ADMINISTRATION OF ALK2 NEUTRALIZING ANTIBODIES TO CYNOMOLGUS MONKEYS LED TO A SUSTAINED DECREASE IN HEPCIDIN, INCREASE IN CIRCULATING IRON AND INCREASE IN ERYTHROCYTE HEMOGLOBIN

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INTRODUCTION

- Hepcidin is a key endocrine mediator of iron metabolism, regulating dietary iron uptake and serum iron levels. Hepcidin binds to and activates the degradation of the iron exporter, ferroportin, in peripheral tissues.[1]
- When hepcidin levels are high, iron is sequestered within cells, increasing tissue iron levels and reducing serum iron availability.
- Elevated hepcidin levels are associated with certain conditions of iron deficiency anemia, including iron-refractory iron deficiency anemia (IRIDA) and anemia of inflammation.[2]
- ALK2 is a type I TGF-β receptor that, upon activation with its BMP ligand(s) and hemojuvelin co-receptor, increases expression of hepcidin.[1]
- KTI-016 and KTI-018 are two novel human monoclonal antibodies that are designed to specifically bind to and neutralize ALK2.
- We have previously reported that inhibition of ALK2 with KTI-016 suppressed hepcidin levels and increased serum iron in healthy mice and in mice with iron deficiency anemia.

OBJECTIVE

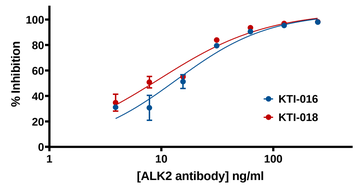
Our objective was to determine the pharmacokinetics and iron-related pharmacodynamic properties of KTI-016 and KTI-018 in cynomolgus monkeys.

METHODS

- **Animals**
  - Female 2-4-year-old cynomolgus monkeys (n=3/group).
- **Procedure**
  - KTI-016 or KTI-018 administered as a single 3 mg/kg subcutaneous dose.
  - Blood was assessed intermittently over a 56-day period for drug exposure, hepcidin, iron content, reticulocyte hemoglobin (RET-Hgb), red blood cell hemoglobin (RBC-Hgb), and mean corpuscular hemoglobin concentration (MCHC).
- **Statistics**
  - Data were analyzed using Prism 9 (GraphPad Software, San Diego, CA, USA) using one-way ANOVA with Fisher's LSD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Error bars = SEM.

RESULTS

KTI-016 and KTI-018 inhibited ALK2 signaling in a cell reporter assay



IC50 (ng/mL)	
KTI-016	14
KTI-018	9.6

Figure 1. *KTI-016 and KTI-018 inhibited ALK2 signaling in a BRE-Luc SMAD1/5/8 C2C12 reporter cell line, with comparable IC50 values of 14 ng/mL for KTI-016 and 9.6 ng/mL for KTI-018. BMP6 at 50 ng/mL was used as the agonist.*

Observed PK profiles of KTI-016 and KTI-018

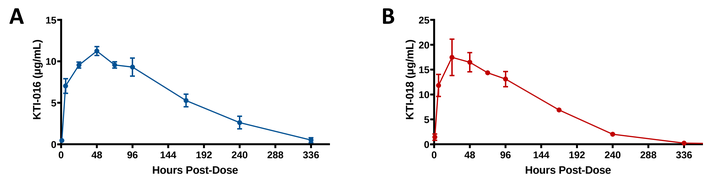


Figure 2. *KTI-016 and KTI-018 were rapidly absorbed, reaching Cmax within 48 hours. **(A) KTI-016 showed a half-life of 49.1 hr. (B) KTI-018 showed a half-life of 33.9 hr.** Half-lives were calculated using a standard non-compartmentalized model.*

ALK2 antibodies reduced serum hepcidin

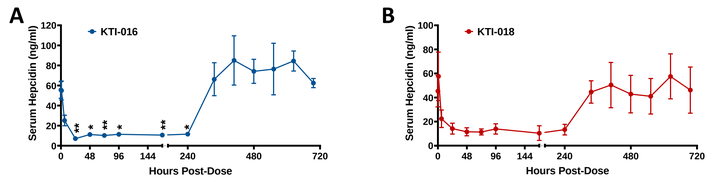


Figure 3. *KTI-016 and KTI-018 had rapid effects on serum hepcidin that were sustained through 10 days. **(A) KTI-016 treatment resulted in a 50.3% decrease at 6 hours post-dose, with a maximal decrease of 77.8% starting at 48 hours post-dose. (B) KTI-018 treatment resulted in a 55.6% decrease at 6 hours post-dose, with a maximal decrease of 77.2% starting at 48 hours post-dose.***

ALK2 antibodies increased serum iron levels

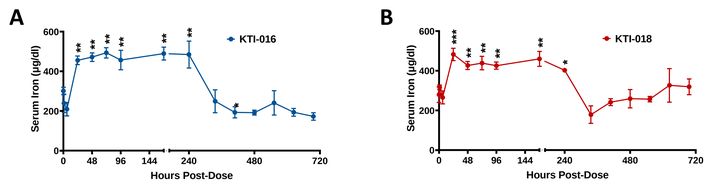


Figure 4. *KTI-016 and KTI-018 also had rapid effects on serum iron levels that were observed starting at 24 hours post-dose and sustained through 10 days. **(A) KTI-016 treatment resulted in a maximal increase of 63.9%. (B) KTI-018 treatment resulted in a maximal increase of 72.4%.***

RESULTS

ALK2 antibody-mediated iron mobilization increased Hgb incorporation into RETs and RBCs

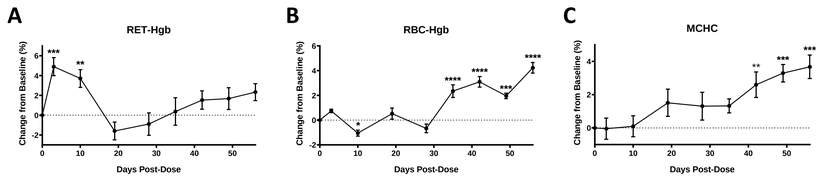


Figure 5. *KTI-016 and KTI-018 had comparable effects on increasing RET-Hgb, RBC-Hgb, and MCHC (data from two antibodies were combined). **(A)** RET-Hgb content reached a maximal increase of 4.9% at 3 days post-dose, remaining elevated through at least 10 days. **(B)** Increases in RBC-Hgb content were observed starting at 35 days post-dose, with a 4.2% increase at study termination on day 56. **(C)** Increases in MCHC were observed starting at 42 days post-dose, with a 3.7% increase at study termination on day 56.*

CONCLUSIONS

- KTI-016 and KTI-018 are novel human monoclonal antibodies designed to inhibit ALK2 signaling, potentially resulting in decreased hepcidin and increased serum iron availability.
- Similar to observed effects in mice, KTI-016 and KTI-018 robustly suppressed hepcidin levels and increased circulating iron in monkeys by as much as 72%.
- In addition, the mobilized iron was incorporated into hemoglobin, as evidenced by observed increases in RET-Hgb, RBC-Hgb, and MCHC.
- These data demonstrate that the effects of KTI-016 and KTI-018 on iron parameters translate to non-human primates, a preclinical model highly representative of human biology.
- These results provide evidence that treatment with KTI-016 or KTI-018 may be a potential treatment for anemias arising from elevated hepcidin, such as IRIDA and anemia of inflammation.

REFERENCES

1. **Sangkhae V and Nemeth E.** Regulation of the Iron Homeostatic Hormone Hepcidin. *Adv Nutr 2017; 8: 126-36*

2. **Pagani A et al.** Hepcidin and Anemia: A Tight Relationship. *Front Physiol 2019; 10: 1294*