Targeting the topoisomerase 1 enzyme in cancer cells with acquired resistance to SN-38

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Abstract # A182

De novo or acquired resistance to anti-cancer drugs represents a major obstacle to successful treatment of cancer patients. In order to improve future cancer drug development we have established the DEN-50R cell line panel which when fully developed will consist of isogenic pairs of drug sensitive and resistant human cancer cell lines representing the 5 most common cancer types.
Based on the DEN-50R we have tested a number of novel anti-cancer drugs in this panel of cell lines. One class of drugs that includes the indenoisoquinolines LMP400 and LMP776, which have topoisomerase 1 inhibitory activity and recently passed phase 1 clinical studies, showed very interesting features in the cell line studies.
When tested in SN-38 (the active metabolite of irinotecan) -sensitive and -resistant isogenic colorectal cancer cell lines and breast cancer cell lines, LMP400 showed significant dose related cytotoxicity independent of the state of SN-38-resistance. As these resistant cell lines have significant up-regulation of either the BCRP and/or mdr-1 protein, it can be concluded that the indenoisoquinoline LMP400 can target topoisomerase 1 enzyme without being a substrate for these drug efflux pumps.
We are now planning to initiate two clinical phase II studies (Simon's two stage design) with LMP400; one in irinotecan-failed metastatic colorectal cancer patients and one in late stage metastatic breast cancer patients. Patients for these studies will be preselected based on an LMP400-responsiveness profile we generated by gene expression data where associations between gene expression profiles and growth inhibition were compared in a panel of cell lines exposed to LMP400. A second step included filtering the identified gene expression profile against mRNA expression from a collection of 3200 human tumors. Only genes being differentially expressed in the clinical tumor material were retained in the model.

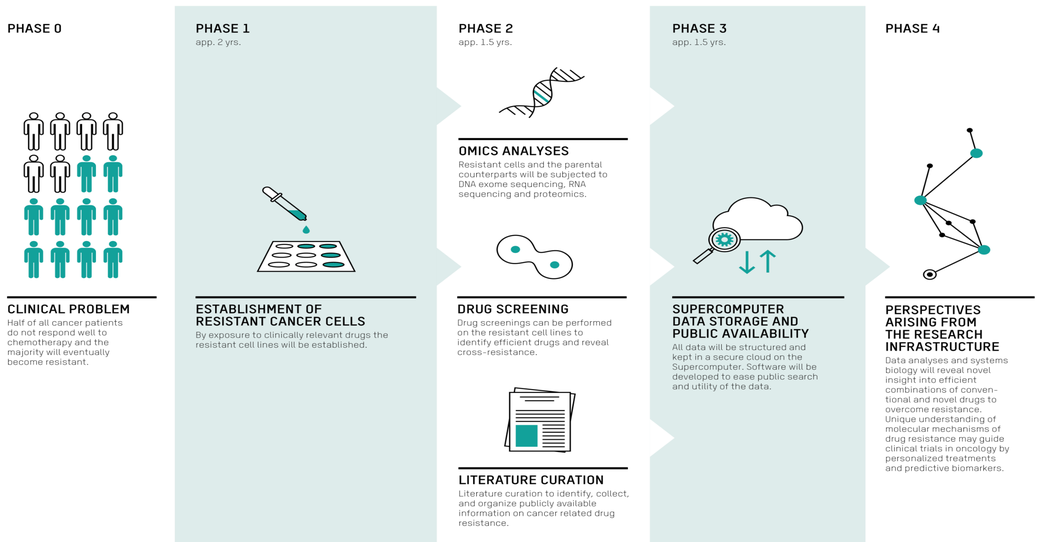
Materials and Methods

We have established several SN-38 (the active metabolite of irinotecan) resistant cancer cell lines by exposing them to gradually increasing drug concentrations. By MTT assays we have analyzed the sensitivity and/or cross resistance to the indenoisoquinolines LMP400 and LMP776, which have topoisomerase 1 inhibitory activity. Analyses of *TOP1* copy number, mRNA levels, protein amounts, Top1-DNA cleavage complexes and Top1 enzyme activity assays were employed to characterize Top1 in the resistant cells. Additionally, drug response (DRP) profiles for irinotecan (SN-38) and LMP400 were establishes to predict drug response to these two drugs.

Key references:

1) **Jensen et al, Mol Oncol. 2015 Jun;9(6):1169-85. Establishment and characterization of models of chemotherapy resistance in colorectal cancer: Towards a predictive signature of chemoresistance.**
2) **Hansen SN et al. Tumour Biol. 2015 Jun;36(6):4327-38. Acquisition of docetaxel resistance in breast cancer cells reveals upregulation of ABCB1 expression as a key mediator of resistance accompanied by discrete upregulation of other specific genes and pathways.**
3) **Beck DE, et al., 2014 J Med Chem. 27;57(4):1495-512. Synthesis and biological evaluation of new carbohydrate-substituted indenoisoquinoline topoisomerase I inhibitors and improved syntheses of the experimental anticancer agents indotecan (LMP400) and indimitecan (LMP776).**
4) **Stenvang J et al. Front Oncol. 2013 Dec 25;3:313. Biomarker-guided repurposing of chemotherapeutic drugs for cancer therapy: a novel strategy in drug development.**
5) **Knudsen S, et al. PLoS One. 2014 Feb 5;9(2):e87415. Development and validation of a gene expression score that predicts response to fulvestrant in breast cancer patients.**

DEN50-R platform



Chemical structures

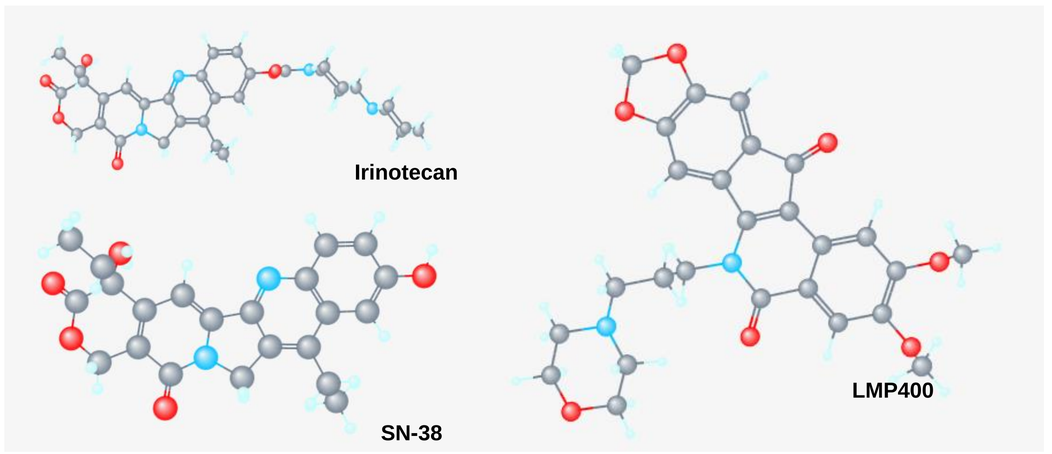


Figure1. Chemical structures of Irinotecan, SN-38 and LMP400 (Indotecan).

Results: Sensitivity of SN-38 resistant cell lines to LMP400

Cell line	SN-38		NSC 725776 / LMP776		NSC 743400 / LMP400		Epirubicin		Etoposide	
	IC50	*RR*	*IC50*	*RR*	*IC50*	*RR*	*IC50*	*RR*	*IC50*	*RR*
HCT116-Wt	0.05 ± 0.01	1	0.06 ± 0.03	1	0.17 ± 0.1	1	0.09 ± 0.01	1	6.3 ± 2.8	1
HCT116-SN38	3.4 ± 0.6	67	47 ± 46 *	782	47 ± 46	280	0.2 ± 0.03	2.1	4.4 ± 3.5	0.7
HT29-Wt	0.13 ± 0.06	1	0.03 ± 0.01	1	0.07 ± 0.04	1	0.18 ± 0.02	1	9.9 ± 3.7	1
HT29-SN38	7.3 ± 1.7	55	1.2 ± 0.7	36	0.14 ± 0.04	2	2.0 ± 0.9	11	38 ± 17	4
LoVo-Wt	0.02 ± 0.004	1	0.02 ± 0.01	1	0.06 ± 0.02	1	0.11 ± 0.03	1	1.8 ± 1.9	1
LoVo-SN38	0.44 ± 0.2	20	0.09 ± 0.03	4.1	0.05 ± 0.03	0.8	0.95 ± 0.4	9	2.7 ± 1.6	1.5

Table 1. Drug sensitivity IC50-values and relative resistances in colorectal cancer cells.
Mean IC50-value (µM) ± standard deviation of three experiments. RR; relative resistance is the IC50-value of the resistant cell line divided by the IC50-value of the parental (wild-type, Wt) cell line. *Did not reach IC50, so the actual IC50-value is larger than this. IC50-values for SN-38 are provided for comparison.

Anti- cancer drugs	Epirubicin	RR	Docetaxel	RR	Cisplatin	RR	LMP776	RR	LMP400	RR
MDA_{acq} DMSO	0.4 ± 0.2		22.0 ± 9.9		62.8 ± 13.7		8.9 ± 11.7		10.5 ± 9.7	
MDA_{acq}	1.7 ± 0.3	4.3	21.0 ± 5.3	1.0	70.6 ± 4.1	1.1	44.6 ± 45.7	5.0	12.4 ± 12.2	1.2
$MCF\text{-}7_{acq}$ DMSO	0.9 ± 0.5		20.4 ± 2.3		26.1 ± 9.5		*		*	
$MCF\text{-}7_{acq}$	1.7 ± 0.9	1.9	25.8 ± 8.2	1.3	65.6 ± 53.4	2.5	*		*	
$MDA_{de\ novo}$ DMSO	5.2 ± 3.2		18.0 ± 3.7		34.7 ± 7.03		11.0 ± 6.2		15.8 ± 6.3	
$MDA_{de\ novo}$	17.6 ± 1.4	3.4	27.3 ± 2.1	1.5	95.1 ± 4.9	2.7	22.5 ± 4.3	2.0	25.8 ± 3.5	1.6
$MCF\text{-}7_{de\ novo}$ DMSO	8.1 ± 9.1		24 ± 7.5		45.6 ± 5.4		40.0 ±14.2		42.6 ± 36.3	
$MCF\text{-}7_{de\ novo}$	10.0 ± 7.9	1.2	34.9 ± 10.2	1.5	53.7 ± 13.0	1.2	55.9 ± 28.6	1.4	58.2 ± 36.5	1.4

Table 2. Drug sensitivity IC50-values and relative resistances in breast cancer cells.
Mean IC50-value (µM) ± standard deviation of three experiments. RR; relative resistance is the IC50-value of the resistant cell line divided by the IC50-value of the parental (wild-type, Wt) cell line. *Did not reach IC50, so the actual IC50-value is larger than this. IC50-values for SN-38 are provided for comparison.

Drug response profiles (DRPs) tested in clinical samples

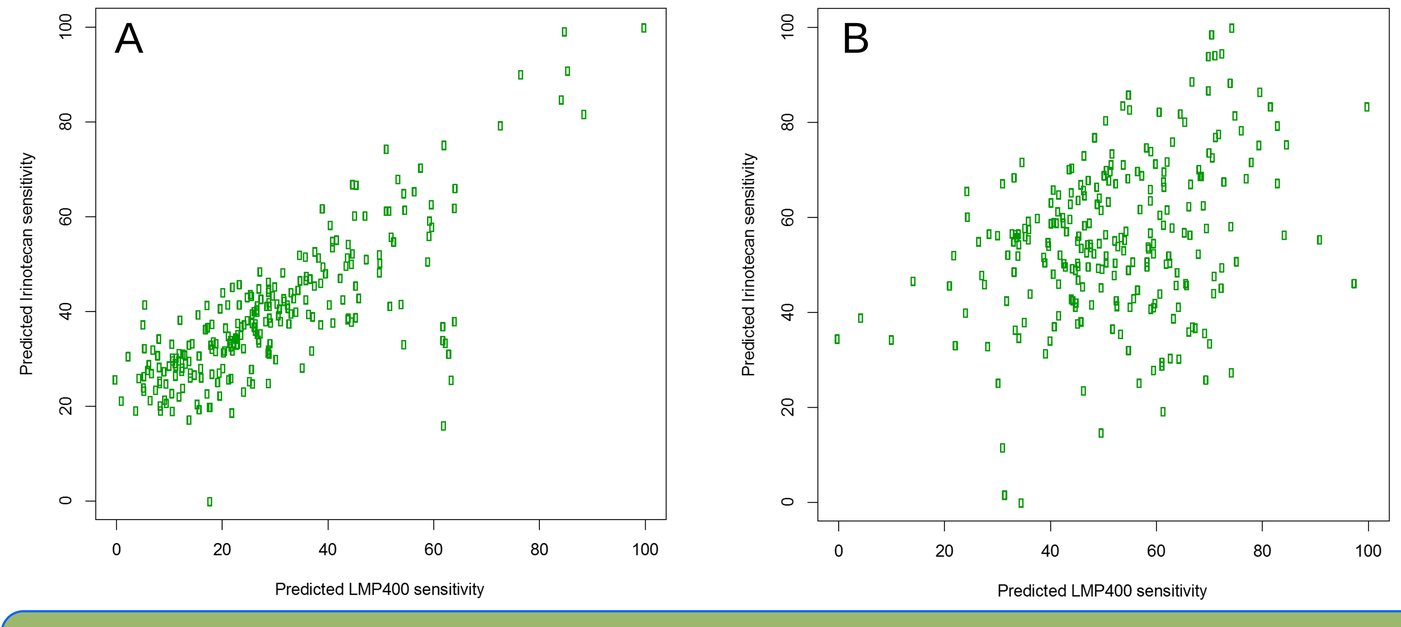


Figure 1. DRPs were established for irinotecan/SN-38 and LMP400 as previously described (Knudsen et al., 2014).
The DRP for LMP400 consists of 165 genes whose expression can be measured in cancer cell lines, in xenografts, in patient biopsies (FFPE or fresh) in order to make predictions of sensitivity to LMP400.
A and B: DRP prediction of the sensitivity to irinotecan/SN-38 and LMP400 in 243 breast cancer patients (A) and 226 non-small cell lung cancer patients (B).

Invitation and contact info (www.den50-r.org): We kindly invite you to contact us if you would like to test any drugs or explore potential biomarkers.
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