Further developments in instrumentation to acquire data from a complete TMA are necessary. Conventional microscopy methods must be significantly adapted to acquire, in an unsupervised manner, images from the complete array, as is required to preserve spatial information and relationships. Large numbers of images are eventually 'stitched' together to provide a complete image. Continuous automatic focusing methods are essential to preserve sub-cellular resolutions. Although the work is currently slow, results are very precise and accurate.

THE FUTURE: Currently, we are moving away from normal microscopy platforms for this type of work. Under development is a prototype imaging station dedicated to high-throughput work. This arrangement eliminates traditional approaches and is equally suited to acquiring data from other 'large-area' samples. An obvious example is the use of high-density multiwell plates for assays involving drug action. One of the current rate-limiting steps is the use of scanned optical beams and single-point detection.[10–13] Highly parallel time-resolved detectors are however under development and the future of this technique will be dependant on complementary highly parallel signal processing instrumentation. Furthermore, data handling and analysis requires multiprocessor computer architectures and a high level of automation.

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HOW CAN PROTEOMICS HELP IN THE DEVELOPMENT OF MOLECULARLY TARGETED THERAPY OF CANCER?

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Matrix-assisted laser desorption/ionisation (MALDI) is a 'soft' ionisation technique that most often uses a time-of-flight (TOF) mass spectrometer. The advantage of MALDI-TOF TOF mass spectrometry (MS) over alternative approaches are ease of use, simple sample preparation and high throughput, rendering it an ideal tool for large scale clinical application.[1] Using MALDI-TOF MS for clinical specimens can yield important information about disease state, drug response and/or efficacy, and drug toxicities. It can also be used to analyse biomolecules that could serve as classifiers to determine which patients will most likely respond to certain molecularly targeted therapies. The use of MALDI-TOF MS as a clinical tool has been overshadowed by some notable failures. Focusing on reproducibility and validation, we have shown that MALDI-TOF MS can be successfully applied to protein profiling in a clinical setting.

MASS SPECTROSCOPY AS A DIAGNOSTICS TOOL: Achieving sufficient reproducibility in mass spectrometry for use in a clinical setting is hardly a trivial issue. Mass spectra can be highly var-

iable for a multitude of reasons: First, variations in instrument settings and performance, and operator performance. Second, variations in sample preparation (freeze/thaw cycles), open time, and pre-treatment (fractionation) can lead to non-reproducible data. Third, biological and population variations (age, gender, race, digestive state, etc.) need to be considered, identified and quantitatively measured.

The success of MALDI-TOF MS profiling hinges on sensitive and robust data analysis algorithms to render mass spectra comparable across different laboratories and instruments.

If the goal is to use mass spectroscopy in clinical practice instead of a research only tool, the methods used must be transparent to the physician and to the patient. A 'black box' approach will not be acceptable. Work flow is another consideration for the clinical setting; the system should be uncomplicated and robust, bearing in mind that this is a high-throughput technique.

VERISTRAT DEVELOPMENT: As an example of using proteomics technique Dr. Roder presented the development of VeriStrat, a mass spec based test, to select beneficiaries of EGFR-TKIs from pre-treatment serum or plasma samples. Competing tests are based on genomic analysis. These include EGFR copy number, determined by fluorescent *in situ* hybridisation (FISH), and mutation analysis, both of which requires large tissue samples. FISH appears to be more predictive for survival than mutation analysis; however, it is time intensive, costly and technically intensive. Clearly, a need exists for alternative means of identifying the patients who would most likely respond to EGFR-TKIs.

VeriStrat is a serum/plasma diagnostics system for stratifying non-small-cell lung cancer (NSCLC) patients based on response to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) such as erlotinib (Tarceva®) or gefitinib (Iressa®). Patient stratification is important because only a subset of patients derive clinical benefit from EGFR-TKIs, treatment cost is high, and alternatives exist. Additionally, increasing evidence indicates that treatment with EGFR-TKIs could be potentially harmful for non-responders. Stratification is also important for possibly extending use of these anticancer agents as first-line treatments. Approval would depend on a true end-point, not tumour response, because it does not correlate with overall survival.

To test the MALDI-TOF/VeriStrat system, serum or plasma samples from six institutions were used to assemble a training sample set, a test set and two control sets. Two laboratories tested the samples independently. Using the training set, a classifier of disease progression was developed (stable disease, progressive disease, partial response, early progressive disease). It was found that if the classifier was trained for extreme cases (stable disease classified as 'good' and early progressive disease as 'bad') it had a strong correlation with survival. Subsequent testing showed that patient classification was very reproducible in two laboratories. Spectra were generated at two institutions and VeriStrat was applied independently. The classification results showed very good agreement despite use of different instruments and lack of standardised operating procedures.[2]

Other patient subgroups have also been studied and the ability of VeriStrat to select patients with good prognosis on EGFR-TKIs was also possible for the subgroups of smokers and squamous cell patients. Concordance was very good, both for tests performed in different institutions on the same samples, and when plasma and serum samples were compared. Multivariate analysis of data based on VeriStrat testing of plasma samples from patients treated with erlotinib (Tarceva®) as a first-line drug showed that weight loss, performance status and the number of involved sites were independently significant, but VeriStrat remained a significant predictor even under the inclusion of these co-factors.

CONCLUSION: In summary, a simple mass spectroscopy-based pre-treatment patient selection system was established that is highly reproducible and capable of classifying patients by survival. It is specific for EGFR-TKI treatment and is capable of distinguishing patient subgroups with respect to covariates. Prospective trials are being planned based upon use of VeriStrat. In further studies we plan to combine proteomic and genomic markers, develop markers earlier and find reliable prognostic tools for other indications. The chance for success using VeriStrat is based on its low cost and high throughput. Commercialisation efforts are under way, including discussions with commercial laboratories and regulators. It would probably be advisable to use a centralised laboratory model at this point for upcoming trials. Other types of samples, based upon where one would expect to find markers for a specific disease may also be used. In general, it is important to consider how abundant the material is.

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