Integrated analysis of preclinical data to support the design of the first-in-human study of LY2181308 a second generation antisense oligonucleotide.

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Introduction:

A modified 2'-O-methoxyethyl 18-mer second generation ASO, LY2181308 specifically inhibits survivin mRNA, a gene coding for survivin protein.

Survivin protein, a member of the inhibitor of apoptosis protein (IAP) family, is specifically expressed in a wide range of human cancer tissues.
Over-expression of survivin protein is associated with a poorer prognosis.

Hence survivin is an attractive molecular target for therapeutic intervention.

Aims:

To predict clinical efficacious dose range
To select appropriate dosing regimen (dosing schedule and amount) for the first-in-human dose study of LY2181308.

To predict LY2181308 plasma and tissue concentration and target inhibition profiles in human.

To predict the range of LY2181308 concentration level needed for relevant efficacious target inhibition.

Material:

•Mice tissue and tumor LY2181308 PK data, and pharmacodynamic (PD) data (i.e survivin inhibition data) and efficacy (tumor growth delay) data
•Monkey plasma and tissue PK data.
•Human LY2181308 plasma and tumor biopsies and [^{11}C]LY2181308 PET concentration data and survivin target inhibition data (from tumor biopsies).

Method:

Non-linear mixed effect modeling technique (NONMEM V) was used to built

•An indirect pharmacodynamic (PD) model describing survivin mRNA and protein inhibition using preclinical target inhibition and tumor growth delay data from mouse xenografts.
• An integrated multi-compartmental plasma-tissue PK model using monkey preclinical data.

Monkey PK parameter were used to predict human PK parameters (allometric scaling).
Assumption that PD parameters values in human would be the same as the ones determined for mice

Clinical PK/PD profiles were simulated (1000 Monte-Carlo simulations).

Results:

Average LY2181308 concentration ≥ 10 µg/g predicted to lead to relevant target inhibition for anti-tumor activity
Model predicted LY2181308 tumor concentrations 18.8 to 54 µg/g (range) and target inhibition 50 to 90 % (5th-95th percentiles) following LY2181308 750 mg in humans.

Clinical data showed LY2181308 tumor concentrations 13.9 to 52.8 µg/g (range, n=4 patients), median survivin mRNA and protein inhibition of 20 % +/- 34 (SD) (n=9) and 23 % +/- 63 (SD) (n=10), respectively following 750 mg.

Two patients data showed little survivin protein expression at baseline hence no reduction post treatment. When these data are excluded, the results are median survivin mRNA and protein inhibition of 34 % +/- 21 (SD) (n=7) and 41 % +/- 38 (SD) (n=8), respectively following 750 mg.

Multi-compartmental PK model adequately described LY2181308 PK data. LY2181308 has an extensive volume of distribution (> 10000 L) and low-moderate clearance (23.1 L/h) leading to a long terminal half life 32.7 days (range 22-52 days).

The model over-estimated PD effect and adequately predicted PK parameters (median difference observed - predicted PK parameters value 20 % (range 1-55).

Conclusion

Integration of LY2181308 PK and PD preclinical data help design the first-in-human study of LY2181308 and predict with reasonable accuracy its outcome.

Survivin mechanism of action

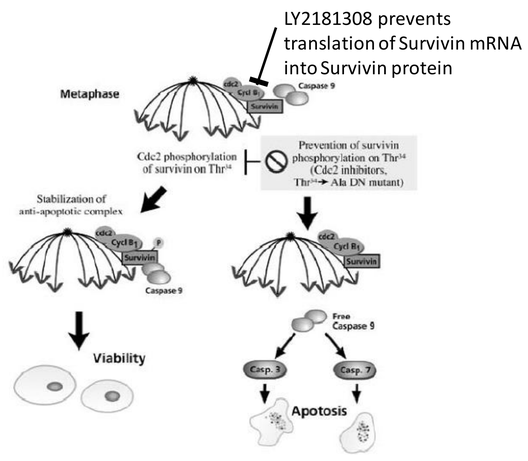


Figure 1: Schematic representation of Survivin activity in the mitosis (adapted from Altieri, 2003)

Role of Survivin in mitosis and cell division
Immunochemically distinct survivin pools are localize to multiple components of the mitotic apparatus, including centrosomes, metaphase and anaphase spindle microtubules and midbodies (telophase).

Material

Table 1: dose administered and sampling scheme for PK and PD data

	Doses administered
Mice single dose+**	5, 20 and 50 mg/kg IV bolus
Mice Multiple dose**#	50 mg/kg IP as a loading dose 25 mg/kg IP every other day for 12 doses (maintenance dose)
Monkey Single dose	20 mg/kg over 3 hours IV infusion; n=3 animals
Monkey Multiple dose Loading dose	45 mg/kg over 72 hours IV infusion (n=6 animals)
Monkey Multiple dose Maintenance dose	4 mg/kg over 3 hours IV infusion twice a week for 7 doses (starting day 12 until day 33) (n=6 animals)
	Sampling scheme Time relative to the start of the last infusion administered
Mice single dose+**	4, 8 , 24, 48, 72, 96 h**
Mice Multiple dose**#	Tumor volume measured every 4 days for the 25-days-treatment period and then every 5 days until 11 days after treatment discontinuation
Monkey Single dose	Predose, 1, 3*, 3.25, 3.5, 4, 4, 6, 10, 27[&] h
Monkey Multiple dose Loading dose	Predose, 24, 48, 72*, 72.25, 72.5, 74, 78, 82, 96, 120 h
Monkey Multiple dose Maintenance dose	On day 18 : Predose, 3*, 24 h On day 33 : Predose, 3*, 24, 120[&] h
	First in human dose PK and PD data
Dose levels (# of patients)	100 (1), 200 (1), 400 (4), 600 (3), 750 (26), 900 (3), 1000 (2) mg 3 hours infusion on day1, day2 and day3 (loading doses) and then weekly afterward (maintenance doses starting day8).
PK Sampling	Day 1: 3 hours (end infusion) post dose Day 3: predose, 3 (end of infusion), 3.25, 3.5, 4, 6, 7 to 8, and 27 to 60 hours post dose) Day8: predose Day 15: predose, 3 (end of infusion), 9 and 24 hours post Day22: predose
Tumor biopsy sampling	Within 14 days prior to dosing 48-to-96-hours time window (study days 5-7) following end of dose on day 3

* End of infusion samples; + plasma PK and PD (tumors) sampling scheme (6 animals per time points), ** various tissues sampled for PK analysis (6 animals per time points); #tumor growth delay experiment; & tissue collected for PK assessment. 3 and 6 animals in the single and multiple dose monkey study, respectively.

Methods

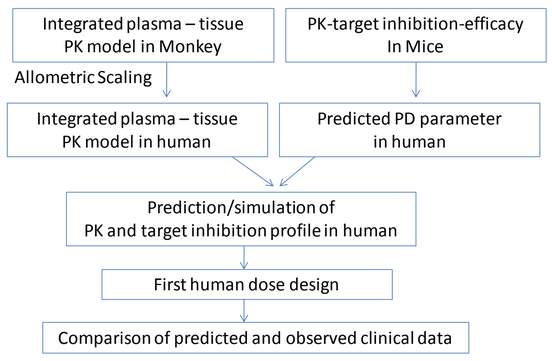


Figure 2: Modelling analysis strategy

Results preclinical mice PK/PD

•A 50 mg/kg loading dose followed by 25 mg/kg every other day for 12 doses led to efficacy with significant tumor growth delay.

•The same loading dose of 50 mg/kg lead to
- •A) significant inhibition, 60 to 70 %, of survivin protein expression within 24 to 48 hours post dose.
- •B) maximum LY2181308 concentration of 13 and 19 µg/g, in muscle and lung (low uptake tissue for ASOs) respectively.

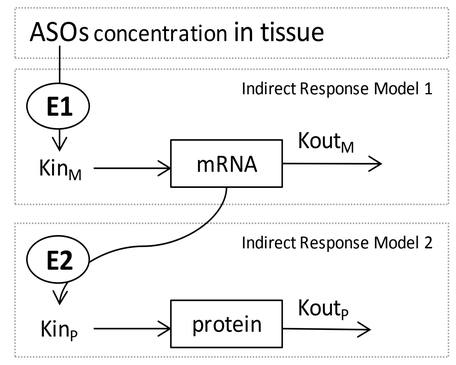
•Hence, average LY2181308 tumor concentration of ~ 10 to 20 µg/g should be expected to lead pharmacologically relevant target inhibition (~ 60%) for anti-tumor activity.

•LY2181308 peak tissue concentration and maximum target inhibition occured at approximately the same time (~ 24 h post administration). Hence survivin mRNA and survivin protein must have rapid half-lives or turn-over rate.

Table 2: Preclinical PD parameters

Parameters	Value	
EC50[a] on Survivin mRNA	20 µg/g	
Kout M, rate constant of degradation of survivin mRNA [b]	1.386 h^{-1}	Tkout M = 30 min
Kout P, rate constant of degradation of survivin protein [b]	0.347 h^{-1}	Tkout P = 2 h

[a] EC50: concentration leading to 50 % inhibition of the target;
[b] Tkout M, survivin mRNA half life; Tkout P, survivin mRNA half life



•KinM and KoutM: rate constant of synthesis and degradation of survivin mRNA assumed to be equal in the absence of LY2181308

•KinP and KoutP: rate constant of synthesis and degradation of survivin protein assumed to be equal in the absence of LY2181308

•In the presence of treatment by LY2181308, KinM is decreased and KinP is decreased.
•E1 and E2 effect of the treatment sigmoidal Emax relationships.

Figure 3: Schematic representation of Survivin PD model

Results: Preclinical Monkey PK model

•Low uptake tissues included, muscle, lung, jejunum, lymphatic nodes, prostate, pancreas, spleen and skin - with LY2181308 concentrations ranging from 10 to 70 µg/g.

• High uptake tissues included the liver and kidney medulla – with LY2181308 concentrations ranging from 100 to 400 µg/µg (concentration in the kidney cortex was even higher (700 to 800 µg/g)

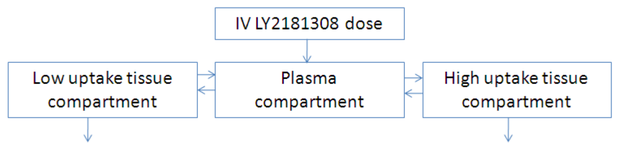


Figure 4: Schematic representation of Survivin PD model

Figure 5 and table 3 below illustrate that the preclinical monkey PK model adequately describes the data

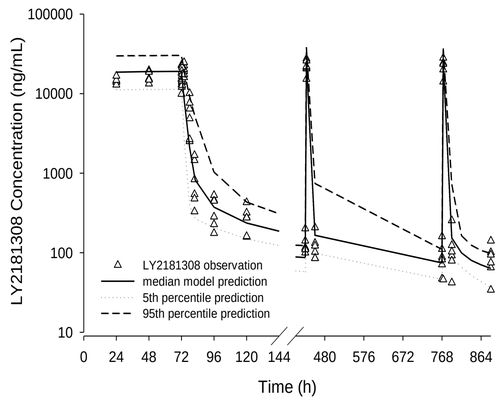


Figure 5 : LY2181308 plasma versus time profile in monkey (Posterior predictive check for LY2181308 monkey PK model)

Table 3: LY2181308 tissue concentration in monkey (observation and model simulation).

Peripheral (tissue) Pharmacokinetics			
Parameter	**Unit**	**observed**	**Model Simulated**
Low uptake tissues	µg/g	10 to 70	49 to 119 (median 70)
High uptake tissues	µg/g	335 to 688	83 to 558 (median 220)

Prediction to human

Allometric scaling with a coefficient of 1 was used to predict the human PK parameter based on the monkey PK model (see table 4 and 5).

Table 4: Predicted LY2181308 PK parameters based on the preclinical monkey model

Plasma pharmacokinetics : distribution clearance from central to peripheral compartments central volume of distribution		
Parameter	**Unit**	**Mean Value**
Central volume of distribution	L	6.27
Distribution clearance #1 +	L/h	2.51
Distribution clearance #2 +	L/h	0.0648
Peripheral (tissue) Pharmacokinetics : elimination clearance and peripheral volumes of distribution		
Parameter	**Unit**	**Mean Value**
Peripheral volume of distribution #1	L	17925
Peripheral volume of distribution #2	L	1.97
Elimination Clearance	L/h	51.8
Terminal half-life	days	9

Table 5: Predicted LY2181308 exposure in human .

	PREDICTIONS (mean 5th- 95th percentile)		
Dose	Cmax plasma	AUC plasma	$C_{average}$ at steady state* in low uptake tissues
mg	ng/mL	ng*h/mL	µg/g
100	8732 5651 – 12920	37830 17856 – 77605	4.4 2.5 – 7.2
200	17464 11302 – 25839	75660 35713 -155211	8.8 5.0 – 14.4
400	34927 22604 – 51679	151320 17425 - 310422	17.7 10.0 – 28.8
600	52391 33907 – 77518	226980 107137 - 465633	26.5 15.0 – 43.2
750	65489 42838 – 96897	283725 133922 - 582041	33.2 18.8 – 54.0

Results clinical PK/PD model

Figure 6: schematic representation of the clinical PK model.

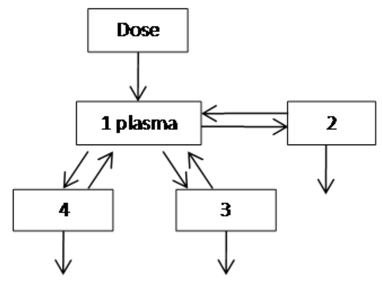


Table 6 : LY2181308 plasma PK parameters in human .

Plasma pharmacokinetics : distribution clearance from central to peripheral compartments central volume of distribution			
Parameter	**Unit**	**Mean Value (SEE %)[a]**	**CV (%) (SEE %)[a]**
Central volume of distribution	L	4.09 (8.95)	18.0 (143)
Distribution clearance #1 +	L/h	2.54 (4.06)	15.4 (44.2)
Distribution clearance #2 +	L/h	0.0608 (29.6)	NE[a]
Distribution clearance #3 +	L/h	1.67 (23.8)	NE[a]
Peripheral (tissue) Pharmacokinetics : elimination clearance and peripheral volumes of distribution			
Parameter	**Unit**	**Mean Value (SEE %)[a]**	**CV (%) (SEE %)[a]**
Peripheral volume of distribution #1	L	25900 (16.3)	38.6 (31.6)
Peripheral volume of distribution #2	L	0.936 (22.5)	NE[a]
Peripheral volume of distribution #3	L	2.51 (11.6)	45.2 (51.9)
Elimination Clearance*	L/h	23.1	19.2
Terminal half-life	days	32.7 (22 – 52)[b]	20.1

Residual proportional variability, 28.8 % (32.9 %)

a SEE standard error on the estimates – CV coefficient of variation – NE non estimated
b Mean (range)
+distribution clearance #1, #2 and #3 = LY2181308 distribution from central compartment to first, second and third peripheral compartment, respectively.* not estimated directly by the model but derived from model estimated parameters hence no SEE available.

Table 7: Observed LY2181308 plasma exposure in human .

		OBSERVATIONS (mean (CV %))	
Dose	N a	Cmax	AUC
mg		ng/mL	ng*h/mL
100	1	7060	27161
200	1	12815	69733
400	4	31370 (23.6)	152637 (21.1)
600	3	45654 (13.1)	211467 (23.9)
750	24	69120 (34.2) 39923 – 155514[b]	342794 (31.5) 187344 – 603944[b]
900	1 d	86787	251031
1000	1 c	84037	425279

a Number of patients;
b Range;
c Following a 4-hour infusion, instead of 3 hours, in order to prevent peak related toxicity;
d On Day 3, this patient was dosed over 1.17 hours instead of the 4 hour prescribed for the higher doses.

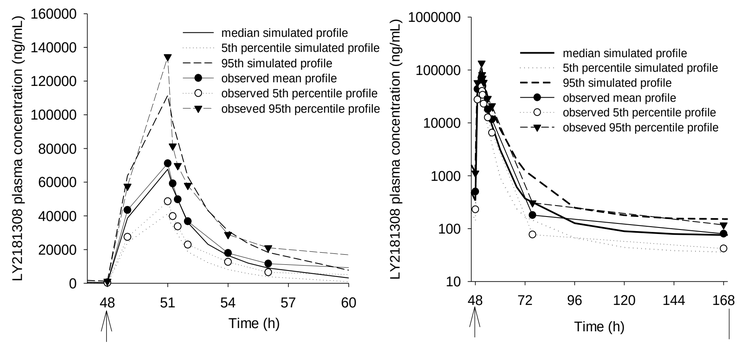


Figure 7 : Posterior predictive check for LY2181308 clinical PK model

Table 8: Predicted and observed exposure in human tumor tissue.

Tumor concentration Pharmacokinetics			
Parameter	**Unit**	**Observed (n=4)**	**Model prediction**
C average	µg/g	(13.9 – 52.8) (median 32.5)	18.8 - 54 (median 33.2)

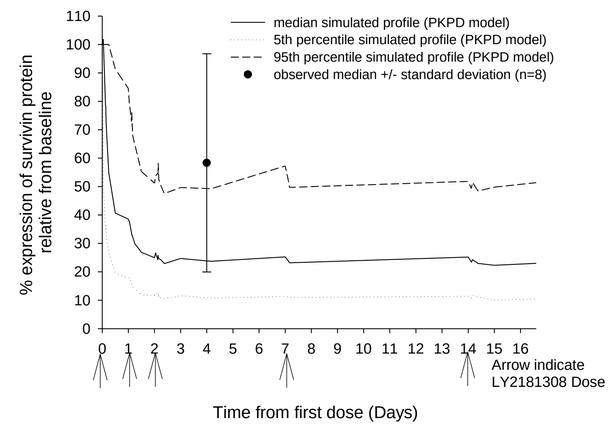


Figure 8 : Comparison of predicted and observed PD response in human.

Discussion Conclusion

•The preclinical PK model accurately predicted human plasma exposure and tumor concentrations. The terminal half-life which accounts for only <10% of the overall plasma AUC was less reliably estimated.
•Overall, the difference between the predicted and observed PK parameter values remained reasonable (median 20% (range 1.4 to 55 %)).

•The PD model predicted that LY2181308 dose > 400 mg, should lead to significant > 60% target inhibition. The observed clinical data indicated that target inhibition was indeed observed in the patient population following 750 mg dose.

•The integration of LY2181308 PK and PD preclinical data within a quantitative model did help design the first-in-human study of LY2181308 and predict with reasonable accuracy.

Lilly Answers That Matter