Tissue Adenosine Distribution-Guided Gene Selection for the Development of a Composite Biomarker in Immuno-Oncology Therapy

Corinne Ramos Ph.D., Sandra Delebecq MS,
Raphael Legouffe MS, Mathieu Gaudin Ph.D.

Aliri BIOANALYSIS

Introduction

The burgeoning field of Immuno-Oncology (IO) has revolutionized cancer treatment by harnessing the body's immune system to fight cancer. Despite the remarkable successes of IO therapies, including checkpoint inhibitors and CAR-T cell therapies, a significant proportion of patients do not respond to these treatments, underscoring the critical need for predictive biomarkers. Biomarkers that can accurately predict patient response to IO therapy are essential for optimizing treatment strategies, minimizing unnecessary exposure to potentially ineffective treatments, and enhancing patient outcomes.

We set out to introduce a novel approach that transcends traditional methods of predicting patient responses. Recognizing the limitations inherent in relying solely on genetic or proteomic biomarkers, our strategy integrates the spatial distribution of adenosine within the tumor microenvironment (TME) with comprehensive spatial genomic profiling. This innovative methodology aims not only to identify patients who are most likely to benefit from IO therapies but also to unveil the complex interplay between tumor metabolism and the immune system's capacity to combat cancer.

Method

Our methodological approach employs a synergistic integration of Mass Spectrometry Imaging (MSI) and Spatial Transcriptomics to unravel the complex landscape of the tumor microenvironment (TME), focusing on the distribution of adenosine and its relationship with gene expression patterns (Figure 1).

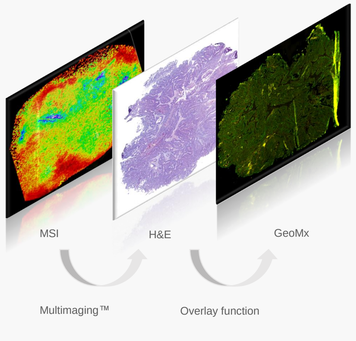


Figure 1 Workflow integrating metabolites analysis and transcriptome assessment; GeoMx: Digital Spatial Transcriptomic Profiler.

Standardized and validated MSI protocol for Adenosine was applied to the tumor sections. Based on the quantitative adenosine maps, tissue regions were selected for subsequent gene expression analysis. This selection was guided by the hypothesis that areas with high adenosine concentration are associated with immune suppression and evasion mechanisms, while low adenosine areas may represent different immunological states.

Adenosine Distribution

MSI data were acquired and analysed with FlexImaging (Bruker Daltonics), Data Analysis software (Bruker Daltonics) and proprietary software MultimagingTM (Aliri France SAS v1.2.6.1). Figure 2 accentuates the adenosine distribution in different human tumor samples, with the molecular signal's intensity scales in each image finely adjusted. This ensures a clear distinction between noise and genuine molecular signals and enhances the overall clarity of the signal's depiction across the sections.

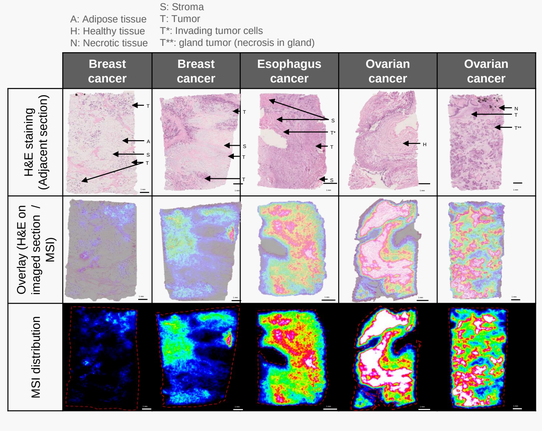


Figure 2 Quantitative mapping of adenosine distribution in tumor tissues, showing the levels of adenosine in various regions of the tumor and the tumor microenvironment (TME).

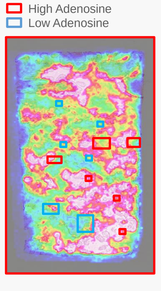
Based on the MSI data set on the tissue sections and the dilution series, the quantitation of the test items was performed with the internal standard approach. MultimagingTM software was used to normalize the signal of the test item in the spectra by the signal of the internal standard (stable isotopic labeled compound sprayed onto the tissue sections in the MALDI matrices). A correlation between the calibration curve and the signal obtained on the tissues was then performed to determine the concentration of the test items per histological structure in µM (Table 1).

Indication	Histological Structure	Adenosine quantification obtained by qMSI* (µM)
Bladder cancer	Entire tissue	70,9
	invading tumor cells	48,6
	Healthy tissue	71,7

Table 1 Adenosine quantification in tissue samples

ROIs Informed Transcriptomics Analysis

Utilizing the data from the quantitative maps, specific tissue regions of interest (ROIs) have been chosen for further gene expression analysis. The selection process is informed by the theory that regions exhibiting high concentrations of adenosine are linked to mechanisms of immune suppression and evasion, whereas regions with lower levels of adenosine could indicate varied immune states.



Indication	ROIs	Adenosine quantification obtained by MSI* (µM)
Bladder	High-1	162,3
	High-2	159,9
	High-3	148,0
	High-4	139,8
	Low-1	69,8
	Low-2	77,3
	Low-3	50,6
	Low-4	50,0

Figure 3 Selection of ROIs Based on Adenosine Levels for Transcriptome Analysis.

Comparative whole transcriptome analyses were conducted between areas with high and low adenosine (ADO) concentrations. Principal Component Analysis (PCA) and clustering were applied to each condition separately, emphasizing the gene expression divergence or similarity between the high and low ADO regions of interest (ROIs). PCA plots reveal a distinct segregation between regions of high and low adenosine (ADO) concentration in ovarian cancer samples.

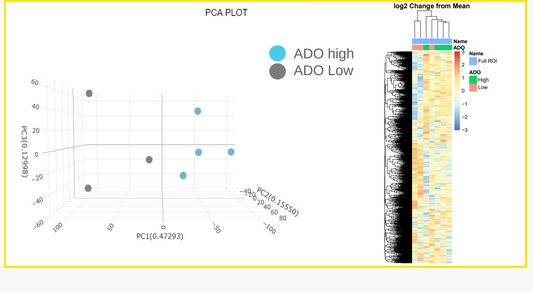
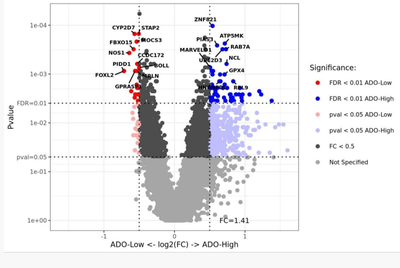


Figure 4 PCA and Heat map representing the unsupervised clusterization of the samples into the two phenotypes ADO high and ADO low.

Gene Expression Profile

Integrating adenosine concentration data with gene expression profiles, we performed a correlation analysis to identify adenosine-driven gene signatures. This analysis pinpointed a set of genes whose expression levels were significantly correlated with adenosine concentrations, particularly those involved in T-cell exhaustion and metabolic adaptation. A LMM (Linear Mixed Models) statistical test, accompanied by a Benjamin-Hochberg (BH) correction, was successfully conducted to identify statistical differences in individual targets between the two phenotypes: ADO high and ADO low.

A



B

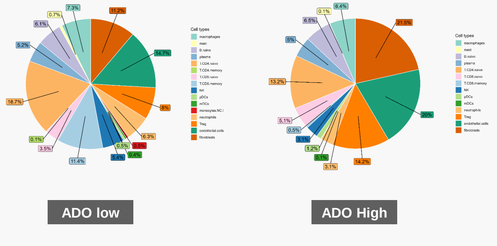


Figure 5 A. Volcano plot representing the gene expression modulation between the two phenotypes ADO high and ADO low. **B.** Pie plot of cell type proportion in the TME of each tested patient.

Conclusions

Our results illustrate the profound impact of adenosine concentration on the immune landscape of TME and underscore the utility of integrating metabolic and transcriptomic data to identify predictive biomarkers for IO therapy. The identified adenosine-driven gene signatures offer a novel avenue for patient stratification and highlight potential targets for therapeutic intervention, paving the way for more personalized and effective cancer treatment strategies.