

Accepted Manuscript

Enhanced Plasmacytoid Dendritic Cell Antiviral Responses After Omalizumab

Michelle A. Gill, MD, Andrew H. Liu, MD, Agustin Calatroni, MS, Rebecca Z. Krouse, MS, Baomei Shao, PhD, Allison Schiltz, BA, James E. Gern, MD, Alkis Togias, MD, William W. Busse, MD



PII: S0091-6749(17)31357-X

DOI: [10.1016/j.jaci.2017.07.035](https://doi.org/10.1016/j.jaci.2017.07.035)

Reference: YMAI 12985

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 18 January 2017

Revised Date: 21 June 2017

Accepted Date: 12 July 2017

Please cite this article as: Gill MA, Liu AH, Calatroni A, Krouse RZ, Shao B, Schiltz A, Gern JE, Togias A, Busse WW, Enhanced Plasmacytoid Dendritic Cell Antiviral Responses After Omalizumab, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.07.035.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Title: Enhanced Plasmacytoid Dendritic Cell Antiviral Responses After Omalizumab

Running Title: Omalizumab Restores *ex vivo* Antiviral IFN α Responses

Authors: Michelle A. Gill, MD^{a, b}, Andrew H. Liu, MD^c, Agustin Calatroni, MS^d, Rebecca Z. Krouse, MS^d, Baomei Shao, PhD^b, Allison Schiltz, BA^c, James E. Gern, MD^f, Alkis Togias, MD^e, William W. Busse, MD^f

Affiliations:

A- Departments of Pediatrics and Immunology, University of Texas Southwestern Medical Center, Dallas, TX

B- Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX

C- Children's Hospital Colorado and University of Colorado School of Medicine, Aurora, CO

D- Rho Inc, Federal Systems Division, Chapel Hill, NC

E- National Institute of Allergy and Infectious Diseases, Bethesda, MD

F- University of Wisconsin School of Medicine and Public Health, Madison, WI

Corresponding Author:

Michelle A. Gill, MD, PhD

UT Southwestern Medical Center

5323 Harry Hines Boulevard

Dallas, TX 75390-9063

Phone: 214-648-8448 or 214-648-4282

Fax: 214-648-2096

Email: Michelle.Gill@UTSouthwestern.edu

Sources of Funding:

This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract and grant numbers NIH NIAID 5R01AI098077, HHSN272200900052C, HHSN272201000052I, 1UM1AI114271-01, and UM2AI117870. Additional support was provided by the National Center for Research Resources, and National Center for Advancing Translational Sciences, National Institutes of Health, under grants, NCATS/NIH UL1TR000150, NCRR/NCAT/NIH UL1TR000077-04, UL1TR000451, UL1TR001105, UL1TR000040, UM1AI109565, UL1TR000075, 1UL1RR025780, UL1TR000154 and UL1TR001082. The following were donated: omalizumab and matching placebo by Novartis, and fluticasone and matching placebo by GSK, under a clinical trial agreement with the University of Wisconsin-Madison; EpiPens by Mylan; and Ayr nasal rinse by B.F. Ascher & Company, Inc. None of these companies had a role in the development or approval of the protocol, conduct of the trial, data analysis, manuscript preparation, or the decision to submit for publication.

Word Count: 3750 (recommended limit 3500)

Abstract (250 words max): 247

Background: Atopy and viral respiratory infections synergistically promote asthma exacerbations. IgE cross-linking inhibits critical viral-induced IFN α responses of plasmacytoid dendritic cells (pDCs), which can be deficient in allergic asthma.

Objective: To determine whether reducing IgE *in vivo* with omalizumab treatment increases pDC antiviral IFN α responses in inner city children with asthma.

Methods: PBMCs and pDCs isolated from children with exacerbation-prone asthma before and during omalizumab treatment were stimulated *ex vivo* with rhinovirus and influenza in the presence or absence of IgE cross-linking. IFN α was measured in supernatants and mRNA of IFN α pathway genes determined by qRT-PCR in cell pellets. Fc ϵ RI α protein and mRNA expression were measured in unstimulated cells by flow cytometry and qRT-PCR respectively. Changes in these outcomes and associations with clinical outcomes were analyzed and statistical modeling utilized to identify risk factors for asthma exacerbations.

Results: Omalizumab treatment increased rhinovirus and influenza-induced PBMC and rhinovirus-induced pDC IFN α responses in the presence of IgE cross-linking, and reduced pDC surface Fc ϵ RI α expression. Omalizumab-induced reductions in pDC Fc ϵ RI α were significantly associated with lower asthma exacerbation rate during the outcome period and correlated with increases in PBMC IFN α responses. PBMC Fc ϵ RI α mRNA measured upon study entry significantly improved an existing model of exacerbation prediction.

Conclusions: These findings indicate that omalizumab treatment augments pDC IFN α responses and attenuates pDC Fc ϵ RI α protein expression, and provide evidence that these effects are related. These results support a potential mechanism underlying clinical observations that allergic sensitization is associated with increased susceptibility to virus-induced exacerbations of asthma.

Key Messages:

- Treatment with omalizumab restores *ex vivo* IFN α responses to rhinovirus and influenza virus in the presence of IgE cross-linking and decreases pDC surface Fc ϵ RI α expression.
- The relationship between increases in RV-induced IFN α and reduced pDC surface Fc ϵ RI α expression in the omalizumab group suggests one potential mechanism underlying the link between atopy and viruses in promoting exacerbations of asthma.
- The enhancement of exacerbation prediction by addition of baseline PBMC Fc ϵ RI α mRNA measurements to an existing model of known clinical risk factors highlights the critical role of the IgE high affinity receptor, Fc ϵ RI α , in promoting asthma exacerbations in children.

Capsule Summary: (35 words) Omalizumab enhanced *ex vivo* antiviral IFN α responses and reduced pDC surface Fc ϵ RI α . Both effects were significantly associated with lower asthma exacerbation rate, highlighting a potential mechanism underlying the atopy-virus connection in promoting exacerbations of asthma.

Key Words: plasmacytoid dendritic cells, rhinovirus, interferon- α , asthma, IgE, omalizumab, Fc ϵ RI α

ABBREVIATIONS:

Flu	Influenza
ICAC	Inner City Asthma Consortium
ICS	Inhaled corticosteroids
IFN- α	Interferon-alpha
IRF7	Interferon regulator factor 7
NIAID	National Institute of Allergy and Infectious Diseases
PBMCs	Peripheral blood mononuclear cells
pDCs	Plasmacytoid dendritic cells
PROSE	Preventative Omalizumab or Step-Up Therapy for Fall Exacerbations
RV	Rhinovirus
TLR	Toll-like receptor
IgE	Immunoglobulin E
AUC	Area under the curve
ROC	Receiver operating characteristic

1 Introduction

2 Allergic sensitization is a risk factor in the development of acute asthma exacerbations with viral
3 respiratory infections. Heymann *et al.* demonstrated that allergic sensitization and elevated
4 serum IgE levels to house dust mite enhance the likelihood that a rhinovirus respiratory infection
5 will cause an asthma exacerbation [1]. Furthermore, allergen exposure in allergic individuals
6 increases the risk for respiratory virus induced wheezing and asthma exacerbations [2, 3]. A
7 critical link between IgE and suppression of TLR9-induced plasmacytoid dendritic cell (pDC)
8 IFN α responses was first described by Schroeder *et al.*, suggesting that pDC antiviral responses
9 may be similarly suppressed in the setting of atopy [4]. We observed that the magnitude of pDC
10 IFN α responses to *ex vivo* viral challenge is inversely related to serum IgE levels [5]. In
11 addition, we showed that stimulation of the IgE/Fc ϵ RI pathway in pDCs via IgE cross-linking
12 abrogates viral and TLR7-induced pDC IFN α production [5, 6], a finding that could explain why
13 pDCs isolated from individuals with allergic asthma have impaired IFN α responses to viruses.
14 Furthermore, surface expression of Fc ϵ RI on pDCs significantly correlates with serum IgE
15 concentrations [7] and is associated with diminished viral-induced IFN α responses in these cells
16 [5]. Reduction of IgE with omalizumab can significantly reduce pDC Fc ϵ RI expression [8, 9], a
17 finding that could potentially translate to improved antiviral responses in these cells.
18 Collectively, these data suggest a significant interaction between IgE, Fc ϵ RI expression and
19 asthma exacerbations, and also that reducing IgE and Fc ϵ RI expression *in vivo* could restore
20 IFN α responses and possibly contribute to the prevention of virus provoked asthma
21 exacerbations.

22 The PROSE (Preventative Omalizumab or Step-up Therapy for Severe Fall Exacerbations) study
23 (clinicaltrials.gov: #NCT01430403) was designed to evaluate the effect of reducing IgE with
24 omalizumab *in vivo* on asthma exacerbations as well as on *ex vivo* pDC antiviral IFN α responses.
25 Children treated with omalizumab had a significant restoration of *ex vivo* IFN α responses in
26 PBMCs, and the group with a greater restoration in IFN α responses had a lower asthma
27 exacerbation rate [10]. In this report, we tested the hypothesis that omalizumab treatment would:
28 1) boost antiviral IFN α responses of both PBMCs and purified pDCs in the presence and absence
29 of IgE cross-linking, and 2) attenuate pDC Fc ϵ RI α expression. We also investigated whether
30 these omalizumab-induced cellular changes were associated with clinical outcomes. Finally, we
31 used multivariate modeling to determine whether the cellular phenotypes and IFN α responses in

32 our study would improve the predictive value of a previously developed model for asthma
33 exacerbations in this population of high-risk children.

Methods

Mechanistic study design. In participants from 2 of the eight sites of the PROSE clinical trial (UT Southwestern Medical Center, Dallas, Texas; National Jewish Health, Denver, Colorado), blood for *ex vivo* assays was drawn before randomization and 12-16 weeks after initiation of treatment. These assays were designed to measure the effect of IgE cross-linking on virus- (rhinovirus and influenza) and TLR7-agonist- (gardiquimod) induced IFN α in cultures of PBMCs (all participants) and pDCs (in a subset of participants) and to determine the impact of omalizumab versus placebo treatment on these IFN α responses.

Isolation of PBMCs, pDCs, culture conditions and reagents. PBMCs were isolated by density centrifugation with Ficoll-Paque (GE Healthcare, Fairfield, Conn). In a subset of participants, pDCs were purified from PBMCs by negative selection using Ab-coated magnetic particles (EasySep™ Human Plasmacytoid DC Enrichment Kit, Catalog #19062; Stem Cell Technologies) according to the manufacturer's Manual EasySep™ Protocol and as previously utilized in our studies [11]. Purity of isolated pDCs (defined as Lineage⁻HLA-DR⁺CD11c⁺CD123⁺ events) was greater than 80%. pDCs were distinguished from basophils by HLA-DR expression (as pDCs express high levels of HLA-DR while basophils lack HLA-DR expression). Basophil contamination in purified pDC samples was minimal (median <0.1% in pDC samples obtained during both the Pre- and Post randomization phase of the study). Aliquots of PMBCs and pDCs were preserved in Cyto-Chex solution (Streck, Omaha, NE) for subsequent antibody staining and flow cytometry analysis. Cells were cultured at 0.5 X 10⁶/0.2mL (PBMCs) or 0.1 X 10⁵/0.2mL (pDCs) in 96-well round-bottom plates in complete RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% penicillin-streptomycin, 1% sodium pyruvate, 1% HEPES buffer solution, 1% nonessential amino acids, 1% glutamate, 100umol/L β -mercaptoethanol, and 10ng/mL IL-3. Cells were cultured for 18 hours in the presence or absence of an IgE cross-linking antibody (rabbit anti-human IgE, 1ug/mL, Bethyl Laboratories). This antibody differs from omalizumab in its ability to bind and cross-link IgE on cell-surface Fc ϵ RI α receptors, whereas omalizumab only binds to free IgE. After 18 hours, PBMCs were stimulated with RV-A16 (10⁶ plaque-forming units/mL; a gift from Wai-Ming Lee and Yury Bochkov, University of Wisconsin-Madison), influenza (A/PR/8/34 [H1N1], 0.1 PFU/cell; Charles River Laboratories) or gardiquimod (Gard, a TLR7 agonist, 1 μ g/ml; Invivogen) for 24 hours. Cells

and supernatants were then harvested by centrifugation and stored at -80 degrees C for RNA extraction/qRT-PCR and IFN α ELISA, respectively.

Flow cytometry. PBMCs were stained [5] with the following fluorochrome-conjugated anti-human Abs: HLA-DR APC-Cy7, Lineage-FITC mixture, CD14-Pacific Blue, CD123-PeCy5 (BD Biosciences) and Fc ϵ RI α -PE (eBiosciences), acquired on a BD LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star). pDCs were identified as lineage-negative, HLA-DR-positive, CD123-positive cells. The mean fluorescence intensity of Fc ϵ RI α was determined by flow cytometry and subsequently converted to Molecules of Equivalent Soluble fluorochrome (MESF) using the Ultra Rainbow Calibration kit (BD Biosciences).

IFN α quantification. IFN- α was measured using the Human IFN- α (pan-specific, to detect 12 IFN α subtypes) ELISA kit (MabTech, Inc., Cincinnati, OH) and analyzed on an ELISA reader DTX 880 Multimode detector (Beckman Coulter).

RNA isolation and quantitative RT-PCR for detection of Fc ϵ RI α and mRNA of interferon signaling and response genes. Total pDC RNA was extracted from 2×10^4 pDCs using RNA-Bee (Tel-test, Friendswood, TX) and chloroform as previously described and stored at -80°C[5]. Total PBMC RNA was extracted from $1.5-2 \times 10^6$ PBMCs using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). The cDNA synthesis was carried using a High Capacity cDNA Reverse Transcriptase kit (Applied Biosystems, Foster City, CA). Expression of *TLR7* and *Fc ϵ RI α* mRNA in unstimulated pDCs and PBMCs and Fc ϵ RI γ mRNA in unstimulated PBMCs was quantified by performing quantitative RT-PCR, using the CFX384TM Real Time system (BIO-RAD, Hercules, CA). *TLR7*, *IFNB1*, *IFNA1*, *IFIT1*, *IRF7*, and *DDX58* were also measured in cultured PBMCs. Sequences for TLR-7 and Fc ϵ RI α probes/primers (TaqMan) shown in Table E1 were used as previously reported [5]. The reporter dye and quencher were FAM and TAMRA, respectively. Applied BiosystemsTM TaqManTM Assays (ThermoFisher Scientific) shown in Table E2 were used for *IRF7*, *IFNB1*, *IFNA1*, *DDX58*, and *IFIT1*. The mRNA expression for all genes in cultured PBMCs was normalized to hypoxanthine-guanine phosphoribosyltransferase mRNA (ThermoFisher Scientific). The mRNA expression for all genes in unstimulated PBMC and pDCs was normalized to hypoxanthine-guanine

phosphoribosyltransferase mRNA or PPIA mRNA (ThermoFischer Scientific). Results were based on $2^{-\Delta\Delta Ct}$ values.

Statistical analysis. Cell values were skewed and therefore log-transformed for statistical tests and models. To compare differences between pre and post-randomization within treatment group (placebo, omalizumab), paired t-tests were used and geometric mean ratios were reported. Change between pre and post-randomization was calculated using a log-difference. In order to compare exacerbation rates by the level of pre- vs. post-randomization change, change was dichotomized at the median value. Logistic regression was performed (Firth's method was used in the case of zero counts) using dichotomized change as the predictor and any exacerbation as the outcome; associated odds ratios were reported. Pearson correlations were used to compare continuous change between variables.

Due to the larger sample size required for multivariate modeling of exacerbations, participants from the third arm of the PROSE clinical trial, an arm that received an inhaled corticosteroid boost, were pooled together with placebo participants. Notably, the primary outcome in the inhaled corticosteroid arm did not differ from placebo in the PROSE trial [10]. The multivariate modeling process consisted of three steps. First, pre-randomization mechanistic variables were compared between exacerbators and non-exacerbators using t-tests within treatment group (non-omalizumab, omalizumab). Due to the exploratory nature of the analysis, multiple comparisons were not accounted for. Next, an elastic net procedure was performed to select the most relevant baseline mechanistic outcomes after adjusting for known baseline predictors of exacerbations [12]. Finally, logistic models were fit using known baseline predictors of exacerbations (age, total IgE, atopy, blood eosinophils, exacerbations in the previous 90 days, FEV1/FVC, treatment step)[13] stratified by treatment group. Similar models were fit using the known predictors of exacerbations and the PBMC mechanistic outcomes selected by the elastic net procedure [14]. All cellular phenotype and IFN α response variables with <40% missing data were included as candidate predictors. Prior to the multivariate modeling, any missing data among the candidate predictors (which amounted to 5% of the values), were imputed using an imputation based on a random forest approach. Nested models were compared using likelihood ratio tests and area under the ROC curve. SAS 9.3 and R version 3.3.2 were used for analyses.

Results

IFN α responses were evaluated in participants receiving omalizumab and placebo treatment (N=92) at two clinical sites. Participants had similar demographic and laboratory characteristics as those in the multi-site clinical trial, including low income, predominant Hispanic ethnicity, elevated peripheral blood eosinophils and total serum IgE (Table E3). No significant differences in baseline characteristics were found between subjects from the two sites (data not shown).

Effect of omalizumab treatment on *ex vivo* antiviral IFN α responses in PBMCs and purified pDCs

Ex vivo antiviral IFN α responses were first measured in cultures of PBMCs (N=92) obtained at 2 time points in the study: prior to randomization and 12-16 weeks after treatment was initiated. As previously reported, PBMC generation of IFN α to RV in the presence of IgE cross-linking was significantly increased in the post-omalizumab treatment group compared with the post-treatment placebo group [10]. We now report that omalizumab treatment significantly increased IFN α responses following IgE cross-linking and stimulation with either RV (geometric mean ratio 2.06, 95% CI [1.20, 3.55]; $p=0.01$) or influenza virus (geometric mean ratio 1.57, 95% CI [1.09, 2.25]; $p=0.02$) (Figure 1 and Table 1). In contrast, omalizumab treatment did not significantly affect IFN α responses to RV, Flu or gardiquimod in the absence of *ex vivo* IgE cross-linking.

To determine whether omalizumab impacted plasmacytoid dendritic cell (pDC) antiviral IFN α responses, pDCs were purified from a subset of participants (n=43). Similar to effects noted with PMBCs, omalizumab significantly enhanced RV-induced IFN α responses of purified pDCs in the presence of IgE cross-linking treatment (Figure 1 and Table 1; geometric mean ratio 4.15, 95% CI [1.76, 9.77]; $p=0.003$). In contrast, omalizumab treatment had no significant effects on pDC-secreted IFN α responses in the absence of IgE cross-linking. Due to the low frequency of pDCs in blood samples, insufficient numbers of pDC were available to evaluate Flu- or gardiquimod-stimulated pDC IFN α responses in the presence of IgE cross-linking.

We previously reported that the risk for asthma exacerbation following treatment with omalizumab was related to restoration of RV-induced IFN α responses of PBMC [10]. Here, we

investigated whether omalizumab effects on IFN α responses, as induced by other *ex vivo* stimulation conditions in PBMCs or pDCs, had a similar relationship to asthma exacerbations (Table E4). We found no additional significant relationships between IFN α restoration and proportions of exacerbations.

Impact of omalizumab on TLR7 mRNA expression in unstimulated PBMCs and pDCs

TLR7, a sensor of single-stranded viral RNA, plays an important role in viral-induced pDC IFN α secretion [15, 16] and IgE cross-linking has been shown to impair viral-induced TLR7 expression [15]. We hypothesized that omalizumab might induce TLR7 expression, and that this effect could contribute to observed increases in IFN α responses. Unexpectedly, treatment with omalizumab resulted in decreased expression of TLR7 mRNA in unstimulated PMBCs ($p=0.002$) and pDCs ($p=0.008$) as shown in Table 2. In addition, there were nonsignificant trends for lower exacerbation rates in the group with the greatest omalizumab-associated change/decrease in PBMC and pDC TLR7 expression (Table E5). Omalizumab did not affect baseline PBMC expression of interferon regulatory factor 7 (IRF7), a master regulator of pDC IFN α secretion [17] (data not shown).

Effect of omalizumab on transcription of genes related to IFN α responses.

We next tested the hypothesis that omalizumab was associated with upregulation of other genes involved in Type I IFN signaling, including Toll-like receptor 7 (*TLR7*), interferon regulatory factor 7 (*IRF7*) [17], *IFN- β 1*, the first member of the type I IFN (IFN- α/β) family to be secreted, *IFNA1*, one of at least 12 distinct IFN α subtypes [18], *IFIT1*, an interferon-stimulated gene [18], and *DDX58* (RIG-I; retinoic acid-inducible gene I), a cytoplasmic sensor of pathogen-associated molecular patterns (PAMPs) within viral RNA. Cells obtained before and during omalizumab treatment were stimulated with RV and IgE cross-linking. Omalizumab significantly increased transcription of IFN- β 1 (geometric mean ratio 1.95, 95% CI [1.04, 3.66], $p=0.04$) and DDX58 (RIG-I; geometric mean ratio 1.43, 95% CI [1.03, 1.99], $p=0.04$) mRNA (Table 3). Transcription of *TLR7*, *IRF7*, *IFNA1*, and *IFIT1* was not significantly affected.

Effects of omalizumab treatment on surface FcεRIα expression on pDCs. As previously demonstrated [8], omalizumab treatment significantly reduced FcεRIα surface expression on pDCs (Table 4; Figure 2A). We next tested whether omalizumab affected FcεRIα mRNA levels in either PBMCs or pDCs, and found that this was not the case (Table 4). Similarly, omalizumab treatment did not affect mRNA levels of the FcεRI receptor gamma chain (FcεRIγ), a transmembrane adaptor of the FcεRI complex (data not shown). FcεRIγ mRNA was measured only in PBMCs due to limited availability of pDC RNA.

We next tested whether omalizumab-induced reductions in pDC FcεRIα surface expression were related to increased RV-induced IFNα secretion in the presence of IgE cross-linking and found a modest inverse correlation (Figure 2B, left panel; Pearson correlation -0.26; p=0.05). There was a similar trend in parallel studies with purified pDCs (Figure 2B, right panel). Finally, among omalizumab participants, a significant association between changes in pDC FcεRIα surface expression and exacerbation rate was observed (Figure 2C and Table E5; odds ratio 0.17, 95% CI [0.02, 0.74]; p=0.02).

Sample sizes for all of the mechanistic variables included in Tables 1-4 and Figures 1-2C are displayed in Table E6 by treatment group and asthma exacerbation status. Figure E1 provides a visual representation of the overlap between participants with available mechanistic data and exacerbation status.

Prediction of asthma exacerbations. In order to investigate the association between mechanistic variables and exacerbations within treatment group (omalizumab, non-omalizumab), we first explored univariate associations (Table E7). Mechanistic variables assessed during the pre-randomization period included PBMC IFNα responses; unstimulated PBMC expression of FcεRIα, TLR7 and IRF7 mRNA; and pDC surface FcεRIα expression. Only variables with <40% missing data were included. Two variables were identified as risk factors for exacerbations in the non-omalizumab group, including PBMC IRF7 mRNA after stimulation with RV and IgE cross-linking (geometric mean ratio exacerbators: non-exacerbators 0.44, p=0.04) and FcεRIα mRNA expression in unstimulated PBMCs (geometric mean ratio 0.61; p=0.03).

We next investigated whether addition of these baseline mechanistic measurements could improve an existing predictive model for asthma exacerbations. Using a multivariate model that includes clinical and laboratory parameters [12] [13] we obtained a significant AUC (0.89 and 0.87 and p-value 0.01 and 0.008 for the “non-omalizumab” group (combined placebo and inhaled corticosteroid boost) and the omalizumab group, respectively (Model 1, Table 5). Using the variable selection procedure, we next added mechanistic variables measured at pre-randomization (baseline) to this model. In the non-omalizumab group, baseline PBMC FcεRIα mRNA expression was found to be a significant predictor of exacerbations after accounting for the set of clinical and laboratory parameters in Model 1. In particular, addition of baseline PBMC FcεRIα mRNA expression increased the AUC by 0.08 to 0.97 (p=0.002) and reduced the proportions of false positive and false negative predictions by 50% ([19] Table E8). No mechanistic parameters were found to be significant additions to known clinical predictors of exacerbations in the omalizumab group.

Discussion

Overexpression of FcεRIα and cross-linking this receptor can disrupt virus-induced IFNα responses [5, 6], and this represents a potential mechanism for more severe viral respiratory illnesses in allergic asthma. We previously demonstrated that omalizumab treatment of urban children with moderate asthma restores *ex vivo* PBMC IFNα responses, and that these improved responses were related to a lower risk for asthma exacerbation [10]. In the present study, we extend these findings by showing that omalizumab treatment *in vivo* restores IFNα responses to both RV and influenza. Furthermore, we demonstrated omalizumab effects on pDCs, including reduced expression of FcεRIα on the cell surface and increased virus-induced IFNα responses in the presence of IgE cross-linking. These effects on pDCs, which are the major source of virus-induced IFNα among immune cells [20], were inversely correlated. Type I IFNs suppress development and stability of Th2 lymphocytes; increasing IFNα could thus diminish Th2-mediated allergic inflammation associated with asthma pathogenesis [21]. In support of this, inhaled IFNβ has shown promise as a potential treatment for viral-induced exacerbations of asthma [22]. These findings provide additional insights into IgE-mediated mechanisms that suppress antiviral responses.

Our findings suggest that omalizumab improves IFNα responses through reduction of FcεRIα, the alpha chain of the high affinity IgE receptor, on the surface of pDCs. Elegant studies of pDC signaling performed by Cao and colleagues suggest that the mechanism for this effect may involve FcεRIγ, an ITAM-containing transmembrane adaptor of the FcεRI complex that can transduce inhibitory signals through pDC surface receptors ILT7 and BDCA2 [23, 24]. FcεRIγ signaling through these receptors inhibits TLR 7/9-induced pDC IFN responses via a B Cell Receptor-like pathway involving Lyn/BLK, Syk and BLNK [24]. This ITAM-mediated inhibition of pDC IFN is effectively reversed by Syk inhibition in primary human pDCs [23] and by knockdown of Syk and FcεRIγ in a pDC cell line [25], and is likely also involved in IgE-mediated inhibition of pDC IFNα responses through FcεRIα-induced FcεRIγ signaling. Therefore, reduction of surface FcεRIα could diminish subsequent FcεRIγ signaling in the presence of IgE cross-linking conditions, and lead to increased capacity of pDCs to secrete IFNα in an allergic environment, although we were unable to assess this in our study. The increased RV-induced PBMC IFN-β1 and RIG-I (*DDX58*) mRNA levels observed in the post-treatment

omalizumab group suggest that pathways downstream of FcεRIγ and upstream of IFNα signaling may be affected. Although we observed no differences in IRF7 transcription in the post-treatment omalizumab group, it is possible that critical signaling events such as IRF7 phosphorylation, required for IRF7 activation, nuclear translocation and induction of Type I IFN expression, could be enhanced in the setting of reduced IgE and FcεRIα. Other posttranslational modifications known to regulate IRF7, including ubiquitination [26], could similarly contribute to the observed effects, but were not assessed in our study. As RIG-I (*DDX58*) signaling also promotes Type I IFN production[27], increased RV-induced RIG-I mRNA levels after omalizumab could also contribute to enhanced IFNα responses. Proof of this concept would require demonstration of diminished FcεRIγ signaling in pDCs from omalizumab-treated participants, which was not feasible given the limited numbers of pDCs available for these analyses. Investigating the impact of omalizumab on pDC FcεRIγ signaling could be tested in future omalizumab treatment studies in adults, from whom higher blood volumes could be obtained.

The finding that omalizumab reduces pDC surface FcεRIα protein levels without regulating FcεRIα transcription suggests that this effect is likely related to reduction of free serum IgE. Consistent with this idea, free IgE levels have been shown to correlate with pDC surface FcεRIα expression both before and after omalizumab treatment [9]. The duration of reduced pDC FcεRIα expression after discontinuation of omalizumab was not measured in our study. Basophil surface FcεRIα protein expression has been shown to increase after omalizumab withdrawal in atopic subjects, paralleling the rise free serum IgE [19]. Given that serum IgE similarly regulates surface FcεRIα protein expression on pDCs, it is reasonable to hypothesize that pDC FcεRIα levels would also increase after omalizumab withdrawal as free serum IgE levels rise to pre-omalizumab levels. Whether such a return to pre-omalizumab pDC FcεRIα protein expression levels would result in loss of the observed improved IFNα responses also remains undetermined, and represents an area for future investigation.

In addition to FcεRIα and FcεRIγ – mediated effects, other mechanisms of allergic inflammation-induced inhibition of antiviral responses have been reported. Aeroallergen-induced IL-33 can suppress pDC IRF7 expression and impair IFN-α production, and promote a respiratory virus-induced asthma phenotype in a murine model of asthma [28]. TLR7

hyporesponsiveness and reduced function have been reported in both murine and human studies of allergic disease [28-30] and likely contribute to associated defective IFN α antiviral responses. Reduced RV-induced PBMC expression of other critical signaling molecules including IRF1, IRF7, NF- κ B family members and STAT1 have also been reported in individuals with allergic asthma [30], and suggest roles for both IFN-dependent and IFN-independent pathways in the synergistic contributions of allergens and viruses to exacerbations of atopic asthma. In addition, Th2 cytokines IL-4 and IL-13 inhibit TLR3 expression and IRF3 activation, resulting in impaired RV-induced IFN responses in human bronchial epithelial cells [31].

The clinical relevance of omalizumab's effect on pDC surface Fc ϵ RI α expression is underscored by the association of a lower exacerbation rate with omalizumab-induced change in Fc ϵ RI α surface protein expression. Based on this, one might expect that higher baseline Fc ϵ RI α expression would have predicted exacerbations in this population of high-risk children. Surprisingly, this was not the case; pDC surface Fc ϵ RI α expression did not improve the ability to predict exacerbations when added to an existing prediction model, which can be attributed to its high correlation with total IgE. In contrast, baseline Fc ϵ RI α mRNA expression in unstimulated PBMCs significantly enhanced the accuracy of an existing predictive model of asthma exacerbations [12] in our study population, although this factor was not influenced by omalizumab treatment. The finding that mRNA levels for Fc ϵ RI α fits and improves this asthma exacerbation prediction model supports the concept that the high affinity IgE receptor plays an important role in exacerbations.

We have demonstrated that omalizumab therapy results in improved viral-induced pDC IFN α responses and have linked omalizumab-induced changes in surface pDC Fc ϵ RI α expression to improved clinical outcomes (decreased asthma exacerbation rate). Our finding that Fc ϵ RI α mRNA expression is not impacted by omalizumab, yet can be used to improve prediction of asthma exacerbations, is intriguing and underscores the complexity of Fc ϵ RI α regulation in immune cells [32]. Future studies investigating the connection between viral-induced pDC IFN α responses and Fc ϵ RI α regulation are needed to further define the mechanisms underlying the connection between atopy, antiviral response and allergic disease. Our findings support the concept that targeting the IgE pathway in pDCs represents a promising strategy to increase IFN responses, improve anti-viral activity in the airways and reduce exacerbations of allergic asthma.

References

1. Heymann, P.W., Carper, H.T, Murphy, D.D., Platts-Mills T.A., Patrie, J., McLaughlin, A.P., et al., *Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing*. J Allergy Clin Immunol, 2004. **114**(2): p. 239-47.
2. Green, R.M., Custovic, A., Sanderson, G., Hunter, J., Johnston, S.L., and Woodcock, A., *Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study*. BMJ, 2002. **324**(7340): p. 763.
3. Murray, C.S., Poletti, G., Keadze, T., Morris, J., Woodcock, A., Johnston, S.L., et al., *Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children*. Thorax, 2006. **61**(5): p. 376-82.
4. Schroeder, J.T., Bieneman, A.P., Xiao, H., Chichester, K.L., Vasagar, K., Saini, S., et al., *TLR9- and FcepsilonRI-mediated responses oppose one another in plasmacytoid dendritic cells by down-regulating receptor expression*. J Immunol, 2005. **175**(9): p. 5724-31.
5. Gill, M.A., Bajwa, G., George, T.A., Dong, C.C., Dougherty, I.I., Jiang, N., et al., *Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells*. J Immunol, 2010. **184**(11): p. 5999-6006.
6. Durrani, S.R., Montville, D.J., Pratt, A.S., Sahu, S., DeVries, M.K., Rajamanickam, V., et al., *Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children*. J Allergy Clin Immunol, 2012. **130**(2): p. 489-95.
7. Foster, B., Metcalfe, D.D., and Prussin, C., *Human dendritic cell 1 and dendritic cell 2 subsets express FcepsilonRI: correlation with serum IgE and allergic asthma*. J Allergy Clin Immunol, 2003. **112**(6): p. 1132-8.
8. Prussin, C., Griffith, D.T., Boesel, K.M., Lin, H., Foster, B., and Casale, T.B., *Omalizumab treatment downregulates dendritic cell FcepsilonRI expression*. J Allergy Clin Immunol, 2003. **112**(6): p. 1147-54.
9. Schroeder, J.T., Bieneman, A.P., Chichester, K.L., Hamilton, R.G., Xiao, H., Saini, S.S., et al., *Decreases in human dendritic cell-dependent T(H)2-like responses after acute in vivo IgE neutralization*. J Allergy Clin Immunol, 2010. **125**(4): p. 896-901 e6.
10. Teach, S.J., Gill, M.A., Togias, A., Sorkness, C.A., Arbes, S.J. Jr., Calatroni, A., et al., *Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations*. J Allergy Clin Immunol, 2015.
11. Bajwa, G., DeBerardinis, R.J., Shao, B., Hall, B., Farrar, J.D., and Gill, M.A., *Cutting Edge: Critical Role of Glycolysis in Human Plasmacytoid Dendritic Cell Antiviral Responses*. J Immunol, 2016. **196**(5): p. 2004-9.
12. Teach, S.J., Gergen, P.J., Szeffler, S.J., Mitchell, H.E., Calatroni, A., Wildfire, J., et al., *Seasonal risk factors for asthma exacerbations among inner-city children*. J Allergy Clin Immunol, 2015. **135**(6): p. 1465-73 e5.
13. Hoch H.E., Calatroni, A., West, J.B., Liu, A.H., Gergen, P.J. Gruchalla, R.S., et al., *Predicting Fall Seasonal Asthma Exacerbations in Inner-City Children in the Absence and Presence of Omalizumab Therapy*. J Allergy Clin Immunol . In press.

14. Friedman, J., Hastie, T., and Tibshirani, R., *Regularization Paths for Generalized Linear Models via Coordinate Descent*. J Stat Softw, 2010. **33**(1): p. 1-22.
15. Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., and Reis e Sousa, C., *Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA*. Science, 2004. **303**(5663): p. 1529-31.
16. Gilliet, M., Cao, W., and Liu, Y.J., *Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases*. Nat Rev Immunol, 2008. **8**(8): p. 594-606.
17. Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., et al., *IRF-7 is the master regulator of type-I interferon-dependent immune responses*. Nature, 2005. **434**(7034): p. 772-7.
18. Pestka, S., Krause, C.D., and Walter, M.R., *Interferons, interferon-like cytokines, and their receptors*. Immunol Rev, 2004. **202**: p. 8-32.
19. Saini, S.S., MacGlashan, D.W. Jr., Sterbinskym S.A., Togias, A., Adelman, D.C., Lichtenstein, L.M., et al., *Down-regulation of human basophil IgE and FC epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo*. J Immunol, 1999. **162**(9): p. 5624-30.
20. Liu, Y.J., *IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors*. Annu Rev Immunol, 2005. **23**: p. 275-306.
21. Huber, J.P., Ramos, H.J., Gill, M.A., and Farrar, J.D., *Cutting edge: Type I IFN reverses human Th2 commitment and stability by suppressing GATA3*. J Immunol, 2010. **185**(2): p. 813-7.
22. Djukanovic, R., Harrison, T., Johnston, S.L., Gabbay, F., Wark, P., Thomson, N.C., et al., *The effect of inhaled IFN-beta on worsening of asthma symptoms caused by viral infections. A randomized trial*. Am J Respir Crit Care Med, 2014. **190**(2): p. 145-54.
23. Cao, W., Rosen, D.B., Ito, T., Boyer, L., Bao, M., Watanabe, G., et al., *Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production*. J Exp Med, 2006. **203**(6): p. 1399-405.
24. Cao, W., Zhang, L., Rosen, D.B., Bover, L., Watanabe, G., Bao, M., et al., *BDCA2/Fc epsilonRI gamma complex signals through a novel BCR-like pathway in human plasmacytoid dendritic cells*. PLoS Biol, 2007. **5**(10): p. e248.
25. Bao, M., Hanabuchi, S., Facchinetti, V., Du, Q., Bover, L., Plumas, J., et al., *CD2AP/SHIP1 complex positively regulates plasmacytoid dendritic cell receptor signaling by inhibiting the E3 ubiquitin ligase Cbl*. J Immunol, 2012. **189**(2): p. 786-92.
26. Ning, S., Pagano, J.S., and Barber, G.N., *IRF7: activation, regulation, modification and function*. Genes Immun, 2011. **12**(6): p. 399-414.
27. Loo, Y.M. and Gale, M. Jr., *Immune signaling by RIG-I-like receptors*. Immunity, 2011. **34**(5): p. 680-92.
28. Lynch, J.P., Werder, R.B., Simpson, J., Loh, Z., Zhang, V., Haque, A., et al., *Aeroallergen-induced IL-33 predisposes to respiratory virus-induced asthma by dampening antiviral immunity*. J Allergy Clin Immunol, 2016.
29. Roponen, M., Yerkovich, S.T., Hollams, E., Sly, P.D., Holt, P.G., and Upham, J.W., *Toll-like receptor 7 function is reduced in adolescents with asthma*. Eur Respir J, 2010. **35**(1): p. 64-71.

30. Pritchard, A.L., White, O.J., Burel, J.G., Carroll, M.L., Phipps, S., and Upham, J.W., *Asthma is associated with multiple alterations in anti-viral innate signalling pathways*. PLoS One, 2014. **9**(9): p. e106501.
31. Contoli, M., Ito, K., Padovani, A., Poletti, D., Marku, B., Edwards, M.R., et al., *Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells*. Allergy, 2015. **70**(8): p. 910-20.
32. MacGlashan, D., Jr., *IgE and FcepsilonRI regulation*. Clin Rev Allergy Immunol, 2005. **29**(1): p. 49-60.

Table 1: Impact of Omalizumab on PBMC and pDC IFN α ¹ secretion²

Mechanistic Parameter		Omalizumab					Placebo				
Cell type	<i>Ex vivo</i> Stimulus	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	P-value	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	P-value
PBMC	RV	64	1365.8 (6.1)	1035.8 (7.0)	0.76 [0.47, 1.22]	0.25	23	1443.1 (6.3)	764.2 (7.9)	0.53 [0.21, 1.32]	0.16
PBMC	Anti-IgE+RV	56	201.7 (6.9)	415.7 (8.2)	2.06 [1.20, 3.55]	0.01	23	169.7 (5.5)	129.0 (6.4)	0.76 [0.34, 1.71]	0.49
PBMC	Flu	64	7304.7 (2.2)	7301.8 (2.4)	1.00 [0.80, 1.24]	>0.99	23	6864.2 (3.7)	6371.6 (2.6)	0.93 [0.50, 1.74]	0.81
PBMC	Anti-IgE+Flu	38	4352.1 (2.6)	6814.9 (2.3)	1.57 [1.09, 2.25]	0.02	10	4043.6 (5.6)	4637.1 (3.9)	1.15 [0.20, 6.51]	0.86
PBMC	Gardiquimod	64	856.4 (3.8)	1167.3 (4.1)	1.36 [1.00, 1.87]	0.05	23	1314.3 (4.7)	985.4 (3.4)	0.75 [0.49, 1.16]	0.18
pDC	RV	25	125.2 (5.3)	237.5 (7.1)	1.90 [0.83, 4.34]	0.12	12	216.3 (7.6)	260.4 (8.0)	1.2 [0.26, 5.64]	0.80
pDC	Anti-IgE+RV	14	94.8 (4.4)	393.6 (6.4)	4.15 [1.76, 9.77]	0.003	7	174.8 (7.2)	277.7 (6.7)	1.59 [0.19, 13.14]	0.61
pDC	Flu	25	236.6 (3.7)	317.5 (3.5)	1.34 [0.87, 2.08]	0.18	11	171.6 (4.7)	340.2 (3.4)	1.98 [0.84, 4.70]	0.11
pDC	Gardiquimod	21	936.6 (6.5)	1900.2 (4.7)	2.03 [0.85, 4.83]	0.10	11	1280.3 (6.6)	1955.4 (2.7)	1.53 [0.33, 7.14]	0.55

¹IFN α concentrations (pg/ml) measured in supernatants after depicted *ex vivo* stimulations²Means are geometric means, and SDs are geometric standard deviations

Table 2: Impact of Omalizumab on TLR7¹ expression in unstimulated PBMCs and pDCs²

Mechanistic Parameter			Omalizumab					Placebo				
Cell type	Method measured	Readout ¹	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	P-value	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	P-value
PBMC	rt-PCR	TLR7 mRNA	64	1.2 (1.6)	1.0 (1.8)	0.81 [0.71, 0.92]	0.002	23	1.3 (1.6)	1.2 (2.0)	0.92 [0.72, 1.18]	0.50
Purified pDC	rt-PCR	TLR7 mRNA	18	0.3 (1.7)	0.2 (1.5)	0.79 [0.66, 0.93]	0.008	7	0.2 (1.4)	0.2 (1.8)	0.89 [0.62, 1.29]	0.47

¹TLR7 mRNA values represent expression measured prior to *ex vivo* stimulation and normalized to HPRT expression ($2^{-\Delta Ct}$)

²Means are geometric means, and SDs are geometric standard deviations.

Table 3: Impact of Omalizumab on Rhinovirus-induced PBMC expression of IFN α signaling and responsiveness genes¹

mRNA Expression ²	Omalizumab					Placebo				
	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	p-value	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	p-value
TLR7	56	2.5 (2.2)	3.0 (2.3)	1.21 [0.94, 1.55]	0.13	22	2.6 (2.1)	2.4 (2.0)	0.94 [0.66, 1.34]	0.73
IRF7	56	7.1 (3.4)	7.6 (2.7)	1.07 [0.75, 1.54]	0.70	22	8.0 (2.8)	5.9 (2.8)	0.74 [0.45, 1.21]	0.21
IFNB1	55	23.8 (11.6)	46.4 (8.8)	1.95 [1.04, 3.66]	0.04	20	27.2 (7.2)	20.7 (8.2)	0.76 [0.28, 2.05]	0.57
IFNA1 ³	32	17.9 (11.5)	32.0 (12.5)	1.79 [0.65, 4.94]	0.25	13	65.4 (6.5)	24.2 (5.6)	0.37 [0.12, 1.15]	0.08
DDX58 (RIG-I)	56	6.2 (3.6)	8.9 (2.8)	1.43 [1.03, 1.99]	0.04	22	9.1 (2.7)	6.4 (3.3)	0.7 [0.41, 1.22]	0.20
IFIT1	56	44.8 (13.2)	75.5 (8.5)	1.69 [0.85, 3.34]	0.13	22	65.9 (8.0)	24.5 (10.9)	0.37 [0.14, 1.01]	0.05

¹Means are geometric means, and SDs are geometric standard deviations.

²Cells were stimulated *ex vivo* with anti-IgE + RV and mRNA expression of demonstrated genes normalized to HPRT expression ($2^{-\Delta Ct}$)

³Measuring transcriptional levels of total IFN α was not feasible given the existence of at least 12 distinct IFN α subtypes.

Table 4: Impact of Omalizumab on FcεRIα expression¹ in unstimulated PBMCs and pDCs²

Mechanistic Parameter			Omalizumab					Placebo				
Cell type	Method measured	Readout ¹	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	p-value	N	Pre-Randomization Mean (SD)	Post-Randomization Mean (SD)	Geometric Mean Ratio [95% CI]	p-value
pDCs ³	Flow cytometry	Surface FcεRIα expression	64	3231.2 (1.7)	1381.7 (1.6)	0.43 [0.37, 0.49]	<0.001	23	3540.0 (1.6)	3974.9 (1.6)	1.12 [0.85, 1.48]	0.40
PBMCs	rt-PCR	FcεRIα mRNA	64	1.9 (1.8)	1.9 (1.7)	1.02 [0.91, 1.13]	0.78	23	1.5 (1.8)	1.3 (2.1)	0.85 [0.67, 1.08]	0.17
Purified pDCs	rt-PCR	FcεRIα mRNA	18	0.4 (1.7)	0.4 (1.9)	1.07 [0.80, 1.42]	0.62	7	0.3 (2.1)	0.2 (1.7)	0.69 [0.53, 0.9]	0.02

¹FcεRIα mRNA values represent expression measured prior to *ex vivo* stimulation, normalized to HPRT expression ($2^{-\Delta\text{ct}}$).

²Means are geometric means, and SDs are geometric standard deviations

³pDCs and basophils gated in total PBMCs for flow cytometry analysis (unstimulated cells)

Table 5: Multivariate modeling¹ to predict asthma exacerbations

Model	Non-Omalizumab ²			Omalizumab		
	Variables ^{3,4}	Area under the ROC curve (AUC)	p-value	Variables ^{3,4}	Area under the ROC curve (AUC)	p-value
1 <i>Known clinical predictors</i>	Age, total IgE, atopy, blood eosinophils, exacerbations in previous 90 days, FEV1/FVC, Treatment step	0.89	0.01	Age, total IgE, atopy, blood eosinophils, exacerbations in previous 90 days, FEV1/FVC, Treatment step	0.87	0.008
2 <i>Known clinical predictors + New selected mechanistic variables</i>	<i>Known clinical predictors</i> + PBMC FcεRIα mRNA expression	0.97	0.002	N/A	N/A	N/A

¹The set of mechanistic variables considered by the feature selection procedure included data obtained from unstimulated PMBCs (FcεRIα protein and mRNA expression, TLR7 and IRF7 mRNA expression) and from *ex vivo* RV-stimulated PBMC assays (IFNα response). The new mechanistic variables selected by the feature selection procedure were then included in Model 2.

²Includes participants receiving Placebo (N=23) and ICS Boost (N=36) treatments.

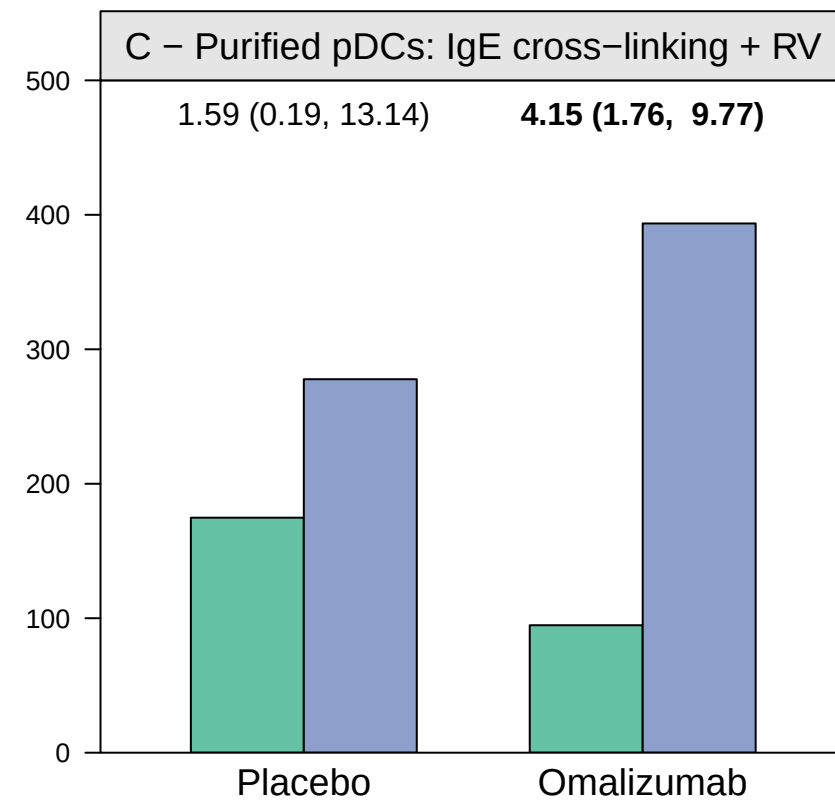
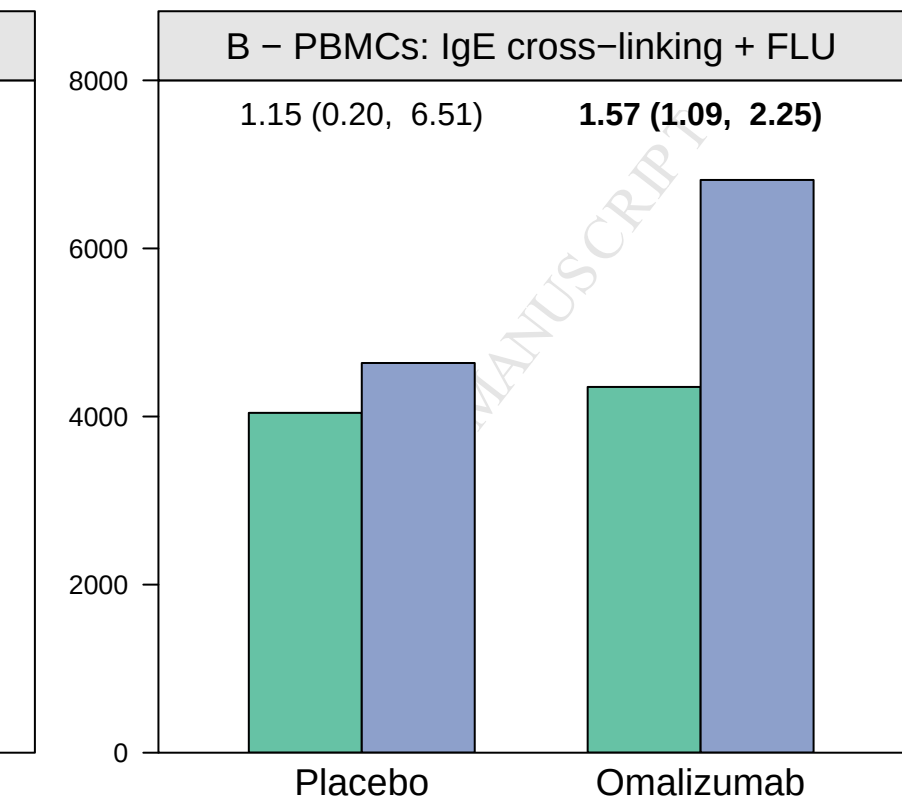
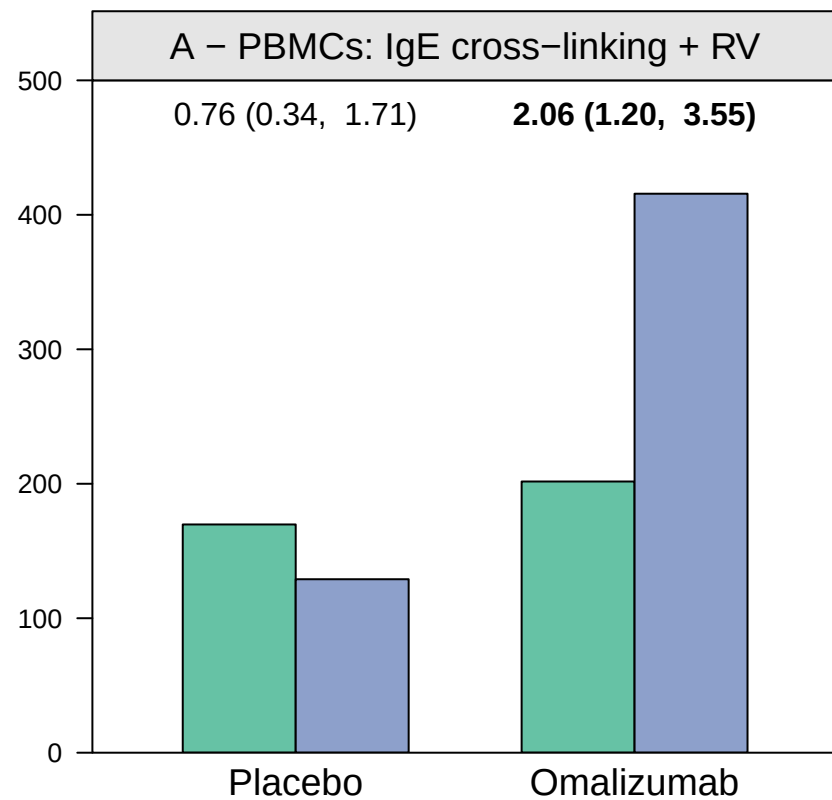
³*Known clinical predictors* include: Age, total IgE, atopy, blood eosinophils, exacerbations in previous 90 days, FEV1/FVC, Treatment step

⁴N/A indicates that no variables were selected by the feature selection algorithm

Figures Legends

Figure 1. Enhanced PMBC and pDC IFN- α responses after omalizumab. PBMC and pDC IFN- α responses to RV and FLU significantly increased during the intervention phase of the study in the omalizumab group. Bars represent geometric mean IFN- α concentrations (pg/mL) measured in supernatants. Geometric mean ratios comparing post vs. pre within each group are annotated above the bars, along with associated 95% confidence intervals. Bolding indicates a p-value < 0.05 as determined by the paired t-test. **A**, A 2.06-fold increase in PBMC IFN- α response to RV + IgE cross-linking (P= 0.01), **B**, a 1.57-fold increase in PBMC IFN- α response to FLU + IgE cross-linking (P=0.02) and **C**, a 4.15-fold increase in pDC IFN- α response to RV + IgE cross-linking (P=0.003) was observed between pre (green bars) and post (blue bars) randomization in the omalizumab group.

Figure 2. Loss of surface pDC Fc ϵ RI α surface expression is associated with restoration of IFN α responses and decreased exacerbations. **A**, pDC surface Fc ϵ RI α expression are expressed as mean equivalent soluble fluorochrome (MESF) values in the omalizumab group (represented in pink) and placebo group (represented in blue). Points represent geometric mean values at corresponding time point; vertical segments cover the 95% confidence interval around the geometric mean. **B**, Correlation between change in IFN α and change in pDC Fc ϵ RI α surface expression in the omalizumab group. IFN α was measured in PBMC (left) or pDC (right) supernatants after stimulation with anti-IgE+RV; pDC Fc ϵ RI α was measured in unstimulated cells. Change is calculated as a log-difference. Blue line and shading represent a linear model fit and its 95% confidence interval. **C**, Proportion of exacerbations in omalizumab group is associated with Fc ϵ RI α change. Bars represent proportion of exacerbations during the study for each group (below and above the median level of change in Fc ϵ RI α) and error bars represent standard errors. Numbers annotated on the bars represent the number of subjects. Odds ratio, associated 95% confidence interval, and p-value are annotated above the bars.

Geometric Mean IFN – α (pg/mL)

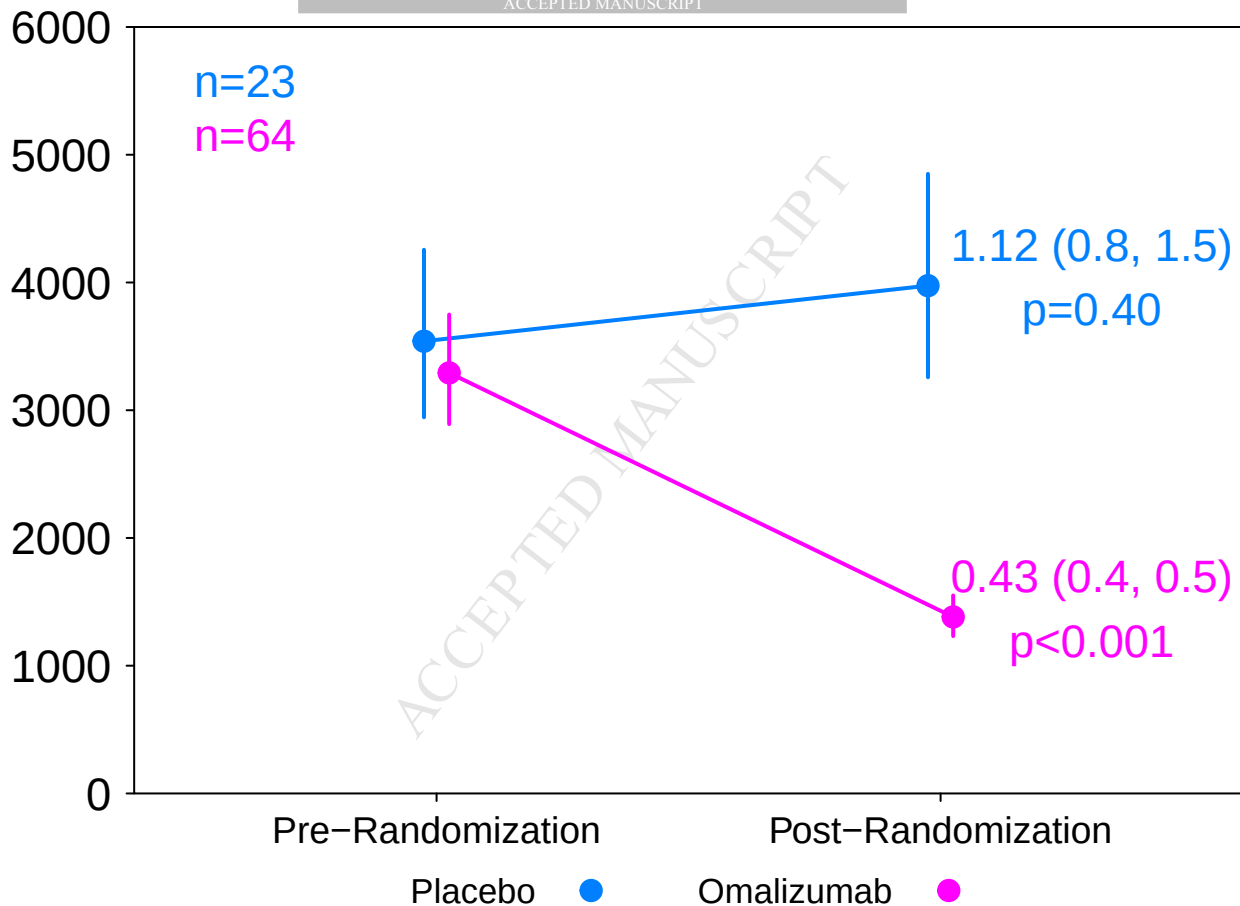
Pre-Randomization

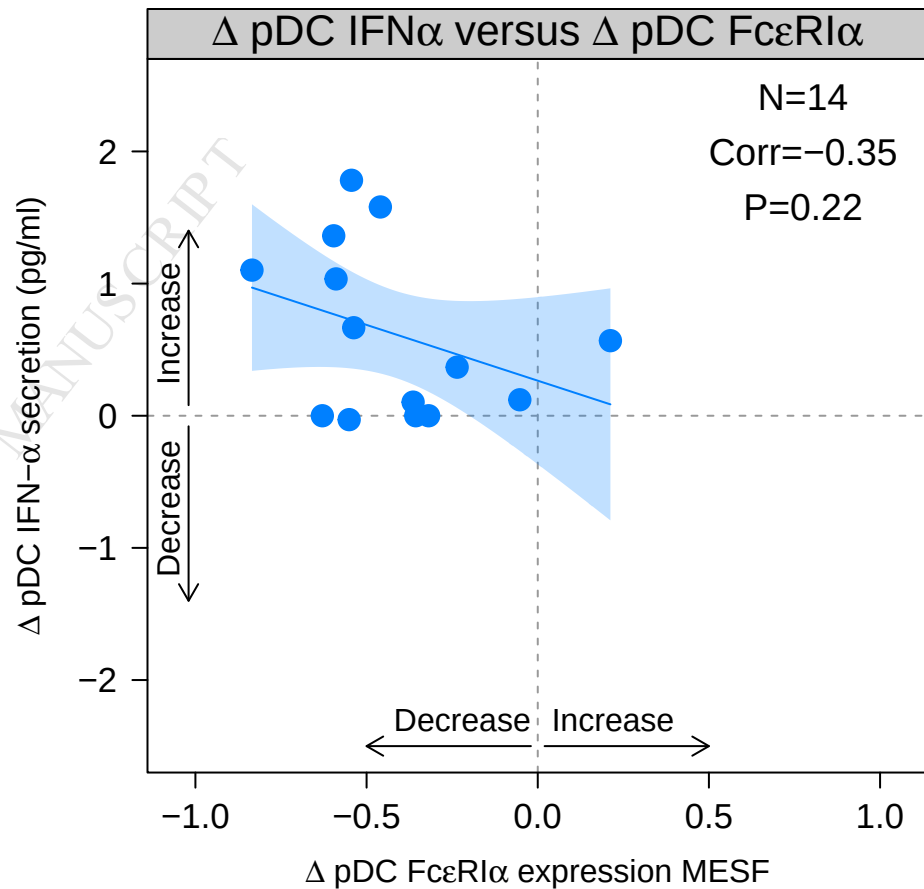
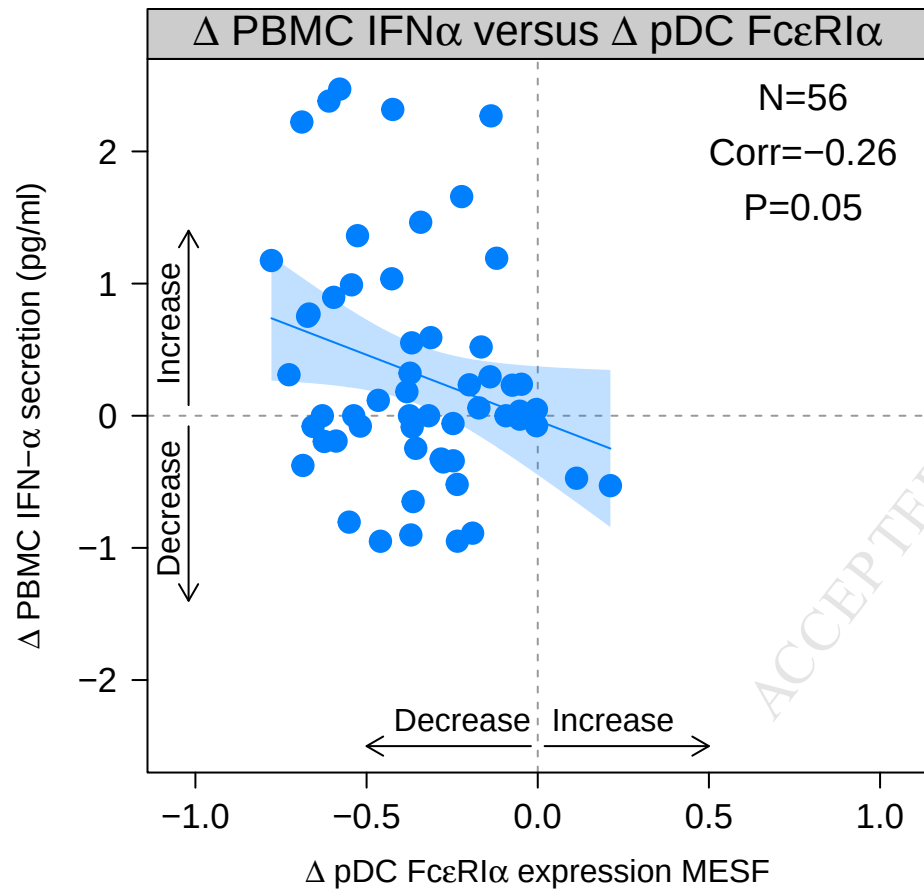


Post-Randomization



Geometric mean value





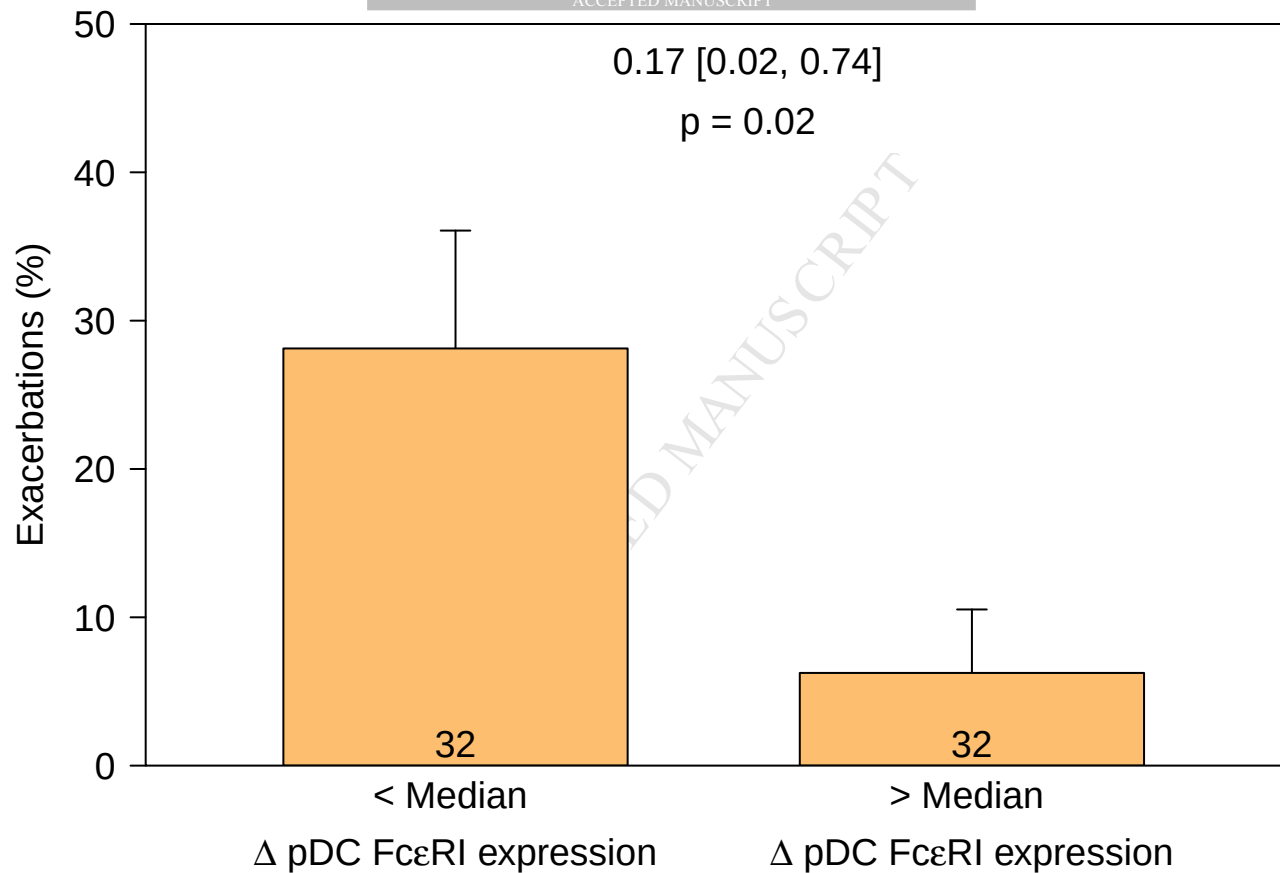


Table E1: Sequences of Primers/Probes for qPCR Analysis

Gene	Sequence
<i>TLR-7</i>	Forward: 5-TTA CCT GGA TGG AAA CCA GCT ACT-3
	Reverse: 5-TCA AGG CTG AGA AGC TGT AAG CTA-3
	Probe: *6FAM-AGA TAC CGC AGG GCC TCC CGC-TAMRA
<i>FcεR1a</i>	Forward: 5-GTG AAC CTG TGT ACC TGG AAG TCT T-3
	Reverse: 5-CAT CCC AGT TCC TCC AAC CA-3
	Probe: *6FAM-TGA CTG GCT GCT CCT TCA GGC CTC-TAMRA

* 6FAM: 6-fluorescein amidite, and TAMRA: tetramethylrhodamine

Table E2: TaqMan Probe and Primer Sets used for qPCR Analysis

Gene	Assay ID*	NCBI Reference sequences
<i>IRF7</i>	Hs01014809_g1	https://www.ncbi.nlm.nih.gov/nuccore/NM_001572.3
<i>IFNB1</i>	Hs01077958_s1	https://www.ncbi.nlm.nih.gov/nuccore/NM_002176.3
<i>IFNA1</i>	Hs00855471_g1	https://www.ncbi.nlm.nih.gov/nuccore/NM_024013.2
<i>DDX58</i>	Hs00204833_m1	https://www.ncbi.nlm.nih.gov/nuccore/NM_014314.3
<i>IFIT1</i>	Hs00356631_g1	https://www.ncbi.nlm.nih.gov/nuccore/NM_001548.4
<i>HPRT</i>	Hs99999909_m1	https://www.ncbi.nlm.nih.gov/nuccore/NM_000194.2
<i>PPIA</i>	Hs99999904_m1	https://www.ncbi.nlm.nih.gov/nuccore/NM_001300981.1

* Assays purchased from ThermoFisher Scientific

Note: ThermoFisher Scientific does not provide sequence information for pre-designed commercial gene assays

Table E3: Demographics and Clinical Data¹

	Overall Mechanistic N=92	Placebo Mechanistic N=23	Omalizumab Mechanistic N=69	p-value
Study Cohort - no. (%):				0.90
2012	47 (51.1%)	11 (47.8%)	36 (52.2%)	
2013	45 (48.9%)	12 (52.2%)	33 (47.8%)	
Injection schedule - no. (%):				0.80
Once per 2 weeks	32 (34.8%)	9 (39.1%)	23 (33.3%)	
Once per 4 weeks	60 (65.2%)	14 (60.9%)	46 (66.7%)	
Race or ethnic group - no. (%):				0.69
African American	31 (33.7%)	9 (39.1%)	22 (31.9%)	
Hispanic	54 (58.7%)	12 (52.2%)	42 (60.9%)	
White, mixed or other	7 (7.61%)	2 (8.70%)	5 (7.25%)	
Annual household income <\$15,000 - no. (%)	57 (63.3%)	13 (56.5%)	44 (65.7%)	0.59
Age – yr.	10.0 [8.00;13.0]	10.0 [7.50;12.5]	10.0 [8.00;13.0]	0.70
Male sex - no. (%)	63 (68.5%)	14 (60.9%)	49 (71.0%)	0.52
Duration of asthma – yr.	6.75 [4.29;9.25]	5.08 [3.67;7.79]	7.42 [4.92;9.92]	0.05
C-ACT score in the previous month, age 4 to 11 yr. ²	21.2 (4.16)	21.2 (4.03)	21.2 (4.25)	0.96
ACT score in the previous month, age 12 yr. or older ²	20.8 (2.91)	19.7 (4.46)	21.1 (2.28)	0.48
Asthma-related symptoms - days in prior 2 weeks ³	2.27 (2.94)	2.09 (2.37)	2.33 (3.11)	0.69
FEV1 - % of predicted value	90.2 [82.0;101]	94.0 [83.0;101]	90.0 [81.5;99.3]	0.61
FEV1:FVC ×100	78.2 [72.8;84.9]	79.2 [76.7;85.4]	77.2 [72.8;83.9]	0.40
Medication - no. (%) ⁴				0.81
Step level 2 to 4	44 (47.8%)	12 (52.2%)	32 (46.4%)	
Step level 5	48 (52.2%)	11 (47.8%)	37 (53.6%)	
1+ Asthma Exacerbation	33 (35.9%)	9 (39.1%)	24 (34.8%)	0.90
Composite Asthma Severity Index at Randomization	5.38 (2.60)	5.45 (2.56)	5.36 (2.63)	0.88
Asthma-related symptoms - days in prior 2 weeks	2.27 (2.94)	2.09 (2.37)	2.33 (3.11)	0.69
Total IgE (kU/l)	230 [135;442]	316 [152;452]	203 [125;442]	0.30
ECP at Visit 4T [ug/L]	938 [290;2542]	722 [246;1631]	1003 [318;2677]	0.21
Eosinophils (K/uL)	0.37 (0.24)	0.38 (0.31)	0.37 (0.22)	0.90

¹Values are counts (percentages) , means (SDs) or medians [IQR]²Scores on the Childhood Asthma Control Test (C-ACT) and the Asthma Control Test (ACT) were measured on scales of 0 to 27 and 5 to 25, respectively. A score of 19 or less on either test indicates that asthma is not well controlled. The minimally important difference for ACT equals 3 points; for the C-ACT, a 3-point increase suggests a clinically relevant improvement in asthma control, whereas a 2-point decrease suggests a clinically relevant worsening.

³The number of days with symptoms was calculated as the largest of the following variables during the previous 2 weeks: number of days with wheezing, chest tightness, or cough; number of nights of sleep disturbance; and number of days when activities were affected. This symptom scale ranges from 0 to 14 days per 2-week period.

⁴Six treatment steps were established, which is consistent with report 3 of the National Asthma Education and Prevention Program guidelines to standardize prescribing patterns according to levels of asthma severity summarized here. Steps 1 and 2 apply to mild asthma, step 3 to moderate asthma, and steps 4 through 5 to severe asthma. At step 0, the recommendation is for no asthma control medication or albuterol as needed; at step 1, the recommendation is for 50 µg of fluticasone twice a day; at step 2, the recommendation is for 100 µg of fluticasone twice a day; at step 3, the recommendation is for 250 µg of fluticasone twice a day; at step 4, the recommendation is for 250 µg of fluticasone and 50 µg of salmeterol twice a day (Advair, GlaxoSmithKline); and at step 5, the recommendation is for 500 µg of fluticasone and 50 µg of salmeterol twice a day (Advair, GlaxoSmithKline).

Table E4: Proportion of exacerbations by IFN α change – Omalizumab group

Cell type	<i>Ex vivo</i> stimulation	Exacerbations: n (%)		Odds ratio [95% CI]	P-value
		Below median IFN α change	Above median IFN α change		
PBMCs	RV	6 (18.8%)	5 (15.6%)	0.80 [0.21, 2.98]	0.74
	Anti-IgE + RV	6 (21.4%)	1 (3.6%)	0.14 [0.01, 0.88]	0.03
	FLU	4 (12.5%)	7 (21.9%)	1.96 [0.53, 8.23]	0.32
	Anti-IgE + FLU	0 (0.0%)	2 (10.5%)	5.57 [0.42, 789.38]	0.21
	Gardiquimod	5 (15.6%)	6 (18.8%)	1.25 [0.34, 4.80]	0.74
pDCs	RV	3 (23.1%)	6 (50.0%)	3.33 [0.63, 21.03]	0.16
	Anti-IgE + RV	3 (42.9%)	2 (28.6%)	0.53 [0.05, 4.86]	0.58
	FLU	3 (23.1%)	6 (50.0%)	3.33 [0.63, 21.03]	0.16
	Gardiquimod	3 (27.3%)	5 (50.0%)	2.67 [0.45, 18.35]	0.28

Table E5: Proportion of exacerbations by TLR7 and FcεRIα change in unstimulated cells – Omalizumab group

Cell type	Variable ¹	Exacerbations: n (%)		Odds ratio [95% CI]	P-value
		Below median change	Above median change		
PBMCs	TLR7 mRNA	8 (25.0%)	3 (9.4%)	0.31 [0.06, 1.20]	0.09
pDCs	TLR7 mRNA	5 (55.6%)	2 (22.2%)	0.23 [0.02, 1.62]	0.14
pDCs	FcεRIα surface expression	9 (28.1%)	2 (6.3%)	0.17 [0.02, 0.74]	0.02

¹TLR7 mRNA values represent expression normalized to HPRT (for PBMCs) and PPIA (for pDCs) expression ($2^{-\Delta\text{ct}}$); FcεRIα measured by flow cytometry.

Table E6: Sample size in mechanistic population by treatment group and exacerbation status^{1,2}

Mechanistic Parameter			Omalizumab			Placebo		
Cell type	<i>Ex vivo</i> stimulus	Readout	Overall	Non-exac	Exac	Overall	Non-exac	Exac
PBMCs	RV	IFN α secretion	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)
	Anti-IgE + RV	IFN α secretion	56	49 (87.5%)	7 (12.5%)	23	17 (73.9%)	6 (26.1%)
	Flu	IFN α secretion	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)
	Anti-IgE + Flu	IFN α secretion	38	36 (94.7%)	2 (5.3%)	10	9 (90%)	1 (10%)
	Gardiquimod	IFN α secretion	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)
pDC	RV	IFN α secretion	25	16 (64%)	9 (36%)	12	7 (58.3%)	5 (41.7%)
	Anti-IgE + RV	IFN α secretion	14	9 (64.3%)	5 (35.7%)	7	4 (57.1%)	3 (42.9%)
	Flu	IFN α secretion	25	16 (64%)	9 (36%)	11	7 (63.6%)	4 (36.4%)
	Gardiquimod	IFN α secretion	21	13 (61.9%)	8 (38.1%)	11	7 (63.6%)	4 (36.4%)
PBMCs	Anti-IgE + RV	TLR7 mRNA	56	49 (87.5%)	7 (12.5%)	22	16 (72.7%)	6 (27.3%)
		IRF7 mRNA	56	49 (87.5%)	7 (12.5%)	22	16 (72.7%)	6 (27.3%)
		IFNB1 mRNA	55	48 (87.3%)	7 (12.7%)	20	15 (75%)	5 (25%)
		IFNA1 mRNA	32	26 (81.2%)	6 (18.8%)	13	11 (84.6%)	2 (15.4%)
		DDX58 (RIG-I) mRNA	56	49 (87.5%)	7 (12.5%)	22	16 (72.7%)	6 (27.3%)
		IFIT1 mRNA	56	49 (87.5%)	7 (12.5%)	22	16 (72.7%)	6 (27.3%)
PBMCs	None	TLR7 mRNA	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)
		Fc ϵ RI α mRNA	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)

pDCs	None	TLR7 mRNA	18	11 (61.1%)	7 (38.9%)	7	4 (57.1%)	3 (42.9%)
		FcεRIα mRNA	18	11 (61.1%)	7 (38.9%)	7	4 (57.1%)	3 (42.9%)
pDCs	None	Surface FcεRIα protein expression (MESF)	64	53 (82.8%)	11 (17.2%)	23	17 (73.9%)	6 (26.1%)

¹ Ns reflect the number of participants with both pre-randomization and post-randomization measures

² Table applies to all analyses except the prediction model, which combines placebo and ICS (n=36) groups into a non-omalizumab group. All variables entered in the prediction model have full sample size (N=128) due to imputation.

Table E7 Difference in baseline mechanistic variables between Exacerbators and Non- Exacerbators¹

	Non-Omalizumab (N=59)				Omalizumab (N=69)			
Variable measured pre-randomization	Non-exacerbators: Mean (SD) (n=49)	Exacerbators: Mean (SD) (n=10)	Geometric Mean Ratio [95% CI]	P-value	Non-exacerbators: Mean (SD) (n=57)	Exacerbators: Mean (SD) (n=12)	Geometric Mean Ratio [95% CI]	P-value
PBMC IFN α (pg/ml) - RV	1287.09 (5.5)	757.58 (10)	0.59 [0.11, 3.18]	0.50	1485.08 (6.5)	969.23 (5.4)	0.65 [0.21, 2.06]	0.44
PBMC IFN α (pg/ml) - Anti-IgE + RV	111.56 (5.7)	95.14 (4)	0.85 [0.29, 2.5]	0.76	207.84 (6.6)	75.15 (4.5)	0.36 [0.13, 1.04]	0.06
PBMC IFN α (pg/ml) - FLU	5850.31 (3)	7085.5 (3.2)	1.21 [0.51, 2.86]	0.64	7499.6 (2.3)	7191.63 (1.7)	0.96 [0.64, 1.43]	0.83
PBMC IFN α (pg/ml) - Gardiquimod	927.01 (4)	1203.52 (7.1)	1.3 [0.31, 5.43]	0.70	822.08 (4.3)	1156.59 (3.1)	1.41 [0.64, 3.1]	0.38
PBMC TLR7 mRNA - unstimulated	1.32 (1.7)	1.27 (1.6)	0.96 [0.67, 1.37]	0.80	1.36 (1.6)	0.95 (1.6)	0.7 [0.51, 0.96]	0.03
PBMC IRF7 mRNA - unstimulated	8.44 (1.5)	9.84 (1.6)	1.17 [0.81, 1.67]	0.37	8.6 (1.6)	6.83 (1.4)	0.79 [0.62, 1.02]	0.07
PBMC TLR7 mRNA - RV	4.45 (1.7)	3.29 (2.4)	0.74 [0.39, 1.4]	0.32	4.28 (2.1)	4.56 (1.8)	1.07 [0.71, 1.6]	0.75
PBMC TLR7 mRNA - Anti-IgE + RV	2.75 (2.2)	2.1 (1.9)	0.76 [0.46, 1.25]	0.26	2.46 (2.2)	2.11 (1.6)	0.86 [0.59, 1.24]	0.40
PBMC IRF7 mRNA - RV	11.3 (2.4)	7.07 (2.5)	0.63 [0.32, 1.24]	0.16	10.63 (3)	12.45 (1.5)	1.17 [0.8, 1.72]	0.41
PBMC IRF7 mRNA - Anti-IgE + RV	9.62 (2.6)	4.23 (2.9)	0.44 [0.2, 0.98]	0.04	7.43 (3.2)	6.66 (2.8)	0.9 [0.44, 1.83]	0.75
PBMC IFN β 1 mRNA - RV	118.07 (6.8)	55.57 (8.4)	0.47 [0.1, 2.28]	0.32	105.97 (7.4)	137.19 (3.8)	1.29 [0.49, 3.43]	0.59
PBMC IFN β 1 mRNA - Anti-IgE + RV	22.03 (7.7)	12.21 (6)	0.55 [0.14, 2.18]	0.37	23.36 (11)	14.58 (5.7)	0.62 [0.18, 2.14]	0.43
PBMC DDX58 mRNA - RV	11.88 (2.3)	7.22 (2.6)	0.61 [0.3, 1.25]	0.16	10.4 (2.6)	14.14 (1.5)	1.36 [0.96, 1.92]	0.08
PBMC DDX58 mRNA - Anti-IgE + RV	8.22 (2.8)	4.51 (2.5)	0.55 [0.27, 1.11]	0.09	6.42 (3.1)	5.35 (4.9)	0.83 [0.29, 2.37]	0.71
PBMC IFIT1 mRNA -	121.18 (5)	49.75 (9.6)	0.41	0.26	122.6 (5.5)	175.52 (2.1)	1.43	0.25

	Non-Omalizumab (N=59)				Omalizumab (N=69)			
Variable measured pre-randomization	Non-exacerbators: Mean (SD) (n=49)	Exacerbators: Mean (SD) (n=10)	Geometric Mean Ratio [95% CI]	P-value	Non-exacerbators: Mean (SD) (n=57)	Exacerbators: Mean (SD) (n=12)	Geometric Mean Ratio [95% CI]	P-value
RV			[0.08, 2.15]				[0.77, 2.66]	
PBMC IFIT1 mRNA - Anti-IgE + RV	67.21 (6.5)	16.57 (8.4)	0.25 [0.05, 1.19]	0.08	40.28 (12.5)	38.53 (5.1)	0.96 [0.29, 3.15]	0.94
pDC FcεRIα MESF - unstimulated	3504.17 (1.6)	3643.04 (1.6)	1.04 [0.74, 1.46]	0.81	3168.2 (1.7)	3950.26 (1.9)	1.25 [0.81, 1.92]	0.29
PBMC FcεRIα mRNA - unstimulated	1.68 (1.6)	1.03 (1.8)	0.61 [0.4, 0.93]	0.03	1.98 (1.7)	1.47 (2.1)	0.74 [0.46, 1.21]	0.21

¹Means are geometric means, and SDs are geometric standard deviations.

Table E8: Threshold values for the index for differentiation between exacerbations

	Non-Omalizumab ¹ (Model 1) ²	Non-Omalizumab ¹ (Model 2) ³	Omalizumab (Model 1) ²
Area under the ROC curve (AUC)	0.89	0.97	0.87
Coefficient of discrimination	0.34	0.59	0.29
Sensitivity ⁴	0.80	0.90	0.75
Specificity ⁵	0.80	0.90	0.75
Positive predictive value ⁶	0.44	0.64	0.39
Negative predictive value ⁷	0.95	0.98	0.93
False Positive	10	5	14
False Negative	2	1	3

¹Includes participants receiving Placebo (N=23) and ICS Boost (N=36) treatments.

²Model 1: *Known* clinical predictors

³Model 2: *Known* clinical predictors + *New* mechanistic predictors

⁴Sensitivity = True Positive / Total Positive

⁵Specificity = True negatives / (False positives + True negatives)

⁶Positive predictive value = True positive / (False positives + True negatives)

⁷Negative predictive value = True negative / (False positives + True negatives)

PBMC IFNα secretion: Anti-IgE + RV

pDC IFNα secretion: RV

pDC IFNα secretion:
Anti-IgE + RV

Exacerbations

PBMC IFNα secretion: RV

pDC surface FcεR1α expression

