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POSTER ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

JAK2 ^{V617F} Impairs Lymphoid Differentiation in Myeloproliferative Neoplasms

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Myeloproliferative neoplasms (MPNs) arise from the clonal acquisition of a driver mutation in a hematopoietic stem cell (HSC), often decades before the emergence of clinical disease. A defining characteristic of an HSC is its potential to give rise to both myeloid and lymphoid hematopoietic lineages. Yet the most common MPN driver mutation, JAK2 ^{V617F}, has only rarely been observed in lymphocytes. We conducted this study to determine why the JAK2 ^{V617F} clone does not contribute to mature lymphocytes.

We directly measured myeloid and lymphoid lineage contribution of normal and MPN HSCs in 133 patients with JAK2 ^{V617F} MPN. Peripheral blood subpopulations were fractionated via fluorescence-activated cell sorting (FACS) and JAK2 ^{V617F} mutation allele frequency (MAF) was measured in each subpopulation by droplet digital PCR. JAK2 ^{V617F} was enriched in neutrophils and maintained in erythroid progenitors (EPs) relative to HSCs. In contrast, JAK2 ^{V617F} alleles were largely absent in T and B cells relative to HSCs.

Lymphopoiesis declines with age, potentially explaining the absence of JAK2 ^{V617F} in lymphocytes from older patients with MPN even if JAK2 ^{V617F} has no effect on lymphopoiesis. This "JAK2 ^{V617F} neutral" hypothesis predicts that patients who acquired the mutation at a younger age, and those who harbor the mutation for a longer duration, have a higher likelihood of accumulating JAK2mutated lymphocytes. However, we found that JAK2 ^{V617F} MAF in T and B cells across our cohort does not correlate with either patient age at diagnosis or duration of clinical disease. Thus, predictions of the "JAK2 ^{V617F} neutral" hypothesis are not supported by patient data.

The alternative explanation is that JAK2 ^{V617F} impairs lymphocyte differentiation from mutated progenitors. We tested this "JAK2 ^{V617F} adverse" hypothesis by tracking JAK2 ^{V617F} during various stages of lymphopoiesis. To assess the relative bias of JAK2 ^{V617F} HSCs during the early stages of lymphoid and myeloid commitment, we measured JAK2 ^{V617F} MAF in common lymphoid progenitors (CLPs), common myeloid progenitors (CMPs) and HSCs isolated by FACS from the peripheral blood of 79 patients with JAK2 ^{V617F} MPN. We found that while myeloid commitment from HSC to CMP was associated with an increase in mean JAK2 ^{V617F} MAF, there was no statistically significant change in mean MAF between CLPs and the parent HSCs across the cohort. Therefore, JAK2 ^{V617F} HSCs are not completely excluded from the earliest stages of lymphoid differentiation.

To further define the extent to which JAK2 ^{V617F} affects lymphopoiesis, we used FACS to isolate CLPs and CMPs from 17 patients with JAK2 ^{V617F} polycythemia vera (PV) and differentiated each subpopulation *in vitro* into T cell progenitors (pro- and pre-T cells) and erythroblasts (EBs), respectively. We found that all CLP samples could be differentiated into T cell progenitors. However, we observed a marked depletion of JAK2 ^{V617F} alleles in progressively mature T cell progenitors (Figure 1). In contrast, JAK2 ^{V617F} alleles were further enriched in progressively mature erythroblasts. The *in vitro* differentiation potential of MPN progenitors thus supports the "JAK2V617F adverse" hypothesis.

To determine the effect of JAK2 ^{V617F} on lymphopoiesis *in vivo*, we performed competitive transplantations of lethally irradiated CD45.1 mice with congenic CD45.2 donor whole bone marrow (WBM) cells harboring JAK2 ^{V617F} or wild type JAK2 (JAK2 ^{WT}). Measuring CD45.2 chimerism by FACS in lymphocytes and neutrophils from engrafted mice revealed that only CD45.2 cells harboring JAK2 ^{V617F} were preferentially depleted in lymphocytes (Figure 2). Therefore, JAK2 ^{V617F} HSCs displayed a competitive disadvantage relative to their JAK2 ^{WT} counterparts during lymphopoiesis *in vivo*.

Myeloid proliferation in MPN driven by JAK2 ^{V617F} is well recognized but does not fully explain certain MPN complications such as increased risk of infections and second malignancies. Our findings demonstrate impaired lymphoid differentiation from JAK2 ^{V617F} stem and progenitor cells that may also contribute to clinical MPN phenotypes. Further study is ongoing to define actionable mechanisms through which JAK2 ^{V617F} interferes with T cell differentiation.

Disclosures No relevant conflicts of interest to declare.

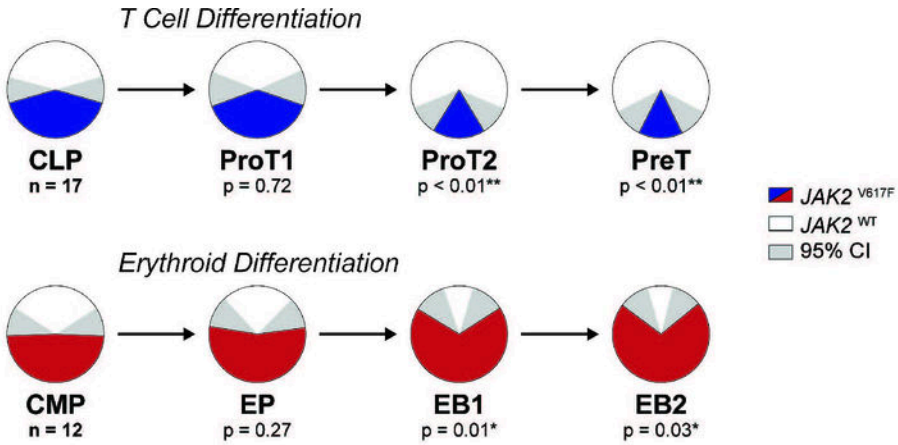

Figure 1. JAK2^{V617F} MAF declines with progressive T cell differentiation of PV CLPs *in vitro* (top). In contrast, JAK2^{V617F} MAF is enriched *in vitro* with progressive erythroid differentiation (bottom). Mean MAF for each subpopulation is shown as blue or red wedges with the 95% confidence intervals (upper bound) shown in gray. Statistically significant differences in mean MAF for a subpopulation relative to starting CLP or CMP MAF were identified using Wilcoxon matched-pairs signed rank tests.

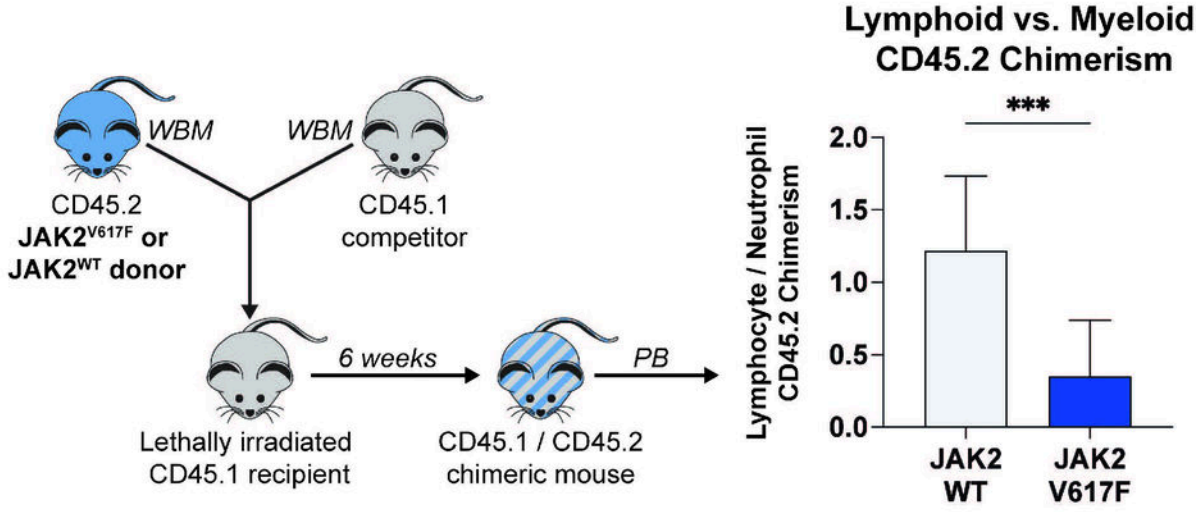

Figure 2. (Left) Schematic representation of competitive transplantation experiments to model lymphopoiesis from JAK2^{V617F} or JAK2^{WT} whole bone marrow (WBM) *in vivo*. PB = peripheral blood. (Right) CD45.2 donor cell chimerism measured by flow cytometry in lymphocytes, normalized to engraftment in neutrophils, is significantly lower in JAK2^{V617F} transplanted mice (n = 26) relative to JAK2^{WT} transplanted mice (n = 23) (*** p < 0.001, Mann-Whitney u-test).

Figure 1

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