Impact of concentrations below the limit of quantification on pharmacodynamic predictions. A preclinical example, using new features of NONMEM VI.

Celine Dartois, Jean-Louis Steimer, Mick Looby and Colin Pillai

Novartis, Modeling and Simulation

Muncie, May 19, 2009

NOVARTIS

Study objective

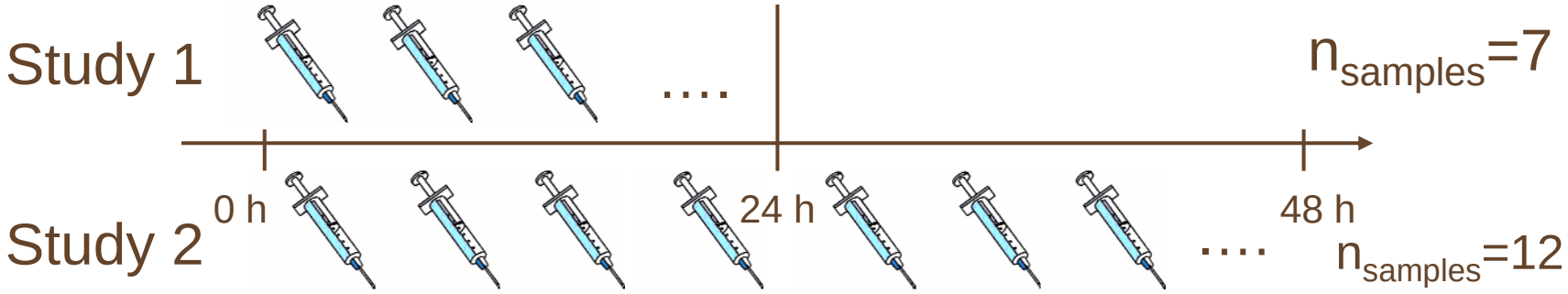
- To compare 2 drugs, a lead compound and its backup, on a pharmacodynamic endpoint based on their relative potency in animals
- These 2 drugs are from the same therapeutic class
- PK and PD collected for the two drugs
- The pharmacodynamic endpoint studied is a biomarker activity inhibition

Design

PK and PD data

- Dose levels, time collections, number of animals

	Lead					Backup			
Dose mg/kg	0.1	0.3	1	2	10	2	10	30	100
Study 1				N=4 animals					
Study 2	N= 6 animals				N= 6 animals		N= 6 animals		

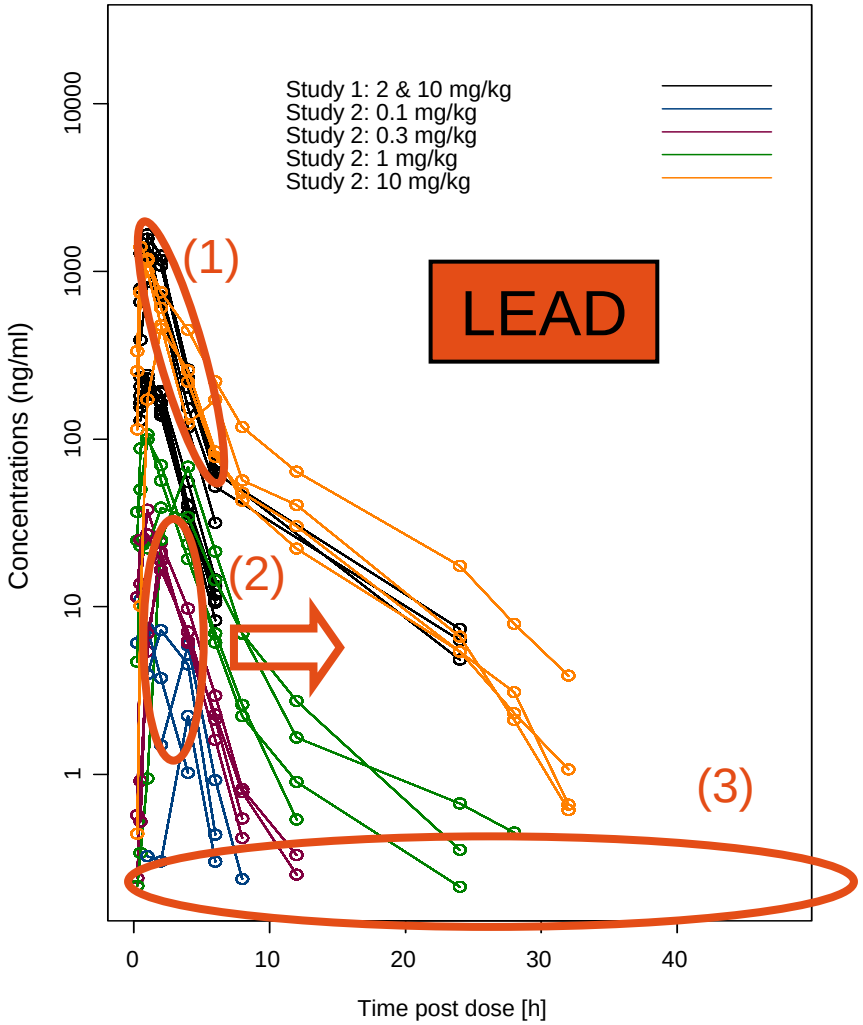


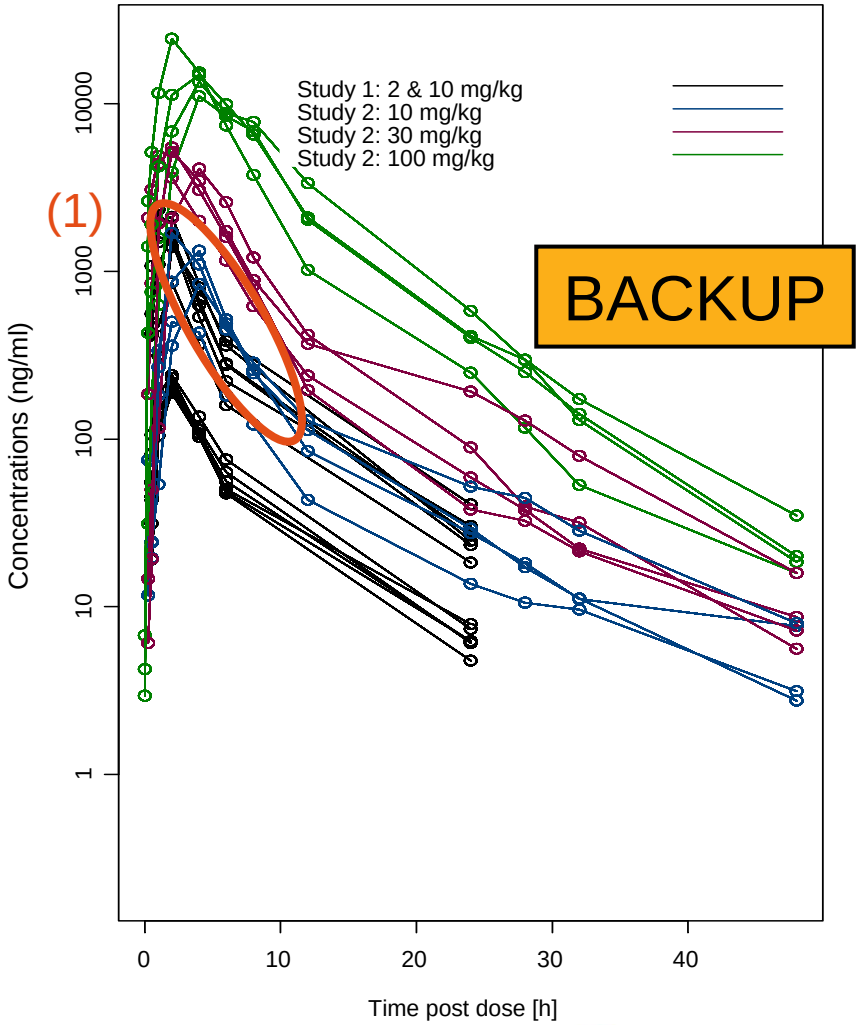
PK collected at the same time as PD

NOVARTIS

PK data

Exploratory analysis



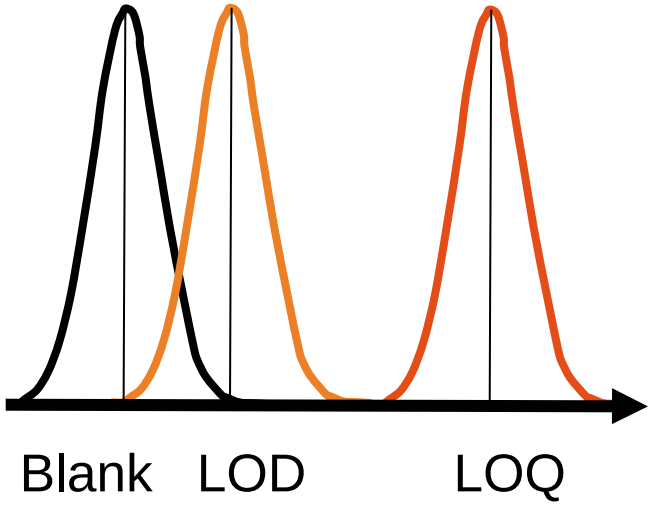


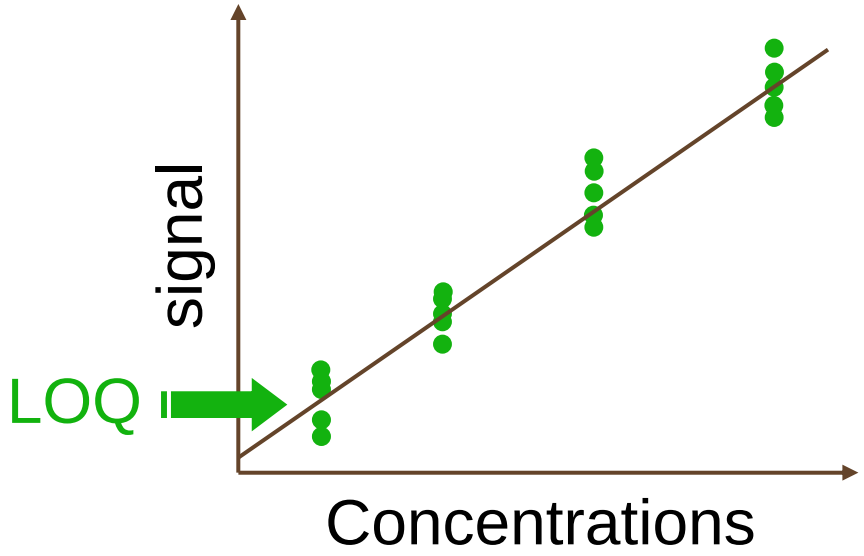
NOVARTIS

PK data

limit of quantification (LOQ) definition

- LOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision.*
- Should be established using at least five samples independent of standards and determining the coefficient of variation and/or appropriate confidence interval (<20 %) *





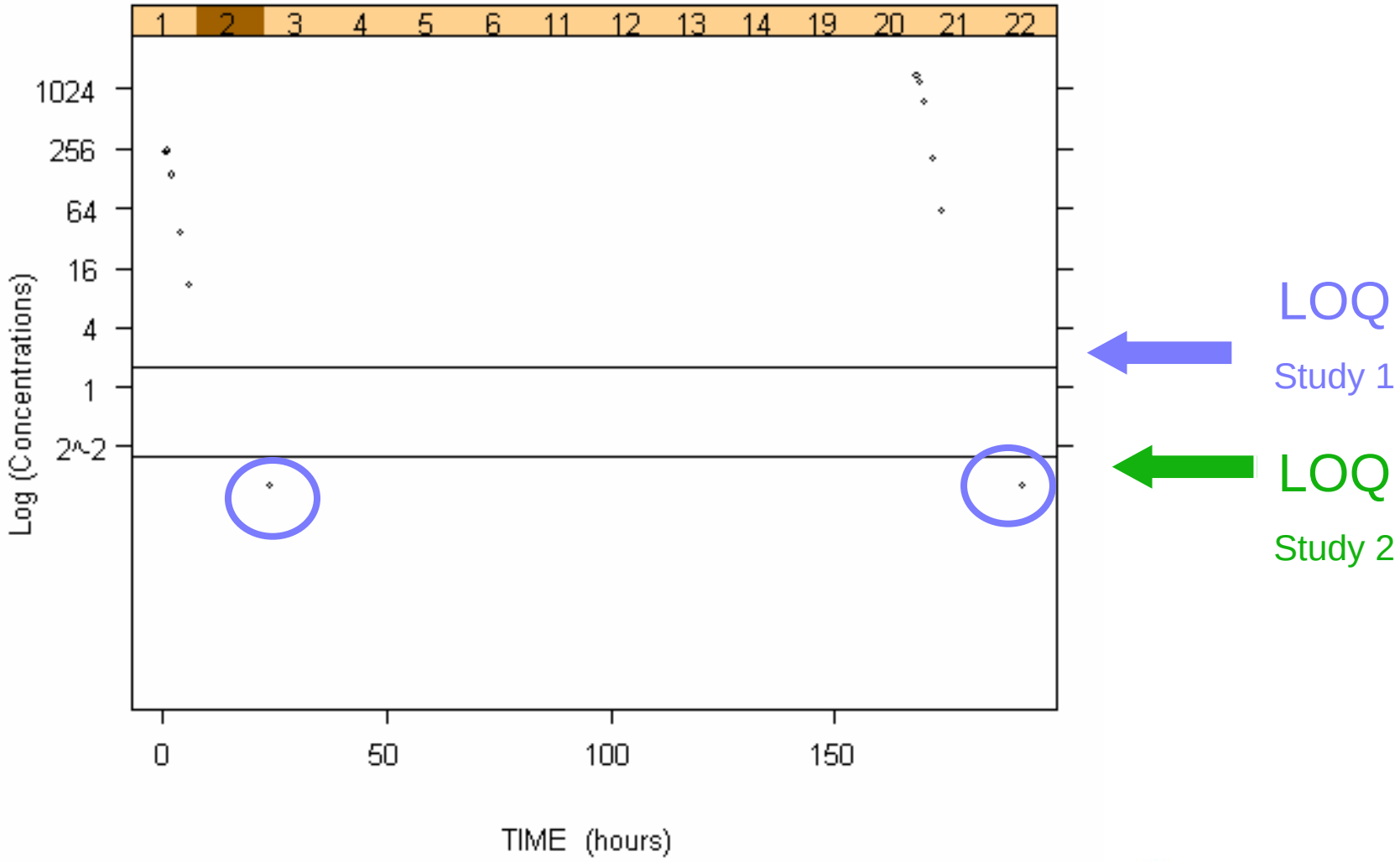
* *Source: FDA guidance.* Bioanalytical Method Validation

NOVARTIS

PK data

Exploratory analysis – data below the limit of quantification (BLQ)

LEAD

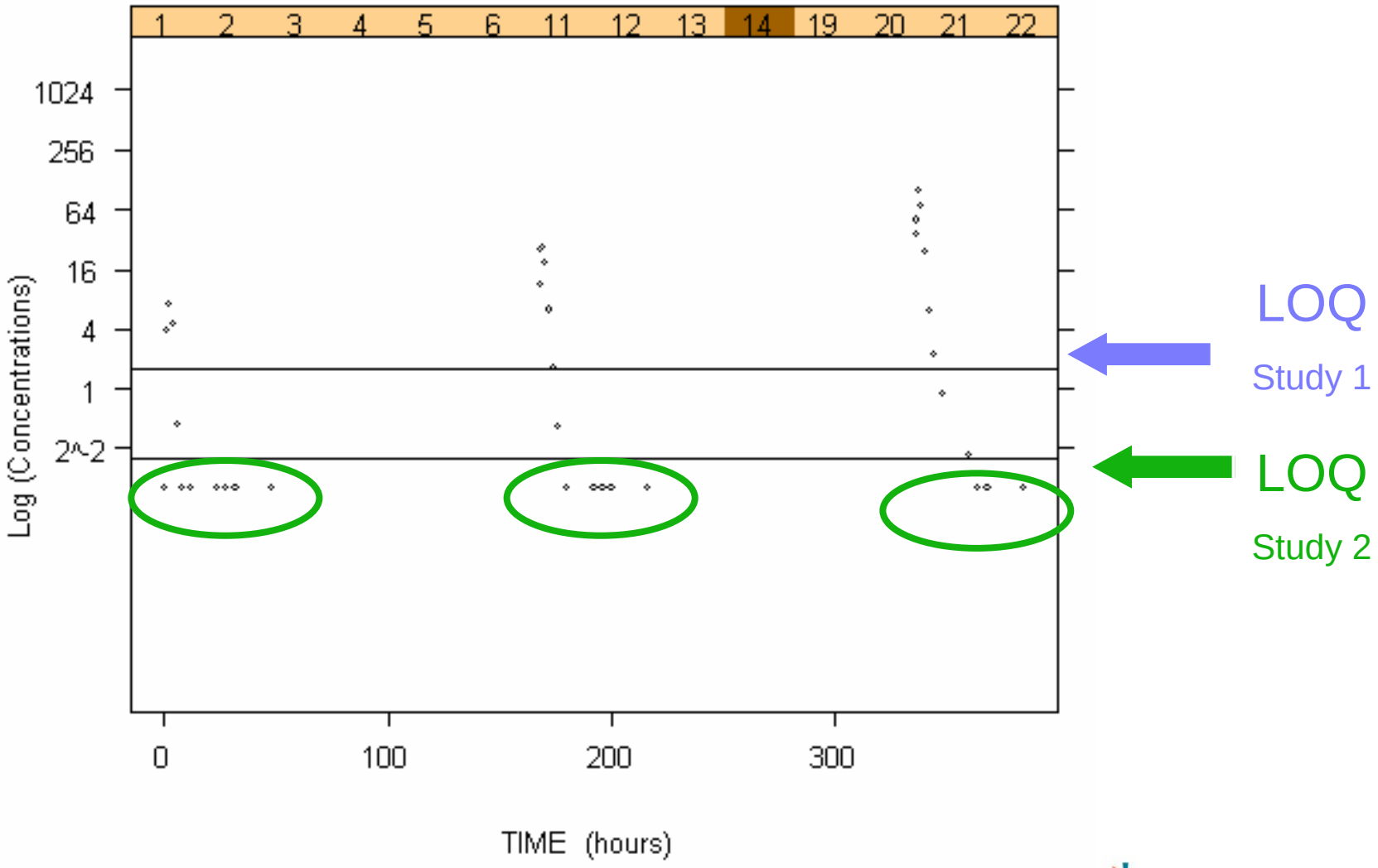


NOVARTIS

PK data

Exploratory analysis – data below the limit of quantification (BLQ)

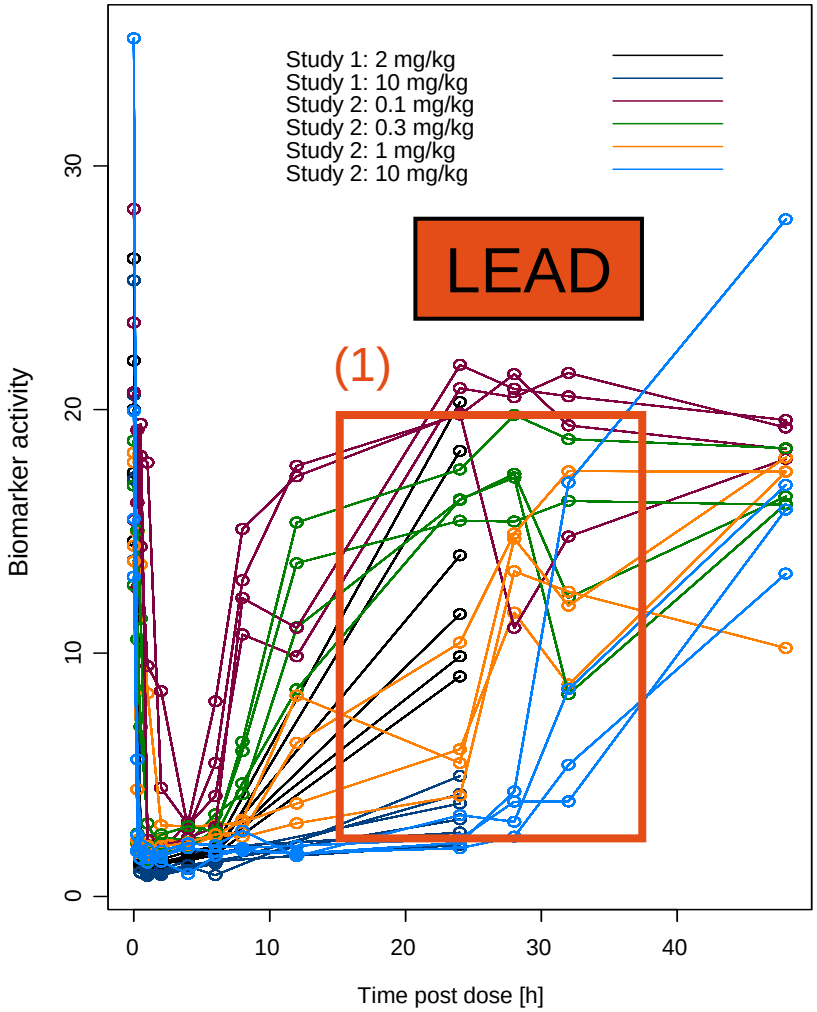
LEAD

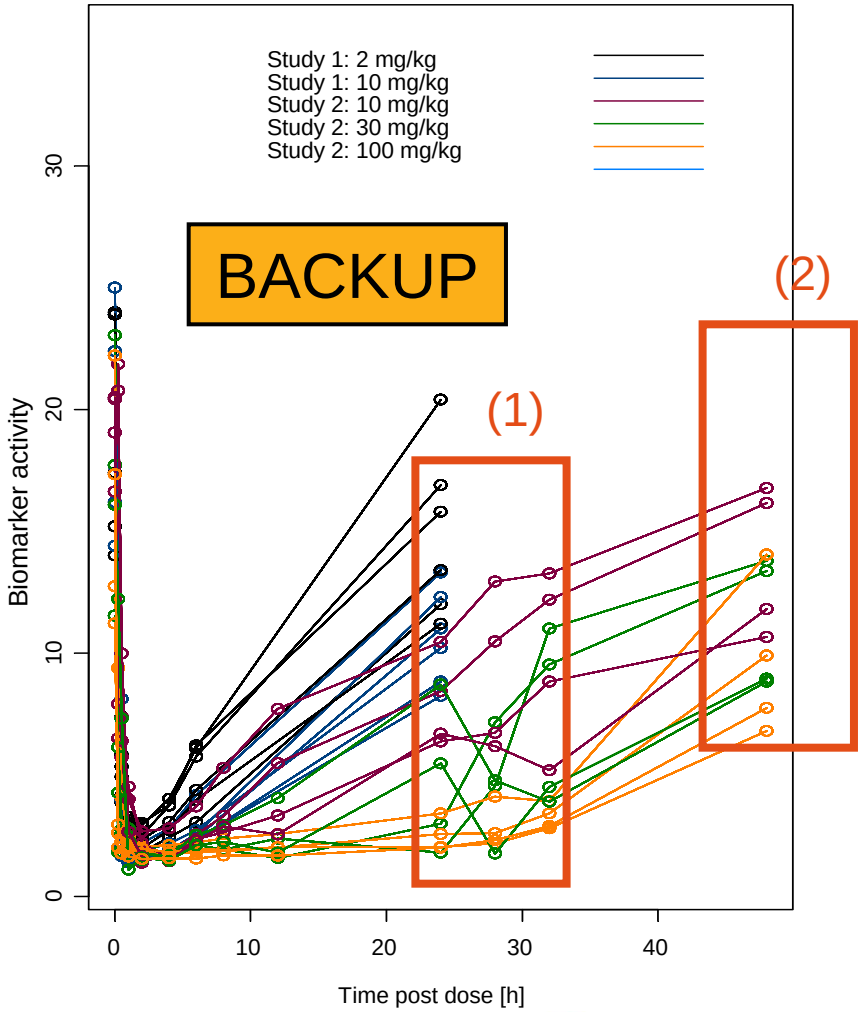


NOVARTIS

PD data

Exploratory analysis

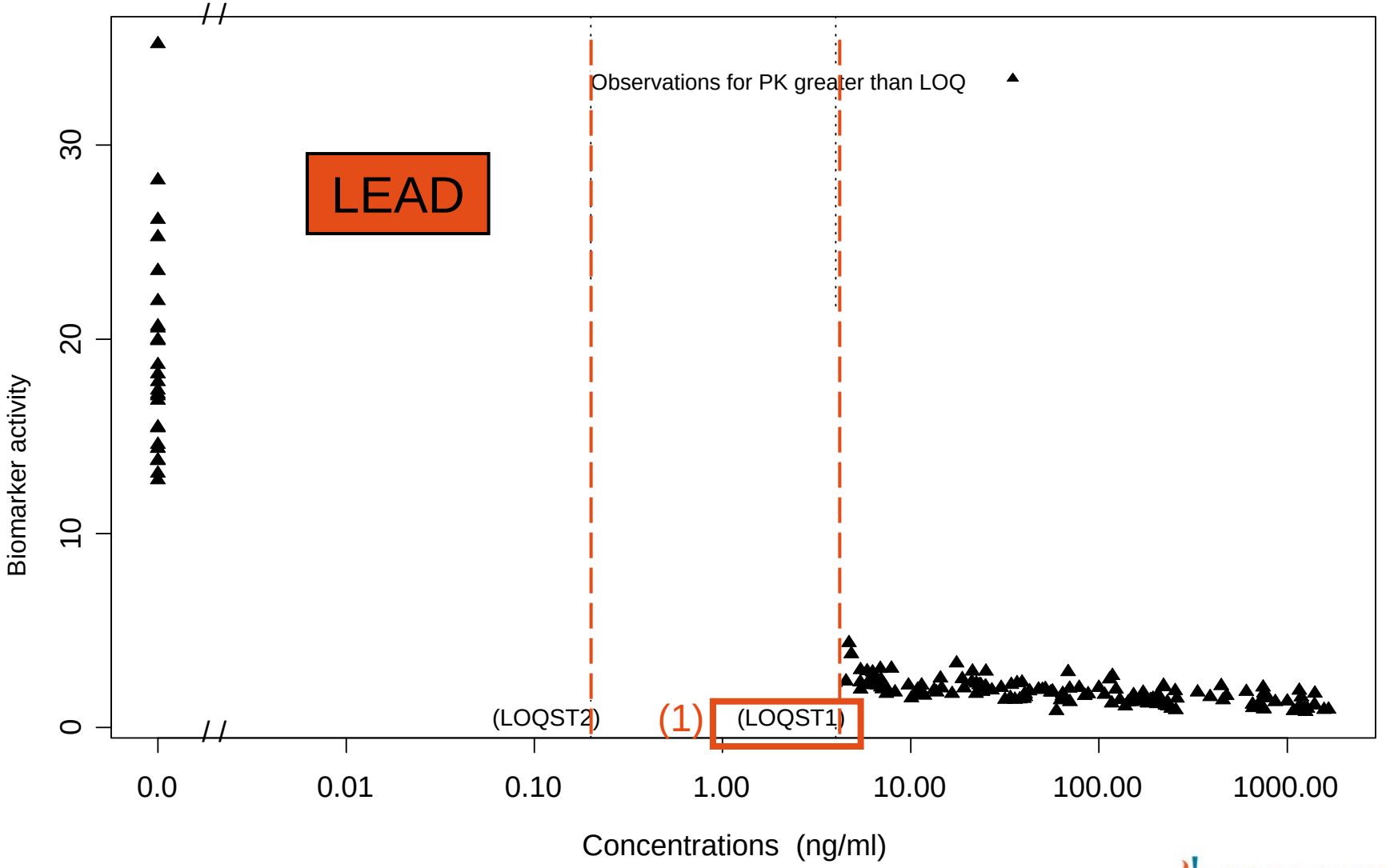




NOVARTIS

PKPD data

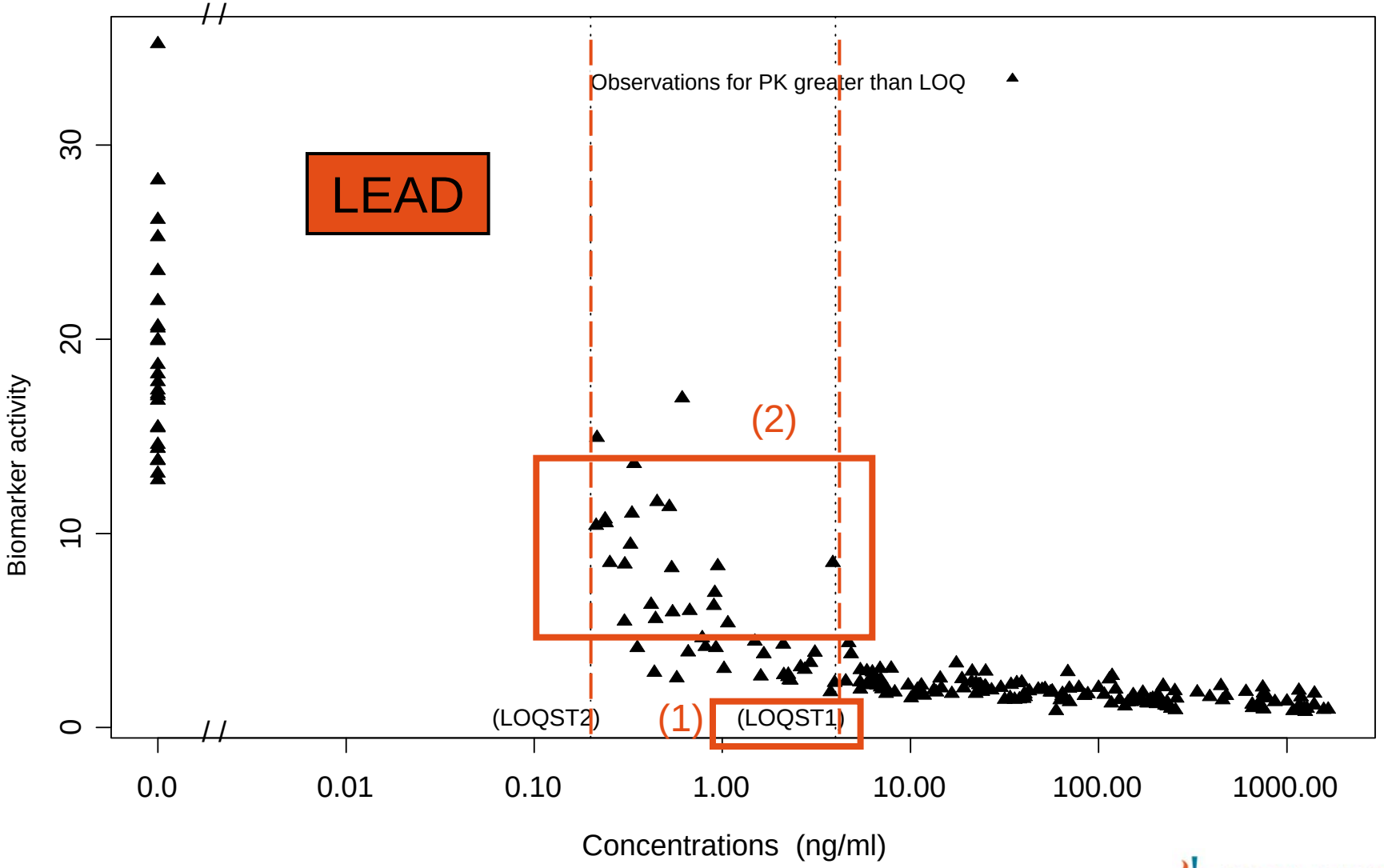
Exploratory analysis



NOVARTIS

PKPD data

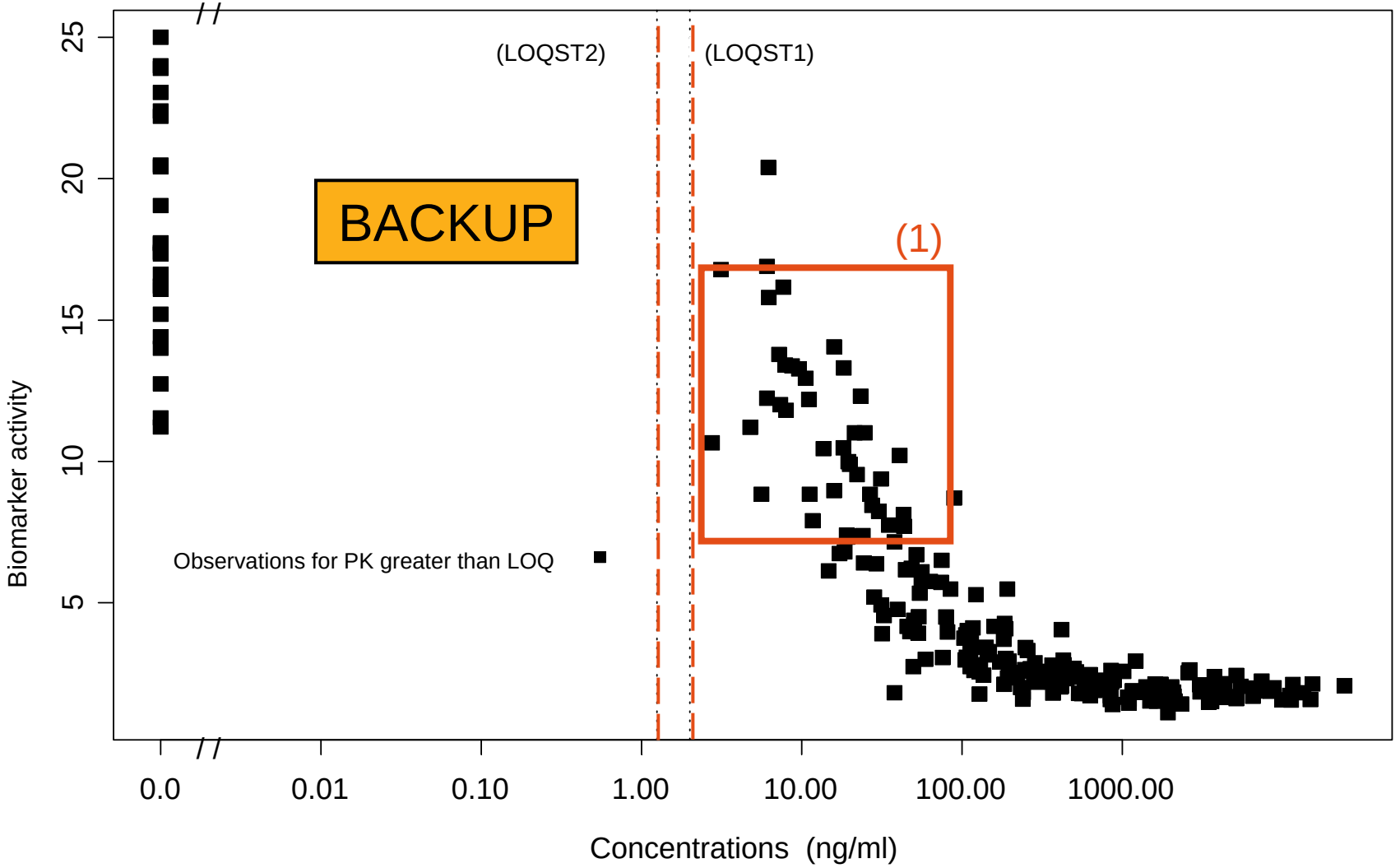
Exploratory analysis



NOVARTIS

PKPD data

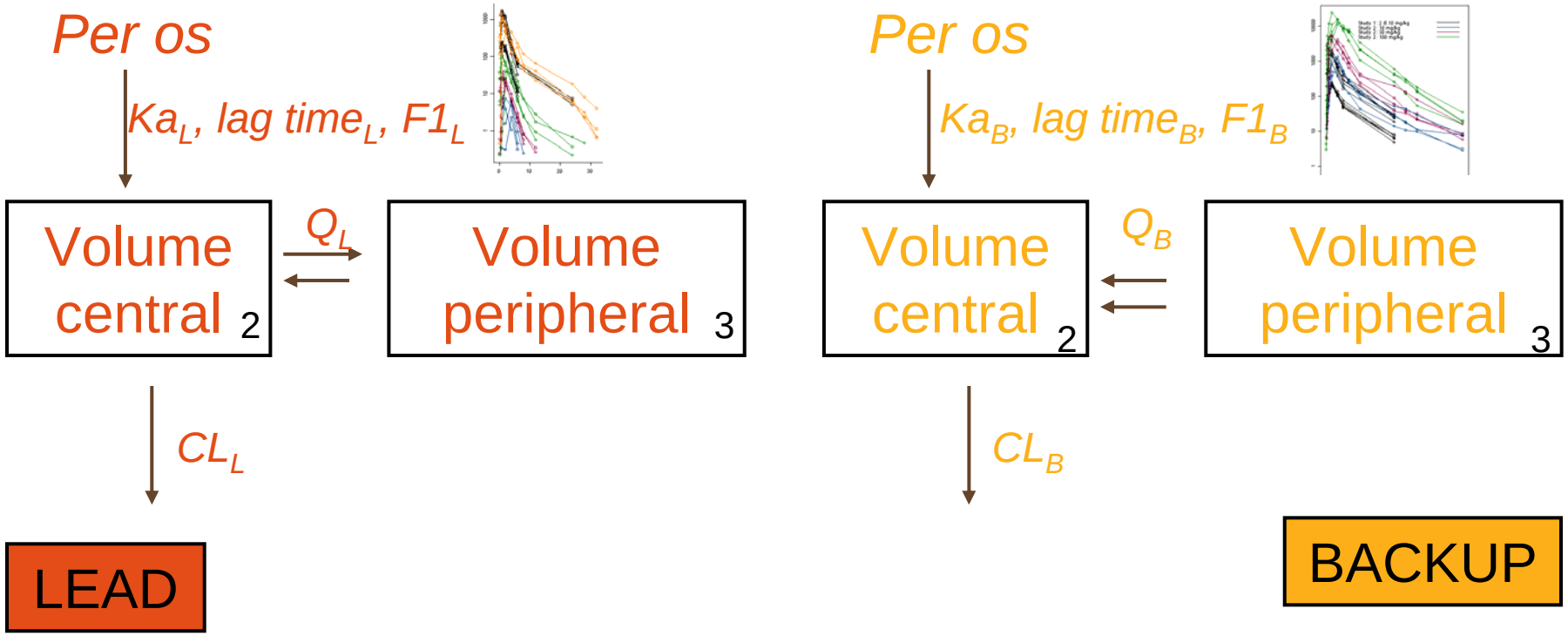
Exploratory analysis



NOVARTIS

Method

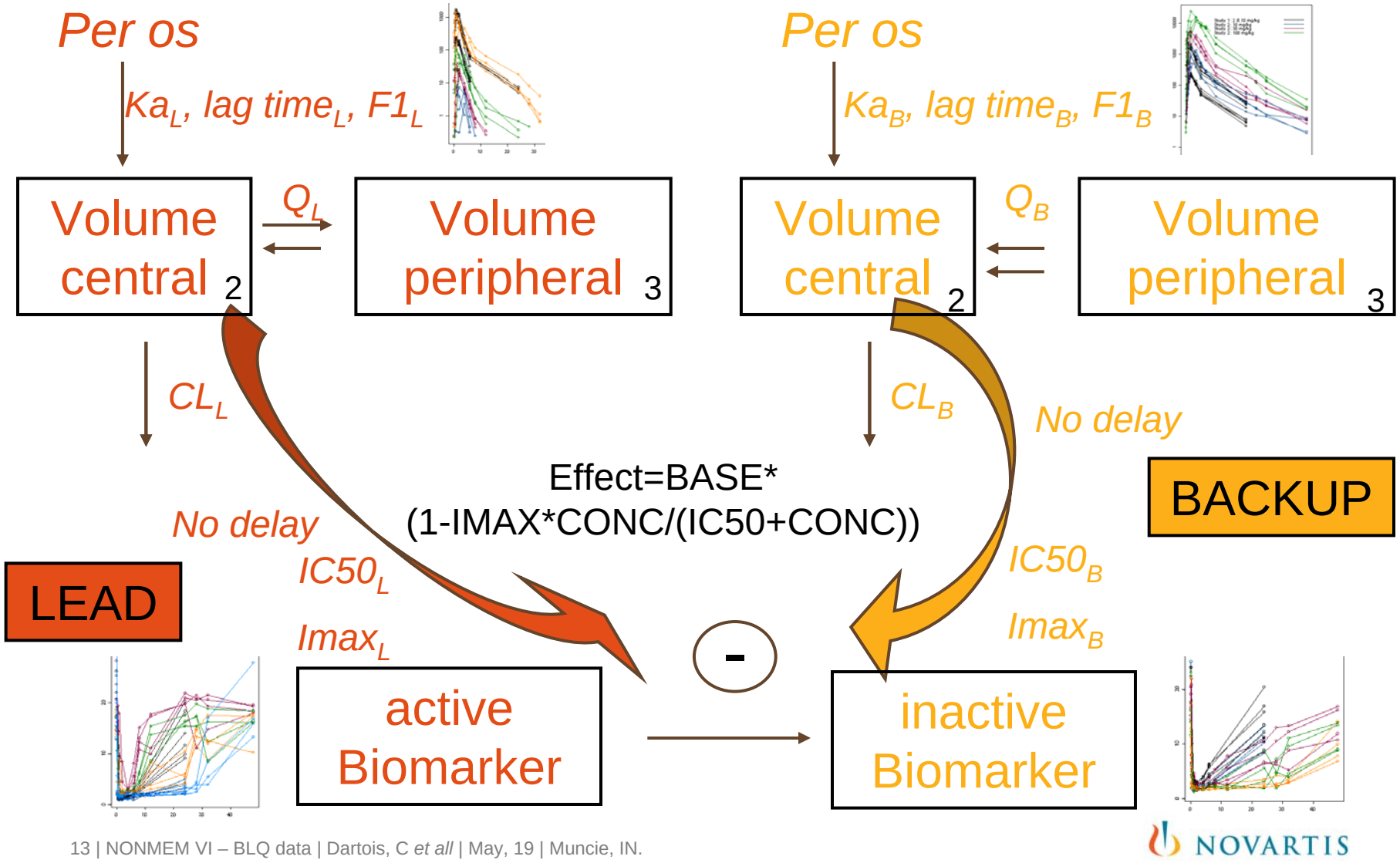
model structure




```
DADT(1)= -KA*A(1)
DADT(2)= KA*A(1)-K23*A(2)+K32*A(3)-K*A(2)
DADT(3)= K23*A(2)-K32*A(3)
```

Method

model structure



NOVARTIS

Method

Modeling approach

- Sequential PKPD
  - A lot of data below the LOQ in PK for the LEAD (75/292), only two for the backup
  - A few PK data in IC50 region
  - Noisy PD data in the recovery phase
- PK estimation
  - Handling the BLQ data

Method

*Modeling approach – BLQ data**

- Method 1 (M1): Discard BLQ observations and apply extended least squares to the remaining observations.
- Method 2 (M2): Discard BLQ observations and apply the method of maximum conditional likelihood to the remaining observations.
- Method 3 (M3): Maximize the likelihood for all the data treating BLQ observation as censored.
- Method 4 (M4): Like M3 but the likelihoods for data above and below the LOQ are conditioned on the observations being greater than zero.
- Method 5 (M5): Replace BLQ observations with LOQ/2 and apply extended least squares estimation.
- Method 6 (M6): Replace first BLQ observation with LOQ/2 and discard the rest of them as in M1.
- Method 7 (M7): Replace first BLQ observation with 0 and discard the rest of them.

**source: Beal. JPP, 2001,28(5):481-504*

Method

*Modeling approach – BLQ data**

- Method 1 (M1): Discard BLQ observations and apply extended least squares to the remaining observations.
  - The lower of the remaining observations misrepresent the true lower concentrations; the lower remaining observations are selectively too high.*

**source: Beal. (2001) and Ahn et al (2008)*

Method

*Modeling approach – BLQ data**

- Method 5 (M5): Replace BLQ observations with LOQ/2 and apply extended least squares estimation.
- Method 6 (M6): Replace first BLQ observation with LOQ/2 and discard the rest of them as in M1.
  - Replacement withtout real justification, arbitrary. May yield greater bias and imprecision in estimates for IIV. Sampling schedule important for performance of LOQ/2 substitution.*

**source: Beal. (2001) and Ahn et al (2008)*

NOVARTIS

Method

*Modeling approach – BLQ data**

- Method 7 (M7): Replace first BLQ observation with 0 and discard the rest of them.
  - Replacement is always too low, induces the greatest bias *

**source: Beal. (2001) and Ahn et al (2008)*

NOVARTIS

Method

*Modeling approach – BLQ data**

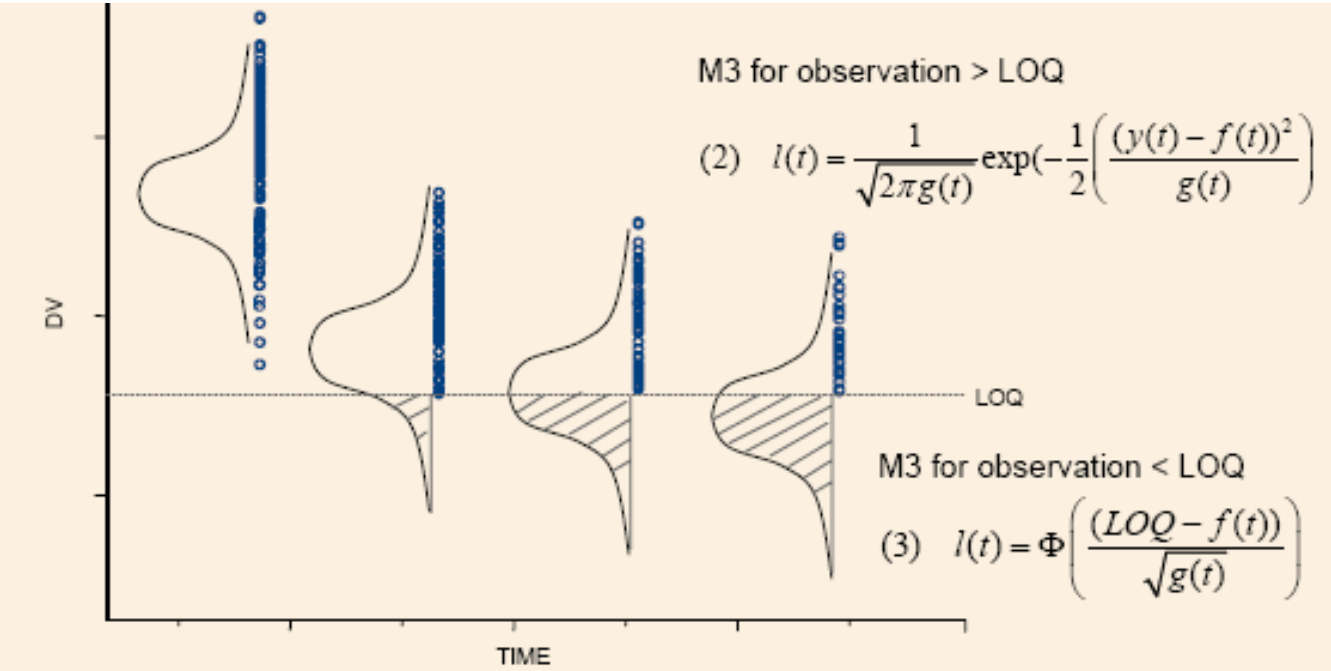
- Method 2 (M2): Discard BLQ observations and apply the method of maximum conditional likelihood to the remaining observations.
  - When time of BLQ data not available, the best method.*
- Method 3 (M3): Maximize the likelihood for all the data treating BLQ observation as censored.
  - Data above the LOQ (extended least square method)
  - Data below the LOQ (integrating density function from –inf to LOQ*
- Method 4 (M4): Like M3 but the likelihoods for data above and below the LOQ are conditioned on the observations being greater than zero.
  - No much superiority despite its complexity and longer computation time.*

**source: Beal. (2001) and Ahn et al (2008)*

Method

Modeling approach

- NONMEM VI M3 method *



**Source: Bergstrand et al, PAGE 2007.*

NOVARTIS

NONMEM version VI

Method M3

- NONMEM VI has a new functionality to simplify simultaneous modelling of continuous and categorical data
- Likelihood of data above/below the LOQ is included in the calculation of the objective function
- Laplacian method should be used
- A F_FLAG = indicator variable whether Y (or F) should be set to a prediction or a likelihood
  - Prediction: F_FLAG=0 (default)
  - Likelihood: F_FLAG=1
- For data above LOQ – continuous data – log transformed

```
IF (TYPE.EQ.0) THEN
   F_FLAG=0
   Y=LOG(F)+ERR(1)
ENDIF
```

Source: Ahn et al (2008)

NONMEM version VI

Method M3 - code

- For data below LOQ – categorical data - approximation of the cumulative normal density

```
IPRED=LOG(F)
DUM=(LLOQ-IPRED)/SIG
ARG=ABS(DUM)
W1=0.39894228
W2=0.2316419
B1=1.330274429
B2=-1.821255978
B3=1.781477937
B4=-0.356563782
B5=0.319381530
AA=EXP(-0.5*ARG**2)
R=1./(1.+W2*ARG)
AUC=((((B1*R+B2)*R+B3)*R+B4)*R+B5)*R
PHITL=AA*AUC*W1
```

```
IF (DUM.LT.0) CUMD=PHITL
IF (DUM.GT.0) CUMD=1-PHITL
IF (DUM.EQ.0) CUMD=0.5

IF (TYPE.EQ.1) THEN
     F_FLAG=1
     Y=CUMD
ENDIF
```

Source: Ahn et al (2008)

NOVARTIS

Method

Modeling approach

- PK estimation
  - Log transformation of the data, add a lag time
  - NONMEM VI ® - F_FLAG – best model
    - IIV and IOV on Ka and F1
  - Winbugs ® - Upper – Lower
    - each occasion = a different animal
- PD estimation
  - Same baseline
  - Different Imax for the two drugs
  - IC50 ratio estimated
  - Error model different for each study

Results

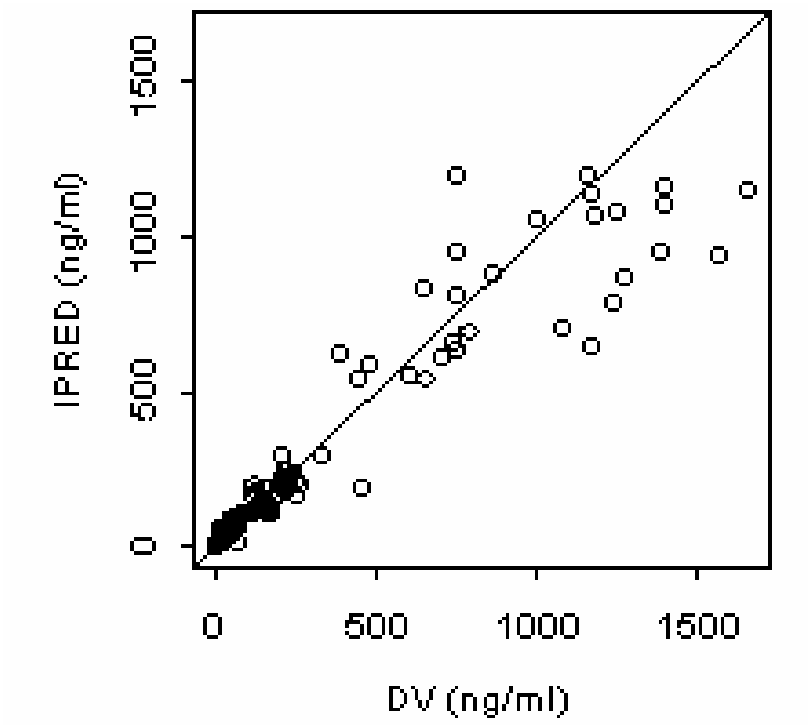
PK goodness of fit

LEAD

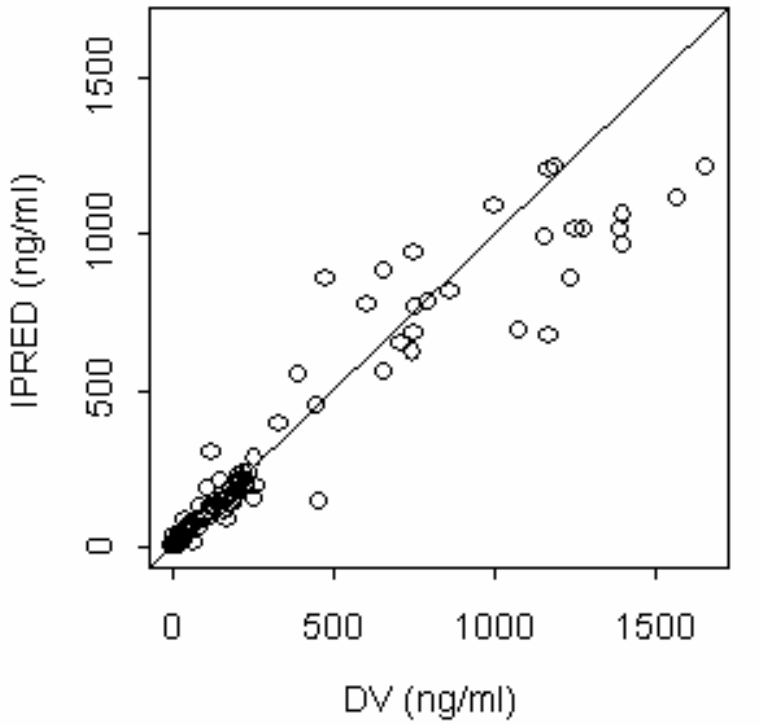
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



NOVARTIS

Results

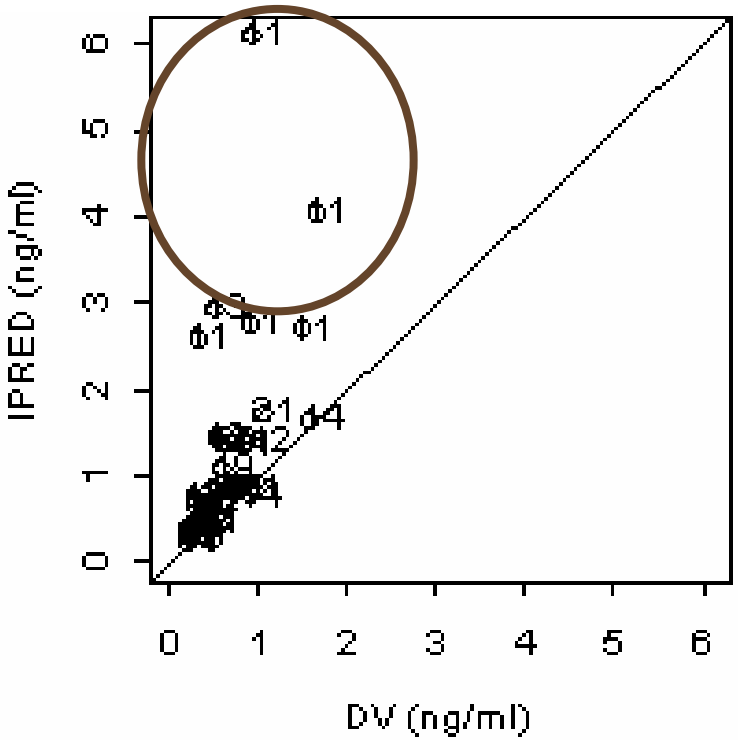
LEAD

PK goodness of fit – observed data <= 2 ng/ml (IC 50 region)

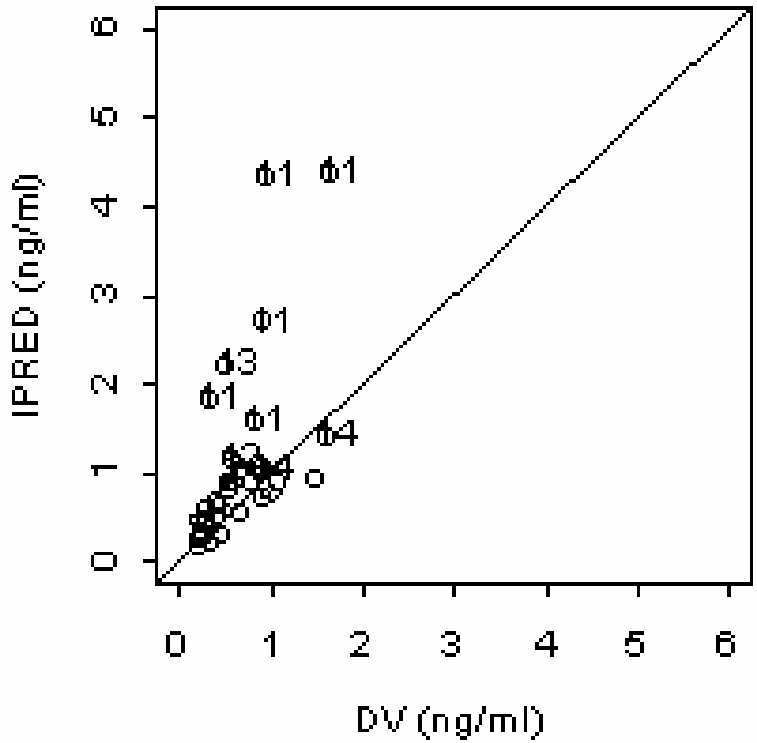
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



NOVARTIS

Results

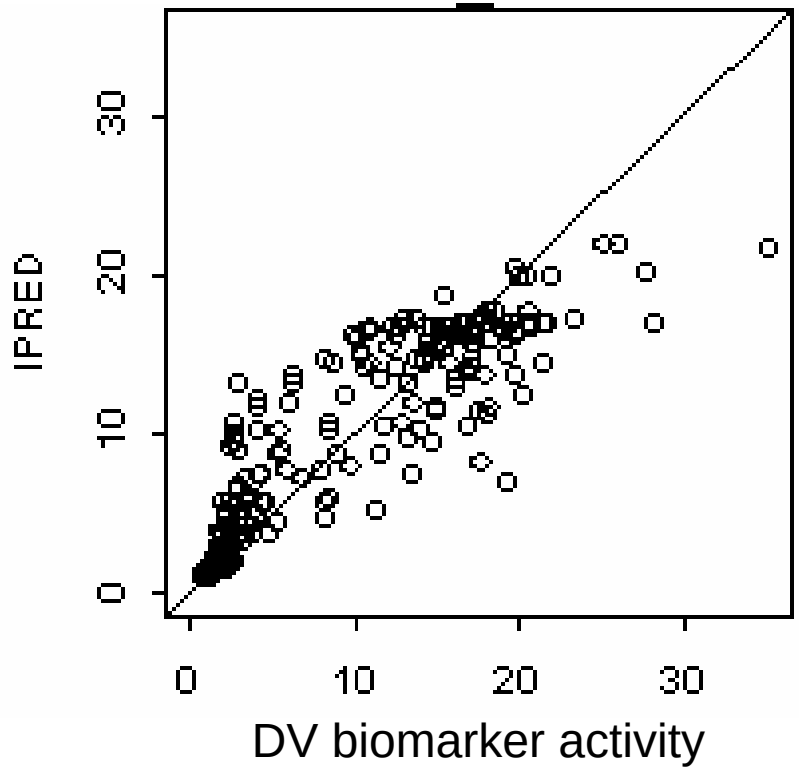
PD – goodness of fit

LEAD

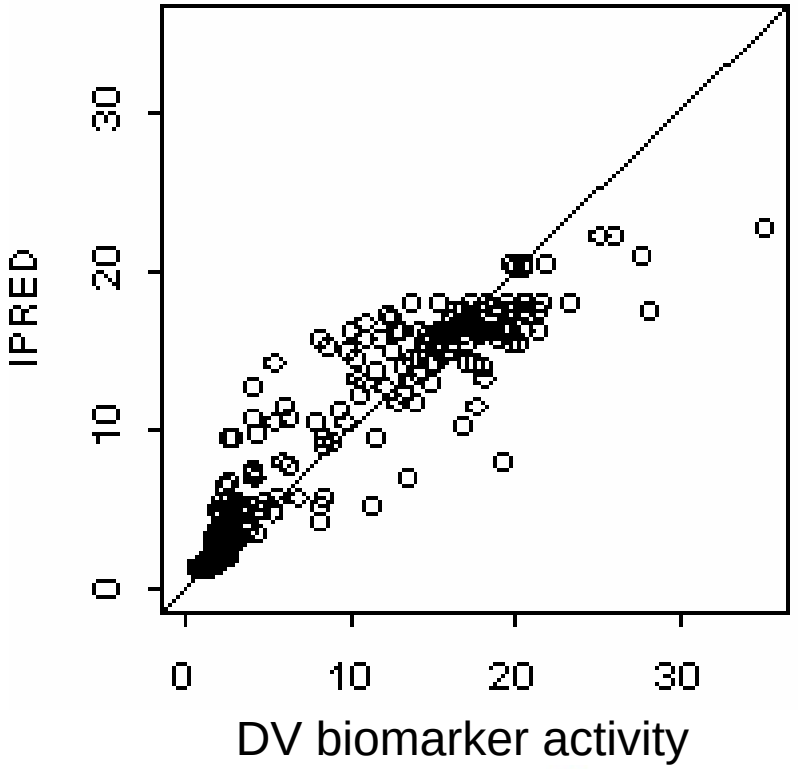
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



NOVARTIS

Results

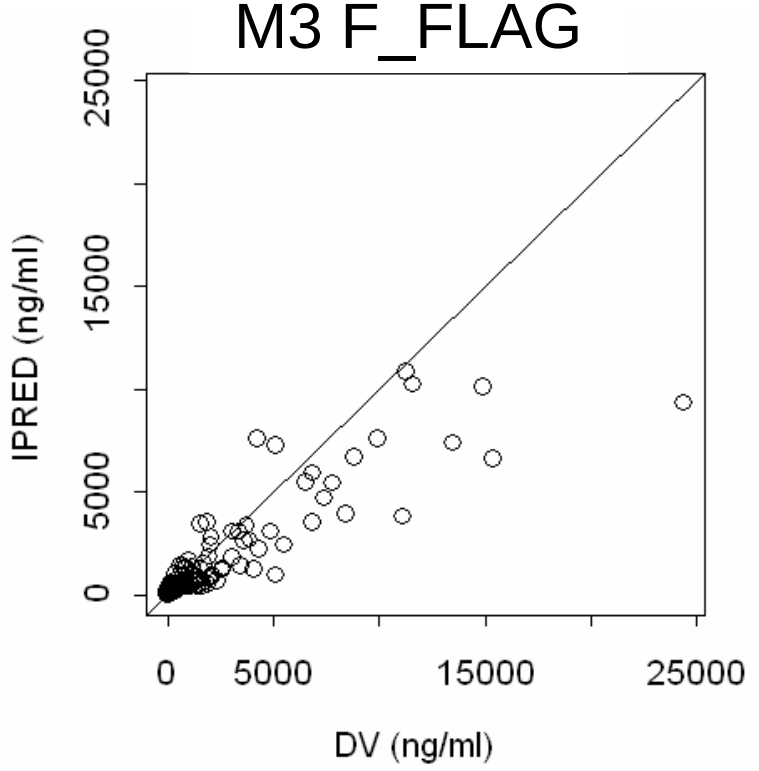
PK goodness of fit

BACKUP

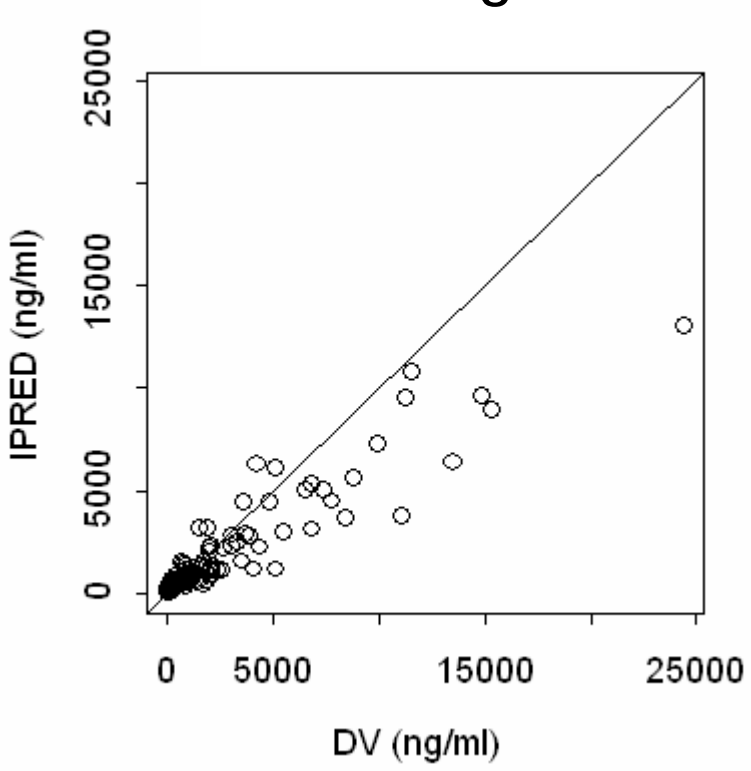
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



NOVARTIS

Results

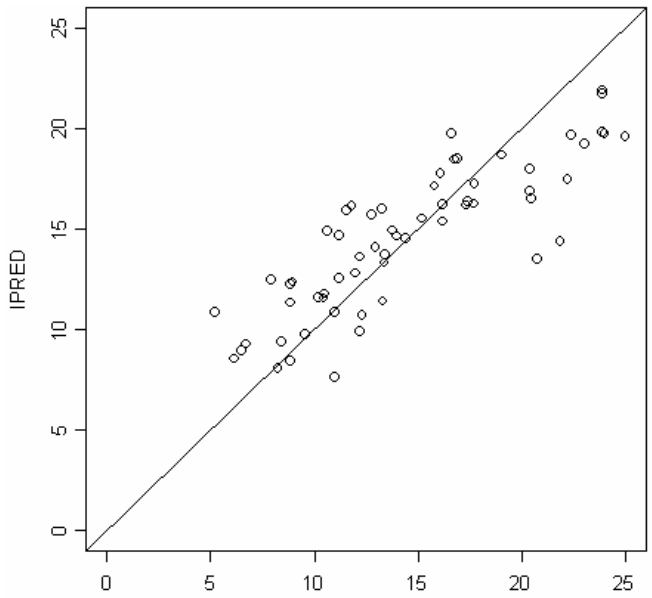
PD – goodness of fit

BACKUP

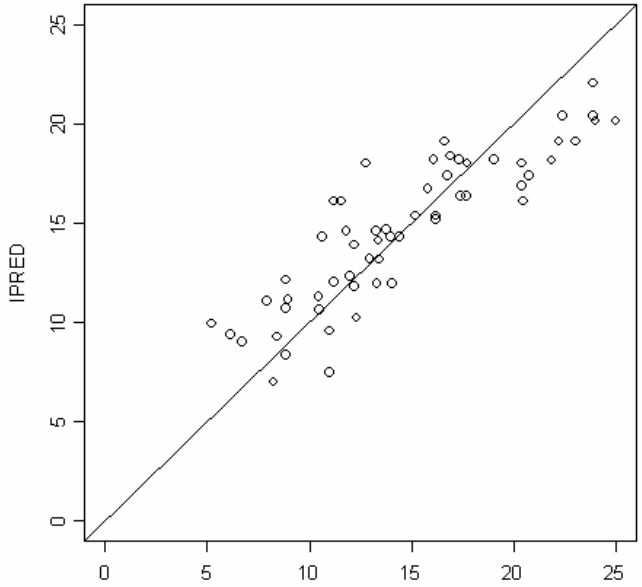
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



NOVARTIS

Results

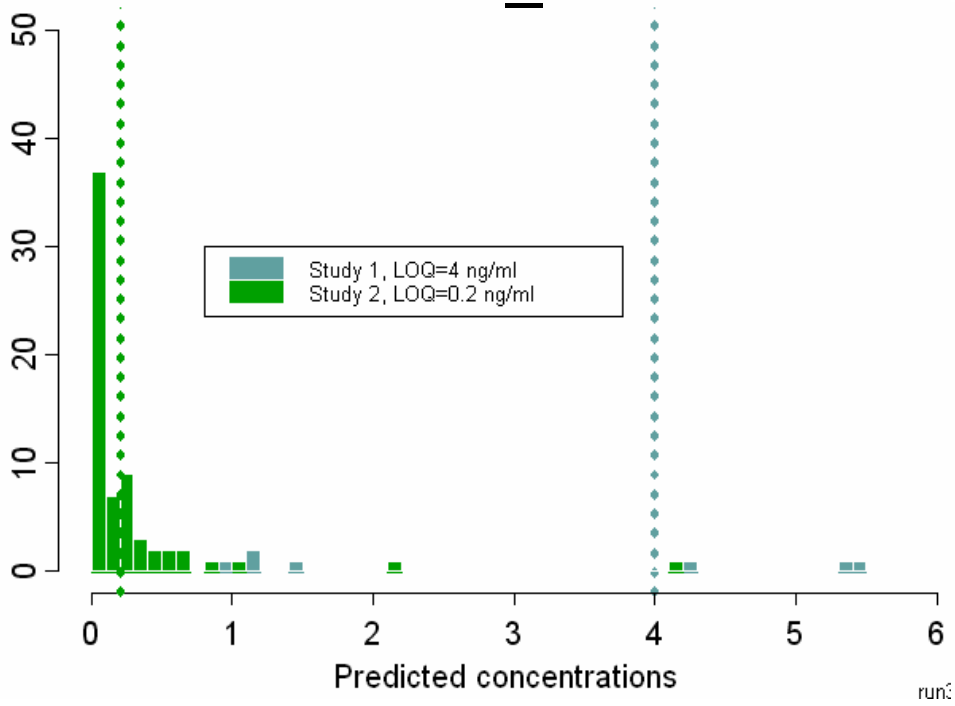
PK distribution – non observed data

LEAD

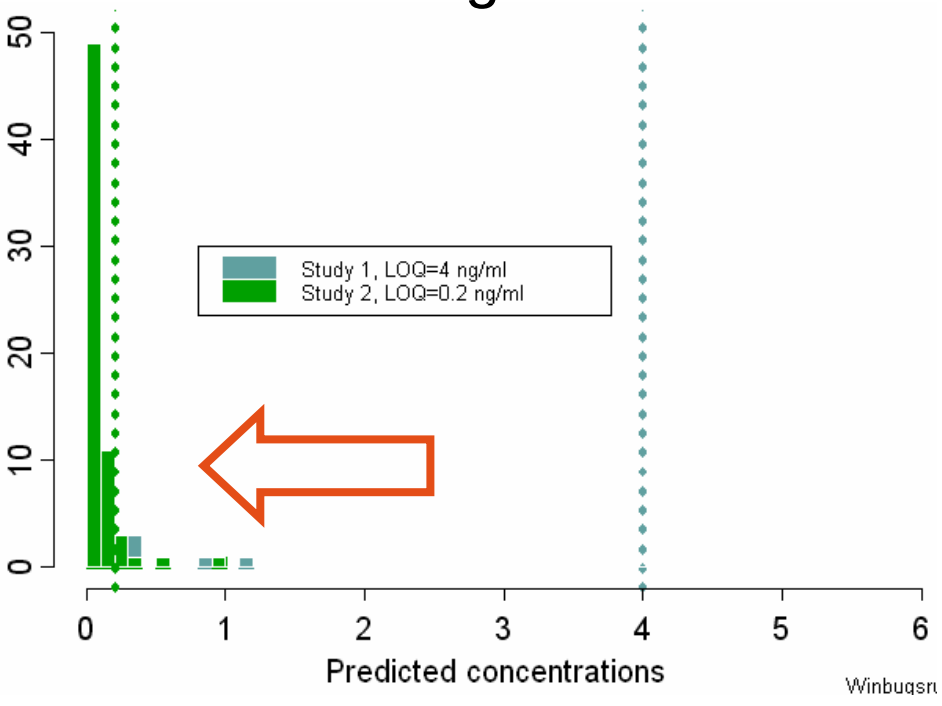
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



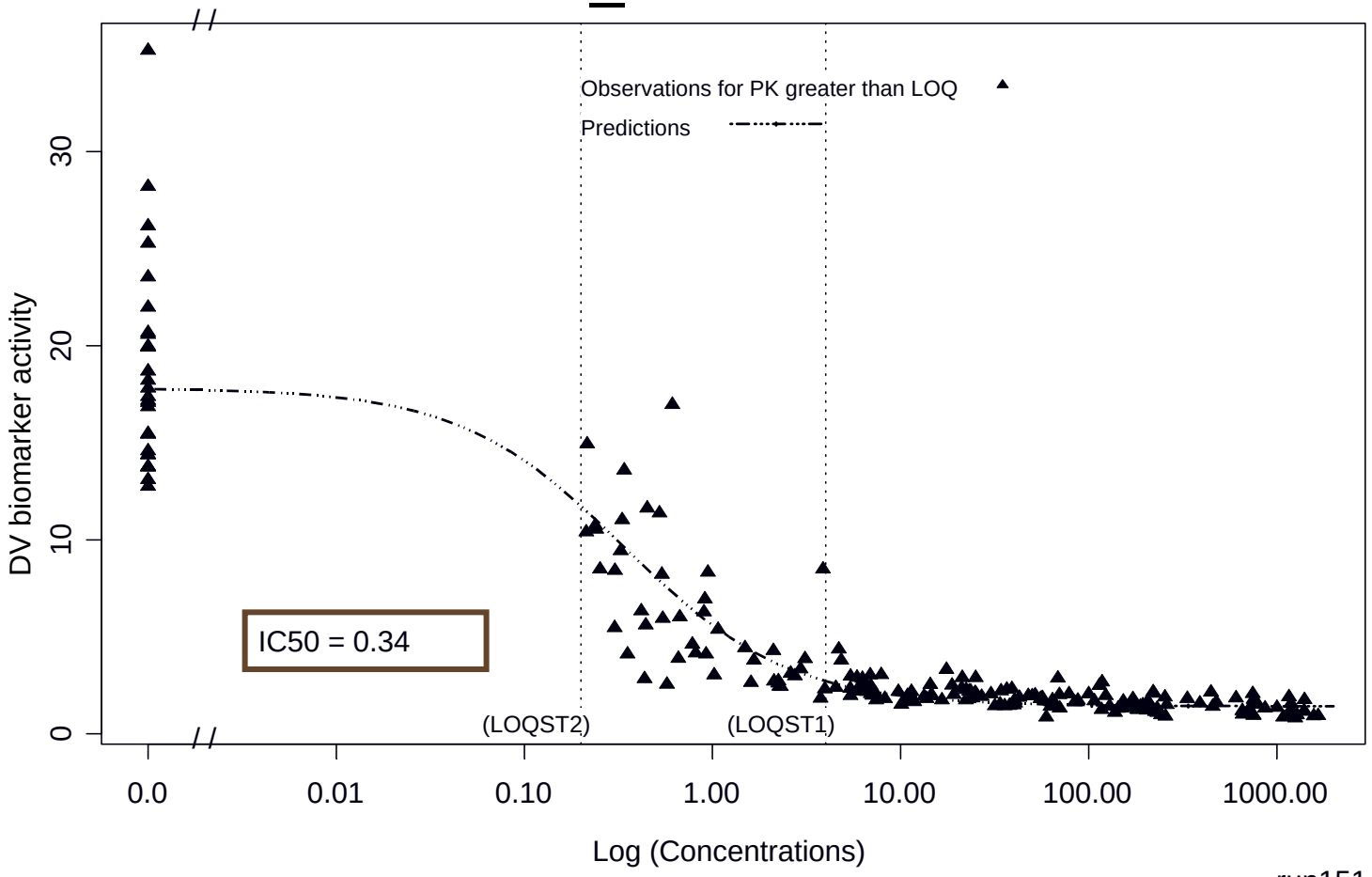
NOVARTIS

Results

PKPD – observed PK data only

LEAD

- NONMEM VI - M3 F_FLAG



run151.txt

NOVARTIS

Results

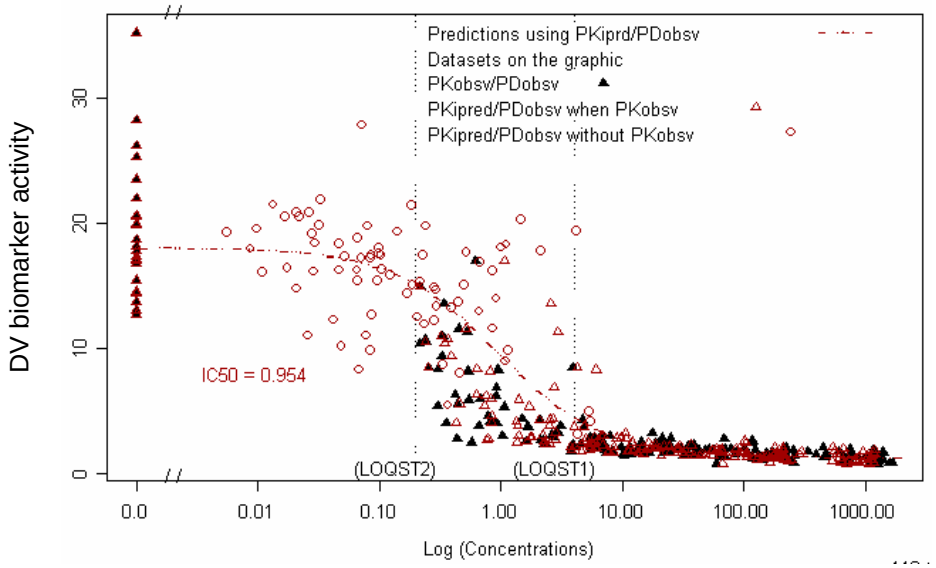
PKPD – goodness of fit

LEAD

- NONMEM VI - Winbugs

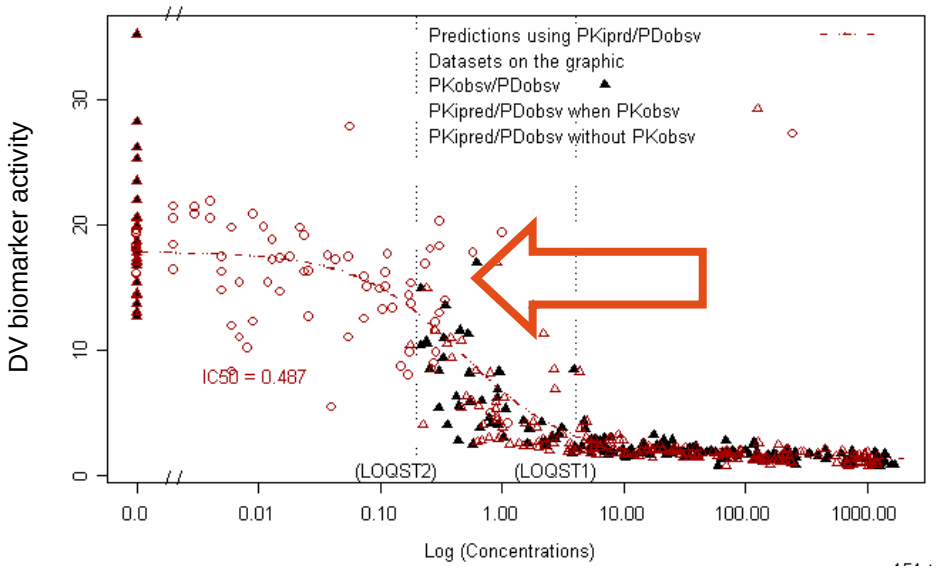
NONMEM VI

M3 F_FLAG



IC50=0.95

Winbugs



IC50=0.49

NOVARTIS

Results

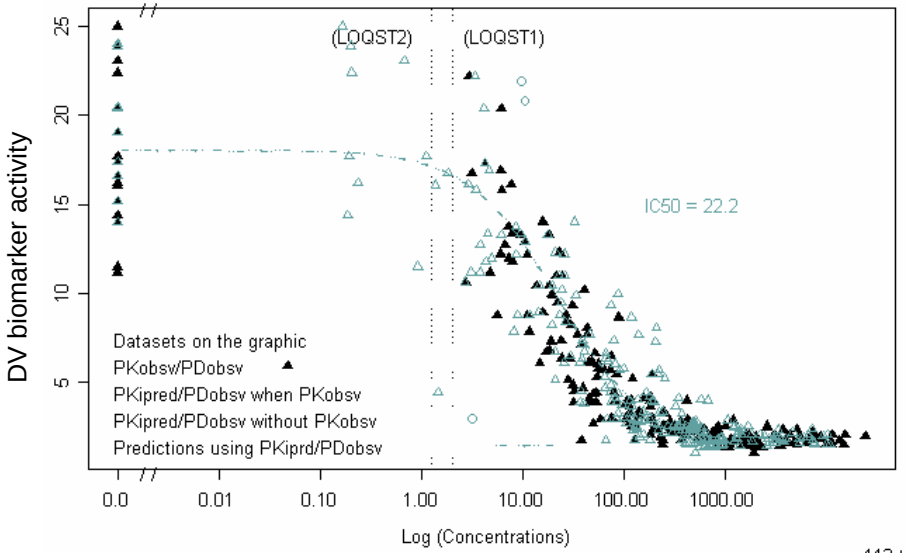
PKPD – goodness of fit

BACKUP

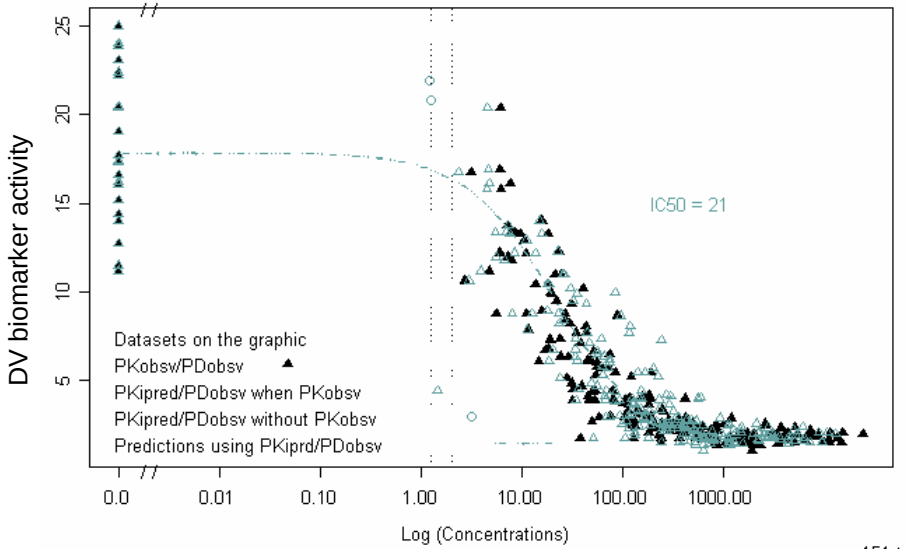
- NONMEM VI - Winbugs

NONMEM VI

M3 F_FLAG



Winbugs



IC50=22

IC50=21

NOVARTIS

Results

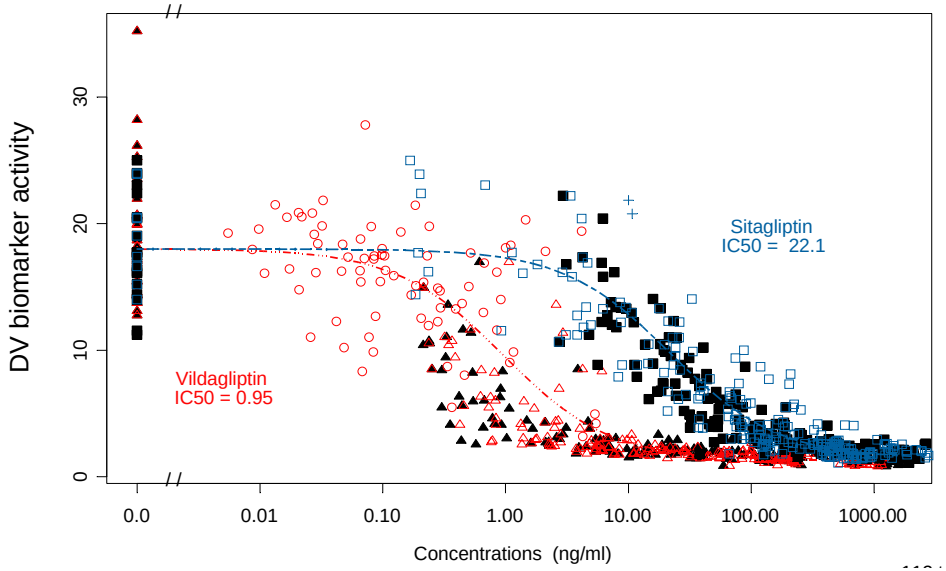
PKPD – goodness of fit

LEAD BACKUP

- NONMEM VI - Winbugs

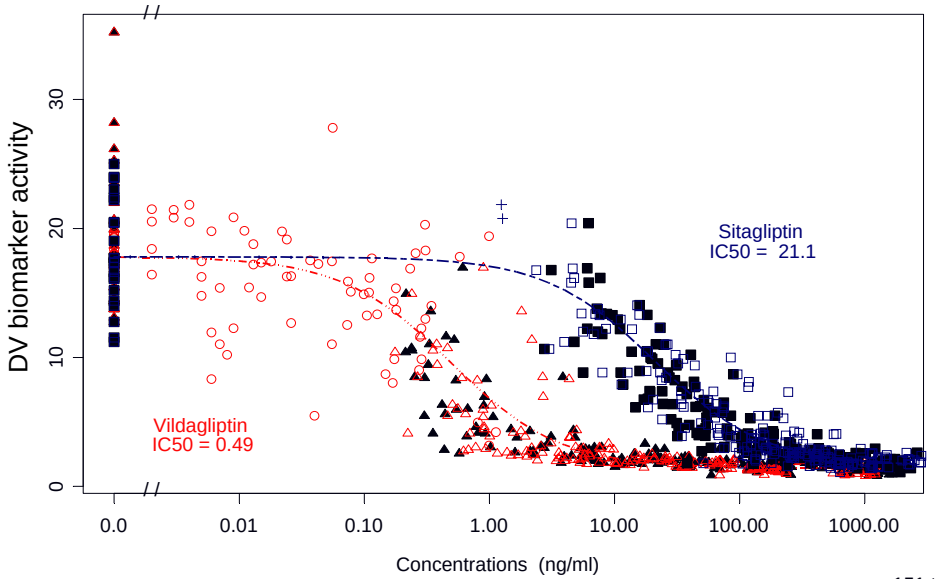
NONMEM VI

M3 F_FLAG



IC50 ratio = 23 (SE=5.7 (24 %))

Winbugs



IC50 ratio = 43 (SE=7.6 (18 %))

NOVARTIS

Conclusion

- Handling BLQ data, already important for PK, can be crucial for PD estimation when IC50 around LOQ
- A large diversity of methodologies exists *
- Until recently, due to implementation complexity and no big difference in efficiency, the simplest method (M1: discarding all BLQ data) was encouraging in NONMEM V. *
- Now NONMEM VI ,with the F-FLAG option, allows to apply very easily a more efficient method (method M3). *
- In this study, this method is tested against Winbugs (for which LOQ is also taken into account to explain BLQ data)

** Beal. (2001) and Ahn et al (2008)*

Conclusion

- The only graphic allowing for distinguishing the methods was the BLQ distribution against the LOQ : Winbugs showed better results on PK, predicting only a few concentrations above LOQ.
- PK results had a great impact on PD estimation and reduced bias on relative potency (ratio of 43 instead of 23)
- As drugs are more and more potent and analytical methods cannot always quantify with accuracy the concentrations of interest, a real need exists for handling BLQ data in PKPD modeling.
- For this example, NONMEM VI did not show very convincing results.

Acknowledgments

- Jerry Nedelman (Novartis, Modeling and Simulation)
- David James (Novartis, Modeling and Simulation)

NOVARTIS