Phosphodiesterase 4 inhibitors have wide-ranging activity in B-cell malignancies
Jeffrey D. Cooney\textsuperscript{1,2} and Ricardo C. T. Aguiar\textsuperscript{1,3-5}

\textsuperscript{1}Division of Hematology and Medical Oncology, Department of Medicine, \textsuperscript{2}MD/PhD Program, \textsuperscript{3}Cancer Therapy and Research Center, and \textsuperscript{4}Greehey Children’s Cancer Research Institute, University of Texas Health Sciences Center at San Antonio, San Antonio, TX; and \textsuperscript{5}Audie L. Murphy Memorial VA Hospital, South Texas Veterans Health Care System, San Antonio, TX

Phosphodiesterase 4 (PDE4) inhibition restores the suppressive effects of 3',5'-cyclic adenosine monophosphate in lymphocytes. In this concise review, we detail how PDE4 inhibition down-modulates the B-cell receptor (BCR)-related kinases spleen tyrosine kinase and phosphatidylinositol 3-kinase and inhibits vascular endothelial growth factor A secretion by tumor cells, inducing cancer cell apoptosis and blocking angiogenesis in the microenvironment. We describe the successful clinical re-purposing of PDE4 inhibitors in B-cell malignancies, and propose that given their anti-inflammatory/immunomodulatory activity, these agents will suppress BCR signals without the toxicity associated with other targeted biological doublets. (Blood. 2016;128(25):2886-2890)

Introduction

The second messenger 3',5'-cyclic adenosine monophosphate (cyclic-AMP) uses effector proteins to influence cell function and fate.\textsuperscript{1} The synthesis and degradation of cyclic-AMP are tightly controlled by 2 classes of enzymes, adenylyl cyclases and phosphodiesterases (PDEs), respectively.\textsuperscript{2,3} The therapeutic utility of controlling the intracellular levels of cyclic-AMP, and of cyclic guanosine monophosphate (cyclic-GMP), with PDE inhibitors is well established.\textsuperscript{4} These agents are in clinical testing or have been US Food and Drug Administration (FDA)-approved for the treatment of multiple conditions, from cardiac failure to fertility, from neurodegeneration to inflammatory/autoimmune conditions and erectile dysfunction (see Maurice et al\textsuperscript{5} for a comprehensive review on PDE inhibitors). However, notably absent from these efforts is a detailed examination of PDE inhibitors in cancer therapeutics. Here, we review a decade-long exploration of the contribution of cyclic-AMP and PDE4 to the pathogenesis of B-cell lymphoma,\textsuperscript{5-9} culminating with the first-in-cancer clinical trial of a PDE4 inhibitor in advanced B-cell malignancies.\textsuperscript{10} In particular, we highlight the interplay between cyclic-AMP/PDE4 and B-cell receptor (BCR) signals, discuss the antiangiogenic activity of PDE4 inhibitors, and, considering their anti-inflammatory/immunomodulatory activity, we suggest that adding these agents to rationally devised therapeutic combinations will prevent the development of inflammatory/autoimmune toxicity that associates with the simultaneous use of multiple biological agents, the so-called biological doublets (eg, spleen tyrosine kinase [SYK] and phosphatidylinositol 3-kinase \delta [PI3K\delta] inhibitors) and triplets (eg, anti-CD20, lenalidomide, and Bruton tyrosine kinase [BTK] or PI3K\delta inhibitors).\textsuperscript{11,12}

Preclinical data

The cyclic-AMP/PDE4 axis in B-cell lymphoma: mapping the relevant signaling nodes

The association between this signaling axis and diffuse large B-cell lymphoma (DLBCL) was first noted when we found high PDE4B expression in a 13-gene signature that distinguished curable from fatal DLBCL.\textsuperscript{5} The association between high PDE4B expression and poor DLBCL outcome was subsequently confirmed in larger independent series.\textsuperscript{6,13,14} To advance these early observations, preclinical models were used to show that genetic or pharmacological inhibition of PDE4 results in growth suppression and apoptosis in DLBCL.\textsuperscript{5} Mechanistically, PDE4 inhibition resulted in elevation of intracellular cyclic-AMP levels and suppression of PI3K and AKT activity.\textsuperscript{6} These data linked the cyclic-AMP/PDE4 axis to the essential tonic BCR signals, highlighting the potential impact of PDE4 modulation in malignant B cells (Figure 1).

In this initial characterization, it was unclear how cyclic-AMP inhibited PI3K activity. Further investigation showed that cyclic-AMP, in a PDE4B-dependent manner, terminated the BCR-induced SYK activation,\textsuperscript{7} which can block phosphorylation of p85 and PI3K activity.\textsuperscript{15} This inhibitory effect was present in DLBCL as well as in normal mature B cells, and it was specific to SYK, as other proximal BCR kinases (eg, c-SRC, LYN) appeared unaffected by cyclic-AMP.\textsuperscript{7} These data suggested that the combination of PDE4 inhibitors with compounds that target BCR-dependent kinases may represent a synergistic approach for the treatment of DLBCL (see “Prospects”). This combinatorial strategy is likely to be relevant to additional mature B-cell tumors, as the benefits of PDE4 (as well as PI3K\delta and SYK) inhibition have also been preclinically and clinically validated in chronic lymphocytic leukemia (CLL).\textsuperscript{16,17}

Cyclic-AMP is a known suppressor of T-cell receptor (TCR) signaling.\textsuperscript{20,21} The data discussed in the previous paragraphs showed that this second messenger is also an important negative regulator of the BCR, a physiologic safeguard that is lost in B-cell malignancies that display elevated PDE4 expression and activity.\textsuperscript{5,6,13,14,16}

PDE4 and B-cell lymphoma angiogenesis

The proangiogenesis crosstalk between lymphoma cells and the tumor microenvironment is poorly understood. Addressing this problem is important because high vascular endothelial growth factor (VEGF) levels and angiogenesis are associated with poor outcome in DLBCL.\textsuperscript{22,23} Additionally, clinical trials of the anti-VEGFA agent bevacizumab with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) were negative, at least in part due to prohibitive cardiotoxicity.\textsuperscript{26,27} Thus, understanding the putative proangiogenic role of the lymphoma cell may uncover novel actionable targets and improve survival.

In nonneoplastic models, cyclic-AMP is known to attenuate vessel development.\(^\text{26-31}\) To address the contribution of PDE4B expression/activity to lymphoma angiogenesis, a compound mouse combining Pde4b knockout with the Eμ-Myc allele was recently created.\(^\text{9}\) In this model, lymphomas arising in a Pde4b-null background displayed significantly suppressed angiogenesis in association with decreased PI3K/AKT activity and VEGFA expression in the tumor cells. PI3K/AKT signals are important hypoxia-inducible factor-independent regulators of VEGF expression in epithelial malignancies,\(^\text{32-34}\) explaining the relationship between PDE4 levels, cyclic-AMP-modulated PI3K/AKT activity, VEGF secretion, and lymphoma angiogenesis (Figure 1). Importantly, the findings from this genetically engineered mouse were recapitulated with the FDA-approved PDE4 inhibitor roflumilast.\(^\text{9}\) These data suggest that PDE4 is as an actionable antiangiogenic target in B-cell lymphomas, which likely does not induce the cardiotoxicity found with the bevacizumab/anthracycin combination.\(^\text{26-27}\) Remarkably, given their anti-inflammatory activity, PDE4 inhibitors have been independently shown to actually protect against doxorubicin-induced cardiomyopathy in rats.\(^\text{35}\) Although lenalidomide has also been suggested to inhibit lymphoma angiogenesis,\(^\text{36}\) the proposed use of PDE4 inhibitors in this setting is grounded in a more robust mechanistic understanding of its effects on the tumor and endothelial cells,\(^\text{9,28-31}\) which may be leveraged for the development of successful antiangiogenesis initiatives.

## Clinical data

In an unbiased gene expression and chemical genomics screen, PDE4B was found as one of the highest expressed genes in glucocorticoid-resistant acute lymphoid leukemia (ALL).\(^\text{37}\) Furthermore, PDE4B variants were associated with a higher rate of relapse in childhood ALL.\(^\text{38}\) Corroborating these observations, genes associated with glucocorticoid resistance in ALL were enriched in DLBCLs that express high levels of PDE4B,\(^\text{8}\) whereas PDE4 inhibitors restored glucocorticoid sensitivity and reduced tumor burden in in vivo preclinical models of human lymphoma.\(^\text{8}\) In these studies, the effects of PDE4 on glucocorticoid sensitivity were associated with modulation of

---

**Figure 1.** The cyclic-AMP/PDE4B axis in the lymphoma cell and its microenvironment. Antigen binding activates the BCR, a process initiated by phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) on CD79A and CD79B, and amplified by a cascade of events that involve multiple protein and lipid kinases. These “upstream” events culminate in the activation of downstream, prosurvival, signaling pathways, including among others NF-κB, MAPK, and the AKT/mTOR complex 1 (mTORC1).\(^\text{2,3}\) Cyclic-AMP (cAMP) downmodulates this positive signaling wave by suppressing SYK and PI3K activity.\(^\text{3-5}\) cAMP may also use its effector PKA to activate CSK, which inhibits SFKS via C-terminal phosphorylation. Decreased SFK activity may directly, or via SYK, limit p85 phosphorylation and thus PI3K function.\(^\text{15}\) This PKA-CSK-SFK interplay has been demonstrated in T lymphocytes and endothelial cells, but not yet in normal and malignant B cells.\(^\text{21,31}\) In B-cell lymphoma and related malignancies, the inhibitory effects of cAMP are abrogated by PDE4, which hydrolyzes this second messenger to the inactive adenosine monophosphate (AMP), thus sustaining BCR activity and defining a prosurvival profile.\(^\text{5-6,16}\) Downstream to PI3K/AKT, the cAMP/PDE4 axis also impinges on the lymphoma microenvironment. In B-cell lymphomas with high PDE4B levels, cAMP is hydrolyzed to AMP, resulting in higher AKT-driven VEGFA expression in the tumor cell, excessive secretion in the tumor milieu, and enhanced angiogenesis.\(^\text{9}\) cAMP, in a PDE4-dependent manner, also suppresses the survival and proliferation of endothelial cells,\(^\text{28}\) downmodulates the secretion of various proinflammatory cytokines, and increases the number of Tregs.\(^\text{31-34}\) The PDE4 inhibitor roflumilast abrogates cAMP hydrolysis, elevates the intracellular levels of this second messenger, and restores its suppressive effects in the lymphoma cell and the microenvironment. PLC, phospholipase C; TNF, tumor necrosis factor.
P13K/akt signals. These data informed the design of a first-in-cancer clinical trial of the PDE4 inhibitor roluflastin (FDA-approved for severe chronic obstructive pulmonary disease). In this single-arm, pharmacokinetics and pharmacodynamics phase Ib study (clinicaltrials.gov identifier NCT01888952), the combination of roluflastin with prednisone was tested in patients with advanced B-cell malignancies (B-cell lymphoma, CLL, and multiple myeloma) who had failed multiple prior therapies, and for whom no standard curative regimen was available. In addition to safety and tolerability, the role of P13K/akt as biomarkers for PDE4 inhibition and clinical activity were also examined.

In the trial, patients received roluflastin orally once daily (500 mcg) for 21 consecutive days (21-day cycle), with prednisone (60 mg/m² daily, up to a maximum of 100 mg per day) added on days 8 through 14 (cycle 1). If patients tolerated cycle 1, and had at least stable disease, additional cycles of roluflastin and prednisone were administered starting on day 22, with prednisone taken on days 1 through 7. The median number of cycles administered was 4 (range, 1-13) and the median number of days in trial until progression of the disease, death, or withdrawal of consent was 105 days (range, 28-315 days). Treatment was well tolerated and the majority of adverse events were reversible and/or clinically manageable. One patient experienced a transitory episode of suicidal ideation, a known potential side-effect of roluflastin, and was removed from the study; the adverse event resolved upon cessation of the study drug. Among the evaluable patients, objective partial response or stable disease was detected in 66% of cases, including 1 high-risk (17p deletion) CLL patient with stable disease after 8 cycles, who withdrew from the study. The adverse event resolved upon cessation of the study drug. The subsequent withdrawal of consent was 105 days (range, 28-315 days). Treatment was well tolerated and the majority of adverse events were reversible and/or clinically manageable. One patient experienced a transitory episode of suicidal ideation, a known potential side-effect of roluflastin, and was removed from the study; the adverse event resolved upon cessation of the study drug. Among the evaluable patients, objective partial response or stable disease was detected in 66% of cases, including 1 high-risk (17p deletion) CLL patient with stable disease after 8 cycles, who withdrew consent to join an ibritinib trial. Treatment with roluflastin as a single agent for 7 days (cycle 1) suppressed P13K/akt activity in 77% of the cases studied. Patients with P13K/akt suppression stayed in trial longer than those without this biomarker response (156 vs 91 days), a trend that may become firmly established with larger studies. Notably, 3 nonresponders had earlier failed Btk, P13K6, and/or AKT inhibitors. Thus, considering that PDE4 inhibitors downmodulate P13K/akt in malignant B lymphocytes, lack of response to agents directed at these kinases may be considered a predictor of negative response to roluflastin and inform the design of future clinical trials. Lastly, in this pilot study, nearly all responders were considered glucocorticoid-resistant, supporting the premise that PDE4 inhibition may restore glucocorticoid sensitivity.

**Prospects**

**PDE4 inhibition: targeting BCR dependency and angiogenesis**

Following antigen binding, C-terminal tyrosine residues of CD79A/B are phosphorylated by SRC-family kinases (SFKs), recruiting SYK, which amplifies these initial signals by phosphorylating B-cell linker (BLNK) and p85, activating BTK and P13K5, respectively. The subsequent engagement of NF-κB, AKT/mechanistic target of rapamyacin (mTOR), and MAPK and other signaling pathways promotes proliferation and survival of B cells. Malignant mature B cells hijack these signals, creating a BCR dependency that has been exploited with SYK, BTK, and P13K5 inhibitors. However, even with the successful deployment of BTK and P13K5 inhibitors, limitations persist as complete remission is rarely achieved, and acquired resistance can develop, and toxicity has emerged as a serious limitation to the targeting of P13K5 with idelalisib, especially when in combination. Preclinical and clinical data confirmed that PDE4 inhibitors suppress the activity of components of the BCR-signaling machinery in mature B-cell malignancies. Notably, although FDA-approved adenosine triphosphate–competitive kinase inhibitors (eg, entospletinib, idelalisib) block the amplification of BCR signals, targeting PDE4 increases the intracellular levels of cyclic-AMP and actively promotes the termination of BCR activity. In addition, PDE4 inhibitors have a wider reach than individual kinase inhibitors, as they concomitantly downmodulate SYK and P13K signals. Furthermore, although not yet fully characterized in B cells, PDE4 inhibition, in a protein kinase A (PKA)/c-terminus Src kinase (CSK)-dependent manner, may also suppress the activity of SFKs, a well-established mode of cyclic-AMP-mediated termination of the T-cell receptor activity (Figure 1).

Thus, we suggest that the combinatorial vertical targeting of the BCR with kinase and PDE4 inhibitors should be tested in a phase 1/2 context. We predict that by using different pharmacological classes, this biologically sound strategy will synergistically suppress the growth of BCR-dependent tumors. Furthermore, by targeting multiple nodes in the BCR network, this combination may limit the emergence of resistant clones, and allow for more flexible dosing and diminished toxicity. Importantly, as defined in in vivo preclinical models, in addition to downmodulating the BCR, PDE4 inhibitors also block angiogenesis in the tumor microenvironment. Thus, considering the adverse impact of angiogenesis in DLBCL outcome, we advocate that roluflastin be tested in combination with standard of care, R-CHOP. We suggest that, differently from the bevacizumab/R-CHOP combination, PDE4 inhibition will not add prohibitive toxicity to anthracycline-containing regimens. This prediction stems from the unique dual antiangiogenic effects of PDE4 inhibition, that is, toward the tumor and endothelial cell, and the reported protection against doxorubicin-induced cardiomyopathy in animal models.

**PDE4 inhibition: limiting the toxicity of targeted biological agents**

One of the most vexing problems facing drug development in lymphoid malignancies is the unexpected severe toxicity associated with the combined use of biological agents, the so-called biological doublets (eg, SYK and P13K6 inhibitors) and triplets (eg, anti-CD20, lenalidomide, and BTK or P13K6 inhibitors). Although less frequent and ominous, serious adverse events have also been reported in single-agent idelalisib studies. While the precise mechanistic basis for these events remains undefined, the connection to T-cell–mediated autoimmunity is substantial. CD8+ infiltrates, increases in the T helper 1 cytokines such as interferon γ (IFNγ), interleukin-6 (IL-6), IL-18, CCL3, and CCL4, and reduction in the number and function of regulatory T cells (Tregs), have all been reported in the affected tissues or serum of idelalisib-treated patients. The anti-inflammatory/immunomodulatory activity of roluflastin and apremilast (a PDE4 inhibitor FDA-approved for psoriatic arthritis) associates with suppression of numerous proinflammatory cytokines, including IL-6, IL-10, IL-17A, tumor necrosis factor α (TNFα), IFNγ, granulocyte-macrophage colony-stimulating factor, as well as an increase in Treg numbers. For these reasons, we suggest that inclusion of a PDE4 inhibitor in association with, or replacing, idelalisib in doublets and triplets of targeted agents will significantly limit toxicity while effectively inhibiting BCR-related signals. Testing this concept is a “low hanging fruit” in the developmental therapeutics of B-cell malignancies.

**Conclusions**

Preclinical and clinical data provide compelling biological rationale for the repurposing of roluflastin for the treatment of B-cell malignancies,
with focus on BCR-dependent tumors in which angiogenesis plays a pathogenic role. In light of the negative bevacizumab/R-CHOP trial, testing the combination of roflumilast with R-CHOP in DLBCL is a scientifically sound strategy. Additional clinical initiatives are warranted to validate the concept that the immuno-modulatory activity of roflumilast will contribute to BCR suppression, while preventing the autoimmunity associated with biological combinations. Finally, the development of novel PDE4 inhibitors with improved therapeutic indexes should increase the likelihood that these strategies are successful.

Acknowledgments

J.D.C. was supported by the National Institutes of Health, National Cancer Institute (1F30CA206343-01). R.C.T.A. was supported in part by an award from the Cancer Prevention and Research Institute of Texas (CPRIT: RP15077), the Owens’ Foundation, and the Leukemia & Lymphoma Society (TRP-6524).

Authorship

Contributions: R.C.T.A. conceived and designed the outline of the review; and J.D.C. and R.C.T.A. performed literature review and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Ricardo C. T. Aguiar, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78229; e-mail: aguiar@uthscsa.edu.

References


Phosphodiesterase 4 inhibitors have wide-ranging activity in B-cell malignancies

Jeffrey D. Cooney and Ricardo C. T. Aguiar