Lees s.v.p. zorgvuldig de handleiding.
Klik bovenaan de 1e pagina in de cel 'Coordinating investigator 1'. Gebruik de tab-toets om binnen pagina 1 naar een ander veld te gaan. Klik bovenaan de volgende pagina weer in de 1e cel (vraag 1.3) om verder te gaan. Gebruik daarna weer de tab-toets.

Final report

LF 2013
Projectnr:
3.2.09.034

1. General information

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1.3	Title of project:	
	English	The novel cAMP effector Epac: new avenues in the treatment of inflammation, tissue remodelling and airway narrowing in COPD
	Dutch	De cAMP effector Epac: nieuwe wegen in de behandeling van ontstekingen, tissue remodelling en luchtwegvernauwing in COPD
	Project number	3.2.09.034
1.4	Time schedule:	
	Start of project	15-10-2009
	Duration of project	48 (months)
	Period of funding	from 15-10-2009 till 15-10-2013
1.5	Grant	€ 245.396
1.6	Short description of the project for public information (in Dutch, see guidelines) (max. 250 words):	

"Chronic obstructive pulmonary disease" (COPD) is een chronische ontstekingsziekte van de longen die meestal veroorzaakt wordt door tabaksrook of luchtvervuiling. Miljoenen mensen lijden wereldwijd aan COPD, wat grote medische en economische schade veroorzaakt. COPD wordt gekarakteriseerd door een progressieve en irreversibele vermindering van de longfunctie, die gepaard gaat met ontstekingsprocessen, structurele veranderingen en emfyseem ontwikkeling in de luchtwegen. De moleculaire en cellulaire processen die de ziekte kenmerken en veroorzaken zijn nauwelijks bekend. Deze kennis zou echter een doorbraak in de behandeling en genezing van COPD kunnen betekenen.

In dit project hebben wij ons tot doel gesteld om de rol van Epac1 en Epac2 (**e**xchange **p**rotein directly **a**ctivated by **c**AMP) in ontstekingsreacties en bij structurele verandering van de luchtwegen te bepalen. Onze studies in luchtweggladde spiercellen hebben aangetoond dat Epac de tabaksrook-geïnduceerde ontsteking remt. Bij COPD patiënten is deze functie verdwenen en is ook de expressie van Epac1 verminderd. Dit gebeurt vermoedelijk door de productie van miRNA7. Verder blijkt in epitheelcellen dat zowel E-cadherin als AKAP9 (A-kinase anchoring protein9), dat Epac kan binden, de tabaksrook-geïnduceerde daling van de epitheliale barrière functie kan tegenhouden. Tot slot geven onze studies in Epac-deficiënte muizen aan dat Epac1 en Epac2 verschillende processen sturen die van belang zijn voor de pathogenese van COPD. Samengevat tonen onze studies aan dat Epac een belangrijke pathofysiologische rol speelt bij ontstekingsreacties en structurele verandering van de luchtwegen bij COPD. Onze studies kunnen leiden tot een betere inzetbaarheid van farmaca die zich richten op de intracellulaire signaalstof cyclisch AMP.

2. Report

2.1	Summary:	
	Title	The novel cAMP effector Epac: new avenues in the treatment of inflammation, tissue remodelling and airway narrowing in COPD
	Authors	Anouk Oldenburger, Prof. Dr. W.Timens, Prof. Dr. H. Meurs, Prof. Dr. M. Schmidt

	Dept./Institute(s)	University of Groningen, Department of Molecular Pharmacology; University of Groningen, University Medical Center Groningen, Department of Pathology
	Keywords (max. 6)	COPD; cyclic AMP; exchange protein directly activated by cAMP (Epac)

Abstract (max. 250 words):

"Chronic obstructive pulmonary disease" (COPD) is a chronic inflammatory disease of the lungs, that is generally caused by tobacco smoke or air pollution. Millions of people worldwide suffer from COPD, which causes profound medical and economic damage. COPD is characterized by a progressive and irreversible decline in lung function caused by airway inflammation, small airway remodeling, and emphysema development. The molecular and cellular processes that cause development and progression of disease are not known, and new breakthroughs in the causes, treatment and cure of COPD are urgently required.

In this project, we aimed to establish the role of "exchange protein directly activated by cAMP" (Epac1 and Epac2) in airway inflammation and remodeling. Our studies in airway smooth muscle cells have demonstrated that Epac activation inhibits cigarette smoke extract (CSE)-induced inflammation but that this suppressive function is reduced in COPD patient most likely caused by an reduced Epac1 expression. Furthermore, our studies show that CSE-induced production of miRNA7 decreases Epac1 expression. In epithelial cells stabilization of both E-cadherin and "A-kinase anchoring protein9" (AKAP9), the latter known to interact with Epac, decreases CSE-induced decline of the epithelial barrier function. Finally, *in vivo* studies in Epac-deficient mice indicate that Epac1 and Epac2 differentially regulate processes known to be important in the pathogenesis of COPD. Overall, our studies demonstrate an important pathophysiological role of Epac1 and Epac2 in airway inflammation and remodeling in COPD. Our studies provide better insight into drugs that target the intracellular messenger cyclic AMP.

2.2 Description of original question/aim (max. 150 words):

We investigated the hypothesis that protective Epac signalling is reduced in COPD, resulting in inflammation and tissue remodelling thereby contributing to airway narrowing and emphysema. Within this central hypothesis, the following specific research aims have been pursued:

Aim #1 To investigate the role of Epac1 and Epac2 in pro-inflammatory and remodelling responses of airway smooth muscle and epithelial cells.
Aim #2 To establish the role of Epac1 and Epac2 in beta$_2$-agonist/PGE$_2$-induced airway smooth relaxation.
Aim #3 To determine the contribution of Epac1, Epac2 and the Epac effector to inflammation and tissue remodelling in animal models of COPD.
Aim #4 To study alterations of Epac1 and Epac2 expression and function in pulmonary tissues of patients with COPD.
Aim #5 To elucidate potential molecular mechanisms underlying the reduced expression of Epac1 in airway smooth muscle cells and tissue of COPD patients.

2.3 Results (max. 2500 words, please submit a maximum of 4 figures and diagrams separately):

Introduction
Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease of the airways and the lung parenchyma, further characterized by airway obstruction and remodeling. Currently, COPD is the third leading cause of death worldwide. Chronic inflammation and tissue remodeling, including loss of epithelial barrier, small airway fibrosis, mucus hypersecretion, increased airway smooth muscle mass and parenchymal destruction (emphysema), contribute to the progressive and irreversible decline in lung

function in COPD. No curative treatment for COPD exists and symptoms are treated with glucocorticosteroids, anticholinergics, β_2-agonists or phosphodiesterase (PDE)-4 inhibitors alone or in combination.

The mechanism of action of both β_2-agonists and PDE4 inhibitors involves elevation of the second messenger cyclic AMP (cAMP), although by distinct mechanisms. Whereas binding of β_2-agonists to the G_s protein-coupled β_2-adrenoceptors activates adenylyl cyclase subsequently leading to the formation of cAMP, PDE4 inhibitors prevent the breakdown of cAMP into the inactive 5'-AMP. cAMP has been implicated in the development and progression of chronic diseases due to its ability to modulate gene transcription and secretion. The two main effectors of cAMP are protein kinase A (PKA) and the exchange protein directly activated by cAMP (Epac), which consists of the two isoforms Epac1 and Epac2. Several recent studies have focused on the role of cAMP-regulated Epac in cell biology in general and in pulmonary disease in particular, which has resulted in intriguing, unexpected findings. We reviewed novel aspects of the "old" second messenger cAMP in several recent manuscripts Schmidt et al., Pharmacol. & Therap. (2012) 137:248; Oldenburger et al., Pharmaceuticals (2012) 5, 1291; Schmidt et al., Pharmacol Rev. (2013) 65:670.

We aimed to establish the functional role of Epac in airway inflammation and remodeling in COPD using in vitro studies in airway smooth muscle and epithelial cells, in vivo models and clinical samples. We propose that our studies may lead to the development of new drugs to optimize the treatment of COPD.

Epac1 and Epac2: Inflammation

A major characteristic of COPD is inflammation, a process characterized by infiltration and activation of diverse inflammatory cells including macrophages, monocytes, lymphocytes and particularly neutrophils, and subsequently leading to the secretion of cytokines by these cells. An important cytokine that is increased in COPD is interleukin-8 (IL-8). The secretion of IL-8 correlates with pulmonary neutrophil levels in COPD, underscoring the importance of this cytokine in COPD. The effect of cigarette smoke, the main risk factor for COPD, on IL-8 release has been investigated *in vitro*. Bronchial epithelial cells, macrophages, fibroblasts and airway smooth muscle cells all secrete IL-8 after stimulation with cigarette smoke. We demonstrated in our initial studies that Epac1, Epac2 and PKA act in concert to modulate the release of IL-8 from airway smooth muscle cells via signaling to the main Epac effector Rap1 and extracellular signal-regulated kinases (ERK1/2). These results were published in Roscioni et al., Respir. Res. (2009) 10:88.

Our first aim was to report on the roles of Epac and PKA in cigarette smoke-induced IL-8 release. We demonstrated that cigarette smoke extract (CSE)-induced release of IL-8 from human airway smooth muscle was almost fully inhibited by the β_2-agonist fenoterol. Direct pharmacological activation of either Epac or PKA mimicked the effect of fenoterol on CSE-induced IL-8 release. Silencing of both Epac1 and Epac2 resulted in a reduction of the anti-inflammatory effects of Epac activation. We investigated the underlying mechanisms, and reported that Epac and PKA decreased CSE-induced IL-8 release via inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and ERK1/2, respectively. In addition Epac1, but not Epac2 or PKA, protein expression was down regulated in CSE-exposed human airway smooth muscle cells as well as in lung tissue of COPD patients (see also Figure 1). The down-regulation of Epac1 protein in COPD patients might be due to irreversible protein structure alterations caused by cigarette smoke-induced oxidative stress which is also responsible for lung damage and chronic inflammation. As a potential effector in cAMP-driven and β_2-adrenergic receptor-induced signaling and a newly discovered inhibitor of NF-κB-dependent inflammatory response, Epac1 down-regulation by cigarette smoke may provide an additional explanation for the variable anti-inflammatory capacities of β_2-agonists in the treatment of COPD. The result of these studies were published in Oldenburger et al., Plos One (2012) 7:e31574.

To investigate the potential cause for the downregulation of Epac1, we studied next the

possible involvement of microRNAs (miRNAs). MicroRNAs are epigenetic regulators involved in fine-tuning of cellular activities by posttranscriptional repression of mRNA, by direct degradation of mRNA and by inhibition of the translation process. In COPD, miRNAs have been implicated in the regulation of inflammatory processes and miRNA-7 is increased in serum of COPD patients. Interestingly, *in silico* analysis revealed that Epac1 might represent a putative target of miRNA-7. We have reported on a potential interaction of Epac1 and miRNA-7. CSE induced miRNA-7 specifically in human airway smooth muscle cells. In line with the specific induction of miRNA-7 in human airway smooth muscle cells by CSE, miRNA-7 was increased in bronchial smooth muscle of COPD patients isolated by laser dissection compared to controls. Importantly, Epac1 expression was reduced in miRNA-7 overexpressing human airway smooth muscle cells (see Figure 2). Our data implicate that upregulation of miRNA-7 by cigarette smoke correlates with the downregulation of Epac1 in COPD. This interaction might be involved in Epac related inflammation. The results of these studies were published in Oldenburger et al., Naunyn-Schmiedeberg's Arch. Pharmacol. (2014), doi:10.1007/s00210-014-1015-z.

Both Epac1 and Epac2 seem to be implicated in the reduction of CSE-induced IL-8 release from human airway smooth muscle cells as shown by pharmacological activation of Epac by 8-pCPT-2'-*O*-Me-cAMP and silencing of both Epac1 and Epac2. However, the Rap-activated phospholipase Cε (PLCε), a direct effector of Epac, has been linked to the production of pro-inflammatory mediators including keratinocyte-derived chemokine (KC), the murine functional homolog of interleukin (IL)-8, IL-1β and tumor necrosis factor (TNF)-α. To translate our findings on inflammation *in vitro* to the *in vivo* situation and to make a distinction between the effects of Epac1, Epac2 and the effector PLCε, Epac1-/-, Epac2-/- and PLCε-/- mice were exposed to cigarette smoke for 5 days. An acute model of cigarette smoking was used, as recently reported by our group. Using this model we were able to induce inflammation and remodeling after acute cigarette smoke exposure. In this model we demonstrated that compared to wild-type (WT) mice exposed to cigarette smoke, the number of total inflammatory cells, macrophages, and neutrophils as well as IL-6 release were lower in Epac2-/- mice, which was also the case for neutrophils and IL-6 in PLCε-/- mice. Again compared to WT mice exposed to cigarette smoke, the number of macrophages was reduced in Epac1-/- mice. Whereas, the numbers of lymphocytes, only present in low numbers in bronchoalveolar lavage fluid (BALF) of air-exposed WT mice, were increased in Epac1-/- mice compared to WT mice exposed to either fresh air or cigarette smoke (see Figure 3). Together our data indicated that particularly Epac2 acts pro-inflammatory *in vivo*. In line with our findings presented in this thesis showing either a pro- or an anti-inflammatory role for Epac1, it has been reported in other studies that the effect of Epac1 on inflammation seems to be cell-type specific. The results of these studies are under revision in Oldenburger et al., FASEB J (2014).

Aberrant epithelial repair of damage caused by cigarette smoke is also regarded as a pathophysiological feature of COPD. Cigarette smoke-induced inflammation, together with oxygen radicals present in cigarette smoke may disturb epithelial repair and barrier function. With respect to such barrier function it is of interest that the A-kinase anchoring protein (AKAP) family member AKAP9 enhanced the endothelial barrier function in concert with Epac1. Members of the AKAP family compartmentalize cellular cAMP upon generation of multiprotein complexes consisting of either the β_2-adrenoceptor, PDE4, PKA or Epac or a combination of these proteins. We demonstrated that CSE reduces the barrier function in human bronchial epithelial cells as well as the membrane expression of E-cadherin and AKAP9. Next to the expression of PKA, Epac1 and Epac2, CSE did also not alter the expression of AKAP5 and AKAP12, which are known to interact both with the β_2-adrenergic receptor showing a specific role for AKAP9 in CSE-induced effects on the barrier in this thesis. Silencing of AKAP9 reduced the functional epithelial barrier and prevented the ability of st-Ht31, an inhibitor of AKAP-PKA interactions, to restore membrane localization of E-cadherin (see Figure 4). Our data indicated that AKAP proteins, most likely AKAP9, maintain the bronchial epithelial barrier function and may be important in the pathophysiology of COPD. The results of these studies were published in Oldenburger et al., Am. J. Physiol. (2014), 15; 306:C585-597.

Epac1 is related to anti-inflammatory effects both in CSE-exposed human airway smooth muscle cells and in bronchial smooth muscle of COPD patients. In contrast, our studies performed in Epac2-/- demonstrated that Epac2 acts primarily pro-inflammatory. The pro-inflammatory role of cAMP-regulated Epac2 seems to be contradicting in the context of the anti-inflammatory effects of cAMP-elevating drugs. Compartmentalization of cAMP driven by AKAP family members, may be responsible for the distinct biological effects of cAMP.

Remodeling: Epac1 and Epac2

Tissue remodeling is another feature of COPD and covers different characteristics of the disease such as mucus hypersecretion, airway fibrosis and emphysema. The role of cigarette smoke in remodeling processes has been well established and an increased mucus secretion, onset of airway wall extracellular matrix (ECM) protein deposition and onset of emphysema by cigarette smoke has been observed. The majority of ECM proteins, including collagens and fibronectin, are produced by fibroblasts, but can also be produced by other structural lung cells like smooth muscle cells and epithelial cells. Alterations in the tightly controlled balance of production and degradation of ECM proteins causes structural changes in the lung such as emphysema, characterized by excessive degradation of parenchymal ECM, and (small) airway fibrosis characterized by excessive deposition of ECM protein. Matrix metalloproteases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs) regulate the balance between production and degradation of ECM proteins. In COPD patients, both MMP9 and its main inhibitor TIMP1 are elevated, making the final outcome with regard to ECM production and/or degradation more difficult to predict in COPD.

In view of these results, it has been reported that cAMP-elevating drugs reduced synthesis of collagen I in human lung fibroblasts. In addition, it has been reported that both PKA and Epac inhibited the production of the ECM proteins collagen I and III. Binding of Epac1 to the activated TGF-β1 type I receptor subsequently decreased the phosphorylation of Smad2 and Smad2-dependent transcription raising the possibility that Epac1 inhibited collagen production via TGF-β1. A PDE inhibitor, adenosine and the cAMP analog 8-Br-cAMP have been shown to reduce MMP9 and TIMP1 gene expression and activity in different cell types. Although a role for cAMP in the regulation of remodeling has been shown, we studied next the exact role of the cAMP effectors Epac and PKA on the different aspects of remodeling. We demonstrated that CSE exposure of the human bronchial epithelial cell line 16HBE14o- leads to increase of MMP9 mRNA and thereby of the MMP9/TIMP1 ratio. Induction of MMP9 mRNA was reduced by specific PKA activation. Pro-MMP9 levels induced by CSE were reduced by the β_2-agonist fenoterol, an effect specifically mimicked by pharmacological activation of PKA. So, inhibition of PKA may enhance the MMP9/TIMP1 ratio and thereby reduce emphysema. In contrast, activation of PKA may reduce emphysema by a decrease of the MMP9/TIMP1 ratio. These results were included in chapter 5 of the thesis of Anouk Oldenburger, and will be submitted for publication in the near future.

As Epac did not seem to be involved in the regulation of MMP we tried to identify the role of Epac1 and Epac2 in other remodeling processes. Growth factors are able to transform a contractile phenotype of airway smooth muscle (ASM) into a proliferative, hypo-contractile phenotype. We demonstrated that Epac, next to PKA, prevented the growth factor-induced reduction of airway smooth muscle strip contractility and contractile protein expression (α-smooth muscle actin), and thereby reversed the phenotypic modulation of airway smooth muscle. The results of these studies were published in Roscoini et al., Brit. J. Pharmacol. (2011), 162, 193-209; 164, 958-969. As β_2-agonists induce bronchodilation of the obstructed airways in COPD, we studied the relative contribution of Epac and PKA to this process. We demonstrated that Epac shifts the balance between Rho and Rac towards Rac, thereby inducing relaxation of airway smooth muscle. The results of these studies were published in Roscoini et al., J. Cell. Mol. Med. (2011), 15, 1551-1563. In subsequent studies in Epac1-/- and Epac2-/- mice we demonstrated that both proteins are differentially involved in airway smooth muscle relaxation. These results are currently not

	in the thesis of Anouk Oldenburger, and will be submitted for publication in the near future. In the lung by exposure of Epac1-/- and Epac2-/- mice to cigarette smoke. We reported that Epac1-/- mice expressed higher levels of the pro-fibrotic cytokine TGF-β1 (mRNA), collagen I (mRNA and protein) and fibronectin (mRNA and protein). Based on the findings demonstrated in this thesis, we propose that particularly Epac1, but not Epac2, acts anti-fibrotic. Mucus hypersecretion represents another factor of remodeling effects in the airways. Interestingly, Epac1-/- and Epac2-/- were characterized by a constitutively higher expression of MUC5AC mRNA at basal level. We observed that goblet cells tended to be increased in Epac2-/- and PLCε-/- mice, whereas primarily Epac1-/- mice tended to stain positive for the inducer of goblet cell differentiation SPDEF. Interestingly, we demonstrated in total lung homogenates of Epac1-/- mice, MUC5AC and matrix remodeling parameters, (transforming growth factor-β1, collagen I and fibronectin), were increased at baseline (see Figure 3). Overall, we identified distinct roles of Epac1 and Epac2 in remodeling processes as seen in COPD patients. The results of these studies are under revision in Oldenburger et al., FASEB J (2014).
2.4	Did the study solve the original question? yes/no (explain) (max. 250 words): YES. In this project we identified a role for Epac1 and Epac2 in airway inflammation and remodelling *in vitro*, *in vivo* and in clinical samples. In airway smooth muscle cells we have demonstrated that Epac activation inhibits cigarette smoke extract (CSE)-induced inflammation but that this suppressive function is reduced in COPD patient most likely caused by an reduced Epac1 expression. Furthermore, our studies show that CSE-induced production of miRNA7 decreases Epac1 expression. In epithelial cells stabilization of both E-cadherin and "A-kinase anchoring protein9" (AKAP9), the latter known to interact with Epac, decreases CSE-induced decline of the epithelial barrier function. Finally, *in vivo* studies in Epac-deficient mice indicate that Epac1 and Epac2 differentially regulate processes known to be important in the pathogenesis of COPD. Overall, our studies demonstrate an important pathophysiological role of Epac1 and Epac2 in airway inflammation and remodeling in COPD. Our studies provide better insight into drugs that target the intracellular messenger cyclic AMP.
3	**Papers (see instructions)**
3.1	All publications (published or submitted peer-reviewed manuscripts): **In preparation** Oldenburger A, van Basten B, Elzinga CRS, Maarsingh H, Meurs H, Timens W, Schmidt M. Cyclic AMP modulates cigarette smoke-induced MMP9/TIMP1 balance in human bronchial epithelial cells. Oldenburger A, Elzinga CRS, Timens W, Meurs H., Schmidt M, Maarsingh H. Epac1 and Epac2 are differentially involved in airway smooth muscle relaxation. **In revision** Oldenburger A, Timens W, Bos S, Smit M, Alan Smrcka, Anne-Coline Laurent, Junjun Cao, Machteld Hylkema, Herman Meurs, Harm Maarsingh, Frank Lezoualc'h, Martina Schmidt, Epac1 and Epac2 are differentially involved in inflammatory and remodeling processes induced by cigarette smoke, *The FASEB Journal*, in revision **Published** Oldenburger A, van Basten B, Kooistra W, Meurs H, Maarsingh H, Krenning G, Timens W, Schmidt M. Interaction between Epac1 and miRNA-7 in airway smooth muscle cells, *Naunyn Schmiedebergs Arch Pharmacol.* 2014, doi:10.1007/s00210-014-1015-z Oldenburger A, Poppinga WJ, Kos F, de Bruin HG, Rijks WJ, Heijink IH, Timens W, Meurs

	H, Maarsingh H, Schmidt M. A-kinase anchoring proteins contribute to the loss of E-cadherin and bronchial epithelial barrier by cigarette smoke. *Am.J.Physiol.* 2014, 15;306(6):C585-97 Schmidt, M, Dekker F, Maarsingh H (2013) Exchange protein directly activated by cAMP (Epac): a multidomain cAMP mediator in the regulation of diverse biological functions. *Pharmacol. Rev.* 65, 670-709 Oldenburger A, Maarsingh M, Schmidt M. Multiple Facets of cAMP Signalling and Physiological Impact: cAMP Compartmentalization in the Lung. *Pharmaceuticals* 2012, 5(12), 1291-1331 Oldenburger A, Roscioni SS, Jansen E, Menzen MH, , Timens W, Halayko AJ, Meurs H, Schmidt M, Potential anti-inflammatory role of the cAMP effectors Epac and PKA: implications in chronic obstructive pulmonary disease, *PLoS ONE* 2012;7(2):e31574 Schmidt M, Dekkers BGJ, Racké K (2012) Distinct PKA and Epac compartmentalization in airway plasticity and function. *Pharmacol. & Therap.* 137, 248-265 Roscioni SS, Maarsingh H, Elzinga CRS, Schuur J, Menzen M, Halayko AJ, Meurs H, Schmidt M (2011) Epac as a novel effector of airway smooth muscle relaxation. *J.Cell.Mol.Med.,* 15, 1551-1563 Roscioni SS, Dekkers BGJ, Prins AG, Meurs H, Schmidt M, Maarsingh H (2011) cAMP inhibits airway smooth muscle phenotype modulation. *Brit. J. Pharmacol*. 162, 193-209 Roscioni SS, Prins AG, Dekkers BGJ, Elzinga CRS, Halayko AJ, Meurs H, Schmidt M (2011) Functional roles of Epac and PKA in human airway smooth muscle phenotype plasticity. *Brit. J. Pharmacol*., 164, 958-969 Roscioni SS, Kistemaker LEM, Menzen M, Elzinga CRS, Gosens R, Halayko AJ, Meurs H, Schmidt M (2009) PKA and Epac cooperate to augment bradykinin-induced interleukin-8 release from human airway smooth muscle. *Resp. Res.*, 10,88
3.2	All publications (not peer-reviewed like abstracts, newspapers, websites, etc.): Schmidt M, Oldenburger A, Timens W, Bos IST, Smit M, Meurs H, Maarsingh H. Epac1 and Epac2 are differentially involved in inflammatory and remodeling processes induced by cigarette smoke. *Am. J. Respir. Crit. Care Med.* 189:A2060. Schmidt M, Oldenburger A, Poppinga WJ, Heijink, IH, Timens W, Maarsingh H, Meurs H. A-kinase anchoring proteins contribute to loss of E-cadherin and bronchial epithelial barrier by cigarette smoke. *Am. J. Respir. Crit. Care Med.* 189:A2061. Oldenburger A, Bos S, Smrcka A, Meurs H, Maarsingh H, Schmidt M. Epac2 and PLCε contribute to the inflammatory response to cigarette smoke *in vivo*. *FASEB J.* 27:1107.7 Oldenburger A, Poppinga W, Kos F, Rijks W, Heijink I, Timens W, Meurs H, Maarsingh H. Role for A-kinase anchoring proteines in cigarette smoke-induced barrier dysfunction. *FASEB J.* 27:1107.6 Oldenburger A, Rijks W, Kos F, Sewbalaksing VD, Heijink IH, Maarsingh H, Schmidt M. Role for A-kinase anchoring proteins in cigarette smoke-induced barrier dysfunction. Figon DMD 2012. Oldenburger A, Rijks WJ, Kos F, Poppinga WJ, Sewbalaksing VD, Timens W, Meurs M, Heijink IH, Maarsingh M, Schmidt M. A-kinase anchoring proteins (AKAPs) as potential novel therapeutic targets to improve cigarette smoke-induced loss of barrier function.

	Proceed. Brit. Pharmacol. Soc. 2012, 101:P407 Oldenburger A, Rijks W, Sewbalaksing VD, Heijink IH, Maarsingh H, Schmidt M. Repair of cigarette smoke-induced loss of barrier function: Role of AKAPs. ERS, Lung science conference 2012 Poppinga WJ, Oldenburger A, Skroblin P, Klussmann E, Michel MC, Halayko AJ, Maarsingh H, Schmidt M. Coordination of airway smooth muscle secretory functions by A-Kinase anchoring proteins, Figon DMD 2011. A Oldenburger, W Rijks, WJ Poppinga, SS Roscioni, IH Heijink, H Maarsingh, M Schmidt, Interaction between cigarette smoke and cyclic AMP signaling in human bronchial epithelial function. *The FASEB Journal*. 2011;25:659.13 Oldenburger A, Roscioni SS, Jansen E, Menzen MH, Halayko AJ, Meurs M, Schmidt M. Epac and PKA inhibit cigarette smoke-induced production of IL-8 in airway smooth muscle cells. World Congress on Basic and Clinical Pharmacology, 2010, Copenhagen, Denmark. Roscioni SS, Jansen E, Menzen MH, Oldenburger A, Timens W, Halayko AJ, Meurs H, Schmidt M. Epac and PKA inhibit cigarette smoke-induced production of interleukin-8 in airway smooth muscle cells. *Am J Respir Crit Care Med* (2010), A3601.
4.	**Implementation (see instructions)**: **Follow-up studies** The findings of these studies will be followed up in Longfonds project 3.2.11.015 to evaluate the role of A-kinase anchoring proteins as novel drug targets in the development and progression of airway obstruction in COPD. Further, the impact of compartmentalized cAMP signaling will be studied in the Science without Border Grant, "Histone and NOX in asthma, pulmonary fibrosis and chronic obstructive pulmonary disease" (CNPq, Proc40187/2013-6), as well as in the CAPES/NUFFIC Grant "Activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and antioxidant response elements (ARE) as a therapeutic target for tissue repair in chronic degenerative lung disease" (EDITAL068/2013). No patent applications have resulted from this project.

Ondertekening	
Datum: 14-07-2014	Handtekening aanvrager: