CpG DEPLETION RESULTS IN INCREASED DURATION OF GENE EXPRESSION FROM PLASMID DNA VECTORS IN VIVO

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\section*{Introduction.}

\subsection*{Gene therapy is being developed as a treatment for Cystic Fibrosis.}

\subsection*{Treatments will likely require long-term gene expression and/or repeated administration.}

\subsection*{We are developing plasmid-based vectors which may be less immunogenic than viral vectors.}

\subsection*{CpG motifs in plasmids can cause a host inflammatory response when complexed with liposomes.}

\subsection*{The host inflammatory response may result in transient gene expression.}

\subsection*{We investigated the effects of CpG depletion on \textit{in vivo} gene expression.}

\section*{Aims.}

\subsection*{To investigate reporter gene expression from plasmids with a reduced CpG content.}

\subsection*{To test the persistence of gene expression following aerosol delivery.}

\section*{Construction of Plasmid Vectors}

\textbf{A:} pCIKLux and pUbLux share a CpG-rich backbone and Luciferase gene. pCIKLux contains the CMV promoter, pUbLux contains the UbC promoter.

\textbf{B:} pGM CMV Lux and pGM UbC Lux share a CpG-depleted backbone (Kanamycin resistance gene, BGH PolyA, ColEl origin) and a CpG-rich Luciferase gene.

\textbf{C:} These plasmids share a CpG-depleted backbone. pGM GZB Lux contains a CpG-depleted CMV enhancer-promoter, pGM CUBI/CUCI Lux plasmids contain the CMV enhancer and UbB/UbC promoter respectively.

\section*{CpGs Per Plasmid}

<table>
<thead>
<tr>
<th>pCIKLux</th>
<th>pUbLux</th>
<th>pGM CMV Lux</th>
<th>pGM UbC Lux</th>
<th>pGM GZB Lux</th>
<th>pGM CUBI Lux</th>
<th>pGM CUCI Lux</th>
</tr>
</thead>
<tbody>
<tr>
<td>317 245</td>
<td>369 146</td>
<td>193 231 262</td>
<td></td>
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</tr>
</tbody>
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\section*{Aerosol Delivery of Plasmids to Mice}

Plasmid DNA (2mg) was complexed with polyethyleneimine (PEI) at an N:P ratio of 10:1 in water for injection to give 10ml total dose per experiment. The aerosol was delivered to female BALB/c mice housed in a polycarbonate box (left) using an Aerotech II nebuliser. Whole lungs were harvested and homogenised and the homogenates assayed for Luciferase expression at the timepoints shown.

\section*{Conclusions.}

\subsection*{CpG depletion of the backbone does not have a significant effect on the level or duration of Luciferase expression from plasmids which contain the CMV Promoter.}

\subsection*{Incorporation of the CMV enhancer into a plasmid containing the UbB promoter to generate pGM CUBI Lux results in increased gene expression at Day 1 and expression which is still detectable at Day 28.}

\subsection*{For persistent high-level gene expression following aerosol delivery, a plasmid containing the UbC promoter in a CpG-depleted backbone is optimal.}

\textbf{Luciferase Expression in Whole Lung Lysates of Aerosol Dosed Mice}

\begin{itemize}
  \item \textbf{A:} pGM CMV Lux and pGM GZB Lux give a similar expression profile to pCIKLux, peaking at Day 1 and dropping to background levels by Day 7.
  \item \textbf{B:} pGM CUBI Lux and pGM CUCI Lux follow a similar expression profile to pUbLux, peaking at Day 1 then falling to a low level which persists to Day 28. pGM CUBI Lux generally gives increased levels of expression compared to pGM CUCI Lux.
  \item \textbf{C:} Overall pGM UbC Lux generally gives increased levels and duration of expression compared to the other plasmids.
\end{itemize}