Abstract 5217: 9-ING-41, a GSK-3β-selective small molecule inhibitor, in combination with ruxolitinib in *JAK2*617F primary myelofibrosis

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Abstract

Introduction: Primary myelofibrosis (MF) is a chronic clonal malignancy which is compounded by the presence of inflammation, bone marrow fibrosis/scarring and risk of transformation to acute myeloid leukemia (AML). Ruxolitinib (Rux), a JAK2 inhibitor, is beneficial for reduction of symptoms and splenomegaly, however, significant myelosuppression is also a toxic side effect of this treatment, limiting optimal drug dosage in many patients. 9-ING-41 is a small molecule GSK-3β inhibitor which has significant clinical activity in patients with advanced malignancies and causes no myelosuppression. We thus assessed the effect of 9-ING-41in MF alone or in combination with Rux. We performed ex-vivo colony assays with primary cells from cases with untreated MF along with normal bone marrow (BM). The experiments were designed to assess the effect of drug on the ability of MF stem/progenitor cells to proliferate/differentiate by evaluating the number, size, and the morphology of colonies, both with and without treatment.
Methods: Primary peripheral blood mononuclear cells from MF patients and BM from controls were plated in methylcellulose in duplicate, containing cytokines promoting hematopoietic growth, in the presence of either no drug, Rux only (0.05 μM), 9-ING-41 only (range: 0.125 μM – 10 μM), or a combination of 9-ING-41 (range: 0.125 μM - 10 μM) and Rux (0.05 μM). Colonies were counted ten days later, and colony growth frequency, distribution, and morphology were calculated.
Results: We observed an increasing presence of large primitive granulocytic/erythroid/macrophage/monocyte (GEMM) colonies proportional to no drug treatment and linear to increasing concentrations of 9-ING-41 in both MF cases relative to other colony types and normal BM, suggestive of a selective primitive proliferative and/or differentiation effect of GSK-3β inhibition on MF hematopoietic progenitor growth. We did not observe this effect in the combinatory treatments (9-ING-41 + Rux), and there was no significant difference on MF colony number or size in colonies grown without treatment or with Rux alone (Figure 1 A/C/E). However, the morphology of the colonies within different treatments was remarkable. In both MF cases, either without treatment or addition of Rux only (0.05μM), the colonies appeared similar to each other - irregular and disorganized relative to normal bone marrow (Figure 1, B/D/F). In the presence of 9-ING-41 alone, we observed morphologically very large, fully differentiated primitive colonies in both MF cases. However, the most striking observation was the comparison of colonies after combination of both inhibitors. These colonies appeared normal and healthy-looking -discreet, ro cases relative to other colony types and normal BM, suggestive of a selective primitive proliferative and/or differentiation effect of GSK-3β inhibition on MF hematopoietic progenitor growth. We did not observe this effect in the combinatory treatments (9-ING-41 + Rux), and there was no significant difference on MF colony number or size in colonies grown without treatment or with Rux alone (Figure 1 A/C/E). However, the morphology of the colonies within different treatments was remarkable. In both MF cases, either without treatment or addition of Rux only (0.05μM), the colonies appeared similar to each other -irregular and disorganized relative to normal bone marrow (Figure 1, B/D/F). In the presence of 9-ING-41 alone, we observed morphologically very large, fully differentiated primitive colonies in both MF cases. However, the most striking observation was the comparison of colonies after combination of both inhibitors. These colonies appeared normal and healthy-looking -discreet, round size and similar in morphology to normal bone marrow colonies. Additional studies are currently underway to look at the signaling consequences, including proliferation, differentiation, and cell cycle effect. Our preliminary results suggest this same mechanism might be occurring in MF cases and could explain the selective presence of large differentiated primitive colonies in MF vs. normal BM in the presence of 9-ING-41. Combining 9- ING-41 with Rux may reduce the dose needed for optimal therapeutic response and/or reverse myelosuppression. A Phase 2 study of 9-ING-41 in patients with advanced MF will be conducted.