TumorMapr™ Analytical Platform

An Unbiased Spatial Analytics and Explainable Artificial Intelligence Platform for Generating Data, Extracting Information and Creating Knowledge from Multi to Hyperplexed Fluorescence and/or Mass Spectrometry Image Datasets

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The current computational analyses of multi to hyperplexed fluorescence and/or mass spectrometry image datasets from patient pathology samples are not powerful enough to extract the maximum amount of information or to create the detailed knowledge that is required to advance precision medicine in pathology, including the development of personalized therapeutic strategies, identification of potential novel targets for drug discovery, selection of optimal patient cohorts for clinical trials, and improvement of the predictive power of prognostics/diagnostics. SpIntellx meets this challenge by harnessing the computational power of proprietary, unbiased spatial analytics, spatial systems pathology, and explainable artificial intelligence (xAI) to extract information and to create knowledge from patient primary disease pathology samples imaged on any of the existing fluorescence and/or mass spectrometry imaging platforms. SpIntellx provides TumorMapr-Basic and TumorMapr-Advanced as a pipeline of critical solutions to the current challenges for precision medicine applied to solid tumors and other diseases.

I. **Background**

There is a consensus that for precision medicine to fully succeed, the field must look beyond genomics in developing personalized therapeutic strategies, identifying potential novel drug targets, selecting the optimal patient cohorts for clinical trials and improving the predictive power of diagnostics and prognostics (*1*). In the case of solid tumor cancers, a major issue is that patients do not respond predictably to treatment. It is estimated that 90% of drugs are effective for fewer than 50% of patients (*2*), leading to unnecessary side effects while failing to prevent disease-related morbidities and mortalities. A fundamental driver of these unpredicted inefficiencies is the heterogeneity in cellular composition and signaling networks among cells that is intrinsic to every cancer and is observed both across patients with the "same" tumor type (i.e., intertumor heterogeneity) and within a single tumor specimen (i.e., intratumor heterogeneity). The evolving cellular microenvironments and their dynamic molecular pathway interactions (*3*) determine the extent of spatial intratumor heterogeneity, which is key to an individual tumor's ability to evade immunity or hinder therapy to progress and eventually metastasize. To date such changes have been coarsely profiled at the population (bulk) level through genomics for developing prognostic and drug response biomarkers, but this can mask subtle spatial intercellular variations that are both functional and clinically relevant (*4-5*). In solid tumor cancers, substantial evidence exists across several tumor types to indicate that spatial intratumoral heterogeneity among malignant and non-malignant cells, and their interactions within the tumor microenvironment, can cause treatment failures (*4*).

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Figure 1. Spatial Intelligence and Explainable Artificial Intelligence Revolutionizes Computational and Systems Pathology for Precision Medicine. SpIntellx holds a very strong intellectual property position in Unbiased Spatial Analytics (Functional Cell Phenotyping, Microdomain Discovery), Spatial Systems Pathology, and Explainable AI (xAI) that allows the creation of deep knowledge and powerful applications in precision medicine.

Recent advances in regional and single cell genomics have demonstrated tumor heterogeneity even within a single solid tumor (*6*). However, there are important limitations to using regional/single cell genomic methods. The most significant limitation is the importance of preserving spatial microenvironments (i.e., microdomains) within tumors that can contain distinct cancer cells, stromal cells and immune cells, as well as cells in distinct states or in transition. Therefore, the spatial context of all of these cells is critical for the proper analysis of each patient's tumors. Removing tissue regions or single cells for genomic analysis perturbs intra- and intercellular regulation, along with the spatial context upon which this regulation is dependent. In addition, the process of regional and/or single cell genomics from tumor samples adds time and cost to perform the patient analysis.

The field of Digital Pathology was initially developed based on the projected value of acquiring digitized images of clinical specimens to allow pathologists to "read" hematoxylin and eosin stained (H&E) and immunohistochemistry (IHC) pathology slides on a high-resolution monitor in order to reduce eyestrain and to efficiently share images for consults and telemedicine. Generating digital image data opened the way for the field of Computational Pathology that started by performing simple image analysis on transmitted light images based on H&E and IHC, such as detecting nuclei, counting cells and cellular structures and labeling specific cell types. Currently, multiple companies offer transmitted light and fluorescence/mass spectrometry reagents that identify specific cells/structures and imaging platforms with very basic computational software. Major pathology organizations (DPA and USCAP) have shown tremendous interest in computational pathology, starting with applications applying transmitted light with H&E and IHC labeling.

Digital pathology is developing rapidly with the advent of clinical-grade imaging systems approved by FDA as class II medical devices and with increased utilization during the COVID pandemic. Accelerating this trend is the emergence of more powerful computational pathology machine learning algorithms using artificial intelligence, that pathologists are beginning to recognize as an important new aid in the workflow. Pathologists previously had access to basic digital image analysis tools for transmitted light microscopy for both H&E and IHC, but these early tools were of limited clinical utility, due to factors such as a lack of a digital pathology infrastructure and insufficient gains in efficiency. Further, while multiplexed fluorescence applications were

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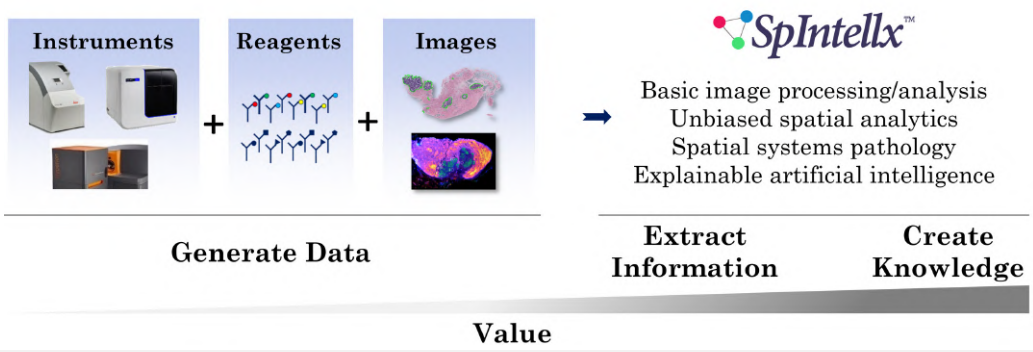

Figure 2. Strategies Involved in Developing the SpIntellx TumorMapr Analytical Platform. There are now multiple imaging and reagent platforms that can generate huge multi-hyperplexed image datasets, but they have very limited analytical software tools. SpIntellx offers TumorMapr-Basic that performs basic image processing and analyses on raw image datasets. SpIntellx then applies TumorMapr-Advanced that harnesses the computational power of proprietary **unbiased spatial analytics** with automated functional cell phenotyping and microdomain discovery, **spatial systems pathology,** and **explainable artificial intelligence (xAI)** to extract information from pathology samples imaged on any fluorescence and/or mass spectrometry platform to create predictive knowledge that is required to develop personalized therapeutic strategies, identify potential novel targets for drug discovery, optimally select patient cohorts for clinical trials, and improve the predictive power of prognostics/diagnostics.

previously possible, adequate computational pathology tools for sophisticated analyses or correlation with transmitted light features were not yet available. Now there are many multi- to hyperplexed platforms available based on fluorescence and mass spectrometry imaging and there is a clear opportunity for advanced computational pathology applied to precision medicine (*7*).

Despite the great potential, the current approach of computational pathology with multi-hyperplexed datasets usually involves simple quantitation and "black box" artificial intelligence (AI). This article discusses the importance of advanced spatial analytics and explainable AI (xAI) for computational and systems pathology applied to fluorescence and/or mass spectrometry image datasets in order to create the knowledge required to develop personalized therapeutic strategies, identify potential novel targets for drug discovery, optimally select patient cohorts for clinical trials, and improve the predictive power of prognostics/diagnostics.

As a pioneering force, SpIntellx has made significant contributions to advance the application of spatial intelligence and xAI in computational and systems pathology and holds a significant intellectual property position in unbiased spatial analytics, spatial systems pathology and xAI for fluorescence/mass spectrometry imaging and transmitted light applications that are creating powerful predictive knowledge for precision medicine **(Figure 1)**. A white paper on HistoMapr™ (*8*), the SpIntellx platform for transmitted light, will be released separately.

Multiple commercial platforms that generate unprecedented, complex multi-hyperplexed image data sets are now available in the market, but for the most part the extraction of information and creation of knowledge is left largely under-explored as the complexity of the analytics are either significantly reduced and/or are impenetrable to the simple analysis tools that have been available. SpIntellx came to the market at this critical time with a systematic solution for the multiple challenges facing computational and now computational and systems pathology applied to multi to

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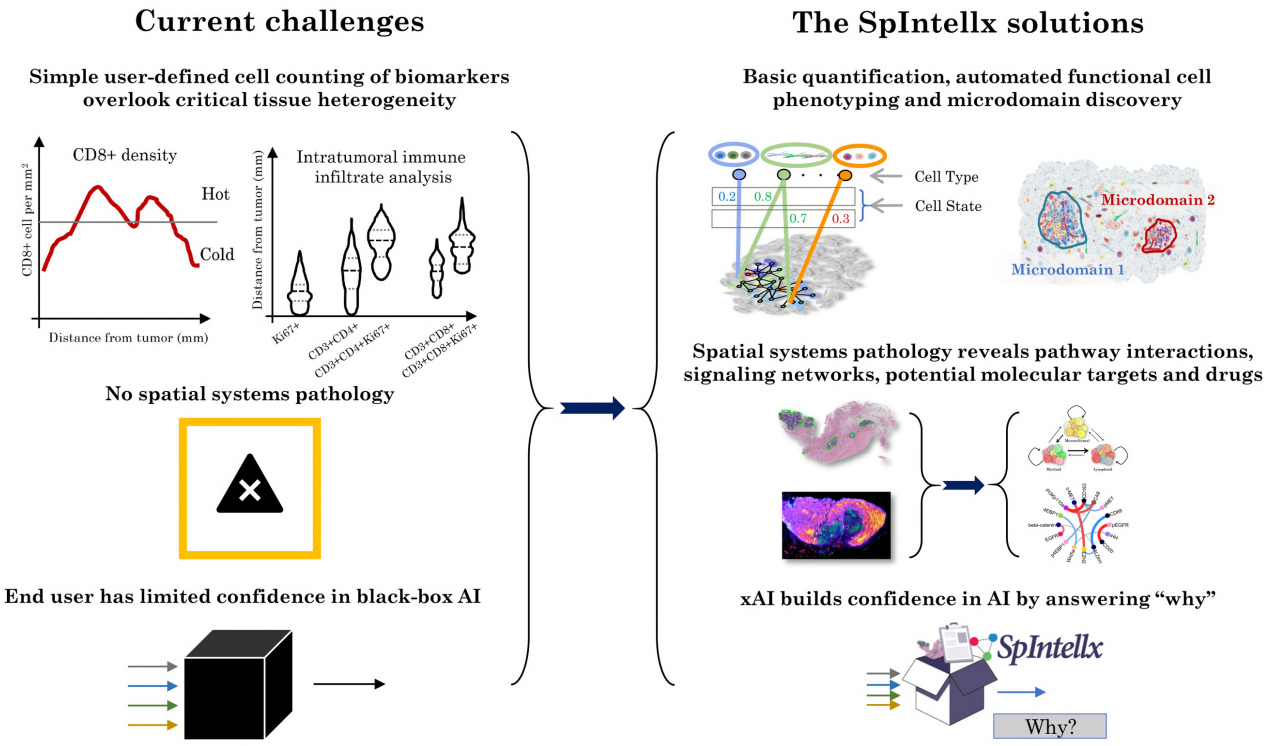

Figure 3. SpIntellx Approach to Precision Medicine. (Left panel) Current genomic and simple computational pathology software present a series of challenges that hinder the advance of precision medicine. (Right panel) The application of unbiased spatial analytics (automated functional cell phenotyping and microdomain discovery which define spatial relationships and identify critical tissue heterogeneity), spatial systems pathology and xAI provide a series of critical solutions to the challenges and meet rigorous clinical needs for precision medicine applied to solid tumors and other diseases.

hyperplexed labeling of tissue samples. TumorMapr, developed by SpIntellx, integrates basic image processing/analyses applied to raw image datasets **(TumorMapr-Basic),** as well as unbiased spatial analyses, spatial systems pathology, and xAI **(TumorMapr-Advanced)** applied to the TumorMapr-Basic processed images **(Figure 2)**.

In contrast to simple measurements of user-defined biomarkers based on an arbitrary threshold for determining expression of the biomarker as yes (presence) / no (absence), SpIntellx applies unbiased spatial analytics to quantify the spatial relationships between cells and tissue structures in whole slide images and/or large bore tumor microarrays (TMA's). This approach generates data that accurately reflects the true complexity and heterogeneity of the clinical specimen. Next, through spatial systems pathology and xAI, these high-resolution data enable the extensive and automatic characterization of the clinical sample at the molecular level to allow information on molecular events and network interactions to be extracted, thus creating the high-confidence predictive knowledge that is required to develop personalized therapeutic strategies, identify potential novel targets for drug discovery, optimally select patient cohorts for clinical trials, and improve the predictive power of prognostics/diagnostics **(Figure 3).** In summary, SpIntellx solutions go beyond simply **generating** data and **extracting** information from data by **creating** the high-value predictive knowledge essential to precision medicine.

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II. The TumorMapr Platform from SpIntellx with Unbiased Spatial Analytics and Explainable Artificial Intelligence is Revolutionizing Computational and Systems Pathology for Precision Medicine

Despite the progress in computational pathology applied to multi-hyperplexed fluorescence and mass spectrometry imaging, serious challenges remain in three major areas: 1) biased simple user-defined cell counting of biomarkers fail to capture critical aspects of spatial tissue heterogeneity; 2) inability to identify key molecular events and signaling networks in the absence of spatial systems pathology; and 3) reliance on black-box AI, whose inputs and operations are invisible to users, and the subsequent lack of confidence by clinical experts in the "decisions" suggested by the AI software **(Figure 3**).

The challenges identified above hinder the implementation and progression of precision medicine by failing to recommend personalized therapeutic regimens, lacking the ability to identify potential novel molecular targets, having unsatisfactory prediction accuracy for diagnostics and prognostics and resulting in inefficient clinical practice/workflow **(Figure 4, upper panel).** Rising to address these challenges, SpIntellx has built a computational and systems pathology analytical software pipeline that directly address all of the challenges mentioned above **(Figure 3, right panel, Figure 4 lower panel).** The SpIntellx unbiased spatial analytics approach combines basic quantification, automated functional cell phenotyping and microdomain discovery to define spatial relationships of various cell types to identify and characterize tissue heterogeneity. The unbiased spatial analytics approach goes beyond basic quantitation by performing automated functional cell phenotyping and microdomain discovery. A single tumor sample may contain multiple, distinct microdomains. The number and the type of microdomains determine the extent of heterogeneity of a clinical specimen. A thorough and accurate characterization of tissue heterogeneity based on microdomain discovery is absolutely necessary for the subsequent spatial systems pathology analyses.

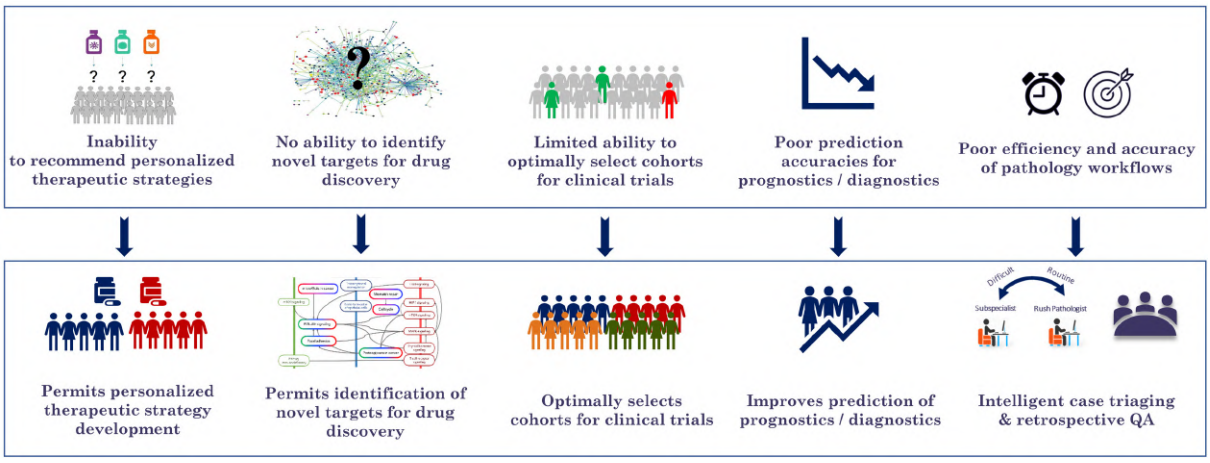

Figure 4. Performance Comparison – Competition (Top) vs SpIntellx (Bottom). The SpIntellx Approach Results in New and Improved Applications for Precision Medicine and Drug Discovery. SpIntellx allows for the development of personalized therapeutic strategies, identification of novel targets in drug discovery, optimization of cohort selection for clinical trials, improvement of disease prognoses/diagnoses, and increased efficiency and accuracy of pathology workflows.

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Analytics	Competitors/Limitations	SpIntellx
Basic image processing and image analyses	• Most platforms and software providers have this capability	• TumorMapr-Basic is the front end of the analytics that performs the basic image processing and simple analyses
Spatial identification of cell types and states (phenotypes)	• Based only on positive and negative signals from pre-selected biomarkers • Cell types identified without complete spatial context • Cannot identify hybrid and transitional cells	• Automated functional cell phenotyping based on the full intensity scale of the complete panel of biomarkers and their spatial relationships • Identifies a continuum of cell types and states including cells undergoing transformation (epithelial -mesenchymal transition, hybrid cell -fusion) critical to disease progression
Identification of microdomains	• Quantify the density of cell types derived from selected biomarkers using a grid -based analysis of the whole slide image • Microdomains identified without accounting for spatial relationships between biomarkers • Limited ability to identify critical intratumor heterogeneity associated with disease outcomes	• Based on spatial network analysis of cell -cell interactions • Discovers unique spatial collection of functional cell types and cell states that may share a common program — e.g., geographical feature in the tissue that recruits and activates adaptive immune cells affecting overall survival and responsiveness to therapy
Characterization of pathway interactions and network biology in microdomains	• No microdomain -specific pathway interactions and network biology revealed across all biomarkers within a pathology sample	• Identifies patient -specific pathway interactions, signaling networks, potential molecular targets and drugs defined within microdomains driving disease progression
Use of AI to create knowledge	• "Black box AI" simply presents results produced by algorithms with no explanation of how it was derived • End-users must blindly trust the algorithms • Summary "reports" only yield data and information with limited interpretable and predictive knowledge	• Explainable AI presents results produced by algorithms but also explains why a particular recommendation has been made • End-user can accept or reject the results which builds trust and confidence in the algorithms • Summary "guides" yield actionable knowledge in clinically recognized language

Table 1. Computational and Systems Pathology Analytics from SpIntellx vs Competitors.

Next, in order to arrive at the most optimal recommendations based on machine learning and AI, critical molecular, signaling and cell-to-cell interactions must be determined using spatial systems pathology based on the high-resolution data produced from unbiased spatial analytics. Finally, the xAI supported interface allows seamless interactions between end-users (oncologists/pathologists) and the software, to provide explanations for recommendations made by the algorithms and to build confidence in the platform **(Figure 3, right panel)**. These powerful technologies are revolutionizing the impact of pathology on precision medicine **(Figure 4, lower panel).**

The SpIntellx TumorMapr platform consists of five integrated key components, **basic image processing and image analysis (TumorMapr-Basic)**, as well as **unbiased spatial analytics** for generating data from clinical samples, **spatial systems pathology** for extracting critical information from the data and an **explainable artificial intelligence** powered user interface **(TumorMapr-Advanced)** for creating the knowledge that ultimately provides high confidence recommendations to clinical experts. The advantages of these elements are described below in detail and are summarized in **Table 1**.

Why Unbiased Spatial Analytics Matters?

The biology of human disease is highly complex. For example, heterogeneity in cellular composition and signaling networks among cells is the intrinsic nature of every cancer, even within a single tumor specimen. The evolving cellular configuration and dynamic molecular and network interactions determine the extent of intratumor heterogeneity, which is critical to disease progression and response to therapy.

SpIntellx has developed an unsupervised machine learning algorithm to build a hierarchy of functional cell phenotypes on a continuum (*9*). In combination with the pointwise mutual information (PMI) (*10*) algorithm, microdomains are identified. These algorithms allow the capture

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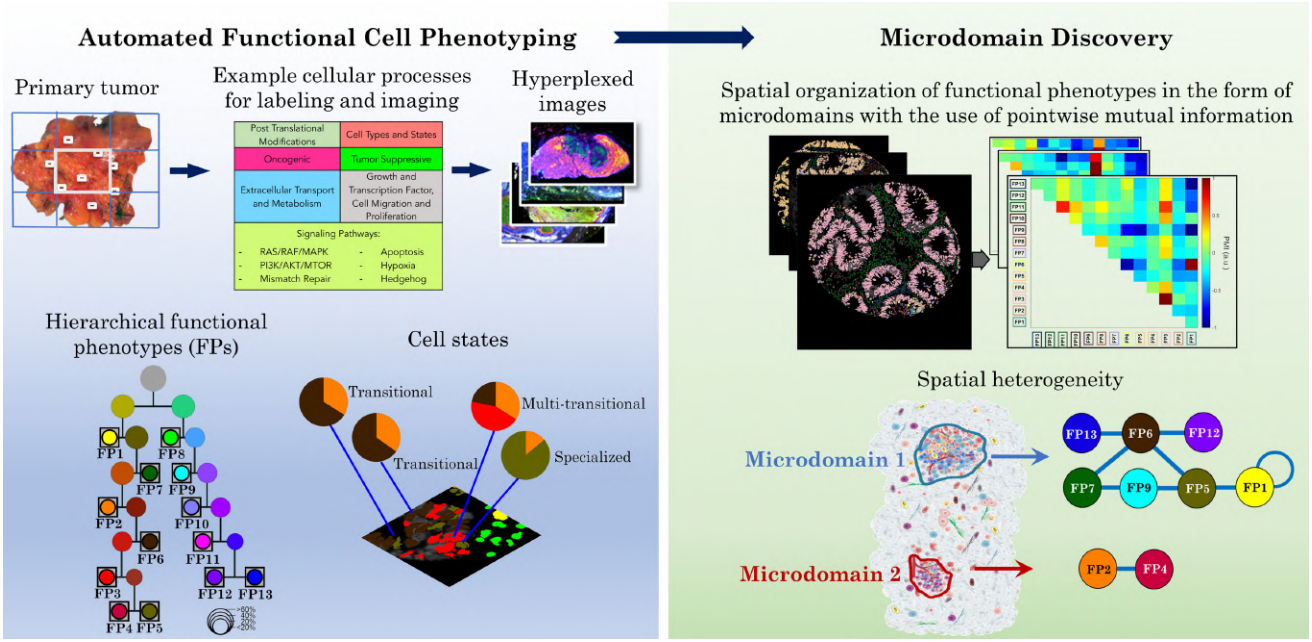

Figure 5. Why Unbiased Spatial Analytics Matters? Biological spatial heterogeneity as an example in primary tumors impacts disease progression, and therefore clinical decisions and selection of therapeutic strategies. Basic quantitation, characterization of functional phenotypes and microdomain discovery allow the SpIntellx platform to identify and to characterize complex tissue heterogeneity.

of a phenotypic continuum comprised of specialized, transitional, and multi-transitional cell states that accurately reflect the complexity of solid tumor heterogeneity **(Figure 5, left panel).** More importantly, using spatial heterogeneity data determined by PMI, the SpIntellx approach enables the discovery of microdomains, characterized by distinct compositions and spatial configurations of cancer and non-cancer cell populations **(Figure 5, right panel).**

The identification of heterogenous microdomains within the same primary tumor, and the in-depth evaluation of the distinct cell composition, spatial interactions between cancer cells and stromal cells (including immune cells and functionally intermediate cell types across multiple cell states) are critical to generate accurate predictions of disease progression and outcomes. The molecular nature and the extent of heterogeneity determines the response to a specific therapy. Thus, a thorough understanding and evaluation of the heterogenous biology of a tumor by generating and analyzing spatially imaged data are essential for the applications described here.

Why Spatial Systems Pathology Matters?

The rapid progress in biomedical research has led to an enormous collection of knowledge that expands our understanding of the diverse mechanisms driving human diseases. The complex molecular and network interactions form the basis of disease mechanisms. Therefore, a systematic characterization of the molecular and pathway interaction networks holds the key to unlock the benefits of existing knowledge to healthcare. Recent advancements in imaging technology have opened the door to unprecedented, complex high dimensional data sets. However, the knowledge that can potentially be extracted has been left largely unexplored as the complexity in the data is either reduced or else remains impenetrable to simple analytical tools that are widely used.

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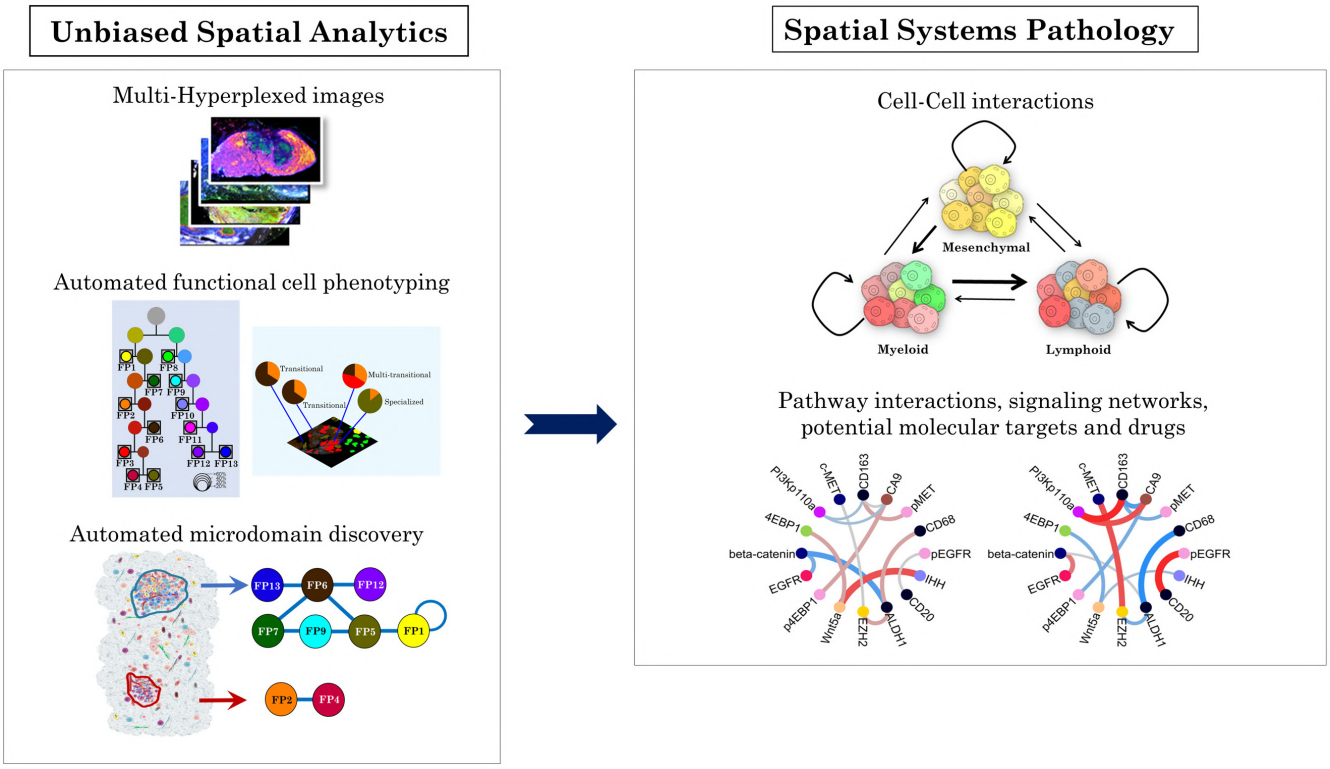

Figure 6. Why Spatial Systems Pathology Matters? Starting with information from unbiased spatial analytics (left) application of spatial systems pathology (right) reveals patient-specific pathway interactions, signaling networks, potential molecular targets and drugs defined within microdomains that drive tumor progression, predict untreated disease outcomes, and predict optimal therapeutic strategies. Colored lines in the right panel connecting biomarkers indicate interactions between molecules (red for cooperativity, blue for inhibition) and thickness of the lines indicate strength of the interaction. Each of the molecules is given a color circle to denote the cellular process that it better represents.

To solve this problem, the SpIntellx spatial systems pathology analysis taps into the current network biology knowledge databases to derive interaction networks in a spatial context. Using information extracted with unbiased spatial analytics and spatial systems pathology, patient-specific network interactions, signaling networks and potential molecular targets and drugs, as well as cell to cell interactions within and across microdomains are defined **(Figure 6).** Key networks that drive tumor progression need to be accurately determined in order to predict disease outcomes, optimal therapeutic strategies and optimal patient cohorts for clinical trials. Ultimately, spatial systems pathology has the power to create knowledge sufficient to predict the natural course of a disease, as well as the optimal therapeutic strategies customized to individual patients.

Why Explainable Artificial Intelligence (xAI) Matters?

Pathologists and disease experts require transparency regarding why a certain decision or recommendation is made by the AI software while applying computational and systems pathology. However, without the ability to show how and/or why a certain decision or recommendation is made by the algorithms, the standard "black box" nature of AI algorithms makes it very difficult to establish confidence with clinical experts. The lack of confidence in "black box" AI decisions has been a major roadblock to the adoption of computational pathology, since additional studies may be required to verify an AI decision and may introduce inefficiency involving extra time and resources.

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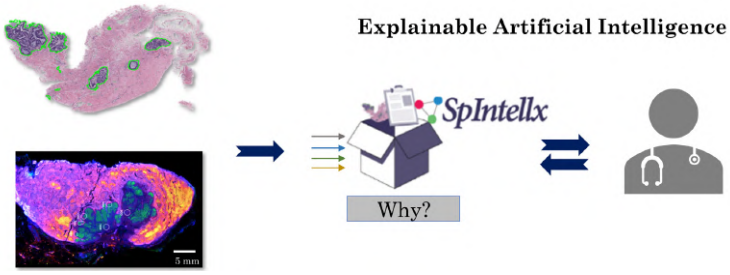

Figure 7. Why Explainable Artificial Intelligence (xAI) Matters? Pathologists and disease experts require **transparency** in applying computational and systems pathology algorithms to their data. The TumorMapr platform offered by SpIntellx using xAI provides confidence in decisions recommended by the algorithms by answering "Why?".

TumorMapr utilizes a user interface based on explainable artificial intelligence (xAI), thus, it allows critical interactions with oncologists/pathologists and it is capable of providing explanations for the decisions recommended by the AI algorithms **(Figure 7).** In addition, novel findings and supporting evidence provided by xAI can be studied to further advance our understanding of diseases. The application of xAI builds confidence in the algorithms and extends its utility in clinical and biomedical research. Therefore, xAI plays a critical role in facilitating wider adoption of computational and systems pathology **(Figure 7).**

III. TumorMapr Pipeline

How does TumorMapr work?

TumorMapr applies cutting edge artificial intelligence to gather and analyze spatial data from patient specimens using from a few to dozens of biomarkers analyzed with an unbiased approach, resulting in deeper interrogation of tissue heterogeneity. This platform is capable of analyzing data sets from any multi-hyperplexed imaging platform, reagent type (e.g., DNA, RNA or protein biomarkers) and any disease that involves solid tissue. Within intact tissue sections, the data generated by the platform preserves *in situ* spatial context **(Figure 8).**

In the initial step, TumorMapr automatically collects information regarding the presence and levels of distinct biomarkers from all cells within the sample, generating an impartial, unbiased analysis of the specimen. This point is especially relevant, as current competitive platforms collect and analyze information from a subset of cells within each sample. TumorMapr instead generates data and extracts information from all cells within the tissue sample.

Two components are part of the TumorMapr pipeline: TumorMapr- Basic and TumorMapr- Advanced. The first offers rapid and basic measurements and is indicated to customers primarily interested in basic quantitation of multiplexed data. The second, TumorMapr- Advanced, offers advanced features that include automated functional phenotyping and microdomain discoveries, systems biology and xAI guides.

TumorMapr-Basic **generates** data from the image datasets and provides basic quantitation and feature extraction from multi-hyperplexed imaging for initial analysis and is a valuable first step in differentiating pathological findings and responses to therapies. It is designed to perform basic image processing, cell segmentation and quality control. In addition, it performs simple biomarker quantification and simple, biased spatial analytics based on location of cells on a grid **(Figure 8). TumorMapr-Basic** is similar to the software used by most competitors **(Table 1**).

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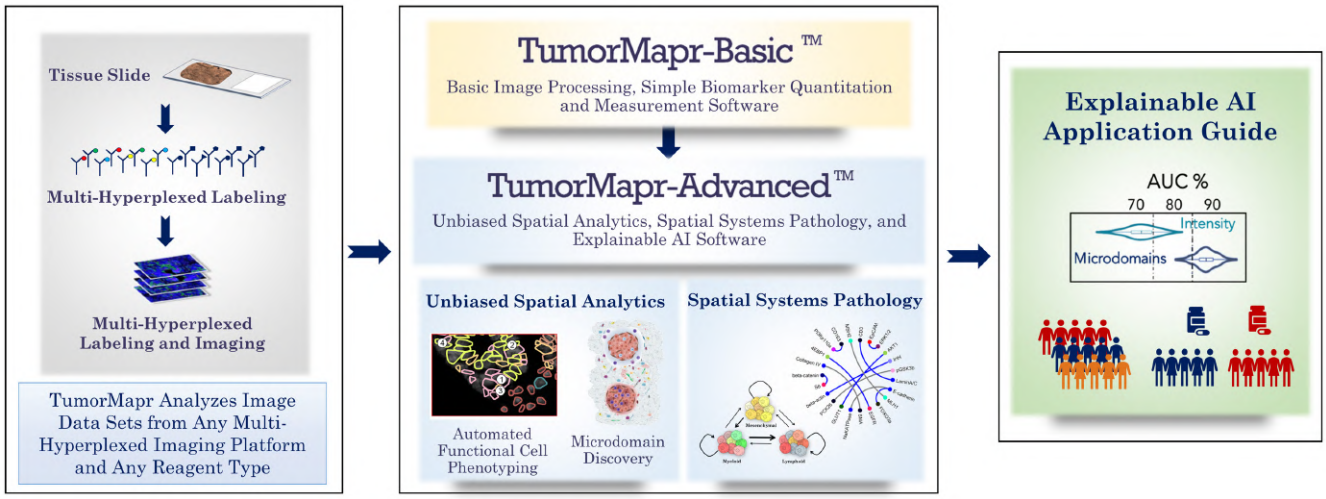

Figure 8. TumorMapr Pipeline. TumorMapr combines TumorMapr-Basic™ and TumorMapr-Advanced™ to develop powerful applications in the fields of personalized therapeutic strategies, drug target identification, clinical trial stratification and prognostics/diagnostics.

Building on the initial steps of TumorMapr-Basic, the more powerful TumorMapr-Advanced is designed to analyze images derived from TumorMapr-Basic in further depth by extracting information through unbiased spatial analytics. TumorMapr-Advanced takes advantage of recent dramatic improvements in the capacity of imaging platforms, which can now image multiple dozens of biomarkers, including specific proteins and nucleic acids, within the same tissue section. TumorMapr-Advanced is scalable with the ability to work with any number of biomarkers. Analysis of multi-hyperplexing biomarkers within their *in situ* spatial context maximizes the information that can be extracted from a single specimen. This is critical for the subsequent thorough characterization of tissue heterogeneity.

Based on the information extracted through unbiased spatial analytics, TumorMapr-Advanced then determines cell types and cell states by automated functional phenotyping. A hierarchy of functional phenotypes can be derived based on the expression of the biomarkers and the cellular processes in which they are involved. Specialized and non-specialized cell types and additional transitional cell states that are critical to disease progression are then automatically defined. Furthermore, using spatial organization of functional phenotypes, the software can perform unsupervised automated discovery of data-driven microdomains **(Figure 5).** Drawing information from advanced network biology, the software has the ability to infer microdomain-specific pathway interaction and signaling networks that drive disease progression, immune evasion, therapeutic responses and drug resistance to allow optimization of clinical decisions, including therapeutic strategies.

The automated functional phenotyping and microdomain discovery accurately reflect the intratumoral heterogeneity in an individual patient tumor. Parallel analysis of microdomain-specific signaling networks from patient samples before and after treatment supports the existence of a continuum of phenotypic states and the emergence of functional plasticity in response to clinical interventions. TumorMapr, used in combination with non-destructive hyperplexed imaging platforms, allows mechanistic hypotheses to be tested through iterative interrogation of the same microdomains with additional biomarkers inferred by the spatial systems pathology analyses. This

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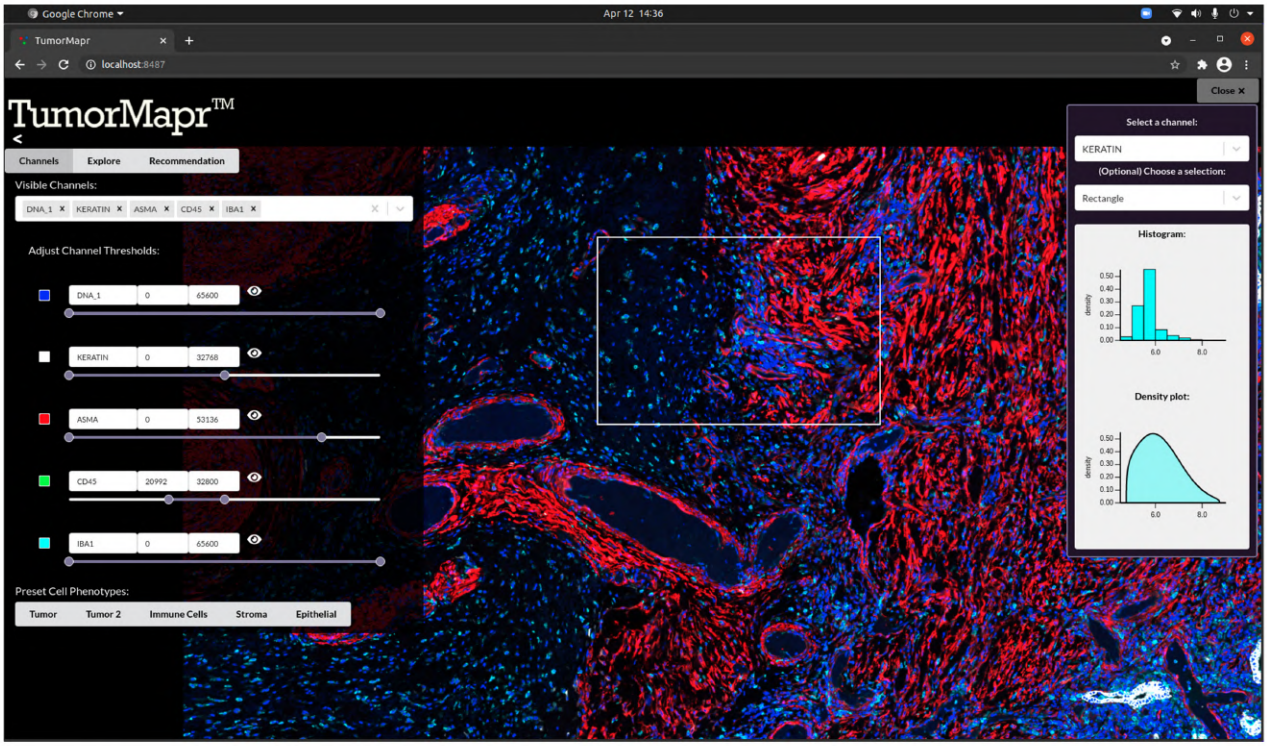

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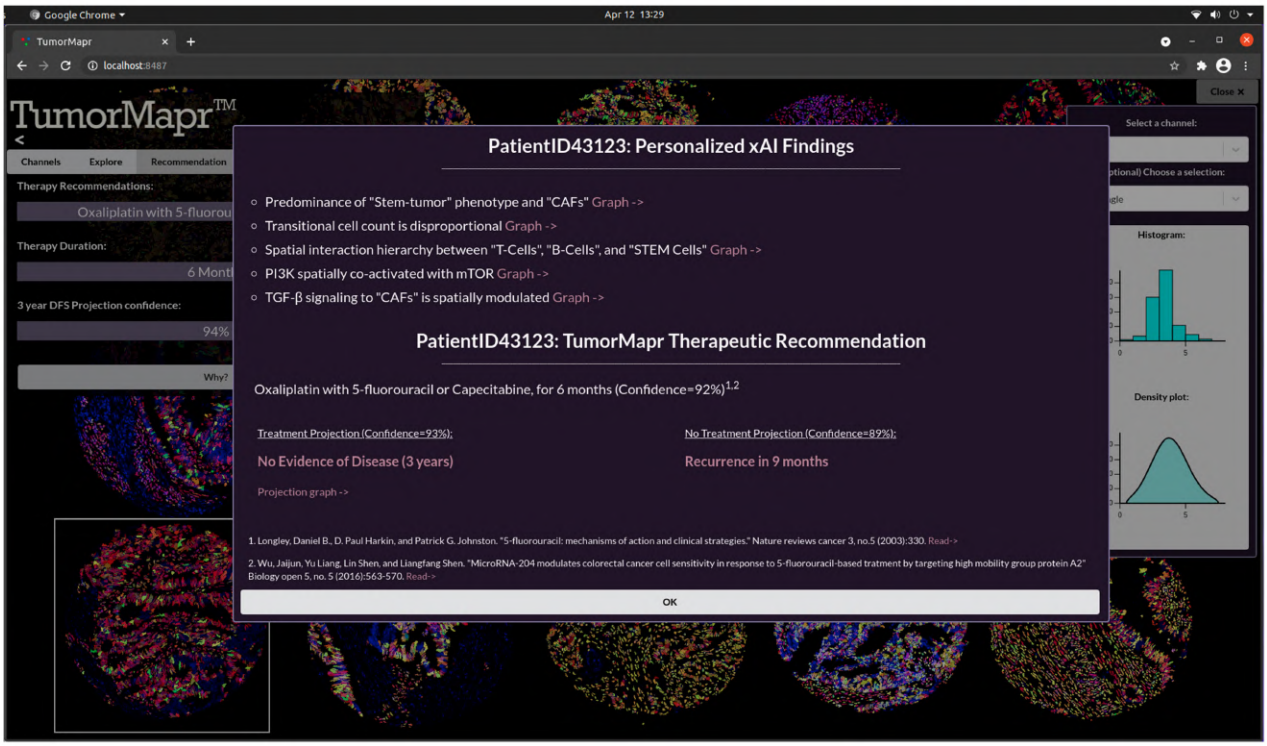

Figure 9. TumorMapr User Interface and the Transformative Impact of the "Why" Button. A. Web browser-based, user-friendly, interactive, image viewer and analytical engine for multiplexed and hyperplexed image datasets. B. TumorMapr makes specific recommendations that can be questioned by the oncologists and clinicians with a click of the Why? button. TumorMapr presents the key findings to justify each recommendation and the clinician remains in full control of the entire process and makes final decisions (Figure 10). TumorMapr mitigates the risk of high discordance rate between clinicians.

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iterative process enables more specific mechanistically linked biomarkers to emerge, essentially individualizing each set of biomarkers as required for highly specific prognostics and predictive analytics. Importantly, by enabling the testing of mechanistic hypotheses in patient samples directly connected to a specific clinical outcome, TumorMapr informs personalized therapeutic strategies. Therefore, TumorMapr-Advanced is extremely robust and powerful at guiding treatment strategies, identifying drug targets, selecting patients for clinical trials, and building powerful prognostic/diagnostic tests. The SpIntellx TumorMapr-Advanced platform outperforms existing software in both accuracy of diagnosis and clinical risk assessment (see below).

TumorMapr xAI user interface

The user-interface in TumorMapr is both a visualization tool and a functional tool **(Figure 9).** First, spatial image datasets originating from any multi-hyperplexed imaging platform can be retrieved and observed by pathologists/clinicians. The SpIntellx TumorMapr platform presents to the oncologists and pathologists critical knowledge created from the extracted information through an xAI enabled interface.

TumorMapr-Advanced presents the pathologists/clinicians with an interactive and user-friendly interface with the "Why?" button **(Figure 9)**. This tool provides explanations for the recommendations made by the algorithms to the pathologists/clinicians. The pathologist can question the software with a click of the "Why?" button and TumorMapr-Advanced presents the key findings to justify each recommendation. This allows collaborations and conversations between pathologists and other clinical experts, particularly in atypical cases, and allows the pathologist/clinician to remain in full control during the entire process. The pathologist/clinician makes the final decision and the potential of a high discordancy rate between pathologists can be reduced or eliminated.

IV. Example Applications of TumorMapr in Cancer Precision Medicine

There is emerging consensus that the complexity and diversity of tumor microdomains (tumor microenvironments) are key determinants of an individual's response to therapy. Different types of tissue microenvironments have a marked influence on tumor initiation, progression and response to therapy, thus explaining the observation that patients with the same general diagnosis do not all respond in the same way to unique therapies and reinforcing the fact that we need personalized/precision treatments according to each patient's specific characteristics.

The powerful SpIntellx TumorMapr platform fulfills the urgent need to identify and characterize the microdomains existing within each single tumor. The computational power to capture and characterize critical aspects of spatial tissue heterogeneity and predict disease progression increases our ability to address current clinical needs. All of the applications and advantages can be extended not only to solid tumors, but to other conditions like autoimmune disease, organ transplantation, organ-specific and infectious diseases.

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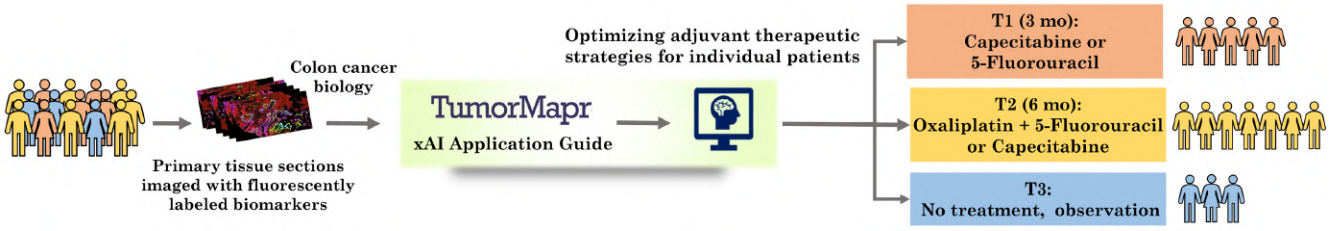

Figure 10. Personalizing Adjuvant Therapeutic Strategies for Cancer Patients. TumorMapr will analyze primary resected samples based on fluorescently labeled biomarkers to personalize existing adjuvant chemotherapy strategies for stage II high-risk and stage III colon cancer patients. The xAI interface (Figure 9B) presents the key findings to justify each recommendation and the clinician remains in full control of the entire process and makes the final decision.

1. Personalizing adjuvant therapeutic strategies for cancer patients

SpIntellx is validating TumorMapr-Colon, which is TumorMapr applied to hyperplexed image datasets of colorectal carcinoma primary tumors, in collaboration with Roswell Park Comprehensive Cancer Center. The goal is to personalize the existing adjuvant therapeutic strategies for stage II high-risk and stage III colon cancer patients **(Figure 10).** Only 4% of the stage II high-risk and 25% of the stage III colon cancer patient populations truly benefit from adjuvant chemotherapy, leaving the rest with unnecessary toxicities and financial burden (*11*). Consequently, there is an urgent need to more optimally evaluate the tumor biology, specifically the critical component involving host cell-tumor cell interactions, to predict an individual patient's response to adjuvant chemotherapy. TumorMapr-Colon employs an adaptable and diverse set of fluorescently labeled biomarkers to harness unbiased spatial analytics, spatial systems pathology, and explainable AI (xAI) to extract in-depth information on the heterogeneous tumor biology from spatially imaged tumor samples to predict response to a subset of adjuvant chemotherapy options currently in use (including no treatment) for stage II high-risk / III colon cancer patients. This approach is applicable to any solid tumor.

2. Building powerful prognostics and diagnostics tests

TumorMapr-Colon shows transformational improvement over the state-of-the-art in predicting the five-year risk of recurrence in stage I, II and III CRC patients (*12*). The accuracy of risk prediction of 5-year recurrence in colon cancer patients using the current pathology workflow, including basic cell counting and the presence/absence of biomarkers, is not significantly better (AUC ~ 0.76) than the prediction using just the clinical information (AUC ~ 0.72) **(Figure 11).** In contrast, TumorMapr-Colon applied to a fluorescence-based, hyperplexed (starting with 55 biomarkers) image dataset of colorectal carcinoma primary tumors (N=432) is consistent and stable in identifying patients in whom risk of colorectal cancer recurrence is high for Stages I through III **(Figure 11),** with mean AUC of bootstrapped ROC curves ~0.9. The results are far superior to the tissue-based tests currently available in the market, including those applying genomics. The key advantage in using spatial analytics over biomarker intensity-based approaches is to achieve the same prognostic performance result using a significantly smaller list of biomarkers (from 55 biomarkers to ~12-15 biomarkers in the CRC dataset). In a different gastrointestinal case study, TumorMapr achieved dramatic improvement in predicting the risk of progression to cancer using spatial analytics, increasing the AUC values by ~30% and significantly reducing the number of biomarkers by 40%.

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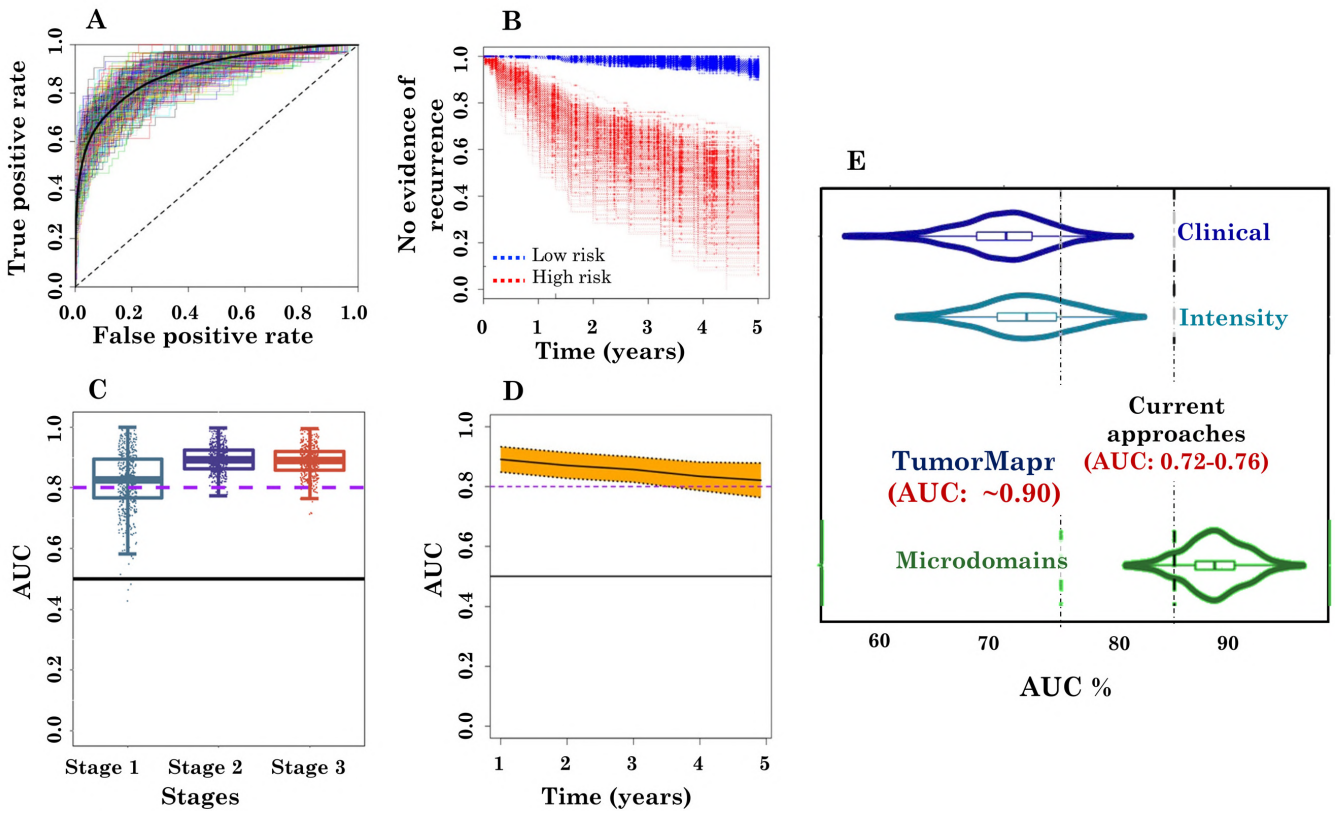

Figure 11. Building Powerful Prognostics and Diagnostics Tests. TumorMapr-Colon analyzes primary resected samples based on fluorescently labeled biomarkers to predict the risk of disease recurrence in individual patients. **A.** Receiver operating characteristics (ROC) curves for predicting risk of 5-year CRC recurrence in patients (n = 432) with resected CRC primary tumor samples imaged with fluorescently labeled biomarkers (= 55). Plots for 500 bootstrap runs with independent training and validation sets are shown. Area under the mean ROC curve, shown as a black solid curve, is 88.5% with a standard error of 0.1%. **B.** Kaplan-Meier recurrence-free survival curves for each of the 500 bootstrap runs for patients a priori identified by TumorMapr at low and high-risk of five-year CRC recurrence. **C.** Boxplots of stage-based area under the 500 bootstrapped ROC curves demonstrating stable stage-based clinical performance. **D.** Stable temporal performance using time-dependent AUC values plotted as a function of time. 95% confidence interval is shown in orange. **E.** Superior performance predicting 5-yr risk of recurrence of colon cancer with spatial analytics & xAI. The key advantage in using spatial analytics over biomarker intensity-based approaches is to achieve the same prognostic performance result using a significantly smaller list of biomarkers (from 55 biomarkers to ~12-15 biomarkers in the CRC dataset).

Thus, TumorMapr has been proven to be highly accurate in predicting patient outcomes with a small number of biomarkers.

3. Identifying novel drug targets

TumorMapr provides mechanistic insights into tumor biology in multiple aspects, 1) by establishing correlations between specific spatially defined biomarkers with pathways and the potential molecular targets and high risk microdomains; 2) by detecting spatial tissue heterogeneity regarding specific metabolic pathways activation or inhibition and; 3) by correlating specific biomarkers/modifications and particular microdomains to disease outcome (**Figure 12**). Application of this information can aid the drug discovery pipelines by providing more precise recommendations for drug targets, suggesting candidate repurposed drugs and informing potential

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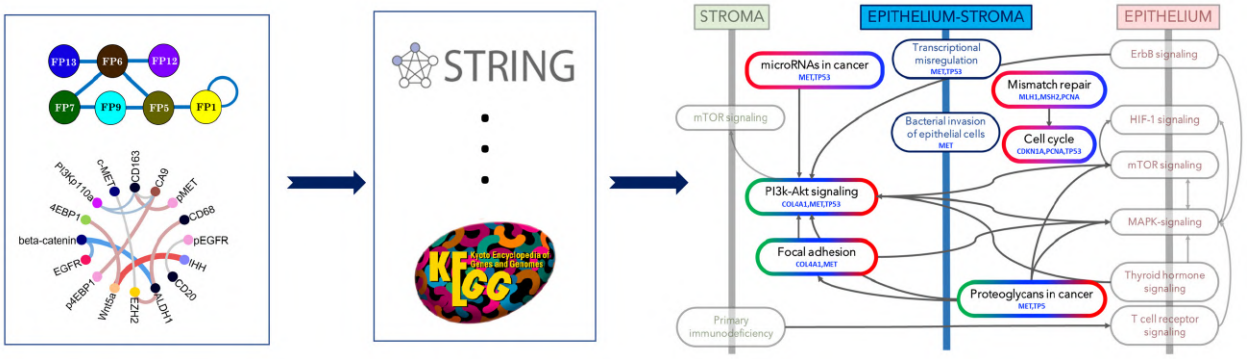

Figure 12. Novel Drug Target Identification. (Left) From the networks each specific to a microdomain, (Middle) TumorMapr interrogates protein-protein interaction databases and pathway databases, (Right) to infer microdomain-specific pathways and their interactions, signaling networks, and potential molecular targets and drugs within microdomains driving disease progression.

interactions between the drug and the cellular pathways. Novel drug therapies could be developed as better prediction and guided diagnoses arise, creating and streamlining robust precision/companion diagnostics to benefit each specific patient.

V. Integration of TumorMapr and HistoMapr for Selected Applications

At present, manual light microscopy techniques including H&E staining and additional immunohistochemistry labeling and imaging to evaluate clinical specimens are the gold standard for pathology. The Whole Slide Imaging systems approved by the FDA provide the automated processing capacity to facilitate the workflow. In the past few years, the field has observed a large increase in digital pathology imaging and expansion of imaging platform vendors, both of which greatly increased during the COVID pandemic. Despite the great potential and increased utilization of digital pathology, the current approaches involve mostly simple quantitation, some instances of multiplexed fluorescence and "black box" AI, thus limiting the clinical utility that can be achieved through computational pathology. Moreover, sophisticated computational analyses of the images or correlation with transmitted light features are not yet available. The SpIntellx approach to computer-assisted clinical decision is novel and unique in its ability to address these needs, offering a continuum of solutions to maximize the extraction of information from spatial data and to create predictive knowledge **(Figure 13).**

At one end of the spectrum, the HistoMapr™ platform analyzes datasets from traditional staining and labeling of transmitted light images (e.g., H&E, immunohistochemistry), but HistoMapr™ is a much more advanced solution than those currently used for disease diagnosis. A separate article will discuss HistoMapr™ in detail but, briefly, the software analyzes the images, presents the key findings and makes recommendations for prognoses/diagnoses to the pathologist. Besides the application of unbiased spatial analytics and spatial systems pathology, the transformative impact of HistoMapr™ resides in the "Why?" button: The pathologist can click and question the software about each recommendation and the platform provides its key findings to justify each decision. The pathologist thus remains in full control of the entire process and decision making, and can override the software. SpIntellx first tested HistoMapr™-Breast, the initial/prototype xAI enabled software, to evaluate breast core biopsies and, in 2020, published their review describing the successful first application of xAI for Anatomic Pathology to analyze breast biopsies and to assist anatomic pathologists.

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Figure 13. TumorMapr and HistoMapr Can Be Used Separately or Together. SpIntellx has developed powerful applications to analyze image data sets from any imaging platform and reagent type based on fluorescent or mass spectrometry-based labeling (TumorMapr) or from transmitted light images such as conventional H&E and immunohistochemistry (HistoMapr). SpIntellx products are backed by a very strong intellectual property position in unbiased spatial analytics (automated functional cell phenotyping, microdomain discovery), spatial systems pathology, and xAI.

At the other end of the spectrum, TumorMapr represents a continuum with the HistoMapr™ system and, through a rich, interactive and user-friendly interface, expands the platform beyond analyzing information obtained from transmitted light images. TumorMapr is SpIntellx's most powerful platform and capable of analyzing images from multi to hyperplexed fluorescence and/or mass spec biomarker datasets (scalable to any number of biomarkers). These two platforms can be used individually or in combination and are applicable to any solid tumor or tissue disease pathology. Thus, TumorMapr/HistoMapr™ can handle both current and archival datasets which are a combination of multiplexed fluorescence/mass spec and transmitted light datasets. In consequence, TumorMapr / HistoMapr™ represent an invaluable tool for clinical experts and an essential resource for health care providers. Adoption of TumorMapr/HistoMapr™ will greatly boost the efficiency of clinical experts to achieve better care of patients.

VI. TumorMapr Summary

TumorMapr is able to process and analyze images acquired by any existing multi-hyperplexed imaging platform to generate extensive spatial data. The data are based on measurements of multi to hyperplexed biomarkers within their *in situ* spatial context through an unbiased spatial analytics approach. These spatial data drive automated functional phenotyping of the cells and discovery of microdomains, which provide a thorough and accurate picture of tissue heterogeneity within the sample. Next, using a spatial systems pathology approach and applying the most advanced network biology, key information regarding microdomain specific networks can be extracted to create the predictive knowledge that ultimately supports reliable recommendations for clinical decisions. Finally, the xAI-enabled user interface eases interactions between the software and clinical experts, increases confidence on the recommendations proposed by the software and facilitates communication between experts in the field.

Implementation of TumorMapr in both research and clinical settings will bring innovations needed to improve reliability in diagnosis/prognosis, optimize personalized treatment regimens, boost efficiency of pathology and oncology workflow, and optimize stratification of patients for clinical trials. In addition, the knowledge created by TumorMapr with unbiased spatial analytics and

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spatial systems pathology analysis serves as a solid foundation for identification of novel biomarkers, signal pathways and drug targets. Finally, the TumorMapr pipeline is applicable to additional diseases besides solid tumors, such as autoimmune, transplant or infectious diseases. TumorMapr, as a sophisticated and powerful computational pathology platform, has the potential to revolutionize precision medicine.

VII. TumorMapr Services and Contacts

The combination of TumorMapr-Basic and TumorMapr-Advanced is presently offered as a software as a service (SaaS), where users can access our interface online to upload their data and interact with the results. Raw multi-hyperplexed image datasets from the image platform and/or datasets generated by the platform and partially processed and analyzed can serve as the starting point for TumorMapr. Data are securely shared in the cloud and the processed analyzed images are returned to the customer along with an xAI guide customized to the project (e.g., personalized therapeutic strategy, identification of potential novel targets for drug discovery, selection of patient cohorts for clinical trials and prognostic and/or diagnostic tests).

Our initial focus is on solid tumors, but the platform is applicable to other diseases (e.g., autoimmune, transplantation medicine, infectious diseases, organ specific diseases, etc.). SpIntellx is entertaining requests to license TumorMapr-Basic to include on imaging platforms. Additionally, based on customer input, SpIntellx is evaluating the future potential to perform the complete service in-house including sample preparation and generation of the image datasets.

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Glossary (in alphabetical order)

Artificial Intelligence (AI) – The ability of a computerized system to perform tasks commonly associated with natural beings. The term is frequently applied to the project of developing systems endowed with the intellectual processes characteristic of humans, such as the ability to reason, discover meaning, generalize, or learn from experience.

Biomarker– A measurable biological substance, e.g., a labeled antibody, whose presence and relative quantity are indicative of physiological and/or pathological states

Computational Pathology– Application of computing algorithms and AI, to pathology data to **extract** information. This includes both image data such as whole slide images, and non-image data such as patient demographics, clinical information, or pathologists' observations.

Digital Pathology– The use of digital imaging in pathology. Initial efforts focused on remote viewing of microscopy with both manual and robotic remote-controlled microscopes. Whole slide images are a more recent area of digital pathology, both for earlier applications (e.g., telepathology, image analysis) and for more recent primary diagnosis applications.

Explainable Artificial Intelligence (xAI) –xAI algorithms are programmed to describe its purpose, rationale and decision-making process in a way that can be understood by the end user. xAI plays an important role in the fairness, accountability and transparency in machine learning.

Machine Learning – A computer science discipline in artificial intelligence research that is mainly concerned with the implementation of computer software that can learn data patterns autonomously.

Microdomains – A microdomain is a localized niche or microenvironment with distinct composition and spatial configuration of multiple cell populations within a tissue sample.

Multiplex to Hyperplex Labeling and Imaging – Use of either fluorescence or mass spectrometry-based biomarker labeling and imaging to detect from a few to several dozen (multiplex < 9 and hyperplex >= 9) targeted proteins and nucleic acids in tissue sections and/or tumor microarrays at subcellular resolution. The use of routine formalin-fixed paraffin-embedded (FFPE) pathology and in some cases frozen sections that are labeled with biomarkers including antibodies to investigate the complexity in tissue samples.

Network Biology – Ability to model biology as a network of components with emergent properties using an integrative and systems approach.

Pointwise Mutual Information (PMI) – Two-dimensional maps for relative co-occurrences and anti-associations of spatially distributed cellular phenotypes in a tissue sample. A PMI map with strong diagonal entries and weak off-diagonal entries describes a tumor sample that is locally homogeneous but heterogeneous with respect to the spatial distribution of the cellular phenotypes. PMI maps with strong off-diagonal entries describe a tumor with many localized interactions between different cellular phenotypes, thus signifying a tumor exhibiting strong local heterogeneity.

Precision Medicine – The goal of precision medicine is to deliver the right therapeutic to the right patient at the right dose and the right time.

Spatial Intelligence – To quantify the spatial relationships between cells and tissue structures in whole slide images and /or tumor microarrays in an unbiased manner.

Systems Pathology – Study of the complicated interplay of spatial interactions between the various cell types to infer molecular signaling networks and network interactions driving disease progression.

Tumor Microarray (TMA) – Tumor microarrays are core samples of tumors arrayed on a slide to allow the investigation of multiple samples per slide.

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