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applicability of posttransplant pharmacologic modulation of JAKs in order to both inhibit GVHD and control tumor burden by a drug-mediated effect or by making myeloid and lymphoid diseases more susceptible to allo-responses. Importantly, we found that ruxolitinib not only yields significant anti-GVHD activity, but also preserves the GVT effect against two different tumor cell lines and that this was not due to a direct antitumor effect but rather was likely the result of a sustained posttransplant T-cell alloreactivity. This is consistent with the fact that A20-mediated malignancy is driven by NF-κB activation rather than JAK–STAT signaling (34); consistent with this biology, we observed initial recurrence of GVHD at target sites on day +30 post-BMT after suspending ruxolitinib. Therefore, our results suggest that patients with a broad spectrum of hematologic malignancies may benefit from treatment with ruxolitinib after transplant.

In this study, we provide further strength to the recent findings by Choi and colleagues (35): that is, pharmacologic inhibition of the JAK1–JAK2 pathway by ruxolitinib was associated with a preserved GVT effect. Building upon this prior study, we now show that the preserved GVT activity by ruxolitinib is associated with maintained *in vivo* polarization of donor T cells toward the Th1 and Th17 phenotypes that are generally recognized as efficient mediators of antitumor effects (36, 37). Moreover, our observations are in agreement with a previous report from Choi and colleagues (19) that described JAK1/JAK2 inhibition by ruxolitinib as an approach to block the IFNγR–CXCR3 axis and prevent T-cell migration into GVHD organs. Indeed, we also found a reduction of CXCR3 expression, but this effect was more evident on $CD8^+$ cells, and limited on $CD4^+$ subsets. This modest effect of ruxolitinib on chemokine expression in our model suggests that other factors may have contributed to the reduced overall infiltration of donor T cells in the GVHD target tissues. Given the pathophysiologic three-step model of acute GVHD (38), reduced tissue infiltration may also have been a result of reduced alloreactivity and T-cell proliferation. For instance, Betts and colleagues (18) showed that JAK2 inhibition reduced DC-mediated T-cell activation, thereby impairing the activation of central and effector memory T cells as well as the expansion of responder Th1 and Th17 cells. We did not investigate DC activation in this study, but the fact that spleen T-cell infiltration, circulating T-cell numbers, Th1 and Th17 polarization were unchanged in mice receiving ruxolitinib suggests that this might not be the most relevant mechanism in our mouse model.

Of note, prevention of acute GVHD in our experiments was more pronounced in the skin and the intestine. Whether this effect was due to a different modulation of chemokines involved in liver GVHD, such as CXCR6 (39) and CCR5 (40), would be the object of further investigations. Interestingly, our findings partially confirm and also extend a recent study by Spoerl and colleagues (24) who reported a similar anti-GVHD effect of ruxolitinib in a mouse model of transplant. In fact, we demonstrated that ruxolitinib could preserve an immunologic GVT effect, but, differently from the earlier report, we could not identify increased Treg differentiation *in vivo*. It is possible that methodologic differences between the two experimental models may explain the fact that potential mechanisms of ruxolitinib mechanism of action against GVHD were identified.

In conclusion, our work provides further rationale for evaluating the effect of ruxolitinib on GVHD and GVT effects in clinic trials for patients with hematologic malignancies undergoing allogeneic HSCT. Although our findings point to chemokine modulation as a potential mechanism of action, further mechanistic studies will be required to better understand the precise mechanism of action of ruxolitinib for GVHD prevention. Our results open the way to test the potential application of new JAK inhibitors, such as pacritinib, both for regulating GVHD and directly inhibiting lymphoma cell proliferation after HSCT.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** C. Carniti, P. Corradini, J. Mariotti
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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** C. Carniti, S. Gimondi, A. Vendramin, C. Recordati, P. Corradini, J. Mariotti, D. Confalonieri
**Writing, review, and/or revision of the manuscript:** C. Carniti, C. Recordati, P. Corradini, J. Mariotti
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** A. Bermema
**Study supervision:** C. Carniti, P. Corradini, J. Mariotti

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