

## Review of The FDA Pharmacogenomics Guidance for Industry: Role of *StaRT-PCR*™ in Increasing Value of Pharmacogenomic Data

### Summary

The promise of pharmacogenomics in facilitating development of new drugs and making individualized medicine a reality will be realized, in part, through development of known valid biomarkers comprising transcript abundance values. Gene Express, Inc. plays an integral role in this effort through its proprietary technology, *StaRT-PCR*™. *StaRT-PCR*™ performs this role by generating transcript abundance data that are of sufficient quality to be valid biomarkers. As stated in the FDA Guidance for Industry Pharmacogenomic Data Submissions:

*“For the purposes of this [FDA] guidance, a pharmacogenomic test result may be considered a valid biomarker if*

*(1) it is measured in an analytical test system with well-established performance characteristics and*

*(2) there is an established scientific framework or body of evidence that elucidates the physiologic, pharmacologic, toxicologic or clinical significance of the test results.”*

*StaRT-PCR*™ is a properly controlled transcript abundance measurement method with well-established performance characteristics that is suitable to qualify potential or probable biomarkers. Specific characteristics important for this purpose include:

- i. An established scientific framework and a body of evidence supporting the excellent performance characteristics of the method
- ii. Generation of standardized, sensitive, biomarker data for drug development that are reproducible across institutions.

Following are specific applications for which *StaRT-PCR*™ is used by the industry to generate data that meet the criteria and goals described in the FDA pharmacogenomics submission guidance:

1. Generating transcript abundance data that meet the criteria for valid biomarkers described in the FDA Guidance.
2. Developing valid biomarkers comprising *StaRT-PCR*™ transcript abundance data from genes first identified through high-throughput screening.
3. Converting probable valid biomarkers into known valid biomarkers through multi-institutional clinical validation studies.
4. Developing valid biomarkers in the form of interactive transcript abundance indices

*StaRT-PCR*™ is suitable for generating probable valid biomarkers for the following purposes:

1. Early efficacy/safety assessment
2. Pre-clinical potency/dose selection information on human samples
3. Potential new target identification
4. Refinement of animal models
5. Potential theranostic, prognostic and diagnostic biomarkers
6. Supporting IND, NDA, and BLA submissions

Recently, the Food and Drug Administration (FDA) released a new final industry guidance addressing the submission of pharmacogenomic data<sup>1</sup>. The formal release of the guidance for pharmacogenomic data submission was preceded by much discussion between the FDA and the industry (including a pharmaceutical working group). This included a draft release of the document<sup>2</sup> with solicitation by the agency for input and concerns from the industry. In addition, at least two workshops were conducted to address this topic, one in May of 2002<sup>3,4</sup> and one in November of 2003<sup>5</sup>. The expressed intent of the guidance was to provide information to the industry regarding when to submit pharmacogenomic data, what information to provide, and

the mechanisms through which pharmacogenomic data could be submitted to the agency. In the guidance the FDA clarifies under what circumstances, and what kind of, pharmacogenomic data will be used in regulatory review of IND, NDA, and/or BLA submissions. In addition, it states what kind of data are of insufficient quality to be used in regulatory review, but are solicited for submission in the form of voluntary submissions.

Pharmacogenomics involves the identification and measurement of certain types of biomarkers and the associated attempts to relate these measurements to various parameters regarding the exposure, efficacy, safety, toxicity, dosing, and patient stratification relative to drugs under development. The general definition of a biomarker includes “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids” that can reflect the indication of normal or pathogenic processes or of a response to therapy.<sup>6,7</sup> Biomarkers have a long history in biology but relatively short life-span in the diagnostics arena. The category of biomarkers relevant to pharmacogenomics is the molecular biomarkers. The measurement of these biomarkers requires relatively new techniques. The general types of molecular biomarkers are listed in Table 1 in the general order relative to the stage of development. Of these types of molecular biomarkers, two are defined as pharmacogenomic (gene-based and transcript-based) and are specifically addressed in the guidance.

In the guidance, a distinction is made “between pharmacogenomic tests that may be considered either probable or known valid biomarkers, which may be appropriate for regulatory decision making, and other less well-developed tests that are either observational or exploratory biomarkers that, alone, are insufficient for making regulatory decisions.” For a pharmacogenomic test to be considered either a probable or known valid biomarker, it “must be measured in an analytical test system with well-established performance characteristics”. The implication is that transcript abundance data that are generated during drug development and/or clinical validation and that are submitted with the intent of supporting an IND, NDA, or BLA will be used in regulatory decision making only if they are measured in an analytical test system with well-established performance characteristics.

There is specific discussion regarding the classification of biomarkers that have relevance to IND, NDA and BLA. The classes are known valid biomarkers, and biomarkers that have the potential to be known valid biomarkers. The classification is as follows:

*“Valid biomarker: A biomarker that is (1) measured in an analytical test system with well-established performance characteristics and for which there is (2) an established scientific framework or body of evidence that elucidates the physiologic, toxicologic, pharmacologic, or clinical significance of the test results. The classification of biomarkers is context specific. Likewise, validation of a biomarker is context-specific and the criteria for validation will vary with the intended use of the biomarker. The clinical utility (e.g., predict toxicity, effectiveness or dosing) and use of epidemiology/population data (e.g., strength of genotype-phenotype associations) are examples of approaches that can be used to determine the specific context and the necessary criteria for validation.*

*Known valid biomarker:* A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiologic, toxicologic, pharmacologic, or clinical significance of the results

*Probable valid biomarker:* A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is a scientific framework or body of evidence that appears to elucidate the physiologic, toxicologic, pharmacologic, or clinical significance of the test results. A probable valid biomarker may not have reached the status of a known valid marker because, for example, of any one of the following reasons:

- The data elucidating its significance may have been generated within a single company and may not be available for public scientific scrutiny.
- The data elucidating its significance, although highly suggestive, may not be conclusive.
- Independent verification of the results may not have occurred.”<sup>8</sup>

Either class of biomarker may be appropriate for regulatory decision-making. At this time, the majority of pharmacogenomic data generated during drug development does not meet the criteria for valid biomarkers and are in the process of investigation and/or validation.

The guidance states that known valid biomarker data will be used in regulatory decision-making and to be in compliance with 21 CFR Sections § 312.23(a), 314.50, and 601.2 they must accompany IND, new NDA or BLA, or previously approved NDA or BLA submissions. Further, probable valid biomarker data will be used in regulatory decision making for new or previously approved NDA or BLA, and are welcomed for IND. Pharmacogenomic data that are not classified as known or probable valid biomarkers, are recommended for submission in support of new NDA or BLA, and are encouraged, but not required, for IND applications. Although it appears that it is not required to submit data obtained during investigational or screening studies to identify potential biomarkers, the guidance indicates that voluntary submission of such data is encouraged because it will serve to inform the agency of the types of studies and technologies that are being used to measure the genomic parameters.

The guidance specifically is intended for either genotype analyses (such as single nucleotide polymorphism analyses) or gene expression analyses (such as microarrays, RT-PCR). It is indicated that pharmacoproteomics or multiplexed protein analyte based technologies are not specifically covered by the guidance. Apparently, metabolomics is also not covered in the guidance. However, the agency does indicate that the latter types of data may be submitted voluntarily in a manner similar to that described in the guidance. Since *StaRT-PCR*<sup>™</sup> is a gene expression or transcript abundance measurement technology; the remainder of this discussion will focus on this area of pharmacogenomics.

<b>Table 1. Categories of Molecular Biomarkers</b>			
<b>Molecular Level</b>	<b>Description</b>	<b>“Analyte” detected</b>	<b>Examples of appropriate technologies</b>
Gene (DNA level, genetics, genomics) 25,000 genes	Determination of specific alterations in the DNA sequence of specific genes that may affect a relevant phenotype	Specific DNA sequences Insertions Deletions Translocations Single nucleotide polymorphisms	FISH CGH Microarray (“SNP chips”) PCR
Transcript (mRNA level, genomics, transcriptomics) 100,000 mRNA's	Determination of differences in the transcript abundance of specific genes that may affect the relevant phenotype	mRNA, cDNA	DNA microarrays RNAse protection QPCR ( <i>StaRT-PCR</i> <sup>TM</sup> )
Protein (proteomics) 1,000,000 proteins	Determination of qualitative and/or quantitative differences in specific proteins produced by specific genes that may affect the relevant phenotype	Protein	ELISA Mass spectrometry Antibody microarrays Antigen microarrays
Small Molecule (metabolomics) 2500 small molecule metabolites	Determination of the amount of specific metabolites in body tissues or fluids that may reflect the relevant phenotype	Intermediary metabolites (lipid, carbohydrates, amino acids)	Mass spectrometry Chromatography Biosensors

At the current time, the level of sophistication of most measurement technologies and the small number of known, valid pharmacogenomic biomarkers prevents the widespread application of pharmacogenomics in the clinic. Pharmacogenomic technologies are very varied and span a broad range with respect to reliability, reproducibility, robustness, target gene specificity, and lower limit of detection. Specific biomarkers have not yet been defined for many diseases or phenotypes and only very few have reached the status of known, valid biomarker. However, there are many potential biomarkers and measurement technologies in various stages of development and the agency is highly encouraging continued research and development and the eventual validation of pharmacogenomic tests. This R&D process often parallels the development and characterization of pharmaceutical drugs. Thus, it is appropriate that the agency requires the submission of pharmacogenomic data in the case where the biomarkers are known, valid biomarkers and encourages the submission of pharmacogenomic data as a VGDS when the biomarkers and associated measurement technologies are investigative or experimental in nature (see page 9 of reference 1 for the specific guidance on submission of pharmacogenomic data).

### **Performance Characteristics of Pharmacogenomic Measurement Tools**

For pharmacogenomic data to qualify as known or probable biomarkers, they must have been obtained with a measurement method with known analytical performance characteristics. For a biomarker to become a known valid biomarker, it must be reproducible in multiple different laboratories. *StaRT-PCR*<sup>TM</sup> has well-defined performance characteristics and enables comparison of data across multiple different laboratories<sup>9</sup>.

Presently, several potential valid biomarkers are in clinical trials. For example, a biomarker to improve accuracy of lung cancer diagnosis (*MYC x E2F/p21*)<sup>10,11</sup> is being studied in a clinical study funded by the NCI (CA 103594). Other biomarkers measured by *StaRT-PCR*<sup>TM</sup> are in the investigational stage<sup>12,13,16,17</sup>. In addition, *StaRT-PCR*<sup>TM</sup> has been evaluated in a study intended to begin to define the normal transcript abundance levels of blood for a focused set of genes. Thus, in both the

short-term and long-term it is clear that *StaRT-PCR*<sup>™</sup> will play a significant role in 1) identifying potential biomarkers, 2) validating potential biomarkers, and 3) converting probable valid biomarkers to known valid biomarkers.

The particular advantages of *StaRT-PCR*<sup>™</sup> in achieving each of these steps are as follows. *StaRT-PCR*<sup>™</sup> facilitates initial identification of potential biomarkers in part by enabling mathematical interaction among individual transcript abundance measurements. It does this by generating standardized, numerical transcript abundance values that are easily comparable in the same database. This approach has been used effectively to identify several clinically relevant biomarkers, many of which are already published<sup>10,11,12,13,16,17</sup>. *StaRT-PCR*<sup>™</sup> facilitates validation of potential biomarkers because the performance characteristics are known and are optimal. *StaRT-PCR*<sup>™</sup> facilitates conversion of probable valid biomarkers to known valid biomarkers because data from multiple institutions are easily compared.

One critical issue relevant to pharmacogenomics relates to the validity and performance characteristics of the measurement technology employed for any particular biomarker. The guidance specifically identifies the need to detail the performance characteristics of any tool used to generate data that accompany a submission. No specific performance criteria are defined in the Pharmacogenomics Data Submission Guidance. However, it is reasonable to expect that standard criteria identified for quantitative analysis in analytical chemistry would be appropriate and a separate FDA guidance<sup>27</sup>, also issued earlier this year, lists relevant performance characteristics as follows:

- Analytical Sensitivity and Assay Limits
  - Lower detection threshold
  - Linear dynamic range
  - Signal-to-Analyte response
- Interference
  - Cross-contamination
  - Interfering substances (e.g. Inhibitors of reverse transcription and/or PCR amplification)
- Precision
  - Repeatability/Reproducibility
- Analytical specificity
- Quality Control; control for analytical false negatives and false positives, control for inhibitors of signal (e.g. inhibitors of RT and/or PCR)

Similarly, the Clinical Laboratory Improvement Amendments (CLIA) indicate that "...a laboratory that introduces a new procedure for patient testing using: a method developed in-house; a modification of the manufacturer's test procedure; or a method (instrument, kit, or test system) that has not been cleared by FDA as meeting the CLIA requirements for general quality control, must, prior to reporting patient test results...verify or establish for each method the performance specifications for the following performance characteristics, as applicable:

- Accuracy
- Precision
- Analytical sensitivity (lower detection threshold)
- Analytical specificity to include interfering substances
- Reportable range of patient test results
- Reference range(s); and
- Any other performance characteristics required for test performance<sup>28</sup>

*StaRT-PCR*<sup>TM</sup> performance is optimal by the criteria set forth by both agencies (Table 2). The listed performance characteristics of *StaRT-PCR*<sup>TM</sup> have been demonstrated in a number of studies published by multiple independent laboratories<sup>14-25</sup>.

<b>Table 2. Performance Characteristics of <i>StaRT-PCR</i><sup>TM</sup></b>	
<b>Methodological Analytical Performance Characteristics</b>	<b><i>StaRT-PCR</i><sup>TM</sup> Performance</b> Validated in multiple independent studies <sup>9</sup> Published in peer-review literature <sup>14-25*</sup>
1.) Precision (Reproducibility)	Intra sample reproducibility CV 5-10% Day-to-day Reproducibility Monitoring <15%
2.) Lower Detection Threshold	< 10 molecules (and/or as low as one transcript in 30,000 cells)
3.) Quality Control	SMIS <sup>TM</sup> in each measurement controls for interference and all other known sources of variation
4.) Assay Specificity	Primers and method designed and validated to ensure specificity of transcript and Internal Standard bp size validated
5.) Effective Assay Range	>10 <sup>7</sup> (<10 <sup>1</sup> to >10 <sup>7</sup> molecules/10 <sup>6</sup> β-actin molecules)
6.) Ability to identify small differences	As little as 20% differences due to high signal-to-analyte response (sensitivity) and reproducibility
* Validated in multiple independent studies published in peer-reviewed journals <sup>14-25</sup>	

*StaRT-PCR*<sup>TM</sup> achieves optimal performance due to integrated quality control enabled by the inclusion of a defined internal standard within a standardized mixture of internal standards (SMIS<sup>TM</sup>) in each transcript abundance measurement.

Currently, the most popular paradigm for developing transcript abundance based biomarkers is to initially screen samples from affected and unaffected individuals for expression of a large number of genes by a semi-quantitative, high throughput method such as microarrays. This is done either by using large "genome-wide arrays", such as the Affymetrix Human Genome U133 (HG-U133) GeneChip<sup>®</sup> Set, that represents approximately 33,000 human genes, or by using "focused arrays" that contain probes for a defined subset of genes suspected of having an association with the disease of interest. Comparison of the hybridization results obtained using probes prepared from samples from affected individuals with those obtained with samples from unaffected individuals allow for the identification of subsets of genes that are either up- or down-regulated in the affected samples. Following this, it is the strategy of some companies

to further develop microarray-based platforms using arrays that contain a subset of probes for the defined biomarker genes. Other companies, including Gene Express, limit biomarkers to those target genes that can be confirmed by an independent method, such as *StaRT-PCR*<sup>TM</sup>, and that have the required information content for optimum clinical specificity and clinical sensitivity and thus have a higher probability of becoming known, valid biomarkers.

Even though the guidance does not address the issues regarding the development of molecular diagnostics, it is clear that biomarkers developed for use in pre-clinical evaluation and clinical trials associated with drug evaluation will be the ideal candidates for implementation as diagnostic tests in the clinic. Because of this, it is prudent to use transcript abundance measurement methods that generate valid molecular biomarker data suitable for rapid approval as molecular diagnostic tests with little or no modification.

Recently, two workshops organized by the Centers for Disease Control (CDC) have developed recommendations to address quality control materials for genetic testing<sup>26</sup>. In their recommendations, controls and reference materials used for genetics testing were addressed with respect to traceability, validation processes, and other parameters. It was noted that “for genetic testing, current CLIA regulations contain...no specific requirements for molecular or biochemical genetic testing. Therefore, laboratories performing molecular and/or biochemical genetic testing are at present subject to the applicable general CLIA requirements.” And that “general, professional guidelines recommend the use of appropriate QC materials in all assays and the inclusion of positive controls when available.”<sup>26</sup>

A reasonable interpretation of this statement is that materials and analytical methods used during drug development should meet performance criteria such as those applied to diagnostic tests. Because *StaRT-PCR*<sup>TM</sup> incorporates standards in the form of SMIS<sup>TM</sup> into the analytical method, it has performance characteristics that meet the intent of both the FDA Pharmacogenomic Data Submissions guidance and the recommendations of the CDC study group.

Appropriately, the FDA guidance does not support any specific technology for pharmacogenomic testing. DNA microarrays are used as an example a number of times, but it is clearly indicated that the state of development, optimum application, and/or advantages of microarrays or many of the potential technologies are not yet known by the agency. It is stated that one of the goals of encouraging voluntary submission of pharmacogenomic data is so the agency can gain knowledge regarding the various technologies that are used and are being developed. Even in the case of biomarkers measured by DNA microarrays, it is stated in the guidance that “genes of importance should be confirmed with secondary assays” including quantitative RT-PCR. *StaRT-PCR*<sup>TM</sup> is the ideal quantitative RT-PCR method for this confirmation. Additionally, *StaRT-PCR*<sup>TM</sup> could be used throughout the experimental, pre-clinical, and clinical phases of drug development to develop and validate select biomarkers and mature the specific biomarkers from probable to known valid biomarker status so that they could be used in the clinic.

*StaRT-PCR*<sup>TM</sup> is a properly controlled transcript abundance measurement method with well-established performance characteristics that is suitable to qualify potential or probable

biomarkers. Specific characteristics important for this purpose include:

1. An established scientific framework and a body of evidence supporting the excellent performance characteristics of the method
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**Abbreviation used:**

FDA – Food and Drug Administration

IND – investigational new drug

NDA – new drug application

BLA – biologics license applications

VGDS – voluntary genomic data submissions

*StaRT-PCR*<sup>™</sup> – Standardized reverse transcriptase polymerase chain reaction

ELISA – enzyme linked immunosorbant assay

FISH – fluorescent in situ hybridization

CGA – comparative genomic hybridization

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