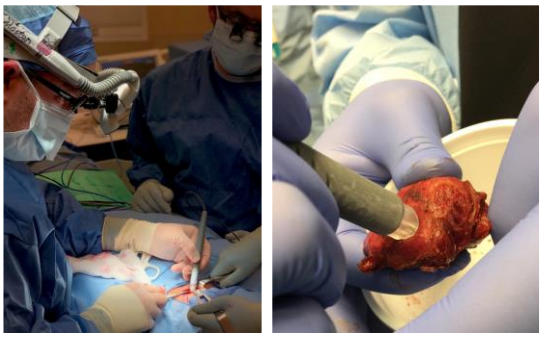
Title: Clinical translation of the MasSpec Pen technology for surgical margin evaluation of pediatric sarcomas

PI: Livia Schiavinato Eberlin, Ph.D.

Summary: For children battling sarcoma, the best and most effective treatment is still full surgical removal of the tumor. However, this can be difficult because sarcoma tumors closely resemble critical surrounding tissue. Dr. Eberlin proposes using the MasSpec Pen to better delineate between tumor and tissue. The MasSpec Pen will be used by the surgeon during the operation to quickly molecularly analyze tissue to ensure safe margins are achieved and the full tumor is removed.

Pediatric sarcomas are a heterogeneous group of tumors accounting for about 10% of childhood solid tumors, which are divided into soft tissue sarcomas and bone tumors. Soft tissue sarcomas are further delineated into rhabdomyosarcoma and nonrhabdomyosarcoma, while the most common types of bone sarcomas are osteosarcomas and Ewing's sarcoma.

For all pediatric sarcoma tumors, surgical resection remains the main treatment option as it provides patients with the highest chances of a cure (1-3). In pediatric sarcoma surgeries, complete tumor resection is of utmost importance as complete resection is strongly associated with improved prognosis and increased likelihood of a cure for patients. Yet, in practice, achieving clear resection margins is difficult because sarcomas often closely resemble healthy adjacent tissues and may also be adjacent to critical soft tissue or bone structures serving various vital functions. Failure to discern the margins between sarcoma and healthy tissues can have devastating consequences for pediatric patients including increased risk of recurrence and the need of more aggressive adjuvant treatment (4). Intraoperative assessment of tumor margins is conventionally performed through histopathologic frozen section analysis (5). Although valuable, frozen sectioning is time- and labor-intensive and the results can be subjective, especially for sarcoma tissues that can be difficult to freeze and/or section for histologic evaluation. Thus, new molecular technologies that allow cancer-specific biomarkers to be incorporated into intraoperative decision-making are highly needed to improve surgical treatment for pediatric sarcoma patients.



Intraoperative MSPen *in vivo* (left) and *ex vivo* (right) in thyroidectomy surgery.

We propose to test the MasSpec Pen (MSPen) technology in its first use in pediatric oncology for identification of sarcomas and intraoperative surgical margin evaluation. The MSPen is an innovative handheld device integrated to a high-performance mass spectrometer for rapid and non-destructive tissue diagnosis (6). The MSPen uses a single droplet of water to gently extract a wealth of diagnostic molecules from tissues upon contact, which are then directly analyzed by mass spectrometry (MS) and statistical classifiers to provide a diagnosis in seconds. The MSPen capitalizes on MS's unparalleled sensitivity and specificity for molecular analysis and cancer diagnosis, and on its biocompatibility, non-destructiveness, and ease-of-use for intraoperative implementation. In previous studies focused on adult cancers, we reported that the MSPen allows accurate diagnosis of cancer tissues and shown its clinical use for *ex vivo* and *in vivo* tissue analysis in breast, thyroid, and pancreatic cancer surgeries (6-8). Now, we will explore the usefulness of the MSPen technology in identifying pediatric sarcoma tissues and guiding surgical decision-making. Our expectation is that with further development and validation, the MSPen could be routinely used in pediatric oncology to increase precision in sarcoma resections and improve outcomes and survival for patients.

Specific Aims: *Our hypothesis is that the molecular information provided by the MSPen allows accurate and real-time identification of pediatric sarcomas and can be used to improve surgical margin evaluation in pediatric sarcoma surgeries*. In collaboration with Dr. Sanjeev Vasudevan (Department of Surgery, Division of Pediatric Surgery, BCM), and Dr. Nino Rainusso (Department of Pediatrics-Oncology, BCM), whose clinical practices are focused on pediatric surgical oncology and pediatric sarcomas, respectively, we propose to conduct a translational study to test this hypothesis and demonstrate the utility of the MSPen for identification of pediatric sarcomas and surgical guidance. Our team has the expertise and complementary strengths to carry out the research proposed. *Our specific aims are:*

Aim 1. Identify molecular signatures diagnostic of soft and osseous pediatric sarcomas: Pediatric sarcomas are a heterogeneous group of tumors that presents high molecular and histologic diversity. We will use the MSPen to identify diagnostic molecular profiles of the most common types of soft tissue sarcomas (rhabdomyosarcoma and nonrhabdomyosarcoma) and bone sarcomas (osteosarcomas and Ewing's sarcoma) using patient-derived tumor xenografts (PDXs), developed by Dr. Rainusso (9), and tissues from BCM's tissue bank. We have already obtained tissues from the BCM tissue bank and started to characterize molecular signatures in our laboratory. Using machine learning, we will develop and validate predictive models for sarcoma diagnosis. Sensitivity, specificity, and accuracy for test and validation sample sets will be defined to evaluate method performance.

Aim 2. Test the MSPen for intraoperative surgical margin evaluation of pediatric sarcomas: The demonstration that the MSPen allows rapid intraoperative detection of molecular predictors of sarcomas directly from tissues has profound clinical implications and potential to significantly improve surgical management and outcomes for pediatric patients with sarcomas. We will evaluate the usefulness of MSPen as a clinical tool for surgical guidance in pediatric sarcoma surgeries performed at Texas Children's Hospital. This critical validation step will be carried out during surgeries using sarcoma tissues freshly excised from patients.

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