MOLECULAR RESPONSE TO HYDROXYUREA AND ROPEGINTERFERON ALFA-2B IN THE PROUD-PV RANDOMIZED PHASE 3 TRIAL

Jean-Jacques Kiladjian\textsuperscript{1,2,3}, Bruno Cassinat\textsuperscript{2,4}, Juliette Soret-Dulphy\textsuperscript{1}, Emmanuelle Verger\textsuperscript{4}, Lydia Roy\textsuperscript{5}, Jerome Rey\textsuperscript{6}, Nabih Maslah\textsuperscript{4}, Barbara Grohmann-Izay\textsuperscript{7}, Christoph Klade\textsuperscript{7}, Heinz Gisslinger\textsuperscript{8}

\textsuperscript{1}Clinical Investigations Center, Hospital Saint-Louis, \textsuperscript{2}INSERM UMRS-1131, \textsuperscript{3}Paris Diderot University, \textsuperscript{4}Hospital Saint-Louis, Paris, \textsuperscript{5}Hospital Henri Mondor, Créteil, \textsuperscript{6}Institut Paoli Calmettes, Marseille, \textsuperscript{7}AOP Orphan Pharmaceuticals AG, \textsuperscript{8}Medical University of Vienna, Vienna, France

Please indicate all of which apply to the presenting author: MD, PhD

**Background:** Interferon alfa (IFNa) has been successfully used to treat myeloproliferative neoplasms (MPN) for many years and several phase 2 studies have independently shown high rates of hematological and molecular responses assessed by the quantification of mutant JAK2 allele burden (%JAK2V617F) in peripheral blood. However, direct in vivo studies investigating the impact of IFNa treatment on proliferation of bone marrow (BM) normal and malignant hematopoietic progenitors are lacking.

**Aims:** We took advantage of the randomized controlled phase III trial (PROUD-PV) comparing the novel, long-acting Ropeginterferon alfa-2b (AOP2014) with hydroxyurea (HU) in polycythemia vera (PV) patients (pts) to assess correlation between evolution of %JAK2V617F in peripheral blood and the impact of therapy on malignant clones by functional assays testing mutant BM hematopoietic progenitors in the French study population.

**Methods:** Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Ropeginterferon Alfa-2b in PV pts (NCT01949805). The primary endpoint was non-inferiority of AOP2014 vs. HU at 12 months (mos) of therapy in terms of complete hematological response (CHR) according to ELN criteria and normal spleen size. As important secondary endpoint the effect of treatment on %JAK2V617F was assessed as rate of complete and partial molecular response (C/PMR) according to modified ELN criteria. In the group of pts enrolled in France, we could study BM progenitors clonogenic potential by cultures with or without Erythropoietin (EPO) at baseline and after 12 months of therapy. The presence of colonies without EPO, namely Endogenous Erythroid Colonies (EECs) is a hallmark of PV. After 14 days, erythroid colonies were enumerated and picked for molecular analyses.

**Results:** A total of 257 pts were randomized in 13 European countries including 13 pts in France. Non-inferiority of AOP2014 versus HU regarding CHR could be demonstrated in the whole study population (43.1 vs 45.6 %). In the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46.5%, respectively, reduced to 29.1% and 25.8% after 6 mos, and 13.8% and 33.2% at 12 mos. No complete MR was achieved at 12 mos, but PMR was observed in 40% and 25% of pts in AOP2014 and HU arms (p= ns), respectively. BM progenitors could be studied in 10/13 French pts, 3 treated with AOP2014 and 7 with HU. AOP2014 treatment induced an important decrease of the proportion of EEC (median decrease 64%) between samples collected at baseline and after 12 months of therapy compared to HU (median decrease 25%). In addition, clonal architecture studies showed that the % of JAK2V617F mutant colonies before and after treatment profoundly decreased in all AOP2014-treated pts (mean ratio of mutant vs. wild type JAK2 colonies decreased from 96% at baseline to 46% at 12 mos). Among HU-treated pts, only 1 experienced a decrease in the % of mutated colonies while mean ratio of mutant vs. wild type JAK2 colonies didn’t significantly decrease (from 87% at baseline to 79% after 12 mos).

**Summary/Conclusion:** In this phase 3 trial comparing Ropeginterferon alfa-2b versus HU, we found a different impact of both drugs on hematopoietic cells. Although both treatment induced a decrease of JAK2 mutant allele burden at 12 mos in peripheral blood, BM clonogenic assays suggest that AOP2014 is able to specifically target JAK2 mutant progenitors, an effect not seen in HU treated pts. Such targeted impact of AOP2014 may account for the strikingly different kinetics in allele burden reduction and suggests that sustained long-term molecular response may only be achieved with IFNa based treatment.

**Keywords:** Interferon alpha, Molecular response, Polycythemia vera