THYMIDINE KINASE 1
IN THE STUDY OF HEMATOLOGICAL MALIGNANCIES

Thymidine Kinase 1 (TK1) has been long known as a valuable biomarker of cellular proliferation. However, previous methods have been based on enzyme activity measurements that are complex and require specialised equipment.

The AroCell TK 210 ELISA kit is a new valuable assay for TK1 that brings the simplicity and robustness to this field and makes it widely available.

The AroCell TK 210 ELISA kit will provide new opportunities for studying cellular proliferation, tumor cell turnover and therapy response in subjects with hematological malignancies.

Thymidine Kinase 1 in Studying Hematological Malignancies

Tissue TK1

DNA replication requires the production of phosphorylated thymidine. There are two enzymes that can perform this, Thymidine Kinase 2 (TK2), found in mitochondria, which is constitutively expressed and Thymidine Kinase 1 (TK1) the expression of which varies during the cell cycle. TK1 concentrations in the cell are low in the G0/G1 phase (resting phase) of the cell cycle but increase during the S/G2 phases when DNA synthesis occurs and then decrease during mitosis. The presence of TK1 in cells is an indicator of active cellular proliferation.

TK1 up-regulation may be an early event in cancer development and TK1 may even be elevated in pre-cancerous conditions[1]. Increased TK1 expression is often associated with increased expression of cell proliferation markers such as the Ki-67 antigen and proliferating cell nuclear antigen (PCNA)[2,3], although studies have shown that TK1 may be a more useful as a proliferation marker than either of them[4]. TK1 has the further practical advantage in that it is measurable in both tissues and serum, simplifying serial testing.

Increased cellular proliferation is a hallmark of malignancies and TK1 has been found to be significantly over-expressed compared to circulating lymphocytes in subjects with aggressive Chronic Lymphocytic Leukemia (CLL) compared with lymphocytes from healthy individuals and subjects with indolent disease[5].

Tissue TK1 activities in subjects with NHL were found to increase in parallel with the occurrence of less well differentiated cells and the clinical Rappaport score[5]. An increase in intracellular TK1 was associated with a change to more aggressive disease[5] and increased cellular TK1 could be a predictive biomarker.

Intracellular TK1 is comprised of a tetramer of four identical 25kD monomers, however, in serum, it is mainly found in the form of a range of high molecular weight complexes[6].

TK1 activity in serum

95% of serum TK1 activity in cancer patients seems to be tumor derived, making TK1 an excellent indicator of cellular proliferation[7] and a useful complement to immunohistological testing for proliferation biomarkers. TK1 enzyme activity has been shown to be elevated in subjects with many forms of cancer, including leukemia, lymphoma, prostate, breast, lung, sarcoma and colon cancer patients[8].

Elevations in serum TK1 are associated with more aggressive hematological malignancies and elevated serum TK1 activities in subjects with chronic lymphocytic leukemia are of increased risk of disease progression[9]. While subjects with lower serum TK1 values may have improved survival[10].

Serum TK1 can be profitably combined with other biomarkers. In CLL the addition of TK1 added value to diagnostic staging based on clinical criteria and the well-known serological biomarker $\beta_2$microglobulin[10]. Serum TK1 activities may reflect a combination of tumor mass and proliferation rate[11,12].

Serum TK1 activity has been widely used and proven to be a valuable biomarker in hematological malignancies but, being an enzyme activity assay, it has limitations regarding assay complexity and serum contains inhibitors of TK1 activity[13]. Furthermore, there are multiple forms of serum TK1 with differing specific activities and which differ between healthy and subjects with hematological malignancies[14].

Current assays for TK1 enzyme activities require specialist equipment and, in the case of the reference method, radioactive reagents, restricting their use. The development of a standard immunoassay can solve the above problems, but producing sensitive and specific antibodies against TK1 has proven difficult.

However, all TK1 forms express the unique TK 210 epitope (amino acid sequence 194-225) and this offers the potential to develop immunoassays capable of detecting all TK1 forms.

The AroCell TK 210 ELISA kit, based on two monoclonal antibodies specific for the TK 210 epitope, brings the specificity, sensitivity and robustness of ELISA to the study of serum TK1[15].

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AroCell TK 210 ELISA as a Cell Proliferation Biomarker in Hematological Malignancies

Correlation with the TK1 Enzyme Radioassay

There are many years' experience of the use of TK1 enzyme activity measurements to study hematological malignancies and it is important that new improved methods can be related to the existing knowledge base. The AroCell TK 210 ELISA kit has been compared with the existing gold-standard TK1 enzyme activity radioassay.

Figure 1 presents a study comparing TK1 enzyme activity to the molecular weights of the TK1 complexes and the expression of the TK 210 antigen (25 kDa monomer)[14]. A serum sample from a subject with myelodysplastic syndrome was fractionated and the enzyme activity compared with the presence of the TK 210 antigen. Western blot analysis, using the TK 210 antibody as a probe, showed that TK1 units (25 kDa) were found in all of the fractions showing TK1 activity and so are quantifiable with the AroCell TK 210 ELISA kit. This is important as some enzyme activity assays for TK1 may under-estimate the TK1 activity of low molecular weight fractions[16].

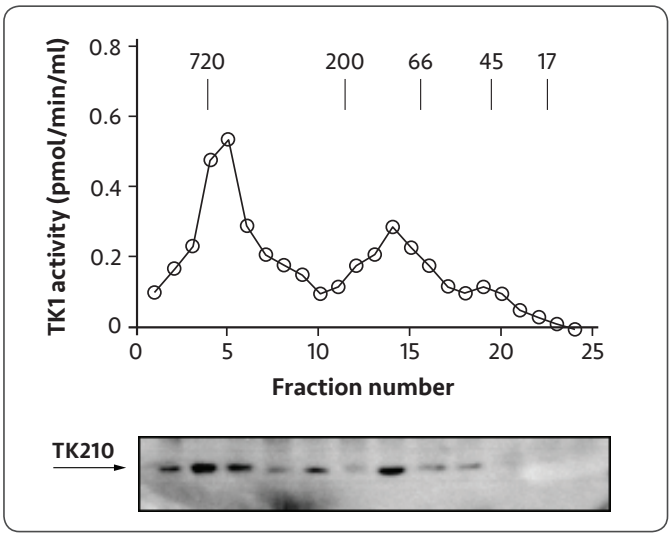


Figure 1

Furthermore, AroCell TK 210 ELISA results correlate very highly with the TK1 activity radioassay values, facilitating comparisons with historical data (Figure 2) while having the accuracy and convenience of an immunoassay[17].

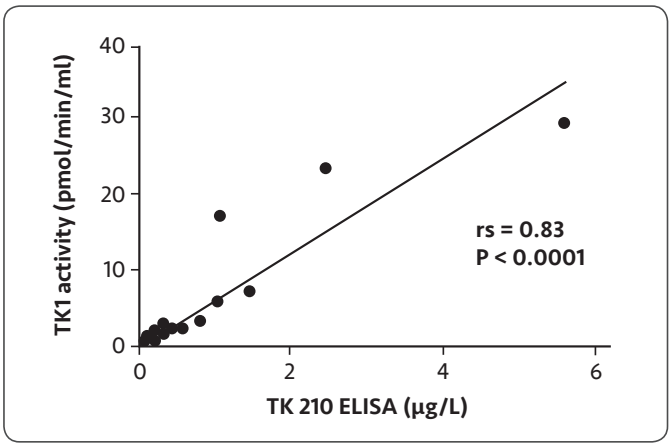


Figure 2

Comparison of AroCell TK 210 ELISA with TK1 Activity Radioassay

This equivalence is con irmed by studies on clinical material where the AroCell TK 210 ELISA kit and the Gold-Standard radioassay had similar diagnostic accuracy to distinguish subjects with myelodysplasias from healthy subjects[14]. See figure 3.

Comparison of TK 210 ELISA and TK1 Radioassay in Subjects with Myelodysplasias

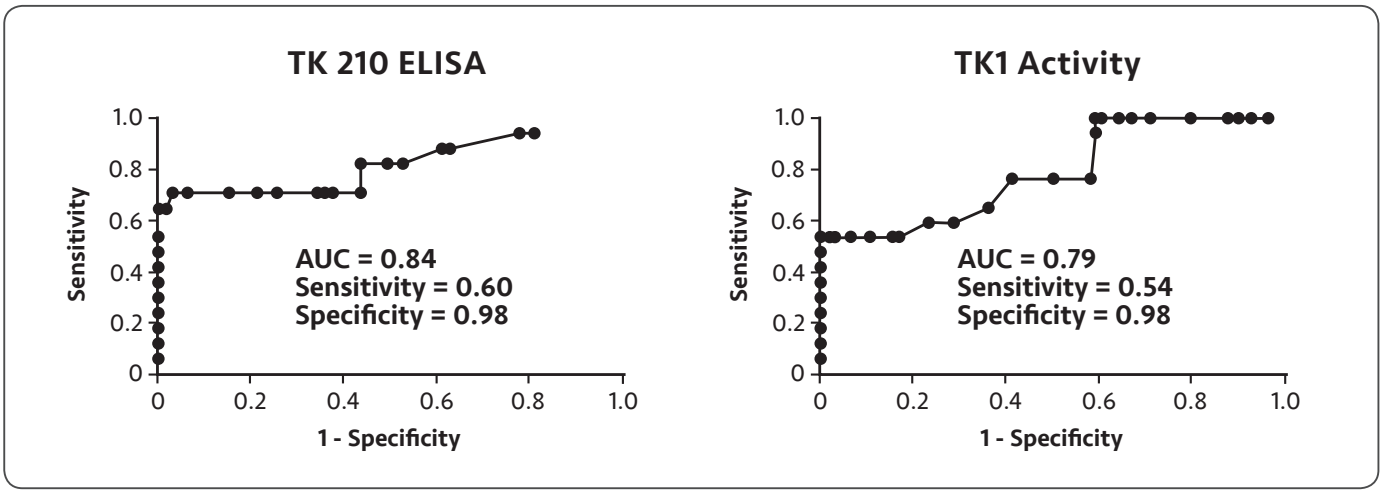
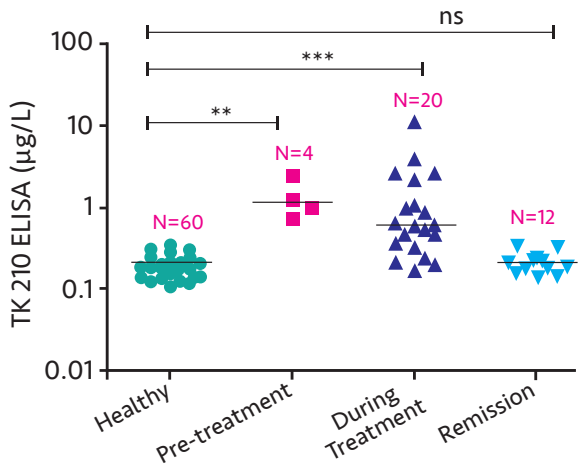


Figure 3

Serum TK1/TK 210 Levels in Subjects with Hematological Malignancies



** p< 0.01; *** p < 0.0001

		TK 210-ELISA (µg/L)		
Group	**No of samples**	**Mean**	**Median**	**SD**
Healthy	60	0.22	0.21	0.08
Before Treatment	4	1.35	1.15	0.72
During Treatment	20	1.02	0.61	1.07
Remission	12	0.22	0.21	0.09

Mean and median TK1 (TK 210) protein levels in different patient groups and in controls.

Figure 4

Further studies have shown that serum TK 1 concentrations measured in subjects with hematological malignancies are elevated and tend to return to near normal in subjects in remission[18].

The Arocell TK 210 ELISA Kit Brings The Accuracy and Convenience of Immunoassay to a Well-Known Biomarker

AroCell TK 210 ELISA as a Biomarker for Hematological Malignancies

- Measures all TK1 forms (both active and inactive)
- Unaffected by serum inhibitors
- TK1 is an early biomarker of uncontrolled proliferation
- Serum levels reflect tumor proliferation and cell turnover
- TK1 can be used to study many tumors
- TK1 may aid in prognoses and therapy selection
- Standard ELISA procedure
- Good correlation with reference radioassay
- Open system
- CE marked

AroCell TK 210 ELISA is for Research Use Only in the USA. Not for use in diagnostic procedures.

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