



Structural Characterisation Reveals Mechanism of IL-13-Neutralising Monoclonal Antibody Tralokinumab as Inhibition of Binding to IL-13R α 1 and IL-13R α 2

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<http://dx.doi.org/10.1016/j.jmb.2016.12.005>

Edited by Thomas J. Smith

Abstract

Interleukin (IL)-13 is a pleiotropic T helper type 2 cytokine frequently associated with asthma and atopic dermatitis. IL-13-mediated signalling is initiated by binding to IL-13R α 1, which then recruits IL-4R α to form a heterodimeric receptor complex. IL-13 also binds to IL-13R α 2, considered as either a decoy or a key mediator of fibrosis. IL-13-neutralising antibodies act by preventing IL-13 binding to IL-13R α 1, IL-4R α and/or IL-13R α 2. Tralokinumab (CAT-354) is an IL-13-neutralising human IgG4 monoclonal antibody that has shown clinical benefit in patients with asthma. To decipher how tralokinumab inhibits the effects of IL-13, we determined the structure of tralokinumab Fab in complex with human IL-13 to 2 Å resolution. The structure analysis reveals that tralokinumab prevents IL-13 from binding to both IL-13R α 1 and IL-13R α 2. This is supported by biochemical ligand-receptor interaction assay data. The tralokinumab epitope is mainly composed of residues in helices D and A of IL-13. It is mostly light chain complementarity-determining regions that are driving paratope interactions; the variable light complementarity-determining region 2 plays a key role by providing residue contacts for a network of hydrogen bonds and a salt bridge in the core of binding. The key residues within the paratope contributing to binding were identified as Asp50, Asp51, Ser30 and Lys31. This study demonstrates that tralokinumab prevents the IL-13 pharmacodynamic effect by binding to IL-13 helices A and D, thus preventing IL-13 from interacting with IL-13R α 1 and IL-13R α 2.

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Introduction

Interleukin (IL)-13 is a cytokine secreted predominantly by CD⁺ T helper type 2 (Th2) cells that share a receptor component and many biological properties with IL-4. IL-13 mediates its biological effects by initially binding IL-13R α 1 on one side and then recruiting IL-4R α on the opposite side to form a signal transducer and activator of transcription 6 signalling complex in which IL-13 is in the middle of the two receptors [1]. IL-13 also binds very tightly to IL-13R α 2

[2]. IL-13R α 2 lacks a significant cytoplasmic tail and is generally considered to be a decoy [3,4] involved in removing IL-13 by internalisation [5]. Supporting this decoy hypothesis, we have not yet identified a heterodimeric partner for IL-13R α 2 although other data have suggested IL-13:IL-13R α 2 interactions with activator protein 1 signalling and fibrosis [6].

Asthma is a complex, chronic and heterogeneous inflammatory disease characterised by airway hyper-responsiveness in association with airway inflammation. IL-13 has been shown to drive key

disease mechanisms in asthma including airway hyper-responsiveness, mucus hypersecretion, eosinophilia and fibrosis development [7]. Hence, there has been great interest in neutralising IL-13 as a therapeutic strategy for treating asthma. A key subtype of human asthma is termed Th2-high, characterised by both the presence of lung IL-13 and responsiveness to IL-13 neutralisation with therapeutic antibodies [8–11].

Atopic dermatitis is a chronic and pruritic inflammatory skin disease characterised by atopy, elevated Th2 responses, a defective skin barrier and a predisposition to infection by viruses and bacteria [12]. IL-4 and IL-13 have been implicated as central mediators in atopic dermatitis. Clear clinical benefit has been found following the administration of the IL-4R α blocking antibody dupilumab [13] in trials of patients with atopic dermatitis. Trials evaluating the role of IL-13-neutralisation alone in atopic dermatitis are on-going (NCT02347176, NCT02340234).

Tralokinumab (CAT-354) is a fully human, IL-13-neutralising IgG4 monoclonal antibody with a very high affinity [14] for IL-13. Alanine scanning mutation analysis demonstrated that tralokinumab bound to IL-13 in helix D (PCT/GB2004/003059).

Tralokinumab functionally neutralises IL-13 in a range of cell-based assays (IL-13R α 1:IL-4R α interactions [15]) and has shown efficacy in moderate–severe asthma [10,11]. It is currently in pivotal phase 3 trials for moderate–severe asthma (STRATOS 1 and 2 [NCT02161757, NCT02194699]) as well as a phase 2 trial for atopic dermatitis (NCT02347176).

To support the ongoing clinical development of tralokinumab in both asthma and atopic dermatitis, we crystallised tralokinumab in complex with IL-13 and performed receptor–ligand interaction assays.

Results

Tralokinumab inhibits binding of IL-13 to both IL-13R α 1 and IL-13R α 2

Tralokinumab is a potent inhibitor of IL-13 pharmacodynamics [15]. To define the mechanism by which this occurs, we employed biochemical receptor–ligand interaction assays. Tralokinumab dose-dependently prevented 600 pM IL-13 from interacting with 10 nM IL-13R α 1 with a geometric mean 50% inhibitor

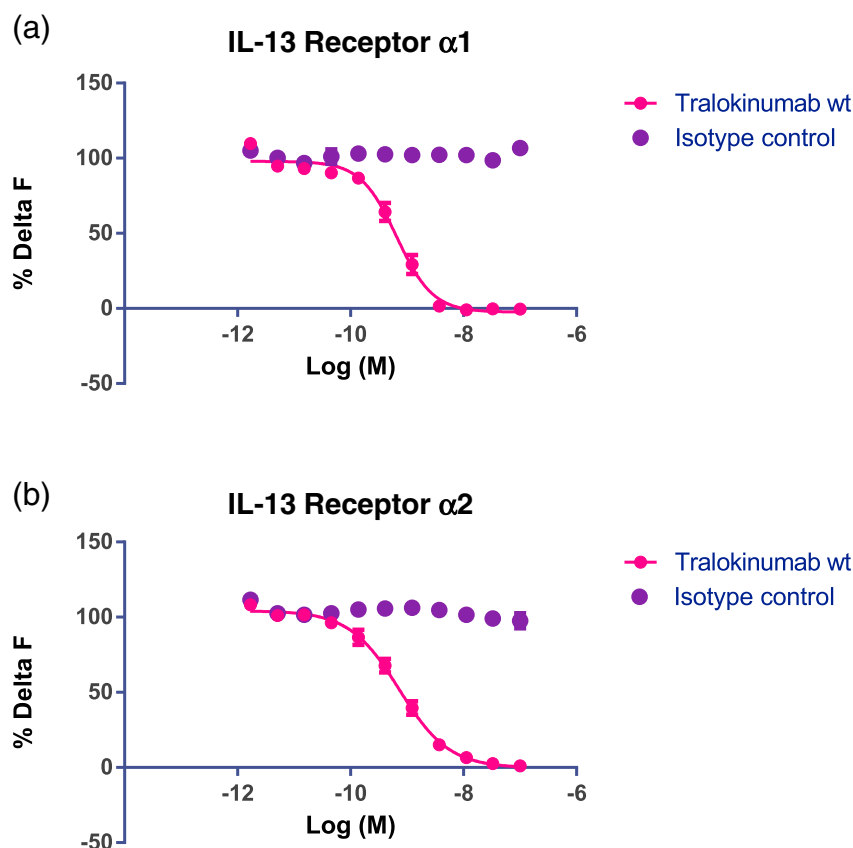


Fig. 1. Tralokinumab prevents IL-13 from interacting with both (a) IL-13R α 1 and (b) IL-13R α 2. Data are shown as mean % DeltaF (standard error of the mean) from two independent experiments each performed in duplicate.

concentration (IC_{50}) of 660 (95% CI range: 402–1086) pM ($n = 4$; Fig. 1a). Tralokinumab also dose-dependently prevented 50 pM IL-13 from interacting with 2.5 nM IL-13R α 2 with a geometric mean IC_{50} of 716 (95% CI range: 464–1107) pM ($n = 4$; Fig. 1b). These data confirm that tralokinumab binding to IL-13 prevents IL-13 from interacting with both IL-13R α 1 and IL-13R α 2 in a concentration-dependent fashion.

Structure of the tralokinumab:IL-13 complex determined by X-ray crystallography

In order to determine the atomic resolution details of the interactions in the tralokinumab:IL-13 complex, we determined the structure of a tralokinumab Fab fragment in complex with IL-13 by X-ray crystallography (Fig. 2). The structure was solved and refined to 2 Å resolution (Table 1). The residues at the interface were well resolved in the electron density map, allowing for detailed analysis and interpretation of binding.

The interface of the antibody–antigen complex was analysed. The IL-13 antigen presents a flat binding surface for binding to tralokinumab. The IL-13 structure in the complex has the characteristic four-helix bundle, typical for this family of proteins, where helices A and B are antiparallel to helices C and D. The structure of IL-13 in this complex is very similar to IL-13 in crystal structures from the Protein

Table 1. X-ray data collection and refinement for tralokinumab–IL-13 complex

	Tralokinumab–IL-13
SPG/Unit cell	P1 50.99 53.19 62.05 $\alpha = 107.9^\circ$ $\beta = 101.4^\circ$ $\gamma = 96.9^\circ$ 49.6–1.99 (2.1–1.99)
Resolution	$R_{\text{merge}} = 0.13$ (0.32)
Data R_{factor}	419,281/39,821
Observations/Unique	97.7 (93.4)
Completeness	10.5 (7.1)
Multiplicity	11.7 (2.1)
Signal-to-noise	0.189/0.211
R/R_{free}	0.02/1.2
RMSD bonds/angles	97/3/0/0
Ramachandran %	

Data Bank (PDB) 3G6D [16], 4I77 [17] and 4PS4 [18]. Superimposing these PDB IL-13 structures with that of the IL-13–tralokinumab complex reported here demonstrates high structural similarity with root mean square deviation (RMSD) values of 0.5, 0.45 and 0.53, respectively. There is a small (20°) but notable movement of helix D in the tralokinumab-bound IL-13 structure, as compared with other structures such as the IL-13 NMR structure [19]. The overall structure of the IL-13 four-helix bundle in the IL-13–tralokinumab complex is consistent with the previously solved crystal structures of IL-13. The structure of the tralokinumab

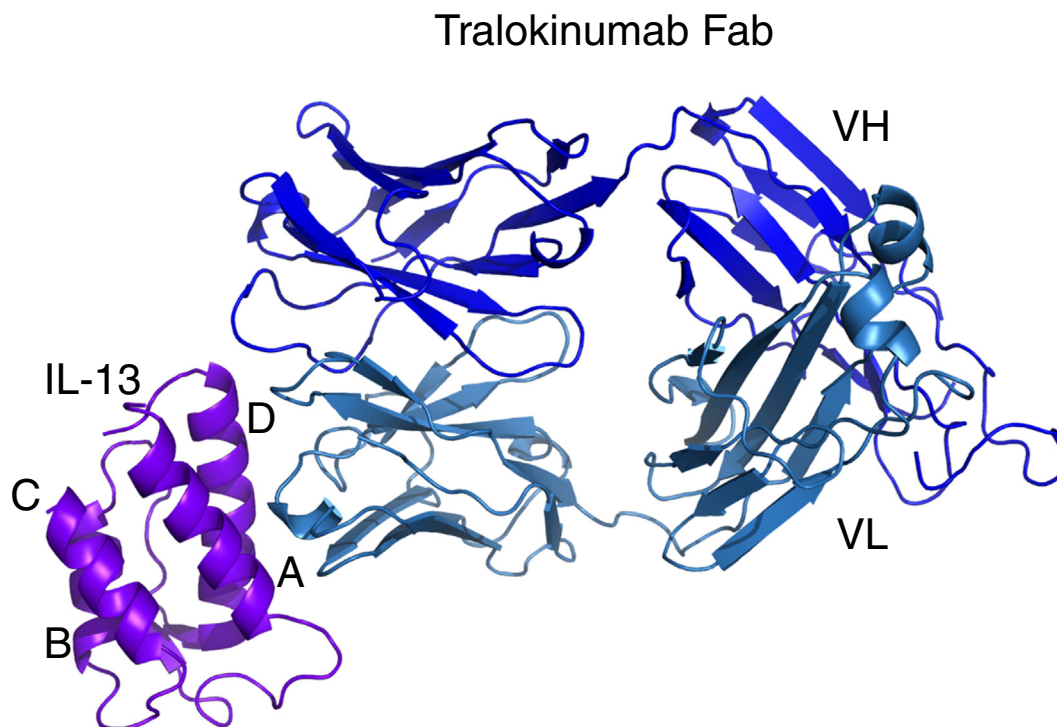


Fig. 2. Overall structure of the tralokinumab:IL-13 complex. Heavy chain (VH) is coloured in dark blue, light chain (VL) is coloured in light blue, and IL-13 is shown in purple. Helices A–D of IL-13 are also labelled.

Fab fragment bound to IL-13 forms a classical immunoglobulin fold characterised by an antiparallel beta-sheet structure.

The tralokinumab:IL-13 crystal structure shows the atomic resolution detail of the interaction interface between IL-13 and tralokinumab (Fig. 3). Tralokinumab binds IL-13 at the interface formed by IL-13 helices A and D via epitope composed of 16 amino acids. There are several basic residues, mostly in helix D, that make the surface positively charged. These include Lys 103, Lys 104 and Arg 107. The complementary surface on tralokinumab is negatively charged, highlighting that charge complementarity is

one of the factors driving complex formation. Negatively charged tralokinumab residues that complement the basic residues from IL-13 include Asp 50, Asp 51 and Asp 53.

Moreover, 20 residues from the tralokinumab Fab paratope—composed of 5 variable heavy (VH) residues and 15 variable light region (VL) residues—interact with 16 residues from the IL-13 epitope (Fig. 3). These are located in VL, VH, complementary-determining regions (CDRs) and within the framework (FW) of the antibody. Residues from VLCDR1, VLCDR2, VLCDR3, VLFW3 and VHCDR3 make up the paratope. The residues contributing to the

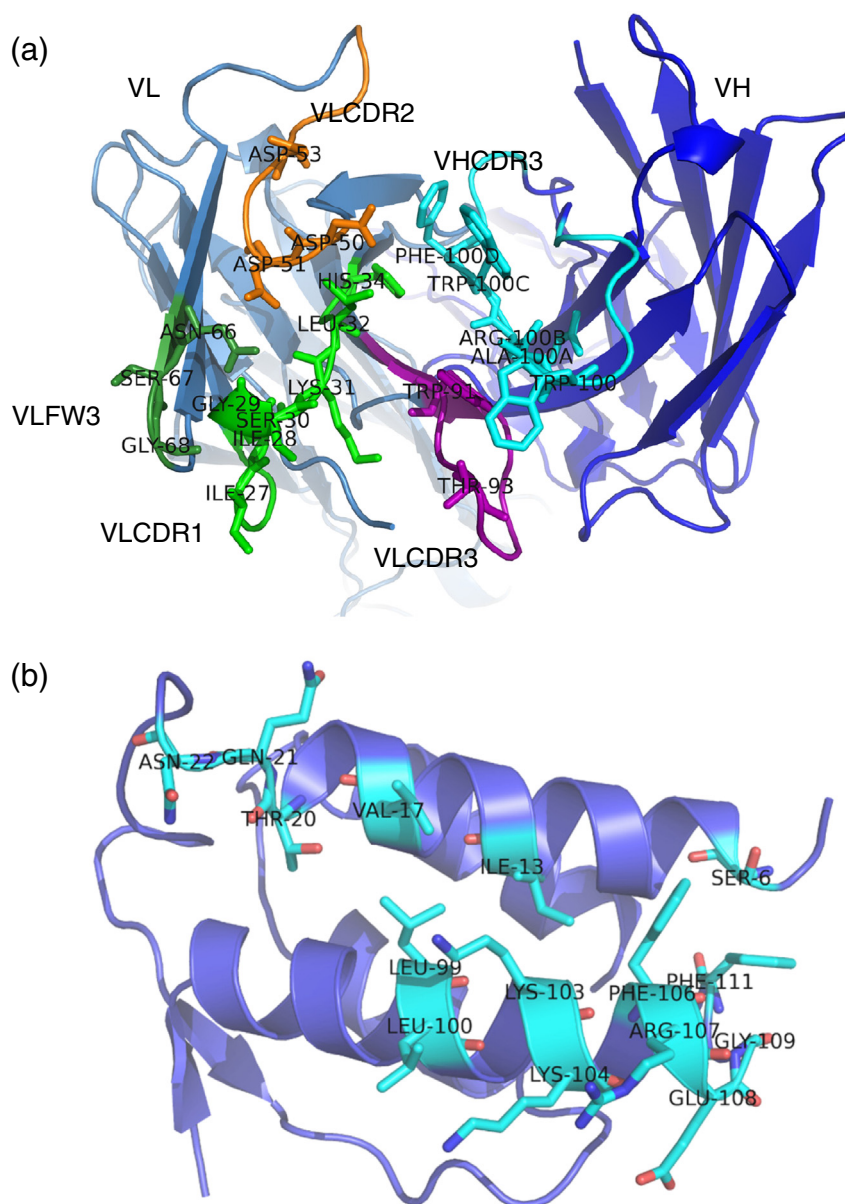


Fig. 3. The structural epitope and paratope of tralokinumab:IL-13 complex. (a) The tralokinumab structural paratope. VH is dark blue and VL is light blue. VLCDR1 is green, VLCDR2 is orange, VL FW3 is dark green, VLCDR3 is purple, and VH CDR3 is cyan. (b) The structural epitope of IL-13 showing the details of the epitope.

paratope are predominantly from the light chain with VLCDR2 at the core of the binding interface. The interactions at the antibody:antigen binding interface include hydrogen bonds, salt bridges and non-polar interactions. Residues in VLCDR1, VLCDR3 and VHCDR3 contribute to non-polar interactions including hydrophobic interactions. The contributions of charged residues involved in strong interface interactions are from VLCDR2 and VLFW3, including three salt-bridge interactions. Salt bridges are formed by IL-13:VLCDR2 interactions between Arg107 and Asp50, and Lys103 and Asp51. The core of the interface is characterised by these polar interactions, and they are shielded by non-polar interactions provided by VLCDR3 and VHCDR3 (Fig. 4). Shielding of these polar contacts excludes water molecules from the binding interface and lowers the dielectric constant. In total, the interface has 24 Pi interactions that are attractive but non-covalent, 15 hydrogen bonds and three salt-bridge interactions. The binding interface buries 2101 Å² [2]. The combined contribution of VHCDR3 and VLCDR2 makes up the biggest contribution; buried surface area is from VHCDR3, and its non-polar interactions and polar contacts are from VLCDR2.

A further factor in driving the binding is shape complementarity of the interacting surfaces that facilitate hydrophobic interactions. The topographic complementarity of the IL-13 and tralokinumab Fab surfaces is typical of antibody–antigen interactions

with a calculated shape complementarity statistic of 0.8. Published figures for antibody–antigen shape complementarity values range from 0.6 to 0.8 [20,21], where a surface complementarity of 1.0 signifies the interfaces with a geometrically perfect fit. This suggests that the interface between the tralokinumab Fab and IL-13 shows high surface complementarity.

Impact of tralokinumab mutations on binding to IL-13

The tralokinumab:IL-13 crystal structure clearly reveals key contacts in the binding interface, namely Lys103 and Arg107 on IL-13. Lys103 binds three residues on tralokinumab, Ser30, Lys31 and Asp51, creating a hydrogen bond network, and Arg107 binds Asp50 from tralokinumab, creating a salt bridge. Of note is that Asp50 is shielded by a number of hydrophobic interactions in the binding interface.

To understand the role these residues play in tralokinumab:IL-13 binding, we generated IgG1 alanine mutants of tralokinumab (Asp50Ala single mutant, and Ser30Ala, Lys31Ala and Asp51Ala triple mutant). These were tested in a competition assay to assess any potential impact on IL-13 binding to the IL-13 receptors (Fig. 5). The single and triple mutant variants greatly reduce the ability of tralokinumab to prevent IL-13 from interacting with either IL-13Rα1 or IL-13Rα2. To further understand the effect of the IgG1

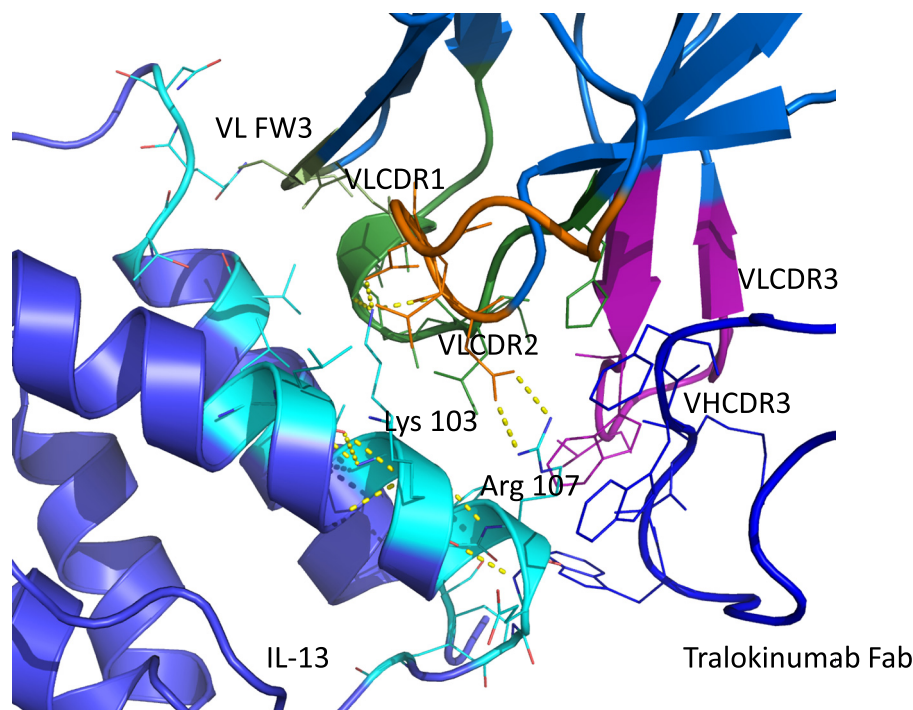


Fig. 4. Tralokinumab:IL-13 interaction interface showing key residues responsible for binding affinity. VLCDR1 is green, VLCDR2 is orange, VLFW3 is dark green, VLCDR3 is purple, and VHCDR3 is cyan. At the core of binding interface are Lys 103 and Arg 107. Lys 103 is coordinating three hydrogen bonds on the antibody between VLCDR1 and VLCDR2. Arg 107 is creating a salt bridge with Asp50 from VLCDR2 of the antibody.

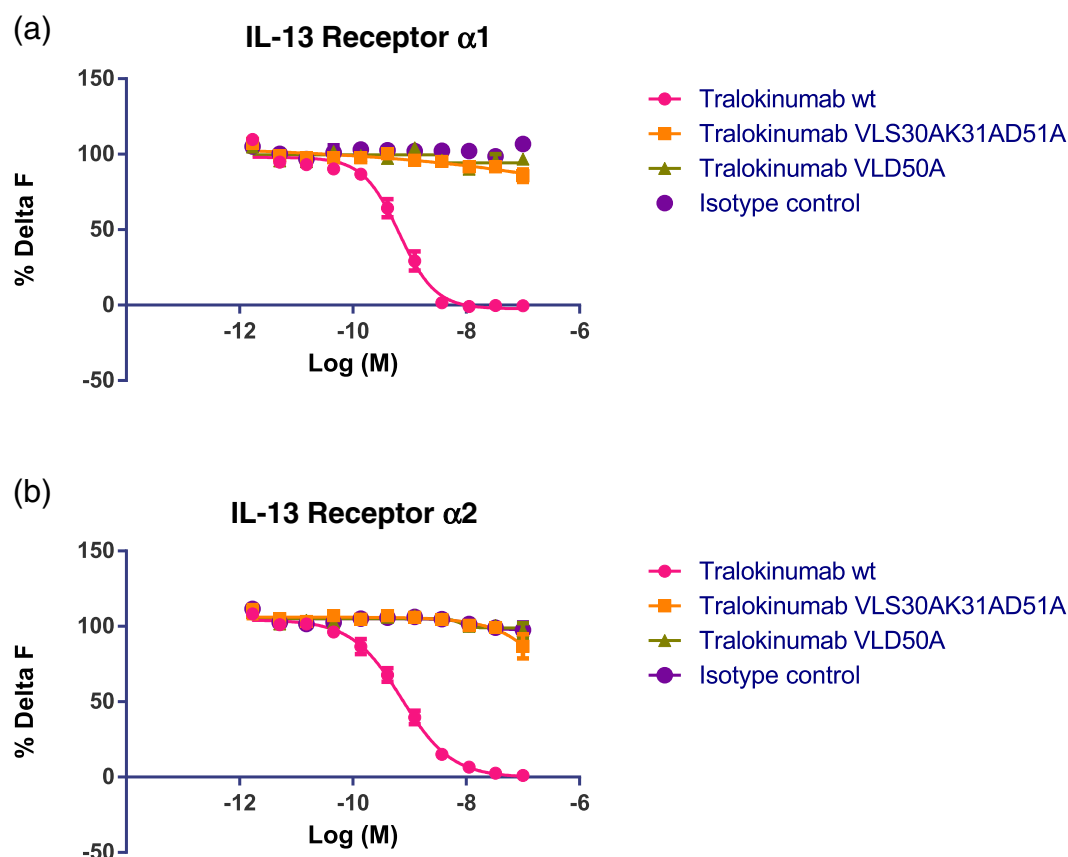


Fig. 5. Effects of tralokinumab and binding interface mutants on the binding of IL-13 to (a) IL-13R α 1 or (b) IL-13R α 2 using a receptor-ligand competition assay. Tralokinumab potently inhibits IL-13 binding to both IL-13R α 1 and IL-13R α 2, but the binding interface mutants show little or no activity. Data are shown as mean % DeltaF (standard error of the mean) from two independent experiments each performed in duplicate.

tralokinumab alanine mutations on the strength of binding, we determined the affinity of the tralokinumab variants for IL-13 using surface plasmon resonance (Table 2). The clinical candidate tralokinumab IgG4 has an affinity of 58 pM and tralokinumab IgG1 has an approximately equal affinity of 63 pM for IL-13. The single and triple mutant Tralokinumab IgG1 variants had an affinity for IL-13 of 2.7 μ M and 2.8 μ M, respectively. Taken together, the competition assay data and affinity measurements show that the key contact residues in tralokinumab identified by the crystal structure are driving the binding affinity for IL-13. Tralokinumab potently inhibits IL-13 binding to both IL-13R α 1 and IL-13R α 2, but the binding interface

mutants lose the ability to prevent IL-13 from interacting with either IL-13R α 1 or IL-13R α 2 at the concentrations tested.

Discussion

Tralokinumab is a phage display-derived antibody that specifically binds IL-13 with a 58 pM affinity (Table 2). In this study, we present the structure of tralokinumab Fab in complex with IL-13. The structure helps us to elucidate the epitope and paratope, the mechanism of action of tralokinumab and the prevention of IL-13 binding to both IL-13R α 1

Table 2. Biacore affinities of tralokinumab mutants for IL-13

	IgG captured (RUs)	k_a ($M^{-1} s^{-1}$)	k_d (s^{-1})	K_D	R_{max} (RUs)
VL S30A K31A D51A IgG1	134	3.6E+5	1.0	2.8 μ M	18.1
VL D50A IgG1	155	6.0E+5	1.6	2.7 μ M	7.75
Tralokinumab IgG1	145	5.2E+6	3.2E -4	63 pM	23.4
Tralokinumab IgG4	42	5.6E+6	3.2E -4	58 pM	7.5

and IL-13R α 2. Interestingly, the structure described here shows that the light chain of the antibody drives the interactions. This is somewhat different from that reported for previous analyses of antibody–antigen complexes [22] where the authors describe that VHCDR2 plays a central role in providing polar contacts for many antibody–antigen complexes. In contrast, we show here that the light chain CDR2 and heavy chain CDR3 are at the core of the binding interface mediating both polar and hydrophobic interactions with IL-13.

The data presented here suggest that tralokinumab inhibits the formation of the tertiary complex responsible for signalling among IL-13, IL-13R α 1, and IL-4R α . Furthermore, tralokinumab also inhibits complex formation between IL-13 and IL-13R α 2. When the structure of tralokinumab is overlaid with that of the IL-13 tertiary complex with IL-13R α 1 and IL-4R α [1] (Fig. 6), a significant steric clash is present between IL-13R α 1 and tralokinumab. It is clear that both these molecules cannot bind to IL-13 at the same time. The binding site for IL-4R α is sufficiently distant that it would not be expected to interfere with IL-13R α 1.

Previously [14], we reported an affinity of 164 pM for tralokinumab IgG4 (BAK1.1) that was amine coupled directly to the CM5 chip surface on a Biacore 2000. Using the same commercial source IL-13, we found that the updated 58 pM affinity

presented here is a result of deploying an optimised Protein G' IgG capture method on a more sensitive Biacore T100 instrument. By minimising IgG capture levels and performing antigen titrations at higher flow rate, mass transport effects were minimised. The resulting improvements in data quality allowed us to fit the whole dataset to the simplest 1:1 model.

There is a specific genetic variant of IL-13 that contributes to the risk of asthma, namely 2044A/G (amino acid R130 where the arginine residue is substituted with glutamine [Q130R]) [23–25]. In the structure described in this paper, we have investigated the location of this change in IL-13 with respect to the tralokinumab-binding site. The amino acid R130 is located at the C terminus of helix D (Fig. 7). As described earlier, helix D is part of the binding interface. However, the mutation at position R130 is located at the opposite side of helix D with respect to the side that engages antibody binding. The location of this variant in the crystal structure suggests that the polymorphism at this position would have minimal impact on tralokinumab binding this genetic variant of IL-13. This hypothesis is confirmed by previous data demonstrating that tralokinumab equipotently neutralises wild-type IL-13 and the Q130 variant IL-13 [15].

Aside from tralokinumab, there is another antibody targeting IL-13 in late-stage clinical development,

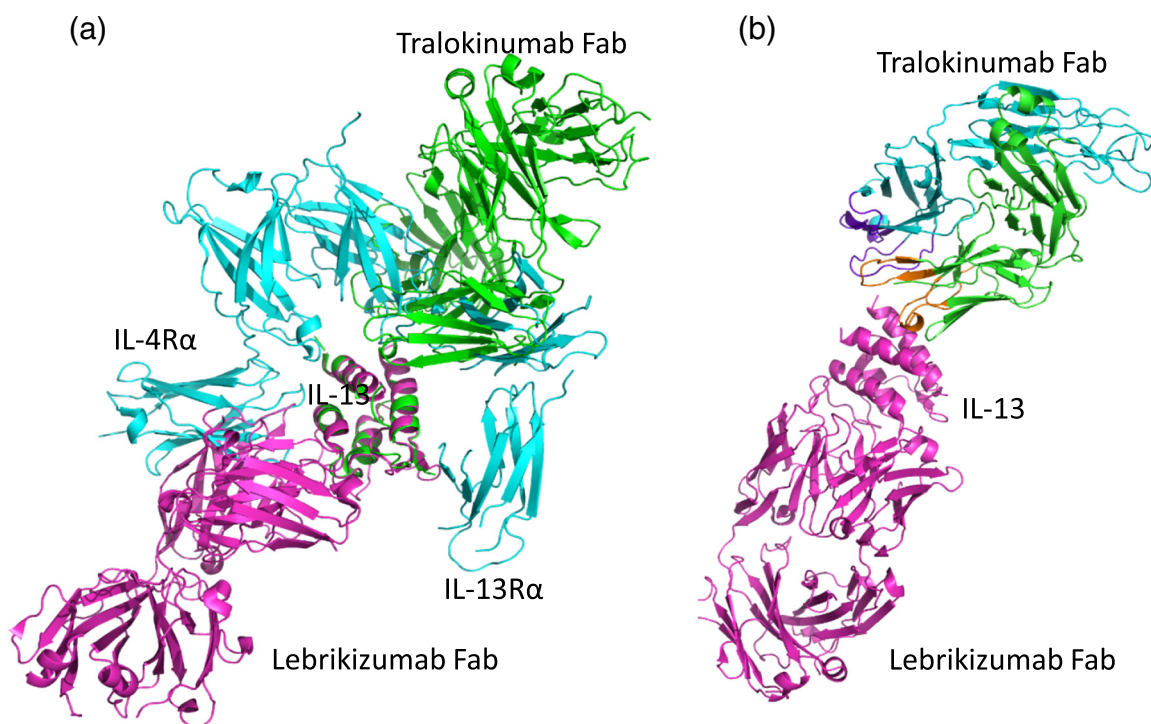


Fig. 6. Comparison of the tralokinumab and lebrikizumab binding sites on IL-13. (a) shows tertiary complex of IL-13, IL-13R α 1 and IL-4R α with tralokinumab and lebrikizumab. (b) shows the details of lebrikizumab binding as compared with tralokinumab on IL-13. The two antibodies bind on the opposite faces of IL-13 and have different mechanisms of action.

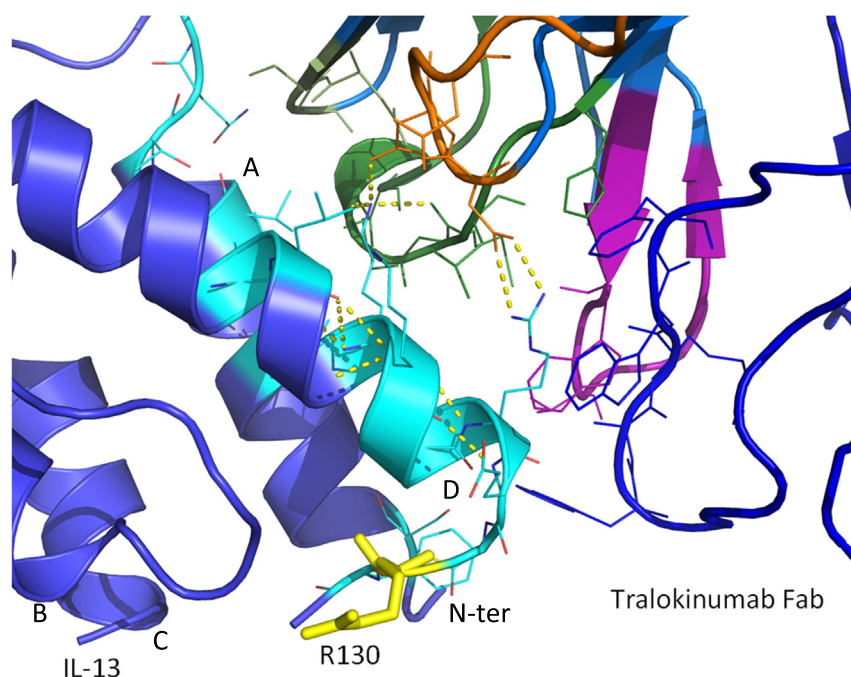


Fig. 7. The location of the IL-13 polymorphism 2044A/G (R130) in context of tralokinumab binding IL-13. The residues at the C terminus of helix D are unlikely to impact tralokinumab:IL-13 binding. VLCDR1 is green, VLCDR2 is orange, VLFW3 is dark green, VLCDR3 is purple, and VHCDR3 is cyan.

lebrikizumab (Genentech) [8,9]. The overall structure of the tralokinumab:IL-13 complex is very similar to other structures of IL-13 targeting antibodies in complex with IL-13 [16,17]. The mechanisms of binding and the nature of the interactions are very similar. For example, the binding surface in the lebrikizumab–IL-13 (PDB 4I77) interaction buries 1780 Å² that is comparable to the tralokinumab-binding interface that buries 2101 Å². However, epitopes for the two antibodies on IL-13 are distinct, and consequently, the mode of action of these antibodies is significantly different. As shown in Fig. 6, the two antibodies target two different epitopes on IL-13 and interfere with IL-13 signalling via different mechanisms. The binding epitope on lebrikizumab interferes with IL-4Rα binding [17], whereas tralokinumab inhibits signalling by blocking IL-13 binding to both IL-13Rα1 and IL-13Rα2. Both modes of action effectively prevent signalling through IL-13Rα1; the key difference is that tralokinumab, but not lebrikizumab, prevents IL-13 binding to IL-13Rα2. It is noteworthy that whilst tralokinumab and lebrikizumab neutralise IL-13 in fundamentally different ways, both have been reported to improve lung function and reduce the rate of asthma exacerbation in patients with uncontrolled severe asthma and evidence of type 2 inflammation [9,11]. Recently, airway-remodelling data following 1 year's treatment with tralokinumab were published (a sub-study from the 12-month efficacy study) [11]. Using quantitative computed tomography, Brightling *et al.* found that tralokinumab treatment was shown to increase airway lumen area and decrease

percentage wall area compared with placebo [26]. Whether these effects on airway remodelling were driven through IL-13Rα1 or IL-13Rα2 is not known. The literature characterises IL-13Rα2 either as a decoy receptor [3] with no identified function aside from acting as a “sink” removing IL-13 from circulation [5] or as a key driver of remodelling [6]. More extensive and longer clinical trials including airway remodelling endpoints and using both IL-13Rα2 blocking (e.g., tralokinumab) and IL-13Rα2 sparing (e.g., lebrikizumab) antibodies will be necessary to elucidate whether IL-13Rα2 plays an important role.

In summary, we have described the crystal structure of the tralokinumab Fab:IL-13 complex and elucidated key residues in the epitope and paratope that drive the protein:protein interaction. These data, alongside complementary biochemical interaction assay data, confirm that tralokinumab prevents IL-13 from interacting with both IL-13Rα1 and IL-13Rα2 and explain why tralokinumab inhibits IL-13 and the asthma-associated IL-13 coding variant Q130R.

Materials and Methods

Cloning of tralokinumab mutants

The QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent cat #210514) was used to introduce the following mutations into the tralokinumab

VL according to manufacturer's recommended conditions using the oligos below:

VL(S30A K31A D51A)

PrimerF1 5' GGAAACATCATTGGAGCTGCACTT
GTACACTGGTACC 3'.

PrimerF2 5' GGTCATCTATGATGCTGGCGACC
GGCCCTCAGGGATCC 3'.

The QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent cat #210518) was used to introduce the following mutations into the tralokinumab VL according to manufacturer's recommended conditions using the oligos below:

VLD50A

PrimerF3 5' CCTGTGCTGGTCATCTATGCTGAT
GGCGACCGGCC 3'.

PrimerR3 5' GGGCCGGTCGCCATCAGCATAGAT
GACCAGCACAGG 3'.

Transformed colonies were screened for the correct sequence using primers within the human lambda expression vector, and positive clones were grown overnight in 2×TY media supplemented with 100 mg/ml carbenicillin at 37 °C with shaking at 240 rpm. Plasmid DNA was prepared using Qiagen plasmid Plus maxi kit (25) (Agilent cat # 12963).

Clones were reconverted into human lambda antibody format to eliminate any non-specific mutations within the expression vector caused by the mutagenesis reaction. The conversion process is essentially as described previously [27] with the following modification: an OriP fragment was included in the expression vectors to facilitate the use with CHO- transient cells and to allow episomal replication.

Transfection methods

Transient transfection was carried out as outlined previously [28] with a temperature shift at 34 °C 4 h post transfection.

Purification methods

IgGs were purified from culture supernatant using an AKTApur (GE healthcare) and 1 ml MabSelect Sure columns (GE healthcare, cat #11-0034-93). IgGs were eluted from the columns using 0.1 M sodium citrate (pH 3.0) and buffer exchanged into phosphate-buffered saline (PBS) (Live Technologies, Carlsbad, CA; 14190) using PD10 columns (GE healthcare, cat #17-0851-01). The concentration of the antibodies was determined spectrophotometrically using extinction coefficients based upon the individual amino acid sequences of the IgGs. Full quality control was performed including SDS-PAGE

and high-performance liquid chromatography–size-exclusion chromatography (HPLC-SEC).

Fab generation, purification, and complex formation for X-ray crystallography

In order to produce the tralokinumab Fab, the full IgG4 antibody [14] was digested enzymatically. The antibody was diluted to a concentration of 10 mg/ml in PBS (pH 7.2) and DL-cysteine hydrochloride (Sigma-Aldrich, cat #C8256) added to a final concentration of 30 mM. Papain (Sigma-Aldrich, cat #P4762) was activated by preparing the Papain at a concentration of 10 mg/ml in a 30 mM DL-cysteine hydrochloride digest buffer.

A reaction was set up by adding 0.5 mg of the activated Papain to each 100 mg of the tralokinumab IgG to be digested. Digestion progress was monitored by HPLC-SEC until the level of intact antibody remaining was between 10% and 20% before the reaction was stopped using E64 solution (Sigma-Aldrich, cat #E3132) at a concentration of 1 mg/ml.

To purify the Fab, we loaded the reaction mixture onto a pre-equilibrated 4 ml Protein A column (Amersham Biosciences #DSPM 00121) at a flow rate of 300 cm/h in order to bind any Fc fragments or undigested antibody. The flow through containing the Fab was collected and polished by size exclusion using a preparation grade Superdex 75 16/60 column (Amersham Biosciences, cat #17-1043-10). Confirmation of the target protein was determined by SDS-PAGE and HPLC-SEC analyses with concentration determined spectrophotometrically.

Purified recombinant human IL-13 was sourced from R&D Systems, (cat #213-ILB/CF). Optimal conditions for complex formation between the tralokinumab Fab and IL-13 were determined to allow the complete separation of the intact complex from any remaining, non-complexed Fab. Complex reactions were monitored by HPLC-SEC. The final complex reaction was set up using a Fab to antigen molar ratio of 1:1.4 and incubated at room temperature for 1 h. The resulting complex was then purified by size exclusion using a HiLoad 16/600 Superdex 75 pg column (GE Healthcare, cat #28989333) and an isocratic elution with a flow rate of 0.15 ml/min. Eluted fractions were analysed by HPLC-SEC and SDS-PAGE.

Fractions were selected by purity of the complex and pooled and concentrated to a final concentration of 28.61 mg/ml in order for X-ray crystallography studies to be performed.

Crystallisation

Protein, prepared as described above, was subject to crystallisation screening by sitting drop vapour diffusion method using both in-house and commercial screens at 277 K and 293 K. Crystals suitable

for diffraction studies were obtained by introducing a seed stock derived from crystal clusters grown at 3.5–4 M formate concentrations into pre-equilibrated drops over a reservoir of 2.0–2.5 M sodium formate at 293 K. Data from a number of crystals were collected, merged and scaled. The best crystals from across the range were used. Single needle-shaped crystals grow to approximately 700 μm in length over 3–4 days. Crystals were cryoprotected in 7 M sodium formate and then frozen directly into liquid nitrogen.

Crystal structure determination

Data were collected on beam line i03 of the Diamond Light Source synchrotron (Harwell Science and Innovation Campus in Oxfordshire). Data from several crystals were scaled and merged using XDS to give a dataset in P1 complete to 2 Å. Data from several crystals were scaled and merged to give a dataset in P1 complete to 2 Å. The structure was solved by molecular replacement using IL-13 from PDB entry 3L5X and a Fab variable region (CDR loops truncated) and constant region from a previously solved in-house Fab structure. Model building and refinement were completed using Phaser, Coot and Buster software that gave a final model with agreement factors R of 0.189 and R_{free} of 0.211 [29–34]. Ramachandran plot was created within CCP4 using default parameters. For structural comparison with lebrikizumab, we used PDB 4I77 [17].

Affinity measurements

Experiments were performed on a Biacore T100. The instrument was controlled and the resulting data were evaluated using Biacore T100 control and evaluation software version 2.0.1. CM5 S-Series biosensor chips, amine coupling kits, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffered saline based buffer concentrates, and regeneration buffers were from GE Healthcare and were used according to the manufacturer's instructions.

Biosensor affinity measurements were performed using protein G'-mediated capture immobilisation of the human antibodies, and the recombinant human IL-13 was flowed (analyte). Recombinant protein G' was from Sigma (P-4689) and human IL-13 (PeproTech). Preparation of protein G' surfaces on a CM5 chip was performed essentially as previously described [35]. For the protein G' capture surface, the Sigma recombinant protein G' was reconstituted in water and buffer exchanged into Dulbecco's PBS via a PD-10 column (GE Healthcare) to remove Tris. This protein G' was further diluted into 10 mM sodium acetate (pH 3.6) and amine coupled to the chip.

During each analysis cycle, after titration of between 42 and 155 reference units of IgG onto the protein G' surface at 5 $\mu\text{L min}^{-1}$, the human

IL-13 (PeproTech) binding analysis was then performed at a higher 50 $\mu\text{L min}^{-1}$ flow rate with an association time of 5 min. Depending on the strength of the interaction, dissociation was followed for 10–30 min. Regeneration of protein G' surfaces was performed with two consecutive 20-s injections of 6 M guanidinium hydrochloride in Dulbecco's PBS.

Homogeneous time resolved fluorescence (HTRF) assay

All HTRF experiments were performed in 384-well white shallow well nonbinding plates (Corning Incorporated, Corning, NY; 4513) in assay buffer containing PBS, 0.1% (vol/vol) bovine serum albumin (Sigma, St. Louis, MO; A9576), and 0.4 M potassium fluoride (VWR International, Radnor, PA; 26820). Time-resolved fluorescence at 590 and 665 nm was measured following excitation at 320 nm on an Envision plate reader (PerkinElmer, Waltham, MA) after the indicated incubation periods. Ratio values ($[\text{665 nm emission}/\text{590 nm emission}] \times 10,000$) were used to calculate % Delta F according to the following equation:

$$\text{Delta F} = \frac{([\text{sample ratio} - \text{negative control ratio}])}{[\text{negative control ratio}]} \times 100.$$

Curves were analysed and IC_{50} values determined using GraphPad Prism software (GraphPad, La Jolla, CA) using a four-parameter logistic curve-fitting equation.

Ligand–receptor IC_{50} determination assay

Recombinant IL-13R α 1 and IL-13R α 2 (R&D Systems Europe, Abingdon, UK) were labelled using a DyLight 650 microscale antibody labelling kit (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. Test and control antibodies were serially diluted into HTRF assay buffer to give an 11-point, 1-in-3 serial titration of sample. Then, 2.5 μL of each dilution point was added in duplicate to the assay plate with 10 nM DyLight650-labelled IL-13R α 1 or 2.5 nM DyLight650-labelled IL-13R α 2 and Flag-tagged human IL-13 at 0.6 nM for the R α 1 assay or 0.05 nM for the R α 2 assay and 0.5 nM anti-Flag europium cryptate (Cisbio, Codolet, France) in a total assay volume of 10 μL . For the determination of negative 665/590 nm ratio, control wells containing DyLight650-labelled IL-13 receptor and anti-Flag europium cryptate only were also analysed.

Accession numbers

The coordinates and structure factors of tralokinumab Fab in complex with IL-13 have been deposited in the PDB with accession number **5L6Y**.

Acknowledgements

We would like to thank Lauren Smith, BA (QXV Comms, an Ashfield Business, part of UDG Healthcare plc, Macclesfield, UK), who provided editing assistance funded by AstraZeneca.

Received 12 October 2016;

Received in revised form 2 December 2016;

Accepted 5 December 2016

Available online xxxx

Keywords:

tralokinumab;
crystal structure;
antibody–antigen complex;
mutagenesis;
antibody structure

Abbreviations used:

IL, interleukin; Th2, T helper type 2; PDB, Protein Data Bank; VH, variable heavy; VL, variable light region; CDR, complementary-determining region; FW, framework; PBS, phosphate-buffered saline; HPLC-SEC, high-performance liquid chromatography–size-exclusion chromatography; HTRF, homogeneous time resolved fluorescence.

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