# Targeting Neuroblastoma's Weak Spots with GD2-Directed siRNA-Lipid Nanoparticles

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**Introduction:** Neuroblastoma is an aggressive paediatric cancer requiring more effective and less toxic therapies. One promising strategy involves using short interfering RNA (siRNA) to silence cancer dependency genes, which are crucial for cancer cell survival. To deliver siRNA effectively to tumours, improvements to the gold standard delivery vehicle, lipid nanoparticles (LNPs), are needed. Here, we developed neuroblastoma targeted LNPs using a bispecific antibody (BsAb) that recognizes the antigen GD2 and polyethylene glycol (PEG), to improve the delivery of siRNAs that target neuroblastoma dependency genes.
**Methods:** Microfluidic mixing was used to make siRNA-LNPs. To generate αGD2-siRNA-LNPs, we combined siRNA-LNPs with our GD2 BsAb via simple mixing. The targeting of CHP-134 neuroblastoma cells by αGD2-siRNA-LNPs was assessed by two methods: flow cytometry for 2D cultures and confocal microscopy for 3D bioprinted tumouroids (1). To assess therapeutic delivery, siRNA against a pan-cancer dependency gene, polo-like kinase 1 (PLK1), was incorporated into LNPs (αGD2-siPLK1-LNPs), and tumouroid viability was measured by AlamarBlue™ cytotoxicity assay. Next, analysis of publicly available gene dependency maps, expression and survival data (2-4) was conducted to identify neuroblastoma-specific dependency genes.
**Results:** GD2 targeting improved cell association >10-fold in comparison to untargeted siPLK1-LNPs in 2D cultures ($p<0.0001$), whilst a marked increase in cell association was also observed in 3D neuroblastoma tumouroids. Untargeted siPLK1-LNPs did not impact cell viability, whereas a 50% reduction in viability was observed following αGD2-siPLK1-LNP treatment. Next, the 20 genes with the highest gene dependency scores for neuroblastoma were identified, including HAND2 and MSI2. Importantly, both of these genes were found to be overexpressed in neuroblastoma patient tumours compared to other organs. Further, overexpression of HAND2 and MSI2 were both found to be correlated with poor overall survival in neuroblastoma ($p<0.021$). Finally, siRNA sequences targeting HAND2 and MSI2 were validated for target gene silencing using qPCR, and will next be incorporated into our GD2 targeted LNP formulations. In conclusion, bispecific antibodies have great potential to enhance targeted LNP uptake and improve delivery of siRNA against cancer dependency genes in neuroblastoma.

**References:**

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