(54) Title: METHOD OF TREATING PULMONARY HYPERTENSION BY ADMINISTRATION OF NATRIURETIC PEPTIDE RECEPTOR C ACTIVATORS

(57) Abstract: The present invention is based upon the observation that a loss of function or mutations of a gene encoding the C-type natriuretic peptide receptor (NPR-C) or the inhibition of NPR-C pathway may lead to the development of pulmonary arterial hypertension (PAH). Accordingly, the invention proposes a method of using synthetic analogs of NPR-C pathway, specifically synthetic C-type atrial natriuretic factor or intermediates for or modulators of NPR-C pathway as anti-pulmonary vasculopathy agents. Activators to NPR-C pathway, as well as polymorphisms encoding NPR-C or positively regulating the expression of NPR-C, are disclosed to treat or prevent vasculopathy, including but not limited to PAH and other types of pulmonary hypertension, peripheral vascular disease, critical limb ischemia, coronary artery disease, and diabetic vasculopathy. The invention also proposes methods of diagnosis, prediction of treatment response, and screening for alleles of the NPR-C gene that may cause the development of PAH and associated disorders.
FIELD OF THE INVENTION

The present invention relates to loss of function or mutations of NPR3 gene, particularly loss of function or mutations of gene encoding the C-type natriuretic peptide receptor (NPR-C) or the inhibition of NPR-C pathway that may cause the development of pulmonary hypertension (PH) and other disorders related to pulmonary vasculopathy. The invention further proposes methods of treating PH and disorders related to vasculopathy by administration of NPR-C activators or polynucleotides encoding a NPR-C or positively regulating the expression of NPR-C. Additionally, the invention proposes methods of diagnosis, prediction of treatment response, and screening for alleles of the NPR-C gene that may cause the development of pulmonary arterial hypertension.

BACKGROUND

Pulmonary arterial hypertension (PAH) is a progressive lung disorder which, untreated, leads to death on average within 2.8 years after being diagnosed. PAH is one of the five classes of pulmonary hypertension (PH). The other four types of PH are venous, hypoxic, thromboembolic and miscellaneous PH. PAH generally affects young and otherwise healthy individuals and strikes women twice as frequently as men. The average age of diagnosis has been estimated to be 36 years, and only 10% of the patients were over 60 years of age (G. E. DAlonzo et al, Ann. Intern. Med. 1991, 115:343-349; A.J. Peacock et al. Eur Respir J 2007, 30:104–9.). Each year, between 15 and 50 people per million population are diagnosed with the disease, although this is certainly a low estimate (A.J. Peacock et al. Eur Respir J 2007, 30:104–9.).

PAH is a condition in which the progressive obliteration of the pulmonary vasculature leads to increased resistance to blood flow through the lungs. In turn, this obstruction leads to increased stress on the right heart, which may develop into right heart failure and, ultimately, death. Although the “trigger” that leads to the disease is still unknown, a complex interplay among different types of cells occurs and multiple alterations are verified: (i) intimal hyperplasia; (ii) medial hypertrophy and hyperplasia; (iii) adventitia proliferation; (iv) neointima formation and (v) occurrence of plexiform lesions. In addition, these changes are accompanied by vasoconstriction, local inflammation and in situ thrombi of the small pulmonary arteries and arterioles (R. Nogueira-Ferreira et al. Biochim Biophys Acta. 2014 pii: S0167-4889(14)00042-1. doi: 10.1016/j.bbamcr.2014.01.030.).

Idiopathic pulmonary arterial hypertension (IPAH) is the best described form of PAH and its pathophysiology may include loss of function mutations in the morphogenetic protein receptor 2

There are also heritable forms of the disease (HPAH), which have been often linked to the BMPR2 gene mutations (J.R. Thomson et al. J Med Genet 2000, 37:741-745.).

Standard therapies available on the market (e.g. prostacyclin analogs, endothelin receptor antagonists, phosphodiesterase inhibitors) provide symptomatic relief and improves prognosis, but falls short as to re-establishment of structural and functional lung vascular integrity, as a basis for handicapped free long-term survival. The principles of these therapies are primarily hemodynamic, influencing vessel tone but having, as mentioned above, no direct influence on the pathogenic remodeling processes, characteristic of this devastating disease, as most patients with PAH may already have full established pulmonary vascular remodelling at diagnosis. Moreover, by orders of magnitude more frequent, the unmet clinical need is even more pronounced in pulmonary vascular disorders outside the PAH group, e.g., those with underlying heart or lung disease and thromboembolic disease, for all of which no single medical treatment is currently approved. In addition, the possibility of using these medicaments is restricted through the sometimes serious side effects and/or complicated types of administration. Despite all the advances in the therapy of PAH there is as yet no prospect of cure of this deadly disease and the majority of patients continue to progress to right ventricular failure, albeit at a slower pace. Hence deciphering the molecular mechanisms, which drive the maladaptive inward remodeling processes in PH, as well as developing novel agents capable of preventing the progression or reversing pulmonary vascular remodelling would represent an important step in the treatment of patients with PAH and other subtypes.

Natriuretic peptides (NPs), including atrial (ANP), B-type (BNP), C-type (CNP) and dendraospis (DNP) NPs constitute a family of at least four structurally related hormones that may play a relevant role in cardiovascular homeostasis, including regulation of vascular tone, blood volume, endothelial permeability, and cardiac hypertrophy (Casserly et al. Drug Des Devel Ther. 2009, 3: 269–287; G.E. Woodard et al. Int rev Cell Mol. Biol. 2008, 268:59-93). Among the four family members, ANP and CNP have been demonstrated to suppress the signaling of vascular endothelial growth factor, a key regulator of PH (I. Dijkgraaf et al. Cancer Biother. Radiopharm. 2009, 24: 637-647). The NPs exert their biological effects by binding to three specific receptors on the cell membrane denoted NPs’ receptors A, B and C (NPR-A, NPR-B and NPR-C) (T. Maack et al., Science 1987, 238: 675-678). NPR-A (which binds ANP and BNP) and NPR-B (which binds CNP) increase intracellular cyclic guanosine monophosphate (cGMP) levels following activation of a
membrane bound guanylyl cyclase (GC) enzyme, which may mediate most of the biological actions of NPs (S. Suga et al. Endocrinology 1992, 130(1): 229-239.). NPR-C, which binds all NPs with approximately equal affinity, does not contain a GC domain and was originally classified as a ‘clearance receptor’ with no signalling function (L.R. Potter et al. Endocr Rev. 2006, 27:47-72; D.G. Johns et al. Biochem Biophys Res Commun. 2007, 358:145-149). This receptor is called the NPs’ clearance receptor in that it has been shown to participate in the local clearance of the NPs. The receptor binds the ligand and is internalized. The ligand is degraded and the receptor is retroendocytosed back to the cell surface (Nussenzveig, et al. J. Biol. Chem. 1990, 265:20952-20958). The NPR-C accounts for approximately 50% of NPs’ clearance, the other half being carried out by cell surface neutral endopeptidases. Although still commonly called a clearance receptor (and thus largely ignored), it has been suggested that NPR-C has other biological activity other than simply NPs’ clearance. Several groups have shown that NPR-C is coupled to a pertussis toxin sensitive inhibitory G protein (Gi) and mediates a reduction in adenyl cyclase (AC) activity and intracellular cAMP levels (Palaparti, et al. Biochem. J. 2000, 346:313-320; Pagano et al. J. Biol. Chem. 2001, 276:22064-22070). Recently, it has been postulated that the vasodilatory effects of endothelium-derived hyperpolarizing factor may be attributed to such NPR-C mediated adenyl cyclase inhibition (Chauhan et al. Proc. Natl. Acad. Sci. USA 2003, 100:1426-1431). Specific mice lacking NPR-C (NPR-C (-/-)) were made to determine the effect of an absence of NPR-C on water balance, salt balance, and blood pressure (Matsukawa, et al., Proc. Natl. Acad. Sci. USA 1999, 96:7403-7408). The animals have a moderately but statistically significantly lowered blood pressure and with age show an increase in daily water uptake with a significant increase in urinary output. The NPR-C (-/-) mice also have a defect in the ability to concentrate their urine. The observed alterations in renal function were interpreted as being the result of a failure of local clearance of NPs in the glomerular and post-glomerular structures resulting in an increase in filtered volume and a decrease in water reabsorption. The decrease in blood pressure was attributed to simple hypovolemia. Unexpectedly, the authors also found that mice lacking NPR-C exhibit striking skeletal abnormalities, including hunched backs, dome-shaped skulls, elongated tails, increased body length, decreased weight, elongated femurs, tibias, metatarsal, and digital bones as well as a more constricted thoracic cages than their counterpart wild type. However, the authors did not perform any evaluation of the heart nor did they make any examination of the pulmonary vasculature.
Several spontaneously occurring mutants in the NPR3 gene have been identified, the first of which was called longjohn (lgj) due to the skeletal defects described above. A French group studied them to examine and compare the skeletal defects among the three strains (Jaubert et al., Proc. Natl. Acad. Sci. USA 1999, 96:10278-10283). Again, the authors did not perform any evaluation of the heart nor did they make any examination of the pulmonary vasculature.

A US20040898490 patent disclosed a method of using synthetic analogs of NPs as pro-lipolytic, as anti-obesity agents. Again, the inventors did not perform any evaluation of the heart nor did they make any examination of the pulmonary vasculature.

Several basic and clinical researches have been carried out to understand the roles of NPs in regulating pulmonary vascular tone and remodeling, as well as their roles in the pathogenesis of hypoxia or monocrotaline-induced PH. All the antimitogenic, antifibrotic, and antihypertrophic effects of NPs on pulmonary vascular remodeling and maladaptive hypertrophic responses in the right ventricle were reported to be linked to the GC-linked NPs’ receptors. Even the often observed down regulation of NPR-C in hypoxia-associated PH were repeatedly reported to be part of a compensatory mechanism of the lungs aimed at reducing NPs’ clearance from the circulation, thus enhancing the biological effects of NPs and mitigating the severity of hypoxia-induced PH (J.Z. Sun et al. Am J Physiol Lung Cell Mol Physiol. 2000, 279(3):L511–519; Casserly et al. Drug Des Devel Ther. 2009, 3: 269–287; Itoh et al. Am J Respir Crit Care Med. 2004, 170:1204-11.) Again, the impaired NPR-C pathway as the underlying cause of PH has never been mentioned in the literature. In summary, there has been no suggestion in the art that NPR-C pathway or modulators of the gene encoding NPR-C or positively regulating the expression of NPR-C, would be useful as therapeutics for PH.

**SUMMARY OF THE INVENTION**

The present invention relates to loss of function or mutations of NPR3 gene, particularly loss of function or mutations of gene encoding NPR-C or the inhibition of NPR-C pathway that may cause the development of PH and other disorders related to pulmonary vasculopathy.

The present invention is also directed to the use of synthetic analogs of NPR-C pathway, specifically the synthetic C-type atrial natriuretic factor (cANF), or intermediates for or modulators of NPR-C pathway as anti-pulmonary hypertension or anti-pulmonary vasculopathy agents. Activators to NPR-C pathway, as well as polynucleotides encoding NPR-C or positively regulating the expression of NPR-C, are disclosed to treat or prevent vasculopathy, including but not limited to PAH and other
types of PH, peripheral vascular disease, critical limb ischemia, coronary artery disease, and diabetic vasculopathy.

Also proposed are methods of diagnosis PAH using genetic testing or other suitable techniques, prediction of treatment response, as well as screening for a genetic predisposition by detecting mutant NPR-C proteins and/or nucleic acids.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a schematic representation of a cascade of events following an impairment of NPR-C pathway. The impaired NPR-C pathway may be the result of several factors, including but not limited to an abnormal NPR3 gene, an inhibition of NPR3 gene expression, or an inhibition or abnormal NPR-C protein. Impaired activation of this signaling pathway may lead to failure of the antiproliferative effect of NPR-C in the pulmonary vasculature, which in turn may result in vascular pulmonary injury, including endothelial dysfunction, vascular smooth muscle dysfunction, matrix changes, and platelets, as well as inflammatory cell activation. The proliferation of smooth muscle in pulmonary arterioles, secondary to remodelling, would then ultimately lead to PAH.

Figure 2 illustrates the loss of homeostasis with NPR-C pathway. The imbalance between impaired activation and physiological activation may cause or prevent the development of PAH. Impaired activation of NPR-C signaling pathway may lead to failure of the antiproliferative effect of NPR-C in the pulmonary vasculature, which in turn may result in PAH. Activators to NPR-C pathway may initiate signaling that results in the inhibition of cell proliferation in pulmonary artery smooth muscle cells and therefore reverses the remodelling that is typical to PAH.

DETAILED DESCRIPTION OF THE INVENTION

The present inventor has discovered that the NPR-C (-/-) mice may be PAH-prone and, therefore, may represent an experimental animal model for PAH. The NPR-C (-/-) mice may, therefore, have right atrial dilation, tricuspid regurgitation as well as echocardiographic signs of right ventricular pressure overload, including paradoxical bulging of the septum into the left ventricle during systole, and hypertrophy of the right ventricular free wall and trabeculae. The left ventricular systolic and diastolic function would be expected to be within normal limits. Doppler echocardiography assessment may reveal a higher right ventricular systolic pressure (RVSP) and thus a higher pulmonary artery pressure (PAP) in NPR-C (-/-) mice compared with wild-type littermates. This significantly increase in PAP, in NPR-C (-/-) mice, may be confirmed with right heart catheterization.
Accordingly, the invention relates to loss of function or mutations of NPR3 gene, particularly loss of function or mutations of gene encoding NPR-C, to mutants of the NPR-C protein, as well as related derivatives, fragments and homologs thereof, and NPR-C nucleic acids encoding them, that may cause PAH.

The invention also calls for the use of activators to the NPR-C pathway, as well as polynucleotides encoding NPR-C or positively regulating the expression of NPR-C for the treatment and prevention of PH and disorders related to vasculopathy. These compounds and compositions may be administered to humans in a manner similar to other therapeutic agents. Therapeutics of the invention may be administered either alone or in combination with other therapies, e.g., therapeutics effective PAH and PH. Therapeutics of the invention may also be administered with drugs, which may modulate NRP-C expression. Acute administration of the cANF may reduce significantly RVSP and PAP without alteration of the systemic arterial pressure. The magnitude of this reduction may be greater in subjects with concomitant diabetes or coronary artery disease including heart failure, who may have endothelial dysfunction. This observation may be supported by the finding that, in the coronary vasculature, the vasorelaxant effect of NPR-C pathway may be increased in the presence of nitric oxide (NO) synthase inhibition (Hobbs et al. Circulation 2004, 110:1231-5). This observation suggests that they may be synergistic and complementary functions for NPR-C pathway and NO-mediated signaling. The inhibition of one pathway may thus be compensated for by the upregulation of the other. This may be of particular clinical significance in patients with PAH who are known to have endothelial dysfunction and thus reduced or loss of NO pathway (K.A. Fagan et al. Am J Physiol, 1999, 277; L472–L478; H.C. Champion et al. Proc Natl Acad Sci U S A, 2002, 99;13248–13253).

The role of NPR-C pathway in pulmonary vasculopathy is unclear. Evidence suggests that chronic hypoxia causes a significant down regulation of NPR-C expression in several tissues, including pulmonary vascular smooth muscle, independently of changes in NPs levels and expression of other NPs’ receptors (Sun JZ et al. Am J Physiol Lung Cell Mol Physiol. 2000, 279:L511-9). This down regulation of NPR-C expression and associated impaired NPR-C pathways may lead to failure of the antiproliferative effect in the pulmonary vasculature, which would then ultimately lead to PAH (Figure 1). Therefore, an impaired NPR-C pathway may be a common underlying cause of all hypoxia-associated vasculopathy including but not limited to pulmonary vasculopathy. The most common causes of PH are chronic lung and left sided heart disease. The development of PH in these conditions occurs, at least partially, as the result of chronic hypoxia. This observation suggests that
NPR-C pathway may, therefore, represent a therapeutic target to inhibit pulmonary vascular remodeling and maladaptive increases in PH.

How the loss of normal functioning of NPR-C pathway leads to PH still remains to be elucidated. The NPR-C pathway may prevent cellular proliferation in some types of cells and the hypothesis is that abnormal NPR-C pathway activity may permit excess cell growth and proliferation in response to a variety of injuries. The proliferation of smooth muscle in pulmonary arterioles would then ultimately lead to PAH. These affirmations are supported by the proposed observation that transgenic mice with genetic deletion of NPR-C may have PAH. Therefore, the fundamental mechanism of NPR-C-related PAH may be an imbalance of growth signaling caused, at least partially, by an impaired or reduction in the braking function of NPR-C. (Figures 1 and 2). In summary, abnormal NPR-C pathway may play an important role in the pathogenesis of PAH and in particular IPAH, and may causally be linked to some cases in familial PAH (FPAH) and a substantial percentage of IPAH patients.

Also proposed are methods of diagnosis PAH using genetic testing or other suitable techniques, prediction of treatment response, as well as screening for a genetic predisposition by detecting mutant NPR-C proteins and/or nucleic acids.
Claims

1. A method of treating or preventing a vasculopathy in a subject comprising administering to the subject a therapeutically effective amount of activator to NPR-C pathway.

2. The method of claim 1, wherein said vasculopathy comprises pulmonary arterial hypertension.

3. The method of claim 1, wherein said vasculopathy comprises pulmonary hypertension.

4. The method of claim 3, wherein said pulmonary hypertension is a complication of left sided heart disease.

5. The method of claim 3, wherein said pulmonary hypertension is a complication of heart failure.

6. The method of claim 3, wherein said pulmonary hypertension is a complication of chronic hypoxia.

7. The method of claim 3, wherein said pulmonary hypertension is a complication of thromboembolic disease.

8. The method of claims 1, 2, 3, 4, 5, 6 and 7, wherein the subject is a mammal.

9. The method of claims 1, 2, 3, 4, 5, 6 and 7, wherein the subject is a human.

10. The method of claims 1, 2, 3, 4, 5, 6, 7, 8 and 9, wherein the NPR-C pathway activator is polynucleotides encoding a NPR-C.

11. The method of claims 1, 2, 3, 4, 5, 6, 7, 8 and 9, wherein the NPR-C pathway activator is polynucleotides positively regulating the expression of NPR-C.

12. The method of claims 1, 2, 3, 4, 5, 6, 7, 8 and 9, wherein the NPR-C pathway activator is synthetic analogs of or intermediates for or modulators of NPR-C pathway.

13. The method of claim 1, 2, 3, 4, 5, 6, 7, 8 and 9, wherein the NPR-C pathway activator is cANF.


15. The use of claim 14, wherein said vasculopathy comprises pulmonary arterial hypertension.

16. The use of claim 14, wherein said vasculopathy comprises pulmonary hypertension.

17. The use of claim 16, wherein said pulmonary hypertension is a complication of left sided heart disease.
18. The use of claim 16, wherein said pulmonary hypertension is a complication of heart failure.

19. The use of claim 16, wherein said pulmonary hypertension is a complication of chronic hypoxia.

20. The use of claim 16, wherein said pulmonary hypertension is a complication of thromboembolic disease.

21. The use of claims 14, 15, 16, 17, 18, 19 and 20, wherein the subject is a mammal.

22. The use of claims 14, 15, 16, 17, 18, 19 and 20, wherein the subject is a human.

23. Use of NPR3 gene polymorphism for screening of vasculopathy in a subject.

24. The use of claim 23, wherein said vasculopathy comprises pulmonary arterial hypertension.

25. The use of claim 23, wherein said vasculopathy comprises pulmonary hypertension.

26. The use of claim 25, wherein said pulmonary hypertension is a complication of left sided heart disease.

27. The use of claim 25, wherein said pulmonary hypertension is a complication of heart failure.

28. The use of claim 25, wherein said pulmonary hypertension is a complication of chronic hypoxia.

29. The use of claim 25, wherein said pulmonary hypertension is a complication of thromboembolic disease.

30. The use of claims 23, 24, 25, 26, 27, 28 and 29, wherein the subject is a mammal.

31. The use of claims 23, 24, 25, 26, 27, 28 and 29, wherein the subject is a human.

32. Use of NPR3 gene testing for predicting the treatment response in a vasculopathy.

33. The use of claim 32, wherein said vasculopathy comprises pulmonary arterial hypertension.

34. The use of claim 32, wherein said vasculopathy comprises pulmonary hypertension.

35. The use of claim 34, wherein said pulmonary hypertension is a complication of left sided heart disease.

36. The use of claim 34, wherein said pulmonary hypertension is a complication of heart failure.

37. The use of claim 34, wherein said pulmonary hypertension is a complication of chronic hypoxia.
38. The use of claim 34, wherein said pulmonary hypertension is a complication of thromboembolic disease.

39. The use of claims 32, 33, 34, 35, 36, 37 and 38, wherein the subject is a mammal.

40. The use of claims 32, 33, 34, 35, 36, 37 and 38, wherein the subject is a human.

41. Use of subject lacking NPR-C to study or as a model of a vasculopathy.

42. The use of claim 41, wherein said vasculopathy comprises pulmonary arterial hypertension.

43. The use of claim 41, wherein said vasculopathy comprises pulmonary hypertension.

44. The use of claim 43, wherein said pulmonary hypertension is a complication of left sided heart disease.

45. The use of claim 43, wherein said pulmonary hypertension is a complication of heart failure.

46. The use of claim 43, wherein said pulmonary hypertension is a complication of chronic hypoxia.

47. The use of claim 43, wherein said pulmonary hypertension is a complication of thromboembolic disease.

48. The use of claims 41, 42, 43, 44, 45, 46 and 47, wherein the subject is a mammal.

49. The use of claims 41, 42, 43, 44, 45, 46 and 47, wherein the subject is a human.
Figure 1

Tunica Adventitia (Fibroblast)
Tunica Media (Smooth Muscle Cells)
Tunica Intima (Endothelial Cells)

Impaired NPR-C Activation → Failure of Antiproliferative Effects → Vascular Pulmonary Injury → Remodeling → Pulmonary Artery Hypertension

- Abnormal NPR3 gene
- Inhibition of NPR-C
- Abnormal NPR-C protein
- Inhibition of NPR3 gene expression

- Endothelial dysfunction
- Vascular smooth muscle dysfunction
- Matrix changes, platelets and inflammatory cells activation
Figure 2

Impaired activation

Normal pulmonary artery

Opposes Pulmonary Hypertension

Mutant NPR-C

Favors Pulmonary Hypertension

Activation

Progressive narrowing of pulmonary artery

Progressive narrowing of pulmonary artery
# INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**

| IPC: | A61K 38/22 (2006.01), A61P 11/00 (2006.01), A61P 9/12 (2006.01) |

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

| IPC: | A61K 38/22 (2006.01), A61P 11/00 (2006.01), A61P 9/12 (2006.01) |

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)


Keywords: NPR-C, NPR3, vasculopathy, cANF, hypertension, knockout, activator

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C.

- Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means of publication prior to the international filing date but later than the priority date claimed
  - "P" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - "X" document of particular relevance; the claimed invention cannot be combined with one or more other such documents
  - "Y" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - "&" document member of the same patent family

Date of the actual completion of the international search:

31 October 2014 (31-10-2014)

Date of mailing of the international search report:

21 November 2014 (21-11-2014)

Name and mailing address of the ISA/CA

Canadian Intellectual Property Office

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Authorized officer:

Keely Ingrey (819) 994-8923

Form PCT/ISA/210 (second sheet) (July 2009)
### INTERNATIONAL SEARCH REPORT

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**Remark on Protest**

- ☐️ The additional search fees were accompanied by the applicant=s protest and, where applicable, the payment of a protest fee.
- ☑️ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐️ No protest accompanied the payment of additional search fees.

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