In the context of biomarkers, antibodies fall into four main categories, each of which carries a different level of risk from an economic development point of view (Fig. 1). Those markers used for routine diagnostic purposes entail little or no risk, and prognostic markers involve high risk. Predictive markers associated with established therapies are low risk whereas those for new therapies involve high risk.

The focus of the presentation was on development of antibodies for detecting cancer biomarkers. It is crucial to use prospectively defined criteria to select patients who are most likely to respond to a specific molecularly targeted therapy. Proper patient selection enables efficient clinical trial design for targeted therapies and ensures that the number of individuals exposed to the risks of anticancer therapy is minimised.

Patient selection can be facilitated through the use of systems, such as pharmDx, Dako's complete diagnostic assays that enable selection of patients more likely to benefit from targeted therapy. Herceptest® was the first such system developed. It is used to identify patients whose tumours overexpress Her-2/ERB2 and, therefore, who would be mostly likely to respond to treatment with trastuzumab (Herceptin®), a humanised antibody targeting the HER-2 receptor. By screening with the pharmDx system, the response rate is greater than if the general patient population were treated with trastuzumab. Semiquantitative scaling was used for registration of the pharmDx technique and is the basis for its labelling.

The quality of antibodies under development in terms of sensitivity and specificity is extremely important. Antibodies can be developed internally or acquired from external sources, usually from the university research community. No matter how antibodies are developed, they must be of the best quality with no tolerance for variation between batches.

Screening systems are required to ensure quality and researchers use additional quality testing to ensure that the antibodies actually recognise the specific moieties. Epitope mapping is necessary to ensure specificity. Many antibodies on the market have different specificities; this fact must be taken into account when using them to select patients. The shelf life of Dako antibodies is usually about 2 years. Detection of activated (phosphorylated) proteins has received increasing interest during the last few years. It is challenging to develop phospho-specific antibodies that do not display cross-reactions with the backbone sequence. The specificity, functionality, and the absence of cross-reactions are verified by different methodologies to ensure quality and performance of antibodies.

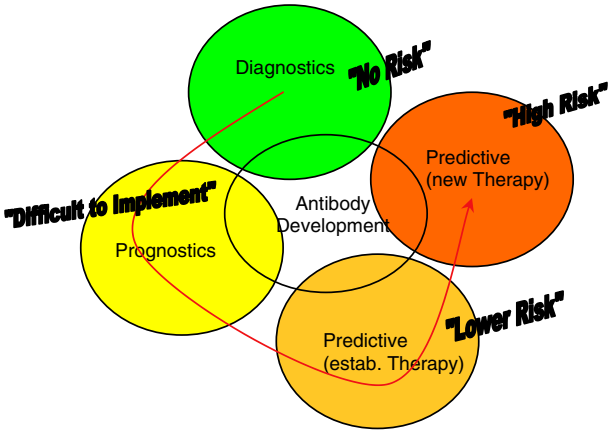

Fig. 1 – Strategies and challenges for cancer biomarker and antibody development.

The antibodies must work on different types of tissues. This needs to be confirmed by testing in multi-tissue arrays to make sure that background staining is not problematic. The final step is standardization of the assay to ensure consistency across laboratories.

In conclusion, the keys to successful development of antibodies for use in patient selection are high quality – in terms of specificity, functionality, and sensitivity – and standardisation of reagents (no batch-to-batch variation), automated protocols, and use of imaging as a means of interpreting the response. Developing antibody-based testing for biomarkers is a high-risk area, but the potential benefits are significant. Regulatory authorities throughout the world strongly advocate standardization of testing to minimise the number of patients who experience adverse side effects from treatment. Proper patient selection can also optimise treatment expenditures by selecting the patient population most likely to respond.

CONFLICT OF INTEREST STATEMENT: Dr. Henrik Winther Ph.D. the author of this paper is employed by Dako A/S in Clinical Research & Development. There is no conflict of interest involved with any matters presented in this paper.

doi:10.1016/j.ejcsup.2007.09.013

HOW TO INCORPORATE MULTIPLE MARKERS IN CLINICAL TRIALS

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The goal of incorporating biomarkers into cancer treatment and clinical trials is to manage a patient's disease by administering effective and well-tolerated therapies, based on an understanding of the patient's unique genetic and molecular profile. For targeted therapies that might only benefit a proportion of patients, failure to select patients correctly has the potential to dilute trial outcomes. Challenges in identifying those patients most likely to benefit might risk wrongly concluding that therapeutically beneficial drugs are ineffective.

There are many types of biomarkers of potential interest in the field of targeted anticancer therapy. These can mainly be divided into those that present in histopathological tissues and blood-borne biomarkers. Significant advances in imaging (e.g., positron emission tomography [PET] scans) have also improved the ability to monitor treatment effects. The focus of the presentation was on acquisition of histopathological tissues.

GEFITINIB (IRESSA®) CLINICAL DEVELOPMENT: The clinical development of gefitinib, an orally-available epidermal growth factor tyrosine kinase inhibitor (EGFR TKI) was reviewed: Phase I and II development showed dramatic and unexpected tumour regressions in approximately 10% of patients with advanced

non-small-cell lung cancer (NSCLC). Data from early-phase trials did not show a clear correlation between patient outcome and EGFR expression in archived tissue.[1] Subsequently, however, data emerged indicating that EGFR mutations and increased gene copy number, as measured by fluorescence *in situ* hybridisation (FISH) are associated with clinical response to gefitinib treatment.[2–5] Other potential biomarkers of gefitinib outcome have also been identified.

One of the challenges in the development of gefitinib was that knowledge of potential biomarkers emerged during the conduct of the pivotal trials. Indeed, increased EGFR gene copy number measured by FISH was shown in 2003 to be a prognostic biomarker for outcome after surgery in patients with NSCLC,[3] and subsequently shown to be predictive of response to gefitinib.[4] In 2004, EGFR mutations also emerged as predictors of response to EGFR TKIs in patients with advanced NSCLC.[5]

Evolution of biomarkers during the conduct of large randomised trials might become the rule rather than the exception. Although initial candidate biomarkers are evaluated early in development, knowledge increases exponentially as research and clinical experience become more widespread and increased clinical data with which to correlate the translational work becomes available.

THE CRITICAL IMPORTANCE OF TISSUE SAMPLES: The IRESSA Survival Evaluation in Lung Cancer (ISEL) phase III trial highlighted many of the challenges in acquiring tissue samples in large multinational randomised phase III trials. The phase I studies of gefitinib involved collaboration of just a few academic centres that were very devoted to collecting tissues. In phase II, 40 centres were involved, but the sample acquisition rate dropped to 80%. The ISEL phase III study accrued very quickly worldwide, but only 33% of patients' samples were available (Fig. 1). Of these, 177 samples were evaluable for all three of the following biomarkers: FISH, EGFR expression and EGFR mutations.

Dr. Botwood outlined the challenges encountered in collecting tissues for such studies. In ISEL, more than 25% of tissue samples were inadequate (insufficient quantity or fixation) for any sort of analysis, more than 60% were inadequate for mutational studies, and 80% required remounting. Documentation of samples also proved to be quite challenging as many were incorrectly labelled and could not be validated.

The informed consent process also presented some challenges with fewer than 40% of patients consenting to tissue sampling overall. Local changes to, and interpretation of, the consent by ethics committees internationally also meant that it was not possible to analyse all available samples from all countries.

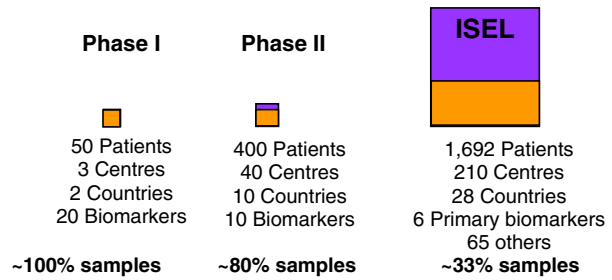

Fig. 1 – Collecting markers in phase III was more difficult than earlier phase trials of gefitinib in NSCLC patients.

Genetic testing is a critical consideration for informed consent. Dr. Botwood emphasised the need for consent documents to adequately explain the difference between hereditary and somatic mutations (i.e. tumour-specific mutations that are not part of the host's genome). Investigators must be prepared to respond to questions from regulators and ethics boards in this regard.

PATHOLOGISTS AS RESEARCH PARTNERS: Acquisition of tissue samples is increasingly central to research molecularly targeted therapies. Protocols should be developed with tissue collection and analysis in mind. Pathologists are critical partners in clinical trials to ensure that samples are properly fixed, labelled and shipped.

The situation is complex because security is a key concern to pathologists when providing samples to investigators outside their institutions. Therefore, every measure should be taken to ensure that samples are promptly returned to pathologists, as necessary, and their contributions should be recognised as co-investigators. A study-initiation visit should be undertaken to offer pathologists clear guidance on what is needed, with an emphasis on the importance of biomarker work.

In terms of documentation, case report forms and requisition documents should be complete and clear and should adhere to data-reporting and protection provisions of the study protocol. Documents should be subject to 100% source and data validation. The data management plan for biomarkers should include such issues as format, transfer and destination.

CHALLENGES ASSOCIATED WITH TISSUE SAMPLES: A host of regulatory, ethical, and cultural challenges must be overcome. Dr. Botwood pointed out that routine clinical practice does not necessarily generate the samples required for biomarker analysis. Many referral centres lack functioning systems for obtaining research samples. Some countries prohibit export of DNA-containing material, and several have a cultural preference for conducting their own national research. Some countries prohibit 'genetic' analyses and do not make a clear distinction between host and tumour research. Regulations on this are continually evolving; what is permissible today might not be tomorrow.

Dr. Botwood noted that 95% of patients in the INVITE trial, a randomised phase II study of gefitinib versus vinorelbine, provided tumour samples, as provision of a tissue sample was mandatory requirement for trial entry. In the IMEX trial comparing gefitinib and methotrexate for treatment of head and neck cancer, 56% of patients provided tumour samples, most of which were evaluable. In this and other studies, an implicit assumption is made that the diagnostic biopsy reflects current tumour status. This is not necessarily the case however, as tumours and their markers evolve during subsequent therapies. In addition, while markers of disease progression are also potentially very interesting, re-biopsy at this time is very challenging to patients.

MOVING FORWARD: Obtaining samples from multicentre, multinational phase III trials is complex because sample quality varies and informed consent processes present different challenges in different countries. Novel assays should ideally be carried out at a single institution, or if this is not possible, by locally-validated laboratories. Assays that can be carried out on cytology or blood specimens are preferable to tissue biopsies.