White Paper

Validating the efficacy of a compound with spatial imaging

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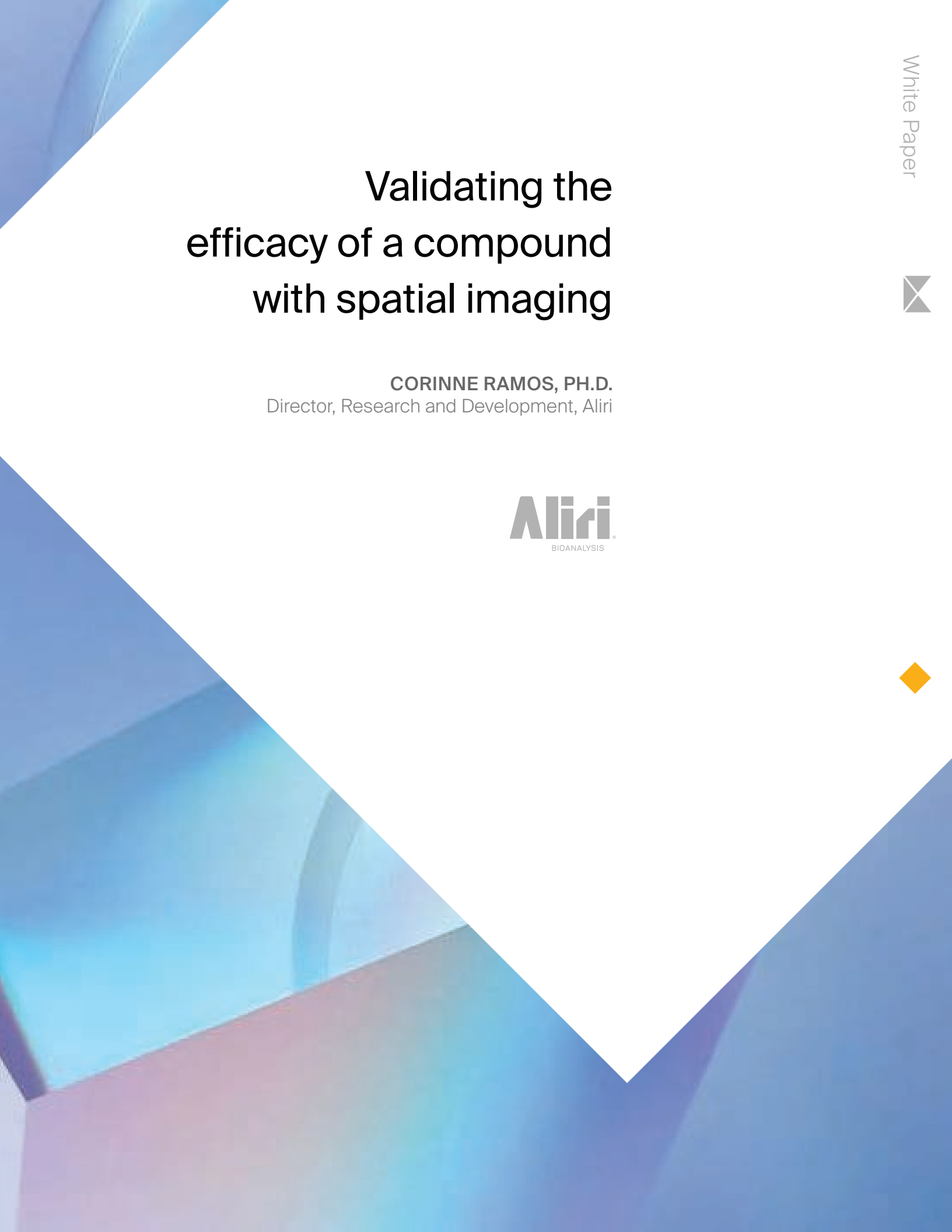
BIOANALYSIS



SUMMARY

In the current drug development environment, sponsors need to efficiently generate quality efficacy data to assess drug candidacy. In precision medicine, and in oncology specifically, this process requires an understanding of the potential disease heterogeneity across and within patients and the changes that occur upon drug administration within the tumor microenvironment. Spatial analysis tools offer a molecular look into the interactions between cells within the context of the disease and provide powerful visualizations into the changes that occur upon drug administration. The benefits of efficient target and efficacy validation can translate into substantial savings for sponsors and a substantial market advantage over non-precision medicines due to shortened development and approval timelines.

CONTENTS

PRECISION MEDICINE DEVELOPMENT AND THE ADDED VALUE OF SPATIAL ANALYTICS

Precision medicine is founded upon the understanding that a patient's individual genetic and other specific characteristics affect their disease, response to treatment, and outcomes. Ultimately, the goal of precision medicine is to provide the right therapy to the right patient at the right time.[1]

The development of a strategy for establishing drug mechanism of action (MOA) is vital to the success of precision medicine. Spatial analysis supports drug evaluation strategy and comprises three pillars: 1) Advanced imaging technologies, 2) In-context multi-omics, and 3) Use of artificial intelligence (AI) to find patterns and make predictions.

Next-generation spatial imaging tools (e.g., mass spectrometry imaging and NanoString's GeoMx Digital Spatial Profiler) facilitate the deep-profiling of tissues and provide options for visualizing RNA, protein and peptides, small molecules, and metabolites. Data from each of these tools can be mined through data analysis supported by AI to improve understanding of the disease and the impact of drugs within the spatial and clinical contexts.

In the discovery phase, the added value of spatial omics and spatial technology is realized during disease characterization and target identification, as well as in the evaluation of drug distribution. In the development phase, spatial analysis tools inform the understanding of a drug's MOA, pharmacodynamics relative to dose response, and drug and metabolite biodistribution for determining toxicity in targeted or other tissues. In the clinical phase, the added value of spatial technology comes from the elucidation of the prognostic relationship, and the identification of patients with the best chance for response.

DISEASE HETEROGENEITY: WHAT IS IT? WHY IS IT IMPORTANT? HOW DO WE VISUALIZE IT?

To understand the importance of spatial technology, one must understand the impact of disease heterogeneity. Most human solid tumors display differences in many features, including cellular morphology, gene expression, metabolism, motility, and angiogenic, proliferative, immunogenic, and metastatic potential. These differences can predict disease development and therapeutic potential.[2]

Spatial analysis supports the understanding of disease heterogeneity by allowing deep-profiling and comparison of biologically relevant regions of interest on the tissue biologically relevant. Regions of interest are identified through fluorescent markers and findings can be compared across areas of the tumor or surrounding tissue (see Figure 1). Imaging can offer important information on the upregulation and/or downregulation of specific genes and differences across samples from a single tumor or multiple patients.

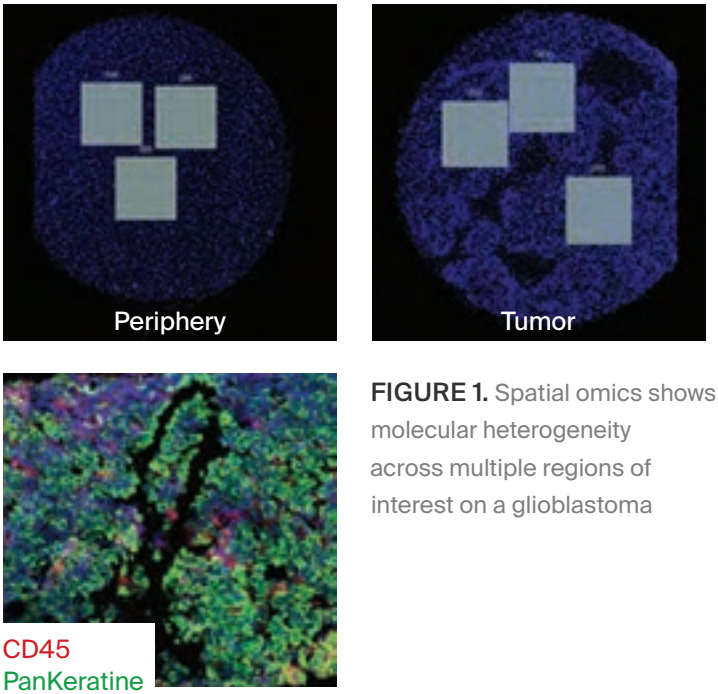


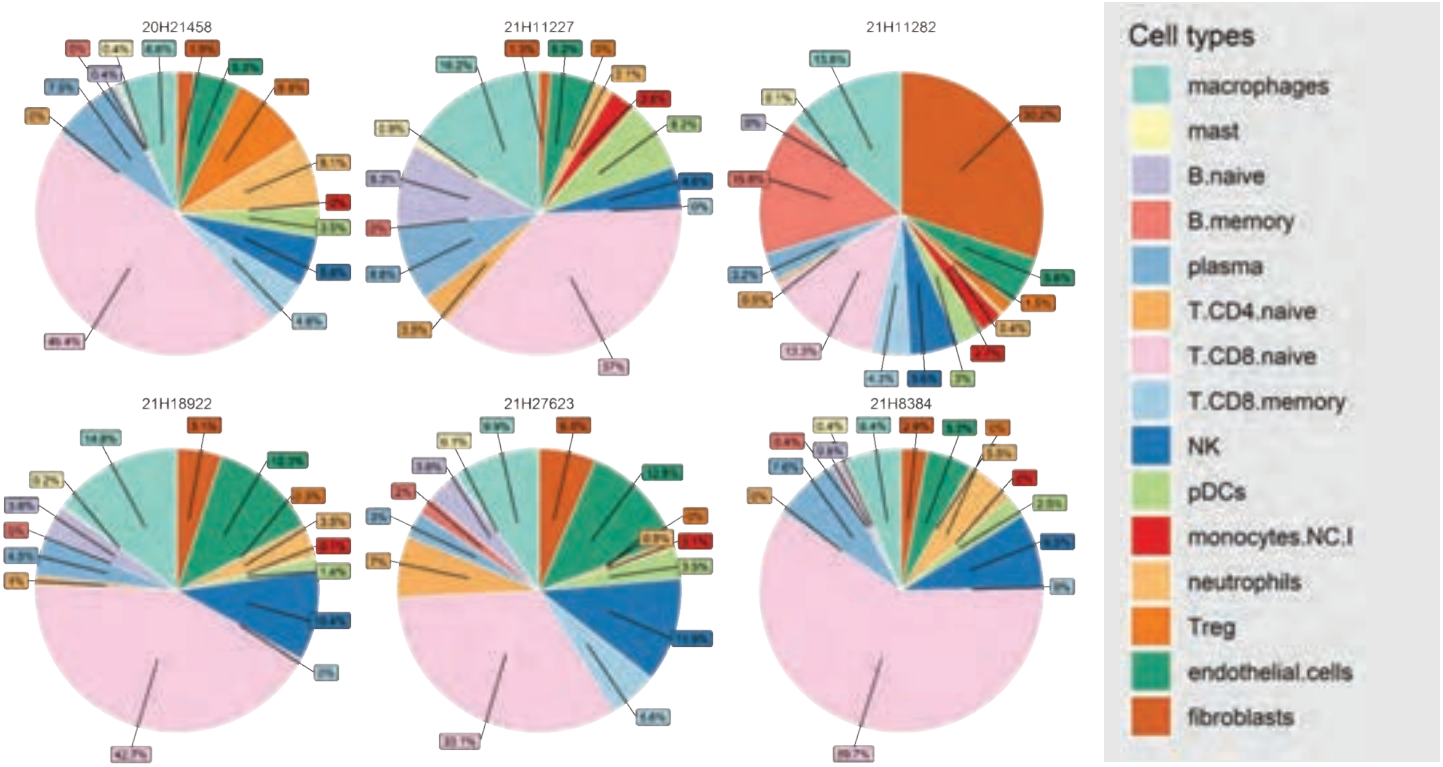
FIGURE 1. Spatial omics shows molecular heterogeneity across multiple regions of interest on a glioblastoma

TUMOR CELL INFILTRATION HETEROGENEITY

Further study of the example in Figure 1 showed little heterogeneity of gene expression in the periphery cells and much heterogeneity within the tumor. This heterogeneity can be translated to an understanding of the tumor microenvironment which can be visualized through deconvolution, computational techniques aiming at estimating the proportions of different cell types in samples collected from a tissue[3] (see Figure 2).

Deconvolution can improve the interpretation of omics data and mitigates the effects of heterogeneity.[4] Cell type composition across samples can vary greatly and inform the choices for drugs to pursue as candidate therapies for the disease. In the example provided below, spatial analysis of the tumor microenvironment demonstrated that one patient with glioblastoma had a marked infiltration of fibroblasts. Fibroblasts are not part of the current treatment focus for glioblastoma, but could eventually become a new drug target if supported by further research.

FIGURE 2. Deconvolution shows heterogeneity of the microenvironment across 6 glioblastoma samples



TARGET DISCOVERY AND DEVELOPMENT OF COMPOSITE BIOMARKERS

A major challenge for most drug development companies is the need to evaluate potential molecules while at the same time maximizing investments. Failed targets are costly in dollars and time, and solutions to identify drug targets more efficiently save companies time and money and bring needed treatments to patients faster. Spatial imaging analytics tools provide added value to the early development process in that they intrinsically support more efficient target identification. Imaging Mass Cytometry platform can analyze more than 40 protein markers on the same tissue section. Staining different sections of the biopsy with antibodies results in a heat map and can show clusters of differentiated up- and downregulated protein expression across the tissue. Dimensionality reduction methods such as uniform manifold approximation and projection (UMAP) can clearly show clustering and have demonstrated that expression of certain proteins is very specific to the indication (e.g., bone, lung, brain, or prostate cancers). This approach further aids new potential drug target identification within the context of the complexity of a specific disease.

Spatial imaging analytics tools provide added value to the early development process in that they intrinsically support more efficient target identification. Imaging Mass Cytometry platform can analyze more than 40 protein markers on the same tissue section.

Interestingly, target discovery can be further bolstered by combining data from multiple platforms to generate a composite biomarker. For example, mass spectrometry imaging combined with the GeoMx DSP allows the user to visualize the tissue, identify regions where drug accumulation is observed, and spatially profile the molecular heterogeneity in terms of genes and protein content (see Figure 3).

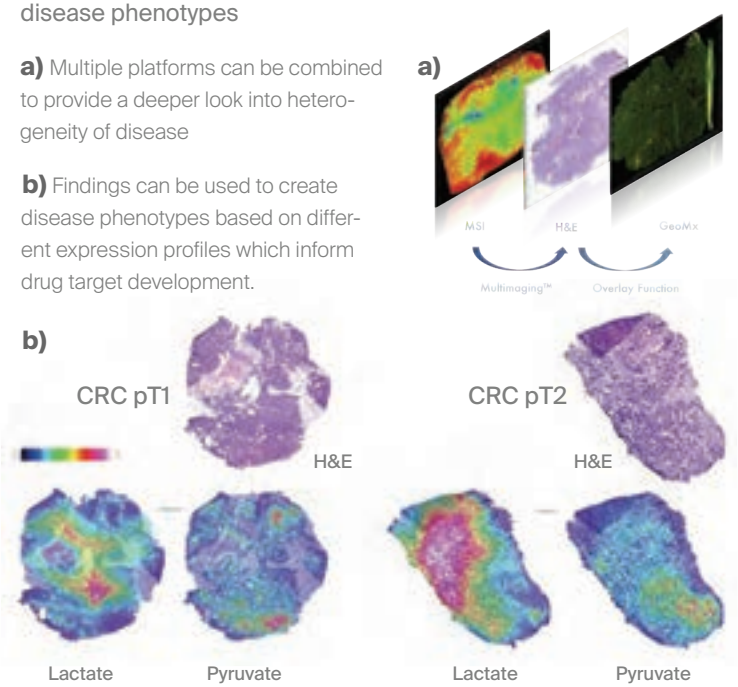
The example provided in Figure 3 highlights distribution of two different metabolites (lactate and pyruvate) in two patients with different stages of colorectal cancer (CRC). The molecular images generated through mass spectrometry imaging showed that pyruvate and lactate were differentially expressed across the samples, revealing two distinct metabolic phenotypes. When phenotypes like these are identified, they can undergo deep genomic and protein profiling using the GeoMx platform, resulting in multiple protein profiles and gene expression data. A heat map can demonstrate differential expression of proteins using protein panels related to the immune subtype, as well as immune activation. This allows for isolation of the "hot phenotype," or that which has high immune cell infiltration. This is an incredibly powerful finding, because it contributes to an understanding of the resistance mechanism and provides information on the efficacy of treatments at the site of action. Identifying a new potential drug target that could convert "cold phenotypes" to "hot phenotypes" may make patients more susceptible to the drug.[5]

The combined platforms facilitate deconvolution of gene expression, allowing developers to profile up to 18,000 genes within the region of interest and evaluate the impact of metabolite distribution on the tumor microenvironment cell distribution.

FIGURE 3. Multiple platforms allow for definition of disease phenotypes

a) Multiple platforms can be combined to provide a deeper look into heterogeneity of disease

b) Findings can be used to create disease phenotypes based on different expression profiles which inform drug target development.



CRC: colorectal cancer, H&E: hematoxylin and eosin imaging, MSI: mass spectrometry imaging

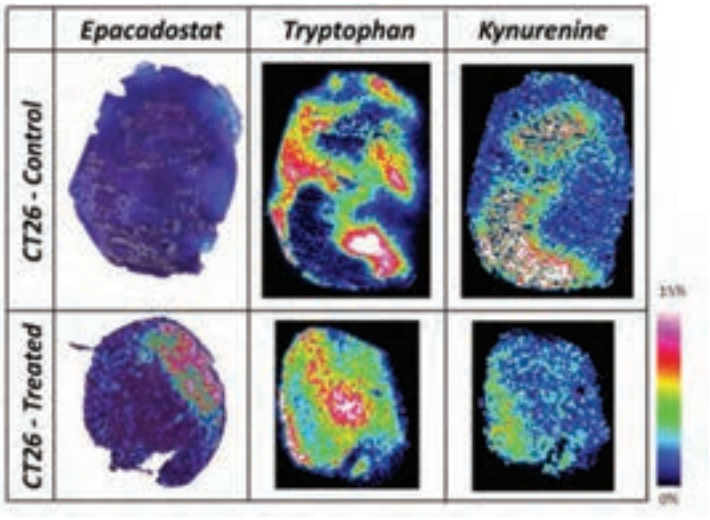
EARLY DEVELOPMENT WHOLE BODY PHARMACOKINETICS

Of course, developers must ensure that their potential drug is viable from a safety perspective. Mass spectrometry imaging brings a powerful capability to very early-stage development through its imaging of drug distribution and direct target engagement. Whole body tissue distribution of drugs and metabolites has been successfully demonstrated in mouse and rat models.[6,7] This process provides monitoring capabilities over several organs in one dataset at different time points. Understanding drug accumulation in the organ of interest is very important, but it is also important to identify unexpected accumulation in other organs to ensure safety concerns are addressed early.

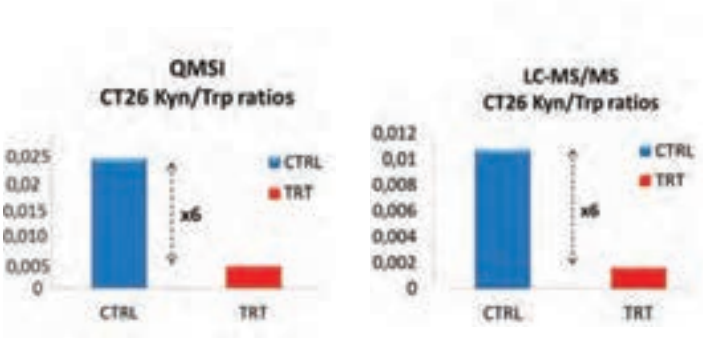
ASSESSING DRUG EFFICACY: AN EXAMPLE IN ID01 INHIBITORS

Spatial relationships and interaction of drugs with their targets are critical to understanding the potential for efficacy in a new precision medicines candidates and for reducing early development time. In one example, spatial analysis was used to elucidate relationships between metabolites transformed by the enzyme ID01 to ultimately determine the efficacy of an ID01 inhibitor drug. ID01 transforms tryptophan into kynurenine. Using a mouse model, mass spectrometry imaging and liquid chromatography with tandem mass spectrometry (LC-MS/MS) were used to not only monitor drug distribution, metabolite distribution, and endogenous quantity in the mouse model,[8] but also to estimate the pharmacological efficacy of the ID01 inhibitor through a target exposure study in various regions of interest. Interestingly, the LC-MS/MS and quantitative mass spectrometry imaging (QMSI) showed similar results in terms of quantification of the drug, but the QMSI was also able to provide insight into the heterogeneity and distribution. A tool was then developed to assess the ratio of kynurenine to tryptophan in treated and untreated tissue samples to evaluate efficacy of the ID01 inhibitor (see Figure 4).

FIGURE 4. Spatial imaging supports a tool for determining efficacy of an ID01 inhibitor by monitoring ratios of effected metabolites



Kynurenine to tryptophan ratios as an efficacy tool



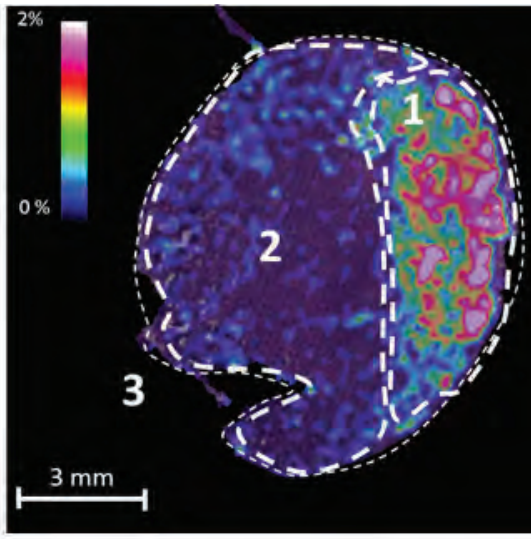
Kyn/Trp ratio shows the IDO1 activity level

Overlay of immunohistochemistry (IHC) data with the target shows different regions of distribution and absolute quantification at the site of action (see Figure 5). With heterogeneous tissues come heterogeneous distributions of drugs and biomarkers; thus, this capability of monitoring whether the drug reaches its site of action is highly useful in directing drug development strategy.

FIGURE 5. Overlay of QMSI and IHC imaging provides data on drug quantification and distribution

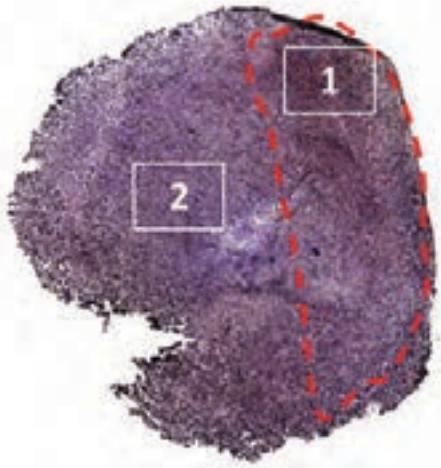
QMSI & IMMUNO-HISTOCHEMISTRY

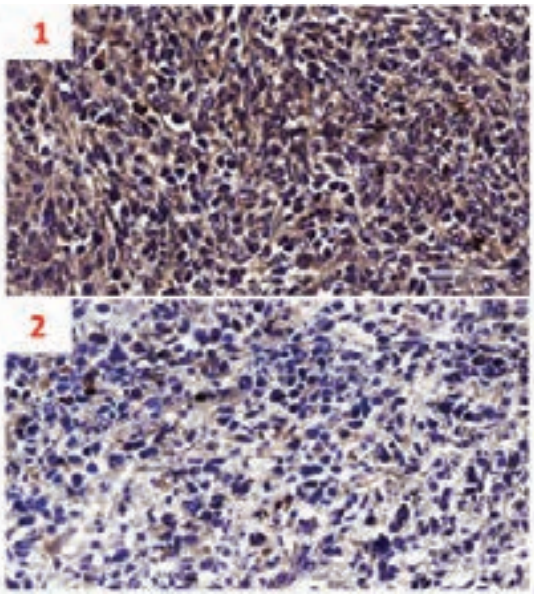
A



	EPA		
	ROI 1	ROI 2	ROI 3
Surface percentage	38 %	62 %	100 %
Quantity per ROI	68 µg/g	25 µg/g	42 µg/g
Quantity in entire section	61 %	39 %	100 %

B





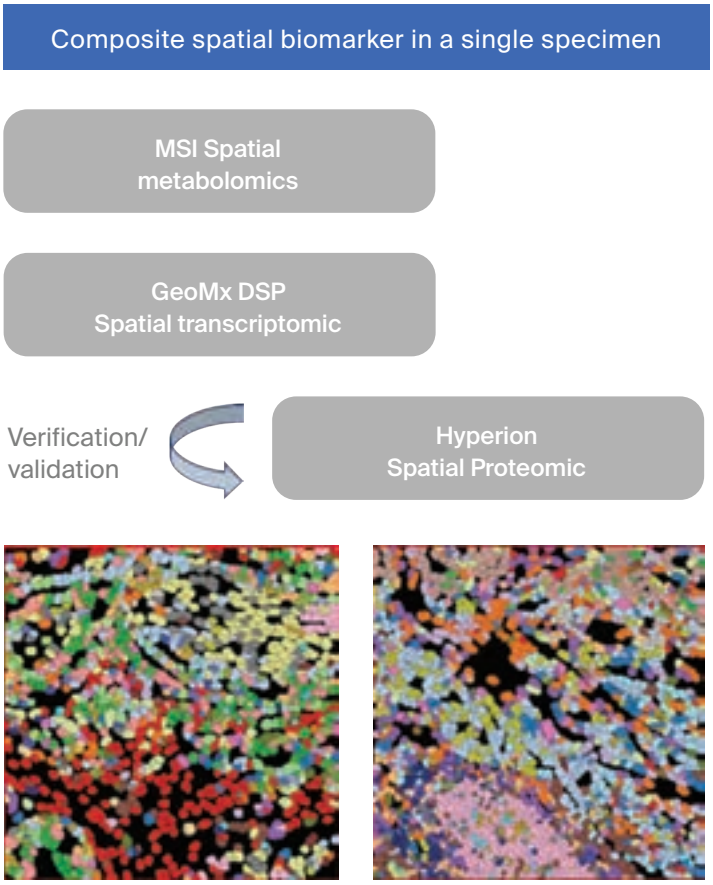
In summary, spatial bioanalysis can provide information on the bioavailability, drug biodistribution, targeted tissue exposure at the cellular level, and on-tissue pharmacokinetics and pharmacodynamics. It allows for monitoring of the drug effects through biomarkers and visualization of drug compound concentrations in tumor samples.

RESPONSE PREDICTION

A critical part of precision medicine is predicting the treatment response and finding ways to ensure or improve effectiveness for a given patient or type of patient. Spatial imaging platforms provide data that inform the understanding of what molecular mechanism(s) could be responsible for the non-response of the treatment. These platforms offer visualization of upregulation and downregulation of genes and changes in cell type distribution related to the treatment response. The visualizations further allow developers to determine the exact mechanism of action in the context of the disease.

Toward precision medicine, the identification of specific signatures that are associated with patients that are likely to respond to the therapy can be achieved through staining of samples from responders and non-responders. The signatures can comprise genes that are related to the prediction of response to immune checkpoint inhibitor therapies and can aid in the identification of targetable pathways in non-responders. Spatial analysis (see Figure 6) further elucidates where and how cells are interacting around the site of action to better predict response to therapy.

FIGURE 6. Imaging mass cytometry showing differences in responder and non-responder specimens



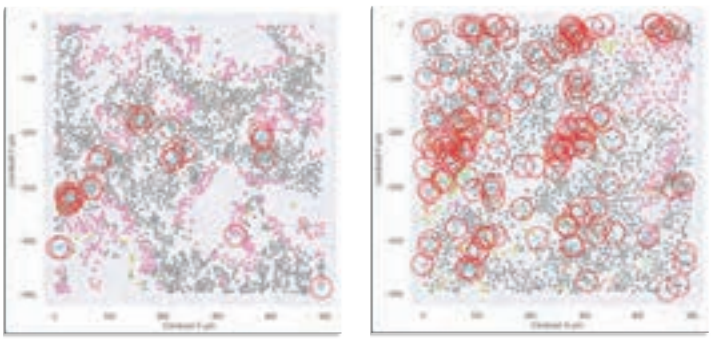


FIGURE 7. Spatial analysis shows cell interactions which are predictive of therapy response

Phenotype hub to identify therapeutic target

SUPPORTING REGULATORY SUBMISSIONS FOR PRECISION MEDICINES USING SPATIAL ANALYSIS

This paper has discussed several powerful spatial analysis tools that help drive more efficient drug development in both early and late development. It is important to understand the value of these analytic approaches in terms of their ability to produce high quality evidence to support filing documents with the speed demanded by today's regulatory environment. The US Food and Drug Administration acknowledges the potential of precision medicine to create "powerful new discoveries and FDA-approved treatments that are tailored to specific characteristics of individuals..."[9] Indeed, the proportion of FDA drug approvals and the speed at which they have been approved over the past decade demonstrates FDA's commitment to addressing integrating targeted therapies into clinical care.[10]

From a regulatory perspective, careful validation of the drug target, patient population and drug efficacy are important aspects of successful Investigational New Drug Applications (IND), New Drug Applications (NDA), and Clinical Trial Applications (CTA). While other precision medicine techniques have supported regulatory filings for some time now, the accuracy with which spatial analytic tools can visualize these data *in the context of patient response* facilitates rapid, high-quality evidence generation to support the regulatory agencies' understanding of the potential benefits of the drug. From a business perspective, investments into spatial analysis can direct investment decisions when it comes to choosing potential new precision medicines, and the market advantages gained through shortened development and regulatory review for precision medicines compared to non-precision medicines cannot be overlooked.

Most importantly, the power of spatial analysis to identify which patients will benefit most from a precision medicine suggests that patients could streamline some of the trial-and-error inherent in the treatment journey. Ultimately, the overall success of a drug is dependent upon its success in providing positive outcomes for patients. The more quickly and accurately those outcomes can be reached, the better for everyone.

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