IN VIVO DELIVERY OF TRANSCRIPTIONALLY TARGETED GENE THERAPY OF SMALL CELL LUNG CARCINOMA

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Aim

To deliver suicide gene therapy to cancer cells* in vivo *by non-viral methods

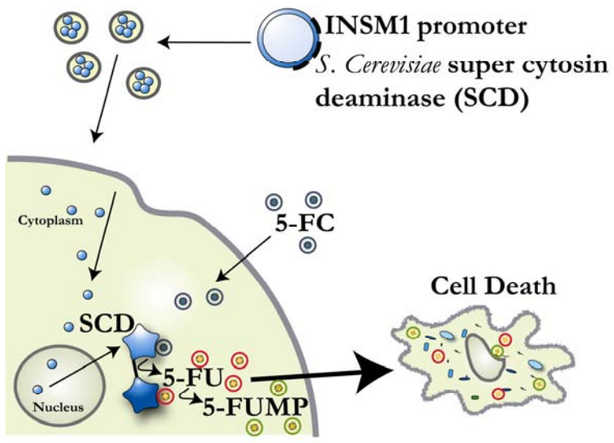
Background

•Small cell lung cancer (SCLC) is characterised by aggressive progression, dissemination at the prevalent time of diagnosis and a poor prognosis of survival.

•Requirement for new therapies; systemic treatment of SCLC by transcriptionally targeted suicide gene therapy.

•Using GeneChip transcript profiling we have identified SCLC specific gene promoters for expression of suicide genes in SCLC cells – the most promising promoters is from the human INSULINOMA-ASSOCIATED 1 (INSM1) gene.

•A promising suicide gene strategy is the Yeast Cytosine Deaminase (YCD) gene fused with the Yeast Uracil Phosphoribosyl Transferase (YUPRT) gene (fusion gene designated SCD) in combination with the prodrug 5-fluorocytosin (5-FC).



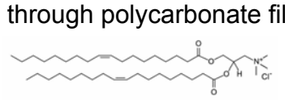
•SCD converts 5-FC into the chemotherapeutic agent 5-fluorouracil (5-FU)

•5-FU and derived cytotoxins can spread to nearby cancer cells and induce cytotoxicity “local bystander effect”

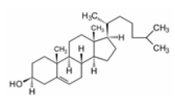
Results

Non-viral delivery by cationic lipoplex: DOTAP/chol/DNA*

1:1 molar ratio, extruded to 100 nm in diameter through polycarbonate filters



Dioleoyl trimethylammonium propane chloride-salt



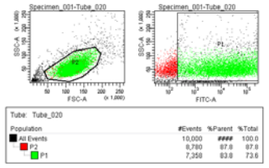
Cholesterol

*Method is FDA-approved for clinical trial: Gene therapy against non-SCLC

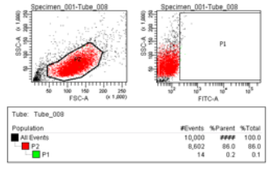
Advantages: Easy and cheap preparation in comparison to viral methods

Transfection *in vitro* with gene reporter, EGFP flow cytometry

DOTAP/chol lipoplex w/pEGFP-N1 DNA

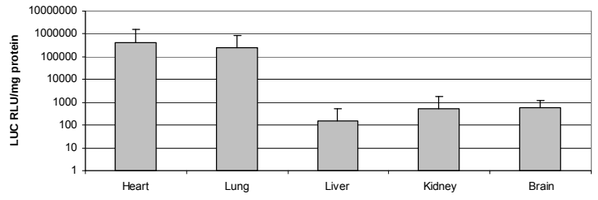


DOTAP/chol lipoplex w/pCMV-luc DNA



Systemic delivery by tailvein injection, luciferase activity

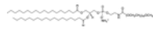
Luciferase activity in tissue of mice sacrificed day 4, tailvein-injections of DOTAP/chol/pCMV-luc, day 1,2,3 (n=8)



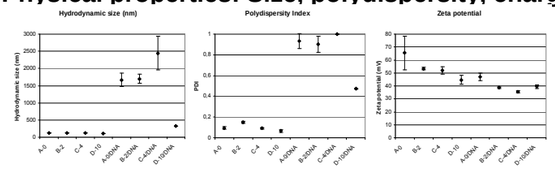
Mainly lung and heart transfected, significant weight loss during treatment – ca. 10 %

Improving systemic stability by PEGylation of DOTAP/chol

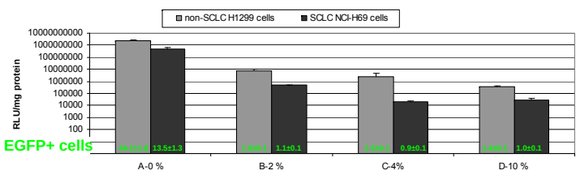
DOTAP/chol particles were prepared with 0 % (A-0); 2 % (B-2); 4 % (C-4) or 10 % (D-10) DSPE-PEG2000 (and with ^{3}H-CHE or NBD-chol tracers)



Physical properties: Size, polydispersity, charge

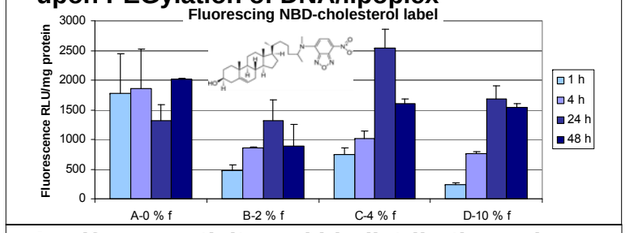


DNA/lipoplex-PEG transfection of cells *in vitro*



DNA: Mix of pEGFP-N1/pCMV-luc

Cellular uptake kinetics *in vitro* is changing upon PEGylation of DNA/lipoplex

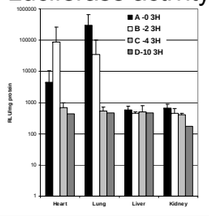


Luciferase activity and biodistribution using ^{3}H-cholesteryl-hexadecyl ether as tracer

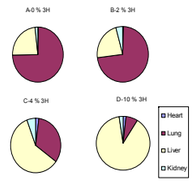
DNA/lipoplex with PEG (0,2,4 or 10 %) and 3H-CHE lipid (1 µCi) was injected in the tail vein of male NMRI mice (n=20).

Periorbital blood samples were collected at 15 min, 2 h, 5 h and 24 h before animals were sacrificed and organ samples isolated. In all 4 formulations blood samples contained 3H counts marginally above the background, presumably due to retention in internal organs.

Luciferase activity

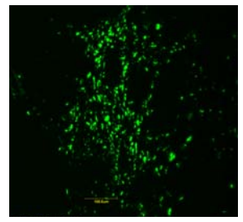


Biodistribution



Intratumoral injection

EGFP expression

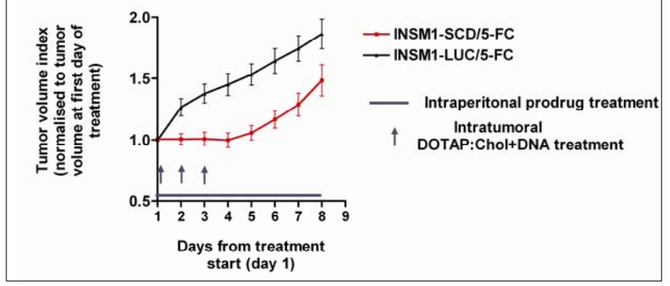


DOTAP/chol/pEGFP-N1 injected into SCLC xenograft tumor on the flank of nude mouse
Cryosection of tumour one day after injection, confocal laser scanning microscopy

SCD expression/prodrug treatment

DOTAP/chol/ Treatment of SCLC xenografts on nude mice with INSM1-YCD-YUPRT/5-FC results in significant tumor growth delay compared to mock treated xenografts

NMRI Nude mouse treatment with DNA encapsulated in DOTAP:CHOLESTEROL



DOTAP/chol conclusion

Systemic delivery
- Lung/heart transfection
- Poor stability and circulation

Intratumoral delivery
- EGFP expression detectable in center of tumor
- Tumor growth retardation *in vivo* by intratumoral injection of suicide gene/prodrug treatment

PEGylation
- Abrogate transgene expression *in vitro* and *in vivo*
- May alter the mechanism of DNA/lipoplex uptake
- Biodistribution indicates decreasing of lung retention of DNA/lipoplex when increasing PEGylation