Defining best practices for tissue procurement in immuno-oncology clinical trials: Consensus statement from the Society for Immunotherapy of Cancer Surgery Committee

Supplemental Information

Cutaneous Biorepository Figures and Legends

Figure 1a
This is the skin and subcutaneous tissue specimen from a patient who underwent wide local excision of a Merkel Cell Carcinoma. The red line encircles the tumor. The blue dye is for the sentinel node mapping, and the black stitches are marking lateral and superior margins.
Figure 1b

Here we are cutting into the tumor. One section is taken to confirm microscopic presence of actual tumor instead of scar/fibrosis.
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Figures 2a and 2b

A portion of tumor tissue (Figure 2a) and a portion of normal adjacent tissue (NAT, Figure 2b) have been removed for processing.

Figure 3

This shows the schematic for recording how tissue is accessioned and saved. For this particular patient, tumor and NAT are banked for our institutional repository and tumor and NAT are being sent to ORIEN which is a national consortium.
Figures 4a and 4b

The specimens have been placed in individual containers with the appropriate labelling (Figure 4a) and then are placed in liquid nitrogen for storage (Figure 4b)

The authors would like to acknowledge Dr. Parisa Javidian from the Department of Anatomic Pathology at Robert Wood Johnson University Hospital for these figures.

**Excisional Biopsy of Vaccine-site Draining Lymph Node (Sentinel Immunized Lymph Node)**

*Sample protocol text from the Mel63 protocol (NCT02425306) University of Virginia Cancer Center and Human Immune Therapy Center, written primarily by Kimberly A. Chianese-Bullock, PhD, and Craig L Slingluff Jr. MD.*
Procedure

The node (sentinel immunized node, SIN) will be identified by radiocolloid (usually technetium 99 sulfur colloid) injection, with or without lymphoscintigraphy imaging, and with use of a handheld gamma probe during the procedure. This will be performed under local anesthesia in the clinic, in conjunction with the vaccine site biopsy, by a qualified surgeon.

Lymphatic mapping will be initiated, usually in the nuclear medicine suite, after intradermal injection with radiocolloid (typically technetium 99-sulfur colloid). The node excision will be performed under local anesthesia (usually lidocaine HCl 1-2%, with or without epinephrine 1:100,000 injection, with or without 0.84% sodium bicarbonate), in the outpatient clinic or comparable procedure room, using sterile technique. A handheld gamma probe will be used.

When possible, the node will be sectioned into 5 sections: a central section (10-20% of the node), leaving two adjacent sections of about 40% each. These latter two sections will be bisected. They will be allocated into various preservation conditions:

a) 1 central section will be fixed in formalin, then paraffin-embedded (for histology/immunohistology)
b) 1 section will be placed in RNA-later. (for RNA/RT PCR)
c) 1 section will be quick-frozen (for immunohistology/protein studies)
d) 2 sections (40%) will be processed for single cell suspension by mechanical disaggregation, then (if necessary) enzymatic digestion (collagenase, hyaluronidase, DNAase). The resulting suspensions will be cryopreserved in 90% fetal bovine serum and 10% DMSO (for cellular immune function and flow cytometry).

If there is excess tissue, it may be processed for additional immunologic or angiogenic studies.

The incisions will be sutured closed.

Toxicities related to the biopsies will be recorded.

Tumor Biopsies (applicable to biopsies of palpable lymph node metastases or other palpable tumor masses)

Sample protocol text from the Mel64 protocol (NCT02515227) University of Virginia Cancer Center and Human Immune Therapy Center, written primarily by Kimberly A. Chianese-Bullock, PhD, and Craig L Slingluff Jr. MD.

Tumor biopsies will be completed in subjects who have adequate and accessible metastatic tumor in addition to at least one site of measurable disease. Biopsy sites may be in nodes, skin, soft tissue that can be accessed by needle biopsy, incisional or excisional biopsy. Biopsies may be completed with or without image guidance.

Size Requirements

A critical component of this protocol is the histologic and cytologic evaluation of changes in immune effectors and the tumor microenvironment. A minimum of 0.16 cm$^3$ but ideally 0.3 cm$^3$ or more of tumor tissue will be needed for each biopsy time point as described in the inclusion criteria. Biopsies
may be taken from a single lesion or multiple lesions at each of the time points depending on the size of each lesion. If taken from multiple lesions, those lesions should be similar. For example, three non-ulcerated skin metastases would be considered similar; one bleeding small bowel metastasis would not be considered similar to a subcutaneous nodule).

**Sampling**

The biopsies will vary based on the clinical scenario and may include six core biopsies, an incisional biopsy or an excisional biopsy as outlined in the inclusion criteria.

**Procedure**

When appropriate (and we anticipate the majority of cases) the biopsies will be performed under local anesthesia (typically lidocaine HCl 1% and epinephrine 1:100,000 injection + or − 0.84% sodium bicarbonate), in the outpatient clinic or comparable procedure room, using sterile technique. In cases when clinical standard of care requires a larger procedure the biopsies may be performed in the operating room under standard technique.

To minimize errors in analysis due to sampling error and specimen heterogeneity, each study biopsy specimen will be divided into several components and randomly allocated into various preservation conditions. Ideally, tissue will be divided into the following preservation conditions, using core needle biopsies (19 mm long and 2 mm diameter; about 80 mm³), or incisional or excisional biopsies with at least the same minimum tissue volume:

It is most critical to obtain the following:

**Formalin-fixed paraffin-embedded (FFPE):** 1 core biopsy or similar tissue volume (about 80 mm³ or greater) will be fixed in formalin, then paraffin-embedded (for histology/immunohistology)

**Quick-frozen (QF):** 2 core biopsies or similar tissue volume (each about 80 mm³ or greater) quick-frozen processed for protein studies, histology, or nucleic acid studies. If only one core can be obtained, this portion should be provided as two specimens (eg: cut the core biopsy specimen in half).

When sufficient tissue is available, the following should also be obtained:

**RNA-later:** 1 core biopsy or similar tissue volume (about 80 mm³ or greater) will be placed in RNA-later (for RNA/RT PCR)

**Viable cell suspension:** 2 core biopsies or similar tissue volume (total about 160 mm³ or greater) will be processed for single-cell suspension by mechanical disaggregation, then enzymatic digestion (collagenase, hyaluronidase, DNAase). The resulting suspensions will be cryopreserved in FBS serum and DMSO (for cellular immune function and flow cytometry).

If there is additional tissue, it may be processed for additional immunologic studies.
The incisions will be sutured closed. Toxicities related to the biopsies will be recorded.

A 5 micron section of each tumor specimen will be stained by H&E and reviewed to assess the extent and quality of viable tumor. For FFPE specimens, only those with at least 4 mm² viable tumor (on cross-section) will be considered evaluable for histologic and immunohistologic studies. FFPE tissue will be evaluated for immune cell infiltrates. For QF specimens, those with at least 70% tumor will be considered evaluable.

**Evaluations**

Tissue samples may be screened for antigen expression or protein profiles using tests such as Western blots, immunohistochemistry, PCR, flow cytometry or gene chip analysis.

Tumor escape mechanisms may also be evaluated.

Specimens will be used in immunological assays to assess T cell function or antibody response. Assays generally used for this type of testing include, but are not limited to, ELIspot assays, ELISAs, chromium-release assays, proliferation assays, intracellular cytokine staining, and T cell receptor sequencing.

Specimens may be used to study the immunologic aspects of the tumor microenvironment or as targets or controls in laboratory assays.

Specimens may be used to establish cell lines for long-term studies.