Pulmonary Pathology Journal Club – AC Roden
May, 2017 (April 2017 Articles)

Articles for discussion

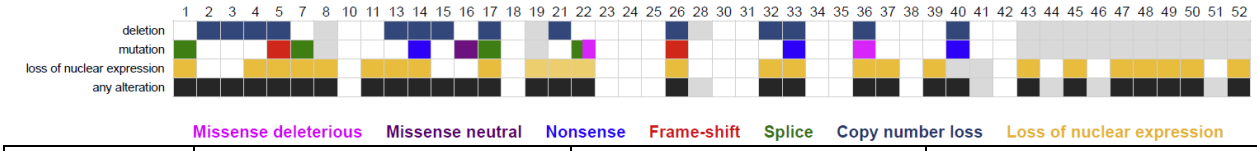
Leblay N et al. BAP1 Is Altered by Copy Number Loss, Mutation, and/or Loss of Protein Expression in More Than 70% of Malignant Peritoneal Mesotheliomas. 2017. JTO.12:824-33.

Summarized and presented by Dr. Roger Hsu

Background:
Germline and somatic inactivation of *BAP1* are well-reported in pleural mesothelioma, but limited data exist on the *BAP1* status in peritoneal mesothelioma.

Methods:
A cohort of 46 patients with peritoneal mesotheliomas is identified through the French National Reference Centers for the Diagnosis of Malignant Pleural Mesothelioma and Rare Peritoneal Tumors and for the Treatment of Rare Peritoneal Surface Malignancies. *BAP1* DNA sequencing and BAP1 IHC were performed on available materials.

Results:
- 26 male and 20 female, median age 62.5 and 60, respectively.
- Histologic types include 41 epithelioid, 3 biphasic, and 2 sarcomatoid.
- *BAP1* mutation or loss of BAP1 expression, but not CN correlates with better survival.



	BAP1 expression		*BAP1* deletion		*BAP1* mutation	
Histological type	Lost (n=25)	Retained (n=19)	None (n=19)	Deletion (n=14)	None (n=23)	Mutation (n=11)
Epithelioid	25 (100%)	15 (79%)	15 (89%)	13 (93%)	19 (82%)	10 (91%)
Biphasic	0	2 (10.5%)	2 (10.5%)	1 (7%)	2 (9%)	1 (9%)
Sarcomatoid	0	2 (10.5%)	2 (10.5%)	0	2 (9%)	0

Discussion:
Mismatch between deletion or mutation and the loss of BAP1 nuclear expression, suggests heterozygous inactivation of *BAP1* in those with retained expression or additional mechanisms resulting in the inactivation of *BAP1* in those with loss of BAP1 expression without CN loss or mutation. Integrative analyses of different technologies are required to capture the full spectrum of *BAP1* gene alterations in malignant mesotheliomas, although biologic significance of reduced *BAP1* activity is unclear.

Take home point:
Alterations in *BAP1* gene occur in 73% of malignant peritoneal mesotheliomas, while loss of BAP1 expression was demonstrated in 57%. Malignant mesotheliomas with *BAP1* mutation or loss of BAP1 may have a better prognosis.

Shinozaki-Ushiku A et al. Diagnostic utility of BAP1 and EZH2 expression in malignant mesothelioma. Histopathol. 2017. 70:722-33.

Background:

- Loss of BRCA1-associated protein 1 (BAP1) might be specific to mal. mesothelioma (MM)
- BAP1 loss promotes cell proliferation in vitro through up-regulation of enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2). EZH2 encodes a histone-lysine N-methyltransferase that acts as a transcriptional repressor. EZH2 is not organ-specific.
- EZH2 mRNA expression also increased in MM

Purpose:

- To evaluate the diagnostic utility and prognostic significance of BAP1 and EZH2 in MM.

Methods:

- BAP1 (clone C-4) and EZH2 (clone D2C9) expression by IHC
- BAP1: retained vs lost; EZH2 expression: low (score 0–1; <50%) vs high (score 2–3; ≥ 50%)

Results:

- 32 MM (27 pleura, 5 peritoneal; 23 epithelioid, 7 biphasic, 2 sarcomatoid), 44 benign mesothelial proliferative lesions (4 WDPM, 22 mesothelial inclusion cysts, 18 reactive mesothelial hyperplasia).
- BAP1 loss in 17/32 (53%) MM (61% epithelioid MM, 43% biphasic); in most biphasic MM, epithelioid and sarcomatoid components scored similar except 1 case with BAP1 loss in epithelioid and retained BAP1 expression in sarcomatoid component
- All benign mesothelial lesions had retained BAP1 expression
- High EZH2 expression in 21/32 (66%) MM (57% epithelioid, 100% biphasic, 50% sarcomatoid). Same score in epithelioid & sarcomatoid component of biphasic MM
- No high EZH2 expression in benign mesothelial lesions.

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
All histological types				
BAP1 loss	53.1 (34.7–70.9)	100 (88.2–100)	100 (72.7–100)	74.6 (61.6–85.0)
EZH2-high	65.6 (46.8–81.4)	100 (88.2–100)	100 (77.2–100)	80.0 (67.0–89.6)
BAP1 loss or EZH2-high	87.5 (71.0–96.5)	100 (88.2–100)	100 (82.2–100)	91.7 (80.0–97.7)
Epithelioid/biphasic				
BAP1 loss	56.7 (37.4–74.5)	100 (88.2–100)	100 (72.7–100)	77.2 (64.2–87.3)
EZH2-high	66.7 (47.2–82.7)	100 (88.2–100)	100 (76.2–100)	81.5 (68.6–90.7)
BAP1 loss or EZH2-high	90.0 (73.5–97.9)	100 (88.2–100)	100 (81.7–100)	93.6 (82.5–98.7)

- No associations between clinical parameters (age, sex of patients, tumor location, asbestos exposure, treatment, histology) and BAP1 or EZH2 expression.
- Follow-up for MM, mean 530 days (3-2213). 15 died from disease, 3 alive with disease, 14 lost. No patient with reactive mesothelial proliferation developed MM or died from disease.
- BAP1 loss, but not high EZH2 expression - independent predictor of better prognosis.

Take Home Points:

- EZH2 might be a good marker to have although findings still need to be validated

- Whether addition of testing for homozygous deletion of *CDKN2A* might add value was not tested
- Several recently developed EZH2-specific inhibitors are predicted to improve prognosis of MM; some in phase 1 trials

Buerger T et al. Metastatic type A thymoma: morphological and genetic correlation. Histopathology. 2017. 70:704-10

Background:

- Most type A thymomas are diagnosed at stage 1 or 2; metastases exceedingly rare; stage IV disease in only 1% of type A thymomas
- 2015 WHO: introduction of "atypical type A thymoma" (type A thymoma with ≥4 mitoses/10hpf, mild to moderate nuclear atypia and/or scattered small foci of coagulative tumor necrosis).

Purpose:

- To describe clinicopathologic features and genetic findings in metastatic type A thymoma

Methods:

- All cases independently reviewed by 3 TET-expert pathologists
- Comparative genomic hybridization (CGH).
- Sequencing of TP53 transcript variant one, exons 5-8 in 2 cases with losses on chromosome 17

Results:

- 5 metastatic type A thymomas (2 atypical type A thymomas)
- All Masaoka stage IVb with metastases to lung at presentation
- Cases 1-3 - type A thymoma. No mitoses (although 1 case did not have original resection specimen available for review). 2 patients alive after 12 months (no f/u on case 3), Ki-67 LI 5-10%; tumor size, 2.8-7 cm, age 64-72 years
- Cases 4, 5 – atypical type A thymoma. 4 mites in case 4, necrosis in case 5; Ki-67 LI <5-12.5%, 1 patient alive after 45 months (no f/u on case 5), tumor size, 7 cm (not available in case 5), age 66, 67 years
- CGH: partially recurrent alterations in 4 cases (with or w/o atypical morphology):
- gains on chr 17q (n=2 typical), 19 (n=2 typical, n=1 atypical), 22q (n=1 atypical), 1q (n=1 atypical)
- loss on chr 17q (n=2 typical), 22q (n=1 atypical)

Take Home Message:

- Type A thymomas (as all other thymomas) are considered malignant!
- Metastatic type A thymomas have tendency to slightly higher genetic instability than what was found previously in type A thymomas (although no direct comparison in this study)
- Possible implications for the management and treatment of these tumors as the low proliferation rate and the still few identified chromosomal alterations may predict poor response to conventional adjuvant therapies such as cisplatin-based chemo.

Kwon D et al. MET exon 14 skipping mutation in triple-negative pulmonary adenocarcinomas and pleomorphic carcinomas: An analysis of intratumoral MET status heterogeneity and clinicopathological characteristics. Lung Cancer. 2017. 106:131-7

Background:

- MET-receptor TK – activated upon binding to hepatocyte growth factor/scatter factor ligand → promotion of cell survival, proliferation, angiogenesis, invasion, metastasis
- MET alterations (protein overexpression, gene amplification) - more aggressive NSCLC
- MET considered as potential therapeutic target for NSCLC (HGF antagonists, anti-MET monocloncal antibodies, MET TKIs); recent phase III trial with anti-MET antibody failed
- *MET* mutations in NSCLC usually involve the splicing sites adjacent to exon 14 which precipitate exon 14 skipping → sustained activation of MET
- NSCLC patients with MET exon 14 skipping mutation responded to MET TKIs
- *MET* mutations leading to exon 14 skipping rarely occur in NSCLC (3%)

Purpose:

- To elucidate clinicopathological characteristics of patients with NSCLC with *MET* mutation
- To evaluate protein expression and gene amplification in *MET*-mutated NSCLC
- To examine the intratumoral heterogeneity of MET alterations in *MET*-mutated NSCLC.

Methods:

- Surgical resection specimens of pulmonary adenoCa (EGFR-/ALK-/KRAS-) and pleomorphic Ca (irrespective of mutation status), No neoadjuvant chemotherapy.
- All of Korean descent
- MET exon 14 skipping mutations by quantitative RT-PCR
- *MET* mutation and gene copy numbers also evaluated in microdissected tissues from tumor areas with heterogeneous MET expression.

Results:

- 102 adenoCa, 45 pleomorphic Ca
- *MET* mutations in 8.8% (9/102) of adenoCa; Patients with *MET* mutation were older (71.6 vs 64.2 years), tended to be female and never smoker; no difference in TNM stage or outcome; all *MET*-mutated adenoCa - acinar predominant, most with associated lepidic patterns
- *MET* mutations in 20% (9/45) of pleomorphic Ca; Patients with *MET* mutation tended to be male and ever-smoker; no difference in TNM and outcome. The carcinoma component of *MET*-mutated pleomorphic Ca – mostly adenoCa of acinar pattern (8/9); sarcomatous component – mostly spindle cell pattern; *EGFR* mutation in 9.3% (4/43) w/o *MET* mutation;
- Heterogeneous MET expression in 5/9 *MET*-mutated adenoCa and 6/9 mutated pleomorphic Ca. *MET* mutation detected by qRT-PCR in all areas irrespective of IHC intensity that were sampled from 5 cases with heterogeneous MET expression in *MET*-mutated adenoCa
- *MET* gene amplification only detected in tumor areas expressing high MET protein levels in *MET*-mutated adenoCa.

Take Home Points:

- *MET*-mutation might be therapeutic target in elderly patients with adenoCa of acinar subtype
- *MET* mutation does not seem to differ with ethnicity as findings are similar in western literature

Patterson KC. Interstitial lung disease in the elderly. Chest; 2017. 151(4):838-44.

Background:

- IPF rare at < 50 yo; associated with telomerase mutations – related to premature aging?
- Not much else is known about the epidemiology of ILD in the elderly.

Purpose:

- To describe diagnoses, clinical characteristics, and outcomes of elderly patients.

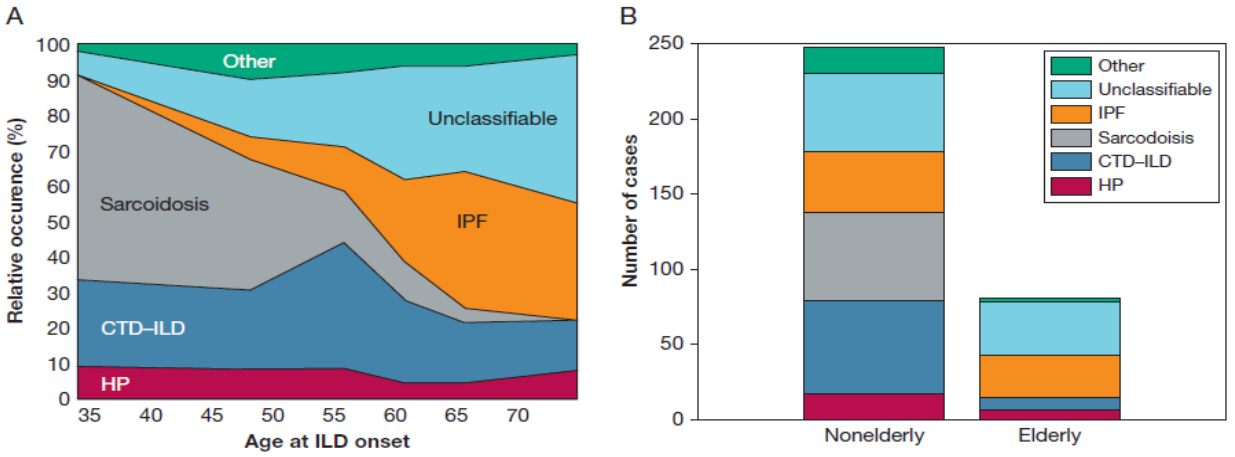
Methods:

- Subjects from a prospective cohort study of ILD who were ≥ 70 years
- Diagnoses from multidisciplinary review (radiologist blinded to clinical info; only few had SLB, explant or autopsy; PFTs, 6-min walk test; clinical review) – 2 pulmonologists, 1 thoracic radiologist, 1 pulmonary pathologist
- IPF diagnosed according to ATS/ERS/JRS/LATA criteria.
- Control group = non-elderly

Results:

- 80 of 327 (24%) patients were elderly; 94% white, 68% men.
- Most common diagnoses in elderly: unclassifiable ILD (45%), IPF (34%), CTD-ILD (11%), HP (8%).
- Most elderly (74%) with unclassifiable ILD had imaging pattern inconsistent with UIP including patients with inadequate data (mainly lack of SLB) (59%) or discordant data (15%)
- No differences in follow up PFT or 3-year mortality between nonelderly and elderly combined or in a subgroup analysis of those with IPF.

Characteristic	Nonelderly (n=247)	Elderly (n=80)	P-value
Mean age at ILD onset	54	76	<0.001
Male sex, %	47	68	<0.001
Ancestry, White (%) Black Other	69 23 8	94 1 3	<0.001
Surgical lung bx, %	17	9	0.09
Mean walking distance, m	370	320	0.02
Hypoxemia, %	26	40	0.01



Limitations:

- Only few patients had SLB in elderly- risk to benefit ratio of a SLB in older patients is often unfavorable because rates of comorbidities and surgical risks are higher.
- Follow up not available in all patients
- Possible referral bias (cohort might be enriched for patients with atypical features of ILD)
- Small sample size

Take Home Points:

- Majority of elderly have non-IPF ILD $\rightarrow$ every patient with new-onset ILD needs to be surveyed for exposures and findings of CTD, regardless of age.
- Although the effect of ILD may be more pronounced in the elderly due to reduced global functionality, ILD was not more severe or aggressive in this group.

Articles of Neoplastic Lung/Mediastinal Disease for Notation

Cox ML et al. The role of extent of surgical resection and lymph node assessment for clinical stage I pulmonary lepidic adenocarcinoma: an analysis of 1991 patients. 2017. JTO. 12 (4):689-96.

Background:

- Lung adenoCa with lepidic growth are more accepted as presenting at earlier stages and behaving less aggressively than other adenoCa subtypes – is sublobar resection sufficient?

Purpose:

- To examine the association of extent of lung resection, pathologic nodal evaluation, and survival for patients with clinical stage I (cT1–2N0M0) adenoCa with lepidic histologic features in the National Cancer Data Base (NCDB).

Methods:

- NCDB – retrospective database of American Cancer Society and American College of Surgeons Commission on Cancer – collecting data from >1500 cancer facilities in US
- Inclusion: all patients treated with lobectomy or sublobar resection for clinical stage I lepidic disease, 2003-2006; 6th AJCC staging system used

Results:

- 1991 patients (1544 [77.5%] lobectomies, 447 sublobar resections – 374 (83.7%) wedge resection, 73 segementectomies).
- Patients with sublobar resection were older (median 70 vs 69 years), more often female (68.9% vs 63%), higher comorbidity scores, smaller tumors (median diameter 1.6 vs 2.4 cm), lower T stage (proportion of T1 disease, 80.5% vs 67.6%).
- Upstaging because of + LN in 6% of lobectomies (LN sampling in 94.6% of cases) and 3.5% of sublobar resection (LN sampling performed in 45%)
- Lobectomy had better OS than sublobar resection in univariate (median 9.2 versus 7.5 years, 5-year survival 70.5% vs 67.8%) and multivariate analysis.
- In patients with sublobar resection – LN sampling vs no sampling had better OS (median not reached vs 6.5 years) and 5-yr survival (71.1% vs 65.1%).
- No survival difference between lobectomy and sublobar resection with LN sampling but worse survival for sublobar resection w/o LN sampling; in multivariable analysis including lobectomies and sublobar resection with LN sampling – extent of resection was not associated with survival

Limitations:

- No disease-free survival, No molecular data
- No knowledge whether some patients had multiple nodules
- Retrospective, non-randomized, reasoning for treatment decisions unclear
- Segmentectomies and wedge resections lumped together (too few segmentectomies)
- Degree of lepidic pattern unclear; AIS, MIA, invasive adenoCa, all lumped together

Take Home Points:

- LN evaluation in sublobar resection improves survival (LN sampling therapeutic?; patients with +LN receive adjuvant therapy?; selection bias of patients in regards to surgery?)
- Data from randomized trials still awaited

Pfister F et al. Vascular architecture as a diagnostic marker for differentiation of World Health Organization thymoma subtypes and thymic carcinoma. Histopathol. 2017;70:693-703.

Background:

- Blood vessel type (immature, intermediate, mature) and endothelial cell proliferation correlates with invasiveness of thymomas; Tumor angiogenesis correlates with invasiveness
- VEGF expression is associated with invasiveness of TET

Purpose:

- To compare vascularization, pericyte coverage and expression of angiogenic growth factors in different WHO subtypes of thymoma

Methods:

- Vascular density, diameter, architecture and expression of SMA, PDGFRb, VEGFR1 and VEGFR2 in thymomas and TSQCCs
- IHC, quantitative morphometry, tumor vessel isolation by trypsin digestion.
- Quantitative RT-PCR for expression levels of angiopoietin 1 (Ang-1), Ang-2, VEGF-A, PDGF-B and Hif-1a

Results:

- Vascular density from densest to least: thymoma (A>AB>B [B1>B2, B3]) > TSQCC; B>normal thymic cortex and medulla
- Vascular diameter: A and AB similar to thymic cortex; larger in B2 and B3 thymomas, TSQCC; comparable to thymic medulla; B thymomas had larger vessels than A and AB thymomas; within B thymomas, stepwise increase from B1 to B3
- SMA and PDGFRb expression high in A thymomas; strong but concentrated in larger, less numerous vessels in B thymomas. Total staining intensities of SMA and PDGFRb higher in A thymomas than B1 and B2 thymomas; almost identical in A and B3 thymomas; highest expression in TSQCC; ratio of SMA and PDGFRb to vascular density low in A and AB, high in B2/B3 thymomas and TSQCC
- VEGFR1 expression in endothelial cells of all TET; most expression in tumor epithelial cells; high in A, B2, B3 and TSQCC; low in AB and B1
- VEGFR2 strongly expressed in endothelial cells of all TET; in tumor epithelial cells strong in B3 and TSQCC; weak in A and AB; lowest in B1; highest in B3
- Vascular architecture in A and B3 thymomas: A: tumor vessels smooth and straight capillary tubes, composed of capillary-like endothelial cells and compact pericytes; B3: tortuous vessels, with irregular diameters and disorganized arrangement of endothelial cells; pericytes are irregularly arranged
- mRNA of Ang-2, but not of Ang-1, up-regulated in all TET, highest levels in A lowest in B3 thymomas. PDGFb and VEGF upregulated in A, AB, B3 but not in B2
- In TSQCCs, Ang-1 and VEGF were the predominantly up-regulated growth factors. Hif-1a was only up-regulated in B3 thymomas and TSQCCs.

Take Home Points:

- Thymomas and TSQCCs differ in vascular architecture and expression of angiogenic growth factors.

- Quantitation of vascular density and diameter and expression of SMA and PDGFRb might serve as additional criteria for differentiation of WHO thymoma subtypes; especially A vs B3
- Possible different mechanisms of tumor angiogenesis and functional differences of tumor vessels of major thymoma subtypes and TSQCCs.

Dimmler A et al. Molecular analysis of *BRAF* V600E mutation in multiple nodules of pulmonary Langerhans cell histiocytosis. Virchows Arch. 2017; 470:429-35.

Background:

- PLCH - recently shown to harbor *BRAF*V600E mutations in a significant subset of cases
- LCH cells can also harbor genetic alterations in the RAS-RAF-MEK pathway
- Inhibition of *BRAF* by vemurafenib or dabrafenib used in *BRAF*V600E mutated tumors

Purpose:

- To better understand PLCH pathogenesis and to analyze *BRAF*V600E mutation in multiple PLCH nodules of a single patient

Methods:

- *BRAF* mutation analysis by pyrosequencing (microdissected nodules, limit of detection 10.74%) and allele-specific quantitative PCR (AS-PCR) (sensitivity limit, 0.1%)

Results:

- 38 PLCH nodules of 9 patients
- 5 of 9 (55.5%) men; mean age, 54.4 years (range, 29-75); nodule size, 0.1 – 0.8 cm
- All nodules showed typical PLCH morphology, expressed S100 and CD1a
- Pyrosequencing: *BRAF* V600E mutations in 16/38 (42%) nodules
  Concordant mutation status in all nodules in 6 of 9 (66.7%) patients
  In all 13 nodules of 3 patients – no mutation
  In all 7 nodules of 3 patients – *BRAF*V600E mutation
  In 3 patients – mutated and non-mutated nodules within a patient including 1 patient with predominant mutated nodules (7/9), 1 patient with predominant non-mutated nodules (6/7), 1 patient equal number of mutated and non-mutated nodules
  No histologic difference between mutated and non-mutated nodules
- AS-PCR: *BRAF* V600E mutations in 31/37 (84%) (AS-PCR) nodules
  In all nodules of 7/8 (87.5%) patients – concordant mutation status
  In all 23 nodules of 6 patients – *BRAF*V600E mutation
  In 5 nodules of 1 patient – no mutation
  In 1 patient – predominant mutated nodules (8/9)

Limitations:

- IHC for *BRAF*V600E mutation has been shown to correlate with mutation status in most cases – however, IHC was not performed in this study

Take Home Points:

- Sensitivity of the method used is crucial in analyzing *BRAF* mutation status
- AS-PCR is more sensitive in detecting *BRAF* V600E mutations than pyrosequencing
- *BRAF* mutation is frequent and might play a key role in the pathogenesis of PLCH
- *BRAF* mutation might guide treatment with vemurafenib or dabrfenib

Dagaonkar RS et al. Significance of coexistent granulomatous inflammation and lung cancer. J Clin Pathol. 2017; 70:337-41.

Background:

- Granulomatous inflammation in neoplastic disease thought to be due to immunological hypersensitivity to antigens derived from tumor cells
- Occurs in 4.4% of cancers including 1.3 – 11% of lung cancers, 13.8% of Hodgkin lymphoma, 50% of seminomas, 0.4% of sarcomas
- In Singapore, annual incidence of lung cancer and pulmonary TB similar

Purpose:

- To identify the prevalence, clinicopathological features, treatment outcomes and prognosis in patients with coexisting granulomatous inflammation undergoing curative lung resection for lung cancer, in a TB-endemic country.

Methods:

- Patients with lung cancer undergoing curative resection in a tertiary centre in Singapore.

Results:

- 127 cases, median age, 68 years; 58.2% males; overall median survival, 451 days.; 18 (14%) patients died at median duration of 271 days after surgery; 64.5% adenoCa.
- 19 of 127 (14.9%) patients who underwent lung resection for cancer had coexistent granulomatous inflammation in the resected specimen with or w/o involving LNs.
- Ziehl-Neelsen stain negative in all 19 cases; 2 cases had history of NTM, 2 of TB
- 1 case in non-granulomatous group had history of NTM, 2 of TB
- No differences in age, gender, location of cancer, radiological features, type of cancer, chemotherapy, history of TB or survival in patients with or without coexistent granulomatous inflammation.

Limitations:

- No mention whether necrotizing or non-necrotizing granulomas
- Findings might be different in countries with even higher TB number

Take Home Points:

- Coexistence of granulomas with lung cancer not unusual
- Incidental detection of granulomatous inflammation in patients undergoing lung resection for cancer, even in a TB-endemic country, may not require any intervention.
- Such findings may be due to either mycobacterial infection in the past or ‘sarcoid reaction’ to cancer. – In US – coexistence with fungal infection (Histoplasma, Coccidiomycosis) likely more prevalent
- Although AFB cultures should be performed (in Singapore), tumor treatment can get started as it does not affect their outcome adversely – chemo is tolerated safely in these patients

Augert A et al. Small cell lung cancer exhibits frequent inactivating mutations in the histone methyltransferase KMT2D/MLL2: CALGB 151111 (Alliance). JTO. 2017;12(4):704-13.

Background:

- SCLC: Mutations in *TP53* and *RB1*-tumor suppressor genes; *MYC* family members are frequently amplified; a vast smoking-induced mutational burden

Purpose:

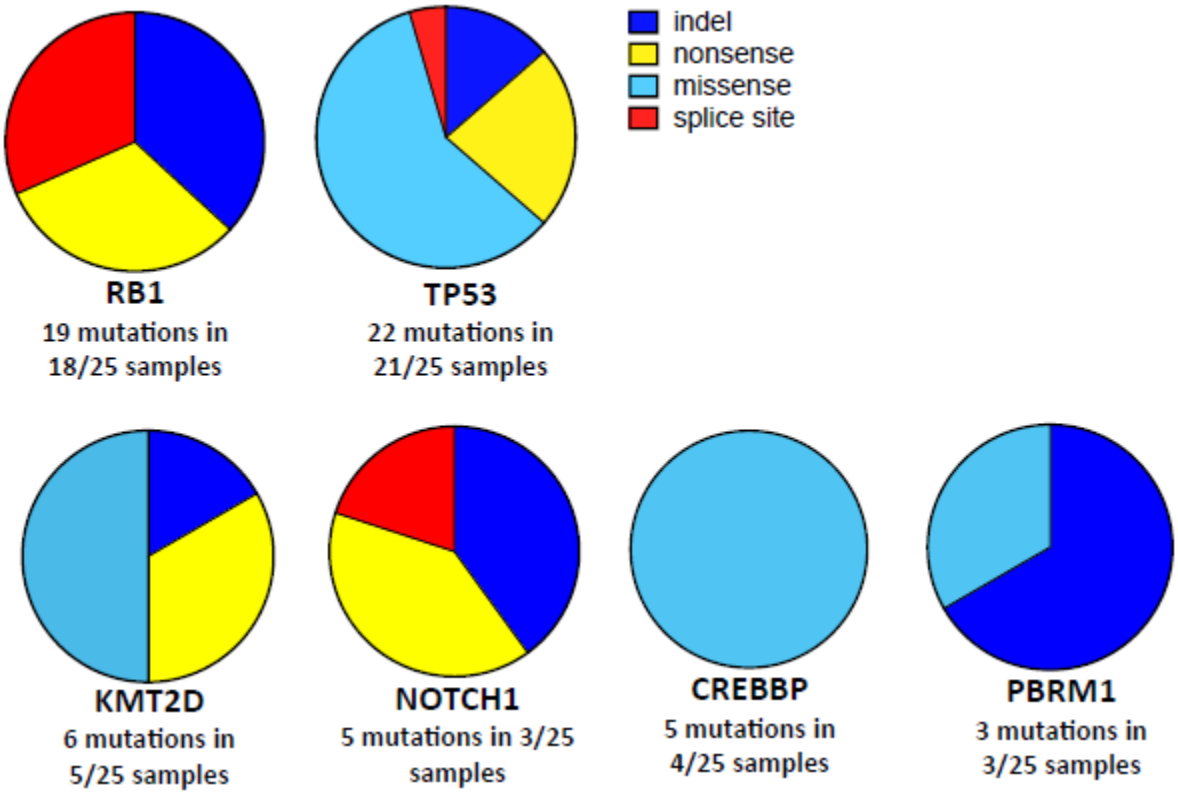
- To expand our knowledge on driver mutations in SCLC

Methods:

- Whole genome sequencing of 18 primary resected SCLCs (mostly limited stage) and matched normal controls (adjacent lung tissue or blood); 7 human SCLC cell lines (extensive stage) with matched EBV-transformed lymphoblast cells derived from same patients
- Resequenced a panel of genes across 40 SCLCs and 48 cell lines.

Results:

- 224 protein-altering mutations in tumors and 283 in cell lines identified.
- C:G>A:T transversions and C:G>T:A transitions = most prevalent alterations in cell lines and primary tumors = alterations that are associated with carcinogens from tobacco smoke



- Frequent mutations in *the lysine methyltransferase 2D* gene (*KMT2D*) (a.k.a. MLL2), a key regulator of transcriptional enhancer function. *KMT2D* exhibited truncating nonsense/frameshift/splice site mutations in 8% of SCLC and 17% of SCLC cell lines.
- Resequencing - *TP53* and *RB1* are the most frequently mutated genes, *KMT2D* and *NOTCH1* mutations also identified; Identified mutations in other genes associated with transcriptional

enhancer control, including *CREB* binding protein gene (*CREBBP*), E1A binding protein p300 gene (*EP300*), and *chromodomain helicase DNA binding protein 7* gene (*CHD7*); mutations in additional chromatin remodeling genes such as *polybromo 1* gene (*PBRM1*).→ also found that chromatin-modifying genes are frequently mutated in SCLC

- *KMT2D* mutation in SCLC cell lines was associated with reduced expression or loss of KMT2D protein levels in most cell lines and reduced monomethylation of histone H3 lysine 4, a mark associated with transcriptional enhancers.

Take Home Points:

- *KMT2D* might be one of the major mutated genes in SCLC, and they point to perturbation of transcriptional enhancer control as potentially contributing to SCLC.
- An inhibitor of the H3K4 demethylase LSD1 is being tested in a clinical trial for SCLC.

Krencz et al (Andras Khoor – coauthor). Expression of mTORC1/2-related proteins in primary and brain metastatic lung adenocarcinoma. Human Pathol. 2017;62:66-73.

Background:

- Dysregulated activity of mammalian target of rapamycin (mTOR) might influences the metastatic potential of various tumors
- PI3K/AKT/mTOR pathway can be activated by EGFR, insulin-like GFR, VEGF, PDGF receptor membrane receptor families and various mutations in *PI3KCA* gene – activation may lead to tumor progression.
- Raptor and Rictor – elements of mTORC1 and mTORC2; targets of mTORC1 – S6K, 4EBP-1 protein through S6 phosphorylation
- Expression of p-mTOR and p-S6 correlates with mTORC1 activity, expression of p-mTOR and Rictor correlates with mTORC2 activity.
- mTORC1 is sensitive, mTORC2 is resistant to rapamycin
- next gen mTOR inhibitors (everolimus, temsirolimus) can penetrate the blood-brain barrier.

Purpose:

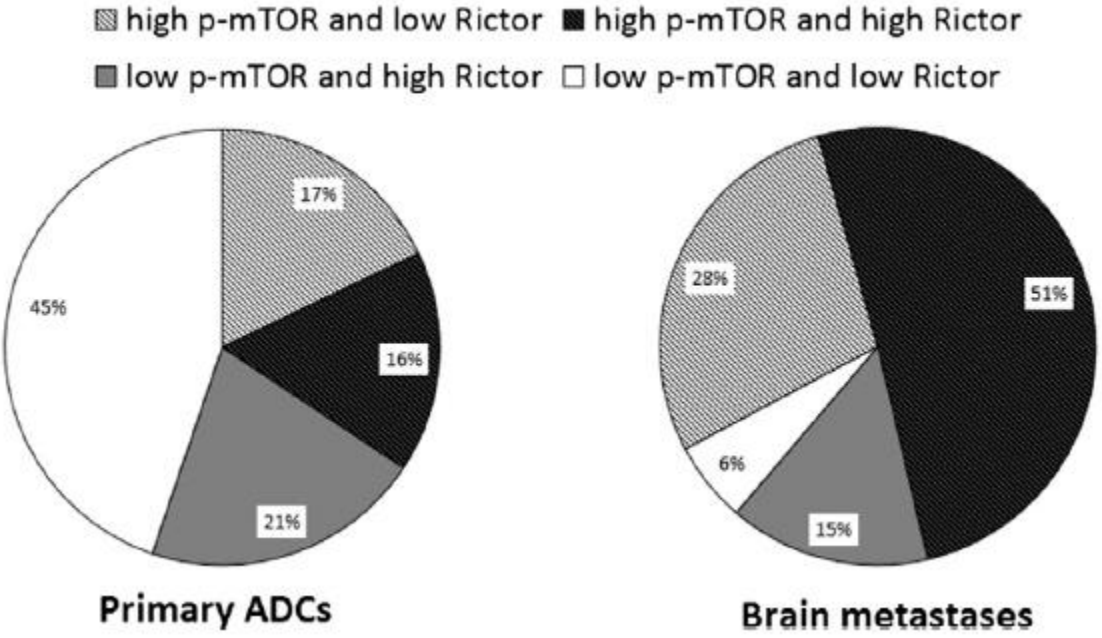
- To identify potential predictive biomarkers to targeted therapy by mTOR inhibitors

Methods:

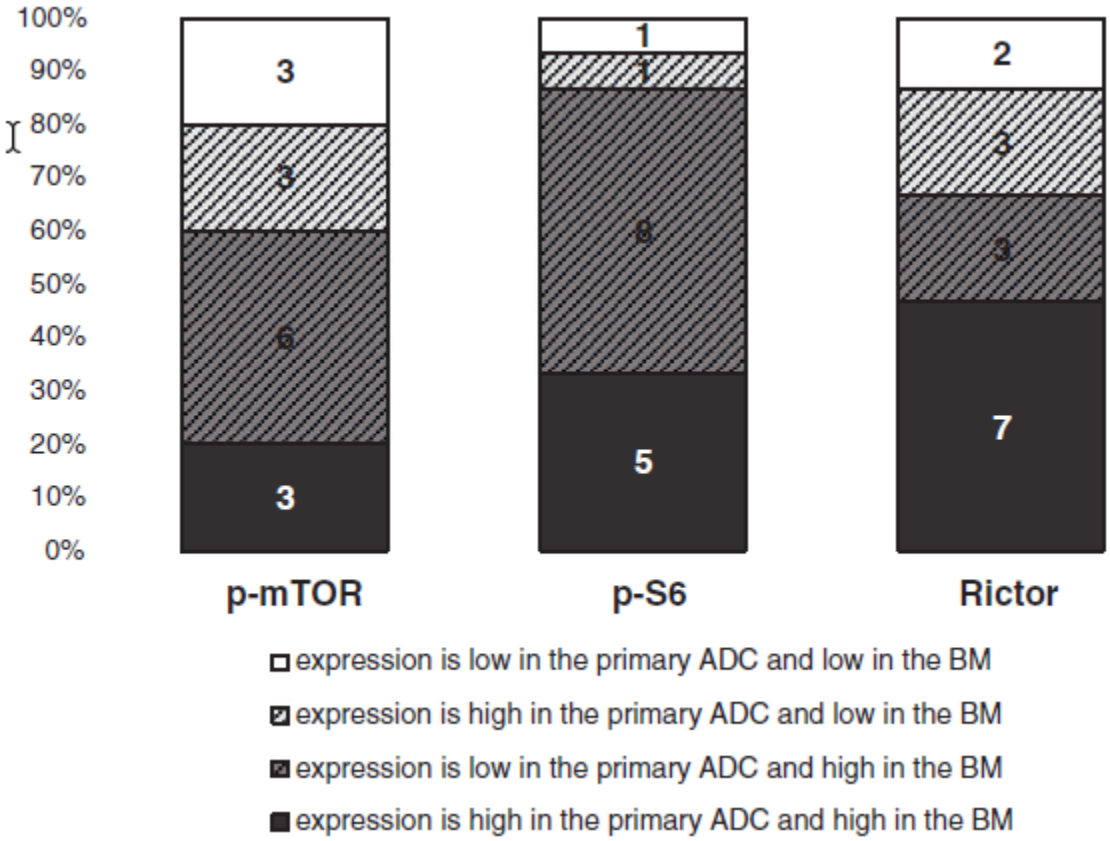
- Expression of mTOR-related proteins (mTORC1: p-mTOR, p-S6; mTORC2: p-mTOR, Rictor) in primary (n = 67) and brain mets (n = 67) of lung adenoCa, including 15 paired tissue samples by IHC; all resection specimens
- TMAs with double or triple cores
- IHC semiquantitative using H score.

Results:

- Increased p-mTOR, p-S6, and Rictor expressions in 34%, 33%, and 37% of primary adenoCa and in 79%, 70%, and 66% of brain mets.
- Expression of these markers higher in brain mets vs primary adenoCa



- Rictor expression higher in primary adenoCa of the paired cases with brain mets as compared with primary adenoCa without brain mets (67% vs 28%). No other correlations between mTOR activity and clinicopathological parameters.



Take Home Points:

- Potential efficacy of mTOR pathway inhibition in both primary and brain metastatic lung adenoCa with high mTOR activity
- The mTOR inhibitor treatment could slow down the tumor growth
- Stratifying patients by mTORC1/2 activity may lead to better patient selection and a more effective targeted therapy

Gniadek et al. Heterogeneous expression of PD-L1 in pulmonary squamous cell carcinoma and adenocarcinoma: implications for assessment by small biopsy. Mod Pathol. 2017; 30:530-8.

Background:

- PD-L1 expression is geographically heterogeneous within many tumors

Purpose:

- To identify whether small tissue samples (biopsies) might be sufficiently representative of PD-L1 expression for evaluating this marker in lung cancer tumors.

Methods:

- Score PD-L1 expression by IHC in TMA cores from 79 lung SQCC and 71 lung adenoCa.
- 4 cores per case, 0.6mm diameter of core
- PD-L1 clone SP142; membranous staining of tumor cells - % + tumor cells
- Scores: 0%, 1-5%, 5-10%, 10-20%, 20-50%, >50%; if not concordant between 3 pathologists performing a visual estimate, cells were manually counted
- For sensitivity and specificity different thresholds were used: >1%, >10%, >50%
- Sensitivity at each threshold level = # cores positive divided by the total # cores from those tumors that were determined to be true + for PD-L1 at that threshold level.
- Specificity at each threshold level = 1 minus false-positive rate based on cores scored as positive at particular threshold in tumors that were not true positive at that threshold.
- Gold standard – TMA cores if all cores showed same result or whole tissue section

Results:

- Substantial inconsistencies for % cells + for PD-L1 among different TMA cores in many cases of both adenoCa and SQCC
- Variable scoring seen at both high and low levels of PD-L1 expression
- Cases with discordant results were evaluated on full-face sections - discordant results that were identified among different TMA cores reflected geographic variation of PD-L1 expression in those tumors.
- 39% of adenoCa + for PD-L1 in at least 1 TMA core, but only 11% of cases + in >50% of tumor cells for all TMA cores.
- 54% of SQCC + in at least 1 TMA core and 22% + in >50% of tumor cells for all TMA cores
- High variation in staining between cores from same case:
  <50% of tumor cells + PD-L1 in 5/13 adenoCa and 6/23 SQCC that could be classified as + in >50% of cells for at least 1 TMA core. 21/100 cases (adenoCa and SQCC combined) that showed no PD-L1 staining in ≥ 1 TMA did also have some tumor cells + for PD-L1 in at least 1 TMA core.
- Highly heterogeneous staining across different areas of tumor

Overall score threshold	*Sensitivity*	*Specificity*
Adenocarcinoma		
>1%	87.2% (89/102)	99% (1–1/102)
>10%	100% (74/74)	100% (1–0/74)
>50%	85.1% (40/47)	97.9% (1–1/47)
Squamous cell carcinoma		
>1%	89.9% (151/168)	99% (1–1/168)
>10%	100% (109/109)	100% (1–0/109)
>50%	94.9% (75/79)	97.5% (1–2/79)

Take Home Points:

- Many cases of lung cancer could be inaccurately or variably scored for PD-L1 expression with a single biopsy sample. Accordingly, lung cancer patients can be inconsistently classified for PD-L1 expression status, particularly when a threshold for the % of positive cells is used to determine eligibility for checkpoint blockade therapy.
- In a substantial percentages of lung cancers, the classification of PD-L1 staining with small biopsy samples could be highly inconsistent, depending on the particular area of tumor sampled which is due to heterogeneous expression of PD-L1 across the tumor

Articles of Non-Neoplastic Lung Disease for Notation

Best DH et al. EIF2AK4 mutations in patients diagnosed with pulmonary arterial hypertension. Chest. 2017; 151(4):821-8

Background:

- Differentiating PVOD and PCH from idiopathic PAH (IPAH) or heritable PAH (HPAH) is clinically important
- Mutations in *eukaryotic translation initiation factor 2 alpha kinase 4 (EIF2AK4)* cause heritable PVOD and PCH
- According to current guidelines, the identification of homozygous or compound heterozygous *EIF2AK4* mutations is sufficient to confirm PVOD or PCH. The absence of *EIF2AK4* mutations does not exclude PVOD or PCH.
- The identification of biallelic *EIF2AK4* gene mutations may allow identification of PVOD or PCH among patients diagnosed with IPAH or HPAH.

Purpose:

- To describe the frequency of pathogenic *EIF2AK4* mutations in patients diagnosed clinically with IPAH or HPAH.

Methods:

- Sanger sequencing and deletion/duplication analysis to detect *BMPR2* gene mutations
- *BMPR2* mutation-negative patients were sequenced for mutations in *ACVRL1, ENG, CAV1*, and *KCNK3* genes; genes that are known to cause HPAH.
- Patients that were negative for all these genes were sequenced for mutations in *EIF2AK4.*

Results:

- 81 patients with IPAH (n = 72) or HPAH (n = 9).
- Both groups predominantly comprised of women (IPAH, 82%; HPAH, 56%) at an age at diagnosis of 39+/-13.4 (IPAH) and 45.5+/-16.4 (HPAH). Most white ethnicity (IPAH, 85%; HPAH, 89%)
- The inheritance patterns of 7/9 families was compatible with the inheritance patterns of HPAH (ie, autosomal dominant with incomplete penetrance). 2 family pedigrees were compatible with either autosomal dominant with incomplete penetrance or autosomal recessive inheritance
- *BMPR2* mutations were most commonly identified: 8/72 (11.1%) IPAH, 6/9 (66.7%) HPAH.
- Other mutations in patients with IPAH: 1 pathogenic *ENG* mutation, 1 likely pathogenic variation in *ACVRL1,* 1 likely pathogenic *KCNK3* mutation and a variant of uncertain clinical significance. No pathogenic CAV1 variant.
- 66 patients were negative for *BMPR2, ACVRL1, ENG, CAV1, KCNK3*
- A homozygous *EIF2AK4* mutation (c.257þ4A>C) was identified in 1/9 (11.1%) HPAH that did not have any other mutation. This mutation was homozygous in 2 sisters who had severe PAH and died at 64 and 62 yo. The inheritance pattern of PH in the family suggested either autosomal recessive inheritance (which would be consistent with the inheritance pattern of PVOD or PCH due to *EIF2AK4* mutations) or autosomal dominant inheritance with incomplete penetrance (which would be consistent with the inheritance pattern of PAH due to mutations in *BMPR2, ACVRL1, ENG, CAV1,* or *KCNK3*).
- None of the 72 patients with IPAH had biallelic *EIF2AK4* mutations.

- 2 additional *EIF2AK4* gene variants, 1 likely pathogenic, 1 of uncertain clinical significance, identified in two unrelated patients with IPAH. Both were heterozygous mutations.

Take Home Points:

- Pathogenic biallelic *EIF2AK4* mutations are rarely identified in patients diagnosed with HPAH. Identification of pathogenic biallelic *EIF2AK4* mutations can aid clinicians in differentiating HPAH from heritable PVOD or PCH.

Reviews

Calvayrac O et al. Molecular biomarkers for lung adenocarcinoma. Eur Respir J 2017; 49: 1601734

- Good reference for techniques used to discover each of the candidate oncogenes and their prevalence in NSCLC. Provides some good algorithms on techniques for obtaining tissue, cytology specimen and/or liquids for tumor sampling
- Provides a decision tree algorithm for tumor sampling technique
- Outline of the epidemiological features of the major oncogenes and ways in which their identification can determine therapeutic strategies.
- Shows nice graphs with mutation rates of NSCLC based on geographic regions
- Describes methods to detect molecular alterations including screening assays (Saenger sequencing, pyrosequencing, high resolution melting, next generation sequencing), targeted assays (Competitive allele-specific TaqMan® PCR, Therascreen® mutation kits, Cobas® kit, Peptide nucleic-acid clamp technology, BEAMing, digital PCR); molecular methods used to detect gene rearrangements (FISH, IHC, RT-PCR)
- Lists biomarkers (biology, incidence, therapy, clinical trials) for targeted therapy including EGFR, KRAS, ALK, PI3K/AKT/mTOR pathway, HER2, ROS1
- Discusses recently identified mutations-emerging mutational targets (RET, MET exon 14 mutations)

Methods of tumor sampling

TABLE 1 Advantages and pitfalls of the different methods of cytological sampling for molecular profiling of nonsmall cell lung cancer

Sample	Diagnostic accuracy	Advantages	Pitfalls
EBUS-TBNA	Sensitivity >95%	Histologic diagnostic, staging and molecular profile at the same time	Relatively invasive
cfDNA	Good sensitivities >95% (*EGFR, KRAS, BRAF*)	Noninvasive Rapid, simple monitoring Early detection or acquired resistance (*EGFR* T790M)	Low number of mutated alleles among wild-type alleles
CTCs	Sensitivity 78% (*KRAS*) Sensitivity 92% (*EGFR*)	Noninvasive Possibility of: - Cytomorphological analysis - FISH (*ALK*) - ICC (*ALK*)	Expensive and laborious Lack of standardisation Multiplicity of methods
Pleural fluid	88% sensitivity	Possibility of multiplexed molecular testing if previously centrifuged	Contamination by haematopoietic cell DNA
Bronchoalveolar lavage	Sensitivity 16% (Sanger) to 81% (NGS)	Good sensitivity with NGS	Low number of tumour cells Poor sensitivity with conventional sequencing methods

EBUS-TBNA: endobronchial ultrasound transbronchial needle aspiration; cfDNA: circulating free DNA; CTCs: circulating tumour cells; FISH: fluorescence *in situ* hybridisation; ICC: immunocytochemistry; NGS: next-generation sequencing.

Decision tree algorithm for tumor sampling technique

- Tissue should be 1st choice

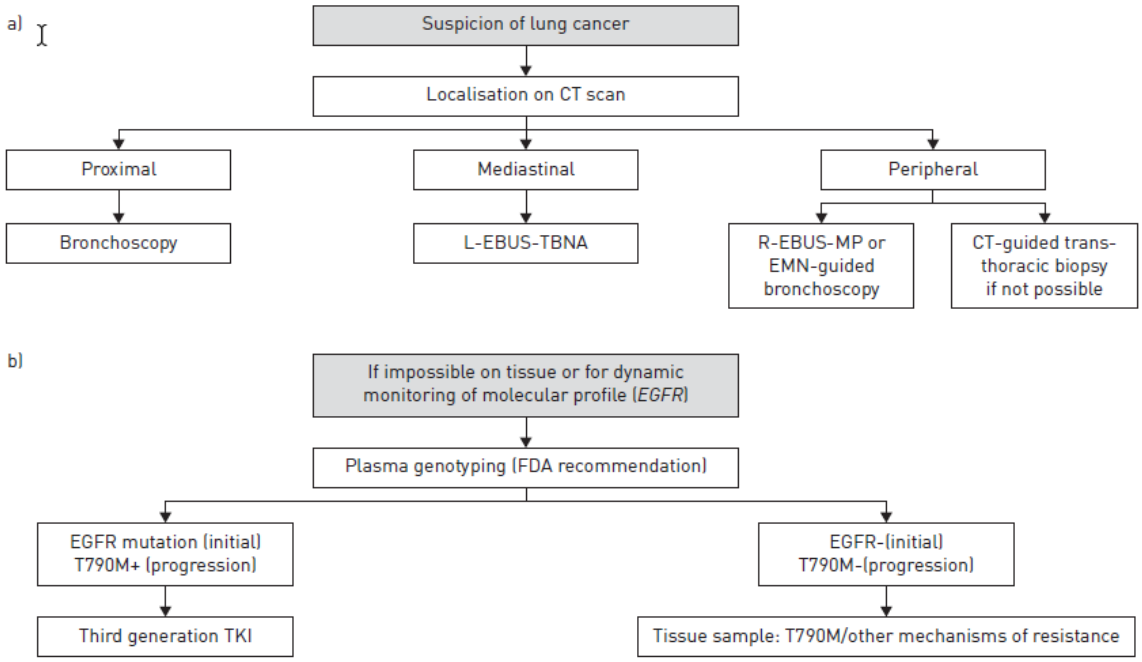


FIGURE 1 Decision tree algorithm for tumour sampling and molecular profiling at initial diagnosis (a) and during progression (b). CT: computed tomography; L-EBUS-TBNA: linear endobronchial ultrasound transbronchial needle aspiration; R-EBUS-MP: radial endobronchial ultrasound miniprobe; EMN: electromagnetic navigation; FDA: US Food and Drug Administration; EGFR: epidermal growth factor receptor; TKI: tyrosine kinase inhibitor.

TABLE 2 Incidence and characteristics of the main biomarkers for nonsmall cell lung cancer

Target	Biology	Caucasian patients %	Approved treatments	Clinical trials
EGFR	Mutation	10–15	Gefitinib, erlotinib, afatinib osimertinib (second-line if T790M)	Rociletinib (second-line if T790M)
ALK	Translocation	3–5	Crizotinib	Ceritinib, alectinib, brigatinib, lorlatinib
BRAF	Mutation	2	NA	Vemurafenib, dabrafenib, dabrafenib+trametinib
ROS1	Translocation	1	Crizotinib	Ceritinib, Lorlatinib
HER2	Mutation	1	NA	Trastuzumab, afatinib, neratinib
KRAS	Mutation	20–25	NA	Trametinib, selumetinib, abemaciclib
PI3K	Mutation	2	NA	PI3K inhibitors, mTOR inhibitors
MET	Amplification mutation	2–5	NA	Crizotinib, INC280, tepotinib
RET	Translocation	1–2	NA	Cabozantinib, sorafenib, vandetanib

NA: not applicable.

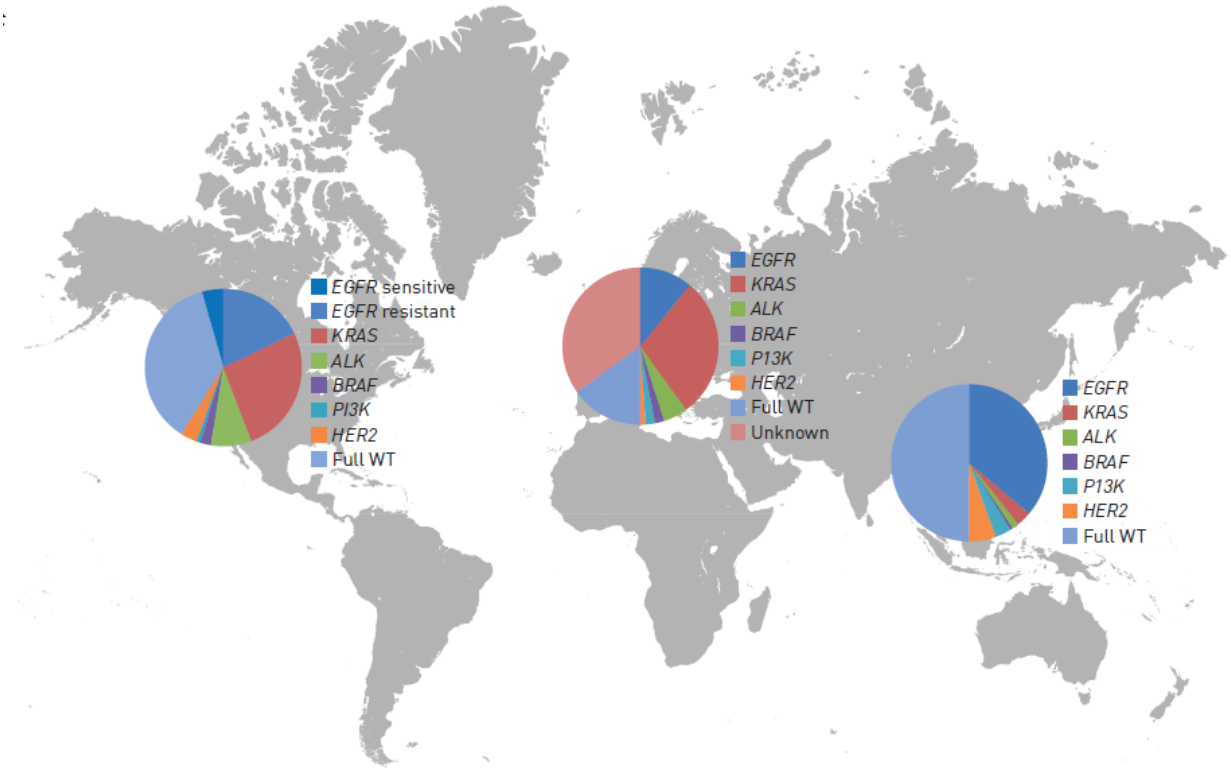


FIGURE 2 Molecular epidemiology of nonsmall cell lung cancer according to geographic origin. Asia: from Li *et al.* [46], Shan *et al.* [47] and Zhao *et al.* [48]. Europe: from Barlesi *et al.* [49], Mazieres *et al.* [50] and Mazieres *et al.* [51]. America: from Kris *et al.* [52].

Castellanos E et al. Driven by Mutations: The predictive value of mutation subtype in EGFR-mutated non–small cell lung cancer. 2017. JTO; 12:612-23

- Review of common and uncommon *EGFR* mutations, clinical significance and outcomes
- *EGFR*-mutated NSCLC is genetically heterogeneous disease; > 200 distinct mutations. Response to EGFR TKIs depends on the mutational subtype of the tumor.
- Exon 19 deletions and L858R mutations – most common, both predict sensitivity to EGFR TKIs;
- Across all EGFR mutation subtypes, treatment with an EGFR TKI showed a 63% reduction in risk for disease progression or death as compared with treatment with chemotherapy.
- Outcomes may be improved in patients with exon 19 deletions. Subgroup analyses demonstrated that patients with exon 19 deletions showed a 50% greater PFS benefit when treated with an EGFR TKI than did patients with exon 21 L858R substitutions
- Sensitizing *EGFR* mutations – most common actionable driver mutations in NSCLC, occur in approximately 10% of white patients and up to 50% of Asian patients.
- Mutations occur within the *EGFR* exons 18 to 21 (encode a portion of the EGFR kinase domain).
- Appr. 90% of patients with *EGFR*-mutated NSCLC have deletions in exon 19 or substitutions of leucine for arginine (L858R) in exon 21 of the *EGFR* gene
- 10% of patients have an uncommon *EGFR* mutation - response to EGFR TKI therapy is highly variable and depends on the mutation.
- With some exception, most mutations involving exons 18, 19, and 21 are considered predictive of sensitivity to EGFR TKI therapy,
- Mutations in exon 20 are typically resistant.
- T790M mutation - most common mechanism of acquired resistance to EGFR TKI therapy; can be de novo, as a germline mutation, and in combination with other genetic aberrations. Occur in approximately 1% of patients with NSCLC; these patients may also have a second activating *EGFR* mutation. Familial studies: patients carrying a germline T790M mutation have a high lifetime risk for development of lung cancer (up to 31% among never smoking genetic carriers).
- Proposed mechanism of adaptive resistance in NSCLC: inhibition of MAPK, a downstream signaling molecule of EGFR, leading to activation of signal transducer and activator of transcription 3 and IL-6, which promotes cell survival and ultimately resistance. Therapies targeting EGFR can immediately activate NFkB to induce an antiapoptotic signaling cascade.
- Novel biopsy-free techniques (digital droplet PCR), in which genomic testing can be performed on circulating tumor DNA, may make mutational testing more convenient
- Compared with chemotherapy, EGFR TKI treatment demonstrated a 27% greater benefit in women than in men and in never-smokers than smokers; no difference for ethnicity, age, histologic subtype, performance status

Chee J et al. Immunotherapy for lung malignancies from gene sequencing to novel therapies. Chest. 2017; 151:891-7.

- Reviews current and potential future approaches to immunotherapy
- Discusses background of immunotherapies and why they are important
- Antitumor immunity can be augmented by checkpoint blockade therapy, which removes the inhibition/brakes imposed on the immune system by the tumor.
- Checkpoint blockade therapy with anti-PD-1)/anti-PDL-1 antibodies for instance causes tumor regression in about 25% of patients with NSCLC.
- In another approach, the immune system is forced or accelerated to attack the tumor through augmentation of the antitumor response against mutations carried by each lung tumor. NGS makes this approach feasible as it identifies the specific mutations of individual lung tumor.
- Lung cancers have high mutation rates, making them logical targets for mutation-directed immune therapies.
- Review how sequencing of lung cancer mutations leads to better understanding of how the immune system recognizes tumors, providing improved opportunities to track antitumor immunity and ultimately leading to the development of personalized vaccine strategies aimed at unleashing the host immune system to attack mutations in the tumor.
- Immune recognition of lung cancer mutations
- How to determine if patients with lung cancer have immune responses against neoantigens
- T-cell recognition of mutations does occur in patients with lung cancer
- DNA sequencing and lung cancer therapy
- How neoantigen vaccination might be used as a treatment strategy for lung cancer
- Tracking neoantigen responses could generate improved lung cancer therapy
- Neoantigen therapy in the clinic (at diagnosis, T-cell testing, vaccination, combination with other therapies, testing host response to neoantigen)

Case Report

Droukas DD et al. A 28-year-old woman with branching opacity and chest pain. Chest. 2017. 151(4):e85-9.

Case:

- 28 yo female – atypical chest pain (pleuritic) and chronic cough
- Leukocytosis
- Imaging: large tubular branching opacity overlying RUL – nonenhancing on CT, surrounded by wedge-shaped area of hyperlucency pointing to the ipsilateral hilum
- Scintigraphy: no ventilation or perfusion in expanded posterior segment of RUL
  $\rightarrow$ posterior segmentectomy of RUL
- Gross: soft pliable mass surrounded by hyperexpanded pulmonary parenchyma
- Bronchi distal to mass dilated and with mucostasis

Take Home Points:

- Bronchial atresia of RUL
- Focal interruption of lobar, sublobar or segmental bronchus
- Peripheral mucus impaction – hyperinflation of distal lung parenchyma
- Apicoposterior segment of LUL most commonly affected > RUL > RML > RLL, maybe multifocal
- Likely congenital
- Most cases identified incidentally in children or adults; if symptomatic – recurrent pulmonary infections, wheezing, atypical chest pain
- Imaging – highly diagnostic – rounded, tubular or branching opacity pointing towards the ipsilateral hilum with surrounding pulmonary hyperlucency
- No FDG uptake on PET
- Scintigraphy – no ventilation or perfusion distally
- No patent lumen between distal and proximal bronchial tree
- Hyperinflation without destruction distally