



Long-Term Stability of Aqueous pCIKLux/PEI Complexes

Graciela A Nunez-Alonso^{1,2}, Lee A Davies^{1,2}, Stephen C Hyde^{1,2}, Deborah Gill^{1,2}

1. Gene Medicine Research Group, NDCLS, John Radcliffe Hospital, University of Oxford, Oxford, UK.
2. The United Kingdom Cystic Fibrosis Gene Therapy Consortium

chela.nunez@ndcls.ox.ac.uk

<http://users.ox.ac.uk/~genemed>

<http://www.cfgenetherapy.org.uk>

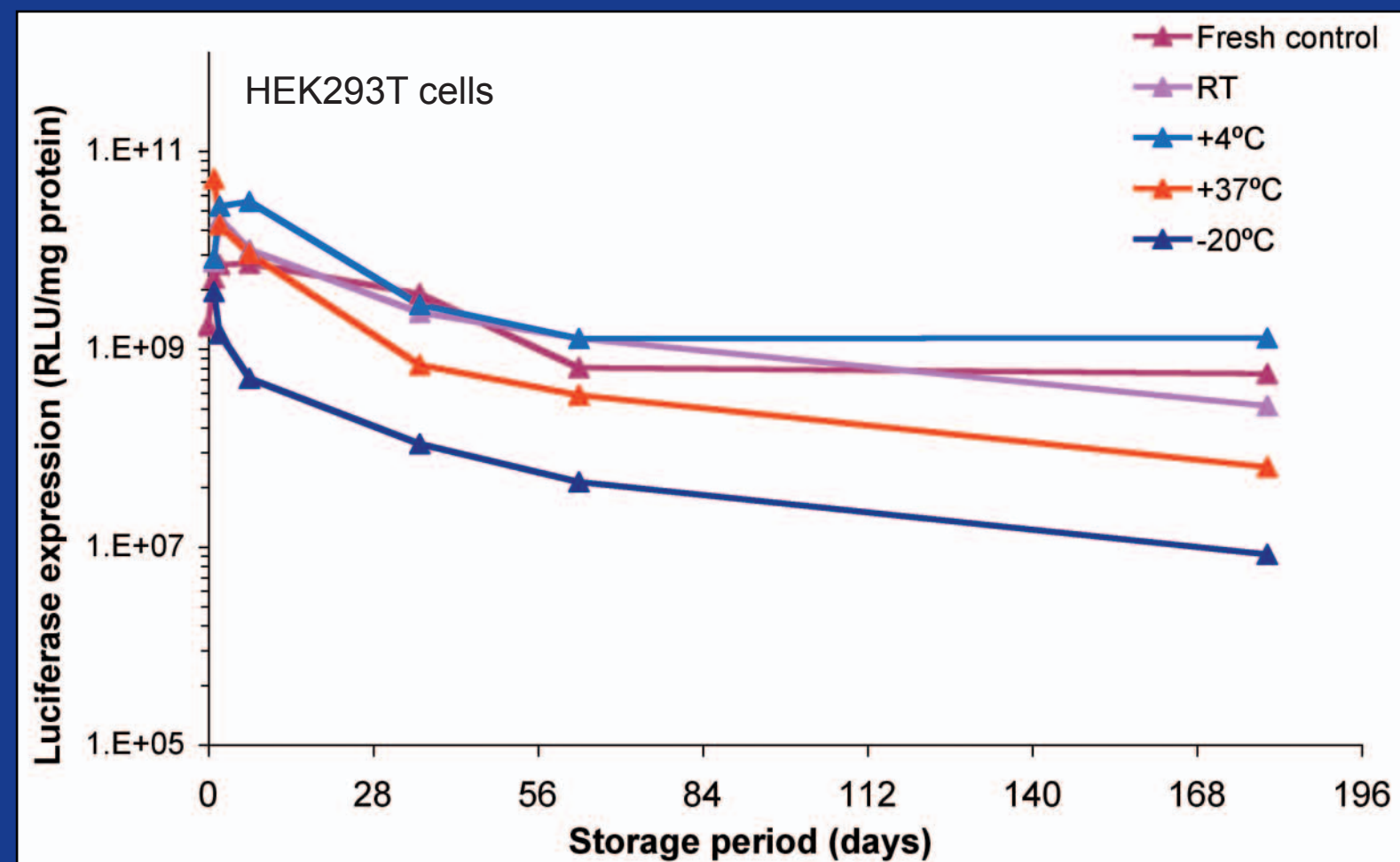
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Introduction

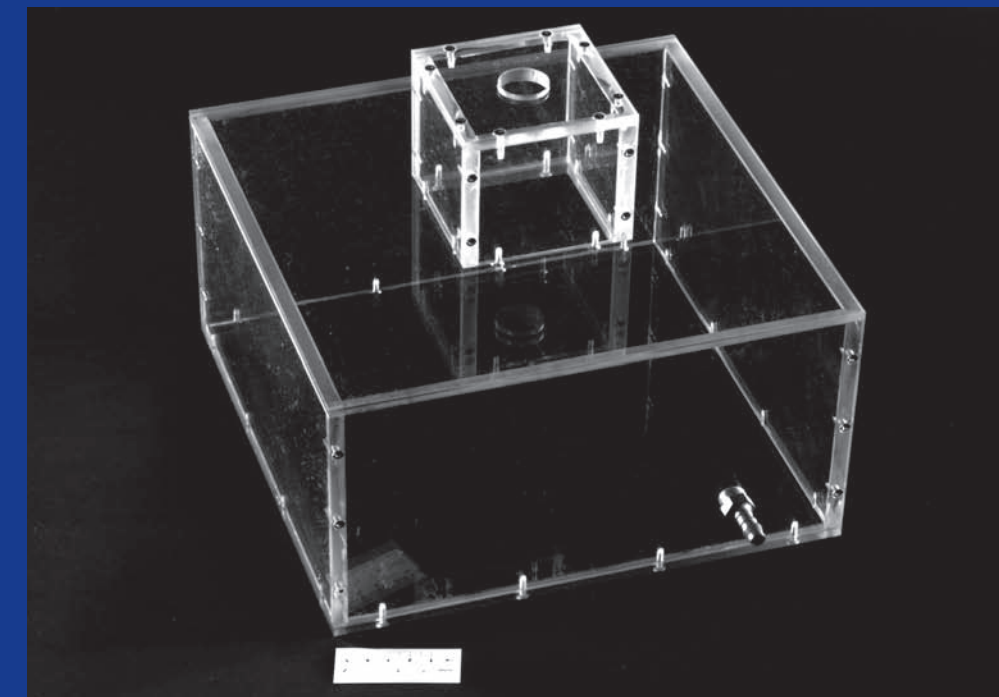
- ▶ The UK Cystic Fibrosis Gene Therapy Consortium is committed to the testing and development of gene therapy vectors for CF clinical trials
- ▶ Successful lung gene therapy vectors will require topical administration to the airway epithelium via aerosol delivery
- ▶ Instability of non-viral gene transfer agents (GTAs) can restrict the suitability of vectors for clinical studies
- ▶ The cationic polymer 25kDa polyethyleneimine (PEI) has demonstrated successful gene expression following aerosol administration to the mouse lung
- ▶ We have investigated the stability of PEI/DNA complexes over time using a mouse aerosol delivery model

Results



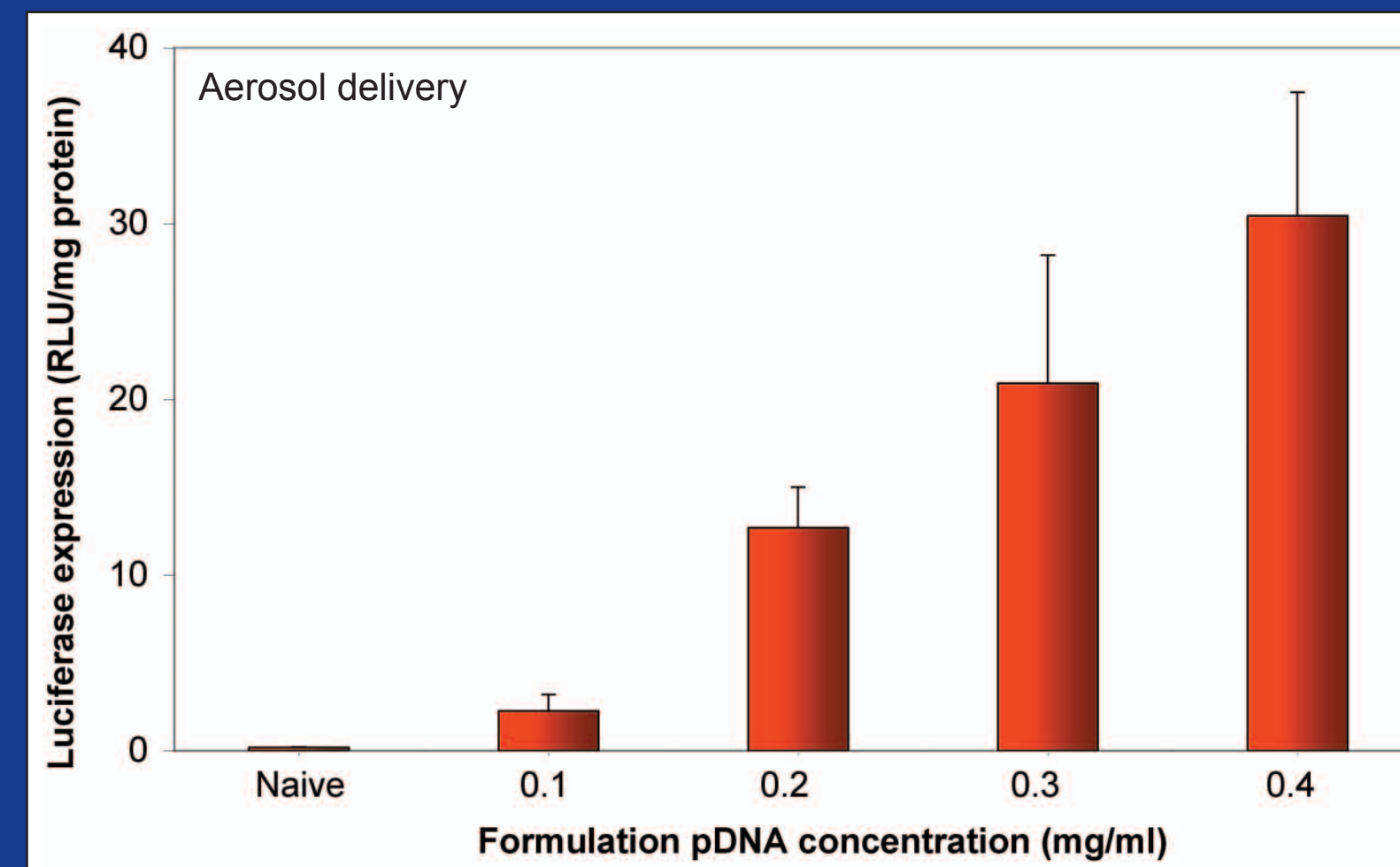
▶ **Figure 1: Stability of PEI/DNA formulations *in vitro***

- ▶ 25kDa PEI was complexed to the plasmid pCIKLux (expressing luciferase) at a concentration of 0.2mg/ml in sterile water and at an N:P ratio of 10:1
- ▶ Aliquots of PEI/DNA complexes were stored at -20°C, 4°C, 20°C and 37°C
- ▶ HEK293T cells were transfected with 0.5µg of stored PEI/pCIKLux complexes after storage for between 1 day and 180 days
- ▶ Luciferase expression was quantified 24 hours later
- ▶ Samples stored at 4°C retained biological activity even after storage for 180 days



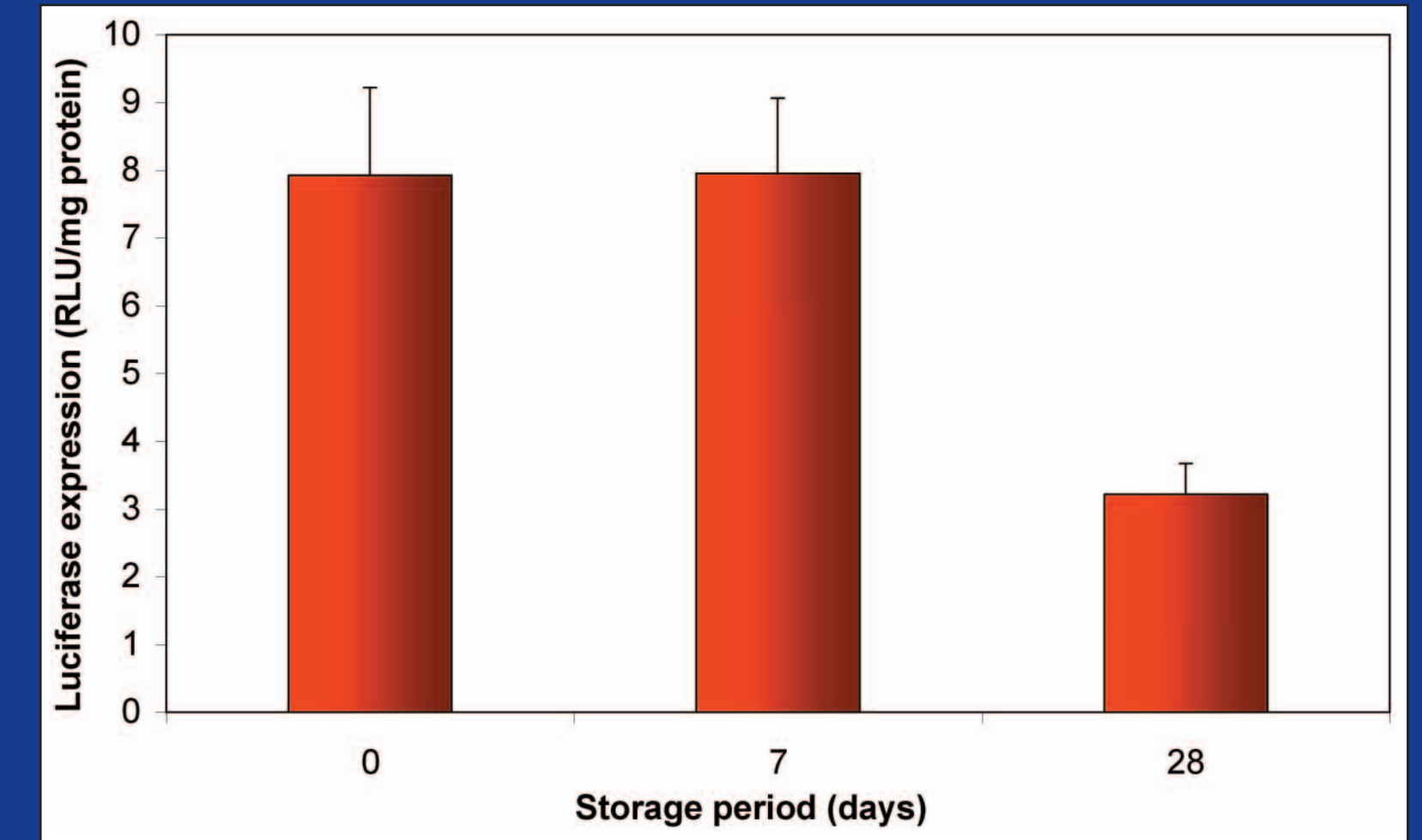
▶ **Figure 2: Aerosol delivery to the mouse lung**

- ▶ Female BALB/c mice (n=6) were exposed to PEI/DNA aerosols using a whole body exposure chamber (above)
- ▶ 10ml of PEI/DNA solution was aerosolised into the chamber using an Aerotech II pneumatic nebuliser (CIS-US Inc, Bedford, MA, USA)
- ▶ Nebuliser was operated at 40psi using 5% CO₂ in air as the driving gas



▶ **Figure 3: Luciferase expression in the mouse lung**

- ▶ Mice were exposed to aerosols containing pCIKLux complexed to 25kDaPEI over a range of pDNA concentrations
- ▶ Luciferase expression in whole lung homogenates was determined 24hr later
- ▶ Dose-dependent luciferase expression was detected following aerosol delivery of formulations containing up to 0.4mg/ml
- ▶ A concentration of 0.2mg/ml was selected for stability studies



▶ **Figure 4: Stability of PEI/DNA formulations in the mouse lung**

- ▶ 25kDa PEI was complexed with pCIKLux (N:P ratio 10:1) at 0.2mg/ml in sterile water and stored at 4°C
- ▶ Aliquots (10ml) were removed after 0, 7 and 28 days and aerosolised to groups of mice (n=6)
- ▶ Luciferase expression was assayed 24hr later
- ▶ Storage of PEI/DNA formulations at 4°C resulted in no loss of biological activity over 7 days but storage for 28 days was associated with a 60% fall in expression

Conclusions

- ▶ PEI is a viable GTA for aerosol gene delivery
- ▶ PEI/DNA complexes can be stored for at least 1 week with no loss of gene transfer efficacy
- ▶ Storage temperature is an important factor in stability of PEI/DNA complexes
- ▶ Improved stability will facilitate translation to clinical studies