Combination Therapy with Ruxolitinib and Ibrutinib Reduces the Severity of Murine Sclerodermatous Chronic Graft-Versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

V. Raguraman[1], S. Mohiyuddin[1], S. Gabure[1], N. Jabbour[2], M. Kesler[2], S. Palaniyandi[1], and G. C. Hildebrandt*[1]

[1]Division of Hematology and Medical Oncology, Department of Medicine, Ellis Fischel Cancer Center, University of Missouri, Columbia, MO, USA.

[2]Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington, Kentucky, USA.

BACKGROUND

- Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potential curative therapy for malignant hematopoietic disorders, but its clinical efficacy is limited by the onset of graft-versus-host disease (GVHD).
- Chronic GVHD (cGVHD) is driven by sustained inflammation and fibrosis mediated by alloreactive donor B- and T-cells.
- Bruton tyrosine kinase (BTK), a key effector of B-cell receptor signaling, is critical for B-cell and mast cell activation.
- JAK/STAT signaling pathways regulate T-cell activation and inflammatory cytokine production, thereby contributing to progression of cGVHD.

AIM

- We hypothesize that dual inhibition of JAK and BTK signaling will reduce mast cell activation and effector immune responses in murine models of sclerodermatous chronic graft-versus-host disease (cGVHD).

METHODS

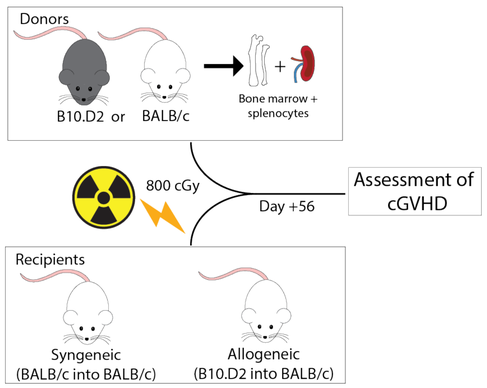

Figure 1. Schematic representation of cGVHD transplant model

RESULTS

Chronic GVHD symptoms are ameliorated by combination treatment

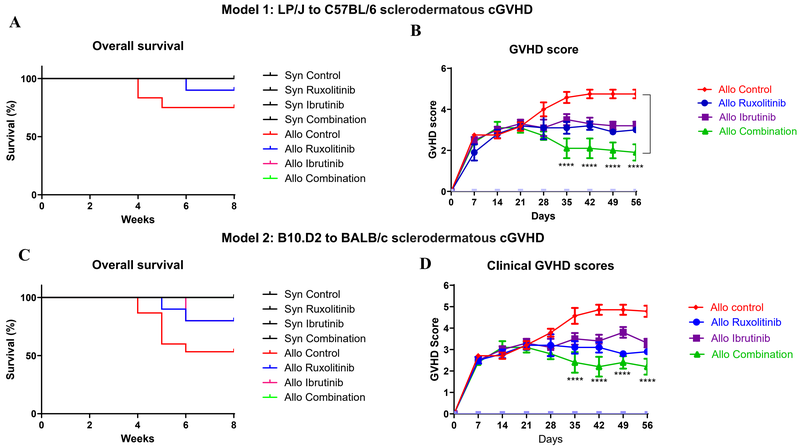

Figure 2:A&C) Kaplan-Meier survival curve of syngeneic (n = 4), syn-ruxolitinib (n = 4), syn-ibrutinib (n = 4), syn- ruxolitinib + ibrutininb (n = 4), allogenic (n= 9), allo-ruxolitinib (n=9), allo- ibrutinib (n=9), allo - ruxolitinib + ibrutininb (n = 10) groups after transplant. B&D) Weekly GVHD scoring of groups after transplant. Error bars are the standard error of the mean (SEM).

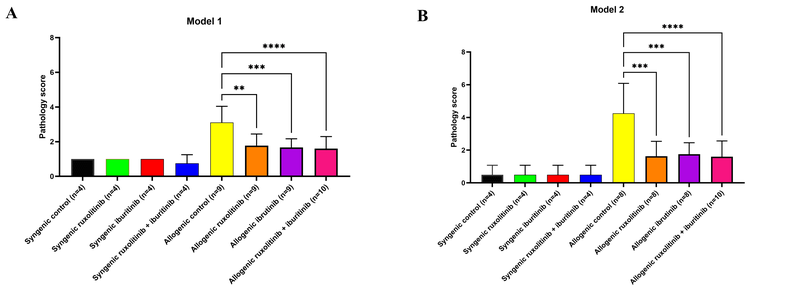

Figure 3:) Pathology scores for skin scored by pathologists in a blinded fashion. A) LP/J to C57BL/6 sclerodermatous cGVHD model and B) B10.D2 to BALB/c sclerodermatous cGVHD model. Data were expressed as mean ±SD. *P = 0.01–0.05, **P = 0.001–0.01, ***P = 0.0001–0.001, ****P < 0.0001.

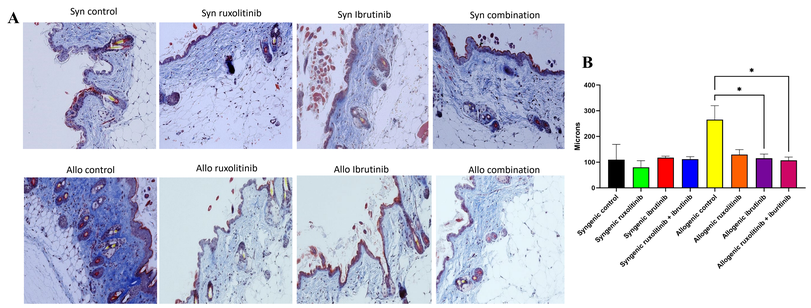

Figure 4: A) Representative images and B) quantification of Masson's trichrome staining of skin from syngeneic (n = 4), syn-ruxolitinib (n = 4), syn-ibrutinib (n = 4), syn-ruxolitinib + ibrutininb (n = 4), allogenic (n= 9), allo- ruxolitinib (n=9), allo- ibrutinib (n=9), allo - ruxolitinib + ibrutininb (n = 10) groups after transplant.

Reduced mast cell infiltration in dermal environment

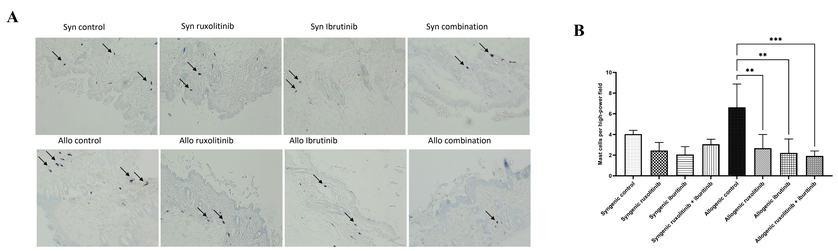

Figure 5: Representative images of (A) toluidine blue-stained skin and B) mast cell counts from syngeneic (n = 4), syn-ruxolitinib (n = 4), syn-ibrutinib (n = 4), syn- ruxolitinib + ibrutininb (n = 4), allogenic (n= 8), allo- ruxolitinib (n=8), allo- ibrutinib (n=8), allo - ruxolitinib + ibrutininb (n = 10) groups after transplant.

Figure 6: Transcriptomic analysis of cGVHD skin samples by RNA-seq analysis A) Heatmap of Pearson correlation plot shows different global gene expression profile between groups B) Heatmap of selected genes across different treatment groups C) Volcano plots identifies differentially expressed genes (DEG) between combination treatment vs Allogeneic control D) Pathway analysis based on DEGs shows multiple inflammatory pathways are altered in combination treatment vs Allogeneic control. Groups: SYN-Syngeneic control, SYN_IB – Syngeneic Ibrutinib, SYN_RX – Syngeneic Ruxolitinib, SYN_CB – Syngeneic Combination, ALLO – Allogeneic control, ALLO_IB – Allogeneic Ibrutinib, ALLO_RX – Allogeneic Ruxolitinib, ALLO_CB – Allogeneic Combination.

CONCLUSIONS

Our results suggest that combination therapy with ruxolitinib and ibrutinib are beneficial in improving the clinical GVHD scores in different mouse models by downregulating multiple inflammatory pathways (chemokine and JAK-STAT signaling) than standard monotherapy and ameliorating sclerodermatous cGVHD progression.

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CONTACT INFORMATION

Please reach out to V.Raguraman (vraguraman@missouri.edu) and Dr.G.C. Hildebrandt (gchhrb@health.missouri.edu) for additional information.