Identifikation prognostischer Marker für T-Zell-Lymphome

unter Verwendung der Tissue Microarray Technik

Dissertation

zur Erlangung des akademischen Grades

Doktor der Medizin (Dr. med.)

vorgelegt

der Medizinischen Fakultät

der Martin-Luther-Universität Halle-Wittenberg

von Franziska Lea Schümann

Betreuer: PD Dr. med. Thomas Weber

Gutachter:

Prof. Dr. Christian Scholz, Berlin

PD Dr. Marco Herling, Leipzig

Datum der Verteidigung: 17.04.2024

Referat

Die T-Zell Non-Hodgkin-Lymphome stellen eine heterogene Gruppe von Erkrankungen dar, die äußerst selten auftreten. Die Prognose der Patient:innen ist aufgrund unzureichend etablierter Therapiestandards bei häufig therapierefraktären Verläufen im Vergleich zu B-Zell-Lymphomen schlecht. Die Identifizierung prognostischer Biomarker sowie effektiver Behandlungskonzepte stellt derzeit ein Desiderat in der Forschung dar. In den meisten T-Zell-Lymphomen zeigt sich eine durch Mutationen oder Expressionsveränderungen alterierte Funktion von Histonmodifikatoren sowie RNA-Bindeproteinen. Jedoch sind die Auswirkungen dieser Alterationen im klinischen Kontext bis jetzt nicht umfassend verstanden.

Ziel dieser beiden explorativen Arbeiten war es, die Proteinexpression von *Enhancer of zeste homolog* 1 und 2 (EZH1/2), von der konsekutiven Trimethylierung am Lysin 27 des Histons H3 (H3K27me3) und von dem *RNA-binding motif protein X-linked* (RBMX) in T-Zell-Neoplasie-Gewebe zu erfassen. Hierzu wurden *Tissue Microarrays* aus Tumormaterialien, die vor Einleitung der Erstlinientherapie gewonnen wurden, in einer Kohorte von insgesamt 56 T-Zell-Neoplasie-Patient:innen konstruiert und die dazugehörigen klinischen sowie histopathologischen Daten erfasst. Anschließend wurden Assoziationen der Proteinexpressionsdaten mit klinisch-pathologischen Daten, dem Therapieansprechen sowie mit dem Gesamtüberleben und dem progressionsfreien Überleben untersucht.

Die EZH1/2-Studie weist in ihrer univariablen Analyse nach, dass eine hohe EZH1-Proteinexpression mit einem verbesserten Gesamtüberleben verbunden ist, während hohe EZH2- und H3K27me3-Proteinexpressionen mit einem schlechteren einhergehen. Diese Assoziationen bestätigen sich in den multivariablen Analysen. In der RBMX-Studie kann gezeigt werden, dass eine hohe RBMX-Proteinexpression mit einem schlechteren Ansprechen auf eine anthrazyklinhaltige Erstlinienbehandlung verbunden ist und ein kürzeres Gesamtüberleben sowie progressionsfreies Überleben in der univariablen Analyse voraussagt. In der multivariablen Analyse ergibt sich, dass RBMX als unabhängiger prognostischer Marker für Gesamtüberleben und progressionsfreies Überleben dienen kann.

Franziska Lea Schümann: Identifikation prognostischer Marker für T-Zell-Lymphome unter Verwendung der Tissue Microarray Technik, Halle (Saale), Univ., Med. Fak.; Diss., 63 Seiten, 2024

Report

T-cell non-Hodgkin lymphomas are a heterogeneous group of diseases that occur extremely rarely. The prognosis of patients is poor compared to B-cell lymphomas due to insufficiently established treatment standards and frequent refractory courses. The identification of prognostic biomarkers as well as effective treatment concepts is currently a desideratum in research. In most T-cell lymphomas, altered function of histone modifiers as well as RNA-binding proteins due to mutations or expression changes is evident. However, the implications of these alterations in the clinical context are not yet fully understood.

The aim of these two exploratory studies was to detect in T-cell neoplasia tissue the protein expression of Enhancer of zeste homolog 1 and 2 (EZH1/2), consecutive trimethylation at lysine 27 of histone H3 (H3k27me3) and RNA-binding motif protein X-linked (RBMX). For this purpose, tissue microarrays were constructed from tumor materials collected before initiation of first-line therapy in a cohort of a total of 56 T-cell neoplasia patients, and the associated clinical and histopathological data were recorded. Associations of the protein expression data with the clinical and histopathological findings as well as with overall survival and progression-free survival were examined.

In univariable analysis, the EZH1/2 study demonstrates that high EZH1 protein expression is associated with improved overall survival, whereas high EZH2 and H3K27me3 protein expressions are associated with worse. These associations are confirmed in multivariable analyses. In the RBMX study, high RBMX protein expression can be shown to be associated with a worse response to first-line anthracycline-containing treatment and to predict shorter overall survival and progression-free survival in univariable analysis. Multivariable analysis here also reveals that RBMX may serve as an independent prognostic marker for overall survival and progression-free survival.

Franziska Lea Schümann: Identifikation prognostischer Marker für T-Zell-Lymphome unter Verwendung der Tissue Microarray Technik, Halle (Saale), Univ., Med. Fak.; Diss., 63 Seiten, 2024

I Inhaltsverzeichnis

II Abkürzungsverzeichnis

1 Einleitung und Zielstellung 1

2 Diskussion 12

3 Literaturverzeichnis 22

4 Thesen 32

5 Publikationen 33

5.1 Publikation 1: Schümann FL, Groß E, Bauer M, Rohde C, Sandmann S, Terziev D, Müller LP, Posern G, Wienke A, Fend F, Hansmann ML, Klapper W, Rosenwald A, Stein H, Dugas M, Müller-Tidow C, Wickenhauser C, Binder M, Weber T (2021) Divergent Effects of EZH1 and EZH2 Protein Expression on the Prognosis of Patients with T-Cell Lymphomas. Biomedicines 9 (12) 33

5.2 Publikation 2: Schümann FL, Bauer M, Groß E, Terziev D, Wienke A, Wickenhauser C, Binder M, Weber T (2021) RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis. Cancers (Basel) 13 (19) 51

III Selbständigkeitserklärung

IV Erklärung über frühere Promotionsversuche

V Danksagung

VI Anlagen

II Abkürzungsverzeichnis

5-JÜR 5-Jahresüberlebensrate

95 %-KI 95 % -Konfidenzintervall

AITL Angioimmunoblastisches T-Zell-Lymphom

ALCL Anaplastisch großzelliges Lymphom

ALK Anaplastische Lymphomkinase

AML Akute myeloische Leukämie

AUC *Area Under Curve*

CD *Cluster of Differentiation*

CHOEP Cyclophosphamid, Doxorubicin, Vincristin, Etoposid und Prednisolon

CHOP Cyclophosphamid, Doxorubicin, Vincristin und Prednisolon

DNA Desoxyribonukleinsäure

ECOG *Eastern Cooperative Oncology Group*

EED *Embryonic ectoderm development*

EGIL *European Group for the Immunological Characterization of Leukemias*

ENKTL Extranodales Natürliche Killerzell (NK)-/T-Zell-Lymphom, nasaler Typ

EZH1 *Enhancer of zeste homolog 1*

EZH2 *Enhancer of zeste homolog 2*

FDA *Food and Drug Administration*

FFPE-Gewebe Formalin-fixiertes Paraffin-eingebettetes Gewebe

H3K27me3 Trimethylierung am Lysin 27 des Histons H3

HCC Hepatozelluläres Karzinom

hnRNP Heterogene nukleare Ribonukleoproteine

IPI Internationaler Prognostischer Index

Ki-67 Kiel-Antigen-67

KM Knochenmark

LDH Lactatdehydrogenase

MEITL Monomorphes epitheliotropes intestinales T-Zell-Lymphom

NHL Non-Hodgkin-Lymphom

NK *Natürliche Killerzellen*

OS Gesamtüberleben

PFS Progressionsfreies Überleben

PRC1 *Polycomb repressive complex 1*

PRC2 *Polycomb repressive complex 2*

PTCL Peripheres T-Zell-Lymphom

PTCL-NOS   Peripheres T-Zell-Lymphom, nicht weiter spezifiziert

RbAp46/48 *Retinoblastoma-binding protein 46/48*

RBMX *RNA-binding motif protein X-linked*

RBP RNA-bindende Proteine

RNA Ribonukleinsäure

ROC *Receiver Operating Characteristic*

SET *Su(var)3-9-Enhancer-of-zeste-Trithorax*

SUZ12 *Zinc finger protein suppressor of zeste 12*

SZT Stammzelltransplantation

T-ALL Akute T-Zell-Leukämie

TCGA *The Cancer Genome Atlas*

T-LBL   Lymphoblastisches T-Zell-Lymphom

TMA *Tissue Microarray*

T-NHL T-Zell Non-Hodgkin-Lymphom

UKH Universitätsklinikum Halle (Saale)

WHO *World Health Organisation*

1 Einleitung und Zielstellung

Non-Hodgkin-Lymphome mit T-Zell Differenzierung (T-NHL) sind eine Gruppe von seltenen Erkrankungen mit einer Inzidenz von ca. einer Neuerkrankung pro 100.000 Personen im Jahr (Vose et al.,2008; Adams et al.,2016). Die *World Health Organisation* (WHO) unterscheidet unreife, akute lymphatische T-Zell-Leukämien (T-ALL) von reifen, postthymischen Natürliche Killerzellen (NK)-/T-Zell-Neoplasien. Klinisch werden die reifen T-NHL je nach ihren prädominanten Organmanifestationen in primär kutan, primär leukämisch und aggressiv verlaufend mit nodaler bzw. extranodaler Manifestation differenziert. Letztere, die peripheren T-Zell-Lymphome (PTCL) im engeren Sinne, machen nur 10-15 % aller Non-Hodgkin-Lymphome (NHL) aus und zeichnen sich durch ihre Heterogenität aus (Anderson et al.,1998; Swerdlow et al.,2017).

Bis vor kurzem wurden die T-Zell-Lymphome anhand der vierten WHO-Klassifikation von 2017 eingeteilt, welche zum Zeitpunkt der Erstellung der beiden Studien die aktuell gültige war (Swerdlow et al.,2017). Seit Juli 2022 gilt die aktualisierte fünfte WHO-Klassifikation, welche nahezu 40 verschiedene T-Zell-Neoplasie Entitäten benennt. Weltweit ist die Inzidenz der nicht weiter spezifizierbaren peripheren T-Zell-Lymphome (PTCL-NOS) (25,9 %) am höchsten, gefolgt vom angioimmunoblastischen T-Zell-Lymphom (AITL) (18,5 %) und der Gruppe der anaplastisch großzelligen Lymphome (ALCL) (12,1 %), wie Abbildung 1 veranschaulicht (Vose et al.,2008).

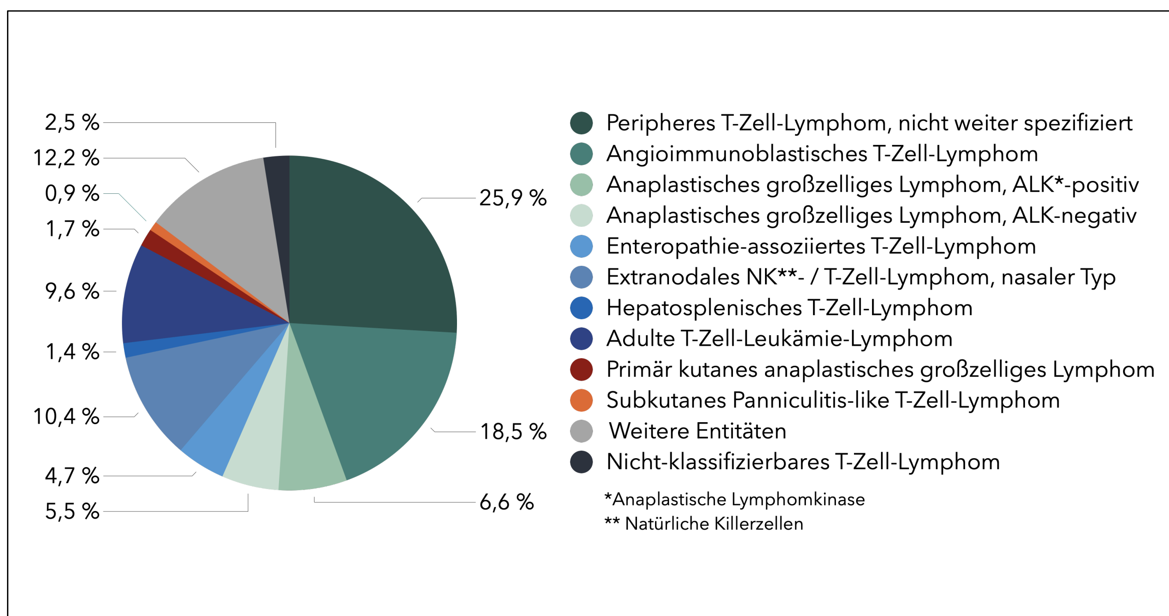

Abbildung 1: Weltweite Verteilung der T-Zell-Lymphom Subentitäten (modifiziert nach Vose et al.,2008)

Während von den T-ALL vielfach Kinder betroffen sind, erkranken an einem reifen T-Zell-Lymphom häufiger Erwachsene (Armitage und Weisenburger,1998; Rudiger et al.,2002). Eine Ausnahme stellt das ALCL mit reziproker Translokation zwischen dem anaplastischen Lymphomkinase (ALK)-Gen (Chromosom 2p23) und dem häufigsten Fusionspartner Nucleophosmin (Chromosom 5q35) dar (Gokbuget,2015). Insgesamt ist das männliche Geschlecht öfter betroffen als das weibliche (Rudiger et al.,2002).

Die Erstlinien-Standardtherapie – ein aus Cyclophosphamid, Doxorubicin, Vincristin, ggf. Etoposid und Prednisolon (CHO(E)P) bestehendes Chemotherapieschema – hat sich seit Jahrzehnten für die meisten T-NHL-Subtypen nicht geändert (d'Amore et al.,2015). Hierunter ist die Prognose des ALK-positiven ALCL mit einer 5-Jahresüberlebensrate (5-JÜR) von 70 % besser als die der anderen Entitäten: Während die 5-JÜR für das ALK-negative ALCL 49 % beträgt, liegt sie sowohl für das PTCL-NOS und das AITL bei nur 32 % (Vose et al.,2008). Damit sind die 5-JÜR im Vergleich zu vielen B-Zell-Lymphomen insgesamt ungünstig. Für die ALCL steht seit der im Jahr 2019 erfolgten europäischen Zulassung des Anti-*Cluster-of-Differentiation* (CD)-30-Antikörper-Chemotherapie-Konjugats Brentuximab-Vedotin in Kombination mit Cyclophosphamid, Hydroxodaunorubicin und Prednisolon eine den CHOP-Regimen überlegene Erstlinientherapie zur Verfügung (Horwitz et al.,2019; Horwitz et al.,2022). Weitere Strategien, das Ansprechen auf und Überleben nach Erstlinentherapie zu verbessern, sehen die Hinzunahme von Etoposid zum CHOP-Schema (CHOEP) (Schmitz et al.,2010) und die konsolidierende autologe Stammzelltransplantation (SZT) vor. Trotzdem sind therapierefraktäre Verläufe und Rezidive häufig: Bei etwa 30 % der Patient:innen liegt eine primär refraktäre Erkrankung vor (Vose et al.,2008; Bellei und Federico,2019) und die meisten refraktären oder rezidivierten T-NHL haben ohne SZT als Teil der Rezidivtherapie ein kurze Überlebenszeit (Mak et al.,2013). In der Rezidivsituation kann eine realistische Chance auf eine dauerhafte Heilung lediglich durch eine konsolidierende allogene SZT erreicht werden (Schmitz et al.,2018; Hamadani et al.,2022).

Ein zunehmend vertieftes Verständnis der Pathogenese und Biologie von T-Zell-Lymphomen in den letzten Jahrzehnten führte bereits zur Untersuchung zahlreicher neuer zielgerichteter Substanzen, mit denen genetische, epigenetische und immunologische Ansätze verfolgt werden. Die Ergänzung der Therapielinien um weitere Wirkstoffe wie Histon-Deacetylase-Inhibitoren, Desoxyribonukleinsäure (DNA)-Methyltransferase-Inhibitoren, Phosphatidylinostol-3-Kinase-Inhibitoren und monoklonale Antikörper hat bisher (außer bei Patient:innen mit ALCL) zu keinem

Paradigmenwechsel der PTCL-Standardtherapie geführt (Schümann und Weber,2021; Schümann und Weber,2023).

Die Prognoseabschätzung der PTCL erfolgt in der klinischen Praxis anhand des Internationalen Prognostischen Index (IPI), welcher ein in der Onkologie gebräuchliches Scoring-System für aggressive NHL darstellt. Für jeden der folgenden Parameter wird bei Zutreffen ein Punkt vergeben: Alter über 60 Jahre, Ann-Arbor-Stadium III oder IV, schlechter Performance Status der *Eastern Cooperative Oncology Group* (ECOG), mehr als ein extranodaler Herd und erhöhte Lactatdehydrogenase (LDH)-Konzentration im Serum. Je nach Score werden vier Risikogruppen unterschieden: 0–1, niedrig; 2, niedrig-intermediär; 3, hoch-intermediär und 4–5, hoch. Durch die Ergänzung weiterer Parameter sollten krankheitsspezifischere Scores mit präziserer Risikoabschätzung entwickelt werden. Diese Scoring-Systeme beinhalteten beispielweise den Knochenmark (KM)-Befall, eine erniedrigte Thrombozytenzahl und den Kiel-Antigen-67-basierten Proliferationsindex (Ki-67) (Übersicht in Gallamini et al.,2004)). Allerdings konnte keine klare Überlegenheit dieser Scores gegenüber des IPI gezeigt werden, sodass letzterer für den klinischen Alltag weiterhin empfohlen wird (d'Amore et al.,2015). Insgesamt beurteilt der IPI die Krankheitsausdehnung und die Gebrechlichkeit der Patient:innen, liefert jedoch keine molekularen krankheitsspezifischen Hinweise, weshalb dieser nur ungenügend für zielgerichtete Therapien genutzt werden kann.

Auf molekularer Ebene ist der Nachweis einer ALK-Translokation in ALCL eindeutig mit einer günstigeren Prognose assoziiert, was bereits in den Therapeischemata Berücksichtigung findet (Vose et al.,2008; Schmitz et al.,2010). Auch bei Patient:innen mit PTCL-NOS konnten durch Mutationsanalysen zwei große molekulare Untergruppen identifiziert werden, die mit unterschiedlichen Prognosen assoziiert sind: Dabei liegt entweder das *GATA-binding protein 3* oder der *T-box transcription factor 21* erhöht vor (Iqbal et al.,2014). Darüber hinaus wurden in den letzten Jahren vielfach Alterationen in epigenetischen Regulationsgenen wie z.B. der Tet-Methylcytosin-Dioxygenase 2, der DNA (Cytosine-5)-Methyltransferase 3A und der Isocitrate Dehydrogenase (NADP(+)) 2 aufgedeckt (Lemonnier et al.,2012; Van Arnam et al.,2018), die eine enge Assoziation von epigenetischen Anomalien und der Pathogenese in T-NHL vermuten lassen.

Einer der in den letzten Jahren am besten untersuchten epigenetischen Regulatoren und gleichzeitig Ziel zahlreicher neuer Therapeutika für verschiedene Neoplasien stellt der *Enhancer of zeste homolog* 1/2 (EZH1/2) dar. Dieses histonmodifizierende Enzym ist eine katalytische Untereinheit des *Polycomb repressive complex 2* (PRC2). Die Proteine der *Polycomb*-Gruppe sind in zwei Familien

von Komplexen zu finden, den PRC1 und PRC2. Sie halten Genexpressionsmuster aufrecht, die während der Entwicklung oder Differenzierung etabliert worden sind (Ito et al.,2018). Der in Abbildung 2 veranschaulichte PRC2 besteht im Wesentlichen aus vier Untereinheiten: *Zinc finger protein suppressor of zeste 12* (SUZ12), *Embryonic ectoderm development* (EED) Protein und *Retinoblastoma-binding protein 46/48* (RbAp46/48) sowie einem der beiden Paraloge EZH1 oder EZH2 (Cao et al.,2002; Czermin et al.,2002; Kuzmichev et al.,2002; Muller et al.,2002; Gan et al.,2018). EZH1/2 enthält am COOH-Terminus eine katalytische Domäne, die den 27. Lysinrest des Histons H3 trimethyliert (H3K27me3). Durch diese Methylierung wird die Chromatinstruktur kompakter, was zur Unterdrückung der Genexpression führt. Auf diese Weise nehmen EZH1/2 als epigenetische Regulatoren hochgradig Einfluss auf Zellentwicklung und Zelldifferenzierung (Danis et al.,2016; Herviou et al.,2016).

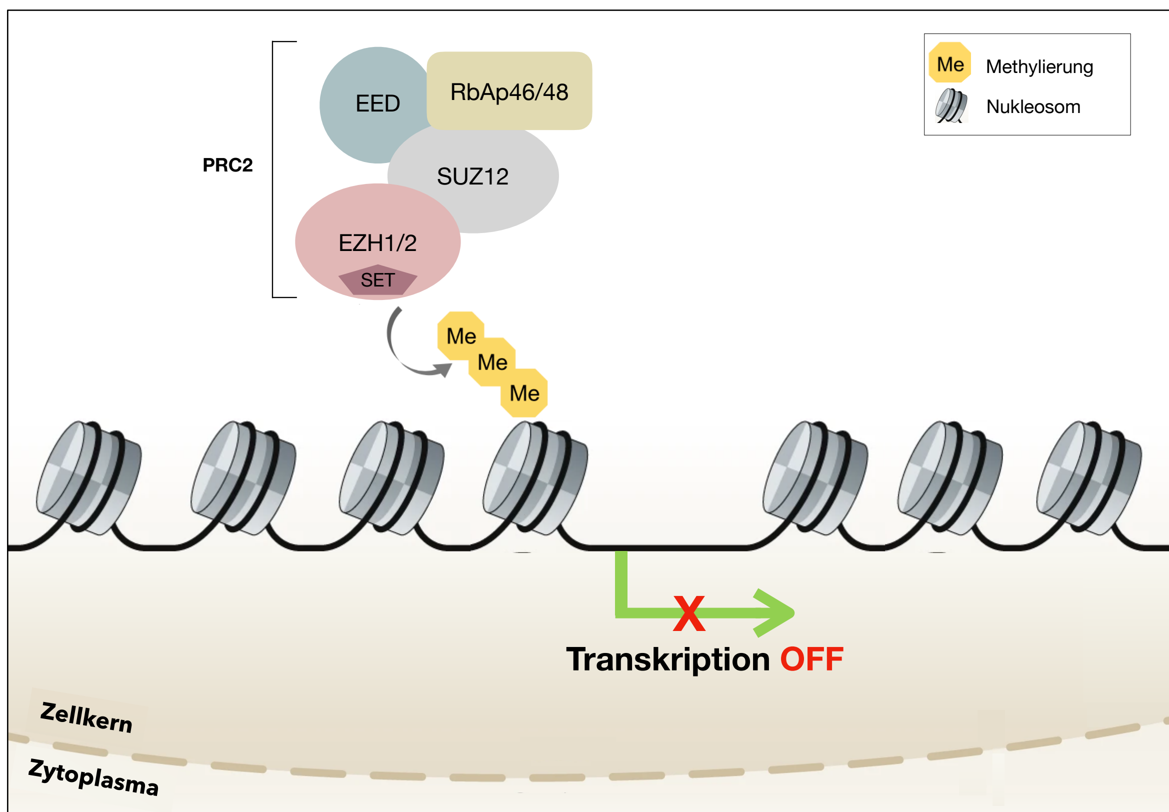

Abbildung 2: Der *Polycomb repressive complex 2* (PRC2), bestehend aus vier Untereinheiten: *Enhancer of zeste homolog 1/2* (EZH1/2) mit *Su(var)3-9-Enhancer-of-zeste-Trithorax* (SET)-Domäne, *Embryonic ectoderm development* (EED), *Zinc finger protein suppressor of zeste 12* (SUZ12) und *Retinoblastoma-binding protein* (RbAp46/48). EZH1/2 vermitteln in ihrer kanonischen Funktion die H3K27-Trimethylierung (H3K27me3) zur Repression ihrer Zielgene (modifiziert nach Herviou et al.,2016; Li und Chng,2019)

Wie neuere Studien zeigen, wirken EZH1/2 über diese kanonischen Funktionen hinaus H3K27me3-unabhängig als transkriptionelle Ko-Aktivatoren (siehe Abbildung 3) in verschiedenen Malignomen wie Brustkrebs (Anwar et al.,2021), Prostatakrebs (Xu et al.,2012) und auch extranodalen NK-/T-Zell-Lymphomen vom nasalen Typen (ENKTL) (Yan et al.,2013; Yan et al.,2016). Die nicht-kanonischen Mechanismen von EZH2 unterscheiden sich je nach Malignom (Huang et al.,2021).

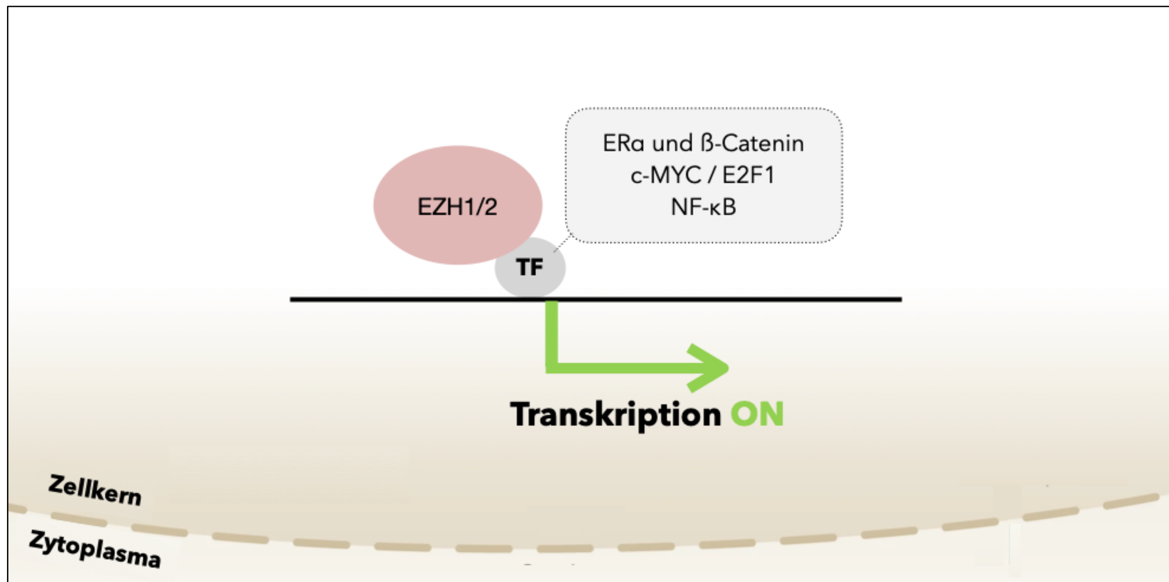

Abbildung 3: Die nicht-kanonische Funktion von *Enhancer of zeste homolog* 2 (EZH2) als transkriptioneller Ko-Aktivator im Komplex mit anderen Transkriptionsfaktoren (TF) wie z.B. Östrogenrezeptor-α (ERα) und β-Catenin, *cellular Myelocytomatosis Oncogene* (c-MYC) und E2F *Transcription Factor* 1 (E2F1) sowie *nuclear factor kappa-light-chain-enhancer of activated B-cells* (NF-κB) (modifiziert nach Gan et al.,2018; Huang et al.,2021)

Die Paraloge von *Enhancer of zeste homolog* weisen unterschiedliche Expressionsmuster auf: Während EZH1 in sich wenig teilenden und differenzierten Zellen vorkommt, findet man EZH2 nur in stark proliferierenden Zellen (Margueron und Reinberg,2011). Weiterhin besitzen PRC2-Komplexe, die EZH2 enthalten, im Vergleich zu denen, die EZH1 aufweisen, eine höhere Methyltransferase-Aktivität (Margueron und Reinberg,2011). Da es bei einer EZH2-Depletion zu keinem vollständigen Verlust der H3K27me3-Expression kommt, wird angenommen, dass EZH1 in der Lage ist, den Verlust von EZH2 zumindest teilweise zu kompensieren (Margueron et al.,2008; Shen et al.,2008). EZH2 und EZH1 haben größtenteils sich überlappende, zu einem geringen Anteil aber auch unterschiedliche Zielgene (Margueron et al.,2008).

Bereits vor über 30 Jahren wurde der erste EZH2-Inhibitor 3-Deazaneplanocin A entwickelt, welcher zu einer indirekten EZH2-Hemmung führt (Glazer et al.,1986). Seitdem etablierten sich zahlreiche weitere EZH2-Inhibitoren, die zum Teil hoch spezfisch wirken und von denen aktuell mehrere

klinisch getestet werden (Li et al.,2021). Tazemetostat wurde als erster im Januar 2020 von der *Food and Drug Administration* (FDA) der Vereinigten Saaten von Amerika für Patient:innen mit fortgeschrittenen oder metastasierten epitheloiden Sarkomen zugelassen (Hoy,2020). Allerdings wird in jüngster Zeit nicht nur die Wirkung von EZH2-selektiven Inhibitoren, sondern auch von dualen EZH1/2-Inhibitoren intensiv klinisch untersucht (Yamagishi et al.,2019). Es konnte bereits gezeigt werden, dass die duale EZH1- und EZH2-Hemmung (z.B. mittels Valemetostat) zu einer deutlich stärkeren Senkung der H3K27me3-Expression führt als eine selektive EZH2-Hemmung (Yamagishi et al. 2019; Kagiyama et al. 2021).

Grundlage für die Entwicklung dieser zielgerichteten Therapien war der Nachweis von *EZH1/2*-Mutationen und/oder EZH1/2-Überexpressionen in verschiedenen Neoplasien, die mittlerweile vielfach mit Tumorproliferation, Metastasierung, schlechter Prognose und Therapieversagen assoziiert worden sind (Varambally et al.,2002; Bachmann et al.,2006; Wang et al.,2010; Duan et al.,2020). Allerdings werden Mutationen von *EZH1* und/oder *EZH2* in T-Zell-Lymphomen als selten oder nicht vorhanden beschrieben (Palomero et al.,2014; Sakata-Yanagimoto et al.,2014; Crescenzo et al.,2015; Roberti et al.,2016; Wang et al.,2017; Watatani et al.,2019; Laginestra et al.,2020; Maura et al.,2020). Währenddessen wird über eine EZH2-Proteinüberexpression (Yan et al.,2013; Shi et al.,2015; Abdalkader et al.,2016; Kim et al.,2016; Yi et al.,2018) und damit assoziiert eine ungünstige Prognose (Zhang et al.,2019) im Kontext von PTCL häufiger berichtet. Expressionsunterschiede zwischen den einzelnen Lymphomsubentitäten wurden bisher nicht beobachtet (Abdalkader et al.,2016). Eine Übersicht derjenigen Studien, die sich in den letzten Jahren mit der EZH1/2-Proteinexpression in T-Zell Neoplasien befassten, liefert Tabelle 1.

Tabelle 1: Ausgewählte Studien zur immunhistochemischen EZH2/1-Proteinxpression mit Anzahl der eingeschlossenen T-Zell-Neoplasien und Expressiosniveaus (eigene Darstellung)

Referenz	Entitäten	EZH1		EZH2	
		n	EZH1+	n	EZH2+
Yan et al. 2013	Extranodales NK-/T-Zell-Lymphom, nasaler Typ			38	61 %
Shi et al. 2015	Anaplastisches großzelliges Lymphom, ALK-positiv			15	93 %
	Anaplastisches großzelliges Lymphom, ALK-negativ			15	100 %
	Peripheres T-Zell-Lymphom, nicht weiter spezifiziert			18	89 %
	Angioimmunoblastisches T-Zell-Lymphom			19	79 %
	Extranodales NK-/T-Zell-Lymphom, nasaler Typ			20	90 %
	Adultes T-Zell-Leukämie-Lymphom			7	100 %
	Akute T-Zell-Leukämie			10	100 %
Abdalkader et al. 2016	Lymphoblastisches T-Zell-Lymphom	5	11,1 %	10	70 %
	Extranodales NK-/T-Zell-Lymphom, nasaler Typ	6	0 %	10	63,3 %
	Adultes T-Zell-Leukämie-Lymphom	3	33,3 %	10	53,3 %
	Peripheres T-Zell-Lymphom, nicht weiter spezifiziert	5	11,1 %	10	53,3 %
	Angioimmunoblastisches T-Zell-Lymphom	4	6,7 %	5	53,3 %
	Anaplastisches großzelliges Lymphom	5	11,1 %	10	46,7 %
	Kutanes T-Zell-Lymphom	3	13,3 %	5	46,7 %
Kim et al. 2016	Extranodales NK-/T-Zell-Lymphom, nasaler Typ			56	
	Peripheres T-Zell-Lymphom, nicht weiter spezifiziert			44	
	Angioimmunoblastisches T-Zell-Lymphom			16	Σ54,7 %
	Anaplastisches großzelliges Lymphom			32	
	Lymphoblastisches T-Zell-Lymphom			19	
Yi et al. 2018	Lymphomatoide Papulose			16	100 %
	Primär kutanes anaplastisches großzelliges Lymphom			13	100 %
	Mycosis fungoides mit großzelliger Transformation			14	100 %
Zhang et al. 2019	Anaplastisches großzelliges Lymphom			15	73,3 %
	Peripheres T-Zell-Lymphom, nicht weiter spezifiziert			43	67,4 %
	Angioimmunoblastisches T-Zell-Lymphom			10	60 %
	Extranodales NK-/T-Zell-Lymphom, nasaler Typ			14	50 %

(ALK: Anaplastische Lymphomkinase, EZH2/1+: Anzahl an Gewebeproben mit positiver EZH2/1-Expression in Prozent. Die *Cutoff*-Werte für eine positive Expression wurden jeweils innerhalb der Studien bestimmt, n: Anzahl der Gewebeproben, NK: Natürliche Killerzellen)

Darüber hinaus fanden einige Studien Assoziationen zwischen erhöhter EZH2-Proteinexpression und verschiedenen klinischen Markern wie dem Vorhandensein von B-Symptomen, erhöhter LDH-Konzentration im Serum, erhöhtem ß2-Mikroglobulin sowie einer Ki-67 Erhöhung (Shi et al.,2015; Abdalkader et al.,2016; Liu et al.,2019; Zhang et al.,2019). Abdalkader et al. untersuchten ferner die Assoziation der beiden Paraloge EZH1 und EZH2 in NK-/T-Zell-Neoplasien und konnten gegensätzliche Proteinexpressionsmuster in sowohl normalem als auch neoplastischem lymphoiden Gewebe sowie eine gegensätzliche Beziehung zur Ki-67-Expression beobachten. Während die Autor:innen über die NK-/T-Zell-Neoplasien hinweg eine Überexpression von EZH2 feststellen konnten, wurde die Expression von EZH1 nur in 12,5 % der Fälle als positiv beschrieben (Abdalkader

et al.,2016). Weitere Daten über EZH1-Proteinexpressionsmuster und/oder eine Assoziation dieser zum Überleben von Patient:innen mit T-NHL sind bisher nicht bekannt.

Die Erkenntnisse zur Assoziation des konsekutiven Transkriptionsrepressionsmarkers H3K27me3 und der Prognose von Krebspatient:innen sind divers: Einerseits wird eine hohe H3K27me3-Proteinexpression in hepatozellulären Karzinomen (HCC) (Cai et al.,2011) und Plattenepithelkarzinomen des Ösophagus (He et al.,2009) mit einer schlechteren Prognose assoziiert. Andererseits zeigt sich bei Ovarial-, Brust- und Pankreaskarzinomen eine schlechtere Prognose bei niedriger Proteinexpression (Wei et al.,2008). Bei Patient:innen mit ENKTL ist eine hohe H3K27me3-Proteinexpression mit einer besseren Prognose und niedrigen klinischen Stadien verbunden (Liu et al.,2019). Bislang ist der H3K27me3 Expressionsstatus und dessen Assoziation mit anderen Prognosemarkern und dem Überleben in weiteren Subtypen von T-Zell-Neoplasien kaum untersucht.

Auch die Korrelation von EZH2 und H3K27me3 stellt sich heterogen dar: Während Kim et al. in einer Studie, die mehrere T-Zell-Lymphom Entitäten integrierte (ENKTL, PTCL-NOS, AITL, ALCL und Akute lymphoblastische T-Zell-Lymphome (T-LBL)), eine mäßig positive Korrelation beschreiben ($r = 0{,}230$, $p = 0{,}002$) (Kim et al.,2016), wird bei ENKTL über eine starke inverse Korrelation der Proteinexpression berichtet ($r = -0{,}652$, $p = 0{,}002$) (Liu et al.,2019). Aufgrund dieser Ergebnisse wird auch eine mögliche nicht-kanonische Funktion von EZH2 in der Onkogenese von T-NHL diskutiert (Yan et al.,2013; Yan et al.,2016; Liu et al.,2019).

Nicht nur epigenetische Modifikationen beeinflussen Genexpressionsmuster in hohem Maße. Ein weiterer entscheidender Aspekt in der Genregulation ist die Feinabstimmung auf posttranskriptionaler Ebene. Das Ribonukleinsäure (RNA)-Transkript ist Ergebnis eines stark regulierten Prozesses, der zur Reifung der mRNA führt (El Marabti und Younis,2018). Die Schlüsselfaktoren der posttranskriptionalen Kontrolle sind microRNAs und RNA-bindende Proteine (RBP). RBP binden doppel- oder einzelsträngige RNA, bilden Ribonukleoproteinkomplexe aus und beeinflussen jeden Aspekt der posttranskriptionellen Regulierung. Abbildung 4 veranschaulicht die nuklearen (Transkription, Spleißen, *Capping*, Polyadenylierung) und zytoplasmatischen (Transport, Lokalisierung, Translation, Abbau) Schritte des mRNA-Metabolismus (Gebauer et al.,2021; Guillemin et al.,2021). Eine Störung dieser präzisen Maschinerie führt zu einem abweichenden Zellverhalten, welche eine bösartige Transformation und damit die Entstehung und Entwicklung von Malignomen verursachen kann (Wurth,2012; Pereira et al.,2017; Wang et al.,2019). Darüber hinaus wird die Rolle von RBP im Zusammenhang mit Therapieresistenzen durch vielfältige Mechanismen wie die

Regulation der RNA-Stabilität, die Translation, das alternative Spleißen und mögliche Interaktionen mit microRNAs vielfach diskutiert (Übersicht in Cen et al.,2023)).

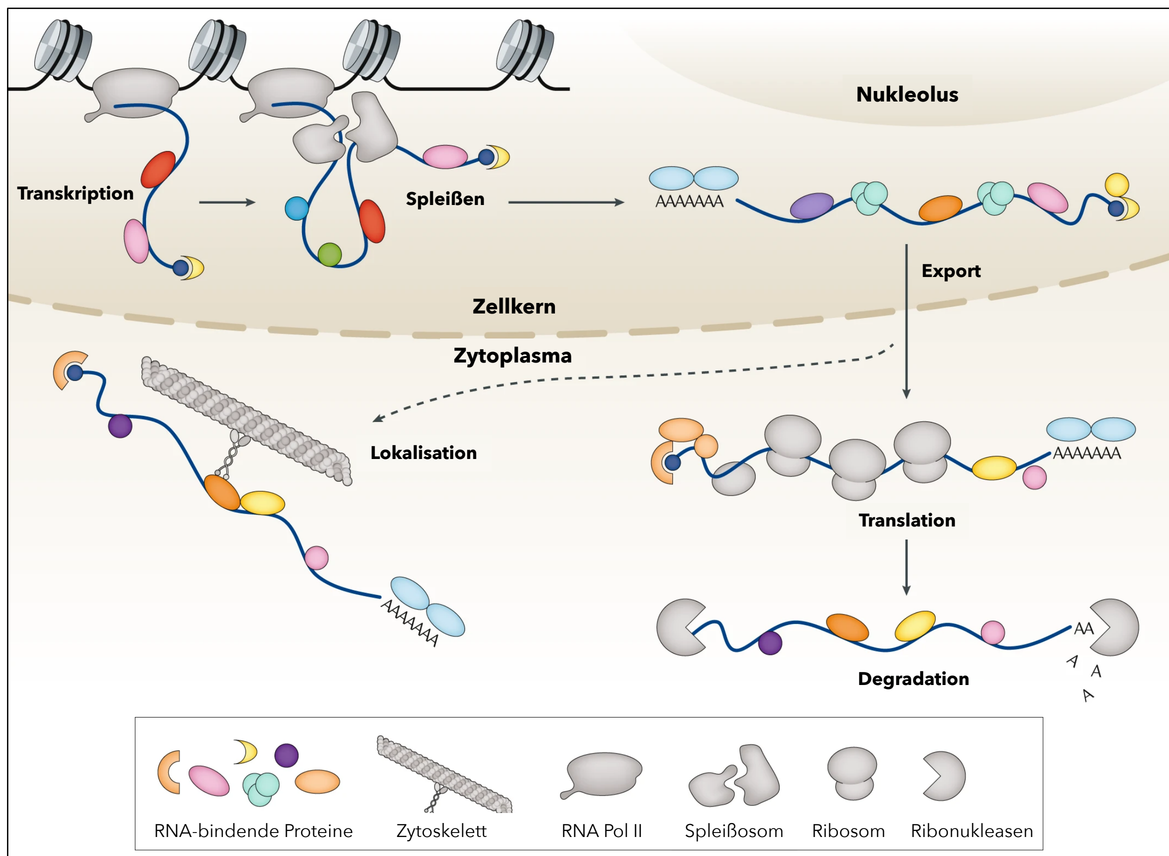

Abbildung 4: Die RNA-bindenden Proteine und ihre zahlreichen Funktionen in der posttranskriptionellen Genregulation. Dargestellt sind nukleare (Transkription, Spleißen, Capping, Polyadenylierung) und zytoplasmatische (Transport, Lokalisierung, Translation, Abbau) Schritte des mRNA-Metabolismus (modifiziert nach Gebauer et al.,2021)

Die Gruppe der heterogenen nuklearen Ribonukleoproteine (hnRNP), die RNA-Moleküle im Zellkern binden und beeinflussen, umfasst mehr als 30 verschiedene Mitglieder (Dreyfuss et al.,1993). Das *RNA-binding motif protein X-linked* (RBMX) (auch hnRNP G genannt) ist einer der Vertreter dieser heterogenen Proteingruppe. RBMX besteht aus 391 Aminosäureresten und weist eine RNA-Bindungsdomäne am N-Terminus auf (Soulard et al.,1993). Ursprünglich ist RBMX als Kernprotein bekannt, welches als Teil des Supra-Spleißosoms die Auswahl alternativer Spleißstellen konzentrationsabhängig steuert (Martinez-Contreras et al.,2007; Heinrich et al.,2009). Weiterhin wird RBMX eine regulatorische Rolle bei der Aufrechterhaltung und dem zentromerischen Schutz der Schwesternchromatidkohäsion während der Zellteilung zugesprochen (Matsunaga et al.,2012). Unlängst veröffentlichte Daten zeigen, dass RBMX in der Lage ist, die Genomstabilität während der Replikation sicherzustellen, indem das Protein an repetitive DNA-Sequenzen bindet (Zheng et

al.,2020). Eine Depletion von RBMX führt deshalb zu Replikationsdefekten und somit Genominstabilität (Munschauer et al.,2018; Zheng et al.,2020).

RBMX wurde bisher als potenzieller Tumorsuppressor bei Plattenepithelkarzinomen der Mundhöhle (Shin et al.,2006; Shin et al.,2008; Shin et al.,2011) und Lungenkrebs (Renieri et al.,2014) vorgeschlagen. Bezüglich der Vermittlung von Chemotherapieresistenzen, welche wie bereits beschrieben bei T-Zell-Neoplasien sehr häufig vorkommen, liefern mehreren Studien Hinweise auf eine entscheidende Rolle von RBMX. So wird berichtet, dass eine RBMX-Depletion zu einer Sensibilisierung gegenüber DNA-Schäden, die durch ionisierende Strahlung und verschiedene genotoxische Medikamente induziert wurden, führt (Adamson et al.,2012). Weiterhin weist eine Studie in HCC-Zellen eine Sorafenib-Resistenz bei gleichzeitiger RBMX-Überexpression nach (Song et al.,2020). Bei Exom-Sequenzierungsanalysen wurden *RBMX-Missense*-Mutationen in Vemurafenib-resistenten papillären Schilddrüsenkrebszellen nachgewiesen (Antonello et al.,2017). Die Funktion von RBMX bei der Krebsentstehung und der Förderung von Therapieresistenz wurde allerdings insbesondere im Kontext von T-Zell-Lymphomen lediglich sporadisch untersucht und ist bisher unzureichend geklärt.

Die Ergebnisse der Assoziation der RBMX-Proteinexpressionsmuster mit klinischen Parametern in verschiedenen Neoplasien sind heterogen: Hohe RBMX-Expressionswerte werden mit einem verbesserten Überleben in Endometriumkarzinomen (Ouyang et al.,2011; Hirschfeld et al.,2015) und Urothelkarzinomen (Yan et al.,2021) assoziiert, während eine erhöhte RBMX-Expression in HCC (Song et al.,2020) und bei Kopf-Hals-Tumoren (Guo et al.,2020) mit einem schlechteren Überleben in Verbindung gebracht werden. Erst kürzlich veröffentlichte Daten zeigen, dass RBMX bei Patient:innen mit akuter myeloischer Leukämie (AML) im Vergleich zu Gesunden überexprimiert ist und der Verlust von RBMX die Leukämieentwicklung verzögert (Prieto et al.,2021). Aktuelle Studien der Arbeitsgruppe Weber haben gehäuft Mutationen von *RBMX* in PTCL nachgewiesen (unpubliziert). Weitere veröffentlichte Daten über die Proteinexpression von RBMX in reifzelligen T-Zell-Lymphomen existierten bis zum Zeitpunkt der Einreichung und Publikation der RBMX-Studie nach bestmöglichem Kenntnisstand nicht.

Insgesamt zeigt sich ein heterogenes und bisher unzureichend kartiertes Bild der EZH1/2-, H3K27me3- und RBMX-Proteinexpressionen im Kontext von Therapieresistenz und Prognoseabschätzung von an T-NHL erkrankten Patient:innen. Diese Proteinexpressionen anhand eigens hergestellter *Tissue Microarrays* (TMA) aus Tumorgewebe, welches vor Einleitung der Erstlinientherapie entnommen wurde, zu erfassen und mit den dazugehörigen klinischen und

histopathologischen Patient:innendaten in Beziehung zu setzen, war Gegenstand der beiden Studien.

Es ergeben sich somit drei wesentliche Ziele der explorativen Arbeiten:

1. Erfassung der Proteinexpression von EZH1, EZH2, H3K27me3 und RBMX sowie der klinisch-pathologischen Daten einer Kohorte von T-Zell-Lymphom Patient:innen
2. Analyse potenzieller Expressionsunterschiede zwischen den T-Zell-Lymphom Subentitäten sowie Korrelation der immunhistochemischen Proteinexpressionsmuster untereinander
3. Untersuchung der Assoziationen zwischen den erhobenen Proteinexpressionsmustern und klinisch-pathologischen Daten, dem Therapieansprechen sowie dem Gesamtüberleben (OS) und dem progressionsfreien Überleben (PFS) der Patient:innen

2 Diskussion

Die vorgelegten Studien untersuchen die Proteinexpression von EZH1, EZH2, H3K27me3 und RBMX anhand von TMAs, um deren prognostische Bedeutung in T-Zell-Lymphomen zu evaluieren. Die Studienkohorte, welche aus 56 Patient:innen besteht, beinhaltet 14 verschiedene T-NHL Subentitäten und bildet damit die immanente Heterogenität der T-Zell-Lymphome ab. In Tabelle 1 ist die Verteilung der Entitäten im Vergleich zu den bereits oben gezeigten weltweiten Daten von Vose et al. aufgeführt (Vose et al.,2008). Es zeigt sich, dass sowohl bei Vose et al. als auch in den beiden vorgelegten Studien PTCL-NOS, AILT und ALCL die größten Subentitäten ausmachen. Das durchschnittliche Alter der Patient:innen bei Diagnose liegt bei 60,26 Jahren (Median: 60,33 Jahre), wobei die Altersspanne von 19 bis 92 Jahren reicht. Es wurden 18 (32,1 %) Patientinnen und 38 (67,9 %) Patienten eingeschlossen.

Tabelle 2: Verteilung der T-Zell Non-Hodgkin-Lymphome (T-NHL) und akuten T-Zell-Leukämien (T-ALL) innerhalb der Studienkohorte im Vergleich zu Vose et al. 2008 (eigene Darstellung)

Entitäten		Studien-kohorte		Vose et al.
		n	%	%
Nodale T-NHL	Peripheres T-Zell-Lymphom, nicht weiter spezifiziert	11	19,6 %	25,9 %
	Angioimmunoblastisches T-Zell-Lymphom	16	28,6 %	18,5 %
	Anaplastisches großzelliges Lymphom, ALK-negativ	7	12,5 %	5,5 %
	Nodales peripheres T-Zell-Lymphom mit T-Helferzell Phänotyp	3	5,4 %	
Extranodale T-NHL	T-Zell Prolymphozyten Leukämie	1	1,8 %	
	T-Zell-Leukämie der groß-granulierten Lymphozyten	2	3,6 %	
	Enteropathie-assoziiertes T-Zell-Lymphom	2	3,6 %	9,1 %
	Monomorphes epitheliotropes intestinales T-Zell-Lymphom	1	1,8 %	
	Extranodales NK-/T-Zell-Lymphom, nasaler Typ	2	3,6 %	4,3 %
	Subkutanes *Panniculitis-like* T-Zell-Lymphom	1	1,8 %	0,5 %
	Mycosis Fungoides	1	1,8 %	
	Sézary Syndrom	1	1,8 %	
	Zerebrales T-Zell-Lymphom	1	1,8 %	
	Lymphoproliferative Erkrankung nach Transplantation, vom polymorphen Subtyp	1	1,8 %	
T-ALL	*Early* T-Zell akute lymphatische Leukämie	3		
	Thymic T-Zell akute lymphatische Leukämie	3		
	Σ	56	100 %	

(ALK: Anaplastische Lymphomkinase, n: Anzahl der Patient:innen, NK: Natürliche Killerzellen)

Als erstes Ergebnis der EZH1/2-Studie kann festgehalten werden, dass im Vergleich zu normalem Lymphknotengewebe die EZH2- und H3K27me3-Proteinexpression in T-Zell-Lymphomen erhöht, während die EZH1-Proteinexpression vermindert ist

(EZH1 vs. normales Lymphknotengewebe: Median = 5 vs. 45; p = 0,001; EZH2 vs. normales Lymphknotengewebe: Median = 85 vs. 30; p = 0,016; H3K27me3 vs. normales Lymphknotengewebe: Median = 185 vs. 130; p = 0.054). Dieser Befund ist vereinbar mit den Erkenntnissen aus bereits besprochenen früheren Veröffentlichungen (Yan et al.,2013; Shi et al.,2015; Abdalkader et al.,2016; Kim et al.,2016; Yi et al.,2018). Die vorgelegte Studie kann ebenfalls keine Expressionsunterschiede zwischen den Lymphomsubentitäten nachweisen, wobei die Aussagekraft dieses Ergebnisses bei der kleinen Anzahl an Patient:innen pro Subentität eingeschränkt belastbar ist.

Weiterhin zeigen die Ergebnisse analog zu denen früherer Veröffentlichungen (Abdalkader et al.,2016; Zhang et al.,2019), dass eine hohe EZH2-Proteinexpression in der Kohorte der nodalen T-NHL mit zwei der erfassten Marker assoziiert ist, die für einen ungünstigeren Verlauf bekannt sind. Denn die EZH2-Proteinexpression ist bei Patient:innen mit B-Symptomen (Median: 130) im Vergleich zu denen ohne B-Symptome (Median: 53,8) erhöht (p = 0,031). Eine erhöhte EZH2-Proteinexpression ist ebenfalls bei Patient:innen mit hohem Ki-67-Index (Median: 150) im Vergleich zu denen mit niedrigem Index (Median: 47,5) zu beobachten (p = 0,059). Jedoch besteht insbesondere mit dem Ansprechen auf Erstlinientherapie keine Assoziation.

Die univariable Überlebenszeitanalyse zeigt in beiden Kohorten bei Patient:innen mit hoher EZH2-Proteinexpression ein kürzeres OS im Vergleich zu denen mit niedriger Expression (T-NHL: medianes OS 78,0 (95 %-Konfidenzintervall (KI): 0,0–178,0) vs. 16,0 Monate (95 %-KI: 0,0– 38,5); p = 0,011; nodale T-NHL: medianes OS 124,0 (95 %-KI: 13,8–234,2) vs. 16,0 Monate (95 %-KI: 0,0–40,0); p = 0,012). Weiterhin ist eine hohe EZH2-Proteinexpression mit einem kürzeren PFS verbunden (T-NHL: medianes PFS 29,0 (95 %-KI: 16,3–41,7) vs. 9,0 Monate (95 %-KI: 4,2–13,8); p = 0,016; nodale T-NHL: medianes PFS 22,0 (95 %-KI: 0,0–71,0) vs. 9,0 Monate (95 %-KI: 4,8–13,2); p = 0,042).

In einem Cox-Regressionsmodell, welches die bekannten prognostischen Faktoren Geschlecht, Alter, ggf. Ann Arbor Stadium, B-Symptome, KM-Infiltration und die erfassten Proteinexpressionen von EZH1, EZH2 und H3K27me3 beinhaltete, ist EZH2 ebenfalls ein unabhängiger prognostischer Marker für das OS (T-NHL: *Hazard Ratio* (HR) = 8,245; 95 %-KI: 1,898-35,826; p = 0,005; nodale T-NHL: HR = 28,398; 95 %-KI: 2,166-372,334; p = 0,011) und PFS (T-NHL: HR = 3,754; 95 %-KI: 1,233-11,426; p = 0,020; nodale T-NHL: HR = 5,147; 95 %-KI: 1,472-17,998; p = 0,010). Damit sind die in dieser Studie vorgelegten Ergebnisse bezüglich der EZH2-Proteinexpression und deren Assoziationen zu Überlebenszeiten und klinischen Markern kohärent zu denen der bereits genannten Studien. Sie unterstützen die Annahme, dass eine höhere EZH2-Proteinexpression ein geeigneter prognostischer Marker zur Vorhersage einer ungünstigen Prognose für Patient:innen mit

T-Zell-Lymphomen ist. Zudem deuten die Daten darauf hin, dass EZH2 ein potenzielles Therapieziel in diesen T-Zell-Neoplasien sein könnte. Dieser Befund macht weitere Untersuchungen zu den zellbiologischen und molekularen Auswirkungen einer erhöhten EZH2-Expression im Kontext von T-NHL dringend erforderlich.

Auch H3K27me3 kann im Vergleich zu normalem Lymphknotengewebe im untersuchten Lymphomgewebe überexprimiert nachgewiesen werden. Lymphome mit hoher Trimethlyierung sind in der univariablen Analyse mit einem schlechteren Überleben assoziiert (T-NHL: medianes OS 58,0 (95 %-KI: 0,0–165,0) vs. 36,0 (95 %-KI: 0,0–73,7) Monate; p = 0,014; nodale T-NHL: medianes OS 124,0 (95 %-KI: 8,6–239,4) vs. 11,0 (95 %-KI: 0,0–50,4) Monate; p = 0,033). Allerdings ist H3K27me3 in der multivariablen Cox-Regressionsanalyse kein unabhängiger prognostischer Marker. Es lassen sich zudem keine Assoziationen zu klinischen oder pathologischen Befunden der Patient:innen feststellen. Eine mögliche Korrelation zwischen der EZH2- und der H3K27me3-Proteinexpression wurde ebenfalls betrachtet, kann jedoch in den Daten nicht beobachtet werden (r = 0,175; p = 0,224). Aus mehreren Gründen legen daher die Ergebnisse dieser Studie nahe, dass EZH2 die Prognose von Patient:innen mit T-Zell-Lymphomen möglicherweise durch seine nicht-kanonischen Funktionen beeinflussen könnte. Denn es besteht keine Korrelation der Proteinexpression von EZH2 und H3K27me3 sowie kein Zusammenhang zwischen der H3K27me3-Proteinexpression und den untersuchten klinisch-pathologischen Markern. Darüber hinaus ist die H3K27me3-Proteinexpression in der multivariablen Analyse kein unabhängiger prognostischer Marker für das Überleben der untersuchten Patient:innen. Dies steht im Einklang mit vorliegenden Daten aus ENKTL-Studien, bei denen eine EZH2-Überexpression ebenfalls nicht mit der H3K27-Trimethylierung assoziiert war und die Autor:innen auf eine nicht-kanonische Funktion von EZH2 in ENKTL schließen (Yan et al.,2013; Yan et al.,2016).

Anders als bei den bereits beschriebenen Proteinen zeigt sich die EZH1-Proteinexpression im Vergleich zum untersuchten Normalgewebe über die gesamte Kohorte hinweg sehr gering. In der univariablen Analyse ist eine niedrige EZH1-Proteinexpression mit einem schlechten OS verbunden (T-NHL: medianes OS 16,0 (95 %-KI: 7,6-24,4) vs. 124,0 (95 %-KI: 16,4-232,0) Monate; p = 0,016; nodale T-NHL: medianes OS 16,0 (95 %-KI: 2,5-29,5) vs. 124,0 (95 %-KI: 0,0 -272,9) Monate; p = 0,020). Darüber hinaus ist die EZH1-Proteinexpression im Cox-Regressionsmodell ein unabhängiger prognostischer Marker für das OS (T-NHL: HR = 0,183; 95 %-KI: 0,044-0,767; p = 0,020; nodale T-NHL: HR = 0,085; 95 %-KI: 0,008-0,859; p = 0,037). Eine Assoziation zwischen der EZH1-Proteinexpression und klinischen oder pathologischen Markern kann allerdings ebenso wenig wie

eine Korrelation zwischen EZH1 und EZH2 nachgewiesen werden. Einschränkend ist den Ergebnissen hinzuzufügen, dass die Proteinexpression wie bereits beschrieben insgesamt sehr gering war, was die Beurteilung der Färbungen und das Festlegen des H-Scores für die Untersucher:innen erschwerte. Dies könnte auch eine mögliche Erklärung für die fehlenden Korrelationen zwischen EZH1 und EZH2 sowie den klinisch pathologischen Markern sein.

Die Arbeitsgruppe Weber erhob parallel mittels *Next Generation Sequencing* den *EZH1/2*-Mutationsstatus in einer Kohorte von monomorphen epitheliotropen intestinalen T-Zell-Lymphomen (MEITL). Klinische Daten dieser Kohorte waren nicht vorhanden. Insgesamt wurden 11 Fälle auf *EZH1*-Mutationen und 33 Fälle auf *EZH2*-Mutationen untersucht. Die Analysen weisen lediglich eine *EZH2*-Mutation, die in der Su(var)3-9-Enhancer-of-zeste-Trithorax (SET)-Domäne lokalisiert ist, nach. In den untersuchten Proben kann keine einzige *EZH1*-Mutation identifiziert werden. Auf Grundlage der singulären *EZH2*-Mutation lassen sich potenzielle Zusammenhänge zur Proteinexpression von EZH1, EZH2 oder H3K27me3 nicht untersuchen. Diese Ergebnisse stehen im Einklang mit früheren Studien, in denen *EZH2*-Mutationen bei T-NHL selten waren: einer von 36 Fällen (2,7 %) PTCL-NOS und ein AITL-Fall von 84 Fällen (1,2 %) in einer Kohorte bestehend aus AITL, PTCL-NOS, ALCL und MEITL, die sich als Einzelnukleotidvarianten präsentieren (Maura et al.,2020). Darüber hinaus haben mehrere Gruppen auch mittels *Whole exome sequencing* keine *EZH1*-Mutationen feststellen können (Palomero et al.,2014; Sakata-Yanagimoto et al.,2014; Crescenzo et al.,2015; Roberti et al.,2016; Wang et al.,2017; Watatani et al.,2019; Laginestra et al.,2020; Maura et al.,2020).

Mechanistische Studien zeigen, dass im Vergleich zu etablierten EZH2-spezifischen Inhibitoren (GSK126 oder Tazemetostat) die duale EZH1- und EZH2-Hemmung (z.B. Valemetostat) zu einer deutlich stärkeren Senkung der Trimethylierung führt (Yamagishi et al.,2019; Kagiyama et al.,2021). Bei selektiver EZH2-Hemmung kommt es nicht zu einer vollständigen Herunterregulierung von H3K27me3, vermutlich weil EZH1 in der Lage ist, Teilaspekte der EZH2-Funktion bei deren Verlust zu kompensieren (Shen et al.,2008; Yamagishi et al.,2019). Die duale Hemmung von EZH2 und EZH1 führt über eine effektive H3K27me3-Hemmung zu einer starken Letalität in verschiedenen Lymphomen, darunter auch PTCL (Yamagishi et al.,2019). Diese Erkenntnisse stehen in Diskrepanz zu den bereits besprochenen Daten zur nicht-kanonischen Rolle von EZH2 bei der Entstehung von ENKTL (Yan et al.,2013; Yan et al.,2016). Welche Funktionen von EZH1/2 – die kanonische oder die nicht-kanonische – in welchem Ausmaß in der Pathogenese von T-Zell-Lymphomen eine Rolle

spielen oder ob beide Mechanismen gleichzeitig aber abhängig vom zellulären Kontext agieren, gilt es zukünftig zu beleuchten.

Zusammenfassend lässt sich konstatieren, dass EZH1 und EZH2 in den untersuchten T-Zell-Lymphomen prognostische Marker sind, wobei eine erhöhte EZH2-Expression und eine erniedrigte EZH1-Expression jeweils mit einem ungünstigen Krankheitsverlauf assoziiert sind. In Ermangelung bereits publizierter Daten und aufgrund ihrer Heterogenität zu den Auswirkungen der EZH1/2-Expression auf Überlebensparameter in anderen Malignomen wurden in einem nächsten Schritt mithilfe des *The Cancer Genome Atlas* (TCGA) (https://tcga-data.nci.nih.gov/tcga/) die EZH1/2-Genexpressionen in der aus mehreren Neoplasien zusammengesetzten TCGA *Pan-Cancer* (PANCAN)-Studie betrachtet. Auch hier zeigt sich ein divergenter Zusammenhang der EZH1- bzw. EZH2-Expression zu den Überlebensraten. Für die weitere Verifikation der prognostischen Bedeutung von EZH1/2 in T-NHL sind prospektive Studien mit größeren Kohorten erforderlich. Darüber hinaus sind weitere experimentelle Untersuchungen wichtig, um die molekularen Mechanismen von EZH2 insbesondere im Zusammenspiel mit EZH1 in T-Zell-Lymphomen aufzuklären. Dieses Verständnis kann dabei helfen, die Effektivität der Therapie mit spezifischen EZH2- und/ oder dualen EZH1/2-Inhibitoren in biologisch rational definierten Gruppen von T-NHL zu überprüfen.

Die RBMX-Studie betrachtet die immunhistochemische Proteinexpression von RBMX in T-Zell-Lymphomen, dessen Alteration in verschiedenen Malignomen mit Tumorgenese und Resistenzentwicklung assoziiert ist (Cai et al.,2021). Es lassen sich keine Unterschiede der RBMX-Proteinexpression zwischen Tumor- und Normalgewebe feststellen. Weiterhin können erneut keine Proteinexpressionsunterschiede zwischen den Subentitäten gefunden werden. Dieses Ergebnis muss ebenfalls vor dem Hintergrund der kleinen Anzahl an Patient:innen pro Subentität betrachtet werden.

Eine hohe RBMX-Proteinexpression ist mit klinischen Parametern assoziiert, die für einen moderaten Verlauf der Krankheit bekannt sind (Leukozytenzahl innerhalb des Referenzbereiches vs. Leukozytenzahl erhöht: Mittelwert 193 vs. 118, ($p = 0.023$), keine KM-Infiltration vs. KM-Infiltration: Mittelwert 152 vs. 107, ($p = 0.090$)). Im Gegensatz dazu zeigt sich in den ersten Betrachtungen dieser Studie eine hohe RBMX-Proteinexpression bei Patient:innen, die auf die Erstlinienbehandlung nicht ansprachen (*Stable Disease* oder *Progressive Disease*) und zwar unabhängig vom verwendeten Therapieregime (Resistent vs. sensitiv: Mittelwert 178 vs. 128; $p = 0,029$). Auch in weiterführenden Analysen, in die nur Patient:innen, die in Erstlinientherapie CHOP bzw. CHOEP erhielten,

eingeschlossen wurden, sind hohe RBMX-Proteinexpressionswerte mit einer Therapieresistenz auf die anthrazyklinhaltige Chemotherapie bei Patient:innen mit T-NHL assoziiert (Resistent vs. sensitiv: Mittelwert 185 vs. 127; p = 0,018; *Receiver Operating Characteristic (*ROC)-Analyse *Area Under Curve* (AUC) = 0,725; 95 %-KI: 0,550-0,901; p = 0,012). Allerdings können diese Ergebnisse in der kleineren Untergruppe der primär nodalen T-NHL nicht beobachtet werden, was möglicherweise auf die geringe Größe der Untergruppe zurückzuführen ist. Die Daten stimmen mit den kürzlich von Song et al. veröffentlichten überein, die ebenfalls eine Chemotherapieresistenz bei gleichzeitiger RBMX-Überexpression in HCC zeigen (Song et al.,2020).

Frühere Studien zeichnen wie bereits beschrieben ein indifferentes Bild zwischen RBMX-Expressionsniveau und Prognose bei verschiedenen Krebsarten (Ouyang et al.,2011; Hirschfeld et al.,2015; Guo et al.,2020; Song et al.,2020; Prieto et al.,2021; Yan et al.,2021). In der univariablen Analyse ist eine hohe RBMX-Proteinexpression mit einem kürzeren OS assoziiert (T-NHL: medianes OS 78,0 (95 %-KI: 0,0-160,2) vs. 11,0 (95 %-KI: 5,5-16,5) Monate; p < 0,001; nodale T-NHL: medianes OS 124,0 (95 %-KI: 14,0-233,9) vs. 13,0 (95 %-KI: 8,7-17,3) Monate; p = 0,001). Weiterhin besteht eine Assoziation zwischen hoher RBMX-Proteinexpression und einem kürzeren PFS bei der Kohorte von T-NHL Patient:innen (medianes OS 17,0 (95 %-KI: 2,6-31,4) vs. 7,0 (95 %-KI: 0,0-14,4) Monate; p = 0,012). Im Cox-Regressionsmodell, welches Geschlecht, Alter, ggf. Ann Arbor Stadium, B-Symptome und KM-Infiltration einschloss, zeigt sich ebenfalls, dass RBMX in T-NHL ein unabhängiger prognostischer Marker für das OS (HR = 0,204; 95 %-KI: 0,064-0,646; p = 0,007) und das PFS (HR = 0,235; 95 %-KI: 0,083-0,666; p = 0,006) ist. Bei Patient:innen mit nodalem T-NHL-Phänotyp war die RBMX-Proteinexpression ein unabhängiger prognostischer Marker ausschließlich für das OS (HR = 0,149; 95 %-KI: 0,025-0,898; p = 0,038).

Die Ergebnisse deuten an, dass die RBMX-Proteinexpression zur Chemotherapieresistenz beiträgt und somit bei hoher Ausprägung das Überleben von Patient:innen mit T-NHL negativ beeinflusst. Bisher veröffentlichte Studien zeigen wie bereits ausgeführt ein inhomogenes Bild im Kontext verschiedener Neoplasien. Eine bereits nach dieser Studie erschienene Veröffentlichung legt Daten vor, die die tumorsuppressiven Eigenschaften von RBMX untermauern, und zeigt, dass RBMX die Produktion von pro-apoptotischen Proteinen fördert und gleichzeitig die Produktion von Onkogenen herunterfährt (Hao et al.,2022). Diese Daten legen eine vom tumorbiologischen Kontext abhängige Auswirkung einer alterierten RBMX-Expression nahe. Translationale Studien mit größeren Kohorten könnten zu einem besseren Verständnis der Funktion von RBMX bei Krebsentstehung sowie Chemotherapieresistenz und dessen Relevanz als prognostischer Biomarker

führen. Darüber hinaus sind weitere mechanistische Zell- und molekularbiologische Experimente in Modellen mit definierten tumorbiologischen Hintergründen unabdingbar, um die molekularen Mechanismen und biologischen Funktionen von RBMX-Alterationen zu verstehen.

Aufgrund des retrospektiven Charakters der beiden vorgelegten explorativen Studien, der großen Heterogenität der Kohorte bei gleichzeitig geringer Fallzahl weisen die Ergebnisse einige Limitationen auf. Aufgrund der Seltenheit von T-Zell-Neoplasien ist eine referenzpathologische Begutachtung klinischer Standard, weshalb die Diagnosen aller Patient:innen vor Einschluss in die Studie referenzpathologisch nach den Kriterien der zum Zeitpunkt der Studie gültigen vierten WHO- bzw. *European Group for the Immunological Characterization of Leukemias* (EGIL)-Klassifikationen begutachtet wurden. Dabei kam es zur Reklassifizierung von vier Patient:innen. Ein Patient, dessen Diagnose nicht eindeutig zu stellen war, musste von der Studie ausgeschlossen werden.

Letztlich konnten Gewebeproben von 56 Patient:innen mit T-Zell-Neoplasien (T-NHL und T-ALL Fälle) zur Konstruktion der TMAs genutzt werden. Um deren Vergleichbarkeit zu gewährleisten, mussten weitere vier Patient:innen von den Expressionsanalysen ausgeschlossen werden. Denn von ihnen war ausschließlich Gewebematerial vorhanden, welches erst nach Einleitung der Erstlinientherapie – zum Beispiel im Rahmen von Rezidivdiagnostik – entnommen worden war. Von verbliebenen 52 Patient:innen war Tumorgewebe, welches vor Einleitung der Erstlinientherapie gewonnen worden war, vorhanden. In den Veröffentlichungen wurden zusätzlich die Daten der Patient:innen mit T-ALL (n=6) aufgrund der stark von den T-NHL abweichenden Tumorbiologie ausgeschlossen, was die Fallzahl auf 46 (EZH1/2-Studie) bzw. 43 (RBMX-Studie) T-NHL Patient:innen mit vollständigen klinischen Daten und auswertbaren Tumormaterial reduzierte. Aufgrund der geringen Fallzahl unterliegt die explorative Studie einem hohen Risiko von statistischen Fehlern bei multiplen Tests. Die Interpretation der Ergebnisse ist im Sinne des Ansatzes der Studie explorativ zu werten.

Die TMAs wurden aus Formalin-fixiertem und paraffineingebettetem (FFPE) Lymphom- und Leukämiegewebe konstruiert. Aus jedem Tumorblock wurden zwei Stanzen entnommen und bei der Auswertung der Mittelwert der H-Scores (McCarty et al.,1985) gebildet, um die Repräsentativität der Gewebestanzen für das Tumorgewebe zu gewährleisten. Verwendet wurden im Institut für Pathologie des Universitätsklinikums Halle (Saale) (UKH) lagernde Gewebeblöcke von nach abgeschlossener Diagnostik verbliebenen Tumorprobenresten. Die Gewebeproben befanden sich zum Teil mehrere Jahre im hiesigen Archiv, sodass trotz optimaler Lagerungsbedingungen grundsätzlich die Gefahr einer Antigendegradierung besteht (Xie et al.,2011). Es wurden je nach

Verfügbarkeit der Gewebeblöcke verschiedene Gewebearten integriert. Den größten Anteil machen Lymphknotengewebe (52,8 %) und KM-Gewebe (18,9 %) aus. Weiterhin wurden in absteigender Reihenfolge Bindegewebe (5,7 %), Nasopharynx (5,7 %), Darmgewebe (5,7 %), Hautgewebe (3,8 %), Milzgewebe (1,9 %), Tonsille (1,9 %) und Mediastinum (1,9 %) eingeschlossen. Insbesondere das entkalkungsbedürftige knöcherne Gewebe lässt, bedingt durch den Entkalkungsprozess, eine gewisse Alteration der Antigene erwarten. Obwohl Lymphknotengewebe den größten Anteil ausmachte, ist die Vergleichbarkeit der verschiedenen Materialarten zu hinterfragen. Weiterhin ist bei den Proben, die vor 2014 im Institut für Pathologie bearbeitet wurden, nicht bekannt, ob in jedem Fall gepufferter Formaldehyd zur Anwendung kam. Um die Auswertung der Stanzen mittels H-Score zu objektivieren, wurden sie unabhängig von zwei Untersucher:innen (Franziska Lea Schümann, Dr. med. Marcus Bauer) ausgewertet. Zur Qualitätskontrolle der immunhistochemischen Färbungen wurden Gewebeproben mit bekanntem Färbestatus mitgeführt. Zusätzlich halfen die nachträglich durchgeführten Großflächenschnitte dabei, die Färbereaktion von *Bystander*-Immun- sowie Stroma-Zellen einordnen zu können. Es ist zu beachten, dass bei den immunhistochemischen Färbungen die EZH1-, EZH2-, H3K27me3- und RBMX-Expression auf Proteinlevel nachgewiesen wird, sodass potentielle Mutationen, Spleißvarianten oder andere posttranslationale Modifikationen nicht erfasst werden können.

Neben den Stärken der TMA Technik (große Probenanzahl, geringe Kosten bei Materialersparnis, Erhalt der Gewebeproben für spätere Untersuchungen, standardisierte Auswertungsbedingungen (Bubendorf et al.,2001; Kallioniemi et al.,2001)) zeigten sich im Verlauf der Arbeit in der Methode begründete Hindernisse. Aufgrund des kleinen Durchmessers der in die TMAs eingebrachten Gewebestanzen gingen einzelne Areale beim Schneide- und Färbeprozess gehäuft verloren. Erst der Einsatz hochadhäsiver Objektträger (Apex BOND, Leica) führte zu einer verbesserten Adhäsion der Gewebestanzen auf den Objektträgern. Letztlich erwiesen sich im Durchschnitt 11,9 % der Stanzen als nicht auswertbar, was den Angaben der Literatur entspricht (Gillett et al.,2000).

Die klinischen Patient:innendaten wurden retrospektiv von der Verfasserin erhoben. Teilweise unvollständig waren insbesondere die Daten von Patient:innen, die erst im späteren Verlauf ihrer Erkrankung im UKH behandelt worden waren, da detaillierte klinische Daten ihrer Erstdiagnose zum Teil nicht in den jeweiligen Akten vermerkt waren. Insgesamt waren Daten zu Laborwerten, dem ECOG-Status und dem Ki-67-Index inkomplett, weshalb eine Beurteilung der Zusammenhänge zu den Proteinexpressionsdaten kritisch zu hinterfragen ist.

Die äußerste Seltenheit der Erkrankung und deren Heterogenität stellen Studien zu T-Zell-Lymphomen vor große Herausforderungen, denn meist können nur wenige Patient:innen einer Tumorentität integriert werden. Die Studienkohorte ist sehr heterogen und musste für die Analysen in Subgruppen (T-NHL und nodale T-NHL), die wiederum in sich sehr divers sind, unterteilt werden. In früheren Studien, die sich primär mit der EZH2-Proteinexpression befassten, konnten 2016 Abdalkader et al. 25 Patient:innen, 2015 Shi et al. 57 Patient:innen, und 2019 Zhang et al. 68 Patient:innen mit primär nodalen T-NHL einschließen. Bei einer Fallzahl von 34 nodalen T-NHL kann also durchaus ein Vergleich zu älteren Studien stattfinden. Bisher sind der Verfasserin keine Daten zur RBMX-Proteinexpression in T-NHL bekannt, sodass diese explorative Betrachtung erste wichtige Hinweise in diesem Forschungsfeld gibt. Die geringe Stichprobengröße, die bei der Untersuchung von T-Zell-Lymphomen leider üblich ist, stellt Wissenschaftler:innen vor große Herausforderungen und ist einer der Gründe, aus dem sich Patient:innen mit T-Zell-Lymphomen einem unbefriedigendem Ansprechen auf Standardtherapie und einer schlechten Langzeitprognose ausgesetzt sehen. Um diese Lücke zu schließen, befindet sich derzeit ein deutschlandweites Register sowie eine Biomaterialdatenbank für reifzellige systemische T-Zell-Lymphome der German Lymphoma Alliance und der Ostdeutschen Studiengruppe Hämatologie und Onkologie im Aufbau. Die für dieses Dissertationsvorhaben erhobenen Daten wurden bereits in dieses Register integriert.

Zusammenfassend weist die EZH1/2-Studie entgegengesetzte Expressionsmuster der untersuchten Proteine nach und zeigt einen divergenten prognostischen Einfluss in den Überlebensanalysen. In der univariablen Analyse ist eine hohe EZH1-Proteinexpression mit einer verbesserten Prognose verbunden, während eine hohe EZH2- und H3K27me3-Proteinexpression mit einer schlechteren assoziiert ist. Die multivariable Analyse zeigt, dass die EZH2- und EZH1-Proteinexpression unabhängige, in unterschiedliche Richtungen weisende prognostische Parameter für das Überleben sind. Diese Ergebnisse deuten darauf hin, dass neben dem epigenetischen Modifikator EZH2, der bereits in zahlreichen Studien untersucht wurde, auch sein Paralog EZH1 insbesondere im Zusammenspiel mit EZH2 in der Entstehung von T-Zell-Neoplasien eine wichtige Rolle spielen könnte. Die RBMX-Studie belegt, dass eine hohe RBMX-Proteinexpression ein schlechtes Ansprechen auf eine anthrazyklinhaltige Erstlinientherapie vorhersagt und mit einer schlechteren Prognose verbunden ist.

Beide Studien zeigen, dass die Proteinexpressionen von häufig alterierten epigenetischen Modifikatoren und RNA-Bindeproteinen in der Lage sind, die Prognose von Patient:innen mit T-Zell-Neoplasien zu bestimmen. Sowohl EZH1/2 als auch RBMX sind nicht nur vielversprechende

prognostische Marker, sondern auch mögliche therapeutische Ziele im Kontext von T-Zell-Lymphomen. Die weitere experimentelle Untersuchung der molekularen Mechanismen ist deshalb unerlässlich, um die noch stark begrenzten Therapiemöglichkeiten der Patient:innen zu erweitern und deren Prognose damit langfristig zu verbessern.

3 Literaturverzeichnis

Abdalkader L, Oka T, Takata K, Sato H, Murakami I, Otte AP, Yoshino T (2016) Aberrant differential expression of EZH1 and EZH2 in Polycomb repressive complex 2 among B- and T/NK-cell neoplasms. Pathology 48 (5):467-482.

Adams SV, Newcomb PA, Shustov AR (2016) Racial Patterns of Peripheral T-Cell Lymphoma Incidence and Survival in the United States. J Clin Oncol 34 (9):963-971.

Adamson B, Smogorzewska A, Sigoillot FD, King RW, Elledge SJ (2012) A genome-wide homologous recombination screen identifies the RNA-binding protein RBMX as a component of the DNA-damage response. Nat Cell Biol 14 (3):318-328.

Anderson JR, Armitage JO, Weisenburger DD (1998) Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 9 (7):717-720.

Antonello ZA, Hsu N, Bhasin M, Roti G, Joshi M, Van Hummelen P, Ye E, Lo AS, Karumanchi SA, Bryke CR, Nucera C (2017) Vemurafenib-resistance via de novo RBM genes mutations and chromosome 5 aberrations is overcome by combined therapy with palbociclib in thyroid carcinoma with BRAF(V600E). Oncotarget 8 (49):84743-84760.

Anwar T, Gonzalez ME, Kleer CG (2021) Noncanonical Functions of the Polycomb Group Protein EZH2 in Breast Cancer. Am J Pathol 191 (5):774-783.

Armitage JO, Weisenburger DD (1998) New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 16 (8):2780-2795.

Bachmann IM, Halvorsen OJ, Collett K, Stefansson IM, Straume O, Haukaas SA, Salvesen HB, Otte AP, Akslen LA (2006) EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. J Clin Oncol 24 (2):268-273.

Bellei M, Federico M (2019) The outcome of peripheral T-cell lymphoma patients failing first-line therapy: a report from the prospective International T-Cell Project. Haematologica 104 (4):e178.

Bubendorf L, Nocito A, Moch H, Sauter G (2001) Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput in situ studies. J Pathol 195 (1):72-79.

Cai MY, Hou JH, Rao HL, Luo RZ, Li M, Pei XQ, Lin MC, Guan XY, Kung HF, Zeng YX, Xie D (2011) High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. Mol Med 17 (1-2):12-20.

Cai T, Cinkornpumin JK, Yu Z, Villarreal OD, Pastor WA, Richard S (2021) Deletion of RBMX RGG/RG motif in Shashi-XLID syndrome leads to aberrant p53 activation and neuronal differentiation defects. Cell Rep 36 (2):109337.

Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298 (5595):1039-1043.

Cen Y, Chen L, Liu Z, Lin Q, Fang X, Yao H, Gong C (2023) Novel roles of RNA-binding proteins in drug resistance of breast cancer: from molecular biology to targeting therapeutics. Cell Death Discov 9 (1):52.

Crescenzo R, Abate F, Lasorsa E, Tabbo F, Gaudiano M, Chiesa N, Di Giacomo F, Spaccarotella E, Barbarossa L, Ercole E, Todaro M, Boi M, Acquaviva A, Ficarra E, Novero D, Rinaldi A, Tousseyn T, Rosenwald A, Kenner L, Cerroni L, Tzankov A, Ponzoni M, Paulli M, Weisenburger D, Chan WC, Iqbal J, Piris MA, Zamo A, Ciardullo C, Rossi D, Gaidano G, Pileri S, Tiacci E, Falini B, Shultz LD, Mevellec L, Vialard JE, Piva R, Bertoni F, Rabadan R, Inghirami G, European T-Cell Lymphoma Study Group TCPPCoDiPwPTCL, the AxCG-DTMoLM (2015) Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. Cancer Cell 27 (4):516-532.

Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V (2002) Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 111 (2):185-196.

d'Amore F, Gaulard P, Trumper L, Corradini P, Kim WS, Specht L, Bjerregaard Pedersen M, Ladetto M, Committee EG (2015) Peripheral T-cell lymphomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 26 Suppl 5:v108-115.

Danis E, Yamauchi T, Echanique K, Zhang X, Haladyna JN, Riedel SS, Zhu N, Xie H, Orkin SH, Armstrong SA, Bernt KM, Neff T (2016) Ezh2 Controls an Early Hematopoietic Program and Growth and Survival Signaling in Early T Cell Precursor Acute Lymphoblastic Leukemia. Cell Rep 14 (8):1953-1965.

Dreyfuss G, Matunis MJ, Pinol-Roma S, Burd CG (1993) hnRNP proteins and the biogenesis of mRNA. Annu Rev Biochem 62:289-321.

Duan R, Du W, Guo W (2020) EZH2: a novel target for cancer treatment. J Hematol Oncol 13 (1):104.

El Marabti E, Younis I (2018) The Cancer Spliceome: Reprograming of Alternative Splicing in Cancer. Front Mol Biosci 5:80.

Gallamini A, Stelitano C, Calvi R, Bellei M, Mattei D, Vitolo U, Morabito F, Martelli M, Brusamolino E, Iannitto E, Zaja F, Cortelazzo S, Rigacci L, Devizzi L, Todeschini G, Santini G, Brugiatelli M, Federico M, Intergruppo Italiano L (2004) Peripheral T-cell lymphoma unspecified (PTCL-U):

a new prognostic model from a retrospective multicentric clinical study. Blood 103 (7):2474-2479.

Gan L, Yang Y, Li Q, Feng Y, Liu T, Guo W (2018) Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. Biomark Res 6:10.

Gebauer F, Schwarzl T, Valcarcel J, Hentze MW (2021) RNA-binding proteins in human genetic disease. Nat Rev Genet 22 (3):185-198.

Gillett CE, Springall RJ, Barnes DM, Hanby AM (2000) Multiple tissue core arrays in histopathology research: a validation study. J Pathol 192 (4):549-553.

Glazer RI, Knode MC, Tseng CK, Haines DR, Marquez VE (1986) 3-Deazaneplanocin A: a new inhibitor of S-adenosylhomocysteine synthesis and its effects in human colon carcinoma cells. Biochem Pharmacol 35 (24):4523-4527.

Gökbuget N (2015) Aktuelle Therapie der akuten lymphatischen Leukämie des Erwachsenen. Internist (Berl) 56 (4):344-353.

Guillemin A, Kumar A, Wencker M, Ricci EP (2021) Shaping the Innate Immune Response Through Post-Transcriptional Regulation of Gene Expression Mediated by RNA-Binding Proteins. Front Immunol 12:796012.

Guo J, Wang X, Jia J, Jia R (2020) Underexpression of SRSF3 and its target gene RBMX predicts good prognosis in patients with head and neck cancer. J Oral Sci 62 (2):175-179.

Hamadani M, Ngoya M, Sureda A, Bashir Q, Litovich CA, Finel H, Chen Y, Boumendil A, Zain J, Castagna L, Cashen AF, Blaise D, Shadman M, Pastano R, Khimani F, Arat M, Dietrich S, Schmitz N, Glass B, Kharfan-Dabaja MA, Corradini P, Sauter CS, Montoto S, Kwon M, Herrera AF, Dreger P (2022) Outcome of allogeneic transplantation for mature T-cell lymphomas: impact of donor source and disease characteristics. Blood Adv 6 (3):920-930.

Hao C, Zheng Y, Jonsson J, Cui X, Yu H, Wu C, Kajitani N, Schwartz S (2022) hnRNP G/RBMX enhances HPV16 E2 mRNA splicing through a novel splicing enhancer and inhibits production of spliced E7 oncogene mRNAs. Nucleic Acids Res.

He LR, Liu MZ, Li BK, Rao HL, Liao YJ, Guan XY, Zeng YX, Xie D (2009) Prognostic impact of H3K27me3 expression on locoregional progression after chemoradiotherapy in esophageal squamous cell carcinoma. BMC Cancer 9:461.

Heinrich B, Zhang Z, Raitskin O, Hiller M, Benderska N, Hartmann AM, Bracco L, Elliott D, Ben-Ari S, Soreq H, Sperling J, Sperling R, Stamm S (2009) Heterogeneous nuclear ribonucleoprotein G regulates splice site selection by binding to CC(A/C)-rich regions in pre-mRNA. J Biol Chem 284 (21):14303-14315.

Herviou L, Cavalli G, Cartron G, Klein B, Moreaux J (2016) EZH2 in normal hematopoiesis and hematological malignancies. Oncotarget 7 (3):2284-2296.

Hirschfeld M, Ouyang YQ, Jaeger M, Erbes T, Orlowska-Volk M, Zur Hausen A, Stickeler E (2015) HNRNP G and HTRA2-BETA1 regulate estrogen receptor alpha expression with potential impact on endometrial cancer. BMC Cancer 15:86.

Horwitz S, O'Connor OA, Pro B, Illidge T, Fanale M, Advani R, Bartlett NL, Christensen JH, Morschhauser F, Domingo-Domenech E, Rossi G, Kim WS, Feldman T, Lennard A, Belada D, Illes A, Tobinai K, Tsukasaki K, Yeh SP, Shustov A, Huttmann A, Savage KJ, Yuen S, Iyer S, Zinzani PL, Hua Z, Little M, Rao S, Woolery J, Manley T, Trumper L, Group E-S (2019) Brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma (ECHELON-2): a global, double-blind, randomised, phase 3 trial. Lancet 393 (10168):229-240.

Horwitz S, O'Connor OA, Pro B, Trumper L, Iyer S, Advani R, Bartlett NL, Christensen JH, Morschhauser F, Domingo-Domenech E, Rossi G, Kim WS, Feldman T, Menne T, Belada D, Illes A, Tobinai K, Tsukasaki K, Yeh SP, Shustov A, Huttmann A, Savage KJ, Yuen S, Zinzani PL, Miao H, Bunn V, Fenton K, Fanale M, Puhlmann M, Illidge T (2022) The ECHELON-2 Trial: 5-year results of a randomized, phase III study of brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma. Annals of Oncology 33 (3):288-298.

Hoy SM (2020) Tazemetostat: First Approval. Drugs 80 (5):513-521.

Huang J, Gou H, Yao J, Yi K, Jin Z, Matsuoka M, Zhao T (2021) The noncanonical role of EZH2 in cancer. Cancer Sci 112 (4):1376-1382.

Iqbal J, Wright G, Wang C, Rosenwald A, Gascoyne RD, Weisenburger DD, Greiner TC, Smith L, Guo S, Wilcox RA, Teh BT, Lim ST, Tan SY, Rimsza LM, Jaffe ES, Campo E, Martinez A, Delabie J, Braziel RM, Cook JR, Tubbs RR, Ott G, Geissinger E, Gaulard P, Piccaluga PP, Pileri SA, Au WY, Nakamura S, Seto M, Berger F, de Leval L, Connors JM, Armitage J, Vose J, Chan WC, Staudt LM, Lymphoma Leukemia Molecular Profiling P, the International Peripheral TcLP (2014) Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. Blood 123 (19):2915-2923.

Ito T, Teo YV, Evans SA, Neretti N, Sedivy JM (2018) Regulation of Cellular Senescence by Polycomb Chromatin Modifiers through Distinct DNA Damage- and Histone Methylation-Dependent Pathways. Cell Rep 22 (13):3480-3492.

Kagiyama Y, Fujita S, Shima Y, Yamagata K, Katsumoto T, Nakagawa M, Honma D, Adachi N, Araki K, Kato A, Inaki K, Ono Y, Fukuhara S, Kobayashi Y, Tobinai K, Kitabayashi I (2021) CDKN1C-mediated growth inhibition by an EZH1/2 dual inhibitor overcomes resistance of mantle cell lymphoma to ibrutinib. Cancer Sci 112 (6):2314-2324.

Kallioniemi OP, Wagner U, Kononen J, Sauter G (2001) Tissue microarray technology for high-throughput molecular profiling of cancer. Hum Mol Genet 10 (7):657-662.

Kim SH, Yang WI, Min YH, Ko YH, Yoon SO (2016) The role of the polycomb repressive complex pathway in T and NK cell lymphoma: biological and prognostic implications. Tumour Biol 37 (2):2037-2047.

Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D (2002) Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev 16 (22):2893-2905.

Laginestra MA, Cascione L, Motta G, Fuligni F, Agostinelli C, Rossi M, Sapienza MR, Righi S, Broccoli A, Indio V, Melle F, Tabanelli V, Calleri A, Novero D, Facchetti F, Inghirami G, Sabattini E, Bertoni F, Pileri SA (2020) Whole exome sequencing reveals mutations in FAT1 tumor suppressor gene clinically impacting on peripheral T-cell lymphoma not otherwise specified. Mod Pathol 33 (2):319.

Lemonnier F, Couronne L, Parrens M, Jais JP, Travert M, Lamant L, Tournillac O, Rousset T, Fabiani B, Cairns RA, Mak T, Bastard C, Bernard OA, de Leval L, Gaulard P (2012) Recurrent TET2 mutations in peripheral T-cell lymphomas correlate with TFH-like features and adverse clinical parameters. Blood 120 (7):1466-1469.

Li B, Chng WJ (2019) EZH2 abnormalities in lymphoid malignancies: underlying mechanisms and therapeutic implications. J Hematol Oncol 12 (1):118.

Li C, Wang Y, Gong Y, Zhang T, Huang J, Tan Z, Xue L (2021) Finding an easy way to harmonize: a review of advances in clinical research and combination strategies of EZH2 inhibitors. Clin Epigenetics 13 (1):62.

Liu J, Liang L, Huang S, Nong L, Li D, Zhang B, Li T (2019) Aberrant differential expression of EZH2 and H3K27me3 in extranodal NK/T-cell lymphoma, nasal type, is associated with disease progression and prognosis. Hum Pathol 83:166-176.

Mak V, Hamm J, Chhanabhai M, Shenkier T, Klasa R, Sehn LH, Villa D, Gascoyne RD, Connors JM, Savage KJ (2013) Survival of patients with peripheral T-cell lymphoma after first relapse or progression: spectrum of disease and rare long-term survivors. J Clin Oncol 31 (16):1970-1976.

Margueron R, Li GH, Sarma K, Blais A, Zavadil J, Woodcock CL, Dyniacht BD, Reinberg D (2008) Ezh1 and Ezh2 Maintain Repressive Chromatin through Different Mechanisms. Molecular Cell 32 (4):503-518.

Margueron R, Reinberg D (2011) The Polycomb complex PRC2 and its mark in life. Nature 469 (7330):343-349.

Martinez-Contreras R, Cloutier P, Shkreta L, Fisette JF, Revil T, Chabot B (2007) hnRNP proteins and splicing control. Adv Exp Med Biol 623:123-147.

Matsunaga S, Takata H, Morimoto A, Hayashihara K, Higashi T, Akatsuchi K, Mizusawa E, Yamakawa M, Ashida M, Matsunaga TM, Azuma T, Uchiyama S, Fukui K (2012) RBMX: a regulator for maintenance and centromeric protection of sister chromatid cohesion. Cell Rep 1 (4):299-308.

Maura F, Dodero A, Carniti C, Bolli N, Magni M, Monti V, Cabras A, Leongamornlert D, Abascal F, Diamond B, Rodriguez-Martin B, Zamora J, Butler A, Martincorena I, Tubio JMC, Campbell PJ, Chiappella A, Pruneri G, Corradini P (2020) <i>CDKN2A</i> deletion is a frequent event associated with poor outcome in patients with peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). Haematologica Online ahead of print.

McCarty KS, Jr., Miller LS, Cox EB, Konrath J, McCarty KS, Sr. (1985) Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch Pathol Lab Med 109 (8):716-721.

Muller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, Miller EL, O'Connor MB, Kingston RE, Simon JA (2002) Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 111 (2):197-208.

Munschauer M, Nguyen CT, Sirokman K, Hartigan CR, Hogstrom L, Engreitz JM, Ulirsch JC, Fulco CP, Subramanian V, Chen J, Schenone M, Guttman M, Carr SA, Lander ES (2018) The NORAD lncRNA assembles a topoisomerase complex critical for genome stability. Nature 561 (7721):132-136.

Ouyang YQ, zur Hausen A, Orlowska-Volk M, Jager M, Bettendorf H, Hirschfeld M, Tong XW, Stickeler E (2011) Expression levels of hnRNP G and hTra2-beta1 correlate with opposite outcomes in endometrial cancer biology. Int J Cancer 128 (9):2010-2019.

Palomero T, Couronne L, Khiabanian H, Kim MY, Ambesi-Impiombato A, Perez-Garcia A, Carpenter Z, Abate F, Allegretta M, Haydu JE, Jiang X, Lossos IS, Nicolas C, Balbin M, Bastard C, Bhagat G, Piris MA, Campo E, Bernard OA, Rabadan R, Ferrando AA (2014) Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. Nat Genet 46 (2):166-170.

Pereira B, Billaud M, Almeida R (2017) RNA-Binding Proteins in Cancer: Old Players and New Actors. Trends Cancer 3 (7):506-528.

Prieto C, Nguyen DTT, Liu Z, Wheat J, Perez A, Gourkanti S, Chou T, Barin E, Velleca A, Rohwetter T, Chow A, Taggart J, Savino AM, Hoskova K, Dhodapkar M, Schurer A, Barlowe TS, Vu LP, Leslie C, Steidl U, Rabadan R, Kharas MG (2021) Transcriptional control of CBX5 by the RNA binding proteins RBMX and RBMXL1 maintains chromatin state in myeloid leukemia. Nat Cancer 2:741-757.

Renieri A, Mencarelli MA, Cetta F, Baldassarri M, Mari F, Furini S, Piu P, Ariani F, Dragani TA, Frullanti E (2014) Oligogenic germline mutations identified in early non-smokers lung adenocarcinoma patients. Lung Cancer 85 (2):168-174.

Roberti A, Dobay MP, Bisig B, Vallois D, Boechat C, Lanitis E, Bouchindhomme B, Parrens MC, Bossard C, Quintanilla-Martinez L, Missiaglia E, Gaulard P, de Leval L (2016) Type II enteropathy-associated T-cell lymphoma features a unique genomic profile with highly recurrent SETD2 alterations. Nat Commun 7:12602.

Rudiger T, Weisenburger DD, Anderson JR, Armitage JO, Diebold J, MacLennan KA, Nathwani BN, Ullrich F, Muller-Hermelink HK, Non-Hodgkin's Lymphoma Classification P (2002) Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 13 (1):140-149.

Sakata-Yanagimoto M, Enami T, Yoshida K, Shiraishi Y, Ishii R, Miyake Y, Muto H, Tsuyama N, Sato-Otsubo A, Okuno Y, Sakata S, Kamada Y, Nakamoto-Matsubara R, Tran NB, Izutsu K, Sato Y, Ohta Y, Furuta J, Shimizu S, Komeno T, Sato Y, Ito T, Noguchi M, Noguchi E, Sanada M, Chiba K, Tanaka H, Suzukawa K, Nanmoku T, Hasegawa Y, Nureki O, Miyano S, Nakamura N, Takeuchi K, Ogawa S, Chiba S (2014) Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. Nat Genet 46 (2):171-175.

Schmitz N, Lenz G, Stelljes M (2018) Allogeneic hematopoietic stem cell transplantation for T-cell lymphomas. Blood 132 (3):245-253.

Schmitz N, Trumper L, Ziepert M, Nickelsen M, Ho AD, Metzner B, Peter N, Loeffler M, Rosenwald A, Pfreundschuh M (2010) Treatment and prognosis of mature T-cell and NK-cell lymphoma: an analysis of patients with T-cell lymphoma treated in studies of the German High-Grade Non-Hodgkin Lymphoma Study Group. Blood 116 (18):3418-3425.

Schümann FL, Weber T (2021) Periphere T-Zell-Lymphome - Neue zielgerichtete Therapien auf dem Vormarsch in die Klinik. Onkologie heute 20 (06/21):34-39.

Schümann FL, Weber T (2023) Periphere T-Zell-Lymphome – Ein Update zu Klassifikation und Behandlungsmöglichkeiten. Onkologie heute 20 (02/23):8-13.

Shen X, Liu Y, Hsu YJ, Fujiwara Y, Kim J, Mao X, Yuan GC, Orkin SH (2008) EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. Mol Cell 32 (4):491-502.

Shi M, Shahsafaei A, Liu C, Yu H, Dorfman DM (2015) Enhancer of zeste homolog 2 is widely expressed in T-cell neoplasms, is associated with high proliferation rate and correlates with MYC and pSTAT3 expression in a subset of cases. Leuk Lymphoma 56 (7):2087-2091.

Shin KH, Kang MK, Kim RH, Christensen R, Park NH (2006) Heterogeneous nuclear ribonucleoprotein G shows tumor suppressive effect against oral squamous cell carcinoma cells. Clin Cancer Res 12 (10):3222-3228.

Shin KH, Kim RH, Kim RH, Kang MK, Park NH (2008) hnRNP G elicits tumor-suppressive activity in part by upregulating the expression of Txnip. Biochem Biophys Res Commun 372 (4):880-885.

Shin KH, Kim RH, Yu B, Kang MK, Elashoff D, Christensen R, Pucar A, Park NH (2011) Expression and mutation analysis of heterogeneous nuclear ribonucleoprotein G in human oral cancer. Oral Oncol 47 (11):1011-1016.

Song Y, He S, Ma X, Zhang M, Zhuang J, Wang G, Ye Y, Xia W (2020) RBMX contributes to hepatocellular carcinoma progression and sorafenib resistance by specifically binding and stabilizing BLACAT1. Am J Cancer Res 10 (11):3644-3665.

Soulard M, Della Valle V, Siomi MC, Pinol-Roma S, Codogno P, Bauvy C, Bellini M, Lacroix JC, Monod G, Dreyfuss G, et al. (1993) hnRNP G: sequence and characterization of a glycosylated RNA-binding protein. Nucleic Acids Res 21 (18):4210-4217.

Swerdlow SH, World Health Organization, International Agency for Research on Cancer (2017) WHO classification of tumours of haematopoietic and lymphoid tissues. World Health Organization classification of tumours, Revised 4th edition. edn. International Agency for Research on Cancer, Lyon.

Van Arnam JS, Lim MS, Elenitoba-Johnson KSJ (2018) Novel insights into the pathogenesis of T-cell lymphomas. Blood 131 (21):2320-2330.

Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, Chinnaiyan AM (2002) The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419 (6907):624-629.

Vose J, Armitage J, Weisenburger D, International TCLP (2008) International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol 26 (25):4124-4130.

Wang CG, Ye YJ, Yuan J, Liu FF, Zhang H, Wang S (2010) EZH2 and STAT6 expression profiles are correlated with colorectal cancer stage and prognosis. World J Gastroenterol 16 (19):2421-2427.

Wang E, Lu SX, Pastore A, Chen X, Imig J, Chun-Wei Lee S, Hockemeyer K, Ghebrechristos YE, Yoshimi A, Inoue D, Ki M, Cho H, Bitner L, Kloetgen A, Lin KT, Uehara T, Owa T, Tibes R, Krainer AR, Abdel-Wahab O, Aifantis I (2019) Targeting an RNA-Binding Protein Network in Acute Myeloid Leukemia. Cancer Cell 35 (3):369-384 e367.

Wang M, Zhang S, Chuang SS, Ashton-Key M, Ochoa E, Bolli N, Vassiliou G, Gao Z, Du MQ (2017) Angioimmunoblastic T cell lymphoma: novel molecular insights by mutation profiling. Oncotarget 8 (11):17763-17770.

Watatani Y, Sato Y, Miyoshi H, Sakamoto K, Nishida K, Gion Y, Nagata Y, Shiraishi Y, Chiba K, Tanaka H, Zhao L, Ochi Y, Takeuchi Y, Takeda J, Ueno H, Kogure Y, Shiozawa Y, Kakiuchi N, Yoshizato T, Nakagawa MM, Nanya Y, Yoshida K, Makishima H, Sanada M, Sakata-Yanagimoto M, Chiba S, Matsuoka R, Noguchi M, Hiramoto N, Ishikawa T, Kitagawa J, Nakamura N, Tsurumi H, Miyazaki T, Kito Y, Miyano S, Shimoda K, Takeuchi K, Ohshima K, Yoshino T, Ogawa S, Kataoka

K (2019) Molecular heterogeneity in peripheral T-cell lymphoma, not otherwise specified revealed by comprehensive genetic profiling. Leukemia 33 (12):2867-2883.

Wei Y, Xia W, Zhang Z, Liu J, Wang H, Adsay NV, Albarracin C, Yu D, Abbruzzese JL, Mills GB, Bast RC, Jr., Hortobagyi GN, Hung MC (2008) Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. Mol Carcinog 47 (9):701-706.

Wurth L (2012) Versatility of RNA-Binding Proteins in Cancer. Comp Funct Genomics 2012:178525.

Xie R, Chung JY, Ylaya K, Williams RL, Guerrero N, Nakatsuka N, Badie C, Hewitt SM (2011) Factors influencing the degradation of archival formalin-fixed paraffin-embedded tissue sections. J Histochem Cytochem 59 (4):356-365.

Xu K, Wu ZJ, Groner AC, He HH, Cai C, Lis RT, Wu X, Stack EC, Loda M, Liu T, Xu H, Cato L, Thornton JE, Gregory RI, Morrissey C, Vessella RL, Montironi R, Magi-Galluzzi C, Kantoff PW, Balk SP, Liu XS, Brown M (2012) EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. Science 338 (6113):1465-1469.

Yamagishi M, Hori M, Fujikawa D, Ohsugi T, Honma D, Adachi N, Katano H, Hishima T, Kobayashi S, Nakano K, Nakashima M, Iwanaga M, Utsunomiya A, Tanaka Y, Okada S, Tsukasaki K, Tobinai K, Araki K, Watanabe T, Uchimaru K (2019) Targeting Excessive EZH1 and EZH2 Activities for Abnormal Histone Methylation and Transcription Network in Malignant Lymphomas. Cell Rep 29 (8):2321-2337 e2327.

Yan J, Li B, Lin B, Lee PT, Chung TH, Tan J, Bi C, Lee XT, Selvarajan V, Ng SB, Yang H, Yu Q, Chng WJ (2016) EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. Blood 128 (7):948-958.

Yan J, Ng SB, Tay JL, Lin B, Koh TL, Tan J, Selvarajan V, Liu SC, Bi C, Wang S, Choo SN, Shimizu N, Huang G, Yu Q, Chng WJ (2013) EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone methyltransferase activity. Blood 121 (22):4512-4520.

Yan Q, Zeng P, Zhou X, Zhao X, Chen R, Qiao J, Feng L, Zhu Z, Zhang G, Chen C (2021) RBMX suppresses tumorigenicity and progression of bladder cancer by interacting with the hnRNP A1 protein to regulate PKM alternative splicing. Oncogene.

Yi S, Sun J, Qiu L, Fu W, Wang A, Liu X, Yang Y, Kadin ME, Tu P, Wang Y (2018) Dual Role of EZH2 in Cutaneous Anaplastic Large Cell Lymphoma: Promoting Tumor Cell Survival and Regulating Tumor Microenvironment. J Invest Dermatol 138 (5):1126-1136.

Zhang H, Lv H, Jia X, Hu G, Kong L, Zhang T, Li L, Pan Y, Zhai Q, Meng B, Wang X, Wang H, Wang X (2019) Clinical significance of enhancer of zeste homolog 2 and histone deacetylases 1 and 2 expression in peripheral T-cell lymphoma. Oncol Lett 18 (2):1415-1423.

Zheng T, Zhou H, Li X, Peng D, Yang Y, Zeng Y, Liu H, Ren J, Zhao Y (2020) RBMX is required for activation of ATR on repetitive DNAs to maintain genome stability. Cell Death Differ 27 (11):3162-3176.

4 Thesen

1. Im Vergleich zu normalem Lymphknotengewebe zeigt sich in T-Zell-Lymphomgewebe die EZH2- und H3K27me3-Proteinexpression erhöht, während die EZH1-Proteinexpression vermindert ist.

2. Patient:innen mit hoher EZH2-Proteinexpression weisen in der univariablen Analyse ein kürzeres Gesamtüberleben und progressionsfreies Überleben auf.

3. Patient:innen mit niedriger EZH1-Proteinexpression weisen in der univariablen Analyse ein kürzeres Gesamtüberleben auf.

4. Im multivariablen Cox-Regressionsmodell sind eine erniedrigte EZH1- und eine erhöhte EZH2-Proteinexpression unabhängige prognostische Marker für ein kürzeres Gesamtüberleben.

5. Während Patient:innen mit hoher H3K27me3-Proteinexpression in der univariablen Analyse ein kürzeres Gesamtüberleben aufweisen, zeigt sich die H3K27me3-Proteinexpression im multivariablen Cox-Regressionsmodell nicht als unabhängiger prognostischer Marker für das Überleben.

6. Eine hohe RBMX-Proteinexpression ist mit einem schlechteren Ansprechen auf anthrazyklinhaltige Erstlinienchemotherapie assoziiert.

7. Patient:innen mit hoher RBMX-Proteinexpression weisen in der univariablen Analyse ein kürzeres Gesamtüberleben und progressionsfreies Überleben auf.

8. Im multivariablen Cox-Regressionsmodell ist eine erhöhte RBMX-Proteinexpression ein unabhängiger prognostischer Marker für ein kürzeres Gesamtüberleben.

5 Publikationen

5.1 Publikation 1: Schümann FL, Groß E, Bauer M, Rohde C, Sandmann S, Terziev D, Müller LP, Posern G, Wienke A, Fend F, Hansmann ML, Klapper W, Rosenwald A, Stein H, Dugas M, Müller-Tidow C, Wickenhauser C, Binder M, Weber T (2021) Divergent Effects of EZH1 and EZH2 Protein Expression on the Prognosis of Patients with T-Cell Lymphomas. Biomedicines 9 (12)

biomedicines

MDPI

Article

Divergent Effects of EZH1 and EZH2 Protein Expression on the Prognosis of Patients with T-Cell Lymphomas

Franziska Lea Schümann [1], Elisabeth Groß [1], Marcus Bauer [2], Christian Rohde [3], Sarah Sandmann [4], Denis Terziev [1], Lutz P. Müller [1], Guido Posern [5], Andreas Wienke [6], Falko Fend [7], Martin-Leo Hansmann [8], Wolfram Klapper [9], Andreas Rosenwald [10], Harald Stein [11], Martin Dugas [12], Carsten Müller-Tidow [3], Claudia Wickenhauser [2], Mascha Binder [1] and Thomas Weber [1,*]

[1] Department of Internal Medicine IV, Hematology and Oncology, University Hospital Halle (Saale), Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany; franziska.schuemann@uk-halle.de (F.L.S.); elisabeth.gross@uk-halle.de (E.G.); denis.terziev@uk-halle.de (D.T.); lutz.mueller@uk-halle.de (L.P.M.); mascha.binder@uk-halle.de (M.B.)
[2] Institute of Pathology, University Hospital Halle (Saale), Martin-Luther-University Halle-Wittenberg, 06112 Halle, Germany; marcus.bauer@uk-halle.de (M.B.); claudia.wickenhauser@uk-halle.de (C.W.)
[3] Department of Medicine V, Hematology, Oncology and Rheumatology, University Hospital Heidelberg, 69120 Heidelberg, Germany; christian.rohde@med.uni-heidelberg.de (C.R.); carsten.mueller-tidow@med.uni-heidelberg.de (C.M.-T.)
[4] Institute of Medical Informatics, University of Münster, 48149 Münster, Germany; sarah.sandmann@uni-muenster.de
[5] Institute for Physiological Chemistry, Medical Faculty, Martin Luther University Halle-Wittenberg, 06114 Halle (Saale), Germany; guido.posern@medizin.uni-halle.de
[6] Institute of Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University Halle-Wittenberg, 06112 Halle, Germany; andreas.wienke@uk-halle.de
[7] Institute of Pathology, University Hospital Tübingen, 72076 Tübingen, Germany; falko.fend@medizin.uni-tuebingen.de
[8] Institute of Pathology and Molecular Pathology, Helios University Hospital Wuppertal, 42283 Wuppertal, Germany; m.l.hansmann@em.uni-frankfurt.de
[9] Department of Pathology, Hematopathology Section and Lymph Node Registry, University Hospital Schleswig-Holstein, Christian-Albrechts-University, 24105 Kiel, Germany; wolfram.klapper@uksh.de
[10] Institute of Pathology, University Würzburg, 97080 Würzburg, Germany; rosenwald@uni-wuerzburg.de
[11] Pathodiagnostik Berlin, 12099 Berlin, Germany; h.stein@pathodiagnostik.de
[12] Institute for Medical Informatics, University Hospital Heidelberg, 69120 Heidelberg, Germany; martin.dugas@med.uni-heidelberg.de
* Correspondence: thomas.weber@uk-halle.de

Citation: Schümann, F.L.; Groß, E.; Bauer, M.; Rohde, C.; Sandmann, S.; Terziev, D.; Müller, L.P.; Posern, G.; Wienke, A.; Fend, F.; et al. Divergent Effects of EZH1 and EZH2 Protein Expression on the Prognosis of Patients with T-Cell Lymphomas. *Biomedicines* **2021**, *9*, 1842. https://doi.org/10.3390/biomedicines9121842

Academic Editors: Danilo Fiore and Luca Vincenzo Cappelli

Received: 30 October 2021
Accepted: 3 December 2021
Published: 5 December 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: T-cell lymphomas are highly heterogeneous and their prognosis is poor under the currently available therapies. Enhancers of zeste homologue 1 and 2 (EZH1/2) are histone H3 lysine-27 trimethyltransferases (H3K27me3). Despite the rapid development of new drugs inhibiting EZH2 and/or EZH1, the molecular interplay of these proteins and the impact on disease progression and prognosis of patients with T-cell lymphomas remains insufficiently understood. In this study, *EZH1/2* mutation status was evaluated in 33 monomorphic epitheliotropic intestinal T-cell lymphomas by next generation sequencing and EZH1/2 and H3K27me3 protein expression levels were detected by immunohistochemistry in 46 T-cell lymphomas. Correlations with clinicopathologic features were analyzed and survival curves generated. No *EZH1* mutations and one (3%) *EZH2* missense mutation were identified. In univariable analysis, high EZH1 expression was associated with an improved overall survival (OS) and progression-free survival (PFS) whereas high EZH2 and H3K27me3 expression were associated with poorer OS and PFS. Multivariable analysis revealed EZH1 (hazard ratio (HR) = 0.183; 95% confidence interval (CI): 0.044–0.767; p = 0.020;) and EZH2 (HR = 8.245; 95% CI: 1.898–35.826; p = 0.005) to be independent, divergent prognostic markers for OS. In conclusion, EZH1/2 protein expression had opposing effects on the prognosis of T-cell lymphoma patients.

Keywords: T-cell non-Hodgkin's lymphomas; PTCL; epigenetics; EZH1; EZH2; H3K27me3; immunohistochemistry; next generation sequencing

1. Introduction

Lymphoid neoplasms with T-cell differentiation are a heterogeneous group of rare diseases that are classified by the World Health Organization (WHO) in immature acute T-cell lymphoblastic leukemias (T-ALL) and mature post-thymic T-/NK-cell neoplasms (T-NHL) [1]. The latter, also known as peripheral T-cell lymphomas (PTCL), are classified into primary cutaneous, primary leukemic, and aggressive with nodal or extranodal manifestation, depending on the clinical presentation. For decades, the first-line standard therapy for most T-NHL subtypes has not changed. CHO(E)P (cyclophosphamide, doxorubicin, vincristine, (etoposide), and prednisone), a standard chemotherapy for B-cell lymphomas, is usually chosen as first-line treatment, although refractoriness and relapse are common [2,3].

Understanding of epigenetics in cancer development and tumor progression has improved rapidly in recent years, leading to the development of targeted therapies. Enhancer of zeste homologue 1 and 2 (EZH1/2), the catalytic subunit of polycomb repression complex 2 (PRC2), is one of the best studied histone-modifying enzymes and thus the target of numerous new therapeutics. EZH1/2 contains a catalytic domain at the COOH terminus that trimethylates the 27th lysine residue of histone H3 (H3K27me3) [4–7]. H3K27me3 is widely known as a marker for transcriptional repression [6–8]. The paralogues EZH1 and EZH2 have different expression patterns: while EZH1 is present in dividing and differentiated cells, EZH2 is found only in highly proliferative cells [9]. Furthermore, PRC2 complexes containing EZH2 have higher methyltransferase activity than those containing EZH1 [9]. Assuming that EZH1 compensates for the loss of EZH2 [10,11], not only the effect of EZH2-selective inhibitors, but also dual EZH1/2 inhibitors are currently under intense clinical investigation [12].

The basis for these new developments was the detection of *EZH1/2* alterations and/or EZH1/2 overexpression in many different neoplasms and their association with metastasis, poor prognosis, and treatment failure [13–16]. Mutations of *EZH1* and/or *EZH2* are described as rare or absent in T-cell lymphomas by a number of groups [17–24]. Furthermore, EZH1 expression has been reported to be low in T-NHL [25]. Even less is known about its association with progression or prognosis. In contrast, EZH2 is frequently overexpressed in T-NHL [25–30] and additionally appears to be associated with an unfavorable prognosis [30]. Several studies have shown associations between elevated EZH2 protein expression and various clinical markers such as the presence of B symptoms, elevated serum lactate dehydrogenase (LDH) levels, elevated ß2-microglobulin, and an increase in the proliferation marker Ki-67 [25,29–31].

In contrast to EZH2, several studies have demonstrated an inconsistent correlation between histone lysine modification H3K27me3 and cancer prognosis [32–34]. The conflicting results may suggest that methylation of different target genes occurs depending on its cellular context [35]. In patients with extranodal natural killer/T-cell lymphoma, nasal type (NKTCL), high H3K27me3 protein expression was associated with better prognosis and low clinical stages [31]. To date, expression in further subtypes of T-cell neoplasms has been studied less. A correlation between EZH2 and H3K27me3 in T-cell neoplasms was previously investigated by some research groups with conflicting results [26,31]. Thus, a possible non-canonical function (H3K27-specific histone methyltransferase) of EZH2 in oncogenesis is currently discussed [28,31].

Overall, T-cell lymphomas show a heterogeneous and so far insufficiently characterized profile of EZH1, EZH2, and H3K27me3 expression as well as a poorly studied correlation of these proteins with each other and with clinicopathological markers. To improve our understanding of these epigenetic factors, the present study

evaluated the *EZH1/2* mutation status in a cohort of 33 monomorphic epitheliotropic intestinal T-cell lymphomas (MEITL) by next generation targeted sequencing and detected EZH1/2 and H3K27me3 expression levels by immunohistochemistry in 46 T-cell lymphomas.

2. Materials and Methods

2.1. Patients and Samples

Thirty-three MEITL samples, classified by hematopathologists, were collected. While *EZH1* and *EZH2* mutation analysis by WES was performed in 11 samples, the mutation status of *EZH2* was analyzed in all 33 samples by targeted sequencing. Available patient characteristics are summarized in Table 1, but no survival data or further clinical data were available for these cases, so they were not included in further analyses of clinical and survival data.

Table 1. Characteristics of mutation analysis patients.

Characteristics		MEITL *n* = 33 *n* (%)
Sex	Female	16 (49)
	Male	12 (36)
	Not evaluable	5 (15)
Age	Median (years) (range)	62 (38–92)
Histopathology	CD3^{positive}	33 (100)
	CD4^{negative}	31(94)
	CD8^{positive}	27 (82)
	CD58^{positive}	31 (94)
T-cell Receptor	$\alpha\beta^{positive}$	10 (30)
	$\gamma\delta^{positive}$	9 (27)
	Not evaluable	14 (42)

Abbreviations: MEITL—monomorphic epitheliotropic intestinal T-cell lymphoma; n —number; CD—cluster of differentiation.

A cohort of 46 patients with T-NHL and available survival data who received treatment at University Hospital Halle (Saale) between 2006 and March 2020 met inclusion criteria for tissue microarray (TMA) construction. T-NHL tissue samples were provided for TMA construction by the Institute of Pathology of the University Hospital Halle (Saale). The patients were identified through a review of the internal hospital database and those with available formalin fixated paraffin embedded (FFPE) T-cell lymphoma tissue were included in the sense of a convenience sample. Patients with an age below 18 years at initial diagnosis were excluded. The diagnosis of all patients who met the inclusion criteria was verified by two pathologists (C.W. and M.B. (Marcus Bauer)) according to the 2017 WHO criteria [1]. Five out of 57 original samples had to be excluded because the integrated tissue samples were not from the primary diagnosis or the diagnosis was not confirmed. Clinicopathological characteristics at the time of primary diagnosis including age, sex, histologic phenotype, B symptoms, Ann Arbor stage, International Prognostic Index (IPI), Eastern Cooperative Oncology Group (ECOG) status, bone marrow involvement (BMI), LDH level, white blood cell (WBC) count, Ki-67 expression, (response to) first-line chemotherapy, occurrence of relapses, and follow-up data were recorded. The study included 19 cases of peripheral T-cell lymphomas with T-helper phenotype (angioimmunoblastic T-cell lymphoma (AITL) and nodal peripheral T-cell lymphoma with T follicular helper phenotype (PTCL-TFH)), eight cases of peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS), seven cases of anaplastic large-

cell lymphomas, ALK-negative (ALCL, ALK-negative), three cases of intestinal T-NHL, two cases of NKTCL, two cases of T-cell large granular lymphocytic leukemia (T-LGL), and five cases of other subtypes (Mycosis fungoides (MF) *n* = 1, subcutaneous panniculitis-like T-cell lymphoma (SPTCL) *n* = 1, T-cell prolymphocytic leukemia (T-PLL) *n* = 1, cerebral T-NHL *n* = 1, and polymorphic post-transplant lymphoproliferative disorder (PTLD) *n* = 1). The recorded clinicopathological characteristics are summarized in Table 2.

Table 2. Characteristics of tissue microarray patients.

Characteristics		T-NHL *n* = 46	Nodal T-NHL *n* = 34
		***n* (%)**	***n* (%)**
Sex	Female	15 (33)	12 (35)
	Male	31 (67)	22 (65)
Age	Median (years) (range)	64.1 (36–92)	64.4 (51–92)
B symptoms	Absent	27 (59)	20 (59)
	Present	18 (39)	14 (41)
	Not evaluable	1 (2)	0 (0)
Bone marrow involvement	Absent	32 (70)	25 (74)
	Present	10 (22)	7 (21)
	Not evaluable	4 (9)	2 (6)
Ann Arbor stage	Stages I and II	8 (17)	6 (18)
	Stages III and IV	28 (61)	25 (74)
	Not evaluable	10(22)	3 (9)
IPI	0–2	18 (39)	15 (44)
	3–5	19 (41)	17 (50)
	Not evaluable	9 (20)	2 (6)
ECOG	0–1	20 (43)	16 (47)
	2–5	6 (13)	5 (15)
	Not evaluable	20 (43)	13 (38)
WBC	Normal	13 (28)	8 (24)
	Upper limit of normal	11 (24)	7 (21)
	Not evaluable	22 (48)	19 (56)
LDH	Normal	6 (13)	2 (6)
	Upper limit of normal	19 (41)	17 (50)
	Not evaluable	21 (46)	15 (44)
Ki-67 expression	<65%	16 (35)	12 (35)
	≥65%	14 (30)	10 (29)
	Not evaluable	19 (35)	12 (35)
Relapse	Absent	23 (50)	14 (41)
	Present	23 (50)	20 (59)
First-line treatment	(R)-CHO(E)P	37 (80)	33 (97)
	Others	9 (20)	1 (1)

Abbreviations: T-NHL—T-cell non-Hodgkin's lymphoma; IPI—International Prognostic Index, ECOG—Eastern Cooperative Oncology Group status; WBC—white blood cell count; LDH—lactate dehydrogenase level; (R)-CHO(E)P—(rituximab)-cyclophosphamide, doxorubicin, vincristine, (etoposide) and prednisone containing chemotherapy.

2.2. Sequencing and Mutation Analysis

Whole exome sequencing (WES) was performed in a total of 11 MEITL and the available corresponding normal tissue from one tumor sample. Genomic DNA was

extracted from FFPE tumor tissues and subsequently treated with uracil DNA glycosylase (UGD; GeneRead DNA FFPE Kit, Qiagen, Hilden, Germany) to suppress FFPE induced sequencing artifacts. After using the Agilent AllHuman V5 kit, sequencing was accomplished on an Illumina sequencing platform with paired end 100 base-pair reads. An FFPE-tissue optimized analysis pipeline was used for the analysis of single nucleotide polymorphisms (SNP) and copy number variations (CNV). SNP occurring in the sequenced normal tissue sample, dbSNP137, or in the 1000 genome database with a frequency of more than 1% were excluded. Mutations with allele frequencies below 10% were excluded because of the low frequency. MutSig and DOTS-Finder algorithms were used to identify the most significant and functional relevant mutations.

Targeted sequencing was performed on the WES-cohort and 22 additional cases. A custom Haloplex panel (Agilent Technologies Inc., Santa Clara, CA, USA) targeting 71 genes, exomes, or hotspots of mutated T-cell neoplasms was used for mutational analysis. Libraries were sequenced using an Illumina system. Variants were called using the appreci8 pipeline [36]. Read depth was set to a minimum of 20, the minimum number of variant allele read to 5 and the minimum variant allele frequency (VAF) to 5%. The pipeline automatically filtered artifacts and SNPs based on call characteristics. These include, but are not limited to, a variant's base quality, presence of a variant in common databases (e.g., dbSNP, 1000 genomes, and COSMIC) and the effect of a variant based on in silico prediction. Additionally, manual investigation of all borderline calls was performed using the integrated genome viewer (IGV).

2.3. Tissue Microarray Construction

TMAs were prepared using a manual tissue arrayer (Beecher Instruments Inc., Sun Prairie, WI, USA), as described previously [37]. Hematoxylin and eosin (H&E) staining was obtained from each donor block, and representative tumor regions were morphologically identified and marked by a pathologist (M.B. (Marcus Bauer)). Two 0.6-mm-diameter tissue cores were extracted from these marked areas and arranged on recipient paraffin blocks. Adequate controls for specific antibodies including liver tissue, tonsil tissue, breast cancer, seminoma, prostate carcinoma, and osteosarcoma were added.

2.4. Immunohistochemistry and Scoring

Immunohistochemistry (IHC) for EZH1, EZH2, and H3K27me3 was performed following a standard protocol using a Bond III automated immunostainer (Leica Biosystems Nussloch GmbH, Wetzlar, Germany) and the Bond Polymer Refine Detection Kit (DS9800-CN). In addition to the tumor samples, 10 normal lymph node tissues were stained to compare the expression levels of healthy and tumor tissues. The primary antibodies used in this study were the following: EZH1 (1:100, Abcam, Cambridge, UK; ab137693), EZH2 (1:100; Cell Signaling, Danvers, MA; 3147s), and H3K27me3 (1:200, Cell Signaling, Danvers, 9733s). Immunostaining was assessed by two investigators (M.B. (Marcus Bauer) and F.L.S.) using the Zeiss Axioscope 5 microscope (Carl Zeiss Mikroskopie GmbH, Jena, Germany). The two investigators were blinded to pathologic and clinical data. Staining was evaluated semiquantitatively using the H-scoring method [38]. The H-score for one patient was calculated from the mean of two stains. In 89% of the cases, the results of the two examiners agreed, confirming the reproducibility of the used evaluation method.

2.5. Statistical Analysis

Analyses were performed for the entire study cohort, followed by nodal T-NHL phenotypes. Comparison of continuous variables between two groups was evaluated with the Mann-Whitney *U* test and between multiple groups with the Kruskal–Wallis test using Bonferroni's correction. Associations between the protein expressions were assessed using the Spearman correlation. Univariable overall survival (OS) and

progression-free survival (PFS) analyses were performed with the Kaplan–Meier method. Statistical comparisons between groups were made by log rank tests. Multivariable analysis was performed using a Cox proportional hazards model with the Enter method to evaluate the impact of previously defined variables (age, sex, Ann Arbor stage, B symptoms, BMI, and protein expression of EZH1, EZH2, and H3K27me3) on PFS and OS. OS was defined as the time from primary diagnosis until last follow-up or death from any cause. PFS was defined as the time from primary diagnosis until lymphoma progression or death from any cause. Receiver operating characteristic (ROC) and Youden Index were used to determine a cutoff value for protein expression to divide the samples into two groups of high and low expression, respectively. Patients alive at the last follow-up date were censored. All *p*-values were interpreted exploratorily.

3. Results

3.1. EZH1 and EZH2 Mutations

In the MEITL cohort, the mutational status of *EZH1* and *EZH2* was investigated by next generation sequencing (NGS; Figure 1). *EZH1* mutation status was analyzed in 11 MEITL cases, but no mutation could be detected. *EZH2* mutation status was analyzed in 33 MEITL cases. A missense mutation with unknown biological impact located in the SET-domain (Figure 1b) was found to be present in one sample (3%). Immunohistochemical EZH1 (n = 16), EZH2 (n = 27), and H3k27me3 (n = 31) expression was also evaluated for the cases studied (Figure 1). Based on the one mutation found, no association with EZH1, EZH2, or H3K27me3 protein expression could be detected.

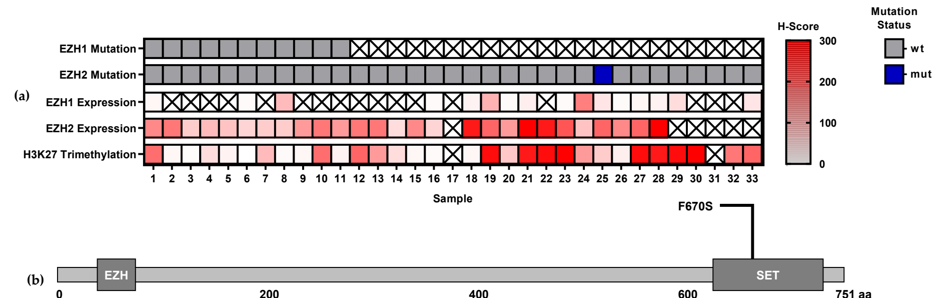

Figure 1. *EZH1* and *EZH2* mutations in monomorphic epitheliotropic intestinal T-cell lymphomas (MEITL): (**a**) *EZH1* and *EZH2* mutations and its association with EZH1, EZH2 and H3K27m3 protein expression. (**b**) Localization of the *EZH2* mutation in the SET-domain. Abbreviations: wt—wild type; mut—mutation.

3.2. EZH1, EZH,2 and H3K27me3 Protein Expression Levels in T-Cell Lymphomas

We compared the protein expression levels of T-cell lymphomas ($n \leq 46$) with normal non tumor lymph node tissue samples (n = 10; Figure 2). EZH1 expression was found to be strongly decreased in tumor samples (median = 5; interquartile range (IQR) = 33) compared to normal lymphoid tissue (median = 45; IQR = 65; p = 0.001). In contrast, the expression of EZH2 (median = 85; IQR = 116) was increased compared to the controls (median = 30; IQR = 33; p = 0.016). Increased expression was also detected for H3K27me3 in tumor samples (median = 185; IQR = 70) compared to normal tissues (median = 130; IQR = 55; p = 0.054).

Representative immunohistochemical staining showing different levels of nuclear EZH1, EZH2, and H3K27me3 protein expression are illustrated in Figure 3.

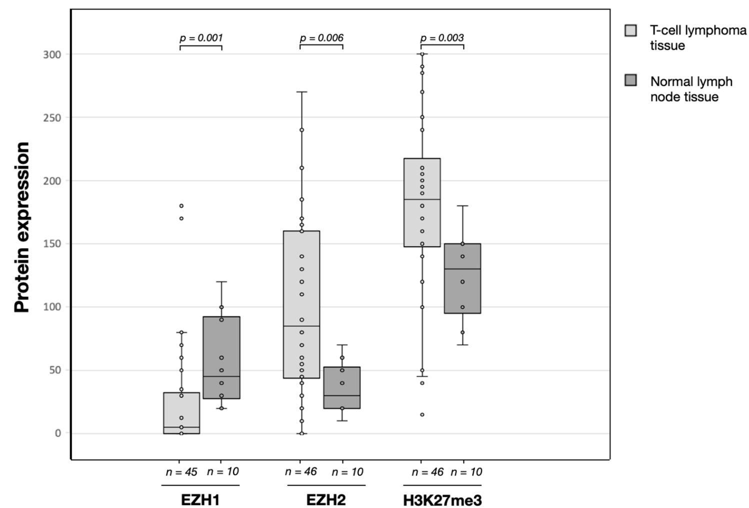

Figure 2. EZH1, EZH2, and H3K27me3 protein expression (H-score) in T-cell lymphomas and non-tumor tissues analyzed by immunohistochemistry. Boxplots represent the median and interquartile range of protein level (H-score). The number (*n*) of tissue samples is given below. *p*-values are shown above.

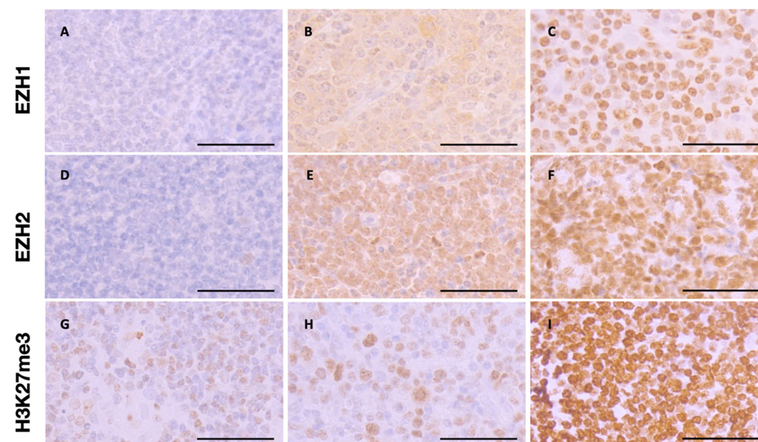

Figure 3. Representative immunohistochemical features of EZH1, EZH2, and H3K27me3. (**A**–**C**) Immunohistochemical staining showing low (**A**), middle (**B**), and high (**C**) levels of nuclear EZH1 expression. (**D**–**F**) Immunohistochemical staining showing low (**D**), middle (**E**), and high (**F**) levels of nuclear EZH2 expression. (**G**–**H**) Immunohistochemical staining showing low (**G**), middle (**H**), and high (**I**) levels of nuclear H3K27me3 expression. Original magnification ×400, the scale bars are 50 μm.

3.3. Associations between EZH1, EZH2 and H3K27me3 Protein Expression

Correlations between EZH1, EZH2, and H3K27me3 protein expression (H-score) were analyzed in the entire study cohort. No correlation was observed between EZH1 and EZH2 (r = 0.127; p = 0.406; n = 45), EZH1 and H3K27me3 (r = 0.020; p = 0.899; n = 45), or

EZH2 and H3K27me3 (r = 0.175; p = 0.224; n = 46). Visually, no other associations were detected between EZH1, EZH2, and H3K27me3 protein expression (Figure 4).

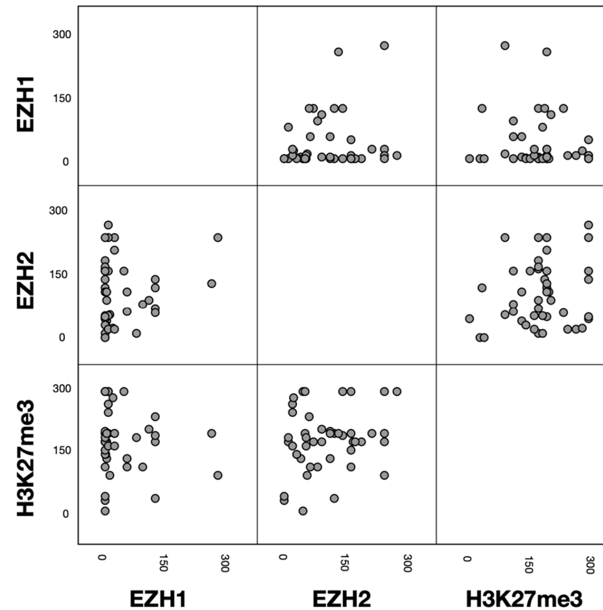

Figure 4. Scatterplot matrix visualizing the associations between EZH1, EZH2, and H3K27me3 immunohistochemical protein expression (H-score).

3.4. Associations between EZH1, EZH2 and H3K27me3 Protein Expression and Clinicopathological Characteristics

Possible associations between protein expression of EZH1, EZH2, and H3K27me3 with clinicopathological characteristics including sex, age, B symptoms, Ann Arbor stage, IPI, BMI, ECOG status, WBC, LDH, Ki-67 expression, the occurrence of relapses and response to first-line chemotherapy were investigated. No relevant associations were seen in the entire study cohort. In the nodal T-NHL cases, high EZH2 protein expression was associated with both the presence of B symptoms (B symptoms vs. no B symptoms: median 130 vs. 53.8; p = 0.031; Figure 5a) and with a high Ki-67 Index (Ki-67 Index ≥ 65 vs. Ki-67 Index < 65: median 150 vs. 47.5; p = 0.059; Figure 5b). A normal white blood cell count (WBC) was also associated with high EZH2 protein expression (WBC normal vs. upper limit of normal: median 165 vs. 70; p = 0.040; Figure 5c). No associations with sex, age, Ann Arbor stage, IPI, BMI, ECOG status, LDH, the occurrence of relapses, and response to first-line chemotherapy were noted. Furthermore, no associations between EZH1 and H3K27me3 with clinicopathological characteristics were observed in nodal T-NHL.

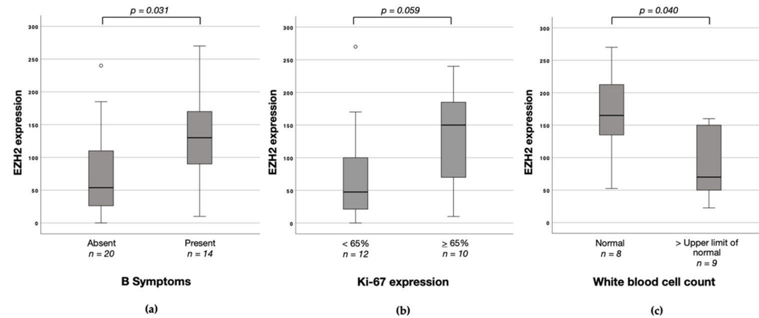

Figure 5. EZH2 protein expression (H-score) according to clinicopathological characteristics in nodal T-cell non-Hodgkin's lymphomas (T-NHL). (**a**) Corresponding boxplot of EZH2 protein expression depending on the presence of B symptoms. (**b**) Corresponding boxplot of EZH2 protein expression depending on Ki-67 expression. (**c**) Corresponding boxplot of EZH2 protein expression depending on white blood cell count.

3.5. Divergent Effects of EZH1 and EZH2/H3K27me3 Protein Expression on Patient Prognosis

Survival analyses were performed separately for all markers in the two cohorts (entire study cohort and nodal T-NHL cohort). The cohorts were divided into two groups using ROC-analysis: Patients with high protein expression and patients with low protein expression. Using the 1-year landmark as the end point, the cutoff point was set at an H-score of 4 for EZH1, of 85 for EZH2, and of 203 for H3K27me3. At the time of analysis, median follow-up time for living patients was 25.0 months (range, 0 to 142). Overall, 21 patients (45.7%) had died.

In univariable analysis, $EZH1^{low}$ expression was associated with poorer OS rates in the entire cohort ($EZH1^{low}$ vs. $EZH1^{high}$: median OS 16.0 (95% CI: 7.6–24.4) vs. 124.0 (95% CI: 16.4–232.0) months; p = 0.016; Figure 6a) and in nodal T-NHL cases ($EZH1^{low}$ vs. $EZH1^{high}$: median OS 16.0 (95% CI: 2.5–29.5) vs. 124.0 (95% CI: 0.0–272.9) months; p = 0.020; Figure 6b). However, EZH1 protein expression was not associated with PFS in univariable analysis.

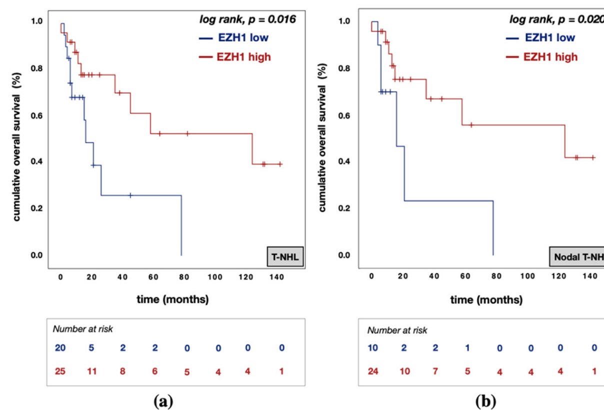

Figure 6. Kaplan–Meier (KM) curves for overall survival (OS) according to EZH1 protein expression. (**a**) KM curve for OS according to EZH1 protein expression in T-NHL; (**b**) KM curve for OS according to EZH1 protein expression in nodal T-NHL. Abbreviations: T-NHL —T-cell non-

Hodgkin's lymphomas; EZH1^{low}—EZH1 protein expression with an H-score below 4; EZH1^{high}—H3K27me3 protein expression above or equal to an H-score of 4.

In contrast, EZH2^{high} expression was associated with poorer OS rates in the entire cohort (EZH2^{low} vs. EZH2^{high}: median OS 78.0 (95% CI: 0.0–178.0) vs. 16.0 (95% CI: 0.0–38.5) months; p = 0.011; Figure 7a). This finding agrees with observations in the nodal T-NHL subtypes (EZH2^{low} vs. EZH2^{high}: median OS 124.0 (95% CI: 13.8–234.2) vs. 16.0 (95% CI: 0.0–40.0) months; p = 0.012; Figure 7b). Furthermore, EZH2^{high} expression was associated with inferior PFS rates in the entire study cohort (EZH2^{low} vs. EZH2^{high}: median PFS 29.0 (95% CI: 16.3–41.7) vs. 9.0 (95% CI: 4.2–13.8) months; p = 0.016; Figure 7c) and in nodal T-NHL cases (EZH2^{low} vs. EZH2^{high}: median PFS 22.0 (95% CI: 0.0–71.0) vs. 9.0 (95% CI: 4.8–13.2) months; p = 0.042; Figure 7d).

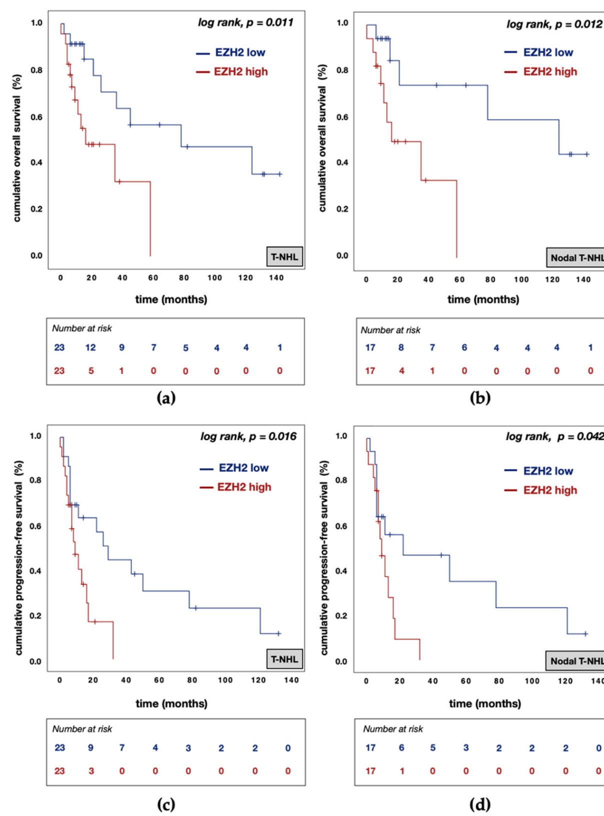

Figure 7. Kaplan–Meier (KM) curves for overall survival (OS) and progression-free survival (PFS) according to EZH2 protein expression. (**a**) KM curve for OS according to EZH2 protein expression in T-NHL; (**b**) KM curve for OS according to EZH2 protein expression in nodal T-NHL; (**c**) KM curve for PFS according to EZH2 protein expression in T-NHL; (**d**) KM curve for PFS according to EZH2 protein expression in nodal T-NHL. Abbreviations: T-NHL—T-cell non-Hodgkin's lymphomas; EZH2^{low}—EZH2 protein expression with an H-score below 85; EZH2^{high}—EZH2 protein expression above or equal to an H-score of 85.

Following the epigenetic modifiers EZH1/2 themselves, the influence of their consecutive histone lysine modification H3K27me3 on patient survival was investigated. In univariable analysis, H3K27me3^{high} expression was associated with poorer OS rates in the entire cohort (H3K27me3^{low} vs. H3K27me3^{high}: median OS 58.0 (95% CI: 0.0–165.0) vs. 36.0 (95% CI: 0.0–73.7) months; p = 0.014; Figure 8a) and in nodal T-NHL cases (H3K27me3^{low} vs. H3K27me3^{high}: median OS 124.0 (95% CI: 8.6–239.4) vs. 11.0 (95% CI: 0.0–50.4) months; p = 0.033; Figure 8b).

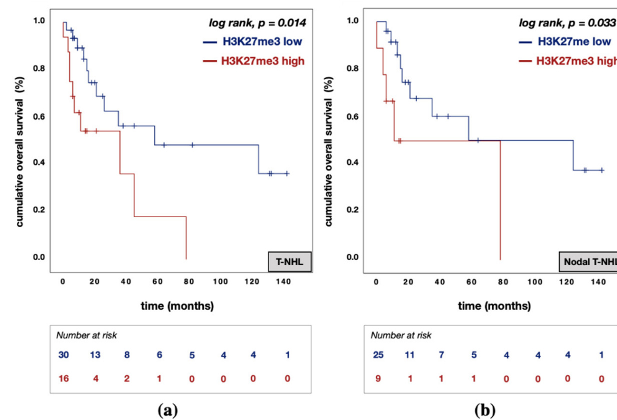

Figure 8. Kaplan–Meier (KM) curves for overall survival (OS) according to H3K27me3 protein expression. (**a**) KM curve for OS according to H3K27me3 protein expression in T-NHL. (**b**) KM curve for OS according to H3K27me3 protein expression in nodal T-NHL. Abbreviations: T-NHL—T-cell non-Hodgkin's lymphomas; H3K27me3 ^{low}—H3K27me3 protein expression with an H-score below 203; H3K27me3 ^{high}—H3K27me3 protein expression above or equal to an H-score of 203.

Multivariable Cox regression analysis of age, sex, Ann Arbor stage (only for nodal T-NHL), B symptoms, BMI, and protein expression of EZH1, EZH2, and H3K27me3 was performed for OS and PFS. This analysis revealed EZH1 expression (HR = 0.183; 95% CI: 0.044–0.767; p = 0.020; Table 3) and EZH2 expression (HR = 8.245; 95% CI: 1.898–35.826; p = 0.005; Table 3) to be independent prognostic markers for OS in the entire cohort. In nodal T-NHL cases, EZH1 (HR = 0.085; 95% CI: 0.008–0.859; p = 0.037; Table 3) and EZH2 (HR = 28.398; 95% CI: 2.166–372.334; p = 0.011; Table 3) were also independent prognostic markers for OS.

In terms of PFS, BMI (T-NHL: HR = 4.621; 95% CI: 1.706–12.515; p = 0.003; nodal T-NHL: HR = 3.750; 95% CI: 1.252–11.231; p = 0.018; Table 3) and EZH2 expression (T-NHL: HR = 3.754; 95% CI: 1.233–11.426; p = 0.020; nodal T-NHL: HR = 5.147; 95% CI: 1.472–17.998; p = 0.010; Table 3) were independent prognostic markers in both cohorts. Age, sex, Ann Arbor stage, B symptoms, and H3K27me3 expression were not associated with OS or PFS in multivariable analysis.

Table 3. Multivariable analysis of overall survival (OS) and progression-free survival (PFS).

Variable	Categories	T-NHL (n = 41) HR	95% CI LL	95% CI UL	p-Value	Nodal T-NHL (n = 30) HR	95% CI LL	95% CI UL	p-Value
Overall Survival									
Sex	female vs. male	3.816	0.990	14.699	0.052	2.900	0.462	18.198	0.256
Age in years		1.053	0.998	1.111	0.059	1.070	0.979	1.171	0.137
Ann Arbor stage	III–IV vs. I–II					0.148	0.021	1.042	0.055
B Symptoms	present vs. absent	1.182	0.411	3.397	0.756	1.066	0.166	6.822	0.947
Bone marrow involvement	present vs. absent	2.148	0.614	7.513	0.232	1.312	0.220	7.814	0.766
EZH1 expression	high vs. low	0.183	0.044	0.767	0.020	0.085	0.008	0.859	0.037
EZH2 expression	high vs. low	8.245	1.898	35.826	0.005	28.398	2.166	372.334	0.011
H3K27me3 expression	high vs. low	2.322	0.688	7.836	0.175	3.500	0.735	16.652	0.115
Progression-Free Survival									
Sex	female vs. male	1.607	0.630	4.097	0.321	0.959	0.289	3.179	0.945
Age in years		1.008	0.966	1.051	0.728	0.987	0.932	1.046	0.660
Ann Arbor stage	III–IV vs. I–II					0.933	0.249	3.495	0.918
B Symptoms	present vs. absent	1.299	0.512	3.298	0.582	0.888	0.250	3.159	0.855
Bone marrow involvement	present vs. absent	4.621	1.706	12.515	0.003	3.750	1.252	11.231	0.018
EZH1 expression	high vs. low	0.668	0.246	1.814	0.428	0.391	0.104	1.471	0.165
EZH2 expression	high vs. low	3.754	1.233	11.426	0.020	5.147	1.472	17.998	0.010
H3K27me3 expression	high vs. low	1.326	0.546	3.218	0.533	1.537	0.469	5.038	0.478

Abbreviations: T-NHL—T-cell non-Hodgkin's lymphomas; HR—hazard ratio; CI—confidence interval; LL—lower limit; UL—upper limit; n—number; EZH1 high—EZH1 protein expression above or equal to an H-score of 4; EZH1 low—EZH1 protein expression with an H-score below 4; EZH2 high—EZH2 protein expression above or equal to an H-score of 85; EZH2 low—EZH2 protein expression with an H-score below 85; H3K27me3 high—H3K27me3 protein expression above or equal to an H-score of 203; H3K27me3 low—H3K27me3 protein expression with an H-score below 203.

4. Discussion

Aberrant epigenetic regulation has been shown to play a central role in the development of multiple malignancies. EZH2 functions as an important histone methyltransferase to regulate DNA methylation and control gene expression [12]. Over the past decade, studies have established that EZH2 is overexpressed in malignancies and that its high expression is associated with tumor progression and poor prognosis. In contrast, the function of EZH1 and expression status is a less studied field. In this study, we evaluated the *EZH1/2* mutation status in a cohort of 33 MEITL by NGS and investigated the immunohistochemical protein expression of EZH1, EZH2, and H3K27me3 in combination with the clinical outcomes in 46 patients with T-cell lymphomas.

In NGS analysis, *EZH2* mutations were found to be present in just one MEITL case, which was located in the SET-domain. Furthermore, no *EZH1* mutation could be identified. On the basis of the single mutation found, we could not detect any associations with protein expression of EZH1, EZH2, or H3K27me3. Our results are in line with previous studies reporting that mutations of *EZH2* are rare in T-NHL: one in 36 cases (2.7%) in PTCL-NOS and one AITL case in 84 cases (1.2%) in a cohort consisting of AITL, PTCL-NOS, ALCL, and MEITL presented as nonsynonymous single nucleotide variants (SNV) [21]. In addition, several other groups have not identified mutations of *EZH1* by WES [17–24]. Mutations of other PCR2 complex members such as embryonic ectoderm development (*EED*) and suppressor of Zeste 12 (*SUZ12*) were not studied, but also not detected in other T-NHL studies [17–24].

Moreover, compared with normal lymph node tissues, EZH2 and H3K27me3 proteins were found to be overexpressed in T-cell lymphomas, whereas EZH1 was underexpressed, which is consistent with previous reports [25–29]. Our further findings, along with those of others [14–17] show that high EZH2 protein expression was associated with poor prognosis in T-cell lymphomas and markers that are known for cancer progression. Strong EZH2 protein expression was associated with the presence of B symptoms (median 130 vs. 53.8; $p = 0.031$) and high Ki-67 expression (median 150 vs. 47.5; $p = 0.059$) in nodal T-NHL. The univariable analysis showed poorer OS rates in both cohorts exhibiting high EZH2 protein expression compared with those exhibiting low expression (T-NHL: median OS 78.0 vs. 16.0 months; $p = 0.011$; nodal T-NHL: median OS 124.0 vs. 16.0 months; $p = 0.012$). Furthermore, high EZH2 protein expression was associated with inferior PFS rates (T-NHL: median PFS 29.0 vs. 9.0 months; $p = 0.016$; nodal T-NHL: median PFS 22.0 vs. 9.0 months; $p = 0.042$). In multivariable analysis, EZH2 was also an independent prognostic marker for OS (T-NHL: HR = 8.245; 95% CI: 1.898–35.826; $p = 0.005$; nodal T-NHL: HR = 28.398; 95% CI: 2.166–372.334; $p = 0.011$) and PFS (T-NHL: HR = 3.754; 95% CI: 1.233–11.426; $p = 0.020$; nodal T-NHL: HR = 5.147; 95% CI: 1.472–17.998; $p = 0.010$).

H3K27me3 was also overexpressed and high protein expression also led to poor survival in univariable analysis (T-NHL: median OS 58.0 vs. 36.0 months; $p = 0.014$; nodal T-NHL: median OS 124.0 vs. 11.0 months; $p = 0.033$). However, H3K27me3 was not an independent prognostic marker in multivariable analysis and no associations with patient characteristics were observed. The correlation between EZH2 and its consecutive trimethylation of lysine 27 of histone H3 has previously been studied. In the context of T-cell lymphomas, results have been inconclusive so far: on one hand, a moderate positive correlation integrating multiple T-cell lymphoma entities (NKTCL, PTCL-NOS, AITL, ALCL, and T-LBL) was found [26], on the other hand, a strong inverse correlation of protein expression was reported in NKTCL [31]. Our analysis showed no correlation between EZH2 and H3K27me3 protein expression ($r = 0.175$; $p = 0.224$). Since there was no correlation between EZH2 and H3k27me3, no association between H3k27me3 and the studied clinicopathological markers, and the impact of H3K27me3 protein expression on OS could not be confirmed in multivariable analysis, we concluded that EZH2 may also influence the outcome of T-cell lymphomas by non-canonical functions.

Compared to the proteins already described, EZH1 was weakly expressed and associated with poorer survival when expressed at low levels. In univariable analysis, low EZH1 protein expression was associated with poor prognosis (T-NHL: median OS 16.0 vs. 124.0 months; $p = 0.016$; nodal T-NHL: median OS 16.0 vs. 124.0 months; $p = 0.020$). Furthermore, EZH1 protein expression was also an independent prognostic marker for OS (T-NHL: HR = 0.183; 95% CI: 0.044–0.767; $p = 0.020$; nodal T-NHL: HR = 0.085; 95% CI: 0.008–0.859; $p = 0.037$) in multivariable analysis. Thus, we can conclude that EZH1 and EZH2 have the opposite impact on the OS in the T-cell lymphomas cohorts studied. These results were replicated using The Cancer Genome Atlas (TCGA) (TCGA, https://tcga-data.nci.nih.gov/tcga/) with data from the TCGA Pan-Cancer (PANCAN) study and an inverse association to survival rates could also be observed (Supplementary Figure S1, available at Biomedicines online). Furthermore, Abdalkader et al. examined the association between EZH1 and EZH2 in T/NK-cell neoplasms and observed opposing protein expression patterns in both normal and neoplastic lymphoid tissues as well as an opposing relationship with Ki-67 expression [25]. Restrictively, it is to be added that our correlation analysis showed no association between the two paralogs EZH1 and EZH2, which could have been due to the extremely low or negative EZH1 expression.

Counterintuitively to our correlative study, in which EZH2 and EZH1 expression was not associated and low EZH1 expression conferred a poor prognosis, mechanistic studies revealed that dual inhibition of EZH1 and EZH2 is required for strong lethality in lymphomas due to increased EZH1 occupancy with focal H3K27me3 upregulation [12]. Valemetostat is a dual inhibitor of EZH1 and EZH2 that prevents trimethylation of H3K27,

leading to altered gene expression patterns, which suppresses proliferation of EZH1/2-dependent cancer cells. Compared with established EZH2-specific inhibitors (GSK126), dual EZH1 and EZH2 inhibitors showed a significantly stronger reduction in H3K27me3 levels [12,39]. In an ongoing open-label phase 1 study (NCT 02732275, November 2 2020 data cut-off) evaluating valemetostat tosylate monotherapy, the objective response rate (ORR) was 55.6% in 45 relapsed/refractory (r/r) PTCL patients and 50% in 14 r/r adult T-cell leukemia/lymphoma (ATL) patients [40]. The most frequently reported related treatment-emergent adverse events (TEAE) were decreased platelet count, dysgeusia, and anemia [40]. Based on these findings, a global phase 2 study of valemetostat tosylate monotherapy in patients with r/r peripheral T-cell lymphomas (n = 176) is currently ongoing (NCT 04703192).

Because of the retrospective nature of this analysis and the large heterogeneity of the studied cohort, the results have some limitations. The small sample size, which is unfortunately common in the study of T-cell lymphomas, carries the risk of statistical error. In addition, the extremely low protein expression of EZH1 posed a problem because it made staining evaluation difficult. Prospective studies with larger cohorts are therefore urgently needed to investigate the function of EZH1/EZH2 during carcinogenesis. In addition, further mechanistic experiments are required to elucidate the molecular mechanisms of EZH1 and EZH2 in T-cell lymphomas.

In conclusion, we have demonstrated that *EZH1* and *EZH2* mutations are rare in MEITL and therefore do not appear to be of particular significance. Nevertheless, the proteins studied in T-cell lymphomas had opposite expression patterns and effects in survival analyses. In univariable analysis, high EZH1 protein expression was associated with an improved OS and PFS, whereas high EZH2 and H3K27me3 protein expression were associated with poorer OS and PFS. Multivariable analysis showed that EZH2 and EZH1 protein expression were independent, divergent prognostic markers for OS. EZH2 protein expression was also an independent prognostic marker for PFS. Targeting of both EZH1 and EZH2 enzyme activity may serve as a target for anticancer therapy in T-cell lymphomas.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/biomedicines9121842/s1, Figure S1: Kaplan–Meier (KM) curves for overall survival (OS) according to EZH1 and EZH2 protein expression in the TCGA Pan Cancer (PANCAN) study. (**a**) KM curve for OS according to EZH1 protein expression. (**b**) KM curve for OS according to EZH2 protein expression.

Author Contributions: Conceptualization, T.W.; Methodology, A.W., M.B. (Mascha Binder), L.P.M., G.P., C.M.-T. and T.W.; Validation, A.W. and T.W.; Formal analysis F.L.S., E.G., M.B. (Marcus Bauer) and T.W.; Investigation, M.B. (Marcus Bauer), F.L.S., A.W., S.S., C.R., G.P., F.F., M.-L.H., W.K., A.R., H.S. and T.W., Data curation, F.L.S., D.T., E.G. and T.W.; Writing—original draft preparation, F.L.S., E.G. and T.W.; Writing—review and editing, F.L.S., E.G., M.B. (Marcus Bauer), C.R., S.S., D.T., L.P.M., G.P., A.W., F.F., M.-L.H., W.K., A.R., H.S., M.D., C.M.-T., C.W., D.T., M.B. (Mascha Binder), and T.W.; Visualization, F.L.S., E.G. and M.B. (Marcus Bauer); Supervision, T.W.; Project administration, T.W.; Funding acquisition, T.W., M.B. (Mascha Binder) and F.L.S. All authors have read and agreed to the published version of the manuscript.

Funding: F.L.S. was supported by a grant from the German Cancer Aid, grant number 70114141. E.G. was supported by a grant from the Wilhelm-Roux program of the Martin-Luther-University Halle-Wittenberg, grant number TIF-Roux FKZ31/45.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Martin-Luther-University of Halle-Wittenberg (#2020–033, 10 May 2020).

Informed Consent Statement: Patient consent was waived due to the study's retrospective nature.

Data Availability Statement: All relevant data are within the paper and its Supplementary Materials.

Acknowledgments: We would like to thank Janin Siebert and Sarah Vogtländer for their excellent support in the laboratory.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Swerdlow, S.H.; World Health Organization; International Agency for Research on Cancer. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th ed.; International Agency for Research on Cancer: Lyon, France, 2017.
2. Vose, J.; Armitage, J.; Weisenburger, D.; International, T.C.L.P. International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. *J. Clin. Oncol.* **2008**, *26*, 4124–4130.
3. Bellei, M.; Federico, M. The outcome of peripheral T-cell lymphoma patients failing first-line therapy: A report from the prospective International T-Cell Project. *Haematologica* **2019**, *104*, e178. https://doi.org/10.3324/haematol.2019.218305.
4. Czermin, B.; Melfi, R.; McCabe, D.; Seitz, V.; Imhof, A.; Pirrotta, V. Drosophila Enhancer of Zeste/ESC Complexes Have a Histone H3 Methyltransferase Activity that Marks Chromosomal Polycomb Sites. *Cell* **2002**, *111*, 185–196. https://doi.org/10.1016/s0092-8674(02)00975-3.
5. Kuzmichev, A.; Nishioka, K.; Erdjument-Bromage, H.; Tempst, P.; Reinberg, D. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev.* **2002**, *16*, 2893–2905. https://doi.org/10.1101/gad.1035902.
6. Müller, J.; Hart, C.; Francis, N.J.; Vargas, M.L.; Sengupta, A.; Wild, B.; Miller, E.L.; O'Connor, M.; Kingston, R.E.; Simon, J.A. Histone Methyltransferase Activity of a Drosophila Polycomb Group Repressor Complex. *Cell* **2002**, *111*, 197–208. https://doi.org/10.1016/s0092-8674(02)00976-5.
7. Cao, R.; Wang, L.; Wang, H.; Xia, L.; Erdjument-Bromage, H.; Tempst, P.; Jones, R.S.; Zhang, Y. Role of Histone H3 Lysine 27 Methylation in Polycomb-Group Silencing. *Science* **2002**, *298*, 1039–1043. https://doi.org/10.1126/science.1076997.
8. Barski, A.; Cuddapah, S.; Cui, K.; Roh, T.-Y.; Schones, D.E.; Wang, Z.; Wei, G.; Chepelev, I.; Zhao, K. High-Resolution Profiling of Histone Methylations in the Human Genome. *Cell* **2007**, *129*, 823–837. https://doi.org/10.1016/j.cell.2007.05.009.
9. Margueron, R.; Reinberg, D.; Margueron, R.; Reinberg, D. The Polycomb complex PRC2 and its mark in life. *Nature* **2011**, *469*, 343–349. https://doi.org/10.1038/nature09784.
10. Shen, X.; Liu, Y.; Hsu, Y.-J.; Fujiwara, Y.; Kim, J.; Mao, X.; Yuan, G.-C.; Orkin, S.H. EZH1 Mediates Methylation on Histone H3 Lysine 27 and Complements EZH2 in Maintaining Stem Cell Identity and Executing Pluripotency. *Mol. Cell* **2008**, *32*, 491–502. https://doi.org/10.1016/j.molcel.2008.10.016.
11. Margueron, R.; Li, G.; Sarma, K.; Blais, A.; Zavadil, J.; Woodcock, C.L.; Dynlacht, B.D.; Reinberg, D. Ezh1 and Ezh2 Maintain Repressive Chromatin through Different Mechanisms. *Mol. Cell* **2008**, *32*, 503–518. https://doi.org/10.1016/j.molcel.2008.11.004.
12. Yamagishi, M.; Hori, M.; Fujikawa, D.; Ohsugi, T.; Honma, D.; Adachi, N.; Katano, H.; Hishima, T.; Kobayashi, S.; Nakano, K.; et al. Targeting Excessive EZH1 and EZH2 Activities for Abnormal Histone Methylation and Transcription Network in Malignant Lymphomas. *Cell Rep.* **2019**, *29*, 2321–2337.e7. https://doi.org/10.1016/j.celrep.2019.10.083.
13. Bachmann, I.M.; Halvorsen, O.J.; Collett, K.; Stefansson, I.M.; Straume, O.; Haukaas, S.A.; Salvesen, H.B.; Otte, A.P.; Akslen, L.A. EZH_2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J. Clin. Oncol.* **2006**, *24*, 268–273. https://doi.org/10.1200/JCO.2005.01.5180.
14. Duan, R.; Du, W.; Guo, W. EZH2: A novel target for cancer treatment. *J. Hematol. Oncol.* **2020**, *13*, 104. https://doi.org/10.1186/s13045-020-00937-8.
15. Varambally, S.; Dhanasekaran, S.M.; Zhou, M.; Barrette, T.R.; Kumar, C.; Sanda, M.G.; Ghosh, D.; Pienta, K.; Sewalt, R.G.A.B.; Otte, A.P.; et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nat. Cell Biol.* **2002**, *419*, 624–629. https://doi.org/10.1038/nature01075.
16. Wang, C.-G.; Ye, Y.-J.; Yuan, J.; Liu, F.-F.; Zhang, H.; Wang, S. EZH2 and STAT6 expression profiles are correlated with colorectal cancer stage and prognosis. *World J. Gastroenterol.* **2010**, *16*, 2421–2427. https://doi.org/10.3748/wjg.v16.i19.2421.
17. Wang, M.; Zhang, S.; Chuang, S.-S.; Ashton-Key, M.; Ochoa, E.; Bolli, N.; Vassiliou, G.; Gao, Z.; Du, M.-Q. Angioimmunoblastic T cell lymphoma: Novel molecular insights by mutation profiling. *Oncotarget* **2017**, *8*, 17763–17770. https://doi.org/10.18632/oncotarget.14846.
18. Palomero, T.; Couronné, L.; Khiabanian, H.; Kim, M.-Y.; Ambesi-Impiombato, A.; Perez-Garcia, A.; Carpenter, Z.; Abate, F.; Allegretta, M.; Haydu, J.E.; et al. Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. *Nat. Genet.* **2014**, *46*, 166–170. https://doi.org/10.1038/ng.2873.
19. Sakata-Yanagimoto, M.; Enami, T.; Yoshida, K.; Shiraishi, Y.; Ishii, R.; Miyake, Y.; Muto, H.; Tsuyama, N.; Sato-Otsubo, A.; Okuno, Y.; et al. Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. *Nat. Genet.* **2014**, *46*, 171–175. https://doi.org/10.1038/ng.2872.
20. Crescenzo, R.; Abate, F.; Lasorsa, E.; Gaudiano, M.; Chiesa, N.; Di Giacomo, F.; Spaccarotella, E.; Barbarossa, L.; Ercole, E.; Todaro, M.; et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. *Cancer Cell* **2015**, *27*, 516–532.
21. Maura, F.; Dodero, A.; Carniti, C.; Bolli, N.; Magni, M.; Monti, V.; Cabras, A.; Leongamornlert, D.; Abascal, F.; Diamond, B.; et al. *CDKN2A* deletion is a frequent event associated with poor outcome in patients with peripheral T-cell lymphoma not otherwise specified (PTCL-NOS). *Haematologica* **2021**, *106*, 2918.

22. Roberti, A.; Dobay, M.P.; Bisig, B.; Vallois, D.; Boéchat, C.; Lanitis, E.; Bouchindhomme, B.; Parrens, M.- C.; Bossard, C.; Quintanilla-Martinez, L.; et al. Type II enteropathy-associated T-cell lymphoma features a unique genomic profile with highly recurrent SETD2 alterations. *Nat. Commun.* **2016**, *7*, 12602. https://doi.org/10.1038/ncomms12602.
23. Watatani, Y.; Sato, Y.; Miyoshi, H.; Sakamoto, K.; Nishida, K.; Gion, Y.; Nagata, Y.; Shiraishi, Y.; Chiba, K.; Tanaka, H.; et al. Molecular heterogeneity in peripheral T-cell lymphoma, not otherwise specified revealed by comprehensive genetic profiling. *Leukemia* **2019**, *33*, 2867–2883. https://doi.org/10.1038/s41375-019-0473-1.
24. Laginestra, M.A.; Cascione, L.; Motta, G.; Fuligni, F.; Agostinelli, C.; Rossi, M.; Sapienza, M.R.; Righi, S.; Broccoli, A.; Indio, V.; et al. Whole exome sequencing reveals mutations in FAT1 tumor suppressor gene clinically impacting on peripheral T-cell lymphoma not otherwise specified. *Mod. Pathol.* **2020**, *33*, 319.
25. Abdalkader, L.; Oka, T.; Takata, K.; Sato, H.; Murakami, I.; Otte, A.P.; Yoshino, T. Aberrant differential expression of EZH1 and EZH2 in Polycomb repressive complex 2 among B- and T/NK-cell neoplasms. *Pathology* **2016**, *48*, 467–482. https://doi.org/10.1016/j.pathol.2016.05.002.
26. Kim, S.H.; Yang, W.I.; Min, Y.H.; Ko, Y.H.; Yoon, S.O. The role of the polycomb repressive complex pathway in T and NK cell lymphoma: Biological and prognostic implications. *Tumor Biol.* **2015**, *37*, 2037–2047. https://doi.org/10.1007/s13277-015-3977-y.
27. Yi, S.; Sun, J.; Qiu, L.; Fu, W.; Wang, A.; Liu, X.; Yang, Y.; Kadin, M.E.; Tu, P.; Wang, Y. Dual Role of EZH2 in Cutaneous Anaplastic Large Cell Lymphoma: Promoting Tumor Cell Survival and Regulating Tumor Microenvironment. *J. Investig. Dermatol.* **2018**, *138*, 1126–1136. https://doi.org/10.1016/j.jid.2017.10.036.
28. Yan, J.; Ng, S.-B.; Tay, J.L.-S.; Lin, B.; Koh, T.L.; Tan, J.; Selvarajan, V.; Liu, S.-C.; Bi, C.; Wang, S.; et al. EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone methyltransferase activity. *Blood* **2013**, *121*, 4512–4520. https://doi.org/10.1182/blood-2012-08-450494.
29. Shi, M.; Shahsafaei, A.; Liu, C.; Yu, H.; Dorfman, D.M. Enhancer of zeste homolog 2 is widely expressed in T-cell neoplasms, is associated with high proliferation rate and correlates with MYC and pSTAT3 expression in a subset of cases. *Leuk. Lymphoma* **2014**, *56*, 2087–2091. https://doi.org/10.3109/10428194.2014.968780.
30. Zhang, H.; Lv, H.; Jia, X.; Hu, G.; Kong, L.; Zhang, T.; Li, L.; Pan, Y.; Zhai, Q.; Meng, B.; et al. Clinical significance of enhancer of zeste homolog 2 and histone deacetylases 1 and 2 expression in peripheral T-cell lymphoma. *Oncol. Lett.* **2019**, *18*, 1415–1423. https://doi.org/10.3892/ol.2019.10410.
31. Liu, J.; Liang, L.; Huang, S.; Nong, L.; Li, D.; Zhang, B.; Li, T. Aberrant differential expression of EZH2 and H3K27me3 in extranodal NK/T-cell lymphoma, nasal type, is associated with disease progression and prognosis. *Hum. Pathol.* **2019**, *83*, 166–176. https://doi.org/10.1016/j.humpath.2018.08.025.
32. Cai, M.-Y.; Hou, J.-H.; Rao, H.-L.; Luo, R.-Z.; Li, M.; Pei, X.-Q.; Lin, M.C.; Guan, X.-Y.; Kung, H.-F.; Zeng, Y.-X.; et al. High Expression of H3K27me3 in Human Hepatocellular Carcinomas Correlates Closely with Vascular Invasion and Predicts Worse Prognosis in Patients. *Mol. Med.* **2010**, *17*, 12–20. https://doi.org/10.2119/molmed.2010.00103.
33. He, L.-R.; Liu, M.-Z.; Li, B.-K.; Rao, H.-L.; Liao, Y.-J.; Guan, X.-Y.; Zeng, Y.-X.; Xie, D. Prognostic impact of H3K27me3 expression on locoregional progression after chemoradiotherapy in esophageal squamous cell carcinoma. *BMC Cancer* **2009**, *9*, 461. https://doi.org/10.1186/1471-2407-9-461.
34. Wei, Y.; Xia, W.; Zhang, Z.; Liu, J.; Wang, H.; Adsay, V.; Albarracin, C.; Yu, D.; Abbruzzese, J.L.; Mills, G.B.; et al. Loss of trimethylation at lysine 27 of histone H3 is a predictor of poor outcome in breast, ovarian, and pancreatic cancers. *Mol. Carcinog.* **2008**, *47*, 701–706. https://doi.org/10.1002/mc.20413.
35. Holm, K.; Grabau, D.; Lövgren, K.; Aradottir, S.; Gruvberger-Saal, S.; Howlin, J.; Saal, L.H.; Ethier, S.P.; Bendahl, P.-O.; Stål, O.; et al. Global H3K27 trimethylation and EZH2 abundance in breast tumor subtypes. *Mol. Oncol.* **2012**, *6*, 494–506. https://doi.org/10.1016/j.molonc.2012.06.002.
36. Sandmann, S.; Karimi, M.; O De Graaf, A.; Rohde, C.; Göllner, S.; Varghese, J.; Ernsting, J.; Walldin, G.; A Van Der Reijden, B.; Müller-Tidow, C.; et al. appreci8: A pipeline for precise variant calling integrating 8 tools. *Bioinformatics* **2018**, *34*, 4205–4212. https://doi.org/10.1093/bioinformatics/bty518.
37. Schümann, F.L.; Bauer, M.; Groß, E.; Terziev, D.; Wienke, A.; Wickenhauser, C.; Binder, M.; Weber, T. RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis. *Cancers* **2021**, *13*, 4788. https://doi.org/10.3390/cancers13194788.
38. Detre, S.; Jotti, G.S.; Dowsett, M. A "quickscore" method for immunohistochemical semiquantitation: Validation for oestrogen receptor in breast carcinomas. *J. Clin. Pathol.* **1995**, *48*, 876–878. https://doi.org/10.1136/jcp.48.9.876.
39. Kagiyama, Y.; Fujita, S.; Shima, Y.; Yamagata, K.; Katsumoto, T.; Nakagawa, M.; Honma, D.; Adachi, N.; Araki, K.; Kato, A.; et al. CDKN1C-mediated growth inhibition by an EZH1/2 dual inhibitor overcomes resistance of mantle cell lymphoma to ibrutinib. *Cancer Sci.* **2021**, *112*, 2314–2324.
40. Ishitsuka, K.; Izutsu, K.; Maruyama, D.; Makita, S.; Jacobsen, E.D.; Horwitz, S.; Kusumoto, S.; Allen, P.; Porcu, P.; Imaizumi, Y.; et al. First-in-human Study of the EZH1 and EZH2 Dual Inhibitor Valemetostat Tosylate (Ds-3201b) in Patients with Relapsed or Refractory Non-Hodgkin Lymphomas. *Hematol. Oncol.* **2021**, *39*, 1–520. https://doi.org/10.1002/hon.14_2879.

Ergänzende Abbildung 1

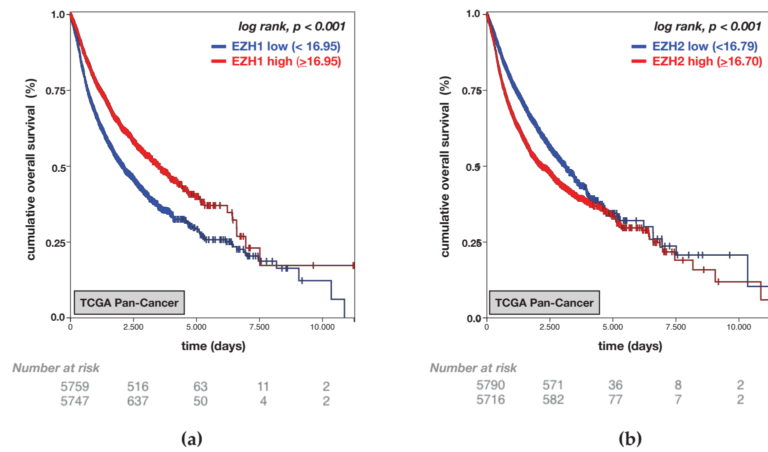

Figure S1: Kaplan–Meier (KM) curves for overall survival (OS) according to EZH1 and EZH2 protein expression in the TCGA Pan Cancer (PANCAN) study. (a) KM curve for OS according to EZH1 protein expression. (b) KM curve for OS according to EZH2 protein expression.

5.2 Publikation 2: Schümann FL, Bauer M, Groß E, Terziev D, Wienke A, Wickenhauser C, Binder M, Weber T (2021) RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis. Cancers (Basel) 13 (19)

cancers

MDPI

Article

RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis

Franziska Lea Schümann [1,†], Marcus Bauer [2,†], Elisabeth Groß [1], Denis Terziev [1], Andreas Wienke [3], Claudia Wickenhauser [2], Mascha Binder [1] and Thomas Weber [1,*]

[1] Department of Internal Medicine IV, Haematology and Oncology, University Hospital Halle (Saale), Martin-Luther-University Halle-Wittenberg, 06120 Halle, Germany; franziska.schuemann@uk-halle.de (F.L.S.); elisabeth.gross@uk-halle.de (E.G.); denis.terziev@uk-halle.de (D.T.); mascha.binder@uk-halle.de (M.B.)
[2] Institute of Pathology, University Hospital Halle (Saale), Martin-Luther-University Halle-Wittenberg, 06112 Halle, Germany; marcus.bauer@uk-halle.de (M.B.); claudia.wickenhauser@uk-halle.de (C.W.)
[3] Institute of Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University Halle-Wittenberg, 006112 Halle, Germany; andreas.wienke@uk-halle.de
* Correspondence: thomas.weber@uk-halle.de
† Contributed equally to this article.

Simple Summary: Patients with T-cell non-Hodgkin's lymphomas (T-NHL) are often chemotherapy refractory and subsequently have poor prognosis. So far, mechanisms leading to this primary chemotherapy refractoriness and factors identifying such cases are not well established. This study investigated the prognostic relevance of the RNA binding protein X (RBMX) in 53 T-NHL cases using conventional immunohistochemistry. As shown, low RBMX expression was associated with better response to anthracycline-containing first-line treatment. Furthermore, low RBMX expression predicted an improved overall survival (OS) and progression-free survival (PFS). These results suggest that RBMX protein expression levels might be a contributing factor towards chemotherapy resistance and thus affect prognosis of patients with T-cell lymphomas.

Abstract: T-cell non-Hodgkin's lymphomas (T-NHL) are a heterogeneous group of lymphomas with a mature T-cell phenotype. While in some hematological diseases the prognosis improved over the last decades, T-NHL cases often relapse early or present with an initially refractory course. Recently, it has been shown that RNA binding proteins have a crucial role for malignant tumor initiation, progression and treatment response while contributing to chemotherapy resistance. Therefore, we investigated the protein expression of the RNA binding protein X (RBMX), which has been shown to be of great relevance in disease initiation and progression in hematological diseases in 53 T-NHL cases using conventional immunohistochemistry. Low RBMX expression was associated with better response to anthracycline-containing first-line treatment. Furthermore, low RBMX expression predicted an improved overall survival and progression-free survival in univariate analysis. Multivariable Cox regression revealed RBMX as an independent prognostic marker for overall survival ($p = 0.007$; hazard ratio (HR) = 0.204; 95% confidence interval (CI): 0.064–0.646) and progression-free survival ($p = 0.006$; HR = 0.235; 95% CI: 0.083–0.666). The study identifies low RBMX expression to predict better chemotherapy response, overall survival and progression-free survival in patients with T-cell non-Hodgkin's lymphomas. These results suggest that RBMX protein expression levels might be a contributing factor towards chemotherapy resistance and thus affect prognosis. Hence, RBMX may be a potential therapeutic target and prognostic marker in T-cell lymphomas.

Keywords: T-cell non-Hodgkin's lymphomas; PTCL; RBMX; hnRNP G; drug resistance; immunohistochemistry

Citation: Schümann, F.L.; Bauer, M.; Groß, E.; Terziev, D.; Wienke, A.; Wickenhauser, C.; Binder, M.; Weber, T. RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis. *Cancers* **2021**, *13*, 4788. https://doi.org/10.3390/cancers13194788

Academic Editor: Pierre Brousset

Received: 4 August 2021
Accepted: 20 September 2021
Published: 24 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

T-cell non-Hodgkin's lymphomas (T-NHL) arise from post-thymic lymphocytes and represent 10–15% of all non-Hodgkin's lymphomas in western countries [1]. T-NHL are highly heterogeneous in their clinical presentation, histologic features, and pathogenesis [2]. The disease is characterized by frequent relapse, and an initially refractory course is not uncommon. For most subtypes, the first-line treatment regime is typically an anthracycline-containing chemotherapy combination, such as CHO(E)P (cyclophosphamide, doxorubicin, vincristine, (etoposide), and prednisone) [3]. Approximately 30% of patients face primary refractory disease [4,5], and most patients with refractory or relapsed T-NHL have poor outcomes with short survival [6]. Therefore, it urgently requires further research for precise prognostic indicators and novel treatment options in order to improve the survival of affected patients.

Recently, it has been reported that RNA-binding proteins play an important role in cancer progression, and even contribute to chemotherapy resistance [7]. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a large family of chromatin-associated RNA-binding proteins with more than 30 different members. Among these, the X-linked RNA-binding motif protein (RBMX, also named hnRNP G) is one of the least characterized proteins concerning its biological functions [8]. RBMX was originally recognized as a nuclear protein that is part of the supraspliceosome, where it regulates alternative splice site selection depending on its concentration [9,10]. RBMX is also known to regulate the proper cohesion of sister chromatids during cell division [11]. Recently, several studies have reported replication defects in cells lacking RBMX, and the authors pointed to a key role of RBMX in genome stability [12,13].

The function of RBMX during carcinogenesis and promoting therapy resistance has only sporadically been studied and so far has remained insufficiently understood. RBMX has been proposed as a potential tumor suppressor in several cancer types, including oral squamous carcinoma [8,14,15] and lung cancer [16]. However, reports of RBMX expression levels in cancer samples are more contradictory. Low RBMX expression levels have been associated with poor outcome in endometrial cancer [17,18] and bladder cancer [19]. In contrast, recent studies interestingly show poor outcome in hepatocellular carcinomas (HCC) [7] as well as head and neck cancers [20] when RBMX was highly expressed. Furthermore, RBMX was overexpressed in individuals with acute myeloid leukemia (AML) compared to healthy ones, and loss of RBMX delayed leukemia development [21].

In addition, RBMX was intimately involved in chemoresistance according to previous studies. In 2012, it was shown by Adamson et al. that the depletion of RBMX sensitizes cells to DNA damage caused by ionizing radiation and several genotoxic drugs (mitomycin C, chlorambucil, oxaliplatin, and carboplatin). Moreover, exome sequencing analysis show mutations in the RBMX gene in vemurafenib-resistant thyroid carcinoma cells [22]. On the contrary, it has recently been shown that in HCC cells, sorafenib resistance was increased when RBMX was overexpressed [7]. To further understand the role of RBMX in chemotherapy resistance and cancer prognosis of T-cell lymphomas, the present study investigates the immunohistochemical protein expression of RBMX in combination with the clinical outcomes in patients with T-NHL.

2. Materials and Methods

2.1. Patients and Tissue Samples

This is a retrospective single-center analysis of 53 patients forming a convenience sample who were treated between 2006 and March 2020 at the University Hospital Halle (Saale). Only patients with available formalin-fixed, paraffin-embedded (FFPE) T-cell leukemia and lymphoma tissues were included. The tissue samples originated from 43 patients that had been integrated into a tissue microarray (TMA) for a previous study and from 10 large tissue sections obtained for this study. Patients were identified by a review of the internal hospital database records. Patients were included in the analysis if they

were ≥ 18 years of age with a biopsy-proven diagnosis of T-NHL according to the WHO classification. All samples were histopathological reviewed by two pathologists (C.W. and M.B.) to verify the diagnosis according to WHO criteria 2017 [2]. Five samples were excluded since the integrated tissue samples did not originate from the primary diagnosis or the diagnosis was not confirmed. Clinicopathological characteristics at the time of primary diagnosis, including age, sex, histological phenotypes, B symptoms, Ann Arbor stage, international prognostic index (IPI), Eastern Cooperative Oncology Group (ECOG) status, bone marrow involvement (BMI), lactate dehydrogenase (LDH) level, white blood cell (WBC) count and Ki-67 expression along with treatment regime, chemotherapy response, the occurrence of relapses, and follow-up data were recorded in the TMA cohort ($n = 43$). Chemotherapy resistance was defined as stable disease (SD) or progressive disease (PD) after first-line treatment, while chemosensitive patients had a complete response (CR) or a partial response (PR) according to RECIST [23]. In accordance with the Declaration of Helsinki, this study was performed and approved by the Ethics Committee at the Martin-Luther-University of Halle-Wittenberg (#2020–033).

2.2. *Tissue Microarray Construction*

FFPE leukemia and lymphoma tissues from 53 non-selected patients were obtained from the Institute of Pathology, University Hospital Halle (Saale). Tissue microarrays (TMAs) containing two 0.6 mm tissue cylinders of each donor block were constructed using a manual tissue arrayer (Beecher Instruments Inc., Sun Prairie, WI, USA). A slide stained with hematoxylin and eosin (H&E) was prepared from each donor block and representative tumor regions were morphologically identified and marked on each slide by a pathologist (M.B). From these defined areas, two tissue cores with a diameter of 0.6 mm were taken and arrayed on a recipient paraffin block. Adequate control tissues, including liver tissue, tonsil tissue, breast carcinoma, seminoma, prostate carcinoma, and osteosarcoma for specific antibodies were also included.

2.3. *Immunohistochemistry*

Immunohistochemistry (IHC) analysis was performed on a Bond III automated immunostainer (Leica Biosystems Nussloch GmbH, Wetzlar, Germany) using the Bond Polymer Refine Detection Kit (DS9800-CN). The primary antibody for RBMX (1:150; Abcam, Cambridge, UK; ab190352) was applied as recommended by the manufacturer. Immunostaining was assessed by two investigators (M.B. and F.L.S.) blinded to additional pathological and clinical data using Zeiss Axioscope 5 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Semi-quantitative H-scoring [24] was performed in adherence to the following steps: Brown immunoreactivity of cell nuclei was taken as positive and the proportion of negative cells (P0) as well as staining at low (P1), moderate (P2), or high (P3) levels of intensity were scored. The H-score for one patient (H-score = (% of cells stained at intensity category 1 × 1) + (% of cells stained at intensity category 2 × 2) + (% of cells stained at intensity category 3 × 3)) was calculated from the mean of two stains. The conclusions of the two inspectors were in complete agreement in approximately 88% of the cases confirming this scoring method as reproducible. In addition, 10 normal lymph node tissues were stained to compare the RBMX expression levels of healthy and tumor tissues.

2.4. *Survival and Statistical Analysis*

IBM SPSS statistic software, version 27.0, for Mac (International Business Machines Corporation, Armonk, NY, USA) was used for data analysis. All analyses were performed for T-NHL overall, followed by nodal T-NHL phenotypes. Comparison of continuous variables between two groups was computed with unpaired t-tests and between multiple groups with one-way ANOVA with Bonferroni's correction. Overall survival (OS) and progression-free survival (PFS) rates were obtained using the Kaplan–Meier method. Statistical comparisons between groups were made by log rank tests. Receiver operating

characteristic (ROC)-analysis was used to determine a cutoff value for RBMX expression to divide the samples into two groups: $RBMX^{high}$ and $RBMX^{low}$. The resulting cutoff point was an H-score of 175. Additionally, ROC was used to evaluate RBMX as a predictive factor for chemotherapy response. Multivariable analysis was performed using a Cox proportional hazards model to assess the independent effect of prognostic variables on PFS and OS. OS was defined as the time from primary diagnosis until last follow-up or death from any cause. PFS was defined as the time from primary diagnosis until lymphoma progression or death from any cause. Patients alive at the last follow-up date were censored. All *p*-values were interpreted exploratorily.

3. Results

3.1. Patient and Treatment Characteristics

A total of 43 patients with T-NHL fulfilled the inclusion criteria and had available clinical data (TMA cohort). In 10 cases, only the H-score was available but not the clinical data. The median age of all the evaluated patients was 66 years (range, 36–92 years), with a male-to-female ratio of 2.1:1. At the time of analysis, median follow-up time for living patients was 25.0 months (range, 0 to 142). Overall, 20 patients (46.5%) had died. The study included 19 cases of peripheral T-cell lymphomas with T-helper phenotype (angioimmunoblastic T-cell lymphoma (AITL)) and nodal peripheral T-cell lymphoma with T follicular helper phenotype (PTCL-TFH)), eight cases of peripheral T-cell lymphomas, not otherwise specified (PTCL-NOS), seven cases of anaplastic large-cell lymphomas, ALK-negative (ALCL, ALK-negative), 12 cases of intestinal T-NHL, two cases of extranodal natural killer/T-cell lymphoma, nasal type (NKTCL) and five cases of other subtypes (Mycosis fungoides (MF) *n* = 1, Sézary syndrome (SS) *n* = 1, subcutaneous panniculitis-like T-cell lymphoma (SPTCL) *n* = 1, T-cell prolymphocytic leukemia (T-PLL) *n* = 1, and T-cell large granular lymphocytic leukemia (T-LGL) *n* = 2). The recorded clinicopathologic characteristics are summarized in Table 1.

Table 1. Characteristics of patients.

Characteristic		T-NHL *n* = 43	Nodal T-NHL *n* = 34
		***n* (%)**	***n* (%)**
Sex	Female	14 (33)	12 (35)
	Male	29 (67)	22 (65)
Age (years)	<65	18 (42)	14 (41)
	≥65	25 (58)	20 (59)
B Symptoms	Absent	24 (56)	20 (59)
	Present	19 (44)	14 (41)
Bone marrow involvement	Absent	29 (67)	25 (74)
	Present	10 (23)	7 (21)
	Not evaluable	4 (9)	2 (6)
Ann Arbor stage	Stages I and II	8 (19)	6 (18)
	Stages II and IV	28 (65)	25 (74)
	Not evaluable	7 (16)	3 (9)
IPI	0–2	18 (42)	15 (44)
	2–4	19 (44)	17 (50)
	Not evaluable	6 (14)	2 (6)
ECOG	0–1	19 (44)	16 (47)
	2–5	5 (12)	5 (15)
	Not evaluable	19 (44)	13 (38)
WBC	Normal	12 (28)	8 (24)
	Upper limit of normal	11 (26)	7 (21)

	Not evaluable	20 (47)	19 (56)
LDH	Normal	6 (14)	2 (6)
	Upper limit of normal	18 (42)	17 (50)
	Not evaluable	19 (44)	15 (44)
Ki-67 expression	<65%	15 (35)	12 (35)
	≥65%	13 (30)	10 (29)
	Not evaluable	15 (35)	12 (35)
Relapse	Absent	21 (49)	14 (41)
	Present	22 (51)	20 (59)
First-line treatment	R-CHO(E)P	34 (79)	33 (97)
	Others	9 (21)	1 (1)

Abbreviations: T-NHL: T-cell non-Hodgkin's lymphoma; *n*: number; IPI: International Prognostic Index, ECOG: Eastern Cooperative Oncology Group status; WBC: white blood cell count; LDH: lactate dehydrogenase level.

3.2. Expression of RBMX in T-cell Lymphomas

IHC for RBMX was analyzed on a total of 53 T-cell lymphomas and ten normal non-tumor lymph nodes using FFPE tissues. The overall mean of RBMX protein expression (H-score) was 145 (standard deviation (SD) = 68). No relevant differences in the RBMX expression levels among the histological phenotypes or compared to normal lymph nodes were observed (ANOVA; $p = 0.994$; Figure 1). Representative immunohistochemical staining showing different levels of nuclear RBMX expression are illustrated in Figure 2.

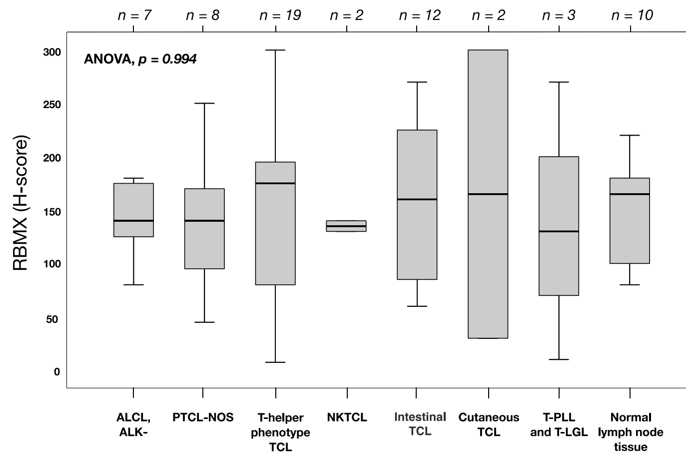

Figure 1. RBMX expression in T-cell lymphoma and non-tumor tissues analyzed by immunohistochemistry. Boxplots represent the median and interquartile range of protein levels. *p*-values were calculated using ANOVA and post-hoc tests for comparison between histological phenotypes and non-tumor tissues. The number of tissue samples is shown at top. Abbreviations: ALCL, ALK-: anaplastic large-cell lymphoma, ALK-negative; TCL: T-cell lymphoma; PTCL-NOS: peripheral T-cell lymphoma, not otherwise specified; NKTCL: extranodal natural killer/T-cell lymphoma, nasal type; T-PLL: T-cell prolymphocytic leukemia; T-LGL: T-cell large granular lymphocytic leukemia.

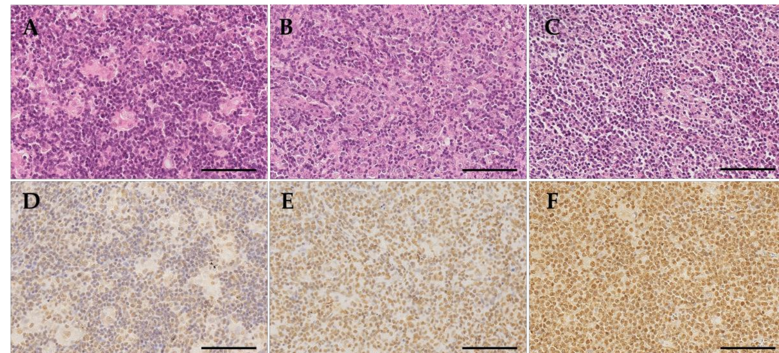

Figure 2. Histomorphology and RBMX immunohistochemistry. All samples were stained with hematoxylin and eosin (H&E) as exemplary shown in micrographies (**A–C**). The immunohistochemical stains for RBMX were examined and evaluated in conjunction with the H&E stains. Immunohistochemical staining showing low (**D**), middle (**E**), and high (**F**) levels of nuclear RBMX expression. Original magnification ×40, the scale bars are 100 µm.

3.3. Association between RBMX Expression and Clinicopathological Characteristics

We investigated the association between nuclear RBMX protein expression and clinicopathological characteristics including sex, age, B symptoms, Ann Arbor stage, IPI, BMI, ECOG status, WBC, LDH, Ki-67 expression, the occurrence of relapses, and response to first-line chemotherapy (regardless of the regime used). The results of unpaired t-tests are summarized in Table 2. In patients with T-NHL, high RBMX expression was associated with a normal white blood cell count (WBC normal vs. upper limit of normal: mean 193 vs. 118; p = 0.023) and non-response to first-line chemotherapy (resistant vs. sensitive: mean 178 vs. 128; p = 0.029). Furthermore, no strong association between high RBMX expression and the absence of bone marrow involvement (noBMI) (noBMI vs. BMI: mean 152 vs. 107; p = 0.090) was observed. No relevance with sex, age, B symptoms, Ann Arbor stage, IPI, ECOG status, LDH level, Ki-67 expression, and the occurrence of relapses was noted in T-NHL. In patients with nodal T-NHL, high RBMX expression was associated with noBMI (noBMI vs. BMI: mean 153 vs. 94; p = 0.034). In addition, no strong associations between high RBMX expression with a normal white blood cell count (WBC normal vs. upper limit of normal: mean 186 vs. 126; p = 0.089) and non-response to first-line chemotherapy (resistant vs. sensitive: mean 167 vs. 130; p = 0.119) were observed. No relevant association with sex, age, B symptoms, Ann Arbor stage, IPI, ECOG status, LDH level, Ki-67 expression, and the occurrence of relapses was noted in nodal T-NHL.

Table 2. Correlations between RBMX expression (H-score) and the clinicopathological characteristics in T-NHL and nodal T-NHL.

Characteristic		T-NHL		Nodal T-NHL	
		RBMX Expression (Mean)	***p*-Value**	**RBMX Expression (Mean)**	***p*-Value**
Sex	Female	131	0.524	132	0.544
	Male	146		146	
Age (years)	<65	155	0.310	154	0.308
	≥65	132		131	
B Symptoms	Absent	133	0.570	132	0.328
	Present	146		154	
	Absent	152	0.090	153	0.034

Bone marrow involvement	Present	107		94	
Ann Arbor stage	Stages I and II	153	0.402	158	0.494
	Stages II and IV	130		137	
IPI	0–2	140	0.433	154	0.295
	2–4	122		129	
ECOG	0–1	140	0.726	145	0.634
	2–5	128		128	
WBC	Normal	193	0.023	186	0.089
	Upper limit of normal	118		126	
LDH	Normal	126	0.741	195	0.304
	Upper limit of normal	137		144	
Ki-67 expression	<65%	130	0.375	154	0.493
	≥65%	155		135	
Relapse	Absent	145	0.762	148	0.603
	Present	139		136	
Response to first-line treatment	Resistant	178	0.029	167	0.119
	Sensitive	128		130	

Abbreviations: T-NHL: T-cell non-Hodgkin's lymphoma; IPI: International Prognostic Index; ECOG: Eastern Cooperative Oncology Group status; WBC: white blood cell count; LDH: lactate dehydrogenase level.

3.4. Predictive Value of RBMX Expression to Anthracycline-Containing First-Line Treatment

To assess RBMX expression as a predictive factor for response to anthracycline-containing first-line treatment, ROC-analysis and t-tests were used. In patients with T-NHL, high RBMX expression was associated with non-response (resistant vs. sensitive: mean 185 vs. 127; p = 0.018; Figure 3a) and the area under the curve (AUC) was 0.725 (95% CI: 0.550–0.901; p = 0.012; Figure 3b) (specificity 66.7%, sensitivity 72.7%). In nodal T-NHL, high RBMX expression was not strongly associated with non-response (resistant vs. sensitive: mean 169 vs. 130; p = 0.120). The AUC was 0.662 (95% CI: 0.463–0.861; p = 0.111) (specificity 60.0%, sensitivity 71.4%).

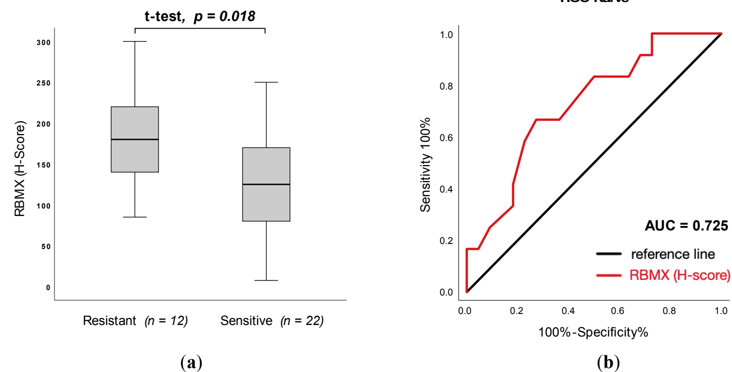

Figure 3. RBMX expression according to response following anthracycline-containing first-line treatment in T-NHL. (**a**): Corresponding histogram of RBMX expression (H-score) in resistant and Scheme 0. (**b**): ROC analysis of RBMX expression (H-score) to discriminate between resistant and sensitive groups. AUC (area under the curve) = 0.725 (95% confidence interval: 0.550–0.901; p =

0.012). Abbreviations: CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone content chemotherapy; CHOEP: cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone content chemotherapy.

3.5. RBMX Expression Predicts OS and PFS in T-Cell Lymphomas

In univariate analysis, $RBMX^{high}$ expression was associated with a poor OS rate in T-NHL ($RBMX^{low}$ vs. $RBMX^{high}$: median OS 78.0 (95% CI: 0.0–160.2) vs. 11.0 (95% CI: 5.5–16.5) months; $p < 0.001$; Figure 4a). This finding agrees with our observations in the nodal T-NHL subtypes ($RBMX^{low}$ vs. $RBMX^{high}$: median OS 124.0 (95% CI: 14.0–233.9) vs. 13.0 (95% CI: 8.7–17.3) months; $p = 0.001$; Figure 4c). Furthermore, the $RBMX^{high}$ expression was associated with a poor PFS rate in patients with T-NHL ($RBMX^{low}$ vs. $RBMX^{high}$: median OS 17.0 (95% CI: 2.6–31.4) vs. 7.0 (95% CI: 0.0–14.4) months; $p = 0.012$; Figure 4b). In nodal T-NHL, RBMX expression was not significantly associated with a poor PFS ($RBMX^{low}$ vs. $RBMX^{high}$: median OS 16.0 (95% CI: 4.8–27.2) vs. 9.0 (95% CI: 5.6–12.4) months; $p = 0.152$; Figure 4d).

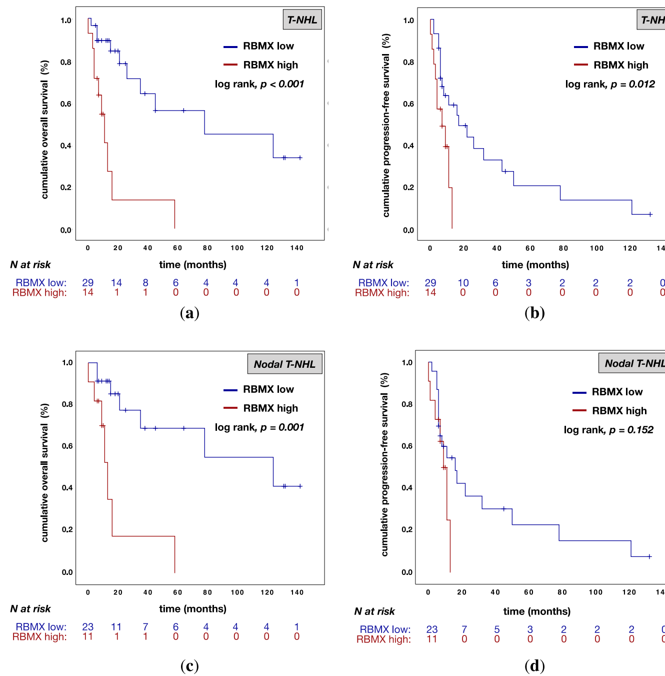

Figure 4. Kaplan–Meier (KM) curves for overall survival (OS) and progression-free survival (PFS) according to RBMX expression. (**a**): KM curve for OS according to RBMX expression in T-NHL; (**b**): KM curve for PFS according to RBMX expression in T-NHL; (**c**): KM curve for OS according to RBMX expression in nodal T-NHL: (**d**) KM curve of PFS according to RBMX expression in nodal T-NHL. Abbreviations: T-NHL: T-cell non-Hodgkin's lymphomas; RBMX low: RBMX expression with an H-score below 175; RBMX high: RBMX expression above or equal to an H-score of 175; *N*: number of patients.

Multivariable analysis of age, sex, B symptoms, BMI, Ann Arbor stage (only for nodal T-NHL), and RBMX expression was performed for OS and PFS. Table 3 summarizes the

results of all tested variables. It turned out that RBMX expression was associated with a poor OS ($p = 0.007$; HR = 0.204; 95% CI: 0.064–0.646; Table 3) in T-NHL. In addition, RBMX expression ($p = 0.006$; HR = 0.235; 95% CI: 0.083–0.666; Table 3) and BMI ($p = 0.004$; HR = 0.243; 95% CI: 0.094–0.628; Table 3) were associated with a poor PFS in T-NHL. In patients with T-NHL, B symptoms ($p = 0.087$; HR = 0.466; 95% CI: 0.194–1.118; Table 3) were not strongly associated with poor PFS. In patients with nodal T-NHL phenotypes the RBMX expression was an independent prognostic marker only for OS ($p = 0.038$; HR = 0.149; 95% CI: 0.025–0.898).

Table 3. Multivariable analysis of overall survival (OS) and progression-free survival (PFS) in T-NHL and nodal T-NHL.

Multivariable Analysis		**Overall Survival**				**Progression-Free Survival**			
Variable	**Categories**	**HR**	**95% CI**		***p*-Value**	**HR**	**95% CI**		***p*-Value**
			LL	**UL**			**LL**	**UL**	
T-NHL cohort (*n* = 39)									
Sex	female vs. male	1.996	0.618	6.445	0.248	1.329	0.536	3.295	0.539
Age in years		1.046	0.986	1.110	0.138	1.002	0.956	1.051	0.924
B Symptoms	absent vs. present	0.592	0.179	1.957	0.390	0.466	0.194	1.118	0.087
Bone marrow involvement	absent vs. present	0.623	0.192	2.020	0.431	0.243	0.094	0.628	0.004
RBMX expression	low vs. high	0.204	0.064	0.646	0.007	0.235	0.083	0.666	0.006
Nodal T-NHL cohort (*n* = 34)									
Sex	female vs. male	1.777	0.376	8.402	0.469	0.796	0.258	2.455	0.691
Age in years		1.026	0.964	1.093	0.416	0.989	0.937	1.043	0.68
Ann Arbor stage	I-II vs. III-IV	5.461	0.945	3.155	0.058	0.928	0.248	3.482	0.912
B Symptoms	absent vs. present	0.365	0.056	2.371	0.291	0.606	0.180	2.038	0.419
Bone marrow involvement	absent vs. present	0.497	0.072	3.414	0.477	0.248	0.080	0.771	0.016
RBMX expression	low vs. high	0.149	0.025	0.898	0.038	0.361	0.107	1.221	0.101

Abbreviations: HR: hazard ratio; CI: confidence interval; LL: lower limit; UL: upper limit; *n*: number; RBMX low: RBMX expression with an H-score below 175; RBMX high: RBMX expression above or equal to an H-score of 175.

4. Discussion

To the best of our knowledge, we present the first analysis of the prognostic impact of RBMX protein expression in patients with T-cell lymphomas. RBMX (also called hnRNP G) is an RNA-binding-motif gene located on the X-chromosome. Beyond its function as a splicing factor, RBMX plays an important role in DNA-damage protection [25], chromosome segregation [11], and genome stability [12,13]. Still, the function of RBMX during carcinogenesis constitutes a desideratum of research. In this study, we investigated the immunohistochemical expression of RBMX in combination with the clinical outcomes in 53 patients with T-cell non-Hodgkin's lymphomas.

RBMX was expressed homogeneously across the histological phenotypes. RBMX expression was examined in normal non-tumor lymph node tissue and T-cell lymphoma, showing lower expression in healthy tissues compared to tumor samples with high RBMX expression (non-tumor vs. $RBMX^{high}$: mean 150 (SD = 49) vs. 220 (SD = 44); $p = 0.024$). Subsequently, we assessed the association of RBMX expression and clinicopathological characteristics at the time of primary diagnosis. Interestingly, high RBMX expression was associated with markers that are known for moderate disease progression (normal WBC count, noBMI). In contrast, high RBMX expression was associated with resistance to first-line treatment, regardless of the regime used. Due to the functions of RBMX presented above, we examine the hypothesis that the protein might be contributing to drug resistance. Previously, several studies investigated the association between RBMX expression and drug resistance, pointing out that high expression levels led to non-response to various drugs [7]. We investigated the correlation between RBMX expression and the response to anthracycline-containing first-line treatment. In agreement with the previous

reports, low RBMX expression levels predict better response to anthracycline-containing chemotherapy in patients with T-NHL (t-test p-value = 0.018; ROC-analysis AUC = 0.725). Former studies indicated an opposite association of RBMX expression levels and prognosis in different cancer types [7,17,18,20,21]. In our univariate analysis, low RBMX levels predict improved overall survival in patients with T-NHL (median OS 78.0 vs. 11.0 months; $p < 0.001$) and nodal T-NHL (median OS 124.0 vs. 13.0 months; $p = 0.001$) and also better progression-free survival in patients with T-NHL (median OS 17.0 vs. 7.0 months; $p = 0.012$). These results are consistent with a previous report which describes that RBMX controls myeloid leukemogenesis by regulating the chromatin state [21]. As a caveat, all patients with high RBMX expression died within 58 months after initial diagnosis. Continuously, the multivariable analysis has been showing RBMX to be an independent prognostic marker for overall survival ($p = 0.007$; HR; 0.204; 95% CI: 0.064–0.646) and progression-free survival ($p = 0.006$; HR = 0.235; 95% CI: 0.083–0.666) in T-NHL. In patients with nodal T-NHL phenotype, the RBMX expression was an independent prognostic marker solely for overall survival ($p = 0.038$; HR = 0.149; 95% CI: 0.025–0.898). As shown above, our results are not significant in the smaller subgroup of primary nodal T-NHL; this could be due to the small size of the subgroup ($n = 34$).

Our results suggest that RBMX expression contributes to chemotherapy resistance and thus affects prognosis in patients with T-NHL. These findings are consistent with previous mentioned studies. However, the current results are subject to several limitations mainly caused by the retrospective character of this analysis and the high heterogeneity in the investigated cohort. Furthermore, the small sample size increases the risk of statistical errors. Therefore, studies with larger cohorts might provide a better understanding on the function of RBMX during carcinogenesis and chemotherapy resistance. Moreover, further cellular experiments are needed to elucidate this issue. In conclusion, our study found that low RBMX protein expression predicted better response to anthracycline-containing first-line treatment, overall survival, and progression-free survival in patients with T-cell non-Hodgkin's lymphomas. Hence, RBMX may be a potential therapeutic target and prognostic marker in T-cell lymphoma.

5. Conclusions

In conclusion, this study showed that low RBMX expression was associated with better response to anthracycline-containing first-line treatment and an improved overall survival (OS) and progression-free survival (PFS) in patients with T-cell non-Hodgkin's lymphomas.

Author Contributions: Conceptualization, T.W.; methodology, A.W., M.B. (Mascha Binder), and T.W.; validation, A.W. and T.W.; formal analysis F.L.S., M.B. (Marcus Bauer), E.G., D.T. A.W., and T.W.; investigation, M.B. (Marcus Bauer), F.L.S., T.W., and A.W.; data curation, F.L.S., D.T., and T.W.; writing—original draft preparation, F.L.S., M.B. (Marcus Bauer), and T.W.; writing—review and editing, F.L.S., M.B. (Marcus Bauer), E.G., D.T. A.W., C.W., M.B. (Mascha Binder), and T.W.; visualization, F.L.S.; supervision, T.W.; project administration, T.W.; funding acquisition, T.W., M.B. (Mascha Binder) and F.L.S. All authors have read and agreed to the published version of the manuscript.

Funding: F.L.S. was supported by a grant from the German Cancer Aid, grant number 70114141.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Martin-Luther-University of Halle-Wittenberg (#2020–033, 10 May 2020).

Informed Consent Statement: Patient consent was waived due to the study's retrospective nature.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Acknowledgments: We would like to thank Janin Siebert and Sarah Vogtländer for excellent support in the laboratory.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Anderson, J.R.; Armitage, J.O.; Weisenburger, D.D. Epidemiology of the non-Hodgkin's lymphomas: Distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. *Ann. Oncol.* **1998**, *9*, 717–720.
2. Swerdlow, S.H.; World Health Organization; International Agency for Research on Cancer. *WHO Classification of Tumours of Haematopoietic And Lymphoid Tissues*, 4th ed.; International Agency for Research on Cancer: Lyon, France, 2017.
3. D'Amore, F.; Gaulard, P.; Trumper, L.; Corradini, P.; Kim, W.S.; Specht, L.; Bjerregaard Pedersen, M.; Ladetto, M.; ESMO Guidelines Committee. Peripheral T-cell lymphomas: ESMO Clinical Prac-tice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2015**, *26*, v108–v115.
4. Vose, J.; Armitage, J.; Weisenburger, D. International TCLP. International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. *J. Clin. Oncol.* **2008**, *26*, 4124–4130.
5. Bellei, M.; Federico, M. The outcome of peripheral T-cell lymphoma patients failing first-line therapy: A report from the prospective International T-Cell Project. *Haematologica* **2019**, *104*, e178.
6. Mak, V.; Hamm, J.; Chhanabhai, M.; Shenkier, T.; Klasa, R.; Sehn, L.H.; Villa, D.; Gascoyne, R.D.; Connors, J.M.; Savage, K.J. Survival of Patients with Peripheral T-Cell Lymphoma After First Relapse or Progression: Spectrum of Disease and Rare Long-Term Survivors. *J. Clin. Oncol.* **2013**, *31*, 1970–1976, doi:10.1200/jco.2012.44.7524.
7. Song, Y.; He, S.; Ma, X.; Zhang, M.; Zhuang, J.; Wang, G.; Ye, Y.; Xia, W. RBMX contributes to hepatocellular carcinoma progression and sorafenib resistance by specifically binding and stabilizing BLACAT1. *Am. J. Cancer Res.* **2020**, *10*, 3644–3665.
8. Shin, K.-H.; Kang, M.K.; Kim, R.H.; Christensen, R.; Park, N.-H. Heterogeneous Nuclear Ribonucleoprotein G Shows Tumor Suppressive Effect against Oral Squamous Cell Carcinoma Cells. *Clin. Cancer Res.* **2006**, *12*, 3222–3228, doi:10.1158/1078-0432.ccr-05-2656.
9. Heinrich, B.; Zhang, Z.; Raitskin, O.; Hiller, M.; Benderska, N.; Hartmann, A.M.; Bracco, L.; Elliott, D.; Ben-Ari, S.; Soreq, H.; et al. Heterogeneous nuclear ribonucleoprotein G regulates splice site selection by binding to CC(A/C)-rich regions in pre-mRNA. *J. Biol. Chem.* **2009**, *284*, 14303–14315.
10. Martinez-Contreras, R.; Cloutier, P.; Shkreta, L.; Fisette, J.-F.; Revil, T.; Chabot, B. hnRNP Proteins and Splicing Control. *Cancer Biol. Nucl. Envel.* **2007**, *623*, 123–147, doi:10.1007/978-0-387-77374-2_8.
11. Matsunaga, S.; Takata, H.; Morimoto, A.; Hayashihara, K.; Higashi, T.; Akatsuchi, K.; Mizusawa, E.; Yamakawa, M.; Ashida, M.; Matsunaga, T.M.; et al. RBMX: A Regulator for Maintenance and Centromeric Protection of Sister Chromatid Cohesion. *Cell Rep.* **2012**, *1*, 299–308, doi:10.1016/j.celrep.2012.02.005.
12. Zheng, T.; Zhou, H.; Li, X.; Peng, D.; Yang, Y.; Zeng, Y.; Liu, H.; Ren, J.; Zhao, Y. RBMX is required for activation of ATR on repetitive DNAs to main-tain genome stability. *Cell Death Differ.* **2020**, *27*, 3162–3176.
13. Munschauer, M.; Nguyen, C.T.; Sirokman, K.; Hartigan, C.R.; Hogstrom, L.; Engreitz, J.M.; Ulirsch, J.C.; Fulco, C.P.; Subramanian, V.; Chen, J.; et al. The NORAD lncRNA assembles a topoisomerase complex critical for genome stability. *Nature* **2018**, *561*, 132–136, doi:10.1038/s41586-018-0453-z.
14. Shin, K.H.; Kim, R.H.; Yu, B.; Kang, M.K.; Elashoff, D.; Christensen, R.; Pucar, A.; Park, N.-H. Expression and mutation analysis of heterogeneous nu-clear ribonucleoprotein G in human oral cancer. *Oral Oncol.* **2011**, *47*, 1011–1016.
15. Shin, K.-H.; Kim, R.H.; Kim, R.H.; Kang, M.K.; Park, N.-H. hnRNP G elicits tumor-suppressive activity in part by upregulating the expression of Txnip. *Biochem. Biophys. Res. Commun.* **2008**, *372*, 880–885, doi:10.1016/j.bbrc.2008.05.175.
16. Renieri, A.; Mencarelli, M.A.; Cetta, F.; Baldassarri, M.; Mari, F.; Furini, S.; Piu, P.; Ariani, F.; Dragani, T.; Frullanti, E. Oligogenic germline mutations identified in early non-smokers lung adenocarcinoma patients. *Lung Cancer* **2014**, *85*, 168–174, doi:10.1016/j.lungcan.2014.05.020.
17. Ouyang, Y.Q.; Hausen, A.Z.; Orlowska-Volk, M.; Jäger, M.; Bettendorf, H.; Hirschfeld, M.; Tong, X.W.; Stickeler, E. Expression levels of hnRNP G and hTra2-beta1 correlate with opposite outcomes in endometrial cancer biology. *Int. J. Cancer* **2010**, *128*, 2010–2019, doi:10.1002/ijc.25544.
18. Hirschfeld, M.; Ouyang, Y.Q.; Jaeger, M.; Erbes, T.; Orlowska-Volk, M.; Zur Hausen, A.; Stickeler, E. HNRNP G and HTRA2-BETA1 regu-late estrogen receptor alpha expression with potential impact on endometrial cancer. *BMC Cancer* **2015**, *15*, 86.
19. Yan, Q.; Zeng, P.; Zhou, X.; Zhao, X.; Chen, R.; Qiao, J.; Feng, L.; Zhu, Z.; Zhang, G.; Chen, C. RBMX suppresses tumorigenicity and progression of bladder cancer by interacting with the hnRNP A1 protein to regulate PKM alternative splicing. *Oncogene* **2021**, *40*, 2635–2650, doi:10.1038/s41388-021-01666-z.
20. Guo, J.; Wang, X.; Jia, J.; Jia, R. Underexpression of SRSF3 and its target gene RBMX predicts good prognosis in patients with head and neck cancer. *J. Oral Sci.* **2020**, *62*, 175–179, doi:10.2334/josnusd.18-0485.
21. Prieto, C.; Nguyen, D.T.T.; Liu, Z.; Wheat, J.; Perez, A.; Gourkanti, S.; Chou, T.; Barin, E.; Velleca, A.; Rohwetter, T.; et al. Transcriptional control of CBX5 by the RNA-binding pro-teins RBMX and RBMXL1 maintains chromatin state in myeloid leukemia. *Nat. Cancer* **2021**, *2*, 741–757.
22. Antonello, Z.A.; Hsu, N.; Bhasin, M.; Roti, G.; Joshi, M.; Van Hummelen, P.; Ye, E.; Lo, A.S.; Ananth Karumanchi, S.; Bryke, C.R.; et al. Vemurafenib-resistance via de novo RBM genes mutations and chromosome 5 aberrations is overcome by combined therapy with palbociclib in thyroid carcinoma with BRAF(V600E). *Oncotarget* **2017**, *8*, 84743–84760.

23. Eisenhauer, E.A.; Therasse, P.; Bogaerts, J.; Schwartz, L.H.; Sargent, D.; Ford, R.; Dancey, J.; Arbuck, S.; Gwyther, S.; Mooney, M.; et al. New response evaluation criteria in solid tu-mours: Revised RECIST guideline (version 1.1). *Eur. J. Cancer* **2009**, *45*, 228–247.
24. Detre, S.; Saclani Jotti, G.; Dowsett, M. A "quickscore" method for immunohistochemical semiquantitation: Validation for oes-tro-gen receptor in breast carcinomas. *J. Clin. Pathol.* **1995**, *48*, 876–878.
25. Adamson, B.; Smogorzewska, A.; Sigoillot, F.D.; King, R.W.; Elledge, S.J. A genome-wide homologous recombination screen identi-fies the RNA-binding protein RBMX as a component of the DNA-damage response. *Nat. Cell Biol.* **2012**, *14*, 318–328.

III Selbständigkeitserklärung

Ich erkläre hiermit, die Arbeit selbstständig geschrieben und keine anderen als die angegebenen Quellen genutzt zu haben.

Halle (Saale), der 21.04.2024 Franziska Lea Schümann

IV Erklärung über frühere Promotionsversuche

Ich erkläre hiermit, diese Arbeit im Rahmen meines ersten Dissertationsversuches verfasst zu haben. Ich habe diese Arbeit ausschließlich an der Medizinischen Fakultät der Martin-Luther-Universität Halle-Wittenberg als Dissertationsschrift eingereicht.

Halle (Saale), der 21.04.2024 Franziska Lea Schümann

V Danksagung

Zuvorderst möchte ich meinem Doktorvater Herrn PD Dr. med. Thomas Weber meinen großen und herzlichen Dank für die Überlassung des Themas und die hervorragende Betreuung aussprechen. Zahlreiche Diskussionen, konstruktive Hilfestellungen und seine fortwährende Unterstützung haben das Projekt in den letzten Jahren maßgeblich geformt. Des Weiteren gilt mein besonderer Dank Frau Prof. Dr. med. Mascha Binder, die zunächst gerne die formale Betreuung der Arbeit übernommen hatte.

Äußerst danken möchte ich Herrn Dr. med. Marcus Bauer, ohne den die Durchführung des Projekts nicht möglich gewesen wäre. Von seinen praktischen Erfahrungen sowie vielen, unverzichtbaren Ratschlägen habe ich sehr profitiert. Ebenso gilt mein Dank Herrn Prof. Dr. rer. nat. Andreas Wienke, der mir stets bei allen statistischen Fragen zum Projekt zur Seite stand. Darüber hinaus danke ich Denis Terziev und Elisabeth Groß für deren tatkräftige Unterstützung.

Mein außerordentlicher Dank gilt der Deutschen Krebshilfe sowohl für die materielle als auch die immaterielle Unterstützung des Promotionsvorhabens. Darüber hinaus möchte ich dem Halleschen Promotionskolleg Medizin für die strukturierte inhaltliche Betreuung danken.

Abschließend danke ich von ganzem Herzen meinen Freunden und meiner Familie für ihre stetige moralische Unterstützung.

VI Anlagen

Anlage A: Genehmigung des *Multidisciplinary Digital Publishing Institute* zur Publikation der Artikel innerhalb dieser Dissertation vom 11. Mai 2022 in originaler Zeichensetzung

Von: Ioana Craciun <ioana.craciun@mdpi.com>
Gesendet: Mittwoch, 11. Mai 2022 18:03
An: Weber, Thomas <thomas.weber@uk-halle.de>
Cc: Schümann, Franziska <Franziska.Schuemann@uk-halle.de>
Betreff: Re: Permission for use of publication in cancers and biomedicines in MD/PhD work

Dear Dr. Webber,

For all articles published in MDPI journals, copyright is retained by the authors. Articles are licensed under an open access Creative Commons CC BY 4.0 license, meaning that the article may be reused and quoted provided that the original published version is cited.

Permission is not necessary and Franziska Schümann can use her two publications bellow as part of her publication, given that the original publication is correctly cited.

Paper 1: Schümann FL, Groß E, Bauer M, Rohde C, Sandmann S, Terziev D, Müller LP, Posern G, Wienke A, Fend F, Hansmann ML, Klapper W, Rosenwald A, Stein H, Dugas M, Müller-Tidow C, Wickenhauser C, Binder M, Weber T
(2021) Divergent Effects of EZH1 and EZH2 Protein Expression on the Prognosis of Patients with T-Cell Lymphomas. Biomedicines 9 (12)

Paper 2: Schümann FL, Bauer M, Groß E, Terziev D, Wienke A, Wickenhauser C, Binder M, Weber T (2021) RBMX Protein Expression in T-Cell Lymphomas Predicts Chemotherapy Response and Prognosis. Cancers (Basel) 13 (19)"

Kind regards,
Ioana

https://webmail.uk-halle.de/owa/#viewmodel=ReadMessageItem&...2F3%2FbWzbX1AACgxwY9AAA%3D&IsPrintView=1&wid=96&ispopout=1 Seite 1 von 3