targeted therapy. However, development of therapies targeting *MET*amp has been hampered in part due to poor genomic stratification of patients. We investigated the natural distribution of the size of the *MET* amplicon and associated genomic characteristics. **Method:** Hybrid-capture based comprehensive genomic profiling (CGP) was performed prospectively on DNA isolated from FFPE samples from NSCLC. Tumor mutational burden (TMB) was calculated from 1.1 Mbp of sequenced DNA and reported as mutations/Mb, as previously described (PMID: 28420421). **Result:** We identified 545 NSCLC cases with focal, defined as <20 Mbp (n = 457, 84%), or non-focal (n = 88, 16%) amplification of the *MET* gene using CGP. Within this set, the size of the *MET* amplicon ranged from 0.095 – 158 Mbp; 25th, 50th and 75th quartiles were 1.63 Mbp, 3.46 Mbp, and 11.66 Mbp, respectively. In cases with focal *MET*amp the median *MET* copy number was 11, compared to a median of 7 copies for cases with non-focal *MET*amp (P <0.001). Median TMB in cases with focal vs. non-focal *MET*amp was 10.8 and 9.0, respectively (P=0.47). *MET* exon 14 splice site alterations co-occurred with *MET*amp in 45 cases (8%), of which 80% had focal *MET*amp (median amplicon size of 2.02 Mbp). *EGFR* mutations co-occurred with *MET*amp in 93 cases (17%) in this dataset, of which 78% had focal *MET*amp (median amplicon size: 3.77 Mbp). In contrast, cases with other co-occurring alterations described in the NSCLC NCCN guidelines (*ALK*, *ROS1* or *RET* rearrangements, BRAF V600E, or *ERBB2* mutations) *MET*amp was more commonly non-focal (3 focal and 6 non-focal cases), with a median amplicon size of 25.5 Mbp. Clinical outcomes will be presented, including a subset of cases in the setting of resistance to EGFR inhibitors. **Conclusion:** The size of the *MET* amplicon in *MET*-amplified NSCLCs is largely variable. Focal amplification is associated with a higher estimate of *MET* copy number. Neither TMB or co-occurring *MET* or *EGFR* mutations significantly correlated with size of the *MET* amplicon; however, other co-occurring known drivers were associated with non-focal *MET*amp. Additional investigation is warranted to determine the clinical significance of the size of the *MET* amplicon in NSCLC. **Keywords:** focal amplification, MET, NSCLC

OA 13.01
CD38-Mediated Immunometabolic Suppression as a Mechanism of Resistance to PD-1/PD-L1 Axis Blockade

L. Chen,[1] L. Diao,[2] Y. Yang,[1] X. Yi,[3] B.L. Rodriguez,[1] Y. Li,[3] J. Rodriguez-Canales,[4] X. Liu,[3] A. Huang,[5] Q. Zhao,[5] D. Peng,[5] J. Fradette,[3] P. Tong,[2] C. Ungewiss,[3] Y. Fan,[1] D. Peng,[3] P. Villalobos,[3] E. Dmitrovsky,[3] V. Papadimitrakopoulou,[1] J. Wang,[2] L.A. Byers,[1] J. Heymach,[1] S. Ullrich,[3] I. Wistuba,[6] X. Qin,[5] D. Gibbons[1] *[1]Thoracic/Head and Neck Medical Oncology, MD Anderson, Houston, TX/US, [2]Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX/US, [3]MD Anderson Cancer Center, Houston, TX/US, [4]Translational Molecular Pathology, MD Anderson, Houston, TX/US, [5]Center for Systems Medicine, Institute of Basic Medical Sciences, Beijing/CN, [6]Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX/US*

Background: Although immune checkpoint inhibitors of the PD-1/PD-L1 axis provide significant clinical benefit for patients with lung cancer, effective use of these agents is encumbered by a high rate of primary or acquired resistance. Strategies for optimal therapeutic application of immunotherapy require a thorough understanding of resistance mechanisms. To date, there have been only a few studies reporting potential mechanisms of resistance to PD-1/PD-L1 blockade. **Method:** In multiple immunocompetent syngeneic and spontaneous animal models of *K-ras/p53* mutant lung cancer, we explored the resistance mechanisms to PD-1/PD-L1 blockade using both pharmacologic and genetic approaches (therapeutic antibody treatment and CRISPR/Cas9-mediated editing). The molecular and immune profiles of the tumor microenvironment were evaluated. Additionally, to determine the applicability to patients with lung cancer, we analyzed 259 tumor specimens with IHC staining and mRNA expression, and further confirmed the analyses in publically-available TCGA datasets. **Result:** In multiple models of antibody blockade and genetic knockout of PD-L1, we identified the up-regulation of CD38 on tumor cells as a marker of treatment resistance. Furthermore, by manipulating CD38 on a panel of lung cancer cell lines we demonstrated *in vitro* and *in vivo* that CD38 expression inhibits $CD8^+$ T cell proliferation, anti-tumor cytokine secretion, and tumor cell killing capability. The T cell suppressive effect is dependent upon the ectoenzyme activity of CD38 that regulates the extracellular levels of adenosine. To test whether CD38 blockade might be therapeutically efficacious to prevent anti-PD-L1/PD-1 resistance, we applied combination therapy with anti-CD38 and anti-PD-L1 and demonstrated dramatic therapeutic benefit on primary tumor growth and metastasis. Additionally, in a set of 259 resected lung cancer specimens, ~15% exhibited positive staining for CD38 on tumor cells, and the expression correlated with cytolytic T cell score and an immune/inflammatory signature across multiple large datasets. **Conclusion:** CD38 was found to be a novel mechanism for tumor escape from immune checkpoint PD-1/PD-L1 inhibitor therapy. Targeting this resistance pathway may broaden the benefit of PD-L1/PD-1 axis blockade for lung cancer treatment.

OA 13.02
Distribution of PD-L1 Tumor Expression by Assay Type in Patients with Metastatic NSCLC (MNSCLC)

V. Velcheti,[1] X. Cao,[2] F. Liu,[2] P. Patwardhan[2] *[1]Cleveland Clinic, Pepper Pike/US, [2]2Center for Observational and Real-World Evidence (Core), Merck & Co., Inc., Kenilworth, NJ/US*

Background: Pembrolizumab was initially approved as a single agent by the US FDA on October 2, 2015, for treating patients with mNSCLC who have disease progression on or after platinum-containing chemotherapy and PD-L1 tumor expression ≥50%, as determined by the FDA-approved test (Dako 22C3). Subsequent approvals for first-line therapy and expanded second-line therapy followed in October 2016. Our aim was to study PD-L1 testing patterns in US Oncology practices from October 2015 through March 2017 and the potential impact of the PD-L1 IHC assay type on measurement of PD-L1 tumor expression. **Method:** This retrospective, observational study drew on de-identified, longitudinal data from a large electronic medical record database (Flatiron Health) representing 17% of incident oncology cases in the US. Eligible patients were adults (≥18 years) with histologically/cytologically confirmed initial diagnosis of mNSCLC (stage IV) or metastatic recurrence from October 2015 through March 2017. We determined the rate of PD-L1 testing (test date defined as the result date) and distribution of PD-L1 tumor expression (percentage of tumor cells staining for PD-L1) by IHC assay type. **Result:** The 7879 eligible patients included 4111/3768 (52%/48%) men/women; 5123 (65%) were >65 years old, and 6706 (85%) had a history of smoking. The rate of PD-L1 testing increased consistently over time from 15% in Q4/2015 to 70% in Q1/2017. Of 1728 patients with mNSCLC tested for PD-L1, 77%, 5%, 4%, and 19% were tested using Dako 22C3, Dako 28-8, Ventana SP142, and laboratory-developed tests (LDTs), respectively. Measured PD-L1 expression varied significantly (χ^2 p<0.0001) across the four assay types, although there was no significant difference (p=0.053) among the remaining three assays when the SP142 assay was excluded (Table**)**. **Conclusion:** We found no significant differences in measuring PD-L1 tumor expression using Dako 22C3, Dako 28-8, and LDTs; however, results of the SP142 assay appeared discordant. **Keywords:** Biomarker, PD-L1, non-small cell lung cancer

PD-L1 biomarker immunohistochemical (IHC) assay results for 1728 patients with mNSCLC whose tumors were tested from October 2015 through March 2017, by assay type.*

PD-L1 tumor expression, categorized‡	FDA-approved IHC assay, n (%)			Laboratory-developed tests, n (%)
	Dako 22C3 (N=1335)	Dako 28-8 (N=90)	Ventana SP142† (N=75)	(N=323)
<1%	478 (35.8)	37 (41.1)	46 (61.3)	127 (39.3)
1–49%	376 (28.2)	25 (27.8)	16 (21.3)	107 (33.1)
≥50%	481 (36.0)	28 (31.1)	13 (17.3)	89 (27.6)

*Some patients had more than one test and are represented in more than one column.
†Ventana SP142 results represent percentage of tumor cells staining for PD-L1.
‡$p<0.0001$ for χ^2 test comparing results across the four assay types, and $p=0.053$ for χ^2 test comparing results across three assay types, excluding the Ventana SP142 assay.
FDA, Food and Drug Administration.

OA 13.03

Wide Expression of Alternative Immune Checkpoint Molecules, B7x and HHLA2, in PD-L1 Negative Human Lung Cancers

H. Cheng,[1] A. Borczuk,[1] M. Janakiram,[1] X. Ren,[1] J. Lin,[1] A. Assal,[2] B. Halmos,[1] R. Perez-Soler,[1] X. Zang[1] *[1]Albert Einstein College of Medicine/Montefiore Medical Center, New York, NY/US, [2]Columbia Medical Center, New York, NY/US*

Background: Immunotherapy targeting the PD-1/PD-L1 pathway has dramatically changed the treatment landscape of non-small-cell lung carcinoma (NSCLC). We previously demonstrated that HHLA2, a recently identified B7 family immune inhibitory molecule, was widely expressed in NSCLC. To better understand the immune evasion mechanisms within the tumor microenvironment, we compared the expression profiles and functional roles of PD-L1 with potential alternative immune checkpoints, B7x and HHLA2. **Method:** Expression was assessed by immunohistochemistry using tissue microarrays consisting of 392 NSCLC tumor tissues (mostly resected stage I to III), including 195 tumors in the discovery (D) set and 197 cases in the validation (V) set. Positive PD-L1 cases were defined as samples with percentage of tumor cells revealing membranous staining of PD-L1 $\geq$1% with SP142 antibody. Human T cells were purified from eleven donors. Control human IgG, human PD-L1-Ig, human B7x-Ig and human HHLA2-Ig were used to determine the effects of PD-L1, B7x and HHLA2 on T cell proliferation and cytokine production [Human Th Cytokine Panel: IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, IFN-γ, TNF-α, IL-17F, IL-17A, IL-4, IL-21 and IL-22]. **Result:** PD-L1 expression was detected in 25% and 31% of tumors in the D and V sets respectively, and was associated with higher stage and lymph node involvement in both cohorts. Multivariate analysis further showed that stage, TIL status and lymph node involvement were independently associated with PD-L1 expression. B7x was expressed in 69% and 68% of cases, while HHLA2 was positive in 61% and 64% of tumors in the two sets. Triple positive expression was detected in 13% whereas triple negative in 15% of cases. The double-expression of PD-L1 with B7x or HHLA2 was rare, 6% and 3% respectively. Interestingly, the majority (78%) of PD-L1 negative cases expressed B7x, HHLA2 or both. Moreover, the triple positive group correlated with more TIL infiltration as compared to the triple negative group ($P = 0.0175$). At the same concentration, B7x-Ig and HHLA2-Ig inhibited TCR-mediated proliferation of both CD4 and CD8 T cells significantly more robustly than PD-L1-Ig. All three significantly suppressed a variety of cytokine production by T cells. **Conclusion:** The majority of PD-L1 negative lung cancer cases express alternative immune checkpoint molecules (B7x, HHLA2 or both). The potential role of the B7x/HHLA2 pathway in mediating immune evasion in PDL1 negative tumors deserves to be explored to provide the rationale for an effective immunotherapy strategy in these tumors. **Keywords:** Lung cancer, immune checkpoint, PD-L1

OA 13.05

Immune, Molecular and T Cell Repertoire Landscape of 235 Resected Non-Small Cell Lung Cancers and Paired Normal Lung Tissues

A. Reuben,[1] R. Gittelman,[2] J. Zhang,[3] R. Chen,[3] K. Quek,[3] L. Vence,[3] I. Fernandez-Cubelo,[3] C. Behrens,[4] J. Gao,[3] E. Yusko,[2] R. Emerson,[2] S. Benzeno,[2] M. Vignali,[2] C. Tipton,[2] A. Jalali,[3] W. Lee,[3] C. Wu,[3] J. Li,[3] X. Wu,[3] Y. Ye,[3] A. Eterovic,[3] L. Little,[3] C. Gumbs,[3] C. Bernatchez,[3] C. Haymaker,[3] M. Forget,[3] L. Federico,[3] T. Cascone,[3] H. Robins,[2] E. Roarty,[4] J. Rodriguez,[4] E. Parra,[4] J. Wargo,[3] J. Allison,[3] P. Sharma,[3] J. Zhang,[3] J. Lee,[3] B. Sepesi,[5] S. Swisher,[4] D. Gibbons,[6] J. Heymach,[7] A. Futreal,[1] I. Wistuba,[8] J. Zhang[9] *[1]Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX/US, [2]Adaptive Biotechnologies, Seattle, WA/US, [3]MD Anderson Cancer Center, Houston TX/US, [4]Icon Study Team, Houston, TX/US, [5]Thoracic and Cardiovascular Surgery, MD Anderson Cancer Center, Houston, TX/US, [6]Thoracic/Head and Neck Medical Oncology, MD Anderson, Houston, TX/US, [7]Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX/US, [8]Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX/US, [9]Department of Thoracic/Head and Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX/US*

Background: Non-small cell lung cancer (NSCLC) is characterized by a high mutational load. Accordingly, it is also among the tumor types responding to immune checkpoint blockade, likely through harnessing of the anti-tumor T cell response. However, the lung is continuously exposed to the outside environment, which may result in a continuous state of inflammation against outside pathogens unrelated to the tumor microenvironment. Therefore, further investigation into the T cell repertoire and T cell phenotypes across normal lung and tumor is warranted. **Method:** We performed T cell receptor (TCR) sequencing on peripheral blood mononuclear cells (PBMC), normal lung, and tumor from 225 NSCLC patients, among which, 96 patients were also subjected to whole exome sequencing (WES) of PBMC, tumor and normal lung tissues. We further performed Cytometry by Time-of-Flight (CyTOF) on 10 NSCLC tumors and paired normal lung tissues to phenotype immune and T cell subsets. **Result:** Comparison of the T cell repertoire showed 9% (from 4% to 15%) of T cell clones were shared between normal lung and paired tumor. Furthermore, among the top 100 clones identified in the tumor, on average 57 (from 0 to 95) were shared with paired normal lung tissue. Interestingly, T cell clonality was higher in the normal lung in 89% of patients suggesting potential differences in the immune response and immunogenicity. A substantial number of somatic mutations were also identified not only in NSCLC tumors (average 566; from 147 to 2819), but also in morphologically normal lung tissues (average 156; from 50 to 2481). CyTOF demonstrated striking differences in the immune infiltrate between normal lung and tumor, namely a lower frequency of PD-1+CD28+ T cells (both CD4+ and CD8+) in the normal lung (2.7% versus 3.0% in tumor). In addition, a unique GITR+ T cell subset (0.96%) was entirely restricted to the normal lung. Conversely, increases in regulatory T cell frequency (CD4+FoxP3+) were observed in the tumor (10.4% vs 1.7% in normal lung), further highlighting the differences in T cell phenotype and response across normal lung and tumor. **Conclusion:** These results suggest that a substantial proportion of infiltrating T cells in NSCLC tumors may be residential T cells associated with response to environmental factors. However, normal lung and NSCLC tumors carry T cells of distinct phenotypes including increases in immunosuppressive T cells within the tumor which may further highlight the differences in the anti-tumor immune response. **Keywords:** NSCLC, TCR Repertoire, molecular profiling