TARGET MEDIATED DRUG DISPOSITION MODEL TO DESCRIBE THE EXPRESSION AND KINETICS OF IL12 AND IFNγ IN GENE THERAPY

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Introduction

Interleukin-12 (IL_{12}) has shown to have a great therapeutical potential in the treatment of chronic hepatic diseases [1]. Nevertheless its in vivo efficacy is hampered by a negative feedback mediated by the interferon γ (IFNγ) produced in response to this cytokine [2].

A model able to describe the relationship between IL_{12} and IFNγ has already been developed when constant doses of Mifepristone (RU, inductor of the gene expression of IL_{12}) were administered [3]. The aim of the study is to challenge an improved the previously developed model when increasing doses of the Mifepristone are administered under different dosing regimens.

Methododology

I. Animal Experimentation

Wild type mice were infected with two different doses (DNA=1 or DNA=2.5) of gutless adenoviral vectors containing a Mifepristone (RU) -inducible system for liver-specific expression of interleukin-12. Daily induction of constant or increasing doses of RU (Table I) was performed and levels of IL_{12} and IFNγ were measured.

II. Mathematical Model

Data from Treatments (TTO) 0-5 (Table I) were used to develop a kinetic-pharmacodynamic model (Figure 1).The quasi-equilibrium model proposed by Mager *et al.*[4] was implemented and a K-PD [5] an Emax model was introduced to account for Mifepristone kinetic and its effect over IL_{12}. Non parametric bootstrap was performed to calculate the 90% confidence interval of parameter estimates.

III. Internal validation

Visual Predictive Checks (VPCs) were performed: 1000 simulated individuals, for each of the treatment groups (TTO) included in the analysis, were obtained and 5th, 50th and 95th percentiles were calculated and plotted against the observed data.

IV. External validation

The model developed was used to simulate the dosing protocols not included in the analysis (TTO 6 and 7). VPCs were used to evaluate the validity of the model

Berkeley-Madonna, R and NONMEM VII and PsN softwares were used to develop the model

Table I: Summary of the different experimental protocols

TTO	RU Dose (times)	Nº mice	DNA
0	250 (10)	10	2.5
1	250 (10)	31*	1
2	500(10)	12	1
3	125(2)/250(3)/500(3)/1000(3)	21*	1
4	125(1) 250(1) 500(1) 1000(1) 4000(1)	5 5 5 5 5	1
5	125(2)/500(3)/1000(3)/2000(3)	29*	1
6	125(2)/1000(3)/2000(3)/4000(3)	30*	1
7	125(2)/1000(3)/2000(6)	1	1

* IFNγ data available

Results

I. Mathematical Model

$$\frac{dRU}{dt} = DOSE - \beta \times RU$$

$$Ct = IL_{12} + R_{IL12} IL_{12}$$

$$\frac{dCt}{dt} = K_{SIL} \times \left(1 + \frac{SLRU \times RU \times DNA}{(RU + RU50) \times \left(1 + \frac{REG}{IC/1000}\right)} \right) - K_{INT} \times (Ct - IL_{12})$$

$$Rt = R_{IL12} + R_{IL12} IL_{12}$$

$$\frac{dRt}{dt} = K_{SYN\_} - K_{DEG} \times (Rt - Ct + IL_{12}) - K_{INT} \times (Ct - IL_{12})$$

$$IL_{12} = \frac{1}{2} \times \left[(Ct - Rt - K_D) + \sqrt{(Ct - Rt - K_D)^2 + 4 \times Ct \times K_D} \right]$$

$$\frac{dIFN\gamma}{dt} = K_{SIF} \times (R_{IL12} - R_{IL12\_}0) - K_{DIF} \times IFN\gamma$$

$$\frac{dREG}{dt} = K_{REG} \times IFN\gamma - K_{REG} \times REG$$

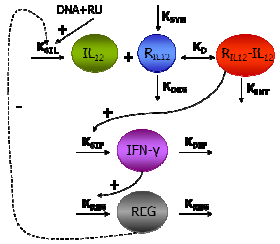

Figure 1. Scheme and mathematical equations of the model. Ct: total amount of IL_{12}; Rt: total amount of IL_{12} receptor; β: RU elimination rate constant; RU50: half stimulatory amount of RU; SLRU: Slope induced by the administration of RU; K_{SIL}: IL_{12} zero order synthesis rate constant; K_{SYN}: zero order receptor synthesis rate constant; K_{DEG}: zero order receptor degradation rate constant; K_D: $R_{IL12}IL_{12}$ dissociation rate constant, K_{INT}: internalization rate constant; K_{SIF}: IFNγ synthesis; K_{DIF}: IFNγ degradation rate constant and K_{REG}: modulator rate constant; IL_0: basal IL_{12} levels; R_{IL12}_0: basal amount of free receptor; R_{IL12} IL_{12}_0: basal amount of bound receptor; RU_0 = IFNγ_0 = REG_0 = 0

Table II Model Parameters

Parameter (units)	Estimate (5th-95th)
IL_{12}_0 (pmol)	0.0043 (0.00294 - 0.00636)
K_D (pmol/day)	0.0451 (0.02089 - 0.1423)
SLRU	130 (62.43 – 404)
K_{SIF} (day-1)	0.0821 (0.04168-0.2441)
K_{DIF} (day^{-1})	1.61 (0.5622-3.193)
K_{DEG} (day^{-1})	6.33 (3.378 - 8.56)
K_{INT} (day^{-1})	1.44 (0.7781 – 7.011)
R_{IL12}_0 (pmol)	1 FIX
IC (pmol)	0.00384 (0.00017-0.00611)
K_{REG} (day^{-1})	1.36x10^{-5} (1.38x10^{-6}-2.09x10^{-5})
β (day^{-1})	3.3 FIX [6A]
RU50(pmol)	71200 (38340 -200100)
Residual Error IL12 (log(ng))	2.25 (1.43-3.42)
Residual Error IFNγ	0.0228 (0.01608-0.02994)

II. Internal Validation

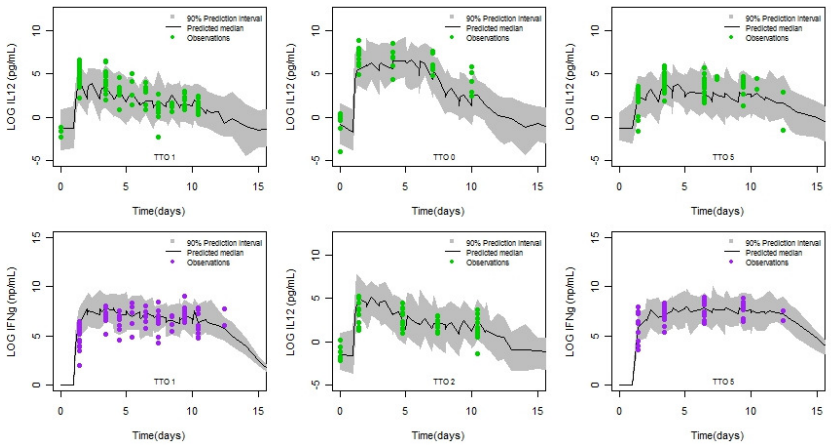

Fig 2. VPCs of some of the studies included in the model development. Grey shadow represents the 90% prediction interval, black line the predicted median and the points corresponds to IFNγ and IL_{12} observations (purple and green respectively)

III. External Validation

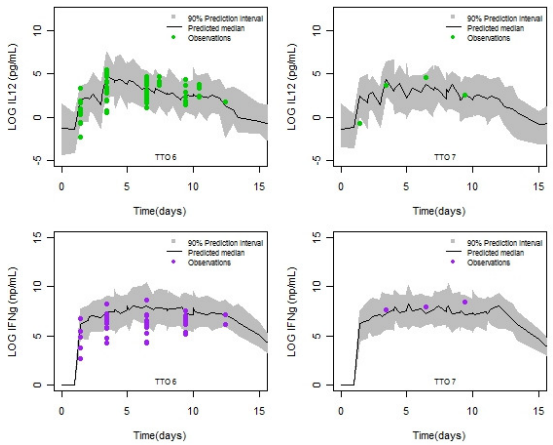

Fig 3. VPCs of the studies not included in the model development. Grey shadow represents the 90% prediction interval, black line the predicted median and the points corresponds to IFNγ and IL_{12} observations (purple and green respectively)

Conclusions

A kinetic- pharmacodynamic model able to describe jointly the IL_{12} and IFNγ profiles has been developed by introducing the target mediated drug disposition quasi-equilibrium model to account for the observed dose dependent disposition of IL_{12}.

The different experimental protocols with increasing doses of RU were satisfactorily described by incorporating a monoexponential decay of RU and an Emax model to describe its effect over IL_{12} gene expression.

References

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