# Comprehensive genomic and immune profiling of non-small cell lung cancer brain metastases reveals low tumor inflammation and elevated cancer testis antigen burden

R.J. Seager[1], Sarabjot Pabla[1], B. R. Achyut[2], Mary K. Nesline[1], Geoffrey Kannan[2], Anjen Chenn[2], Shengle Zhang[1], Roger Klein[1], Jeffrey Conroy[1], Mark Sausen[2], Kamal S. Saini[2], Taylor J. Jensen[2], Prasanth Reddy[2], Eric Severson[2], Shakti Ramkissoon[2]

[1]Omniseq Inc. (a Labcorp subsidiary), 700 Ellicott Street, Buffalo, NY 14203, US
[2]Labcorp, Burlington, NC 27215, US

## Introduction

- Non-small cell lung cancer (NSCLC) accounts for ~50% of brain metastases.
- Many individual biomarkers describe the complexity of each tumor and its interactions with the tumor microenvironment (TME).
- We compare the genomic and immune biomarker landscapes of two cohorts of patients: one with primary NSCLC (pNSCLC) and another with metastatic NSCLC to the brain (mNSCLC).

## Methods

- Standard-of-care comprehensive genomic and immune profiling was performed on FFPE tumors representing 39 histologic types, assessing expression levels of 395 immune genes and >500 tumor-associated genes [1,2].
- From this data, three previously published gene expression signatures were calculated: cell proliferation (CP), tumor immunogenic signature (TIGS), and cancer testis antigen burden (CTAB) [3,4,5].
- PD-L1 status of each tumor was assessed by IHC and designated as positive when ≥1% tumor proportion score (TPS), and tumor mutational burden (TMB) was calculated and designated as high when ≥10Mut/Mb was observed.
- We analyzed 137 mNSCLC patient tumors (ages 40-85y [mean 65y], 52% female, 48% male) and 5533 primary NSCLC (pNSCLC) patient tumors (ages 24-100y [mean 71y], 51% female, 49% male) with comprehensive genomic and immune biomarker profiling, including PD-L1 IHC, TMB, TIGS, CP, and CTAB.

**Table 1:** Biomarker and demographic composition of pNSCLC and mNSCLC cohorts.

| Demographics | mNSCLC Cohort (n=137) | | pNSCLC Cohort (n=5533) | |
|---|---|---|---|---|
| | Number of Patients | Percentage of Total Cohort | Number of Patients | Percentage of Total Cohort |
| | 137 | 100.00% | 5533 | 100.00% |
| **Gender** | | | | |
| Female | 71 | 51.82% | 2823 | 51% |
| Male | 66 | 48.18% | 2710 | 49% |
| **TMB (≥10Mut/Mb)** | | | | |
| High | 77 | 56.20% | 1581 | 28.57% |
| Not High | 58 | 42.34% | 3134 | 56.64% |
| Missing | 2 | 1.46% | 818 | 14.78% |
| **PD-L1 IHC (≥1% TPS)** | | | | |
| Positive | 75 | 54.74% | 3678 | 66.47% |
| Negative | 62 | 45.26% | 1833 | 33.13% |
| Missing | 0 | - | 22 | 0.40% |
| **Tumor Immunogenic Signature (TIGS)** | | | | |
| Strong | 34 | 24.82% | 1952 | 35.28% |
| Moderate | 31 | 22.63% | 1699 | 30.71% |
| Weak | 72 | 52.55% | 1882 | 34.01% |
| **Cell Proliferation (CP)** | | | | |
| High | 10 | 7.30% | 470 | 8.49% |
| Moderate | 58 | 42.34% | 2042 | 36.91% |
| Poor | 69 | 50.36% | 3021 | 54.60% |
| **Cancer Testis Antigen Burden (CTAB)** | | | | |
| High | 94 | 68.61% | 3185 | 57.56% |
| Low | 43 | 31.39% | 2348 | 42.44% |

## Results

- PD-L1 expression (%TPS) for all cases by IHC was not significantly different. However, pNSCLC cases were more likely to be PD-L1 positive (≥1%TPS) (p=0.00506) and mNSCLC cases were more likely to be PD-L1 negative (p=0.0037) [Fig. 1].

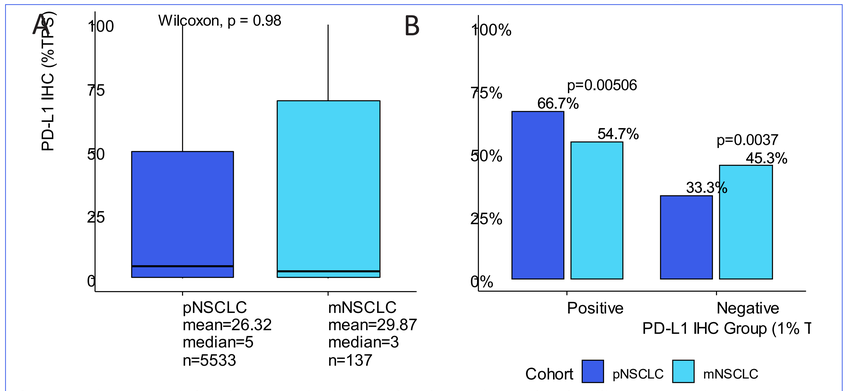


**Figure 1:** A) PD-L1 IHC distributions (%TPS) in each cohort; B) Bar plot detailing PD-L1 IHC group (Positive: PD-L1 IHC ≥ 1%TPS) composition of each cohort with overrepresentation test p-values indicated.

- Genomic alteration (GA) frequency in mNSCLC and pNSCLC were similar; only KRAS was significantly increased (39.9% vs 25.5%, p<0.0005). Mean TMB was significantly higher in mNSCLC versus pNSCLC (p=7.8e-10). Additionally, mNSCLC cases were more likely to have high TMB (TMB≥10 mut/Mb) (p=3.33e-11) and pNSCLC cases were more likely to not have high TMB (TMB<10 mut/Mb) (p=0.000943) [Fig. 2].

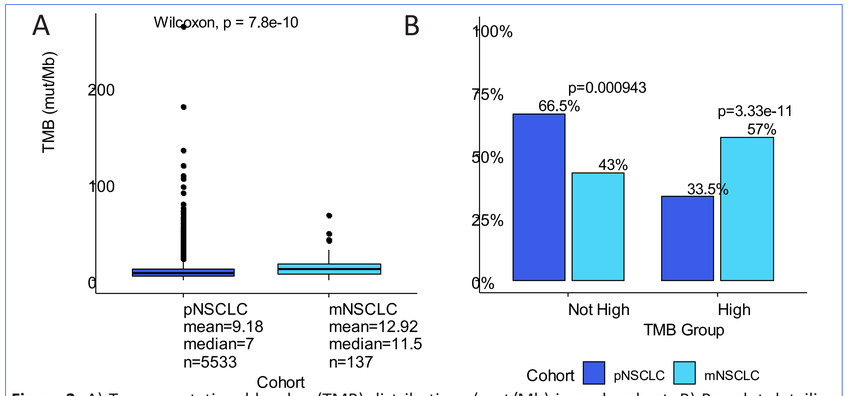


**Figure 2:** A) Tumor mutational burden (TMB) distributions (mut/Mb) in each cohort; B) Bar plot detailing TMB group (High: ≥10 mut/Mb) composition of each cohort with overrepresentation test p-values indicated.

- mNSCLC cases had a significantly higher mean CP score (p=0.025) [Fig. 3].

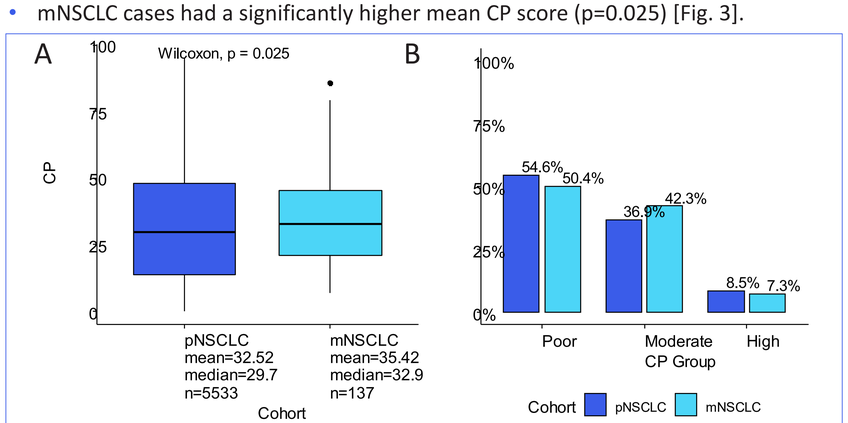


**Figure 3:** A) Cell proliferation signature (CP) distributions in each cohort; B) Bar plot detailing CP group composition of each cohort with overrepresentation test p-values indicated.

- The TIGS score was significantly higher for pNSCLC cases (p=3.9e-6). mNSCLC cases were more likely to be weakly inflamed (p=1.19e-5) while pNSCLC cases were more likely to be moderately (p=0.0392) or strongly (p=0.00979) inflamed [Fig. 4].

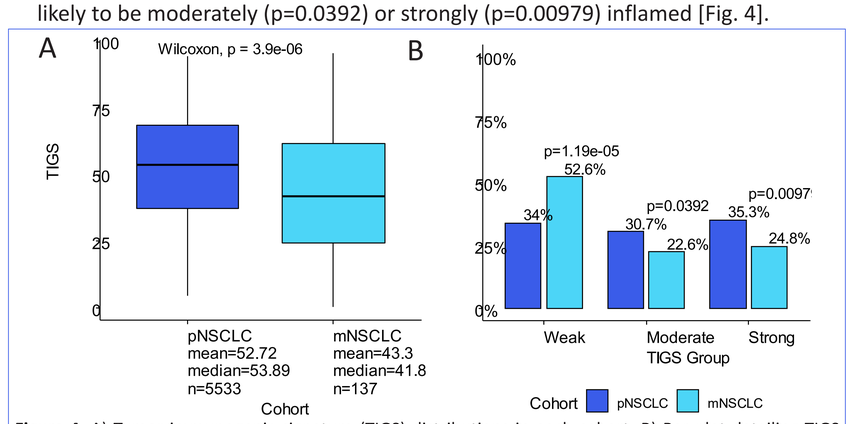


**Figure 4:** A) Tumor immunogenic signature (TIGS) distributions in each cohort; B) Bar plot detailing TIGS group composition of each cohort with overrepresentation test p-values indicated.

- The CTAB score was significantly higher in mNSCLC cases (p=2e-5). Additionally, mNSCLC cases were more likely to have high (≥171) CTAB (p=0.00902) while pNSCLC cases were more likely to have low (<171) CTAB (p=0.00902) [Fig. 5].

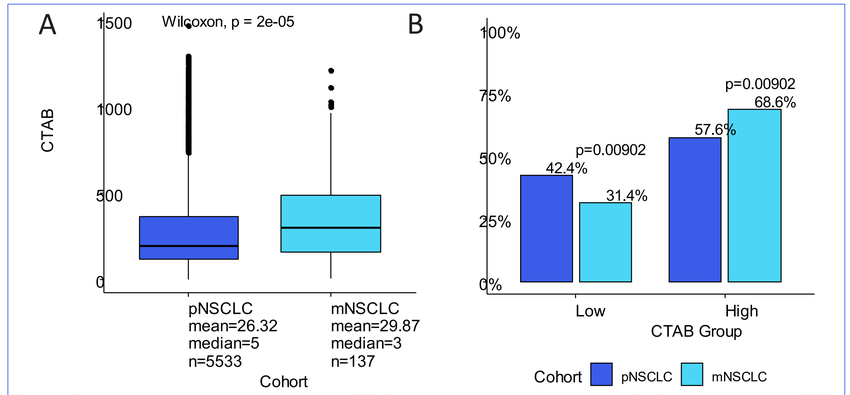


**Figure 5:** A) Cancer testis antigen burden (CTAB) distributions in each cohort; B) Bar plot detailing CTAB group composition of each cohort with overrepresentation test p-values indicated.

## Conclusions

- Comprehensive genomic and immune profiling (CGIP) facilitates the interrogation of tumor immunity biomarkers in real-world NSCLC brain metastasis specimens.
- CGIP reveals that mNSCLC cases have a larger antigen burden, with increased TMB and CTAB, likely due to the immune privileged nature of the brain, which is reflected in the lower TIGS scores and PD-L1 positivity.
- Despite lower overall PD-L1 positivity, mNSCLC with negative PD-L1 IHC may potentially benefit from immunotherapy including cancer vaccine and adoptive cell therapy strategies given the high TMB and CTAB.

## References

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OmniSeq® 700 Ellicott Street | Buffalo NY, 14203

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