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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, 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, 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.

Keywords

PDE; PDE1; PDE2; PDE3; PDE4; PDE5; PDE6; PDE7; PDE8; PDE9; PDE10; PDE11; PDE1A; PDE1B; PDE1C; PDE2A; PDE3A; PDE3B; PDE4A; PDE4B; PDE4C; PDE4D; PDE5A; PDE7A; PDE8A; PDE9A; PDE10A; PDE11A; drug development; therapeutics; biomarker; optogenetics; activator; inhibitor; post-translational modification; protein-protein binding; protein-protein interactions; compartmentalization; signalosome; microdomains; nanodomains; cAMP; cGMP; cyclic nucleotide

Introduction

Conventional 3',5'-cyclic nucleotide phosphodiesterases (PDEs) are members of a highly conserved superfamily of enzymes that degrade the canonical cyclic nucleotides 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP)[1], as well as the non-canonical cyclic nucleotides 3',5'-cCMP, 3',5'-cUMP, 3',5'-cIMP and c-di-GMP[2–4] (Figure 1). As extensively reviewed elsewhere[1], 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-

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terminal regulatory domains (Figure 2). PDEs do not simply control the total cellular content of cyclic nucleotides, but rather create individual pockets or nanodomains of cyclic nucleotide signaling. It is this subcellular compartmentalization of cyclic nucleotide signaling 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 a number of pathophysiological states including those affecting the nervous system, cardiovascular system, fertility, immunity, cancer, and metabolism (Box 1). Because the location of a PDE is just as important to its overall function as is 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 signaling, or elevations in cyclic nucleotides—is of paramount importance when considering the therapeutic potential of a given PDE isoform (e.g.,[5–12]; for full review, see Table S1).

Importantly, no two PDE isoforms share the exact same combination of substrate specificity, tissue expression profile and subcellular localization (Table S1). This is quite important because there are a number of diseases where compartment-specific defects in cyclic nucleotide signaling have been identified. For example, the function of soluble guanylyl cyclase, but not particulate guanylyl cyclase, is significantly impaired in brains of Alzheimer's patients and *in vitro* models of Alzheimer's disease 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/suppressed[16] and membrane-enriched PDE10A appears to be overexpressed[17], 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 cells[17], and—at least in 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 signaling, studies examining brain tissue from patients with bipolar disorder show no change in membrane but increased signaling in the cytosol, which may be normalized by the classic mood stabilizer lithium (e.g.,[20, 21]). Other disease states where compartment-specific defects in cyclic nucleotide signaling have been implicated include—but are not limited to—erectile dysfunction[22], hypertension[23], cardiac hypertrophy[18, 24], acrodysostosis[25, 26], and Huntington's disease[27, 28]. The unique substrate/localization profile offered by each 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 signaling in affected compartments (i.e., provide efficacy) without disturbing cyclic nucleotide signaling elsewhere (i.e., avoid side effects).

As reviewed in detail below, there has been and continues to be strong interest in developing PDE-targeted therapeutics for a number of 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 sub-family. That said, novel therapeutic strategies are currently being explored to increase the selectivity and specificity with which PDEs are targeted (e.g., by

targeting protein-protein interactions). Further, PDE activators are now being considered as agents for treating select diseases, as are 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 a number of 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, as little is known about the pathophysiology of non-canonical cyclic nucleotides and what is known has been recently reviewed elsewhere[4].

Therapeutic PDE inhibition

Several PDE family-selective inhibitors have successfully reached the market, targeting diseases such as psoriasis, COPD and erectile dysfunction (Table 1). Conversely, many potent and selective PDE inhibitors have also 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. Given the clinical successes of some PDEi's as discussed, traditional PDEi's are still very much being pursued as potential therapeutics, particularly in the context of the central nervous system (CNS), cardiovascular system, reproductive system, cancer and metabolic disorders (Table 3).

Marketed PDE inhibitors

The non-selective PDE1 inhibitor (PDE1i) vinpocetine is not FDA approved but is available in over-the-counter supplements (e.g., Cavinton or Intelectol, Richter Gedeon; Cognitex, Life Extension) claiming to improve memory and recovery from stroke, likely due to increasing vasodilation[29]. As extensively reviewed elsewhere[29], a number of clinical trials have examined the cognition-enhancing effects of vinpocetine—either alone or in combination with another compound (e.g., caffeine or Ginko Biloba)—and have generally found improvement in healthy volunteers, individuals with cerebral hypofusion, and possibly aged individuals, but no improvement in AD patients. Reports of side effects associated with vinpocetine have generally been minimal (Table 1,[30]).

Several PDE3i's 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 stenosis (c.f.,[31]). Cilostazol improves function across a number of domains, but it is associated with serious side effects (Table 1) and so is contraindicated for patients with severe heart failure, hepatic impairment, or renal impairment[32]. 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 transplant[33]. 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 (i.e., generally not used in patients with renal failure)[33].

Three so-called “second-generation” PDE4i’s are currently FDA approved, with a 4th compound marketed as an over-the-counter supplement. Roflumilast is an add-on therapy for chronic obstructive pulmonary disorder (COPD; Table 1). Although it causes gastrointestinal and weight loss side effects, making it a third line treatment for COPD, it improves sugar metabolism in obese patients and may decrease cardiovascular events in patients with COPD[34]. Apremilast is used in the treatment of moderate to severe plaque psoriasis and psoriatic arthritis[35–37] 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 PDE4i’s are the same that plagued first-generation PDE4i’s (i.e, gastrointestinal disturbances; Table 1), albeit with much improved therapeutic windows[35]. 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 amongst these FDA-approved PDE4i’s. Zembrin in a non-selective PDE4 inhibitor (also acts as a 5-HT uptake inhibitor) that is a component of a number of herbal supplements claiming calming or mood-stabilizing properties (e.g., Calm, Doctor’s Best; Mood, Procera; Nutri-calm, Nature’s Sunshine)[38]. fMRI imaging of the amygdala in humans supports an anxiolytic-like effect of Zembrin[38]. Further, a Phase I trial found Zembrin was well tolerated and improved cognitive flexibility, executive function, mood and sleep[39]. As noted below, a number of PDE4i’s are currently being pursued to improve cognitive functioning (see below).

There are 4 PDE5i’s currently FDA approved and marketed in the U.S., with 2 additional PDE5i’s marketed outside the U.S.. All 6 PDE5i’s 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 a secondary approval for pulmonary hypertension (contraindicated for pediatric patients, veno-occlusive disease, or sickle cell disease), as did tadalafil. These PDE5i’s are generally considered safe and well tolerated, with no increase in cardiac mortality or myocardial infarction[40]. They share largely similar side effect profiles (Table 1) with headache, flushing, dyspepsia, and vision disturbances being the most common adverse events[40]. Interestingly, udenafil (Zydena, Mezzion Pharma) —one of the PDE5i’s 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 a number of marketed PDEi’s 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 PDEi clinical trials—Despite the successes noted above, a number of PDEi’s that entered the clinic failed to make it to market. The selective PDE2i PF-05180999 was originally considered a candidate for cognitive impairments associated with schizophrenia based on its preclinical profile[45]; however, it was brought into the clinic for migraine.

Despite the completion of earlier Phase 1 safety and tolerability studies, additional trials were terminated early due to safety concerns (Table 2). Exisulind inhibits both PDE2A and PDE5A (which are overexpressed in a number of precancerous and cancerous cell types) and triggers apoptosis in precancerous/cancerous cells with minimal effects on healthy cells (c.f., [46, 47]). Despite promising findings in multiple clinical trials, exisulind failed to secure FDA approval due to deficiencies in safety and efficacy (Table 2, c.f.,[46, 47]).

As noted above, Cilastozol has gained FDA approval for intermittent claudication; however, clinical trials for other indications, such as type 2 diabetes mellitus peripheral neuropathy, have failed[48]. That said, cilostazol significantly improved walking speed in these patients, suggesting improved peripheral blood flow as would be expected based on its current approved indication[48].

The PDE4i cilomilast (Ariflo, GlaxoSmithKline) gained FDA approval in 2003 as a second-line treatment for COPD in patients who are poorly responsive to salbutamol[49]. However, cilomilast never made it to market due to the severely dose-limiting nature of gastrointestinal side effects (e.g., nausea and vomiting, diarrhea, and abdominal pain[35]). The fact that cilomilast elicited more pronounced side effects relative to the other systemically-delivered PDE4i's described above may be related to preferential inhibition of the PDE4D family relative to the other PDE4 subtypes[49]. A novel PDE4i 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 disease[50]. As target engagement in the organ of interest was not confirmed[50], the reasons underlying the lack of efficacy remain unclear.

A number of clinical trials have attempted to extend therapeutic indications for PDE5i's, but have failed. As reviewed extensively elsewhere[29], a number of 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 risperidone[56]. Several studies were initiated to study sildenafil and/or tadalafil in patients with Duchenne or Becker Muscular dystrophy, with the hopes that the vasodilatory properties of the drugs would improve muscular ischemia; however, clinical trial outcomes have been mixed (Table 2;[57, 58]).

Two PDE9i's 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 behavioral disturbances in a Phase II clinical trial[59]. Similarly, BI 409306 was reported as safe and well tolerated in healthy subjects as well as patients with AD or schizophrenia; however, no positive effects on cognition were observed in either patient population (https://www.boehringer-ingelheim.com/PDE9-Inhibition-in-AD, accessed 05/28/19;[60–63]). 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 PDE9i's to improve functioning in AD may be related to the fact that brain PDE9A is enriched in the nucleus and membrane[5] and, thus, is not in a position to directly regulate the cytosolic pools of cGMP that appear to be dysregulated in Alzheimer's disease[13–15].

A number of clinical trials have tested the PDE10i PF-02545920 in schizophrenia and Huntington's disease (Table 2–3). Despite widely replicated efficacy in a number of preclinical assays (e.g.,[64, 65]), PF-02545920 failed to improve symptoms in patients with either exacerbated, stable, or sub-optimally treated schizophrenia (Table 2;[66]). Further, in at least 1 trial, there were reports of motoric side effects such as dystonia[66]. Similarly, despite decreased striatal expression of PDE10A being found in patients with Huntington's disease (Box 2) and promising efficacy of PDE10i's in preclinical models of the disease[28, 67, 68], PF-02545920 failed to improve symptoms in patients with Huntington's (https://clinicaltrials.gov/ct2/show/results/NCT02197130?sect=X70156#outcome1, accessed 05/28/19), and so efforts for this disease indication were terminated (https://clinicaltrials.gov/ct2/show/NCT02342548, accessed 05/28/19). Several other PDE10i's have also been pursued in the clinic for schizophrenia and/or HD, with some efforts subsequently suspended or terminated (Table 2) and others ongoing (Table 3—see more below).

The unsuccessful translation of these PDEi's from promising preclinical data to human testing suggest 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, 69, 70]. 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 PDEis 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 chemistry efforts to optimize structure-activity relationships, have yielded recent vast improvements in terms of selectivity and potency (e.g., see work related to PDE10is and PDE4is[71–73]). These efforts have also yielded PDEis with novel modes of action in some cases (i.e., acting as a negative allosteric modulator instead of direct catalytic inhibitor[72]). In addition, there are still significant efforts to repurpose older PDEi's. 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 indication (c.f.,[74]). Indeed, the PDE5i 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 development[74]. That said, there are a number of 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[74] for further discussion).

Non-selective inhibitor

The non-selective PDE3-4-10-11 inhibitor Ibudilast, which also inhibits glial cell activation, is approved for use in Japan as a bronchodialator and has long been of interest as a therapeutic approach for neuropathic pain and substance abuse/withdrawal[75, 76]. Recent clinical trials have tested Ibudilast in the context of amyotrophic lateral sclerosis, pain, as well as opiate, methamphetamine, and alcohol abuse (Table 3) and positive effects have been reported for all trials completed to date[77–80].

PDE1 inhibitors

The broad PDE1 inhibitor ITI-214, which shows picomolar IC50s for PDE1A, PDE1B and PDE1C in enzymatic assays and >1000-fold selectivity versus its nearest neighbor PDE4[81, 82], 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[81–83], mimicking effects of a dopamine receptor 1 (D1) agonist[83] and occurring at doses that leave efficacy of the antipsychotic risperidone intact[81]. Although the target mediating the cognition-enhancing effects of ITI-214 remains undetermined, PDE1B may be the most likely candidate given its expression in D1-expressing neurons[83], along with the fact that a PDE1B-selective inhibitor showed similar cognition-enhancing effects[84]. ITI-214 was moved into the clinic, with potential applications for cognitive deficits associated with schizophrenia, AD, and Parkinson's Disease[82], 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 () given its ability to improve cardiac function in dog and rabbit models of heart failure[85] as a consequence of its inhibition of PDE1C[85].

PDE2 inhibitors

A number of highly selective PDE2i's have demonstrated cognition-enhancing, anxiolytic and anti-depressant like-effects in animal models (c.f.,[86]). 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[87–89] (Table 3). Looking beyond the brain, PDE2i's may hold relevance for cardiovascular function since elevated PDE2A expression has been found in failing human hearts as well as a large number of animal models of heart disease (c.f.,[90]). Further, PDE2i's 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 pathogen (c.f.,[91]).

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 04/30/2019) 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, 92, 93]. This PDE3i also elicited some improvement in chronic tinnitus[94]. 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[29] for review). The mechanism by which cilostazol elicits improved cognition has yet to be determined empirically. Given there is very little expression of PDE3A or PDE3B in the brain[70, 95], it may be likely that 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 (e.g.,[96]).

Novel therapeutic applications of cilostazol are also being explored in preclinical studies. For example, oral cilostazol (30 mg/kg) improved retinal stress, ischemia, and ganglion cell death in a rat model of diabetic retinopathy[97]. In addition, PDE3A knockout (KO) mice are infertile[98] and chronic administration of cilostazol blocks pregnancy in naturally-cycling swine[99], suggesting potential utility of PDE3i's 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 maturation[100].

Due to the promise of PDE3 as a therapeutic target, coupled with concerns over side effect associated with cilostazol, alternative PDE3 inhibitors are currently being developed[101–103].

PDE4 inhibitors

The PDE4 family is arguably the most studied of all the PDE families. A number of 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 apremilast dramatically improved repigmentation in a woman with treatment-resistant Vitiligo[104]. Multiple case reports have also described an ability of apremilast to improve symptoms in patients with treatment-resistant erosive oral lichen planus[105–107], 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 roflumilast reduced fat mass and, thus, body weight in obese women with polycystic ovary syndrome (PCOS); however, these reductions were smaller than those elicited by liraglutide (Table 3;[108, 109]). The PDE4 inhibitor TAK-648 is being tested in the clinic in patients with Type 2 diabetes, based on preclinical data[110]. 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 effects[111]. 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 memory[112]. Given these positive findings, roflumilast was tested in elderly subjects who demonstrated no change in spatial memory but improved verbal word memory with roflumilast treatment[113]. Numerous preclinical studies have supported the therapeutic potential of PDE4i's in the context of schizophrenia and cognition[114–117].

Cognition-enhancing effects have also been reported for the PDE4i HT-0712, which improved long-term memory for word-lists without serious adverse events in elderly

subjects experiencing cognitive decline (http://www.dartneuroscience.com/press_release/july_22_2008.pdf). The cognition-enhancing effect of HT-0712 in humans is consistent with previous reports in mice[118, 119]. 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, healthy volunteers with scopolamine-induced cognitive impairment, and 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 working memory in healthy elderly adults (http://tetradiscovery.com/wp-content/uploads/2016/11/FINAL-Tetra-Phase-1-121616-FINAL.pdf; accessed 05/28/19). These effects in humans are consistent with preclinical studies showing BPN14770 improved a number of behaviors in a mouse model of Fragile-X Syndrome and antagonized the amnestic effects of scopolamine in mice[120, 121]. Based on preclinical studies showing anxiolytic and cognition-enhancing effects[122], the PDE4i GSK356278 entered Phase I safety trials for Huntington's disease but adverse events limited the highest dose to that achieving only ~50% occupancy in brain (Table 3;[122]). Other nervous system disorders for which preclinical evidence suggests a therapeutic potential of PDE4i's include ischemic stroke[118, 123–126], traumatic brain injury[127], axon regeneration[128], and substance abuse disorders (both causes and consequences,[129–132]).

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 alpha subunit Gαs (Gαs*). Preclinical studies show that while Gαs* triggers increased cAMP levels in some tissues, it actually results in decreased cAMP levels in other tissues due to a PKA-dependent upregulation of PDE activity, particularly that of PDE1 and PDE4[115, 133, 134]. Consistent with this upregulation of PDE4 activity, the PDE4i rolipram was able to reverse deficits in Gαs mouse models[115, 116]. 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 is examining PDE4 in the context of cancer. Both preclinical and clinical data suggest roflumilast may exhibit anti-tumor activity for B-cell lymphomas[135]. The PDE4i rolipram, in combination with cAMP-elevating agents, has been shown to suppress triple negative breast cancer both *in vitro* and *in vivo* in mice[136]. Apremilast similarly induced tumor regression in mouse models of colorectal cancer[137]. Perhaps even more interesting, specific inhibition of PDE4D, either with genetic tools or the PDE4Di Gebr-7b, resensitized chemotherapy-resistant ER-positive breast cancer cells[138]. These early studies provide promise for the chemotherapeutic potential of PDE4is.

PDE5 inhibitors

A number of recent clinical trials have explored additional disease indications that might benefit from the vasodilatory properties of PDE5i's. A cream version of sildenafil has recently been tested in a Phase II study examining female sexual arousal disorder (Table 3) as well as a study in which improved blood flow in patients with secondary Raynaud phenomenon was observed[139]. International consortiums are investigating the effects of sildenafil in intrauterine growth restriction, due to anticipated improvements in

uteroplacental perfusion[140, 141]. Initial results suggest sildenafil improves fetal growth and maternal blood pressure across species, including human, sheep, rabbit, and rodents[142, 143]. 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 signaling 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 men[144], and chronic sildenafil improved glycometabolic control, ameliorated visceral adiposity, and prevented remodeling in diabetic cardiomyopathy[145–148]. That said, vardenafil failed to reduce cardiovascular risk in men with type 2 diabetes[149]. 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 signaling and/or a reduction of inflammatory chemokines[145–148]. 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 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 suggest PDE5i's could be an effective medical expulsive therapy for distal ureteral calculi, albeit not significantly improved relative to tamsulosin[150]. 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 PDE4i's, a number of trials are exploring the therapeutic potential of PDE5i's as chemopreventives for solid tumors, multiple myeloma, and head and neck squamous cell carcinoma. Early reports suggest combining sildenafil with the chemotherapeutic regorafenib is safe in patients with solid tumors[151]. Further, a number of *in vitro* and animal models of colorectal cancer suggest that PDE5i's, either alone or as part of a multi-chemotherapeutic regimen, demonstrate an ability to prevent tumor growth (e.g., [17, 151, 152]). Similarly, reports suggest tadalafil promotes tumor immunity in patients with head and neck squamous cell carcinoma (Table 3,[153, 154]). However, particularly with regard to colorectal cancer, PDE5is do not produce complete anti-tumorigenic effects[16]. This lack of complete efficacy may be related to the fact that membrane GCs are inhibited in colorectal cancer[16], and PDE5 may be primarily regulating cytosolic rather than membrane GCs[18, 19]. Alternatively, the overexpression of both PDE5 and PDE10A in colorectal cancer cells—the latter a membrane-enriched PDE[69, 155]—may be involved[17]. Indeed, PDE10i's also inhibit growth of colorectal cancer cells[156, 157]; however, when both PDE5 and PDE10 are inhibited, anti-tumor efficacy is improved in preclinical models[17]. Although enthusiasm for PDE5i's as chemopreventives is growing[158], it should be noted that PDE5i's similarly prevented prostate carcinogenesis in preclinical models but did not appear to reduce risk or recurrence in clinical studies[159]. Perhaps even more concerning, PDE5A appears to suppress melanoma cell invasion in mice[160] yet a recent systematic review and meta-analysis showed that PDE5i's actually increase risk for melanoma and basal cell carcinoma in humans[161].

PDE9 inhibitors

Although PDE9i's 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 PDE9i BAY73–6691 exerted immediate benefits on acute vaso-occlusive events[162], and a Phase 1 clinical trial looking at safety, tolerability and PK/PD of the PDE9i PF-04447943 in patients with sickle cell anemia has recently been completed (Table 3). PDE9i's may also hold therapeutic potential for cardiovascular indications as PDE9A expression is upregulated by cardiac hypertrophy and cardiac failure. Indeed, the PDE9i PF-04449613 reverses heart disease in animal models by controlling pools of cGMP downstream of pGCs[18].

PDE10i inhibitors

Despite the PDE10i 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 tolerated[163], altering the effects of ketamine on brain activity in healthy controls, particularly in the striatum, substantia nigra, and ventrolateral prefrontal cortex (https://clinicaltrials.gov/ct2/show/results/NCT01892189?sect=X70156#outcome1, accessed 05/28/19). In patients with schizophrenia, although TAK-063 failed to demonstrate a significant effect on the total PANSS score, there was a trend that mirrored effects sizes normally seen with risperidone[164]. Furthermore, TAK-063 did significantly improve a number of secondary endpoints relative to placebo[164]. It is not entirely clear why TAK-063 was able to succeed where PF-2545920 failed. While 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[165], other studies have reported that PF-254920 activates these pathways equally[64, 166]. It is notable that TAK-063—but not PF-254920—increased sensorimotor gating in rodents as measured by prepulse inhibition of acoustic startle (PPI)[165], suggesting PPI may more accurately predict antipsychotic-like effects of novel compounds. Preclinical studies are also exploring the therapeutic potential of PDE10i's in the context of L-DOPA-induced dyskinesias[167] and alcohol abuse disorders[130].

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 PDE7i's and PDE8i's may have positive effects in diseases where cognition, neuroprotection, neuroinflammation, and/or motor function are impaired (e.g., multiple sclerosis and/or Parkinson's disease;[168–172]). Similarly, PDE11i's may hold potential for treating age-related cognitive decline[70, 173] or as an adjunctive treatment to improve lithium responsiveness in patients with bipolar disorder[174, 175]. PDE7i's may also hold promise in treating leukemia[176, 177], and PDE8i's may have potential for treating disorders associated with reduced androgen production in males as PDE8i's, particularly when applied in combination with PDE4i's, stimulate Leydig cell steroidogenesis[178, 179].

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 signaling have been implicated in select disease states, including some age-related deficits[180, 181], Huntington's disease[182], social isolation[183], migrane[184–188], retinitis pigmentosa[189], infertility[98], prostate cancer[190], melanoma and basal cell carcinoma[161], cardiac hypertrophy[24, 191], acrodysostosis[25], and polycystic kidney disease[192]. 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 empiracly established. Indeed, Mironid have developed PDE4 longform-specific activators (mechanism as yet unknown; Table 4) for the treatment of polycystic kidney disease where increased adenylate cyclase activity caused by overexpression of vasopressin V2R receptors results in elevated cAMP levels that drive cyst growth and disease progression[193]. There are several natural mechanisms by which PDE activity can be activated (Figure 3), and it is our contention that these avenues could be manipulated phamacologically 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 E. Coli FhlA) domains[194] (Figure 3B). Although GAF domains have been identified in over 7400 proteins, in mammals they are only are found in the PDE families 2, 5, 6, 10 and 11[195, 196]. 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 (Figure 3). PDE2 and PDE5 are activated when cGMP binds the GAF domain[197–199], and PDE10 is activated by cAMP binding the GAF domain[200]. In the context of activation, binding of cyclic nucleotides to GAF domains is thought to cause structural changes that relieve autoinhibition of the PDEs (Figure 3). In contrast, cGMP binding the GAF-A domain of PDE6 enhances protein-protein interactions that inhibit PDE6 catalytic activity[201]. This suggests that blocking cGMP binding of the PDE6 GAF domain may provide a means of promoting PDE6 activity. It 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 analog—but not cGMP itself—binds the GAF domain[200]. Further, even though cGMP binding of the GAF domain activates PDE5[197], a number of other types of molecules that bind the GAF domain inhibit PDE5 in its activated—but not basal—state[202]. This is consistent with the fact that GAF domains are known to bind a diverse array of small molecules that are unrelated to cyclic nucleotides[194]. The fact that GAF domains are only found in PDEs in mammals[196] makes GAF domains of high interest in the context of drug targeting[203]. Importantly, mammalian GAF domains are sufficiently structurally divergent from one another (e.g., low degree of homology between PDE families and the tandem GAF domains are preceded by variable N-terminal stretches) as to allow selective pharmacological targeting of individual PDE families[197]. Together, this suggests 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 PKG phosphorylation of PDE3[204, 205], PDE4[204, 206], PDE5[207], and PDE8[208] 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[72, 209] (Figure 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 state[72]. Notably, select PDE4Di's allosterically inhibit catalytic activity by promoting "trans-capping"[127]; whereas, phosphatidic acid activates PDE activity by inhibiting trans-capping in a similar but mutually exclusive manner to PKA[210–212]. Furthermore, the dominant negative peptide "UCR1C", which corresponds to UCR1 sequence, also activates PDE4 activity by inhibiting trans-capping[213]. 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 site[214] (Figure 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 subunits[215]. The full crystal structure of PDE6 is not yet available[216]; however, recent cryo-EM work[217] has confirmed the predicted structural organisation of the holo-enzyme, albeit without sufficient detail to inform pharmacological targeting. The success in upregulating PDE6 activity via gene transfer to combat retinitis pigmentosa[189] (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 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 (e.g. antisense, silencing or microRNAs)[160, 218–222], or gene editing (e.g., Crispr/Cas9)[223] might also prove a useful means of therapeutically targeting individual PDE isoforms (Figure 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, vision[224]. Expression of recombinant PDE6α in the retina via an adeno-associated virus (AAV-PDE6α) preserved retinal structure, photo-transduction, and vision in retinal degeneration (rd) mice, as did AAV-PDE6β[225, 226]. AAV-PDE6α similarly rescued retinal deficits in a mouse model that mimics human retinitis pigmentosa mutations[227]. Experiments injecting AAV-PDE6γ into the retina have also proven successful in mice[228]. In dogs, delivery of recombinant PDE6α using a tyrosine capsid-mutant AAV8 was able to stabilize cGMP levels and improve survival of photoreceptor rods and cones in PDE6α-mutant dogs; however, several adverse effects related to the AAV injection were identified[189]. The recent development of synthetic AAV vectors that target the retina in non-human primates may provide the answer to these problems in the future[229]. Notably, two clinical trials are underway testing the safety and

efficacy of PDE6 gene therapy in retinitis pigmentosa (PhI , PhII ; clinicaltrials.gov accessed 5/29/19).

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 proteins[230]. With the field of personalized bioelectronic implants quickly evolving, optogenetic-based biomedical approaches are being pursued for neurological diseases, cancer, cardiovascular disease and metabolic disorders[230]. Given that optogenetic-based approaches have now entered clinical trials[230], it is worth noting here that light-activated PDEs have been identified in lower organisms[231–233] and engineered in the lab[234]. 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 signaling is down regulated in one tissue yet upregulated in another (e.g., aging; c.f.,[235]). It might also provide a means of avoiding side-effects associated with targeting PDE activity in a specific tissue (e.g., nausea/emesis associated with inhibiting PDE4 in the area postrema).

Targeting location

As production of cAMP is utilized by a variety of different Gs-coupled receptors to transduce signals, compartmentalization of signaling intermediates is crucial to define physiological outcomes specific to each receptor[236]. This compartmentalization of cyclic nucleotide signaling is achieved by virtue of PDEs being tethered to a precise cellular location via binding partners (Table S1). 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 (Figure 4).

Dominant negative PDEs—Proof of principle for such an approach first emerged with studies using dominant negative (DN) PDEs, catalytically inactive mutants that would displace their endogenous PDE. Using specific DN-PDE4 isoforms, *in vitro* studies have successfully altered perinuclear cAMP signaling[237], β-arrestin-dependent desensitization of the beta2-adrenergic receptor[238, 239], growth control of prostate cancer cells[190], prostanoid receptor-mediated cAMP signaling[240], glucagon-like peptide-1 release[241], and cAMP gradients at the centrosome[242]. 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 signaling deficits and hippocampus-dependent memory impairments that were caused by sleep deprivation[243–245]. 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 mice[246]. 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

behaviors[247]. 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 dead[248].

One point to consider in adopting a DN 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 signaling complexes, which involve mutually exclusive protein-protein interactions[249] (Table S1; Figure 4). For instance, PDE4D5 is involved in a number of 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[250]. 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 (e.g., RACK1 at leading edge of cells, beta-arrestin at transmembrane receptors, and HSP20 in the cytosol; Figure 4;[250]). It is clear that this is also the case for a number of other isoforms based on proven protein-protein binding interactions (e.g., in heart tissue/cells, PDE4D3 can bind to either the ryanodine receptor, HSP20, or an AKAP9/Potassium channel complex—see Table S1) or based on inference from the fact that the exact same isoform can be found localized to multiple subcellular domains (e.g., ~50% of PDE11A4 in neurons localizes to the cytosol while 25% is localized to the nuclear fraction and another 25% to the membrane compartment[69]). Thus, a non-selective DN 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 site(s) that mediates a particular protein-protein interaction. Mutating isoform-specific binding sites has also proven a useful approach, revealing that an integrin α5-PDE4A5 complex regulates endothelial inflammation[251], a PDE3A1-SERCA complex regulates myocardium contractility[252], and a DISC1-mediated sequestering of PDE4B regulates hippocampal function[247]. An alternative approach is to develop a peptide or small molecule that specifically competes for a given protein-protein binding site[253]. This approach would displace only a specific “pool” of a given PDE isoform, while leaving the vast remainder unfettered in their respective signaling complexes (Figure 4). Efforts have begun to identify small molecules capable of promoting or interfering with protein-protein interactions, but 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 manner[253] and evidence continues to build. More recently, a PDE4D-FAK disrupting peptide prevented direction sensing and invasion of melanoma cells[254, 255] and a PDE8A-Raf1 disruptor retarded cancer cell growth promoted by a Ras mutation[208]. 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 signaling[254]. The effectiveness of PDE displacement has also been demonstrated *in vivo*, where intraperitoneal injections of a PDE4-HSP20 disruptor significantly attenuated hypertrophy-induced cardiac remodeling[256].

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 responsivity to the mood stabilizer lithium[174] or age-related cognitive decline[70, 173]. Conversely, a peptide or mutation that could stabilize PDE11A4 homodimerization might prove useful in treating the deleterious psychological effects of social isolation[183]. Targeting homodimerization of PDE2, PDE4, or PDE5 may also relocate the enzymes by virtue of changing susceptibility to regulatory post-translational modifications[196, 257]. 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 homodimerization[258]. Such complex-specific targeting of PDE function may be required to achieve efficacy in absence of unwanted side effects, particularly in cases where multiple subfamily isoforms orchestrate a variety of physiologic responses by virtue of different protein-protein interactions (e.g., targeting various PDE3 isoforms for cardiovascular disease[259]).

Targeting post-translational modifications

As post-translational modifications (PTM) directly regulate PDE activity and location, PTMs could be considered a point of therapeutic control (Figure 5).

Phosphorylation—PKA or PKG phosphorylation of PDE3[204, 205], PDE4[204, 206], PDE5[207], and PDE8[208] will stimulate catalytic activity. Phosphorylation can also influence PDE cellular location by virtue of preventing other PTMs that promote membrane association (e.g., 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 dendrites[155]. If, however, PDE10A2 undergoes isoform-specific phosphorylation by PKA on Thr16[260], palmitoylation of PDE10A2 is attenuated and the specific membrane localization is lost[155]. Interestingly, phosphorylation on the same site also interferes with the scaffolding of PDE10A2 by AKAP150[261]. 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 membrane[262]. PKA phosphorylation of PDE4D3 drives an association with the mAKAP signaling complex to evoke rapid signal termination in the muscle compartment[263], which may have therapeutic implications given that polymorphisms in the PDE4D3-mAKAP binding site lead to a higher susceptibility to cardiovascular disease[264]. PDE4D enzymes also get phosphorylated by both casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3β) in the catalytic region on a motif known as a “phosphodegron”[265]. This action increases the affinity of the PDE for a ubiquitin ligase complex (Cullin 1 containing SCF E3 ligase) which promotes proteosomal degradation of

the enzyme[265]. Hence PDE phosphorylation not only affects activity, localization, and protein-protein interactions, it also regulates protein turnover.

Ubiquitination and sumoylation—It has been known for some time that increases in PKA activity promote the proteosomal degradation of short-lived proteins, an action that can be enhanced by PDE inhibitors (c.f.,[266]). However, we are just starting to understand that the stability of PDEs can themselves be influenced by the ubiquitin-proteosome system (Figure 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 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 an SCF E3[265], whereas PDE4B levels can be down-regulated by a different E3, Smurf2, to promote anti-fibrotic signaling in the liver[267]. PDE4D5 can also be targeted for ubiquitin modification by the RING type MDM2 E3 ligase; however, this beta-adrenergic driven ubiquitination of PDE4D does not signal a degradation of the enzyme. Instead, it shifts PDE4 from binding RACK1 to binding β-arrestin[268]. PDE4s are similarly regulated by the ubiquitin-like protein SUMO (small ubiquitin-like modifier)[269]. SUMO-conjugation tends to alter the location, activity or protein-protein interactions of a protein rather than tagging for destruction via the ubiquitin-proteosome system[270]. Unlike ubiquitination, 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)[270]. PDE4s from subfamilies A and D contain the consensus motif, whereas subfamilies B and C do not. This adds an extra layer of sub-family-specific regulation as SUMOylation serves both to protect against the inhibitory phosphorylation by ERK/MAP kinases[271] and further enhances activity of the PKA phosphorylated longform PDE4 by locking it in the “open” non-UCR inhibited conformation[269].

S-nitrosylation and proline hydroxylation—Two additional PTMs that trigger PDEs for destruction include S-nitrosylation and proline hydroxylation (Figure 5). PDE5 can be S-nitrosylated by NO on Cys220[272], which targets the enzyme to the proteasome and reduces PDE activity. Under conditions of reduced NO bioavailability, as in heart disease, PDE5 is upregulated due to a loss of this S-nitrosylation-induced degradation[272]. Proline hydroxylation has also been identified as a modification that can tag substrates for recognition by E3 ligase complexes[273]. In the heart, proline hydroxylases domain-containing proteins (PHDs) hydroxylate surface-associated prolines on PDE4D enzymes, triggering their degradation[274]. In this way, direct binding of PHDs to PDE4s increase cAMP without affecting adenylate cyclase activity.

Challenges

Although this is clearly an exciting time in the PDE field, there is much work that remains to be done. For therapeutics to be efficiently developed, we need to more thoroughly understand precisely where cyclic nucleotide signaling is disrupted in a given disease—in which tissue, cell types, 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 (e.g.,[5–12]; for full review, see Table S1). 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 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 (i.e., target only its cAMP- or cGMP-hydrolytic activity, see[203] 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 studies[4]. Cyclic cytidine monophosphate (cCMP) and cyclic uridine monophosphate (cUMP) are synthesized by soluble guanylate and soluble adenylate cyclases in mammalian systems, although an as-yet-to-be identified generator likely accounts for the majority of production given the dissociation between sGC/sAC and cCMP/cUMP expression patterns[4]. ExoY, a bacterial nucleotidyl cyclase, is known to generate cUMP in non-mammalian systems[2]. Hydrolysis of cCMP is catalyzed by PDE7A whereas cUMP is broken down by PDE3A, PDE3B and PDE9A[2]. Functionally both nucleotides have been shown to activate PKA/PKG[275], cyclic nucleotide gated channels[276], and cCMP is described as a partial agonist of EPAC[277]. In a disease context, the non-canonical cyclic nucleotides play roles in promoting virulence of pseudomonas aeruginosa infections[278] and triggering apoptosis of cancer cells[279]; however much more research is needed to accurately characterize the pathophysiology involving these signaling molecules. Difficult issues facing the field will be defining specific non-canonical cyclic nucleotide signaling systems that are aberrantly regulated in disease, determining the mechanisms by which PDEs might preferentially degrade non-canonical versus canonical cyclic nucleotides, and visualising the compartmentalisation of non-canonical signalosomes in specific locations within cells and tissues.

Finally, development of PDE-targeted therapeutics faces the same challenges as does all drug discovery—namely the high rate of failure in clinical trials. A recent study suggests that from 2000–2015, only 13.8% of all compounds made it from Phase I to approval[280]. 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 made it from Phase 1 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%, respectively[280]. 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 benefited[280]. 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 signaling has proven 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 sub-families of PDE4 are showing promising results in mouse models of learning and memory and translation to human disease would be a game-changing advance[121]. 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 (e.g.,[281, 282]). 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 sub-optimal doses of PDEi's 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 (e.g.,[283]). Indeed, PKA, PKG, and Epacs have been implicated as therapeutic targets in their own right for a number of indications for which PDEi's are being pursued, including diseases of the cardiovascular, immune, metabolic and nervous systems as well as cancer (e.g.,[115, 116, 284–289]). Further, several studies in a variety of tissues have attributed the beneficial effects of PDEi's to the activation of PKA, PKG and/or Epac (e.g., [121, 290–292]). 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Maurice DH et al. Advances in targeting cyclic nucleotide phosphodiesterases. Nat Rev Drug Discov 13, 290–314 (2014). [PubMed: 24687066]
2. Schneider EH & Seifert R Inactivation of Non-canonical Cyclic Nucleotides: Hydrolysis and Transport. Handb Exp Pharmacol 238, 169–205 (2017). [PubMed: 28204955] Helpful review on the role PDEs play in regulating non-canonical cyclic nucleotides.
3. Herbst S et al. Transmembrane redox control and proteolysis of PdeC, a novel type of c-di-GMP phosphodiesterase. EMBO J 37 (2018).
4. Seifert R cCMP and cUMP Across the Tree of Life: From cCMP and cUMP Generators to cCMP- and cUMP-Regulated Cell Functions. Handb Exp Pharmacol 238, 3–23 (2017). [PubMed: 28181008]
5. Patel NS et al. Identification of new PDE9A isoforms and how their expression and subcellular compartmentalization in the brain change across the life span. Neurobiol Aging 65, 217–234 (2018). [PubMed: 29505961] First report showing that the subcellular compartmentalization of a PDE family differs depending on the specific isoform, age, and tissue investigated.
6. Huston E, Gall I, Houslay TM & Houslay MD Helix-1 of the cAMP-specific phosphodiesterase PDE4A1 regulates its phospholipase-D-dependent redistribution in response to release of Ca2+. J Cell Sci 119, 3799–810 (2006). [PubMed: 16940352] This study provides the first evidence that PDEs can be relocated by Ca2+, providing a potential mechanism by which cAMP, phospholipase D and Ca2+-signaling pathways interact.
7. Nagel DJ et al. Role of nuclear Ca2+/calmodulin-stimulated phosphodiesterase 1A in vascular smooth muscle cell growth and survival. Circ Res 98, 777–84 (2006). [PubMed: 16514069]
8. Houslay MD & Baillie GS Beta-arrestin-recruited phosphodiesterase-4 desensitizes the AKAP79/PKA-mediated switching of beta2-adrenoceptor signalling to activation of ERK. Biochem Soc Trans 33, 1333–6 (2005). [PubMed: 16246112]
9. Perera RK et al. Microdomain switch of cGMP-regulated phosphodiesterases leads to ANP-induced augmentation of beta-adrenoceptor-stimulated contractility in early cardiac hypertrophy. Circ Res 116, 1304–11 (2015). [PubMed: 25688144] Important work showing that the subcellular compartmentalization of a PDE may change with disease.
10. Penmatsa H et al. Compartmentalized cyclic adenosine 3',5'-monophosphate at the plasma membrane clusters PDE3A and cystic fibrosis transmembrane conductance regulator into microdomains. Mol Biol Cell 21, 1097–110 (2010). [PubMed: 20089840]
11. Ahmad F et al. Differential regulation of adipocyte PDE3B in distinct membrane compartments by insulin and the beta3-adrenergic receptor agonist CL316243: effects of caveolin-1 knockdown on formation/maintenance of macromolecular signalling complexes. Biochem J 424, 399–410 (2009). [PubMed: 19747167] Results suggest elevations in cAMP can trigger a subcellular redistribution of PDEs.
12. Al-Tawashi A & Gehring C Phosphodiesterase activity is regulated by CC2D1A that is implicated in non-syndromic intellectual disability. Cell Commun Signal 11, 47 (2013). [PubMed: 23826796]
13. Bonkale WL, Winblad B, Ravid R & Cowburn RF Reduced nitric oxide responsive soluble guanylyl cyclase activity in the superior temporal cortex of patients with Alzheimer's disease. Neurosci Lett 187, 5–8 (1995). [PubMed: 7617301] Example of a human study showing cyclic nucleotide signaling deficits associated with disease can be specific to a particular subcellular compartment.
14. Baltrons MA, Pifarre P, Ferrer I, Carot JM & Garcia A Reduced expression of NO-sensitive guanylyl cyclase in reactive astrocytes of Alzheimer disease, Creutzfeldt-Jakob disease, and multiple sclerosis brains. Neurobiol Dis 17, 462–72 (2004). [PubMed: 15571982]

15. Baltrons MA, Pedraza CE, Heneka MT & Garcia A Beta-amyloid peptides decrease soluble guanylyl cyclase expression in astroglial cells. Neurobiol Dis 10, 139–49 (2002). [PubMed: 12127152]
16. Yarla NS et al. Targeting the paracrine hormone-dependent guanylate cyclase/cGMP/phosphodiesterases signaling pathway for colorectal cancer prevention. Semin Cancer Biol (2018).
17. Li N et al. Suppression of beta-catenin/TCF transcriptional activity and colon tumor cell growth by dual inhibition of PDE5 and 10. Oncotarget 6, 27403–15 (2015). [PubMed: 26299804]
18. Lee DI et al. Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. Nature 519, 472–6 (2015). [PubMed: 25799991] This study clarifies that, in heart, PDE9 regulates pools of cGMP downstream of particulate GCs while PDE5 regulates pools of cGMP downstream of soluble GCs.
19. Hartell NA, Furuya S, Jacoby S & Okada D Intercellular action of nitric oxide increases cGMP in cerebellar Purkinje cells. Neuroreport 12, 25–8 (2001). [PubMed: 11201085]
20. Rahman S et al. Reduced [3H]cyclic AMP binding in postmortem brain from subjects with bipolar affective disorder. J Neurochem 68, 297–304 (1997). [PubMed: 8978738] Another example of a human study showing cyclic nucleotide signaling deficits associated with disease can be specific to a particular subcellular compartment.
21. Mori S et al. Effects of lithium on cAMP-dependent protein kinase in rat brain. Neuropsychopharmacology 19, 233–40 (1998). [PubMed: 9653711]
22. Francis SH & Corbin JD Phosphodiesterase-5 inhibition: the molecular biology of erectile function and dysfunction. Urol Clin North Am 32, 419–29, vi (2005). [PubMed: 16291034]
23. Schwartz BG & Kloner RA Drug interactions with phosphodiesterase-5 inhibitors used for the treatment of erectile dysfunction or pulmonary hypertension. Circulation 122, 88–95 (2010). [PubMed: 20606131]
24. Zoccarato A et al. Cardiac Hypertrophy Is Inhibited by a Local Pool of cAMP Regulated by Phosphodiesterase 2. Circ Res 117, 707–19 (2015). [PubMed: 26243800]
25. Kaname T et al. Heterozygous mutations in cyclic AMP phosphodiesterase-4D (PDE4D) and protein kinase A (PKA) provide new insights into the molecular pathology of acrodysostosis. Cell Signal 26, 2446–59 (2014). [PubMed: 25064455]
26. Motte E, Le Stunff C, Briet C, Dumaz N & Silve C Modulation of signaling through GPCR-cAMP-PKA pathways by PDE4 depends on stimulus intensity: Possible implications for the pathogenesis of acrodysostosis without hormone resistance. Mol Cell Endocrinol 442, 1–11 (2017). [PubMed: 27908835]
27. Boscutti G, R. E,A & Plisson C. PET Radioligands for imaging of the PDE10A in human: current status. Neurosci Lett (2018).
28. Russell DS et al. Change in PDE10 across early Huntington disease assessed by [18F]MNI-659 and PET imaging. Neurology 86, 748–54 (2016). [PubMed: 26802091]
29. Heckman PRA, Blokland A, Bollen EPP & Prickaerts J Phosphodiesterase inhibition and modulation of corticostriatal and hippocampal circuits: Clinical overview and translational considerations. Neurosci Biobehav Rev 87, 233–254 (2018). [PubMed: 29454746]
30. Vinpocetine Monograph. Altern Med Rev 7, 240–243 (2002). [PubMed: 12126465]
31. Asal NJ & Wojciak KA Effect of cilostazol in treating diabetes-associated microvascular complications. Endocrine 56, 240–244 (2017). [PubMed: 28293857]
32. Chapman TM & Goa KL Cilostazol: a review of its use in intermittent claudication. Am J Cardiovasc Drugs 3, 117–38 (2003). [PubMed: 14727939]
33. Chong LYZ, Satya K, Kim B & Berkowitz R Milrinone Dosing and a Culture of Caution in Clinical Practice. Cardiol Rev 26, 35–42 (2018). [PubMed: 29045285]
34. Celli BR Pharmacological Therapy of COPD: Reasons for Optimism. Chest (2018).
35. Cada DJ, Ingram K & Baker DE Apremilast. Hosp Pharm 49, 752–62 (2014). [PubMed: 25477601]
36. Papp K et al. Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, in patients with moderate to severe plaque psoriasis: Results of a phase III, randomized, controlled trial (Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis [ESTEEM] 1). J Am Acad Dermatol 73, 37–49 (2015). [PubMed: 26089047]

37. Edwards CJ et al. Apremilast, an oral phosphodiesterase 4 inhibitor, in patients with psoriatic arthritis and current skin involvement: a phase III, randomised, controlled trial (PALACE 3). Ann Rheum Dis 75, 1065–73 (2016). [PubMed: 26792812]
38. Terburg D et al. Acute effects of Sceletium tortuosum (Zembrin), a dual 5-HT reuptake and PDE4 inhibitor, in the human amygdala and its connection to the hypothalamus. Neuropsychopharmacology 38, 2708–16 (2013). [PubMed: 23903032]
39. Chiu S et al. Proof-of-Concept Randomized Controlled Study of Cognition Effects of the Proprietary Extract Sceletium tortuosum (Zembrin) Targeting Phosphodiesterase-4 in Cognitively Healthy Subjects: Implications for Alzheimer's Dementia. Evid Based Complement Alternat Med 2014, 682014 (2014). [PubMed: 25389443]
40. Campbell HE Clinical monograph for drug formulary review: erectile dysfunction agents. J Manag Care Pharm 11, 151–71 (2005). [PubMed: 15766322]
41. Cho MC & Paick JS Udenafil for the treatment of erectile dysfunction. Ther Clin Risk Manag 10, 341–54 (2014). [PubMed: 24868160]
42. Kang SG & Kim JJ Udenafil: efficacy and tolerability in the management of erectile dysfunction. Ther Adv Urol 5, 101–10 (2013). [PubMed: 23554845]
43. Shim YS et al. Effects of daily low-dose treatment with phosphodiesterase type 5 inhibitor on cognition, depression, somatization and erectile function in patients with erectile dysfunction: a double-blind, placebo-controlled study. Int J Impot Res 26, 76–80 (2014). [PubMed: 24285284]
44. Shim YS et al. Effects of repeated dosing with Udenafil (Zydena) on cognition, somatization and erection in patients with erectile dysfunction: a pilot study. Int J Impot Res 23, 109–14 (2011). [PubMed: 21544084]
45. Helal CJ et al. Identification of a Potent, Highly Selective, and Brain Penetrant Phosphodiesterase 2A Inhibitor Clinical Candidate. J Med Chem 61, 1001–1018 (2018). [PubMed: 29293004]
46. Exisulind: Aptosyn, FGN 1, Prevatac, sulindac sulfone. Drugs R D 5, 220–6 (2004). [PubMed: 15230629]
47. Griffiths GJ Exisulind Cell Pathways. Curr Opin Investig Drugs 1, 386–91 (2000).
48. Rosales RL, Santos MM & Mercado-Asis LB Cilostazol: a pilot study on safety and clinical efficacy in neuropathies of diabetes mellitus type 2 (ASCEND). Angiology 62, 625–35 (2011). [PubMed: 21733952]
49. Giembycz MA An update and appraisal of the cilomilast Phase III clinical development programme for chronic obstructive pulmonary disease. Br J Clin Pharmacol 62, 138–52 (2006). [PubMed: 16842388]
50. Ratziu V et al. Lack of efficacy of an inhibitor of PDE4 in phase 1 and 2 trials of patients with nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 12, 1724–30 e5 (2014). [PubMed: 24530600]
51. Schultheiss D et al. Central effects of sildenafil (Viagra) on auditory selective attention and verbal recognition memory in humans: a study with event-related brain potentials. World J Urol 19, 46–50 (2001). [PubMed: 11289570]
52. Grass H et al. Sildenafil (Viagra): is there an influence on psychological performance? Int Urol Nephrol 32, 409–12 (2001). [PubMed: 11583362]
53. Reneerkens OA et al. The effects of the phosphodiesterase type 5 inhibitor vardenafil on cognitive performance in healthy adults: a behavioral-electroencephalography study. J Psychopharmacol 27, 600–8 (2013). [PubMed: 23427190]
54. Reneerkens OA et al. The PDE5 inhibitor vardenafil does not affect auditory sensory gating in rats and humans. Psychopharmacology (Berl) 225, 303–12 (2013). [PubMed: 22855271]
55. Goff DC et al. A placebo-controlled study of sildenafil effects on cognition in schizophrenia. Psychopharmacology (Berl) 202, 411–7 (2009). [PubMed: 18716761]
56. Akhondzadeh S et al. Sildenafil adjunctive therapy to risperidone in the treatment of the negative symptoms of schizophrenia: a double-blind randomized placebo-controlled trial. Psychopharmacology (Berl) 213, 809–15 (2011). [PubMed: 20949350]
57. Nelson MD et al. PDE5 inhibition alleviates functional muscle ischemia in boys with Duchenne muscular dystrophy. Neurology 82, 2085–91 (2014). [PubMed: 24808022]

58. Martin EA et al. Tadalafil alleviates muscle ischemia in patients with Becker muscular dystrophy. Sci Transl Med 4, 162ra155 (2012).

59. Schwam EM et al. A multicenter, double-blind, placebo-controlled trial of the PDE9A inhibitor, PF-04447943, in Alzheimer's disease. Curr Alzheimer Res 11, 413–21 (2014). [PubMed: 24801218] Despite proving target engagement by measuring a change in CSF cGMP levels, this clinical trial failed to detect any significant behavioral effect of a PDE9i in patients with Alzheimer's disease.

60. Brown D et al. Evaluation of the Efficacy, Safety, and Tolerability of BI 409306, a Novel Phosphodiesterase 9 Inhibitor, in Cognitive Impairment in Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled, Phase II Trial. Schizophr Bull (2018).

61. Moschetti V et al. The safety, tolerability and pharmacokinetics of BI 409306, a novel and potent PDE9 inhibitor: Overview of three Phase I randomised trials in healthy volunteers. Eur Neuropsychopharmacol 28, 643–655 (2018). [PubMed: 29567399]

62. Brown D, Daniels K, Pichereau S & Sand M A Phase IC Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Cognitive Outcomes of BI 409306 in Patients with Mild-to-Moderate Schizophrenia. Neurol Ther 7, 129–139 (2018). [PubMed: 29177699]

63. Wunderlich G et al. Study design and characteristics of two phase II proof-of-concept clinical trials of the pde9 inhibitor BI 409306 in early alzheimer's disease. Alzheimers Dementia: J Alzheimers Associ 12, P820–P821 (2016).

64. Grauer SM et al. Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J Pharmacol Exp Ther 331, 574–90 (2009). [PubMed: 19661377]

65. Schmidt CJ et al. Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Ther 325, 681–90 (2008). [PubMed: 18287214]

66. DeMartinis N et al. Results of a Phase 2A Proof-of-Concept Trial with a PDE10A Inhibitor in the Treatment of Acute Exacerbation of Schizophrenia. Schizophr Res 136, S262 (2012).

67. Russell DS et al. The phosphodiesterase 10 positron emission tomography tracer, [18F]MNI-659, as a novel biomarker for early Huntington disease. JAMA Neurol 71, 1520–8 (2014). [PubMed: 25322077]

68. Beaumont V et al. Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington's Disease Models. Neuron 92, 1220–1237 (2016). [PubMed: 27916455]

69. Kelly MP in Encyclopedia of Signaling Molecules (ed. Choi S) 3804–3826 (Springer International Publishing, Cham, 2018).

70. Kelly MP et al. Select 3',5'-cyclic nucleotide phosphodiesterases exhibit altered expression in the aged rodent brain. Cell Signal 26, 383–97 (2014). [PubMed: 24184653] This study directly compares expression patterns of all brain-expressed PDEs using both in situ hybridizaton and PCR in mouse and rat brains.

71. Raheem IT et al. Discovery of pyrazolopyrimidine phosphodiesterase 10A inhibitors for the treatment of schizophrenia. Bioorg Med Chem Lett 26, 126–32 (2016). [PubMed: 26602277]

72. Burgin AB et al. Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. Nat Biotechnol 28, 63–70 (2010). [PubMed: 20037581] Rare example of a PDEi that does not simply compete for the catalytic site.

73. Hagen TJ et al. Discovery of triazines as selective PDE4B versus PDE4D inhibitors. Bioorg Med Chem Lett 24, 4031–4 (2014). [PubMed: 24998378] Important example of how knowledge of biology can drive more sophisticated medicinal chemistry efforts that end up achieving more selective pharmacological targeting of a given PDE.

74. Pushpakom S et al. Drug repurposing: progress, challenges and recommendations. Nat Rev Drug Discov (2018).

75. Ledeboer A, Hutchinson MR, Watkins LR & Johnson KW Ibudilast (AV-411). A new class therapeutic candidate for neuropathic pain and opioid withdrawal syndromes. Expert Opin Investig Drugs 16, 935–50 (2007).

76. Schwenkgrub J, Zaremba M, Mirowska-Guzel D & Kurkowska-Jastrzebska I Ibudilast: a nonselective phosphodiesterase inhibitor in brain disorders. Postepy Hig Med Dosw (Online) 71, 137–148 (2017). [PubMed: 28258674]
77. DeYoung DZ et al. Safety of Intravenous Methamphetamine Administration During Ibudilast Treatment. J Clin Psychopharmacol 36, 347–54 (2016). [PubMed: 27269956]
78. Worley MJ, Swanson AN, Heinzerling KG, Roche DJ & Shoptaw S Ibudilast attenuates subjective effects of methamphetamine in a placebo-controlled inpatient study. Drug Alcohol Depend 162, 245–50 (2016). [PubMed: 26993372]
79. Cooper ZD et al. Effects of ibudilast on oxycodone-induced analgesia and subjective effects in opioid-dependent volunteers. Drug Alcohol Depend 178, 340–347 (2017). [PubMed: 28688296]
80. Ray LA et al. Development of the Neuroimmune Modulator Ibudilast for the Treatment of Alcoholism: A Randomized, Placebo-Controlled, Human Laboratory Trial. Neuropsychopharmacology 42, 1776–1788 (2017). [PubMed: 28091532]
81. Snyder GL et al. Preclinical profile of ITI-214, an inhibitor of phosphodiesterase 1, for enhancement of memory performance in rats. Psychopharmacology (Berl) 233, 3113–24 (2016). [PubMed: 27342643]
82. Li P et al. Discovery of Potent and Selective Inhibitors of Phosphodiesterase 1 for the Treatment of Cognitive Impairment Associated with Neurodegenerative and Neuropsychiatric Diseases. J Med Chem 59, 1149–64 (2016). [PubMed: 26789933]
83. Pekcec A et al. Targeting the dopamine D1 receptor or its downstream signalling by inhibiting phosphodiesterase-1 improves cognitive performance. Br J Pharmacol 175, 3021–3033 (2018). [PubMed: 29726015]
84. Dyck B et al. Discovery of Selective Phosphodiesterase 1 Inhibitors with Memory Enhancing Properties. J Med Chem 60, 3472–3483 (2017). [PubMed: 28406621]
85. Hashimoto T et al. Acute Enhancement of Cardiac Function by Phosphodiesterase Type 1 Inhibition: A Translational Study in the Dog and Rabbit. Circulation (2018).
86. Zhang C, Lueptow LM, Zhang HT, O'Donnell JM & Xu Y The Role of Phosphodiesterase-2 in Psychiatric and Neurodegenerative Disorders. Adv Neurobiol 17, 307–347 (2017). [PubMed: 28956338]
87. Mikami S et al. Discovery of a Novel Series of Pyrazolo[1,5-a]pyrimidine-Based Phosphodiesterase 2A Inhibitors Structurally Different from N-((1S)-1-(3-Fluoro-4-(trifluoromethoxy)phenyl)-2-methoxyethyl)-7-methoxy-2-oxo-2, 3-dihydropyrido[2,3-b]pyrazine-4(1H)-carboxamide (TAK-915), for the Treatment of Cognitive Disorders. Chem Pharm Bull (Tokyo) 65, 1058–1077 (2017). [PubMed: 29093293]
88. Mikami S et al. Discovery of Clinical Candidate N-((1S)-1-(3-Fluoro-4-(trifluoromethoxy)phenyl)-2-methoxyethyl)-7-methoxy-2-oxo-2, 3-dihydropyrido[2,3-b]pyrazine-4(1H)-carboxamide (TAK-915): A Highly Potent, Selective, and Brain-Penetrating Phosphodiesterase 2A Inhibitor for the Treatment of Cognitive Disorders. J Med Chem 60, 7677–7702 (2017). [PubMed: 28796496]
89. Mikami S et al. Discovery of an Orally Bioavailable, Brain-Penetrating, in Vivo Active Phosphodiesterase 2A Inhibitor Lead Series for the Treatment of Cognitive Disorders. J Med Chem 60, 7658–7676 (2017). [PubMed: 28759228]
90. Weber S et al. PDE2 at the crossway between cAMP and cGMP signalling in the heart. Cell Signal 38, 76–84 (2017). [PubMed: 28668721]
91. Zhang C et al. The roles of phosphodiesterase 2 in the central nervous and peripheral systems. Curr Pharm Des 21, 274–90 (2015). [PubMed: 25159070]
92. Bundhun PK, Qin T & Chen MH Comparing the effectiveness and safety between triple antiplatelet therapy and dual antiplatelet therapy in type 2 diabetes mellitus patients after coronary stents implantation: a systematic review and meta-analysis of randomized controlled trials. BMC Cardiovasc Disord 15, 118 (2015). [PubMed: 26450578]
93. Neel JD, Kruse RL, Dombrovskiy VY & Vogel TR Cilostazol and freedom from amputation after lower extremity revascularization. J Vasc Surg 61, 960–4 (2015). [PubMed: 25595396]

94. Lim HW et al. Effect of a 4-Week Treatment with Cilostazol in Patients with Chronic Tinnitus: A Randomized, Prospective, Placebo-controlled, Double-blind, Pilot Study. J Int Adv Otol 12, 170–176 (2016). [PubMed: 27716604]

95. Lakics V, Karran EH & Boess FG Quantitative comparison of phosphodiesterase mRNA distribution in human brain and peripheral tissues. Neuropharmacology 59, 367–74 (2010). [PubMed: 20493887] Head to head comparison of PDE expression patterns in a variety of human tissues.

96. Mochizuki Y, Oishi M & Mizutani T Effects of cilostazol on cerebral blood flow, P300, and serum lipid levels in the chronic stage of cerebral infarction. J Stroke Cerebrovasc Dis 10, 63–9 (2001). [PubMed: 17903802]

97. Jung KI, Kim JH, Park HY & Park CK Neuroprotective effects of cilostazol on retinal ganglion cell damage in diabetic rats. J Pharmacol Exp Ther 345, 457–63 (2013). [PubMed: 23536314]

98. Masciarelli S et al. Cyclic nucleotide phosphodiesterase 3A-deficient mice as a model of female infertility. J Clin Invest 114, 196–205 (2004). [PubMed: 15254586]

99. Taiyeb AM et al. Cilostazol blocks pregnancy in naturally cycling swine: An animal model. Life Sci 142, 92–6 (2015). [PubMed: 26477292]

100. Taiyeb AM, Muhsen-Alanssari SA, Dees WL, Ridha-Albarzanchi MT & Kraemer DC Improvement in in vitro fertilization outcome following in vivo synchronization of oocyte maturation in mice. Exp Biol Med (Maywood) 240, 519–26 (2015). [PubMed: 25245076]

101. Duan LM, Yu HY, Li YL & Jia CJ Design and discovery of 2-(4-(1H-tetrazol-5-yl)-1H-pyrazol-1-yl)-4-(4-phenyl)thiazole derivatives as cardiotonic agents via inhibition of PDE3. Bioorg Med Chem 23, 6111–7 (2015). [PubMed: 26319621]

102. Kim KY, Lee H, Yoo SE, Kim SH & Kang NS Discovery of new inhibitor for PDE3 by virtual screening. Bioorg Med Chem Lett 21, 1617–20 (2011). [PubMed: 21330134]

103. Nikpour M et al. Design, synthesis and biological evaluation of 6-(benzyloxy)-4-methylquinolin-2(1H)-one derivatives as PDE3 inhibitors. Bioorg Med Chem 18, 855–62 (2010). [PubMed: 20005117]

104. Huff SB & Gottwald LD Repigmentation of Tenacious Vitiligo on Apremilast. Case Rep Dermatol Med 2017, 2386234 (2017). [PubMed: 29234546]

105. AbuHilal M, Walsh S & Shear N Treatment of recalcitrant erosive oral lichen planus and desquamative gingivitis with oral apremilast. J Dermatol Case Rep 10, 56–57 (2016). [PubMed: 28400896]

106. Hafner J et al. Apremilast Is Effective in Lichen Planus Mucosae-Associated Stenotic Esophagitis. Case Rep Dermatol 8, 224–226 (2016). [PubMed: 27721755]

107. Bettencourt M Oral Lichen Planus Treated With Apremilast. J Drugs Dermatol 15, 1026–8 (2016). [PubMed: 27538007]

108. Jensterle M, Kocjan T & Janez A Phosphodiesterase 4 inhibition as a potential new therapeutic target in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 99, E1476–81 (2014). [PubMed: 24823465]

109. Jensterle M, Salamun V, Kocjan T, Vrtacnik Bokal E & Janez A Short term monotherapy with GLP-1 receptor agonist liraglutide or PDE 4 inhibitor roflumilast is superior to metformin in weight loss in obese PCOS women: a pilot randomized study. J Ovarian Res 8, 32 (2015). [PubMed: 26032655]

110. Plock N, Vollert S, Mayer M, Hanauer G & Lahu G Pharmacokinetic/Pharmacodynamic Modeling of the PDE4 Inhibitor TAK-648 in Type 2 Diabetes: Early Translational Approaches for Human Dose Prediction. Clin Transl Sci 10, 185–193 (2017). [PubMed: 28088839]

111. Van Duinen MA et al. Acute administration of roflumilast enhances immediate recall of verbal word memory in healthy young adults. Neuropharmacology 131, 31–38 (2018). [PubMed: 29241652] One of the first studies to demonstrate a pro-cognitive effect in humans of a commerically-avaible PDE4i.

112. Gilleen J et al. An experimental medicine study of the phosphodiesterase-4 inhibitor, roflumilast, on working memory-related brain activity and episodic memory in schizophrenia patients. Psychopharmacology (Berl) (2018).

113. Blokland A et al. Acute treatment with the PDE4 inhibitor roflumilast improves verbal word memory in healthy old individuals: a double-blind placebo-controlled study. Neurobiol Aging 77, 37–43 (2019). [PubMed: 30776650]

114. Kanes SJ et al. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. Neuroscience 144, 239–46 (2007). [PubMed: 17081698]

115. Kelly MP et al. Constitutive activation of Galphas within forebrain neurons causes deficits in sensorimotor gating because of PKA-dependent decreases in cAMP. Neuropsychopharmacology 32, 577–88 (2007). [PubMed: 16738544] Important study showing that chronic signaling through Galphas will lead to PKA-dependent compensatry increases in PDE activity in some brain regions but not others.

116. Kelly MP et al. Developmental etiology for neuroanatomical and cognitive deficits in mice overexpressing Galphas, a G-protein subunit genetically linked to schizophrenia. Mol Psychiatry 14, 398–415, 347 (2009). [PubMed: 19030002]

117. Rodefer JS, Saland SK & Eckrich SJ Selective phosphodiesterase inhibitors improve performance on the ED/ID cognitive task in rats. Neuropharmacology 62, 1182–90 (2012). [PubMed: 21856317]

118. MacDonald E et al. A novel phosphodiesterase type 4 inhibitor, HT-0712, enhances rehabilitation-dependent motor recovery and cortical reorganization after focal cortical ischemia. Neurorehabil Neural Repair 21, 486–96 (2007). [PubMed: 17823313]

119. Peters M et al. The PDE4 inhibitor HT-0712 improves hippocampus-dependent memory in aged mice. Neuropsychopharmacology 39, 2938–48 (2014). [PubMed: 24964813]

120. Gurney ME, Cogram P, Deacon RM, Rex C & Tranfaglia M Multiple Behavior Phenotypes of the Fragile-X Syndrome Mouse Model Respond to Chronic Inhibition of Phosphodiesterase-4D (PDE4D). Sci Rep 7, 14653 (2017). [PubMed: 29116166]

121. Zhang C et al. Memory enhancing effects of BPN14770, an allosteric inhibitor of phosphodiesterase-4D, in wild-type and humanized mice. Neuropsychopharmacology 43, 2299–2309 (2018). [PubMed: 30131563]

122. Rutter AR et al. GSK356278, a potent, selective, brain-penetrant phosphodiesterase 4 inhibitor that demonstrates anxiolytic and cognition-enhancing effects without inducing side effects in preclinical species. J Pharmacol Exp Ther 350, 153–63 (2014). [PubMed: 24784567]

123. Munshi A & Das S Genetic Understanding of Stroke Treatment: Potential Role for Phosphodiesterase Inhibitors. Adv Neurobiol 17, 445–461 (2017). [PubMed: 28956342]

124. Chen J et al. The phosphodiesterase-4 inhibitor, FCPR16, attenuates ischemia-reperfusion injury in rats subjected to middle cerebral artery occlusion and reperfusion. Brain Res Bull 137, 98–106 (2018). [PubMed: 29155261]

125. Soares LM et al. Rolipram improves cognition, reduces anxiety- and despair-like behaviors and impacts hippocampal neuroplasticity after transient global cerebral ischemia. Neuroscience 326, 69–83 (2016). [PubMed: 27058148]

126. Li LX et al. Prevention of cerebral ischemia-induced memory deficits by inhibition of phosphodiesterase-4 in rats. Metab Brain Dis 26, 37–47 (2011). [PubMed: 21327879]

127. Titus DJ et al. A negative allosteric modulator of PDE4D enhances learning after traumatic brain injury. Neurobiol Learn Mem 148, 38–49 (2018). [PubMed: 29294383]

128. Knott EP, Assi M, Rao SN, Ghosh M & Pearse DD Phosphodiesterase Inhibitors as a Therapeutic Approach to Neuroprotection and Repair. Int J Mol Sci 18 (2017).

129. Olsen CM & Liu QS Phosphodiesterase 4 inhibitors and drugs of abuse: current knowledge and therapeutic opportunities. Front Biol (Beijing) 11, 376–386 (2016). [PubMed: 28974957]

130. Logrip ML Phosphodiesterase regulation of alcohol drinking in rodents. Alcohol 49, 795–802 (2015). [PubMed: 26095589]

131. Avila DV et al. Dysregulation of hepatic cAMP levels via altered Pde4b expression plays a critical role in alcohol-induced steatosis. J Pathol 240, 96–107 (2016). [PubMed: 27287961]

132. Avila DV et al. Phosphodiesterase 4b expression plays a major role in alcohol-induced neuro-inflammation. Neuropharmacology 125, 376–385 (2017). [PubMed: 28807677]

133. Favilla C, Abel T & Kelly MP Chronic Galphas signaling in the striatum increases anxiety-related behaviors independent of developmental effects. J Neurosci 28, 13952–6 (2008). [PubMed: 19091983]

134. Kelly MP et al. Constitutive activation of the G-protein subunit Galphas within forebrain neurons causes PKA-dependent alterations in fear conditioning and cortical Arc mRNA expression. Learn Mem 15, 75–83 (2008). [PubMed: 18230676]

135. Cooney JD & Aguiar RC Phosphodiesterase 4 inhibitors have wide-ranging activity in B-cell malignancies. Blood 128, 2886–2890 (2016). [PubMed: 27756749]

136. Wang W et al. Triple negative breast cancer development can be selectively suppressed by sustaining an elevated level of cellular cyclic AMP through simultaneously blocking its efflux and decomposition. Oncotarget 7, 87232–87245 (2016). [PubMed: 27901486]

137. Nishi K et al. Apremilast Induces Apoptosis of Human Colorectal Cancer Cells with Mutant KRAS. Anticancer Res 37, 3833–3839 (2017). [PubMed: 28668883]

138. Mishra RR et al. Reactivation of cAMP Pathway by PDE4D Inhibition Represents a Novel Druggable Axis for Overcoming Tamoxifen Resistance in ER-positive Breast Cancer. Clin Cancer Res 24, 1987–2001 (2018). [PubMed: 29386221]

139. Wortsman X, Del Barrio-Diaz P, Meza-Romero R, Poehls-Risco C & Vera-Kellet C Nifedipine cream versus sildenafil cream for patients with secondary Raynaud phenomenon: A randomized, double-blind, controlled pilot study. J Am Acad Dermatol 78, 189–190 (2018). [PubMed: 29241781]

140. Maged M, Wageh A, Shams M & Elmetwally A Use of sildenafil citrate in cases of intrauterine growth restriction (IUGR); a prospective trial. Taiwan J Obstet Gynecol 57, 483–486 (2018). [PubMed: 30122565]

141. Pels A et al. STRIDER (Sildenafil TheRapy in dismal prognosis early onset fetal growth restriction): an international consortium of randomised placebo-controlled trials. BMC Pregnancy Childbirth 17, 440 (2017). [PubMed: 29282009]

142. El-Sayed MA, Saleh SA, Maher MA & Khidre AM Utero-placental perfusion Doppler indices in growth restricted fetuses: effect of sildenafil citrate. J Matern Fetal Neonatal Med 31, 1045–1050 (2018). [PubMed: 28345432]

143. Paauw ND et al. Sildenafil During Pregnancy: A Preclinical Meta-Analysis on Fetal Growth and Maternal Blood Pressure. Hypertension 70, 998–1006 (2017). [PubMed: 28893896]

144. Aversa A et al. Tadalafil improves lean mass and endothelial function in nonobese men with mild ED/LUTS: in vivo and in vitro characterization. Endocrine 56, 639–648 (2017). [PubMed: 28133708]

145. Giannetta E et al. Chronic Inhibition of cGMP phosphodiesterase 5A improves diabetic cardiomyopathy: a randomized, controlled clinical trial using magnetic resonance imaging with myocardial tagging. Circulation 125, 2323–33 (2012). [PubMed: 22496161]

146. Di Luigi L et al. Phosphodiesterase Type 5 Inhibitor Sildenafil Decreases the Proinflammatory Chemokine CXCL10 in Human Cardiomyocytes and in Subjects with Diabetic Cardiomyopathy. Inflammation 39, 1238–52 (2016). [PubMed: 27165639]

147. Fiore D et al. PDE5 Inhibition Ameliorates Visceral Adiposity Targeting the miR-22/SIRT1 Pathway: Evidence From the CECSID Trial. J Clin Endocrinol Metab 101, 1525–34 (2016). [PubMed: 26964730]

148. Mandosi E et al. Endothelial dysfunction markers as a therapeutic target for Sildenafil treatment and effects on metabolic control in type 2 diabetes. Expert Opin Ther Targets 19, 1617–22 (2015). [PubMed: 26178526]

149. Santi D et al. Could chronic Vardenafil administration influence the cardiovascular risk in men with type 2 diabetes mellitus? PLoS One 13, e0199299 (2018). [PubMed: 29953477]

150. Montes Cardona CE & Garcia-Perdomo HA Efficacy of phosphodiesterase type 5 inhibitors for the treatment of distal ureteral calculi: A systematic review and meta-analysis. Investig Clin Urol 58, 82–89 (2017).

151. Booth L et al. Neratinib augments the lethality of [regorafenib + sildenafil]. J Cell Physiol (2018).

152. Islam BN et al. Sildenafil Suppresses Inflammation-Driven Colorectal Cancer in Mice. Cancer Prev Res (Phila) 10, 377–388 (2017). [PubMed: 28468928]

153. Califano JA et al. Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. Clin Cancer Res 21, 30–8 (2015). [PubMed: 25564570]

154. Weed DT et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. Clin Cancer Res 21, 39–48 (2015). [PubMed: 25320361]

155. Charych EI, Jiang LX, Lo F, Sullivan K & Brandon NJ Interplay of palmitoylation and phosphorylation in the trafficking and localization of phosphodiesterase 10A: implications for the treatment of schizophrenia. J Neurosci 30, 9027–37 (2010). [PubMed: 20610737] Key finding showing 2 post-translational modifications can compete to regulate the subcellular compartmentalization of a PDE.

156. Lee K et al. beta-catenin nuclear translocation in colorectal cancer cells is suppressed by PDE10A inhibition, cGMP elevation, and activation of PKG. Oncotarget 7, 5353–65 (2016). [PubMed: 26713600]

157. Li N et al. Phosphodiesterase 10A: a novel target for selective inhibition of colon tumor cell growth and beta-catenin-dependent TCF transcriptional activity. Oncogene 34, 1499–509 (2015). [PubMed: 24704829]

158. Barone I, Giordano C, Bonofiglio D, Ando S & Catalano S Phosphodiesterase type 5 and cancers: progress and challenges. Oncotarget 8, 99179–99202 (2017). [PubMed: 29228762]

159. Aoun F et al. Association between phosphodiesterase type 5 inhibitors and prostate cancer: A systematic review. Prog Urol 28, 560–566 (2018). [PubMed: 30201551]

160. Arozarena I et al. Oncogenic BRAF induces melanoma cell invasion by downregulating the cGMP-specific phosphodiesterase PDE5A. Cancer Cell 19, 45–57 (2011). [PubMed: 21215707]

161. Feng S et al. Are phosphodiesterase type 5 inhibitors associated with increased risk of melanoma?: A systematic review and meta-analysis. Medicine (Baltimore) 97, e9601 (2018). [PubMed: 29504984] Important reminder that attention must be paid not only to acute side effect profiles, but also long-term risks of PDEi treatment.

162. Almeida CB et al. Hydroxyurea and a cGMP-amplifying agent have immediate benefits on acute vaso-occlusive events in sickle cell disease mice. Blood 120, 2879–88 (2012). [PubMed: 22833547]

163. Goldsmith P et al. A Randomized Multiple Dose Pharmacokinetic Study of a Novel PDE10A Inhibitor TAK-063 in Subjects with Stable Schizophrenia and Japanese Subjects and Modeling of Exposure Relationships to Adverse Events. Drugs R D 17, 631–643 (2017). [PubMed: 29103081]

164. Macek TA et al. A phase 2, randomized, placebo-controlled study of the efficacy and safety of TAK-063 in subjects with an acute exacerbation of schizophrenia. Schizophr Res (2018).

165. Suzuki K, Harada A, Suzuki H, Miyamoto M & Kimura H TAK-063, a PDE10A Inhibitor with Balanced Activation of Direct and Indirect Pathways, Provides Potent Antipsychotic-Like Effects in Multiple Paradigms. Neuropsychopharmacology 41, 2252–62 (2016). [PubMed: 26849714]

166. Wilson JM et al. Phosphodiesterase 10A inhibitor, MP-10 (PF-2545920), produces greater induction of c-Fos in dopamine D2 neurons than in D1 neurons in the neostriatum. Neuropharmacology 99, 379–86 (2015). [PubMed: 26256420]

167. Padovan-Neto FE & West AR Regulation of Striatal Neuron Activity by Cyclic Nucleotide Signaling and Phosphodiesterase Inhibition: Implications for the Treatment of Parkinson's Disease. Adv Neurobiol 17, 257–283 (2017). [PubMed: 28956336]

168. Jankowska A, Swierczek A, Chlon-Rzepa G, Pawlowski M & Wyska E PDE7-Selective and Dual Inhibitors: Advances in Chemical and Biological Research. Curr Med Chem 24, 673–700 (2017). [PubMed: 28093982]

169. Redondo M et al. Effect of phosphodiesterase 7 (PDE7) inhibitors in experimental autoimmune encephalomyelitis mice. Discovery of a new chemically diverse family of compounds. J Med Chem 55, 3274–84 (2012). [PubMed: 22385507]

170. Morales-Garcia JA et al. Silencing phosphodiesterase 7B gene by lentiviral-shRNA interference attenuates neurodegeneration and motor deficits in hemiparkinsonian mice. Neurobiol Aging 36, 1160–73 (2015). [PubMed: 25457552]

171. Morales-Garcia JA et al. Phosphodiesterase7 Inhibition Activates Adult Neurogenesis in Hippocampus and Subventricular Zone In Vitro and In Vivo. Stem Cells 35, 458–472 (2017). [PubMed: 27538853]

172. Tsai LC et al. Inactivation of Pde8b enhances memory, motor performance, and protects against age-induced motor coordination decay. Genes Brain Behav 11, 837–47 (2012). [PubMed: 22925203]

173. Pilarzyk K et al. Loss of function of phosphodiesterase 11A4 shows that recent and remote long term memories can be uncoupled. Curr Biol (accepted).

174. Pathak G et al. PDE11A negatively regulates lithium responsivity. Mol Psychiatry 22, 1714–1724 (2017). [PubMed: 27646265] This study not only identifies PDE homodimerziation as a molecular mechanism regulating PDE protein stability and subcellular compartmentalization, it also provides proof of principle for how homodimerization could be therapeutically targeted using a biologic.

175. Mertens J et al. Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. Nature 527, 95–9 (2015). [PubMed: 26524527]

176. Dong H, Zitt C, Auriga C, Hatzelmann A & Epstein PM Inhibition of PDE3, PDE4 and PDE7 potentiates glucocorticoid-induced apoptosis and overcomes glucocorticoid resistance in CEM T leukemic cells. Biochem Pharmacol 79, 321–9 (2010). [PubMed: 19737543]

177. de Medeiros AS et al. Identification and characterization of a potent and biologically-active PDE4/7 inhibitor via fission yeast-based assays. Cell Signal 40, 73–80 (2017). [PubMed: 28867658] This study reports a novel yeast-based assay for screening PDE modulators, which not only reads out changes in PDE activity but also proves compounds are cell permeable, are chemically-stable, and do not exhibit wide-spread protein binding.

178. Tsai LC, Shimizu-Albergine M & Beavo JA The high-affinity cAMP-specific phosphodiesterase 8B controls steroidogenesis in the mouse adrenal gland. Mol Pharmacol 79, 639–48 (2011). [PubMed: 21187369]

179. Shimizu-Albergine M, Tsai LC, Patrucco E & Beavo JA cAMP-specific phosphodiesterases 8A and 8B, essential regulators of Leydig cell steroidogenesis. Mol Pharmacol 81, 556–66 (2012). [PubMed: 22232524]

180. Jang IS et al. Lysophosphatidic acid-induced changes in cAMP profiles in young and senescent human fibroblasts as a clue to the ageing process. Mech Ageing Dev 127, 481–9 (2006). [PubMed: 16516270]

181. Ramos BP et al. Dysregulation of protein kinase a signaling in the aged prefrontal cortex: new strategy for treating age-related cognitive decline. Neuron 40, 835–45 (2003). [PubMed: 14622586]

182. Giralt A et al. Increased PKA signaling disrupts recognition memory and spatial memory: role in Huntington's disease. Hum Mol Genet 20, 4232–47 (2011). [PubMed: 21835884]

183. Hegde S et al. PDE11A regulates social behaviors and is a key mechanism by which social experience sculpts the brain. Neuroscience 335, 151–69 (2016). [PubMed: 27544407]

184. Guo S, Olesen J & Ashina M Phosphodiesterase 3 inhibitor cilostazol induces migraine-like attacks via cyclic AMP increase. Brain 137, 2951–9 (2014). [PubMed: 25161294]

185. Holland PR & Strother L Cilostazol as a chemically induced preclinical model of migraine. Cephalalgia 38, 415–416 (2018). [PubMed: 28952338]

186. Khan S, Deen M, Hougaard A, Amin FM & Ashina M Reproducibility of migraine-like attacks induced by phosphodiesterase-3-inhibitor cilostazol. Cephalalgia 38, 892–903 (2018). [PubMed: 28677994]

187. Hansen EK, Guo S, Ashina M & Olesen J Toward a pragmatic migraine model for drug testing: I. Cilostazol in healthy volunteers. Cephalalgia 36, 172–8 (2016). [PubMed: 25934317]

188. Birk S, Kruuse C, Petersen KA, Tfelt-Hansen P & Olesen J The headache-inducing effect of cilostazol in human volunteers. Cephalalgia 26, 1304–9 (2006). [PubMed: 17059437]

189. Mowat FM et al. Gene Therapy in a Large Animal Model of PDE6A-Retinitis Pigmentosa. Front Neurosci 11, 342 (2017). [PubMed: 28676737]

190. Henderson DJ et al. The cAMP phosphodiesterase-4D7 (PDE4D7) is downregulated in androgen-independent prostate cancer cells and mediates proliferation by compartmentalising cAMP at the

plasma membrane of VCaP prostate cancer cells. Br J Cancer 110, 1278–87 (2014). [PubMed: 24518597]

191. Abi-Gerges A et al. Decreased expression and activity of cAMP phosphodiesterases in cardiac hypertrophy and its impact on beta-adrenergic cAMP signals. Circ Res 105, 784–92 (2009). [PubMed: 19745166]
192. Wang X, Ward CJ, Harris PC & Torres VE Cyclic nucleotide signaling in polycystic kidney disease. Kidney Int 77, 129–40 (2010). [PubMed: 19924104]
193. Malekshahabi T, Khoshdel Rad N, Serra AL & Moghadasali R Autosomal dominant polycystic kidney disease: Disrupted pathways and potential therapeutic interventions. J Cell Physiol 234, 12451–12470 (2019). [PubMed: 30644092]
194. Zoraghi R, Corbin JD & Francis SH Properties and functions of GAF domains in cyclic nucleotide phosphodiesterases and other proteins. Mol Pharmacol 65, 267–78 (2004). [PubMed: 14742667]
195. Schultz JE Structural and biochemical aspects of tandem GAF domains. Handb Exp Pharmacol, 93–109 (2009). [PubMed: 19089327]
196. Francis SH, Blount MA & Corbin JD Mammalian cyclic nucleotide phosphodiesterases: molecular mechanisms and physiological functions. Physiol Rev 91, 651–90 (2011). [PubMed: 21527734]
197. Jager R, Schwede F, Genieser HG, Koesling D & Russwurm M Activation of PDE2 and PDE5 by specific GAF ligands: delayed activation of PDE5. Br J Pharmacol 161, 1645–60 (2010). [PubMed: 20698857]
198. Martinez SE et al. The two GAF domains in phosphodiesterase 2A have distinct roles in dimerization and in cGMP binding. Proc Natl Acad Sci U S A 99, 13260–5 (2002). [PubMed: 12271124]
199. Beavo JA, Hardman JG & Sutherland EW Stimulation of adenosine 3',5'-monophosphate hydrolysis by guanosine 3',5'-monophosphate. J Biol Chem 246, 3841–6 (1971). [PubMed: 4327188]
200. Jager R et al. Activation of PDE10 and PDE11 phosphodiesterases. J Biol Chem 287, 1210–9 (2012). [PubMed: 22105073] First demonstration that PDEs can be pharmacologically activated by a molecule other than cAMP or cGMP itself (i.e., PDE11A can be activated by a cGMP analog binding the GAF-A domain).
201. D'Amours MR & Cote RH Regulation of photoreceptor phosphodiesterase catalysis by its non-catalytic cGMP-binding sites. Biochem J 340 (Pt 3), 863–9 (1999). [PubMed: 10359674]
202. Schultz JE, Dunkern T, Gawlitta-Gorka E & Sorg G The GAF-tandem domain of phosphodiesterase 5 as a potential drug target. Handb Exp Pharmacol, 151–66 (2011).
203. Kelly MP Does phosphodiesterase 11A (PDE11A) hold promise as a future therapeutic target? Curr Pharm Des 21, 389–416 (2015). [PubMed: 25159071] This review introduces the unique idea that it may be possible to therapeutically target dual-specificty PDEs in a functionally selective manner (i.e., target only their cAMP or cGMP hydrolytic activity).
204. Murthy KS, Zhou H & Makhlouf GM PKA-dependent activation of PDE3A and PDE4 and inhibition of adenylyl cyclase V/VI in smooth muscle. Am J Physiol Cell Physiol 282, C508–17 (2002). [PubMed: 11832336]
205. Manganiello VC, Taira M, Degerman E & Belfrage P Type III cGMP-inhibited cyclic nucleotide phosphodiesterases (PDE3 gene family). Cell Signal 7, 445–55 (1995). [PubMed: 8562305]
206. MacKenzie SJ et al. Long PDE4 cAMP specific phosphodiesterases are activated by protein kinase A-mediated phosphorylation of a single serine residue in Upstream Conserved Region 1 (UCR1). Br J Pharmacol 136, 421–33 (2002). [PubMed: 12023945]
207. Corbin JD, Turko IV, Beasley A & Francis SH Phosphorylation of phosphodiesterase-5 by cyclic nucleotide-dependent protein kinase alters its catalytic and allosteric cGMP-binding activities. Eur J Biochem 267, 2760–7 (2000). [PubMed: 10785399] One of the first studies to identify phosphorylation as a post-translational modification capable of changing PDE catalytic activity.
208. Brown KM, Lee LC, Findlay JE, Day JP & Baillie GS Cyclic AMP-specific phosphodiesterase, PDE8A1, is activated by protein kinase A-mediated phosphorylation. FEBS Lett 586, 1631–7 (2012). [PubMed: 22673573]

209. Jacobitz S, McLaughlin MM, Livi GP, Burman M & Torphy TJ Mapping the functional domains of human recombinant phosphodiesterase 4A: structural requirements for catalytic activity and rolipram binding. Mol Pharmacol 50, 891–9 (1996). [PubMed: 8863835]

210. Marcoz P, Nemoz G, Prigent AF & Lagarde M Phosphatidic acid stimulates the rolipram-sensitive cyclic nucleotide phosphodiesterase from rat thymocytes. Biochim Biophys Acta 1176, 129–36 (1993). [PubMed: 8452869]

211. Norambuena A et al. Phosphatidic acid induces ligand-independent epidermal growth factor receptor endocytic traffic through PDE4 activation. Mol Biol Cell 21, 2916–29 (2010). [PubMed: 20554760]

212. Grange M et al. The cAMP-specific phosphodiesterase PDE4D3 is regulated by phosphatidic acid binding. Consequences for cAMP signaling pathway and characterization of a phosphatidic acid binding site. J Biol Chem 275, 33379–87 (2000). [PubMed: 10938092]

213. Wang L et al. UCR1C is a novel activator of phosphodiesterase 4 (PDE4) long isoforms and attenuates cardiomyocyte hypertrophy. Cell Signal 27, 908–22 (2015). [PubMed: 25683917]

214. Guo LW et al. The inhibitory gamma subunit of the rod cGMP phosphodiesterase binds the catalytic subunits in an extended linear structure. J Biol Chem 281, 15412–22 (2006). [PubMed: 16595671]

215. Zhang XJ, Skiba NP & Cote RH Structural requirements of the photoreceptor phosphodiesterase gamma-subunit for inhibition of rod PDE6 holoenzyme and for its activation by transducin. J Biol Chem 285, 4455–63 (2010). [PubMed: 19948718]

216. Slep KC et al. Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 A. Nature 409, 1071–7 (2001). [PubMed: 11234020]

217. Zhang Z et al. Domain organization and conformational plasticity of the G protein effector, PDE6. J Biol Chem 290, 12833–43 (2015). [PubMed: 25809480]

218. Lynch MJ et al. RNA silencing identifies PDE4D5 as the functionally relevant cAMP phosphodiesterase interacting with beta arrestin to control the protein kinase A/AKAP79-mediated switching of the beta2-adrenergic receptor to activation of ERK in HEK293B2 cells. J Biol Chem 280, 33178–89 (2005). [PubMed: 16030021]

219. Ren L et al. MiR-541–5p regulates lung fibrosis by targeting cyclic nucleotide phosphodiesterase 1A. Exp Lung Res 43, 249–258 (2017). [PubMed: 28816543]

220. Zhang DD, Li Y, Xu Y, Kim J & Huang S Phosphodiesterase 7B/microRNA-200c relationship regulates triple-negative breast cancer cell growth. Oncogene (2018).

221. Yang G et al. Phosphodiesterase 7A-deficient mice have functional T cells. J Immunol 171, 6414–20 (2003). [PubMed: 14662840]

222. Ding B et al. Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure. Circulation 111, 2469–2476 (2005). [PubMed: 15867171]

223. Wu WH et al. CRISPR Repair Reveals Causative Mutation in a Preclinical Model of Retinitis Pigmentosa. Mol Ther 24, 1388–94 (2016). [PubMed: 27203441] Proof-of-concept study showing how gene editing might be used to therapeutically target a PDE.

224. Wensel TG et al. Structural and molecular bases of rod photoreceptor morphogenesis and disease. Prog Retin Eye Res 55, 32–51 (2016). [PubMed: 27352937]

225. Deng WT et al. Cone phosphodiesterase-6alpha' restores rod function and confers distinct physiological properties in the rod phosphodiesterase-6beta-deficient rd10 mouse. J Neurosci 33, 11745–53 (2013). [PubMed: 23864662]

226. Pang JJ et al. AAV-mediated gene therapy for retinal degeneration in the rd10 mouse containing a recessive PDEbeta mutation. Invest Ophthalmol Vis Sci 49, 4278–83 (2008). [PubMed: 18586879] One of the first studies showing viral constructs can be used to therapeutically target a PDE.

227. Wert KJ, Davis RJ, Sancho-Pelluz J, Nishina PM & Tsang SH Gene therapy provides long-term visual function in a pre-clinical model of retinitis pigmentosa. Hum Mol Genet 22, 558–67 (2013). [PubMed: 23108158]

228. Deng WT et al. Cone Phosphodiesterase-6gamma' Subunit Augments Cone PDE6 Holoenzyme Assembly and Stability in a Mouse Model Lacking Both Rod and Cone PDE6 Catalytic Subunits. Front Mol Neurosci 11, 233 (2018). [PubMed: 30038560]

229. Carvalho LS et al. Synthetic Adeno-Associated Viral Vector Efficiently Targets Mouse and Nonhuman Primate Retina In Vivo. Hum Gene Ther 29, 771–784 (2018). [PubMed: 29325457]

230. Ye H & Fussenegger M Optogenetic Medicine: Synthetic Therapeutic Solutions Precision-Guided by Light. Cold Spring Harb Perspect Med (2018).

231. Yoshida K, Tsunoda SP, Brown LS & Kandori H A unique choanoflagellate enzyme rhodopsin exhibits light-dependent cyclic nucleotide phosphodiesterase activity. J Biol Chem 292, 7531–7541 (2017). [PubMed: 28302718] One of the first studies to identify a light-dependent PDE with potential utility for optogenetic applications.

232. Lamarche LB et al. Purification and Characterization of RhoPDE, a Retinylidene/Phosphodiesterase Fusion Protein and Potential Optogenetic Tool from the Choanoflagellate Salpingoeca rosetta. Biochemistry 56, 5812–5822 (2017). [PubMed: 28976747] One of the first studies to identify a light-dependent PDE with potential utility for optogenetic applications

233. Tian Y, Gao S, Yang S & Nagel G A novel rhodopsin phosphodiesterase from Salpingoeca rosetta shows light-enhanced substrate affinity. Biochem J 475, 1121–1128 (2018). [PubMed: 29483295]

234. Gasser C et al. Engineering of a red-light-activated human cAMP/cGMP-specific phosphodiesterase. Proc Natl Acad Sci U S A 111, 8803–8 (2014). [PubMed: 24889611]

235. Kelly MP Cyclic nucleotide signaling changes associated with normal aging and age-related diseases of the brain. Cell Signal 42, 281–291 (2018). [PubMed: 29175000] This review underscores the fact that any targeting of PDE activity in the context of age-related disease will likely need to be done in a tissue-specific manner, as some tissues see increased cyclic nucleotide signaling with age awhile others see decreased signaling.

236. Fertig BA & Baillie GS PDE4-Mediated cAMP Signalling. J Cardiovasc Dev Dis 5 (2018).

237. McCahill A et al. In resting COS1 cells a dominant negative approach shows that specific, anchored PDE4 cAMP phosphodiesterase isoforms gate the activation, by basal cyclic AMP production, of AKAP-tethered protein kinase A type II located in the centrosomal region. Cell Signal 17, 1158–73 (2005). [PubMed: 15905070]

238. Bolger GB et al. Scanning peptide array analyses identify overlapping binding sites for the signalling scaffold proteins, beta-arrestin and RACK1, in cAMP-specific phosphodiesterase PDE4D5. Biochem J 398, 23–36 (2006). [PubMed: 16689683] First report of a key technique in the field (i.e., the scanning peptide array) that has been successfully used to identiy many PDE protein-protein binding partners.

239. Baillie GS et al. beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from Gs to Gi. Proc Natl Acad Sci U S A 100, 940–5 (2003). [PubMed: 12552097] Seminal finding showing how PDEs can trigger negative feedback loops upstream (i.e., by making a receptor switch from Gs to Gi signaling).

240. Willoughby D et al. Dynamic regulation, desensitization, and cross-talk in discrete subcellular microdomains during beta2-adrenoceptor and prostanoid receptor cAMP signaling. J Biol Chem 282, 34235–49 (2007). [PubMed: 17855344]

241. Ong WK et al. The role of the PDE4D cAMP phosphodiesterase in the regulation of glucagon-like peptide-1 release. Br J Pharmacol 157, 633–44 (2009). [PubMed: 19371330]

242. Terrin A et al. PKA and PDE4D3 anchoring to AKAP9 provides distinct regulation of cAMP signals at the centrosome. J Cell Biol 198, 607–21 (2012). [PubMed: 22908311]

243. Vecsey CG et al. Sleep deprivation impairs cAMP signalling in the hippocampus. Nature 461, 1122–5 (2009). [PubMed: 19847264]

244. Havekes R et al. Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. Elife 5 (2016).

245. Havekes R et al. Compartmentalized PDE4A5 Signaling Impairs Hippocampal Synaptic Plasticity and Long-Term Memory. J Neurosci 36, 8936–46 (2016). [PubMed: 27559174]

246. Campbell SL, van Groen T, Kadish I, Smoot LHM & Bolger GB Altered phosphorylation, electrophysiology, and behavior on attenuation of PDE4B action in hippocampus. BMC Neurosci 18, 77 (2017). [PubMed: 29197324]

247. McGirr A et al. Specific Inhibition of Phosphodiesterase-4B Results in Anxiolysis and Facilitates Memory Acquisition. Neuropsychopharmacology 41, 1080–92 (2016). [PubMed: 26272049]

248. Johnston LA et al. Expression, intracellular distribution and basis for lack of catalytic activity of the PDE4A7 isoform encoded by the human PDE4A cAMP-specific phosphodiesterase gene. Biochem J 380, 371–84 (2004). [PubMed: 15025561] This report identifies a catalytically dead isoform of PDE4A, showing that nature has developed its own dominant-negative approach.

249. Lynch MJ, Baillie GS & Houslay MD cAMP-specific phosphodiesterase-4D5 (PDE4D5) provides a paradigm for understanding the unique non-redundant roles that PDE4 isoforms play in shaping compartmentalized cAMP cell signalling. Biochem Soc Trans 35, 938–41 (2007). [PubMed: 17956250]

250. Wills L, Ehsan M, Whiteley EL & Baillie GS Location, location, location: PDE4D5 function is directed by its unique N-terminal region. Cell Signal 28, 701–5 (2016). [PubMed: 26808969]

251. Yun S et al. Interaction between integrin alpha5 and PDE4D regulates endothelial inflammatory signalling. Nat Cell Biol 18, 1043–53 (2016). [PubMed: 27595237]

252. Ahmad F et al. Regulation of sarcoplasmic reticulum Ca2+ ATPase 2 (SERCA2) activity by phosphodiesterase 3A (PDE3A) in human myocardium: phosphorylation-dependent interaction of PDE3A1 with SERCA2. J Biol Chem 290, 6763–76 (2015). [PubMed: 25593322]

253. Lee LC, Maurice DH & Baillie GS Targeting protein-protein interactions within the cyclic AMP signaling system as a therapeutic strategy for cardiovascular disease. Future Med Chem 5, 451–64 (2013). [PubMed: 23495691]

254. Delyon J et al. PDE4D promotes FAK-mediated cell invasion in BRAF-mutated melanoma. Oncogene 36, 3252–3262 (2017). [PubMed: 28092671]

255. Serrels B et al. A complex between FAK, RACK1, and PDE4D5 controls spreading initiation and cancer cell polarity. Curr Biol 20, 1086–92 (2010). [PubMed: 20493699]

256. Martin TP et al. Targeted disruption of the heat shock protein 20-phosphodiesterase 4D (PDE4D) interaction protects against pathological cardiac remodelling in a mouse model of hypertrophy. FEBS Open Bio 4, 923–7 (2014).

257. Keravis T & Lugnier C Cyclic nucleotide phosphodiesterases (PDE) and peptide motifs. Curr Pharm Des 16, 1114–25 (2010). [PubMed: 20030615]

258. MacMullen CM, Vick K, Pacifico R, Fallahi-Sichani M & Davis RL Novel, primate-specific PDE10A isoform highlights gene expression complexity in human striatum with implications on the molecular pathology of bipolar disorder. Transl Psychiatry 6, e742 (2016). [PubMed: 26905414]

259. Movsesian M Novel approaches to targeting PDE3 in cardiovascular disease. Pharmacol Ther 163, 74–81 (2016). [PubMed: 27108947]

260. Kotera J et al. Subcellular localization of cyclic nucleotide phosphodiesterase type 10A variants, and alteration of the localization by cAMP-dependent protein kinase-dependent phosphorylation. J Biol Chem 279, 4366–75 (2004). [PubMed: 14604994]

261. Russwurm C, Koesling D & Russwurm M Phosphodiesterase 10A Is Tethered to a Synaptic Signaling Complex in Striatum. J Biol Chem 290, 11936–47 (2015). [PubMed: 25762721]

262. Kelly MP A Role for Phosphodiesterase 11A (PDE11A) in the Formation of Social Memories and the Stabilization of Mood. Adv Neurobiol 17, 201–230 (2017). [PubMed: 28956334]

263. Carlisle Michel JJ et al. PKA-phosphorylation of PDE4D3 facilitates recruitment of the mAKAP signalling complex. Biochem J 381, 587–92 (2004). [PubMed: 15182229]

264. Suryavanshi SV et al. Human muscle-specific A-kinase anchoring protein polymorphisms modulate the susceptibility to cardiovascular diseases by altering cAMP/PKA signaling. Am J Physiol Heart Circ Physiol 315, H109–H121 (2018). [PubMed: 29600899]

265. Zhu H et al. Evolutionarily conserved role of calcineurin in phosphodegron-dependent degradation of phosphodiesterase 4D. Mol Cell Biol 30, 4379–90 (2010). [PubMed: 20647544]

266. VerPlank JJS & Goldberg AL Regulating protein breakdown through proteasome phosphorylation. Biochem J 474, 3355–3371 (2017). [PubMed: 28947610]

267. Cai Y et al. Smurf2, an E3 ubiquitin ligase, interacts with PDE4B and attenuates liver fibrosis through miR-132 mediated CTGF inhibition. Biochim Biophys Acta Mol Cell Res 1865, 297–308 (2018). [PubMed: 29100790]

268. Li X, Baillie GS & Houslay MD Mdm2 directs the ubiquitination of beta-arrestin-sequestered cAMP phosphodiesterase-4D5. J Biol Chem 284, 16170–82 (2009). [PubMed: 19372219] The

first study to identify a post-translational modification that regulates PDE signaling via degradation.

269. Li X et al. Selective SUMO modification of cAMP-specific phosphodiesterase-4D5 (PDE4D5) regulates the functional consequences of phosphorylation by PKA and ERK. Biochem J 428, 55–65 (2010). [PubMed: 20196770]
270. Hay RT Decoding the SUMO signal. Biochem Soc Trans 41, 463–73 (2013). [PubMed: 23514139]
271. Hoffmann R, Baillie GS, MacKenzie SJ, Yarwood SJ & Houslay MD The MAP kinase ERK2 inhibits the cyclic AMP-specific phosphodiesterase HSPDE4D3 by phosphorylating it at Ser579. EMBO J 18, 893–903 (1999). [PubMed: 10022832]
272. Wang Y et al. S-nitrosylation of PDE5 increases its ubiquitin-proteasomal degradation. Free Radic Biol Med 86, 343–51 (2015). [PubMed: 26093192]
273. Garcia JA Aiming straight for the heart: prolyl hydroxylases set the BAR. Sci Signal 2, pe70 (2009). [PubMed: 19903936]
274. Huo Z et al. Prolyl hydroxylase domain protein 2 regulates the intracellular cyclic AMP level in cardiomyocytes through its interaction with phosphodiesterase 4D. Biochem Biophys Res Commun 427, 73–9 (2012). [PubMed: 22975349]
275. Lorenz R, Bertinetti D & Herberg FW cAMP-Dependent Protein Kinase and cGMP-Dependent Protein Kinase as Cyclic Nucleotide Effectors. Handb Exp Pharmacol 238, 105–122 (2017). [PubMed: 27885524]
276. VanSchouwen B & Melacini G Regulation of HCN Ion Channels by Non-canonical Cyclic Nucleotides. Handb Exp Pharmacol 238, 123–133 (2017). [PubMed: 28181007]
277. Rehmann H Interaction of Epac with Non-canonical Cyclic Nucleotides. Handb Exp Pharmacol 238, 135–147 (2017). [PubMed: 27900608]
278. Hauser AR The type III secretion system of Pseudomonas aeruginosa: infection by injection. Nat Rev Microbiol 7, 654–65 (2009). [PubMed: 19680249]
279. Wolter S et al. cCMP causes caspase-dependent apoptosis in mouse lymphoma cell lines. Biochem Pharmacol 98, 119–31 (2015). [PubMed: 26300059]
280. Wong CH, Siah KW & Lo AW Estimation of clinical trial success rates and related parameters. Biostatistics 20, 273–286 (2019). [PubMed: 29394327]
281. Liu FC et al. Use of cilomilast-loaded phosphatiosomes to suppress neutrophilic inflammation for attenuating acute lung injury: the effect of nanovesicular surface charge. J Nanobiotechnology 16, 35 (2018). [PubMed: 29602314]
282. Yu S et al. Targeted Delivery of an Anti-inflammatory PDE4 Inhibitor to Immune Cells via an Antibody-drug Conjugate. Mol Ther 24, 2078–2089 (2016). [PubMed: 27731313]
283. Raymond DR, Wilson LS, Carter RL & Maurice DH Numerous distinct PKA-, or EPAC-based, signalling complexes allow selective phosphodiesterase 3 and phosphodiesterase 4 coordination of cell adhesion. Cell Signal 19, 2507–18 (2007). [PubMed: 17884339]
284. Sapio L et al. Targeting protein kinase A in cancer therapy: an update. EXCLI J 13, 843–55 (2014). [PubMed: 26417307]
285. Raker VK, Becker C & Steinbrink K The cAMP Pathway as Therapeutic Target in Autoimmune and Inflammatory Diseases. Front Immunol 7, 123 (2016). [PubMed: 27065076]
286. Kumar N et al. Insights into exchange factor directly activated by cAMP (EPAC) as potential target for cancer treatment. Mol Cell Biochem 447, 77–92 (2018). [PubMed: 29417338]
287. Parnell E, Palmer TM & Yarwood SJ The future of EPAC-targeted therapies: agonism versus antagonism. Trends Pharmacol Sci 36, 203–14 (2015). [PubMed: 25744542]
288. Holland NA et al. Cyclic Nucleotide-Directed Protein Kinases in Cardiovascular Inflammation and Growth. J Cardiovasc Dev Dis 5 (2018).
289. Kleppe R, Krakstad C, Selheim F, Kopperud R & Doskeland SO The cAMP-dependent protein kinase pathway as therapeutic target: possibilities and pitfalls. Curr Top Med Chem 11, 1393–405 (2011). [PubMed: 21513494]
290. Bollen E et al. Improved long-term memory via enhancing cGMP-PKG signaling requires cAMP-PKA signaling. Neuropsychopharmacology 39, 2497–505 (2014). [PubMed: 24813825]

291. Joshi R et al. Phosphodiesterase (PDE) inhibitor torbafylline (HWA 448) attenuates burn-induced rat skeletal muscle proteolysis through the PDE4/cAMP/EPAC/PI3K/Akt pathway. Mol Cell Endocrinol 393, 152–63 (2014). [PubMed: 24973766]
292. Miller CL et al. Cyclic nucleotide phosphodiesterase 1A: a key regulator of cardiac fibroblast activation and extracellular matrix remodeling in the heart. Basic Res Cardiol 106, 1023–39 (2011). [PubMed: 22012077]
293. Zhang HT et al. Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. Neuropsychopharmacology 27, 587–95 (2002). [PubMed: 12377395]
294. Salpietro V et al. A homozygous loss-of-function mutation in PDE2A associated to early-onset hereditary chorea. Mov Disord 33, 482–488 (2018). [PubMed: 29392776]
295. Iribarne M & Masai I Neurotoxicity of cGMP in the vertebrate retina: from the initial research on rd mutant mice to zebrafish genetic approaches. J Neurogenet 31, 88–101 (2017). [PubMed: 28812418]
296. Savai R et al. Targeting cancer with phosphodiesterase inhibitors. Expert Opin Investig Drugs 19, 117–31 (2010).
297. Ahmad F et al. Cyclic nucleotide phosphodiesterases: important signaling modulators and therapeutic targets. Oral Dis 21, e25–50 (2015). [PubMed: 25056711]
298. Zuo H, Cattani-Cavalieri I, Musheshe N, Nikolaev VO & Schmidt M Phosphodiesterases as therapeutic targets for respiratory diseases. Pharmacol Ther (2019).
299. Pincelli C, Schafer PH, French LE, Augustin M & Krueger JG Mechanisms Underlying the Clinical Effects of Apremilast for Psoriasis. J Drugs Dermatol 17, 835–840 (2018). [PubMed: 30124722]
300. Gupta A, Tiwari M, Prasad S & Chaube SK Role of Cyclic Nucleotide Phosphodiesterases During Meiotic Resumption From Diplotene Arrest in Mammalian Oocytes. J Cell Biochem 118, 446–452 (2017). [PubMed: 27662514]
301. Drobnis EZ & Nangia AK Phosphodiesterase Inhibitors (PDE Inhibitors) and Male Reproduction. Adv Exp Med Biol 1034, 29–38 (2017). [PubMed: 29256125]
302. Vasta V et al. Identification of a new variant of PDE1A calmodulin-stimulated cyclic nucleotide phosphodiesterase expressed in mouse sperm. Biol Reprod 73, 598–609 (2005). [PubMed: 15901640]
303. Alves de Inda M et al. Validation of Cyclic Adenosine Monophosphate Phosphodiesterase-4D7 for its Independent Contribution to Risk Stratification in a Prostate Cancer Patient Cohort with Longitudinal Biological Outcomes. Eur Urol Focus 4, 376–384 (2018). [PubMed: 28753810]
304. van Strijp D et al. The Prognostic PDE4D7 Score in a Diagnostic Biopsy Prostate Cancer Patient Cohort with Longitudinal Biological Outcomes. Prostate Cancer 2018, 5821616 (2018). [PubMed: 30147955]
305. Bottcher R et al. Human phosphodiesterase 4D7 (PDE4D7) expression is increased in TMPRSS2-ERG-positive primary prostate cancer and independently adds to a reduced risk of post-surgical disease progression. Br J Cancer 113, 1502–11 (2015). [PubMed: 26575822] The first of 3 key studies (see 2 additional studies immediately above) that led to the development of a PDE4D7-based prostate cancer biomarker.
306. Bottcher R et al. Human PDE4D isoform composition is deregulated in primary prostate cancer and indicative for disease progression and development of distant metastases. Oncotarget 7, 70669–70684 (2016). [PubMed: 27683107]
307. Nazir M et al. Targeting tumor cells based on Phosphodiesterase 3A expression. Exp Cell Res 361, 308–315 (2017). [PubMed: 29107068]
308. Vandenberghe P et al. Phosphodiesterase 3A: a new player in development of interstitial cells of Cajal and a prospective target in gastrointestinal stromal tumors (GIST). Oncotarget 8, 41026–41043 (2017). [PubMed: 28454120]
309. Fryknas M et al. Phenotype-based screening of mechanistically annotated compounds in combination with gene expression and pathway analysis identifies candidate drug targets in a human squamous carcinoma cell model. J Biomol Screen 11, 457–68 (2006). [PubMed: 16928983]

310. Ahmad R et al. PET imaging shows loss of striatal PDE10A in patients with Huntington disease. Neurology 82, 279–81 (2014). [PubMed: 24353339]
311. Wilson H et al. Loss of extra-striatal phosphodiesterase 10A expression in early premanifest Huntington's disease gene carriers. J Neurol Sci 368, 243–8 (2016). [PubMed: 27538642]
312. Niccolini F et al. Loss of phosphodiesterase 10A expression is associated with progression and severity in Parkinson's disease. Brain 138, 3003–15 (2015). [PubMed: 26210536] Important study showing how in vivo imaging of a PDE may prove a useful biomarker for disease diagnosis and monitoring.
313. Boscutti G, R. E,A & Plisson C PET Radioligands for imaging of the PDE10A in human: current status. Neurosci Lett 691, 11–17 (2019). [PubMed: 30099081]
314. Schroder S, Wenzel B, Deuther-Conrad W, Scheunemann M & Brust P Novel Radioligands for Cyclic Nucleotide Phosphodiesterase Imaging with Positron Emission Tomography: An Update on Developments Since 2012. Molecules 21 (2016).
315. Neves SR, Ram PT & Iyengar R G protein pathways. Science 296, 1636–9 (2002). [PubMed: 12040175]
316. Chen J, Martinez J, Milner TA, Buck J & Levin LR Neuronal expression of soluble adenylyl cyclase in the mammalian brain. Brain Res 1518, 1–8 (2013). [PubMed: 23611875]
317. Kobialka M & Gorczyca WA Particulate guanylyl cyclases: multiple mechanisms of activation. Acta Biochim Pol 47, 517–28 (2000). [PubMed: 11310956]
318. Brand T The Popeye Domain Containing Genes and Their Function as cAMP Effector Proteins in Striated Muscle. J Cardiovasc Dev Dis 5 (2018).
319. Gamanuma M et al. Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. Cell Signal 15, 565–74 (2003). [PubMed: 12681444]
320. Goraya TA & Cooper DM Ca(2+)-calmodulin-dependent phosphodiesterase (PDE1): Current perspectives. Cellular Signalling 17, 789–797 (2005). [PubMed: 15763421]
321. Pandit J, Forman MD, Fennell KF, Dillman KS & Menniti FS Mechanism for the allosteric regulation of phosphodiesterase 2A deduced from the X-ray structure of a near full-length construct. Proc Natl Acad Sci U S A 106, 18225–30 (2009). [PubMed: 19828435] Seminal finding revealing the 3-dimensional nature by which cyclic nucleotide binding and protein-protein interactions at a PDE GAF domain can influence the ability of the regulatory N-terminal to control access of the catalytic pocket.
322. Qureshi BM et al. It takes two transducins to activate the cGMP-phosphodiesterase 6 in retinal rods. Open Biol 8 (2018).
323. Francis SH, Houslay MD & Conti M Phosphodiesterase inhibitors: factors that influence potency, selectivity, and action. Handb Exp Pharmacol, 47–84 (2011). [PubMed: 21695635]
324. Berthouze-Duquesnes M et al. Specific interactions between Epac1, beta-arrestin2 and PDE4D5 regulate beta-adrenergic receptor subtype differential effects on cardiac hypertrophic signaling. Cell Signal 25, 970–80 (2013). [PubMed: 23266473]
325. Bolger GB RACK1 and beta-arrestin2 attenuate dimerization of PDE4 cAMP phosphodiesterase PDE4D5. Cell Signal 28, 706–12 (2016). [PubMed: 26257302]
326. Govindan R et al. A phase II study of carboplatin, etoposide, and exisulind in patients with extensive small cell lung cancer: CALGB 30104. J Thorac Oncol 4, 220–6 (2009). [PubMed: 19179900]
327. Dawson NA et al. A phase II study of estramustine, docetaxel, and exisulind in patients with hormone-refractory prostate cancer: results of cancer and leukemia group B trial 90004. Clin Genitourin Cancer 6, 110–6 (2008). [PubMed: 18824434]
328. Yu EY et al. A pilot study of high-dose exisulind in men with biochemical relapse (BCR) of prostate cancer after definitive local therapy treated with intermittent androgen deprivation (IAD). Journal of Clinical Oncology 31, 209–209 (2013).
329. Curiel-Lewandrowski C et al. Randomized, double-blind, placebo-controlled trial of sulindac in individuals at risk for melanoma: evaluation of potential chemopreventive activity. Cancer 118, 5848–56 (2012). [PubMed: 22605570]

330. Leung DG et al. Sildenafil does not improve cardiomyopathy in Duchenne/Becker muscular dystrophy. Ann Neurol 76, 541–9 (2014). [PubMed: 25042693]

331. Victor RG et al. A phase 3 randomized placebo-controlled trial of tadalafil for Duchenne muscular dystrophy. Neurology 89, 1811–1820 (2017). [PubMed: 28972192]

332. McDonald CM et al. The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. Muscle Nerve 41, 500–10 (2010). [PubMed: 19941337]

333. Ramirez CE et al. Treatment with Sildenafil Improves Insulin Sensitivity in Prediabetes: A Randomized, Controlled Trial. J Clin Endocrinol Metab 100, 4533–40 (2015). [PubMed: 26580240]

334. Frolich L et al. Evaluation of the efficacy, safety and tolerability of orally administered BI 409306, a novel phosphodiesterase type 9 inhibitor, in two randomised controlled phase II studies in patients with prodromal and mild Alzheimer's disease. Alzheimers Res Ther 11, 18 (2019). [PubMed: 30755255]

335. Targum SD et al. Application of external review for subject selection in a schizophrenia trial. J Clin Psychopharmacol 32, 825–6 (2012). [PubMed: 22388170]

336. Targum SD et al. Impact of BPRS interview length on ratings reliability in a schizophrenia trial. Eur Neuropsychopharmacol 25, 312–8 (2015). [PubMed: 25554563]

337. Delnomdedieu M et al. In vivo measurement of PDE10A enzyme occupancy by positron emission tomography (PET) following single oral dose administration of PF-02545920 in healthy male subjects. Neuropharmacology 117, 171–181 (2017). [PubMed: 28122201] Example of how clinical trials are attempting to verify target engagement when testing PDEis in the context of nervous system disorders.

338. Metz VE et al. Effects of Ibudilast on the Subjective, Reinforcing, and Analgesic Effects of Oxycodone in Recently Detoxified Adults with Opioid Dependence. Neuropsychopharmacology 42, 1825–1832 (2017). [PubMed: 28393896]

339. Zhang F et al. Vinpocetine Inhibits NF-kappaB-Dependent Inflammation in Acute Ischemic Stroke Patients. Transl Stroke Res 9, 174–184 (2018). [PubMed: 28691141]

340. Ma XW et al. A randomized, open-label, multicentre study to evaluate plasma atherosclerotic biomarkers in patients with type 2 diabetes mellitus and arteriosclerosis obliterans when treated with Probucol and Cilostazol. J Geriatr Cardiol 9, 228–36 (2012). [PubMed: 23097651]

341. Lee JY et al. Efficacy of Cilostazol Administration in Alzheimer's Disease Patients with White Matter Lesions: A Positron-Emission Tomography Study. Neurotherapeutics (2019).

342. Heckman PRA et al. Acute administration of roflumilast enhances sensory gating in healthy young humans in a randomized trial. Psychopharmacology (Berl) 235, 301–308 (2018). [PubMed: 29098341]

343. Wouters EF et al. Effect of the phosphodiesterase 4 inhibitor roflumilast on glucose metabolism in patients with treatment-naive, newly diagnosed type 2 diabetes mellitus. J Clin Endocrinol Metab 97, E1720–5 (2012). [PubMed: 22723325]

344. Gonzalez-Ortiz M, Martinez-Abundis E, Hernandez-Corona DM & Ramirez-Rodriguez AM Effect of tadalafil administration on insulin secretion and insulin sensitivity in obese men. Acta Clin Belg 72, 326–330 (2017). [PubMed: 28245726]

345. Sbardella E et al. Cardiovascular features of possible autonomous cortisol secretion in patients with adrenal incidentalomas. Eur J Endocrinol 178, 501–511 (2018). [PubMed: 29510982]

346. Pauls MMH et al. Perfusion by Arterial Spin labelling following Single dose Tadalafil In Small vessel disease (PASTIS): study protocol for a randomised controlled trial. Trials 18, 229 (2017). [PubMed: 28532471]

347. Charnigo RJ et al. PF-04447943, a Phosphodiesterase 9A Inhibitor, in Stable Sickle Cell Disease Patients: A Phase Ib Randomized, Placebo-Controlled Study. Clin Transl Sci 12, 180–188 (2019). [PubMed: 30597771]

348. Moschetti V et al. First-in-human study assessing safety, tolerability and pharmacokinetics of BI 409306, a selective phosphodiesterase 9A inhibitor, in healthy males. Br J Clin Pharmacol 82, 1315–1324 (2016). [PubMed: 27378314]

349. Boland K et al. A phase I, randomized, proof-of-clinical-mechanism study assessing the pharmacokinetics and pharmacodynamics of the oral PDE9A inhibitor BI 409306 in healthy male volunteers. Hum Psychopharmacol 32 (2017).

350. Fazio P et al. Patterns of age related changes for phosphodiesterase type-10A in comparison with dopamine D2/3 receptors and sub-cortical volumes in the human basal ganglia: A PET study with (18)F-MNI-659 and (11)C-raclopride with correction for partial volume effect. Neuroimage 152, 330–339 (2017). [PubMed: 28254508]

351. Macek TA et al. A phase 2, randomized, placebo-controlled study of the efficacy and safety of TAK-063 in subjects with an acute exacerbation of schizophrenia. Schizophr Res 204, 289–294 (2019). [PubMed: 30190165]

Box 1.

Physiological roles of PDEs.

Because 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 (Table S1).

Most PDEs families are expressed in the **nervous system** where they regulate neurodevelopment and apoptosis, neuronal excitability, synaptic transmission and neuroplasticity[70, 95]. Every brain region expresses more than one PDE, but no two PDEs exhibit the exact same regional distribution[70]. 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 behaviors[174]. In contrast, PDE4D is expressed in most brain regions and does appear to regulate basal depression-related behaviors (e.g.,[293]). PDE1B, PDE2A, PDE7B, PDE8B and PDE10A are enriched in the striatum relative to other brain regions[70], and each has been implicated in regulating basic motor function; whereas, PDE5A and PDE9A that are enriched in the cerebellum have not (for review, see[70, 294]). 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[224, 295].

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 signaling has been associated with disease (for review, see[1]). For example, heart failure has been associated with reduced levels of PDE3A and PDE4D, which results in myocyte apoptosis and cardiac arrhythmias, respectively[222, 236].

Many **cancers** have been associated with reduced levels of cAMP and/or cGMP secondary to an elevation in PDE activity. For example, chronic lymphocytic leukemia cells exhibit increased PDE7B expression; leukemia, colon cancer, and glioma cells overexpress one or more isoforms of PDE4; and colon cancer cells and adenocarcinmoas exhibit elevated PDE5 activity[16, 296, 297].

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-hydrolyzing enzyme in the immune system—is elevated in the context of various inflammatory diseases, including psoriasis, COPD and asthma[298, 299].

PDEs are also implicated in **reproductive health**. Several isoforms are present in granulosa cells as well as in oocytes in preovulatory follicles of mammalian ovary regulating the meiotic cell cycle[300]. Furthermore, many PDEs are expressed in cells of the spermatogenic pathway where they may regulate sperm motility[301, 302], and PDE5 is

expressed in the contractile tissues of the male excurrent tract and accessory where its increased activity contributes to erectile dysfunction (e.g.,[23]).

Box 2.

PDEs as disease biomarkers.

PDEs are also being explored as both diagnostic and patient-selection biomarkers. This super family of enzymes, like other genes, can be genotyped from blood samples to assess risk for specific diseases (e.g., high suicide risk,[303]; PDE8A,). PDE mRNA and protein expression can be measured *ex vivo* in an isoform-specific manner from biopsied samples (e.g, excised tumors). Further, imaging compounds can be engineered with relative ease to selectively target a given PDE family/sub-family *in vivo*. Thus, PDEs can be explored as biomarkers in diseases where tissue is readily biopsied (e.g,. cancers) and diseases where tissue is not (e.g., 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 treatment decisions[303, 304] (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 (e.g., in castration-resistant tumours)[305, 306], consistent with *in vitro* reports using prostate cancer cells[190]. 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 tumors and, thus, inform post-biopsy/post-surgery treatment decisions (https://mdxhealth.com/press-release/mdxhealth-launch-agreement-philips-prognostic-prostate-cancer-biomarker; accessed 12/03/18).

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 (GIST) cells[307, 308]. Furthermore, cancer cell lines with the highest PDE3A expression proved the most susceptible to the chemotherapeutic effects of PDE3i's[309]. 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 signaling has been implicated in a variety of age-related diseases of the brain (c.f.,[235]). PDE10A is widely reported as downregulated in both the striatum and cortex of patients with Huntington's disease, with the extent of PDE10A loss corresponding to the genetic burden associated with the disease[28, 67, 310, 311]. A loss of PDE10A expression has also been observed in the basal ganglia of patients with Parkinson's disease[312]. Importantly, highly-specific PDE10A positron emission tomography (PET) tracers shows that PDE10A expression in HD patients continues to decline over years[28]. Thus, PET imaging of PDE10A could be a useful biomarker for assessing the initial diagnosis and subsequent progression of these neurodegenerative diseases[313]. PET ligands also exist for PDEs 2, 4, 5, and 7[314].

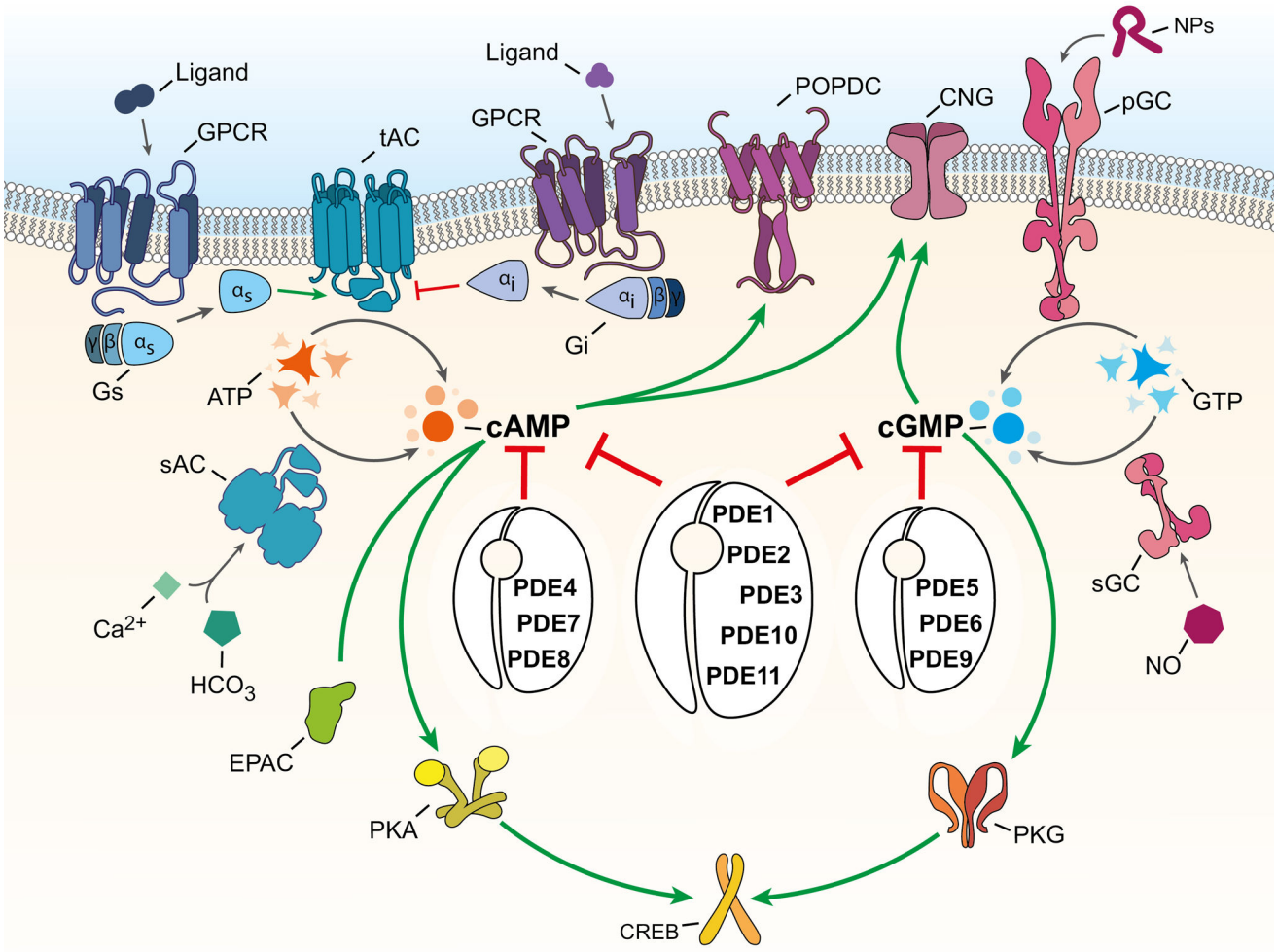

Figure 1. 11 families of PDEs degrade cyclic nucleotides.

Both of the canonical cyclic nucleotide signaling 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 (ACs) that are activated by Gαs and inhibited by Gαi[315] as well as soluble ACs that are activated by bicarbonate and calcium[316]. cGMP is synthesized by particulate guanylyl cyclases (pGCs) that are activated by natriuretic peptides and soluble guanylyl cyclases (sGCs) that are activated by nitric oxide (NO)[317]. Both cAMP and cGMP activate cyclic nucleotide gated channels and allosterically modulate activity of select PDEs[196]. 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 proteins (POPDC)[318]. Signaling 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 hydrolyze the non-canonical cyclic nucleotides (not included) cUMP (PDE3A, PDE3B, PDE9A), cCMP (PDE7A), and c-di-GMPa (bacterial PDEs), albeit with much lower affinity[2, 3].

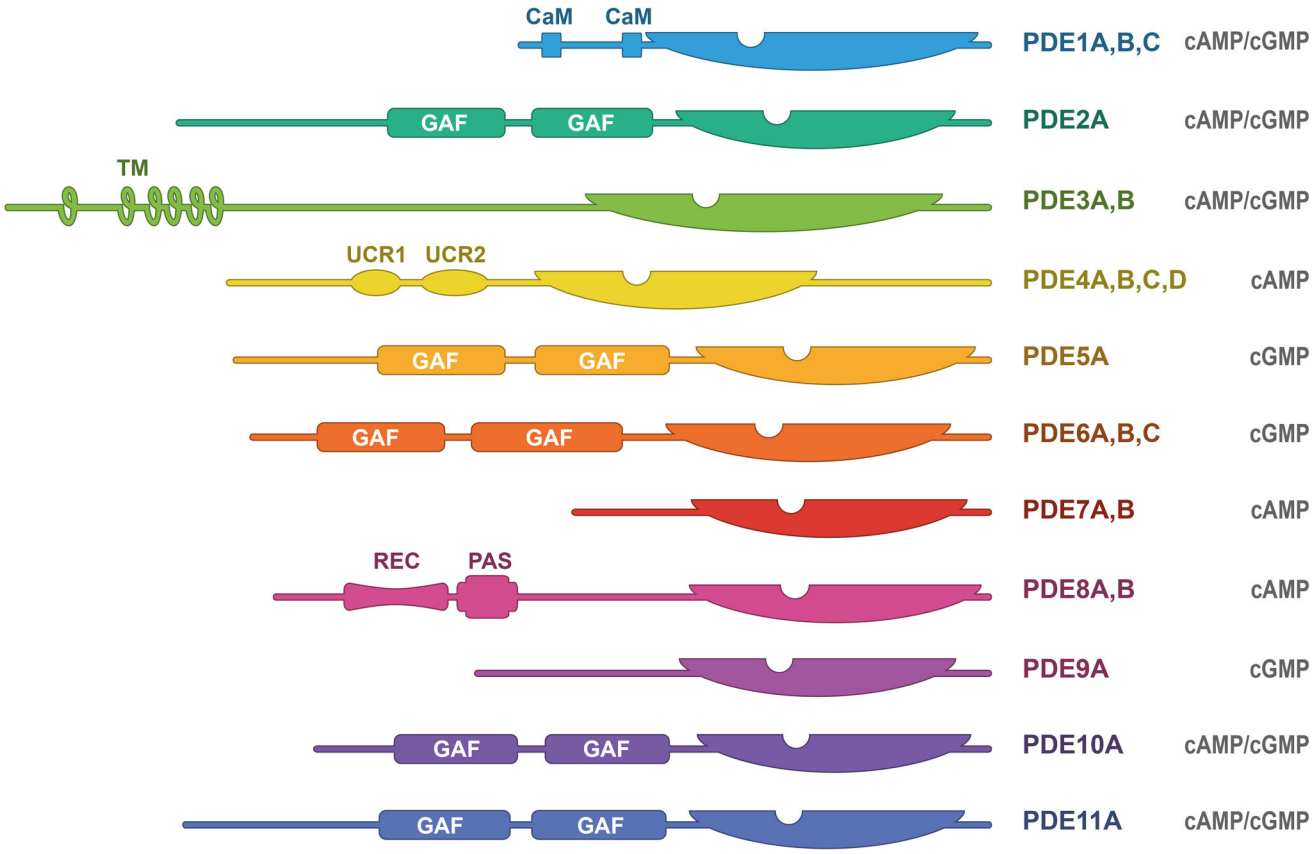

Figure 2. The 21 phosphodiesterase (PDE) genes are grouped into families (name and substrate specificity listed to right of each illustration) based on the homology of their C-terminal catalytic domain (represented as a semi-ellipse).

Due to alternate promoters and splicing events, each 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 REC domain of PDE8 (estimated from[319]) and the second CaM domain of PDE1 (estimated from[302]). Illustrations represent the longest isoform for gene A of each PDE family. CaM, calmodulin-binding domain; GAF, cGMP-binding PDEs Anabaena adenylyl cyclases and E. coli FhlA; TM, transmembrane domain of PDE3; UCR, upstream conserved region; REC, signal receiver domain; PAS, Per-Arnt-Sim domain.

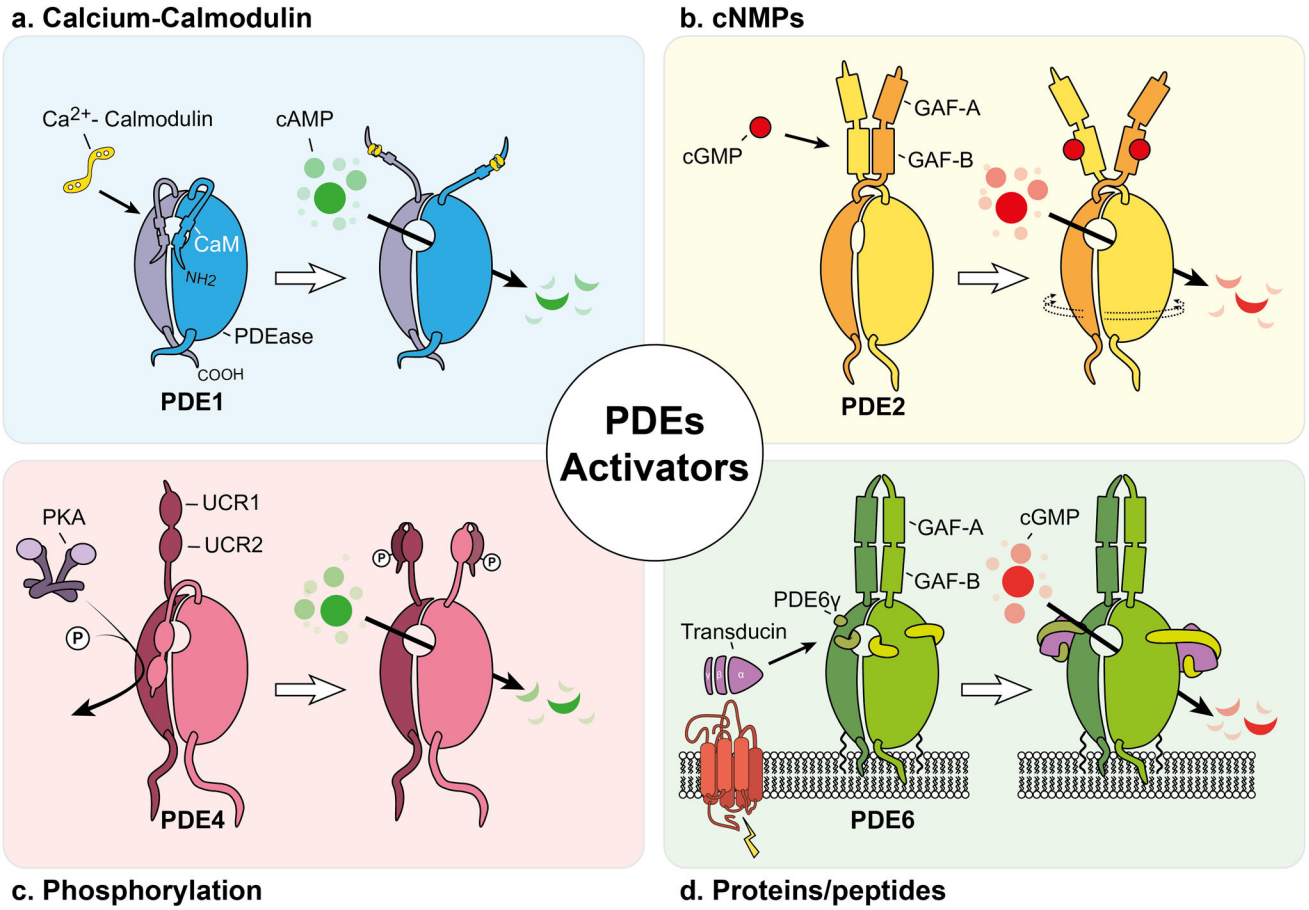

Figure 3. Mechanisms that activate phosphodiesterase (PDE) catalytic activity.

A) Calcium-calmodulin (CaM) binding to the CaM domains of PDE1 relieves N-terminal auto-inhibition of the catalytic site, thereby promoting enzymatic activity[320]. B) Cyclic nucleotides binding to GAF domains of dimeric 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 substrates[321]. C) Phosphorylation by PKA or PKG activates several PDEs[196]. 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 PDE6γ C-termini from the catalytic sites on PDE6αβ, thus, promoting cGMP hydrolysis[322].

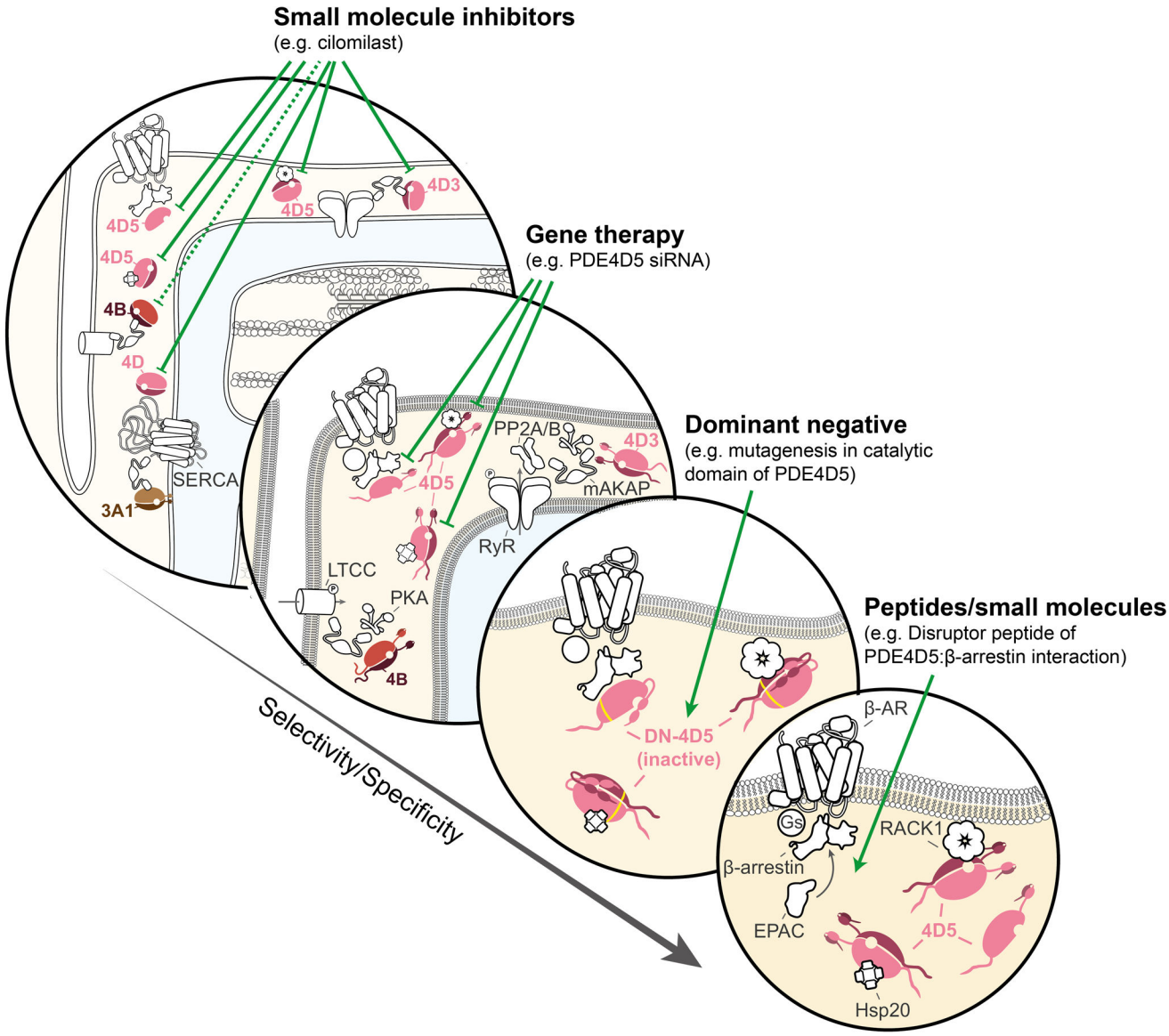

Figure 4. Methods for targeting phosphodiesterase signaling with increasing specificity.

Given the vast diversity of 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 (e.g.,** cilomilast) are readily developed with family-specific selectivity (e.g., targeting PDE4 over PDE3); however, isoform specificity remains a challenge (e.g., cilomilast inhibits PDE4D with only 7-fold selectivity versus PDE4B)[323]. Conversely, **gene therapy** (i.e., expressing a recombinant construct to knock down or restore expression of a given PDE isoform) and **dominant negative** approaches (i.e., expressing a catalytically inactive PDE4D5 that displaces the endogenous isoform from its interacting partners) can target isoform subtypes exclusively (e.g., 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 signaling 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 β2 adrenergic receptors (βAR), but would leave PDE4D5 regulation of heat shock protein 20 (HSP20) and RACK1 complexes intact[236, 324, 325].

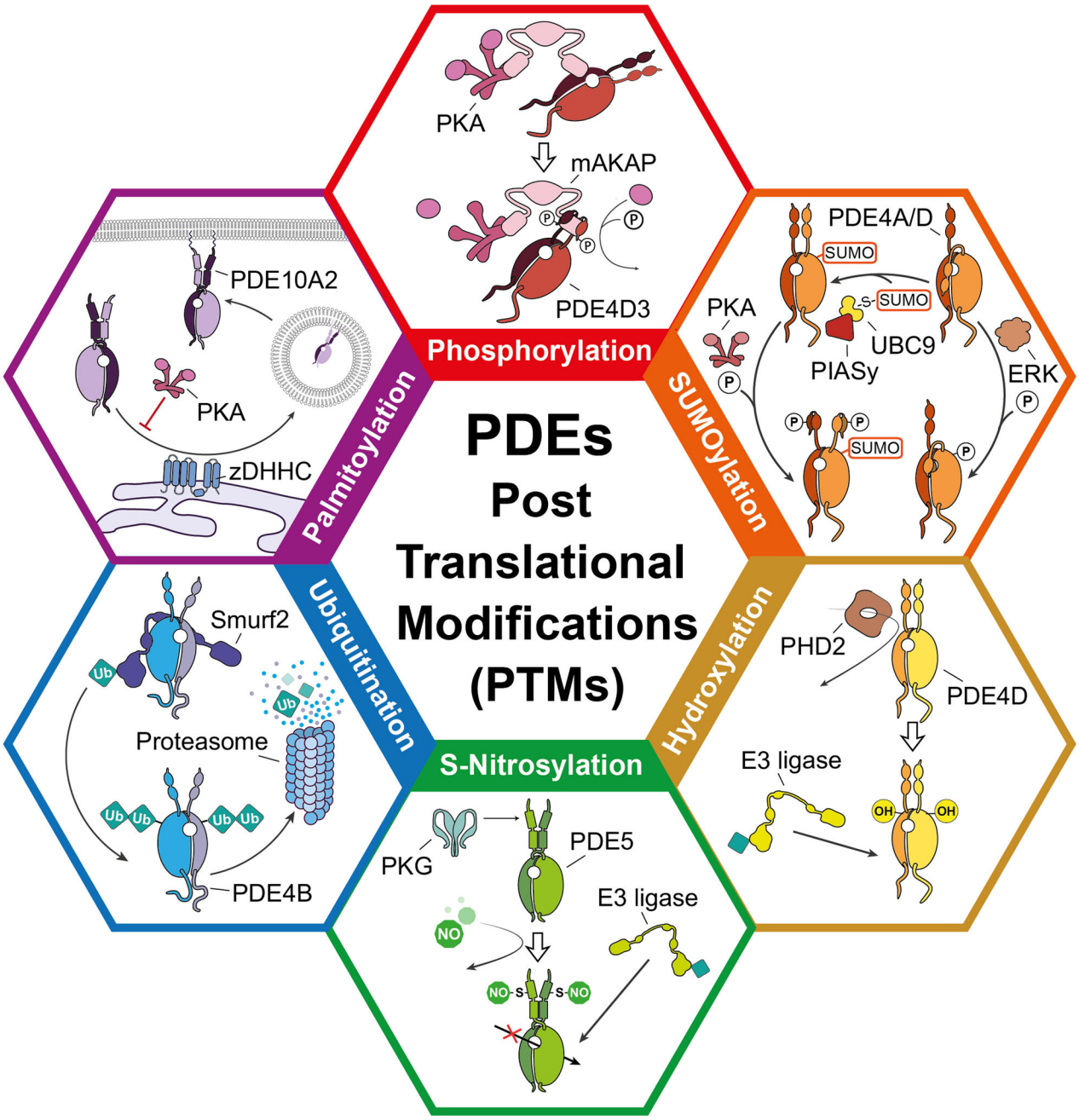

Figure 5. Phosphodiesterase (PDE) regulation by post-translation modification (PTM).

Cyclic nucleotide dynamics can be modulated by the addition of different functional groups to PDEs. **Phosphorylation** is a very common mechanism to control PDE activity as depicted by the action of PKA on PDE4D3. Both enzymatic activity and binding affinity of PDE4D3 for mAKAP are increased by PKA phosphorylation, allowing a faster signal termination in myocytes[263]. **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)[155]. **Ubiquitination** can influence PDE function by controling stability. For example, the E3 ubiquitin ligase Smurf2

targets PDE4B for degradation which leads to the attenuation of liver fibrosis[267]. **S-nitrosylation** can also tag PDEs for destruction. Thus, the covalent incorporation of nitric oxide (NO) to the GAF-A domain of a PKG-phosphorylated and active PDE5, directs the enzyme to the proteasome[272]. **Hydroxylation** of proline residues has emerged as another PTM to stimulate turnover of PDEs. Prolyl hydroxylase domain protein 2 (PHD2) action on PDE4D increases its recognition by E3 ligase complexes in cardiomyocytes[274]. Finally, **SUMOylation** can intensify the activity of PDE4A and PDE4D. The 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 ERK activity[269].

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 (e.g. Spain, 1922), Asia (e.g. India, 1969)	Nausea, vomiting, diarrhea, headache, irritability, flushing and palpitations.
Aminophylline *[inhibits PDE3, 4, 7, adenosine 2 receptor]* (Phyllocontin)	Asthma and Bronchoconstriction	FDA (1940), Europe (e.g. Hungary, 1935), Asia (e.g. India, 1950)	Stomach pain, diarrhea, 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, diarrhea, 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 (e.g. Spain 1968) and Asia (e.g. Japan, 1952)	Stomach pain, nausea, vomiting, diarrhea, 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 (e.g. Spain, 1978) and Asia (e.g. India, 1975)	Belching, bloating, upset stomach, nausea, vomiting, indigestion, dizziness, and flushing, 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, diarrhea and abdominal pain, depression, rash and fatigue
	Allergic conjunctivitis	Japan (2000)	
Tofisopam *[highest affinity for PDE4, PDE10, 3, 2]* (Emandaxin, Grandaxin)	Anxiety	Some countries in Europe (e.g. Hungary, 1974) and Asia (e.g. 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 (e.g. Spain 1986) and Asia (e.g. India, 1964)	Headache, dizziness, nausea, diarrhea, muscle pain and vomiting
PDE1			
Vinpocetine (Cavinton)	Cerebral vascular disorders and memory impairment	Some countries in Europe (e.g. Spain, 1997) and Asia (e.g. India 2002). USA as an over-the-counter dietary supplement	Flushing, rashes, and minor gastrointestinal disturbances
PDE3			
Cilostazol (Pletal, Ekistol)	Intermittent Claudication	FDA (1999), some countries in Europe (e.g. UK, 2000) and Asia (e.g. South Korea, 1990)	Headache, palpitations, diarrhea, dizziness, nasal irritation and pharyngitis
Milrinone (Primacor, Corotrope)	Congestive Heart Failure	FDA (1987), EMA (2016), Asia (e.g. Japan, 1996)	Ventricular/supraventricular arrhythmias, hypotension and headache
Amrinone (Inamrinone, Inocor)	Congestive heart failure	FDA (1984), some countries in Asia (e.g. India, 1988)	Thrombocytopenia, nausea, diarrhea, hepatotoxicity, arrhythmias and fever
Enoximone (Perfan)	Congestive heart failure	Some countries in Europe (e.g. France, 1987)	Headache, diarrhoea, insomnia, hypotension, vomiting, nausea, tachycardia and arrhythmias

Compound (popular trade names)	Indication	Approval date (USA, Europe and Asia markets)	Side effects
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)	Thrombocythemia	FDA (1997), EMA (2004), some countries in Asia (e.g. South Korea, 2004)	Headache, diarrhea, unusual weakness/ fatigue, hair loss, nausea and dizziness
PDE4			
Roflumilast (Daliresp, Daxas)	Chronic Obstructive Pulmonary Disease (COPD)	FDA (2011), EMA (2010), some countries in Asia (e.g. India, 2014)	Diarrhea, weight loss, nausea, headache, insomnia, decreased appetite
Apremilast (Otezla)	Psoriasis and psoriatic disorders	FDA (2014), EMA (2014), some countries in Asia (e.g. Japan, 2016)	Diarrhea and vomiting, weight loss, mood changes
Crisaborole (Eucrisa)	Moderate Atopic dermatitis (patients >2 years old)	FDA (2016)	Hypersensitivity reactions of the skin
Drotaverine *[also inhibits L-type voltage-operated calcium channel]* (No-Spa, Doverin)	Functional bowel disorders and alleviating pain caused by smooth muscle spasm	Some countries in Europe (e.g. Hungary, 1963) and Asia (e.g. China, 1999)	Fainting, nausea, vomiting and dry mouth
PDE5			
Sildenafil (Viagra, Revatio)	Erectile Dysfunction	FDA (1998), EMA (1998), Asia (e.g. Japan, 1999)	Headache, flushing, dyspepsia, nasal congestion, and impaired vision, including photophobia and blurred vision
	Pulmonary arterial hypertension (PAH)	FDA (2014), EMA (2005), Asia (e.g. Japan, 2008)	
Vardenafil (Levitra, Staxyn, Vivanza)	Erectile Dysfunction	FDA (2003), EMA (2003), some countries in Asia (e.g. Japan, 2004)	Headache, flushing, and dyspepsia
Tadalafil (Cialis, Adcirca)	Erectile Dysfunction, benign prostatic hyperplasia	FDA (2003), EMA (2002), some countries in Asia (e.g. India, 2003)	Headache, dyspepsia, back pain and myalgia
	PAH	FDA (2009), EMA (2008), some countries in Asia (e.g. India, 2009)	
Avanafil (Stendra, Spedra)	Erectile Dysfunction	FDA (2012), EMA (2013), some countries in Asia (e.g. South Korea, 2011)	Headache, flushing, and nasopharyngitis
Udenafil (Zydena)	Erectile dysfunction and hypertension	Some countries in Asia (e.g. 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 and vasospasm and erectile dysfunction	FDA, Europe (e.g. Hungary, 1933), Asia (e.g. Japan, 1953)	Ventricular tachycardia, diarrhea, somnolence, vertigo, flushing and headache

Source: Drugs.com, drugcentral.org, drugbank.ca, kegg.jp, and approval dates by **FDA** (Food and Drug Administration), **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).

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	Cause of failure
PDE2				
PF-05180999 / Pfizer	Healthy volunteers (schizophrenia)	Phase I / Completed 2011		
	Healthy volunteers	Phase I / Completed 2012		
	Migraine	Phase I / Terminated early 2014		Safety concerns
	Migraine	Phase I / Withdrawn prior to enrollment 2014		
PDE2/5				
Exisulind (Aptosyn) / Cell Pathways	Breast cancer neoplasms and metastases	Phase I/II / Completed 2003		
	Breast cancer	Phase II / Completed 2008		
	Non-small cell lung cancer	Phase I/II/III / Completed 2003–2007	, ,	
	Small cell lung cancer	Phase II / Completed 2008	[326]	Lack of efficacy
	Prostate cancer and prostatic neoplasms	Phase II / Completed 2006–2011	, , , , [327, 328]	Safety and efficacy deficiencies
	Melanoma	Phase II / Completed 2011	[329]	Lack of efficacy
PDE3				
Cilastozol (Pletal) / Otsuka Pharmaceuticals	Type 2 diabetes polyneuropathy	Phase IV / Completed 2009	[48]	Lack of efficacy
PDE4				
ASP9831 / Astellas Pharma	Non-alcoholic steatohepatitis (NASH)	Phase II / Completed 2010	[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	, [57]	
		Phase II/III / Terminated early 2014–2017	, [330–332]	Lack of efficacy
		Phase IV / Completed 2012	[58]	
	Impaired glucose tolerance	Phase IV / Terminated early 2016	[333]	Safety concerns
Vardenafil (Levitra) / Bayer/GSK	Type 2 diabetes	Phase II / Completed 2014	[149]	Lack of efficacy
PDE9				
BI 409306 / Boehringer Ingelheim	AD	Phase II / Completed 2017	, [63, 334]	Lack of efficacy
PF-04447943 / Pfizer	AD	Phase II / Completed 2010	[59]	Lack of efficacy
PDE10				
PF-02545920 (a.k.a. MP-10) / Pfizer	Schizophrenia	Phase I / Completed 2007		
	Schizophrenia	Phase II / Terminated early 2008		Safety concerns
	Healthy volunteers (glucose metabolism)	Phase I / Completed 2011		

Compound / Manufacturer	Indication	Phase / Status	Clinical trial ID	Cause of failure
	Schizophrenia	Phase II / Completed 2011	66, 335	Lack of efficacy
	Schizophrenia	Phase I / Terminated early 2012		Results from other clinical study
	Schizophrenia	Phase I / Completed 2013		
	Schizophrenia	Phase II / Terminated early 2014	336	Lack of efficacy
	Healthy male volunteers (PET imaging)	Phase I / Completed 2014	337	
	Huntington's Disease	Phase II / Completed 2015–2016	,	Lack of efficacy
	Huntington's Disease	Phase II / Terminated early 2017		Results from
OMS643762 / Omeros	Schizophrenia	Phase II / Completed 2014		
	Huntington's Disease	Phase II / Terminated early 2016		Results from
PBF-999 / Palobiofarma	Huntington's Disease	Phase I / Completed 2015		
	Huntington's Disease	Phase I / Terminated early 2016		Change in therapeutic indication (cancer)

Reported on Clinicaltrials.gov (accessed 05/28/19). Information is included for all clinical trials involving molecules whose pursuit was terminated after April 2009.

Table 3.

Selected clinical trials involving PDE inhibitors pursued for new indications

Compound / Manufacturer	Indication	Phase / Status	Clinical trial ID (Refs)
PDE3, 4, 10, 11			
Ibudilast (AV-411, MN-166) / MediciNova	Opioid withdrawal	Phase II / Completed 2012–2017	,
	Methamphetamine-dependence	Phase I / Completed 2013	77, 78
	Alcohol use disorder	Phase I / Completed 2015	80
	Alcohol use disorder	Phase II / Recruiting	
	Opioid abuse	Phase II / Completed 2017	79, 338
	Amyotrophic lateral sclerosis (ALS) (Biomarker study)	Phase II / Active, not recruiting	
PDE1			
ITI-214 / Intracellular Therapies	Schizophrenia	Phase I / Terminated early 2014	
	PD	Phase I/II / Completed 2018	
	Healthy volunteers (CNS engagement)	Phase I / Recruiting	
	Systolic heart failure	Phase I/II / Recruiting	
Vinpocetine / Rxmidas Pharmaceuticals/ Nootrobox	Ischemic stroke	Phase II/III / Completed 2013–2015	, 339
	Cognition enhancement	Not Applicable / Completed 2017	
PDE2			
TAK-915 / Takeda	Healthy volunteers (PET imaging, schizophrenia)	Phase I / Completed 2016	
	Healthy volunteers	Phase I / Completed 2016	
PDE3			
Cilostazol (Pletal) / Otsuka Pharmaceuticals	Type 2 diabetic atherosclerosis	Phase IV / Completed 2010–2012	, 340
	Chronic tinnitus	Not applicable / Completed 2013	94
	Alzheimer's Disease	Phase IV / Completed 2013	341
	Atherosclerotic events in type 2 diabetes	Phase IV / Unknown	
	Mild Cognitive Impairment	Not applicable / Completed 2015	
	Ischemic events in type 2 diabetic artery obstruction	Phase IV / Recruiting	
	Antiplatelet aggregation in type 2 diabetes	Phase IV / Active, not recruiting	
	Antiplatelet aggregation in type 2 diabetes	Phase IV / Recruiting	
	Antiplatelet aggregation in type 2 diabetes	Phase IV / Unknown	
PDE4			
Apremilast (Otezla) / Celgene Corp.	Vitiligo	Phase II / Active, not recruiting	,

Compound / Manufacturer	Indication	Phase / Status	Clinical trial ID (Refs)
	Lichen Planus of Vulva	Phase II / Not yet recruiting	,
BPN14770 / Tetra Discovery	Alzheimer's Disease	Phase I / Completed 2016–2017	,
	Alzheimer's Disease	Phase II / Now recruiting	
	Fragile X Syndrome (FXS)	Phase II / Recruiting	
Crisaborole (Eucrisa) / Pfizer	Morphea	Phase II / Recruiting	
GSK356278/ GlaxoSmithKline	Huntington's Disease	Phase I / Completed 2012	,
Roflumilast (Daxas or Daliresp) / Astrazeneca	Polycystic Ovary Syndrome	Phase IV / Completed 2014	, [108, 109]
	Cognitive deficits in schizophrenia	Phase II / Completed 2015	[112]
	Cognition (Dementia)	Phase II / Completed 2013–2015	, 2013–001223–39 (EudraCT)[111, 113, 342]
	Insulin and Blood Sugar Levels in Prediabetic Overweight and Obese Individuals	Phase I/II / Completed 2017	[343]
HT-0712 / Dart Neuroscience	Age-associated memory impairment (AAMI)	Phase II / Completed 2015	
N/A	McCune-Albright Syndrome (PET imaging)	Phase I/II / Recruiting	
TAK-648 / Takeda	Type 2 diabetes	Phase I / Completed 2015	, ,
Zembrin / ND	Aged Individuals	Phase I / Completed 2012	[39]
PDE5			
ND	Contrast Media-induced Nephropathy (CMN)	Not Applicable / Unknown	
ND	Diabetic nephropathy	Not Applicable / Unknown	
Sildenafil (Viagra or Revatio) / Pfizer	Diabetic cardiomyopathy (type 2 diabetes)	Phase IV / Completed 2009	[145–148]
	Metabolic syndrome (Skeletal muscle insulin signaling)	Phase IV / Completed 2016	
	Urolithiasis/urinary stones	Phase IV / Active, not recruiting	
	Migraine aura	Early Phase I / Recruiting	
	Solid tumors	Phase I / Active, not recruiting	[151]
	mTBI or concussion	Phase I / Recruiting	,
Sildenafil cream (SST-6007) / Strategic science and technologies/ Dare	Sexual arousal disorder	Phase II / Completed 2017	
Tadalafil (Cialis or Adcirca) / Eli Lilly	Type 2 Diabetes (Postprandial Hyperglycemia)	Phase I / Terminated early 2011	
	Head and neck squamous cell carcinoma	Phase II / Completed 2012–2016	[153]
	Head and neck squamous cell carcinoma	Phase II / Active, not recruiting	
	Obesity	Phase IV / Completed 2015	[144]
	Insulin secretion/ sensitivity in obesity	Phase IV / Completed 2015	[344]
	Aortic stenosis (AS) left ventricular remodeling/hypertrophy	Phase IV / Terminated early 2017	

Compound / Manufacturer	Indication	Phase / Status	Clinical trial ID (Refs)
	Multiple myeloma (MM)	Phase II / Terminated early 2017	
	Insulin Resistance in Type 2 Diabetes	Phase II / Recruiting	
	Diabetic cardiomyopathy (DC)	Phase IV / Recruiting	
	Endocrine cardiomyopathy in Cushing Syndrome (CS)	Phase II / Recruiting	[345]
	Access sheath deployment (Nephrolithiasis/kidney stones)	Phase IV / Enrolling 2019	
	Lower urinary tract symptoms (prostatic hyperplasia)	Phase IV / Recruiting	
	Obesity-related cardiometabolic dysfunction	Phase II / Recruiting	
	Anti-tumor Mucin 1 vaccine efficacy in head and neck squamous cell carcinoma (HNSCC)	Phase I/II / Recruiting	
	Small Vessel Disease	Phase II / Active, not recruiting	[346]
PDE9			
PF-04447943 / Pfizer	Stable sickle cell disease	Phase I / Completed 2016	[347]
BI 409306 / Boehringer Ingelheim	Healthy volunteers	Phase I / Completed 2011–2018	, , , , , , , , [61, 348, 349]
	Alzheimer's disease, schizophrenia	Phase I / Completed 2017	
	Schizophrenia	Phase I / Completed 2013–2016	, [60, 62]
	Schizophrenia or attenuated psychosis syndrome	Phase II / Recruiting	,
	Drug-drug interactions	Phase I / Completed 2016–2017	, , , ,
PDE10			
[18F]MNI-659	Huntington's Disease (PET imaging)	Early Phase I / Completed 2016/2017	, , 2012–003808–13 (EudraCT)[350]
PBF-999 / Palobiofarma	Cancer	Phase I / Recruiting	
EVP-6308 (now FRM-6308) / En Vivo Pharmaceuticals (now Forum Pharmaceuticals)	Schizophrenia	Phase I / Completed 2014	,
RO5545965 / Hoffmann-La Roche	Schizophrenia	Phase I / Completed 2013–2017	, , , ,
TAK-063 / Takeda	Schizophrenia	Phase I / Completed 2014	, , [163]
	Schizophrenia	Phase II / Completed 2016	[351]

Reported on Clinicaltrials.gov (accessed 05/28/19) with an end date after April 2009

PK/PD, Pharmacokinetics and Pharmacodynamics; ND, not described

Table 4.

Selected patents involving PDEs published in the last 5 years.

Patent # (Priority date d/m/y)	Subject	Assignee	Author
PDE3			
US2019046528 (08/08/2017)	Method of preventing hair loss or promoting hair growth by using PDE3 inhibitor	Seoul Nat Univ Hospital	Kwon O, Choi HI, Jo SJ, Kim KH
WO2017186103 (26/04/2016)	Applications of PDE3A in determination of tumor treatment effect of Anagrelide	Shanghai Inst Materia Medica Cas	Yu Q, Liu J
PDE4			
CN108904493A (12/08/2018)	PDE4 inhibitor and purpose for preparing novel anti-inflammatory drugs	Hu Y	Hu Y
WO2018167142 (16/03/2017)	Treatment of idiopathic pulmonary fibrosis [with a PDE4 inhibitor]	Takeda GMBH	Hanauer G, Nikam S, Hazama M
WO2017133713 (05/02/2016)	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 (28/09/2016)	Compounds and their use as PDE4 activators for the treatment of disorders requiring a reduction of cAMP	Mironid Ltd	Adam JM, Adams DR
WO2018037109 (26/08/2016)	Treatment of nonalcoholic fatty liver disease with PDE4 inhibitors	Takeda GMBH	Hanauer G, Nagabukuro H, Amano Y
CN107412214A (31/07/2017)	Application of PDE4 Inhibitor (FCPR16) for the treatment of PD	Guangzhou Lanssonpharm Jianzhi Tech Co Ltd	Xu L
US2017051291 (28/12/2005)	RNAi-mediated inhibition of PDE4 for treatment of cAMP-related ocular disorders	Arrowhead Pharmaceuticals Inc	Yanni JM, Chatterton JE, Gamache DA, Miller ST
WO2017017165 (29/07/2015)	PDE4 inhibitor for the treatment of diabetic nephropathy	Takeda GMBH	Hanauer G, Vollert S, Hazama M, Matsuo T
WO2016075543 (13/11/2014)	Treatment of multiple sclerosis with the combination of laquinimod and a PDE4 inhibitor	Teva Pharma, Piryatinsky V, Kaye J	Piryatinsky V, Kaye J
WO2015022417 (16/08/2013)	Treatment of cognitive impairment with the combination of a PDE4 inhibitor and an acetylcholinesterase inhibitor	Univ Maastricht	Yamada T, Prickaerts J, Van Duinen M, Sambeth A, Blokland A
PDE5			
US2018221373 (16/09/2015)	Method of treating insomnia with PDE5 inhibitors	Rosenberg LI	Rosenberg LI
CA2975049 (10/08/2016)	PDE inhibitors (sildenafil) to repair brain and/or retinal injury in human newborns	Wintermark P	Wintermark P
CN107163052A (18/04/2017)	Immunodetection method for various PDE5 inhibitor drugs	Univ South China Agricult	Shen Y, Hua Y, Xu Z, Yang J, Wang H, Sun Y, Lei H
WO2014088326 (04/12/2012)	Composition comprising PDE5 inhibitor for inhibiting apoptosis of nerve cells	Aribio Inc, Sk Chemicals Co Ltd	Kim MH, Choung JJ, Ku SK
PDE6			
JP2019047763A (12/09/2017)	Rhodopsin PDE as an optogenetic tool for light control of intracellular cyclic nucleotides	Nagoya Institute Of Technology	Kandori H, Tsunoda SP, Yoshida K
CN107287239 (11/04/2016)	Gene therapy vector and medicine (adenovirus encoding PDE6B) for retinal pigment degeneration	Shenyang Fuming Biological Tech Co Ltd	Pang J
PDE7			
WO2018055140 (23/09/2016)	T cells with increased immunosuppression resistance [expressing PDE4C or 7A for the treatment of cancer]	Adaptimmune Ltd	Laugel B, Skibbe K

Patent # (Priority date d/m/y)	Subject	Assignee	Author
PDE9			
WO2017070293 (20/10/2015)	PDE9 inhibitor and levodopa therapy for treating PD or Parkinsonism	Ironwood Pharmaceuticals Inc	Leventhal L, Townsend TM
PDE10			
WO2019067955 (29/07/2017)	Compositions and methods for regulating let-7 microRNA targets, such as PDE10A, for treatment of cancers	Univ California	Roos M, Lowry W

Source: Espacenet and Google patents (accessed 05/28/19)