T cells and epigenetic drugs: A useful merger in cancer immunotherapy?

Jaydeep Bhat and Dieter Kabelitz*
Institute of Immunology; University of Kiel and UKSH Campus Kiel; Kiel, Germany

\(\gamma\delta\) T cell-based immunotherapeutic strategies in cancer patients are as yet of limited success. Drugs targeting epigenetic mechanisms including histone acetylation and DNA methylation trigger cell death in tumor cells but in addition have immunomodulatory activity. Here, we discuss the potential benefit of combining both strategies in cancer immunotherapy.

\(\gamma\delta\) T Cells: Limited T-Cell Receptor Diversity, Multiple Tumor Target Molecules

In view of their unique features, \(\gamma\delta\) T cells have recently raised substantial interest as a cellular target in cancer immunotherapy. Major subsets of human \(\gamma\delta\) T cells can be categorized on the basis of their expressed V\(\gamma\)/V\(\delta\) gene usage. V\(\gamma\)9V\(\delta\)2 T cells (hereafter termed V\(\delta\)2 T cells) dominate in the peripheral blood where they usually account for 50 to 95% of all \(\gamma\delta\) T cells.1 \(\gamma\delta\) T cells expressing other V\(\delta\) genes (mostly V\(\delta\)1 or V\(\delta\)3) that can be paired with various V\(\gamma\) elements are here collectively termed non-V\(\delta\)2 T cells. These non-V\(\delta\)2 T cells are usually localized in the mucosal tissues where they constitute a substantial proportion of intraepithelial lymphocytes.2 \(\gamma\delta\) T cells kill a broad range of tumor cells of epithelial origin as well as many leukemia/lymphoma cells in a T-cell receptor and/or Natural Killer Group 2 Member D (NKG2D)-dependent manner.3,6 V\(\delta\)2 T cells recognize tumor cells due to their enhanced production of isopentenyl pyrophosphate (IPP), an intermediate of the mevalonate pathway which is frequently dysregulated in tumor cells.7 Importantly, the IPP production can be easily manipulated by nitrogen-containing bisphosphonates (N-BP), drugs that are widely used in the clinics for the treatment of bone fragility disorders. N-BP inhibit an enzyme of the mevalonate pathway downstream of IPP synthesis, thereby inducing accumulation of IPP and enhanced sensitivity of tumor cells to V\(\delta\)2 T cell cytotoxicity.8 In addition to the T-cell receptor-dependent recognition of IPP, tumor cell killing by V\(\delta\)2 T cells can be triggered through antibody-dependent cellular cytotoxicity9 and tumor-targeting bispecific antibodies.10 Tumor surface-expressed antigens that are specifically recognized by the T-cell receptor of non-V\(\delta\)2 T cells include the endothelial protein C receptor11 and constitutively expressed NKG2D ligand MICA.12 Both V\(\delta\)2 and non-V\(\delta\)2 T cells are thus of interest for cancer immunotherapy.13

Clinical Application of Human \(\gamma\delta\) T Cells

Two strategies to exploit \(\gamma\delta\) T cells for immunotherapy can be envisaged, i.e., the in vivo activation of \(\gamma\delta\) T cells by selective stimulation, or the adoptive transfer of in vitro expanded \(\gamma\delta\) T cells. Wilhelm et al. were the first time to attempt in vivo activation of \(\gamma\delta\) T cells by giving N-BP (pamidronate) plus low-dose IL-2 to patients with lymphoid malignancies including relapsed/refractory low-grade non-Hodgkin lymphoma or multiple myeloma. They demonstrated the feasibility of this approach and reported some anti-lymphoma efficacy.14 Five years later, Kondo et al.15 established an efficient protocol for large-scale ex vivo expansion of...
γδ T cells from patients with advanced non-small cell lung cancer (NSCLC), bone metastatic breast or prostate cancer, or lung metastatic colorectal cancer. These two lines of clinical application have been explored by several groups since then in clinical phase I/II studies, including the combination of in vivo activation plus adoptive transfer of γδ T cells (Fig. 1A).

In vivo activation of γδ T cells by N-BP (mostly zoledronate) plus low-dose IL-2 has been investigated in various cancer entities. In a phase I clinical trial with zoledronate and IL-2 in patients with metastatic hormone-refractory prostate cancer, Dieli et al. observed in vivo activation of Vδ2 T cells that produced TNF-Related Apoptosis Ligand (TRAIL). In accordance, increased serum levels of TRAIL were present in the serum, and clinical responses were observed in some of the patients. Using a similar zoledronate plus low-dose IL-2-based protocol in a phase I study in advanced metastatic breast cancer, Meraviglia et al. observed a strong correlation between a clinical response (i.e., reduction of carcinoma progression) and the peripheral Vδ2 T cell numbers. A similar approach was extended to patients with metastatic renal cell carcinoma. Interestingly, however, the repeated administration of zoledronate and IL-2 reduced the proliferative capacity of Vδ2 T cells. This might in fact be related to an inhibitory effect of granulocytes on Vδ2 T cell activation that we observed in vitro upon uptake of N-BP by granulocytes. Taken together, administration of zoledronate plus low-dose IL-2 emerges as a novel, safe and feasible approach for γδ T cell-based immunotherapy. However, treatment regimens need to be optimized for the therapeutic in vivo augmentation of Vδ2 T cells. Moreover, potential adverse effects of the in vivo activation of Vδ2 T cells need to be carefully considered.

The adoptive transfer of zoledronate plus (in this case high dose) IL-2-activated and in vitro expanded γδ T cells was first applied by Abe et al. to six patients with multiple myeloma. No serious treatment-related adverse effects were observed. A detailed analysis revealed an increase in CD27−CD45RA− effector memory (TEM) Vδ2 T cell subset in the patients, and soluble NKG2D ligand MICA was

![Figure 1. An overview of human γδ T cell-based immunotherapies and epigenetic inhibitors used in the clinics.](image-url)
detected in the serum of some patients with elevated myeloma (M-) protein. Other studies performed e.g., in patients with NSCLC also supported the safety and feasibility of adoptive transfer of γδ T cells. Importantly, another phase I study in patients with advanced solid tumors performed by Nicol et al.\textsuperscript{23} provided preliminary clinical evidence for the migration of adoptively transferred γδ T cells to tumor sites. A systematic review of all clinical trials with γδ T cells using both treatment options (i.e., in vivo activation and/or adoptive transfer) was performed by Fisher et al.\textsuperscript{24} Their analysis of pooled data of 12 clinical trials with 157 patients highlighted the promising clinical efficacy of human γδ T cell immunotherapy. It is obvious, however, that a therapeutic benefit of γδ T cell-targeted immunotherapy might best be expected in combination with other therapeutic strategies.\textsuperscript{25}

Shedding of NKG2D ligands from the tumor cell surface is a known tumor escape mechanism, due to the inhibitory effect of soluble NKG2D ligands on NKG2D receptor expressing killer cells including γδ T cells.\textsuperscript{26,27} There is substantial heterogeneity in the mechanisms and the extent of NKG2D ligand shedding among various tumor entities, thus making a prediction of the consequences with respect to γδ T cell therapy in a given patient difficult.\textsuperscript{28} Therefore, a personalized approach with novel treatment regimens might be required to enhance the efficacy of γδ T cell-based therapy.

**Epigenetic Modifiers in The Clinic**

The epigenetic modification of protein expression based on the inhibition of enzymes that introduce and remove chromatin modifications is an established treatment option in oncology. Enzymes involved in chromatin modifications include DNA methyltransferases (DNMTs), histone lysine acetyltransferases (KATs), histone lysine methyltransferases (KMTs), histone lysine demethylases (KDMs), histone deacetylases (HDACs), protein arginine methyltransferases (PRMTs) as well as proline isomerases, kinases and ubiquitylases, many of which are represented by families of proteins.\textsuperscript{29}

One of the well-established epigenetic inhibitors used in clinical and basic research is 5-azacytidine (Aza-CR) and its derivative 5-aza-2′-deoxycytidine (5-AzaCdR). Both were synthesized by Piskala and Sorm\textsuperscript{30} and were tested as nucleoside antimetabolites with clinical application in acute myelogenous leukemia.\textsuperscript{31} Later on, Flatau et al.\textsuperscript{32} demonstrated that the concentration-dependent cytotoxic effect is related to the effects on DNA methylation. 5-AzaCdR (decitabine) is an established drug for the therapy of leukemias. In a study involving 130 patients with chronic myelogenous leukemia (CML), the therapeutic efficacy and toxicity of decitabine were analyzed in different stages of CML.\textsuperscript{33} Therapy with decitabine has also been extended to certain solid tumors. Several studies in patients with NSCLC have shown remarkable therapeutic efficacy.\textsuperscript{34,35} Based on available preclinical and clinical data, Karahoca and Momparler\textsuperscript{36} proposed a novel dose regimen for the use of decitabine in oncology. The molecular mechanism responsible for the efficacy varies substantially from inhibitor to inhibitor.\textsuperscript{37} Among others mechanism, decitabine induces apoptosis but also promotes differentiation, senescence and autophagy.\textsuperscript{38} Hence, after extensive studies on efficacy and safety, various analogs of DNMTs inhibitors have been recently approved as antitumor agents.

Most commonly known as antiepileptic drug, mood stabilizing agent and anti-convulsant drug, the prototypic HDAC inhibitor valproic acid represents another class of therapeutic epigenetic drugs. The generic name of valproic acid is Dipropylacetate (DPA), synthesized in 1881. However, its biological effect on leukocytes and platelets was studied in Sprague-Dawley Rats only in 1985.\textsuperscript{39} Several studies have shown that class I HDAC inhibitors such as valproic acid increase NK cell mediated killing of tumor cells primarily via the NKG2D-NKG2D ligand axis.\textsuperscript{40,41} Valproic acid exerts anti-tumor activity on multiple lymphoid malignancies like B cell-, T cell- and NK cell- lymphomas. The study revealed increased efficacy when combined with the proteasome inhibitor Bortezomib.\textsuperscript{32} Based on these results, valproic acid has been studied many fold in clinical trials. Ten patients with Castration-Resistant Prostate Cancer (CRPC) were included in a phase II trial performed by Sharma et al.\textsuperscript{43} This study revealed intolerance of valproic acid administration with significant levels of toxicity. The authors concluded that oral application of valproic acid should be avoided in CRPC patients. In contrast, however, valproic acid resulted in a tumor response and increased survival associated with enhanced tumor marker production and Notch1 activation in a pilot phase II study of low-grade neuroendocrine carcinoma.\textsuperscript{44} In view of such discrepancy, an interventional, randomized phase II study is currently ongoing in patients with breast cancer (NCT01900730). In this study, valproic acid is expected to stop cancer cell division and differentiation, thereby ultimately leading to less pleural fluid production. Considering the diverse molecular and physiological effects of epigenetic modifiers, treatment protocols have been designed combining potent inhibitors of both DNMT and HDAC (Fig. 1B).

Along this line, several reports revealed superior effects of combined epigenetic inhibitor application as opposed to single agents. In a phase I/II study in patients with advanced leukemia, the combination of 5-AzaCdR with valproic acid was safe and effective, and was associated with a transient reversal of aberrant epigenetic markers (NCT00075010).\textsuperscript{35} Leclercq et al.\textsuperscript{46} studied the anticaner effect of 5-AzaCdR combined with valproic acid in an aggressive tumor, malignant pleural mesothelioma. In addition to tumor cell death, they observed immunomodulatory effects at the level of cytokine production including interferon-γ and TGFβ expression of CD8+ cytotoxic T lymphocytes. A non-randomized, phase I study of low-dose DNMT inhibitor 5-AzaCtidine (Aza-CR) combined with HDAC inhibitor valproic acid in patients with advanced cancers was recently performed to evaluate adverse effects and maximum tolerated doses (Razelle Kurzrock, MD Anderson Cancer Centre, NCT00496444). The combination of decitabine and valproic acid turned out to be effective in patients...
with NSCLC, with limited neurological toxicity at relatively high dosage.\textsuperscript{47} Furthermore, a phase II study of Aza-CR in combination with HDAC inhibitor entinostat is ongoing in women with advanced triple negative or hormone-refractory breast cancer (Vered Stearns, Sidney Kimmel Comprehensive Cancer Centre, Australia and National Cancer Institute, USA; NCT01349959).

Taken together, targeting different pathways of epigenetic modification (DNMT, HDAC) promises to be more effective in cancer therapy than the inhibition of single pathways.

**γδ T Cells and Epigenetic Modifiers – A Useful Liaison?**

Cellular processes including differentiation and cell type-specific functions are determined by the digital language of covalent histone modification. A multitude of modifications act as guide for every cell fate decision, for sensing of microenvironmental signals and for cellular adaptation under pathophysiological conditions.\textsuperscript{48} Not surprisingly, this also applies to T cell differentiation. Accordingly, histone modification marks characterize early T cell development from multipotent progenitors to committed T cells,\textsuperscript{49} primary human leukocyte differentiation into CD4\textsuperscript{+}, CD8\textsuperscript{+}, CD14, CD16, CD19 subsets,\textsuperscript{50} memory T cell differentiation,\textsuperscript{51,52} but also transition from normal lymphocyte development into neoplastic transformation and lymphomagenesis.\textsuperscript{53}

With regard to tumor immunotherapy, the potential application of epigenetic drugs needs to be carefully evaluated. For instance, 5-Aza-2′-deoxycytidine or decitabine efficiently demethylate FoxP3 CpG loci and thereby induce a strong and stable suppressive activity of Treg which is unwanted in the context of cellular antitumor immunity.\textsuperscript{54} On the other hand, class I HDAC inhibitor entinostat was found to reduce FoxP3 expression and to reduce suppressive Treg activity associated with better efficacy of immunotherapies in murine cancer models.\textsuperscript{55} Moreover, HDAC inhibitors modulate CD4\textsuperscript{+} T cell polarization in vitro and in vivo\textsuperscript{56} which again might have an impact on tumor immunity. So far, limited information is available on the epigenetic signature of γδ T cells. A recent study has shed some light on the epigenomic analysis of IL-17-producing CD27\textsuperscript{−} and IFNγ-producing CD27\textsuperscript{−} murine γδ T cells. The detailed analysis emphasizes the effect of subset-specificity on key transcriptional regulators of differentiation and peripheral function in murine thymus-derived γδ T cells.\textsuperscript{57} It is also known that IFNγ production in splenic γδ T cells is controlled through hypomethylation with simultaneous requirement for Eomes and T-bet transcription factors.\textsuperscript{58} Our own preliminary studies reveal an overall differential methylation status of human peripheral blood γδ T cells as compared to αβ T cells. Furthermore, we noted a selective modulation of some surface markers when activated human γδ T cells are treated with valproic acid (Bhat et al., unpublished).

Given the constitutive expression of the activating NKG2D receptor on virtually all γδ T cells, enhanced and/or stable expression of NKG2D ligands on tumor cells should greatly facilitate their killing by γδ T cells. NKG2D ligands are highly methylated and thus transcriptionally suppressed in many lymphoma and tumor cells.\textsuperscript{59} Consequently, treatment with 5-aza or decitabine restores NKG2D ligand expression.\textsuperscript{59} Similarly, NKG2D ligand expression is induced or enhanced on diverse tumor entities including liver cancer, osteosarcoma, pancreatic carcinoma, myeloma, leukemias by HDAC inhibitors.\textsuperscript{60-64} Importantly, increased surface expression of NKG2D ligands occur in the absence of enhanced shedding which might negatively interfere with NKG2D receptor activation.\textsuperscript{62} Thus, we consider the increase of NKG2D ligand expression by epigenetic drugs a straightforward and doable approach to enhance γδ T cell immunotherapy.

At this stage it is unclear, however, how epigenetic drugs will affect the functional program of human γδ T cells, an issue that requires further investigation. NKG2D is demethylated in NK cells and CD8\textsuperscript{+} T cells (and presumably in γδ T cells as well, although this was not explicitly studied here).\textsuperscript{65} Importantly, treatment with the histone acetyltransferase inhibitor Curcumin reduced NKG2D transcription and reduced the lytic capacity of a NK cell line,\textsuperscript{65} which again would be a serious disadvantage in the setting of γδ T cell immunotherapy. Based on all these considerations, we believe that treatment of cancer patients with epigenetic drugs combined with an adoptive transfer of in vitro expanded γδ T cells currently holds the best promise for a useful liaison.

**Concluding Remarks**

γδ T cells are important players in cellular immunotherapy. Accumulating evidence suggests that γδ T cell immunotherapy needs to be combined with other strategies for improved clinical results. Among others, these additional strategies might include chemotherapeutics, antibody constructs, and epigenetic drugs. While the impact of epigenetic drugs on human γδ T cell transcriptional regulation and functions needs to be investigated, the proposed two-step approach (treatment with epigenetic drugs plus adoptive transfer of in vitro expanded γδ T cells) is ready for clinical evaluation.

**Disclosure of Potential Conflicts of Interest**

This work forms part of the PhD thesis of JB. There are no other potential conflicts of interest.

**Funding**

Work from the authors laboratory is supported by the Cluster of Excellence “Inflammation-at-Interfaces” (EXC 306 Project N) funded by the Deutsche Forschungsgemeinschaft.

**References**

1. Hinz T, Wesch D, Halay F, Marx S, Choudhary A, Aeden B, Janssen O, Bonneville M, Kabatitz D. Identification of the complete expressed human TCR V gamma repertoire by flow cytometry. Int Immunol 1997; 9:1065–72; PMID:9263003; http://dx.doi.org/10.1093/intimm/9.8.1065

2. Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. Nat Rev Immunol 2013; 13:88–100; PMID:23348415; http://dx.doi.org/10.1038/nri3384

3. Wrobel P, Shojaei H, Schiteck B, Giseler F, Wollenberg B, Kalthoff H, Kabatitz D, Wesch D. Lysis of a broad range of epithelial tumour cells by human γδ T cells: involvement of NKG2D ligands and T-cell
16. Dieli F, Vermijlen D, Fulfaro F, Caccamo N, Meraviello A, et al. Targeting human γδ T cells in antitumor immunity. J Exp Med 2003; 197:163–8; PMID:12538656; http://dx.doi.org/10.1084/jem.2002172296

17. Meraviglia S, Eberl M, Vermijlen D, Todorao M, Bucci S, Caccio G, La Mendola C, Guggino G, D’Auro M, O’Hare GM. In vitro manipulation of Vγ9Vδ2 T cells with zoledrone and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. Clin Exp Immunol 2010; 161:290–7; PMID:20491785; http://dx.doi.org/10.1111/j.1365-2179.2010.03617.x

18. Lang J, Kajokob M, Wallace M, Staab M, Horvath D, Wilding G, Liu G, Eichkoff J, McNeel D, Malkovsky M. Pilot trial of interleukin-2 and zoledronic acid to augment γδ T cells as treatment for patients with refractory renal cell carcinoma. Cancer Immunol Immunother 2011; 60:1447–60; PMID:21647691; http://dx.doi.org/10.1007/s00262-011-1049-8

19. Kalyan S, Chandrasekaran V, Quabuis E, Lindhorst T, Kabelitz D. Neutrophil uptake of nitrogen-bisphosphonates leads to the suppression of human peripheral blood γδ T cells. Cell Mol Life Sci 2014; 71:2325–46; PMID:24162933; http://dx.doi.org/10.1007/s00018-014-1495-x

20. Kuznetsov X, Smetak M, Kimmig B, Weigang-Koeberl K, Goebele M, Birkenm J, Becker J, Schmide-Wolf I, Einsele H, Willem M. Tumor-promoting versus tumor-antagonizing roles of γδ T cells in cancer immunotherapy: results from a prospective phase III trial. J Immunother 2009; 32:320; PMID:23300909; http://dx.doi.org/10.1097/JI.0b013e3182435b1b

21. Abe Y, Muto M, Nieda M, Nakagawa Y, Nicola A, Kaneko T, Goto S, Yokogawa K, Suzuki K. Clinical and immunological evaluation of zoladroxate-activated Vγ9Vδ2 T cell-based immunotherapy for patients with multiple myeloma. Exp Hematol 2009; 37:956–8; PMID:19409955; http://dx.doi.org/10.1016/j.exphem.2009.04.008

22. Nakajima J, Murakawa T, Fukami T, Goto S, Kaneko T, Yodoi J, Segawa M, Saitoh K, Kimura H. A phase I trial of adoptive immunotherapy for recurrent non-small-cell lung cancer patients with autologous γδ T cells. Eur J Cardiothorac Surg 2010; 37:1191–7; PMID:20931378; http://dx.doi.org/10.1093/ejcts/ezq076

23. Nicola A, Tokayama H, MatarrHallo S, Hagi T, Suzuki K, Yokokawa K, Nieda M. Clinical evaluation of autologous γδ T cell-based immunotherapy for metastatic solid tumors. Br J Cancer 2011; 105:1784–91; PMID:21847128; http://dx.doi.org/10.1038/bjc.2011.293

24. Fisher J, Heuijerjens J, Yan M, Gustafsson K, Anderson P, Amsz J, et al. DNA methylation in 5-Aza-2’-deoxycytidine-resistant var-iants of C3H 1OT1/2 C18 cells. Mol Cell Biol 1984; 4:2098–102; PMID:2609555; http://dx.doi.org/10.1111/j.1365-2966.2009.tb05968.x

Cakir A. Biological effects of 5-azacytidine in eukaryotes. Oncology 1974; 30:405–2; PMID:4142650; http://dx.doi.org/10.1159/0000229481

Flatau E, Gonzales F, Michalowsky L, Jones D. DNA methylation in 5-Aza-2’-deoxycytidine-resistant variants of C3H 1OT1/2. C18 cells. Mol Cell Biol 1984; 4:2098–102; PMID:2609555; http://dx.doi.org/10.1111/j.1365-2966.2009.tb05968.x

Kanzian JH, O’Brien S, Cortes J, Giles F, Faderl S, Issa J, Garcia-Manero G, Rios M, Shan J, Andreff M, et al. Results of decitabine (5-aza-2’-deoxycytidine) therapy in 130 patients with chronic myelogenous leukemia. Cancer 2003; 98:522–8; PMID:12879469; http://dx.doi.org/10.1002/cncr.11641

Mompour R, Boulard D, Mompour L, Dionne J, Belanger K, Ayoub J, Pilot phase I-II study on 5-aza-2’- deoxycytidine (decitabine) in patients with metastatic lung cancer. Anticancer Drugs 1997; 8:358–68; PMID:9200889; http://dx.doi.org/10.1097/00001813-199704000-00008

Mompour R. Epigenetic therapy of non-small cell lung cancer using decitabine (5-aza-2’-deoxycytidine). Front Oncol 2013; 3:188; PMID:23908569; http://dx.doi.org/10.3389/fonc.2013.00188

Karakasheva M, Mompour R. Pharmacokinetic and pharmacodynamic analysis of 5-aza-2’-deoxycytidine (decitabine) in the design of its dose-schedule for cancer therapy. Clin Epigenetics 2013; 5:3; PMID:23639223; http://dx.doi.org/10.1186/2041-1243-5-3

Streensam C, Brueckner B, Musch T, Stopper H, Lyko F. Functional diversity of DNA methyltransferase inhibitors in human cancer cell lines. Cancer Res 2006; 66:5784–800; PMID:16516061; http://dx.doi.org/10.1158/0008-5472.CAN-05-2821

Schnekenburger M, Grandjenette C, Gheffl J, Karius T, Folgiate B, Miedier D. Miedier S. Supported exposure to the DNA demethylating agent, 5-aza-2’-deoxycytidine leads to apoptosis in chronic myeloid leukemia by promoting differentiation, senescence, and autophagy. Biochem Pharmacol 2011; 81:364–78; PMID:21044612; http://dx.doi.org/10.1016/j.bcp.2010.10.013

Abedini S, Aghaie C, Naziforouz A, Basistdas-Ramirez B, Mota-Navarro M, Gonzalez J. Pathway-specific effects of 5-aza-2’-deoxycytidine (decitabine) on leukocytes and platelets of Sprague-Dawley rats. Gen Pharmacol 1985; 16:423–6; PMID:3930436; http://dx.doi.org/10.1016/0306-3623(85)90210-1

Suzuki T, Terao A, Achiwa R, Nase M, Yamamoto S, Okamura H, Gotoh A. The antimitotic effect of 5-aza T cells is enhanced by valproic acid-induced up-regulation of NKG2D ligands. Anti-Cancer Res 2010; 30:4509–14; PMID:21119908

Chavez-Blancano A, De La Cruz-Hernandez E, Domi-quez G, Rodriguez-Cortez O, Alanorte B, Perez-Car-denas E, Chacon-Salinas R, Trejo-Becerril C, Tajay-Chelay L, Trujillo JE, et al. Upregulation of NKG2D ligands and enhanced natural killer cell cytotoxicity by histone deacetylase and valproate. Int J Oncol 2011; 39:1491– 9; PMID:21805029; http://dx.doi.org/10.3892/ijo.2011.1144

Iwata S, Saito T, Ito Y, Kamakura M, Gotoh K, Kawada J, Nishiyama Y, Kimura H. Antitumor activities of valproic acid on Epstein-Barr virus-associated T and natural killer lymphoma cells. Cancer Sci 2012; 103:375–81; PMID:22173677; http://dx.doi.org/10.1111/j.1349-7006.2011.02127.x

Sharma S, Symanskiowa J, Wang B, Dino P, Manza P, Vogelzang N. A phase II clinical trial of oral valproic
acid in patients with castration-resistant prostate cancers using an intensive biomarker sampling strategy. Transl Oncol 2008; 1:141–7; PMID:18795124; http://dx.doi.org/10.1593/doi:08136

44. Muhammed T, Holen K, Jaska-Smil M, Mulkern D, Lubner S, Schelman W, Eickhoff J, Chen H, Locante N. A pilot II phase study of valproic acid for treatment of low-grade neuroendocrine carcinoma. Oncologist 2011; 16:835–43; PMID:21632454; http://dx.doi.org/10.1612/j.oncol.2011-00317.002

45. Garcia-Manero G, Kantarjian H, Sanchez-Gonzalez B, et al. A genome-wide analysis of histone methylation reveals polymerase state-based regulation of gene transcription and function of memory CD8+ T cells. Immunity 2009; 30:912–5; PMID:19523850; http://dx.doi.org/10.4161/epi.23115

46. Ramming A, Drude D, Leipe J, Schulze-Koops H, Ska- penko A. Maturation-related histone modifications in the PU.1 promoter regulate Th9 cell development. Blood 2012; 119:4665–74; PMID:22454686; http://dx.doi.org/10.1182/blood-2012-02-392589

47. Viscus C, Wasserkott R, Lesche R, Dong J, Stein T, Thié A, Eckhardt F. DNA methylation profiling of transcription factor genes in normal lymphocyte development and lymphomas. Int J Biochem Cell Biol 2007; 39:1523–38; PMID:17453759; http://dx.doi.org/10.1016/j.biocel.2007.02.006

48. Lai G, Zhang N, van der Touw W, Ding Y, Ju W, Bot- tenger E, Reid S, Levy D, Bromberg J. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J Immunol 2009; 182:259–73; PMID:19109157; http://dx.doi.org/10.4161/25051

49. Laume R, Wu X, Tao Y, Hou J, Meng X, Shi J. Valproic acid sensitizes pancreatic cancer cells to natural killer cell-mediated lysis by upregulating MICA and MICB via the PI3K/Akt signaling pathway. BMC Cancer 2014; 14:370; PMID:24885711; http://dx.doi.org/10.1186/1471-2407-14-370

50. Shi P, Yin T, Zhai F, Cui P, Gos W, et al. Sodium valproate and its impact on the susceptibility of leukemic cells to the cytotoxicity of NKGD2-expressing cells. Leukemia 2007; 21:2103–8; PMID:17625602; http://dx.doi.org/10.1038/leu.2004862

51. Armeanu S, Bitzer M, Lauer U, Ventura S, Pathil A, et al. Natural killer cell-mediated lysis of hepatoma cells via specific induction of NKGD2 ligands by the histone deacetylase inhibitor sodium valproate. Cancer Res 2005; 65:6321–9; PMID:16024634; http://dx.doi.org/10.1158/0008-5472.CAN-04-4252

52. Fernández-Sánchez A, Baragaño Raneros A, Carvajal Palao R, Sanz A, Ortiz A, Artega F, Suarez-Alvarez B, Lopez-Larrea C. DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKGD2 gene in human CD8+ T and NK cells. Epigenetics 2013; 8:66–78; PMID:23255109; http://dx.doi.org/10.4161/epi.23115