Complete But Not Partial Reduction of Plasmid CpG Content Increases Transgene Expression And Eliminates The Inflammatory Response Associated With Delivery Of Non-Viral Vectors To The Lung

S.C. Hyde[1,2], A.E. Lawton[1,2], A. Varathalingam[1,2], S.H. Cheng[3], L.A.Davies[1,2], I.A.Pringle[1,2], D.R. Gill[1,2]

1. GeneMedicine Research Group, Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, University of Oxford, Oxford, UK.
2. The United Kingdom Cystic Fibrosis Gene Therapy Consortium
3. Genzyme Corporation, Cambridge, MA, USA

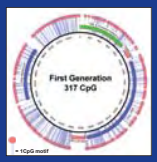
steve.hyde@ndcls.ox.ac.uk http://users.ox.ac.uk/~genemed http://www.cfgenetherapy.org.uk

Poster download available

Overview

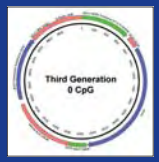
- Non-viral gene therapy is being developed as a treatment for the lung disease associated with Cystic Fibrosis (CF).
- CpG motifs found in plasmid DNA (pDNA) cause a potent host inflammatory response *in vivo*.
- The host imflammatory response may result in gene silencing and therefore transient gene expression.
- We investigated the effects of CpG depletion on *in vivo* gene expression and the host inflammatory response.

Inflammatory Responses Following GL67/pDNA Delivery To The Mouse Lung

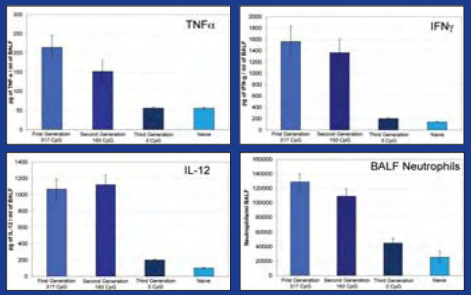


pDNA backbone similar to that used in multiple Phase I trials in mid 1990s.

Based on Genzyme minimal CpG backbone pOri-K-syn (Yew, Mol Ther 1:255 (2000)).



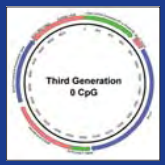
Based on InvivoGen zero CpG backbone pCpGLacZ.

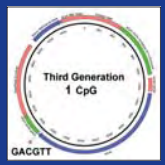


80µg pDNA (< 5EU/mg) complexed with GL67 was delivered to the lungs of BALB/c mice (n=10/group) by nasal insufflation (Lee *et al* , Human Gene Therapy 7:1701 (1996)). At 24hr post-dosing BALF (3ml) was recovered and assayed for cytokines by ELISA.

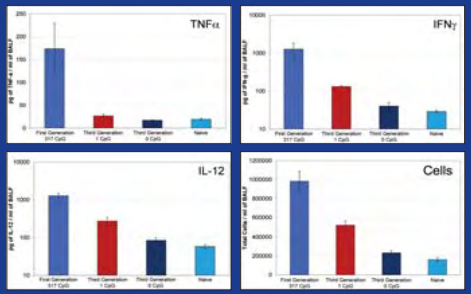
- Delivery of GL67/First Generation pDNA (317 CpG) results in increased inflammatory cytokines and BALF neutrophils.
- Partial reduction of pDNA CpG content (Second Generation pDNA) does not result in a decrease in lung inflammation.
- Abolition of pDNA CpG content (Third Generation pDNA) eliminates lung inflammation associated with non-viral gene delivery.

Inflammatory Responses Following Delivery of GL67/pDNA Containing 1 CpG Motif To The Mouse Lung





Based on InvivoGen zero CpG backbone pCpGLacZ.

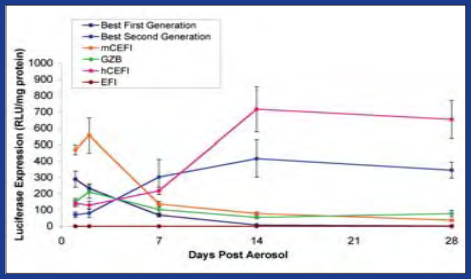


80µg pDNA (< 5EU/mg) complexed with GL67 was delivered to the lungs of BALB/c mice (n=10/group) by nasal insufflation as described previously. At 24hr post-dosing BALF (3ml) was recovered and assayed for cytokines by ELISA.

- A single pDNA CpG motif is sufficient to direct lung inflammation following non-viral gene delivery.

Expression Profile of Zero CpG pDNAs Following Aerosol Delivery To The Mouse Lung

Enhancer	Promoter	Code
-	Elongation Factor 1α	EFI
Mouse CMV	Elongation Factor 1α	mCEFI
Human CMV	Elongation Factor 1α	hCEFI
Human CMV	Human CMV	GZB



25mg pDNA (< 5EU/mg) complexed with GL67 was delivered to the lungs of BALB/c mice (n=6/group) by aerosol delivery (Eastman *et al* , Human Gene Therapy 8:765 (1997)). Lung luciferase activity was determined at the indicated time post aerosol delivery.

- A variety of Fourth Generation pDNAs have been constructed containing alternative CpG-free enhancers (mouse CMV, human CMV) and promoters (human CMV, human Elongation Factor 1α).
- Zero CpG Fourth Generation pDNAs containing the hCEFI enhancer/promoter direct sustained lung transgene expression following aerosol-mediated non-viral gene delivery.
- Fourth Generation pDNAs are suitable for use in clinical trials.

Conclusions.

- Partial CpG reduction does not reduce the CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.
- A single plasmid-derived CpG is sufficient to direct a CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.
- Complete CpG depletion abolishes the CpG inflammatory response in mouse lungs following delivery of GL67/pDNA complexes.
- Zero-CpG plasmids harbouring the hCEFI enhancer/promoter direct sustained lung transgene expression following aerosol delivery of GL67/pDNA complexes.

Produced by Anna E. Lawton, GeneMedicine Group & The Medical Informatics Unit, NDCLS, University of Oxford, Telephone +44 (0)1865 220545