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

Jan Stenvang, Niels Frank Jensen, Hafsha Janda, Steen Knudsen, Yves Pommier, Peter Buhl Jensen, Thomas Jensen, Anker Hansen, Mark Cushman and Nils Brünner

1Department of Molecular Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark, 2Medical Prognosis Institute A/S, Hoersholm, Denmark, 3Laboratory of Molecular Pharmacology, National Cancer Institute, NHL, Bethesda, USA, 4Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, and the Purdue University Center for Cancer Research, USA

Abstract # A182

Drug resistance/ 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-01R cell line panel which when fully developed will consist of logistic pairs of drug sensitive and resistant human cancer cell lines representing the 5 most common cancer types. Based on the DEN-01R we have tested a number of novel anti-cancer drugs in this panel of cell lines. One class of drugs that includes the indenoquinacridines 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. The panel of cell lines was treated with Irinotecan, a topoisomerase 1 enzyme inhibitor, and breast, colon and lung cancer cell lines, and LMP showed significant dose related cytotoxicity independent of the state of SN-38 resistance. As these resistant cell lines have significant up-regulation of both the BCIP and/or mdr-1 protein, it can be concluded that the indenoquinacridine LMP400 can target topoisomerase 1 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 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 2000 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 indenoquinacridines 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), LMP776 and LMP400 were established to predict drug response to these two drugs.

Key references:

Chemical structures

Figure 1. Chemical structures of Irinotecan, SN-38 and LMP400 (Indotecan).

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (nM)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN-38</td>
<td>3.4 ± 0.5</td>
<td>1</td>
</tr>
<tr>
<td>LMP400</td>
<td>0.09 ± 0.1</td>
<td>3.7</td>
</tr>
<tr>
<td>LMP776</td>
<td>0.33 ± 0.1</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 1. Drug sensitivity IC50-values and relative resistance in colorectal cancer cells. Mean IC50-value ± 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).

Chemical structures

Figure 1. Chemical structures of Irinotecan, SN-38 and LMP400 (Indotecan).

Table 2. Drug sensitivity IC50-values and relative resistances in breast cancer cells. Mean IC50-value ± 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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (nM)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN-38</td>
<td>3.4 ± 0.5</td>
<td>1</td>
</tr>
<tr>
<td>LMP400</td>
<td>0.09 ± 0.1</td>
<td>3.7</td>
</tr>
<tr>
<td>LMP776</td>
<td>0.33 ± 0.1</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2. Drug sensitivity IC50-values and relative resistances in breast cancer cells. Mean IC50-value ± 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.