TK 210™ ELISA has potential for monitoring of patients suffering from hematological malignancies

Eriksson S., Jagarlamudi K. K., AroCell AB

Hamberg Levedahl K., Höglund M., Venge P.

Uppsala University Hospital, Dep. of Medical Sciences, Uppsala, Sweden

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   – for monitoring during drug therapy of patients suffering from DLBCL.

   – for determination of overall survival rate of patients suffering from DLBCL.

Note: DLBCL = Diffuse Large B-cell Lymphoma

Disclosure of conflict of interest

Staffan Eriksson is a founder, board member and consultant to AroCell AB, Uppsala, Sweden.

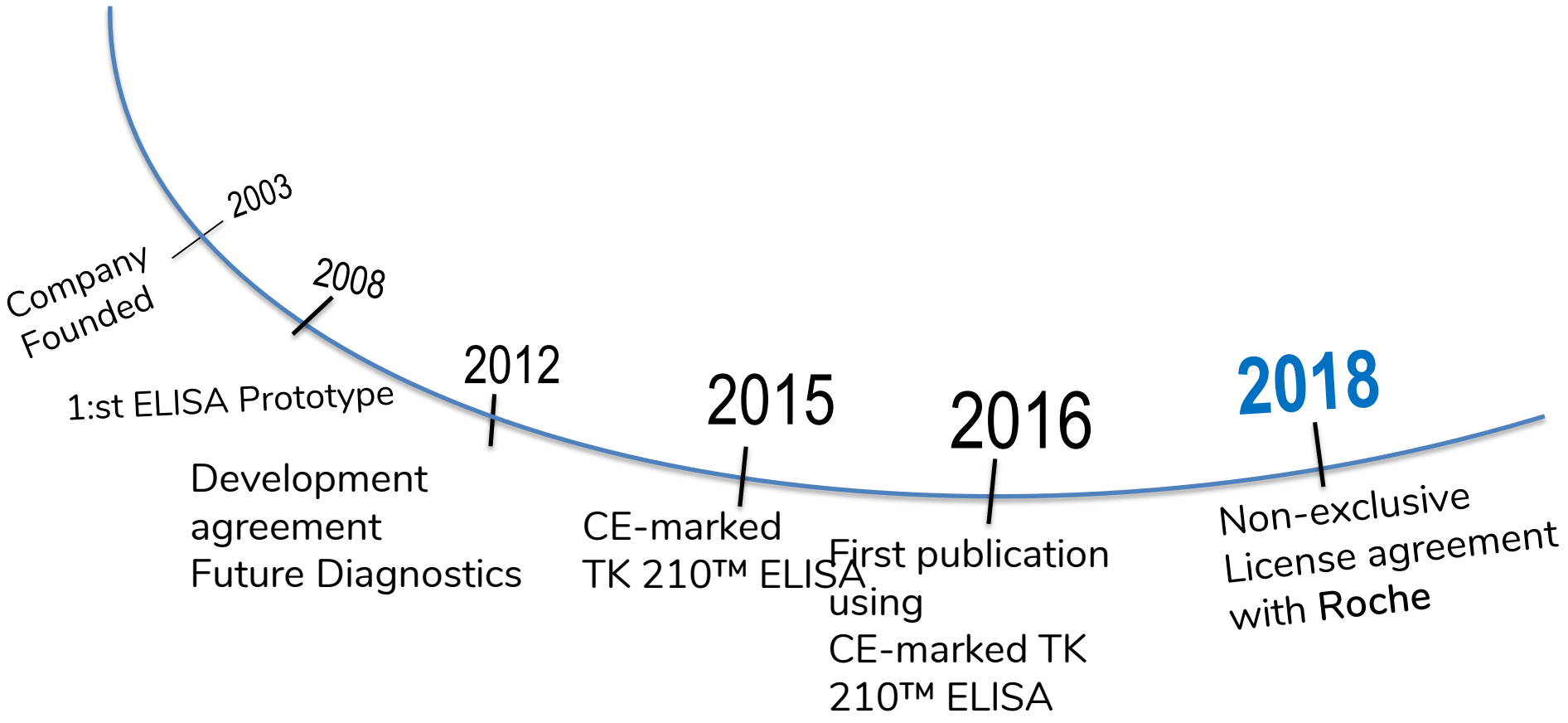
AroCell AB

A Swedish *in vitro* diagnostics company involved in the development, manufacturing, and sale of products for the measurement of human Thymidine Kinase 1 (TK1) in blood.

Clinical focus is hematological malignancies, breast cancer and other solid tumors.

AroCell's proprietary product, TK 210™ ELISA, is CE-marked and designed for simple, robust, reproducible and scalable concentration measurements of human thymidine kinase 1 in blood and cell culture.

Company history



TK1 can be present in the blood as a stable aggregate and is derived from dividing and disrupted cells

To determine the amounts of TK leaked to the blood, TK activity assays have been developed and used for many years.

Serum TK1 is a biomarker for studying increased cell proliferation and cell disruption.

Clinical significance of TK1 is in hematological malignancies such as leukemia, lymphoma and multiple myeloma, in breast cancer and in some additional solid tumor diseases.

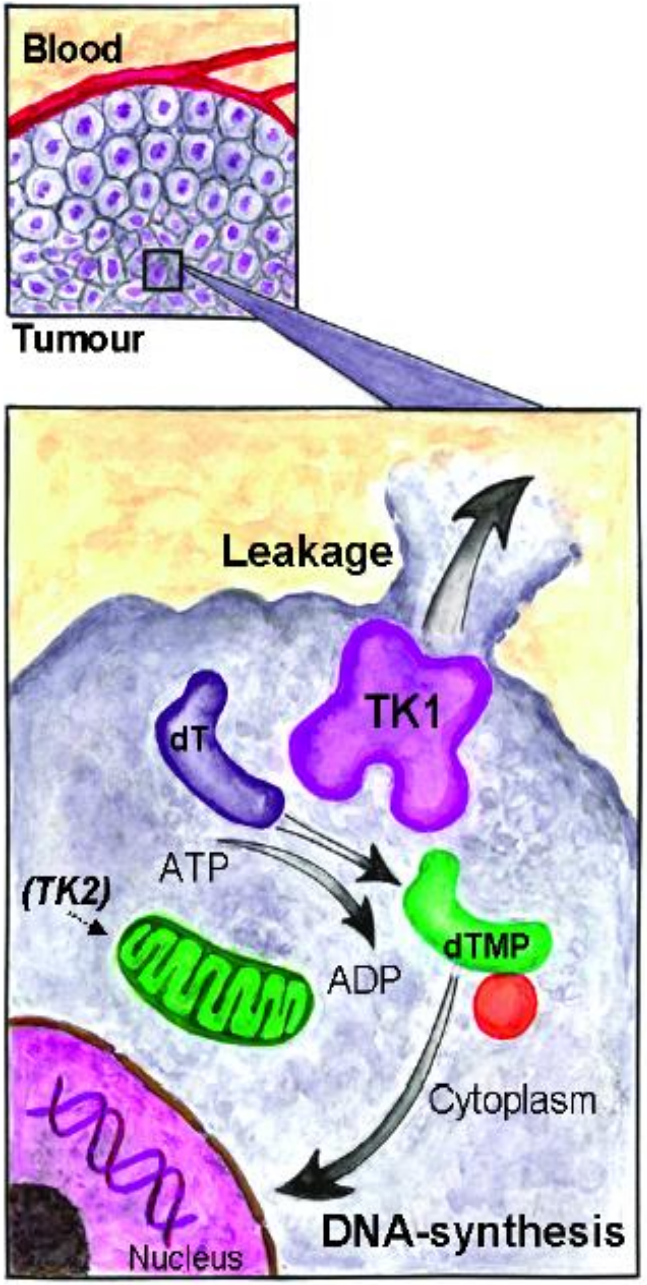


Fig 1

Note: Illustration from H. von Euler, R. Einarsson, U. Olsson, A-S Lagerstedt and S Eriksson 2003

TK1 assays for research and *in vitro* diagnostics

Assay	Comments
Prolifigen TK-REA	An activity assay based on ^{125}I-dUrd phosphorylation, sold by DiaSorin Inc., and Immunotech. *Ref. Gronowitz, J. S. et al., 1983.*
LIAISON™ Thymidine kinase	A non-radiometric activity assay based on AzidoThd phosphorylation, sold by DiaSorin Inc. *Ref. Öhrvik, A. et al., 2004.*
DiviTum	A TK1 activity assay based on series of phosphorylations of BrdUrd leading to its incorporation and detection in oligonucleotides. Provided by Biovica AB, Uppsala. *Ref. Gronowitz, J. S. et al., 2010.*
^{3}H-Thd phosphorylation	A classic research assay optimized for serum TK determinations. *Ref. Sharif H. et al., 2012.*
TK immunoassays	A dot blot ECL assay based on a polyclonal antibodies, sold by SSTK Biotech, China. *Ref. He Q. et al., 2004.*
TK 210 ELISA	A simple, robust and scalable sandwich ELISA based on two specific monoclonal antibodies, sold by AroCell AB, Sweden. CE-marked. *Ref. Jagarlamudi K. K. et al., 2016.*

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Potential clinical use for TK 210 as biomarker

Primary care/ Health center

Routine screening

Local hospital or cancer institute to undergo tests

Cancer not diagnosed

Monitoring of progession and/or regression of disease

Diagnosis of cancer

Monitoring of relapse of disease

Treatment

Palliative care

End of treatment

Continuous treatment

Long-term monitoring and follow-up

Relaps

Terminal care

Cure

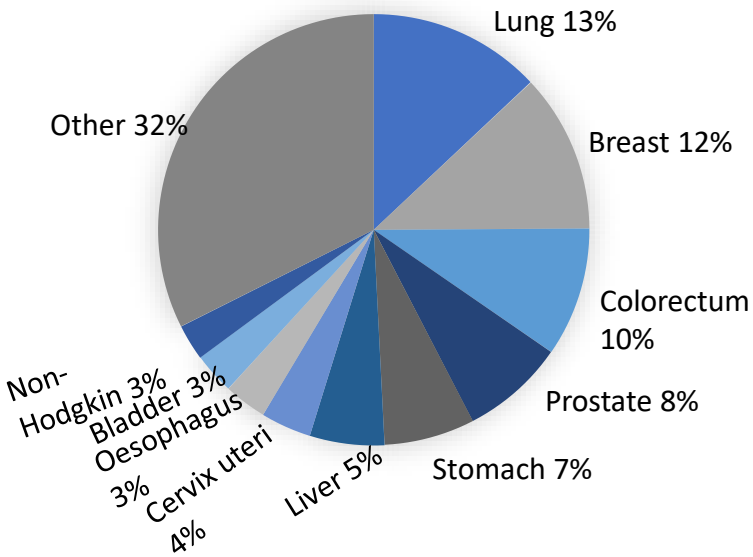
Long term survival

Ref: Fitch, 2003. From: Healthcare Quarterly, 8(3) May 2005.doi:10.12927/hcq.2005.17194

Epidemiology - seven cancers

Cancer	Incidences ('000)	3-y Preval. ('000)	5-y Preval. ('000)
Lung Cancer	1 824	1 450	1 893
Breast Cancer	1 670	4 020	6 232
Colorectal cancer	1 360	2 409	3 543
Prostate cancer	1 094	2 581	3 857
Hematological Cancers	844	1 203	1 751
Cervical cancer	524	1 041	1 547
Sarcoma	260	N/A	630

Global Cancer Incidence share, By Cancer Type (2012)



- AroCell has focused its clinical research efforts targeting breast cancer, prostate cancer, hematological malignancies and Sarcoma.

Note: Incidence = Number of new cases per year, Prevalence = Number of people alive of previously diagnosed cases, after 3 years and 5 years respectively.
Source: Global Cancer Biomarkers Market 2018-2015; DataM Intelligence, ***2018.***

Examples of drugs and drug candidates

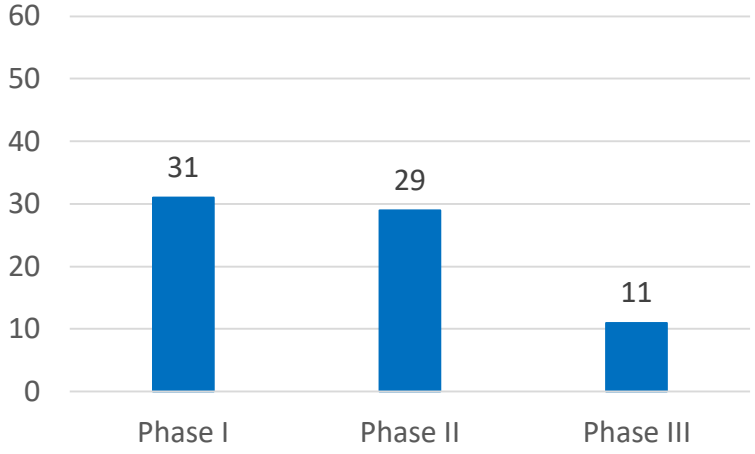
Need for patient monitoring

Prostate cancer drugs: marketed and R&D pipelines

Marketed drugs; testosterone reducing:

- Zytiga® ($10,691/month, 24m), Yonsa® ($9,623/month)
- Xtandi® ($11,394/month , 8m), Erleada®($11,409/month)
- Other marketed drugs: Keytruda®, Lynparza®, Rubraca®, NanoPac®, Opdivo®, Provenge, Tecentriq®

R&D pipeline in the US (# of projects):

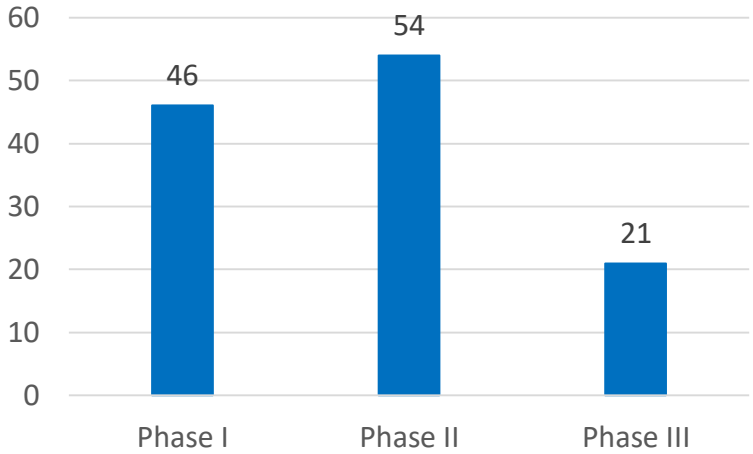


Breast cancer drugs: marketed and R&D pipeline

Marketed drugs; CDK inhibitors:

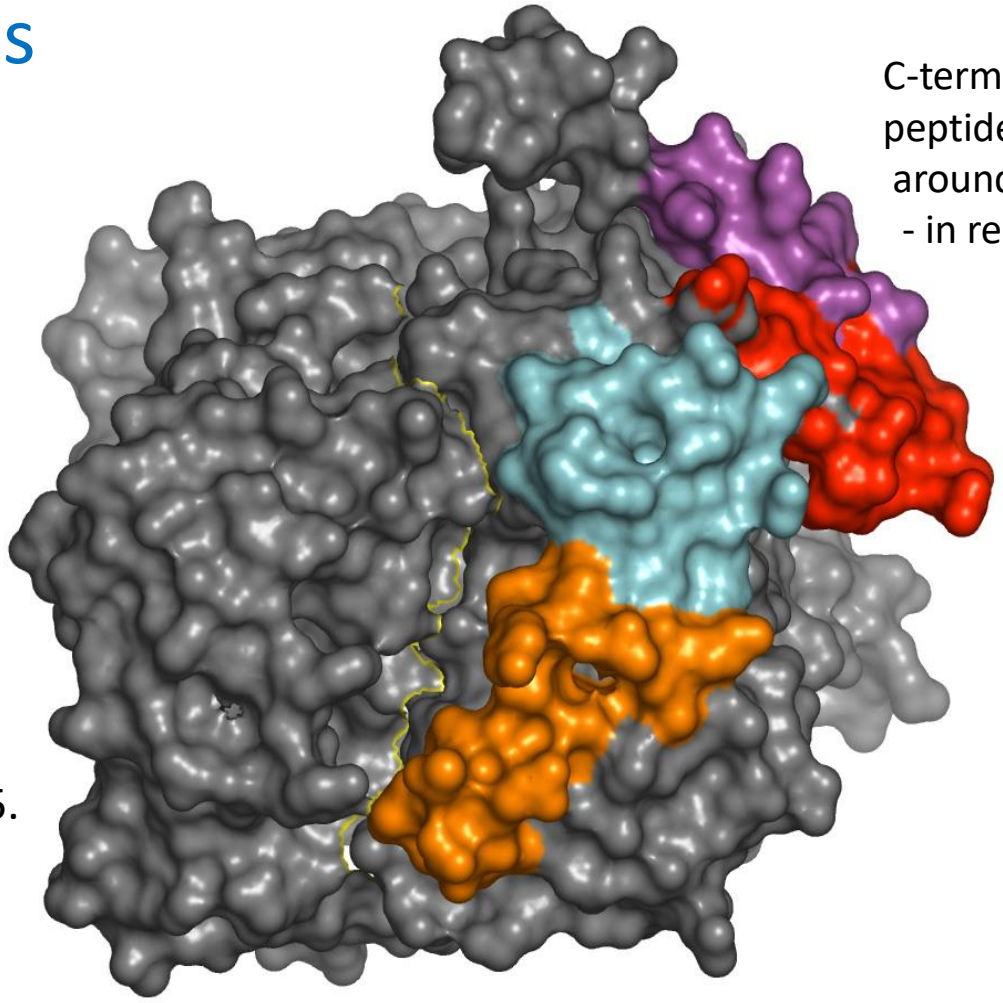
- Ibrance (Pfizer) → ($11,797/ treatment – 28 days 14m)
- Kisqali® (Novartis) → ($12,490/ treatment – 28 days)
- Verzenio™ (Eli Lilly) → ($11,812/ 28 days, 12m)

R&D pipeline in the US (# of projects):



Source: phrma.org., 2018

TK-210 ELISA is based on monoclonal anti-TK1 peptide antibodies



C-terminal peptides around aa 210 - in red

Peptides in the C-term TK1 region can give antibodies that reactive with serum TK1 (He Q et al, 1996). Two Anti XPA 210 Mabs are used by AroCell AB to produce the **TK 210™ ELISA**, sandwich assay.
CE- marked as of October 2015.

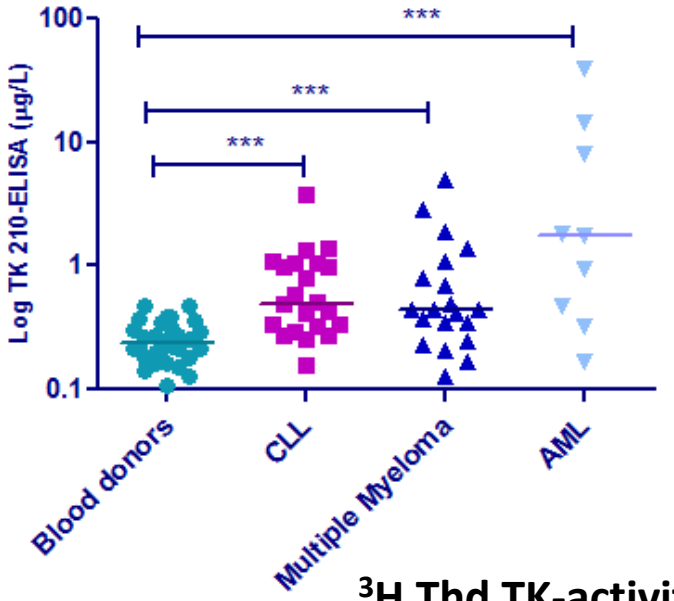
Ref. Welin M, et al. PNAS 102, 17970, 2004.

Comparison of three assays

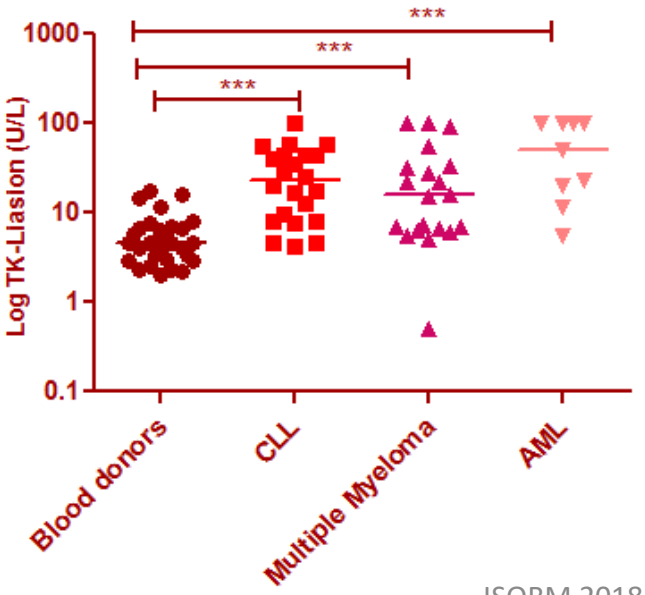
Assay comparison using samples from:

- Blood donors (N=30)
- Chronic Lymphatic Leukemia (N=22)
- Multiple Myeloma (N=20)
- Acute Myeloid Leukemia (N=9)

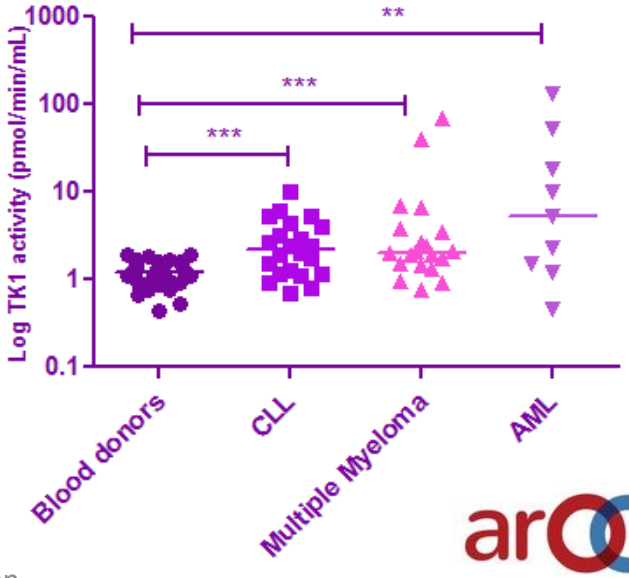
TK 210™ ELISA



LIAISON™ Thymidine kinase

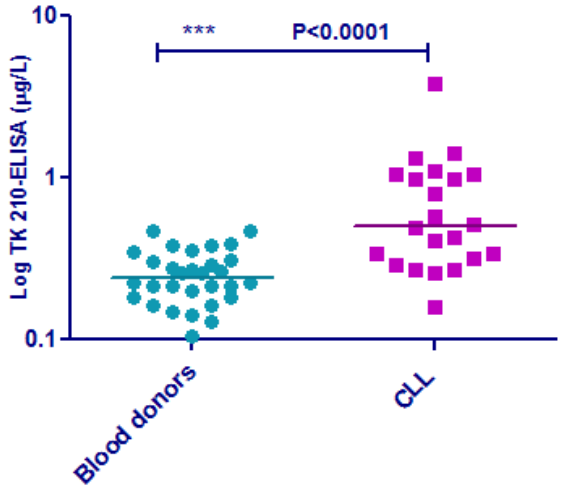


^{3}H Thd TK-activity

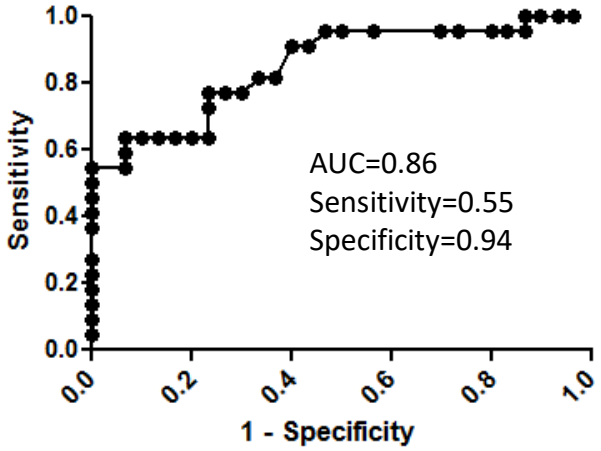


TK1 conc. in Chronic Lymphocytic Leukemia (N=22)

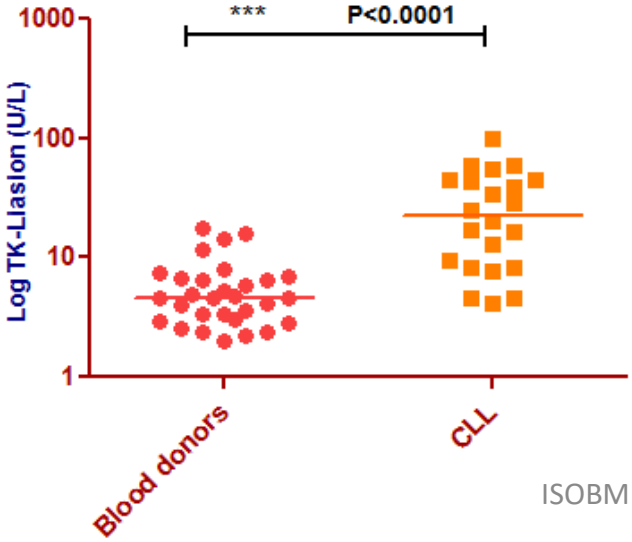
TK 210™ ELISA

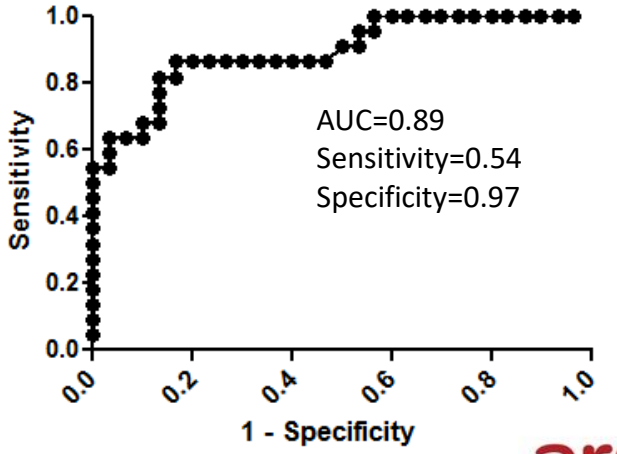


ROC curve analysis



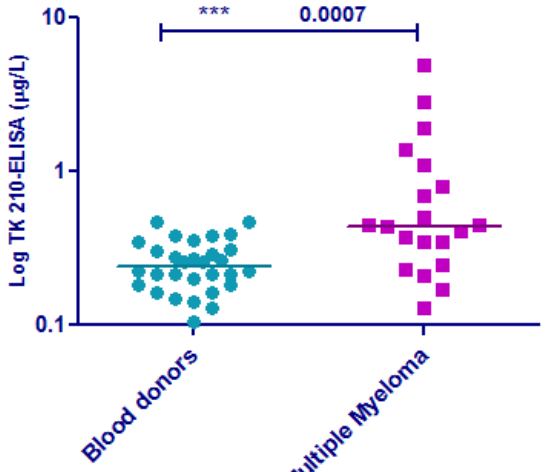
LIAISON™ Thymidine kinase



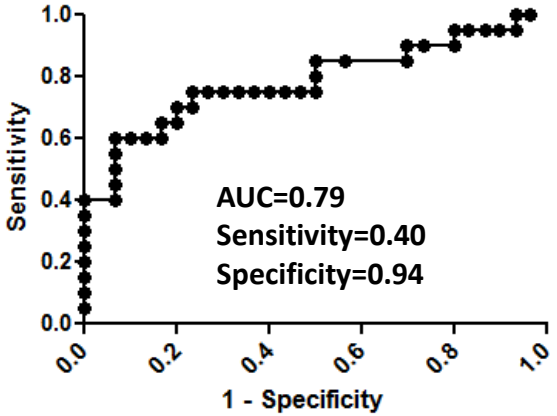


TK1 concentrations in sera from patients suffering from Multiple Myeloma (N=20)

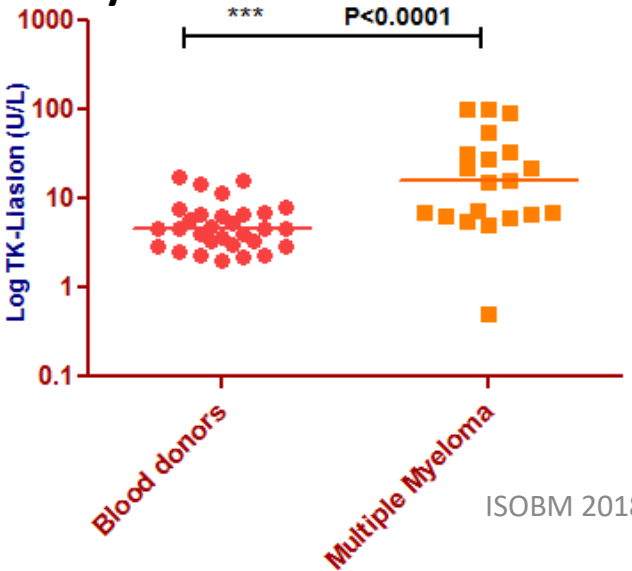
TK 210™ ELISA

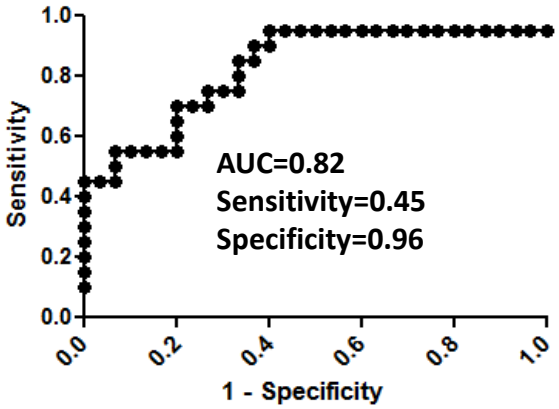


ROC curve analysis



LIAISON™ Thymidine kinase





The correlation between the different TK assays

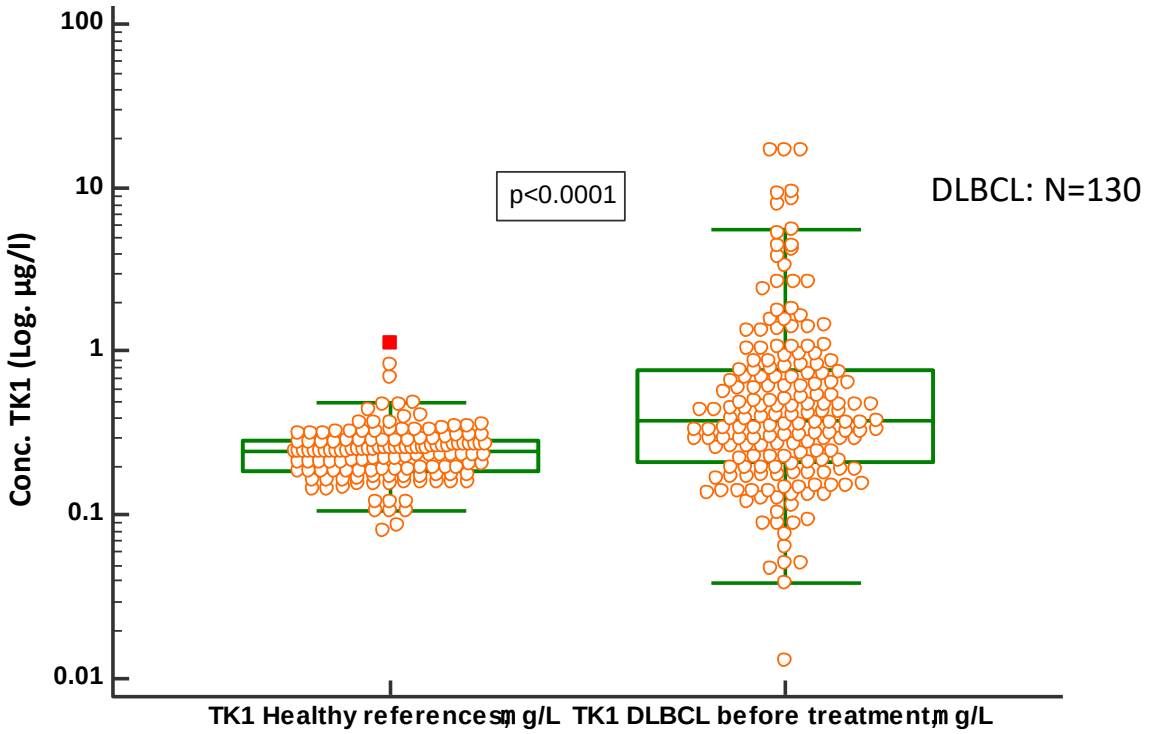
Assays	Patient group		
	CLL (N=22)	MM (N=20	AML (N=9)
TK 210 ELISA vs LIAISON TK	rs=0.94 P<0.0001	rs=0.68 P<0.0001	rs=0.92 P=0.0002
TK 210 ELISA vs ^{3}HThd phos.	rs=0.88 P<0.0001	rs=0.88 P<0.0001	rs=0.98 P<0.0001
LIAISON TK vs ^{3}HThd phos.	rs=0.85 P<0.0001	rs=0.69 P<0.0001	rs=0.95 P=0.0002

Summary of the method comparison

Patient group	No of samples	LIAISON™ Thymidine kinase	TK 210™ ELISA	^{3}HThd assay
		Cut-off value: >17 U/L)	Cut-off value: >0.47 ng/mL)	Cut-off value: >3 pmol/min/mL)
CLL	22	13/22	12/22	12/22
Multiple Myeloma	20	9/20	8/20	7/20
AML	9	7/9	7/9	7/9

Conclusion: The three TK1 assays have similar analytical performance when analyzing serum samples from patients suffering from hematologic malignancies.

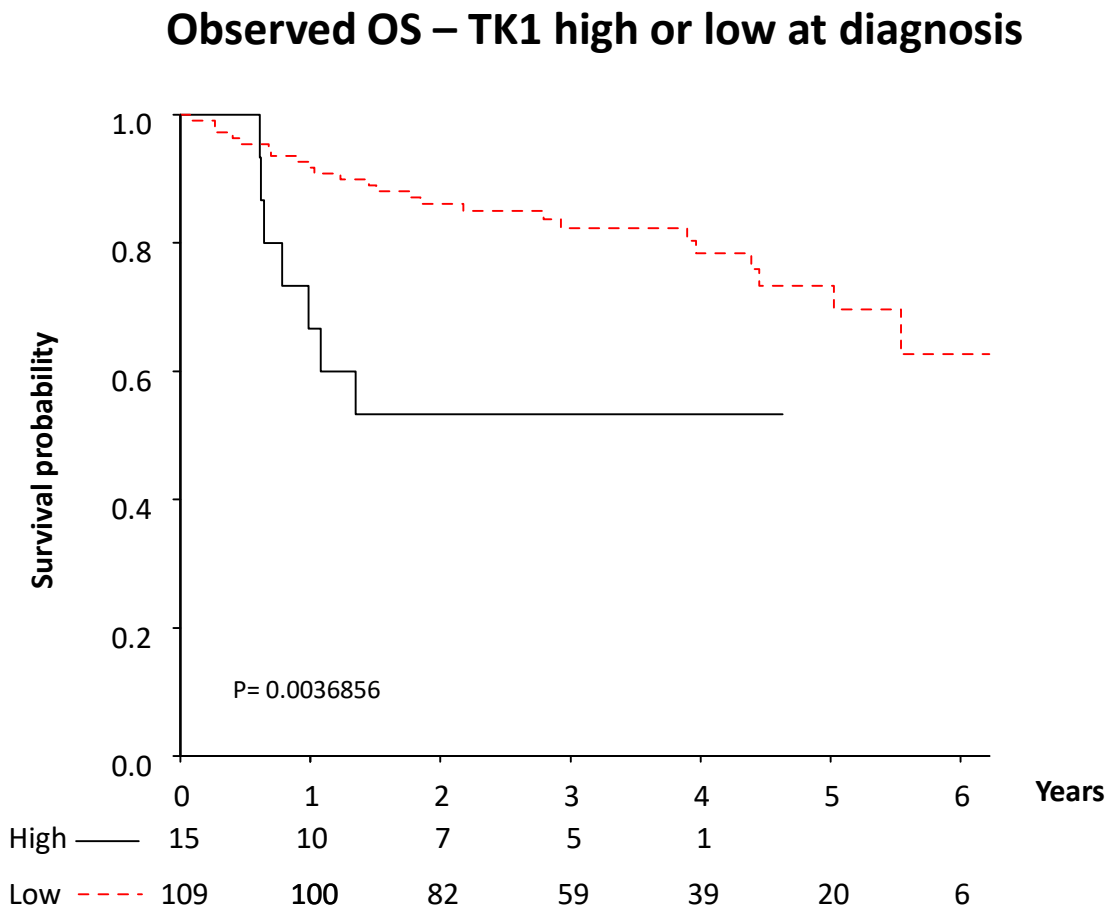
Preliminary results from UCAN study: TK1 conc. in patients suffering from DLBCL



Reference to and permission from: Gustaf Hedström, Per Venge, Gunilla Enblad, Uppsala University, Dept Medical Sciences, Akademiska Hospital, Uppsala, Sweden.

Note: DLBCL = Diffuse Large B-cell Lymphoma

TK1 conc. >1.5 µg/l is prognostic for short overall survival of DLBCL patients



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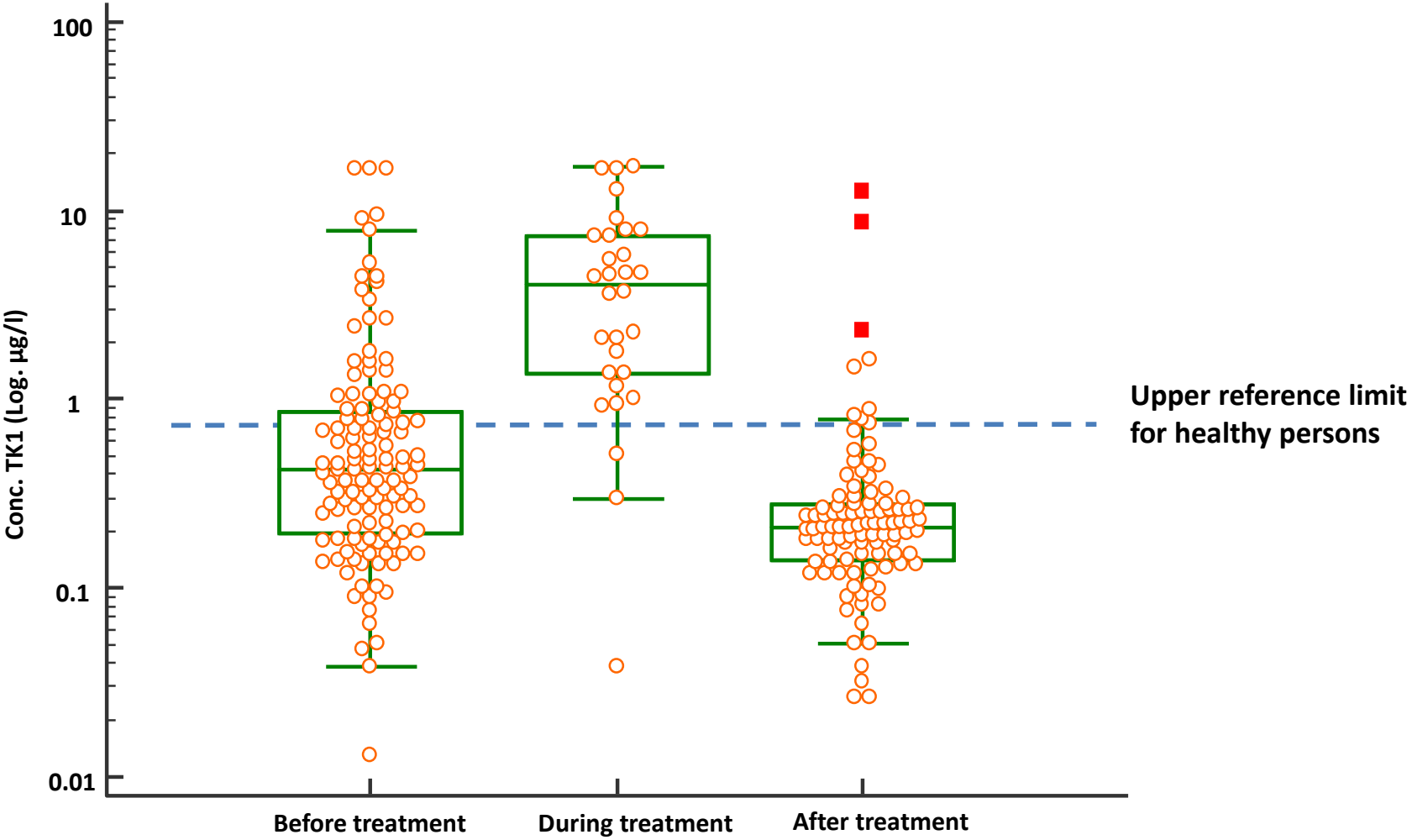
Patient monitoring of DLBCL during treatment

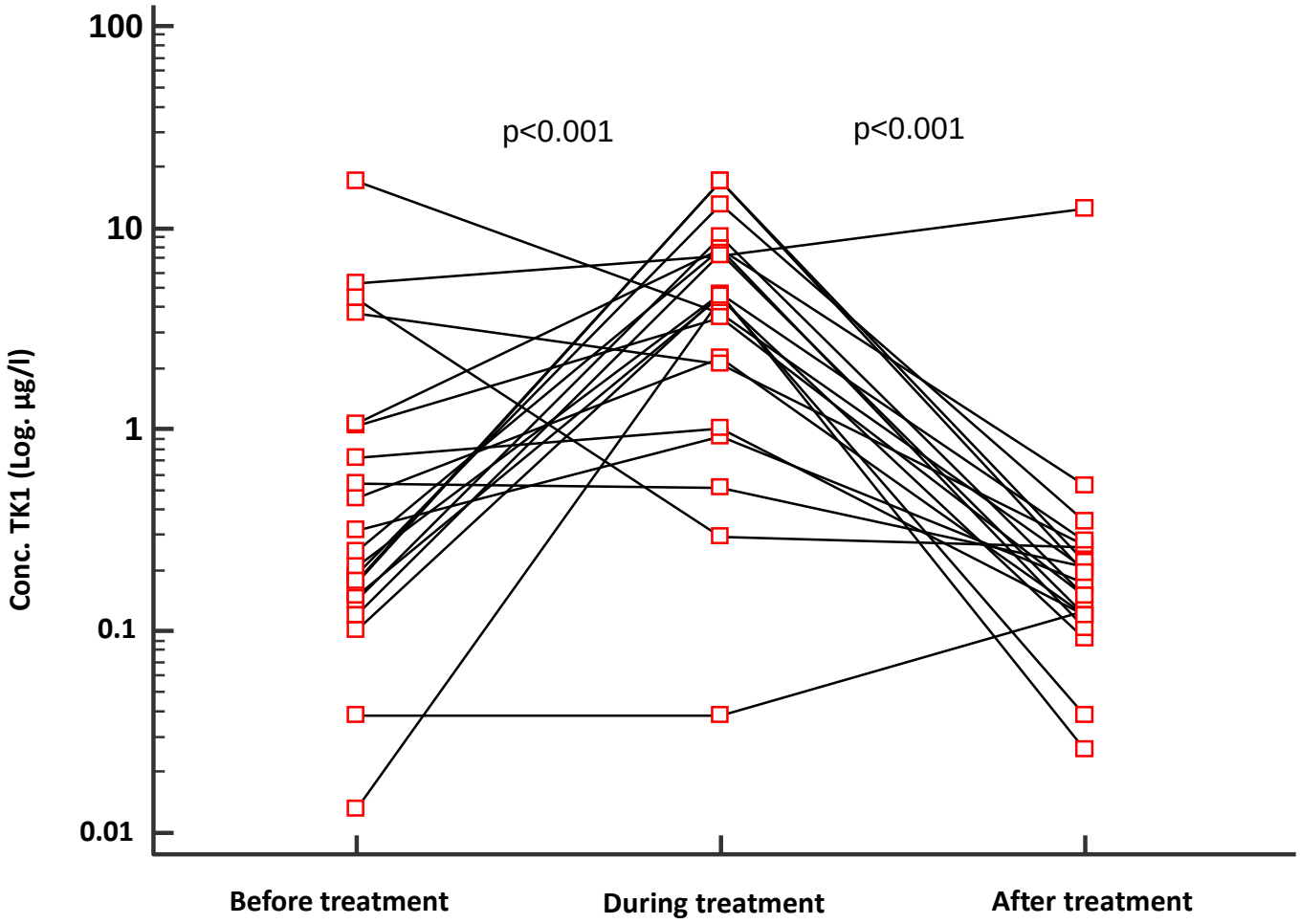
TK1 conc. in DLBCL patients during (6 courses) rituximab + CHOP therapy



Reference to and permission from: Gustaf Hedström, Per Venge, Gunilla Enblad, Uppsala University, Dept Medical Sciences, Akademiska Hospital, Uppsala, Sweden.

Patient monitoring of DLBCL during treatment

TK1 conc. in DLBCL in individual patients during CHOP+rituximab



Reference to and permission from: Gustaf Hedström, Per Venge, Gunilla Enblad, Uppsala University, Dept Medical Sciences, Akademiska Hospital, Uppsala, Sweden.

The first TK 210™ ELISA publication in 2016

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

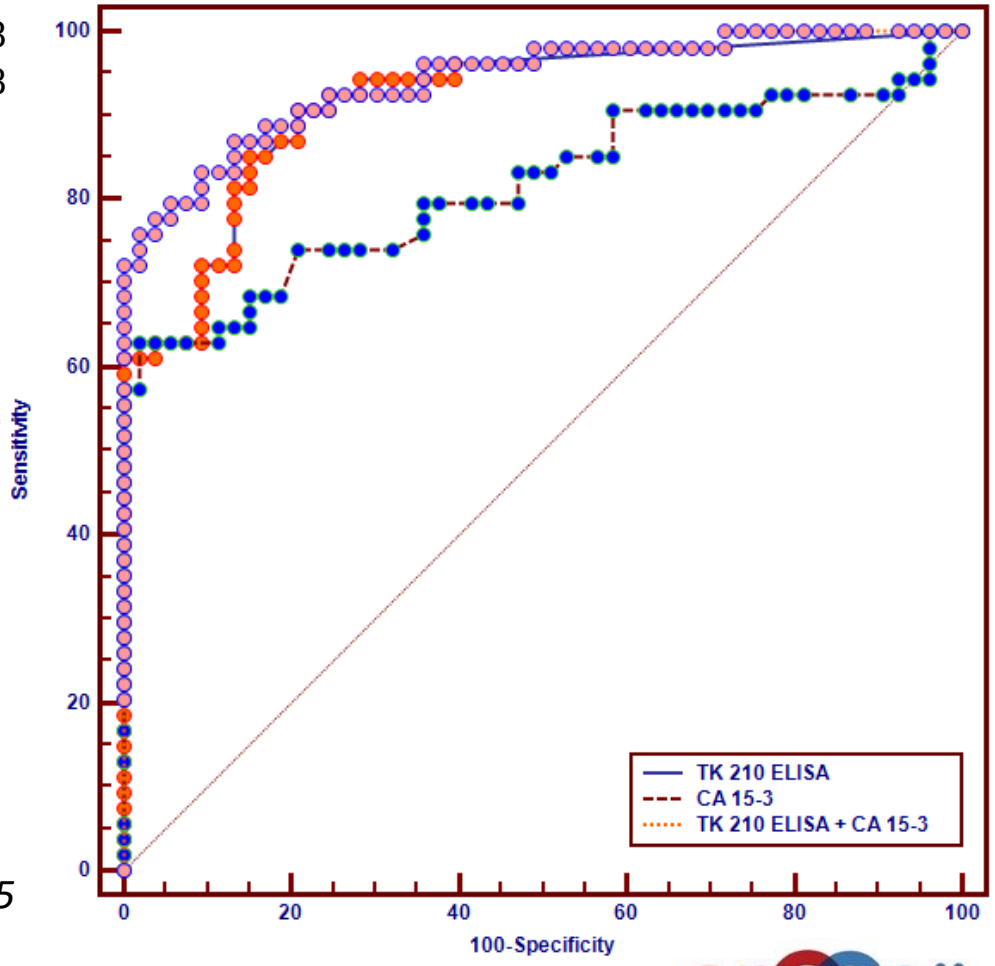
J. Kiran Kumar[1,2] · A. C. Aronsson[2] · G. Pilko[3] · M. Zupan[4] · K. Kumer[5] · T. Fabjan[5] · J. Osredkar[5] · S. Eriksson[1,2]

TK 210™ ELISA and CA15-3

Comparison and the combination

Assay	AUC	Sensitivity	Specificity
TK 210 ELISA	0.92	0.61	0.98
CA 15-3	0.82	0.63	0.98
TK 210 ELISA + CA 15-3	0.94	0.76	0.98

Healthy (N=53)
T2 breast cancer patients (N=54)



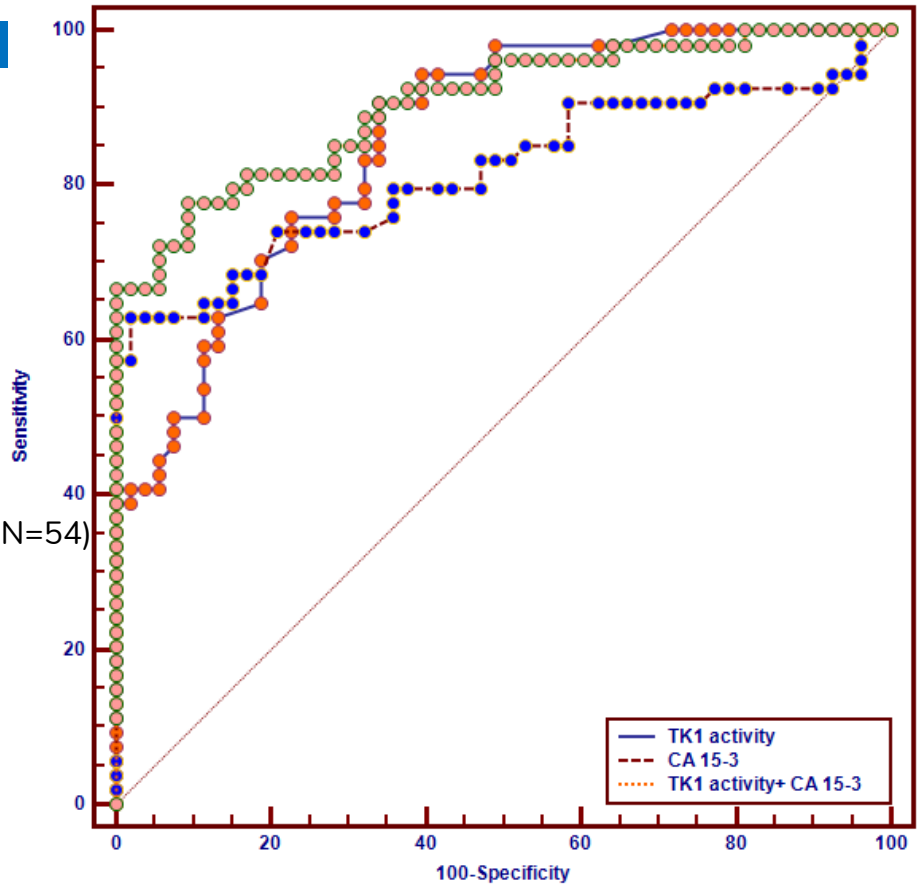
Ref: Tumor Biology 2016 , 37:11937-11945

^{3}H-Thd TK1 activity and CA15-3

Comparison and the combination

Assay	AUC	Sensitivity	Specificity
TK1 activity assay	0.86	0.40	0.98
CA 15-3	0.82	0.63	0.98
TK1 activity + CA 15-3	0.91	0.66	0.98

Healthy (N=53)
T2 breast cancer patients (N=54)



Conclusion: TK 210 in breast cancer shows higher sensitivity than TK activity both alone and in combination with CA 15-3.

Ref. Jagarlamudi, K. K. et al., unpublished.

Summary

- TK 210™ ELISA shows high analytical performance and similar to commercially available activity-based methods.
- TK 210 ™ ELISA is easy-to-use, reproducible, robust and scalable on standard hardware platforms commonly used in clinical laboratories.
- Preliminary results suggest that TK210 ELISA may be useful monitoring the effect of therapy of DLBCL patients.
- Preliminary results suggest that TK210 ELISA may be useful for determination of overall survival rate of DLBCL patients.
- Based on results earlier published (Tumor Biology, 2016), TK 210 ™ ELISA has the potential to be a valuable biomarker in the management of breast cancer.