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## Shedding New Light on the Use of Imaging Technology for Glioblastoma Tumor Resection

John Nemunaitis<sup>1-4</sup> and Neil Senzer<sup>1,3</sup>

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Complete surgical resectability is highly correlated with positive prognosis in all of solid-tumor management. It is particularly relevant in glioblastoma multiforme (GBM), where resectability of wide margins of the tumor is virtually impossible owing to the difficulty of distinguishing adjacent functional structures, such as the so-called “eloquent” areas controlling language and movement. The use of fluorescence-guided surgery (FGS) results in the differential labeling of tumor cells and thereby effects a more accurate delineation of the margins than does the use of visual bright-light surgical (BLS) techniques. In this issue of *Molecular Therapy*, Yano *et al.* describe a modification of the fluorescence-guided assistance approach that enabled use of a conditionally replicating adenovirus as both a delivery/diagnostic agent and an oncolytic therapeutic.<sup>1</sup>

The standard treatment for GBM comprises extensive surgical resection followed by external-beam radiation and concomitant chemotherapy with the DNA-alkylating agent temozolomide (TMZ), followed by additional cycles of TMZ administration. However, the median survival time is still less than 12 months even after intensive combination therapy, and in 90% of cases tumors recur at the primary site.

Like other tumor types, GBM is more effective than adjacent normal tissue at converting 5-aminolevulinic acid (5-ALA; Gliolan) to protoporphyrin, which is fluorescent under blue light. Diez Valle *et al.* recently described results in 36 consecutive patients with glioblastoma who underwent surgery guided by 5-ALA fluorescence.<sup>2</sup>

<sup>1</sup>Mary Crowley Cancer Research Centers, Dallas, Texas, USA; <sup>2</sup>Texas Oncology, P.A., Dallas, Texas, USA; <sup>3</sup>Gradalis, Inc., Dallas, Texas, USA; <sup>4</sup>Medical City Dallas Hospital, Dallas, Texas, USA

Correspondence: John Nemunaitis, 12222 Merit Drive, Suite 1500, Dallas, Texas 75251, USA. E-mail: jnemunaitis@marycrowley.org

Tumor volume was quantified with volumetric magnetic resonance imaging (MRI) before and after resection to differentiate grossly normal from tumor tissue. MRI is noninvasive, has no known side effects, and allows consecutive measurements in longitudinal studies. In addition, MRI has intrinsic contrast abilities that can be used to assess tumor tissue characteristics. On the basis of the MRI data, the authors report that strong fluorescence identified solid tumor with 100% positive predictive value. Normal tissue beyond the solid-tumor mass that had been invaded by tumor cells was identified by vague fluorescence with 97% correlation with the presence of tumor (i.e., a low rate of false-positive results) but with a 66% negative predictive value (i.e., ~34% chance of false-negative results) as measured against standard hematoxylin–eosin examination, which stains the nuclear and cytoplasmic components. A subsequent retrospective study compared GBM patients from 27 hospitals comprising centers using FGS-5-ALA with centers not doing so.<sup>3</sup> Although there were some disparities between the groups, there was significant improvement in achieving complete resection in the FGS-5-ALA group (using surgical + MRI criteria), accompanied by a significant prolongation of 6-month progression-free survival.

Although a recent randomized phase III study<sup>4</sup> of FGS-5-ALA also showed improvement in resections guided by 6-month progression-free survival compared with those guided by bright light, improvement in overall survival was not observed. Subsequently, Eyupoglu *et al.*<sup>5</sup> used intraoperative MRI (iMRI) to verify completeness of resection in 21 glioblastoma patients following FGS-5-ALA and compared the results achieved with FGS-5-ALA imaging to the combination of FGS-5-ALA imaging in conjunction with iMRI. Fourteen patients whose surgeries showed complete resection using 5-ALA

imaging were found to have residual tumor that could be identified by iMRI. The combination of FGS-5-ALA and iMRI during surgery significantly increased the extent of tumor resection—from 61.7% to 100%—in the subgroup of patients with malignant gliomas located adjacent to eloquent areas of the brain with highly functional importance. 5-ALA imaging alone proved to be insufficient in attaining gross total resection without neurological deficit due to damage to eloquent areas.

Using an implanted orthotopic glioblastoma (U87MG) immunodeficient nude mouse model, Yano *et al.*,<sup>1</sup> tested a replication-competent serotype 5-based adenoviral vector expressing green fluorescent protein (GFP) that incorporates a human telomerase reverse transcriptase gene (*hTERT*) promoter to drive the expression of the viral E1A and E1B factors (OBP-401).<sup>6</sup> This product has previously demonstrated specificity, safety, and antitumor activity in TERT-expressing cancers<sup>7</sup> and was used in the new study to mark tumor tissue following intratumoral injection.

The authors demonstrated the superiority of low-dose ( $1 \times 10^8$  plaque-forming units (PFU)) OBP-401-FGS over the BLS technique in terms of the presence of residual postsurgical GFP-labeled cells and survival rates. They also showed greater efficacy in achieving improved progression-free survival with less extensive surgery using high-dose ( $2 \times 10^8$  PFU) vs. low-dose OBP-401.

Even though the authors showed evidence of dose-dependent effectiveness of oncolytic high-dose OBP-401-FGS > low-dose OBP-401-FGS >> BLS, there are some reservations that could impact the translational potential of this study. The U87MG orthotopic xenograft is not an optimal model for spontaneous GBM<sup>8</sup> in that it grows centrifugally with smooth rather than poorly demarcated borders, has limited areas of spontaneous tumor cell death, has limited sites of noncontiguous invasion, and lacks irregular microvascular proliferation. Furthermore, before telomerase is expressed in only ~60% of GBM tissues, a significant immunogenic bystander effect would be needed to overcome tumor heterogeneity.<sup>9</sup>

Tumor recurred in 12 of 14 mice that underwent bright light–assisted glioblastoma surgery but not in any of the mice that underwent surgery assisted by high-

dose GFP-OBP-401. In addition, all the mice in the GFP-OBP-401 group remained alive at 120 days after treatment. Despite some reservations related to methodology, these results justify further evaluation in GBM and demonstrate an advantage over standard practice with FGS-5-ALA. In addition, GFP-OBP-401 may have much broader application in other solid tumors.

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