

# An Association between Neutrophils and Immunoglobulin Free Light Chains in the Pathogenesis of Chronic Obstructive Pulmonary Disease

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**Rationale:** Neutrophils are key players in chronic obstructive pulmonary disease (COPD), and increased numbers of neutrophils are present in sputum and lung tissue of patients with COPD. Interestingly, immunoglobulin free light chains (IgLC) are able to prolong the life of neutrophils; therefore, IgLC may contribute to the chronic state of inflammation.

**Objectives:** In this study, the relation between IgLC and COPD has been investigated.

**Methods:** We investigated the presence of IgLC in different murine lung emphysema models. IgLC levels in serum from mice and patients with COPD were examined by Western blot analysis and ELISA, respectively. IgLC levels in lung tissue were determined by immunohistochemistry. Fluorescence-activated cell sorter and immunofluorescent analysis were used to detect binding between IgLC and human neutrophils. Interleukin-8 (CXCL8) release by neutrophils after IgLC incubation was measured by ELISA. The effect of F991, an IgLC antagonist, was examined on the neutrophil influx in murine lungs after 5 days of smoke exposure.

**Measurements and Main Results:** Increased levels of IgLC in serum of cigarette smoke-exposed and cigarette smoke extract-treated mice compared with control mice were observed. Patients with COPD showed increased serum IgLC and expression of IgLC in lung tissue compared with healthy volunteers. Interestingly, IgLC bound to neutrophils and activated neutrophils to release CXCL8. F991 inhibited the IgLC binding to neutrophils and reduced the smoke-induced neutrophil influx in murine lungs after smoke exposure.

**Conclusions:** This study describes for the first time an association between neutrophils and IgLC in the pathophysiology of COPD, which could open new avenues to targeted treatment of this chronic disease.

**Keywords:** lung emphysema; cigarette smoke; CXCL8; F991

## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

B cells have been suggested to contribute to the ongoing inflammation and development of emphysema in chronic obstructive pulmonary disease (COPD). B cells produce and secrete tetrameric immunoglobulins, but in addition also release a substantial amount of immunoglobulin free light chains (IgLC). The presence of IgLC is often associated with an increased inflammatory status. To date, little is known about a possible role of IgLC in COPD.

### What This Study Adds to the Field

We provide evidence of production of IgLC after cigarette smoke exposure in various murine models for COPD. Serum IgLC concentrations and local expression of IgLC were also found increased in human patients with COPD. IgLC were found to bind to neutrophils, and cross-linking induces CXCL8 production. Antagonizing the action of IgLC inhibited the IgLC binding to neutrophils and partly reduced the cigarette smoke-induced neutrophil influx and activation in murine lungs. This study suggests that IgLC may serve as a potential novel therapeutic target in the treatment of COPD.

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Chronic obstructive pulmonary disease (COPD) is characterized by an incompletely reversible, progressive airflow limitation and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, most commonly cigarette smoke (1, 2). The relation between neutrophils and COPD has been extensively investigated, and several studies provide suggestive evidence that neutrophils are important key players in the development of COPD (3, 4). Increased numbers of neutrophils are present in sputum, lung tissue, and bronchoalveolar lavage fluid (BALF) of patients with COPD (5, 6). Neutrophils are critical effector cells during acute inflammatory processes, and recruitment and activation of neutrophils can be initiated and regulated via a multitude of mechanisms. An example is the interaction of interleukin-8 (CXCL8) with the chemokine receptor CXCR2, which has been claimed to be a major mechanism of neutrophil activation in inflammatory lung diseases (7). However, neutrophils also express receptors for different immunoglobulin isotypes. These so-called Fc receptors are important triggers of neutrophil effector functions and may modulate airway inflammatory reactions. Human neutrophils constitutively express the IgG receptors, FcγRIIIB and FcγRIIA, whereas expression of

Fc $\gamma$ RI is increased during inflammatory conditions (8). Moreover, neutrophils from asthmatic subjects express the IgE high-affinity receptor (Fc $\epsilon$ RI), and the cross-linking of this receptor is involved in the release of CXCL8 (9). It is widely accepted that Fc $\epsilon$ RI cross-linking by IgE bound to its antigen results in biological effects; however, Saffar and colleagues (10) demonstrated an antiapoptotic effect exerted by monomeric IgE alone on human neutrophils *in vitro*. Interestingly, immunoglobulin free light chains (IgLC) are also able to prolong the life of neutrophils, suggesting that IgLC could contribute to the chronic state of inflammation (11). It has been long recognized that B cells not only produce and secrete tetrameric immunoglobulins, but also secrete a substantial amount of IgLC. In the mammalian immune system, two isotypes of IgLC are produced:  $\kappa$  and  $\lambda$  (12). The ratio in which both types are produced varies significantly among species. For instance, in humans, half to two-thirds of the IgLC produced is the kappa isotype, whereas mice predominantly produce kappa IgLC (95%) (13, 14). IgLC can exert various biological activities: enzymatic activities, binding to intracellular and extracellular proteins, and cellular interactions (15). Different inflammatory disorders and autoimmune diseases are accompanied by elevated IgLC levels in different body fluids (16–21), and the increased IgLC concentrations correlate with relapses of disease and enhanced activity of the immune system (12, 22–25).

In this study, we investigated the involvement of IgLC in COPD and focused on the neutrophil, the major inflammatory cell involved in the pathogenesis of COPD. Our data suggest a role of IgLC in COPD, because increased levels of IgLC in sera were found in three different murine models of lung emphysema. Furthermore, IgLC levels were increased in serum and lung tissue from patients with COPD. Interestingly, we determined that IgLC bind to human neutrophils and cross-linking of IgLC resulted in the production CXCL8 *in vitro*. Finally, antagonizing the action of IgLC reduced the cigarette smoke-induced neutrophil influx in murine lungs.

## METHODS

### Murine Lung Emphysema Models

In the first model, C57Bl/6 mice were exposed nose-only to cigarette smoke twice every weekday for 3 months. In the second model, A/J mice were subjected to whole-body cigarette smoke exposure 5 days/week for 20 weeks. In the third model, C57Bl/6 mice were injected intraperitoneally with cigarette smoke extract (CSE), which was prepared as described before (26), plus adjuvant at Day 1, and at Day 11, CSE was administered intratracheally (*see online supplement*).

The mice lungs were fixed, embedded, cut, and stained as described before (27). Morphometric assessment of emphysema was estimated by the mean linear intercept (Lm) analysis (28).

The right ventricle was removed from lower heart after removal of the atria. The ratio of the weights was calculated as follows: (right ventricle)/(left ventricle + septum) (29).

### F991, IgLC Antagonist, Study

Balb/c mice were whole-body exposed to cigarette smoke for 5 consecutive days and received the IgLC antagonist F991 by oropharyngeal administration twice daily. The mice's lungs were lavaged, and total cell counts and the absolute number of neutrophils were calculated as described before (30) (*see online supplement*).

### IgLC Measurement in Murine Serum and Thoracic Lymph Node Cells

Murine serum samples were precipitated to deplete high amounts of albumin as previously described (31). The IgLC proteins in precipitated serum and thoracic lymph node (TLN) supernatants were characterized by Western blot analysis with horseradish peroxidase (HRP)-labeled goat anti-mouse  $\kappa$ IgLC as previously described (32) (*see online supplement*).

### Patient Characteristics

Patient characteristics of the six patients with COPD included in the study are presented in Table 1.

### IgLC Measurement in Serum and Lung Tissue of Patients with COPD

The concentration of  $\kappa$ IgLC in serum samples obtained from patients with COPD and healthy volunteers was measured by ELISA as described previously (33, 34). IgLC expression in lung tissue of patients with COPD was visualized by an immunohistological staining, according to a procedure previously described (35) (*see online supplement*).

### Flow Cytometry and Immunofluorescence Staining for IgLC on Polymorphonuclear Leukocytes

Polymorphonuclear leukocytes (PMNs) were isolated from buffy coats via a standard protocol as described before (36). PMNs were incubated with fluorescein isothiocyanate (FITC)-labeled  $\kappa$ IgLC or F991 (kindly produced by Steffen van der Wal, Utrecht University, Utrecht, The Netherlands) was preincubated with FITC-labeled  $\kappa$ IgLC for 30 minutes before incubation with PMNs. Binding of FITC-labeled  $\kappa$ IgLC to PMNs was analyzed via flow cytometry and immunofluorescence (*see online supplement*).

### PMN Stimulation Assay

Ninety-six-well plates were coated with human  $\kappa$ IgLC and incubated with buffy coat-isolated PMNs for 4 hours (*see online supplement*). After incubation, the cells were centrifuged and supernatant was collected.

### CXCL8 ELISA

CXCL8 levels were measured using a human CXCL8 ELISA kit (BD Biosciences, Breda, The Netherlands) according to the manufacturer's instructions.

### Statistical Analysis

Experimental results are expressed as mean  $\pm$  SEM. Differences between groups were statistically determined by an unpaired two-tailed

TABLE 1. PATIENT CHARACTERISTICS

| Patient with COPD | Sex (M/F) | Age (yr) | Current Smoker | Smoking History (pack-years) | FEV <sub>1</sub> (%predicted) | FEV <sub>1</sub> /FVC (%) |
|-------------------|-----------|----------|----------------|------------------------------|-------------------------------|---------------------------|
| 1                 | m         | 67       | —              | 37                           | 40                            | 45                        |
| 2                 | m         | 67       | —              | 35                           | 57                            | 58                        |
| 3                 | m         | 52       | +              | 31                           | 33                            | 23                        |
| 4                 | m         | 59       | +              | 42                           | 35                            | 42                        |
| 5                 | m         | 63       | +              | 50                           | 42                            | 41                        |
| 6                 | m         | 62       | —              | 45                           | 55                            | 58                        |

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; FEV<sub>1</sub> = forced expiratory volume in 1 second; FVC = forced vital capacity.

Student's *t* test and differences between (*in vitro*) data were analyzed using two-way analysis of variance, with *post hoc* Bonferroni's *t* test for multiple comparisons using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA). Results were considered statistically significant when  $P < 0.05$ .

## RESULTS

### Increased Production of IgLC after Nose-Only Cigarette Smoke Exposure

IgLC levels were measured in serum of C57Bl/6 mice that were nose-only exposed to cigarette smoke for 1, 3, 6, 9, and 12 weeks. The concentrations of IgLC in serum of cigarette smoke-exposed mice were significantly enhanced compared with the air-exposed mice, which was dependent on the duration of cigarette smoke exposure (Figure 1A). This nose-only exposure model leads to characteristics of lung emphysema, because airspace enlargement (observed by patchy emphysema in some parts of the lung) and right ventricle heart hypertrophy were observed in mice after 12 weeks of cigarette smoke exposure compared with the air-exposed mice (Table 2). To investigate whether production of IgLC occurred in local lymph nodes of cigarette smoke-exposed C57Bl/6 mice, cells from the TLNs ( $n = 6$  pooled) were cultured and supernatants were analyzed for presence of IgLC. These supernatants showed complete immunoglobulins (about 180–200 kD), IgLC dimers (about 45 kD), and IgLC monomers (20 kD). The TLN cells of cigarette smoke-exposed mice produced higher levels of IgLC compared with TLN cells of control animals (Figures 1B and 1C).

### Increased IgLC Levels in Serum after Whole-Body Cigarette Smoke Exposure

In the next experiments, IgLC production was monitored in a different model for experimental emphysema. Different lung emphysema models were used to confirm that increased

production of IgLC was a general response to cigarette smoke exposure and not strain dependent or dependent on other factors like exposure system, cigarettes, and exposure time. A/J mice were whole-body exposed to cigarette smoke for 1, 4, and 20 weeks, and after killing the animals, the IgLC levels were measured in serum. The IgLC concentrations in serum of cigarette smoke-exposed mice were increased (1 wk:  $48.54 \pm 7.9$  optical density [OD]/mm<sup>2</sup>, 4 wk:  $65.65 \pm 11.1$  OD/mm<sup>2</sup>; 20 wk:  $126.34 \pm 11.4$  OD/mm<sup>2</sup>) compared with the air-exposed mice (1 wk:  $27.68 \pm 9.0$  OD/mm<sup>2</sup>, 4 wk:  $20.98 \pm 7.2$  OD/mm<sup>2</sup>; 20 wk:  $73.97 \pm 20.0$  OD/mm<sup>2</sup>) at all time points (1, 4, and 20 wk) (Figures 2A–2C). After smoking cessation for 8 weeks, the IgLC serum levels were significantly decreased ( $100.66 \pm 2.0$  OD/mm<sup>2</sup>) compared with the smoke-exposed mice ( $126.34 \pm 11.4$  OD/mm<sup>2</sup>).

Air space enlargement and right ventricle heart hypertrophy was observed in the mice after 20-week cigarette smoke exposure compared with the air-exposed mice (Table 2). The increased Lm and the heart hypertrophy ratio was not reduced after a period of 8 weeks without cigarette smoke exposure, demonstrating irreversible lung damage (37).

### Increased IgLC Levels in Serum of Mice Intraperitoneally and Intratracheally Injected with Cigarette Smoke Extract

To induce an (auto)immune response leading to the pathogenesis of COPD as suggested earlier (38), mice were exposed to cigarette smoke extract via intraperitoneal and intratracheal administration leading to an immune response to components of cigarette smoke. We found that serum IgLC concentrations in CSE-treated mice were significantly enhanced compared with control mice (control:  $171.8 \pm 10.2$  OD/mm<sup>2</sup>, CSE:  $228.2 \pm 16.9$  OD/mm<sup>2</sup>;  $P < 0.05$ ) (Figure 3). Characteristics of lung emphysema, alveolar enlargement, and right ventricle heart hypertrophy were evident in the CSE-treated mice but not in control mice (Table 2).

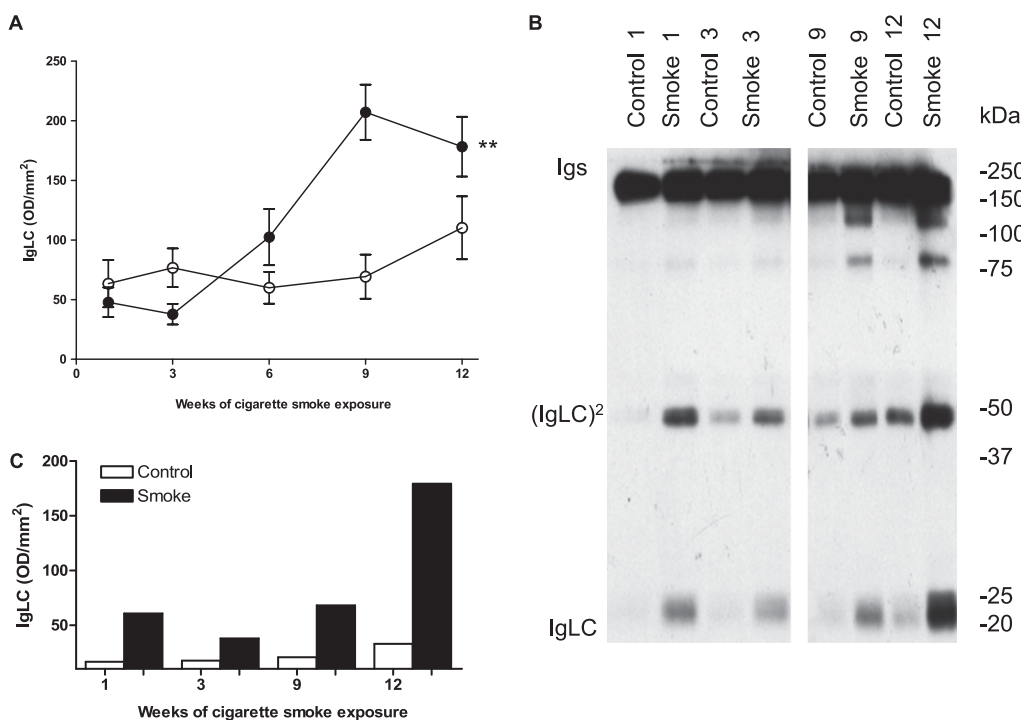


**Figure 1.** Increased levels of immunoglobulin free light chains (IgLC) in serum and supernatant of thoracic lymph node cells after nose-only cigarette smoke exposure. (A) Densitometric analysis after Western blotting of IgLC in serum of C57Bl/6 mice after 1, 3, 6, 9, and 12 weeks of nose-only cigarette smoke exposure (closed symbols) compared with air-exposed mice (open symbols).  $n = 4$ –6 animals per group. Values are expressed as mean (optical density [OD]/mm<sup>2</sup>)  $\pm$  SEM. \*\* $P \leq 0.01$ ; significantly different from the control group. (B) Representative Western blot of IgLC levels in supernatants of thoracic lymph node cells of C57Bl/6 mice after 1, 3, 9, and 12 weeks of nose-only cigarette smoke exposure compared with air-exposed mice. The two images were obtained from different regions of the same gel. Igs = total immunoglobulins (IgA, IgD, IgE, IgG, and IgM); IgLC<sup>2</sup> = IgLC dimers; IgLC = monomers. (C)

Quantification of the thoracic lymph node supernatant samples of the air-exposed mice ( $n = 6$  pooled, white bars) and the smoke-exposed mice ( $n = 6$  pooled, black bars) used for Western blotting via densitometric analysis. Values are expressed as OD/mm<sup>2</sup>.

**TABLE 2. LUNG EMPHYSEMA CHARACTERISTICS IN THE DIFFERENT EMPHYSEMA MODELS**

| Animal model        | Groups          | Mean Linear Intercept | Heart Hypertrophy   |
|---------------------|-----------------|-----------------------|---------------------|
|                     |                 | ( $\mu\text{m}$ )     |                     |
| Nose-only exposure  | Control         | 38.9 $\pm$ 0.5        | 0.18 $\pm$ 0.005    |
|                     | Smoke           | 40.1 $\pm$ 0.4        | 0.21 $\pm$ 0.004*** |
| Whole-body exposure | Control         | 42.5 $\pm$ 0.8        | 0.21 $\pm$ 0.010    |
|                     | Smoke           | 52.6 $\pm$ 1.7**      | 0.27 $\pm$ 0.010*** |
|                     | Smoke cessation | 49.6 $\pm$ 1.4**      | 0.26 $\pm$ 0.008*** |
| CSE administration  | Control         | 38.7 $\pm$ 0.1        | 0.17 $\pm$ 0.010    |
|                     | CSE             | 41.9 $\pm$ 0.5**      | 0.21 $\pm$ 0.007**  |

Definition of abbreviation: CSE = cigarette smoke extract.

Mean linear intercept values and heart hypertrophy measurement [(weight of the right ventricle)/(weight of the left ventricle + septum)] of mice nose-only or whole-body exposed to air or cigarette smoke or after smoking cessation and of phosphate-buffered saline- and CSE-treated mice.  $n = 4$ –6 animals per group. Values are expressed as mean  $\pm$  SEM. \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ ; significantly different from the control group.

### IgLC in Serum and Lung Tissue Specimens of Patients with COPD

To investigate whether IgLC are also involved in the pathogenesis of human emphysema, IgLC levels were examined in serum samples obtained from patients with COPD and healthy control subjects. As depicted in Figure 4A, the IgLC serum levels of patients with COPD were significantly increased compared with the healthy control subjects. Immunohistochemical staining with antibodies specific for  $\kappa$  and  $\lambda$  free light chain was performed to analyze the localization of IgLC in lung tissue specimens of patients with COPD. Figure 4 illustrates representative photomicrographs of  $\kappa$ IgLC (Figure 4B) and  $\lambda$ IgLC (Figures 4C and 4D) staining in lung tissue of patients with COPD. Positive IgLC staining was observed around the larger airways and within follicles.

### IgLC Binding to PMNs Inhibited by F991 and Cross-linking Bound IgLC Induces CXCL8 Production

Because neutrophils are key players in the development of COPD and previous studies showed that IgLC could influence neutrophil activity (11), we first analyzed whether IgLC could bind to human

PMNs. Human PMNs show extensive binding of FITC-labeled IgLC as determined via fluorescence-activated cell sorter analysis (Figure 5A). The IgLC binding to these human PMNs can be reduced by F991, because PMNs incubated with IgLC in presence of 50  $\mu\text{g}$  F991 showed diminished binding of IgLC, whereas incubation with a lower dose of F991 (5  $\mu\text{g}$ ) did not lead to a diminished binding of IgLC (Figure 5A). The PMNs of the three donors used in this study showed reduced binding of IgLC of  $22.8 \pm 0.3\%$ . In addition, the immunofluorescent analysis showed focal binding of IgLC on the cell membrane of PMNs (Figure 5B). To determine whether cross-linking bound IgLC induced activation of PMNs, PMNs were incubated in IgLC-coated wells to mimic cross-linking. CXCL8 release by PMNs was significantly increased in the wells coated with  $\kappa$ IgLCs compared with the noncoated wells (Figure 5C), and this CXCL8 increase was dose dependent. In addition, incubating PMNs with soluble  $\kappa$ IgLC (100  $\mu\text{g}/\text{ml}$ ) did not result in an increased release of CXCL8 (data not shown).

### Cigarette Smoke-induced Neutrophil Influx in the BALF Is Significantly Decreased after F991 Administration

To investigate whether IgLC in the airways were of functional importance in COPD, we explored whether antagonizing its biological action with a specific IgLC antagonist F991 affected cigarette smoke-induced neutrophil migration and activation in the airways. When mice were exposed for 5 days to cigarette smoke, a significant increase of BALF neutrophils was observed compared with the air-exposed mice. The cigarette smoke-induced neutrophil influx in the BALF was significantly decreased after F991 administration (Figure 6). The TLNs of cigarette smoke-exposed mice produced higher levels of IgLC compared with control animals (IgLC levels: control: 166.51 OD/ $\text{mm}^2$  vs. smoke: 245.43 OD/ $\text{mm}^2$ ). After F991 administration *in vivo*, the IgLC levels produced by TLNs *ex vivo* were reduced to normal level (IgLC levels: 171.96 OD/ $\text{mm}^2$ ). Furthermore, we observed significantly increased KC (the murine CXCL8 homolog) levels in the BALF of smoke-exposed mice compared with air-exposed (KC levels: control:  $44.5 \pm 7.3$  pg/ml vs. smoke:  $191.1 \pm 18.2$  pg/ml,  $P \leq 0.001$ ), whereas after F991 administration, the cigarette smoke-induced KC levels were decreased ( $169.5 \pm 17.5$  pg/ml KC). To measure PMN activation in the lung after F991 treatment, we assessed the lung homogenates for two relevant granule products, matrix metalloproteinase-9 (MMP-9)

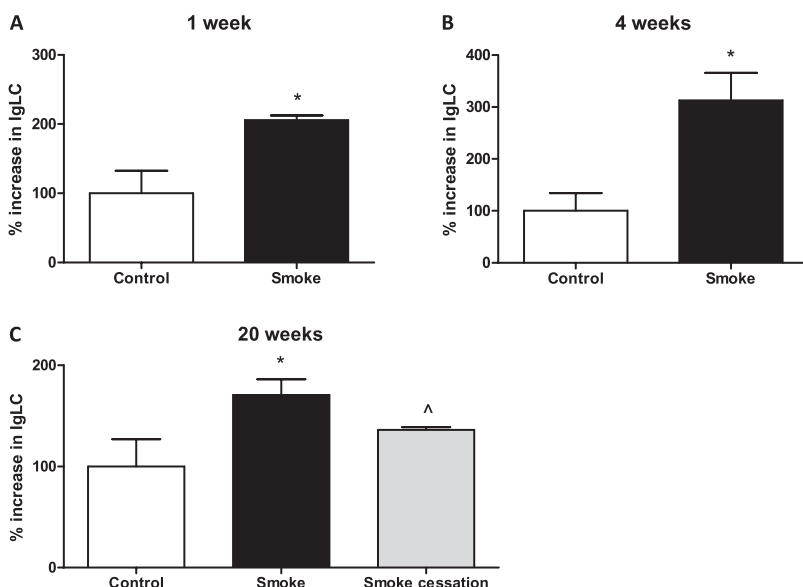


**Figure 2.** Increased levels of immunoglobulin free light chains (IgLC) in serum after whole-body cigarette smoke exposure. Densitometric analysis after Western blotting of IgLC in serum of A/J mice after whole-body cigarette smoke exposure (black bars) for (A) 1 week, (B) 4 weeks, and (C) 20 weeks and 20 weeks plus 8 weeks smoking cessation (gray bar), all compared with air-exposed mice (white bars).  $n = 4$ –6 animals per group. Values are expressed as percentage increase in IgLC (optical density/ $\text{mm}^2$ )  $\pm$  SEM. \* $P \leq 0.05$ ; significantly different from the control group. ^ $P \leq 0.05$ ; significantly different from the smoke group.

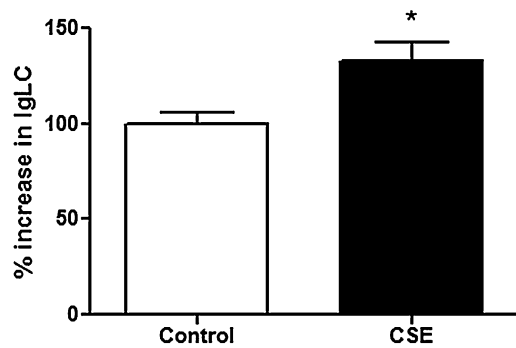


**Figure 3.** Increased levels of immunoglobulin free light chains (IgLC) in serum after cigarette smoke extract (CSE) administration. Shown is densitometric analysis after Western blotting of IgLC in serum of C57Bl/6 mice after an intraperitoneal injection of CSE + adjuvant at Day 1 followed by an intratracheal administration of CSE at Day 11 (black bar). Mice were killed at Day 21 and compared with phosphate-buffered saline-treated mice (white bar).  $n = 5$  animals per group. Values are expressed as percentage increase in IgLC (optical density/mm<sup>2</sup>)  $\pm$  SEM. \* $P \leq 0.05$ ; significantly different from the control group.

and myeloperoxidase (MPO). The mice exposed to cigarette smoke for 5 days showed increased total MMP-9 levels (total MMP-9 levels: control:  $7.4 \pm 0.8$  ng/mg vs. smoke:  $15.0 \pm 1.4$  ng/mg), and the active form of MMP-9 was also significantly elevated after smoke exposure (active MMP-9 levels: control:  $0.5 \pm 0.3$  ng/mg vs. smoke:  $2.3 \pm 0.5$  ng/mg). After F991 treatment, the total and active MMP-9 levels (total MMP-9 levels:  $13.4 \pm 1.2$  ng/mg; active MMP-9 levels:  $1.6 \pm 0.3$  ng/mg) showed a tendency to decrease, but this did not reach statistical significance. No clear differences in MPO levels were observed in the lung homogenates after F991 treatment compared with the smoke-exposed mice (data not shown).

## DISCUSSION

In this study, we describe a possible role of increased IgLC production in the development of COPD. First, IgLC serum levels were

enhanced in cigarette smoke-exposed mice and CSE-treated mice compared with control animals. In addition, local lymph nodes of smoke-exposed mice showed enhanced production of IgLC *ex vivo* compared with air-exposed mice. Furthermore, the concentration of IgLC is significantly higher in sera of patients with COPD than in that of healthy control subjects, and positive IgLC staining was found in lung tissue of patients with COPD. To date, little is known about the role of immunoglobulins in COPD. The presence of B cells has been demonstrated in human and in murine lung tissue in association with COPD (39–41). Increased IgG and IgA levels were observed in serum from patients with COPD (42) as well as higher levels of IgA-positive cells in blood (43). A recent study by Singh and colleagues (44) suggests that IgE serum levels correlate with clinical aspects of disease severity in COPD and that production of IgE in the airways of patients with COPD may be related to smoking. Additionally, COPD is associated with a higher prevalence of antinuclear autoantibodies (45). Nevertheless, the biological role of these immunoglobulins in the development of COPD remains unclear. To our knowledge, the role of IgLC has never been investigated in the pathogenesis of COPD. Increased IgLC serum or plasma levels have been assessed in other (auto)immune/inflammatory diseases (18, 19, 32, 46–49). Rises in IgLC levels appear to be associated with relapses of disease, enhanced activity of the immune system, disease progression, and severity, and this may indicate increased polyclonal B-cell activity, hypersecretion of (auto)antibodies, or increased local synthesis (20). Many of these inflammatory diseases are also associated with increased numbers of activated PMNs. Because PMNs are one of the major inflammatory cell types implicated in COPD, and because it has been shown that whole immunoglobulins can induce CXCL8 production by PMNs, we investigated the relation between IgLC and neutrophils in COPD.

We observed that IgLC can bind to the cell membrane of human PMNs. The fluorescent signal suggests a patchy distribution of IgLC-binding proteins on the neutrophil membrane. Such clustered distribution has also been demonstrated previously for mast cells (50). In earlier studies, we described that cross-linking of receptor-bound IgLC by an antigen leads to mast cell degranulation, release of proinflammatory mediators, and the induction of a local inflammatory response (50). The current

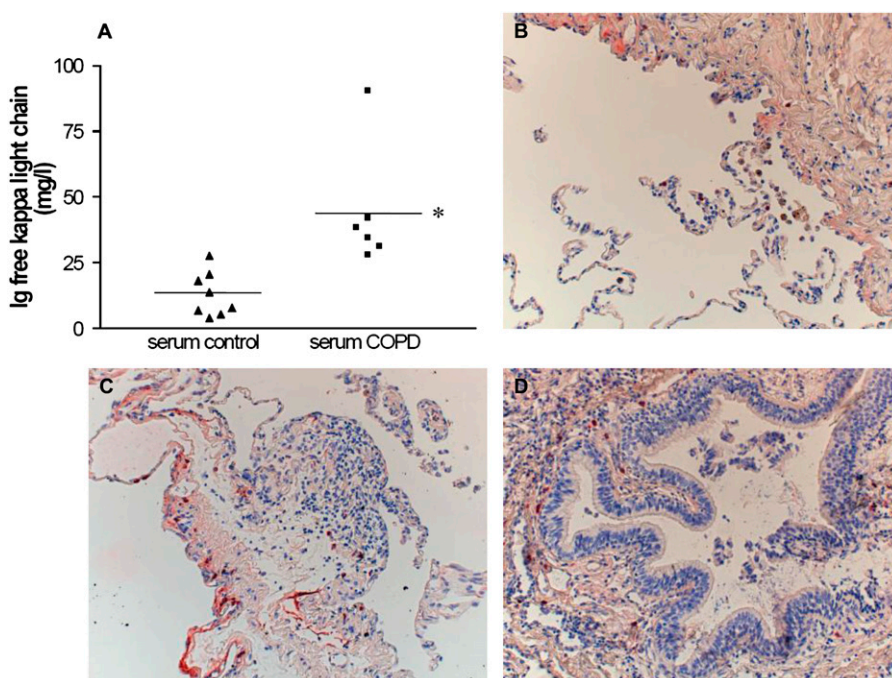
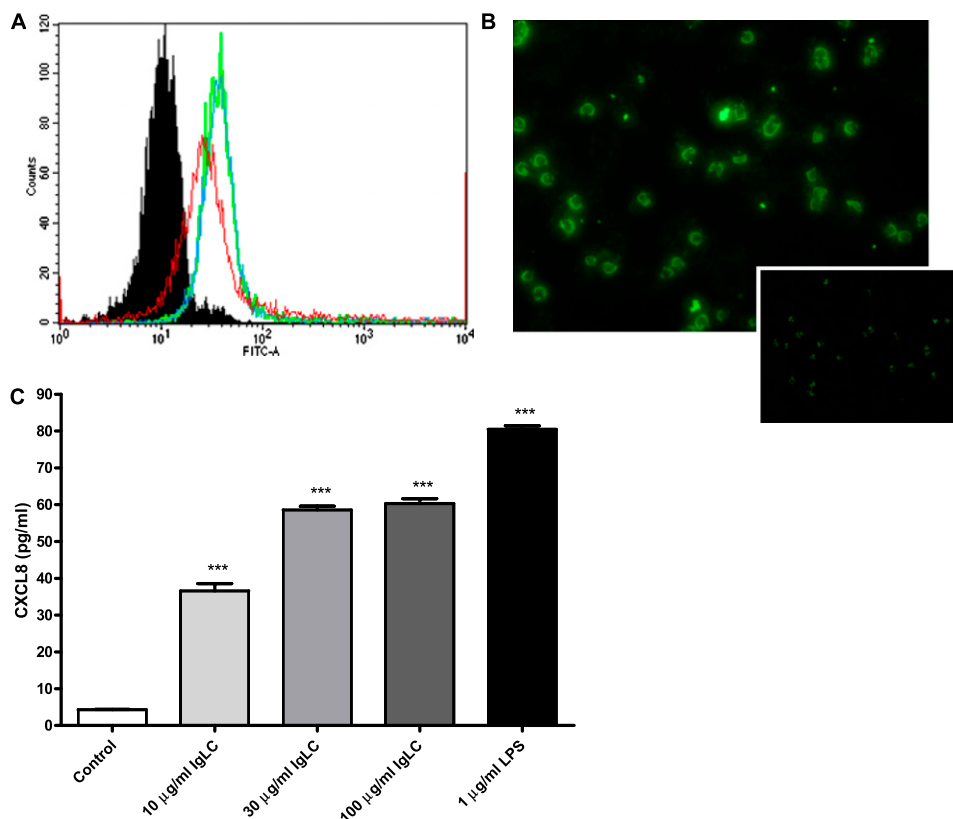


**Figure 4.** Increased levels of immunoglobulin free light chains (IgLC) in serum and lung tissue of patients with chronic obstructive pulmonary disease (COPD). (A) IgLC levels in serum of healthy control subjects (triangles,  $n = 8$ ) and patients with COPD (squares,  $n = 6$ ) were analyzed using a specific ELISA. \* $P < 0.05$ ; significantly different from the healthy control subjects. (B–D) Representative photomicrographs of an immunohistological staining for IgLC (red color, AEC staining) in human lung tissue of patients suffering from COPD. Magnification, 200 $\times$ . The presence of both (B) kappa and (C and D) lambda IgLC was demonstrated around the larger airways and within follicles.



LPS (1  $\mu$ g/ml, black bars) was added to the PMNs and also incubated for 4 hours. CXCL8 values of one donor are expressed as mean  $\pm$  SEM. \*\*\* $P \leq 0.001$ ; significantly different from the control group. Similar results were obtained in two independent experiments using two different donors.

study provides evidence that IgLC may also act on neutrophils, and *in vitro* studies demonstrate that cross-linking of IgLC on neutrophils resulted in an increase of the inflammatory mediator CXCL8. In COPD, CXCL8 is a prominent and selective attractant of neutrophils. CXCL8 levels in sputum from patients with COPD correlate with the extent of neutrophilic inflammation and disease severity (4, 6, 51). Interestingly, Gounni and colleagues (9) showed that cross-linking of the high-affinity IgE receptor on human neutrophils (10) leads to the release of CXCL8. Furthermore, *in vitro* IgE alone (without cross-linking) can delay programmed cell death of neutrophils (10). Our study suggests that the presence of IgLC could be responsible for a sustained neutrophilic inflammation as observed in COPD (41, 52). Moreover, it is described that IgLC may contribute to a prestimulation of PMNs, because IgLC increase the basal levels of neutrophil oxidative metabolism (11) and interfere with essential PMN functions, such as the activation of glucose uptake and chemotaxis (11, 53). Cohen and colleagues (11) demonstrated that IgLC increase the percentage of viable PMNs, by reducing the PMN apoptosis, thereby inducing increased PMN survival. It was further investigated that tyrosine phosphorylation was essential for this IgLC-induced increase of PMNs survival (11, 54–56). These data are in line with our results and suggest the presence of a possible IgLC receptor on the cell membrane of the neutrophil. To date, no receptor for IgLC proteins has been characterized, and the identity of this protein is currently under investigation.

IgE levels are increased in patients with COPD (9–11, 44), and we now also demonstrate increased IgLC levels. This is of interest, because both IgE and IgLC can exert similar proinflammatory effects via PMNs. This remarkable common functionality leads to the speculation whether these immunoglobulins and IgLC play a crucial role in the onset and development of neutrophil-related

diseases, such as COPD. It is yet unknown which antigen(s) in cigarette smoke or in the airways may be responsible for PMN activation through either IgE or IgLC.

An important finding of our study is that binding of immunoglobulin free light chains to PMNs can be blocked by F991. The association between neutrophils and IgLC is substantiated by the *in vivo* study, where intraairway treatment with the antagonist of IgLC, F991, reduces the cigarette smoke-induced neutrophil influx and activation in the BALF of mice. This suggests that IgLC may play a role in the smoke-induced neutrophil influx in the lungs of mice. Possible explanations for the F991-induced decreased smoke-induced neutrophil influx could be related to a reduction of the IgLC-mediated decrease in PMN apoptosis (11) and/or an inhibition of the IgLC-mediated activation of neutrophils. To measure PMN activation after F991 treatment, KC, MMP-9, and MPO levels were assessed in the lung. After F991 treatment, the KC levels in the BALF and the total and active MMP-9 levels in the lung homogenates showed a tendency to decrease compared with the smoke-exposed mice, but did not reach statistical significance. No clear differences in MPO levels in lung homogenates were observed after F991 treatment compared with the smoke-exposed mice. Interestingly, earlier studies showed that F991 effectively inhibits the development of non-atopic asthma in mice (19) by inhibition of IgLC-induced mast cell activation, but it cannot be excluded that F991 may also have additional inhibitory effects on the associated neutrophil influx in this model.

Although we have not yet performed experiments to address the effects of F991 on the development of lung emphysema, the reduction of neutrophil numbers by F991 as observed in our acute COPD model will presumably lead to less lung emphysema, because neutrophil numbers in bronchial biopsies and sputum are correlated with COPD disease severity in humans

**Figure 5.** IgLC binding to polymorphonuclear leukocytes (PMNs) is inhibited by F991, and cross-linking bound immunoglobulin free light chains (IgLC) induces CXCL8 production. Freshly isolated human PMNs were incubated with fluorescein isothiocyanate (FITC)-labeled  $\kappa$ IgLC. (A) Subsequent flow cytometric analysis of the live cell population showed significant binding of IgLC to PMNs (green line) compared with unstained cells (black line). After incubation with 5  $\mu$ g F991 (blue line), no reduced binding was observed, whereas 50  $\mu$ g F991 (red line) showed a reduced binding of PMNs to IgLC. Similar results were obtained in three independent experiments using three different donors. (B) Representative photomicrograph of an immunofluorescent staining on cytopsin preparations of PMNs incubated with FITC-labeled  $\kappa$ IgLC compared with (B, lower right corner) untreated PMNs. IgLC binding was detected along the cell membrane of PMNs. Magnification, 400 $\times$ . This experiment was repeated in two different donors with similar results. (C) Interleukin-8 (CXCL8) production of freshly isolated human PMNs incubated in 96-well plates coated with medium (control, white bars) and  $\kappa$ IgLC (10, 30, or 100  $\mu$ g/ml, gray bars) for 4 hours. As a positive control,

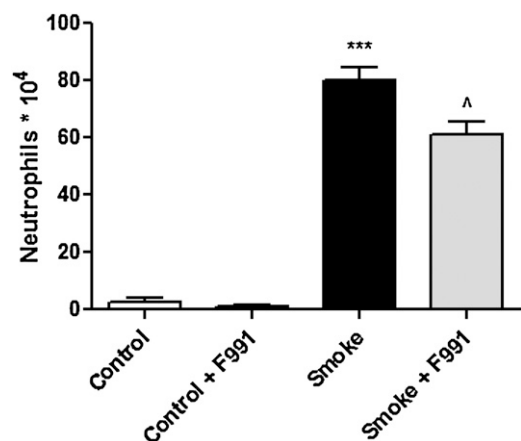


**Figure 6.** Cigarette smoke-induced neutrophil influx in the bronchoalveolar lavage fluid was significantly decreased after F991 administration. Shown are absolute neutrophil numbers in the bronchoalveolar lavage fluid of mice exposed to air (white bar), F991-treated mice exposed to air (light gray bar), mice exposed to cigarette smoke (black bar), and F991-treated mice exposed to cigarette smoke (gray bar) for 5 days. The mice received vehicle (phosphate-buffered saline [PBS]) or F991 by oropharyngeal aspiration (100  $\mu$ g F991/70  $\mu$ l sterile PBS) twice daily.  $n = 4$ –6 animals per group. Values are expressed as mean  $\pm$  SEM. \*\*\* $P \leq 0.001$ ; significantly different from the control group. ^ $P \leq 0.05$ ; significantly different from the smoke group.

(57–59). The inhibitory effect of F991 on neutrophil influx was limited *in vivo*, which may be explained by the short half-life of F991. Further research is needed to investigate the therapeutic effects of F991 on the development of lung emphysema at different dosages and to clarify the mechanism of IgLC-induced neutrophil activation.

In conclusion, our studies demonstrate production of IgLC after cigarette smoke exposure in various experimental models of COPD. This IgLC phenomenon is also observed in sera from patients with COPD and in lung tissue of patients with COPD. Antagonizing the action of IgLC reduces the cigarette smoke-induced neutrophil influx and activation in murine lungs. IgLC were shown to bind to and activate PMNs. This can serve as an attractive therapeutic target and may open new avenues toward effective treatment of COPD.

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## References

- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;176:532–555.
- Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for diagnosis, management, and prevention of chronic obstructive pulmonary disease [Internet; updated 2009]. NHLBI/WHO workshop report [accessed 2010 Dec]. Available from: [www.goldcopd.org](http://www.goldcopd.org)
- Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008;8:183–192.
- Barnes PJ. Mechanisms in COPD: differences from asthma. *Chest* 2000; 117:10S–14S.
- Pesci A, Balbi B, Majori M, Cacciani G, Bertacco S, Alciato P, Donner CF. Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease. *Eur Respir J* 1998;12:380–386.

- Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor- $\alpha$  in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
- Thatcher TH, McHugh NA, Egan RW, Chapman RW, Hey JA, Turner CK, Redonnet MR, Seweryniak KE, Sime PJ, Phipps RP. Role of cxcr2 in cigarette smoke-induced lung inflammation. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L322–L328.
- Selvaraj P, Fidadara N, Nagarajan S, Cimino A, Wang G. Functional regulation of human neutrophil Fc gamma receptors. *Immunol Res* 2004;29:219–230.
- Gounni AS, Lamkhioed B, Koussih L, Ra C, Renzi PM, Hamid Q. Human neutrophils express the high-affinity receptor for immunoglobulin E (Fc epsilon RI): role in asthma. *FASEB J* 2001;15:940–949.
- Saffar AS, Alphonse MP, Shan L, Hayglass KT, Simons FE, Gounni AS. IgE modulates neutrophil survival in asthma: role of mitochondrial pathway. *J Immunol* 2007;178:2535–2541.
- Cohen G, Rudnicki M, Deicher R, Horl WH. Immunoglobulin light chains modulate polymorphonuclear leucocyte apoptosis. *Eur J Clin Invest* 2003;33:669–676.
- Redegeld FA, Nijkamp FP. Immunoglobulin free light chains and mast cells: pivotal role in T-cell-mediated immune reactions? *Trends Immunol* 2003;24:181–185.
- Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, Kyle RA. Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem* 2002;48:1437–1444.
- Woloschak GE, Krco CJ. Regulation of kappa/lambda immunoglobulin light chain expression in normal murine lymphocytes. *Mol Immunol* 1987;24:751–757.
- Thio M, Blokhuis BR, Nijkamp FP, Redegeld FA. Free immunoglobulin light chains: a novel target in the therapy of inflammatory diseases. *Trends Pharmacol Sci* 2008;29:170–174.
- Rijnierse A, Redegeld FA, Blokhuis BR, Van der Heijden MW, Te Velde AA, Pronk I, Hommes DW, Nijkamp FP, Koster AS, Kraneveld AD. Ig-free light chains play a crucial role in murine mast cell-dependent colitis and are associated with human inflammatory bowel diseases. *J Immunol* 2010;185:653–659.
- Groot Kormelink T, Thio M, Blokhuis BR, Nijkamp FP, Redegeld FA. Atopic and non-atopic allergic disorders: current insights into the possible involvement of free immunoglobulin light chains. *Clin Exp Allergy* 2009;39:33–42.
- Schouten B, van Esch BC, van Thuijl AO, Blokhuis BR, Groot Kormelink T, Hofman GA, Moro GE, Boehm G, Arslanoglu S, Sprickelman AB, et al. Contribution of IgE and immunoglobulin free light chain in the allergic reaction to cow's milk proteins. *J Allergy Clin Immunol* 2010; 125:1308–1314.
- Kraneveld AD, Kool M, van Houwelingen AH, Roholl P, Solomon A, Postma DS, Nijkamp FP, Redegeld FA. Elicitation of allergic asthma by immunoglobulin free light chains. *Proc Natl Acad Sci USA* 2005; 102:1578–1583.
- van der Heijden M, Kraneveld A, Redegeld F. Free immunoglobulin light chains as target in the treatment of chronic inflammatory diseases. *Eur J Pharmacol* 2006;533:319–326.
- Powe DG, Groot Kormelink T, Sisson M, Blokhuis BJ, Kramer MF, Jones NS, Redegeld FA. Evidence for the involvement of free light chain immunoglobulins in allergic and nonallergic rhinitis. *J Allergy Clin Immunol* 2010;125:139–145.e3.
- Mehta PD, Cook SD, Troiano RA, Coyle PK. Increased free light chains in the urine from patients with multiple sclerosis. *Neurology* 1991;41: 540–544.
- Gottenberg JE, Aucouturier F, Goetz J, Sordet C, Jahn I, Busson M, Cayuela JM, Sibilia J, Mariette X. Serum immunoglobulin free light chain assessment in rheumatoid arthritis and primary Sjogren's syndrome. *Ann Rheum Dis* 2007;66:23–27.
- Gottenberg JE, Miceli-Richard C, Ducot B, Goupille P, Combe B, Mariette X. Markers of B-lymphocyte activation are elevated in patients with early rheumatoid arthritis and correlated with disease activity in the ESPOIR cohort. *Arthritis Res Ther* 2009;11:R114.
- Constantinescu CS, Mehta PD, Rostami AM. Urinary free kappa light chain levels in chronic progressive multiple sclerosis. *Pathobiology* 1994;62:29–33.

26. Mortaz E, Braber S, Nazary M, Givi ME, Nijkamp FP, Folkerts G. ATP in the pathogenesis of lung emphysema. *Eur J Pharmacol* 2009;619:92–96.
27. Braber S, Verheijden KA, Henricks PA, Kraneveld AD, Folkerts G. A comparison of fixation methods on lung morphology in a murine model of emphysema. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L843–L851.
28. Thurlbeck WM. Measurement of pulmonary emphysema. *Am Rev Respir Dis* 1967;95:752–764.
29. Weathington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, Folkerts G, Nijkamp FP, Blalock JE. A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. *Nat Med* 2006;12:317–323.
30. Braber S, Koelink PJ, Henricks PA, Jackson PL, Nijkamp FP, Garssen J, Kraneveld AD, Blalock JE, Folkerts G. Cigarette smoke-induced lung emphysema in mice is associated with prolyl endopeptidase, an enzyme involved in collagen breakdown. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L255–L265.
31. Chen YY, Lin SY, Yeh YY, Hsiao HH, Wu CY, Chen ST, Wang AH. A modified protein precipitation procedure for efficient removal of albumin from serum. *Electrophoresis* 2005;26:2117–2127.
32. van Esch BC, Schouten B, Blokhuis BR, Hofman GA, Boon L, Garssen J, Knippels LM, Willemsen LE, Redegeld FA. Depletion of CD4+CD25+ T cells switches the whey-allergic response from immunoglobulin E to immunoglobulin free light chain-dependent. *Clin Exp Allergy* 2010;40:1414–1421.
33. Abe M, Goto T, Kosaka M, Wolfenbarger D, Weiss DT, Solomon A. Differences in kappa to lambda (kappa:lambda) ratios of serum and urinary free light chains. *Clin Exp Immunol* 1998;111:457–462.
34. Groot Kormelink T, Tekstra J, Thurlings RM, Boumans MH, Vos K, Tak PP, Bijlsma JW, Lafeber FP, Redegeld FA, van Roon JA. Decrease in immunoglobulin free light chains in patients with rheumatoid arthritis upon rituximab (anti-CD20) treatment correlates with decrease in disease activity. *Ann Rheum Dis* 2010;69:2137–2144.
35. Davern S, Tang LX, Williams TK, Macy SD, Wall JS, Weiss DT, Solomon A. Immunodiagnostic capabilities of anti-free immunoglobulin light chain monoclonal antibodies. *Am J Clin Pathol* 2008;130:702–711.
36. Overbeek SA, Henricks PA, Srien AI, Koelink PJ, de Kruijf P, Lim HD, Smit MJ, Zaman GJ, Garssen J, Nijkamp FP, et al. N-Acetylated proline-glycine-proline induced G-protein dependent chemotaxis of neutrophils is independent of cxcl8 release. *Eur J Pharmacol* 2011;668:428–434.
37. Braber S, Henricks PA, Nijkamp FP, Kraneveld AD, Folkerts G. Inflammatory changes in the airways of mice caused by cigarette smoke exposure are only partially reversed after smoking cessation. *Respir Res* 2010;11:99.
38. Chen Y, Hanaoka M, Droma Y, Chen P, Voelkel NF, Kubo K. Endothelin-1 receptor antagonists prevent the development of pulmonary emphysema in rats. *Eur Respir J* 2010;35:904–912.
39. van der Strate BW, Postma DS, Brandsma CA, Melgert BN, Luinge MA, Geerlings M, Hylkema MN, van den Berg A, Timens W, Kerstjens HA. Cigarette smoke-induced emphysema: a role for the B cell? *Am J Respir Crit Care Med* 2006;173:751–758.
40. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
41. Gosman MM, Willems BW, Jansen DF, Lapperre TS, van Schadewijk A, Hiemstra PS, Postma DS, Timens W, Kerstjens HA. Increased number of B-cells in bronchial biopsies in COPD. *Eur Respir J* 2006;27:60–64.
42. Chauhan S, Gupta MK, Goyal A, Dasgupta DJ. Alterations in immunoglobulin & complement levels in chronic obstructive pulmonary disease. *Indian J Med Res* 1990;92:241–245.
43. Brandsma CA, Hylkema MN, Geerlings M, van Geffen WH, Postma DS, Timens W, Kerstjens HA. Increased levels of (class switched) memory B cells in peripheral blood of current smokers. *Respir Res* 2009;10:108.
44. Singh B, Arora S, Khanna V. Association of severity of COPD with IgE and interleukin-1 beta. *Monaldi Arch Chest Dis* 2010;73:86–87.
45. Bonarius HP, Brandsma CA, Kerstjens HA, Koerts JA, Kerkhof M, Nizankowska-Mogilnicka E, Roozendaal C, Postma DS, Timens W. Antinuclear autoantibodies are more prevalent in COPD in association with low body mass index but not with smoking history. *Thorax* 2011;66:101–107.
46. Elovaara I, Seppala I, Kinnunen E, Laaksovirta H. Increased occurrence of free immunoglobulin light chains in cerebrospinal fluid and serum in human immunodeficiency virus-1 infection. *J Neuroimmunol* 1991;35:65–77.
47. Goffette S, Schluep M, Henry H, Duprez T, Sindic CJ. Detection of oligoclonal free kappa chains in the absence of oligoclonal IgG in the CSF of patients with suspected multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2004;75:308–310.
48. Solling K, Solling J, Romer FK. Free light chains of immunoglobulins in serum from patients with rheumatoid arthritis, sarcoidosis, chronic infections and pulmonary cancer. *Acta Med Scand* 1981;209:473–477.
49. Romer FK, Solling K. Repeated measurements of serum immunoglobulin-free light chains in early sarcoidosis. *Eur J Respir Dis* 1984;65:292–295.
50. Redegeld FA, van der Heijden MW, Kool M, Heijdra BM, Garssen J, Kraneveld AD, Van Loveren H, Roholl P, Saito T, Verbeek JS, et al. Immunoglobulin-free light chains elicit immediate hypersensitivity-like responses. *Nat Med* 2002;8:694–701.
51. Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokuyama T, Tsukaguchi K, Narita N. Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest* 1997;112:505–510.
52. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir Res* 2006;7:53.
53. Cohen G, Haag-Weber M, Mai B, Deicher R, Horl WH. Effect of immunoglobulin light chains from hemodialysis and continuous ambulatory peritoneal dialysis patients on polymorphonuclear leukocyte functions. *J Am Soc Nephrol* 1995;6:1592–1599.
54. Yousefi S, Green DR, Blaser K, Simon HU. Protein-tyrosine phosphorylation regulates apoptosis in human eosinophils and neutrophils. *Proc Natl Acad Sci USA* 1994;91:10868–10872.
55. Hebert MJ, Takano T, Holthofer H, Brady HR. Sequential morphologic events during apoptosis of human neutrophils: modulation by lipoxygenase-derived eicosanoids. *J Immunol* 1996;157:3105–3115.
56. Sweeney JF, Nguyen PK, Omann GM, Hinshaw DB. Lipopolysaccharide protects polymorphonuclear leukocytes from apoptosis via tyrosine phosphorylation-dependent signal transduction pathways. *J Surg Res* 1998;74:64–70.
57. Barnes PJ. Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev* 2004;56:515–548.
58. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996;51:267–271.
59. Tsoumakidou M, Tzanakis N, Siafakas NM. Induced sputum in the investigation of airway inflammation of COPD. *Respir Med* 2003;97:863–871.

**An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD**

***Immunoglobulin light chains in COPD***

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**Online data supplement**

## **Material and methods**

### **Animals**

Female A/J mice, 9-14 weeks old, male C57Bl/6 mice, 6-8 weeks old, male Balb/c mice, 6-8 weeks old (Charles River Laboratories) were housed under controlled conditions in standard laboratory cages. They were provided free access to water and food. All *in vivo* experimental protocols were approved by the local Ethics Committee and were performed under strict governmental and international guidelines on animal experimentation.

### **Nose-only cigarette smoke exposure**

C57Bl/6 mice were nose-only exposed to the diluted mainstream and sidestream smoke from commercially available Lucky Strike cigarettes without filter (British-American Tobacco, Groningen, The Netherlands), using the TE-10z smoking machine (Teague Enterprises, Davis, CA) which is programmed to smoke cigarettes according to the Federal Trade Commission protocol (35 ml puff volume drawn for 2 seconds once per minute). Before starting smoke exposure, mice were accustomed to the nose-only exposure chambers (In-Tox Products Inc., Albuquerque, NM) by gradually prolonging their stay in the tubes over the course of one week. Smoke exposures were conducted twice every weekday for 3 months after an adaptation period of 2 weeks, starting with 1×1 cigarette, increasing the dosage to 2×3 cigarettes within two weeks. Control mice underwent the same procedures, but were allowed to breathe room air throughout the whole exposure period. The average total suspended particulates (TSP) concentration of the smoke inside the exposure chamber was 85 mg/m<sup>3</sup> determined by gravimetric analysis of Emfab filter samples (Pall Corporation, East Hills, NY, USA). The carbon monoxide (CO) content of the smoke inside the exposure chamber was measured by sampling with the Monoxor II CO analyzer (Bacharach Inc, New Kensington, PA, USA) at 15 seconds intervals during 2 runs and was around 1200 ppm. The mice were sacrificed 16-24 hours after the last air or smoke exposure.

### **Whole body cigarette smoke exposure**

A/J mice were divided into three groups. The first group was exposed to room air for 20 weeks, the second group was exposed to cigarette smoke for 20 weeks and the third group was exposed to cigarette smoke for 20 weeks followed by a period of 8 weeks without cigarette smoke exposure. The mice were exposed in whole-body chambers to air (sham) or to diluted mainstream cigarette smoke from the reference cigarettes 2R4F (University of Kentucky, Lexington, Kentucky) using a smoking apparatus. Exposures were conducted 4h/day (with a 30/60-minutes fresh air break after each hour of exposure), 5 days/week for 20 weeks to a target cigarette smoke concentration of 750 µg total particulate matter/l (TPM/l). This TPM concentration was reached after an adaptation period of 2 weeks, starting

with a TPM concentration of 125 µg TPM/l. The mass concentration of cigarette smoke TPM was determined by gravimetric analysis of Cambridge filter samples. Carbon monoxide levels were monitored continuously and were around 800 ppm. The nicotine concentration in the smoke was approximately 40 µg/l. The sample sites were located in the middle of the exposure chamber at the breathing zone. The carboxyhemoglobin level measured via blood gas analysis was 50% on average in the smoke-exposed mice. The mice were sacrificed 16-24 hours after the last air or smoke exposure, or after the smoke-free period of 8 weeks.

### **Cigarette smoke extract administration**

Cigarette smoke extract (CSE) was prepared as described before [25]. Briefly, TE-10z smoking machine was used to direct mainstream and sidestream smoke from one cigarette through 5 ml PBS. Hereafter, absorbance was measured spectrophotometrically and the OD of the CSE-PBS solution was 11 on average. Freshly prepared cigarette smoke extract was used for each injection. The control C57Bl/6 mice were intraperitoneally injected with 200 µl PBS at day 1. The CSE group was injected intraperitoneally with 100 µl CSE-PBS plus 100 µl adjuvant (Freunds Complete Adjuvant H37Ra, Difco) at day 1 and 50 µl CSE-PBS was administered intratracheally at day 11. At day 21 the mice were sacrificed.

### **F991, IgLC antagonist, study**

Balb/c mice were exposed in whole-body chambers to air (sham) or to diluted mainstream cigarette smoke from the reference cigarettes 2R4F (University of Kentucky, Lexington, Kentucky) using a peristaltic pump. Just before the experiments, filters were cut from the cigarettes. Each cigarette was smoked in 5 minutes at a rate of 5 l/hour in a ratio with 60 l/hour air. The mice were exposed to cigarette smoke using 5 cigarettes twice daily for 5 consecutive days, except for the first day when they were exposed to 3 cigarettes. The mice were sacrificed 16 hours after the last air or smoke exposure. In this cigarette smoke exposure model, cigarette smoke- and air-exposed mice received F991 (100µg F991 in 70µl sterile PBS) by oropharyngeal administration twice daily for 5 consecutive days during isoflurane anesthesia. The IgLC antagonist F991, a 9-mer peptide (AHWSGHCCCL), was synthesized by Fmoc chemistry (Ansynth, Roosendaal, The Netherlands).

### **Bronchoalveolar lavage**

Immediately after i.p. injection with an overdose of pentobarbital, the lungs of mice were lavaged 4 times through a tracheal cannula with 1 ml saline (NaCl 0.9%), pre-warmed at 37 °C. After centrifuging the bronchoalveolar lavage fluid at 4°C (400 g, 5 min), the cell pellets of the 4 lavages were used for neutrophil cell counts. The 4 cell pellets, kept on ice, were pooled per animal and resuspended in 150 µl cold saline.

### **Neutrophil cell count in BALF**

After staining with Türk solution, total cell counts per lung lavage were made under light microscopy using a Burker-Türk chamber. Differential cell counts were performed on air-dried cytopsin preparations stained by DiffQuick™ (Dade A.G., Düdingen, Switzerland). Cells were identified according to standard morphology. At least 200 cells were counted and the absolute number of neutrophils was calculated.

### **Histology and morphometric analysis**

Mice used for morphometric analysis, were sacrificed by an i.p. injection with an overdose of pentobarbital (Nembutal™, Ceva Santé Animale, Naaldwijk, The Netherlands). The lungs were fixed with a 10% formalin infusion through a tracheal cannula at a constant pressure of 25 cm H<sub>2</sub>O [27]. The left lung was immersed in fresh fixative for at least 24h, after which it was embedded in paraffin. After paraffin embedding, 5 µm sections were cut and stained with hematoxylin/eosin (H&E) according to standard methods. Morphometric assessment of emphysema, including determination of the average inter-alveolar distance, was estimated by the mean linear intercept (Lm) analysis. The Lm was determined by light microscopy at a total magnification of 100x, whereby 24 random photomicroscopic images per left lung tissue section were evaluated by microscopic projection onto a reference grid. By dividing total grid length by the number of alveolar wall-grid line intersections, the Lm (in µm) was calculated.

### **Right ventricular heart hypertrophy measurement**

The right ventricle was removed from lower heart after removal of the atria. The right ventricle and the left ventricle plus septum were weighed and the ratio of the weights was calculated as follows: (right ventricle)/(left ventricle + septum).

### **Serum collection**

Directly after the i.p. injection with an overdose of pentobarbital, blood was obtained by heart puncture and collected in MiniCollect Z Serum Sep tubes (Greiner Bio-one, part no.450472). After 1 hour the blood samples were centrifuged for 10 min at 20.000 g and the sera were stored at -20°C for IgLC measurement.

### **Culture of thoracic lymph node (TLN) cells**

Lung-draining lymph nodes were collected from the thorax and transferred to 1 ml sterile RPMI 1640 without L-glutamine and phenol red (Lonza Verviers SPRL, Belgium), supplemented with 1% Pen-Strep. TLNs from six mice were pooled and were filtered through a 70 µm nylon cell strainer (BD Falcon, Belgium) with 4 ml RPMI 1640 without L-glutamine

and phenol red, supplemented with 1% Pen-Strep to obtain a single-cell suspension. The total number of cells in the TLN-cell suspension was determined using a Burker-Turk counting-chamber. TLN cells ( $1 \times 10^6$  cells/ml) were cultured in a culture flask at 37°C in 5% CO<sub>2</sub>. After 24 hours, the supernatant was harvested, and stored at -20°C until free immunoglobulin light chains were determined.

### **Western Blot analysis**

Before use, serum samples were precipitated to deplete high amounts of albumin by using trichloroacetic acid/acetone, as previously described [30]. Equal amounts of proteins (precipitated serum and TLN supernatants) of boiled nonreduced samples were separated electrophoretically (SDS-PAGE 12%) and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Veenendaal, The Netherlands). The membrane was blocked with Tween-PBS containing 2% milk proteins. HRP-labeled goat anti-mouse κIgLC (1:10.000) was applied for 1 h at room temperature as primary Ab. Blots were washed in Tween-PBS three times for 10 min, incubated in commercial ECL reagents (Amersham Biosciences, Roosendaal, The Netherlands), and exposed to photographic film. Films were scanned on a GS710 Calibrated Imaging Densitometer (Bio-Rad Laboratories) and the optical density (OD) of the immunoreactive bands was quantified.

### **Patient characteristics**

All patient material collection was performed according to the local Medical Ethical Committees of the University Medical Center Groningen and all patients gave their informed consent before participation in the study.

### **ELISA for kappa IgLC proteins**

The concentration of κIgLC in serum samples obtained from patients with COPD and healthy volunteers was measured by ELISA with mAbs specific for human IgLC (purchased from A. Solomon, University of Tennessee, Knoxville, TN) as described previously [31, 32].

### **Immunohistochemical staining for IgLC**

Paraffin sections of lung tissue were routinely deparaffinized and blocked for endogenous peroxidase for 30 min at room temperature. Sections were incubated with the primary mouse antibodies directed against human κ and λ IgLC [33] for 60 min at room temperature. Post-incubation with the primary antibody, the sections were incubated with anti-mouse HRP-labeled polymer (Dakocytomation, Heverlee, Belgium) for 30 min at room temperature. Color was developed with AEC substrate chromogen. Between incubation steps, the sections were

intensively rinsed with 0.05 M TBS containing 0.05% Tween. Within each test, negative controls were included, and they were all found not to contain any specific staining.

### **Isolation of human PMNs**

PMNs were isolated from buffy coats, which were purchased from Sanquin Blood Bank (Amsterdam, The Netherlands). The PMNs were obtained by gradient centrifugation on Ficoll-Paque<sup>TM</sup> PLUS (density: 1.077 g/ml, GE Healthcare, Diegem, Belgium), followed by hypotonic lysis of erythrocytes with sterile lysis buffer (0.15 M NH<sub>4</sub>Cl, 0.01 M KHCO<sub>3</sub> and 0.1 mM EDTA, pH 7.4) on ice. Contamination by monocytes was prevented by incubating the isolated PMN fraction at 37°C in RPMI/10%FBS medium for 1h. After incubation, the PMNs were washed with PBS and finally resuspended in RPMI 1640 medium (without L-glutamine and phenol red) supplemented with 1% heat-inactivated FBS. Resulting PMN preparations consisted of approximately 95-97% PMNs.

### **Flowcytometry**

Freshly isolated PMNs (1x10<sup>5</sup> cells/100 µl) were incubated with 3 µg FITC labeled κIgLC (κIgLC isolated and purified from the urine of multiple myeloma patients were kindly donated by dr B. Bast, UMC Utrecht, the Netherlands) for 1 hour at 37°C or 5 µg / 50 µg F991 (kindly produced by Steffen van der Wal, Utrecht University) was preincubated with FITC labeled κIgLC for 30 min before incubation with PMNs. In the *in vitro* experiments oxidized F991 was used, because recent experiments showed that F991 is rapidly oxidized *in vivo* into its possible biological active molecular conformation (Van der Wal et al, manuscript in preparation). After incubation, the cells were washed to remove the unbound FITC-IgLC and resuspended in PBS/1%BSA. Binding of FITC labeled κIgLC to PMNs was analyzed using a FACS Calibur (BD Biosciences, Breda, the Netherlands).

### **Immunofluorescence staining for IgLC on PMNs**

Freshly isolated PMNs (1x10<sup>6</sup> cells/ml) were incubated with 7 µg FITC labeled κIgLC for 30 min on ice. After incubation, the cells were washed to remove the unbound FITC-IgLC. The labeled cells were spun onto slides by using a cytospin centrifuge. The FITC signal was amplified using a FITC signal amplification kit (Invitrogen, Breda, the Netherlands). Fluorescent imaging was performed on a Zeiss microscope.

### **PMN stimulation assay**

96-well plates (Greiner, Alphen a/d Rijn, the Netherlands) were coated with κIgLC (10, 30 and 100 µg/ml) (isolated and purified from the urine of a myeloma patient, purity > 95%) in carbonate buffer (pH 9.6) by overnight incubation. Prior to adding the cells, the coated

surface was blocked with RPMI containing 10% immunoglobulin free BSA (Sigma Aldrich, Zwijndrecht, the Netherlands) for 1h at room temperature. Buffy coat isolated PMNs (200  $\mu$ l per well, cell density adjusted to  $1 \times 10^6$  cells/ml using RPMI/10%FBS) were added to the 96-well plate coated with klgLC and incubated for 4 hours. As a positive control 1  $\mu$ g/ml LPS (Sigma Aldrich, Zwijndrecht, the Netherlands) was added to the isolated PMNs and incubated for 4 hours. After incubation the cells were centrifuged at 450 g for 5 min and the supernatant was collected for CXCL8 measurement.

### **CXCL8 ELISA**

CXCL8 levels were measured using a human CXCL8 ELISA kit (BD Biosciences, Breda, the Netherlands) according to manufacturer's instructions.

### **Statistical analysis**

Experimental results are expressed as mean  $\pm$  S.E.M. Differences between groups were statistically determined by an unpaired two-tailed Student's *t*-test and differences between (*in vitro*) data were analyzed using two-way ANOVA, with post hoc Bonferroni's *t*-test for multiple comparisons using GraphPad Prism (Version 4.0). Results were considered statistically significant when  $P < 0.05$ .

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

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#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                   |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Gillina                                                                                      | 2. Surname (Last Name)<br>Bezemer | 3. Effective Date (07-August-2008)<br>28-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                   | Corresponding Author's Name<br>Saskia Braber        |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                   |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                   |                                                     |

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#### The Work Under Consideration for Publication

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|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
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|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
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|                                                               |                                     |                          |                            |        |          | ADD |
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|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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### Section 1. Identifying Information

|                                                                                                                            |                                    |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Bart                                                                                         | 2. Surname (Last Name)<br>Blokhuys | 3. Effective Date (07-August-2008)<br>28-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                    | Corresponding Author's Name<br>S.Braber             |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                    |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                    |                                                     |

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|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
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|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
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|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
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|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
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|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

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|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
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|                                                               |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
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|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Saski

2. Surname (Last Name)  
Braber

3. Effective Date (07-August-2008)  
28-April-2011

4. Are you the corresponding author? ☐ Yes ☒ No Corresponding Author's Name  
Saskia Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section 4.

#### Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☒ No other relationships/conditions/circumstances that present a potential conflict of interest

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

## ICMJE Form for Disclosure of Potential Conflicts of Interest

---

### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

#### 2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes". Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

#### 3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

#### 4. Other relationships.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                    |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Gert                                                                                         | 2. Surname (Last Name)<br>Folkerts | 3. Effective Date (07-August-2008)<br>28-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                    | Corresponding Author's Name<br>S.Braber             |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                    |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                    |                                                     |

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

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Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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SAVE

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Johan

2. Surname (Last Name)  
Garssen

3. Effective Date (07-August-2008)  
28-April-2011

4. Are you the corresponding author? ☐ Yes ☒ No Corresponding Author's Name  
S. Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section 4.

#### Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☒ No other relationships/conditions/circumstances that present a potential conflict of interest

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

## ICMJE Form for Disclosure of Potential Conflicts of Interest

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### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

#### 2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes". Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

#### 3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

#### 4. Other relationships.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                    |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|------------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Paul A.J.                                                                                    | 2. Surname (Last Name)<br>Henricks | 3. Effective Date (07-August-2008)<br>28-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                    | Corresponding Author's Name<br>Saskia Braber        |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                    |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                    |                                                     |

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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Hide All Table Rows Checked 'No'

SAVE

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Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

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Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Huib A.M.

2. Surname (Last Name)  
Kerstjens

3. Effective Date (07-August-2008)  
29-April-2011

4. Are you the corresponding author? ☐ Yes ☒ No  
Corresponding Author's Name  
Saskia Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

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| Relevant financial activities outside the submitted work |                          |                          |                                     |                      |          |     |
|----------------------------------------------------------|--------------------------|--------------------------|-------------------------------------|----------------------|----------|-----|
| Type of Relationship (in alphabetical order)             | No                       | Money Paid to You        | Money to Your Institution*          | Entity               | Comments |     |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | AstraZeneca          |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Novartis             |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Nycomed              |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | GSK                  |          | X   |
|                                                          |                          |                          |                                     |                      |          | ADD |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Pfizer               |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Novartis             |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Astellas             |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | AstraZeneca          |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | GSK                  |          | X   |
|                                                          |                          |                          |                                     |                      |          | ADD |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                                     |                      |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|-------------------------------------|----------------------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution*          | Entity               | Comments |     |
| 3. Employment                                                               | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 4. Expert testimony                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 5. Grants/grants pending                                                    | <input type="checkbox"/>            | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 6. Payment for lectures including service on speakers bureaus               | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 7. Payment for manuscript preparation                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X   |
|                                                                             |                                     |                          |                                     |                      |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

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### Section 4.

#### Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- ☒ No other relationships/conditions/circumstances that present a potential conflict of interest
- ☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

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Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

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### Instructions

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#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

#### 2. The work under consideration for publication.

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Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Pim

2. Surname (Last Name)  
Koelink

3. Effective Date (07-August-2008)  
29-September-2011

4. Are you the corresponding author? ☐ Yes ☒ No Corresponding Author's Name  
Saskia Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section 4.

#### Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☒ No other relationships/conditions/circumstances that present a potential conflict of interest

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

## ICMJE Form for Disclosure of Potential Conflicts of Interest

---

### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

#### 2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes". Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

#### 3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

#### 4. Other relationships.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Tom

2. Surname (Last Name)  
Groot Kormelink

3. Effective Date (07-August-2008)  
29-April-2011

4. Are you the corresponding author? ☐ Yes ☒ No Corresponding Author's Name  
S.Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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Hide All Table Rows Checked 'No'

SAVE

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Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                           |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Tom                                                                                          | 2. Surname (Last Name)<br>Groot Kormelink | 3. Effective Date (07-August-2008)<br>29-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                           |                                                     |
|                                                                                                                            |                                           | Corresponding Author's Name<br>?                    |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                           |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                           |                                                     |

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section 4. Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- ☒ No other relationships/conditions/circumstances that present a potential conflict of interest
- ☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

## ICMJE Form for Disclosure of Potential Conflicts of Interest

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### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

#### 1. Identifying information.

Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

#### 2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes". Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

#### 3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                  |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|----------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Dirkje S.                                                                                    | 2. Surname (Last Name)<br>Postma | 3. Effective Date (07-August-2008)<br>29-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                  | Corresponding Author's Name<br>Saskia Braber        |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                  |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                  |                                                     |

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

| Relevant financial activities outside the submitted work |                          |                          |                                     |                      |          |     |
|----------------------------------------------------------|--------------------------|--------------------------|-------------------------------------|----------------------|----------|-----|
| Type of Relationship (in alphabetical order)             | No                       | Money Paid to You        | Money to Your Institution*          | Entity               | Comments |     |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | AstraZeneca          |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Novartis             |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Nycomed              |          | X   |
| 1. Board membership                                      | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | GSK                  |          | X   |
|                                                          |                          |                          |                                     |                      |          | ADD |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Pfizer               |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Novartis             |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Astellas             |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | AstraZeneca          |          | X   |
| 2. Consultancy                                           | <input type="checkbox"/> | <input type="checkbox"/> | <input checked="" type="checkbox"/> | GSK                  |          | X   |
|                                                          |                          |                          |                                     |                      |          | ADD |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                                     |                      |          |          |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|-------------------------------------|----------------------|----------|----------|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution*          | Entity               | Comments |          |
| 3. Employment                                                               | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 4. Expert testimony                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 5. Grants/grants pending                                                    | <input type="checkbox"/>            | <input type="checkbox"/> | <input checked="" type="checkbox"/> | Boehringer Ingelheim |          | X<br>ADD |
| 6. Payment for lectures including service on speakers bureaus               | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 7. Payment for manuscript preparation                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>            |                      |          | X<br>ADD |

\* This means money that your institution received for your efforts.

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## ICMJE Form for Disclosure of Potential Conflicts of Interest

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### Section 4.

#### Other relationships

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Hide All Table Rows Checked 'No'

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### Instructions

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Enter your full name. If you are NOT the corresponding author please check the box "no" and a space to enter the name of the corresponding author in the space that appears. Provide the requested manuscript information. Double-check the manuscript number and enter it.

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Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name)  
Frank

2. Surname (Last Name)  
Redegeld

3. Effective Date (07-August-2008)  
28-April-2011

4. Are you the corresponding author? ☐ Yes ☒ No Corresponding Author's Name  
S.Braber

5. Manuscript Title  
An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD

6. Manuscript Identifying Number (if you know it)  
Blue-201104-0761OC

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

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### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

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| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

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Hide All Table Rows Checked 'No'

SAVE

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#### 4. Other relationships.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

|                                                                                                                            |                                |                                                     |
|----------------------------------------------------------------------------------------------------------------------------|--------------------------------|-----------------------------------------------------|
| 1. Given Name (First Name)<br>Marco                                                                                        | 2. Surname (Last Name)<br>Thio | 3. Effective Date (07-August-2008)<br>28-April-2011 |
| 4. Are you the corresponding author? <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No                   |                                | Corresponding Author's Name<br>Saskia Braber        |
| 5. Manuscript Title<br>An association between neutrophils and immunoglobulin free light chains in the pathogenesis of COPD |                                |                                                     |
| 6. Manuscript Identifying Number (if you know it)<br>Blue-201104-0761OC                                                    |                                |                                                     |

### Section 2. The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc...)?

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#### The Work Under Consideration for Publication

| Type                                                                                                                                    | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|-----------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| 1. Grant                                                                                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 2. Consulting fee or honorarium                                                                                                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 3. Support for travel to meetings for the study or other purposes                                                                       | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 4. Fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 5. Payment for writing or reviewing the manuscript                                                                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                                                                                                                         |                                     |                          |                            |                |            | ADD |
| 6. Provision of writing assistance, medicines, equipment, or administrative support                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| The Work Under Consideration for Publication |                                     |                          |                            |                |            |     |
|----------------------------------------------|-------------------------------------|--------------------------|----------------------------|----------------|------------|-----|
| Type                                         | No                                  | Money Paid to You        | Money to Your Institution* | Name of Entity | Comments** |     |
|                                              |                                     |                          |                            |                |            | ADD |
| 7. Other                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |                |            | X   |
|                                              |                                     |                          |                            |                |            | ADD |

\* This means money that your institution received for your efforts on this study.

\*\* Use this section to provide any needed explanation.

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking "No" or providing the requested information. If you have more than one relationship click the "Add" button to add a row. Excess rows can be removed by clicking the "X" button.

| Relevant financial activities outside the submitted work      |                                     |                          |                            |        |          |     |
|---------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                  | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
| 1. Board membership                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 2. Consultancy                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 3. Employment                                                 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 4. Expert testimony                                           | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 5. Grants/grants pending                                      | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 6. Payment for lectures including service on speakers bureaus | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                               |                                     |                          |                            |        |          | ADD |
| 7. Payment for manuscript preparation                         | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |

## ICMJE Form for Disclosure of Potential Conflicts of Interest

| Relevant financial activities outside the submitted work                    |                                     |                          |                            |        |          |     |
|-----------------------------------------------------------------------------|-------------------------------------|--------------------------|----------------------------|--------|----------|-----|
| Type of Relationship (in alphabetical order)                                | No                                  | Money Paid to You        | Money to Your Institution* | Entity | Comments |     |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 8. Patents (planned, pending or issued)                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 9. Royalties                                                                | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 10. Payment for development of educational presentations                    | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 11. Stock/stock options                                                     | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed** | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |
| 13. Other (err on the side of full disclosure)                              | <input checked="" type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/>   |        |          | X   |
|                                                                             |                                     |                          |                            |        |          | ADD |

\* This means money that your institution received for your efforts.

\*\* For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

### Section 4.

#### Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

☒ No other relationships/conditions/circumstances that present a potential conflict of interest

☐ Yes, the following relationships/conditions/circumstances are present (explain below):

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

Hide All Table Rows Checked 'No'

SAVE

## ICMJE Form for Disclosure of Potential Conflicts of Interest

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### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.