Establishing the preclinical PKPD relationship for NM32-2668 a ROR1 targeting T cell engager

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Introduction

NM32-2668 is a fragment-based multispecific antibody therapeutic [1] that has been designed to activate T-cells (via CD3) in the presence of tumour antigen receptor tyrosine kinase-like orphan receptor 1 (ROR1). The structure and the mechanism of action of NM32-2668 are shown in Figure 1.

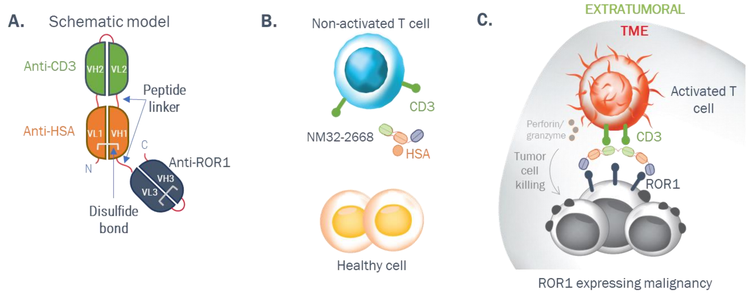


Figure 1. A. Structural model of NM32-2668. B. NM32-2668 does not activate T cells in the absence of target. C. In the presence of target, CD3 T cells are engaged and activated to kill ROR1+ cells.

The objective of this work was to build a mathematical model to establish a PKPD relationship using both *in vitro* and *in vivo* data for NM32-2668.

Material and methods

Data were collected from *in vitro* studies measuring CD4/8 activation and cytotoxicity from a panel of cell lines and patient samples with increasing concentrations of NM32-2668, and from *in vivo* tumour growth inhibition (TGI) data from a humanised mouse model in one cell line with two different donors with increasing doses of NM32-2668. See *in vitro* and *in vivo* sections for details:

***In vitro* analysis**. T cell-mediated depletion of solid tumour cell lines and haematological tumour cell lines was assessed. Tumour cell lines were co-cultured together with T cells for 40 h at an E:T ratio of 10:1, and cytotoxicity was assessed using lactate dehydrogenase release relative to controls. T cell activation was assessed within these experiments by flow cytometry via the upregulation of CD69 as a marker for activation. The average number of ROR1 proteins on the surface of cancer cell lines was quantified by flow cytometry using an anti-ROR1-PE labelled antibody (clone 2A2, Biolegend). Receptor density values are reported as the antibody binding capacity (ABC). ABC values were derived from standard curves generated with Quantum Simply Cellular beads anti-mouse IgG (Bangs Laboratories, Inc.).

***In vivo* analysis**. The anti-tumour efficacy of NM32-2668 was assessed in immunodeficient NCG mice engrafted with the mantle cell lymphoma xenograft cell line JeKo-1 and human PBMCs from healthy donors (A & C). Mice were inoculated subcutaneously with 1.5 x 10e6 JeKo-1 cells on day 0 and subsequently were implanted with 1x10e7 PBMC on day 3. Mice were randomized into 8 animals per group when the mean tumour size reached 80-90 mm3 and test article treatment was initiated on the same day. Mice were administered NM32-2668 at 1, 0.2 or 0.04 mg/kg every 5 days for 8 repeat doses. Control groups of irrelevant protein (palivizumab), saline and no PBMCs were also included in the study.

Modelling techniques. Nonlinear mixed-effects models were used to assess the variability in cytotoxicity as a function of drug concentration activation. The nlme package in R v4.1.3 was used for this analysis [2]. The translatability of *in vitro* potency values for immune and immune system activation was assessed by linking PK to *in vitro* data and to TGI *in vivo* data.

In vitro analysis

ROR1 density in combination with CD8 activation fully captures the concentration response of cytotoxicity.

To address this statement we developed the following step-wise modelling procedure to analyse these data. A hierarchical concentration response model, shown below, was first fit to the CD8 activation data across a panel of 14 cell-lines:

$$R_{ij} = B_i + Emax_i \frac{[D]_{ij}^{h_i}}{EC50_i^{h_i} + [D]_{ij}^{h_i}} + e_{ij}$$

$$\exp(B_i) \sim N(\mu_0, \sigma_0^2)$$
$$\exp(Emax_i) \sim N(\mu_1, \sigma_1^2)$$
$$\exp(EC50_i) \sim N(\mu_2, \sigma_2^2)$$
$$\exp(h_i) \sim N(\mu_3, \sigma_3^2)$$
$$e_{ij} \sim N(0, \sigma_4^2)$$

Response for cell-line *i* at concentration *j*, R_{ij}, is equal to the baseline value for cell-line *i*, B_i, plus concentration response, $EC50_i$ value for cell-line *i* and hill coefficient h_i for each cell-line. The error term e_{ij} is the unexplained variance. We assume the parameter values are log-normally distributed with unknown mean and variance. The final model fits to the CD8 activation data are shown below in Figure 2, all parameter values showed good precision (% relative standard error (RSE) <25).

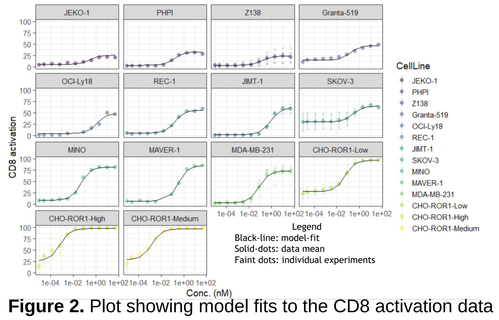


Figure 2. Plot showing model fits to the CD8 activation data

In order to assess if ROR1 density values (see Table in Figure 3) and CD8 activation, as measured via EC50, can be used as a surrogate for the EC50 of cytotoxicity the equation for EC50 was changed to,

$$\exp(EC50_i) \sim N(a_0\, EC50_{CD8i} + a_1 * \log(ROR1_i), 0)$$

Which assumes that the cytotoxicity EC50 value can be calculated using a cell-lines CD8 EC50 and ROR1 density value. The final model described the data well, see Figure 3. The parameters had good precision (% RSE <25). Thus, the combination of CD8 EC50 and ROR1 density can be used as a surrogate for cytotoxicity EC50.

Cell-line	ROR1 Density
CHO-High	4676200
CHO-Medium	354800
CHO-Low	14900
Granta-519	6800
JEKO-1	55900
JIMT-1	23300
MDA-MB-231	62000
OCI-Ly18	32300
MINO	17900
REC-1	33400
SKOV-3	18400
Z138	12600

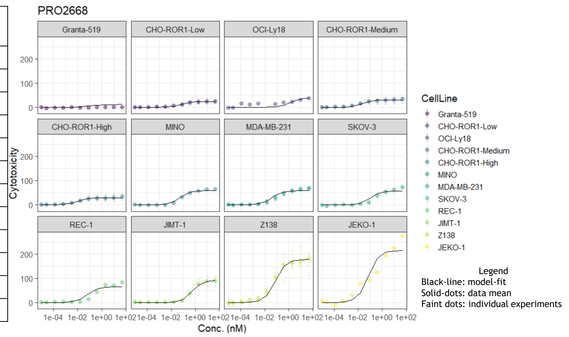


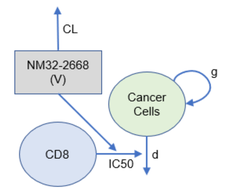
Figure 3. Plot showing the ROR1 density values and model fits to the cytotoxicity data

In vivo analysis

***In vitro in vivo* correlation in JEKO-1: PK-(*in vitro*)-PD-TGI model captures the dose-response observed using different donors**

To address this statement a mathematical model linking the PK to TGI was done using the *in vitro* estimate of CD8 activation of JEKO-1 as the link between drug levels and efficacy. The model structure and equations are shown at the top of Figure 4. To account for the effect of different donors, a different growth rate, g. was estimated for each control. The decay rate, d, was held constant for both donors. Finally the model was regressed against tumour radius assuming the tumour to be spherical.

The final model fits to the data can be seen in the bottom panel of Figure 4. The model described the data well thus, the *in vitro* CD8 EC50 value can be used to link *in vivo* PK to TGI.



$$\frac{dC}{dt} = -\frac{CL}{V}C \qquad \text{C: amount of drug in plasma (mg)}$$

$$[D] = 1e^6 \frac{C/V}{MW} \qquad \text{Plasma [NM32-2668] in nM}$$

$$\frac{dR}{dt} = g - d\frac{[D]}{EC50_{CD8} + [D]} \qquad \text{Change in tumour size}$$

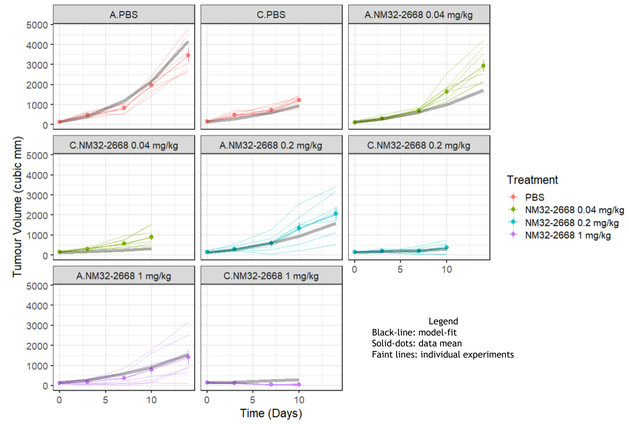


Figure 4. Top panel shows the model structure and equations. Bottom-panel shows the model fit (black line) to the TGI data (coloured lines) for different donors, A and C.

Results

The combination of ROR1 expression and CD8 activation fully explained the variance in cytotoxicity across all *in vitro* data. The estimated *in vitro* potency for CD8 activation could successfully be used to provide a link between PK and TGI *in vivo*.

Conclusions

A PK-PD-Efficacy model based on the *in vitro* data was established showing that the cytotoxicity response was strongly correlated to ROR1 expression and CD8 activation. Building on this *in vitro* model, we developed an *in vivo* PK-TGI model that can link immune system activation to TGI. The final model will support the starting dose justification in Phase 1 studies and also be combined with human PK predictions to assist in the design of the trial.

References:
[1] Egan TJ et al., Novel multispecific heterodimeric antibody format allowing modular assembly of variable domain fragments. MABS, 2017, VOL. 9, NO. 1, 68–84.
[2] Pinheiro J, Bates D, R Core Team (2022). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-159.