



# Application Notes

## Advances in Clinical Research with Affymetrix GeneChip® RNA Expression and DNA Analysis Microarrays

The success of GeneChip® microarrays in advancing molecular medicine has been demonstrated by a growing number of published studies. This application note reviews some key clinical studies in the areas of leukemia, inflammatory diseases and diabetes that have used GeneChip arrays for gene expression and DNA analyses. These studies are intended as examples of the kinds of discoveries that are possible with microarrays, from basic discovery to the later stages in the drug development pipeline, and highlight the importance of microarrays toward optimizing patient care in the future.

Scientists need a better understanding of the molecular mechanisms responsible for complex biological processes to develop more effective drugs and diagnostic tools for the detection and treatment of disease. The Affymetrix GeneChip® System, which supports both DNA and RNA analysis on a single, integrated platform, provides a global view of molecular pathways and genetic polymorphisms critical to understanding disease. This ability to study the entire expressed genome, as we know it, has led to insights into the involvement of diverse molecular interactions as well as the pathologies that result from their disruption. This may ultimately lead to new drug targets, therapeutics with fewer side effects, and better diagnostic tools to detect disease.

The success of microarrays in advancing molecular medicine has been demonstrated by the large numbers of recent publications. Many of the early discoveries with microarrays on human disease were focused on cancers, specifically leukemias and lymphomas, and thus a significant body of work has accumulated in this area. In recent years, the spectrum of diseases studied with microarrays has expanded considerably. New insights have been gained into solid tumors as well as several non-cancerous diseases such as lupus, psoriasis, diabetes, hypertension, and infectious diseases, to name a few (Ref 1).

Affymetrix GeneChip arrays are now being used to generate gene expression data in clinical trials by pharmaceutical companies, thus extending their importance in the drug development process beyond drug target discovery and target validation studies. In drug development,

pharmaceutical companies are using the Affymetrix GeneChip platform to increase certainty in drug development via improved target identification, to gain a better understanding of mechanism-of-action, and identify signatures or specific biomarkers that can form the basis of patient stratification and pharmacogenomic approaches.

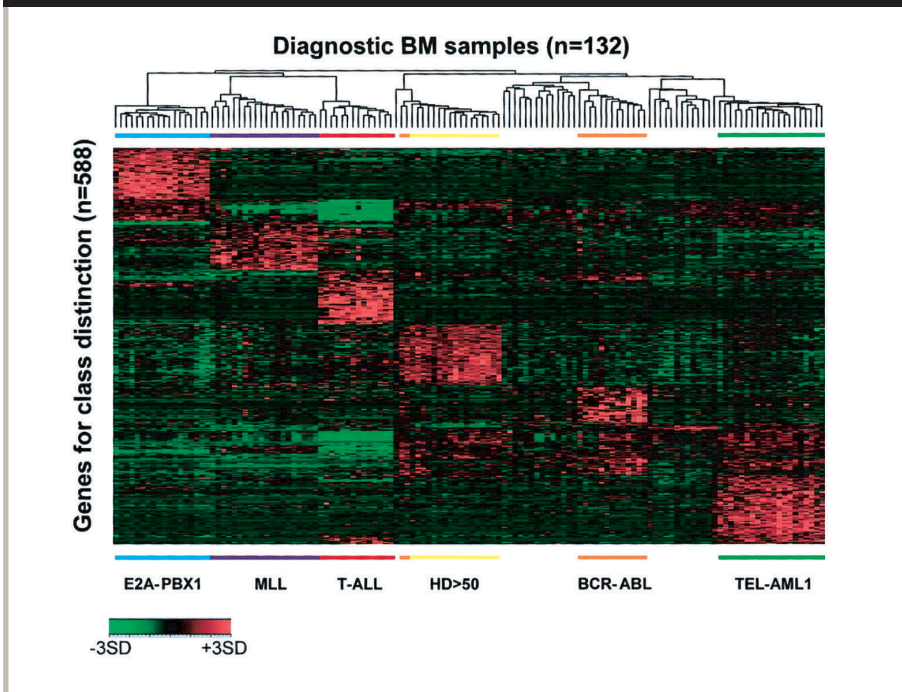
This paper reviews studies that have used GeneChip arrays and serve as examples of the kinds of discoveries that are possible with microarrays, from basic discovery to the later stages in the drug development pipeline.

### Leukemias

In 1995, researchers at the Whitehead Institute demonstrated that gene expression profiles could distinguish between Acute Myeloblastic Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL). Their results showed that microarray analysis was able to differentiate between these pathologically similar diseases with as much accuracy as the battery of time-consuming and labor intensive tests that are conventionally used (Ref 2).

A series of publications from St. Jude Children's Research Hospital in Tennessee reports the use of gene expression profiles to further illuminate the field of pediatric ALL. In 2002, Yeoh and colleagues showed how gene expression profiles classified the known prognostic subtypes of ALL with high accuracy, identified a novel subtype that was not uncovered by conventional methods, and predicted outcome for some subtypes (Ref 3). They studied 327 patients using the HG-U95Av2 arrays,

**Figure 1:** Expression profile of pediatric ALL diagnostic bone marrow blasts. Shown is a 2-dimensional hierarchic cluster of 132 pediatric ALL diagnostic bone marrow samples (columns) versus the top 100 chi-square ranked probe sets (rows) for each of the 6 diagnostic subgroups of ALL. There were 12 probe sets identified as useful in discriminating more than one class and they are represented only once in the diagram. Probe set signal values are normalized to the mean for the dataset, and values for each individual case are represented by a color, with red representing deviation above the mean and green representing deviation below the mean. Genetic subtypes are indicated across the bottom of the panel. (Figure provided by Ross *et al*, Ref 4)



bearing probes representing approximately 12,000 distinct transcripts. More recently, Ross *et al* chose 132 representative cases from the same patient cohort, and performed classification studies using the next generation human arrays, namely the HG-U133 A and B set, bearing probes representing an estimated 39,000 transcripts (Ref 4). As shown in Fig 1, the previously identified novel subtype (split into two groups here) was easily identified with these arrays, as were the known prognostic subtypes.

The candidate genes reported for pediatric ALL by Yeoh *et al* were validated in an independent patient cohort of 34 adult leukemia patients at the Ludwig-Maximilians-University in Germany by Kohlmann *et al* (Ref 5). They demonstrated that the childhood ALL signatures were also capable of distinguishing the respective adult ALL subtypes. This finding sug-

gests that there may exist common therapeutic targets in pediatric and adult ALL patients.

Cheek *et al* from St. Jude Children's Research Hospital in Tennessee revealed insights into the **mechanism of action** of commonly used chemotherapeutic agents, and demonstrated through gene expression profiles that these agents acted through different pathways when used in combination than when applied singly (Ref 6). Fig 2 shows the distinct groups of genes that are altered by these drugs when used in combination. Valk *et al* at the Erasmus University Medical Center in Rotterdam studied 285 patients using HG-U133A arrays. They used gene expression signatures from peripheral blood or bone marrow to perform a comprehensive classification of AML into 16 distinct groups consisting of known subtypes as well as a novel subtype that indicated poor treatment outcome (Ref 7).

The importance of microarray analysis in providing optimal patient care in the future is stressed by Louis Staudt of the NIH, who, in review articles on lymphoid malignancies (Ref 8, 9), advocates using gene expression profiling in new clinical trials.

## Inflammatory Diseases

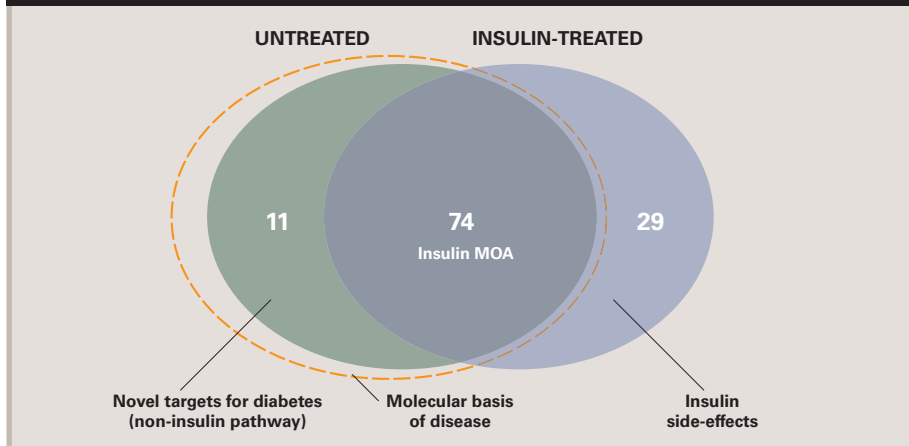
Psoriasis and systemic lupus erythematosus (SLE) are chronic inflammatory diseases whose etiology and pathogenesis have challenged researchers for years. Some recent genome-wide gene expression profiling studies (Ref 10-15) have provided new insights into these diseases.

Although treatment options for psoriasis have not changed much over the past 20 years, many new anti-inflammatory or immune-modulating drugs are now being tested. In a study that compared involved and uninvolved skin from psoriatic patients to skin from unaffected controls, Oestreicher *et al* (Ref 10) used HuGeneFL arrays to identify a set of differentially expressed genes that were specific to psoriasis. Next, by obtaining samples from patients in a longitudinal study who were treated with an experimental immunomodulatory drug or with a commonly used immunosuppressant, the authors identified gene expression changes that precede clinical improvement and thus may play a more causal role in disease progression. They showed that some of these changes may be targets for therapeutic intervention, and others may serve as markers of **treatment efficacy and outcome**.

Zhou *et al* (Ref 12) used the HG-U95 A-E set of arrays and revealed the perturbation of a wide range of biological processes including several immune signaling pathways, and also provided possible explanations for the **mechanism of action** of therapeutic agents that are currently under evaluation. More recently, Nomura *et al* (Ref 13) used the HG-U95Av2 array to generate gene expression profiles that distinguish between psoriasis and atopic dermatitis (AD), showing the disease-specific pattern



**Figure 4:** A schematic representation of the results obtained by Sreekumar *et al* (Reference 16). The green circle shows 85 transcripts altered in type 2 diabetes patients. These may be involved in the molecular basis of disease. The blue circle shows 103 transcripts that were altered on insulin treatment. 74 transcripts altered in disease (shown in the overlapping region) showed restored expression levels after insulin treatment and may be responsible for preventable chronic complications. They may play a role in the mechanism of action of insulin. 11 transcripts were not altered by insulin treatment, and may represent candidate genes for the pathogenesis of muscle insulin resistance. They may also include drug targets in the non-insulin pathway. 29 transcripts were altered specifically by insulin treatment and may be involved in treatment-induced complications.



diabetes mellitus (DM2). They devised an analytical strategy termed Gene Set Enrichment Analysis that allowed them to identify a biological pathway, namely genes involved in oxidative phosphorylation, which showed reduced expression in diabetic muscle. If further studies show that these genes are indeed responsible for the clinical characteristics of DM2, they would be important targets for DM2 prevention and therapy.

## DNA Analysis

Although microarrays have been predominantly used for performing gene expression studies, they can also be used for the **study of genetic variation** among individuals, the most common of which are single nucleotide polymorphisms (SNPs). SNP analysis is useful for a variety of applications, such as **linkage analysis** to identify disease markers, loss of heterozygosity (LOH) analysis to identify tumor suppressor genes, and **association studies** to link SNPs to patient drug responses.

A novel gene locus for neonatal diabetes was identified by Sellick *et al* (Ref 18) by genome-wide **linkage analysis** of a large consanguineous family on the GeneChip Human Mapping 10K Array Xba 131. LOH regions have been identified in bladder cancer, prostate cancer and small-cell lung carcinomas samples (Ref 19-21) with HuSNP arrays bearing probes representing 1500 SNPs. More recently, researchers at the Dana Farber Cancer Institute have identified LOH regions in lung and breast cancer cell lines and lung tumors using the Mapping 10K array (Ref 22). Bignell *et al* (Ref 23) used an Affymetrix SNP research array bearing oligonucleotides representing approximately 8500 SNPs to identify genotype information as well as changes in DNA copy number in 20 cancer cell lines. The ability to perform both analyses on a single platform allows greater insight into cancer genetics. Further information regarding LOH analysis using GeneChip Mapping 10K arrays can be found at the Affymetrix web site.\*

\*Under the data sheet entitled "Affymetrix solutions for Cancer Research".

## Summary

Whole genome analyses at both RNA and DNA levels have already brought about significant advances in biomedical research. The GeneChip System provides the global view of the genome necessary for studying disease states, identifying therapeutic targets, and can form the basis for assessing treatment efficacy and patient stratification approaches. The depth and breadth of information that is being generated with GeneChip technology ensures its role in the practice of molecular medicine.

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