A physiology-based model predicts pharmacokinetics, target occupancy in the tumour, and HSP70 biomarker response in serum for the **HSP90 inhibitor**, 17-AAG

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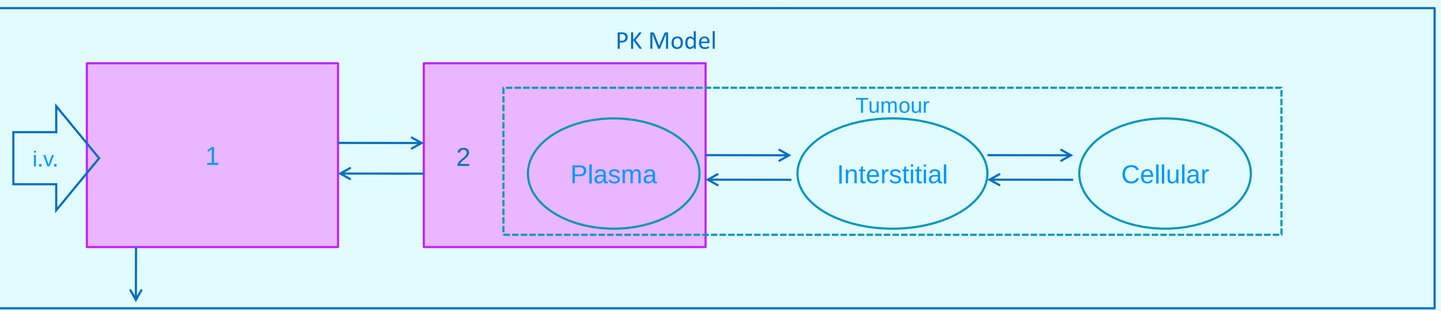
Objectives: HSP90 inhibitors of various structures (geldamycin analogs, ATP analogs) are currently in clinical evaluation [1]. The front-runner, 17-allylamino-17-demethoxygeldamycin, 17-AAG, was in phase 2 [2] but its development has been stopped. All HSP90 inhibitors lack a reliable biomarker to guide dose selection in the absence of typical cytotoxic effects. This simulation study uses published data to extrapolate from a mouse xenograft model to cancer patients, predicting drug concentrations and HSP90 occupancy in tumour cells and the increase in HSP70 levels in serum.

17-*N*-Allylamino-17-demethoxygeldanamycin, Tanespimycin

Methods:

- *Analytics in Plasma*: A LC-MS/MS method determined concentrations of 17-AAG which reflected a redox-equilibrium between 17-AAG and its hydroquinone analog, 17-AAGH2.
- *Plasma PK in cancer patients*: A two-compartment PK model [3] for cancer patients participating in a phase 1 study provided the parameters for the simulation of the plasma 17-AAG level in patients of 80 kg body weight (BSA=1.96m2, HCT=0.4) bearing a 500g solid tumour and being treated with six weekly i.v. infusions of 450 mg/m2 over 2 h. That was the regimen in the last phase 2 study in melanoma [2].
- *PK in tumour*: Tumour plasma compartment was assumed within the peripheral compartment of the PK model. The uptake of 17-AAG into tumour cells was predicted from the scaled (based on tumour size) permeability surface-area product (0.26 L/h)[4], from the ratio of tumour plasma (0.02 L) versus peripheral volume (90 L), and from the rate constants between interstitial and cellular space (Kitc = 0.186 h-1, Kcit = 0.062 h-1)[4].
- *Occupancy of HSP90*: Tumour tissue concentrations of 17-AAG, CT, were calculated from the amounts and volumes of the tumour interstitial and cellular space. The relationship between CT and % occupancy of HSP90, OCC, was obtained by fitting digitised published data [5] with a three-parameter log-logistic function (LL.3 in R).
- *Tumour and serum HSP70*: The relationship between intracellular concentration of 17-AAG, CC, and levels of HSP70 was obtained by fitting digitised published data [6]. Similarly, secreted HSP70 was predicted from intracellular HSP70 [6] and the former was diluted by the ratio of tumour interstitial volume (0.27 L) to serum volume (4.4 L) to arrive at the HSP70 serum level (measureable by ELISA [6]).



How to merge a human popPk model [3] with a mouse xenograft popPBPK model [4]?

- Derive composition of human tumour from xenograft: 6.5% of volume is plasma, 54.3% of volume is interstitial space.
- Use rate constants between tumour interstitial space and cellular space (Kitc, Kcit) unscaled because xenograft is human.
- Make tumour plasma kinetically identical to peripheral compartment of PK model, and calculate fraction entering tumour as ratio of tumour plasma over V2, the peripheral volume.
- Scale the permeability surface area product (PSVIT=0.26 L/h) alometrically from xenograft (0.3 g) to human tumour (500 g).

HSP90 Occupancy (%)

CT...tumour tissue conc., e=5000 ng/mL

$$OCC = \frac{100}{1 + \exp\left(b \cdot \left(log(CT) - log(e)\right)\right)}$$

Intracellular HSP70 (ng/mL)

CC...tumour cellular conc.

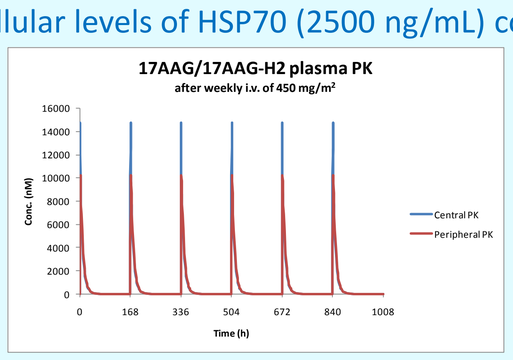
$$HSP70_{intr} = \frac{Emax}{1 + \exp\left(b \cdot \left(log(CC) - log(e)\right)\right)}$$

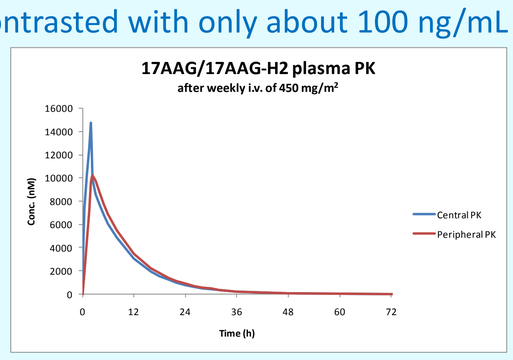
Secreted HSP70 (ng/mL)

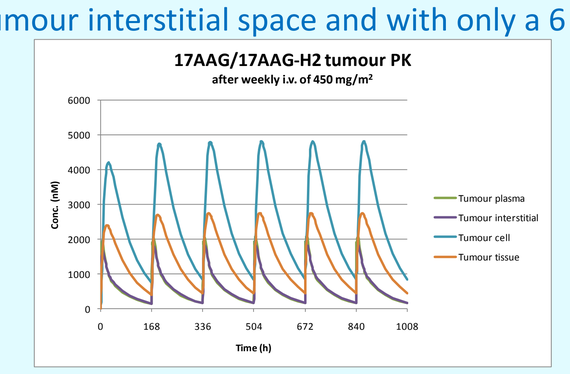
$$HSP70_{secr} = \frac{Emax}{1 + \exp\left(b \cdot \left(log(HSP70_{intr}) - log(e)\right)\right)}$$

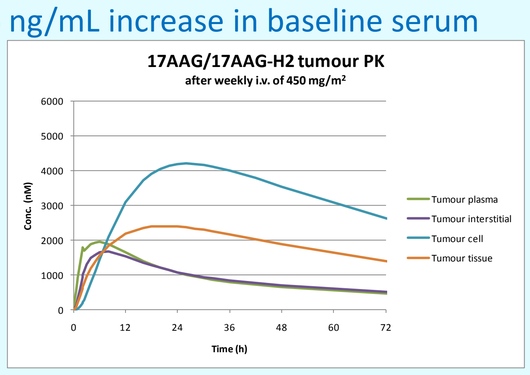
Results:

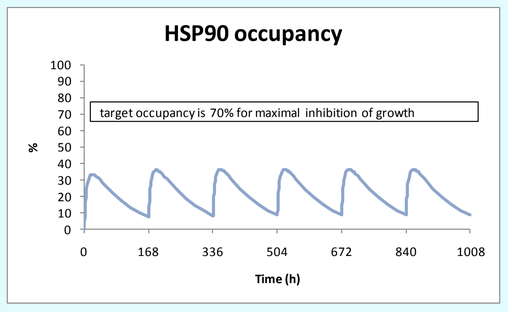
Simulated plasma concentrations of 17-AAG reached 15000 nM at the end of the infusion and were undetectable after 48 h. Interstitial and cellular 17-AAG levels reached 1800 nM and 4800 nM at 6 h and 24 h after the end of the infusion, respectively. CT reached 2700 nM at 14 h after the infusion. There was little accumulation of 17-AAG tumour concentrations between the first and sixth week of treatment. OCC varied between 35% and 10% throughout the six weeks of treatment. High intracellular levels of HSP70 (2500 ng/mL) contrasted with only about 100 ng/mL in the tumour interstitial space and with only a 6 ng/mL increase in baseline serum HSP70.

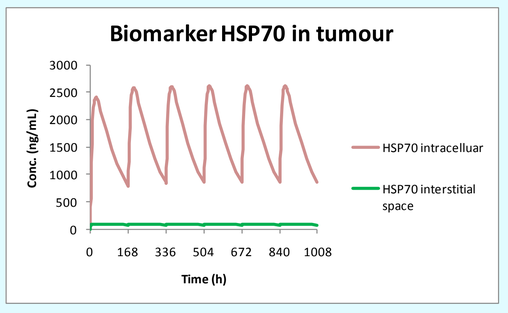


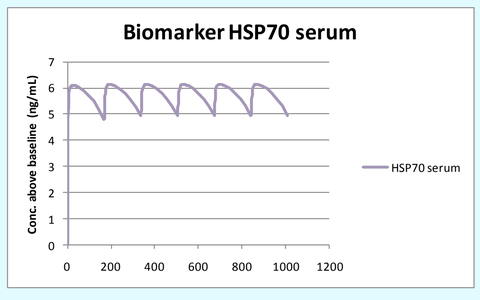












Discusion:

- Abundance of HSP90, e.g. 10µM in lymphoma cells (PNAS 1986).
- Slow off rate of pharmacologically active inhibitors: $k_{off} = 2.5 \cdot 10^{-3}$ min^{-1} , T½ = 4.6 h [7]
- High affinity K_i = 10 nM for most inhibitors; specifically 50 nM for 17-AAG-H_2 , the active species of 17-AAG in vivo.
- Long exposure of HSP90 to inhibitors required to reach occupancy at steady state. About 70% would be required for growth inhibition.
- For a successful therapy, one has to expose the tumour cells high and long enough with HSP90 inhibitors.

References:
[1] Trepel J et al. Nature Rev Cancer 2010;10:537-549.
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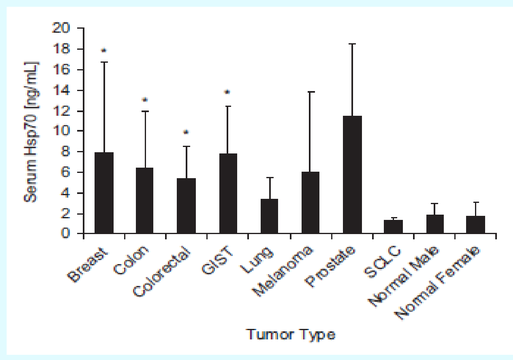
[3] Chen X et al. Cancer Chemother Pharmacol 2005;55:237-243.
[4] Xu L et al. J Pharmacokin Pharmacodyn 2003;30:185-219.
[5] Tillotson B et al. J Biol Chem 2010;285:39835-43.
[6] Dakappagari N et al. Biomarkers 2010;15:31-38.
[7] Gooljarsingh LT et al. PNAS 2006;103:7625-7630.

Reality Check:

Baseline HPS70 in serum of cancer patients [6]



Bars show mean (SD)

The utility of serum HSP70 by ELISA as biomarker for the target interaction of an HSP90 inhibitor is limited by the variability of the baseline level in patients suffering from a specific cancer.
In the current simulation the secreted HSP70 reached already its maximum, despite that fact that HSP90 was far from being saturated with the inhibitor. Thus more successful occupancy of HSP90 would not necessarily increase serum HSP70 levels.