WHILE questions persist as to the delineable influence for extent of glioma resection (EOR) on outcome measures such as quality of life and survival, equally persistent are our efforts to improve rates of resection, despite the controversy. Ultimately, there are 2 pervasive and recognizable barriers to the “gross-total resection” (GTR): insecurity regarding the borders between tumor and normal tissue, and fear of the eloquent brain that lies beyond that border. Truly then, the first is made more relevant by the second and is contextually dependent on location. Still, the confidence that could come with “foolproof” demarcation of tumor borders intraoperatively (and importantly in real time) offers assuredly intrinsic value to the surgeon, some would likely advance, independent of arguments surrounding statistical connections between EOR and outcomes. Importantly, such demarcation is perhaps increasingly a realizable goal, and, in addition to advances amidst technologies, such as intraoperative MRI and ultrasound, a burgeoning nidus for its realization may lie with fluorescence-guided surgery.

It is near common knowledge now that 5-aminolevulinic acid (5-ALA) is approved for use in Canada, Europe, and Japan, and is undergoing clinical testing presently in the US. What is less recognized, however, are the alternative compounds under examination; their relative mechanisms, advantages, and limitations; their impact for objective outcome measures; and the information needed to properly assess them in isolation or in tandem. Notably, one alternative to 5-ALA is a dye long familiar to the neurosurgical field: fluorescein sodium, whose seemingly specific accumulation within gliomas has been recognized admittedly since the 1940s, and whose testing in humans has reached a new advent in the wake of growing attention for fluorescence-guided surgery.

In this issue of the Journal of Neurosurgery, Diaz et al. advance a degree of new insight into the “old” mechanism for fluorescein’s glioma homing. Indeed, it is similarly long-presumed and noted that fluorescein accumulates in areas of disrupted blood-brain barrier (BBB), but Diaz et al. began with a hypothesis that the dye would perhaps additionally specifically label glioma cells. What they found instead, perhaps not surprisingly, was that the dye accumulated merely in the extracellular spaces around glioma cells in vitro and in vivo, with their further experiments suggesting passive accumulation within areas of BBB disruption.

How should we evaluate the impact and context for this study? On the most apparent level, this study provides evidence for a “passive” means for fluorescein glioma “labeling,” whose accuracy is then dependent upon evidence for BBB disruption in glioma and other tumors that are brain situated. This should be contrasted with 5-ALA, which “actively” labels neoplastic cells based upon its metabolic conversion to the fluorescent molecule protoporphyrin IX (PpIX), a heme-synthesis pathway substrate that accumulates preferentially within certain tumor cells, including those of high-grade gliomas. The authors and other advocates of fluorescein, however, will be encouraged by recent work that more definitively characterizes the phenomenon of BBB disruption in glioma and may indirectly support glioma specificity for fluorescein as a result. Watkins et al., in a 2014 study, directly visualized the displacement of astrocytic foot processes from preexisting vascular endothelium by glioma cells, which highly associated with blood vessels, particularly when migrating away from the main mass (i.e., the invasive components). Furthermore, focal disruptions in the BBB were precipitated by even a single perivascular glioma cell. Although a leaky BBB is historically recognized among newly formed glioma vessels lacking tight junction proteins, this focal disruption of a previously intact BBB was unprecedented, as were the forward disruptions solicited by distant invading cells. If indeed fluorescein proves a sensitive indicator for BBB disruption, these findings imply that it may be a capable
marker for “forward” areas of microinvasion. Conversely, its freedoms within the extracellular space may instead permit it to diffuse along white matter tracts and stain edematous normal parenchyma, perhaps severely limiting its specificity.

Truthfully, the deeper questions that studies such as Diaz et al. elicit are for fluorescein sodium and for fluorescence-guided surgery more broadly: 1) Can these compounds veritably improve resection? 2) If so, will improving resection translate to improved outcomes? 3) How ultimately should we evaluate and/or compare fluorescence guidance systems? 4) Will implementation be feasible?

Regarding the first question, the only related and reliable data in patients to date come from clinical testing of 5-ALA, which indeed does appear to possess the capacity for improving EOR. A meta-analysis of the literature on 5-ALA, following the guidelines of the Cochrane Collaboration, ultimately selected 5 studies for analysis and concluded significant benefit with regard to EOR, 6-month progression-free survival (PFS), and even overall survival (OS). The most prominent individual 5-ALA study (also included in the meta-analysis) is of course Stummer’s Phase III trial published in 2006, in which 322 patients with newly diagnosed glioma were randomized to either conventional white light microsurgery or 5-ALA–guided resection. 5-ALA improved the rate of GTR from 36% in white light controls to 65%, and the 6-month PFS from 21.1% to 41.0%. OS did not reach significance (p = 0.1), reflecting lack of power to detect an effect. The study has been criticized for not permitting intraoperative use of neuronavigation (in actuality, neuronavigation was permitted for tumor localization, but not resection; however, many cite this fault as a reason for the low GTR rate in the white light group); for utilizing PFS as a primary endpoint; and for the likely unavoidable non-blinding of surgeons. An ongoing trial of 5-ALA in the US (BALANCE, clinicaltrials.gov: NCT01502280) permits the use of neuronavigation and may provide further insight into utility in the context of standard/optimized surgical conditions.

No such analysis is available for fluorescein, which remains in feasibility testing, particularly when used in conjunction with a newly designed, and likely mandatory, Yellow 560-nm microscope filter that permits better visualization at lower dosages (clinicaltrials.gov: NCT02028325). As a molecule with innate fluorescent properties, however, fluorescein can (in contrast to 5-ALA) be visualized at high doses under standard white light without the aid of a filter and has been formally studied in that capacity since the 1990s as a resection aid. A nonrandomized prospective study of use in this context was published in 2003 and demonstrated GTR rates in the fluorescein group of 84.4%, compared to only 30.1% in controls. No survival benefit was revealed (the study enrolled just 32 patients, however). Similar results were obtained in subsequent studies. Ultimately, large prospective randomized trials are still wanted for any application of fluorescein guidance.

On the second question of links between EOR and outcomes (the objective utility for fluorescent-guidance is seemingly inevitably tied to this question), a full discussion of this argument is likely beyond the scope of this editorial but is, needless to say, speckled throughout the neurosurgical literature and deserves comment. The greatest limitation for resolution is that prospective randomization of glioma patients to subtotal versus gross-total resection is unlikely to ever be accomplished, although in viciously cyclic fashion, ethical arguments preventing such studies are inextricably founded in a “gestalt” that has not been satisfactorily backed by the literature. Through a number of retrospective observational studies, EOR has been identified as a predictor of survival in newly diagnosed and recurrent high-grade gliomas. In general, the strongest evidence for this association comes with complete resection, although one well-known study demonstrated a benefit, at least in newly diagnosed glioblastoma, of any resection greater than 78%. Others have demonstrated a continuous nonlinear association with EOR and survival, suggesting that even a less rigid resection threshold might be adoptable for these tumors.

Based on these data, EOR has perhaps mistakenly taken a place alongside age and Karnofsky Performance Scale (KPS) as being a “cause” of survival in this patient population. A “predictor” of survival, however, must be differentiated from a “cause.” For example, a younger age, or a better KPS score, may push surgical decision making toward achieving a greater EOR, and thus age or performance status may directly influence (as an upstream determinant) the degree of resection. Such is the limitation of retrospective observational studies. As Barker pointed out in his comments on Sanai and Berger’s article, these studies are often confounded by indication, as the “extent of resection” is frequently determined in turn by the tumor’s “resectability,” a limitation typically left unaddressed in a nonrandomized study. Of importance, these biases are not fixed by a multivariate analysis, which will still demonstrate EOR to be important, insomuch as the degree of resection was in turn influenced by patient age or performance status. Barker also pointed out that there is a selective loss to follow-up of those patients who do poorly after surgery. Ironically, some of these biases are well revealed in Stummer’s 5-ALA trial, where the age of patients proved lower overall in those receiving complete resection, a subgroup that also proved to have fewer tumors located near eloquent cortex. This imbalance would typically confound any extrapolation of the data on EOR to a survival end point. What all this means for fluorescent guidance, in the end, is that EOR, much like PFS, may be a somewhat misleading primary end point for study if we are going to require objective outcome-driven justification for its use. Compounding the issue, the most encouraging correlative data between EOR and outcome is likely for low-grade glioma, where neither 5-ALA nor fluorescein proves useful.

The next question then does indeed become how to properly evaluate and compare such technology. The low-hanging fruit is safety, concerns for which to date have mostly surrounded the consequences of obtaining more complete and aggressive resections, including into areas of lower fluorescence whose specificity for tumor remains somewhat indeterminate, as well as dependent on the agent used. If we search instead for acceptable objective outcomes measures, we may be left chasing the grail of overall survival differences, which similarly becomes difficult to adequately design studies to reveal. If easier-to-obtain