INTEGRATED *IN VITRO* AND *IN VIVO* APPROACHES TO DRUG METABOLISM INVESTIGATION – A STUDY CASE

Massimiliano Fonsi

Meet the Experts Transporter Conference
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EVERY STEP OF THE WAY

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Introduction

An ideal *in vitro* DMPK model should include all the pathways that are relevant for the PK *in vivo*. Quantitative scaling to whole organ/body is essential for the prediction of exposure of parent drug and its metabolites *in vivo*.

The liver is generally considered the organ most involved in metabolic transformations and in vitro screening systems are based essentially on liver tissue fractions. More frequently, metabolism in the gut is also considered. Conversely, metabolism in other organs is rarely taken into consideration.

The case study presented today will offer an example of

1) The experimental evidence that should trigger the investigation of extrahepatic metabolism
2) The differences that may exist between preclinical species and humans*
3) The impact of extrahepatic metabolism on screening cascades and decisions process in a drug discovery project.

*Extrahepatic metabolism may complicate the IVIVC in rats.
Fonsi M - Drug Metab Lett. 2014;8(1):51-66.

Discovery of MK-4827 (Niraparib)

Poly(ADP-ribose)polymerase (PARP) as a context specific anti-cancer target

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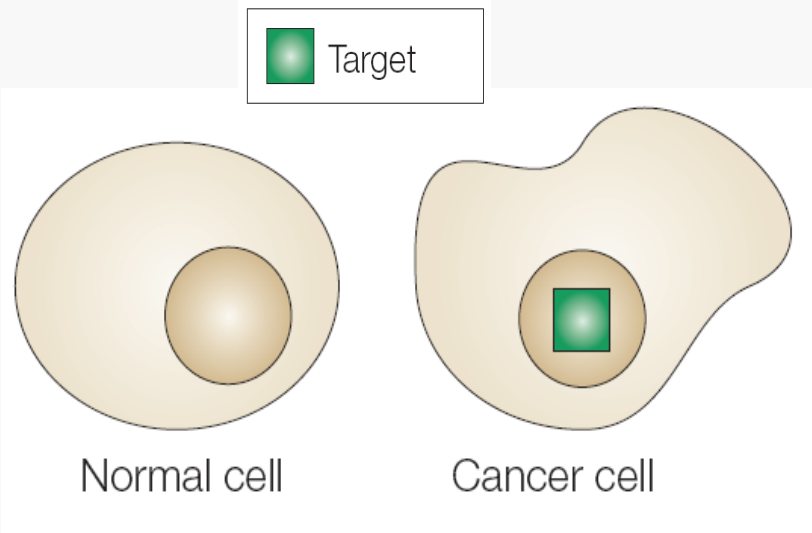
Can we selectively kill tumor cells?
The Synthetic Lethality approach

- **Most anticancer drugs in use today have a low therapeutic index**

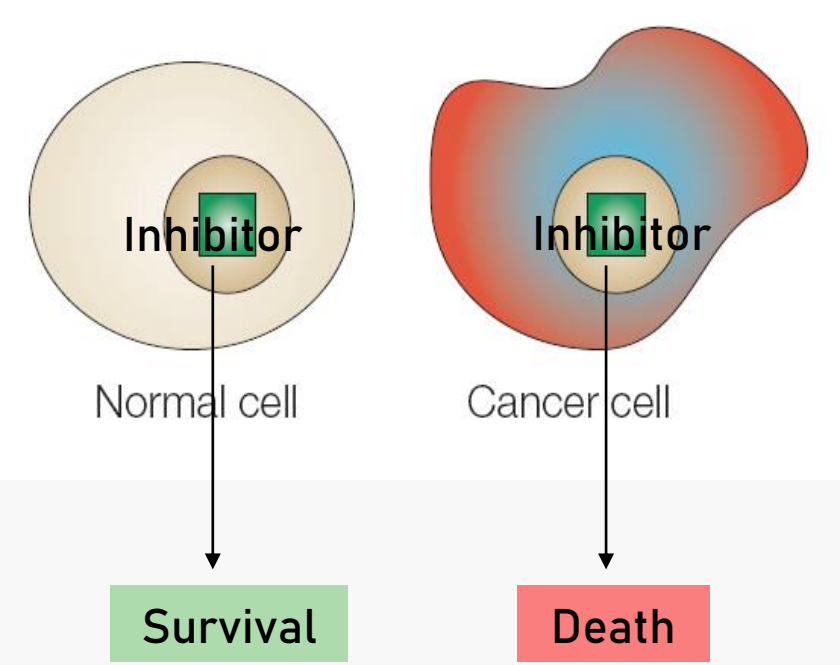
- **Two strategies to improve high therapeutic index**

Target-driven

- Target only in cancer cells



Context-driven



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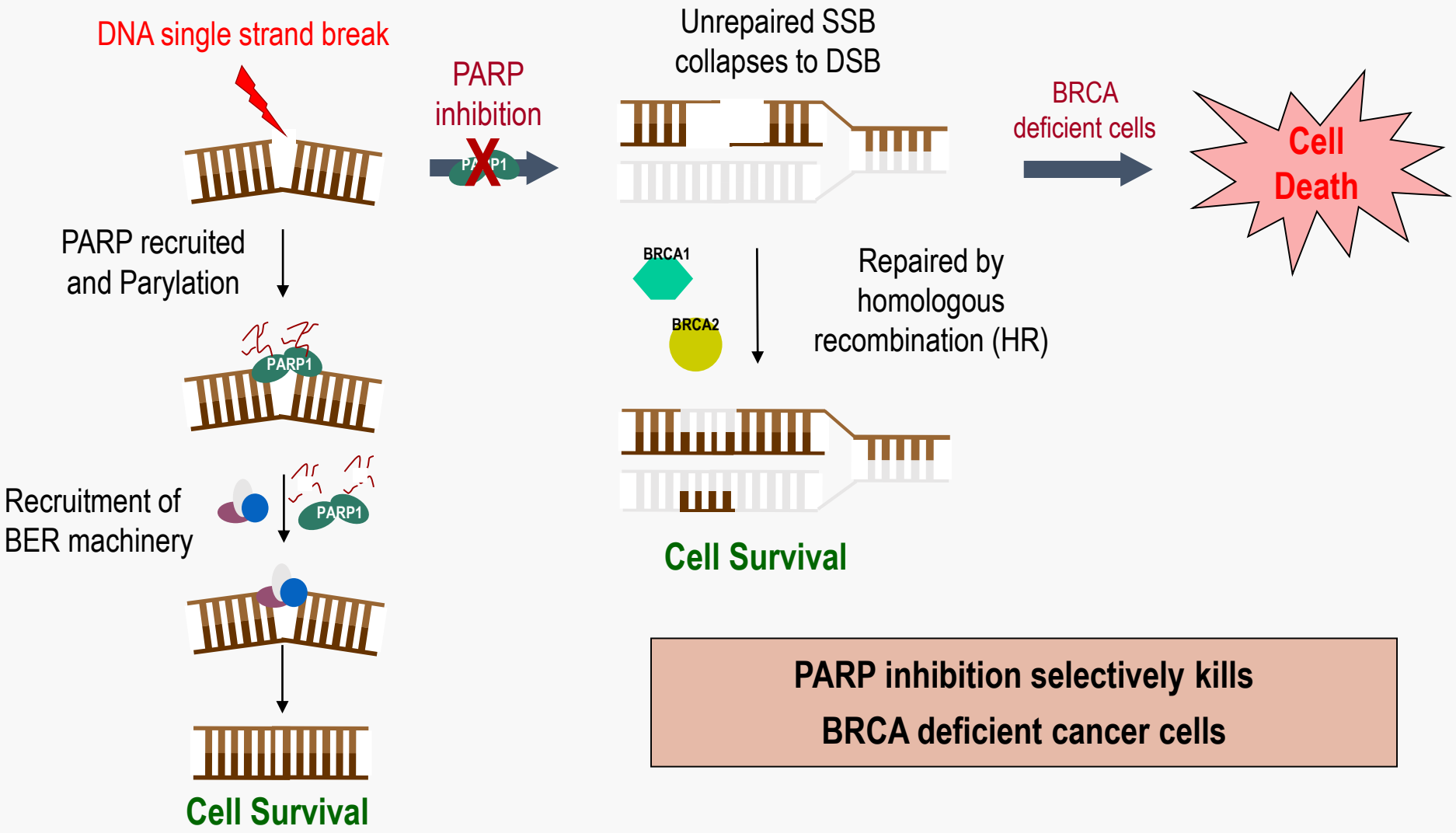
PARPi as context-specific anti-cancer agents



PARP inhibition selectively kills BRCA deficient cancer cells

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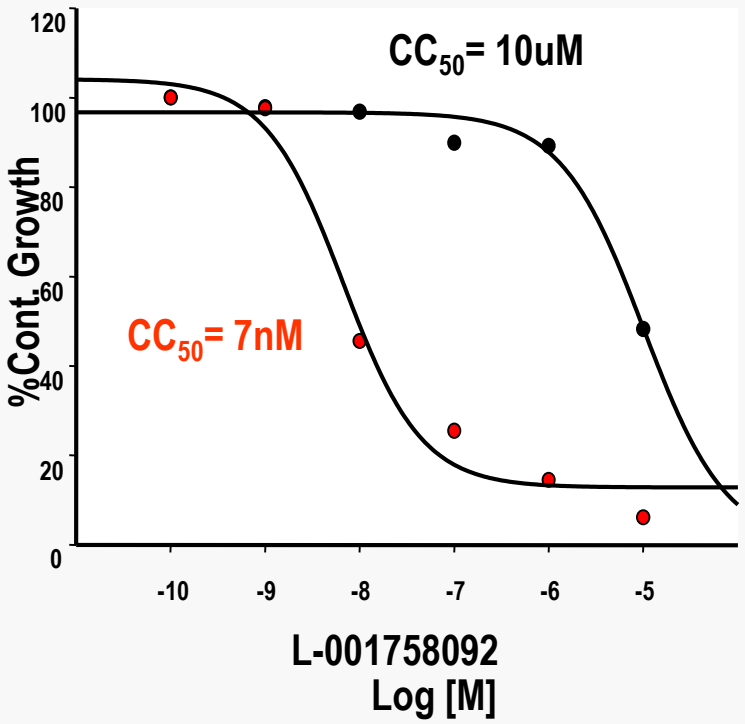
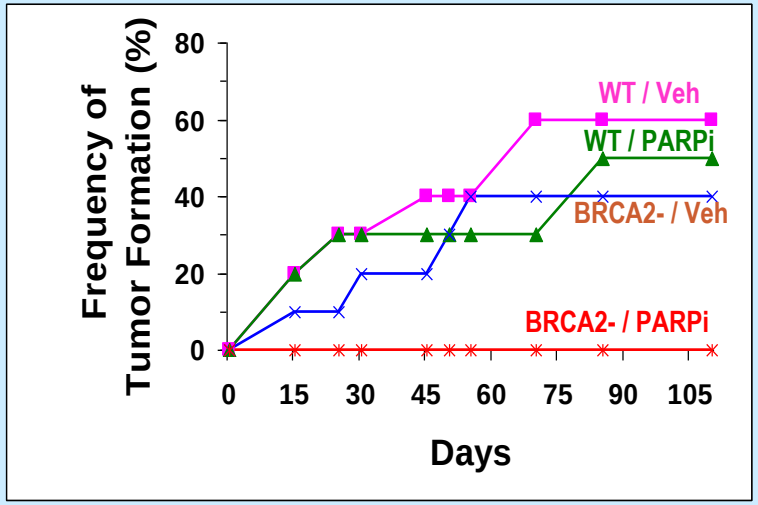
PARPi's in BRCA-deficient cells

Selectively kill BRCA-deficient cells *in vitro*



- HeLa wt
- HeLa shBRCA1

PARP inhibition selectively blocks growth of BRCA2-deficient tumors



Farmer, Nature 2005

Prevents tumor formation in mice when implanted with BRCA -/- cells but not WT cells

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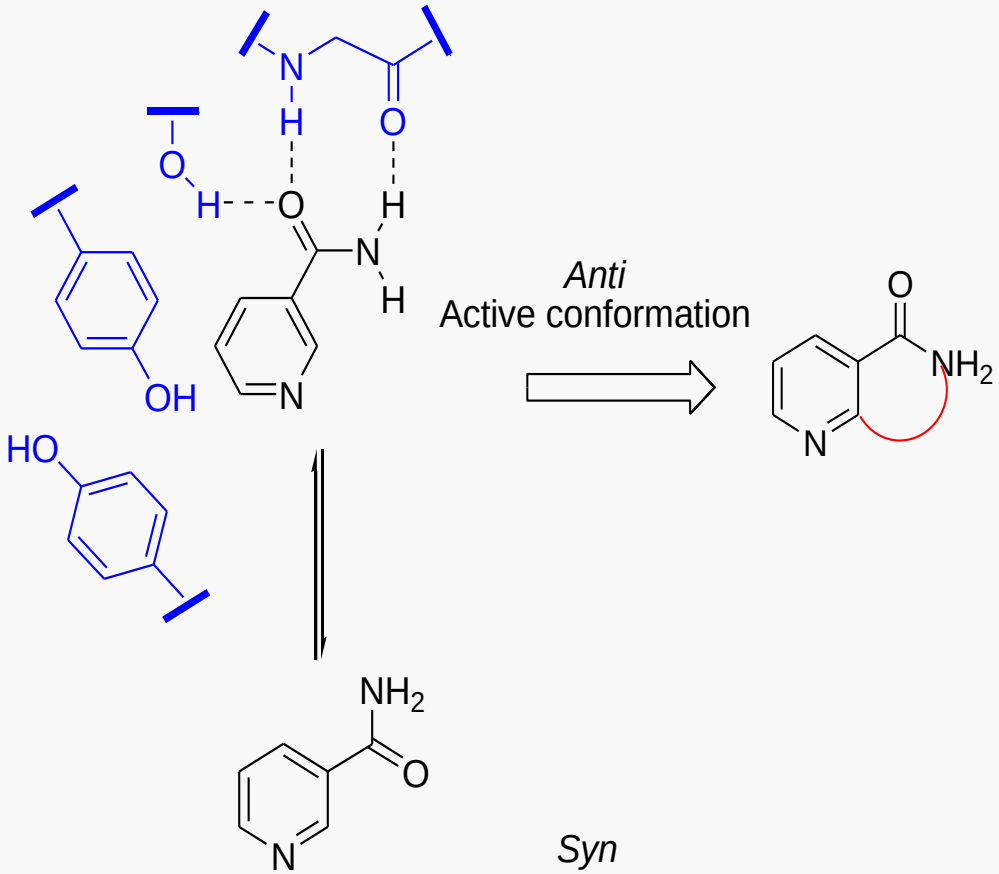
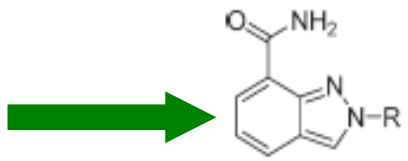
First Generation of PARP inhibitors

- First PARPi were based on the cleavage product - nicotinamide





Indazole series

R	PARP-1 IC_{50} (nM)	PARylation EC_{50} (nM)	PARylation EC_{90} (nM)
Ph	24	3700	6200
benzyl	130	> 5000	> 5000
o-Cl-C_6H_4-	100	ND	ND
m-Cl-C_6H_4-	14	450	5600
p-Cl-C_6H_4-	24	720	6700
	71	ND	ND
	55	ND	ND
	270	ND	ND

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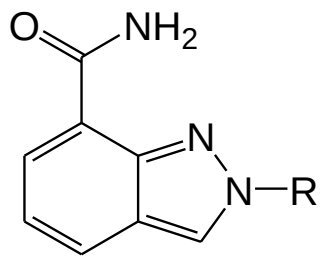
Initial profiling of the prototype PARP inh: early detection of series releted liabilities



Where R= Ph

Solubility 0.02 mg/mL (water);
LogP = 2.4 – TPSA=61

RLM Cl_{int} ((μL/min)/ mgP)	HLM Cl_{int} ((μL/min)/ mgP)	rat Cl ((mL/min)/ kg)	*F* (%)
123	138	30	41

Permeability (Papp) = 5 (cm*10E-6/s)) (LLC-PK1);
ER (BA/AB in LLC-PK1 humMDR1) = 12
ER (BA/AB in LLC-PK1 mouseMdr1) = 17

Enzyme Inhibition
CYP450 (IC_{50})
3A4, 2D6, 2C9, 2C19, 1A2 >50 μM
Enzyme Induction
hPXR 74% rifampicin effect @ 25 μM (weak inducer)
No CYP3A4 induction in HH @ 30 μM

Off-Target
hERG 9.7 μM
$Na_v1.5$ (0.2 + 3 Hz) 19μM

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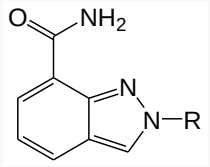

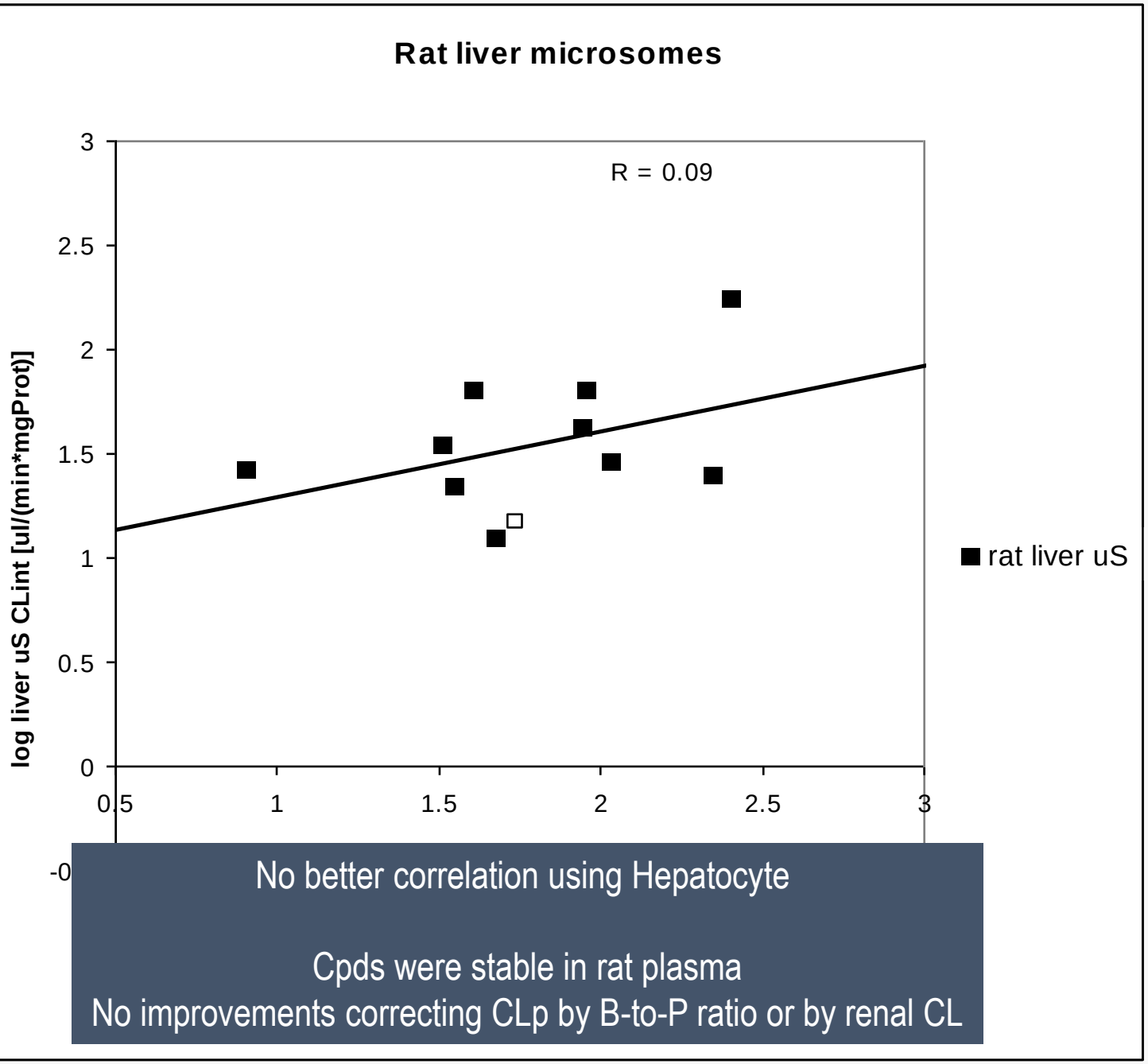

No better correlation using Hepatocyte

Cpds were stable in rat plasma
No improvements correcting CLp by B-to-P ratio or by renal CL

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Investigating mechanism of extrahepatic CL in vivo of cpd 10…

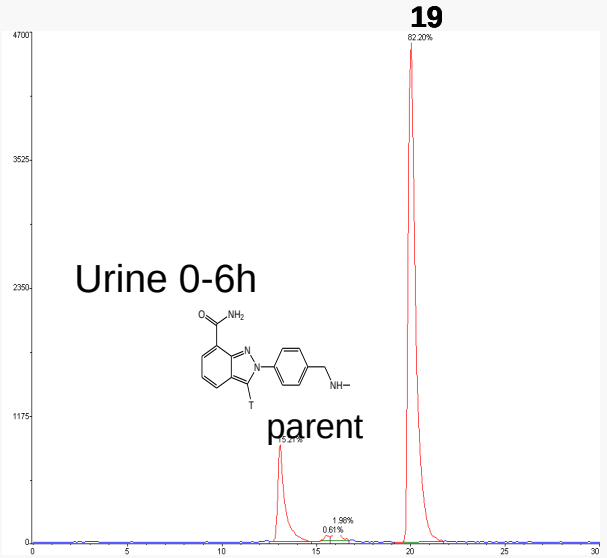
IV (treatment) Dose: 3 mg/kg
Formulation: DMSO/PEG400/Water (20%/60%/20%)

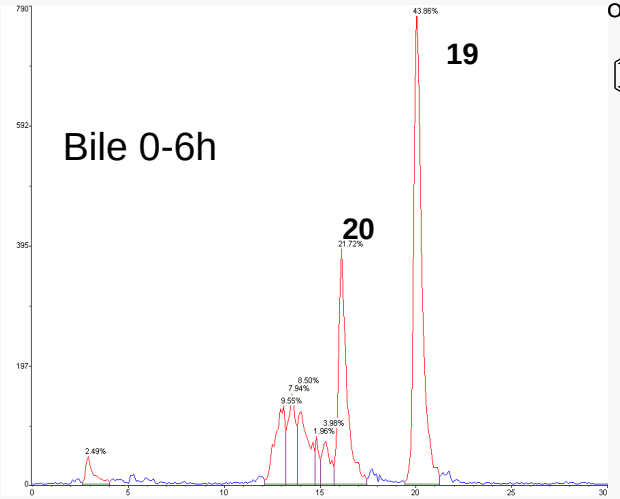
		Mean	std. Dev.
$AUC_{(0-\infty)}$	µM·hr	**0,83**	0,08
Cl	mL/min/kg	**217**	20
t½	hr	**1,4**	0,2
Vd_{ss}	L/kg	**22,1**	0,8

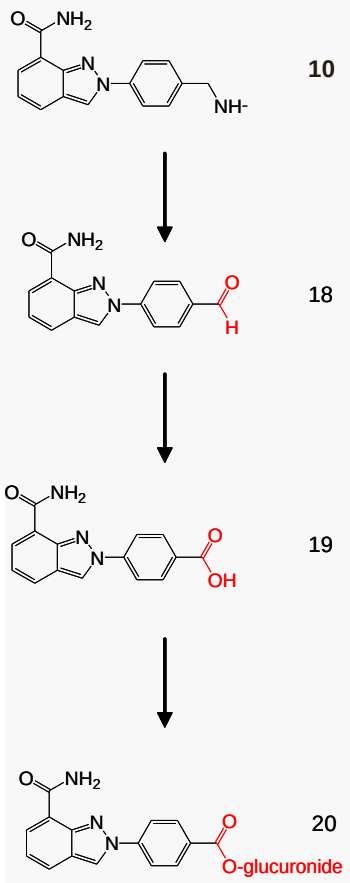
PO (treatment) Dose: 3 mg/kg
Formulation: PEG 400 (SUSPENSION)

		Mean	std. Dev.
C_{max}	µM	**0,07**	0,04
T_{max}	hr	**2,2**	2,5
$AUC_{(0-\infty)}$	µM·hr	**0,37**	0,14
t½	hr	**5,4**	3,2
$F_{(0-\infty)}$	%	**45**	17,0

	% of dosed radioactivity	% of the dose recovery transformed into 19 or 20
dose	100%	
urine 0-6h	20%	19%
urine6-24h	21%	19%
bile 0-6h	30%	16%
bile 6-24h	10%	6%
recovery	**81%**	**59,4%**







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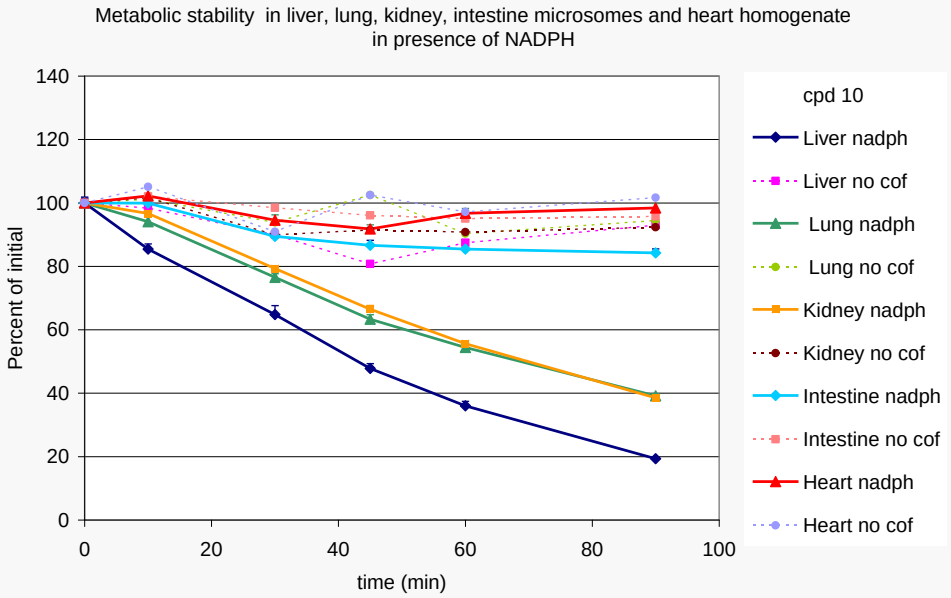
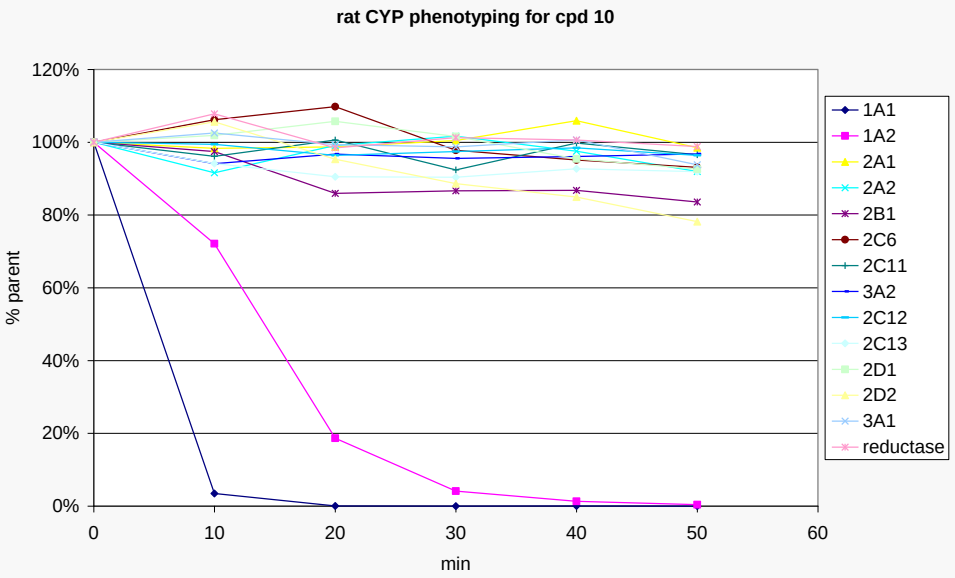
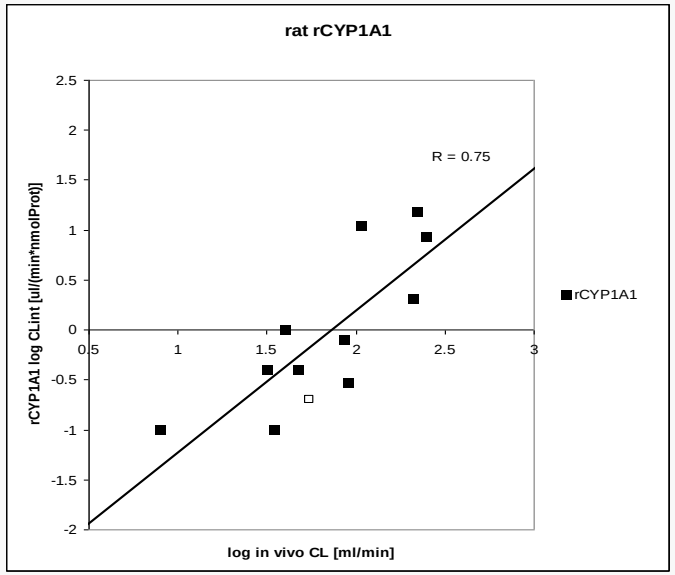
Trying to establish an in vitro model of extrahepatic metabolism





compd	RLM Cl_{int} ((μL/min)/mgP)	HLM Cl_{int} ((μL/min)/mgP)	CYP1 a 1 Cl_{int} ((μL/min)/mgP)	rat Cl ((mL/min)/kg)
8	123	138	ND	30
37	177	1	8.7	450
39	29	3	3.8	107
41	34	2	< 0.1	8
43	28	3	6	220
47	18	1	0.8	131
54	32	5	5.4	69
48	9	1	2.2	58
50	11	< 1	0.4	30
13	52	4	0.7	87
14	36	3	0.7	30
15	22	4	2.5	ND
16	11	1	0.3	47
17	15	5	< 0.1	ND
56	16	4	0.3	28
57	7	3	< 0.1	24



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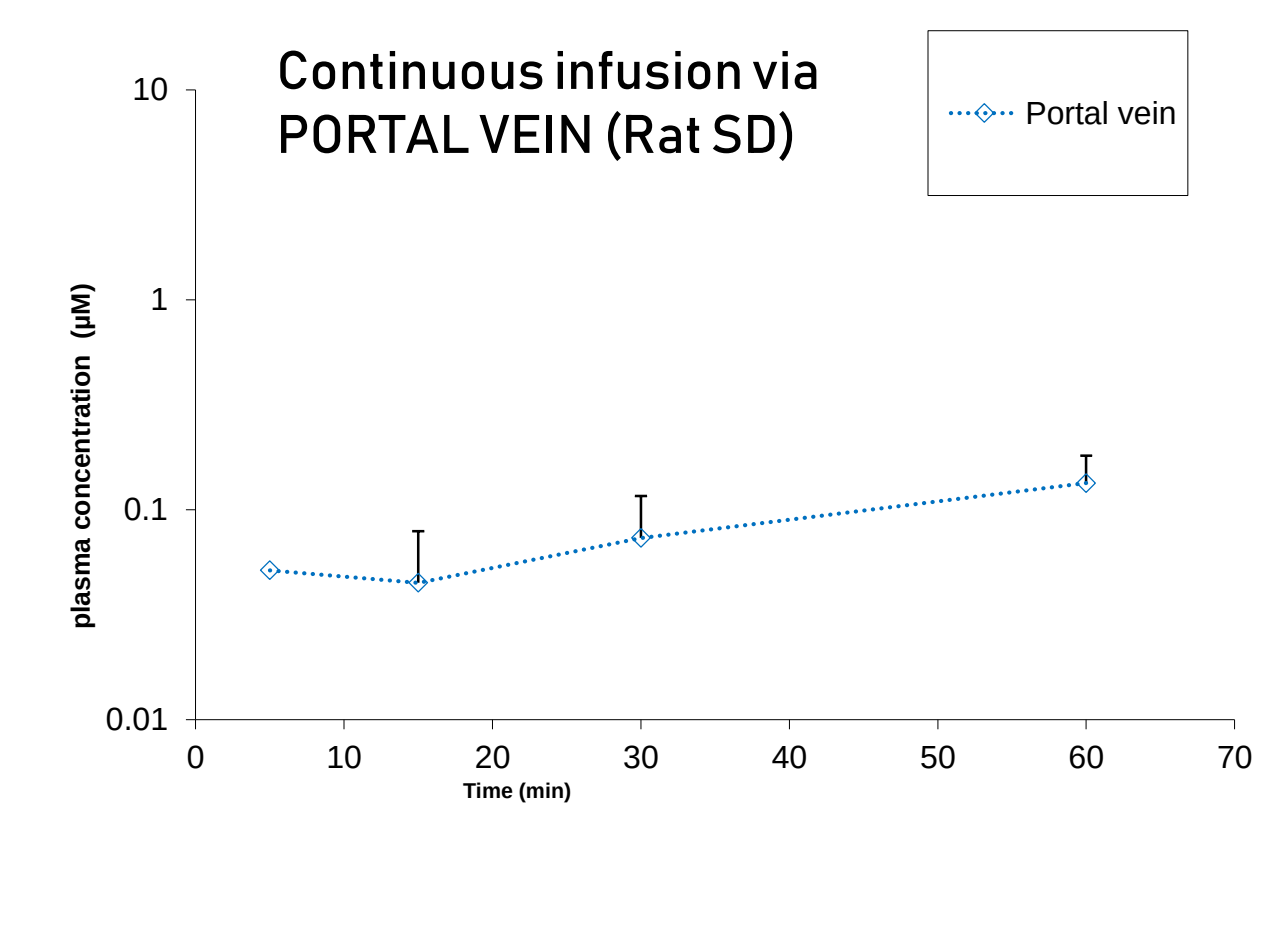
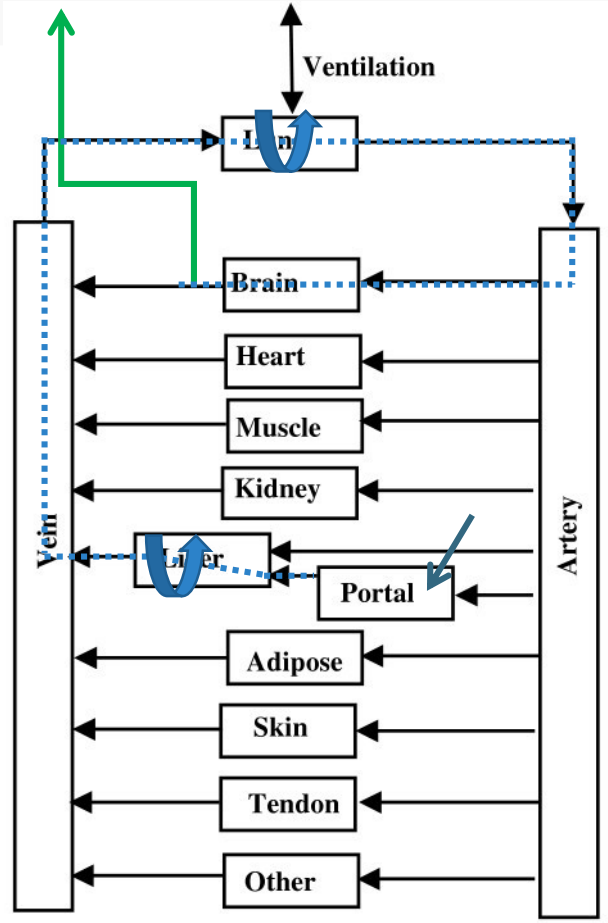
Mechanistic in vivo PK to confirm hepatic and liver organ extraction



Sampling site = jugular vein



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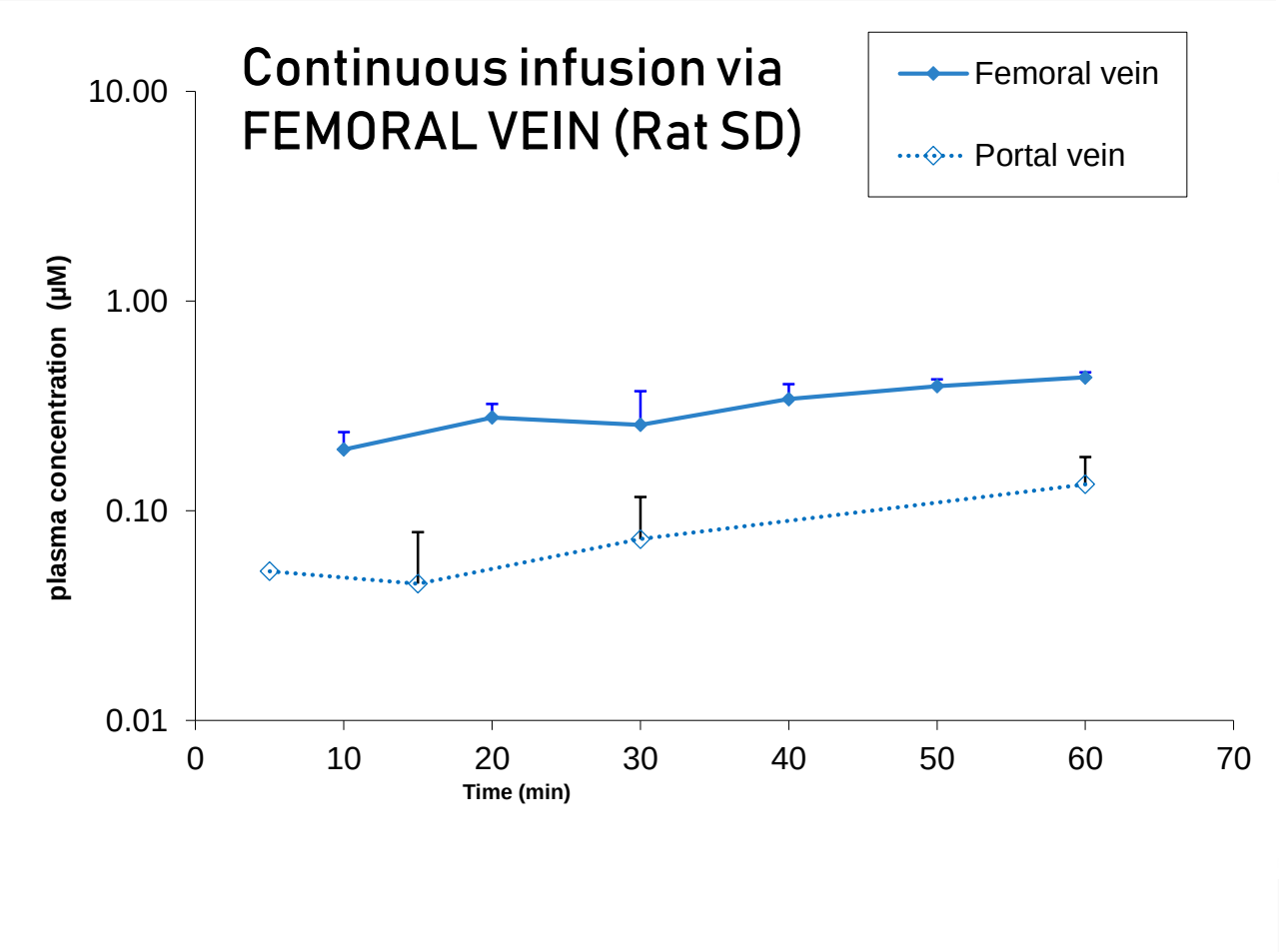
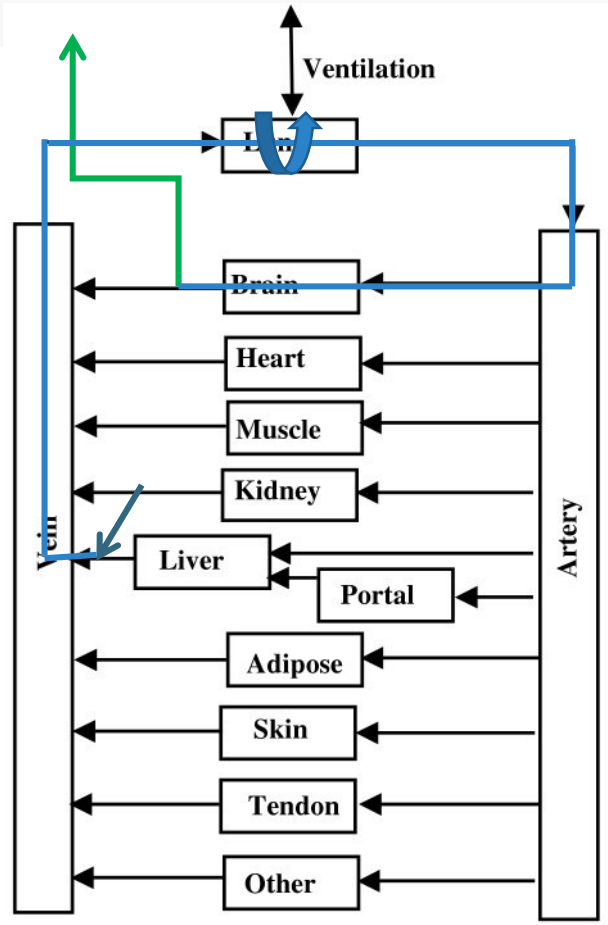


Sampling site = jugular vein



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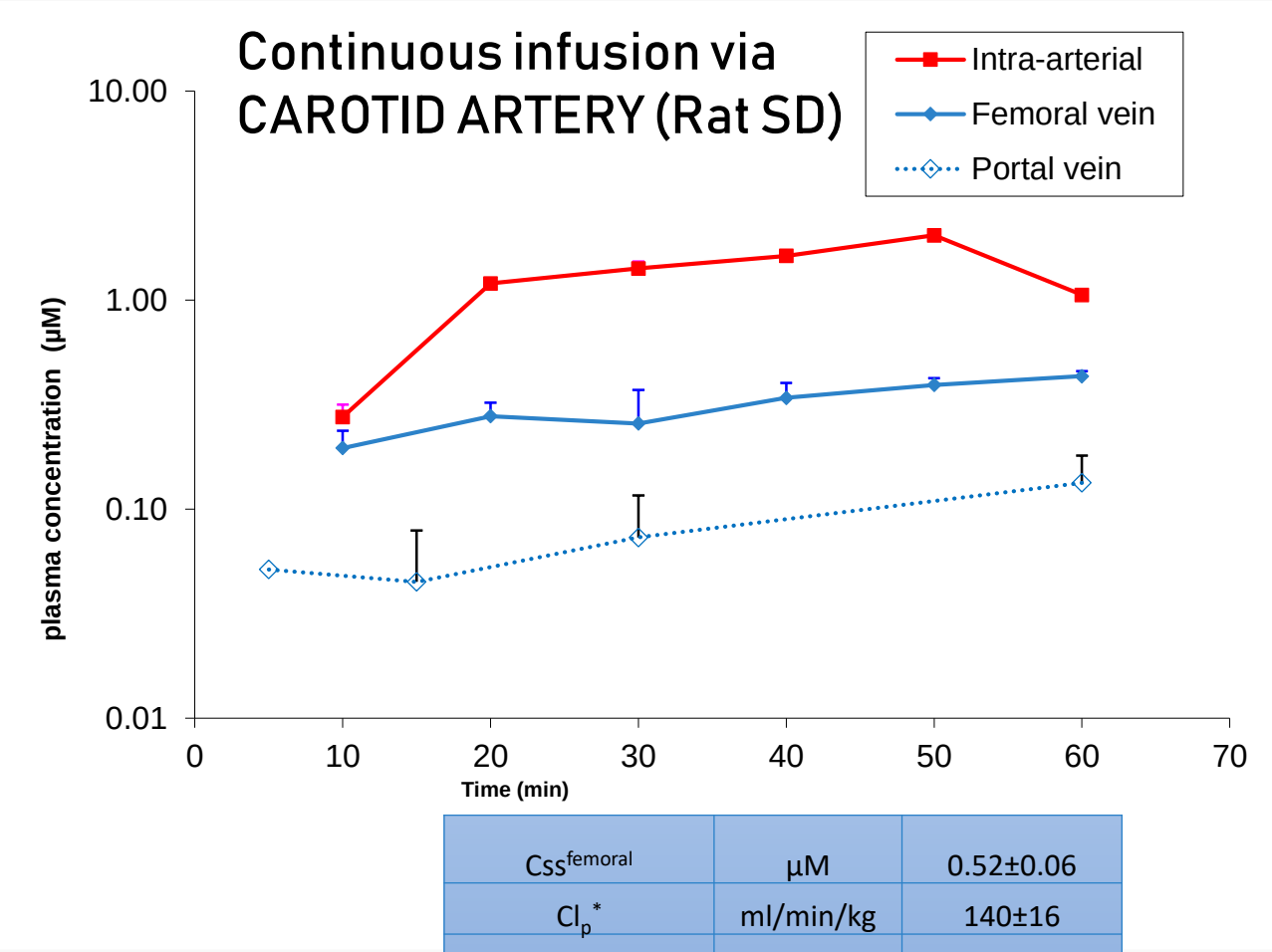
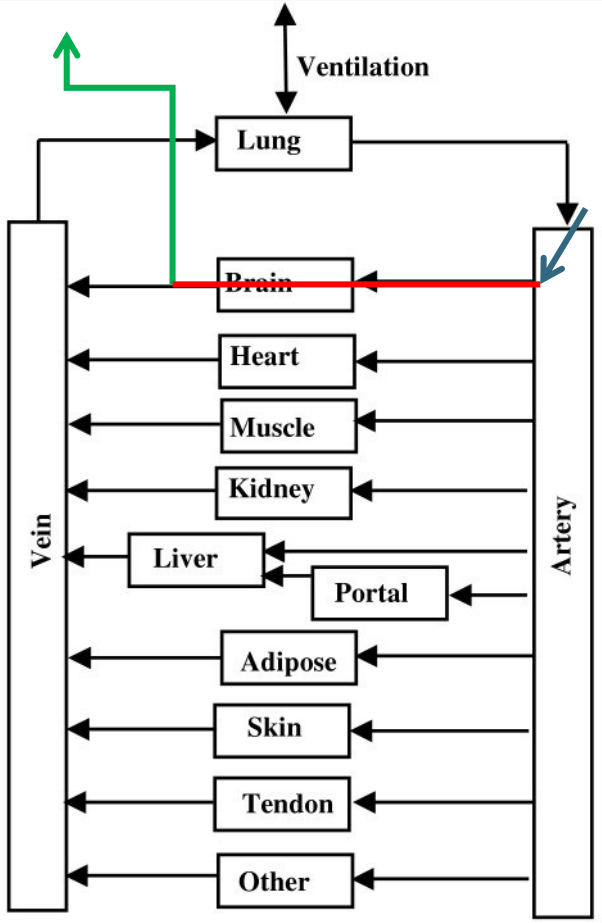
Sampling site = jugular vein



$Css^{femoral}$	µM	0.52±0.06
Cl_p^*	ml/min/kg	140±16
Css^{portal}	µM	0.21±0.06
$E\%^{hepatic}$	%	59±19
$Css^{arterial}$	µM	2.0±0.5
$E\%^{lung}$	%	78±23



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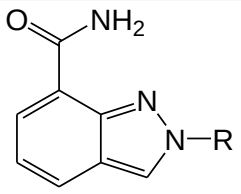
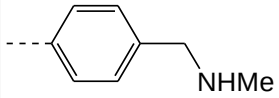
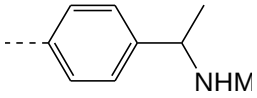
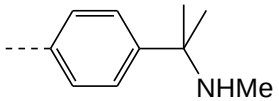
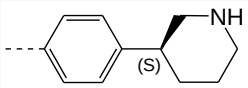
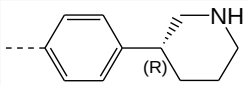
SPR *in vitro & in vivo*



					
	NHMe	NHM	NHMe	NH (S)	NH (R)
CLint rCYP1a1 (uL/min/nmol)	6000	2200	400	300	<100
CL_b (mL/min/kg)	220	58	30	46	24
PARylation EC90 (nM)				30	280

MK-4827 (Niraparib)

J Med Chem. 2009 Nov 26;52(22):7170-85.

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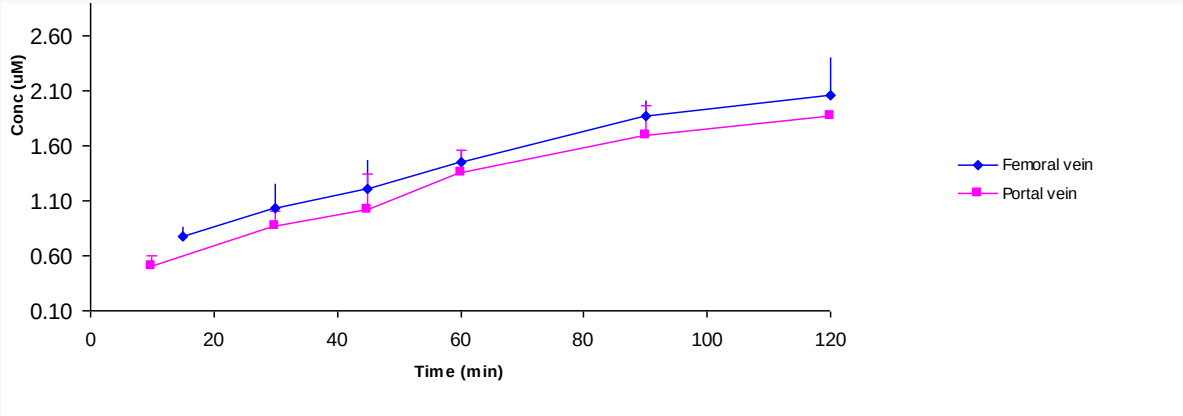
MK-4827 IVIVC: Rat liver data

TOTAL blood CL = 46 mL/min/Kgbw – F% = 92%



Hep Intrinsic Clearance
(µl/min/million cells)

Rat	Hum
2.2	1.6

Infusion via ***Femoral vein***

Time (min)	Concentration (µM) Rat 1	Rat 2	Rat 3	Average	Std. Dev.
15	0.80	0.85	0.67	0.78	0.09
30	1.16	1.17	0.79	1.04	0.22
45	1.36	1.36	0.90	1.21	0.26
60	1.53	1.52	1.30	1.45	0.13
90	1.89	2.00	1.69	1.86	0.15
120	2.29	2.22	1.64	2.05	0.36
AUC (µM * h)	174.8	177.9	140.5	164.4	20.8
Css (µM)				1.8	

Average 60 and 120 min

Infusion via ***Portal vein***

Time (h)	Concentration (µM) Rat 4	Rat 5	Rat 6	Average	Std. Dev.
15	0.51	0.41	0.59	0.50	0.09
30	0.84	0.78	0.97	0.86	0.09
45	1.02	0.87	1.15	1.01	0.14
60	1.61	0.99	1.48	1.36	0.33
90	1.82	1.47	1.79	1.69	0.19
120	1.85	1.60	2.15	1.87	0.28
	152.9	121.7	159.2	144.6	20.1
				1.6	

Predicted Rat hepatic CL[1]

ER_H = 11%

CL_H= 7.1 (ml/min)/kg

Predicted hum hepatic CL[1]

ER_H = 14%

CL_H= 2.8 (ml/min)/kg

Measured hepatic CL *(in vivo)*

ER_H = 10% $CL_H = ER_H * Q_H$ = 6.8 ((ml/min)/kg)[1]

[1] Rat Q_H = 68 ((ml/min)/kg) - Hum Q_H = 20 ((ml/min)/kg).
Ring B.J.et al. *Pharmaceutical Sciences*, **2011** DOI 10.1002/jps

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Mechanism of Clearance in rats

TOTAL blood CL = 46 mL/min/Kgbw – F% = 92%

- 35% urinary excretion

=> CL_R= 0.35*CL_B = 0.35*46= 16.1 ml/min/kg ; > GFR_{rat} (5-6 ml/min/kg)-Rat Kidney BF (40-52 ml/min/kg)

- 2 % biliary excretion
- 10 % hepatic extraction (determined by portal/femoral infusion)
- ~ 50 % extrahepatic metabolism

Transporters Data

Compound	$P_{app}B\text{-}A/P_{app}A\text{-}B$					
	LLC-PK1 Papp (cm*10E-6/s)	Human MDR1	Mouse Mdr1a	Rat Mdr1a	Beagle MDR1	Monkey MDR1
MK-4827 (5 μM)	3	12	22	17	10	ND
Verapamil (1 μM)	1	6	12	7	4	ND

MK-4827 is a Pgp substrate

Solubility >20 mg/mL

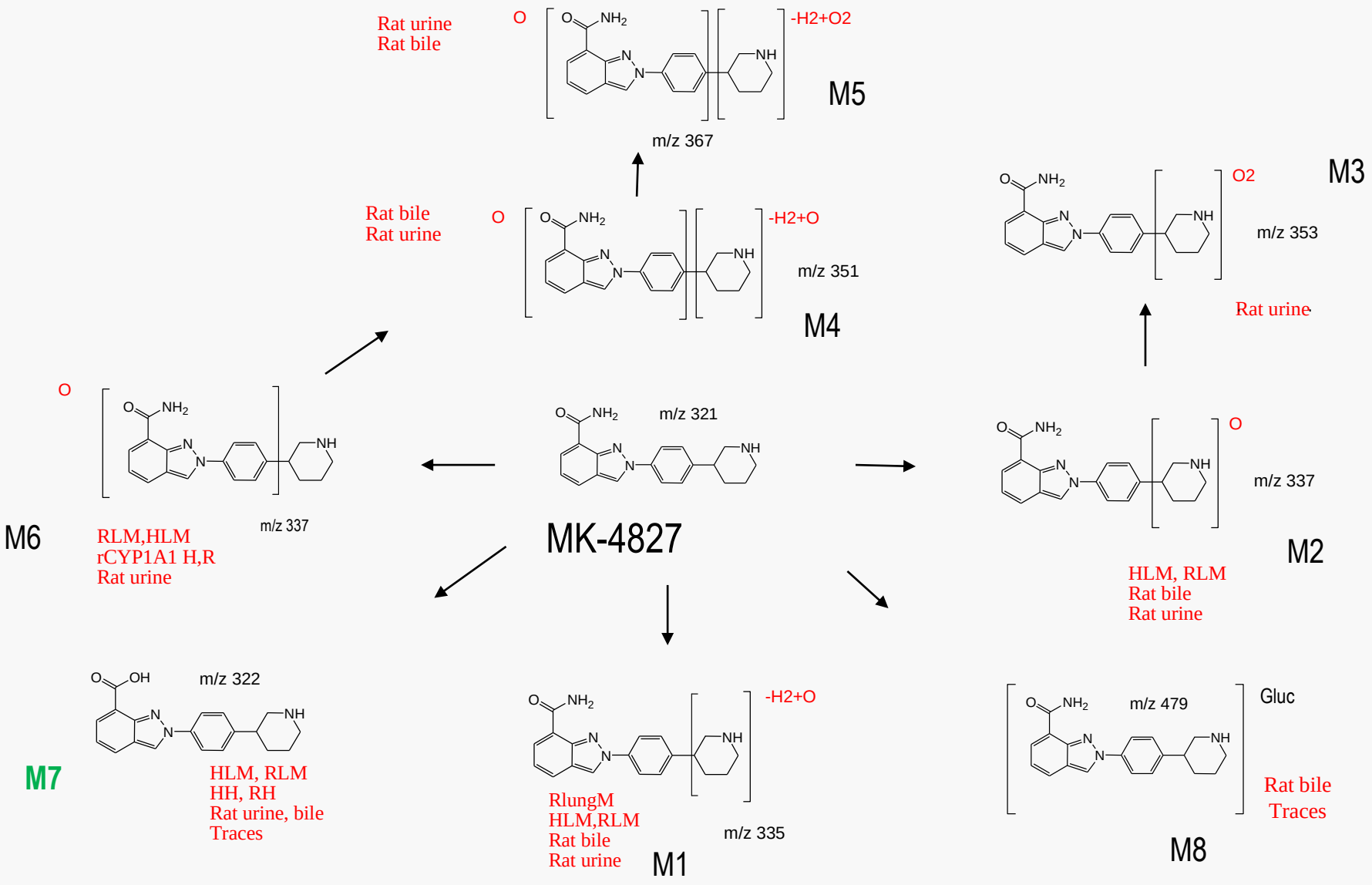
Turnover in lung and kidney preparations

MK-4827 in NADPH-supplemented preparations:

- rat lung microsomes (40 % turnover in 90 min)
- rat kidney S9 fraction (50% turnover in 90 min)
- human lung microsomes, from both non-smokers and smokers (stable)
- human kidney S9 fraction (stable).

- These results suggest low contribution of extra-hepatic metabolism in humans.
- Predicted low hepatic CL in human (IVIVE using HH)
- Potential renal CL in human due to Low PPB – Mdr1 active efflux
- Expected good F%

Proposed Metabolite Scheme (Rat and Human)



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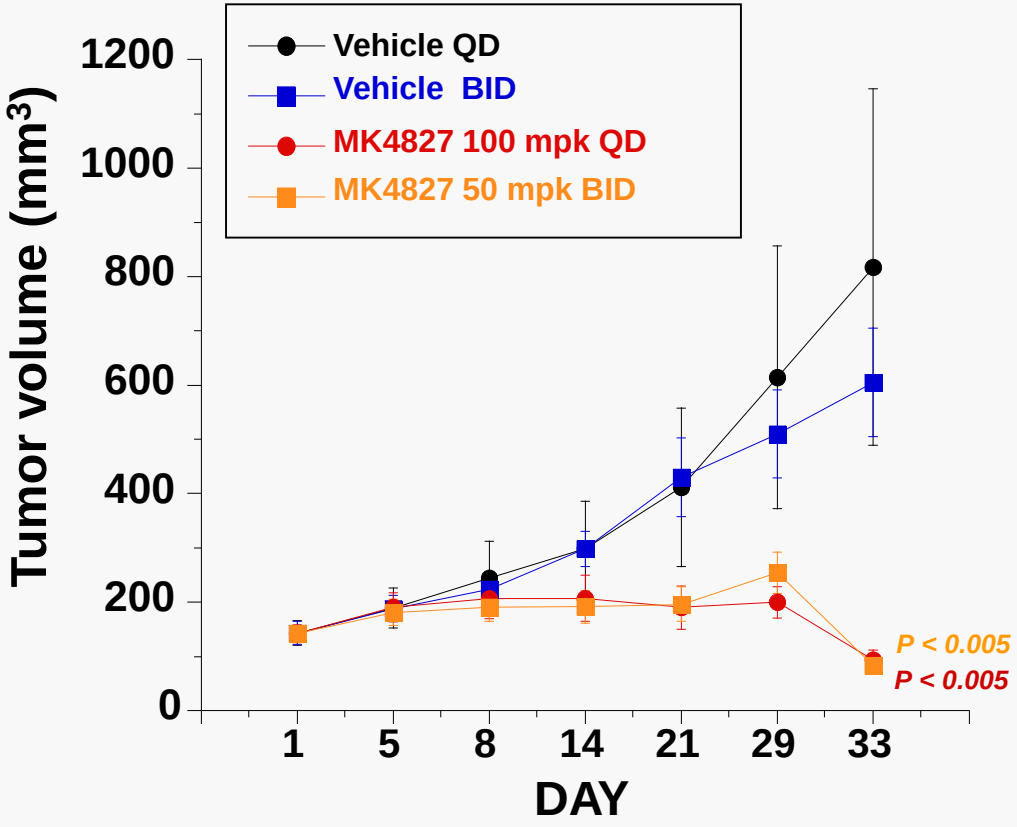
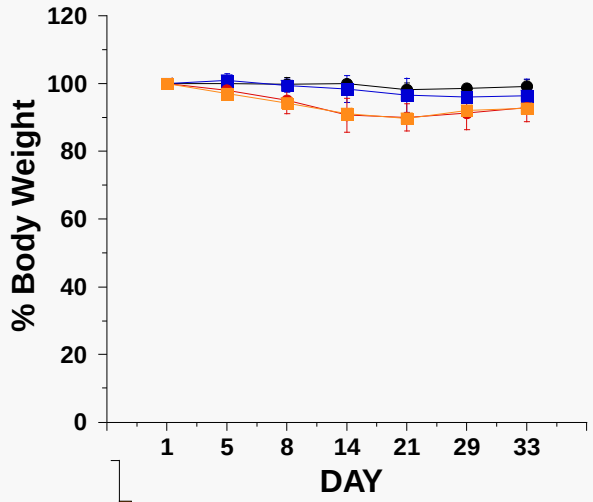
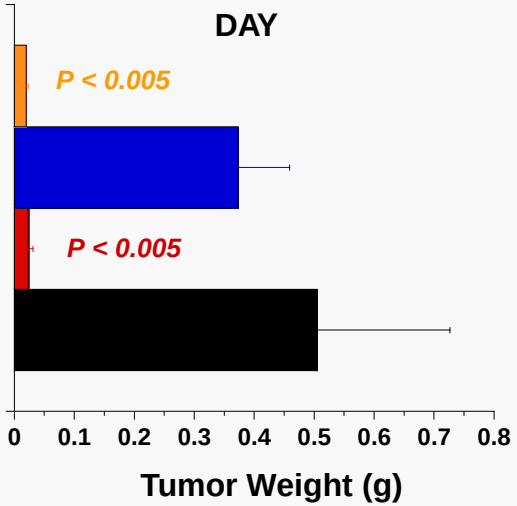
Activity in MDA-MB-436 (BRCA1-) xenografts

Administered orally







Approved as PCC April 2007 (MK-4827)

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MK-4827 (Niraparib) preclinical profiling - PK

IV (male, n=3, saline)

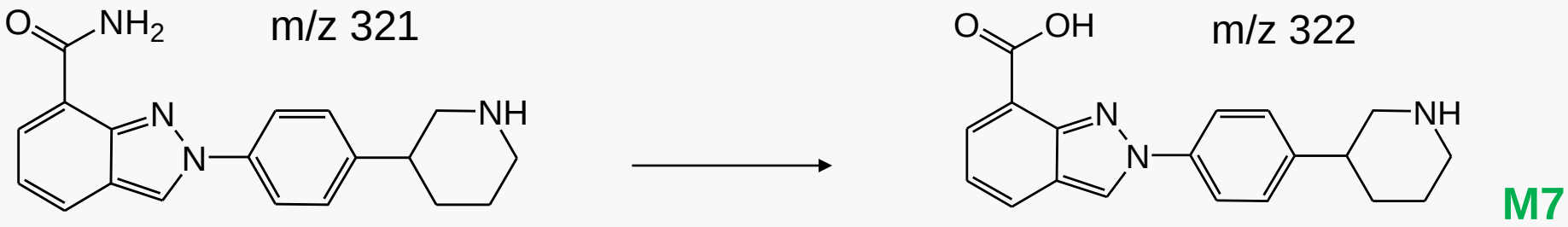
Species	Dose (mg/kg)	Cl_b (mL/(min*kg))	Vd (L/kg)	$T_{1/2}$ (hr)	AUC $(\mu M*hr)_{0-x}$
Rat	3	46±3	22.8±8.7	5.1±2.0	2.9±0.1
Dog	1	89±6	29.3±5.2	5.3±0.3	0.6±0.0
Monkey	1	17.5±6	17.1±2.5	6.8±1.0	1.5±0.2
		±	±	±	±

P.O. (male, n=3, water)

Species	Dose (mg/kg)	C_{max} (μM)	T_{max} (hr)	AUC $(\mu M*hr)_{0-x}$	%F
Rat	4	0.3±0.1	4.0±1.0	3.7±1.8	92±29
Dog	2	0.2±0.0	0.9±0.3	0.8±0.1	80±14
Monkey	2	0.3±0.1	1.3±0.6	1.9±0.5	70±17
		±	±	±	±

Dose proportional PK in rat – MK-4827

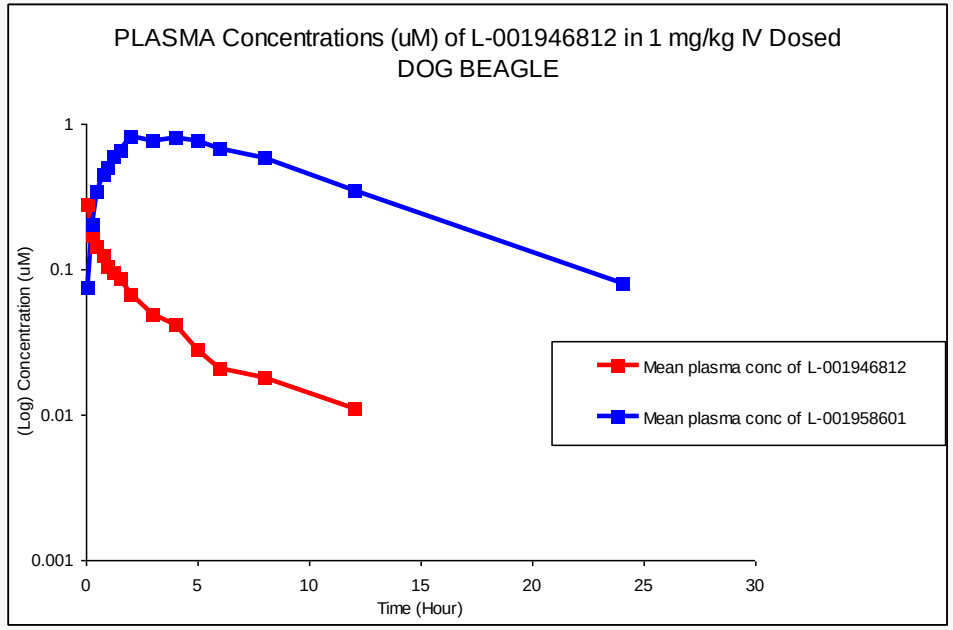
Proposed Metabolite Scheme (Dog)



MK-4827

L-00958601

hepatocytes
microsomes
urine
plasma



Rat/Dog Excretion Study 3 mg/kg iv (n=3)

	Percent of Dose Recovered (0-48h)		
	urine	bile (0-24h)	total
Total	48±1	32 ±4	80±4
Parent	35±2	2±2	37±3
Water	2±1	2±1	

Data obtained using MK4827 labeled with tritium in position 3 of the indazole ring.
Low tritium loss was detected both in vivo and in vitro

In dogs only 2 % of parent excreted in urine

Hum CL – t1/2 prediction for MK4827

- Total hum CL = $CL_{metab\ liver} + CL_R + CL_{metab\ extrahep}$
  - $CL_{metab\ liver}$: IVIV scaling from HH = 2.8 ml/min/kg (range 1.8-3.8)
  - $CL_R = CL_{R\text{-}rat} * (fu_{hum} / fu_{rat}) * KBF_{hum} / KBF_{rat} * ER_{hum}/ER_{rat}$ = 4.5 ml/min/kg (range 3.5-7)
  - $CL_{metab\ extrahep}$: expected to be negligible in human
  - Vdss = Vdss hum = Vd (animal)*f_u(hum)/f_u(animal) = 26 (L/kg) range (17-37)

			Vd (L/kg)		
			17	26	37
total CL (mL/min/kg)			min	avrg	max
	5.5	min	28	43	61
	8.3	avrg	21	**36**	46
	11	max	16	25	36

Predicted half-life (hr)

Values used for rat, and human KBF were 52 (Hsu et al., 1975) and 16 ml/min/kg (Wolf et al., 1993)

25

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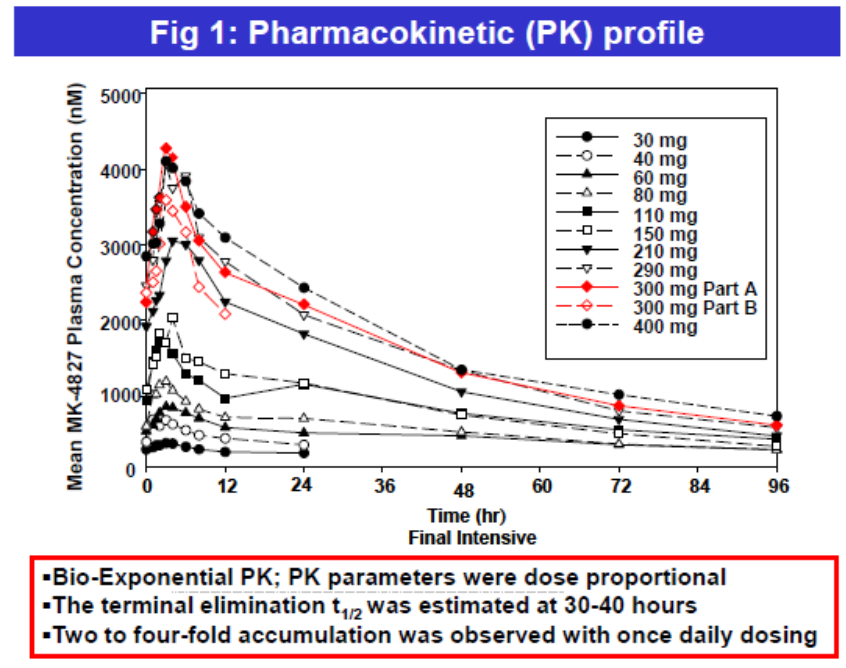
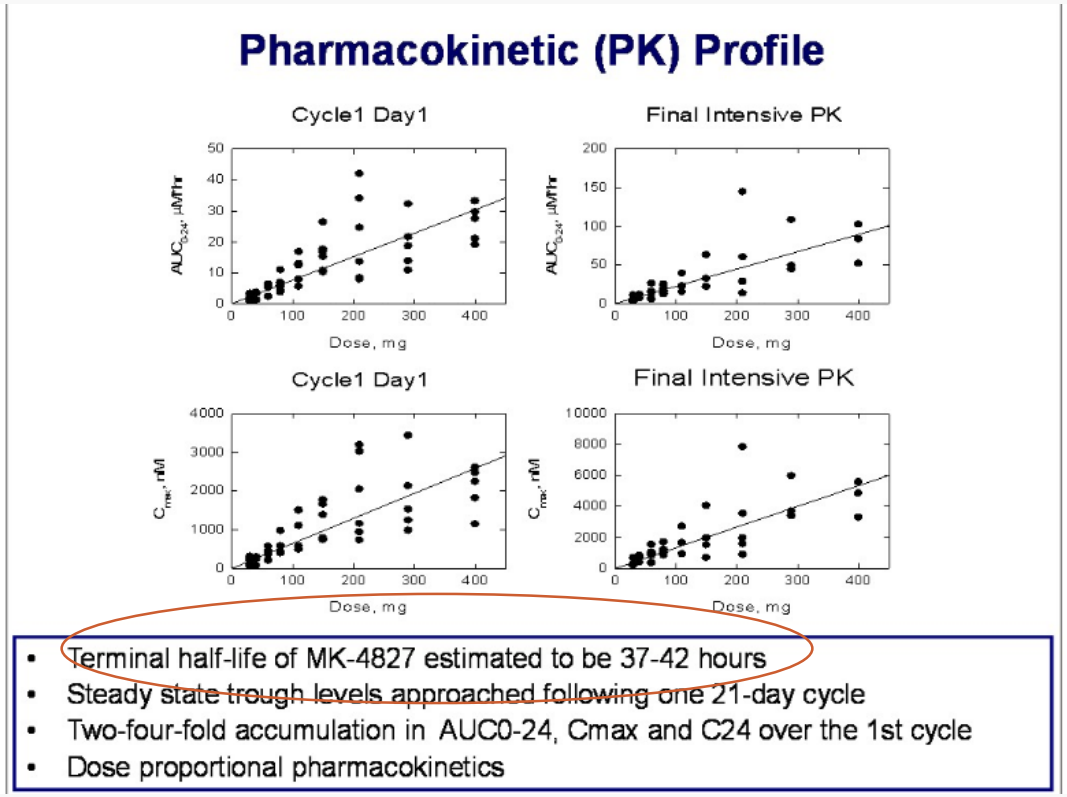
Human PK data (Phase II)





http://meetinglibrary.asco.org/content/62393?format=posterImg

Niraparib was approved in 2017

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Conclusion

Case studies in drug discovery have been reported showing relevant lungs and generalized extrahepatic metabolism in rats. The large extrahepatic contribution appeared to be rat specific with no analogy to human metabolism. Rat CYP1a1 was identified as the major enzyme responsible

The extraction fraction (CL/Q) in the lung is in general small, as a result of its modest metabolic activity but due to the fact that the lung blood flow is equivalent to the total cardiac output, in some cases, drug metabolism in the lungs can be substantial.

In general, xenobiotic metabolizing enzymes (XMEs) are expressed in lower levels in the extrahepatic tissues than in the liver, making the former less relevant for the clearance of xenobiotics. Local metabolism, however, may lead to tissue-specific adverse responses, e.g. organ toxicities, allergies or cancer.*

Fonsi M - Drug Metab Lett. 2014;8(1):51-66.
* U. Gundert-Remy et al. Drug Metab Rev, Early Online: 1–34

Thank you very much!