Bertilimumab Cambridge Antibody Technology Group
Changhai Ding1,2*, Jun Li2 & Xuejue Zhang2

Addresses
1Menzies Research Institute
University of Tasmania
Hobart 7000
Australia
Email: changhai.ding@utas.edu.au
2Anhui Medical University
Hefei 230032
China
*To whom correspondence should be addressed

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Cambridge Antibody Technology (CAT) is developing bertilimumab, an anti-eotaxin-1 monoclonal antibody, for the potential treatment of allergic disorders. By September 2003, CAT had released results from phase I/II trials, and was seeking to outlicense rights for bertilimumab.

Introduction
The chemokine eotaxin-1 is an 8.4-kDa protein that was initially purified from the bronchoalveolar lavage fluid of allergen-challenged sensitized guinea pigs [557608], [557609]. This 74-amino-acid protein can be produced by a variety of cell types including eosinophils, epithelial cells, fibroblasts, endothelial cells, T-lymphocytes, monocytes and macrophages, after induction by pro-inflammatory mediators such as tumor necrosis factor (TNF)α, interferon, interleukin (IL)-1, IL-13 and IL-4 [390207], [557614], [557616], [561366]. The release of eotaxin-1 from these cells is thought to contribute to the local accumulation of eosinophils in inflammatory conditions [381281]. The marked accumulation of eosinophils, a condition known as eosinophilia, can occur in many disorders such as asthma, allergic rhinitis and conjunctivitis. Eotaxin-1 levels are significantly with eosinophil counts [557629]. Therefore, therapies involving the suppression of eotaxin-1 function, which can inhibit the recruitment of eosinophils to sites of inflammation, may constitute a new therapeutic strategy.

Eotaxin-1 binds with high affinity to Cys-Cys chemokine receptor 3 (CCR3), which is expressed on cells such as eosinophils, basophils, mast cells, dendritic cells and T-helper type 2 (Th2) cells [557631], [557632], [557633]; therefore, these cells constitute the effector cells for eotaxin-1. On binding to eosinophilic CCR3, eotaxin-1 causes intracellular calcium mobilization, initiation of intracellular actin polymerization, upregulation of integrin expression and the induction of oxygen radical production [557634], [557635].

Bertilimumab (CAT-213) is a human immunoglobulin (Ig)G4 monoclonal antibody against eotaxin-1 that effectively and specifically inhibits its function, and is under development by Cambridge Antibody Technology (CAT) for the potential treatment of allergic disorders such as asthma, allergic rhinitis and conjunctivitis [367167], [398555].

Synthesis and SAR
The production of bertilimumab was described in detail in the patent application that first disclosed the antibody [WO-00166754]. Single-chain fragment variables (scFvs) specific to eotaxin-1 were isolated in three rounds of selection from a B-lymphocyte-derived phage display library. Only four distinct scFvs were identified by phage Enzyme Linked ImmunoSorbent Assay (ELISA), reflecting the relative difficulty in isolating scFvs specific to eotaxin-1, compared with other antigens. The clone identified for further characterization (named 3G3) showed low-to-moderate potency (IC50 value of 800 nM) in an eotaxin-1-mediated chemotaxis assay, making this antibody relatively unsuitable for therapeutic application [398555].

 Modifications were made to 3G3 to produce the scFv-derived antibody CAT-212, and its DNA sequence was amplified by PCR, and V<sub>H</sub> and V<sub>L</sub> gene sequences added. The V<sub>H</sub> and V<sub>L</sub> DNA fragments were digested in parallel with their acceptor vectors (HindIII/ApaI and BstBI/BsiWI, respectively), and fragments were ligated to construct the
Preclinical Development

Bertilimumab is highly specific to human eotaxin-1 and does not bind to a number of other chemokines and cytokines, including eotaxin-2 and -3 (both of which have only approximately 37% homology with eotaxin-1, and approximately 10-fold lower potency at CCR3), monocyte chemotactic proteins (MCPs) 1 to 4 or TNFα. [WO-00166754]. Bertilimumab (0.01, 0.1, 1 and 10 mg/kg) received 30 min prior to ipo injection of human eotaxin (1 µg) in the same mouse model. A significant dose-dependent inhibition of eosinophil recruitment (46, 73, 79 and 91%, respectively) was observed relative to non-specific control antibody treatment, and eosinophilia was blocked at 6 h. At the above doses, the chemotaxis of mononuclear cells was inhibited by 7, 49, 101 and 156%, respectively, and that of neutrophils by 9, 37, 41 and 61%, respectively, relative to null control [WO-00166754]. Thus, bertilimumab is approximately 10-fold more potent when administered locally (ipo) than systemically (iv), although systemic (but not local) administration has the ability to block neutrophil influx.

The effects of bertilimumab on the recruitment of eosinophils were also tested in vivo in cynomolgus monkeys. Local intradermal (10 µg) or systemic (20 and 100 mg/kg iv) administration of bertilimumab inhibited human eotaxin-1-mediated recruitment of eosinophils, and the higher intravenous dose inhibited IL-13-induced cell recruitment to the dermis [543999].

Metabolism and Pharmacokinetics

The pharmacokinetics of bertilimumab were assessed in an ascending single dose, single-blind study in healthy male volunteers (n = 25) [471969]. Treatment groups received bertilimumab (0.01, 0.1, 1, 5 and 10 mg/kg) or placebo, infused intravenously over 30 min. While 0.01 mg/kg of bertilimumab could not be detected in serum at any time after infusion, the antibody remained present in serum in an active form for over 60 days after 5 and 10 mg/kg infusions. Following doses of 0.1, 1, 5 and 10 mg/kg, pharmacokinetic parameters were, respectively, as follows: C_{max} = 21, 24.6, 122.7 and 291.9 mg/kg; volume of distribution = 58.3, 138.1, 277.8 and 220.9 ml/kg; and terminal t_{1/2} = 0.6, 2.1, 8.5 and 8.4 days. The observed t_{1/2} values for the lower doses of bertilimumab were short, possibly because concentrations decrease to an undetectable level before the terminal phase is established [471969].

Toxicology

The systemic toxicity of bertilimumab was evaluated in rhesus monkeys. After single doses (10 or 100 mg/kg) and repeat doses (10, 30 or 100 mg/kg), twice weekly for 4 weeks, no target organ or non-specific toxicology was observed. As a preliminary for the treatment of ocular indications, the ocular tolerance of bertilimumab was studied in the rabbit. Repeat doses of bertilimumab (0.1 ml or 10 mg/ml tid) were well tolerated when administered locally to the eye for 7 days [559592].

Clinical Development

Phase I

An initial tolerance and pharmacokinetic study of a single
dose of bertilimumab (0.01, 0.1, 1, 5 and 10 mg/kg) or placebo, infused intravenously over 30 min, was conducted in healthy male volunteers (n = 25). No serious adverse events were reported, and three events were of moderate severity [471969].

**Phase II**

To investigate the effects of bertilimumab on allergen-induced nasal response, cell infiltration and activation, a randomized, double-blind, placebo-controlled and parallel clinical trial was performed in patients with seasonal allergic rhinitis out of season. Patients (n = 52) were challenged with grass pollen 30 min after drug administration (a single dose of 50, 200 or 500 mg iv, or 10 mg intranasally) or matching placebo [518824], [518825]. Bertilimumab reduced post-allergy nasal obstruction, as determined by the nasal cross-sectional area AUC data from 48 patients, by 45.5, 26.7 and 12.5 cm²·min in the 10-mg intranasal, 200- and 500-mg intravenous groups, respectively. Neither peak nasal inspiratory flow nor symptoms were significantly altered, by either intravenous or intranasal treatment [518825].

The infiltration and activation of cells from nasal lavage samples collected pre-dosing and at five timepoints from 30 to 480 min, and nasal biopsy samples from 6 h were assessed from this study. Beneficial trends in reduced eosinophil number and tryptase were noted following bertilimumab treatment, but these failed to reach statistical significance; possibly due to the small sample size. Both intranasal and intravenous administration significantly reduced the infiltration of submucosal mast cells, by 40, 46 and 42% in the 50- and 500-mg intravenous groups and 10-mg intranasal group, respectively. The infiltration of submucosal eosinophils was also reduced by intranasal, but not intravenous, administration. Changes in cell counts in the epithelium did not achieve statistical significance, but the trends were similar to those seen for effects on submucosal cells [518824].

A phase I/IIa allergen challenge study of a topically applied single dose of bertilimumab has also been carried out in patients with allergic conjunctivitis. CAT stated that bertilimumab had no effect on symptoms, although detailed results have not been published. Analysis showed that allergen challenge did not provoke a large enough late-phase response involving eosinophils, thus the drug did not demonstrate effectiveness [513568].

**Side Effects and Contraindications**

In the initial tolerance and pharmacokinetic study no serious adverse events were reported, and only three events were of moderate severity. There was no clear dose-response relationship for events, with 1, 3, 7, 13, 0 and 3 events in the placebo, 0.01, 0.1, 1, 5 and 10 mg/kg bertilimumab treatment groups, respectively. No details of these events are available [471969].

In the phase II clinical trial, no serious adverse events were observed, and again there appeared to be no dose-response relationship for adverse events. One patient experienced influenza-like symptoms, including pyrexia, on the same day as receiving a 200-mg intravenous infusion, one patient suffered a vasovagal attack and wheezing (thought to be a late reaction to pollen) and epistaxis was noted in another patient. No antibodies against bertilimumab were detected [518825].

**Patent Commentary**

The only apparent patent application relating to bertilimumab is the above-mentioned WO-00166754, published by CAT Group plc in March 2000, which discloses in detail the method by which bertilimumab was produced, as well as several preclinical studies confirming its activity as an inhibitor of eotaxin-1.

**Current Opinion**

As a chemokine highly specific to eosinophils, eotaxin-1 has been proposed to play a central role in the pathogenesis of allergic diseases such as asthma, rhinitis and conjunctivitis, and has been identified as a potential therapeutic target for these conditions. Anti-eotaxin-1 treatment also has the potential to provide therapeutic benefit for other eosinophil-mediated diseases, including some skin conditions (such as eczema, psoriasis, urticaria and prurigo nodularis), inflammatory bowel diseases (ulcerative colitis, Crohn’s disease and gastroenteritis), vasculitis, eosinophilic pneumonitis, malignant diseases (especially lymphomas, leukemias and gastrointestinal cancer) and parasitic infections [559483], [WO-00166754]. Furthermore, as effector cells of eotaxin-1, basophils, mast cells and Th2 lymphocyte-mediated diseases such as scleroderma, viral infections [559483] and multiple sclerosis [559485], [559486] may also benefit from anti-eotaxin-1 treatment.

Preclinical studies have shown that bertilimumab inhibits the eotaxin-1-mediated chemotaxis of eosinophils from both healthy and asthmatic donors, and the recruitment of eosinophils in vivo in mice. Bertilimumab also neutralized eosinophil chemotaxis in induced sputum from moderate and severe asthma, but not mild asthma [557636], suggesting that this drug is potentially more effective for the treatment of asthmatics with severe disease.

In early clinical studies, bertilimumab was well tolerated and reduced allergen-induced infiltration of submucosal eosinophils and mast cells in patients with seasonal allergic rhinitis, but its clinical efficacy was not significant. This may be due to the small sample size in this study, but one cannot exclude the possibility that anti-eosinophilic treatment for these allergic conditions has little or no efficacy, as the results of recent clinical trials with monoclonal antibodies directed against IL-5 question the role of eosinophils in mediating the symptoms of asthma and allergic diseases [467752], [561367].

However, a potential added value of an anti-eotaxin antibody is that eotaxin is also a chemoattractant for basophils, mast cells and Th2 lymphocytes, cells which also play important roles in asthma and other allergic diseases. Therefore, bertilimumab is possibly more efficient than some anti-eosinophilic agents, eg, GlaxoSmithKline plc’s mepolizumab, a monoclonal antibody directed against IL-5 that is in phase II trials as a potential treatment for asthma, hypereosinophilic syndrome and atopic dermatitis. Compared with CCR3 antagonists (eg, GlaxoSmithKline
plc’s 766994 or Bristol-Myers Squibb Co’s DPC-168), however, bertilimumab may have less pronounced efficacy in some indications, as CCR3 antagonists inhibit not only the effects of eotaxin-1, but also those of other chemokines such as eotaxin-2 and -3, MCP-2, -3 and -4, and regulated on activating normal T-cell expressed and secreted [559490]. Further clinical trials with larger sample sizes will be required to determine the true efficacy of bertilimumab in the treatment of allergic asthma and rhinitis, and any other diseases.

### Commercial Opinion

In November 2000, Lehman Brothers predicted a 2007 launch for bertilimumab, with estimated peak sales of $250 million in 2014, and a 5% probability of reaching the market [394921].

### Development history

Phase I trials of bertilimumab in healthy volunteers commenced in June 2001 [412413], and were completed by September of that year. Consequently, CAT received authorization to begin a phase I/IIa double-blind trial of bertilimumab at two UK sites in patients with allergic rhinitis challenged with a nasal allergen. This trial in 48 patients was underway by January 2002 and completed by May of that year.

<table>
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<th>Status</th>
<th>Indication</th>
<th>Date</th>
<th>Reference</th>
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<td>Cambridge Antibody Technology Group plc</td>
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### Literature classifications

#### Chemistry

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<thead>
<tr>
<th>Study Type</th>
<th>Results</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Antibody synthesis and production.</td>
<td>Single-chain fragment variables (scFvs) specific to eotaxin-1 were isolated, and the 3G3 clone was modified to produce CAT-212, which inhibited eotaxin-1-mediated chemotaxis (IC50 value = 0.65 nM). CAT-212 was converted into the IgG4 type whole antibody bertilimumab using the VH and VL expression vectors pGamma4 and pMR15.1. Transformed cells were ELISA screened to detect IgG expression, adapted to serum-free medium and grown to produce bertilimumab, which was affinity purified.</td>
<td>560445</td>
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</table>

#### Biology

<table>
<thead>
<tr>
<th>Study Type</th>
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<th>Experimental Model</th>
<th>Result</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>In vitro</td>
<td>Inhibition of eotaxin-1-mediated chemotaxis.</td>
<td>CCR3-transfected L1.2 cells (incubated with eotaxin-1) treated with bertilimumab (0.01 to 100 nM).</td>
<td>IC50 = 0.7 nM.</td>
<td>423724</td>
</tr>
<tr>
<td>In vitro</td>
<td>Inhibition of eotaxin-1-mediated chemotaxis.</td>
<td>Eotaxin-1-incubated eosinophils from healthy human donors treated with bertilimumab (0.01 to 100 nM).</td>
<td>IC50 = 1.5 nM.</td>
<td>423724</td>
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<tr>
<td>In vitro</td>
<td>Inhibition of eotaxin-1-mediated chemotaxis.</td>
<td>Eosinophils incubated with 30 nM eotaxin-1 from the airway secretions of asthmatic human donors pre-incubated for 30 min with bertilimumab (0.03 to 33 nM).</td>
<td>Bertilimumab concentration-dependently neutralized the chemotactic activity of the eosinophils (IC50 = 0.95 nM).</td>
<td>557636</td>
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<tr>
<td>In vitro</td>
<td>Inhibition of eotaxin-1-mediated chemotaxis.</td>
<td>Induced sputum supernatants from moderate and severe asthmatic patients (n = 12) pre-incubated for 30 min with 100 nM bertilimumab.</td>
<td>Bertilimumab inhibited the chemotactic activity of eosinophils, with a median inhibition of 51.9, 77.5 and 86.1% in stable-moderate, unstable-moderate and severe asthmatic samples, respectively.</td>
<td>557636</td>
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<tr>
<td>In vitro</td>
<td>Antichemotactic efficacy.</td>
<td>Eotaxin (3 nM)-induced shape change of human peripheral eosinophils from five healthy donors treated with bertilimumab (0.1 to 500 nM).</td>
<td>Bertilimumab treatment inhibited shape change with an IC50 value of 0.71 nM.</td>
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<td>In vivo</td>
<td>Allergen-induced inflammation.</td>
<td>An IL-5-treated, ovalbumin-sensitized murine air pouch model. Mice were given both human eotaxin-1 (1 µg ipo) and bertilimumab (0.001, 0.01, 0.1 and 1 mg/kg ipo) or null IgG4 control.</td>
<td>Bertilimumab dose-dependently inhibited eosinophilia. The maximum inhibition of 94% was observed with 1 mg/kg of bertilimumab, relative to control.</td>
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### Biology (continued)

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<td>In vivo</td>
<td>Allergen-induced inflammation.</td>
<td>An IL-5-treated, ovalbumin-sensitized murine air pouch model. Mice were pretreated with bertilimumab (0.01, 0.1, 1 and 10 mg/kg iv) or null IgG4 control, 30 min prior to ipo injection of human eotaxin (1 µg).</td>
<td>A dose-dependent inhibition of eosinophil recruitment (46, 73, 79 and 91%, respectively) was observed, relative to controls. The chemotaxis of mononuclear cells was also inhibited by 7, 49, 101 and 156%, respectively, and that of neutrophils by 9, 37, 41 and 61%, respectively.</td>
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<tbody>
<tr>
<td>In vivo</td>
<td>Allergen-induced inflammation.</td>
<td>Cynomolgus monkeys treated with eotaxin-1 and either local intradermal (10 µg) or systemic (20 and 100 mg/kg iv) bertilimumab.</td>
<td>Bertilimumab inhibited recruitment of eosinophils by both routes of administration, and the higher intravenous dose inhibited IL-13-induced cell recruitment to the dermis.</td>
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### Metabolism

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<th>Model Used</th>
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<tr>
<td>In vivo</td>
<td>Pharmacokinetics.</td>
<td>Healthy male volunteers (n = 25) received bertilimumab (0.01, 0.1, 1, 5 and 10 mg/kg) or placebo, as a 30-min intravenous infusion.</td>
<td>The 0.01-mg/kg bertilimumab dose could not be detected in serum, but the antibody remained active in serum for &gt; 60 days after infusions of 5 and 10 mg/kg. The respective pharmacokinetic parameters following doses of 0.1, 1, 5 and 10 mg/kg were: $C_{\text{max}} = 2.1, 24.6, 122.7$ and $291.9$ mg/kg; volume of distribution = 58.3, 138.1, 277.8 and 220.9 ml/kg; terminal $t_{1/2} = 0.6, 2.1, 8.5$ and 8.4 days.</td>
<td>471969</td>
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### Clinical

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<th>Model Used</th>
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<td>Safety.</td>
<td>Healthy male volunteers (n = 25) received bertilimumab (0.01, 0.1, 1, 5 and 10 mg/kg) or placebo, as a 30-min intravenous infusion.</td>
<td>No serious adverse events were reported, and three events were of moderate severity.</td>
<td>471969</td>
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<tr>
<td>Safety and efficacy in allergic asthma.</td>
<td>A randomized, double-blind, placebo-controlled trial in patients with seasonal allergic rhinitis (n = 52) out of season. Patients were challenged with grass pollen 30 min after drug administration (a single dose of 50, 200 or 500 mg iv, or 10 mg intranasally) or matching placebo.</td>
<td>Bertilimumab reduced post-allergy nasal obstruction, as determined by the nasal cross-sectional area AUC data from 48 patients, by 45.5, 26.7 and 12.5 cm².min in the 10-mg intranasal, 200- and 500-mg iv groups, respectively. Peak nasal inspiratory flow or symptoms were not significantly altered by either iv or intranasal treatment. Both intranasal and iv administration significantly reduced the infiltration of submucosal mast cells by 40, 46 and 42% in the 50- and 500-mg iv groups, and 10-mg intranasal group, respectively. The infiltration of submucosal eosinophils was also reduced by intranasal, but not iv, administration.</td>
<td>518824 518825</td>
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<tr>
<td>Safety and efficacy in allergic conjunctivitis.</td>
<td>A phase I/IIa allergen challenge study of a topically applied single dose of bertilimumab in patients with allergic conjunctivitis.</td>
<td>Bertilimumab had no effect on symptoms, although detailed results have not been published. Analysis showed that allergen challenge did not provoke a large enough late phase response involving eosinophils, thus the drug did not demonstrate effectiveness.</td>
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### Associated patent

**Title** Human antibodies against eotaxin and their use.

**Assignee** Cambridge Antibody Technology Limited

**Publication** WO-00166754 13-SEP-01

**Priority** US-2000 187246 03-MAR-00

**Inventors** Vaughan TJ, Wilton AJ, Smith S.

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infiltration of submucosal eosinophils


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