Optimizing Delivery of Molecular Targeting Agents to Glioblastoma

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Statement of Purpose: Molecular targeting agents are most useful when the location of the cancer cells is unknown and undetectable by conventional methods. Although large constructs (such as nano-particles) can accumulate in large tumors by the enhanced permeability and retention (EPR) effect, such large tumors can most likely be located using conventional imaging techniques.

In glioblastoma multiforme the challenge is not detection and location of the primary tumor – diagnosis usually comes when the tumor is sufficiently developed for detection via MRI. The challenge is that, by the time the primary tumor is detected and removed, cells have invaded other parts of the brain and formed nascent secondary tumors. We and others have developed bio-macromolecular targeting constructs designed to target these secondary tumors by intracranial injection and perfusion through brain tissue. The very small size (~6kDa) of these constructs allows them to perfuse freely in tissue although they are orders-of-magnitude too large to pass through intact blood-brain barrier (which is the case for nascent secondary tumors).

Here we show *in vitro* proof-of-principle for a delivery scheme that has potential to successfully deliver bio-macromolecular targeting constructs to secondary tumors. A finite element model simulating injection of these constructs into brain tissue can help us determine the number of catheters, catheter placement, and injection duration necessary to successfully infuse an entire hemisphere of the brain in order to detect and treat all secondary tumors created by invasive cells.

Methods & Results: Mathematical modeling was performed using a finite difference scheme to incorporate diffusion and convection into the model of multivalent construct targeting developed by Caplan & Rosca (Ann Biomed Eng, 2005). Figure 1 shows trivalent construct (1 pM) injected at 3μl/min followed by a wash of artificial cerebrospinal fluid (aCSF) at the same flow rate.

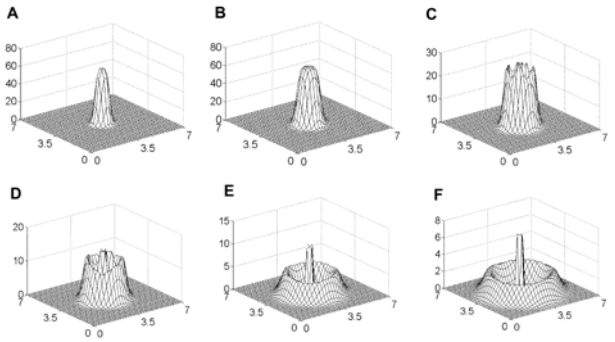

Figure 1. Number of targeting constructs (vertical axis) vs. position (horizontal axes) for injection of targeting constructs (A,B) followed by washing with aCSF (C-F).

The tumor, which is offset 0.75cm from the injection site, expresses the targeted receptor at 5x the density of the normal cells. As the convective wash continues for several hours (D-F), the contrast between construct bound to tumor vs. bound to normal tissue becomes more pronounced, but the total number of constructs decreases.

In vitro characterization of this prediction has been performed by injecting trivalent constructs (synthesis and characterization described in Rosca et al., Biomacromolecules, 2007) into 0.3%w/0.3%w agar/collagenI gels containing regions encapsulating glioblastoma cells (SF767) or normal human astrocytes (NHAs). Figure 2 shows that the SF767 cells are bound by the construct, but the NHAs are not (the two bright spots are not co-localized with cells by phase image).

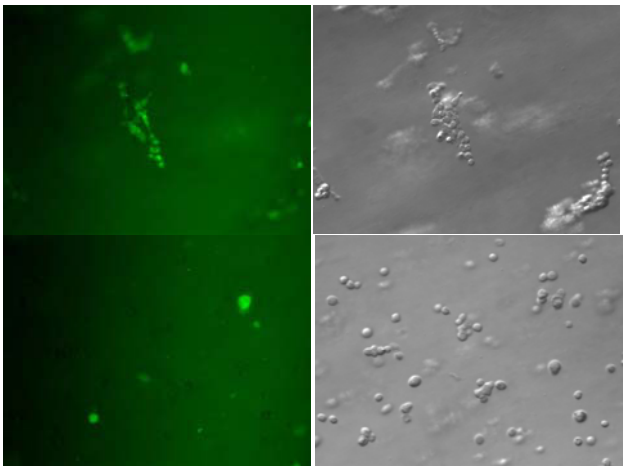

Figure 2. Fluorescently-labeled constructs (left) binding to SF767s (top) and NHAs (bottom). Phase contrast images (right) show that NHAs are not targeted.

This work is being translated to the clinic in collaboration with a neurosurgeon at Barrows Neurologic Institute, Kris Smith, who is involved in a clinical trial of an antibody-conjugated radioisotope. The challenge is to perfuse an entire hemisphere of the brain with adequate dose of the targeting construct. We are developing a finite element simulation based on the equations used in the model shown in Figure 1, but in this case for realistic geometry and anisotropy of brain tissue (data not shown).

Conclusions: Bio-macromolecular constructs are needed to target nascent secondary tumors. Delivery of these constructs requires a convective wash to achieve specific targeting of cancer cells. *In vitro* experiments have demonstrated proof-of-principle that a trivalent construct targeted to the $\alpha_6\beta_1$-integrin can achieve specific targeting of glioblastoma cells. Further work modeling realistic geometry and anisotropy of brain tissue has potential to aid translation of this work from the bench to the bedside.

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