ABSTRACT

Thymidine Kinase 1 (TK1) is one of the essential nucleotide salvage pathway enzymes that is up-regulated during cell division and DNA repair. In normal cell division, TK1 is not released into the blood and is consumed intracellularly. In pre-neoplastic and malignant cell division, proportionately elevated levels of TK1 are released into the blood serum and can be measured by the ultra-sensitive Red Drop TK Test to indicate the rate of abnormal cell division.

These two things – accelerated division of cells with damaged DNA – are the hallmark features associated with cancer, its aggressiveness, and its progression. TK1 has been shown through hundreds of research articles around the world over the past 30 years to correlate to a wide range of cancers. This award-winning, patented, and ultrasensitive test out of Sweden is now made available as a discrete, affordable test from Red Drop.

The Red Drop TK Test is the ultimate biomarker for quantification of pathological cell division in the whole body. This simple blood test is a quantifiable and reliable way to monitor systemic abnormal cell division rates which when tracked over time indicate risk of developing cancer, the aggressiveness of the cancer, and treatment efficacy for many solid tumors and blood malignancies.

BACKGROUND

Today, the lives of millions of people around the world are affected both directly and indirectly by cancer. It is a ruthless and indiscriminate disease, affecting the rich and famous as well as the poor in virtually every country in the world.

The World Health Organization reports that approximately 25 million people live with cancer in Japan, Europe and North America. More startling is the fact that every year more than 7.6 million people die from cancer with another 12 million new cases diagnosed [1]. The case load is increasing and apparent to everyone that there needs to be dramatic improvements in how we prevent, diagnose and treat cancer.

The general public is slowly coming to understand that cancer is not a single disease, but hundreds of different diseases that fall within a similar category where mutated cells undergo uncontrolled cell growth, with the rate of cell growth linked directly to the aggressiveness of the cancer.

Numerous methods for detecting cancer are currently being employed, using assays that detect the presence of tumor-specific markers. All of these markers, including imaging techniques, are based on tumor mass. Well known tumor markers are CEA, HER2/neu, PSA, Beta-HCG, CA15-3, and CA19-9 [2].

Researchers have developed a biomarker, thymidine kinase 1 (TK1), which detects cancerous cell proliferation that is directly related to the aggressiveness of the tumor and represents an important complementary tool for cancer monitoring. This biomarker targets continually dividing cells, because very few cell types in the body divide at the same frequency as cancerous cells.

Targeting cell division is a major priority for candidate drugs, and monitoring the efficacy of treatment to enable personalized medicine is a major priority for oncologists worldwide.
**TK1 FAST FACTS**

**FACT: TK1 Correlates to Cancer Progression**
TK1 levels are elevated in a stage-like manner, meaning the more advanced the tumor or metastasis, the higher the level of TK1 activity. It has been shown through serial sampling that it is possible to identify recurrence months before clinical symptoms appear [13, 18].

**FACT: TK1 Correlates to Patient’s Response to Treatment**
TK1 has been demonstrated to accurately reflect patient’s response to treatment. In patients who respond to therapy, TK1 levels decrease. In those that do not respond, TK1 activity levels continue to increase [13-17].

**FACT: TK1 Correlates to Aggressiveness of Cancer**
TK1 is a measure a malignant cell division which gives clear indications on the aggressiveness of the cancer and provides data which can guide how aggressively to treat a patient [23, 24, 30].

**FACT: TK1 Useful as Health Screening Tool**
In “normal” individuals, TK1 levels have been shown to be very low, but rising through stages of hyperplasia, dysplasia, and severe dysplasia in pre-neoplastic conditions, which can show and allow for monitoring of patients pre-cancer [19-20, 22]. This allows patients time to modify lifestyle and make changes to possibly prevent cancer from forming [51-52].

By regularly measuring and tracking the prognostic trend of accelerated proliferation of cells with DNA damage – both hallmarks of cancer – TK1 is elevated proportionately as tissue progresses through hyperplasia, dysplasia, and various stages of cancer.

**FACT: TK1 Short Biological Half-Life**
The TK1 enzyme has a short biological half-life, which makes it possible to follow rapid changes in TK1 activity levels [46]. Case studies on individuals who have monitored changes in TK1 activity levels as often as once per week have been able to track the progression of cancer and efficacy of their treatment.

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**THYMIDINE KINASE (TK1) AND CANCER CELL PROLIFERATION**

One way of measuring abnormal cell proliferation is the serum assay of the metabolic enzyme, thymidine kinase (TK1) which is involved in DNA synthesis during cell division and DNA repair [3, 30]. Research has shown TK1 to have widespread use as a diagnostic and prognostic indicator for cancer progression [4-6].

Tumor cell proliferation is one of the most commonly used variables in the evaluation of prognosis in breast cancer, for example. A classic, simple measure of cellular proliferation, mitotic count, is incorporated in important breast tumor grading systems [7]. Another measure of proliferation which is now widely used is Ki67/MIB1 labeling index [8]. This has prognostic value for many types of malignant tumors, including breast cancer. However, the evaluation of all of these parameters requires tissue, either from a biopsy or surgical samples.

Therefore, the development of the minimally invasive Red Drop TK1 assay that gives pre-operative information on proliferative tumor potential is imperative, as the information obtained by these assays could aid in the decision regarding systemic adjuvant treatment [9, 30].

TK1 levels typically only increase during the S-phase of normal cell division. It has been discovered that TK1 is over-expressed in many malignant carcinomas during all phases of cell division, significantly raising the serum TK1 activity of patients with cancer [10-12].
THE MECHANISM OF TK1 –
CELL PROLIFERATION RATE FROM A SIMPLE BLOOD TEST

Cell division requires the four bases that build the genetic code in the DNA-synthesis. One of them is thymidine and to make it present in body fluids available for DNA synthesis, it must be phosphorylated to thymidine monophosphate. This is then further phosphorylated and used for the growing DNA chains. For this critical phosphorylation step, all mammalian cells use the enzyme thymidine kinase 1 (TK1), the enzyme that the Red Drop TK Test measures [44].

TK1 is not typically present in resting cells. It is synthesized just before cell division in cell phase S to assist in the synthesis of DNA [Fig 1]. In phase G2, the enzyme is degraded intracellularly and is not released into circulation. Aside from TK1’s role in DNA replication, TK1 is also up-regulated to repair DNA damage [Fig 2].

In situations where the DNA is not able to be repaired, apoptosis or programmed cell death is triggered [Fig 3].

In certain situations, the DNA damage results in the disabling of apoptosis, resulting in a cell which will then continue to divide with these mutations intact in the daughter cells [Fig 4]. This begins the pre-neoplastic carcinogenesis process. Over time, DNA mutations will continue to accumulate in these eternal cells and their daughter cells until the right combination occurs and cancer is formed [Fig 5].

Immature cells which are malformed and less differentiated begin going through more rapid cell division. During malignant cell division, TK is over-expressed in every phase of cell division, and ultimately released into the blood stream during this process [12].

The Red Drop TK Test measures the serum TK1 activity which is proportional to the amount of dividing cells with damaged DNA. These things – accelerated division of cells with damaged DNA – are the hallmark features of cancer, its aggressiveness, and its progression.

THE NEW GENERATION TK1 TEST
THE WORLD-WIDE GOLD STANDARD

Early techniques for measuring serum TK1 activity using a radioactive substrate analog have been used for more than 30 years. However, the use of the radioactive assay hampered the wide use of the TK1 assay in routine clinical medicine. Over time, a kit form of the radioactive immunoassay was developed by Dr. Simon Gronowitz called the “TK-REA” test. Later, DiaSorin introduced the “Liaison” test, a fully automated competitive chemiluminescence TK1 assay. However, because of limited analytical sensitivity, these tests were primarily used for hematological cancers and advanced carcinomas.

The ultrasensitive Red Drop TK Test now allows accurate measurements of TK1 activity more than 100 times more sensitive than previous tests. This functionality expands the applicability of the TK1 test for use with early stage and solid tumors, as well as a health screening tool for monitoring those at risk for developing cancer.
The TK1 test has enjoyed a long, well-established history around the world in monitoring blood malignancies. One of the most important applications for the TK1 test has historically been in non-Hodgkin’s lymphoma and in chronic lymphocytic leukemia [31-32]. TK1 levels in acute lymphocytic leukemia (ALL) have been found to decrease upon remission and increase upon relapse of the disease [49], thus giving an early warning of recurrence of the disease and allowing timely treatment. It has been shown that patients with high TK1 activity seemed to have a shorter survival than the patients with low TK1 activity [50]. In myelodysplastic syndrome, the TK1 value is fundamental for the treatment of choice, as it gives a reliable indication for transformation to acute myeloid leukemia [53].

**HISTORY OF USE IN BLOOD MALIGNANCIES**

**CORRELATION TO TK-REA**

Strong correlation is obtained between the Red Drop TK Test and the traditional Prolifigen TK-REA assay as seen in this experiment, which is a cohort of sera from cancer patients. This shows that all published data on the application of blood malignancies expressing high TK1 activity levels are valid for the Red Drop TK Test.

**SENSITIVITY VERSUS TK-REA**

This experiment made by successively diluting a suspension of TK-positive CEM cells shows that the Red Drop TK Test is capable of detecting TK activity levels a hundred times smaller than in the traditional TK-REA assay.

**COMPARISON WITH LIAISON TK ASSAY**

In a test on 368 women which included 149 healthy donors for control, 59 donors with benign breast disease, and 160 donors with primary breast cancer, both assays showed similar prognostic information, with pre-operative enzyme activity in primary breast cancer patients correlating with aggressive features of the cancer as well as identifying low risk breast cancer patients.

The Red Drop TK Test was able to give more valuable clinical information than the Liaison assay, detecting smaller increases in malignant cell division. The TK1 activity measured was associated with tumor size, T-stage, presence of vascular invasion, and estrogen and progesterone receptor status. The assay measured a significant increase in TK1 activity of patients with triple-negative tumors. The sensitivity of the Red Drop TK Test was also useful for breaking down the benign breast disease patients into sub-groups. TK1 activity was higher in patients with fibro adenoma than those with non-proliferative breast lesions [9].

With the increased sensitivity of the Red Drop TK Test, the benefits of measuring serum TK1 activity broaden to increase their use in solid tumors. The latest clinical studies demonstrate the usefulness in a wide range of common cancers.

FOR MORE RESEARCH AND INFORMATION WWW.REDDROP.COM
For the past 30 years, researchers from over 30 countries have documented the direct correlation of TK1 to over 18 different major categories of cancer – thyroid, cervical, colon, bladder, pancreatic, melanoma, ovarian, gastric, and more. The evidence is clear and overwhelming – rising TK1 activity levels show a clear link to development and spread of cancer. For more information, go to www.reddrop.com/research.

**BREAST CANCER**

A recent study of 161 patients with localized breast cancer who were followed for at least 3 years after surgery (lumpectomy or mastectomy) concluded that the Red Drop TK Test was found to be an independent factor for recurrence-free survival. A cut-off value was documented and women with an initial score below 134Du/L had significantly less risk of disease recurrence [23]. In a study of 1,692 breast cancer patients, TK1 levels were found to be an independent prognostic factor for local recurrence [30].

**COMPLEMENT TO CA 15-3**

One of the many advantages of the Red Drop TK Test is that it measures the tumor growth rate which is valuable information to complement other tumor markers such as PSA and CA 15-3 which measure tumor volume. The TK Test can provide a more comprehensive picture of the disease and give important prognostic and monitoring information [23, 27-28].

**RENAL CELL CARCINOMA**

Researchers in a recent study of 77 patients with localized RCC who were followed over a period of at least 12 months (median of 45 months) concluded that pre-operative TuM2-PK and TK1 could predict disease recurrence after nephrectomy, especially when used in combination. Patients with a score below 170 Du/L had significantly less risk of disease recurrence [24].

**LUNG CANCER**

Studies have shown in NSCLC that preoperative TK1 levels were higher than normal individuals, and increased in a stage-like manner from T1 to T4 tumors. Apart from its prognostic value, TK1 is suggested as a reliable marker for responding to treatment [29, 54-55]. Studies on SCLC have demonstrated that TK1 activity could divide patients into groups with highly significant difference in survival time. Of the various markers studied, only TK1 was judged useful for estimation of disease spread and prognosis of the patient [56-57].

**PROSTATE CANCER**

Recent studies showed that metastatic prostate cancer is connected to pathological levels of TK1. The trial also showed that TK1 does not correlate to PSA. This means that the tumor growth rate of the TK Test as a complement to the PSA adds valuable information enhancing the prognostic picture of the disease [3, 25-26].

**TK1 CORRELATES TO WIDE RANGE OF CANCERS**

For the past 30 years, researchers from over 30 countries have documented the direct correlation of TK1 to over 18 different major categories of cancer – thyroid, cervical, colon, bladder, pancreatic, melanoma, ovarian, gastric, and more. The evidence is clear and overwhelming – rising TK1 activity levels show a clear link to development and spread of cancer. For more information, go to www.reddrop.com/research.
TK1 LEVELS IN NORMAL INDIVIDUALS – USE AS HEALTH SCREENING TOOL

Based on a study of 240 individuals in an asymptomatic control group ranging split equally between male and female, ages ranging from 20 - 80, as well as in the control groups from the various research studies - the TK1 activity level in “normal” range in both male and female donors is quite low [21]. In another health screening study of 35,365 people demonstrated elevated serum TK1 correlated to and is a reliable marker for risk assessment of pre-/early cancerous progression [22].

Based on this research, corresponding Risk Zones are established based on the percentile distribution of results. As individuals go through hyperplasia, low grade dysplasia, and severe dysplasia, you see a proportionate rise in TK1 activity to the number of abnormal, immature, cells going through more rapid cell division. The data from studies shows that Red Drop TK Scores over 90 are in the 97th percentile and above 120 would be at severe risk for malignancy.

The Red Drop TK Test can provide valuable information for health conscious individuals to validate their lifestyle, individuals who are at risk for cancer, and individuals who are in remission wondering if their cancer is returning.

There are several known causes of elevated TK1 activity other than malignant cells growth. These factors must be considered and eliminated as causal factors when evaluating a patient’s TK Score [45, 47].

1. Major Injury healing [43]
2. Active viral infection [41-44]
3. Pernicious anemia (Vitamin B-12 deficiency) [36-40, 47]
4. Certain drugs that interfere with thymidine metabolism (methotrexate, fluorouracil, etc.) [46].
5. Autoimmune disease [5].

Elevated levels of TK1 must always be interpreted together with detailed knowledge of the patient’s condition, because nonspecific elevations of serum levels must be excluded.

TK1 AS PROGNOSTIC INDICATOR FOR CANCER PREVENTION

Most individuals will find the real value of the Red Drop TK Test when they take the test periodically and regularly to establish a baseline trend and monitor their results over time. The TK Score gives the person an indication of abnormal cell division in a pre-cancerous situation, giving the person time to change behaviors before cells become neoplastic.

Case studies have shown that the tracking their TK Score can be a motivating factor for individuals to implement changes such as diet modification, cut backs in alcohol and tobacco consumption, nutritional supplementation, and lifestyle changes to reduce their risk of developing cancer. The TK Score gives the patient accessible information on a regular basis, empowering them to take control of their health and go through traditional cancer screening as necessary.

CONCERN ABOUT FALSE NEGATIVES

The result from the determination of a biological marker, such as TK1, should always be considered in conjunction with other diagnostic techniques and in consultation with a health care professional. In some situations such as - 1) brain cancer where the active TK1 stays in the cerebral spinal fluid, 2) slow growing cancers such as sarcomas, or even 3) advanced cancers which are slow growing or in an indolent phase - a low TK1 Score may give an individual a false sense of security.
PROACTIVE CANCER PREVENTION
Use of the TK1 test as a healthy screening and monitoring tool empowers the individual to take control of their life to reduce and measure progress in cancer prevention [50-51].

Stop cancer before it starts.

EARLY CANCER DETECTION
Research clearly shows that the earlier cancer is detected, the better the chances of survival. The Red Drop TK Test is one of the tools that can provide valuable information.

Stop cancer before it takes root.

VALIDATE EFFECTIVENESS OF TREATMENT
The TK1 test is a simple, non-invasive way to measure and monitor the success of cancer treatments and aggressiveness of the cancer, allowing the patient to focus time and resources on things that work.

Stop cancer before it is unstoppable.

EARLY INDICATION OF RELAPSE
For patients who have had cancer and been through treatment successfully, there is a perpetual nagging worry about relapse of cancer. Patients have a simple tool to monitor systemic changes.

Stop worrying and find peace of mind.

FOR MORE RESEARCH AND INFORMATION
For the 30 years of research, use of the radioactive immunoassay, the TK-REA test (Prolifigen), and the Liaison (DiaSorin) has provided a wealth of insight for researchers and doctors on the role of thymidine kinase (TK1) and cancer. Hundreds of papers have been published from researchers in over 20 countries. While the TK1 technology may appear to be old or outdated, the release of the new ultra-sensitive Red Drop TK Test opens new doors to fresh research on health screening applications, progression levels of TK1 through hyperplasia and dysplasia leading up to cancer, early stage solid tumor detection, indications of metastatic spread, etc. The role of TK1 as an important biomarker will expand significantly in practice.

The research articles listed here are only a sampling of what is available. We invite you to visit www.reddrop.com/research for a more comprehensive library of research articles by cancer type.

REFERENCES

FOR MORE RESEARCH AND INFORMATION WWW.REDDROP.COM
REFERENCES CONT’D.


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