Pulmonary delivery of siRNA targeting EGFR and PD-L1 in *in vivo* traceable NSCLC models

Rico Chi Hang Man[1,2], Susan Wai Sum Leung[1], Jenny Ka Wing Lam[1,3] & Gilbert O. Fruhwirth[2]

[1] Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR
[2] School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom
[3] Department of Pharmaceutics, UCL School of Pharmacy, University College London, London, United Kingdom

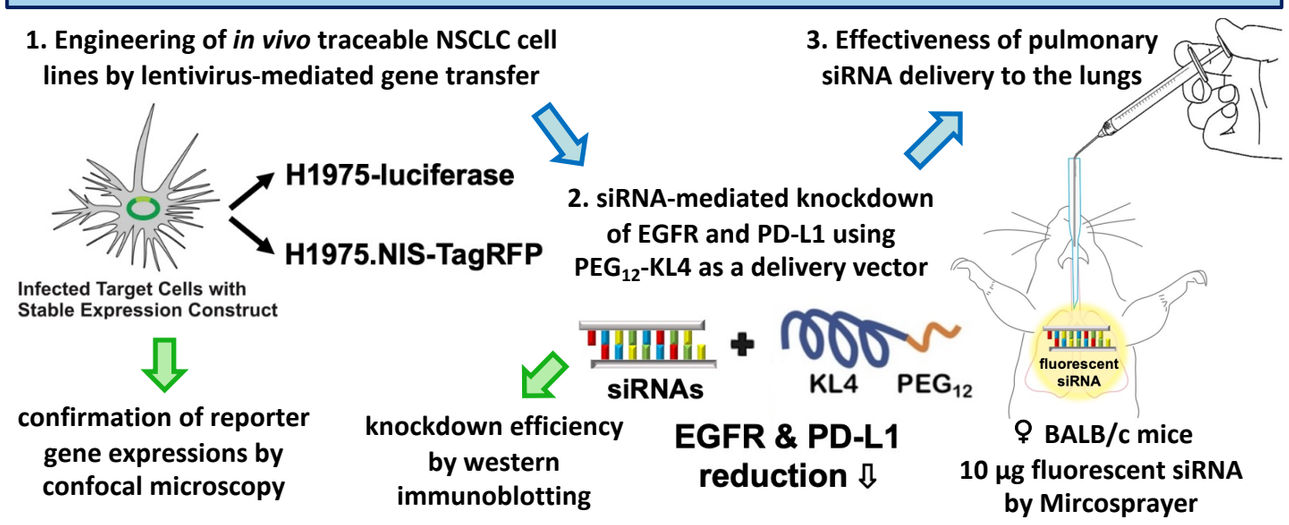
Background

- Non-small cell lung cancer (NSCLC) is a leading cause of mortality worldwide, resulting in a heavy burden on the healthcare system.
- Over-expression of epidermal growth factor receptor (EGFR) and programmed death-ligand 1 (PD-L1) are frequently observed in NSCLC, in which EGFR promotes tumour survival and PD-L1 prevents cancer cells from immune detection [1].
- The small interfering RNA (siRNA)-based therapies emerge as a novel and and attractive anti-cancer therapeutic approach [2].
- Different *in vivo* traceable NSCLC cell lines are engineered to track the tumour progression and evaluate the therapeutic efficacy following pulmonary delivery of siRNAs targeting EGFR and PD-L1 [3].

Aims

- To engineer and validate *in vivo* traceable NSCLC cell models which express either luciferase (for 2D bioluminescence imaging) or NIS-TagRFP (sodium iodide symporter reporter gene coupled with a fluorescent protein for 3D radionuclide tomography).
- To investigate the efficiency of siRNAs in inhibiting EGFR and PD-L1 expressions in the established NSCLC cells.
- To explore the effectiveness and safety of pulmonary delivery of siRNA therapeutics to the lungs.

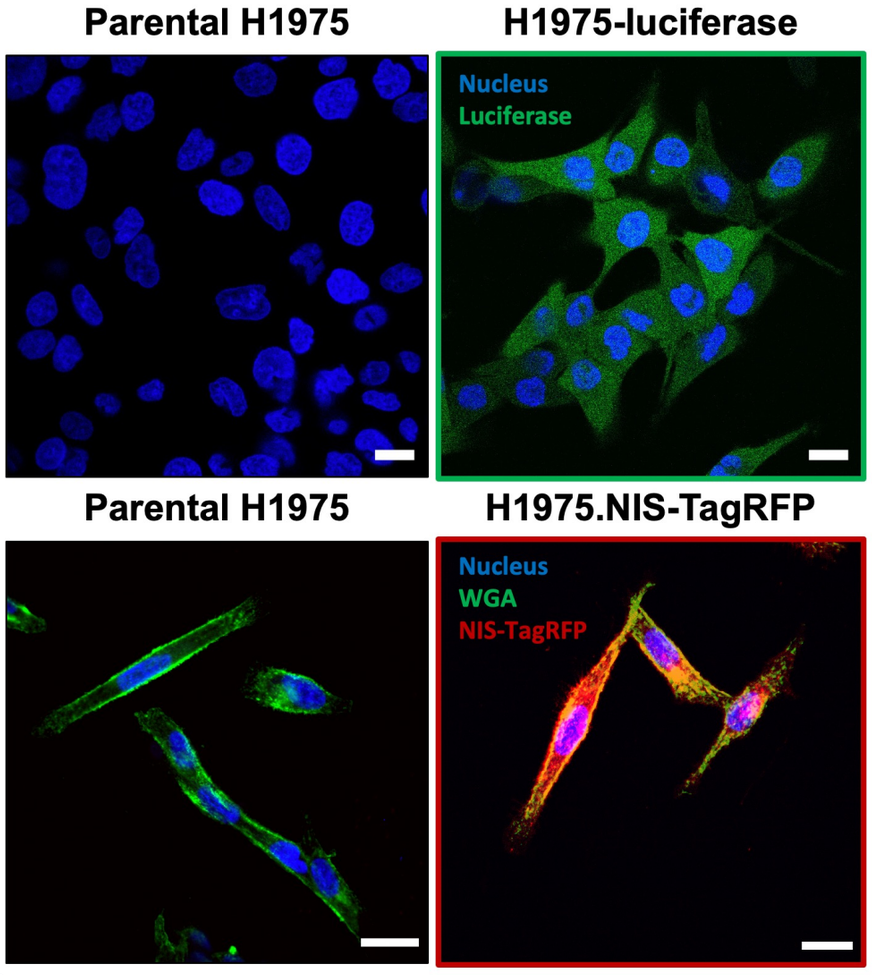
Methods



(The animal work was approved by Committee on the Use of Live Animals for Teaching and Research, HKU)

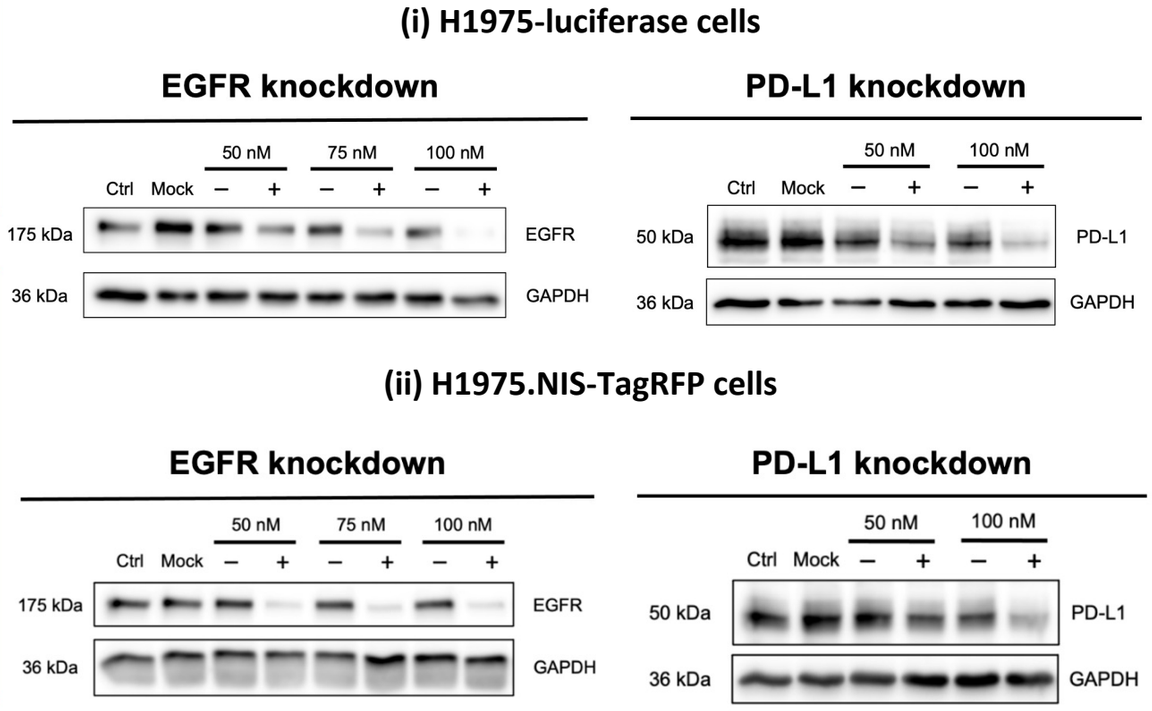
Results

A Establishment of *in vivo* traceable NSCLC cell models



◄ **Figure 1.** Representative confocal microscopy images of H1975 cells expressing firefly luciferase and NIS-TagRFP reporter gene. (Upper panel) The parental and luciferase-expressing cells were stained with primary firefly luciferase antibody, followed by secondary antibody staining with AlexaFluor488 dye. (Lower panel) Analysis of subcellular reporter localisation: plasma membrane was visualised with WGA conjugated to AlexaFluor488 dye and NIS-TagRFP by its intrinsic red fluorescence. Scale bar = 20 µm.

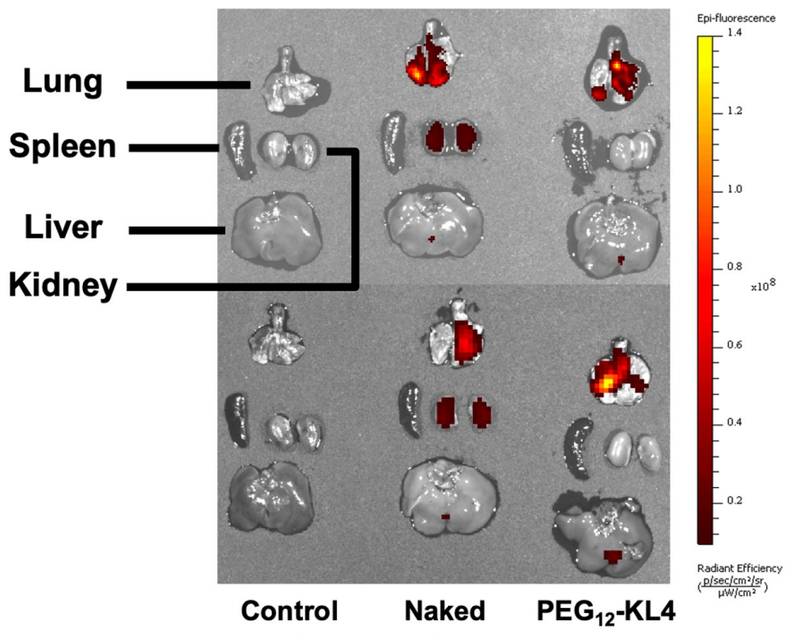
B Knockdown efficiency of EGFR & PD-L1 using PEG_{12}-KL4 as a delivery vector



◄ **Figure 2.** The knockdown effects of EGFR and PD-L1 using a synthetic delivery vector, PEG_{12}-KL4 peptide in (i) H1975-luciferase and (ii) H1975.NIS-TagRFP cells. The cells were transfected with PEG_{12}-KL4 only (mock control), EGFR or PD-L1 siRNA (+) or scramble siRNA (-) at 50, 75 or 100 nM. At 48-h post-transfection, EGFR or PD-L1 and GAPDH (as an internal control) protein expressions were analysed by western immunoblotting.

C Pulmonary delivery of fluorescent siRNA

4-h post intratracheal administration



▲ **Figure 3.** Biodistribution of DY-547 fluorescently labelled siRNA following intratracheal administration. Female BALB/c mice were administered intratracheally with (i) PBS, (ii) naked siRNA or (iii) PEG_{12}-KL4/siRNA complexes containing 10 µg siRNA in 75 µL PBS using Microsprayer® Aerosolizers. At 4 h post-administration, the lung, liver, kidneys and spleen tissues were isolated and the DY-547 red fluorescence signal of the tissues was measured (*n*=2).

Conclusions

- H1975 cells expressing firefly luciferase or NIS-TagRFP were successfully established.
- A significant EGFR and PD-L1 knockdown was attained using PEG_{12}-KL4 peptide as a delivery vector in the established cell lines.
- Intratracheal administration was a reliable and effective method to achieve high siRNA localisation in the lungs.

Key messages

This study demonstrates successful engineering of two *in vivo* traceable H1975 cell models. They now serve as a tool to visualise and evaluate the anti-tumour effects of EGFR and PD-L1 dual inhibition by siRNAs.

Acknowledgement

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References

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