

Therapeutic targeting of 3',5'-cyclic nucleotide phosphodiesterases: inhibition and beyond

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Abstract | Phosphodiesterases (PDEs), enzymes that degrade 3',5'-cyclic nucleotides, are being pursued as therapeutic targets for several diseases, including those affecting the nervous system, the cardiovascular system, fertility, immunity, cancer and metabolism. Clinical development programmes have focused exclusively on catalytic inhibition, which continues to be a strong focus of ongoing drug discovery efforts. However, emerging evidence supports novel strategies to therapeutically target PDE function, including enhancing catalytic activity, normalizing altered compartmentalization and modulating post-translational modifications, as well as the potential use of PDEs as disease biomarkers. Importantly, a more refined appreciation of the intramolecular mechanisms regulating PDE function and trafficking is emerging, making these pioneering drug discovery efforts tractable.

Nanodomains

Subcellular compartments within which intracellular signalling is discretely localized largely due to anchoring of molecules within macromolecular complexes.

Acrodysostosis

A rare congenital disorder characterized by abnormal bone growth leading to very short fingers/toes, underdeveloped facial bones, a small nose and short stature, as well as developmental and intellectual disabilities.

Conventional 3',5'-cyclic nucleotide phosphodiesterases (PDEs) are members of a highly conserved superfamily of enzymes that degrade the canonical cyclic nucleotides 3',5'-cAMP and 3',5'-cGMP¹, as well as the non-canonical cyclic nucleotides 3',5'-cCMP, 3',5'-cUMP, 3',5'-cIMP and c-di-GMP^{2–4} (FIG. 1). As extensively reviewed elsewhere¹, there are 11 families of PDEs that are grouped based on the homology of their C-terminal catalytic domain, and each PDE family has multiple isoforms that differ in terms of the length and complexity of their N-terminal regulatory domains (FIG. 2). PDEs do not simply control the total cellular content of cyclic nucleotides, but rather create individual pockets or nanodomains of cyclic nucleotide signalling. It is this subcellular compartmentalization of cyclic nucleotide signalling that enables a single cell to respond discretely to multiple extracellular and intracellular signals. Thus, PDEs regulate a myriad of physiological processes, and their dysfunction has been associated with numerous pathophysiological states including those affecting the nervous system, the cardiovascular system, fertility, immunity, cancer and metabolism (BOX 1). Because the location of a PDE is just as important to its overall function as its catalytic activity, how the location of a given PDE isoform can change based on tissue type, age or disease status — possibly due to factors such as activation of receptors, alterations in calcium signalling or elevations in cyclic nucleotides — is of paramount importance when considering the therapeutic potential of a given PDE isoform (for example, see REFS^{5–12}; for a full review, see Supplementary Table 1).

Importantly, no two PDE isoforms share the exact same combination of substrate specificity, tissue expression profile and subcellular localization (Supplementary Table 1). This is quite important because there are several diseases in which compartment-specific defects in cyclic nucleotide signalling have been identified. For example, the function of soluble guanylyl cyclase, but not particulate guanylyl cyclase, is substantially impaired in brains of patients with Alzheimer disease (AD) and in *in vitro* models of AD pathology^{13–15}, which would be expected to decrease cytosolic pools of cGMP. In contrast, in colon cancer, membrane-bound guanylyl cyclase appears to be dysregulated/suppressed¹⁶ and membrane-enriched PDE10A appears to be overexpressed¹⁷, both of which would decrease membrane-proximal pools of cGMP. That said, cytosolic pools of cGMP may also be decreased in colorectal cancer cells, as PDE5A is also overexpressed in these cells¹⁷, and — at least in the heart and brain — PDE5 regulates pools of cGMP that are downstream of soluble guanylyl cyclases^{18,19}.

With regard to compartment-specific changes in cAMP signalling, studies examining brain tissue from patients with bipolar disorder show no change in the membrane but increased signalling in the cytosol, which may be normalized by the classic mood stabilizer lithium (for example, see REFS^{20,21}). Other disease states where compartment-specific defects in cyclic nucleotide signalling have been implicated include — but are not limited to — erectile dysfunction²², hypertension²³, cardiac hypertrophy^{18,24}, acrodysostosis^{25,26} and Huntington disease^{27,28}. The unique substrate/localization profile offered by each

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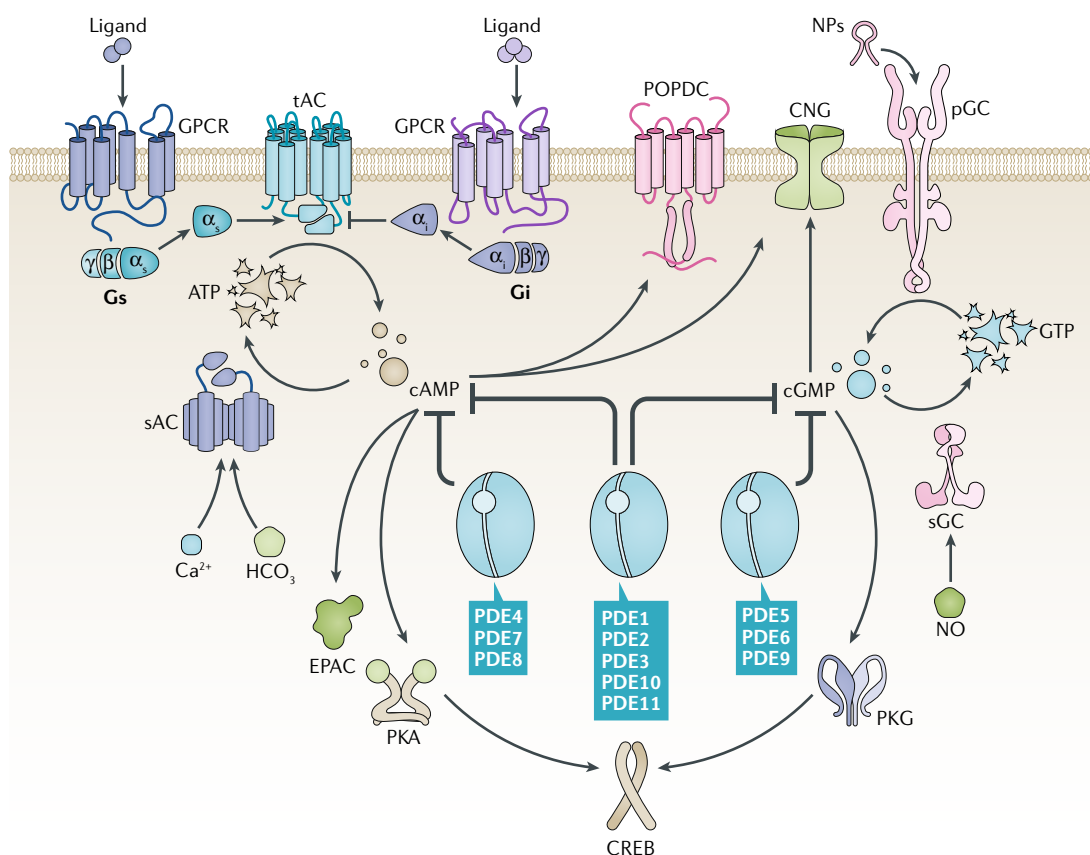


Fig. 1 | Cyclic nucleotide signalling pathways. Both of the canonical cyclic nucleotide signalling pathways (cAMP and cGMP) are composed of numerous molecules responsible for the synthesis, execution and breakdown of their signals. cAMP is synthesized by transmembrane adenylyl cyclases (tACs) that are activated by G α_s and inhibited by G α_i ³¹⁸ as well as soluble adenylyl cyclases (sACs) that are activated by bicarbonate and calcium³¹⁹. cGMP is synthesized by particulate guanylyl cyclases (pGCs) that are activated by natriuretic peptides (NPs) and soluble guanylyl cyclases (sGCs) that are activated by nitric oxide (NO)³²⁰. Both cAMP and cGMP activate cyclic nucleotide gated channels (CNGs) and allosterically modulate the activity of select phosphodiesterases (PDEs)¹⁹⁹. In contrast, only cGMP stimulates protein kinase G (PKG), whereas cAMP activates protein kinase A (PKA), exchange protein activated by cAMP (Epac) and popeye domain-containing (POPDC) proteins³²¹. Signalling through either the cAMP or cGMP pathways ultimately leads to phosphorylation of a myriad of downstream targets, including the transcription factor cAMP response element binding protein (CREB). In addition to cAMP and cGMP, several PDEs also hydrolyse the non-canonical cyclic nucleotides (not included) cUMP (PDE3A, PDE3B, PDE9A), cCMP (PDE7A) and c-di-GMPa (bacterial PDEs), albeit with much lower affinity²⁻³. GPCR, G protein-coupled receptor.

PDE isoform offers multiple degrees of freedom in the context of therapeutic targeting. As such, isoform-specific targeting could enable selective restoration of cyclic nucleotide signalling in affected compartments (that is, provide efficacy) without disturbing cyclic nucleotide signalling elsewhere (that is, avoid side effects).

As reviewed in detail below, there has been and continues to be strong interest in developing PDE-targeted therapeutics for numerous diseases. Unfortunately, the majority of PDE-targeted therapeutics on the market are simply competitive blockers of substrate binding at the catalytic site that lack the ability to selectively target a specific isozyme within a single PDE family or subfamily. That said, novel therapeutic strategies are currently being explored to increase the selectivity and specificity with which PDEs are targeted (for example, by targeting protein–protein interactions). Further, PDE activators are now being considered as agents for treating select diseases, as is the exploitation of PDEs as biomarkers for diagnosis and/or patient selection (BOX 2). Here, we

review the clinical successes and failures of PDE inhibitors to date and describe several ways in which the field is moving beyond pharmacological inhibition of PDEs for therapeutic gain. This review largely focuses on PDE function as it relates to the canonical cyclic nucleotide substrates, cAMP and cGMP, because little is known about the pathophysiology of non-canonical cyclic nucleotides and what is known has been recently reviewed elsewhere⁴.

Therapeutic PDE inhibition

Several PDE family-selective inhibitors have successfully reached the market, targeting diseases such as psoriasis, chronic obstructive pulmonary disorder (COPD) and erectile dysfunction (TABLE 1). Conversely, many potent and selective PDE inhibitors have failed when tested in clinical trials (TABLE 2). Below, we will summarize the main achievements and pitfalls in the development of marketed PDE inhibitors to consider factors that currently limit the effectiveness of such therapeutic agents.

Cerebral hypofusion
Reduces blood flow to/throughout the brain.

Coronary stent stenosis
A gradual re-narrowing of a coronary artery leading to restricted blood flow that occurs after angioplasty and stenting have been performed to relieve a prior blockage.

Given the clinical successes of some PDE inhibitors as discussed, traditional PDE inhibitors are still very much being pursued as potential therapeutics, particularly in the context of the central nervous system (CNS), the cardiovascular system, the reproductive system, cancer and metabolic disorders (TABLE 3).

Marketed PDE inhibitors

The non-selective PDE1 inhibitor vinpocetine is not US Food and Drug Administration (FDA) approved but is available in over-the-counter supplements (for example, Cavinton or Intelectol, Richter Gedeon; Cognitex, Life Extension) claiming to improve memory and recovery from stroke, likely due to increasing vasodilation²⁹. As extensively reviewed elsewhere²⁹, numerous clinical trials have examined the cognition-enhancing effects of vinpocetine — either alone or in combination with another compound (for example, caffeine or Ginkgo biloba) — and have generally found improvement in healthy volunteers, individuals with cerebral hypofusion and possibly aged individuals, but no improvement in patients with AD. Reports of side effects associated with vinpocetine have generally been minimal³⁰ (TABLE 1).

Several PDE3 inhibitors are currently marketed, with cilostazol and milrinone perhaps the most widely known. Cilostazol received FDA approval in 1999 for

intermittent claudication, but off-label uses include secondary prevention of cerebrovascular accident, percutaneous coronary intervention and coronary stent stenosis³¹. Cilostazol improves function across several domains, but is associated with serious side effects (TABLE 1) and, thus, is contraindicated for patients with severe heart failure, hepatic impairment or renal impairment³². Milrinone increases contractility of the heart and is FDA approved for short-term management of severe congestive heart failure. It is particularly used in the context of end-stage heart failure for patients who prove resistant to optimal therapy and for those awaiting cardiac transplant³³. That said, the clinical utility of milrinone has been limited by significant side effects (TABLE 1) and the fact that it is cleared through the kidneys (that is, generally not used in patients with renal failure)³³.

Three so-called ‘second-generation’ PDE4 inhibitors are currently FDA approved, with a fourth compound marketed as an over-the-counter supplement. Roflumilast is an add-on therapy for COPD (TABLE 1). Although it causes gastrointestinal and weight-loss side effects, making it a third-line treatment for COPD, roflumilast improves sugar metabolism in obese patients and may decrease cardiovascular events in patients with COPD³⁴. Apremilast is used in the treatment of moderate to severe plaque psoriasis and psoriatic arthritis^{35–37}

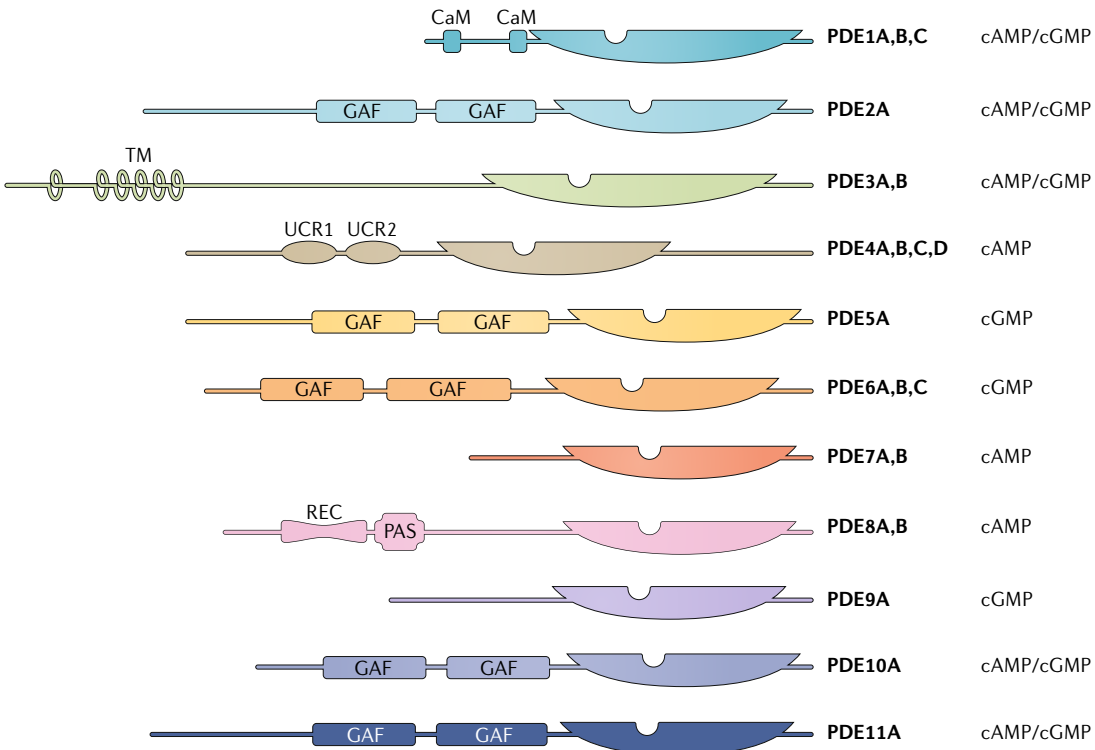


Fig. 2 | **The 21 PDE genes, grouped into families.** Family designation is based on homology of the C-terminal catalytic domain (represented as a semi-ellipse). The name and substrate specificity of each family is shown to the right of each illustration. Due to alternate promoters and splicing events, each phosphodiesterase (PDE) family has multiple isoforms that differ in terms of the length and complexity of their N-terminal regulatory domains (depicted with different shapes), which are thought to regulate subcellular trafficking, substrate affinity and catalytic activity. The relative size and domain distances were drawn based on estimations from the Pfam/Uniprot database, with the exception of the signal receiver (REC) domain of PDE8 (estimated from³²²) and the second calmodulin-binding (CaM) domain of PDE1 (estimated from³⁰⁵). Illustrations represent the longest isoform for gene A of each PDE family. GAF, cGMP-binding PDEs *Anabaena* adenylyl cyclases and *Escherichia coli* FhlA; PAS, Per-Arnt-Sim domain; TM, transmembrane domain; UCR, upstream conserved region.

and is also being tested in a phase IV trial for active ankylosing spondylitis (see below). The most common side effects for both of these orally administered PDE4 inhibitors are the same as those that plagued first-generation PDE4 inhibitors (that is, gastrointestinal disturbances; TABLE 1), albeit with much improved therapeutic windows³⁵. Crisaborole is a topically applied ointment for treatment of moderate atopic dermatitis in patients >2 years old. Given the topical nature of the drug, gastrointestinal side effects are avoided and, instead, hypersensitivity reactions are the major possible side effect. Clearly, there is an anti-inflammatory theme shared among these FDA-approved PDE4 inhibitors. Zembrin is a non-selective PDE4 inhibitor (also acts as a 5-hydroxytryptamine uptake inhibitor) that is a component of numerous herbal supplements claiming calming or mood-stabilizing properties (for example, Calm, Doctor's Best; Mood, ProCera; Nutri-calm, Nature's Sunshine)³⁸. Functional magnetic resonance imaging of the amygdala in humans supports an anxiolytic-like effect of Zembrin³⁸. Further, a phase I trial found that

Zembrin was well tolerated and improved cognitive flexibility, executive function, mood and sleep³⁹. Numerous PDE4 inhibitors are currently being pursued to improve cognitive functioning (see below).

There are four PDE5 inhibitors currently FDA approved and marketed in the USA, with two additional PDE5 inhibitors marketed outside the USA. All six PDE5 inhibitors were originally marketed for erectile dysfunction (TABLE 1), with the most recent approval for avanafil based on its much more rapid onset of action. Sildenafil later received secondary approval for pulmonary hypertension (contraindicated for paediatric patients, veno-occlusive disease or sickle cell disease), as did tadalafil. These PDE5 inhibitors are generally considered safe and well tolerated, with no increase in cardiac mortality or myocardial infarction⁴⁰. They share largely similar side-effect profiles (TABLE 1), with headache, flushing, dyspepsia and vision disturbances being the most common adverse events⁴⁰. Interestingly, udenafil (Zydena, Mezzion Pharma) — one of the PDE5 inhibitors used to treat erectile dysfunction in Korea, Russia and Philippines^{41,42} — has also been reported to improve cognitive function in patients with erectile dysfunction^{43,44}.

The success of several marketed PDE inhibitors validates PDEs as appropriate therapeutic targets in many pathological conditions. However, the presence of unwanted side effects resulting from the inability to target individual isoforms is the major limiting factor to success. It is notable that of the 11 PDE families, only agents that attenuate the activity of PDEs 1, 3, 4 and 5 have made it to market, despite significant efforts targeting the inhibition of other PDE families (see next).

Failed PDE inhibitor clinical trials

Despite the successes noted above, numerous PDE inhibitors that entered the clinic failed to make it to market. The selective PDE2 inhibitor PF-05180999 was originally considered a candidate for cognitive impairments associated with schizophrenia based on its pre-clinical profile⁴⁵; however, it was brought into the clinic for migraine. Even with the completion of earlier phase I safety and tolerability studies, additional trials were terminated early due to safety concerns (TABLE 2). Exisulind inhibits both PDE2A and PDE5A (which are over-expressed in numerous precancerous and cancerous cell types) and triggers apoptosis in precancerous/cancerous cells with minimal effects on healthy cells^{46,47}. Despite promising findings in multiple clinical trials, exisulind failed to secure FDA approval due to deficiencies in safety and efficacy^{46,47} (TABLE 2).

As noted above, cilostazol has gained FDA approval for intermittent claudication; however, clinical trials for other indications, such as type 2 diabetes mellitus peripheral neuropathy, have failed⁴⁸. That said, cilostazol significantly improved walking speed in these patients, suggesting improved peripheral blood flow as would be expected based on its current approved indication⁴⁸.

The PDE4 inhibitor cilomilast (Ariflo, GlaxoSmith-Kline) gained FDA approval in 2003 as a second-line treatment for COPD in patients who are poorly responsive to salbutamol⁴⁹. However, cilomilast never made it to market due to the severely dose-limiting nature of

Box 1 | Physiological roles of PDEs

Because phosphodiesterases (PDEs) are ubiquitously distributed and are the only enzymes to degrade 3',5'-cyclic nucleotides, this superfamily of enzymes plays a role in numerous biological processes in health and disease. That said, the biological roles played by a given PDE isoform are distinct due to its unique expression pattern at the level of tissue/organ, cell type and subcellular compartment (Supplementary Table 1).

Most PDE families are expressed in the nervous system where they regulate neurodevelopment and apoptosis, neuronal excitability, synaptic transmission and neuroplasticity^{71,96}. Every brain region expresses more than one PDE, but no two PDEs exhibit the exact same regional distribution⁷¹. For instance, PDE11A is the only PDE with brain expression restricted to the hippocampus. While PDE11A regulates how well an individual responds to the mood stabilizer lithium, it does not regulate basal anxiety and depression-related behaviours¹⁷⁷. In contrast, PDE4D is expressed in most brain regions and does appear to regulate basal depression-related behaviours (for example, see REF.²⁹⁶). PDE1B, PDE2A, PDE7B, PDE8B and PDE10A are enriched in the striatum relative to other brain regions⁷¹ and each has been implicated in regulating basic motor function, whereas PDE5A and PDE9A that are enriched in the cerebellum have not (for a review, see REFS^{71,297}). In the retina, PDE6 function is central in mediating activation of the light response in rod and cones photoreceptors, and PDE6 mutations cause photoreceptor degeneration in retinitis pigmentosa^{227,298}.

In the cardiovascular system, PDE2, PDE3 and PDE4 isoforms control different subcellular pools of cyclic nucleotides to regulate important cardiac functions from myocardial contraction/relaxation to chronic cell growth and survival, and disruption of this PDE signalling has been associated with disease (for a review, see REF.³). For example, heart failure has been associated with reduced levels of PDE3A and PDE4D, which results in myocyte apoptosis and cardiac arrhythmias, respectively^{225,239}.

Many cancers have been associated with reduced levels of cAMP and/or cGMP secondary to an elevation in PDE activity. For example, chronic lymphocytic leukaemia cells exhibit increased PDE7B expression; leukaemia, colon cancer and glioma cells overexpress one or more isoforms of PDE4; and colon cancer cells and adenocarcinomas exhibit elevated PDE5 activity^{16,299,300}.

Inflammation of numerous tissue types can be enhanced by a drop in cAMP levels that is caused by an increase in cAMP-PDE activity. For example, activity of PDE4 — the predominant cAMP-hydrolysing enzyme in the immune system — is elevated in the context of various inflammatory diseases, including psoriasis, chronic obstructive pulmonary disorder and asthma^{301,302}.

PDEs are also implicated in reproductive health. Several isoforms are present in granulosa cells as well as in oocytes in preovulatory follicles of the mammalian ovary regulating the meiotic cell cycle³⁰³. Furthermore, many PDEs are expressed in cells of the spermatogenic pathway where they may regulate sperm motility^{304,305}, and PDE5 is expressed in the contractile tissues of the male excurrent tract and accessory where its increased activity contributes to erectile dysfunction (for example²³).

Box 2 | PDEs as disease biomarkers

Phosphodiesterases (PDEs) are also being explored as both diagnostic and patient-selection biomarkers. This superfamily of enzymes, like other genes, can be genotyped from blood samples to assess risk for specific diseases (for example, high suicide risk³⁰⁶; PDE8A, NCT02855918). PDE mRNA and protein expression can be measured *ex vivo* in an isoform-specific manner from biopsied samples (for example, excised tumours). Further, imaging compounds can be engineered with relative ease to selectively target a given PDE family/subfamily *in vivo*. Thus, PDEs can be explored as biomarkers in diseases where tissue is readily biopsied (for example, cancers) and in diseases where tissue is not (for example, diseases of the brain).

Ex vivo biomarkers

Measurement of PDE4D7 is seen as a valuable biomarker for both pre-surgical and post-surgical risk stratification to optimize prostate cancer treatment decisions^{306,307} (TABLE 4). Studies in patient samples showed that PDE4D7 expression is initially upregulated with the development of primary tumours but then is downregulated when the disease progresses to an androgen-independent state (for example, in castration-resistant tumours)^{308,309}, consistent with *in vitro* reports using prostate cancer cells¹⁹³. The analysis of PDE4D7 expression in biopsy/surgery samples has been applied to develop InformMDx (licensed by MDxHealth from Philips), a tissue-based prognostic prostate cancer biomarker test to stratify patients by risk of disease progression and secondary tumours and, thus, inform post-biopsy/post-surgery treatment decisions³¹⁰.

PDE3A may also represent a useful cancer biomarker as it is greatly expressed in certain cancer cell types such as squamous carcinoma cell lines or gastrointestinal stromal tumour cells^{311,312}. Furthermore, cancer cell lines with the highest PDE3A expression proved the most susceptible to the chemotherapeutic effects of PDE3 inhibitors³¹³. Thus, PDE3A expression could qualify as a biomarker for patient selection which improves patient care by reducing exposure to ineffective drugs and accelerates clinical development of novel therapeutic agents by testing them in targeted populations.

In vivo biomarkers

Altered cyclic nucleotide signalling has been implicated in various age-related diseases of the brain²³⁸. PDE10A is widely reported as downregulated in both the striatum and the cortex of patients with Huntington disease, with the extent of PDE10A loss corresponding to the genetic burden associated with the disease^{28,68,314,315}. A loss of PDE10A expression has also been observed in the basal ganglia of patients with Parkinson disease³¹⁶. Importantly, highly-specific PDE10A positron emission tomography (PET) tracers show that PDE10A expression in patients with Huntington disease continues to decline over years²⁸. Thus, PET imaging of PDE10A could be a useful biomarker for assessing the initial diagnosis and subsequent progression of these neurodegenerative diseases²⁷. PET ligands also exist for PDEs 2, 4, 5 and 7 (REF. 317).

gastrointestinal side effects (for example, nausea and vomiting, diarrhoea and abdominal pain³⁵). The fact that cilomilast elicited more pronounced side effects relative to the other systemically delivered PDE4 inhibitors described above may be related to preferential inhibition of the PDE4D family relative to the other PDE4 subtypes⁴⁹. The novel PDE4 inhibitor ASP9831 was tested in phase I and II trials for non-alcoholic steatohepatitis based on preclinical findings, but failed to improve biochemical biomarkers of the disease⁵⁰. As target engagement in the organ of interest was not confirmed⁵⁰, the reasons underlying the lack of efficacy remain unclear.

Several clinical trials have attempted to extend therapeutic indications for PDE5 inhibitors but failed. As reviewed extensively elsewhere²⁹, numerous trials have tested the effects of sildenafil or vardenafil on various measures of cognition in healthy volunteers or patients with schizophrenia and have largely found no effects^{51–55}. That said, one report from an Iranian clinical trial did report an improvement in negative symptoms in patients with chronic schizophrenia when sildenafil was administered in addition to risperidone⁵⁶. Several studies were initiated to study sildenafil and/or tadalafil in patients

with Duchenne or Becker muscular dystrophy, with the hope that the vasodilatory properties of the drugs would improve muscular ischaemia; however, clinical trial outcomes have been mixed^{57,58} (TABLE 2).

Two PDE9 inhibitors have been tested in the clinic for cognition-enhancing effects. Although PF-04447943 was found to be safe and well tolerated, it failed to improve either cognition or dementia-related behavioural disturbances in a phase II clinical trial⁵⁹. Similarly, BI 409306 was reported as safe and well tolerated in healthy subjects as well as in patients with AD or schizophrenia; however, no positive effects on cognition were observed in either patient population^{60–64}. BI 409306 is still being tested in the clinic for prevention of schizophrenia relapse and prevention of first psychotic episode (TABLE 3). The failure of PDE9 inhibitors to improve functioning in AD may be related to the fact that brain PDE9A is enriched in the nucleus and membrane⁵ and, thus, is not in a position to directly regulate the cytosolic pools of cGMP that appear to be dysregulated in AD^{13–15}.

Numerous clinical trials have tested the PDE10 inhibitor PF-02545920 in schizophrenia and Huntington disease (TABLES 2, 3). Despite widely replicated efficacy in several preclinical assays (for example, see REFS^{65,66}), PF-02545920 failed to improve symptoms in patients with either exacerbated, stable or suboptimally treated schizophrenia⁶⁷ (TABLE 2). Further, in at least one trial, there were reports of motoric side effects such as dystonia⁶⁷. Similarly, despite decreased striatal expression of PDE10A being found in patients with Huntington disease (BOX 2) and promising efficacy of PDE10 inhibitors in preclinical models of the disease^{28,68,69}, PF-02545920 failed to improve symptoms in patients with Huntington disease (NCT02197130; accessed 28 May 2019), and so efforts for this disease indication were terminated (NCT02342548; accessed 28 May 2019). Several other PDE10 inhibitors have also been pursued in the clinic for schizophrenia and/or Huntington disease, with some efforts subsequently suspended or terminated (TABLE 2) and others ongoing (TABLE 3; see more below).

The unsuccessful translation of these PDE inhibitors from promising preclinical data to human testing suggests that therapeutic approaches targeting PDEs need to extend beyond occlusion of the enzyme's catalytic site. Of particular note are the numerous failures seen in nervous system disorders, even when target engagement was verified. Expression of PDEs in the brain is particularly complex, with PDE isoforms differentially expressed across circuits, cell types and subcellular domains^{5,30,70,71}. Thus, the challenge in evaluating the clinical potential for the next generation of PDE-modulating drugs is to gain novel insights about disease-related changes in PDE structure, function and regulation to understand how PDEs should be targeted in a compartment-specific manner for therapeutic gain.

PDE inhibitors in development

Ongoing efforts in the development of novel PDE inhibitors include creation of new chemical entities as well as the repurposing of existing entities. Advances in our understanding of structural differences that exist between PDEs, coupled with extensive medicinal

Dystonia

A movement disorder characterized by repetitive or twisting movements due to involuntary muscle contraction.

chemistry efforts to optimize structure–activity relationships, have yielded recent vast improvements in terms of selectivity and potency (for example, see work related to PDE10 inhibitors and PDE4 inhibitors^{72–74}). These efforts have also yielded PDE inhibitors with novel modes of action in some cases (that is, acting as a negative allosteric modulator instead of a direct catalytic inhibitor⁷³). In addition, there are still significant efforts to repurpose older PDE inhibitors. Drug-repurposing efforts can be driven by computational or experimental approaches; however, most drug-repurposing efforts have been driven either by a better understanding of pharmacology or by a retrospective analysis of clinical effects that were observed during trials or marketed use of a drug for its original indication⁷⁵. Indeed, the PDE5 inhibitor sildenafil was originally brought into clinical trials for angina but — following observations made by clinicians in that trial — was later repurposed for erectile dysfunction. Drug repurposing has several advantages including reduced risk and substantially reduced timelines and cost due to the fact that the drug would already have passed preclinical and phase I safety testing, and possibly even formulation development⁷⁵. That said, there are numerous barriers to recouping expenses incurred by drug-repurposing trials, particularly when they are carried forward by an entity other than the patent holder or following the expiration of the original patent (see REF.⁷⁵ for further discussion).

Non-selective inhibitor. The non-selective PDE3, 4, 10 and 11 inhibitor Ibudilast, which also inhibits glial cell activation, is approved for use in Japan as a bronchodilator and has long been of interest as a therapeutic approach for neuropathic pain and substance abuse/withdrawal^{76,77}. Recent clinical trials have tested Ibudilast in the context of amyotrophic lateral sclerosis and pain, as well as opiate, methamphetamine and alcohol abuse (TABLE 3), and positive effects have been reported for all trials completed to date^{78–81}.

PDE1 inhibitors. The broad PDE1 inhibitor ITI-214, which shows picomolar half-maximal inhibitory concentration (IC₅₀) values for PDE1A, PDE1B and PDE1C in enzymatic assays and >1000-fold selectivity versus its nearest neighbour PDE4 (REFS^{82,83}), is being explored for CNS and non-CNS indications. ITI-214 demonstrates cognition-enhancing effects in rodent models of long-term memory and working memory deficits^{82–84}, mimicking effects of a dopamine 1 receptor (D1R) agonist⁸⁴ and occurring at doses that leave efficacy of the antipsychotic risperidone intact⁸². Although the target mediating the cognition-enhancing effects of ITI-214 remains undetermined, PDE1B may be the most likely candidate given its expression in D1R-expressing neurons⁸⁴, along with the fact that a PDE1B-selective inhibitor showed similar cognition-enhancing effects⁸⁵. ITI-214 was moved into the clinic, with potential applications for cognitive deficits associated with schizophrenia, AD and Parkinson disease⁸³, with safety and tolerability established in healthy volunteers and patients with schizophrenia (TABLE 3). ITI-214 is also being explored in the clinic for heart failure (NCT03387215)

given its ability to improve cardiac function in dog and rabbit models of heart failure⁸⁶ as a consequence of its inhibition of PDE1C⁸⁶.

PDE2 inhibitors. Several highly selective PDE2 inhibitors have demonstrated cognition-enhancing, anxiolytic and antidepressant-like effects in animal models⁸⁷. TAK-915 entered phase I trials to correlate plasma exposures with central target engagement, with the purpose of informing dose selection for future trials targeting cognitive impairment in schizophrenia^{88–90} (TABLE 3). Looking beyond the brain, PDE2 inhibitors may hold relevance for cardiovascular function as elevated PDE2A expression has been found in failing human hearts as well as a large number of animal models of heart disease⁹¹. Further, PDE2 inhibitors may hold promise as an antifungal treatment for moderate to severe candidiasis infections, given that genetic deletion of *Pde2a* reduces virulence and biofilm integrity of the fungal pathogen⁹².

PDE3 inhibitors. Despite its existing FDA approval, the efficacy and safety of cilostazol is still very much a topic of investigation, with 27 active clinical trials registered on ClinicalTrials.gov (accessed 30 April 2019) and 54 more drawn to a close within just the past 10 years. Numerous recent phase IV studies appear focused on broadening the therapeutic indications of cilostazol to include vasculature-related insults and nephropathies associated with type 2 diabetes (TABLE 3), and recent reports suggest largely positive effects^{31,48,93,94}. This PDE3 inhibitor also elicited some improvement in chronic tinnitus⁹⁵. Several prospective and retrospective studies have examined cilostazol as a primary or adjunctive treatment for cognitive deficits associated with AD and schizophrenia; the majority of which demonstrated positive effects on cognition (see REF.²⁹ for a review). The mechanism by which cilostazol elicits improved cognition has yet to be determined empirically. Given that there is very little expression of PDE3A or PDE3B in the brain^{71,96}, it may be likely that the cognition-enhancing effects of cilostazol are driven by increased cerebral blood flow that comes with chronic — but not acute — dosing as opposed to inhibition of PDE3 isoforms directly in the brain (for example, see REF.⁹⁷).

Novel therapeutic applications of cilostazol are also being explored in preclinical studies. For example, oral cilostazol improved retinal stress, ischaemia and ganglion cell death in a rat model of diabetic retinopathy⁹⁸. In addition, *Pde3a*-knockout mice are infertile⁹⁹ and chronic administration of cilostazol blocks pregnancy in naturally cycling swine¹⁰⁰, suggesting potential utility of PDE3 inhibitors for birth control or regulating in vivo oocyte maturation in the context of assisted reproduction. Indeed, administration of cilostazol to superovulated mice improved in vitro fertilization rates of subsequently harvested oocytes, possibly by virtue of synchronizing the oocyte maturation¹⁰¹.

Due to the promise of PDE3 as a therapeutic target, coupled with concerns over side effects associated with cilostazol, alternative PDE3 inhibitors are currently being developed^{102–104}.

Table 1 | **Marketed PDE inhibitors**

Compound (popular trade names)	Indication	Approval date (USA, Europe and Asia markets)	Side effects
Non-selective			
Theophylline (inhibits PDE3, 4, 7, adenosine 2 receptors) (Theolair, Slo-Bid, Theo 24)	Asthma and bronchoconstriction	FDA (1937), Europe (for example, Spain, 1922), Asia (for example, India, 1969)	Nausea, vomiting, diarrhoea, headache, irritability, flushing and palpitations
Aminophylline (inhibits PDE3, 4, 7, adenosine 2 receptors) (Phyllocontin)	Asthma and bronchoconstriction	FDA (1940), Europe (for example, Hungary, 1935), Asia (for example, India, 1950)	Stomach pain, diarrhoea, headache, irritability, restlessness and insomnia
Oxtriphylline (inhibits PDE3, 4, 7, adenosine 2 receptors) (Choledyl)	Asthma and bronchoconstriction	FDA (1981), Europe (only in Greece, 2003)	Stomach pain, nausea, vomiting, diarrhoea, headache, irritability, restlessness, insomnia, flushing and increased urination
Dyphylline (inhibits PDE3, 4, 7, adenosine 2 receptors) (Dilor, Lufyllin)	Asthma and bronchoconstriction	FDA (1951), some countries in Europe (for example, Spain, 1968) and Asia (for example, Japan, 1952)	Stomach pain, nausea, vomiting, diarrhoea, headache, irritability, restlessness, insomnia, flushing and increased urination
Pentoxifylline (inhibits PDE4, 5, adenosine 2 receptors) (Trental, Pentoxil)	Intermittent claudication	FDA (1984), some countries in Europe (for example, Spain, 1978) and Asia (for example, India, 1975)	Belching, bloating, upset stomach, nausea, vomiting, indigestion, dizziness, flushing and headache
Ibudilast (highest affinity for PDE10A, 4, 11, 3) (Ketas, Pinatos, Eyevinal)	Asthma and dizziness related to cerebral infarction	Asia (Japan, 1989; South Korea, 1998; China, 2003)	Nausea, diarrhoea and abdominal pain, depression, rash and fatigue
	Allergic conjunctivitis	Japan (2000)	Nausea, diarrhoea and abdominal pain, depression, rash and fatigue
Tofisopam (highest affinity for PDE4, 10, 3, 2) (Emandaxin, Grandaxin)	Anxiety	Some countries in Europe (for example, Hungary, 1974) and Asia (for example, Japan, 1985)	Nausea, stomach discomfort, dry mouth, skin rash, insomnia, vomiting and drowsiness
Dipyridamole (highest affinity for PDE8, 1, 3, 2, adenosine deaminase and ENT1) (Persantine)	Post-operative thromboembolism	FDA (1961), some countries in Europe (for example, Spain, 1986) and Asia (for example, India, 1964)	Headache, dizziness, nausea, diarrhoea, muscle pain and vomiting
PDE1			
Vinpocetine (Cavinton)	Cerebral vascular disorders and memory impairment	USA as an over-the-counter dietary supplement, some countries in Europe (for example, Spain, 1997) and Asia (for example, India, 2002)	Flushing, rashes and minor gastrointestinal disturbances
PDE3			
Cilostazol (Pletal, Ektisol)	Intermittent claudication	FDA (1999), some countries in Europe (for example, UK, 2000) and Asia (for example, South Korea, 1990)	Headache, palpitations, diarrhoea, dizziness, nasal irritation and pharyngitis
Milrinone (Primacor, Corotrope)	Congestive heart failure	FDA (1987), EMA (2016), Asia (for example, Japan, 1996)	Ventricular/supraventricular arrhythmias, hypotension and headache
Amrinone (Inamrinone, Inocor)	Congestive heart failure	FDA (1984), some countries in Asia (for example, India, 1988)	Thrombocytopenia, nausea, diarrhoea, hepatotoxicity, arrhythmias and fever
Enoximone (Perfan)	Congestive heart failure	Some countries in Europe (for example, France, 1987)	Headache, diarrhoea, insomnia, hypotension, vomiting, nausea, tachycardia and arrhythmias
Olprinone (Coretec)	Heart failure	Japan (1996)	Cardiac dysrhythmias and thrombocytopenia
Pimobendan (Acardi)	Heart failure	Japan (1994)	Headache, palpitation, nausea and ventricular arrhythmias
Anagrelide (also inhibits phospholipase A2) (Agrylin, Xagrid)	Thrombocythaemia	FDA (1997), EMA (2004), some countries in Asia (for example, South Korea, 2004)	Headache, diarrhoea, unusual weakness/fatigue, hair loss, nausea and dizziness
PDE4			
Roflumilast (Daliresp, Daxas)	Chronic obstructive pulmonary disease	FDA (2011), EMA (2010), some countries in Asia (for example, India, 2014)	Diarrhoea, weight loss, nausea, headache, insomnia and decreased appetite
Apremilast (Otezla)	Psoriasis and psoriatic disorders	FDA (2014), EMA (2014), some countries in Asia (for example, Japan, 2016)	Diarrhoea and vomiting, weight loss and mood changes
Crisaborole (Eucrisa)	Moderate atopic dermatitis (patients >2 years old)	FDA (2016)	Hypersensitivity reactions of the skin

Table 1 (cont.) | Marketed PDE inhibitors

Compound (popular trade names)	Indication	Approval date (USA, Europe and Asia markets)	Side effects
PDE4 (cont.)			
Drotaverine (also inhibits L-type voltage-operated calcium channel) (No-Spa, Doverin)	Functional bowel disorders; pain caused by smooth muscle spasm	Some countries in Europe (for example, Hungary, 1963) and Asia (for example, China, 1999)	Fainting, nausea, vomiting and dry mouth
PDE5			
Sildenafil (Viagra, Revatio)	Erectile dysfunction	FDA (1998), EMA (1998), Asia (for example, Japan, 1999)	Headache, flushing, dyspepsia, nasal congestion, and impaired vision, including photophobia and blurred vision
	Pulmonary arterial hypertension	FDA (2014), EMA (2005), Asia (for example, Japan, 2008)	Headache, flushing, dyspepsia, nasal congestion, and impaired vision, including photophobia and blurred vision
Vardenafil (Levitra, Staxyn, Vivanza)	Erectile dysfunction	FDA (2003), EMA (2003), some countries in Asia (for example, Japan, 2004)	Headache, flushing and dyspepsia
Tadalafil (Cialis, Adcirca)	Erectile dysfunction, benign prostatic hyperplasia	FDA (2003), EMA (2002), some countries in Asia (for example, India, 2003)	Headache, dyspepsia, back pain and myalgia
	Pulmonary arterial hypertension	FDA (2009), EMA (2008), some countries in Asia (for example, India, 2009)	Headache, dyspepsia, back pain and myalgia
Avanafil (Stendra, Spedra)	Erectile dysfunction	FDA (2012), EMA (2013), some countries in Asia (for example, South Korea, 2011)	Headache, flushing and nasopharyngitis
Udenafil (Zydena)	Erectile dysfunction and hypertension	Some countries in Asia (for example, South Korea, 2005)	Headache, dizziness, reddening, nasal congestion, dyspepsia and impaired vision
Mirodenafil (Mvix)	Erectile dysfunction	South Korea (2007)	Flushing, headache, nasal congestion, eye redness, nausea and dizziness
PDE10A			
Papaverine (Pavabid, Pavagen)	Visceral spasm, vasospasm and erectile dysfunction	FDA, Europe (for example, Hungary, 1933), Asia (for example, Japan, 1953)	Ventricular tachycardia, diarrhoea, somnolence, vertigo, flushing and headache

Source: Drugs.com, drugcentral.org, drugbank.ca, kegg.jp and approval dates by the US Food and Drug Administration (FDA), EMA (European Medicines Agency), ANSM (French Agency for the Safety of Health Products), MHRA (Medicines and Healthcare products Regulatory Agency), AEMPS (Spanish Agency of Medicines and Medical Products), EOF (Greek National Organization for Medicines), OGYÉI (National Institute of Pharmacy and Nutrition), PMDA (Pharmaceuticals and Medical Devices Agency), CDSCO (Central Drugs Standard Control Organisation), MFDS (Ministry of Food and Drug Safety) and CFDA (China Food and Drug Administration). PDE, phosphodiesterase.

PDE4 inhibitors. The PDE4 family is arguably the most studied of all PDE families. Numerous clinical trials have tested the effect of apremilast for indications beyond psoriasis and arthritis. Two phase II studies are testing the efficacy of apremilast in combination with phototherapy to produce repigmentation in patients with vitiligo (TABLE 3). Interestingly, a recent case report showed that apremilast dramatically improved repigmentation in a woman with treatment-resistant vitiligo¹⁰⁵. Multiple case reports have also described an ability of apremilast to improve symptoms in patients with treatment-resistant erosive oral lichen planus^{106–108}, perhaps motivating the recently registered phase II study that will test the ability of apremilast to improve genital erosive lichen planus (TABLE 3).

Additional indications are also being explored for roflumilast. Phase IV studies showed that roflumilast reduced fat mass and, thus, body weight in obese women with polycystic ovary syndrome; however, these reductions were smaller than those elicited by liraglutide^{109,110} (TABLE 3). The PDE4 inhibitor TAK-648 is being tested in the clinic in patients with type 2 diabetes, based on preclinical data¹¹¹. Roflumilast has also been tested for its ability to improve cognition and information processing in healthy humans, with promising results observed

at a dose previously indicated as being devoid of side effects¹¹². Patients with stabilized schizophrenia receiving adjuvant roflumilast in a small phase II trial showed no improvement in working memory but did show some improvement in verbal learning and memory¹¹³. Given these positive findings, roflumilast was tested in elderly subjects who demonstrated no change in spatial memory but improved verbal word memory with roflumilast treatment¹¹⁴. Numerous preclinical studies have supported the therapeutic potential of PDE4 inhibitors in the context of schizophrenia and cognition^{115–118}.

Cognition-enhancing effects have also been reported for the PDE4 inhibitor HT-0712, which improved long-term memory for word lists without serious adverse events in elderly subjects experiencing cognitive decline¹¹⁹. The cognition-enhancing effect of HT-0712 in humans is consistent with previous reports in mice^{120,121}. The PDE4D negative allosteric modulator BPN14770 is also being pursued for improving cognitive impairment and has been tested for safety and/or efficacy in healthy elderly subjects, in healthy volunteers with scopolamine-induced cognitive impairment and in adult males with fragile X syndrome (TABLE 3). In a press release, BPN14770 was described as having good safety and oral bioavailability and an ability to improve

Lichen planus

An inflammatory condition characterized by purplish, itchy bumps on/around the skin, hair, nails and mucous membrane (due to unknown causes).

Table 2 | Clinical trials involving PDE inhibitors that failed to reach the market for the described indication

Compound/manufacturer	Indication	Phase/status	Clinical trial ID (refs)	Cause of failure
PDE2				
PF-05180999/Pfizer	Healthy volunteers (schizophrenia)	Phase I/completed 2011	NCT01429740	–
	Healthy volunteers	Phase I/completed 2012	NCT01530529	–
	Migraine	Phase I/terminated early 2014	NCT01981499	Safety concerns
	Migraine	Phase I/withdrawn prior to enrolment 2014	NCT01981486	–
PDE2/5				
Exisulind (Aptosyn)/Cell Pathways	Breast cancer neoplasms and metastases	Phase I/II/completed 2003	NCT00037609	–
	Breast cancer	Phase II/completed 2008	NCT00039520	–
	Non-small cell lung cancer	Phase I/II/III/completed 2003–2007	NCT00072618, NCT00085826, NCT00041314	–
	Small cell lung cancer	Phase II/completed 2008	NCT00041054 (REF. ³²⁹)	Lack of efficacy
	Prostate cancer and prostatic neoplasms	Phase II/completed 2006–2011	NCT00078910, NCT00166478, NCT00166426, NCT00052845, NCT00283803 (REFS ^{330,331})	Safety and efficacy deficiencies
	Melanoma	Phase II/completed 2011	NCT00841204 (REF. ³³²)	Lack of efficacy
PDE3				
Cilastazol (Pletal)/Otsuka Pharmaceuticals	Type 2 diabetes polyneuropathy	Phase IV/completed 2009	NCT01076478 (REF. ⁴⁸)	Lack of efficacy
PDE4				
ASP9831/Astellas Pharma	Non-alcoholic steatohepatitis	Phase II/completed 2010	NCT00668070 (REF. ⁵⁰)	Lack of efficacy
PDE5				
Sildenafil (Viagra or Revatio) or tadalafil (Cialis or Adcirca)/Pfizer and Eli Lilly, respectively	Duchenne or Becker muscular dystrophy	Early phase I/phase I/completed 2013	NCT01359670, NCT01580501 (REF. ⁵⁷)	–
		Phase II/III/terminated early 2014–2017	NCT01168908, NCT01865084 (REFS ^{333–335})	Lack of efficacy
		Phase IV/completed 2012	NCT01070511 (REF. ⁵⁸)	–
	Impaired glucose tolerance	Phase IV/terminated early 2016	NCT01409993 (REF. ³³⁶)	Safety concerns
Vardenafil (Levitra)/Bayer/GlaxoSmithKline	Type 2 diabetes	Phase II/completed 2014	NCT02219646 (REF. ¹⁵²)	Lack of efficacy
PDE9				
BI 409306/Boehringer Ingelheim	Alzheimer disease	Phase II/completed 2017	NCT02240693, NCT02337907 (REFS ^{64,337})	Lack of efficacy
PF-04447943/Pfizer	Alzheimer disease	Phase II/completed 2010	NCT00930059 (REF. ⁵⁹)	Lack of efficacy
PDE10				
PF-02545920 (also known as MP-10)/Pfizer	Schizophrenia	Phase I/completed 2007	NCT00463372	–
	Schizophrenia	Phase II/terminated early 2008	NCT00570063	Safety concerns
	Healthy volunteers (glucose metabolism)	Phase I/completed 2011	NCT01103726	–
	Schizophrenia	Phase II/completed 2011	NCT01175135 (REFS ^{67,338})	Lack of efficacy
	Schizophrenia	Phase I/terminated early 2012	NCT01244880	Results from another clinical study
	Schizophrenia	Phase I/completed 2013	NCT01829048	–
	Schizophrenia	Phase II/terminated early 2014	NCT01939548 (REF. ³³⁹)	Lack of efficacy
	Healthy male volunteers (PET imaging of PDE10A)	Phase I/completed 2014	NCT01918202 (REF. ³⁴⁰)	–
	Huntington disease	Phase II/completed 2015–2016	NCT01806896, NCT02197130	Lack of efficacy
	Huntington disease	Phase II/terminated early 2017	NCT02342548	Results from NCT02197130

Table 2 (cont.) | Clinical trials involving PDE inhibitors that failed to reach the market for the described indication

Compound/manufacture	Indication	Phase/status	Clinical trial ID (refs)	Cause of failure
PDE10 (cont.)				
OMS643762/Omeros	Schizophrenia	Phase II/completed 2014	NCT01952132	–
	Huntington disease	Phase II/terminated early 2016	NCT02074410	Results from NCT02197130
PBF-999/Palobiofarma	Huntington disease	Phase I/completed 2015	NCT02208934	–
	Huntington disease	Phase I/terminated early 2016	NCT02907294	Change in therapeutic indication (cancer)

Reported on ClinicalTrials.gov (accessed 28 May 2019). Information is included for all clinical trials involving molecules whose pursuit was terminated after April 2009. PDE, phosphodiesterase; PET, positron emission tomography.

working memory in healthy elderly adults¹²². These effects in humans are consistent with preclinical studies showing that BPN14770 improved numerous behaviours in a mouse model of fragile X syndrome and antagonized the amnesic effects of scopolamine in mice^{123,124}. Based on preclinical studies showing anxiolytic and cognition-enhancing effects¹²⁵, the PDE4 inhibitor GSK356278 entered phase I safety trials for Huntington disease but adverse events limited the highest dose to that achieving only ~50% occupancy in the brain¹²⁵ (TABLE 3). Other nervous system disorders for which preclinical evidence suggests a therapeutic potential of PDE4 inhibitors include ischaemic stroke^{120,126–129}, traumatic brain injury¹³⁰, axon regeneration¹³¹ and substance abuse disorders (both causes and consequences^{132–135}).

McCune–Albright syndrome is a disease affecting endocrine tissues, skin and bones and is caused by a mutation that results in constitutive activation of the G-protein α -subunit G_{α_s} ($G_{\alpha_s}^*$). Preclinical studies show that whereas $G_{\alpha_s}^*$ triggers increased cAMP levels in some tissues, it actually results in decreased cAMP levels in other tissues due to a protein kinase A (PKA)-dependent upregulation of PDE activity, particularly that of PDE1 and PDE4 (REFS^{116,136,137}). Consistent with this upregulation of PDE4 activity, the PDE4 inhibitor rolipram was able to reverse deficits in G_{α_s} mouse models^{116,117}. A clinical trial measuring PDE4 expression in the brain and peripheral organs of patients with McCune–Albright syndrome is currently underway (TABLE 3).

More recent work examined PDE4 in the context of cancer. Both preclinical and clinical data suggest that rolipram may exhibit antitumor activity for B cell lymphomas¹³⁸. The PDE4 inhibitor rolipram, in combination with cAMP-elevating agents, has been shown to suppress triple-negative breast cancer both in vitro and in vivo in mice¹³⁹. Apremilast similarly induced tumour regression in mouse models of colorectal cancer¹⁴⁰. Perhaps even more interesting, specific inhibition of PDE4D, either with genetic tools or the PDE4D inhibitor Gebr-7b, resensitized chemotherapy-resistant oestrogen receptor-positive breast cancer cells¹⁴¹. These early studies provide promise for the chemotherapeutic potential of PDE4 inhibitors.

PDE5 inhibitors. Several recent clinical trials have explored additional disease indications that might benefit from the vasodilatory properties of PDE5 inhibitors. A cream version of sildenafil has recently been tested in

a phase II study examining female sexual arousal disorder (TABLE 3) as well as in a study in which improved blood flow in patients with secondary Raynaud phenomenon was observed¹⁴². International consortiums are investigating the effects of sildenafil in intrauterine growth restriction, due to anticipated improvements in uteroplacental perfusion^{143,144}. Initial results suggest sildenafil improves fetal growth and maternal blood pressure across species, including human, sheep, rabbit and rodent^{145,146}. Several studies have also explored the effects of sildenafil, tadalafil or vardenafil in the context of metabolic disorders such as type 2 diabetes and obesity, assessing glucose tolerance and insulin signalling as well as effects on elevated body weight, nephropathy and cardiomyopathy (TABLE 3). Tadalafil improved insulin secretion, endothelial function and abdominal lean mass content in non-obese men¹⁴⁷, and chronic sildenafil improved glycometabolic control, ameliorated visceral adiposity and prevented remodelling in diabetic cardiomyopathy^{148–151}. That said, vardenafil failed to reduce cardiovascular risk in men with type 2 diabetes¹⁵². Interestingly, the positive effects of sildenafil on adiposity and diabetic cardiomyopathy are suggested to be independent of sildenafil's vasodilatory properties, rather, being mediated by epigenetic signalling and/or a reduction of inflammatory chemokines^{148–151}. It is important to note, however, that one of the studies examining the effect of sildenafil on glucose homeostasis was terminated early due to safety concerns (TABLE 2). With regard to other indications related to nephropathy and cardiomyopathy, sildenafil has also been tested against contrast media-induced nephropathy, and tadalafil is being explored in the context of kidney stones and endocrine cardiomyopathy (TABLE 3). A meta-analysis of older clinical studies suggests PDE5 inhibitors could be an effective medical expulsive therapy for distal ureteral calculi, albeit not significantly improved relative to tamsulosin¹⁵³. With regard to the brain, two early-stage clinical trials are testing the ability of sildenafil to reverse concussion-related reductions in cerebrovascular reactivity (TABLE 3).

As described for PDE4 inhibitors, numerous trials are exploring the therapeutic potential of PDE5 inhibitors as chemopreventives for solid tumours, multiple myeloma and head and neck squamous cell carcinoma. Early reports suggest that combining sildenafil with the chemotherapeutic regorafenib is safe in patients with solid tumours¹⁵⁴. Further, numerous in vitro and animal

Table 3 | Selected clinical trials involving PDE inhibitors pursued for new indications

Compound/manufacture	Indication	Phase/status	Clinical trial ID (refs)
PDE3, 4, 10 and 11			
Ibutilast (AV-411, MN-166)/ MediciNova	Opioid withdrawal	Phase II/completed 2012–2017	NCT00723177, NCT01740414
	Methamphetamine dependence	Phase I/completed 2013	NCT01217970 (REFS ^{78,79})
	Alcohol use disorder	Phase I/completed 2015	NCT02025998 (REF. ⁸¹)
	Alcohol use disorder	Phase II/recruiting	NCT03489850
	Opioid abuse	Phase II/completed 2017	NCT01740414 (REFS ^{80,341})
	Amyotrophic lateral sclerosis (biomarker study)	Phase II/active, not recruiting	NCT02714036
PDE1			
ITI-214/Intracellular Therapies	Schizophrenia	Phase I/terminated early 2014	NCT01900522
	Parkinson disease	Phase I/II/completed 2018	NCT03257046
	Healthy volunteers (central nervous system engagement)	Phase I/recruiting	NCT03489772
	Systolic heart failure	Phase I/II/recruiting	NCT03387215
Vinpocetine/Rxmidas Pharmaceuticals/ Nootrobox	Ischaemic stroke	Phase II/III/completed 2013–2015	NCT01400035, NCT02878772 (REF. ³⁴²)
	Cognition enhancement	Not applicable/completed 2017	NCT02857829
PDE2			
TAK-915/Takeda	Healthy volunteers (schizophrenia)	Phase I/completed 2016	NCT02584569
	Healthy volunteers	Phase I/completed 2016	NCT02461160
PDE3			
Cilostazol (Pletal)/Otsuka Pharmaceuticals	Type 2 diabetic atherosclerosis	Phase IV/completed 2010–2012	NCT01252056, NCT00823849 (REF. ³⁴³)
	Chronic tinnitus	Not applicable/completed 2013	NCT01378650 (REF. ⁹⁵)
	Alzheimer disease	Phase IV/completed 2013	NCT01409564 (REF. ³⁴⁴)
	Atherosclerotic events in type 2 diabetes	Phase IV/unknown	NCT00886574
	Mild cognitive impairment	Not applicable/completed 2015	NCT01872858
	Ischaemic events in type 2 diabetic artery obstruction	Phase IV/recruiting	NCT02983214
	Antiplatelet aggregation in type 2 diabetes	Phase IV/active, not recruiting	NCT03248401
	Antiplatelet aggregation in type 2 diabetes	Phase IV/recruiting	NCT02983214
	Antiplatelet aggregation in type 2 diabetes	Phase IV/unknown	NCT02933788
PDE4			
Apremilast (Otezla)/ Celgene Corp.	Vitiligo	Phase II/active, not recruiting	NCT03036995, NCT03123016
	Lichen planus of vulva	Phase II/not yet recruiting	NCT03656666, NCT03836885
BPN14770/Tetra Discovery	Alzheimer disease	Phase I/completed 2016–2017	NCT02840279, NCT03030105
	Alzheimer disease	Phase II/now recruiting	NCT03817684
	Fragile X syndrome	Phase II/recruiting	NCT03569631
Crisaborole (Eucrisa)/Pfizer	Morphea	Phase II/recruiting	NCT03351114
GSK356278/ GlaxoSmithKline	Huntington disease	Phase I/completed 2012	NCT01573819, NCT01602900
Roflumilast (Daxas or Daliresp)/Astrazeneca	Polycystic ovary syndrome	Phase IV/completed 2014	NCT02037672, NCT02187250 (REFS ^{109,110})
	Cognitive deficits in schizophrenia	Phase II/completed 2015	NCT02079844 (REF. ¹¹³)
	Cognition (dementia)	Phase II/completed 2013–2015	NCT01433666, 2013–001223-39 (EudraCT) ^{112,114,345}
	Insulin and blood sugar levels in prediabetic overweight and obese individuals	Phase I/II/completed 2017	NCT01862029 (REF. ³⁴⁶)

Table 3 (cont.) | Selected clinical trials involving PDE inhibitors pursued for new indications

Compound/manufacture	Indication	Phase/status	Clinical trial ID (refs)
PDE4 (cont.)			
HT-0712/Dart Neuroscience	Age-associated memory impairment	Phase II/completed 2015	NCT02013310
NA	McCune-Albright Syndrome (PET imaging of PDE4)	Phase I/II/recruiting	NCT02743377
TAK-648/Takeda	Type 2 diabetes	Phase I/completed 2015	NCT02480439, NCT02684396, NCT02430870
Zembrin/ND	Aged individuals	Phase I/completed 2012	NCT01805518 (REF. ³⁹)
PDE5			
ND	Contrast media-induced nephropathy	Not applicable/unknown	NCT01564303
ND	Diabetic nephropathy	Not applicable/unknown	NCT01566006
Sildenafil (Viagra or Revatio)/Pfizer	Diabetic cardiomyopathy (type 2 diabetes)	Phase IV/completed 2009	NCT00692237 (REFS ^{148–151})
	Metabolic syndrome (skeletal muscle insulin signalling)	Phase IV/completed 2016	NCT02129725
	Urolithiasis/urinary stones	Phase IV/active, not recruiting	NCT02519153
	Migraine aura	Early phase I/recruiting	NCT02795351
	Solid tumours	Phase I/active, not recruiting	NCT02466802 (REF. ¹⁵⁴)
	Mild traumatic brain injury or concussion	Phase I/recruiting	NCT03417492, NCT02990078
Sildenafil cream (SST-6007)/Strategic Science and Technologies/Dare	Sexual arousal disorder	Phase II/completed 2017	NCT02570282
Tadalafil (Cialis or Adcirca)/Eli Lilly	Type 2 diabetes (postprandial hyperglycaemia)	Phase I/terminated early 2011	NCT01238224
	Head and neck squamous cell carcinoma	Phase II/completed 2012–2016	NCT00894413 (REF. ¹⁵⁶)
	Head and neck squamous cell carcinoma	Phase II/active, not recruiting	NCT01697800
	Obesity	Phase IV/completed 2015	NCT02554045 (REF. ¹⁴⁷)
	Insulin secretion/ sensitivity in obesity	Phase IV/completed 2015	NCT02595684 (REF. ³⁴⁷)
	Aortic stenosis left ventricular remodelling/hypertrophy	Phase IV/terminated early 2017	NCT01275339
	Multiple myeloma	Phase II/terminated early 2017	NCT01374217
	Insulin resistance in type 2 diabetes	Phase II/recruiting	NCT02601989
	Diabetic cardiomyopathy	Phase IV/recruiting	NCT01803828
	Endocrine cardiomyopathy in Cushing syndrome	Phase II/recruiting	NCT02611258 (REF. ³⁴⁸)
	Access sheath deployment (nephrolithiasis/kidney stones)	Phase IV/enrolling 2019	NCT03229889
	Lower urinary tract symptoms (prostatic hyperplasia)	Phase IV/recruiting	NCT02252367
	Obesity-related cardiometabolic dysfunction	Phase II/recruiting	NCT02819440
	Anti-tumour mucin 1 vaccine efficacy in head and neck squamous cell carcinoma	Phase I/II/recruiting	NCT02544880
	Small vessel disease	Phase II/active, not recruiting	NCT02450253 (REF. ³⁴⁹)
PDE9			
PF-04447943/Pfizer	Stable sickle cell disease	Phase I/completed 2016	NCT02114203 (REF. ³⁵⁰)
BI 409306/Boehringer Ingelheim	Healthy volunteers	Phase I/completed 2011–2018	NCT01343706, NCT01493570, NCT01505894, NCT01611311, NCT01841112, NCT02597998, NCT03505151, NCT02222168, NCT02438683 (REFS ^{62,351,352})
	Alzheimer disease, schizophrenia	Phase I/completed 2017	NCT02392468
	Schizophrenia	Phase I/completed 2013–2016	NCT01892384, NCT02281773 (REFS ^{61,63})
	Schizophrenia or attenuated psychosis syndrome	Phase II/recruiting	NCT03351244, NCT03230097
	Drug–drug interactions	Phase I/completed 2016–2017	NCT03151499, NCT03193307, NCT02853136, NCT02635750, NCT02248259

Table 3 (cont.) | Selected clinical trials involving PDE inhibitors pursued for new indications

Compound/manufacture	Indication	Phase/status	Clinical trial ID (refs)
PDE10			
[¹⁸ F]MNI-659 (PDE10A-selective PET tracer)	Huntington disease (PET imaging)	Early phase I/completed 2016/2017	NCT02061722, NCT02956148, 2012–003808-13 (EudraCT) ³⁵³
PBF-999/Palobiofarma	Cancer	Phase I/recruiting	NCT03786484
EVP-6308 (now FRM-6308)/En Vivo Pharmaceuticals (now Forum Pharmaceuticals)	Schizophrenia	Phase I/completed 2014	NCT02001389, NCT02037074
RO5545965/Hoffmann-La Roche	Schizophrenia	Phase I/completed 2013–2017	NCT01923025, NCT01711801, NCT01864226, NCT02019329, NCT02824055
TAK-063/Takeda	Schizophrenia	Phase I/completed 2014	NCT02370602, NCT01892189, NCT01879722 (REF. ¹⁶⁶)
	Schizophrenia	Phase II/completed 2016	NCT02477020 (REF. ¹⁶⁷)

Reported on ClinicalTrials.gov (accessed 28 May 2019) with an end date after April 2009. NA, not applicable; ND, not described; PDE, phosphodiesterase; PET, positron emission tomography.

models of colorectal cancer suggest that PDE5 inhibitors, either alone or as part of a multi-chemotherapeutic regimen, demonstrate an ability to prevent tumour growth (for example, see REFS^{17,154,155}). Similarly, reports suggest tadalafil promotes tumour immunity in patients with head and neck squamous cell carcinoma^{156,157} (TABLE 3). However, particularly with regard to colorectal cancer, PDE5 inhibitors do not produce complete antitumorigenic effects¹⁶. This lack of complete efficacy may be related to the fact that membrane guanylyl cyclases are inhibited in colorectal cancer¹⁶, and PDE5 may be primarily regulating cytosolic rather than membrane guanylyl cyclases^{18,19}. Alternatively, the overexpression of both PDE5 and PDE10A in colorectal cancer cells — the latter a membrane-enriched PDE^{70,158} — may be involved¹⁷. Indeed, PDE10 inhibitors also inhibit growth of colorectal cancer cells^{159,160}; however, when both PDE5 and PDE10 are inhibited, antitumor efficacy is improved in preclinical models¹⁷. Although enthusiasm for PDE5 inhibitors as chemopreventives is growing¹⁶¹, it should be noted that PDE5 inhibitors similarly prevented prostate carcinogenesis in preclinical models but did not appear to reduce risk or recurrence in clinical studies¹⁶². Perhaps even more concerning, PDE5A appears to suppress melanoma cell invasion in mice¹⁶³ and a recent systematic review and meta-analysis showed that PDE5 inhibitors actually increase risk for melanoma and basal cell carcinoma in humans¹⁶⁴.

PDE9 inhibitors. Although PDE9 inhibitors thus far have failed in the clinic for brain diseases, they may hold therapeutic potential for cardiovascular diseases. In a mouse model of sickle cell disease, the PDE9 inhibitor BAY73-6691 exerted immediate benefits on acute vaso-occlusive events¹⁶⁵, and a phase I clinical trial looking at safety, tolerability and pharmacokinetics/pharmacodynamics of the PDE9 inhibitor PF-04447943 in patients with sickle cell anaemia has recently been completed (TABLE 3). PDE9 inhibitors may also hold therapeutic potential for cardiovascular indications as PDE9A expression is upregulated by cardiac hypertrophy and cardiac failure. Indeed, the

PDE9 inhibitor PF-04449613 reverses heart disease in animal models by controlling pools of cGMP downstream of particulate guanylyl cyclases¹⁸.

PDE10 inhibitors. Despite the PDE10 inhibitor clinical failures described above, TAK-063 was tested in healthy controls and patients with schizophrenia. In healthy controls, TAK-063 was reported to be safe and well tolerated¹⁶⁶, altering the effects of ketamine on brain activity in healthy controls, particularly in the striatum, substantia nigra and ventrolateral prefrontal cortex (NCT01892189; accessed 28 May 2019). In patients with schizophrenia, although TAK-063 failed to demonstrate a significant effect on the total Positive and Negative Syndrome Scale score, there was a trend that mirrored effect sizes normally seen with risperidone¹⁶⁷. Furthermore, TAK-063 did significantly improve numerous secondary endpoints relative to placebo¹⁶⁷. It is not entirely clear why TAK-063 was able to succeed where PF-254920 failed. Whereas one study suggested that TAK-063 activates the striatal direct and indirect pathways in a balanced manner and PF-254920 activates the direct pathway more so than TAK-063 (REF.¹⁶⁸), other studies have reported that PF-254920 activates these pathways equally^{65,169}. It is notable that TAK-063 — but not PF-254920 — increased sensorimotor gating in rodents as measured by prepulse inhibition of acoustic startle¹⁶⁸, suggesting that prepulse inhibition of acoustic startle may more accurately predict antipsychotic-like effects of novel compounds. Preclinical studies are also exploring the therapeutic potential of PDE10 inhibitors in the context of L-DOPA-induced dyskinesias¹⁷⁰ and alcohol abuse disorders¹³³.

Inhibition of PDE7, PDE8 or PDE11. Studies describing the physiological function of the PDE7, PDE8 and PDE11 families are now emerging; however, inhibitors have not yet reached the clinic. Like many of the PDE families discussed above, early research suggests that PDE7 and PDE8 inhibitors may have positive effects in diseases where cognition, neuroprotection, neuroinflammation and/or motor function are impaired

Dyskinesias
Diminished voluntary movements or uncontrollable involuntary movements (for example, tics or chorea).

Retinitis pigmentosa

A rare genetic disorder characterized by black pigmentation and retinal degeneration.

Trans-capping

The process whereby the regulatory domains from one phosphodiesterase (PDE) enzyme physically bind — and, thus, block substrate access to — the C-terminal catalytic domain of another PDE molecule.

(for example, multiple sclerosis and/or Parkinson disease^{171–175}). Similarly, PDE11 inhibitors may hold potential for treating age-related cognitive decline^{71,176} or as an adjunctive treatment to improve lithium responsiveness in patients with bipolar disorder^{177,178}. PDE7 inhibitors may also hold promise in treating leukaemia^{179,180}, and PDE8 inhibitors may have potential for treating disorders associated with reduced androgen production in males as PDE8 inhibitors, particularly when applied in combination with PDE4 inhibitors, stimulate Leydig cell steroidogenesis^{181,182}.

Therapeutic strategies beyond inhibition**PDE activation**

There are several disease states where PDE activation may be warranted. Tissue, brain region and subcellular domain-specific decreases in PDE expression/activity and/or increases in cyclic nucleotide signalling have been implicated in select disease states, including some age-related deficits^{183,184}, Huntington disease¹⁸⁵, social isolation¹⁸⁶, migraine^{187–191}, retinitis pigmentosa¹⁹², infertility⁹⁹, prostate cancer¹⁹³, melanoma and basal cell carcinoma¹⁶⁴, cardiac hypertrophy^{24,194}, acrodysostosis²⁵ and polycystic kidney disease¹⁹⁵. PDE activators would be expected to have a greater impact in cells with higher cyclic nucleotide levels (either basal or stimulated) as opposed to cells with low cyclic nucleotide levels, although this remains to be empirically established. Indeed, Mironid have developed PDE4 longform-specific activators (mechanism as yet unknown; TABLE 4) for the treatment of polycystic kidney disease where increased adenylyl cyclase activity caused by overexpression of vasopressin V2R receptors results in elevated cAMP levels that drive cyst growth and disease progression¹⁹⁶. There are several natural mechanisms by which PDE activity can be activated (FIG. 3), and it is our contention that these avenues could be manipulated pharmacologically to trigger PDE activation.

Targeting GAF domains. One route to PDE activation is by way of tandem GAF (cGMP-specific and stimulated PDE, *Anabaena* adenylyl cyclases and *Escherichia coli* FhlA) domains¹⁹⁷ (FIG. 3b). Although GAF domains have been identified in over 7400 proteins, in mammals they are only found in the PDE 2, 5, 6, 10 and 11 families^{198,199}. For a vast majority of non-PDE GAF domains, the activating ligand is unknown; however, for PDEs it is known that cyclic nucleotides bind to these pockets (FIG. 3). PDE2 and PDE5 are activated when cGMP binds the GAF domain^{200–202}, and PDE10 is activated by cAMP binding the GAF domain²⁰³. In the context of activation, binding of cyclic nucleotides to GAF domains is thought to cause structural changes that relieve autoinhibition of the PDEs (FIG. 3). In contrast, cGMP binding the GAF-A domain of PDE6 enhances protein–protein interactions that inhibit PDE6 catalytic activity²⁰⁴. This suggests that blocking cGMP binding of the PDE6 GAF domain may provide a means of promoting PDE6 activity. This also suggests it may be possible to both activate and inhibit GAF-containing PDEs with small molecules at a site distinct from the catalytic domain. Indeed, PDE11A is activated when a cGMP analogue — but not cGMP

itself — binds the GAF domain²⁰³. Further, even though cGMP binding of the GAF domain activates PDE5 (REF.²⁰⁰), several other types of molecule that bind the GAF domain inhibit PDE5 in its activated — but not basal — state²⁰⁵. This is consistent with the fact that GAF domains are known to bind a diverse array of small molecules that are unrelated to cyclic nucleotides¹⁹⁷. The fact that GAF domains are only found in PDEs in mammals¹⁹⁹ makes GAF domains of high interest in the context of drug targeting²⁰⁶. Importantly, mammalian GAF domains are sufficiently structurally divergent from one another (for example, low degree of homology between PDE families and the tandem GAF domains are preceded by variable N-terminal stretches) to allow selective pharmacological targeting of individual PDE families²⁰⁰. Together, these observations suggest that the GAF domains may provide an inroad for targeting reagents that selectively activate a given PDE isoform while avoiding off-target activity.

Preventing trans-capping. PKA or protein kinase G (PKG) phosphorylation of PDE3 (REFS^{207,208}), PDE4 (REFS^{207,209}), PDE5 (REF.²¹⁰) and PDE8 (REF.²¹¹) is also known to activate catalytic activity in a negative feedback loop. In the case of PDE4, for example, catalytic activity is inhibited when the UCR2 regulatory domain ‘trans-caps’ the catalytic site, thus occluding cAMP from reaching the enzymatic core of PDE4 (REFS^{73,212}) (FIG. 3c). PKA phosphorylation of the UCR1 regulatory domain blocks the ability of UCR2 to trans-cap the catalytic site, which locks PDE4 in the active state⁷³. Notably, select PDE4D inhibitors allosterically inhibit catalytic activity by promoting ‘trans-capping’¹³⁰, whereas phosphatidic acid activates PDE activity by inhibiting trans-capping in a similar but mutually exclusive manner to PKA^{213–215}. Furthermore, the dominant negative peptide ‘UCR1C’, which corresponds to the UCR1 sequence, also activates PDE4 activity by inhibiting trans-capping²¹⁶. These results provide proof of principle that activation of PDE4 may be achieved by either small molecules or biologics that prevent UCR2 from adopting a trans-capping conformation.

Manipulating protein–protein binding. PDEs may also be activated by manipulating protein–protein binding interactions. PDE6 is unique in the fact that the heterodimeric holo-enzyme includes two inhibitory subunits that span the catalytic pockets of the dimer, thus occluding cGMP from the catalytic site²¹⁷ (FIG. 3d). Binding of the GTP-bound α -subunit of the heterotrimeric G-protein transducin relieves PDE6 inhibition by binding to the C-terminal region of PDE6 and its inhibitory subunits²¹⁸. The full crystal structure of PDE6 is not yet available²¹⁹; however, recent cryo-EM work²²⁰ has confirmed the predicted structural organization of the holo-enzyme, albeit without sufficient detail to inform pharmacological targeting. The success in upregulating PDE6 activity via gene transfer to combat retinitis pigmentosa¹⁹² (see following section) suggests that PDE6 activation could be a viable therapeutic strategy for the treatment of vision loss. As discussed in greater detail below, it may also be possible to increase PDE activity

Table 4 | Selected patents involving PDEs published in the last 5 years

Patent ID (priority date)	Subject	Assignee	Authors
PDE3			
US2019046528 (8 August 2017)	Method of preventing hair loss or promoting hair growth by using PDE3 inhibitor	Seoul National University Hospital	Kwon O, Choi HI, Jo SJ, Kim KH
WO2017186103 (26 April 2016)	Applications of PDE3A in determination of tumour treatment effect of anagrelide	Shanghai Institute of Materia Medica, Chinese Academy of Sciences	Yu Q, Liu J
PDE4			
CN108904493A (12 August 2018)	PDE4 inhibitor and purpose for preparing novel anti-inflammatory drugs	Hu Y	Hu Y
WO2018167142 (16 March 2017)	Treatment of idiopathic pulmonary fibrosis (with a PDE4 inhibitor)	Takeda GMBH	Hanauer G, Nikam S, Hazama M
WO2017133713 (5 February 2016)	Application of PDE4 inhibitor ZL-N-91 in preparation of medications for lung cancer proliferation and metastasis	Guangzhou Sinogen Biomedical Tech Ltd	Zhao AZ, Gong S, Lin Y, Li F, Li X
WO2018060704 (28 September 2016)	Compounds and their use as PDE4 activators for the treatment of disorders requiring a reduction of cAMP	Mironid Ltd	Adam JM, Adams DR
WO2018037109 (26 August 2016)	Treatment of non-alcoholic fatty liver disease with PDE4 inhibitors	Takeda GMBH	Hanauer G, Nagabukuro H, Amano Y
CN107412214A (31 July 2017)	Application of PDE4 Inhibitor (FCPR16) for the treatment of Parkinson disease	Guangzhou Lanssonpharm Jianzhi Tech Co Ltd	Xu L
US2017051291 (28 December 2005)	RNA interference-mediated inhibition of PDE4 for treatment of cAMP-related ocular disorders	Arrowhead Pharmaceuticals Inc	Yanni JM, Chatterton JE, Gamache DA, Miller ST
WO2017017165 (29 July 2015)	PDE4 inhibitor for the treatment of diabetic nephropathy	Takeda GMBH	Hanauer G, Vollert S, Hazama M, Matsuo T
WO2016075543 (13 November 2014)	Treatment of multiple sclerosis with the combination of laquinimod and a PDE4 inhibitor	Teva Pharma, Piryatinsky V, Kaye J	Piryatinsky V, Kaye J
WO2015022417 (16 August 2013)	Treatment of cognitive impairment with the combination of a PDE4 inhibitor and an acetylcholinesterase inhibitor	University of Maastricht	Yamada T, Prickaerts J, Van Duinen M, Sambeth A, Blokland A
PDE5			
US2018221373 (16 September 2015)	Method of treating insomnia with PDE5 inhibitors	Rosenberg LI	Rosenberg LI
CA2975049 (10 August 2016)	PDE inhibitors (sildenafil) to repair brain and/or retinal injury in human newborns	Wintermark P	Wintermark P
CN107163052A (18 April 2017)	Immunodetection method for various PDE5 inhibitor drugs	South China Agricultural University	Shen Y, Hua Y, Xu Z, Yang J, Wang H, Sun Y, Lei H
WO2014088326 (4 December 2012)	Composition comprising PDE5 inhibitor for inhibiting apoptosis of nerve cells	Aribio Inc., Sk Chemicals Co Ltd	Kim MH, Choung JJ, Ku SK
PDE6			
JP2019047763A (12 September 2017)	Rhodopsin PDE as an optogenetic tool for light control of intracellular cyclic nucleotides	Nagoya Institute of Technology	Kandori H, Tsunoda SP, Yoshida K
CN107287239 (11 April 2016)	Gene therapy vector and medicine (adenovirus encoding PDE6B) for retinal pigment degeneration	Shenyang Fuming Biological Tech Co Ltd	Pang J
PDE7			
WO2018055140 (23 September 2016)	T cells with increased immunosuppression resistance (expressing PDE4C or 7A for the treatment of cancer)	Adaptimmune Ltd	Laugel B, Skibbe K
PDE9			
WO2017070293 (20 October 2015)	PDE9 inhibitor and levodopa therapy for treating Parkinson disease or Parkinsonism	Ironwood Pharmaceuticals Inc	Leventhal L, Townsend TM
PDE10			
WO2019067955 (29 July 2017)	Compositions and methods for regulating let-7 microRNA targets, such as PDE10A, for treatment of cancers	University of California	Roos M, Lowry W

Source: Espacenet and Google patents (accessed 28 May 2019). PDE, phosphodiesterase.

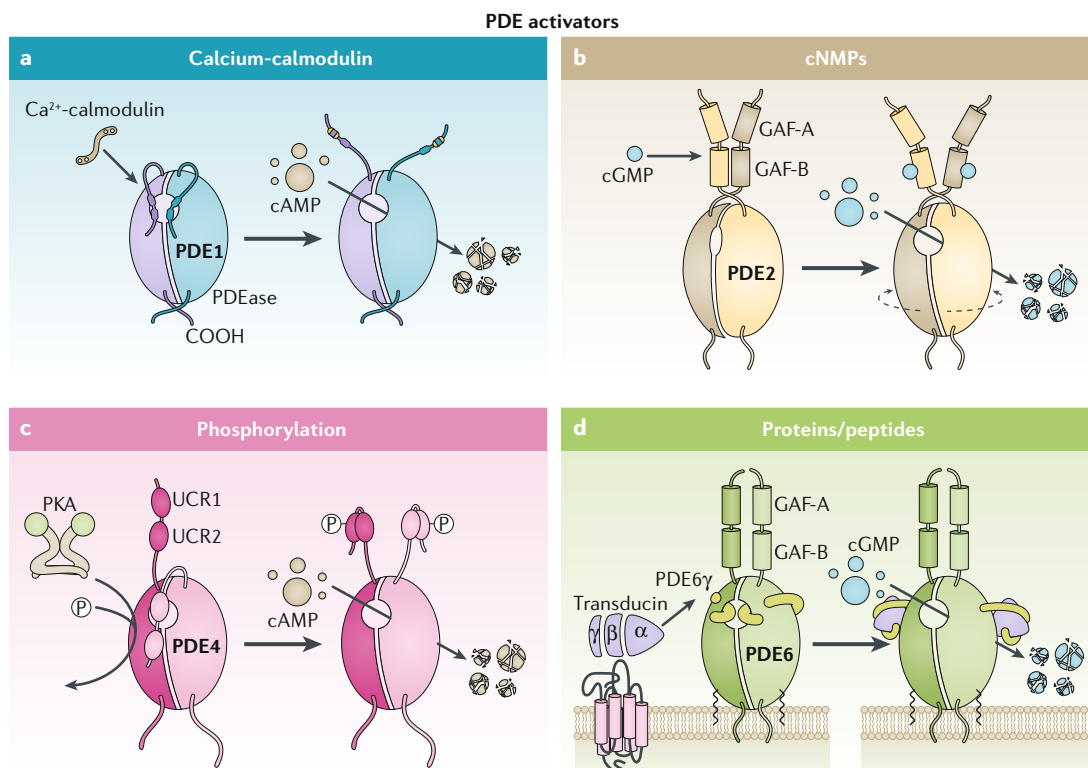


Fig. 3 | Mechanisms that activate PDE catalytic activity. **a** | Calcium-calmodulin (CaM) binding to the CaM domains of PDE1 relieves N-terminal auto-inhibition of the catalytic site, thereby promoting enzymatic activity³²³. **b** | Cyclic nucleotides (cNMPs) binding to GAF domains of dimeric phosphodiesterases (PDEs) (shown here: cGMP binding the GAF-B domain of PDE2) are thought to promote catalytic activity by inducing an outward rotation of the catalytic domains and, thus, enabling access to substrates³²⁴. **c** | Phosphorylation by protein kinase A (PKA) or protein kinase G (PKG) activates several PDEs¹⁹⁹. In the case of PDE4D, phosphorylation of the UCR1 domain by PKA causes UCR1 to bind its own UCR2 domain instead of the catalytic site of the other monomer, thereby locking the enzyme in an active state. **d** | PDE activity can also be modulated by protein–protein binding interactions. One such well-characterized example involves membrane-bound PDE6, where the rhodopsin-activated G-protein α -subunit transducin displaces the inhibitory PDE6 γ C-terminus from the catalytic sites on PDE6 $\alpha\beta$, thus promoting cGMP hydrolysis³²⁵. GAF, cGMP-binding PDEs *Anabaena* adenylyl cyclases and *Escherichia coli* FhlA; UCR, upstream conserved region.

by preventing the binding of PDEs to binding partners that sequester or suppress activity.

Gene therapy

Viral transfer of PDE genes, agents that silence PDE gene expression (for example, antisense, silencing or microRNAs)^{163,221–225} or gene editing (for example, CRISPR–Cas9)²²⁶ might also prove a useful means of therapeutically targeting individual PDE isoforms (FIG. 4). The best-characterized PDE gene therapy approach to date targets PDE6 activity in the retina. A loss of transducin-mediated activation of PDE6 results in elevated cGMP levels, which causes the loss of primary rods and, ultimately, vision²²⁷. Expression of recombinant PDE6 α in the retina via an adeno-associated virus (AAV-PDE6 α) preserved retinal structure, photo-transduction and vision in retinal degeneration mice, as did AAV-PDE6 β ^{228,229}. AAV-PDE6 α similarly rescued retinal deficits in a mouse model that mimics human retinitis pigmentosa mutations²³⁰. Experiments injecting AAV-PDE6 γ into the retina have also proven successful in mice²³¹. In dogs, delivery of recombinant PDE6 α using a tyrosine capsid-mutant AAV8 was able

to stabilize cGMP levels and improve survival of photo-receptor rods and cones in PDE6 α -mutant dogs; however, several adverse effects related to the AAV injection were identified¹⁹². The recent development of synthetic AAV vectors that target the retina in non-human primates may provide the answer to these problems in the future²³². Notably, two clinical trials are underway testing the safety and efficacy of PDE6 gene therapy in retinitis pigmentosa (phase I NCT02759952 and phase II NCT03328130; accessed 29 May 2019).

A rapidly evolving approach within the gene therapy field is optogenetic medicine, which combines viral delivery of recombinant, light-activated proteins with biomedical devices that emit light of the specific intensity and wavelength needed to activate those proteins²³³. With the field of personalized bioelectronic implants quickly evolving, optogenetic-based biomedical approaches are being pursued for neurological diseases, cancer, cardiovascular disease and metabolic disorders²³³. Given that optogenetic-based approaches have now entered clinical trials²³³, it is worth noting here that light-activated PDEs have been identified in lower organisms^{234–236} and engineered in

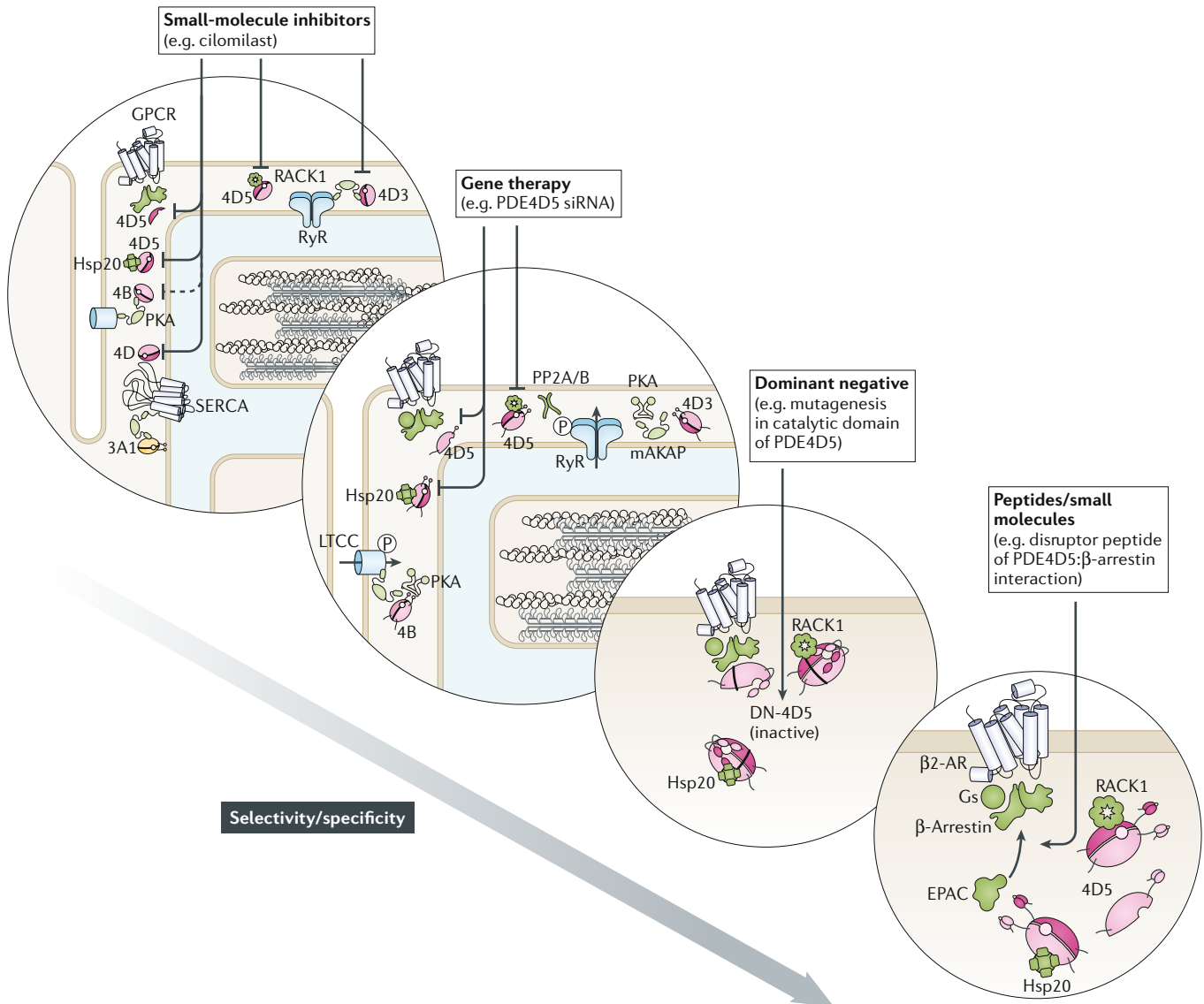


Fig. 4 | Methods for targeting PDE signalling with increasing specificity. Given the vast diversity of phosphodiesterase (PDE) isoforms, each with unique tissue expression profiles, subcellular compartmentalization and protein–protein interactions, it is becoming clear that selective targeting of PDE function will be required to achieve efficacy while diminishing undesirable side effects. Small-molecule inhibitors (for example, cilomilast) are readily developed with family-specific selectivity (for example, targeting PDE4 over PDE3); however, isoform specificity remains a challenge (for example, cilomilast inhibits PDE4D with only sevenfold selectivity versus PDE4B)³²⁶. Conversely, gene therapy (that is, expressing a recombinant construct to knock down or restore expression of a given PDE isoform) and dominant-negative approaches (that is, expressing a catalytically inactive PDE4D5 that displaces the endogenous isoform from its interacting partners) can target isoform subtypes exclusively (for example, targeting PDE4D5 but not PDE4D3 nor PDE4B). That said, dominant-negative approaches would influence signalling within all microdomains regulated by that isoform. The greatest specificity can be achieved with peptide/small-molecule binding disruptors or mutagenesis approaches (not shown) that are designed to prevent a specific PDE isoform from binding a specific partner, thus altering signalling only within one specific complex. As shown here, a disruptor peptide that specifically prevents the interaction between PDE4D5 and β -arrestin would lead to the recruitment of EPAC1 to β 2 adrenergic receptors (β 2-AR), but would leave PDE4D5 regulation of heat shock protein 20 (HSP20) and RACK1 complexes intact^{239,327,328}. EPAC, exchange protein activated by cAMP.

the laboratory²³⁷. Both are being explored as biological tools in higher organisms. Activating or inhibiting a given PDE by a spatially and temporally restricted light emission, as opposed to a systemically administered pharmacological agent, may prove an ideal approach for treating diseases where cyclic nucleotide signalling is

downregulated in one tissue yet upregulated in another (for example, ageing; see REF.²³⁸). This technique might also provide a means of avoiding side effects associated with targeting PDE activity in a specific tissue (for example, nausea/emesis associated with inhibiting PDE4 in the area postrema).

Targeting location

As production of cAMP is utilized by various different Gs-coupled receptors to transduce signals, compartmentalization of signalling intermediates is crucial to define physiological outcomes specific to each receptor²³⁹. This compartmentalization of cyclic nucleotide signalling is achieved by virtue of PDEs being tethered to a precise cellular location via binding partners (Supplementary Table 1). Thus, promoting or disrupting isoform-specific protein–protein interactions may prove a viable approach to therapeutically target PDEs in an isoform-specific manner, a level of specificity that has not been achieved with pharmacological inhibitors to date (FIG. 4).

Dominant-negative PDEs. Proof of principle for such an approach first emerged with studies using dominant-negative PDEs (DN-PDEs), which are catalytically inactive mutants that would displace their endogenous PDE. Using specific DN-PDE4 isoforms, *in vitro* studies have successfully altered perinuclear cAMP signalling²⁴⁰, β -arrestin-dependent desensitization of the β 2-adrenergic receptor^{241,242}, growth control of prostate cancer cells¹⁹³, prostanoid receptor-mediated cAMP signalling²⁴³, glucagon-like peptide-1 release²⁴⁴ and cAMP gradients at the centrosome²⁴⁵. DN-PDE4 tools have also yielded beneficial effects *in vivo*. For example, viral delivery of a DN-PDE4A5 to the mouse hippocampus was able to rescue localized cAMP signalling deficits and hippocampus-dependent memory impairments that were caused by sleep deprivation^{246–248}. In contrast, overexpression of a DN-PDE4B1 in the forebrain of mice did not affect hippocampus-dependent memory, although it did enhance hippocampal long-term potentiation in male mice²⁴⁹. This finding underscores the importance of understanding the role of specific PDE isoforms, because a homozygous mutation in PDE4B (Y358C) that greatly reduces activity of all PDE4B isoforms by virtue of attenuating interactions with the scaffold protein disrupted in schizophrenia 1 (DISC1) improves both long-term potentiation and memory as well as other mood-related behaviours²⁵⁰. It is interesting to note that nature has developed its own dominant-negative approach with PDE4A7, a PDE isoform that is targeted to specific subcellular compartments but is catalytically dead²⁵¹.

One point to consider in adopting a dominant-negative approach is the fact that a single PDE isoform can contribute to more than one function in a cell via its participation in multiple distinct signalling complexes, which involve mutually exclusive protein–protein interactions²⁵² (Supplementary Table 1; FIG. 4). For instance, PDE4D5 is involved in numerous processes common in almost all cells, such as cell growth, cell orientation, desensitization of Gs-coupled receptors and inactivation of the phosphorylation of the ubiquitous chaperone HSP20 (REF.²⁵³). The ability for PDE4D5 to have all these functions in a cell is a result of it being localized in different compartments by different anchors (for example, RACK1 at the leading edge of cells, β -arrestin at transmembrane receptors and HSP20 in the cytosol²⁵³; FIG. 4). It is clear that this is also the case for several other isoforms based on proven protein–protein binding interactions (for example, in heart tissue/cells, PDE4D3 can bind to either

the ryanodine receptor, HSP20 or an AKAP9/potassium channel complex; see Supplementary Table 1) or based on inference from the fact that the exact same isoform can be found localized to multiple subcellular domains (for example, ~50% of PDE11A4 in neurons localizes to the cytosol whereas 25% is localized to the nuclear fraction and another 25% to the membrane compartment⁷⁰). Thus, a non-selective dominant-negative approach has the potential to influence multiple domains within the cell. To achieve a compartment-specific manipulation of a given PDE isoform, one could mutate the binding sites that mediate a particular protein–protein interaction. Mutating isoform-specific binding sites has also proven a useful approach, revealing that an integrin α 5–PDE4A5 complex regulates endothelial inflammation²⁵⁴, a PDE3A1–SERCA complex regulates myocardium contractility²⁵⁵ and a DISC1-mediated sequestering of PDE4B regulates hippocampal function²⁵⁰. An alternative approach is to develop a peptide or small molecule that specifically competes for a given protein–protein binding site²⁵⁶. This approach would displace only a specific ‘pool’ of a given PDE isoform, leaving the vast remainder unfettered in their respective signalling complexes (FIG. 4). Efforts have begun to identify small molecules capable of promoting or interfering with protein–protein interactions, but the results have not yet been published so it is too early to speculate on the required design characteristics at this stage.

Disrupting protein–protein interactions with peptides.

A recent review suggests cell-permeable peptide disruptors effectively manipulate specific PDE isoforms in a compartment-specific manner²⁵⁶, and evidence continues to build. More recently, a PDE4D-FAK disrupting peptide prevented direction sensing and invasion of melanoma cells^{257,258} and a PDE8A-Raf1 disruptor retarded cancer cell growth promoted by a Ras mutation²¹¹. Interestingly, the same PDE8A-Raf1 peptide has also been used to target T cell adhesion and migration and was more potent than a PDE8-specific inhibitor in reducing inflammatory signalling²⁵⁷. The effectiveness of PDE displacement has also been demonstrated *in vivo*, where intraperitoneal injections of a PDE4-HSP20 disruptor significantly attenuated hypertrophy-induced cardiac remodelling²⁵⁹.

Disrupting PDE homodimerization. Disrupting PDE homodimerization (that is, a PDE monomer binding to itself) may also prove an effective way to target PDE function in a domain-specific manner. Disrupting PDE11A4 homodimerization using a peptide recognizing its GAF-B domain was shown to selectively remove PDE11A4 from membrane-bound complexes but not the cytosol, which may hold utility for improving responsiveness to the mood stabilizer lithium¹⁷⁷ or age-related cognitive decline^{71,176}. Conversely, a peptide or mutation that could stabilize PDE11A4 homodimerization might prove useful in treating the deleterious psychological effects of social isolation¹⁸⁶. Targeting homodimerization of PDE2, PDE4 or PDE5 may also relocate the enzymes by virtue of changing susceptibility to regulatory post-translational modifications (PTMs)^{199,260}.

Indeed, nature appears to have taken advantage of dimerization as a mechanism to regulate PDE trafficking. For example, when PDE10A2 heterodimerizes with PDE10A19, PDE10A2 is prevented from trafficking to the membrane as it normally does under conditions of homodimerization²⁶¹. Such complex-specific targeting of PDE function may be required to achieve efficacy in the absence of unwanted side effects, particularly in cases where multiple subfamily isoforms orchestrate various physiologic responses by virtue of different protein–protein interactions (for example, targeting various PDE3 isoforms for cardiovascular disease²⁶²).

Targeting post-translational modifications

As PTMs directly regulate PDE activity and location, PTMs could be considered a point of therapeutic control (FIG. 5).

Phosphorylation. PKA or PKG phosphorylation of PDE3 (REFS^{207,208}), PDE4 (REFS^{207,209}), PDE5 (REF.²¹⁰) and PDE8 (REF.²¹¹) will stimulate catalytic activity. Phosphorylation can also influence PDE cellular location by virtue of preventing other PTMs that promote membrane association (for example, palmitoylation) or changing protein–protein binding interactions. A great example to illustrate these principals is PDE10A2. PDE10A2 is palmitoylated in its N-terminal region, which directs membrane targeting and trafficking to dendrites¹⁵⁸. If, however, PDE10A2 undergoes isoform-specific phosphorylation by PKA on Thr16 (REF.²⁶³), palmitoylation of PDE10A2 is attenuated and the specific membrane localization is lost¹⁵⁸. Interestingly, phosphorylation on the same site also interferes with the scaffolding of PDE10A2 by AKAP150 (REF.²⁶⁴). Hence, although PDE10A catalytic activity is not directly affected by this PTM, cyclic nucleotide levels should increase within this compartment due to the absence of PDE10A2. Preliminary evidence also suggests that PDE11A4 can similarly be shuttled between membrane and cytosolic compartments by virtue of phosphorylation of N-terminal serines, although this is likely by virtue of altering protein–protein interactions as opposed to a direct insertion into the membrane²⁶⁵. PKA phosphorylation of PDE4D3 drives an association with the mA-KAP signalling complex to evoke rapid signal termination in the muscle compartment²⁶⁶, which may have therapeutic implications given that polymorphisms in the PDE4D3-mA-KAP binding site lead to a higher susceptibility to cardiovascular disease²⁶⁷. PDE4D enzymes also get phosphorylated by both casein kinase 1 (CK1) and glycogen synthase kinase 3 β (GSK3 β) in the catalytic region on a motif known as a ‘phosphodegron’²⁶⁸. This action increases the affinity of the PDE for a ubiquitin ligase complex (cullin 1 containing SCF E3 ligase) which promotes proteasomal degradation of the enzyme²⁶⁸. Hence, PDE phosphorylation not only affects activity, localization and protein–protein interactions but also regulates protein turnover.

Ubiquitylation and SUMOylation. It has been known for some time that increases in PKA activity promote the proteasomal degradation of short-lived proteins, an action that can be enhanced by PDE inhibitors²⁶⁹.

However, we are just starting to understand that the stability of PDEs can themselves be influenced by the ubiquitin–proteasome system (FIG. 5). Ubiquitin conjugation is known to target proteins for degradation by the proteasome and specificity is introduced at the level of E3 ligase–substrate interaction. We now know of multiple instances where PDEs interact definitively with one of the over 600 E3 ligase types to dramatically shorten the PDE half-life and this could be a new point at which to direct innovative therapeutics. As mentioned above, PDE4Ds can be degraded by virtue of the SCF E3 (REF.²⁶⁸), whereas PDE4B levels can be downregulated by a different E3, Smurf2, to promote anti-fibrotic signalling in the liver²⁷⁰. PDE4D5 can also be targeted for ubiquitin modification by the RING-type MDM2 E3 ligase; however, this β -adrenergic-driven ubiquitylation of PDE4D does not signal a degradation of the enzyme. Instead, it shifts PDE4 from binding RACK1 to binding β -arrestin²⁷¹. PDE4s are similarly regulated by the ubiquitin-like protein SUMO (small ubiquitin-like modifier)²⁷². SUMO conjugation tends to alter the location, activity or protein–protein interactions of a protein rather than tagging for destruction via the ubiquitin–proteasome system²⁷³. Unlike ubiquitylation, sites of SUMOylation can be predicted in the amino acid sequence of putative substrates as conjugation usually occurs on a lysine residue within the consensus h-K-X-D/E (where h is a bulky hydrophobic and X is any residue)²⁷³. PDE4s from subfamilies A and D contain the consensus motif, whereas subfamilies B and C do not. This adds an extra layer of subfamily-specific regulation as SUMOylation serves both to protect against the inhibitory phosphorylation by extracellular signal-regulated/mitogen-activated protein kinases²⁷⁴ and further enhances activity of the PKA-phosphorylated longform PDE4 by locking it in the ‘open’ non-UCR inhibited conformation²⁷².

S-nitrosylation and proline hydroxylation. Two additional PTMs that trigger PDEs for destruction include S-nitrosylation and proline hydroxylation (FIG. 5). PDE5 can be S-nitrosylated by nitric oxide on Cys220 (REF.²⁷⁵), which targets the enzyme to the proteasome and reduces PDE activity. Under conditions of reduced nitric oxide bioavailability, as in heart disease, PDE5 is upregulated due to a loss of this S-nitrosylation-induced degradation²⁷⁵. Proline hydroxylation has also been identified as a modification that can tag substrates for recognition by E3 ligase complexes²⁷⁶. In the heart, proline hydroxylase domain-containing proteins hydroxylate surface-associated prolines on PDE4D enzymes, triggering their degradation²⁷⁷. In this way, direct binding of proline hydroxylase domain-containing proteins to PDE4s increases cAMP without affecting adenylyl cyclase activity.

Challenges

Although this is clearly an exciting time in the PDE field, much work remains to be done. For therapeutics to be efficiently developed, we need to more thoroughly understand precisely where cyclic nucleotide signalling is disrupted in a given disease — in which tissue, cell types

Phosphodegron

A phosphorylation motif that alters the rate of degradation by attracting ubiquitin ligases to the phosphorylated protein.

and subcellular compartments. We then need to target a PDE in a defined locale, with the understanding that subcellular compartmentalization of a given PDE may vary depending on species, age, tissue type or disease status (for example, see REFS^{5–12}; for a full review, see Supplementary Table 1). This consideration is equally

important in the evaluation of potential efficacy and potential side effects. To maximize potential efficacy while minimizing potential side effects, one would target a PDE that is enriched, if not exclusively expressed, in the tissue of interest and that controls the same pool of cyclic nucleotides that is altered by the disease. At the

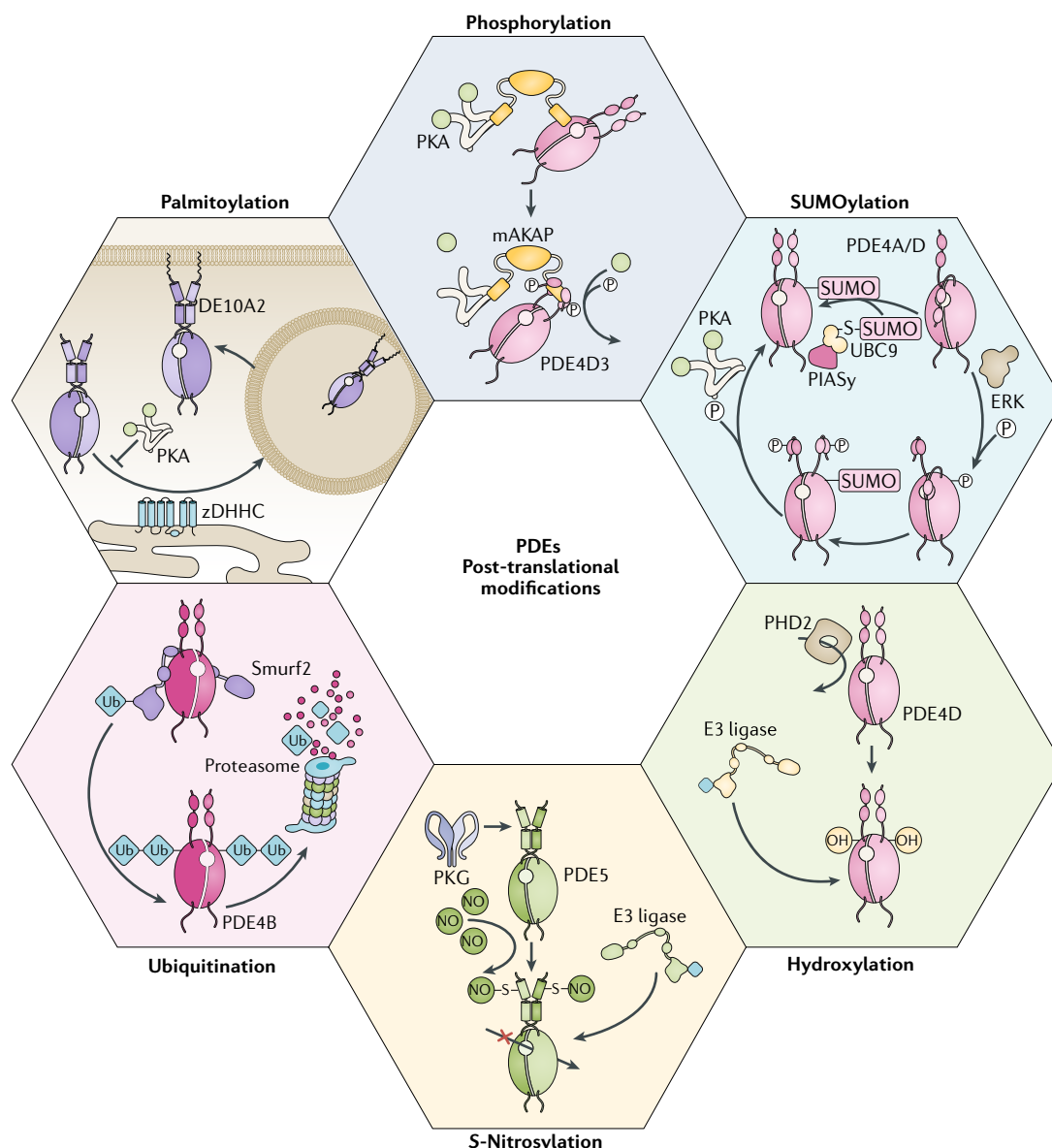


Fig. 5 | PDE regulation by post-translational modification. Cyclic nucleotide dynamics can be modulated by the addition of different functional groups to phosphodiesterases (PDEs). Phosphorylation is a very common mechanism to control PDE activity as depicted by the action of protein kinase A (PKA) on PDE4D3. Both enzymatic activity and binding affinity of PDE4D3 for mAKAP are increased by PKA phosphorylation, allowing a faster signal termination in myocytes²⁶⁶. Palmitoylation of PDE10A2 in its N-terminal region translocates the enzyme to the plasma membrane, although its phosphorylation by PKA can prevent the action of the palmitoyl acyltransferase (zDHHC)¹⁵⁸. Ubiquitination can influence PDE function by controlling stability. For example, the E3 ubiquitin ligase Smurf2 targets PDE4B for degradation which leads to the attenuation of liver fibrosis²⁷⁰. S-Nitrosylation can also tag PDEs for destruction. Thus, the covalent incorporation of nitric oxide (NO) to the GAF-A domain of a protein kinase G (PKG)-phosphorylated and active PDE5 directs the enzyme to the proteasome²⁷⁵. Hydroxylation of proline residues has emerged as another post-translational modification to stimulate turnover of PDEs. Prolyl hydroxylase domain protein 2 (PHD2) action on PDE4D increases its recognition by E3 ligase complexes in cardiomyocytes²⁷⁷. Finally, SUMOylation can intensify the activity of PDE4A and PDE4D. The small ubiquitin-like modifier (SUMO) transfer from the E2 conjugase UBC9-E3 enzyme PIASy complex to the PDEs enhances their activation by PKA phosphorylation and represses their inhibition induced by extracellular signal-regulated kinase (ERK) activity²⁷².

same time, efforts to unravel the intramolecular signals responsible for trafficking each PDE also need to continue to inform more sophisticated therapeutic approaches that can preferentially target a given PDE in a given subcellular compartment. Along these same lines, we need to grow our understanding of how to stimulate PDE activity and how to target the PDE catalytic activity of dual-specificity PDEs in a functionally selective manner (that is, target only its cAMP or cGMP-hydrolytic activity; see REF.²⁰⁶ for further discussion). Perhaps by increasing the specificity of our approach, we can retain efficacy while mitigating the numerous side effects described above that have plagued PDE inhibitors to date.

An additional challenge is to gain a better understanding of which physiological/disease processes are governed by PDE regulation of canonical versus non-canonical cyclic nucleotides. Research into the role of non-canonical cyclic nucleotides is rapidly evolving as new techniques and reagents facilitate functional studies⁴. cCMP and cUMP are synthesized by soluble guanylate and soluble adenylyl cyclases in mammalian systems, although an as-yet-to-be identified generator likely accounts for the majority of production given the dissociation between soluble guanylyl cyclase/soluble adenylyl cyclase and cCMP/cUMP expression patterns⁴. ExoY, a bacterial nucleotidyl cyclase, is known to generate cUMP in non-mammalian systems². Hydrolysis of cCMP is catalysed by PDE7A whereas cUMP is broken down by PDE3A, PDE3B and PDE9A². Functionally, both nucleotides have been shown to activate PKA/PKG²⁷⁸ and cyclic nucleotide gated channels²⁷⁹, and cCMP is described as a partial agonist of EPAC²⁸⁰. In a disease context, the non-canonical cyclic nucleotides play roles in promoting virulence of *Pseudomonas aeruginosa* infections²⁸¹ and triggering apoptosis of cancer cells²⁸²; however, much more research is needed to accurately characterize the pathophysiology involving these signalling molecules. Difficult issues facing the field will be defining specific non-canonical cyclic nucleotide signalling systems that are aberrantly regulated in disease, determining the mechanisms by which PDEs might preferentially degrade non-canonical versus canonical cyclic nucleotides, and visualizing the compartmentalization of non-canonical signalosomes in specific locations within cells and tissues.

Finally, the development of PDE-targeted therapeutics faces the same challenge as does all drug discovery — the high rate of failure in clinical trials. A recent study suggests that from 2000 to 2015, only 13.8% of all compounds made it from phase I to approval¹²⁸³. When considering success rates for individual indications, we may gain insight into the likelihood that a PDE-targeted therapeutic will achieve success within a given disease area. For example, oncology saw only 3.4% of compounds make it from phase I to approval, perhaps not surprising given the resistance and heterogeneity that dogs this area. In contrast, metabolic/endocrinology, cardiovascular, CNS, autoimmune/inflammation, genitourinary and ophthalmology saw a success rate of 19.6%, 25.5%, 15%, 15.1%, 21.6% and 32.6%, respectively²⁸³. This certainly paints a grim picture for pursuing any type of therapeutic in the context of cancer; however, this failure rate also underscores the desperate need to

develop novel therapeutic options. It is then important to note that success rates were doubled for cancer compounds when patient selection biomarkers were employed in the trials; the success rate for cardiovascular compounds similarly benefited¹²⁸³. Importantly, expression or activity of PDEs themselves may prove viable biomarkers in this context (BOX 2).

Outlook

Although the challenge of targeting localized pools of PDEs for purposes of correcting pockets of aberrant cyclic nucleotide signalling has proved difficult in the past, there are indications that innovative approaches and technological advances are making headway (see TABLE 4 for recent patent activity that includes PDE activators, biomarkers and viral approaches). For example, agents that show remarkable selectivity for subfamilies of PDE4 are showing promising results in mouse models of learning and memory, and translation to human disease would be a game-changing advance¹²⁴. Additionally, novel delivery systems are being developed that can transport PDE inhibitors to precise tissues or cell types, thereby abrogating complications associated with systemic distribution (for example, see REFS^{284,285}). In the future, novel delivery systems such as these could be employed to deliver agents that specifically disrupt the anchoring of single isoforms. Another potential approach is the intelligent design of a new generation of PDE inhibitors that preferentially accumulate in or segregate from certain tissues or organs. The mild side-effect profile of apremilast is largely due to its inability to penetrate the brain and engineering of similarly restricted distribution profiles may unlock the latent abilities of other PDE inhibitors. Gene therapy that seeks to abrogate or enhance activity of single PDE isoforms in a cell type-specific manner may provide a way to combat disease or fight complications associated with ageing. There is also the possibility that an improved therapeutic window might be achieved by combining suboptimal doses of PDE inhibitors with ineffective doses of downstream-target activators, which would result in an effective combined dose only in tissues and subcellular compartments where both molecules were present (for example, see REF.²⁸⁶). Indeed, PKA, PKG and Epacs have been implicated as therapeutic targets in their own right for numerous indications for which PDE inhibitors are being pursued, including diseases of the cardiovascular, immune, metabolic and nervous systems as well as cancer (for example, see REFS^{116,117,287–292}). Further, several studies in various tissues have attributed the beneficial effects of PDE inhibitors to the activation of PKA, PKG and/or Epac (for example, see REFS^{124,293–295}). As we learn more about the functional role and molecular interactions of each PDE splice variant, and how the function and/or localization of an individual variant may be altered in a given disease, it will become clearer how we can successfully target PDEs in a specific fashion to achieve efficacy while avoiding side effects. Only with this detailed level of knowledge will we be able to realize the full potential of PDEs as therapeutic targets.

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Competing interests

G.S.B. is co-founder and director of Portage Glasgow Limited. The other authors declare no competing interests. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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