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. $k_{\text{in}25}/k_{\text{out}25}$ and $k_{\text{in}26}/k_{\text{out}26}$ 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.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY26 extracellular concentration</td>
<td>$FY_{26\text{out}}$</td>
</tr>
<tr>
<td>FY25 extracellular concentration</td>
<td>$FY_{25\text{out}}$</td>
</tr>
<tr>
<td>FY26 intracellular concentration</td>
<td>$FY_{26\text{in}}$</td>
</tr>
<tr>
<td>FY25 intracellular concentration</td>
<td>$FY_{25\text{in}}$</td>
</tr>
<tr>
<td>Nuclear factor concentration</td>
<td>$N$</td>
</tr>
<tr>
<td>Messenger RNA concentration</td>
<td>$R$</td>
</tr>
<tr>
<td>Uptake transporter concentration</td>
<td>$UT$</td>
</tr>
<tr>
<td>Efflux transporter concentration</td>
<td>$ET$</td>
</tr>
</tbody>
</table>

**Table S2. Model Parameters.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY25 passive uptake/efflux rate (h(^{-1}))</td>
<td>$k_{25}$</td>
</tr>
<tr>
<td>FY26 passive uptake/efflux rate (h(^{-1}))</td>
<td>$k_{26}$</td>
</tr>
<tr>
<td>FY26 active uptake rate (h(^{-1}))</td>
<td>$k_{26}^{\text{in, active}}$</td>
</tr>
<tr>
<td>FY25 active uptake rate (h(^{-1}))</td>
<td>$k_{25}^{\text{in, active}}$</td>
</tr>
<tr>
<td>FY26 active efflux rate (h(^{-1}))</td>
<td>$k_{26}^{\text{out, active}}$</td>
</tr>
<tr>
<td>FY25 active efflux rate (h(^{-1}))</td>
<td>$k_{25}^{\text{out, active}}$</td>
</tr>
<tr>
<td>FY26 to FY25 transformation rate (h(^{-1}))</td>
<td>$k_m$</td>
</tr>
<tr>
<td>Critical activation threshold</td>
<td>$\text{Thres}$</td>
</tr>
<tr>
<td>Hill coefficient for the nuclear factor</td>
<td>$n$</td>
</tr>
<tr>
<td>Maximal effect rate of the nuclear factor (h(^{-1}))</td>
<td>$k_{\text{induction}}$</td>
</tr>
</tbody>
</table>
1.1. FY26 PK model including “Enhanced Efflux”

**FY25 extracellular concentration:**
\[
\frac{d FY25_{out}}{dt} = (-k^{in}_{25}FY25_{out} + k^{out}_{25} ET FY25_{in}) \frac{V_{in}}{V_{out}}
\]

**FY25 intracellular concentration:**
\[
\frac{d FY25_{in}}{dt} = k^{in}_{25}FY25_{out} - k^{out}_{25} ET FY25_{in} + k_m FY26_{in}
\]

**FY26 extracellular concentration:**
\[
\frac{d FY26_{out}}{dt} = (-k^{in}_{26}FY26_{out} + k^{out}_{26} ET FY26_{in}) \frac{V_{in}}{V_{out}}
\]

**FY26 intracellular concentration:**
\[
\frac{d FY26_{in}}{dt} = k^{in}_{26}FY26_{out} - k^{out}_{26} ET FY26_{in} - k_m FY26_{in}
\]

**Generic nuclear factor concentration:**
\[
\frac{d N}{dt} = k_{induction}(FY25_{in} + FY25_{in})^n \frac{(FY25_{in} + FY25_{in})^n + Thres^n}{(FY25_{in} + FY25_{in})^n + Thres^n}
\]

**Efflux transporter mRNA concentration:**
\[
\frac{d R}{dt} = k^R_f (1 + N) - k^R_d R
\]

**Efflux transporter protein concentration:**
\[
\frac{d ET}{dt} = k^P_f R - k^P_d ET
\]

1.2. FY26 PK model including “Reduced Uptake”

**FY25 extracellular concentration:**
\[
\frac{d FY25_{out}}{dt} = (-k^{in}_{25} UT FY25_{out} + k^{out}_{25} FY25_{in}) \frac{V_{in}}{V_{out}}
\]

**FY25 intracellular concentration:**
\[
\frac{d FY25_{in}}{dt} = k^{in}_{25} UT FY25_{out} - k^{out}_{25} FY25_{in} + k_m FY26_{in}
\]

**FY26 extracellular concentration:**
\[
\frac{d FY26_{out}}{dt} = (-k^{in}_{26} UT FY26_{out} + k^{out}_{26} FY26_{in}) \frac{V_{in}}{V_{out}}
\]
FY26 intracell concentration:

\[
\frac{d \text{FY26}_\text{in}}{dt} = k_{26}^{\text{in}} \text{UT} \text{FY26}_\text{out} - k_{26}^{\text{out}} \text{FY26}_\text{in} - k_m \text{FY26}_\text{in}
\]

Unknown species concentration:

\[
\frac{d N}{dt} = k_{\text{induction}} \frac{(\text{FY25}_\text{in} + \text{FY26}_\text{in})^n}{(\text{FY25}_\text{in} + \text{FY26}_\text{in})^n + \text{Thres}^n}
\]

Uptake transporter mRNA concentration:

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

Uptake transporter protein concentration:

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

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**

<table>
<thead>
<tr>
<th>Parameter Description</th>
<th>Reduced uptake</th>
<th>Enhanced efflux</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA steady state value (RNAss)</td>
<td>1.5214e-4</td>
<td>3.9771e-005</td>
<td>µM</td>
</tr>
<tr>
<td>Protein steady state (Protss)</td>
<td>0.4948</td>
<td>0.1623</td>
<td>µM</td>
</tr>
<tr>
<td>$k_f^R$</td>
<td>1.1624e-5</td>
<td>3.3710e-006</td>
<td>µM.h$^{-1}$</td>
</tr>
<tr>
<td>$k_f^P$</td>
<td>97.23</td>
<td>188.84</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$k_d^R$ (=kf_RNA/RNA_ss)</td>
<td>0.0764</td>
<td>0.0848</td>
<td>h$^{-1}$</td>
</tr>
<tr>
<td>$k_d^P$ (=RNA_ss*kf_prot/Prot_ss)</td>
<td>0.0299</td>
<td>0.0463</td>
<td>h$^{-1}$</td>
</tr>
</tbody>
</table>
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 \(\theta\), and \(\sigma_i\) 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 \(\theta_j\), 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 \(\theta_j\) is defined as:

\[
\{\theta_j | C_{PL}(\theta_j) - C_{PL}(\theta_j^*) < \Delta_\alpha \}
\]

where \(\theta_j^*\) is the parameter optimal value which minimizes \(C(\theta)\). \(\Delta_\alpha\) is the \(\alpha\) quantile of the \(\chi^2\) 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