STATISTICAL ANALYSIS PLAN

A Feasibility Study for the use of Multispectral Optoacoustic Tomography in the Detection of Solid Tumors and Lymph Nodes (MSOT)

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Abbreviations

CI	Confidence interval
FDA	Food and Drug Administration
IDE	Investigational Device Exemption
MSOT	Multispectral optoacoustic tomography
SAP	Statistical analysis plan

Contents

1. **Objectives**
    a. **Primary Objectives**
        i. To collect safety data on patients in whom MSOT was used to image tumor or lymph nodes
        ii. To evaluate skin temperature pre and post imaging (pre- and post-surgery) as part of the safety evaluation of MSOT
    b. **Secondary Objectives**
        i. To detect tumor or lymph node by MSOT before and after surgical removal
        ii. To determine tumor positivity based on tissue pathology and detection of oxy- and deoxy-hemoglobin using MSOT localization
        iii. To determine tumor volume by MSOT before and after surgical resection

2. **Study Endpoints**
    a. **Primary Endpoint**
        i. Adverse events as characterized by CTCAE v5.0 in patients that may result from MSOT imaging (≥44^{o}C)
        ii. Measurement of skin temperature pre- and post-MSOT imaging (pre- and post-surgery 4 measurements total) with a touch thermometer as part of the safety evaluation of the MSOT device. The thermometer will be placed onto the skin until a temperature appears, about 1 minute, and the temperature will be recorded.

    b. **Secondary Endpoints**
        i. Tumor positivity as based on standard clinical laboratory techniques and signal detection of oxy- and deoxy-hemoglobin using MSOT localization
        ii. Identification of tumor and/or lymph nodes based upon either Isosulfan Blue or IC Green dye

3. **Design information**
    a. **General statistical considerations**

    This IDE FDA approved study will involve the interaction with patients that are scheduled for routine standard of care surgery. It will be a single-arm study that is designed to provide safety information regarding the use of the Acuity MSOT device in the clinical setting and the ability of MSOT imaging data to correlate with clinical findings identified via pathology.

    b. **Sample Size and Power**

    Study will initially accrue 10 patients for safety analysis regarding the use of the MSOT device pre- and post-surgery. If there are no grade III-V AEs observed in the patients as defined in CTCAE v5.0 per Section 8.2, we will submit an IDE supplement to request an enrollment expansion for an additional 40 patients, for a total of 50 patients. Up to an additional 55 patients will be accrued (to account for patient dropouts) to enroll 40 patients.

4. Study Populations

Inclusion and exclusion criteria are sometimes modified during the duration of the protocol. Before beginning analysis, the statistical team will review the criteria in the SAP to confirm it is up to date with the current criteria.

a. Inclusion Criteria

i. Patients with an identified solid tumor, i.e. breast (Stage I-IV), melanoma (Stage I-IV), HNSCC (Stage I-III), pancreatic (Stage I-III), ovarian (Stage I-IV) that is scheduled for surgical removal of the tumor and completed standard imaging prior to surgery
ii. Have acceptable hematologic status [total hemoglobin (tHb) ≥ 10 mg/dL]
iii. Patients ≥ 18 yrs of age
iv. Patient provided a signed and dated informed consent
v. Willing to comply with study procedures and be available for the duration of the study
vi. Ability to understand and the willingness to sign an IRB-approved informed consent document.

b. Exclusion Criteria

i. Patients with central nervous system tumors
ii. Patients with a tattoo over the surgical site
iii. Pregnant women
iv. Women who are breastfeeding
v. Systemic or local infection
vi. Any systemic anomaly during the pre-op assessment preventing patient participation in the study
vii. Any febrile illness that precludes or delays participation preoperatively
viii. Anything that would put the participant at increased risk or preclude compliance with the study
ix. Patients with Stage IV pancreatic cancer, Stage IV HNSCC are not surgical candidates and therefore excluded from this study

5. Analysis Populations

• **Safety population:** All subjects who received MSOT readings will be included in the overall safety analysis, with toxicities graded according to NCI CTCAE v5.0. This is primarily a safety analysis study.

6. Summary of Study Population

a. Patient Disposition

i. Descriptive statistics on the demographics and presenting features will be calculated. Proportions will be calculated for categorical variables and medians with the minimum and maximum will be calculated for continuous variables.

7. Safety Analyses

a. Adverse Events

i. The severity of the AE will be graded by the Investigator using the NCI CTCAE, version 5.0.
ii. The adverse events will be estimated by grade, type and relatedness. The first analysis will pool all grade 3 unexpected events that are possibly, probably or

definitely related to MSOT together and then construct a 95% exact Clopper-Pearson binomial confidence interval for this estimate.

b. Temperature Analysis

i. Measurement of skin temperature will be evaluated where skin temperature greater than 44^{o}C will be recorded and a 95% exact Clopper-Pearson binomial confidence interval constructed.

c. Secondary Analysis

i. Tumor positivity from pathology reports and MSOT readings will be compared and Pearson correlation coefficient created.

ii. Deoxy- and oxy-genation levels will be compared to non-tumor values from the same patient. Paired t-tests will be used to compare the values and a Wilcoxon Signed Rank test performed. Median and interquartile range will be reported.

8. Handling of Missing Data

Every effort will be made to collect information at all defined visits including at early withdrawal or dropout. Reasons for missing data will be summarized. However, there will be no imputation of missing data.