# Cancer testis antigen burden: A novel predictive biomarker for immunotherapy in solid tumors

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## Introduction

- When expressed in cancer cells, cancer testis antigens (CTAs) are highly immunogenic and have the capacity to elicit cancer-specific immune responses in diverse malignancies.
- With their expression limited to tumor cells, CTAs have become a prime target of natural T cell response, immune cell-based therapy, and cancer vaccines.
- In this study, we investigated CTA burden in real-world clinical tumors spanning multiple histologies, revealing a novel prognostic gene expression-based biomarker.

## Methods

- Targeted RNA-seq was performed on 5450 FFPE tumors representing 39 histologic types, predominantly composed of lung cancer (40.4%) followed by colorectal cancer (10.6%) and breast cancer (Figure 1).
- Expression levels of 17 CTA genes were classified as positive (nRPM≥20) or negative (nRPM<20) across all tumors.
- CTAs were ranked against a reference population[1]. Cancer Testis Antigen Burden (CTAB) was calculated as the sum of the gene expression rank for each CTA gene. The median CTAB of ≥ 171 was used as cutoff for CTAB High versus Low classification.
- We estimated Pearson's correlation for all CTA genes to discover co-expression patterns between CTAs and histologies. Overall survival (OS) analysis was performed using CoxPh regression model whereas response analysis was performed using logistic regression model with p-values reported.

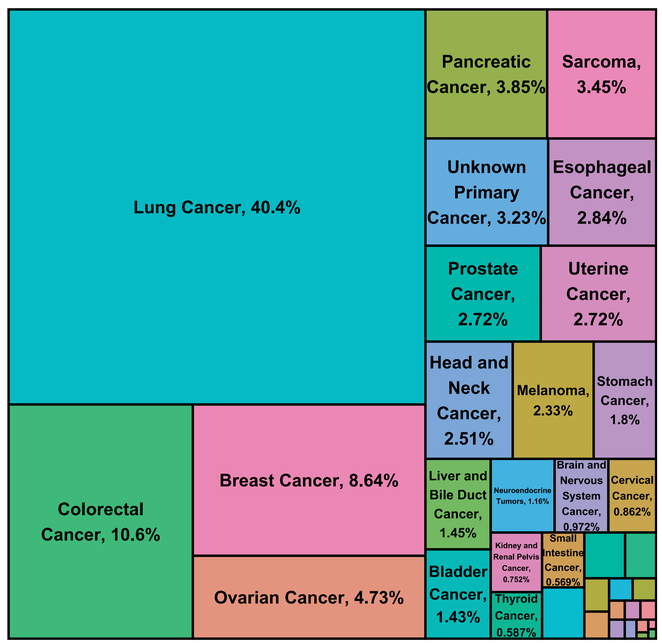


**Figure 1:** 5,450 samples from clinically tested FFPE tumors spanning 39 cancer types. Cohort primarily consists of lung cancer followed by colorectal, breast, and ovarian cancer. Inclusion criteria for the samples was based on clinical QC parameters for RNA-seq.

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## Results

In total, positive CTA expression prevalence ranged from 3% (GAGE13) to 31.5% (XAGE1B) across the 5450 tumors studies (Figure 2).

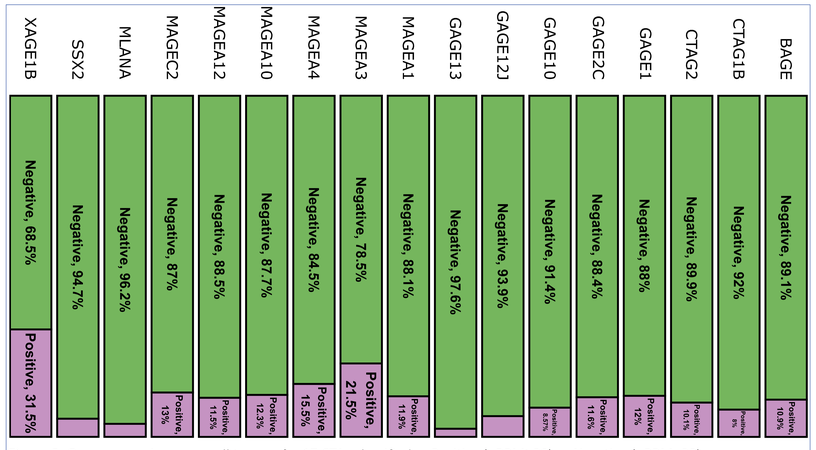


**Figure 2:** Gene expression across all tumors for 17 CTAs classified as Positive (nRPM≥20) or Negative (nRPM<20)

Within the tumor samples, CTAB values ranged from 0-1700, with kidney cancer demonstrating overall lowest mean CTAB (110) and melanoma the highest (550). NSCLC had an average CTAB of 283 (Figure 3). The median CTAB was 171, and was used as the cutoff for CTAB High versus Low classification (Figure 4).

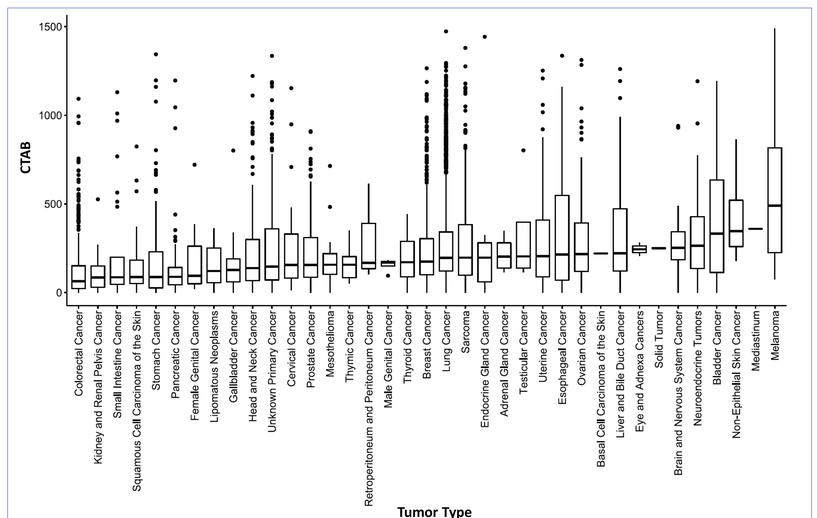


**Figure 3:** Distribution of cancer testis antigen burden (CTAB) for 5450 samples of 37 histologies.

Co-expression of CTAS was observed across several histologies with highest levels of co-expression observed within the MAGEA and GAGE families (Figure 5).

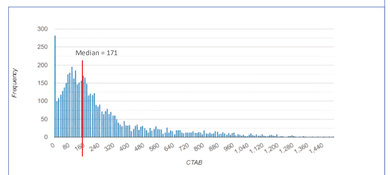


**Figure 4:** CTAB distribution across 5450 samples. Median value of 171 indicated by red vertical bar.

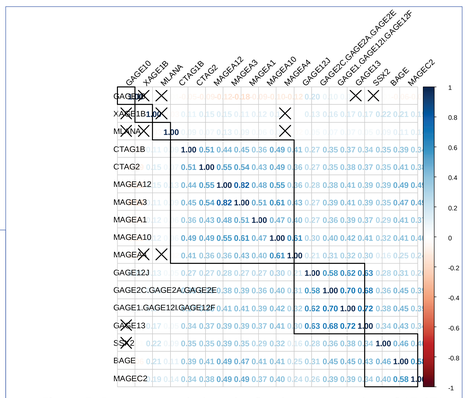


**Figure 5:** Pearson correlation plot for the coexpression of 17 CTAs across 5450 tumors. Insignificant (p<0.05) correlations = X.

In an immune checkpoint blockade treated retrospective cohort of 110 NSCLC patients, High CTAB showed better OS compared to Low CTA (HR: 0.55, p=0.07). Additionally, when combined with tumor inflammation and cell proliferation biomarkers[2], highly inflamed but poorly proliferative tumors with High CTAB had improved OS (HR: 0.27, p=0.05). A significant association with higher response (HR=1.84; p=0.05) was detected for the retrospective cohort (Table 1).

| Group | Predictor | Survival Comparison | Overall Survival | | | |
|---|---|---|---|---|---|---|
| | | | Hazard Ratio | CI Low | CI High | P Value |
| Retrospective Cohort (n=242) | CTAB MEDIAN | Above Median vs Below Median | 0.63 | 0.43 | 0.94 | **0.02** |
| NSCLC (n=110) | CTAB MEDIAN | Above Median vs Below Median | 0.5 | 0.29 | 1.04 | **0.07** |
| NSCLC (N=110) | CTAB MEDIAN | TIGS=Strong CP=High | 0.28 | 0.03 | 2.69 | 0.27 |
| | | TIGS=Strong CP=Moderate | 0.66 | 0.20 | 2.20 | 0.49 |
| | | TIGS=Strong CP=Poor | 0.27 | 0.08 | 0.99 | **0.05** |
| **Group** | **Predictor** | **ORR Comparison** | **Hazard Ratio** | **CI Low** | **CI High** | **P Value** |
| Retrospective Cohort (n=242) | CTAB MEDIAN | Above Median vs Below Median | 1.84 | 1.07 | 3.36 | **0.05** |
| NSCLC (N=110) | CTAB MEDIAN | TIGS=Moderate CP=High | 0.66 | 0.03 | 14.05 | 0.79 |
| | | TIGS=Moderate CP=Moderate | 0.00 | 0.00 | NA | 0.98 |
| | | TIGS=Moderate CP=Poor | 6.86 | 0.83 | 56.60 | **0.07** |

**Table 1:** Overall Survival (OS) and objective response rate (ORR) differences for entire retrospective cohort (n=242, NSCLC, Melanoma, RCC) for High versus Low CTAB.

## Conclusions

Our studies show that co-expression of multiple CTA genes occurs in many tumor types and can be reliably detected using a targeted RNA-seq approach. Utilization of this co-expression pattern to calculate CTAB reveals tumor-type associated signatures, which in a small NSCLC cohort is associated with the overall survival. The findings suggest that these immunogenic antigens expose the tumor cells to natural or immunotherapy augmented cell-based immune response, and that CTAB is a potential predictive marker for therapeutic response to checkpoint inhibitors. Further studies are needed to establish the predictive value in other tumor types, as well as the role of CTAB in immune cell therapies and vaccinations.

## References

1. Conroy JM, Pabla S, Glenn ST. Analytical validation of a next generation sequencing assay to monitor immune responses in solid tumors. J Mol Diagn. 2018;20:95–109.
2. Pabla, S., Seager, R.J., Van Roey, E. et al. Integration of tumor inflammation, cell proliferation, and traditional biomarkers improves prediction of immunotherapy resistance and response. Biomark Res 9, 56 (2021).

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