ANB032, a BTLA Checkpoint Agonist Monoclonal Antibody, Reduced T Cell Proliferation, Inflammatory Cytokine Secretion and Prevented Graft versus Host Disease (GvHD) in a Mouse Model

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Disclosures

• All authors are employees of AnaptysBio, Inc., San Diego, CA, USA
Checkpoint Receptors Modulate Immune Cells

Checkpoint antagonists: “release the brakes”

Checkpoint agonists: “tap the brakes”

Immune cells (e.g., T, B, dendritic)

Treat cancers: Unleash immune response

Treat inflammation: Attenuate overactive/persistent immune response
B and T lymphocyte attenuator (BTLA) is a potent modulator of T cells, B cells and dendritic cells (DC)

Expressed only on immune cells and preferentially on activated immune cells

Dysregulation of BTLA pathway accelerates onset and exacerbates disease

Adapted from Xu et al, J. Cell Biol. 2020 Vol. 219 No. 6
BTLA is a Key Node Of Immune Regulation

B and T lymphocyte attenuator (BTLA) is a potent modulator of T cells, B cells and dendritic cells (DC).

Expressed only on immune cells and preferentially on activated immune cells.

Dysregulation of BTLA pathway accelerates onset and exacerbates disease.

Adapted from Xu et al, J. Cell Biol. 2020 Vol. 219 No. 6
Proposed Mechanism of Action for ANB032

ANB032: IgG4 antibody (non-depleting)
- Binds to BTLA on epitope proximal to immune cell
- Fc receptor binding profile contributes to differentiated potency
- Non-blocking of HVEM engagement with optimized antigen binding affinity

ANB032’s agonist signal modulates immune cells
- Inhibits activated T cell proliferation
- Reduces inflammatory cytokine secretion
- Modulates DC function, including inducing Tregs
Fc Receptor Engagement by ANB032 Enhances BTLA Agonism, Measured by SHP2 Recruitment Assay

Jurkat BTLA SHP2 Recruitment Assay methodology: BTLA and SHP2 are fused with complementary enzyme fragments, when SHP2 is recruited to activated phosphorylated BTLA, the enzyme donor and enzyme acceptor form active β-gal that is detected by chemiluminescence.
**Agonism of BTLA in a Preclinical Mouse Dermatitis Model**

**BTLA knock-out mice have exacerbated T cell-mediated skin disease**

- Infiltrating CD4 T cells (similar for CD8)
- Proliferating CD8 T cell IFNγ

**BTLA agonist-treated WT mice have reduced T cell-mediated skin disease**

- Infiltrating CD4 T cells (similar for CD8)
- Infiltrating CD8 T cell IFNγ

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Nakagomi et al, Journal of Investigative Dermatology, 2013; Surrogate Murine BTLA agonist third-party data
Determining the Efficacy and Immune Regulatory Effects of ANB032 in a Humanized Mouse Model of Graft-versus-host Disease (GvHD)

Endpoints:
- Weight loss
- Death
- GvHD disease activity index (DAI) scores (Fur, skin, posture, activity)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Test Agent</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>Isotype Control</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>ANB032</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>BTLA Agonist Ref1</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>BTLA Agonist Ref2</td>
<td>10 mg/kg</td>
</tr>
</tbody>
</table>

Study Day

Endpoints:
- Weight loss
- Death
- GvHD disease activity index (DAI) scores (Fur, skin, posture, activity)
Evaluating the Contribution of Both Epitope Binding and FcR Engagement to Efficacy

<table>
<thead>
<tr>
<th></th>
<th>ANB032 IgG4</th>
<th>Ref1 Mutated IgG4</th>
<th>Ref2 Mutated IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Binding epitope is HVEM sparing</strong></td>
<td>✓</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td><strong>FcR engagement</strong></td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>
Quantification of circulating human CD45+ cells in whole blood of mice surviving at Study Day 18 (SD18 solid fill) and 35 (SD35 dashed fill)
Quantification of circulating human CD45+ cells in whole blood of mice surviving at Study Day 18 (SD18 solid fill) and 35 (SD35 dashed fill)
ANB032 Reduced Human T Cell Expansion

Human T cell Frequency
(Day 18)

- Isotype Control
- ANB032
- BTLA Agonist Ref1
- BTLA Agonist Ref2
ANB032 Treatment Resulted in Prolonged Survival and Reduced Disease Activity Index
ANB032 Reduced Serum Inflammatory Cytokines

Study Day 18

- IFNγ
- TNFα
- GM-CSF
Elevated T Cells and Dendritic Cells are Hallmarks of Atopic Dermatitis (AD) Skin

Broad T Cell (Th1, Th2, Th17 and Th22) Signatures Characterize AD

PBMC Assay for Assessment of Pharmacology in Patient-Derived Samples

**PBMC isolation and CFSE labeling**
- PBMCs from Healthy or Disease Donors
- CFSE labeling

**T cell activation and proliferation**
- CFSE labeled PBMCs (200K/well)
- sCD3/CD28
- ANB032 vs. comparators, Isotype control
- 3 days
- sup
- cells

**Analysis**
- MSD
- IFN-γ, IL-17A, IL-13, IL-22, etc.
- 17 color FACS
ANB032 Reduced T Cell Proliferation in AD Patient-Derived PBMCs

Hsu et al. Presented at the Antibody Engineering & Therapeutics Conference US, San Diego, Dec 12-16, 2021
ANB032 Reduced Th1, Th2, Th17 and Th22 Cytokine Secretion in AD Patient-Derived PBMCs In Vitro
B and T lymphocyte attenuator (BTLA) is a potent modulator of T cells, B cells and dendritic cells (DC).

Expressed only on immune cells and preferentially on activated immune cells.

Dysregulation of BTLA pathway accelerates onset and exacerbates disease.
BTLA is Highly Expressed on Mature DCs

Inflammatory stimuli induce DC maturation

LPS*

Pathogens Cytokines
PAMPs DAMPs

Immature DC

Mature DC

Co-stimulatory molecules
MHC II Expression
Secretion of pro-inflammatory cytokines

Co-stimulatory molecules
MHC II Expression
Secretion of pro-inflammatory cytokines

BTLA

LPS stimulated DCs

CD11c vs MHC II

Immature DC 50%
Mature DC 92%

Mature DC

CD11c vs BTLA

Immature DC

Adapted from Frontiers in Immunology. 2019 Jan 21;9:3176; * LPS=Lipopolysaccharide (a TLR4 agonist).
ANB032 Reduced DC Maturation, Antigen Presentation, and Co-stimulatory Molecule Expression

ANB032-treated DCs Induced Functional Tregs and Reduced Inflammatory Cytokines in a MLR Assay

Conclusion

• In a human xenograft GvHD mouse model, ANB032:
  • Reduced T cell expansion
  • Reduced inflammatory cytokines in plasma
  • Demonstrated superior in vivo efficacy on key endpoints, including prolonged survival, maintained body weight and an overall reduced disease activity index (DAI), compared to reference BTLA agonist antibodies

• ANB032 reduced Th1, Th2, Th17 and Th22 inflammatory cytokine secretion from atopic dermatitis patient-derived PBMCs

• ANB032 reduced the maturation of DCs, reduced MHC II, reduced co-stimulatory molecules and induced Tregs in vitro

• ANB032 is currently being evaluated in an ongoing Phase 2 study in moderate-to-severe atopic dermatitis (NCT05935085)