

Metabolomics, The Iceberg of Complexity and Clinical Utility

by Robert G. Hunter MBA

The genomic revolution continues to deliver research and clinical breakthroughs (CRISPR/Cas, WGS) that suggest greater precision and prediction is just ahead. And despite extraordinary complexities proteomics performance/cost has evolved into a \$10- 20B annual market, spanning research to clinical applications on common new MS-based platforms, certainly one key for the validation and standardization essential to enable biomarkers robust enough for prime time diagnostics.

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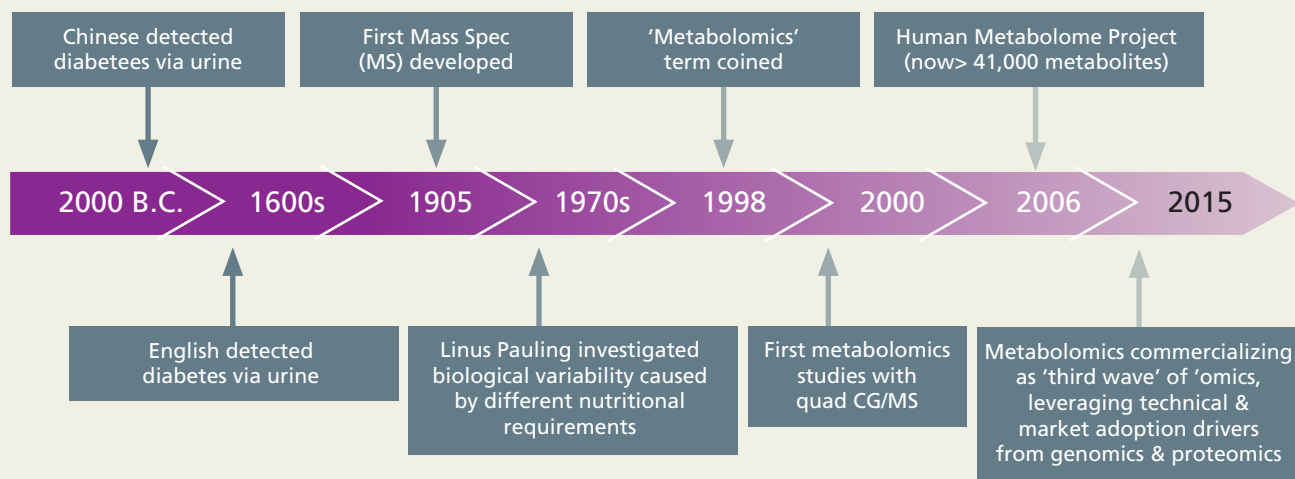
However complex diseases involve not only multiple proteins but also full pathway modifications, and require subtle but critical biochemical markers to detect patient-specific: gene protein toxin/drug/metabolome (microbiome) interactions. Naturally this is key for precision medicine.

In a recent BCC-sponsored webinar, I compared genomics as being like the tip of the iceberg to metabolomics being like the iceberg itself,

with respect to complexity, clinical utility, and unmet need for precision medicine. (See Figure 1). According to a new BCC Research report, the global metabolomics market is estimated at \$6.8 billion in 2015 and forecast to grow at 13% CAGR (compound annual growth rate) to \$12.5 billion by 2020. Genomics or transcriptomics involves DNA/RNA molecules with similar chemistry and limited modifications, and proteomics can identify approximately 20

amino acids, but the study of metabolomics involves thousands of different compounds with a wide chemical diversity. Not surprisingly, metabolomics brings tremendous data processing and interpretation challenges, evolving closely with systems biology. Also, in practice genomics is actively helping drive adoption of metabolomics, and proteomics has also helped pave the way technically and commercially.

LONG HISTORY, BUILDING MOMENTUM



\$12B

annual market

Clinical Biomarkers Unmet Needs

The main clinical application of metabolomics is in developing biomarkers for the diagnosis of disease and for monitoring therapies. Much of the promise of future precision medicine rests on robust biomarkers, and market forecasts continue to reflect big expectations, but actual clinical biomarker performance and use for treatment decisions has been questionable and new approvals have been slow.

Interestingly, the challenges begin with a large gap in awareness of what is practical. Most drug developers and physicians believe that most, if not all, biomarker assays can be standardized to produce consistent results, but the clinical laboratory community recognizes that even among decades-old tests, only a few qualify as standardized in vitro diagnostics: total cholesterol, creatinine

and glycosylated hemoglobin. They know widely variant results are possible if tests are performed in different labs using different methodologies or platforms, or even within the same labs using the same but using different lots of reagents. And beyond laboratory developed tests (LDTs), significantly higher variability is expected with in vitro diagnostic (IVD) kits utilized across different labs. They understand that even in the best cases such as HER2 biomarker, the odds of identifying HER2-positive patient candidates can vary significantly. This remains despite substantial improvements in quality systems, oversight via certifications such as Clinical Laboratory Improvement Amendments (CLIA) and via accreditation and proficiency testing provided by organizations such as the College of American Pathologists (CAP).

Metabolomics Advantage

Measuring metabolites has long been a mainstay of clinical labs. However, complex diseases are often multifaceted and require biochemical markers that integrate different aspects, rather than being defined by a limited menu of standalone markers. Also a given metabolic pathway may be involved in more than one disease.

Metabolomics is uniquely poised to add resolution and sensitivity at the pathway level and to thus identify biomarkers that are predictive of complex disease or response to therapeutic and nutritional intervention. And metabolomics is also enabling critical multiplexing to conserve sample volumes and drive down costs.

Furthermore the metabolome is highly conserved between different species, so metabolomics-based results are translatable from early pharmacology through to the clinical patient setting.

Metabolomics is being used to produce key new insights into important disease areas such as cancer, liver and metabolic disease, importantly translating across both clinical and research applications. (See Figure 3). Thus metabolomics is poised to dramatically expand the scope and utility of the clinical reference laboratory, and emerging metabolomic-derived tests are expected to play significant roles in precision medicine and patient management of the future.

BROAD MARKETS & POWERFUL DRIVERS

	Research	Clinical
Technologies	Mass Spectrometry Extraction Separation (HPLC) Systems Biology	Mass Spectrometry
Other Drivers	Genomics (CRISPR/Cas) Proteomics Precision Medicine Cell-Based Assays	Whole Genome Sequencing (WGS) Non-Invasive Prenatal Testing (NIPT)
Markets	Drug Discovery ADME-Toxicity Testing Basic Research	Cancer, Rare Diseases, Cardiac, Metabolic Diseases incl. Liver, Pharmacogenomics

Metabolomics Applications

Overview

Using metabolomics for biomarkers often begins with a discovery phase, utilizing untargeted metabolomics to identify novel metabolite biomarkers. Following the discovery phase, focused assay panels can be used to measure sets of metabolically related compounds allowing valuable insight into pathway regulation, underlying disease biology and biomarker verification. In clinical studies, a specific patient population is often compared with appropriately matched controls to define previously unrecognized associations between metabolites and clinical biomarkers. Finally, targeted assays are utilized that narrow in on a subset of biomarkers to facilitate validation of the metabolite biomarker.

60 Targeted metabolomic assay platforms are extremely useful for hypothesis testing. However they require extensive planning to define the key metabolites in biochemical pathways of interest. Rate-limiting precursors, end-product pools, critical intermediates, and potential alternate precursors provide valuable references to metabolic dynamics, and they can sometimes provide insight into the balance between product synthesis and transport rates.

No single analytical platform can sample all metabolites, so this generally requires the design of multiple platforms that provide the greatest selectivity and sensitivity for specific types. The use of previous findings from non-targeted studies, along with transcriptomics and proteomics, greatly improves the performance of individual assay platforms.

Alternately, untargeted or global approaches to metabolomics reveal subtle but relevant metabolic changes with a broad survey, offering the opportunity to identify meaningful clinical events that may be important for smaller sub-populations.

A key principle of modern metabolomics states that if a significant perturbation in a metabolic pathway is detected in an untargeted analysis,

other metabolites in that pathway, or in alternate pathways where metabolites can be diverted should also be detected in a more targeted metabolomic analysis.

Clinical Biomarkers Applications

Metabolomics is playing a key role in solving major unmet medical needs. (see figure 4).

Even in genome-wide association studies (GWAS), the majority of associations only explain a small proportion of the trait of interest. Often metabolomics is used in concert with genomics and proteomics.

Metabolomics stands to benefit greatly from adoption of genomics. The ultimate adoption of genomics is a complex issue, in part stemming from consumer issues of privacy concerns, responsibility and action-ability. Greater adoption, however, is already being driven by the technology development, cost reduction and available resources of companies such as Life Technologies, now part of Thermo Fisher. Genomic innovators have recently begun actively partnering with other players, including those in metabolomics. In a large sense, genomics and metabolomics are co-developing the market.

An example is the testing for inborn errors of metabolism (IEMs). These are relatively rare but serious conditions some of which have helped pave precision medicine chops within

Orphan Drug commercial approaches, for example lysosomal storage disorders. MS-based screening for IEM in neonates is routinely done in most industrialized countries. Non-invasive prenatal testing (NIPT) alone is poised to become a \$1-billion market, and metabolomics is playing a key role in developing new and more cost-effective IEM tests.

Naturally cancer is another active application for the dynamic duo, and research clinical translational experience gained here holds important insights for the broader development of precision medicine. Other clinical areas in which metabolomics adoption is proceeding more readily include next-generation cardiovascular, pre-diabetes/metabolic syndrome and liver disease biomarkers.

In pharmacogenomics, metabolomics is helping to deduce serious adverse reactions of certain individuals to various medications, not only across the disease areas mentioned above, but also important others like mental health and neural degeneration.

IEMs

Inborn errors of metabolism (IEMs) are inherited metabolic disorders that prevent the body from converting one chemical compound to another, or from transporting a compound in or out of cells or from one cellular compartment to another, essentially

CLINICAL APPS MEETING MAJOR MEDICAL NEEDS

Disease Category	Metabolomics Contribution	Opportunity
Cancer	Cancer Metabolism	Many metabolisms, drug targeted, in vitro models (3D)
Rare Diseases (Orphan Drugs)	Inborn Errors of Metabolism (EMs)	Several hundred recognized, only-50 tested at birth> Multiplex
Pharmacogenomics (PGx)	Adverse Drug Reaction (ADR) Tests	100,000 deaths = \$30-100B/yr. Statins, Warfarin, Pain, Depression and Mental Health
Cardio & Metabolic Disease	Early Diagnosis, On-going Monitoring	Heart Attack and Stroke, Diabetes, Liver Disease
Liver	Functional Diagnosis and even Basic Liver Biology	Liver Disease expected to be as large as Diabetes

necessary for all body functions. Most IEMs are caused by defects in the enzymes that help process nutrients, which result in an accumulation of toxic substances or a deficiency of substances needed for normal body function. Making a swift, accurate diagnosis of an IEM and prescribing the appropriate diet or medication are critical in preventing brain damage, organ damage and even death. Some examples of IEMs are represented within the category of Orphan Drugs, including lysosomal storage disorders such as Gaucher's disease, Tay-Sachs, Niemann-Pick disease.

There are several hundred recognized IEMs, but according to the National Institutes of Health, state public health programs only screen for nearly 40 disorders or fewer at birth. Multiple specimen types and analytic approaches are currently required to screen for the compendium of IEMs, but now untargeted or global metabolic profiling is enabling a single test to screen for dozens of IEMs that might otherwise require ordering multiple unique biochemical tests. In fact, the collaboration between Baylor Miraca Genetics Laboratories and Metabolon is able to identify all common IEMs studied to date that are screened on plasma amino acids, urine organic acids and acylcarnitine profiles. Novel IEMs continue to be discovered, assisted in recent years by whole-exome sequencing. Some studies have identified more than 400 agents that could not be detected in a traditional clinical biochemical genetics laboratory, even when using the full repertoire of available tests, including urine analyses.

Cancer

The cellular metabolism of cancer cells is a key characteristic that is altered when compared to normal, differentiated cells. This phenomenon was observed in early days of cancer research by Otto Warburg and became known as Warburg paradox, but little of the substantial research was done until the late 1990s because of the lack of basic

detection assays and in vitro tumor models.

Seahorse Bioscience Innovation.

Seahorse developed their XF "plate reader for metabolism" platform over about 15 years', at a time when the only other alternative was the Clark Electrode, which was a time-consuming and labor intensive technique, that primarily only hard core scientists studying mitochondria were willing to use. Seahorse was innovative and developed their technology working closely with KOLs from research institutes, and focused on software, and developing instruments that were robust and reliable. Seahorse focused on outreach to mitochondrial cell biologists, and with instrument sales tripling in 2008, there were over 450 scientists worldwide using the technology. With a focus on metabolic diseases such as diabetes, in 2008 Seahorse developed their first-in-class glycolysis assay; and at that point research (and funding) in cancer metabolism took off. They collaborated with lots of researchers, and by 2015 there were approximately 1,800 papers with Seahorse XF data. On September 9, 2015 Seahorse announced a \$235 million acquisition by Agilent Technologies, revealing that the privately-held Seahorse Bioscience FY15 revenue was estimated to be \$49 million, and that their cell-based assay tools are now an established standard, enabling the exploration of bioenergetics in living cells by nearly 10,000 scientists worldwide.

Cancer research has traditionally focused on transformations in signaling molecules that turn homeostatic cells into malignant ones, targeted approaches such as for tyrosine kinase inhibitors and targets of cell proliferation such as epidermal growth factor receptor (EGFR) and HER receptor. Recent realities of genetic mutations and the heterogeneity of individual tumors has led to a belief that understanding cancer biology at the systems level is key, and that more general approaches such as metabolomics and systems biology provide critical overarching support to assess the effect

of genomic and transcriptomic alterations on cell-signaling architectures and to investigate network modifications alongside changes in the relevant proteins.

Altered metabolism within cancer cells are driven by altered gene expression and altered activity of metabolic enzymes, engaging metabolic pathways that may not occur within normal cells. This increases their biosynthesis of certain macromolecules in order to grow tumors.

One-carbon metabolism, which encompasses a complex metabolic network that is based on the chemical reactions of folate compounds, is essentially the basis for most chemotherapy over the past 60 years. Some of the most popular cancer drugs utilize cancer metabolism, including folate agonist, and nucleotide inhibitors (antimetabolites) like 5-fluorouracil (5-FU).

Recently there has been a surge of interest in the study of the metabolic processes associated with cancer, much of it focused on the roles of glucose and glutamine in supporting energy metabolism and anabolic processes. Serine and glycine metabolic pathways have also been studied extensively. Recent data have suggested that many of the growth factor signaling pathways commonly perturbed in cancer impinge on metabolic enzymes, and that these metabolic enzymes may even act as oncogenes and even transform cells.

While glucose and glutamine serve as the primary catabolic substrates of proliferative metabolism, lipids such as FAO can also be used as an additional or alternative energy source in some leukemia cells and lung tumors.

In addition, lipids may play catalyst roles in providing an extra source of free fatty acids in some higher-grade tumors. Lipidomic analysis has also suggested that elevated lipid activity was particularly responsible for the increased production of specific lipid messengers known to promote tumor cell aggressiveness.

Newer approaches target metabolic enzymes and also mitochondrial folate metabolism. Metabolism in the mitochondria can differ in many respects from metabolism in the main part of the cell. Researchers have found that enzymes of the mitochondrial folate metabolic pathway, which are ordinarily low or absent in normal adult tissues, are highly upregulated in cancer.

One of the downstream consequences of cancer metabolism is the conversion of a large percentage of glycolytic pyruvate to secreted lactate, which in turn triggers additional metabolic responses as a result of local acidification within the tumor microenvironment. It has been suggested that this acidification can promote both tumor invasion and immune evasion, which are among the hallmarks of cancer. Also, lactate secretion may have a functional role described as a two-compartment model of tumor metabolism, in which malignant cells extract high-energy metabolites such as glutamine and fatty acids from neighboring cells and thus stimulate tumor proliferation and metastasis.

Pharmacogenomics

Pharmacogenomics (PGX) combines pharmacology with genomics to develop safe, effective medications and doses suited to a person's genetic makeup.

At the same dose, one group of patients can experience no therapeutic effect, whereas others develop serious adverse drug reactions (ADRs). This can lead to expensive and potentially life-threatening consequences.

Each year in the U.S. alone, approximately 2 million people suffer drug-related adverse events, accounting for 7% of all hospital admissions. More importantly, serious drug toxicities cause more than 100,000 deaths with costs estimated to be between \$30 billion and \$100 billion annually.

Initial pharmacogenomic studies have focused on candidate genes that encode proteins hypothesized to be involved in the absorption, distribution, metabolism and excretion (ADME) of specific drugs.

Major medical centers are beginning to implement pharmacogenomic tests in patients undergoing certain procedures or taking certain medications. This is particularly important for cardiovascular drugs because individual responses to medications are so varied.

There are now several common, well-documented genetic variations for many commonly prescribed cardiovascular drugs that can have a dramatic effect on an individual's metabolism, producing either too high an effective dose with increased risk of an adverse drug reaction or too low with reduced efficacy. Several classes of medications, including blood pressure and cholesterol-lowering drugs, anti-platelet agents, anti-arrhythmic drugs, and agents to control bleeding and clotting, can also produce adverse effects in certain individuals. A patient taking statins may experience muscle aches, commonly termed myalgia, and some patients have a small chance of developing a serious, life-threatening adverse drug reaction called statin-induced myopathy.

Other important applications include chronic pain medications, mental health medications, and polypharmacy where many concurrent medications increase the risk of interactions. However, most physicians do not receive pharmacogenetic training, and a big gap in awareness and know-how remains.

Ten percent of FDA approved drugs or approximately 200 drug labels carry pharmacogenomic information in their labels, and metabolizing enzymes account for the basis of 80% of these drugs. More than 650 drug-related variants have been identified for their clinical relevance.

Pharmacogenomic successes have been realized in management of warfarin (Coumadin), statins, and cancer drugs such as cisplatin chemotherapy and anthracyclines. An important body of work has been accomplished around azathioprine (Imuran and Imurel), an immunosuppressive drug used in organ transplantation and autoimmune diseases. Originally developed as a cancer

drug in the 1950s, it has also been widely used as an immunosuppressant. The main adverse effect of azathioprine is bone marrow suppression, which can be life-threatening especially in people with a genetic deficiency of a specific enzyme.

Another key application of metabolomics in pharmacogenomics is mental health, where the first treatment option doesn't work for nearly two-thirds of psychiatric patients, and half are still suffering after three more treatment attempts. Personalized medicine has the potential to both increase treatment effectiveness and lower costs of mental health.

One of the key drivers of this unpredictable response is personal variation in liver enzymes. The liver enzyme cytochrome P450 (CYP) 2C19 contributes to the metabolism of a large number of clinically relevant drugs, including antidepressants, benzodiazepines, mephenytoin, some proton pump inhibitors and clopidogrel. Like many other CYP450 super family members, the CYP2C19 gene varies highly among the population, with more than 25 variant alleles known.

Cardiac Disease

For decades, measures of metabolites such as glucose, lipids, creatinine, urea and uric acid have been used to assess an individual's disease condition. In fact the field of cardiology has been a frontrunner in the use of biomarkers in the diagnosis and management of various cardiac diseases.

These include diagnostic markers of acute changes (e.g., troponin I and troponin T for myocardial infarction, B-type natriuretic peptide for decompensated congestive heart failure), as well as prognostic markers (e.g., LDL cholesterol). Both types require differentiating subtle clinical phenotypes, acutely and over time, and to control for a wide range of variables including diet, medications and co-morbidities. Metabolomic studies in humans have begun to address these challenges, using both experimental and epidemiological study designs. Metabolomics provides better prognostic value versus

conventional biomarkers like B-type natriuretic peptide (BNP) in estimating heart failure-related metabolic disturbance.

In cardiology metabolomics has thus far been focused predominantly on improving the established markers associated with the common pathologies in cardiovascular diseases, including myocardial ischemia, atrial fibrillation, developing atherosclerosis, chemotherapy-induced cardiotoxicity and pulmonary hypertension related to advanced heart failure. Researchers have used both untargeted and targeted metabolomic approaches to identify, describe and verify metabolic differences between disease and non-disease conditions, independent of traditional risk factors, including those at risk for heart attack or myocardial infarction, stroke or death.

Early attempts to define metabolic profiles of cardiovascular disease (CVD) involved NMR-based profiling to compare the serum from individuals with severe coronary artery disease (CAD) with individuals having normal coronary arteries. The spectral profiles differed significantly and provided greater than 90% predictive power for discrimination between the two groups. However many cardiovascular biomarkers detect only the consequences of cardiovascular injury or ischemia, typically based on quantifying protein-based end products identified in biospecimens. The potential detection of metabolic changes that may occur before myocardial ischemia remains an important unmet need in medical diagnostics.

To this end, metabolomics has been used to study various forms of cardiovascular risk factors. Much of this work has occurred in either a context such as exercise stress testing (called planned myocardial ischemia) or a therapeutic procedure called planned myocardial infarction. These are two unique clinical cardiology scenarios in which serial sampling can be performed in patients both before and after a controlled trigger, thereby

allowing each patient to serve as his or her own biological control. In turn, the cardiology field has been a frontrunner in the adoption of early diagnostics, including those for assessing the risk of developing diabetes, obesity and metabolic syndrome, and also including in various settings relating to wellness and functional medicine.

One of the most important opportunities for metabolomics remains to uncover the key metabolic pathways that drive early cardiovascular disease, and much of this work has been conducted in the context of atherosclerosis, or hardening of the arteries: the build-up of fats, cholesterol and other substances in and on the artery walls. Various researchers have used metabolomics to identify risk of atherosclerosis and its associated impact in patients. Prior to 2008, most research focused solely on lowering cholesterol (LDL-C) with statin therapy to reduce cardiovascular risk. The Jupiter Trial was the first landmark trial to go beyond this and introduce the importance of a multi-marker strategy in assessing cardiovascular risk, looking at inflammation levels via systemic inflammation markers called hsCRP and vascular-specific markers for vulnerable plaque formation called MPO. And then with advanced lipid testing it became increasingly important to know the type and characteristics of the lipoprotein particles. Cleveland HeartLab is somewhat unique in looking at inflammation markers, but other suppliers of advanced lipid testing include Quest Diagnostics through its acquisition of Berkeley HeartLab, Athrotech and LipoScience, whose Liposcale test measures lipoprotein particles.

Metabolomics is being used in various ways to enhance cardiovascular biomarkers and to develop next-generation multi-markers to enable earlier detection. These have ranged from studies of phosphatidylcholine and choline metabolism to the arginine-NO metabolic pathways. The trimethylamine N-oxide (TMAO) assay is a test for TMAO, a gut flora-dependent metabolite that contributes

to heart disease. TMAO is a metabolite produced by the liver after intestinal bacteria have digested animal protein. Its value as a novel biomarker of increased cardiac risk was identified by Stanley L. Hazen, MD, PhD, at the Cleveland Clinic and the Lerner Research Institute. The assay was commercialized through LipoScience now part of LabCorp.

Diabetes and Metabolic Disease

A major clinical application of metabolomics is in the emerging area of early detection of diabetes and metabolic syndrome. Pre-diabetes can exist for a long time without triggering the most common diabetes symptoms which include frequent urination, continual thirst, and blurred vision. Standard methods of detecting insulin resistance or pre-diabetes using glucose tolerance tests or A1C percentage often show false negatives, because the pancreas is still able to produce enough insulin to overcome insulin resistance. In fact it is common that someone diagnosed with type 2 diabetes has already had the disease for as much as five years.

There are now ways to identify insulin resistance using blood biomarkers as a normal part of an annual or semi-annual general checkup. These include tests showing the degree of pancreatic output and pancreatic stress, such as fasting insulin and fasting glucose, C-Peptide test and a pro-insulin test, and a homeostasis model assessment (HOMA) that measures beta cell function and insulin sensitivity. Biomarkers that evaluate inflammation, including cardiac-specific C-reactive protein measurement (CRP), and tests that quantify fatty acid metabolism are also used. There are also biomarkers of lipid hormones such as leptin and adiponectin, which can give insight into a person's unique fat metabolism and insulin management. The target for these tests are individuals having a family history of diabetes and who have other indicators of metabolic imbalance, such as elevated cholesterol or hypertension. The tests facilitate patient education and motivation

METABOLOMICS IS THE ICEBERG

Genomics is the tip of the iceberg
DNA and RNA (A,C,T,G)

Proteomics
-20 Amino Acids

Metabolomics
2,800 to well more than 200,000 biochemical compounds, polar & nonpolar, myriad functional groups, chirality and isomer

Classifications and subclassification:
lipodome, glycome

***Established Metabolomes:**
Gut, food, human urine, human serum, cerebral spinal fluid, microbiome...

COMPLEXITY

CLINICAL IMPACT

FOUNDATION OF
PRECISION
MEDICINE

According to a new BCC Research report, the global metabolomics market is estimated at \$6.8 billion in 2015 and forecast to grow at 13% CAGR (compound annual growth rate) to \$12.5 billion by 2020.

to modify diet and lifestyle earlier, potentially avoiding insulin resistance.

Companies offering insulin-resistance screening tests include Genova Diagnostics and Metabolon, whose new insulin-resistance test Quantose IR is being marketed in the U.S., Mexico and Europe. Quantose IR is a laboratory-developed test (LDT) using a metabolomic approach that reflects insulin resistance based on insulin and three non-glycemic biomarkers, to assess an individual's risk of progression to prediabetes earlier than traditional glycemic measures such as A1C.

Metabolomics is used for the assessment of whole-body and target tissue metabolic responses to exercise stimulus, both short and long term, and for assessing the mechanistic aspects of exercise training. For more than a decade, the Studies of a Targeted Risk Reduction Intervention Through Defined Exercise (STRRIDE) trial has examined the impact of low, moderate and high-intensity exercise interventions on cardiac risk and insulin resistance markers. Targeted metabolic profiling revealed an association of branched-chain amino acids (BCAAs) and related metabolites with insulin resistance. Interestingly, obese subjects undergoing gastric bypass surgery have a much more dramatic decline in circulating BCAAs, acylcarnitines and key amino acids than found in response to dietary intervention, despite equal weight loss. This may help explain why gastric bypass causes a greater improvement in glucose homeostasis than dietary intervention. This is only one example of the emerging appreciation for altered amino acid metabolism in cardio & metabolic disease risk.

Liver Disease

Metabolomics is playing a very important role in diagnosing liver disease, which is envisioned to become as big a problem as diabetes within the next few years, and thus warrants a more thorough description.

NASH

Nonalcoholic liver disease (NASH) resembles alcoholic liver disease, but occurs in people who drink little or no alcohol. The major feature in NASH is fat in the liver, along with inflammation and damage. Most people with NASH feel well and are unaware that they have liver problems, but NASH can be severe and lead to cirrhosis in which the liver is permanently damaged, scarred and no longer able to work properly.

Patients generally only begin to have symptoms such as fatigue, weight loss and weakness once the disease is more advanced or cirrhosis develops, where the liver becomes seriously scarred, hardened and unable to function normally. This can take years or even decades, and not every person with NASH develops cirrhosis, but once serious scarring or cirrhosis is present, few treatments can halt the progression. A person with cirrhosis experiences fluid retention, muscle wasting, bleeding from the intestines and liver failure. Liver transplantation is the only treatment for advanced cirrhosis with liver failure, and transplantation is increasingly performed in people with NASH.

NASH ranks as one of the major causes of cirrhosis behind hepatitis C and alcoholic liver disease. It is estimated that NASH affects 2% to 5% of Americans. An additional 10% to 20% of Americans have fat in their livers, a condition called "fatty liver." If fat is suspected based on blood tests or liver scans, this condition is called nonalcoholic fatty liver disease (NAFLD). If a liver biopsy is performed, it will show that some people have NASH whereas others have simple fatty liver. Both NASH and NAFLD are becoming more common, in part because in the past 10 years the rate of obesity has doubled in adults and tripled in children. Obesity also contributes to diabetes and high blood cholesterol, which can further complicate the health of someone with NASH. In Europe, it is estimated that 20% to 25% of the population suffer from NAFLD, and

up to 33% in the U.K., where the government has made it a priority to fund research into better diagnosis and treatment.

Diagnosis

NASH is usually first suspected in a person when found to have elevations in liver tests included in routine blood test panels, such as alanine aminotransferase (ALT) or aspartate aminotransferase (AST). When further evaluation rules out more obvious reasons for liver disease, including medications, viral hepatitis and excessive use of alcohol, and when X-rays or imaging studies of the liver show fat, NASH is suspected.

The only means of proving a diagnosis of NASH and separating it from simple fatty liver is a liver biopsy. For a liver biopsy, a needle is inserted through the skin to remove a small piece of the liver. NASH is diagnosed when examination of the tissue with a microscope shows fat along with inflammation and damage to liver cells. If the tissue shows fat without inflammation and damage, simple fatty liver (NAFLD) is diagnosed. An important piece of information learned from the biopsy is whether scar tissue has developed in the liver. No current blood tests or scans can reliably provide this information.

NASH most often occurs in persons who are middle-aged and overweight or obese. Many patients with NASH have elevated blood lipids, such as cholesterol and triglycerides, and many have diabetes or prediabetes, but not every obese person or every patient with diabetes has NASH. Furthermore, some patients with NASH are not obese, do not have diabetes, and have normal blood cholesterol and lipids. NASH can occur without any apparent risk factor, and it can even occur in children. Thus, NASH is not simply obesity that affects the liver. The underlying reason for the liver injury that causes NASH is not known, but the several factors suspected include insulin resistance, release of toxic inflammatory proteins by fat cells (cytokines) and oxidative stress (deterioration of cells) inside liver cells.

Treatment

No specific therapies for NASH currently exist. The most important recommendations given to persons with this disease are to reduce their weight (if obese or overweight), follow a balanced and healthy diet, increase physical activity, and avoid alcohol and unnecessary medications.

Experimental approaches under evaluation in patients with NASH include antioxidants such as vitamin E, selenium and betaine. These medications act by reducing the oxidative stress that appears to increase inside the liver in patients with NASH. Whether these substances actually help treat the disease is not known, but the results of clinical trials should become available in the next few years. Another experimental approach to treating NASH is the use of newer antidiabetic medications, even in persons without diabetes. Most patients with NASH have insulin resistance, meaning that the insulin normally present in the bloodstream is less effective for them in controlling blood glucose and fatty acids in the blood than it is for people without NASH. The newer antidiabetic medications make the body more sensitive to insulin and may help reduce liver injury in patients with NASH. These medications include metformin, rosiglitazone and pioglitazone, and studies are ongoing.

Research

Due to the complexities of NAFLD, robust and coordinated basic and applied research is needed to find the molecular mechanisms and translate them into the clinic. From the simple starting point for triacylglycerol (TG) accumulation in the liver to the more complex implications of phospholipids in membrane biophysics, researchers are finding that the influence of lipids may be the key to understanding NAFLD, to enable non-invasive diagnosis, and to stop, revert or even prevent disease. Researchers are focusing on finding the metabolic fluxes that underlie membrane integrity in NAFLD by merging metabolomics with systems biology and other techniques. In addition to clinical liver disease as currently defined by NASH and NAFLD, metabolomics is playing a key role in liver research and has potential to play important clinical roles in the future such as for liver regeneration and

treatment for inflammation.

The National Institute of Diabetes and Digestive and Kidney Diseases funds the NASH Clinical Research Network, which comprises eight clinical centers located throughout the U.S. and a coordinating center at Johns Hopkins University. The NASH network researches the nature and underlying cause of NASH and conducts clinical studies on prevention and treatment. In the EU, the research program Elucidating Pathways of Steatohepatitis (EPoS) was funded by the first round of the European Union Horizon 2020 framework for health research and innovation. It is bringing together scientists and clinicians from nine leading centers in Europe to further understand the disease.

Near-term, liver research is closely aligned with the cutting edge of testing new drug candidates for efficacy and safety, where ADME Tox is a well-proven and commercially important market.

Metabolomics in the Age of the Microbiome

Metabolomics also seems poised to make an impact in the nascent field of microbiome research and therapeutics.

There is a growing acceptance that the microbes living symbiotically with our bodies play a fundamental role in our health. The microbiome is highly differentiated for each individual, and dynamic based on factors like diet, infection or antibiotic use. In fact one of the biggest challenges currently is understanding the basics of what a healthy microbiome looks like. Significant research has been published correlating microbiome changes to disease states, but little is known about the cause or effect of these changes.

Much of the initial focus is on the gut microbiota, partly because its size of up to 100 trillion cells exceeds the size of all the body's other microbe communities. Also the gut mediates a lot of other areas in the body, with an estimated one-third of circulating metabolites being a product of the gut microbiota.

Thus far the main proof of concept in the field has been the successful use of fecal microbiota transplantation (FMT) in which fecal matter is taken from a healthy donor and given to critically ill *Clostridium difficile* patients to

restore the good bacteria in their intestines. FMT has already advanced significantly from a DIY therapy using minimally screened individual donors to a highly standardized process, using universal donors subject to rigorous screening. As an example, the first public stool bank called OpenBiome has delivered over 6000 treatments to 400 hospitals in 5 countries. Less than 3% of prospective donors pass the 109-point clinical assessment and 30-item laboratory screening panel required for enrollment.

Naturally, another major area of therapeutic interest is on immune dysregulation and specifically local autoimmune diseases like inflammatory bowel disease, Crohn's disease and ulcerative colitis. Research also involves metabolic disorders, including obesity and type 2 diabetes, and recent microbiome transplant studies have demonstrated that introduction of specific microbes can influence host biology to drive weight loss or gain, e.g. possibly useful to treat obesity. Microbiome research is turning convention on its' head, finding in many cases a lack of microbial diversity being associated with disease states!

Concluding Remarks

Genomics can be viewed as the high-level road map and metabolomics the last mile details complete with dynamic updates, with the microbiome being only one of the more recently studied drivers. Many major medical challenges are being addressed by metabolomics.

We look forward to monitoring research and clinical developments as well as those in various settings relating to wellness and functional medicine. We expect to find metabolomics at the heart of the complexity as well as the clinical utility that is essential for advancing precision medicine.

Robert G. Hunter, MBA. is a life sciences market and technology analyst, management consultant and entrepreneur with over 20 years' experience. He authored BCC Research reports on Metabolomics (BIO055B), In Vitro Testing (PHM017E), and 3D Cell Culture (BIO140A). Recent consulting includes market analysis and revenue forecasting for a high content screening (HCS) leader. Bob has founded Predict-Medicine, drawing on experiences with disease management and integrative health & wellness, and inspired by the visions of Dr. Leroy Hood and others. See www.predict-medicine.com.