Pulmonary Journal Club August 2023 (Articles from July 2023)

Presented by

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Articles for Discussion

Dagogo-Jack I et al. B-cell infiltrate in the tumor microenvironment is associated with improved survival in resected lung adenocarcinoma. JTO Clinical and Research Reports 2023;7:100527

Background/Purpose

- Biological and molecular diversity within the same stage groups
- Molecular and immunologic profile of tumors modulates survival outcomes
- To better understand the immunologic and molecular contexture for insights on drivers of heterogeneous outcomes among patients
- Comprehensive analysis of more than 1,500 resected lung ADCs from 3 distinct cohorts

Materials and Methods

- Three cohorts for analysis:
    - TCGA (cohort 1): Transcriptomic data from the USCS XENA portal
    - Other external data sets (cohort 2): Pooled cohort downloaded for array expression analysis (more crude form; no RNA-seq information)
    - MGH stage II cohort (cohort 3):
        - primary lung tumor and adjacent normal lung tissues
        - 32 patients who underwent resection of stage II lung ADC at MGH during 4/2009- 10/2016
        - Demographics, Tx hx, survival/outcome data, updated as of 11/2021
- Molecular analysis:
    - Cohort 3: Whole exome and RNA sequencings, transcriptomic analysis
    - Cohort 1 and 2: RNA expression analysis
    - Identification of B-cell subsets done on cohorts 1 and 3
- Histopathologic analysis: On MGH (cohort 3) cases, lymphocyte infiltration, histologic grade of tumor, and lymphoid aggregates

Results

- Cohort 1: TCGA resected lung ADC RNA-seq data set (stage I to IV) analyzed with "RNA-seq deconvolution algorithm" (published in Cancer Cell 2022;40:879-94.e16)
    - Divided the data set into tertiles on the basis of the degree of B cell infiltration

    - No statistically significant difference in survival across tertiles (p=0.08)
    - But if divide the group into two groups (low and high) based on 10% absolute B cells that corresponded to 66th percentile, OS was significantly higher in high B cell content group (p=0.01), but not in relapse-free survival (p=0.10)
    - No association with OS and either TMB or PD-L1 expression
- Cohort 2: Validation in microarray data sets
    - A meta-cohort of 1422 resected lung ADC captured in publicly available microarray data sets (stage I to III; 54% stage I)
    - B-cell expression that exceeded the median was considered high B-cell abundance
    - High B-cell abundance was associated with longer median OS ($p < 0.01$), consistent with findings from bulk RNA sequencing in the TCGA cohort
    - Prognostic association was apparent in all stages but only reached statistical significance in the larger stage I subgroup
- Cohort 3: MGH group of stage II resected ADC, to compare with stage I ADC predominating cohorts 1 and 2; stage III tumors were not used given their low availability and more aggressive behavior
    - Of 32 cases, 29 cases have sufficient quality to support RNA-Seq.
    - Most tumors (62%) were stage IIB; median age 69, female 62%; 7 (24%) were never smokers, 17 (55%) had adjuvant chemotherapy; 20 of 29 had driver mutations including KRAS (n=12) and EGFR (n=8)
    - Median follow up of 4.9 years (1.5-12.4) after surgery
    - Two groups on the basis of B cell infiltration per the 10% threshold
    - High B-cell content significantly associated with improved OS (p=0.04) and trend toward improved RFS (Figure 4A and B)
    - No statistically significant association with lymphoid aggregates or density of immune infiltrate (either B or T cells)
    - High B cell content was identified in ADC with or without lymphoid aggregate
    - Tumor PD-L1 expression (by RNA-seq) did not predict relapse risk or affect survival but lower TMB (by WES) was associated with longer survival
    - Impact of distinct B-cell subsets on survival outcomes (Fig. 5A and 5B)
        - Naïve, class-switch memory, non switched memory, and secreting (plasmablasts) based on mining of RNA-seq data using Kassandra algorithm in cohort 1 and 3 with 10% threshold as high and low – naïve B-cells have some association in RFS or OS in cohorts 3 and 1
- B-cell infiltration is enriched in tumor compared with adjacent lung tissue (Fig 6)

Take Home Points/Discussion

- High tumor infiltrating B-cell content is a favorable prognostic marker
- May need to look beyond the traditional focus on T-cells and pay attention to B-cells as potential prognostic immunologic biomarker

Sun D et al. Classification of tumor immune microenvironment according to programmed death-ligand 1 expression and immune infiltration predicts response to immunotherapy plus chemotherapy in advanced patients with NSCLC. J Thorac Oncol 2023;18:869-81

Background/Purpose

- 4 tumor immune microenvironment (TIME): 1) PD-L1^{-}/ TIL^{-} (type 1), 2) PD-L1^{+}/TIL^{+} (type 2), 3) PD-L1^{-}/TIL^{+} (type 3), and 4) PD-L1^{+}/TIL^{-} (type 4)
- Relationship between the TIME classification model and immunotherapy efficacy in a large-scale randomized controlled clinical trial among advanced NSCLC patients

Materials and Methods

- ORIENT-11 trial: randomized, double-blind, phase 3 study in 47 centers in China
- Previously untreated, locally advanced or metastatic nonsquamous NSCLC without sensitizing EGFR or ALK genomic aberration
- Randomized (2:1 ratio) to receive combination [sintilimab (anti-PD-1) plus chemo (pemetrexed and platinum)] or chemo alone, followed by the same maintenance therapy
- PFS (by blinded radiology review by RECIST) and OS/ORR as primary and secondary end points, respectively
- All tumor specimens: obtained through core bx
- Gene expression level quantified using PPFE samples
- Tumor immune infiltration evaluated on the bases of RNA-seq, using R immuno-oncology biological research package that integrates 8 open source cell type quantification methodologies such as ESIMATE, quanTIseq, xCell and so on
- Scores of CD8+ T cells, TILs and TIICs calculated to identify the best predictive biomarker reflecting tumor immune infiltration
- Evaluation of PD-L1 expression by IHC and RNA-seq
- Median scores of CD8+ T cells, TILs, TIICs from RNA seq data as the cutoff points for high or low TILs density in the combination group and chemotherapy group
- Median level of PD-L1 mRNA expression: set as the cutoff point for high or low PD-L1
- IHC of PD-L1: TPS cut points of 1%, 5%, 10%, 50%, to distinguish between high and low PD-L1 expression
- MHC class II antigen presentation pathway analysis: a predictive biomarker in the previous research of the ORIENT-11 trial, so they further investigated antgen presentation in the relationship with TIME subtypes including 15 MHC class II
- Survival and statistical analysis: predictive value of a variable defined as the ratio of HRs across each variable in two groups; KM method to analyze PFS and OS

Results

- Patients characteristics: Combination group (n=113), Chemo alone (n=56)
- Predictive values of biomarkers reflecting immune infiltration:
    - Estimation of STromal and Immune cells in MAlignant Tumors using Expression data (ESTIMATE): an algorithm using the unique properties of transcriptional

profiles of cancer samples to infer tumor cellularity and the infiltrating stroma and immune cells
    - Immune score by ESTIMATE method representing levels of TIICs (Fig. 1) performed best for prediction
    - TIL by xCell and quanTIseq methods and CD8+ T cells with xCell and quanTIseq methods also did fine
- Predictive values of biomarkers reflecting PD-L1 expression:
    - PD-L1 mRNA was more predictive than TPS: HR of 0.21 in the high expression group; HR of 0.71 in low expression group, yielding a predictive value of 2.38 for PFS; similar results for OS analysis
    - 50% cut off point was most predictive for the efficacy of combination therapy among the TPS cutoff points at 1%, 5%, 10%, 50%,
- PD-L1 mRNA expression and immune score (calculated by ESTIMATE): the highest predictive values for the efficacy of combination therapy, so used for TIME classification
    - 4 groups according to the median levels of PD-L1 mRNA and median immune scores by ESTIMATE method: type I (PD-L1$^-$/TIL$^-$), type II (PD-L1$^+$/TIL$^+$), type III (PD-L1$^-$/TIL$^+$), and type IV (PD-L1$^+$/TIL$^-$)
    - Type I (n=61; 36.1%), II (n=62; 36.7%), III (n=23; 13.6%), IV (23; 13.6%)
- Survival analyses based on the basis of TIME classification model: Fig. 3 and 4
    - Only type II was statistically significant association with improved PFS and OS when compared to between combination vs. chemotherapy alone
    - In the combination group, type II had much longer survival time
        - PFS was the same among type I, III and IV,
        - OS was longer in types III and IV than type I
    - In chemotherapy group, TIME subtypes did not show differences in PFS or OS
    - Multivariate regression analyses for TIME subtypes, type II correlated with improved PFS and OS in combination but not in chemotherapy group
- MHC class II antigen presentation correlated with immune infiltration but not with PD-L1 expression

Take Home Points/Discussion

- Only type II (with both high PD-L1 expression and high immune infiltration) benefits from chemotherapy plus immunotherapy

Hwang S et al. Whole-section landscape analysis of molecular subtypes in curatively resected small cell lug cancer: Clinicopathologic features and prognostic significance. Mod Pathol 2023;36:100184

Background/Purpose

- Classification of SCLC based on the differential expression of transcription regulators ASCL1, NEUROD1, POU2F3, and YAP1 has been introduced recently

- Different subtypes exhibit distinct biology and therapeutic responses
- Most studies have been based on small biopsy samples, tissue microarrays or cell lines which could be misleading due to heterogeneous molecular subtypes within any given tumors
- In this study, they performed whole-section IHC and multiplexed immunofluorescence in curatively resected SCLCs to elucidate the clinicopathologic relevance and prognostic significance of the molecular subtypes

Materials and Methods

- Patients: 98 stage IA to IIIB lung SCLC patients from a single institution who underwent surgical resection with curative intent between 1/1998- 12/2019
    - Exclusion criteria: inadequate tissue for IHC, neoadjuvant therapy before surgery, multiple primary cancers within 5 years
    - Cut off value for data analysis was 10/31/2021
- Total of 73 patients were included in the final analysis
- Histologic evaluation: combined small cell and large cell neuroendocrine ca was defined for cases showing ≥10% of tumor cells with large cell morphology; reviewed by 3 thoracic pathologists
- Molecular subtyping with IHC: ASCL1, NEUROD1, POU2F3, and YAP1
- All were also stained for Rb and p53 to determine the clonal relationship of both components in combined SCLC and that all transcription factor-expressing cells, especially YAP1-expressing cells, were true SCLC cells, but not entrapped benign cells
- Digitally scanned whole slide image images were to correlate with spatial distribution of subtype markers; ASCL1 and NEUROD1 displayed discernible geographic heterogeneity, while expression of YAP1 was mostly scattered among the tumor cells
- So, to define spatial distribution of YAP1 in relation to ASCL1 and NEUROD1, multiplexed IF for cases exhibiting a significant overlap of ASCL1, NEUROD1 and YAP1 (n=2)
- H-score for IHC interpretation: 1 x (% weak positive cells) + 2 x (% moderately positive cells) + 3 x (% strongly positive cells); H score <50 – negative, ≥50 – positive; for all markers except for YAP1
- YAP1: 0, complete absence; +, H score 0-10; ++ 10-100, +++ >100
- In combined SCLC cases, all IHC scores (including YAP1) were measured exclusively in the SCLC component
- TTF1 (8G7G3/1 clone): diffuse + (>70%), partially + (10-70%) or negative (<10%)
- Rb1 loss (all tumor cells lost expression); p53 recorded as complete absence or over expression (>50% of tumor cells showing moderate strong nuclear staining) or equivocal
- For tumors showing both ASCL1 and NEUROD1 positivity, tumors with a higher ASCL1 H score classified as ASCL1 predominant (SCLC-A) and those with a higher NERUOD1 H score as NEURO D1 predominant (SCLC-N); tumors with negative ASCL1, NEUROD1 and POU2F3 as a triple-negative subtype (SCLC-TN); POU2F3 predominant as SCLC-P

- To confirm the notion of SCLC-TN enriched from inflamed phenotype, MHC I and CD8 IHC stains
- Multiplex IF: ASCL1, NEUROD1, YAP1, p53, pancytokeratin, vimentin; ASCL1, NEUROD1, and p53 were used to identify SCLC cells; YAP1 can be positive in non-SCLC cells (stromal cells and vascular smooth muscle cells), which were labeled with vimentin to exclude from the analysis
- Survival analysis: OS, RFS, recurrence (no, locoregional, distant); hazard ratio (HR) by Cox proportional hazards model; multivariable HRs
- External validation: To independently evaluate the association of YAP1 expression with survival, RNA sequencing data (George J et al. Nature 2015;524:47-53), were retrieved from the published resected SCLC cohort and 77 patients with available OS were included in the analysis

Results

- 73 patients (median age 66; 60-72); 84.9% had adjuvant chemo or radiation; stage I 47.9%, II 23.3%, III 28.8%; pure SCLC 65.8%, combined SCLC 34.2%; NSCLC component was adenoca 16, sqcc 4, LCNEC 5
- Molecular subtypes and clinicopathologic correlation:
    - SCLC-A 54.8% (n=40), SCLC-N 31.5% (n=23), SCLC-P 6.8% (n=5), and SCLC-TN 6.8% (n=5)
    - No survival difference among molecular subtypes (SCLC-A, -N, -P, -TN), or between pure and combined SCLC
    - YAP1 expression was low throughout all 4 molecular subtypes
    - 23 of 25 combined SCLC showed the same pattern of Rb loss and p53 aberrations in SCLC and NSCLC components, while 2 cases showed Rb loss only in the small cell component
    - Significant enrichment of SCLC-N in the combined SCLC as compared to pure SCLC (48% vs. 22.9% $p < 0.004$); SCLC combined with ADC, SCLC-N comprised 56.3% (9/16)
    - SCLC-TN was associated with higher MHC I expression and CD8+ lymphocyte infiltration compared to those in SCLC-A or SCLC-N, but statistically not significant
- Distribution and morphologic plasticity of YAP1-expressing cells
    - YAP-1 was positive in 54.8% but mostly at a low level
    - Increased YAP-1 level was in SCLC-P and SCLC-TN ($p = .033$)
    - In a subset, YAP1-expression highlighted clusters of tumor cells with vague tubular structures and non-small cell like morphology
- YAP1 expression is reciprocal with ASCL1 and/or NEUROD1 at the cellular level on multiplex IF
    - YAP1 expression tumor cells show co-expression of pan cytokeratin and diminished expression of neuroendocrine markers (INSM1 and CD56) (i.e. neuroendocrine low phenotype)

- YAP1 expression is an independent poor prognostic factor in resected SCLC
    - Also validated in external surgical cohort

Take Home Points/Discussion

- Although YAP1 is not a subtype delineator, YAP1 relates to the phenotypic plasticity of SCLC and may serve as a poor prognostic factor in resected SCLC

Hong TH et al. Programmed Death-Ligand 1 copy number alteration as an adjunct biomarker of response to immunotherapy in advanced NSCLC. J Thorac Oncol 2023;18:896-906

Background/Purpose

- Current companion diagnostic tests for anti-PD-(L)1 therapy are only moderately effective in predicting responders and ineffective in predicting nonresponders
- As high-grade immune-related adverse effects are increasingly recognized, the lack of effective biomarkers to identify patients who should not be treated with ICI poses a problem
- In this study, two PD-L1-related biomarkers were evaluated in parallel: PD-L1 copy number (CN) loss by NGS and conventional PD-L1 IHC (22C3)

Materials and Methods

- Tumor PD-L1 CN alteration was assessed by whole-exome sequencing data and compared with IHC (22C3) TPS as $\geq$50, 1-49%, or 0, prior to ICI monotherapy in 291 advanced-stage NSCLC (8/2014 – 11/2021)
- PFS and OS were correlated with both biomarkers
- Two independent cohorts using NGS panel were evaluated for the impact of CN alteration

Results

- TPS $\geq$50 by IHC distinguished the best responsive group
- The CN based classification distinguished the worst responsive group (CN loss) from the others (PFS p = .020; OS p = .004)
- After adjusting for IHC results, CN loss was an independent risk factor for progression (adjusted HR = 1.32, p = .049) and death (adjusted HR = 1.39, p = .022)
- A risk classification system on the basis of IHC and CN profiles outperformed the conventional IHC system
- In validation cohorts, CN loss by NGS was independently associated with worse PFS after ICI treatment, with its practical value

Take Home Points/Discussion

- Tumor PD-L1 CN alteration may complement the shortcomings of PD-L1 IHC in patients with advanced NSCLC
- On the basis of survival analysis, tumor PD-L1 CN loss may be useful in predicting the lack of response to ICI therapy in patients NSCLC, especially in the PD-L1 IHC TPS 1-49% group

Articles for Notation

NEOPLASTIC

Tian J et al. Genomic characteristics and prognosis of lung cancer patients with MSI-H: A cohort study. Lung Cancer 2023;181:107255

Background/Purpose

- MSI-H has been thought to be a third possible predictive biomarker for ICIs following PD-L1 and TMB
- MSI-H is a hypermutator phenotype occurring in tumors with deficient mismatch repair system (dMMR), mainly due to mutations in mismatch repair genes MLH1, MSH2, MSH6, or PMS2
- MSI-H frequency is very low in the lung cancers (~0.5%)
- This study analyzed genetic profiles of 66 MSI-H lung cancer s identified from 1 total of 12,484 lung cancer cases

Materials and Methods

- NGS and IHC were used to detect MSI status, TMB and PD-L1 expression

Results

- Compared to microsatellite stability (MSS), TMB was higher in MSI-H lung cancers, while PD-L1 expression did not show significant differences between MSI-H and MSS cases
- The most common companion mutations in MSI-H cases were TP53, BRCA2, PTEN and KMT2C
- In MSH-H lung ADC with EGFR mutation, TGFBR2, ERBB2 had higher mutation frequency than MSS

Take Home Points/Discussion

- This study showed some genetic characteristics of MSI-H lung cancer

Mahmood K et al. High yield of pleural cell-free DNA for diagnosis of oncogenic mutations in lung adenocarcinoma. Chest 2023;164:252-261

Background/Purpose

- A prospective study to answer the following question: can pleural cell-free DNA (cfDNA) be used to assess targetable mutations in lung ADC patients with malignant pleural effusions (MPE)?

Materials and Methods

- Lung ADC patients with MPE during 1/2017-9/2021 (n=54)
- Cytologic exam for dx with or without bx as clinically needed, followed by NGS of 50 gene panel at Duke or 324-gene panel at Foundation Medicine as a clinical workflow
- cfDNA isolation and quantification from pleural fluid and plasma for NGS using the InvisionFirst Lung platform (Inivata) to target 37 oncogenic genes including SNVs, indels, copy number amplifications, and structural rearrangement (gene fusions)
- Interpretation of pleural cfDNA (n=54), cytology (n=33), bx (n=25), plasma cfDNA (n=32): true negative – if all available specimens are negative in the same pt; false negative – if the test was negative in a specimen type but positive in other corresponding specimens; insufficient – cannot perform mutational analysis due to poor quality; positive – all positive tests were assumed to be true positives and cross-referenced between all corresponding types

Results

- 54 pleural fluid samples collected from 42 patients
- Diagnostic yields for oncogenic mutations: pleural cfDNA 49/54 (90.7%); pleural cytology 16/33 (48.5%); bx 22/25 (88%); plasma cfDNA 24/32 (75%)
- Pleural cfDNA vs. bx: true negative 6 (11.1%) vs. 2 (8%); false negative 2 (3.7%) vs. 2 (3.7%) vs. 0; insufficient 3 (5.5%) vs. 3 (12%)

Take Home Points/Discussion

- Diagnostic yield of pleural cfDNA NGS for oncogenic mutations in lung ADC patients is comparable to tumor biopsies and higher than pleural cytology and plasma cfDNA.
- Pleural cfDNA can be useful for longitudinal testing, without having to do biopsy

Wells K et al. Unique correlation between GTF21 mutation and spindle cell morphology in thymomas (type A and AB thymomas). J Clin Pathol 2023;76:463-466

Background/Purpose

- Frequent GTF21 mutation has been observed in a recent study with the highest in types A and AB, followed by B1, B2, B3, and thymic carcinomas
- To investigate the relation between GTF21 mutation status and histology subtype

Materials and Methods

- 111 thymic epithelial tumors were tested for GTF21 mutation by Sanger sequencing
- Correlation between GTF21 mutation status and clinicopathological parameters

Results

- Histology: Type A (n=16), AB (n=37), B1 (n=13), B2 (n=23), B3 (n=9), micronodular (n=6), metaplastic (n=2), thymic carcinoma (n=5)
- GTF21 mutation: Type A 78.6%, AB 83.9%, micronodular 75%; not expressed in type B1-3, metaplastic, or thymic carcinoma
- GTF21 mutation showed a trend towards a favorable px, likely due to association with indolent histologic types (A and AB)

Take Home Points/Discussion

- GTF21 mutation is unique in type A and AB thymomas, including those with atypical features and micronodular type, all of which share spindle cell morphology, suggesting they represent a group biologically distinct from type B thymomas

Almici E et al. Quantitative image analysis of fibrillar collagens reveals novel diagnostic and prognostic biomarkers and histotype-dependent aberrant mechanobiology in lung cancer. Mod Pathol 2023;36:100155

Background/Purpose

- Growing awareness of the role of tumor microenvironment (TME) surrounding carcinoma cells
- Major component of TME in lung ADC and sqcc is a prominent desmoplastic/fibrotic stroma, rich in tumor associated fibroblasts in the background of excessive deposition of fibrillar collagens
- Among 7 fibrillar collagens (type I, II, III, V, XI, XXIV, and XXVII), type I and III are most abundant in TME
- Expression and deposition of fibrillar collagens have been associated with a poor px in lung cancer and other cancer types
- Fibrillar collagens as an important source of cancer-relevant biomarkers and has drawn therapeutic interest in understanding their pathologic functions in tumor progression
- The aims of this study:
    - Optimize CT-FIRE software settings by analyzing fibers in computer-generated phantom images and randomly selected picrosirius red (PSR) stained slides in polarized light (PL) (PSR-PL) images from lung cancer samples
    - These preoptimized settings to retrospectively define the changes in collagen architecture between tumor and non-malignant tissue samples within tissue microarrays (TMAs) from patients with surgical lung cancer
    - Examine the relationship between collagen fibro descriptors and clinicopathologic patient characteristics and defined the potential of these collagen descriptors as novel diagnostic and/or prognostic biomarkers
    - Association of fibrillar collagens with a panel of mechanobiology-related processes commonly associated with tumor progression to shed light on the

pathologic effects and underlying mechanisms of the aberrant collagen organization in lung cancer

- To evaluate the suitability of a digital pathology approach based on PSR-PL imaging and an optimized CT-FIRE analysis to assess quantitative collagen structure and topology descriptors in histologic samples from surgical lung cancer patients

Materials and Methods

- Retrospective analysis of tumor (n=205) and paired uninvolved pulmonary tissues (n=133) from patients with surgical NSCLC gathered from multiple Spanish hospitals
- Minimum of 3 year follow up; histologic dx and staging
- TMA: cores selected by 3 pathologists
- Fibrillar collagens stained with PSR; HE and IHC for α-SMA, Ki-67, and PD-L1 (22C3)
- PSR-PL analyzed with CT-FIRE system
- % of α-SMA computed with imageJ software
- Ki-67 DIA; HE for vascular or lymphatic invasion, tumor grading; PD-L1
- Phantom fiber images: CT-FIRE parameters were pre-optimized on computer-generated phantom images of fibers with synfiber software
- mRNA expression of genes coding for fibrillar collages were analyzed using level 3 RNA-seq expression data for tumor and normal tissue downloaded from TCGA database
- Survival analysis of fibrillar collagen genes (as 2 groups of high and low expression)
- Differential correlation between collagen genes and the YAP/TAZ transcriptional signature

Results

- 106 ADC, 89 sqcc analyzed
- Straightness as the single high-accuracy diagnostic collagen fiber descriptor and fiber density as the single descriptor consistently associated with a poor prognoses in both ADC and SqCC independently of TNM staging (HR 2.69; $p < 0.01$)
- Collagen fibers are much straighter, longer and more aligned in tumor samples compared to uninvolved lung tissue, especially in lung ADC
- Increase in a panel of stiffness-associated processes in the high collagen fiber density patient group in ADC, including venous/lymphatic invasion, fibroblast activation (by α-SMA) and immune evasion (PD-L1)
- Transcriptional correlation analysis supported the potential involvement of the major YAP/TAZ pathway in ADC

Take Home Points/Discussion

- Proof-of-principle to use CT-FIRE analysis of PSR-PL to assess new collagen fiber-based diagnostic and prognostic biomarkers
- An aberrant stiff micro-environment in lung ADC may foster immune evasion and dissemination

Grenier K et al. Routine clinically detected increased ROS1 transcripts are related with ROS expression by imunohistochemistry and associated with EGFR mutations in lung adenocarcinoma. JTO Clinical and Research Reports 2023;4:100530

Background/Purpose

- Translocations of ROS1 gene: driver tumorigenesis in 1-2% of lung ADC
- ROS IHC has been used as screening method for ROS1 rearrangement
- Significant minority of equivocal or positive ROS1 IHC case without ROS1 translocation
- A real-world retrospective study to analyze the relationships between ROS1 protein expression, ROS1 mRNA transcripts, and molecular characteristics obtained from NGS data to clarify the impact of ROS1 IHC positive but not rearranged cases in lung ADC

Materials and Methods

- Retrospective analysis of 1021 cases of nonsquamous NSCLC with both ROS1 IHC and NGS molecular data

Results

- ROS1 IHC was negative in 938 cases (91.9%), equivocal in 65 cases (6.4%), positive in 18 cases (1.7%)
- Among 83 equivocal (2+) or positive (3+) cases, only 2 cases had ROS1 rearrangements (low positive predictive value at 2%)
- ROS1 IHC 2+ or 3+ positive cases, however, correlated with an increased mRNA levels
- Significant relationship between ROS1 IHC expression and other molecular alterations, especially EGFR gene mutations, and to a lesser extent MET driver mutations, compared with cases without an identified driver mutation ($p = .0001$ and $p = .0162$, respectively)

Take Home Points/Discussion

- ROS1 IHC may represent true ROS1 mRNA expression, independent of ROS1 gene rearrangement
- ROS1 expression is associated with other driver mutations, especially with EGFR mutation, suggesting a broader role in NSCLC, independent of ROS1 rearrangement
- *It is noted that they did not look into the ROS1 IHC negative cases for false negative status*

Ilie M et al. Lack of correlation between MET and PD-L1 expression in non-small cel lung cancer revealed by comparative study of matched biopsies and surgical resection samples. Lung Cancer 2023;181:107230

Background/Purpose

- Limited available data on the impact of anti-PD1/PD-L1 and anti-MET inhibitors on NSCLC patients with different expression profiles of these molecules

- To evaluate the rate of correlation between MET expression and the PD-L1TPS in matched bx's and surgically resected specimens from NSCLC patients

Materials and Methods

- Retrospective analysis of the prevalence and correlation between MET expression by IHC (SP44 clone) and PD-L1 TPS (22C3), with molecular alterations determined by targeted NGS in matched lung bx and surgically resected lung specimens from 70 NSCLC cases

Results

- Significant correlation between the MET H-score in surgical samples and matched bx ($p < .0001$), and between PD-L1 TPS in paired bx and surgical samples ($p < .0001$)
- No significant correlation between MET H-score or expression subgroups and the PD-L1 TPS in both types of paired samples
- MET H-score was significantly higher in ADC than in sqcc
- Mutational analysis showed that MET H-score was significantly higher in in NSCLC cases with targetable molecular alterations, but no such correlation found for PD-L1 TPS

Take Home Points/Discussion

- The use of MET and PD-L1 expression levels as predictive biomarkers for NSCLC is limited by the intratumor heterogeneity of these markers, which may interfere with biomarker-based tx decisions
- Standardization of MET levels is still lacking and the MET expression threshold required for anticancer therapy has not yet been validated

NON-NEOPLASTIC

Valenzi E et al. Single-nucleus chromatic accessibility identifies a critical role for TWIST1 in idipathic pulmonary fibrosis myofibroblast activity. Eur Respir J 2023;62:2200474

Background/Purpose

- Myofibroblasts are key effectors of fibrosis and architectural distortion in IPF, by excessive deposition of extracellular matrix and their acquired contractile capacity
- Single-cell RNA-seq (scRNA-seq) can define the IPF myofibroblast transcriptome but identifying critical transcription factor activity by this approach is imprecise
- Multiomic single-cell analyses combined with in vivo murine disease models to investigate transcription factor networks critical to IPF myofibroblasts

Materials and Methods

- Single-nucleus assay for transposase-accessible chromatin sequencing on explanted lungs from 3 IPF patients and 2 donor controls

- Integrated this data with a larger scRNA-seq dataset (10 IPF, 8 controls) to identify differentially accessible chromatin regions and enriched transcription factor motifs within lung cell populations
- RNA-sequencing on pulmonary fibroblasts of bleomycin-injured *Twist*1-overexpressing COL1A2 Cre-ER mice to examine alterations in fibrosis-relevant pathways following *Twist*1 overexpression in collagen-producing cells

Results

- TWIST1, and other E-box transcription factor motifs, were significantly enriched in open chromatin of IPF myofibroblasts, compared to both IPF nonmyogenic and control fibroblasts
- TWIST1 expression was selectively upregulated in IPF myofibroblasts with two regions of TWIST1 having significantly increased accessibility in IPF myofibroblasts
- Overexpression of Twist1 in COL1A2-expressing fibroblasts of bleomycin-injured mice resulted in increased collagen synthesis and upregulation of genes with enriched chromatic accessibility in IPF myofibroblasts

Take Home Points/Discussion

- TWIST1 activity seen in myofibroblasts in IPF lung tissue may be one of the factors with critical regulatory function in fibrotic lung

Shapanis A et al. Topological data analysis identifies molecular phenotypes of idiopathic pulmonary fibrosis. Thorax 2023;78:682-9

Background/Purpose

- Heterogeneous clinical course of IPF suggests the possibility of distinct subphenotypes
- This study combined multiple publicly available peripheral blood mononuclear cell datasets of IPF and other diseases to create a prediction model that could predict IPF in a diseased background to a high degree using a panel of 44 genes

Materials and Methods

- Publically available peripheral blood mononuclear cell expression datasets (n=1,318): 219 IPF, 411 asthma, 362 TB, 151 healthy, 92 HIV, 83 others
- Two groups: 871 train set, 477 test set
- Utility of machine learning model for predicting IPF
- Topological data analysis to identify IPF subphenotypes

Results

- A panel of 44 genes predicted IPF in a background of healthy, TB, asthma and HIV with an area under the curve of 0.9464 (sensitivity of 0.865, specificity of 0.89)
- 5 molecularly characteristic subphenotypes were identified using bioinformatic and pathway analysis tools

- One group corresponded to a phenotype enriched for death/transplant
- One with suggestion for extrapulmonary or systemic fibrotic disease

Take Home Points/Discussion

- Blood test for dx of IPF and distinct subphenotypes of IPF?

Reviews

Harms PW. Multiplex immunohistochemistry and immunofluorescence: A practical update for pathologists. Mod Pathol 2023;36:100197

- Nice review that covers many practical details and issues on this complex and rapidly advancing field by a group of experienced authors
- Update on the current state of the art for tissue multiplexing, including the capabilities and limitations of different techniques with an emphasis on potential relevance to clinical diagnostic practice

Lenskaya V et al. Pleural mesothelioma: current practice and approach. Adv Anat Pathol 2023;30:243-52

- One of the reviews in the current issue of Adv Anat Pathol dedicated to all types of mesotheliomas along with other reviews listed below
- This review does not seem to refer to 2021 WHO book

Strange CD, et al. Imaging of malignant pleural, pericardial, and peritoneal mesothelioma. Adv Anat Pathol 2023;30:280-91

Zambrano E et al. Mesotheliomas in children. Adv Anat Pathol 2023;30:275-9

Malpica A. Peritoneal mesothelioma – An update. Adv Anat Pathol 2023;30:262-74

Iczkowski KA. Malignant mesothelioma of tunica vaginalis testis. Update for 2022. Adv Anat Pahtol 2023;30:259-61

Arossi AV. Pericardial mesotheliomas. Adv Anat Pathol 2023;30:253-8

Editorial/Commentary

Moran CA. Mesothelioma: A tumor of ubiquitous distribution. Adv Anat Pathol 2023;30:241-2

- Mentions about the difficulties and issues on CDKN2A FISH interpretation at length, but no comment on MTAP IHC either here or in the pleural mesothelioma review paper listed above (Lenskaya et al) he coauthored

- Raised doubts on mesothelioma in situ in both review paper (Lenskaya et al) and in this commentary

Eberth JM et al. Changing recommendations for lung cancer screening: National Lung Cancer Roundtable member perspectives. Cancer 2023;129:1953-8

- US preventive Services Task Force (USPSTF) issued updated guidance for lung cancer screening using low-dose CT in March 2021
- National Lung Cancer Round Table (NLCRT) members discussed diverse perspectives about the potential impacts and opportunities resulting from the 2021 USPSTF recommendations
- A doubling of the eligible population for screening since under the guidances issued in 2013, many strategic challenges are noted including disparities and risk assessments, decision making about screening, etc

Case Reports

Zhang T et al. Waxing and waning cysts and nodules. Am J Pulm Crit Care Med 2023;208:101-2

- A case of vascular Ehlers-Danlos syndrome
- 24 year old woman with intermittent hemoptysis and chest pain; hx of easy bruising
- Chest CT shows multiple nodules and cysts with halo signs, that waxed and waned over time