Development of Targeted Liposomal Nanocarriers for Delivery of siRNA to Neuroblastoma

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Children's Cancer Institute

UNSW Australian Centre for NanoMedicine

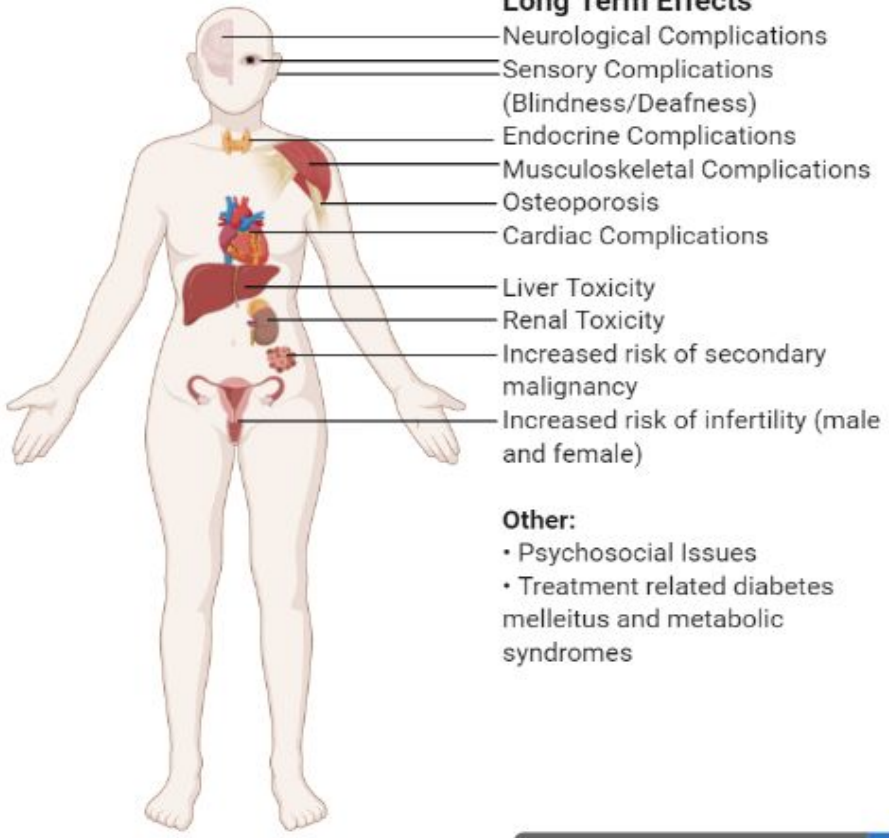
UNSW SYDNEY

UNSW RNA Institute

High Risk Neuroblastoma

- Current therapies include chemotherapy, radiotherapy, and immunotherapy.
- 5 year survival in high-risk patients <50%.

Short Term Effects
- Infections
- Fever
- Fluid Retention
- Dose Limiting Toxicity
- Kidney Toxicity
- Nausea and vomiting
- Loss of appetite
- Mouth Sores
- Cardiac Problems
- Bleeding Problems
- Digestive Problems
- Neuropathy
- Lung Problems



Polo-like kinase 1 (PLK1) as a therapeutic target for neuroblastoma

- Promotes and regulates cell cycle progression.
- PLK1 overexpression associated with poor survival in neuroblastoma.
- Inhibition of PLK1 leads to potent DNA damage, cell cycle arrest and cell death.
- **PLK1 inhibitors exhibit off target effects against other PLK members.**

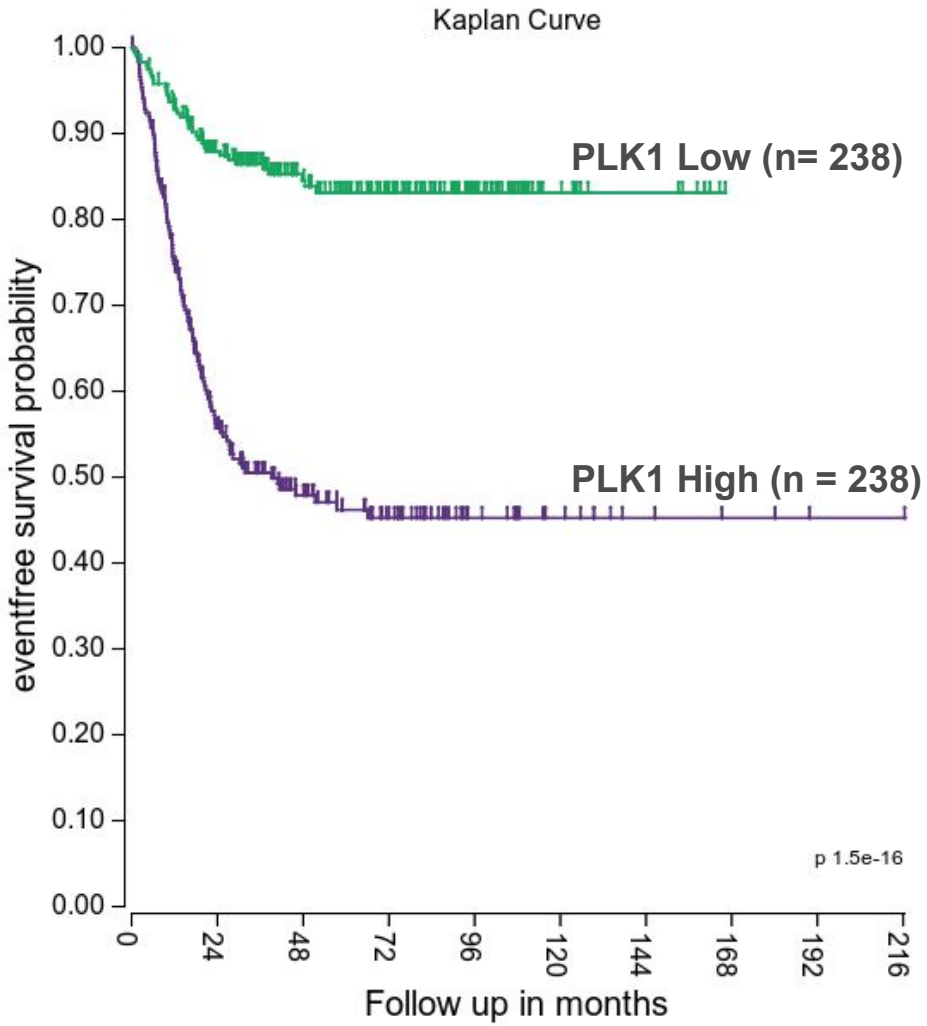
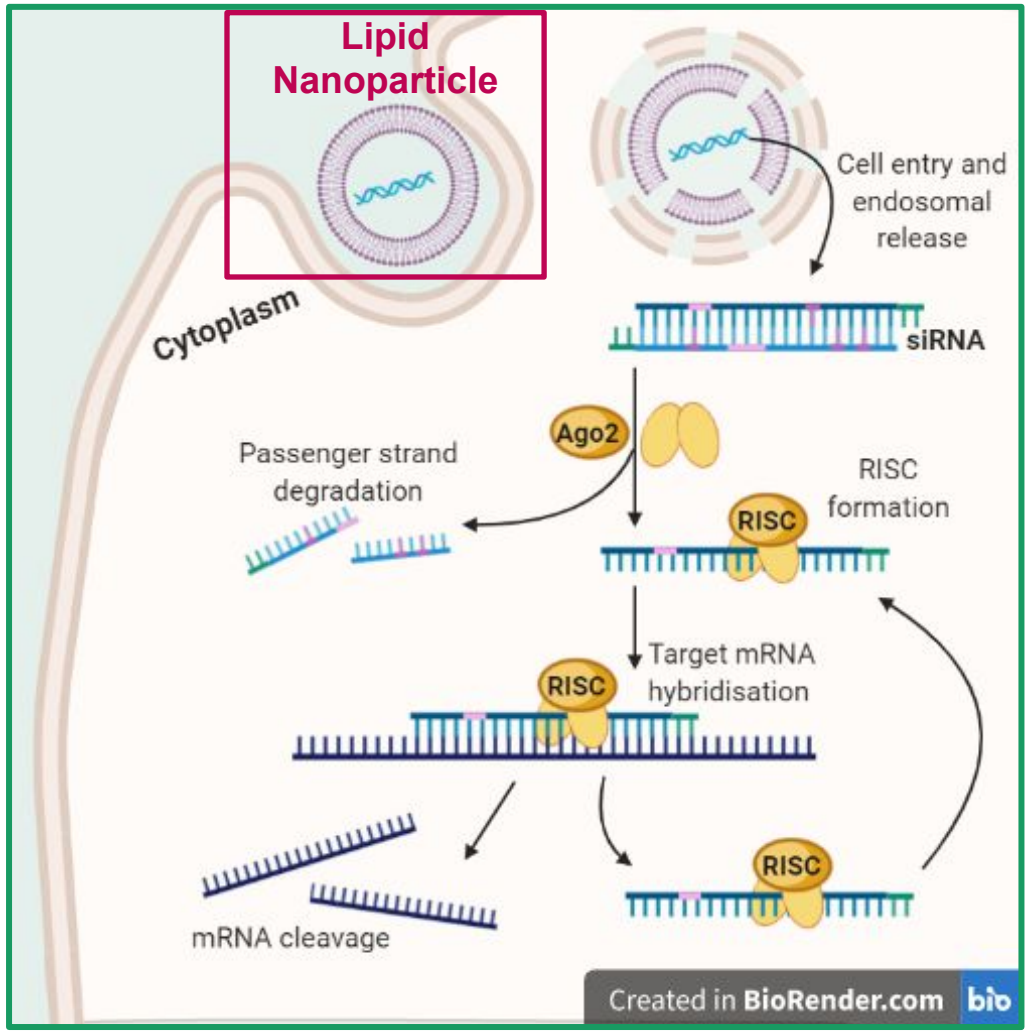


Figure generated in R2 visualisation platform using Kocak (n=649) dataset.

Short Interfering RNA (siRNA)



Main issue with siRNA is delivery.

- Naked siRNA is rapidly broken down in serum by RNAses.
- Cannot pass through cell membranes due to negative charge.

Lipid Nanoparticles for siRNA delivery

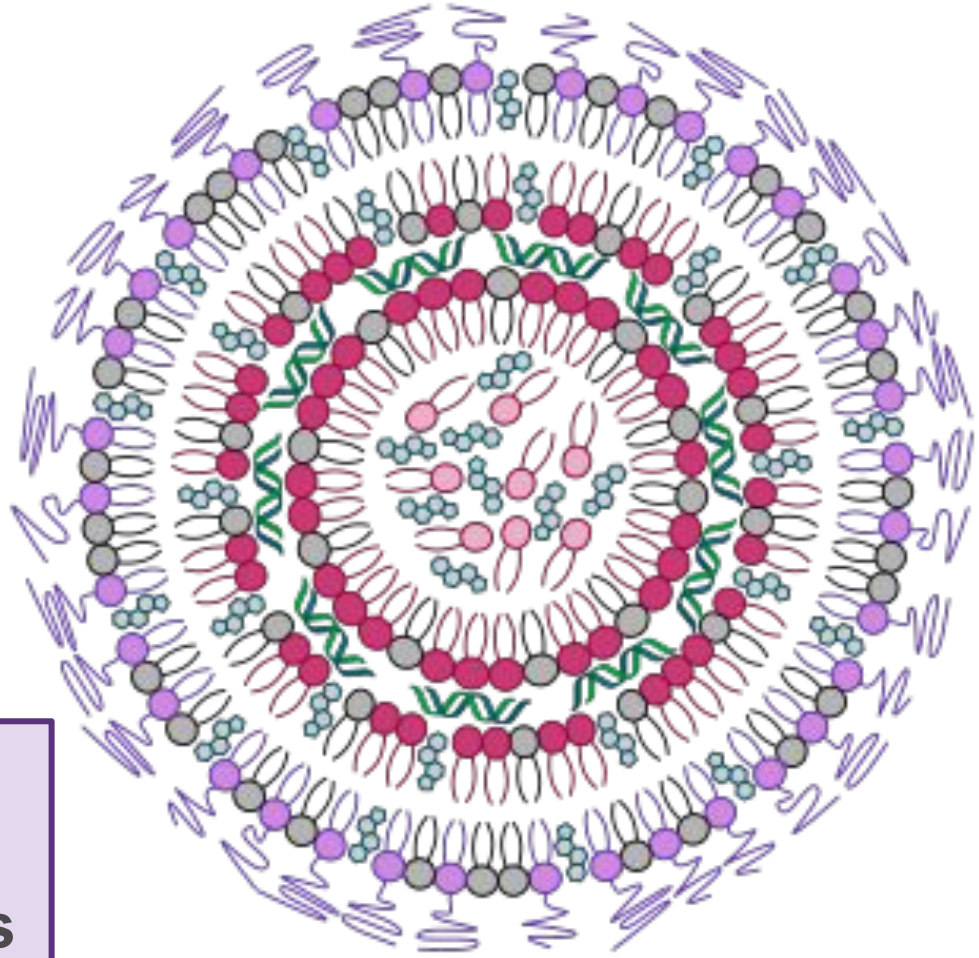
Lipid Nanoparticles (LNPs) are comprised of:

Ionizable Cationic lipid (40-50 mol%) - to bind with negatively charged siRNA.

Helper lipid (10 mol%) – help with endosomal fusion or stability.

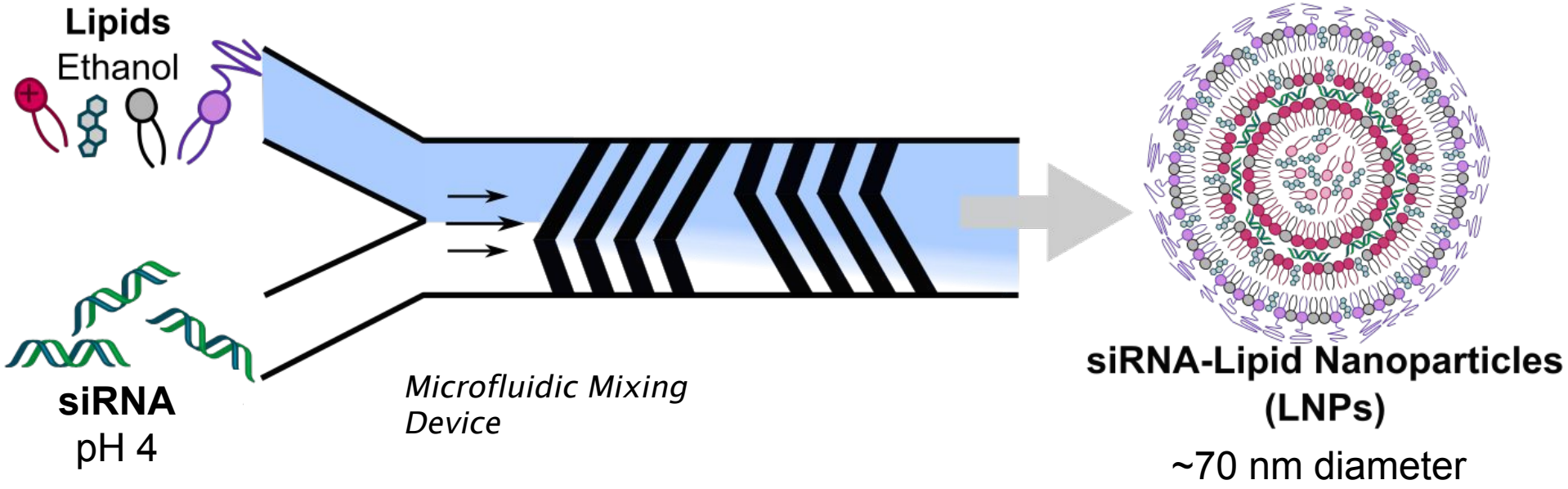
Cholesterol (30-40 mol%) – to increase stability.

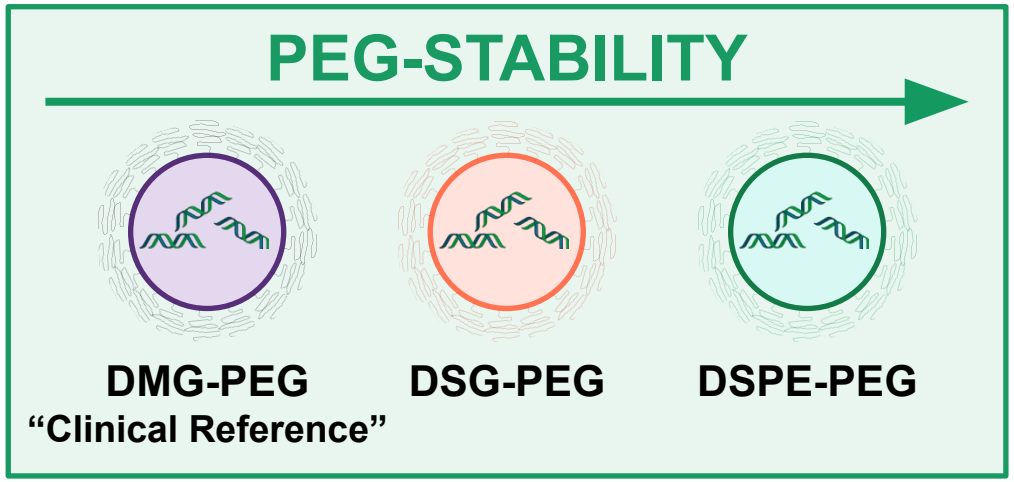
PEG lipid (1-10 mol%) – Coats the surface of the particle. Contains polyethylene glycol (PEG), to improve stability and reduce immunogenicity of particle.



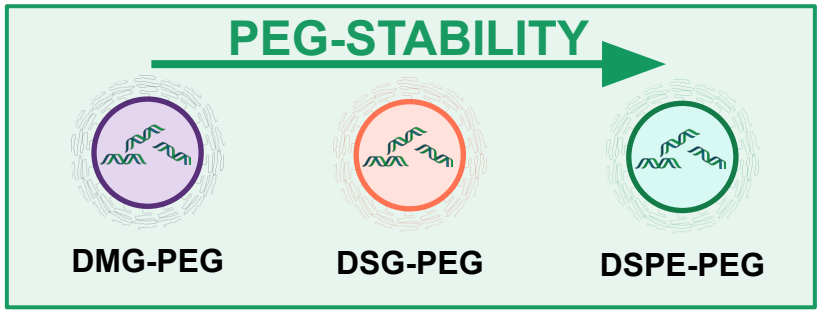
Since 2018, 4 siRNA lipid nanoparticles (Patisiran, Lumasiran, Givosiran, Inclisiran) have been approved for treatment of diseases involving the liver.

Development of siRNA Lipid Nanoparticles

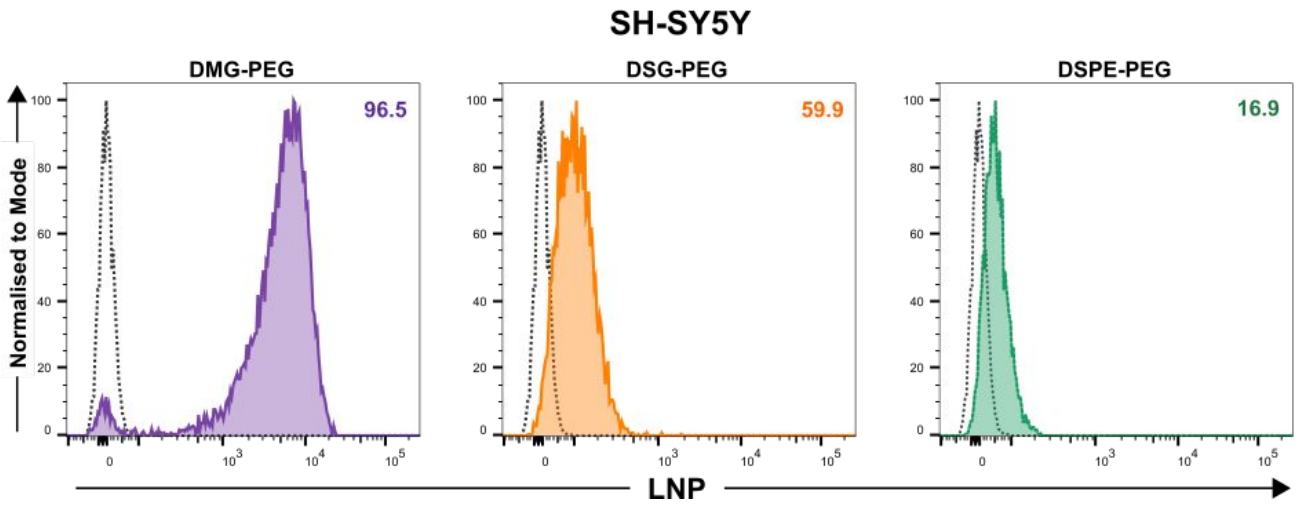




Choice of PEG-lipid affects association of lipid nanoparticle with neuroblastoma cell lines

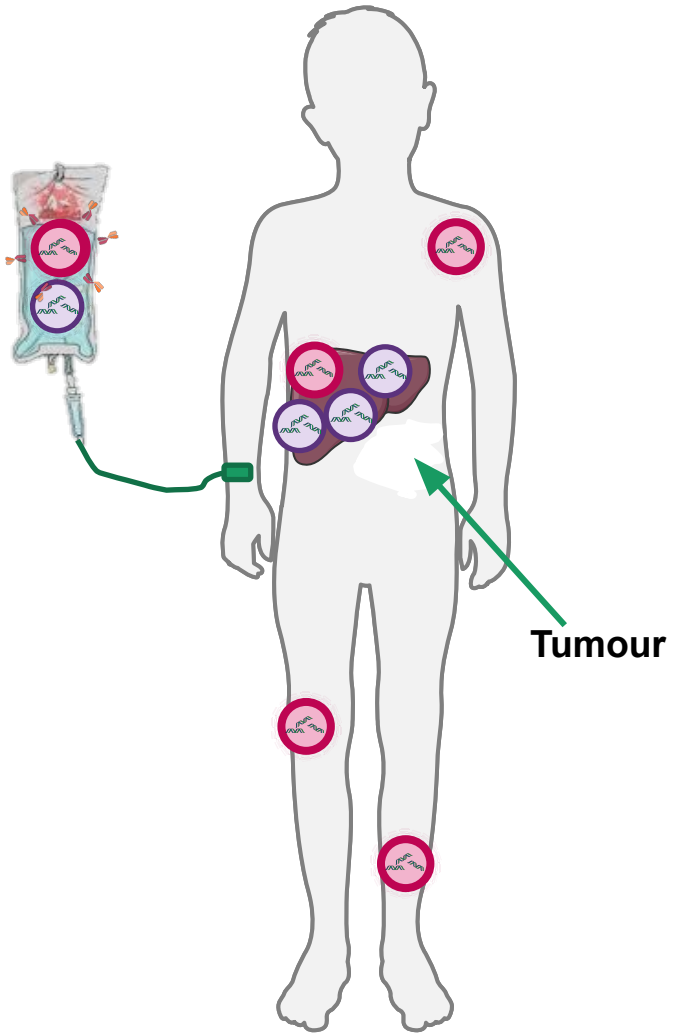


PEG-Lipid	DMG-PEG	DSG-PEG	DSPE-PEG
Z-Average size (nm)	71.2 ± 4.5	79.5 ± 8.6	64.8 ± 5.3
Polydispersity index (PDI)	0.14 ± 0.05	0.10 ± 0.05	0.11 ± 0.03
siRNA encapsulation efficiency (%)	91.6 ± 3.2	91.2 ±3.9	92.5 ± 3.5
Zeta potential (mV)	-16.2 ± 10.4	-17.0 ± 7.6	-18.7 ± 5.5



Similar results were obtained in SKNBE(2), Chp134 and Kelly neuroblastoma cells.

Targeted siRNA-lipid nanoparticles for delivery to neuroblastoma

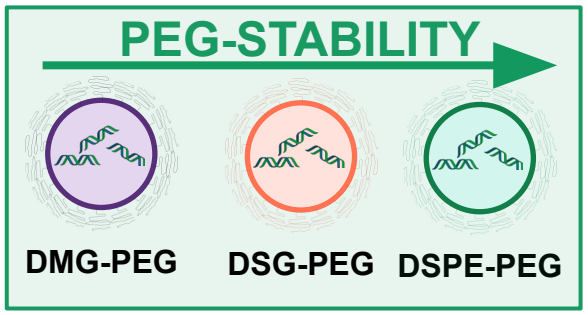


Bispecific Antibody (BsAb) Targeted siRNA-LNP

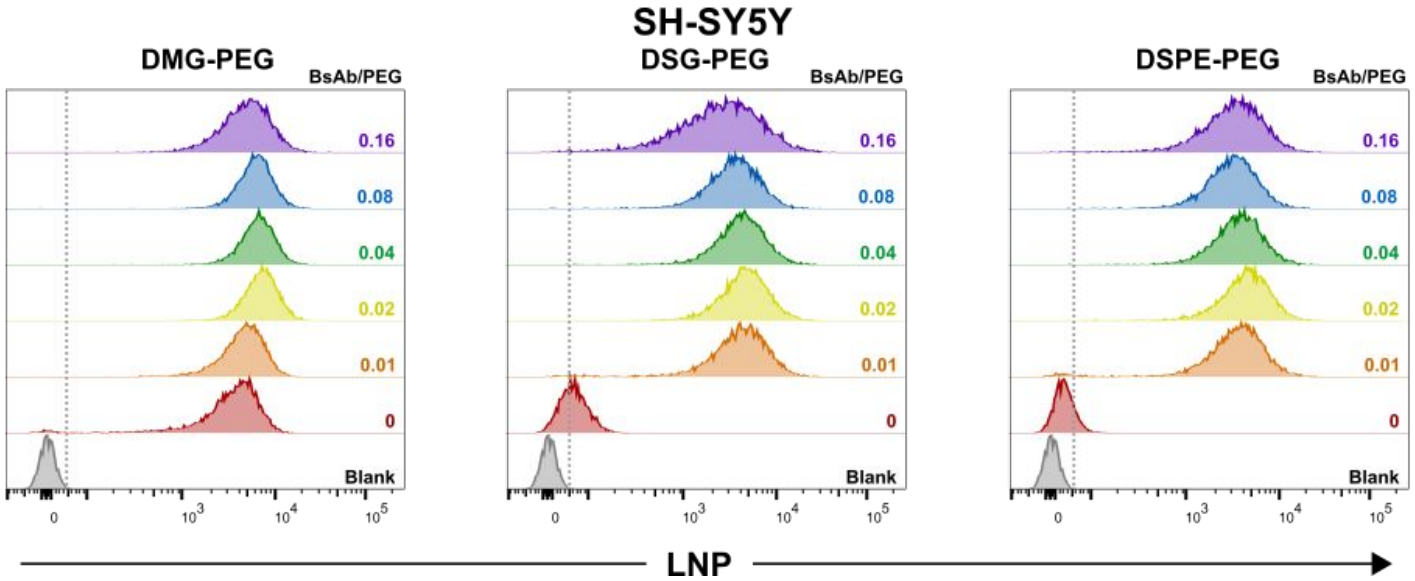
Epidermal growth factor receptor (EGFR) binding domain

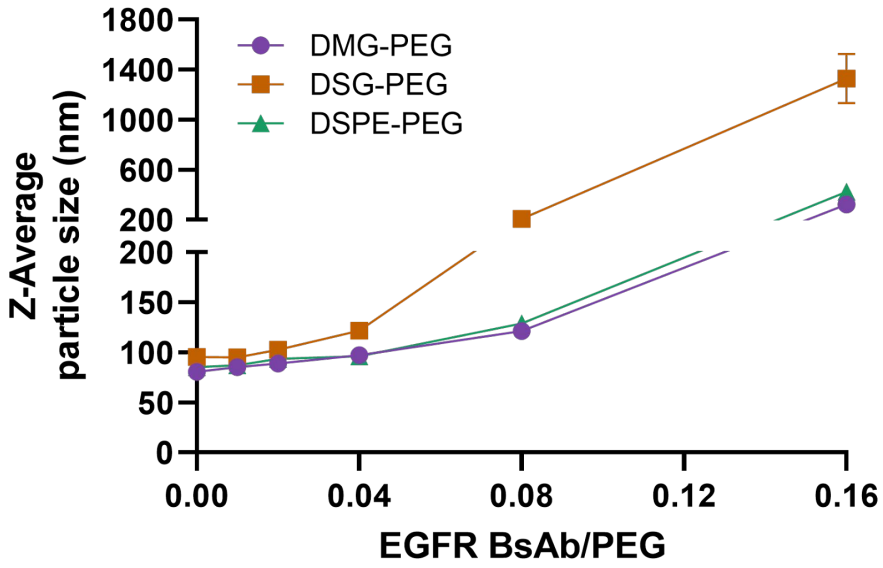
PEG binding domain

Epidermal growth factor receptor (EGFR) is overexpressed in a range of cancers, including neuroblastoma.



EGFR BsAb targeting improves association of stabilised LNPs with neuroblastoma cells

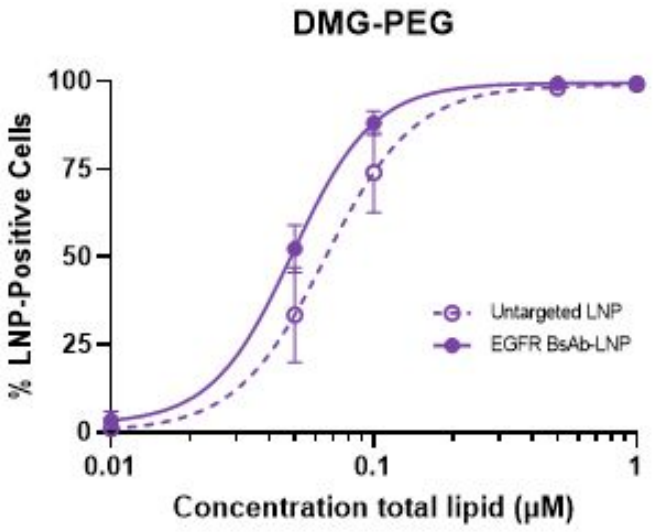




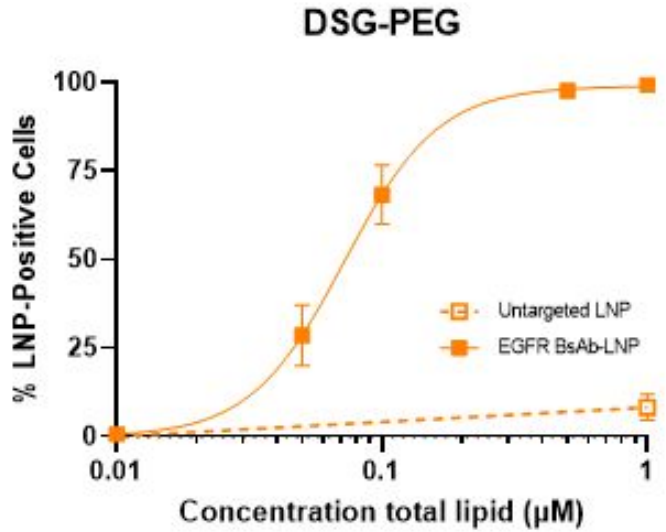
Z-average size (nm)	DMG-PEG LNP	DSG-PEG LNP	DSPE-PEG LNP
Untargeted	80.5 ± 3.5	85.0 ± 3.3	85.3 ± 2.3
EGFR BsAb-LNP	97.1 ± 9.2	121.4 ± 7.5	96.0 ± 7.2

LNP uptake in SH-SY5Y cells following 4 hr incubation. Similar results obtained in SKNBE(2) neuroblastoma cells.

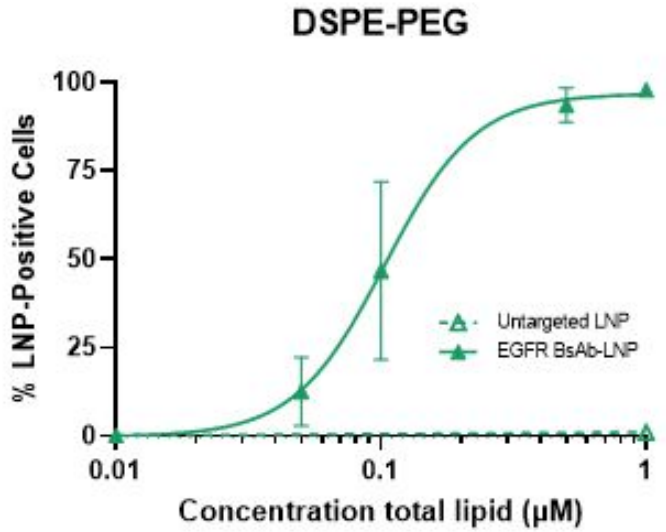
EGFR BsAb targeting improves association of stabilised LNPs with neuroblastoma cells



DMG-PEG LNP	EC50 (μM)
Untargeted	0.065
EGFR BsAb-LNP	0.049



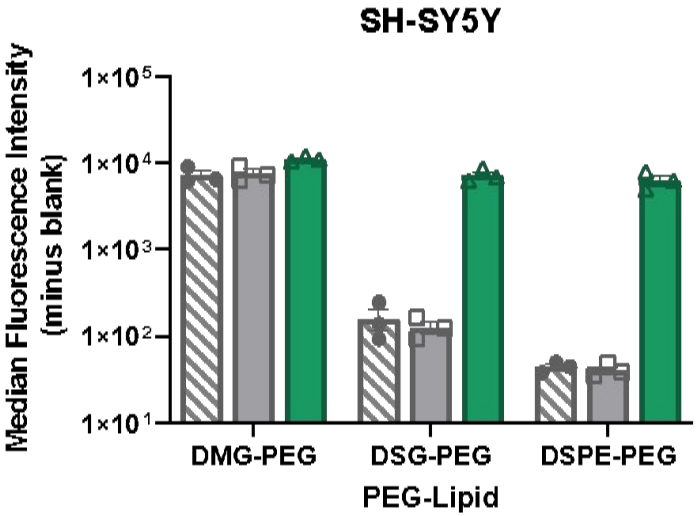
DSG-PEG LNP	EC50 (μM)
Untargeted	>1
EGFR BsAb-LNP	0.072



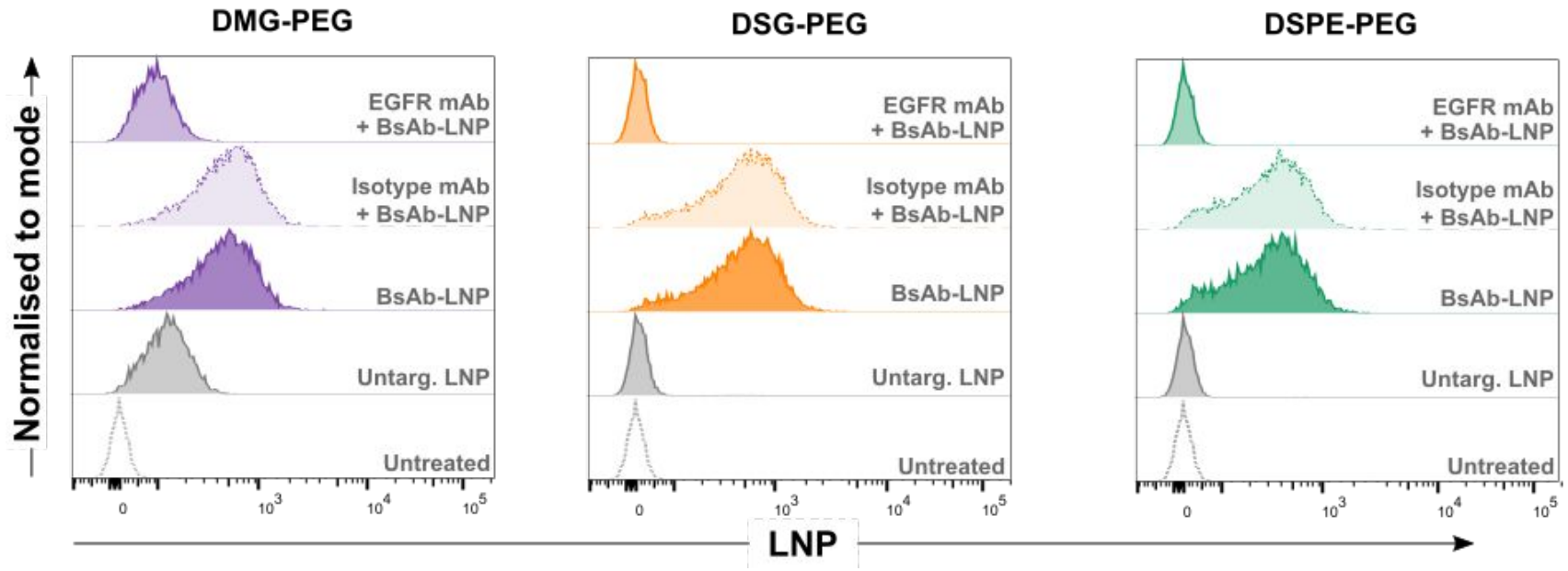
DSPE-PEG LNP	EC50 (μM)
Untargeted	>1
EGFR BsAb-LNP	0.1032

LNP uptake in SH-SY5Y cells following 4 hr incubation. Similar results obtained in SKNBE(2) neuroblastoma cells.

Improvement in BsAb-LNP cell association is target specific

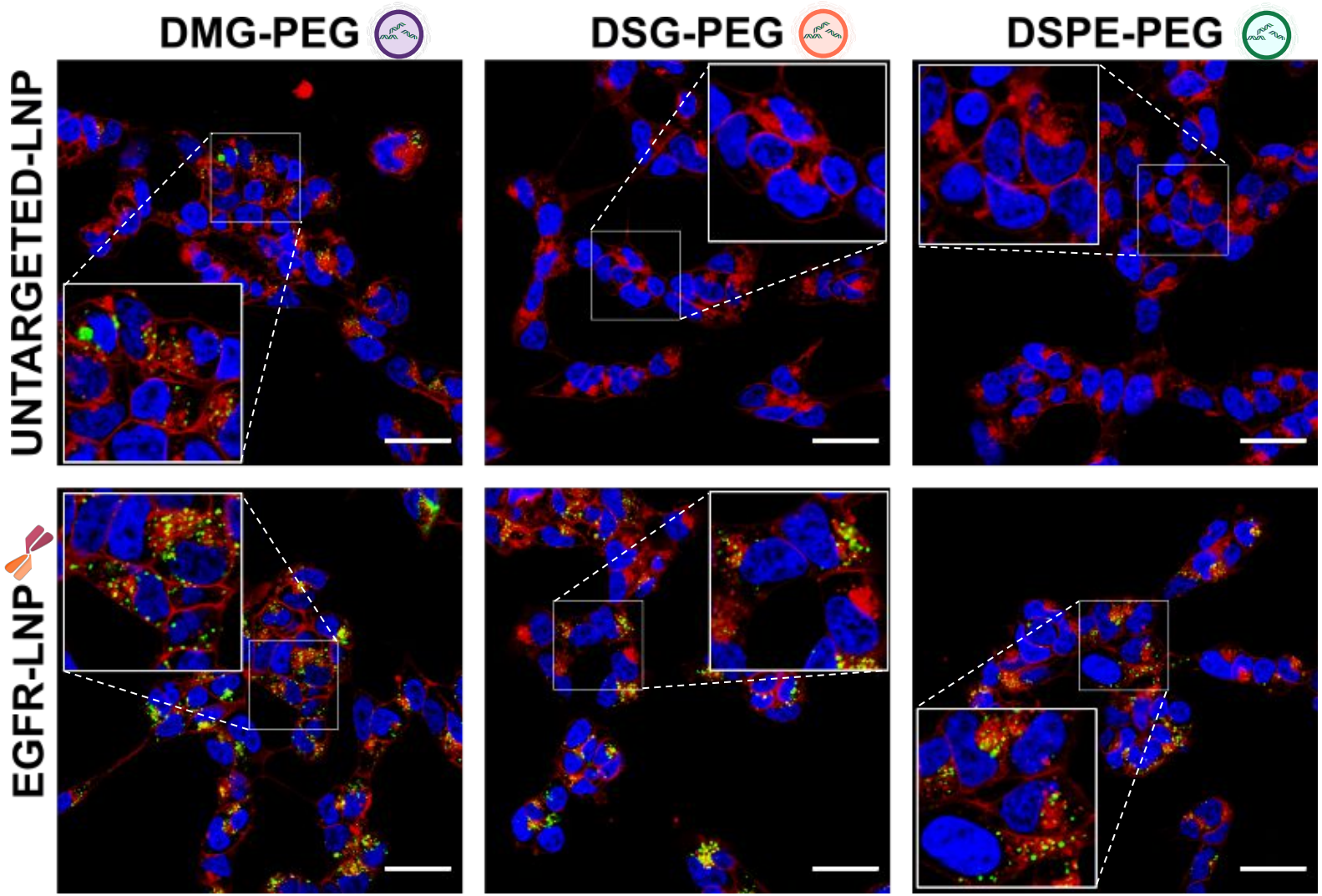


LNP uptake in SH-SY5Y cells following 4 hr incubation. CD-19 BsAb used as isotype control.

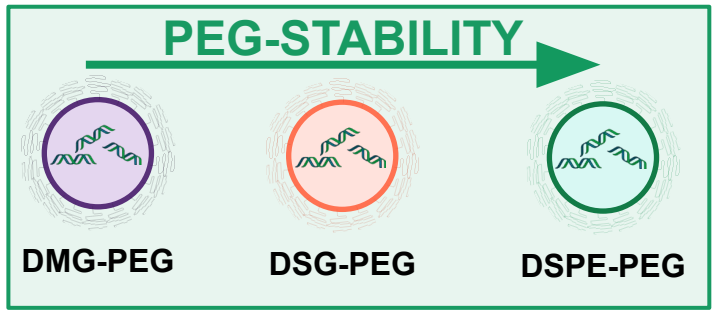


EGFR or Isotype mAb were added at a 1:20 ratio mAb scFv: BsAb scFv. Similar results obtained in SKNBE(2) neuroblastoma cells.

EGFR BsAb targeting improves internalisation of stabilised LNPs

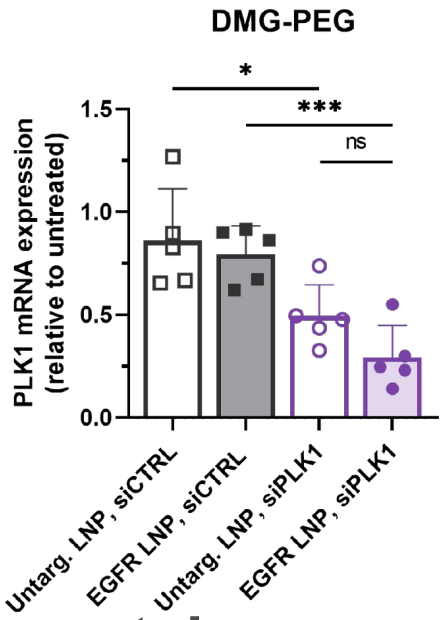


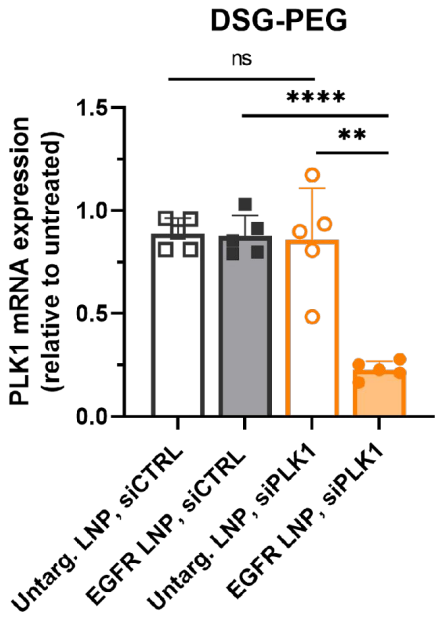
Nuclei are stained in blue, **cell membrane** in red, and **nanoparticles** in green. Scale bar represents 30 μm.

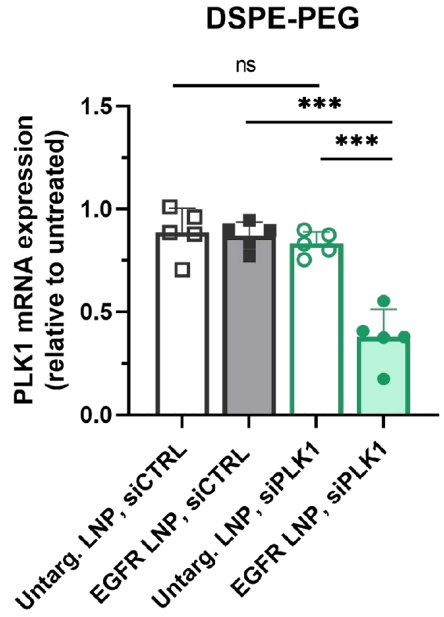


LNP targeting improves knockdown capacity of siRNA-LNPs

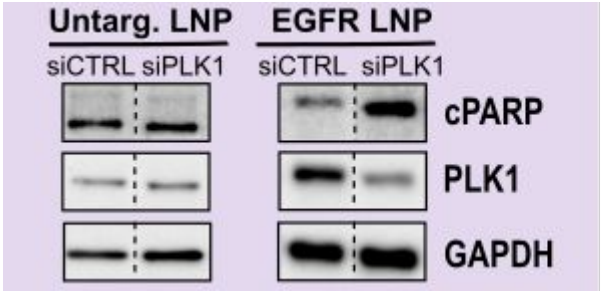
PLK1 mRNA expression

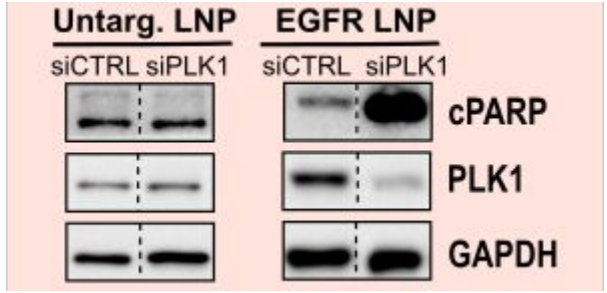


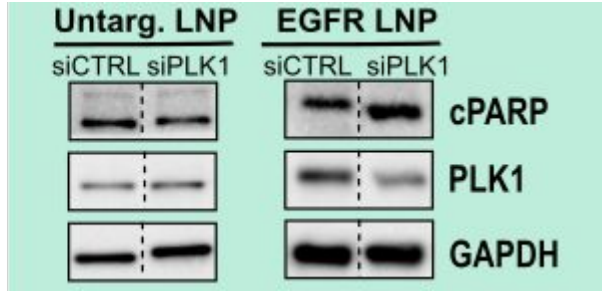




PLK1 protein expression



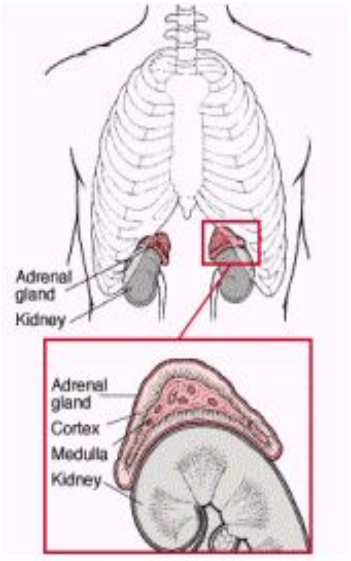


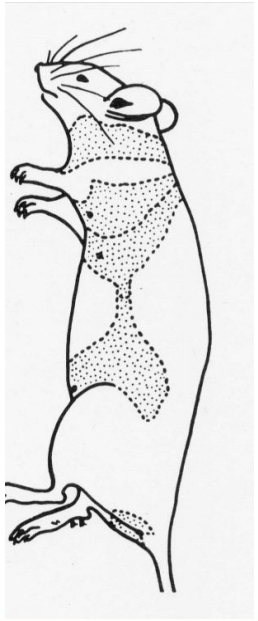


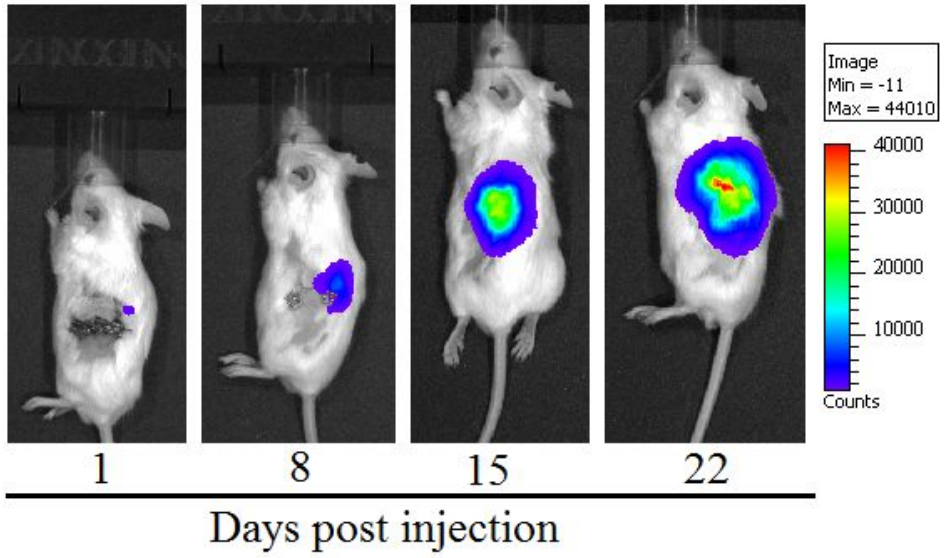
Neuroblastoma SH-SY5Y cells were treated with siRNA-LNPs at a concentration of 90 nM siRNA for 48 hrs.

Conclusions and Next steps

- EGFR targeting via bispecific antibodies improves cell association and gene silencing efficiency of siRNA-LNPs formulated with less diffusible PEG-Lipids.
- Biodistribution of targeted siRNA lipid nanoparticle formulations to commence soon.
- Trial other siRNA and bispecific antibody combinations to develop “mix and match” system for neuroblastoma treatment.
- siRNA lipid nanoparticle formulations will then be trialled in an orthotopic neuroblastoma model to assess efficacy.







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