AG-946, an activator of pyruvate kinase, improves ineffective erythropoiesis in the bone marrow of mouse models of myelodysplastic syndromes

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BACKGROUND

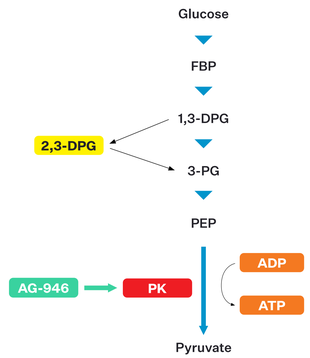
Myelodysplastic syndromes

- Myelodysplastic syndromes (MDSs) are a heterogeneous group of hematologic malignancies characterized by ineffective erythropoiesis[1–4]
- Anemia, the most common cytopenia of MDS, represents a major clinical problem, as it is experienced by >90% of patients with MDS at either diagnosis or throughout the course of disease[5–7]
- Anemia in MDS is associated with numerous complications, such as transfusional iron overload and debilitating fatigue, which contribute to a negative impact on patient quality of life and can predispose patients to additional morbidities[1,6,8]
- Defective erythroid maturation and acquired pyruvate kinase (PK) deficiency have been reported in patients with MDS
- Preliminary data have shown decreased glycolytic activity in MDS red blood cells (RBCs), indicating a potential mechanism in the pathogenesis of MDS-associated anemia[9–14]
- Treatment options for anemia in MDS are limited, and there continues to be a need for novel therapies[1,7]

AG-946

- AG-946 is an investigational, potent, small-molecule, allosteric activator of PK (**Figure 1**) that has the potential to:
  - Increase RBC glycolysis and ATP production to enhance RBC functionality[16]
  - Improve anemia driven by ineffective erythropoiesis through improved maturation of erythroid cells in bone marrow (BM)[17,18]
- AG-946 has been demonstrated to increase PK activity in RBCs of patients with MDS[13,19]; however, further research is required to understand the effects of AG-946 as an activator of PK targeting this mechanism of anemia

Figure 1. Mechanism of action of AG-946, an investigational, oral activator of PK[9,10,15,16,20–22]



1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; ADP, adenosine diphosphate; FBP, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvate; PK, pyruvate kinase

OBJECTIVE

- **To evaluate the effect of AG-946 treatment on impaired erythropoiesis in the BM of 2 mouse models of MDS**

METHODS

Study design

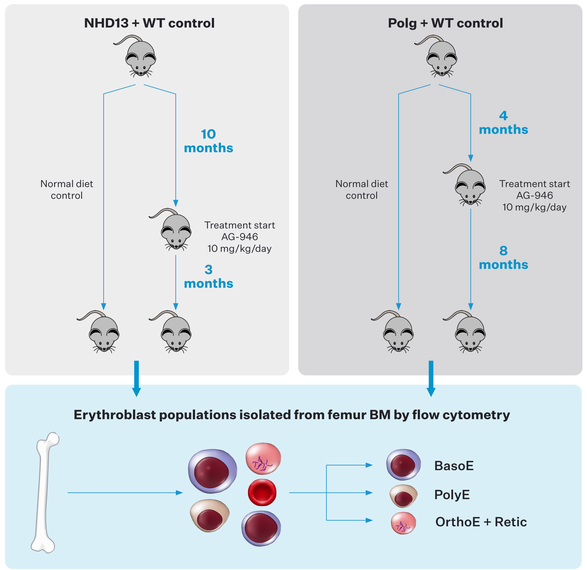
- 2 mouse models that recapitulated key aspects of MDS, including impaired erythropoiesis and progressive anemia, were used (**Figure 2**):
  - NUP98-HOXD13 (NHD13), a fusion gene mutation observed in some patients with MDS
  - Polg^{D257A} (Polg), a mutation not observed in patients with MDS
- Male and female non-carrier (wild-type [WT] littermate control) and C57BL/6J mice with NHD13 were placed on an ad libitum AG-946-formulated diet (an approximate dose of 10 mg/kg/day), started at 10 months of age and continued for 3 months
- Male and female non-carrier (WT) and Polg mice were placed on the same diet, started at 4 months of age and continued for 8 months
- NHD13, Polg, and WT mice fed on a diet without AG-946 were used as a control

Analyses

- BM erythroblast populations were gated using Single Cells/Live/B220-/Ter119+/CD44 vs forward scatter
- The selected populations were then gated into 3 further populations:
  - Basophilic erythroblasts (BasoE) (early-stage erythroblasts)
  - Polychromatic erythroblasts (PolyE) (early-stage erythroblasts)
  - Orthochromatic erythroblasts and reticulocytes (OrthoE + Retic) (late-stage erythroblasts)
- Samples with <1000 Ter119+ events were removed from the analysis due to insufficient cell count
- At the end of the study, whole blood was collected from mice 1 hour after lights were switched on (12 hours light/dark cycle), with plasma isolated for PK evaluation
- AG-946 concentration was determined by liquid chromatography in tandem with mass spectrometry
- BM from 1 femur per mouse was collected and analyzed by flow cytometry, with BM cell pellets washed and treated with ammonium–chloride–potassium lysis buffer prior to staining
- Flow cytometry analysis was performed on BM, and the mean (standard error of the mean) proportion of Ter119+ cells in the gated erythroblast populations at the end of AG-946 treatment was assessed (**Figure 2**)
  - Differences between groups were analyzed by ordinary 1-way ANOVA (analysis of variance)

Figure 2. Study design and methods

Characteristic	Anemia	Pancytopenia	Dysplasia	N–H BM cellularity	Survival	MDS or MDS-like
NHD13	✓	✓	✓	✓	14 months	✓
Polg	✓	✓	✓		15 months	✓

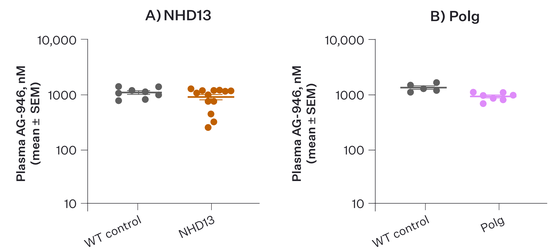


BasoE, basophilic erythroblasts; BM, bone marrow; MDS, myelodysplastic syndrome; N–H, normal–hyper; NHD13, NUP98-HOXD13; OrthoE + Retic, orthochromatic erythroblasts and reticulocytes; Polg, Polg^{D257A}; PolyE, polychromatic erythroblasts; WT, wild-type

RESULTS

- AG-946 plasma concentrations were generally similar between control and MDS model mice (**Figure 3**)
  - Larger within-group variations were observed for NHD13 mice, potentially due to reduced food intake as a result of disease burden

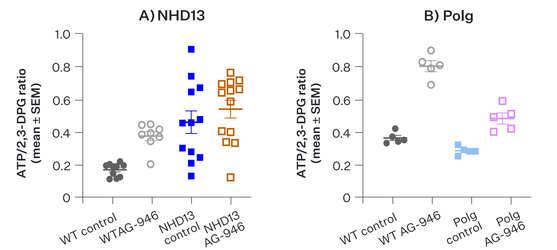
Figure 3. AG-946 plasma concentrations at end of study in (A) NHD13 mice and (B) Polg mice



NHD13, NUP98-HOXD13; Polg, Polg^{D257A}; SEM, standard error of the mean; WT, wild-type

- NHD13: AG-946 increased the ratio of ATP/2,3-diphosphoglycerate (2,3-DPG) vs control in WT mice, but the effect was minimal in NHD13 mice (**Figure 4A**)
  - Larger within-group variations were observed for NHD13 mice than with other groups
- Polg: AG-946 increased ATP/2,3-DPG ratio vs control in both WT and Polg mice (**Figure 4B**)

Figure 4. AG-946 pharmacodynamics in (A) NHD13 mice and (B) Polg mice



2,3-DPG, 2,3-diphosphoglycerate; NHD13, NUP98-HOXD13; Polg, Polg^{D257A}; SEM, standard error of the mean; WT, wild-type

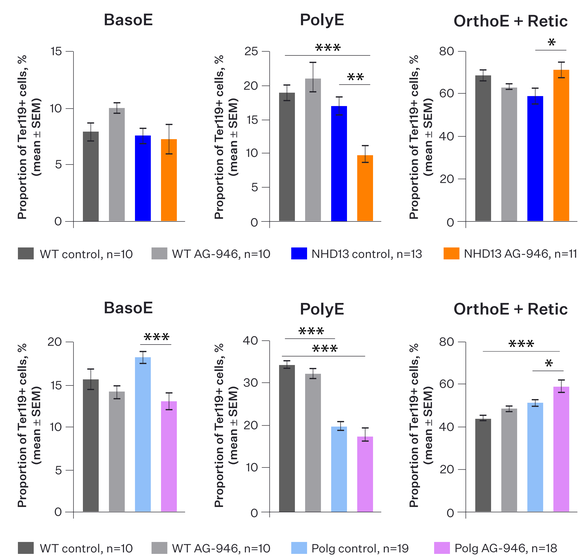
NHD13 mice

- No significant differences were observed in any erythroblast population between untreated WT and NHD13 mice (**Figure 5**)
- NHD13 mice treated with AG-946 showed significantly decreased PolyE and significantly increased OrthoE + Retic populations compared with untreated NHD13 mice (p=0.0021 and 0.0275, respectively)

Polg mice

- No significant differences in BasoE or OrthoE + Retic populations were observed between untreated WT and Polg mice; the PolyE population was significantly decreased in untreated Polg mice compared with WT (p<0.0001) (**Figure 5**)
- AG-946-treated Polg mice had significantly decreased BasoE and significantly increased OrthoE + Retic populations compared with untreated Polg mice (p=0.0002 and 0.0304, respectively)
- No significant difference in the PolyE population was observed in Polg mice following treatment with AG-946
- Similar trends were observed when CD71 cells were assessed (data not shown)

Figure 5. Effect of AG-946 on erythroblast maturation in mouse models of MDS



*p≤0.05; **p≤0.01; ***p≤0.001

BasoE, basophilic erythroblasts; MDS, myelodysplastic syndrome; NHD13, NUP98-HOXD13; OrthoE + Retic, orthochromatic erythroblasts and reticulocytes; Polg, Polg^{D257A}; PolyE, polychromatic erythroblasts; SEM, standard error of the mean; WT, wild-type

CONCLUSIONS

- **Our data suggest AG-946 treatment improves erythroblast maturation in 2 MDS mouse models, as demonstrated by a reduction in early-stage erythroblasts (BasoE, PolyE) coupled with an increase in late-stage erythroblast populations (OrthoE + Retic)**

These data are the first to suggest that PK activation by AG-946 could improve ineffective erythropoiesis in MDS

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