

Modeling of Erlotinib Cancer Cell Growth Inhibition Measured by *in vitro* Impedance-based Real-Time Cell Analysis System

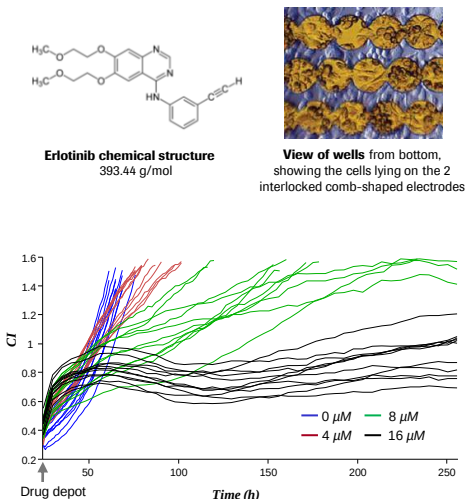


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Introduction

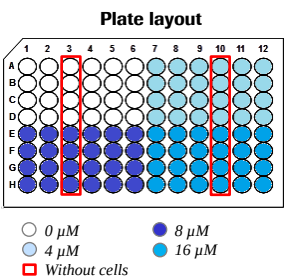
- Erlotinib is an EGFR tyrosine kinase inhibitor used in advanced Non-Small Cell Lung Cancer [1].
- The xCELLigence Real-Time Cell Analysis (RTCA) system allows continuous *in vitro* monitoring of drug effects on cancer cell count and morphology [2]. The readout *Cell Index* (CI) is derived from the impedance measurement between two electrodes at the bottom of cell culture wells.
- Mathematical models have been earlier developed to describe the time-dependent effects of anti-cancer drugs *in vitro* [2] and *in vivo* [3]. Our objective was to explore the potential benefit of RTCA data to describe the effect of Erlotinib on the A431 epidermoid human carcinoma cell line.



Material & Methods

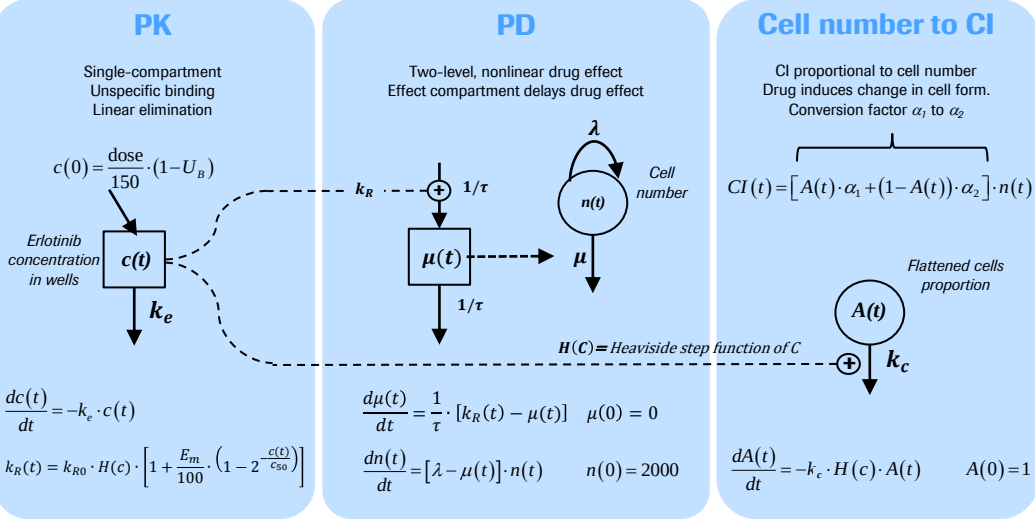
Experimental setting

- Growth dynamics measurement in xCELLigence system [4].
- 4 Erlotinib concentration groups: 0, 4, 8, 16 μM (4 quadrants/plate).
- 3 plates of 96 wells seeded each with 2000 A431 cells at t=0, excluding columns 3 and 10.
- Dosing at t = 24h after seeding.
- One plate was harvested at 24, 144, and 240h, for PK measurement by LC-MS/MS.



Methodology

- PK/PD population approach using the software Monolix 4.1.3 [5].
- As the statistical unit the cell culture well was chosen.
- PK analyzed separately and fixed for PD analysis.
- Selected CI range: 0 to 1.6 to avoid signal saturation.



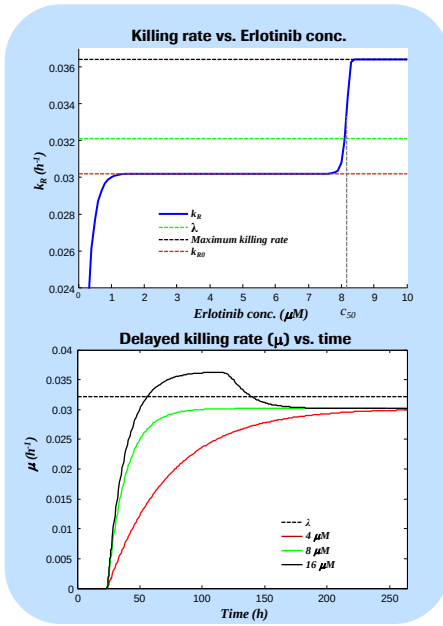
Discussion

Killing rate model:

- Input rate k_R of the effect compartment is a nonlinear function of drug concentration with two-level, nonlinear drug effect.
- Drug concentrations ranging from 1.25 to 8 μM resulted in a k_R equal to 94% of the growth rate λ of A431 cells confirming the cytostatic properties of Erlotinib.
- Drug concentrations above 8 μM led to a cell killing rate exceeding the growth rate by 13%.

Effect delay τ :

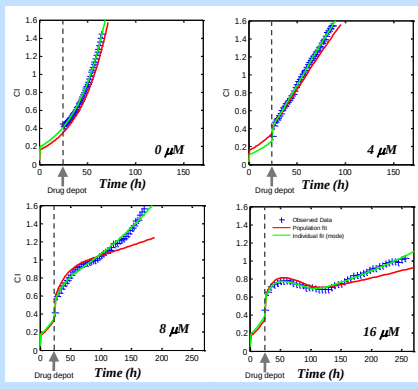
- Significant difference in τ were observed between [0,4] and [8,16] μM concentration groups.
- Continuous relationship between τ and PK could be established.



Results

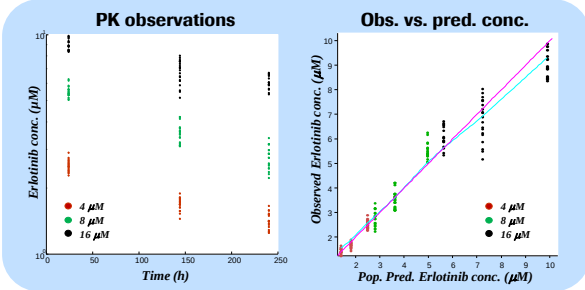
Parameters	Units	Fixed effects		Random effects		Description
		Estimation	Estimation	Estimation	Estimation	
k_e	h^{-1}	0.00263	3	0.0708	38	Drug elimination rate
U_B	-	0.342	2	-	-	Fraction bound (unspecific)
b_{PK}	-	0.1	-	-	-	PK proportional measurement error
k_{R0}	h^{-1}	0.0302	5	0.205	16	Sub-cytostatic killing rate
E_m	%	20.5	13	0.246	34	Maximum increase of k_{R0}
C_{50}	μM	8.16	1	-	-	Transition from sub-cytostatic (k_{R0}) to cytotoxic effect
h	-	100	-	-	-	Sigmoid factor
$\tau_{0 \text{ and } 4 \mu M}$	h^{-1}	49.8	16	0.311	18	Effect delay for 4 μM conc. group
$\tau_{8 \text{ and } 16 \mu M}$	h^{-1}	14.8	8	0.311	18	Effect delay for 8 and 16 μM conc. groups
λ	h^{-1}	0.0321	2	0.105	15	A431 cells growth rate
α_1	$cell^{-1}$	7.95E-05	4	0.197	13	Conversion factor from number of cells to CI for control
$\alpha_{2, 4 \mu M}$	$cell^{-1}$	9.94E-05	5	0.111	13	Maximum conversion factor for 4 μM
$\alpha_{2, 8 \text{ and } 16 \mu M}$	$cell^{-1}$	1.30E-04	4	0.111	13	Maximum conversion factor for 8 and 16 μM
k_c	h^{-1}	2.07	3	-	-	Cells shape change rate
a_{PD}	-	0.025	5	-	-	PD additive measurement error
b_{PD}	-	0.014	13	-	-	PD proportional measurement error
ρ	-	0.87	1	-	-	PD autocorrelation in measurement error

Some examples of Cell Index individual fits



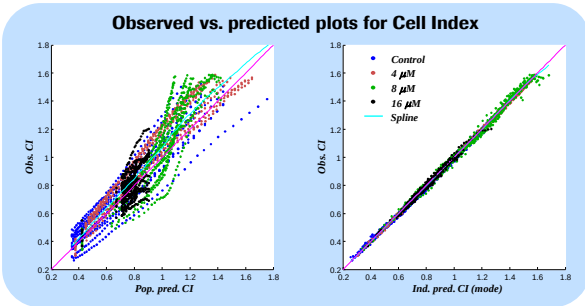
PK results

- Concentration range: 1.25 to 9.88 μM.
- Fraction of drug amount available: 66%.
- Typical half-life: 114.5h.
- Low estimated k_e dispersion (0.07).
- No difference between wells with and without cells.
- Proportional measurement error ($b_{PK} = 0.1$).

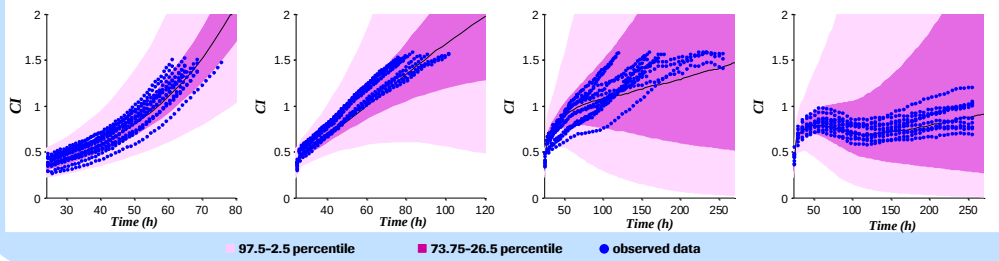


PK/PD results

- Significant autocorrelation (0.87) of measurement error was determined.
- Low additive ($a_{PD} = 0.025$) and proportional measurement error ($b_{PD} = 0.014$).
- Concentration group was determined as a categorical covariate on τ and α_2 .
- Close values for λ (0.0321 h^{-1}) and k_{R0} (0.0302 h^{-1}).



Predictions distribution stratified by conc. group

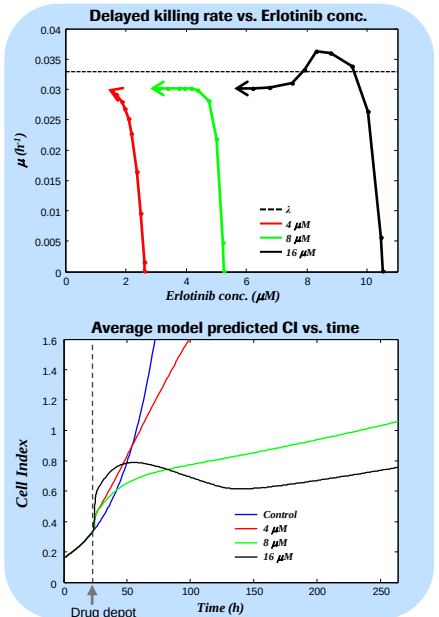


Cell Index and cell number relationship

- Just after drug administration, conversion factor α was increased up to 65% from the control value.
- In agreement with known cell morphology change with EGFR tyrosine kinase inhibitor [6].

Final PK-PD model

- The proposed PK/PD model well described the loss of drug effect after 130h in the 16μM concentration group.
- Observed drug loss over time allowed identification of a double-step $k_R = f([Erlotinib])$ model.
- Initial drug concentration was reduced by unspecific binding and drug loss occurred as a function of time. Additionally, incubation medium protein binding was explored and free fraction of Erlotinib was determined to be 63 ± 4 %.



Conclusion

- The rich dynamic data collected in this RTCA assay combined with the model based analysis allow both testing hypotheses on drug action and quantifying drug effect kinetics at a cellular level.
- The loss of exposure confirmed by PK measurement allows transformation of the *in vitro* system in a PK/PD system. We recommend a control for effective exposure in this type of in vitro systems
- Our model suggests that drug concentration drives the effect from sub-cytostatic (close to growth rate λ) to cytotoxic (above λ) levels and influences the delay in the cell killing rate signal.

- This model based approach helped to build and test hypotheses of drug action as well as quantify drug effect kinetics at a cellular level.
- A dynamic conversion factor model has been developed to take into account the change of cell shape and the resulting modification in Cell Index.
- As a perspective, additional experiments (as cell count) could be designed to confirm drug-induced cell shape changes.

References

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