unacceptably high. Identified biomarkers could also be a basis for monitoring treatment and identifying resistance mechanisms.

One example for the need to identify biomarkers is immunotherapy. It is important to bear in mind that immune-mediated anti-tumour reactivity is the result of a well-orchestrated interaction of multiple factors and multiple pathways. Redundant pathways and interactions present a set of challenges when designing immunotherapies or identifying surrogates. Studying any of the multiple immune mediators in isolation offers little about efficacy because of these interactions. Therefore a set of biomarkers is expected to more and more replace single ones. Methods such as proteomics need to be better standardised and validated as they may speed up the identification of biomarkers and thus drug development as a whole.

As soon as biomarkers have been fully validated, they have the potential to be developed to surrogate markers. Surrogate endpoints should be based on functional parameters of critical importance for cancer control. Although functional parameter techniques have been around for many years, they can provide very useful information for molecularly targeted drug development. Such parameters include cell proliferation, cell death, inflammatory infiltration, and tumour regression.

For example, the immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) trial relied upon a measure of proliferation – immunohistochemical assessment of the nuclear antigen Ki-67 – as an endpoint.[2,3] The method was highly reproducible and predictive of therapeutic efficacy. Mohsin et al.[4] used another functional parameter – apoptosis – in a trial of the neoadjuvant trastuzumab in 35 patients with locally advanced Her-2/neu overexpressing breast cancers. They found that induction of apoptosis correlated with tumour regression.

Tumour biopsies can be examined for inflammatory lymphocyte migration as another means of monitoring treatment efficacy using functional parameters, as shown in one study involving stage III melanoma patients treated with interferon-alpha.[5] Patients whose tumours demonstrated $\geqslant$2% CD4+ tumour-infiltrating lymphocytes (TIL's) had prolonged time to progression and improved overall survival compared with patients whose tumours had < 2% TIL's. Finally, tumour regressive changes correlate with long-term survival. Overall survival in metastatic melanoma patients has been shown to correlate with tumour regressive changes.[6]

Trials that rely on functional parameter endpoints obviously depend on the availability of tumour biopsies. Obtaining such samples is critical for developing molecularly targeted therapies. Stated otherwise, 'No tissue – no trial'.[7] For example, with biopsy specimens, it would be possible to compare all phosphorylated proteins in the tumours before and after treatment to observe potential changes. Moreover, it is becoming more possible to predict response to immunotherapy based on tumour biopsies. Therefore prognostic biomarkers also provide means for identifying patients who might be at high risk of disease recurrence after radical surgery and might be candidates for adjuvant therapy.

Predictive biomarkers could be used to discern which patients would be more likely to respond to a particular therapy. Interleukin-6 (IL-6) production by peripheral blood mononuclear cells (PBMC's) preoperatively collected from patients with primary colorectal cancer predicts survival. Eight of 13 patients with >5000 pg/mL IL-6 died from cancer within the 54-month follow-up period, whereas no cancer-related deaths were recorded among 21 patients with 5000 pg/mL IL-6 or less. A multivariate Cox regression analysis, stratified for tumour and node stage, identified IL-6 production as an independent prognostic factor.[8]

In conclusion, molecularly targeted treatment of cancer is sometimes criticised for poor therapeutic efficacy. Among the reasons that it has not met with greater success are reliance upon suboptimal dosing, the fact that molecular targets are not always the therapeutic target, pathway redundancy, and resistance mechanisms. However, molecularly targeted treatment of cancer is still at a very early stage; there is a great need to identify relevant therapeutic targets and establish molecular and functional surrogate endpoints. The techniques are available the time to design the respective clinical trials is now.

CONFLICT OF INTEREST STATEMENT: Professor Leif Håkansson, MD Ph.D. the author of this paper can confirm that there is no conflict of interest involved with any matters presented in this paper.

References:

1. Sheehan KM, Calvert VS, Kay EW, et al. Use of reverse phase protein microarrays and reference standard development for molecular network analysis of metastatic ovarian carcinoma. *Mol Cell Proteom* 2005;**4**:346–55.
2. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol* 2005;**23**:7212–20.
3. Smith IE, Dowsett M, Ebbs SR, et al. IMPACT Trialists Group. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) multicentre double-blind randomized trial. *J Clin Oncol* 2005;**23**:5108–16.
4. Mohsin SK, Weiss HL, Gutierrez MC. Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. *J Clin Oncol* 2005;**23**:2460–8.
5. Håkansson A, Gustafsson B, Krysander L, et al. Effect of interferon-alpha on tumour-infiltrating mononuclear cells and regressive changes in metastatic malignant melanoma. *J Interferon Cytokine Res* 1998;**18**:33–9.
6. Håkansson A, Håkansson L, Gustafsson B, et al. On the effect of biochemotherapy in metastatic malignant melanoma: an immunopathological evaluation. *Melanoma Res* 2003;**13**:401–7.
7. Baselga J, Arteaga CL. Critical update and emerging trends in epidermal growth factor receptor targeting in cancer. *J Clin Oncol* 2005;**23**(11):2445–59.
8. Clinchy B, Fransson A, Druvefors B, et al. Preoperative interleukin-6 production by mononuclear blood cells predicts survival after radical surgery for colorectal carcinoma. *Cancer* 2007;**109**:1742–9.

doi:10.1016/j.ejcsup.2007.09.012

HOW TO SELECT BIOMARKERS FOR MULTITARGETED THERAPIES

H. Winther. *Antibodies & Reagents, Dako, Produktionsvej 42, DK-2600, Glostrup, Denmark*

E-mail address: Henrik.Winther@Dako.com

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.

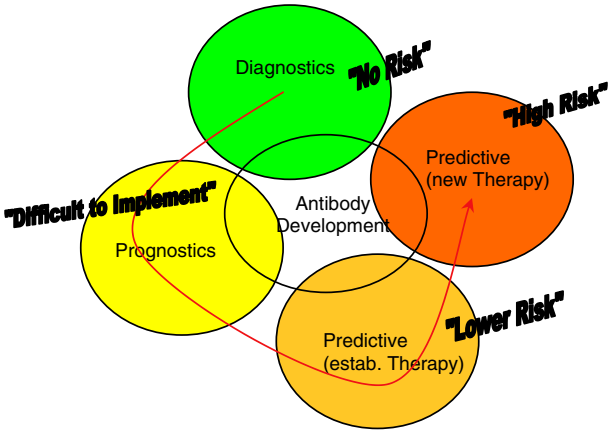

Fig. 1 – Strategies and challenges for cancer biomarker and antibody development.

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

N. Botwood. *AstraZeneca Research and Development, Alderley Park, Cheshire SK10 4TG, England, United Kingdom*

E-mail address: Nick.Botwood@AstraZeneca.com

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