Population semi-mechanistic modelling of tumour response elicited by immune-stimulatory based therapeutics in mice

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Background

Mathematical modelling approach represents a useful tool to better understand complex systems such as the interactions established between immune and tumour cells after immune-stimulatory therapies, a growing therapeutic strategy in oncology. However its use in this area is still scarce, especially during preclinical stages.

The aim of this work were:

(i) to develop a semi-mechanistic population pharmacodynamic model to describe the effects of a vaccine (CyaA-E7) able to trigger a potent and specific immune response in xenograft mice, when administered alone or in combination with CpG (a TLR9 ligand) and/or cyclophosphamide (CTX).

(ii) to assess the applicability of the model under different immune-based treatments.

Methodology

Animal experimentation

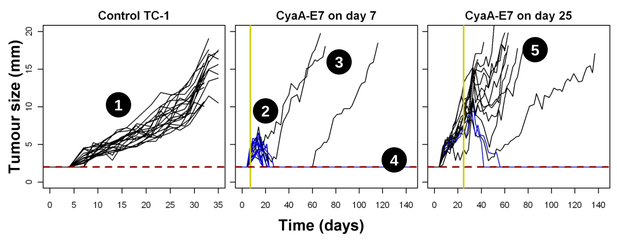
- Berraondo *et al.* [1] published data were used to develop the model. Briefly, $5x10^5$ tumour cells expressing HPV16-E7 proteins were injected into the shaved back of C57BL/6 mice in 200uL of PBS and different regimens were followed:
  - *Monotherapy:* A single dose of 50µg of vaccine or PBS (control group) was administered to mice on different days after tumour inoculation (day 4,7,11 ,18, 25 or 30).
  - *Bitherapy:* A single dose of 50µg of vaccine on day 25 was administered in combination with 30 µg of CpG on the same day or 2.5 mg of CTX on the day before. CpG and CTX in monotherapy administered with the same dosing schedule were also included as reference.
  - *Tritherapy:* A single dose of 50µg of vaccine on day 25, 30 or 40 was administered in combination with 30 µg of CpG on the same day and 2.5 mg of CTX on the day before. A group receiving only CpG and CTX in combination on day 25 and 24 respectively was also included.
- Medina-Echeverz *et al.* [2] published data were used to test model applicability to other immune-stimulatory scenarios. Briefly, $5x10^5$ MC38 cells were subcutaneously injected to 5-week-old female C57BL/6 mice. Subsequently, mice were treated with either PBS (control group) or with 10µg of a plasmid codifying for murine interleukin 12 (IL12) administered by hydrodynamic injection on day 23, alone or in combination with 2.5 mg dose of CTX on the previous day.

Tumour size, presented as the average of two perpendicular diameters (mm), was measured at regular intervals. Mice were euthanized if tumour size reached 20mm.

Model development

Sequential model developement was followed:

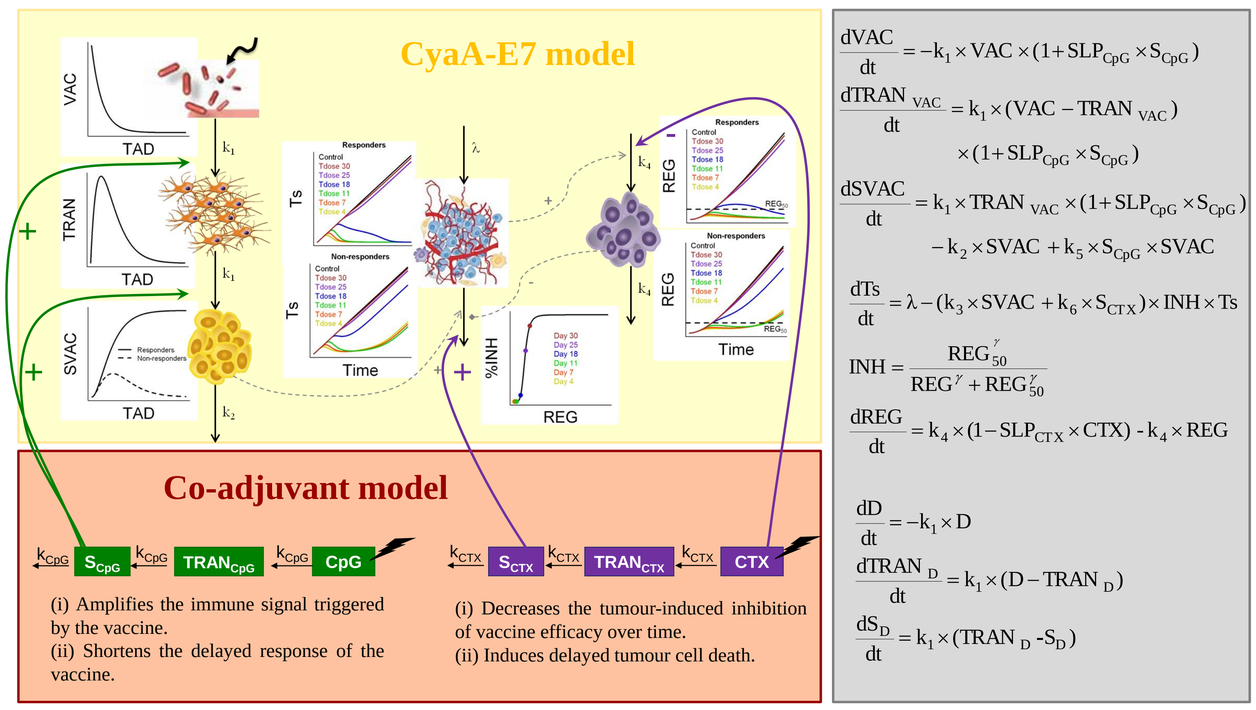
- *CyaA-E7 model:* Analysis of the monotherapy data revealed five main aspects (Fig. 1) considered during model building (Fig. 2, yellowed area) [3].



1. Unperturbed tumour growth
2. Delay drug effect
3. Data below the limit of quantification (BQL)
4. Mixture model to account for non-responders
5. Drug resistance

Figure 1. Individual raw data. Tumour size profiles for control mice and CyaA-E7 administered on day 7 or 25 (yellow line) dosing groups

- *Co-adjuvant model* was developed using bitherapy data (Fig. 2) (red area).
- Finally, both bitherapy models were coupled to simulate the different tritherapy dosing groups.



$$\frac{dVAC}{dt} = -k_1 \times VAC \times (1 + SLP_{CpG} \times S_{CpG})$$

$$\frac{dTRAN_{VAC}}{dt} = k_1 \times (VAC - TRAN_{VAC}) \times (1 + SLP_{CpG} \times S_{CpG})$$

$$\frac{dSVAC}{dt} = k_1 \times TRAN_{VAC} \times (1 + SLP_{CpG} \times S_{CpG}) - k_2 \times SVAC + k_5 \times S_{CpG} \times SVAC$$

$$\frac{dTs}{dt} = \lambda - (k_3 \times SVAC + k_6 \times S_{CTX}) \times INH \times Ts$$

$$INH = \frac{REG^{\gamma}_{50}}{REG^{\gamma} + REG^{\gamma}_{50}}$$

$$\frac{dREG}{dt} = k_4 \times (1 - SLP_{CTX} \times CTX) - k_4 \times REG$$

$$\frac{dD}{dt} = -k_1 \times D$$

$$\frac{dTRAN_D}{dt} = k_1 \times (D - TRAN_D)$$

$$\frac{dS_D}{dt} = k_1 \times (TRAN_D - S_D)$$

Figure 2. Scheme and mathematical equations of the model. After vaccine (VAC) administration and through a transit compartment ($TRAN_{VAC}$) , the vaccine induces a signal (SVAC) able to decrease tumours size (Ts). An inhibition of vaccine efficacy induced by a regulator compartment (REG) controlled by tumour size was detected. Regarding the co-adjuvant therapies, after CpG administration and through a transit compartment ($TRAN_{CpG}$), the drug triggers a signal (S_{CpG}) able to increase transit between vaccine compartments and induce the proliferation of SVAC. On the other hand CTX is able to directly inhibit regulator compartment (REG) proliferation and also generate, through a delay compartment ($TRAN_{CTX}$), a signal (S_{CTX}) able to induce tumour death. k_1: first order rate constant controlling vaccine elimination and transit between compartments; k_2: first order rate constant accounting for SVAC degradation; λ: zero order rate constant of tumour growth; k_3: vaccine efficacy second order rate constant; k_4: first order rate constant controlling the regulator compartment dynamics; Ts_{50}: tumour size able to inhibit the 50% of the vaccine response ; γ: shape parameter; k_{CTX} and k_{CpG}: first order rate constants controlling co-adjuvant drug elimination and transit between compartments. SLP_{CpG}: linear effect triggered by CpG over the vaccine compartment dynamics; SLP_{CTX}: linear effect triggered by CTX over the REG compartment dynamics; k_6: CTX efficacy second order rate constant . In the equations, D stands for either, CpG or CTX.

Data analysis and Model Evaluation

- Non parametric bootstrap was used to evaluate precision of parameter estimates (Tables I and II).
- Goodness of fit plots and individual fits were represented (Fig 3).
- Visual Predictive Checks (VPCs) were performed by simulating 1000 individuals for each of the treatment groups included in the analysis. 5th, 50th and 95th percentiles were calculated and plotted against the observed data (Fig. 4, upper panels).
- Percentage of simulations above the LOQ were obtained and plotted against raw data (Fig. 4, lower panels).
- 1000 thousand studies were simulated. The simulated probability of cure for each group was calculated and compared with the experimental one (Fig. 5).

During the analysis, M3 method [4] was used to account for the BQL, considering 2 mm as the lowest measurable value. Berkeley-Madonna, R, NONMEM VII and PsN softwares were used to develop the model.

Model Applicability

The model structure was used to described IL12 data, estimating only the IL12 model specific parameters (see Table I) and evaluated as previously described.

Results

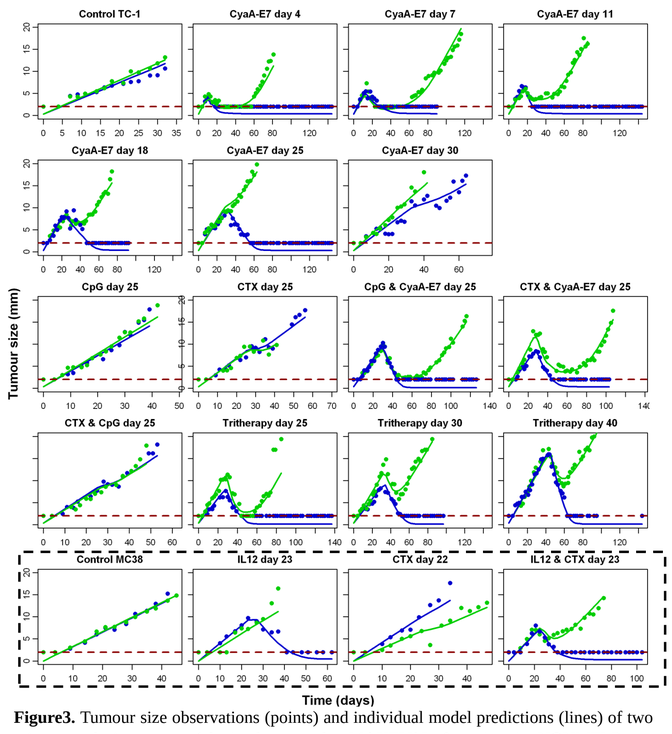


Figure3. Tumour size observations (points) and individual model predictions (lines) of two mice per dosing group (obtained using the MAXEVAL=0 option in NONMEM) are presented for CyaA-E7 and IL12 (framed plots). 2 mm was considered as the limit of quantification (dashed line).

Table I: Monotherapy model parameter estimates

	CyaA-E7		IL12	
Parameter	Mean Value [2.5th-97.5th]	IAV (%) [2.5th-97.5th]	Mean Value [2.5th-97.5th]	IAV (%) [2.5th-97.5th]
Ts_0 (mm)	0.324 [0.0796-0.572]	-	$1.16x10^{-6}$ [$5.57x10^{-7}$ -$2.35x10^{-6}$]	-
λ (mm·day^{-1})	0.354 [0.325-0.381]	10.1 [4.9-13.4]	0.335 [0.306-0.362]	19.3 [11.3-24.4]
k_1 (day^{-1})	0.0907 [0.0842-0.118]	-	0.189 [0.101-0.615]	-
P(1)	0.844 FIX	-	0.844 FIX	-
k_{2_pop1} (day^{-1})	0 FIX	-	0 FIX	-
k_{2_pop2} (day^{-1})	0.0907 [0.0842-0.118]	-	0.189 [0.101-0.615]	-
k_3 (day^{-1})	1.08 [0.870-1.378]	-	1.08 FIX	-
k_4 (day^{-1})	0.0390 [0.0193-0.0771]	-	0.0390 FIX	-
REG_{50} (mm)	3.18 [1.767-4.422]	33.8 [25.4-53.2]	2.08 [1.387-2.966]	36.1 [6.4-60.2]
γ	5.24 [3.673-6.781]	-	5.24 FIX	-
Residual error [Log (mm)]	0.206 [0.184-0.228]	-	0.168 [0.128-0.215]	-

[a] Parameter fix to a previous result obtained when analysing only groups of days 4,7 and 11, where no tolerance was observed.

Table II: Combination model parameter estimates

	CpG	CTX
Parameter	Mean Value [2.5th-97.5th]	Mean Value [2.5th-97.5th]
k_D (day^{-1})	0.268 [0.0533-0.420]	0.302 [0.233-0.483]
SLP_D (au^{-1})	9.01 [3.52-62.3]	2.30 [1.25-4.48]
k_5 (au^{-1}·day^{-1})	0.478 [0.0847-1.65]	-
k_6 (au^{-1}·day^{-1})	-	0.189 [0.0606-0.283]
Residual error [Log (mm)]	0.166 [0.144-0.189]	0.153 [0.133-0.166]

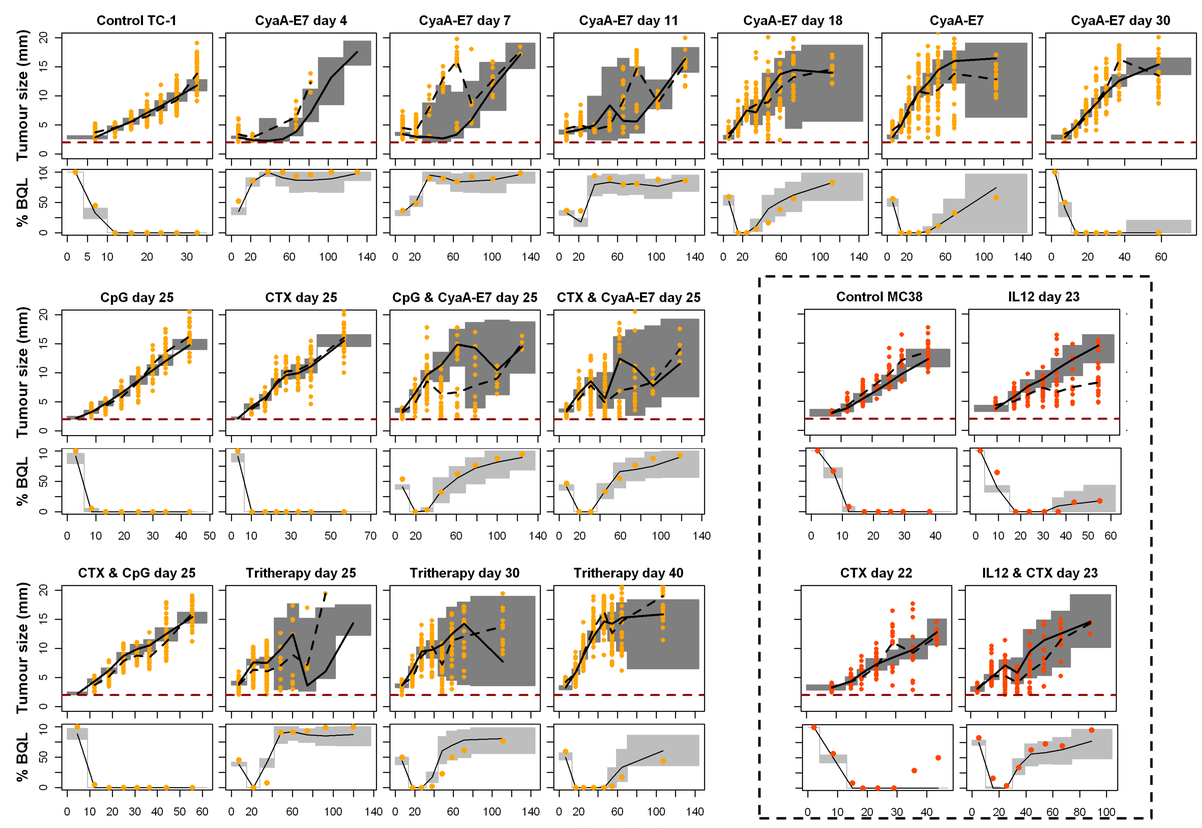


Figure 4. Visual and numerical predictive check to evaluate final model performance. Simulated tumour size measurements above the limit of quantification (upper panels) and percentage of data below the limit of quantification (lower panel) versus raw data (points) are plotted over time for CyaA-E7 (orange) or IL12 (red) dosing groups (framed plots) . Grey areas in the upper panels represent the 90% prediction interval of the simulated median. Grey areas in the lower panels represent the 90% prediction interval of the simulated percentage of data below the limit of quantification. Solid and dashed black lines are the simulated and raw median respectively. 2 mm was considered as the limit of quantification (darkred dashed line).

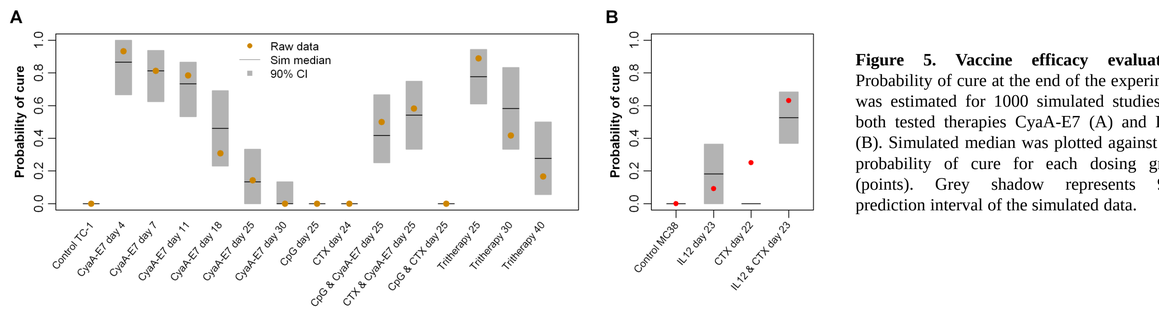


Figure 5. Vaccine efficacy evaluation. Probability of cure at the end of the experiment was estimated for 1000 simulated studies for both tested therapies CyaA-E7 (A) and IL12 (B). Simulated median was plotted against raw probability of cure for each dosing group (points). Grey shadow represents 90% prediction interval of the simulated data.

Conclusions

A novel mathematical model integrating different modelling strategies, such as censored data and mixture model, within the population approach has been successfully developed to describe the different outcomes obtained after CyaA-E7 vaccine administration. In addition, the model structure was applied to describe tumour size outcome after administration of a different immunotherapeutic agent, IL12.

The pharmacodynamic effects of two widely used co-adjuvants in immunotherapy, CTX and CpG, were incorporated into the model considering biologically plausible mechanism of action.

The final model was used to satisfactorily predict tumour response under different immunotherapy scenarios.

This model can be used to maximize the information obtained from preclinical cancer immunotherapy experiments , being useful for the design of better clinical trials of immune modulating drugs.

References

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