Determinants of Gemcitabine-Pemetrexed Synergism in Pancreatic Cancer Cell Lines

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Mechanism of action of gemcitabine and pemetrexed

- Pemetrexed (MTA)
- FPGS
- Pemetrexed (Glu)
- dUMP
- TS
- Thymidylate biosynthesis

- PRPP + Glutamine
- purine de novo biosynthesis
- 10 CHO-FH₄
- GARFT
- 5-10CH₂-FH₄
- Folate metabolism
- GAR
- IMP
- HX + PRPP
- purine salvage biosynthesis
- Orotate
- UMP
- UMP

- Gemcitabine (dFdC)
- CDA
- dFdU
- Deoxycytidine
- pirimidine salvage biosynthesis
- dCK/5'NT
- dCMP
- dCDP
- CDP
- RR
- CDP
- dFdCDP
- dFdCTP
- dFdCMP
- (-)
- dFdCDP
- dFdCTP

- DNA

Adjei et al., J Clin Oncol 2000; 8:1748
Shih et al., Cancer Res 1997; 57:1116
Tonkinson et al., Cancer Res 1999; 59:3671
Tesei et al., Clin Cancer Res 2002; 8:233
Cytotoxicity and pharmacologic interaction between gemcitabine and pemetrexed

**Capan-1**

- dFdC (IC$_{50}$) = 4.75 µg/ml
- MTA (IC$_{50}$) = 7.33 µg/ml
- dFdC-MTA (IC$_{50}$) = 0.03 µg/ml
- MTA-dFdC (IC$_{50}$) = 0.02 µg/ml

**MIA PaCa-2**

- dFdC (IC$_{50}$) = 2.90 µg/ml
- MTA (IC$_{50}$) = 1.58 µg/ml
- dFdC-MTA (IC$_{50}$) = 0.12 µg/ml
- MTA-dFdC (IC$_{50}$) = 0.04 µg/ml

**PANC-1**

- dFdC (IC$_{50}$) = 42.21 µg/ml
- MTA (IC$_{50}$) = 2.42 µg/ml
- dFdC-MTA (IC$_{50}$) = 0.75 µg/ml
- MTA-dFdC (IC$_{50}$) = 0.09 µg/ml

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**Graphs:**

- MIA PaCa-2
- PANC-1
- Capan-1

- Log [Drug] _µg/ml_
- % Cells surviving respect to control
- Combination Index (CI)
- Fraction affected
### Cell cycle modulation by gemcitabine and pemetrexed

#### MIA PaCa-2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1 (%)</th>
<th>S (%)</th>
<th>G2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.01</td>
<td>15.30</td>
<td>7.69</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>46.36</td>
<td>49.29</td>
<td>4.35</td>
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<tr>
<td>Pemetrexed</td>
<td>30.12</td>
<td>46.63</td>
<td>23.25</td>
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</tbody>
</table>

#### PANC-1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1 (%)</th>
<th>S (%)</th>
<th>G2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88.25</td>
<td>10.55</td>
<td>1.20</td>
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<tr>
<td>Gemcitabine</td>
<td>66.98</td>
<td>29.29</td>
<td>3.73</td>
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<tr>
<td>Pemetrexed</td>
<td>17.21</td>
<td>80.13</td>
<td>2.66</td>
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</table>

#### Capan-1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1 (%)</th>
<th>S (%)</th>
<th>G2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.73</td>
<td>31.13</td>
<td>18.14</td>
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<tr>
<td>Gemcitabine</td>
<td>54.19</td>
<td>36.40</td>
<td>9.41</td>
</tr>
<tr>
<td>Pemetrexed</td>
<td>31.10</td>
<td>63.21</td>
<td>5.69</td>
</tr>
</tbody>
</table>

*a* Mean percent values of total number of cells examined in three independent experiments.
Induction of apoptosis by gemcitabine, pemetrexed and their combination

Columns, mean values obtained from three independent experiments; bars, SE
*Statistically significantly different from controls (P<0.05)
Modulation of dCK expression by pemetrexed

<table>
<thead>
<tr>
<th>Gene</th>
<th>MIA PaCa-2</th>
<th>PANC-1</th>
<th>Capan-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine</td>
<td>12.29</td>
<td>53.43</td>
<td>29.90</td>
</tr>
<tr>
<td>+dCyd</td>
<td>86.11</td>
<td>503.97</td>
<td>272.53</td>
</tr>
<tr>
<td>+DEPC</td>
<td>10.15</td>
<td>28.03</td>
<td>13.71</td>
</tr>
<tr>
<td>+THU</td>
<td>7.54</td>
<td>10.52</td>
<td>9.40</td>
</tr>
</tbody>
</table>

*a Mean values ±SE of at least three independent experiments.

Gene expression (C_T)

Log [cDNA]

dCK expression (2^-ΔΔC_T)

MIA PaCa-2  PANC-1  Capan-1
Enhancement of dCK/RRM1×RRM2 expression ratio after pemetrexed treatment

- Gemcitabine IC₅₀ (µg/ml)
  - PANC-1
  - Capan-1
  - MIA PaCa-2

- dCK/RRM1×RRM2 expression ratio
  - Control
  - Pemetrexed

- $R^2 = 0.95$
Conclusions

Gemcitabine and pemetrexed were cytotoxic against MIA PaCa-2, PANC-1 and Capan-1 cells and the combination index demonstrated that the drug sequence showing the maximum degree of synergism was pemetrexed → gemcitabine in all cell lines.

Flow cytometric studies demonstrated that pemetrexed and gemcitabine enhanced cellular population in S phase in all cell lines.

Gemcitabine-pemetrexed combinations increased the occurrence of apoptosis.

Quantitative RT-PCR analysis showed that pemetrexed significantly enhanced dCK expression in all cell lines, while there was only a minor increase of RR expression.

These data provide evidence that the combination of gemcitabine and pemetrexed displays schedule-dependent synergistic cytotoxic activity against various pancreatic cancer cells, associated with favorable modulation of cell cycle, induction of apoptosis and inducible dCK gene expression.