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Anti-β_c mAb CSL311 inhibits human nasal polyp pathophysiology in a humanized mouse xenograft model

To the Editor,

Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory disease of the nose and paranasal sinuses characterised by eosinophilia, elevated levels of local IgE and Type 2 (T2) inflammatory cytokines(1) and mucus production. Interleukin (IL)-5, together with the other T2 beta common (β_c) cytokines IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), support the survival, and enhance the differentiation and activation of myeloid cells, which play a key role in the pathogenesis of this disease(2, 3).

We have developed a fully-human therapeutic monoclonal antibody (mAb), CSL311, with specificity for the common cytokine binding site of the human β_c receptor(4). The ability of CSL311 to block IL-3, IL-5 and GM-CSF signals simultaneously may offer distinct advantages in the treatment of inflammatory diseases compared to mAbs targeting single cytokines. Preclinical testing of CSL311 is limited in animal models due to the species-specificity of CSL311. To overcome this constraint we modified the human nasal polyp (NP) xenograft model described previously(5), and utilised *Rag2*$^{-/-}$*Il2rg*$^{-/-}$hIL-3/GM-CSF knock-in mice, which express human IL-3 and GM-CSF to support longer-term survival, growth and differentiation of human myeloid cells to evaluate, for the first time, the *in vivo* efficacy of CSL311, on NP progression in a preclinical proof-of-concept study.

NPs from 12 patients, who were withdrawn from any medication one month prior to polypectomy, were used in this study. The most frequent comorbidities in these patients were allergic rhinitis (50%) and asthma (41.7%) (Table S1); consistent with atopy, histological analysis revealed that the NPs were highly eosinophilic (11/12), demonstrating characteristic T2 inflammation (data not shown). High levels of β_c cytokines were detected in the NP compared to normal sinus tissues (Figure S1), suggesting a role of β_c cytokines in contributing to the pathogenesis of CRSwNP. NP from each patient was engrafted into 4–10 mice with all treatment groups included in each independent experiment (Table S2). CSL311 significantly suppressed NP progression in recipient mice after 5 weeks compared with isotype control mAb (Figure 1A, Figure S3). We found that treatment with CSL311 for one week resulted in an immediate reduction of NP volume measured externally, which was not observed with positive control prednisolone treatment. After 5 weeks treatment, CSL311 significantly suppressed NP volume in recipient mice when compared with isotype control mAb.

To understand how CSL311 treatment inhibited NP growth, we examined the immune cell profiles in the xenografted NPs collected at the end of the 5-week engraftment. The percentage of eosinophils, neutrophils and plasma B cells (Figure 1B, Figure S2, Table S3), as well as the number of mast cells (Figure 1C) were significantly reduced in the presence of CSL311 when compared with isotype control mAb treatment. These cells are known to express β_c receptor and when activated by β_c cytokines produce chemical mediators that directly promote tissue remodelling and oedema. Next, we assessed the effect of CSL311 on mucous gland hyperplasia, a general feature of NPs(6). Histological examination revealed a significant decrease in mucous gland number and mucus production in NPs treated with CSL311 that was not observed in those treated with isotype control mAb (Figure 1D, Figure S3). The stroma of NPs contains increased levels of fibroblasts(7). Fibroblast-specific protein 1(FSP-1) is expressed in fibroblasts in different organs that undergo tissue remodelling and is commonly used as a marker to identify fibroblasts(8). Using immunofluorescence staining, we found that CSL311 significantly decreased the percentage of FSP-1 positive fibroblasts compared to isotype control mAb (Figure 1E, Figure S3).

We also performed transcriptome analysis by RNA sequencing to understand the impact of CSL311 on the global gene expression profile in the xenografted NPs. Treatment with CSL311 resulted in differential expression of 29 genes with a fold change >2 ($|\log_2 FC| > 1$)

and false discovery rate < 0.05. The top 25 differentially expressed (DE) genes (Figure 2A) were used to calculate a gene score based on the average counts per gene, which confirmed that CSL311 significantly reduces expression of this gene set compared to isotype control mAb; no significant effect of prednisolone was observed on expression of these genes (Figure 2B). The average log fold change (log_2FC) in gene expression, induced by CSL311 vs isotype control mAb and prednisolone vs saline, is shown in Figure 2C. These DE genes encode: i) surface markers associated with macrophage/dendritic cells e.g. *CD14, CD206, CD209* and granulocytic cell subsets e.g. *CLEC4A, CD32, FCERG1*, ii) chemokines that recruit T2 cells (basophils, eosinophils, TH2 cells) e.g. *CCL7, CCL13, CCL18 CCL23* and iii) inflammatory response proteins including complement components *C1QA, C1QB* and *C1QC*. Ingenuity Pathway Analysis (IPA) upstream regulator tool, which predicts the most likely regulators of the DE genes, identified GM-CSF (Figure 2D) and IL-3 (Table S4) as regulators of genes down-regulated by CSL311. In addition, this analysis identified other cytokines that may contribute to airway inflammation, such as IL-4, IL-13, IFNγ, IL-17A and IL1β as contributing to gene expression down-regulated by CSL311(Figure 2D and Table S4). This is consistent with reduced IL-4 and IL-13 protein production observed in allergic $\beta_c^{-/-}$ mice and point to a potentially important link between the β_c-signaling cytokines and the IL-4/IL-13 axis in T2 cytokine-mediated diseases(9).

mAbs that target T2 inflammation have emerged as important alternative therapeutics for patients with CRSwNP. Clinical trials using omalizumab (anti-IgE), mepolizumab (anti-IL-5), and dupilumab (anti-IL-4 receptor α) have demonstrated that these mAbs shrink NPs and improve clinical symptoms (10). The key mechanisms of these drugs on blocking the IgE-dependent mast cell activation, IL-5-driven eosinophil activities, and IL-4/IL-13-mediated inflammation were replicated by CSL311 treatment in our xenograft model. Our study provides the first demonstration that targeting β_c with a therapeutic mAb may be an effective strategy for treating CRSwNP. Intrapolyp local drug delivery, as has been utilised in this model, has been reported to be an effective and safe method for treatment of NPs(11). Systemic efficacy of CSL311 treatment in this mouse model, as well as clinical trials will be required to assess the safety and therapeutic potential of CSL311 in improving clinical outcomes of patients with nasal polyposis.

AUTHOR CONTRIBUTIONS

K.H.Y, N.J.W, M.A.G. and C.M.O were involved in study concept and design. K.H.Y, N.J.W, C.L.B, H.P., M.S., S.B., M.N, M.A. and N.W. contributed to the acquisition, analysis or interpretation of data. G.V., A.F.L., A.D.N. D.J.T. and M.J.W. provided intellectual input; K.H.Y, N.J.W and C.M.O drafted the manuscript. All authors approved the manuscript for submission

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CONFLICTS OF INTEREST

K.H.Y., H.P., C.L.B., M.S. D.J.T declare no conflict of interest. N.J.W., M.N., M.A., N.W., G.V., A.D.N, M.J.W. and C.M.O. are employees of CSL Limited. S.B. is a former employee of CSL Limited and declares no conflict of interest. A.F.L. and M.A.G. receive funding from CSL Limited.

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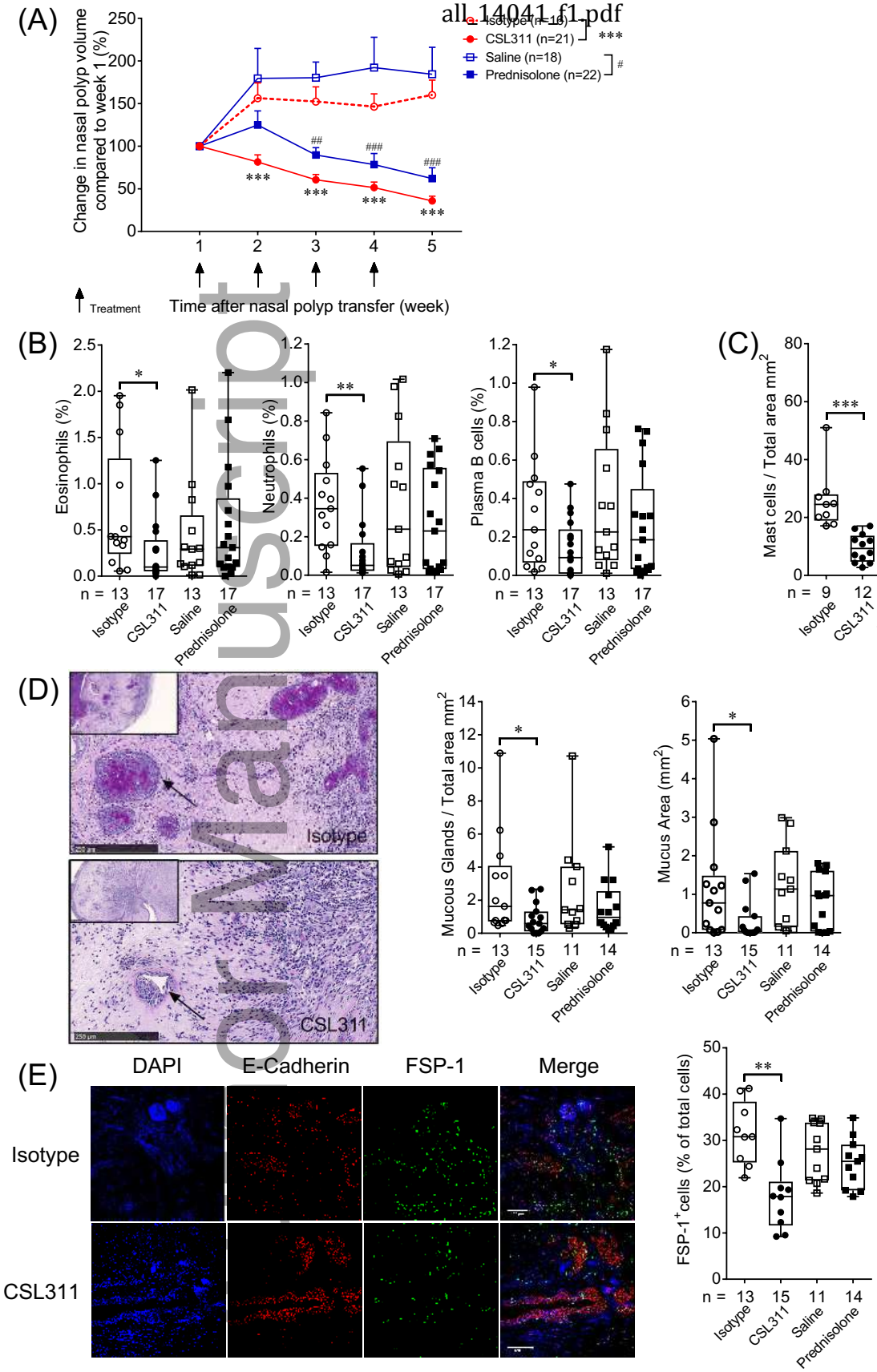
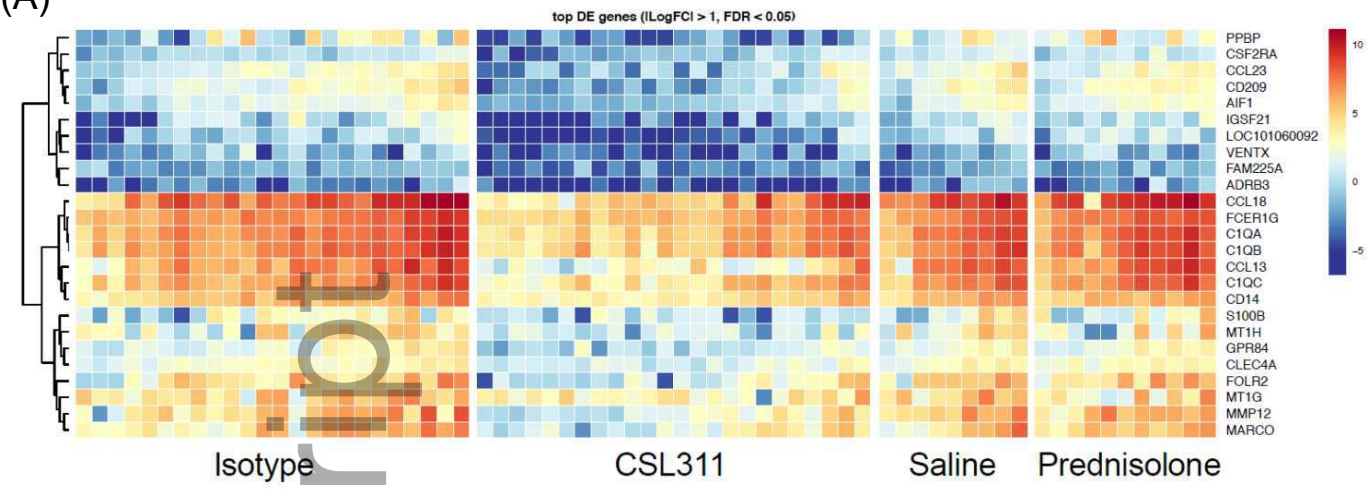


FIGURE 1. CSL311 restrains human nasal polyp xenograft progression and pathophysiology *in vivo*. CSL311 mAb treatment reduces (A) volume of nasal polyp measured externally every week, (B) granulocytes and plasma B cell populations, (C) mast cell numbers, (D) mucous gland numbers and mucus production and (E) fibroblast numbers in xenografted nasal polyps compared to isotype control mAb treatment. #/*p<0.01, ##/**p<0.01, ###/***p<0.001, (A) data are mean ± S.E.M of 12 independent experiments with n = 16 to 22 mice for each group. Two-way ANOVA with Bonferroni post-test for comparison on indicated time points between isotype control mAb vs CSL311 and saline vs prednisolone treatments; (B – E) data: median ± range, with the number of mice in each group indicated below x-axis. Mann-Whitney U test for indicated comparisons.

(A)



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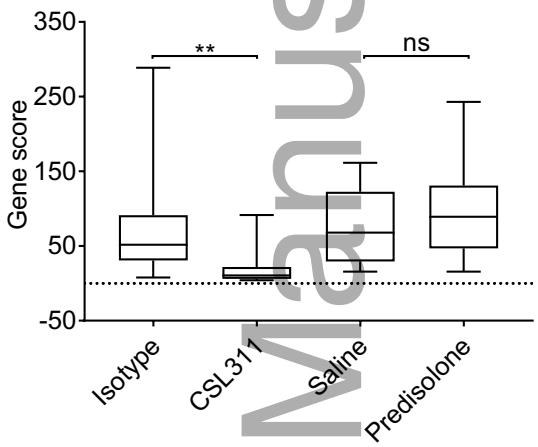
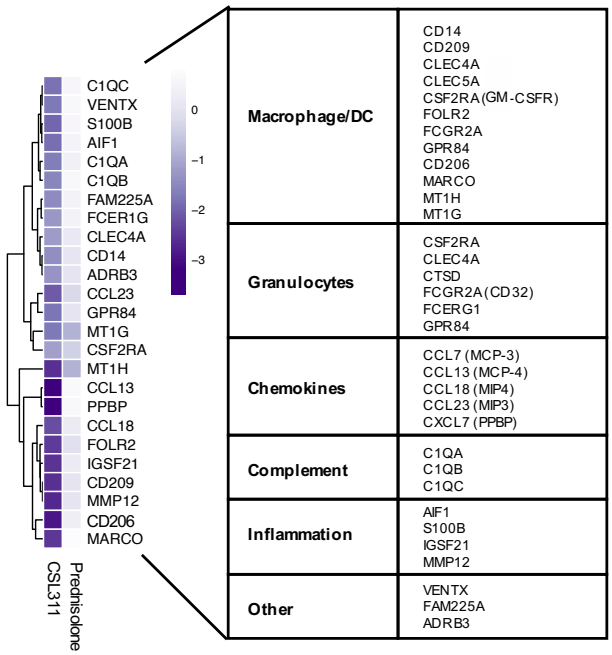


(C)



(D)

IPA Analysis generated with a threshold of Adj p-value <0.1			
Top 5 Upstream Regulator	Molecule Type	p-value of overlap	Target molecules in dataset
SPI1	transcription regulator	4.9E-14	C1QC,C3AR1,CD209,CSF2RA,GPR84,IL1B,IL24,MT1G,MT1H
Gm-csf	group	3.57E-08	CCL23,CD14,CD209,IL1B,MRC1
IL4	cytokine	0.000000375	CCL18,CCL23,CD14,CD209,IL1B,MRC1
IL13	cytokine	0.000000557	C3AR1,CCL18,CD14,CD209,CLEC4A,IL1B,MRC1
Immunoglobulin	complex	0.00000259	C3AR1,CCL7,FCGR2A,IL1B

FIGURE 2. RNA sequencing and Ingenuity Pathway Analysis (IPA) reveal CSL311 targets type 2 inflammation-associated genes in xenografted human NPs. (A) Hierarchical clustering of top 25 differentially expressed (DE) genes. (B) The average expression of the top 25 DE genes was calculated for each treatment and expressed as a "gene score". The difference in gene score between isotype control and CSL311 mAb and between saline control and prednisolone was determined (** $p<0.01$, one-way ANOVA with Tukey's multiple comparison tests). (C) The heat map shows average fold change in DE genes (isotype control mAb vs CSL311 and saline control vs prednisolone) and genes annotated for cellular expression or function. (D) IPA Upstream Regulator Analysis reveals top 5 targeted upstream regulators by CSL311 treatment in xenografted NPs.

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