

# 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, the disease 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 (WHO). There is thus an urgent need for more efficient therapies for treating cancer, and research laboratories in academia and pharmaceutical companies are working hard on developing these therapies.

In this research, pharmaceutical companies need to find not only good targets for therapies and good candidate drugs for these targets, they also 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.

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## 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. A vast number of potential therapeutic compounds have been identified. These compounds take many different forms and target many different mechanisms associated with cell proliferation. But developing a new drug is a long, difficult, complicated, and enormously expensive process. To evaluate their efficacy, these compounds are first tested in the laboratory and if passing these test, eventually in clinical trials. Very few candidate compounds make it through the drug-approval process to become routine treatment.

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 how efficient a certain drug candidate is are crucial in this aspect.

### Strategies for Determining Drug Efficacy

How can the efficacy of a candidate drug be quickly and accurately determined? One strategy is to detect disease progression as soon as possible, especially before clinical symptoms appear. Another is to determine as soon as possible which patients seem to benefit from the drug and which do not. Still another is to determine 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 is available. However, many cancer drugs, including cell-cycle inhibitors, have no established biomarkers that reliably monitor disease progression or that accurately predicts response to therapy (Asghar, Witkiewicz et al. 2015).

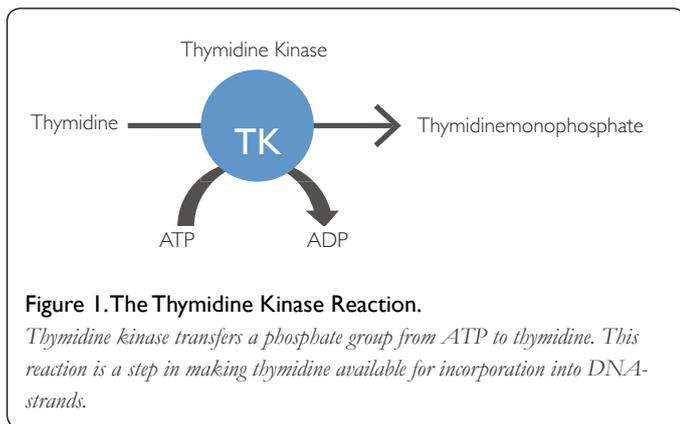
Serum thymidine kinase activity is a biomarker closely related to cell proliferation. Accumulating evidence indicates that this biomarker can provide response information useful

in cancer therapy development (Bjohle, Bergqvist et al. 2013, Nisman, Nechushtan et al. 2014). More importantly, a new technology—the DiviTum assay—now allows even small changes in serum thymidine kinase activity to be measured accurately, quickly, and noninvasively. As such, it has several attractive features as a tool in drug development:

- Thymidine kinase activity is closely associated with cell proliferation and DNA repair, meaning that the assay can indicate cancer progression;
- The DiviTum assay can measure thymidine kinase activity from a serum sample, eliminating the need for biopsies; and
- The 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 (TK) is an important enzyme for re-cycling de-phosphorylated thymidine back into a form that can be used in DNA-synthesis. TK phosphorylates thymidine to thymidine-monophosphate (Figure 1). This is an important step in making thymidine useful as a building block in DNA-synthesis. Cells have two types of TK: TK-1 and TK-2. TK-1 is a cytosolic enzyme primarily expressed and active in the G1/S-phases of the cell cycle, during which its activity increases more than 15 fold (Sherley and Kelly 1988) (Kauffman and Kelly 1991). TK-2 is a mitochondrial enzyme whose activity varies little over the cell cycle (Arner and Eriksson 1995, Topolcan and Holubec 2008).

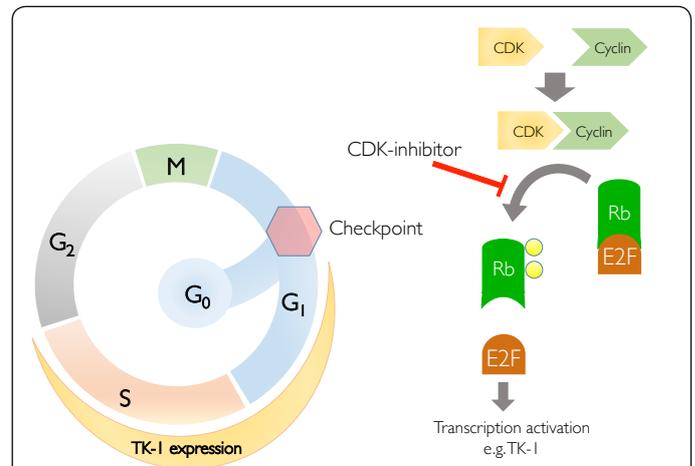


TK-1 activity can also be detected in the blood, and its level of activity is elevated in patients with conditions associated with increased cell proliferation or cell death, such as cancer, immune responses, and viral diseases. Elevated activity levels reflect an increase in cell proliferation and death of proliferating cells, but the mechanisms behind the release of TK into serum are not yet fully investigated. The tumor microenvironment is a complex mixture of normal cells and tumor cells, and tumor growth involves processes

of inflammation and angiogenesis (Hanahan and Weinberg 2011). Therefore, the increase in serum thymidine kinase activity observed 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 (Topolcan and Holubec 2008).

## 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 it is dependent on the transcription factor E2F (Figure 2). This transcription factor is activated by the CDK4/6 kinases as one of many functions that must be activated to prepare cells for replicating chromosomes in the S-phase (Giacinti and Giordano 2006).



**Figure 2. The cell cycle and function of palbociclib and other CDK4/6 inhibitors**

*Palbociclib blocks the CDK-cyclin complex from phosphorylating the 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.*

The central function of CDK4/6 kinases in cell proliferation has made these enzymes attractive targets for cancer therapy (Asghar, Witkiewicz et al. 2015). More than 15 clinical trials with candidate drugs based on CDK4/6 inhibition have been registered (ClinicTrials). 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 (Figure 2). This blocks the cycle from progressing to DNA replication because phosphorylation of the retinoblastoma protein activates E2F transcription factors, which is a critical step in chromosomal replication. The E2F transcription factors activate several genes associated with DNA replication, such as those for DNA-polymerase  $\alpha$  and  $\delta$  and, notably, also the TK-1 gene (Dou, Zhao et al. 1994).

Early clinical trials have reported that palbociclib has been beneficial in treating patients with mantle-cell lymphoma (Leonard, LaCasce et al. 2012), estrogen-receptor positive advanced breast cancer (Finn, Crown et al. 2015), and well-differentiated liposarcoma (Dickson, Tap et al. 2013). Other major pharmaceutical companies, such as Eli Lilly (abemaciclib), Novartis (LEE-011), and Bayer (ronidociclib) also have candidate drugs targeting CDK4/6 in clinical trials.

In developing and evaluating candidate CDK4/6-inhibitor drugs, serum TK-activity may be the missing biomarker. The close connection between CDK4/6 and TK1 expression and the large and well documented increases in TK activity in serum samples 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.

#### 4. Serum Thymidine Kinase Activity as a Biomarker in Cancer

The first commercial TK-activity assays were based on monitoring the turnover of a radiolabelled 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 haematopoietic cancers.

The research on TK as a biomarker in cancer has been extensively reviewed (Alegre, Robinson et al. 2013) (Zhou, He et al. 2013) (Topolcan and Holubec 2008). The new DiviTum assay measures serum TK-activity without the need for a radiolabelled substrate, with higher sensitivity and over a wider range of values 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 in blood malignancies (BiovicaWebpages).

TK 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 <sup>3</sup>H-thymidine phosphorylation and incorporation into DNA have established a thymidine-labelling index for estimating proliferative activity in biological tissues (Meyer and Connor 1977) (Kamel, Franklin et al. 1989).

A new technique attracting considerable attention is 3-deoxy-3-<sup>18</sup>F-fluorothymidine positron emission tomography (FLT-PEIT) (Sanghera, Wong et al. 2014). Using this imaging technique it is possible to estimate the activity of thymidine kinase in the tumors in the patients. 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 support our opinion that serum TK-activity determination with the DiviTum assay is useful in evaluating cancer therapy efficacy. The first investigated the clinical relevance of pre-treatment serum TK-activity in patients with advanced breast cancer (Bjohle, Bergqvist et al. 2013). High TK activity before chemotherapy treatment 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).

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

*Patients with high pretreatment serum TK-activity level generally had progressive disease and little benefit from the therapy, whereas patients with low levels responded much better (Bjohle, Bergqvist et al. 2013).*

Response	Serum TKI levels, median, Du/L	Patients, n
Objective Response	193	77
Stable disease	110	91
Progressive disease	1,492	16

In the second study (Nisman, Nechushtan et al. 2014), 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.

In conclusion, accumulating evidence continues to show that DiviTum 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 two studies. The first is the PREDIX study, a Phase II randomized trial evaluating response-guided treatment in luminal A breast cancer without lymph node metastases. This study is being done in collaboration with the Karolinska Institute, Stockholm, Sweden.

The second study is a collaboration between Biovica and the Dana Farber Cancer Institute, Boston, USA. The patients have either metastatic or unresectable lung cancer with CCND1 amplification or CCND1 amplified metastatic or unresectable tumors at any site and in which CDK4/6 amplification or CCND1 mutation or a splice variant is expected to lead to nuclear retention of cyclin D1 protein. This study is also using the DiviTum assay to monitor serum TK-activity as marker of progression, survival, and response rate.

What other studies would benefit from evaluating DiviTum as a biomarker? With external partners, we are presently designing new studies on various cell-cycle inhibitors. An application we find particularly interesting is the use of DiviTum as a companion diagnostic — a diagnostic test used to guide a specific treatment — to new cancer therapies targeting the mechanisms of cell-cycle regulation, e.g. CDK4/6 inhibitors.

In addition to these efforts, we document the potential of DiviTum to be useful with other types of targeted therapies where serum TK-activity can be expected to provide clinically useful information, (e.g. immunotherapy and cancer vaccines), and we explore the associations between serum TK-activity levels and clinical events in many different types of cancer, with breast and lung cancer as current areas of emphasis.

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