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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.

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30 NPs from 12 patients, who were withdrawn from any medication one month prior to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

45 To understand how CSL311 treatment inhibited NP growth, we examined the immune cell
46 profiles in the xenografted NPs collected at the end of the 5-week engraftment. The
47 percentage of eosinophils, neutrophils and plasma B cells (Figure 1B, Figure S2, Table S3),
48 as well as the number of mast cells (Figure 1C) were significantly reduced in the presence of
49 CSL311 when compared with isotype control mAb treatment. These cells are known to
50 express β_c receptor and when activated by β_c cytokines produce chemical mediators that
51 directly promote tissue remodelling and oedema. Next, we assessed the effect of CSL311 on
52 mucous gland hyperplasia, a general feature of NPs(6). Histological examination revealed a
53 significant decrease in mucous gland number and mucus production in NPs treated with
54 CSL311 that was not observed in those treated with isotype control mAb (Figure 1D, Figure
55 S3). The stroma of NPs contains increased levels of fibroblasts(7). Fibroblast-specific protein
56 1(FSP-1) is expressed in fibroblasts in different organs that undergo tissue remodelling and is
57 commonly used as a marker to identify fibroblasts(8). Using immunofluorescence staining,
58 we found that CSL311 significantly decreased the percentage of FSP-1 positive fibroblasts
59 compared to isotype control mAb (Figure 1E, Figure S3).

61 We also performed transcriptome analysis by RNA sequencing to understand the impact of
62 CSL311 on the global gene expression profile in the xenografted NPs. Treatment with
63 CSL311 resulted in differential expression of 29 genes with a fold change >2 ($|\log_2FC| >1$)

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*, *FCER1*, ii) chemokines that recruit T2 cells (basophils, eosinophils, TH2 cells) e.g. *CCL7*, *CCL13*, *CCL18*, *CCL23* and iii) inflammatory response proteins including complement components *CIQA*, *CIQB* and *CIQC*. 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, IFN γ , IL-17A and IL1 β 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 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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