Development of Tumour Specific Therapy for Treatment of Diffuse Intrinsic Pontine Glioma (DIPG)

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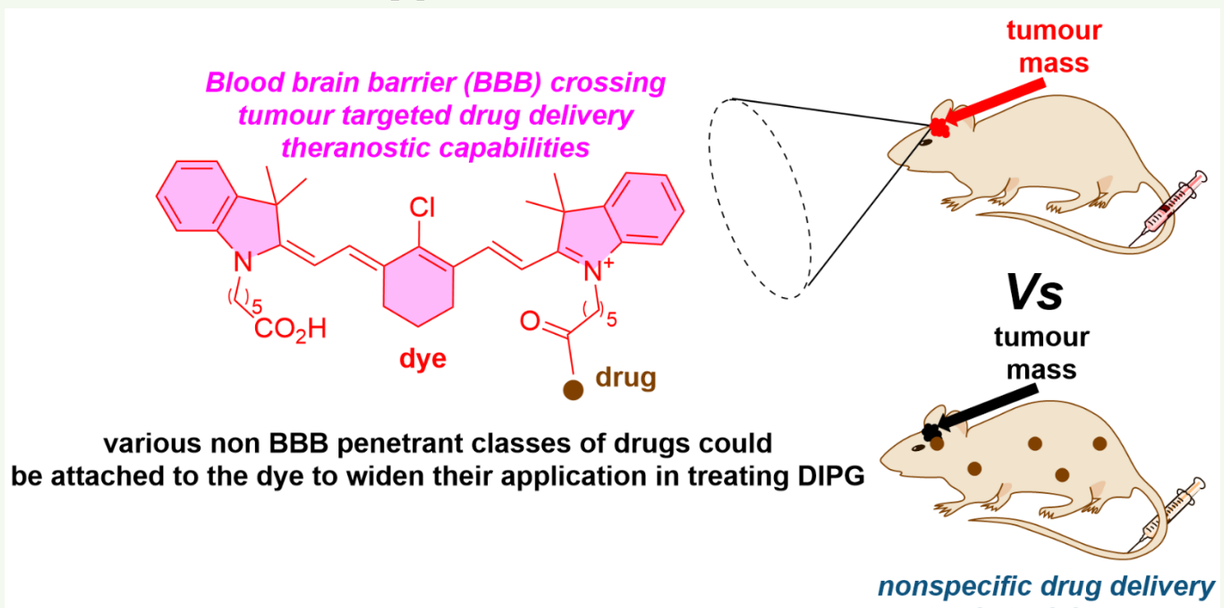
Introduction

DIPG primarily affects children between the ages of 5 and 10 years but can occur in younger children and teens, with statistically higher numbers in male children. It is the second most common type of primary high grade brain tumour in children. It is a lethal brain tumour that starts in the part of the brain stem called the pons, which controls various functions such as blood pressure, heart rate, breathing, and the nerves and muscles that control hearing, walking, talking, eating, and vision. The diffuse nature of the disease does not warrant surgery; therefore, radiation therapy is the current standard, but there are various cytotoxic drugs trialled for treatment with limited success. The average life span of children suffering from DIPG is between 9-12 months. The worldwide incidence is approximately 1-2 per 100,000 population, with the disease almost always proving to be lethal. On a cellular level, mutations in histone genes were found to be driver mutations for DIPG especially the H3.1K27M and H3.3K27M mutations.

Various chemotherapy agents have been tested and shown to be effective against DIPG cell lines and patient-derived xenograft (PDX) mouse models. However, their clinical translation has been challenging due to poor efficacy and system toxicity. A major hindrance is the blockage of most drugs by the blood-brain barrier (BBB), the barrier that protects the brain from various pathogens and toxins. Our group has shown that a certain class of near infra red emitting cyanine dyes can cross the BBB and specifically accumulate in tumour tissues in brain.[1] We hypothesized chemical attachment of approved chemotherapy agents to such dyes could enable selective delivery of drugs to DIPG tumours.

Hypothesis



Results

Table 1: Molecular characteristics of the primary brain tumour cultures employed for evaluating new therapeutic agents.

Primary Culture	Molecular aberrations
HSJD-DIPG007	H3K27M, PI3Kmut, ACVR1mut
SU-DIPGVI	H3K27M
RA055	H3K27M, PDGFRa amplification
VUMC-DIPG010	MYCN amplification

Table 2: IC_{50} concentrations (μM) derived from in vitro cytotoxicity assays performed across a panel of neurosphere-forming DIPG cultures. PDGFRa and PARP inhibitors were conjugated with the dye SN38741.

Primary cultures	SN39699		SN39704		SN38741
	Imatinib-Dye	Imatinib	PARPi-dye	PARPi	Dye
HSJD-DIPG007	0.11	>10	0.611	7.12	8.63
SU-DIPGVI	0.1	>10	0.040	>10	5.35
RA055	0.002	>10	0.028	>10	5.26
VUMC-DIPG010	0.1	>10	0.064	>10	5.52

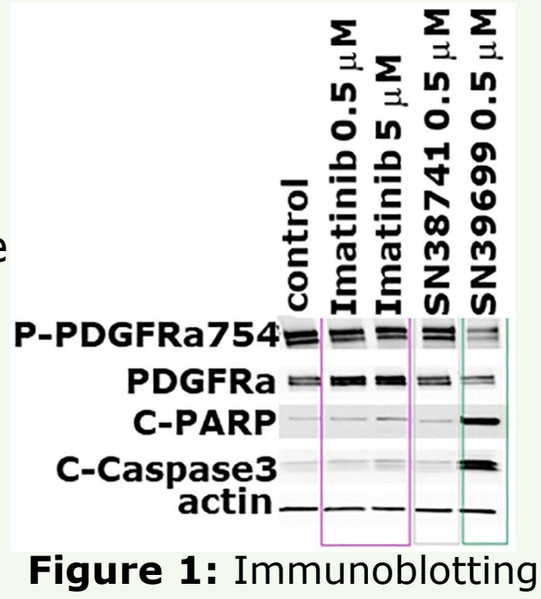


Figure 1: Immunoblotting analysis performed in RA055 cells treated with Imatinib, dye and drug-dye conjugate SN39699.

Table 3: Drug penetration studies of drug, and the drug-dye conjugate SN39699 in DIPG cell lines.

Compound	Concentration (nM)
Imatinib	5.9
SN39699	48.03

Mass spectrometric analysis revealed a remarkable nearly 8-fold increase in the conjugate cell penetration concentration compared to the drug alone after a three hour treatment period.

Discussion

Besides H3K27M mutation, DIPG tumor cells commonly harbor copy number losses and gains with platelet-derived growth factor receptor A (PDGFRA).[2] Elevated DNA damage repair enzyme PARP1 levels have also been reported in DIPG cell lines.[3] Conjugation of PDGFRA inhibitor Imatinib and PARP1 inhibitor Rucaparib to cyanine dye SN38741 resulted in conjugates SN39699 and SN39704, both of which showed potent cytotoxic effects in various DIPG cell lines (Table 1&2). A detailed study of the effect of SN39699 on DIPG cell lines was carried out to progress the compound to in vivo studies (Table 3 and Figure 1).

Conclusions and outlook

Cyanine dyes were used as a drug transport system to improve cytotoxic effect of drugs targeting abnormal signalling pathways in DIPG.

Data presented here enables us to progress these drug-dye systems to be tested in an *in vivo* model of DIPG.

References

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Acknowledgement: