Received: 3 January 2020 | Revised: 18 March 2020 | Accepted: 19 March 2020

DOI: 10.1111/ejh.13419

ORIGINAL ARTICLE

European Journal of Haematology WILEY

Thioredoxin-1 as a biological predictive marker for selecting diffuse large B-cell lymphoma patients for etoposide-containing treatment

Esa Jarkko Mikael Kari[1] | Milla Elvi Linnea Kuusisto[1] | Päivi Honkavaara[1] | Anna Hakalahti[1] | Kirsi-Maria Haapasaari[2] | Risto Bloigu[3] | Peeter Karihtala[1,4] | Hanna-Riikka Teppo[2] | Risto Pirinen[5] | Taina Turpeenniemi-Hujanen[1,4] | Outi Kuittinen[6]

[1]Department of Oncology and Radiotherapy, Medical Research Center, University of Oulu, Oulu University Hospital, Oulu, Finland

[2]Department of Pathology, University of Oulu and Oulu University Hospital, Oulu, Finland

[3]Medical Informatics and Statistics Research Group, University of Oulu, Oulu, Finland

[4]Cancer and Translational Medicine Research Unit, University of Oulu, Oulu, Finland

[5]Department of Pathology, North Karelia Central Hospital, Joensuu, Finland

[6]Department of Oncology, Faculty of Health Medicine, Institute of Clinical Medicine, Kuopio University Hospital, University of Eastern Finland, Kuopio, Finland

Correspondence
Esa Jarkko Mikael Kari, Tuumoribiologinen tutkimuslaboratorio / OYS, Kajaanintie 50, 90029 OYS, Finland.
Email: esa.kari@student.oulu.fi

Funding information
This study was supported by research funding provided by the Cancer Society of Northern Finland to Kuusisto, the Finnish Medical Association Duodecim to Karihtala and Kuittinen and the Finnish Medical Foundation to Kuusisto. Teppo would like to acknowledge the financial support from the Finnish Medical Foundation, the Finnish medical society Duodecim, the Thelma Mäkikyrö foundation and the Finnish Society for Oncology. Kari and Turpeenniemi-Hujanen would like to acknowledge the financial support provided by the Väisänen Fund in Terttu Foundation.

Abstract

Objective: In diffuse large B-cell lymphoma (DLBCL), there is an unmet medical need to select patients who would benefit from intensified frontline treatments such as adding etoposide to an R-CHOP regimen.

Methods: The present work included a retrospective clinical analysis of two patient cohorts and an in vitro study. Primary biopsy samples from DLBCL patients treated with an etoposide-containing high-dose regimen (n = 37) and etoposide-containing frontline treatment (n = 69, R-CHOEP) were studied using immunohistochemical thioredoxin-1 (Trx1) staining. Two DLBCL cell lines expressing Trx1 were cultured, and their expression was silenced using the small interfering RNA knockdown technique. Chemoresistance was tested with doxorubicin, etoposide, vincristine, prednisolone and carboplatin.

Results: Thioredoxin-1 knockdown sensitised DLBCL cells to doxorubicin ($P < .0001$) but decreased etoposide-induced cell death ($P < .00001$). In DLBCL patients who received etoposide-containing frontline treatment, low cytoplasmic Trx1 expression was associated with inferior 5-year overall survival (46% vs 76%, $P = .026$) and disease-specific survival (68% vs 90%, $P = .026$).

Conclusions: Strong Trx1 expression appears to increase drug resistance to doxorubicin but sensitises cells to etoposide. This implies that Trx1 expression might be the first predictive biological marker to select the patients who might benefit from adding etoposide to R-CHOP immunochemotherapy.

Esa Jarkko Mikael Kari and Milla Elvi Linnea Kuusisto equally contributed.

KEYWORDS
diffuse large B-cell lymphoma, doxorubicin, etoposide, R-CHOEP, thioredoxin-1

1 | INTRODUCTION

Mature B-cell neoplasms are the fifth most common cancers in men and the seventh most common in women, comprising 5.4% of all cancers in Finland.[1] Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma subtype. It is an aggressive subtype of non-Hodgkin lymphoma with a potentially fatal outcome.[2] The standard care of DLBCL is immunochemotherapy, with the R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) or R-CHOP combined with etoposide (R-CHOEP). During the pre-rituximab era, CHOEP was shown to be superior to CHOP in young high-risk patients. There are no randomised studies evaluating its impact after adding rituximab to the therapy and its exact role in the therapy calls for further definition.[3] There are currently no biological predictive factors in clinical use for selecting patients for R-CHOEP therapy. In a retrospective work based on immunohistochemistry, we previously showed that thioredoxin-1 (Trx1) is associated with an adverse outcome in DLBCL.[4] However, this does not prove causality because of the retrospective study setting.

Trx1 plays a major role in the antioxidant system and has been implicated in chemoresistance to cisplatin, docetaxel and anthracyclines. The exact role of Trx1 in cancer cells is a matter of debate.[5,6] Previous work by Li et al[6] suggested that Trx1 is involved in the development of chemoresistance against doxorubicin, which is widely used in front-line therapy for DLBCL. It has also been suggested[7] that knockdown of Trx1 might sensitise the cells to the influence of reactive oxygen species (ROS) and thereby to DNA damage and eventually cell death. ROS play an important role in cell proliferation, differentiation and angiogenesis, and this might give a survival advantage to tumour cells.[7,8]

The aim of the present work was to study the causal relationship between high Trx1 expression and adverse disease outcome with different treatment regimens. We also wanted to explore the influence of Trx1 to several chemotherapeutics' cytotoxicity by using an in vitro knockdown model. Our hypothesis was that Trx1 might influence the development of chemoresistance in DLBCL.

2 | MATERIALS AND METHODS

See Appendix S1.

3 | RESULTS

3.1 | Knockdown of Trx1 in cultured cells

In the Western blot analysis, Trx1 knockdown was detected 48 hours post-transfection. It was, however, most obvious after 72 hours, when Trx1 expression was found to decrease by 95% compared to the expression seen in 24-hour samples. The result was verified using β-actin as loading control (Figure 1). Negative controls were used as comparison. At 48 hours, Trx1 expression decreased by 47% in comparison with the negative control sample and Trx1 knockdown sample. In the 72-hour samples, the decrease was 91%.

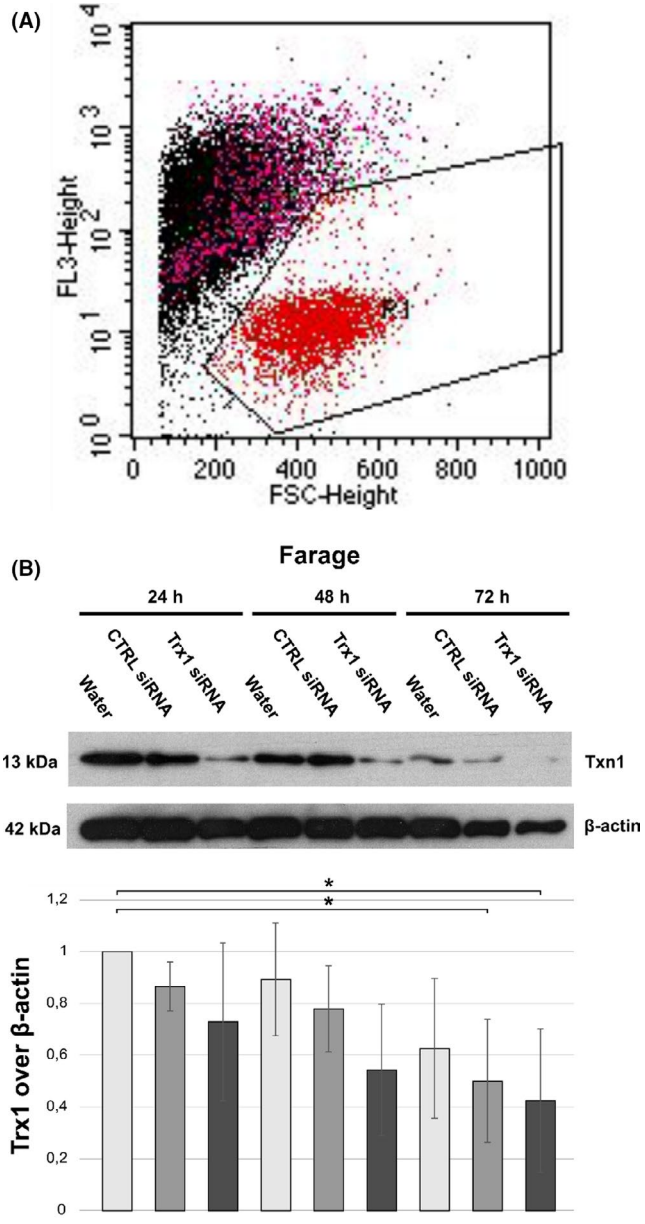

FIGURE 1 A, Transfection success was verified by using fluorescence-activated cell sorting. *Y*-axis represents the number of cells and *x*-axis represents wavelength (534-545 nm for green). Red colour indicates live cells. Approximately 21% of cells (range 8%-48%) survived transfection process. B, Western blot analysis of thioredoxin-1 knockdown in Farage cells. Cell lines with water and negative siRNA were used as control. Knockdown of thioredoxin-1 is observed during the next three post-transfection days. Chemotherapeutic agents were added at day 2 [Colour figure can be viewed at wileyonlinelibrary.com]

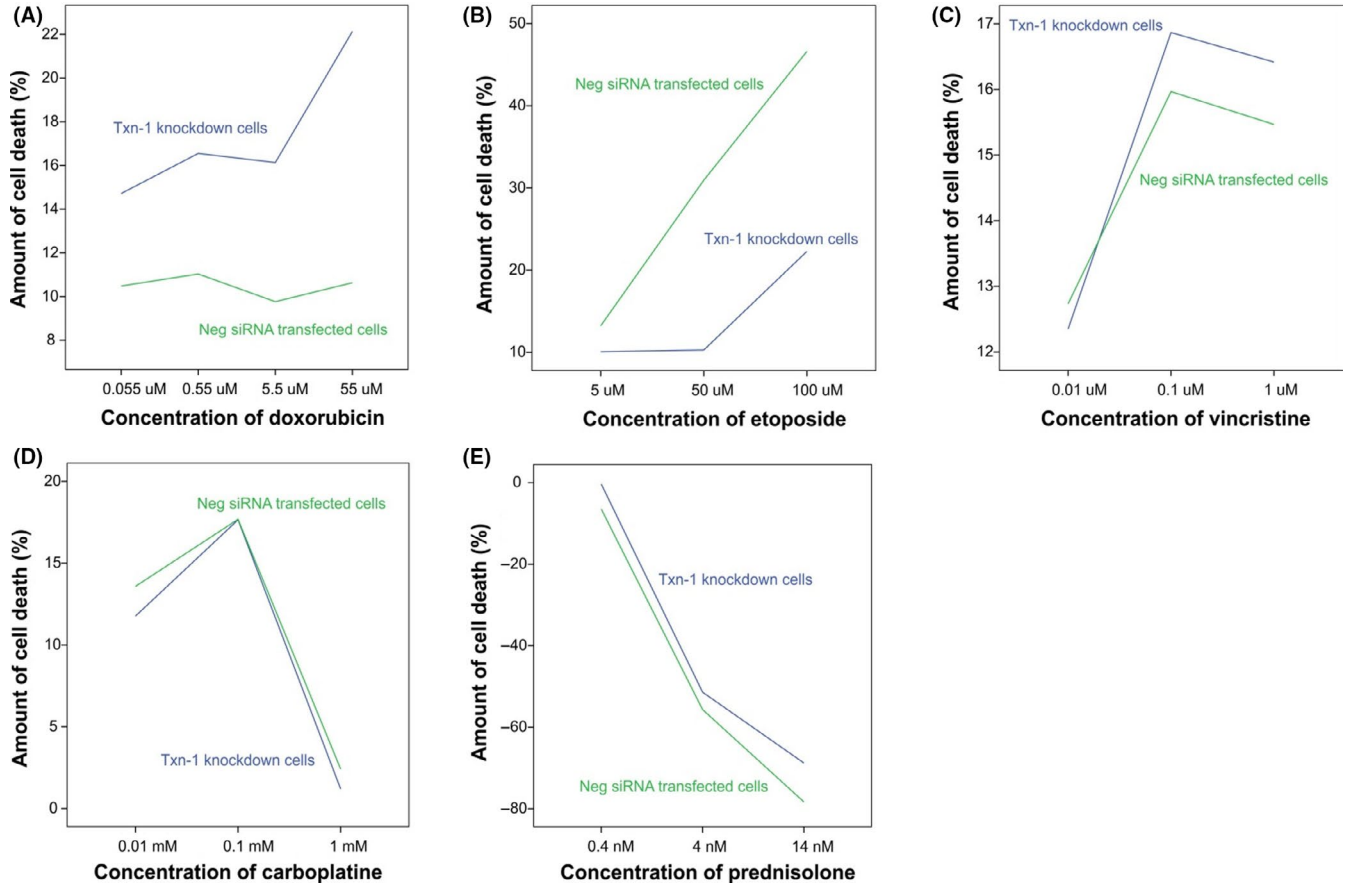

FIGURE 2 Different chemotherapeutic agents tested after thioredoxin-1 knockdown. Data represent mean values. *Y*-axis represents percentage of cell death, and *x*-axis represents agent concentration. Blue indicates knockdown cell line, and green indicates control cell line. Results differ significantly with doxorubicin and etoposide. A, Doxorubicin. B, Etoposide. C, Vincristine. D, Carboplatine. E, Prednisolone [Colour figure can be viewed at wileyonlinelibrary.com]

The success of the transfection was verified both in FACS assay and qRT-PCR. The amount of cells alive after the transfection was 21% in total (range: 8%-48%). Approximately 43% of the cells were successfully transfected (range: 20%-79%). The amount of live transfected cells was 75% (range: 43%-93%) (Figure 1). In qRT-PCR, the mean purity (260/280) was 2.00.

3.2 | Chemotherapeutic agent testing

Three tests with five parallel samples were conducted in each case and the results were calculated by defining the mean values and later comparing them with each other. The variance analysis was performed with ANOVA and, because of the multiple differences found with challenging analysis, the results were reassessed with the student *t* test (n = 900).

3.3 | Doxorubicin

Doxorubicin-induced cell death increased from 10.5% to 14.7% in 0.055 µmol/L solution when the Trx1 knockdown was performed. The same trend was also seen with higher concentrations of doxorubicin; 11.0%-16.6% in 0.55 µmol/L solution, 9.8%-16.0% in 5.5 µmol/L solution and 10.6%-22.1% in 55 µmol/L solution, respectively (Figure 2). The increased doxorubicin-induced cell death with Trx1 knockdown was statistically highly significant ($P = .000093$).

3.4 | Etoposide

Etoposide-induced cell death decreased from 13.2% to 10.1% in 5 µmol/L solution of etoposide when the Trx1 knockdown was performed. A similar effect was seen in more concentrated solutions: 31.0%-10.3% in 50 µmol/L solution and 46.6%-22.3% in 100 µmol/L solution (Figure 2B). The higher amount of cell death caused by etoposide in native cells was statistically highly significant ($P = .000004$).

3.5 | Vincristine, carboplatin and prednisolone

No effect in vincristine-, carboplatin- or prednisolone-induced cell death was seen when the native and knockdown cells were compared (Figure 2) ($P = .355$).

TABLE 1 Patient demographics with known clinical risk factors for diffuse large B-cell lymphoma

	n	%	Primary high IPI score	Primary refractory disease	Relapsed DLBCL	*P*-value	n	%	*P*-value[a]
Sex									
Male	24	65	8	3	13		42	67	
Female	13	35	1	1	11	.148	21	33	1.000
B symptoms									
None	7	19	1	0	6		20	32	
Yes	13	35	5	1	7	.428	38	60	.775
Missing	17	46					5	8	
Age									
≤60	24	65	6	4	14		57	90	
>60	13	35	3	0	10	.388	6	10	.010
LD levels									
Normal	6	16	1	0	5		7	11	
Elevated	15	41	6	1	8	.539	52	83	.272
Missing	16	43					4	6	
Stage									
I-II	3	8	0	0	3		4	6	
III-IV	28	76	9	2	17	.620	58	92	.673
Missing	6	16					1	2	
WHO performance status									
0-1	8	22	1	1	6		28	44	
>1	9	24	3	0	6	.576	29	46	1.000
Missing	20	54					4	10	
Extranodal affusion									
0-1	11	30	3	2	6		29	46	
>1	23	62	6	2	15	.761	30	48	.076
Missing	3	8					4	6	
IPI									
0-2	5	14	1	0	4		7	11	
3-5	20	54	6	2	12	1.000	55	87	.467
Missing	12	32					1	2	
DLBCL subtype									
GC	12	32	1	1	10		18	29	
non-GC	17	46	4	2	11		27	42	
THRLBCL[b]	8	22	4	1	3	.244	18	29	.737

Note: Median age of patient cohort I was 54 y, range: 21-67. Median age of patient cohort II was 54 y, range: 19-69. *P*-values represent Fisher's exact test. No statistical differences existed between demographic groups except for age groups in cohort II.

Abbreviations: DLBCL, diffuse large B-cell lymphoma; GC, germinal centre; IPI, international prognostic index; LD, lactate dehydrogenase; THRLBCL, T-cell histiocyte-rich large B-cell lymphoma.

[a]Difference between cohorts.

[b]Including one primary mediastinal B-cell lymphoma in cohort I.

3.6 | Expression of Trx1 in patient cohorts and its association with clinical outcome

Patient characteristics are presented in Table 1. In patient cohort I in all samples, the malignant cells generally expressed Trx1. In nine samples (24.3%), there were some malignant cells that did not express Trx1 at all (Figure 3) and in 28 cases all malignant cells expressed some amount of Trx1 (75.7%) (Figure 3). Trx1 positivity was detectable in the nuclei of malignant cells in 29 cases (78.4%) and in nuclear membranes in seven samples (18.9%). Based on the overall mortality-associated ROC curve, 24 patients were graded as having weak cytoplasmic Trx1 expression (range: 0-420) and

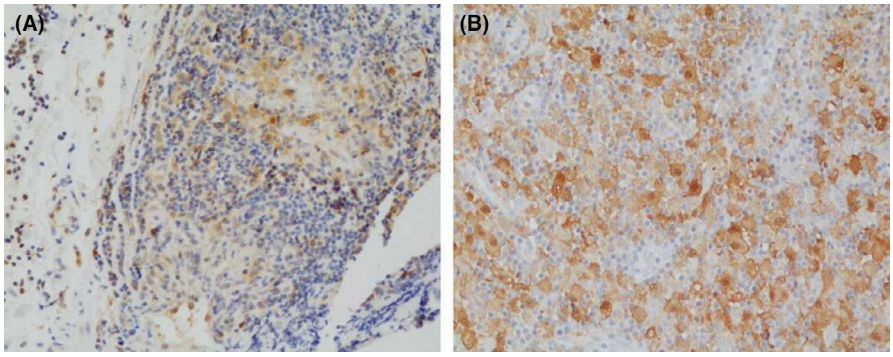

FIGURE 3 Thioredoxin-1 (Trx) immunohistochemical staining samples with ×20 magnification. A, Negative immunohistochemical staining of Trx1 of malignant cells in a lymph node. B, Strong intensity of cytoplasmic Trx1 staining of malignant cells in a lymph node [Colour figure can be viewed at wileyonlinelibrary.com]

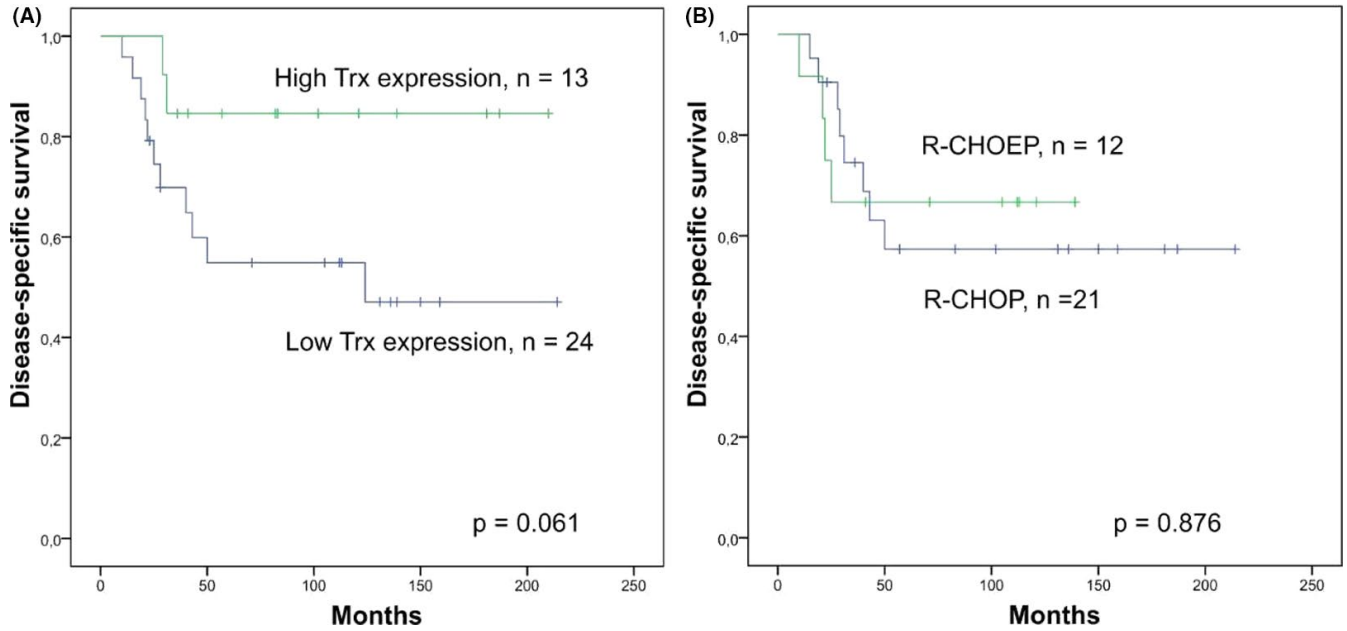

FIGURE 4 Survival in patient cohort I. Patients received mainly R-CHOP or R-CHOEP as frontline treatment and high-dose therapy as salvage chemotherapy after relapse. A, Disease-specific survival according to Trx-1 expression. B, Disease-specific survival according to frontline treatment [Colour figure can be viewed at wileyonlinelibrary.com]

24 patients as having strong cytoplasmic Trx1 expression (range: 50-800).

In patient cohort II, Trx1 expression was predominantly seen in cytoplasm. Cytoplasmic expression was totally absent in two cases and nuclear expression in 18 cases. Based on the ROC curve, 16 patients were graded with weak cytoplasmic Trx1 expression (range: 0-40) and 47 patients with strong cytoplasmic Trx1 expression (range: 50-240). In patient cohort I, there were no statistically significant correlations of Trx1 staining intensity or localisation when compared to the known clinical risk markers of DLBCL (stage, International Prognostic Index [IPI], elevated lactate dehydrogenase, WHO performance status, extranodal effusion, age over 60 or B symptoms). In Kaplan-Meier analysis, an association between low Trx1 cytoplasmic expression and an adverse prognosis was found (Figure 4). At the 5-year follow-up, disease-specific survival (DSS) was 54.9% in the patient group with low Trx1 cytoplasmic expression and 84.6% in the group with high Trx1 cytoplasmic expression intensity in lymphoma cells (P = .061). In Cox regression analysis, low Trx1 expression was associated with OS (HR 12.408; 95% CI 1.787-86.133, P = .011), when compared to non-GC phenotype (HR 5.463; 95% CI 1.318-22.639; P = .019) and high IPI (HR 1.123; 95% CI 0.212-5.940; P = .892), and also with DSS (Trx1: HR 28.075; 95% CI 2.188-360.282; P = .010; non-GC phenotype: HR 6.267; 95% CI 1.254-31.319; P = .025; high IPI: HR 1.699; 95% CI 0.308-9.380; P = .543) and RFS (Trx1: HR 56.546; 95% CI 2.896-1104.066; P = .008; non-GC phenotype: HR 15.353; 95% CI 1.634-144-287; P = .017; high IPI: 18.217; 95% CI 1.296-255.992; P = .031).

In patient cohort II, strong cytoplasmic Trx1 expression was associated with age under 60 years (P = .032). There were no other statistically significant associations with cytoplasmic or nuclear Trx1 expression. Low cytoplasmic Trx1 expression was associated with inferior 5-year OS (46% vs 76%, P = .026), DSS (68% vs 90%, P = .026) and non-significantly with RFS (64% vs 81%, P = .076) compared to the group with a high cytoplasmic Trx1 expression with known DLBCL risk markers (Figure 5). In Cox regression analysis, cytoplasmic Trx1 expression was an independent risk factor from IPI

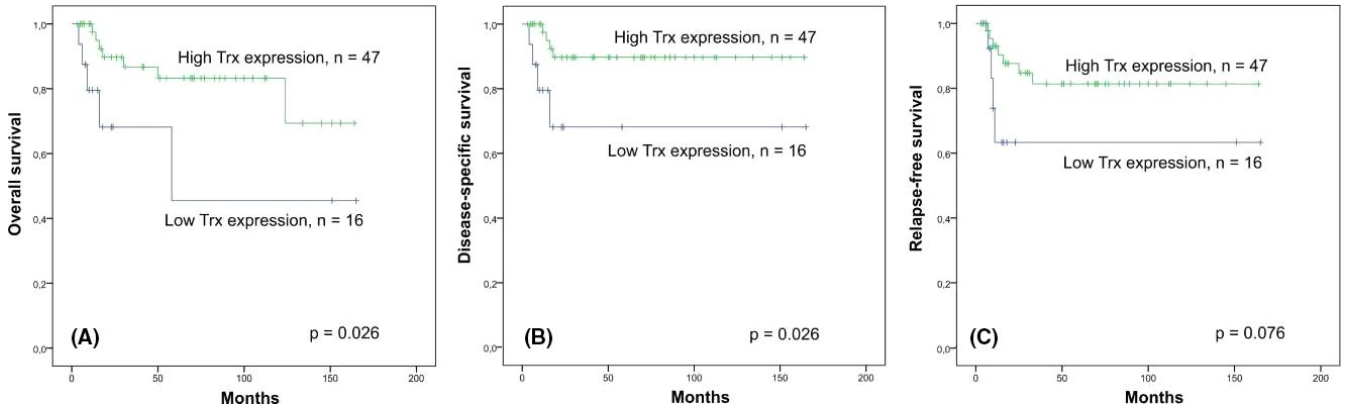

FIGURE 5 Survival in patient cohort II based on thioredoxin-1 (Trx1) expression. All patients received R-CHOEP as frontline treatment. High Trx1 expression is associated with better overall (A), disease-specific (P = .026) (B) and relapse-free survival (P = .026) (C) among patients treated with R-CHOEP (P = .076). A, Overall survival. B, Disease-specific survival. C, Relapse-free survival [Colour figure can be viewed at wileyonlinelibrary.com]

but not from GC phenotype. Low cytoplasmic Trx1 expression was associated with OS (HR 5.531; 95% CI 1.522-20.101; P = .009) when compared to IPI (HR 1.580; 95% CI 1.580-22.347; P = .008) and with DSS (Trx1: HR 5.842; 95% CI 1.334-25.588; P = .019; IPI: HR 4.405; 95% CI 0.800-24.246; P = .088).

4 | DISCUSSION

In the present work, we have shown that high cytoplasmic Trx1 expression seems to induce a resistance to doxorubicin but sensitises cells to etoposide in a lymphoma cell culture model. We and others[4,6] have previously shown that Trx1 is a negative prognostic factor in DLBCL and the findings of the present study suggest strong evidence of the causality of Trx1 being involved in this adverse outcome. Moreover, here we demonstrate in clinical material that high Trx1 expression appears to sensitise lymphoma to etoposide-containing treatment. These results strongly suggest that high Trx1 expression in lymphoma cells might serve as a biological predictive factor to select the patients who may benefit from adding etoposide to their treatment regimen.

There are multiple antioxidant systems with a purpose of maintaining the intracellular redox state[9] and neutralising ROS caused by oxidative phosphorylation and other sources.[8] One of these effectors is the Trx/TrxR1 system, containing Trx1 protein, thioredoxin reductase 1 (TrxR1) and nicotinamide adenine dinucleotide phosphate (NADPH). Trx1 reduces other antioxidant enzymes but requires TrxR1 and NADPH reserves to remain functional.[10,11] The system defends the cells against oxidative stress and these enzymes are also involved in apoptosis, DNA synthesis and redox signalling.[10,12] Trx1 expression has often been reported to be at higher levels in cancer cells compared to normal cells. However, Trx1 is ubiquitously present in all cells, also under physiological circumstances.[5] In cancer cells, Trx1 protects cells from apoptosis by neutralising the toxic effects of ROS.[7,10] It may promote cell growth[12] and correlates with higher metastatic potential of the cancer cells.[7] The key effect of Trx1 in cisplatin resistance has been demonstrated in a lung cancer model.[13]

The Trx/TrxR1 system has an essential role in tumorigenesis and malignant behaviour, but their mutual relationship is still being investigated.[11] Li et al[6] suggest that blocking any component of the Trx/TrxR1 system has essentially the same effect on cancer cells and this has been demonstrated in in vitro studies with dimethyl fumarate[14] and auranofin.[15-17] However, TrxR1 has been shown to have tumour-promoting functions independent of Trx1.[11,18] TrxrR1 has also been postulated to be the master regulator of the system as it crosstalks with the Nrf2-Keap1 axis, the main regulator of antioxidant response, and inhibition of TrxR1 also inhibits Trx1.[19] Inhibitors of the Trx/TrxR1 system are being investigated in clinical trials.[11]

Many chemotherapeutic agents such as doxorubicin[20] induce oxidative stress, which leads to sub-lethal DNA damage, which subsequently induces cell death via apoptosis. Etoposide, on the other hand, inhibits topoisomerase II. The cytotoxicity of etoposide is dependent on the cell cycle.[21] Doxorubicin also acts via topoisomerase II inhibition, but it has other key effects such as intercalation between DNA base pairs, which evidently leads to inhibition of DNA transcription.[20] In the present study, we found that Trx1 knockdown can sensitise the lymphoma cells to doxorubicin. When using etoposide, the effect was found to be opposite: native cells were more prone to etoposide-induced toxicity compared to those cells that had undergone Trx1 knockdown. We also evaluated the effect of other chemotherapeutic agents used in standard combinations to treat lymphoma, but no significant differences in the cell death were found between the native and Trx1 knockdown cells.

Before the rituximab era, a German study group published a randomised study in which a comparison of CHOP and CHOEP regimens as DLBCL frontline treatment was performed.[3] They found that the CHOEP regimen might give a survival advantage when compared to the standard CHOP therapy in those patients that were younger than 60 years and had an IPI score of one or more. Since establishing the efficacy of rituximab in B-cell lymphomas, R-CHOP has evolved as the gold standard for DLBCL frontline therapy. However, some patients are still refractory to or relapse after R-CHOP therapy. Some of these patients might

benefit from more aggressive frontline treatment[22] such as R-CHOEP. Since R-CHOEP is more toxic than R-CHOP, there is an unmet clinical need to find a predictive marker, probably a biological one, to identify the patients that might benefit from adding etoposide to the treatment regimen.

It has previously been shown in clinical data[4] that Trx1 is associated with an adverse outcome seen in a DLBCL population treated with R-CHOP chemotherapy. It was shown that strong Trx1 intensity correlated with poor progression-free survival (PFS) and poor DSS. In the present study, we assessed the Trx1 expression rate in primary biopsy samples from DLBCL patients who were treated with etoposide-containing ASCT and frontline R-CHOEP. We wanted to explore Trx1 expression in two differently treated and analysed patient populations, and the results were similar. Overall, in the ASCT patient group, Trx1 lost its biological predictive value, while among R-CHOEP-treated patients there was a strong association with better outcome in cases with high Trx1 expression. In the in vitro model, we found that the knockdown of Trx1 increases the cytotoxic effect of doxorubicin, whereas etoposide was more effective in the native cells with intact Trx1 expression. Based on these data, we suggest that immunohistochemically detected high Trx1 expression in lymphoma might serve as a predictive indicator to select patients who might benefit from adding etoposide to their frontline R-CHOP treatment.

These conclusions are mainly based on results obtained from a cell culture model and two relatively small patient cohorts. The cell lines used represent selected cell material, and therefore, definitive conclusions for clinical decision-making need further investigation. Nevertheless, the repeatability was good. Our study also included clinical material supporting the results obtained from the cell culture model. If these results could be confirmed in future studies, they might indicate that the treatment regimen for DLBCL could be chosen based on the Trx1 expression rate in primary biopsy samples. In the future, it would be crucial to verify our findings prospectively in a large patient cohort treated with R-CHOP and R-CHOEP. These results should also be correlated with expression of TrxR1, which could be another potential target for therapy.

ACKNOWLEDGEMENTS

We would like to thank Mirka Peränen for her technical assistance in cell culturing and Anne Bisi for her skilful technical assistance in immunohistochemical staining. We would also like to thank Elina Jokinen for her support and guidance in cell culture and Jarkko Lackmann for assistance with FACS.

CONFLICT OF INTEREST

The authors state no conflicts of interest.

AUTHOR CONTRIBUTIONS

EJMK participated in clinical data and biopsy sample collection, analysed immunohistochemistry and wrote the manuscript. MELK collected the lymphoma patient data and samples, participated in pathological analysis of immunohistochemical staining, conducted the cell culture examinations, optimised the chemotherapeutic agent testing, carried out statistical analysis and wrote the manuscript. PH founded the cell culture, participated in optimising the cell culture surroundings and wrote the manuscript. AH established the cell culture and siRNA transfection methods and participated in optimising them as well as SDS-PAGE and Western blot. KMH analysed the immunohistochemistry. RB assisted with statistical analysis. PK assisted with statistical analysis. HRT assisted with statistical analysis and analysed immunohistochemistry. RP participated in clinical data and biopsy sample collection. TTH participated in planning the study and analysing the data and funded the study. OK planned the study. All authors reviewed the manuscript.

NOVELTY STATEMENT

Strong expression of Trx1 is associated with better survival in DLBCL patients treated with R-CHOEP but not with R-CHOP. A low level of Trx1 sensitises DLBCL cells to doxorubicin but decreases etoposide-induced cell death in vitro. Trx1 is a possible marker for selecting DLBCL patients for etoposide-containing treatment.

ORCID


Esa Jarkko Mikael Kari https://orcid.org/0000-0002-7165-8120

REFERENCES

1. Suomen Syöpärekisteri (Finnish Cancer Registry) 2016. 2018.
2. Tome ME, Johnson DBF, Rimsza LM, et al. A redox signature score identifies diffuse large B-cell lymphoma patients with a poor prognosis. *Blood*. 2005;106:3594-3601.
3. Wunderlich A, Kloess M, Reiser M, et al. Practicability and acute haematological toxicity of 2- and 3-weekly CHOP and CHOEP chemotherapy for aggressive non-Hodgkin's lymphoma: results from the NHL-B trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNSL). *Ann Oncol*. 2003;14:881-893.
4. Peroja P, Pasanen AK, Haapasaari K, et al. Oxidative stress and redox state-regulating enzymes have prognostic relevance in diffuse large B-cell lymphoma. *Exp Hematol Oncol*. 2012;1:2.
5. Mukherjee A, Martin SG. The thioredoxin system: a key target in tumour and endothelial cells. *Br J Radiol*. 2008;81:S57-S68.
6. Li C, Thompson MA, Tamayo AT, et al. Over-expression of Thioredoxin-1 mediates growth, survival, and chemoresistance and is a druggable target in diffuse large B-cell lymphoma. *Oncotarget*. 2012;3:314-326.
7. Zhang T, Liu H, Zhu C, et al. Silencing thioredoxin induces liver cancer cell senescence under hypoxia. *Hepatol Res*. 2012;42:706-713.
8. Karihtala P, Puistola U. Hypoxia and oxidative stress in the pathogenesis of gynecological cancers and in therapeutical options. *Curr Cancer Ther Rev*. 2011;7:37-55.
9. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS*. 2007;115:81-103.
10. Berghella AM, Pellegrini P, Del Beato T, Ciccone F, Contasta I. The potential role of thioredoxin 1 and CD30 systems as multiple pathway targets and biomarkers in tumor therapy. *Cancer Immunol Immunother*. 2011;60:1373-1381.
11. Zhang J, Li X, Han X, Liu R, Fang J. Targeting the thioredoxin system for cancer therapy. *Trends Pharmacol Sci*. 2017;38:794-808.
12. Holmgren A, Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem Biophys Res Commun*. 2010;396:120-124.

13. Wangpaichitr M, Sullivan EJ, Theodoropoulos G, et al. The relationship of thioredoxin-1 and cisplatin resistance: Its impact on ROS and oxidative metabolism in lung cancer cells. *Mol Cancer Ther*. 2012;11:604-615.
14. Schroeder A, Warnken U, Röth D, et al. Targeting Thioredoxin-1 by dimethyl fumarate induces ripoptosome-mediated cell death. *Sci Rep*. 2017;7:43168.
15. Raninga PV, Di Trapani G, Vuckovic S, Tonissen KF. TrxR1 inhibition overcomes both hypoxia-induced and acquired bortezomib resistance in multiple myeloma through NF-κβ inhibition. *Cell Cycle*. 2016;15:559-572.
16. Fan C, Zheng W, Fu X, Li X, Wong Y, Chen T. Enhancement of auranofin-induced lung cancer cell apoptosis by selenocystine, a natural inhibitor of TrxR1 in vitro and in vivo. *Cell Death Dis*. 2014;5:e1191.
17. Hwang-Bo H, Jeong J, Han MH, et al. Auranofin, an inhibitor of thioredoxin reductase, induces apoptosis in hepatocellular carcinoma Hep3B cells by generation of reactive oxygen species. *Gen Physiol Biophys*. 2017;36:117-128.
18. Dong C, Zhang L, Sun R, et al. Role of thioredoxin reductase 1 in dysplastic transformation of human breast epithelial cells triggered by chronic oxidative stress. *Sci Rep*. 2016;6:36860.
19. Cebula M, Schmidt EE, Arnér ESJ. TrxR1 as a potent regulator of the Nrf2-Keap1 response system. *Antioxid Redox Signal*. 2015;23:823-853.
20. Danesi R, Fogli S, Gennari A, Conte P, Del Tacca M. Pharmacokinetic-pharmacodynamic relationships of the anthracycline anticancer drugs. *Clin Pharmacokinet*. 2002;41:431-444.
21. You B, Tranchand B, Girard P, et al. Etoposide pharmacokinetics and survival in patients with small cell lung cancer: a multicentre study. *Lung Cancer*. 2008;62:261-272.
22. Holte H, Leppä S, Björkholm M, et al. Dose-densified chemoimmunotherapy followed by systemic central nervous system prophylaxis for younger high-risk diffuse large B-cell/follicular grade 3 lymphoma patients: Results of a phase II Nordic lymphoma group study. *Ann Oncol*. 2013;24:1385-1392.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kari EJM, Kuusisto MEL, Honkavaara P, et al. Thioredoxin-1 as a biological predictive marker for selecting diffuse large B-cell lymphoma patients for etoposide-containing treatment. *Eur J Haematol*. 2020;105:156–163. https://doi.org/10.1111/ejh.13419