CHARACTERIZATION AND TRANSFECTION EXPERIMENTS OF POLYPLEXES TARGETING HDAC7

Antoine Frère1, Michal Kawalec2, Laurence Collard3, Nicolas Matheus2, Paul Peixoto2, Brigitte Evrard1, Philippe Dubois3, Laetitia Mespouille3, Denis Mottet2, Geraldine Piel1

1 Laboratory of Pharmaceutical Technology - CIRM, 2 Metastasis Research Laboratory – GIGA Cancer, University of Liege, Liege, Belgium; 3 Laboratory of Polymeric and Composite Materials, University of Mons, Mons, Belgium
E-mail: antoine.frere@ulg.ac.be

PURPOSE
• To develop polyplexes with new cationic biodegradable aliphatic polycarbonate polymers bearing or not polyethyleneoxide (PEO) and guanidinium functions; C93, C98, C106 and C122.
• These polymers are applied to the complexation of siRNA directed specifically against the histone deacetylase 7 (HDAC7). HDAC7 is a rational target for anti-angiogenic therapy.
• The aim of this study is to determine the best N/P ratio according to the size, the charge and the incorporation level of polyplexes. Tests on HeLa cells are performed with the selected ratio to evaluate cellular internalization and transfection efficiency.

Polycarbonate polymers + siRNA → Polyplexes

<table>
<thead>
<tr>
<th>Polycarbonate</th>
<th>Mn (nmol/µg)</th>
<th>N (nmol/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C93</td>
<td>44362</td>
<td>2.66</td>
</tr>
<tr>
<td>C98</td>
<td>30102</td>
<td>2.59</td>
</tr>
<tr>
<td>C106</td>
<td>28720</td>
<td>2.78</td>
</tr>
<tr>
<td>C122</td>
<td>20250</td>
<td>2.47</td>
</tr>
</tbody>
</table>

Figure 1: Effect of N/P ratio on the size (A), charge (zeta potential) (B), and the percentage of siRNA incorporated (C) in C93, C98, C106 and C122 polyplexes. Polyplexes were prepared at a siRNA concentration of 100nM in TE buffer with 5% mannitol; n=3, s.d. are not shown on the graphs for more clarity.

N/P 15 was selected for each polyplex.

Transfection experiments on HeLa cells

A

C93 (10x)
C93 polyplexes with non-fluorescent siRNA (10x)
C93 polyplexes with fluorescent siRNA (20x)

B

Polyplexes % fluorescent cells
C93 75.49 %
C98 84.67 %
C106 80.45 %
C122 46.53 %

C

PEI + siRNA HDAC7
C106 + siRNA HDAC7
C122 + siRNA HDAC7

HDAC7
Normalization:

CONCLUSION
These results show that C93, C98, C106 and C122 polycarbonates are able to form polyplexes with good physicochemical parameters and high cellular internalization. C93, C98 (results not shown) and C106 polyplexes were not able to shut down the expression of HDAC7. C122 polyplexes show a promising partial decrease in the protein expression despite a lower % of transfected cells measured. This result may be explained by a better configuration of the polymer. Future studies will try to further improve the decrease in HDAC7 expression by an optimization of the polyplex characteristics and of the cell culture conditions.