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P1391 Briquilimab Potently Inhibits SCF/c-Kit Signaling, Which Does Not Induce Healthy HSC Apoptosis, But Skews HSC Differentiation Potential

Minyoung Youn[1], Song-Eun Lee[1], Hye-Sook Kwon[1], Wendy W. Pang[1] [1]Jasper Therapeutics, Redwood City, United States of America

INTRODUCTION

- Hematopoietic stem cells (HSCs) are responsible for the production of all blood cell lineages.
- The HSC compartment is tightly regulated by intrinsic and extrinsic signals, including cytokines and growth factors, during both steady state and stress hematopoiesis.
- The net output of signaling pathways determines the fate of HSC for self-renewal, differentiation, migration, or apoptosis.
- Stem cell factor (SCF) and its signaling via the c-Kit receptor plays an important role in the regulation of HSC fate.
- Briquilimab is a humanized monoclonal antibody that binds to c-Kit and inhibits SCF signaling.

AIM

We evaluated the molecular basis of briquilimab inhibition of the SCF/c-Kit signaling pathway and its functional impact on healthy human HSC survival, proliferation, and differentiation.

METHOD

- Briquilimab binding to c-Kit, phosphorylation and receptor internalization of c-Kit were evaluated using the M-07e cell line, which expresses endogenous SCF-responsive human c-Kit.
- Healthy long term primary human HSCs (CD34+CD38-CD90+CD45RA-) were sorted from bone marrow mononuclear cells and cultured ex vivo in X-vivo media containing Thrombopoietin (TPO), Flt3-ligand (Flt3-L), and SCF, with or without briquilimab.
- The proliferation, apoptosis, and differentiation of primary human HSCs were monitored by flow cytometry and automated cell imaging.

RESULTS

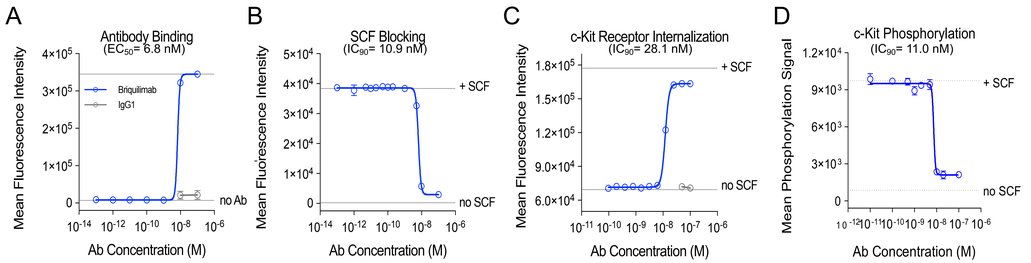

Figure 1. Briquilimab blocks SCF binding to c-Kit, SCF mediated c-Kit internalization, and c-Kit phosphorylation in M-07e cells. M-07e cells were incubated with increasing amounts of briquilimab prior to each assay for 30 min to 1 hr. **(A)** Briquilimab binding to c-Kit was detected by Alexa Flour 488 anti-human IgG1 antibody. **(B)** SCF binding to c-Kit was detected by Alexa-647-conjugated SCF. **(C)** Cells were further incubated with SCF (10 ng/mL) for 30 min, followed by incubation with briquilimab. Cell surface c-Kit was measured by PE conjugated to a non-competing and non-neutralizing antibody to c-Kit (104D2 clone). Fluorescence was analyzed by flow cytometry. **(D)** Cells were stimulated with SCF (100 ng/mL) for 2 min followed by incubation with briquilimab. After cell lysis, phospho-c-kit levels were detected by the Meso Scale Discovery (MSD) immunoassay platform using an anti-c-Kit capture antibody (clone 104D2) and a SULFO-TAG anti-phosphotyrosine detection antibody.

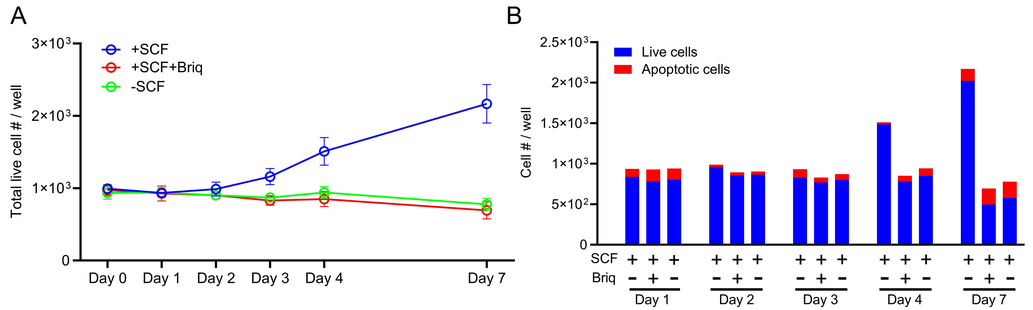

Figure 2. Briquilimab does not induce apoptosis of healthy HSC. 1000 purified HSC were cultured with TPO (50 ng/mL) and Flt3-L (50 ng/mL) in the presence or absence of SCF (25 ng/mL) and briquilimab (1 □g/mL). **(A)** Total live cells were counted by PICO automated cell imaging system on the indicated day of *ex vivo* culture. **(B)** Cells were stained with Annexin V and Propidium Iodide (PI) and analyzed by flow cytometry to determine live and Annexin V$^+$PI$^-$ apoptotic cell numbers.

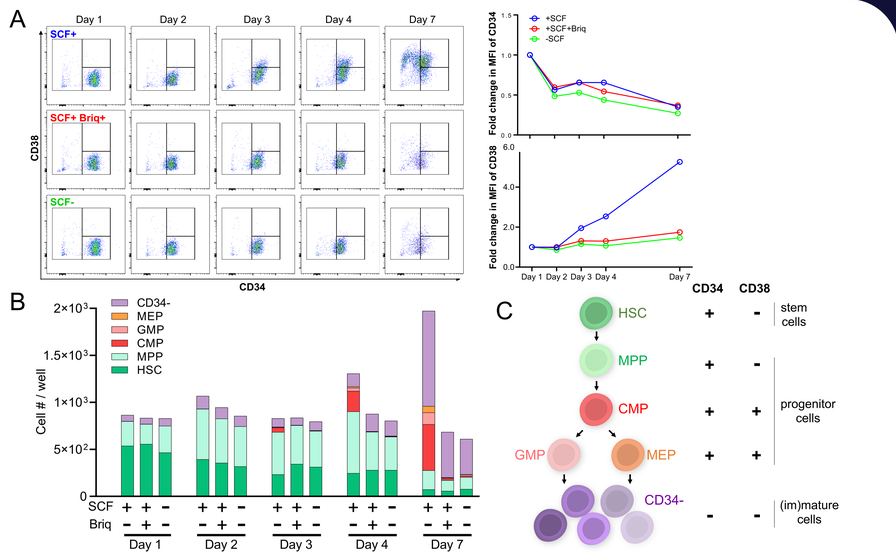

Figure 3. Briquilimab skews HSC differentiation into an alternative pathway. The cultured HSC were stained with CD34, CD38, CD90, CD45RA, CD123, and c-Kit on the indicated day of *ex vivo* culture, and then analyzed by flow cytometry. **(A)** The flow cytometry plots of CD34 and CD38 expression (left panel) and fold change of Mean Fluorescence Intensity (MFI) of CD34 and CD38 expression on the cell surface (right panel) at indicated timepoints are shown. **(B)** The HSC and each progenitor cell number were defined based on cell markers. Multipotent progenitor: MPP (CD34+CD38-CD90-CD45RA-), Common myeloid progenitor: CMP (CD34+CD38+CD45RA-CD123low), Granulocyte-monocyte progenitor: GMP(CD34+CD38+CD45RA+ CD123low), Megakaryocyte-erythrocyte progenitor: MEP(CD34+CD38+CD45RA-CD123-) **(C)** Classic scheme of HSC differentiation into the myeloid lineage and expression pattern of CD34 and CD38 on HSC and progenitor cells. **(D)** The flow cytometry plot of c-Kit expression in each group (left panel) and fold change of c-Kit expression on the cell surface (right panel) at the indicated timepoints are shown.

CONCLUSIONS

- Briquilimab potently blocks SCF/c-Kit signaling by binding to c-Kit.
- Blocking of SCF/c-Kit signaling by briquilimab does not cause HSC apoptosis
- *Ex vivo* cultured HSC with SCF, TPO, Flt3-L have the capability to differentiate into different progenitors (CMP, GMP, MEP), evidenced by increased CD38 expression on differentiated cells by Day 7.
- HSC cultured with SCF, TPO, Flt3-L in the presence of briquilimab differentiated directly into CD34- cells with higher c-Kit expression and without increased CD38 expression by Day 7.

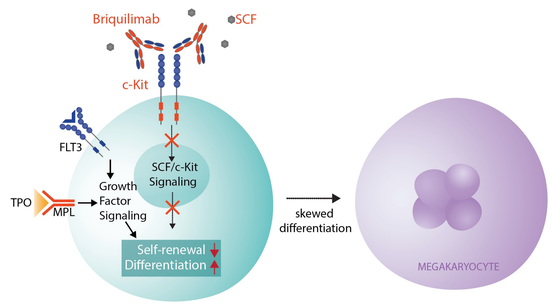

Figure 4. A hypothetical model. Blocking of SCF/c-Kit signaling by briquilimab skews HSC towards a distinct alternative HSC differentiation pathway in ex vivo culture. Briquilimab binds to c-Kit and blocks SCF/c-Kit signaling that plays an essential roles in self-renewal and differential potential. Given previously published findings showing that c-Kit high expressing HSC exhibits a megakaryocytic lineage differentiation bias (Shin JY et al.) and megakaryocytic progenitors are enriched in CD34-CD38-/low cells (Steevels T et al.), briquilimab may skew HSC differentiation bias towards the megakaryocytic lineage.

REFERENCES

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CONTACT INFORMATION

EHA2024@jaspertherapeutics.com