Food and Drug Administration
Center for Biologics Evaluation and Research

The Development of Diagnostic Immunohistochemistry (IHC) and Fluorescence *in situ* Hybridization (FISH) Assays Intended to Identify Patients Who Might Benefit from Treatment with a Particular Therapeutic Product

Focus on Characterization and Interpretation of Assay Results

Briefing Document for the Oncologic Drugs Advisory Committee Meeting
December 5, 2001
Morning Session

Development and Characterization of Immunohistochemistry and Fluorescence *in situ* Hybridization Diagnostic Assay Kits for Use in the Selection of Patients for Treatment with a Particular Therapeutic Agent

With the advent of targeted therapies, medical oncology enters into the arena of "customized" treatment of cancer based on the presence of molecular targets or particular patient/tumor characteristics. The fields of pharmacogenetics and pharmacogenomics have emerged and are rapidly expanding. The long term view on drug development appears to indicate that therapies to treat cancer will proceed down a path of selection based upon these molecular targets. The ability to efficiently and precisely identify these targets is more critical now than ever before in the cancer therapy development process. The Food and Drug Administration (FDA) seeks the advice of the Oncologic Drugs Advisory Committee (ODAC) at this time in regard to two major areas of concern: (1) the systematic and science based incorporation of molecular target assay development into anti-cancer therapy development programs and (2) the identification of information which clinicians need to know in order to select the proper testing modality and to interpret those results.

Until very recently, biomarkers were scientifically interesting, but not crucial to the drug development process, as they did not have a major impact on treatment decisions. Clearly, that is no longer the case. Assays now need to be developed prior to and in parallel with the therapeutics. Ideally, there should be a good understanding of the target and the mechanism of action of the therapeutic to better design the diagnostic; however, the reality is that relatively little is understood about the targets and the issues surrounding the diagnostic have been more of an afterthought. Diagnostic assays can be the rate limiting step in the selection of therapy for patients; therefore, patients and their clinicians need to know the advantages and limitations of each assay. Of necessity, medical oncologists will need to become more familiar with tools of the pathologist and pathologists will need to provide oncologists with assurance of the reproducibility and reliability of their assays.

In order to approve therapies which are intended for use in patients whose tumors express a particular molecular trait (e.g. protein overexpression, gene amplification, or genetic polymorphism), the FDA, Center for Biologics Evaluation and Research (CBER) has required that the diagnostic assay for identification of that molecular trait be available to physicians either through central laboratory testing or as a Center for Devices and Radiological Health (CDRH) approved PMA (Pre-Market Approval) for a device or test kit. Such was the case with the approval of denileukin diftitox (Ontak) in 1999 and trastuzumab (Herceptin) in 1998. In the case of denileukin diftitox, indicated for cutaneous T-cell lymphoma, central testing for CD25 (IL-2Rα) was provided by the sponsor and in the case of trastuzumab, DAKO developed a commercial IHC test kit, HercepTest which was filed as a PMA and approved by CDRH. CBER felt it necessary to include information about the testing modalities in the labels for these therapeutics.

The focus of this ODAC session will be on two types of assays: (1)immunohistochemistry (IHC), a technology now widely available in most pathology

laboratories, and a preferred method for identification of protein overexpression, and (2)fluorescence *in situ* hybridization (FISH), a technique less widely available, but rapidly becoming a preferred method for identification of gene amplification. In the best of circumstances, both methods are semi-quantitative, but in the real world, both methods may be merely qualitative. Neither serves as a gold standard. Clinical outcome is the only gold standard currently available to confirm the predictive value between an assay result and the effectiveness of a therapeutic.

Difficulties with the performance and interpretation of IHC and FISH have come to light in the medical oncology community over the last 3 years; in particular, attention has been focused on the detection of estrogen and progesterone receptors and on the detection of HER2/*neu* targets. Problems encountered include, but are not limited to, identification of cutoff points in assay scores to define positive vs. negative results, broad interlaboratory variability in performance of the assays, discrepancies between laboratories with high volume vs low volume throughput, use of "home brew" antibodies for IHC, deviations from recommended methods in the package insert leading to altered performance characteristics of the assays, conflicting data in the published literature, and lack of data from prospectively conducted studies.

FDA anticipates that the impact of these problems, if left unaddressed, will expand. With the advent of inhibitors of epidermal growth factor receptors (EGFR's) and tyrosine kinases, diagnostic assays of other molecular targets will also come under closer scrutiny. In addition, there are many agents in preclinical and early Phase 1 development, for which selection of patients based upon the presence of a target is crucial.

We also expect that as our understanding of the targets deepens, so should our ability to identify the most relevant aspect of the target in question. For example: It may be more important to detect the activated form of a protein, rather than simply the presence of the protein. It may be more important to identify mutations in particular exons of a gene, rather than simply mutant genes. It may be important to detect co-expressed proteins. We recognize that medical science's understanding of molecular targets is in its infancy and, over time, refinements will be made to both the diagnostic assays employed and the therapies developed. Drug development programs will, of necessity, need to remain flexible to these advances and be prepared to address the additional questions that these advances will bring to light.

CBER feels it is crucial to elicit the opinions of medical oncologist, statistician, and patient representative members on the ODAC regarding the issues outlined above. An altered format will be used during this ODAC meeting with the intent of optimizing the discussion of these issues. Experts in the fields of IHC and FISH will provide, for the committee, overviews of the science and methods behind these assays, their advantages, and their limitations. "Pathology case studies" will be presented by members of the two cooperative groups conducting adjuvant trials employing HER2 detection methods. These will serve as examples of some of the problems encountered and are intended to spur discussion. This will be followed by a Q&A discussion between the ODAC

members and a separate panel of experts in pathology and test development. Questions from the FDA will then be addressed by the ODAC. Expert pathologists and members from CDRH along with CBER representatives will be on hand to answer questions from the ODAC as needed.

Included in this briefing document is information regarding IHC and FISH. In addition, there are summary reports from two cooperative groups (NSABP and the Breast Intergroup) regarding results of their early phase pathology testing from their adjuvant breast cancer trials using trastuzumab and chemotherapy. They serve as examples of some of the difficulties encountered with IHC and FISH testing. Information on the role of the National Institute of Standards and Technology in standard development for DNA-Based testing modalities is also provided. Lastly, included is a concept sheet addressing the issue of assay development from the perspective of the National Cancer Institute.

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