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News & Views

Protein Shakes — the future is not here yet for proteomic—based companion diagnostics, but it's coming

Michael Dumiak

In the corner of her lab deep in a brightly-colored postindustrial campus on the south side of Amsterdam, Connie Jimenez can measure the strides being made in her field of proteomics: it sits there in the form of a new mass spectrometer.

The new instrument is the third mass spectrometer to go online at Jimenez's OncoProteomics Laboratory, part of the industrious VUmc Cancer Center Amsterdam at the sprawling Free University. "This machine—we don't use it for discovery," she says. It is designed for high or semi-high throughput for verification and validation of candidates. "We are really moving from the discovery phase to the validation phase in things like colon cancer."

Applied research in proteomics still lags behind its sister field of genetics in producing companion diagnostics and drugs tailored to specific types of patients in the ongoing push toward 'personalized medicine,' an imaginative and compelling vision affecting pharma research, development and business strategies on a multitude of fronts. Stiff challenges still very much lay ahead and there is no shortage of scientists who are skeptical of the utility of proteomics in developing companion diagnostics.

But the same public-private partnerships which are producing genetics-based companion diagnostics to accompany drug development are starting to show up in the proteomics field. Funders seem a little more keen overall to back diagnostics and molecular-based drug development, and fundamental proteomic research is a fast-moving field, now moving past what were probably premature hopes attached to a proteomics-based diagnostic test for ovarian cancer. "The field of proteomics is maybe 10 years behind that of genomics," Jimenez says. "But I think the proteomic and mass spec techniques are maturing and in only recent years have gotten to the point where they can be applied and yield really powerful information."

It maybe a matter of time, Jimenez says, but only just. "In the end most drugs target proteins. It makes most sense to 1574-7891/\$ – see front matter
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Connie Jimenez, head of the OncoProteomics Laboratory and Associate Professor at the Dept. of Medical Oncology of the VU University Medical Center in Amsterdam.

put substantial effort into protein work to find biomarkers for companion diagnostics."

1. Push for Personal Diagnostics

Personalized medicine is many things to many people, but broadly speaking it is the use of new methods of molecular analysis to better manage a patient's disease or predisposition to disease, according to parameters set out by the US nonprofit advocacy group The Personalized Medicine Coalition (<http://www.personalizedmedicinecoalition.org/about>). The pairing of specialized diagnostics with drugs is part of this idea, as ideally this practice could tailor therapeutic and preventive treatment—dosages could be adjusted, or doctors could base choices of drug therapies based on a patient's genetic and molecular makeup.

QIAGEN, Manchester, UK.

It's now been more than a decade since the first companion diagnostics started coming on to the markets paired with drugs. The Dako-produced immunohistochemistry assay HercepTest launched in 1998 identified HER2-positive breast cancer patients; Genentech's Herceptin worked more effectively when paired with patients showing amplification of the protein. There are now more than 30 pairings of niche companion diagnostics outfits working with firms such as Roche, Pfizer, Bayer Schering Pharma, GlaxoSmithKlein and other big drug developers, according to published reports and an overview of companion diagnostics by Stephen Naylor, Percorso Life Sciences advisor and founder of a diagnostics startup called D2D.

The field of companion diagnostics is broad. Their use can result in drug treatment recommendations and on-label information; some researchers link early detection or early diagnosis kits to potential therapies, or see tests to detect biomarkers as potential guides to treatment. Researchers say the available companion diagnostics paired with drugs, however, are based in genetics, not proteomics, at least for now. These include QIAGEN's diagnostics—its TheraScreen®: EGFR29 Mutation Kit is used to test the mutation status of a patient's EGFR oncogene to identify their eligibility for treatment with AstraZeneca's IRESSA—or Amgen's Vectibix, for example; or LabCorp testing for HIV-infected patients for potential hypersensitivity to the GSK drug Ziagen. Merck is working with Celera in the development of diagnostics to pair with potential RNAi-based therapeutics.

2. Challenges...

Researchers are bullish on the future for proteomics and the use of proteomics tools in companion diagnostics, but there are more basic hurdles to overcome first, says Stephen Little, vp of the Venlo, Netherlands- and Hilden, Germany-based

molecular diagnostics firm QIAGEN. First there's the challenge for all companion diagnostics, which is demonstrating the clinical utility of the tests. Clinical tests are already intensive logistical, financial and reputation risks—companion diagnostics just complicate the picture.

Solutions are starting to come into play here: notably, partnerships among academic and institutional labs, diagnostics firms and pharma companies. Still, there's another step for proteomics. Little uses the example of an immunohistochemistry test, or real-time PCR test.

"You've got the drug approved, you've got the diagnostic approved. Now it's a pretty straightforward proposition to sell that diagnostic, because many labs have instrumentation or the platform or expertise to run the assay," he says. "With a proteomic test you have a whole new level of complexity because not only are you introducing a new diagnostic, you're introducing a new diagnostic on what is effectively a new platform."

He's referring to mass spectrometers. "It's not impossible. It's just another layer of complexity."

For Timothy Haystead, associate professor of pharmacology at Duke University Medical Center, part of the Duke Comprehensive Cancer Center, the barriers are taller.

"There is no effective way to globally separate the human proteome in a quantitative way. I think there are ways to do it, but I don't think anyone is really doing it," he says. Haystead is the former scientific founder of the proteomic tools firm Serenex—Pfizer snapped it up in 2008—which developed an inhibitor drug using a chemoproteomic platform for anti-cancer small molecule target Hsp90. The phase I inhibitor showed promise in treating solid tumors and hematological malignancies, a report in *Contract Pharma* said at the time. "Before the company was bought,

there was a lot of searching for the next validated target for cancer,” he says. “We couldn’t find a single one where you could make an argument to invest in it as a target and be sure that when you hit that target you would have a positive therapeutic outcome. That’s still true.”

Serenex was founded, Haystead says, to solve cell separation issues. “We don’t really have anything in hand that can separate out a cellular milieu so everything is separate from everything else, and you can measure it,” he says. “The best thing about protein is if you can do that, it’s much more readily quantifiable than other techniques like with RNA. They always use enzymes and amplification. With protein, you’ll get much more of an accurate evaluation and it is less dynamic in terms of its turnover. But nobody’s really cracked that nut.”

3. ...and Promises

Connie Jimenez’s OncoProteomics Lab (OPL) in Amsterdam came into being with two high-end tandem mass spectrometers, one experienced post-doc level mass spectrometrist, one experienced post-doc level informatician, a biochemist and a sense of calm perseverance.

Interest in proteomics picked up due to a 2002 paper from Emanuel Petricoin and Peter J. Levine in the *Lancet* about a National Cancer Institute-funded study outlining the use of the Seldi-TOF (surface-enhanced laser desorption / ionization, time-of-flight) mass spectrometer and the use of serum peptide patterns for a diagnostic to detect ovarian cancer. It first prompted the US Congress to weigh in with a resolution supporting funding for the clinic; after the *Lancet* paper, other researchers attacked it and even questioned the validity of any diagnostic test based on proteomics. The research became embroiled in a bitter conflict-of-interest imbroglio spilling far outside the lab as Congress pressed for reform in the National Institutes of Health. (As a post-script to the controversy, the OvaCheck diagnostic got approval from EU regulators in June 2010 for distribution and sale.)

In the midst of this the Cancer Center Amsterdam went ahead with the OPL project. “There was overpromise in that *Lancet* paper. But it generated the interest of oncology researchers in using mass spec-based approaches for pattern diagnostics,” Jimenez says. “It was the background of...not really companion diagnostics, but the hope for early detection and personalized treatments”.

Jimenez’s background is in neurobiology, using mass spectrometry to study neuropeptides in snail brains. Today, she says, this field would be called peptidomics and it grew up around her—things that took her years can now be done in a matter of hours. In 2005 she was working in the biology faculty and, in the wake of the *Lancet* paper and given that the Free University was building a new cancer research building, the oncology department asked whether Seldi-TOF was the way to go in mass spectrometry. Jimenez recommended Maldi-TOF (matrix-assisted laser desorption / ionization) instead, and came over to start the OPL in 2006. She added another instrument, a hybrid ion trap Fourier transform mass spectrometer, and started profiling serum peptides.

“Our workflow yields large sets of identified proteins—in the thousands of proteins per experiment—that we can identify and quantify. The nice thing about that approach is that we can feed the differential proteins, usually a few hundred in these comparisons, into pathway analysis tools to couple the differential proteins to biological functions and pathways. This can lead to more insight into potential mechanisms, for example during progression of colon adenomas to carcinoma. Drug resistance is another topic of interest.”

Progress can be seen in the development of proteomics partnerships—already further established among institutions pursuing genetics-based diagnostics and molecular-based drug development. Jimenez’s lab is talking with Roche about a prototype panel test for early detection of colon cancer. “It’s not perfect; working with our candidates, it may get better,” she says. “They say they already need quantitative assays for the proteins. If they believe there is potential, they will send a blinded serum set or a blinded sample set, and we have to show the performance in their blinded sets. If the markers have good performance, they are interested.”

4. Partners

Several factors are driving the emerging partnerships among these different players—research institutions, pharma, diagnostics firms—and even though each will have colliding interests, as will patient, doctor and insurer, enough of them align to make the business models compelling. QIAGEN’s Little says diagnostics is traditionally seen as low risk, low return; pharma, high risk, high return. If diagnostics are moved to a higher reward class and still maintain lower risk, investors are going to be interested. A drug discovery partnership for inflammatory disease like that struck by GSK and Cellzome, a European biotech employing chemical proteomics tools, shows a sample pricing mechanism:

At work with arrays and robots in the Cellzome labs.

Cellzome gets €33 million up front along with a piece of equity and a chance for milestone payments. The milestone payments could reach €475 million—if the alliance programs are developed and commercialized. The companies are working together using Cellzome's Episphere™ technology platform to identify selective small molecule drug candidates against targets from four different epigenetic target classes. The companies share operational responsibility for the programs until identification of drug candidates, at which stage GSK will assume responsibility for any further preclinical and clinical development and commercialisation. Cellzome research triggered two milestone payments in the year since the collaboration started, the most recent milestone coming in February.

A survey of the proteomics field shows converging interest, especially in oncology but in other fields as well. The US National Cancer Institute in July said it would invest \$120 million into centers conducting cancer proteomic studies and biomarker verification. Smaller and varied institutions like Cellzome and QIAGEN are also mulling the future of both proteomics and companion diagnostics. Cellzome vp of research Gitte Neubauer says the firm is starting an epigenetics drug discovery project targeting oncology, and is also considering using its chemical proteomics platform for the development of companion diagnostics to be paired with drugs. "Epigenetic regulation plays such a pivotal role in this disease area. We use proteomics assays to assess the potency and selectivity of our compounds directly in the lysate of cells or tissues," she says. "With advances in proteomics and the possibility to not only look at protein expression patterns but also at post-translational modifications, proteomics will play a role in the discovery of markers for pathway activity, for example. We ourselves are considering an approach where we use our chemical proteomics platform to measure, directly in patient material, the interaction of drugs with their targets including their modification status."

Stephen A. Williams,
chief medical officer of Boulder,
CO-based SomaLogic.

Boulder, CO-based SomaLogic is using a proteomics platform to develop a lung cancer detection diagnostic and is using its array to pinpoint markers for pancreatic cancer and mesothelioma. SomaLogic's chief medical officer, Stephen Williams, has a reminder about companion diagnostics, though. "It shouldn't be forgotten that measures of early response soon after initiating a drug are almost as useful and much easier to find," he says. "If we can do that in oncology it would be fantastic, yet it's looked down upon as somewhat less clever than making a prediction based on a specific target—even though the presence of a target or mutation is only one way of enriching targeted therapy."

New partnerships such as Strand Life's recent deal with Bangalore's MSCC to use proteomics tools in developing diagnostics for head and neck cancer show developments are active further afield than just western research centers. William Cho at the Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong, outlines an argument in his paper *Contribution of oncoproteomics to cancer biomarker discovery* that oncoproteomics still has the potential to revolutionize clinical practice. "It's because of the throughput, the sensitivity and quantitative—e.g. MRM—characteristics," he says. "But it's not fully explored yet."

Maybe not today, maybe not tomorrow, but soon.