

A NonLinear Mixed Effect model of the LNA-i-miR-221 kinetics in rats for predicting key parameters of human kinetics via allometric scaling

Hadija Marchiori¹, Michele Schiavon¹, Chiara Roversi², Simone Zannoni², Massimiliano Fonsi³, Maria Teresa di Martino⁴, Alessia Tagliavini², and Chiara Dalla Man¹

¹Department of Information Engineering, University of Padova, Padova, Italy;

² Clinical Development and translational medicine, Aptuit an Evotec Company, Verona, Italy;

³ADME & Developability Assessment, Evotec, Toulouse, France;

⁴Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro, Italy

BACKGROUND AND AIM

MicroRNAs (miRs) are small non-coding regions of single-stranded RNA that can impact gene and protein expression, regulating many biological pathways, some of which are disrupted in **cancer cells**. Recently, **miR-221** has been investigated, both for its oncogenic potential and as a promising target of RNA-based therapies [1]. Its **inhibition** can be performed by the antisense oligonucleotide **LNA-i-miR-221** that has shown a favorable toxicokinetic profile and no organ-related toxicity in rats and monkeys [2].

AIM: To develop a NonLinear Mixed Effects (NLME) model, able to describe the pharmacokinetics of LNA-i-miR-221 in rats, and use it to predict key parameters of human kinetics via different allometric scaling approaches.

DATASET

ANIMALS

- Subjects:** 42 Sprague Dawley rats, 21 females and 21 males, age = 6 ± 1 weeks, mean weight = 0.256 Kg at the beginning of the study.
- Protocol:** two administration cycles, each lasting 4 days, interspersed with a 10-day washout period. A bolus of LNA-i-miR-221 was intravenously injected at the beginning of days 1, 2, 3, 4, 15, 16, 17, and 18. Blood samples were collected both after each administration and after the end of the administration cycles.

HUMANS

- Subjects:** Progressive cancer patients (aged ≥ 18 years) with ECOG 0–2 were enrolled into 5 cohorts.
- Protocol:** 30-min IV infusion of LNA-i-miR-221 on 4 consecutive days, followed by an observation period [3].

METHODS

MODEL DEVELOPMENT

- Structural model:** The model structure is represented in Fig. 1. It assumes a **delayed** (T_{lag}) **three-compartment kinetics**. The central compartment (Q_1) represents both the site of drug injection (i.e. plasma) and the elimination organs (i.e. liver and kidneys), whereas the second compartment (Q_2) represents the organs exhibiting high concentrations of LNA-i-miR-221 (i.e. bone marrow, adipocytes, and lymph nodes). The third compartment (Q_3) is representative of the remaining organs and tissues reached by LNA-i-miR-221. The volume of each compartment is annotated using V_i . The flux between Q_1 and Q_2 is defined as BCL_2 whereas the flux between Q_1 and Q_3 is defined as BCL_3 .

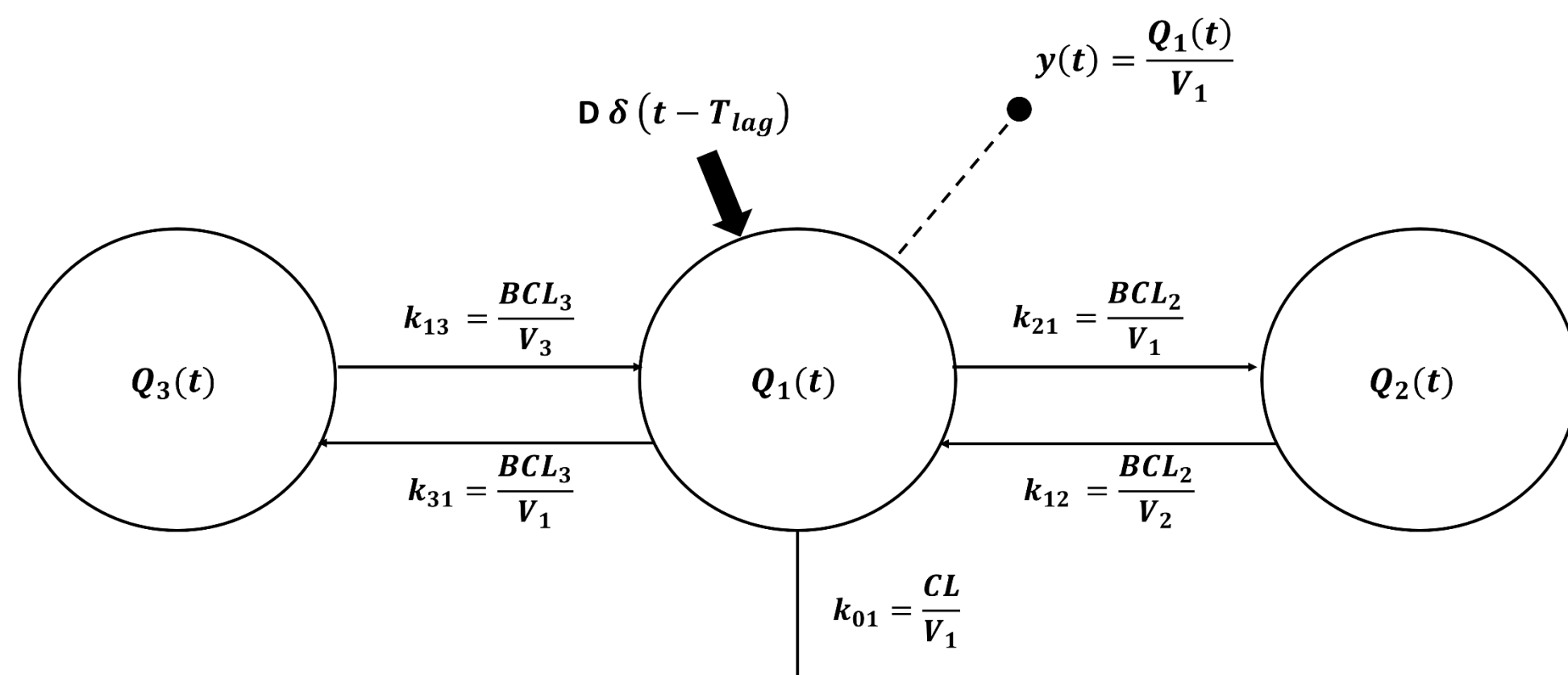


Fig. 1: Schematic representation of the structural model. Circles represents kinetic compartments of the model. Continuous arrows are fluxes of substances. The dashed line ending with a dot identifies the site of measurement.

- Stochastic model:** The distribution of each parameter was assumed to be **log-normal**, with covariance matrix Ω , whose diagonal element ω_{ii}^2 represents the variance associated to the i -th parameter. Off-diagonal correlations between CL and V_2 (ρ_{CL,V_2}), CL and BCL_3 (ρ_{CL,BCL_3}), and V_2 and BCL_3 (ρ_{V_2,BCL_3}), were included in Ω . No random effects were associated with the time delay parameter T_{lag} .
- Error model:** Standard deviation of the residual unexplained variability was assumed to be the sum of a constant (a) and a proportional (through parameter b) component.

IDENTIFICATION STRATEGY

Model identification was performed in Monolix (version 2023R1, Lixoft, Antony, France) using the **stochastic approximation of expectation maximization** for parameter estimation.

ALLOMETRIC SCALING

Several **fixed exponent** (β) allometric scaling methods were applied to the scaling of clearance and distribution volume [4][5]. **Human data** were used to evaluate the performance by comparing the predicted pharmacokinetics variables with the observed human parameters through the **fold error**, defined as the ratio between predicted parameter and its observed value, obtained applying compartmental analysis methods on [3]. The difference between the scaled and observed parameter was assessed using the Wilcoxon test: if the difference between the two was not statistically significant, the allometric scaling method was considered suitable for its intended purpose.

RESULTS

Tab. 1 shows the results of model identification. Parameter estimates were **physiologically plausible** and estimated with **good precision** (max RSE=48.2%).

Parameter		Value	RSE [%]
Name	Unit		
Fixed Effects			
T_{lag}	h	0.04	22.2
CL	L/h	0.11	7.26
V_1	L	0.047	8.60
BCL_2	L/h	0.22	15.1
V_2	L	0.15	8.99
BCL_3	L/h	0.03	8.59
V_3	L	1.85	27.3
Standard deviation of the random effects			
ω_{CL}	dimensionless	0.38 (39%)	12.8
ω_{V_1}	dimensionless	0.27 (28%)	18.1
ω_{BCL_2}	dimensionless	0.13 (13%)	48.2
ω_{V_2}	dimensionless	0.43 (45%)	16.0
ω_{BCL_3}	dimensionless	0.32 (33%)	37.2
ω_{V_3}	dimensionless	0.61 (66%)	37.3
Correlation			
ρ_{CL,V_2}	dimensionless	0.87	8.79
ρ_{CL,BCL_3}	dimensionless	0.8	17.3
ρ_{V_2,BCL_3}	dimensionless	0.86	14.0
Error model parameters			
b	dimensionless	0.24	4.77
a	ng/mL	3.51	37.5

Tab. 1: Parameter estimates. RSE = Relative Standard Error. The Coefficient of Variation (CV%) is reported into brackets if available.

Tab.2 shows, for each pharmacokinetic parameter of interest, the best prediction, the fold error, and the scaling method used.

Parameter		Prediction	Fold Error	p-value	Method
Name	Unit				
CL	L/h	14.8	1.07	0.28	Simple allometry $\beta = 0.85$
V_1	L	4.75	1.51	E-10	Simple allometry $\beta = 0.8$
V_2	L	15.0	1.08	0.09	Simple allometry $\beta = 0.8$
V_3	L	186	2.87	E-13	Simple allometry $\beta = 0.8$
V	L	205	2.5	E-13	Simple allometry $\beta = 0.8$

Tab. 2: Best estimate for each pharmacokinetic parameter. The text is highlighted in bold if the fold error is not significantly different from one.

- Scaling of **clearance was successful**, as a statistically reliable scaling algorithm was identified.
- Among the **volume** terms, only V_2 was successfully scaled. Therefore, it was inferred that predicting the distribution volume with single species allometric scaling algorithms is overall **not satisfactory**.
- However, it is important to note that all the predictions computed using a fixed exponent simple allometry with $\beta = 0.8$ lay in the 3-fold error interval.

RESULTS: Using fixed-exponent single species allometric scaling algorithms, good results were obtained for the scaling of the clearance, whereas predictions of the distribution volumes were inaccurate.

CONCLUSIONS

A **delayed three-compartment model** describing the LNA-imir-221 kinetics in Sprague Dawley rats plasma following an IV injection has been developed in a NLME framework. Furthermore, this study indicates that single-species allometric scaling is a robust method for the prediction of **humans clearance** from rat data, whereas predictions of the volumes of distribution likely requires multiple species data.

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