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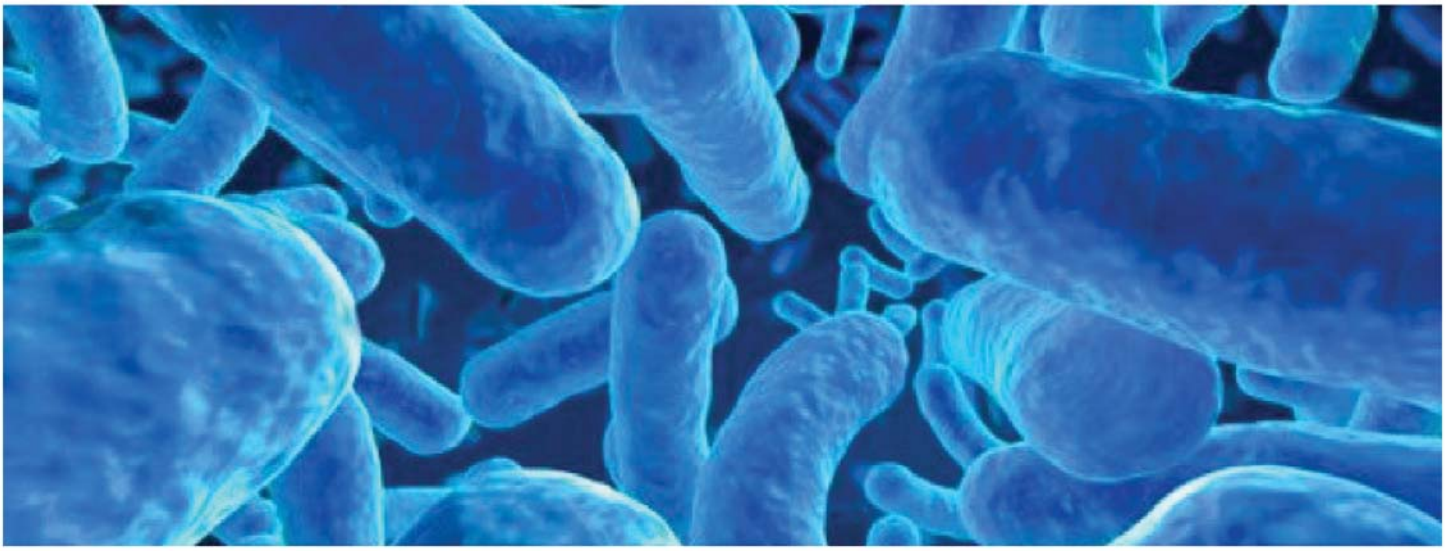
From complexity to clarity in the microbiome

WHITE PAPER



How to Use Microbiome Signatures as Biomarkers

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EXECUTIVE SUMMARY

Validated molecular biomarkers have become an increasingly important part of developing and prescribing drugs more efficiently as well as diagnosing physiological states earlier or more reliably. Enabled by improvements in next generation sequencing technologies, the microbiome (the entire collection of bacteria, viruses, and fungi that naturally occupy various niches on or in a given environment) has become a strategic source for biomarker discovery in the drug development and diagnostic industry. In this paper, we provide an introduction to microbiome-based biomarkers and the use of microbiome data as one component of a larger data profile for individuals in this era of precision medicine. We discuss the application of biomarkers enabling "targeted therapies" or serving as surrogate efficacy markers to shorten otherwise lengthy clinical trials. We explore the role biomarkers play in reimbursement decisions for new therapies based on cost-effectiveness[1]. Finally, we provide an example process for biomarker identification and validation. Details regarding Diversigen's unique approach and expertise are provided for consideration.

THE CHALLENGE

The latest analysis of the average cost to develop and win marketing approval for a new drug has been estimated as being higher than 2.5 billion USD with an approximate even split between out-of-pocket costs and time costs[2]. Innovative approaches are needed to increase efficiency in the drug development process in order to get new, more efficacious treatments to patients more quickly and at lower costs.

The stratification of both healthy individuals and patients through the use of validated biomarkers is an integral part of precision medicine. The application of these biomarkers, enabling "targeted therapies", range from predicting drug response, adverse effects, disease predisposition and disease progression as well as serving as surrogate efficacy markers to shorten otherwise lengthy clinical trials. To date, the majority of pharmacogenomic biomarkers for various types of therapies are "host" mutations/polymorphisms in either drug target receptor genes, e.g. EGFR, or drug metabolism enzyme genes like CYPs, but other factors such as the microbiome community structure have to be taken into account when developing a targeted therapy.

Besides improving the standard of care and increasing patient benefit, biomarkers play an increasingly important role when it comes to reimbursement decisions for new therapies based on cost-effectiveness[1]. Of note, the ten highest-grossing drugs currently in the market have a patient benefit ratio of 25%, at best, to an appallingly low ratio of 4% (Figure 1)[3]. Interestingly, the target indications for many of the highest-grossing drugs have all been recently linked to dysbiosis in the microbiome[4,5,6].

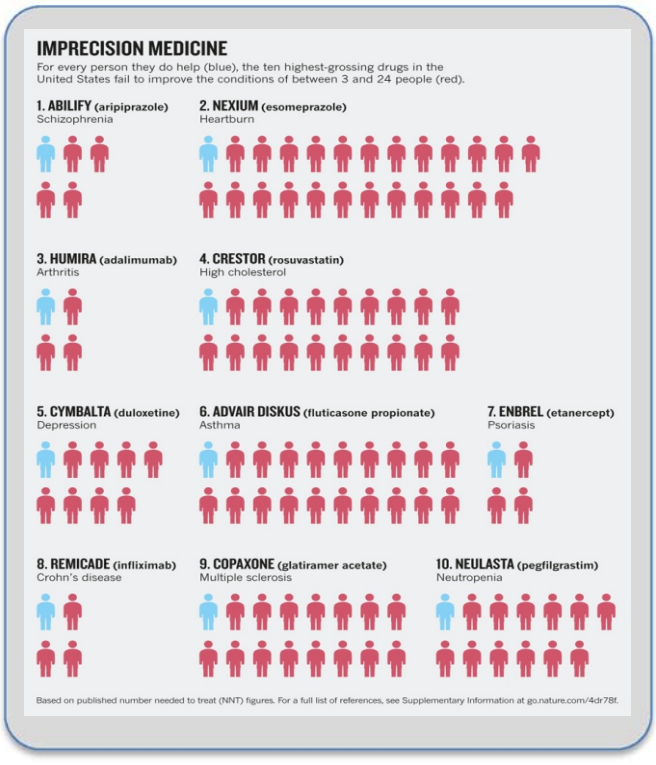

Fig. 1: Drug Response Rate for the ten highest grossing drugs[3]

THE MICROBIOME – AN EMERGING FIELD FOR BIOMARKERS

The field of microbiome research has grown exponentially over the past several years, catalyzed by the US-led Human Microbiome Project (HMP)[7] and the European-led MetaHIT[8] project. Study design and methods are evolving from strictly descriptive approaches to those that include comprehensive functional analysis of relevant microbial communities and constituents[9]. Microbial cells in the human body not only outnumber human cells by approximately ten to one[1] but their genetic load has been estimated between 100 to 200:1 greater than the human genome. So their influence on human development as well as disease pathogenesis and progression is a significant area of interest[10]. Countless efforts are now underway to develop microbiome-based diagnostics, therapeutics, and services in both the academic and commercial arenas.

Indeed, a healthy symbiotic microbiome has now been shown to have a direct role in the:

- Establishment of innate immunity
- Host metabolism (Energy, Vitamins, Digestion)
- Drug metabolism
- Production of signaling molecules with local or distant targets

Therefore, one area of marked focus is the ability to use changes in microbial community signatures, e.g. dysbiosis, to interrogate potential biomarkers associated with risk, onset or exacerbation of disease[11] as well as for prediction of treatment success.

Several mechanisms of action associated with a dysbiotic microbial community have been implicated in many disease states, such as:

- Disruption of microbial community homeostasis (e.g. *C.diff.*[12,13,14], *H.pylori*, viral upper respiratory infections[15,16])
- Interference with immune regulation and maturation[17] (e.g. diabetes[18], IgA secretion[18], T cell over-reactivity[19])
- Stimulation of host-gene expression (e.g. cancer[20,21])
- Shift in nutrient metabolism (e.g. malnutrition[22], obesity[23])

One of the most highly studied indications is ***Clostridium difficile*** **infections**, in which microbial dysbiosis has been directly linked to onset as well as chronic recurrence. FMT treatment has become more common, but its outcome/success seems under developed. [12,13,14].

In a model of **Type 1 diabetes** (T1D), there is evidence that certain shifts in the microbial signature can be linked to both onset and progression of the disease[13]. To better understand this link in humans and establish a microbiome causality in the development of T1D, a sub-study was established in the multi-national endeavor, TEDDY (The Environmental Determinant of Type 1 Diabetes in the Young), to analyze over 22,000 GI, plasma, and nasal swab samples from birth to disease onset. Dr. Joseph Petrosino, Chief Science Officer at Diversigen, is spearheading the microbiome component of this NIH-funded project, the largest clinical microbiome study to-date.

For various forms of **cancer**[20], enrichment or augmentation of specific bacteria compared to a control as well as links with other clinical endpoints, including BMI and gene profile changes, are paving the way for better preventative and diagnostic tools[21].

Additionally, a gut microbial profile linked to **obesity** has been shown to be transmissible, unveiling a unique way to identify risk markers of the disease[23].

Studies indicate that mitochondrial dysfunction impairs synaptic function and plays an important role in **neurodegenerative disease** pathology[24]. Given that certain microbial community structures result in an overproduction of propionic acid, which – when metabolized to methyl-malonyl-CoA – negatively impacts mitochondrial efficiency[25], stratification of subjects for clinical trials in the neurodegenerative field based on such community structures has the potential to significantly reduce cost and time.

Environmental factors, such as diet and prolonged antibiotic use are suggested triggers of microbial dysbiosis and other microbial shifts that may cause an increase in disease risk for, and/or an earlier onset and more aggressive progression of disease[11-13,17]. Linking the human commensal microbiota and the mechanisms and triggers underlying disease, in the context of biomarker identification, is quickly becoming the most clinically relevant use of microbiome data. This information, which may be one component of a larger data profile for individuals in this era of personalized or precision medicine, may be used for risk prediction and/or diagnosis of disease or disease progression and may also be used for stratification of subjects in clinical drug trials.

A CUSTOMIZED APPROACH FOR BIOMARKER PROJECTS

At Diversigen, we utilize our extensive knowledge base, flexibility in sequencing platforms, and an agnostic approach to analysis to identify the appropriate method for microbial community or metabolic profiling for each project. With an emphasis on reaching adequate sequencing depth and delivery of high quality data, we are able to infer metabolic potential for more targeted hypothesis generation under which specific biomarkers could be identified and validated.

We offer approaches to address every level of microbiome query. We begin with amplicon-based approaches, such as 16S rDNA sequencing, which yield a high-level overview of the structure of a microbial community. We then have the flexibility to utilize multiple different strategies, including whole genome shotgun (WGS) DNA and/or RNA sequencing to profile the species in a microbial community, as well as map the functional and metabolic potential of the microbes in a particular sample. When a strain of interest is isolated, we then can use long read sequencing platforms and library construction strategies to assemble accurate, complete bacterial genomes.

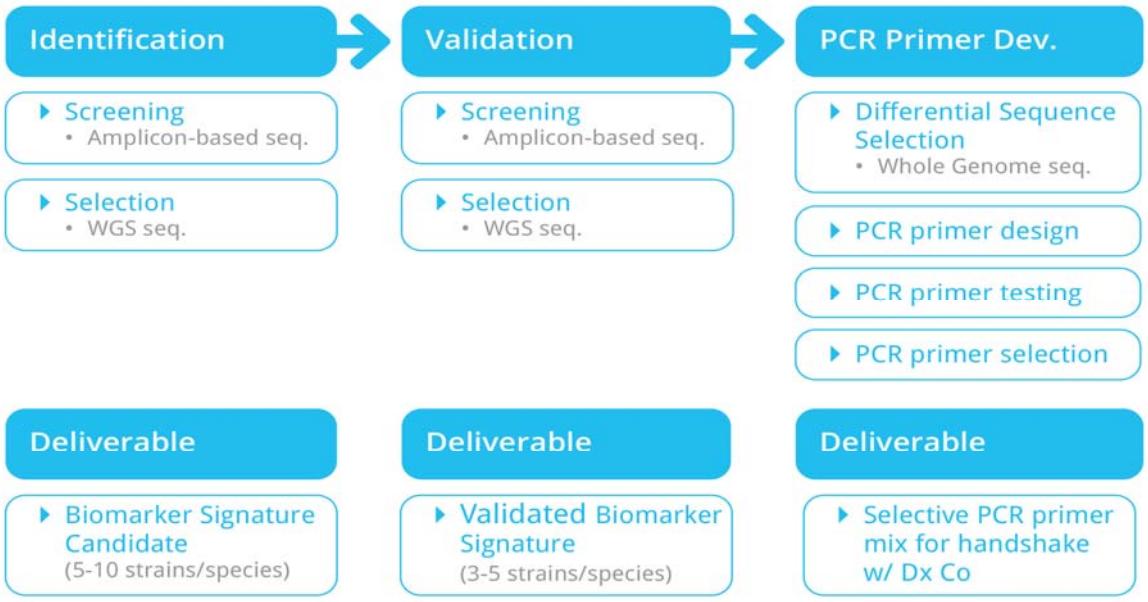

Fig 2: Example process for identifying, validating and developing microbiome-based biomarkers

Our customers benefit from our experience in both human clinical trials and animal model studies of numerous human diseases, as well as our experience linking other 'omics data with microbiome/metagenomic data. For example, our expertise enables us to link unique metabolite signatures to various bacteria of interest based on metabolomics data.

The value of microbiome-based biomarkers is clear when considering that the majority of samples taken for microbiome analysis can be either non- or minimally invasive without the need for biopsies. This will help with increased patient compliance when it comes to testing for various diseases.

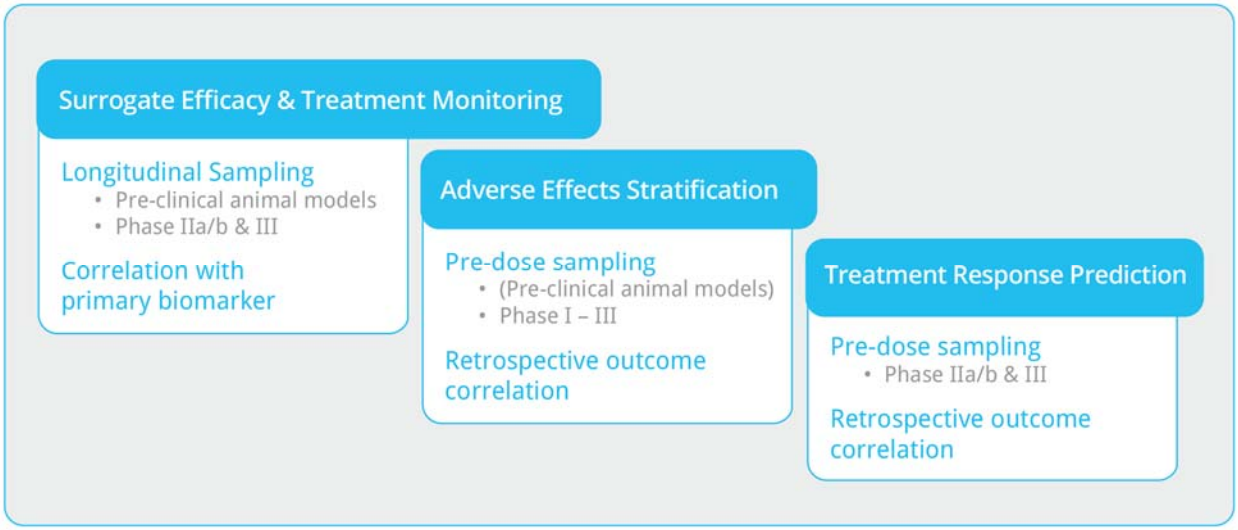

Fig. 3: Sampling strategies for microbiome-based biomarker discovery and validation

CONCLUSION

The future looks bright as research efforts continue to utilize microbiome data for biomarker discovery across multiple disease states. Potential impact of this research includes the development of novel approaches associated to diagnose risk, onset, or exacerbation of disease resulting in significantly increased patient benefit ratios for successful drugs. Microbiome information, which may be one component of a larger data profile for individuals in this era of personalized medicine, may be used for risk prediction and/or diagnosis of disease or disease progression and for stratification of subjects in clinical drug trials.

Key factors in successful microbiome-based biomarker identification and validation include stringent sampling protocols, high quality metagenomic data with sequencing depth adequate to identify all key players, and the ability to identify bacteria of interest. When properly employed, these factors can also result in the successful linking of metabolic and functional profiles to human or animal phenotypic data.

Diversigen offers extensive experience, a full-service lab, and a customized approach to maximize the potential of biomarker projects. For more information, contact the authors of this paper at info@diversigen.com.

ABOUT DIVERSIGEN

With years of experience, flexible sequencing, and expert bioinformatic capacity, Diversigen is building on research conducted at Baylor College of Medicine in the Alkek Center for Metagenomics and Microbiome Research. Our highly qualified and experienced personnel are recognized as thought leaders at the forefront of the microbiome field. Diversigen provides comprehensive services that include a variety of

amplicon-based and metagenomic sequencing, expert bioinformatics with timely turnaround of high quality sequencing data, in-depth data analysis, customized project design, professional project management and customer-focused service. Diversigen's lab is soon be CLIA-certified.

References

[1] McCabe C et al.; The NICE cost-effectiveness threshold: what it is and what it means; *Pharmacoecon*, 2008; 26(9):733-44

[2] DiMasi J; Innovation in the Pharmaceutical Industry: New Estimates of R&D Costs; http://csdd.tufts.edu/files/uploads/Tufts_CSDD_briefing_on_RD_cost_study_-_Nov_18,_2014..pdf; accessed on Sept. 18th, 2015.

[3] Schork J; Personalized Medicine: Time for one-person trials; *Nature*, 2015, 520:609-611

[4] Huang YJ, Boushey, HA; The microbiome in asthma, *J Allergy Clin Immunol*, 2015, 135(1):25-30

[5] Tinan TG et al.; Genomics of schizophrenia: time to consider the gut microbiome?, *Molecular Psychiatry*, 2014, 19:1252-57

[6] Cho I, Blaser MJ; Human Microbiome: at the interface of health and disease, *Nat Rev Genet.*, 2012, 13(4):260-270

[7] s. www.hmpacc.org

[8] Qin J et al.: A human gut microbial gene catalogue established by metagenomic sequencing; *Nature*, 2010, 464:59-65

[9] The iHMP Network Consortium; The Integrative Human Microbiome Project: Dynamic Analysis of Microbiome-Host Omics Profiles during Periods of Human Health and Disease; *Cell Host & Microbe*; 2015, 16: 276-289

[10] Gross L; Human Gut Hosts a Dynamically Evolving Microbial Ecosystem, *PLoS Biol*; 2007, 5(7): e199

[11] Dietart RR at al.; Biomarkers for the 21st century: listening to the microbiome; *Toxicol Sci*, 2015, 144(2): 208-216

[12] Seekatz AM et al; *Clostridium difficile* and the microbiota; *J Clin Invest*; 2014, 124(10: 4182-4189

[13] Koenigsknecht MJ et al.; Faecal microbiota transplantation for the treatment of recurrent *Clostridium difficile* infection: current promise and future needs; *Curr Opin Gastroenterol*, 2013, 29(6): 628-632

[14] Taur Y, Pamer EG; Harnessing microbiota to kill a pathogen: Fixing the microbiota to treat *Clostridium difficile* infections; *Nat Med*, 2014, 20(3): 246-247

[15] Allen EK et al.; Characterization of the nasopharyngeal microbiota in health and during rhinovirus challenge, *Microbiome*, 2014, 25;2:22

[16] Yi H et al.; Profiling bacterial community in upper respiratory tracts, BMC Infect Dis., 2014, 14:583

[17] Kau AL et al; Human nutrition, the gut microbiome and immune system: envisioning the future; *Nature*, 2012, 474(7351): 327-336

[18] Brown K et al.; Prolonged antibiotic treatment induces a diabetogenic intestinal microbiome that accelerate diabetes in NOD mice; *ISME J*, 2015, 10: 114

[19] Yu B et al.; Dysbiosis of gut microbiota induced the disorder of helper T cells in influenza virus-infected mice, Hum Vaccin Immunother., 2015, 11(5):1140-6

[20] Narayanan V et al; Human Fecal Microbiome-Based Biomarkers for Colorectal Cancer; *AACR J*, 7(11): 1108-1111

[21] Gui QF et al; Well-balanced commensal microbiota contributes to anti-cancer responses in a lung cancer mouse model; *Genet Mol Res*; 2015, 14(2): 5642-5651

[22] Preidis GA et al.; Composition and function of the undernourished neonatal mouse intestinal microbiome, *J Nutr Biochem.*, 2015, 26(10):1050-7

[23] Turnbaugh PJ et al; An obesity-associated gut microbiome with increased capacity for energy harvest; *Nature*, 2006, 444: 1027-1031

[24] D. Pathak et al.; The Role of Mitochondrially Derived ATP in Synaptic Vesicle Recycling; *J Biol Chem*; 2015 290(37): 22325-22336

[25] R. Frye et al.; Gastrointestinal Dysfunction in Autism Spectrum Disorder: The Role of the Mitochondria and the Enteric Microbiome; *Microb Ecol Health Disease*; 2015, 26: 27458-74