Duration of Expression from CpG-Free Plasmids Following Hydrodynamic Delivery to the Mouse Liver

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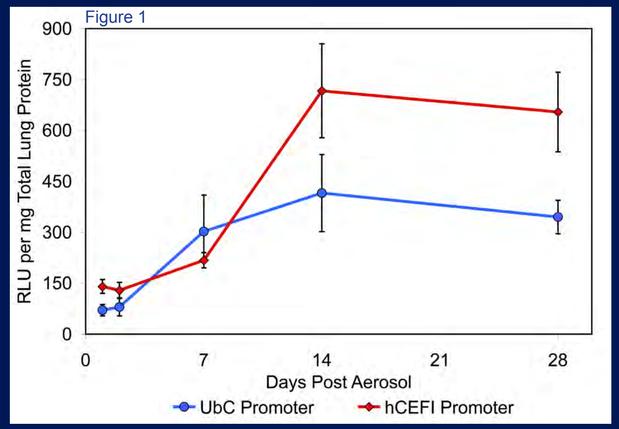
Overview

- CpG-Free plasmids are being used to minimise gene-transfer mediated inflammation in several organs including the lung[1].
- Expression from a CpG-Free[2] clinical plasmid complexed with Genzyme Lipid 67 (GL67A) is being evaluated in the lungs of CF patients following aerosol delivery.
- Only two promoters have been shown to direct persistent expression from GL67A/pDNA complexes after aerosol delivery to the mouse lung.
  1. The human polyubiquitin C promoter[3].
  2. The CpG-Free hCEFI promoter (human CMV enhancer and EFI promoter)[1].

Duration of Expression Following Aerosol Delivery of GL67A/pDNA

Figure 1



Aims of the study

1. Assess the duration of expression from UbC and hCEFO in the liver following hydrodynamic (HPTV) delivery.

   Key Question 1
   Do the UbC and hCEFI promoters exhibit persistent expression in other organs?

2. We have previously identified a range of novel CpG-Free promoters and have established that these express in mouse lung. Their expression profile following HPTV delivery will now also be established.

   Key Question 2
   Can CpG-Free promoters be used widely in gene therapy models?

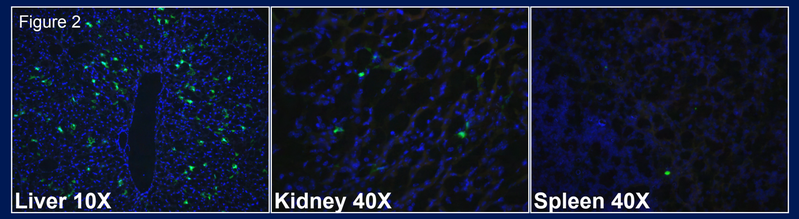
Method

1. Plasmid DNA was diluted to 10 µg/ml in D-PBS.
2. Female BALB/c mice (6-8 wks) were anaesthetised with isoflurane.
3. 10% v/w (typically 1.8 ml) pDNA was injected into the mouse tail vein over 5 secs.
4. Reporter gene expression was determined at various timepoints by Luciferase assay or direct analysis of GFP fluorescence.

Confirmation of Expression Following HPTV Delivery

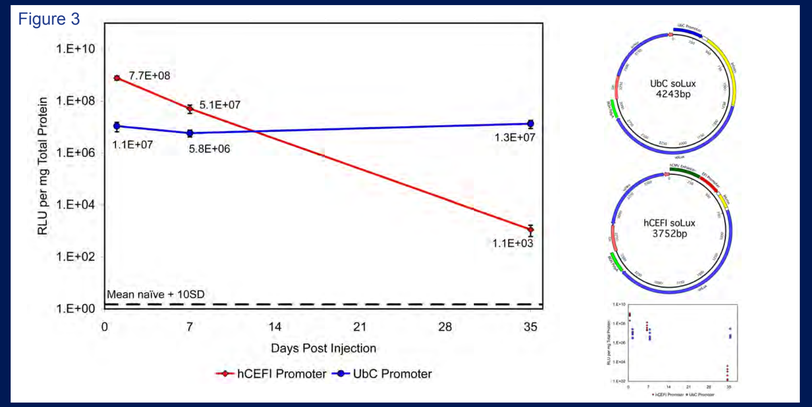
- Direct visualisation of GFP was used to confirm gene expression following HPTV delivery.
- GFP was clearly observed in the liver at very high levels and at much lower levels in the spleen, kidney and lungs (Figure 2) (d1 post pEGFP-N1 delivery)
- When using Luciferase as the reporter gene, high levels of Luciferase can be detected in the serum
- Therefore Luciferase is unsuitable for the assessment of reporter gene expression in the internal organs other than the liver

Figure 2



Duration of Expression from hCEFI and UbC in the Mouse liver

Figure 3



Key Question 1:- Outcome

- The UbC promoter directs persistent expression at a stable level in the mouse liver.
- At early timepoints (d1,d7) expression from hCEFI was 70X and 9X higher than expression from UbC.
- Expression from the hCEFI promoter does not persist and by d35 was 5-logs lower than at d1.

Novel CpG-Free Promoter Plasmids

Figure 4

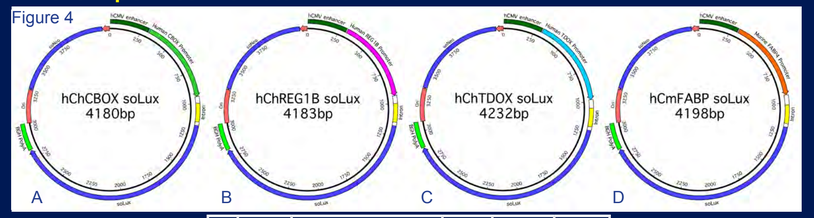
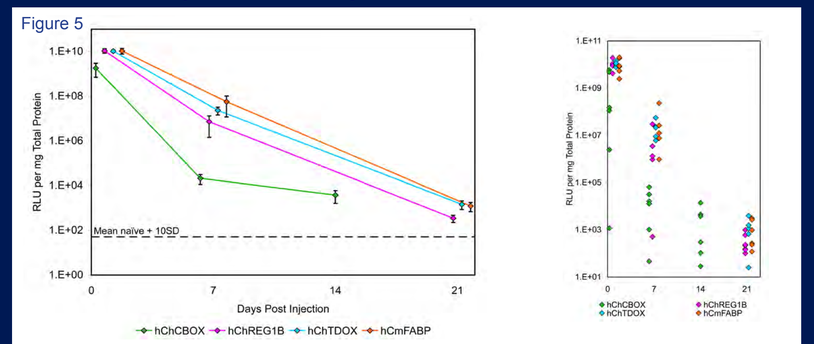


Fig 4	Abbr	Promoter Name	Species	5' Enhancer	Transgene
A	hChCBOX	Carboxypeptidase P	Human	Human CMV	Luciferase
B	hChREG1B	Regenerating islet derived protein 1B	Human	Human CMV	Luciferase
C	hChTDOX	Tryptophan 2,3 dioxygenase	Human	Human CMV	Luciferase
D	hCmFABP	Fatty acid binding protein4	Mouse	Human CMV	Luciferase

Duration of Expression from CpG-Free Promoter Plasmids in the Liver

Figure 5



Key Question 2:- Outcome

- At d1 post injection all 4 CpG-Free promoters had similar high levels of expression, significantly higher than levels achieved with hCEFI or UbC.
- By d7 levels from three REG1B, TDOX & FABP had fallen approx 1000X and CBOX had fallen 80,000X.
- By d21 expression had continued to fall but was still higher than background.

Conclusions

- The hCEFI promoter does not persist in the liver, in contrast to the lung.
- The UbC promoter persists in both the lung and the liver.
- The novel CpG-Free promoters tested here express at very high levels in the liver, but do not exhibit persistent expression.
- Duration of expression from pDNA is therefore dependent on the mode of delivery and the target organ rather than simply the promoter sequence.
- Theories aimed at identifying factors affecting promoter performance need to be studied in a wide range of model systems.

1. Hyde et al., 2008, Nature Biotechnology, 26, 549-551
2. Utilizing CpG-Free plasmid technology developed by Cayla Invivogen (Toulouse, France).
3. Gill et al., 2001, Gene Therapy, 8, 1539-1546

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