LUNG-MAP PATHOLOGY WEBINAR

SESSION 2: March 20th, 2017

David Gandara, MD
S1400 Study Co-Chair/UC Davis Comprehensive Cancer Center

James Suh, MD
Associate Medical Director and Molecular Pathologist/Foundation Medicine

Roy Herbst, MD, PhD
S1400 Study Co-Chair/Yale Cancer Center

Mary Redman, PhD
S1400 Lead Biostatistician/Fred Hutchinson Cancer Research Center

SWOG
Leading cancer research. Together.

NCI National Clinical Trials Network
a National Cancer Institute program

NCI Community Oncology Research Program
A program of the National Cancer Institute of the National Institutes of Health

FNIH
Foundation for the National Institutes of Health

WEBINAR OVERVIEW

Dr. David Gandara

S1400 Study Co-Chair

UC Davis Comprehensive Cancer Center

WEBINAR OVERVIEW

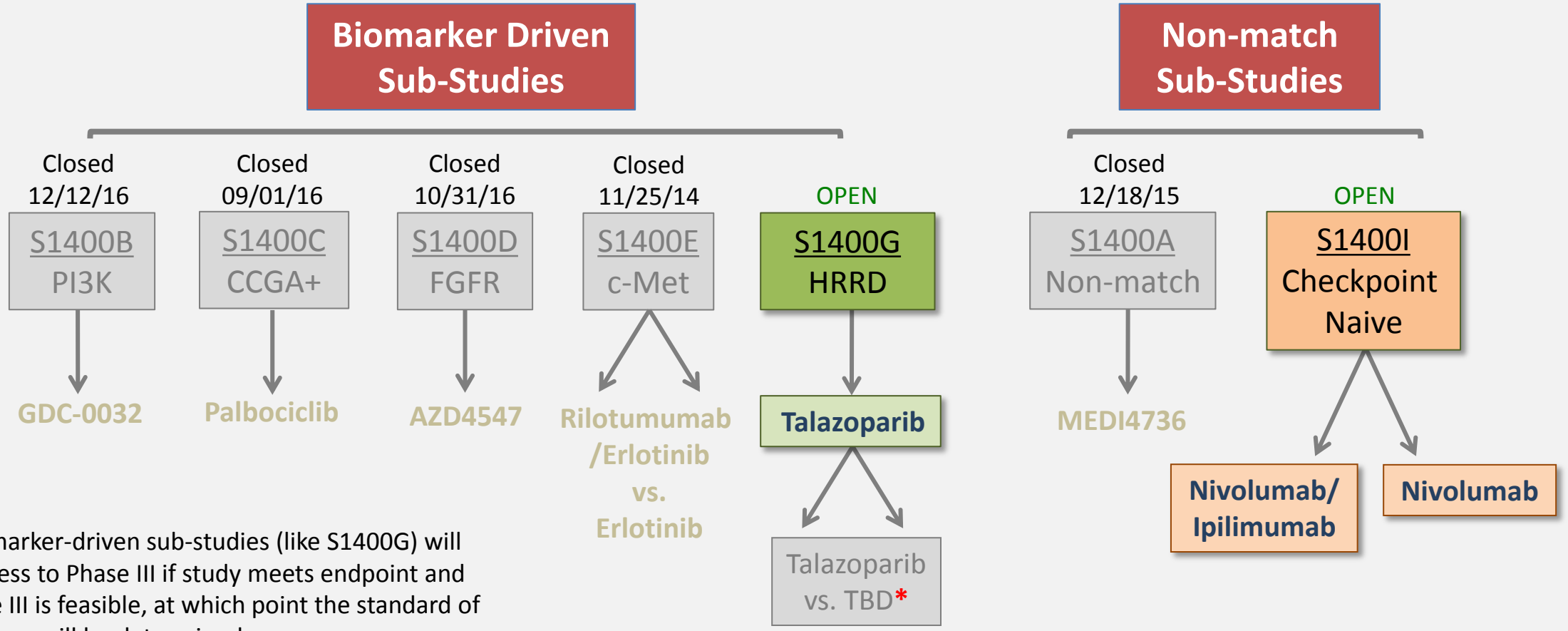
Topics

- Selecting the best specimen
- Preparing specimens
- Specimen Enrichment
- Sample acquisition
- FNAs, core biopsies, cell blocks
- Submission summary
- Inadequacy rates and reasons
- Fresh vs archival tissue
- Biomarker results
- Tips and interactive Q+A

Goals

- Review guidelines for quality sample acquisition and processing
- Encourage discussion between S1400 site Pathologists
- Learn from sites about any challenges you face related to tissue submissions

Current Schema



*Biomarker-driven sub-studies (like S1400G) will progress to Phase III if study meets endpoint and Phase III is feasible, at which point the standard of care arm will be determined.

TISSUE COLLECTION GUIDELINES

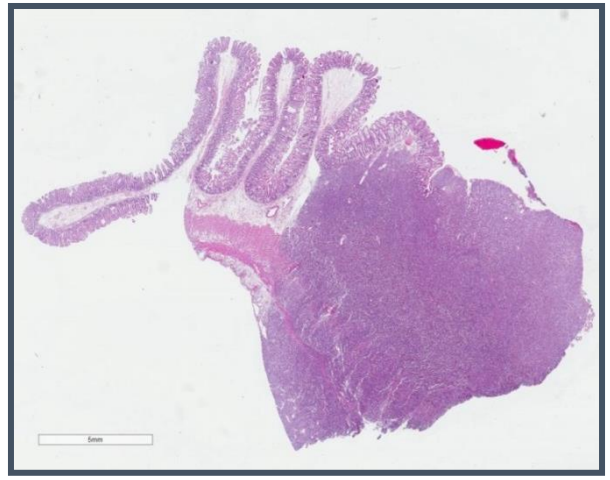
Dr. James Suh

Senior Pathologist and Associate Medical Director

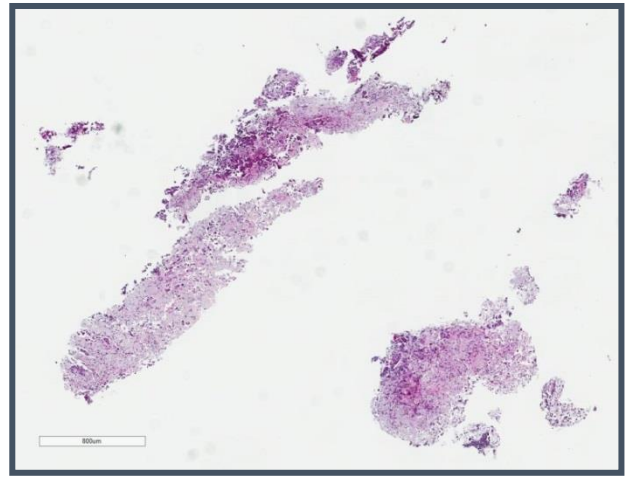
Foundation Medicine

Appropriate Samples

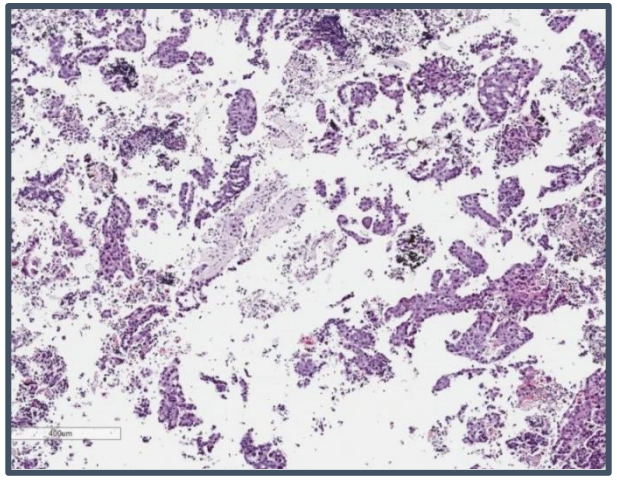
Comprehensive genomic profiling on real world tissues



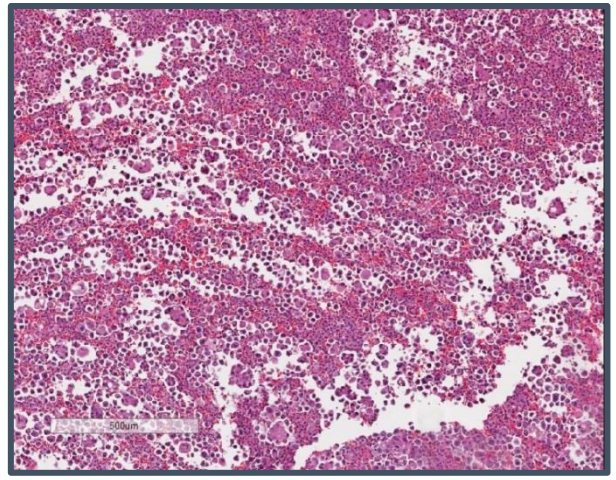
Resection



Small Biopsy



Fine Needle Aspiration (cell block)



Fluid Exfoliative Cytology (cell block)

FOUNDATION MEDICINE®

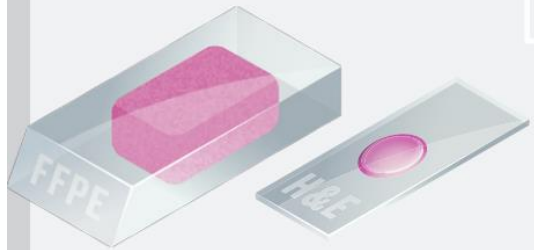
Specimen Guidelines

FOUNDATIONONE®

Optimizing for success

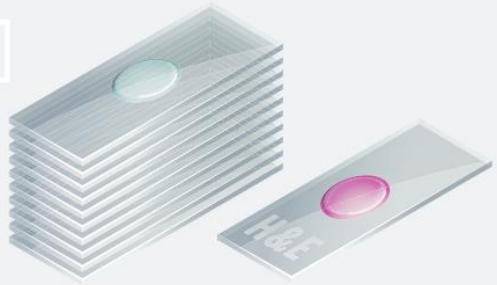
SAMPLE SIZE

When feasible, please send the block + 1 original (not recut) H&E slide.



OR

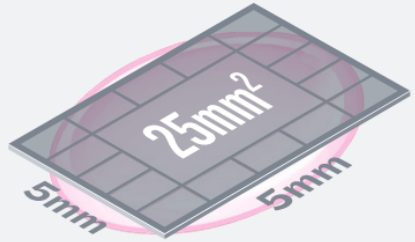
12-20 unstained slides (positively charged and unbaked at 4-5 microns thick) + 1 original (not recut) H&E Slide.



SAMPLE SIZE SURFACE AREA

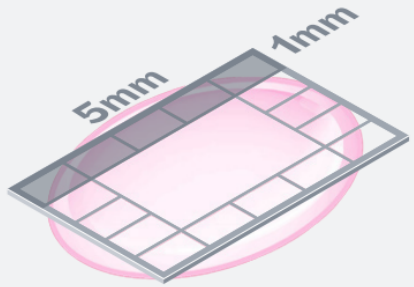
Optimal: 25 mm²

If sending slides, provide **12-20** unstained slides cut at 4-5 microns thick.



Minimal: 5 mm²

For small (<25mm²) or impure samples, additional unstained slides may be needed to extract sufficient DNA for testing.



TUMOR NUCLEI PERCENTAGE

Optimal: 30% Minimal: 20%

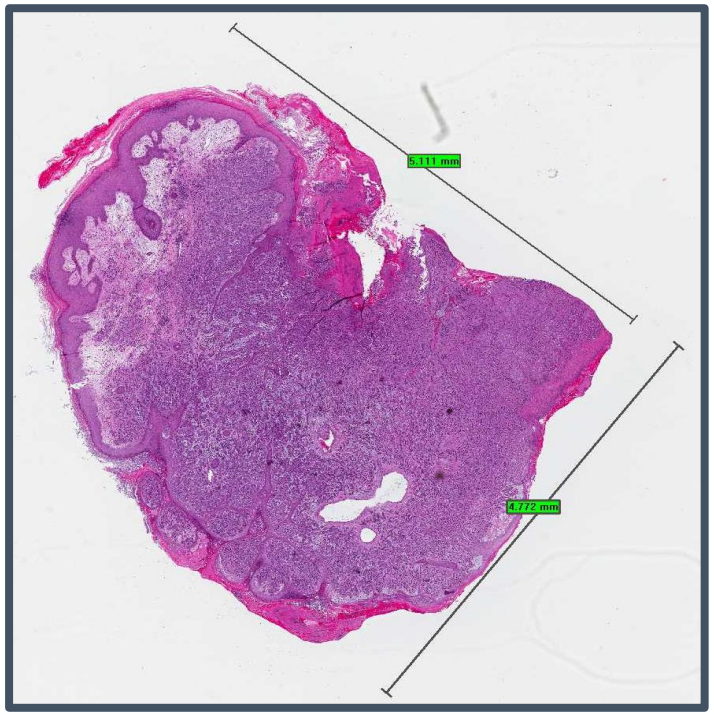
Percent tumor nuclei = number of tumor cells divided by total number of all cells with nuclei (Liver specimens may require additional tumor)

FOUNDATION MEDICINE®

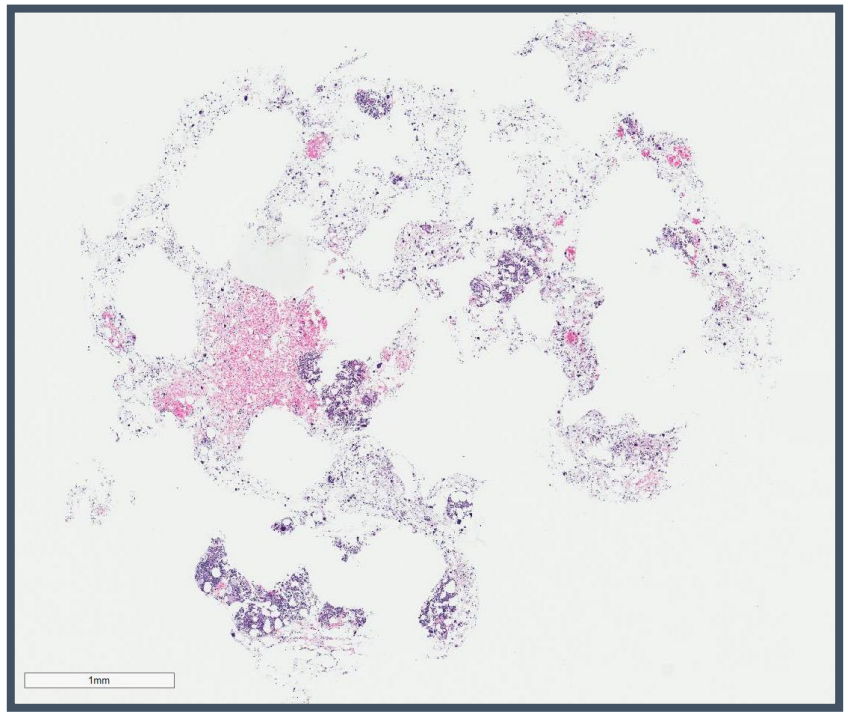
Specimen Guidelines

Optimizing for success

Optimal



Suboptimal: <12 USS slides received

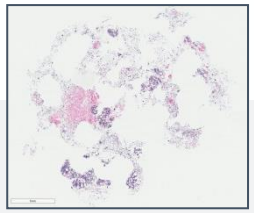


Pathologist initiated intervention:
Request FFPE block to increase tissue volume
Result:
Adequate DNA extracted → successful report

FOUNDATION MEDICINE®

Specimen Guidelines

Percent tumor requirements



TUMOR NUCLEI PERCENTAGE

Optimal: 30% Minimal: 20%

Percent tumor nuclei = number of tumor cells divided by total number of all cells with nuclei (Liver specimens may require additional tumor)

≥40% Recommended for liver specimens
Normal liver nuclei are 4n, so hepatocytes count double when calculating tumor content

≥30% Optimal
(for non-liver specimens)

≥20% Acceptable
Percentage can be of an area compatible with macrodissection (residual area must meet previous size criteria)

<20% Unacceptable for Lung-MAP

Computational models sort tumor sequence signal from normal

The greater the tumor content the higher the signal to noise ratio

% Tumor is most important for detecting copy number changes & subclonal events

Low tumor purity makes it difficult to isolate low level copy number changes (amplifications of 5-6 or homozygous loss)

FOUNDATION MEDICINE®

Specimen Enrichment

Pathologist directed sample processing

Boost tumor purity:

- to reach the 20% tumor purity threshold
- sometimes macroenrichment can eliminate normal tissue to reach threshold in otherwise suboptimal specimen

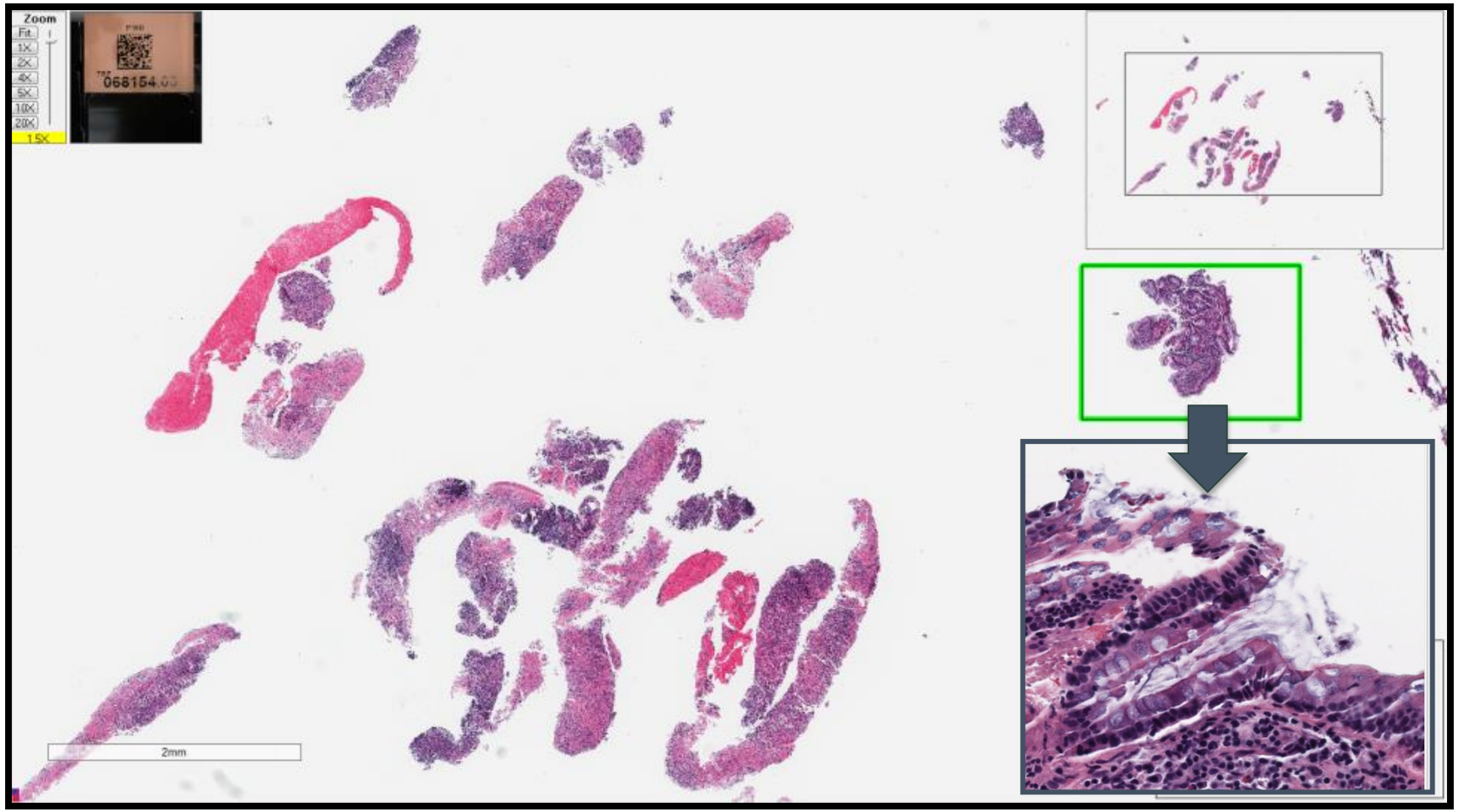
Eliminate contamination from original routine AP processing:

- small contaminating tissue fragments from other individuals are not uncommon in routine specimens
  "floaters"
- contaminating tissues contributes to noise in the sequencing data;
  guidance to practice good grossing and tissue processing techniques to prevent cross-contamination
- when floaters are recognized, macroenrichment can eliminate from material submitted for extraction

FOUNDATION MEDICINE®

Specimen Enrichment

Pathologist directed sample processing



Optimizing sample acquisition

Small biopsies and cytology specimens

Collect additional tumor upfront:

- Perform multiple passes for all needle biopsies
- Create cell blocks for all cytology specimens

Apply tissue preservation protocols:

- Reduce number of H&E sections/levels and IHC stains
- Cut unstained slides for IHC with H&E or simultaneously with slides for molecular studies*

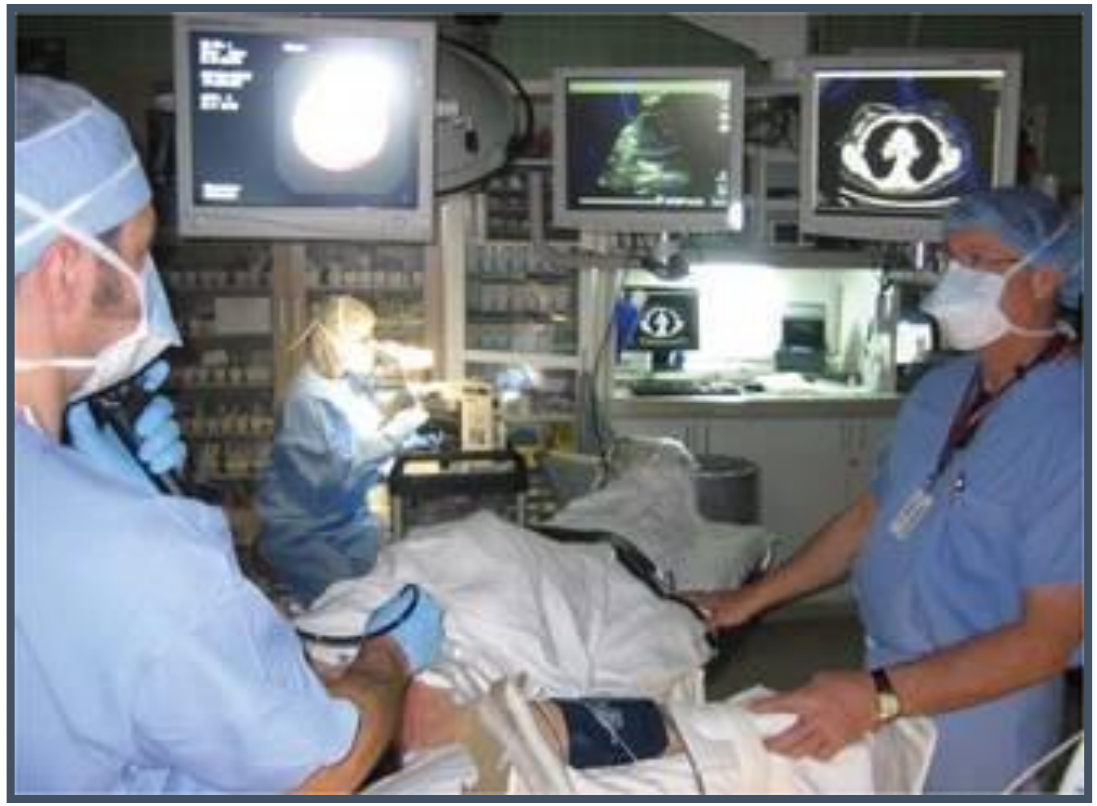
***Do not cut the block more than twice from start to finish**

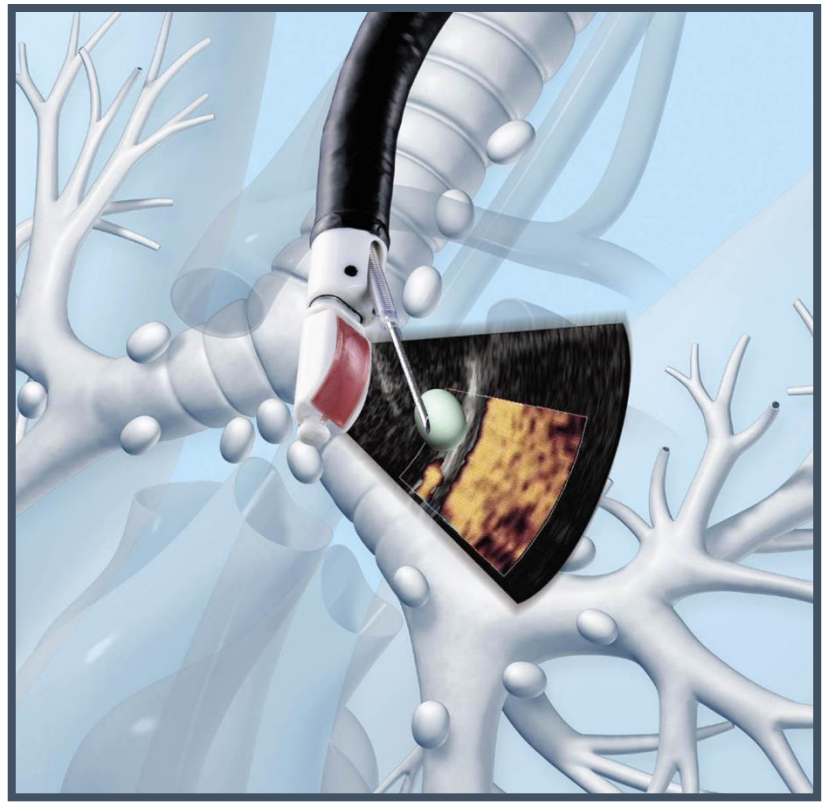
FOUNDATION MEDICINE®

Pulmonologists and fine needle aspiration

Perform multiple needle passes

As soon as target acquisition is confirmed by rapid on-site assessment, all subsequent passes using 20-25 g needles (minimum of 4) should be placed in the cell block container

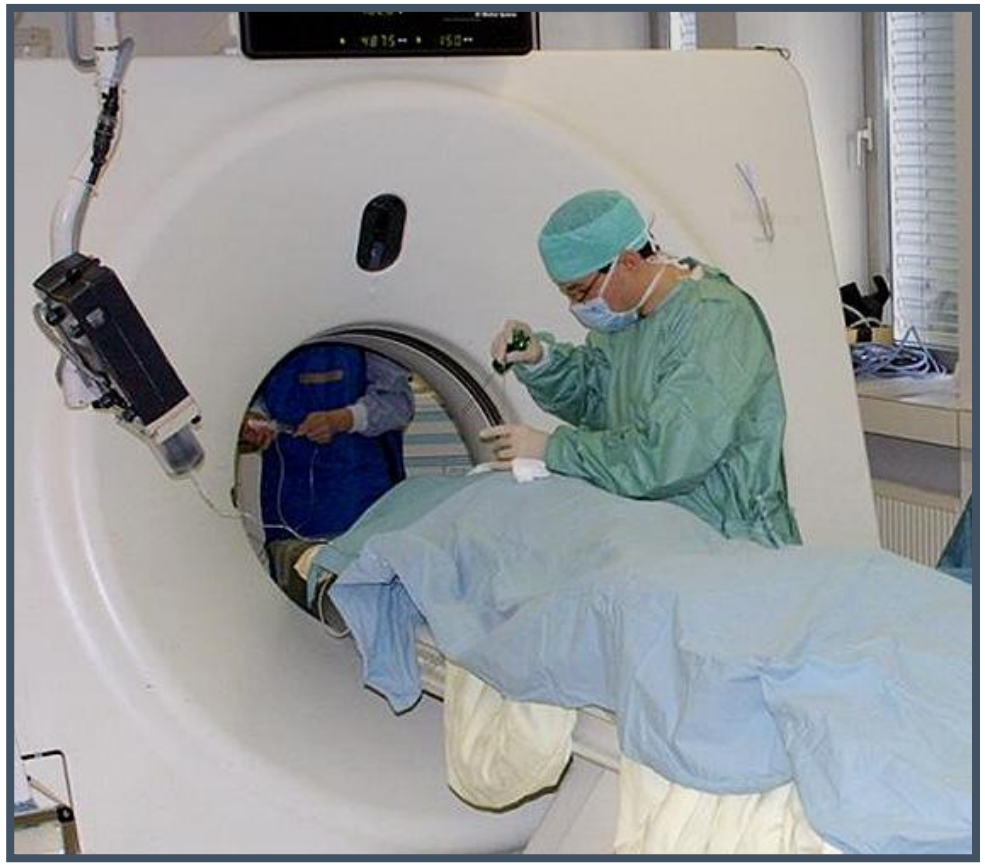


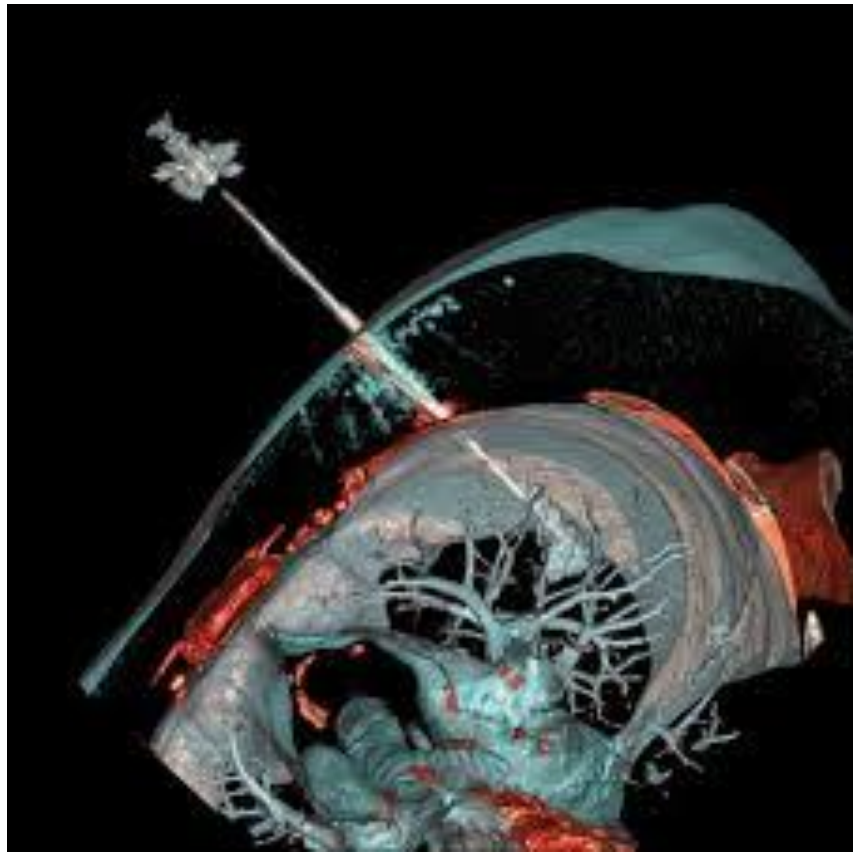


Radiologists and core biopsies

Perform multiple needle passes

Acquire 4 or more core needle biopsies using 18-20 g needles rather than or in addition to fine-needle aspirates

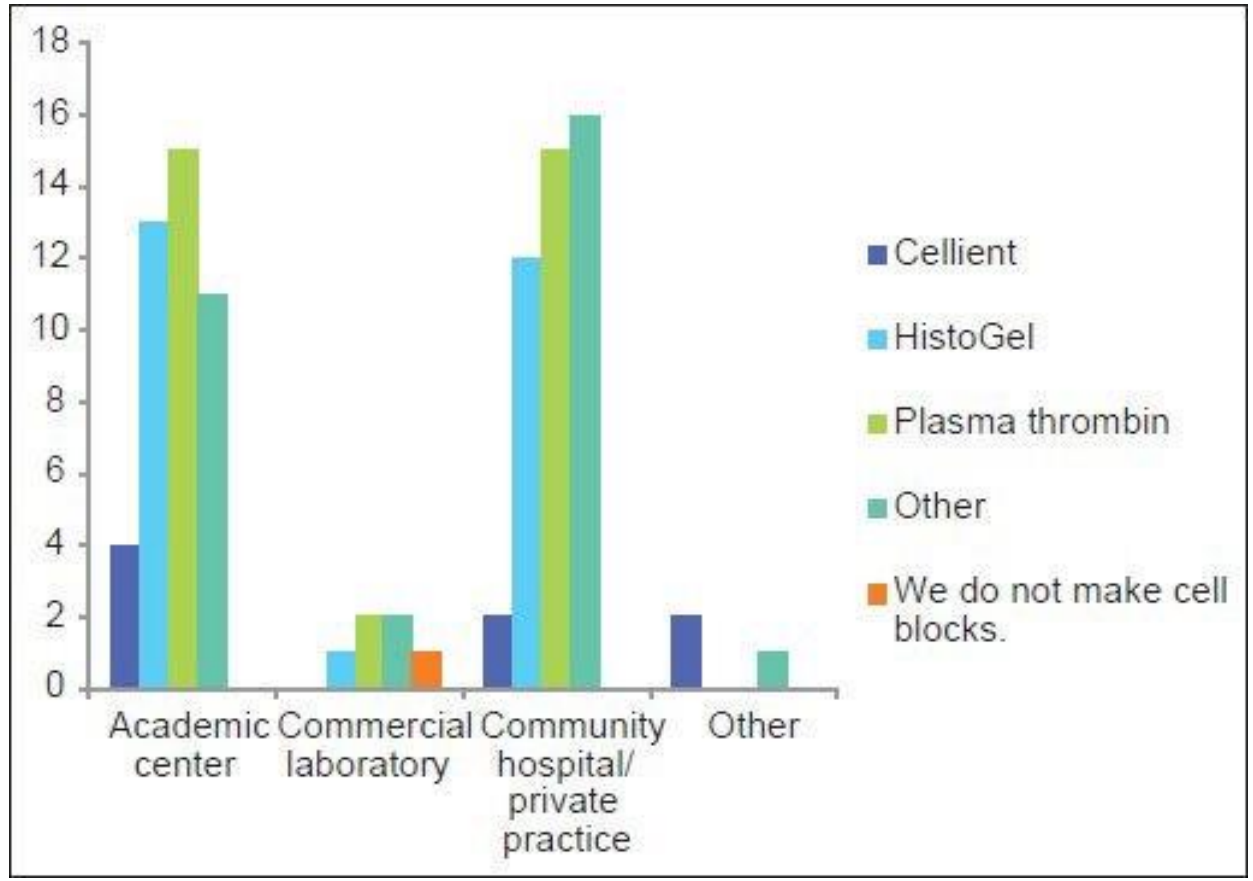




Solomon S et al Am J Roentgenol 194 (1): 266-269, 2010

Create cell blocks for all cytology specimens

Use an enrichment method to increase the yield of tumor cells in the cell block for all types of cytology specimens



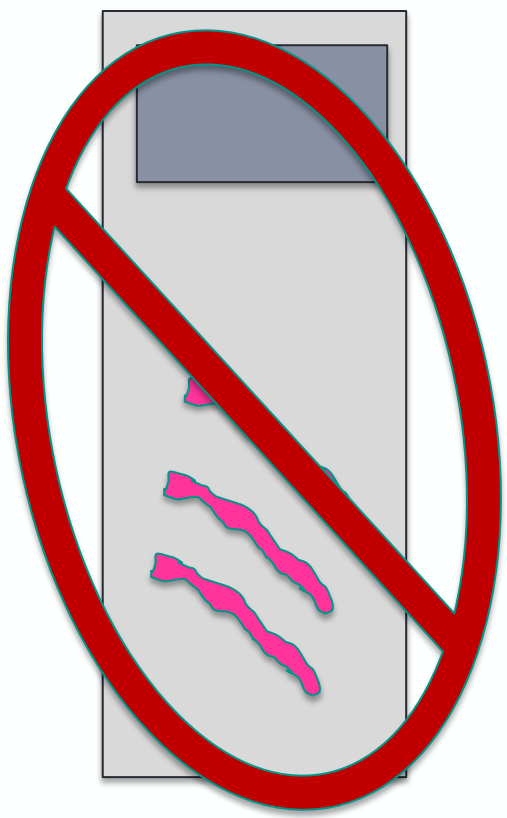
Crapanzano J et al CytoJournal 11 (7):, 2014

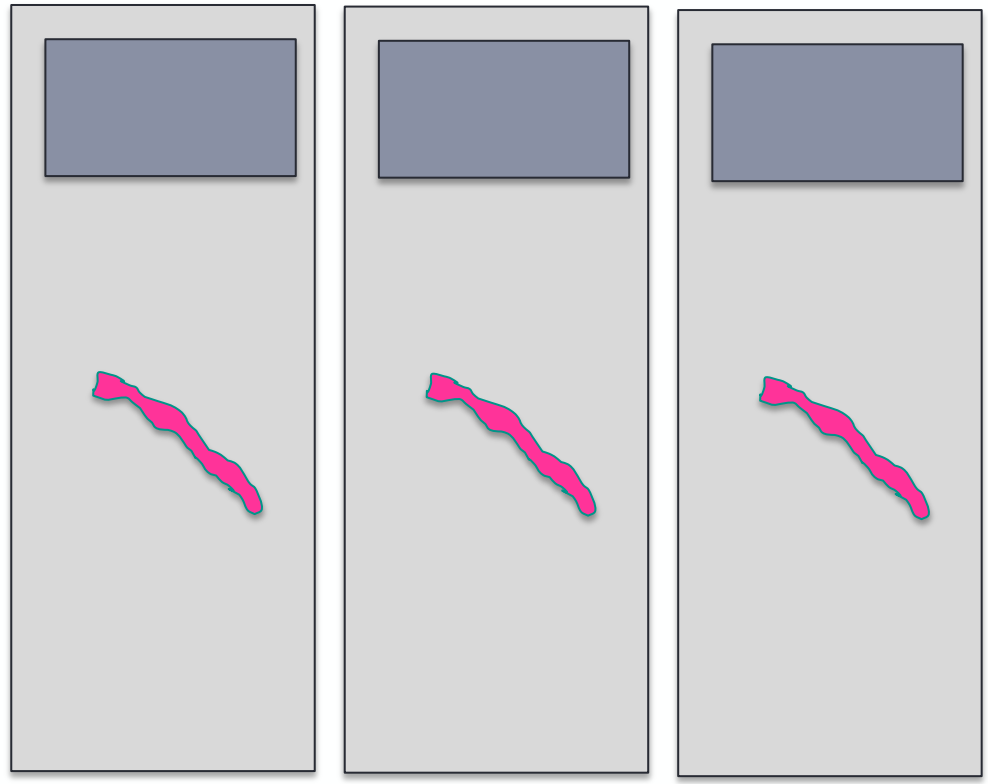
Minimize tissue use for diagnostic H&E staining

Know the goal of biopsy and use minimum tissue necessary to achieve it

- Primary diagnosis versus confirm known tumor/adequacy
- Embed & section FFPE blocks like prostate biopsies (3 H&E levels and 6 unstained slides) with one section per slide

(Note: FMI testing for Lung-MAP requires an FFPE block or 12-20 unstained +1 H&E slide)





FOUNDATION MEDICINE®

Preserve FFPE blocks

Do not deplete FFPE blocks on first pass

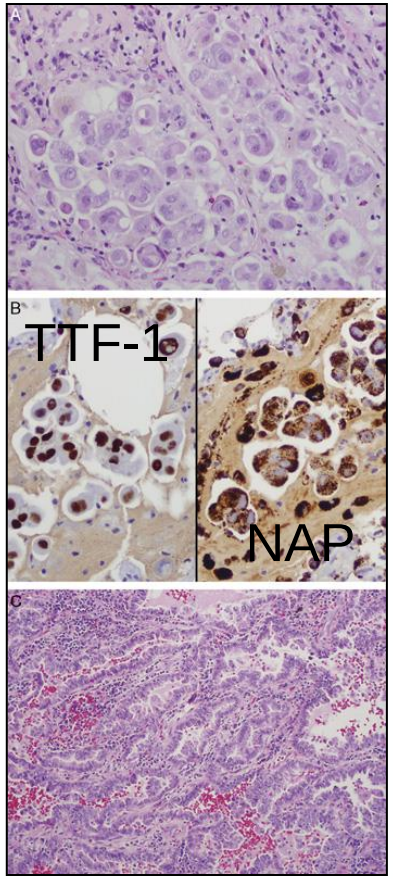
Instruct histotechnologists to stop cutting blocks halfway through the tissue, preserving the remainder for molecular studies

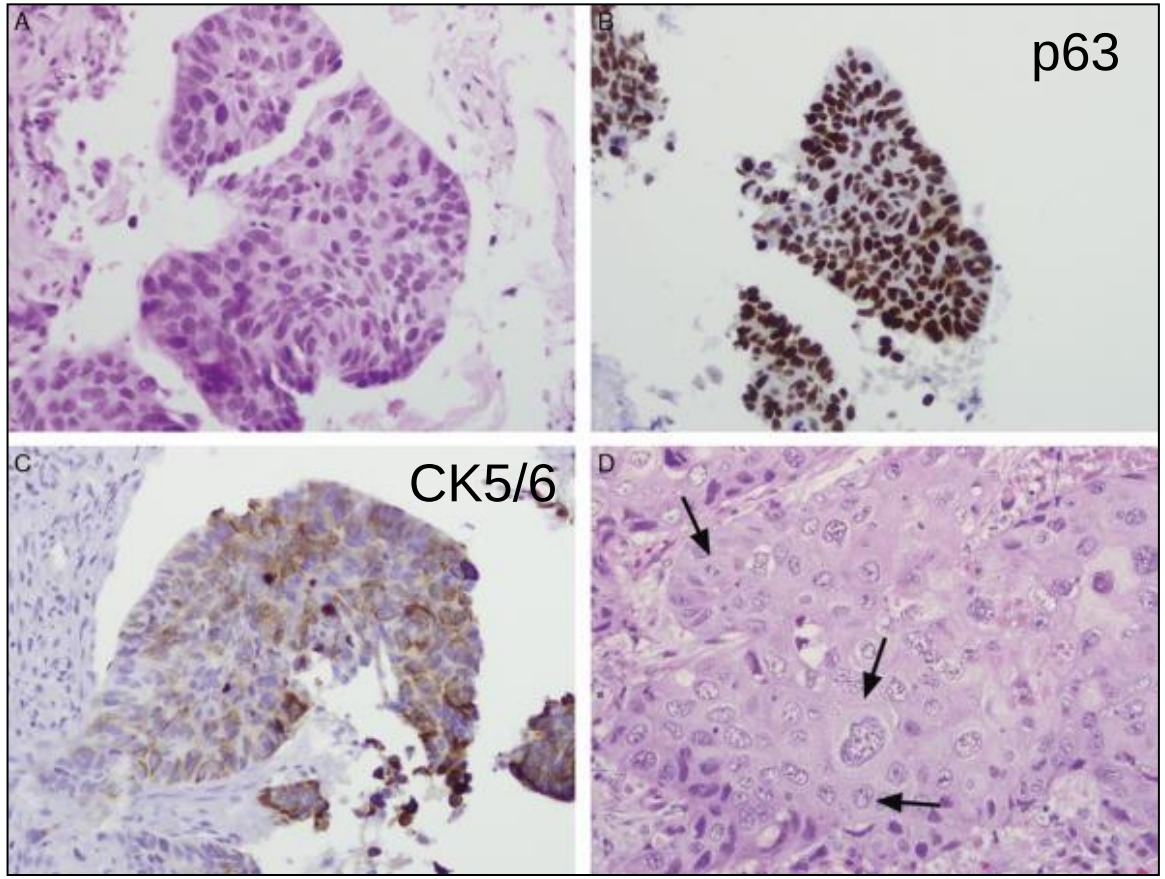
FOUNDATION MEDICINE®

Minimize diagnostic IHC

Perform multiple needle passes

Use unstained slides for limited IHC workup: TTF-1 and p40 for adenocarcinoma vs squamous cell carcinoma (Napsin-A, CK5/6 and mucin, if necessary)





Mukhopadhyay S et al AJSP 35; 15-25, 2011

If not submitting the FFPE block…

Cut additional unstained, positively charged, unbaked slides with one section per slide for molecular studies

TISSUE SUBMISSION UPDATE

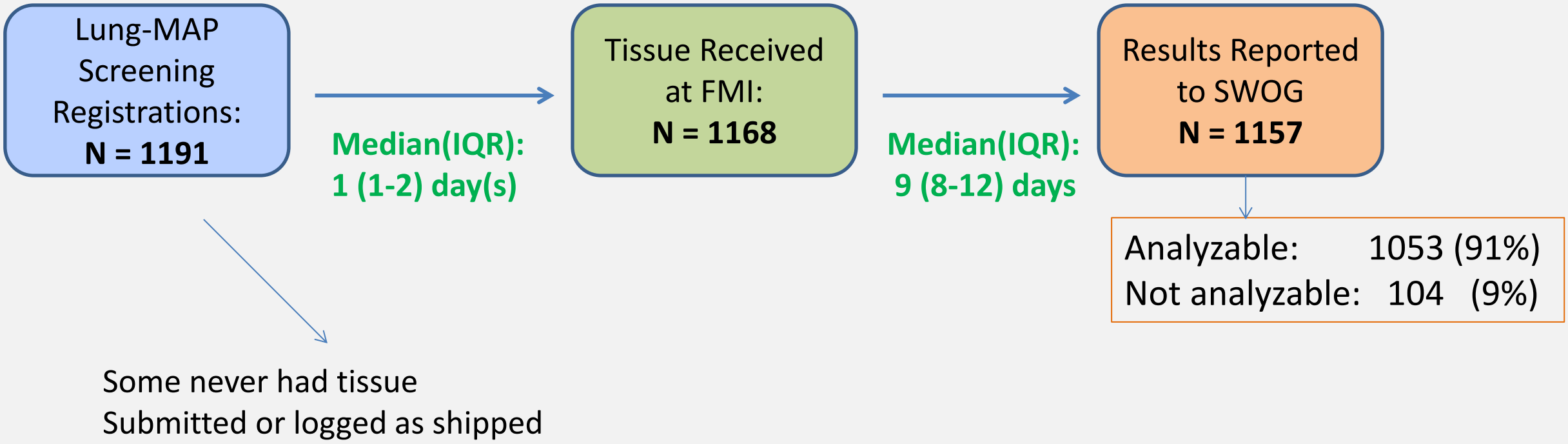
Dr. Roy Herbst

S1400 Study Co-Chair

Yale Cancer Center

TISSUE SUBMISSION UPDATE: Dr. Fred Hirsch

As of Mar 15, 2017



Go to: http://www.swogstat.org/accrual/lungmap.pdf for current status

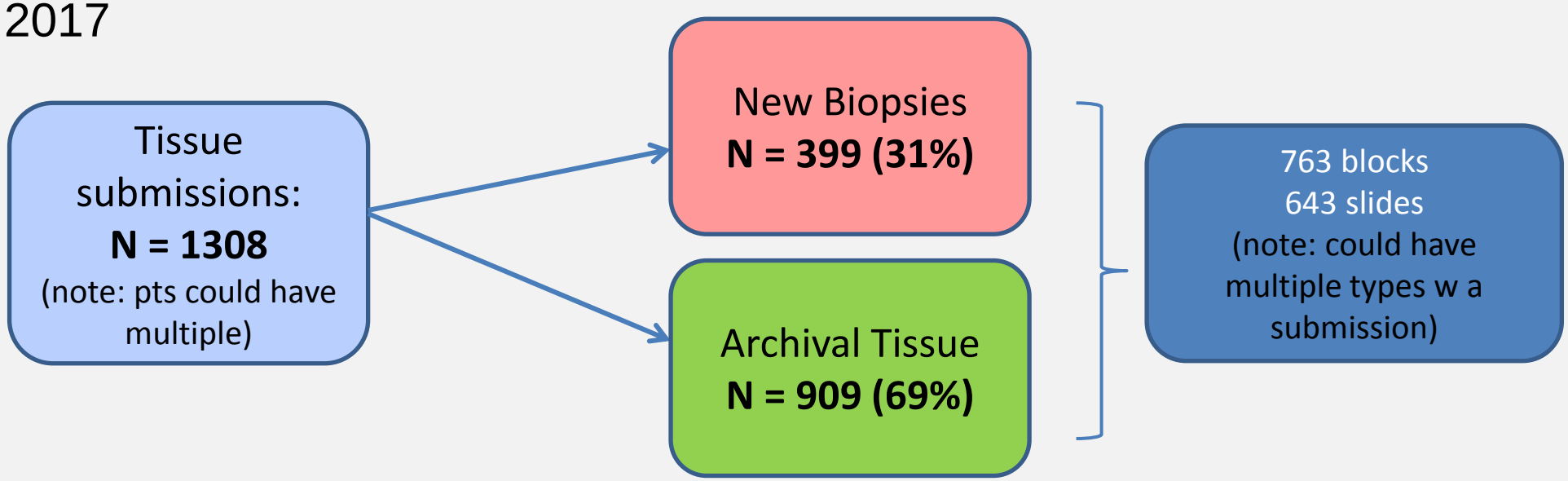
Lung-MAP Biomarker Results

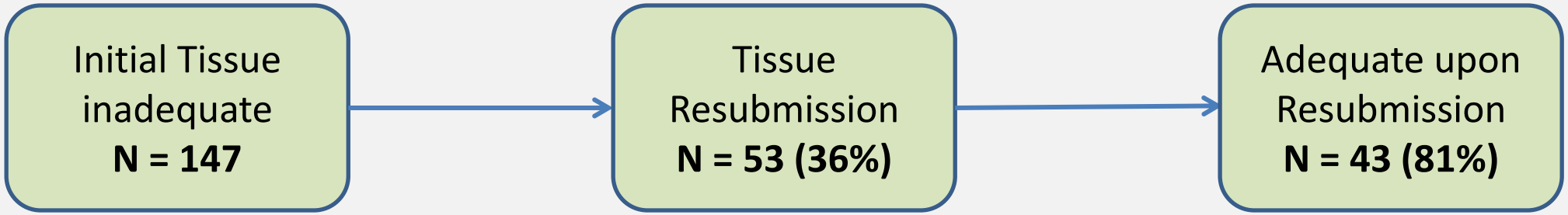
As of Mar 15, 2017

Total Screening/Pre-screening registrations:	N=1191
• Pre-screened prior to PD	410 (34%)
• Screened at PD	781 (66%)
Biomarker testing results:	N=1053
Pi3K+ (S1400B biomarker)	82 (8%)
CCGA+ (S1400C biomarker)	197 (19%)
FGFR+ (S1400D biomarker)	167 (16%)
HRRD+ (S1400G biomarker)	159 (15%)
Multiple Biomarkers	103 (10%)
Others (non-eligible biomarkers):	
EGFR	7 (1%)
ALK	1 (<1%)

New Biopsies vs. Archival

As of Mar 15, 2017





"The Tissue is the Issue"

As of Mar 15, 2017

~ 13% of tissue submissions are inadequate

Reasons for inadequacy (a sample could have multiple reasons):

- **48% Insufficient amount of tissue**
- **35% Insufficient tumor cells**
- **37% Insufficient DNA**
- **19% Insufficient tumor size**
- **14% Failed Sequencing**
- **5% Other Reasons**

When tissue resubmissions are accounted for,
9% of patients had inadequate tissue

Tissue Tips

"There must be local quality control at every step: acquisition, procession and submitting the tissue, to get good quality." - Dr. Fred Hirsch, Pathology Study Chair

1. Meeting with the Chief of Pathology to review the tissue requirements and timeline, discuss barriers and resolutions
2. Identifying a pathology staff member to work with
3. Checking the status of available tissue as soon as a potential patient is identified

- Naomi L. Hullinger, RN, Supervisor, San Antonio Military Medical Center

S1400 TISSUE LOGISTICS

Dr. Mary Redman

S1400 Lead Biostatistician

Fred Hutchinson Cancer Research Center

S1400 Tissue Logistics

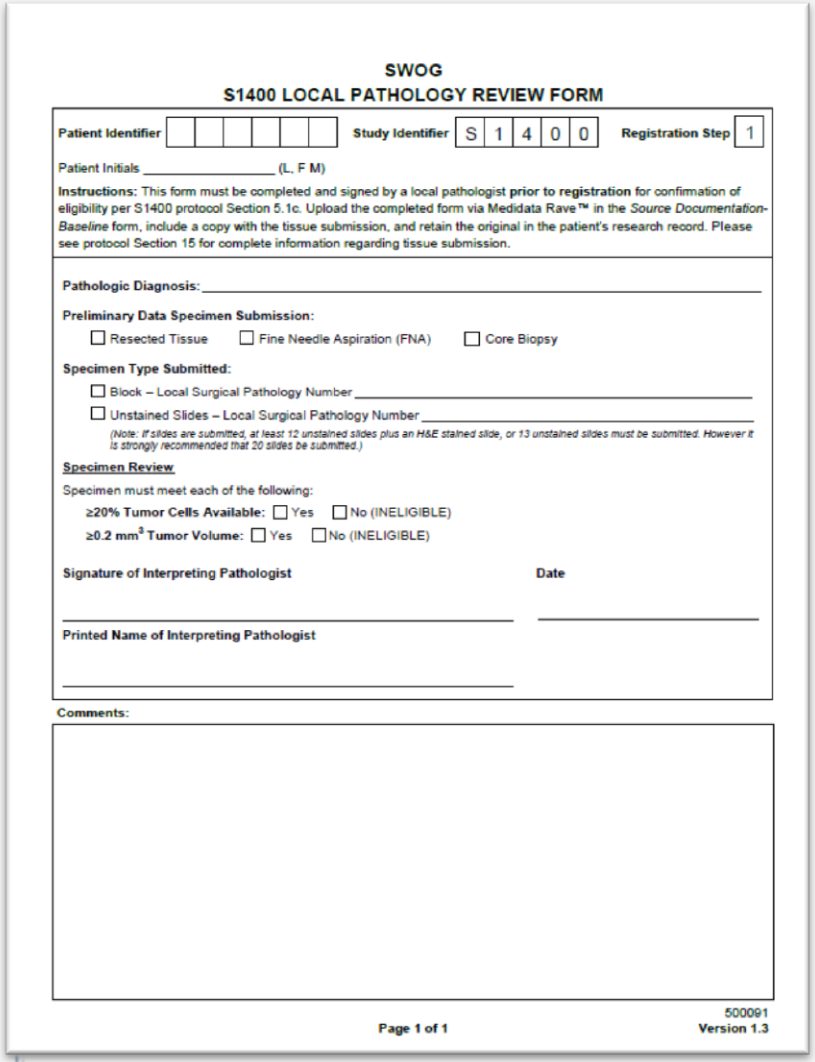
Evaluate eligibility, consent patient*, and confirm evaluable tissue**
Need Pathology Form sign-off

S1400 requires adequate tissue for biomarker profiling.

Tumor Content ≥ 20% including tumor volume ≥ 0.2 mm³

For details, refer to the **S1400** protocol Section 5 for eligibility requirements and Section 15 for a complete description of tissue requirements. Specimens must be submitted using the SWOG Specimen Tracking System, a process outlined in the **S1400** protocol Section 15.

S1400 Tissue Logistics



SWOG
S1400 LOCAL PATHOLOGY REVIEW FORM

Patient Identifier | | | | | | | Study Identifier | S | 1 | 4 | 0 | 0 | Registration Step | 1 |

Patient Initials _____ (L, F M)

Instructions: This form must be completed and signed by a local pathologist **prior to registration** for confirmation of eligibility per S1400 protocol Section 5.1c. Upload the completed form via Medidata Rave™ in the *Source Documentation-Baseline* form, include a copy with the tissue submission, and retain the original in the patient's research record. Please see protocol Section 15 for complete information regarding tissue submission.

Pathologic Diagnosis: _____

Preliminary Data Specimen Submission:

☐ Resected Tissue ☐ Fine Needle Aspiration (FNA) ☐ Core Biopsy

Specimen Type Submitted:

☐ Block – Local Surgical Pathology Number _____

☐ Unstained Slides – Local Surgical Pathology Number _____

(Note: If slides are submitted, at least 12 unstained slides plus an H&E stained slide, or 13 unstained slides must be submitted. However it is strongly recommended that 20 slides be submitted.)

Specimen Review

Specimen must meet each of the following:

≥20% Tumor Cells Available: ☐ Yes ☐ No (INELIGIBLE)

≥0.2 mm³ Tumor Volume: ☐ Yes ☐ No (INELIGIBLE)

Signature of Interpreting Pathologist **Date**

_____ _____

Printed Name of Interpreting Pathologist

_____

Comments:

500091
Page 1 of 1 Version 1.3

Patients must have an adequate tissue specimen confirmed by the local pathologist on the **S1400 Local Pathology Review Form.**

Register to S1400 in OPEN

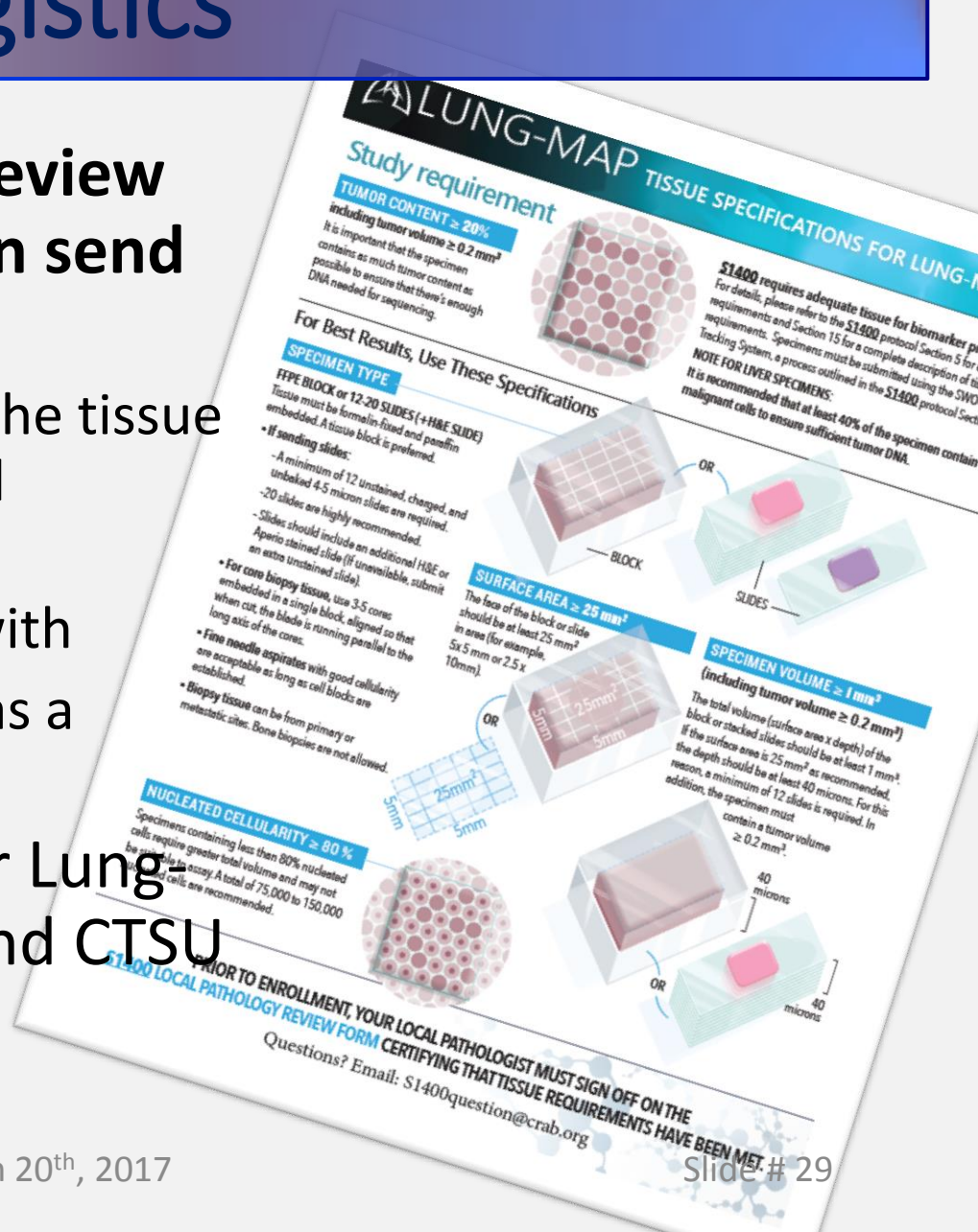
Submit tissue to FMI **within 1 day** after registration

S1400 Tissue Logistics

How to educate pathologists about the path review and requirements? Is there a fact sheet we can send to them?

- Meeting with the Chief of Pathology to review the tissue requirements and timeline, discuss barriers and resolutions
- Identifying a pathology staff member to work with
- Checking the status of available tissue as soon as a potential patient is identified

- Yes, there is a Tissue Specifications Sheet for Lung-MAP. The sheet is available on the SWOG and CTSU websites.



S1400 Tissue Logistics

If biopsies are needed, sites will receive
$3,000/$6,000 for the biopsies performed at
screening and/or progression after initial response on protocol therapy

Interactive Q+A Session

***Please note**: We will take additional questions and comments as time allows.*

Please use the "Raise your hand" function in WebEx if you'd like to speak, or, use the chat function if you'd like to submit a comment or question while on mute.

A record of the Q+A will be compiled and provided to all study sites.

Thank you for participating!

Comments or questions?

Please write to Sarah Basse at the SWOG Stats Center:

sarahb@crab.org