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Commentary

# Tanovea® for the treatment of lymphoma in dogs

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ABSTRACT

Tanovea® (first named GS-9219, then VDC-1101, generic name: rabacfosadine) is a pro-prodrug or "double" prodrug of PMEG [9-(2-phosphonylmethoxyethyl)guanine], which has been conditionally approved by the US FDA (Food and Drug Administration) for the treatment of lymphoma in dogs. Tanovea has been demonstrated to be effective against non-Hodgkin's lymphoma (NHL) in dogs, as well as canine cutaneous T-cell lymphoma, spontaneous canine multiple myeloma, naïve canine multicentric lymphoma and relapsed canine B-cell lymphoma. As a double prodrug of PMEG, GS-9219 is first converted intracellularly by hydrolysis to cPr-PMEDAP, then deaminated to PMEG, which is then phosphorylated twice to its active metabolite PMEGpp, acting at the level of the cellular DNA polymerases.

## 1. Introduction

The acyclic nucleoside phosphonates (ANPs: [1]) have received major attention and clinical usefulness as antiviral drugs in the treatment of HIV (human immunodeficiency virus) and HBV (hepatitis B virus) infections. Foremost have been TDF (tenofovir disoproxil fumarate) [2] and TAF (tenofovir alafenamide) [3], which became the cornerstone for the treatment of HIV and HBV infections; TDF and TAF in combination with various other anti-HIV drugs, in the treatment of HIV infections, and TDF (Viread®) and TAF (Vemlidy®) as such for the treatment of HBV infections. In combination with emtricitabine, TDF has also been approved, as Truvada®, in the US in 2012, and 4 years later in the EU, for the prevention (PrEP: pre-exposure prophylaxis) of HIV infections.

What is widely accepted is that, being ANPs, both TDF and TAF, which qualify as antiviral agents for the treatment of HIV and HBV infections, stem from Dr. Antonín Holý's legacy [4]. What is hardly recognized, however, is that the first anti-tumor drug, Tanovea® (rabacfosadine), ever approved by the US Food and Drug Administration (FDA) for the treatment of lymphoma in dogs also originates from the same class of compounds, referred to as ANPs [1]. Where did Tanovea® come from? What are its credentials? How does it work? And where it may lead to? This will be the subject of the present Commentary.

## 2. History

The era of the ANPs started with the demonstration that (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] and its closely related analogue PMEA [9-(2-phosphonylmethoxyethyl)adenine] had broad-spectrum anti-DNA virus activity [5]. In the follow-up paper [6], various other phosphonylmethoxyalkyl derivatives of purines and pyrimidines, including the guanine counterpart of PMEA, 9-(2-phosphonylmethoxyethyl)guanine (PMEG), were described as antiviral agents, PMEG being the most cytotoxic of the series.

PMEG was subsequently found effective against Shope papilloma virus infection in rabbits and human papilloma virus type 11 infection in human foreskin xenografts in athymic nude mice [7]. Kreider noted that "drug toxicity paralleled the therapeutic effects in rabbits but there was much less toxicity in athymic mice". Of (*S*)-HPMPA, PMEA and PMEG, the latter was the most active in inhibiting intraperitoneal P388 leukemia in mice [8]. The *in vivo* antitumor activity of PMEG was noteworthy as the compound was considered "representative of a new class of antitumor antimetabolites heretofore recognized only for their antiviral properties".

PMEG was then shown to owe its cytotoxic activity to the inhibitory effects of its diphosphate (PMEGpp) on the cellular DNA polymerases, particularly α, δ and ε [9], and the incorporation of PMEG into DNA by DNA polymerases δ and ε [10].

Holý reported on the inhibition of murine lymphocyte proliferation by $N^6$-substituted acyclic purine nucleoside phosphonates [11]. One of these $N^6$-substituted derivatives, cPr-PMEDAP [9-(2-phosphonylmethoxyethyl)-$N^6$-cyclopropyl-2,6-diaminopurine] was then found to block choriocarcinoma in rats, thereby acting as a prodrug of PMEG [12]. Apparently cPr-PMEDAP was converted to PMEG through an as yet unidentified cellular enzyme, as both adenosine deaminase and adenylate deaminase proved unable to deaminate cPr-PMEDAP [13]. Later on, Schinkmanová et al. [14,15] would identify the enzyme responsible for converting cPr-PMEDAP to PMEG. It would be the same

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enzyme as that responsible for converting abacavir 5′-monophosphate to carbovir 5′-monophosphate. Unlike cPr-PMEDAP that is converted to PMEG, PMEDAP would not be deaminated to PMEG but directly phosphorylated to PMEDAPp and PMEDAPpp [16].

In addition to cPr-PMEDAP, a few other $N^6$-substituted PMEDAP derivatives have been described [17], but they were not considered superior to the parental PMEDAP for the treatment of hematological malignancies. Yet, transdermal or topical application of cPr-PMEDAP has been considered as an attractive alternative route for the administration of this potentially useful antitumor/antiviral agent [18].

## 3. The origin of GS-9219

Non-Hodgkin's lymphoma (NHL) is the second fastest growing form of cancer and the fifth leading cause of cancer deaths in the US [19]. In 2006, the estimated number of deaths due to NHL in the US was 18,840 [20]. As, on the one hand, lymphoid malignancies served as the primary target, and, on the other hand, PMEG as an anticancer agent was limited by its poor cellular permeability and toxicity, especially for the kidney and gastrointestinal tract, the strategy developed at Gilead Sciences was to identify a PMEG prodrug that effectively loaded peripheral blood mononuclear cells (PBMCs) with PMEGpp and resulted in minimal plasma levels of PMEG [19]. This led to the selection of cPr-PMEDAP as the $N^6$-substituted prodrug of PMEG [12,13,16], which was then further equipped with a phosphonoamidate moiety to increase the efficiency of lymphoid cell loading, thus leading to the creation of a pro-prodrug (or double prodrug) of PMEG, GS-9219 (diethyl N,N′-[({2-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]ethoxy}methyl)phosphonoyl]di-L-alaninate) [19]. This compound would later also be known as VDC-1101 and rabacfosadine, and marketed by VetDC as Tanovea®-CA1. A novel, efficient one-pot synthesis of GS-9219 has been reported by Jansa et al. [21].

## 4. Intracellular metabolism of GS-9219 (Tanovea®)

GS-9219 is widely taken up by lymphoid cells, i.e. PBMCs, and intracellularly metabolized to cPr-PMEDAP (Fig. 1). The enzyme responsible for this hydrolysis is probably cathepsin A, a lysosomal carboxypeptidase, which is also responsible for the initial hydrolysis of TAF (tenofovir alafenamide, GS-7340) [3,22]. The $N^6$-substituted PMEDAP derivative cPr-PMEDAP would then be deaminated, apparently by the enzyme described by Schinkmanová et al. [14,15] to PMEG, which would yield PMEGpp, the active metabolite, through two consecutive phosphorylation steps (Fig. 1). The fact that cPr-PMEDAP and PMEGpp are the predominant GS-9219 metabolites in lymphocytes suggests that deamination rather than phosphorylation is the rate-limiting step in these cells [19]. Reiser et al. further noted that GS-9219, but not PMEG, depleted the germinal centers of lymphoid tissues of normal beagle dogs at doses that were tolerated.

## 5. GS-9219 (Tanovea®) in a pet dog with Non-Hogdkin's lymphoma (NHL)

Following the initial results of Hans Reiser et al. [19] on the efficacy of GS-9219 in five dogs with spontaneous NHL, a phase I/II trial was conducted in 38 pet dogs with naturally occurring NHL using different dose schedules of GS-9219. The compound was generally well tolerated and showed significant antitumor activity [23]: antitumor responses were observed in 79% of dogs, occurring in previously untreated dogs and dogs with chemotherapy-refractory NHL. A subset of dogs was evaluated with 3′-deoxy-3′-$^{18}$F-fluorothymidine positron emission tomography/computed tomography imaging before and after treatment (Fig. 2). The data showed a significant decrease of the tracer after treatment ($P = 0.016$). The compound was administered intravenously over a 30 min period, following 4 different schedules: (a) daily × 5 d every 21 d at 0.20 mg/kg or 0.29 mg/kg, (b) once every 7 d at 0.66 mg/kg or 0.82 mg/kg, (c) once every 14 d at 0.66 mg/kg or 0.82 mg/kg, and (d) once every 21 d at 0.66 mg/kg or 0.82 mg/kg. Dose-limiting toxicities were generally manageable and reversible and included dermatopathy, neutropenia, and gastrointestinal signs [23].

## 6. Efficacy of Tanovea® in canine T- and B-cell lymphoma and multiple myeloma

Tanovea® (alias VDC-1101 and GS-9219) was shown to be effective against spontaneous canine multiple myeloma at well-tolerated doses [24]. *In vitro*, it exhibited antiproliferative activity against several human multiple myeloma-derived cell lines (H-929, RPMI-8226, and U-266) (Fig. 3) [24]. *In vivo*, it proved effective against canine cutaneous T-cell lymphoma (CTCL), a malignant disease with a poor prognosis, where it achieved an objective response rate (ORR) of 45% [25].

The current standard of care for the treatment of dogs with multicentric lymphoma is combination chemotherapy including cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) [26]. Objective response rates (ORR) of ≥85% and median response durations of 6–10 months, and median response durations of 8–12 months have been reported with CHOP-based protocols (see Section 1 in Thamm et al. [27]). Most dogs that achieve remission after a CHOP-based chemotherapy protocol eventually relapse, with < 25% of dogs experiencing survival times > 2 years. Since treatment of lymphoma in dogs is not curative, owners may be hesitant to commit the time and financial resources necessary to complete a lengthy multidrug protocol [28].

Dogs with naïve multicentric lymphoma that received alternating Tanovea® (rabacfosadine) (1 mg/kg iv at weeks 0, 6, 12) and doxorubicin (30 mg/m$^2$ iv at weeks 3, 9, 15) showed an overall response rate of 84% and an overall median progression-free interval (PFI) of 194 days [27]. Alternating rabacfosadine/doxorubicin generally was well tolerated, with a PFI comparable to standard doxorubicin-based treatment regimens (CHOP), and fewer treatment visits. Most adverse events were mild and moderate and self-limiting. Fig. 4 illustrates the effect of the immunophenotype on the progression-free survival of dogs with naïve multicentric lymphoma following treatment with alternating rabacfosadine/doxorubicin [27].

In dogs with relapsing canine B-cell lymphoma, rabacfosadine, dosed at either 0.82 mg/kg or 1.0 mg/kg as a 30-min iv infusion every 21 days for up to 5 treatments, achieved an overall response rate of 74%, with 45% of dogs experiencing a complete response, with PFIs up to 203 days [29]. It was concluded that rabacfosadine is an effective treatment for dogs with B-cell lymphoma that relapsed following an initial doxorubicin-based chemotherapy regimen.

## 7. Perspectives

In general, chemotherapy protocols, i.e. CHOP, are first evaluated in humans before, if successful, to be applied in animals, i.e. pet dogs. Here, the compound Tanovea® was conditionally approved by the US FDA, without being pursued for its potential usefulness in humans. The question to be raised is whether the information gathered by the use of Tanovea in dogs would facilitate its application in the appropriate conditions in humans?

The compound has been indicated for the treatment of lymphoma in dogs. Its efficacy has been demonstrated in the treatment of NHL (Non-Hodgkin's lymphoma) and multiple myeloma. Should it also find utility in the chemotherapy of other malignant diseases in dogs and other pet animals?

For the treatment of HIV infections in human, ANPs are, as a rule, combined with other anti-HIV drugs, whereas for the treatment of HBV infections the antivirals are generally used as single drugs. For the treatment of malignancies, so as to reduce the likelihood of relapses, it may be advocated to use Tanovea in combination with other antitumor drugs, either simultaneously or sequentially (as has already been done

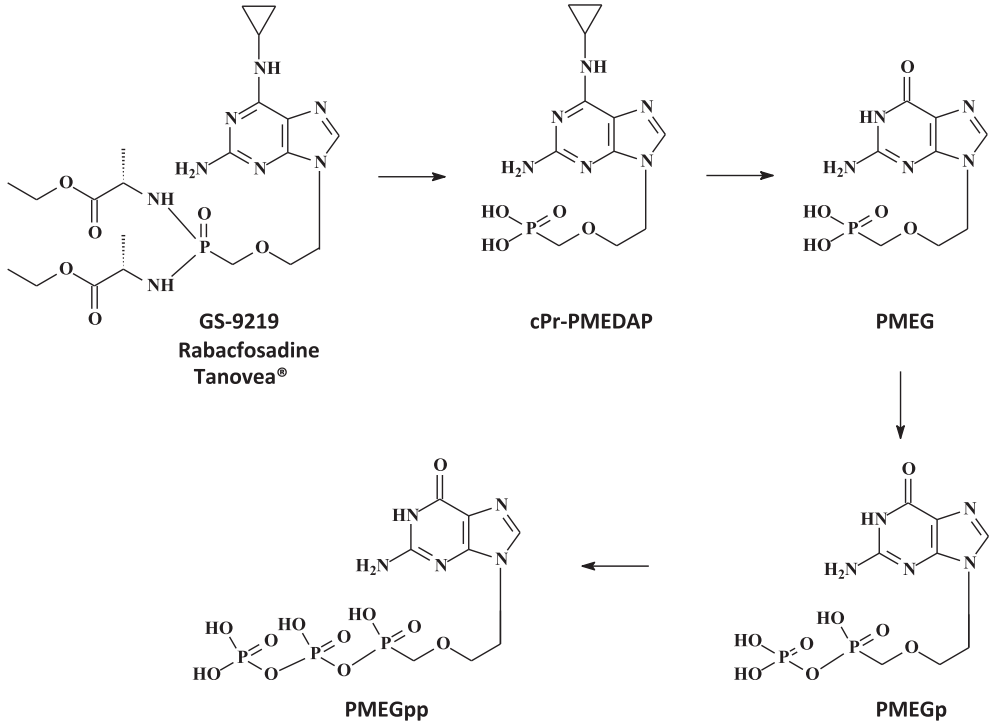


**Fig. 1.** Metabolism of GS-9219 (Tanovea®), *via* cPr-PMEDAP, to PMEG and its active form, PMEGpp, the diphosphate of PMEG. PMEGpp then inhibits the DNA polymerase(s) and/or is incorporated, as PMEG, into DNA [9,10].

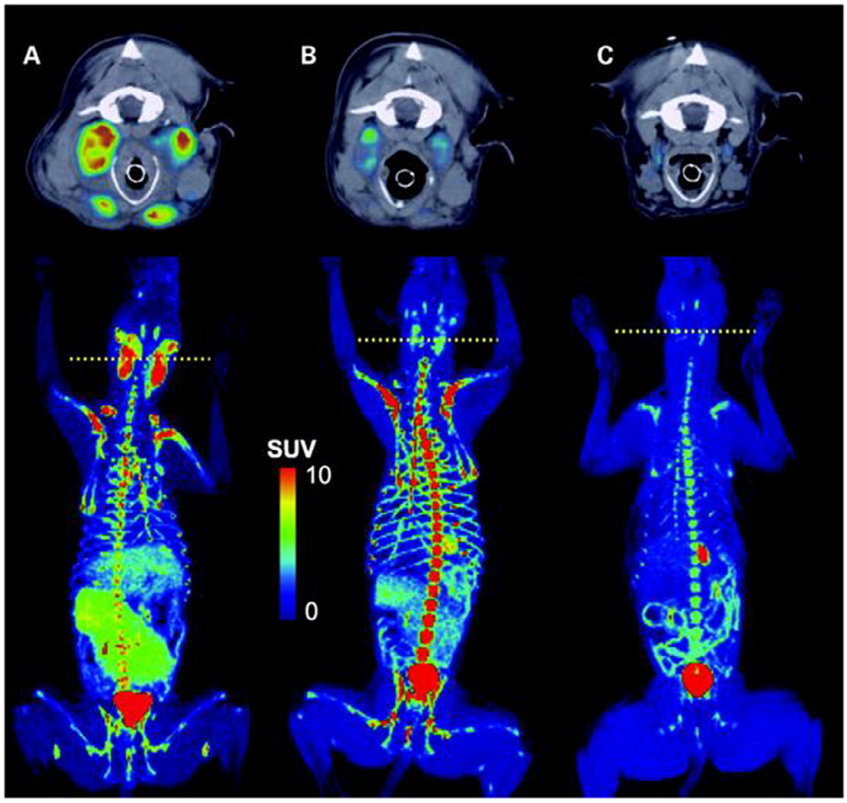


**Fig. 2.** Representative FLT-PET/CT of a dog with non-Hodgkin's lymphoma before and after GS-9219 treatment. FLT-PET/CT scan before (A) and 5 d after a single 0.66 mg/kg dose of GS-9219 (B) in a dog with stage V, B-cell non-Hodgkin's lymphoma. A third scan was done 3 wk following completion of five cycles of GS-9219 (C). Bottom, whole-body FLT-PET/CT scans at three time points; top, cross-sectional FLT-PET/CT. Whole-body PE scan before therapy (A) shows significant proliferative response in affected lymphoid tissues (popliteal, mesenteric, mediastinal, prescapular, submandibular lymph nodes, and spleen). Scans repeated 5 d after initial therapy (B) and 3 wk following completion of all treatment cycles (C) clearly indicate biological response as measured by significantly diminished uptake of tracers. Data taken from Vail et al. [23].

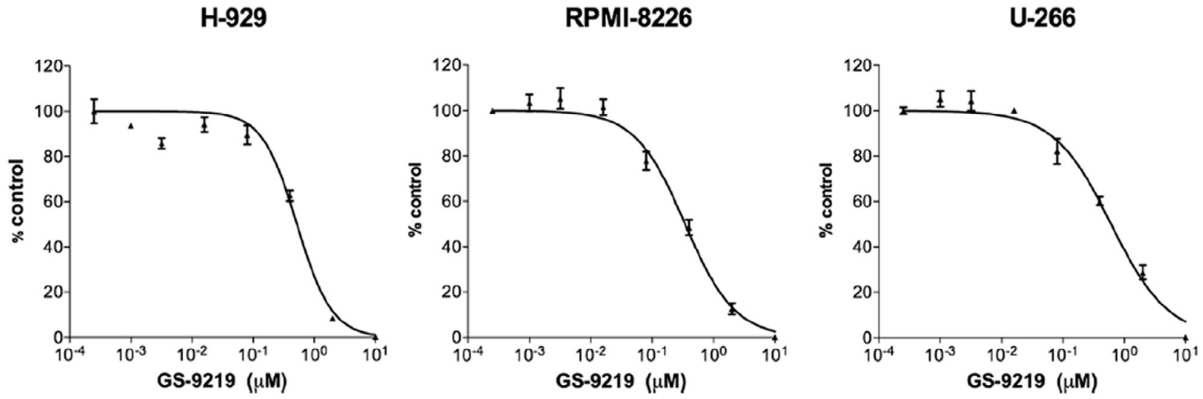


**Fig. 3.** *In vitro* antiproliferative effects of VDC-1101 (Tanovea®) against human multiple myeloma-derived cell lines. The three cell lines, designated H-929, RPMI-8226 and U-266, were incubated with varying concentrations of drug for 5 days, followed by determination of relative viable cell number using a luminescent cell viability assay. VDC-1101 (Tanovea®) demonstrated dose-dependent inhibition of cell growth in all cell lines examined. Error bars indicate SEM. Data taken from Thamm et al. [24].

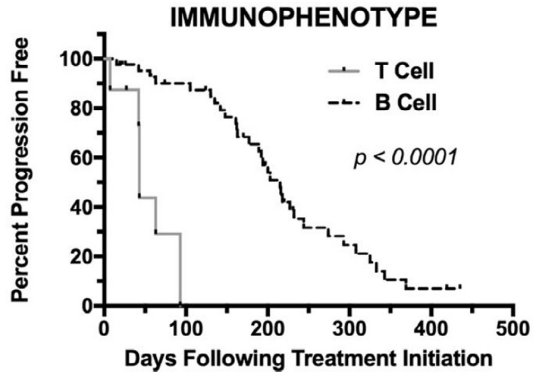


**Fig. 4.** Kaplan-Meier curves depicting effect of immunophenotype on progression-free survival (dogs with naïve multicentric lymphoma treated with alternating rabacfosadine/doxorubicin). P values indicate univariate log-rank values. Data taken from Thamm et al. [27].

with alternating rabacfosadine/doxorubicin [27]).

Tanovea has so far been used only for iv infusion, as GS-9219 was specifically formulated for this purpose. Yet, the use of a permeation enhancer DDAK (dodecyl 6-dimethylaminohexanoate) may provide an attractive alternative route of topical or transdermal administration for cPr-PMEDAP, the prodrug of PMEG to which GS-9219 is converted [18].

Finally, novel acyclic nucleotide analogues GS-343074 and GS-424044, both prodrugs of PMEG, for which the chemical structures were not revealed, have been reported to demonstrate antiproliferative and pro-apoptotic activity in canine neoplastic cell lines [30]. Hence, compounds similar to Tanovea® are at the horizon, which could be further explored for their potency and/or safety.

## 8. Conflict of interest

The author is co-inventor of Tanovea.

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## References

[1] E. De Clercq, A. Holý, Acyclic nucleoside phosphonates: a key class of antiviral drugs, Nat. Rev. Drug Discovery 4 (2005) 928–940.
[2] E. De Clercq, Where rilpivirine meets with tenofovir, the start of a new anti-HIV drug combination era, Biochem. Pharmacol. 84 (2012) 241–248.
[3] E. De Clercq, Role of tenofovir alafenamide (TAF) in the treatment and prophylaxis of HIV and HBV infections, Biochem. Pharmacol. (2018) in press.
[4] E. De Clercq, The acyclic nucleoside phosphonates (ANPs): Antonín Holý's legacy, Med. Res. Rev. 33 (2013) 1278–1303.
[5] E. De Clercq, A. Holý, I. Rosenberg, T. Sakuma, J. Balzarini, P.C. Maudgal, A novel selective broad-spectrum anti-DNA virus agent, Nature 323 (1986) 464–467.
[6] E. De Clercq, T. Sakuma, M. Baba, R. Pauwels, J. Balzarini, I. Rosenberg, A. Holý, Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines, Antiviral Res. 8 (1987) 261–272.
[7] J.W. Kreider1, K. Balogh, R.O. Olson, J.C. Martin, Treatment of latent rabbit and human papillomavirus infections with 9-(2-phosphonylmethoxy)ethylguanine (PMEG), Antiviral Res. 14 (1990) 51–58.
[8] W.C. Rose, A.R. Crosswell, J.J. Bronson, J.C. Martin, In vivo antitumor activity of 9-[(2-phosphonylmethoxy)ethyl]-guanine and related phosphonate nucleotide analogues, J. Natl. Cancer Inst. 82 (1990) 510–512.
[9] P. Kramata, I. Votruba, B. Otová, A. Holý, Different inhibitory potencies of acyclic phosphonomethoxyalkyl nucleotide analogs toward DNA polymerases alpha, delta and epsilon, Mol. Pharmacol. 49 (1996) 1005–1011.
[10] P. Kramata, K.M. Downey, L.R. Paborsky, Incorporation and excision of 9-(2-phosphonylmethoxyethyl)guanine (PMEG) by DNA polymerase delta and epsilon in vitro, J. Biol. Chem. 273 (1998) 21966–21971.
[11] A. Holý, Z. Zidek, I. Votruba, Inhibition of murine lymphocyte proliferation by $N^6$-substituted acyclic purine nucleoside phosphonates, Collect. Czech. Chem. Commun. 61 (1996) S182–S187.
[12] L. Naesens, S. Hatse, C. Segers, E. Verbeken, E. De Clercq, M. Waer, J. Balzarini, 9-(2-phosphonylmethoxyethyl)-$N^6$-cyclopropyl-2,6-diaminopurine: a novel prodrug of 9-(2-phosphonylmethoxyethyl)guanine with improved antitumor efficacy and selectivity in choriocarcinoma-bearing rats, Oncol. Res. 11 (1999) 195–203.
[13] S. Hatse, L. Naesens, E. De Clercq, J. Balzarini, $N^6$-cyclopropyl-PMEDAP: a novel derivative of 9-(2-phosphonylmethoxyethyl)-2,6-diaminopurine (PMEDAP) with distinct metabolic, antiproliferative, and differentiation-inducing properties, Biochem. Pharmacol. 58 (1999) 311–323.
[14] M. Schinkmanová, I. Votruba, A. Holý, $N^6$-methyl-AMP aminohydrolase activates N6-substituted purine acyclic nucleoside phosphonates, Biochem. Pharmacol. 71 (2006) 1370–1376.
[15] M. Schinkmanová, I. Votruba, R. Shibata, B. Han, X. Liu, T. Cihlar, A. Holý, Human $N^6$-methyl-AMP/DAMP aminohydrolase (abacavir 5'-monophosphate deaminase) is capable of metabolizing $N^6$-substituted purine acyclic nucleoside phosphonates, Collect. Czech. Chem. Commun. 73 (2008) 275–291.
[16] M.L. Compton, J.J. Toole, L.R. Paborsky, 9-(2-Phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine (cpr-PMEDAP) as a prodrug of 9-(2-phosphonylmethoxyethyl)guanine (PMEG), Biochem. Pharmacol. 58 (1999) 709–714.
[17] M. Valeriánová, I. Votruba, A. Holý, V. Mandys, B. Otová, Antitumour activity of N6-substituted PMEDAP derivatives against T-cell lymphoma, Anticancer Res. 21 (2001) 2057–2064.
[18] K. Vávrová, P. Kovaříková, B. Skolová, M. Líbalová, J. Roh, R. Cáp, A. Holý, A. Hrabálek, Enhanced topical and transdermal delivery of antineoplastic and antiviral acyclic nucleoside phosphonate cPr-PMEDAP, Pharm. Res. 28 (2011) 3105–3115.
[19] H. Reiser, J. Wang, L. Chong, W.J. Watkins, A.S. Ray, R. Shibata, G. Birkus, T. Cihlar, S. Wu, B. Li, X. Liu, I.N. Henne, G.H. Wolfgang, M. Desai, G.R. Rhodes, A. Fridland, W.A. Lee, W. Plunkett, D. Vail, D.H. Thamm, R. Jeraj, D.B. Tumas, GS-

9219–a novel acyclic nucleotide analogue with potent antineoplastic activity in dogs with spontaneous non-Hodgkin's lymphoma, Clin. Cancer Res. 14 (2008) 2824–2832.
[20] A. Jemal, R. Siegel, E. Ward, T. Murray, J. Xu, C. Smigal, M.J. Thun, Cancer statistics, 2006, CA Cancer J. Clin. 56 (2006) 106–130.
[21] P. Jansa, O. Baszczyňski, M. Dračínský, I. Votruba, Z. Zídek, G. Bahador, G. Stepan, T. Cihlar, R. Mackman, A. Holý, Z. Janeba, A novel and efficient one-pot synthesis of symmetrical diamide (bis-amidate) prodrugs of acyclic nucleoside phosphonates and evaluation of their biological activities, Eur. J. Med. Chem. 46 (2011) 3748–3754.
[22] G. Birkus, R. Wang, X. Liu, N. Kutty, H. MacArthur, T. Cihlar, C. Gibbs, S. Swaminathan, W. Lee, M. McDermott, Cathepsin A is the major hydrolase catalyzing the intracellular hydrolysis of the antiretroviral nucleotide phosphonoamidate prodrugs GS-7340 and GS-9131, Antimicrob. Agents Chemother. 51 (2007) 543–550.
[23] D.M. Vail, D.H. Thamm, H. Reiser, A.S. Ray, G.H. Wolfgang, W.J. Watkins, D. Babusis, I.N. Henne, M.J. Hawkins, I.D. Kurzman, R. Jeraj, M. Vanderhoek, S. Plaza, C. Anderson, M.A. Wessel, C. Robat, J. Lawrence, D.B. Tumas, Assessment of GS-9219 in a pet dog model of non-Hodgkin's lymphoma, Clin. Cancer Res. 15 (2009) 3503–3510.
[24] D.H. Thamm, D.M. Vail, I.D. Kurzman, D. Babusis, A.S. Ray, N. Sousa-Powers, D.B. Tumas, GS-9219/VDC-1101–a prodrug of the acyclic nucleotide PMEG has antitumor activity in spontaneous canine multiple myeloma, BMC Vet. Res. 10 (2014) 30.
[25] M.A. Morges, J.H. Burton, C.F. Saba, D.M. Vail, K.E. Burgess, D.H. Thamm, Phase II evaluation of VDC-1101 in canine cutaneous T-cell lymphoma, J. Vet. Intern. Med. 28 (2014) 1569–1574.
[26] D.M. Vail, M.E. Pinkerton, K.M. Young, Hematopoietic tumors, in: S.J. Withrow, D.M. Vail, R.L. Page (Eds.), Withrow & MacEwen's Small Animal Clinical Oncology, fifth ed., Elsevier, St. Louis, MO, 2013, pp. 608–678.
[27] D.H. Thamm, D.M. Vail, G.S. Post, T.M. Fan, B.S. Phillips, S. Axiak-Bechtel, R.S. Elmslie, M.K. Klein, D.A. Ruslander, Alternating rabacfosadine/doxorubicin: efficacy and tolerability in naïve canine multicentric lymphoma, J. Vet. Intern. Med. 31 (2017) 872–878.
[28] R.J. Mellanby, M.E. Herrtage, J.M. Dobson, Owners' assessments of their dog's quality of life during palliative chemotherapy for lymphoma, J. Small Anim. Pract. 44 (2003) 100–103.
[29] C.F. Saba, K.R. Vickery, C.A. Clifford, K.E. Burgess, B. Phillips, D.M. Vail, Z.M. Wright, M.A. Morges, T.M. Fan, D.H. Thamm, Rabacfosadine for relapsed canine B-cell lymphoma: efficacy and adverse event profiles of 2 different doses, Vet. Comp. Oncol. 16 (2018) E76–E82.
[30] J.A. Lawrence, M.K. Huelsmeyer, D.H. Thamm, D.B. Tumas, G. Birkus, I. Kurzman, D.M. Vail, Novel acyclic nucleotide analogues GS-343074 and GS-424044 demonstrate antiproliferative and pro-apoptotic activity in canine neoplastic cell lines, Vet. Comp. Oncol. 13 (2015) 246–254.