Establishing the preclinical/translational PK/PD relationship for BT7480, a Nectin-4/CD137 *Bicycle* tumor-targeted immune cell agonist™ (*Bicycle* TICA™)

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Introduction

A new class of modular synthetic drugs, termed Bicycle® tumor-targeted immune cell agonists (Bicycle® TICAs), based on constrained bicyclic peptides has been developed as agonists of immune costimulatory receptors in cancer therapeutics [1]. One example is BT7480 which binds simultaneously to Nectin-4 on tumor cells and CD137 on primed immune cells with activation (agonism) of CD137 being dependent on co-ligation of Nectin-4.

In Vitro analysis

In vitro CD137 reporter activity and cytokine secretion data were generated using *Bicycle* TICA™ BT7480 (Figure 1).

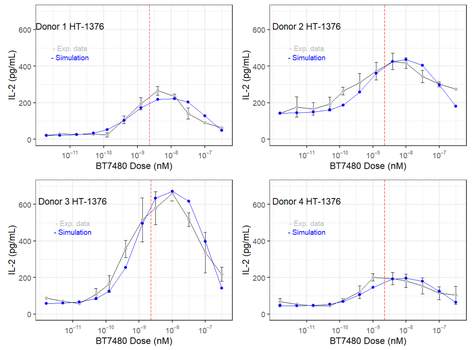

Figure 1. BT7480 concentration dependent driven IL-2 secretion across a panel of 4 PBMC donors, using hPBMC/HT-1376 co-culture assay (grey color) stimulated with anti-CD3 to induce CD137 expression. We fit a 3-body binding model to the experimental data (blue color). Vertical dashed lines correspond to the product of the square-root of CD137 and Nectin-4 dissociation constants.

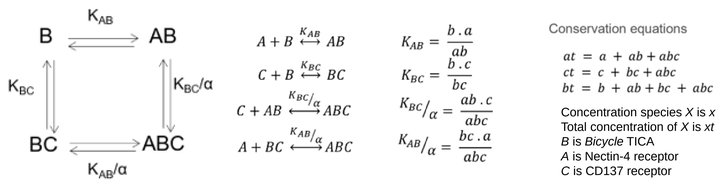

Figure 2. Reaction system for a 3-body binding problem with representations of disassociation constants in terms of free species concentrations and conservation equations

Perelson explored the dose-response curve for a 3-body binding problem [2], Figure 2. A key result is that turning point in the dose response occurs when $b = \sqrt{K_{AB}} . \sqrt{K_{BC}}$. We adapted the scheme to model the effect of BT7480.

We found that the model describes the IL-2 activation profile using the same parameter value set but total CD137 levels vary between hPBMC donors.

Pharmacokinetic modelling

BT7480 plasma and tumor pharmacokinetics (PK) in CT26-Nectin-4 tumor-bearing mice is shown in Figure 3. A two-compartment open model describes the drug plasma profile after intravenous dosing and the tumor profile was described by a single effect compartment model.

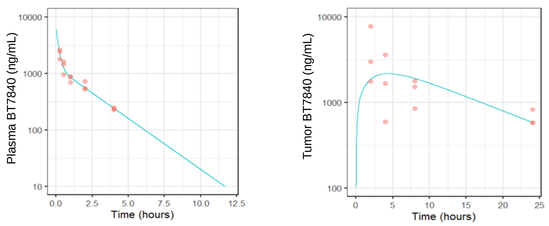

Figure 3. Plasma and tumor pharmacokinetics for BT7480 for a single 5 mg/kg dose in CT26 model (pink dots). The solid cyan line correspond to the modelling simulation.

Pharmacodynamics – Tumor growth analysis

A linear model for tumor growth was calibrated using preclinical data (Figure 4) by placing the model within a mixed-effects framework,

$$R_{ij} = a_i + b_i t_{ij} + e_{ij} \qquad a_i \sim N(\mu_1, \sigma_1^2) \qquad b_i \sim N(\mu_2, \sigma_2^2) \qquad e_{ij} \sim N(0, \sigma_3^2) \qquad \text{(M1)}$$

where *Rij* is the observed radius of tumor *i* at time *j*, a_i is the value of the radius at time 0 for tumor *i*, b_i is the rate of growth of the radius for tumor *i*, t_{ij} is the time-point for tumor *i* at time *j* at which the observation was recorded and e_{ij} is the residual error for tumor *i* at time *j*.

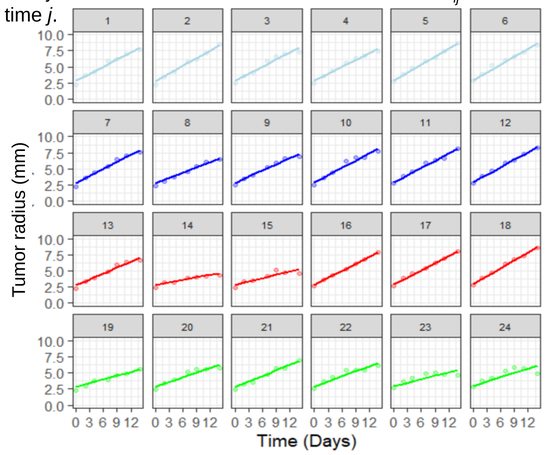

Figure 4. Tumor growth in CT26 xenograft model. The plots show the model fit (solid line) to the raw data (open-circles) to each treatment group: control (light-blue), 1 mg/kg Q3D (dark-blue), 1 mg/kg QD (red) and 5mg/kg Q3D (green). *QD: Once a day; Q3D: every three days*

Pharmacodynamics: Biomarker-Tumor growth analysis

We assessed how the tumor growth rate correlates to the immune system markers collected. We derived a rank correlation coefficient between every pair of immune system markers and growth rate. These were visualized within a heatmap, Figure 5.

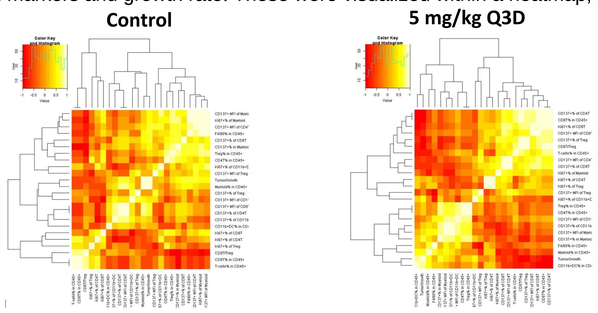

Figure 5. Heatmaps of the rank correlation coefficient across all immune markers and tumor growth in the control group (left-hand side) and the high dose, 5 mg/kg Q3D, treated group (right-hand side).

Next, we assessed whether one of these biomarkers could capture the treatment effect on tumor growth inhibition induced by BT7840 (Figure 6). We modified b_i in the M1 equation shown above to include a parameter *c* which accounts for the biomarker (BM) effect on the growth rate, $b_i \sim N(\mu_2 + cBM_i, \sigma_2^2)$ (M2)

Next, we introduced a population parameter, *d*, which accounts for average daily dose, such $b_i \sim N(\mu_2 + cBM_i + dD_i, \sigma_2^2)$ (M3)

We found that % total T-cells and %CD8+ T-cells were strong correlates to tumor growth (M2 vs M1: p<0.001) and were closest to fully capturing the treatment effect (M3 vs M2: %T-cells p = 0.12 & %CD8+ T-cells p = 0.08).

Figure 6. Schematic highlighting the paths explored with the mixed-effects models. PD: Pharmacodynamics, TGI: tumor growth inhibition.

PK-PD-TGI model

From the mixed-effects analysis we have identified a surrogate biomarker of efficacy for BT7840, %CD8+ T-cells which can be used for development of a PK-PD-TGI model for exploring dosing/scheduling. We then moved onto developing the PK-PD-TGI model. Figure 7 shows the structure of the final model.

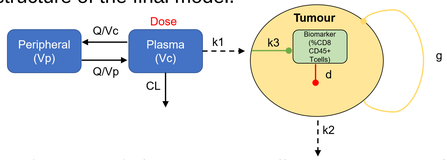

Figure 7. Schematic of the final Pharmacokinetic-Pharmacodynamic-Tumor-Growth-Inhibition model. *g: control growth rate; k3: %CD8 CD45+ T-cell expansion rate, d: CD8 CD45+ T-cell's killing rate of tumour cells. K1 and k2: drug effect compartment. Vc, Vp, Q and CL are the plasma PK parameters for BT7480.*

We determined the %CD8+ T-cell expansion rate, *k3*, and the killing rate of tumor cells, d. These two parameters are estimated by fitting the model to the day 15 biomarker data and the tumor growth inhibition data in a sequential manner, Figure 8 and 9. The model PD parameters and tumor growth inhibition are shown in Table 1.

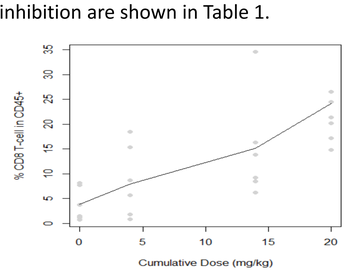

Figure 8. %CD8+ T-cells at Day 15 for each tumor (grey dots) and model fit (solid line).

Parameter	Value (% RSE)
k3 (%CD8/hour)	0.088 (14)
g (mm/hour)	0.016 (3)
d (mm/hour)/%CD8	6e-4 (10)

Table 1. Parameter values for the pharmacodynamic and tumor growth inhibition aspects of the model. *RSE: residual standard error.*

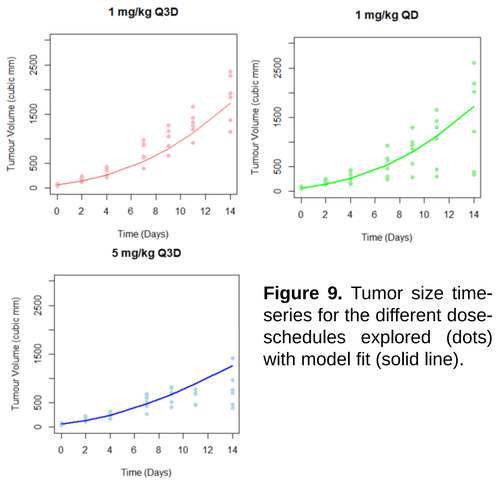

Figure 9. Tumor size time-series for the different dose-schedules explored (dots) with model fit (solid line).

Conclusions

We assessed the predictions of the in vitro model against the experimental observations and found that the position of the inflection point could be predicted from the dissociation constants (Kd's).

The combined BT7480 pharmacokinetic model shows that the elimination rate of BT7480 from plasma is faster than that from the tumor. We hypothesized that this results from BT7480 binding to Nectin-4 in the tumor.

We found that the level of tumor infiltrating CD8+ T-cells fully captures the treatment effect of BT7480 on tumor growth in the syngeneic mouse model. Therefore, we established a potential causal link: from dose/pk to CD8+ T-cell infiltration changes and ultimately to tumor growth inhibition.

A PK/PD modelling framework was developed that predicts preclinical biomarker level and tumor growth inhibition in response to changes in the BT7480 dose and dosing schedule. In addition, plasma and tumor drug concentration levels can be associated with the target concentration estimated using in vitro data [2]. Namely, the product of the square-root of the two target Kds is likely to be the free drug concentration at which maximal activity of the trimer [T-Cell—BT7480—Tumor-Cell] is achieved.

References:
[1] Upadhyaya P, Anticancer immunity induced by a synthetic tumor-targeted CD137 agonist. Journal for ImmunoTherapy of Cancer. 2021;9:e001762.
[2] Perelson AS, Receptor clustering on a cell surface. III. theory of receptor cross-linking by multivalent ligands: description by ligand states. Mathematical Biosciences. 1981;53:1-39