Pharmacological Evaluation of a First-in-Class Hemoglobin Elevating Agent (HbEA) AND017 in a Mouse MDS Model of an Inducible Mutant c-Myc Knock-in

Gang Huang[1], Jie Bai[1], Mingzhe Pan[1], Dong Liu[2], Min Xiong[2], and Shaojiang Deng[2]

1. Department of Cell Systems & Anatomy, UT Health San Antonio; 2. Kind Pharmaceuticals LLC, Redwood City, CA, USA

INTRODUCTION

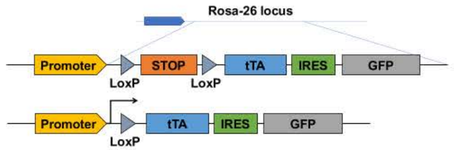
Myelodysplastic syndromes (MDS) are a group of diseases of hematopoietic stem cells (HSCs) that occur primarily in the aging population either de novo or secondarily following therapy for unrelated cancers. Among the pan-cytopenia, anemia-related symptoms caused by ineffective erythropoiesis and reduced RBC and hemoglobin levels are a serious condition by most MDS patients that has yet to be resolved by transfusion and/or erythropoiesis-stimulating agents (ESAs).

AND017 is a first-in-class hemoglobin elevating agent (HbEA), which has been shown excellent efficacy in a wild type rat model, rat 5/6 nephrectomy chronic kidney disease (CKD) model, the Hbbd3th β-thalassemia mouse model, and Townes sickle cell disease (SCD) mouse model. In this study, we evaluated hemoglobin elevating agent (HbEA), AND017, in our MDS mouse model.

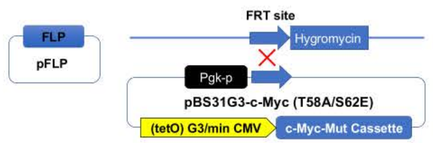
METHOD

To mimic low c-Myc-driven MDS-like phenotypes in mice, we generated a knock-in (KI) mouse model by introducing a doxycycline (DOX)-inducible double mutations c-Myc (T58A/S62E) expression cassette at Collagen 1A1 (Col1a1) locus. This KI allele was further combined with Vav1-Cre and ROSA-26-LSL-rtTA alleles in C57 mice. In this mouse model, Vav1-Cre expression induces cleavage in LSL casettte, resulting expression of rtTA. After DOX administration, rtTA binds to Tet promoter of c-Myc (T58A/S62E) cassette and promotes its expression.

A. When expressing specific Cre (*Vav1*-Cre), tTA and GFP will express

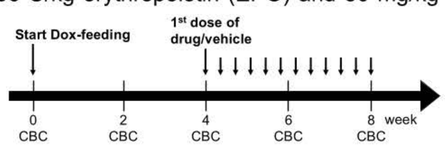


B. KI into ColA1 locus (KH2 ES cells)

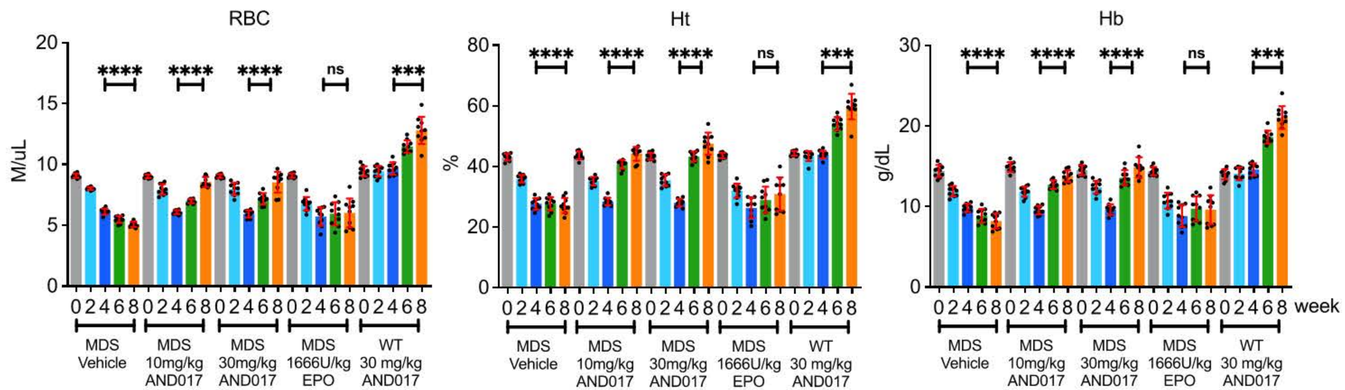


C. Adding Doxycycline, tTA binds to tet-promoter and turns on c-Myc (T58A/S62E), dominant negative c-Myc mutant leads to MDS

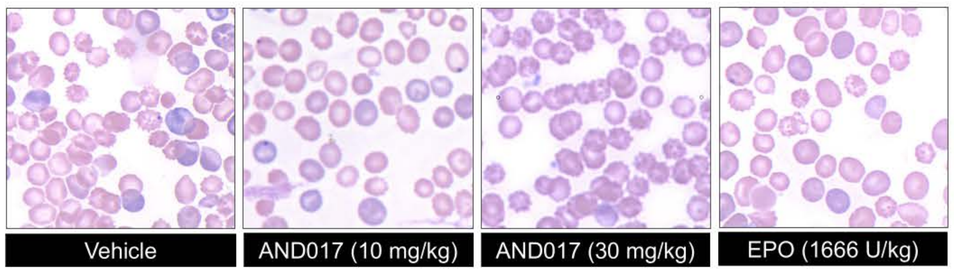
Forty Myc mutant KI mice (female/F: 20; male/M: 20; 8-12 weeks) were randomly divided into 4 groups: G1, G2, G3 and G4 (5F + 5M in each group). Ten Vav1-Cre/LSL-tTA mice (female/F: 5; male/M: 5; 8-12 weeks) were in G5. Mice in all 5 groups were fed with chaws containing 200 mg/kg Doxycycline. After 4 weeks, mice in G1, G2, G3, G4 and G5 were orally dosed three times per week with vehicle, 10 mg/kg AND017, 30 mg/kg AND017, 1666 U/kg erythropoietin (EPO) and 30 mg/kg AND017 respectively for 28 days. RBC, HGB, and HCT were measured and calculated by a hematology system on days 0, 14, and 28, respectively. The level of statistical significance was set at 5% or P < 0.05; the data were compared to G1 at the same time point.



RESULTS



AND017 was well tolerated in all groups. HGB levels (g/dL) for G1 to G5 were 9.67, 9.52, 9.54, 8.77 and 15.08 respectively on day 0; were 8.72, 10.97****, 13.56****, 9.76 and 18.48**** on day 14; and were 8.19, 11.77****, 14.91****, 9.57 and 20.62**** on day 28. The RBC numbers ($\times 10^6$cells/μL) for G1 to G5 were 6.01, 6.03, 5.90, 5.65 and 9.61 on day 0, were 5.57, 6.33***, 7.11****, 5.91 and 11.39**** on day 14; and were 5.27, 7.17****, 8.46****, 5.95 and 12.57**** on day 28. The hematocrits (%) for G1 to G5 were 27.47, 28.12, 28.01, 26.15 and 43.78 on day 0; were 27.69, 34.36****, 43.13****, 30.14 and 53.57**** on day 14; and were 27.13, 37.52****, 47.41****, 30.69 and 59.25**** on day 28. (*, **, ***, and ****: for p < 0.05, 0.01, 0.001, and 0.0001 respectively)



Microscopic examination of blood smears showed enhanced erythrocyte staining, and decreased ratio of abnormal red blood cells obviously after treatment with AND017 dose dependently, indicating marked improvement in the morphology of red blood cells.

CONCLUSIONS

AND017 elevated important several parameters related to anemia significantly and dose-dependently, including Hb levels, RBC numbers, and HCTs in a c-Myc mutant KI MDS mouse model, indicating that AND017 has the potential to be a safe, efficacious and convenient treatment for MDS patients in the future. Future clinical trials for AND017 in MDS are warranted.

CONTACT INFO

Gang Huang, PhD: huangg1@uthscsa.edu
Jie Bai, PhD: baij4@uthscsa.edu
Dong Liu, PhD: liudong@kindpharmaceutical.com
Shaojiang Deng: sdeng@kindpharmaceutical.com