released at the level of gut (by the action of the enterobacteriaceae [85] or bacteroides [86], whereupon it is absorbed and distributed through the organism, so as to enhance the toxicity of 5-fluorouracil at the intestinal mucosa as well as the whole organism. In the case of BVDU, BVU is primarily formed at the site of the liver, where, in fact, it can be converted again to BVDU through the reversible 2′-deoxyribosyl transfer reaction noted above [41]. It would, therefore, be worth examining pharmacokinetically whether BVDU, as compared to BvaraU, indeed exhibits differential kinetics of BVU release.

7. The (*E*)-5-(2-bromovinyl)uracil connection

The hallmark of BVDU has remained its exquisite activity against varicella-zoster virus, and it is noteworthy, therefore, that its in vivo efficacy, first demonstrated against the simian counterpart (SVV) in monkeys [35], was recently corroborated in two novel mouse models for VZV infection, viz. umbilical cord cushion and hollow fiber model: in both models, BVDU significantly reduced VZVtiters [91].

The pharmacophore or structural determinant for the exquisite anti-VZV activity of BVDU is the (*E*)-5-(2-bromovinyl) substituent. It has to be *E* (for ‘‘Entgegen’’, or *trans*), since the isomeric Z (‘‘Zusammen’’, or *cis*) configuration for the bromine group makes the compound much less active [92]. Numerous pyrimidine nucleoside analogues (Fig. 9) have been described, all equipped with the (*E*)-5-(2-bromovinyl) substituent, that show remarkable antiviral activity, particularly against VZV. The most potent is BVaraU, with an EC_{50} against VZV in the subnanomolar range (0.1 ng/ml) [59]. For BVDC [26] and its 4-(l,2,4-triazol-l-yl) derivative [93], EC_{50} values of about 0.02 (g/ml have been recorded.

Carbocyclic (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (C-BVDU) (which is not a substrate for thymidine phosphorylase) is a unique example of a chiral molecule where the two enantiomeric (+)- and (−)-forms are antivirally active [94], apparently because they can both be recognized as substrate by the virus-encoded thymidine kinase [94]. Also, the L-enantiomer of BVDU can be recognized by the viral (HSV-1) TK, resulting in antiviral activity comparable to that of the D-enantiomer [95]. The L-dioxolane derivative of (*E*)-5-(2-bromovinyl)uracil (L-BVODDU) inhibits VZV at an EC_{50} value of about 0.07 ng/ml [96]. *S*-BVDU, or (*E*)-5-(2-bromovinyl)-2′-deoxy-4′-thio-uridine [97], is equipotent with BVDU [$EC_{50} \sim 1$ ng/ml against VZV] [98]. The 2-deoxy-2-*C*-methylene derivative thereof (*S*-BVMDU) was found to be active against VZV at an EC_{50} of 0.013 (g/ml [99]. For 4′-methyl BVDU an EC_{50} of 0.8 ng/ml was recorded, but this compound also appeared to be rather cytotoxic [cytotoxic concentration (CC_{50}): 0.45 (g/ml] [100].

Various branched-chain C-hydroxymethyl nucleoside analogues containing (*E*)-5-(2-bromovinyl)uracil have been described. (*E*)-5-(2-bromovinyl)-l-[3-deoxy-3-C-(hydroxymethyl)-β-D-*arabino*-pentofuranosyl]uracil inhibited VZV only at a rather high concentration (EC_{50}: 5–15 (g/ml) [101]. For its 4′-thio counterpart, no antiviral data were provided [102]. BMS-181,165, or [3*S*-(3α,4β,5α)]-(*E*)-5-(2-bromovinyl)-l-[tetrahydro-4,5-bis(hydroxymethyl)-3-furanyl]-2,4(l*H*,3*H*)-pyrimidinedione, was found to inhibit VZV at an EC50 of *circa* 0.01 (g/ml [103]. This compound also proved efficacious against SVV infection in African green monkeys when administered orally at 4, 16 or 64 mg/kg per day [104], and quoted as of potential value in therapy of VZV infections in humans [104]. For the cyclopropyl derivative AV-100, or (l′*S*,2′R)-5-[(*E*)-2-bromovinyl]-l-[[l′,2′-bis(hydroxymethyl)cycloprop-r-yl]methyl]-2,4-(l*H*,3*H*)-pyrimidinedione, an EC_{50} of about 0.03 (g/ml, that is 100 times higher than for BVaraU, against VZV was noted [105]; in this study, it was ascertained that the cyclopropyl derivative, unlike BVaraU, did not release BVU in plasma after oral administration (in rats) [105]. This means that AV-100 must have been resistant to cleavage by thymidine phosphorylase. BVisoDDU represents another BVDU analogue, whereby BVU is linked via a N–glycosidic bond with C-2′ [106]. BVisoDDU showed pronounced activity against HSV-1, but, for reasons that still remain to be clarified, did not show activity against VZV.

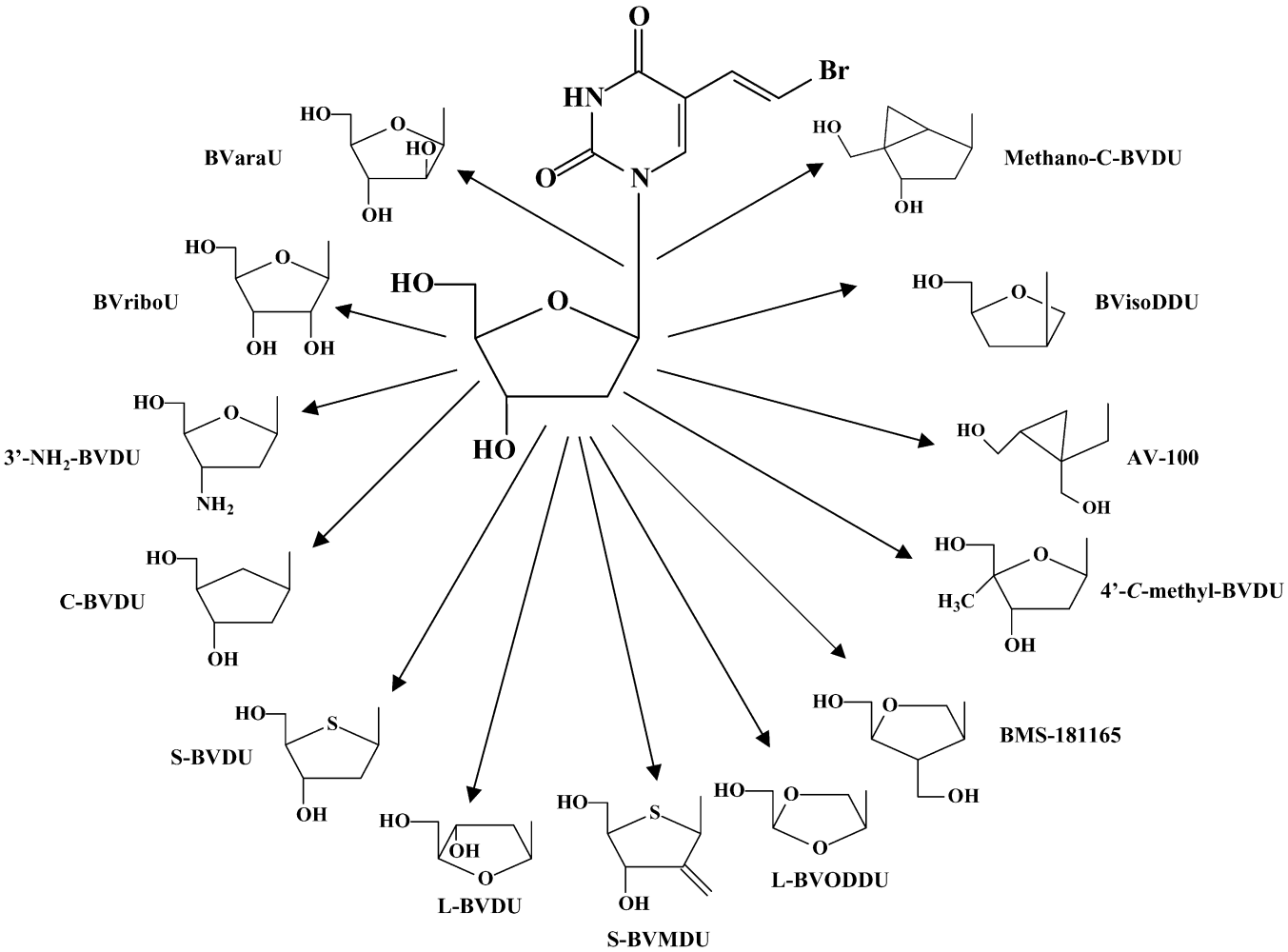

Fig. 9. BVDU derivatives all based on the (*E*)-2-bromovinyl substituent as the pharmacophore: (*E*)-5-(2-bromovinyl)-l-β-D-*arabino*-pentofuranosyluracil (BVaraU), (*E*)-5-(2-bromovinyl)uridine (BVriboU), 3′-NH_2-BVDU, (±)-carbocyclic BVDU (C-BVDU), (*E*)-5-(2-bromovinyl)-2′-deoxy-4′-thiouridine (*S*-BVDU), (*E*)-5-(2-bromovinyl)-2′-deoxy-L-uridine (L-BVDU), (*E*)-5-(2-bromovinyl)-l-(2-deoxy-2-*C*-methylene-4-thio-β-D-*erythro*-pentofuranosyl)uracil (*S*-BVMDU), the L-dioxolane derivative of (E)-5-(2-bromovinyl)uracil (L-BVODDU), [3*S*-(3α,4β-5α)]-(*E*)-5-(2-bromovinyl)-l-[tetrahydro-4,5-bis(hydroxymethyl)-3-furanyl]-2,4(l*H*,3*H*)-pyrimidinedione (BMS-181165), 4′-*C*-methyl-BVDU, (1′*S*,2′*R*)-5-[(*E*)-2-bromovinyl]-[[1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl-2,4-(l*H*,3*H*)-pyrimidinedione (AV-100), (*E*)-5-(2-bromovinyl)isodideoxyuridine (BVisoDDU) and the north-locked methanocarba-(*E*)-5-(2-bromovinyl)uarcil (methano-C-BVDU).

As another witness of the BVDU connection, the conformationally locked nucleoside, (north)-methanocarba-(*E*)-5-(2-bromovinyl)uracil (Methano-C-BVDU) was recently shown to be equipotent as BVDU in its activity against VZV [107]. (*E*)-5-(2-bromovinyl)uridine, the riboside counterpart of BVDU, has also been accredited with antiviral activity, with an activity spectrum that was similar but an antiviral potency that was inferior to that of BVDU [108]. BVriboU could be phosphorylated by the HSV-1-encoded thymidine kinase, and upon conversion to its 2′-deoxy counterpart [probably at the 5′-diphosphate level (BVriboU-DP → BVDU-DP)] interact in its 5′-triphosphate form and be incorporated (as BVDU-MP) into the viral DNA within the HSV-1-infected cell [109].

A number of phosphotriesters [110] and cyclic phosphoramidates [111] were designed in attempts to release the 5′-monophosphate form of BVDU into the cells: however, these conjugates reacted as prodrugs of BVDU, rather than BVDU-MP, as could be judged from their inactivity against thymidine kinase deficient (TK) virus strains [110,111]. Another prodrug of BVDU, namely 3′-*O*-benzyl-(*E*)-5-(2-bromovinyl)-2′-deoxyuridine, showed activity against HSV-1 infection in vivo but not in vitro, probably because it was readily metabolized in vivo, but not in vitro, to BVDU [112].

8. New furo[2,3-*d*]pyrimidine nucleoside analogues

The bicyclic furo[2,3-*d*]pyrimidine nucleoside analogues represent an entirely new class of fused furopyrimidine derivatives with unprecedented selectivity for varicella-zoster virus (VZV). From extensive structure-activity relationship (SAR) studies, the 6-alkyl- and 6-

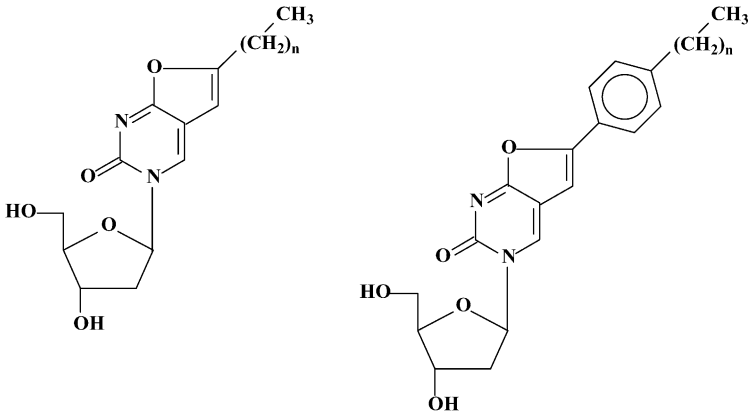

Fig. 10. Prototype furo[2,3-*d*]pyrimidine nucleoside analogues: Cf 1368, Cf 1369, Cf 1742 and Cf 1743.

(*p*-alkylphenyl)-substituted furopyrimidine derivatives Cf 1368, Cf 1369, Cf 1742 and Cf 1743 (Fig. 10) emerged as the most potent inhibitors of VZV replication: they were found to inhibit both laboratory VZV strains and clinical VZV isolates at subnanomolar concentrations, while not being toxic to the host cells at 100,000-fold higher concentrations [113]. Although the precise mechanism of action of these compounds remains to be elucidated, it is clear that for their antiviral activity they depend on phosphorylation by the VZV-encoded thymidine kinase (TK). However, phosphorylation by the viral TK is necessary but not sufficient for the furo[2,3-*d*] pyrimidine nucleoside analogues to display antiviral activity, as recently demonstrated by the inactivity of these compounds against simian varicella virus [114]. The furo[2,3-*d*] pyrimidine nucleoside analogues are not susceptible to degradation by human or bacterial thymidine phosphorylase, which may otherwise release the free aglycone. Also, the latter is not inhibitory to dihydropyrimidine dehydrogenase (DPD), the enzyme that, as explained above, is critically involved in the degradation of thymine, uracil and the anticancer agent 5-fluorouracil. Further development of the furo[2,3-*d*]pyrimidine nucleoside analogues as new therapeutic modalities for the treatment of VZV infections (i.e., varicella and herpes zoster) seems highly warranted.

9. Present clinical situation for treatment of herpes zoster (in Europe)

BVDU (Brivudin) was originally marketed under the trade name of Helpin® in East Germany, and then the whole of Germany, for the treatment of severe VZV and HSV-1 infections in immunosuppressed patients, where it was administered orally at 4× 125 mg per day for 5 days. Later BVDU has become available in Germany and some other European Countries under the trade name of Zostex®, Zerpex® or Brivirac®, for the treatment of herpes zoster in immunocompetent patients, where it is given orally as a single dose of 125 mg daily for 7 days. This makes that in Europe there are four ''standard'' treatments available for varicella-zoster virus (VZV) infections: brivudin (Zostex®, Zerpex®, Brivirac®), acyclovir (Zovirax®), valaciclovir (Valtrex®, Zelitrex®) and famciclovir (Famvir®) (Fig. 11) [115]. The recommended dosage schedules [115] are as follows:

- Brivudin orally 125 mg 1× daily for 7 days.
- Acyclovir orally 800 mg 5× daily for 7 days.
- Acyclovir intravenously 8–10 mg 3× daily for 7–10 days (in immunocompromised patients).
- Valaciclovir orally 1000 mg 3× daily for 7 days.
- Famciclovir orally 250 mg 3× daily for 7 days.

Of these drug regimens, brivudin, with a single (oral) daily administration of 125 mg, is obviously the most convenient. However, because of the interaction of BVU, the degradation product of brivudin, with the catabolism of 5-fluorouracil, concomitant use of 5-fluorouracil and BVDU should be avoided.

Note: At some points in the development of BVDU as an antiviral drug questions have been raised about the potential genotoxicity and carcinogenicity of this nucleoside analogue as well as other antiviral nucleoside analogues (such as acyclovir, penciclovir and ganciclovir) that are used in the treatment of herpesvirus infections. Induction of chromosomal aberrations [i.e., sister chromatid exchanges (SCEs)] has been reported for BVDU and for other antiviral nucleoside analogues [116], but the significance thereof is unclear. The carcinogenic activity of BVDU is essentially unknown [116]. As there is no conclusive evidence that BVDU or any of the other nucleoside analogues mentioned above can cause cancer in humans, the use of these nucleoside analogues in antiviral therapy remains a pragmatic option that seems justified by the risk/benefit assessment [116].

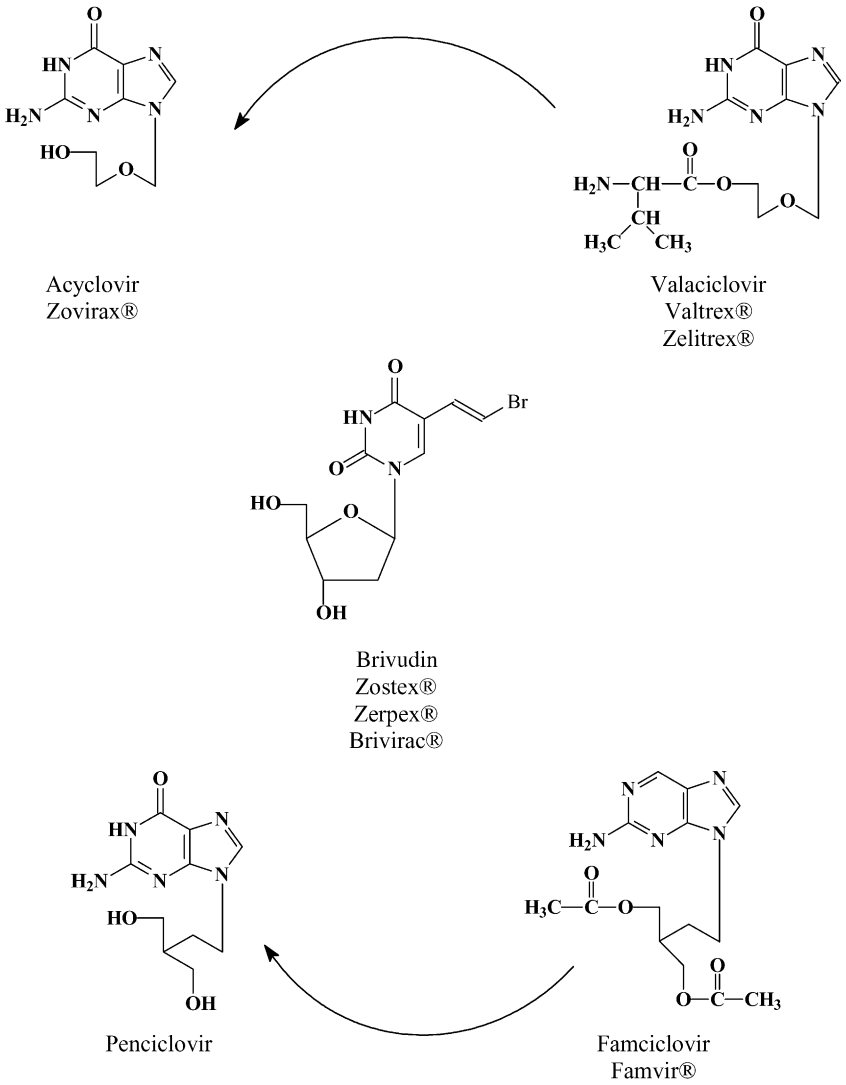

Fig. 11. Antiviral drugs currently available for treatment of VZV infections. Valaciclovir acts as oral prodrug of acyclovir, whereas famciclovir serves as oral prodrug form of penciclovir.

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