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First-in-human Clinical Trial Results of a Small-Molecule Fluorophore Applied to Soft-Tissue Sarcomas

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Background:
Soft-tissue sarcomas are cancers originating from mesoderm-derived connective tissues (fat, muscle, tendon, ligament, peripheral nerve, and fascia). Soft-tissue sarcomas demonstrate generally poor responses to chemotherapy and only modest responses to radiation; complete surgical excision of a non-metastatic primary tumor is therefore the only reliably curative treatment. Fluorescence-guided surgery (FGS) is nascent technology that seeks to improve the safety and effectiveness of invasive medical procedures through the use of targeted fluorescent reporters—fluorophores—that bind to important tissues. The purpose of this manuscript is to report our results from this completed trial.

Questions/Purposes:
Based upon our preclinical murine results, we hypothesized that ABY-029 would demonstrate tumor-to-background contrast (>2.0), capable of providing useful guidance for discriminating sarcoma from non-sarcoma tissues. The primary objectives of this study were to determine whether a biological variability ratio (BVR, analogous to signal-to-noise ratio) of 10 is achievable with microdose administration on day of surgery, and whether fluorescence intensity in tissue correlates with EGFR expression.

Patients and Methods:
In a Phase 0/1 clinical trial, patients with soft-tissue sarcomas were recruited based on positive immunohistochemical staining for EGFR of diagnostic biopsy tissue. Patients were administered a microdose (237 g, 30 nanomoles) of ABY-029 and tumor resection was performed within 3-6 hours; two rounds of dose escalation occurred. Following resection, *ex vivo* tumor tissue was scanned to determine BVR and tumor to background ratio (TBR). Samples were correlated to histopathological staining. Blood plasma was collected at intervals after administration to evaluate ABY-029 plasma clearance.

Results:
Soft-tissue sarcomas positive for EGFR on histopathological staining demonstrated whole tumor BVR and TBR of 5.4 ± 0.3 and 3 ± 1, respectively, and regionally selected tissue samples yielded values of 8 ± 5 and 5 ± 3, respectively. ABY-029 fluorescence from the *ex vivo* sarcoma tissue sections with confirmed pathology were linearly correlated with immunohistochemistry stain intensity with $r = 0.84$ and $r = 0.64$, respectively. No adverse events were observed.

Conclusions:
ABY-029 administered at microdose demonstrated contrast values in soft-tissue sarcoma that are encouraging for translation to clinical practice. Contrast ratios found in this pilot study are similar to those observed with

fluorescent antibodies, but with significantly reduced dose and infusion-to-excision time, and no agent-related adverse events.

FIGURES

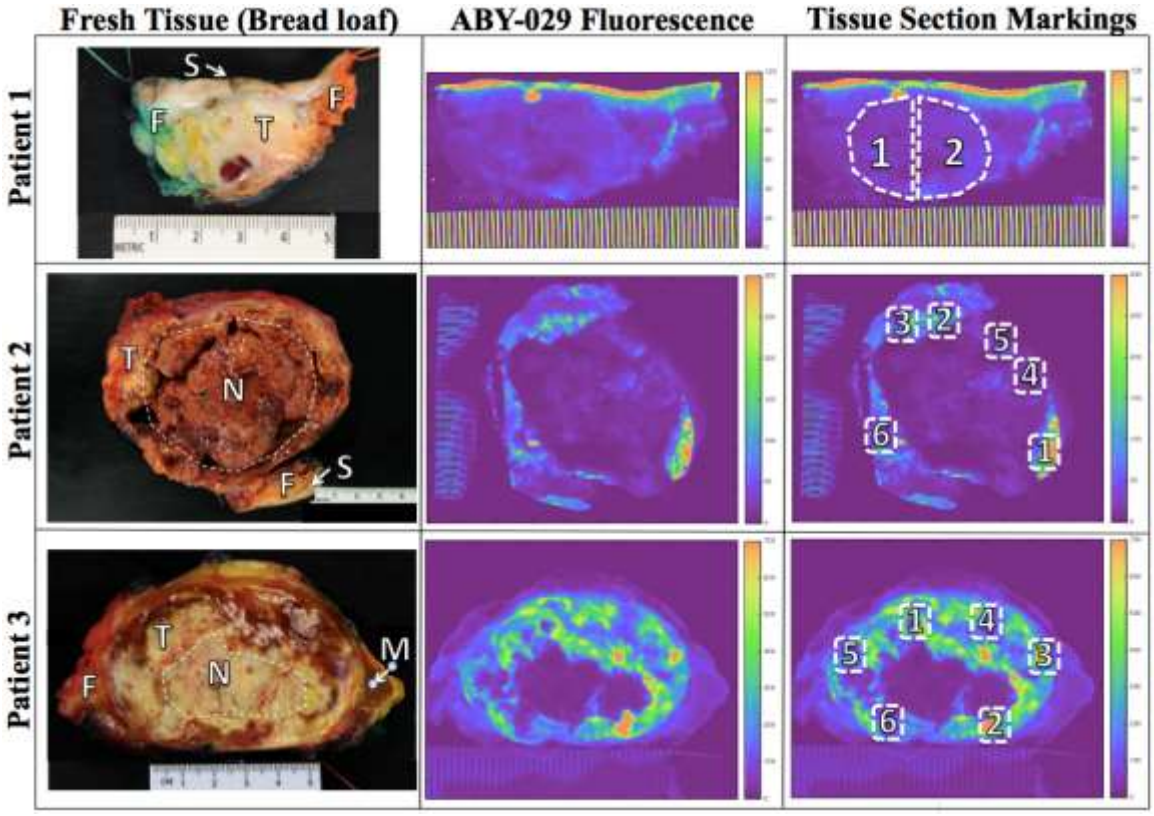

Figure 1. Whole breadloafed sarcoma sections with corresponding ABY-029 fluorescence. The white ruler in the fresh tissue images corresponds to 5 cm. Note the fluorescence scale is not the same in each image, in order to visualize regions of interest, as Patient 3 required a significantly higher scale. The tissue sections that were removed from the breadloafed slice for high resolution scanning and histopathological analysis are shown in the final column. T = tumor, N = area of necrosis (outlined with dashed line), F = fat, M = muscle, and S = skin.

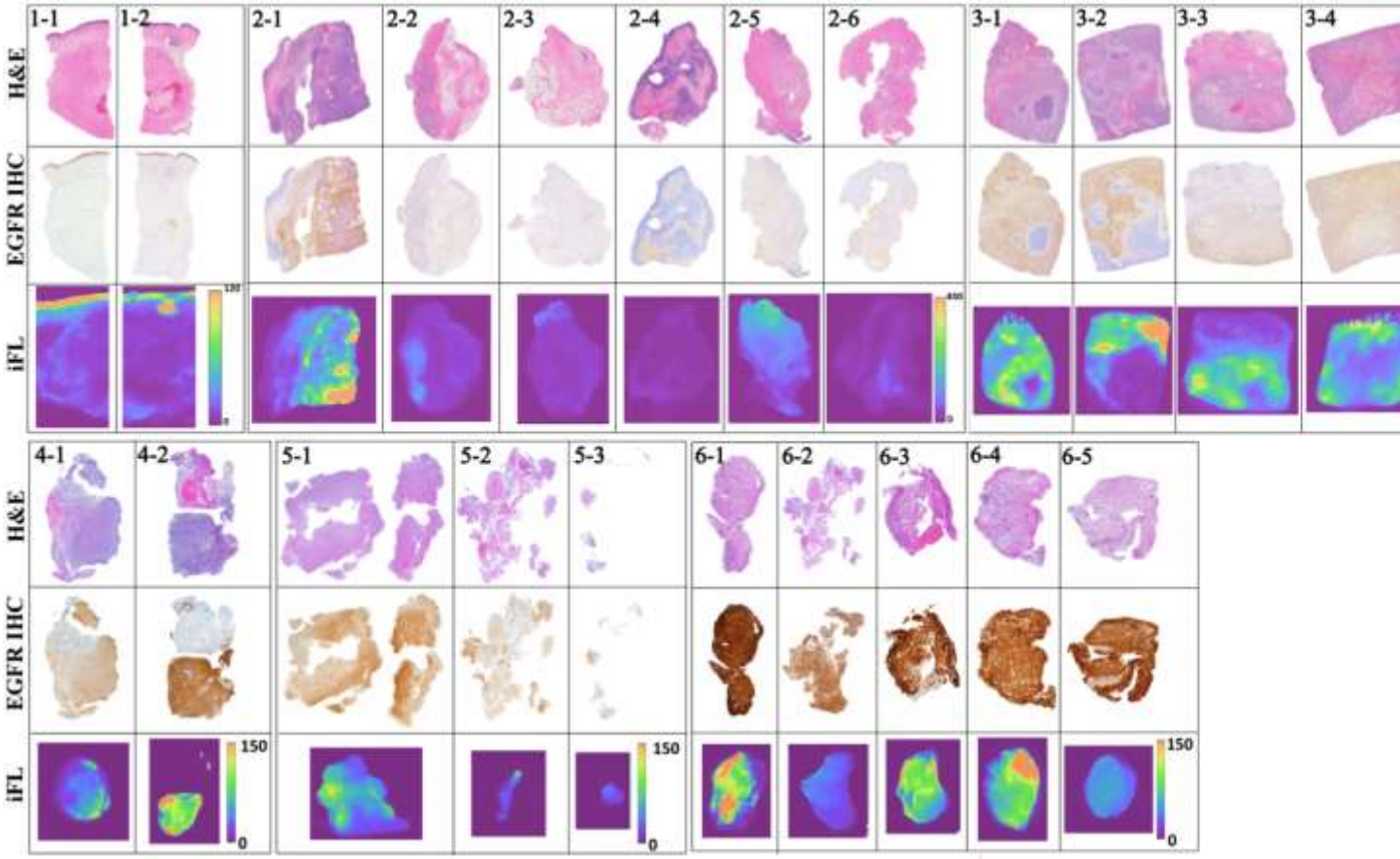

Figure 2. Patient H&E, EGFR immunohistochemistry (IHC) and ABY-029 calibrated fluorescence (iFL) for each collected tissue sample, where the numbers indicate patient-tissue numbers (Tables 1 and 2). The sarcoma patient sections (1-1 to 3-6, correspond to the numbers identified in Figure 1)