MALDI-IHC high-plex protein biomarker imaging for fast, non-iterative identification of lung carcinoma subtypes

Catherine A. Kita[1], Leonardo G. Dettori[1], Kristina Schwamborn[2], Gargey Yagnik[1], Kenneth J. Rothschild[1,3], Mark J. Lim[1]

[1] AmberGen Inc, Billerica, MA, USA
[2] Technische Universität München, München, Germany
[3] Boston University, Boston, MA, USA

Introduction

Lung cancer is the second most common cancer, and the leading cause of cancer-related deaths worldwide. Most lung cancer (80-85%) is non-small cell (NSCLC), comprising mostly adenocarcinoma and squamous cell carcinoma, while roughly 10-15% of lung cancer is small cell lung cancer (SCLC), also termed neuroendocrine lung cancer. Subtype identification is critical for determining treatment, and with limited samples, it is critical to get the most data possible from each tissue section.

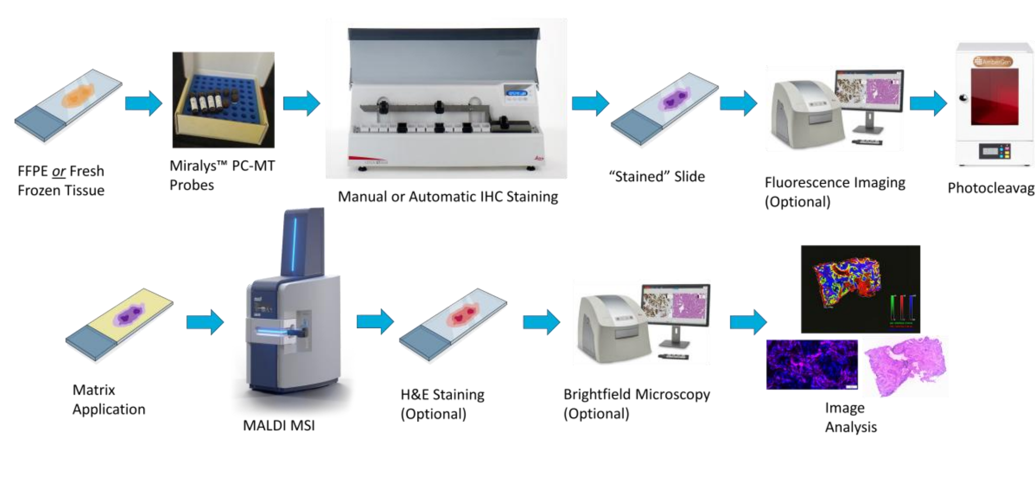
Traditional immunohistochemical (IHC) confirmation remains a prolonged and often iterative (i.e. requires cycling). To overcome this, we have developed a new IHC approach using novel photocleavable mass-tag (PC-MT) labeled antibodies combined with matrix laser desorption ionization (MALDI) mass spectrometry imaging.

Here, we present a 23-plex MALDI-IHC panel for the determination of subtype in lung cancer. This panel includes markers for adenocarcinoma (TTF-1, Napsin A), squamous cell carcinoma (p40, CK5), and small cell lung cancer (NCAM1, Synaptophysin, Chromogranin A), as well as general carcinoma markers for T cells, B cells, macrophages and fibroblasts. Antibodies are first validated individually by immunofluorescence and compared to MALDI-IHC on annotated tissues, we present an overview of the validation here.

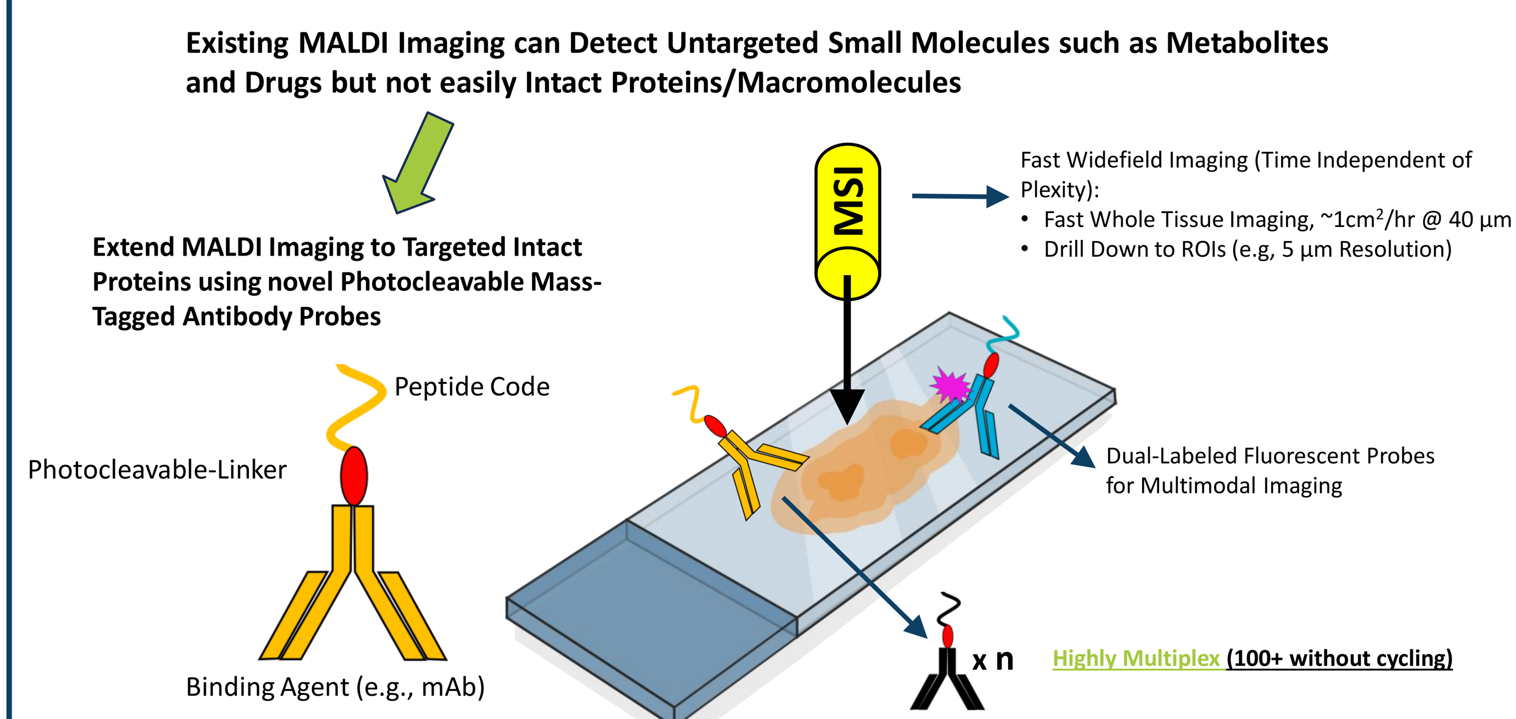
We report application of the MALDI-IHC approach to the simultaneous determination of pathologist determined lung carcinoma subtypes on a 100-core human lung tissue microarray (TMA) comprised of normal, normal adjacent, cancer adjacent, and cancerous tissues. Given the non-destructive nature of MALDI-IHC, it can be followed by H&E staining, allowing for accurate co-registration of traditional pathology annotations with the highly multiplexed MALDI-IHC results.

Methods

To assess the utility of our 23-plex lung carcinoma panel in determining subtype, we imaged a variety of healthy and diseased human lung tissue using a highly multiplexed, noniterative, multimodal method termed MALDI-IHC[1,2]. This method combines the advantages of mass spectrometry imaging (MSI) and immunohistochemistry (IHC) using novel photo-cleavable mass-tagged antibodies to yield spatial information of intact proteins. MSI was performed on a Bruker rapifleX or timsTOF fleX at 20 µm resolution. Multimodal imaging combines fluorescence and/or brightfield microscopy with MALDI-IHC, all performed on the same tissue section.



Key Features of MALDI-HIPLEX-IHC

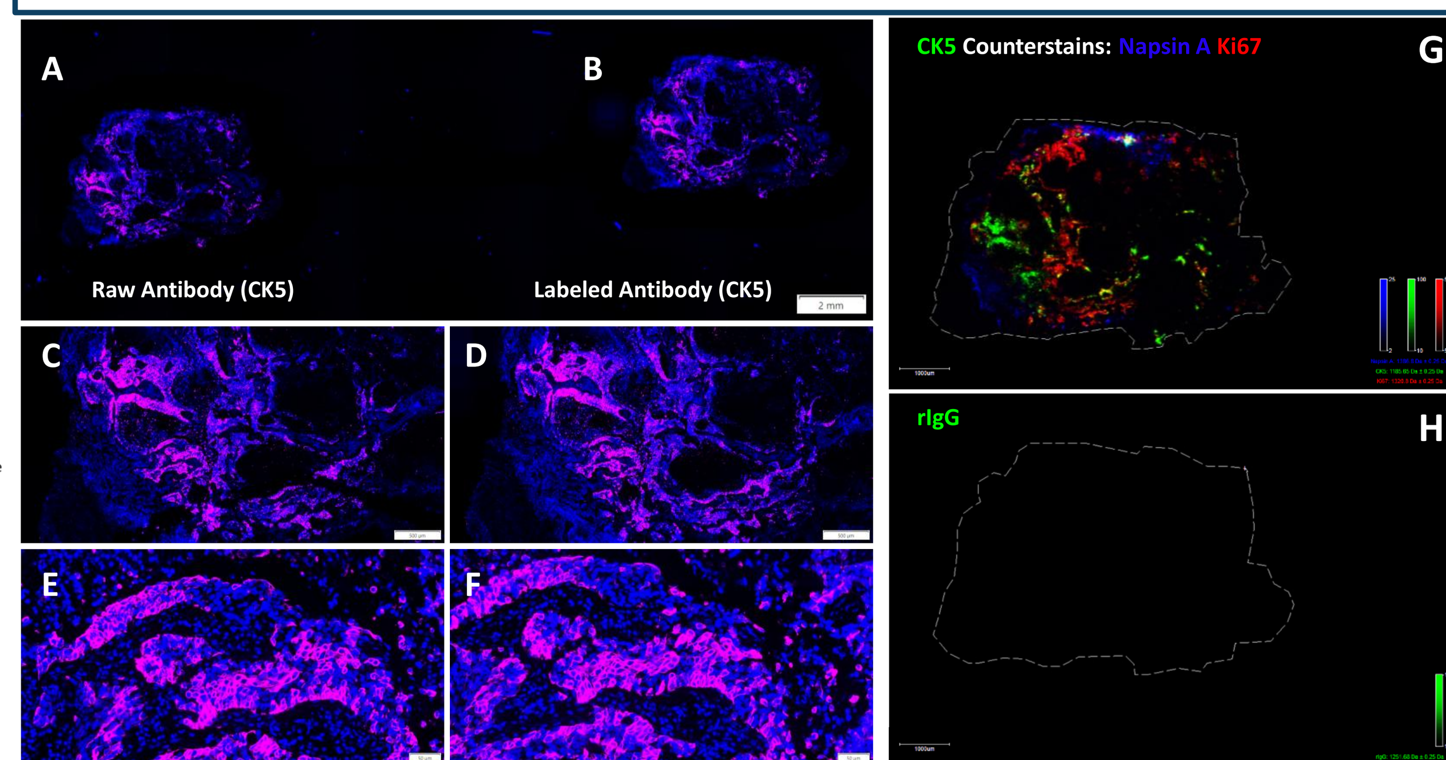


PC-MT Labeled Antibody Validation

PC-MT-antibody probe validation consists of several steps. First, confirmation of the antibody labeling with the PC-MT is performed using protein G purification followed by MS-based photocleavage assay. Next, immunofluorescence and MALDI imaging is performed on selected tissue to assess functionality. These results are assessed for correct histology, subcellular localization, and staining sensitivity/specificity.

Immunofluorescence validation is performed on each antibody probe by staining each of 3 serial tissue sections on a slide with one of the following primary antibody conditions, followed by whole slide staining with a fluorescent secondary antibody (pink = secondary antibody signal, blue = DAPI nuclear counterstain): i) the unlabeled raw antibody (A, C, E), ii) the PC-MT labeled antibody (B, D, F), iii) no primary antibody (negative controls lacking the primary antibody confirm lack of non-specific binding of the secondary antibody (data not shown)).

MALDI validation is achieved by following the Miralys™ staining protocol with MALDI-MSI imaging on each of 2 serial tissue sections on the same slide stained with one of the following: i) PC-MT labeled antibody (G) or ii) species-matched isotype control immunoglobulin (IgG) labeled with PC-MT (H), as a negative control.

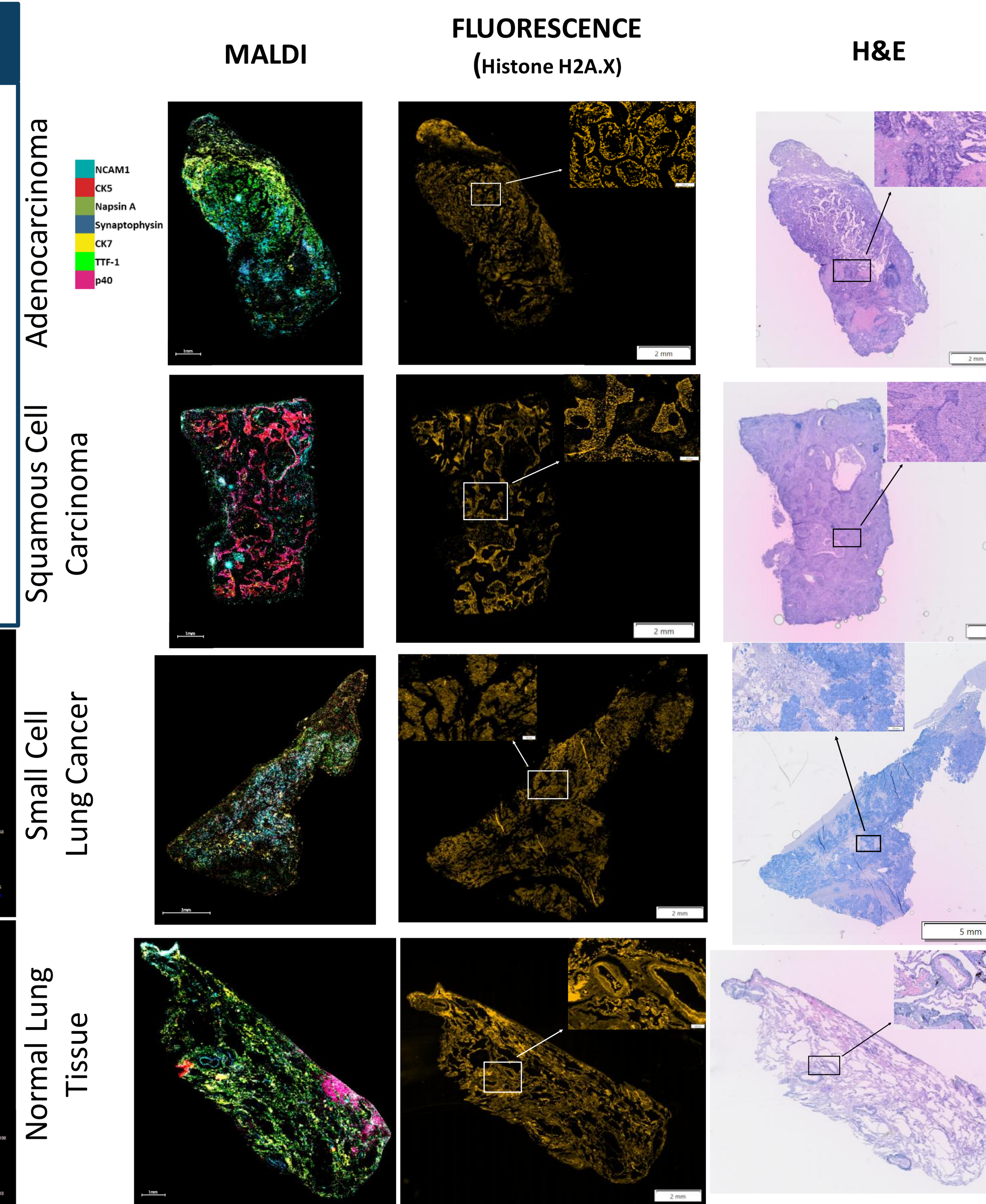


Non-Iterative Lung Cancer Subtyping

Two common markers were chosen for NSCLC subtyping of adenocarcinoma (TTF-1, Napsin A) and squamous cell carcinoma (p40, CK5), as well as two additional markers for neuroendocrine/ small cell lung cancer (NCAM1, Synaptophysin), and CK7 as a marker for prognosis and tumor origin.

Along with several other general tissue morphology markers (Ki67, PanCK, Histone H2A.X), this panel of subtyping markers was used to stain and image sections of at least 3 different pathologist verified FFPE tissues of each of the following: normal lung, adenocarcinoma, squamous cell carcinoma, and mesothelioma, as well as 2 tissues of SCLC.

As expected, adenocarcinoma tissues had a higher signal for TTF-1 and Napsin A, with low to no signal for p40 and CK5. Conversely, SCC tissues were all positive for p40 or CK5 and had weak to no signal for TTF-1. The SCLC tissues were both positive for NCAM1 and Synaptophysin (SYP), while the tissue annotated as SCC-SCLC was also positive for both p40 and CK5. Normal tissues ranged from strong to no signal for TTF-1, p40, CK5 and Ki67, with a strong signal for Napsin A. All mesothelioma tissues had a strong CK5 signal as expected.



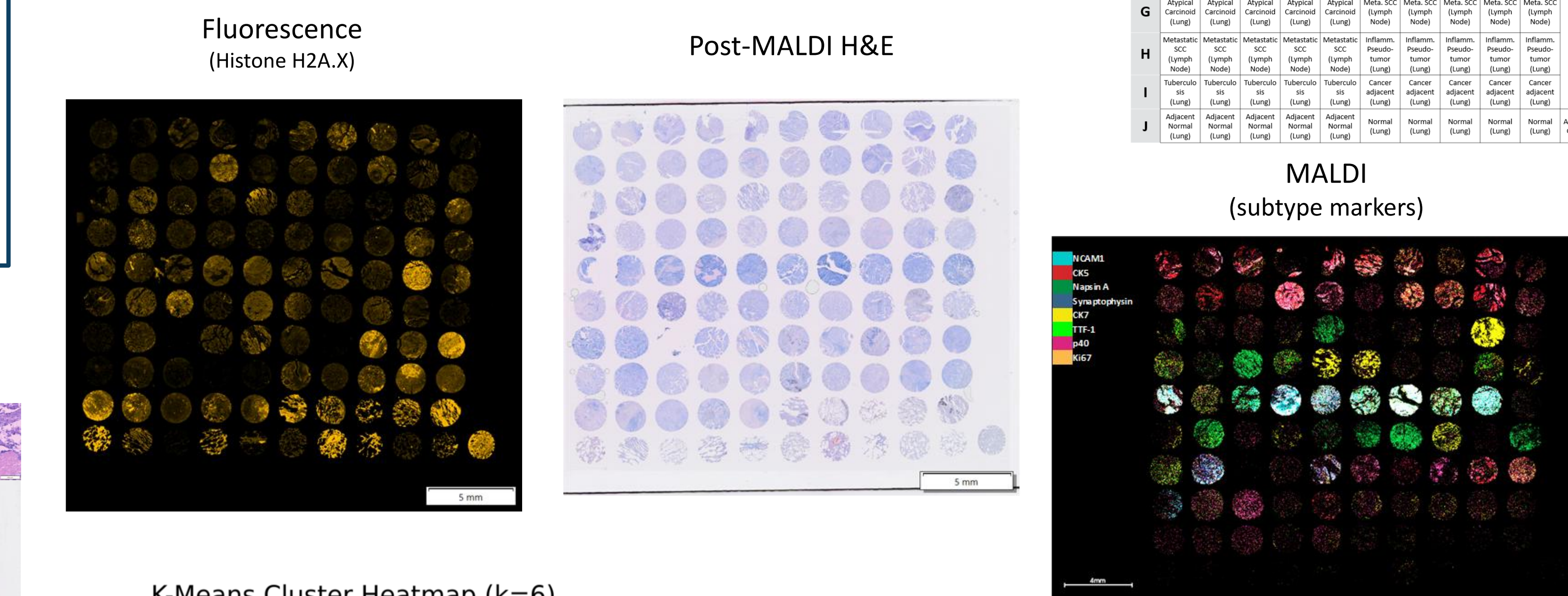
100-Core Human Lung TMA

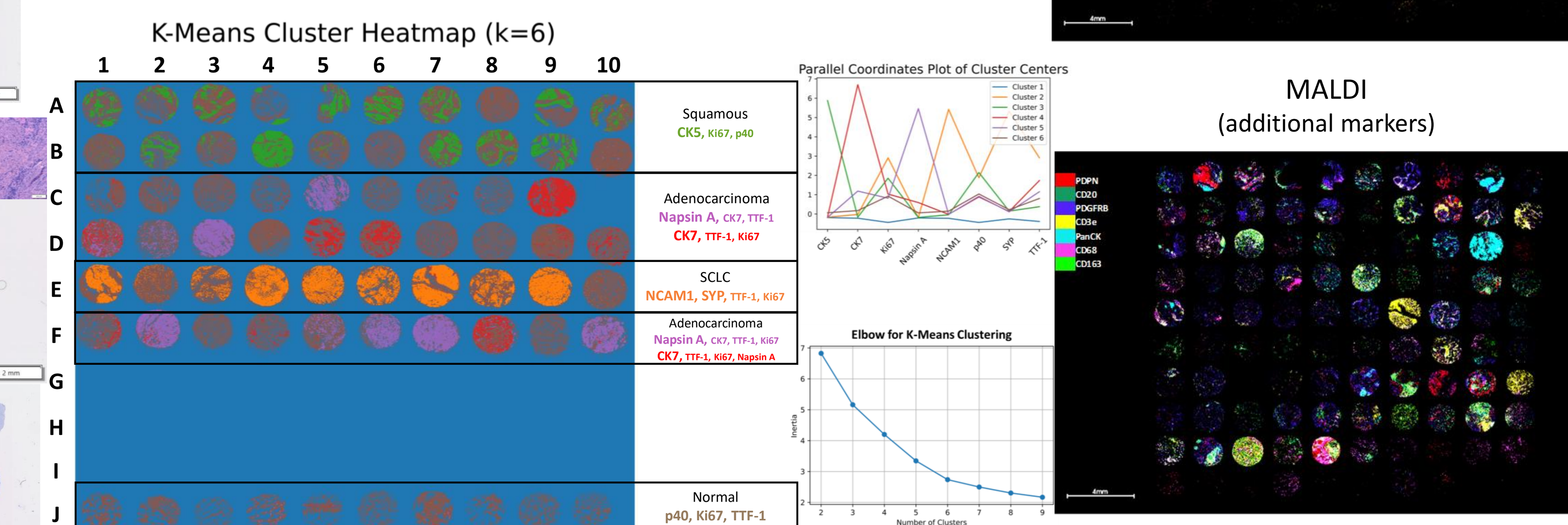
A commercially available 100-core FFPE human lung TMA containing a variety of diseased and normal state tissues was stained with a 23-plex lung cancer panel. The TMA was imaged by fluorescence, MALDI and brightfield microscopy, with the combined staining and run times equivalent to 27.5 hours, including a 15.25-hour MALDI scan overnight at 20 µm.

For subtype analysis, K-Means clustering was performed in Python on the relevant diseased and normal cores using the following 8 markers: TTF-1, Napsin A, p40, CK5, NCAM1, SYN, Ki67, CK7. The elbow method was then used to determine the appropriate k value. Using this method, we were able to compare subtype with clusters and their component markers.

TMA Map

	1	2	3	4	5	6	7	8	9	10	
A	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	
B	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	SCC (Lung)	
C	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	
D	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	
E	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	SCLC (Lung)	
F	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	ADENOCA (Lung)	Papillary ADENOCA (Lung)	Papillary ADENOCA (Lung)	Papillary ADENOCA (Lung)	
G	Atypical Carcinoid (Lung)	Atypical Carcinoid (Lung)	Atypical Carcinoid (Lung)	Atypical Carcinoid (Lung)	Atypical Carcinoid (Lung)	Meta. SCC (Lymph Node)	Meta. SCC (Lymph Node)	Meta. SCC (Lymph Node)	Meta. SCC (Lymph Node)	Meta. SCC (Lymph Node)	
H	Metastatic SCC (Lymph Node)	Metastatic SCC (Lymph Node)	Metastatic SCC (Lymph Node)	Metastatic SCC (Lymph Node)	Metastatic SCC (Lymph Node)	Inflamm. Pseudo-tumor (Lung)	Inflamm. Pseudo-tumor (Lung)	Inflamm. Pseudo-tumor (Lung)	Inflamm. Pseudo-tumor (Lung)	Inflamm. Pseudo-tumor (Lung)	
I	Tuberculo sis (Lung)	Tuberculo sis (Lung)	Tuberculo sis (Lung)	Tuberculo sis (Lung)	Tuberculo sis (Lung)	Cancer adjacent (Lung)	Cancer adjacent (Lung)	Cancer adjacent (Lung)	Cancer adjacent (Lung)	Cancer adjacent (Lung)	
J	Adjacent Normal (Lung)	Adjacent Normal (Lung)	Adjacent Normal (Lung)	Adjacent Normal (Lung)	Adjacent Normal (Lung)	Normal (Lung)	Normal (Lung)	Normal (Lung)	Normal (Lung)	Normal (Lung)	Adrenal Gland





Conclusions

An overview on the validation of antibody probes for MALDI-IHC by immunofluorescence and MALDI is presented.

Use of a 7-plex lung cancer subtyping panel, including TTF-1, Napsin A, p40, CK5, NCAM1, SYP, and CK7 was used in determining lung cancer subtype by MALDI-IHC on annotated tissues.

An extended 23-plex panel for the simultaneous determination of subtype on a 100-core human lung TMA, with K-Means clustering analysis, was able to provide good differentiation between subtypes in this preliminary study, while also providing additional fluorescence and H&E staining results in under 28 hours.

Further research will be conducted to extend the sample set, further utilizing H&E and fluorescence data, and performing pixel-by-pixel analysis of MALDI-IHC data.

Contact

Catherine Kita
AmberGen
ckita@ambergen.com
(978)-362-1131
www.ambergen.com

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