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Current Understanding of Membrane Transporters as Regulators or Targets for Cisplatin-Induced Hearing Loss

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Abstract

Cisplatin is a platinum-based drug which remains among the most efficacious anticancer treatment options. Unfortunately, use of cisplatin is hindered by dose-limiting toxicities, including irreversible hearing loss, which can grossly affect patient quality of life. Cisplatin-induced ototoxicity is the result of cochlear hair cell damage through a mechanism that is poorly understood. However, cisplatin cytotoxicity is reliant on intracellular accumulation, a process that is largely dependent on the presence of particular membrane transporters. This review will provide an update on our current understanding of the various transporters known to be involved in the disposition and cytotoxicity of platinum drugs or their metabolites, as well as their role in mediating cisplatin-induced hearing loss. We also provide a summary of the successes and opportunities in therapeutically targeting membrane transporters to alleviate platinum-induced hearing loss. Moreover, we describe how this approach could be used to reduce the severity or onset of other adverse events associated with exposure to various forms of platinum drugs, without diminishing anti-tumor efficacy.

Significance statement

Cisplatin-induced hearing loss is a dose limiting and irreversible adverse event with no current preventative or curative treatment measures. Pharmacological targeting of membrane transporters that regulate platinum uptake into cochlear hair cells, if conducted appropriately, may alleviate this devastating side effect and could be applied to alleviate other platinum-induced toxicities.

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Introduction

Cisplatin is considered among the most widely used therapeutics for various forms of cancer in children and adults. This is due to high efficacy in malignancies of tissues that include bladder, ovaries, testis, head and neck, and neuroblastoma (Gold and Raja, 2020; Harrach and Ciarimboli, 2015; Rabik and Dolan, 2007). Unfortunately, despite its widespread efficacy, cisplatin-based therapy is often limited by the onset of dose-restricting toxicities.

The major complications associated with cisplatin include neuro-, nephro-, and oto-toxicity. These adverse events (AEs) are not only dose-limiting, but common, as nephrotoxicity affects 25-33% of patients and nearly 70% suffer from ototoxicity (Langer et al., 2013; Madias and Harrington, 1978). Moreover, cisplatin-induced ototoxicity (CIO) is more debilitating in children, where at least 60% experience permanent bilateral hearing loss (Langer et al., 2013). This can have devastating long-term impacts on quality of life and development, as impaired auditory function can impact communication, socialization, cognition, performance, and lifetime earnings (Gurney et al., 2007; Langer et al., 2013; Paken et al., 2016; Wilson et al., 2017). Unfortunately, despite uncovering remedies to mitigate nephrotoxicity (Crona et al., 2017), CIO is poorly understood and there remains no applicable preventative or curative resolutions (Freyer et al., 2019; Freyer et al., 2020).

Currently, it is understood that CIO is confined to the inner ear or cochlea, where inner- and outer-hair cells (IHCs and OHCs), stria vascularis supporting cells, and spiral ganglion cells are affected (Paken et al., 2016). Collectively, these cells manage mechanotransduction required to perceive sound, however cisplatin exposure promotes destruction of IHCs and OHCs. Details of factors regulating hair cell damage is limited, but cell death is attributed to platinum-DNA adduct formation (Basu and Krishnamurthy, 2010) or generation of reactive oxygen species, two attributes that also contribute to cisplatin antineoplastic effects (Bragado et al., 2007; Choi et al., 2015). As such, strategies to reduce CIO have sought to preserve hair cells by mitigating

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formation of DNA adducts or reactive oxygen species, although, these approaches have not yielded clinical improvements. Meanwhile, cellular accumulation of cisplatin is largely dependent on specific membrane transporters, a characteristic that is considered understudied.

The human genome consists of more than 400 membrane transporters that are separated into two major superfamilies: the ATP-binding cassette (ABC) transporters, and solute carriers (SLC) (Borst and Elferink, 2002; He et al., 2009; Nigam, 2015). Collectively, these proteins are among the least studied family of proteins despite involvement in regulating cellular uptake or efflux of endogenous compounds and xenobiotics (Borst and Elferink, 2002; Nigam, 2015). The majority of membrane transporter investigations associated with xenobiotics focus on select proteins and their involvement of mediating drug absorption, distribution, metabolism, and excretion (ADME) in organs such as the intestine, liver, kidney, and blood-brain-barrier (Giacomini et al., 2010). However, all tissues express a variety of transporters, and knowledge of those responsible for cisplatin accumulation into hair cells would uncover key strategies to ameliorate CIO. This is especially advantageous in the event that cisplatin uptake into tumors is mediated by distinctively different transporters from those in hair cells. Furthermore, inhibition of transporters that mediate platinum disposition could be expanded to alleviate other platinum-based toxicities. For example clinical use of oxaliplatin is often associated with peripheral neurotoxicity that worsens with higher cumulative doses into dorsal root ganglia (Grothey, 2005), and many patients exposed to carboplatin suffer from thrombocytopenia due to death of megakaryoctes (Markman et al., 2004; van der Vijgh, 1991). As mentioned above, cisplatin also promotes nephro- and neurotoxicity (de Jongh et al., 2003; van den Bent et al., 2002). Considering that each of these AEs are a result of cellular platinum accumulation which is dependent on transport-mediated uptake, it is not a stretch to imagine targeting uptake transporters may have widely impactful benefits.

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Over the past two decades, there has been literary expansion of transporters reportedly involved in platinum drug disposition and CIO, along with other forms of platinum-mediated toxicities.

SLCs involved in cisplatin disposition and CIO

Numerous SLCs have been reported to regulate cellular uptake of cisplatin (Figure 1), serving as the primary step of cytotoxicity, and as contributors to CIO. Below describes the transporters identified to date:

Organic Cation Transporter 2 (OCT2; SLC22A2)

OCT2 has been implicated as a promoter of tubular secretion and urinary elimination of cisplatin and oxaliplatin both *in vitro* and *in vivo* (Filipski et al., 2008; Filipski et al., 2009; Sprowl et al., 2013a), as well as oxaliplatin accumulation into rodent dorsal root ganglia (Sprowl et al., 2016). It is currently understood that the metabolically active, cationic form of cisplatin is transported into cells via OCT2 inciting cytotoxicity (Browning et al., 2017). Interestingly, OCT2 expression is highly tissue specific, with the greatest found in proximal tubules (Filipski et al., 2009). OCT2 is also expressed in human and murine dorsal root ganglia (Sprowl et al., 2013a), as well as in IHC, OHC and the stria vascularis of the cochlea in mice (Ciarimboli et al., 2010; More et al., 2010). In agreement with tissue expression, several studies support the role of OCT2 in mediating CIO. Genetic deficiency of Oct2 in mice is protective of CIO (Ciarimboli et al., 2010; Sprowl et al., 2014), and rs316019 (Table 1; (c.808G>T; p.Ser270Ala)), a reduced functional OCT2 variant that exists within approximately 7 - 13% of Caucasians (Filipski et al., 2008; Filipski et al., 2009), has been associated with reduced incidence of CIO (Table 2; (Lanvers-Kaminsky, 2015)). In contrast, one investigation was unable to confirm a protective role of rs316019 in patients (Table 2; (Drogemoller et al., 2017)), but a minor CIO protective effect of rs316019 was recently confirmed in a large patient population (Table 2;(Langer et al., 2020)). These findings are encouraging, as cisplatin remains effective in xenograft or *in vitro* tumor

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models deficient of OCT2, indicating that pharmacological inhibition of this transporter has no impact on therapeutic efficacy (Ciarimboli, 2012; Ciarimboli et al., 2010; Sprowl et al., 2013b).

Copper Transporters 1 and 2 (CTR1/2; SLC31A1/2)

Consistent with its role in cellular uptake of heavy metals, *in vitro* overexpression of CTR1 increases cellular accumulation of cisplatin and consequential cytotoxicity (Holzer et al., 2004; Pabla et al., 2009). Similarly, CTR1 deficiency is associated with cisplatin resistance *in vitro* (More et al., 2010; Pabla et al., 2009). The role of CTR1 in managing cisplatin disposition and sensitivity is further strengthened by numerous *in vivo* and *ex vivo* studies. For example, genetic knockdown of CTR1 in mice diminishes platinum uptake into proximal and distal tubules, and reduces the severity of cisplatin-induced nephrotoxicity (Pabla et al., 2009). CTR1 inhibition has also resulted in reduced cisplatin accumulation into isolated mouse cochlea (More et al., 2010). Clinical evidence also exists, as two reduced functional CTR1 variants in patients have been associated with cisplatin tumor resistance, while an increased functional variant is linked to more severe cisplatin-induced toxicity (Table 1; Table 2; (Xu et al., 2012a; Xu et al., 2012b)). As CTR1 is also expressed in IHCs, OHCs and the stria vascularis of the cochlea of mice (More et al., 2010), a role for this transporter in mediating CIO is logical. However, CTR1 is widely expressed throughout human tissues, including various tumors (Ishida et al., 2010), implying that targeting this transporter pharmacologically to alleviate CIO could jeopardize anti-tumor efficacy.

In addition to CTR1, CTR2 has also been investigated as a regulator of cisplatin disposition. CTR2 is similar in homology and function to CTR1 but is associated with cellular efflux of cisplatin. Overexpression of CTR2 *in vitro* conveys cisplatin resistance, while decreased expression allows greater platinum accumulation and elevated cell death (Blair et al., 2011; Blair et al., 2009).

Organic Anion Transporter 1 and 3 (OAT1/3; SLC22A6/8)

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The OAT1 and OAT3 transporters are highly specific to the basolateral membrane of kidney proximal tubules and were shown to contribute to cisplatin-induced renal toxicity *in vitro* (Hu et al., 2017; Nigam et al., 2015). These findings emerged following the observation that genetic deficiency of murine Oct2 provides only partial protection to cisplatin-induced nephrotoxicity *in vivo*. Meanwhile, OAT1 and OAT3 have been shown *in vitro* to mediate transport of the mercapturic acid cisplatin-metabolite, N-acetylcysteine S-conjugate (NAC-1), which is responsible for OCT2-independent cisplatin-toxicity (Hu et al., 2017). NAC-1 is a powerful reducing agent with the propensity to interact with DNA (Hu et al., 2017; Townsend et al., 2003). This metabolite is also a precursor to a highly potent and reactive thiol molecule that can further elevate toxicity. Consistent with the above observations, Oat1 and Oat3 deficiency in mice is reportedly protective of nephrotoxicity, as determined by a noticeable reduction in blood urea nitrogen and serum creatinine after cisplatin exposure (Hu et al., 2017).

Recently, the involvement of OAT1 and OAT3 was challenged by Nieskens et al. who reported that cisplatin sensitivity was not increased in a proximal tubule cell line overexpressing OAT1 and OAT3 (Nieskens et al., 2018), although the investigators did not assess cellular uptake or sensitivity to NAC-1, implying further investigation is warranted. Regardless, targeting OAT1 and OAT3 appears to be a viable approach considering that inhibition of these transporters does not compromise the efficacy of cisplatin *in vitro* (Hu et al., 2017). As such, the involvement of OAT1 and OAT3 in CIO and other cisplatin-induced AEs should be further explored.

Monocarboxylate Transporter 6 (MCT6; SLC16A5)

MCTs are generally well characterized, have broad tissue expression, and mediate the disposition of various drugs. However, less is known about MCT6, a suspected orphan transporter. To date, no endogenous MCT6 substrates are known, but several xenobiotic substrates have been identified *in vitro* (Fisel et al., 2018; Murakami et al., 2005) and *in vivo* (Fisel et al., 2018; Jones et al., 2020; Murakami et al., 2005). Similar to other MCT family

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members, MCT6 is reportedly expressed across numerous tissues, including in the gut, kidney, and liver in humans. Interestingly, MCT6 protein expression has also been reported in murine cochlea (Drogemoller et al., 2017).

MCT6 has been implicated as a regulator of CIO since Drogemoller et al. identified a MCT6 single nucleotide polymorphism (SNP) in adult testicular cancer patients that was associated with protection from hearing loss (Table 2; (Drogemoller et al., 2017)). This particular variant, rs4788863 (c.121T>C; p.Leu41Leu), is predicted to be associated with lower mRNA expression, and is expected to reduce cisplatin uptake and sensitivity (Table 1). Moreover, *in vitro* evidence demonstrates that cisplatin exposure appears to increase MCT6 mRNA expression, and siRNA mediated knockdown of MCT6 expression offers protection from cisplatin cytotoxicity, although only at lower concentrations (Drogemoller et al., 2017). Unfortunately, the genetic association of MCT6 and CIO has not been replicated in follow up genome-wide association studies (Langer et al., 2020; Lui et al., 2018), implying that its true role requires focused investigation. This includes the mechanism by which MCT6 could contribute to platinum disposition, as cisplatin is not a prototypical substrate. In contrast, MCT6, recognized as an anion transporter, has substrate overlap with OATs and may be involved in disposition of cisplatin metabolites such as NAC-1 (Fisel et al., 2018; Jones et al., 2020; Murakami et al., 2005). To date, no literature exists demonstrating MCT6 expression in tumors, signifying that inhibition of MCT6 would likely not decrease antitumor efficacy; however, more research must be done to verify the absence of MCT6-dependent drug uptake across multiple tumor types.

Efflux transporters and cisplatin disposition

In addition to the SLCs described above, efflux transporters are also known to regulate cellular sensitivity to platinum drugs. The ABC transporters P-glycoprotein (ABCB1; MDR1) and Breast Cancer Resistant Protein (ABCG2; BCRP), which are commonly major suspects of clearing

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cytotoxic molecules from cells, do not recognize cisplatin (Vesel et al., 2017; Wang et al., 2014; Yin et al., 2009). However, other proteins have been identified as mediators of cisplatin efflux:

ATP7A and ATP7B

Consistent with the role of CTR1, the copper efflux transporters ATP7A and ATP7B are reportedly involved in cisplatin sensitivity. The presence and/or upregulation of ATP7A/B transporters are associated with platinum-based tumor resistance both *in vitro* (Samimi et al., 2004) and *in vivo* (Nakagawa et al., 2008; Samimi et al., 2004). Similar to CTR1, ATP7A/B are widely expressed throughout human tissues, including the cochlea, where ATP7A is highly localized to pillar cells in the organ of Corti and ATP7B is found in hair cells (Ding et al., 2011). Although ATP7A/B are hypothesized to play a role in CIO, no evidence exists to provide a direct association.

Multidrug and toxin extrusion protein 1 and 2K (MATE1/2K; SLC47A1/2)

MATE1 and MATE2K mediate the efflux of various cationic molecules, including numerous xenobiotics (Nies et al., 2016). Cisplatin has been recognized as a substrate for both MATE1 and MATE2K in overexpressing cells (Sauzay et al., 2016), and MATE1 is classified as a protector against cisplatin-induced nephrotoxicity *in vivo* (Nakamura et al., 2010). Its role in renal efflux and protection of proximal tubules is not surprising, considering that MATE1 is expressed among a variety of tissues, including the liver and kidney, while MATE2K is currently classified as kidney specific. Neither MATE1 nor MATE2K expression has been reported in IHC or OHC and as such, a contribution of MATE1 and MATE2K to CIO has not been discovered.

Multidrug Resistance-associated Protein 2 and 3 (ABCC2/3; MRP2/3)

Unlike P-glycoprotein and BCRP, the ABCC-subfamily members ABCC2 and ABCC3, which are known glutathione conjugate transporters, reportedly mediate efflux of cisplatin—or more likely, cisplatin conjugates (Chen and Kuo, 2010; Cui et al., 1999; Taniguchi et al., 1996). More

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research is necessary to better define these interactions, however ABCC2-overexpressing cells have greater tolerance to cisplatin as a result of increased cellular efflux *in vitro* (Kawabe et al., 1999), and genetic knockdown reverses cisplatin resistance in tumor cells (Chen et al., 2020). The role of ABCC2 is further strengthened by *in vivo* evidence, whereby ABCC2-deficient mice have greater renal and hepatic accumulation of platinum, along with increased nephrotoxicity, while reintroduction of human ABCC2 can remedy these events (Wen et al., 2014). These findings are consistent with ABCC2 expression that is largely localized to tissues associated with absorption or elimination, such as hepatic and gastrointestinal tissues, as well as renal proximal tubules (Jedlitschky et al., 2006). Furthermore, the discoveries in murine models are also consistent with human observations, where the reduced functional ABCC2 variant rs11597282 (Table 1; (n.-24C>T)) has been associated with increased response to cisplatin and toxicity (Table 2; (Sun et al., 2010)). However, this variant has also been reported to have no effect on cisplatin disposition, or prognostic outcomes (e.g. progression free outcome, overall survival, and toxicity) in patients with different tumor types (Table 2; (Campa et al., 2012; Sprowl et al., 2012)), indicating that this particular variant warrants further study to clarify its role. This includes assessing a potential role in CIO, as there is currently no association of ABCC2 with this AE.

Although less is known about ABCC3 compared to the other ABCC-family members, this transporter has garnered recent interest in regulating cisplatin sensitivity. Several studies have postulated that rs1051640, an increased functional variant of ABCC3 (Table 1; (c.4509A>G; p.Glu1503Glu)) may provide protection from CIO (Table 2; (Pussegoda et al., 2013; Tserga et al., 2019)). Unfortunately, its true clinical impact and the mechanism responsible remains unclear, as the involvement of ABCC3 in CIO is currently purely speculative. Furthermore, while ABCC3 is widely expressed throughout human tissues, expression has not yet been reported in cochlear cells.

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Clinical outcome of transporter-targeting strategies to alleviate CIO

As described above, genetic variation of a few transporters involved in platinum disposition have been associated with clinical protection from CIO (Figure 1 and Table 1). These observations have sparked enthusiasm for strategies to manipulate transporter activity and reduce platinum accumulation into tissues linked with AEs. The challenge in alleviating CIO, however, is to regulate transporter function that limits cisplatin disposition into cochlear cells without sacrificing sufficient accumulation into tumors to maintain treatment efficacy. This has proven to be particularly challenging with efflux transporters implicated in cisplatin disposition, as there have been no pharmacological strategies to selectively target these proteins to prevent cisplatin-associated toxicities. In contrast, various investigations have been conducted to pharmacologically manipulate select SLCs involved in cisplatin uptake and CIO using *in vivo* and *ex-vivo* models (Figure 1).

Early attempts to alleviate CIO involved administering competitive inhibitors of CTR1 to restrict access into cochlear hair cells. *In vitro*, and murine *ex vivo* evidence supports the utility of copper sulfate, a CTR1 substrate, to reduce platinum accumulation and offer protection from cisplatin cytotoxicity (More et al., 2010). However, contradictory findings describing the limitations of copper sulfate to reduce CIO in *ex vivo* models have also been reported (Ding et al., 2011). Furthermore, this strategy is complicated by the widespread expression of CTR1 and its involvement in tumor uptake (More et al., 2010), as it is anticipated that application of copper sulfate or even copper chelators would simultaneously alter tumoral response. Ultimately, future studies would benefit from analyzing local application of CTR1 modulators.

In contrast to CTR1, tissue specific expression of OCT2 provides a selective target to alleviate cisplatin-induced AEs without impacting clearance or efficacy in patients (Katsuda et al., 2010; Sprowl et al., 2013b). Multiple studies using *in vitro, ex vivo* and *in vivo* models have reported that numerous OCT2 inhibitors, including cimetidine, pifithrin-α, and tyrosine kinase inhibitors

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alleviate cisplatin-mediated toxicities, including ototoxicity (Ciarimboli et al., 2010; Ciarimboli et al., 2005; Martins et al., 2013; Sprowl et al., 2014; Sprowl et al., 2013b). Unfortunately, clinical evidence of this approach to reduce CIO is limited. Lower incidence of CIO was observed in patients that were co-administered the OCT2 substrate, erlotinib, although this was not the primary focus of the study (Martins et al., 2013). In contrast, pantoprazole did not offer protection from CIO, despite being administered as an OCT2 inhibitor (Fox et al., 2018). However, it is important to highlight that pantoprazole is reportedly a weak OCT2 inhibitor and would likely not provide CIO protection (Franke et al., 2010). Accordingly, the findings by Fox *et al.* may be irrelevant to this strategy and future investigations need to include stronger candidate inhibitors.

In addition to use of potent transporter inhibitors, another ideal approach to provide maximum protection from CIO is to inhibit multiple transporters. As described above, loss of OCT2-mediated cisplatin accumulation into proximal tubules does not provide complete nephrotoxic protection, but instead requires simultaneous inhibition of OAT1 and OAT3 to reduce NAC-1 uptake (Hu et al., 2017). In fact, probenecid reportedly inhibits uptake of NAC-1 by OAT1 and OAT3 *in vitro*, and also provides protection from cisplatin-induced nephrotoxicity in patients (Hu et al., 2017; Jacobs et al., 1991). To date however, NAC-1 and the transporters that mediate its disposition, including OAT1 and OAT3, have not been implicated in CIO. Furthermore, probenecid does not appear to offer protection from CIO (Jacobs et al., 1991). However, lack of protection from CIO may be the result of insufficient inhibition or involvement of other transporters. Regardless, accumulation of toxic metabolites or involvement of multiple transporters should be considered. Indeed, the otoprotective effect of cimetidine witnessed *in vivo* may involve its ability to simultaneously inhibit OCT2 and MCT6 (Drogemoller et al., 2017; Murakami et al., 2005). Therefore, clinical improvements would likely result from investigation of inhibitors targeting multiple transporters involved in cisplatin accumulation in IHCs and OHCs.

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For example, many tyrosine kinase inhibitors, such as erlotinib or nilotinib, may be attractive options given their ability to reduce the activity of multiple SLCs (Hu et al., 2017; Martins et al., 2013).

Future Directions, Applications and Considerations

This review highlights various studies that have collectively advanced our understanding of platinum disposition and impact on CIO or other toxicities. However, the lack of clinical success stresses the need for further detailed investigations. Primarily, it is important to inspect the role of each transporter involved in platinum disposition, as there are likely additional transporters to be discovered. It is also particularly important to characterize the interplay of multiple transporters, as well as the involvement of metabolism, to identify all toxic platinum metabolites and the transporters that recognize these molecules. There is a high probability, based on the studies described above, that multiple transporters will need to be considered simultaneously to regulate platinum disposition and prevent accumulation into sensitive tissues. Strategies to target multiple transporters may carry significant risk, including altered homeostasis in tissues that express these proteins, or consequential reduced tumor uptake and efficacy. Clearly these events will require consideration throughout the development of such strategies, however various investigations described above indicate that these risks may be minimal. For example, the competitive OCT2 inhibitor cimetidine is known to competitively inhibit multiple transporters and can be safely administered in patients without major risk of unintended effects in organs (Sprowl et al., 2013b). Moreover, *in vivo* data indicates that cimetidine has no effect on anti-tumor efficacy, which collectively suggests that certain transporter inhibitors can be used to selectively diminish toxicity without impacting tumor response.

Identification of ideal inhibitors is the most crucial task regarding the strategy to alleviate CIO by targeting transporters. Future inhibitors for clinical considerations must have well characterized properties regarding inhibition potential and success in alleviating CIO in preclinical models. The

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lack of success in ameliorating CIO with pantoprazole is not surprising considering that past literature reported mixed potential of this drug as an OCT2 inhibitor at clinically relevant doses (Fox et al., 2018; Franke et al., 2010; Hacker et al., 2015; Nies et al., 2011), and there was a lack of evidence showing that pantoprazole could prevent CIO *in vivo*. Instead investigators should consider inhibitors that show promise at clinically relevant doses in reducing platinum-induced AEs within *in vitro* and *in vivo* models, such as cimetidine (Ciarimboli et al., 2010), or particular tyrosine kinase inhibitors (Ateyya et al., 2017; Bielefeld et al., 2013; Hu et al., 2017; Huang et al., 2020; Sprowl et al., 2016). Cyclin-dependent kinase inhibitors have also recently shown promise in alleviating platinum-induced toxicities *in vivo* through inhibition of transport (Pabla et al., 2015; Teitz et al., 2018) and could be further investigated. Additionally, as indicated above, ideal future inhibitors may also need to target multiple transporters, as long as tumor efficacy is unaffected. Unfortunately, due to expression across multiple tissues and decreases in efficacy, inhibiting CTR1 or CTR2 is not recommended as it is anticipated that a marked decrease in antitumor response or other off-site, unintended toxicities would occur. Manipulation of efflux transporters is also not recommended at this time due to their ubiquitous expression, difficulty in select targeting, and the potential to promote cellular accumulation of platinum agents. Currently, evidence suggests that OCT transporters would be optimal primary targets based on hair cell expression and involvement in cisplatin disposition; although simultaneous inhibition of transporters such as OATs may provide more wholistic protection from cisplatin toxicity, effects of OATs on CIO are unknown. With this in mind, some tyrosine kinase inhibitors, such as nilotinib, are emerging as agents capable of reducing function of multiple transporters (Hu et al., 2017; Sprowl et al., 2016) and could be investigated as compounds to maximize protection from CIO. While further work is needed to assure patient safety, the potential of using tyrosine kinase inhibitors in combination with cisplatin is supported by long-term use of tyrosine kinase inhibitors in the clinic, and evidence of TKIs either having no impact, or greater anti-tumor efficacy when given with cisplatin (Hu et al., 2017; Huang et al.,

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2020). In fact, use of TKIs to ameliorate CIO is expected to only require a single dose before exposure to cisplatin, and therefore may avoid AEs associated with this class of drugs (Hu et al., 2017; Huang et al., 2020). Finally, additional inhibitors could be identified or synthesized based on structure-activity explorations of known transporter substrates or inhibitors, which may yield improved clinical options.

Although optimal inhibitors will certainly advance clinical success of reducing CIO, it is also imperative to improve models that would accelerate translation to clinical application. For example, throughout cisplatin treatment, a consistent evaluative test of hearing loss associated with CIO needs to be established, as various methods and timing can yield differing sensitivity that could mask pharmacological benefits. Ideally, this common evaluative test would be applicable within *in vivo* models which could then be used to test new strategies and accurately predict clinical success. Furthermore, the field would largely benefit from the generation of simulations with transporter modulators based on physiologically-based pharmacokinetic and pharmacodynamic mathematical modeling at clinically achievable concentrations to guide future research or strategies.

Although the current review focused widely on CIO, it is important to remember that numerous studies described above, in which transporters were investigated as mediators of platinum disposition, involved application to other forms of platinum-induced AEs. As such, the strategy of reducing platinum uptake into cells responsible for AEs can be applied to other platinum AEs as well. To date there are no reports of successful clinical abolishment of platinum-induced nephrotoxicity, neuropathy or thrombocytopenia. In fact, cimetidine has been the only transporter inhibitor administered to patients in combination with cisplatin, which was conducted in a study that revealed no risk of changes to cisplatin pharmacokinetics (Sprowl et al., 2013b). While the study did not report on platinum-associated AEs, the lack of platinum pharmacokinetic changes, along with *in vivo* reports, indicates that targeting OCT2 to ameliorate cisplatin-

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induced nephrotoxicity or oxaliplatin-induced neurotoxicity, along with CIO, is highly promising (Filipski et al., 2009; Huang et al., 2020; Sprowl et al., 2013a; Sprowl et al., 2016). Moreover, these findings have initiated a recent clinical trial to assess the potential of dasatinib to prevent oxaliplatin-induced neuropathy (Noonan et al., 2020). Certainly, the results of this clinical trial will highlight the feasibility of this strategy, however, data generated to date indicates that targeting select transporters can be beneficial to diminish all forms of platinum-induced AEs. Nonetheless, further study and clinical applications are required to assess the protective potential of this application to other platinum-induced AEs.

Concluding Remarks

Due to its anti-tumor efficacy (Brown et al., 2019), cisplatin is likely to remain the standard of care for various cancers throughout the foreseeable future. As cancer rates rise (Ferlay et al., 2015), this means that quality of life altering AEs, such as CIO, will become increasingly burdensome. While strategies to abolish CIO through targeting cytotoxic properties are attractive, there remains high risk of reducing anti-tumor activity. However, evidence indicates that various membrane transporters—some highly tissue specific—mediate platinum disposition and could be pharmacologically regulated to selectively reduce CIO without sacrificing anti-tumor efficacy. Despite success using *in vitro* and *in vivo* models, current approaches have not been adequately expanded to yield ideal clinical benefits.

Yet, the success of *in vitro*, *ex vivo*, and *in vivo* investigations indicate that regulating transporter function is a viable approach to mitigate platinum-induced AEs, including CIO. Clearly though, additional research is needed to unveil the true benefits or potential detriments of transporter-based strategies to alleviate CIO or other forms of toxicity. However, given the complications, as well as the emotional and financial burden of CIO and other platinum-induced AEs, future studies and strategies to control platinum disposition should be expanded clinically, as

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regulation of these events will improve quality of life and treatment success by alleviating major dose-limiting AEs.

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Wrote or contributed to the writing of the manuscript: K.Z.P., J.M.D., J.A.S

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Footnotes

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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Figure Legend

Figure 1. Transporters implicated in the disposition of cisplatin or its metabolites *in vitro* (Blue) and *in vivo* or *ex vivo* (Green). Relevant transporters with polymorphisms known to change cisplatin disposition, found from genome association studies, are also shown (Red). A combination of the above colors means the transporter was found to interact with cisplatin across several experimental models, respective to their representative color. This image was created with BioRender online software.

Table 1. Transporter polymorphisms directly involved in cisplatin disposition and cisplatin-induced toxicities.

TRANSPORTER	IDENTIFICATION	SUBSTRATE	TRANSLATION	FUNCTIONAL CHANGE	TOXICITY CHANGE	REFERENCE
OCT2	rs316019	Cationic, Activated Cisplatin	c.808T>G; p.Ser270>Ala	Decreased	Decreased	Filipski et al., 2009
CTR1	rs12686377	Cationic, Activated Cisplatin	c.-35-14361C>A	Decreased	Decreased	Xu et al., 2012a
	rs7851395	Cationic, Activated Cisplatin	c.-35-15930A>G	Decreased	Decreased	Xu et al., 2012a
	rs10981694	Cationic, Activated Cisplatin	c.-36+2451T>G	Increased	Increased	Xu et al., 2012b
MCT6	rs4788863	Anionic NAC-1 metabolite	c.121T>C; p.Leu41> Leu	Decreased	Decreased	Drogemoller et al., 2017
ABCC2	rs11597282	Anionic NAC-1 metabolite	n.-24C>T *	Decreased	Increased	Sun et al., 2010
ABCC3	rs1051640	Anionic NAC-1 metabolite	c.4509A>G; p.Glu1503>Glu	Increased	Decreased	Pussegoda et al., 2013

Table 2. Summary of existing clinical studies reporting on transporter-based treatments or variants.

TRANS-PORTER	STUDY	OUTCOME MEASURED	NUMBER OF PTS	AGES (YRS)	DOSES	OUTCOME
OCT2	Filipski et al., 2008	Rs316019 association with cisplatin nephrotoxicity	106	18 – 75	50 – 100 mg/m^2	rs316019 was identified and had no effect on cisplatin pharmacokinetics
	Drogemoller et al., 2017	GWAS association with CIO	188	24 – 39	200 – 920 mg/m^2	No retrospective involvement of rs316019 in CIO protection
	Langer et al., 2020	SNP association with platinum toxicity	900	N/R	30 – 1650 mg/m^2	rs316019 provides minor CIO protection but is a poor clinical marker in this model
CTR1	Xu et al., 2012a	SNP association with platinum resistance	282	33 – 78	100 mg/m^2	rs12686377 and rs7851395 decrease tumor efficacy of cisplatin
	Xu et al., 2012b	SNP association with platinum toxicity and survival	204	33 – 77	100 mg/m^2	rs10981694 is associated with increased severe CIO
MCT6	Drogemoller et al., 2017	GWAS association with CIO	188	24 – 39	200 – 920 mg/m^2	Retrospective analyses showed rs4788863 confers protection from CIO
	Langer et al., 2020	SNPs association with platinum toxicity	900	N/R	30 – 1650 mg/m^2	No association of rs4788863 with CIO
	Lui et al., 2018	SNP association with CIO	106	0.2 – 16.9	N/R	No association of rs4788863 with CIO
ABCC2	Sun et al., 2010	SNP association with overall survival	248	N/R	N/R	rs11597282 is associated with increased overall survival in SCLC patients
	Campa et al., 2012	SNP association with overall survival and toxicity	377	N/R	N/R	rs11597282 associated with decreased efficacy and shorter overall survival in SCLC, but not NSCLC, patients
	Sprowl et al., 2012	SNP association with cisplatin pharmacokinetics, efficacy, and toxicity	237	N/R	N/R	rs11597282 has no influence on nephrotoxicity, cisplatin response, or overall survival
ABCC3	Pussegoda et al., 2013	SNPs as genetic markers for CIO	155	0 – 25	92 – 800 mg/m^2	rs1051640, when integrated into a mathematical model, helped predict CIO
	Tserga et al., 2019	Literature review of SNP association with CIO	N/R	N/R	N/R	rs1051640 is protective from CIO

N/R = Not reported; SNP = Single nucleotide polymorphism; PTs = Patients

Figure 1