Selective Targeting of Immunosuppressive Tumor Macrophage from Systemic Administration

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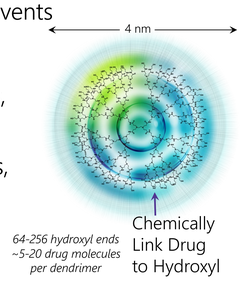
Abstract: 2595

Background Rationale & Objectives

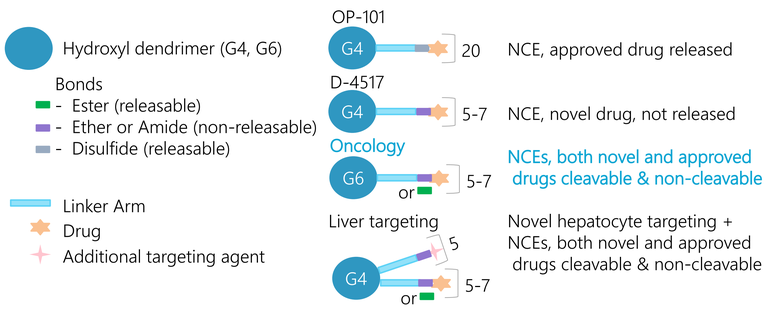
- Tumor associated macrophages (TAMs) in the M2 immunosuppressive state reduce the effectiveness of immunotherapy & facilitate metastases
- Current approaches to manipulate TAMs are not selective causing systemic inflammation and other side effects
- Hydroxyl dendrimers selectively target reactive macrophages as demonstrated over 30 animal models
- Objective: Determine the optimal hydroxyl dendrimer for systemic targeting of solid tumor M2 TAMs and effect of CSF1R inhibitor on TAM uptake
- Hydroxyl dendrimer-drug conjugates are currently being synthesized and tested in preclinical oncology animal models

Hydroxyl Dendrimer for Hyper-Targeted Drugs

- Selective targeting to activated cells (cells actively endocytosing) at sites of inflammation (cross tissue barriers, e.g. BBB)
- Proof of concept in 30+ animal models, 6 species including dogs and monkeys (>$30 M in NIH funding)
- No off-target toxicity – no toxicity of platform at 1000 mg/kg
- Human safety (up to 40 mg/kg) – no clinical adverse events
- Flexible dosing – oral or injectable
- Wide range of drugs (>65) – small molecules, proteins, RNA/DNA
- Tunable functionality – multiple linkers and chemistries, different size dendrimers to alter PK/distribution
- Inexpensive to manufacture at large scale (>1 kg) (1 kg GMP lots)



Hyper Targeting Building Blocks – New Chemical Entity, NCE



Unlimited number of combinations including multiple drugs on same dendrimer

Study Design & Methods

- Syngeneic murine colon cancer cell line, MC38, in C57BL/6 mice (n=10/group)(**Flank tumors**)
  - Tumor size range at dosing of 80 to 120 mm3
  - Two Studies were conducted under the same conditions
  - Dose groups (single dose IV, 55 mg/kg dendrimer, 10 mL/kg)
    - Saline or PBS control
    - Dendrimer (G4; 14,000 Da, 4 nm) conjugated with Cy5 (D4-Cy5)
    - Dendrimer (G6; 58,000 Da, 7 nm) conjugated with Cy5 (D6-Cy5)
    - Dendrimer (G4) conjugated with Cy5 and CSF1R inhibitor (C-D4-Cy5)
  - Sacrifice 48 hr post-dose (dendrimers systemically cleared within 48 hr)
  - Tumor analysis: Total radiant fluorescence & FACS
- Orthotopic GBM Model (C57BL/6 mice) (**Brain tumors**)
  - Inoculated intracranially with 100,000 GL261 murine glioblastoma cells
  - 14 days post-inoculation, mice were given single IV dose of 55 mg/kg C-D4-Cy5 followed by sacrifice 48 hr post-dose

MC38 Model Results

Tumor Cy5 Signal

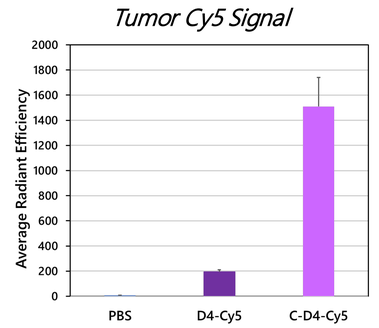
Greater uptake of generation 6 (D6-Cy5)) in MC38 tumors, compared to Generation 4 (D4-Cy5). A similar increase is seen in the GBM Model (Liaw et al., 2020).

Tumor Immune Cell Populations (FACS):
~56% CD45+
subsets of CD45+ cells:
~20-25% M2 Macrophage
~13% M1 Macrophage
~8% monocytic myeloid-derived suppressor (mMDSC)
(other subsets < 5%)

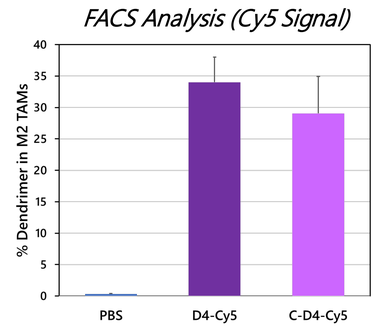
Dendrimer Selectively Targets M2 TAMs
>60% of M2 TAMs contain D6-Cy5 after a SINGLE systemic dose

MC38 Model Results

Tumor Cy5 Signal

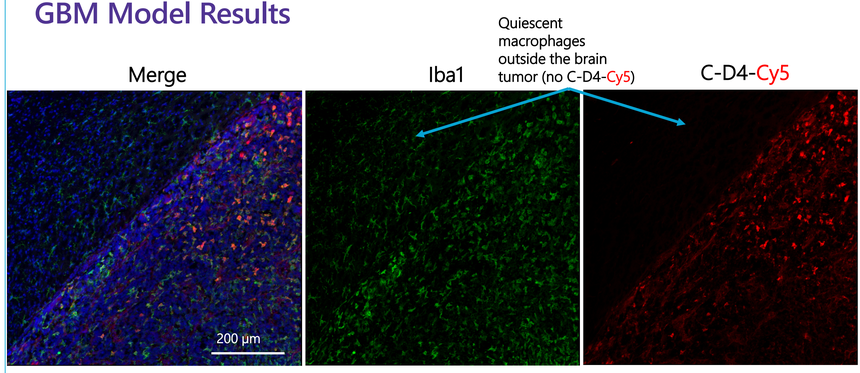


FACS Analysis (Cy5 Signal)



Greater tumor uptake of C-D4-Cy5 with same % of cells having Cy5 signal = more C-D4-Cy5 per cell

GBM Model Results



C-D4-Cy5 clearly delineates tumor border
Note that the macrophages outside the tumor have a ramified morphology and do NOT contain C-D4-Cy5

Uptake Exclusive to Only Reactive Microglia & Macrophage

Conclusions

- The larger hydroxyl dendrimer (D6) has significantly greater tumor uptake compared to the smaller hydroxyl dendrimer (D4)
- A single intravenous dose of D6 results in >60% of the M2 TAMs endocytosing the D6 demonstrating efficient targeting
- M1 TAMs, mMDSCs and gMDSCs also take up the hydroxyl dendrimer to a lesser extent
- CSF1R inhibitor increases uptake of D4 in individual tumor immune cells
- Highly specific uptake of C-D4-Cy5 into TAMs with no detectable uptake by adjacent non-tumor associated macrophages/microglia
- These results support the development of hydroxyl dendrimer-drug conjugates with D6 to target and manipulate M2 TAMs

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Reference: Liaw K, Zhang F, Mangraviti A, Kannan S, Tyler B, Kannan RM*, Bioengineering and Translational Medicine, In press, https://doi.org/10.1002/btm2.10160, April (2020)