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MALDI-MS Detection Schemes Hasten Drug Development

Novel Method to Finding New Drug Candidates with Promising Therapeutic Applications

Rohan Thakur, Ph.D.

Over the last few decades, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has proven its usefulness and robustness in many applications. Recent innovations in MALDI-MS utilize advances in two detection schemes being applied to accelerate preclinical drug discovery: one in ultra-high-throughput screening (uHTS) programs, the other in drug-distribution MALDI imaging studies.

The recurring principle—fail early, fail cheap—drives innovation in pharma who seek to rapidly generate more compound leads or “hits” as well as involve ADME/TOX (absorption, distribution, metabolism, excretion/toxicity) earlier in the discovery process to quickly eliminate candidates. New initiatives have undoubtedly driven the growth (2015–2016) in drug pipelines to an estimated 11.5 %.[1]

Ultra-High-Throughput Screening

The ideal analytical tool for small-molecule R&D is label-free characterization and mapping along with the ability to measure directly and quantitatively. Rapid, robust, easy-to-use, cost-effective, and automation-ready are also features on most wish lists. New generation mass spectrometry inherently delivers on many of these criteria, and opened up a new arena for MALDI–MS within the last years. MALD-MS is helping researchers identify the most promising small-molecule leads, and is now expanding into the ultra-high-throughput, compound-screening programs that Big Pharma relies on.

When configured for uHTS, as with Bruker’s rapifleX MALDI-PharmaPulse™ instrument, the result is a system that is up to 50 times faster than a traditional instrument, with improved robustness, sensitivity, and extended mass range. Presented at the ASMS conference in 2016, a poster from Dr. Peter

Marshall *et al.* (GlaxoSmithKline, Stevenage, U.K.) described how their use of MALDI-PharmaPulse, coupled with nanoliter liquid handling, enabled them to screen more than 1 million samples per week.[2] The work was possible in large part because of the revolutionary 10 kHz laser in rapifleX MALDI–PharmaPulse.

Mass spectra were acquired in the range of either m/z 80–400 or 700–3,500, with 200 laser shots per sample. Under these experimental conditions, the system processed a 1,536-well plate in 7.36 minutes. With a vast in-house collection of candidate compounds, simple scale-up calculations indicate that to analyze 2 million compounds would require 7.85 days. An assessment of the robustness of the system and the methodology was made—with good correlation of results before and after 108 measurements.

They conclude that the technology and approach for uHTS is robust and can deliver very fast analysis times. The group has measured more than 1 million samples in a week and found that it is possible to measure more than 2 million samples without having to clean the instrument lens stack. Finally, looking forward to even higher throughput, the group achieved similar assay performance using 6,144-well plates. If adopted into routine, this could cut the time required to screen 2 million compounds to 2.39 days.

Valuable Tool in Small-Molecule R&D

Pharma companies are increasingly aware of the disconnection between outcomes in clinical programs and early-stage development work. As part of the industry's response, ADME/TOX investigations are now integrated as early as possible, in order to understand essential details of drug distribution, metabolism, and toxicology while still in preclinical phase.

The current best practice methods for determining tissue distribution of the parent drug and its metabolites are quantitative whole-body autoradiography (QWBA) and liquid chromatography–mass spectrometry (LC–MS), but these don't provide a complete distribution picture. QWBA studies are expensive and cannot distinguish parent drug from metabolite, presenting severe limitations for researchers looking for early insight into biochemical pathways and mechanisms.

LC–MS analysis is performed on extracts from tissue homogenates which inherently do not convey any spatial information and, just as importantly, can be misleading. For example, if a metabolite is localized to very small compartments, average amount in tissue reported by LC–MS doesn't accurately represent the true distribution and a researcher could draw incorrect conclusions about toxicity.

MALDI–MSI has emerged as a test that provides quantitative, spatially resolved data on the tissue distribution of drugs, drug metabolites, and other endogenous species. MALDI–MSI is helping

developers understand the spatial distribution and tissue physiology of a candidate drug and related metabolites before costly QWBA experiments, thereby allowing for informed decisions whether to move the candidate to the next development stage.

A range of mass spectrometers are utilized for MALDI-MSI, depending on application needs. MALDI-TOF systems, for example, offer highest throughput while magnetic resonance mass spectrometers (MALDI-MRMS) provides the ultimate in measurement accuracy and mass resolving power. Additionally, MALDI-MRMS can often yield unique molecular formula identification for compounds imaged.

In MALDI-MS imaging (MALDI-MSI), a conventional, fresh-frozen tissue section is treated with a solution of MALDI matrix solution, which extracts molecules from the tissue but retains the spatial relationships found in the underlying tissue. MALDI-MSI analysis generates a panel of label-free intensity/distribution maps of compounds detected from the sample. MALDI-MSI does not destroy underlying cellular features, which can subsequently be histologically stained to facilitate co-registration of histology with the MSI images for histopathological context.

Images shown in *Figure 1* illustrate the potential of this new technology for providing deeper insight into ADME/TOX mechanisms. In an early work by Castellino *et al.*,[3] lapatinib-dosed dog liver was analyzed by MSI at 50 µm spatial resolution. A correlation with histology revealed a metabolite having molecular weight of 649.14 Daltons localized to areas of inflammation.

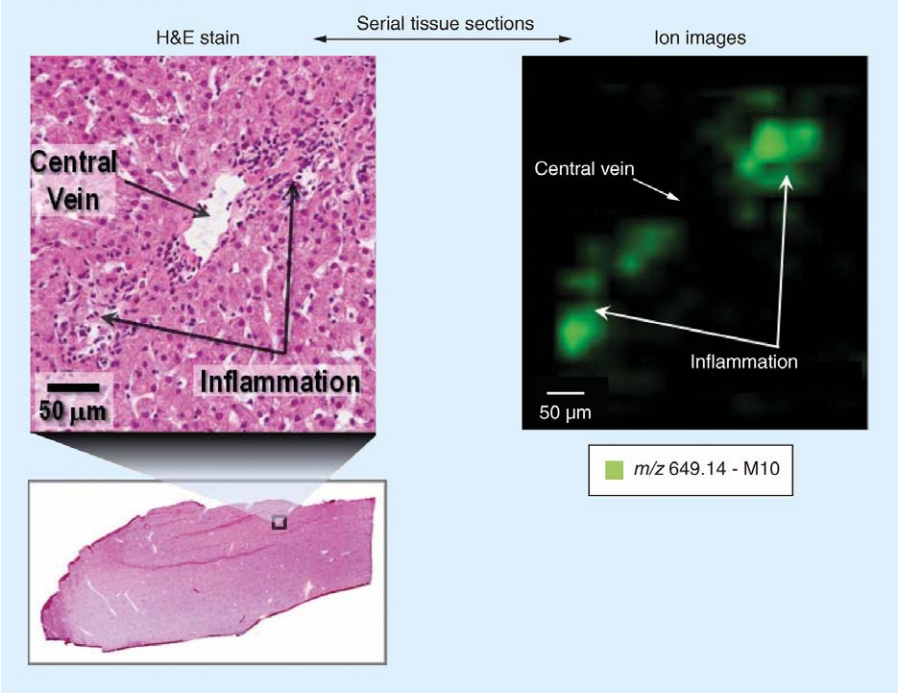

Figure 1. Images from histology and MALDI–MSI correlate to show that a specific metabolite is localized to areas of inflammation in a dosing study.[2]

In another recent publication from the same group,[4] MALDI-MSI was able to answer a question that had eluded conventional LC-MS analysis. Studying nephrotoxicity of the anticancer drug Dabrafenib in juvenile rats, tubular deposits were observed in kidney sections that did not present in kidneys of adult rats. MALDI-MSI was used to directly analyze the crystals and determined them to be calcium phosphate, providing a more complete risk assessment of Dabrafenib than would have been possible using traditional LC–MS or QWBA.

Conclusion

New technology and innovative approaches built around MALDI-MS are emerging that facilitate more rapid filling of discovery pipelines with new candidates, as well as the ability to probe ADME/TOX of these candidates at earlier stages in a more cost-effective manner. Already, many pharma companies have recognized the value in these new technologies and have invested in the latest instrumentation. With new applications being published monthly, anticipation is high that MALD-MS technology will provide additional insight and a deeper understanding of candidate compounds early in the discovery process.