ALK2 INHIBITION, A NOVEL THERAPEUTIC APPROACH TO IRON OVERLOAD

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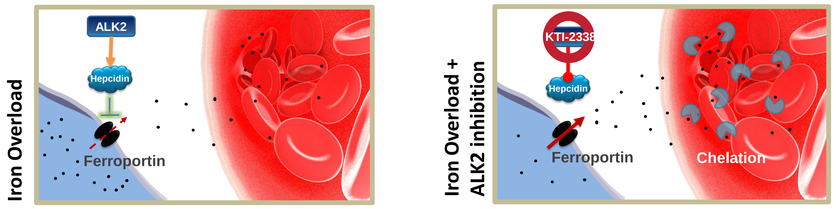
INTRODUCTION

- Hepcidin is a key endocrine regulator of iron which is expressed, at least in part, in response to signaling through the ALK2 receptor, a type I TGF-β receptor, that mediates dietary iron uptake and iron levels in blood. Hepcidin circulates to peripheral tissues and binds to and activates the degradation of the iron exporter, ferroportin. Dysregulation results in defective iron metabolism.
  - High hepcidin: iron is sequestered within cells resulting in increased tissue iron levels, reduced absorption from diet and reduced serum iron availability.
  - Low hepcidin: ferroportin-mediated iron efflux results in the mobilization of iron out of tissues and enhanced absorption from diet which, in its extreme, can result in iron overload.
- Functional iron deficiency anemia and iron-refractory iron deficiency anemia are a result of elevated hepcidin which reduces ferroportin and sequesters iron in tissues. The result is insufficient circulating iron for adequate erythropoiesis in bone marrow.
- Our preclinical data demonstrated that ALK2 inhibition resulted in mobilization of iron into serum and increased RBC hemoglobin in mouse models of functional iron deficiency.
- Phase 1 clinical trial data demonstrated that inhibition of ALK2 with KER-047, a small molecule ALK2 kinase inhibitor, elicited rapid, robust and sustained dose-related increases in serum iron that were associated with decreases in hepcidin. Changes were also associated with decreased serum ferritin, supporting that KER-047 was, at least in part, mobilizing iron out of tissue stores to elicit its effect on serum iron.
- Iron overload is a condition where excess iron accumulates in peripheral tissues and circulation. It can be caused by genetic mutation of the HFE gene or introduced via blood transfusions, hemolysis or diet. The increase in unbound free iron in serum and tissues causes oxidative stress, tissue damage and reduces the efficiency of hematopoiesis.
- Current therapies for iron overload include iron chelators that remove iron from circulation; however, this process is inefficient and requires prolonged therapeutic periods.
- Although potentially counterintuitive, we considered the possibility that the activity of ALK2 inhibition in mobilizing iron out of tissues could be beneficial in iron overload. Under these conditions, reduction of circulating hepcidin and mobilization of the excess iron from tissues should aid in the removal of iron via chelation, or excretion.

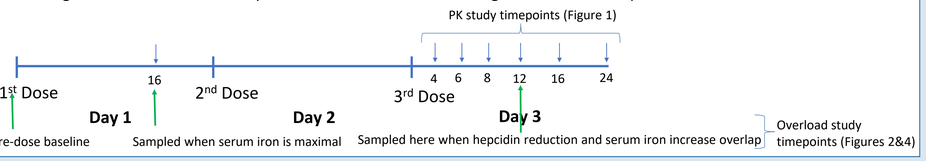


OBJECTIVE

To optimize inducible models of iron overload and assess the effect of ALK2 inhibition on regulation of hepcidin and hepatic iron content in iron overload.

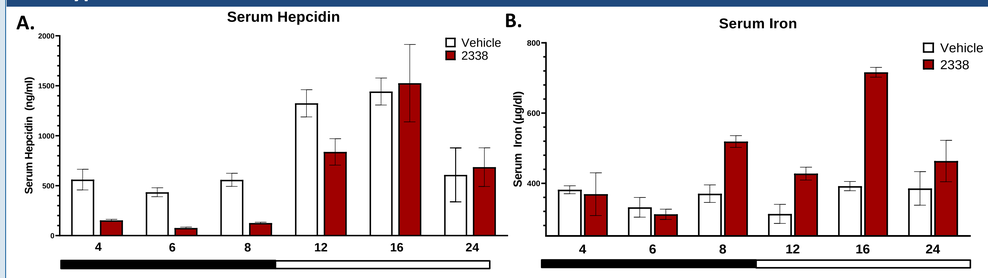
METHODS

- For PK/PD analysis, mice were gavaged, QD with either KTI-2338, a small molecule selective ALK2 kinase inhibitor (5 mg/kg), or vehicle. On the third day, all samples were assayed at the timepoints indicated on the diagram below.
- To induce iron overload, CD1 mice were dosed QD with 100 mg/kg of iron dextran or vehicle, IP. After 20 days, a subgroup of mice was sacrificed to confirm iron overload. The remaining mice were dosed QD with either KTI-2338, or vehicle. Iron dextran administration continued throughout the therapeutic period. Mice were sacrificed 16hr post the 1st dose (16hr) and 12hr post the 3rd dose (63hr) to assess timepoints where hepcidin and iron were both regulated.
- Non-heme tissue iron was extracted with either of two protocols: One, where tissues were homogenized and hydrolyzed in 30% HCl 10% Trichloroacetic acid mixture before incubating at 65° C for 20hr. The other, optimized to mitigate iron dextran interference, where tissues were processed in 9.5% HCl 10% Trichloroacetic acid.
- Homogenates from both techniques were assessed for iron using a standard bathophenanthroline disulfonate method.



RESULTS

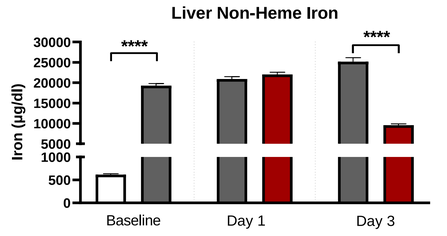
Figure 1: Treatment with KTI-2338 reduced circulating hepcidin levels and increased serum iron in wild-type mice.



Time course assessment of serum hepcidin and iron levels with and without KTI-2338 demonstrates:

- 2338 treatment reduced hepcidin as early as the first time point, 4 hours post administration – and sustained through 12hr
- 2338 treatment increased serum iron as early as 8 hours post administration, peaking at 16hr at 716.31µg/dl
- **These timepoints were subsequently used to assess whether 2338 acts to increase serum iron, at least in part, through mobilization of iron out of tissues and whether 2338 could reduce liver iron content in iron overload**

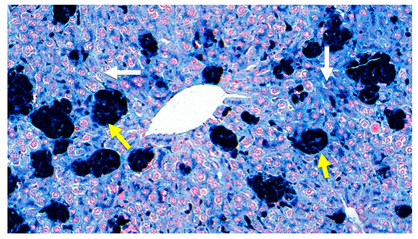
Figure 2: ALK2 inhibition resulted in a reduced liver iron content from overloaded livers.



Iron dextran resulted in a 30-fold increase in hepatic iron confirming iron overload. No differences were observed in hepatic iron at 16hr post dose. However, by 63hr of KTI-2338 dosing, significant reductions in liver non-heme iron content were observed.

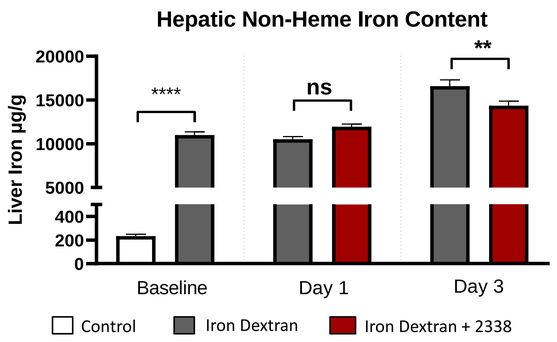
Control Iron Dextran Iron Dextran + 2338

Figure 3: Iron dextran overload resulted in the formation of siderotic nodules that may confound the ability to assess iron stored within hepatocytes.



Micrograph of iron loaded liver with Prussian blue staining. Yellow arrows highlight iron dextran-burdened Kupffer cells in intrasinusoidal and portal siderotic nodules (1,2). White arrows indicate staining of intra-hepatocyte/parenchymal iron. Iron dextran requires phagocytic breakdown of dextran to liberate labile iron within the Kupffer cell before release and storage within hepatocytes. Due to the supraphysiologic level of iron dextran contained, these nodules have been described as permanent (2).

Figure 4: ALK2 inhibition mobilized labile iron out of hepatic parenchymal cells.



Further optimization including reducing acid hydrolysis to 9.5% HCl may mitigate the extraction of iron from iron dextran within Kupffer cells. This may have reduced the likelihood of iron dextran and the processes of iron dextran breakdown within the Kupffer cell confounding the ability to assay intra-hepatocyte iron. Data shows a reduced but significant reduction in iron that is likely more reflective of intra-hepatic iron. Data is shown as average.

All data are shown as average ± SEM. N=10 per group. Stats calculated using 1-way ANOVA and individual comparisons done with a Tukey post test. ns= not significant, * P≤0.05, ** P<0.01, *** P<0.001, **** P<0.0001

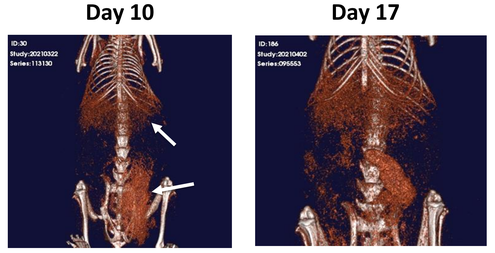
CONCLUSIONS

This proof-of-concept study has demonstrated that, in this supraphysiologic model of iron overload, ALK2 inhibition was able to mobilize iron from livers loaded with an excess.

FUTURE DIRECTIONS

Future work will assess the mobilization of iron from tissues in genetic and diet-induced models of iron overload. The effect of systemic iron chelation to aid in excretion of the iron liberated from tissues will also be investigated.

Tissue iron resolves on µCT and may be used to track iron overload in mice



µCT image of a mouse after 10 and 17 days of loading IP with iron dextran. Arrows highlight iron dextran resolving in the liver and peritoneum. Future studies will clarify the nature of the iron visualized and assess whether the approach can be used to track hepatic iron overload in live mice.

ACKNOWLEDGEMENTS

Keros Therapeutics Research Team

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