POLYMERIC MICELLES FOR NOSE-TO-BRAIN DELIVERY OF CRIZOTINIB-IR786 IN THE TREATMENT OF GLIOBLASTOMA

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BACKGROUND

Glioblastoma (GBM) is one of the most challenging tumours to treat, with considerable intra-and inter-tumoral heterogeneity and limited treatment options. Tyrosine kinase inhibitors (TKIs) can target multiple pathways aberrantly activated in GBM; however, their clinical application in GBM treatment is largely limited by their poor blood-brain barrier (BBB) permeability and low tumour specificity. Heptamethine cyanine dyes (HMCDs), a class of near-infrared-emitting molecules, have been recently utilised to improve BBB penetration and tumour tissue specificity of drugs (1). A conjugate of crizotinib (a TKI) and a HMCD (IR786) has been reported to have improved cytotoxic activity in GBM cells and resulted in tumour growth reduction in a rodent model (2, 3). But it undergoes first pass metabolism and have off-target effects.

HYPOTHESIS

- Nose-to-brain delivery provides localized delivery to brain tumours and minimize the off-target effects.
- Encapsulation in nanocarriers increases the metabolic stability and shelf life of conjugate.

AIM

Development and characterisation of crizotinib-IR786 loaded polymeric micelles for nose-to-brain delivery.

METHODS

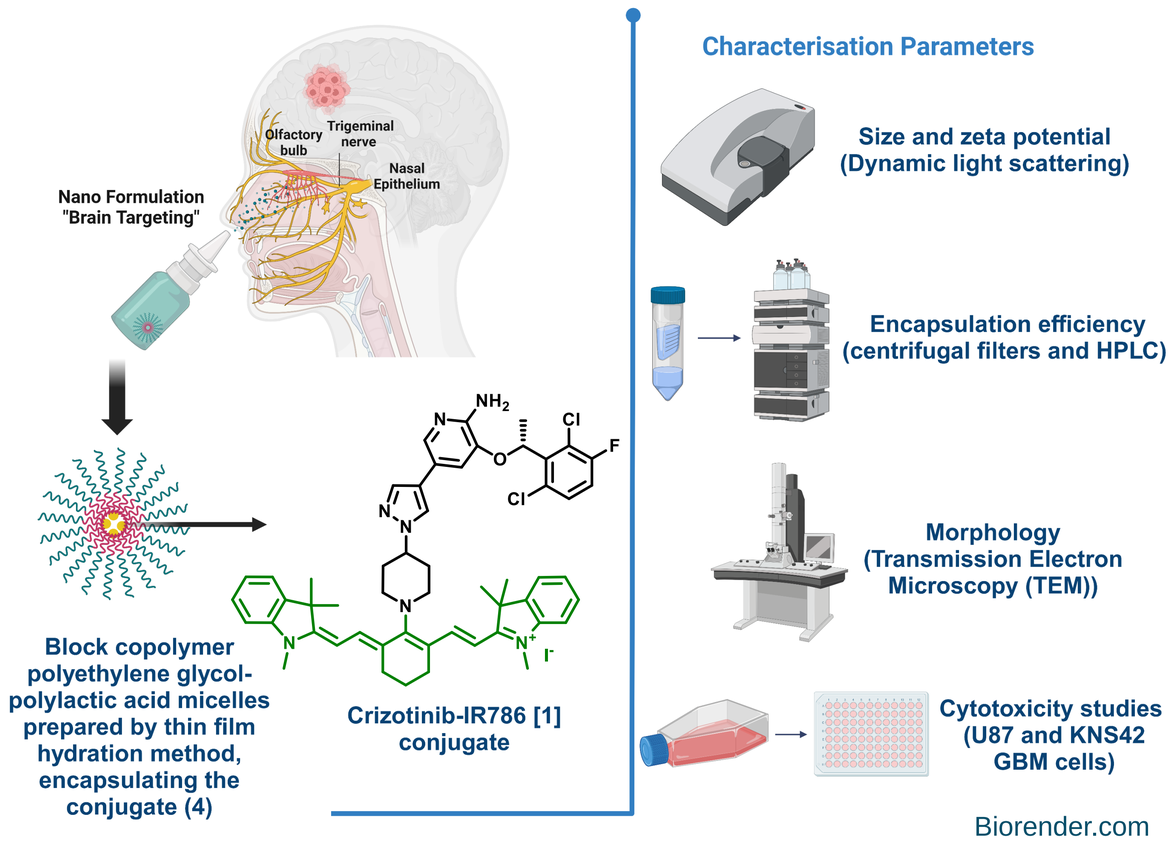

Figure 1: Preparation and characterisation of crizotinib-IR786 loaded polymeric micelles.

RESULTS

Table 1: Characterisation of crizotinib-IR786 loaded micelles (n=3, mean ± SEM).

Diameter (nm)	99.6 ± 9.1
Polydispersity index	0.5 ± 0.01
Zeta potential (mV)	12.8 ± 2.2
Encapsulation efficiency (%)	99.6 ± 0
Drug loading (%)	2.9 ± 0

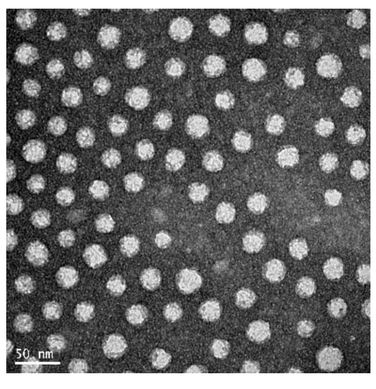

Figure 2: TEM image of crizotinib-IR786 loaded micelles by negative staining with 2% aqueous Uranyl acetate.

Table 2: EC_{50} values of free compounds and micelle formulations in U87 and KNS42 GBM cells. Nuclei detected using Hoechst and EC_{50} values estimated from sigmoid-like dose-response curve. Results expressed as mean ± SEM (n=3) P<0.05 considered significant.

	EC50 (µM)	
	U87	**KNS42**
Crizotinib	3.03 ± 0.15	9.1 ± 2.8
IR786	1.06 ± 0.21	2.94 ± 0.66
Crizotinb-IR786	0.45 ± 0.05	0.45 ± 0.09
Crizotinib micelles	2.04 ± 0.51	4.77 ± 1.29
Crizotinib-IR786 micelles	0.37 ± 0.10	0.44 ± 0.01

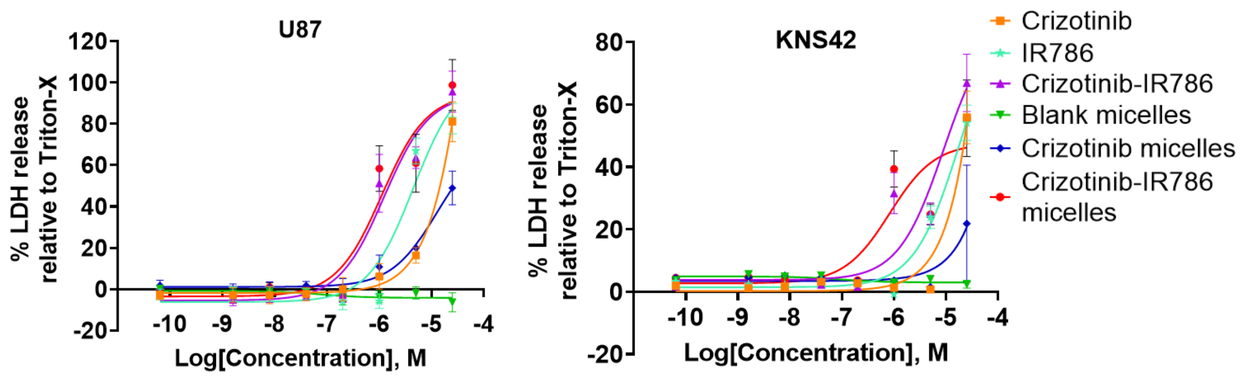

Figure 3: Extracellular LDH release in U87 and KNS42 cells. Results expressed as mean ± SEM (n=3).

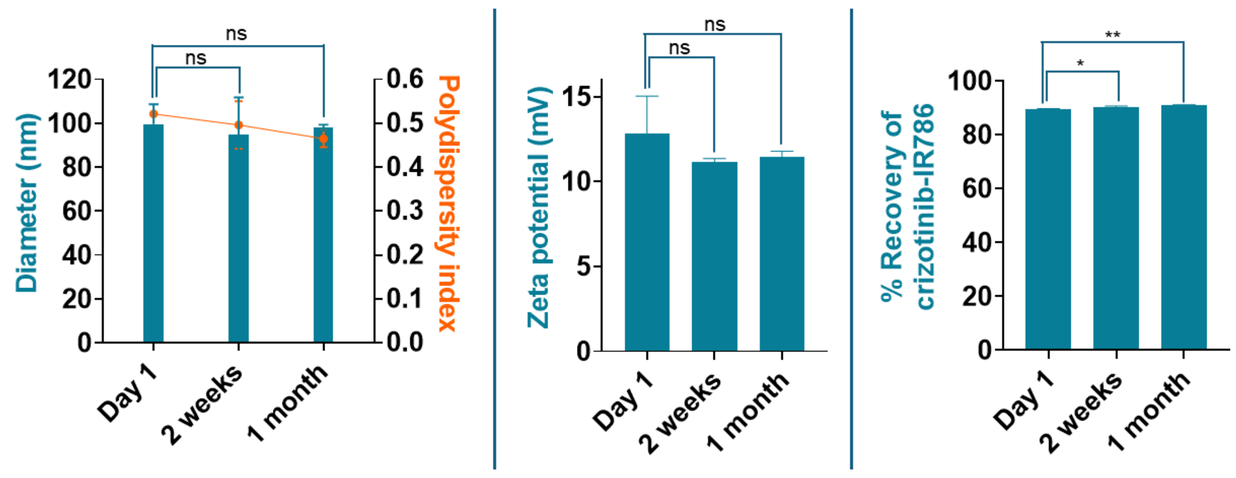

Figure 5: Micelle diameter, polydispersity index, zeta potential, and percentage recovery of crizotinib-IR786 over a period of one month when stored at 4°C. Results expressed as mean ± SEM (n=3). P<0.05 considered significant, one way ANOVA and Tukey's multiple comparison test.

DISCUSSION

- Size and zeta potential of micelles was suitable for nose-to-brain delivery via olfactory receptor neuron with acceptable encapsulation efficiency.
- TEM revealed spherical morphology of micelles with uniform size distribution.
- Crizotinib-IR786 was 6.7 times more potent than crizotinib in U87 cells and 20 times more potent in KNS42 cells.
- Crizotinib-IR786 was 2.4 times more potent than IR786 in U87 cells and 6.5 times more potent in KNS42 cells.
- Potency of free crizotinib-IR786 was similar to that encapsulated in micelles in both U87 and KNS42 cells.
- Micelles were stable for a period of one month and there was no considerable change in micelle size and zeta potential and % recovery of Crizotinib-IR786.

CONCLUSION

The developed polymeric micelles have the potential to be used as a suitable delivery vehicle for crizotinib-IR786. The future studies include exploring similar TKI-HMCD conjugates for encapsulation in polymeric micelles and delivery to brain tumours and in vivo application of polymeric micelles using GBM mouse model.

REFERENCES

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