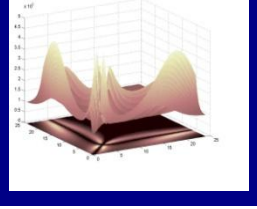

Pharmacokinetics of methotrexate in red blood cells

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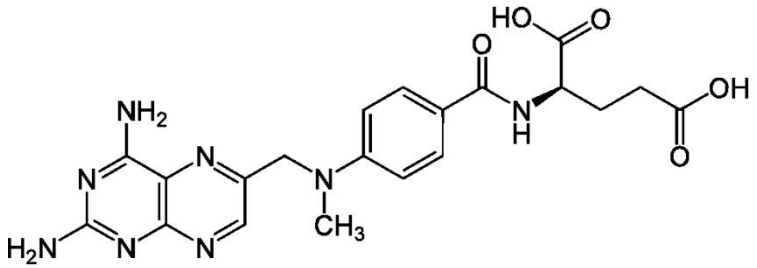
Introduction

Methotrexate (MTX)

- Folate antagonist
  - Anti-inflammatory activity



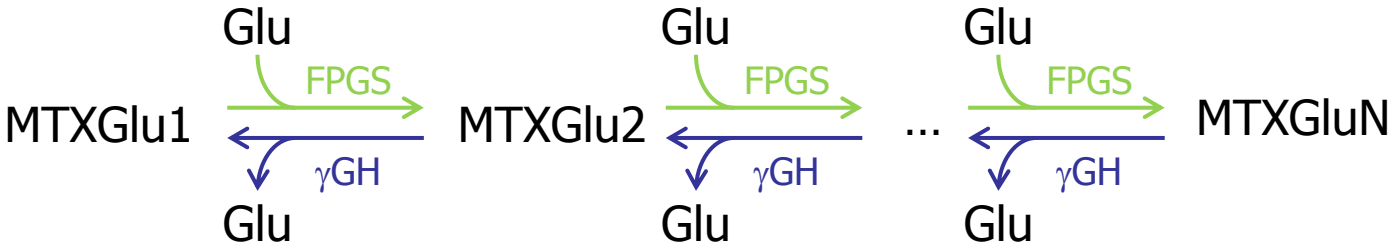
- Gold standard in treatment of rheumatoid arthritis (RA):
  - Weekly doses of 5 - 20mg oral or subcutaneous (sc)
  - Fast disease control needed to prevent further joint damage ⇨ required doses hard to predict due to high variability between patients
  - Severe side effects possible:
    - GI irritation, liver toxicity, myelosuppression
  - TDM desirable, but plasma MTX concentrations are unsuitable as biomarker [1]

[1] Angelis-Stoforidis P *et al.* (1999). Clinical and Experimental Rheumatology 17(3);313-320

MTX polyglutamates (MTXPGs)

- Intracellular metabolism of MTX via polyglutamation
- MTXPGs accumulate inside the cells

- 2 enzymes involved:
  - Folyl-polyglutamyl synthetase (FPGS):
    - Successive addition of 1 glutamate moiety at a time
  - γ-Glutamate hydrolase (γGH):
    - Successive cleaving of 1 and/or 2 glutamate moieties at a time



MTXPG concentrations in RBCs

An alternative TDM biomarker for MTX?

- Red blood cells (RBCs) are easily accessible
- HPLC assay for individual MTXPGs well established

- Ambiguous results in literature linking RBC MTXPGs with disease outcomes and side effects in RA [2,3]

- Intracellular pharmacokinetics (PK) / time course of accumulation of MTXPGs poorly understood

[2] Dervieux T *et al*. (2004) Arthritis & Rheumatism 50(9):2766-2774
[3] Stamp L *et al*. (2010) Arthritis & Rheumatism 62(2):359-368

Objective

To assess the predictive performance of RBC MTXPGs on disease outcomes and side effects of low-dose MTX therapy in RA based on a population PKPD model.

Specific aim of current work:

To develop a parent-metabolite population PK model for MTX and MTXPGs measured in RBCs.

Materials & Methods

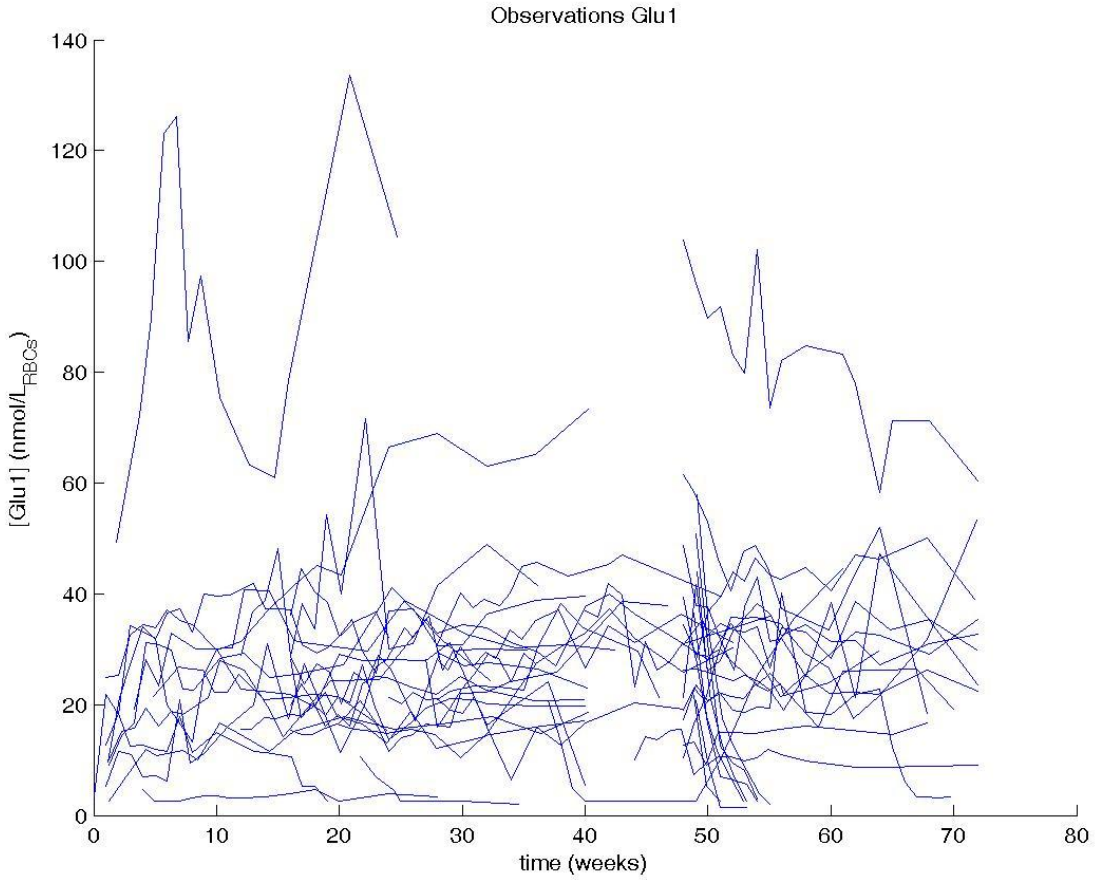
The data

- Data of 48 patients with RA pooled from 2 studies [4,5]
  - 5 - 20mg MTX per week, oral or sc
  - 10 patients started MTX at study begin
  - 10 patients stopped MTX at study begin / during the study
  - 28 patients received continuous therapy with MTX
- No plasma MTX concentrations available
- Individual RBC concentrations measured for parent drug (MTXGlu1) and all metabolites up to MTXGlu5

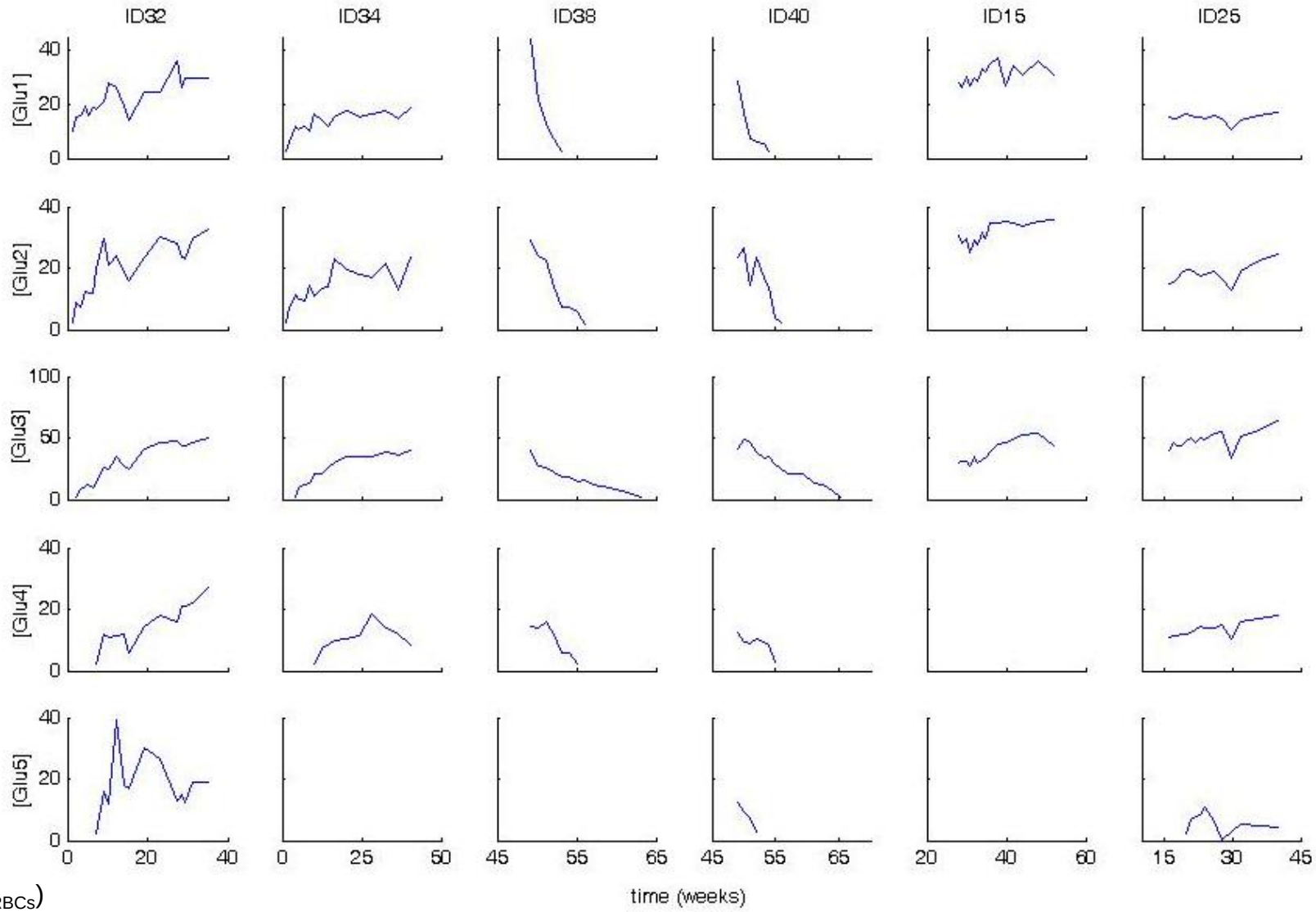
- Analysis using NONMEM® 7.2 – ADVAN5

[4] Dalrymple J *et al.* (2008) Arthritis & Rheumatism 58(11):3299-3308
[5] Stamp L *et al.* (2011) The Journal of Rheumatology (Online first)

The data – Parent drug MTXGlu1



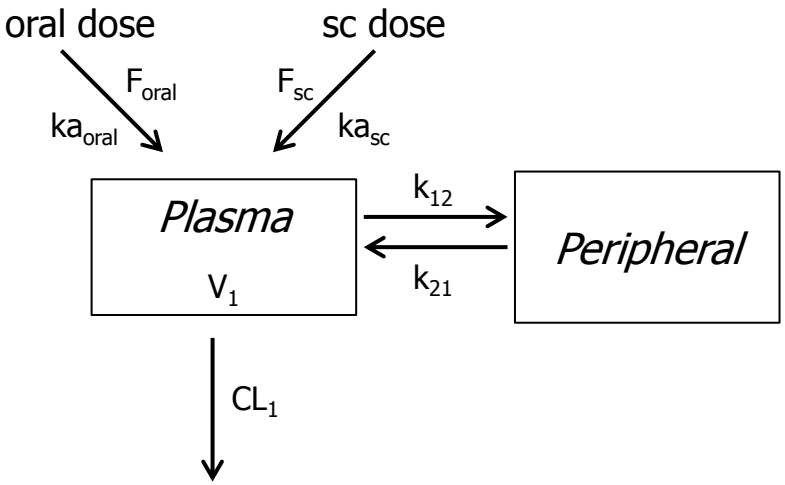
The data – Individual patients



(nmol / L_{RBCs})

Plasma PK model

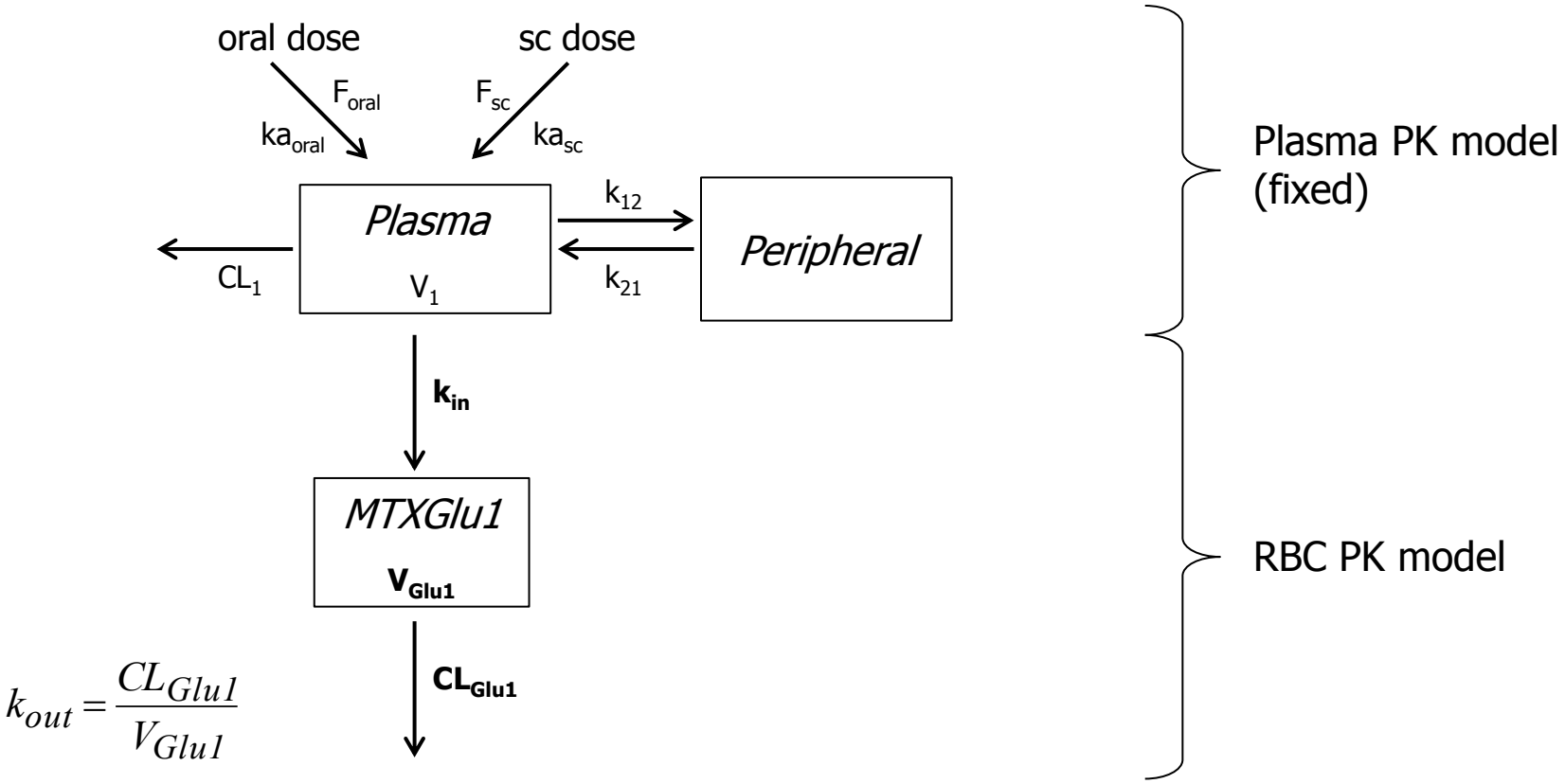
- MTX plasma PK predicted based on previously published 2-compartment model with absorption lag time [6]



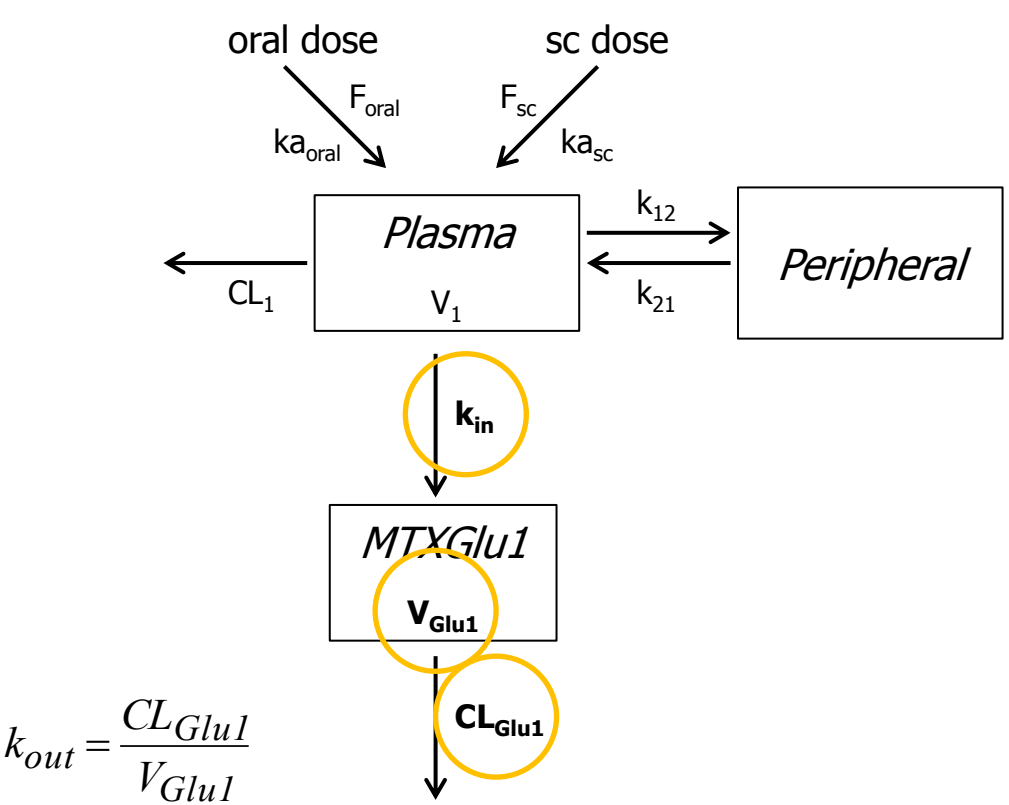
Parameter	Population mean
ka_{oral} (hr^{-1})	0.87
$tlag_{oral}$ (hrs)	0.36
F_{oral}	0.70
ka_{sc} (hr^{-1})	0.36
$tlag_{sc}$ (hrs)	0.06
F_{sc}	1.0
V_1 (L)	9.6
CL_1 (L/hr)	8.4
k_{12} (hr^{-1})	0.81
k_{21} (hr^{-1})	0.55

[6] Hoekstra M *et al.* (2004). The Journal of Rheumatology 31(4):645-648

RBC PK model – Parent drug MTXGlu1



RBC PK model – Parent drug MTXGlu1

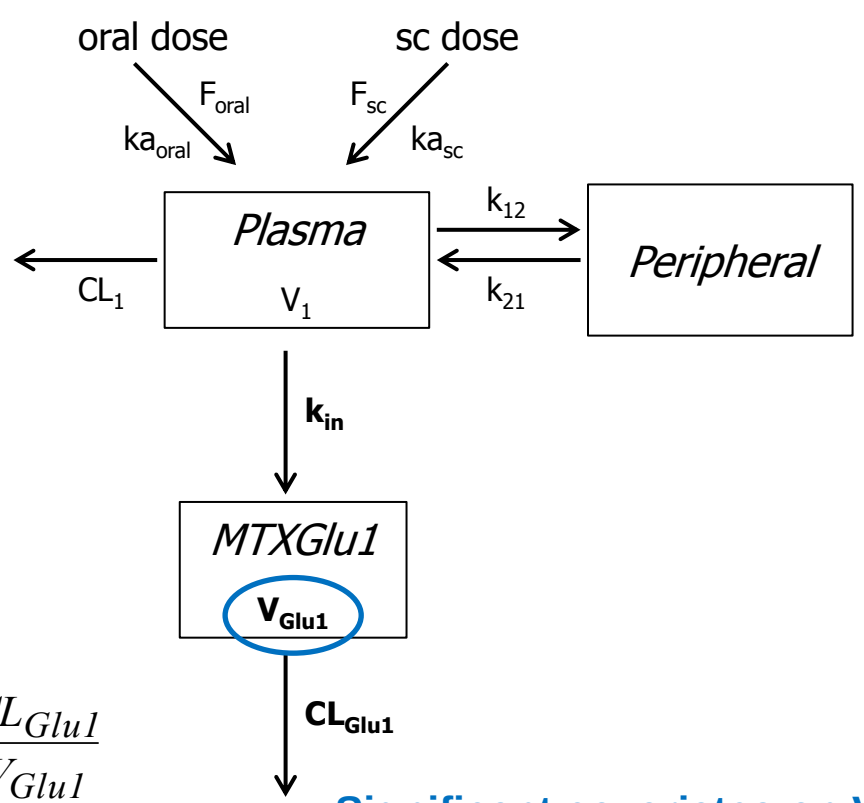


Plasma PK model (fixed)

RBC PK model

estimated parameters

RBC PK model – Parent drug MTXGlu1



Plasma PK model (fixed)

RBC PK model

$$k_{out} = \frac{CL_{Glu1}}{V_{Glu1}}$$

Significant covariates on V_{Glu1}:

$$\hat{V}_{Glu1} = TVV_{Glu1} \cdot \frac{LBW}{45} \cdot \frac{[Hb]}{130} \cdot \frac{MCV}{90}$$

Parent-metabolite RBC PK model

- Adding an additional catenary RBC compartment for each MTXPG metabolite ⇨ 5 RBC compartments in total

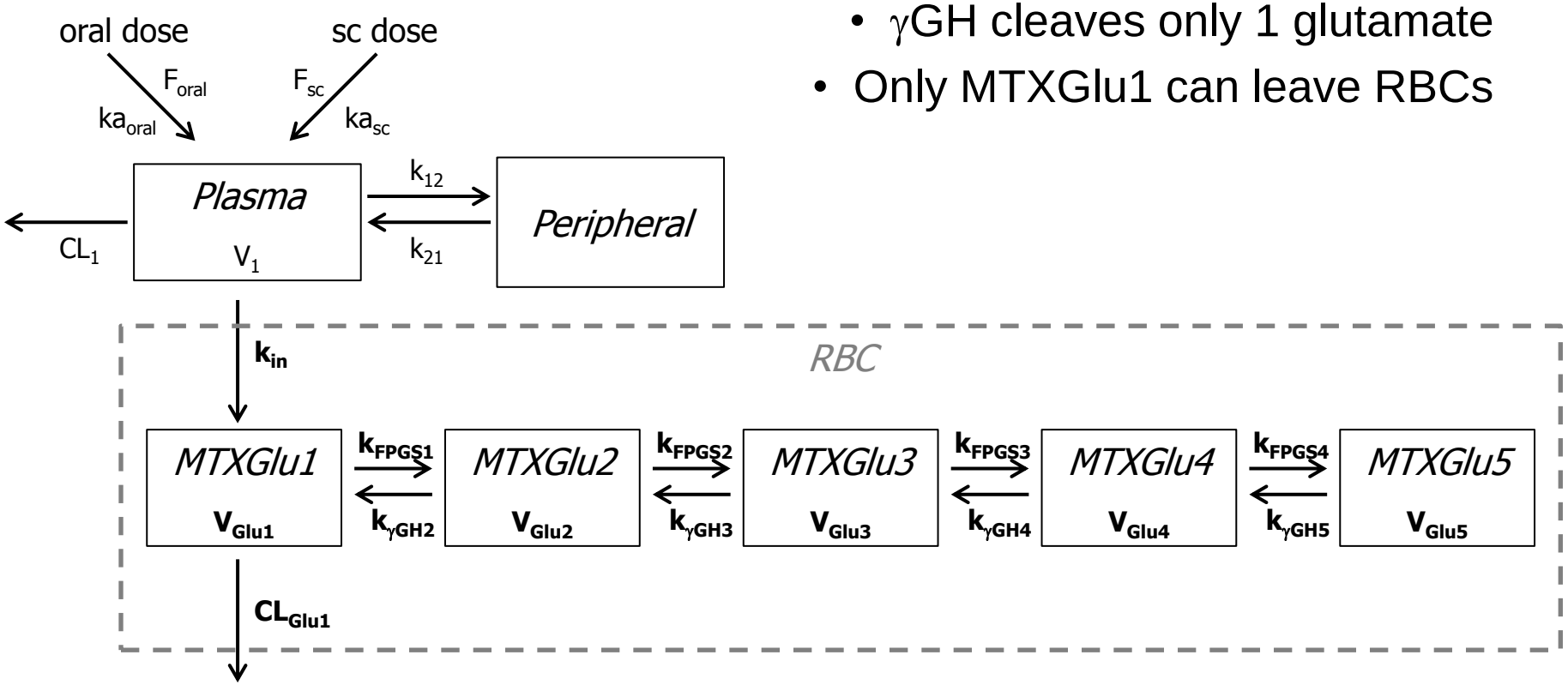
- Questions to be addressed:
  - Cleaving mechanism of γGH:
    - 1, 2 or 1&2 glutamate moieties at a time?
  - Are MTXPGs lost from the system, e.g. is the death of RBCs observable from the data?

- Assumptions:
  - All enzymatic reactions, transport processes and loss from RBCs approximated as first-order kinetics

    ⇨ allows use of ADVAN5 ⇨ computation time ↓

Parent-metabolite RBC PK model

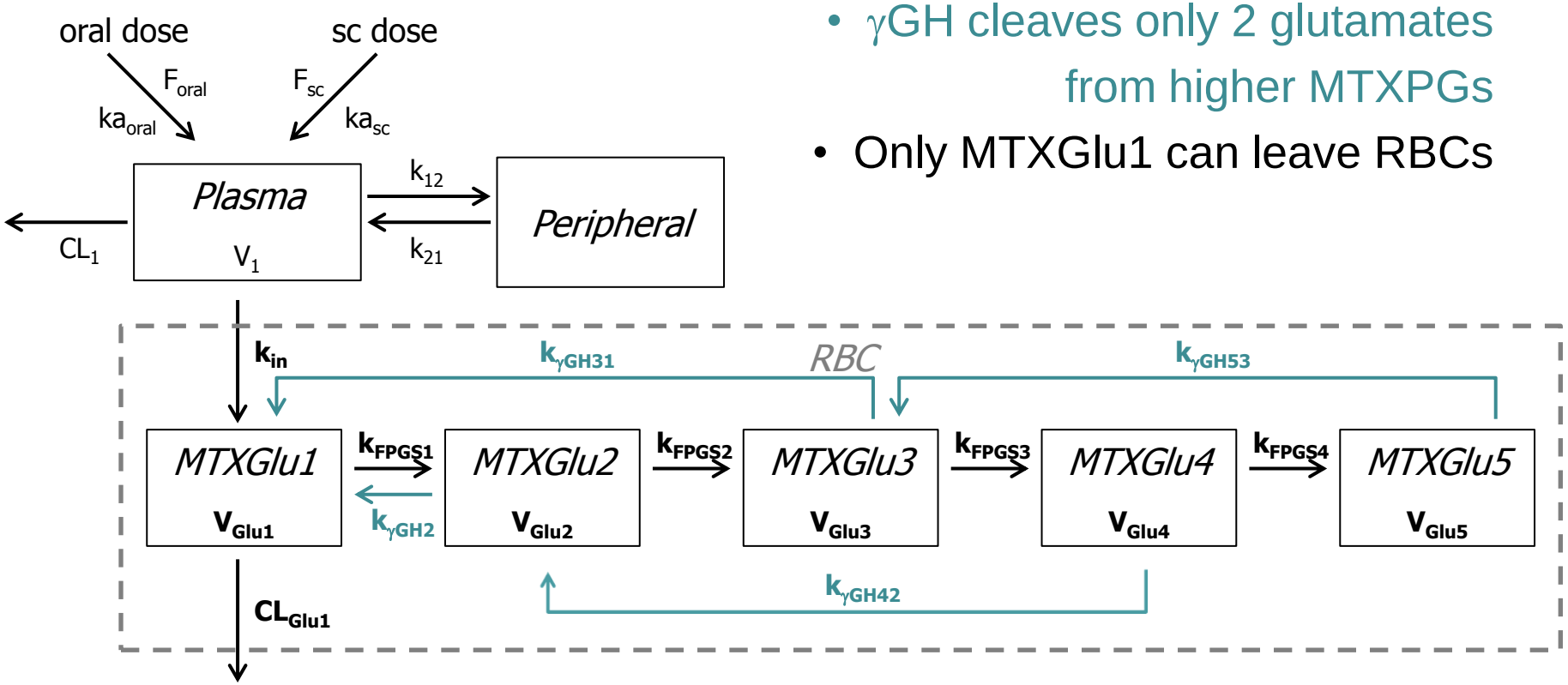
Scenario 1



- γGH cleaves only 1 glutamate
- Only MTXGlu1 can leave RBCs

Parent-metabolite RBC PK model

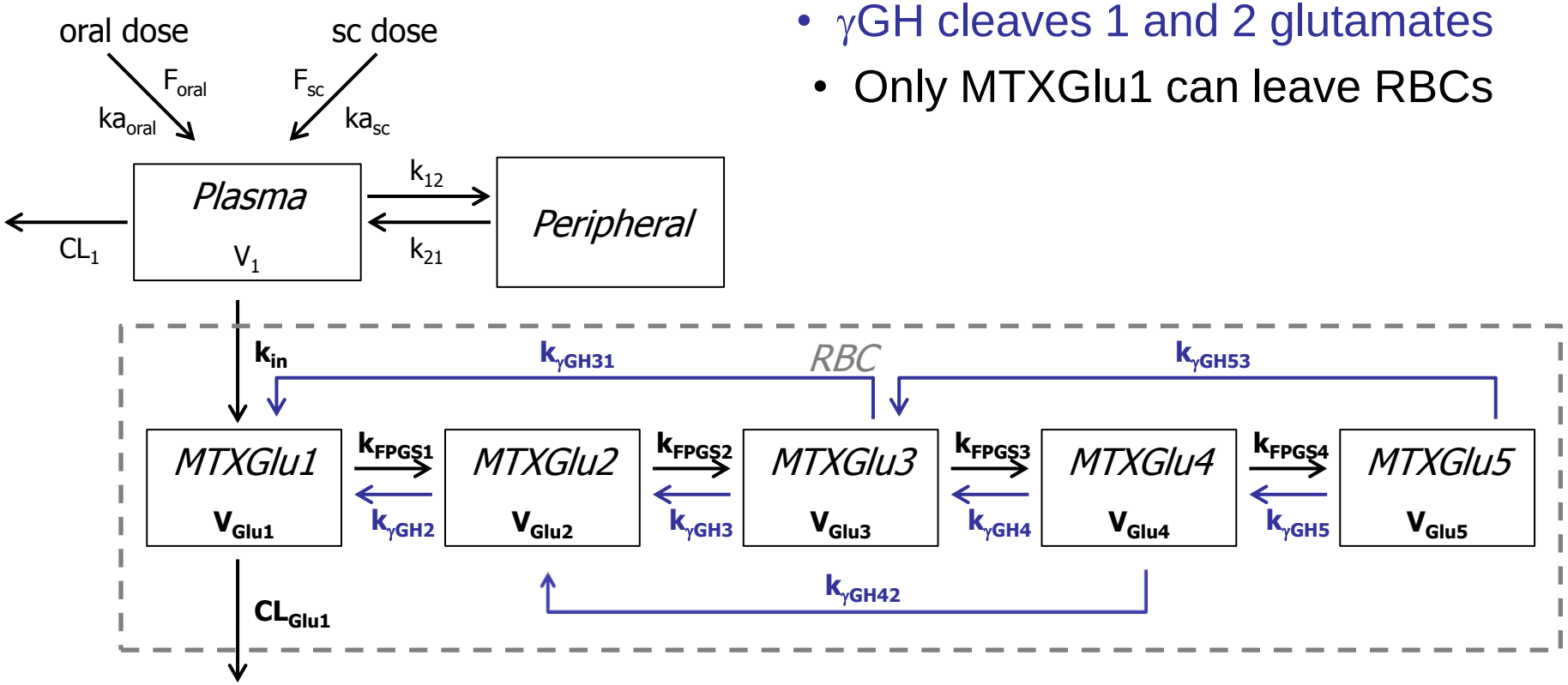
Scenario 2



- γGH cleaves only 2 glutamates from higher MTXPGs
- Only MTXGlu1 can leave RBCs

Parent-metabolite RBC PK model

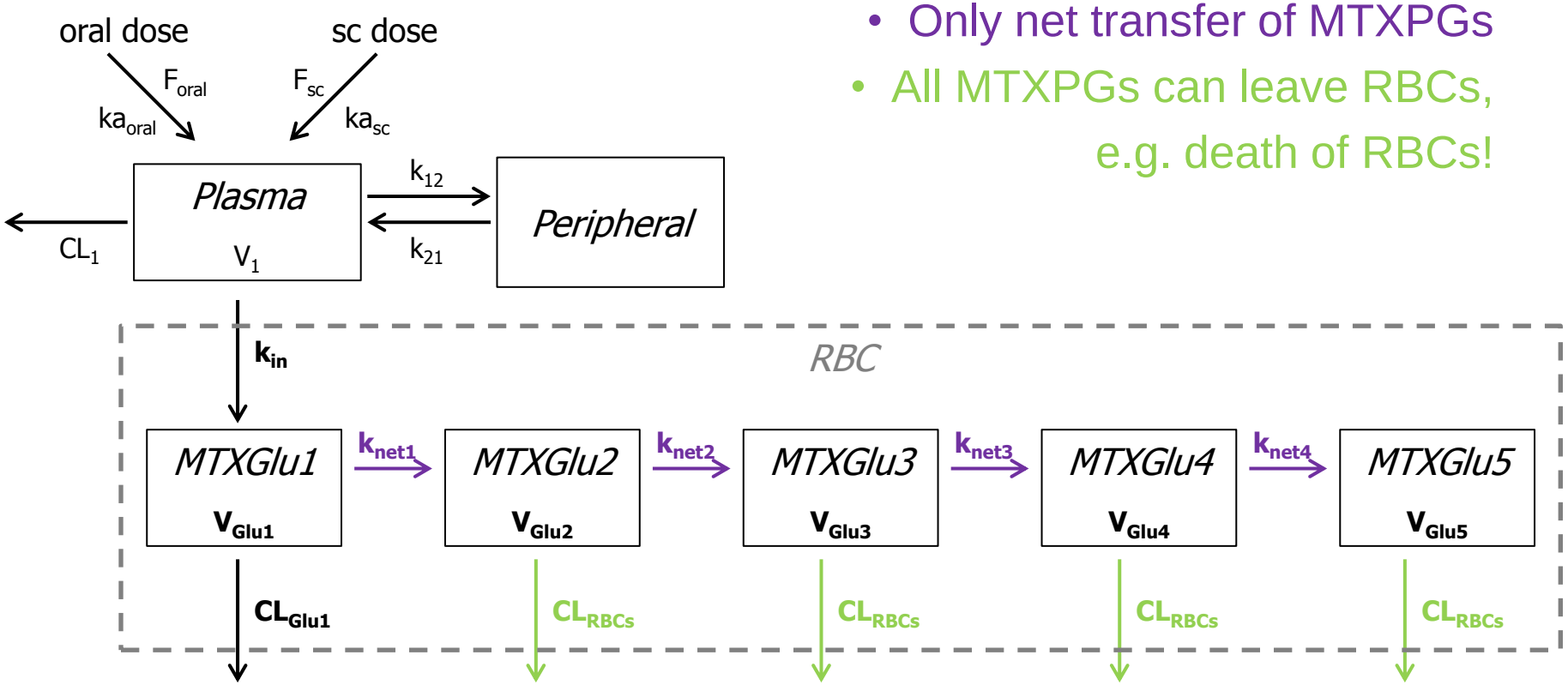
Scenario 3



- γGH cleaves 1 and 2 glutamates
- Only MTXGlu1 can leave RBCs

Parent-metabolite RBC PK model

Scenario 4



- Only net transfer of MTXPGs
- All MTXPGs can leave RBCs, e.g. death of RBCs!

Results

Parent - metabolite RBC PK model

	Scenarios	OFV (AIC)
1	• γGH cleaves only 1 glutamate • Only MTXGlu1 can leave RBCs	15132 (15232)
2	• GH cleaves only 2 glutamates • Only MTXGlu1 can leave RBCs	15598 (15678)
3	• γGH cleaves 1 and 2 glutamates • Only MTXGlu1 can leave RBCs	16222 (16314)
4	• Only net transfer of MTXPGs • All MTXPGs are lost from RBCs	15260 (15324)

Parent - metabolite RBC PK model

	Scenarios	OFV (AIC)
1	• γGH cleaves only 1 glutamate • Only MTXGlu1 can leave RBCs	15132 (15232)
2	• GH cleaves only 2 glutamates • Only MTXGlu1 can leave RBCs	15598 (15678)
3	• γGH cleaves 1 and 2 glutamates • Only MTXGlu1 can leave RBCs	16222 (16314)
4	• Only net transfer of MTXPGs • All MTXPGs can leave RBCs	15260 (15324)

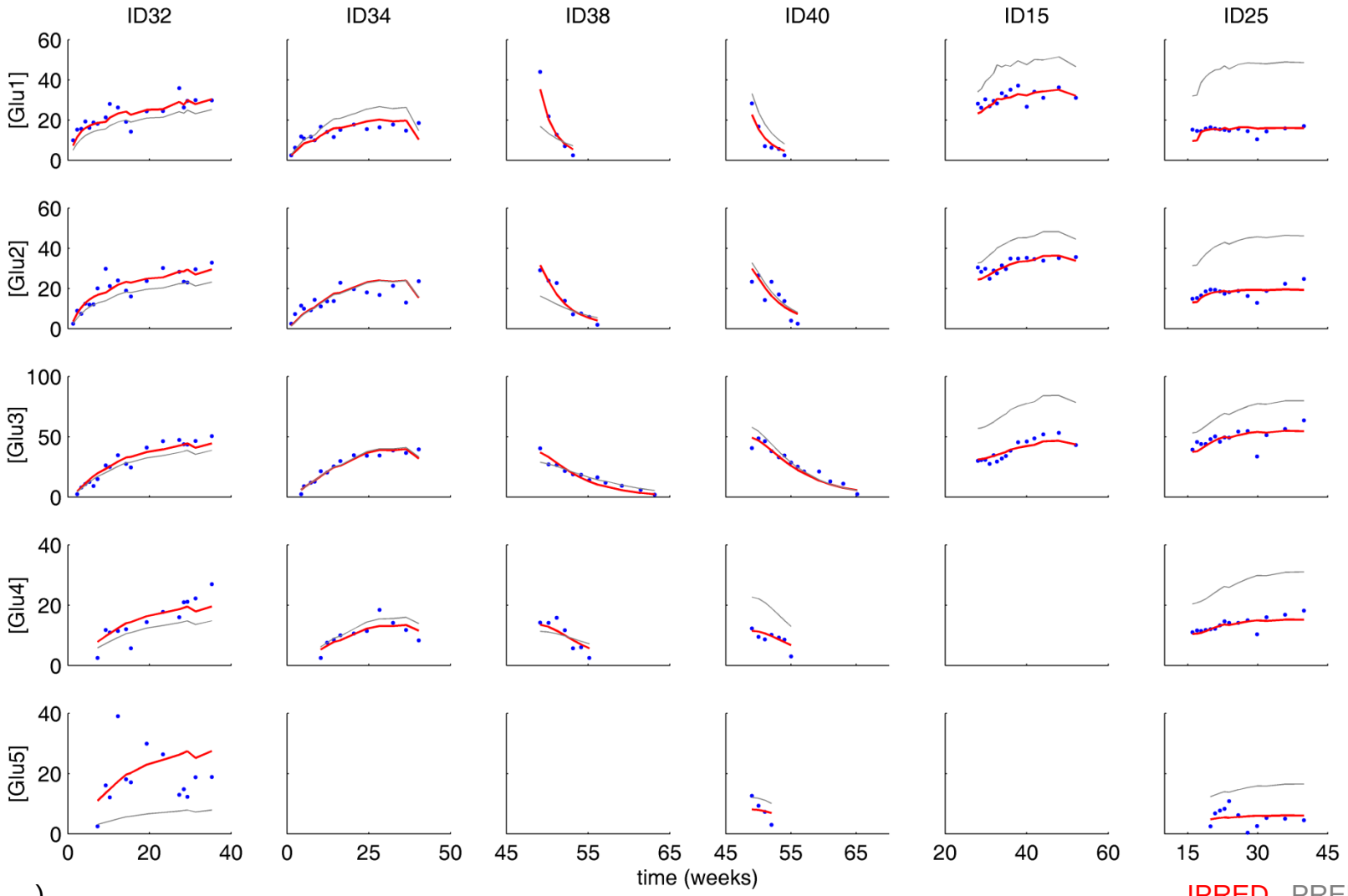
Parent - metabolite RBC PK model

Pop. mean (CV%)	MTXGlu1	MTXGlu2	MTXGlu3	MTXGlu4	MTXGlu5
k_{in} (hr^{-1})	1.06×10^{-4} (38.8)	-	-	-	-
CL_{GluX} (L/hr)	5.65×10^{-4} (50.8)	-	-	-	-
V_{GluX} (L)	0.270 (142)	0.061 (111)	0.004 (109)	0.023 (119)	0.054 (132)
$k_{FPGS(X,X+1)}$ (hr^{-1})	1.0×10^{-3} (24.3)	1.7×10^{-3} (2.1)	0.036 (0.3)	6.01 (1.6)	-
$k_{\gamma GH(X+1,X)}$ (hr^{-1})	-	5.2×10^{-3} (28.1)	0.017 (27.7)	0.014 (28.9)	4.67 (3.0)
CV_{prop} (%)	20.9	21.5	12.8	24.0	25.7
σ_{add} (nmol/L)	3.64	2.33	5.66	1.99	1.94

- Covariance for V_{GluX}: Correlation 65 - 85%
- Shrinkage >90% for k_{FPGS23}, k_{FPGS34} k_{FPGS45} & $k_{\gamma GH54}$

⇨ Fine tuning required!

Parent - metabolite RBC PK model



(nmol / L_{RBCs})

IPRED PRED DATA

Discussion & Conclusions

RBC PK model for MTX and MTXPGs

- The developed population PK model is able to describe the time course of MTX and MTXPGs in RBCs

- MTX and MTXPG concentrations << Km values reported for enzymes & transporters ⇨ approximation as first-order kinetics valid

- Cleaving of 2 glutamate moieties by γGH not supported in parent-metabolite model although reported in literature
- Loss of MTXPGs from RBCs not supported ⇨ death of RBCs not the rate limiting step ⇨ unobservable

RBC MTXPGs as potential TDM biomarker

- RBCs are not on the postulated pathway of action of MTX:
  - Debated correlation with PD outcomes not based on causality
- RBCs are quite different from other cells:
  - Finite lifespan
  - Limited enzyme & transporter capacity
  - ⇨ MTXPG kinetics in RBCs are different from kinetics in other cells, e.g. leukocytes
- However, RBC concentrations have a potential as a measure of cumulative MTX exposure (similar to AUC)

Future directions

- Test of genotypic covariates in the population PK parent-metabolite model
  - Polymorphisms in γGH and transporters reported

⇨ A full population PKPD model would allow to test the value of RBC MTXPGs as TDM biomarker for MTX.

To be continued…

Acknowledgments

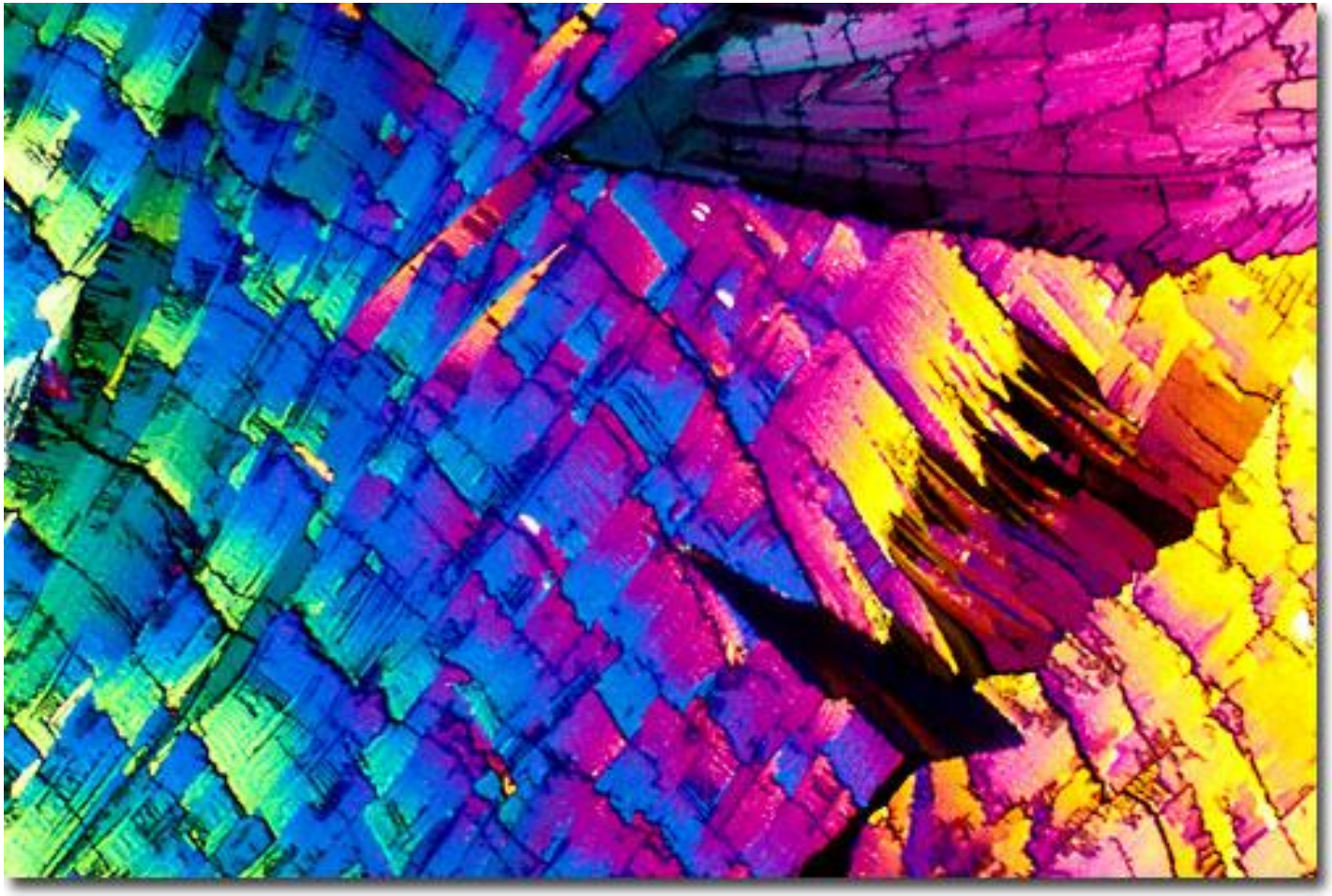
- Prof. Steve Duffull
- Assoc. Prof. Lisa Stamp

Rheumatology Group, Christchurch, NZ:

- Judith Dalrymple
- Prof. Murray Barclay
- Dr Mei Zhang
- Jill Drake

- Friends of the Otago Pharmacometrics Group
- School of Pharmacy, University of Otago

Thank you!



MTX crystal - http://micro.magnet.fsu.edu/pharmaceuticals/pages/methotrexate.html

Plausibility of covariates on V_{Glu1}

- Final model with LBW, [Hb] & MCV as covariates on V_{RBCs}

$$\hat{V}_{Glu1} = TVV_{Glu1} \cdot \frac{LBW}{45} \cdot \frac{[Hb]}{130} \cdot \frac{MCV}{90}$$

- LBW · [Hb] · MCV ≈ true V_{RBCs}:

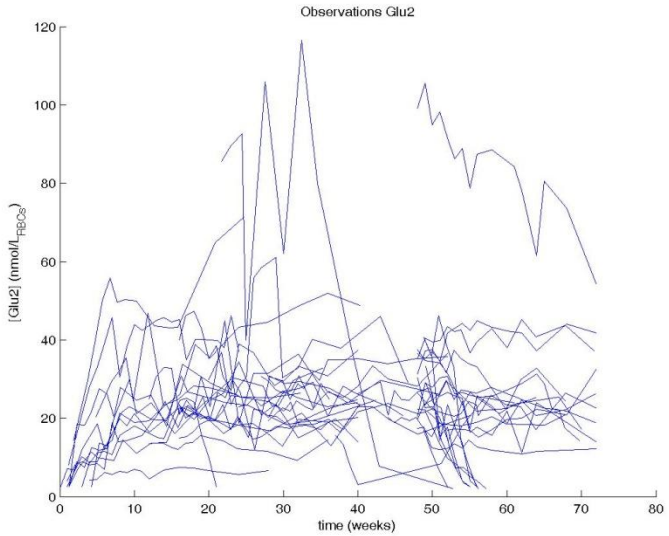
$$\text{with} \quad MCV = \frac{V_{RBCs}}{RBC_{count}} \quad \text{and} \quad [Hb] = \frac{A_{Hb}}{V_{blood}}$$

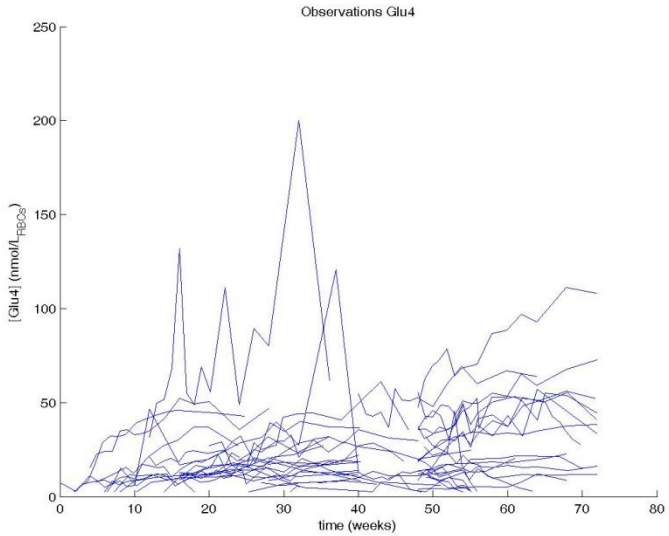
$$\text{if} \quad V_{blood} \sim LBW \quad \text{then} \quad [Hb] \sim \frac{A_{Hb}}{LBW} \quad \text{and} \quad A_{Hb} \sim [Hb] \cdot LBW$$

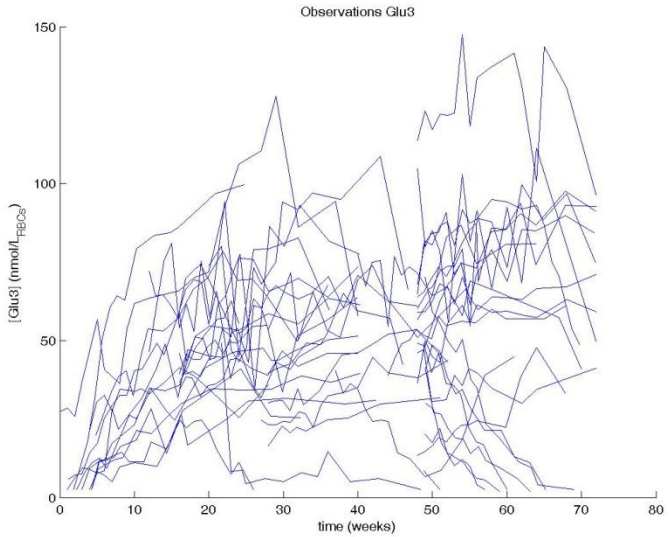
$$\text{if} \quad RBC_{count} \sim A_{Hb} \quad \text{then} \quad MCV \sim \frac{V_{RBCs}}{A_{Hb}} \sim \frac{V_{RBCs}}{[Hb] \cdot LBW}$$

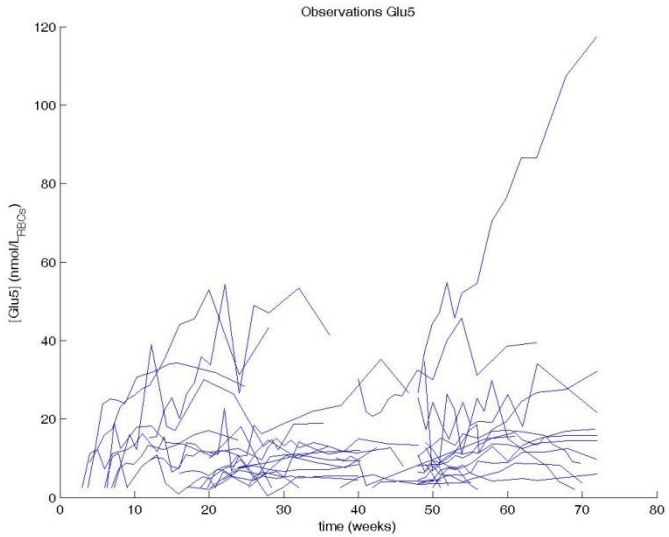
$$\text{therefore} \quad V_{RBCs} \sim MCV \cdot [Hb] \cdot LBW$$

The data – Metabolites MTXGlu2-5



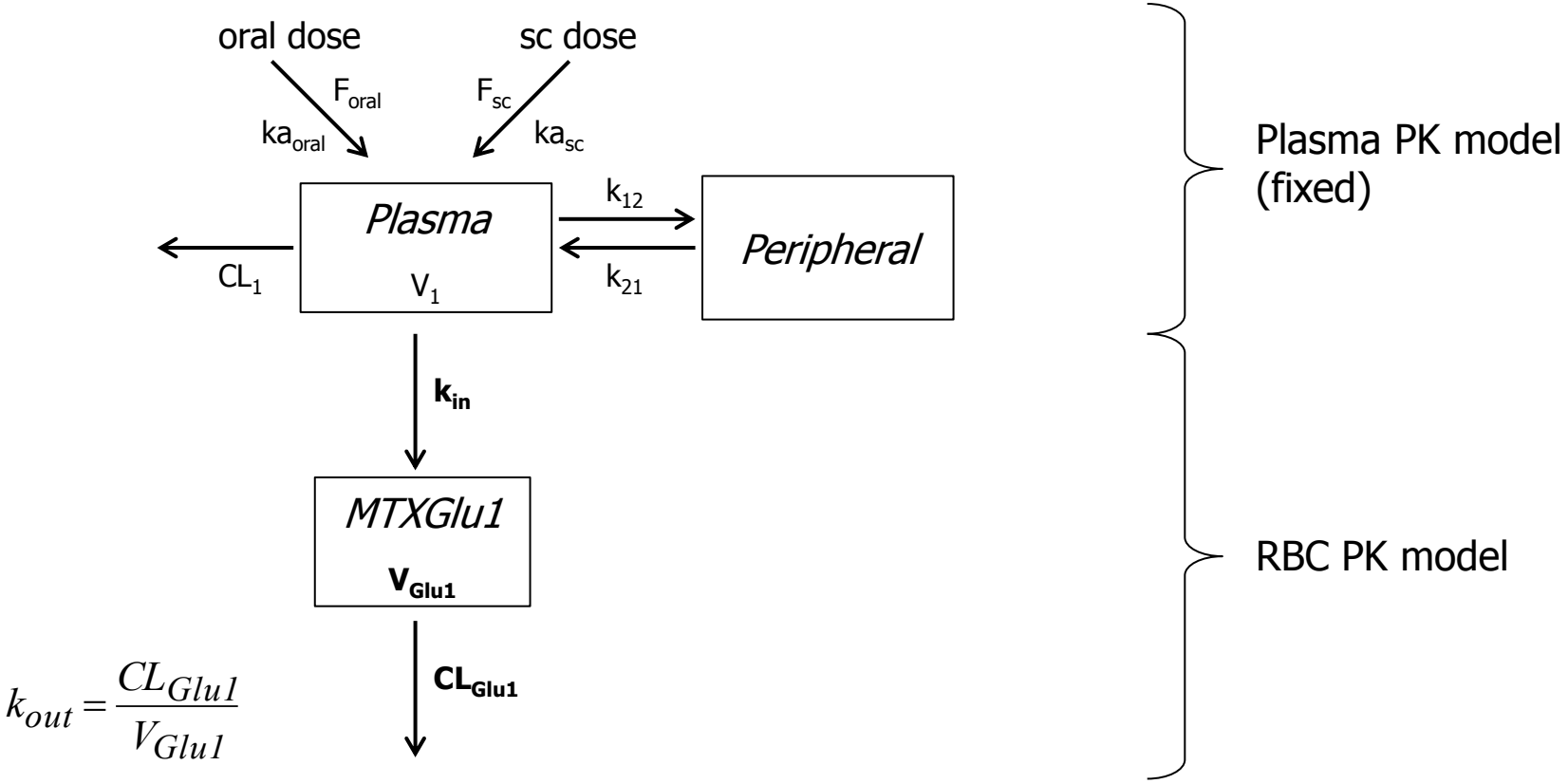




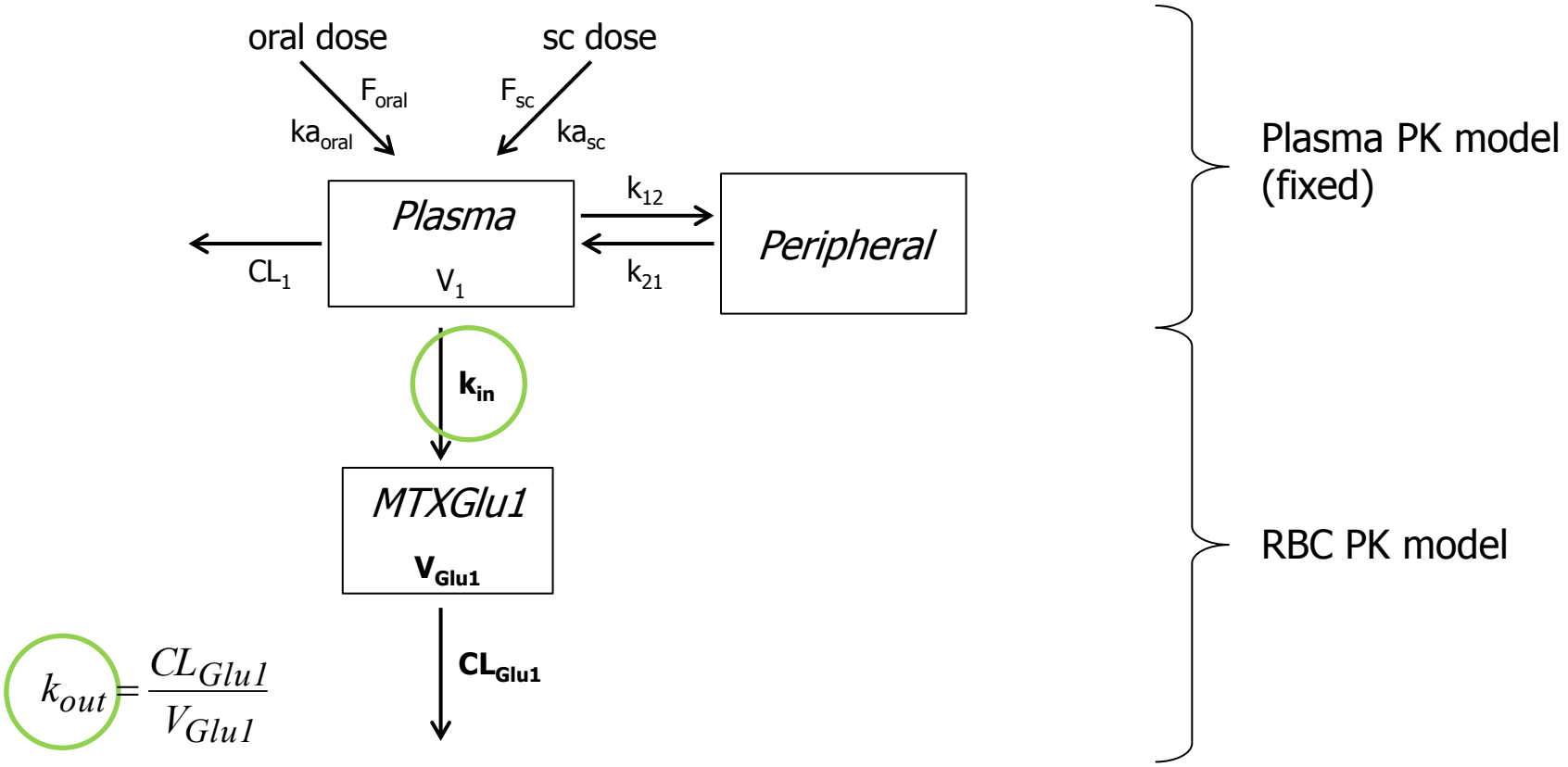


Parent model

RBC PK model – Parent drug MTXGlu1

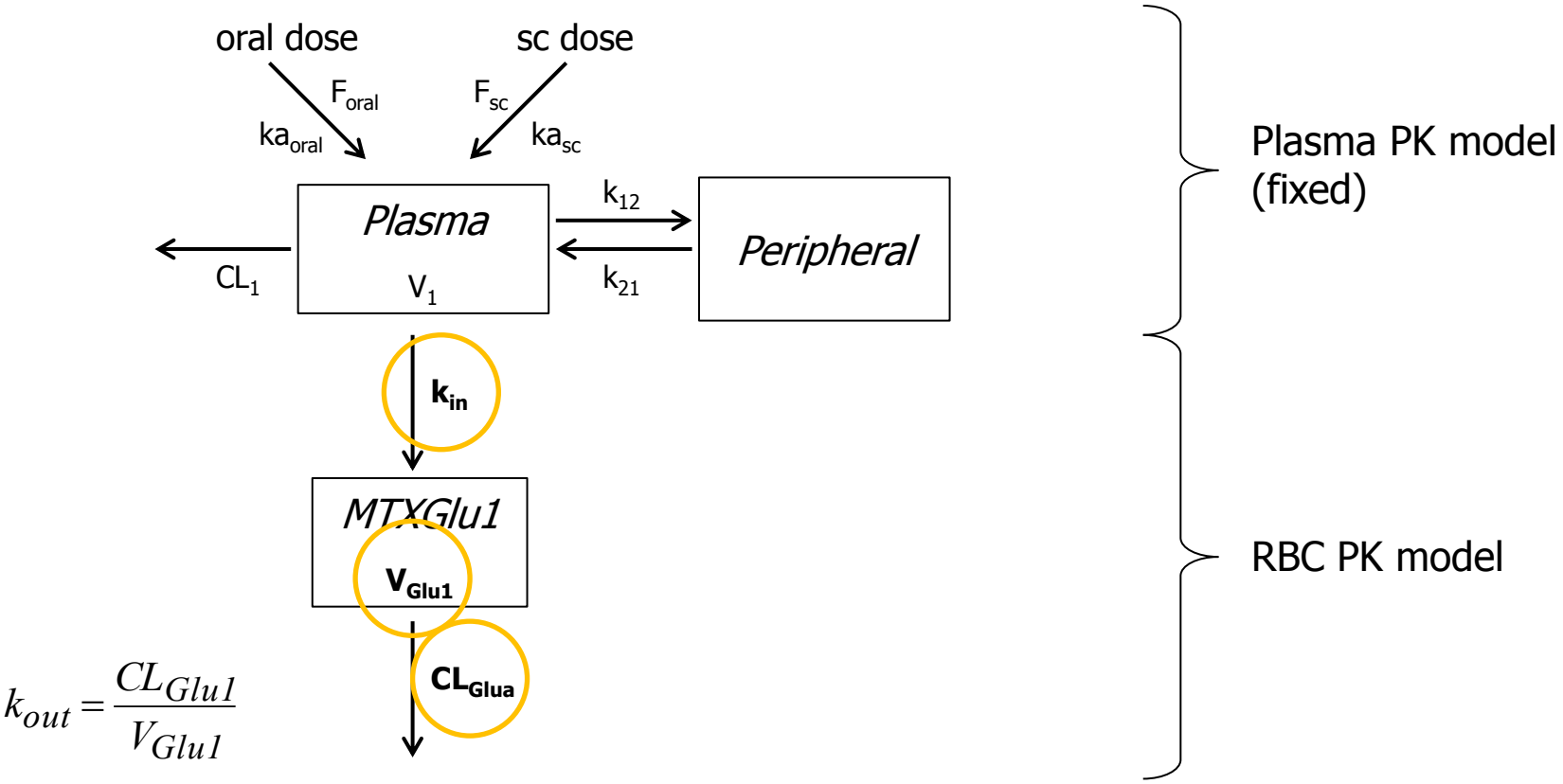


RBC PK model – Parent drug MTXGlu1



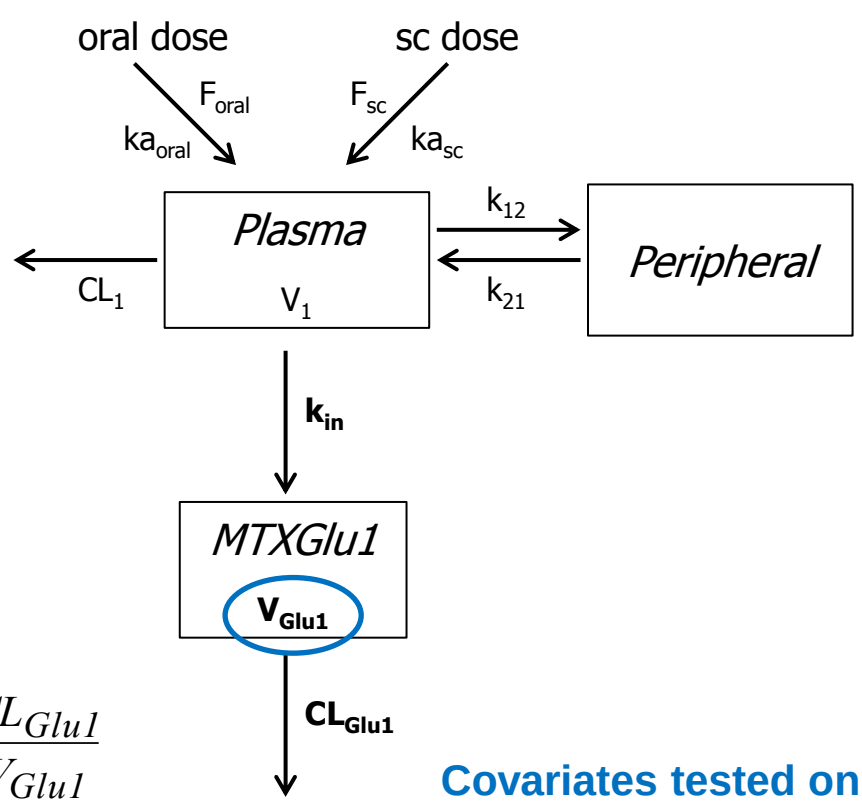
Active transport into and out of RBCs approximated as first-order kinetics

RBC PK model – Parent drug MTXGlu1



estimated parameters

RBC PK model – Parent drug MTXGlu1



$$k_{out} = \frac{CL_{Glu1}}{V_{Glu1}}$$

Plasma PK model (fixed)

RBC PK model

Covariates tested on V_{Glu1}:

- total body weight (WT)
- lean body weight (LBW)
- blood haemoglobin concentration ([Hb])
- mean corpuscular volume (MCV)

MTXGlu1 parent RBC PK model

Final model with LBW, [Hb] & MCV as covariates on V_{RBCs}

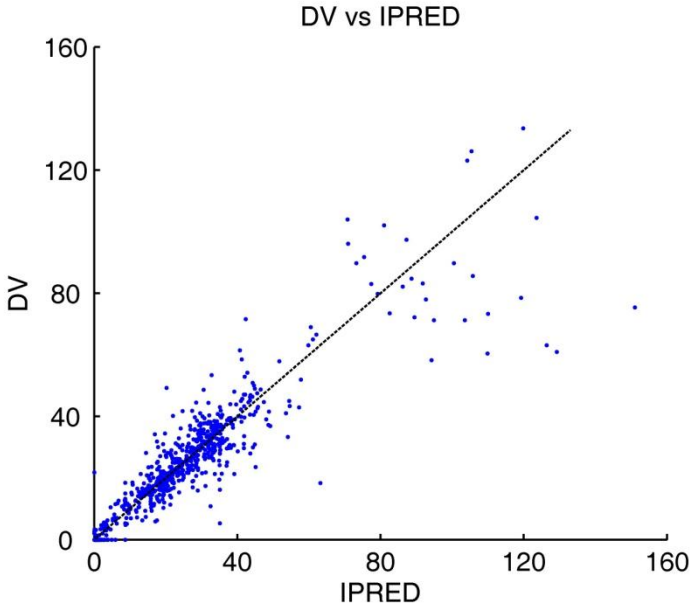
$$\hat{V}_{RBCs} = TVV_{RBCs} \cdot \frac{LBW}{45} \cdot \frac{[Hb]}{130} \cdot \frac{MCV}{90}$$

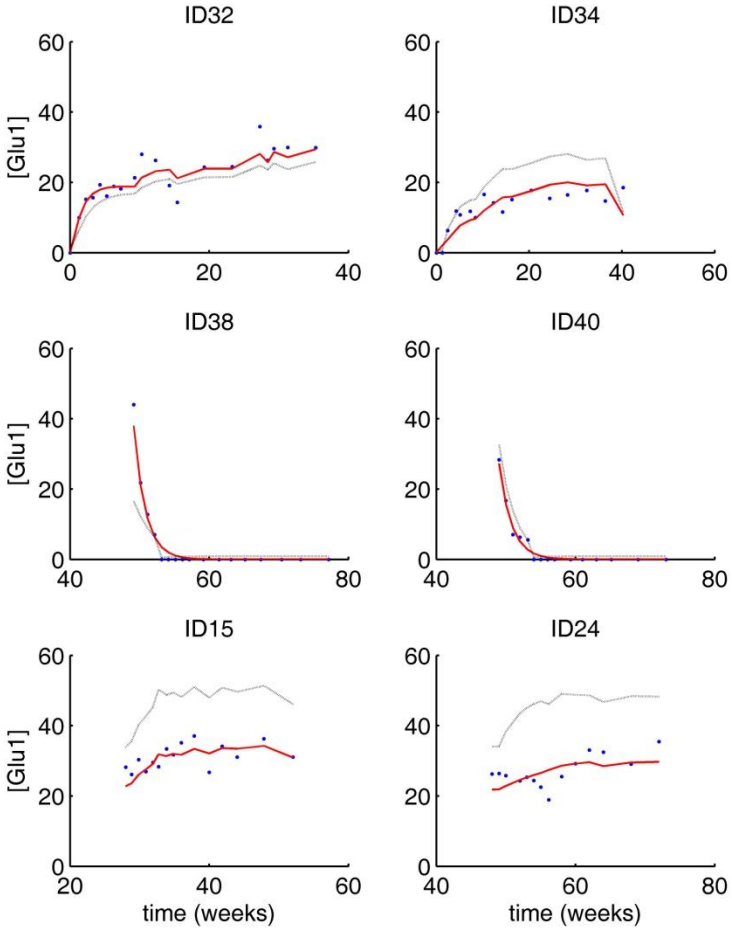
Parameter	Population Mean	BSV (%)	Median [a]	95% Prediction Interval [a,b]
k_{in} (hr^{-1})	1.35×10^{-4}	63.7	1.40×10^{-4}	9.09×10^{-5} – 2.00×10^{-4}
CL_{Glu1} (L/hr)	7.05×10^{-4}	-	7.00×10^{-4}	4.90×10^{-4} – 1.03×10^{-3}
V_{Glu1} (L)	0.287	110.1	0.286	0.184 – 0.434
CV_{prop} (%)	20.3	-	20.3	16.3 – 24.8
σ_{add} (nmol/L)	3.58	-	2.43	1.32 – 5.21

[a] Bootstrap statistics based on 1126 runs with successful minimisation out of 2000 runs (success rat 56.3%)
[b] Non-parametric 95% prediction interval constructed from the 2.5th and 97.5th percentiles

MTXGlu1 parent RBC PK model

Goodness-of-fit plot and
Individual fits for 6 patients





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