

Brief Communication



Higher Binding Affinity and *In Vitro* Potency of Reslizumab for Interleukin-5 Compared With Mepolizumab

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ABSTRACT

Reslizumab and mepolizumab are recently approved monoclonal antibodies for the treatment of severe (uncontrolled) eosinophilic asthma. Both are effective in neutralizing the function of interleukin-5 (IL-5). This study is the first to compare the binding affinity and *in vitro* potency of both antibodies in head-to-head assays. Two assays assessed binding affinity (using the equilibrium dissociation constant [K_D]) of each drug for human IL-5. In the Biacore surface plasmon resonance assay, the association constant (k_{on}) values for human IL-5 for reslizumab and mepolizumab were 3.93×10^6 and 1.83×10^5 , respectively. The dissociation constant (k_{off}) values were 4.29×10^{-4} and 2.14×10^{-4} , respectively. Calculated K_D values for human IL-5 for reslizumab and mepolizumab were 109 and 1,170 pM, respectively, representing an approximately 11-fold stronger binding affinity with reslizumab. In the Kinetic Exclusion Assay, the k_{on} values for human IL-5 for reslizumab and mepolizumab were 3.17×10^6 and 1.32×10^5 , respectively. The k_{off} values were 1.36×10^{-5} and 1.48×10^{-5} , respectively. Measured K_D values for human IL-5 for reslizumab and mepolizumab were 4.3 and 112 pM, respectively, representing an approximately 26-fold stronger binding affinity for reslizumab. A human-IL-5-dependent cell proliferation assay was developed to assess *in vitro* potency, based on a human cell line selected for enhanced surface expression of IL-5 receptor-alpha and consistent proliferation response to IL-5. The concentration at which 50% inhibition occurred (IC_{50}) was determined for both antibodies. Reslizumab and mepolizumab inhibited IL-5-dependent cell proliferation, with IC_{50} values of approximately 91.1 and 286.5 pM, respectively, representing on average 3.1-fold higher potency with reslizumab. In conclusion, comparative assays show that reslizumab has higher affinity binding for and *in vitro* potency against human IL-5 compared with mepolizumab. However, these results do not take into consideration the different methods of administration of reslizumab and mepolizumab.

Keywords: Reslizumab; mepolizumab; antibody affinity; interleukin-5; drug evaluation, preclinical

Laurie Pukac <https://orcid.org/0000-0001-5767-2172>Jacquelyn Lyons <https://orcid.org/0000-0003-0437-6307>**Disclosure**

There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Approximately 20%–32% of patients with difficult-to-treat and severe asthma have persistent eosinophilia in sputum and elevated blood eosinophil levels.¹ Patients with eosinophilic asthma usually have adult-onset disease, a good response to systemic corticosteroids, poor lung function, and a high risk of severe exacerbations,² with worse severity and poorer asthma control at higher levels of blood eosinophils.³

Interleukin-5 (IL-5) is the key mediator of eosinophil differentiation, migration, activation and survival, which has led to the development of therapies targeted to IL-5 for the treatment of eosinophilic asthma.^{4–6} Reslizumab and mepolizumab are both humanized monoclonal antibodies that target IL-5.^{7,8} Reslizumab is approved as add-on maintenance therapy in patients aged ≥ 18 years with severe uncontrolled eosinophilic asthma and elevated eosinophil levels.⁹ Mepolizumab has a similar indication in patients aged ≥ 12 years in the US or in adults in the EU.¹⁰ Findings from randomized, placebo-controlled trials demonstrated that reslizumab¹¹ and mepolizumab^{12,13} are effective in reducing the risk of asthma exacerbation in patients with eosinophilic asthma; however, to date there is limited direct comparative information on the 2 agents.¹⁴

The aim of the current study was to investigate the binding affinity of reslizumab and mepolizumab for human IL-5, and the *in vitro* potency of the 2 agents for suppressing cell proliferation in response to human IL-5 in the same assay systems. These methods, therefore, allow for head-to-head, non-clinical comparison of reslizumab and mepolizumab.

MATERIALS AND METHODS

Sources of antibodies

All studies were conducted with drug product antibody. Reslizumab was manufactured by Teva Pharmaceutical Co., Sydney, Australia. Mepolizumab (Nucala) was purchased from GlaxoSmithKline.

Binding affinity assays

Two assays were used to assess binding affinity of anti-IL5 antibodies for human IL-5. The first method used the Biacore™ T200 system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The system was equilibrated to 30°C prior to performing experiments. Approximately 50 response units (RU) of each test antibody and isotype control antibody were captured on a Biacore Series S sensor chip Protein A (GE Healthcare Bio-Sciences AB), and two-fold serial dilutions (beginning at 10 $\mu\text{g}/\text{mL}$) of recombinant human IL-5 (in running buffer: 1 \times HBS-EP+, GE Healthcare Bio-Sciences AB) were injected at a flow rate of 60 $\mu\text{L}/\text{min}$ for 70 seconds and were allowed to dissociate in running buffer for 300 seconds. Sensorgrams were fitted to a global 1:1 Langmuir binding model with the maximal capacity (R_{max}) fitted locally using Biacore T200 Evaluation Software (version 3.1; GE Healthcare Life Sciences, Chicago, IL, USA). Data were double referenced by subtracting both isotype and buffer blanks. Binding affinity (using the equilibrium dissociation constant [K_D]) was calculated by dividing the dissociation constant by the association constant ($k_{\text{off}}/k_{\text{on}}$).

The second method used the Kinetic Exclusion Assay (KinExA™) 3200 system (Sapidyne Instruments, Boise, ID, USA), in which the reaction components are combined and allowed

to reach equilibrium prior to affinity measurement. As a first step, the IL-5 k_{on} rate was measured for both antibodies to determine the length of time required to reach equilibrium. Solutions were prepared using serially diluted human IL-5 and 3 concentrations of each antibody (reslizumab: 2.5, 12.5 and 25 pM; mepolizumab: 50, 250 and 500 pM); each was incubated for the time determined in the first step to reach equilibrium. Concentrations above and below the expected K_D for each molecule were selected to provide a full concentration response and enable direct comparison of the antibodies. The n-curve analysis tool within the KinExA™ Pro Software version 4.2.10 was used to integrate the data from the 3 equilibrium curves to obtain one K_D value for each experiment by finding the least squares best fit of a 1:1 binding model to the data. The k_{off} rate was estimated by multiplying the K_D value with the k_{on} rate.

Cell proliferation assay

A luminescent cell proliferation potency assay was developed for the assessment of antibodies which bind to and neutralize IL-5 activity. The assay utilized a sub-clone from a human erythroleukemic TF-1 cell line, which was selected to enhance the expression of the IL-5 receptor-alpha on the cell surface. These cells respond to human IL-5 with increased cell proliferation. Cells were prepared for a proliferation assay by withdrawing cytokines for 16–20 hours. Prior to the assay, cultured cells were washed, centrifuged and resuspended in assay media to achieve a final concentration of 1×10^6 viable cells/mL. The assay media consisted of RPMI-1640 (Life Technologies, Paisley, UK), 10% fetal bovine serum (AusGeneX, Gold Coast, Australia), 2 mM L-glutamine (Life Technologies), 100 U/mL penicillin-streptomycin (Life Technologies), and 55 μ M β -mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA). A total of 50 μ L of this cell suspension and 25 μ L of human IL-5 at a constant concentration was added to each well of a 96-well assay plate and pre-incubated at 37°C, 5% CO₂ for 30–45 minutes. Following pre-incubation, 25 μ L of serially diluted antibodies were added to each well of assay plates containing cells and cytokine in 2 split replicates. Plates were incubated in a humidified 37°C/5% CO₂ incubator for approximately 48 hours.

The number of viable cells was assessed using CellTiter-Glo® 2.0 Reagent (Promega Corporation, Madison, WI, USA). Assay plates were incubated in minimal light at room temperature for approximately 10 minutes. Luminescence was then assessed using a GloMax® 96 Microplate Luminometer (Promega Corporation). Two independent assays were performed. All proliferation and inhibition curves were fitted using a 4-parameter dose-response curve using SoftMax® Pro version 5.3 (Molecular Devices Corporation, Sunnyvale, CA, USA). The concentration at which 50% inhibition occurred (IC_{50}) was determined for both antibodies.

RESULTS

Binding affinity and kinetics

The measured k_{on} rates showed remarkable similarity in the 2 assay formats for both antibodies. The average k_{on} values for reslizumab were 3.93×10^6 and 3.17×10^6 , and for mepolizumab were 1.83×10^5 and 1.32×10^5 using the Biacore assay (**Table 1**) and KinExA™ (**Table 2**), respectively. This demonstrates an approximately 20-fold higher k_{on} rate for IL-5 with reslizumab compared with mepolizumab. In the Biacore assay, the more rapid binding to IL-5 for reslizumab compared with mepolizumab is clearly observed in the sensorgrams (**Supplementary Fig. S1**).

Table 1. Summary of binding affinity and kinetics for reslizumab and mepolizumab with the Biacore assay

Repeat	k_{on} (1/Ms)		k_{off} (1/s)		K_D (pM)		Chi ² (RU ²)	
	Reslizumab	Mepolizumab	Reslizumab	Mepolizumab	Reslizumab	Mepolizumab	Reslizumab	Mepolizumab
1	3.7×10^6	1.83×10^5	3.92×10^{-4}	2.17×10^{-4}	106	1,180	0.022	0.015
2	4.16×10^6	1.82×10^5	4.65×10^{-4}	2.11×10^{-4}	112	1,160	0.032	0.017
Average	3.93×10^6	1.83×10^5	4.29×10^{-4}	2.14×10^{-4}	109	1,170	N/A	N/A
SD	0.33×10^6	0.01×10^5	0.52×10^{-4}	0.04×10^{-4}	4.2	14.1	N/A	N/A

Two independent experimental repeats were performed for each antibody (all data is shown in the table). The χ^2 values (≤ 0.032) from the global sensorgram curve fits used to calculate K_D values indicate high confidence.

K_D , equilibrium dissociation constant (binding affinity); k_{off} , dissociation rate constant; k_{on} , association rate constant; N/A, not applicable.

Table 2. Summary of binding affinity and kinetics for reslizumab and mepolizumab with the KinExA™ assay

Repeat	k_{on} (1/Ms)		K_D (pM)		Residual Error (%) on K_D	
	Reslizumab	Mepolizumab	Reslizumab	Mepolizumab	Reslizumab	Mepolizumab
1	2.50×10^6	1.90×10^5	3.1	104	1.3	2.6
2	3.90×10^6	1.03×10^5	4.9	111	2.0	2.9
3	3.10×10^6	1.02×10^5	5.2	71	2.0	2.4
4	N/A	N/A	3.9	163	2.4	4.0
Average	3.17×10^6	1.32×10^5	4.3	112	N/A	N/A
SD	0.70×10^6	0.51×10^5	0.9	38	N/A	N/A

Four independent experimental repeats were performed for each antibody (all data is shown in the table). The residual errors for the least squares best fit of the binding model to the data within a 95% confidence interval are shown for the K_D .

K_D , equilibrium dissociation constant (binding affinity); k_{on} , association rate constant; N/A, not applicable; SD, standard deviation.

With the Biacore assay, the average k_{off} values for the 2 agents were: 4.29×10^{-4} with reslizumab and 2.14×10^{-4} with mepolizumab (**Table 1**). With KinExA™, the average k_{off} value was calculated to be 1.36×10^{-5} with reslizumab and 1.48×10^{-5} with mepolizumab.

The average K_D values for human IL-5 were 109 pM with reslizumab and 1,170 pM with mepolizumab as measured by the Biacore assay (**Table 1**); they were 4.3 pM with reslizumab and 112 pM with mepolizumab as measured by KinExA™ (**Table 2**). This difference in binding affinity between antibodies was also clearly evident by the shift in concentration curves with KinExA™ (**Supplementary Fig. S2**).

Cell proliferation assay

Reslizumab was approximately 3.1-fold more potent than mepolizumab to inhibit IL-5-dependent cell proliferation (**Figure**). The average IC_{50} values were 91.1 pM for reslizumab (90.9 pM in experiment 1 and 91.3 pM in experiment 2) compared with 286.5 pM with mepolizumab (315 pM in experiment 1 and 258 pM in experiment 2).

DISCUSSION

This study is the first to show in head-to-head assays that reslizumab has higher binding affinity for and greater *in vitro* potency against human IL-5 compared with mepolizumab.

Two different assays, Biacore and KinExA™, were used to accurately determine the binding affinity and kinetics of reslizumab and mepolizumab. Within both assays, findings between repeats for each parameter were similar, supporting the robustness and reproducibility of each assay. In addition, consistent findings, in terms of faster and stronger binding affinity with reslizumab compared with mepolizumab, were seen using the 2 different assays. However, k_{off} values with KinExA™ were approximately 10-fold lower than what was determined in the Biacore assay. The difference in k_{off} rates between the 2 technologies may

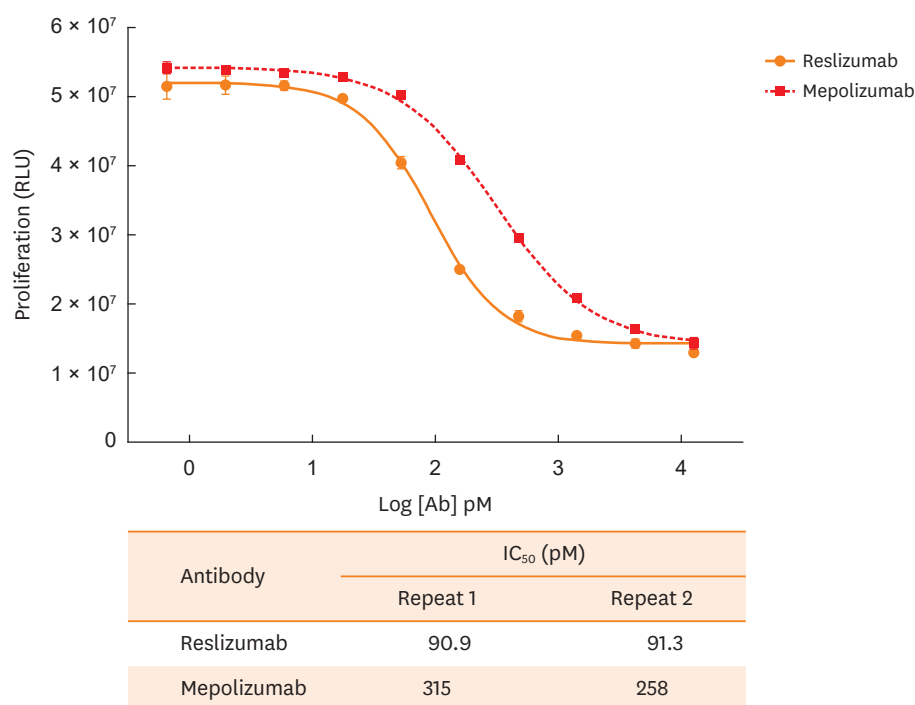


Figure. Inhibition of IL-5-induced proliferation by reslizumab and mepolizumab in the presence of 45 pM human IL-5. Two independent experimental replicates were made for each antibody and the fits for the calculated IC₅₀ values showed high R² values (> 0.99) in each replicate, indicating high confidence. Data shown for the dose-response curves were from a single representative experiment. IC₅₀, concentration at which 50% inhibition occurred; IL-5, interleukin-5.

be attributed to the use of a soluble platform with KinExA™ compared with a surface-based platform with the Biacore assay.¹⁵ In addition, with the Biacore assay the k_{off} is a measured value, while with KinExA™ it is calculated using the measured K_D and k_{on} rates.

The higher binding affinity of reslizumab for IL-5 is predominantly due to the more rapid antigen binding (approximately 20-fold higher k_{on} rates with reslizumab compared with mepolizumab in both assay formats). A recent population pharmacokinetic study with weight-based dosing of intravenously administered reslizumab reported a steady-state serum concentration of 45 µg/mL in patients of different body weights.¹⁶ This concentration corresponds to approximately 300 nM antibody in the serum, *i.e.*, approximately 66,700-fold higher than the serum concentration of IL-5 (physiological IL-5 serum concentrations are approximately 150 pg/mL [4.5 pM] in severe asthmatics).¹⁷ Thus, for both reslizumab and mepolizumab, based on the affinity constants from our study and likely serum IL-5 concentrations, all available serum IL-5 is likely to be bound by antibody, thereby maximizing the potential therapeutic effect. Further theoretical benefit derived from the high k_{on} rate with reslizumab may potentially result from the blocking of the IL-5 autocrine loop. It has previously been demonstrated that eosinophils from patients with asthma release IL-5 in an autocrine system, in which the intracellular IL-5 protein synthesized and released by eosinophils is upregulated by the IL-5 alpha-receptors of circulating eosinophils, thereby preventing apoptosis.¹⁸ In viral infections, faster k_{on} rates of monoclonal antibodies are associated with better viral neutralizing activity by decreasing the time the virus has to infect another cell before it is bound by neutralising antibody.¹⁹ Similarly, in an IL-5 driven autocrine loop it is possible that the faster k_{on} rate of reslizumab (21.5-fold and 24-fold faster in the

Biacore and KinExA™ assays, respectively, compared with mepolizumab) could decrease the time that the IL-5 has to stimulate a nearby cell and protect it from apoptosis before it is bound and neutralized by reslizumab.

The cell proliferation assay demonstrated a 3.1-fold higher potency for IL-5 inhibition with reslizumab than with mepolizumab. The measure of biological activity and potency using a suitably quantitative biological assay should be based on the attribute of the product which is linked to the relevant biological properties and mechanisms of action.²⁰ We therefore identified the cell proliferation assay as a suitable biological assay to determine the inhibition of IL-5-dependent cell proliferation using reslizumab and mepolizumab as the consistent proliferation response of the TF-1 cell line to IL-5 is analogous to eosinophils.

Both reslizumab and mepolizumab have been shown to provide significant clinical benefit in patients with severe eosinophilic asthma compared with placebo^{11,13,21-23}; however, there have been no direct clinical or PK comparisons of these antibodies to date. As the study designs and study populations in the 2 phase III programs with these drugs are different, it is not appropriate to directly compare the efficacy and safety data. However, an indirect network analysis of randomized, placebo-controlled studies has shown therapeutic effects in favor of reslizumab, such as reduced exacerbations and improved lung function.¹⁴ Although the exact mechanisms for these potential treatment differences are unknown, our *in vitro* data suggest that stronger IL-5 binding and more potent inhibition of IL-5 activity by reslizumab may, in part, contribute. However, this needs to be confirmed with clinical studies.

In conclusion, this head-to-head study involving comparative assays shows that, in these assays, reslizumab has higher binding affinity for human IL-5 and higher potency to suppress cell proliferation in response to human IL-5 compared with mepolizumab.

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SUPPLEMENTARY MATERIALS

Supplementary Fig. S1

Biacore assay sensorgrams from a single experiment showing binding of human IL-5 at a range of concentrations to (A) reslizumab and (B) mepolizumab. Two independent experimental replicates were performed for each antibody. All sensorgrams showing a sufficient reference-subtracted signal (≥ 1 RU; 10 IL-5 concentrations for reslizumab and top 7 IL-5 concentrations for mepolizumab) were used to calculate kinetic constants. Fits to a 1:1 Langmuir binding model are shown as dotted lines over measured data (coloured lines). The χ^2 values (≤ 0.032) from the global sensorgram curve fits used to calculate K_D values indicate high confidence.

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Supplementary Fig. S2

KinExA™ concentration curves for the binding affinity of IL-5 with reslizumab and mepolizumab. Four independent experimental replicates were performed for each antibody. The residual errors for the least squares best fit of the binding model to the data within a 95% confidence interval for the effective K_D were all $\leq 4\%$ (see **Table 2** for individual values), indicating high confidence. Data shown are from a single representative experiment.

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