

# Viewpoint

## Managing Iron Overload: A Gut Check

### Abstract

Divalent metal transporter 1 (DMT1) is the major importer of ferrous iron at the apical surface of enterocytes in the duodenum. Multiple groups have tried to design specific inhibitors for DMT1 both to study its contributions to iron (and metal ion) homeostasis and to provide a pharmacological means to treat iron overload disorders like hereditary hemochromatosis and thalassemias. This task faces challenges because many tissues express DMT1 and DMT1 transports other metals adding to standard risks in making specific inhibitors. Xenon Pharmaceuticals have published several papers on their efforts. Their latest paper in this issue of the journal culminates their efforts with compounds named XEN601 and XEN602 but implies that these very effective inhibitors have sufficient toxicity for them to halt development. This Viewpoint evaluates their efforts and briefly considers alternative routes to the goal.

### Significance Statement

This Viewpoint briefly reviews the paper on inhibitors of DMT1 that appears in this issue of the journal and commends the effort and research utility of those developed by Xenon. The inhibitors have proven to be valuable research tools for studying metal ion homeostasis particularly for iron. If Xenon is ceasing to try to develop them for treatment of iron overload disorders, then new alternatives need to come to the fore.

Cutts et al. (2023) has just published their evaluation of a series of related inhibitors of divalent metal transporter 1 (DMT1, also known as Nramp2/DCT1/SLC11A2) developed to target intestinal versions of DMT1 with the aim of managing several hereditary forms of iron overload. DMT1, located in the proximal duodenum, enables ferrous iron uptake from the diet (Fleming et al., 1997; Gunshin et al., 1997). Because no regulated process exists for removal of iron once ingested, body stores of iron are regulated via control of duodenal levels of DMT1 as an entry transporter for enterocytes (Mackenzie and Garrick, 2005) and ferroportin as the transporter that promotes iron exit from enterocytes into circulation (Mackenzie and Garrick, 2005). Elevation of DMT1 levels during iron overload in hereditary hemochromatosis (HH) or  $\beta$ -thalassemia intermedia ( $\beta$ TI) should clearly contribute to the imbalance (Anderson and Frazer, 2017). Iron loading also occurs in  $\beta$ -thalassemia major and sickle cell disease where the need for repeated transfusions provides another source of excess iron although anemia-induced increases of intestinal DMT1 should also contribute to overload. Current clinical management of the loading involves repeated phlebotomy for HH (Anderson and Frazer, 2017), but the existing anemia leads to the use of iron chelators for  $\beta$ TI, thalassemia major, and (occasionally) sickle cell disease (Anderson and Frazer, 2017). Targeting DMT1 is an obvious way to intervene, but DMT1 is present in many cells other than enterocytes where it contributes to iron metabolism. A notable example is erythroid progenitors where iron entry via the transferrin cycle relies on DMT1 and accounts for a major fraction of overall iron flux (Fleming et al., 1998). DMT1 potentially transports other metal ions than ferrous iron (Garrick et al., 2003), a property that could complicate choosing it as a target. Hence, it is desirable that duodenal iron uptake be selectively inhibited and to assure that the inhibitor does not severely impact metal homeostasis for other DMT1 substrates.

Linking monomers from their prior screens to form dimers, Cutts et al. (2023) designated one candidate XEN601 and a related one XEN602 then focused on their features to achieve a goal of inhibiting enterocyte DMT1 yet minimizing blocking of systemic DMT1 particularly in the erythroid cells that require high iron flux for hemoglobin synthesis. The group assayed cell DMT1 expression models for divalent metal ion influx by both fluorescence quenching and voltage clamp studies where they sorted out proton and divalent metal

**Address correspondence to:** Michael D. Garrick, Department of Biochemistry, University at Buffalo, 3A Cary Hall, Buffalo, NY, 14214-3000. E-mail: mgarrick@buffalo.edu  
dx.doi.org/10.1124/jpet.123.001645.

**ABBREVIATIONS:**  $\beta$ TI,  $\beta$ -thalassemia intermedia; DCT1, divalent cation transporter 1; DMT1, divalent metal transporter 1; HH, hereditary hemochromatosis; Nramp2, natural resistance associated macrophage protein 2; siRNA, small interfering RNA; SLC11A2, solute carrier family 11 member 2.

ion influx due to DMT1 from each other and leak currents. They also evaluated the two inhibitors by experiments on outbred Sprague–Dawley rats (after both normal and iron deficient diets, the latter increasing dependency on DMT1) and weanling pigs (where rapid growth leads to high demand for iron again ordinarily resolved by dependency on DMT1 expression). Both inhibitors were nearly 1000× more potent than ferristatin, reported as a candidate for blocking DMT1 after screens by a different group (Buckett and Wessling-Resnick, 2009). In yet another similar screen that examined some members of a similar family of inhibitors, the lead candidate, given the acronym CISMBI, was only slightly better than ferristatin (Montalbetti et al., 2014). In a study designed to establish that DMT1 was present and functional on the outer mitochondrial membrane, Wolff et al. (2018) also found that XEN602 was much more effective than CISMBI.

Thus, are Cutts and collaborators ready to proceed into testing XEN601 and XEN602 for ameliorating iron overload in human patients or perhaps at least next in rodent models with iron overloading? As an academic, I have little experience relevant to the circumstances that the group, all but one directly associated with Xenon Pharmaceuticals, must address to decide on this issue. Although they recognize that inhibition of DMT1 is a promising means to treat iron overload disorders and that XEN602 not only potently blocked iron uptake *in vivo* but also might be restricted to the gut at a dose ~100× higher than the ED<sub>50</sub>, they also note a steep dose response relationship and signs of clinical toxicity at the highest doses. Given that DMT1 can transport other physiologically relevant metal ions like Mn<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, Cd<sup>2+</sup>, and Cu<sup>+</sup> (Gunshin et al., 1997; Garrick et al., 2003), they look for tissue perturbations in these metal ions and others. They mention that only splenic Co<sup>2+</sup> was elevated; but the direct expectation for DMT1 substrates would be lower levels. Ultimately, weight loss at the highest doses combined with some mortality, and the dose curve noted above, led them to halt clinical development. One is left wondering about the toxicity's cause. Perhaps the poly cationic nature of the inhibitors affects another transporter. Given the risks of taking a compound to the pharmaceutical market, one must acknowledge their choice.

They note the utility of the XEN601/2 inhibitors for continuing study of how DMT1 participates in metal ion homeostasis and disruptions thereof. Our application illustrating a role for DMT1 in mitochondrial metal ion import (Wolff et al., 2018) represents an example of the company's past willingness to share their compounds as probes. One can foresee multiple applications of the XEN601/2 blockers for understanding metal ion metabolism if they remain available to inspire investigators in that field of research.

The authors mention three ways to try for improvements: 1) developing a biomarker for toxicity so that one could intervene early enough to prevent adverse effects; 2) relying on XEN602's brief half-life to design an extended-release formulation; and 3) appending larger substituents to decrease systemic exposure. They also suggest combining strategies. Perhaps inhibitors that are even more effective can now be developed after the recent publication of work characterizing the structural interaction of *bis*-isothiourea compounds with human and bacterial DMT1 (Manatschal et al., 2019). If there were a better understanding of XEN602's toxicity, rationale design might be facilitated.

Perhaps one needs to develop better pharmacologic means of delivery for these potent inhibitors. In a mouse model of HH (Wang et al., 2019, 2021), oral gavage of ginger nanoparticles targeted to the duodenum and delivering DMT1 small interfering RNA (siRNA) to lower DMT1 levels mitigates iron overload without apparent toxicity. Although the surface design of these nanoparticles enables duodenal, intracellular delivery of siRNA, perhaps nanoparticles can be redesigned to deliver XEN602, a smaller polycation, in a similarly targeted fashion but to the cell surface, improving it as a therapeutic agent. I am unsure if the skill set of nanoparticle designers currently includes a way to get local, extracellular release of contents. Alternatively, perhaps, nanoparticle delivery of DMT1 siRNA is the next route to try. Experiments with this approach have also shown that supplying DMT1 siRNA in this fashion is effective against iron overload in a mouse model of βTI (unpublished data). The two publications and as yet unpublished data validate the strategy of trying to inhibit intestinal DMT1 without inhibiting systemic DMT1, implying that problems with Xenon's inhibitors are more likely toxicity issues than strategic. I am also curious about whether one could find a dose range for Xenon's reagents that would be effective in either mouse model without toxicity given that both models have iron overload while both their models were healthy, but relatively iron-deficient.

Michael D. Garrick

*Department of Biochemistry, University at Buffalo, Buffalo, New York*

## Acknowledgments

The author thanks Laura M. Garrick for critically reviewing a draft of the manuscript. Each of the two anonymous reviewers provided useful insights. Recent research from the Garrick Laboratory cited here was supported by grant R01DK109717 from the National Institute of Diabetes and Digestive and Kidney Diseases and the Office of Dietary Supplements and the Laura and Michael Garrick Fund. Earlier research was supported by specific National Institutes of Health grants acknowledged in the citations.

## Data Availability

The author declares that the paper, being a Viewpoint, contains commentary and opinion, not new data *per se*.

## References

- Anderson GJ and Frazer DM (2017) Current understanding of iron homeostasis. *Am J Clin Nutr* **106** (Suppl 6):1559S–1566S.
- Buckett PD and Wessling-Resnick M (2009) Small molecule inhibitors of divalent metal transporter-1. *Am J Physiol Gastrointest Liver Physiol* **296**:G798–G804.
- Cutts AJ, Chowdhury S, Ratkay L, Eyers M, Young C, Namdari R, Cadieux J, Chahal N, Grimwood M, Zhang Z et al. (2023) Potent, gut-restricted inhibitors of divalent metal transporter 1 (DMT1): preclinical efficacy against iron overload and safety evaluation. *J Pharmacol Exp Ther* **386**:4–14.
- Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, and Andrews NC (1998) Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci USA* **95**:1148–1153.
- Fleming MD, Trenor 3rd CC, Su MA, Foernzler D, Beier DR, Dietrich WF, and Andrews NC (1997) Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* **16**:383–386.
- Garrick MD, Dolan KG, Horbinski C, Ghio AJ, Higgins D, Porubcin M, Moore EG, Hainsworth LN, Umbreit JN, Conrad ME et al. (2003) DMT1: a mammalian transporter for multiple metals. *Biometals* **16**:41–54.
- Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, and Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**:482–488.
- Mackenzie B and Garrick MD (2005) Iron imports. II. Iron uptake at the apical membrane in the intestine. *Am J Physiol Gastrointest Liver Physiol* **289**:G981–G986.
- Manatschal C, Pujol-Giménez J, Poirier M, Reymond J-L, Hediger MA, and Dutzler R (2019) Mechanistic basis of the inhibition of SLC11/NRAMP-mediated metal ion transport by bis-isothiourea substituted compounds. *eLife* **8**:e51913.
- Montalbetti N, Simonin A, Dalghi MG, Kovacs G, and Hediger MA (2014) Development and validation of a fast and homogeneous cell-based fluorescence screening assay for divalent metal transporter 1 (DMT1/SLC11A2) using the FLIPR tetra. *J Biomol Screen* **19**:900–908.
- Wang X, Zhang M, Flores SRL, Woloshun RR, Yang C, Yin L, Xiang P, Xu X, Garrick MD, Vidyasagar S, Merlin D and Collins JF (2019) Oral gavage of ginger nanoparticle-derived lipid vectors carrying Dmt1 siRNA blunts iron loading in murine hereditary hemochromatosis. *Mol Ther* **27**:493–506.
- Wang X, Zhang M, Woloshun RR, Yu Y, Lee JK, Flores SRL, Merlin D, and Collins JF (2021) Oral administration of ginger-derived lipid nanoparticles and Dmt1 siRNA potentiates the effect of dietary iron restriction and mitigates pre-existing iron overload in *Hamp* KO mice. *Nutrients* **13**:1686.
- Wolff NA, Garrick MD, Zhao L, Garrick LM, Ghio AJ, and Thévenod F (2018) A role for divalent metal transporter (DMT1) in mitochondrial uptake of iron and manganese. *Sci Rep* **8**:211.