WHY SERUM THYMIDINE KINASE ACTIVITY CAN BE A USEFUL BIOMARKER IN CANCER THERAPY DEVELOPMENT

The need for tools to predict and monitor efficacy during drug development

Despite great efforts to cure cancer, it remains one of the world’s major causes of death. The annual number of cancer-related deaths worldwide in 2015 is estimated to be more than 14 million (1). Thus, there is an urgent need for more effective treatments for cancer, and research laboratories in academia and pharmaceutical companies are working hard on developing these treatments.

In this research, pharmaceutical companies need to find not only good targets for therapies and good candidate drugs for these targets, they need techniques to provide accurate and clinically meaningful data on the efficacy of these drugs. These techniques are what Biovica is developing. In particular, our DiviTum assay can be used both for determining the proliferation rate in cell cultures during pre-clinical evaluation and the in vivo efficacy of candidate drugs in clinical trials. Furthermore, we believe this assay can be a useful companion diagnostic, particularly for therapies targeting the cell cycle, which is the topic of this Perspective on Biomarkers.

I. The Need to Determine Efficacy in Drug Development

Advances in science have produced new treatment strategies based on understanding the molecular mechanisms of cell growth and metabolism. Molecular modelling and high-throughput screening of small compounds are useful tools that have greatly improved rational drug design and a vast number of potential therapeutic compounds have been identified. These compounds take many different forms and many target different mechanisms associated with cell proliferation. However, developing a compound into a new drug is a long, difficult, and complicated process that is also enormously expensive. The efficacy of these compounds is first tested in the laboratory and, if successful, in clinical trials. Very few candidate compounds actually make it to or through the drug-approval process to become routine treatment. This means that for each successfully developed drug, the pharma industry also have costs for work on many failing drug candidates. Nevertheless, the number of drugs approved for cancer treatment is constantly growing, a process driven by the potential profits associated with a new, useful therapy.

The urgent need for new drugs and their high development costs mean that pharmaceutical companies are keenly interested in technologies that shorten the time to regulatory drug approval. Techniques that provide conclusive information on the efficiency of a candidate drug are crucial in this aspect.

Strategies for Determining Drug Efficacy

How can a biomarker aid in evaluating the efficacy and benefit of a candidate drug? One way is to indicate disease progression in patients in therapy as early as possible, especially before clinical symptoms appear. Another is to indicate the response to therapy. Still another is to predict which individual patients may benefit from the drug before it is administered. Each of these strategies can be realized if a suitable, validated, and sensitive biomarker could be included in the development programme. However, many cancer drugs, including cell-cycle inhibitors, have no established biomarkers that reliably monitor disease progression, accurately predict response to therapy, or serve as pharmacodynamic markers (2).
Serum thymidine kinase activity is a biomarker closely related to cell proliferation. Accumulating evidence indicates that this biomarker can provide response information useful in cancer therapy development (3, 4). The DiviTum assay allows even small changes in thymidine kinase (TK) activity to be measured accurately, quickly, and non-invasively, both in cell cultures and serum. As such, it has several attractive features as a tool in drug development:

- TK activity is closely associated with cell proliferation and DNA repair, meaning that the assay can indicate cancer progression;
- The DiviTum assay can measure TK activity from a serum sample, eliminating the need for biopsies; and
- The DiviTum assay can provide important information during both the pre-clinical and clinical phases of drug development.

2. Thymidine Kinase Activity in cells and serum

Thymidine kinase is an important enzyme for re-cycling de-phosphorylated thymidine back into a form that can be used in DNA synthesis. Thymidine kinase phosphorylates thymidine to thymidine monophosphate (Figure 1). This step is important in making thymidine useful as a building block in DNA-synthesis. Cells have two types of TK: TK-1 is a cytosolar enzyme primarily expressed and active in the G1/S-phases of the cell cycle, during which its activity increases more than 15 fold (5) (6), and TK-2 is a mitochondrial enzyme whose activity varies little over the cell cycle (7, 8).

Thymidine kinase-1 activity can also be detected in the blood, and its activity is elevated in patients with conditions associated with increased cell proliferation or cell death, such as cancer, immune responses, and viral diseases (9) (8). Elevated activity levels reflect an increase in cell proliferation and death of proliferating cells, but the mechanisms behind the release of TK into serum are not yet fully understood. The tumor microenvironment is a complex mixture of normal and tumor cells, and tumor growth involves processes of inflammation and angiogenesis (10). Therefore, the increase in serum thymidine kinase activity in cancer is unlikely to occur solely because of proliferating tumor cells. Nevertheless, the relationship between cancer aggressiveness and increases in serum TK-1 activity is clear (8).

3. The rationale for Thymidine Kinase Activity being a proliferation marker

The expression of the TK-1 gene increases in the late G1-phase of the cell cycle, and expression is dependent on the transcription factor E2F (Figure 2). This transcription factor is activated by the cyclin-dependent kinases (CDK) 4/6 as one of many functions necessary to prepare cells for replicating chromosomes in the S-phase (11).

The central function of CDK4/6 kinases in cell proliferation has made these enzymes attractive targets for cancer therapy (2). More than 15 clinical trials with candidate drugs based on CDK4/6 inhibition have been registered (12). In February, 2015, one of these drugs, Ibrance (palbociclib) by Pfizer, obtained an accelerated FDA approval for estrogen-receptor-positive advanced breast cancer. Palbociclib inhibits CDK4-catalyzed phosphorylation of retinoblastoma protein (Rb), which inhibits the release of the transcription factor E2F from the retinoblastoma protein and, in turn, stops the transcription of many replication-associated genes. Thus, the cell cycle is stalled and tumor cells will not proliferate.
lymphoma (14), estrogen-receptor-positive advanced breast cancer (15), and well-differentiated liposarcoma (16). Other major pharmaceutical companies also have candidate drugs targeting CDK4/6 in clinical trials: Eli Lilly (abemaciclib), Novartis (LEE-011), and Bayer (roninib).

The close connection between CDK4/6 and TK-1 expression and the large and well-documented increases in TK activity in serum from patients with aggressive cancers provide a strong rationale for evaluating serum TK activity as a marker of the efficacy of CDK4/6 inhibitors in cancer therapy. In developing and evaluating candidate CDK4/6-inhibitor drugs, serum TK activity may well be the missing biomarker.

4. Serum Thymidine Kinase Activity as a Biomarker in Cancer

The first commercial TK activity assays were based on monitoring the turnover of a radiolabeled substrate. In the 1980s, studies performed with these assays established that serum TK activity could reflect tumor activity. Since then, serum TK activity measurements have been used clinically to obtain information about the status of the cancer, particularly haematopoetic cancers. The research on TK as a biomarker in cancer has been extensively reviewed (17) (18) (8).

The new DiviTum assay measures serum TK activity without the need for a radiolabeled substrate and with higher sensitivity and a broader working range than that of other tests. Measuring TK activity has therefore become of interest in investigating solid tumors, in which serum TK activity levels are lower than they are in blood malignancies (19).

Thymidine kinase activity has also been used as a marker of cell proliferation with other types of assays, both in vivo and in vitro. Scintigraphic measurements of the rate of 3H-thymidine phosphorylation and incorporation into DNA have established a thymidine-labelling index for estimating proliferative activity in biological tissues (20) (21).

A new technique attracting considerable attention is 3-deoxy-3-18F-fluorothymidine positron emission tomography (FLT-PET) (22). This imaging technique can estimate the TK activity in tumors non-invasively. Such in vivo imaging techniques are powerful tools for tumor examination, but they are markedly more expensive than a serum assay, and the patient needs to go through a special examination at an imaging facility.

Two recent studies have documented that determining serum TK activity with the DiviTum assay is useful in evaluating the efficacy of cancer therapy. The first investigated the clinical relevance of serum TK activity in women with advanced breast cancer before treatment (3). High TK activity before chemotherapy was begun was associated with poorer therapeutic response, shorter progression-free survival, and shorter overall survival. A frequently used serum biomarker for breast cancer, CA 15-3, also predicted response, but not progression-free or overall survival (Table 1).

In the second study (4), high pre-treatment levels of serum TK activity were associated with a poorer therapy response in patients with lung cancer. This study also determined that changes in serum TK activity, and hence response to treatment, could be monitored during therapy.

Table 1. Pre-Treatment Serum TK activity Levels and Response to Treatment among 184 Women with Advanced Breast Cancer.

<table>
<thead>
<tr>
<th>Response</th>
<th>Serum TK level, median, Du/L</th>
<th>Patients, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Response</td>
<td>193</td>
<td>77</td>
</tr>
<tr>
<td>Stable disease</td>
<td>110</td>
<td>91</td>
</tr>
<tr>
<td>Progressive disease</td>
<td>1,492</td>
<td>16</td>
</tr>
</tbody>
</table>

In conclusion, accumulating evidence continues to show that the DiviTum assay can provide important clinical data based on serum TK activity for solid tumors as well as for hematological malignancies.

5. Ongoing and Future Studies

Biovica is currently evaluating serum TK activity as a biomarker for CDK-inhibitors in cancer therapy in several clinical studies. The PREDIX study is a Phase II randomized trial evaluating response-guided treatment in luminal A breast cancer (23). This study is being done in collaboration with the Karolinska Institute in Stockholm, Sweden.

Another clinical study evaluating serum TK activity as a biomarker for CDK-inhibitor therapy in advanced breast cancer is the PYTHIA study, set up jointly by the International Breast Cancer Study Group/Breast International Group, a multicentre cancer research consortium. The purpose of this randomized, double-blind, placebo-controlled, Phase II clinical trial is to determine whether palbociclib in combination with fulvestrant is more effective than placebo plus fulvestrant in prolonging progression-free survival in post-menopausal women with hormone-receptor-positive advanced breast cancer.

Further, in collaboration with the Dana Farber Cancer Institute, Boston, Massachusetts, USA, serum TK activity is being evaluated as a biomarker for CDK-inhibitor efficacy in patients with either metastatic or unresectable lung cancer or solid tumors with CCND1 amplification or CCND1 mutation or a splice variant. Serum TK activity is being evaluated as a marker of disease progression, survival, and treatment response.
What other studies would benefit from evaluating DiviTum as a biomarker? A wide range of drug candidates targeting CDKs are now being evaluated by pharma companies. In addition to CDKs that are directly involved with cell cycle control, CDKs with other functions are also targeted (24). Inhibition of these CDKs may result in cell death, and, as mentioned, several studies show that killing proliferating cancer cells is reflected in changes in serum TK activity. Therefore, we are now designing studies to determine whether serum TK activity has applications for these types of CDK inhibitors.

We are also investigating the relationship between serum TK activity and the effects of other types of therapies in which serum TK activity can be expected to provide clinically useful information. The PREDIX lumA study will give us more than information on how serum TK activity responds to the CDK-inhibitor. Patients in the control arm receive only standard endocrine therapy. Because the estrogen receptor is targeted, and because it activates the cyclin D1 gene, this therapy alone can potentially change serum TK activity. Of special interest is whether endocrine therapy and CDK-inhibitor therapy will provide additive changes in serum TK activity.

A final note: Biovica is presently also addressing interesting potential applications for TK-activity in pre-clinical drug efficacy evaluation and data on these applications will be presented soon.

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References: