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Evaluation von prädiktiven Biomarkern für Therapieansprechen und Prognose bei Hochrisiko-Weichgewebesarkomen

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Abkürzungsverzeichnis

AI	Doxorubicin + Ifosfamid
AJCC	American Joint Commission on Cancer
AMPKα1	AMP-activated protein kinase α1
ATP	Adenosintriphosphat
BAK	Bcl-2 homologous antagonist killer
Bcl-2	B-cell lymphoma 2
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
CPI	Checkpoint-Inhibitor
CSC	Cancer stem cell
CTLA-4	Cytotoxic T-lymphoyte-associated protein 4
DNS	Desoxyribonukleinsäure
DSB	Double-strand break
ECOG	Eastern Cooperative Oncology Group
ESHO	European Society of Hyperthermic Oncology
EIA	Etoposid + Ifosfamid + Doxorubicin
FDA	United States Food and Drug Administration
FNCLCC	Fédération Nationale des Centres de Lutte Contre le Cancer
FOXO3	Forkhead-Box-Protein O3
H&E	Hematoxylin&Eosin-Färbung
HPF	High-power field
HR-STS	High-risk soft tissue sarcoma
IHC	Immunhistochemie
IL	Interleukin
LMS	Leiomyosarkom
MAGE	Melanoma antigen gene protein
MDR	Multiple drug resistance
Mesna	2-Mercaptoethansulfonat-Natrium
miRNA	microRNA
MPNST	Maligner peripherer Nervenscheidentumor
NY-ESO	New York esophageal squamous cell carcinoma-1
OS	Overall survival
PD-1	Programmed cell death 1

PD-L1	Programmed cell death ligand 1
PFS	Progression-free survival
PI3K	Phosphoinositid-3-Kinase
PRAME	Preferentially expressed antigen in melanoma
RHT	Regionale Tiefenhyperthermie
RNS	Ribonukleinsäure
ROS	Reactive oxygen species
siRNA	Small interfering RNA
SIRT1	Sirtuin 1
TIL	Tumor-infiltrierende Lymphozyten
TKI	Tyrosinkinase-Inhibitor
TIM-3	T cell immunoglobulin and mucin domain-containing protein 3
TMA	Tissue microarray
TMB	Tumor mutational burden
TNF	Tumor necrosis factor
TOP2A	Topoisomerase IIα
TOP2B	Topoisomerase IIβ
TP53	Tumor protein p53
UICC	Union for International Cancer Control
UPS	Undifferenziertes pleomorphes Sarkom
VEGF	Vascular endothelial growth factor

Publikationsliste

1. **Berclaz LM**, Altendorf-Hofmann A, Dürr HR, Klein A, Angele MK, Albertsmeier M, Schmidt-Hegemann NS, Di Gioia D, Knösel T, Lindner LH. Expression Patterns of TOP2A and SIRT1 Are Predictive of Survival in Patients with High-Risk Soft Tissue Sarcomas Treated with a Neoadjuvant Anthracycline-Based Chemotherapy. *Cancers.* 2021; 13(19):4877. doi: 10.3390/cancers13194877.

2. **Berclaz LM**, Altendorf-Hofmann A, Lindner LH, Burkhard-Meier A, Di Gioia D, Dürr HR, Klein A, Albertsmeier M, Schmidt-Hegemann N-S, Klauschen F, Knösel T. TIM-3 Qualifies as a Potential Immunotherapeutic Target in Specific Subsets of Patients with High-Risk Soft Tissue Sarcomas (HR-STS). *Cancers*. 2023; 15(10):2735. Doi: 10.3390/cancers15102735.

Eigener Beitrag zu den Veröffentlichungen

1.1 Beitrag zu Publikation Nr. 1

Als Erstautor dieser Publikation war ich an allen Teilen der Studie mitbeteiligt:

Mittels digitaler Literaturrecherche zu Chemoresistenz und potenziellen Biomarkern in Hochrisiko-Weichgewebesarkomen habe ich die getesteten Marker TOP2A und SIRT1 identifiziert. Nach ausführlicher Diskussion der Biomarker in Kooperation mit meinen Betreuern und dem Pathologischen Institut der LMU München habe ich den Ethikantrag für dieses Projekt verfasst und eingereicht. Nach umfassender Datenbankrecherche am Sarkomzentrum der LMU München (SarKUM) habe ich das anonymisierte Patientenkollektiv in einer bestehenden Datenbank erweitert und zur Bildung eines neuen *Tissue Microarrays* (TMA) am Pathologischen Institut der LMU München unter der Aufsicht von Prof. Thomas Knösel beigetragen. Zusammen mit Prof. Knösel habe ich die mittels Immunhistochemie gewonnenen Ergebnisse an den Tissue Microarrays begutachtet und die Expression der Biomarker quantifiziert. Anschließend habe ich die Ergebnisse unter der Aufsicht von PD Dr. Annelore Altendorf-Hofmann am Universitätsklinikum Jena statistisch analysiert und in den Kontext der klinischen Datenbank gebracht. Als Erstautor der eingereichten Publikation habe ich das Manuskript verfasst und Tabellen / Abbildungen gestaltet. Das endgültige Manuskript habe ich in einem Q1-Journal eingereicht und anschließend den Peer-Review-Prozess unter der Aufsicht von Prof. Lindner, Prof. Knösel und PD Dr. Altendorf-Hofmann geleitet.

1.2 Beitrag zu Publikation Nr. 2

Als Erstautor dieser Publikation war ich an allen Teilen der Studie mitbeteiligt:

Mittels digitaler Literaturrecherche zu Chemoresistenz, immuntherapeutischen Ansätzen und potenziellen Biomarkern in Hochrisiko-Weichgewebesarkomen habe ich den getesteten Marker TIM-3 identifiziert und in den Kontext mit weiteren Immunmarkern wie PD-1, PD-L1 und TILs gebracht. Nach ausführlicher Diskussion der Biomarker in Kooperation mit meinen Betreuern und dem Pathologischen Institut der LMU München habe ich den Ethikantrag für dieses Projekt verfasst und eingereicht. Nach umfassender Datenbankrecherche am Sarkomzentrum der LMU München (SarKUM) habe ich das anonymisierte Patientenkollektiv in einer bestehenden Datenbank erweitert und zur Bildung eines neuen *Tissue Microarrays* (TMA) am Pathologischen Institut der LMU München unter der Aufsicht von Prof. Thomas Knösel beigetragen. Zusammen mit Prof. Knösel habe ich die mittels Immunhistochemie gewonnenen Ergebnisse an den Tissue Microarrays begutachtet und die Expression der Biomarker quantifiziert. Anschließend habe ich die Ergebnisse unter der Aufsicht von PD Dr. Annelore Altendorf-Hofmann am Universitätsklinikum Jena statistisch analysiert und in den Kontext der klinischen Datenbank gebracht. Als Erstautor der eingereichten Publikation habe ich das Manuskript verfasst und Tabellen / Abbildungen gestaltet. Das endgültige Manuskript habe ich in einem Q1-Journal eingereicht und anschließend den Peer-Review-Prozess unter der Aufsicht von Prof. Lindner, Prof. Knösel und PD Dr. Altendorf-Hofmann geleitet.

2. Einleitung

Ziel dieser Einleitung ist ein kurzer Überblick über die Definition von (Hochrisiko-)Weichgewebesarkomen, aktuelle Therapiestandards und relevante Wirkmechanismen der verwendeten Chemotherapeutika in dieser seltenen Tumorentität. In einem zweiten Abschnitt wird auf Chemotherapie-Resistenzmechanismen, alternative Therapieoptionen sowie die Rolle der *Tissue Microarrays* in der Identifikation von Biomarkern eingegangen.

2.1 Weichgewebesarkome

2.1.1 Epidemiologie und Ätiologie

Weichgewebesarkome bestehen aus einer heterogenen Gruppe von malignen Tumoren mesenchymalen Ursprungs. Sie umfassen ca. 80 verschiedene histopathologische Subtypen[1,2]. Zu den häufigsten histologischen Subtypen gehören das undifferenzierte pleomorphe Sarkom (UPS), das Liposarkom sowie das Leiomyosarkom. Bei Erwachsenen machen sie weniger als 1% aller Tumoren aus, die Inzidenzrate liegt in Europa bei 4-5 Erkrankungen pro 100.000 Einwohner pro Jahr. Das durchschnittliche Erkrankungsalter liegt zwischen 70 und 80 Jahren, wobei die Zahl der Neuerkrankungen bereits ab dem 20. Lebensjahr linear zunimmt[3].

Weichgewebesarkome können aufgrund ihres mesenchymalen Ursprungs überall am Körper auftreten. Analog zur Verteilung der Körpermasse tritt ein großer Teil an den Extremitäten (60%), im Retroperitonealraum (20%) oder am Rumpf (10%) auf[1]. Ein Tumor, der die oberflächliche Faszie infiltriert oder unter ihr liegt wird als tief bezeichnet und geht mit einer schlechteren Prognose einher. Retroperitoneale, intraabdominale und pelvine Tumoren, sowie Tumoren der Kniekehle, der Achselhöhle und der Ellenbeuge gelten per definitionem als tief gelegen[4]. Der Hauptteil der Sarkome entsteht sporadisch. Mit Ausnahme von Personen mit erblich bedingten Syndromen werden keine allgemeinen Früherkennungsmaßnahmen für die Bevölkerung empfohlen[5].

2.1.2 Definition und Prognose von Hochrisiko-Weichgewebesarkomen

Als Hochrisiko-Weichgewebesarkome werden Tumoren bezeichnet, die mit einem hohen Metastasierungs- und Rezidivrisiko einhergehen[4,6,7]. Dazu gehören Weichgewebesarkome mit einem Grading von 2 und 3 nach dem dreistufigen Grading-System der *Fédération Nationale des Centres de Lutte Contre le Cancer* (FNCLCC), tief gelegene Tumoren und Tumoren ≥5cm[1]. Die Prognose unterscheidet sich stark zwischen den unterschiedlichen histologischen Subtypen[8]. Weitere prädiktive Faktoren für eine schlechte Prognose und das Auftreten von Fernmetastasen sind der Differenzierungsgrad nach FNCLCC, das Patientenalter, die Lokalisation (Extremitäten vs. Nicht-Extremitäten), die Tumorgröße, die Lage (oberflächlich vs. tief) und der Resektionsstatus[4,8,9]. Zur besseren Einschätzung des individuellen Risikos wurden prädiktive Nomogramme etabliert, die bei der Risikostratifizierung von Patienten mit Hochrisiko-Weichgewebesarkomen unterstützen und die o.g. Risikofaktoren einschließen[6]. So können beispielsweise Patienten identifiziert werden, die von einer neoadjuvanten Systemtherapie profitieren und ein hohes Rezidiv- und Fernmetastasierungsrisiko besitzen. Verschiedene Studien haben die Anwendung dieser Nomogramme in Sarkomen validiert[10–12]. Hochrisiko-Weichgewebesarkome metastasieren häufig hämatogen in die Lunge[13]. Trotz aktueller lokaler und systemischer Therapieoptionen versterben

bis zu 50% aller Patienten mit Hochrisiko-Weichgewebesarkomen in den ersten fünf Jahren nach Erstdiagnose[6,7].

2.2 Therapie von Hochrisiko-Weichgewebesarkomen

2.2.1 Multimodales Therapiekonzept

Der einzige kurative Ansatz in der Therapie von Weichgewebesarkomen liegt in der vollständigen Resektion des Tumors. Die sogenannte R0-Resektion wird in allen Fällen angestrebt. Hierzu kann eine en bloc-Resektion, welche den Tumor als Ganzes zusammen mit umliegenden Strukturen entfernt, erforderlich werden[14]. Im Vergleich zu Niedrigrisiko-Weichgewebesarkomen, welche häufig mit einer alleinigen chirurgischen Resektion behandelt werden können, wird bei Hochrisiko-Weichgewebesarkomen aufgrund der hohen Lokalrezidiv- und Fernmetastasierungsrate ein multimodales Therapiekonzept empfohlen[15]. In einem interdisziplinären Sarkom-Tumorboard wird hier eine Therapieempfehlung unter Hinzunahme der Chemotherapie sowie ggf. der regionalen Tiefenhyperthermie und/oder Strahlentherapie erarbeitet.

2.2.2 Chemotherapie

Die Chemotherapie ist ein wichtiger Bestandteil der multimodalen Therapie in ausgewählten Patienten mit Weichgewebesarkom und hohem Rezidiv- und Fernmetastasierungsrisiko[14,16–18]. Ziel der neoadjuvanten Chemotherapie ist die systemische Tumorkontrolle durch eine Mitbehandlung von potenziellen, radiologisch nicht nachweisbaren Mikrometastasen. Als präoperative Chemotherapie soll sie zudem zu einer Verbesserung der Resektabilität des Primärtumors führen. Die adjuvante Chemotherapie soll mögliche verbliebene Tumorzellen entfernen und wird in ausgewählten Fällen angewendet[14]. Standardsubstanz in der Systemtherapie von Weichgewebesarkomen ist eine Anthrazyklin-basierte Chemotherapie mit Doxorubicin. Trotz bekannter Unterschiede in der Tumorbiologie der verschiedenen Sarkom-Subtypen ist nach aktuellem Wissensstand eine Anthrazyklin-basierte Standardtherapie gegenüber einer Subtypen-spezifischen Systemtherapie überlegen[19]. Nach aktueller Studienlage wird in Hochrisiko-Weichgewebesarkomen eine Kombinationstherapie mit Doxorubicin und Ifosfamid gegenüber einer Monotherapie mit Doxorubicin bevorzugt[19–23]. Die Anwendung von Doxorubicin in Kombination mit Dacarbazin bildet eine Ausnahme in fortgeschrittenen Leiomyosarkomen[24].

Insgesamt konnte durch die Hinzunahme einer systemischen Chemotherapie das Gesamtüberleben in bisherigen Studien in Weichgewebesarkomen um ca. 4 - 19% verbessert werden[17,25–27]. Die Ansprechraten einer Chemotherapie in Weichgewebesarkomen liegen je nach Patientenpopulation und Chemotherapie-Protokoll zwischen 10 - 49%[28–33]. Aufgrund der limitierten Datenlage in dieser seltenen Tumorentität wird die Anwendung einer Systemtherapie in mehreren prospektiven Studien überprüft. Ein Beispiel bildet hier die aktuelle Phase-III-Studie von Bonvalot et al., welche die Rolle der neoadjuvanten Chemotherapie in retroperitonealen Weichgewebesarkomen überprüft (NCT04031677).

In Patienten mit palliativer Therapiestrategie, signifikanten Komorbiditäten oder marginalem Performance-Status nach *Eastern Cooperative Oncology Group* (ECOG) wird eine Monotherapie mit Doxorubicin als Erstlinien-Therapie empfohlen[34,35]. Als Zweitlinien-Therapien in fortgeschrittenen und metastasierten Weichgewebesarkomen werden Protokolle mit Gemcitabin und Docetaxel, Trabectedin, Pazopanib oder Eribulin eingesetzt[32,36–39].

2.2.2.1 Doxorubicin

Doxorubicin gehört zu der Gruppe der Anthrazykline und ist ein wichtiger Bestandteil von Chemotherapie-Protokollen in hämatologischen und soliden Tumorerkrankungen[40]. Doxorubicin interagiert innerhalb des Nukleus der Tumorzelle mit der Topoisomerase IIα (TOP2A). Durch diese Interaktion kann TOP2A seiner Funktion als DNS-Reparaturprotein nicht mehr nachkommen[41]. Als Folge entstehen DNS-Schäden (sog. *Double-strand breaks*), die nicht mehr adäquat repariert werden können[42]. Durch weitere Downstream-Mechanismen, beispielsweise durch die Entstehung von pro-apoptotischen Bcl-2-Proteinen, aktiviert die Tumorzelle ein Apoptose-Programm[43,44]. Als zusätzliche Wirkungsmechanismen von Doxorubicin wurden in experimentellen Studien die Bildung von mitochondrialen *Reactive oxygen species* (ROS), eine TOP2A-unabhängige Bildung von DNS-Addukten sowie eine direkte Inhibition der DNS-/RNS-Synthese in Tumorzellen beschrieben[45,46]. Alle genannten Mechanismen führen zu einem programmierten Zelltod.

2.2.2.2 Ifosfamid

Ifosfamid gehört zu der Gruppe der Alkylanzien. Ifosfamid ist ein Oxazaphosphorin-Analogon von Cyclophosphamid und ist in Hochrisiko-Weichgewebesarkomen neben Doxorubicin das am weitesten verbreitete Zytostatikum[30,47]. Aktive Metaboliten von Ifosfamid alkylieren DNS-Stränge durch eine Bindung von elektrophilen Gruppen an die N-7-Position von Guanin. Hierdurch kommt es zu einer kovalenten Verknüpfung von DNS-Basen, sog. *Inter-/Intrastrand Crosslinks*, die zytotoxisch wirken und zum Zelltod führen[48]. In der Vergangenheit wurde die Therapie mit Ifosfamid durch eine wichtige Nebenwirkung, die hämorrhagische Zystitis, limitiert. Durch die Verwendung von 2-Mercaptoethansulfonat-Natrium (Mesna) kann diese Nebenwirkung weitgehend verhindert werden, indem toxische Metaboliten von Ifosfamid in der Blase neutralisiert werden[49,50].

2.2.3 Regionale Tiefenhyperthermie

In Kombination mit einer systemischen Chemotherapie hat sich die regionale Tiefenhyperthermie (RHT) in den letzten Jahren zu einer zusätzlichen Therapiemodalität in Hochrisiko-Weichgewebesarkomen etabliert[27,51–53]. Issels et al. wiesen in einer randomisierten Phase-III-Studie einen Überlebensvorteil in Patienten nach, die zusätzlich zur neoadjuvanten Chemotherapie einer RHT zugeführt wurden: So konnte das 5- und 10-Jahres-Gesamtüberleben im Vergleich zur alleinigen Chemotherapie um 11.4% beziehungsweise 9.9% gesteigert werden[26].

Im Rahmen der regionalen Tiefenhyperthermie wird der Tumor unter externer Wärmeapplikation auf ca. 40-43°C erwärmt. Erzeugt wird die Hyperthermie durch elektromagnetische Wellen, die mittels Viel-Antennen-Systemen um den tumortragenden Querschnitt zirkulär angeordnet werden. Die multidisziplinäre Planung der regionalen Tiefenhyperthermie erfolgt analog zu den Qulitätsrichtlinien der *European Society of Hyperthermic Oncology* (ESHO)[54]. Die Hyperthermie-Behandlung erfolgt gleichzeitig mit der Gabe der neoadjuvanten oder adjuvanten Chemotherapie. Ziel ist eine Erwärmung des vollständigen Tumorgewebes für 60 Minuten. Neben dem zelltoxischen Effekt der Wärmeapplikation durch eine direkte Proteindenaturierung führt die regionale Tiefenhyperthermie zu einer Chemo- und Strahlensensibilisierung im Bereich des Tumors sowie zu einer Immunmodulation des Tumors[55,56]. Mögliche Mechanismen für eine Chemosensibilisierung sind ein beschleunigter Transport und eine gesteigerte metabolische Aktivierung der Chemotherapie in der Tumorzelle sowie eine verstärkte Reaktivität bei der Interaktion mit zellulären Zielstrukturen (z.B. DNS-Alkylierung), was zu einer Blockade von Zellreparaturmechanismen führt[57,58].

2.2.4 Strahlentherapie

Ziel der Strahlentherapie im Rahmen des multimodalen Therapiekonzepts ist die Verbesserung der lokalen Kontrolle im Bereich des Primärtumors ab einem UICC-Stadium II[14,59]. Der genaue Zeitpunkt sowie die Dosierung der Strahlentherapie wird anhand des histologischen Subtyps im Rahmen des interdisziplinären Tumorboards festgelegt. Eine Verbesserung der lokalen Kontrolle durch eine additive Bestrahlung konnte in Hochrisiko-Weichgewebesarkomen der Extremitäten in mehreren Studien nachgewiesen werden[59–62]. In Kontrast wird die neoadjuvante Bestrahlung in viszeralen und retroperitonealen Weichgewebesarkomen nach aktuellem Wissensstand nur in ausgewählten Fällen empfohlen[63]. Der genaue Zeitpunkt der Bestrahlung hatte in bisherigen Studien keinen direkten Einfluss auf die lokale Kontrolle sowie das Gesamtüberleben in Patienten mit Weichgewebesarkomen[64–66]. Der Vorteil der neoadjuvanten Bestrahlung liegt in einer insgesamt geringeren Langzeitmorbidität wie Fibrosen oder Ödeme. Wundheilungsstörungen werden jedoch nach einer neoadjuvanten Bestrahlung signifikant häufiger beobachtet[64,67,68].

2.3 Chemoresistenz in Hochrisiko-Weichgewebesarkomen

2.3.1 Molekulare Mechanismen

Bis zu 90% aller Therapieversagen in metastasierten Tumorstadien werden auf eine Chemotherapie-Resistenz zurückgeführt[69]. Weichgewebesarkome können intrinsische Resistenz-Mechanismen besitzen oder eine Resistenz im Verlauf der Chemotherapie entwickeln. Eine Herausforderung in der erworbenen Chemotherapie-Resistenz ist die Fähigkeit des Tumors, eine Art Kreuzresistenz mit weiteren Chemotherapeutika zu entwickeln[69].

2.3.1.1 Multiple Drug Resistance (MDR)

In Kontrast zu Tumor- und Medikamenten-spezifischen Resistenzmechanismen sind manche Signalwege für eine Resistenz gegenüber einer Vielzahl von Zytostatika-Gruppen verantwortlich. Die sogenannten *Multiple Drug Resistance* (MDR)-Signalwege beinhalten spezifische Transporter, die eine Akkumulation der Zytostatika in der Tumorzelle verhindern. Einen wichtigen Bestandteil der MDR-Gruppe bilden die *ATP-Binding Cassette* (ABC)-Transporter[70]. Neben physiologischen Aufgaben, beispielsweise im Epithel der Niere sowie in Hepatozyten, können diese Transporter aktiv Zytostatika aus der Tumorzelle befördern. P-Glycoprotein (P-gp), auch *Multiple Drug Resistance 1* (MDR1) genannt, ist ein Bestandteil der ABC-Transporter-Gruppe und eine wichtige Ursache für eine unspezifische Chemotherapie-Resistenz[71]. Die Expression von MDR1 korreliert mit einer starken Resistenz gegenüber Anthrazyklin-haltigen Chemotherapie-Protokollen und wurde in verschiedenen Tumoren mit einer schlechteren Prognose assoziiert[72–74]. Abolhoda et al. wiesen eine Zunahme der MDR1-Expression unter laufender Anthrazyklin-haltiger Chemotherapie nach, was die intrinsische sowie die erworbene Komponente dieser Resistenzmechanismen unterstreicht[75].

2.3.1.2 Anthrazyklin-spezifische Resistenzmechanismen

Neben unspezifischen Resistenzmechanismen sind multiple Zytostatika-spezifische Signalwege bekannt. Anthrazyklin-spezifische Resistenzmechanismen zielen auf einen verringerten DNS-Schaden sowie auf eine Blockierung der Signalwege, die zu einem programmierten Zelltod führen[41]. Die Wichtigsten Mechanismen werden in den nächsten Abschnitten genannt.

Topoisomerase IIα (TOP2A): TOP2A gilt als Hauptangriffspunkt von Doxorubicin. Die Interaktion zwischen Doxorubicin und TOP2A führt im Normalfall zu einer Akkumulation von *Double-strand Breaks* (DSB) in der Tumorzelle und somit zum programmierten Zelltod. Durch eine Modifikation der TOP2A-Expression können Tumorzellen diese Mechanismen verändern. So können Doxorubicin-resistente Zellen die Expression von TOP2A reduzieren, was zu einer kompensatorischen Überexpression der weniger Doxorubicin-sensitiven Topoisomerase IIβ (TOP2B) führt[76,77]. Eine Überexpression von TOP2A korrelierte in mehreren Studien ebenfalls mit einer Chemotherapie-Resistenz: Der Zusammenhang zwischen einer TOP2A-Überexpression und einer Chemotherapie-Resistenz wurde noch nicht gänzlich geklärt. Eine mögliche Erklärung ist die Zunahme von TOP2A-Mutationen i.R. der Überexpression, die die Sensitivität für Doxorubicin herabsetzen[41,78]. Die Überexpression von TOP2A wurde in mehreren Studien mit einem schlechten Gesamtüberleben und einer Chemotherapie-Resistenz assoziiert[79–81]. Gleichzeitig korrelierte eine Überexpression von TOP2A mit einer geringeren Rate an Lokalrezidiven in fortgeschrittenen Weichgewebesarkomen[82]. Die genaue Rolle von TOP2A ist insgesamt noch nicht gänzlich verstanden.

Sirtuine: Sirtuine sind NAD-abhängige Histon-Deacetylasen und ADP-Ribosyltransferasen[83]. Die physiologische Rolle der Sirtuine liegt in der Zelladaptation an oxidative, metabolische oder gentoxische Stress-Signale durch eine Chromatin-Regulation und die Aktivierung von DNS-Reparaturmechanismen[84,85]. Sirtuine bestehen aus einer Gruppe von insgesamt sieben Mitgliedern (SIRT1-SIRT7), die sowohl als Onkogene als auch als Tumorsuppressor-Gene agieren können[86,87]. Sirtuine führen zu einer Inaktivierung von Tumorsuppressor-Genen sowie zu einer De-Acetylierung und Inaktivierung von Proteinen, die für die Apoptose in Tumorzellen mitverantwortlich sind[88–90]. Eine Überexpression von Sirtuinen inklusive SIRT1 wurde in mehreren Studien mit einem Tumorwachstum, Chemotherapie-Resistenz und einem schlechten Gesamtüberleben in malignen Erkrankungen inklusive Weichgewebesarkomen in Verbindung gebracht[91–93]. Umgekehrt führte die selektive Hemmung von SIRT1 und SIRT2 zum programmierten Zelltod in Sarkom-Zellen einer Studie von Ma et al.[94]. In Kontrast zu diesen Ergebnissen wurde die Überexpression von SIRT1 in Studien von Firestein et al. sowie Wang et al. mit einer Hemmung der Zellteilung in Kolon- und BRCA1-assoziierten Karzinomen assoziiert[95,96]. Die Rolle der Sirtuine ist ebenfalls noch nicht gänzlich verstanden und variiert stark zwischen verschiedenen Tumorentitäten.

Weitere Resistenzmechanismen: Eine Anthrazyklin-Resistenz kann durch weitere zelluläre Mechanismen verursacht werden. Neben TOP2A und SIRT1 kann beispielsweise eine Überexpression des Transkriptionsfaktors FOXO3 zu einer Doxorubicin-Resistenz führen: in resistenten Zelllinien aktiviert FOXO3 die unspezifische Efflux-Pumpe *Multiple Drug Resistance 1*[97]. Ein weiterer Mechanismus von Anthrazyklin-resistenten Tumoren ist die Überaktivierung von wachstumsstimulierenden Signalwegen wie PI3k/Akt oder MAP-Kinase[98,99]. Zudem können Micro-RNAs (miRNAs) eine Chemotherapie-Resistenz durch eine Modulation von Zielgenen wie TP53, AMPKα1 oder BAK verursachen[100,101]. Tumorstammzellen (Cancer Stem Cells, CSC), gelten als besonders resistent und können die pro-apoptotische Wirkung von Doxorubicin durch eine Überexpression von MDR1 oder spezifischen miRNAs verhindern[102,103]. Diese Beispiele illustrieren die Diversität der Mechanismen für eine Anthrazyklin-Resistenz.

2.4 Immuntherapie in Hochrisiko-Weichgewebesarkomen

2.4.1 Aktueller Wissensstand

Aufgrund des schlechten Gesamtüberlebens von Patienten mit Hochrisiko-Weichgewebesarkomen werden neben der konventionellen Chemotherapie weitere systemische Therapieoptionen in fortgeschrittenen Tumorstadien benötigt. Ein potenzieller Ansatz liegt hier in der Immuntherapie[104]. Immuntherapeutische Ansätze wie beispielsweise Checkpoint-Inhibitoren haben die Rolle der Systemtherapie in anderen Tumorentitäten wie das maligne Melanom oder das nicht-kleinzellige Lungenkarzinom (NSCLC) revolutioniert[105,106]. In Sarkomen haben immuntherapeutische Ansätze wie Immuncheckpoint-Inhibitoren in bisherigen Studien kaum zu einer Verbesserung des Gesamtüberlebens geführt, sind jedoch tumor-agnostisch in ausgewählten Patienten mit hoher Mikrosatelliteninstabilität (MSI-high) oder hoher Tumormutationslast (TMB high) zugelassen[107–112]. Zudem wurde eine Kombination aus Checkpoint-Inhibitoren mit einer konventionellen Doxorubicin-basierten Chemotherapie mit einer Verbesserung des Gesamtansprechens (ORR) sowie des Progressions-freien Überlebens (PFS) in Patienten mit Weichgewebesarkomen assoziiert[113,114]. Die Kombination aus Checkpoint-Inhibitoren mit etablierten Tyrosinkinase-Inhibitoren (TKI) ist ebenfalls ein vielversprechender Ansatz: Eine Kombinationtherapie mit Pembrolizumab und dem Tyrosinkinase-Inhibitor Axitinib führte zu einem signifikanten Therapieansprechen in Patienten mit alveolären Weichteilsarkomen und wird momentan in weiteren Studien überprüft[115]. Die vorläufigen Ergebnisse führten im Dezember 2022 zu einer Zulassung von Atezolizumab in der Therapie des alveolären Weichteilsarkoms.

2.4.2 Potenzielle immuntherapeutische Ansätze

Neben den o.g. immuntherapeutischen Ansätzen werden verschiedene Therapieoptionen in aktuellen Studien geprüft:

2.4.2.1 Alternative Checkpoint-Inhibitoren

Neben besser etablierten Immuncheckpoint-Rezeptoren wie PD-1 oder CTLA-4 wird eine Hemmung von alternativen Checkpoint-Rezeptoren in experimentellen und klinischen Studien getestet: *T cell immunoglobulin and mucin domain-containing protein 3* (TIM-3) ist ein Checkpoint-Rezeptor der TIM-Familie und wird hauptsächlich auf CD4+ und CD8+ T-Zellen exprimiert[116]. TIM-3 wird mit anderen Checkpoint-Rezeptoren co-stimuliert und co-reguliert. Lymphozyten mit einer Co-Expression von TIM-3 und PD-1 wurden in bisherigen Studien als besonders inaktive T-Zell-Subpopulation eingestuft[117]. Immunstimulierende Zytokine wie Interferon-γ, *tumor necrosis factor* (TNF) sowie Interleukin-2 werden in dieser Population kaum exprimiert, was zu einer unzureichenden Immunantwort und dadurch zu einem Tumorwachstum führen kann[118]. Die Expression von TIM-3 in Tumorzellen ist in bisherigen Studien kaum evaluiert worden, und der prognostische Einfluss von TIM-3 in Patienten mit Weichgewebesarkomen ist ebenfalls nicht klar definiert[119–122]. In anderen Tumorentitäten konnte die Überexpression von TIM-3 mit einem schlechteren Gesamtüberleben und fortgeschrittenen Tumorstadien assoziiert werden, und in ersten klinischen Studien führte eine selektive Blockade von TIM-3 zu einem Therapieansprechen in einzelnen Patienten[123–125]. Mehrere klinische Studien prüfen aktuell eine Co-Blockade von TIM-3 mit etablierten Checkpoint-Inhibitoren (NCT03446040, NCT03744468).

2.4.2.2 Vakzinierungen

Ein weiterer immuntherapeutischer Ansatz in Weichgewebesarkomen liegt in therapeutischen Vakzinen: Ziel ist die Bildung und Stimulation einer körpereigenen Immunantwort durch eine Präsentation von Tumorantigenen. Bisherige Studien zeigen nur minimale Ansprechraten durch therapeutische Vakzine in Sarkomen. Eine Ausnahme bildet eine Studie von Takahashi et al., in der personalisierte Peptidvakzinierungen für HLA-gematchte Peptide verwendet wurden. Hier konnte ein medianes Gesamtüberleben von 9.6 Monaten in Patienten mit Chemotherapie-refraktären Knochen- und Weichgewebesarkomen gezeigt werden[126]. Mehrere klinische Studien prüfen diesen immuntherapeutischen Ansatz durch eine Stimulation von dendritischen Zellen und Peptiden wie NY-ESO-1, MAGE-A1 oder MAGE-A3, die beispielsweise im Synovialsarkom verbreitet sind (NCT01803152, NCT01242262)[127,128].

2.4.2.3 Adoptive Zelltherapien

Unter adoptiven Zelltherapien versteht man Therapien, in denen Immunzellen zur Bekämpfung des Tumors modifiziert und selektiert werden. Es existieren mehrere therapeutische Strategien, unter anderem *Engineered T-Cell-Receptor* (TCR)-Therapien, *Chimeric Antigen Receptor* (CAR)-Therapien sowie *Tumor-infiltrating Lymphocyte* (TIL)-Therapien. Durch eine Kombination von körpereigenen Lymphozyten mit tumor-spezifischen Antigenen soll die Immunantwort verstärkt werden[129–132]. In malignen Erkrankungen wie Leukämien, Lymphomen und dem multiplen Myelom werden beispielsweise CAR-T-Cell-Therapien breit angewendet und sind Teil nationaler und internationaler Leitlinien[133]. In Sarkomen werden CAR- und TCR-Therapien mit verschiedenen Antigenen geprüft: MAGE, NY-ESO und PRAME gehören zu der Gruppe der *Cancer Testis Antigens* (CTA) und werden in verschiedenen Weichgewebesarkom-Subtypen exprimiert[134]. MAGE- sowie NY-ESO-spezifische Zelltherapien wurden in mehreren klinischen Studien getestet und zeigten speziell im Synovialsarkom ein Therapieansprechen[129]. Ergebnisse aus größeren Studien werden in den nächsten Jahren erwartet (NCT02992743, NCT04044768). Im Vergleich zu TCR-Therapien haben CAR-T-Cell-Therapien in Sarkomen noch keine vielversprechenden Daten gezeigt: In einer Studie von Ahmed et al. wurden anti-Her-2 CAR-T-Cells in Her-2-positiven Sarkomen getestet, zeigten jedoch in keinem Patienten ein ausreichendes Therapieansprechen (NCT00902044). Auch im Rahmen der adoptiven Zelltherapien werden aktuell mehrere klinische Studien durchgeführt (NCT03365782, NCT03132922).

2.5 Biomarker in Sarkomen

Im Vergleich zu anderen Tumorentitäten sind prognostische und prädiktive Biomarker in Weichgewebesarkomen kaum etabliert[135]. Im klinischen Alltag werden deshalb Parameter wie das TNM-Staging-System, die Tumorgröße, Resektionsränder und die Lokalisation der Hochrisiko-Weichgewebesarkome genutzt[136,137]. Diese klinischen Parameter sind jedoch unzureichend, um prädiktive Aussagen über ein Chemotherapie-Ansprechen und die genaue Prognose in dieser Entität zu treffen. Zudem werden durch die Heterogenität und Seltenheit der Sarkome vergleichsmäßig wenige Biomarker als potenzielle pharmakologische Ziele getestet.

Ziel dieses Promotionsvorhabens ist die Identifikation und Analyse von prädiktiven und prognostischen Biomarkern in Hochrisiko-Weichgewebesarkomen. TOP2A und SIRT1 sind Moleküle, die wie bereits beschrieben wichtige Faktoren für die Entstehung einer Chemotherapie-Resistenz sind und in bisherigen Studien die Prognose von Patienten mit malignen Erkrankungen negativ

beeinflusst haben[79–81,91,92]. TIM-3 wurde als Checkpoint-Rezeptor mit einer schlechteren Prognose in verschiedenen Tumoren assoziiert und wird aktuell in mehreren klinischen Studien als neuer immuntherapeutischer Ansatz geprüft[123,125]. Diese Arbeit liegt der Hypothese zugrunde, dass die o.g. Biomarker einen signifikanten prognostischen Einfluss in der gesamten Gruppe der Hochrisiko-Weichgewebesarkome haben und somit unabhängig vom histologischen Subtyp relevant sind. Langfristig sollen Biomarker dabei unterstützen, individuelle Therapieentscheidungen zu treffen und Patienten nicht wie bisher nach einem „*One-size-fits-all*"-Ansatz zu behandeln.

2.5.1 Tissue Microarrays zur Identifikation von Biomarkern

Tissue Microarrays (TMAs) wurden 1998 erstmals als Array-basierte Technologie zur Analyse von multiplen Gewebeproben beschrieben[138]. Durch TMAs können hunderte Proben auf Proteinebene gleichzeitig analysiert werden[139]. TMAs werden heutzutage breit eingesetzt und haben einen hohen Stellenwert in der Krebsforschung, beispielsweise für die Validierung von Ergebnissen, die auf DNS- oder RNS-Ebene identifiziert wurden[140,141]. Zur Analyse der o.g. Biomarker eignet sich die Kombination aus TMAs und immunhistochemischen Analysen. Auf die Methodik wird ausführlich in Publikation Nr. 1 und Nr. 2 eingegangen.

3. Zusammenfassung

Hintergrund: Patienten mit Hochrisiko-Weichgewebesarkomen (HR-STS) werden aktuell nach einem „One-size-fits-all"-Ansatz mit einer Kombinationschemotherapie mit Doxorubicin und Ifosfamid behandelt. Molekulare Prädiktoren für Therapieansprechen und Prognose sind in diesem Patientenkollektiv nicht etabliert. Topoisomerase IIα (TOP2A) und Sirtuin 1 (SIRT1) sind für die Wirkungs- und Resistenzmechanismen von Doxorubicin mitverantwortlich und somit mögliche Biomarker für eine Chemotherapieresistenz. Als Alternative zu einer systemischen Chemotherapie werden immuntherapeutische Ansätze und zielgerichtete Therapieoptionen in mehreren prospektiven Studien in HR-STS getestet. T cell immunoglobulin and mucin domain-containig protein 3 (TIM-3), ein Checkpoint-Rezeptor, ist ein möglicher alternativer Therapieansatz in diesem Kollektiv.

Methoden: Die Proteinexpression von TOP2A, SIRT1 und TIM-3 wurde in prä-therapeutischen Biopsien von Patienten mit HR-STS mittels *Tissue microarrays* (TMAs) und Immunhistochemie analysiert. TIM-3 wurde mit der Expression von programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1) und Tumor-infiltrierenden Lymphozyten (TILs) korreliert. Die analysierten Biomarker wurden mit klinischen Daten, Tumordaten und einem Langzeit-Follow-Up korreliert.

Ergebnisse: Die Expression der o.g. Biomarker wurde in 179 Patienten mit HR-STS analysiert. Die Proteinexpression von TOP2A, SIRT1 und TIM-3 wurde in 47%, 60% sowie 56% aller Patienten gemessen. Während TOP2A mit einem schlechteren Gesamtüberleben ($p = 0.039$) und einem höheren Tumor-Grading ($p = 0.001$) korrelierte, konnte SIRT1 erstmalig mit einem verlängerten Gesamtüberleben in Patienten mit HR-STS in Zusammenhang gebracht werden ($p = 0.025$). Es konnte eine „*Top survivor*" Kohorte bestehend aus Patienten mit niedriger TOP2A-Expression und hoher SIRT1-Expression identifiziert werden (10-Jahres-Gesamtüberleben 67% vs. 40%, $p = 0.002$). Die Expression von TIM-3 korrelierte signifikant mit undifferenzierten pleomorphen Sarkomen ($p < 0.001$), der Expression von PD-1 ($p < 0.001$), PD-L1 ($p < 0.001$) und TILs ($p < 0.001$). Zusammenhänge zwischen dem Gesamtüberleben und der Expression von TIM-3 waren statistisch nicht signifikant ($p = 0.339$).

Schlussfolgerungen: TOP2A, SIRT1 und TIM-3 werden innerhalb der Hochrisiko-Weichgewebesarkome sehr heterogen exprimiert. Die Expression von SIRT1 konnte erstmalig mit einem besseren Gesamtüberleben in HR-STS korreliert werden. Sollte sich die prognostisch signifikante „*Top survivor*"-Kombination aus niedriger TOP2A- und hoher SIRT1-Expression in weiteren Studien als robust erweisen, könnte sie zukünftige individuelle Therapieentscheidungen mitunterstützen. TIM-3 wurde erstmals auf HR-STS-Tumorzellen analysiert und zeigt ein signifikantes Expressionsmuster in ausgewählten Patienten. Unsere Ergebnisse unterstützen die Auswahl von potenziellen Studienpatienten mit signifikanter TIM-3-Expression im Rahmen von zukünftigen klinischen Studien.

4. Abstract (English)

Background: Patients with high-risk soft tissue sarcomas (HR-STS) are currently treated in a “one-size-fits-all” approach with doxorubicin and ifosfamide. Molecular predictors of therapy response and survival are not established in this patient collective. Topoisomerase IIα (TOP2A) and Sirtuin 1 (SIRT1) have implications for the mechanisms of action and resistance to doxorubicin and are therefore potential biomarkers for chemotherapy resistance. As an alternative to systemic chemotherapy, immunotherapeutic approaches in HR-STS are currently being tested in multiple clinical trials. T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), a checkpoint receptor, is a potential therapeutic candidate.

Methods: Protein expression of TOP2A, SIRT1 and TIM-3 was analyzed in pre-treatment biopsies of patients with HR-STS with tissue microarrays (TMAs) and immunohistochemistry. TIM-3 was correlated with the expression of programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1) and tumor infiltrating lymphocytes (TILs). Results were correlated with clinicopathological parameters and a long-term follow-up.

Results: 179 patients with HR-STS were included in this study. The expression of TOP2A, SIRT1 and TIM-3 was measured in 47%, 60% and 56% of all patients. The expression of TOP2A was associated with a shorter overall survival ($p = 0.039$) and higher tumor grading ($p = 0.001$). SIRT1 was correlated with a prolonged overall survival (OS) in this cohort ($p = 0.025$). The combination of high SIRT1 and low TOP2A (“Top survivors”) significantly predicted a better overall survival compared to other biomarker combinations (10-year-OS 67% vs. 40%, $p = 0.002$). The expression of TIM-3 was significantly more often observed in undifferentiated pleomorphic sarcomas compared to other histological subtypes ($p < 0.001$) and correlated with high TIL counts ($p < 0.001$), high PD-1 ($p < 0.001$) and PD-L1 ($p < 0.001$) expression. It did not have a prognostic impact on survival in patients with HR-STS ($p = 0.339$).

Conclusions: TOP2A, SIRT1 and TIM-3 expression varies between specific subgroups of patients with HR-STS. This is the first study to correlate SIRT1 expression with a better outcome in patients with HR-STS and demonstrate a “Top survivor” cohort of HR-STS patients with high SIRT1 and low TOP2A expression. If the “Top survivor” biomarker combination remains robust in future studies, it could support individual therapy decisions. In addition, it is the first study to describe significant tumor cell expression of TIM-3 in specific subgroups of patients with HR-STS. TIM-3 qualifies as a potential immunotherapeutic target in this patient cohort.

5. Publikation Nr. 1

Expression patterns of TOP2A and SIRT1 are predictive of survival in patients with high-risk soft tissue sarcomas treated with a neoadjuvant anthracycline-based chemotherapy

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Simple summary: High-risk soft tissue sarcomas (HR-STS) account for less than 1% of all malignancies in adults. Despite optimal local treatment, almost half of patients will die within five years of their diagnosis. Chemoresistance is a major responsible mechanism for treatment failure in advanced tumor stages. In contrast to other cancer types, molecular predictors of response to chemotherapy and survival have not been identified and put into clinical practice by now. We analyzed the predictive value of two molecules involved in the working and resistance mechanisms to doxorubicin, TOP2A and SIRT1, in a large cohort of locally advanced HR-STS who underwent a neoadjuvant anthracycline-based chemotherapy with long-term follow-up. Our results show sarcoma subtype-specific patterns of TOP2A and SIRT1 expression. We demonstrate significant differences in overall survival according to TOP2A and SIRT1 expression status. Both markers can be used as clinically significant predictive indicators for HR-STS patients scheduled for neoadjuvant anthracycline-based chemotherapy.

Abstract:

Background: Molecular predictors of response to chemotherapy and survival have not been put into clinical practice in high-risk soft tissue sarcomas (HR-STS) by now. Expression of TOP2A

and SIRT1 has implications for the mechanism of action of doxorubicin, which is the backbone of chemotherapy in HR-STS.

Methods: Pre-treatment samples of 167 patients with HR-STS were collected. Protein expression levels of TOP2A and SIRT1 were evaluated with tissue microarrays and immunohistochemistry and correlated with clinicopathological parameters including overall survival (OS).

Results: Expression of TOP2A and SIRT1 was seen in 47% and 60% of patients with HR-STS, respectively. TOP2A expression was associated with higher tumor grading and shorter 5-year OS. Expression of SIRT1 was correlated with a better 5- and 10-year OS. The combination of high SIRT1 and low TOP2A ("Top survivors") significantly predicted a better OS compared to other biomarker combinations. Multivariate analysis confirmed the expression of SIRT1 and the "Top survivor" biomarker combination as independent predictive factors of OS.

Conclusions: This is the first study to associate SIRT1 overexpression with a statistically significant prolongation of OS in HR-STS. Both individual markers and their combination can be used as predictive indicators for HR-STS patients scheduled for neoadjuvant anthracycline-based chemotherapy.

Keywords: Soft Tissue Sarcoma; TOP2A; SIRT1; Tissue Microarray; Immunohistochemistry; Biomarker; Precision Oncology

1. Introduction

High-risk soft tissue sarcomas (HR-STS) are defined by a size of 5 cm or more, deep localization in relation to the fascia and grade 2 or 3 according to the French Fédération Nationale de la Lutte Contre le Cancer (FNCLCC) grading system[51]. They have high metastatic potential and mostly metastasize hematogenously with the lung as main target organ[142]. Despite optimal local treatment, almost half of patients with HR-STS will die within 5 years of their diagnosis[6,7]. Drug resistance is believed to cause treatment failure in over 90% of patients with metastatic cancer[69].

Despite the current knowledge about biological and clinical differences in sarcoma subtypes, patients have been treated in a "one-size-fits-all" approach with anthracyclines and alkylating agents for the past 3 decades[143]. Broadly usable, clinically significant biomarkers that predict therapy response and survival have yet to be discovered in sarcomas. In other tumors, biomarkers with prognostic and predictive importance are already found. An important example is the use of hormone receptors in breast cancer[144]. Rare examples in sarcomas are the use of AMPD2 in undifferentiated pleomorphic sarcomas or PD-L1 in soft tissue sarcomas, which were correlated with survival by Orth et al., or MDM2 amplification status in liposarcomas, which correlated with drug sensitivity and clinical outcomes in a study by Bill et al[145–147]. They have no use in clinical routine at present. Prognostic factors currently rely on clinical evaluations, such as TNM staging, topography, tumor size and assessment of surgical margins[136,137]. The lack of molecular predictors leads to a fraction of patients suffering from side effects of chemotherapy without achieving any benefit, as sensitivity to chemotherapeutic agents is not routinely tested despite the common presence of intrinsic and acquired chemoresistance[69]. With novel biomarkers, future treatment plans could be adjusted to the individual patient. In addition, signaling pathways and molecular mechanisms could be specifically targeted to minimize chemoresistance and improve prognosis.

Aim of this study was to analyze expression patterns of two molecular markers involved in the working mechanisms and resistance pathways of doxorubicin: TOP2A, which regulates DNA

structure and cell proliferation and acts as the main target of doxorubicin, and SIRT1, an NAD-dependent histone deacetylase which regulates cellular differentiation and responses to apoptosis, stress and DNA damage.

Variations in expression of TOP2A have been associated with chemoresistance and changes in outcome. The predictive properties of TOP2A have been described in several studies, with varying results: Overexpression of TOP2A was linked to a poor prognosis and unfavorable five year overall survival (OS) in different soft tissue sarcomas[79–81]. In leiomyosarcomas, TOP2A was highly expressed but failed to predict outcomes[148]. TOP2A overexpression was also correlated with better pathohistological response and a decreased risk of relapse in locally advanced soft tissue sarcomas[82]. In bladder cancer, TOP2A downregulation was predictive for poor response to neoadjuvant chemotherapy, making it one of fourteen predictive genes among over 27'000 genes in a genome-wide microarray assay[149] .

Depending on the expression status and cellular context, SIRT1 acts as a tumor suppressor or as an oncogene and resistance mechanism to doxorubicin. In gastric cancers, SIRT1 was associated with lymphatic invasion, vessel invasion, lymph node metastasis and poor cancer-specific survival[91]. SIRT1 was expressed in 71% of 104 sarcoma patients in a study by Kim et al., regardless of histological subtype. SIRT1 was also associated with advanced clinicopathological parameters, including stage, grade and metastasis. In addition, event-free survival and OS were significantly reduced with high SIRT1 levels[92]. The importance of SIRT1 in carcinogenesis was also discussed in a study by Chu et al. SIRT1 was upregulated in five different cell lines including osteosarcoma. Chemoresistance was observed in all cell types, independent of the used chemotherapeutic regimen. In addition, SIRT1 was responsible for the overexpression of other proteins responsible for drug resistance, including MDR1. Chemoresistance was then reversed by selectively inhibiting SIRT1 with small interfering RNAs (SiRNAs), enhancing response to chemotherapy in the drug-resistant cells by an additional 25% to 30%[150].

In the present study, we analyze the expression of TOP2A and SIRT1 in a large and well-characterized cohort of high-risk soft tissue sarcoma patients with long-term follow-up and correlate our findings with clinical tumor characteristics and survival data. We show that TOP2A and SIRT1 display distinct expression patterns in different STS subtypes. TOP2A and SIRT1 expression levels are inversely correlated to patient survival. In multivariate analysis, the expression of SIRT1 and the biomarker combination of high SIRT1 and low TOP2A ("Top survivors") are confirmed as independent predictive factors of OS.

2. Materials and Methods

2.1. Patient selection

An exploratory retrospective cohort study design was chosen to address the research question. Eligible patients had proven soft tissue sarcoma with the following risk criteria: tumor diameter 5 cm or larger, French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade 2 or 3, deep to the fascia, and no evidence of distant metastases. N=167 patients treated between 1989 and 2012 were included in this study. Data on clinical parameters were extracted from original clinical and pathology reports at the LMU University Hospital of Munich, Germany. Clinical data was updated until February 2021.

2.2. Procedures

All patients with HR-STS were to receive a multimodal treatment including neoadjuvant doxorubicin-based chemotherapy, surgery, radiation therapy and adjuvant doxorubicin-based chemotherapy. Chemotherapy was combined with regional hyperthermia (RHT). In the end, nearly all patients underwent surgical resection of the tumor and about two-thirds received radiotherapy.

Neoadjuvant and adjuvant chemotherapy was given in 3-week-intervals consisting of doxorubicin, ifosfamide, and partly etoposide. Etoposide was eliminated from chemotherapy protocols after 2010. In the etoposide containing regimen (EIA), 50mg/m^2 of doxorubicin was given on day 1, combined with 1500mg/m^2 of ifosfamide on days 1 to 4 and 125mg/m^2 of etoposide on days 1 and 3. In the regimen without etoposide (AI), 60mg/m^2 of doxorubicin was given in combination with 3000mg/m^2 of ifosfamide on days 1 to 3 or 1500mg/m^2 on days 1 to 4 (AI60/9 vs. AI60/6).

Chemotherapy was given concurrently with regional hyperthermia (42°C for a 60-minute period on day 1 and 4 of each cycle). Response to neoadjuvant therapy was evaluated by computed tomography or magnetic resonance imaging and chest radiography after two cycles of induction therapy. Definitive surgery occurred within 4 to 6 weeks of neoadjuvant therapy.

Adjuvant treatment was started within six weeks of local therapy. It consisted of external beam radiation therapy (Administered dose between 50.0 and 60.0 Gray, with daily fractions of 1.8 to 2.2 Gray, and a boost up to 66.0 Gray) and another four cycles of adjuvant chemotherapy with regional hyperthermia within 6 weeks of local therapy. Quality of hyperthermia was ensured by current guidelines of the European Society for Hyperthermic Oncology (ESHO)[27,54]. Treatment continued unless progressive disease or unacceptable toxic effects occurred.

2.3. Histopathology and Tissue Microarray Construction

Tumor samples originated from tumor biopsies that were taken before the initiation of neoadjuvant treatment at the Ludwig-Maximilians-University hospitals, Munich. In addition to the original pathology reports, microscopic findings (tumor type according to current WHO classifications and degree of differentiation) were reassessed. For tissue microarray (TMA) assembly, representative tumor areas were marked on H&E stained slides of formalin-fixed, paraffin-embedded tumor samples from all patients according to standard procedures and two 0.6mm punch biopsies were taken from each sample[151]. Normal tonsillar tissue samples were used as controls on the TMA. In the end, 4 tissue microarrays containing 167 pre-treatment tumor samples from 167 patients with high-grade soft tissue sarcomas were constructed.

2.4. Immunohistochemistry

For the immunohistochemical detection of TOP2A and SIRT1, commercially available and validated monoclonal antibodies were used[91,152] (Table 1). Antigen retrieval was carried out by heat treatment with Target Retrieval Solution Citrate (Agilent Technologies, Santa Clara, USA). Staining was performed on a Ventana Benchmark XT Autostainer (Ventana Medical Systems, Tucson, USA) with a DAB+ Kit (Agilent Technologies, Santa Clara, USA). All slides were counterstained with hematoxylin (Vector Laboratories, Burlingame, USA). An ImmPRESS Anti-Rabbit IgG Polymer Kit was used for detection (Vector Laboratories, Burlingame, USA). To exclude unspecific staining, system controls were included. Tonsillar tissue served as a positive control for immunohistochemistry. Immunostaining of cells was evaluated and scored semi-quantitatively (0 = 0-9%, negative; 1 = 10-100%, positive) (Table 2). All immunohistochemical and pathologic evaluations

were carried out independently and blinded together with an experienced pathologist with special expertise in sarcoma pathology (T.K.). In the case of discrepancy, the slides were reevaluated under a multiheaded microscope and consensus reached.

Table 1: Antibodies used for immunohistochemistry.

Antibody	**Dilution**	**Company**
SIRT1 HPA006295 Rabbit IgG monoclonal	1:250	Atlas antibodies (Stockholm, Sweden)
TOP2A D10G9 Rabbit IgG monoclonal	1:200	Cell Signaling Tech. (Danvers, USA)

Table 2: Semi-quantitative evaluation of immunostaining.

Coloration intensity	**Fraction of positive cells**
Negative reaction: 0	0% - 9%
Positive reaction: 1	10% - 100%

2.5. Statistical Analysis

Categorical variables were tested for independence using the Chi square test. Survival was calculated from the date when sarcoma was first diagnosed. OS (patients' death without regarding the cause of death) was used as the endpoint for estimating prognosis. Survival curves were created using the Kaplan–Meier method, and the log-rank test was used to assess differences in survival.

Significant and independent predictors of OS were identified by Cox proportional hazard analysis. The forward stepwise procedure was set to a threshold of 0.05. All statistical analyses were performed using SPSS 26.0 (IBM, Chicago, IL, USA) software. Statistical significance was defined as a p value < 0.05 for all analyses.

3. Results

3.1. Patient cohort

The baseline characteristics of the investigated cohort are summarized in table 1. In total, 167 patients were analyzed. Median age was 53 years (18 – 79 years), 83 patients were female and 84 were male. The most common sarcoma subtypes were undifferentiated pleomorphic sarcomas (UPS, 33%), followed by liposarcomas (21%). Half of tumors were high grade (G3, 50%). 65% of all patients were diagnosed with non-extremity sarcomas. Most patients (84%) underwent a R0 or R1 resection of their primary tumor. Most patients had a stable disease (SD) according to the RECIST tumor response criteria (47%). 100 patients expressed a high level of SIRT1 (60%). TOP2A was positive in 79 patients (47%). We correlated the expression of TOP2A and SIRT1 with all clinical factors in table 3. Of those parameters, correlation of TOP2A and grading was

statistically significant (G2 vs. G3, p=0.001, Fisher's Exact 2-sided Test) (Table 4). The expression of SIRT1 did not significantly correlate with higher FNCLCC tumor grading (G2 vs. G3, p=0.115) (Table 4). Correlation of TOP2A and SIRT1 expression with AJCC staging was not statistically significant (Stage II vs. III, p=0.688 and p=0.417) (Table 4). Correlation of tumor response and TOP2A expression was not statistically significant (p=0.184). In addition, association between tumor response and SIRT1 expression was not statistically significant (p=0.940). Lastly, correlation between high TOP2A and high SIRT1 expression was statistically significant (p=0.001).

Table 3: Patient characteristics.

Factor	**Strata**	**n**	**%**
Total		167	100
Sex	male	84	50
	female	83	50
Histological subtype	Undiff. pleomorphic sarcoma	55	33
	Leiomyosarcoma	30	18
	Synovial sarcoma	20	12
	Liposarcoma	35	21
	Angiosarcoma	5	3
	MPNST	9	5
	Other	13	8
Grading	Intermediate (G2)	84	50
	High (G3)	83	50
Location	Extremities	58	35
	Non-Extremities	109	65
Size	50mm - 80mm	56	33
	≥80mm	93	56
	Missing	18	11
Surgical margins	R0	44	26
	R1	96	58
	R2 or no resection	27	16
AJCC stage	Stage II	31	18
	Stage III	118	71
	Missing	18	11
Tumor response	Complete / Partial response	24	14
	Stable disease	78	47
	Progressive disease	24	14
	Missing	41	25
TOP2A expression	Low (0-9%)	88	53
	High (10-100%)	79	47
SIRT1 expression	Low (0-9%)	67	40
	High (10-100%)	100	60

3.2. TOP2A and SIRT1 expression in sarcoma subtypes

The expression of both biomarkers varied between different sarcoma subtypes (Table 4). Malignant peripheral nerve sheath tumors and synovial sarcomas were strongly correlated with TOP2A expression (67% and 65%, respectively). SIRT1 was mostly expressed in synovial sarcomas and undifferentiated pleomorphic sarcomas (75% and 67%). Examples of immunohistochemistry staining for TOP2A and SIRT1 are shown in figure 1.

Table 4: Candidate biomarker TOP2A and SIRT1 expression according to histological subtype, grading and AJCC stage. The table shows the number and percentage of patients with high TOP2A and SIRT1 expression.

	Total	**TOP2A**		**SIRT1**	
Histological subtype	**n**	**n**	**%**	**n**	**%**
UPS	55	31	56	37	67
Leiomyosarcoma	30	12	40	17	57
Synovial Sarcoma	20	13	65	15	75
Liposarcoma	35	11	31	19	54
Angiosarcoma	5	2	40	3	60
MPNST	9	6	67	3	33
Other	13	4	31	6	46
Grading	**n**	**n**	**%**	**n**	**%**
G2	84	28	33	45	54
G3	83	51	61	55	66
AJCC Stage	**n**	**n**	**%**	**n**	**%**
Stage II	31	16	52	21	68
Stage III	118	55	47	70	59

UPS = Undifferentiated pleomorphic sarcoma, MPNST = Malignant peripheral nerve sheath tumor. Other = 3 Chondrosarcomas, 4 Myxofibrosarcomas, 1 Alveolar sarcoma, 2 Carcinosarcomas, 1 Rhabdomyosarcoma, 2 malignant solitary fibrous tumors.

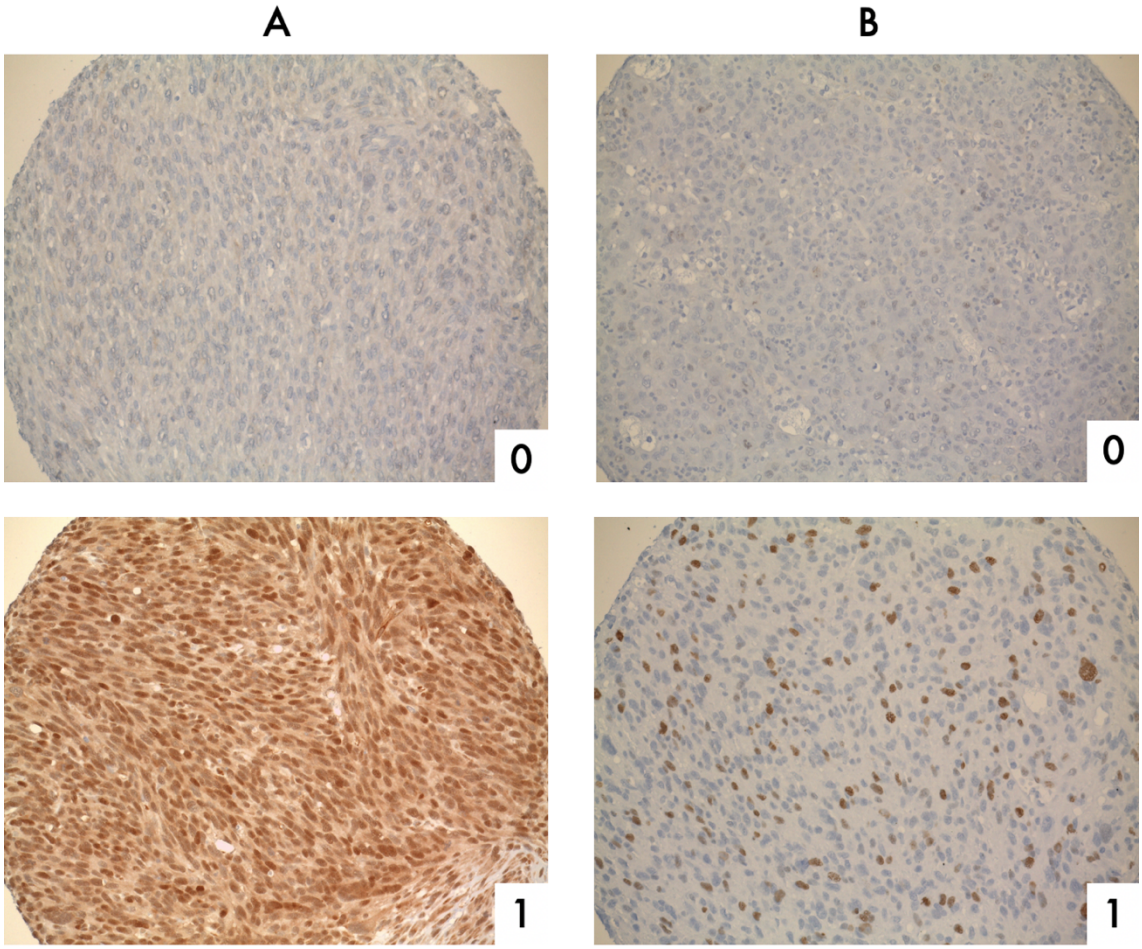

Figure 1: Stained tissue microarray cores. Representative micrographs of cores on a tissue microarray stained for (A) SIRT1 and (B) TOP2A. Numbers represent semiquantitative scoring of immunostaining: 0, negative; 1, positive. Magnification 20x.

3.3. Study population survival analysis

Median Follow-up-time was 120 months. All but 9 patients (95%) were followed until the end of 2020 or until death. The 9 remaining patients were treated in Germany and returned to their home country after treatment, which explains the loss to follow-up. We performed a univariate survival analysis with all clinical parameters mentioned in table 3. Age (<55y median OS >120 months vs. >55y median OS 52 months, p=0.033) and surgical margins (R0/R1 median OS 114 months vs. R2/no resection median OS 19 months, p<0.001) were significant predictors of OS.

OS was significantly worse in angiosarcomas compared to all histologies except undifferentiated pleomorphic sarcomas (p<0.05) (Figure 2). In addition, OS was significantly worse in malignant peripheral nerve sheath tumors compared to liposarcomas (p=0.043). 5- and 10-year OS was not calculated in the “Other” group due to major differences in sarcoma subtypes and low case number. Statistically nonsignificant predictors of OS were sex (Male median OS 68 months vs. female median OS >120 months, p=0.084), grading (G2 median OS >120 months vs. G3 median OS 52 months, p=0.108), tumor location (Extremity median OS >120 months vs. non-extremity median OS 80 months, p=0.222) and tumor size (<80mm median OS 114 months vs. >80mm median OS 68 months, p=0.224).

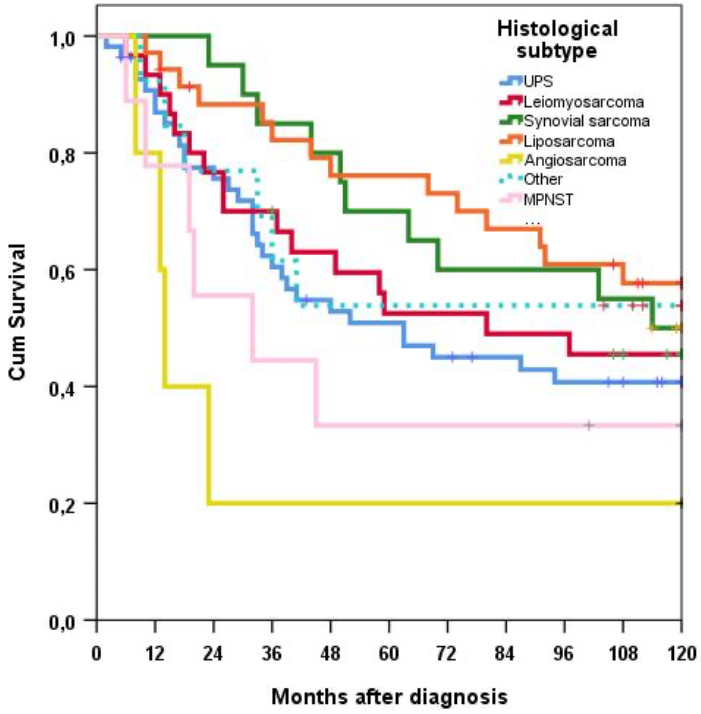

Figure 2: Overall survival according to histological subtype. UPS = Undifferentiated pleomorphic sarcoma. MPNST = Malignant peripheral nerve sheath tumor. Other = 3 Chondrosarcomas, 4 Myxofibrosarcomas, 1 Alveolar sarcoma, 2 Carcinosarcomas, 1 Rhabdomyosarcoma, 2 malignant Solitary Fibrous Tumors.

3.4. Correlation of TOP2A and SIRT1 expression with overall survival

In this study, high TOP2A expression was correlated with a worse prognosis in HR-STS (Figure 3). Median OS was 50 months compared to 108 months in TOP2A-negative tumors, 5-year OS 46% vs. 66% and 10-year OS 42% compared to 50%. The difference in 5-year OS was statistically significant (p=0.039). In contrast, the difference in 10-year OS was not statistically significant (p=0.160).

When analyzing the effect of TOP2A expression on the four most important histological subgroups, differences in OS were only statistically significant in synovial sarcomas (64 months vs. >120 months, p=0.021).

High expression of SIRT1 was correlated with a longer OS in HR-STS (Figure 4). Median OS was >120 months in patients with high expression of SIRT1 compared to 63 months with low SIRT1 levels. Differences in 5- and 10-year OS were also statistically significant (5-year OS 61% vs. 52%, 10-year OS 53% vs. 35%, p=0.025). Differences in OS in the four most important histological subgroups were not statistically significant.

In this study, 39 patients (23%) were diagnosed with HR-STS expressing both high SIRT1 and low TOP2A levels. As mentioned before, high SIRT1 and low TOP2A levels favored OS in this patient cohort. This specific “Top survivor” biomarker combination had a significant positive impact on OS (5-year OS 80% vs. 50%, 10-year OS 67% vs. 40%, p=0.002) (Figure 5).

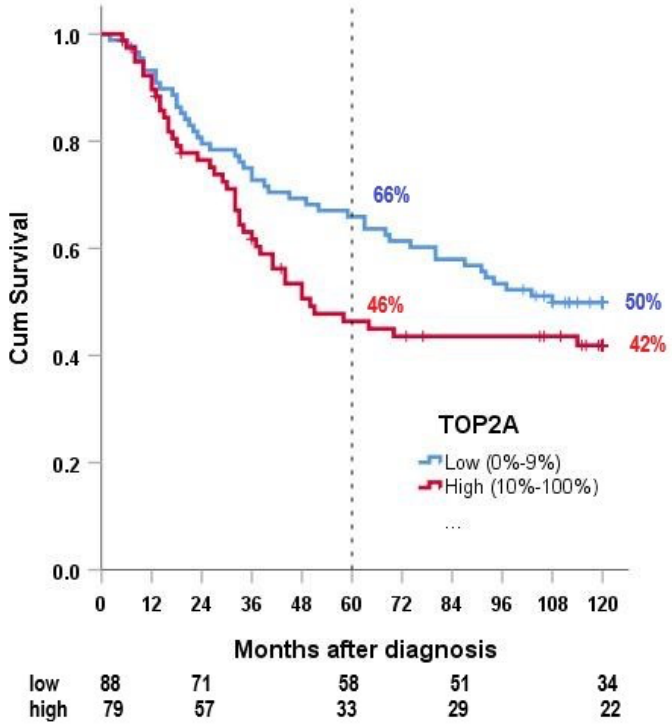

Figure 3: Overall survival according to TOP2A expression.

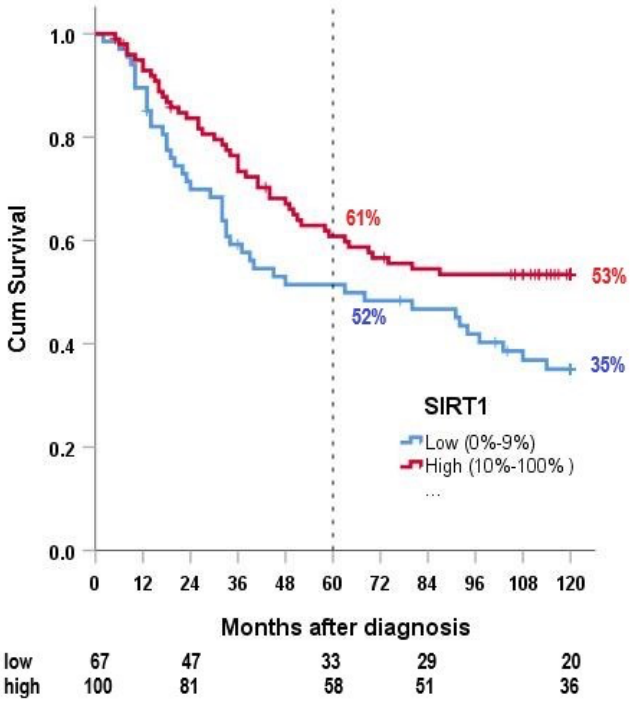

Figure 4: Overall survival according to SIRT1 expression.

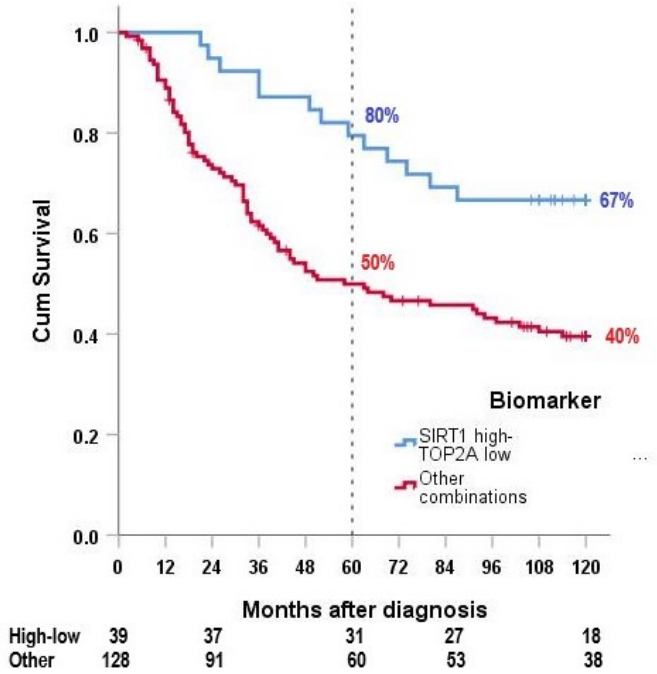

Figure 5: Overall survival with favorable “Top Survivor” biomarker expression (High SIRT1, low TOP2A) compared to other biomarker combinations.

3.5. TOP2A and SIRT1 in multivariate analysis

To control for confounding factors, multivariate analysis was performed with five previously analyzed prognostic factors (Age, surgical resection margins, grade, TOP2A and SIRT1 expression) using a Cox proportional hazards model. Low expression of SIRT1 and high expression of TOP2A were independent predictors of shorter OS. Radical resection, younger age (<55 years) and lower tumor grade were associated with a longer OS in HR-STS. All factors except grade and TOP2A expression proved to be statistically significant (Table 5).

Secondly, we performed a multivariate analysis with several clinical variables (Age, resection margins, grade) and the favorable “Top survivor” combination of high SIRT1 and low TOP2A expression, which was previously correlated with a significant survival benefit. In this setting, a combination of high SIRT1 and low TOP2A expression proved to be an independent statistically significant predictor of OS compared to other biomarker combinations (p=0.002). Patients with other biomarker combinations were 2.547 times more at risk to suffer from a shorter OS (Table 6).

Table 5: Multivariate analysis of overall survival 1. A Cox proportional hazards model for overall survival was calculated including our biomarkers TOP2A and SIRT1 and statistically significant clinical parameters.

Factor	Strata	Significance	Hazard Ratio	95.0% CI
Surgical margins	R0/R1 vs. R2 or no resection	0.0001	2.735	(1.632 - 4.581)
Age		0.020	1.018	(1.003 - 1.033)
Grade	G2 vs. G3	0.136	1.409	(0.897 - 2.213)
TOP2A	Low vs. High	0.058	1.566	(0.985 - 2.489)
SIRT1	High vs. Low	0.004	1.935	(1.240 - 3.018)

Table 6: Multivariate analysis of overall survival 2. A Cox proportional hazards model for overall survival was calculated including the favorable “Top Survivor” biomarker combination (Low TOP2A and high SIRT1) and statistically significant clinical parameters.

Factor	Strata	Significance	Hazard Ratio	95.0% CI
Surgical margins	R0/R1 vs. R2 or no resection	0.0001	2.839	(1.696 - 4.752)
Age		0.022	1.017	(1.003 – 1.032)
Grade	G2 vs. G3	0.153	1.362	(0.891 – 2.081)
Biomarker	High SIRT1 and low TOP2A	0.002	2.547	(1.406 – 4.612)

4. Discussion

In this study, we analyzed the expression of TOP2A and SIRT1 in a well-characterized cohort of high-grade soft tissue sarcoma patients using tissue microarrays and immunohistochemistry. These markers were chosen because of their relationship to doxorubicin and chemoresistance. Our findings suggest that patients with high TOP2A expressing tumors have a shorter OS than patients with low TOP2A levels. In contrast, high expression of SIRT1 correlates with a prolonged OS. In addition to these findings, patients with a combination of high SIRT1 and low TOP2A expression (“Top survivors”) had a significantly longer OS than patients with other biomarker combinations.

Some classically significant clinical predictors of OS including grading, tumor size and tumor location did not reach statistical significance in our study population. This is partly due to the sample size and large number of high-risk soft tissue sarcoma subgroups. Nevertheless, absolute numbers correlate with previous studies on this subject (Grading: G2 median OS >120 months vs. G3 median OS 52 months, p=0.108), tumor location: Extremity median OS >120 months vs. non-extremity median OS 80 months, p=0.222, and tumor size: <80mm median OS 114 months vs. >80mm median OS 68 months, p=0.224).

Our results indicate that a high TOP2A expression leads to a shorter 5-year OS in patients with HR-STS (5-year OS 46% vs. 66%, p=0.039). These findings correlate with current literature about TOP2A expression and cancer prognosis: TOP2A expression was already described as an independent predictor of an unfavorable prognosis in sarcomas in a study conducted by Da Cunha et

al. in 2012. They successfully included TOP2A in a prognostic scoring system along with histologic grade, surgical margins and tumor size[79]. Our results correlate with these findings. A different mix of histological subtypes and other inclusion criteria between their study and ours (only high-risk patients (>5cm, G2/G3, deep to the fascia), no evidence of metastases, different biomarker scoring evaluations) did not change the prognostic use of TOP2A.

Skotheim et al. correlated TOP2A gene and protein overexpression with a poor 10-year OS in malignant peripheral nerve sheath tumors (MPNST)[81]. Our results also indicate that TOP2A expression predicts poor survival in MPNST. However, as this study only included 9 MPNST, our results were not statistically significant. In general, the correlation between TOP2A expression and shorter OS was not statistically significant in our subgroups partly due to the low sample sizes. This underlines the difficulty with research on HR-STS: a statistically significant analysis of histological subgroups requires large patient cohorts, which is only possible in large-volume sarcoma centers over a long period of time.

Interestingly, our results indicate that TOP2A expression only correlates with a shorter 5-year OS compared to a 10-year OS (p=0.039 vs. p=0.160). This might be due to the fact that many patients with low TOP2A expression were censored in the follow-up period between five and ten years, which limits the significance of 10-year OS in this regard.

Molecular mechanisms associated with TOP2A overexpression and tumor progression or chemoresistance still need to be established. Possible explanations are an increase in TOP2A mutations leading to doxorubicin insensitivity and decreased apoptosis signaling in affected cells[41,78]. Another molecular explanation is TOP2A deregulation caused by YB-1 overexpression. YB-1 is a DNA-binding protein which is located on the promoter of the TOP2A gene. YB-1 overexpression results in downstream TOP2A overexpression and tumor progression. Oda et al. demonstrated a shorter OS in synovial sarcomas due to YB-1 overexpression[80]. This example demonstrates the fragility of regulatory mechanisms in sarcomas, as the over- and underexpression of other molecules can also affect downstream TOP2A activity and result in chemoresistance and poor prognosis.

This study identified SIRT1 as an independent predictor of OS in HR-STS. High SIRT1 expression correlated with a better 5- and 10-year OS (10-year OS 53% vs. 35%, p=0.025). Our multivariate analysis suggests that the prognostic value of SIRT1 is robust, even in relation to previously established clinical parameters such as surgical margins or patient age. We did not expect these results. Kim et al. correlated SIRT1 overexpression with advanced clinicopathological parameters and a reduced event-free and OS in soft tissue sarcoma patients[92]. Additionally, selective inhibition of SIRT1 lead to a reduction of chemoresistance in osteosarcoma cell lines in an experimental study by Chu et al.[150]. These findings were consistent with the results of Ma et al.; In their study, selective inhibition of SIRT1 and SIRT2 impaired the survival of pediatric rhabdomyosarcoma and synovial sarcoma cell lines[94]. The correlation between SIRT1 overexpression and poor survival was explained through deacetylation of downstream molecules of apoptosis, which impairs a physiological DNA damage response, and transcriptional inactivity of tumor suppressor genes[88,89]. Another study explained that SIRT1 supports tumor development by making the tumor “addicted” to sirtuins[85].

Only few studies correlate with our findings on a better survival with SIRT1 expression. These studies were conducted on other cancer types including breast, ovarian and colon cancer, which limits their comparison to our results[95,96]. A possible explanation for our results could be the dual role of sirtuins in cancer as described by Bosch-Presegué et al. It is still not clear why SIRT1 acts as a tumor suppressor and as an oncogene depending on the situation in the individual cell. They

postulated that SIRT1 may swap from tumor suppressor to oncogene after reaching a certain stress threshold[85]. This model usually distinguishes healthy cells from tumor cells, and future research should investigate SIRT1 specifically in HR-STS to determine if there is a dual role specifically in malignant sarcoma cells. Lastly, as our patient population is unique in regard to the use of hyperthermia during treatment, the effect of hyperthermia on sirtuin expression and vice versa could be further analyzed *in vitro*.

We identified a subgroup of patients with HR-STS that distinguished themselves from other patients according to their TOP2A and SIRT1 expression status ("Top survivors"). Patients with high SIRT1 and low TOP2A expression had a statistically significant improvement of OS compared to other biomarker combinations (10-year OS 67% vs. 40%, p=0.002). Multivariate analysis proved this combination to be an independent statistically significant predictor of OS (p=0.002). This is the first study to identify such a predictive model. If the predictive property of our biomarker combination remains robust in future studies, it could be used as an additional tool in individual therapy decisions.

Strengths of this study are the analysis of TOP2A and SIRT1 on a large patient cohort of high-grade soft tissue sarcomas. Sarcomas are very rare tumors, a cohort of 167 patients is therefore considered large in this tumor entity. In addition, our inclusion criteria (only high-risk sarcomas, no evidence of metastases, only pre-treatment biopsies) made our population more homogenous. The follow-up of more than ten years is another strength of this study.

A limitation of this study is the chosen cutoff for a positive vs. negative biomarker expression (0-9% vs. 10-100%). The cutoff value varies between studies and should be standardized to better compare results, as there is no consensus about the definition of "high" and "low" expression of TOP2A and SIRT1. Soto Rodrigo et al. used the median percentage of TOP2A expression as a cutoff in their sarcoma cohort, which could be a clever way to distinguish high from low expression[82]. Another limitation of this study is the correlation of chemotherapy response with survival rates. We chose biomarkers specifically related to chemoresistance and used OS as a measurable outcome in our study population. Future studies could include other variables to measure response to chemotherapy. An example is the Salzer-Kuntschik tumor regression grading system used in osteosarcomas[153]: Tumor samples are analyzed after chemotherapy and the degree of tumor necrosis is taken as a surrogate for response to chemotherapy. High rates of necrosis are therefore correlated with a good histopathologic response to chemotherapy. In a second study, these findings were correlated with a better 10-year overall and event-free survival[154]. Such a grading system could be interesting for our patient cohort. Lastly, the prognostic value of TOP2A and SIRT1 in specific histological subtypes (Compared to HR-STS in general) is limited due to the limited number of specific patient cases.

Future studies should compare the expression of TOP2A and SIRT1 in pre- and post-chemotherapy samples. This could lead to new insights in the adaptive properties of chemotherapy resistance in HR-STS. The predictive properties of TOP2A and SIRT1 could also be examined in patients with HR-STS that were not treated with chemotherapy, working as a control group. In addition, future studies could analyze TOP2A and SIRT1 on a genomic level and compare these results to the protein level of the same markers, as some studies described differences in expression between genome and proteome.

5. Conclusions

TOP2A and SIRT1 show distinct expression patterns in different high-risk soft tissue sarcoma subtypes. This study confirms previous results on TOP2A overexpression and shorter OS in HR-STS. It is the first study to associate SIRT1 overexpression with a statistically significant prolongation of OS in HR-STS. Both markers can be used as clinically significant predictive indicators for HR-STS patients scheduled for neoadjuvant anthracycline-based chemotherapy. If the predictive “Top survivor” biomarker combination (High SIRT1, low TOP2A) remains robust in future studies, it could become an additional tool in individual therapy decisions.

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Institutional Review Board Statement: The Internal Review Board and the Ethical Review Committee of the Ludwig-Maximilians-University Munich approved the protocol of this research project (Reference number 20-824).

Informed Consent Statement: All data was irreversibly anonymized.

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

6. Publikation Nr. 2

TIM-3 Qualifies as a Potential Immunotherapeutic Target in Specific Subsets of Patients with High-Risk Soft Tissue Sarcomas (HR-STS)

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Simple Summary: T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) acts as an immune checkpoint on exhausted T cells and has been associated with dismal outcomes in various solid tumors. TIM-3 is currently being evaluated as an immunotherapeutic target in multiple clinical trials. Our study shows the significant tumor cell expression of TIM-3 in specific subsets of patients with high risk soft tissue sarcomas (HR-STS). We demonstrate an interaction between TIM-3, tumor infiltrating lymphocyte (TIL) counts and PD-1/PD-L1 expression in patients with HR-STS. TIM-3 could qualify as a potential immunotherapeutic target in HR-STS.

Abstract:

Background: The expression of T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), an immune checkpoint receptor on T cells, has been associated with dismal outcomes and advanced tumor stages in various solid tumors. The blockade of TIM-3 is currently under examination in several clinical trials. This study examines TIM-3 expression in high-risk soft tissue sarcomas (HR-STS).

Methods: Tumor cell expression of TIM-3 on protein level was analyzed in pre-treatment biopsies of patients with HR-STS. TIM-3 expression was correlated with clinicopathological parameters

including tumor-infiltrating lymphocyte (TIL) counts, programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PDL-1) expression in patients with HR-STS. Survival dependent on the expression of TIM-3 was analyzed.

Results: TIM-3 expression was observed in 101 (56%) out of 179 pre-treatment biopsies of patients with HR-STS. TIM-3 expression was significantly more often observed in undifferentiated pleomorphic sarcomas (UPS) compared to other histological subtypes ($p < 0.001$), high TIL counts ($p < 0.001$), and high PD-1 ($p < 0.001$) and PD-L1 expression ($p < 0.001$). TIM-3 expression did not have a prognostic impact on survival in patients with HR-STS.

Conclusions: This is the first study to demonstrate a significant tumor cell expression of TIM-3 in specific subsets of patients with HR-STS. TIM-3 qualifies as a potential immunotherapeutic target in HR-STS.

Keywords: sarcoma; tumor biomarkers; immune checkpoint inhibitors; immunotherapy; TIM-3

1. Introduction

High-risk soft tissue sarcomas (HR-STS) are rare tumors with multiple distinct histopathological subtypes, the most common being liposarcoma, leiomyosarcoma and undifferentiated pleomorphic sarcomas (UPS). They account for approximately 1% of adult malignancies[155,156]. Despite optimal local treatment, almost half of patients will die within 5 years of their diagnosis[6,7]. In patients with advanced and metastatic soft tissue sarcomas, median survival rates range around 12–18 months[33,157–159]. Standard treatment for locally advanced and metastatic HR-STS is systemic chemotherapy with anthracycline-based regimens[14,22,160]. Different second- or third-line regimens, including trabectedin, or targeted therapies such as pazopanib have been approved in recent years, with only limited effects on PFS and OS[36,38]. Considering the lack of efficient therapy lines and poor survival rates, there is a great need for new systemic treatment strategies.

While checkpoint inhibitors (CPI) revolutionized the treatment of multiple cancers with high somatic mutation rates such as melanoma and lung cancer, they have demonstrated only limited effects in sarcomas and are currently not part of international treatment guidelines[107–111]. T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), an emerging immune checkpoint receptor, is a member of the TIM family and was originally identified as a receptor expressed on interferon-γ-producing CD4+ and CD8+ T cells[116]. The working mechanisms of TIM-3 are not fully understood. In lymphocytes, TIM-3 is recruited to the immunological synapse on T cell activation[161]. Depending on the interplay with its interacting ligands such as CEACAM1 or lectin galectin 9, TIM-3 is differently phosphorylated and either permissive or inhibitory to T cell activation[117,162]. TIM-3 is expressed in different tumor cells, including lung cancer and melanoma[121,122]. It is co-stimulated and co-regulated with other checkpoint receptors, and the co-expression of TIM-3 with PD-1 is associated with a specific subset of particularly dysfunctional or “exhausted” T cells[117]. TIM-3+/PD-1+ cells appear to express significantly lower amounts of effector cytokines such as IFN-γ, TNF and IL-2[118]. Both checkpoint receptors are co-regulated by immunosuppressive cytokines such as IL-27, which finally results in a diminished immune response in cancer and chronic viral infections[118,163,164]. The expression of TIM-3 was associated with a poor overall survival and advanced tumor stages in several solid malignancies, including colorectal and non-small cell lung cancer[125]. In soft tissue and bone sarcomas, TIM-3 expression in tumor-infiltrating lymphocytes (TIL) did not significantly correlate with PFS or OS in previous studies[119,120].

TIM-3 inhibition has shown promising results in pre-clinical models and is currently being evaluated as a novel immunotherapeutic approach in several clinical trials[123,124,165,166]. Clinical trials often combine TIM-3 inhibitors with checkpoint inhibitors targeting PD-1, as pre-clinical models demonstrated a synergistic effect and a better restoration of T cell responses in CPI "co-blockades"[118,167,168]. Ongoing clinical trials include NCT03446040 combining an anti-TIM-3 antibody with Nivolumab, and NCT03744468 combining anti-TIM-3 antibodies with Tislelizumab. In the present study, we analyzed the tumor cell expression of TIM-3 in a large and well-characterized cohort of HR-STS patients with long-term follow-up. We correlated our findings with clinical tumor characteristics, tumor-infiltrating lymphocyte (TIL) counts, PD-1 and PD-L1 expression status, and survival data. Our study demonstrates a significant expression of TIM-3 in specific subsets of patients with HR-STS.

2. Materials and Methods

2.1. Patient Selection

An exploratory retrospective cohort study design was chosen to address the research question. Eligible patients had pathologically confirmed high-risk soft tissue sarcoma (Tumor diameter 5 cm or larger, French Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade 2 or 3, deep to the fascia). Clinical, pathological, and outcomes data were extracted from our clinical sarcoma database. Most patients were to receive a multimodal treatment including neoadjuvant doxorubicin-based chemotherapy and regional hyperthermia (RHT), surgery, adjuvant chemotherapy + RHT and radiotherapy in select cases. Treatment continued unless disease progression or unacceptable toxic effects occurred. Follow-up was performed until December 2022.

2.2. Histopathology and Tissue Microarray Construction

Tumor samples originated from tumor biopsies that were taken before the initiation of neoadjuvant treatment at the Ludwig Maximilians University hospitals, Munich. In addition to the original pathology reports, microscopic findings (tumor type according to current WHO classifications and degree of differentiation) were reassessed. For tissue microarray (TMA) assembly, representative tumor areas were marked on H&E-stained slides of formalin-fixed, paraffin-embedded tumor samples from all patients according to standard procedures, and two 0.6 mm punch biopsies were taken from each sample[151]. Normal tonsillar tissue samples were used as controls on the TMA. In the end, seven tissue microarrays containing 179 pre-treatment tumor samples from 179 patients with high-grade soft tissue sarcomas (HR-STS) were constructed.

2.3. TIM-3 Immunohistochemistry

For the immunohistochemical detection of TIM-3, commercially available and validated monoclonal antibodies were used (TIM-3 D5D5R, Cell Signaling Tech., Danvers, MA, USA). Antigen retrieval was carried out by heat treatment with Target Retrieval Solution Citrate (Agilent Technologies, Santa Clara, CA, USA). Staining was performed on a Ventana Benchmark XT Autostainer (Ventana Medical Systems, Tucson, AZ, USA) with a DAB+ Kit (Agilent Technologies, Santa Clara, CA, USA). All slides were counterstained with hematoxylin (Vector Laboratories, Burlingame, CA, USA). An ImmPRESS Anti-Rabbit IgG Polymer Kit was used for detection (Vector

Laboratories, Burlingame, CA, USA). To exclude unspecific staining, system controls were included. Tonsillar tissue served as a positive control for immunohistochemistry. The immunostaining of cells was evaluated and scored semi-quantitatively (0 = negative; 1 = ≥5% positive and weakly stained, 2 = ≥25% positive and moderately stained, 3 = ≥50% positive and strongly stained). All immunohistochemical and pathologic evaluations were carried out independently and blinded with an experienced sarcoma pathologist (T.K.). In the case of discrepancy, the slides were reevaluated under a multiheaded microscope and a consensus was reached.

2.4. TILs, PD-1 and PD-L1

Tumor-infiltrating lymphocytes (TILs), PD-1 and PD-L1 were previously investigated in our HR-STS cohort[147,169]. TILs were counted per high-power field (HPF) (400× magnification, field of view 0.237 mm^2) in H&E-stained TMA slides. As previously described, slides were pre-treated with heat and Target Retrieval solution (S1699, Agilent, Santa Clara, CA, USA) before incubation with the monoclonal primary anti-PD-1 mouse antibody (315M; 1:80; Cell Marque, Rocklin, CA, USA) for 60 min at room temperature. The Vectastain Elite ABC HRP Kit (Vector Laboratories, Burlingame, CA, USA) and the chromogen DAB+ (Agilent) were used for detection, and Hematoxylin (Vector Laboratories) for counterstaining. For PD-L1 staining, slides were pre-treated with heat and the Epitope Retrieval Solution pH8 Novocastra (Leica Biosystems, Wetzlar, Germany) before incubation with the monoclonal primary anti-PD-L1 rabbit antibody (E1L3N; 1:50; Cell Signaling Technology) for 60 min at room temperature. We used the SignalStain Boost IHC Detection Reagent (Cell Signalling Technology) and the chromogen DAB+ (Agilent) for detection according to previous studies[169].

2.5. Statistical Analysis

Categorical variables were tested for independence using the Chi square test. Binary variables were compared using Fisher's Exact Test, and continuous variables were compared using T-tests. Logistic regression was used for univariate and multivariate analyses. The forward stepwise procedure was set to a threshold of 0.05. Data analysis was performed using SAS 9.4 (SAS Inst Inc, Cary, NC). All *p*-values were based on a two-tailed hypothesis test, with values less than 0.05 considered statistically significant.

3. Results

3.1. Patient Cohort

In total, 179 patients treated between 1997 and 2019 were included in this study. The median age was 54 years (range, 18–79 years), and 87 (48.6%) patients were female. The most common histological subtypes were undifferentiated pleomorphic sarcomas (UPS) (33%), leiomyosarcomas (17%), and liposarcomas (22%). The clinicopathologic characteristics of the study cohort are summarized in Table 1.

Table 1: Patient characteristics.

Factor	**Strata**	***n***	**%**

Total		179	100
Sex	Male	92	51
	Female	87	49
Histological subtype	UPS	59	33
	Liposarcoma	40	22
	Leiomyosarcoma	31	17
	Synovial sarcoma	18	10
	MPNST	12	7
	Angiosarcoma	5	3
	Malignant SFT	2	1
	Dediff. chondrosarcoma	3	2
	Myxofibrosarcoma	5	3
	Other	4	2
Location	Extremities	71	40
	Non-Extremities	108	60
Size of primary tumor (cm)	50–79 mm	46	26
	80–120 mm	62	35
	>120 mm	71	40
Presence of metastases	No	167	93
	Yes	12	7
FNCLCC Grade	Intermediate (G2)	89	50
	High (G3)	90	50
TIM-3 expression (Grades 0–3)	0	78	44
	1	56	31
	2	37	21
	3	8	4
Follow-up status 5 years after initial diagnosis	Alive	108	60
	Dead	71	40
Distant recurrence within 5 years after R0/R1 resection	No distant recurrence	103	68
	Distant recurrence	49	32
Local recurrence within 5 years after R0/R1 resection	No local recurrence	91	60
	Local recurrence	61	40

UPS: Undifferentiated Pleomorphic sarcoma. SFT: Solitary fibrous tumor. MPNST: Malignant peripheral nerve sheath tumor. Other: 1 rhabdomyosarcoma, 1 alveolar soft part sarcoma, 1 carcinosarcoma, 1 extraosseous osteosarcoma.

3.2. TIM-3 Expression in High-Risk Soft Tissue Sarcomas (HR-STS)

TIM-3 expression was observed in 101 (56%) out of 179 pre-treatment biopsies of patients with HR-STS. Examples of immunohistochemistry staining for TIM-3 are shown in Figure 1. TIM-3 was

more often positive in male than female patients (64% vs. 48%, $p = 0.036$) and associated with older age (67% vs. 47%, $p = 0.010$). TIM-3 expression was more common in undifferentiated pleomorphic sarcomas (UPS) compared to other histological subtypes (75% vs. 47%, $p < 0.001$). There was no significant association between TIM-3 expression and FNCLCC grade ($p = 0.229$). A large proportion of patients received neoadjuvant anthracycline-based chemotherapy (80%), and nearly all patients underwent R0/R1 resection (n = 152, 89%) (Table 2).

Table 2: Correlation between TIM3 expression and clinicopathological parameters.

Factor	**Strata**	**Total**	**TIM-3 > 0**		***p*-Value**
		n	**n**	**%**	
All Patients		**179**	**101**	**56**	**--**
Sex	Male	92	59	64	**0.036**
	Female	87	42	48	
Age at initial diagnosis (years)	<55	92	43	47	**0.010**
	≥55	87	58	67	
Histological subtype	UPS	59	44	75	**<0.001**
	Liposarcoma	31	11	35	
	Leiomyosarcoma	40	26	65	
	Other	49	20	41	
Tumor Location	Extremities	71	47	66	**0.045**
	Non-extremities	108	54	50	
FNCLCC Grade	Intermediate (G2)	89	46	52	0.229
	High (G3)	90	55	61	
Surgical margins	R0	69	48	70	**0.011**
	R1	83	41	49	
	R2	14	4	29	
	No resection	13	8	62	
Chemotherapy	Yes	134	80	60	0.164
	No	45	21	47	
Radiotherapy	Yes	30	16	53	0.535
	No	106	48	45	
	Missing	43			
Regional Hyperthermia (RHT)	Yes	139	86	62	**0.007**
	No	40	15	38	
TIL counts (cells/50HPF)	0–5	108	46	43	**<0.001**
	≥6	70	54	77	
	Missing	1			
PD-1 expression	0	61	18	30	**<0.001**
	≥0	77	46	60	
	Missing	41			

PD-L1 expression	0	139	66	47	**<0.001**
	≥0	34	31	91	

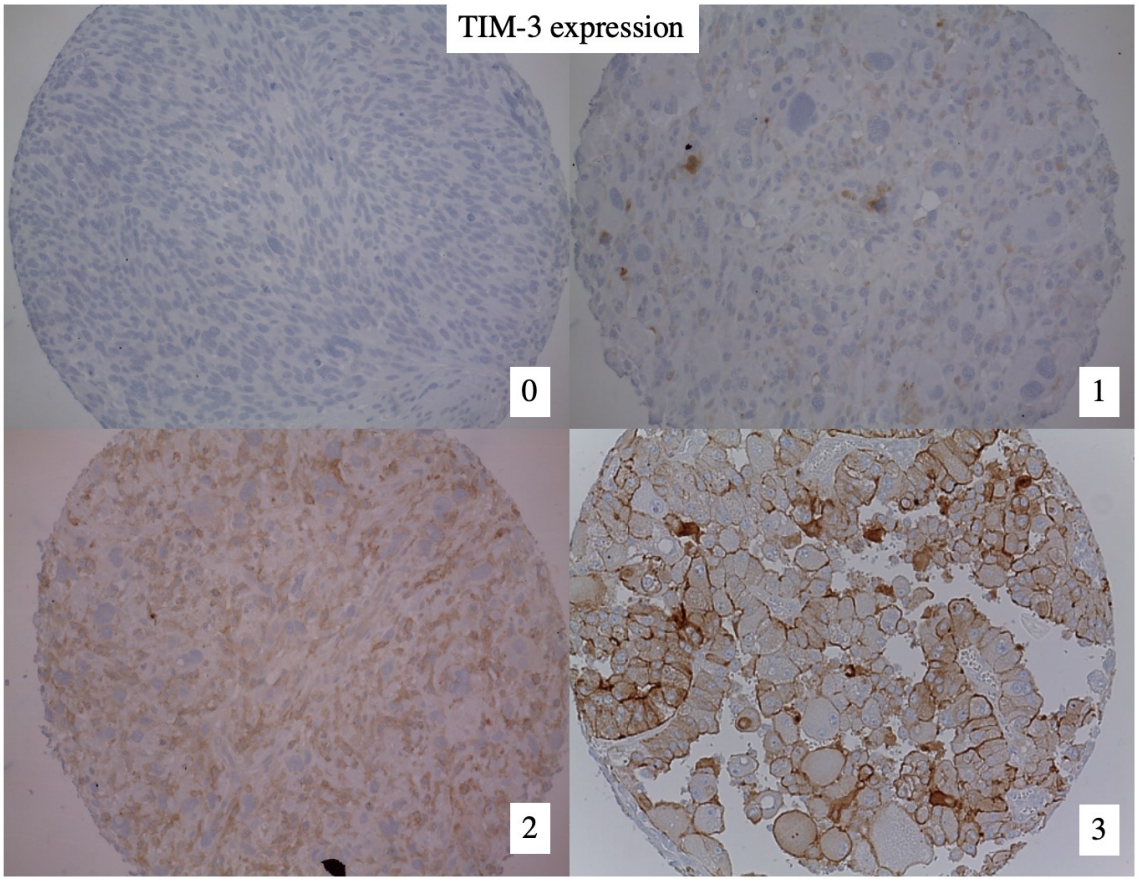

Figure 1: Stained tissue microarray (TMA) cores. Representative micrographs of cores on a TMA stained for TIM-3. Numbers represent semiquantitative scoring of immunostaining: 0, negative. 1–3, positive. Magnification, 20×.

3.3. TIM-3 Expression Is Associated with TILs, PD-1 and PD-L1 Expression Status

TIM-3 expression was associated with high tumor-infiltrating lymphocyte (TIL) counts (77% vs. 43%, $p < 0.001$), high positive PD-1 (60% vs. 30%, $p < 0.001$) and positive PD-L1 expression (91% vs. 47%, $p < 0.001$). We performed a logistic regression analysis of TIM-3 expression using an inclusion approach. Sex, age, increased TIL counts, PD-L1 expression and UPS histological subtype remained statistically significant predictors of TIM-3 expression (Table 3).

Table 3: Multiple logistic regression model of relevant clinicopathological parameters.

Factor	Strata	Significance	Hazard Ratio	95.0% CI
Sex	Male vs. Female	0.026	2.289	(1.106–4.737)
Age	<55 vs. ≥55	0.027	1.030	(1.003–1.056)
TIL counts	0–5 vs. ≥6	0.002	3.499	(1.565–7.823)

PD-L1 expression	0 vs. >0	0.001	9.173	(2.420–34.772)
Histology	UPS vs. other subtypes	0.038	2.316	(1.046–5.128)

3.4. TIM-3 Expression and Survival

The median follow-up duration was 119 months (95% CI 109–128 months). In total, 71 patients (40%) died within 5 years after diagnosis. Statistically significant risk factors for an unfavorable outcome in univariate survival analysis were positive surgical margins ($p < 0.001$), grade ($p = 0.015$), presence of distant metastases ($p < 0.001$) and chemotherapy ($p = 0.010$) (Table 4). Expression of TIM-3 was not associated with statistically significant changes in overall survival ($p = 0.339$) (Figure 2).

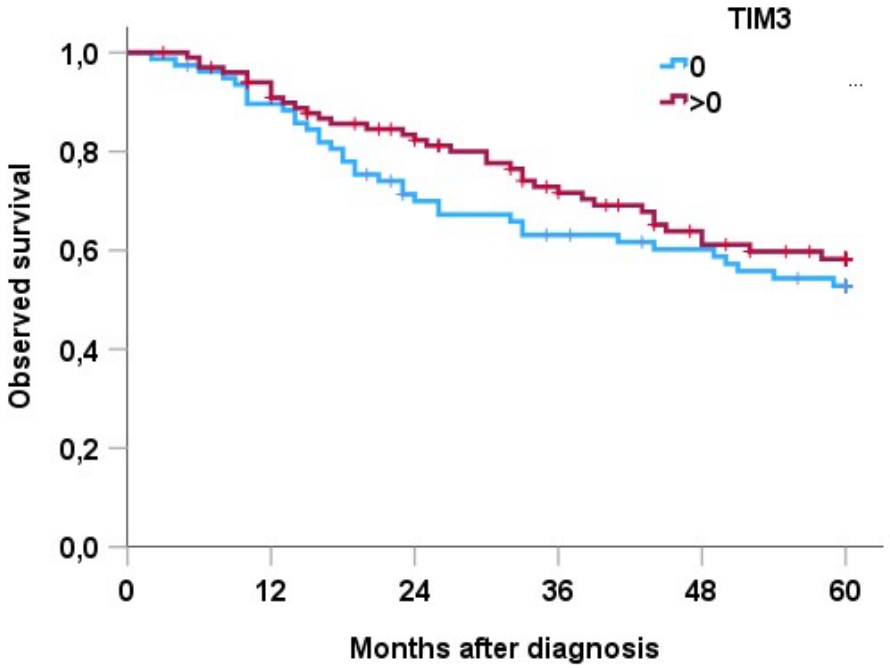

Figure 2. Overall survival according to TIM-3 expression.

Observed 5-year overall survival (OS) was not significantly influenced by TIM-3 expression in different histological subtypes (UPS ($p = 0.207$), leiomyosarcoma ($p = 0.660$), liposarcoma ($p = 0.767$), and other histological subtypes ($p = 0.681$)). All tested immune markers including TIM-3, PD-1, PD-L1 and tumor-infiltrating lymphocytes (TIL) did not have a statistically significant impact on 5-year OS in univariate analysis. In a multivariate Cox proportional hazards model, grade ($p = 0.014$), surgical margins ($p < 0.001$), and presence of distant metastases ($p = 0.003$) remained statistically significant independent predictors of 5-year OS. In conclusion, TIM-3 did not have a statistically significant prognostic impact on overall survival.

Table 4: Univariate and multivariate analysis of overall survival.

		Univariate		Multivariate	
Factor	**Strata**	**Sig.**	**Hazard Ratio**	**Sig.**	**Hazard Ratio**
Sex	Male vs. Female	0.366	0.806 (0.505–1.287)		
Age	1 year step	0.678	1.003 (0.987–1.020)		
Grade	G2 vs. G3	**0.015**	1.812 (1.122–2.926)	**0.014**	1.889 (1.139–3.133)
Surgical margins	R0/1 vs. R2	**<0.001**	7.310 (4.339–12.318)	**<0.001**	6.866 (3.815–12.357)
Distant metastases	M0 vs. M1	**<0.001**	4.187 (2.119–8.273)	**0.003**	3.059 (1.476–6.341)
PD-L1 expression	0 vs. >0	0.180	1.455 (0.840–2.520)	0.542	1.227 (0.636–2.364)
TIL counts	0–5 vs. ≥6	0.830	1.055 (0.649–1.713)	0.247	1.406 (0.790–2.502)
TIM3 expression	0 vs. >0	0.342	0.798 (0.501–1.271)	0.246	1.403 (0.792–2.483)
Histology	UPS vs. other	0.259	0.759 (0.470–1.226)		
Tumor location	Extremities vs. non-Extremities	0.285	1.302 (0.802–2.112)		
Chemotherapy	Yes vs. no	**0.010**	1.912 (1.168–3.129)	0.498	1.212 (0.695–2.114)
Radiotherapy	Yes vs. no	0.241	1.440 (0.783–2.647)		
Regional hyperthermia	Yes vs. no	0.749	1.091 (0.639–1.865)		
PD1 expression	0 vs > 0	0.106	1.521 (0.914–2.530)		
TIM-3 x PDL1	Both 0 vs. both >0	0.690	1.133 (0.613–2.095)		
TIL x TIM-3	TIL ≥ 6 and TIM-3 > 0 vs. TIL < 6 and TIM-3 = 0	0.599	0.848 (0.459–1.566)		
TIM-3 x PD1	Both 0 vs. both >0	0.323	1.372 (0.733–2.569)		

4. Discussion

To our knowledge, this is the first study to analyze the tumor cell expression of TIM-3, a novel immune checkpoint receptor and potential biomarker, in a well-characterized cohort of patients with HR-STS. TIM-3 expression was observed in 56% of patients. Our analysis indicates that patients with undifferentiated pleomorphic sarcomas (UPS), male gender, age ≥55 years and high expression of other immune markers (high TIL counts, positive PD-1 and PD-L1 expression) are more likely to demonstrate strong TIM-3 expression. These results remain significant in a logistic regression model, and indicate that specific subgroups of patients with HR-STS are more likely to express TIM-3.

We demonstrate the strong tumor cell expression of TIM-3 in undifferentiated pleomorphic sarcomas compared to other histological subtypes (75% vs. 47%, $p < 0.001$). UPS belong to non-translocation associated sarcomas and are associated with abundant immune infiltrates due to a higher mutational burden, higher neoantigen counts, and greater intratumoral heterogeneity compared to other entities[170]. Dancsok et al. described higher levels of PD-1, PD-L1 and TIM-3 expression on tumor-infiltrating lymphocytes in non-translocation-associated sarcomas including UPS[120]. In a study by Klaver et al., UPS had the highest fraction of PD-1+/LAG3+/TIM-3+/CD8+ T cell infiltrates, which was comparable to known “immune-dense” tumors such as malignant mel-

anoma[171]. These findings correlate with clinical studies on immune checkpoint inhibitors in sarcomas, where UPS generally were among the best responders[107,111,172]. The strong expression of TIM-3 in UPS tumor cells supports the notion of an immunogenic signature in both tumor cells and immune infiltrates in this entity.

Our results suggest differences in TIM-3 expression according to age and sex. Reitsema et al. have provided evidence that both age and sex modulate the expression of immune checkpoints by human T cells[173]. Interestingly, their results described a decline in PD-1 expression with age and female sex, while our results demonstrate a stronger expression of TIM-3 in male patients ≥55 years of age. Age-related differences in immune checkpoint expression have shown direct effects on the treatment efficacy in other tumors, including head and neck cancer or malignant melanoma[174,175]. In consequence, age- and sex-associated differences in TIM-3 expression should be considered as relevant clinical parameters in ongoing clinical trials.

In addition to TIM-3, 60% of patients demonstrated a significant co-expression of PD-1. The expression of PD-L1 in combination with TIM-3 was observed in 91% of patients. In pre-clinical models, the co-expression of TIM-3 and PD-1 was observed in strongly dysfunctional T cells[118,164,176]. In addition, Koyama et al. demonstrated that TIM-3 can be upregulated as a result of PD-1-directed therapy[167]. With regard to these results, studies in murine models of melanoma, colorectal cancer and AML have analyzed checkpoint co-blockades, and demonstrated greater T cell responses following TIM-3 and PD-1 co-blockades compared to PD-1 inhibition alone[168,177,178]. In metastatic sarcomas, D'Angelo et al. have demonstrated increased response rates in co-blockades with anti-PD-1 and anti-CTLA4 antibodies, while anti-CTLA4 antibodies did not prove effective[111]. Our results provide an additional rationale for checkpoint co-blockades in high-risk soft tissue sarcomas, and support current clinical trials on combinations of anti-TIM-3 and anti-PD-1 antibodies in solid tumors.

We were not able to demonstrate differences in overall survival (OS) in TIM-3+ vs. TIM-3- patients with high-risk soft tissue sarcomas ($p = 0.339$). These results are in line with previous studies on TIM-3 in soft tissue and bone sarcomas: Ligon et al. analyzed tumor-infiltrating lymphocytes in osteosarcoma pulmonary metastases and compared them with primary bone tumors. While PD-L1 and LAG3 significantly predicted progression-free survival (PFS), there was no correlation between TIM-3 status and survival[119]. In addition, Dancsok et al. were not able to correlate TIM-3 expression on tumor-infiltrating lymphocytes of soft tissue and bone sarcomas with OS or PFS[120]. In contrast, a meta-analysis conducted by Zhang et al. reported significantly shorter OS rates and advanced tumor stages in patients with positive TIM-3 expression in various solid tumors including colon cancer, gastric cancer, renal cell carcinoma and non-small cell lung cancer (NSCLC)[125]. Furthermore, Wang et al. associated TIM-3 expression with a shorter OS in esophageal squamous cell carcinoma[179]. It is currently not clear why there seems to be no significant association between survival and TIM-3 expression in high-risk soft tissue sarcomas. Possible reasons could be the large number of histological subtypes and typically small sample size in rare tumors.

Our results demonstrate TIM-3 expression in tumor cells of patients with high-risk soft tissue sarcomas. These findings indicate that tumors with low tumor-infiltrating lymphocyte (TIL) counts can still express TIM-3 and perhaps benefit from future TIM-3 targeting therapies. Currently, there are only limited data on TIM-3 expression in tumor cells: Wiener et al. demonstrated the expression of TIM-3 in melanoma cells, and Zhuang et al. were able to detect TIM-3 in non-small cell lung cancer (NSCLC)[121,122]. In their study, TIM-3 stained positive on tumor cells in 86.7% of patients

with primary NSCLC, and was associated with higher T classification and shorter OS. Interestingly, TIM-3 only stained positive in tumor cells and tumor-infiltrating lymphocytes, but not in normal (control) lung tissue, which adds to the current notion of TIM-3 playing an active role in carcinogenesis.

In addition to the typical limitations of a retrospective study design and immunohistochemical analyses, not all patients underwent the same treatment, which could have an impact on our survival analyses. In conclusion, new systemic therapy options are needed for high-risk soft tissue sarcomas. Immunotherapeutic approaches have become a cornerstone of modern oncology, with many drugs becoming approved for a variety of tumors. This study might help us to better select the patients with HR-STS who might express higher levels of TIM-3, and therefore be candidates for potential new clinical trials.

5. Conclusions

To date, checkpoint inhibitors have shown only limited efficacy in patients with high-risk soft tissue sarcomas (HR-STS). Selective TIM-3 blockade has demonstrated promising results in pre-clinical trials and acts as a potential immunotherapeutic target in combination with established checkpoint inhibitors in ongoing clinical trials. This is the first study to demonstrate a significant tumor cell expression of TIM-3 in specific subsets of patients with HR-STS. We were able to correlate TIM-3 expression with high levels of tumor-infiltrating lymphocytes and PD-1/PD-L1 expression. Our results promote the identification of potential candidates for immunotherapy in HR-STS to expand therapeutic options and move on from the current “one-size-fits-all” paradigm in the therapy of advanced HR-STS.

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