'The promise of pharmacogenomics lies in the potential to personalize medical treatment, matching the right patient to the right drug at the right time.'

editorial


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Expression Analysis

Overcoming challenges of using blood samples with gene expression microarrays to advance patient stratification in clinical trials

Pharmacogenomics is the developing field that correlates an individual's genomic profile to his or her response to drug therapies. Ultimately, it will allow healthcare providers to predict the best possible treatment options, thereby maximizing therapeutic benefits and minimizing potential toxicity risks. The promise of pharmacogenomics lies in the potential to personalize medical treatment, matching the right patient to the right drug at the right time. It offers significant advantages to the healthcare system as a whole, including regulatory agencies, drug developers, physicians, patients and payers.

Many pharmaceutical companies are beginning to evaluate pharmacogenomic data as a means of patient stratification in clinical trials. Traditional clinical trials investigate drug efficacy and toxicity in a diverse pool of participants, which includes both drug responders and drug non-responders. When the number of non-responders to a drug regimen is high, the value of the drug on the responders might be hidden, possibly preventing advancement of the therapeutic. Patient stratification involves the identification of potential responders or non-responders before drug therapy by prescreening the pool of possible participants with a biomarker pattern that has been linked to biologic process or pharmacologic response. The drug is then tested on this enriched pool of individuals with similar genomic profiles. This type of differentiated design could allow for faster and more efficient clinical trials, decreasing the cost of drug development, while improving the probability of a successful new drug application (NDA).

However, the value of stratification in clinical trials relies on the quality of the patient specimens and assays or tests used for screening. For a variety of reasons, RNA isolated from human blood specimens are frequently used in pharmacogenomic testing. However, as with many tissue sources of clinical interest, blood is composed of a mixed populations of cells, which can be difficult to stabilize and dissect. There are many benefits and obstacles inherent in incorporating pharmacogenomic data in clinical trials. Recent technologies have improved the stability of RNA expression profiles and resolved problems associated with excess globin RNA in whole blood tissue. Investigators at Expression Analysis have successfully used these approaches to generate high quality RNA expression profiles from blood specimens.

Patient stratification in clinical trials

Perhaps the most compelling short-term use of patient stratification will be in the field of oncology. In this

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very complex disease model, an undifferentiated clinical trial design could jeopardize a trial's efficacy endpoint, with non-responders potentially masking therapeutic benefit to a genetically responsive subpopulation. For example, the drug Herceptin® might have initially been considered a failed drug because of its impact on only ~25% of the breast cancer patient population during clinical trials. However, testing revealed that the responsive individuals had the same marker, overexpression of the *HER-2* gene. Using *HER-2* overexpression as a means to predict response and stratify breast cancer patients led to a successful NDA, and has allowed Genentech to provide significant therapeutic benefit to these patients, while building a US$500 million Herceptin® franchise.

Additional success stories are beginning to emerge from the use of and the incorporation of pharmacogenomics in drug development. In a Phase III clinical trial designed to evaluate chronic myeloid leukemia patient response to Gleevec™, microarray-based RNA expression profiling identified a 31-gene pharmacogenomic biomarker within the patient population that predicted clinical response with 94% accuracy [1]. In a Phase II clinical trial designed to evaluate myeloma patient response to Velcade™, microarray-based RNA expression profiling identified a 30-gene pharmacogenomic biomarker that predicted responders with 71% accuracy and non-responders with 84% accuracy [2].

Stabilizing RNA expression profiles in blood samples

Although tumor tissue represents a direct measurement of expression activity associated with disease, many factors can have an impact on the overall specimen quality, including sampling (stroma versus tumor), tumor availability, sample storage and sample handling. Blood has been an attractive surrogate tissue for clinical research because of its critical role in immune response, as well as its availability and ease of specimen collection in the clinic. A recent publication by Burczynski *et al.* [3] confirms that transcriptional profiles from microarray assays using peripheral blood mononuclear cells (PBMCs) can predict clinical outcomes in patients with advanced renal cell carcinoma. However, obtaining high-quality samples for gene-expression microarrays in prospective clinical trials has proven to be challenging.

Traditional whole blood samples for gene expression analysis were collected in CPT™ tubes (Becton Dickinson), which contain an anticoagulant. However, RNA expression profiles in CPT tubes can change over time, thus care must be taken to rapidly stabilize the blood specimen between collection and RNA isolation to prevent artifacts from compromising expression profiles. Most studies require PBMCs, which make up <0.1% of the whole blood sample, to be separated and flash frozen to stabilize the expression profile. These techniques are labor intensive and are not practical at most collection sites.

Recently, two companies have developed alternative blood collection tubes: PaxGene™ (PreAnalytix) and Tempus™ (Applied Biosystems). Whole blood specimens collected in these products are immediately lysed so that the RNA is stabilized. According to the manufacturers, the samples can be transported to the laboratory at room temperature, at 4°C or frozen without alterations to the expression profile.

Removing interferences of globin RNA in whole blood samples

Whole blood samples stabilized in PaxGene and Tempus tubes offer significant advantages to blood collected in CPT tubes, but also present their own sets of challenges. In both of these collection tubes, the mRNA from the PBMC subpopulation is overwhelmed by the large quantities of globin transcript contributed by the reticulocytes present in whole blood. This globin RNA tends to reduce the sensitivity of RNA expression assays on microarray platforms, which could potentially obscure clinically relevant information. The significant contribution of globin transcripts is particularly evident when screening cRNA targets for microarray hybridization.

Recently, Affymetrix and PreAnalytiX developed a protocol for globin RNA reduction using four PNA oligomers, which have sequences complementary to the 3′ portions of the α and β hemoglobin RNA transcripts. The PNA oligomers form stable duplex structures with the globin mRNA and block the progression of reverse transcription. The inhibition of globin cDNA synthesis dramatically reduces the relative amount of anti-sense, biotin-labeled cRNA corresponding to the hemoglobin transcripts.

We have tested the PNA protocol using whole blood samples from healthy donors that were collected in PAXgene tubes. After RNA isolation, three 5 μg aliquots of the whole blood RNA were mixed with the four globin PNAs, whereas three aliquots were not pretreated. Biotinylated cRNA targets were generated for all six samples. As expected, electrophoretic traces of the PNA-containing targets showed a reduction in the undesired globin transcript. When fragmented and hybridized to human GeneChips, the samples showed improved sensitivity based on increase in transcripts called Present. The increase in sensitivity is even more dramatic for those transcripts where expression is enriched in PBMCs. Samples demonstrated a 26% increase in sensitivity to PBMC-related probe sets when globin RNA was reduced using PNA oligomers. One unexpected benefit of decreasing the amount of globin mRNA targeted cRNA in the hybridization mixture was an observed reduction in the signal variation across replicate probesets. This phenomenon, which has been consistently obtained, appears to improve the fidelity of the expression profile.

Conclusions

Recently published guidance from the FDA on pharmacogenomic data submissions clarifies how pharmacogenomic data will be evaluated. This guidance describes when and

what data will be required during the application review process, the format of the data and use of this data in regulatory decision making. The FDA has invested in new staff and infrastructure to support these submissions and, as of 21 July 2005, had received 22 pharmacogenomic data submissions (personal conversation with Senior Staff Scientist in Genomics at CDER, FDA). It is the agency's hope to bring pharmacogenomics to physicians and patients to foster personalized medicine.

Pharmacogenomic biomarker identification and validation is a rapidly growing field. Perhaps continued use of pharmacogenomic testing in clinical trials will serve to reduce the costly global population approach to trials by targeting treatments to individuals, moving us another step closer to personalized medicines. The advances in blood protocols that are described here enable improved identification of biomarkers based on RNA expression profiles and might assist us in realizing the promise of pharmacogenomics.

References

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