Evaluation of the Toxicological Risk of targeting FRS (Phenylalanyl-tRNA Synthetase) in the Treatment of Malaria

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1. Background

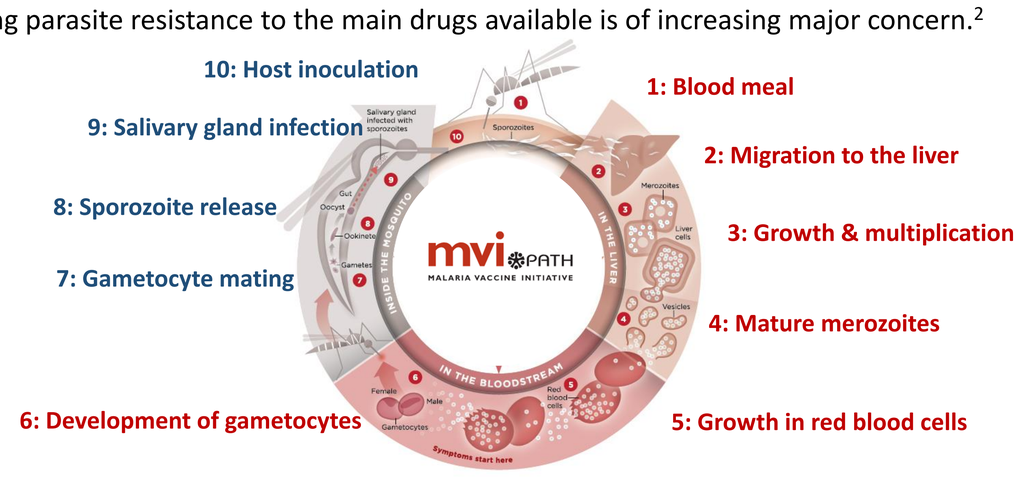
- Malaria is hard to treat effectively due to the life cycle of the Plasmodium parasite and the emergence of drug resistance.
- Drug targeting of phenylalanyl-tRNA synthetase (FRS) presents a unique opportunity to potentially target malaria.

2. Aims

- Conduct a comprehensive target safety review of FRS.
- Create a toxicological assessment plan for the development of an antimalarial FRS inhibitor.

3. Malaria

- Nearly half of the world's population is at risk of malaria.[1]
- In 2017 c.219 million cases of malaria occurred worldwide; with c.435,000 malaria deaths.[1]
- Malaria is caused by the plasmodium parasite which is transmitted to people through infected mosquitoes.[1]
- Emerging parasite resistance to the main drugs available is of increasing major concern.[2]



Symptoms; fever, chills, sweating, headaches, vomiting, seizures in young children

Figure 1 Malaria infection cycle.[3]

4. The target: Phenylalanyl-tRNA synthetase

- Aminoacyl-tRNA synthetases are pivotal in protein synthesis and cell viability, they are part of the translational machinery in all cells.[4]
- Phenylalanyl-tRNA synthetase (FRS) catalyzes the ligation of phenylalanine to its cognate transfer tRNA during protein synthesis.[5]
- Eukaryotic cells harbor 2 different types of FRS: the heterotetrameric cytosolic alpha (FRSA) and beta (FRSB) forms and the monomeric mitochondrial form, FRS2.[6]

- There is a single genomic copy of mitochondrial FRS exclusive to parasites.[7]
- FRS is highly conserved throughout eukaryotes.
- FRS is a ubiquitous enzyme, similarly expressed across cell types and tissues.[8,9]
- Dog, rat and mouse FRS2 have high nucleotide and protein homology to humans (~84%).[8]

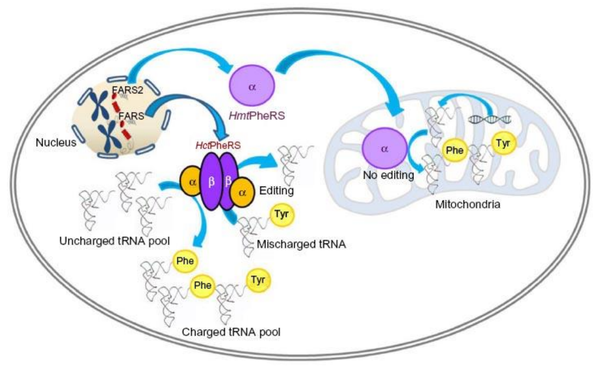


Figure 2 Localization and function of human cytoplasmic FRS and human mitochondrial FRS.[10]

5. Potential risks of mammalian target inhibition

Data from mouse knockouts, human gene mutations and known effects of FRS inhibitors were used to determine risk of mammalian target inhibition.

Increased incidence of epilepsy
FRS2 +/- : Alpers syndrome; seizures, loss of cognitive ability FRS2 -/- : hereditary spastic paraplegia FRS missense mutation in a patient: epilepsy
Medium probability of occurrence (Risk dependent on BBB permeability)
High impact on progression

Myopathy
FRS2 +/- : increased muscle tone in Alpers syndrome patients FRS missense mutation in a patient: myopathy
Medium probability of occurrence
Medium impact on progression

Autoimmunity/ immunosuppression
Ochratoxin A: (FRS inhibitor produced by certain bacterial species) linked to inflammatory pathology, immunosuppression and shown to have deleterious effects on macrophages and neutrophils
Medium probability of occurrence
Medium impact on progression

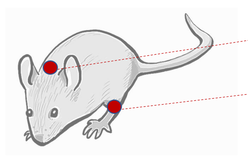
Reduced liver function
FRS2 +/- : liver disease common in Alpers syndrome
Medium probability of occurrence
Medium impact on progression

Perturbed glucose metabolism
Frsa +/- : impaired glucose tolerance; increase body fat
Medium probability of occurrence
Low impact on progression

Nephrotoxicity
Ochratoxin A: nephrotoxic effects in mammals
Medium probability of occurrence
Medium impact on progression

Abnormal bone development
Frsa +/- : abnormal bone morphology; decreased bone mineral density and circulating chloride and sodium levels
Medium probability of occurrence
Low impact on progression (in adults)

Teratogenicity
Ochratoxin A: causes several toxic effects in mammals including being teratogenic
High probability of occurrence
Low impact on progression



	Mouse phenotype
Frsa -/-	Lethal [11]
Frsa +/-	Abnormal bone morphology; impaired glucose tolerance; increased body fat [12]
Frsb & *Frs2*	No knockout phenotype data available

Figure 3 Potential risks of mammalian target inhibition. The likelihood of occurrence and impact on progression were assessed based on the data from mouse knockouts, human gene mutations and known effects of inhibitors.[12-20]

6. Toxicology Assessment Plan

Organ System / Potential Risk	Potential Assessment
Bone / abnormal bone development	In vivo: assess bone formation in preclinical studies with standard histopathological assessment
Endocrine / perturbed glucose metabolism	In vivo: Assess bodyweight, food consumption, glucose and insulin levels
Immune / autoimmunity / immunosuppression	In vivo: include hematology; Immunophenotyping assays using peripheral blood and/or samples of lymphoid tissue to characterize lymphocyte numbers and activation status; cytokine levels from general toxicity studies
Kidney / nephrotoxicity	In vivo: assess kidney histologically, biomarkers of kidney function including Albumin, β2- Microglobulin, Clusterin, Cystatin C, KIM-1, Total Protein, and Trefoil factor-3
Liver / reduced function	In vivo: measure liver transaminases to assess liver function. Assess liver histologically In vitro: assess potential hepatotoxicity in cell lines/primary hepatocytes, compare toxicity to Ochratoxin A, a hepatotoxic FRS inhibitor
Musculoskeletal / myopathy	In vivo: assess muscle formation in preclinical studies with standard histopathological assessment
Nervous / increased incidence of epilepsy	In vivo: Histopathological assessment of brain and electroencephalography (EEG) study In vitro: Hippocampal slice assay to assess changes in epileptogenesis
Reproductive / Teratogenicity	In vivo: Embryo-fetal development studies

7. Conclusions

- Potential target organs of toxicity caused by inhibition of host FRS include bone, immune system, kidney, liver, muscle, and the nervous system.
- Therefore a compound with high specificity for the Plasmodium FRS is essential for the success of an FRS inhibitor in the treatment of malaria.
- An early rodent investigative study looking at in life effects and potential target organs could help identify whether the risks our in silico analysis has identified actually occur in vivo with inhibitors of FRS.
- A target safety assessment (TSA) is vital to determine the toxicity risks and propose a risk mitigation plan to improve the probability of success.

8. References

1. World Health Organisation – malaria fact sheets; 2. 2018 World Malaria Report; 3. Baer, K., et al., (2007). *PLoS Path.* **3**(11)e171; 4. Sissler, M., et al., (2017). *Trends Mol Med.* **23**(8):693-708; 5. Guth, E., et al., (2009). *J Biol Chem*. **284**(31):20753-62; 6. Finarov, I., et al., (2010). *Structure* **18**(3):343-53; 7. Sharma, A., & Sharma, A., (2015). *Biochem J.* **465**(3):459-69; 8. genecards.org; 9. Rodova, M, et al., (1999). *Biochem. Biophys. Res. Commun.* **255**(3): 765-773; 10. Chakraborty S, and Banerjee, R., (2016). *Research & Reports in Biochem.* 6:25-38; 11. Dickinson, M., et al., (2016). *Nature* **537**(7621): 508-514; 12. mousephenotype.org; 13. Elo, J., et al., (2012). *Hum Mol Genet.* 21(20):4521-9; 14. Raviglione, F., et al., (2016). *Am. J. Med. Genet.* **170**(11): 3004-3007; 15. Vernon, H., et al., (2015). *Am. J. Med. Genet*. **167**(5): 1147-1151; 16. Yang, Y., et al., (2016). *Hum Mutat.* **37**(2): 165-9; 17. Almalki, A., et al., (2014). *Acta, Mol. Basis Dis*. **1842**(1), 56-64; 18. Dirheimer, G., and Creppy, E., (1991). *IARC Sci. Publ*. (115): 171-86; 19. Betteridge, Z., et al., (2007). *Rheumatology* **46**(6): 1005-1008; 20. Brennan, K., et al., (2017). *Toxins* ***9***(11), 366.

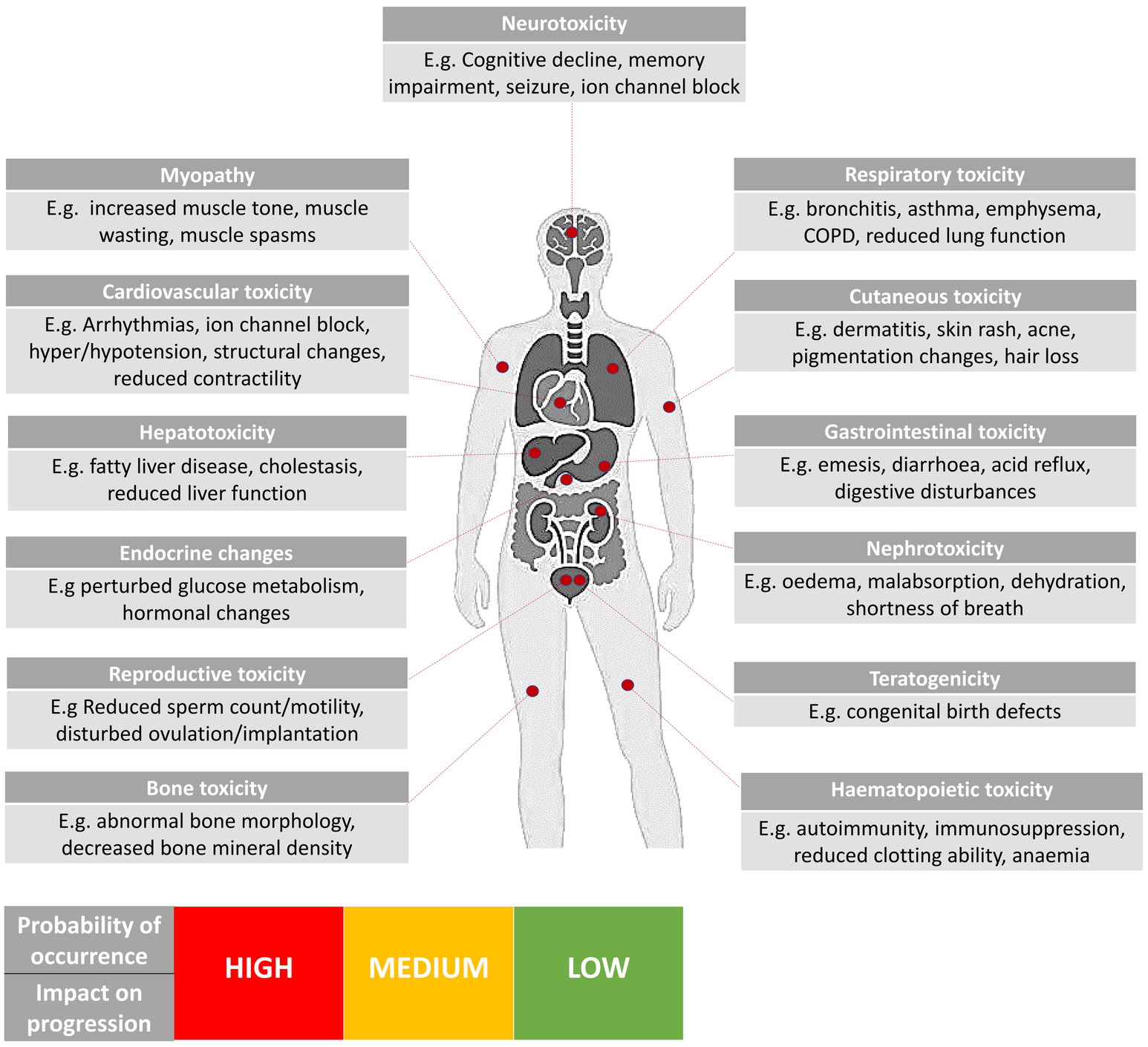


Figure x Potential risks of target inhibition in each organ system

Data from mouse knockouts, human gene mutations and known effects of inhibitors are used to determine the risk of mammalian target inhibition in each organ system. The probability of the identified toxicities occurring in each organ system and the impact on project progression are determined for each organ system and categorized as low, medium or high risk. Other more uncommon toxicities are also appraised in the same way.