GD2 Bispecific Antibody Targeting Enhances Therapeutic siRNA-Lipid Nanoparticle Delivery to Neuroblastoma

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High-risk neuroblastoma is an aggressive, difficult to treat childhood cancer for which more effective and less-toxic therapies are required. Silencing the expression of genes that promote tumour growth, such as polo-like kinase 1 (*PLK1*), using short-interfering RNA (siRNA), is a promising therapeutic strategy. siRNA requires a delivery vehicle to enter cells. Whilst lipid nanoparticles (LNPs) are the most clinically successful siRNA delivery vehicle, these LNPs contain polyethylene glycol (PEG)-lipids on the surface that quickly detach in circulation, leading to rapid uptake by healthy organs, primarily the liver.[1,2] The ability to deliver siRNA to extrahepatic sites, such as neuroblastoma, will be a significant breakthrough. To address this challenge, we have investigated combining LNPs with less-diffusible PEG-lipids, and bispecific antibody targeting to enhance siRNA delivery and *PLK1* gene silencing in neuroblastoma cells.

We used microfluidic mixing to produce siRNA-LNPs with differing surface stabilities through the incorporation of polyethylene glycol (PEG)-lipids with slow (DMG-PEG) or fast (DSG-PEG, DSPE-PEG) detachment kinetics from the LNP.[3] To create GD2-targeted LNPs (αGD2-siRNA-LNPs), we combined siRNA-LNPs with bispecific antibodies (BsAb) that recognise GD2 as well as PEG in the LNP. Cell targeting of αGD2-siRNA-LNPs was examined *in vitro* in high GD2-expressing CHP-134 neuroblastoma cells using flow cytometry. PLK1 gene silencing following treatment with GD2 targeted LNPs containing PLK1 siRNA (αGD2-siPLK1-LNP) was investigated via RT-PCR and viability was measured via AlamarBlue™ cytotoxicity assay. Biodistribution and efficacy studies for our αGD2-siPLK1-LNPs *in vivo* are underway.

Untargeted siRNA-LNPs with more detachable DMG-PEG had greater cell binding than DSG-PEG and DSPE-PEG LNPs. GD2 BsAbs significantly increased cell targeting of all siRNA-LNPs in CHP-134 cells ($p<0.0001$), with greater improvements observed with DSG-PEG and DSPE-PEG formulations. GD2 targeting of siPLK1-LNPs had a modest impact on gene silencing of DMG-PEG formulations (82% gene silencing siPLK1-LNPs vs 91% for αGD2-siPLK1-LNPs), whereas gene silencing efficiency of DSG-PEG and DSPE-PEG siPLK1-LNPs was greatly improved from 11% to 87% and 9% to 84%, respectively, through GD2 targeting. GD2 targeting also reduced the half maximal inhibitory concentration (IC50) of siPLK1-LNPs 5–7-fold. Finally, biodistribution studies of untargeted siPLK1-LNPs demonstrate that exchanging DMG-PEG with less-detachable PEG-lipids increases siPLK1-LNP accumulation in tumour tissue after 48 hours, whilst studies with GD2 targeted siPLK1-LNPs are ongoing. Hence, there is great potential in combining reduced PEG-diffusivity and GD2 targeting to improve therapeutic siRNA delivery to neuroblastoma.

References

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