ScientistSpotlight

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# Biomarker signatures to define the right therapy *the first time*

## Introduction

Personalized medicine research aims to identify molecular biomarkers and translate clinically validated data into improving patient outcomes, defining the right patient treatment *the first time*, and overall, advancing the standard of care. It is fair to say that to date personalized medicine research has been driven by the oncology field; however, such efforts are not limited to cancer and apply to other complex diseases. In the case of autoimmune chronic diseases such as rheumatoid arthritis (RA)—a systemic disease that has a worldwide prevalence of up to 1–2%—stratifying patients based on specific molecular profiles will help physicians determine the most appropriate treatment pathways to stop longer term progression of this potentially very debilitating disease.

Diagnoses are currently made via a combination of medical history, a physical examination of joints, and a series of more than 8 lab tests, including biochemical and hormonal analyses and whole-body imaging. None of these tests, however, are definitive predictors of which therapy to select or the efficacy of the selected therapy.

To date, there are no diagnostic tools available to identify the molecular differences between RA patients and to predict an individual's response to therapy. Being unable to administer the right patient treatment *the first time* can lead to increased healthcare costs and a decrease in the patient's productivity while the disease persists.

Dr. Bruno Stuhlmüller and his team at the Charité Free University and Humboldt University in Berlin are tackling the challenge of identifying and validating mRNA and miRNA biomarker signatures that could define the success of various therapy strategies for RA.

- **Dr. Stuhlmüller, RA is a very common and potentially serious disease. What is the clinical challenge you are trying to address in RA?**

Rheumatoid arthritis is a chronic disease causing the destruction of synovial joints' connective tissues, muscle, tendons, and fibrous tissue. The disease can be very debilitating, as it often affects people in their most productive years, so the disease effects go far beyond the physical and can have significant socioeconomic impact on patients' lives[1,2,3]. The inflamed joints are characterized by cartilage and bone destruction. In RA, not only tissue macrophages, but also blood monocytes are known

**Bruno Stuhlmüller, PhD, is head of the scientific laboratory and team leader at the Institute of the Rheumatology and Clinical Immunology department at the Charité Free University and Humboldt University, Berlin. Dr. Stuhlmüller's team is focused on molecular analyses of autoimmune diseases. The main field of interest concentrates on molecular techniques in basic science and on development of assays for fast diagnosis, therapy monitoring, and the prognosis of rheumatoid arthritis treatment with steroids, methotrexate, and biologics, as well as the prediction of treatment successes for conventional and novel treatment strategies. In close cooperation with Dr. Thomas Häupl and colleagues from the BioRetis, several techniques in bioinformatics have also been established.**

"One of the reasons we selected Affymetrix' GeneChip® U133 Plus 2.0 Array is that it is a proven, highly reproducible technology that can be translated into clinical application."

to be activated systemically, and they spontaneously release inflammatory mediators such as TNF, pro-inflammatory interleukins, and chemokines, which mediate and force the destructive process.

The use of various biologics, such as TNF-inhibitors, for RA therapy is rapidly increasing; however, no diagnostic tools exist to identify molecular differences between patients and therefore predict an individual's response to therapy. This leads to a "trial-and-error" therapy strategy, and included among the many implications to not being able to administer the right patient treatment *the first time* is the impact on the patient's productivity, the risk of infection, and the increased costs of healthcare. Meanwhile, the RA progresses[4].

Currently, we are looking for therapy-predictive mRNA and miRNA biomarkers that would allow us to identify the molecular parameters that will guide successful therapeutic decisions for monotherapies using anti-TNF biologics as well as for non-biologic corticosteroids, MTX (methotrexate) monotherapy, or combination therapy[5]. For molecular analysis of therapy outcome, current industry standards in treatment trials almost exclusively work with whole-blood collection using standardized blood drawing and preservation systems. The aim of our work is to identify and validate a biomarker signature using Affymetrix® microarrays with a high level of automation for expression analysis in whole-blood samples.

**Dr. Stuhlmüller's research group: (back, left to right) Karsten Mans, Sabrina Bolle, and Neeraj Tandon; (front) Bruno Stuhlmüller.**

- **Many translational researchers are faced with a huge range of molecular parameters to study and analysis tools to choose from. Why did you decide to look at mRNA and miRNA markers, and what led you to select Affymetrix' GeneChip® technology to do your work?**

Well, the aim of our studies is to develop a biomarker test for the diagnosis of RA and for prediction of an RA patient's response to anti-TNF therapy. Our instinct was to look for mRNA markers, so we used the GeneChip® U133 Plus 2.0 microarray to analyze the transcription profiles of purified monocytes from RA patients before and after the initiation of therapy to identify the biologically relevant pathways. Our preliminary data indicated that 11 genes were differentially expressed between the 2 conditions and can be used for the prediction of a successful treatment with anti-TNF antibodies[6].

One of the reasons we selected Affymetrix' GeneChip U133 Plus 2.0 Array is that it is a proven, highly reproducible technology that can be translated into clinical application. In addition, it will enable us to validate markers identified by transcription profiling and translate the signature immediately into clinically applicable test systems for diagnosis, prognosis, therapy monitoring, and prediction of successful therapies for RA individuals. One obstacle not related to microarrays is the limitations of using whole blood, with its variability of differential blood count as a confounder of differential gene expression. We've started to address this with the method described below.

After our mRNA studies, we realized that to define diagnostic, predictive, or prognostic biomarker signatures, it is critical to also understand the miRNA regulatory pathways for RA, as this will extend not only the diagnostic power of the test but also provide a deeper understanding of the cellular responses against conventional steroidal and non-steroidal drugs, biologicals, or novel selective small molecules/chemicals used in RA therapy today and in the future. In our studies we therefore included Affymetrix miRNA microarray analyses.

It's also worth mentioning that in recent publications, we demonstrated that monocytes from RA patients exhibit disease-specific gene expression profiles[7] that can be molecularly dissected when compared to *in vitro*-generated cytokine signatures. The results suggest that an assessment of cytokine-response status in monocytes may be helpful for improvement of diagnosis and selection of the best target for therapeutic intervention[6,8].

- **You mention that the standard practice for molecular analyses in RA is to use whole blood. Blood is notoriously difficult to work with; how have you overcome the challenges of working with these important clinical sample types?**

First of all, we isolated monocytes from whole blood, and using the GeneChip® U133 Plus 2.0 microarrays, we identified 11 biomarkers in a comparative screen of the monocyte transcriptome in RA patients that allow the accurate prediction of successful anti-TNF therapy. One of these markers, CD11c, was validated in human-blood monocyte cells with 2 independent groups. Validation using quantitative PCR confirmed this biomarker and resulted in a high sensitivity and specificity[6]. However, in practical terms for clinical applications, working with monocytes is not ideal, as blood samples would need to be transported as quickly as possible to the lab. Monocyte purification may also lead to cell activation, not to mention that the isolation and sorting of monocytes is time consuming, so a test system for whole blood is mandatory and eagerly demanded. When using whole-blood samples collected with the PAXgene blood kit (Qiagen®), it is critical to recalculate the differences in individual cell compositions of the blood samples prior to trancriptome analyses. The recalculation method is called **F**unctional **P**rofile **C**omponent **A**nalysis (FPCA) and was developed in collaboration with Drs. Thomas Häupl and Andreas Grützkau. It allows us to generate a virtual molecular gene expression profile of selected biomarkers in cell mixtures, and it suppresses effects of different changes of blood counts. This in turn allows us to calculate virtual adapted true gene regulation changes.

In summary, we will use a multi-parameter and multi-modality biomarker analysis technology for the characterization of gene networks in whole blood.

- **So you've discovered a sensitive and specific predictive gene signature from a clinically relevant sample type. This sounds like a successful start to your translational research. In order to take your studies further towards the clinic, what are the next steps?**

The next steps are to expand the size of the patient cohort and conduct a larger validation trial. In addition, further studies are needed to define predictive biomarkers for other biologics or drugs used today in the treatment of RA. To do this, we will continue to use whole-genome mRNA and miRNA microarray analyses to define more biomarkers. Once that is completed, we will need to validate the biomarkers in larger patient collectives. Validation might also be performed using other technologies (qPCR, ELISA, flow cytometry, etc.), which will allow not only a technical validation but also gives us more options for translation of basic science knowledge into clinical application.

- **Assuming a positive outcome from your upcoming validation projects, what could the potential impact be on RA patient care? How many patients could this help, and how much money could be saved, uncomfortable treatments avoided, etc.?**

A striking socioeconomic impact leads to direct and indirect costs of ≈€30 billion per year in Germany. Globally, costs are about €700 billion per year. Therefore, an early diagnosis and effective therapies are the most important goals for patients with RA.

Traditionally, RA treatment starts with conventional steroid or non-steroidal drug therapies. If the RA patient doesn't respond to this first-line therapy, in most cases, biological therapy is then considered. These biologics (antibody-based protein drugs) were developed to target specific biomolecules of interest and thereby interfere with their corresponding pathway (e.g., inflammation pathway) and biological function. Biologics are more specific and are generally more successful treatments.

"...and using the GeneChip® U133 Plus 2.0 microarrays, we identified 11 biomarkers in a comparative screen of the monocyte transcriptome in RA patients that allow the accurate prediction of successful anti-TNF therapy."

Discontinuation of therapy for non-responders is usually considered after administering the biologics for 3–6 months. Worldwide, about 500,000 patients are currently treated with TNF-alpha antagonists. Expenses for these patients are close to ≈€8.5 billion per year. Despite this therapy being highly effective in many patients, response rates of 20% improvement or more are achieved only in 60–70% of the patients tested, and this drops to 30–50% when asking for at least 50% improvement. These types of healthcare expenses will increase exponentially within the next few years with many more biologics entering

**Thomas Häupl, MD, established an online database and analysis software algorithm (called FPCA) that recalculates differences in cell compositions in blood prior to transcriptomic analyses, available at www.bioretis.com.**

the market and with the growing evidence that early and effective treatment may reveal the maximum benefit in responding patients.

Furthermore, it is ethically questionable to start a treatment strategy based on molecular pathomechanisms in each patient by "trial-and-error" testing and without previous analysis of individual molecular pathology, which will, in non-responders, contribute to inadequate immunosuppression, increase the risk of infections, and extend the time of insufficient treatment with disease progression. To improve this situation and to reduce costs for the healthcare system, there is an urgent medical need to exploit our current knowledge generated by genomic research.

Our strategy therefore is:

1) to establish tools for the prediction of therapeutic success when using conventional drugs—biologics like anti-TNF or other biologic medication in patients with arthritis
2) to allow a cost-effective tool for transcript detection and quantification that is applicable to whole-blood analysis and includes a quantification of transcripts relative to the fraction of individual leukocyte cell types

For the future, planned routine testing of whole-blood samples has several advantages, and shipment of convenient whole-blood collection systems like PAXgene is easily accessible. It allows the preservation of sample quality and furthermore provides a strategy to bring new molecular markers from expression profiling studies into clinical use.

- **What are your views on the progress made to date on translational research, and what are your views and hopes for the future for personalized medicine?**

High-throughput genomic microarray technology using mRNA and miRNA profiling is the first option of choice to define candidate genes. The identification of biomarkers (mRNA and miRNA markers) needed for diagnosis, prognosis, therapy monitoring, or prediction of medication might open new avenues for personalized medicine in RA, other inflammatory diseases, and cancer. Furthermore, selected biomarkers not only allow insights into molecular processes, cell activities, regulation and intracellular communication, but might also be useful for biological interpretation and therefore allow development of tests as well as novel therapeutic compounds in the future.

This work was supported by the German Research Foundation (DFG) and the German Federal Ministry of Education and Research (BMBF) in cooperation and support of Affymetrix, Inc.

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P/N EMI02629 Rev. 1