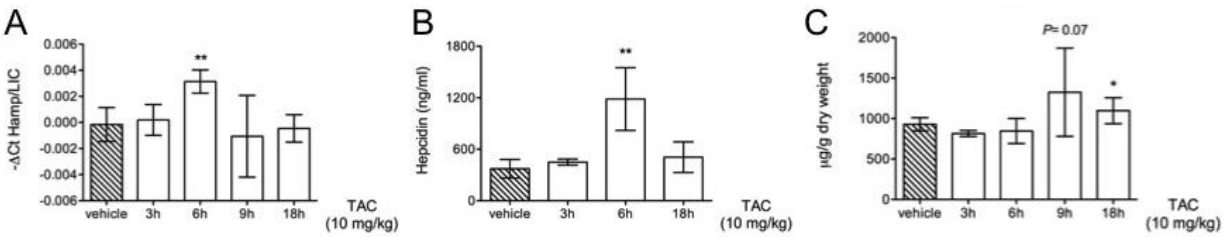
"FKBP12 as druggable target for the treatment of iron-overload diseases"

Background and Description of Invention. Hepcidin is the key regulator of iron homeostasis and it is a short peptide mainly produced by hepatocytes, which, once secreted into the circulation, binds the sole cellular iron exporter ferroportin, triggering its internalization and degradation. Through this mechanism, hepcidin reduces circulating iron by blocking dietary iron absorption in duodenal enterocytes and iron release from other cellular storage systems, mainly macrophages.
Iron is essential for several functions, such as energy production, DNA synthesis and metabolic pathways, but can be toxic if accumulated. Hepcidin expression is tightly regulated in response to multiple stimuli like body iron concentration, erythropoiesis, inflammation, gluconeogenesis, hormones and drugs, including the mTOR inhibitor rapamycin. Hepcidin synthesis is mainly controlled by the Bone Morphogenetic Protein (BMP)–Son of Mother Against Decapentaplegic (SMAD) pathway.
Decreased production of hepcidin leads to iron overload disorders, such as hemochromatosis and β-thalassemia. In hereditary hemochromatosis, defective hepcidin synthesis is caused by mutations in genes that regulate the liver BMP-SMAD pathway. In β-thalassemia, on the other hand, the expanded ineffective erythropoiesis, due to defective β-globin chain synthesis, downregulates hepcidin expression through the erythroid regulator erythroferrone. Current therapies based on phlebotomy in hemochromatosis, and on blood transfusions-iron chelation in β-thalassemia are symptomatic approaches, unsatisfactory or not applicable to all cases.

The present invention identifies a new level of hepcidin regulation, based on the druggable target FKBP12. According to the invention, inhibitors able to displace FKBP12 from the BMP type I receptor ALK2 can be used for the treatment of iron-overload diseases. Tacrolimus (FK506) and rapamycin, by interacting with the same FKBP12-binding pocket, are able to activate hepcidin through the BMP/SMAD pathway; in particular Tacrolimus is able to completely abrogate the interaction between FKBP12 and ALK2.



D

	TAC (10mg/kg)			
Serum Iron (µg/dl)	vehicle	3h	6h	18h
	241±55	331±77	178±44	161±32

Figure 1. Acute tacrolimus treatment upregulates hepcidin *in vivo.* Adult C57BL/6N male wild-type mice were treated with a single subcutaneous injection of tacrolimus (10 mg/kg) or vehicle (DMSO) for different time points (from 3 to 18 hrs). To normalize for variation of liver iron we expressed hepcidin as the hepcidin/LIC (liver iron concentration) ratio. Both hepcidin mRNA (**A**) and serum hepcidin (**B**) were significantly increased by tacrolimus at 6 hrs post-injection. This effect was accompanied by increased spleen iron content (**C**) and trend towards serum iron reduction (**D**) pointing out that the drug modulates hepcidin and iron homeostasis *in vivo*, at least in an acute setting (error bars indicate SD. *p< .05; **p< .01)

Patent information. A US patent application related to the invention was filed (not published yet). A scientific article related to the invention has been published on Blood (Colucci et al., 2017: The immunophilin FKBP12 inhibits hepcidin expression by binding the BMP type I receptor ALK2 in hepatocytes).

Potential Applications and Competitive Advantages. Thus disruption of the FKBP12-ALK2 interaction could be relevant in conditions of iron overload characterized by increased BMP6.

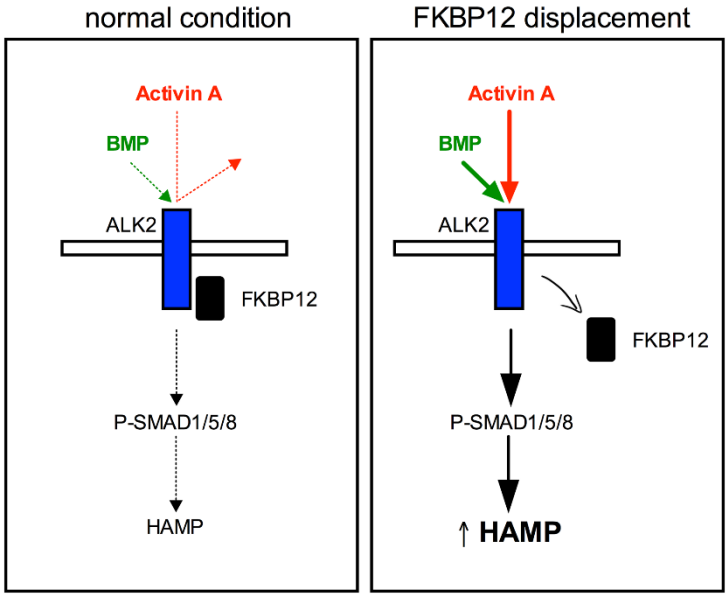


Figure 2. Proposed model for FKBP12 function. When ALK2-FKBP12 interaction is impaired, as in case of the FKBP12 binding drugs rapamycin or tacrolimus, the receptor becomes responsive to the non-canonical ligand Activin A and increases its responsiveness to the canonical ligand BMP6, triggering hepcidin activation through SMAD1/5/8 (HAMP: hepcidin).

Several advantages can be envisaged:

- Repurposing of tacrolimus for the treatment of iron overload disorders.
- Higher specificity in the treatment of iron-overload diseases in view of the specific targeting of FKBP12-ALK2 interaction.
- New druggable target for the development of specific small molecules or biologics.

We seek a potential commercial partner with a pipeline in <u>small molecule therapeutics applied to iron-overload diseases</u>.

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