Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

Kinetic analysis of the accumulation of a half-sandwich organo-osmium pro-drug in cancer cells

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1. Model Equations and Parameters

FY25 or FY26 cellular pharmacokinetics were mathematically investigated taking into account the cellular transport of the complexes and intracellular activation (Figure S1). Two mechanisms were proposed to explain the decrease in Os accumulation during FY25 or FY26 exposure: “enhanced efflux” or “reduced uptake” models. Transporters were assumed to be the same for FY25 and FY26. Equations for both models are presented below. These PK models represent transport of the complexes and their metabolism in a monolayer of one million of cells in culture. The total intracellular volume was estimated to 1e-6 L assuming the volume of a single cell of 1pL. The volume of the culture medium was set to 6mL.

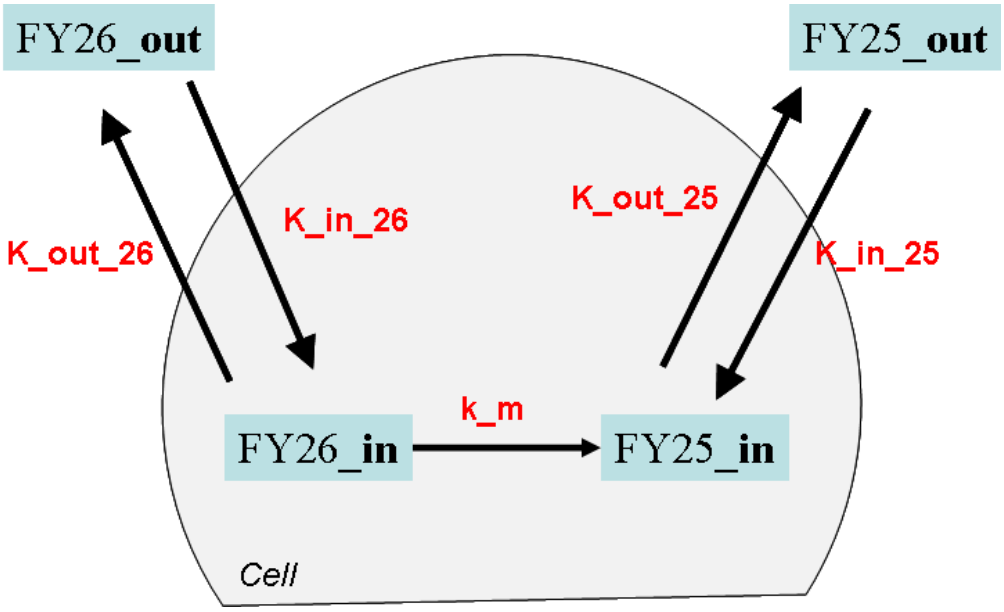

Figure S1: Global model scheme representing the mechanisms considered in the mathematical model of FY26 and FY25 cellular transport. k_in_25/k_out_25 and k_in_26/k_out_26 represent the uptake/efflux rates of FY25 and FY26, respectively. k_m represents the rate of intracellular activation of FY26 into FY25.

Table S1. Model Variables.

FY26 extracellular concentration	$FY26_{out}$
FY25 extracellular concentration	$FY25_{out}$
FY26 intracellular concentration	$FY26_{in}$
FY25 intracellular concentration	$FY25_{in}$
Nuclear factor concentration	*N*
Messenger RNA concentration	*R*
Uptake transporter concentration	*UT*
Efflux transporter concentration	*ET*

Table S2. Model Parameters.

FY25 passive uptake/efflux rate (h^{-1})	k_{25}
FY26 passive uptake/efflux rate (h^{-1})	k_{26}
FY26 active uptake rate (h^{-1})	$k_{26}^{in\_active}$
FY25 active uptake rate (h^{-1})	$k_{25}^{in\_active}$
FY26 active efflux rate (h^{-1})	$k_{26}^{out\_active}$
FY25 active efflux rate (h^{-1})	$k_{25}^{out\_active}$
FY26 to FY25 transformation rate (h^{-1})	k_m
Critical activation threshold	*Thres*
Hill coefficient for the nuclear factor	*n*
Maximal effect rate of the nuclear factor (h^{-1})	$k_{induction}$

1.1. FY26 PK model including "Enhanced Efflux"

FY25 extracellular concentration:

$$\frac{d\ FY25_{out}}{dt} = (-k_{25}^{in} FY25_{out} + \ k_{25}^{out} ET\ FY25_{in}) \frac{V_{in}}{V_{out}}$$

FY25 intracellular concentration:

$$\frac{d\ FY25_{in}}{dt} = k_{25}^{in} FY25_{out} - \ k_{25}^{out} ET\ FY25_{in} + \ k_m\ FY26_{in}$$

FY26 extracellular concentration:

$$\frac{d\ FY26_{out}}{dt} = (-k_{26}^{in} FY26_{out} + \ k_{26}^{out} ET\ FY26_{in}) \frac{V_{in}}{V_{out}}$$

FY26 intracell concentration:

$$\frac{d\ FY26_{in}}{dt} = \ k_{26}^{in} FY26_{out} - \ k_{26}^{out} ET\ FY26_{in} - \ k_m\ FY26_{in}$$

Generic nuclear factor concentration:

$$\frac{d\ N}{dt} = \ k_{induction} \frac{(FY25_{in} + FY25_{in})^n}{(FY25_{in} + FY25_{in})^n + Thres^n}$$

Efflux transporter mRNA concentration:

$$\frac{d\ R}{dt} = k_f^R (1 + N) - k_d^R R$$

Efflux transporter protein concentration:

$$\frac{d\ ET}{dt} = \ k_f^P R - k_d^P ET$$

1.2. FY26 PK model including "Reduced Uptake"

FY25 extracellular concentration:

$$\frac{d\ FY25_{out}}{dt} = (-\ k_{25}^{in} UT\ FY25_{out} + \ k_{25}^{out} FY25_{in}) \frac{V_{in}}{V_{out}}$$

FY25 intracellular concentration:

$$\frac{d\ FY25_{in}}{dt} = \ k_{25}^{in} UT\ FY25_{out} - \ k_{25}^{out} FY25_{in} + \ k_m\ FY26_{in}$$

FY26 extracellular concentration:

$$\frac{d\ FY26_{out}}{dt} = (-\ k_{26}^{in} UT\ FY26_{out} + \ k_{26}^{out} FY26_{in}) \frac{V_{in}}{V_{out}}$$

FY26 intracell concentration:

$$\frac{d\ FY26_{in}}{dt} = \ k_{26}^{in} UT\ FY26_{out} - \ k_{26}^{out} FY26_{in} - \ k_m\ FY26_{in}$$

Unknown species concentration:

$$\frac{d\ N}{dt} = \ k_{induction} \frac{(FY25_{in} + FY26_{in})^n}{(FY25_{in} + FY26_{in})^n + Thres^n}$$

Uptake transporter mRNA concentration:

$$\frac{d\ R}{dt} = k_f^R - k_d^R R$$

Uptake transporter protein concentration:

$$\frac{d\ UT}{dt} = \ k_f^P R - k_d^P (1 + N) UT$$

Transcription, translation and degradation parameters for the intermediate species were set using data for Abcb1 for the efflux transporter *ET* and for ATOX1-ATX1 for the uptake transporter *UT* [1]:

Table S3. Parameter estimates of the "reduced uptake" model

	Reduced uptake	*Enhanced efflux*	*units*
mRNA steady state value (RNAss)	1.5214e-4	3.9771e-005	µM
Protein steady state (Protss)	0.4948	0.1623	µM
k_f^R	1.1624e-5	3.3710e-006	µM.h^{-1}
k_f^P	97.23	188.84	h^{-1}
k_d^R *(=kf_RNA/RNA_ss)*	0.0764	0.0848	h^{-1}
k_d^P *(=RNA_ss*kf_prot/Prot_ss)*	0.0299	0.0463	h^{-1}

2. Model Parameter Identifiability

Parameter practical identifiability was investigated using likelihood profiles [2]. Briefly, the distance between the experimental data and the model is computed by an objective function, here the weighted sum of squared residuals:

$$C(\theta) = \sum_{i=1}^{m} \left(\frac{y_i - f(t_i, \theta)}{\sigma_i} \right)^2$$

where y_i are the data points at the corresponding time points t_i, $f(t_i, \theta)$ are the model values at t_i, with parameters θ, and σ_i the data standard deviations. Minimizing this objective function over parameter values is equivalent to maximizing the likelihood estimator for normally distributed datasets.

For each parameter θ_j, the likelihood profile $C_{PL}(\theta_j)$ is defined as:

$$C_{PL}(\theta_j) = \min_{\theta_k \neq \theta_j} C(\theta)$$

The likelihood-based confidence interval of parameter θ_j is defined as:

$$\{\theta_j \mid C_{PL}(\theta_j) - C_{PL}(\theta_j{}^*) < \Delta_\alpha\}$$

where $\theta_j{}^*$ is the parameter optimal value which minimizes $C(\theta)$. Δ_α is the α quantile of the χ^2 distribution with one degree of freedom:

$$\Delta_\alpha = \chi^2(\alpha, 1) = 3.84$$

We set $\alpha = 0.95$. A parameter is identifiable if its confidence interval is finite [2]. In other words, if the likelihood profile crosses the threshold value $C_{PL}(\theta_j{}^*) + \Delta_\alpha$ twice (i.e. when increasing and decreasing parameter value starting from optimal value), this proves parameter identifiability. The points at which the likelihood profile crosses the threshold are the ranges of the parameter confidence interval.

References

1 Schwanhausser, B. *et al.* Global quantification of mammalian gene expression control. *Nature* **473**, 337-342, doi:10.1038/nature10098 (2011).

2 Raue, A. *et al.* Structural and practical identifiability analysis of partially observed dynamical models by exploiting the profile likelihood. *Bioinformatics (Oxford, England)* **25**, 1923-1929, doi:10.1093/bioinformatics/btp358 (2009).