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REVIEW

A review of prostaglandin analogs in the management of patients with pulmonary arterial hypertension

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Summary
Pulmonary arterial hypertension is a chronic, progressive disease characterized by elevation of pulmonary artery pressure and pulmonary vascular resistance that ultimately results in right ventricular failure and death. Multiple mechanisms are involved in the pathogenesis of pulmonary arterial hypertension, including prostacyclin, endothelin-1, and nitric oxide pathways amongst others. The first agent to be approved for the treatment of pulmonary arterial hypertension was synthetic prostacyclin (epoprostenol), followed by prostaglandin analogs (iloprost, treprostinil, and beraprost [Japan and Korea]), which act on prostaglandin receptors. This article reviews the physiology and pathophysiology of prostanoids, summarizes key clinical studies of prostaglandin analogs for the treatment of pulmonary arterial hypertension, and discusses important pharmacokinetic and pharmacodynamic distinctions between the various prostaglandin analogs. Different prostaglandin analogs have disparate binding affinities for the various prostaglandin receptors and different G-protein-coupled receptor interactions, which may result in varying clinical efficacy and safety depending on the target tissue. Differences in formulation, route of administration, effectiveness, and safety may all play a role in deciding which prostaglandin analog to prescribe for an individual patient. Head-to-head studies will be needed to confirm differences in efficacy and safety for the various prostaglandin analogs.

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Introduction

Pulmonary arterial hypertension (PAH) is a chronic, progressive disease characterized by elevation of pulmonary artery pressure (PAP) and pulmonary vascular resistance (PVR), which ultimately results in right ventricular failure and death.[1] The histopathology of PAH involves changes in both the pulmonary vasculature and the right ventricle that include vasoconstriction, remodeling, and thrombosis *in situ*. Pulmonary arterial hypertension is an incurable disease that is associated with a poor prognosis. In a National Institutes of Health (NIH) sponsored registry including 194 patients with PAH in the 1980s, the estimated median survival was 2.8 years (95% confidence interval [CI], 1.9—3.7 years) and the 1-year survival rate was 68% (95% CI, 61—75%).[2]

Since the time of the original NIH registry and study, treatment options have improved by targeting pathways involved in the pathogenesis of PAH. Each approved therapy with indications for PAH targets one of the following three signaling pathways: prostacyclin, endothelin-1, or nitric oxide.[1] The first agent to be approved by the United States Food and Drug Administration (FDA) for PAH was synthetic prostacyclin (epoprostenol). Intravenous epoprostenol was approved by the FDA in 1995, followed by subcutaneous treprostinil in 2002, intravenous treprostinil in 2004, and inhaled iloprost in 2004. Oral and inhaled formulations of treprostinil and a sustained-release formulation of iloprost are currently in development.[3] A non-prostanoid prostacyclin receptor agonist, NS-304/ACT-293987, is also being developed.[4] The second class of therapeutic agents developed for the treatment of PAH are endothelin receptor antagonists (ERAs) and include bosentan, ambrisentan, and sitaxsentan. The third class of drugs for the treatment of PAH are phosphodiesterase-5 (PDE5) inhibitors, which target the nitric oxide pathway and include sildenafil and tadalafil. Even as novel therapies that target the endothelin receptor and nitric oxide pathways are developed, prostaglandin-based therapies continue to be the front-line therapy for functional class IV patients with PAH.[5,6]

Although early diagnosis (and lead-time bias) may have contributed to better outcomes, these new treatment options have been credited with improving the prognosis for patients with PAH since the late 1980s, with current 1-year survival rates ranging from 85% to 97%.[7—11] The objectives of this manuscript are to review the physiology and pathophysiology of prostanoids, including immune effects, as well as to review some of the clinical studies for the various prostaglandin analogs for the treatment of PAH. This manuscript reviews the prostaglandins that play a role in the pulmonary circulation, including those approved for PAH and those currently in clinical development. Since different analogs may have heterogeneous affinities for the various prostaglandin receptors, there may be differences in the mechanisms of action of these prostaglandin analogs, especially since the route of administration may be different.

Prostanoids and prostanoid receptors

Definitions

Eicosanoids are signaling molecules derived from twenty-carbon essential fatty acids. Prostanoids are the naturally occurring subclass of eicosanoids consisting of the prostaglandins and the thromboxanes. Prostanoids are arachidonic acid metabolites[12] that can be classified into prostaglandins (PG), which contain a cyclopentane ring, and thromboxanes, which contain a cyclohexane ring. The metabolism of arachidonic acid is shown in Fig. 1.

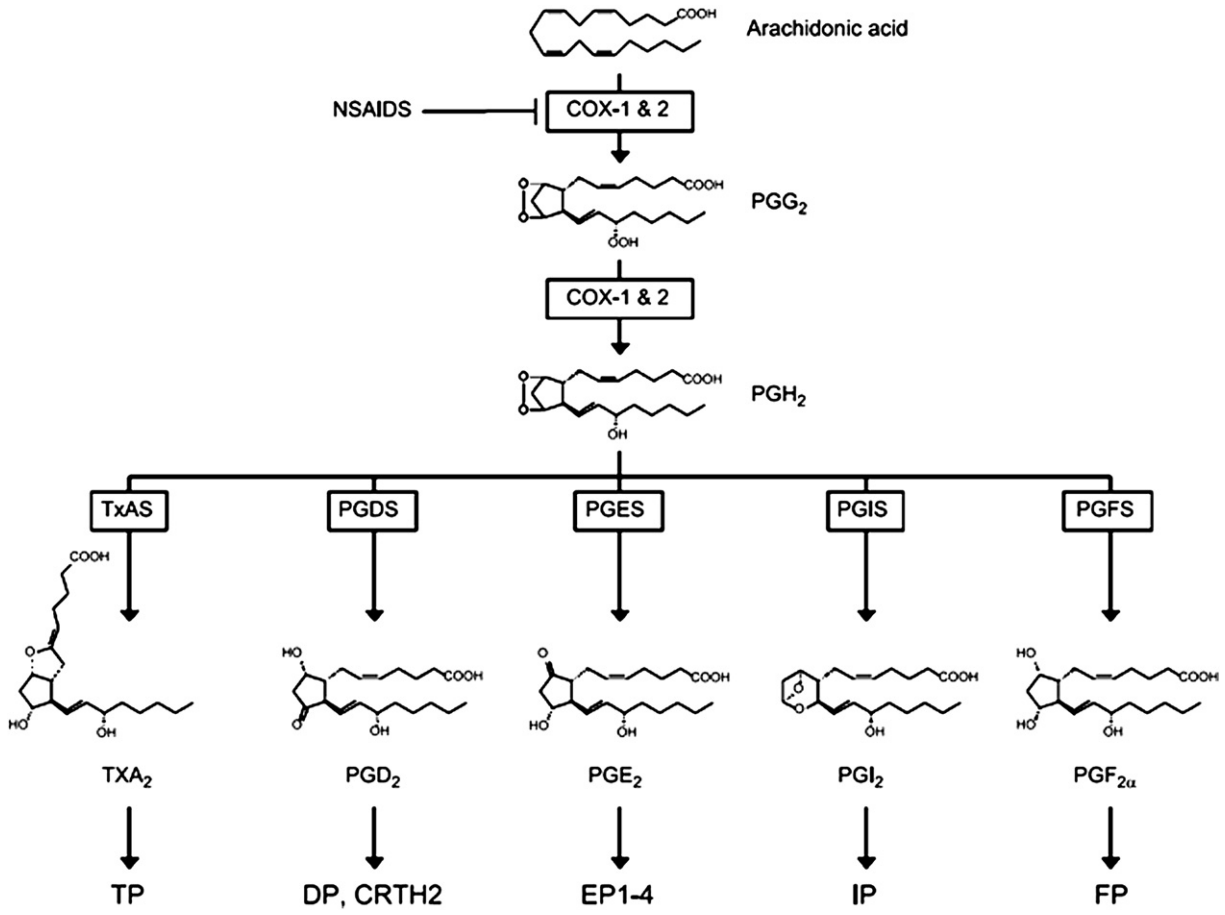

Figure 1 Biosynthesis of prostanoids. Arachidonic acid is metabolized by cyclooxygenase (COX)-1 or -2 to the unstable endoperoxide PGH_2, the common precursor for the five principal prostaglandins. Thromboxane A2, PGD_2, PGE_2, PGI_2, and PGF_{2a} are generated by individual prostaglandin synthase enzymes (TxAS, PGDS, PGES, PGIS, and PGFS) and elicit their biological effects by activating cell surface G-protein-coupled receptors. NSAIDs, non-steroidal anti-inflammatory drugs; PGDS, prostaglandin D synthase; PGES, prostaglandin E synthase; PGIS, prostaglandin I synthase; PGFS, prostaglandin F synthase; TXA_2, thromboxane A_2. Reprinted from *Pharmacol Ther*, 103(2), Hata AN and Breyer RM, Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation, 20, Copyright (2004), with permission from Elsevier.[26]

Naturally occurring prostaglandins can be subdivided into prostaglandin D (PGD), E (PGE), F (PGF), H (PGH), and I (PGI, prostacyclin). The abbreviations are commonly followed by an index (for instance PGE_2), which indicates the number of double bonds present in the various side chains attached to the cyclopentane/cyclohexane ring. Prostaglandin analogs used clinically are listed in Table 1. A summary of the physiological roles of various prostanoids in major organ systems is shown in Table 2.

There are currently nine known prostanoid receptors on various cell types, which are termed DP_{1-2}, EP_{1-4}, FD, IP, and TP (Table 3). These are seven-transmembrane G-protein-coupled receptors (GPCR) that are named for the prostanoids that bind most readily to them; however, cross-binding affinities are largely unknown. Prostanoid receptors can be grouped into different categories, based on the type of heterotrimeric G-protein activated by the different receptors, and thus the cellular response evoked.[12] The first category consists of the relaxant receptors, IP, EP_2, EP_4, and DP, which, in general, activate the G-protein G_s, which stimulates cyclic adenosine monophosphate (cAMP) production by adenylate cyclase. The second category includes the contractile type of prostanoid receptors, TP, EP_1, and FP, which activate the G-protein G_q, mediating enhanced intracellular Ca^{2+} levels by influencing phosphatidylinositol turnover.

The G-protein that is activated by a specific prostanoid receptor may sometimes differ between cell types and may depend upon ligand concentration. For instance, IP, which normally activates G_s, is also capable of activating G_q if the concentration of IP is high enough and has also been suggested to couple to G_i in some cases.[13]

Thromboxane and TP

Thromboxane A_2 (TXA_2) has multiple actions on cardiovascular function. It affects platelet shape and aggregation and defective TP receptor signaling has been linked to bleeding disorders.[14] Increased thromboxane synthesis is associated with acute myocardial ischemia[15] and heart failure.[16] Thromboxane A_2 is also a potent bronchoconstrictor, stimulating airway smooth muscle cell proliferation and potentially leading to asthma.[17] In humans, the TP receptor exists as two

Table 1 Prostaglandin analogs used clinically.

Name	Mechanism of action	Physiologic use(s)
Alprostadil	Prostaglandin E_1 agonist	Erectile dysfunction[a]
Beraprost	**Prostacyclin agonist**	**Arterial occlusive disorders, peripheral vascular disorders, pulmonary hypertension, renal failure**
Bimatoprost	Prostaglandin F_2 alpha agonist	Glaucoma,[a] ocular hypertension[a]
Enprostil	Prostaglandin E_2 agonist	Peptic ulcer
Iloprost	**Prostacyclin agonist**	**Arterial occlusive disorders, Buerger's disease, heart failure, ischemic heart disorders, peripheral vascular disorders, pulmonary arterial hypertension,[a] Raynaud's disease**
Latanoprost	Prostaglandin F_2 alpha agonist	Glaucoma,[a] ocular hypertension[a]
Lubiprostone	Chloride channel agonist	Constipation,[a] irritable bowel syndrome[a]
Misoprostol	Prostaglandin E_1 agonist	Ulcer prevention[a]
Travoprost	Prostaglandin F_2 alpha agonist	Glaucoma,[a] ocular hypertension[a]
Treprostinil	**Prostacyclin agonist**	**Peripheral vascular disorders, pulmonary fibrosis, pulmonary arterial hypertension,[a] transplantation**
Unoprostone	Prostaglandin F_2 alpha agonist	Glaucoma,[a] ocular hypertension[a]

[a] Indication(s) approved by the United States Food and Drug Administration. Analogs with indications specific for PAH or pursuing indications for PAH are bolded.

alternatively spliced variants that may couple to different G-proteins.

Prostacyclin and IP

Prostacyclin is produced by vascular endothelial cells[18] and inhibits vascular smooth muscle cell (VSMC) proliferation and migration,[19] with VSMC proliferation being a major factor in the development of vascular disease. Prostacyclin also plays an important role as an endogenous regulator of vascular homeostasis, inhibits platelet aggregation, stimulates VSMC relaxation, and regulates VSMC differentiation.[19] Prostacyclin also has cardioprotective effects during ischemia-reperfusion injury[20] and is also an important mediator of acute inflammation and inflammatory pain transmission.[21,22]

The IP receptor predominantly couples to the G_s type protein leading to an increase in cAMP, which results in vasodilatory and anti-aggregatory effects (Table 3). The IP receptor can also couple to other G-proteins due to C-terminal modification (G_i and G_q).[23] The IP receptor is thought to undergo agonist-induced desensitization *in vivo*,[24] and agonist-induced internalization and sequestration independently from desensitization.[25] These processes may affect the long-term effectiveness of prostaglandin analogs.

PGE_2 and EP_{1-4}

PGE_2 is the most widely produced prostaglandin in the body and exhibits the most versatile actions. It exerts both pro- and anti-inflammatory effects depending on the receptor subtype, cell population, and context of activation.[26] Therefore, PGE_2 may have either dilator or constrictor effects on vascular smooth muscle.[27,28]

The many different physiological effects of PGE_2 may be accounted for in part by the existence of the four EP receptors (EP_1, EP_2, EP_3, and EP_4) and heterogeneity in the coupling of these receptors to intracellular signal transduction pathways

Table 2 Summary of prostaglandins and their physiologic roles in major organ systems.

System	Mediator(s)	Major Site(s) of Synthesis	Primary Effect(s)
Cardiovascular	Prostacyclin	Endothelial cells	Vasodilatation
	Thromboxane	Platelets	Vasoconstriction
Renal	Prostacyclin	Renal cortex	Vasodilatation
	PGE_2	Renal medulla	Salt and water excretion
Gastrointestinal	PGE_2	Gastric mucosa	Cytoprotection
Hematologic	Prostacyclin	Endothelial cells	Platelet deaggregation
	Thromboxane	Platelets	Platelet aggregation
Respiratory	Prostacyclin	Endothelial cells	Vasodilatation
Musculoskeletal	PGE_2	Osteoblasts	Bone resorption,bone formation
Reproductive	PGE_2	Seminal vesicles	Erection, ejaculation, sperm transport
	PGE_2, PGF_2	Fetal membranes	Parturition/labor, menstruation,
		Uterus	fertilization, ovulation
Neurologic	PGE_2	Unknown	Fever, hyperalgesia

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PGE_2, prostaglandin E_2; and PGF_2, prostaglandin F_2.

Table 3 Signal Transduction of Prostanoid Receptors.

Type	Subtype	Isoform	G protein	Second messenger
DP			G_s	cAMP ↑
EP	EP_1		Unidentified	Ca^{2+} ↑
	EP_2		G_s	cAMP ↑
	EP_4		G_s	cAMP ↑
	EP_3	EP_{3A}	G_i	cAMP ↓
		EP_{3B}	G_s	cAMP ↑
		EP_{3C}	G_s	cAMP ↑
		EP_{3D}	G_i, G_s, G_q	cAMP ↓, cAMP ↑, PI response
FP			G_q	cAMP ↑, PI response
IP			G_s, G_q	cAMP ↑, PI response
TP		TPα	G_q, G_i	PI response, cAMP ↓
		TPβ	G_q, G_s	PI response, cAMP ↑

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cAMP, cyclic adenosine monophosphate; PI, phosphatidylino sitol; ↑ = increase; and ↓ = decrease.

(Table 3). Of the four EP receptors, the EP_3 and EP_4 receptors bind PGE_2 with highest affinity ($K_d < 1$ nM), whereas the EP_1 and EP_2 receptors bind with lower affinity ($K_d > 10$ nM).[29]

The EP_3 receptor has multiple splice variants in its C-terminal tail that cause differential activation of signal transduction pathways.[26] The EP_2 receptor plays a role in ovulation and fertilization, facilitates pain transmission by abolishing glycinergic inhibition, and mediates joint inflammation in collagen-induced arthritis.[30] The EP_4 receptor facilitates closure of ductus arteriosus,[31] induces bone formation, mediates joint inflammation in collagen-induced arthritis, protects against inflammatory bowel disease, and facilitates Langerhans cell migration and maturation.[30]

PGD_2 and DP

PGD_2 is the predominant prostaglandin produced by activated mast cells, which initiate IgE-mediated type I acute allergic responses.[32] PGD_2 may also exacerbate nonimmune pathological processes associated with asthma, such as airway remodeling and bronchoconstriction.[26] The effects of PGD_2 result from binding to two different GPCRs—the DP receptor and the $CRTH_2$ receptor, a seven-transmembrane, G-protein coupled, chemoattractant receptor-homologous molecule expressed on Th2 cells.

PGF_2 and FP

Prostaglandin $F_{2\alpha}$ is produced during the menstrual cycle by secretory endometrium.[33] Changes in $PGF_{2\alpha}$ production have been linked to abnormal menstrual bleeding.[34] $PGF_{2\alpha}$ also affects renal function,[35] cardiac hypertrophy,[36] and intraocular pressure.[37] Selective FP agonists such as latanoprost have been approved by the US FDA for the treatment of ocular hypertension.[38] The FP receptor is the least selective of the prostanoid receptors in binding the endogenous prostaglandins, binding both PGD_2 and PGE_2 at nanomolar concentrations.[29]

Prostanoid receptors and the lung

In the lung, four different prostanoid receptors have been identified that play a role in regulating vascular tone, platelet activation, and immunological cell responses. Vasoconstriction is produced by TX receptors via a signaling pathway coupled with phospholipase C activation and Ca^{2+} mobilization.[39] EP_3 receptors also produce vasoconstriction through two different mechanisms: phosphoinositide metabolism and Ca^{2+} release via G_q proteins and inhibition of cAMP via G_i proteins.[40] The remaining two prostanoid receptors, IP and EP_2, increase plasma cAMP and have vasodilatory effects. Activation of both receptors produces increased intracellular cAMP levels, resulting in the opening of Ca^{2+}-activated K^+ channels, cellular hyperpolarization, and vasodilatation.[41–43]

The distribution of these various prostanoid receptors is not homogeneous throughout the human pulmonary vascular system. The IP receptor is expressed in smooth muscle cells in pulmonary arteries and veins.[43] On the other hand, EP_2 receptors that mediate vasorelaxation are predominantly expressed in pulmonary veins.[44] These distribution differences may influence the results observed in clinical studies for various prostaglandin analogs.

Prostaglandin analogs and immunosuppression

Although clinical studies of infection and prostaglandin analog use are few, there has been a growing body of evidence to implicate immunosuppression in experimental models of prostaglandin analog therapy. Treprostinil inhibits human mixed lymphocyte culture responses similar to cyclosporine,[45] possibly via its agonism of the EP_2 receptor.[46] Phagocytosis of Micrococcus species can be inhibited by prostaglandin analogs, possibly via their agonism on the IP receptor.[47] The differential effects of various prostaglandin analogs (iloprost, carbaprostacyclin, and treprostinil) on macrophage function in rats were recently reported.[46] In this *in vitro* study, the effects of the prostacyclin analogs on the regulation of phagocytosis, bacterial killing, and inflammatory mediator production were determined in alveolar macrophage and peritoneal macrophage populations. Treprostinil inhibited phagocytosis, bacterial killing, and cytokine generation in alveolar macrophages to a much greater degree than the other two prostaglandin analogs. Thus, there are differences in the immunoregulatory properties of these prostaglandin analogs, which may hold important consequences regarding the risk of infection for patients receiving these drugs. Furthermore, differences in receptor affinity may predispose patients receiving chronic prostaglandin therapy to heterogeneous varieties of immunosuppression.

Serious gram-negative bloodstream infections have recently been observed in patients receiving chronic intravenous treprostinil.[48,49] Similar bloodstream infections have been noted with intravenous epoprostenol; however, those have predominantly been of the gram-positive variety, in particular *Micrococcus* species.[50] Micrococcus infections are usually only seen in immunocompromised hosts. Such infections have not been noted with subcutaneous treprostinil. These differences may be due to the higher dose of

treprostinil usually administered via the intravenous route or to differences in catheter care and injection site (intravenous versus subcutaneous). Nonetheless, the differences in bacteriology suggest that there may be immune consequences of chronic prostaglandin analog therapy. This view is buttressed by reports of sepsis with analogs of prostaglandin E_1, including misoprostol[51] and alprostadil,[52] even in the absence of an intravenous catheter.

Prostacyclin and prostaglandin analogs

The vasodilatory effects of prostacyclin constitute the major mechanism of action in PAH. Epoprostenol, a freeze-dried synthetic preparation of prostacyclin, binds to the IP receptor. Prostaglandin analogs have been produced by various companies and these include iloprost, treprostinil, and beraprost (Fig. 2). These analogs are available in different formulations and have different binding affinities for the various prostanoid receptors. Iloprost binds to the IP, EP_1, EP_3 and EP_4 receptors,[53,54] treprostinil binds to the IP[55] and EP_2[46] receptors, and beraprost binds to the IP and EP_3 receptors (Fig. 3).[53] Activity of these analogs on other prostanoid receptors remains unknown.

Epoprostenol

Epoprostenol must be administered by continuous intravenous infusion because of its short half-life of 3–5 min and its instability at pH values <10.5.[56,57] Intravenous administration of the drug requires a permanently implanted central venous catheter and a portable infusion pump, as well as refrigeration of the drug in its high pH glycine buffer during administration. An additional requirement is the fact that the infusion has to be prepared every 24 h. Due to its chemical instability, the pharmacokinetics of epoprostenol are difficult to determine. Recently, a formulation of epoprostenol that uses a higher pH, and therefore affords greater chemical stability, has become available.[58] This new formulation is stable for 24 h without refrigeration at concentrations ≥15,000 ng/mL.

One of the clinical studies that led to US FDA approval of epoprostenol for treatment of PAH was a 12-week prospective, randomized, multicenter, placebo-controlled, open trial that compared the effects of intravenous epoprostenol plus conventional therapy with those of conventional therapy alone in 81 patients with severe PAH (New York Heart Association [NYHA] class III or IV).[59] Exercise capacity (assessed by the 6-min walk distance [6MWD]) at 12 weeks was improved in the 41 patients treated with epoprostenol, but it decreased in the 40 patients treated with conventional therapy alone ($P < 0.002$ for the comparison of the treatment groups). Hemodynamics significantly improved at 12 weeks in the epoprostenol-treated patients (mean PAP, $P < 0.002$; PVR, $P < 0.001$). Serious adverse events included four episodes of catheter-related sepsis and one thrombotic event. Eight deaths occurred during the study, all of whom were receiving conventional therapy ($P = 0.003$). Thus, the continuous intravenous infusion of epoprostenol produced symptomatic and hemodynamic improvement, as well as improved survival in patients with severe PAH. In addition to this study, several subsequent clinical trials have extensively studied the safety profile of long-term administration of epoprostenol.[8,24,60–63]

A randomized, open-label, controlled trial examined the effects of treatment with epoprostenol in 111 patients with PAH associated with scleroderma.[64] Treatment with epoprostenol for 12 weeks improved exercise capacity (change from baseline in median 6MWD, +46 m) but decreased with conventional therapy (change from baseline in median 6MWD, −48 m; $P < 0.001$). Improvement in NYHA functional class only occurred in patients treated with epoprostenol ($N = 21$). There was no significant difference in mortality between the two groups (epoprostenol, four deaths vs conventional therapy, five deaths; *P*-value not significant). Side effects of epoprostenol therapy included jaw pain, nausea, and anorexia. Adverse events that were reported and were related to the epoprostenol delivery system included sepsis, cellulitis, hemorrhage, and pneumothorax (4% for each).

Intravenous epoprostenol requires dose titration to overcome tachyphylaxis. A typical patient may require uptitration for 6 months to reach a dose up to 30–40 ng/kg/min. The American College of Chest Physicians (ACCP) and European Society of Cardiology (ESC) recommend IV epoprostenol in patients with NYHA functional class III and IV (evidence: A).[5,65] Systemic side effects related to the long-term administration of epoprostenol include flushing, warmth, headaches, photosensitivity, jaw pain, abdominal pain, and diarrhea.[66] There may be recurrent infections of the intravenous catheter.[60] Tolerance develops during long-term use of the drug, leading to progressive increases in the dose for several months after initiation.

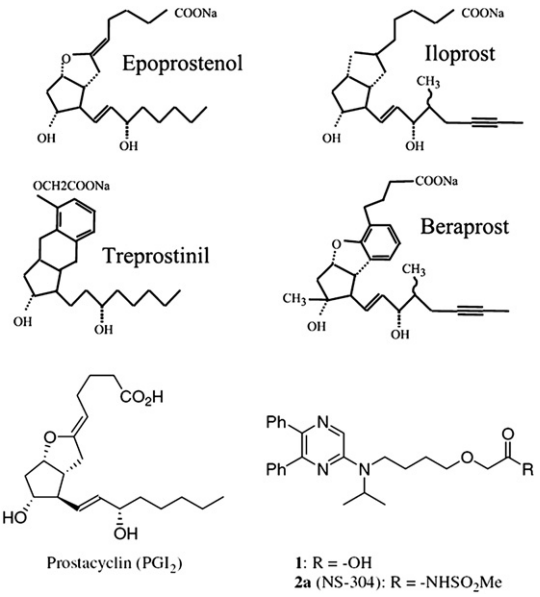

Figure 2 Chemical structure of prostacyclin, epoprostenol, its stable analogs, and a prostacyclin receptor agonist. 1 = MRE-269; 2a = NS-304. Reprinted from *Pharmacol Ther*, 102(2), Olschewski H et al., Prostacyclin and its analogues in the treatment of pulmonary hypertension, 15, Copyright (2004)[43] and *Bioorg Med Chem*, Nakamura A et al, Synthesis and evaluation of *N*-acylsulfonamide and *N*-acylsulfonylurea prodrugs of a prostacyclin receptor agonist, Copyright (2007), with permission from Elsevier.[89]

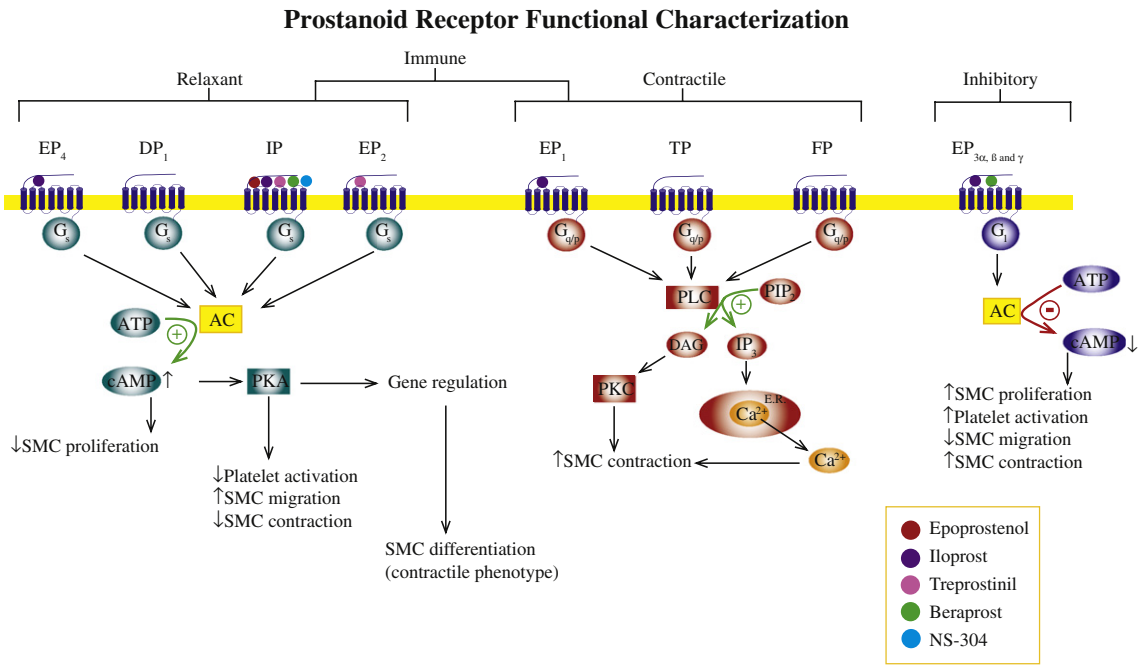

Figure 3 Functional characterization of some of the prostanoid receptors, showing the binding characteristics of different prostaglandin analogs. AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; IP_3, inositol triphosphate; PIP_2, phosphatidylinositol 4,5-biphosphate; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; SMC, smooth muscle cell.

Iloprost

Iloprost is a carbacyclin analog of prostacyclin that has a plasma half-life of 20–30 min. It is available in different countries in intravenous or inhaled formulations. Inhaled iloprost causes pulmonary vasodilation that lasts for 1–2 h[67] and has been approved for the treatment of PAH in Australia, Austria, France, Germany, Ireland, Israel, Italy, the Netherlands, New Zealand, Norway, Switzerland, the United Kingdom, and the USA. The intravenous formulation is approved in New Zealand for PAH and is used elsewhere off-label for PAH. Intravenous iloprost has received regulatory approval in Europe for the treatment of thromboangiitis obliterans.

The pharmacokinetics of a standardized iloprost aerosol dose (5 μg; inhaled within approximately 10 min) was studied in 12 patients with severe pulmonary hypertension (PH) in a crossover design employing three different nebulizers (I-Neb/Aerotrap [Nebu-Tec; Elsenfeld, Germany], Ventstream [Medic-Aid Limited; Bognor Regis, UK], and HaloLite [Profile Therapeutics Ltd.; Bognor Regis, UK]).[68] All three nebulizers produced similar decreases in PVR and PAP. Iloprost entered rapidly into the systemic circulation, with peak concentrations occurring immediately after termination of the inhalation maneuver. The half-lives of serum levels of iloprost ranged from 6.5 min to 9.4 min; however, the ''half-life'' of the pharmacodynamic effects in the pulmonary vasculature (i.e., decrease in PVR) was longer and ranged between 21 and 25 min. An *in vitro* study suggests that tolerance to iloprost may develop rapidly.[69] In perfused rabbit lungs, the pulmonary vasodilatory response to aerosolized iloprost after 3–3.5 h had leveled off, whereas two-thirds of the maximum iloprost perfusate levels were still present.

One of the clinical studies that led to US FDA approval of inhaled iloprost for the treatment of PAH was a 12-week, randomized, placebo-controlled study in 203 patients with severe PAH or chronic thromboembolic PH.[70] The patients received daily inhalations of 2.5 or 5.0 μg of iloprost (six to nine times per day; median inhaled dose, 30 μg per day) or inhalation of placebo. The primary endpoint consisted of a composite endpoint of patient improvement where each patient had to experience an increase of at least 10% in the 6MWD with an improvement in NYHA class by at least one class in the absence of clinical deterioration or death. This clinical response endpoint was met by 17% of the patients receiving iloprost, as compared with 5% of the patients receiving placebo ($P = 0.007$). Hemodynamic values were significantly improved at 12 weeks when measured after iloprost inhalation ($P < 0.001$) and were significantly worse in the placebo group. The most common adverse event was flushing.

Combination therapy of inhaled iloprost with oral bosentan has been studied in 67 patients with PAH.[71] In a randomized, multicenter, double-blind trial, inhaled iloprost (5 μg) or placebo was added to monotherapy with bosentan for 12 weeks. This was primarily a safety study, and secondary efficacy endpoints included change from baseline in 6MWD, NYHA functional class, hemodynamic parameters, and time to clinical worsening. After 12 weeks, patients receiving iloprost had a placebo-adjusted difference in 6MWD of +26 m ($P = 0.051$). The NYHA status improved by one class in 34% of iloprost versus 6% of placebo patients ($P = 0.002$). Treatment with iloprost significantly delayed the time to clinical worsening ($P = 0.0219$). Improvements were noted in post-inhalation placebo-adjusted hemodynamics (mean PAP, $P < 0.001$; PVR, $P < 0.001$). No significant adverse events

were reported. However, these data could not be replicated in a similar study where patients on bosentan were randomized to inhaled iloprost versus placebo. The study was terminated earlier because interim statistical analysis demonstrated futility of outcome.[72]

Combination therapy of inhaled iloprost with oral sildenafil has also been studied. In a randomized, controlled, open-label trial, 30 patients with severe PH were assigned to receive 12.5 mg of oral sildenafil, 50 mg of sildenafil, 12.5 mg of sildenafil plus inhaled iloprost, or 50 mg of sildenafil plus inhaled iloprost.[73] The efficacy endpoints were hemodynamic measurements (PAP, pulmonary arterial occlusion pressure, cardiac output, central venous pressure). The rank order of pulmonary vasodilatory potency (maximum reduction of PVR and increase in cardiac index) was the following: 50 mg of sildenafil plus iloprost > 12.5 mg of sildenafil plus iloprost > iloprost alone, and 50 mg of sildenafil > 12.5 mg of sildenafil and nitric oxide. No serious adverse events were reported. The authors concluded that oral sildenafil acts synergistically with inhaled iloprost to cause pulmonary vasodilatation in severe PH.

The dose of inhaled iloprost is not uptitrated, perhaps because intermittent low dose delivery does not engender tachyphylaxis. The inhaled dose of approximately 0.37 ng/kg/min is considerably lower than when iloprost is used intravenously (off-label) for PAH at about 5 ng/kg/min. The ACCP and ESC recommend inhaled iloprost in patients with NYHA functional class III (ACCP evidence, A; ESC evidence, B) and functional class IV (ACCP evidence, B; ESC evidence, C).[5,65]

Treprostinil

Treprostinil is a prostaglandin analog that is chemically stable at room temperature and has a neutral pH. It has a relatively long terminal half-life but a relatively short effective half-life. It has been approved for the treatment of PAH in Australia, Brazil, Canada, the European Union, Israel, Switzerland, Taiwan, and the USA. Subcutaneous formulation of treprostinil was approved by the FDA in 2002. Subsequently, the same formulation was approved for intravenous use based upon bioequivalence in normal volunteers, without any patient study.

Several studies have examined the pharmacokinetics of treprostinil. The bioequivalence of intravenous and subcutaneous administration of treprostinil to normal volunteers has been studied using a randomized, two-period, crossover study design.[74] Each subject was dosed at 10 ng/kg/min for 72 h by each route, with the infusions separated by a 4-day washout period. The steady-state ratios of the geometric means (intravenous/subcutaneous) for the area under the concentration-time curve and the maximum plasma concentration indicate bioequivalence. The study also showed that, at steady state, the *elimination* half-life of treprostinil was 4.4 and 4.6 h following intravenous and subcutaneous administration, respectively, but the *effective* half-life was less than 10 min and 60 min, respectively. In another study conducted in 15 healthy volunteers, the mean apparent *elimination* half-life of treprostinil (infused at 15 ng/kg/min over 150 min) following subcutaneous administration was 1.38 h, compared to 0.87 h following intravenous administration.[75] Finally, in another study, dose proportionality was shown for treprostinil administered by continuous subcutaneous and intravenous infusion at doses up to 125 ng/kg/min.[76]

A 12-week, double-blind, placebo-controlled multicenter trial was conducted in 470 patients with PAH.[77] The primary endpoint was exercise capacity as given by the 6MWD. Exercise capacity improved with treprostinil and was unchanged with placebo; the between-treatment group difference in median 6MWD was +16 m ($P = 0.006$). Treatment with treprostinil significantly improved indices of dyspnea, signs and symptoms of PH, and hemodynamics. The most common side effect attributed to treprostinil was infusion site pain (85% versus 27% for placebo) leading to premature discontinuation from the study in 8% of patients. Infusion system malfunctions were common and occurred in 24% of the patients in the treprostinil group and in 33% of the patients in the placebo group.

Pilot studies were conducted to evaluate the effects of treprostinil for the management of PAH.[78] In the first trial, intravenous epoprostenol and intravenous treprostinil were compared, with both treatments producing similar acute decreases in PVR (22% and 20%, respectively). In the second trial, intravenous and subcutaneous treprostinil were compared, with both treatments demonstrating similar decreases in PVR (23% and 28%, respectively). In the third 8-week trial, a multicenter, double-blinded, parallel study, subcutaneous treprostinil was compared with placebo infusion in 26 patients. Two patients in the treprostinil group (12%) withdrew from the study because of intolerable side effects (hypotension and intolerable pain at the infusion site). Although not statistically significant, the 6MWD demonstrated a mean improvement of 37 m in patients receiving treprostinil compared with a 6-m reduction in those receiving placebo. Treatment with treprostinil had favorable effects on 6MWD and hemodynamics; however, the differences were not statistically significant. Adverse effects reported included headache, diarrhea, flushing, jaw pain, and foot pain. Injection site reactions, such as erythema (94%) and pain (88%), were frequently observed with subcutaneous treprostinil.

The typical dose of treprostinil when used subcutaneously or intravenously varies from 50 to 100 ng/kg/min. An inhaled dose of 45 μg four times a day equals approximately 1.8 ng/kg/min. The ACCP recommends SC and IV treprostinil in patients with NYHA functional class II (evidence: C and C, respectively), functional class III (evidence: B and C, respectively), and functional class IV (evidence: C and C, respectively)[5] whereas the ESC has equal recommendations for SC and IV treprostinil in patients with NYHA functional class III and IV (evidence: B).[65]

Transitioning long-term epoprostenol patients to IV or SC treprostinil has been investigated. It has been shown that both pediatric and adult patients with PAH who have received IV epoprostenol long-term can be successfully transitioned to IV or SC treprostinil.[79–82] This transition can be rapid (over 24–48 h) or slow (up to 14 days). The safety profile of long-term treprostinil has been shown to be consistent with short-term treprostinil treatment,[83] and the side effects are reported to be much less than those with epoprostenol.[79–83]

Inhaled treprostinil is currently being studied in the TReprostinil Sodium Inhalation Used in the Management of Pulmonary Arterial Hypertension trial (TRIUMPH-1).

Preliminary results of this phase III, 12-week, multicenter, double-blind, randomized, placebo-controlled study in 235 NYHA class III and IV patients with PAH were recently reported in abstract format.[84] Treprostinil (up to 45 μg four times daily) or placebo was inhaled, and the primary endpoint was the change at 12 weeks from baseline in the 6MWD. The peak (10–60 min after inhalation) median change in 6MWD was +20 m compared with placebo ($P < 0.006$) and the placebo-subtracted trough (>4 h after inhalation) change was +14 m. However, there was no improvement in time to clinical worsening or functional class. A serum biomarker, B-type natriuretic peptide, was significantly decreased indicating improvement in right ventricular stretch.

A sustained-release formulation of oral treprostinil is currently under investigation in phase III trials. Results of the FREEDOM-C trial (combination therapy with sildenafil or bosentan) were recently released.[85] The improvement in 6MWD was only 11 m and did not reach statistical significance. Results of the FREEDOM-M trial (monotherapy) have yet to be reported.

Beraprost

Beraprost is an orally administered prostaglandin analog with a half-life in healthy adults of 0.9 to 1.1 h.[57] It has been approved for the treatment of PAH in Japan and South Korea, and phase II studies of a sustained-release formulation are being conducted in the US.

The pharmacokinetics and pharmacodynamics (inhibition of platelet aggregation) of oral beraprost were studied in 12 healthy volunteers in a double-blind, placebo-controlled, dose-escalating crossover study.[86] Beraprost (20, 40, 60 μg) and placebo were administered acutely and repeated three times daily for 3 days. Inhibition of platelet aggregation occurred 1 h after single doses and 0.5–1.0 h after repeated doses for the 40 and 60 μg doses. The terminal half-life of the active enantiomer of beraprost ranged from 0.50 to 0.91 h. The side effects reported were moderate headaches (7 of 12 after single and 8 of 12 after repeated doses) and flushes (6 of 12 and 7 of 12, respectively).

In a double-blind, placebo-controlled study, 130 patients with PAH were randomized to oral beraprost (median dose 80 μg four times a day) or to placebo for 12 weeks.[87] The primary endpoint was the change in 6MWD, and secondary endpoints included changes in hemodynamics and NYHA functional class. The difference between treatment groups in the mean change of 6MWD at Week 12 was +25 m ($P = 0.036$). Improvement in 6MWD only occurred for patients with idiopathic PAH and not PAH associated with different conditions. There were no statistically significant changes in cardiopulmonary hemodynamics or NYHA functional class. Drug-related adverse events, such as headache, flushing, jaw pain, and diarrhea, were more common in the titration phase and decreased in the maintenance period.

In a 12-month double-blind, randomized, placebo-controlled study, a total of 116 patients with World Health Organization functional class II or III idiopathic PAH or PAH related to either collagen vascular diseases or congenital systemic to pulmonary shunts received either oral beraprost (median dose 120 μg four times a day) or placebo.[88] The primary endpoint was disease progression (defined as death, transplantation, epoprostenol rescue, or a >25% decrease in peak oxygen consumption). Patients treated with beraprost exhibited significantly less disease progression at 6 months ($P = 0.002$), but not at shorter or longer follow-up intervals. Similarly, beraprost-treated patients had significantly improved 6MWD at 3 months and at 6 months ($P = 0.010$ and 0.016, respectively) compared with placebo, but not at either 9 or 12 months. Drug-related adverse events were common and were related to the disease or to expected prostacyclin adverse events. These results suggest that treatment with beraprost may produce beneficial effects early but that this effect is not sustained.

NS-304/ACT-293987

NS-304/ACT-293987 is a prostacyclin receptor agonist that is chemically dissimilar from prostanoids. It is a pro-drug that has low affinity for the IP receptor natively, but is metabolized in hepatic microsomes to MRE-269, which selectively binds the IP receptor. By this metabolic step, the native compound avoids causing gastrointestinal side effects when given orally, and leads to sustained release of MRE-269 with a longer half-life.[89]

Preclinical studies of MRE-269 in rats, dogs, and monkeys showed that the compound displayed high oral bioavailability (rat, 102%; dog, 80%) and a long half-life (rat, 3.6 h; dog, 6.2 h; monkey, 5.6 h).[90] MRE-269 has a 130-fold higher affinity for the IP receptor than for the other human prostanoid receptors.[4] NS-304/ACT-293987 is also highly selective for the IP receptor and produces no significant IP receptor desensitization.[4] Finally, a microdose study of oral NS-304/ACT-293987 (100 μg) conducted in five healthy volunteers showed that it was metabolized to MRE-269 in the human body with an elimination half-life of 7.9 h. The only adverse events reported that were thought to be related to the study drug were two headaches that were mild in intensity. In a comparison with oral beraprost, these studies conducted in rats, dogs, and monkeys showed that NS-304/ACT-293987 was superior to beraprost in efficacy, persistence in the blood plasma, and overall safety margin.[4]

NS-304/ACT-293987 showed efficacy in a preclinical study conducted in monocrotaline-induced PH in rats.[91] Treatment with NS-304/ACT-293987 results in improved vascular endothelial function, reduced pulmonary arterial wall hypertrophy, decreased right ventricular systolic pressure, and improved survival. In summary, NS-304/ACT-293987 has characteristics that make it a promising candidate for ideal oral combination therapy in patients with PAH utilizing drugs that target the three major pathways (prostacyclin, nitric oxide, and endothelin-1).

Discussion

This review indicates that there are significant differences among the various prostaglandin analogs. For example, the prostaglandin analogs have different binding affinities for the

various prostaglandin receptors. Iloprost binds to most of the prostaglandin receptors involved in VSMC relaxation; however, less is known about the binding affinities of the other analogs. The different binding affinities and different GPCR coupling interactions for the various prostaglandin analogs may result in different clinical efficacies or safety, depending on the target tissue.[56] The various different prostaglandin analogs have different pharmacokinetics, which may also impact their effectiveness, safety, or ease of use. Head-to-head studies will be needed to confirm implied differences in efficacy or safety.

A small number of preclinical studies have directly compared the effects of several prostaglandin analogs on different physiological systems. One study examined the effects of iloprost, treprostinil, and beraprost on smooth muscle proliferation and cAMP generation in the human pulmonary artery.[55] Serum-induced proliferation produced by a number of prostacyclin analogs acting on pulmonary artery smooth muscle cells was assessed by either counting the cell number or measuring [^{3}H]thymidine incorporation. The ranking in effectiveness in decreasing proliferation by 1 μM concentrations was treprostinil > iloprost > cicaprost > beraprost; however, the EC_{50} values for the first three analogs are not significantly different from each other ($EC_{50} = 8.2$ nM, $EC_{50} = 4.8$ nM, $EC_{50} = 7.1$ nM, and $EC_{50} = 98.2$ nM, respectively). Intracellular cAMP was elevated by all analogs, with treprostinil producing a significantly larger and more sustained increase compared with other analogs and with iloprost producing the smallest increase. However, whether these *in vitro* differences in effectiveness would translate into differences in clinical effectiveness is not known.

The route of administration may be important in deciding upon a particular prostaglandin analog for an individual patient. Intravenous administration requires an ambulatory infusion pump with a central venous catheter, with their attendant risks of sepsis, thrombosis, catheter disruption, and pump failure. Interruption of the infusion or underdosing of prostacyclin and prostaglandin analogs may induce the acute return of PAH symptoms that can be life-threatening.[66,92] Additionally, the high cost of long-term prostacyclin therapy (cost of the drug, the ambulatory pump, and intravenous lines and supplies) and subsequent need for health insurance for lung transplantation must also be considered.[66]

Subcutaneous administration eliminates the need for venous access. The intravenous formulation of treprostinil is associated with more prominent side effects (headaches and leg pain) than the subcutaneous formulation, perhaps because of a higher dose.[93] However, continuous subcutaneous infusion of treprostinil is painful, with the drug causing erythema, induration, and sometimes ulceration of the skin.[77]

Inhaled administration of the prostaglandin analogs decreases the risk of systemic side effects by delivering the drug directly to the alveolar capillaries. However, the dose delivered is a fraction of the dose delivered intravenously, perhaps explaining lesser improvement in 6MWD compared to subcutaneous or intravenous delivery. Iloprost is intended to be delivered in the US by either of two different nebulizer systems (I-Neb™ Adaptive Aerosol Delivery [AAD™] System [Respironics, Inc.; Murrysville, PA, USA] or the Prodose™ AAD™ System [Profile Therapeutics, Ltd.; Bognor Regis, UK] [no longer being manufactured]). These specific nebulizers use AAD to analyze the patient's breathing pattern in order to specifically deliver the aerosol during inhalation. This technology reduces the variability in the delivered dose, and reduces the waste of aerosol to the environment during exhalation.[94] Delivery of the required dosage of iloprost takes approximately 10 min, depending on the patients' breathing patterns, six to nine times a day. Therefore reducing the duration of daily inhalations would be advantageous. Consequently, ongoing clinical trials are evaluating the more rapid delivery of inhaled iloprost.

An oral formulation would have the advantage of being more convenient than either continuous intravenous or subcutaneous infusion or frequent time-consuming inhalations. Oral formulations of treprostinil (FREEDOM clinical trials) and NS-304/ACT-293987 are currently in development.

In addition to route of administration, pharmacoeconomic considerations, including formulary status, are increasingly affecting choice of PAH treatment. On the whole, few rigorous pharmacoeconomic studies have been conducted on prostacyclin-based PAH treatments, in part due to the recent increase in available therapies. Among pharmacoeconomic studies that investigated epoprostenol and treprostinil, results have been mixed. A one-year study of treprostinil plus bosentan vs. epoprostenol indicated that treprostinil was more expensive than epoprostenol with little improvement in quality-adjusted life years.[95] The increased dosing of IV treprostinil vs epoprostenol (approximately 2× epoprostenol dosing) suggests that despite similar efficacy and safety, IV treprostinil will be more expensive than epoprostenol.[96] However, another study that investigated epoprostenol and treprostinil over a three-year period suggested that SC treprostinil was cheaper than epoprostenol due to decreased sepsis.[97]

In summary, prostacyclin has many desirable effects on the pulmonary vasculature that make it effective in the treatment of PAH. Prostaglandin analogs are more chemically stable than endogenous or synthetic prostacyclin, and are approved by the FDA for the treatment of PAH. Differences in formulation, route of administration, effectiveness, and safety may all play a role in deciding on the specific prostaglandin analog to prescribe for an individual patient. Head-to-head studies will be needed to confirm possible differences in efficacy or safety for the various prostaglandin analogs.

Conflict of interest statement

I have previously received Grants/Research Support from Actelion, Gilead, Pfizer; served as a consultant for Actelion, Gilead, United Therapeutics; and I have served on the Speaker's Bureau for Actelion and Gilead.

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