# Hydroxyl Dendrimer Therapeutics Reduce Toxicity in Targeted Delivery to Plexiform Neurofibroma

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## ABSTRACT

Neurofibromatosis type I (NF1), the most common cancer predisposition syndrome, is characterized by the development of slow-growing peripheral nerve sheath tumors called plexiform neurofibromas (PNF). PNF often involve cranial and large peripheral nerves and are characterized by extensive collagen deposition and immune cell infiltrate. Due to the significant morbidity associated with surgical resection, pharmacological strategies play a critical role in the management of these neoplasms. Selumetinib (Koselugo™), a MEK inhibitor, is the only FDA-approved drug for NF1-associated PNF, and though this important inhibitor induces partial responses in both children and adults, there is still a need for agents with improved efficacy and durability. Systemic toxicity remains a challenge in treating PNF, thereby limiting the use of pharmacologic agents in combination. Previous data suggests that the combined targeting of tumorigenic pathways and immunosuppressive cells within the PNF microenvironment (TME) may be an effective strategy to overcome these challenges. Hydroxyl dendrimers (HDs) are small (4-7 nm) hydrophilic compounds that can be conjugated to therapeutics allowing for the delivery of highly toxic drugs specifically to immune cells within the PNF microenvironment. In the preclinical setting, HDTs have demonstrated efficacy in range of pathologies, including Alzheimer's and Glioblastoma. More recently, in human clinical trials HDs exhibited excellent safety and tolerability profiles. HDTs are selectively endocytosed by tumor-associated macrophages (TAMs), which are known to contribute to PNF tumorigenesis. As such, HDTs provide a novel approach for treating these tumors while also limiting toxicity. In preliminary studies, Cy5-labeled HDs were injected into *Nf1$^{flox/flox}$; Postn-cre* mice, an established model of PNF. Postmortem visualization of PNF tumors obtained from these mice revealed striking colocalization of the Cy5-labeled HDs only within tumor-associated macrophages/microglia (TAMs), sparing adjacent normal tissue or neurons. These findings suggest that therapeutic conjugation to HDs to target cells of the PNF TME may be efficacious for the treatment of NF1-associated PNF. Studies are currently underway to evaluate novel HDTs in the *Nf1$^{flox/flox}$; Postn-cre* mouse model.

## INTRODUCTION

- Neurofibromatosis type I (NF1) is characterized by the progressive development of slow-growing tumors called plexiform neurofibromas (PNFs).
- Systemic toxicity and intolerable adverse effects remain major challenges that limit dosing regimens and often result in drug discontinuation.
- PNFs are characterized by significant infiltration of tumor supportive monocytes/macrophages.
- HDTs allow for combined targeting of pro-tumor cells in the TME and key tumorigenic pathways impacting PNF tumor cells.

## BACKGROUND

- Hydroxyl Dendrimer (HD) Therapeutics (HDTs) belong to a new class of drugs in which highly branched HDs, small (4-7 nm) hydrophilic compounds, are chemically linked to a known small molecule drug.
- HDs are preferentially endocytosed by inflammatory cells within the TME.
- HDs are not metabolized and are rapidly cleared intact through the kidneys.
- Colocalization studies performed in orthotopic rat models of glioblastoma (GBM) demonstrate selective uptake of HDTs by tumor-associated macrophages/microglia (TAMs).
- We aim to use HDTs as a strategy to target reactive myeloid cells within the PNF TME to impair tumor growth.

## BACKGROUND

**Adaptability of Hydroxyl Dendrimer Therapeutics (HDTs)**

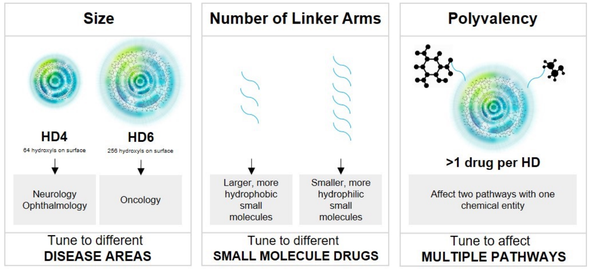


**Figure 1.**

- The number of surface hydroxyls and different sizes of HD4 (4 nm) and HD6 (7nm) equip each for a different disease area: neurology and ophthalmology (HD4) and oncology (HD6).
- HDTs with fewer linker arms on their surface are fit for larger, more hydrophobic small molecules, whereas HDTs with more linker arms facilitate better functionality for smaller, more hydrophilic small molecules.
- HDs can be conjugated to more than one drug, enabling one HDT to affect multiple pathways. (see QR code)

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**Colocalization of Cy5-tagged Hydroxyl Dendrimer and Microglia in Rat Glioblastoma**

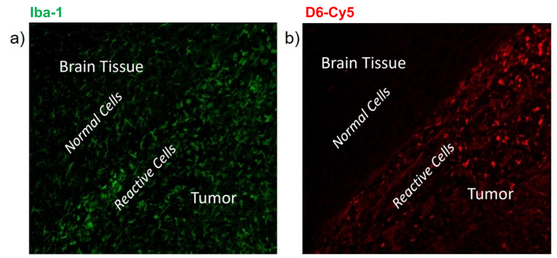


**Figure 2.**

- GBM-bearing rats were injected via tail vein with HD6-Cy5 at a concentration of 55 mg/kg. Brain tissues were harvested, fixed, processed, and cryopreserved in cryomolds using an isopentane bath with dry ice.
- a) Iba-1 (green) serves as a marker of macrophages/microglia within the tumor microenvironment (reactive cells) and surrounding normal cerebral tissue.
- b) Single intravenous injection of Cy5-tagged HD (red) demonstrates uptake of HDs only by cells within the tumor and tumor microenvironment (reactive cells) but not surrounding normal cerebral tissue.
- Colocalization studies demonstrate selective uptake of HDs by actively endocytosing macrophages/microglia in the tumor microenvironment, minimizing off-target toxicity.

## MATERIALS and METHODS

- Tumor-bearing NF1 mouse tissues were fixed, processed, sectioned, and stained with the designated primary (Iba-1, Tuj-1) and secondary antibodies (goat anti-rabbit Cy3). Coverslips were mounted with Prolong Gold Antifade Mountant containing DAPI.
- Images were obtained by high-resolution confocal microscopy to visualize colocalization of Iba+ macrophages and fluorescently labeled hydroxyl dendrimer (Cy5) within the respective tissues.

## RESULTS

**Colocalization of HD4 or HD6 with Iba+ Macrophages in NF1 Mouse Tissue**

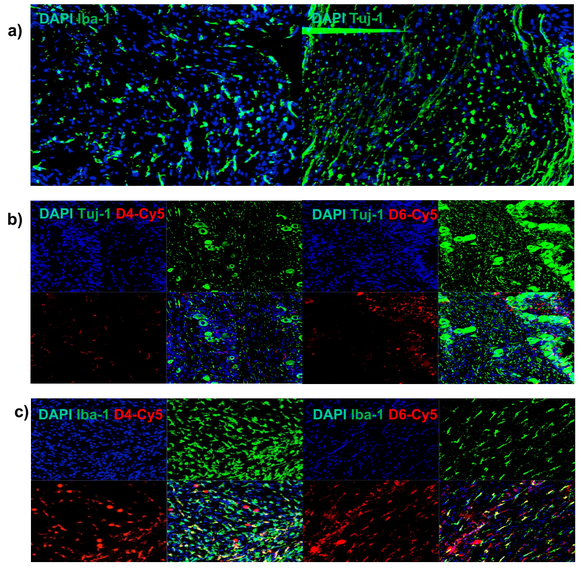


**Figure 3.**

- *Nf1$^{flox/flox}$; Postn-cre* mice were administered a single injection of HD. Tumor-bearing nerve tissues were harvested 48 hours post-injection.
- a) Non-dendrimer injected NF1 tumor tissue demonstrates absence of D4-Cy5 staining (red). Macrophages and neurons are identified by Iba1 or Tuj-1-positivity (green), respectively.
- b) Fluorescently labeled dendrimer (D4-Cy5 or D6-Cy5, red) does not colocalize with neurons (Tuj-1, green) 48 hours after injection demonstrated in merged image (lower right, yellow).
- c) Fluorescently labeled dendrimer (D4-Cy5 or D6-Cy5, red) colocalizes with macrophages (Iba-1) 48 hours after injection demonstrated in merged image (lower right, yellow).

## CONCLUSIONS

- HDs in glioblastoma orthotopic tumor rat models demonstrate selective uptake by TAMs/microglia in the tumor microenvironment.
- Immunofluorescence microscopy demonstrates colocalization of hydroxyl dendrimers and tumor-associated macrophages (TAMs) but not adjacent neurons, suggesting HD endocytosis occurs specifically in tumor-associated phagocytic cells.
- D4-Cy5 colocalization with macrophages is enhanced in tumor-bearing NF1 mouse tissue compared with D6-Cy5 colocalization.

## DISCUSSION

- Monocytes/macrophages reportedly account for 30-50% of cells in PNF tumors.
- Macrophage infiltration correlates with disease progression in NF1-associated PNF.
- HDTs provide a unique opportunity to enhance efficacy and minimize toxicity in the treatment of NF1-associated PNF.
- *In vivo* evaluation of HDT efficacy is currently underway in *Nf1$^{flox/flox}$; Postn-cre* mouse model of PNF.
- Future studies will evaluate combinatorial therapeutic strategies targeting multiple signaling pathways and components of the PNF TME in the *Nf1$^{flox/flox}$; Postn-cre* mouse model and NF1 human patients.

## ACKNOWLEDGMENTS

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