Liquid biopsy for PD-L1 status by analysis of cfRNA in pan-cancer cohort: Preliminary clinical data from a large community practice

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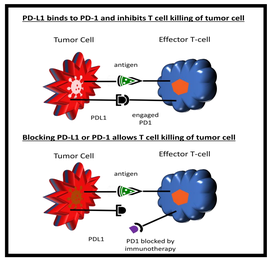
Abstract 3383

Abstract: PD-L1 testing using immunohistochemistry has become a widely used tool to predict response to immune checkpoint inhibitor (ICI) therapy in many tumor types. However, clinical decision-making may suffer from tissue heterogeneity as these results can vary over time due to interference arising from treatment and local factors and cytokine expression. Gene expression profiles of immunologically active genes are being currently studied to more accurately predict response to immune checkpoint inhibitor (ICI) treatment. Identification and measurement of circulating nucleic acids, including both DNA and RNA, represent a less invasive approach to sampling tumor biology and a more accurate way to assess the clinical significance of biomarkers for prediction of response to therapies targeting the immune pathways regardless of the tumor of origin . To this end, we utilized an assay of cfRNA in plasma for PD-L1 , using a real-time PCR based assay (Circulogene, Inc, Birmingham, AL). The demonstrated limit of detection for PD-L1 RNA was 1 copy/ul. Results are reported as either not detected, or low at over 1%, or high at over 50%, using the PCR 30th percentile Ct value corresponding to tissue IHC PD-L1 of >50% and the 66th percentile Ct value corresponding to tissue IHC PD-L1 expression of >1%. We then collected over 400 patients over a 2-year period at a large community practice and a university clinic (Florida Cancer Specialists and Atrium-Wake Forest Baptist Comprehensive Cancer Center) who had a clinical necessity for testing in a commercially available setting. In some cases, corresponding tissue levels of PD-L1 by IHC was available and may have been either positive or negative. To date, 52 patients have been found to have high levels of cfRNA for PD-L1, and their clinical course has been analyzed further using the electronic medical record. Some have received standard of care therapy, and some have been given ICI therapy if the attending oncologist felt it to be the best option. These include tumors such as CRPC, advanced low grade neuroendocrine carcinoma and HR+ metastatic breast carcinoma, that have been rarely treated successfully with ICI therapy.

Introduction: Immune checkpoint therapy

Programmed death-ligand 1 (PD-L1) is a protein overexpressed on the surface of cancer cells. PD-L1 interacts with its receptor, PD-1, on T cells and inhibits the T cell response, enabling cancer cells to evade the immune system. Immune checkpoint therapies, such as PD-1 inhibitors and PD-L1 inhibitors, are designed to block the interaction between PD-L1 and PD-1, thereby enhancing the immune system's ability to recognize and attack cancer cells. Clinical evidence suggest that patients with tumors expressing high levels of PD-L1 are more likely to respond to immune checkpoint therapy than those with low levels of PD-L1 expression. However, PD-L1 expression alone does not always predict response to therapy, and some patients with low PD-L1 expression may still respond to these therapies. Therefore, while PD-L1 expression is a useful biomarker for predicting response to immune checkpoint therapy, it is not entirely reliable and other factors may also play a role in determining treatment efficacy. Ongoing research is aimed at identifying other biomarkers that may be more predictive of response to immune checkpoint therapy.



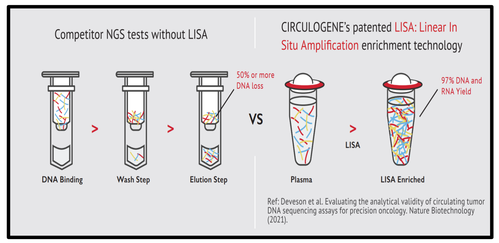
Types of gene regulation:

The following table describe the molecular mechanisms controlling the cellular expression of protein expression. These mechanisms work together to tightly control the expression of proteins in cells, allowing for precise regulation of cellular processes. Several processes are detectable using liquid biopsy assay

Gene activity control	Molecular Mechanism	Detectable in liquid biopsy
Mutational regulation	In both cases, mutations can affect the regulation of gene activity, which can lead to the uncontrolled growth and division of cells, a hallmark of cancer.	✓
Transcriptional regulation	Transcription factors bind to specific DNA sequences in a gene's promoter region to activate or inhibit transcription.	✓
Epigenetic regulation	Control of gene expression through chromatin structure alterations, including DNA methylation and histone modifications.	✓
Post-transcriptional regulation	The regulation of gene expression after transcription has occurred. This can include control of mRNA processing, stability, and translation.	✓
Post-translational regulation	This refers to the control of protein activity after it has been translated. This can include modifications such as phosphorylation, acetylation, and glycosylation.	✗
Protein degradation	This is the process by which proteins are eliminated from the cell. This can be regulated through the ubiquitin-proteasome system or through lysosomal degradation.	✗
Feedback Regulation	Many cellular processes are regulated through feedback mechanisms, where the activity of a protein or pathway regulates the expression of other proteins or pathways.	✗

Methods: Linear in Situ Amplification (LISA):

Patients with inoperable and/or metastatic cancer underwent PCR and NGS somatic testing panel using the LISA method and expression was assessed in a CLIA/CAP accredited laboratory (Circulogene, Pensacola,FL). These patients were distributed across 8 treatment centers and comprised subjects who tested positive for PD-L1 expression were subsequently retested using a novel PD-L1 cfRNA test and a tumor board selected the appropriate treatment based on clinical and pathological factors.



Nucleic acids were specifically isolated from the biological sample using a process that increases the concentration of the analyte. Both cfRNA and cfDNA, were selectively modified and amplified using targeted qPCR directly from the isolated nucleic acid. In the case of circulating RNA, it was previously retrotranscribed to DNA before amplification. Finally, the enriched pool of modified DNA targets were identified and measured using next-generation sequencing (NGS). The detection of specific gene may be potentially used as non-invasive biomarkers for various diseases.

The patented LISA technology provides a successful recovery of 97% of available DNA and RNA from a plasma sample representing a near-full recovery of circulating-free nucleic acid (cfDNA or cfRNA). The simplified laboratory process is easily automated and adapted to high-throughput workflows, while occupying a small footprint of the benchtop. The service provider for LISA enables a prolonged and extended biobank sample usage.

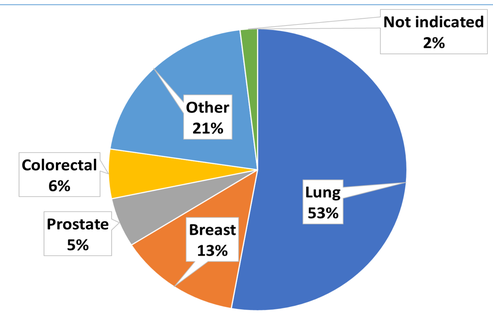
Solid tumor compared to liquid biopsy for predicting ICI response

		At metastatic diagnosis	After subsequent lines of therapy
Tumor biopsy	Advantages	• Key pathological information • Ability to assess non-DNA biomarkers (proteome, RNA, etc.)	• Important for research and discovery • Critical if assessment of non-DNA biomarkers needed
	Disadvantages	• Longer turnaround time for sequencing limits first-line precision-therapy selection • Limited tissue quantities can constrain breadth of testing or cause assay failure	• Requires repeat invasive procedure • Longer turnaround time for sequencing results may hinder rapid selection of therapy
Liquid-biopsy cfDNA	Advantages	• High concordance with tissue biopsy • Ready sample availability • Rapid turnaround to facilitate first-line precision-oncology therapies • Baseline for subsequent liquid biopsy	• Non-invasive, easy to obtain serial samples • Captures heterogeneous resistance alterations • Rapid turnaround time can enhance clinical-trial enrollment
	Disadvantages	• Parallel assessment with tumor testing increases cost • Cannot assess non-DNA biomarkers	• Cannot assess non-DNA biomarkers

In June 2020 the FDA granted accelerated approval for pembrolizumab to treat unresectable or metastatic tumor mutational burden-high (TMB-H), as determined by an FDA-approved test that have progressed following treatment and who have no satisfactory alternative treatment options. However, the utility of this biomarker has not been fully demonstrated across all tumors as TMB-H fails to predict improved or clinically relevant response to ICI in all tumor types. Additionally, cancer types where TMB-H does not predict response generally show no relationship between tumor neoantigen load and CD8 T-cell infiltration. Further studies are needed before the application of TMB-H in all cancer types

Results: PD-L1 status by tumor type:

Tumor Type	PD-L1 positive patients	Treatment
Lung	28	17 of the 28 were/are treated with ICI, 2 went with comfort care after staging, 1 is traveling elsewhere for tx, and 2 have EGFR mutations
Breast	7	5 patients pembrolizumab, 3 were Neoadjuvant + Chemo
Colorectal	3	Unable to identify 1 pt. 2 patients are on clinical trials, one including pembrolizumab
Prostate	3	1- SD on pembrolizumab, 1- PD pembrolizumab, 1- SD casodex + leuprorelin
Breast and Ovarian	1	Multiple Lines of Chemo, not given ICI
Gallbladder	1	Tx with chemo
Cervical Cancer	1	Nivolumab- SD x 1.5 years
Endometrial	1	Treated with oral agents only, still alive.
Cutaneous Melanoma	1	Nivolumab/ipilimumab x 2 cycles, switched to Opdualag SD x 13 cycles
Gastric	1	No ICI
Pharynx	1	Chemo + XRT
Tongue	1	No ICI
Carcinoid	1	Pembrolizumab x 5 cycles –PD
Appendix	1	Received 1 cycle of pembrolizumab, Deceased
Unknown	1	Deceased



PD-L1 cfRNA was detected in patient's plasma for a broad range of types of cancer identified by the origin of the primary tumor. The LISA technology detected the presence of circulating PD-L1 RNA in the plasma samples from fifty-two patients. PD-L1 status of the tumor biopsy is unknown for several patients. Selected patients were subsequently treated with pembrolizumab, and clinical outcomes are forthcoming. No severe adverse effects have been observed for patients expressing PD-L1 in blood and treated with pembrolizumab.

Discussion: Detectable PD-L1 mRNA from blood-plasma has emerged as a potential biomarker for response and resistance to immune checkpoint inhibitors (ICIs) in patients with non-small cell lung cancer (NSCLC). The mRNA levels of PD-L1, a protein that is associated with the activation of pathways for proliferation and maintenance, can be detected using the LISA methodology. This druggable molecular biomarker has a profile that is dependent on the tissue of origin, and a high negative predictive value (NPV) for a molecular test of PD-L1 gene levels can accurately identify patients who are not likely to benefit from ICIs and could be spared the risk of autoimmune toxicity. Researchers have calibrated the mRNA assay according to the tumor proliferation score, with different cut-offs for PD-L1 expression levels (1-49% and greater than or equal to 50%). This is the first study, to the best of our knowledge, to evaluate the potential value of circulating RNA transcripts specific for the translation of the PD-L1 protein. Given the promising results, it would be of both academic and clinical interest to conduct a wider scale clinical comparison of patients with paired IHC and ctRNA measurement of PD-L1. Additionally, different levels of control of gene activity, including epigenomic regulation, gene-regulation, copy number variation, intron-exon splicing, post-translational modification, and targeted protein degradation, should be considered in the evaluation of PD-L1 as a biomarker. Overall, testing cancer patients for PD-L1 mRNA levels in their blood plasma has the potential to improve patient selection for ICI therapy and avoid unnecessary treatment-related toxicity. Genomic and clinical information with patient preferences inform clinical decision-making to improve outcomes by matching each patient to a tailored therapy. Currently, the extent to which NGS contributes to patient outcome improvements is under investigation. Furthermore, the insurance coverage of NGS technology and sequence-matched therapies is variable across healthcare systems, payor policies and geographies.

References:

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Abbreviations:

PD-L1: programmed death ligand 1; ICI: Immune Checkpoint Inhibitor; cfNA: circulating cell-free nucleic acid; cfDNA: circulating cell-free DNA; cfRNA: circulating cell-free RNA; PCR: polymerase chain reaction; IHC: immunohistochemistry; CPRC: castration-resistant prostate cancer; HR: hormone receptor; NGS: next-generation sequencing; LISA: linear in-situ amplification; TMB: tumor mutational burden;