4sc-202 (domatinostat), a novel histone deacetylase inhibitor, improves chemotherapy efficacy and overcomes drug resistance in pancreatic cancer models.

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**BACKGROUND**

Pancreatic cancer is the fourth leading cause of cancer-related death in developed countries. Although new standard first line regimens, such as FOLFIRINOX and gemcitabine combined with nab-paclitaxel, have improved overall survival, the prognosis of this disease is still very poor with a 5-year survival rate of 8%. Thus new treatments for pancreatic cancer represent a critical unmet need (Tannock, 2017). Recently, several pre-clinical and clinical studies showed that new therapeutic strategies based on rational combinations of drugs could valor the most promising therapeutic indexes. Thus, new drugs are required to enhance chemotherapy activity, by modulating the drug targets, metabolism or transport mechanisms. Epigenetic alterations play an important role in inhibition and progression of several cancers, including pancreatic cancer. Moreover, in contrast to DNA mutations, the “epimutations” must be actively modulated by modulating the drug targets, metabolism or transport mechanisms. Epigenetic alterations play an important role in the formation, progression and treatment of cancers, including pancreatic cancer. The “epimutations” must be actively modulated by modulating the drug targets, metabolism or transport mechanisms. Epigenetic alterations play an important role in the formation, progression and treatment of cancers, including pancreatic cancer.

**KEY FINDINGS**

- 4sc-202 (domatinostat), a new epigenetic modulator, inhibits pancreatic tumor growth by an antiproliferative effect and by preventing colon-adenoma formation. Moreover, 4sc-202 blocks cell cycle in G2 phase and induces apoptosis and necrosis.

- Combined treatment with equivalent doses 4sc-202 and standard chemotherapy, such as GP (gemcitabine plus paclitaxel) and CAPIF (5FU/IR and TIC) resulted in synergistic/additive anti-proliferative effect in three pancreatic cancer cell lines. Interestingly, an increase of effect is observed when 4sc-202 is administered with 24h delay to chemotherapy.

- Moreover, the simultaneous and sequential schedule of tested combination treatments show a synergistic pro-apoptotic and anti-clonogenetic effects, suggesting a new therapeutic avenue to increase efficacy of drugs commonly used in clinical practice.

**RESULTS**

**Antimicrobial effect of 4sc-202 as monotherapy on pancreatic cancer cell lines**

*Figure 1. Histone deacetylase inhibitor in pancreatic cancer cell lines (4sc-202). (A) The histology, tumor volume and genetic background of the three cell lines, that recapitulate the hyperplastic of PANC-1 cells, was assessed by RT-PCR. (B) 4sc-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5. (C) 4SC-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5. (D) 4SC-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5.*

**Synergistic antimicrobial effects of 4sc-202 plus Chemotherapeutics in a simultaneous and sequential schedules**

*Figure 3. Synergistic effect of 4sc-202 plus gemcitabine/paclitaxel (GP) in three pancreatic cancer cell lines. (A) The synergistic effect of 4sc-202 plus gemcitabine/paclitaxel is observed by measuring the luminescence in a Multilabel Reader VICTOR X4 2030 (PerkinElmer). (B) The drug combination shows a synergistic effect in all three cell lines when a 24h delay to chemotherapy. In the lower table, as above, we evaluated the combination of 4sc-202 plus gemcitabine/paclitaxel. The sequential scheme showed synergism in PANC1 cell line but not in PANC28 and b35 cell lines. Although the antiproliferative effect of 4sc-202 is similar to that obtained in the combination treatment, the increase of the apoptotic effect, measured as AnnexinV exposition, induced by the sequential treatment approach, is more evident.*

**METHODS**

**Pancreatic cancer cell lines features and 4sc-202 molecular characteristics**

*Table 1. Pancreatic cancer cell lines features and 4sc-202 molecular characteristics.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Histology</th>
<th>Tumor source</th>
<th>Mutational status</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPC1</td>
<td>Adenocarcinoma</td>
<td>Primary Tumour</td>
<td>CDKN2A, TP53, FBXW7</td>
</tr>
<tr>
<td>ASPC2</td>
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</tr>
<tr>
<td>ASPC28</td>
<td>Adenocarcinoma</td>
<td>Primary Tumour</td>
<td>CDKN2A, TP53, FBXW7</td>
</tr>
</tbody>
</table>

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**Cell Culture** The PANC1 cancer cell lines were cultured in CMRL medium–10% FBS (Clonetics). Cell proliferation assay was measured in 96-well plates using an xTremity ecectric proliferation assay (Roche Diagnostics) after 48 h of treatment with 4sc-202 (10 nM) and 5FU (10 μM) at 37°C in 5% CO2 in a humidified atmosphere. The proliferation index was calculated as the ratio of the absorbance of treated cells over untreated cells. Over 72% of proliferative index values were between 0.5 and 1.5. 1% denotes strongly, 0.5 and 1 denotes as moderate, and 0.25 and 0.5 denotes as low. Corresponding index values were used to calculate the IC50 values of each drug treatment combination.

**Loss of Colon-Adenoma** The PANC1 cancer cell lines were cultured in CMRL medium–10% FBS (Clonetics). Cell proliferation assay was measured in 96-well plates using an xTremity ecectric proliferation assay (Roche Diagnostics) after 48 h of treatment with 4sc-202 (10 nM) and 5FU (10 μM) at 37°C in 5% CO2 in a humidified atmosphere. The proliferation index was calculated as the ratio of the absorbance of treated cells over untreated cells. Over 72% of proliferative index values were between 0.5 and 1.5. 1% denotes strongly, 0.5 and 1 denotes as moderate, and 0.25 and 0.5 denotes as low. Corresponding index values were used to calculate the IC50 values of each drug treatment combination. The proliferative index was calculated as the ratio of the absorbance of treated cells over untreated cells. Over 72% of proliferative index values were between 0.5 and 1.5. 1% denotes strongly, 0.5 and 1 denotes as moderate, and 0.25 and 0.5 denotes as low. Corresponding index values were used to calculate the IC50 values of each drug treatment combination.

**Cell Cycle** The PANC1 cancer cell lines were cultured in CMRL medium–10% FBS (Clonetics). Cell proliferation assay was measured in 96-well plates using an xTremity ecectric proliferation assay (Roche Diagnostics) after 48 h of treatment with 4sc-202 (10 nM) and 5FU (10 μM) at 37°C in 5% CO2 in a humidified atmosphere. The proliferation index was calculated as the ratio of the absorbance of treated cells over untreated cells. Over 72% of proliferative index values were between 0.5 and 1.5. 1% denotes strongly, 0.5 and 1 denotes as moderate, and 0.25 and 0.5 denotes as low. Corresponding index values were used to calculate the IC50 values of each drug treatment combination.

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**RESULTS**

**Pancreatic cancer cell lines and 4sc-202 molecular characteristics**

*Figure 1. Histone deacetylase inhibitor in pancreatic cancer cell lines (4sc-202). (A) The histology, tumor volume and genetic background of the three cell lines, that recapitulate the hyperplastic of PANC-1 cells, was assessed by RT-PCR. (B) 4sc-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5. (C) 4SC-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5. (D) 4SC-202 is the tosylate salt of 4SC-202, a histone deacetylase inhibitor with MW of 447.5 g/mol and a pKa of 7.5.*