Briquilimab potently inhibits stem cell factor (SCF)/c-Kit signaling, mast cell (MC) activation and survival

Hind Bouzid, Song Eun Lee, Cheryl Kwan, Mang Yu, Hye-Sook Kwon, Wendy W. Pang.
Jasper Therapeutics Inc., Redwood City, CA, USA

Background:

- Stem Cell Factor (SCF) signaling through c-Kit (CD117) plays a key role in mast cell (MC) differentiation, activation and survival.
- SCF/c-Kit signaling is essential for IgE-dependent and -independent MC activation/degranulation leading to release of mediators including inflammatory cytokines and chemokines.
- Briquilimab, a humanized aglycosylated monoclonal antibody targeting c-Kit, blocks SCF binding to c-Kit and SCF/c-Kit signaling.
- Inhibition of the SCF/c-Kit pathway has the potential to treat mast cell-related disorders.
- JSP084 is a comparative reagent that blocks c-Kit dimerization which was generated from publicly available sequences.
- Imatinib is a registered drug and a small molecule inhibitor targeting multiple tyrosine kinases including c-Kit.
- The effects of briquilimab, JSP084, and imatinib on c-Kit phosphorylation, MC degranulation, and MC survival were compared in the same experimental runs.

Methods:

- SCF mediated c-Kit phosphorylation levels were accessed in the human megakaryoblastic leukemia M-07e cells that express endogenous c-Kit and maintain SCF/c-Kit signaling using Meso Scale Discovery immunoassay platform.
- Human primary mast cells were differentiated from mobilized peripheral blood purified CD34$^+$ HSPCs by *ex vivo* culturing in the presence of SCF (100 ng/mL), IL-6 cytokines (100 ng/mL) and IL-3 (only during the first week of differentiation at 30 ng/mL). >80% of c-Kit$^+$ FcεRI$^+$ mature MC cells were differentiated in 7-10 weeks of culture and used for MC degranulation and survival assays.
- MC degranulation was induced by addition of IgE (100 ng/mL) and anti-IgE (1 µg/mL) in the culture and measured by the release of β-hexosaminidase.
- MC survival was assessed by quantification of live cells and c-Kit$^+$ FcεRI$^+$ frequency using Image Xpress pico and flow cytometry, respectively.
- Antibody-dependent cellular cytotoxicity (ADCC) was evaluated using the ADCC Reporter Bioassay (Promega) containing engineered Jurkat effector cells that stably express the FcγRIIIa receptor and an NFAT response element driving expression of firefly luciferase. The luminescence was measured by the Spectramax Id3.

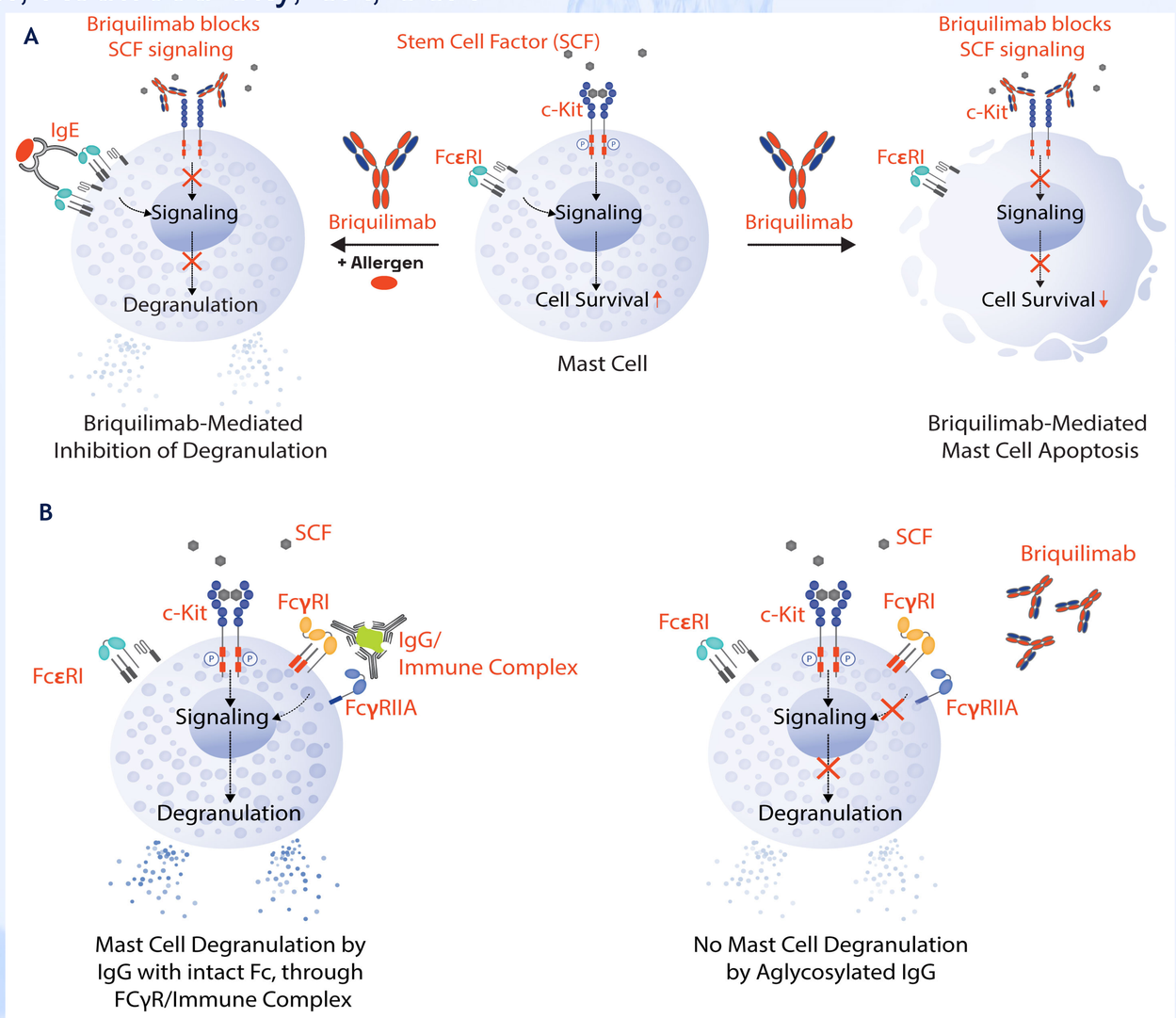

Figure 1. (A) The SCF/c-Kit pathway is essential for mast cell activation/degranulation and survival. Mast cell recognition of allergens by FcεRI-associated IgE induces degranulation and allergic symptoms. Blocking SCF/c-Kit signaling by briquilimab inhibits MC degranulation (short-term effect within an hour) and induces mast cell apoptosis (long-term effect within days to months). (B) The engagement of MC by immune complex and IgG that has an intact Fc region through FcγRs induces MC degranulation. Aglycosylated briquilimab by N297Q mutation cannot bind to FcγRs and thus do not induce FcγR/IgG immune complex mediated MC degranulation.

Results:

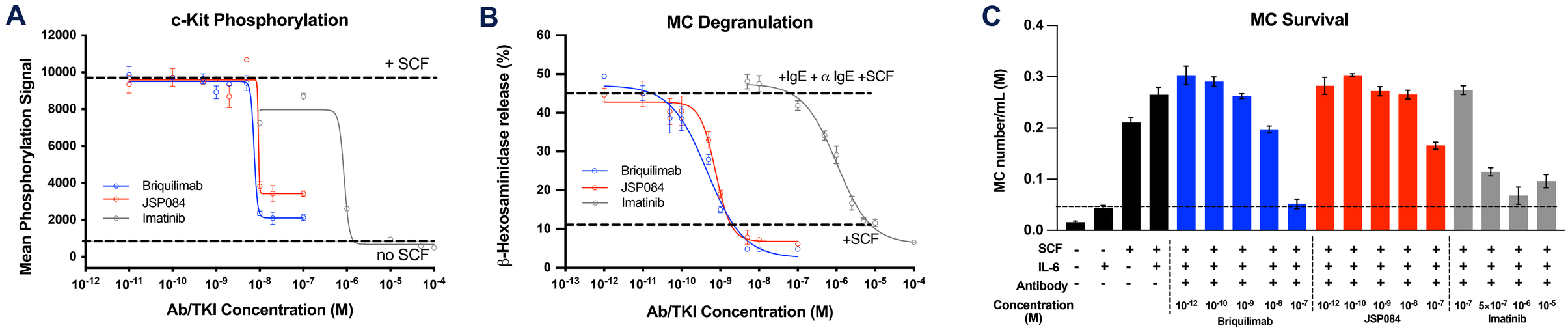

Figure 2. Briquilimab inhibits c-Kit phosphorylation, MC degranulation, and survival more potently than JSP084 and Imatinib. (A) M-07e cells were serum starved in the absence or presence of increasing amounts of briquilimab, JSP084, and imatinib for 30 minutes and stimulated by SCF at 100 ng/mL concentration for 2 minutes. Cells were lysed and analyzed for pan-phosphorylation status of c-Kit using Meso Scale Discovery platform. Briquilimab (IC_{50} = 7.9 nM, 86% inhibition) inhibits pan-cKit phosphorylation more potently than JSP084 (IC_{50} = 9.6 nM, 71% inhibition) and imatinib (IC_{50} = 364.9 nM) (B) Human primary MC were incubated with increasing amount of briquilimab, JSP084 and imatinib for 90 minutes after overnight SCF starvation. Cells were treated with 100 ng/mL of human IgE for 30 minutes followed by continued incubation with SCF (100 ng/mL) and anti-IgE (1 µg/mL) for 30 minutes. The levels of MC degranulation were presented by the percent of β-hexosaminidase released from the total β-hexosaminidase. Briquilimab (IC_{50} = 331 pM) inhibits MC degranulation more potently than JSP084 (IC_{50} = 615 pM) and imatinib (IC_{50} = 0.902×10^6 pM) (C) 25,000 MC per well in 96 well plate were cultured in the presence of increasing amount of briquilimab, JSP084 (up to 100 nM) or imatinib (up to 10 uM) for 6 days. The live cells were counted by Image Xpress Pico and frequency of c-Kit$^+$ FcεRI$^+$ cells were measured by flow cytometry analysis. The MC numbers were calculated based on live cell number and the frequency of c-Kit$^+$ FcεRI$^+$ cells. Briquilimab nearly completely inhibited SCF-dependent MC survival in culture. At 100 nM dose, briquilimab inhibits the survival of MC more potently than JSP084 and imatinib.

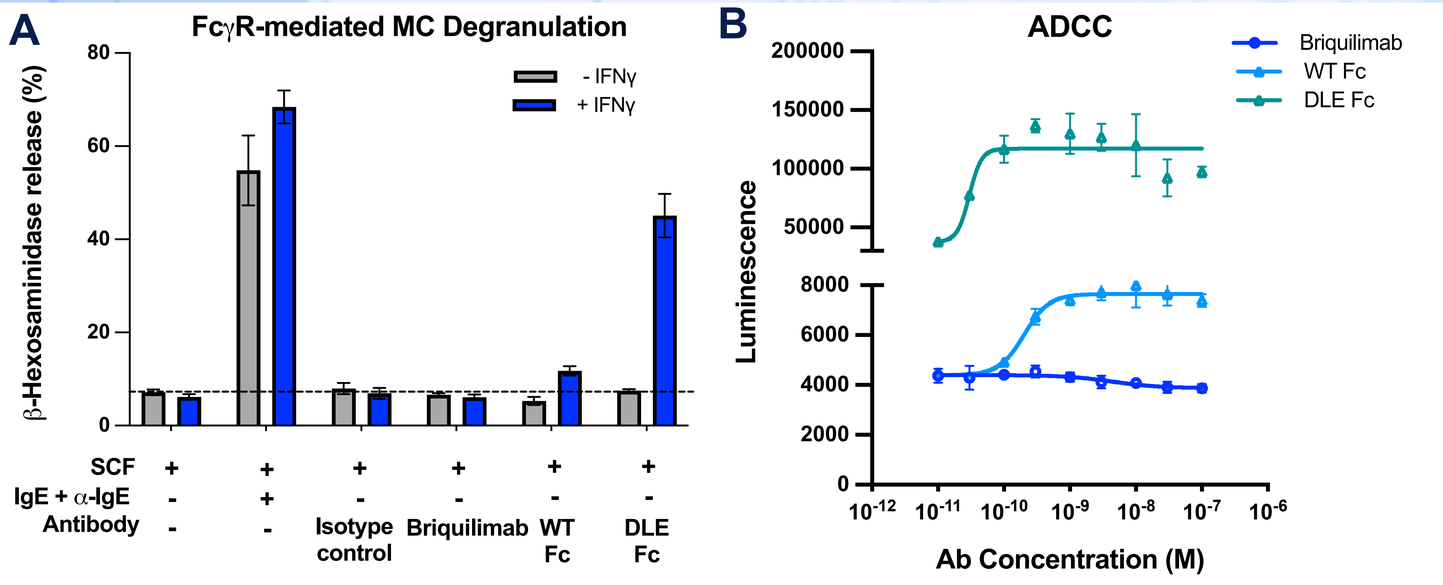

Figure 3: Briquilimab does not elicit FcγR-mediated degranulation or ADCC.
(A) MC were treated with IFNγ (15 ng/mL) for 40 hours followed by either IgE/anti-IgE incubation or each antibody alone (isotype control, briquilimab, WT Fc, and DLE Fc). WT Fc has an intact Fc region in the same antibody backbone as briquilimab. DLE Fc has a modified Fc region by S239D/A330L/I332E mutation leading to increase in affinity to FcγR and enhance ADCC. IFNγ is shown to increase FcγR expression in MC. WT Fc, but not briquilimab, induced MC degranulation in response to IFNγ As expected, DLE Fc induced IFNγ-dependent MC degranulation. (B) M-07e target cells treated with increasing amount of briquilimab, WT Fc or DLE Fc and incubated with Jurkat effector cells that stably express the FcγRIIIa receptor and an NFAT response element driving expression of firefly luciferase for 6 hours. Luciferase activity (luminescence) was measured for ADCC. WT Fc and DLE Fc but not briquilimab, induced ADCC.

Conclusion and Future Directions:

- Briquilimab inhibits c-Kit phosphorylation, MC degranulation, and survival more potently than JSP084 and Imatinib with lower IC_{50} values.
- Briquilimab through an N297Q modification, effectively abolished IgG Fc/FcγR/immune complex mediated degranulation and ADCC.
- Jasper is actively enrolling participants in a phase 1b/2a trial evaluating briquilimab in patients with chronic spontaneous urticaria, (BEACON trial, NCT06162728) and in patients with chronic inducible urticaria (SPOTLIGHT trial, NCT06353971).

JASPER THERAPEUTICS

Briquilimab is an investigational product and not approved for any indication