1.0 PURPOSE

1.1 The purpose of this standard operating procedure (SOP) is to establish the process and criteria for the local BSS pathology review of hematoxylin and eosin (H&E)–stained formalin-fixed, paraffin-embedded (FFPE) sections prepared from the QC FFPE block. This is to confirm diagnosis and confirm adherence to the histological quality criteria for the tissue specimens set by the BPV program.

2.0 SCOPE

2.1 This procedure will be utilized for all H&E stained slides undergoing local pathology QC review at the BSS for the BPV program.

2.2 This procedure applies to the local BSS pathologists involved in reviewing the H&E slides prepared from BPV QC FFPE blocks.

3.0 RESPONSIBILITY

3.1 At the BSS, it will be the responsibility of the site principal investigator (PI) to ensure that this procedure is followed.

3.2 Any deviation or change from this SOP, known before a collection/slide review, should be approved by the BSS technical project manager (TPM) and well documented by the site.

3.3 Any deviation or change that is unexpected or identified during or after a collection/slide review should be well documented by the site in the form of a Nonconformance Report. This deviation should be submitted to the BSS TPM as soon as possible for review by the Quality Management Team.

3.4 It is the responsibility of the project staff that is designated by the PI or BSS to ensure that all the required case report forms (CRFs) in the Comprehensive Data Resource (CDR) are completed.

4.0 DEFINITIONS AND ACRONYMS

4.1 Definitions

Case ID: Identifies study subjects.

Biospecimen ID: Identifies each blood and tissue biospecimen from a study subject.

QC FFPE Sample: A representative FFPE tissue specimen prepared for local (BSS) pathology review to confirm accuracy of tissue diagnosis.

Experimental Samples: The samples prepared from the tumor modules, which have been processed according to the unique preservation and storage conditions (Experimental Protocols).

Deidentification: Removal of text elements or identifiers (per Health Insurance Portability and Accountability Act [HIPAA] Privacy rule) that would make data individually identifiable.

4.2 Acronyms

BSS	Biospecimen Source Site
BPV	Biospecimen Pre-Analytical Variables
CBR	Comprehensive Biospecimen Resource
CRF	Case Report Form
CDR	Comprehensive Data Resource
FFPE	Formalin-Fixed, Paraffin-Embedded
H&E	Hematoxylin and Eosin
PRC	Pathology Resource Center
SOP	Standard Operating Procedure
QA	Quality Assurance
QC	Quality Control
TPM	Technical Project Manager

5.0 ENVIRONMENTAL HEALTH AND SAFETY

None

6.0 MATERIALS AND EQUIPMENT

- Conventional light microscope
- Computer

7.0 PROCEDURE

7.1 Data Entry into the required CRFs in the CDR database:

7.1.1 The **PR-0009-F1 through F4_BPV (Primary Organ) Local Pathology Review Form** in the CDR is required to be completed within 21 days from the date of surgery.

7.1.2 The **PR-0009-F9_BPV Case Quality Review Form** is to be completed within 48 hours of completion of the applicable **BPV Local Pathology Review Form**.

7.2 General Process at BSS

7.2.1 Ensure that all identifying numbers used for study subjects (BPV Case IDs) and associated H&E slides (BPV Biospecimen IDs) properly match.

7.2.2 A trained Surgical Pathologist who is familiar with the study requirements will evaluate the H&E-stained section prepared from the collected QC FFPE block to ensure that the tissue is appropriately identified and classified.

7.2.3 H&E-stained slides will be reviewed using a conventional light microscope by the BSS pathologist.

7.2.4 The CDR database application, accessed through a Web portal, will be the database for entry of all clinical and biospecimen data at the BSS, including pathology review data.

7.3 BSS QC Slide Review

7.3.1 A standardized pathology QC review will be carried out by the pathologist at each BSS for all cancer cases to ensure that the collected biospecimens meet contractual acceptance criteria (as outlined in **BPV Program Protocol, PM-9008**). All pathology review data will be entered into an organ-specific CRF: **PR-0009-F1 through F4_BPV (Primary Organ) Local Pathology Review Form** in the CDR database and/or into the appropriate CRF (see below) for the local pathology review:

7.3.1.1 Kidney biospecimens: **PR-0009-F1_BPV Kidney Local Pathology Review Form**

7.3.1.2 Ovarian biospecimens: **PR-0009-F2_BPV Ovary Local Pathology Review Form**

7.3.1.3 Lung biospecimens: **PR-0009-F3_BPV Lung Local Pathology Review Form**

7.3.1.4 Colorectal biospecimens: **PR-0009-F4_BPV Colon Local Pathology Review Form**

7.3.2 For each BPV slide examined from a unique block, scan the Case and Biospecimen ID on the slide into the appropriate field in corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR. Note: Per the established process flow, two sections are to be cut and H&E stained from each QC block. The second slide is to be used as a backup, and a review is to be conducted in the event the first slide is inadequate for interpretation. The Local Pathology Review Form must be completed for the one slide per module collected. Enter the Histologic Type for the slide being examined on the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR. As applicable, comment on the presence or absence of sarcomatoid features.

7.3.3 Enter information regarding the greatest tumor dimension (in millimeters) and percentage of cross-sectional surface area of the entire slide that is composed of tumor focus, noting the percentage of tumor cellularity by cell count on the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR. In addition, an estimation of percentage of cross-sectional surface area of the entire slide that is composed of necrotic tissue should be recorded.

7.3.3.1 The estimation of surface area involvement by tumor should include necrotic tumor (if present).

7.3.3.2 The assessment of tumor cellularity should include only viable tumor cells (epithelial cells only) as compared to all viable cells of the entire slide.

7.3.4 For each BPV slide examined, perform a histologic profile (see Section 10.1) using specifically the area of the slide involved by tumor. These semi-quantitative assessments will be made by estimates ("eyeballing") rather than by precise measurements. This template will be used for all cancer cases.

Histologic Profile of Tumor
Use for all Cancer types

Histologic Profile
(Semi-Quantitative Visual Assessment)
___ % Viable Tumor by Surface Area (not including stroma)
___ % Necrotic Tumor by Surface Area
___ % Tumor Stroma by Surface Area
___ % Non-Cellular Component by Surface Area (e.g., mucin, hemorrhage, blood clot)

_____
100% Total

7.3.5 If a non-cellular component is present, describe its composition in the area provided on the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR.

7.3.6 Determine and record the Histologic Grade as applicable for the tumor type on the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR.

7.3.7 Copy the tumor staging (per the American Joint Committee on Cancer's TNM Staging System, 7th Edition) from the final surgical pathology report.

Note: Since tumor staging cannot be determined from the slide and additional information is needed to confirm the original tumor staging, this information can be transcribed into the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR.

7.3.8 Note whether the case meets the following contractual acceptance criteria:

7.3.8.1 A review of QC FFPE tumor tissue confirms the histologic type to be eligible.

7.3.8.2 Necrosis is less than 20 percent of the entire tissue section's surface area (semi-quantitative visual assessment), and the tumor content is 50 percent or more tumor nuclei.

7.3.8.2.1 If "No" is selected, specify what findings do not meet the case acceptance criteria in the space provided.

7.3.9 Additional findings may be observed as part of pathology review process: an assessment of secondary tissue changes (e.g., edema, congestion, hemorrhage) as well as the condition of preservation of tissue (e.g., autolysis, staining quality, procedural artifacts). This information and any additional comments should be recorded in the Pathology Review Comments section of the applicable **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR.

7.3.10 If the finding of the local BSS pathologist is in concordance with the diagnostic pathology report, indicate this on the applicable **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR. If the findings are discordant the case should be discussed with the BSS PI for resolution.

Note: It is expected that some variability may be seen between the diagnostic pathology report and local BSS findings given the extent of sampling upon which the diagnostic pathology report is based. This is not invariably to be considered a lack of agreement and should be recorded as such only if there is significant disagreement in the histologic diagnosis.

7.4 Case Release and Shipment of Slides to the BBRB PRC

7.4.1 The BSS pathologist will confirm if the following are completed before releasing the case for shipment to the Comprehensive Biospecimen Resource (CBR) and subsequent review by the Pathology Resource Center (PRC):

7.4.1.1 The deidentified Surgical Pathology report is released by the surgical pathologist and transmitted to the CDR by uploading the document into the CDR.

Note: All HIPAA identifiers must be removed or redacted in advance of uploading the document to the CDR.

7.4.1.2 For each module collected, all pathology review data are entered into the corresponding **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR for both the histologic slide review and the detailed pathology review.

7.4.1.3 All contractual requirements are met.

7.4.1.4 All data entry and the **PR-0009-F9_BPV Case Quality Review Form** are complete in the CDR.

7.4.1.5 The case status has been changed to “BSS QA review complete.”

7.4.2 The BSS pathologist or designee will notify the BSS TPM when a case is ready for release and shipment.

7.4.3 Shipment of the H&E-stained slides to the CBR is governed by a separate standard shipping SOP (**BPV Work Instruction for the Blue Kit, OP-0014-W1**). This SOP defines the optimal shipping logistics for the slides and associated FFPE blocks, which will be delivered to the CBR for storage and for imaging.

7.5 Quality Review by the BBRB PRC

7.5.1 A standardized pathology quality review will be carried out by the PRC for all cases. This review will include both a histologic profile as well as the detailed pathology review that was completed by the BSS pathologist on the applicable **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** in the CDR database.

7.5.2 The BBRB PRC pathologist will review Aperio images of applicable case slides (QC slide(s) and experimental slides), and all collected pathology review data will be recorded on the appropriate **BPV (Primary Organ) Local Pathology Review Form** (**PR-0009-F1 through F4)** within the CDR.

7.6 **Quality Control Plan**

7.6.1 The standard 10 percent pathology quality assurance (QA) review of cases by a second pathologist within the PRC will not be required for the BPV study due to the robust nature of the implemented pathology QC procedure.

7.7 **Disagreement in Diagnosis**

7.7.1 In the event of a significant disagreement in histologic diagnosis (histologic type), meaning disagreement between the local BSS pathology report and the conclusions of the reviewing PRC pathologist, the reviewing PRC pathologist will consult with one or more other PRC pathologists to attempt to resolve the discrepancy.

7.7.2 All discrepancies in diagnosis between the local BSS pathologist and PRC pathologist will be reported to the pathology director of the PRC, the BSS TPM, and the BPV project manager.

7.7.3 In the event of a discrepancy, the PRC and BSS pathologists together will determine the best resolution of the discrepancy and notify the BPV project manager and the BSS TPM of the outcome.

8.0 REFERENCES

8.1 College of American Pathologists Protocol for the Examination of Specimens From Patients With Invasive Carcinoma of Renal Tubular Origin
http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2012/Kidney_12protocol_3101.pdf

8.2 College of American Pathologists Protocol for the Examination of Specimens From Patients With Carcinoma of the Ovary
http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2012/Ovary_12protocol.pdf

8.3 College of American Pathologists Protocol for the Examination of Specimens From Patients With Carcinoma of the Colon and Rectum
http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2012/Colon_12protocol_3200.pdf

8.4 College of American Pathologists Protocol for the Examination of Specimens From Patients With Primary Non-Small Cell Carcinoma, Small Cell Carcinoma, or Carcinoid Tumor of the Lung
http://www.cap.org/apps/docs/committees/cancer/cancer_protocols/2012/Lung_12protocol.pdf

8.5 De-Identifying Protected Health Information Under the Privacy Rule
http://privacyruleandresearch.nih.gov/pr_02.asp

9.0 ATTACHMENTS

9.1 BPV Kidney Local Pathology Review Form, PR-0009-F1
9.2 BPV Ovary Local Pathology Review Form, PR-0009-F2
9.3 BPV Lung Local Pathology Review Form, PR-0009-F3
9.4 BPV Colon Local Pathology Review Form, PR-0009-F4
9.5 BPV Kidney Clinical Data Entry Form, PR-0009-F5
9.6 BPV Lung Clinical Data Entry Form, PR-0009-F6
9.7 BPV Ovary Clinical Data Entry Form, PR-0009-F7
9.8 BPV Colon Clinical Data Entry Form, PR-0009-F8
9.9 BPV Case Quality Review Form, PR-0009-F9

10.0 APPENDIX

10.1 Semi-Quantitative Estimates for Histological Profile

(Excerpt from Microsoft PowerPoint presentation: "Histologic Quality Control Assessment of Tissue Samples Procured for Research Purposes – Procedures used by the Cooperative Human Tissue Network" updated January 2007.)

10.1.1 If a neoplasm (tumor) is present in the tissue section, two different assessments of tumor percentage are made:

10.1.1.1 % cellularity – An estimate of the number of tumor cells in the histologic section relative to the total number of cells

10.1.1.2 % area – An estimate of the area of tissue involved by tumor

10.1.2 The % area and % cellularity may be discrepant if:

10.1.2.1 The tumor is invested by a large number of inflammatory cells

10.1.2.2 The tumor has a high degree of fibrosis

10.1.2.3 The tumor has large areas of acellular mucin

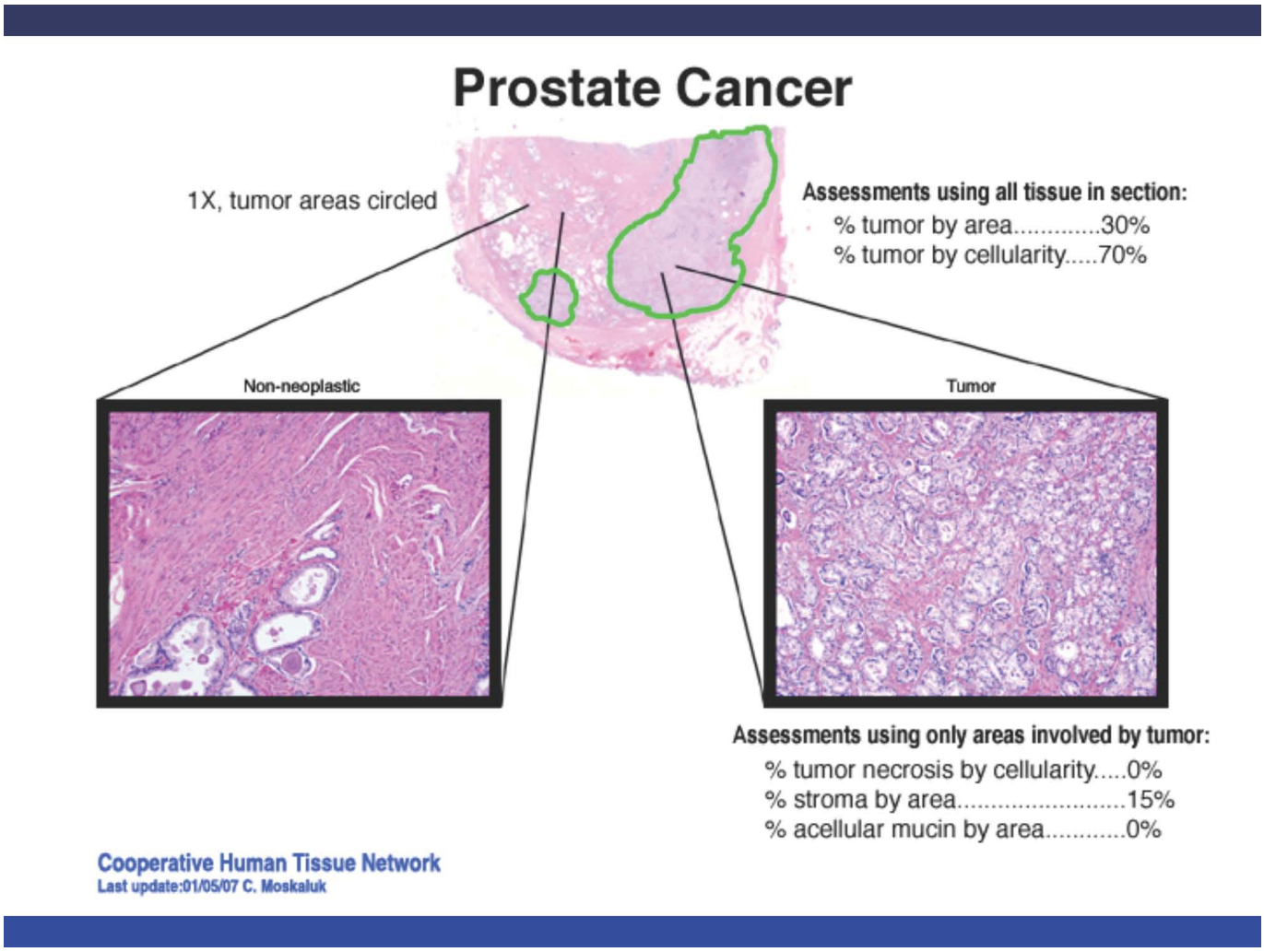
10.1.3 Tumor attributes that are assessed:

10.1.3.1 % tumor necrosis, by cellularity (using only tumor cells as denominator)

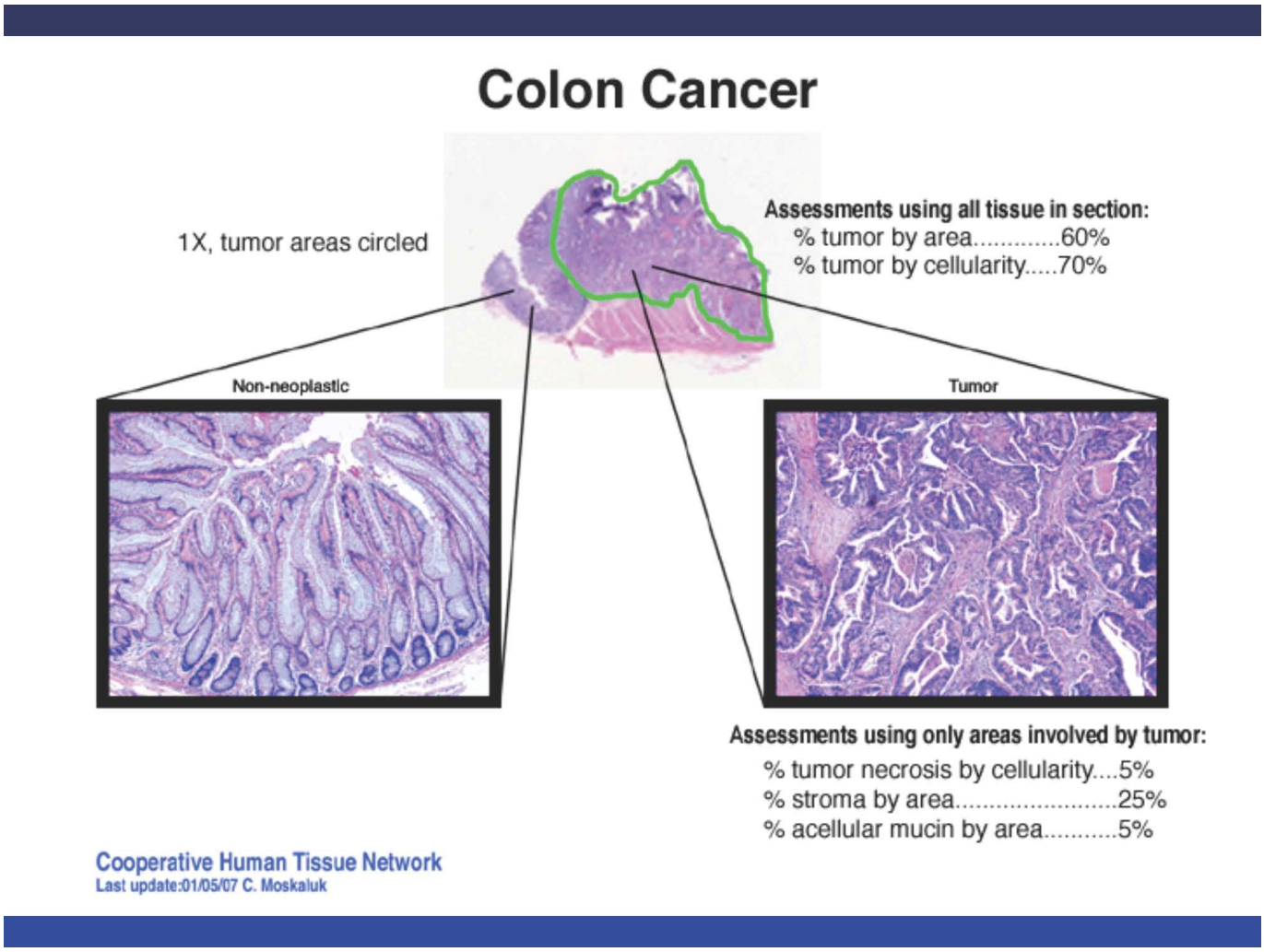
10.1.3.2 % non-neoplastic stroma, by area (using only tumor area as denominator)

10.1.3.3 % acellular mucin, by area (using only tumor area as denominator)

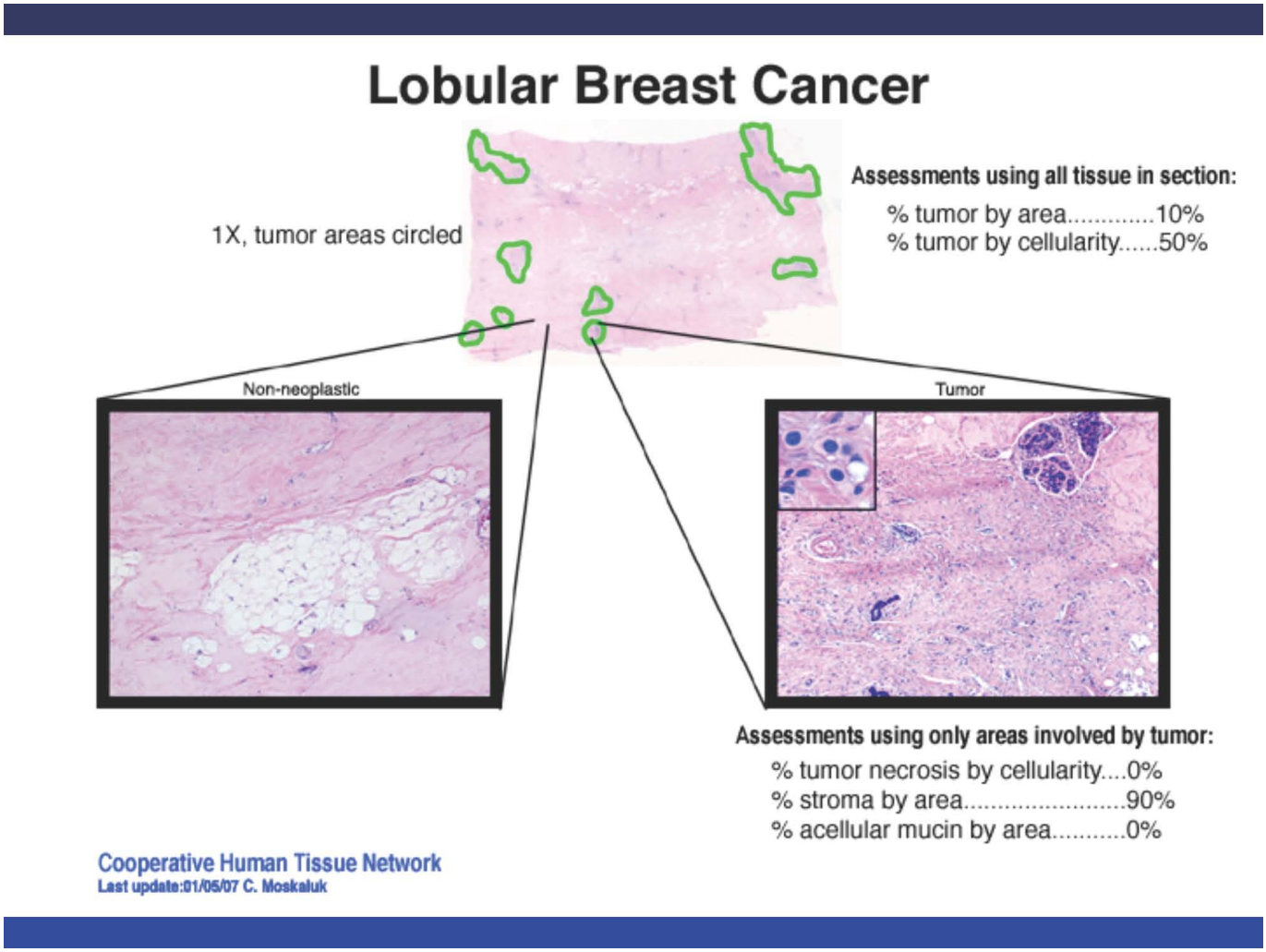
Adenocarcinoma of the Prostate



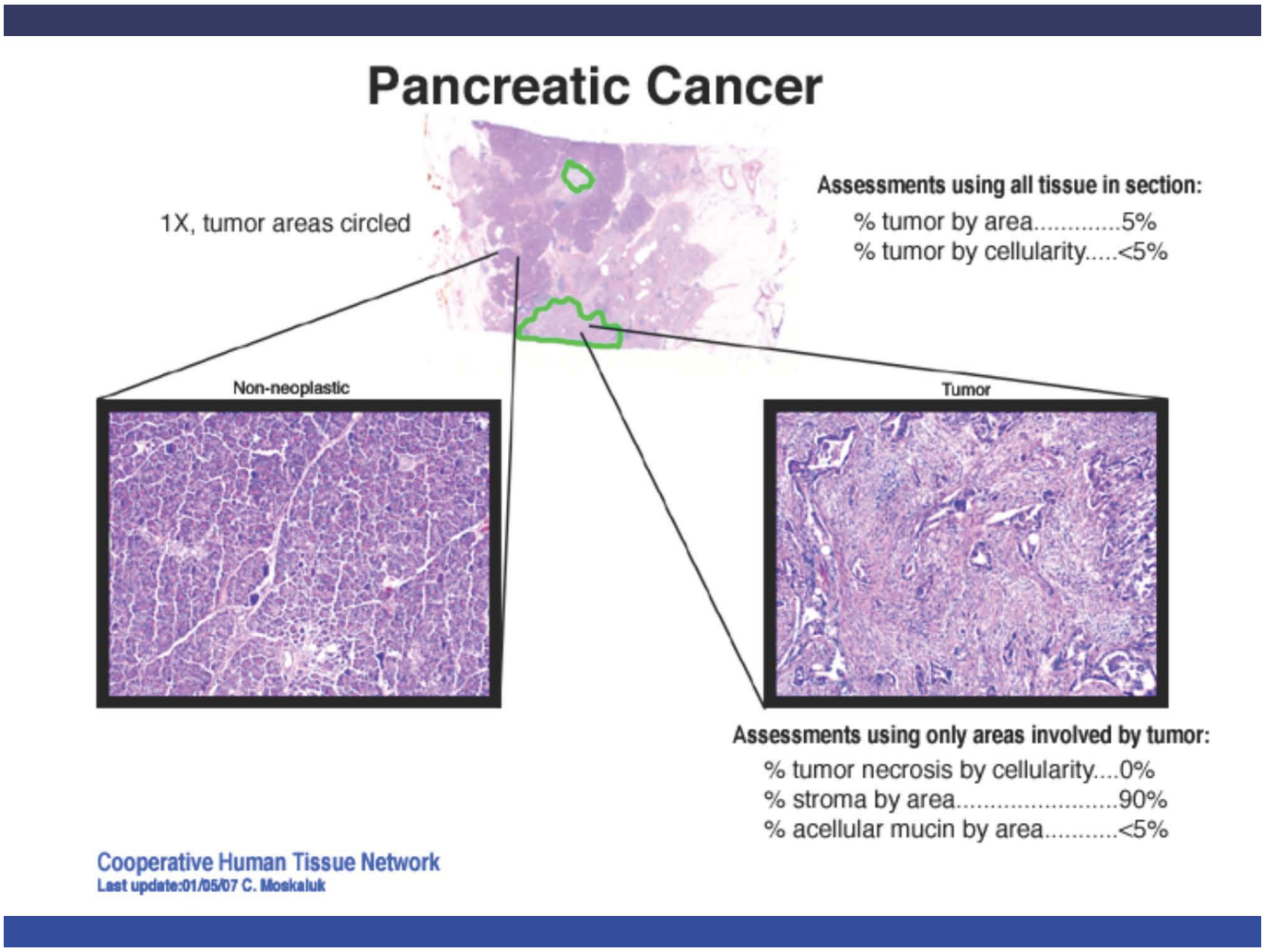
Adenocarcinoma of the Colon



Lobular Breast Carcinoma



Adenocarcinoma of the Pancreas



Mucinous adenocarcinoma of the Colon