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Quantitative adverse outcome pathway modeling of cisplatin-induced nephrotoxicity: developing *in vitro* and *in vivo* models for predictive extrapolation

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Abstract

Nephrotoxicity is a major concern in the safety assessment of chemicals and drugs. Computational modeling, particularly the use of quantitative adverse outcome pathways (qAOPs), offers a promising strategy to improve the translation from *in vitro* to *in vivo*, thereby facilitating reliable predictions of *in vivo* adverse outcomes and potentially reducing the need for animal testing. Platinum-based drugs are widely used in chemotherapy, yet their clinical application is frequently constrained by nephrotoxic effects. Here, we focus on the development of ordinary differential equation (ODE)-based qAOPs for platinum-induced nephrotoxicity by defining both an *in vitro* and an *in vivo* data-driven model. The *in vitro* model incorporates newly generated, time-course gene expression and propidium iodide (PI) staining data from RPTEC/TERT1 cells exposed to cisplatin. The *in vivo* model employs published rat data, including dose-response platinum kinetics as well as single-dose time-course platinum kinetics, gene expression, and histopathology data. Our quantitative approach shows that key processes in the AOP related to immune system activity are nonlinear. Specifically, clearance of necrotic kidney cells by immune system activity counters damage accumulation on a timescale of days, yet low-level inflammation still cumulatively affects kidney failure in the long run. Moreover, we perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) to link the 2 models. With this approach, *in vivo* adverse outcome predictions can be made in the future not only for platinum-based compounds but also for the safety assessment of other chemicals and drugs, reducing the need for animal testing.

Keywords: ODEs; qAOP; cisplatin

Platinum-based compounds are widely employed in chemotherapy, with application in the treatment of malignant tumors such as breast, ovarian, cervical, esophageal, bladder, small cell lung, testicular, and colorectal cancer (Zhang et al. 2022). However, nephrotoxicity—ie deterioration in kidney function caused by drugs and external stresses (Ha 2023)—is a prominent adverse effect and dose-limiting factor of various platinum-based chemotherapeutic agents, including cisplatin, carboplatin, and oxaliplatin (Oun et al. 2018). Specifically, nephrotoxicity stands out as the primary dose-limiting factor for cisplatin, whereas this adverse effect, although still observed, is notably less severe with carboplatin and oxaliplatin (Vijgh 1991; Grothey 2003; Oun et al. 2018). Cisplatin-induced severe renal damage manifests itself as adverse outcomes (AOs) such as acute kidney injury (AKI) and chronic kidney disease (CKD) through common mechanisms of action (Boulikas and Vougiouka 2003; Pabla and Dong 2008). New generation platinum-based compounds were introduced to mitigate this severe effect. Notably, it remains of particular interest

to elucidate how these differences in nephrotoxicity arise among newer agents, especially given their similar mechanisms of action to cisplatin (Sousa et al. 2014; Martinez-Balibrea et al. 2015).

Platinum-based drugs induce cytotoxic effects primarily through the formation of DNA adducts (Rietbroek et al. 1997; Wang and Lippard 2005; Veal et al. 2007; Yao et al. 2007). Particularly, both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) are subject to DNA adduct formation, triggering various forms of molecular stress ultimately converging to common adverse effects (Brivet et al. 2011; Marullo et al. 2013). This molecular stress includes DNA damage, mitochondrial dysfunction, and oxidative stress, and invokes cellular stress pathways such as the DNA damage response that aim to protect cells. Mitochondrial dysfunction and oxidative stress are particularly intensified by mtDNA damage, due to its proximity to ROS production sites and slow DNA repair processes (Yakes and Van Houten 1997; Kowaltowski and Vercesi 1999; Kang and Hamasaki

2003; Picca et al. 2020; Rong et al. 2021). Indeed, mtDNA repair is likely slower than nuclear DNA repair due to the lack of the extensive repair machinery as present in the nucleus (eg nucleotide excision repair) (Larsen et al. 2005; Zinovkina 2018). This suggests that mtDNA damage is often closely interconnected with mechanisms of action such as oxidative stress.

DNA damage and oxidative stress can lead to both apoptotic and necrotic cell death in a dose-dependent manner (Pabla et al. 2008; Florea and Büsselberg 2011; Yousef and Hussien 2015). Indeed, high concentrations of cisplatin primarily induce necrosis, whereas low concentrations tend to induce apoptosis (Lieberthal et al. 1996). In turn, cell death leads to the release of damage-associated molecular pattern molecules (DAMPs), which activates immune responses (which we refer to as “Inflammation”) via Toll-like receptors (TLRs) expressed by innate immune cells (Piccinini and Midwood 2010). Signaling via TLRs invokes the release of proinflammatory chemokines and cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1 β , IL-18, and IL-6 (Kadowaki et al. 2001; Ramesh and Reeves 2002; Faubel et al. 2007; Summers et al. 2011). Increase in the production of chemokines and cytokines as a response to cell death enhances and attracts several types of immune cells, eg dendritic cells, T cells, and macrophages (Liu et al. 2006; Tadagavadi and Reeves 2010), which clear injured and dead cells, mitigating the local damage and promoting organ recovery. However, high concentrations of these cytokines can promote TNF-driven inflammation and can ultimately escalate to organ failure (Zager et al. 2006; Peres and Cunha 2013; Volarevic et al. 2019).

One of the reasons underlying the strong nephrotoxic effects of cisplatin includes its transport into proximal tubular epithelial cells. Cisplatin has a high affinity for organic cation transporter 2 (OCT2) (Filipski et al. 2009), which is located on the basolateral membrane of these cells and transports cisplatin into the cells. Among newer platinum-based compounds, oxaliplatin also shows affinity for OCT2, whereas carboplatin is characterized by non-transporter-mediated uptake (Motohashi and Inui 2013). Once inside the proximal tubular epithelial cell, multidrug and toxin extrusion (MATE) transporters actively pump cisplatin across the apical (brush border) membrane into the tubular lumen, facilitating its excretion (Koepsell et al. 2007). Cisplatin is transported to a lesser extent by MATE compared with OCT2 (Freitas-Lima et al. 2020), whereas oxaliplatin is also a substrate of MATE. In contrast, carboplatin does not rely on MATE for its transport (Motohashi and Inui 2013).

The development of computational models can support understanding how the complex mechanisms of action described above jointly lead to platinum-induced nephrotoxicity, reducing the need for animal testing. However, properly including the biological complexity of the mechanism of action of platinum-induced nephrotoxicity is challenging. In recent years, the development of (quantitative) Adverse Outcome Pathways ((q)AOPs) has emerged as a promising approach to address this challenge (Spinu et al. 2020; Pains et al. 2022). qAOPs provide a mechanistic and computational framework that quantitatively links molecular initiating events (MIEs)—ie the interaction between a toxicant and a biomolecule (Allen et al. 2016)—to AOs, representing the observable effect at the tissue, organ, organism, or population level (Foran et al. 2019), through a series of Key Events (KEs) connected by causal Key Event Relationships (KERs) (Villeneuve et al. 2014). Whereas qualitative AOPs aim to support hazard and risk assessment of compounds (Vinken et al. 2017), quantitative AOPs aim to quantify the KERs, thus providing further confidence in

the AOP and supporting regulatory decision making (Perkins et al. 2015, 2019; Conolly et al. 2017). In terms of the modeling framework, we use ODEs, which are well-suited for capturing time-course data, and follow the computational approach described by Di Tillio and Beltman (2024).

In this study, we develop 2 ODE-based qAOP models to investigate the nephrotoxic mechanisms of cisplatin. The first model is based on newly generated in vitro experimental data acquired from RPTEC/TERT1 cells exposed to cisplatin. This cell line is a widely used human renal proximal tubule epithelial line that serves as a relevant representative for nephrotoxicity studies (Wilmes et al. 2013; Kramer et al. 2015). The second qAOP model utilizes existing in vivo kidney data from rats exposed to cisplatin. As the DNA damage response is recognized as a primary mechanism underlying cisplatin-induced nephrotoxicity (Sahu et al. 2013; Zhu et al. 2015; Tang et al. 2023), we will consider it a key component in our modeling. In constructing these models, we employed transcriptomics, imaging, and histopathology data to capture KE triggering. Specifically, we obtained KE measurements based on transcriptomics data using a mapping procedure grounded in coregulated gene network analysis (implemented as the “TXG-MAPr” (Callegaro et al. 2021), which is based on weighted correlation network analysis (Langfelder and Horvath 2008)). In this approach, gene sets are defined through correlation analysis and subsequently linked to KEs via enrichment analysis and manual annotation. With our mathematical models based on these quantitative data, we show that key processes in the AOP related to immune system activity are nonlinear. Finally, we link the 2 qAOP models through Quantitative In Vitro-In Vivo Extrapolation (QIVIVE) to enable the prediction of in vivo nephrotoxic outcomes for platinum-based drugs using only in vitro data (Yoon et al. 2012).

Materials and methods

Experimental data

For model development, we employed newly acquired time course data from in vitro RPTEC/TERT1 cells and existing time course data from rats, in both cases exposed to cisplatin.

In vitro experimental data

As for the in vitro data, RPTEC/TERT1 cells were routinely cultured and differentiated in culture medium, ie a 1:1 mix of DMEM (ThermoFisher Gibco, 11966-025) and Ham's F12 (ThermoFisher Gibco, 21765-029) (resulting in a final concentration of 5 mM glucose) and supplemented with 2 mM Glutamax (ThermoFisher, 350500038), 10 ng/ml epidermal growth factor (Merck, E9644), 36 ng/ml hydrocortisone (Merck, E9644), 5 μ g/ml insulin, 5 μ g/ml transferrin, 5 ng/ml selenium (Sigma-Aldrich, I1884), 100 U/ml penicillin, and 100 μ g/ml streptomycin (ThermoFisher Gibco, 15070-063). Cells were cultured in a controlled, humidified 37°C, 5% CO₂ environment. For cisplatin exposure, RPTEC/TERT1 cells were seeded in 96-well plates with 25,000 cells per well and cultured for 14 d to reach the mature state of proximal tubule cells, as described by Aschauer et al. (2013). One day before exposure (at $t = -1$), the medium was replaced with fresh culture medium. Upon maturation (at $t = 0$), cells were exposed to cisplatin at 8 concentrations (0.1, 0.5, 1, 2.5, 5, 10, 20, 30, 50 μ M) for 4, 8, 16, 24, 48, or 72 h. Untreated RPTEC/TERT1 cells were taken along as a control. Culture medium has been used as a vehicle. Three replicates were considered for all the treatment groups and time-points.

After exposure, RPTEC/TERT1 cells were washed once with DPBS without calcium and magnesium chloride and lysed with 50 μ l lysis buffer (with a 1:1 ratio for DPBS: TempO-Seq lysis buffer BioSpyder at twice the intended concentration, mixed *ex tempore*). Plates were sealed with an aluminum silver seal (Greiner Bio-One, 676090), incubated for 15 min at room temperature and stored at -80°C . Before shipment, lysates were thawed and transferred to a 96-well conical bottom plate (Thermo Fisher, 249662), and plates were sealed with an aluminum silver seal. Plates were then again stored at -80°C until shipment on dry ice for RNA sequencing.

In separate experiments, the viability of *in vitro* RPTEC/TERT1 cells was assessed following exposure to cisplatin for a duration of 72 h. On the day of the exposure, the cells were stained with 200 ng/ml live Hoechst 33342 (ThermoFisher, H3570) in complete medium for 2 h. Afterward, the medium containing Hoechst was refreshed with complete medium containing 0.2 μM propidium iodide (PI). Moreover, cisplatin was added such that its final concentrations, as mentioned above, were reached. The plates were thereafter placed in the microscope within 1 h after exposure and imaged for 72 h. The images were taken with a Nikon TiE2000 confocal laser microscope (excitation at 540 and 408 nm), equipped with an automated stage and perfect focus system with 2 image points every well. During imaging, the plates were maintained in a humidified atmosphere at 37°C and 5% CO_2 /air mixture. The imaging was done with a 20 $\times$ magnification objective and performed every 1.5 h. Each plate was exposed to the same concentrations of cisplatin, with 3 replicates per condition (each consisting of 2 technical replicates, ie 2 separately imaged positions in a well).

In vivo experimental data

The rat data, from a study conducted by [Wijaya et al. \(2025\)](#), consisted of transcriptomics and histopathology data, and kinetics data measuring platinum accumulation in blood plasma and the kidney. In brief, the transcriptomics and histopathology data consisted of 12 time points for the cisplatin treatment case in the range 1 to 672 h (28 d). For each time point, 3 different rats were sacrificed after intravenous administration of 5 mg/kg of cisplatin (single exposure). In the control experiment, 0.9% NaCl (saline) was administered to 4 groups of 3 rats, which were sacrificed at 1, 8, 15, and 28 d. Gene expression changes were measured by employing the TempO-Seq protocol. Moreover, laser capture microdissection (LCM) was employed to generate transcriptomics data for multiple kidney regions, namely glomerulus, cortical, and outer-medulla proximal tubules (OMPT). Because the OMPT was characterized by strong gene expression changes (ie relevant genes co-expressed in several pathways of interest exhibited high log₂FC values), we only considered data from that specific region. Indeed, analyzing the whole kidney could lead to misaligned risk predictions, as a strong toxic response in the medulla may be masked by weak responses in the rest of the organ.

The histopathology data consisted of expert-based qualitative scoring of H&E staining images across the 12 time points. Rats were graded in the following categories: "Absent", "Minimal", "Mild", "Moderate," and "Marked". In order to provide a quantification of this data, we mapped these categories in a range of values between 0 and 1, in increasing order of severity. In addition to the time-course data at dose 5 mg/kg, we also utilized dose-response data for both transcriptomics and kinetics, consisting of measurements taken 72 h post-exposure at dose levels of 1, 2.5, 5, and 7.5 mg/kg.

Image analysis

In order to extract the number of dead cells from *in vitro* time-lapse images of Hoechst- and PI-stained RPTEC/TERT1 cells, we quantified the number of PI-positive cells with image analysis. First, nuclei were segmented using a trained segmentation pipeline from Ilastik 1.4.0 based on the Hoechst channel. Second, the resulting segmented nuclear images were exported to CellProfiler 3.0 in order to determine PI-positive cells. To quantify the fraction of PI-positive cells, the images from the PI channel were masked with 2-pixel dilated nuclei (as based on the nuclear segmentation), thus excluding background staining noise. PI-positive pixels were determined using the minimum cross-entropy method within the IdentifyPrimaryObjects CellProfiler module, which determines the segmentation threshold based on fitting intensity peaks for background and foreground pixels. For each nucleus, we determined a ratio between the area of PI-positive pixels and that of nuclear pixels. Cells were defined as PI-positive when this ratio exceeded 0.1. Finally, we calculated the fraction of PI-positive (necrotic) cells as the number of PI-positive nuclei divided by the total number of nuclei within the image.

Transcriptomic data analysis

TempO-Seq analysis was performed on both the *in vitro* RPTEC/TERT1 and *in vivo* rat kidney gene expression data following cisplatin exposure.

TempO-Seq analysis of *in vitro* RPTEC/TERT1 data was performed using a whole transcriptome probe panel, and expression data were returned by BioClavis as counts per probe per treatment. To ensure high quality, the data underwent initial quality control measures. A relevance filter, as described by [Verheijen et al. \(2022\)](#), was applied to remove low-quality probes. Subsequently, counts per million (CPM) of probes targeting the same gene were aggregated, resulting in a total expression count for each gene. Samples with fewer than 1,000,000 reads or less than 10% of reads for that sample mapped to genes were excluded from the analysis. Differential expression analysis was conducted using DESeq2. Unlike the standard DESeq2 approach, which combines replicates to obtain a single log₂ fold change (log₂fc) value for each treatment condition, we modified the analysis to calculate log₂fc values for each replicate separately. To achieve this, we compared the expression levels of each individual treatment sample with the combined control replicates ($n = 6$) at the corresponding timepoint.

For the analysis of *in vivo* rat kidney data, we followed the data analysis procedure as performed on the original data in [Wijaya et al. \(2025\)](#) using a Rat Whole Transcriptome panel. This consisted of the following steps: We first filtered out samples with less than 10% of the associated reads properly mapped to a probe, to ensure high data quality for subsequent steps. Raw read count values were summed across technical replicates, and a count of 1 was added to every value to avoid undefined logarithmic values at zero. Probes with base mean values—ie the average of counts normalized using size factors, which are scaling constants that adjust for differences in total read counts across samples—below the first quantile were excluded. For each kidney sample, Pearson correlation analysis was conducted to establish the similarity between the 2 technical replicates and only replicates with a Pearson correlation above 0.8 were retained. We calculated CPM values to assess data distribution and sample comparability, whereas the raw count matrix was provided as input to the DESeq2 package for differential expression analysis and log₂ fold change calculation ([Love et al. 2021](#)). Specifically, focusing on the OMPT kidney segment, the log₂fc

per replicate of the normalized count relative to the control of that segment was computed. For the time-course data, the controls from different time points were aggregated into a single control, as transcriptional variation was minimal. This normalization procedure was already applied by Wijaya et al., with the only difference that here we compute the log2fc per replicate, as having separate estimated values is beneficial for mathematical model calibration.

KE quantification based on gene expression

In order to quantify KERs, one first requires quantitative measures for related KEs representing their “activity,” ie degree of triggering. Such quantification highly depends on the type of available data, which could involve specific assays. Here, we consider how such KE quantification could be achieved on the basis of gene expression data, although this is possibly not feasible for all KEs. In general, the main goal is to link gene expression changes to KE changes. This can be done by employing tools based on gene co-regulation, clustering, and enrichment analysis.

TXG-MAPr

A first approach for KE mapping and quantification is based on the TXG-MAPr, an interactive R-shiny application based on weighted gene co-expression network analysis (WGCNA) (Langfelder and Horvath 2008; Callegaro et al. 2021). Specifically, we employed the rat kidney TXG-MAPr (Kunnen et al. 2025), in which a set of 347 co-regulated gene networks (referred to as modules) was constructed from the TG-GATEs rat kidney toxicogenomics dataset using WGCNA. We uploaded the log2fc data on the rat kidney TXG-MAPr, after which the tool generated, among other outputs, the eigengene (EG) score table based on the log2fc of genes for different conditions. The EG score is a measurement at the module level, which represents the activation of a certain module for each condition (eg dose level, timepoint, replicate). The EG score depends on the Z-scored log2fc of each gene belonging to the module and its correlation eigengene (corEG) value. The latter is given by the correlation between the gene log2fc and the module EG score. Using the functional annotation of modules, which is based on GO terms, pathway, and transcription factor analysis, we performed KE mapping, ie linked modules to relevant KEs. Because both in vitro and in vivo data were uploaded to the rat kidney TXG-MAPr, human genes were converted to rat orthologs using the rat genome database (Smith et al. 2019).

For the present work, we employed module rKID: 7 m to quantify the in vivo KE “Inflammation” and rKID: 160 to quantify both in vitro and in vivo “DNA Damage” KEs. Module rKID: 106 is enriched for Oxidative Stress genes, but not employed in our qAOPs. Note that the module selection process is guided by the enrichment and module tabs within TXG-MAPr. These provide information on GO terms, pathway analysis, and transcription factor (TF)—target gene interactions for each module, allowing selection based on confidence levels. Details on the genes associated with each module, along with statistically significant enrichments (eg GO terms, pathways, diseases, or biological processes) and module annotations, can be found in Tables S1, S3, and S5 of Kunnen et al. (2025). In the case of the KEs relevant for the present work, modules rKID: 160, rKID: 106, and rKID: 7 m contain well-known target genes of the p53, NRF2, and NF- κ B transcription factors, respectively (Ngo et al. 2020; Torrente and DeNicola 2022; Wang et al. 2023).

We chose the rat kidney TXG-MAPr for 2 main reasons. It ensures consistency of gene sets across shared KEs in in vitro and in vivo qAOP models. In addition, because the EG score is based

on Z-scored log2FC values of module genes, differences in expression levels due to the distinct data generation methods for the in vitro and in vivo measurements are mitigated. Importantly, TXG-MAPr only defines module structure and corEG values; the uploaded log2FC data provide the actual input, minimizing inter-platform bias.

Informative gene selection

Similar to the EG score based on TXG-MAPr modules, we devised an alternative approach by calculating the so-called Informative Gene Selection (IGS) score, which also provides a unique quantitative measure of expression among a specific gene set. Given a set of n genes, for which log2fc values per measurement replicate are available, the IGS score is defined as follows:

$$P_j = \frac{\sum_{k=1}^n \log_2 f_{c_{kj}} w_k}{\sum_{k=1}^n w_k} \quad (1)$$

where j is the replicate number and $w_k = 1/n \sum_{i=1}^n |\text{cor}(x_i, x_k)|$, with cor being a gene coexpression similarity matrix for genes i and k . This method can be combined with the output from the TXG-MAPr in order to quantify KEs by applying the IGS score formula, considering gene sets corresponding to modules in the TXG-MAPr. However, in general, one could combine IGS with gene sets selected by any enrichment analysis tool, which makes it a versatile method. Note that when combining IGS with the TXG-MAPr, one can also filter out genes from modules by requiring a minimal correlation eigengene (corEG) value to include specific genes.

In vitro ODE-based qAOP

In order to model the in vitro AOP Fig. 4a), we formulated the following ODE system:

$$\begin{aligned} dQ_{api}/dt &= N_{cell}(F_{outa}Q_{cell}/(N_{cell}V_{cell}) - F_{ina}Q_{api}/V_{api} - K_{el}Q_{cell}/(N_{cell}V_{cell})) \\ dQ_{bas}/dt &= N_{cell}(F_{outb}Q_{cell}/(N_{cell}V_{cell}) - F_{inb}Q_{bas}/V_{bas}) \\ dQ_{cell}/dt &= N_{cell}(F_{ina}Q_{api}/V_{api} + F_{inb}Q_{bas}/V_{bas} - (F_{outa} + F_{outb})Q_{cell}/(N_{cell}V_{cell})) \\ dQ_{inter}/dt &= k_{inter}(Q_{cell} - Q_{inter}) \\ dDD/dt &= k_{cDD}Q_{inter}/(k_{hillDD} + Q_{inter}) - d_{DD}DD \\ dNEC/dt &= \maxdeath(DD^h/(k_{hillnec}^h + DD^h))(p - NEC) \\ Q_{api}(0) &= 10^{-15}C_0M_WV_{api}, Q_{bas}(0) = 10^{-15}C_0M_WV_{bas}, \\ Q_{cell}(0) &= 0, Q_{inter}(0) = 0, DD(0) = 0, NEC(0) = 0. \end{aligned} \quad (2)$$

In system (2), we include the kinetic model and parameters from Wilmes et al. (2015), based on an assay with polarized RPTEC/TERT1 cells (Wilmes et al. 2013). This corresponds to the first 3 equations, describing the time dynamics of the amount of cisplatin in apical and basolateral medium (Q_{api} and Q_{bas} , respectively) and within cells (Q_{cell}). The values of the parameters N_{cell} , V_{cell} , F_{outa} , F_{ina} , F_{outb} , F_{inb} , V_{api} , V_{bas} , K_{el} , and M_W are fixed at previously determined values by Wilmes et al. (2015) and described in Table 1. A limitation of the in vitro PK model is that it was designed for a slightly different experimental setup compared with that of the present study, ie a monolayer separating apical and basolateral medium in the original study, and a monolayer only connected to medium on one side in the current study. This difference in setup could also yield different expression levels of proteins that influence the kinetics, such as OCT2 and MATE transporters.

Table 1. Description of the fixed parameters in model (2), taken from (Wilmes et al. 2015).

Parameter name	Value	Unit	Description
Ncell	2×10^6	–	Number of cells
Vcell	2005	μm^3	Volume of a cell
Fouta	1.24×10^4	$\mu\text{m}^3/\text{h}$	Cisplatin apical cell exit rate
Fina	2.232×10^4	$\mu\text{m}^3/\text{h}$	Cisplatin apical cell entry rate
Fouthb	4.518×10^2	$\mu\text{m}^3/\text{h}$	Cisplatin basal cell exit rate
Finb	2.304×10^4	$\mu\text{m}^3/\text{h}$	Cisplatin basal cell entry rate
Vapi	1×10^{12}	μm^3	Apical volume of assay medium
Vbas	2×10^{12}	μm^3	Basal volume of assay medium
K _{el}	3.024×10^4	$\mu\text{m}^3/\text{h}$	Cisplatin elimination rate from cell system
MW	301.1	g/mol	Molecular weight of cisplatin

Table 2. Description of estimated parameters in model (2).

Parameter name	Unit	Description	Predicted mean	Predicted SD
kinter	1/h	Rate of cisplatin transfer from Cell to Intermediate compartment	5.32×10^{-2}	2.03×10^{-2}
dDD	1/h	Rate of DNA repair	4.35×10^{-2}	7.18×10^{-3}
kcDD	1/h	Rate at which cisplatin accumulation affects DNA Damage response	2.66×10^{-1}	2.57×10^{-2}
khillDD	μg	Amount of cisplatin causing half of the maximal rate of DNA Damage response	3.45×10^{-3}	5.66×10^{-4}
maxdeath	1/h	Maximal rate of cell death	1.00	2.92×10^{-1}
h	–	Hill exponent	26.91	2.61
khillNEC	a.u.	Amount of DNA Damage causing half of the maximal rate of Necrosis	5.09	1.52×10^{-1}
P	–	Maximal detectable fraction of PI stained cells	7.83×10^{-1}	6.62×10^{-2}

Thus, although we employed a published model for cisplatin, the actual in vitro kinetics may deviate from those represented.

We included another intermediate compartment (referred to as Q_{inter}) to account for a delay between rapid cisplatin accumulation in cells, DNA adduct formation, DNA damage in the form of double-strand breaks, and activity of the DNA damage response. Note that Q_{inter} does not refer to an actual cellular compartment, yet it is only included to account for a delay in induction of the DNA damage response due to intermediate events such as adduct formation. Thus, the intermediate compartment connects the kinetic and dynamic parts of model (2). The state variables DD and NEC refer to the KEs “DNA Damage” and “Cell Death” (specifically, “Necrosis”) in the AOP. These are quantified employing EG scores of module rKID: 160 and PI staining data, respectively. The dynamics of DD and NEC were modeled using Michaelis-Menten and Hill-like kinetics, respectively. Specifically, the term $P - \text{NEC}$ regulates the amount of “Cell Death.” Because P is the maximal detectable fraction of PI-stained cells (see Table 2), $P - \text{NEC}$ becomes zero when the maximal detectable levels of necrosis are reached. Note that the fraction of necrotic cells (NEC) does not alter the total cell count (N_{cell}). Thus, we consider necrotic cells to contribute to cisplatin uptake and elimination, because kinetic processes such as partitioning and irreversible protein binding do not require viable cells (Groothuis et al. 2015; Proença et al. 2021). Furthermore, C_0 is the initial concentration of cisplatin, expressed in μM , which varies among 1.0, 2.5, 5.0, 10.0, 20.0, 30.0, and 50.0 (ie the common concentration levels among RPTEC/TERT1 transcriptomics and PI staining data). The remaining model parameters are described in Table 2, along with their estimated values.

In vivo cisplatin kinetics models

To describe the in vivo data, we introduce 2 different pharmacokinetic models. The first one, being a 2-compartmental model (PK2), is defined as:

$$\begin{aligned}
 d\text{Plasma}/dt &= k_{\text{KidPlas}}\text{KidneyPt} - (k_{\text{PlasKid}} + k_{\text{ePlas}})\text{Plasma}, \\
 d\text{KidneyPt}/dt &= k_{\text{PlasKid}}\text{Plasma} - (k_{\text{KidPlas}} + k_{\text{eKid}})\text{KidneyPt}, \\
 \text{Plasma}(0) &= \text{Plasma}_0, \\
 \text{KidneyPt}(0) &= 0.
 \end{aligned} \quad (3)$$

Here, k_{KidPlas} and k_{PlasKid} are the rates at which cisplatin translocates from kidney to plasma, and vice versa, respectively; k_{ePlas} and k_{eKid} are the rates at which cisplatin decays in plasma and kidney, respectively. In model (3), we fit the Plasma and Kidney platinum kinetic data to the *Plasma* and scaled *KidneyPt* variables, respectively. Because the available Kidney platinum data is normalized with the measured amount of protein, it is necessary to define a *scale* parameter to take such normalization into account, ie we fit the Kidney platinum data to

$$\text{scale} \times \text{KidneyPt}.$$

We also define an extended version of model (3), PK3, by adding a third compartment “AccuPt,” which delays the clearance of platinum from the Kidney:

$$\begin{aligned}
 d\text{Plasma}/dt &= k_{\text{KidPlas}}\text{KidneyPt} - (k_{\text{PlasKid}} + k_{\text{ePlas}})\text{Plasma}, \\
 d\text{KidneyPt}/dt &= k_{\text{accuKid}}\text{AccuPt} + k_{\text{PlasKid}}\text{Plasma} \\
 &\quad - (k_{\text{KidPlas}} + k_{\text{KidAccu}} + k_{\text{eKid}})\text{KidneyPt}, \\
 d\text{AccuPt}/dt &= k_{\text{KidAccu}}\text{KidneyPt} - k_{\text{accuKid}}\text{AccuPt}, \\
 \text{Plasma}(0) &= \text{Plasma}_0, \\
 \text{KidneyPt}(0) &= 0, \\
 \text{AccuPt}(0) &= 0.
 \end{aligned} \quad (4)$$

In addition to the parameters specified in system (3), we introduce k_{KidAccu} and k_{accuKid} as the rates at which cisplatin translocates from kidney to the accumulation compartment, and vice versa, respectively. Extending the approach for the 2-compartment model, also for model (4) we fitted the Plasma Platinum

Table 3. Parameter estimates for models (3) and (4).

Parameter name	Unit	Predicted mean PK3	Predicted SD PK3	Predicted mean PK2	Predicted SD PK2
kPlasKid	1/protein × h	2.19×10^0	1.43×10^{-1}	2.10×10^0	1.48×10^{-1}
kePlas	1/h	6.13×10^{-2}	3.20×10^{-2}	7.56×10^{-2}	4.28×10^{-2}
kKidPlas	Protein/h	2.13×10^{-1}	2.10×10^{-2}	1.93×10^{-1}	2.10×10^{-2}
keKid	1/h	4.90×10^{-3}	3.12×10^{-3}	6.74×10^{-3}	3.93×10^{-3}
Plasma ₀	μg	4.99×10^1	9.63×10^{-1}	4.99×10^1	9.62×10^{-1}
kAccuKid	1/h	4.73×10^{-4}	8.59×10^{-5}	—	—
kKidAccu	1/h	4.70×10^{-3}	4.39×10^{-4}	—	—
scale	Protein	3.99×10^0	3.24×10^{-1}	4.25×10^0	2.44×10^{-1}

data to the *Plasma* variable and the Kidney data to the scaled sum of *KidneyPt* and *AccuPt* variables, ie:

$$\text{scale} \cdot (\text{kidneyPt} + \text{AccuPt}), \quad (5)$$

where *scale* is a parameter to be inferred. Below, in Table 3, we list estimated parameters for PK2 and PK3 models.

Generally, integrating a qAOP with a detailed Physiologically Based Pharmacokinetic (PBPK) model that accounts for distribution volume and additional organs would be suitable. However, for the purpose of this study, it is sufficient to develop a model without the complexity of a PBPK framework. The latter would however offer the advantage of rendering predictions for other compounds for which pharmacokinetic parameters are not yet available.

In vivo ODE-based qAOP

Here, we define a qAOP describing the links between in vivo rat cisplatin kinetics, DNA Damage, Cell Death, Inflammation (ie inflammation due to infiltrating immune cells), and Kidney Failure (Fig. 3a).

This qAOP is thus similar as the one based on in vitro data, but extended beyond the KE Cell Death. DNA Damage and Inflammation KEs are quantified employing the EG scores from module rKID: 160 and rKID: 7 m from the in vivo rat kidney TXG-MAPr, respectively. In order to quantify the KE “Cell Death” and the AO “Kidney Failure,” we employed histopathology data related to Necrosis and Fibrosis, respectively. The resulting system of equations takes the following form:

$$\begin{aligned}
 dDD/dt &= k_{\text{kidDD}} \text{scale}(\text{KidneyPt} + \text{AccuPt}) / \\
 &(\text{k}_{\text{hillDD}} + \text{scale}(\text{KidneyPt} + \text{AccuPt})) - d_{\text{DD}} DD, \\
 dCD/dt &= \text{maxdeathDD}^b / (\text{k}_{\text{hillNEC}}^b + DD^b) (1 - CD) - d_{\text{CD}} CD \text{INF}, \\
 d\text{INF}/dt &= k_{\text{CDINF}} CD - d_{\text{INF}} \text{INF}, \\
 d\text{KF}/dt &= k_{\text{INFKF}} \text{INF}^{\lambda+1} / (\text{k}_{\text{hillKF}}^{\lambda+1} + \text{INF}^{\lambda+1}) (1 - \text{KF}), \\
 d\lambda/dt &= -d_{\lambda} \lambda, \\
 DD(0) &= 0, CD(0) = 0, \text{INF}(0) = 0, \text{KF}(0) = 0, \\
 \lambda(0) &= 10.
 \end{aligned} \quad (6)$$

Note that system (6) is coupled to the earlier discussed PK3 model (4). On top of the represented KERs in the AOP scheme (Fig. 3a), we consider a negative feedback loop from Inflammation to Cell Death, representing clearance of dead cells and debris by immune system components. To describe the dynamics of kidney fibrosis (KF), we also required a Hill exponent (λ) that decays over time, and we fixed its rate of decay d_{λ} so that $\lambda + 1$ decays to 1 within the considered time frame. The choice to include a decaying Hill exponent implies that over time, the impact of inflammatory processes on the development of kidney

fibrosis is retained despite the waning of the acute inflammatory response. Thus, the effects of inflammation are not considered immediate but rather cumulative, such that, if sustained over time, it can significantly influence the adverse outcome.

The model parameters are described in Table 4, along with their estimated values.

Model calibration: CmdstanR

We performed model calibration by using the CmdStanR package, which is the R interface for CmdStan (Gabry et al. 2023). CmdStan is the shell interface to Stan (Carpenter et al. 2017), a probabilistic programming language providing Bayesian inference for model calibration, including ODEs, through Markov chain Monte Carlo (MCMC) methods. One Stan file for each model is defined. Those files consist of different blocks: In the “functions” block, we define the ODE model, including the state variables, equations, and parameters. In the “data” and “parameters” block, we define the input quantities and the quantities to be inferred, respectively.

The “transformed parameters” block is employed to run and store the solution of the ODE system. In the “model” block, priors to the inferred parameters and likelihood functions are defined. We employed EG scores per replicate to infer the variability within the likelihood function directly from the data, leading to more accurate and meaningful predictions. To select appropriate priors, an initial simulation is performed using broad priors (ie exponential distributions with a low rate parameter or uniform distributions). Based on the estimates from this first simulation, normal or lognormal priors centered around these values are then chosen, followed by another simulation. Student’s t-distributions are chosen to model the likelihood function. The likelihood function uses both input data and the output of the “transformed parameters” block and quantifies how likely the observed data are given a particular set of parameter values. Finally, the “generated quantities” block is employed to test the model’s out-of-sample performance, using the Pareto smoothed importance sampling (PSIS) (Vehtari et al. 2024) from the Leave-One-Out Cross-Validation (LOO) criteria (Vehtari et al. 2017).

Conversion from dose to in vivo rat kidney concentration

As one of the goals of the current work is to link in vitro to in vivo concentrations, we want to link each in vivo dose to a platinum concentration in the rat kidney. Using model (6), we can already link the in vivo dose to platinum level in the rat kidney, measured in μg/g protein. We translated this into μM units by using the following equation:

Table 4. Description of estimated parameters in model (6).

Parameter name	Unit	Description	Predicted mean	Predicted SD
kkidDD	1/h	Maximal rate of platinum-induced DNA Damage	1.91×10^{-1}	5.70×10^{-2}
khillDD	μg	Platinum concentration in Total Kidney compartment inducing half-maximal rate of DNA Damage	4.07×10^1	2.90×10^0
dDD	1/h	Rate of DNA repair	7.03×10^{-2}	2.32×10^{-2}
maxdeath	1/h	Maximum rate of DNA Damage-induced cell death	1.29×10^1	5.12×10^{-1}
h	–	Hill exponent in the cell death response	8.78×10^0	6.99×10^{-1}
khillNEC	a.u.	DNA Damage levels inducing half-maximal rate of Cell Death	4.31×10^0	3.24×10^{-1}
dCD	1/(a.u. × h)	Rate of inflammation-mediated decrease of dead cells	6.30×10^{-3}	2.30×10^{-3}
kCDINF	1/h	Rate of Cell Death-induced inflammation	2.60×10^{-1}	7.41×10^{-2}
dINF	1/h	Decay rate of INF	1.26×10^{-2}	3.44×10^{-3}
kINFKF	1/h	Rate of Inflammation-induced Kidney Failure	5.57×10^{-2}	2.46×10^{-2}
khillKF	a.u.	Inflammation levels inducing half-maximal rate of Kidney Failure	1.31×10^1	1.89×10^0

$$[Pt]_{\mu M} = C_{Pt}(\mu g g^{-1} \text{protein})P(g \text{proteing}^{-1} \text{tissue})\rho(gtissueL^{-1})/M(\mu g \mu mol^{-1}), \quad (7)$$

where C_{Pt} is the platinum concentration measured in the assay, expressed per gram of protein, P is the protein content of kidney tissue, expressed per gram of tissue, M is the molar mass of cisplatin, and ρ is the density of the kidney tissue. Based on Table 6 in Sysa et al. (2019), we used a mean protein content in rat kidney (P) of 8.103×10^{-2} g proteing⁻¹ tissue. From National Center for Biotechnology Information (2025), we assume the molar mass of cisplatin M to be 300.05 gmol⁻¹. We assume the density of rat kidney tissue ρ to be $1,000$ gl⁻¹ (Rasch and Dørup 1997). Note that we expressed the concentration in terms of cisplatin equivalents by using the molar mass of cisplatin rather than elemental platinum, to facilitate direct comparison with in vitro exposure concentrations that are conventionally reported as nominal cisplatin doses.

qAOP app: predicting key events and adverse outcomes over time

We developed a Flask-based Python web application to provide interactive access to the pre-calibrated in vitro and in vivo qAOP models. The app solves the ODE systems using `scipy.odeint` (version 1.16.2) (Virtanen et al. 2020), samples parameter sets from the estimated posterior distributions, and visualizes results with `matplotlib` (version 3.10.6) (Hunter 2007). The service is deployed on the VHP4Safety platform and is accessible at <https://qaop.vhp4safety.nl/>.

BanffNET

Continuous histological lesion scores were generated with BanffNET, a recently developed framework performing weakly supervised regression analysis of histopathological lesions in kidney transplant pathology. The system was originally designed and validated on large multi-center cohorts of human kidney transplant biopsies and is currently under consideration for a patent application. In the current work, we employed the Interstitial fibrosis and tubular atrophy (ifta) and acute tubular necrosis (atn) scores, as described by Roufosse et al. (2018).

BMD analysis

BMD modeling was performed using the `drc` package in R (Ritz et al. 2015). PI-staining in vitro and histopathology in vivo necrosis data at 67 h were used. For the in vivo analysis, dose levels of 1, 2.5, 5, 7.5, and 10 mg/kg were mapped to kidney concentration using equation (7), assuming linear relationships between intravenous doses and kidney tissue concentrations, with

adverse outcomes taken from our ODE model predictions. For benchmark dose estimation, the following standard dose-response models from the `drc` package were evaluated: LL2, LL3, LL4, LL5, W14, and W24, defined as:

$$f_{LL2}(x) = 1/(1 + \exp(b(\log(x) - \log(e))))$$

$$f_{LL3}(x) = d/(1 + \exp(b(\log(x) - \log(e))))$$

$$f_{LL4}(x) = c + (d - c)/(1 + \exp(b(\log(x) - \log(e))))$$

$$f_{LL5}(x) = c + (d - c)/(1 + \exp(b(\log(x) - \log(e))))^f$$

$$f_{W14}(x) = c + (d - c)(1 - \exp(-\exp(b(\log(x) - \log(e)))))$$

$$f_{W24}(x) = c + (d - c) \exp(-\exp(b(\log(x) - \log(e)))).$$

The best-fitting model was selected based on the lowest Bayesian Information Criterion (BIC).

Results

TXG-MAPr-based EG and IGS scores are suitable for gene-expression-based KE mapping and quantification

As we require KE data to calibrate the qAOPs, it is necessary to select an appropriate method for mapping transcriptomics data into KE data. We first assessed whether TXG-MAPr-based EG and IGS scores are appropriate for KE mapping and quantification. A potential problem with TXG-MAPr-based gene set selection is the presence of modules containing a high number of genes. This could bring noise to the quantitative measurement, as genes that are weakly correlated with their respective module's EG score (ie with low `corEG` value) may not be representative for a specific KE yet could impact the trend of the data.

Therefore, we compared the obtained values for EG scores based on the entire modules within the rat kidney TXG-MAPr

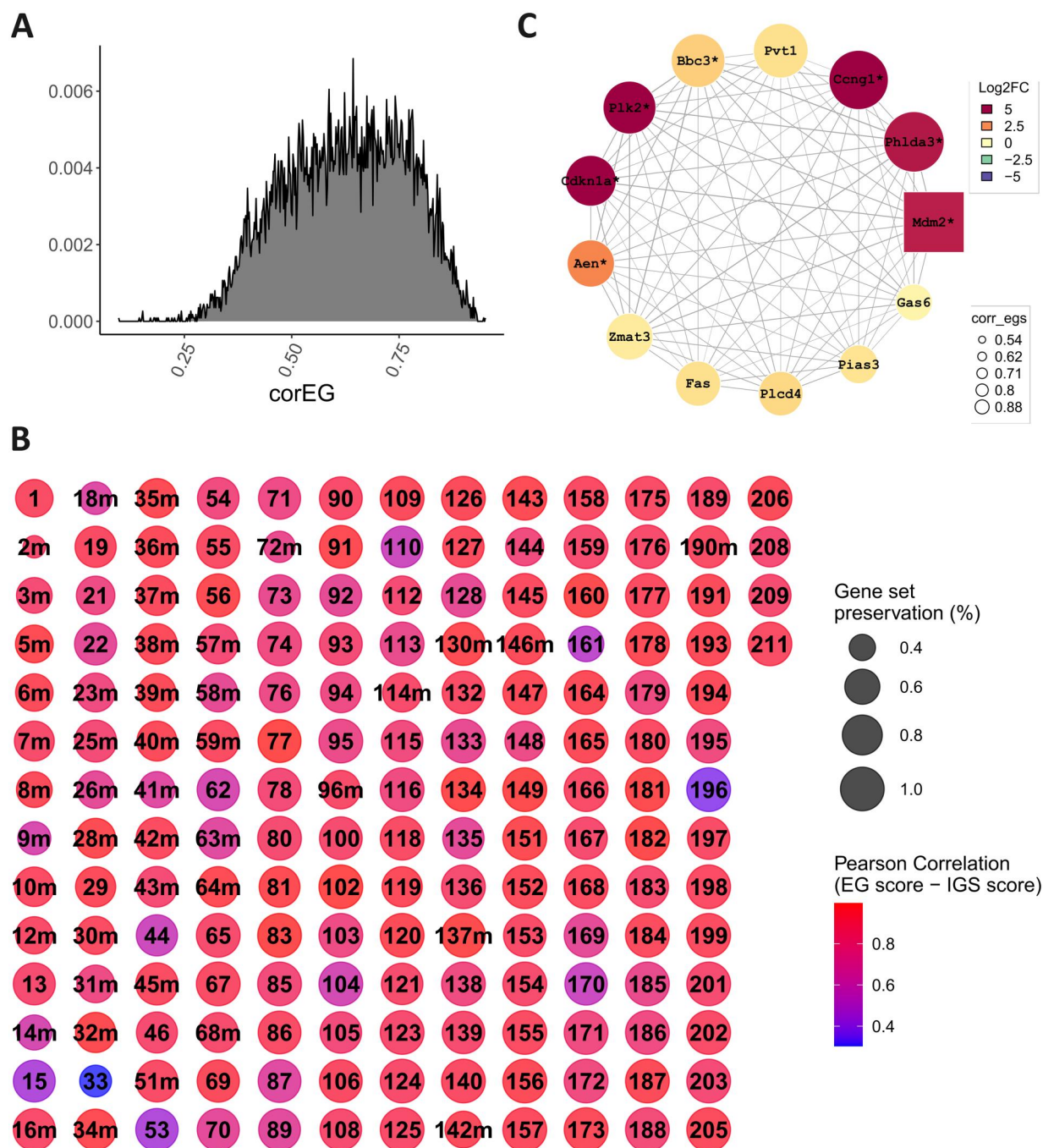


Fig. 1. EG and IGS scores provide a similar estimate of KE activity based on transcriptomics data. a) Distribution of corEG for all rat genes. b) Comparison of EG and IGS scores based on Pearson correlation between the 2 scores and gene set preservation for modules rKID: 1 to rKID: 211. Analysis is based on in vivo rat kidney TXG-MAPr output following cisplatin treatment. The numbers in the circles correspond to the number of a module (ie each circle corresponds to a module); the size and color of each circle correspond to percentage of preserved genes after application of the corEG-based cut-off (corEG > 0.5), and the Pearson correlation between EG and IGS scores, respectively. c) Schematic representation of module rKID: 160, used to quantify the KE "DNA Damage."

with those obtained with the IGS score based on a subset of genes per module by imposing a corEG cut-off. In order to determine an appropriate cut-off value, we considered the distribution of corEG values between all genes in the rat kidney TXG-MAPr (Fig. 1a). This distribution was unimodal and right-skewed, making it unsuitable for deriving a specific cut-off value; hence, we chose an "arbitrary" threshold value of 0.5. In this way, we retain information on a significant portion of the genes.

In order to evaluate the consistency between EG and IGS scores, we computed the Pearson correlation between these scores for each module as well as the gene set preservation after the corEG-based cut-off (Fig. 1b for modules 1-211; Fig. S1a for modules 212 m-399). In order to also compare the activation level of modules with the 2 methods, we calculated, for each dose level, the area under the curve (AUC) of the mean EG and IGS score over time, respectively. Subsequently, we studied the

distribution of the AUC ratio for each dose level separately, which is given by

$$AUC_{ratio} = \frac{AUC_{EGscore}}{AUC_{IGScore}}.$$

For most modules, $AUC_{EGscore} > AUC_{IGScore}$ (Fig. S1b), meaning that the EG score typically predicts a higher activation for most modules than the IGS score.

In the context of our specific qAOP, we aimed to perform KE mapping in order to identify modules relevant for KEs “DNA Damage,” “Inflammation,” and “Oxidative Stress.” When investigating modules related to oxidative stress using the in vitro RPTEC/TERT1 dataset, following the module selection strategy outlined in the Materials and methods section, we found that only module rKID: 106 was activated (Fig. S2a). However, this module did not exhibit a clear concentration-response relationship across the tested concentrations. For instance, at a concentration of 30 μ M, the module was completely deactivated, whereas it showed slight activation at early time points with a low concentration of 5 μ M. Only at a concentration of 50 μ M EG scores exceeded 2. Additionally, rKID: 106 did not exhibit significant activation in the in vivo rat kidney OMPT dataset (Fig. S2b). To further explore oxidative stress in the in vitro setting, we analyzed the time course expression of key oxidative stress-related genes at a concentration

of 50 μ M of cisplatin (Fig. S2c, top). Consistent with the EG scores, this analysis of individual genes revealed significant upregulation of several genes, indicating an oxidative stress response at this concentration. Similarly, most key oxidative stress-related genes were inactive at a concentration of 30 μ M of cisplatin (Fig. S2c, bottom). Because of the lack of consistent activation at low concentrations in vitro and in the in vivo rat kidney OMPT dataset, we opted to exclude oxidative stress as KE in our qAOPs.

With our module selection strategy, we identified modules rKID: 7m and rKID: 160 as representative of the KEs “Inflammation” and “DNA Damage,” respectively. We show a graphical representation of module rKID: 160 in Fig. 1c. Similar to most other modules, EG and IGS scores also correlated strongly for rKID: 7m and rKID: 160 (Fig. 1b). In conclusion, our analysis shows that EG and IGS scores are highly correlated, and both scores could be used for KE quantification. For qAOP modeling, we elected to adopt the EG score as generic KE quantification method.

In vivo PK model identifies cisplatin accumulation in the kidney

To trigger the MIE (DNA adduct formation) leading to the first KE in the AOP (DNA damage), cisplatin first needs to be taken up by cells. In order to describe the kinetics of such cisplatin uptake within cells in the in vivo qAOP, we considered the 3-compartmental kinetic model in equation (4) (schematic representation

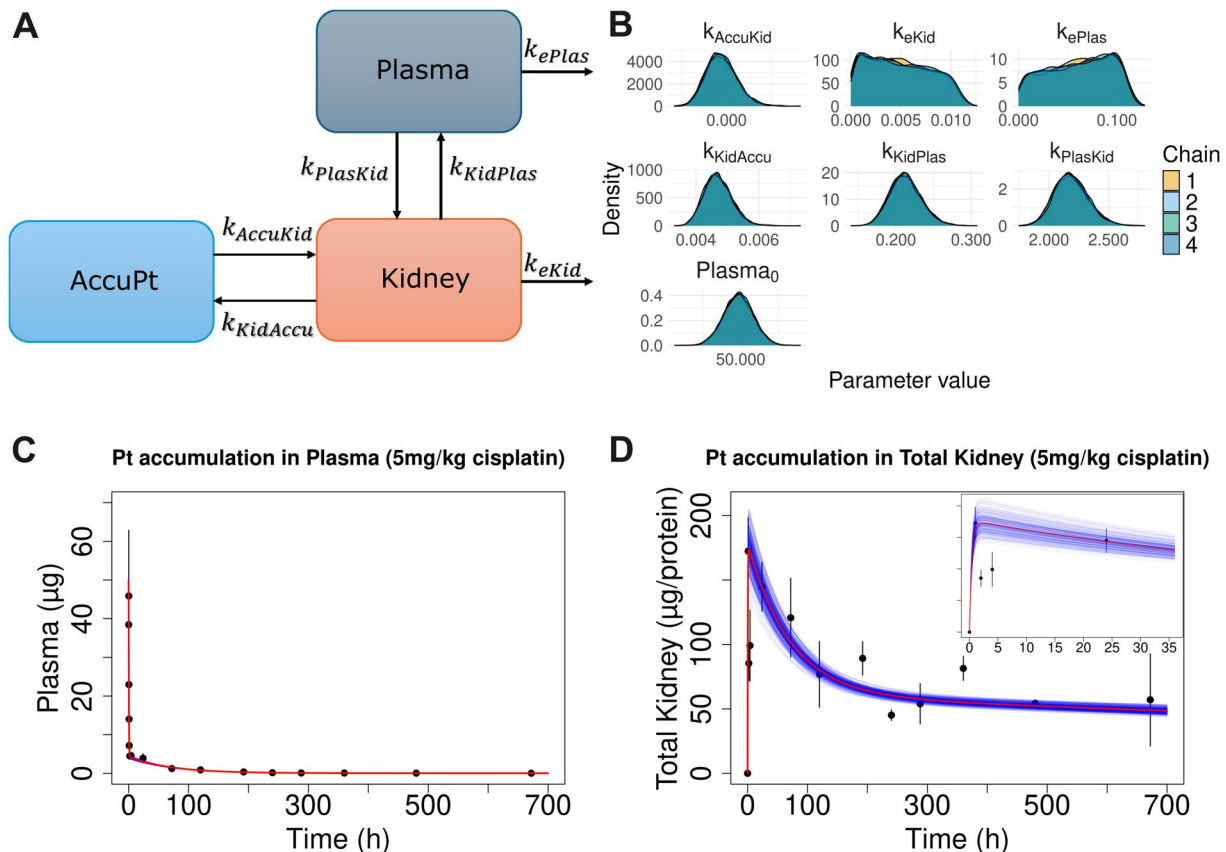


Fig. 2. In vivo 3-compartmental kinetic model fits plasma and kidney kinetic data. a) Schematic representation of the 3-compartmental kinetic model. b) Posterior distribution of the parameters. The overlap of the distributions for different chains entails convergence. c and d) Model fit to the plasma (c) and kidney (d) platinum data. Inset in (d) shows a zoom-in at early time points. Black dots and vertical lines represent data mean and SD, respectively; blue lines (250) represent model simulations corresponding to the results of 250 iterations for randomly sampled parameter combinations from the posterior distributions; red line corresponds to the mean, for each simulated time point, of those 250 simulations.

in Fig. 2a). Here, the function of the AccuPt compartment is to model the slow clearance rate of platinum from the kidney. Note that a 2-compartmental model (equation (3)) (Fig. S3a) could not fully capture these slow kinetics. Despite model fit convergence (Fig. S3b) and an acceptable description of the plasma data (Fig. S3c), the 2-compartment model underpredicted the kidney platinum data (Fig. S3d).

The 3-compartment PK model also converged (Fig. 2b) following parameter calibration of the plasma platinum data to the Plasma compartment (Fig. 2c) and of the kidney platinum data to the scaled sum of the KidneyPt and AccuPt compartments (equation (5); referred to as “Total Kidney” in Fig. 2d). This PK model resulted in an acceptable fit for both plasma (Fig. 2c) and kidney (Fig. 2d) platinum. Note that the 3-compartmental model (4) is not physiologically based, and therefore, not fully able to describe the platinum kinetic data. Indeed, the decrease of platinum in the Kidney between 1 and 4 h, with a second subsequent peak at 24 h (see inset in Fig. 2d), cannot be described by the model. These early kinetics might be caused by interactions with other organs. Nevertheless, because the overall fit was acceptable, especially at late time points, we employed this PK model as a driver for our in vivo qAOP.

In vivo qAOP highlights nonlinear relations induced by inflammation

After establishing an appropriate in vivo PK model, we fixed the estimated parameters from equation (4) to be equal to their predicted mean, and we formulated the in vivo qAOP model (equation (6); scheme in Fig. 3a). On top of the linear cascade of KEs, we included a negative feedback loop from KE Inflammation to KE Cell Death. Thus, we consider immune cells to clear injured or dead cells, therefore, in the long run, decreasing the amount of dead cells. The model fit converged, as visible from the overlapping posterior parameter distributions for all the chains (Fig. 3b) and overlapping traceplots across iterations (Fig. S4a). PSIS analysis predicted overall reliable estimates for our model fit (Fig. S4b). Note that the resulting model can be run in a user-friendly manner at <https://qaop.vhp4safety.nl/>.

This model fit was based on time course measurements of gene expression and histopathology data (Fig. 3c). Specifically, for the KEs DNA Damage and Inflammation, we used EG scores from modules rKID: 160 and rKID: 7 m, respectively. For the KEs Cell Death and Kidney Failure, we used necrosis and fibrosis histopathology data as representative. Note that the histopathology quantifications were expert-based yet not quantitative. In order to confirm the observed time course patterns for these histopathologies in an objective way, we therefore re-assessed these images with self-supervised learning based quantification. Specifically, we focused on scores for general tubular damage (termed *atn*) and interstitial fibrosis and tubular atrophy (termed *ifta*). The trends of the *atn* (Fig. S5a) and *ifta* (Fig. S5b) scores were similar to the trends of the expert-based scores in the sense that the *atn* score increased early and the *ifta* score only late. However, the *atn* score remained high, whereas at late time points, expert-based necrosis estimates over time decreased to slightly increased levels from baseline. This suggests that the early necrosis translates into prolonged tubular damage despite restoration of necrotic tissue. With respect to the fibrosis-related scores, the expert-based qualitative score encompasses all fibrotic changes across different renal compartments, whereas the *ifta* score is restricted to interstitial scarring coupled with tubular structural loss. This likely explains why the former yielded a higher value in the same histopathological specimen.

Nevertheless, the qualitative patterns of expert-based and self-supervised learning based scores are consistent, and thus provide confidence in using the expert-based scores for KE quantification of in vivo data. Therefore, for qAOP modeling, we continued with the expert-based classifications.

The PSIS analysis did indicate high values ($0.7 \leq k < 1$) for 3 data points (Fig. S4b). This could be due to a small sample size and the large difference in variability between transcriptomics and histopathology data. Note that we excluded time point 192 h, as the high standard deviation in the EG scores negatively affected the PSIS prediction, ie it resulted in $k \geq 1$ for that time point (not shown). Interestingly, the employment of a decaying Hill exponent for inflammation-induced kidney failure (dp/dt in model equation (6)) was needed to correctly fit the late time points in the AO (ie the fibrosis histopathology data). Indeed, omitting such decay (by only estimating p_0 and fixing $d_p = 0$) led to a convergent fit (Fig. S6a) with a poor AO estimation (Fig. S6b). This indicates that the threshold with which inflammation induces kidney failure (fibrosis) decreases for prolonged inflammation, ie a low level of ongoing inflammation is sufficient to exacerbate organ failure in the long run. In conclusion, our in vivo qAOP correctly described the response of rat kidneys to cisplatin. Moreover, our quantitative approach suggested additional key processes affecting the KERs in the AOP, ie the clearance of necrotic kidney cells by immune system activity, and a cumulative impact of low-level inflammation on kidney failure in the long run.

In vitro qAOP exhibits a strong threshold for DNA damage-induced cell death

Next, we considered a similar qAOP based on in vitro RPTEC/TERT1 data, modeled by system (2). Here, we combined a predefined in vitro cisplatin PK model from (Wilmes et al. 2013) with a qAOP taking DNA Damage and Cell Death as KE and AO, respectively (Fig. 4a). KE mapping and quantification were performed using the time-course EG score of module 160 for DNA Damage and PI staining data expressed as the percentage of Necrosis. The model fit converged, as visible from overlapping posterior parameter distributions (Fig. 4b) and traceplots (Fig. S7a) for all chains. Note that simulations with the resulting parameters can be run in a user-friendly manner at <https://qaop.vhp4safety.nl/>. The model predicted high values for the Hill exponent (h) describing the impact of DNA damage on necrosis. This is consistent with the absence of PI-stained cells for concentrations below 50 μM of cisplatin. Specifically, due to the high exponent, the term

$$\frac{DD^h}{k_{\text{hillnec}}^h + DD^h}$$

acts as a switch between the complete absence of cell death and a strong increase in cell death based on slight variations in the levels of DNA damage. Model calibration led to an acceptable description of the KE and AO data for almost all concentrations of cisplatin (20 and 50 μM in Fig. 4c; other concentrations in Fig. S8). Also, the PSIS estimates were good, with $k < 0.7$ for all data points (Fig. S7b). Note that for model calibration, we discarded early PI staining data (below 20 h) because they remained near zero across all concentrations. The model will anyway predict an absence of cell death during this period because the variable for cell death (NEC) can only increase over time. Removing these early data thus allows the model fit to place greater emphasis on the DNA Damage data, compensating for the large difference between the number of data points for the 2 observables.

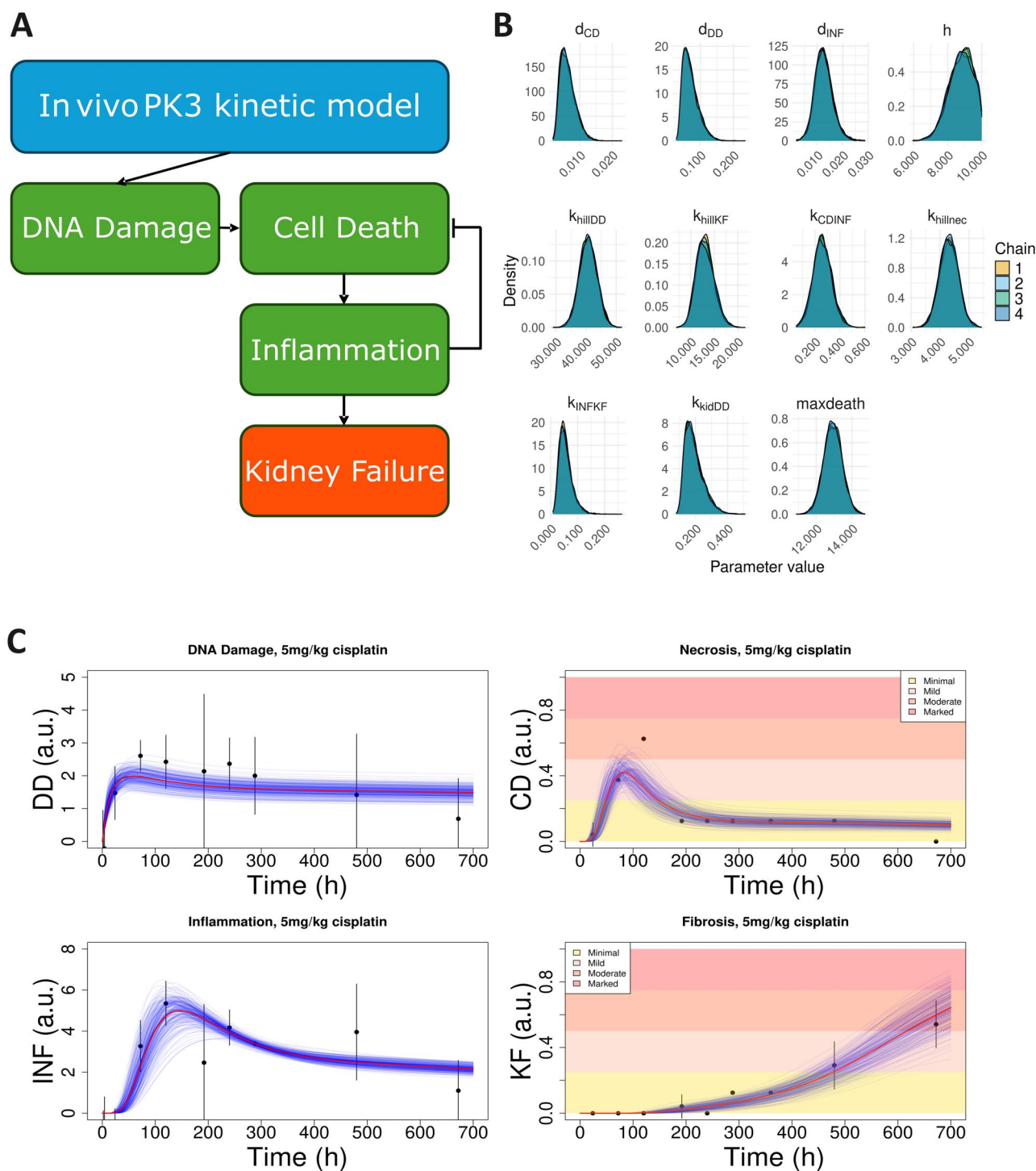


Fig. 3. In vivo qAOP model accurately predicts time-course of KEs DNA Damage, Cell Death, Inflammation and AO Kidney Failure. a) Schematic representation of in vivo PK and qAOP. b) Posterior distribution plots for inferred model parameters. c) Model fit for KEs and AO to time course in vivo rat kidney data following cisplatin exposure. Blue lines (250) represent model simulations corresponding to the results of 250 iterations for randomly sampled parameter combinations from the posterior distributions; red line corresponds to the mean, for each simulated time point, of those 250 simulations. Black dots and vertical lines represent data mean and SD, respectively. a.u.: arbitrary units.

For completeness, we tested whether the in vivo and in vitro qAOPs can also describe the data when utilizing IGS—based instead of EG-based KE mapping. As expected, when KEs were quantified using IGS, the posterior distributions also converged for both in vivo (Fig. S9a) and in vitro (Fig. S9b) qAOPs, and model fits were acceptable (Fig. S9c and d). In conclusion, the qAOP based on in vitro data shows that the KER between DNA

damage and Cell Death is highly nonlinear and exhibits a clear threshold.

QIVIVE links the 2 qAOPs and enables in vivo AO predictions

To connect the 2 qAOPs, we first compared the in vivo and in vitro kinetics, because these drive the MIE and subsequent

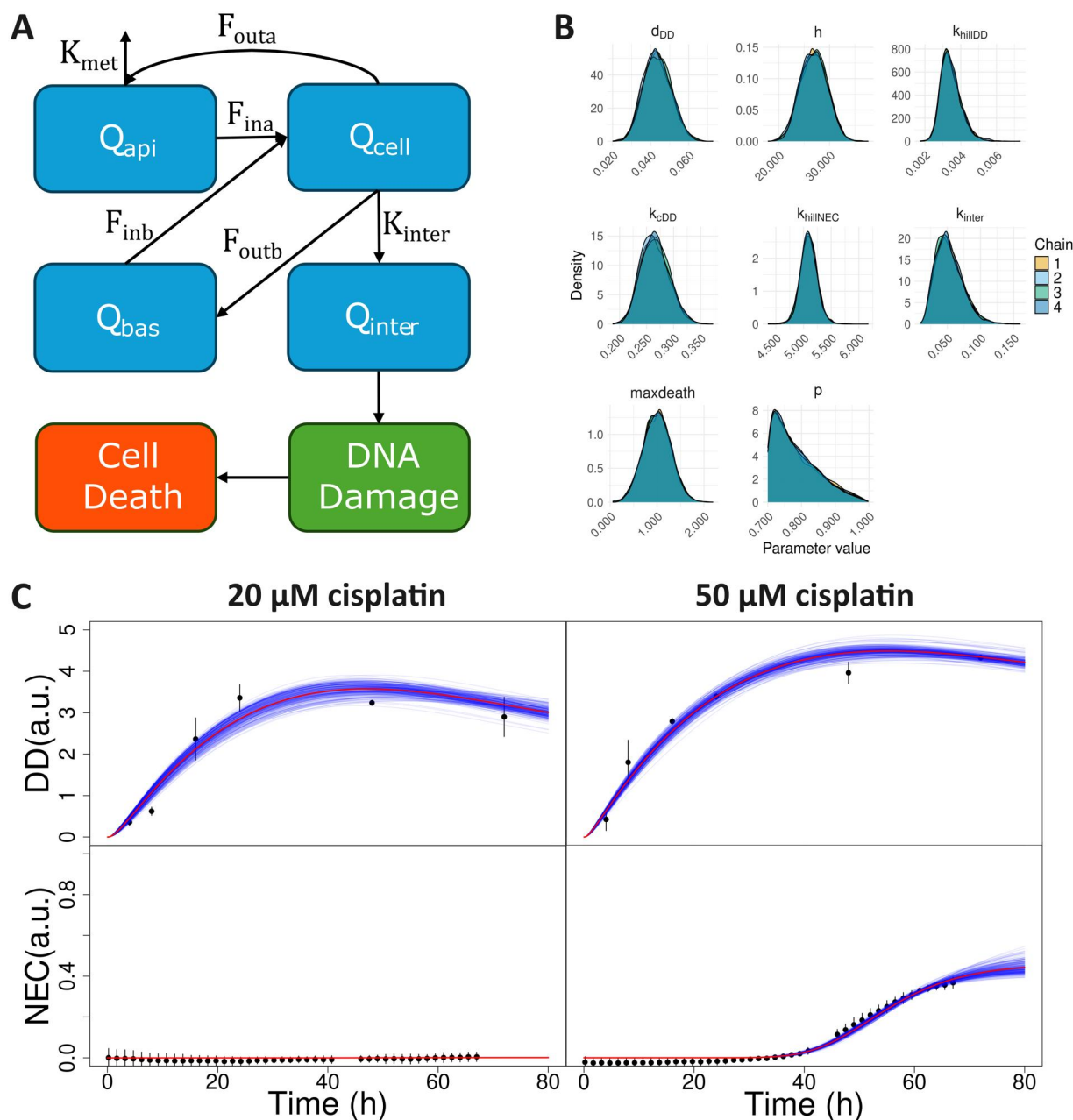


Fig. 4. In vitro qAOP model accurately describes time-course of DNA Damage and Cell Death. a) Schematic representation of in vitro PK and qAOP. b) Posterior density plot for inferred model parameters. The model calibration converges. c) Model fit for concentrations 20 (first column) and 50 (second column) μ of cisplatin. The model predicts the dynamics of KE “DNA Damage” (first row, “DD” variable)—quantified via EG score—and the AO “Cell Death” (second row, “NEC” variable)—quantified based on PI staining data. For each variable and concentration, 250 blue lines, representing model solutions for randomly sampled MCMC iterations, and the mean of those as a red line are plotted. Black dots and vertical lines represent data mean and SD, respectively. a.u.: arbitrary units.

KEs. Specifically, we aligned the in vivo kidney platinum data with the in vitro PK model prediction for the Q_{cell} compartment. We searched for an in vitro concentration where the C_{max} values are similar between the in vitro intracellular concentration as predicted by the PK model and in vivo measured concentration in the kidney. Alternative comparison methods, such as area under the curve (AUC), are impractical due to the large differences in time frames between the in vitro and in vivo data. Selecting an in vitro concentration of 75 μ M and normalizing for protein content yielded a $C_{max} = 175.63 \mu\text{g}/\text{protein}$, which closely aligned with the C_{max} of the in vivo dose of 5 mg/kg (Fig. 5a).

Thus, we took 75 μ M of cisplatin in vitro as representative of the in vivo rat dose of 5 mg/kg (Groothuis et al. 2015). At this concentration, the AO prediction of model (2) accounted for medium to high levels of cell death (Fig. 5b). This is coherent with the mild to moderate cell death observed in the in vivo case at early time points (Fig. 3c), supporting the link between the 2 concentrations.

Next, we analyzed the platinum dose-response data to unravel what is the relationship between the administered dose and the platinum content in the whole kidney. Interestingly, the platinum content linearly depended on the dose in the range

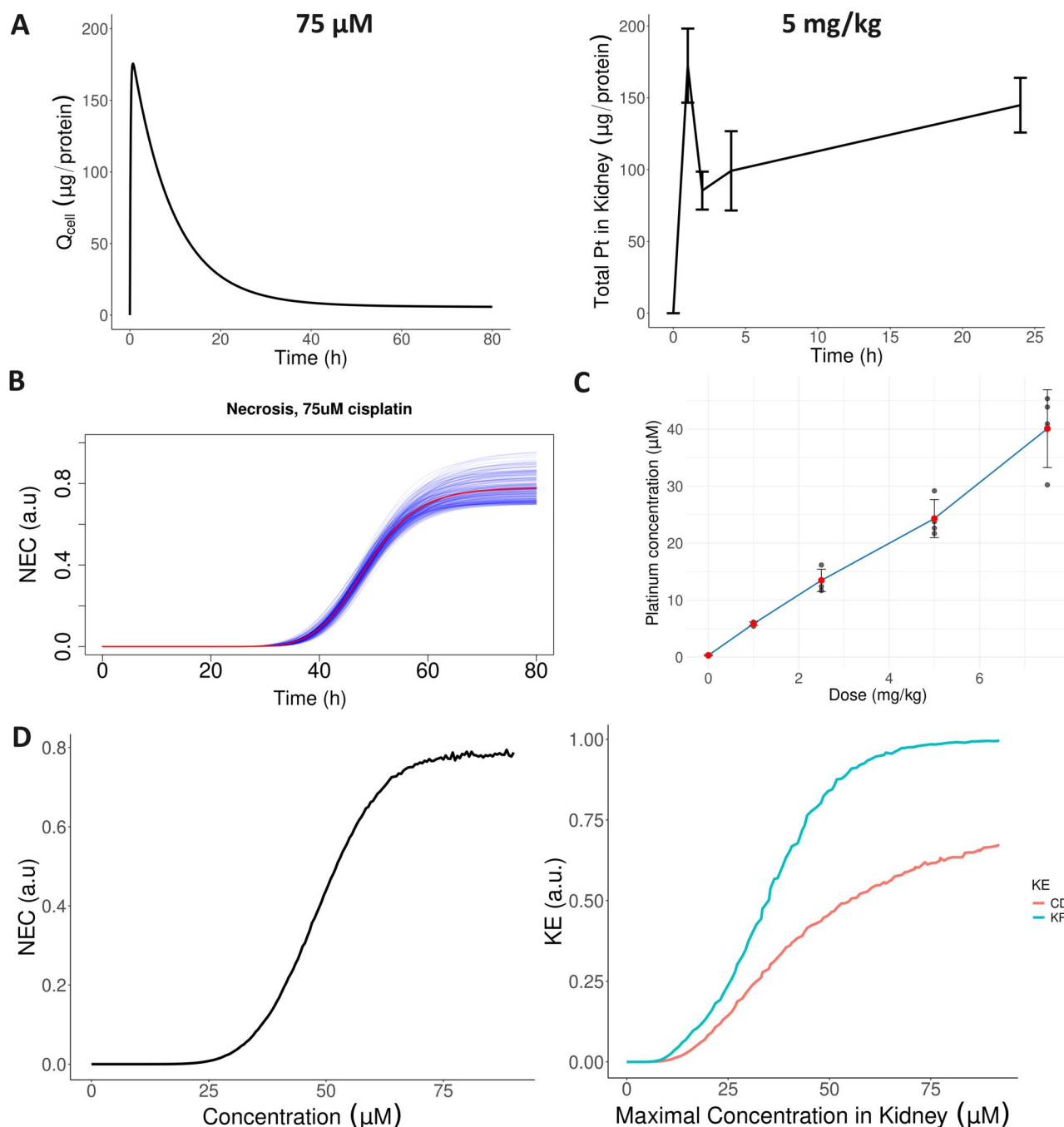


Fig. 5. QAOP-based QIVIVE identifies comparable in vitro concentration and in vivo dose ranges for cisplatin administration. a) PK-model predicted intracellular cisplatin concentrations (Q_{cell}) of in vitro qAOP (left) and platinum concentrations measured in the kidney, resulting in similar C_{max} values at 75 μM and 5 mg/kg cisplatin, respectively. b) Cell death dynamics for 75 μM of cisplatin as predicted by the in vitro qAOP. c) Measured platinum content in rat kidneys for 1, 2.5, 5, and 7.5 mg/kg cisplatin. For each dose level, the red dot indicates the mean of the observations, and the error bars depict the SD. d) Concentration–response curves for cell death in the in vitro qAOP (left), and for cell death (red) and kidney failure (blue) in the in vivo qAOP (right). The in vivo dose range 0 to 10 mg/kg is mapped to the maximal concentration in the kidney (right panel). The variable NEC represents the fraction of necrotic cells (bounded between 0 and 1), and is quantified based on PI staining data; the variables CD and KF (bounded between 0 and 1 as well) are quantified based on histopathology data. a.u.: arbitrary units.

from 0 to 7.5 mg/kg (Fig. 5c). We applied this information to the 3 compartmental kinetic model (4), considering that, for a specific dose $\hat{D}$, the initial condition for the Plasma compartment ($\text{Plasma}(0)$) is set to $\widehat{\text{Plasma}}_0 = \text{Plasma}_0 \cdot \hat{D}/5$ (with Plasma_0 defined in system (4)). Note that this approximation does not necessarily hold for dose levels $\hat{D} \gg 7.5$, hence we studied the responses in the range $\hat{D} \in [0, 10]$ (ie mildly extrapolating). To check whether the

in vivo qAOP (6) described the data for additional dose levels, we apply EG-based KE mapping to the dose-response transcriptomics data measured at single time points. The dose-response data matched the simulation curves at 72h for the DDR, although it overestimated the DDR at a dose of 7.5 mg/kg (Fig. S10a). This might be explained by a negative feedback mechanism becoming operational at this dose in in vivo settings, which could be

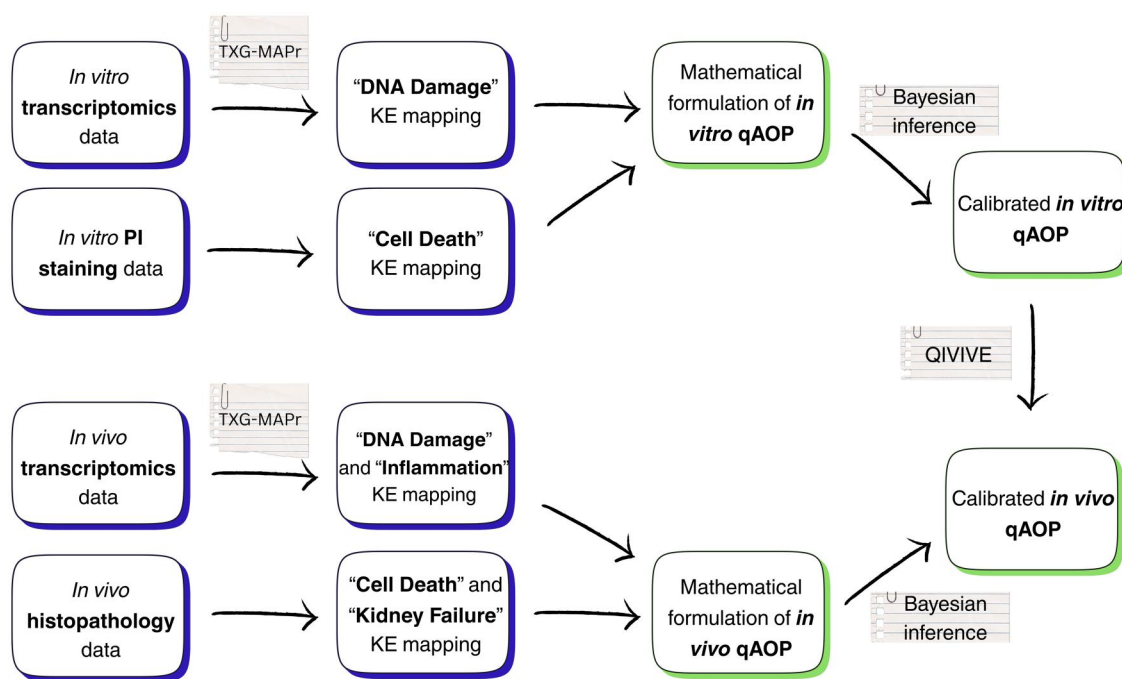


Fig. 6. Workflow scheme summarizing the KE mapping and modeling steps of the study.

Table 5. Summary of in vivo BMD by model with parameter estimates, BIC and ED10 results.

Model	Estimated parameters	BIC	ED10	ED10 LCL	ED10 UCL
LL2	$b = -2.04, e = 6.07 \times 10^1$	-22.00	20.62	14.89	26.35
LL3	$b = -3.03, d = 7.36 \times 10^{-1}, e = 4.43 \times 10^1$	-53.86	21.46	20.91	22.00
LL4	$b = -3.01, c = -1.74 \times 10^{-3}, d = 7.38 \times 10^{-1}, e = 4.43 \times 10^1$	-55.04	21.31	20.41	22.21
LL5	$b = -2.95, c = -1.44 \times 10^{-3}, d = 7.41 \times 10^{-1}, e = 4.36 \times 10^1, f = 1.04$	-53.38	21.39	15.48	27.30
W14	$b = -1.51, c = -6.00 \times 10^{-4}, d = 8.91 \times 10^{-1}, e = 4.01 \times 10^1$	-35.65	23.07	19.71	26.44
W24	$b = 2.42, c = -1.25 \times 10^{-3}, d = 6.65 \times 10^{-1}, e = 4.92 \times 10^1$	-32.30	19.39	13.41	25.38

Table 6. Summary of in vitro BMD by model with parameter estimates, BIC and ED10 results.

Model	Estimated parameters	BIC	ED10	ED10 LCL	ED10 UCL
LL2	$b = -3.89, e = 5.66 \times 10^1$	-35.81	32.20	28.47	35.94
LL3	$b = -9.46, d = 3.86 \times 10^{-1}, e = 3.75 \times 10^1$	-49.83	29.74	27.43	32.05
LL4	$b = -8.76, c = -8.75 \times 10^{-3}, d = 3.91 \times 10^{-1}, e = 3.74 \times 10^1$	-55.17	29.06	27.25	30.87
LL5	$b = -7.02, c = -8.51 \times 10^{-3}, d = 4.05 \times 10^{-1}, e = 3.39 \times 10^1, f = 1.74$	-52.33	29.33	28.57	30.09
W14	$b = -4.83, c = -8.29 \times 10^{-3}, d = 4.38 \times 10^{-1}, e = 3.53 \times 10^1$	-54.15	29.71	28.42	31.00
W24	$b = 7.18, c = -9.10 \times 10^{-3}, d = 3.63 \times 10^{-1}, e = 3.91 \times 10^1$	-56.01	28.54	26.39	30.70

investigated with model extensions when, in the future, more detailed time course data would become available. Nevertheless, the high uncertainty in the experimental measurements at 7.5 mg/kg precludes firm conclusions. Overall, this QIVIVE for the DDR supports the linearity of the PK dependence on applied concentration, although it should be noted that this conclusion is based on very few measurements. Note that the dose-response EG scores at 72h for the KE Inflammation exhibited a slight increase for the highest dose (7.5 mg/kg), but not for lower doses (Fig. S10b). The latter is inconsistent with the time-course data at 72h, where substantial inflammation was triggered at a dose of

5 mg/kg (Fig. 3c). This suggests that late KEs might exhibit a larger response variability than early KEs.

Finally, we performed qAOP-based QIVIVE to derive dose-response and concentration-response curves for the AOs, which are based on the time-resolved data (Fig. 5d). Here, the dose-response curves are obtained directly from the ODE simulations, which allow the calculation of the maximal adverse outcome response obtained during a specific time period for each dose or concentration level. Specifically, for the in vivo model, we considered the dose range 0 to 10 mg/kg, used the linearity assumption from Fig. 5c and, through equation (7), mapped the dose levels to

a concentration in μM . Considering ranges in which 10% to 90% of maximal AO responses are predicted for both systems, we conclude that the in vitro concentration range 35 to 63 μM gives a comparable AO response to the in vivo concentration range 17 to 54 μM . In parallel, conventional BMD analysis at 67 h was carried out with the R package *drc*, using interpolated kidney necrosis data in vivo (Fig. S11a) and PI-staining data in vitro (Fig. S11b). For each case, we selected the model with lowest BIC (in vivo: Model LL4; in vitro: Model W24): this yielded benchmark dose intervals of 20.41 to 22.21 μM in vivo (Table 5) and 26.39 to 30.70 μM in vitro (Table 6). Although both methods yield an effective extrapolation, our qAOP-based QIVIVE (workflow summarized in Fig. 6) is not biased by time point selection, as maximal predicted AO levels over the time frame are selected for each concentration.

Discussion

In this study, we employed computational modeling to quantitatively unravel the mechanisms of platinum-induced nephrotoxicity, based both on newly generated in vitro RPTEC/TERT1 and published in vivo rat kidney data following cisplatin exposure. We achieved this employing an ODE-based qAOP framework (Di Tillio and Beltman 2024), in this case, achievable because time-course data were available to calibrate such models. We integrated various data types, ie transcriptomics, imaging, histopathology, and kinetics data. In order to map transcriptomics data to KEs, we proposed 2 methods: One exclusively based on the TXG-MAPr and another one which integrates it together with IGS. We demonstrated that the 2 methods provide similar outputs in terms of KE data. Consistently, the time-course trend of the mapped data for the KEs of interest is very similar among approaches, showing only a significant quantitative difference in the activation of the KE “Inflammation,” relevant for the in vivo modeling. This is likely due to the difference between how the corEG and IGS weights (w_k in formula (1)) are calculated. Nevertheless, the KE quantifications with both approaches could be described with the same qAOP model structures. In conclusion, both the EG and IGS scores are suitable methods for KE mapping and quantification. The IGS method is more versatile, as it can be combined with any enrichment analysis tool; on the other hand, the EG is based on the module structure of the TXG-MAPr, which independently provides the gene set structure on which KE mapping can be performed. Note that our approach integrates human RPTEC/TERT1 in vitro data with rat kidney in vivo data, thus assuming similar coregulation of gene networks across species. Future work should study whether this is indeed a reasonable approximation, for example by comparing with human kidney biopsies.

By applying our ODE-based framework (Di Tillio and Beltman 2024), we could describe the KE dynamics through time-dependent KERs. This enabled the identification of a strong threshold for DNA damage-induced cell death for the in vitro qAOP, and of a cumulative effect of inflammation on kidney failure for the in vivo qAOP. The strong threshold observed in the in vitro case is likely explained by the type of cell death that the assay quantifies: PI staining primarily detects necrotic cell death, whereas apoptotic cells only become PI-positive during secondary necrosis, when membrane integrity is lost (Honda et al. 2000; Sawai and Domae 2011). Thus, there may be a concentration-dependent threshold below which necrosis is not observed, whereas cells could still be undergoing early apoptosis below this threshold (Leite et al. 1999). A shift from apoptotic to

necrotic cell death induced by cisplatin has previously been demonstrated (Lieberthal et al. 1996; Lau 1999), and our in vitro qAOP model supports this mechanism. As for the in vivo qAOP case, we observed a cumulative effect over time, where prolonged inflammation progressively leads to kidney fibrosis. It has been shown that unresolved inflammation can promote progressive renal fibrosis through the production of profibrotic cytokines and growth factors (Meng et al. 2014; Black et al. 2019). Our modeling supports this evidence and offers a precise quantitative representation of how this process unfolds over time. We chose to represent Kidney Failure using fibrosis histopathology data. Although we acknowledge that these processes are distinct, we emphasize their deep interconnection, as renal fibrosis is widely recognized as the final common pathway to end-stage renal failure. By disrupting and replacing functional kidney tissue, fibrosis ultimately leads to organ dysfunction and failure (Liu et al. 2006; Liu 2011; Sutariya et al. 2016; Chung et al. 2019).

In both in vitro and in vivo qAOPs, we did not include a gene set related to oxidative stress, which has been reported as a KE in the AOP (AOP 274). We based this decision on the limited activation or the absence of a consistent concentration–response relationship in oxidative stress-related modules across both in vivo and in vitro transcriptomics data, rendering them unsuitable for KE quantification. Specifically, module rKID: 106, which is enriched for Nrf2 target genes, was activated only in the in vitro dataset at a high concentration of 50 μM cisplatin. Thus, although rKID: 106 would be the best candidate for quantifying oxidative stress, its use is computationally impractical due to inconsistencies in its concentration–response behavior. For example, we observed some activation of the module after in vitro exposure of RPTEC/TERT cells to 5 μM cisplatin at early time points, whereas activation was lower at initial time points for higher concentrations. Furthermore, this module exhibited no activation for the in vivo rat kidney OMPT dataset. This could be due to the experimental setup, involving a single intravenous administration of cisplatin, and the significant cell death observed around 24 h, which may reduce the counts of specific genes. Consistently, also DNA damage-related genes were somewhat lower expressed in vivo than in vitro. Thus, inclusion of Oxidative Stress KE quantification in the in vitro qAOP would not extrapolate to the in vivo model. The reason for the inconsistent induction between in vitro and in vivo oxidative stress data remains unclear. Additional in vivo time-course data would be needed to determine whether module rKID: 106 is activated at high doses of cisplatin. Because both qAOPs effectively described the dynamics of the considered KEs and AOs, without inclusion of oxidative stress, we consider them sufficiently robust for the scope of this study, ie to provide quantitative predictions for extrapolating from in vitro to in vivo rat kidney. Additional mechanisms would introduce unnecessary complexity and parameter unidentifiability. Thus, we regard the models as sufficiently robust within their domain of applicability, without the need to add additional KEs such as oxidative stress.

Nevertheless, there is ample evidence of oxidative stress to be relevant in the context of platinum-induced nephrotoxicity (Katanić Stanković et al. 2023). Specifically, impairment of mtDNA, especially when affecting the electron transport chain, contributes to ROS formation (Indo et al. 2007). As further evidence, antioxidants such as cannabidiol, fisetin, wogonin, curcumin, and epicatechin gallate, can mitigate the nephrotoxic effects of cisplatin. These compounds reduce oxidative stress, inflammation, and apoptosis, highlighting the role of oxidative stress in nephrotoxicity (Pan et al. 2009; Waly et al. 2011; Sahu

et al. 2014; Malik et al. 2016; Badawy et al. 2019). Moreover, the inhibition of thioredoxin reductase and activation of the MAPK pathway, both associated with oxidative stress and nephrotoxicity, contribute to cellular redox imbalance and inflammation in cisplatin-treated kidneys (Malik et al. 2016; Cheng et al. 2020). In support of the role of oxidative stress in cisplatin-induced toxicity, it is important to recognize that oxidative stress and DNA damage responses are often highly interconnected. The study by Burgers et al. (2025) provides computational evidence that oxidative stress can both result from and influence DNA damage signaling, forming a bidirectional crosstalk that intensifies stress pathway activation. Specifically, their models showed that the DNA damage response (DDR), triggered by agents like etoposide or cisplatin, can upregulate oxidative stress response (OSR) genes such as SRXN1 via p21-mediated stabilization of NRF2. Conversely, oxidative stress induced by DEM leads to increased expression of MDM2—a key DDR regulator—through NRF2 activity, followed by degradation of p53 and delayed DDR activation. The authors concluded that p21 and NRF2 are primary mediators in this crosstalk, and in silico knockdowns confirmed p21 as a critical node for OSR activation in response to DNA damage. These findings highlight that there are circumstances in which oxidative stress responses are tightly linked to DNA damage responses. In the case of cisplatin, an oxidative stress response may occur primarily in response to DNA damage signaling.

Based on the developed qAOPs, we explored whether they are suitable for extrapolating system behavior across different concentration levels and enabling QIVIVE. Such QIVIVE approaches have already been implemented in the context of AOPs. For instance, Jarzina et al. (2022) performed extrapolations in the context of an AOP linking disturbance of lysosomal function to kidney failure. This entailed the formulation of an AOP based on in vitro data, and the use of a PBPK model to simulate human dose levels of polymyxin B leading to a marked AO. More generally, PBPK models have been used as a basis to perform QIVIVE in several contexts, such as neurotoxicity induced by organophosphate pesticides (Chen et al. 2023), lung toxicity induced by paraquat (Noorlander et al. 2023), and impairment of fetal development induced by developmental toxicants (Fragki et al. 2022).

Our approach, although considering both in vitro and in vivo compound kinetics, differs from those previously mentioned. Specifically, we used the PK data and models as an initial step to identify potential in vitro concentration ranges comparable with the in vivo case. To compare between in vitro concentration range and the in vivo dose range, we utilized 2 qAOPs and found ranges that resulted in similar AO responses. Specifically, we selected a cisplatin concentration of 75 μM to approximate in vivo kidney levels observed in rat experiments. Although extrapolation to human kidney concentrations remains challenging due to limited data on platinum accumulation in human tissue, it is important to note that cisplatin-induced nephrotoxicity in patients typically arises after repeated treatment cycles, leading to elevated plasma and tissue exposure. Reported peak plasma concentrations (C_{max}) in patients range from approximately 20 to 60 μM , depending on dosing and individual characteristics (Rajkumar et al. 2016), supporting the plausibility of our chosen concentration in the context of repeated exposure and tissue accumulation. Alternatively to C_{max} -based extrapolation, one could opt for an AUC-based extrapolation, which is inconvenient here as in vitro and in vivo data are measured on different time spans. This approach does not require the development or use of a complex PBPK model, yet sufficiently precise PK models driving each qAOP are required (ie the PK models need not be

physiologically based). Additionally, in this approach, both an in vitro and an in vivo qAOP needs to be formulated and fitted to appropriate AOP quantification data. Once these models are in place, the link between in vitro and in vivo calibrated qAOPs can be utilized to extrapolate information for compounds with a similar mode of action. One could also apply the same method to analyze DNA damage responses in vitro and in vivo (Fig. S10c), and subsequently link the in vivo DNA damage response to Kidney Failure levels. This approach would require selecting different in vitro concentration ranges, as the in vitro DNA damage response is already activated in the absence of detectable cell death. Indeed, even at the lowest in vitro concentration selected based on AO responses, DNA damage is already highly activated. Note that the strong threshold observed between DNA damage and cell death in vitro is not observed in vivo, which complicates interpretation. Thus, although this option of comparing KEs might be more biologically relevant it is less direct than identifying relevant concentration or dose ranges through AOs.

Following this procedure for cisplatin, one can thus in the future consider the potency of other platinum-based compounds, such as carboplatin and oxaliplatin, and use this information to rescale the relevant in vitro cisplatin concentration range. Specifically, one can consider the in vitro AO EC_{50} for other platinum-based compounds, ie the concentration of the compound inducing half-maximal AO (Sebaugh 2011). Assuming the effective concentration of platinum in proximal tubular epithelial cells is the main driver for later toxicity, one could then re-run the qAOPs using the posterior distribution of parameters estimated for cisplatin, by simply rescaling C_0 and $Plasma_0$, which respectively represent the chosen concentration in the in vitro model and the initial condition in plasma for the in vivo model, by the ratio $EC_{50X}/EC_{50\text{cisplatin}}$, where X is an alternative platinum-based compound to investigate. Note that rescaling the effective concentration in Plasma by the EC_{50} ratio is suitable, as the effective concentration in Plasma linearly depends on the in vivo dose. Running the models would then provide AO dose-response curves, identifying new meaningful dose ranges for other compounds of interest. If not known, machine learning models, such as QSAR (Tropsha 2010), can be employed to predict the potency of compounds. This has already been done in the case of platinum-based compounds via testing of their cytotoxicity on an ovarian cancer cell line (Monti et al. 2005). Specifically, regression models were applied to predict $\log(1/IC_{50})$, including 16 different platinum complexes. Similarly, we can use the AO prediction of our models to identify the AO EC_{50} and use that as training data for a regression model aiming to predict EC_{50} of additional platinum-based compounds. As the information on cisplatin alone is not sufficient to appropriately train such models, additional in vitro data can be generated on additional platinum-based compounds, eg carboplatin, nedaplatin, oxaliplatin, and these can be integrated in the test set for more accurate predictions on new generation platinum-based compounds.

Traditional QIVIVE mostly relate an in vitro effect concentration to a plasma or tissue concentration in vivo (Klaren et al. 2019; Valdiviezo et al. 2021). This does not directly translate into a QIVIVE of the downstream response, which can be particularly challenging when the response occurs far downstream. Spinu et al. (2020) previously hypothesized that a qAOP approach could be used to derive a QIVIVE of the response. The main advantage of applying an ODE-based qAOP framework is that predictions arise directly from the model and are not influenced by a somewhat arbitrary selection of specific time points (Di Tillio and Beltman 2024). Although application of a classical BMD analysis

based on a specific time point also gave appropriate QIVIVE predictions, our approach enabled the selection of time points highlighting the maximal possible toxicity that may develop. The added benefit of qAOP to the overall prediction of in vivo bioequivalent doses from in vitro early KE concentration–effect relationships in QIVIVE will need more case studies to assess.

In conclusion, our proposed qAOP-based QIVIVE approach can become highly relevant in risk assessment, as the toxicity of existing and new platinum-based compounds could be tested with the sole employment of in vitro data. Note that this methodology can then be extended to other drugs and chemicals. Indeed, if a group of compounds is known to trigger the same AOP, one can similarly use available data on a subset of those compounds and build qAOPs to then extrapolate information on in vivo outcomes for the remaining compounds in the set, for which data is scarce. This would lead to a further decrease in the need for animal testing.

Supplementary material

Supplementary material is available at Toxicological Sciences online.

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Conflicts of interest. JK received consultancy fees (paid to the hospital) from the following companies, not related to the current work: Aiosyn BV, Novartis AG, Alentis AG. BanffNET (developed by JK) is currently being considered for a patent application. Otherwise, the authors declare no conflicts of interest.

Data availability

The in vivo and in vitro transcriptomics data are deposited at the Annotare (<https://www.ebi.ac.uk/fg/annotare/>) under accession numbers E-MTAB-11934—dose–response study, E-MTAB-11934 and E-MTAB-12016—time–response study, and E-MTAB-11374. The modeling code is available via <https://doi.org/10.5281/zenodo.17861517>. Moreover, simulations with the calibrated qAOP models can be run in a user-friendly manner in the qAOP app available at <https://qaop.vhp4safety.nl/>.

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