

Modeling of Erlotinib and Gefitinib *in vitro* penetration through multiple layers of epidermoid and colorectal human carcinoma cells



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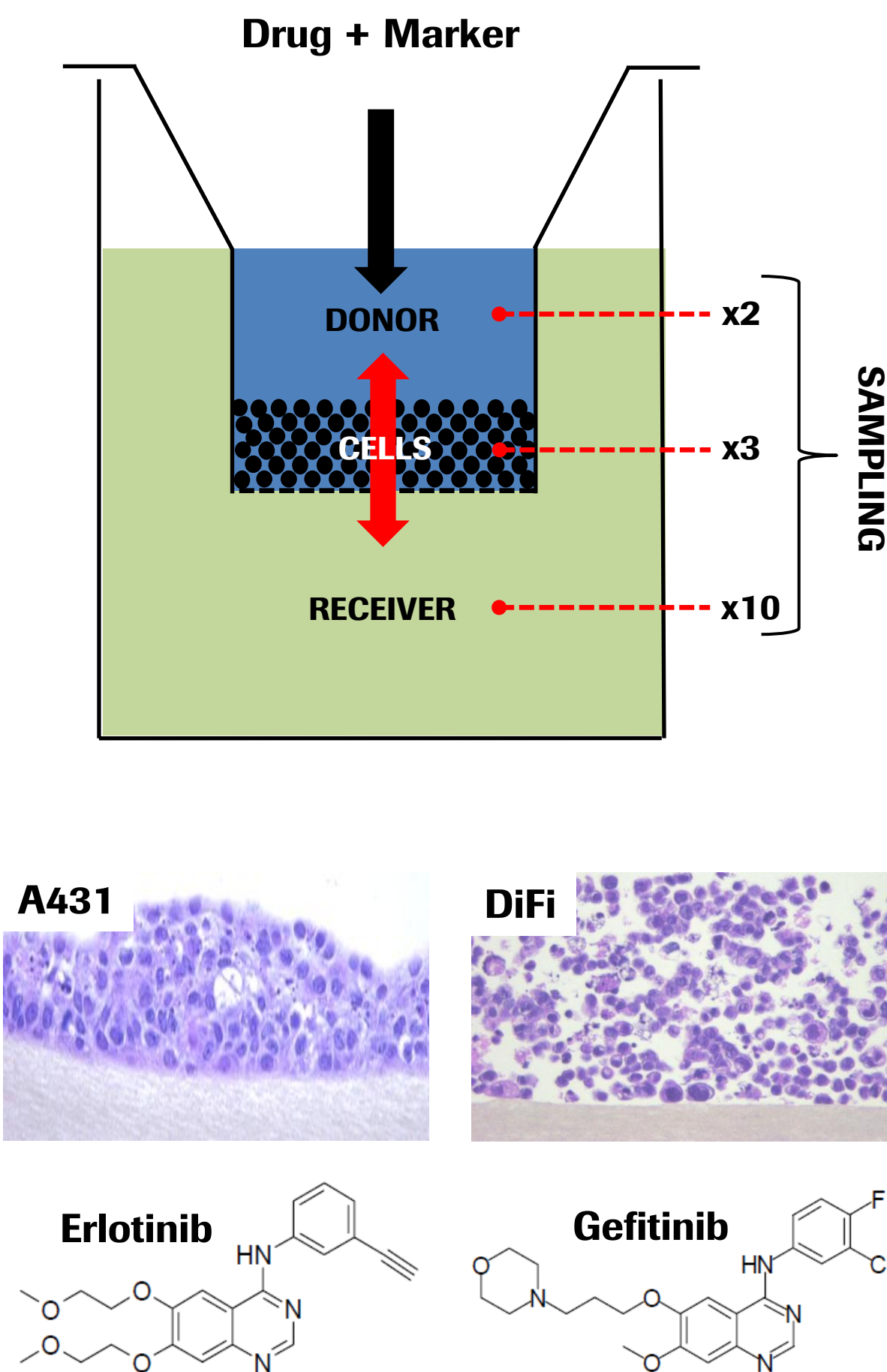
1 – Introduction

Penetration of anticancer drug in tumor tissue is an important factor to achieve exposure of cancer cells to efficacious concentrations and thus a therapeutic effect [1]. Erlotinib and Gefitinib are selective and potent inhibitors of the epidermal growth factor (EGFR) in human [2]. EGFR is expressed in the selected epidermoid (A431) and colorectal (DiFi) human carcinoma cells [3]. The objective of this study was to develop a model based approach to characterize and differentiate the *in vitro* penetration of Erlotinib and Gefitinib for 2 cancer cell lines.

2 – Material and methods

Experimental settings:

- A multicellular layers (MCL) system [4] was used to explore the drug penetration kinetics through a Teflon membrane (control) and cancer cell layers.
 - Control, 3 or 6 cell layers.
 - A431 or DiFi cells.
 - Erlotinib or Gefitinib were co-administered with an integrity marker (Acridone).
 - Drug concentrations (triplicates) were measured in donor, cell lysate and receiver by LC-MS/MS.
- A431 cells: well adherent to the teflon, well organized and pluristratified (6 layers ~75µm).
- DiFi cells: detached by the membrane, disorganized with apoptotic features (6 layers ~165µm).
- Erlotinib: LogD_{7.4} = 2.9; pKa = 5.3
- Gefitinib: LogD_{7.4} = 3.5; pKa = 5.4; 7.2

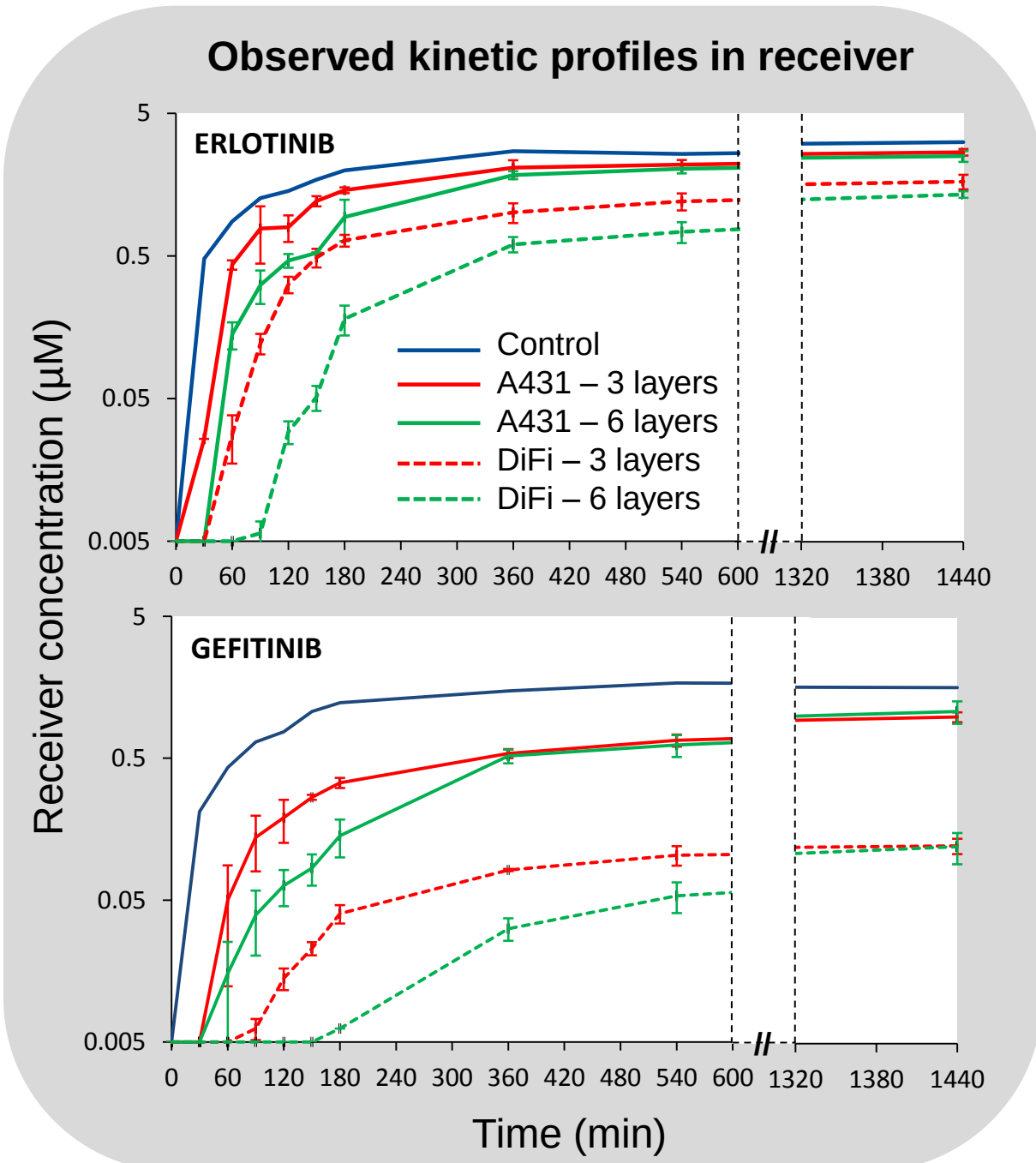


MCL data:

- In vitro* drug penetration kinetics is sensitive to:
 - cell type (A431 vs DiFi)
 - tissue thickness (3 vs 6 layers)
 - drug properties (Erlotinib vs Gefitinib)

- Drug mass balance: $MB = \frac{Q_{Ru(24h)} + Q_{Du(24h)}}{dose} \cdot 100$

Mass Balance (%)	Control	A431	DiFi
Erlotinib	100	94	54
Gefitinib	43	38	7



3 – Model structure

Model assumptions

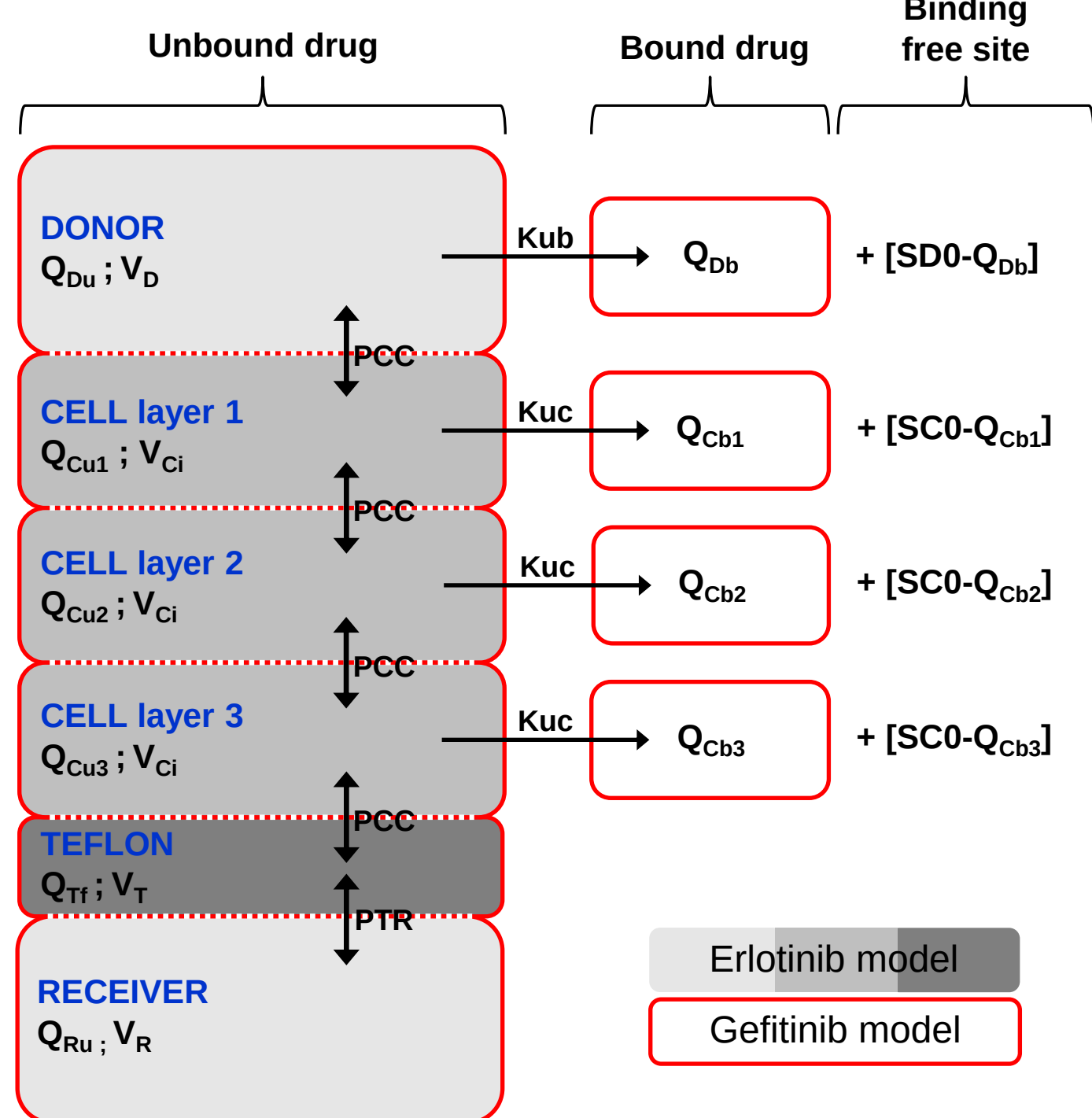
- Drug is transported by passive diffusion according to the Fick's law, based on unbound concentrations gradient.
- Binding explains the drug loss:
 - unspecific: interaction with plastic wall or chemical degradation
 - specific: cell sequestration or metabolism
- Homogeneous cell layers assimilated to compartments.
- Volume and number of cell layers is constant during the MCL experiment.
- Decrease of volume due to sampling is taken into account.

Model parameters

- Permeability of teflon (PTR) and cell (PCC).
- Volume of donor (V_D), one cell layer (V_{Cl}) and receiver (V_R).
- Binding rates in donor (Kub) and cell (Kuc).
- Binding sites in donor (SD0) and cell (SC0).

Parameters estimation

- The well is the statistical unit.
- Population approach using Monolix 4.1.3 [5].
- Simultaneous fit of all data:
 - control, 3 and 6 cell layers
 - donor, cell and receiver concentrations

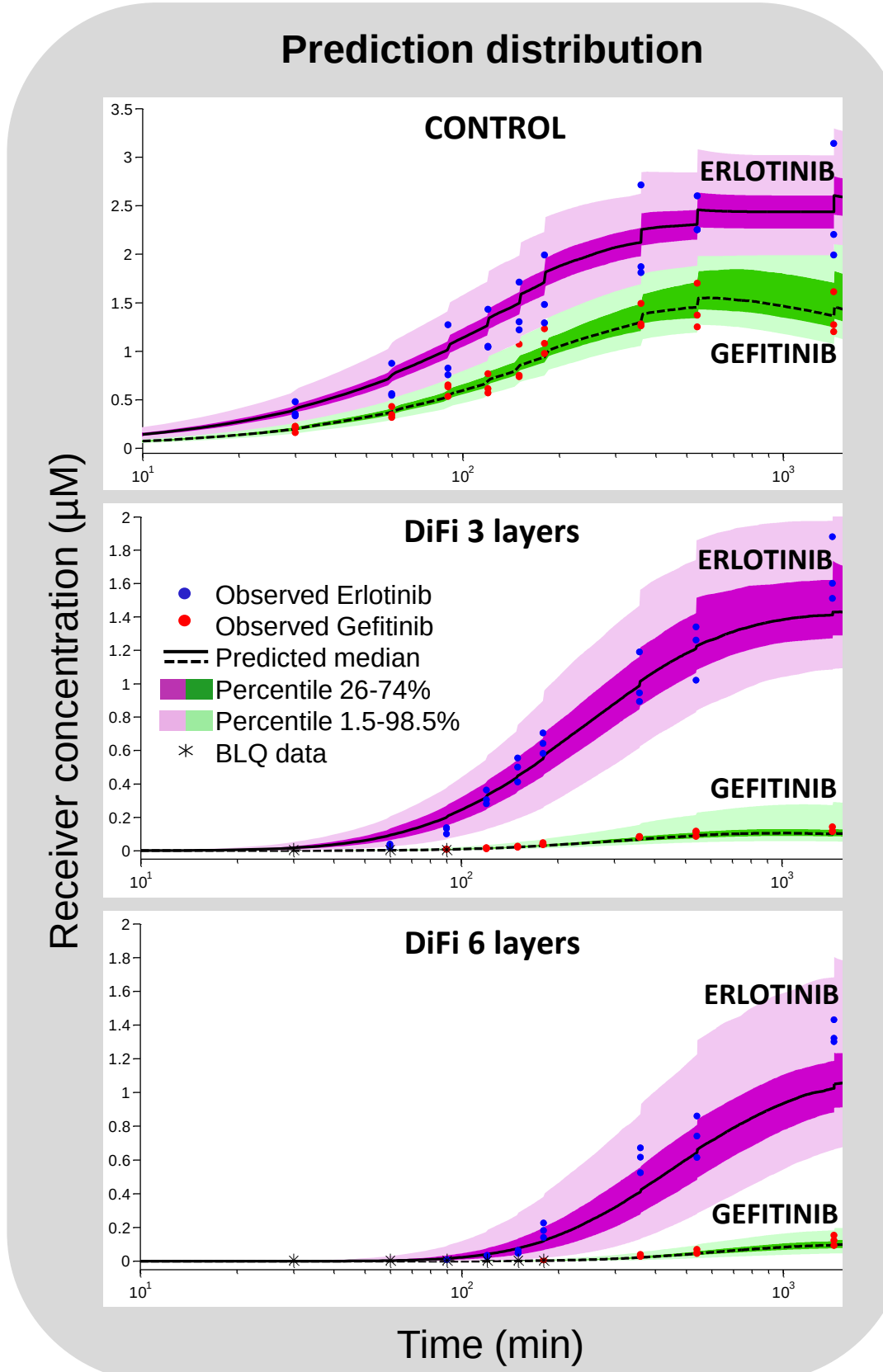
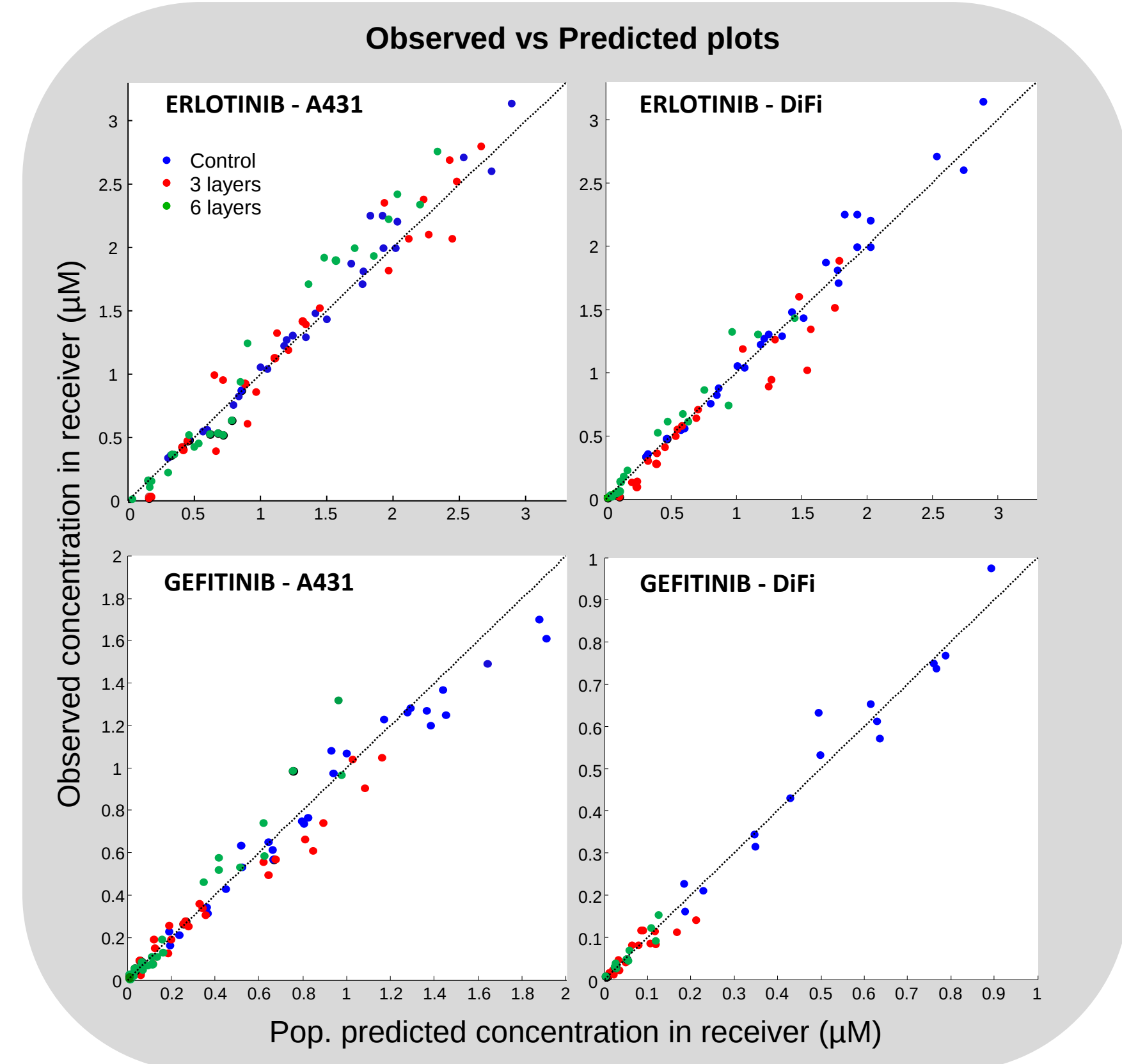
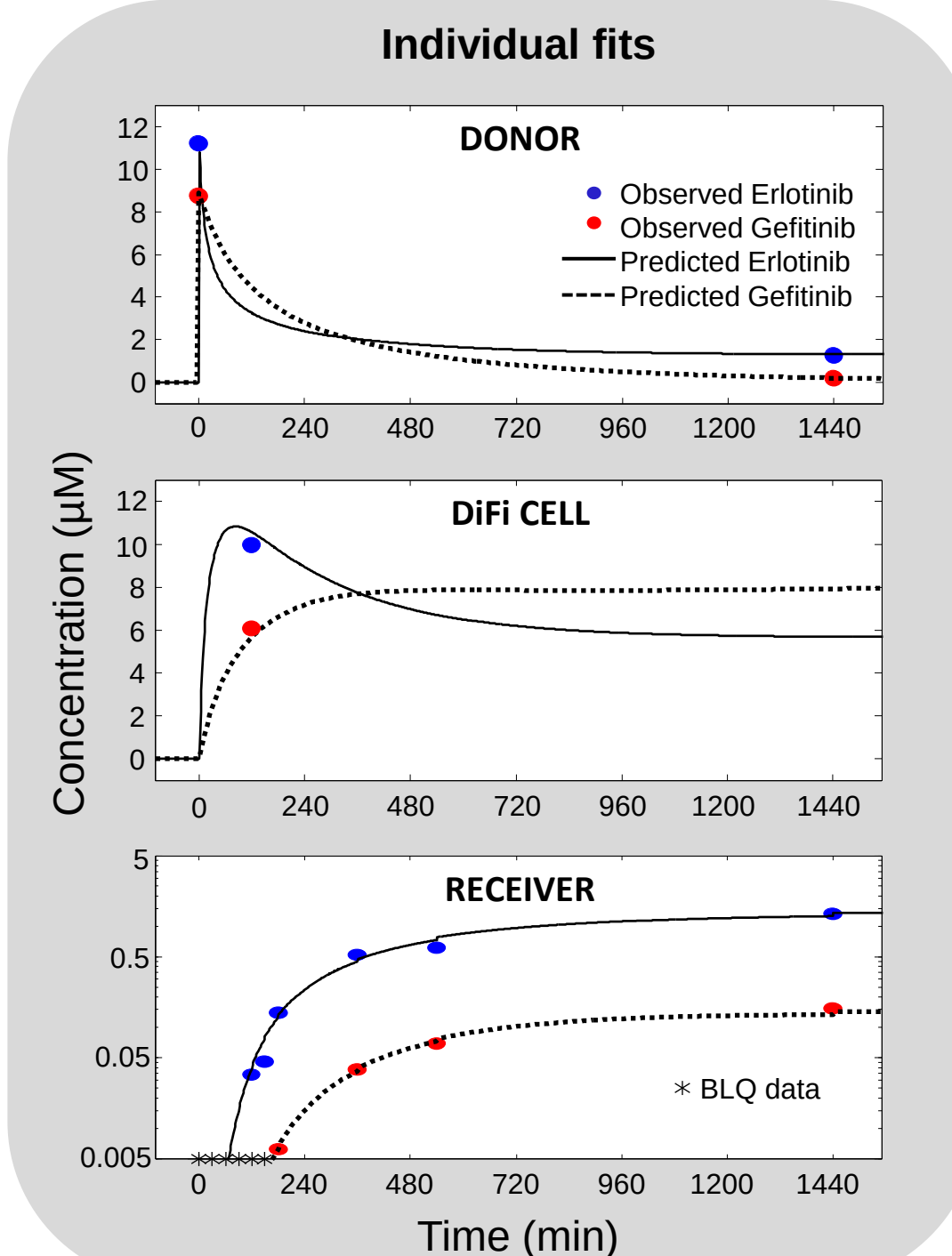


$$\begin{aligned} \frac{dQ_{Du}}{dt} &= PCC \cdot S \cdot \left(\frac{Q_{Cu1}}{V_{Ci}} - \frac{Q_{Du}}{V_D} \right) - Kub \cdot Q_{Du} \cdot (SD0 - Q_{db}) \\ \frac{dQ_{Cu1}}{dt} &= PCC \cdot S \cdot \left(\frac{-2 \cdot Q_{Cu1}}{V_{Ci}} + \frac{Q_{Du}}{V_D} + \frac{Q_{Cu2}}{V_{Ci}} \right) - Kuc \cdot Q_{Cu1} \cdot (SC0 - Q_{cb1}) \\ \frac{dQ_{Tf}}{dt} &= -PCC \cdot S \cdot \left(\frac{Q_{Tf}}{V_T} - \frac{Q_{Cu3}}{V_{Ci}} \right) + PTR \cdot S \cdot \left(\frac{Q_{Ru}}{V_R} - \frac{Q_{Tf}}{V_T} \right) \\ \frac{dQ_{Ru}}{dt} &= -PTR \cdot S \cdot \left(\frac{Q_{Ru}}{V_R} - \frac{Q_{Tf}}{V_T} \right) \end{aligned}$$

4 – Results: population analysis of MCL data

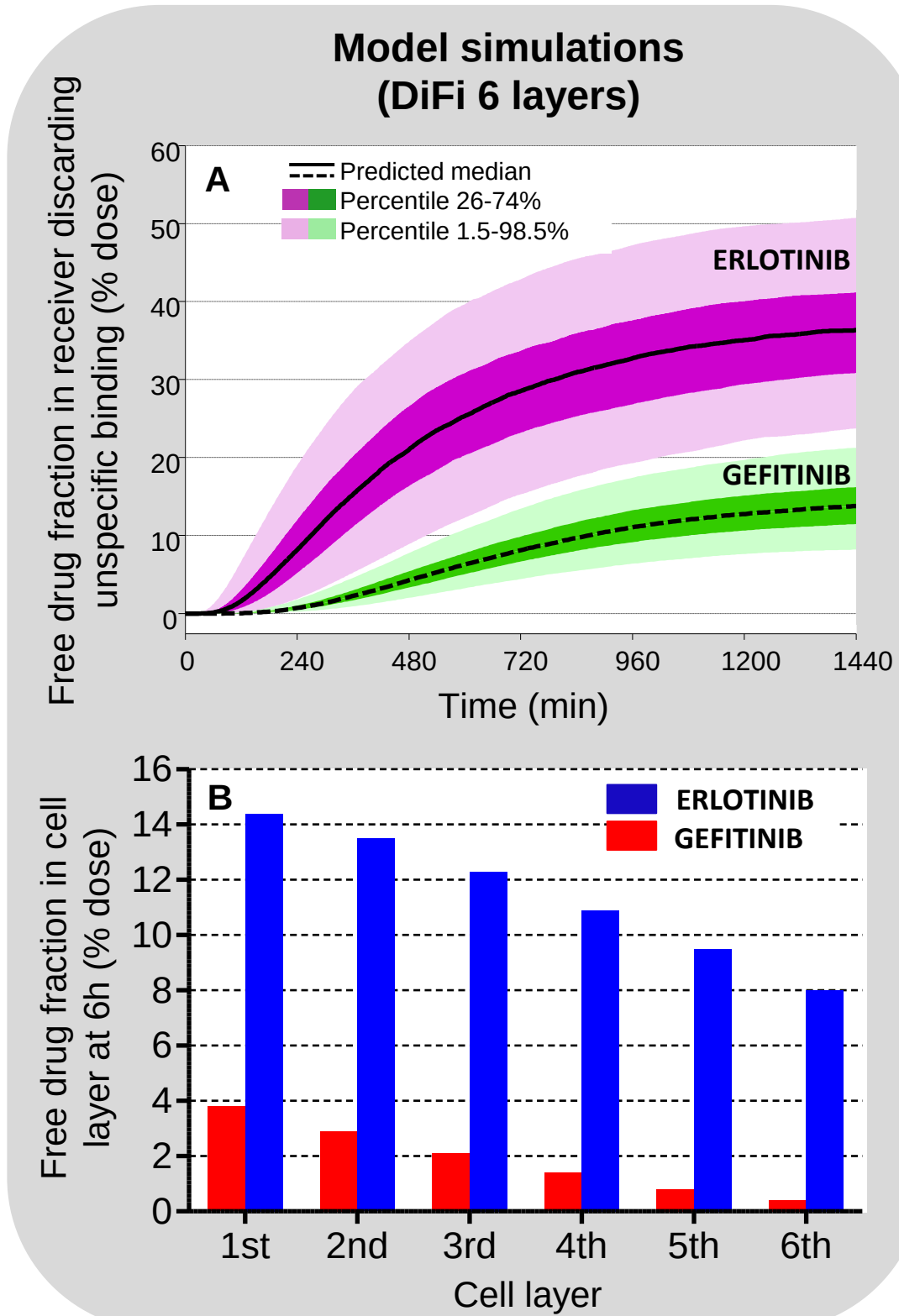
- Limited penetration for Gefitinib compared to Erlotinib especially with DiFi cells.
- Gefitinib permeability values were 2.5 and 10 fold lower than Erlotinib ones for A431 and DiFi cells, respectively.
- Unspecific and specific bindings explained the low mass balance of Gefitinib with DiFi cells. No binding component was required for Erlotinib.
- Drug mass balance was lower with DiFi than with A431. Cell uptake factor (Alp) was only required for DiFi cells.

Model parameters identification		A431 cells				DiFi cells			
Parameter	Unit	Erlotinib	RSE%	Gefitinib	RSE%	Erlotinib	RSE%	Gefitinib	RSE%
Fixed effect									
PTR	nm.min ⁻¹	11.0	6	4.56	4	11.2	5	4.36	6
PCC	cell permeability nm.min ⁻¹	32.4	34	12.6	31	28.5	20	2.68	39
Vci	cell layer volume µL	56.8	27	53.7	18	424	14	75.0	21
Alp	cell uptake factor fold	0.88	25	1.14	22	4.50	13	4.98	22
Kub	unspecific binding rate µmol ⁻¹ .min ⁻¹	-	-	1.26	19	-	-	1.21	6
SD0	total donor binding sites nmol	-	-	3.62	4	-	-	3.97	7
Kuc	cell binding rate µmol ⁻¹ .min ⁻¹	-	-	-	-	-	-	0.71	31
SC0	total cell binding sites nmol	-	-	-	-	-	-	2.44	38
Inter-well variability									
PTR	%	-	-	-	-	-	-	10	-
PCC	%	-	-	-	-	40.4	43	16.3	17
Vci	%	10	-	10	-	33.3	35	20	-
Kub	%	-	-	38.6	37	-	-	-	-
SD0	%	-	-	10	-	-	-	10	-
Kuc	%	-	-	-	-	-	-	10	-
SC0	%	-	-	-	-	-	-	10	-
Error model									
a1	additive in donor µM	0.25	17	0.16	24	0.15	19	0.13	24
b2	proportional in cell %	58.6	29	39.0	37	54.5	28	26.3	17
b3	proportional in receiver %	12.7	12	11.0	12	10.4	12	10.6	6



5 – Discussion

- In vitro* tumor tissue heterogeneity was summarized by a space discretization (compartments).
- Several binding models have been tested for both drugs (reversible/irreversible, saturable/unsaturable).
- Impact of sampling on volume must be considered.
- Acridone data analysis revealed no drug cell-killing effect on penetration kinetics.
- Discarding the unspecific binding, free amount of Gefitinib in receiver [$Q_{Ru}/(dose - Q_{Db})$] at 24h is 2.6 fold lower than Erlotinib (Figure A).
- Free Gefitinib amount in DiFi cells is limited due to extensive cell binding. At 6h, fractions of dose reaching the cell layers were 3.8 to 0.4 % for Gefitinib and 14.4 to 8 % for Erlotinib (Figure B).



6 – Conclusion

- In this MCL system, Gefitinib showed much more limited penetration than Erlotinib, especially with DiFi cells.
- Model based analysis allowed characterization and differentiation of drugs penetration kinetics for both cell lines. These results provide mechanistic insights for *in vivo* PK/PD modeling of cell growth inhibition with drug permeability and cell binding characteristic.
- The modeling approach is proposed as a screening tool to differentiate compounds based on the *in vitro* penetration capabilities.

References

- [1] Minchinton et al. Nat Rev Cancer 2006; 6:583-92.
- [2] Scheffler et al. Clin Pharmacokinet 2011; 50:371-403.
- [3] Untch et al. Cancer Res 1993; 53:1630-36.
- [4] Tannock et al. Clin Cancer Res 2002; 8:878-84.
- [5] Kuhn et al. Computational Statistics & Data Analysis 2005; 49:1020-38.