Abstract N°: LBA-163

Molecular Design & Characterization of GTX-B001: A Bispecific Antibody targeting c-Kit/CD203c for Selective Mast Cell Silencing

Orla Cunningham[1], Emmanuel Vial[1], Jonny Finlay[2], Thibaud Portal[1]

[1] Alys Pharmaceuticals, Boston, United States

[2] WJFinlay Consulting Ltd, Glasgow, United Kingdom

Introduction

Mast cells (MCs) are a key effector in chronic inflammatory signaling pathways and can be activated via both IgE and non-IgE dependent routes leading to granule release, recruitment of other immune cells and local amplification of immune activation. C-kit represents a cardinal mast cell gene, and phosphorylation of c-Kit triggered by SCF controls mast cell differentiation, migration, adhesion, maturation, survival and activation. It has been shown that c-Kit inhibition, via c-Kit targeting antibodies, can effectively deplete mast cells and ameliorate disease in chronic induced and spontaneous urticaria. However, c-Kit has broad global expression and side effects of c-kit targeting include changes in hair and skin pigmentation, taste disorders and neutropenia. We sought to engineer a bispecific antibody, GTX-B001 (ALY-301), that targets c-Kit selectively in mast cells to maintain efficacy while improving the safety profile to enable chronic dosing for chronic inflammatory disease.

Materials and Methods

Recent advances in single cell proteomics have enabled the characterization of the mast cell proteome and revealed several mast cell selective surface markers. CD203c was of particular interest as expression is upregulated at the surface of activated mast cells while being very much restricted to the granulocyte lineage. GTX-B001 was engineered by combining humanized anti-c-Kit and CD203c heavy chain binding domains with a common light chain using classical knob-in-hole (KiH) mutations in the Fc region to ensure effective heterodimeric pairing. Additional engineering was incorporated into the IgG1 Fc region to ablate FcγR interaction and subsequent engagement with immune effector cells. GTX-B001 was expressed and purified from a stable CHO cell line and characterized for purity, stability, selectivity and potency.

Results

GTX-B001 proved highly productive (>4 g/L) and was purified to >99% homogeneity using a platform purification process combining Protein A and ion-exchange chromatography. Purified GTX-B001 showed excellent stability, retaining >99% purity by SEC and 95% potency as measured by dual binding ELISA after 6 months at 25^0C.

Mast cell selectivity experiments demonstrated a classical co-operative binding mechanism for GTX-B001 with minimal binding being observed on KU812 cells engineered to express only a single target. In contrast, high affinity binding of GTX-B001 was observed to the parental KU812 cells expressing both targets. This differential was also clear when comparing GTX-B001 binding to KU812 cells versus primary melanocytes. When compared with the anti-cKit-specific antibody Barzovolimab, GTX-B001 had an affinity >100x lower on primary melanocytes

while both molecules demonstrated equivalent binding to dual target expressing KU812 cells.

Affinities for each targe ranged from 40-80 nM when measured by SPR, and this was equivalent for the cynomologus monkey orthologs, enabling good translation for in vivo safety studies. GTX-B001 blocked fluorescently labelled SCF from binding to c-Kit expressed on KU812 cells with an IC50 of 0.86 nM and inhibited downstream c-Kit phosphorylation with an IC50 of 5 nM. GTX-B001 bound to primary human skin mast cells isolated from healthy donors in a manner that was dose-dependent, with an affinity that was absolutely driven by CD203c expression level, again confirming the dual target selectivity.

Cell based assays measuring FcγR engagement, including Antibody-Dependent Cell mediated Phagocytosis (ADCP) and Cytotoxicity (ADCC) and Complement Dependent Cytotoxicity (CDC) confirmed that GTX-B001 is not effector function competent.

Conclusion

These studies provide evidence that GTX-B001 is an effective MC-selective agent with demonstrated co-operative binding only to cells that express both c-Kit and CD203c targets. This has the potential for safe and potent mast cell targeting without global c-Kit targeting side effects and enables chronic dosing in chronic inflammatory disease settings.