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

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Abstract

Phosphodiesterase 4 (PDE4) inhibition restores the suppressive effects of cyclic-AMP in lymphocytes. In this concise review, we detail how PDE4 inhibition downmodulates the B-cell receptor (BCR)-related kinases SYK and PI3K, inhibits VEGFA secretion by tumor cells, inducing cancer cell apoptosis and blocking angiogenesis in the microenvironment. We describe the successful clinical repurposing 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.
Introduction

The second messenger 3',5'-cyclic adenosine monophosphate (cyclic-AMP) uses effector proteins to influence cell function and fate. The synthesis and degradation of cyclic-AMP are tightly controlled by two classes of enzymes, adenylyl cyclases and phosphodiesterases (PDE), respectively. The therapeutic utility of controlling the intracellular levels of cyclic-AMP, and of cyclic guanosine monophosphate (cyclic-GMP), with PDE inhibitors is well-established. These agents are in clinical testing or have been FDA-approved for the treatment of multiple conditions, from cardiac failure to fertility, from neurodegeneration to inflammatory/auto-immunity conditions and erectile dysfunction (see 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 phosphodiesterase 4 (PDE4) to the pathogenesis of B-cell lymphoma, culminating with the first-in-cancer clinical trial of a PDE4 inhibitor in advanced B-cell malignancies. 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 (e.g., SYK and PI3Kδ inhibitors) and triplets (e.g., anti-CD20, lenalidomide and BTK or PI3Kδ inhibitors).

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. The association between high PDE4B expression and poor DLBCL outcome was subsequently confirmed in larger independent series. To advance these early observations, pre-clinical models were used to show that genetic or pharmacological inhibition of PDE4 result in growth suppression and apoptosis in DLBCL. Mechanistically, PDE4 inhibition resulted in elevation of intracellular cyclic-AMP levels and suppression of PI3K and AKT activity. 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, which can block phosphorylation of p85 and PI3K activity. 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

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kinases (e.g., c-SRC, LYN) appeared unaffected by cyclic-AMP. 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” below). This combinatorial strategy is likely to be relevant to additional mature B-cell tumors, as the benefits of PDE4 (as well as PI3Kδ and SYK) inhibition have also been pre-clinically and clinically validated in chronic lymphocytic leukemia (CLL).

Cyclic-AMP is a known suppressor of T-cell receptor signaling. The data discussed above 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.

**PDE4 and B cell lymphoma angiogenesis**

The pro-angiogenesis crosstalk between lymphoma cells and the tumor microenvironment is poorly understood. Addressing this problem is important because high VEGF levels and angiogenesis are associated with poor outcome in DLBCL. Additionally, clinical trials of the anti-VEGFA agent bevacizumab with R-CHOP were negative, at least in part due to prohibitive cardiotoxicity. Thus, understanding the putative proangiogenic role of the lymphoma cell may uncover novel actionable targets and improve survival.

In non-neoplastic models, cyclic-AMP is known to attenuate vessel development. To address the contribution of PDE4B expression/activity to lymphoma angiogenesis, a compound mouse combining Pde4b knock-out (KO) with the Eµ-Myc allele was recently created. In this model, lymphomas arising in a Pde4b-null background displayed significantly suppressed angiogenesis in association with decreased PI3K/AKT activity and VEGF expression in the tumor cells. PI3K/AKT signals are important HIF-independent regulators of VEGF expression/angiogenesis in epithelial malignancies, explaining the relationship between PDE4 levels, cyclic-AMP-modulated PI3K/AKT activity, VEGFA secretion and lymphoma angiogenesis (Figure 1). Importantly, the findings from this genetically engineered mouse were recapitulated with the FDA-approved PDE4 inhibitor roflumilast. 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/anthracyclines combination. Indeed, given their anti-inflammatory activity, PDE4 inhibitors have been independently shown to actually protect against doxorubicin-induced cardiomyopathy in rats. Although lenalidomide has also been suggested to inhibit lymphoma angiogenesis, 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, which may be leveraged for the development of successful anti-angiogenesis initiatives.

**Clinical data**
In an unbiased gene expression and chemical genomics screen, \textit{PDE4B} was found as one of the highest expressed genes in glucocorticoid resistant acute lymphoid leukemia (ALL)\textsuperscript{38}. Furthermore, \textit{PDE4B} variants were associated with higher rate of relapse in childhood ALL\textsuperscript{39}. Corroborating these observations, genes associated with glucocorticoid resistance in ALL were enriched in DLBCLs that express high levels of \textit{PDE4B}\textsuperscript{8}, while PDE4 inhibitors restored glucocorticoid sensitivity and reduced tumor burden in in vivo pre-clinical models of human lymphoma\textsuperscript{8}. In these studies, the effects of PDE4 on glucocorticoid sensitivity were associated with modulation of PI3K/AKT signals\textsuperscript{8}. These data informed the design of a first-in-cancer clinical trial of the PDE4 inhibitor roflumilast (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 roflumilast 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\textsuperscript{10}. In addition to safety and tolerability, the role of PI3K/AKT as biomarkers for PDE4 inhibition and clinical activity were also examined\textsuperscript{10}.

In the trial, patients received roflumilast orally once daily (500 mcg) for 21 consecutive days (21-day cycle), with prednisone (60mg/m\textsuperscript{2} PO daily, up to a maximum of 100mg/day) added on days 8 through 14 (cycle 1). If patients tolerated cycle 1, and had at least stable disease, additional cycles of roflumilast 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 withdrawn of consent was 105 days (range: 28-315). 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 roflumilast\textsuperscript{40}, 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 one high-risk (17p deletion) CLL patient with stable disease after 8 cycles, who withdrew consent to join an ibrutinib trial. Treatment with roflumilast as a single agent for 7 days (cycle 1) suppressed PI3K/AKT activity in 77% of the cases studied. Patients with PI3K/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, three non-responders had earlier failed BTK, PI3Kδ and/or AKT inhibitors. Thus, considering that PDE4 inhibitors downmodulate PI3K/AKT in malignant B lymphocytes\textsuperscript{6,9}, lack of response to agents directed at these kinases may be considered a predictor of negative response to roflumilast 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\textsuperscript{8}.

**Prospects**
PDE4 inhibition – targeting BCR-dependency and angiogenesis

Following antigen binding, c-terminal tyrosine residues of CD79A/B are phosphorylated by SRC-family kinases (SFK), recruiting SYK, which amplifies these initial signals by phosphorylating BLNK and p85, activating BTK and PI3Kδ, respectively\textsuperscript{15,41,42}. The subsequent engagement of NFκB, AKT/mTOR and MAPK and other signaling pathways promote proliferation and survival of B-cells\textsuperscript{43,44}. Malignant mature B-cells hijack these signals, creating a BCR-dependency that has been exploited with SYK, BTK and PI3Kδ inhibitors\textsuperscript{18,45-47}. However, even with the successful deployment of BTK and PI3Kδ inhibitors, limitations persist as complete remission is rarely achieved\textsuperscript{19,48}, acquired resistance can develop\textsuperscript{49}, and toxicity has emerged as a serious limitation to the targeting of PI3Kδ with idelalisib, especially when in combination\textsuperscript{11,12,50,51}.

Pre-clinical and clinical data confirmed that PDE4 inhibitors suppress the activity of components of the BCR signaling machinery in mature B-cell malignancies\textsuperscript{6-10}. Notably, while FDA-approved ATP-competitive kinase inhibitors (e.g. entosplentinib, ibrutinib, 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 PI3K signals. Further, although not yet fully characterized in B-cells, PDE4 inhibition, in a PKA/CSK-dependent manner, may also suppress the activity of SFKs, a well-established mode of cyclic-AMP-mediated termination of the TCR activity\textsuperscript{21} (Figure 1).

Thus, we suggest that the combinatorial vertical targeting of the BCR with kinase and PDE4 inhibitors should be tested in a phase I/II 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 emergency of resistant clones, and allow for more flexible dosing and diminished toxicity. Importantly, as defined in in vivo pre-clinical 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\textsuperscript{22,25}, we also advocate that roflumilast be tested in combination with standard-of-care, R-CHOP. We suggest that differently from the bevacizumab/R-CHOP combination\textsuperscript{27}, PDE4 inhibition will not add prohibitive toxicity to anthracycline-containing regimens. This prediction stems from the unique dual antiangiogenic effects of PDE4 inhibition, i.e. towards the tumor and endothelial cell\textsuperscript{9,28}, and the reported protection against doxorubicin-induced cardiomyopathy in animal models\textsuperscript{36}.

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 (e.g., SYK and PIKδ inhibitors) and triplets (e.g., anti-CD20, lenalidomide and BTK or PI3Kδ inhibitors)\textsuperscript{11,12}. Although less frequent and ominous, serious adverse events have also been reported in single-agent idelalisib studies\textsuperscript{11}. Although the precise mechanistic basis for these events remains undefined, the connection to T-cell mediated autoimmunity is substantial. CD8\(^+\) infiltrates, increases in the Th1 cytokines such as IFN\(\gamma\), 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\textsuperscript{11,50,51}.

The anti-inflammatory/immunomodulatory activity of roflumilast and apremilast (a PDE4 inhibitor FDA-approved for psoriatic arthritis), associates with suppression of numerous pro-inflammatory cytokines, including IL-6, IL-10, IL-17A, TNF\(\alpha\), IFN\(\gamma\), GM-CSF, as well as an increase in Treg numbers\textsuperscript{52-55}. For these reasons, we suggest that inclusion of this 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**

Pre-clinical and clinical data provide compelling biological rationale for the repurposing of roflumilast 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 immunomodulatory activity of roflumilast will contribute to BCR suppression, while preventing the autoimmunity associated with biological combinations. Lastly, the development of novel PDE4 inhibitors with improved therapeutic indexes should increase the likelihood that these targeted strategies are successful.
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References:


Figure Legend

Figure 1. The cyclic-AMP/PDE4B axis in the lymphoma cell and its microenvironment. Antigen binding activates the B-cell receptor (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, pro-survival, signaling pathways, including among others NF-κB, MAPK and the AKT/mTORC1. Cyclic-AMP (cAMP) downmodulates this positive signaling wave by suppressing SYK and PI3Kδ activity. Cyclic-AMP may also utilize its effector PKA to activate the C-terminal Src kinase (CSK), which inhibits Src family of kinases (SFK) via c-terminal phosphorylation. Decrease in SFK activity may directly, or via SYK, limit p85 phosphorylation and thus PI3K function. This PKA-CSK-SFK interplay has been demonstrated in T lymphocytes and endothelial cells, but not yet in normal and malignant B-cells. In B-cell lymphoma and related malignancies the inhibitory effects of cAMP are abrogated by the phosphodiesterase 4 (PDE4), which hydrolyzes this second-messenger to the inactive AMP, thus sustaining BCR activity and defining a pro-survival profile. 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. Cyclic-AMP, in a PDE4-dependent manner, also suppresses the survival and proliferation of endothelial cells, downmodulate the secretion of various pro-inflammatory cytokines and increase the number of regulatory T-cells (Treg). 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.
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