INFLUENCE OF THE HUMAN AND MURINE CMV ENHANCER ON THE DURATION OF EXPRESSION FROM CpG-FREE PLASMID VECTORS IN THE MOUSE LUNG

Anne-Marie Green[1,3], Ian A. Pringle[1,3], Anna E. Lawton[1,3], Chela Nunez[1,3], Lee A. Davies[1,3], Nelson S. Yew[2], Seng H. Cheng[2], Deborah R. Gill[1,3], Stephen C. Hyde[1,3]

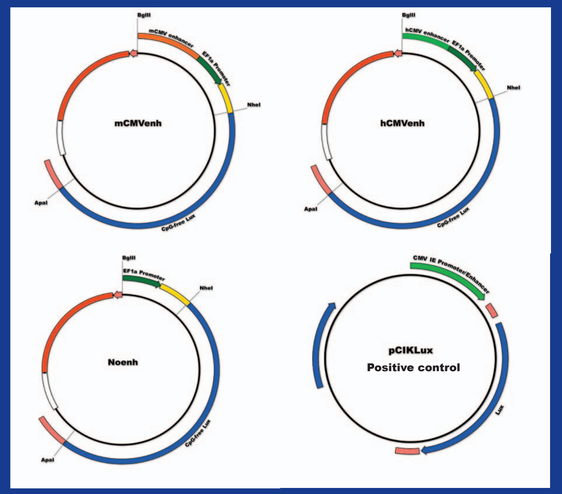
1. Gene Medicine Research Group, NDCLS, John Radcliffe Hospital, University of Oxford, Oxford, UK.
2. Genzyme Corporation, Cambridge, MA, USA 3. The United Kingdom Cystic Fibrosis Gene Therapy Consortium

anne-marie.green@ndcls.ox.ac.uk http://users.ox.ac.uk/~genemed http://www.cfgenetherapy.org.uk

Poster download available

Introduction

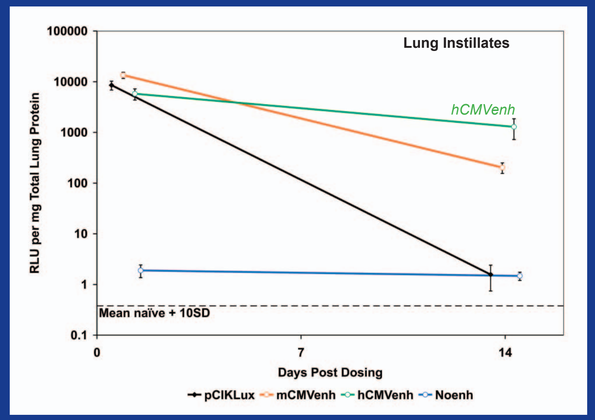
- Non-viral gene therapy is being developed for Cystic Fibrosis (CF)
- Previous plasmid DNA (pDNA) vectors in CF clinical trials have shown:
  - Poor duration of expression
  - Inflammatory responses attributed to CpG motifs
- We have developed a range of novel CpG-free plasmids which show reduced inflammatory responses *in vivo* (Bazzani *et al* poster 1015)
- We are assessing the effects of different enhancer/promoter combinations on duration of expression in these vectors
- We have conducted several studies using the mouse model:
  - Nasal instillation with Genzyme Lipid 67 (GL67)
  - Aerosol delivery to the lung with GL67
  - Aerosol delivery to the lung with 25kDa Polyethylenimine (PEI)
- In particular, we examined the effect on duration of expression of:
  - The human cytomegalovirus (CMV) enhancer
  - The murine CMV enhancer
  - The absence of an enhancer



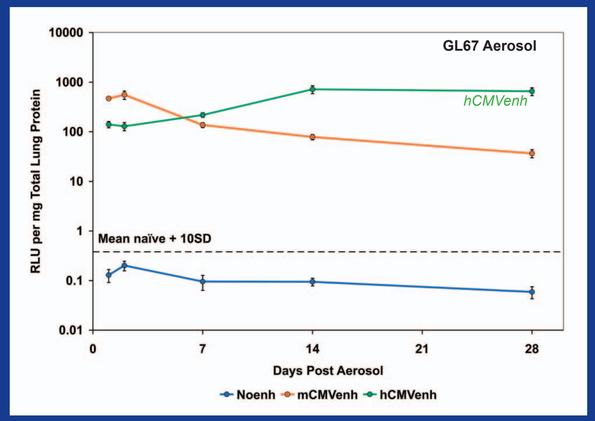
- We used 4 plasmids, constructed using established methods

The 3 test plasmids contained the following key elements:
- CpG-free human elongation factor 1a (hEFIa) promoter
- soLux transgene

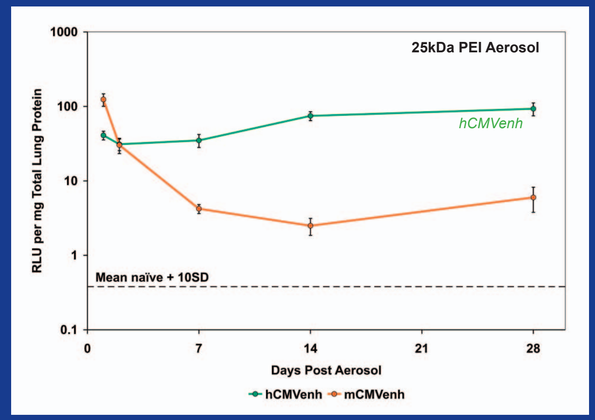
The 3 test plasmids also contained one of either:
- CpG-free hCMV enhancer (hCMVenh)
- CpG-free mCMV enhancer (mCMVenh)
- No enhancer (Noenh)



- Luciferase (Lux) activity following nasal instillation with GL67
  - Plasmid DNA (80µg) complexed with GL67 (100µl total volume)
  - BALB/c mice (n=6/time point) dosed with GL67/pDNA by nasal insufflation
  - Post-dosing Lux activity measured in lung lysates
  - Levels of expression from Noenh were not significant
  - The mCMVenh sequence led to the highest levels of expression initially (p=0.016) but expression declined over time
  - By day 14 levels of expression from hCMVenh were above those from mCMVenh (*p*=0.025)



- Lux activity following aerosol delivery using GL67
  - Plasmid DNA (20mg) complexed with GL67 (10ml total volume)
  - Mice (n=6/time point) dosed with GL67/pDNA by aerosolisation (whole body exposure chamber)
  - Post-aerosol Lux activity measured in lung lysates
  - The mCMVenh plasmid gave the highest levels of expression initially (*p*=0.004) but expression declined over time
  - By day 7 levels of expression from hCMVenh were above those from mCMVenh (*p*=0.004) and continued to be significantly higher until at least day 28 (*p*=0.004)



- Lux activity following aerosol delivery using PEI
  - Plasmid DNA (4mg) complexed with 25kDa branched PEI (Sigma, 10ml total volume)
  - Mice dosed with PEI/pDNA by aerosolisation as previously
  - Post-aerosol Lux activity measured in lung lysates
  - Similar expression pattern to GL67 aerosol

Presentation on PEI aerosol delivery - Sunday 3rd June, 8:30am (Davies *et al*)

Conclusions

- In the absence of an enhancer the EFI promoter alone was unable to generate significantly high levels of expression
- The murine CMV enhancer (mCMVenh) sequence led to the highest levels of expression initially but expression declined over time, offering no advantage over plasmids used in previous clinical trials for CF
- The human CMV enhancer (hCMVenh) can play a crucial role in determining the level and duration of expression from these pDNA vectors in the mouse lung model
- Only when the hCMVenh is combined with the EFI promoter have we observed such sustained high levels of reporter gene expression
- Our clinical trial plasmid is a CFTR expressing version of hCMVenh

Produced by Anne-Marie Green, Gene Medicine Research Group & The Medical Informatics Unit, NDCLS, University of Oxford, Telephone +44 (0)1865 220545