

IMPROVED STUDIES ON MALIGNANCIES WITH A NOVEL PROLIFERATION BIOMARKER - Thymidine Kinase 1



AROCELL TK 210 ELISA

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AGENDA



- 1 Proliferation Biomarkers
- 2 Thymidine Kinase 1 as a Tumor Biomarker
- 3 Thymidine Kinase 1 in the Study of Hematological Malignancies
- 4 Thymidine Kinase 1 in the Study of Solid Tumors
- 5 Thymidine Kinase 1 in *In-Vitro* Drug Discovery
- 6 Immunoassay of TK1 – AroCell TK 210 ELISA
- 7 Summary and Discussion

PROLIFERATION BIOMARKERS



HYPOTHESIS:

- *Most current tumor biomarkers measure what has happened*
 - *Past rate of change (doubling time)*
- *Current situation*
 - *Tumor mass*
- ***What really matters is tumor growth***



MEASURE WHAT REALLY MATTERS



HYPOTHESIS:

PROLIFERATION BIOMARKERS:

- *Provide a measure of tumor growth*
- *Stratify patients / subjects*
- *Are forward looking biomarkers*



MEASURE WHAT REALLY MATTERS



HYPOTHESIS:

PROLIFERATION BIOMARKERS

- *Are forward looking biomarkers*
- *Aid in choice of therapy*
 - *Low: Watchful waiting /
Less aggressive therapy*
 - *High: More aggressive therapy*



MEASURE WHAT REALLY MATTERS



HYPOTHESIS:

Many cancer drugs are:

- *Toxic / have side-effects*
- *Expensive*
- *Drive therapy resistance*



PROLIFERATION BIOMARKERS



IMMUNOHISTOLOGICAL BIOMARKERS

KI 67: Nuclear protein expressed in G1, S, G2 and M phases of cell cycle.

PCNA: (Proliferating Cell Nuclear Antigen). Nuclear protein which is elevated during the G1/S phase of the cell cycle.

TK1: (Thymidine Kinase 1). TK1 concentrations in the cell are low in the G1 phase (resting phase) of the cell cycle, but **increase during the S-phase when DNA synthesis occurs** and then decline rapidly during the G2 phase.

% of cells staining (proliferation index) provides a measure of the rate of tumor growth.

PROLIFERATION BIOMARKERS



IMMUNOHISTOLOGICAL BIOMARKERS

- *Require biopsy - invasive*
- *Only show the status of a small part of the tumor*
- *Low reproducibility*

PROLIFERATION BIOMARKERS



THERE IS A NEED FOR A BIOMARKER THAT:

- *Indicates the total body activity of the malignant disease*
- *That is applicable to a standard serum sample*
- *That can be determined with a routine analytical method*

SERUM THYMIDINE KINASE 1 (TK 210) HAS THE POTENTIAL TO BE SUCH A MARKER

SERUM TK1 AS A TUMOR BIOMARKER



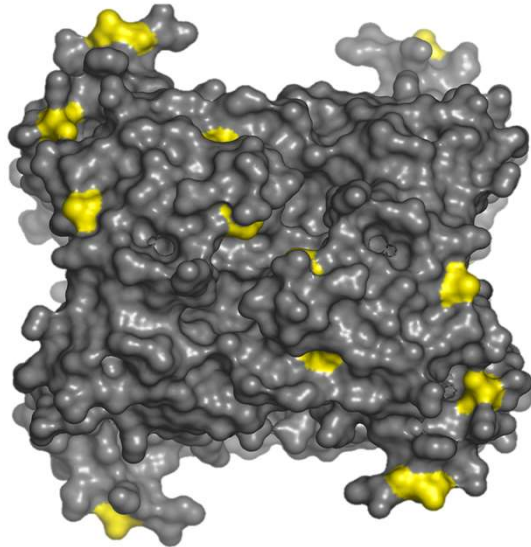
- Serum TK1 *enzyme activity* has been an established biomarker, mainly for hematological malignancies, for 35 years.
- Less studied in solid tumor diseases.
- When TK1 is used in immunohistological staining it is highly associated with the gold-standard proliferation biomarker KI 67.

TK1 PROTEIN IN SERUM



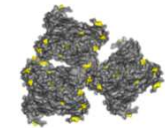
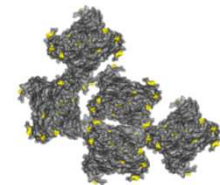
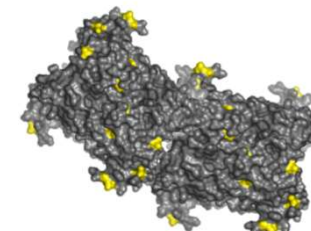
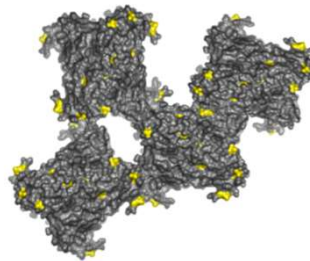
- *Intra-cellular active TK1 is a tetramer*
- *But serum TK1 exists as complexes of different sizes and enzyme activities*
- *Some sera contain TK1 inhibitors*
- *Enzyme activity assays may under-estimate serum TK1*

TK1 PROTEIN IN SERUM



Model of the TK1 tetramer

TK1 in serum forms stable complexes of many molecular weights and enzyme activities



All express the TK 210 antigen

After Welin et al 2004

AROCCELL TK 210 ELISA

MEASURE WHAT REALLY MATTERS



- *Standard microtiter plate sandwich ELISA*
- ***A unique pre-assay treatment breaks up the TK1 serum complexes and exposes the TK 210 antigen sites***



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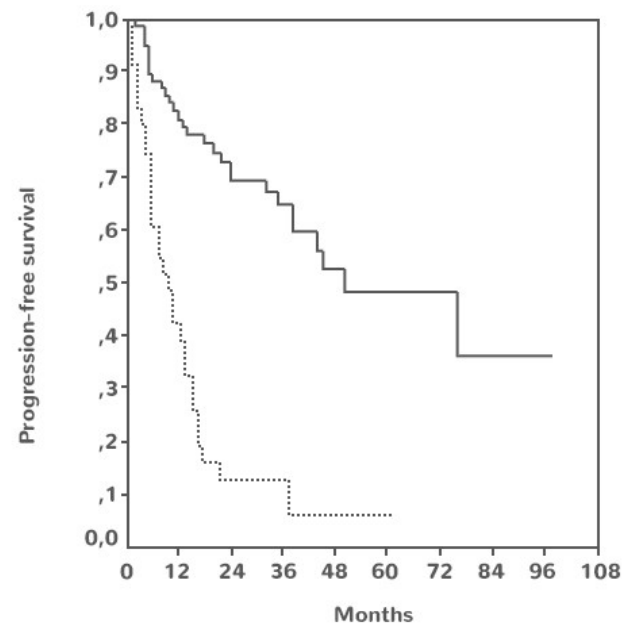
TK1 IN THE STUDY OF HEMATOLOGICAL MALIGNANCIES



SERUM TK1 ENZYME ACTIVITY in Hematological Malignancies:

- Discriminator of disease stage
- Prognostic information
- Disease progression

Serum TK1 Correlates with Prognoses



— TK1 < 7 U/L
.... TK1 > 7 U/L
 $p < 0.001$

Serum TK1 Activity and Progression-Free Survival:
Chronic Lymphocytic Leukemia

Hallek et al
Blood 1999

TK1 IN THE STUDY OF SOLID TUMORS

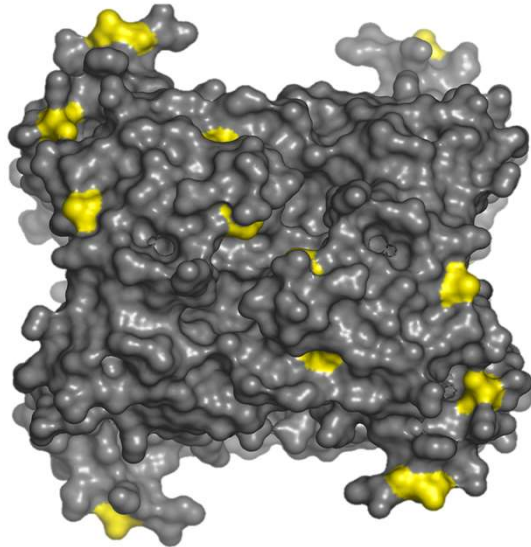


TK1 IMMUNOHISTOCHEMISTRY: Expression in solid tumors:

- *Breast*
- *Ovary*
- *Cervical*
- *Prostate*
- *Lung*
- *Renal Cell Carcinoma*

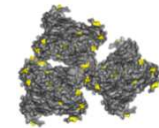
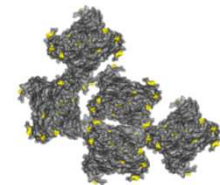
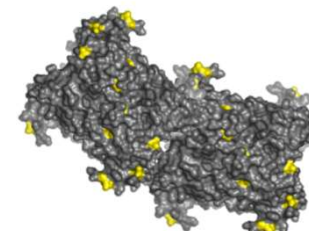
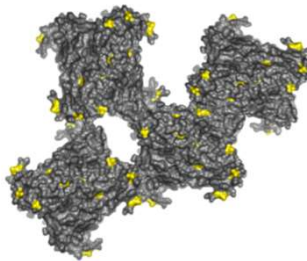
WHY HAS TK1 NOT BEEN WIDELY STUDIED IN SOLID TUMORS?

TK1 PROTEIN IN SERUM



Model of the TK1 tetramer

TK1 in serum forms stable complexes of many molecular weights and enzyme activities



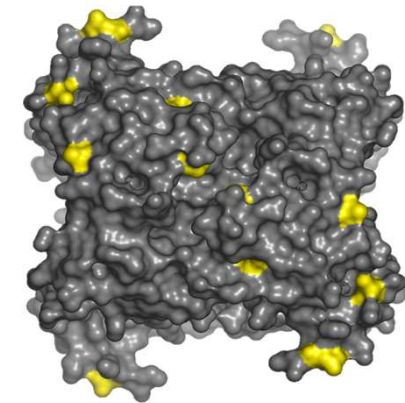
All express the TK 210 antigen

After Welin et al 2004

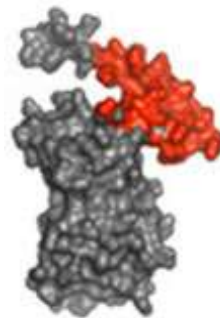
TK1 IS CURRENTLY MEASURED BY ITS ENZYMATIC ACTIVITY



Many of the TK1 aggregates in serum from solid tumor subjects are enzymatically inactive

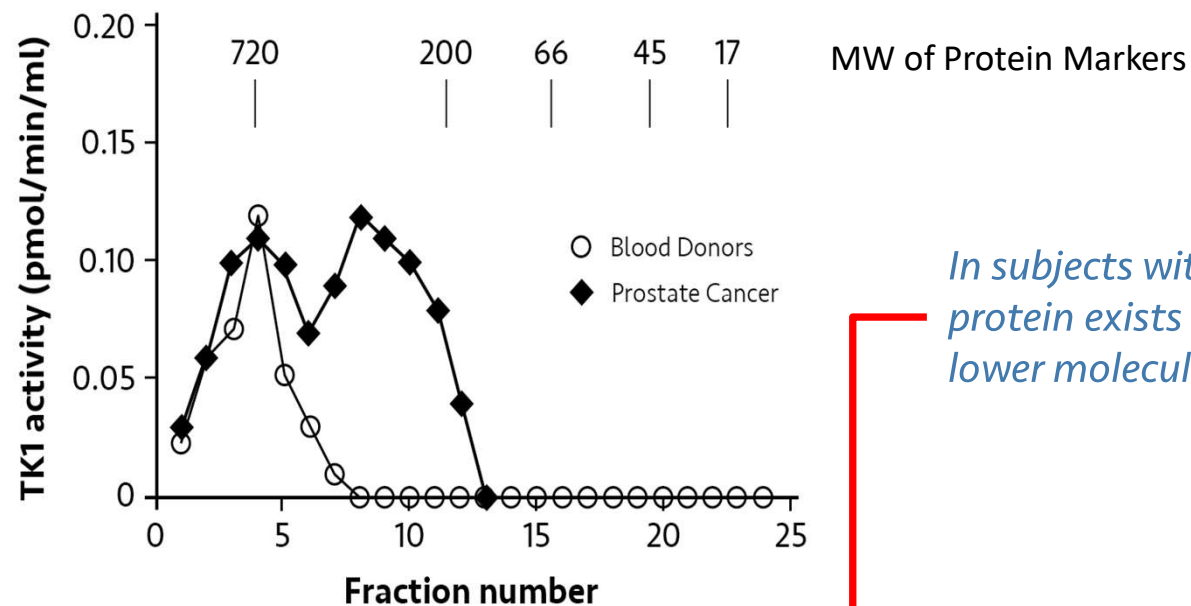


Model of the TK1 tetramer

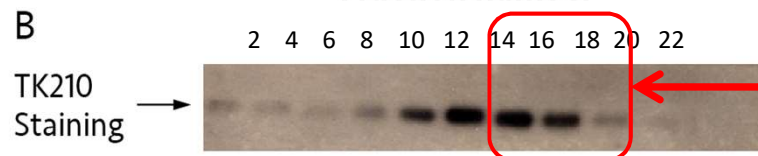


But all express the TK 210 antigen

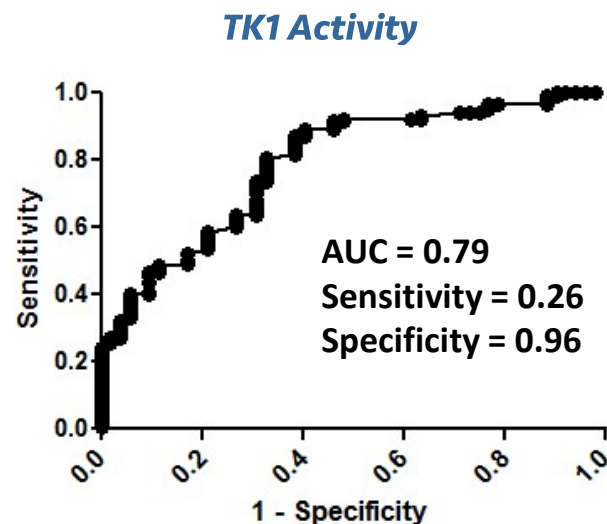
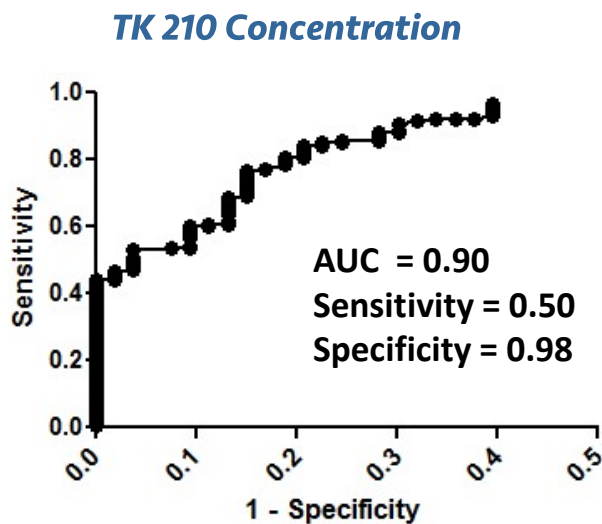
TK1 ACTIVITY AND MOLECULAR WEIGHT IN HUMAN SERUM



In subjects with solid tumors, much of TK1 protein exists as inactive TK1 complexes of lower molecular weight.

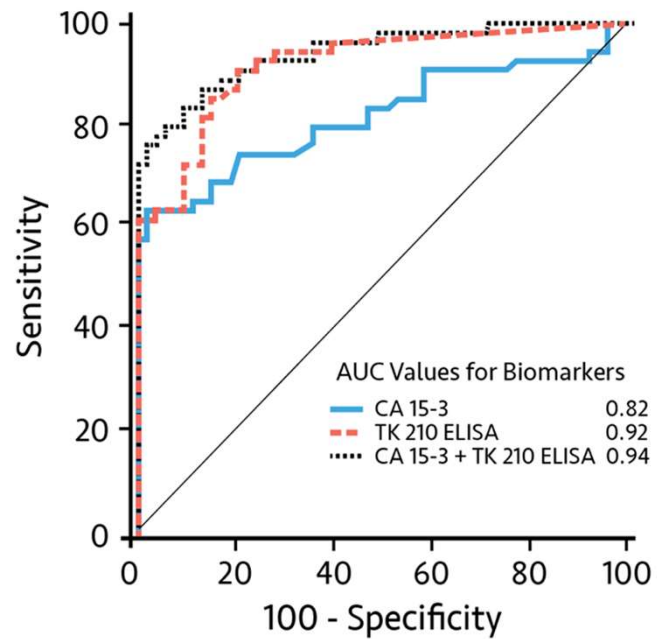


SERUM TK 210 CONCENTRATION / TK1 ENZYME ACTIVITY IN BREAST CANCER



TK 210 ELISA is twice as sensitive

COMBINING WITH OTHER TUMOR BIOMARKERS



TK 210 ELISA and CA15-3 in Breast Cancer (T2) Patients

After Kumar et al.
Tumor Biology 2016



AROCELL TK 210 ELISA

Solid tumors

Improved Sensitivity Compared with
TK1 Activity Assays

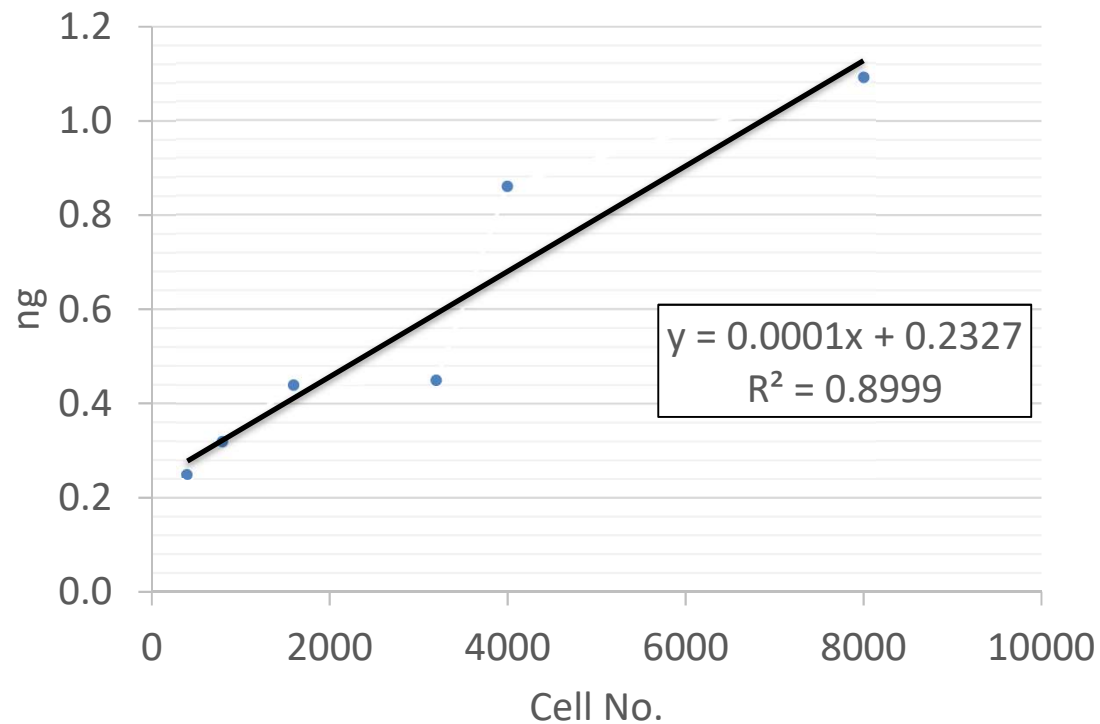
Improved Discrimination between Healthy and
Subjects with Malignancies

Positive Synergy with other Biomarkers

THYMIDINE KINASE 1 IN DRUG DISCOVERY: AROCCELL TK 210 ELISA



TK1 / TK210 IN CELL* CULTURE EXTRACT



*CCRF-CEM,
Acute
Lymphoblastic
Leukemia cell line

TK 1 / TK 210 concentration is linear with cell number

AROCELL TK 210 ELISA

In-vitro Studies

Translational Biomarker

Study of Anti-Proliferative Effects *In-Vitro*

AROCELL TK 210 ELISA

Drug Discovery:

CDK 4/6 inhibitors,
Anti-estrogen therapies

Directly suppress TK1

AROCCELL TK 210 ELISA



A UNIQUE BIOMARKER FOR CELLULAR PROLIFERATION

Assay Procedure



AROCCELL TK 210 ELISA



- *Standard ELISA procedure*
 - *Unique sample preparation*
- *Open system*
- *CE marked*



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TK1 STABILITY IN SERUM



Storage	Time
Room temperature	24 hours
+4° C	5 days
-20° C	2 months
-80° C	Long term storage

AROCCELL TK 210 ELISA

RECENT PUBLICATION



Tumor Biol.
DOI 10.1007/s13277-016-5024-z



ORIGINAL ARTICLE

A clinical evaluation of the TK 210 ELISA in sera from breast cancer patients demonstrates high sensitivity and specificity in all stages of disease

J. Kiran Kumar^{1,2} • A. C. Aronsson² • G. Pilko³ • M. Zupan⁴ • K. Kumer⁵ • T. Fabjan⁵ •
J. Osredkar⁵ • S. Eriksson^{1,2}

THYMIDINE KINASE 1 IN THE STUDY OF MALIGNACIES

CONCLUSIONS

- *TK1 is a biomarker of cell proliferation*
Forward looking biomarker
- *Serum levels reflect disease activity*
 - *Patient / subject classification*
 - *Prognoses*
 - *Monitoring disease progression.*

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THYMIDINE KINASE 1 IN THE STUDY OF MALIGNACIES

CONCLUSIONS

- *TK1 is a biomarker of cell proliferation*
Forward looking biomarker
- *Can be used with many malignancies*
- *Positive synergy with other biomarkers*
- *Translational biomarker*



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AROCCELL TK 210 ELISA



*A NOVEL BIOMARKER OF CELL
PROLIFERATION*

MEASURES WHAT REALLY MATTERS

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www.arocell.com



AROCELL TK 210 ELISA



- *Expands and Simplifies the Study of Cellular Proliferation*
- *New Opportunities in Cancer Research*

OPEN FOR DISCUSSION

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