

SIGNIFICANCE OF TK 210 PROTEIN (TK1) LEVELS IN LYMPHOMA AND LEUKEMIA PATIENTS

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Introduction

- Thymidine kinase 1 (TK1) is a key enzyme in DNA synthesis.
- TK1 activity is cell cycle dependent and is up-regulated in proliferating cells
- Uncontrolled cellular proliferation, which is a major characteristic of malignancies, results in the leakage of TK1 into the blood where it forms large stable aggregates (1, 2).
- Previously, TK1 has been measured using enzyme activity assays that are complicated and may under-estimate TK1 mass.
- The development of TK1 ELISA methods based on antibodies to the XPA 210 peptide sequence of TK1 may offer improved methods for quantifying TK1 (3).
- The AroCell TK 210 ELISA has been demonstrated to provide increased discrimination between TK1 serum levels in healthy subjects and those with malignancies (4).
- This study demonstrates the variation in TK 210 protein levels in lymphoma and leukemia patients before and during treatment and in remission of disease.

Method

- The AroCell TK 210 ELISA utilises antibodies produced against the TK1 antigenic peptide XPA 210 (Fig 1) (5).
- Pre-incubation of samples with a special sample dilution buffer (AroCell AB) releases TK1 from its complexes and is a crucial step for consistent results. Recombinant human TK1 in a serum matrix was used to create a standard curve (Fig 2).

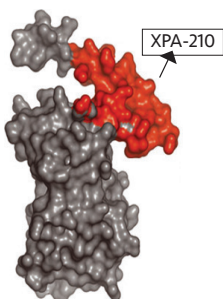


Fig 1. Structural model of TK1

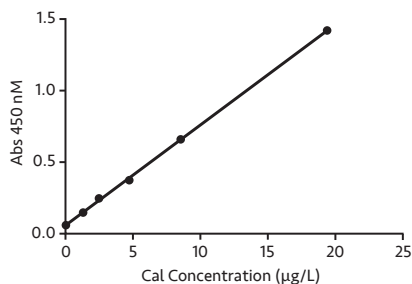


Fig 2. Standard curve

AroCell TK 210 ELISA Procedure

- Calibrators, controls and serum samples were diluted **1:1 in sample dilution buffer** and incubated at room temperature (RT) for **60 min**.
- Plates with coated antibody were prewashed 4 X 3 min with wash buffer (WB).
- Incubate the plate with prepared calibrators, controls and samples **at RT for 2h**.
- Wash 4 x 350 µL Wash Buffer.
- Add Biotinylated anti-TK1 antibody diluted in reagent buffer
- Incubate at RT **60 min**.
- Wash 4 x 350 µL Wash Buffer.
- Add Strep- HRP and incubate for **30 min** at RT.
- Wash 4 x 350 µL Wash Buffer.
- Add Substrate TMB and incubate for **15 min**.
- Add Stop Solution and measure the absorbance at **450 nM**.
- TK1 protein concentrations were determined by 4-PL analysis.

The AroCell TK 210 ELISA is for Research Use Only in the USA. Not for Use in Diagnostic Procedures.

Conclusions

- Serum TK1 levels assayed with the AroCell TK 210 ELISA differ significantly between subjects with different stages of hematological malignancies.
- The AroCell TK 210 ELISA offers new opportunities for the study of these conditions and their therapy.

Study Population

- A total of 36 random serum samples from patients with lymphomas and leukemias were collected to determine the TK1 (TK 210) protein levels.
- The patient groups included pre-treated (N=4), during treatment (N=22), subjects in complete remission (N=12), and 60 healthy individuals as a controls. Serum samples are collected at the University Medical Centre, Ljubljana, Slovenia.

Results

Comparison of Serum TK 210 Protein Levels between Groups

- Serum TK 210 protein levels were significantly higher in the pre-treated (median =1.15 µg/L) and patients undergoing treatment (median =0.61 µg/L) compared to healthy (0.21 µg/L) (Fig 3). Patients under treatment showed a wide distribution of values from 0.22 to 4.1 µg/L, possibly due to the effects of chemotherapy.
- There was no significant difference in TK 210 protein levels between the patients in complete remission (0.21 µg/L) and healthy individuals. Mean and median TK1 protein levels for each group are shown in Table 1.
- As the study involves only 36 patient sera, the prognostic significance of TK 210 levels for patients with who have undergone neoadjuvant chemotherapy should be validated by future prospective clinical trials.

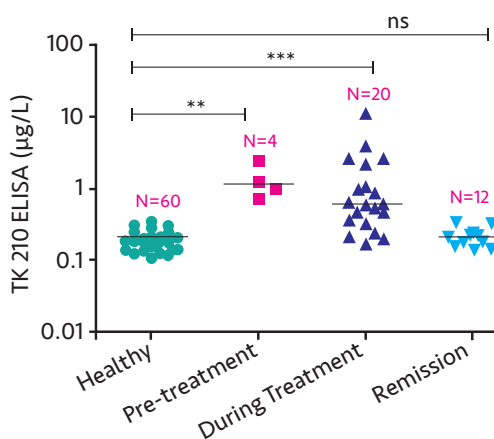


Fig 3: Serum TK1 protein (TK 210) distribution in patients before and during treatment, in remission and in control patients. The line indicates the median value of each group.

Group	No of samples	TK 210-ELISA (µg/L)		
		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

Table 1: Mean and median TK1 (TK 210) protein levels in different patient groups and in controls.

References

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