Inflammatory T cell response is promoted by OX40, whose expression is shown to increase in atopic dermatitis (AD). Targeting OX40 is a rational therapeutic strategy in treating AD.1,2,3,4

We hypothesized that an anti-OX40 monoclonal antibody (mAb) with a silenced antibody-dependent cell-mediated cytotoxicity (ADCC) function would have minimal T cell toxicities, thereby minimizing safety risks.2

IMG-007 is a novel nondepleting anti-OX40 mAb, bioengineered in its Fc region with a N297A mutation to abolish the ADCC function.

Figure 1. IMG-007 was designed for reduced safety risks through N297A bioengineering

Methods

The kinetics of IMG-007’s binding to recombinant human Fcγ receptors was measured using surface plasmon resonance (SPR) Biacore 8K.

The ADCC function was evaluated by measuring cytotoxicity to HEK293-OX40-Luc cells cocultured with primary human NK cells and antibody and quantified by flow cytometry.

The cytokine release potential was assessed by incubating human peripheral blood mononuclear cells (PBMCs) with antibody (0.75, 15.0 and 300 μg/mL) under both solid and solution phases from 10 donors for 4 and 48 hours, followed with measurement of 10 cytokines in the culture supernatant using the Meso Scale Discovery (MSD) Proinflammatory Panel 1.

Results

Figure 2. IMG-007 showed minimal binding affinity to Fcγ receptors, silenced ADCC and cytokine induction

<table>
<thead>
<tr>
<th>Antibody concentration (μg/mL)</th>
<th>ADCC killing of OX40+ cell (%)</th>
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<tbody>
<tr>
<td>Isotype control</td>
<td>0</td>
</tr>
<tr>
<td>IMG-007</td>
<td>0</td>
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<tr>
<td>GBR-830 analog</td>
<td>0</td>
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</table>

A. Kinetic analysis of IMG-007 binding to Human FcγRs via SPR demonstrated that IMG-007 has minimal binding affinity to human FcγRs. B. ADCC assay evaluation using human primary NK cell as effector cells and HEK293-OX40-Luc as target cells showed that IMG-007 is devoid of activity in depleting OX40+ cell, compared with wildtype GBR-830 analog GBR-830 analog. C. Solid phase formal (coating antibody on plate) cytokine release potential evaluation assay in 10 donors’ PBMC using MSD assay panel showed no cytokine secretion from IMG-007 treatment, compared with isotype control. Dose dependent cytokine release from wildtype IGA1 anti-OX40 antagonist GBR-830 analog was observed. ORTs as positive control. Each of 10 donors’ data are presented in 10 rows of each treatment. Data are shown as concentration and also visually by color. D. Solution phase formal (soluble antibody in buffer) cytokine release potential evaluation assay as described above.

Conclusions

IMG-007 exhibited a minimal binding affinity to Fcγ receptors, including FcγRs I, IIa, IIa (V176), IIα (F176), IIIb and IIIb.

At up to the highest concentration tested, IMG-007 did not induce any antibody induced cytotoxicity to the OX40+ HEK293-Luc cells.

Furthermore, it did not induce the release of all 10 proinflammatory cytokines tested in human PBMCs under both solid and solution testing formats.

Silencing ADCC function in IMG-007 via bioengineering resulted in minimal binding to the Fcγ receptors and diminished cytotoxicity to OX40+ cells.

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IMG-007 represents a promising drug candidate for potentially treating AD.