Rationale behind using valproic acid for Non-Hodgkin lymphoma: a biomolecular perspective

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CONCLUSIONS: Based on its biomolecular mechanism of action, VPA appears to be a promising initial treatment before initiating the standard treatment in patients with NHL to overcome resistance.

Key Words: Epigenetics, Histone deacetylase inhibitors, Lymphoma, Non-Hodgkin lymphoma, Valproic acid.

Abstract. – OBJECTIVE: Non-Hodgkin lymphoma (NHL) is a hematological malignancy with a high rate of relapse and refractory cases. It is believed to be caused by resistance to standard treatment modalities. Valproic acid (VPA), previously used as a broad-spectrum anticonvulsant drug, has been proposed for NHL owing to its action of epigenetic modification by inhibiting histone deacetylase. However, VPA studies on NHL are limited. This review describes the rationale behind the use of VPA for NHL treatment, particularly focusing on its molecular mechanism of action.

MATERIALS AND METHODS: This is a narrative review. The literature search strategy for indexed Scopus articles was performed randomly using PubMed and MEDLINE as the primary sources. No specific term was used.

RESULTS: Several mechanisms are responsible for NHL development. VPA can modulate these mechanisms via epigenetic and nonepigenetic modifications. It may also have an impact on the proteins responsible for treatment resistance. The mechanisms of action of VPA in NHL are as follows: the induction of cell cycle arrest via the upregulation of cyclin-dependent protein kinase inhibitors; induction of Apo2 ligand or tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis; inactivation of B-cell lymphoma 6; inhibition of Janus kinase/signal transducer and activator of transcription, phosphoinositide 3-kinase/Akt, and nuclear factor kappa B signaling pathways; upregulation of tumor antigen as the primary target of immunotherapy; and strengthening of tumor immunosurveillance.

CONCLUSIONS: Based on its biomolecular mechanism of action, VPA appears to be a promising initial treatment before initiating the standard treatment in patients with NHL to overcome resistance.

Key Words: Epigenetics, Histone deacetylase inhibitors, Lymphoma, Non-Hodgkin lymphoma, Valproic acid.

Introduction

In 2021, 81,560 estimated cases of incidence and 20,720 estimated cases of mortality have been associated with Non-Hodgkin lymphoma (NHL), a hematological malignancy that is treatable but relatively incurable1. Some patients with NHL experience relapse and are resistant to existing standard therapies2-5. The estimated 5-year survival rate for these patients was 73.2% from 2011 to 20171.

The primary concern regarding NHL treatment is achieving an optimal cure while improving the clinical outcome. Several studies6-9 have been conducted to resolve this concern. Epigenetics is one of the most investigated domains for developing an optimal treatment for NHL because epigenetic dysregulation has been suggested to play a role in lymphomagenesis10.

Several histone deacetylases (HDAC)-inhibiting epigenetic drugs have been studied as po-
tential treatment options for NHL; these drugs may overcome epigenetic dysregulation. Some examples of HDAC inhibitors (HDACis) include romidepsin, vorinostat, chidamide, belinostat, valproic acid (VPA), and others. HDACis can inhibit HDACs that are overexpressed in lymphoma cells. These enzymes are important for regulating certain protein expressions involved in cell growth, proliferation, and survival.

VPA is more accessible and affordable than other HDACis because it is commonly used as a broad-spectrum anticonvulsant drug, mood stabilizer, and migraine prophylactic. Drott et al reported that VPA in combination with rituximab, cyclophosphamide, hydroxydaunorubicin, oneovin, and prednisone (R-CHOP) showed a positive outcome and tolerable safety level in patients with diffuse large B-cell lymphoma (DLBCL) in a phase 1 clinical trial. This trial promises beneficial outcomes for DLBCL. A deeper understanding of the biomolecular aspects of VPA for NHL will help understand the potential of this repurposed drug. This review describes the possibility of using VPA for overcoming resistance to standard treatments in NHL.

**Epigenetic Modification of Non-Hodgkin Lymphoma**

Epigenetic alteration, which is partly involved in the progression of lymphoma, has become the focus of many scientists aiming to treat malignant lymphoma. Epigenetic modification is a process through which gene expression is altered without any changes in the DNA sequence. Three processes are involved in epigenetic modification: DNA methylation by DNA methyltransferases, histone modification by histone acetyltransferases (HATs) and HDACs, and translational repression by noncoding RNA, including microRNA.

Gene expression in epigenetic modification is dependent on chromatin conformations: heterochromatin and euchromatin. Heterochromatin contains many inactive genes that are silenced, whereas euchromatin contains active genes that are accessible for binding by certain transcription factors. Silencing of tumor suppressor genes, DNA repair proteins, and cell cycle control proteins or enzymes plays an important role in lymphomagenesis.

Highly condensed chromatin can repress transcription. This chromatin conformation is supported by the methylation of DNA, primarily at the CpG island, by DNA methyltransferases (DNMT). The methylation of histone tails by the enhancer of zeste homolog 2 (EZH2), a catalyst that adds a methyl group to a histone protein, and deacetylation of histone tails by HDACs promote the heterochromatin topology. DNMT and EZH2 inhibitors can alter highly condensed chromatin into loose chromatin (euchromatin). By contrast, the transcriptionally permissive state of chromatin is endorsed by HDACis and HAT.

Genes encoding HATs, such as CREBBP and EP300, undergo a loss-of-function mutation in 25% of patients with DLBCL and in 60% of patients with follicular lymphoma. This mutation leads to the silencing or repression of some proteins that are essential for inhibiting lymphoma progression. The loss or mutation of CREBBP or EP300 is an important biomarker for identifying patients with lymphoma that can receive HDACis.

**Role of HDACs in NHL**

HDACs are enzymes that catalyze the removal of acetyl groups from the lysine and arginine residues of histones. They can modify histone and several nonhistone proteins in some organisms. Certain proteins that function as apoptotic regulators, including p53 and B cell lymphoma 6 (Bcl-6), have been reported to be directly acetylated by HDACs. Aberrant HDAC activity can alter gene expression and some cellular pathways related to cell death, growth, or survival.

There are four classes of HDACs in the human body, which are categorized based on their function and DNA sequence (Table I). HDACs can regulate certain proteins involved in tumor progression; thus, the presence or overexpression of some HDACs is associated with poor survival in NHL.

Several HDACs may contribute to the inhibition of cell death and progression of the lymphoma cell cycle. HDACs also interfere with immunosurveillance, thereby playing an important role in the growth of lymphoma cells. Furthermore, HDAC6, a class IIb enzyme, can impair CD20 expression as a specific target for immunotherapy, such as that with rituximab; therefore, rituximab resistance may occur in lymphoma cells.

**HDACi for NHL Treatment**

In NHL, HDAC inhibition might be a target for certain treatments. HDACis are epigenetic drugs whose action is mediated by chromatin remodeling via histone modification and by acetylating the nonhistone proteins that impact tumor cell growth and survival. HDACis can alter the expression of several genes involved in lymphomagenesis, thereby promoting tumor cell death, growth arrest, differentiation, and senescence as well as inhibiting angiogenesis.
Several HDACis have been approved by the US Food and Drug Administration (FDA) for treating hematological malignancies. HDACis that have been approved by the US FDA include vorinostat for cutaneous T-cell lymphoma (CTCL; 2006), romidepsin for CTCL (2009) and peripheral T-cell lymphoma (PTCL; 2011), belinostat for PTCL (2014), and panobinostat for multiple myeloma (2015). Each drug generally improves patient outcomes with a modest level of activity. When an HDACi is combined with another chemotherapeutic treatment, it demonstrates better efficacy but with a high rate of moderate-to-severe adverse events. Therefore, therapies combining various HDACis with other treatments should be broadly investigated in clinical trials to determine their efficacy and safety in NHL before approval.

Some HDACis that exhibit antitumor activity in lymphoma by inhibiting HDACs are shown in Table II. Several HDACis have been approved by the FDA for T-cell lymphomas, although some are under investigation for B-cell lymphomas.

**VPA**

VPA has been commonly used as a broad-spectrum anticonvulsant drug, mood stabilizer, and migraine prophylactic. It was first synthesized from *Valeriana officinalis* by Burton. VPA is a short-chain branched fatty acid administered orally or intravenously. VPA has been reported to exert its epigenetic modulation effect via the inhibition of HDAC and help treat various malignancies.

The bioavailability of VPA is >80% after oral administration, and its peak blood concentration is observed within 2 h. Several pathways are employed in VPA metabolism in the human body, including glucuronidation, β-oxidation, and cytochrome P450 (CYP)-mediated oxidation pathways. Oxidation involving cytochrome P450 is employed by only 10% of VPA metabolism pathways. The half-life of VPA varies significantly from 9 to 18 h.

As an antiseizure drug, an appropriate VPA dose for common use is initially 15 mg/kg, with slow titration to the therapeutic dose. Dosages of 25-30 mg/kg/day and even 60 mg/kg/day may be required for some patients. In plasma, the therapeutic levels of VPA should be 350-700 µM for continuous VPA treatment in patients with epilepsy. VPA dose for NHL treatment is relatively different from that used as an antiseizure, which is explained further in the next section.

**Mechanism of Action of VPA**

The mechanism of action of VPA for NHL is shown in Figure 1. Several signaling pathways are affected by VPA. It functions through HDACs.

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**Table I.** Histone deacetylase classification and location.

<table>
<thead>
<tr>
<th>Class</th>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>HDAC1, HDAC2, HDAC3, HDAC8</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Class Ia</td>
<td>HDAC4, HDAC5, HDAC7, HDAC9</td>
<td>Nucleus and cytoplasm</td>
</tr>
<tr>
<td>Class Ib</td>
<td>HDAC6, HDAC10</td>
<td>Cytoplasm</td>
</tr>
<tr>
<td>Class III</td>
<td>SIRT 1-7</td>
<td>Nucleus and cytoplasm</td>
</tr>
<tr>
<td>Class IV</td>
<td>HDAC11</td>
<td>Nucleus and cytoplasm</td>
</tr>
</tbody>
</table>

Abbreviations: HDAC: histone deacetylase; SIRT: Sirtuin.

**Table II.** HDACis in clinical trials for various hematological malignancies.

<table>
<thead>
<tr>
<th>HDACi</th>
<th>HDAC target</th>
<th>Trial phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat[19,31,36–38]</td>
<td>Class I, II, and IV</td>
<td>Marketed CTCL, phase II FL, MZL, MCL, HL</td>
</tr>
<tr>
<td>Romidepsin[17,32,33,39]</td>
<td>Class I, II, and III</td>
<td>Marketed CTCL, PTCL</td>
</tr>
<tr>
<td>Belinostat[40–41]</td>
<td>Class I, II, and IV</td>
<td>Marketed PTCL, phase II B-cell lymphoma</td>
</tr>
<tr>
<td>Panobinostat[42–47]</td>
<td>Class I–IV</td>
<td>Phase III MM, phase II CTCL, DLBCL</td>
</tr>
<tr>
<td>Mocetinostat[48–50]</td>
<td>Class I and IV</td>
<td>Phase II DLBCL, FL, HL</td>
</tr>
<tr>
<td>Valproic acid[51,52]</td>
<td>Class II (except HDAC8 and 9)</td>
<td>Phase II DLBCL</td>
</tr>
<tr>
<td>Chidamide[53]</td>
<td>HDAC 1, 2, 3, 10</td>
<td>Phase I/II B-NHL (ongoing), phase II PTCL</td>
</tr>
<tr>
<td>Abexinostat[54,55]</td>
<td>Class I and II</td>
<td>Phase II CLL, B-NHL, T-NHL</td>
</tr>
<tr>
<td>Quisinostat[54,55]</td>
<td>HDAC 1, 2, 3, 11</td>
<td>Phase II CTCL</td>
</tr>
<tr>
<td>Entinostat[56,57]</td>
<td>Class I–IV</td>
<td>Phase II HL, leukemia</td>
</tr>
</tbody>
</table>

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Rationale behind using valproic acid for Non-Hodgkin lymphoma: a biomolecular perspective

VPA Induces Cell-Cycle Arrest and Apoptosis by Upregulating p21 and p27

The survival of tumor cells depends on the cell cycle control system. The cell cycle is regulated by cyclin-dependent protein kinases (Cdks). Cyclins in a particular phase of the cell cycle can activate Cdks and induce cell cycle entry progression. Cdks inhibitor proteins (CKIs) comprising p27, p21, and p16 can limit cell cycle progression by suppressing G1/S-Cdk and S-Cdk activities after DNA damage, leading to the cessation of the cell cycle.

Newbold et al. reported that p21 (CDKN1A) does not contribute to cell cycle arrest or apoptosis. However, high expression of p27 (CDKN1B) has been reported to induce apoptosis following G1 cell cycle arrest in a mouse model of B-cell lymphoma. This elevated expression occurred at the translational rather than at the transcriptional level. A previous study indicated that an HDACi (romidepsin) upregulated p21 and promoted cell cycle arrest in mantle cell lymphoma (MCL). However, the data from this in vitro study were inconsistent. An in vivo study clarified these results by demonstrating that p21 is not essential in lymphomagenesis, although the aforementioned HDACi strongly influences it. Finally, p27 is considered crucial because it induces apoptosis and cell cycle arrest in B-cell lymphoma through an epigenetic mechanism.

Cyclin D1, which is essential for cell survival regulation, binds to Cdk4 and Cdk6 and promotes the cell cycle. Most patients with MCL exhibit high expression of cyclin D1; however, MCL development cannot adequately occur via a single genetic aberration; it requires another oncogenic aberration, such as disruptions in the ataxia telangiectasia mutated and p53.

A subtype of NHL, MCL, is found in indolent variants with low p21 expression. By contrast, low p27 expression is found in aggressive variants. The implication of low expression of both
CKIs is the progression of the cell cycle and proliferation of B-cell lymphoma. Some studies have demonstrated that the low expression of some CKIs, such as p21 and p57, is associated with HDAC1 and HDAC2 and that the low expression of p27 is associated with a high expression of HDAC3 in activated B-cell (ABC) DLBCL. Eliminating HDAC1 and HDAC2 activities can normalize the expression of these CKIs.

Some studies investigating the effect of VPA as an HDACi on tumor cells suggest that VPA can inhibit tumor cell proliferation and survival by upregulating the expression of p21\(^{\text{WT}}/\text{Cip1}\) and p27\(^{\text{Kip1}}\). The increase in p21 expression is induced through the acetylation of the histone in the p21 promoter region. The enhancement of p21 expression can block retinoblastoma (Rb) phosphorylation, thereby increasing the number of Rb/E2F complex activity. Reduction in free E2F can prevent DNA replication.

**VPA Promotes Apo2-L/Tumor Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL)-Induced Apoptosis by Downregulating Cellular FLICE-Inhibitory Protein**

Apo2-L/TRAIL is a member of the TNF gene superfamily; it can induce apoptosis by binding to the death receptors (DR) on the plasma membrane. As the primary receptors of Apo2-L/TRAIL, DR4 and DR5 have cytoplasmic death domains that transmit the apoptosis signal.

DR4 and DR5 are well known as TRAIL-R1 and TRAIL-R2, respectively. Both mediate apoptosis and are highly expressed in primary human B cells and B-cell lymphoma. Five essential factors increase the sensitivity of this pathway: decoy receptors, cellular FLICE-inhibitory protein (c-FLIP, an inhibitor of apoptosis), proteins, interferons, and nuclear factor kappa B (NF\(\kappa\)B). c-FLIP expression has been detected in 46.7% of patients with DLBCL. c-FLIP, an antiapoptotic protein, inhibits the extrinsic apoptosis signaling pathway by blocking DR signaling, thereby disturbing caspase-8 activation at the death-inducing signaling complex. The dysregulation of extrinsic apoptosis due to c-FLIP overexpression contributes to tumor progression and sensitivity to chemotherapeutic drugs in PTCL.

The suppression of c-FLIP can sensitize tumor cells to TRAIL-mediated apoptosis. VPA, an inhibitor of HDAC, can modulate extrinsic apoptosis via certain mechanisms in various cancer cells. Some studies have reported that inhibiting HDAC1 and HDAC4 by VPA downregulates c-FLIP expression. When c-FLIP is downregulated, DR4 and DR5 are more sensitive to proapoptotic ligands.

**VPA Inhibits the Janus Kinase (JAK)/Signal Transducers and Activators of Transcription (STAT) Pathway Through Epigenetic and Nonepigenetic Mechanisms**

Depending on the signal, tissue, and cellular context, JAK/STAT signaling pathways mediate multiple cellular responses, including cell division, differentiation, migration, death, and cell survival. JAK proteins and phosphorylated STAT are activated after the binding of cytokines or growth factors to B-cell receptors. This leads to STAT dimerization, which then translocates into the nucleus where it activates various effector genes to express Bcl2, myc, cyclin D1, and VEGF.

DLBCL, an aggressive type of B-cell lymphoma, deregulates the JAK/STAT pathway and drives tumor cells toward prolonged survival. The suppressor of cytokine signaling (SOCS) proteins regulates cell survival by inhibiting JAKs. The loss-of-function mutation of SOCS1 functions as a tumor suppressor gene (TSG) and induces tumor cell progression. A group of patients with this mutation showed shorter overall survival (OS). Based on these findings, SOCS1 was studied as a potential target for the treatment of B-cell lymphoma.

Unlike SOCS1, SOCS3 does not function as a TSG in T-cell lymphoma. Although the role of epigenetic mechanisms at the level of SOCS expression in lymphoma cells remains unclear, Kim et al reported that HDACis modulate the expression of SOCS1 and SOCS3 in cervical cancer cells and that histone acetylation by VPA activates TSG SOCS1 and SOCS3 in colon carcinoma cells. Tumor cells subsequently undergo apoptosis. However, the role of epigenetic mechanism of VPA action in modulating SOCS expression in lymphoma remains unclear.

VPA directly inhibits STAT3 through dephosphorylation, thereby inactivating JAK/STAT signaling, which reduces tumor cell proliferation and induces apoptosis. Ni et al reported that the inhibition of HDAC3 by VPA might suppress STAT3 in natural killer cells, suggesting that histone and nonhistone protein modifications by VPA regulate the JAK/STAT pathway in some cancers. However, further studies are warranted to completely elucidate VPA action through JAK/STAT pathway modulation in lymphoma.
VPA Inhibits Akt/mammalian Target of Rapamycin (mTOR) in the Phosphoinositide 3-Kinase (PI3K)/Akt/ mTOR Signaling Pathway

The PI3K/Akt signaling pathway is essential for regulating the cell cycle and is directly associated with cell survival and proliferation in lymphoma\(^9\). It is also associated with the development of human cancer stem cells\(^9\). Multiple aberrations of several genes associated with PI3K/Akt signaling drive cells toward malignancy. The mTOR is a downstream protein of this pathway that can regulate cell growth and death. Gene amplification and mutation of mTOR contribute to malignancy in various cancers\(^10\).

Phosphatase and tensin homolog (PTEN), a protein that can inhibit PI3K and Akt activation, ceases the PI3K/Akt signaling pathway\(^9\). The loss-of-function gene mutation of PTEN plays a vital role in the oncogenic activation of B-cell lymphoma. The hyperactivity of Akt signaling is associated with poor survival in patients with DLBCL\(^10\). The role of HDACs regarding the low expression of PTEN is epigenetically unclear in lymphoma. However, in another type of solid tumor, such as gastric cancer cells, HDAC1/2 inhibition leads to an increased PTEN expression and promotes apoptosis and autophagy. Therefore, PTEN expression might be partly downregulated by HDAC1/2 presence\(^10\).

The overexpression of some HDACs occurs in various cancer cells, including hematological malignancy (such as lymphoma)\(^9\). HDAC1 inhibition by VPA can increase PTEN expression, thereby influencing Akt to follow the Akt/mTOR signaling pathway. Finally, VPA can induce cell death via autophagy and apoptosis, which are associated with Akt and mTOR protein activities\(^9,10\).

The Akt/mTOR pathway is associated with the JAK/STAT signaling pathway. mTOR can promote the dimer form of STAT to activate oncogenic or prosurvival transcription in the nucleus\(^9\). Based on this explanation, VPA directly inhibits cell proliferation and limits cell survival by disrupting several signaling pathways.

VPA Inactivates Bcl-6 and Upregulates p53

p53 protein plays a vital role in response to cellular DNA damage induced by various factors. It drives cellular apoptosis through intrinsic and extrinsic mechanisms. It can also upregulate p21 via cell cycle arrest because it acts as a potent inhibitor of Cdk1, 2, 3, and 4. Many NHL subtypes have low expressions of p53 and p21, which leads to a reduction in DNA damage response, prevention of apoptosis, and continuation of cell cycle progression\(^9\).

Bcl-6 is an oncogene involved in lymphomagenesis, primarily in germinal center B-cell DLBCL. It requires the formation of complexes with certain HDACs to prevent the transcription activation of p53, leading to the expansion of germinal center B cells. Bcl-6 is activated via interaction with HDACs 4, 5, 7, and 9 as corepressors\(^9\). By contrast, Bcl-6 inactivation results in an increase in p53 and apoptosis in DLBCL\(^10\).

VPA plays a vital role in inhibiting most of the class I and II HDACs and disrupting Bcl-6 activities. Without HDACs, Bcl-6 becomes inactive. This condition leads to an increased expression of p53 and p21. Thus, tumor cells may undergo apoptosis\(^9,10\).

VPA Inhibits the NFkB Signaling Pathway

The NFkB signaling pathway is often employed for cell survival. This pathway is of two types: classic and alternative. The canonical pathway of NFkB is initiated by the induction of B-cell receptor and CARD11/MALT1/Bcl-10 complex, which then phosphorylate the inhibitor of NFkB kinase (IKK). This process activates the inhibitor of NFkB alpha. Heterodimer RelA/p50 then translocates into the nucleus and is responsible for cell survival, proliferation, inflammation, and innate immunity\(^9\). NFkB-inducing kinase (NIK) initiates the alternative pathway of NFkB via the activation of BAFF-R, MY88, and IKK. NIK-induced RelB/p50 and RelB/p52 transcription factors then promote lymphoid organogenesis, adaptive immunity, anti-inflammation, and B-cell maturation\(^9\).

The persistent activation of NFkB signaling appears in ABC-DLBCL and is responsible for cell survival\(^9\). This activation occurs because of a gain-of-function mutation in the genes of various proteins involved in the NFkB pathway, including CD79A, CD79B, CARD11, MYD88, Bcl-10, MALT1, and REL; this constitutively activates the downstream signal for cell proliferation and survival\(^9\).

VPA can attenuate NFkB activity by inhibiting the upstream and downstream mediators of the NFkB signaling pathway (Figure 2). It can also reduce the expression of interleukin-1 receptor-associated kinase-1 as an upstream mediator of NFkB activation. Studies\(^9,10\) have shown that VPA downregulates RelA (p65) but upregulates IκBα via nonhistone modifications. The mechanism of this action, particularly in another NHL subtype, should be confirmed in future studies.
VPA Sensitizes CD20-Targeting Immunotherapy by Upregulating CD20 Expression

CD20 is a protein exclusively expressed on the membrane surface of B-cell lymphomas. It is expressed by MS4A1, which is located on chromosome 11q12, and is a strategic therapeutic target of rituximab because of its ability to eliminate B cells by antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and apoptosis.

As a monoclonal antibody (anti-CD20), rituximab can destroy B-cell lymphoma by binding to CD20, which is highly expressed on the cell membrane. However, CD20 protein and mRNA are not highly expressed in some patients with rituximab resistance. Some of the underlying mechanisms of the low expression of CD20 are the downregulation of MS4A1 expression via an epigenetic mechanism, NOTCH1 mutation, and MS4A1 mutation/deletion. However, other factors also contribute to altered MS4A1 transcription, including the positive and negative regulators of transcription (Oct1, Oct2, Foxo1, Smad2/3, and others).

The downregulation of CD20 mRNA is partly because of the recruitment of the HDAC complex. VPA and other HDACis are reported to inhibit some HDACs, primarily HDAC6, thereby significantly increasing the CD20 mRNA and protein levels.

VPA Strengthens Tumor Immunosurveillance by Downregulating Programmed Cell Death Protein 1 (PD1)/Programmed Death-Ligand 1 (PD-L1) and Upregulating Major Histocompatibility Complex Class II (MHCII) and CD1d Expression

Dysregulated PD1/PD-L1 signaling pathway contributes to lymphomagenesis. PD1 is an inhibitory cell surface receptor expressed on the
membrane of the effector T cell, whereas PD-L1 is expressed on antigen-presenting cells (APCs) and overexpressed in several lymphoma cells, including Hodgkin lymphoma, ABC-DLBCL, and primary mediastinal B-cell lymphoma. When PD-L1 is overexpressed in lymphoma cells, it can cause T cell exhaustion and immune escape. High HDAC6 expression has been detected in the B cells of patients with chronic lymphocytic leukemia and DLBCL. The selective inhibition of HDAC6 can reduce the inhibitory molecule PD1/PD-L1. VPA can attenuate the immunosuppressive function of APC through some mechanisms, including interleukin 4 receptor-α/arginase axis, PD-L1, toll-like receptor 4 signaling pathway, and Rb1 derepression, thereby maintaining tumor immunosurveillance.

CD1d is an antigen-presenting molecule at the surface membrane of B cells that can bind specifically to the T-cell receptor on natural killer cells. HDAC2 binds to the CD1d promoter, which dysregulates the presentation of the tumor antigen by CD1d. HDAC2 inhibition by several HDACis can increase CD1d-mediated antigen presentation at the surface membrane of B cells in MCL. VPA having inhibitory activity against HDAC2 is suggested to increase tumor immunosurveillance via this mechanism.

Major histocompatibility complex class II (MHCII) is a protein on the cell membrane that presents antigens to CD4 T cells to initiate antigen-specific immune responses. It is constitutively expressed on dendritic cells, B cells, macrophages, and activated T cells. Therefore, it has an important function in tumor immunosurveillance. MHC class 2 transactivator (CIITA) has been reported to be a key modulator of MHCII transcription. The lack of MHCII partly contributes to the poor survival of patients with DLBCL.

The inhibition of class I HDAC, primarily via the inhibition of HDAC1, can significantly increase the expression of CIITA and MHCII. Therefore, VPA can strengthen immunosurveillance primarily via HDAC1 inhibition.

**Clinical Trials of VPA for NHL**

A case report demonstrated that VPA led to a complete response in a 64-year-old woman with stage IV refractory germinal center DLBCL. This previously untreated patient received R-CHOP as the first-line treatment, but after several months, she suffered recurrence with disease progression. She then received R-ESHAP for two cycles but showed no response. Thereafter, she received R-ICE and salvage (taxol, topotecan, and rituximab) treatments but again showed no response. Approximately 1 year later, she received 250-mg VPA thrice a day for pain relief and as an anti-lymphoma agent. She showed a complete treatment response after 6 months of VPA administration, with no adverse events.

In their VALFRID study, Drott et al. reported that VPA use with R-CHOP resulted in better OS in patients with DLBCL, in which 96.8% of 33 patients showed 2-year OS with tolerable side effects. The study also demonstrated significant improvement in the 2-year progression-free survival rate, which was 84.7% among the patients. While administering VPA to treat patients with DLBCL, the maximum tolerated dose (MTD) of 60 mg/kg/day for 3 days should be considered on days 1–3, which results in the plasma VPA concentration of 600-1200 µM.

Various adverse events associated with VPA administration have been reported. Munster et al. reported that the administration of 120 mg/kg/day VPA might lead to somnolence, confusion, hearing loss, and other neurovestibular disorders. However, the previously reported serious adverse events were not noted in the VALFRID study because the MTD of VPA was only 60 mg/kg/day. However, the dosage did result in hearing disorders; thus, VPA requires close monitoring for adverse events. Other side effects related to VPA administration were tolerable, however, this phase I clinical trial included only 33 patients with DLBCL. Therefore, these findings should be validated in a subsequent phase trial using an appropriate study design with a larger sample size to confirm the efficacy and safety of VPA.

A study evaluating the efficacy and safety of valproate involving 44 patients with CTCL was conducted in combination with hydralazine as a type of DNA methyltransferase inhibitor. The total dose of valproate was 30 mg/kg t.i.d. daily in constitutive 28-day cycles in a phase II clinical trial. The trial concluded that the combination of valproate and hydralazine is safe and effective in CTCL, although it cannot be compared with vorinostat and romidepsin in terms of efficacy. However, this drug combination should be extensively investigated by including more patients with CTCL.

**Future Direction**

Various subtypes of NHL are resistant to some of the available therapies, including chemotherapy, immunotherapy, and radiotherapy. VPA has...
the potential to modulate several proteins associated with resistance to standard therapeutic modalities. The mechanism of resistance varies based on the type of therapy.

Several signaling pathways, such as PI3K/Akt, NFκB, and JAK/STAT3, are responsible for resistance to R-CHOP in NHL. Based on previously described mechanisms, particular proteins in each pathway may be regulated by VPA through epigenetic mechanisms.

Upon activation via phosphorylation, STAT3 contributes to resistance to radiotherapy in B-cell lymphomas. STAT3 inactivation or dephosphorylation induced by VPA may increase the sensitivity of the JAK/STAT3 signaling pathway to radiotherapy.

Low CD20 expression is one of the underlying mechanisms of rituximab resistance. Rituximab can function only by binding to the specific protein CD20. VPA can upregulate CD20 expression in B-cell lymphoma through epigenetic modification.

NHL can be resistant to several PD1 inhibitors. Drug resistance in NHL is caused by the low expression of MHCI and MHCII. MHCs are associated with a strong response to PD1 blockade induced by PD1 inhibitors. VPA can upregulate the expression of MHCI and MHCII, thereby strengthening tumor immunosurveillance.

Based on the biomolecular mechanism of action, the use of VPA with other standard treatments should be further evaluated in well-designed trials. This would clarify whether VPA can significantly optimize the efficacy of standard therapy in clinical settings. Furthermore, this will help overcome resistance to immunotherapy, chemotherapy, and radiotherapy in patients with NHL.

**Conclusions**

The use of VPA for NHL treatment is a topic of interest. The rationale for using VPA to treat NHL can be explained by its mechanism of action. VPA generally functions by regulating the HDAC–target protein axis, as explained previously. The inhibition of HDACs by VPA results in the alteration of multiple signaling pathways that can inhibit lymphomagenesis by modulating several proteins. Moreover, proteins in several signaling pathways also contribute to the resistance of NHL to several standard treatment modalities. VPA appears to be a promising initial treatment before the administration of the standard therapy in patients with NHL. However, further trials should be broadly conducted to establish VPA use for NHL treatment in clinical practice.

**Authors’ Contributions**

H.R. and I.W. conceptualized the study; H.R. wrote first original draft; I.W., M.H.B., B.S.H., and A.H.S.K. reviewed the draft and revised it substantially; B.S.H. acted as the supervisor of the team and performed the final revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest**

The authors declare no conflicts of interest.

**References**


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