JCI The Journal of Clinical Investigation

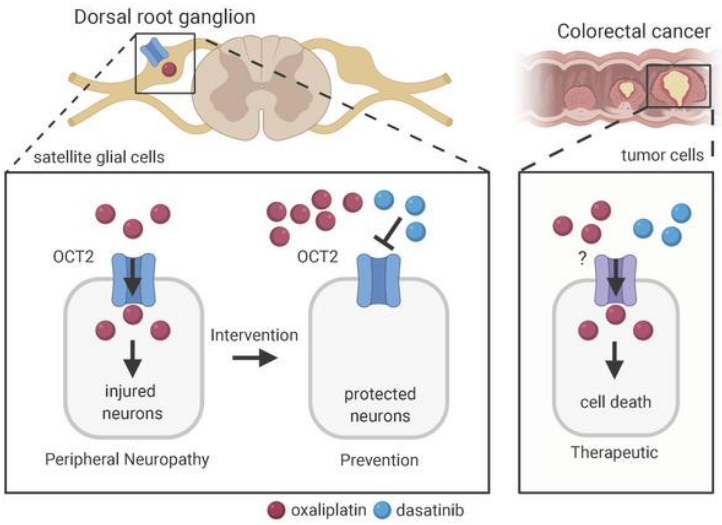
Neuronal uptake transporters contribute to oxaliplatin neurotoxicity in mice

Kevin M. Huang, Alix F. Leblanc, Muhammad Erfan Uddin, Ji Young Kim, Mingqing Chen, Eric D. Eisenmann, Alice Gibson, Yang Li, Kristen W. Hong, Duncan DiGiacomo, Sherry Huinan Xia, Paola Alberti, Alessia Chiorazzi, Stephen N. Housley, Timothy C. Cope, Jason A. Sprowl, Jing Wang, Charles L. Loprinzi, Anne Noonan, Maryam Lustberg, Guido Cavaletti, Navjotsingh Pabla, Shuiying Hu, Alex Sparreboom

J Clin Invest. 2020. https://doi.org/10.1172/JCI136796.

Concise Communication In-Press Preview **Neuroscience** **Oncology**

Graphical abstract



Neuronal uptake transporters contribute to oxaliplatin neurotoxicity in mice

Kevin M. Huang[1,a], Alix F. Leblanc[1,a], Muhammad Erfan Uddin[1], Ji Young Kim[1], Mingqing Chen[1], Eric D. Eisenmann[1], Alice Gibson[1], Yang Li[1], Kristen Hong[1], Duncan DiGiacomo[1], Sherry Xia[1], Paola Alberti[2,3], Alessia Chiorazzi[2,3], Stephen Housley[4], Timothy Cope[4], Jason A. Sprowl[5], Jing Wang[6], Charles L. Loprinzi[7], Anne Noonan[8], Maryam B. Lustberg[8], Guido Cavaletti[2,3], Navjot Pabla[1], Shuiying Hu[1]*, Alex Sparreboom[1]*

[1]Division of Pharmaceutics and Pharmacology, College of Pharmacy & Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210; [2]School of Medicine and Surgery, University of Milano-Bicocca, Monza 20900, Italy; [3]NeuroMI, Milan Center for Neuroscience, Milan, 20126, Italy. [4]School of Biological Sciences & Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30318; [5]Department of Pharmaceutical Sciences, School of Pharmacy & Pharmaceutical Sciences, University at Buffalo, New York 14260; [6]Cancer Biology and Genetics, College of Medicine & Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210; [7]Department of Oncology, Mayo Clinic Comprehensive Cancer Center, Rochester, Minnesota 55905; [8]Division of Medical Oncology, Comprehensive Cancer Center, The Ohio State University Wexner Medical Center, Columbus, OH 43210. [a]Authors contributed equally to the manuscript. *To whom correspondence may be addressed. Email: hu.1333@osu.edu or sparreboom.1@osu.edu

Corresponding authors

Shuiying Hu, Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, BRT, Room 0406, 500 West 12th Avenue, Columbus Ohio 43210, USA. Phone: 614-685-8028; Email: hu.1333@osu.edu

Alex Sparreboom, Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, BRT, Room 0408, 500 West 12th Avenue, Columbus, Ohio 43210, USA. Phone: 614-685-6014; Email: sparreboom.1@osu.edu.

Conflict of interest

MBL has provided consultation regarding chemotherapy-induced peripheral neuropathy to PledPharma and Disarm Therapeutics. CLL has provided consultation regarding chemotherapy-induced peripheral neuropathy to PledPharma, Disarm Therapeutics, Asahi Kasei, and Metys Pharmaceuticals.

Introduction

Clinical use of the chemotherapeutic agent oxaliplatin is associated with a characteristic pattern of peripheral neurotoxicity that affects more than 90% of patients (1). The onset of neurotoxicity may occur immediately after infusion and is characterized by cold-exacerbated paresthesias, muscle spasms, and fasciculations. Although these acute symptoms resolve over time, at higher cumulative doses, oxaliplatin induces dose-limiting sensory neurotoxicity that leads to functional impairment, which can last for years following the discontinuation of treatment and, in more severe cases, result in permanent impairment (2).

The mechanisms underlying oxaliplatin-induced peripheral neurotoxicity (OIPN) remain incompletely understood. Within the nervous system, oxaliplatin preferentially accumulates in peripheral sensory neurons present in the dorsal root ganglia (DRG) (3), and oxaliplatin levels in DRGs remain high for prolonged time periods, even after discontinuation of treatment. Since the severity of OIPN correlates with drug levels in peripheral nerves (4), these studies suggest that oxaliplatin accumulation in DRG is a key trigger for the development of OIPN, and that pharmacological targeting of the transport mechanism regulating the initial accumulation of oxaliplatin could be a promising, previously unexplored, neuroprotective strategy. Studies have demonstrated that the cellular uptake of platinum-based chemotherapeutics such as cisplatin occurs via a facilitated transport mechanism (5). Consequently, distribution patterns and pathological changes following oxaliplatin administration are restricted to cells in organs capable of transporting oxaliplatin from the blood into cells (6, 7). Previous reports have demonstrated that the uptake transporter OCT2 [*SLC22A2*] mediates the intracellular accumulation of both cisplatin and oxaliplatin in mice, rats and humans (7-10), and several other cationic-type transporters (OCTs; OCTNs; MATEs) and organic anion transporting polypeptides (OATPs), may contribute to this process (6, 11-13).

The aim of the present study was to unambiguously identify the DRG transporter mediating oxaliplatin accumulation with the use of engineered knockout mice and rats, and establish the feasibility of a strategic intervention concept with translational potential using transport inhibitors. This approach is derived from the supposition that targeting of an initial uptake mechanism offers conceptual advances over interventions involving intracellular neuronal signaling cascades that may also be relevant to oxaliplatin-mediated antitumor efficacy.

Results & Discussion

In order to gain preliminary insight into the relative contribution of individual uptake transporters to OIPN, we performed a comparative transcriptomic profile of drug transporters in isolated DRGs from untreated mice (**Figure 1A**). Preliminary studies indicated that expression and OIPN profile were similar between sexes and across different strains (**Supplemental Figure 1A-C**). Although copper transporter 1 (CTR1) had the highest expression in DRGs (**Figure 1A**), CTR1 was not considered as a candidate transporter since CRISPR-mediated knockout of CTR1 in cells demonstrates a CTR1-independent mechanism of oxaliplatin accumulation (14). Based on observed expression signatures and putative relevance, OCT1 and OCT2 (hereafter OCT1/2), OCT3, OCTN1, OATP1B2, OATP2B1, and MATE1 were selected for further consideration as *in vivo* transporters of oxaliplatin. A comparative Von Frey Hair test was used in wild-type and transporter-deficient mice to assess neuropathy 24 hours following a single dose of oxaliplatin. OCT1/2-deficiency conferred significant protection against OIPN in both acute and chronic models (**Figure 1B-C**). The observed phenotypic changes occurred without concurrent alteration of oxaliplatin pharmacokinetics caused by OCT1/2 deficiency, as described previously (9). In addition, OCT1/2-deficiency was not associated with compensatory expression changes in phylogenetically-linked transporters (**Figure 2A**), with the exception of OCT3. Although OCT3 is expressed more abundantly than OCT2 in DRGs (**Figure 1A**), OCT3-deficiency failed to offer sustained protection against OIPN 24 hours after treatment (**Supplemental Figure 1D**),

suggesting that OCT3 did not independently contribute to the observed phenotypes. Candidate transporters were next evaluated for their ability to transport oxaliplatin in HEK293 cells overexpressing mouse and human homolog proteins. These studies indicated that OCT2 was the most efficient oxaliplatin transporter (**Figure 1D-E**, and **Supplemental Table 1**), and lack of transport by OCT1 suggest that phenotypic alterations observed in OCT1/2$^{-/-}$ mice are exclusively due to the deficiency of OCT2.

Histological examination of DRG cross-sections revealed the absence of morphological damage in both wild-type and OCT1/2$^{-/-}$ mice, with minimal degeneration of caudal and sciatic nerve fibers (**Supplemental Figure 2A-B**). Taken together with the lack of changes observed in nerve conduction studies (**Supplemental Figure 2C**), these findings suggest the development of mild ganglionopathy and support the notion that functional deficits in sensory transduction and neuronal firing of proprioceptors can continue to exacerbate behavioral outcomes despite the absence of tissue degeneration associated with OIPN (15). Since DRGs represent ganglia of sensory neuronal and glial cells, we next evaluated the contributing cell-type to OIPN. Immunofluorescence staining of untreated DRG cross-sections demonstrated the co-localization of OCT2 with glial fibrillary acidic protein (GFAP) (**Figure 2B**), a satellite glial cell (SGC) marker (16) that has been implicated in the development of chemotherapy-induced neuropathic pain (17). Subsequent investigation with primary cultures of SGCs indicated that OCT2 was highly expressed (**Figure 2A**) compared to non-satellite cells (e.g., sensory neurons). Additionally, primary SGCs from OCT1/2$^{-/-}$ mice have diminished ability to accumulate oxaliplatin (**Figure 2C** and **Supplemental Figure 3A**). Similar findings were obtained in SGCs pretreated with dasatinib, a known inhibitor of OCT2 (**Figure 2C**) (18).

Previously studies have indicated that targeting of OCT2 can confer protection against OIPN in mice (10, 18). In order to demonstrate the translational relevance of this concept (**Supplemental**

Figure 3B), we next sought to validate the importance of OCT2 in OIPN in Sprague dawley rats as a secondary model organism (19). The utility of rats in OIPN was confirmed by the demonstration that rat OCT2 transports oxaliplatin and is highly sensitive to inhibition by dasatinib (**Figure 3A-B**). The translational potential of using an OCT2 inhibitor as an intervention strategy to prevent the onset of OIPN was next evaluated in an acute model using wild-type and OCT2-deficient rats (**Supplemental Figure 4A-C**) pretreated with orally administered dasatinib. These findings indicate that dasatinib significantly protected rats from OIPN (**Figure 3C**) similar to that observed in OCT2-deficient rats, and that OCT2-deficiency or dasatinib pretreatment did not significantly influence urinary excretion or plasma levels of oxaliplatin (**Figure 3D-E**) but diminished accumulation of oxaliplatin in DRGs (**Figure 3F**). Similarly, dasatinib reduced the accumulation of oxaliplatin in primary wild-type rat SGCs (**Figure 3G** and **Supplemental Figure 5A-C**), consistent with a previous murine report (18) and this current study.

In contrast to our current findings, it was previously suggested that the transporter OCTN1 is a possible determinant of OIPN (6, 12, 20). Due to the expression of OCTN1 on the mitochondria, the neuroprotective effects of ergothioneine, an OCTN1-specific substrate, may be in part due to intrinsic antioxidant activity on mitochondrial dysfunction rather than inhibition of oxaliplatin transport into DRGs. Our *in vitro* uptake studies indicate that oxaliplatin is not a transported substrate of mouse or human OCTN1 (**Figure 1D-E**) and deficiency of OCTN1 or pretreatment with ergothioneine in mice did not afford neuroprotection (**Figure 1B** and **Supplemental Figure 6A**). A similar lack of oxaliplatin transport was reported for models overexpressing rat OCTN1 (21). It is conceivable that the neuroprotective effects associated with physiological concentrations of ergothioneine in chronic oxaliplatin treatment regimens are also partially due to effects on transporters other than OCTN1. Support for this comes from a prior study demonstrating that ergothioneine can significantly reduce the cellular uptake of oxaliplatin by more than 50% in DRG neurons from OCTN1-deficient mice (22). This earlier work also indicated

that 80% of oxaliplatin uptake into DRG neurons occurs independently of OCTN1 and that oxaliplatin-induced loss of neuronal cell viability was unchanged in OCTN1-deficient mice (22), in line with our toxicity evaluations using the same mouse model (**Figure 1B** and **Supplemental Figure 6A**). Interestingly, some studies have also suggested that oxaliplatin is a transported substrate of the carnitine transporter OCTN2; however, the significance of this observation is unclear since administration of OCTN2 substrate, L-carnitine, does not afford tissue protection against oxaliplatin (12, 20). Furthermore, we found that dasatinib does not inhibit these transport mechanisms (**Supplemental Figure 6B-C**).

Although the addition of dasatinib to oxaliplatin-based regimens could reduce the incidence and severity of OIPN, it is important to demonstrate that such intervention does not compromise the anticancer efficacy of oxaliplatin. Transcriptional profiling of drug transporters using RNA-seq data (23) revealed that OCT2 is expressed at very low levels in human colorectal tumors and colorectal cancer cell lines (**Supplemental Figure 7A-C**), compared to human DRGs. Consistent with this observation, we found that cytotoxicity and cellular uptake (**Figure 4A** and **Supplemental Figure 7D**) of oxaliplatin in multiple colorectal cancer cell lines was not altered by dasatinib. These findings suggest that oxaliplatin is taken up into cancer cells independently of OCT2 and that this unknown mechanism is insensitive to dasatinib-mediated inhibition. The translational potential of this strategy was further verified by the observation that dasatinib did not influence the plasma levels or anticancer efficacy of oxaliplatin in mice bearing HCT116 tumor cells, while simultaneously preventing OIPN (**Figure 4 B-G** and **Supplemental Figure 7E-F**).

Since dasatinib is known to inhibit transporters of importance to the distribution of oxaliplatin into hepatocytes and renal tubular cells (24, 25), including OATP1B2 and MATE1, we confirmed that deficiency of OATP1B2 did not affect the clearance of oxaliplatin (**Supplemental Figure 8 A-D**). Consistent with a role of MATE1 as a major elimination mechanism of cationic compounds, it is

conceivable that the inhibition of MATE1 efflux may lead to increased renal tubular accumulation of oxaliplatin and increased nephrotoxicity. While MATE1 deficiency in mice was associated with diminished urinary excretion of oxaliplatin, pretreatment with dasatinib did not influence markers of renal dysfunction such as serum creatinine and blood urea nitrogen (**Supplemental Figure 9 A-C**). Furthermore, even high doses of oxaliplatin did not cause renal injury in MATE1-deficient mice, as evaluated by histological and biomarker examination (**Supplemental Figure 9 D-F**).

The combination of SRC kinase inhibitors such as dasatinib with oxaliplatin -containing regimens has been previously evaluated in patients with colorectal cancer (26). Although OIPN was still observed in patients receiving this combination, it is important to point out that the applied dasatinib dosing schedule was not optimized in prior studies to exploit its OCT2-inhibitory properties. Our currently ongoing preclinical and clinical studies are designed to document local (DRG) and systemic (blood) changes in endogenous substrates of OCT2 that could be utilized as a pharmacodynamic biomarker of OCT2 function with inhibitors such as dasatinib.

In the context of our proposed intervention strategy, we acknowledge that several pharmacological approaches have been evaluated previously to prevent OIPN (27). Although duloxetine appears to be a viable lead from this collective work, the utility of this agent in *preventing* OIPN remains unclear (28). In a previously reported small molecule library screen involving >8000 compounds (18), we found more than half of the 433 compounds with potent OCT2 inhibitory properties were neurological compounds (**Supplemental Figure 10A-C**). This is not surprising considering the structural similarities of these compounds with known endogenous substrates of OCT2 such as catecholamines and neurotransmitters. Coincidentally, many of the OCT2 inhibitors identified from this screen, including duloxetine (**Supplemental Figure 10D**) have previously been evaluated in the management of neuropathic pain (27), based on their potential to inhibit intracellular signaling cascades that promote neuronal degeneration.

Collectively, we identified a previously unrecognized, SGC-specific pathway of oxaliplatin-induced neurological injury that is mediated by the transporter OCT2, which can be inhibited by dasatinib without compromising the anticancer properties of oxaliplatin. These findings not only shed light on the etiology of OIPN, but provide a rationale for future development of new targeted interventions using transport inhibitors to mitigate this debilitating side effect.

Methods

See **Supplemental Information** for detailed methods**.**

Animal Studies. All animals were housed in a temperature-controlled environment with a 12-hour light cycle, given standard chow diet and water *ad libitum*, and handled according to the Animal Care and Use Committee of The Ohio State University, under an approved protocol (2015A00000101-R1). For all experiments, age- and gender-matched wild-type or knockout mice (8–12 weeks) or rats (6–8 weeks) were used. Detailed information regarding sources and origins of the rodent models is provided in the supplemental information.

Statistical Analysis. Data presented represents the mean ± SEM before or after normalization to baseline values. All experiments were performed with replicates, unless stated otherwise, and repeated on at least two occasions. An unpaired two-sided Student's *t* test with Welch's correction (2 groups) or a one-way ANOVA with Dunnett's post-hoc test (>2 groups) was used to evaluate statistical significance using $P<0.05$ as the cut-off.

Author contributions

SH and AS conceived and designed the experiments; KMH, AL, MEU, MC, AAG, and DG conducted *in vitro* experiments; KMH, AL, JYK, YL, and SH carried out the *in vivo* experiments; EDE, YL, PA, AC, SNH, TC, JAS, JW, CLL, AN, MBL, GC, and NP suggested experiments and contributed to data interpretation; AAG generated the stable cell models; KMH, AL, SH, and AS analyzed the data; KMH and AS wrote the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

We would like to acknowledge the Small Animal Imaging Core at OSU for providing imaging instrumentation, and tissues procured by the National Disease Research Interchange (NDRI) supported by NIH grant 5U42RR006042. RNA sequencing shown are based upon publicly available data generated by the TCGA Research Network (http://cancergenome.nih.gov). The project was supported in part by NIH grants R01CA215802 (AS), R01CA187176 (AS) and R01CA238946 (MBL and SH), and by the OSU Comprehensive Cancer Center using Pelotonia funds (KMH). The content is solely the responsibility of the authors and does not represent the official views of the funding agencies.

References

1. Argyriou AA, Polychronopoulos P, Iconomou G, Chroni E, and Kalofonos HP. A review on oxaliplatin-induced peripheral nerve damage. *Cancer Treat Rev.* 2008;34(4):368-77.
2. Pachman DR, Qin R, Seisler DK, Smith EM, Beutler AS, Ta LE, Lafky JM, Wagner-Johnston ND, Ruddy KJ, Dakhil S, et al. Clinical course of oxaliplatin-induced neuropathy: results from the randomized phase III trial N08CB (Alliance). *J Clin Oncol.* 2015;33(30):3416-22.
3. Thompson SW, Davis LE, Kornfeld M, Hilgers RD, and Standefer JC. Cisplatin neuropathy. Clinical, electrophysiologic, morphologic, and toxicologic studies. *Cancer.* 1984;54(7):1269-75.
4. Gregg RW, Molepo JM, Monpetit VJ, Mikael NZ, Redmond D, Gadia M, and Stewart DJ. Cisplatin neurotoxicity: the relationship between dosage, time, and platinum concentration in neurologic tissues, and morphologic evidence of toxicity. *J Clin Oncol.* 1992;10(5):795-803.
5. Sprowl JA, Ness RA, and Sparreboom A. Polymorphic transporters and platinum pharmacodynamics. *Drug Metab Pharmacokinet.* 2013;28(1):19-27.
6. Jong NN, Nakanishi T, Liu JJ, Tamai I, and McKeage MJ. Oxaliplatin transport mediated by organic cation/carnitine transporters OCTN1 and OCTN2 in overexpressing human embryonic kidney 293 cells and rat dorsal root ganglion neurons. *J Pharmacol Exp Ther.* 2011;338(2):537-47.
7. Zhang S, Lovejoy KS, Shima JE, Lagpacan LL, Shu Y, Lapuk A, Chen Y, Komori T, Gray JW, Chen X, et al. Organic cation transporters are determinants of oxaliplatin cytotoxicity. *Cancer Res.* 2006;66(17):8847-57.
8. Filipski KK, Mathijssen RH, Mikkelsen TS, Schinkel AH, and Sparreboom A. Contribution of organic cation transporter 2 (OCT2) to cisplatin-induced nephrotoxicity. *Clin Pharmacol Ther.* 2009;86(4):396-402.
9. Sprowl JA, Ciarimboli G, Lancaster CS, Giovinazzo H, Gibson AA, Du G, Janke LJ, Cavaletti G, Shields AF, and Sparreboom A. Oxaliplatin-induced neurotoxicity is dependent on the organic cation transporter OCT2. *Proc Natl Acad Sci USA.* 2013;110(27):11199-204.
10. Sprowl JA, van Doorn L, Hu S, van Gerven L, de Bruijn P, Li L, Gibson AA, Mathijssen RH, and Sparreboom A. Conjunctive therapy of cisplatin with the OCT2 inhibitor cimetidine: influence on antitumor efficacy and systemic clearance. *Clin Pharmacol Ther.* 2013.
11. Li S, Chen Y, Zhang S, More SS, Huang X, and Giacomini KM. Role of organic cation transporter 1, OCT1 in the pharmacokinetics and toxicity of cis-diammine(pyridine)chloroplatinum(II) and oxaliplatin in mice. *Pharm Res.* 2011;28(3):610-25.
12. Nishida K, Takeuchi K, Hosoda A, Sugano S, Morisaki E, Ohishi A, and Nagasawa K. Ergothioneine ameliorates oxaliplatin-induced peripheral neuropathy in rats. *Life Sci.* 2018;207(516-24.
13. Lancaster CS, Sprowl JA, Walker AL, Hu S, Gibson AA, and Sparreboom A. Modulation of OATP1B-type transporter function alters cellular uptake and disposition of platinum chemotherapeutics. *Mol Cancer Ther.* 2013;12(8):1537-44.
14. Liu JJ, Kim Y, Yan F, Ding Q, Ip V, Jong NN, Mercer JF, and McKeage MJ. Contributions of rat CTR1 to the uptake and toxicity of copper and platinum anticancer drugs in dorsal root ganglion neurons. *Biochem Pharmacol.* 2013;85(2):207-15.

15. Vincent JA, Wieczerzak KB, Gabriel HM, Nardelli P, Rich MM, and Cope TC. A novel path to chronic proprioceptive disability with oxaliplatin: distortion of sensory encoding. *Neurobiol Dis.* 2016;95(54-65.
16. Nascimento DSM, Castro-Lopes JM, and Neto FLM. Satellite glial cells surrounding primary afferent neurons are activated and proliferate during monoarthritis in rats: is there a role for ATF3? *PLOS ONE.* 2014;9(9):e108152.
17. Warwick RA, and Hanani M. The contribution of satellite glial cells to chemotherapy-induced neuropathic pain. *Eur J Pain.* 2013;17(4):571-80.
18. Sprowl JA, Ong SS, Gibson AA, Hu S, Du G, Lin W, Li L, Bharill S, Ness RA, Stecula A, et al. A phosphotyrosine switch regulates organic cation transporters. *Nat Commun.* 2016;7(10880.
19. Ellenbroek B, and Youn J. Rodent models in neuroscience research: is it a rat race? *Dis Model Mech.* 2016;9(10):1079-87.
20. Fujita S, Hirota T, Sakiyama R, Baba M, and Ieiri I. Identification of drug transporters contributing to oxaliplatin-induced peripheral neuropathy. *J Neurochem.* 2019;148(3):373-85.
21. Tschirka J, Kreisor M, Betz J, and Grundemann D. Substrate selectivity check of the ergothioneine transporter. *Drug Metab Dispos.* 2018;46(6):779-85.
22. Jong NN-J. *Pharmacology and Clinical Pharmacology*. The University of Auckland; 2012:293.
23. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, and Stuart JM. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* 2013;45(10):1113-20.
24. Minematsu T, and Giacomini KM. Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. *Mol Cancer Ther.* 2011;10(3):531-9.
25. Zimmerman EI, Hu S, Roberts JL, Gibson AA, Orwick SJ, Li L, Sparreboom A, and Baker SD. Contribution of OATP1B1 and OATP1B3 to the disposition of sorafenib and sorafenib-glucuronide. *Clin Cancer Res.* 2013;19(6):1458-66.
26. Strickler JH, McCall S, Nixon AB, Brady JC, Pang H, Rushing C, Cohn A, Starodub A, Arrowood C, Haley S, et al. Phase I study of dasatinib in combination with capecitabine, oxaliplatin and bevacizumab followed by an expanded cohort in previously untreated metastatic colorectal cancer. *Invest New Drugs.* 2014;32(2):330-9.
27. Hu S, Huang KM, Adams EJ, Loprinzi CL, and Lustberg MB. Recent developments of novel pharmacologic therapeutics for prevention of chemotherapy-induced peripheral neuropathy. *Clin Cancer Res.* 2019;25(21):6295-301.
28. Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Fadul CE, Knox C, Le-Lindqwister N, et al. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. *JAMA.* 2013;309(13):1359-67.

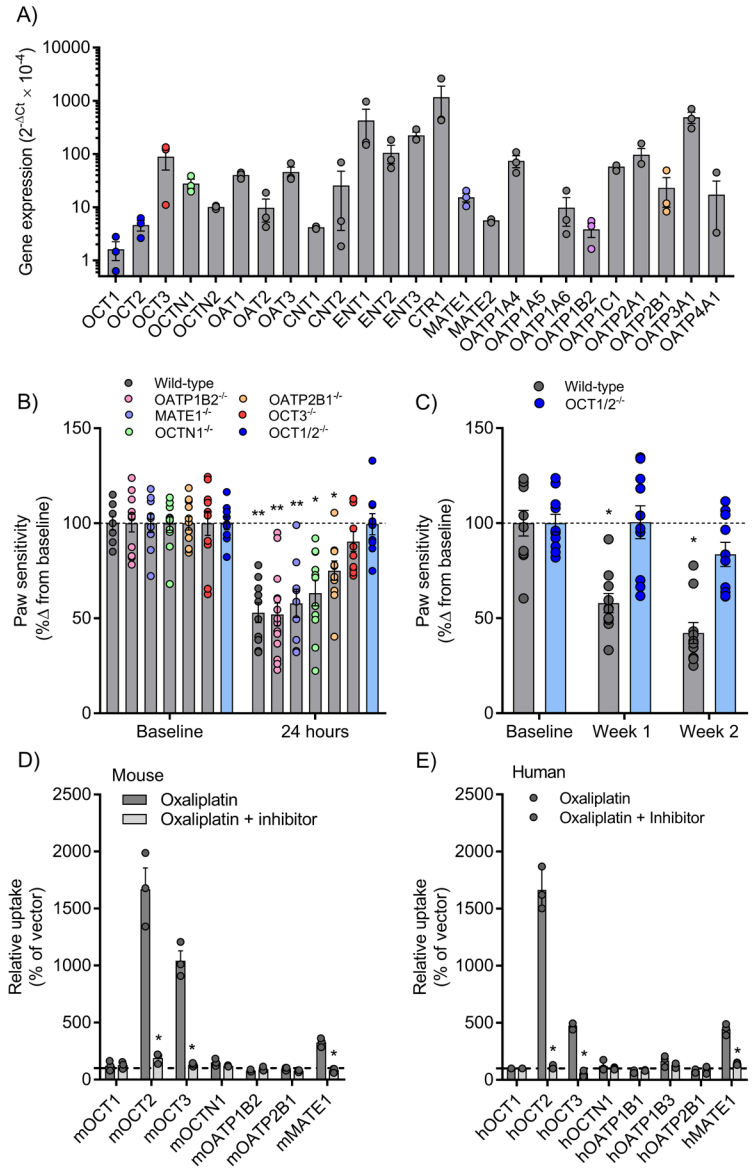


Figure 1. OCT2 is a critical determinant of OIPN. (A) Transcriptomic expression of transporters in DRGs isolated from untreated wild-type FVB mice. **(B)** Acute OIPN wild-type (pooled C57BL/6, FVB or DBA strains) or transporter-deficient mice 24 hours following a single injection of 10 mg/kg oxaliplatin. **(C)** Chronic OIPN in wild-type or OCT1/2$^{-/-}$ mice one or two weeks following multiple injections of 4 mg/kg oxaliplatin, twice a week (cumulative dose 32 mg/kg). Paw sensitivity is expressed as a percent change to baseline values. Intracellular accumulation and inhibition (*for specific inhibitors, please refer to supplemental material*) of oxaliplatin uptake into HEK293 cells overexpressing **(D)** mouse (m) or **(E)** human (h) transporters. Relative uptake is expressed as a percent change compared to empty vector controls. All animal studies contained n=4-8 per group, repeated on two independent occasions. All in vitro studies contained n=3 per group, repeated on three independent occasions. Statistical analysis was performed using an unpaired two-sided Student's *t* test with Welch's correction: *P<0.05, **P<0.01 compared to baseline values.

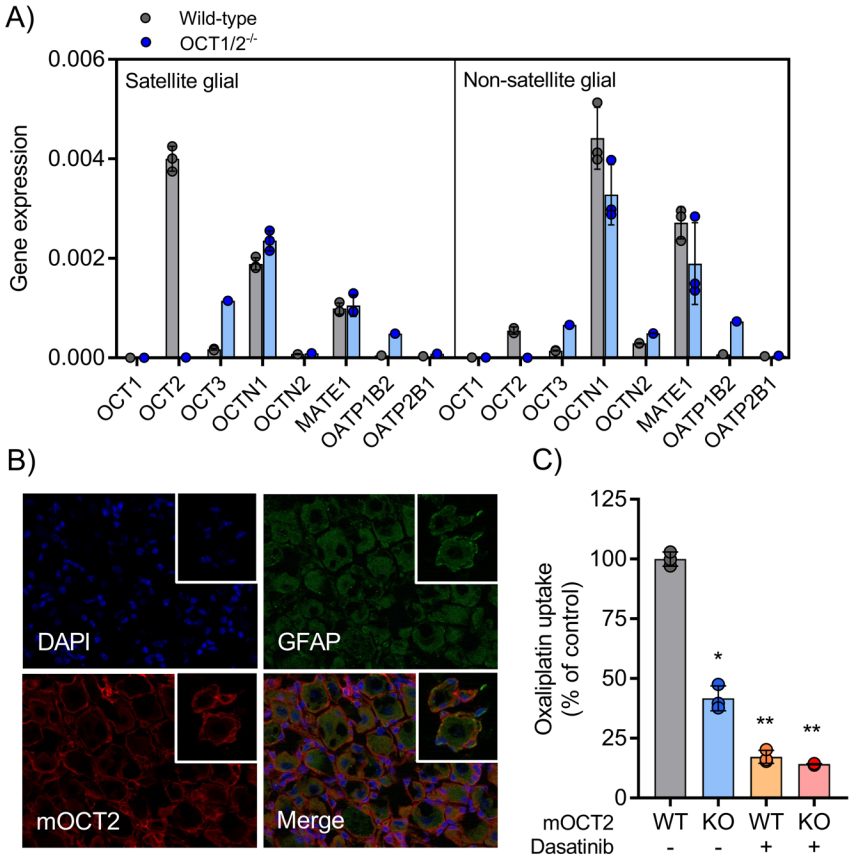


Figure 2. Localization and functional expression of OCT2 in satellite glial cells. (A) Expression of putative oxaliplatin carriers in primary satellite glial cells isolated from DRGs (left panel) compared to non-satellite glial cell fractions (right panel) of untreated wild-type FVB mice. **(B)** Immunofluorescence staining of DRG cross sections with DAPI (blue), mOCT2 (red), or GFAP (green). Representative images at 10x and 100x. **(C)** Accumulation of oxaliplatin in primary SGCs isolated from untreated wild-type FVB or OCT1/2$^{-/-}$ mice with or without 10 μM dasatinib pre-treatment. Oxaliplatin uptake is expressed as a percent change compared to empty vector controls. Statistical analysis was performed using a one-way ANOVA with Dunnett's post-hoc test: *P<0.05, **P<0.01 compared to baseline values.

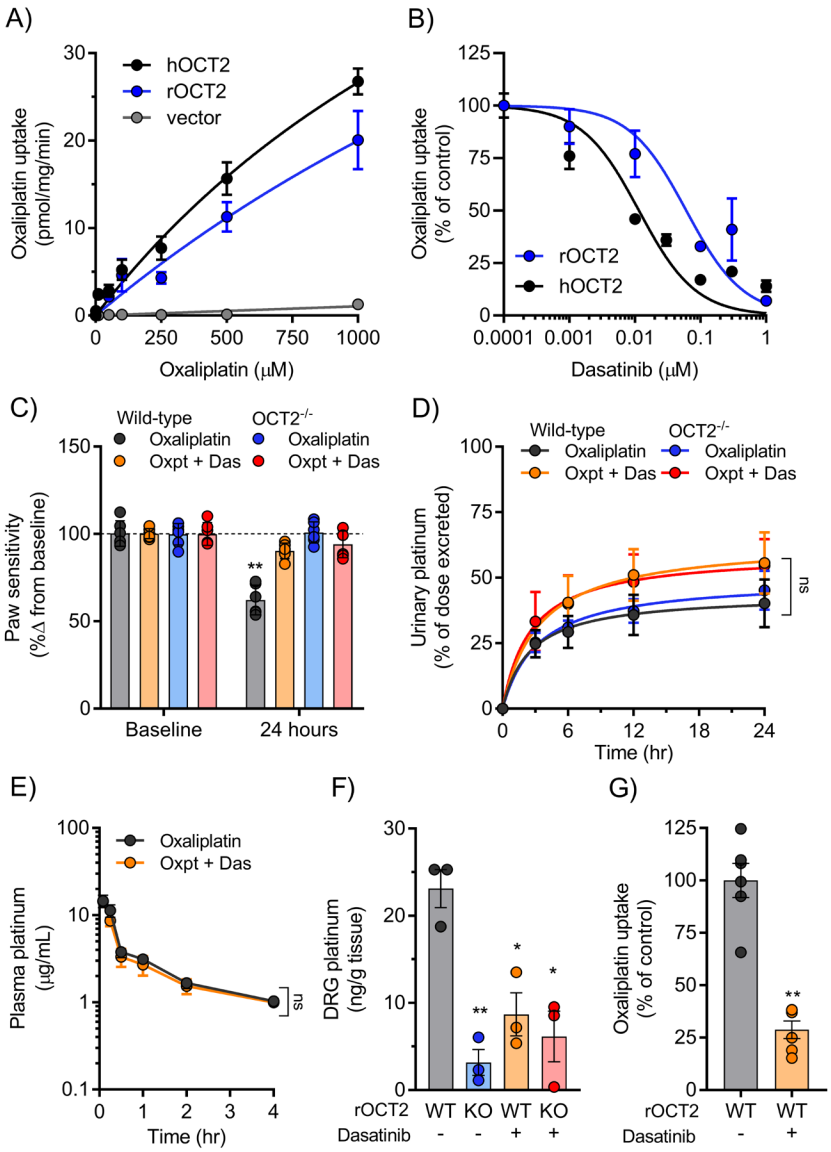


Figure 3. Genetic and pharmacologic targeting of OCT2 protects rats from OIPN. (A) Comparative transport kinetics of HEK293 cells overexpressing rat or human homolog of OCT2 (V_{max}, 83.3 and 94.4 pmol/mg/min, and K_m 2130 and 3726 μM, respectively). **(B)** Sensitivity of rat and human OCT2 to inhibition by dasatinib, IC_{50} 60.4 and 11.5 nM, respectively. **(C)** OIPN in wild-type or $OCT2^{-/-}$rats, expressed as a percentage change to baseline values. **(D)** Urinary excretion, expressed as a percentage of total administered platinum, and **(E)** systemic clearance of oxaliplatin in rats pretreated with vehicle or dasatinib. **(F)** Platinum concentrations from isolated rat DRGs. All studies represented in (C-F) reflect response/exposure following a single injection of 10mg/kg oxaliplatin and/or pretreatment with oral 15 mg/kg dasatinib. **(G)** Accumulation of oxaliplatin in SGCs isolated from untreated wild-type rats. Statistical analysis was performed using a one-way ANOVA with Dunnett's post-hoc test: *P<0.05, **P<0.01 compared to baseline values.

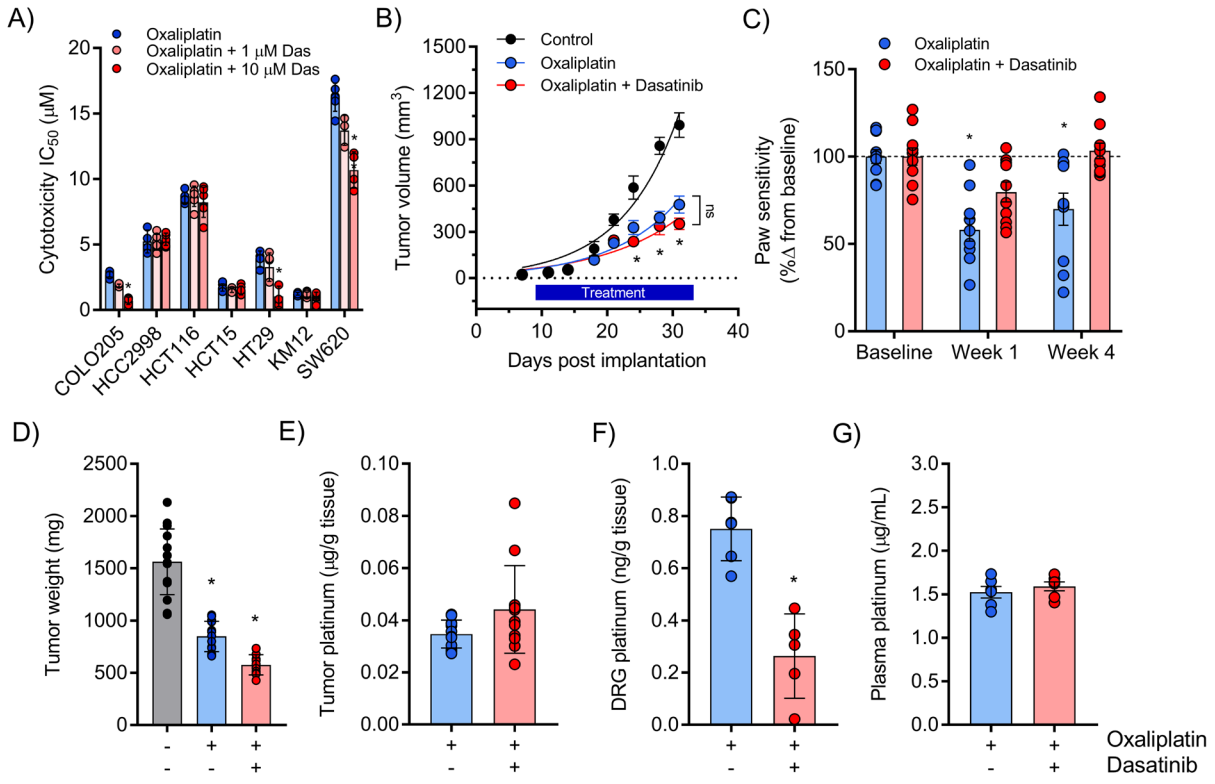


Figure 4. Dasatinib does not antagonize antitumor properties of oxaliplatin. (A) Cytotoxicity of oxaliplatin in the presence or absence of dasatinib (1 or 10μM) following 72 hour continuous exposure. **(B)** Tumor volume of immunocompromised nude mice subcutaneously inoculated with HCT116-luciferase labeled cells. **(C)** OIPN in tumor-bearing mice at baseline, one week, or four weeks through treatment. **(D)** Quantification of tumor weight, and **(E)** platinum concentrations in tumors, **(F)** DRGs, or **(G)** plasma (30 minutes after final injection, representing study state concentrations). 4mg/kg oxaliplatin was injected twice daily for four weeks (cumulative dose 32 mg/kg), pretreatment with oral 15 mg/kg dasatinib 30 minutes before. Paw sensitivity is expressed as a percent change from baseline values. Statistical analysis was performed using an unpaired two-sided Student's *t* test with Welch's correction: *P<0.05, compared to baseline values.