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Methylation Classification: Going Beyond the CNS Classifier

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Disclosures

- I am a molecular pathologist and not a bio informaticist. I will do my best to explain the bioinformatics components of the technology in my talk, but to implement any of these techniques in your laboratory there is a need for coding and bioinformatics skills.
- Much of the work presented here is still unpublished data. Please do not share or post this data on social media websites.

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Objectives

- Brief History of methylation classifiers
- Overview of laboratory workflow and how the methylation array works
- Our approach of how to build and train a classifier
- Clinical cases from our laboratory highlighting the utility and pitfalls of the technology

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History of Methylation Classifiers

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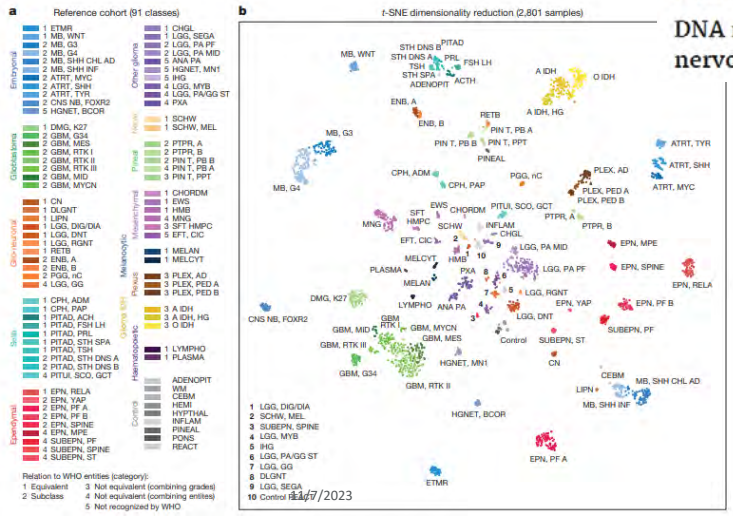
Background on Methylation Array

CNS Methylation classifier- 2018



Nature. 2018 Mar 22;555(7697):469-474. doi: 10.1038/nature26000. Epub 2018 Mar 14.

DNA methylation-based classification of central nervous system tumours

First classifier by the DKFZ group in 2018 published in Nature. Showed there were 91 distinct groups of CNS tumors that cluster on t-SNE. Since then it has expanded to more than 120 subclasses in most recent version of this classifier on their website.

Some of these novel classes have made it into the WHO for CNS tumors in 2021

Northwestern CNS classifier

The Journal of Molecular Diagnostics, Vol. 24, No. 8, August 2022

ELSEVIER

the Journal of Molecular Diagnostics
jmdjournal.org

Validation of Whole Genome Methylation Profiling Classifier for Central Nervous System Tumors

Lucas Santana-Santos,* Kwok Ling Kam,† David Dittmann,* Stephanie De Vito,* Matthew McCord,* Pouya Jamshidi,* Hailie Fowler,† Xinkun Wang,† Alan M. Aalsburg,† Daniel J. Brat,* Craig Horbinski,*‡ and Lawrence J. Jennings*

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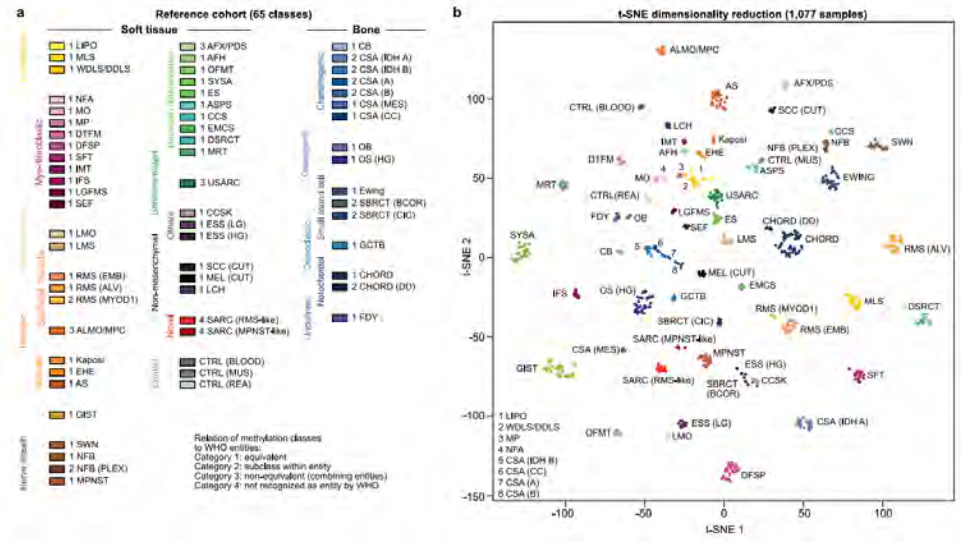
Sarcoma Methylation classifier (2021)

- 65 initial classes of bone and soft tissue tumors
- Many were WHO classified and had two novel entities



Nat Commun. 2021 Jan 21;12(1):498. doi: 10.1038/s41467-020-20603-4.

Sarcoma classification by DNA methylation profiling

Northwestern Experience and Beyond

- Sarcoma Methylation Classifier
  - Utilized DKFZ validation data cohort to re-create classifier in house
  - Also used some in house cases to supplement this data and for additions of additional classes
- Carcinoma of Unknown Primary Classifier
  - Utilization of TCGA methylation array data and in house cases
  - Some carcinomas are not well represented in TCGA data and needed to be added
- Hematologic Neoplasms
  - Other institutions (NIH, UPenn) have been working on this as well- no clinically available test to date

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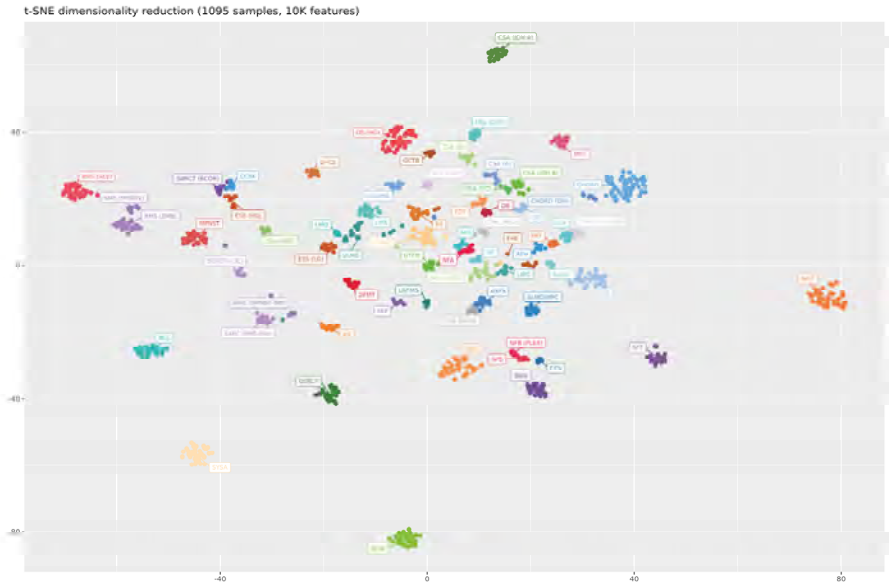
t-SNE and Classifier for Sarcomas



AUC=0.855

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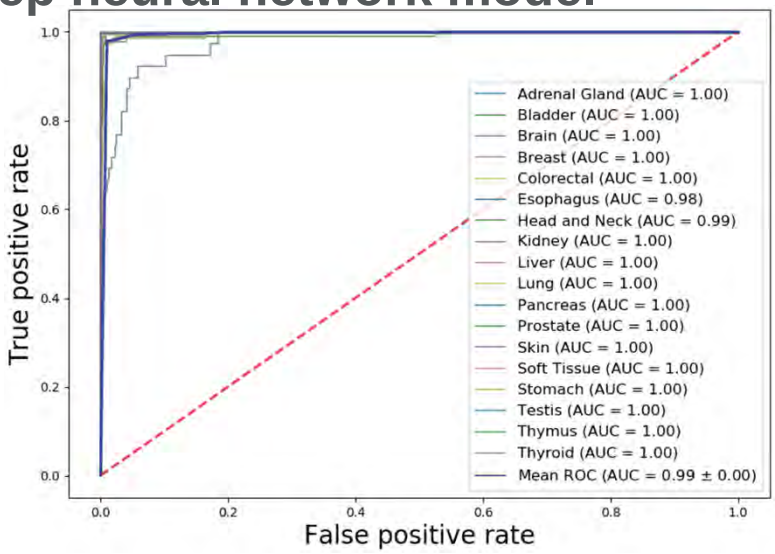
Predicting cancer origins with a DNA methylation-based deep neural network model



Zheng C, Xu R (2020) Predicting cancer origins with a DNA methylation-based deep neural network model. PLOS ONE 15(5): e0226461.

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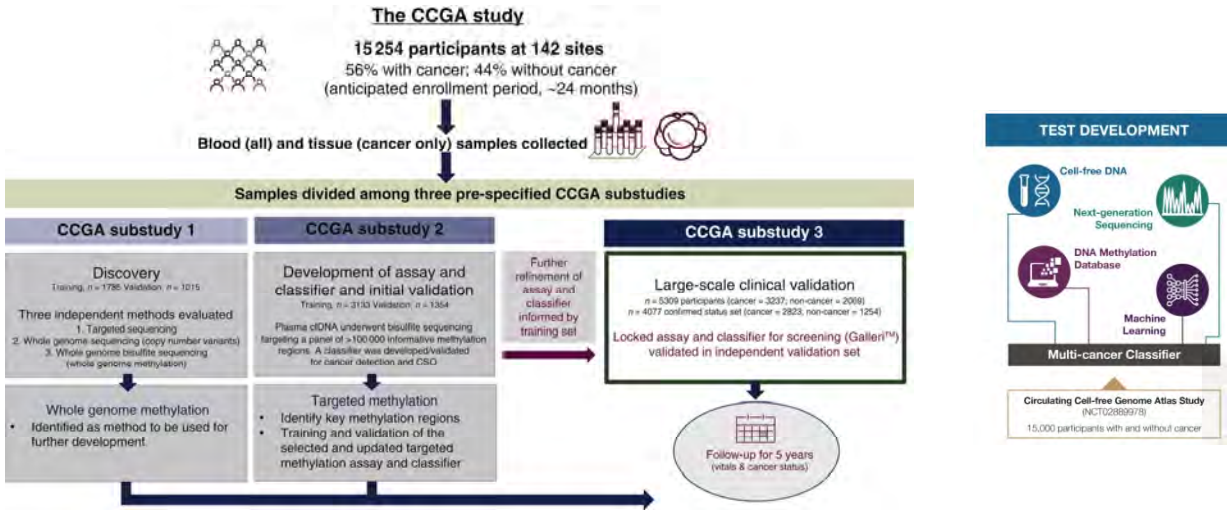
Multi Cancer Early Detection



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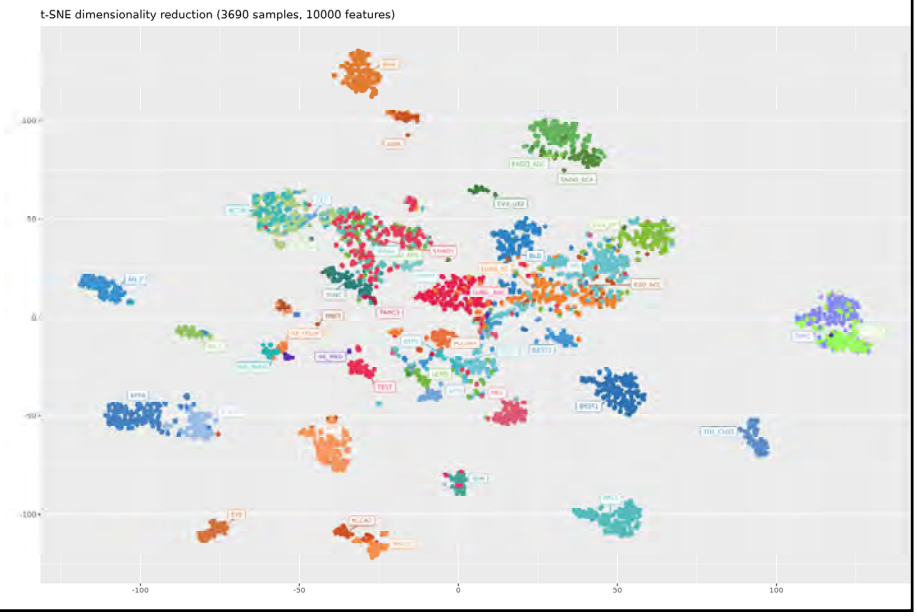
From: *Annals of Oncology* 2021 321167-1177 DOI:(10.1016/j.annonc.2021.05.806) 11

t-SNE and Classifier for Carcinomas of Unknown Primary

AUC=0.952



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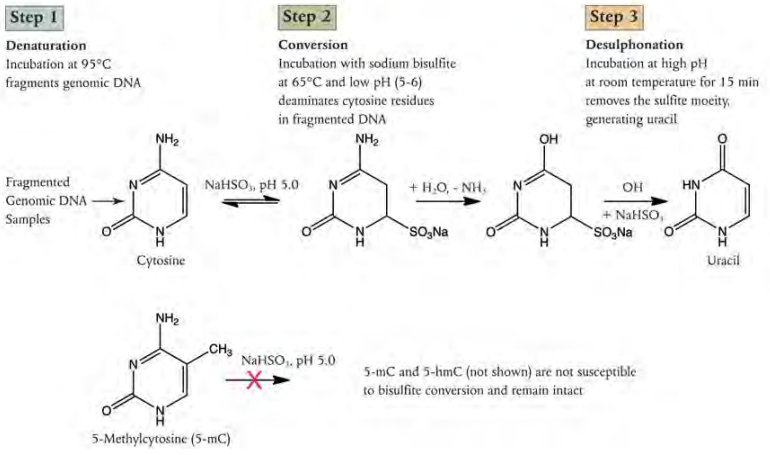
Laboratory Workflow

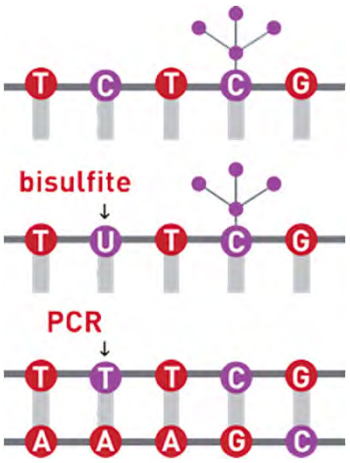
How to Measure DNA Methylation



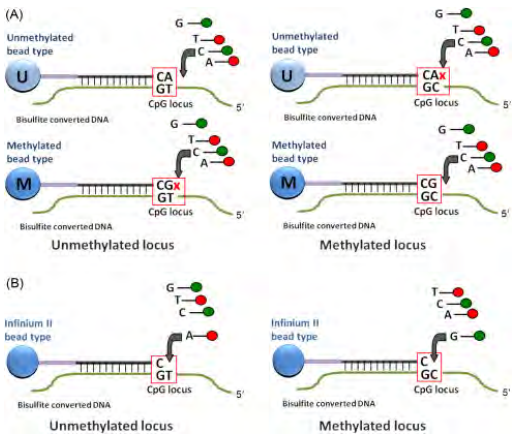
- Modify unmethylated DNA with bisulfonate
- Selectively convert C>U
- Design PCR primers to amplify "methylated" template

Why does the conversion matter?

- Conversion of C ►U in bisulfite conversion process changes C-G pairing to A-T base pair
- This change from C-G to A-T is detectable on PCR, Microarray, and sequencing methods

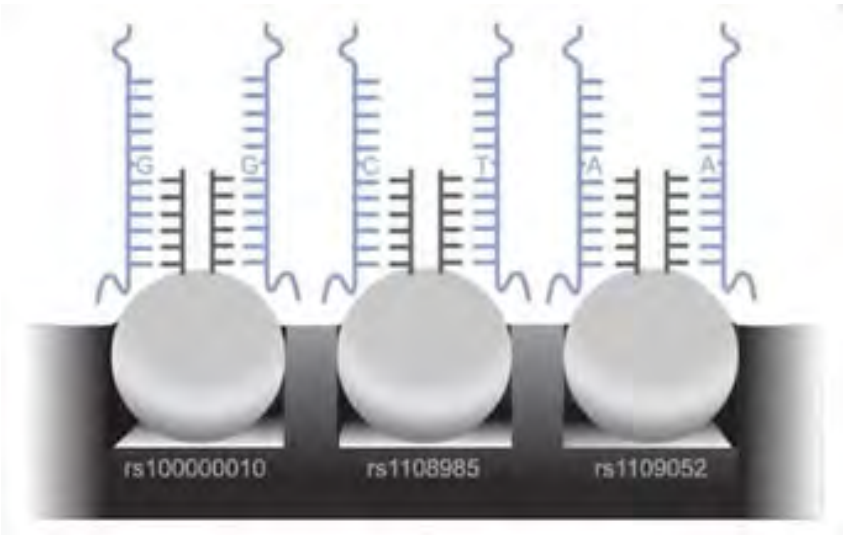


Methylation microarray bead types



- Two types of beads present on array chip
- First type: has beads specific for the methylated and unmethylated sites. Patient DNA fragment must match methylated/unmethylated status of the bead type in order for single base extension to occur
- Second type: The single base extension happens at interrogation site of methylation status. Fluorescence color tells status

Hybridization



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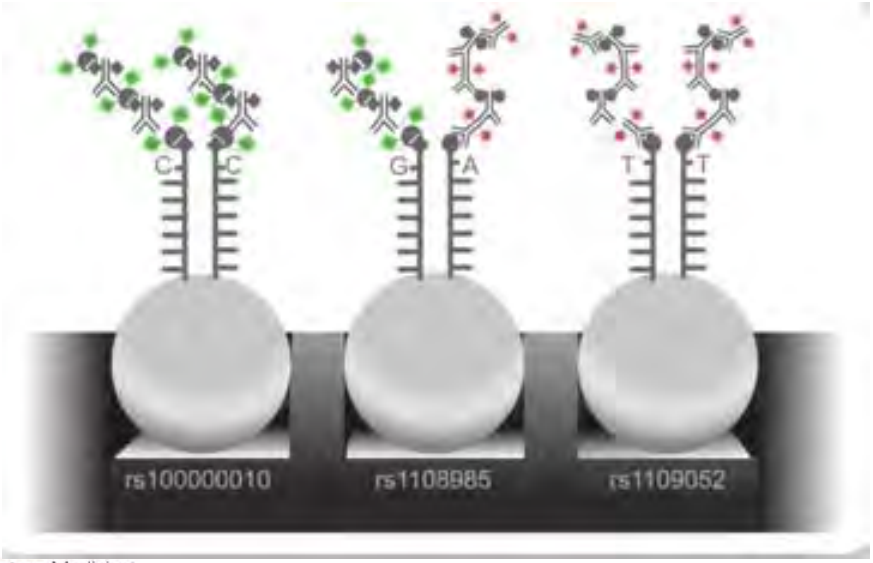
Extension-Staining



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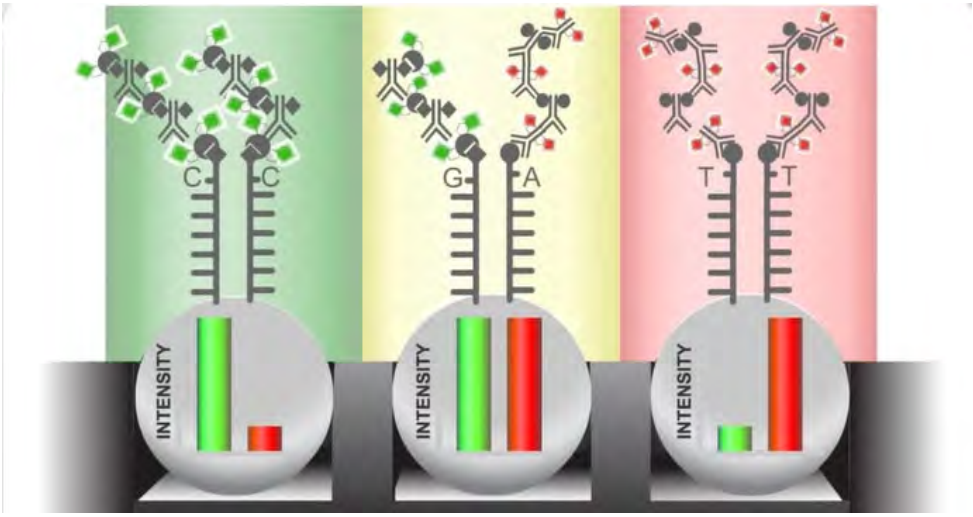
Scanning



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Clinical Workflow

Day 1 (pre-amp)	• QC • Denature gDNA and bisulphite conversion
Day 2 (pre-amp)	• Clean up bisulphite conversion reagent • FFPE restoration protocol • DNA amplification (overnight)
Day 3 (post-amp)	• DNA fragmentation, precipitation and resuspension • Hybridization of DNA to the BeadChips (overnight)
Day 4	• Wash the BeadChips • Single base primer extension and staining of BeadChips • Wash and scan

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Building A Classifier

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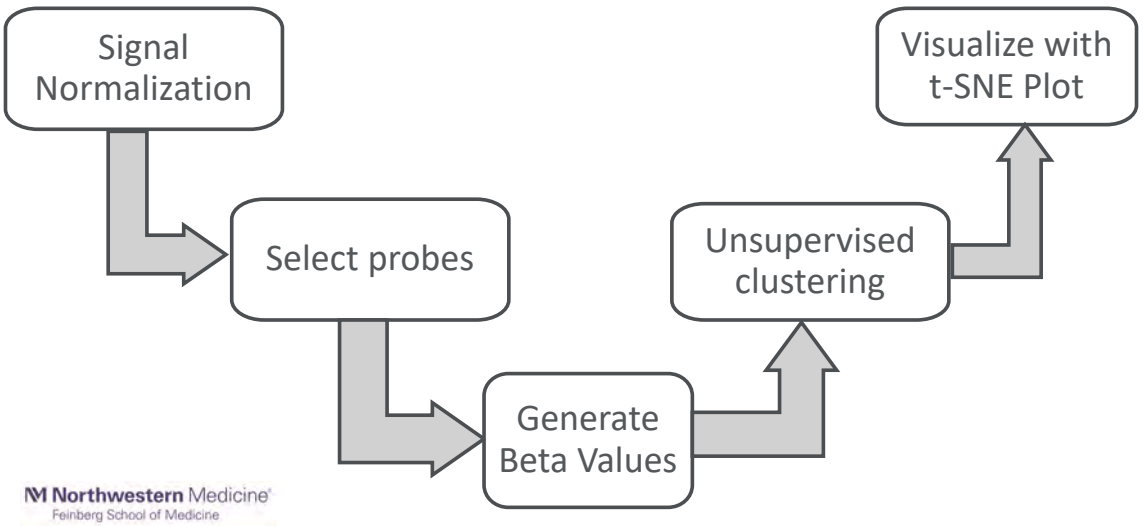
Unsupervised Clustering and Visualization



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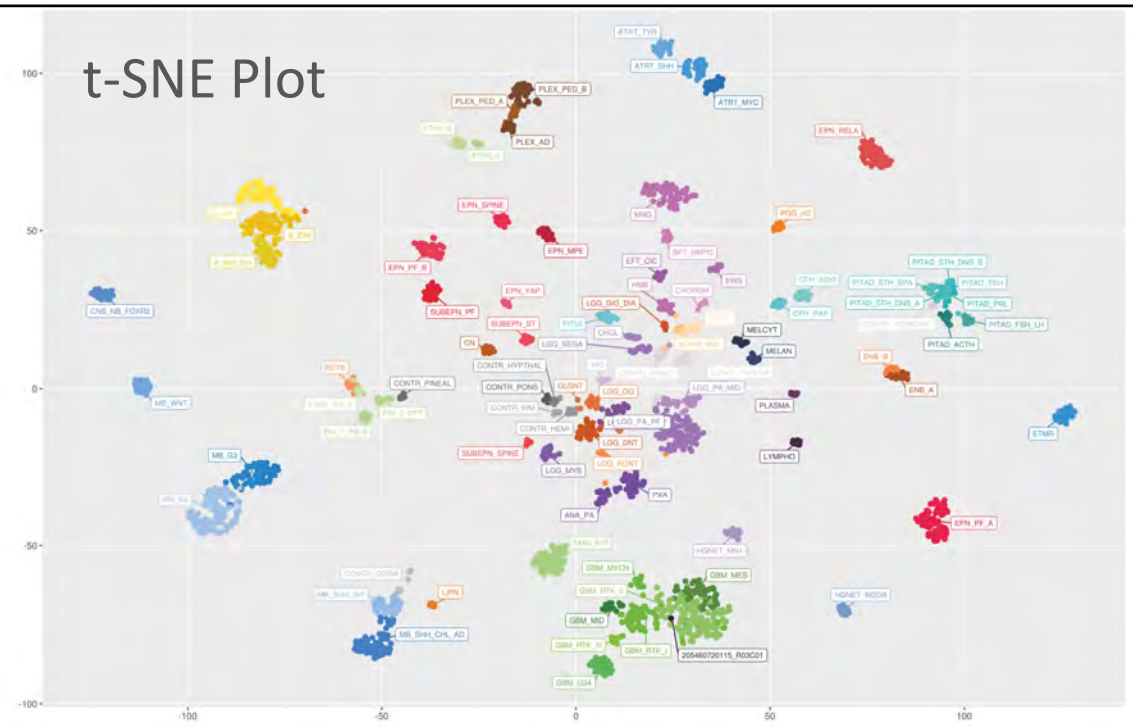
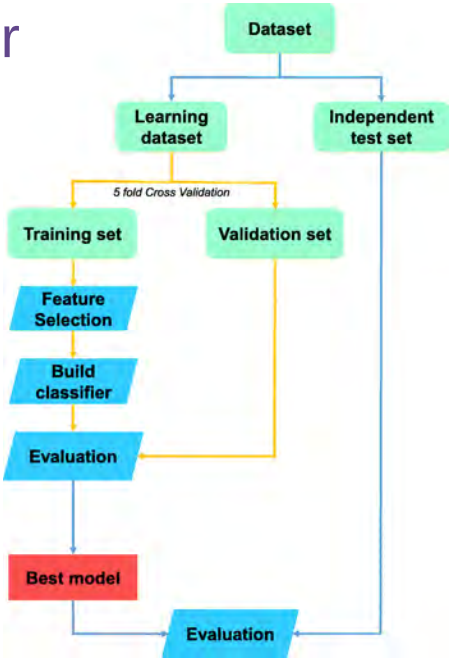
t-SNE Plot



Building a Classifier



Chiesa, M., Maioli, G., Colombo, G.I. *et al.* GARS: Genetic Algorithm for the identification of a Robust Subset of features in high-dimensional datasets. *BMC Bioinformatics* **21,** 54 (2020).

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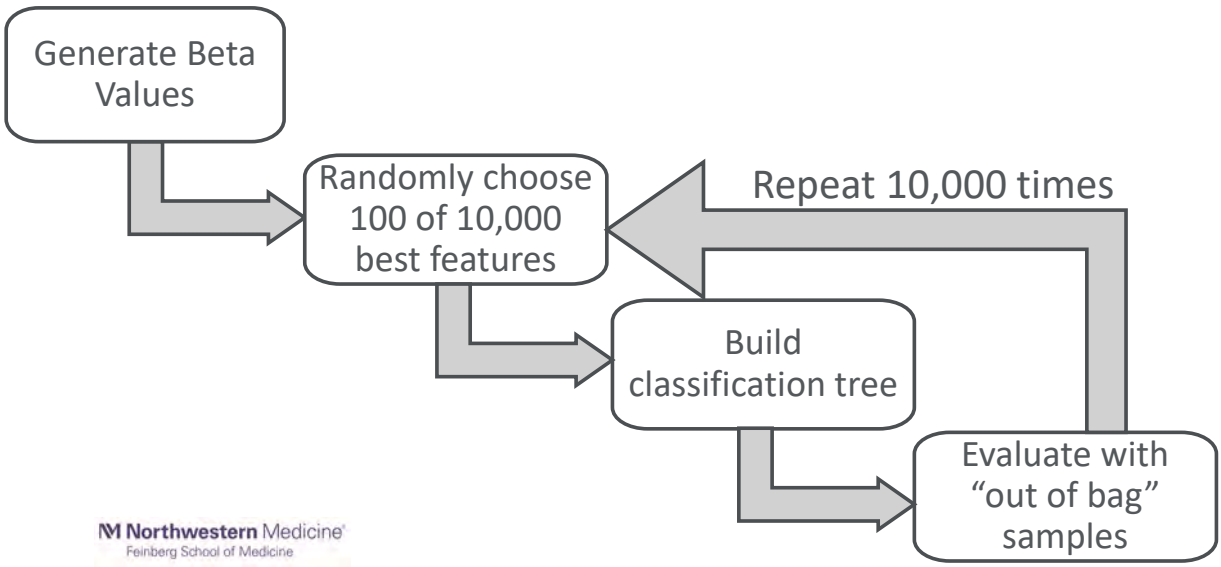
Building a Classifier



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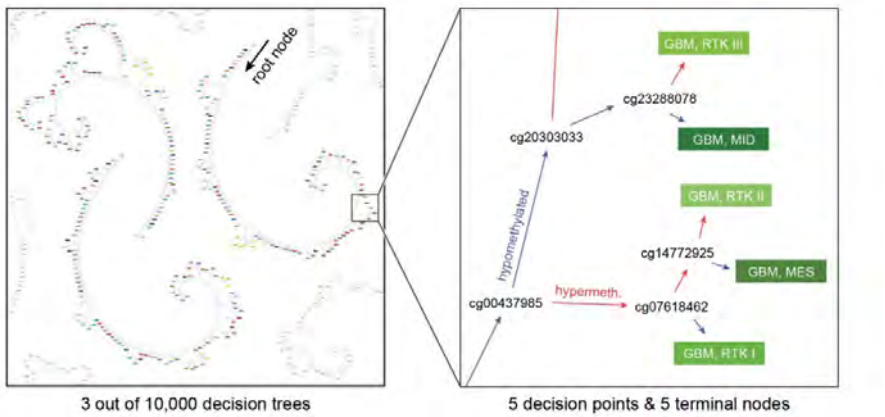
Random Forrest Classifier Generation



3 out of 10,000 decision trees

5 decision points & 5 terminal nodes

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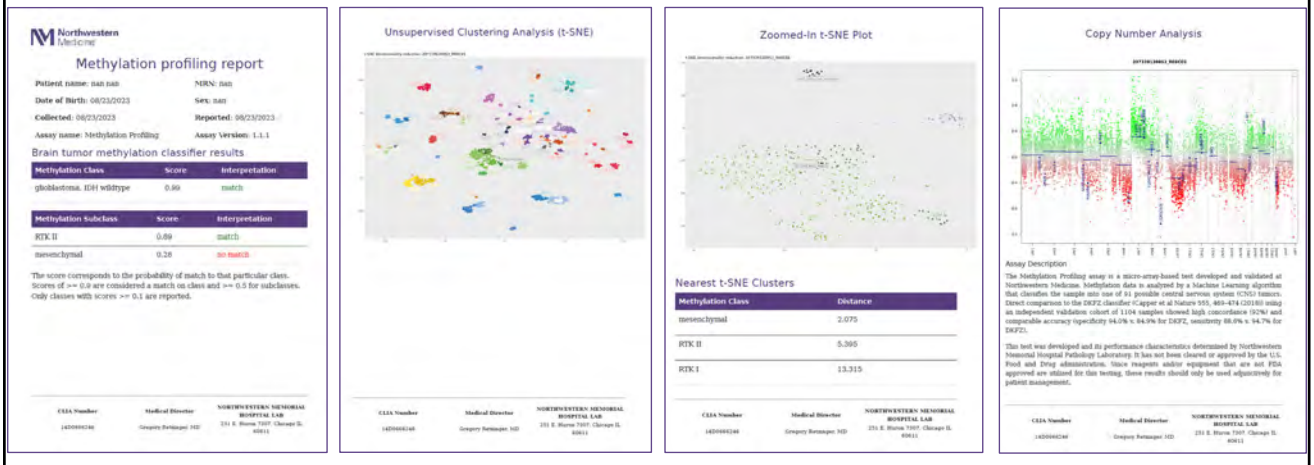
From: DNA Methylation-based classification of central nervous system tumors. Nature 2018

Commonly Asked Questions about Bioinformatics

- How many cases are required for a cluster to be defined as a class?
  - Minimum 12. We use 15 internally, but the more the better!
- What is a class vs. subclass? How are score calculated?
  - Class (superfamily) is a collection of subclasses. The score for this superfamily class is calculated by adding the subclass scores that are within the superfamily together
  - Subclasses are individual diagnostic entities. Examples would be Glioblastoma, RTKII subtype or Lung Adenocarcinoma.
  - In the first example of Glioblastoma: Glioblastoma, IDH-wildtype is the superclass that consists of RTKI, RTKII, RTKIII, Mesenchymal, and midline. The score for the superfamily is the sum of the scores for these subclasses.

Clinical Reporting



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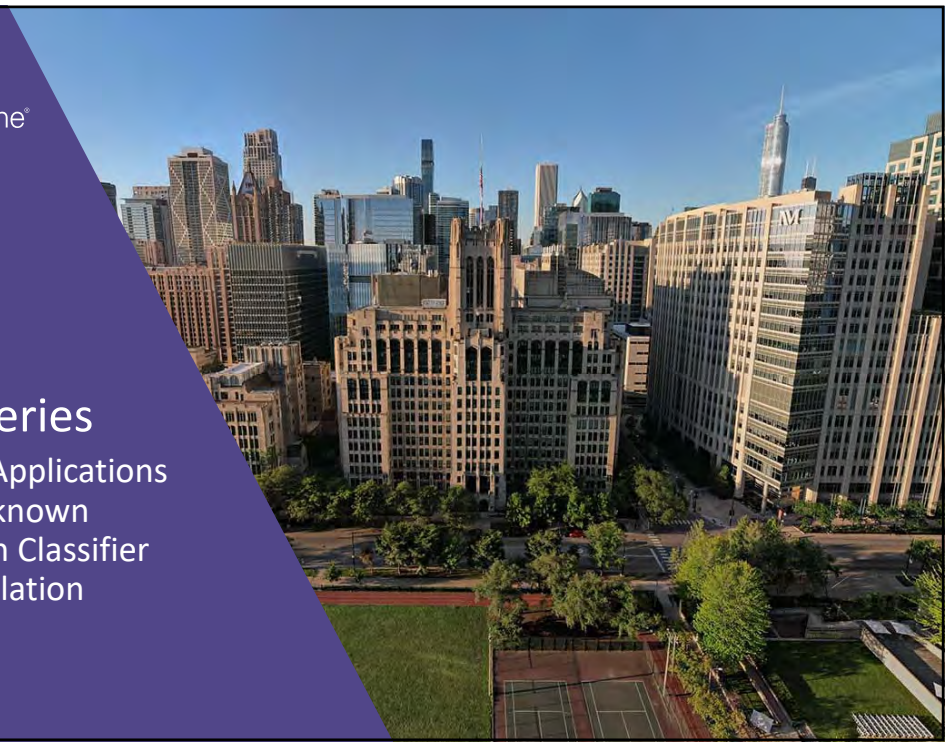


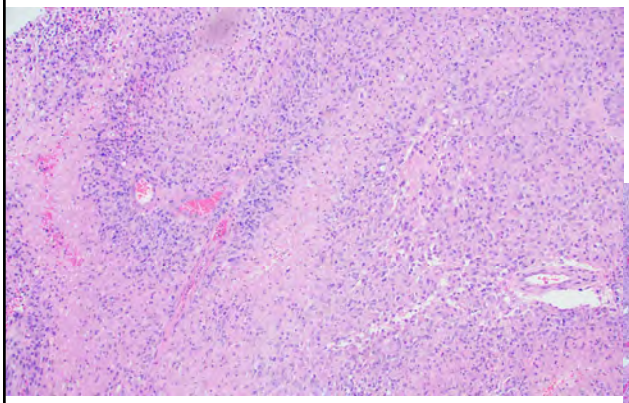
Clinical Case Series

Real World Clinical Applications of Carcinoma of Unknown Primary Methylation Classifier and Sarcoma Methylation Classifier

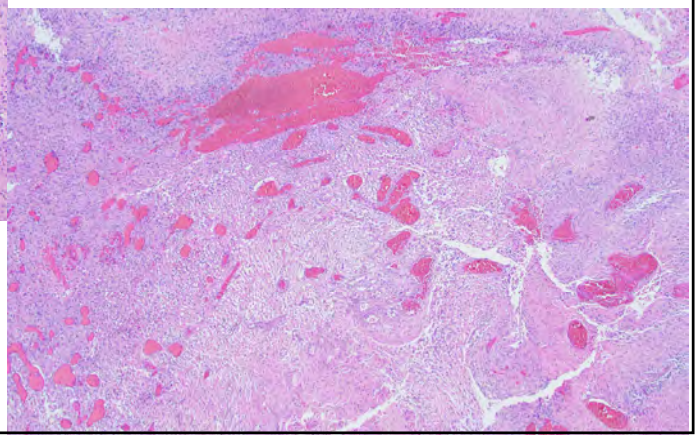
Case #1

- 61 y/o female who has a history of Glioblastoma treated with Resection and temozolomide at initial resection.
- Patient showed progression of lesion on imaging and was scheduled for re-resection.

Re-Resection Specimen



Stain	Result
IDH1 R132H	Negative in tumor cells
ATRX	Retained nuclear expression in tumor cells
Ki-67	Labels ~30-40% of tumor cells
P53	Weak to moderate staining in approximately 10% of tumor cells
Olig-2	Positive in tumor cells



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Methylation Array Results

Brain tumor methylation classifier results

Methylation Class	Score	Interpretation
glioblastoma, IDH wildtype	1.00	match

Methylation Subclass	Score	Interpretation
RTK I	0.94	match

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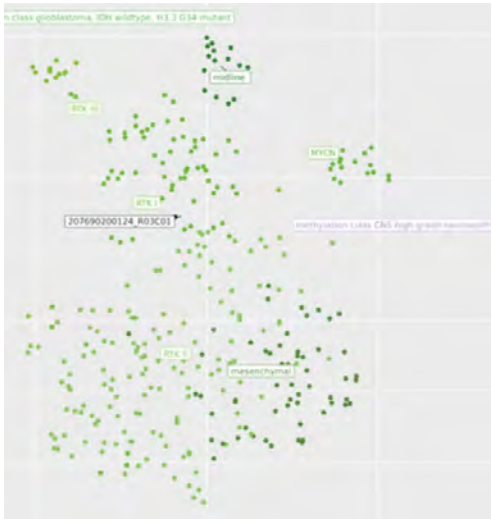
t-SNE



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Additional Molecular Studies

Genomic Signature

Signature	Status/Score
Microsatellite Analysis	MS-Stable
TMB Muts/Mb (Sequenced)	103.1

Variants of known or potential clinical significance

Alteration	Variant	Allele Proportion	Drugs Associated with Sensitivity	Drugs Associated with Resistance
TERT	NM_198253.2 c.-124C>T	37%	None	None
BARD1	NM_000465.2 c.365-1G>A	34%	Olaparib	None
CIC	NM_015125.3 c.4327+1G>A	30%	None	None
EGFR p.(A289V)	NM_005228.3 c.866C>T	7%	None	None
EGFR p.(E709K)	NM_005228.3 c.2125G>A	40%	None	None
RET p.(A883T)	NM_020975.4 c.2647G>A	62%	Pralsetinib, Selpercatinib	None
ERBB2 p.(G660S)	NM_004448.2 c.1978G>A	34%	Ado-Trastuzumab Emtansine, Fam-Trastuzumab Deruxtecan	None
RB1	NM_000321.2 c.1696-1G>A	40%	None	None

Copy Number Variants

Alteration	Variant	Copy Number	Drugs Associated with Sensitivity	Drugs Associated with Resistance
CDK4 Amplification	Amplification	11.0 copies	Palbociclib	None
EGFR Amplification	Amplification	69.0 copies	None	None
MDM2 Amplification	Amplification	85.0 copies	None	None

Rearrangements
None identified

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Case #2

- 67 y/o female sen in neuro-oncology clinic as a second opinion consultation.
- Review of the original resection specimen performed at an outside institution was performed by neuropathology and sent for methylation array testing and additional molecular studies.

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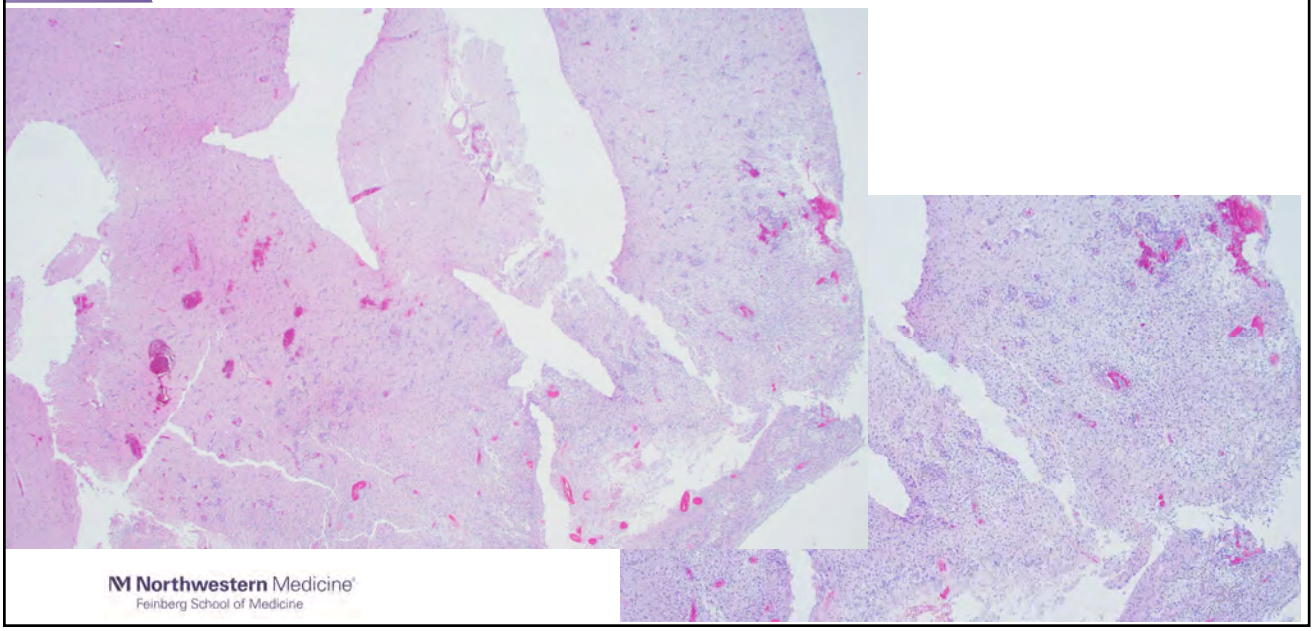
Resection Specimen



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Methylation Classifier

Brain tumor methylation classifier results

Methylation Class	Score	Interpretation
glioblastoma, IDH wildtype	0.84	no match

Methylation Subclass	Score	Interpretation
RTK I	0.41	no match
mesenchymal	0.25	no match
RTK II	0.18	no match

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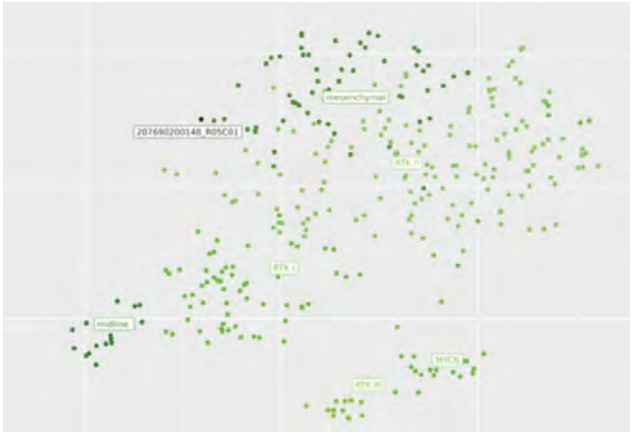
t-SNE



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Additional Molecular Studies

Genomic Signature

Signature	Status/Score
Microsatellite Analysis	MS-Stable
TMB Muts/Mb (Sequenced)	7.7

Variants of known or potential clinical significance

Alteration	Variant	Allele Proportion	Drugs Associated with Sensitivity	Drugs Associated with Resistance
TERT	NM_198253.2 c.-146C>T	18%	None	None

Copy Number Variants
None identified

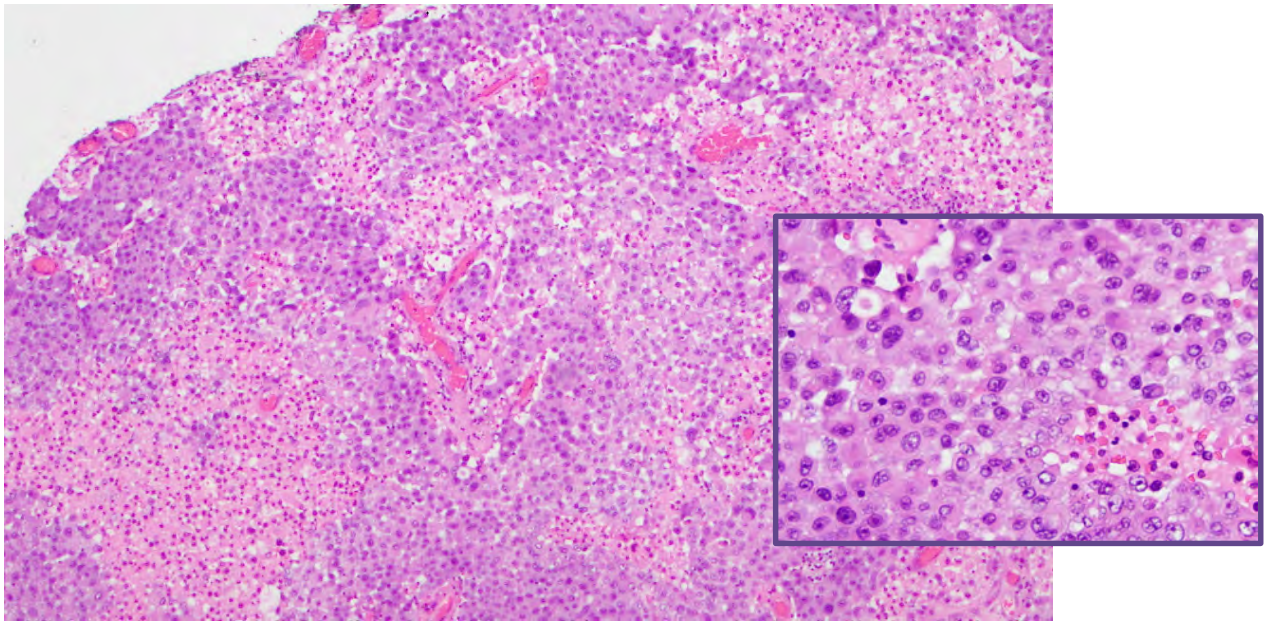
Rearrangements
None identified

Conclusions from Case #1 & 2

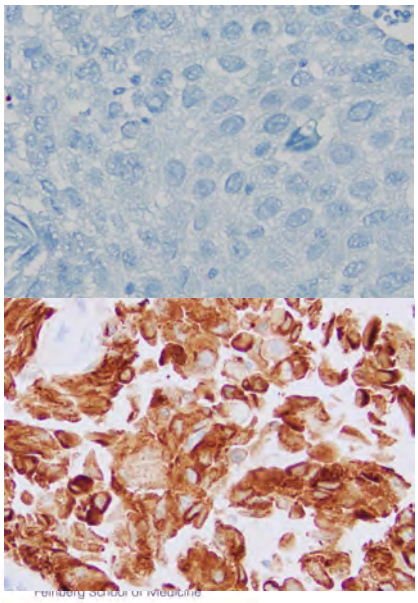
- Tumor percentage matters!
  - The first case had a 80-90% tumor percentage in the area taken for molecular studies, the second case only had about 50%.
- Tumor percentage matters even in really common neoplasms that appear on a methylation classifier!
- Our tumor percentage cutoff for cases is typically 65% for a good match score to occur.

Case #3

- 69 y/o male presented with progressive decreased function of his left arm with progression to his left leg with progressive worsening headaches. Patient had a fall from progressive weakness and presented to ED
- Brain imaging in ED found a right parietal lesion and CT of Chest/Abdomen and Pelvis showed a couple of irregular lymph nodes in the chest
- Patient underwent craniotomy for parietal lesion resection
- We received the tumor resection on our neuropathology service in consult from the outside institution



Immunohistochemical staining



TTF1 IHC

CK7 IHC

Stain	Result
AE1/3	Positive
CK7	Positive
CK20	Negative
CDX2	Negative
PSA	Negative
p40	Negative
TTF1	Negative
Synaptophysin	Negative
GATA3	Negative
Inhibin	Negative
MART1	Negative
CD68	Negative
HepPar-1	Negative

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Methylation Array Results

CUP methylation classifier results

Methylation Class	Score	Interpretation
lung	0.99	match

Methylation Subclass	Score	Interpretation
lung adenocarcinoma	0.96	match

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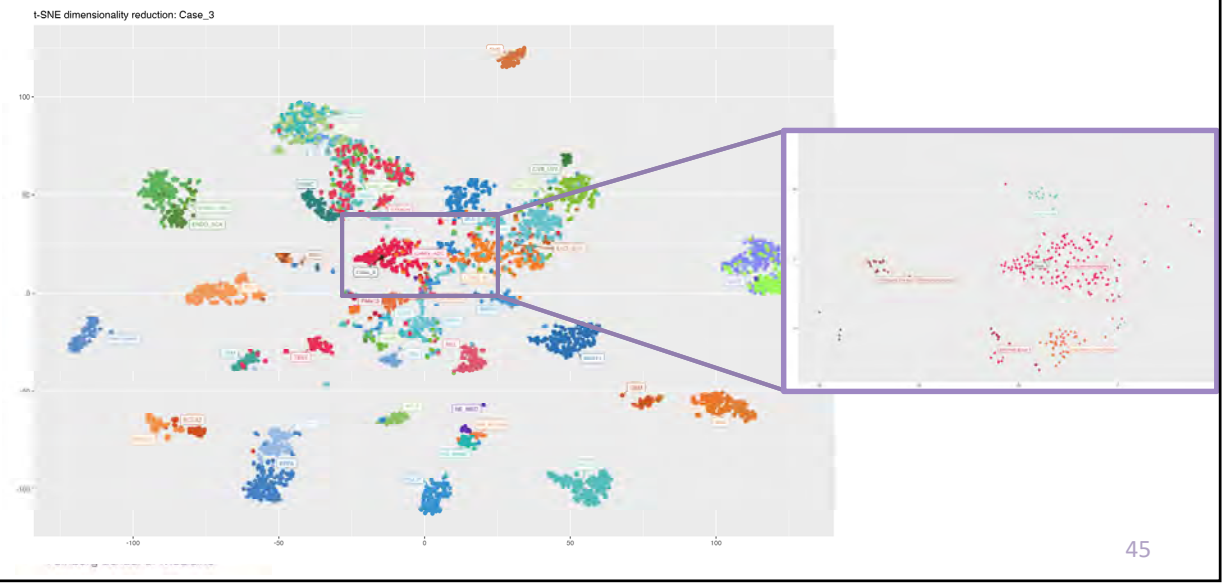
t-SNE plot



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Patient Follow Up

- Patient had a PET scan after pathology sign out showing a right middle lobe hyperintense region
- Patient will be following up at local hospital for biopsy of this lesion and treatment planning

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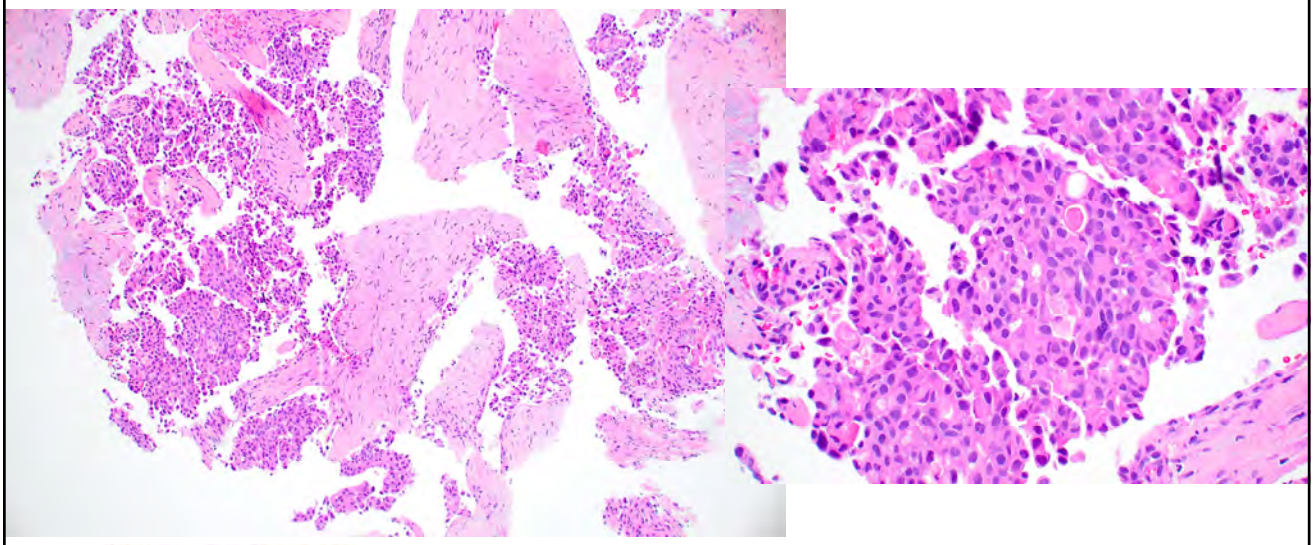
Case #3 Conclusions

- Being able to determine site of origin can change the outcome and prognosis for the patient
- Methylation profiling can be helpful when IHC fails to determine a possible site of origin
- In some instances, a site of origin as well as a tumor type can be identified by methylation profiling, such as in this case
- This can help guide further workup for targeted chemotherapy options

Case #4

- 81 y/o male with history of prostate adenocarcinoma for four years which has been stable on androgen deprivation therapy presents with an enlarging liver mass
- Patient underwent biopsy of the liver mass

Liver Core Biopsy Specimen



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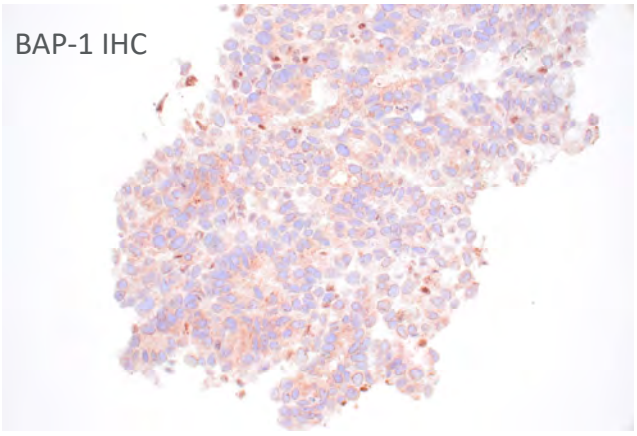
Immunohistochemical workup

BAP-1 IHC



Stain	Result
CK7	**Positive**
CK20	Predominantly Negative
CDX2	Negative
TTF-1	Negative
GATA3	Very rare positive cells
HepPar-1	Negative
Arginase	Negative
NKX3.1	Negative

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Methylation Array Results

CUP methylation classifier results

Methylation Class	Score	Interpretation
liver	0.63	no match

Methylation Subclass	Score	Interpretation
hepatocellular carcinoma	0.38	no match
intrahepatic bile duct (cholangiocarcinoma)	0.25	no match

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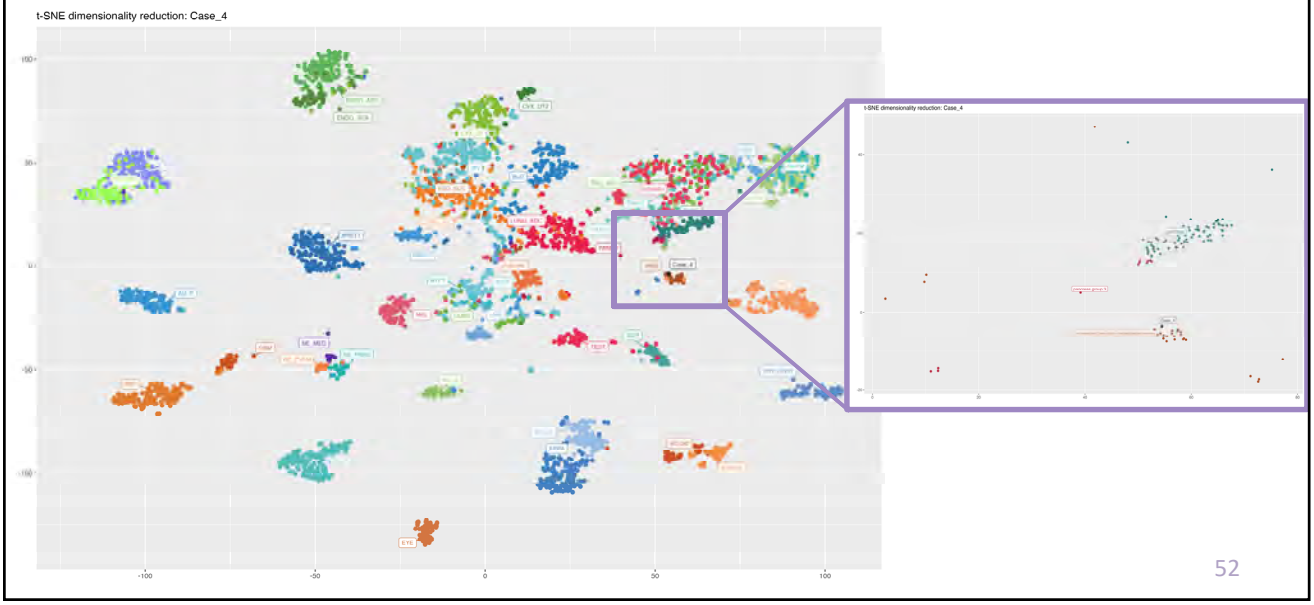
t-SNE unsupervised analysis



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Molecular Sequencing Findings

Genomic Signature

Signature	Status/Score
Microsatellite Analysis	MS-Stable
TMB Muts/Mb (Sequenced)	8.5

Variants of known or potential clinical significance

Alteration	Variant	Allele Proportion	Drugs Associated with Sensitivity	Drugs Associated with Resistance
BAP1 p.(E198fs)	NM_004656.3 c.592dupG	30%	None	None

Copy Number Variants

None identified

Rearrangements

Alteration	Variant	Allele Proportion	Drugs Associated with Sensitivity	Drugs Associated with Resistance
CMSS1-FGFR2 Fusion	Introns 1-17	NA	Pemigatinib, Infigratinib	None

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Patient Follow-up

- Patient continues to have a low PSA and seems to have stable Prostatic disease
- New Liver lesion is being treated as a new cholangiocarcinoma and treatment plan is currently underway with oncology

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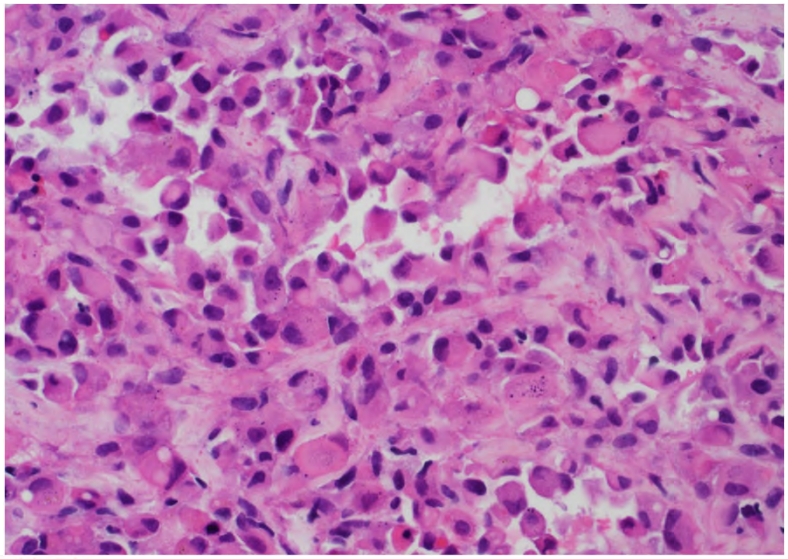
Case #4 Conclusions

- Utilizing methylation profiling in combination with sequencing findings may be helpful when methylation results do not match with a high confidence
- For rarer tumor types, the classifier results paired with the t-SNE may also be helpful to boost confidence in the lower confidence classifier call

Case #5

- 19 y/o female with no PMHx presented with left thigh pain
- Imaging revealed a 7.5 cm mass in the left semitendinosus and biceps femoris muscles
- Case was sent to our institution for consultation (Children's hospital consultation)

Morphology on Biopsy



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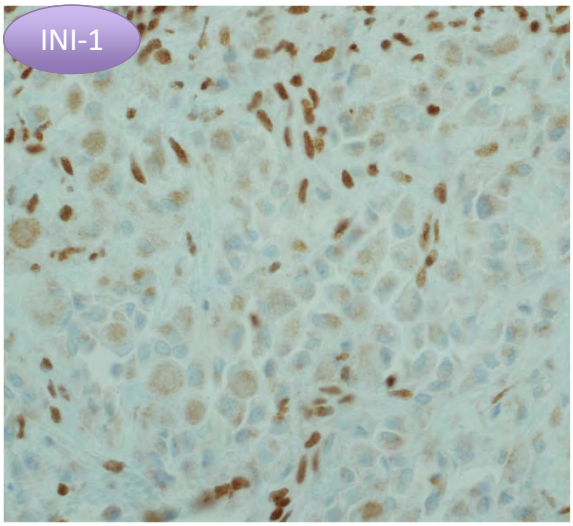
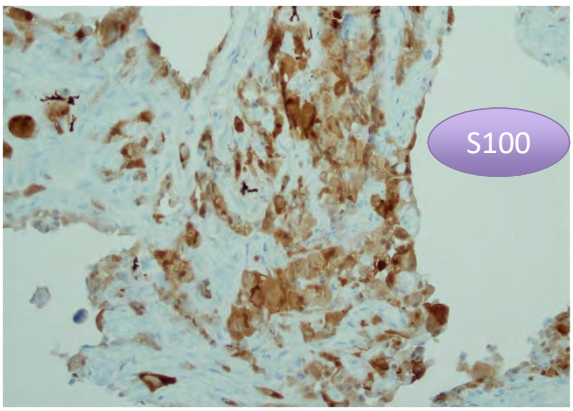
Immunohistochemical Staining





Stain	Result
AE1/AE3	Diffusely positive
S100	Patchy positive
INI-1	Lost nuclear expression

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Methylation Array- Sarcoma Classifier

Sarcoma tumor methylation classifier results

Methylation Class	Score	Interpretation
epithelioid sarcoma	0.61	no match
chordoma (dedifferentiated)	0.11	no match

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t-SNE

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Patient Follow-up

- Patient is currently undergoing treatment following clinical guidelines for treatment of epithelioid sarcoma

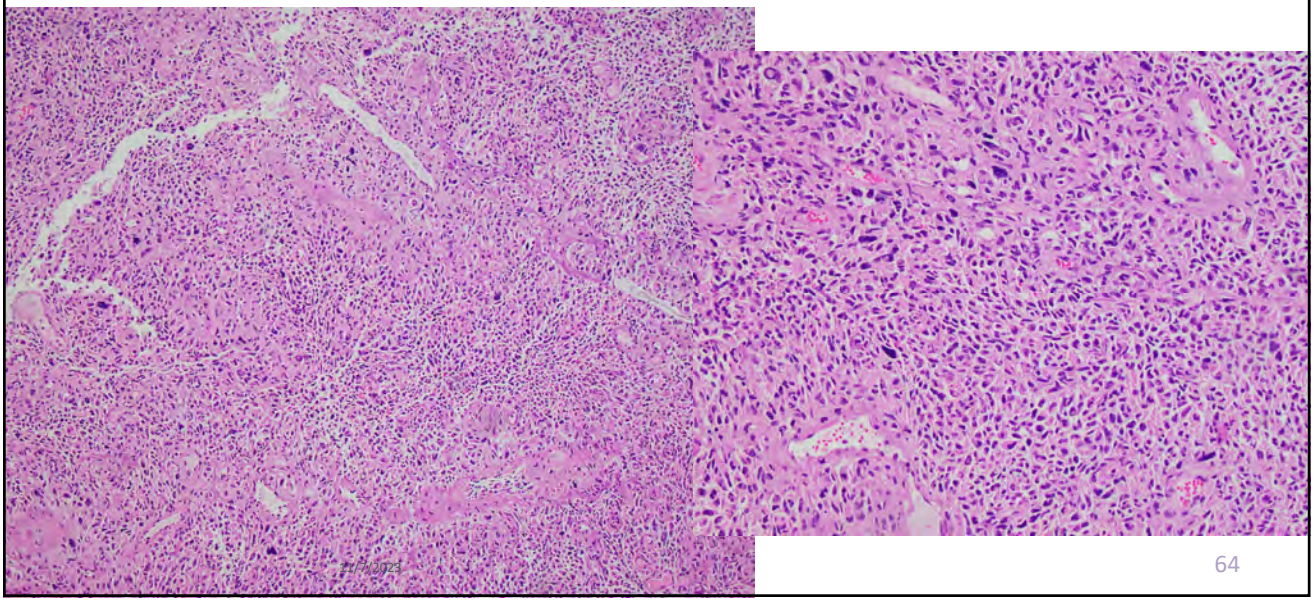
Case #5 Conclusions

- Utilizing methylation profiling in combination with IHC findings may be helpful when methylation results do not match with a high confidence as INI-1 loss is a common finding in epithelioid sarcoma
- Rare sarcomas may not match with as high confidence as more common sarcomas due to the low numbers used in validation of the classifier- your classifier is only as good as the data you use to generate it!

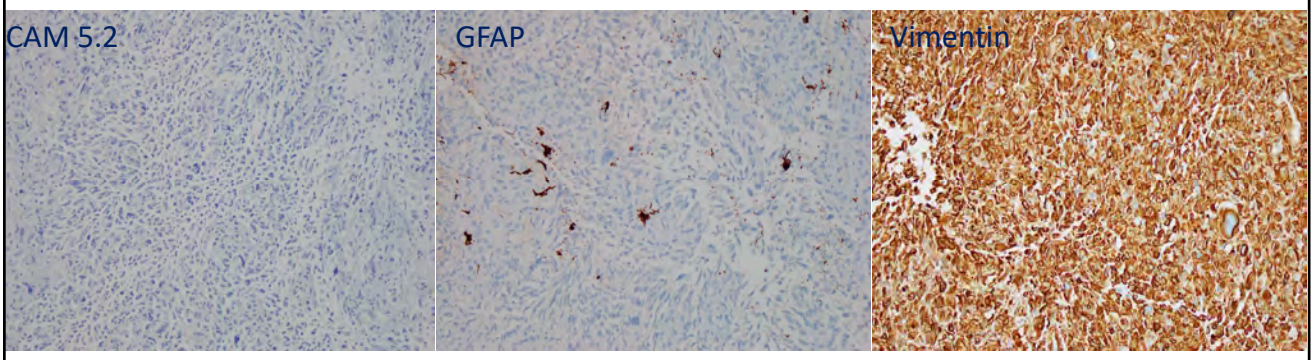
Case #6

- 63 y/o female who presented to ER with lethargy
- History of extra-osseous osteosarcoma of the chest wall about a year prior to presentation that was treated with genzar.
- Molecular findings of the osteosarcoma showed a high tumor mutational burden.
- Imaging in ED showed three lesions in the brain suspicious for metastatic disease
- Resection of two of those lesions was performed and tissue sent for examination.

Morphology



Immunohistochemistry



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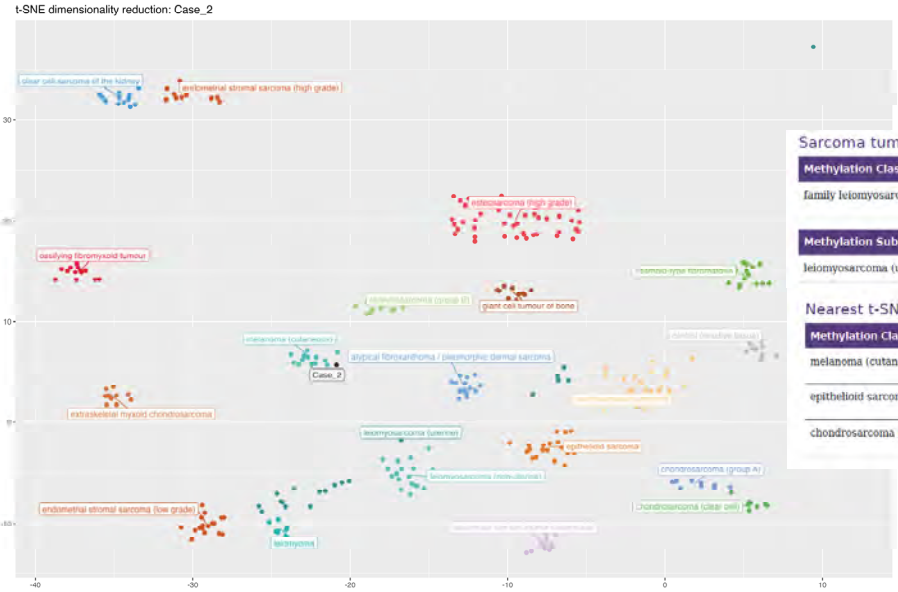
Sarcoma Classifier



Sarcoma tumor methylation classifier results

Methylation Class	Score	Interpretation
family leiomyosarcoma	0.18	no match

Methylation Subclass	Score	Interpretation
leiomyosarcoma (uterine)	0.17	no match

Nearest t-SNE Clusters

Methylation Class	Distance
melanoma (cutaneous)	2.421
epithelioid sarcoma	6.701
chondrosarcoma (group B)	8.628

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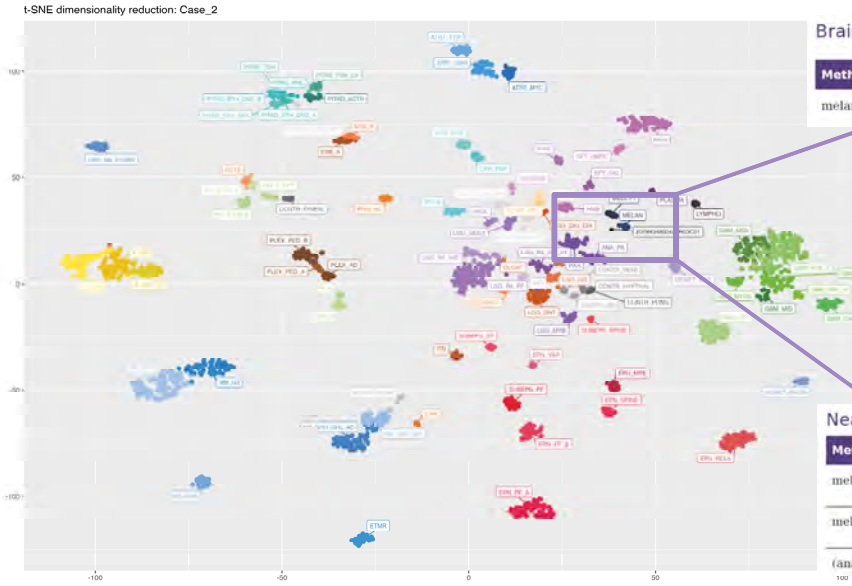
CNS Classifier



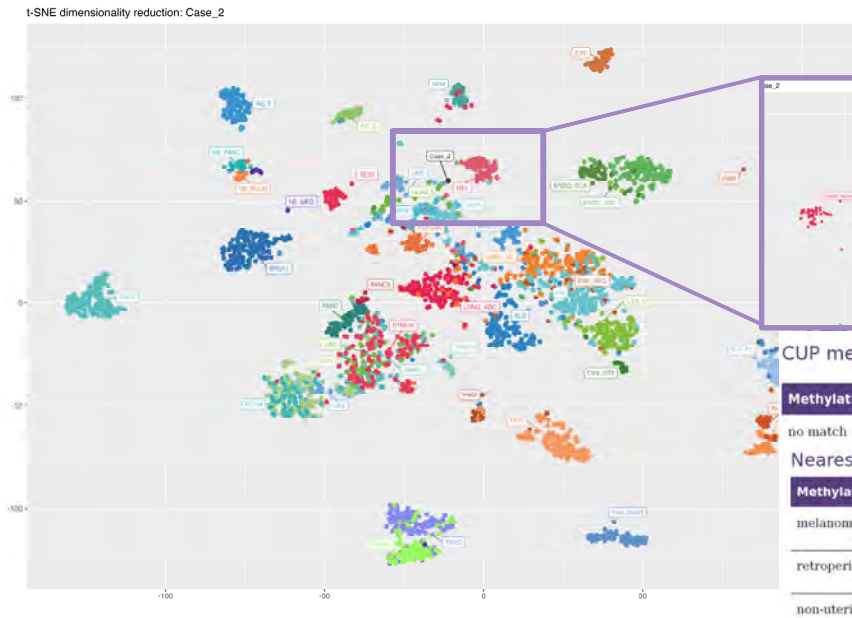
Brain tumor methylation classifier results

Methylation Class	Score	Interpretation
melanoma	0.12	no match

Nearest t-SNE Clusters

Methylation Class	Distance
melanoma	3.163
melanocytoma	6.806
(anaplastic) pleomorphic xanthoastrocytoma	12.223

Carcinoma of Unknown Primary Classifier



CUP methylation classifier results

Methylation Class	Score	Interpretation
no match	0.00	no match

Nearest t-SNE Clusters

Methylation Class	Distance
melanoma	8.870
retroperitoneum	15.702
non-uterine leiomyosarcoma	16.727

Molecular Results

NF1 mutant melanomas show high tumor mutation burden and are mutually exclusive to BRAF and NRAS mutation.

They are more common in desmoplastic melanomas

Patient did have a history of high TMB osteosarcoma as well

Genomic Signature

Signature	Status/Score
Microsatellite Analysis	MS-Stable
TMB Muts/Mb (Sequenced)	43.9

Variants of known or potential clinical significance

Alteration	Variant	Allele Proportion	Drugs Associated with Sensitivity	Drugs Associated with Resistance
TERT	NM_198253.2 c.-146C>T	26%	None	None
ATM p.(R1730*)	NM_000051.3 c.5188C>T	26%	Olaparib	None
NF1 p.(G949*)	NM_001042492.2 c.2845G>T	22%	None	None
NF1 p.(Q282*)	NM_001042492.2 c.844C>T	23%	None	None
HNF1A p.(R200Q)	NM_000545.5 c.599_600delGGinsAA	16%	None	None

Copy Number Variants

Alteration	Variant	Copy Number	Drugs Associated with Sensitivity	Drugs Associated with Resistance
CCND3 Amplification	Amplification	7.0 copies	None	None
ERCC5 Loss	Loss	0.0 copies	None	None
PTCH1 Loss	Loss	0.0 copies	None	None
RASA1 Loss	Loss	0.0 copies	None	None

Rearrangements
None identified

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Patient Follow-up

- After additional clinical history was asked, it was found the patient had a history of melanoma on her back that was excised a few years earlier.
- After molecular studies this case was re-reviewed by surgical pathologists and was found to be positive for HMB45 and MelainA
- Patient was treated for metastatic melanoma and is currently undergoing this therapy

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Case #6 Conclusions

- Utilization of multiple classifiers can aid in determining a diagnosis
- While t-SNE alone is not a clinically validated test it can be very helpful in cases of no matches on the random forest classifier
- Correlation of findings with molecular sequencing results can boost the confidence in a t-SNE and low match on a classifier
- Confident in calling it not metastatic osteosarcoma

Summary

- Building a methylation classifier is possible but needs a lot of well annotated data
- Bioinformatics support for development and maintenance of the classifiers is vital for success
- Expansion of classification to sarcomas and carcinomas of unknown primary have clinical utility and can be helpful in directing patient management
- Should be utilized as an additional ancillary test in the context of the other data present to make a clinical decision

Thank You

Questions?

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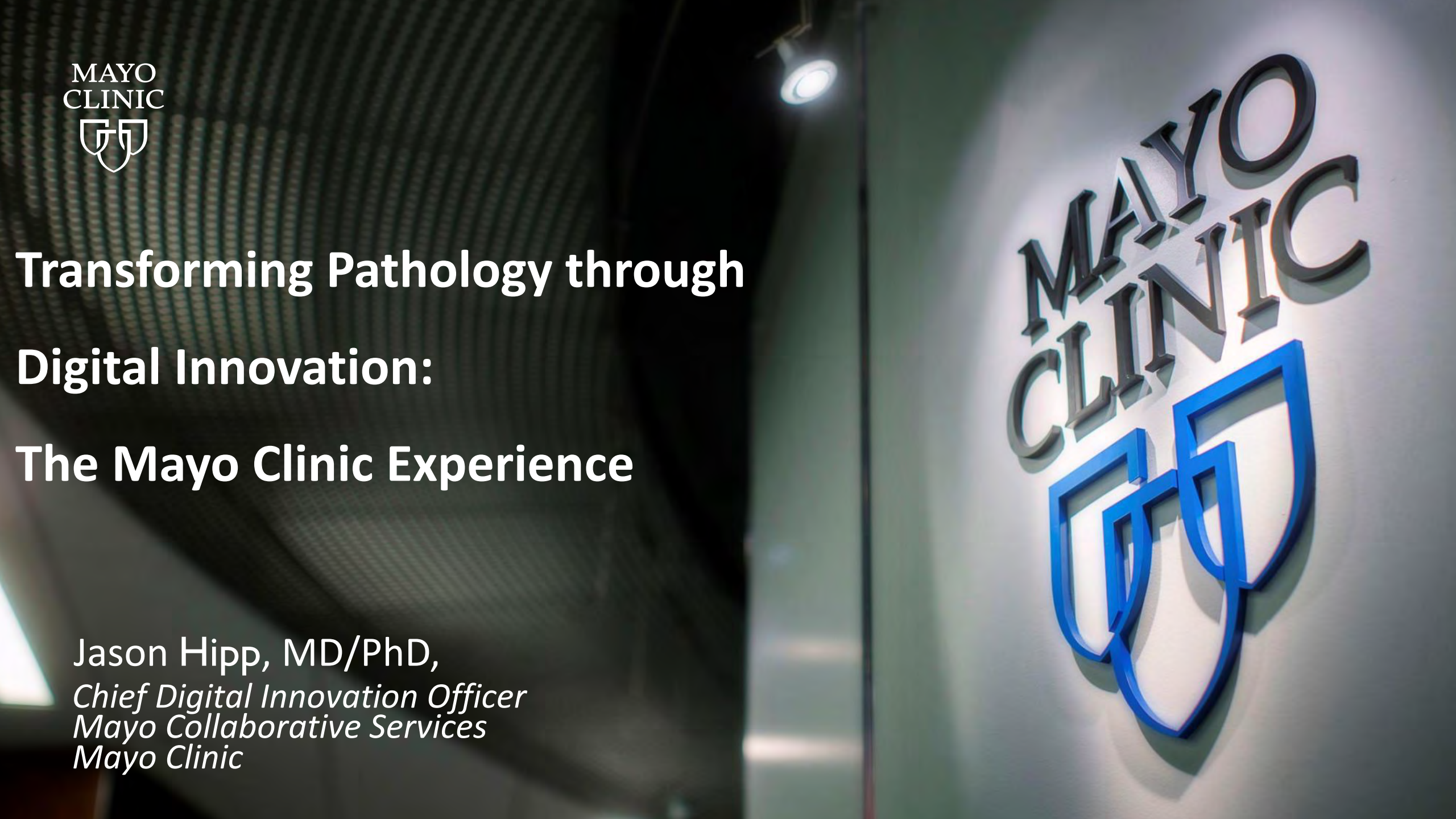

Transforming Pathology through Digital Innovation: The Mayo Clinic Experience

Jason Hipp, MD/PhD,
Chief Digital Innovation Officer
Mayo Collaborative Services
Mayo Clinic

Agenda

- Background & Introduction
- Digital and Computational Pathology at Mayo Clinic
  - Clinical Practice
  - Scanning the tissue archive
- Innovation at Mayo Clinic
  - New division of Computational Pathology & AI
  - AI and colon cancer prognosis
  - A tissue image and molecular searchable atlas
- Future of Digital and Computational Pathology
  - Fresh tissue 3D imaging
  - Multiplex IF and unsupervised learning
- Questions

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- Questions

"The PACS revolution in radiology was much more than digitizing film. It enabled entirely new workflows, AI enabled decision support, and an ecosystem of innovative companies.
Digital Pathology is much more than digitizing glass slides. We will empower specialty consultation at a distance, digital staining, and 3D scanning of tissue which will benefit patients, providers, and pharmaceutical drug discovery."

Dr. John Halamka, MD

THE TRANSITION OF PATHOLOGY TO DIGITAL PATHOLOGY

- **What is digital pathology:**
  - An **imaging sub-specialty** of pathology informatics
  - Is a dynamic, image-based environment enabled by Whole Slide Imaging (WSI) scanners– convert glass slides into digital slides that are viewed, managed and analyzed on a computer monitor

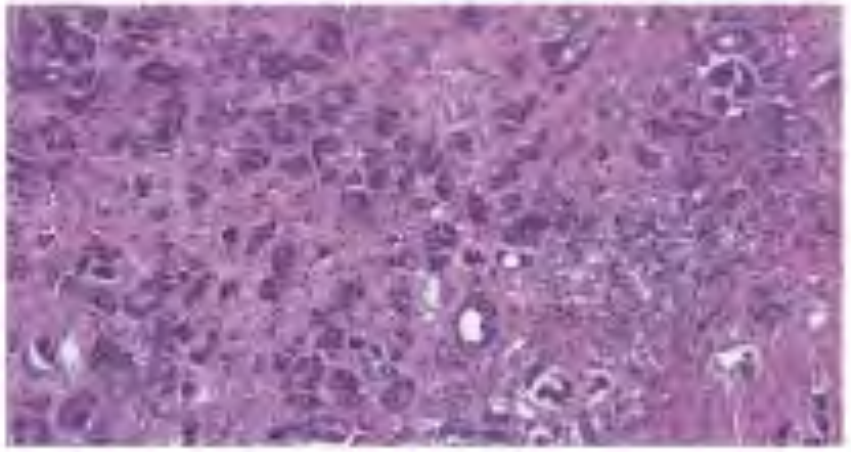
- **What is computational pathology**
  - the ‘omics’ or ‘big-data’ approach to pathology, where multiple sources of patient information including pathology image data and **meta-data** are combined to extract patterns and analyze features*

*https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6852275/

TRADITIONAL PATHOLOGY VS COMPUTATIONAL PATHOLOGY

Traditional pathology is limited and subjective…

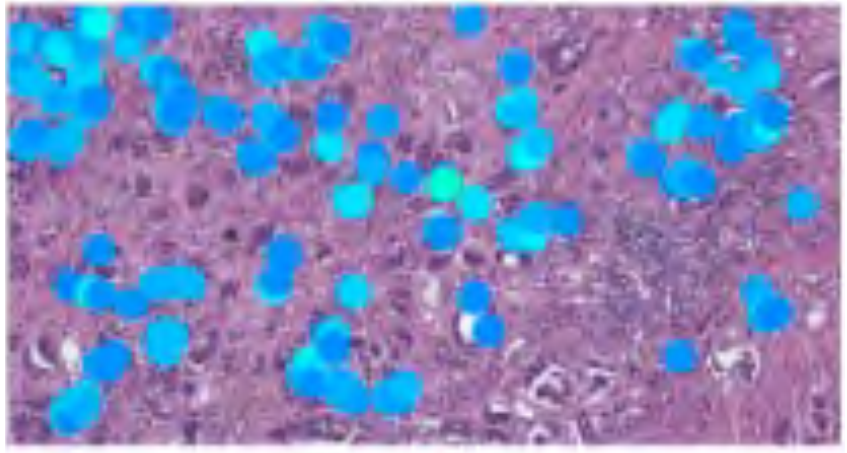
- Tissues and cells are examined under a microscope
- Subjective, qualitative and semi-quantitative assessments about the tissue
- Qualitative and semi-quantitative interpretation in pathology report
- human brain looks for specific patterns and ignores non confirmatory data



Significant amount of data from tissue remains un-utilized or under-utilized

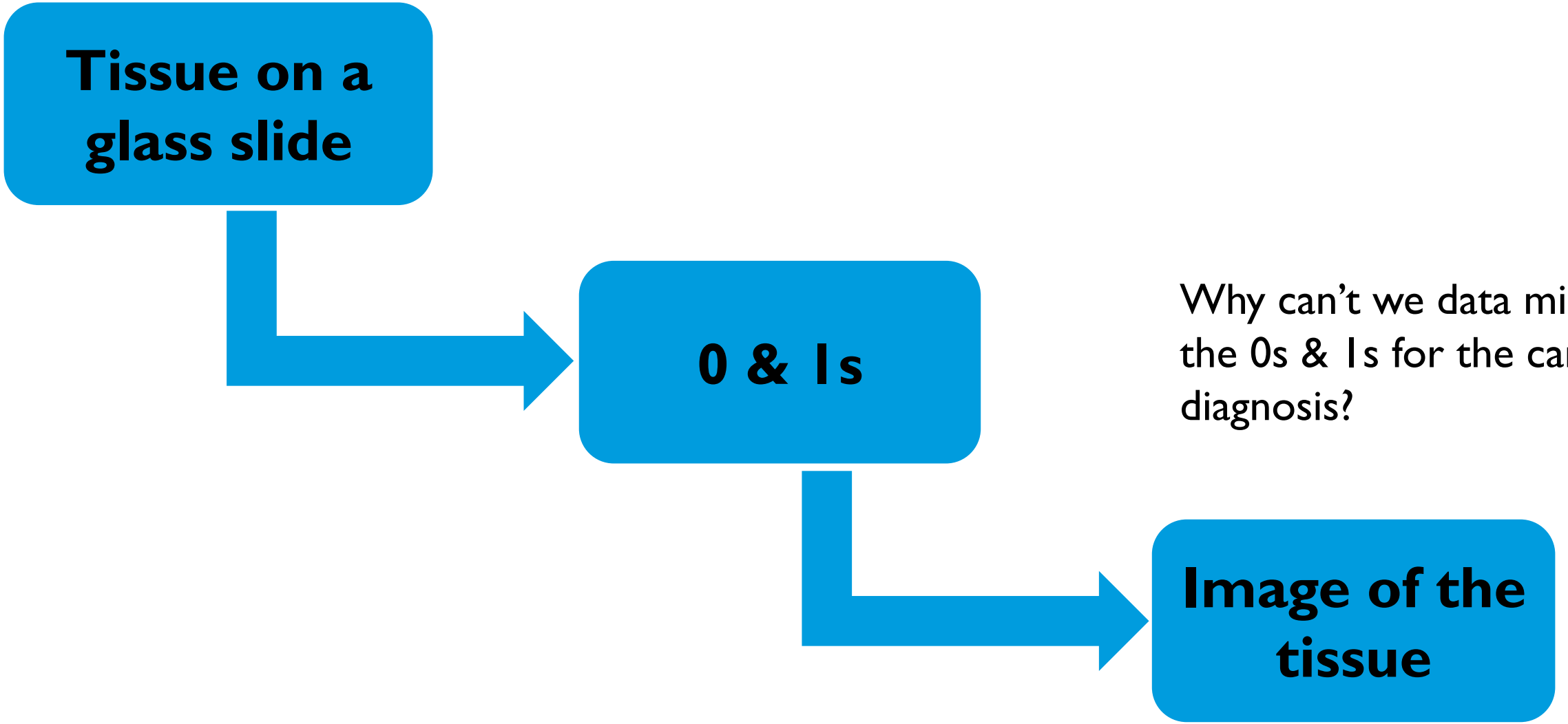
Computational pathology is robust and data-driven

- Tissues and cells are examined by computer algorithms
- Robust, highly quantitative and complex spatial measurements generated for every pixel/feature
- The numerical results integrated with disparate datasets and analyzed by secondary analytic tools
- Computer brain 'looks' at everything, can identify novel features



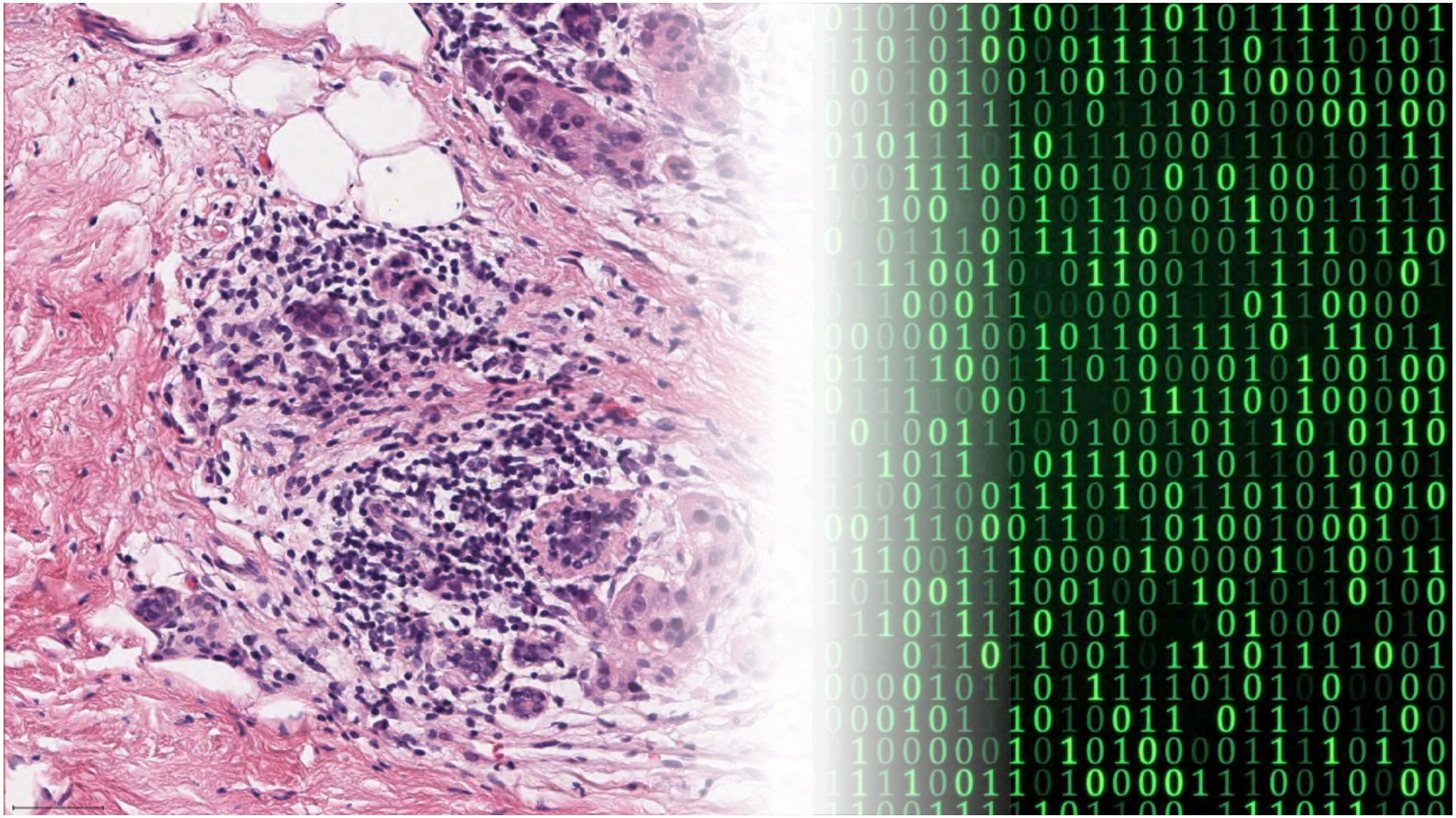
Most data from tissue is being utilized

It Allows New Opportunities To Carry Out Numerical Queries Of Pathology Images



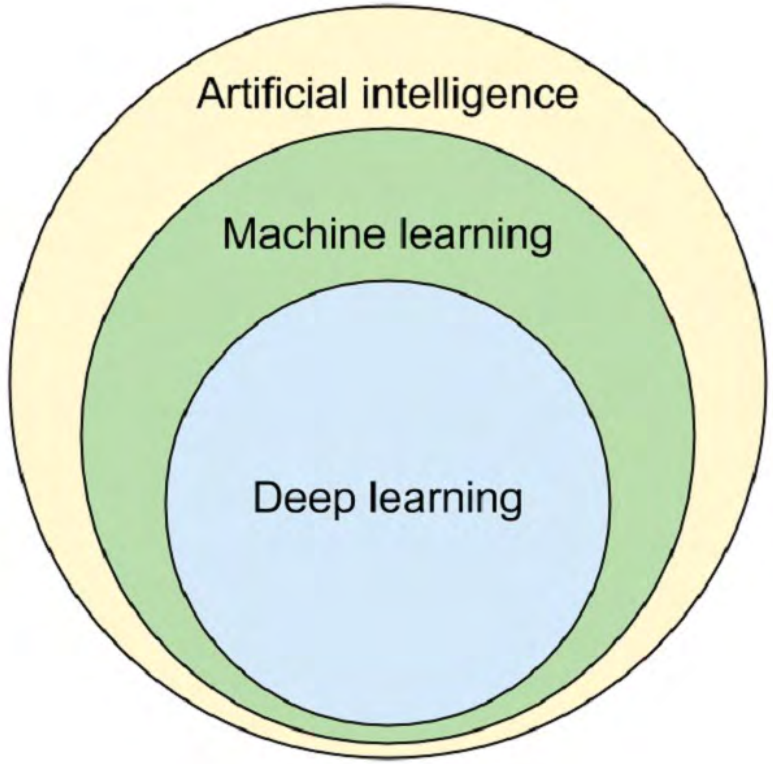
Why can't we data mine the 0s & 1s for the cancer diagnosis?

From Qualitative to Quantitative



THE POTENTIAL WITH COMPUTATIONAL PATHOLOGY

- Whole Slide Imaging (WSI) the production of digital images from pathology glass slides that create giga-pixel images.
- Artificial intelligence (AI) a branch of computer science in which machine-based approaches are used to attempt to make a prediction — emulating what an intelligent human might do in the same situation.
-
- Machine learning (ML)-involves the machine 'learning' from data that are fed into it in order to make a prediction, (falls under the broad umbrella of AI.)
- Deep learning (DL). A DL network typically comprises multiple layers of artificial neural networks and tends to include an input layer, an output layer and multiple hidden layers. The hidden layers are used to generate new representations of the image and, with a sufficient number of training instances, can be used to identify the representations that best distinguish categories of interest.



https://upload.wikimedia.org/wikipedia/commons/f/fe/Fig-X_All_ML_as_a_subfield_of_AI.jpg

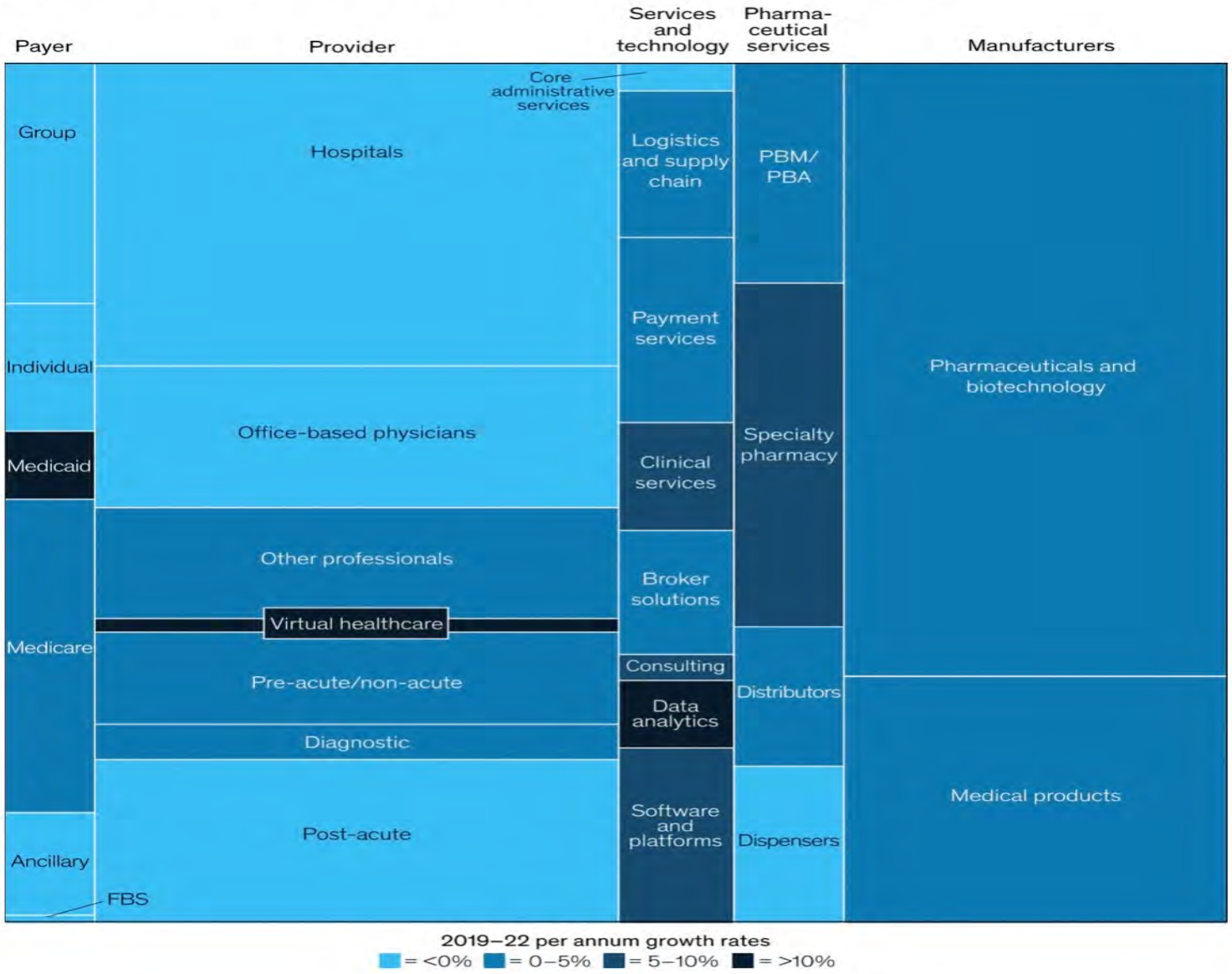
THREE TECHNOLOGICAL FACTORS:
CLOUD + BIG DATA + AI
UNIQUE JUNCTURE IN TIME

https://www.nasa.gov/press-release/nasa-announces-television-coverage-for-aug-21-solar-eclipse

ONE OF FEW AREAS IN HEALTHCARE PREDICTED TO SEE GROWTH

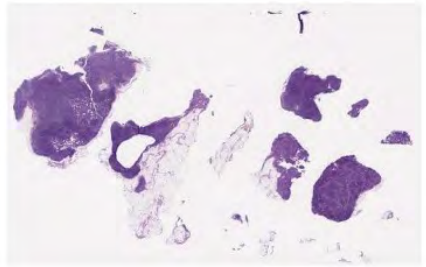
Medicaid, virtual healthcare, and data analytics are among segments offering potential for outpaced growth.

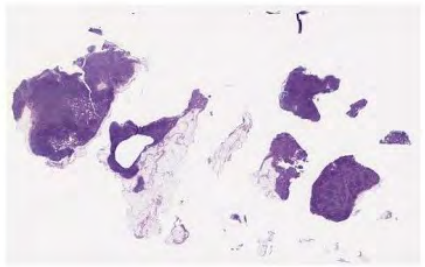
Distribution of projected healthcare EBITDA across healthcare segments, 2022, %

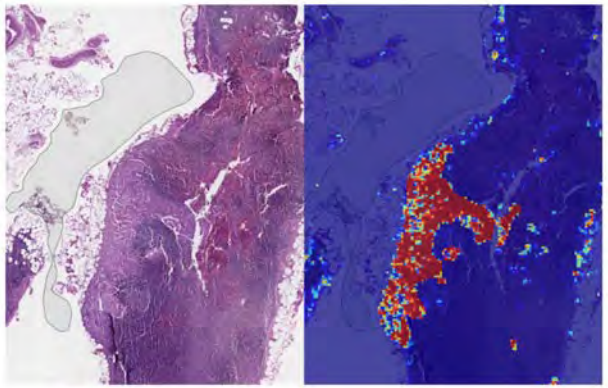


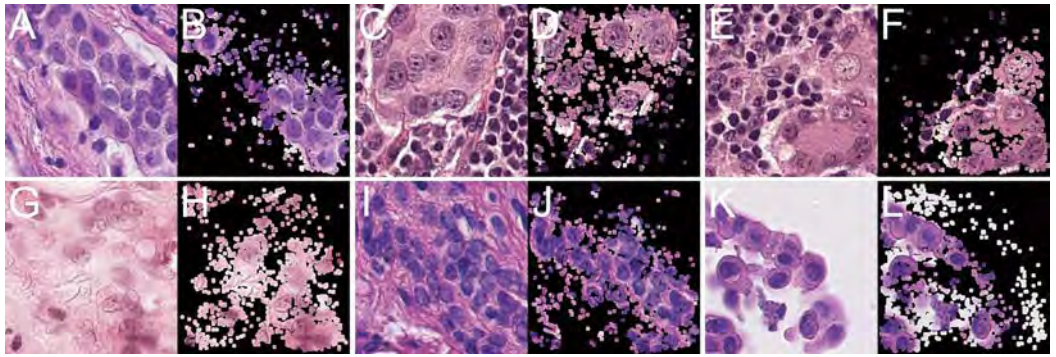
EBITDA, earnings before interest, taxes, depreciation, and amortization; FBS, fixed-benefit and supplemental; PBA, pharmacy benefit administrator; PBM, pharmacy benefit manager.
Source: McKinsey Profit Pools Model

DETECTION OF METASTATIC BREAST CANCER IN LYMPH NODES WITH AI





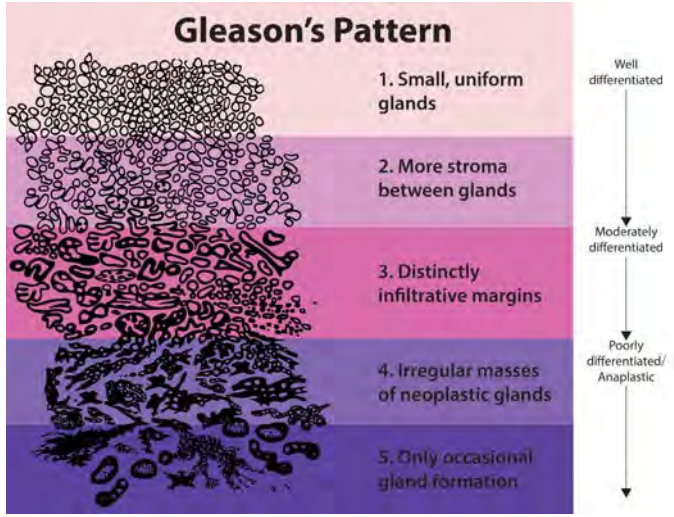


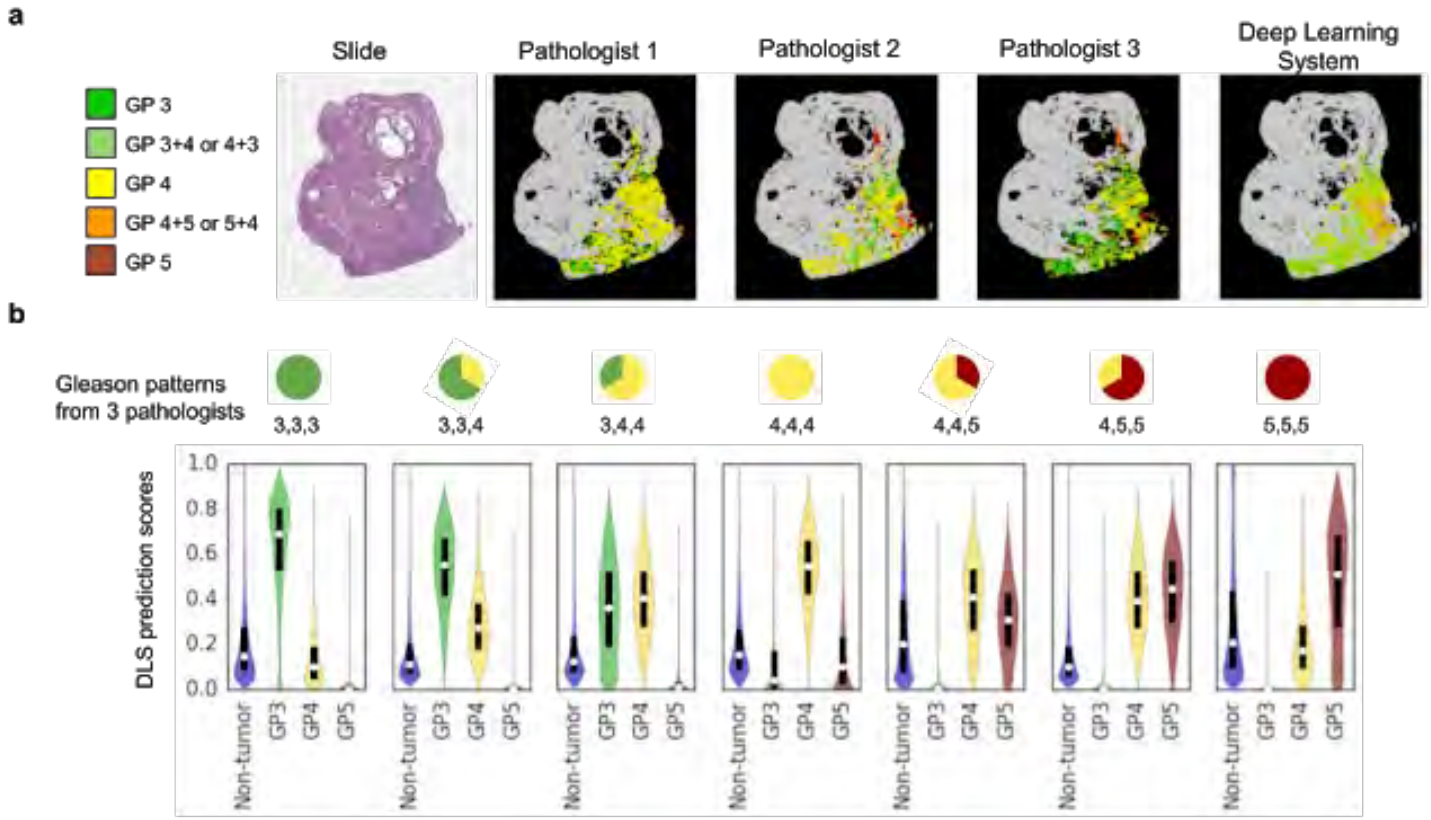


Google Health:

Yun Liu et al. Arch Pathol Lab Med. 2018;143(7):859-868. doi:10.5858/arpa.2018-0147-OA

IMPROVED GRADING OF PROSTATE CANCER WITH AI

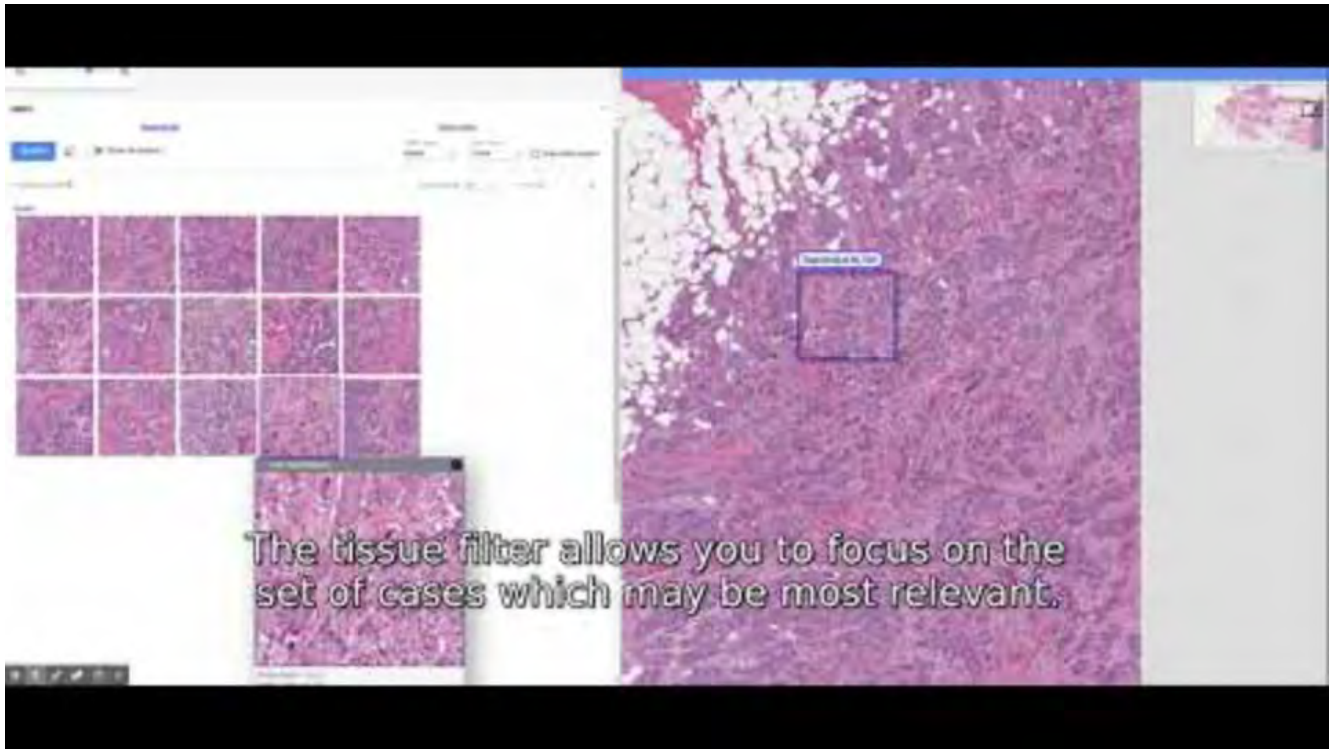


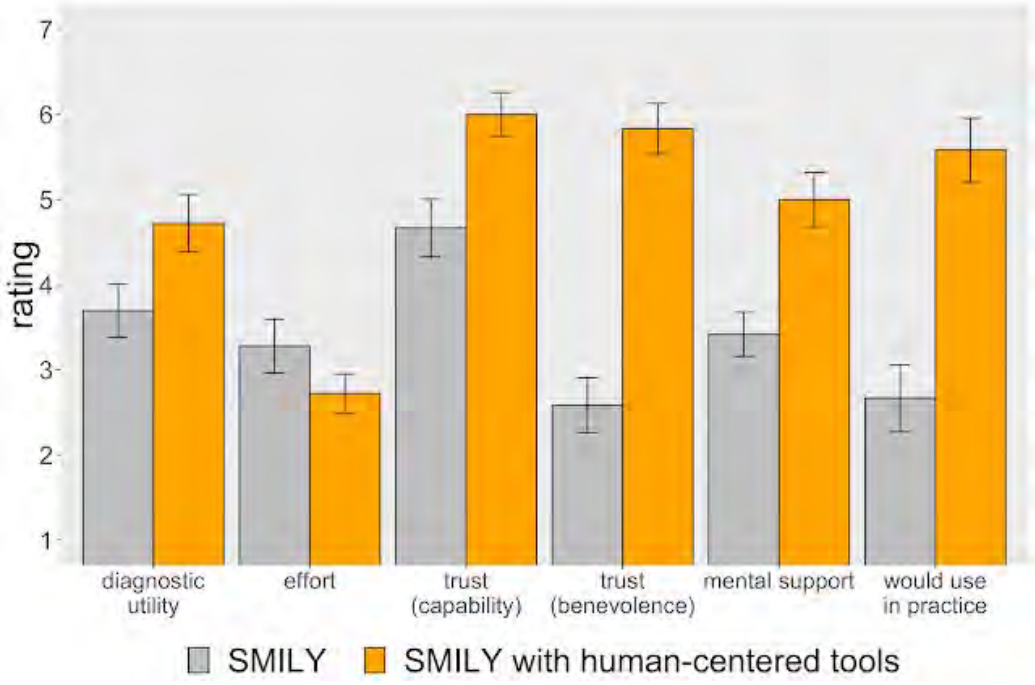


Google Health:

Kunal Nagpal *et al.* Development and validation of a deep learning algorithm for improving Gleason scoring of prostate cancer. *npj Digit. Med.* **2,** 48 (2019)

PATHOLOGY IMAGE SEARCH WITH AI





Google Health:

Narayan Hegde *et al.* Similar image search for histopathology: SMILY. *npj Digit. Med.* **2,** 56 (2019)
https://ai.googleblog.com/2019/07/building-smily-human-centric-similar.html

AUGMENTED REALITY MICROSCOPE

Google Health:

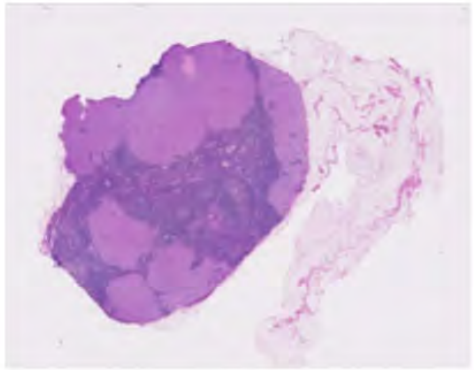
Chen, P.C., Gadepalli, K., MacDonald, R. *et al.* An augmented reality microscope with real-time artificial intelligence integration for cancer diagnosis. *Nat Med* **25,** 1453–1457 (2019)

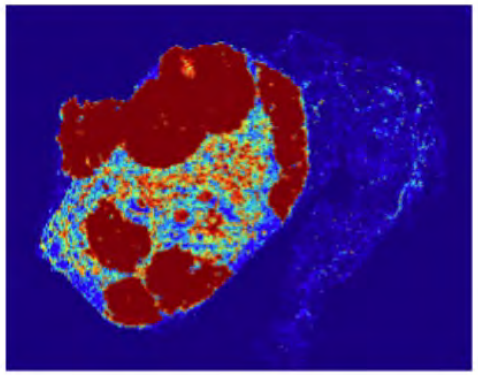
RECENT DEVELOPMENTS IN FIELD OF CPAI

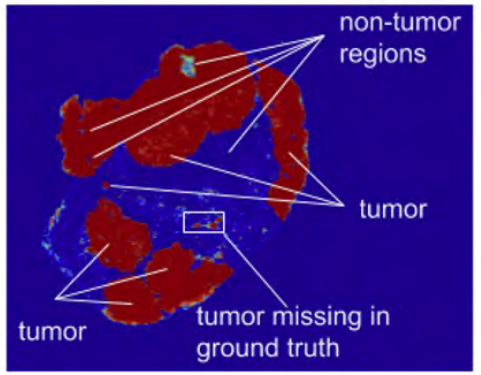
- Identify ROIs
- Presence/absence of cancer
- Tumor Grading
- Quantify IHC
- Identify similar cases

- *Predict*
  - *Mutation status from H&E*
  - *IHC from H&E*
  - *'risk' category*
  - *survival*

The revival of the H&E with AI
Burlutskiy et al. Journal of Clinical & Anatomic Pathology (2020)







Different types of AI algorithms will enhance clinical practice

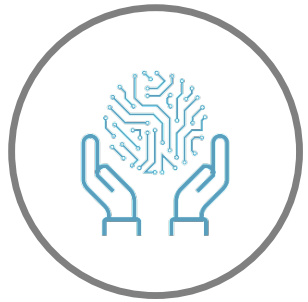
Detect/Triage

Workflow Tools

Quantify

Image Analysis Tools

Predict

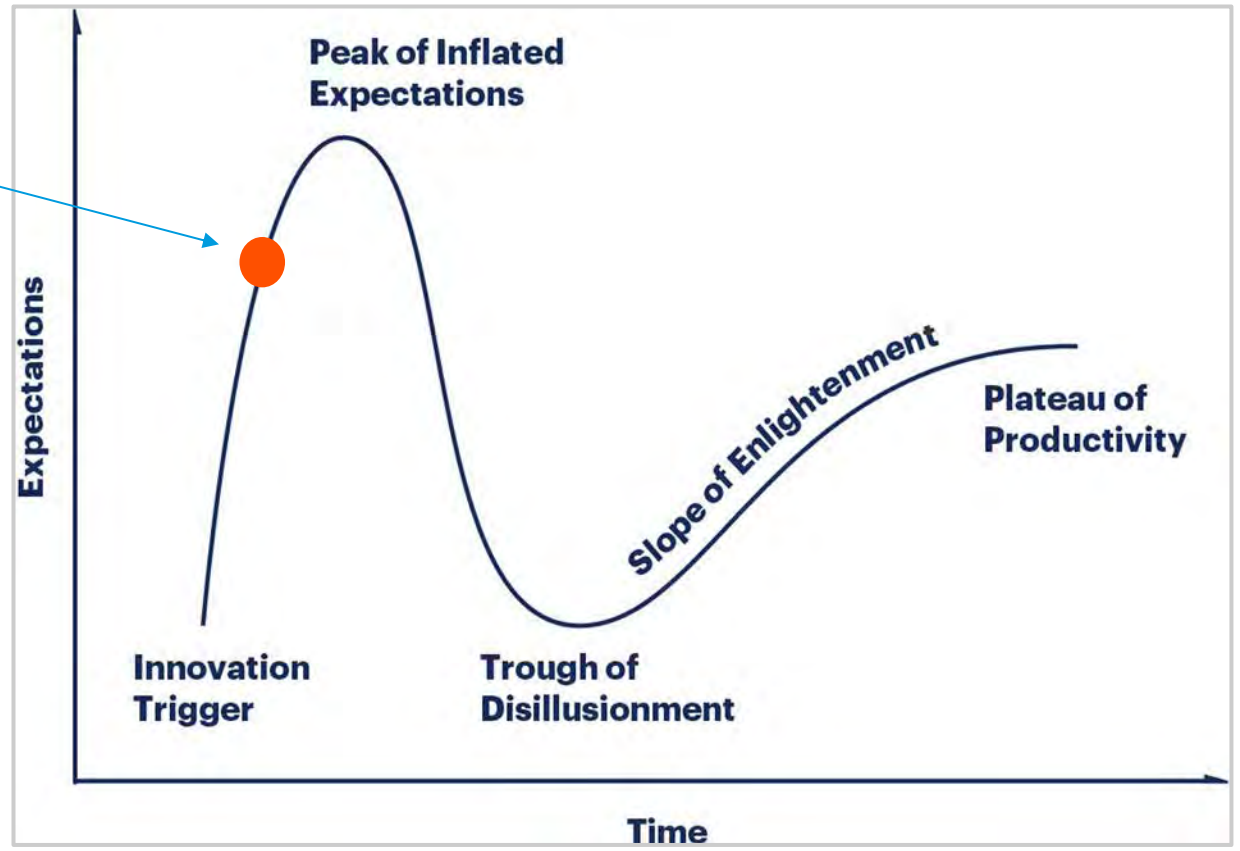


Predictive Tools

Risk/ Reward

Computational Pathology Is Early Stage

We are HERE



https://www.gartner.com/en/research/methodologies/gartner-hype-cycle

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  - Multiplex IF and unsupervised learning
- Questions

History of Innovation & Excellence

1892 : Non-Mayo physicians join, creating first INTEGRATED MULTISPECIALTY GROUP PRACTICE

1905 : Louis Wilson, M.D., Mayo's first researcher, develops FROZEN SECTION PATHOLOGY

1907 : Henry Plummer, M.D., introduces the concept of INTEGRATED MEDICAL RECORDS

1950 : Nobel prize for ISOLATION OF CORTISONE

1969 : First TOTAL HIP in United States

1971 : Creation of MAYO MEDICAL LABORATORIES (MML)

1985 : Creation of MAYO MEDICAL VENTURES

1986 : Creation of MAYO CLINIC FLORIDA (MCF)

1987 : Creation of MAYO CLINIC ARIZONA (MCA)

1992 : Creation of MAYO CLINIC HEALTH SYSTEM (MCHS)

1999 : **Discovery of BASIS OF PD-L1**

2000 : First commercially available rapid ANTHRAX DETECTION KIT DEVELOPED BY MAYO AND ROCHE

2014 : FDA approval of COLOGUARD CANCER DETECTION KIT

2015 : Opening of ONE MAYO SQUARE IN MINNEAPOLIS

2016 : Creation of DEPARTMENT OF BUSINESS DEVELOPMENT

2017 : Partnerships with:
QRATIV – Start-up launched in collaboration with n*f*erence
NDSC – Development of new patient blood-management solution

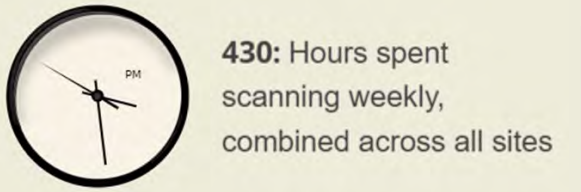
2018 : Unity Biotechnology IPO
WUXI - MedTech Insight Award for Best Partnership/Alliance
CIVICA RX – not-for-profit generic drug coalition

2019 : Partnership with GOOGLE

2020 : Mayo Clinic Platform
Center for Digital Health
Mayo Clinic International
Partnership with N*F*ERENCE

2021: Joint acquisition of Medically Home

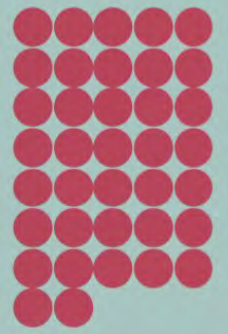
Digital pathology in the Practice



430: Hours spent scanning weekly, combined across all sites

190 Thousand On average, approximately 190,000 slides are scanned monthly across all Mayo Clinic sites

38 Enterprise- wide positions created to support digital pathology



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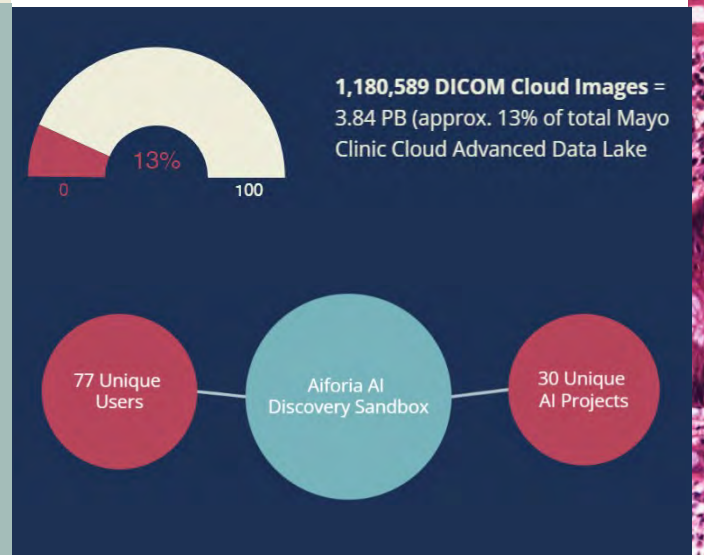
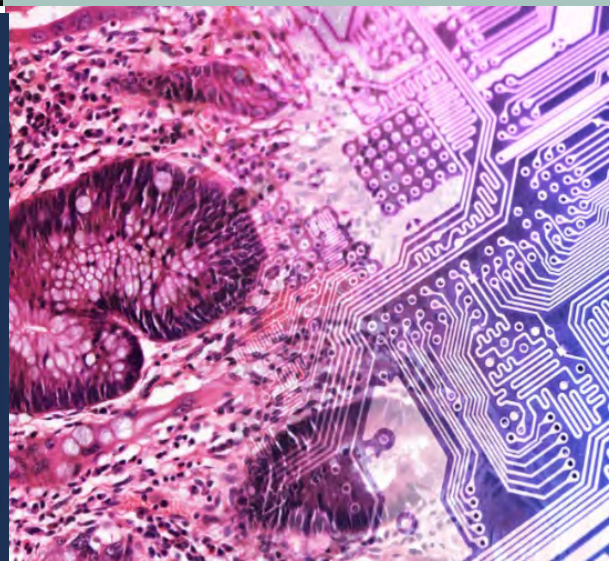
Leica GT450 Scanners Across all sites





State of the art scanning creates the foundation for digital excellence

Image Management Sytem: On premise, Sectra deployment. We have a large multisite single instance shared across our geographically dispersed hospital system

Tissue Registry program is digitizing our slide archive

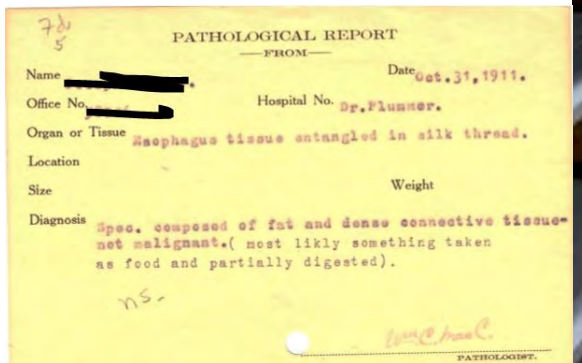
Plus an advanced tissue digitization program

Mayo Clinic has an archive of over 25 million tissue glass slides
The Practice plans to digitize 12 million of these slides in the next 1.5 years.

The digitized data will be stored in a cloud-based image repository to support clinical, research and education use

Clinical: Enhance quality of patient care
Research: Develop AI algorithms to enable automation of highly manual lab processes
Education: Provide ready access to indexed, searchable, high-value content

In addition, 3 million historical paper pathology reports will also be digitized to support longitudinal datasets that match with pathology slides



PATHOLOGICAL REPORT
—FROM—
Name [redacted] Date Oct. 31, 1911.
Office No. [redacted] Hospital No. Dr. Plummer.
Organ or Tissue Esophagus tissue entangled in silk thread.
Location
Size Weight
Diagnosis Spec. composed of fat and dense connective tissue- not maligmant.(most likly something taken as food and partially digested).
ns.
PATHOLOGIST.

Mayo's effort to focus on in-line automated quality assessment and corrections for archival scanning

In-line Automated Scan Quality Assessment and Correction for Archival Histopathology Slide Scanning

Andrew P. Norgan, M.D., Ph.D.[1], Bryan J. Dangott, M.D.[2], Prasanth Perugupalli[3], Jason Ross[3]
Kurt E. Simon, M.B.A., PMP[1], Darin P. Morgan, Stephanie A. Derauf, PMP, and Thomas J. Flotte, M.D.[1]
[1]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, FL; nference, Inc., Cambridge, MA

Abstract

Background:

Academic medical centers maintain large repositories of histopathology slides for regulatory, clinical and scientific purposes. With the advent of digital pathology, there is increasing interest in digitizing archival slides to meet clinical, educational and scientific needs. However, such interest is often tempered by the significant costs associated with digitization and manual quality review of digitized slides.

Quality is a particularly salient issue when discussing archival scanning, because archives typically contain slides with variation in preparatory technique and differences in stain quality, cleanliness, debris, air bubbles, drying artifacts and other confounders that make high-quality scans difficult to achieve.

Correspondingly, archival scanning may require significant manual intervention (including re-scanning) to achieve acceptable successful quality at high throughput. Such a hands-on approach may be cost prohibitive in many situations, and automated quality assessment tools including those with the possibility for in-line correction of a scan may increase the practicality of archival scanning efforts.

Methods:

With a single human operator, we digitized 23,916 slides over 30 days (approximately 800 slides per day) using a research-use-only 4-head scanning system (nference, Inc). Slides were selected from the Mayo Clinic Pathology Tissue Archive and represented periods ranging from the 1950s to present. No cleaning or other preparatory steps were used to prepare slides for scanning.

Using on-scanner quality models, each slide was annotated in real-time for errors detected in focus, stitching, bubbles and folds, and other detectable errors (see Figure 1).

A subset of images from a quality control slide set (100 well-characterized slides) were also reviewed manually.

Automated Scan Error Detection

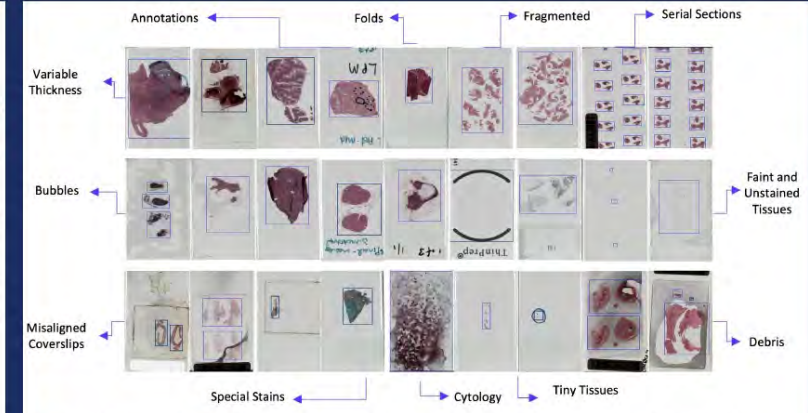


Figure 1. Detectable Features: In-line feature detection was capable of annotating and/or abstracting: 1) areas of variable tissue thickness and/or focus variation, 2) existing hand-written slide annotations, 3) tissue folds, 4) tissue fragmentation, 5) serial sections, 6) bubbles, 7) faded or unstained tissue, 8) misaligned coverslips, 9) special stains, 10) cytology preparations, 11) small or minute tissue fragments, 12) artifacts or debris, and 13) tile stitching artifacts.

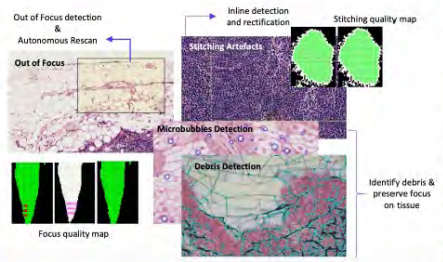


Figure 2. Feature Maps and Automated Correction: Select features, including focus and stitching, are abstracted to tile-based feature overlays. The feature overlays provide an "at a glance" snapshot of slide quality and can also be integrated into a numeric quality metric (if desired) to drive in-line or downstream quality control activities. In our testing, we used real-time on-scanner out-of-focus assessment to provide an automated trigger for autonomous rescan of the image with an increased density of focus points. While use of this quality control feature increased scanning time of out-of-focus scan slides by 3-6 seconds, it provided a significant improvement in 1st scan acceptability without human (manual) intervention.

Scanning Process

Figure 3. High Throughput Cluster: Scanning was accomplished using a a research use only cluster of 4 single slide line scanners using 40X zoom with 0.26 µm/pixel scan resolution. The 4 scanners are fed by an automated robotic arm from a single common slide tray. Each scanner is independent of the others and can complete scanning (or re-scanning as necessary) without disrupting the operation of its cluster partners.

Scanning features:
The scanners capture dynamic Z-stacks of each scan area and utilize Z-stacks to perform real-time focus assessment and continual refinement of the optimal focal plane. Real-time focal quality assessment allows for in-line quality control and autonomous re-scanning as needed. The scanners render final images as DICOM objects with lossy-compressed JPEG2000 pixel data.

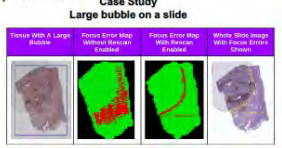


Figure 4. Quality assurance example: Automated in-line error detection initiates automatic re-scan to correct most errors at a tissue level without human intervention

Outcomes

- The system was able to successfully scan 99.4% of archival test slides. In total, 177 slides were rejected for scan quality. The most common reason for scan failure was inability to establish a tissue plane due to faint tissue, debris or protruding labels (n=137).
- Post-scan focus errors were detected algorithmically in 4035 slides (17%), with 4.21% of slides impacted over greater than 1% of the slide pixel area. Similarly, post-scanning stitching errors were algorithmically detected in >1% of pixel areas regions in 2.68% of slides.
- Manual review of 100 slides verified algorithmically detected errors. In rare instances (n=3), manual review detected focus, stitching or other errors that were not flagged by automated review.

Conclusions

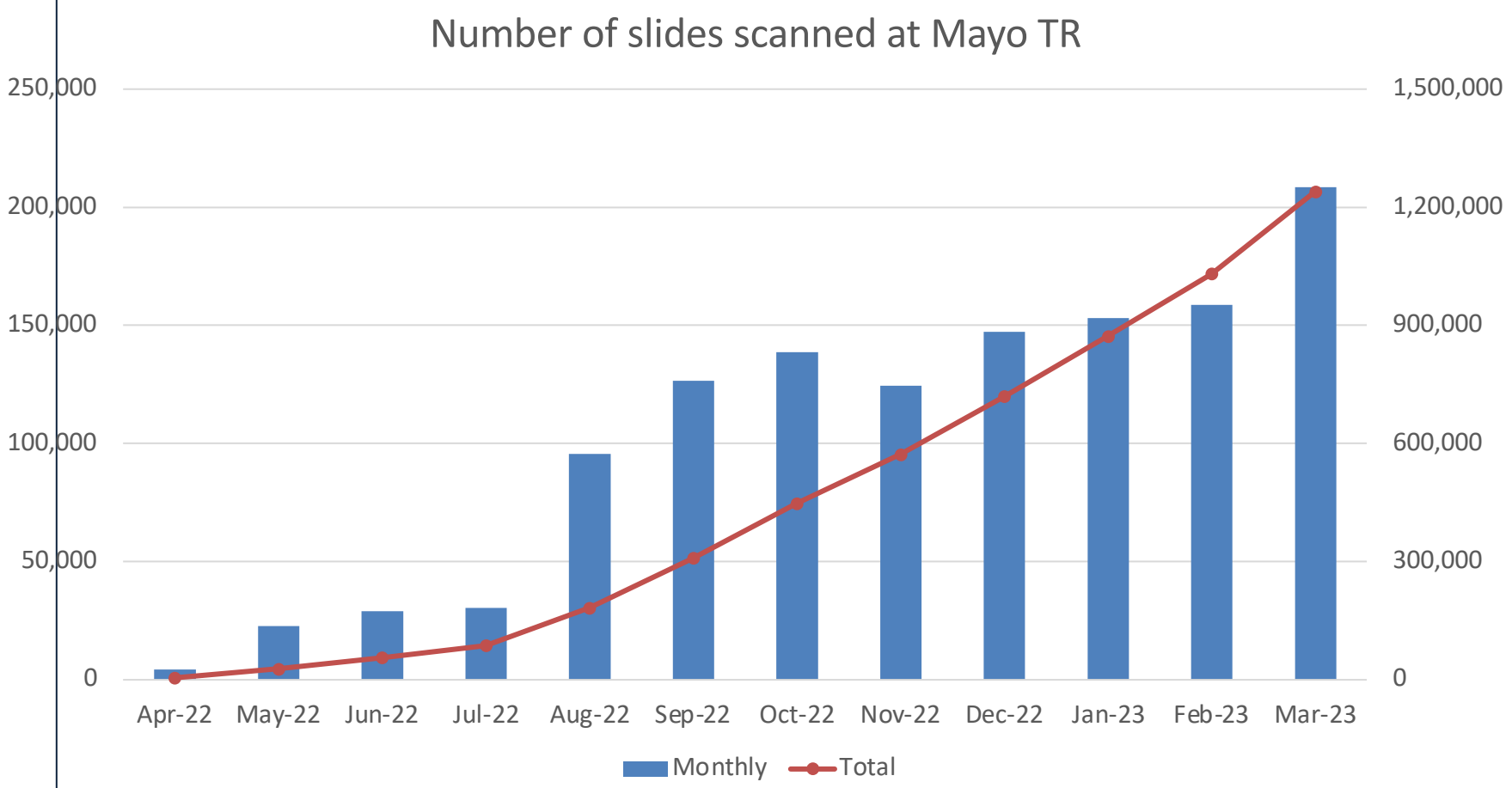
- In-line quality assessment allows for rapid recognition of quality defects in scanned archival tissue slides.
- Autonomous focus assessment can ameliorate focal defects while the slide is still on the scanner, potentially saving significant resources in manual review and slide handling.
- Algorithmically detected errors were verified in manual review, suggesting a high specificity in the error detection algorithms.
- Rare undetected focus & stitching artifacts detected during manual review suggests potential further improvements in algorithm sensitivity are possible.

References

1. Whole slide imaging equivalency and efficiency study: experience at a large academic center. Hanna et al., 2019
2. Complete Digital Pathology for Routine Histopathology Diagnosis in a Multicenter Hospital Network. Retamero et al., 2019
3. A quantitative approach to evaluate image quality of whole slide imaging scanners. Shrestha et al., 2016

Archival Scanning progress at Mayo Clinic Rochester

❑ ***1.2 Million Slides scanned at Mayo TR to date***



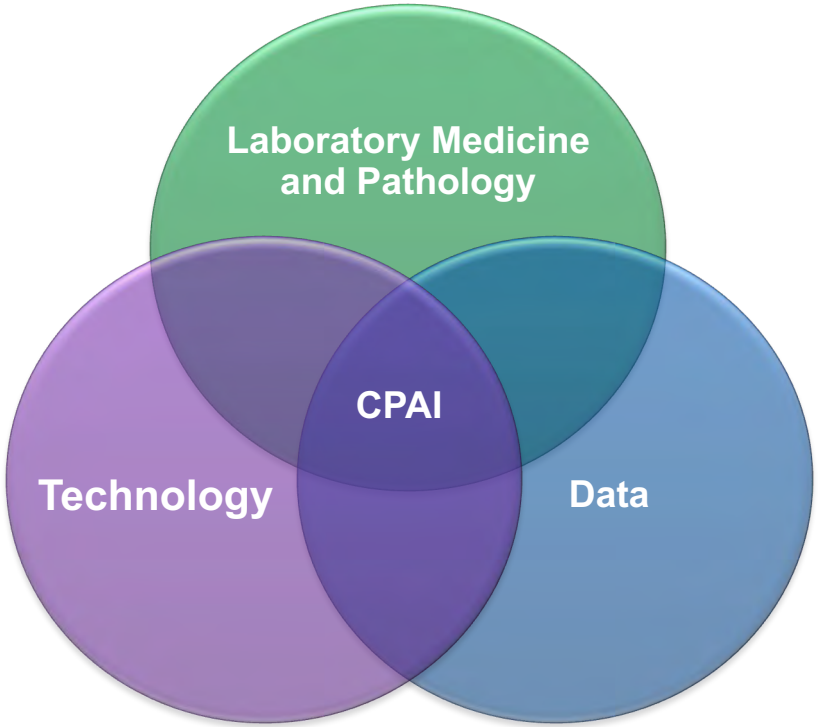
Status	Percentage	Count
Auto QC	85.86%	1,123,158
Manual QC	13.06%	170,866
Rescan	0.86%	11,251
Outliers	0.22%	2,856

Agenda

- Background & Introduction
- Digital and Computational Pathology at Mayo Clinic
  - Clinical Practice
  - Scanning the tissue archive
- **Innovation at Mayo Clinic**
  - **New division of Computational Pathology & AI**
  - **AI and colon cancer prognosis**
  - **A tissue image and molecular searchable atlas**
- Future of Digital and Computational Pathology
  - Fresh tissue 3D imaging
  - Multiplex IF and unsupervised learning
- Questions

Innovation at the Departmental level: The new division of Computational Pathology & AI (CPAI)

- Mission:
  - To transform how Mayo provides answers to people in need around the world
- What we do:
  - We are *change agents*, innovating, educating and implementing computational pathology and artificial intelligence
- How we succeed:
  - *Ambitiously transform the practice of pathology*
  - *Significant patient impact*
  - *Advance the field*
  - *Wise steward of resources*



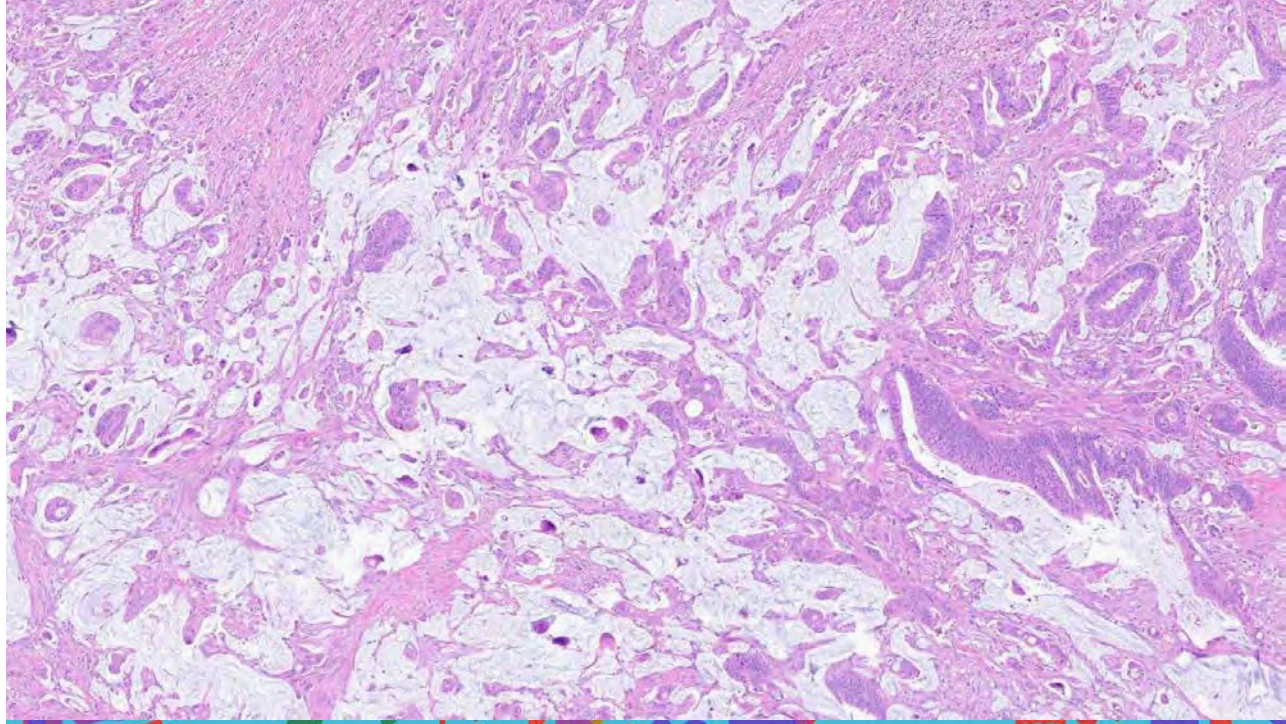
MAYO CLINIC

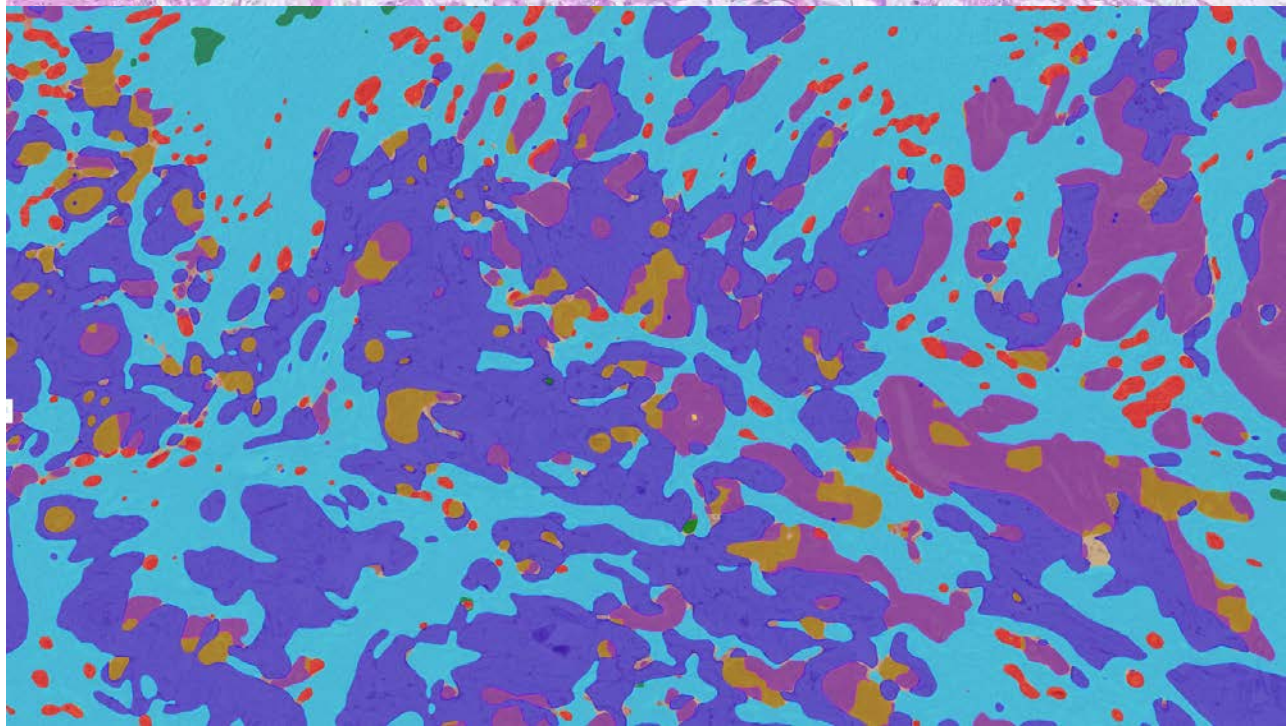
ENABLING PATHOLOGISTS TO DEVELOP AI ALGORITHMS

Dr. Thomas Flotte, MD

AI SANDBOX @ MAYO

- Aiforia and Mayo collaboration established an AI research pipeline where projects can be translated from ideas to practice
- **Seventy-eight investigators** are actively working on AI projects, most of whom had never done an AI project previously
- Foster innovation and upskill pathologists
- Creates a seamless pathway for ideation to clinical deployment





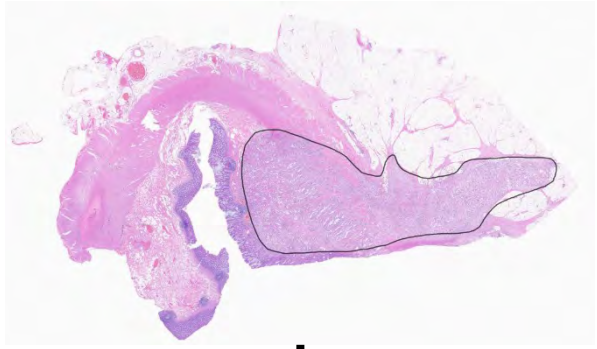
MAYO CLINIC

QUANTCRC: AN AI MODEL TO PREDICT PROGNOSIS IN COLON CANCER

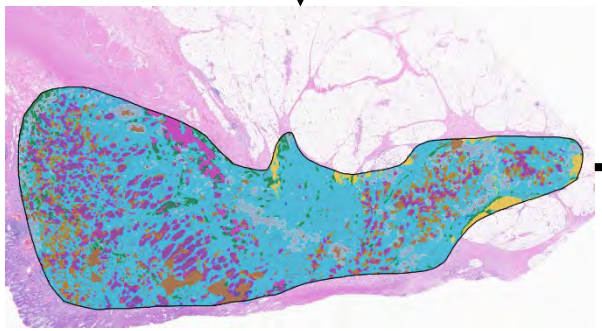
Dr. Rish Pai, MD

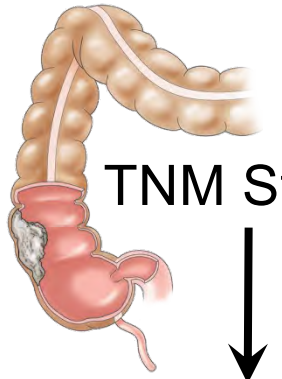
QUANTCRC AI MODEL TO PREDICT PROGNOSIS IN COLON CANCER

QuantCRC Prediction of Recurrence



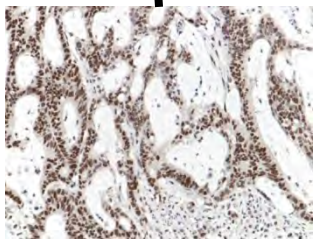
QuantCRC





TNM Stage

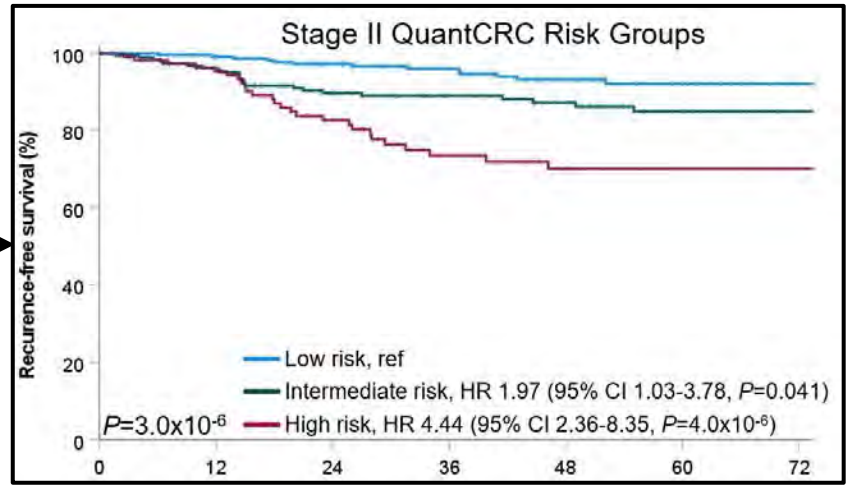
Prognostic Model



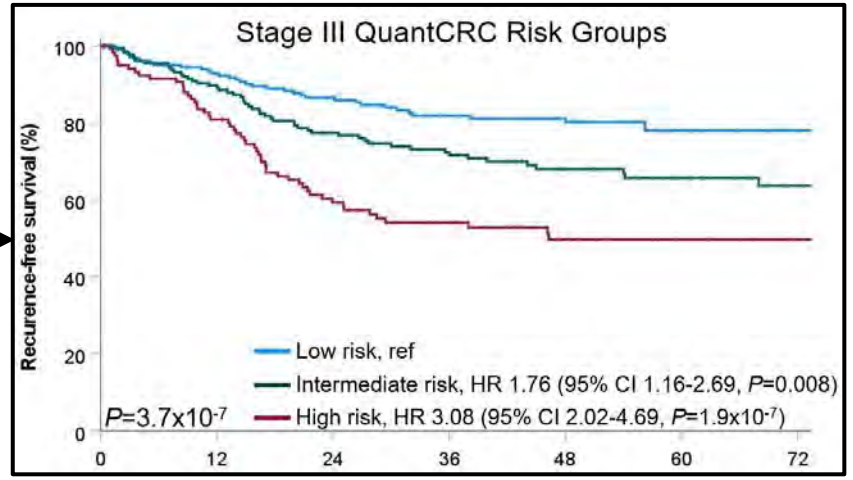
Mismatch repair status

Stage II risk groups

Stage III risk groups



External validation cohorts, c-index 0.75



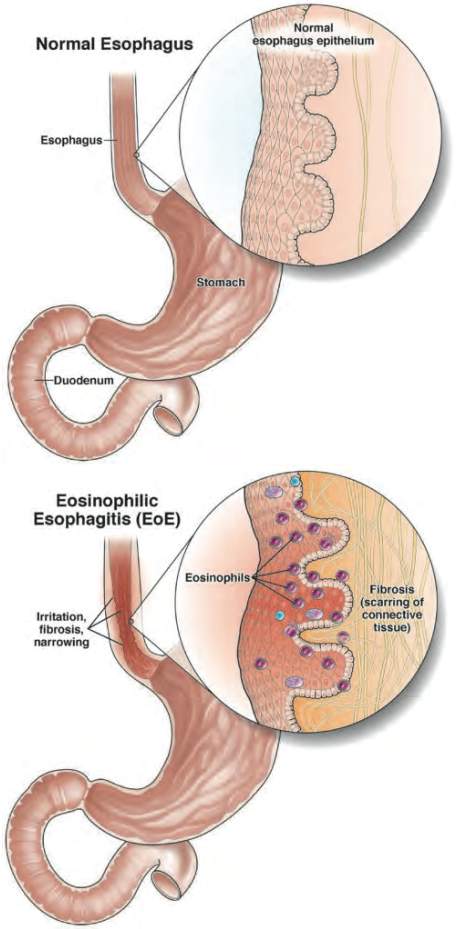
Gastroenterology. 2022 Dec;163(6):1531-1546

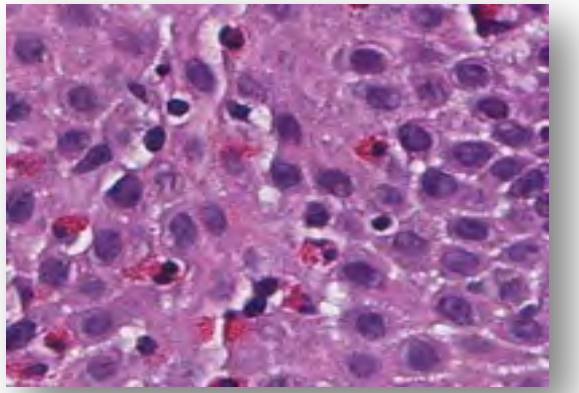
MAYO CLINIC

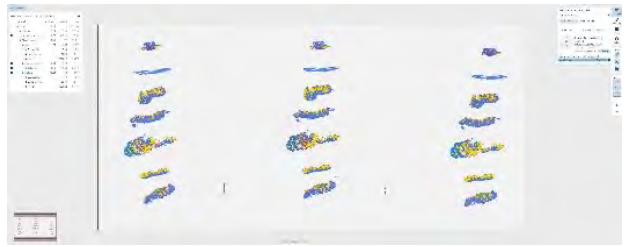
AUTOMATED SCORING OF EOSINOPHILIC ESOPHAGITIS

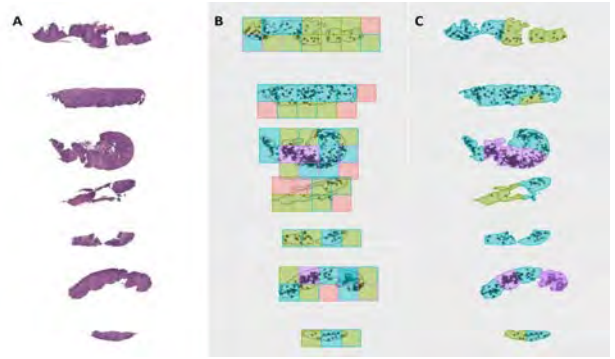
Dr. Roger Moreira, MD

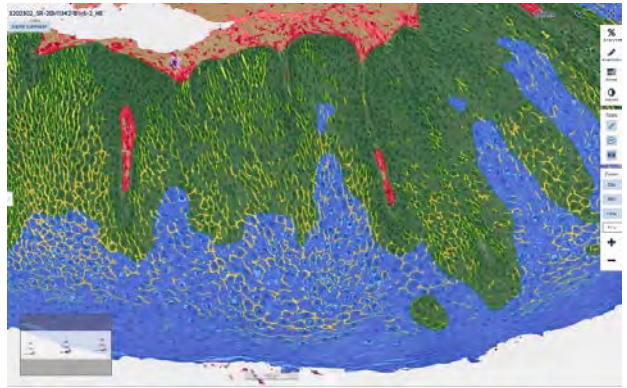
EOSINOPHLIC ESOPHAGITIS AI SCORING











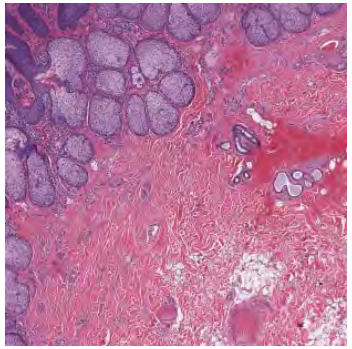
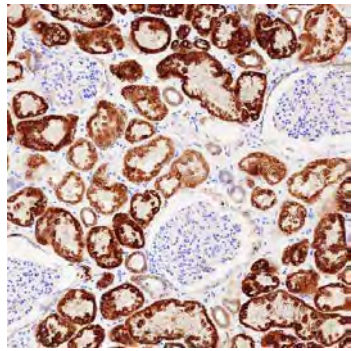
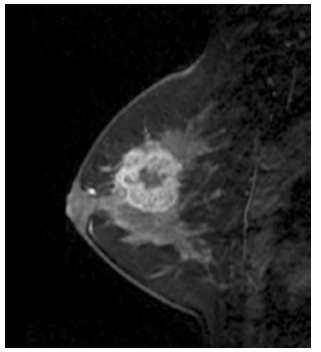
MAYO CLINIC

Cross-modal search in multi-modal archives

Hamid Tizhoosh, Ph.D

Integrating genomics with histopathology utilizing unsupervised AI

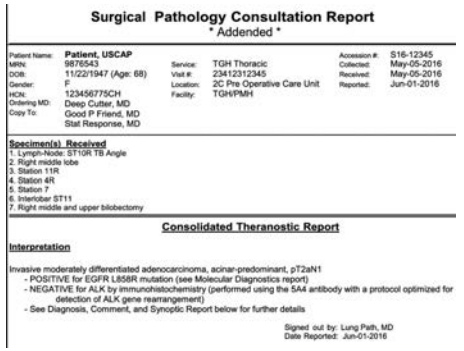
Imaging Data

Molecular Data

	p_val	avg_logFC	pct.1	pct.2	p_val_adj	cluster	gene	gene_name	gene_biotype	description
1	1.969050e-118	0.7613357	0.962	0.615	6.446276e-114	0	ENSG00000111716	LDHB	protein_coding	lactate dehydrogenase B [Source:HGNC Symbol;Acc:H...
2	2.461619e-105	-2.1868976	0.099	0.590	8.058848e-101	0	ENSG00000196126	HLA-DRB1	protein_coding	major histocompatibility complex, class II, DR beta 1 [...
3	7.837515e-94	-2.0285952	0.209	0.631	2.565846e-89	0	ENSG00000223865	HLA-DPB1	protein_coding	major histocompatibility complex, class II, DP beta 1 [...
4	3.121188e-93	0.9574059	0.484	0.108	1.021814e-88	0	ENSG00000126353	CCR7	protein_coding	C-C motif chemokine receptor 7 [Source:HGNC Symb...
5	1.506211e-92	-1.9712872	0.179	0.611	4.931033e-88	0	ENSG00000231389	HLA-DPA1	protein_coding	major histocompatibility complex, class II, DP alpha 1...
6	2.012660e-89	-1.0310573	0.380	0.763	6.589045e-85	0	ENSG00000213719	CLIC1	protein_coding	chloride intracellular channel 1 [Source:HGNC Symbol...
7	1.401968e-88	-2.5481370	0.291	0.655	4.589764e-84	0	ENSG00000204287	HLA-DRA	protein_coding	major histocompatibility complex, class II, DR alpha [...
8	5.699376e-85	-2.0028248	0.177	0.586	1.865862e-80	0	ENSG00000100097	LGALS1	protein_coding	galectin 1 [Source:HGNC Symbol;Acc:HGNC:6561]
9	1.093103e-82	-1.6948946	0.042	0.471	3.578601e-78	0	ENSG00000198502	HLA-DRB5	protein_coding	major histocompatibility complex, class II, DR beta 5 [...
10	1.206028e-68	-1.0474455	0.390	0.695	3.948294e-64	0	ENSG00000122862	SRGN	protein_coding	serglycin [Source:HGNC Symbol;Acc:HGNC:9361]
11	1.019739e-67	-2.3882059	0.133	0.491	3.338420e-63	0	ENSG00000011600	TYROBP	protein_coding	TYRO protein tyrosine kinase binding protein [Source:...

Textual Data



Surgical Pathology Consultation Report

* Addended *

Patient Name: **Patient, USCAP**
MRN: 9876543
DOB: 11/22/1947 (Age: 68)
Gender: F
HCN: 123456775CH
Ordering MD: Deep Cutter, MD
Copy To: Good P Friend, MD; Stat Response, MD

Service: TGH Thoracic
Visit #: 23412312345
Location: 2C Pre Operative Care Unit
Facility: TGH/PMH

Accession #: S16-12345
Collected: May-05-2016
Received: May-05-2016
Reported: Jun-01-2016

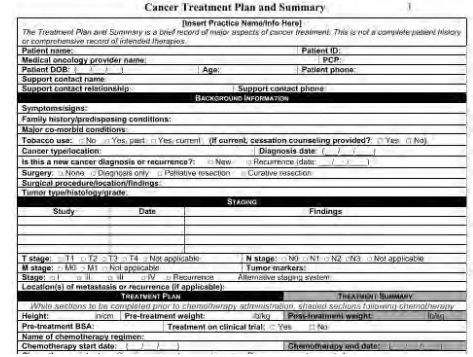
Specimen(s) Received
1. Lymph-Node: ST10R TB Angle
2. Right middle lobe
3. Station 11R
4. Station 4R
5. Station 7
6. Interlober ST11
7. Right middle and upper bilobectomy

Consolidated Theranostic Report

Interpretation

Invasive moderately differentiated adenocarcinoma, acinar-predominant, pT2aN1
- POSITIVE for EGFR L858R mutation (see Molecular Diagnostics report)
- NEGATIVE for ALK by immunohistochemistry (performed using the 5A4 antibody with a protocol optimized for detection of ALK gene rearrangement)
- See Diagnosis, Comment, and Synoptic Report below for further details

Signed out by: Lung,Path, MD
Date Reported: Jun-01-2016



Cancer Treatment Plan and Summary

[Insert Practice Name/Info Here]

The Treatment Plan and Summary is a brief record of major aspects of cancer treatment. This is not a complete patient history or comprehensive record of intended therapies.

Patient name: | Patient ID:
Medical oncology provider name: | PCP:
Patient DOB: (__/__/____) | Age: | Patient phone:
Support contact name:
Support contact relationship: | Support contact phone:

BACKGROUND INFORMATION

Symptoms/signs:
Family history/predisposing conditions:
Major co-morbid conditions:
Tobacco use: ☐ No ☐ Yes, past ☐ Yes, current (If current, cessation counseling provided?: ☐ Yes ☐ No).
Cancer type/location: | Diagnosis date: (__/__/____)
Is this a new cancer diagnosis or recurrence?: ☐ New ☐ Recurrence (date: __/__/____)
Surgery: ☐ None ☐ Diagnosis only ☐ Palliative resection ☐ Curative resection
Surgical procedure/location/findings:
Tumor type/histology/grade:

STAGING

Study	Date	Findings

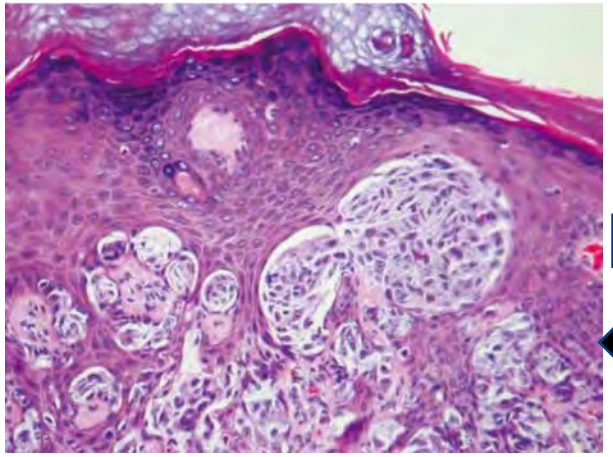
T stage: ☐ T1 ☐ T2 ☐ T3 ☐ T4 ☐ Not applicable | N stage: ☐ N0 ☐ N1 ☐ N2 ☐ N3 ☐ Not applicable
M stage: ☐ M0 ☐ M1 ☐ Not applicable | Tumor markers:
Stage: ☐ I ☐ II ☐ III ☐ IV ☐ Recurrence | Alternative staging system:
Location(s) of metastasis or recurrence (if applicable):

TREATMENT PLAN | **TREATMENT SUMMARY**

White sections to be completed prior to chemotherapy administration, shaded sections following chemotherapy

Height: in/cm | Pre-treatment weight: lb/kg | Post-treatment weight: lb/kg
Pre-treatment BSA: | Treatment on clinical trial: ☐ Yes ☐ No:
Name of chemotherapy regimen:
Chemotherapy start date: (__/__/____) | Chemotherapy end date: (__/__/____)

Cross-Modal Search in Multi-Modal Archives



Input search terms

Pathology Report:
Compound Spitz nevus. Fascicles of spindle and epithelioid melanocytes are present in association with epidermal hyperplasia and dull pink globules at the dermal-epidermal junction.

Molecular Results:

Maleki, Danial, and Hamid R. Tizhoosh. "LILE: Look in-depth before looking elsewhere" *International Conference on Medical Imaging with Deep Learning*. PMLR, 2022.

Agenda

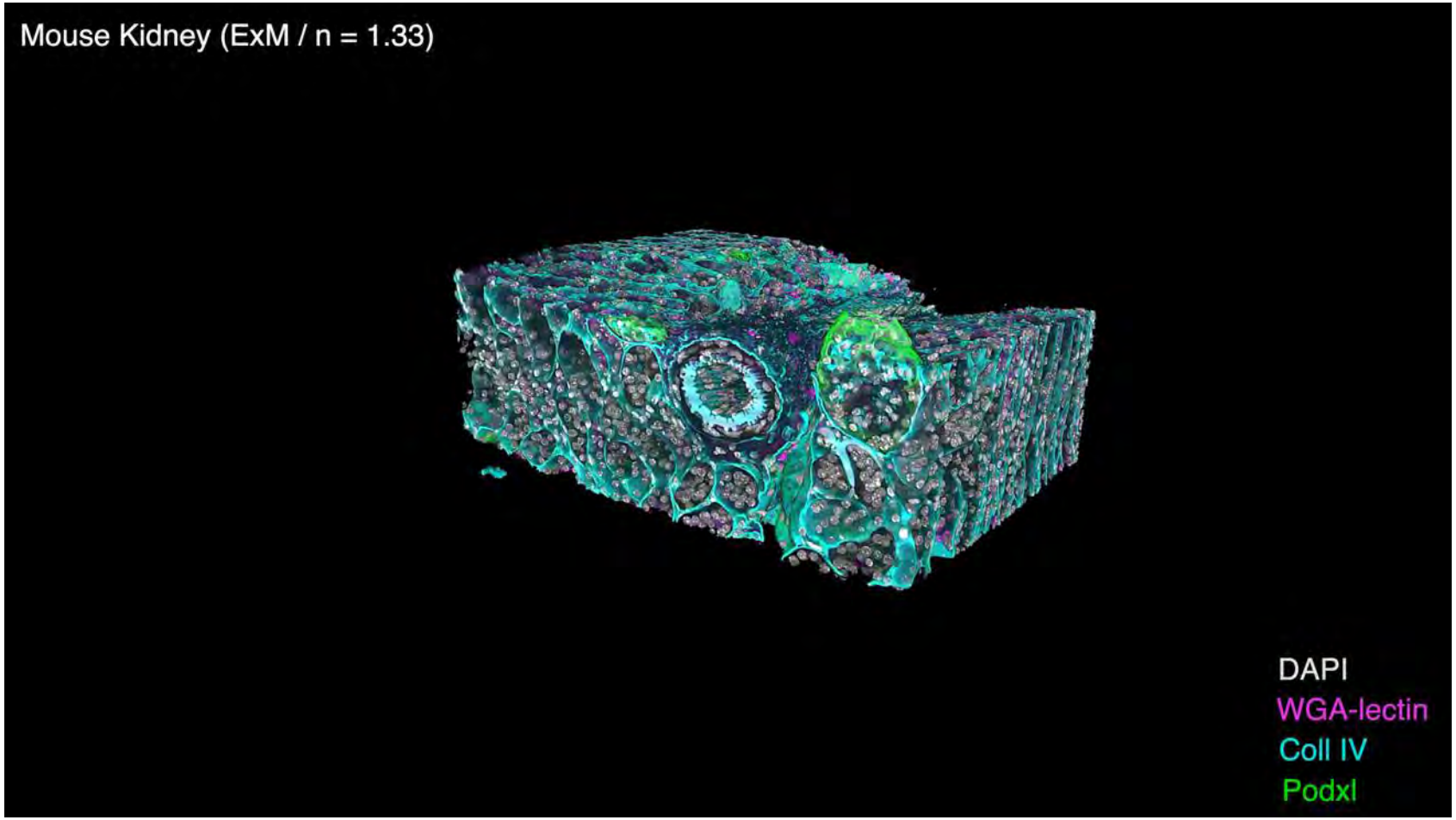
- Background & Introduction
- Digital and Computational Pathology at Mayo Clinic
  - Clinical Practice
  - Scanning the tissue archive
- Innovation at Mayo Clinic
  - New division of Computational Pathology & AI
  - AI and colon cancer prognosis
  - A tissue image and molecular searchable atlas
- **Future of Digital and Computational Pathology**
  - **Fresh tissue 3D imaging**
  - **Multiplex IF and unsupervised learning**
- Questions

MAYO CLINIC

MAYO'S PARTNERSHIP WITH ALPENGLOW BIOSCIENCES

CEO Nick Reder, MD

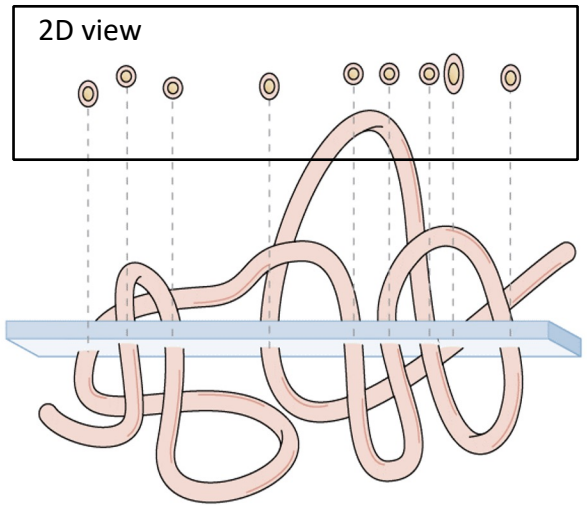
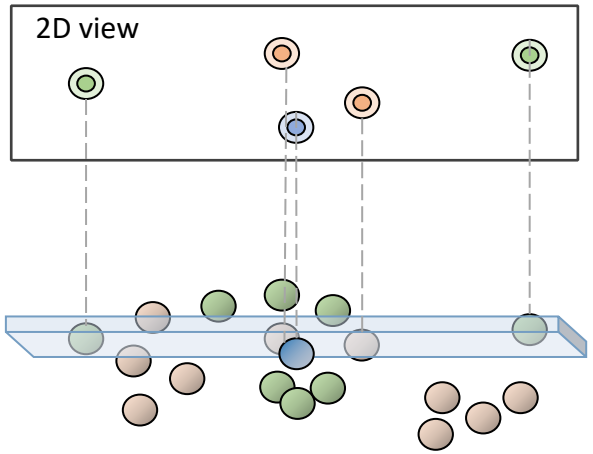
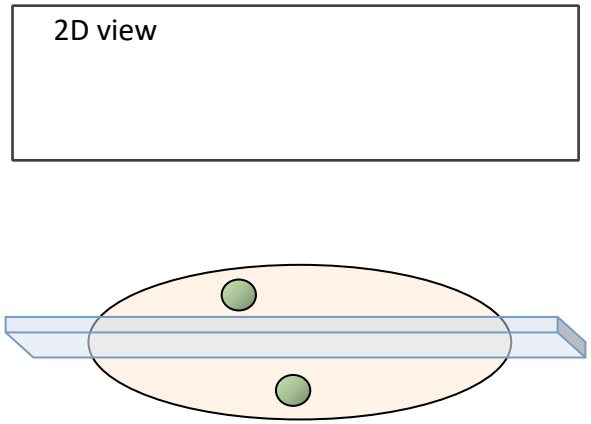
A new frontier in digital pathology



Advantages:

- Tissue is imaged fresh
- No glass slides needed
- Entire tissue is imaged
- No FFPE
- Spatial relationships viewed in 3D
- Unknown, unknowns

3D Spatial resolution is necessary for understanding complex patho-biology

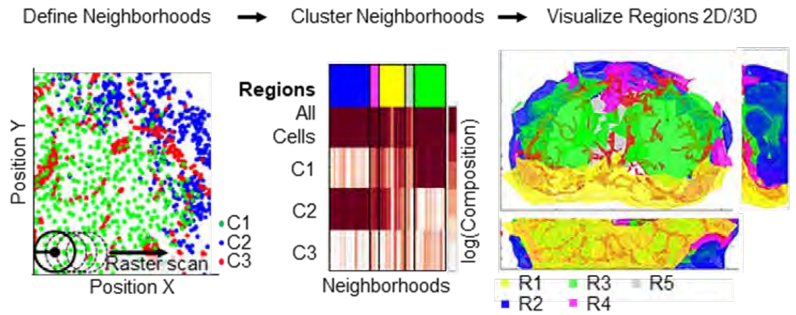
	Convoluted shapes	Complex distributions	Sparse objects
			
Features	**Vasculature, neurons & fibrosis**	**Spatial relationships between cell populations**	**Rare cell populations in large volumes of tissue**
Applications	**NASH, other fibrosis, neuroscience, ophthalmology, organoids**	**Immuno-oncology, immunology, metabolic disease**	**Gene therapy, CRISPR, CAR-T**

2D can lead to inaccurate science and incorrect diagnoses

Converting pathology from semi-quantitative to quantitative will identify novel insights not obtained by the human eye

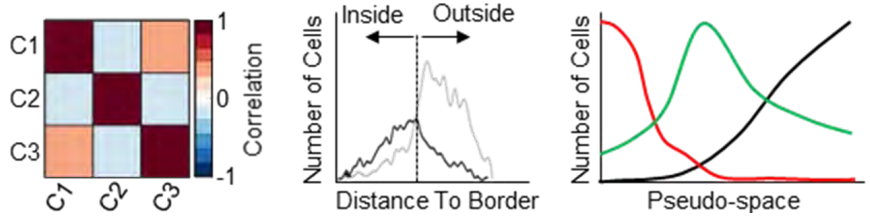
- Cell Calculations
  - Number, Position
  - Volume, Surface area
  - Sphericity
  - Diameter, Length

What cells are present



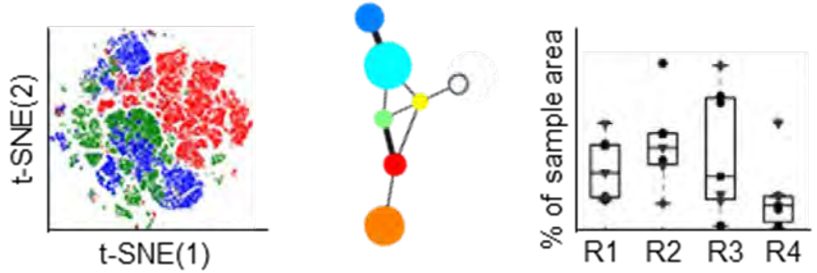
- Spatial Relationships
  - Distance to tissue border, vessels/Nerves, injection site, between cell types
  - Cell density, Cell clustering
  - Spatial correlation between cell types

What are they next to

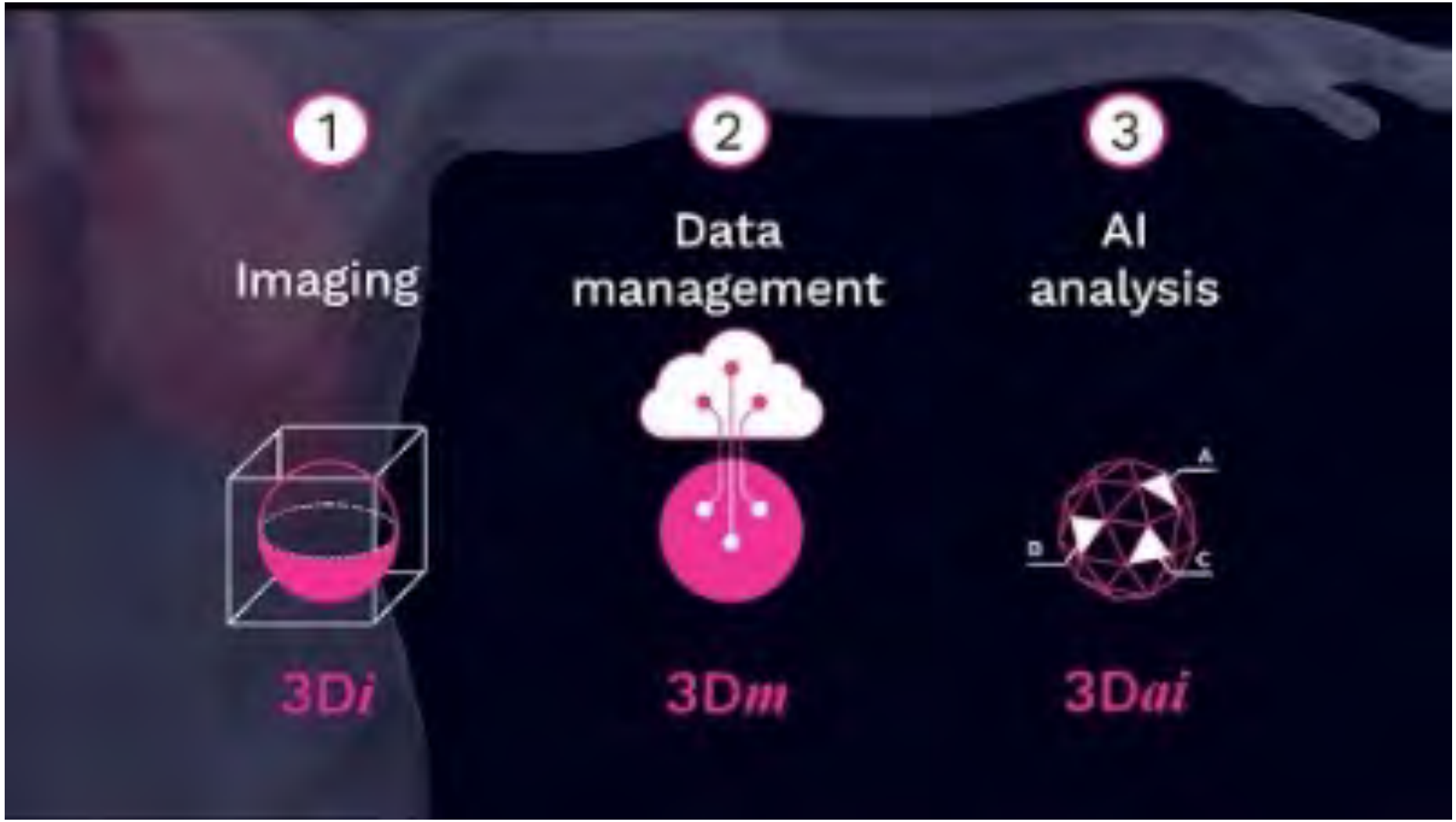


- Group Comparisons
  - Changes in distance to vessels across cancer treatments
  - Changes to cell shape across disease conditions
  - Changes in cell numbers or densities across groups of tissues

How does this change in different conditions



VIDEO OF 3D TERTIAL LYMPHOID STRUCTURES



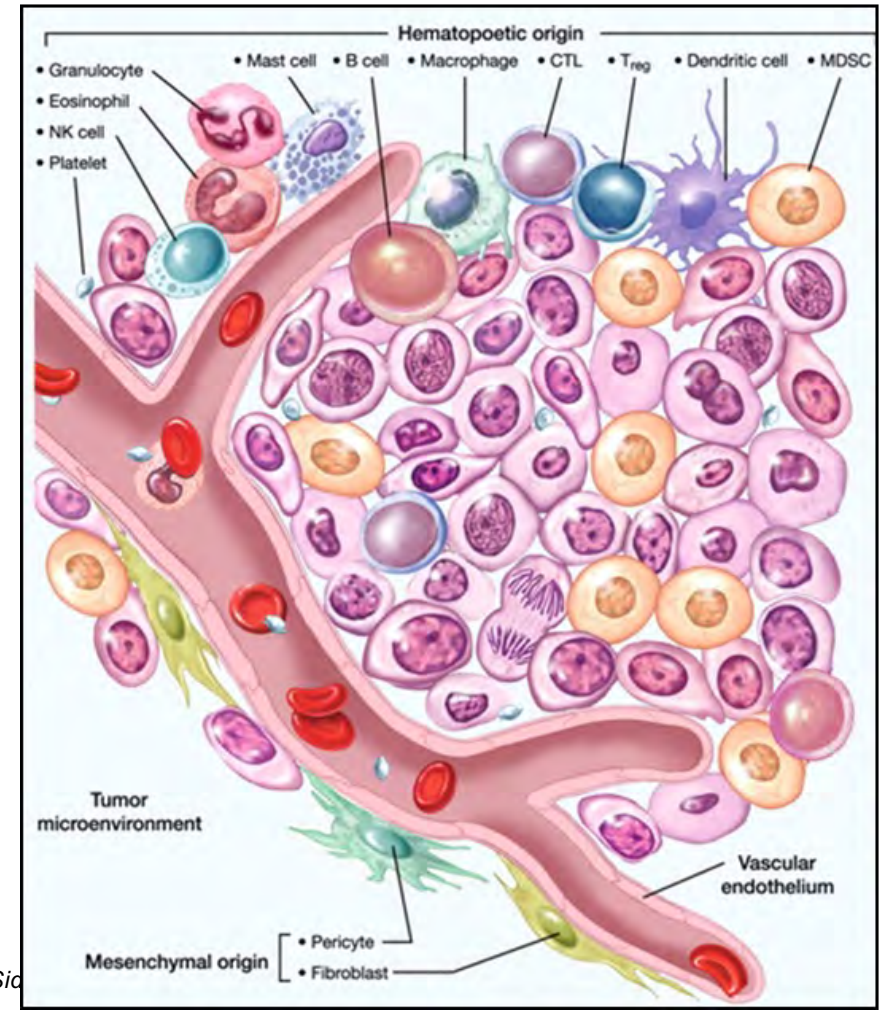
mIF Data are Deep and Broad

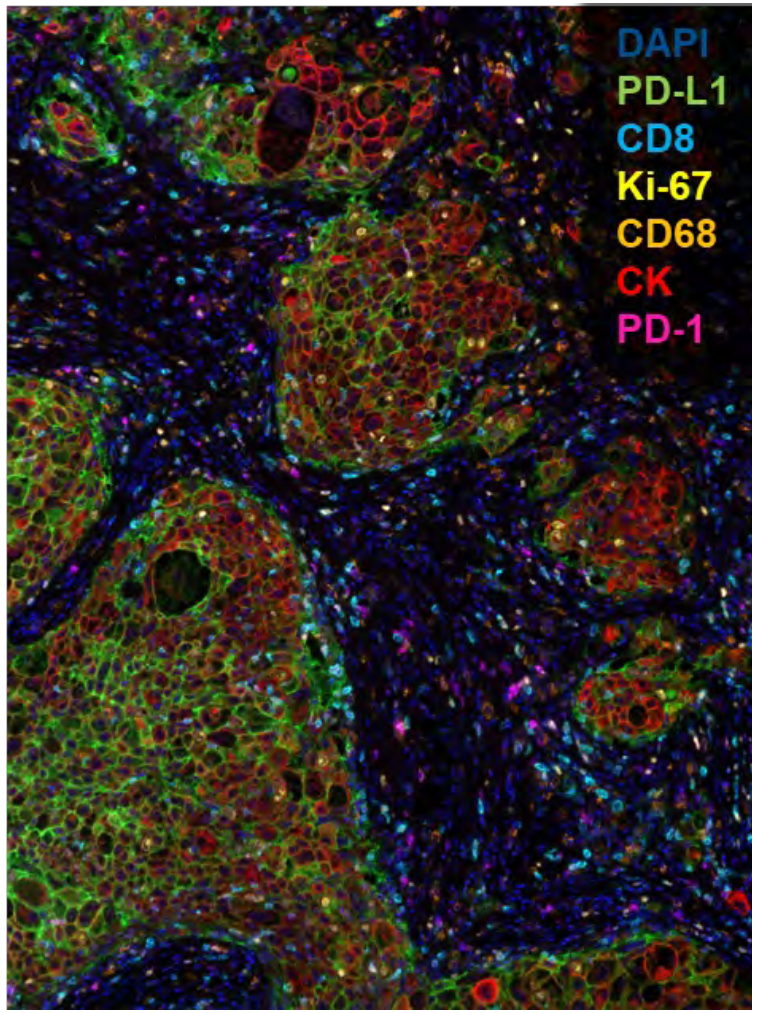
Provide deep information about tumor immune landscape:

- **Multi-marker phenotypes**
- **Spatial features**

In the same data set to help us understand how to select the right therapy for the right patient.

- **Informative & Validated Panel**
- **Consistent Application**
- **Appropriate Data Analysis**





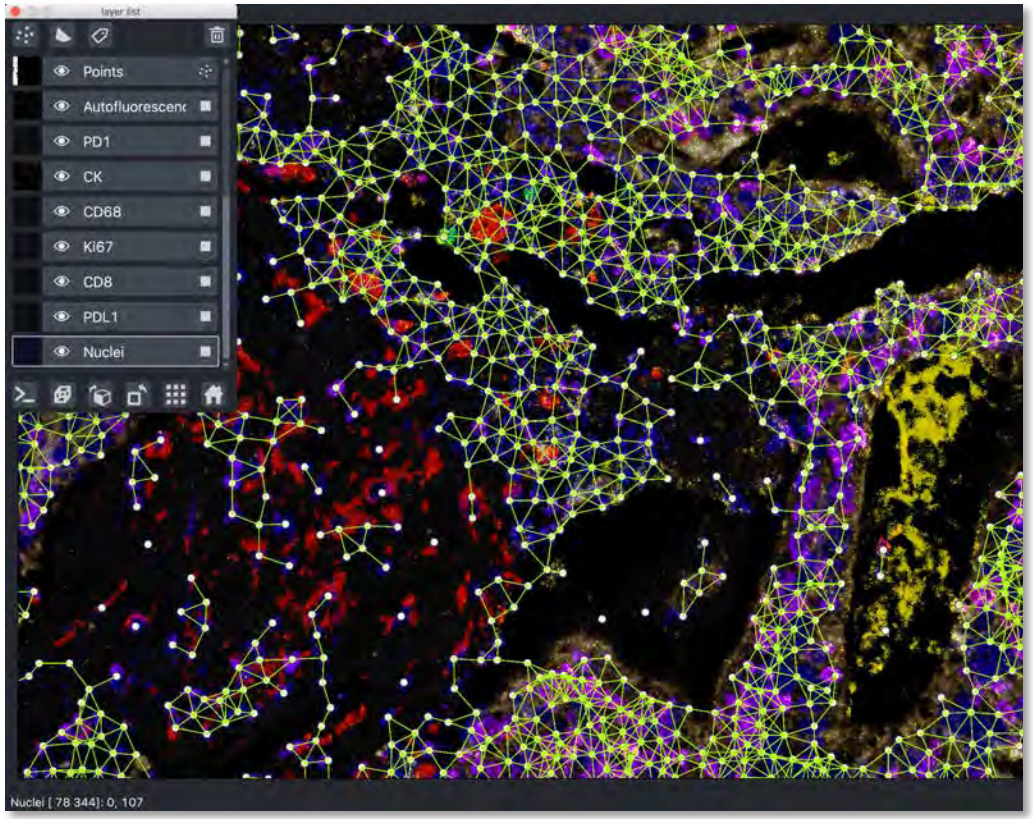
Adapted from Sid P. Kerkar, and Nicholas P. Restifo Cancer Res 2012;72:3125-3130

AstraZeneca Pathology

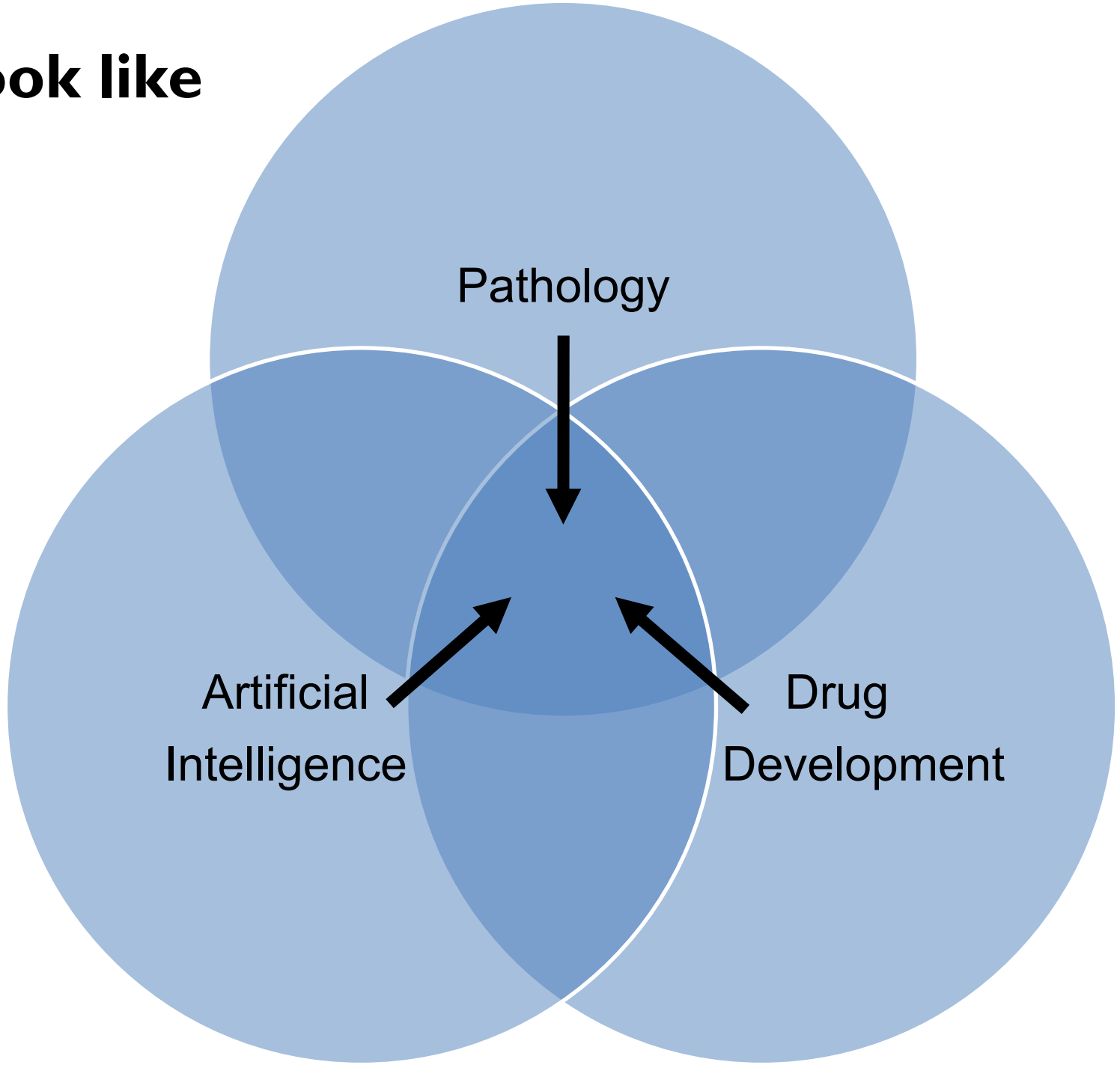
Graphs show connectivity between objects

- Mathematical structures modelling pairwise relations between objects
- Graphs built assuming nearby cells may interact
- Unsupervised learning techniques directly operate on the graph

- **Characterize cellular neighborhoods** in detail (with interactions)
- Support tissue **querying and exploration**
- Derive rich tissue/tumour **representations**
- **Predict *response* and *survival***

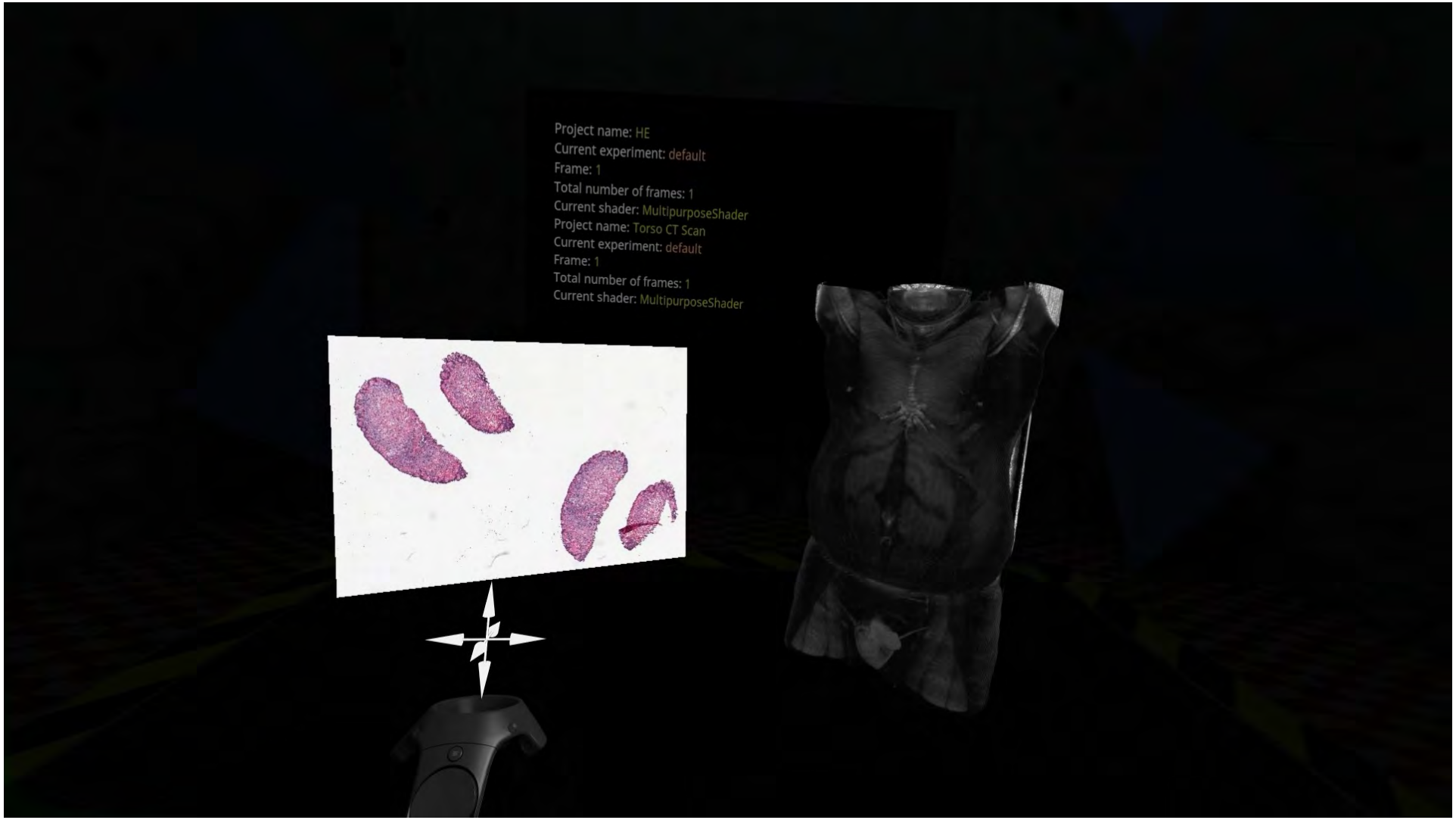


What does the future look like



Convergence to the center will result in a massive expansion opportunity if it is done intelligently

Unlocking the power of digital pathology with AR/VR



https://www.syglass.io/

THANK YOU

QUESTIONS?

Evolving Concepts and Challenges in Thoracic Pathology

Mary Beth Beasley, MD
Professor of Pathology
Icahn School of Medicine at Mount Sinai
New York, New York

Outline

- Talk 1: Current Challenges in Interstitial Lung Disease
- Talk 2: Current Pitfalls and Emerging Topics in Mesothelioma
- Talk 3: Current Challenges in Evaluation of Pulmonary Adenocarcinoma

Current Challenges in Interstitial Lung Disease (ILD)

- Common issues in interstitial lung disease biopsies
- How the ATS guidelines impact pathology
  - Review of usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP)
    - vs fibrotic hypersensitivity pneumonitis (fHP)
    - vs connective tissue disease related ILD (CTD-ILD)
- Interstitial lung abnormalities—what the heck are the radiologists talking about now and what does it mean for me---handout only

An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias

William D. Travis, Ulrich Costabel, David M. Hansell, Talmadge E. King, Jr., David A. Lynch, Andrew G. Nicholson, Christopher J. Ryerson, Jay H. Ryu, Moisés Selman, Athol U. Wells, Jurgen Behr, Demosthenes Bouros, Kevin K. Brown, Thomas V. Colby, Harold R. Collard, Carlos Robalo Cordeiro, Vincent Cottin, Bruno Crestani, Marjolein Drent, Rosalind F. Dudden, Jim Egan, Kevin Flaherty, Cory Hogaboam, Yoshikazu Inoue, Takeshi Johkoh, Dong Soon Kim, Masanori Kitaichi, James Loyd, Fernando J. Martinez, Jeffrey Myers, Shandra Protzko, Ganesh Raghu, Luca Richeldi, Nicola Sverzellati, Jeffrey Swigris, and Dominique Valeyre; on behalf of the ATS/ERS Committee on Idiopathic Interstitial Pneumonias

Am J Respir Crit Care Med Vol 188, Iss. 6, pp 733–748, Sep 15, 2013

TABLE 1. REVISED AMERICAN THORACIC SOCIETY/EUROPEAN RESPIRATORY SOCIETY CLASSIFICATION OF IDIOPATHIC INTERSTITIAL PNEUMONIAS: MULTIDISCIPLINARY DIAGNOSES

Major idiopathic interstitial pneumonias
Idiopathic pulmonary fibrosis
Idiopathic nonspecific interstitial pneumonia
Respiratory bronchiolitis–interstitial lung disease
Desquamative interstitial pneumonia
Cryptogenic organizing pneumonia
Acute interstitial pneumonia
Rare idiopathic interstitial pneumonias
Idiopathic lymphoid interstitial pneumonia
Idiopathic pleuroparenchymal fibroelastosis
Unclassifiable idiopathic interstitial pneumonias*

Am J Respir Crit Care Med Vol 188, Iss. 6, pp 733–748, Sep 15, 2013

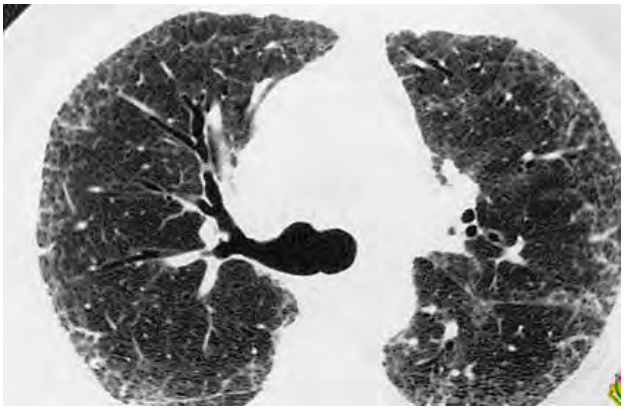
ATS/ERS, et al

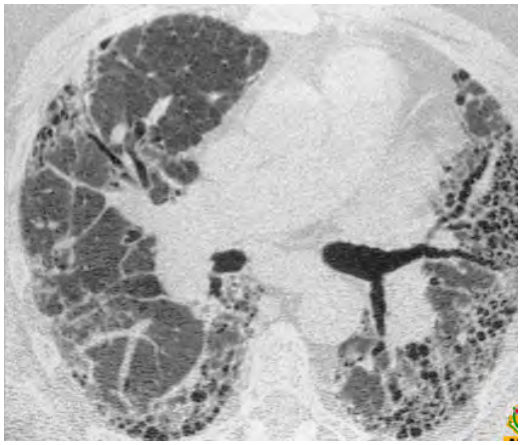
TABLE 2. CATEGORIZATION OF MAJOR IDIOPATHIC INTERSTITIAL PNEUMONIAS

Category	Clinical–Radiologic–Pathologic Diagnoses	Associated Radiologic and/or Pathologic–Morphologic Patterns
Chronic fibrosing IP	Idiopathic pulmonary fibrosis	Usual interstitial pneumonia
	Idiopathic nonspecific interstitial pneumonia	Nonspecific interstitial pneumonia
Smoking-related IP*	Respiratory bronchiolitis-interstitial lung disease	Respiratory bronchiolitis
	Desquamative interstitial pneumonia	Desquamative interstitial pneumonia
Acute/subacute IP	Cryptogenic organizing pneumonia	Organizing pneumonia
	Acute interstitial pneumonia	Diffuse alveolar damage

Am J Respir Crit Care Med Vol 188, Iss. 6, pp 733–748, Sep 15, 2013

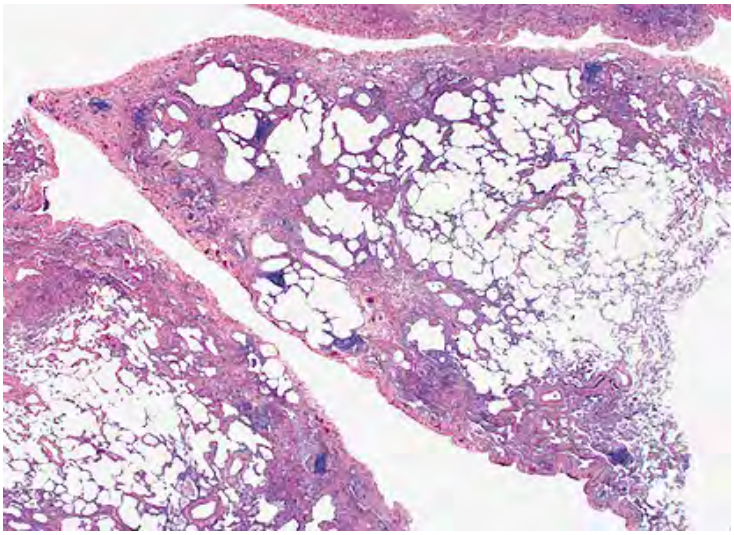
UIP radiology—peripheral fibrosis with honeycombing, lower lobe predominance



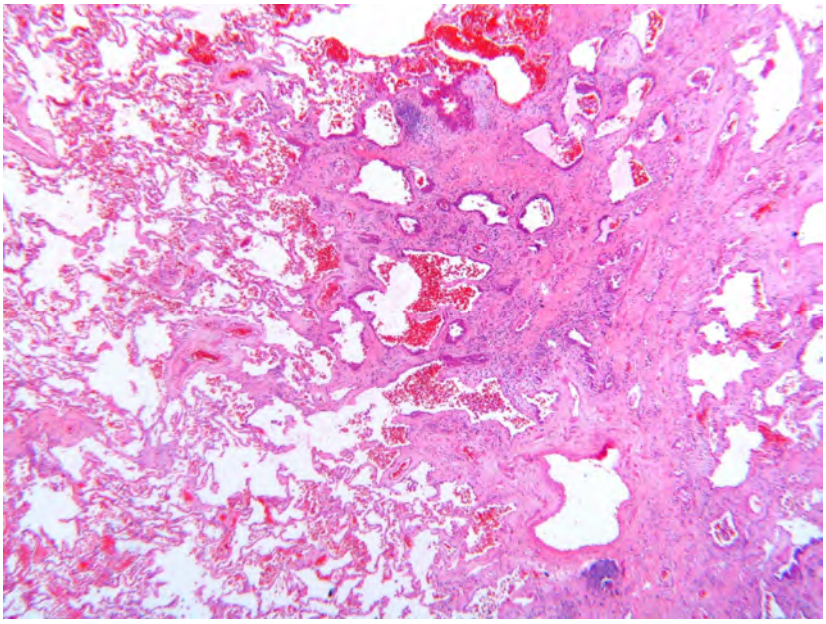


UIP-histology

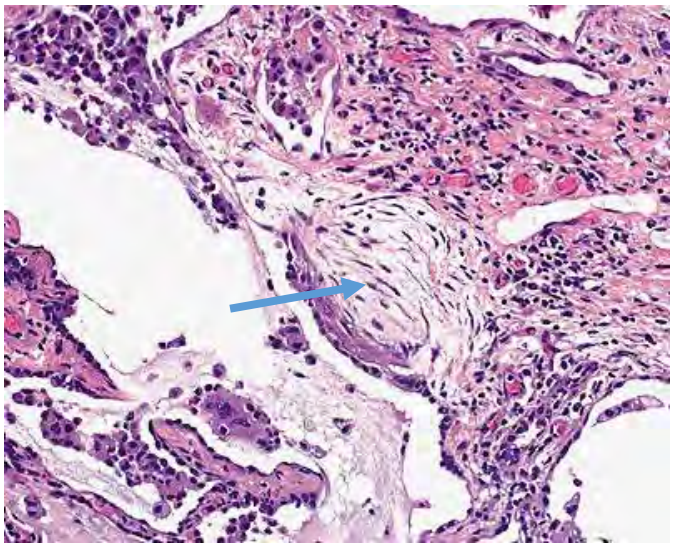
- PATCHY fibrosis, most pronounced beneath pleura and next to interlobular septa
  - Areas of severe fibrosis alternate with areas of relatively spared parenchyma
- The fibrosis itself exhibits TEMPORAL HETEROGENEITY—i.e fibrosis is not all the same age
  - This feature is manifested in the form of the "fibroblast focus"—a region on loose blue-gray connective tissue (new fibrosis) adjacent to an area of dense collagenous scar (old fibrosis)



Patchy fibrosis with subpleural predominance



Patchy fibrosis—severe fibrosis alternates with spared lung



Fibroblast focus (arrow)

Usual Interstitial Pneumonia (UIP)

- **Usual interstitial pneumonia is the histologic diagnosis**
- **The clinician then has to determine if the patient has an underlying disorder that can account for the findings (CTD, fHP, drug reaction)**
- **If known causes are excluded then a CLINICAL diagnosis of idiopathic pulmonary fibrosis is made.**

NSIP-Histology

Cellular pattern

Diffuse chronic inflammatory cell infiltrates without significant alveolar expansion

No fibrosis

Fibrosing Pattern

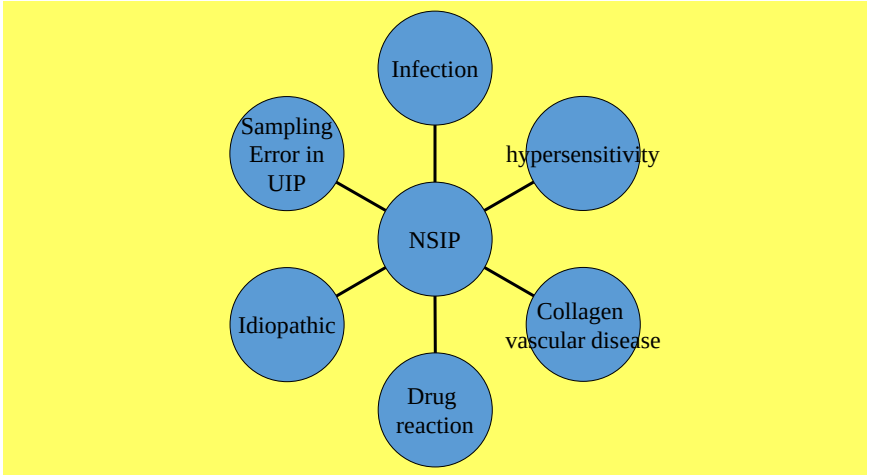
Diffuse interstitial fibrosis with uniform appearance.

Fibrosis all the same age—i.e. “temporally uniform” in contrast to UIP

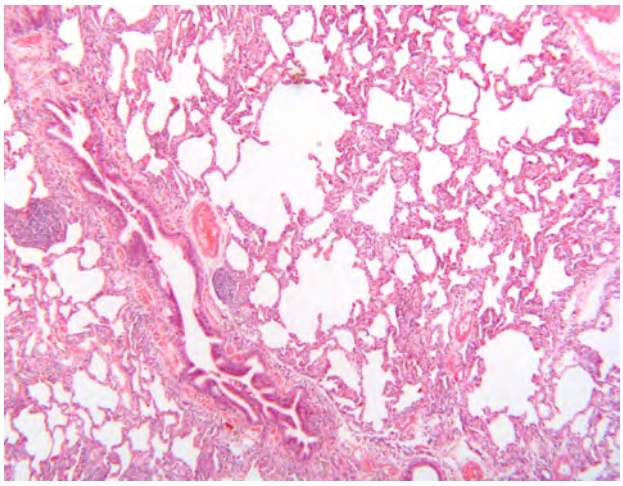
Lung architecture is frequently preserved

Interstitial chronic inflammation—mild or moderate

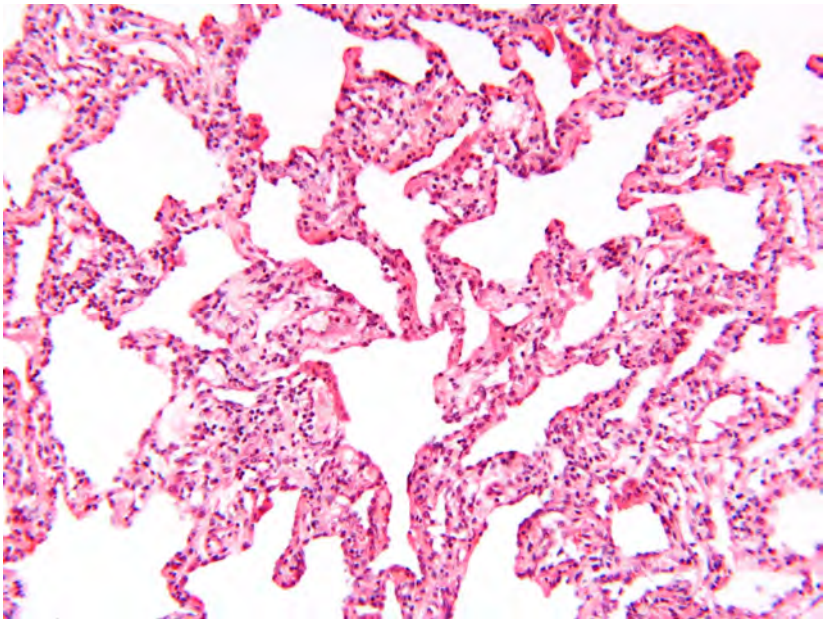
Non-specific Interstitial Pneumonia



Cellular NSIP

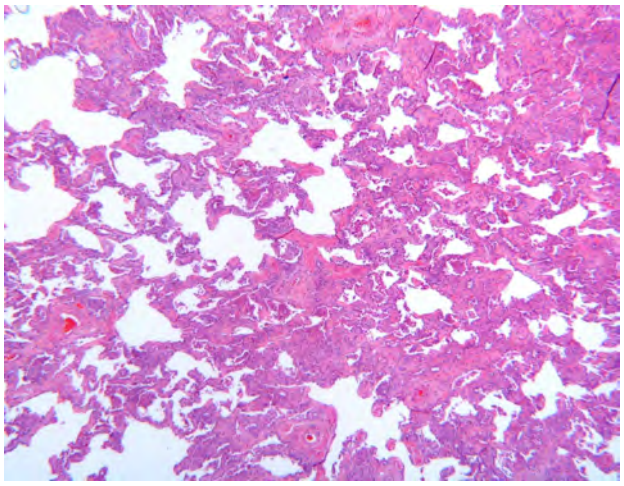


Uniform involvement of lung by chronic interstitial inflammation



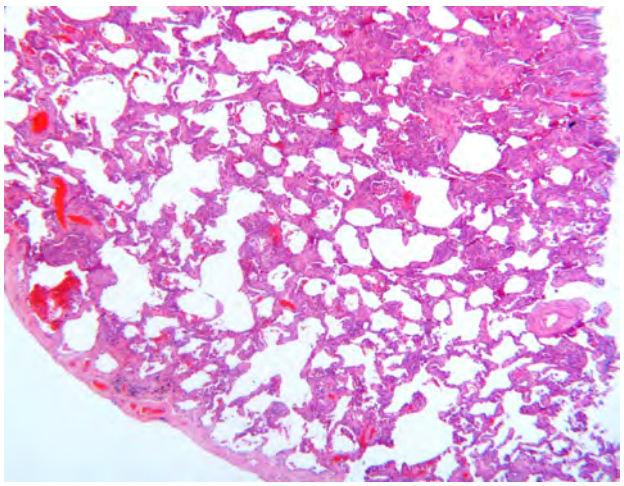
Alveolar septa are involved by lymphocytic infiltrates

Fibrosing NSIP



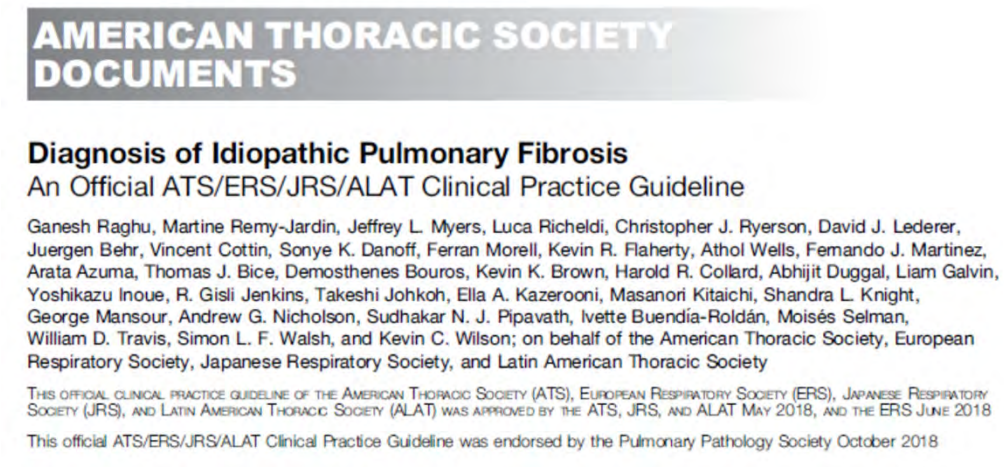
UNIFORM involvement of lung by fibrosis of the same age

Fibrosing NSIP



As my residents always say “that’s really nice but why don’t we ever actually see cases like that….

Because of this….



AMERICAN THORACIC SOCIETY DOCUMENTS

Diagnosis of Idiopathic Pulmonary Fibrosis

An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline

Ganesh Raghu, Martine Remy-Jardin, Jeffrey L. Myers, Luca Richeldi, Christopher J. Ryerson, David J. Lederer, Juergen Behr, Vincent Cottin, Sonye K. Danoff, Ferran Morell, Kevin R. Flaherty, Athol Wells, Fernando J. Martinez, Arata Azuma, Thomas J. Bice, Demosthenes Bouros, Kevin K. Brown, Harold R. Collard, Abhijit Duggal, Liam Galvin, Yoshikazu Inoue, R. Gisli Jenkins, Takeshi Johkoh, Ella A. Kazerooni, Masanori Kitaichi, Shandra L. Knight, George Mansour, Andrew G. Nicholson, Sudhakar N. J. Pipavath, Ivette Buendía-Roldán, Moisés Selman, William D. Travis, Simon L. F. Walsh, and Kevin C. Wilson; on behalf of the American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Society

This official clinical practice guideline of the American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society (JRS), and Latin American Thoracic Society (ALAT) was approved by the ATS, JRS, and ALAT May 2018, and the ERS June 2018

This official ATS/ERS/JRS/ALAT Clinical Practice Guideline was endorsed by the Pulmonary Pathology Society October 2018

Table 4. High-Resolution Computed Tomography Scanning Patterns

UIP	Probable UIP	Indeterminate for UIP	Alternative Diagnosis
Subpleural and basal predominant; distribution is often heterogeneous*	Subpleural and basal predominant; distribution is often heterogeneous	Subpleural and basal predominant	Findings suggestive of another diagnosis, including: • CT features: ° Cysts ° Marked mosaic attenuation ° Predominant GGO ° Profuse micronodules ° Centrilobular nodules ° Nodules ° Consolidation • Predominant distribution: ° Peribronchovascular ° Perilymphatic ° Upper or mid-lung • Other: ° Pleural plaques (consider asbestosis) ° Dilated esophagus (consider CTD) ° Distal clavicular erosions (consider RA) ° Extensive lymph node enlargement (consider other etiologies) ° Pleural effusions, pleural thickening (consider CTD/drugs)
Honeycombing with or without peripheral traction bronchiectasis or bronchiolectasis†	Reticular pattern with peripheral traction bronchiectasis or bronchiolectasis May have mild GGO	Subtle reticulation; may have mild GGO or distortion ("early UIP pattern") CT features and/or distribution of lung fibrosis that do not suggest any specific etiology ("truly indeterminate for UIP")	

Definition of abbreviations: CT = computed tomography; CTD = connective tissue disease; GGO = ground-glass opacities; RA = rheumatoid arthritis; UIP = usual interstitial pneumonia.
*Variants of distribution: occasionally diffuse, may be asymmetrical.
†Superimposed CT features: mild GGO, reticular pattern, pulmonary ossification.

A lot of overlap between "indeterminate" and "alternative", particularly in regard to fibrosing HSP and CTD-ILD

Table 5. Histopathology Patterns and Features

UIP	Probable UIP	Indeterminate for UIP	Alternative Diagnosis
• Dense fibrosis with architectural distortion (i.e., destructive scarring and/or honeycombing) • Predominant subpleural and/or paraseptal distribution of fibrosis • Patchy involvement of lung parenchyma by fibrosis • Fibroblast foci • Absence of features to suggest an alternate diagnosis	• Some histologic features from column 1 are present but to an extent that precludes a definite diagnosis of UIP/IPF *And* • Absence of features to suggest an alternative diagnosis *Or* • Honeycombing only	• Fibrosis with or without architectural distortion, with features favoring either a pattern other than UIP or features favoring UIP secondary to another cause* • Some histologic features from column 1, but with other features suggesting an alternative diagnosis†	• Features of other histologic patterns of IIPs (e.g., absence of fibroblast foci or loose fibrosis) in all biopsies • Histologic findings indicative of other diseases (e.g., hypersensitivity pneumonitis, Langerhans cell histiocytosis, sarcoidosis, LAM)

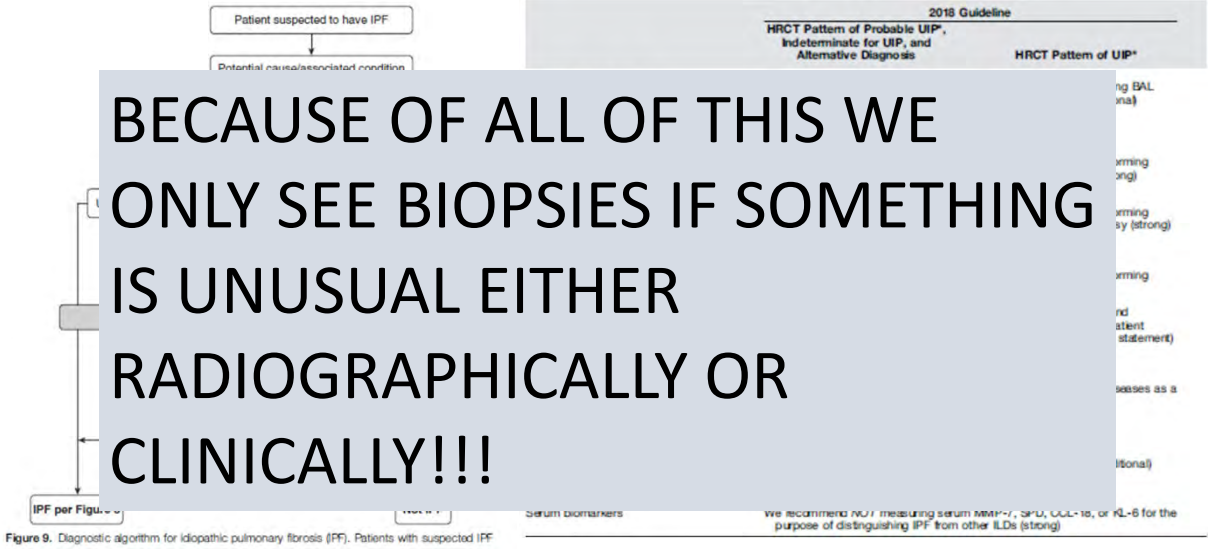
Definition of abbreviations: IIP = idiopathic interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; LAM = lymphangioleiomyomatosis; UIP = usual interstitial pneumonia.
*Granulomas, hyaline membranes (other than when associated with acute exacerbation of IPF, which may be the presenting manifestation in some patients), prominent airway-centered changes, areas of interstitial inflammation lacking associated fibrosis, marked chronic fibrous pleuritis, organizing pneumonia. Such features may not be overt or easily seen to the untrained eye and often need to be specifically sought.
†Features that should raise concerns about the likelihood of an alternative diagnosis include a cellular inflammatory infiltrate away from areas of honeycombing, prominent lymphoid hyperplasia including secondary germinal centers, and a distinctly bronchiolocentric distribution that could include extensive peribronchiolar metaplasia.

IPF suspected*		Histopathology pattern			
		UIP	Probable UIP	Indeterminate for UIP	Alternative diagnosis
HRCT pattern	UIP	IPF	IPF	IPF	Non-IPF dx
	Probable UIP	IPF	IPF	IPF (Likely)**	Non-IPF dx
	Indeterminate for UIP	IPF	IPF (Likely)**	Indeterminate for IPF***	Non-IPF dx
	Alternative diagnosis	IPF (Likely)** /non-IPF dx	Non-IPF dx	Non-IPF dx	Non-IPF dx

Figure 8. Idiopathic pulmonary fibrosis diagnosis based upon HRCT and biopsy patterns.
Clinically suspected of having IPF = unexplained symptomatic or asymptomatic patterns of bilateral pulmonary fibrosis on a chest radiograph or chest computed tomography, bibasilar inspiratory crackles, and age greater than 60 years. (Middle-aged adults [>40 yr and <60 yr], especially patients with risks for familial pulmonary fibrosis, can rarely present with the otherwise same clinical scenario as the typical patient older than 60 years.)
**IPF is the likely diagnosis when any of the following features are present:
- Moderate-to-severe traction bronchiectasis/bronchiolectasis (defined as mild traction bronchiectasis/bronchiolectasis in four or more lobes including the lingual as a lobe, or moderate to severe traction bronchiectasis in two or more lobes) in a man over age 50 years or in a woman over age 60 years
- Extensive (>30%) reticulation on HRCT and an age >70 years
- Increased neutrophils and/or absence of lymphocytosis in BAL fluid
- Multidisciplinary discussion reaches a confident diagnosis of IPF.

***Indeterminate for IPF
- Without an adequate biopsy is unlikely to be IPF
- With an adequate biopsy may be reclassified to a more specific diagnosis after multidisciplinary discussion and/or additional consultation.

dx = diagnosis; HRCT = high-resolution computed tomography; IPF = idiopathic pulmonary fibrosis; UIP = usual interstitial pneumonia.



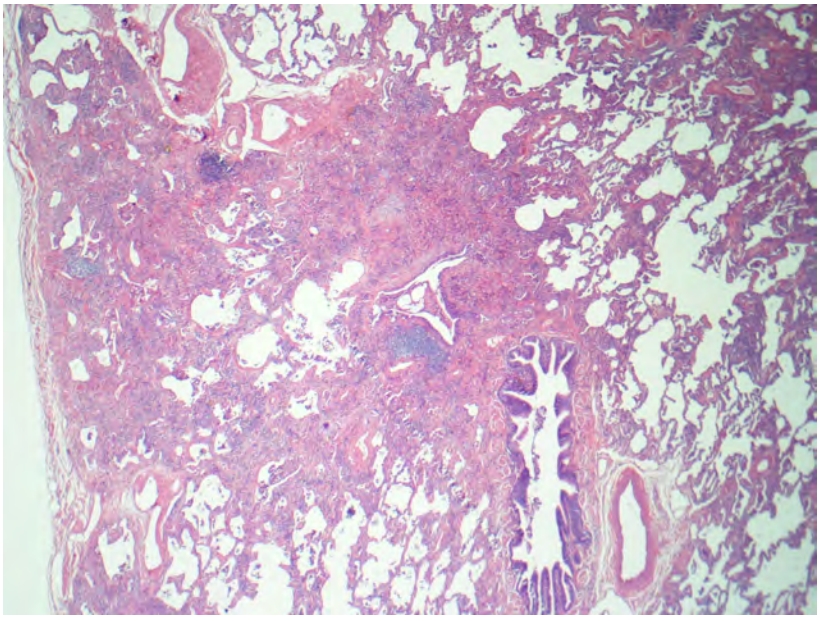
BECAUSE OF ALL OF THIS WE ONLY SEE BIOPSIES IF SOMETHING IS UNUSUAL EITHER RADIOGRAPHICALLY OR CLINICALLY!!!

Figure 9. Diagnostic algorithm for idiopathic pulmonary fibrosis (IPF). Patients with suspected IPF

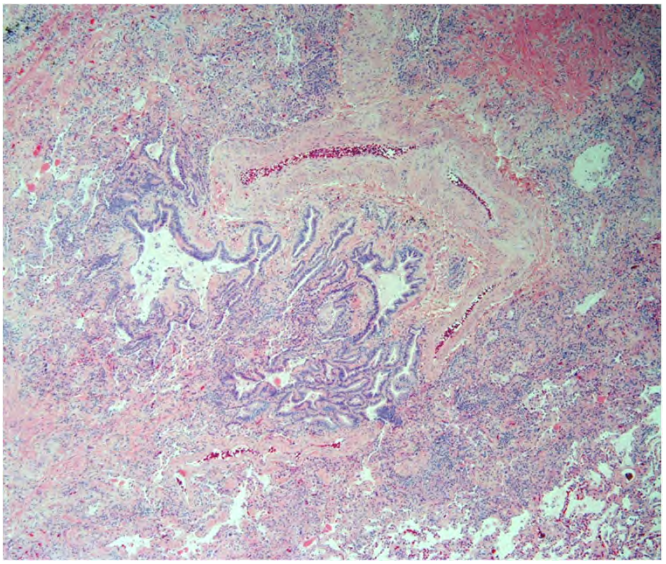
Case 1

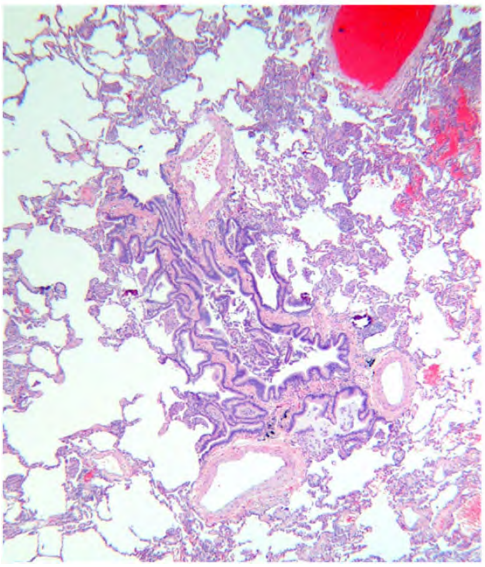
- 55 year old female with progressively worsening shortness of breath for approximately 9 months
- HRCT classified as “Indeterminate for UIP”
- Negative collagen vascular serologies and negative hypersensitivity pneumonitis panel
- Patient underwent surgical lung biopsy

Case 1

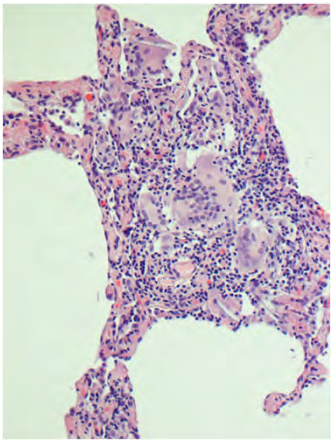


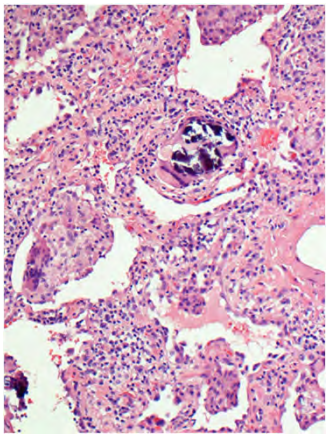
Case 1





Case 1





Patient was later revealed to be an active member of the Long Island Parrot association and neglected to mention this…

Fibrotic hypersensitivity pneumonitis (fHP)

- Sample diagnostic line:
  - Patchy interstitial fibrosis with prominent peribronchiolar component and scattered poorly formed granulomas
  - Note- Findings are most compatible with fibrotic HP, continued clinical and radiographic correlation is recommended
- Other things that need to be excluded clinically:
  - Infection—granulomas often more variable in size, mix of large and small
  - Connective tissue disease—may have granulomas and airway centered disease
  - Drug reaction

HP Background

- Arcane name- extrinsic allergic alveolitis
- Secondary to a variety of agents
  - Thermophilic bacteria
  - Fungi
  - Animal proteins
  - Mycobactera— "Hot tub lung", contaminated metal working fluids

Etiologic Agents of Hypersensitivity Pneumonitis		
Disease	***Antigen***	***Source***
Fungal and bacterial		
Farmer's lung	*Saccharopolyspora rectivirgula*	Moldy hay, grain, silage
Ventilation pneumonitis; humidifier lung; air conditioner lung	*Thermoactinomyces vulgaris, Thermoactinomyces sacchari, Thermoactinomyces candidus Klebsiella oxytoca*	Contaminated forced-air systems; water reservoirs
Bagassosis	*T. vulgaris*	Moldy sugarcane (ie, bagasse)
Mushroom worker's lung	*T. sacchari*	Moldy mushroom compost
Enoki mushroom worker's lung (Japan)	*Penicillium citrinum*	Moldy mushroom compost
Suberosis	*Thermoactinomyces viridis, Aspergillus fumigatus, Penicillium frequentans Penicillium glabrum*	Moldy cork
Detergent lung; washing powder lung	*Bacillus subtilis enzymes*	Detergents (during processing or use)
Malt worker's lung	*Aspergillus fumigatus, Aspergillus clavatus*	Moldy barley
Sequoiosis	*Graphium, Pullularia, and Trichoderma spp. Aureobasidium pullulans*	Moldy wood dust
Maple bark stripper's lung	*Cryptostroma corticale*	Moldy maple bark
Cheese washer's lung	*Penicillium casei, A. clavatus*	Moldy cheese
Woodworker's lung	*Alternaria spp., wood dust*	Oak, cedar, and mahogany dust, pine and spruce pulp
Hardwood worker´s lung	*Paecilomyces*	Kiln-dried wood,
Paprika slicer's lung	*Mucor stolonifer*	Moldy paprika pods
Sauna taker's lung	*Aureobasidium spp., other sources*	Contaminated sauna water
Familial HP	*B. subtilis*	Contaminated wood dust in walls
Wood trimmer's lung	*Rhizopus spp., Mucor spp.*	Contaminated wood trimmings

Selman, et al, AJRCCM Aug 15 2012

Basement shower HP	*Epicoccum nigrum*	Mold on unventilated shower
Hot tub lung	*Mycobacterium avium complex*	Hot tub mists; mold on ceiling
Wine maker's lung	*Botrytis cinerea*	Mold on grapes
Woodsman's disease	*Penicillium spp.*	Oak and maple trees
Thatched roof lung	*Sacchoromonospora viridis*	Dead grasses and leaves
Tobacco grower's lung	*Aspergillus spp.*	Tobacco plants
Potato riddler's lung	Thermophilic actinomycetes, *F. rectivirgula, T. vulgaris, Aspergillus spp.*	Moldy hay around potatoes
Summer-type pneumonitis	*Trichosporon cutaneum*	Contaminated old houses
Dry rot lung	*Merulius lacrymans*	Rotten wood
Stipatosis	*Aspergillus fumigatus; T. actinomycetes*	Esparto dust
Machine operator's lung	*Mycobacterium immunogenum; Pseudomona fluorescens,*	Aerosolized metalworking fluid
Residential provoked pneumonitis Amebae	*Aureobasidium pullulans*	Residential exposure
Humidifier lung	*Naegleria gruberi, Acanthamoeba*	Contaminated water from home
	polyphaga, Acanthamoeba castellani, Bacillus sp., others	humidifier, ultrasonic misting fountains
Shower curtain disease	*Phoma violacea*	Moldy shower curtain
Animal proteins		
Pigeon breeder's or pigeon fancier's disease	Avian droppings, feathers, serum	Parakeets, budgerigars, pigeons, chickens, turkeys
Pituitary snuff taker's lung	Pituitary snuff	Bovine and porcine pituitary proteins
Fish meal worker's lung	Fish meal	Fish meal dust
Bat lung	Bat serum protein	Bat droppings
Furrier's lung	Animal fur dust	Animal pelts
Animal handler's lung; laboratory worker's lung	Rats, gerbils	Urine, serum, pelts, proteins
Insect proteins		
Miller's lung	*Sitophilus granarius (ie, wheat weevil)*	Dust-contaminated grain
Lycoperdonosis	Puffball spores	Lycoperdon puffballs

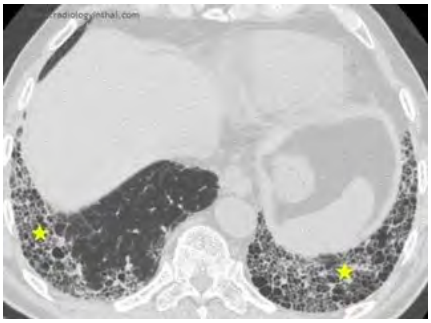
HP background

- fHP generally looks the same microscopically regardless of exposure
- An exposure may not always be identifiable
- Serologic antigen testing may be negative; conversely a positive test is only indicative of exposure not disease

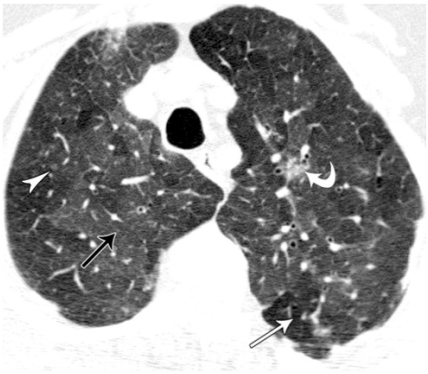
HP Background

- Symptoms, radiology **and pathology** can overlap with other lung diseases
- Accurate diagnosis requires a high degree of clinical suspicious
- Correlation of clinical, radiographic and pathologic findings critical!!!!!!!!!!

UIP vs HP radiology

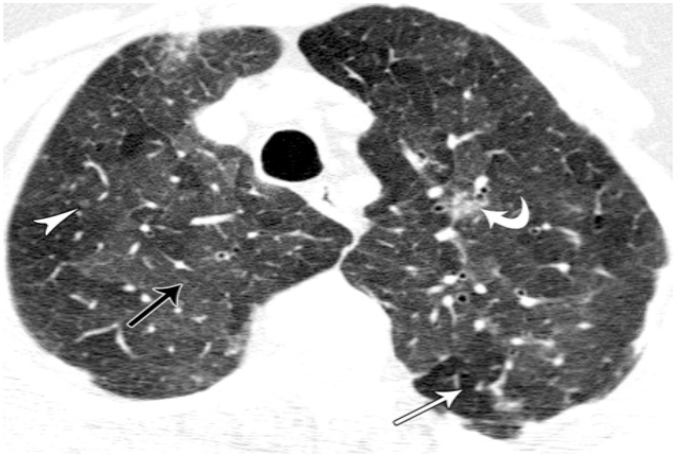


UIP—classically lower lobe predominance, peripheral with honeycomb change



HP may mimic UIP but typically has upper lobe predominance and will also have evidence of air trapping indicating airway disease

HSP radiology



Chronic/Fibrotic HSP

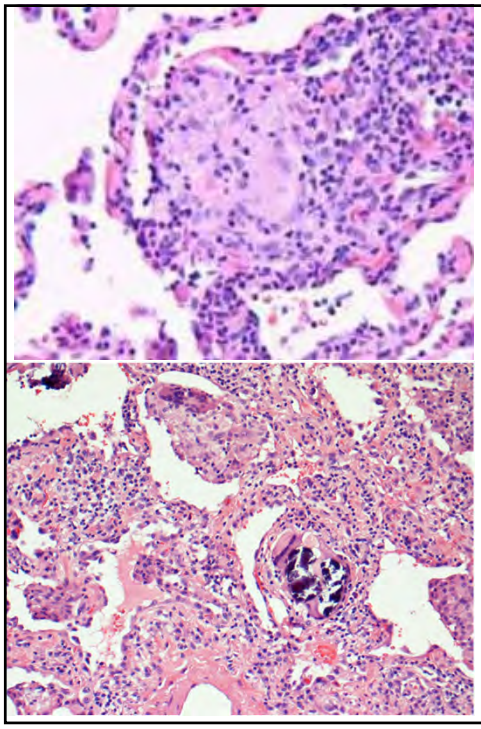
- Churg, et al. AJSP 2006 30(2) 201-208:
  - **Patchy subpleural fibrosis with temporal heterogeneity resembling UIP**

    +/- peribronchiolar fibrosis

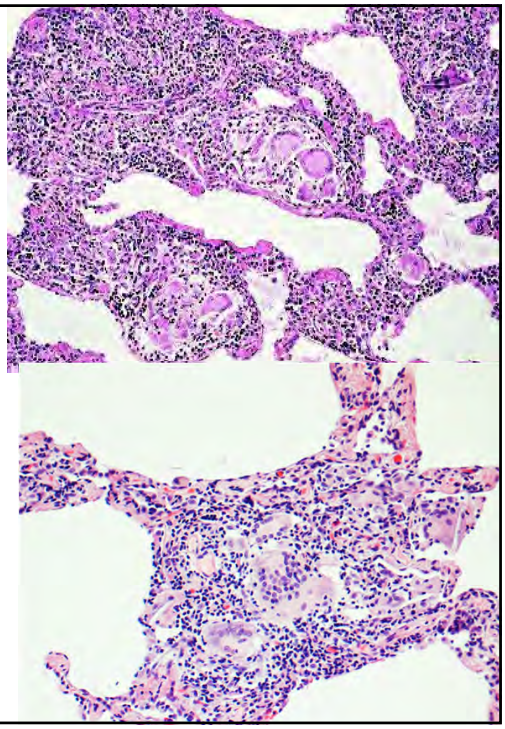
    +/- "NSIP-like areas"
  - Diffuse fibrosis c/w NSIP
  - All cases had identifiable giant cells and/or granulomas and/or Schaumann bodies—usually seen in both fibrotic and non-fibrotic areas

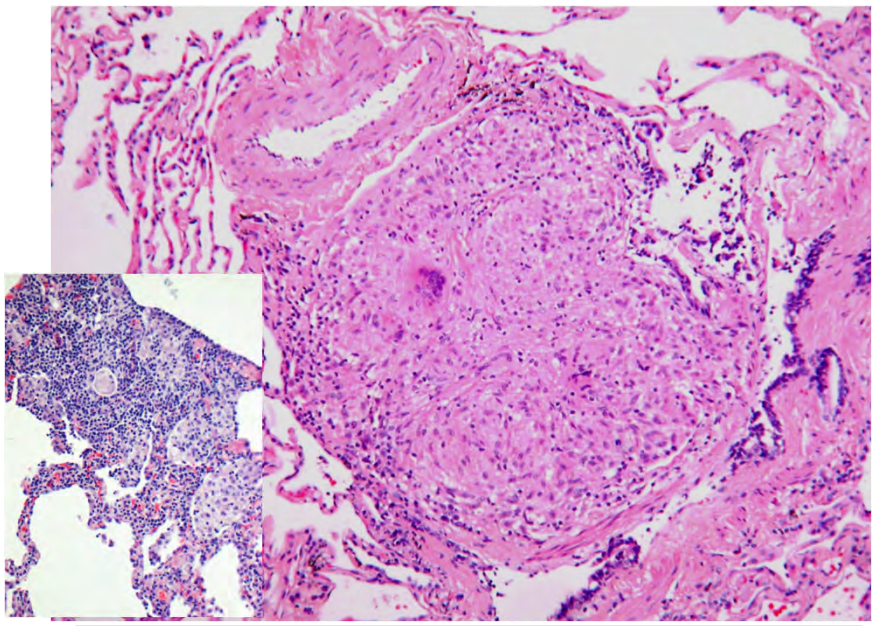
Chronic/Fibrotic HSP

- The finding of significant peribronchiolar fibrosis admixed with a UIP pattern should prompt a careful search for giant cells and granulomas
- The finding of granulomas/giant cells/Schaumann bodies should raise the suspicion of HSP clinically
- Absence of granulomas does not definitively exclude HSP



Poorly formed granulomas





Granuloma is relatively large and well-formed compared with granulomas of HSP (inset)

fHP vs UIP/IPF

Table 2. Separation of Chronic Hypersensitivity Pneumonitis (CHP) With a Usual Interstitial Pneumonia (UIP) Pattern From UIP/Idiopathic Pulmonary Fibrosis (IPF) From Video-Assisted Thoracoscopic Surgery Biopsies

Finding	CHP	UIP/IPF
Zonal predominance	Sometimes upper-zone predominant but can be lower-zone predominant	Always lower-zone predominant
Peribronchiolar fibrosis	Common and frequently associated with fibroblast foci	Uncommon, unless the lobule has been overrun; usually not associated with fibroblast foci
Bridging fibrosis	Common	Rare, unless fibrosis is advanced and the lobule has been overrun
Areas of subacute HP	May be present	Not present by definition
Fibroblast foci	Usually present, if peribronchiolar favors HP	Always present, should not be peribronchiolar unless the disease is very advanced and the lobule has been overrun
Subpleural fibrosis	Can be identical to UIP/IPF but often much less marked and stretches of subpleural sparing may be present	Tends to be very marked with involvement of most or all of the subpleural parenchyma; large blocks of fibrosis with microscopic honeycombing common
Giant cells and granulomas	May be present, but probably most cases do not have either one	The presence of giant cells or granulomas favors another diagnosis
Interstitial inflammation	Plasma cells, lymphocytes, and a few eosinophils common, but some cases are paucicellular	Should be paucicellular
Organizing pneumonia	Common in some series	If present, represents a superimposed process
Microscopic honeycombing	May be present, but many cases do not show honeycombing	Usually present

Abbreviation: HP, hypersensitivity pneumonitis.

(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2017-0173-RA)

AMERICAN THORACIC SOCIETY DOCUMENTS

Diagnosis of Hypersensitivity Pneumonitis in Adults

An Official ATS/JRS/ALAT Clinical Practice Guideline

Ganesh Raghu, Martine Remy-Jardin, Christopher J. Ryerson, Jeffrey L. Myers, Michael Kreuter, Martina Vasakova, Elena Bargagli, Jonathan H. Chung, Bridget F. Collins, Elisabeth Bendstrup, Hassan A. Chami, Abigail T. Chua, Tamera J. Corte, Jean-Charles Dalphin†, Sonye K. Danoff, Javier Diaz-Mendoza, Abhijit Duggal, Ryoko Egashira, Thomas Ewing, Mridu Gulati, Yoshikazu Inoue, Alex R. Jenkins, Kerri A. Johannson, Takeshi Johkoh, Maximiliano Tamae-Kakazu, Masanori Kitaichi, Shandra L. Knight, Dirk Koschel, David J. Lederer, Yolanda Mageto, Lisa A. Maier, Carlos Matiz, Ferran Morell, Andrew G. Nicholson, Setu Patolia, Carlos A. Pereira, Elisabetta A. Renzoni, Margaret L. Salisbury, Moises Selman, Simon L. F. Walsh, Wim A. Wuyts, and Kevin C. Wilson; on behalf of the American Thoracic Society, Japanese Respiratory Society, and Asociación Latinoamericana de Tórax

This guideline is dedicated to the memory of Prof. Jean-Charles Dalphin† (June 2, 1956–October 17, 2019)

This official clinical practice guideline was approved by the American Thoracic Society, Japanese Respiratory Society, and Asociación Latinoamericana de Tórax. May 2020

Table 7. Histopathological Criteria for the Diagnosis of HP (Other than "Hot-Tub Lung"*)

HP	Probable HP	Indeterminate for HP
Nonfibrotic HP (cellular HP) Typical histopathological features of nonfibrotic HP; at least one biopsy site showing all three of the following features: 1. Cellular interstitial pneumonia • Bronchiolocentric (airway-centered) • Cellular NSIP-like pattern • Lymphocyte-predominant 2. Cellular bronchiolitis • Lymphocyte-predominant (lymphs > plasma cells) with no more than focal peribronchiolar lymphoid aggregates with germinal centers • ±Organizing pneumonia pattern with Masson bodies • ±Foamy macrophages in terminal air spaces 3. Poorly formed nonnecrotizing granulomas‡ • Loose clusters of epithelioid cells and/or multinucleated giant cells ± intracytoplasmic inclusions • Situated in peribronchiolar interstitium, terminal air spaces, and/or organizing pneumonia (Masson bodies) *and* Absence of features in any biopsy site to suggest an alternative diagnosis • Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas • Aspirated particulates	Both of the following features (*1* and *2* from first column) in at least one biopsy site: 1. Cellular interstitial pneumonia • Bronchiolocentric (airway-centered) • Cellular NSIP-like pattern • Lymphocyte-predominant 2. Cellular bronchiolitis • Lymphocyte-predominant (lymphs > plasma cells) with no more than focal peribronchiolar lymphoid aggregates with germinal centers • ±Organizing pneumonia pattern with Masson bodies • ±Foamy macrophages in terminal air spaces *and* Absence of features in any biopsy site to suggest an alternative diagnosis • Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas • Aspirated particulates	At least one biopsy site showing one of the following: • *1* or *2* from the first column • Selected IIP patterns ○ Cellular NSIP pattern ○ Organizing pneumonia pattern ○ Peribronchiolar metaplasia *without* other features to suggest fibrotic HP *and* Absence of features in any biopsy site to suggest an alternative diagnosis • Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas • Aspirated particulates
Fibrotic HP§ Typical histopathological features of fibrotic HP; *1* or *2* and *3* in at least one biopsy site: 1. Chronic fibrosing interstitial pneumonia • Architectural distortion, fibroblast foci ± subpleural honeycombing • Fibrotic NSIP-like‖ pattern 2. Airway-centered fibrosis • ±Peribronchiolar metaplasia • ±Bridging fibrosis¶	Both of the following features (*1* or *2* from first column) in at least one biopsy site: 1. Chronic fibrosing interstitial pneumonia • Architectural distortion, fibroblast foci ± subpleural honeycombing • Fibrotic NSIP-like pattern 2. Airway-centered fibrosis • ±Peribronchiolar metaplasia • ±Bridging fibrosis¶ ±Cellular interstitial pneumonia ±Organizing pneumonia pattern ±Cellular bronchiolitis *and* Absence of features in any biopsy site to suggest an alternative diagnosis	Either one of the following features in at least one biopsy site: 1. Chronic fibrosing interstitial pneumonia • Architectural distortion, fibroblast foci ± honeycombing • Fibrotic NSIP-like pattern ±Cellular interstitial pneumonia ±Cellular bronchiolitis ±Organizing pneumonia pattern *and* Absence of features in any biopsy site to suggest an alternative diagnosis • Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas

Table 7. (*Continued*)

HP	Probable HP	Indeterminate for HP
3. Poorly formed nonnecrotizing granulomas[†] ±Cellular interstitial pneumonia ±Cellular bronchiolitis ±Organizing pneumonia pattern *and* Absence of features in any biopsy site to suggest an alternative diagnosis • Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas • Aspirated particulates	• Plasma cells > lymphs • Extensive lymphoid hyperplasia • Extensive well-formed sarcoidal granulomas and/or necrotizing granulomas • Aspirated particulates	• Aspirated particulates

Definition of abbreviations: HP = hypersensitivity pneumonitis; IIP = idiopathic interstitial pneumonias; lymphs = lymphocytes; NSIP = nonspecific interstitial pneumonia; UIP = usual interstitial pneumonia.
[*]Histological findings in hot-tub lung are distinctly different from nonfibrotic and fibrotic forms of classic HP.
[†]Granulomas in HP are smaller, less tightly clustered, and lack the perigranulomatous hyaline fibrosis commonly seen in sarcoidosis.
[‡]Fibrotic HP may show classic features of nonfibrotic HP (cellular HP) in less fibrotic or nonfibrotic areas; if present, this combination of findings is a histological clue to the diagnosis of HP.
[§]Updates to the classification of IIPs by Travis and colleagues (330) and diagnostic guidelines for idiopathic pulmonary fibrosis (20, 128) tightly link a UIP pattern with idiopathic pulmonary fibrosis and an NSIP pattern with idiopathic NSIP.
[‖]Bridging fibrosis spans subpleural and centrilobular or neighboring centrilobular fibrotic foci.

Usual pathologist comment on this: "$%^#@!"

A guideline on how to use the guidelines…. Probably not a good sign….

Integration and Application of Clinical Practice Guidelines for the Diagnosis of Idiopathic Pulmonary Fibrosis and Fibrotic Hypersensitivity Pneumonitis

Check for updates

Daniel-Costin Marinescu, MD; Ganesh Raghu, MD; Martine Remy-Jardin, MD; William D. Travis, MD; Ayodeji Adegunsoye, MD; Mary Beth Beasley, MD; Jonathan H. Chung, MD; Andrew Churg, MD; Vincent Cottin, MD; Ryoko Egashira, MD; Evans R. Fernández Pérez, MD; Yoshikazu Inoue, MD; Kerri A. Johannson, MD; Ella A. Kazerooni, MD; Yet H. Khor, MD; David A. Lynch, MD; Nestor L. Müller, MD; Jeffrey L. Myers, MD; Andrew G. Nicholson, MD; Sujeet Rajan, MD; Ryoko Saito-Koyama, MD; Lauren Troy, MD; Simon L. F. Walsh, MD; Athol U. Wells, MD; Marlies S. Wijsenbeek, MD; Joanne L. Wright, MD; and Christopher J. Ryerson, MD

CHEST 2022; 162(3):614-629

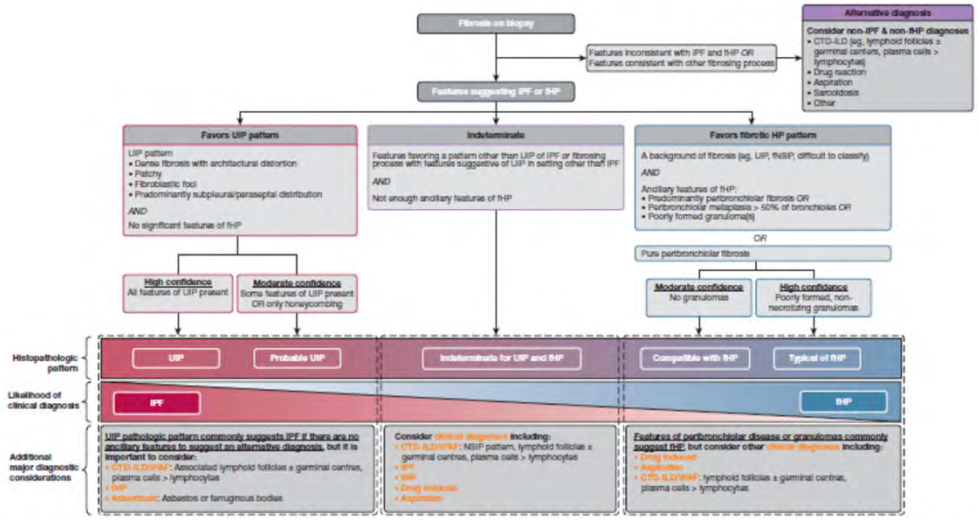


Figure 10 – *Diagram showing an approach to assessment of histopathologic features in patients with IPF, fHP, or both as primary diagnostic considerations. Individual pathologic features are integrated, and the overall pattern is evaluated as favoring UIP, favoring fHP, or indeterminate for both. The presence of specific features further separates high-confidence pathologic patterns as described in recent clinical practice guidelines (eg, UIP suggestive of IPF, typical of fHP), with the collection of features then informing the likelihood of a diagnosis of IPF and fHP across a diagnostic spectrum. Other features occasionally can suggest additional major diagnostic considerations, as shown at the bottom of the figure. CTD-ILD = connective tissue disease-related interstitial lung disease; fHP = fibrotic hypersensitivity pneumonitis; fNSIP = fibrotic nonspecific interstitial pneumonia; IPAF = interstitial pneumonia with autoimmune features; IPF = idiopathic pulmonary fibrosis; UIP = usual interstitial pneumonia.*

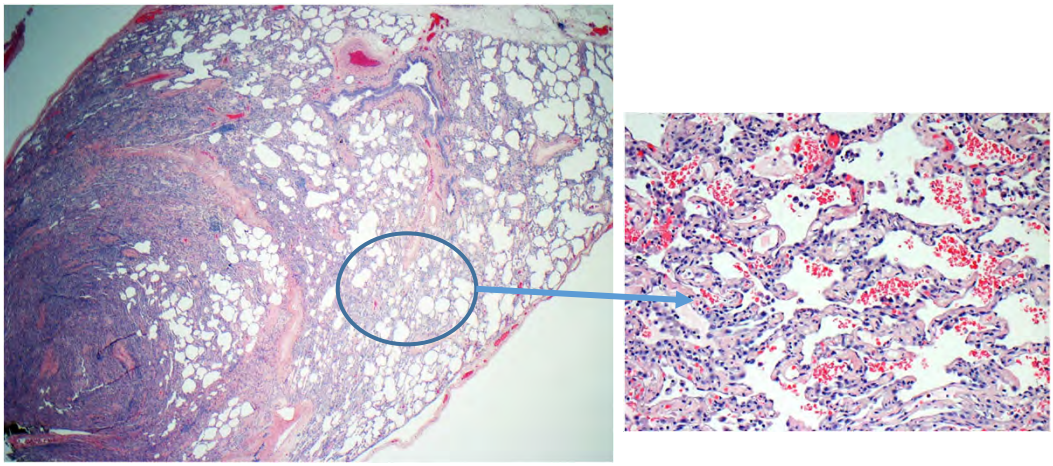
HSP Summary

- May mimic UIP or NSIP
- Prominent peribronchiolar involvement
- Poorly formed granulomas/ single giant cells (may be absent in up to 30%) of cases
- CT with upper lobe predominance and air trapping characteristic
- Identifiable antigen may be lacking

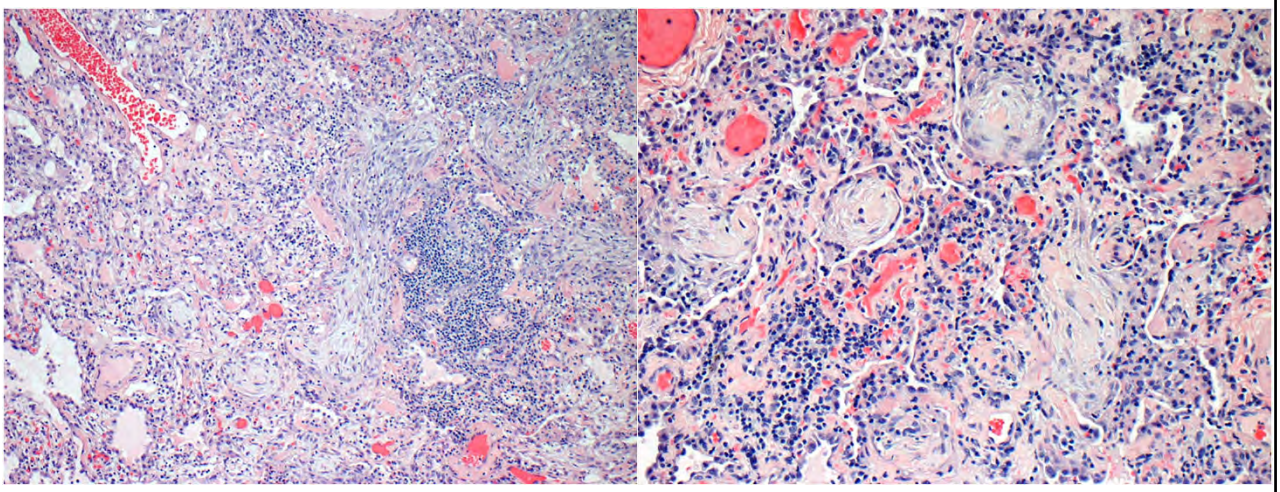
Case2

- 45 year old female with progressively worsening shortness of breath over a several month period
- CT scan with nodular densities and ground glass opacities
- Serology results unavailable but patient denied joint pain, rash, etc
- No known exposures

Case 2



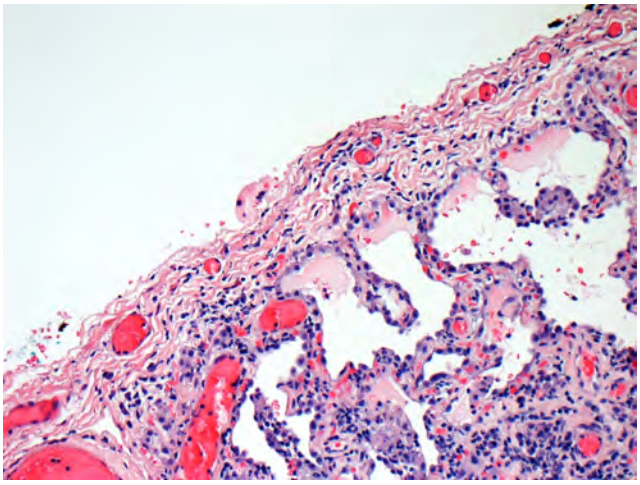
Case 2



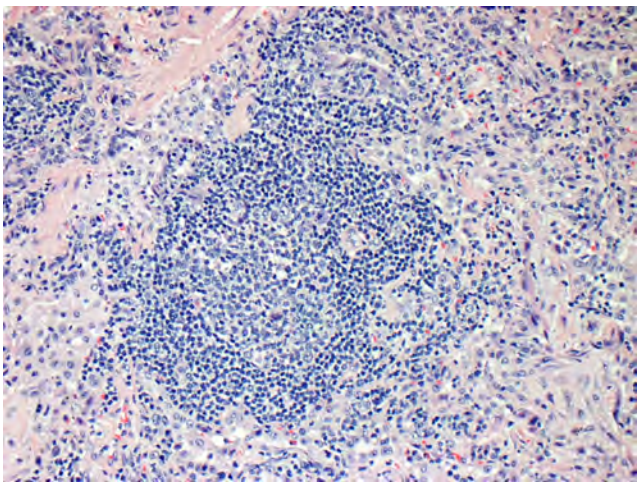
Pathology summary

- Originally called “COP”
  - Organizing pneumonia (OP) is present….however…
- The interstitial chronic inflammation is too diffuse to be classic for “OP pattern” as defined by ATS/ERS classification
- Similarly, this is more than the “occasional foci” of OP one might encounter in cellular NSIP
- ?Cellular NSIP with OP on top or one process i.e CVD explaining both findings?

Mild pleuritis



Lymphoid follicle



Follow up….

- Patient ultimately diagnosed with anti-Jo1 anti-synthetase syndrome
  - "NSIP with too much OP" frequently seen with anti-synthetase disease but not pathognomonic

Connective tissue disease related ILD (CTD-ILD)

- Generally these will require a descriptive diagnosis:
  - Diffuse chronic interstitial pneumonitis with fairly extensive organizing pneumonia
  - Note- The amount of organizing pneumonia present is too extensive to be typical of NSIP as described by the ATS/ERS classification and, conversely, the amount of background interstitial inflammation is too extensive to be typical of OP pattern. Lymphoid follicle formation and pleuritis are also present. An underlying connective tissue disease could explain the totality of findings in this case. Continued clinical and radiographic correlation is recommended.
  - Other considerations: Drug reaction, infection; lymphoid follicles and lack of peribronchiolar disease would be atypical for HP in this case.

Clues that UIP or NSIP may be CTD related

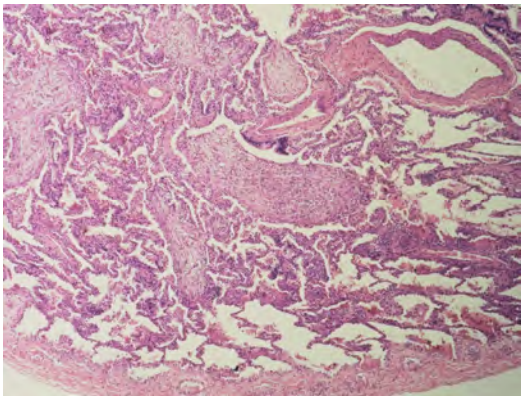
- Younger than 50 years of age
- Female
- Never smoker
- Atypical radiographic findings
- Follicular bronchiolitis/BALT hyperplasia also present
- Mixed pattern of disease (Not quite perfect for either UIP or NSIP, “NSIP with too much OP”)
- Pleuritis
- Issue--Patients may be on immunosuppresive or cytotoxic drugs—infection and drug reaction are also in the differential diagnosis.
- Issue- ILD may precede development of clinically diagnostic features of CTD
- Interstitial pneumonia with autoimmune features (IPAF)- Clinical dx for patient with ILD lacking fully diagnostic features of underlying CTD

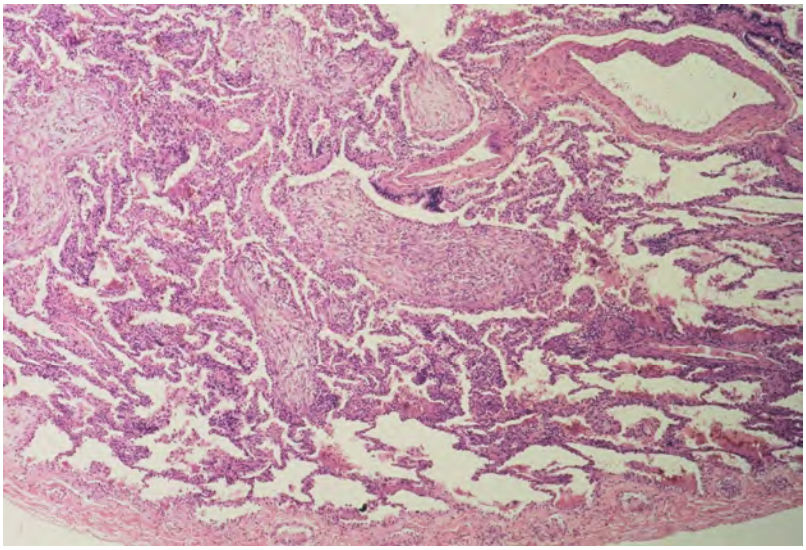
Why is case 2 not OP as defined by ATS/ERS

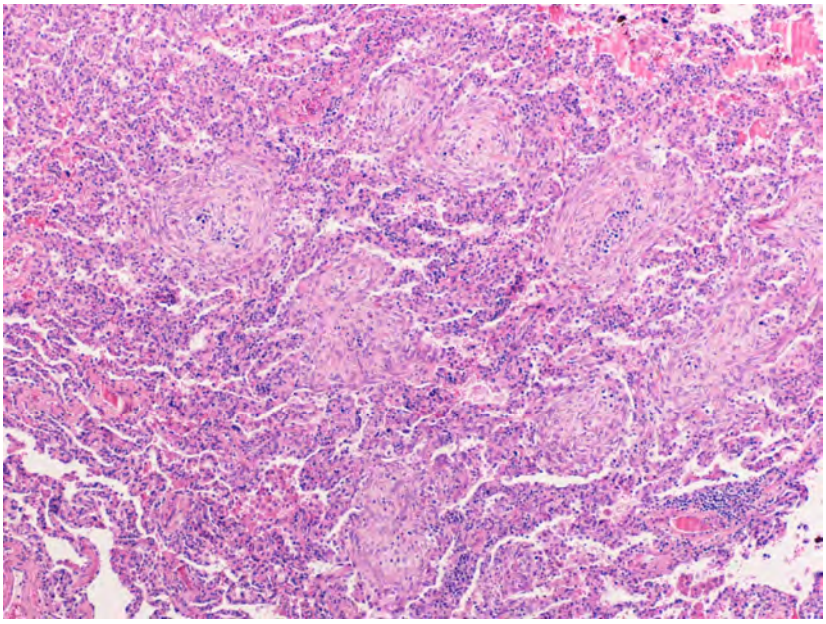
- Background interstitial inflammation is too diffuse and extends far beyond the areas of OP

OP pattern

- Patchy, bronchiolocentric organizing pneumonia +/- fibroblastic plugs in small airway lumens (BO)
- Minimal chronic inflammation in adjacent alveoli
- Intervening lung more or less normal
- NO other findings—i.e. no granulomas, significant acute inflammation, etc
- Need a wedge biopsy to appreciate this pattern



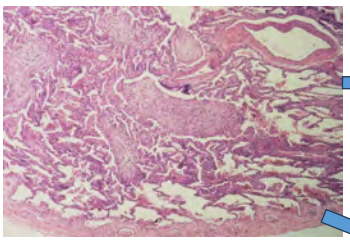




Nomenclature issues

- Organizing pneumonia merely means organizing fibroblastic tissue within an airspace
- The general finding of OP can be due to any number of things
  - Primary disease: OP pattern as defined by ATS/ERS
  - Component of another disease: fHP, vasculitis, bacterial pneumonia
  - Secondary finding adjacent to a completely unrelated process: Tumor, granuloma
- OP pattern histologically PLUS idiopathic disease clinically equals cryptogenic organizing pneumonia (COP)
- OP pattern may be secondary to CTD, drug reaction etc>> recently suggested name “secondary organizing pneumonia” (SOP)

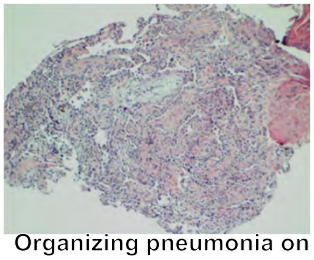
Summary—All OP is not COP



OP/BOOP pattern on wedge

idiopathic disease →  COP!

Not idiopathic → Not COP →  → Suggested term "Secondary OP" (SOP)



Organizing pneumonia on small bx

- Can't assume this is COP
- You don't know what this really is—this could be anything
- MUST correlate with clinical and radiographic findings

Algorithmic Approach to the Diagnosis of Organizing Pneumonia

A Correlation of Clinical, Radiologic, and Pathologic Features

Sujith V. Cherian, MD, FCCP; Dhara Patel, MS; Stephen Machnicki, MD, FCCP; David Naidich, MD, FCCP; Diane Stover, MD, FCCP; William D. Travis, MD; Kevin K. Brown, MD, FCCP; Jason J. Naidich, MD; Akhilesh Mahajan, MD; Michael Esposito, MD; Bushra Mina, MD; Viera Lakticova, MD; Stuart L. Cohen, MD, FCCP; Nestor L. Muller, MD, PhD; Jenna Schulner, PA; Rakesh Shah, MD; and Suhail Raoof, MD, Master FCCP

CHEST 2022; 162(1):156-178

TABLE 4 Classification of OP

Type	Description	
COP	An exhaustive search for different causes for OP has been unrevealing, and the primary pathologic process within the lung is an OP pattern.	
SOP[a]	OP here is secondary to a known pulmonary insult, and the primary pathologic process within the lung is OP.	
	Causes	Examples
	After infection[b]	Bacteria, viruses, parasites, fungi, mycobacteria
	Drug toxicity[c]	Antibiotics, antiepileptics, antiarrhythmics, immunosuppressants
	Inhalation of toxic chemicals or substances	Cocaine inhalation, hydrogen sulfide, industrial gases, electronic nicotine delivery systems with adulterated products (EVALI)
	Aspiration of gastric contents	...
	Organ transplant	Bone marrow transplant, lung transplant, liver transplant
	Radiotherapy	Especially in breast cancer
	Rheumatologic disorders	Rheumatoid arthritis, SLE, Sjögren syndrome, dermatomyositis, polymyositis
	Miscellaneous	Inflammatory bowel disease, polymyalgia rheumatica, CABG

Entities with an OP pattern separate from COP and SOP		
OP pattern according to pathologic examination or CT scanning as a component of other ILDs		
	Causes	Examples
	A minor lesion in the setting of ILD[14,15]	UIP, NSIP, or HP[14]
	A component of organizing acute lung injury such as DAD	...
	A manifestation of acute exacerbation of UIP or IPF or various other ILDs, including NSIP and HP[16]	UIP or IPF or various other ILDs, including NSIP and HP
	In the setting of mixed patterns of lung injury	NSIP and OP or eosinophilic pneumonia and OP[16]
Nonspecific reaction to another process often at the periphery of underlying primary lung disease		
	Causes	Examples
	Primary lung cancers	Any tumor such as lung cancer or lymphomas
	Pulmonary infarct	...
	Aspiration	...
	Lung abscess	...
	Pulmonary vasculitis or hemorrhage	GPA, EGPA, hemorrhage with microscopic polyangiitis, capillaritis

One more issue..

Pathologic Separation of Chronic Hypersensitivity Pneumonitis From Fibrotic Connective Tissue Disease–associated Interstitial Lung Disease

Andrew Churg, MD, Joanne L. Wright, MD,† and Christopher J. Ryerson, MD‡*

(*Am J Surg Pathol* 2017;41:1403–1409)

Evaluated multiple histologic features (peribronchiolar metaplasia, germinal centers, lymphoid aggregates, volume proportion of plasma cells and plasma cell/lymphocyte ratio, number and location of fibroblast foci, location of fibrosis, giant cells/granulomas)

Conclusions:

- No single morphologic feature definitively separated the two
- Numerous foci of peribronchiolar metaplasia favored HSP
- Germinal centers, large numbers of lymphoid aggregates and high plasma cell lymphocyte ratio suggested CTD

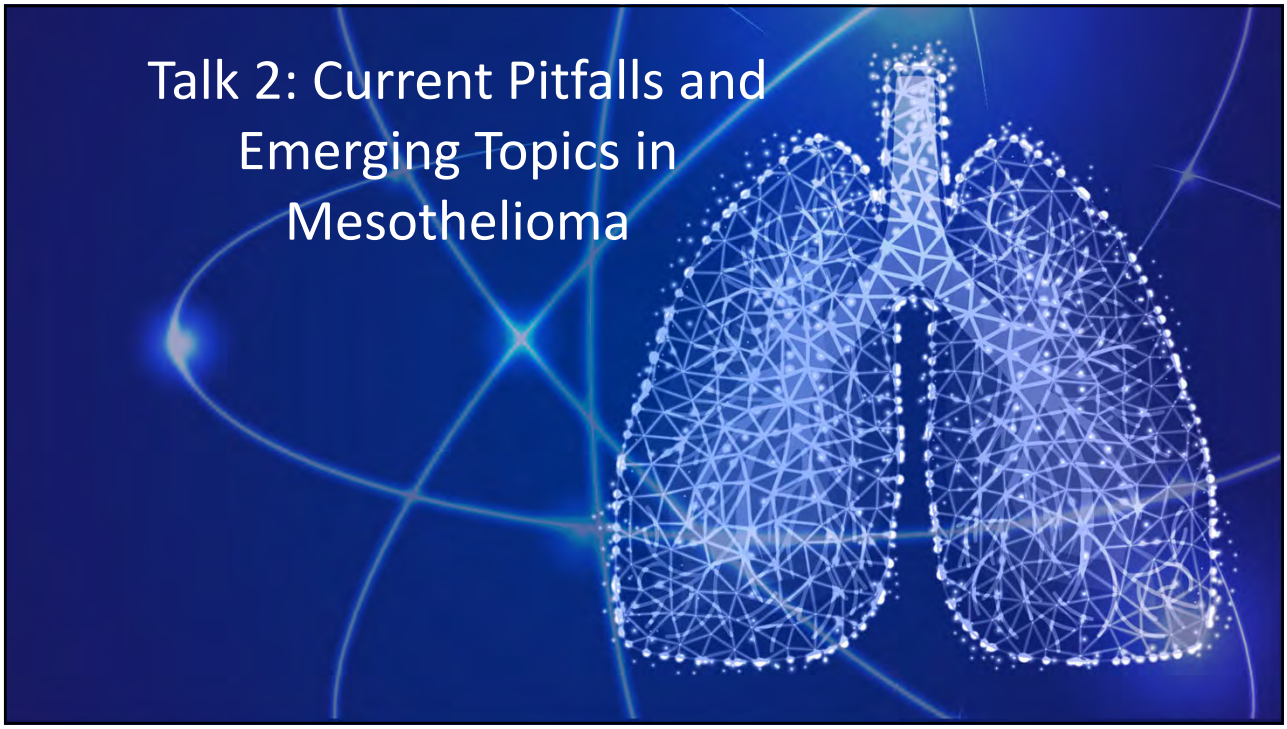
Why do we care?

- Patients with clinical IPF are treated with anti-fibrotics (pirfenidone (Esbriet), nintedanib (Ofev))
- Patient's with fHP and progressive fibrosis may be treated with same but will need to be evaluated for exposures with cessation if possible
- Patient's with CTD-ILD are often treated with immunomodulatory agents +/- antifibrotic

Summary Talk 1

- Decrease in number of classic UIP cases since issuance of clinical guidelines
- Clues for fHP
  - Upper lobe predominance and air trapping radiographically
  - Prominent airway centered disease
  - Giant cells/poorly formed granulomas
- Clues for CTD-ILD
  - Young patient age
  - Female
  - More inflammation than typical for UIP, lymphoid follicle formation, pleuritis
  - Mixture of patterns or patterns not a perfect fit for UIP or NSIP

Talk 2: Current Pitfalls and Emerging Topics in Mesothelioma



Overview

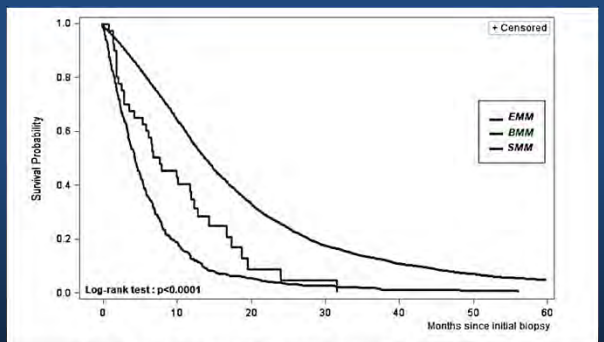
- Epithelioid Morphology:
  - Benign vs malignant
    - Tissue biopsy specimens
      - Invasion vs entrapment/ en face cuts
    - Ancillary techniques
      - Issues with cytology specimens
      - P16 FISH/MTAP and BAP-1 deletion
  - Mesothelioma in situ
  - Epithelioid mesothelioma
    - Diagnosis/Differential
    - Immunostains and pitfalls of immunostains
- Sarcomatoid Morphology
    - Benign vs malignant
    - Sarcomatoid mesothelioma/differential
- Biphasic/transitional/pleomorphic
- Molecular
- Summary

2021 WHO classification of mesothelial tumors

BENIGN AND PREINVASIVE MESOTHELIAL TUMORS
Adenomatoid tumor Well-differentiated papillary mesothelial **tumor** **Mesothelioma in situ**
MESOTHELIOMA
Localized mesothelioma---VERY RARE **Diffuse mesothelioma**

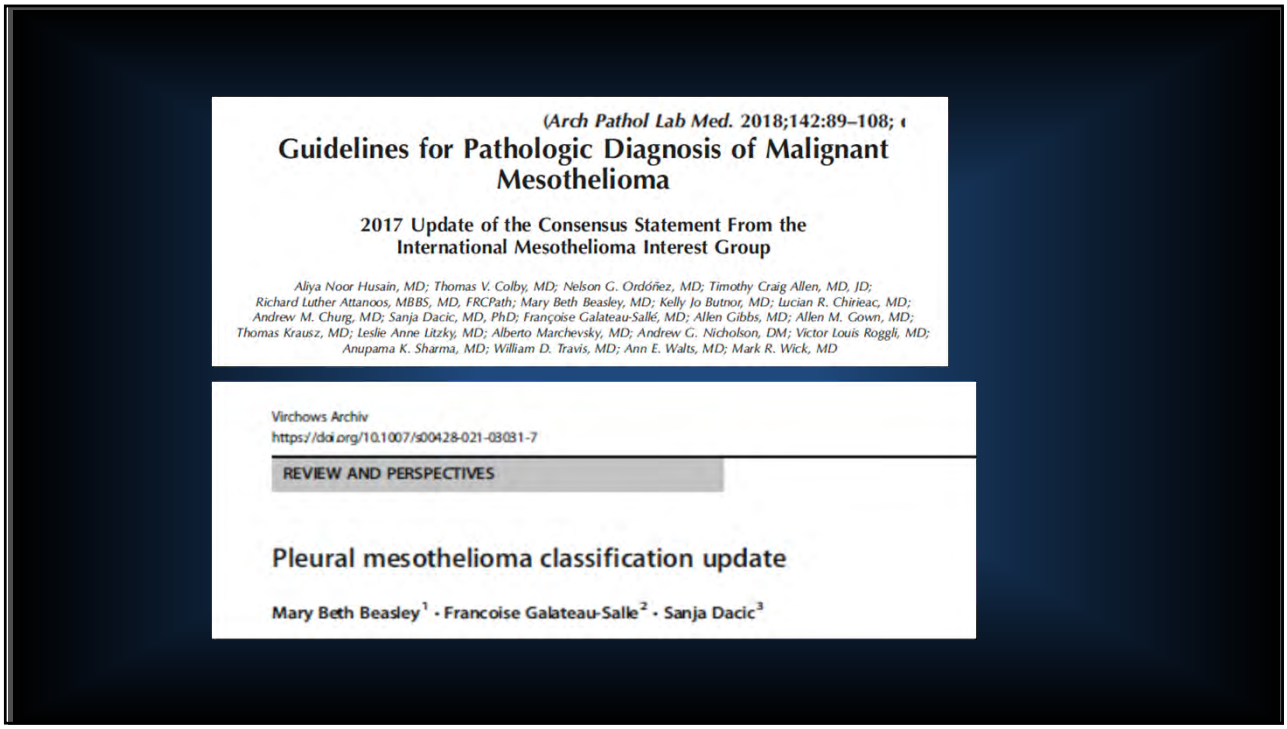
DIFFUSE MESOTHELIOMA SUBTYPES

- Epithelioid
- Sarcomatoid (including desmoplastic)
- Biphasic



	N	Median	1 yr-survival [CI95%]	2 yrs-survival [CI95%]	5 yrs-survival [CI95%]
EMM	5219	14 mos	55% [53%; 57%]	24% [23%; 26%]	4% [3%; 5%]
BMM	42	8 mos	38% [23%; 53%]	8% [0%; 19%]	0%
SMM	465	4 mos	12% [9%; 15%]	3% [1%; 5%]	0%

F. Galateau Salle et al. J Thorac Oncol 2018



Epithelioid Mesothelioma: Issues and Differential

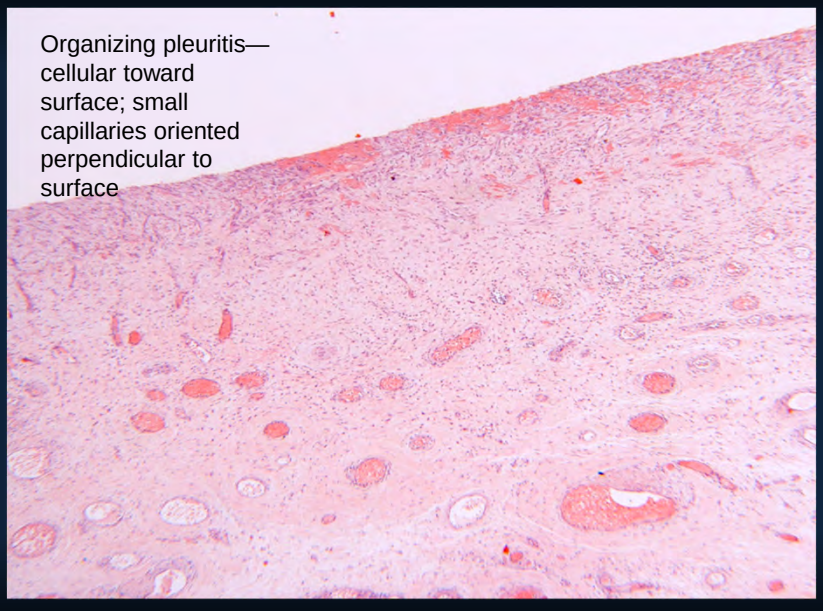
B-9 vs Malignant?

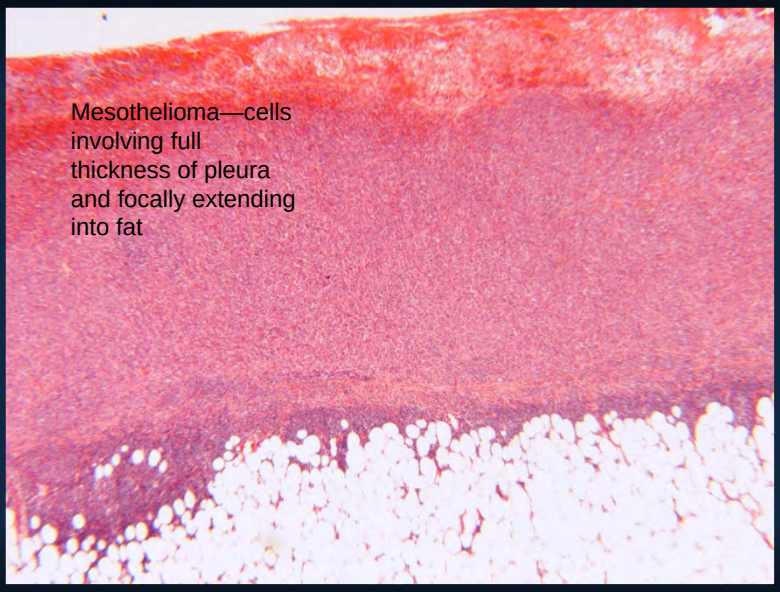
Table 1. Reactive Mesothelial Hyperplasia Versus Mesothelioma

Mesothelial Hyperplasia	Mesothelioma
• Absence of stromal invasion (beware of entrapment and en face cuts)	• Stromal invasion usually apparent (highlight with pancytokeratin staining)
• Cellularity may be prominent but is confined to the mesothelial surface/pleural space and is not in the stroma	• Dense cellularity, including cells surrounded by stroma
• Simple papillae; single cell layers	• Complex papillae; tubules and cellular stratification
• Loose sheets of cells without stroma	• Cells surrounded by stroma ("bulky tumor" may involve the mesothelial space without obvious invasion)
• Necrosis rare	• Necrosis present (occasionally)
• Inflammation common	• Inflammation usually minimal
• Uniform growth (highlighted with cytokeratin staining)	• Expansile nodules; disorganized growth (highlighted on cytokeratin staining)
Usually Not Useful	
• Mitotic activity • Mild to moderate cytologic atypia	

(*Arch Pathol Lab Med.* 2018;142:89–108;

Distribution of mesothelial cells relative to pleura surface:
Cells on the surface, aka “sidedness” of proliferation favors B-9





Main feature discriminating B-9 from malignant mesothelial proliferations

INVASION

INVASION

INVASION

Is it really invasive???

- Pitfalls:
  - Entrapment
  - En face cuts of small, poorly oriented biopsies
  - Additional pitfall— “Fake fat”!!!!!

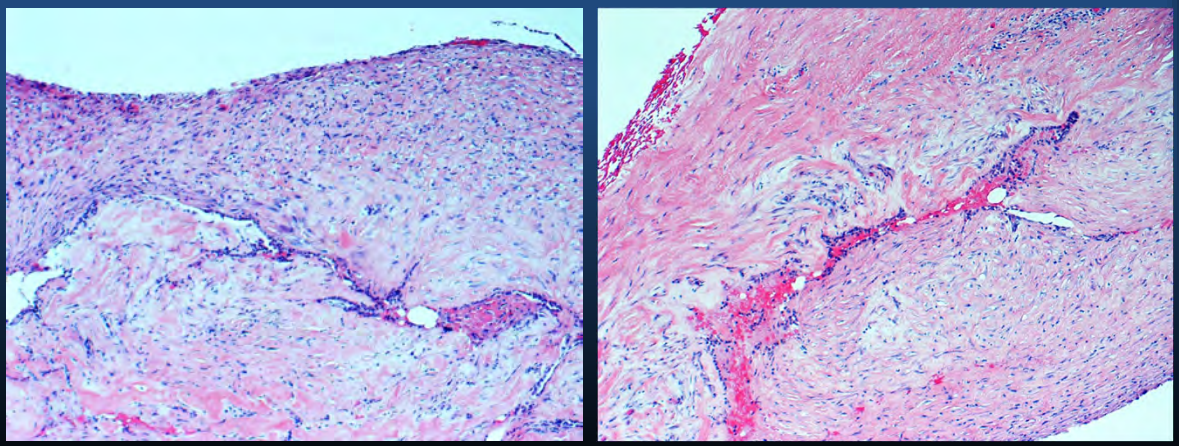
Entrapment vs Invasion

- Entrapment very common in organizing pleuritis
  - Is active inflammation present? Be cautious!!!!!!!
    - Eosinophilic pleuritis s/p pneumothorax can have marked mesothelial hyperplasia
  - Simple tubules (favor B-9) vs complex branched glands and papillae (favor malignant)
  - Linear cellular arrangement (favor B-9) vs irregular pattern (favor malignant)

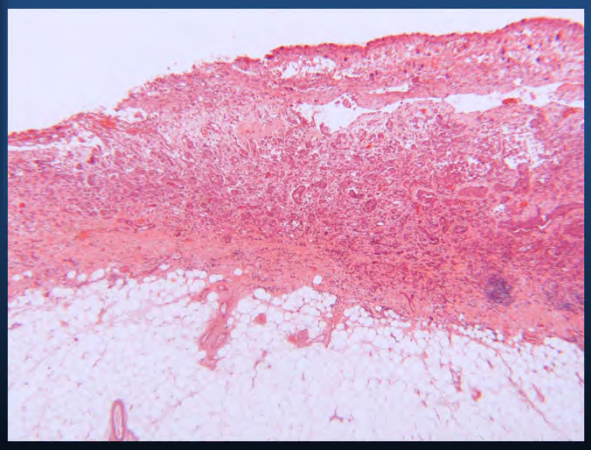
Small Poorly Oriented Specimens

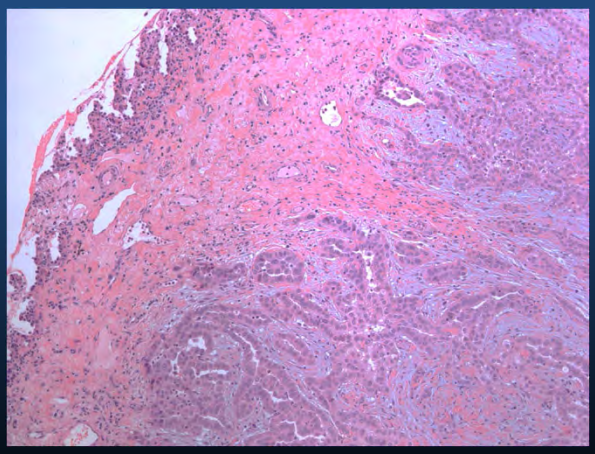
- En Face cuts particularly treacherous
- Closely spaced branching glands (favor malignant) vs simple glands separated by lots of fibrous stroma (favor benign)
- Levels to look for fat or muscle invasion (beware of “fake fat”)
- Use caution if abundant inflammation present
- Beware of linear arrangement (favors benign)

Entrapment—linear meso cells sandwiched between organizing pleuritis.



Definitely malignant—mesothelial cells clearly invasive





Fake fat!—don't confuse with invasion!

The Fake Fat Phenomenon in Organizing Pleuritis: A Source of Confusion With Desmoplastic Malignant Mesotheliomas

Andrew Churg, MD, Philip Cagle, MD,† Thomas V. Colby,‡ Joseph M. Corson, MD,§ Allen R. Gibbs, MD,‖ Samuel Hammar, MD,¶ Nelson Ordonez, MD,# Victor L. Roggli, MD,** Henry D. Tazelaar, MD,‡ William D. Travis, MD,†† Mark Wick, MD,‡‡ and from the US-Canadian Mesothelioma Reference Panel*

(*Am J Surg Pathol* 2011;35:1823–1829)

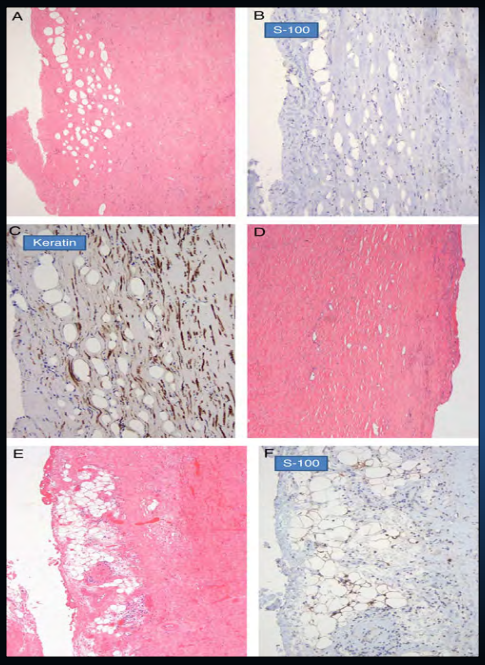


FIGURE 2

The Fake Fat Phenomenon in Organizing Pleuritis: A Source of Confusion With Desmoplastic Malignant Mesotheliomas.
Churg, Andrew; Cagle, Philip; Colby, Thomas; Corson, Joseph; Gibbs, Allen; Hammar, Samuel; Ordonez, Nelson; Roggli, Victor; Tazelaar, Henry; Travis, William; Wick, Mark

American Journal of Surgical Pathology. 35(12):1823-1829, December 2011.

FIGURE 2 . Another example of fat-like spaces deep within a fibrotic pleura. Here, the spaces are both rounded and oblate, with the long axis of the oblate spaces parallel to the pleural surface (A). S-100 stain fails to stain the fat-like spaces (B). Pan-keratin stain (C) shows positive spindled cells between the rounded and oblate spaces. Note in both this figure and in panel B how some of the spaces become fairly narrow slits. Panel D shows another area of the same case with rounded, oblate, and slit-like spaces near the pleural surface, a location where fat should not be found. Panels E and F illustrate real chest wall fat in another part of the same biopsy; the fat cells are S-100 positive.

2

Can stains help?

Table 2. Immunohistochemistry to Separate Reactive Mesothelial Proliferations from Mesothelioma[a]

Antibody	Reactive Mesothelium, No. (% Positive)	Mesothelioma, No. (% Positive)
Desmin	34/40 (85)	6/60 (10)
EMA	8/40 (20)	48/60 (80)
p53	0/40 (0)	27/60 (45)
GLUT-1	5/150 (3)	103/153 (67)
IMP3	0/64 (0)	33/45 (73)

Abbreviations: EMA, epithelial membrane antigen; GLUT-1, glucose transporter 1; IMP3, insulin-like growth factor II messenger RNA–binding protein 3.

[a] Data derived from Kato et al,[5] Acurio et al,[6] Shi et al,[8] Monaco et al,[20] and Attanoos et al.[78]

This is where we were 10+ years ago—
all of these are now defunct

Utility of Methylthioadenosine Phosphorylase Compared With BAP1 Immunohistochemistry, and *CDKN2A* and *NF2* Fluorescence In Situ Hybridization in Separating Reactive Mesothelial Proliferations From Epithelioid Malignant Mesotheliomas

Kyra B. Berg, MD; Sanja Dacic, MD; Caitlyn Miller, BA; Simon Cheung, BSc; Andrew Churg, MD

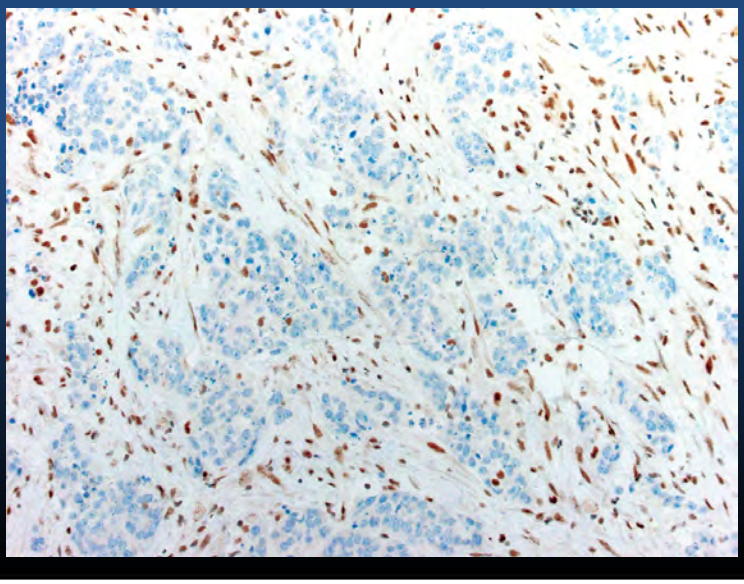
A Combination of MTAP and BAP1 Immunohistochemistry in Pleural Effusion Cytology for the Diagnosis of Mesothelioma

Yoshiaki Kinoshita, MD [1,2]; Tomoyuki Hida, MD, PhD[3]; Makoto Hamasaki, MD, PhD[1]; Shinji Matsumoto, CT, PhD[1]; Ayuko Sato, PhD[4]; Tohru Tsujimura, MD, PhD[4]; Kunimitsu Kawahara, MD, PhD[5]; Kenzo Hiroshima, MD, PhD[6]; Yoshinao Oda, MD, PhD[3]; and Kazuki Nabeshima, MD, PhD[1]

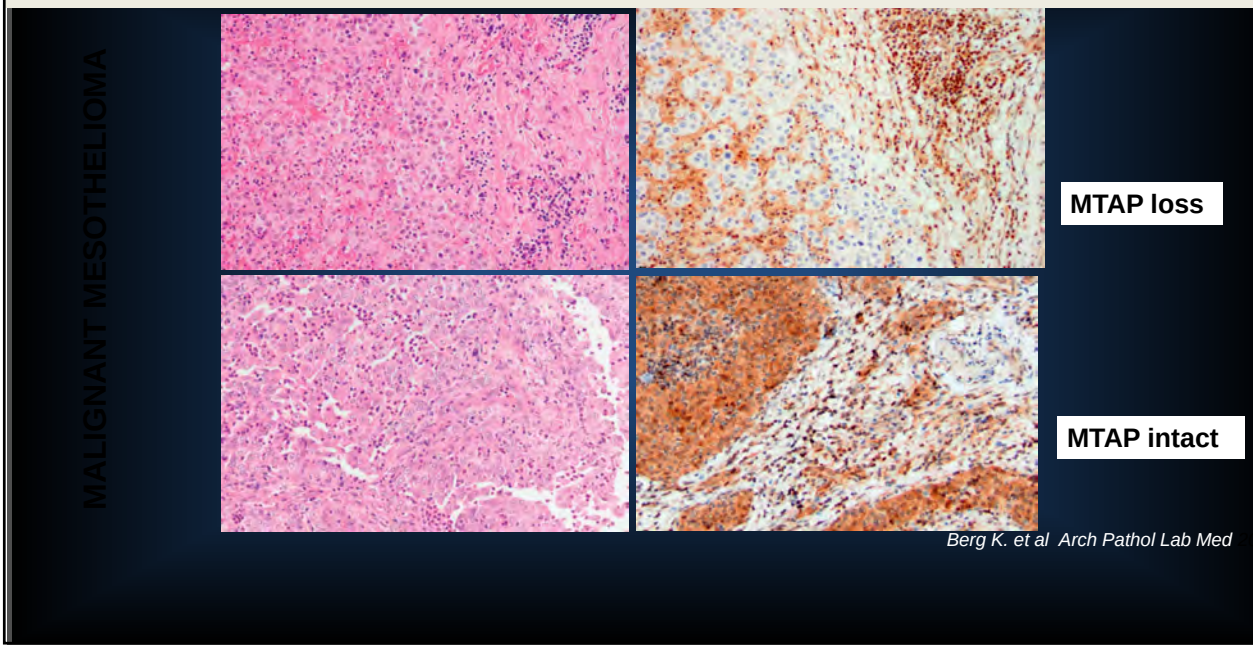
Immunohistochemical detection of MTAP and BAP1 protein loss for mesothelioma diagnosis: Comparison with 9p21 FISH and BAP1 immunohistochemistry

Tomoyuki Hida[a,b,c], Makoto Hamasaki[a], Shinji Matsumoto[a], Ayuko Sato[d], Tohru Tsujimura[d], Kunimitsu Kawahara[e], Akinori Iwasaki[f], Tatsuro Okamoto[g], Yoshinao Oda[b], Hiroshi Honda[c], Kazuki Nabeshima[a,*]

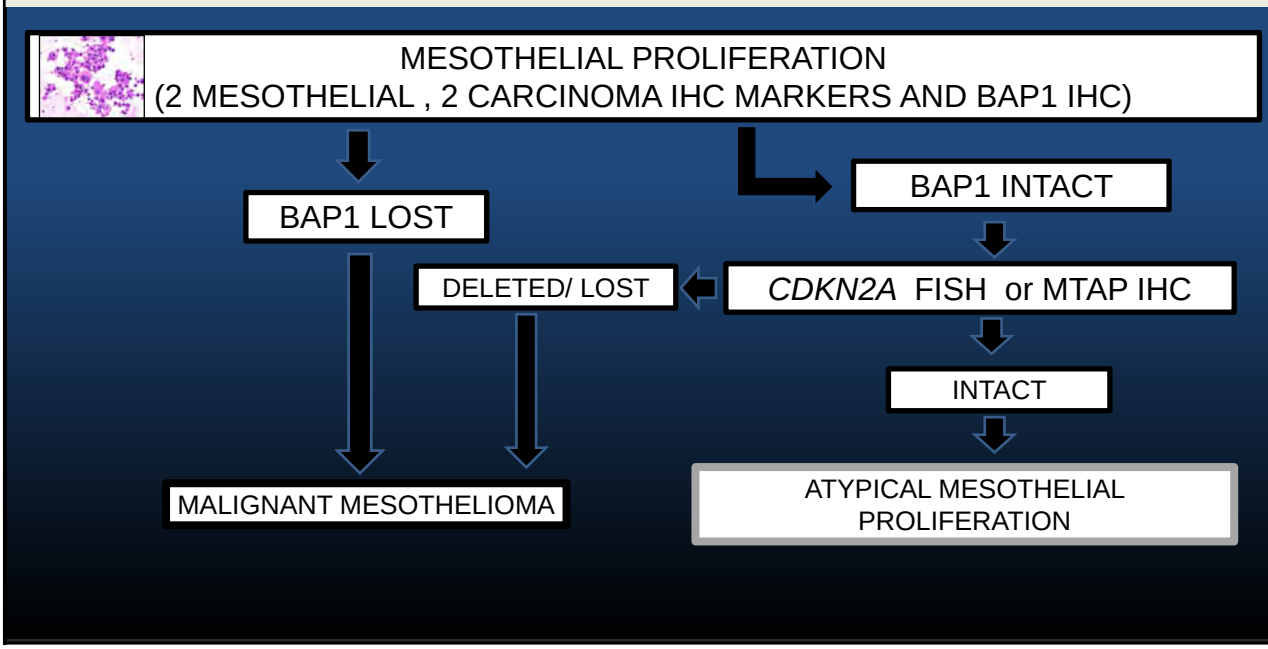
BAP-1 Loss



MTAP IHC AS A SURROGATE FOR *CDKN2A* FISH



DIAGNOSTIC WORK UP OF EFFUSIONS/SMALL SPECIMENS



P16 and BAP-1—summary and caveats

P16 deletion

- Homozygous p16 deletion by FISH is seen in up to 70% of epithelioid pleural mesotheliomas and over 90% of sarcomatoid mesotheliomas
- The generic p16 IHC cannot be used as a substitute for this
- MTAP (methythioadenosine phosphorylase) IHC currently generally shown to be highly sensitive and specific—can be difficult to interpret

BAP-1

- BAP-1 nuclear loss by IHC is detected in 50-80% of epithelioid mesotheliomas and 15-60% of sarcomatoid
- BAP-1 loss by IHC indicates mutation in the tumor but does not mean that the patient has a BAP-1 germline mutation.

BAP-1 nuclear loss plus p16 deletion

- The combination of BAP-1 nuclear loss and homozygous p16 deletion by FISH is essentially 100% specific for mesothelioma over reactive proliferations but sensitivity is variable—generally around 60%
  - Helps if it is lost but not if it is retained
    - Retained does not equal benign as not all mesotheliomas will have BAP-1 or p16 deletion

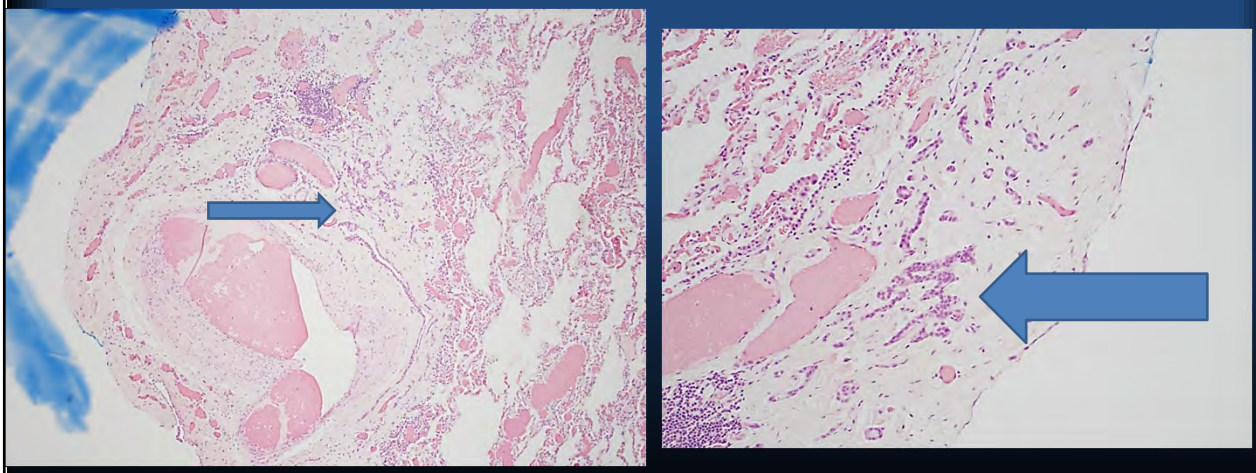
Final caveat

- This applies to cells you KNOW are mesothelial
- P16 abnormalities in particular as well as BAP-1 loss can be seen in tumors other than mesothelioma—loss by itself supports it is malignant not that it is mesothelioma
- Emerging stain: Merlin—correlates with NF2 mutation in mesothelioma---will likely become an additional aid in challenging cases.

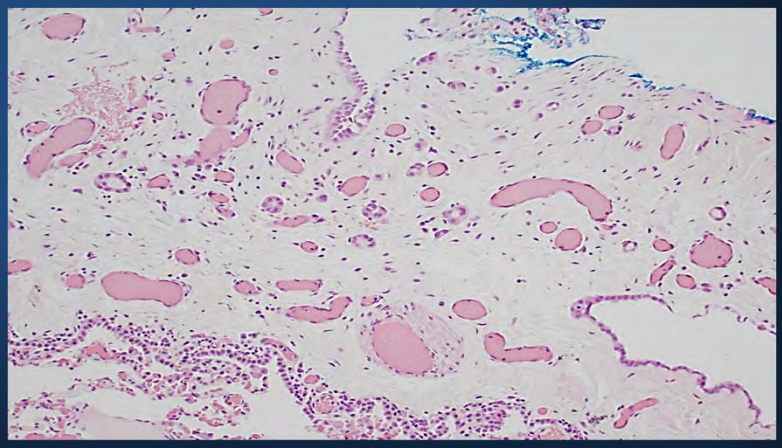
Case 4

- 32 year old male, presented with right pneumothorax, underwent apical wedge resection

Case 4



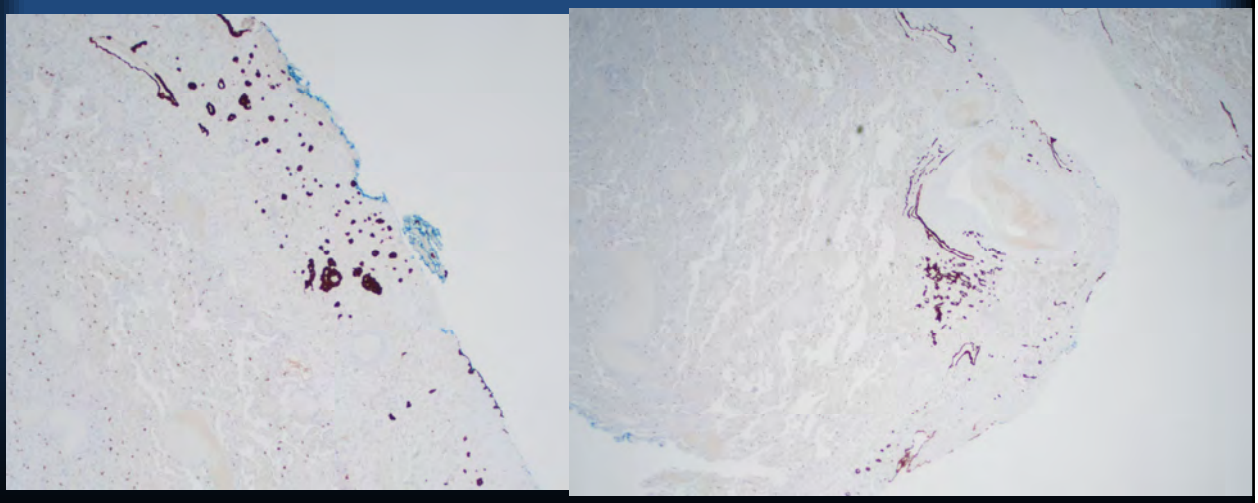
Case 4



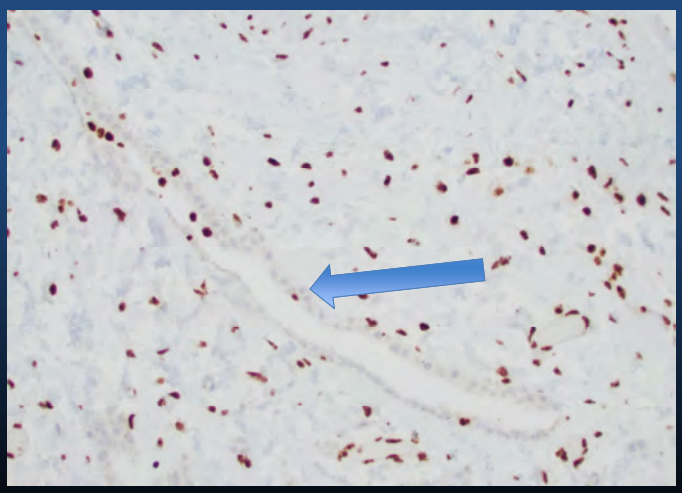
Is this benign or malignant?

Are there any stains that might help with this?

Calretinin—highlights mesothelial pattern and distribution



BAP-1



BAP-1 is lost in the mesothelial cells supporting malignant mesothelioma

Patient's father died from mesothelioma

Patient was found to have a BAP-1 germline mutation

Summary

- Superficial proliferations and cells arranged in a linear fashion parallel to the surface favor benign proliferations
- BAP-1 loss and/or MTAP loss/homozygous p16 deletion support a malignant diagnosis
  - Particularly helpful in subtle cases/biopsies and cytology specimens
- Retention of BAP-1 and MTAP does not mean something is benign---it just doesn't help you

Mesothelioma in situ

- Originally described by Henderson, et al in 1990's however it required the presence of an invasive component and people questioned whether they surface proliferation was just spread of the invasive component as opposed be being truly in situ…

2021 WHO DEFINITION MESOTHELIOMA IN SITU

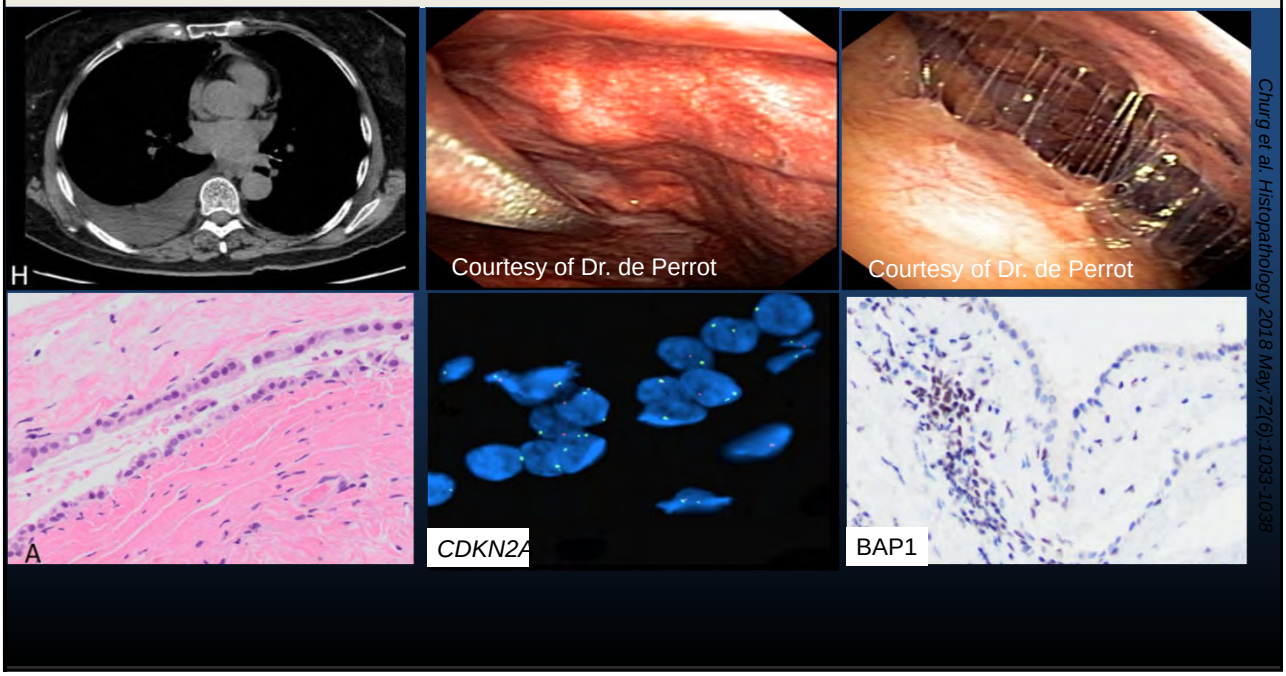
- Mesothelioma in situ is a pre-invasive single layer surface proliferation of neoplastic mesothelial cells.
- This diagnosis requires multidisciplinary information and discussion.

ESSENTIAL AND DESIRABLE DIAGNOSTIC CRITERIA MESOTHELIOMA IN SITU

- Pleural effusion (non-resolving)
- No thoracoscopic or imaging evidence of tumour
- Single layer of atypical mesothelial cells on pleural surface
- Loss of BAP1 and/or MTAP by IHC and/or *CDKN2A* homozygous deletion by FISH
- Multidisciplinary discussion of diagnosis

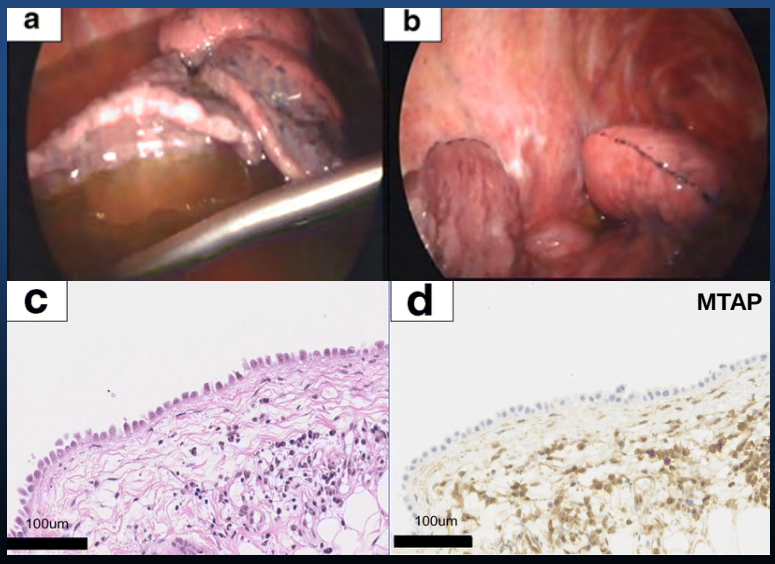
WHO 2021

MESOTHELIOMA IN SITU



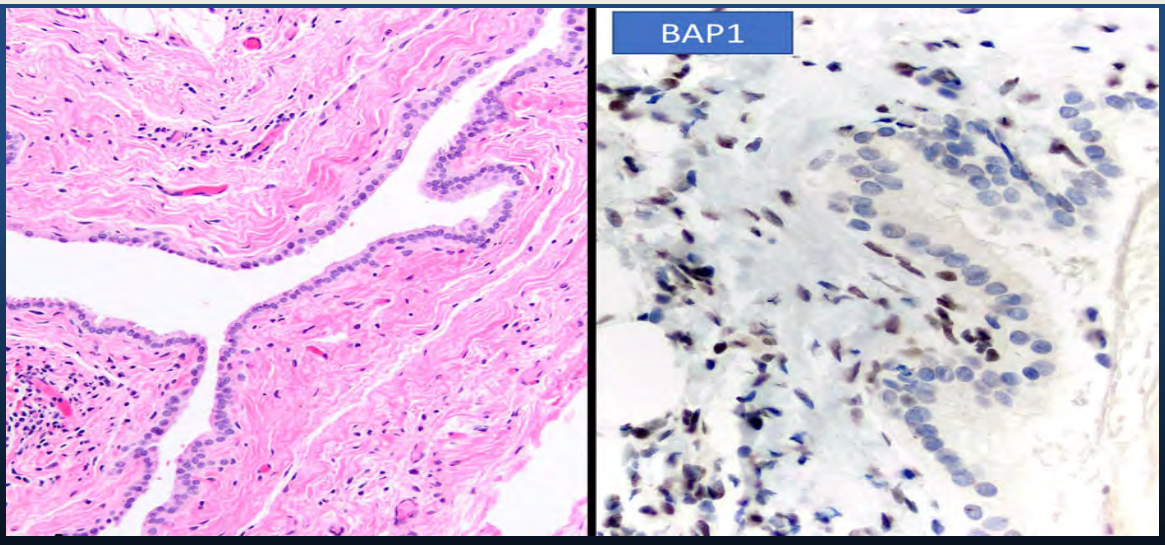
Churg et al. Histopathology 2018 May;72(6):1033-1038

MESOTHELIOMA IN SITU



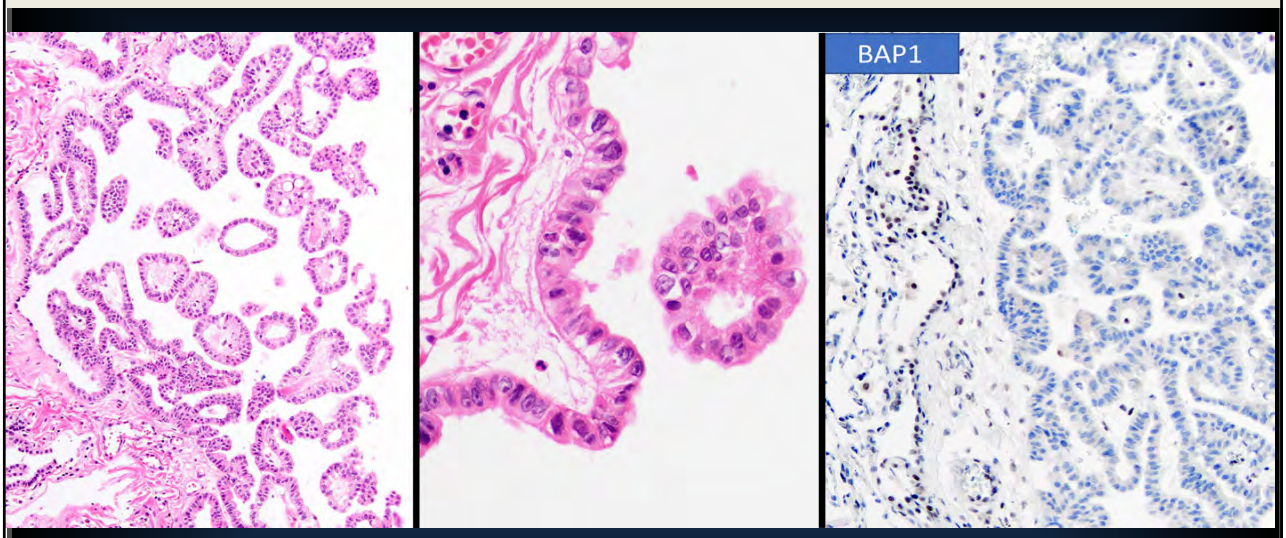
Minami et al. Virchows Arch 2020 Mar; 476 (3);469

MESOTHELIOMA IN SITU



Churg et al. Histopathology 2018 May;72(6):1033-1038

MESOTHELIOMA IN SITU



Churg et al. Histopathology 2018 May;72(6):1033-1038

Papillary tufting like this may be MIS but this is the type of case you really want to worry is actually invasive….

You **must** talk to the clinicians before making this diagnosis

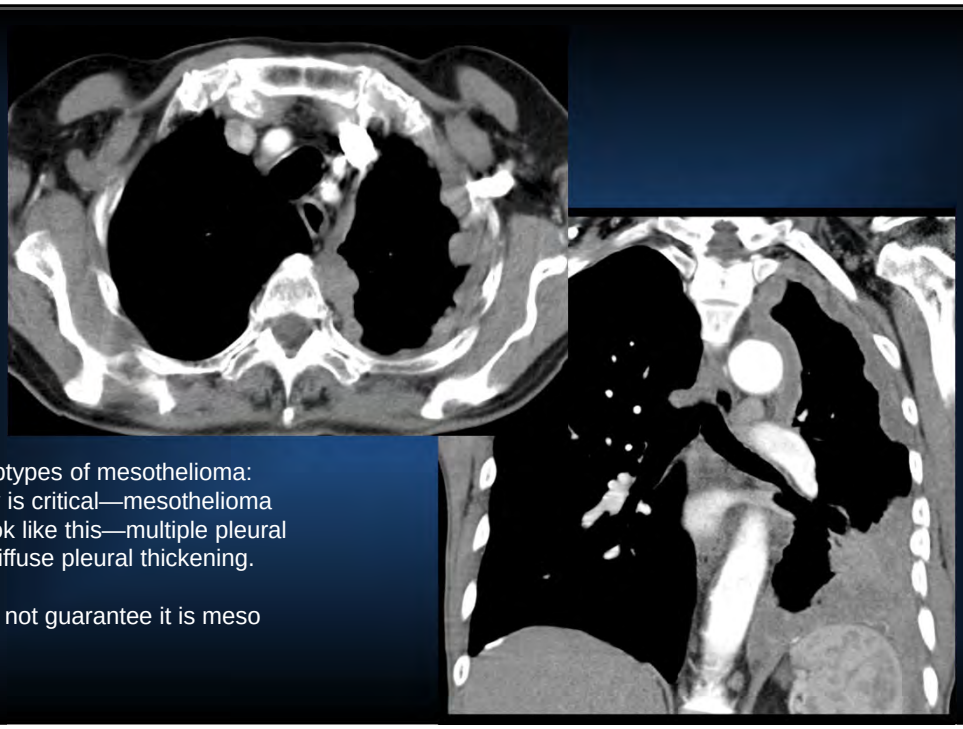
- Pleural effusion (non-resolving)
- No thoracoscopic or imaging evidence of tumor
- Single layer of atypical mesothelial cells on pleural surface
- Loss of BAP1 and/or MTAP by IHC and/or *CDKN2A* homozygous deletion by FISH
- Multidisciplinary discussion of diagnosis

WHO 2021

So, once you are sure it is malignant……
Are you sure what it is???

Mesothelioma differential

- Epithelioid type:
  - Carcinoma
  - Epithelioid vascular tumors
  - Melanoma
  - Lymphoma
  - Thymoma
- Biphasic Type
  - Pleomorphic/Sarcomatoid carcinoma
  - Synovial sarcoma
- Sarcomatoid type
  - Sarcomatoid carcinoma
  - Synovial sarcoma, other sarcomas
  - Spindle cell thymoma



For all subtypes of mesothelioma: Radiology is critical—mesothelioma should look like this—multiple pleural nodules/diffuse pleural thickening.

This does not guarantee it is meso though!

Mesothelioma should **not** look like this!!

A single or intraparenchymal lung mass should give you pause that this is not meso

Mesothelioma vs. Adenocarcinoma

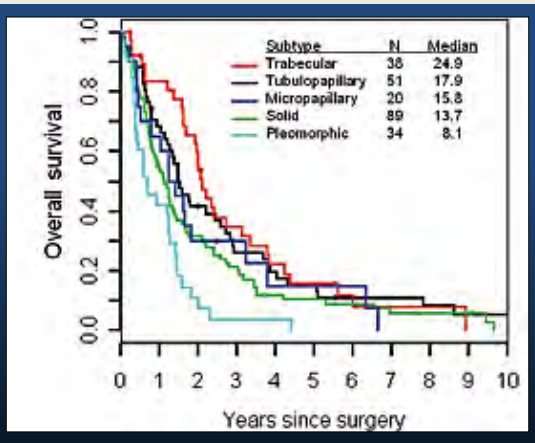
- **Morphology:**
  - **Mesothelioma: relatively uniform cuboidal cells with central nuclei, eosinophilic cytoplasm; tubules, papillary structures, sheets**
  - **Adenocarcinoma: Less uniform pattern, greater cellular pleomorphism, columnar cells**
  - **But not always……**

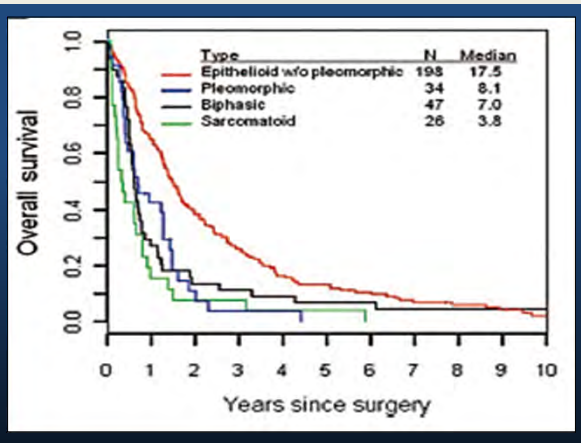
EPITHELIOID MESOTHELIOMA HISTOLOGIC CLASSIFICATION WHO 2021

Architectural patterns	Cytologic features	Stromal variants
▪ Solid ▪ Tubulopapillary ▪ Trabecular ▪ Adenomatoid ▪ Micropapillary	▪ Pleomorphic ▪ Lymphohistiocytoid ▪ Rhabdoid ▪ Deciduoid ▪ Small cell ▪ Clear cell ▪ Signet ring	▪ Myxoid

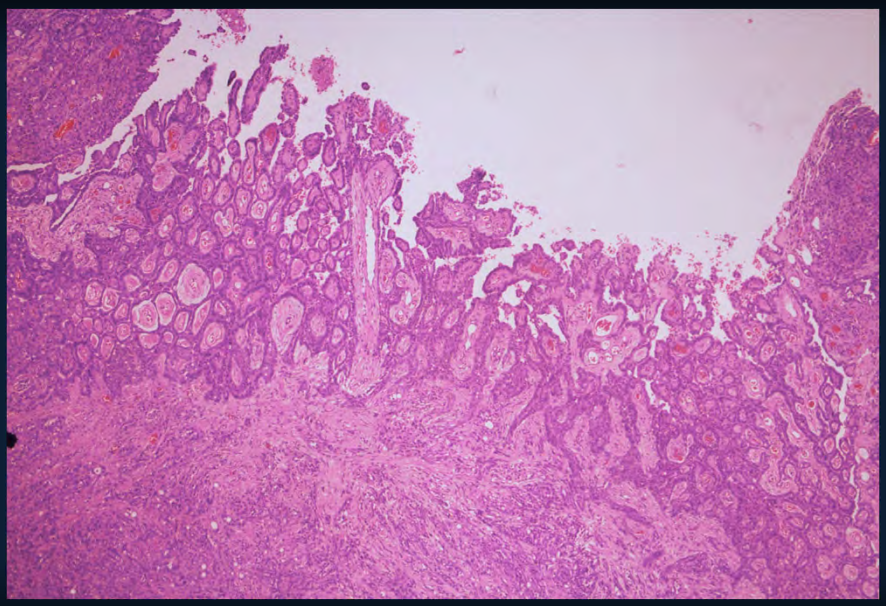
WHO 2021; EURCAN/IASLC proposal; JTO 2020 Jan;15(1):29-49

ARCHITECTURAL PATTERNS OF EPITHELIOID MESOTHELIOMA AND PROGNOSIS



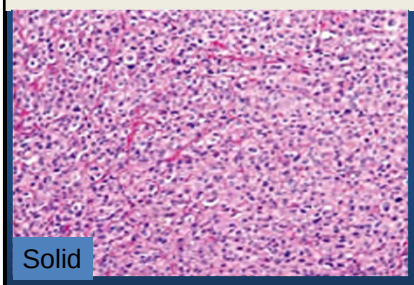


Kadota et al. J Thorac Oncol 2011

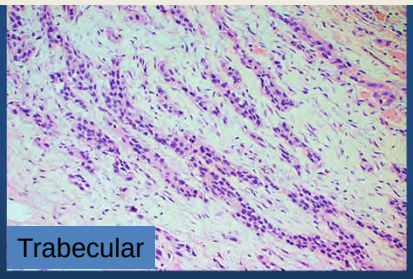


Mesothelioma with classic tubulopapillary growth

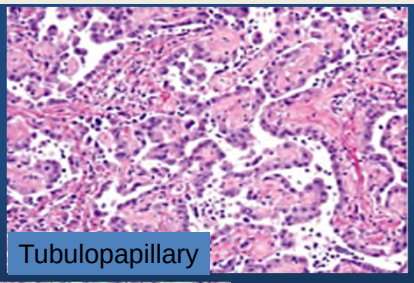
ARCHITECTURAL PATTERNS OF EPITHELIOID MESOTHELIOMA



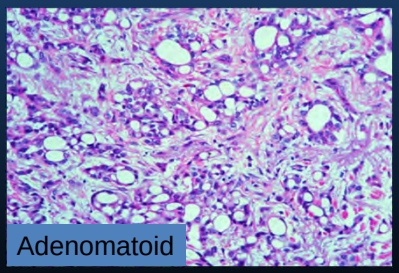
Solid



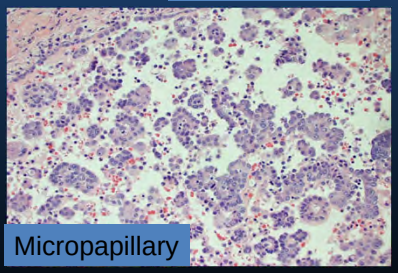
Trabecular



Tubulopapillary

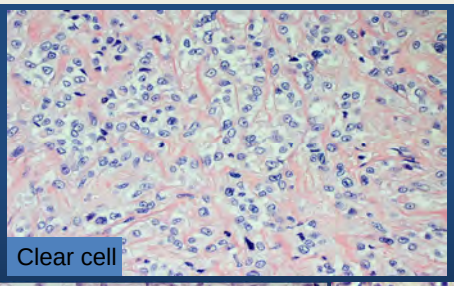


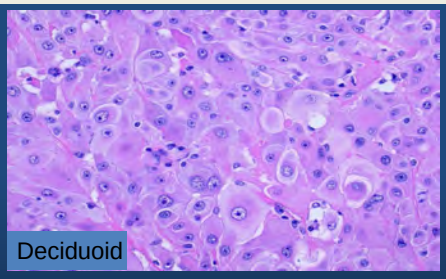
Adenomatoid

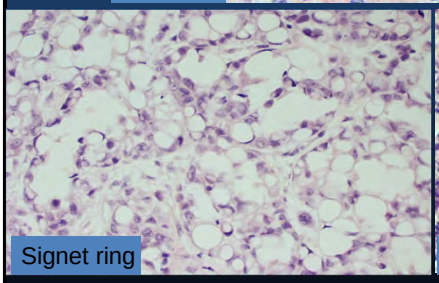


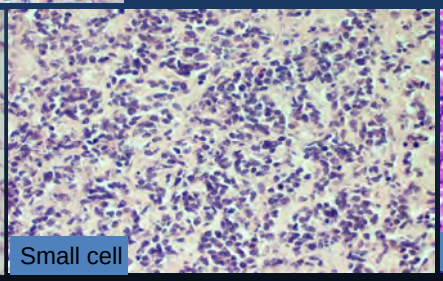
Micropapillary

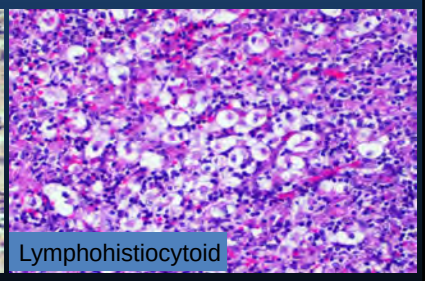
CYTOLOGIC FEATURES OF EPITHELIOID MESOTHELIOMA


Clear cell

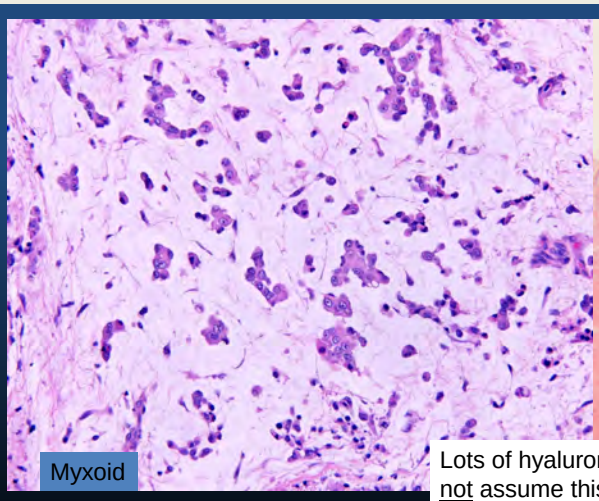

Deciduoid

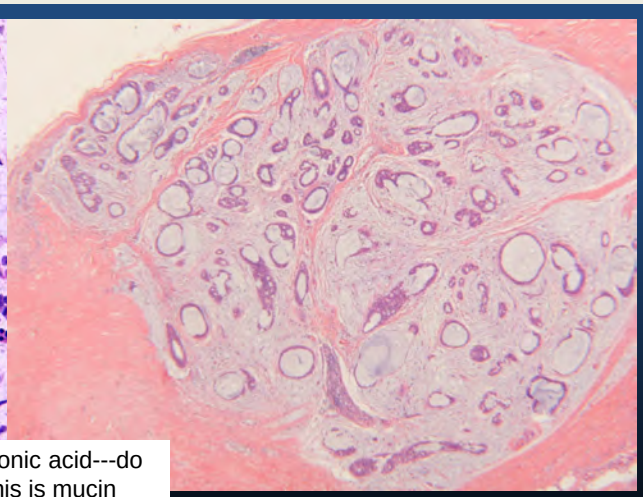

Signet ring


Small cell


Lymphohistiocytoid

STROMAL FEATURES OF EPITHELIOID MESOTHELIOMA


Myxoid



Lots of hyaluronic acid---do not assume this is mucin and call it adenocarcinoma without staining it!!

Mesothelioma vs. Carcinoma Immunohistochemistry

- A panel of antibodies is generally recommended as all of the markers have at least some reported crossover staining
- No single marker is 100% sensitive or specific
- Immunostain results may vary depending on a variety of factors
- Recommended using at least two "carcinoma" markers and two "mesothelioma" markers
- Each marker has potential pitfalls depending on circumstances

Epithelioid mesothelioma vs. carcinoma Immunohistochemistry

- Choice of markers should be based on tumor histology and tumors in the differential
- The "best" markers to use are the ones that work consistently well in your lab
- Keratin (AE1/AE3, Cam 5.2, OSCAR)
  - Helpful to highlight growth pattern and invasive areas not appreciated on H&E
  - Strong diffuse staining with keratin generally supports the tumor is either meso or carcinoma and not something else (i.e malignant vascular tumor, melanoma)
    - Particularly important for tumors with solid growth

Mesothelioma vs carcinoma immunohistochemistry

- "Mesothelial markers"
  - Calretinin—
    - 10% of adeno—focal, cytoplasmic; 40% squamous cell ca (usually focal)
    - May also stain certain ovarian carcinomas
    - May be positive in thymoma
    - Positive in adrenal cortical tumors
    - May be positive in triple negative breast carcinomas
  - CK5/6—important to note this also stains squamous cell carcinoma, breast carcinoma and a significant percentage of gyn malignancies, pancreatic adenoca
  - WT-1---Nuclear staining in meso; also stains ovarian serous tumors and melanoma. Capillaries/lymphatics also positive

Mesothelioma vs carcinoma immunohistochemistry

- "Mesothelial markers"
  - Thrombomodulin-not as sensitive, blood vessels stain also
  - Podoplanin/D2-40---membranous staining; also stains lymphatics and vascular tumors, 50% of squamous cell

-HEG-1—new marker—seems very good so far for pleural mesothelioma, particularly in men; may have some overlap with ovarian tumors in women.

Mesothelioma vs carcinoma immunohistochemistry

- "Carcinoma markers"
  - CEA-stains 85-95% of lung carcinomas
    - Kidney, prostate, ovarian tumors often negative
  - Leu-M1 (CD15)-up to 90% of lung ca, only 60% of others overall
  - BER-EP4-stains nearly all lung ca, most others
    - Up to 25% of mesos pos—usual focal
  - B72.3—85% of lung ca's positive
  - MOC-31
  - Claudin-4—emerging as most optimal glycoprotein marker

Mesothelioma vs carcinoma immunohistochemistry

- "Carcinoma markers"
  - TTF-1—useful if lung ca is the only other consideration, also stains thyroid
  - Napsin-similar to TTF-1—also stains papillary renal cell carcinoma
  - GATA-3 positive in breast ca and bladder ca but is frequently positive in mesothelial cells/mesothelioma—do not use this in isolation to support metastatic breast ca in a pleura specimen
  - P63/p40 are positive in squamous cell and are usually negative in mesothelioma (no diffuse positive staining); also stains thymomas, myoepithelial cells/tumors, etc

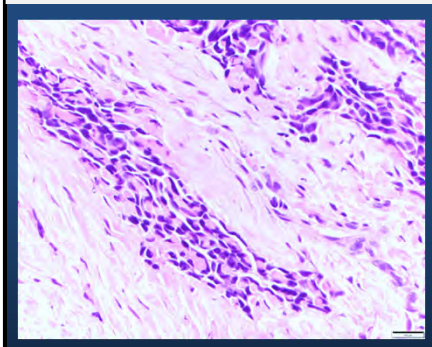
Issues with renal cell carcinoma

- Often negative for traditional "carcinoma markers" i.e CEA, etc
- RCC positive for CD10, RCC-ma, CAIX
  - These markers may be positive in a significant percentage of mesotheliomas
  - PAX-8, PAX-2—positive in RCC
    - PAX-8 recently shown to stain a small percentage of mesotheliomas, particularly peritoneal.
  - RCC rarely positive for WT-1, negative for calretinin, CK5/6 and D2-40 thus far.
  - Metastatic sarcomatoid renal cell carcinoma may be particularly problematic.

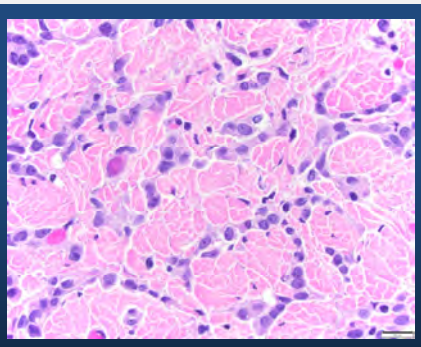
New: Grading

- Mesotheliomas are generally all bad so have not traditionally been graded
- More recently a proposed grading system based on nuclear grade, mitotic count and necrosis has been recommended.
  - Low grade: 16-29 month average survival
  - High grade: 8 month average survival
- Applies ONLY to epithelioid subtype

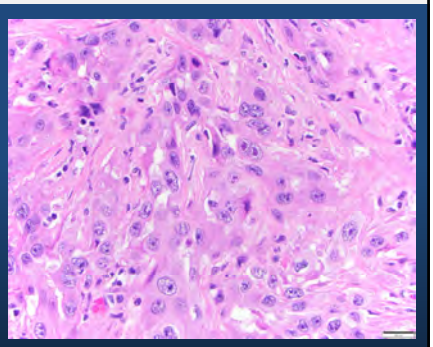
GRADING OF EPITHELIOD MESOTHELIOMA



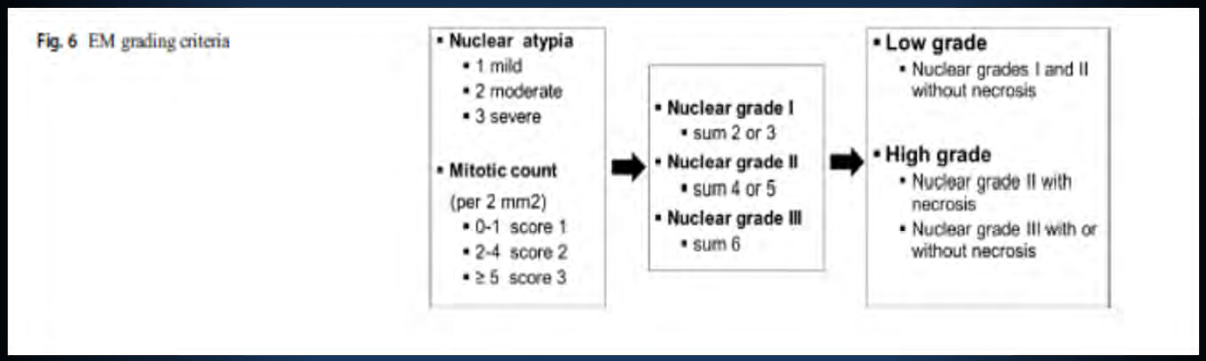
MILD
- Fine granular chromatin
- Indistinct nucleoli



MODERATE
- Intermediate N/C ratio
- Occasional nucleoli



SEVERE
- Low N/C ratio
- Large nucleoli



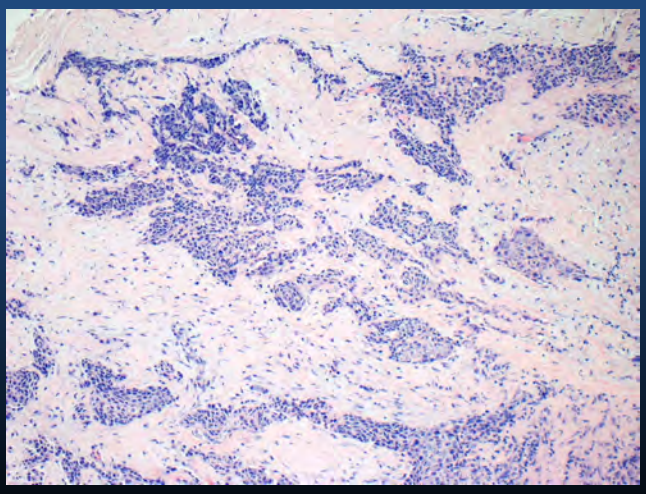

Virchows Archiv
REVIEW AND PERSPECTIVES
Pleural mesothelioma classification update
Mary Beth Beasley[1] · Francoise Galateau-Salle[2] · Sanja Dacic[3]

Staying out of trouble….

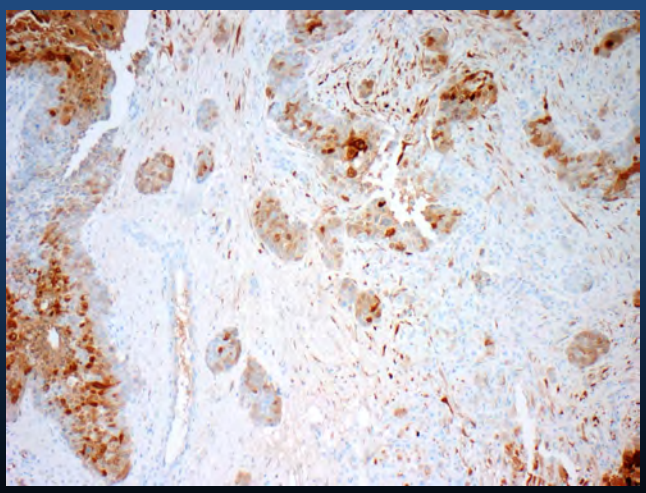
Your Case 3

- 65 year old male with right pleural based masses; underwent pleural biopsy
- ?What is the diagnosis
- ?Are you sure

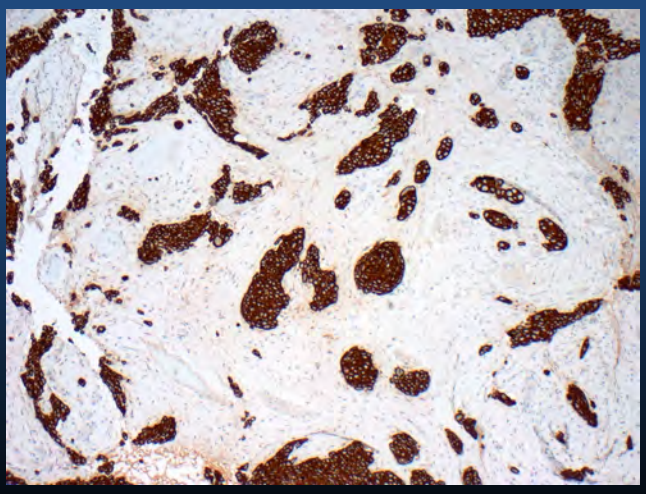
Pitfall case- H&E



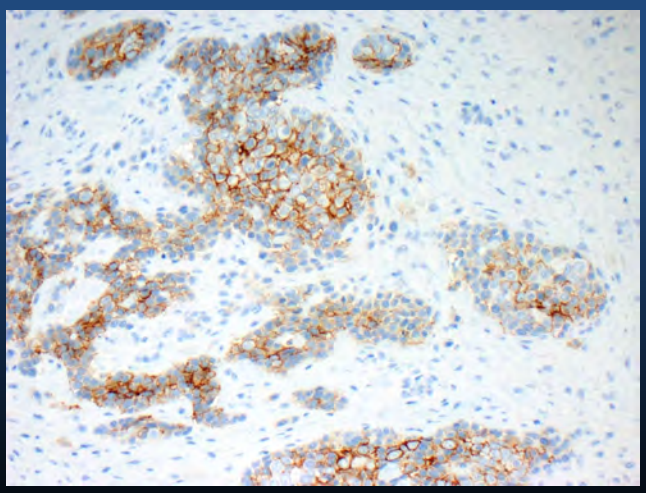
Pitfall case-Calretinin



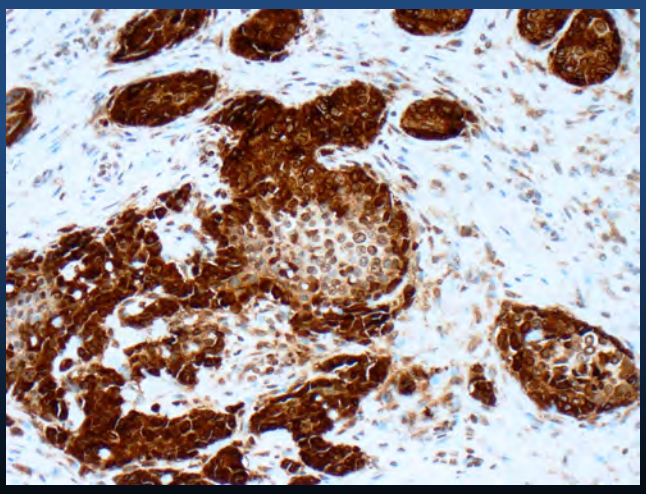
Pitfall case-CK5/6



Pitfall case- BER-EP4



Pitfall case—D2-40

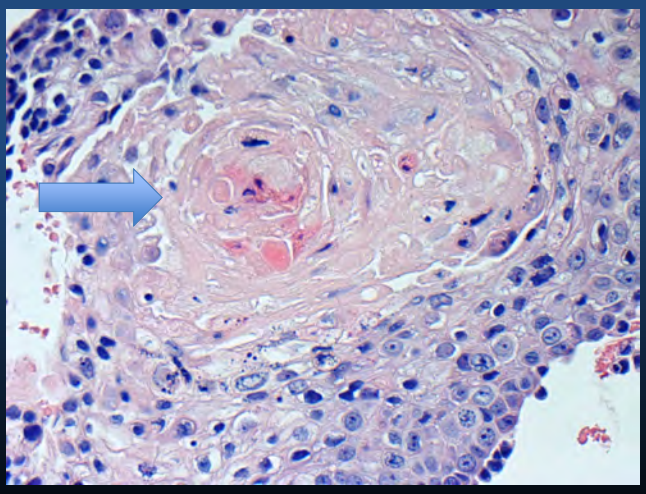


Pitfall case

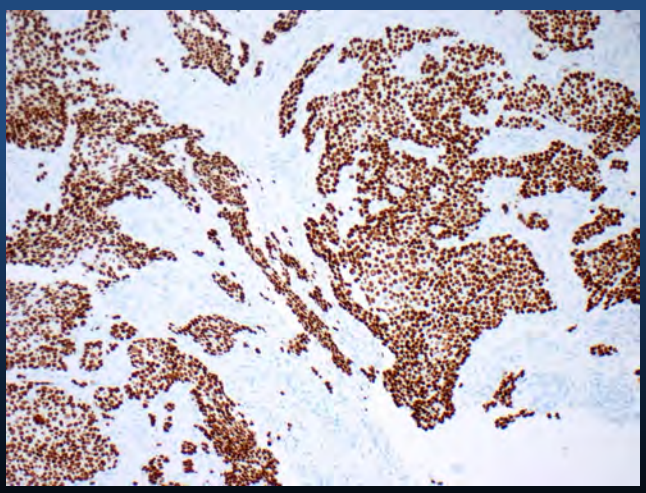
- Positive keratin, calretinin, CK5/6, BER-EP4
  - D2-40 called positive by original pathologist (but staining is not membranous as it should be)
- Negative WT-1, CEA, CD15, B72.3, TTF-1
- Seems like it might be mesothelioma…

Pitfall case

But what is this???!!!



Pitfall case-p63



Critical elements

- Given story of “pleural based masses” and immunostains this could have easily been misdiagnosed—a very small biopsy could have been particularly problematic
- ALWAYS CHECK HISTORY---radiology in this case showed a large intraparenchymal mass with pleural involvement rather than typical distribution of mesothelioma
- Stains must be evaluated in context of histology; be particularly liberal with staining in tumors with solid growth

Part 2-What about spindle cell proliferations…..

Fibrous Pleuritis vs. Desmoplastic Mesothelioma

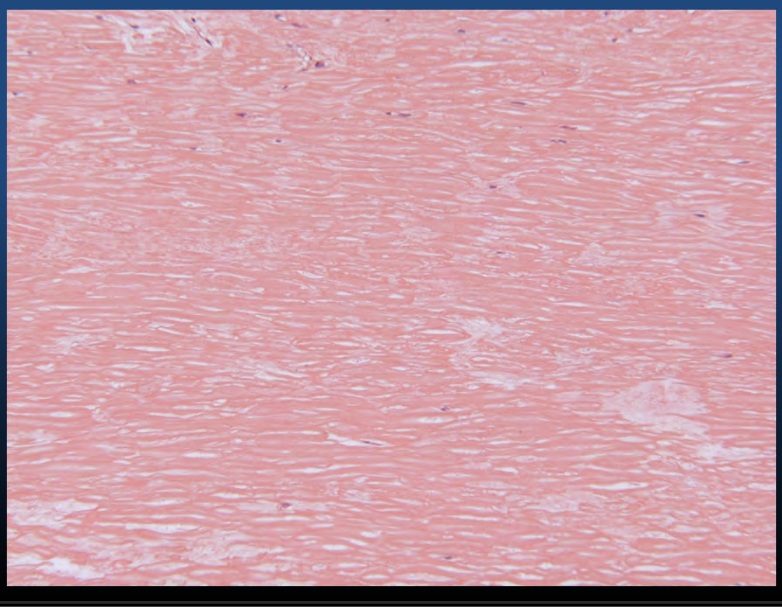
Table 2. Fibrous Pleurisy Versus Desmoplastic Mesothelioma[a]

Fibrous Pleurisy	Desmoplastic Mesothelioma
• Storiform pattern not prominent	• Storiform pattern often prominent
• Absence of stromal invasion	• Stromal invasion present (highlight with pancytokeratin staining)
• Necrosis, if present, is at the surface epithelioid mesothelial cells (where there is often associated acute inflammation)	• Bland necrosis of paucicellular, collagenized tissue
• Uniform thickness of the process	• Disorganized growth, with uneven thickness, expansile nodules, and abrupt changes in cellularity
• Hypercellularity at the surface with maturation and decreased cellularity deep (so-called zonation)	• Lack of maturation from the surface to the depths of the process
• Perpendicularly oriented vessels	• Paucity of vessels, without orientation
Usually Not Useful	
• Cellularity • Atypia (unless severe) • Mitotic activity unless numerous atypical mitotic figures	

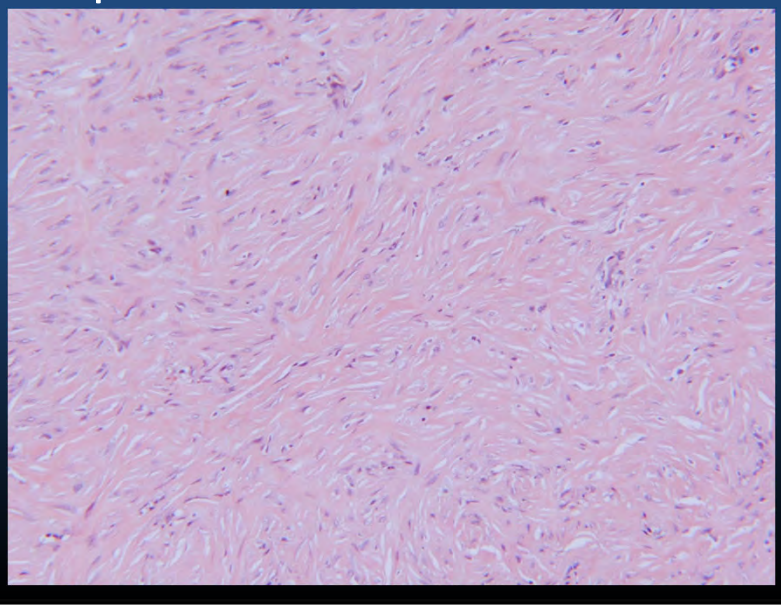
[a] Data derived from Mangano et al,[135] 1998.

(*Arch Pathol Lab Med.* 2018;142:89–108;

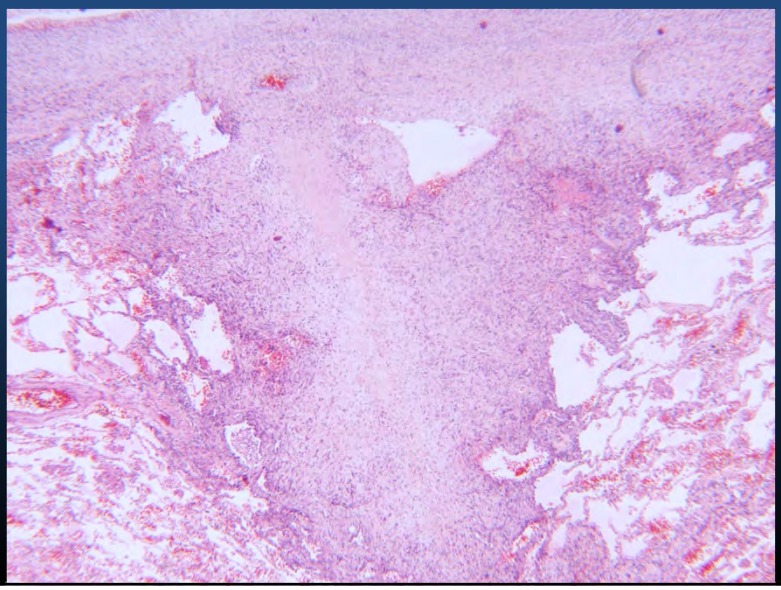
Plaque—linear “basket weave”



Desmoplastic mesothelioma--Storiform



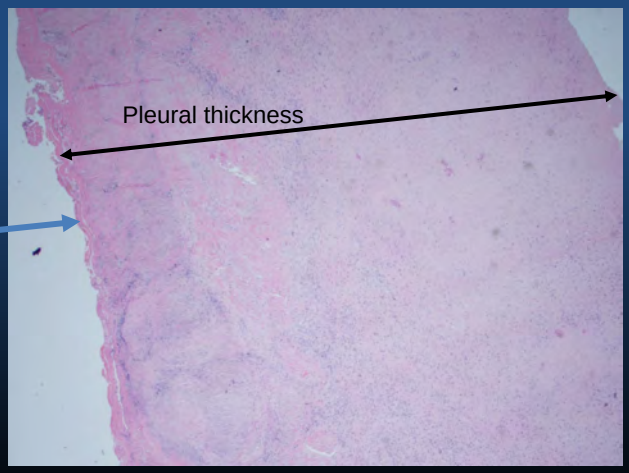
Desmoplastic meso invading lung



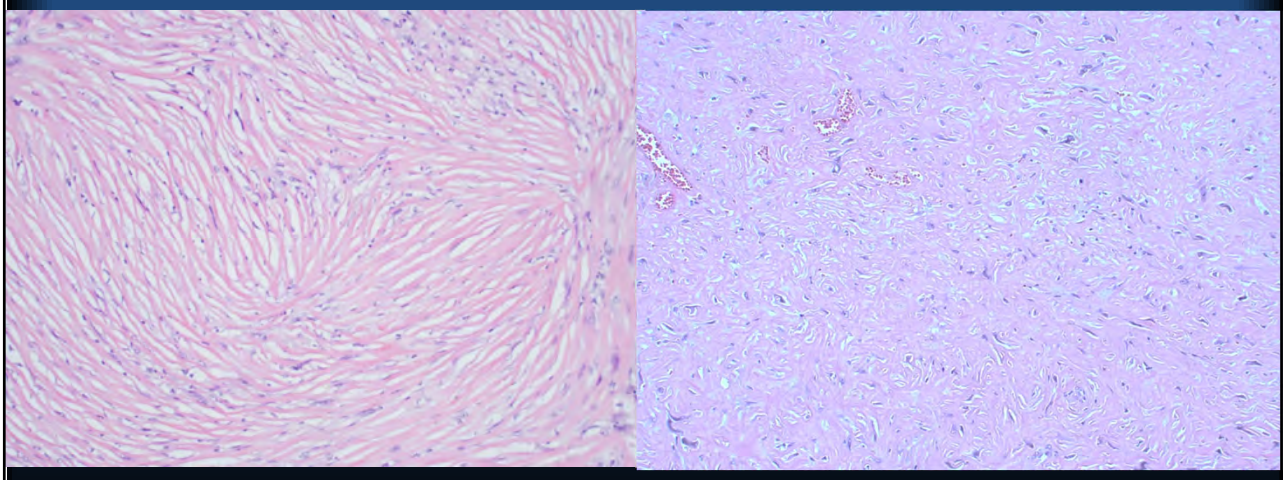
Your Case 5

- 31 year old female with diffuse right sided pleural thickening and nodularity; underwent biopsy
- What is your diagnosis?

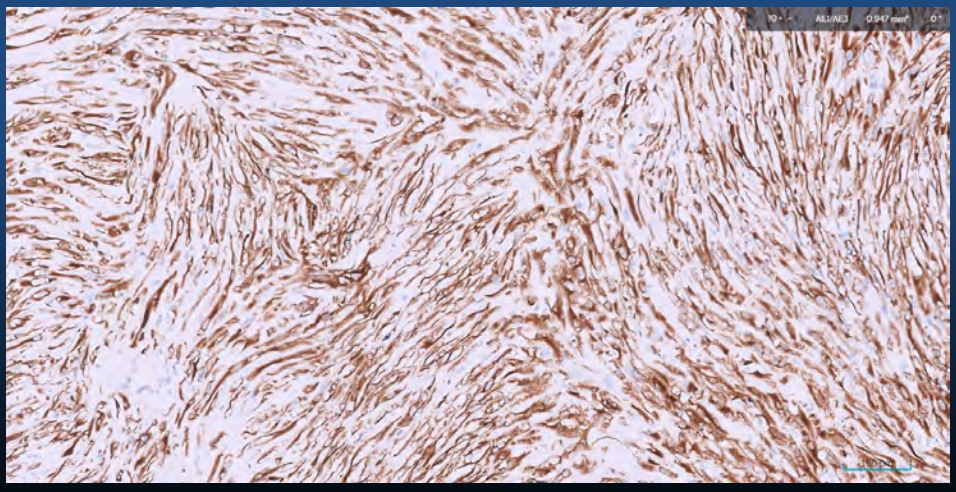
Case 5



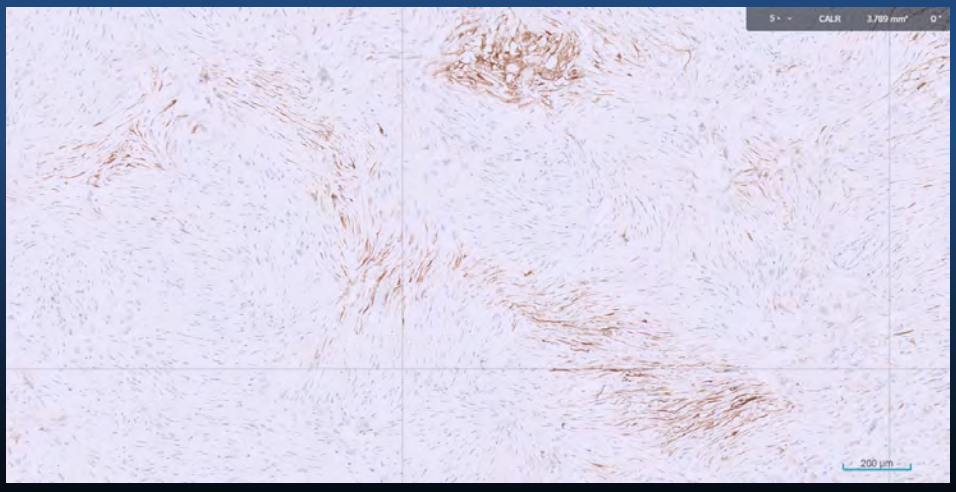
Case 5



Keratin



Calretinin



Case Summary

- Diffuse positive keratin x 2, patchy positive calretinin; negative D2-40, WT-1, CK5/6; retained BAP-1 and MTAP
- Case did have more cellular areas elsewhere
- Disease distribution appropriate
- Desmoplastic mesothelioma
  - Subtype of sarcomatoid; definition should be >50% desmoplastic pattern

Sarcomatoid mesothelioma vs sarcomatoid carcinoma

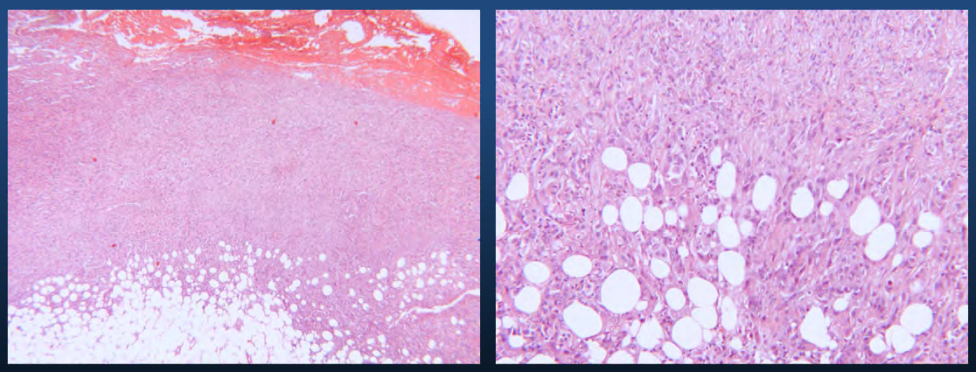
Sarcomatoid mesothelioma

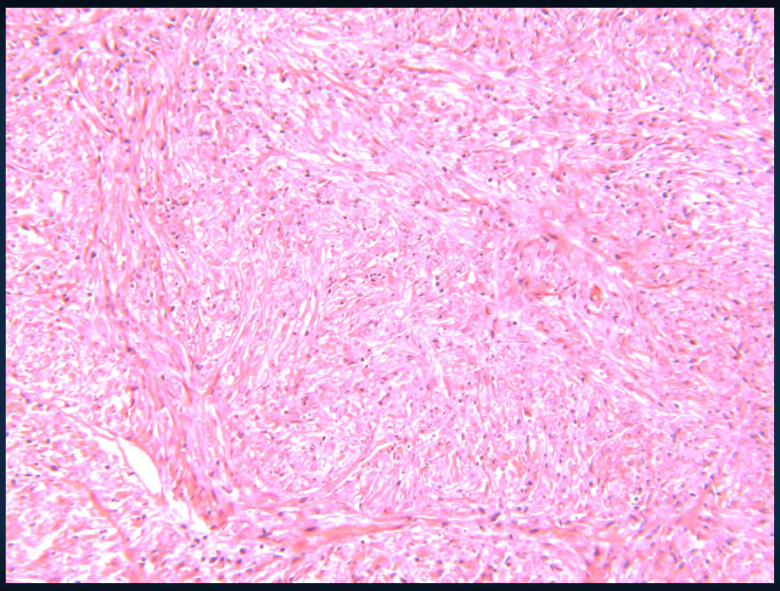
- Differential diagnosis is with sarcomas/sarcomatoid carcinoma rather than conventional carcinoma
- Keratin is a critical marker
  - Strong diffuse staining with cytokeratin supports meso *if disease distribution is correct*
- "Mesothelial markers" not as helpful in sarcomatoid
- A variable number of sarcomatoid mesos will stain for calretinin>>helpful to separate from sarcomatoid carcinoma *if proper disease distribution*
  - Pitfall—calretinin positive in synovial sarcoma, spindle thymoma, some sarcomatoid carcinomas, other sarcomas (CIC-DUX4, rhabdomyosarc, others)
- D2-40, WT-1, CK5/6 variably positive in sarcomatoid meso
- "Carcinoma" markers (CEA LEU-M1, etc) similarly not often helpful here

Sarcomatoid carcinoma

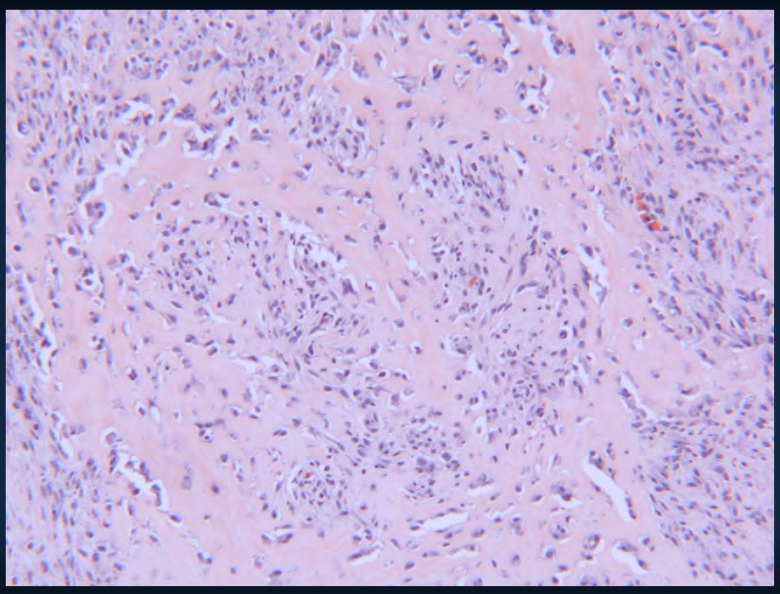
- Keratin often not as strongly positive c/w sarcomatoid meso but can be diffuse
  - May require more than one marker to demonstrate positive staining
  - Small biopsy in particular may still be keratin negative
- "Mesothelioma markers" may be positive
- TTF-1—certain clones may stain some sarcomatoid mesos

Sarcomatoid mesothelioma



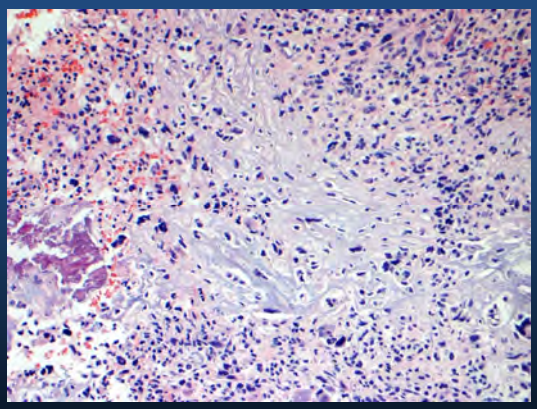


Sarcomatoid mesothelioma



Sarcomatoid mesothelioma may have heterologous differentiation

Sarcomatoid carcinoma



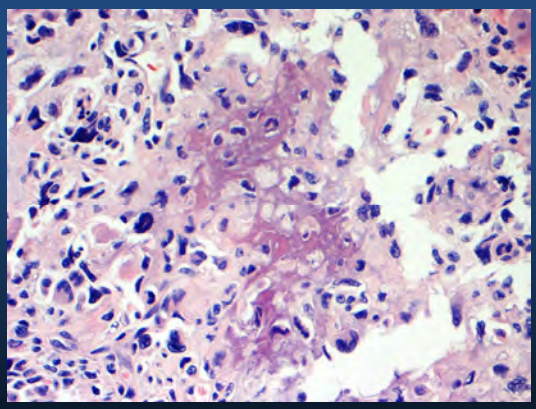


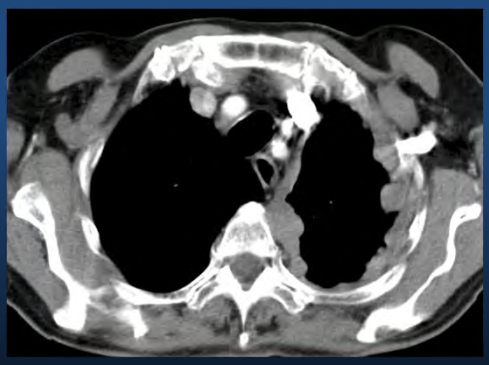
Table 3 Meta-analysis showing the results of various immunostains used for the diagnosis of sarcomatoid mesothelioma versus sarcomatoid carcinoma
Average % immunoreactivity and 95% confidence intervals

Immunostain	Sarcomatoid malignant mesothelioma	Spindle cell/pleomorphic carcinoma
Keratin AE1/AE3	89.4 (85.1-92.6)	88.1 (79.8-93.3)
CAM 5.2	90.7 (81.6-95.6)	91.4 (85.0-95.2)
EMA [a]	45.1 (25.5-66.4)	60.8 (26.4-87.0)
D2-40 [a]	74.0 (47.7-89.9)	20.1 (14.2-27.7)
Thrombomodulin [a]	50.1(28.6-71.6)	40.0 (25.4-56.6)
WT-1 [a]	44.8 (23.7-67.9)	31.4 (18.4-48.2)
CK5/6	26.2 (14.7-42.2)	NA
Calretinin [a]	53.9 (41.8-65.6)	37.1 (14.1-68.0)
MOC-31 [a]	5.6 (0.8-30.8)	29.5 (13.9-52.0)
CEA [a]	1.4 (0.3-5.8)	21.8 (6.7-51.9)
MUC-4 [a]	Only 1 study [b]	59.6 (43.1-74.3)
TTF-1 [a]	4.6 (0.6-28.2)	17.0 (10.3-26.6)

NOTE: Average % immunoreactivity and 95% confidence intervals.
[a] Studies evaluating this antibody showed significant heterogeneity in results.
[b] Amatya et al [21].

Marchevsky, et al. The differential diagnosis between pleural sarcomatoid mesothelioma and spindle cell pleomorphic (sarcomatoid) carcinomas of the lung: evidence-based guidelines from the International Mesothelioma Panel and the MESOPATH National Reference Center. Human Pathology (2017)67, 160-168

Radiology critical particularly if only keratin is positive



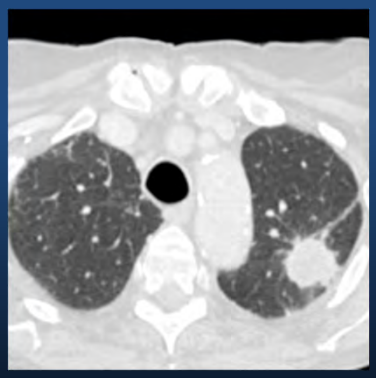


Table 4 Evidence-based criteria for the differential diagnosis between sarcomatoid mesothelioma and spindle cell/pleomorphic carcinoma in patients with diffuse malignant spindle cell pleural tumors

Cytokeratins (use more than 1 antibody)	Mesothelial markers (Calretinin, WT-1, D2-40)	Epithelial markers (Claudin 4, TTF-1, Ber-EP4, other)	Diagnosis
Positive [a]	Positive [a]	Negative [a]	Sarcomatoid mesothelioma
Positive	Negative	Negative	Probable sarcomatoid mesothelioma – exclude possibility of other lesions [b]
Positive	Negative	Positive	Spindle cell/pleomorphic carcinoma
Negative	Positive	Negative	Possible sarcomatoid mesothelioma. Exclude possibility of other lesions [c]
Negative	Positive	Positive	Possible spindle cell/pleomorphic carcinoma or sarcoma. This immunophenotype is unusual, and immunostains should probably be repeated to exclude laboratory errors
Negative	Negative	Positive	Probable spindle cell/pleomorphic carcinoma
Negative	Negative	Negative	Undifferentiated sarcomatoid neoplasm. Evaluate for possible sarcoma differentiation

[a] There is no agreement in literature regarding the minimum % of immunoreactive cells to classify a stain as positive. Most panel members opined that a small number of malignant cells with unequivocal immunoreactivity is sufficient for diagnosis in preparations with adequate controls and no significant background staining.

[b] The possibility of other neoplasms that can exhibit keratin immunoreactivity such as vascular tumors, synovial sarcoma and others need to be excluded with appropriate immunostains and other tests and by careful evaluation of the clinical history and imaging studies. Sarcomas generally appear as localized pleural tumors but in some cases can spread in a diffuse manner that simulates a mesothelioma.

[c] Mesothelial markers are not entirely specific, and their presence needs to be evaluated with caution in cases that stain negatively with several keratin immunostains. Careful review of the clinical history and imaging studies is suggested to exclude the possibility of neoplasms other than sarcomatoid mesothelioma.

Radiology was regarded as extremely important. "A diffuse malignant spindle tumor in a patient without other primary lesions composed of neoplastic cells exhibiting immunoreactivity for keratin and mesothelial markers and negative reactivity for epithelial markers can be definitively diagnosis as sarcomatoid mesothelioma."

Conclusions

- Ultimately sarcomatoid meso vs sarcomatoid carcinoma can be an extremely difficult differential diagnosis
- Make sure all available slides are reviewed
- Make sure good radiology is obtained

Summary and take home points

- Diagnosis must me made in the context of tumor morphology, immunostains, clinical/radiographic information
- Immunostains are not a substitute for thinking!
- Be very judicious with stains in epithelioid tumors with solid growth
- Be cognizant of pitfalls with IHC
- Be cautious of a diagnosis of mesothelioma with inappropriate disease distribution.

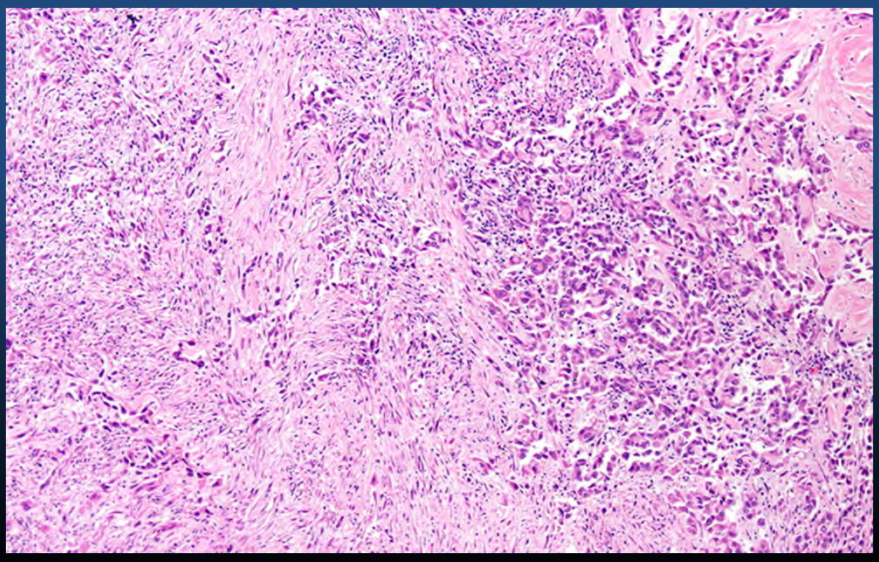
Other things/New Things/Unresolved issues

- Biphasic mesothelioma
  - Transitional morphology
- ??Anything useful with molecular analysis

Biphasic Mesothelioma

- Combination of epithelioid and sarcomatoid morphology
  - Second component must comprise 10% of the tumor
  - Latitude on small biopsies—mention two components if present
- Challenging on small biopsy to discriminate sarcomatoid component from exuberant desmoplastic response
  - BAP-1 and/or MTAP loss may help when present

Biphasic mesothelioma



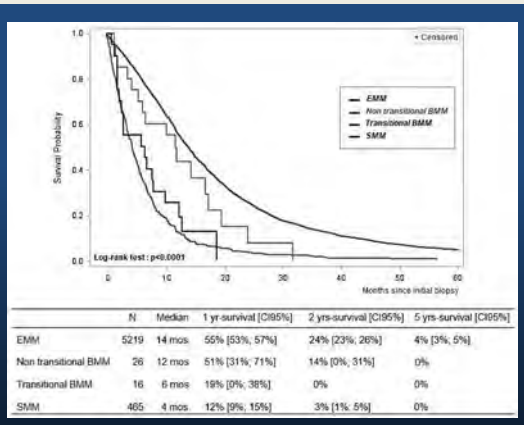
Biphasic mesothelioma

- Since an epithelial component is present, the same immunostaining rules apply as for the epithelioid subtype.
- The percentage of sarcomatoid component correlates with prognosis so you want to record the percentage of each component in your report

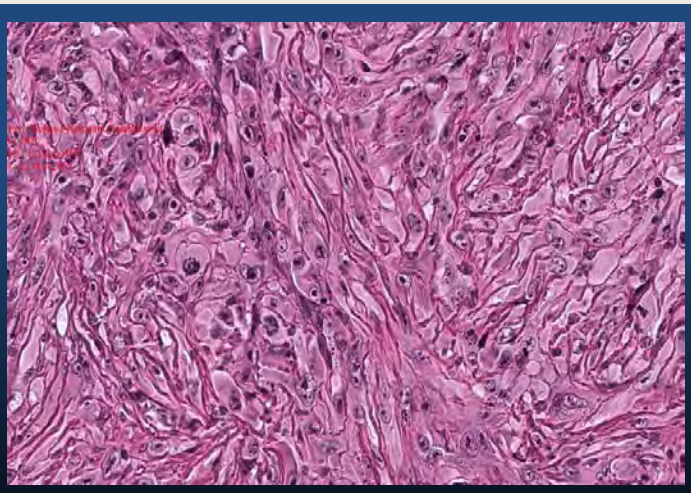
"Transitional morphology"

- Sort of looks epithelioid some of the time
- May look more like sarcomatoid
- Based on reproducibility studies has previously been lumped in with either depending on appearance
- Now clear it belongs in sarcomatoid category and when present in an otherwise epithelioid tumor should change the category to biphasic

TRANSITIONAL FEATURES AND PROGNOSIS

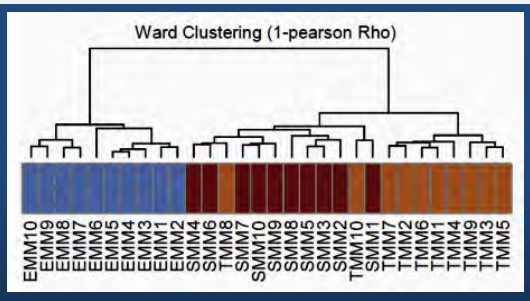


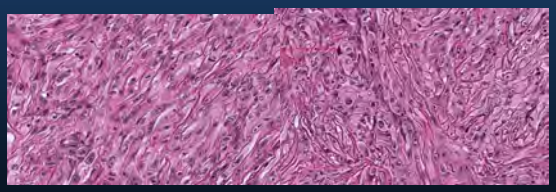
	N	Median	1 yr-survival [CI95%]	2 yrs-survival [CI95%]	5 yrs-survival [CI95%]
EMM	5219	14 mos	55% [53%; 57%]	24% [23%; 26%]	4% [3%; 5%]
Non transitional BMM	26	12 mos	51% [31%; 71%]	14% [0%; 31%]	0%
Transitional BMM	16	6 mos	19% [0%; 38%]	0%	0%
SMM	465	4 mos	12% [9%; 15%]	3% [1%; 5%]	0%



F. Galateau Salle et al. J Thorac Oncol 2020 March 9

TRANSITIONAL FEATURES





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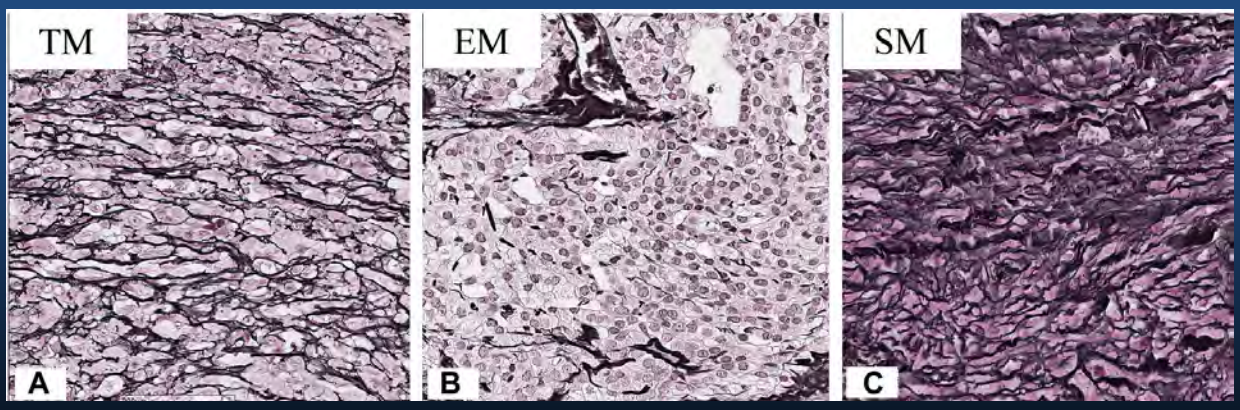
WHO 2015

- The term transitional pattern has been used to describe tumours with a sheet-like growth pattern in which the cells are cohesive, but have elongated morphology

WHO 2020

- Sarcomatoid and biphasic mesothelioma
- Transitional features are signified by elongated yet plump cells appearing intermediate between epithelioid and sarcomatoid in morphology, arranged in a sheet-like pattern, containing moderate cytoplasm and prominent nucleoli.
- These cells appear more round than sarcomatoid cells but more discohesive than epithelioid cells

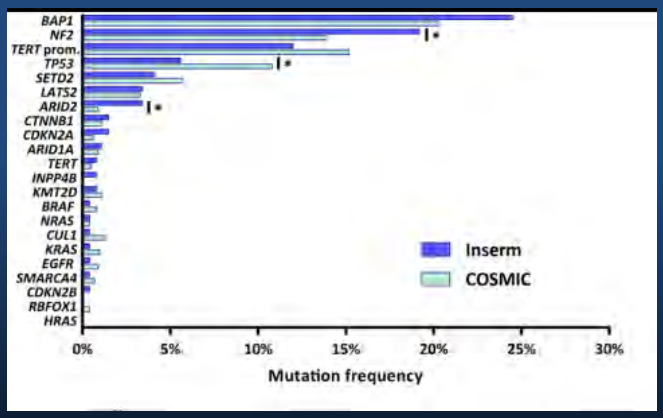
RETICULIN STAIN IN HISTOLOGIC SUBTYPING OF MESOTHELIOMAS



Galateau-Salle F et al. JTO 2020 Mar 9

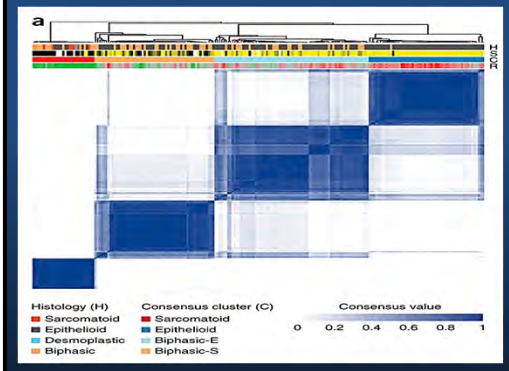
Anything useful re: Molecular?

INSERM AND COSMIC MUTATION FREQUENCIES

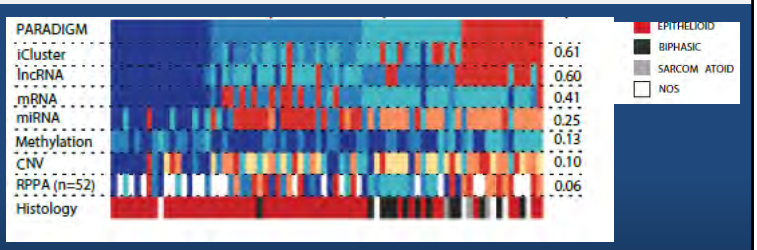


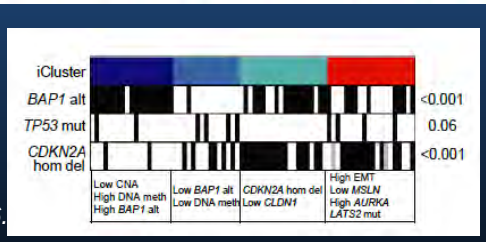
Quetel L et al. Mol Oncol 2020

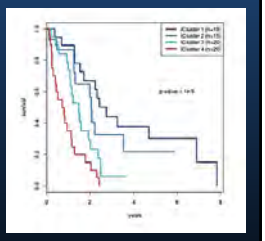
MOLECULAR CLASSIFICATION OF MESOTHELIOMA



Bueno R. et al. Nat Genet 2016; 48 (4):407-16.

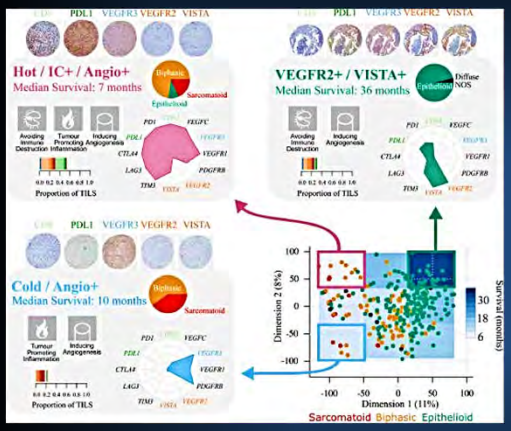




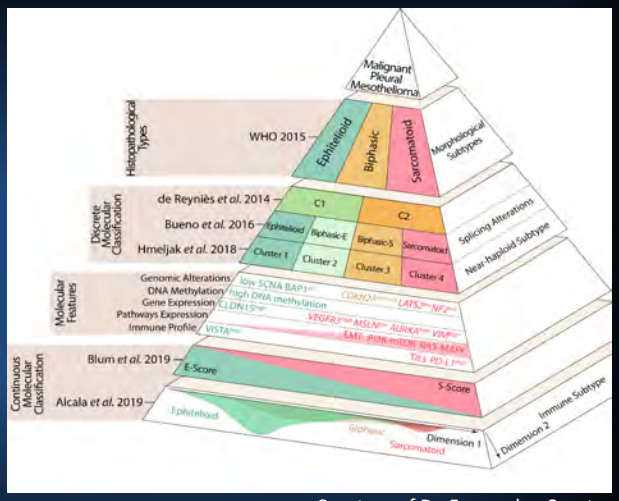


Hmeljak J et al. Cancer Discov. 2018

MOLECULAR CLASSIFICATION AND TREATMENT



Alcala N et al. EBioMedicine 2019



Courtesy of Dr. Fernandez-Cuesta
IARC-WHO, Lyon, France

Molecular

- Lots of tumor heterogeneity
- *BAP-1, CDKN2A, CDKN2B, NF2* mutations frequently reported
- *?Molecular classification-TCGA study (Hmeljak, J, et al; Cancer discovery Dec 2018)—identified histology independent prognostic subsets (TP53 and SETDB1 mutations>Aggressive)*
- Thus far, molecular and immune evaluation have identified prognostic subgroups, generally correlating with histology
  - Impact on treatment has shown some progress but not break out therapies yet

PDL1/immunotherapy

- Trials looking at this demonstrate partial response or stability
- Ongoing evaluation for durability of response.
- Issues:
  - PD-L1—predictive role unclear but a lot of expression correlates with better response; no clear cut off value; we don't even know if we should use TPS or CPS

Summary

- Be aware of pitfalls regarding discrimination of benign vs malignant mesothelial proliferations
- Be aware of the re-introduced concept of mesothelioma in situ
- Understand how to apply an appropriate immunostaining panel depending on tumor histology and recognize pitfalls
- Molecular testing and immunotherapy still an evolving area


Faleminderit
danke
謝謝
teşekkür ederim
спасибо
dank je
thank you
gracias
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Talk 3: Current Challenges in Evaluation of Pulmonary Adenocarcinoma

WHO adenocarcinoma classification

- Pre-invasive lesions:
  - Atypical adenomatous hyperplasia (Lepidic growth less than 5mm in size)
  - Adenocarcinoma in situ (≤ 3.0 cm, formerly BAC)
    - Non-mucinous
    - Mucinous---exceedingly rare
- Minimally invasive adenocarcinoma- ≤ 3.0 cm lepidic pattern predominant tumor with ≤5mm of invasion
  - Non-mucinous
  - Mucinous-exceedingly rare
- MIA is **excluded** if the tumor:
  - 1)invades lymphatics, blood vessels, or pleura (i.e no T2 MIA's) or
  - 2) contains tumor necrosis.
  - 3) contains areas of STAS (spread of tumor through airspaces)

WHO adenocarcinoma classification

- Invasive adenocarcinomas*
  - Lepidic predominant (>5mm of invasive tumor)
  - Acinar predominant
  - Papillary predominant
  - Micropapillary predominant
  - Solid predominant
- Variants
  - Invasive mucinous adenocarcinoma (formerly mucinous BAC)
  - Colloid adenocarcinoma
  - Enteric adenocarcinoma
  - Fetal adenocarcinoma

* Predominant is not necessarily greater than 50%

Lepidic growth

- Growth of tumor cells along existing alveolar walls
  - Term comes from “Scale like”
- Keys
  - abrupt transition from normal lung—helps in discriminating from reactive
  - Architecture preserved—looks like normal lung from low power
- In tumors with mixed lepidic and invasive growth only the invasive component is used for assigning pT---this can be extremely challenging
  - Lepidic growth should ideally be a monolayer
  - Compressed lepidic growth may show a parallel arrangement of cells

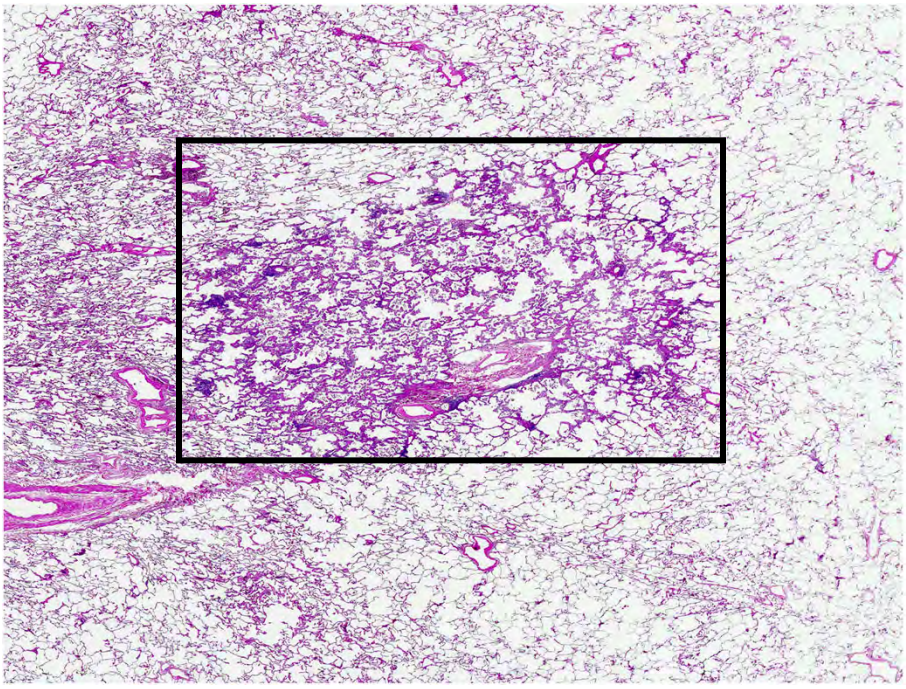
Invasion vs non-invasion not always easy

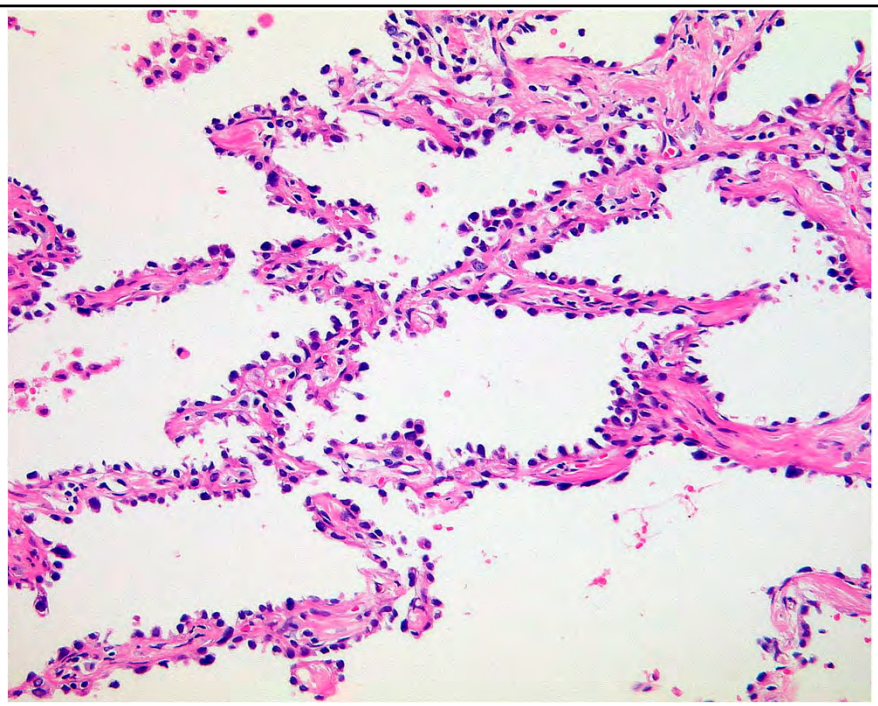
Defining Morphologic Features of Invasion in Pulmonary Nonmucinous Adenocarcinoma With Lepidic Growth: A Proposal by the International Association for the Study of Lung Cancer Pathology Committee

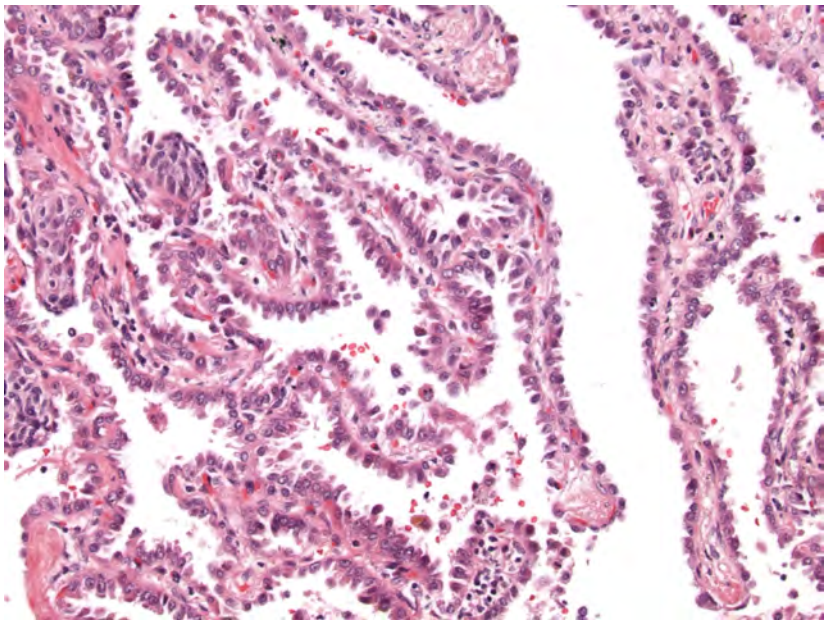
Erik Thunnissen, MD, PhD,[a,*] Mary Beth Beasley, MD,[b] Alain Borczuk, MD,[c] Sanja Dacic, MD, PhD,[d] Keith M. Kerr, MD,[e] Birgit Lissenberg-Witte, PhD,[f] Yuko Minami, MD, PhD,[g] Andrew G. Nicholson, DM,[h] Masayuki Noguchi, MD,[i] Lynette Sholl, MD,[j] Ming-Sound Tsao, MD, FRCPC,[k] John Le Quesne, MD,[l] Anja C. Roden, MD,[m] Jin-Haeng Chung, MD,[n] Akihiko Yoshida, MD, PhD,[o] Andre L. Moreira, MD, PhD,[p] Sylvie Lantuejoul, MD, PhD,[q] Giuseppe Pelosi, MD, MIAC,[r,s] Claudia Poleri, MD,[t] David Hwang, MD, PhD,[u] Deepali Jain, MD,[v] William D. Travis, MD,[w] Elisabeth Brambilla, MD,[x] Gang Chen, MD,[y] Johan Botling, MD, PhD,[z] Lukas Bubendorf, MD,[aa] Mari Mino-Kenudson, MD,[bb] Noriko Motoi, MD, PhD,[cc] Teh Ying Chou, MD,[dd] Mauro Papotti, MD,[ee] Yasushi Yatabe, MD, PhD,[ff] Wendy Cooper, MBBS, PhD,[gg] Invasion Working Group[a,b,c,d,e,g,h,i,j]

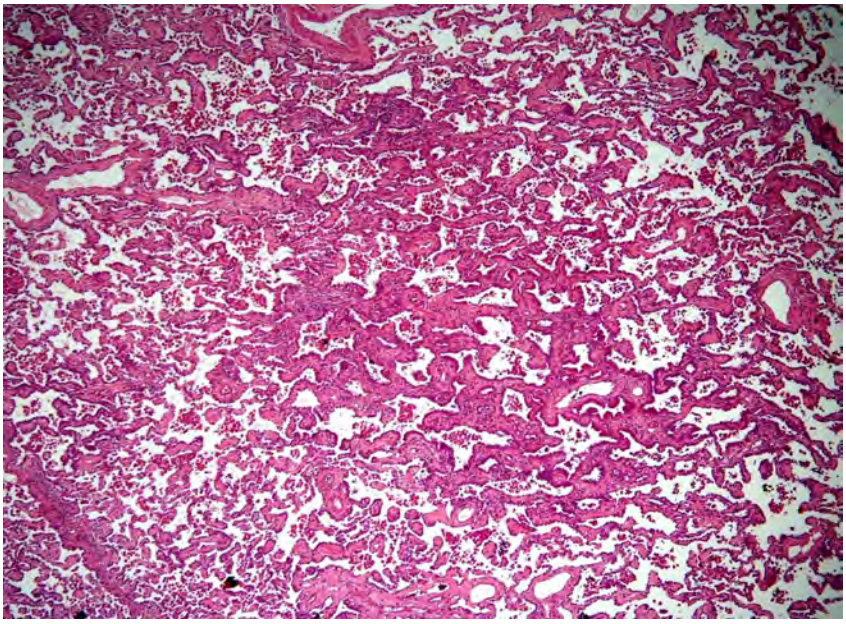
Journal of Thoracic Oncology Vol. 18 No. 4: 447-462

- **Invasion vs collapsed lepidic**
  -Parallel arrangement favors collapse>>implies retained alveolar architecture
- **Lepidic should be a monolayer**
  -Tangential sectioning an issue in discriminating from micropapillary
- **Evaluation can be hampered by handling and processing/fixation issues**
- **When in doubt err on calling invasion**



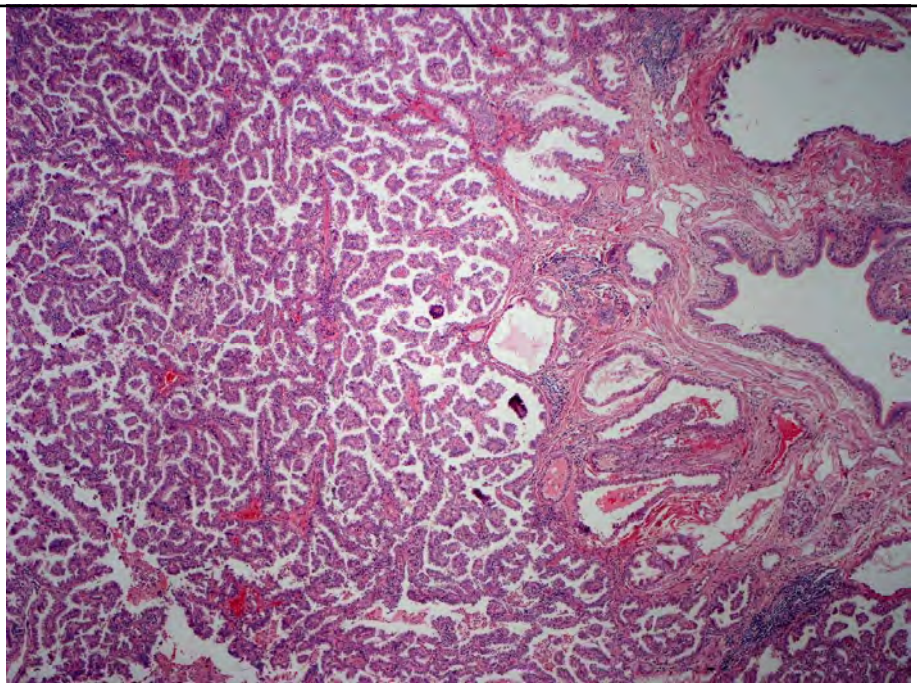






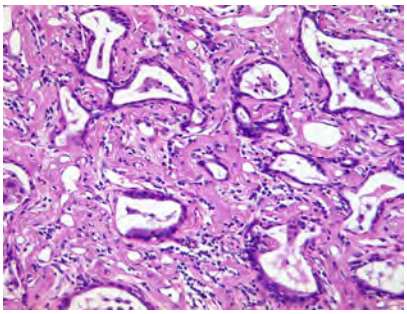
Papillary adenocarcinoma

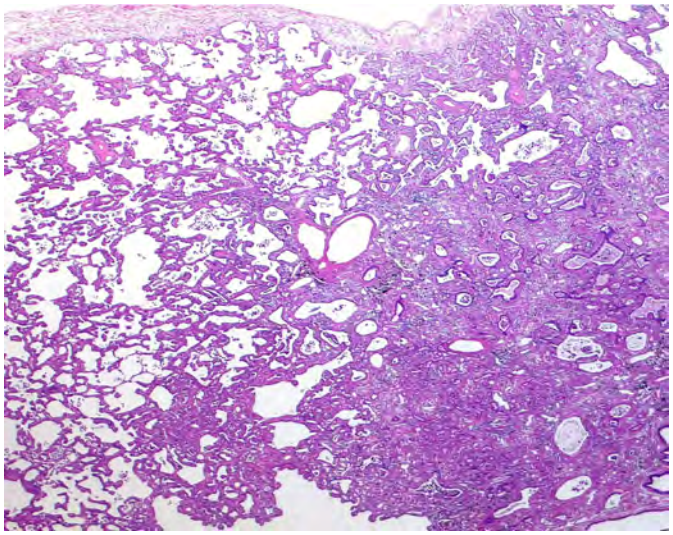
- Papillary adenocarcinoma-Adenocarcinoma with secondary or tertiary branching papillary structures
  - Papillae have fibrovascular cores, lack elastic fibers
- Papillary pattern should be distinguished from tangential sectioning of alveolar walls with lepidic growth.
- If a tumor has lepidic growth but the alveolar spaces are filled with papillary structures the tumor is classified as papillary adenocarcinoma.
- Myofibroblastic stroma is not needed to diagnose this pattern.

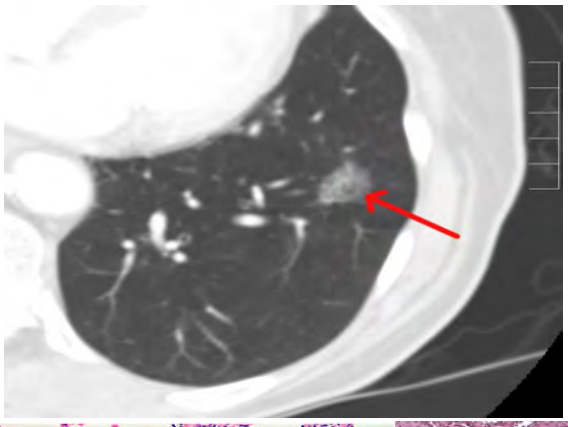


Papillary carcinoma—low power

MIA



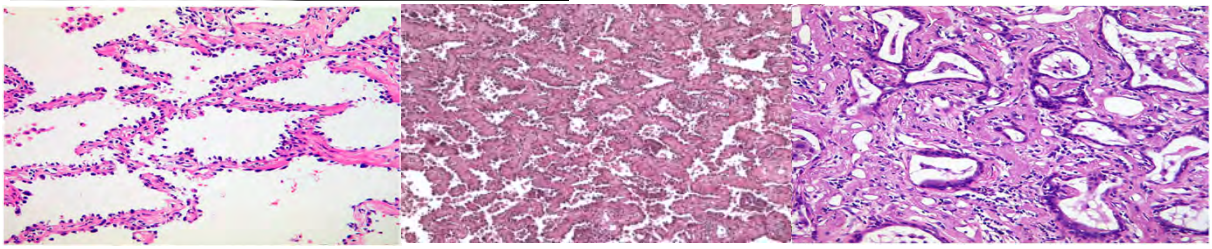


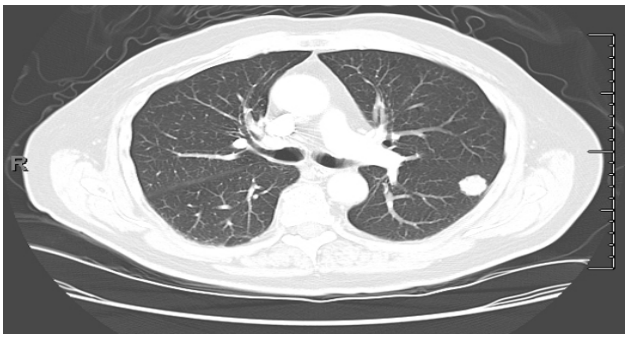


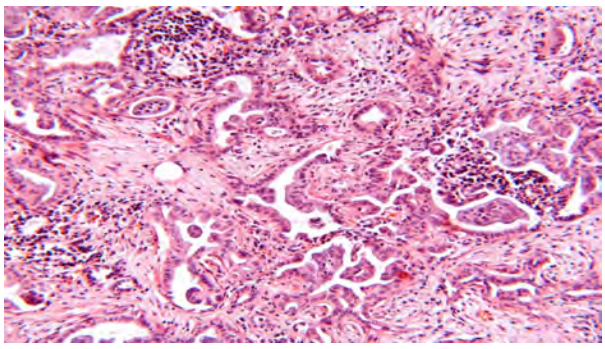
Ground Glass/sub-solid nodules:

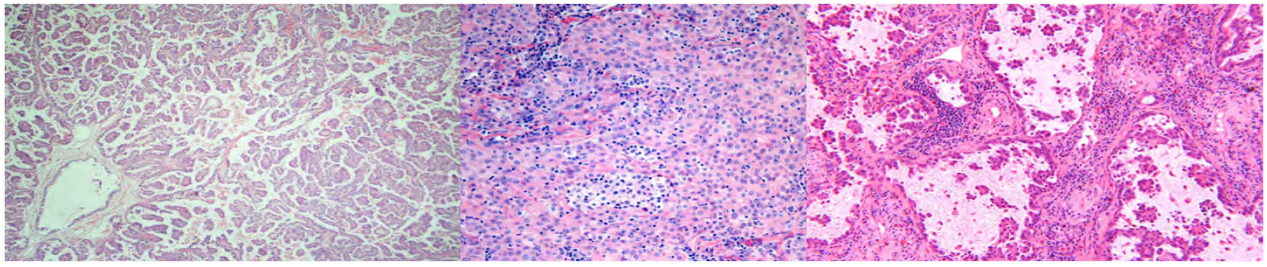
Pure GGO/Sub-solid>>AIS or MIA (Honda, Clin Radiol, 2013)

Mixed solid/Sub-solid>>Lepidic predominant—solid area correlates with invasive area and GG area correlates with lepidic growth (Kodoma, et al;









Does Lung Adenocarcinoma Subtype Predict Patient Survival?

A Clinicopathologic Study Based on the New International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society International Multidisciplinary Lung Adenocarcinoma Classification

J Thorac Oncol. 2011;6: 1496–1504)

Prudence A. Russell, MBBS, FRCPA, Zoe Wainer, BMBS,†‡ Gavin M. Wright, MBBS, FRACS,†‡§ Marissa Daniels, MBBS,§ Matthew Conron, MBBS, FRACP,‖ and Richard A. Williams, MBBS, FRCPA, PhD**

TABLE 3. Pathologic Stage of the 210 Patients According to the 7th Revision TNM Classification and the Predominant Histologic Subtype/Variant

Predominant Subtype	Stage IA	Stage IB	Stage IIA	Stage IIB	Stage III	Total
AIS	1	0	0	0	0	1
MIA	7	0	0	0	0	7
Lepidic	5	5	0	0	0	10
Acinar	27	25	15	6	11	84
Papillary	9	9	5	2	1	26
Solid	12	14	10	5	8	49
Micropapillary	4	3	0	1	6	14
Mucinous	1	4	1	4	0	10
Colloid	1	3	1	0	4	9
Total	67	64	31	18	30	210

AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma.

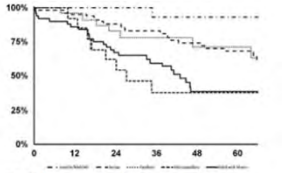


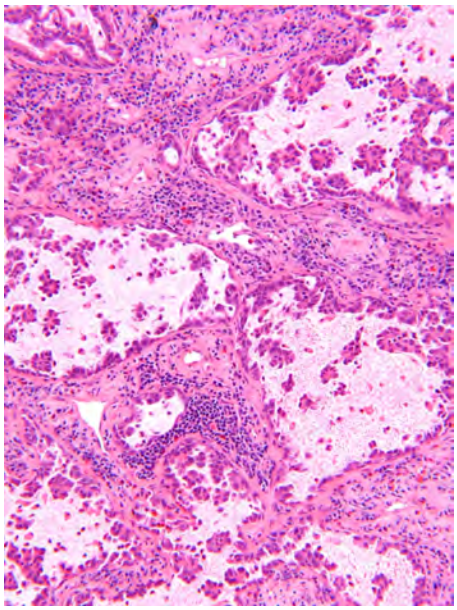
FIGURE 5. Correlation of 5-year survival with the predominant histologic subtype revealed significant differences in survival ($p < 0.0001$) between 18 cases of AIS, MIA, and lepidic-predominant tumors (93% 5-year survival), 26 cases of papillary-predominant (71% 5-year survival), 84 cases of acinar-predominant (68% 5-year survival), 49 cases of solid with mucin-predominant (39% 5-year survival), and 14 cases of micropapillary-predominant (38% 5-year survival) adenocarcinoma.

TABLE 5. Correlation of the Predominant Histologic Subtype or Variant with 5-Year Survival in 210 Patients

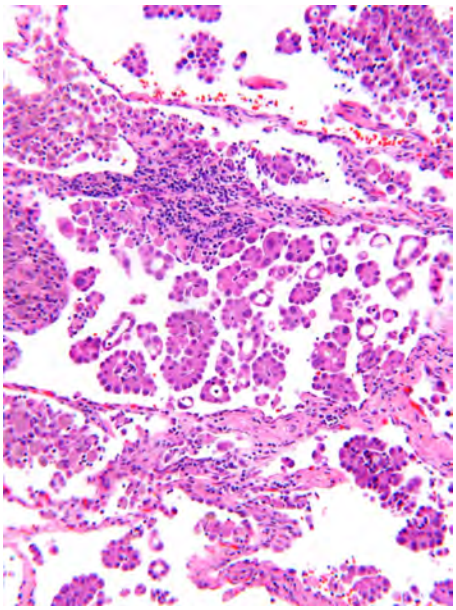
Predominant Histologic Subtype/Variant	No. of Patients	5-Year Survival (%)
Adenocarcinoma in situ	1	100
Minimally invasive adenocarcinoma	7	100
Lepidic predominant	10	86
Papillary predominant	26	71
Acinar predominant	84	68
Invasive mucinous adenocarcinoma	10	51
Colloid predominant	9	51
Solid with mucin predominant	49	39
Micropapillary predominant	14	38

Micropapillary pattern—a deviation from the "predominant" theme

- Early studies suggested ANY amount of micropapillary growth was associated with a worse prognosis and increased incidence of lymph node metastases in pT1 tumors in particular.
- Further work has still supported this contention
  - Yeh YC, et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification predicts occult lymph node metastasis in clinically mediastinal node-negative lung adenocarcinoma. Eur J Cardiothorac Surg. 2015 Sep 15.
  - Tsubokawa N, et al. Negative prognostic influence of micropapillary pattern in stage IA lung adenocarcinoma. Eur J Cardiothorac Surg. 2015 Mar 11.
  - Lee G, et al. Clinicalimpact of minimal micropapillary pattern in invasive lung adenocarcinoma:prognostic significance and survival outcomes. Am J Surg Pathol. 2015May;39(5):660-6.

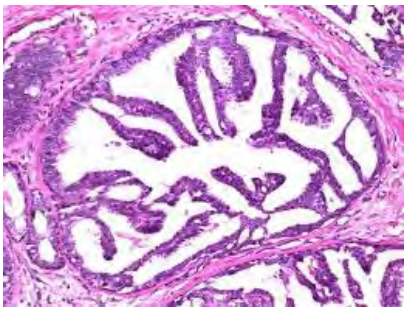


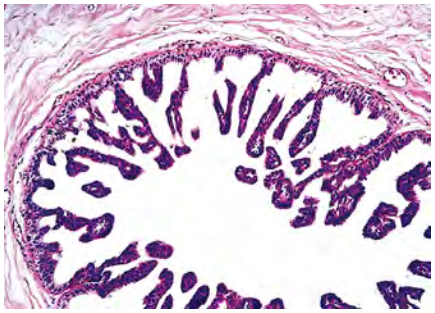
Micropapillary carcinoma within cystic glandular spaces



Micropapillary carcinoma within alveolar spaces

Filigree pattern of micropapillary carcinoma

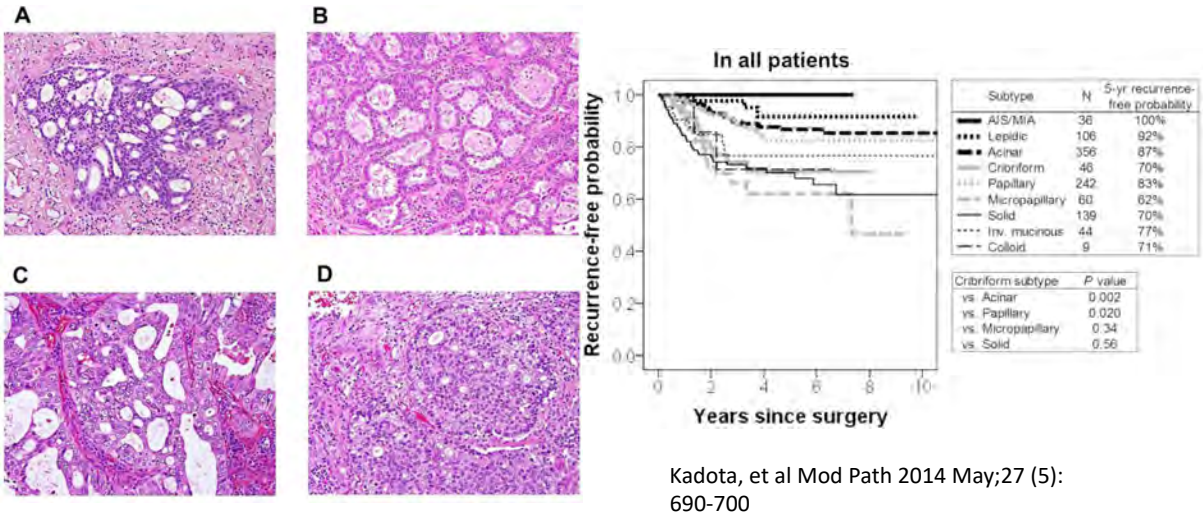




Similar implications for small amounts of other “high grade” growth patterns

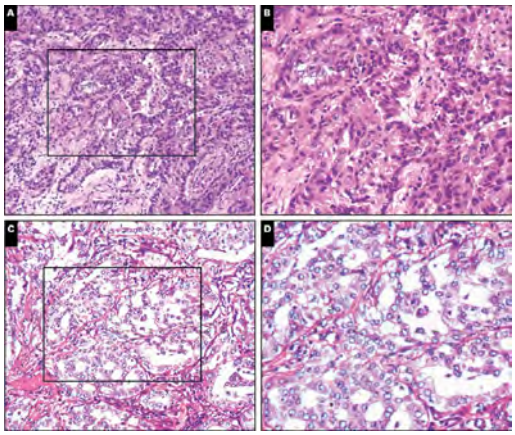
- Solid growth
- Cribriform, complex/fused glands

Cribriform pattern

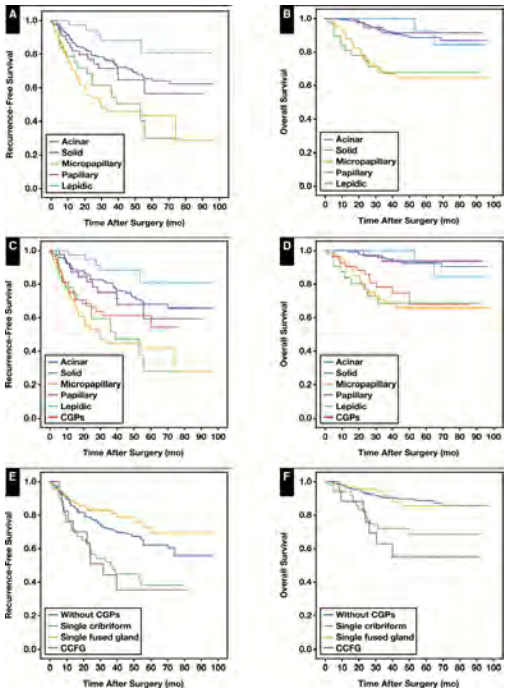


Kadota, et al Mod Path 2014 May;27 (5): 690-700

"Complex glands"—used as a combo of cribriform and fused



Kuang, et al, AJCP 2018 May; 150(1): 65-73



A Grading System for Invasive Pulmonary Adenocarcinoma: A Proposal From the International Association for the Study of Lung Cancer Pathology Committee

Andre L. Moreira, MD, PhD,[a,*] Paolo S. S. Ocampo, MD, PhD,[a] Yuhe Xia,[b] Hua Zhong,[b] Prudence A. Russell, MD,[c] Yuko Minami, MD, PhD,[d] Wendy A. Cooper, MD,[e] Akihiko Yoshida, MD, PhD,[f] Lukas Bubendorf, MD,[g] Mauro Papotti, MD,[h] Giuseppe Pelosi, MD,[i,j] Fernando Lopez-Rios, MD, PhD, FIAC,[k] Keiko Kunitoki, MD,[l] Dana Ferrari-Light, DO,[m] Lynette M. Sholl, MD,[n] Mary Beth Beasley, MD,[o] Alain Borczuk, MD,[p] Johan Botling, MD, PhD,[q] Elisabeth Brambilla, MD, PhD,[r] Gang Chen, MD,[s] Teh-Ying Chou, MD, PhD,[t] Jin-Haeng Chung, MD, PhD,[u] Sanja Dacic, MD, PhD,[v] Deepali Jain, MD,[w] Fred R. Hirsch, MD, PhD,[x] David Hwang, MD, PhD,[y] Sylvie Lantuejoul, MD, PhD,[z] Dongmei Lin, MD,[aa] John W. Longshore, PhD,[bb] Noriko Motoi, MD, PhD,[f] Masayuki Noguchi, MD,[cc] Claudia Poleri, MD,[dd] Natasha Rekhtman, MD, PhD,[ee] Ming-Sound Tsao, MD,[ff] Erik Thunnissen, MD, PhD,[gg] William D. Travis, MD,[ee] Yasushi Yatabe, MD, PhD,[f] Anja C. Roden, MD,[hh] Jillian B. Daigneault, PhD,[ii] Ignacio I. Wistuba, MD,[jj] Keith M. Kerr, MD,[kk] Harvey Pass, MD,[m] Andrew G. Nicholson, MD,[ll,mm] Mari Mino-Kenudson, MD[nn]

Journal of Thoracic Oncology Vol. 15 No. 10: 1599-610

Applies to non-mucinous adenocarcinomas only

How to address the “bad yet not predominant issue”

Table 4. Grading Scheme for Invasive Pulmonary Adenocarcinomas

Grade	Differentiation	Patterns
1	Well-differentiated	Lepidic predominant with no or less than 20% of high-grade patterns
2	Moderately differentiated	Acinar or papillary predominant with no or less than 20% of high-grade patterns
3	Poorly differentiated	Any tumor with 20% or more of high-grade patterns

The model is based on the predominant histologic plus high-grade patterns. The latter includes solid, micropapillary, and complex glandular patterns.

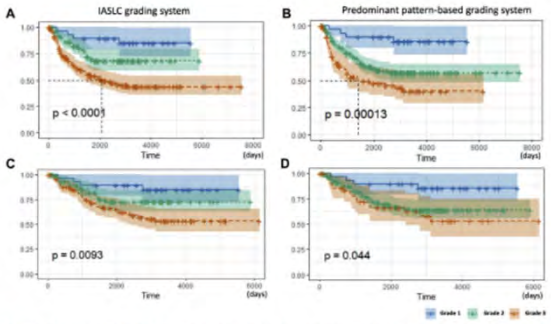


Figure 2. Kaplan-Meier curves for RFS of the test cohort stratified by the IASLC grading system (A: the entire cohort and C: stage I cohort) and predominant pattern-based grading system (B: the entire cohort and D: stage I cohort). For the latter, grade 1 is composed of lepidic predominant tumors; grade 2 of acinar and papillary predominant tumors, and grade 3 of solid, micropapillary, and complex glandular predominant tumors. IASLC, International Association for the Study of Lung Cancer; RFS, recurrence-free survival.

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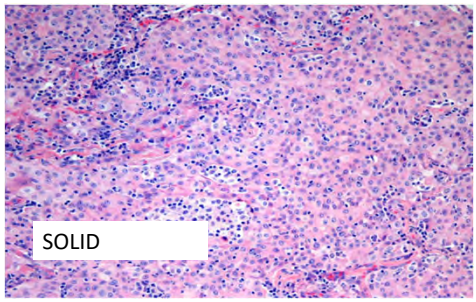
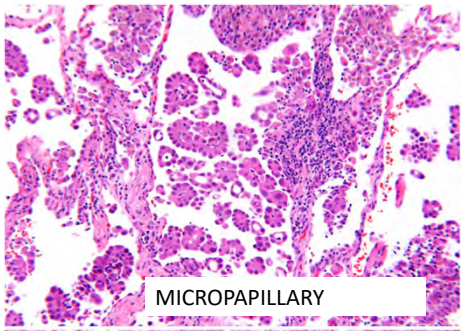
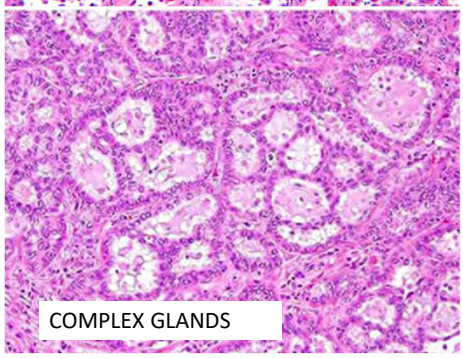
Case 6

- What is the significance of the following growth patterns in regard to <u>grading</u> of non-mucinous lung adenocarcinoma?

Case 6







These are all “high grade” patterns of growth

If any of these are the predominant pattern or comprise 20% or more of a tumor, the tumor is Grade 3

Staging:

- Lepidic vs invasive patterns
- Multiple nodules

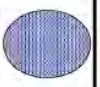
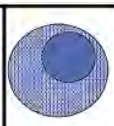
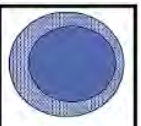
STATE OF THE ART: CONCISE REVIEW

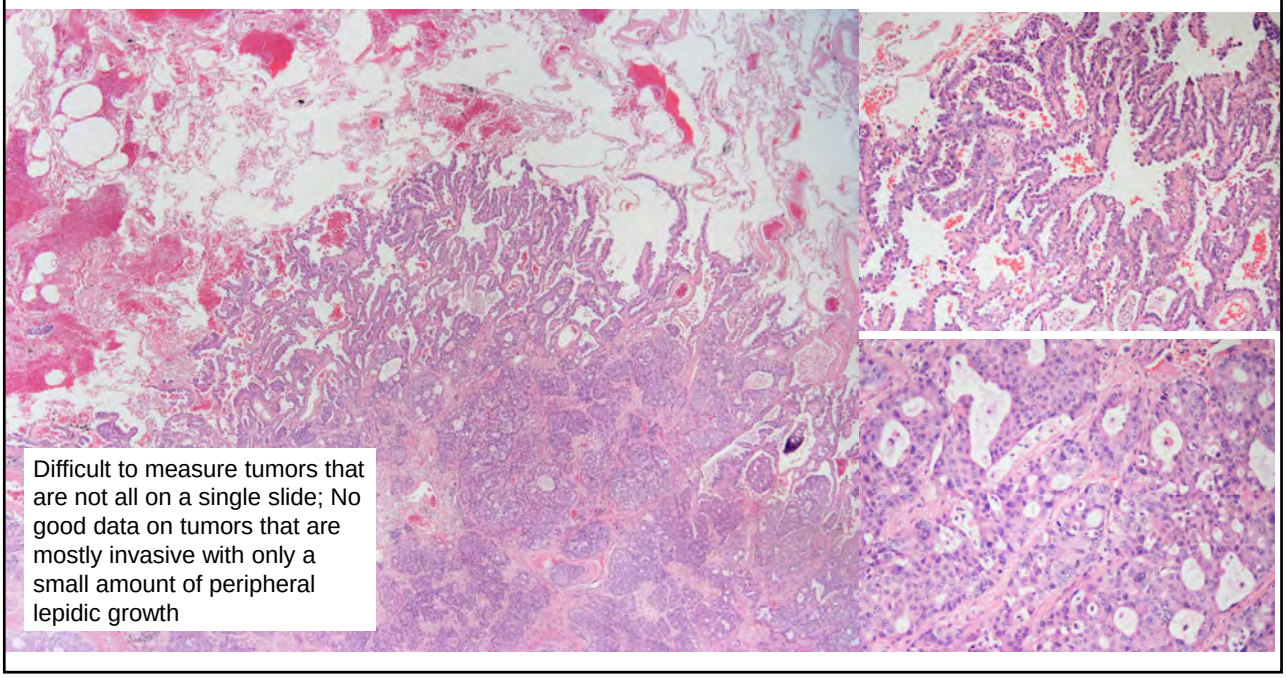
The IASLC Lung Cancer Staging Project: Proposals for Coding T Categories for Subsolid Nodules and Assessment of Tumor Size in Part-Solid Tumors in the Forthcoming Eighth Edition of the TNM Classification of Lung Cancer

William D. Travis, MD,[a,*] Hisao Asamura, MD,[b] Alexander A. Bankier, MD, PhD,[c] Mary Beth Beasley, MD,[d] Frank Detterbeck, MD,[e] Douglas B. Flieder, MD,[f] Jin Mo Goo, MD,[g] Heber MacMahon, MB, BCh,[h] David Naidich, MD,[i] Andrew G. Nicholson, DM, FRCPath,[j] Charles A. Powell, MD,[k] Mathias Prokop, MD,[l] Ramón Rami-Porta, MD,[m,n] Valerie Rusch, MD,[o] Paul van Schil, MD,[p] Yasushi Yatabe, MD,[q] on behalf of the International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee and Advisory Board Members[**]

J Thorac Oncol. 2016 Aug;11(8):1204-23

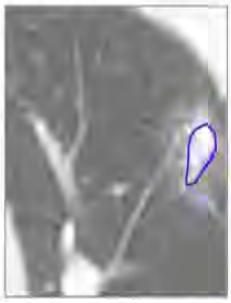
Recommends staging based on size of only the invasive component in non-mucinous adenocarcinomas with a component of lepidic growth

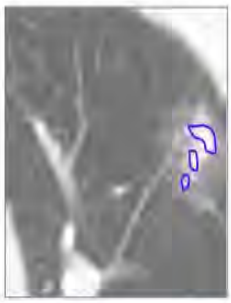
cT*	CT image on HRCT						
	Solid part	0 cm	0 cm	≤0.5 cm†	0.6-1.0 cm†	1.1-2.0 cm†	2.1-3.0 cm†
	Total tumor size including GG	≤0.5 cm	0.6-3.0 cm†‡	≤3.0 cm†‡	0..6-3.0 cm††	1.1-3.0 cm††	2.1-3.0 cm††
	Pathologic Differential Diagnosis	AAH‡, AIS, MIA	AIS, MIA, LPA	MIA, LPA, AIS	LPA, Invasive AD, MIA	LPA, Invasive AD	Invasive AD
	Clinical Stage*		cTis‡‡	cT1mi‡‡	cT1a	cT1b	cT1c
pT	Invasive part	0 cm	0 cm	≤0.5 cm‡‡	0.6-1.0 cm†	1.1-2.0 cm†	2.1-3.0 cm†
	Total tumor size including lepidic growth part	Usually ≤0.5 cm‡	≤3.0 cm†‡	≤3.0 cm†‡	0.6-3.0 cm††	1.1-3.0 cm††	2.1-3.0 cm††
	Pathology	AAH	AIS	MIA	Lepidic predominant AD or Invasive AD with lepidic component	Invasive AD with a lepidic component or lepidic predominant AD	Invasive AD with lepidic component
	Pathologic Stage		pTis‡‡	pT1mi‡‡	pT1a	pT1b	pT1c



Difficult to measure tumors that are not all on a single slide; No good data on tumors that are mostly invasive with only a small amount of peripheral lepidic growth

How to measure invasive size??





From CAP cancer protocols 2017:
cells infiltrating myofibroblastic stroma." In tumors where the invasive component is not a single discrete measurable focus, estimating the percentage of the total tumor that is invasive and then multiplying by the total tumor size to estimate invasive tumor size is recommended.[1]

-Based on all reviewed slides, estimate % lepidic vs % other (invasive)
-Invasive size = total size x % invasive

- Total size: 3.2 cm
- 60% lepidic, 20% acinar, 20% papillary (total 40% invasive)
-Invasive size = 3.2cm x 0.4 = **1.28** cm (pT2a → pT1b)

Travis WD et al J Thorac Oncol. 2016 Aug;11(8):1204-23

What to do with Multiple nodules

- When do you have multiple nodules?
  - By definition these are grossly and/or radiographically distinct
  - A tiny bit of tumor sort of discontinuous with the main tumor in your slide is not a separate primary
- Radiology very important-
  - Multiple ground glass lesions (usually correlating with AIS, MIA or lepidic predominant ADC) are staged as separate primary tumors regardless
  - Multiple solid lesions or a mix of patterns—much more complicated
    - Easy if you have one ADC or one SCC
    - What to do with multiple ADC's….

Multiple nodules--evolution

- Martini and Melamed criteria (1975):
  - Lesions occur in different lobes or different segments of the same lobe
  - Lesions are of different histological type
  - Lesions originate respectively from carcinoma in situ
  - No metastasis in contiguously draining lymphatics or other organs
  - This actually still works pretty well after all this time
- What to do now:
  - AIS, MIA or LPA is ***always*** its own primary
  - Otherwise, look at the patterns present:
    - totally different predominant patterns>>separate primaries
    - Same pattern or "toss up" i.e mixed acinar and papillary and one has slightly more acinar and one has slightly more papillary>> same tumor
      - If these are in different lobes and nodes are negative, the clinicians may still opt to treat these as separate primaries
    - Softer criteria—same pattern but one has much uglier cytology than the other one>>probably different
  - This works pretty well too

Table 1. Schematic Summary of Disease Patterns and TNM Classification of Patients With Lung Cancer With Multiple Pulmonary Sites of Involvement

	Second Primary Lung Cancer	Multifocal GG/L Nodules	Pneumonic-type Adenocarcinoma	Separate Tumor Nodule
Imaging features	Two or more distinct masses with imaging characteristic of lung cancer (eg, spiculated)	Multiple ground-glass or part-solid nodules	Patchy areas of ground glass and consolidation	Typical lung cancer (eg, solid, spiculated) with separate solid nodule
Pathological features	Different histotype or different morphology based on comprehensive histologic assessment	Adenocarcinomas with prominent lepidic component (typically varying degrees of AIS, MIA, LPA)	Same histology throughout (most often invasive mucinous adenocarcinoma)	Distinct masses with the same morphologic features based on comprehensive histologic assessment
TNM classification	Separate cTNM and pTNM for each cancer	T based on highest T lesion, with (#/m) indicating multiplicity; single N and M	T based on size or T3 if in single lobe, T4 or M1a if in different ipsilateral or contralateral lobes; single N and M	Location of separate nodule relative to primary site determines whether T3, T4, or M1a; single N and M
Conceptual view	Unrelated tumors	Separate tumors, albeit with similarities	Single tumor, diffuse pulmonary involvement	Single tumor with intrapulmonary metastasis

AIS, adenocarcinoma in situ; GG/L, ground-glass/lepidic; LPA, lepidic-predominant adenocarcinoma; MIA, minimally invasive adenocarcinoma. From AJCC Cancer Staging Manual, 8th edition. Used with permission.

Multiple nodules—what about molecular

- If possible, you want to get molecular on all of the tumors present
- DIFFERENT MOLECULAR helps you the most
- Same molecular? (i.e same KRAS mutation)
  - Not as useful if part of a single gene analysis or small panel
  - With larger panels there is the opportunity to detect different downstream/additional markers which may be helpful
- Tumors that look alike are usually the same tumor---may get a surprise with different molecular around 10-15% of the time.
- Occasionally, tumors that don't look alike will have the same molecular with comprehensive NGS
  - ?Progression
- Same morphology/same molecular>>probably the same, particularly with large molecular panel. If only small panel done, clinical components may come into play i.e same lobe vs different lobes.

Translational Cancer Mechanisms and Therapy

Clinical Cancer Research

Comprehensive Next-Generation Sequencing Unambiguously Distinguishes Separate Primary Lung Carcinomas From Intrapulmonary Metastases: Comparison with Standard Histopathologic Approach

Jason C. Chang[1], Deepu Alex[1], Matthew Bott[2], Kay See Tan[3], Venkatraman Seshan[3], Andrew Golden[1], Jennifer L. Sauter[1], Darren J. Buonocore[1], Chad M. Vanderbilt[1], Sounak Gupta[1], Patrice Desmeules[1], Francis M. Bodd[1], Gregory J. Riely[4], Valerie W. Rusch[2], David R. Jones[2], Maria E. Arcila[1], William D. Travis[1], Marc Ladanyi[1,5], and Natasha Rekhtman[1]

Clin Cancer Res; 25(23) December 1, 2019

C

		Clonality defined by NGS		Total
		IPM	SPLC	
Prospective prediction	Histologically similar	14 (56%)	6 (12%)	20
	Histologically different	11 (44%)	45 (88%)	56
Total		25	51	76

$P = 0.0002$

Molecularly defined IPMs: histologic challenges in discrepant cases ($n = 11$)

- Histologic progression ($n = 7$)
- Significant lepidic pattern in both tumors ($n = 4$)

D

		NGS testing suggested by pathologist		Total
		Yes	No	
Histology vs. molecular	Concordant	14 (61%)	45 (85%)	59
	Discordant	9 (39%)	8 (15%)	17
Total		23	53	76

$P = 0.034$

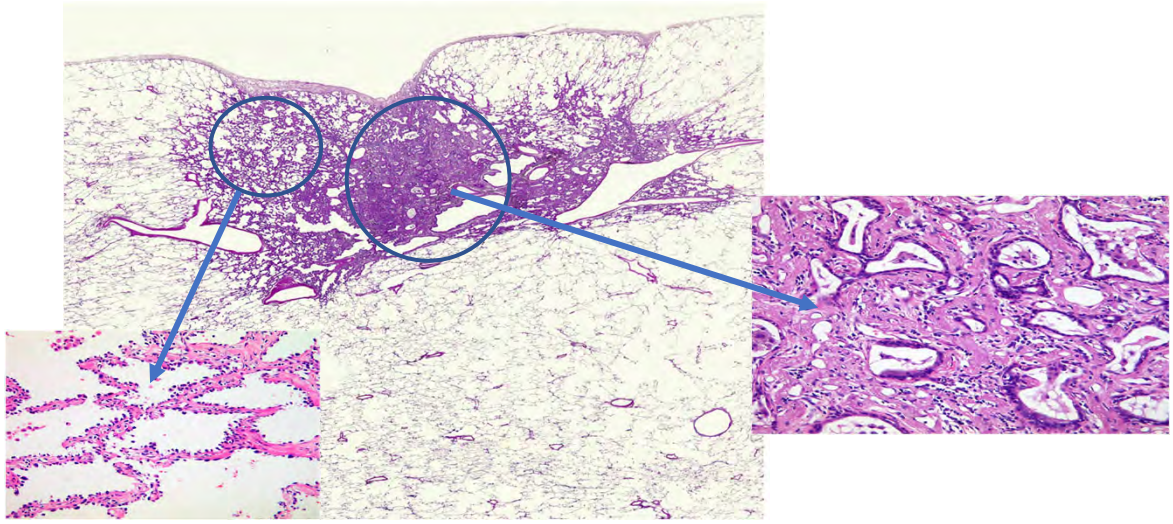
Molecularly defined SPLCs: histologic challenges in discrepant cases ($n = 6$)

- Overlap in architectural patterns ($n = 4$)
- Overlap of cytologic architecture ($n = 2$)

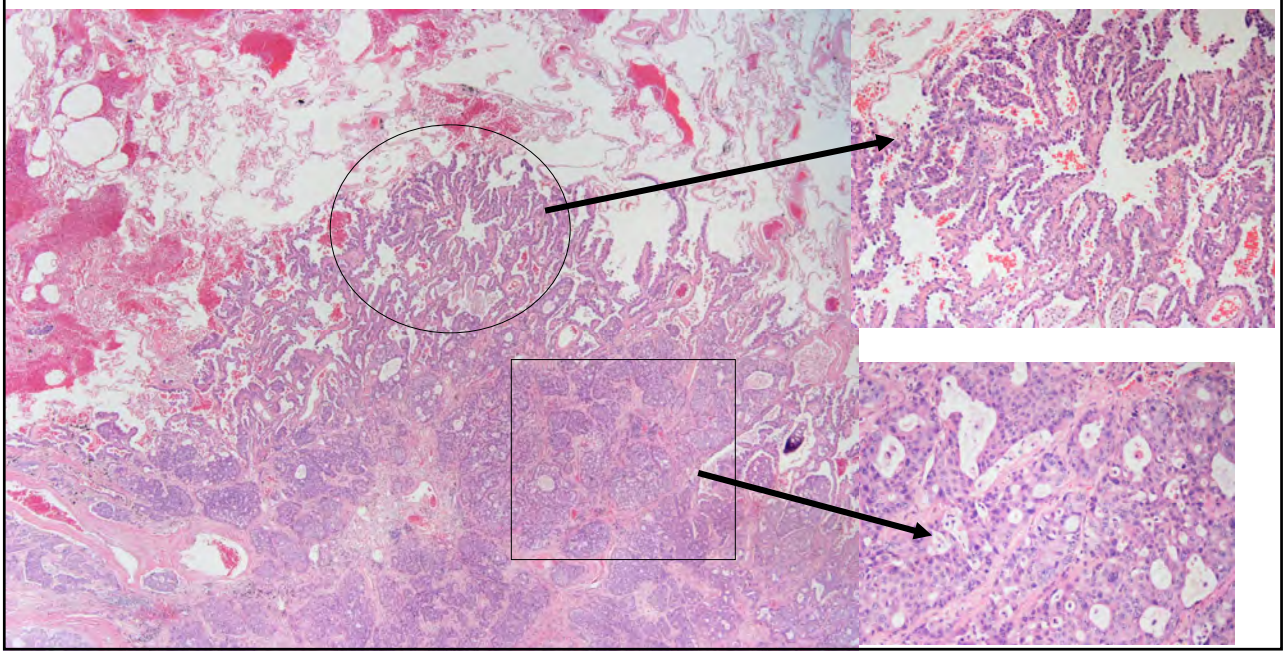
Case 7

- Should a lobectomy specimen with the two tumors shown in the following images be staged as separate primaries or pT3?

Case 7 tumor 1



Case 7 Tumor 2



Evaluation

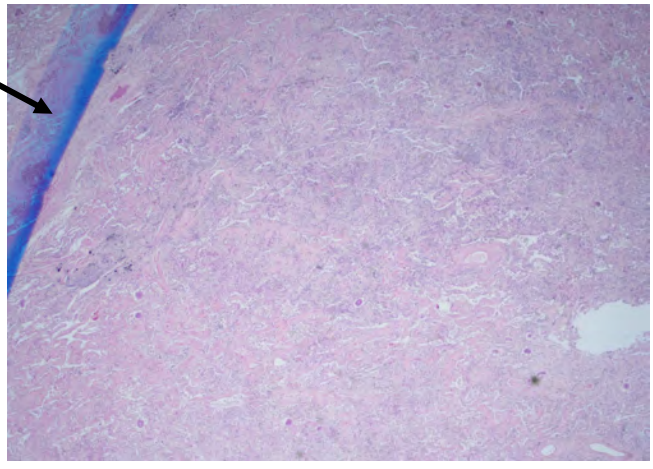
- Tumor 1—Minimally invasive ADC---this is always its own primary
- Tumor 2—has tiny lepidic component but is primarily invasive complex glands
- Morphologically different>>> Stage a separate primaries

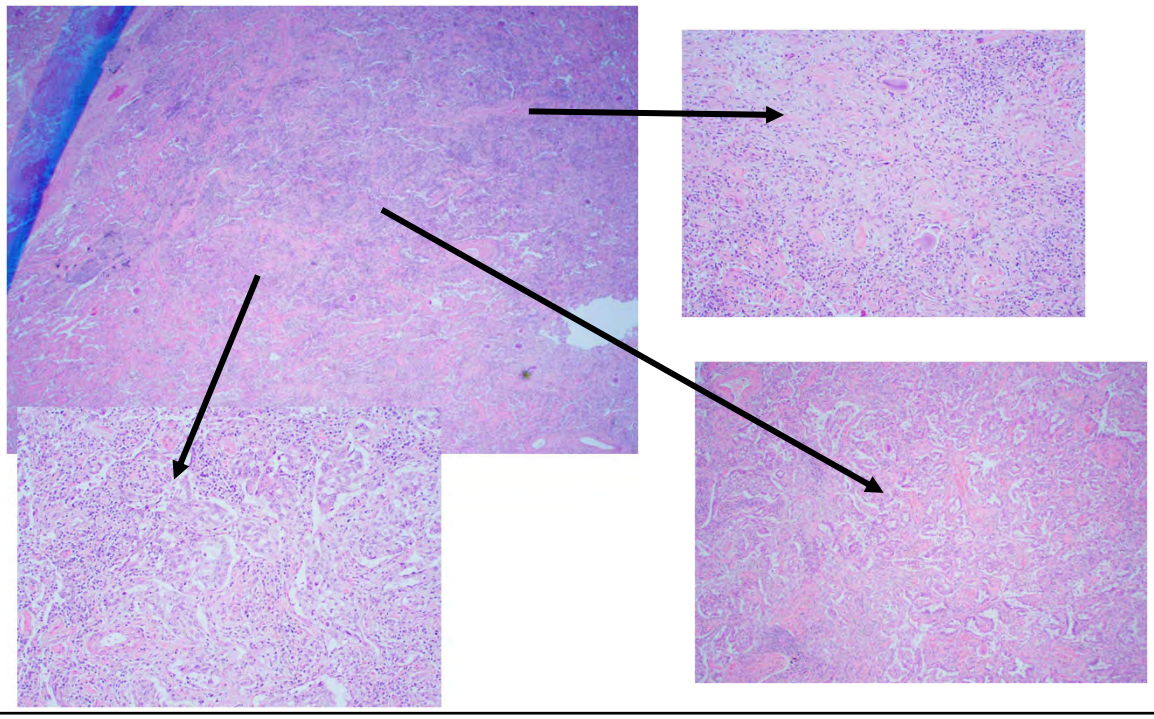
Case 8

- The following images are from a resected tumor following neoadjuvant therapy
- How do you stage this?
- Does this seem to meet criteria for MPR?

Case 8

Edge of tumor bed





How should you evaluate post-neoadjuvant specimens?

Pathological responses after Neoadjuvant Therapy

- **Major Pathological Response (MPR)**
  - Less than or equal to 10% residual viable tumor (NSCLC)
- **Complete Pathological Response (pCR)**
  - NO residual tumor

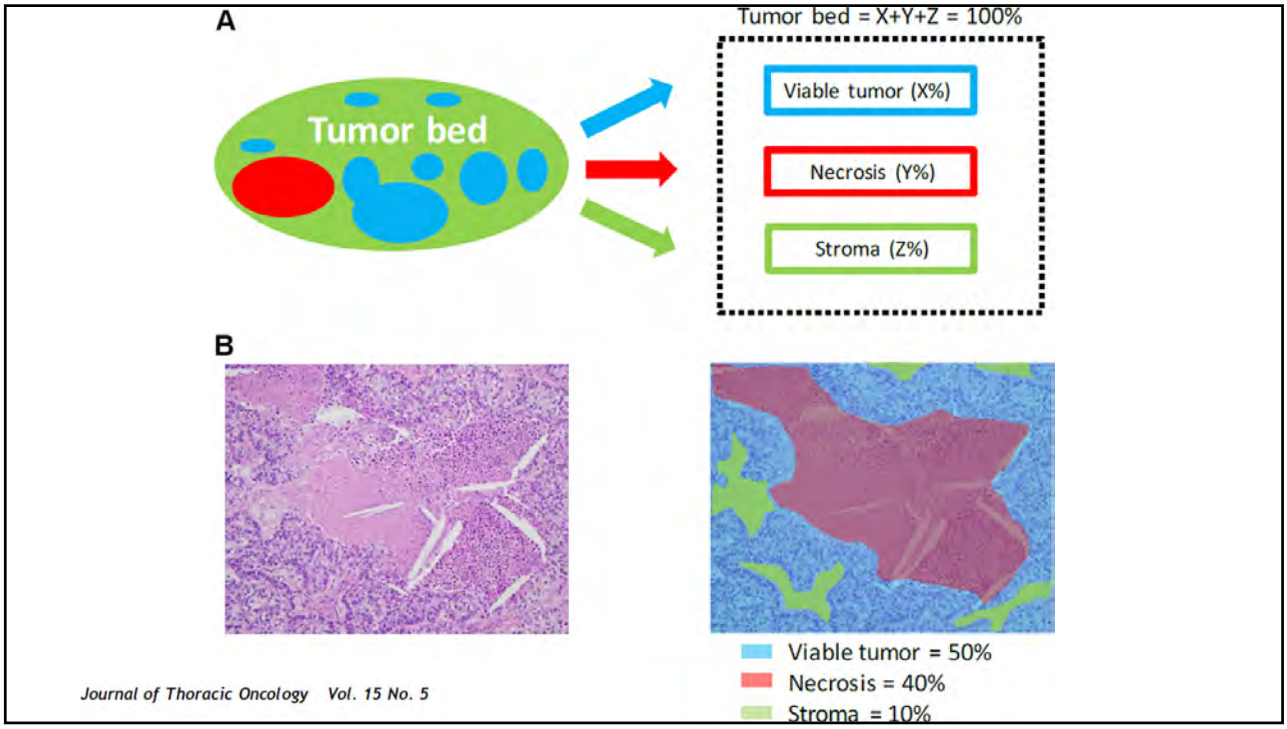
Pathological responses after Neoadjuvant Therapy

- Well established in other tumors: Breast, Rectum, Oesophagus
- MPR definitions based on older studies using chemotherapy
- Recently, cut points of 10% (Squamous cell ca) and 65% (Adenocarcinoma) have been proposed for neoadjuvant chemotherapy.
- Debate about different cut offs for IO vs “traditional” chemo

- Junker K et al. Chest 2001, Pataer A et al. JTO 2012, Qu Y et al. JTO 2019

Core Elements of the Microscopic Pathologic assessment

- Viable Tumor
- Necrosis
- Stroma (includes fibrosis and inflammation)
- Response = viable tumor area/total tumor area x 100%



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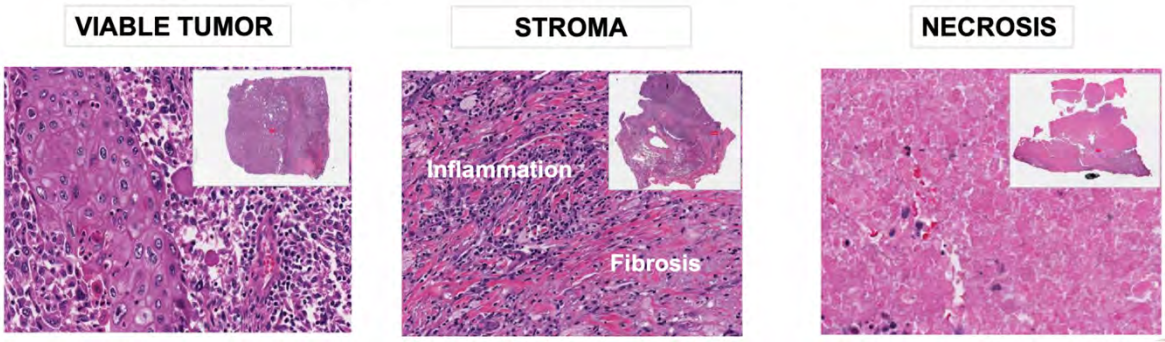
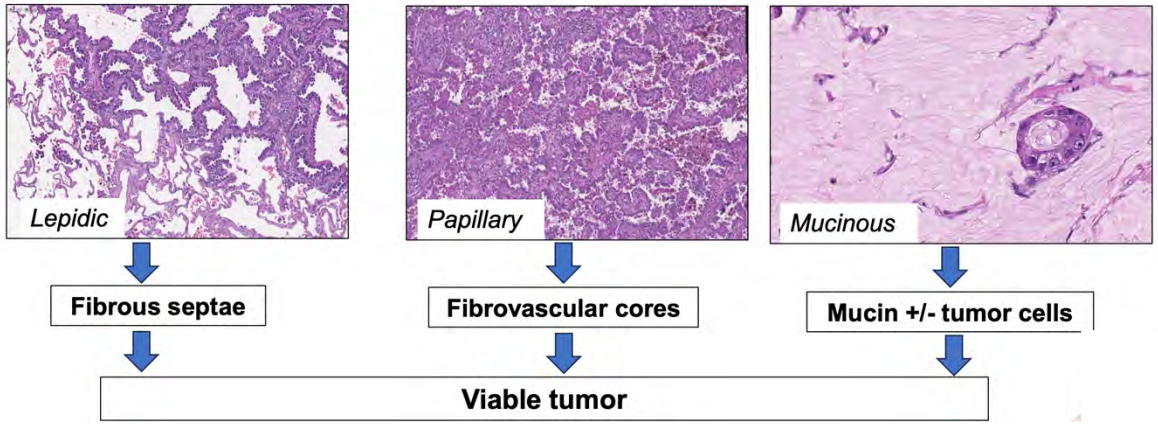
Microscopic Assessment Of The Tumor Bed



Challenging Adenocarcinoma Subtypes in Neoadjuvant Cases



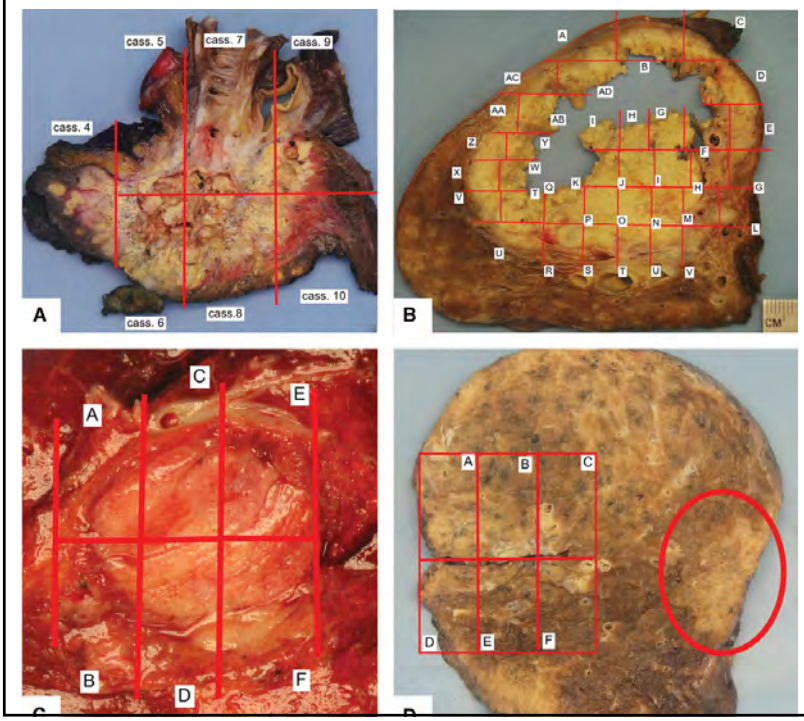
Grossing

- Small (3.0 cm or less) tumor/tumor bed—submit entirely
- IASLC rec for larger tumors is to map a cross sectional slice
- Issues- tumor bed is not always easy to find

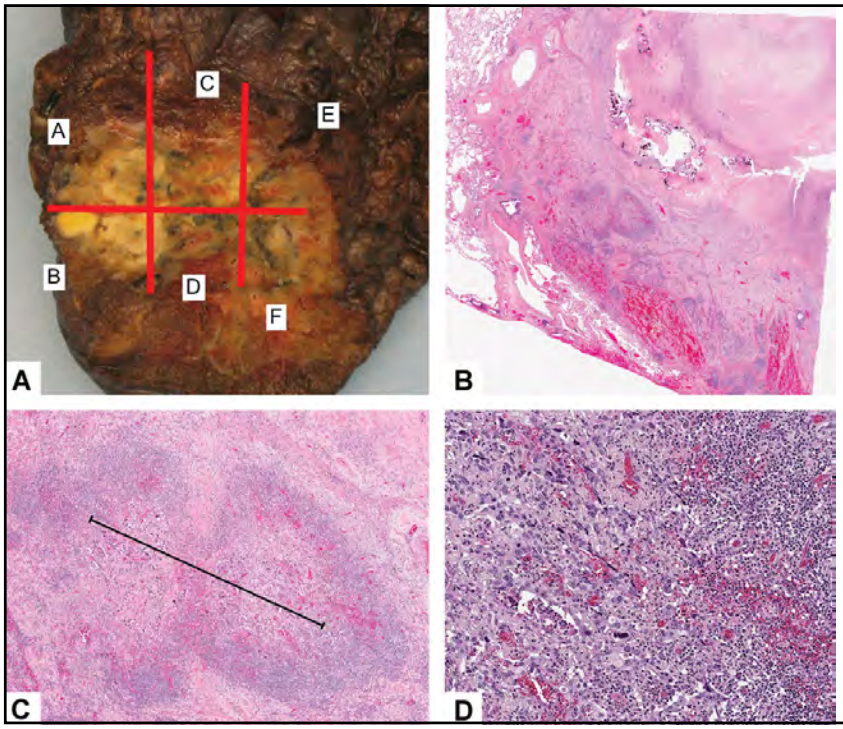
IASLC Multidisciplinary Recommendations for Pathologic Assessment of Lung Cancer Resection Specimens After Neoadjuvant Therapy

William D. Travis, MD,[a,*] Sanja Dacic, MD,[b] Ignacio Wistuba, MD,[c]
Lynette Sholl, MD,[d] Prasad Adusumilli, MD,[e] Lukas Bubendorf, MD,[f] Paul Bunn, MD,[g]
Tina Cascone, MD, PhD,[h] Jamie Chaft, MD,[i] Gang Chen, MD,[j] Teh-Ying Chou, MD,[k]
Wendy Cooper, MD,[l] Jeremy J. Erasmus, MD,[m] Carlos Gil Ferreira, MD,[n]
Jin-Mo Goo, MD,[o] John Heymach, MD, PhD,[h] Fred R. Hirsch, MD,[p]
Hidehito Horinouchi, MD,[q] Keith Kerr, MD,[r] Mark Kris, MD,[i] Deepali Jain, MD,[s]
Young T. Kim, MD,[t] Fernando Lopez-Rios, MD,[u] Shun Lu, MD,[v]

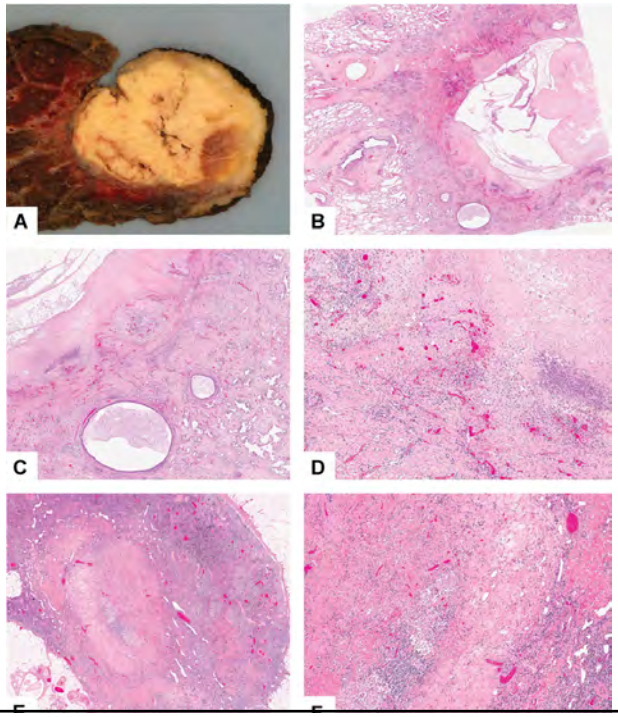
Journal of Thoracic Oncology Vol. 15 No. 5



Gross Appearance and Mapping. A) This adenocarcinoma shows extensive necrosis with both yellow areas and some chalky white tissue within areas of cavitation. In addition, this photograph demonstrates the anatomic relationship of the tumor to the overlying pleura and the proximal bronchus. B) This large necrotic tumor shows a large central area of cavitation. In the cavitated area it is impossible to submit blocks of tissue sections, so this area is not mapped. C) This small tumor is mapped but due to its small size the entire tumor was sampled histologically. D) In this case the mapped area turned out to be granulomatous inflammation, and the tumor was on the opposite side of the specimen (red oval shaped circle). So additional sections from the non-mapped area of tumor needed to be submitted to evaluate for treatment effect.



Major pathologic response: A) This tumor shows a variegated cut surface with yellow and white necrotic areas. B) Low power shows a large area of necrosis surrounded by dense fibrosis and chronic inflammation. C) Only a single 2 mm focus of viable adenocarcinoma was seen. Black line indicates the tumor size measurement. D) This focus of viable tumor cells surrounded by stroma with marked chronic inflammation consists of solid adenocarcinoma that was TTF-1 positive.



Complete pathologic response: A) This tumor is almost entirely necrotic with yellow soft areas and small firm tan areas. B) Low power shows a large necrotic cavitated area surrounded by dense fibrosis. C) The stroma shows marked chronic inflammation, foamy histiocytes and fibrosis with some adjacent alveolar parenchyma showing reactive pneumocytes (bottom) D) High power shows numerous chronic inflammatory cells, foamy histiocytes and dense fibrosis. No viable tumor was seen. E) In addition, no tumor was seen in two lymph nodes with changes consistent with treatment effect. This lymph node shows nodular scar and granulomatous inflammation. F) The tumor bed shows dense fibrosis with focal chronic inflammation and giant cells.

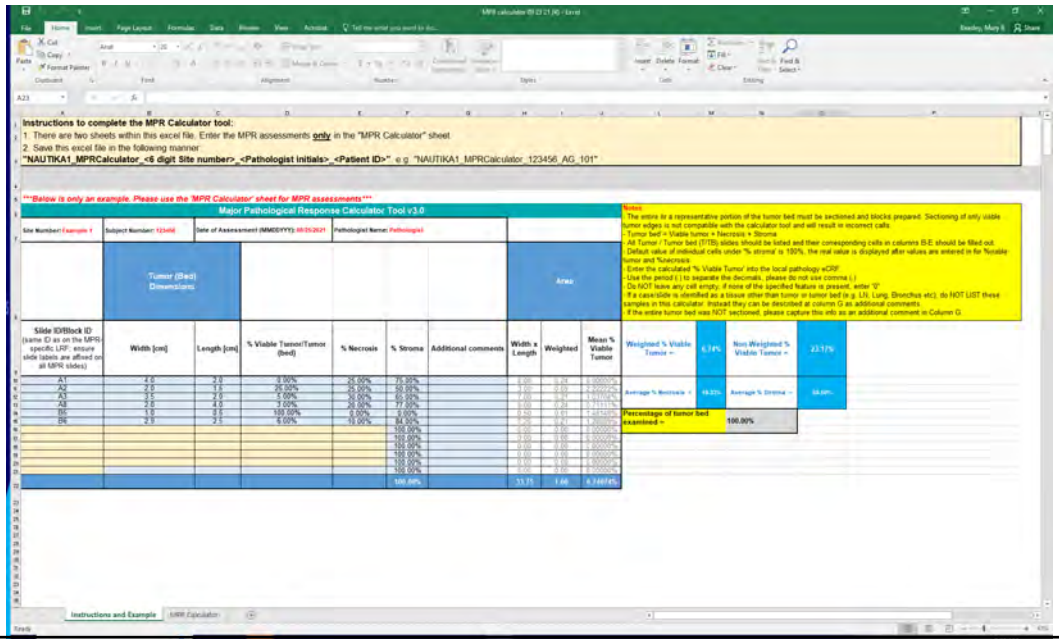
Microscopic Assessment

1. Informal semiquantitative or "eyeball" approach
- Used in all earlier studies that showed the clinical relevance of MPR
- Subjective, but fast for experienced pathologists

2. H&E slide average (unweighted approach)
- Does not take into account that each slide has a different amount of tumor sampled

3. Weighted Approach
- Does take into account that each slide has a different amount of tumor sampled

Response calculator



International Association for the Study of Lung Cancer Study of Reproducibility in Assessment of Pathologic Response in Resected Lung Cancers After Neoadjuvant Therapy

Sanja Dacic, MD, PhD,[a,*] William Travis, MD,[b] Mary Redman, PhD,[c] Anjali Saqi, MD,[d] Wendy A. Cooper, MBBS, PhD, FRCPA,[e,f,g] Alain Borczuk, MD,[h] Jin-Haeng Chung, MD, PhD,[i] Carolyn Glass, MD,[j] Javier Martin Lopez, MD,[k] Anja C. Roden, MD,[l] Lynette Sholl, MD,[m] Annikka Weissferdt, MD,[n] Juan Posadas, BSCS, MBA,[o] Angela Walker, BSCS,[o] Hu Zhu, MSE, MS,[o] Manuja T. Wijeratne, M.S.,[o] Casey Connolly, MPH,[p] Murry Wynes, PhD,[p] Neus Bota-Rabassedas, PhD,[o] Beatriz Sanchez-Espiridion, PhD,[o] J. Jack Lee, PhD,[q] Sabina Berezowska, MD,[r] Teh-Ying Chou, MD, PhD, MBA,[s] Keith Kerr, MBChB, FRCPath,[t] Andrew Nicholson, DM, FRCPath,[u] Claudia Poleri, MD,[v] Kurt A. Schalper, MD, PhD,[w] Ming-Sound Tsao, MD, FRCPC,[x] David P. Carbone, MD, PhD,[y] Neal Ready, MD, PhD,[z] Tina Cascone, MD, PhD,[aa] John Heymach, MD, PhD,[aa]

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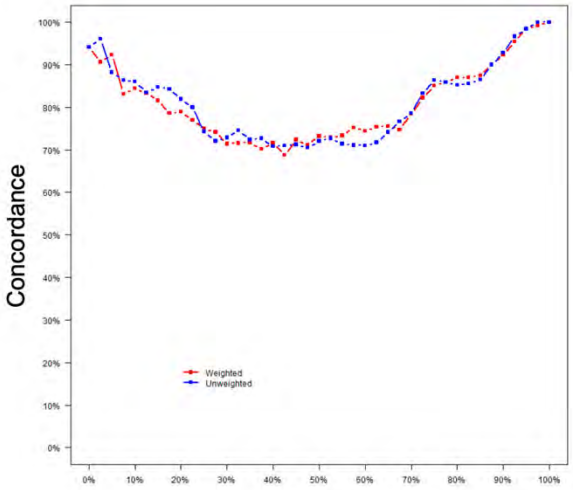
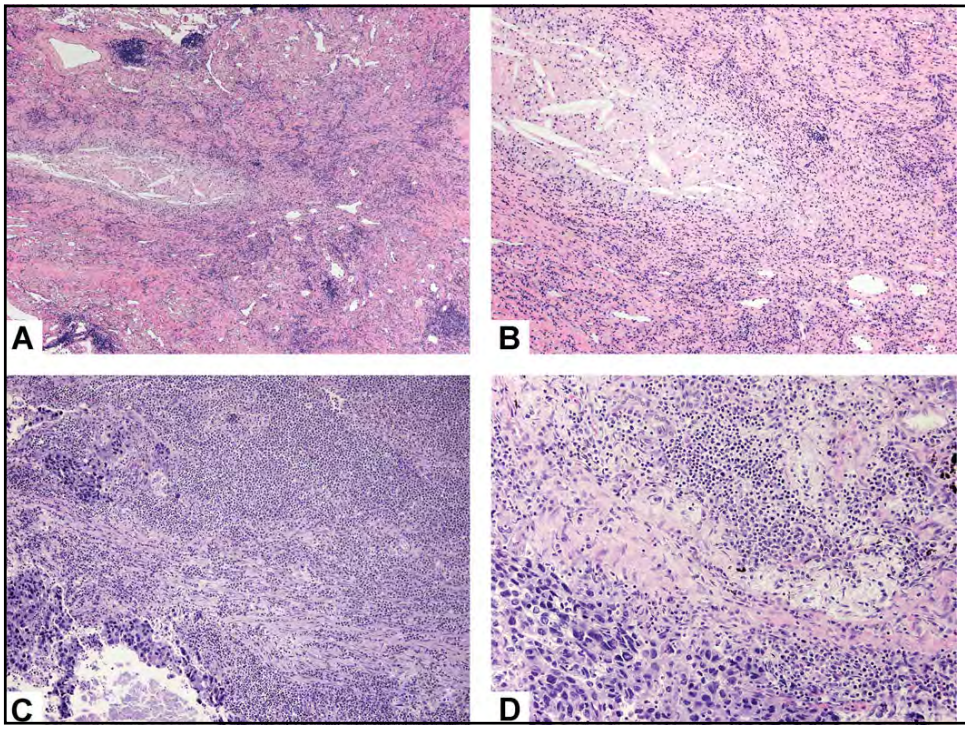
Weighted vs unweighted concordance



Journal of Thoracic Oncology Vol. 18 No. 10: 1290-1302

Of course, there is this problem….



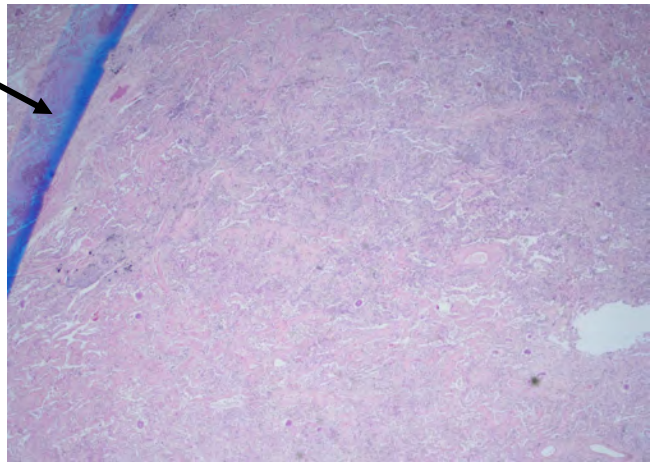
Histologic changes **without neoadjuvant therapy**: A) Only 10% of this tumor was viable with 90% showing fibrosis, chronic inflammation and focal necrosis with cholesterol clefts. B) Higher power shows dense fibrosis with a mild chronic inflammatory infiltrate and an area of necrosis with cholesterol clefts. C) This lung cancer was associated with a large chronic inflammatory infiltrate that overshadowed a small focus of adenocarcinoma. D) This area of solid nests of tumor cells (bottom left) are surrounded by an extensive mixed inflammatory infiltrate of lymphocytes, plasma cells and histiocytes.

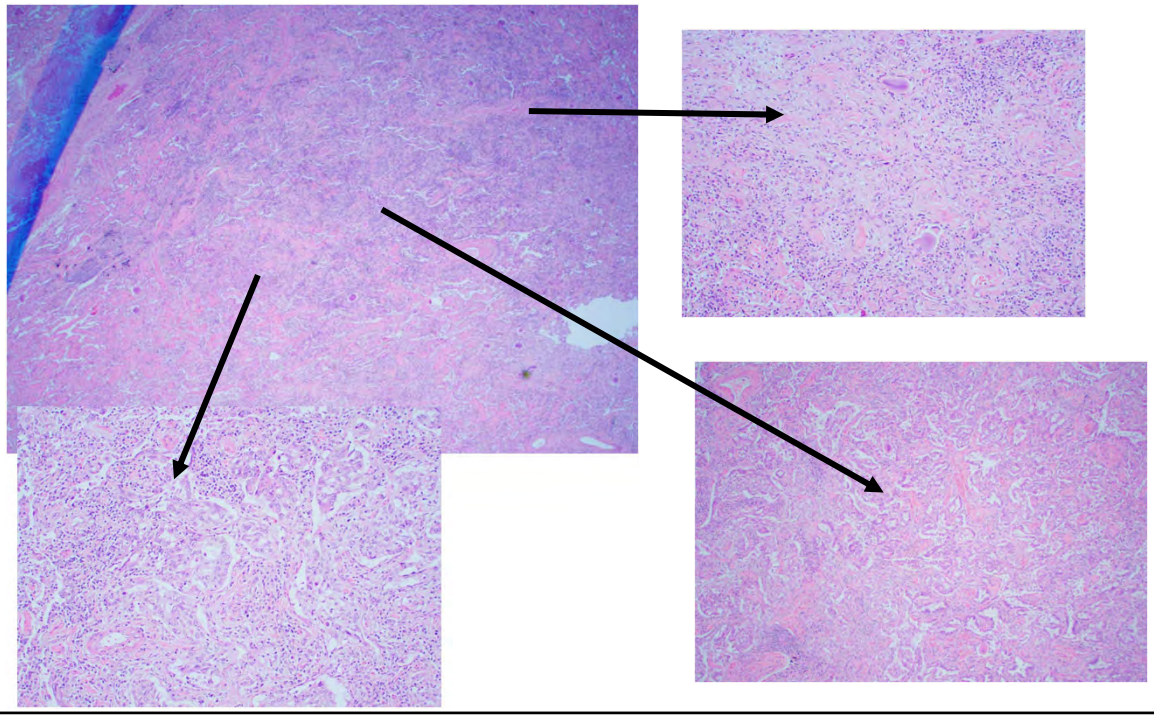
Case 8

- The following images are from a resected tumor following neoadjuvant therapy
- How do you stage this?
- Does this seem to meet criteria for MPR?

Case 8

Edge of tumor bed





Case 8

- Ultimately the tumor bed contained 50% viable tumor, 50% stroma, no necrosis
  - Not MPR
  - Recommended reporting:
    - % viable tumor
    - %necrosis
    - % stroma
- 4.0 cm tumor bed with 50% viable tumor, no pleural invasion
  - 2.0 cm viable tumor>> ypT1b

Summary

- The biggest problem in my experience with post-neoadjuvant cases is that the surgeon usually neglects to tell you a case is post-neo
- That aside:
  - Careful attention must be paid to grossing to identify tumor bed and provide best determination of viable tumor
  - Microscopic evaluation should record % viable tumor, % necrosis and % stroma
  - MPR= 10% or less viable tumor
  - pCR= no residual viable tumor
  - Treatment effect should be recorded in lymph nodes if present >> evolving area of investigation

Summary—Talk 3

- Be aware of high grade histologic patterns (micropapillary, solid, complex glands) and their significance in grading non-mucinous lung adenocarcinomas
- Understand staging of multiple pulmonary nodules
  - Multiple ground glass/subsolid nodules corresponding to AIS, MIA or LPA are always separate primaries
  - Otherwise, comprehensive evaluation of percentage of growth patterns present and comparison of primary patterns is generally recommended and works well
  - Molecular analysis, particularly larger panels, is useful and further complements comprehensive histologic evaluation
    - Optimal number of genes to evaluate not firmly established.

Thank you!