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Metronomische Chemotherapie bei metastasiertem Mammakarzinom

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Abkürzungsverzeichnis

BC	breast cancer – Mammakarzinom
bFGF	basic fibroblast growth factor
BRCA	breast cancer gene
CAPE	capecitabine – Capecitabin
CBR	clinical benefit rate – klinische Benefit-Rate
CCT	conventional chemotherapy – konventionelle Chemotherapie
CDK	cyclin-dependent kinase – Cyclin-abhängige Kinase
CI	confidence interval – Konfidenzintervall
CR	complete remission – komplette Remission
CSC	cancer stem cell – Krebsstammzelle
CTLA	cytotoxic T-lymphocyte-associated protein
CTX	cyclophosphamide – Cyclophosphamid
DCR	disease control rate – Krankheitskontrollrate
DoR	duration of response – Dauer des Ansprechens
ECOG	Eastern Cooperative Oncology Group
EORTC	European Organisation for Research and Treatment of Cancer
FACT-B	Fuctional Assessment of Cancer Therapy Breast
HADS-D	Hospital Anxiety and Depression Scale (deutsche Version)
HER2	humaner epidermaler Wachstumsfaktor-Rezeptor 2
HIF-1α	Hypoxie-induzierbarer Faktor 1α
HR	Hormonrezeptor
HRQoL	Health-Related Quality of Life – gesundheitsbezogene Lebensqualität
MBC	metastatic breast cancer – fortgeschrittenes/metastasiertes Mammakarzinom
MCT	metronomic chemotherapy – metronomische Chemotherapie
MDSC	Myeloid-derived suppressor cell – myeloide Suppressorzelle
MTD	maximum tolerated dose – maximal tolerable Dosis
mTOR	Mammalian Target of Rapamycin
MTX	methotrexate – Methotrexat
ORR	overall response rate - Gesamtansprechrate
OS	overall survival – Gesamtüberleben
PARP	Poly-ADP-Ribose-Polymerase
PD-L1	programmed death-ligand 1

PDGF	platelet-derived growth factor
PFS	progression-free survival – progressionsfreies Überleben
PI3K	Phosphatidylinositol-3-Kinase
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PR	partial remission – partielle Remission
SD	stable disease – stabile Erkrankung
SDF-1	stromal cell-derived factor-1
TNBC	triple-negative breast cancer – triple-negatives Mammakarzinom
Treg	regulatorische T-Zelle
TSP-1	Thrombospondin 1
TTP	time to progression – Zeit bis zum Progress
VEGF	vascular endothelial growth factor
VRL	vinorelbine – Vinorelbin

Metastasiertes Mammakarzinom

Mit einer Inzidenz von 2,3 Millionen und einer Mortalität von 0,7 Millionen Fällen pro Jahr ist das Mammakarzinom (BC) die am häufigsten diagnostizierte Krebserkrankung und die fünfthäufigste Ursache für Krebssterblichkeit bei Frauen weltweit [1]. BC ist für einen von vier Karzinomfällen und einen von sechs Karzinomtodesfällen bei Frauen verantwortlich [1]. Die Sterblichkeitsraten sind in den meisten Industrieländern zurückgegangen und stehen in engem Zusammenhang mit dem BC-Subtyp, der Ethnie und der verfügbaren onkologischen Versorgung [1-3]. Das fortgeschrittene/metastasierte Mammakarzinom (MBC) ist eine behandelbare, aber im Allgemeinen eine unheilbare Erkrankung mit einem medianen Gesamtüberleben (OS) von 2 bis 3 Jahren und einer 5-Jahres-Überlebensrate von etwa 25 % [2, 4, 5]. Das Ziel der Behandlung ist es, eine langfristige Krankheitskontrolle bei einer angemessenen gesundheitsbezogenen Lebensqualität (Health-Related Quality of Life, HRQoL) zu erreichen und so eine Krankheitschronifizierung mit Behandlungsoptionen zu etablieren, die einen optimalen therapeutischen Index bieten [6]. In den letzten Jahren haben die Therapieoptionen für MBC stetig zugenommen. Bei der Wahl der Behandlung sollten mehrere Faktoren berücksichtigt werden. Neben der Bestimmung des Hormonrezeptor (HR)- und des humanen epidermalen Wachstumsfaktor-Rezeptor 2 (HER2)-Status einer metastasierten Läsion sollten auch andere Faktoren, die im Zusammenhang mit einer möglichen Behandlung mit zielgerichteten Substanzen stehen, ermittelt werden. Der breast cancer gene (BRCA)-Keimbahnstatus und der phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)-Mutationsstatus bei HR-positivem sowie der programmed death-ligand 1 (PD-L1)-Status bei triple-negativem Mammakarzinom (TNBC) können für die Behandlungsempfehlung relevant sein. Biologisches Alter, Menopausenstatus, Tumorlast, Komorbiditäten und frühere Therapien mit deren Toxizitäten sind ebenfalls maßgeblich bei der Entscheidungsfindung [2, 4, 7, 8].

Metronomische Chemotherapie

Die metronomische Chemotherapie (MCT), die eine kontinuierliche Verabreichung konventioneller Chemotherapeutika in niedriger Dosierung beschreibt, wurde erstmals Anfang 2000 von Hanahan und Kerbel erwähnt [9, 10]. Seitdem haben zahlreiche Studien mit MCT vielversprechende Ergebnisse gezeigt. Die meisten Daten stammen aus retrospektiven Studien und Phase-II-Studien, wobei BC die am besten untersuchte

Tumorentität darstellt [11]. Aufgrund der nachgewiesenen Wirksamkeit und der guten Verträglichkeit wird die MCT zunehmend als mögliche Behandlungsoption für verschiede Malignome wahrgenommen [2, 4, 12, 13].

In Abgrenzung zur MCT basiert das Konzept der konventionellen Chemotherapie (CCT) auf der maximal tolerablen Dosis (MTD), die in 2- bis 4-wöchigen Abständen verabreicht wird. Das Ziel dieses Therapieschemas ist es, schnell teilende Zellen anzugreifen und so viele maligne Zellen wie möglich abzutöten [9]. Darüber hinaus werden jedoch auch benigne schnell proliferierende Zellen von diesem Therapieschema betroffen. Daher kommen unerwünschte Ereignisse wie Mukositis, Myelosuppression und Alopezie häufig vor und stellen klinisch relevante Folgeerscheinungen mit einer Verschlechterung der HRQoL und Therapielimitierung dar [14]. Insbesondere beim fortgeschrittenen Krebsleiden ist die Krankheitschronifizierung mit einer Verlängerung des progressionsfreien Überlebens (PFS) und OS bei gleichzeitiger Erhaltung der HRQoL das wichtigste Ziel der Behandlung. In diesem Zusammenhang scheint MCT aufgrund ihrer Eigenschaften eine sehr gut praktikable Therapieoption darzustellen. Die orale Verabreichung von MCT ist sicher, einfach zu handhaben und ermöglicht eine flexible Medikamentendosierung [15, 16]. Dosisanhäufungen, die mit nicht tolerierbaren Nebenwirkungen einhergehen, sind selten, sodass die Medikamente über längere Zeiträume verabreicht werden können [17, 18]. Darüber hinaus sind die Wirkmechanismen nicht primär zytotoxisch, sondern eher indirekt und multimodal, insbesondere durch Hemmung der Angiogenese, Immunmodulation und Auswirkungen auf das Tumorstroma [19-22]. Demzufolge sind die typischen zytotoxischen Nebenwirkungen unter MCT deutlich seltener im Vergleich zur CCT [17, 23-26]. Schließlich wird davon ausgegangen, dass MCT nicht einfach nur eine andere Verabreichungsart der CCT ist, sondern eine völlig andere Behandlungsoption darstellt [16, 20, 27-29].

Wirkmechanismen der metronomischen Chemotherapie

Im Vergleich zur CCT, die auf der Zytotoxizität der MTD beruht, sind die zytotoxischen Effekte der MCT, die in viel niedrigeren Dosen verabreicht wird, zwar nachweisbar, aber nicht entscheidend für die antitumorale Antwort [20, 30]. Die Hauptwirkmechanismen von MCT sind sowohl direkte als auch indirekte Effekte auf

Tumorzellen und insbesondere deren Mikroumgebung durch Antiangiogenese und Stimulation der Immunantwort [9, 19, 20, 27, 31].

In der Annahme, dass Zytostatika antiangiogene Eigenschaften aufweisen, verglichen Browder et al. die Wirkung von Cyclophosphamid (CTX) in einer konventionellen und einer kontinuierlichen niedrigen Dosierung. Sie zeigten, dass kontinuierliche niedrig dosierte Verabreichung von CTX bei resistenten BC-Zellen wirksamer war als CTX in einer Standarddosierung. Inzwischen ist bekannt, dass eine niedrig dosierte Chemotherapie die Vermehrung und Zirkulation von Tumorendothelzellen und endothelialen Vorläuferzellen hemmt. Außerdem reduziert sie die Differenzierung unreifer Endothelzellen und moduliert pro- und antiangiogene Moleküle [12, 22, 32, 33]. Es wurde gezeigt, dass die Synthese von Thrombospondin 1 (TSP-1), einem Glykoprotein, das die Angiogenese hemmt, nach der Behandlung mit niedrig dosiertem CTX in Endothelzellen und Plasma eines Mausmodells erhöht war [34]. MCT führt auch zu einer erhöhten Expression anderer antiangiogener Faktoren, wie Vasohibin und Endostatin [22, 35, 36]. Außerdem hemmt MCT die Expression von proangiogenen Faktoren, wie dem vaskulären endothelialen Wachstumsfaktor (VEGF), dem Rezeptor für VEGF 2 (VEGFR)-2, dem aus Thrombozyten gewonnenen Wachstumsfaktor (PDGF), dem basischen Fibroblasten-Wachstumsfaktor (bFGF) und dem aus Stromazellen gewonnenen Faktor 1 (SDF-1) [37-39]. Neben diesen Faktoren wurde gezeigt, dass der Hypoxie-induzierbare Faktor (HIF)-1α, ein Transkriptionsfaktor, der die Mikroumgebung des Tumors reguliert und die Expression von VEGF und VEGFR stimuliert, nach der Verabreichung von MCT verringert wird [40].

Es gibt Hinweise darauf, dass MCT einen positiven Einfluss auf die antitumorale Immunantwort ausübt. Insbesondere die Hemmung von regulatorischen T-Zellen (Tregs), myeloiden Suppressorzellen (MDSCs) und die Stimulierung von dendritischen Zellen könnten zu den positiven Effekten der niedrig dosierten MCT beitragen [12, 41, 42]. Darüber hinaus scheint die MCT die Krebsstammzellen (CSCs) anzugreifen, das einen günstigen Wirkmechanismus von MCT im Gegensatz zu CCT darstellt. Die CSCs spielen eine wichtige Rolle bei Rezidivbildung und Metastasierung, unter anderem weil sie Resistenzen gegenüber der CCT aufweisen und sich regenerieren können [12, 43, 44]. Während die antitumorale Antwort der MCT verzögert eintreten

kann, scheint die Wirkung aufgrund der verminderten Selektion von resistenten Tumorzellklonen mit einem geringeren Rezidivrisiko jedoch nachhaltiger zu sein [30, 45].

Metronomische Chemotherapie bei metastasiertem Mammakarzinom

Trotz der Vielzahl potenzieller Behandlungsoptionen für MBC besteht nach wie vor ein hoher medizinischer Bedarf an neuen Behandlungskonzepten, die eine ausreichende und langfristige antitumorale Wirkung bei einem überschaubaren Toxizitätsprofil bieten. In der letzten Dekade wurden viele neue Substanzen, wie zum Beispiel Cyclin-abhängige Kinase (CDK) 4/6-Inhibitoren, Poly-ADP-Ribose-Polymerase (PARP)-Inhibitoren, Immuncheckpoint-Inhibitoren und Antikörper-Wirkstoff-Konjugate, für die Behandlung von MBC etabliert [6, 46-52].

Nach den derzeitigen Erkenntnissen sollte bei HR-positivem, HER2-negativem MBC ohne Vorliegen einer ausgeprägten viszeralen Beteiligung, die sogenannte viszerale Krise, eine endokrin-basierte Therapie in Kombination mit CDK 4/6-Inhibitoren als erste Wahl durchgeführt werden. Im Falle einer endokrinen Resistenz empfehlen die Leitlinien eine Monochemotherapie als bevorzugte Wahl bei MBC. Eine Polychemotherapie sollte Patient:innen mit einem raschen klinischen Fortschreiten, lebensbedrohlichen viszeralen Metastasen und/oder bei der Notwendigkeit einer raschen Symptom-/Krankheitskontrolle vorbehalten sein [2, 8].

Es ist von großem Interesse, neue Therapieoptionen zu entwickeln, die den Zeitraum zwischen dem endokrinen Versagen und CCT verlängern, wobei letztere mit potenziell schwerwiegenden Nebenwirkungen und einer Beeinträchtigung der HRQoL verbunden ist [6, 7]. In Anbetracht der Tatsache, dass das therapeutische Ziel bei MBC einerseits die Krankheitskontrolle und andererseits die Aufrechterhaltung der HRQoL ist, stellt die MCT eine wertvolle Therapieoption dar. Sie kann insbesondere asymptomatischen Patient:innen ohne einen hohen Remissionsdruck angeboten werden [2, 15, 53].

Verfügbare Substanzen und ihre Wirksamkeit

Die meisten Daten zur MCT beim MBC stammen aus retrospektiven Studien und Phase-II-Studien [11, 14, 16]. Die am häufigsten verwendeten Wirkstoffe sind oral

verfügbare Zytostatika wie CTX, Methotrexat (MTX), Capecitabin (CAPE) und Vinorelbin (VRL), wobei häufig eine Kombination von Wirkstoffen eingesetzt wird. Die Wirksamkeit der MCT wird in vielen Studien durch die Bestimmung der Krankheitskontrollrate (disease control rate, DCR) oder der klinischen Benefit-Rate (clinical benefit rate, CBR) nach 24 Wochen Behandlung definiert. Die DCR/CBR umfasst eine komplette Remission (CR), partielle Remission (PR) und eine stabile Erkrankung (SD). In der größten multizentrischen retrospektiven Studie VICTOR-6, die Daten von 597 MBC-Patientinnen mit MCT gesammelt hat, erhielten die meisten Patientinnen (79,3 %) eine Monotherapie (VRL 34,6 %, CAPE 22,3 % und CTX 20,7 %). Die Verwendung von VRL- und CTX-basierten Schemata nahm während des Beobachtungszeitraums zu (2011: 16,8 % und 17,1 %, 2016: 29,8 % und 25,6 %) [26]. Im deutschsprachigen Raum gehört die Kombination von CTX und MTX zu den am häufigsten verwendeten metronomischen Therapieregimes [8]. Dies konnte auch in unserer Real-World-Analyse **(I)** beoabachtet werden. Für diese retrospektive Analyse wurden MBC-Patientinnen ausgewählt, die zwischen Februar 2009 und Dezember 2021 in der Klinik und Poliklinik für Geburtshilfe und Frauengesundheit der Universitätsmedizin Mainz eine MCT in Form von oralem

CTX 50 mg einmal täglich und MTX 2,5 mg jeden zweiten Tag,
CTX 50 mg täglich und CAPE 500 mg dreimal täglich,
CTX 50 mg einmal täglich alleine oder
VRL 30 mg einmal täglich alleine

für mindestens vier Wochen erhalten hatten. Patientinnen mit HER2-positiven Tumoren und Patientinnen, bei denen eine weitere Krebserkrankung vorlag, wurden ausgeschlossen. Patientinnen, die bis zum Stichtag im März 2022 nicht gestorben waren oder deren Krankheit nicht fortgeschritten war, wurden zensiert.

Insgesamt wurden 72 Patientinnen ausgewertet. CTX/MTX war mit dem Anteil von 86,1 % aller Patientinnen das häufigste metronomische Therapieschema, gefolgt von VRL bei 6,9 %, CTX/CAPE bei 4,2 % und CTX bei 2,8 % der Patientinnen. In unserer Kohorte wurde keine Begleitbehandlung, wie Strahlentherapie, endokrine oder zielgerichtete Therapie, durchgeführt. Der Großteil der Patientinnen (86,1 %) wies vor Beginn der MCT einen Krankheitsprogress unter der vorherigen Therapie auf. In Bezug auf DCR ≥ 24 Wochen Behandlung, PFS und OS konnten wir keine signifikanten Unterschiede zwischen den verschiedenen metronomischen Schemata

feststellen [54]. In einer Meta-Analyse von Liu et al. mit insgesamt 1360 Patient:innen zeigte sich in den Subgruppenanalysen ebenfalls kein signifikanter Unterschied in der CBR zwischen den verschiedenen metronomischen Wirkstoffen sowie zwischen der metronomischen Monochemotherapie und den Kombinationsschemata [55].

Die übliche Verabreichung von MTX in der CTX/MTX-Kombination ist 2,5 mg zweimal täglich am Tag 1 und 4/Woche [18, 23, 38, 56, 57]. In allen unseren Analysen **(I-III)** wurde MTX in einer Dosis von 2,5 mg jeden zweiten Tag kontinuierlich verabreicht, um die Dosierung möglichst zu vereinfachen und die Compliance der Patientinnen zu verbessern. Im Vergleich zur MTX-Verabreichung aus älteren Studien konnten wir vergleichbare Ergebnisse hinsichtlich der Wirksamkeit und des Nebenwirkungsprofils feststellen [54, 58, 59].

VRL weist eine gute Wirksamkeit und ein günstiges Sicherheitsprofil auf und zählt damit zu den Standardtherapieoptionen bei der Behandlung des MBC [60, 61]. Die orale Verabreichung von VRL zeigt gegenüber der intravenösen Gabe eine vergleichbare Wirksamkeit und bietet gleichzeitig die zusätzlichen Vorteile einer oralen Behandlung [62-65]. Die meisten Studien zur MCT mit VRL in Kombination mit anderen metronomischen Substanzen wie CTX und CAPE beziehen sich auf die Gabe von 30-50 mg VRL am Tag 1, 3 und 5/Woche [66-70]. Bei der VRL-Monotherapie werden verschiedene Dosierungen verwendet, wie zum Beispiel VRL 30 mg jeden zweiten Tag in der Studie von De Iuliis et al. [71] oder VRL 70 mg/m^2 am Tag 1, 3 und 5/Woche für drei Wochen gefolgt von einer Woche Pause in den Studien von Addeo et al. [72] und Liu et al. [17]. Bei Patient:innen mit einem nicht-kleinzelligen Lungenkarzinom war die tägliche Gabe von VRL bis zu 40 mg gut verträglich [73, 74]. In unserer prospektiven Phase-II-Studie VinoMetro **(IV)** wurde zum ersten Mal ein metronomisches Schema mit einer täglichen oralen VRL-Gabe von 30 mg bei Patientinnen mit einem HR-positiven, HER2-negativen MBC nach endokriner Resistenz untersucht [75]. VinoMetro war eine multizentrische, offene, einarmige Phase-II-Studie, die von der Universitätsmedizin Mainz gefördert wurde und Patientinnen in zwei Zentren zwischen Januar 2017 und April 2019 eingeschlossen hat. Die VinoMetro-Studie bestätigte die Ergebnisse früherer Studien zum metronomischen VRL nicht, wobei die Studie nach Einschluss von neun Patientinnen aufgrund einer Toxizität Grad 5 frühzeitig beendet wurde [17, 71, 72].

Die verfügbaren Studien zu MCT umfassen oft kleine, heterogene Patientenpopulationen mit unterschiedlichen Wirkstoffen und unterschiedlichen Dosierungen. Darüber hinaus werden die metronomischen Wirkstoffe häufig miteinander und mit anderen Substanzen kombiniert. Aufgrund dieser Tatsachen lässt es sich nicht sagen, welcher Wirkstoff oder welche Wirkstoffkombination am besten für den Einsatz der MCT geeignet ist. Daher werden derzeit alle vier oral verfügbaren Substanzen (CTX, MTX, CAPE und VRL) als Option für eine metronomische Behandlung empfohlen, wobei bisher nicht klar ist, welche Substanz bzw. welche Kombination die höchste Effektivität aufweist und welches Patientenkollektiv am meisten von der Therapie profitieren kann [2, 4, 28].

Zur Beurteilung der Wirksamkeit der metronomischen Kombination CTX/MTX wurden Patientinnen mit MBC, die die orale Kombination von CTX 50 mg einmal täglich und MTX 2,5 mg jeden zweiten Tag in der Klinik und Poliklinik für Geburtshilfe und Frauengesundheit der Universitätsmedizin Mainz zwischen 2009 und 2015 erhalten haben, retrospektiv beurteilt **(II)**. Die Wirksamkeit der MCT wurde mittels DCR ≥ 24 Wochen Behandlung, PFS und Dauer des Ansprechens (duration of response, DoR), definiert als die Zeit von der Dokumentation des Tumoransprechens bis zum Krankheitsprogress, bestimmt.

Insgesamt wurden in der Kohortenanalyse mit der CTX/MTX-Kombination (**II**) 35 Patientinnen ausgewertet. Das mediane Alter betrug 64 (35-83) Jahre und die mediane Anzahl der Chemotherapielinien einschließlich MCT war 2 (1-8). Im Median zeigten die Patientinnen 2 (1-4) verschiedene Metastasenlokalisationen; die häufigsten Lokalisationen waren Knochen (20 Patientinnen), Leber (17 Patientinnen) und Lunge (13 Patientinnen). 24 Patientinnen wiesen eine HR-positive und 11 Patientinnen eine triple-negative Erkrankung auf. Die DCR ≥ 24 Wochen wurde bei 11 (31,4 %) Patientinnen erreicht. Eine (2,9 %), sechs (17,1 %) und vier (11,4 %) Patientinnen erreichten CR, PR und SD. Das mediane PFS betrug 12 (6-86) Wochen. 15 (43 %) Patientinnen zeigten ein Therapieansprechen. Die mediane DoR lag bei 22 (8-74) Wochen [58]. Ähnliche Ergebnisse lieferte auch unsere Real-World-Analyse **(I)** mit einer DCR ≥ 24 Wochen von 31,9 % sowie frühere Studien zur CTX/MTX-Kombinationsbehandlung (DCR ≥ 24 Wochen von 31,2-36,6 %) [18, 23, 54, 76]. In

unserer Analyse **(I)**, in die 86,1 % der Patientinnen nach einem Krankeitsprogress und 6,9 % der Patientinnen aufgrund von unerwünschten Nebenwirkungen unter der vorherigen Therapie eingeschlossen wurden, war CTX/MTX das häufigste metronomische Regime mit einer DCR ≥ 24 Wochen von 30,6 % [54]. Die Ergebnisse der CTX/MTX-Kombinationsbehandlung stimmen mit der DCR ≥ 24 Wochen von 31,2 % in der Studie von Lu et al. überein, wobei 40,3 % der Patientinnen CTX/MTX als Erhaltungstherapie erhielten, ohne dass die Krankheit unter der vorangegangenen Therapie fortgeschritten war [76].

Es gibt jedoch auch Daten mit einer deutlich höheren DCR (74,4-81,0 %) [17, 26, 69, 77]. In der größten retrospektiven Studie VICTOR-6 mit einer DCR von 74,4 % wurde aber nicht erwähnt, ob die DCR erst nach 24 Wochen Behandlung oder sogar früher berechnet wurde [26]. In der retrospektiven Studie von Liu et al. erreichten 78,9 % der Patient:innen eine DCR. Auch in dieser Studie wurde der Zeitpunkt der Bestimmung der DCR nicht genau angegeben. Außerdem hatten nur 28,9 % der Patient:innen einen Krankheitsprogress unter der vorangegangenen Therapie. Der Großteil der Patient:innen hatte vor Beginn der MCT eine PR (50 %) oder SD (15,6 %) [17].

Neben der Tatsache, dass in Studien teilweise auch Patient:innen ausgewertet wurden, die die MCT nicht nur nach Krankheitsprogress sondern auch als Erhaltungstherapie erhielten, sind die Patientenpopulationen häufig heterogen mit unterschiedlichen Vorbehandlungen, einem unterschiedlichen Metastasierungs-ausmaß (viszeral versus nicht-viszeral) und einem unterschiedlichen HR- und HER2-Status. Das kann zur Fehlinterpretation der Ergebnisse führen und erschwert den Vergleich der einzelnen Studien untereinander.

In unserer Real-World-Analyse **(I)** mit insgesamt 72 Patientinnen, die mit 86,1 % der Fälle die CTX/MTX-Kombination erhalten hatten, wurde ein medianes PFS von 17,0 Wochen [95 % Konfidenzintervall (CI) 14,5-19,5] beobachtet [54]. Die metronomische Kombination von CTX und MTX zeigte auch in der Studie von Lu et al. ein vergleichbares PFS von 4,0 Monaten (95 % CI 3,6-4,7) [76]. In unserer Kohortenstudie **(II)** mit Patientinnen, die nur CTX/MTX erhalten haben, war das mediane PFS 12 (6-86) Wochen [58]. Weitere retrospektive Studien zu CTX/MTX-Kombination zeigten ebenfalls ähnliche Ergebnisse mit einer medianen Zeit bis zum Progress (time to

progression, TTP) von 4,2 (2-11) Monaten bzw. einem medianen PFS von 2,6 (0,2-28,9) Monaten [56, 57]. Weadick et al. zeigten mit der Kombination von CTX 50 mg einmal täglich und CAPE 500 mg dreimal täglich eine durchschnittliche Zeit unter Therapie von 9,1 (0,4-67,2) Monaten [25]. CAPE als Monotherapie in einer Dosierung von 1500 mg einmal täglich zeigte eine mediane TTP von 7 (2-36) Monaten [78]. In der Studie VICTOR-2 wurde die metronomische Kombination von CAPE 500 mg 3x/Tag und VRL 40 mg 3x/Woche untersucht. Das mediane PFS betrug 6,7 Monate (95 % CI 4,7-11,3) in der Erstlinienbehandlung und 7,2 Monate (95 % CI 2,8-11,5) in der ≥ Zweitlinienbehandlung [67]. In einer weiteren randomisierten Phase-II-Studie von Brems-Eskildsen et al. wurde die Standard-CAPE-Behandlung mit metronomischem VRL 50 mg 3x/Woche mit Standard-CAPE/VRL-Behandlung verglichen. Die metronomische Verabreichung von VRL führte zu einem medianen PFS von 6,3 Monaten (95 % CI 4,1-8,5) und zeigte keinen signifikanten Unterschied in der Wirksamkeit im Vergleich zur Standardbehandlung [66]. Die Kombination von CTX 50 mg einmal täglich, CAPE 500 mg 3x/Tag und VRL 30-40 mg 3x/Woche zeigte bei Patientinnen mit HR-positivem MBC eine mediane TTP von 25,1 Monaten (95 % CI 14,2-39,1) in der Kohorte ohne Vorbehandlung und eine mediane TTP von 11,2 Monaten (95 % CI 9,2-17,0) in der Kohorte mit Vorbehandlung [69].

Das Behandlungsziel im metastasierten Stadium der Erkrankung ist es, das Überleben unter Erhalt der HRQoL zu verlängern. Unsere metronomisch behandelte Kohorte zeigte in der Real-World-Analyse **(I)** ein medianes OS von 58,0 Wochen (95 % CI 29,0-87,0). Ähnlich wie beim PFS konnte kein signifikanter Unterschied hinsichtlich OS zwischen den vier verschiedenen metronomischen Therapieschemata dokumentiert werden (p=0,127), wobei die CTX/MTX-Kombination mit Abstand am häufigsten verwendet wurde (86,1 % der Patientinnen) und die Fallzahl in anderen Therapiegruppen relativ niedrig war [54]. In der chinesischen, mit der CTX/MTX-Kombination behandelten Bevölkerung, berichtete Lu et al. ein medianes OS von 26,8 Monaten (95 % CI 20,9-37,7). 59,7 % der Patientinnen wiesen vor Beginn der MCT einen Krankheitsprogress auf [76]. Im Gegesatz dazu hatten 86,1 % unserer Patientinnen eine progrediente Erkrankung unter der vorangegangenen Therapie. In der Studie von Miscoria et al. mit 62 Patient:innen war das mediane OS 7,1 Monate (0,2-38,3) [57]. Die Autoren beobachteten einen signifikanten Unterschied im medianen OS durch eine Taxanvorbehandlung. Die Patient:innen unter CTX/MTX-

Kombination mit Taxanvorbehandlung wiesen ein signifikant kürzeres medianes OS von 9,4 Monaten auf im Vergleich zu Patient:innen ohne Taxanvorbehandlung (20,0 Monate) (p=0,04). In der retrospektiven Studie von Gebbia et al. war das mediane OS 14 (4,5-26+) Monate in der CTX/MTX-Kohorte (39 Patient:innen) und 12,8 (4,5-18) Monate in der CTX-Kohorte (22 Patient:innen) ohne einen signifikanten Unterschied [56]. In der Studie VICTOR-6 zeigte die metronomische CTX-Monotherapie ein vergleichbares medianes OS von 14,2 Monaten (95 % CI 9,9-N/A). Die CAPE-Monotherapie, VRL-Monotherapie und VRL-Kombinationstherapie zeigten jeweils ein medianes OS von 28,8 Monaten (95 % CI 23,1-37,0), 22,7 Monaten (95 % CI 13,0-43,5) und 30,0 Monaten (95 % CI 26,2-34,7) [26]. Die randomisierte Studie von Brems-Eskildsen et al. zeigte bei 58 Patientinnen unter der Kombination von metronomischem VRL 50 mg 3x/Woche und Standard-CAPE ein ähnliches medianes OS von 22,3 Monaten (95 % CI 14,3-30,3) [66]. Liu et al. konnte in einer retrospektiven Analyse zeigen, dass die vorangegangene Therapie die Wirksamkeit von metronomischem VRL 70 mg/m^2 3x/Woche beeinflussen kann. Patient:innen, die vor Beginn der MCT CR oder PR aufwiesen, hatten ein medianes OS von 69,4 Monaten und Patient:innen mit SD ein medianes OS von 53,4 Monaten. Im Gegensatz dazu war das mediane OS 41,5 Monate bei Patient:innen, die vor Beginn der MCT einen Krankheitsprogress aufwiesen (p=0,135). Die Daten zur medianen Zeit bis zum Therapieversagen zeigten sogar einen signifikanten Unterschied in Bezug auf die Vorbehandlung (12,5 Monate in der CR/PR-Gruppe, 15,1 Monate in der SD-Gruppe und 6,5 Monate in der Gruppe nach Krankheitsprogress, p=0,015) [17]. In unserer Real-World-Analyse **(I)** wurde die Wirksamkeit der MCT mit der Vor- und Nachbehandlung verglichen. Hinsichtlich der DCR ≥ 24 Wochen und des medianen PFS war die MCT vergleichbar mit der Vorbehandlung und signifikant besser als die Nachbehandlung [54]. Inwieweit die Wirksamkeit der MCT das OS verbessern könnte, bleibt aufgrund mehrerer Einschränkungen spekulativ. Diese einarmige retrospektive Studie wurde konzipiert, um die Erfahrungen einer einzelnen Einrichtung zu beschreiben. Ein studienübergreifender Vergleich mit historischen Kohorten von MBC ist aufgrund der Heterogenität der Kohorte, die sowohl Patientinnen in der Erstlinienbehandlung als auch stark vorbehandelte Patientinnen umfasste, die mit verschiedenen Wirkstoffen behandelt wurden, schwierig.

Wirksamkeit im Vergleich zur konventionellen Chemotherapie

Es gibt nur wenige Studien, in denen die MCT mit den derzeitigen Standardtherapien verglichen wurde [66, 79]. Dies schränkt die Möglichkeit ein, die MCT im Behandlungsalgorithmus für MBC genau zu positionieren [2, 28]. Aus diesem Grund haben wir eine Fall-Kontroll-Studie **(III)** durchgeführt, in der wir die Wirksamkeit der MCT mit der CCT in Bezug auf Alter, Ausmaß der Metastasierung, Anzahl der vorangegangenen Chemotherapielinien und HR-/HER2-Status verglichen haben. Patientinnen mit MBC, die eine MCT in Form vom oralen CTX 50 mg einmal täglich und MTX 2,5 mg jeden zweiten Tag zwischen 2009 und 2018 in der Klinik und Poliklinik für Geburtshilfe und Frauengesundheit der Universitätsmedizin Mainz erhalten haben, wurden für diese retrospektive Analyse ausgewählt. Jede metronomisch behandelte Patientin wurde mit zwei Patientinnen gematcht, die eine CCT erhielt, wenn die Matching-Kriterien (Alter bei Therapiebeginn, Anzahl der Metastasenlokalisationen und der Chemotherapielinien sowie HR-Status) erfüllt waren. Begleitende Behandlungen, wie Strahlentherapie, endokrine oder zielgerichtete Therapien, waren nicht erlaubt. Patientinnen mit HER2-positiven Tumoren und Patientinnen mit einer weiteren Krebserkrankung wurden ausgeschlossen.

Insgesamt wurden 120 Patientinnen (40 Fälle mit MCT und 80 Kontrollen mit CCT) mit MBC hinsichtlich der Wirksamkeit der Chemotherapie ausgewertet. 47,5 % der Patientinnen in der MCT-Gruppe und 51,3 % der Patientinnen in der CCT-Gruppe erhielten eine (neo-)adjuvante Chemotherapie bei der Erstdiagnose von BC (p=0,847). Im metastasierten Stadium erhielten 52,5 % der Patientinnen in beiden Gruppen höchstens zwei Chemotherapielinien vor Beginn der MCT/CCT. In beiden Gruppen wiesen 62,5 % der Patientinnen höchstens zwei verschiedene Metastasenlokalisationen auf und 75 % der Patientinnen hatten eine HR-positive Erkrankung. Sowohl in der MCT- als auch in der CCT-Gruppe waren Knochen (62,5 % versus 70,0 %, p=0,417), Leber (52,5 % versus 52,5 %, p=1,000) und Lunge (40 % versus 40 %, p=1,000) die häufigsten Metastasenlokalisationen. In der CCT-Gruppe wurden am häufigsten CAPE (27,5 %) und pegyliertes liposomales Doxorubicin (26,3 %) eingesetzt. Taxane wurden bei 17,5 % der Patientinnen und Eribulin bei 12,5 % der Patientinnen verwendet.

Der primäre Endpunkt DCR ≥ 24 Wochen unterschied sich nicht signifikant zwischen der MCT- und der CCT-Gruppe (30,0 % versus 22,5 %, p=0,380). 12,5 %, 15,0 % und 2,5 % der MCT-Patientinnen bzw. 18,8 %, 3,8 % und 0,0 % der CCT-Patientinnen zeigten SD, PR bzw. CR. Das mediane PFS betrug 12,0 Wochen in beiden Gruppen, die Hazard Ratio für Progression oder Tod betrug 0,796; 95 % CI 0,541-1,170; p=0,245. Auch bei der DoR (31,0 versus 20,5 Wochen, p=0,383) und beim Therapieansprechen (37,5 % versus 30,0 %, p=0,417) zeigten sich keine signifikanten Unterschiede zwischen der MCT- und CCT-Gruppe. Zusammenfassend konnten wir in unserer retrospektiven Fall-Kontroll-Studie eine vergleichbare Wirksamkeit zwischen MCT und CCT zeigen [59]. Auch wenn der retrospektive Charakter die Aussagekraft der vorgestellten Daten einschränkt, sollten diese Ergebnisse weitere Bemühungen zur Untersuchung der MCT als Therapieoption beim MBC unterstützen. Es ist von großer Bedeutung, prospektive Studien zu MCT nach einer endokrin-basierten Therapie mit CDK 4/6-Inhibitoren zu planen und MCT mit CCT und weiteren Standardtherapieregimes zu vergleichen.

Wirksamkeit in Abhängigkeit von Patientencharakteristika

In allen drei retrospektiven Analysen **(I-III)** wurden Subgruppenanalysen durchgeführt [54, 58, 59]. Das Ziel war es, Subgruppen von Patientinnen zu definieren, die am meisten von dieser Therapieoption profitieren könnten. Für die Subgruppenanalysen wurden die Patientinnen nach Alter bei Beginn der MCT (jünger: ≤ medianes Alter versus älter: > medianes Alter), Anzahl der verschiedenen Metastasenlokalisationen (ohne multiple Metastasen: ≤ 2 verschiedene Metastasenlokalisationen versus multiple Metastasen: > 2 verschiedene Metastasenlokalisationen), Anzahl der vorangegangenen Chemotherapielinien (nicht stark vorbehandelt: < 2 Chemotherapielinien versus stark vorbehandelt: ≥ 2 Chemotherapielinien) und HR-/HER2-Status (HR-positiv: Östrogen-/Progesteronrezeptor-positiv und HER2-negativ versus triple-negativ: Östrogen-/Progesteronrezeptor-negativ und HER2-negativ). Im Gegensatz zur Real-World-Analyse **(I)** und der Fall-Kontroll-Studie **(III)** wurden in der Kohortenstudie mit CTX/MTX **(II)** auch Patientinnen mit einer HER2-positiven Erkrankung eingeschlossen. Bei den statistischen Analysen wurden die Zusammenhänge zwischen den klinisch-pathologischen Merkmalen und dem Therapieansprechen mittels Pearson-Chi-Quadrat-Test analysiert. Für die PFS-, DoR- und OS-Analysen wurden der Kaplan-Meier-Schätzer und der Log-rank-Test

verwendet. Das Cox-Regressionsmodell diente zur Schätzung der Hazard Ratio und des 95 % CI bei den Überlebenszeitanalysen.

Patientenalter

Derzeit sind ältere und gebrechliche Patient:innen mit Begleiterkrankungen, die aufgrund der Toxizität keine CCT erhalten können, die wichtigste Zielgruppe für den Einsatz der MCT [2, 28, 53, 80]. In unserer Real-World-Analyse **(I)** war das mediane Alter der Kohorte bei der Erstdiagnose MBC 59 (33-86) Jahre und beim Beginn der MCT 64,5 (35-87) Jahre. Wir konnten zeigen, dass MCT auch eine Behandlungsoption für jüngere Patient:innen darstellen kann. Das mediane PFS (17,0 Wochen sowohl bei jüngeren als auch bei älteren Patientinnen) und das mediane OS (58,0 Wochen bei jüngeren versus 52,0 Wochen bei älteren Patientinnen) waren hinsichtlich des Alters ohne signifikante Unterschiede (p=0,389 bzw. p=0,237) [54]. In der Fall-Kontroll-Studie **(III)**, die die MCT mit CCT verglichen hat, konnten in den Subgruppen ebenfalls keine signifikanten Unterschiede in Bezug auf das Alter dokumentiert werden. Das mediane Alter bei der Erstdiagnose von MBC betrug 59 (33-82) Jahre in der MCT-Gruppe und 59 (28-81) Jahre in der CCT-Gruppe (p=0,544). Das mediane Alter bei Therapiebeginn betrug 63 (35-83) Jahre und 61 (30-81) Jahre (p=0,230). Das mediane PFS bei jüngeren Patientinnen betrug 15,0 Wochen in der MCT-Gruppe und 14,0 Wochen in der CCT-Gruppe (p=0,212). 40,0 % der jüngeren MCT-Patientinnen und 25,0 % der jüngeren CCT-Patientinnen erreichten DCR ≥ 24 Wochen (p=0,249). Bei älteren Patientinnen waren die Ergebnisse in beiden Gruppen identisch mit einem medianen PFS von 12,0 Wochen (p=0,627) und einer DCR ≥ 24 Wochen von 20,0 % (p=1,000) [59]. Unsere Ergebnisse deuten darauf hin, dass das Alter kein entscheidender Faktor für den Einsatz von MCT sein sollte. Vielmehr müssen die Patient:innen individuell nach Nutzen-Risiko-Abwägung beraten werden, sodass MCT unter bestimmten Voraussetzungen auch für jüngere Patient:innen eine Therapieoption darstellt.

Metastasierung

Die Anzahl und die Lokalisation der Metastasen spielen beim MBC eine wichtige Rolle bei der Therapiewahl und beeinflussen maßgeblich die Prognose der Patient:innen [5, 81-86]. In der Real-World-Analyse **(I)** wiesen unsere Patientinnen im Median 3 (1-7) unterschiedliche Metastasenlokalisationen auf. Die von Metastasen betroffenen Organe waren Knochen (68,1 %), Leber (48,6 %), Lunge (47,2 %), Pleura (27,8 %),

Weichteile (22,2 %), Peritoneum (18,1 %) und Gehirn (9,7 %) [54]. Die Häufigkeit und die Lokalisation der Metastasen stimmte mit den Ergebnissen aus unseren weiteren Studien **(II** und **III)** und der Literatur überein [58, 59, 81, 86]. Insgesamt hatten 80,6 % unserer Patientinnen eine viszerale Metastasierung. In der Gesamtpopulation betrug die DCR ≥ 24 Wochen 31,9 % und das mediane PFS betrug 17,0 Wochen (95 % CI 14,5-19,5). In der Studie haben wir keine Subgruppenanalyse hinsichtlich einer viszeralen Metastasierung durchgeführt. In der VinoMetro-Studie **(IV)** wiesen alle Patientinnen eine hepatische Metastasierung auf und die CBR ≥ 24 Wochen lag bei 22,2 % und das mediane PFS bei 12 Wochen (95 % CI 11,3-12,7) [75].

In der prospektiven Phase-II-Studie von Addeo et al. wurde das metronomische VRL (70 mg/m^2 am Tag 1, 3 und 5/Woche für drei Wochen gefolgt von einer Woche Pause) als Erstlinienbehandlung beim MBC untersucht. 32 % der Patient:innen hatten eine viszerale Metastasierung mit einer CBR ≥ 12 Wochen von 68 % und einem medianen PFS von 7,7 Monaten (95 % CI 6,9-9,1) [72]. Liu et al. untersuchten in der retrospektiven Analyse die gleiche Dosierung von VRL und zeigten bei Patient:innen, die in 81,1 % der Fälle eine viszerale (31,1 % hepatisch) Metastasierung hatten, eine DCR von 78,9 %. In der Phase-II-Studie VICTOR-2 wurde bei Patientinnen mit VRL 40 mg 3x/Woche und CAPE 500 mg 3x/Tag eine Subgruppenanalyse in Bezug auf viszerale Metastasierung durchgeführt. Patientinnen mit einer viszeralen Beteiligung (72,6 % der Patientinnen) hatten eine CBR von 44,8 % (95 % CI 31,7-58,5) und Patientinnen ohne viszerale Beteilung eine CBR von 59,1 % (95 % 36,4-79,3) [67].

Neben der Metastasenlokalisation spielt beim MBC auch die Anzahl der verschiedenen Metastasen eine wichtige Rolle. In den Subgruppenanalysen der Real-World-Daten **(I)** beobachteten wir ein signifikant längeres medianes PFS von 34,0 Wochen versus 13,0 Wochen, p<0,001 und ein signifikant längeres medianes OS von 91,0 Wochen versus 39,0 Wochen, p=0,003 in der Subgruppe ohne multiple Metastasen (≤ 2 verschiedenen Metastasenlokalisationen) im Vergleich zu der Subgruppe mit multiplen Metastasen (> 2 verschiedene Metastasenlokalisationen) [54]. In der Kohortenstudie **(II)** erreichten neun (34,6 %) Patientinnen ohne multiple Metastasen und zwei (22,2 %) Patientinnen mit multiplen Metastasen eine DCR ≥ 24 Wochen (p=0,685) [58]. In der Fall-Kontroll-Studie **(III)** betrug die DCR ≥ 24 Wochen bei Patienten ohne multiple Metastasen 36,0 % in der MCT-Gruppe und 18,0 % in der

CCT-Gruppe (p=0,096) und das mediane PFS betrug 16,0 Wochen versus 12,0 Wochen (p=0,064) mit einem Trend zur Signifikanz. In der Subgruppe mit multiplen Metastasen lag die DCR ≥ 24 Wochen bei 20,0 % in der MCT-Gruppe und 30,0 % in der CCT-Gruppe (p=0,722) und das mediane PFS betrug 12,0 Wochen in beiden Gruppen (p=0,684). Es ist wichtig zu erwähnen, dass beide Kollektive in Bezug auf das Vorhandensein von hepatischen Metastasen (52,5 %) und pulmonalen Metastasen (40,0 %) identisch waren. Bei der Analyse dieser retrospektiven Daten ist es jedoch möglich, dass Patientinnen mit einer eher aggresiven Erkrankung und ausgedehnten Metastasierung eine CCT erhalten haben und die MCT insbesondere bei Patientinnen mit einer stabileren Erkrankung ohne ausgedehnte Metastasierung eingesetzt wurde [59].

Zusammenfassend kann davon ausgegangen werden, dass Patient:innen mit wenigen Metastasen eine weniger aggressive Erkrankung und deshalb tendentiell höhere DCR sowie PFS und OS unter MCT aufweisen als die Patient:innen mit einer aggresiven Erkrankung und einer höheren Wahrscheinlichkeit einer ausgedehnten viszeralen Beteiligung. Dies muss neben den anderen klinisch-pathologischen Faktoren bei der Interpretation der Ergebnisse und beim Vergleich der Studien untereinander unbedingt berücksichtigt werden.

Therapielinie

Eine Meta-Analyse randomisierter Studien mit 2269 MBC-Patient:innen hat gezeigt, dass eine längere Dauer der Erstlinienchemotherapie mit einem geringfügig längeren OS und einem wesentlich längeren PFS assoziiert ist [87]. Des Weiteren ist bekannt, dass die Dauer der Krankheitskontrolle mit zunehmender Anzahl von Chemo-therapielinien abnimmt [88]. Daher ist es von großer Bedeutung, Substanzen zu finden, die über einen langen Zeitraum verabreicht werden können, ohne dass es zu einer Dosisakkumulation und einer Häufung inakzeptabler Nebenwirkungen kommt.

In unserer Real-World-Analyse **(I)** erhielten die Patientinnen im mestastasierten Stadium der Erkrankung vor Beginn der MCT im Median 1,5 (0-6) Chemotherapielinien und 2,0 (0-7) endokrin-basierte Therapielinien. Wir konnten zeigen, dass auch wenig stark vorbehandelte Patientinnen von der MCT profitieren können. In Bezug auf das mediane PFS und OS gab es keinen Unterschied zwischen den nicht stark

vorbehandelten (< 2 Chemotherapielinien) und den stark vorbehandelten Patientinnen (≥ 2 Chemotherapielinien) (17,0 Wochen versus 13,0 Wochen, p=0,764 und 52,0 Wochen versus 58,0 Wochen, p=0,457) [54]. Hinsichtlich der DCR konnten wir in der Kohortenstudie **(II)** ebenfalls keinen signifikanten Unterschied beobachten. Sieben (36,8 %) nicht stark vorbehandelte Patientinnen und vier (25,0 %) stark vorbehandelte Patientinnen erreichten DCR ≥ 24 Wochen (p=0,493) [58]. In der Fall-Kontroll-Studie **(III)** betrug die DCR ≥ 24 Wochen bei den nicht stark vorbehandelten Patientinnen 33,3 % in der MCT- und 26,2 % in der CCT-Gruppe (p=0,568). In der stark vorbehandelten Gruppe erreichten 26,3 % bzw. 18,4 % der Patientinnen eine DCR ≥ 24 Wochen (p=0,509). In der Subgruppe der nicht stark vorbehandelten Patienten betrug das mediane PFS 17,0 Wochen bei den MCT-Patientinnen und 15,0 Wochen bei den CCT-Patientinnen (p=0,531). In der stark vorbehandelten Subgruppe betrug das mediane PFS für beide Behandlungsgruppen 12,0 Wochen (p=0,235) [59].

Die Wirksamkeit der MCT hinsichtlich der Therapielinie wurde auch in der Studie VICTOR-6 mit 597 Patientinnen retrospektiv untersucht. Die Gesamtansprechrate (overall response rate, ORR) und die DCR betrugen 33,8 % bzw. 81,5 % in der ersten Chemotherapielinie und sanken auf 8,8 % bzw. 54,4 % in der vierten Chemotherapie-linie [26]. Ähnliche Ergebnisse zeigte die VEX-Studie, in der metronomisch behandelte MBC-Patientinnen ohne Vorbehandlung mit Patientinnen mit Vorbehandlung verglichen wurden. Die mediane TTP war 25,1 Monate (95 % CI 14,2-39,1) in der therapienaiven Gruppe und 11,2 Monate (95 % CI 9,2-17,0) in der vorbehandelten Kohorte. Das 2-Jahres-OS war 91 % in der therapienaiven Gruppe und 83 % in der vorbehandelten Gruppe [69].

In unserer Phase-II-Studie VinoMetro **(IV)** wurde zum ersten Mal ein metronomisches Schema mit einer täglichen oralen VRL-Gabe als Erstlinienchemotherapie bei HR-positiven, HER2-negativen MBC-Patientinnen nach endokriner Resistenz untersucht. Eine vorherige Behandlung mit Everolimus und/oder dem CDK 4/6-Inhibitor Palbociclib im Rahmen einer endokrin-basierten Therapie war möglich. VRL wurde oral in einer täglichen Dosis von 30 mg bis zum Fortschreiten der Krankheit, dem Auftreten inakzeptabler Toxizität, der Verweigerung der Patientinnen oder der Entscheidung des Prüfarztes, die Behandlung zu beenden, verabreicht. Eine Dosisreduktion auf 20 mg einmal täglich war möglich. Das mediane Alter betrug 63,0 (52,0-77,0) Jahre. Keine

der Patientinnen wies zum Zeitpunkt der Erstdiagnose BC Fernmetastasen auf. Die mediane Anzahl der vorangegangenen endokrinen Therapielinien betrug 3,0 (2,0-4,0). Die VinoMetro-Studie bestätigte die Ergebnisse früherer Studien zum oralen metronomischen VRL nicht, wobei die tägliche orale Verabreichung bei MBC-Patient:innen bisher nicht untersucht wurde [17, 66, 67, 69, 71, 72, 89]. Der primäre Endpunkt CBR ≥ 24 Wochen lag unter metronomischem VRL als Erstlinienchemotherapie bei 22,2 % (90 % CI 4,1-55,0) und damit deutlich unter der erwarteten CBR. Das mediane PFS war 12,0 Wochen (95 % CI 11,3-12,7). Insgesamt starben 3 Patientinnen, sodass das mediane OS nicht berechnet werden konnte. Ein möglicher Grund für diese Diskrepanzen könnte die Tatsache sein, dass alle Patientinnen in der VinoMetro-Studie eine HR-positive Erkrankung und viszerale Metastasierung hatten. Weitere mögliche Gründe könnten in der täglichen Verabreichung von VRL und in der niedrigen Fallzahl liegen. Die Studie wurde nach dem Tod einer Patientin aufgrund der Toxizität Grad 5 mit neun eingeschlossenen Patientinnen frühzeitig beendet [75].

Zusammenfassend kann angenommen werden, dass Patientinnen ohne Symptome und ohne Notwendigkeit eines schnellen Tumoransprechens bereits in den frühen Therapielinien von der MCT profitieren können. Es muss jedoch darauf hingewiesen werden, dass die Mehrheit unserer Patientinnen die MCT vor oder zum Zeitpunkt der Einführung von CDK 4/6-Inhibitoren für das HR-positive, HER2-negative MBC und Immuncheckpoint-Inhibitoren für das triple-negative MBC erhalten hatten, sodass die Interpretation der Ergebnisse eingeschränkt ist.

In unserer Real-World-Analyse **(I)** wurde die MCT im Vergleich zur Vor- und Nachbehandlung untersucht. 40 (59,7 %) Patientinnen erhielten eine CCT als Haupttherapie unmittelbar vor der MCT und 36 (78,3 %) Patientinnen im Anschluss an die MCT. Die MCT zeigte eine ähnliche DCR ≥ 24 Wochen und ein klinisch bedeutsames, aber statistisch nicht signifikant kürzeres medianes PFS im Vergleich zur Vorbehandlung (31,9 % versus 32,8 %, p=0,570 bzw. 17,0 Wochen versus 20,0 Wochen, p=0,093) und eine statistisch signifikant höhere DCR ≥ 24 Wochen und ein längeres medianes PFS im Vergleich zur Nachbehandlung (31,9% versus 17,4%, p=0,038 bzw. 17,0 Wochen versus 12,0 Wochen, p=0,006). Anhand dieser Ergebnisse kann angenommen werden, dass die MCT ähnlich wirksam war wie die vorherige

Standardtherapie und dass die Patientinnen während der anschließenden Therapie eine signifikante Krankheitsprogression erlebten. Es ist wichtig anzumerken, dass die MCT bei fünf (6,9 %) Patientinnen als Erstlinientherapie verabreicht wurde und 24 (33,3 %) Patientinnen nach der MCT aufgrund des schlechten Allgemeinzustandes oder des Todes keine weitere Therapie erhielten. Bei fast 20 % der Patientinnen wurde die MCT aufgrund des schlechten Allgemeinzustandes oder des Todes abgebrochen [54]. Dies deutet darauf hin, dass es in unserer Kohorte viele stark vorbehandelte und schwer kranke Patientinnen gab, was bei der Interpretation und dem Vergleich der Ergebnisse mit anderen Studien berücksichtigt werden sollte.

HR- und HER2-Status

Patient:innen mit einem HR-positiven, HER2-negativen MBC, die gegen eine endokrin-basierte Therapie resistent sind und kein schnelles Ansprechen des Tumors erfordern, sind im Allgemeinen für die MCT geeignet [25, 67, 76, 90]. In den meisten Studien wurden Patient:innen mit einer HER2-negativen Erkrankung eingeschlossen, sodass Patient:innen sowohl mit HR-positiven als auch triple-negativen Tumoren zusammen ausgewertet wurden. Dies kann aufgrund der unterschiedlichen Tumorbiologie, der Vorbehandlung und Prognose unter Umständen zu Einschränkungen bei der Interpretation der jeweiligen Ergebnisse führen und muss deshalb beachtet werden.

Montagna et al. untersuchten das metronomische VEX-Schema (VRL 40 mg dreimal wöchentlich, CTX 50 mg einmal täglich und CAPE 500 mg dreimal täglich) getrennt in zwei Phase-II-Studien bei 108 Patientinnen mit HR-positivem MBC und bei 22 Patientinnen mit TNBC [68, 69]. Die Kohorte mit HR-positiven Tumoren wies eine CBR ≥ 24 Wochen von 81 % in der Erstlinienbehandlung und 74 % bei den Patientinnen mit einer Vorbehandlung auf. Die mediane TTP war jeweils 25,1 Monate (95 % CI 14,2-39,1) und 11,2 Monate (95 % CI 9,2-17,0) [69]. Die Kohorte mit TNBC zeigte eine CBR ≥ 24 Wochen von 50 % und eine mediane TTP von 6,4 Monaten (95% CI 3,6-12,6) in der Erstlinienbehandlung [68]. Daten zur TNBC-Kohorte liefert auch eine Subgruppenanalyse der Studie VICTOR-6, in der 97 Patientinnen mit TNBC eine MCT (45,4 % VRL-basiert, 30,9 % CTX-basiert und 22,7 % CAPE-basiert) erhielten. Die Kohorte war in 40,2 % der Fälle vor Beginn der MCT ohne Vorbehandlung im metastasierten Stadium und erzielte eine ORR von 17,5 % und eine DCR von 64,9 % mit einem medianen PFS von 6,0 Monaten (95 % CI 4,9-7,2) und einem medianen OS

von 12,1 Monaten (95 % CI 9,6-16,7). Sowohl die ORR als auch die DCR lag in der TNBC-Kohorte tiefer als im Gesamtkollektiv mit einer ORR von 29,5 % und einer DCR von 74,6 % [91].

Die CTX/MTX-Kombination zeigte in unserer Kohorte **(II)** keinen signifikanten Unterschied in der DCR hinsichtlich des HR-Status. Acht (33,3 %) Patientinnen mit einem HR-positiven Tumor und drei (27,3 %) Patientinnen mit TNBC erreichten DCR ≥ 24 Wochen (p=1,000) [58]. In der Subgruppenanalyse unserer Real-World-Daten **(I)** konnten keine signifikanten Unterschiede im medianen PFS und OS in Bezug auf den HR-Status dokumentiert werden. Das mediane PFS betrug sowohl in der HR-positiven Subgruppe mit 52 Patientinnen (72,2 %) als auch in der TNBC-Subgruppe mit 20 Patientinnen (27,8 %) 17,0 Wochen (p=0,144). Das mediane OS betrug 58,0 Wochen bei Patientinnen mit einer HR-positiven Erkrankung und 52,0 Wochen bei Patientinnen mit TNBC (p=0,379) [54]. In der Fall-Kontroll-Studie **(III)**, die die MCT mit der CCT verglichen hat, wurde die DCR ≥ 24 Wochen bei 30,0 % versus 28,3 % der Patientinnen mit einem HR-positiven Tumor erreicht (p=1,000). Bei den Patientinnen mit TNBC blieb die DCR in der Gruppe der metronomisch behandelten Patientinnen bei 30,0 %, in der Gruppe der CCT sank die DCR auf 5,0 % (p=0,095) [59]. Das deutet darauf hin, dass die MCT dank der multimodalen Eigenschaften möglicherweise auch beim TNBC wirksam ist. Die Ergebnisse können aber auch dadurch erklärt werden, dass die Patientinnen in der Gruppe mit CCT eine viel aggressivere Erkrankung hatten.

Zusammenfassend lässt sich feststellen, dass die MCT insbesondere bei Patient:innen mit einem HR-positiven MBC den größten Benefit aufweist und für diese Patientenpopulation eine günstige Therapieoption darstellt [15, 25, 67, 76]. Es gibt jedoch zunehmend Daten dafür, dass die MCT auch bei Patient:innen mit TNBC ohne aggressive Erkrankung, die ein günstiges Toxizitätsprofil bevorzugen, ein vielversprechender Ansatz sein kann [2, 28, 92, 93].

In den letzten Jahren wird eine Zunahme an Studien zur MCT bei Patient:innen mit einem HER2-positiven MBC beobachtet [94-97]. In unseren Kohorten **(I-IV)** wurde jedoch nur eine Patientin mit einer HER2-positiven Erkrankung eingeschlossen, die zusätzlich zur CTX/MTX-Kombination Trastuzumab intravenös in der Standarddosierung erhielt und die DCR ≥ 24 Wochen nicht erreichte [58]. Anhand

unserer Daten können wir keine Aussage zur Wirksamkeit der MCT in Kombination mit Anti-HER2-Therapien treffen.

Orlando et al. testeten in der Phase-II-Studie HEX die metronomische Kombination von CTX 50 mg einmal täglich, CAPE 500 mg dreimal täglich und Trastuzumab 4 mg/kg intravenös alle 14 Tage bei 60 unbehandelten HER2-positiven MBC-Patientinnen. 55 % der Patientinnen wiesen eine viszerale Metastasierung auf und 28,3 % der Patientinnen erhielten Trastuzumab bereits im Rahmen der adjuvanten Behandlung. Die Studie zeigte eine Wirksamkeit mit einer ORR von 56,7 % und einer CBR ≥ 24 Wochen von 78,2 % (CR: 8,3 %, PR: 48,3 % und SD: 25,0 %). Das mediane PFS betrug 11,0 Monate (95 % CI 6,3-15,6) und das mediane OS betrug 45,9 Monate (95 % CI 22,7-69,1) [97]. Ähnliche Ergebnisse wurden in einer weiteren Phase-II-Studie mit 20 teilweise vorbehandelten HER2-positiven MBC-Patientinnen gezeigt, die mit metronomischem VRL 40 mg 3x/Woche und Trastuzumab intravenös in der Standarddosierung behandelt wurden. 70 % der Patientinnen hatten eine viszerale Metastasierung und 60 % der Patientinnen waren im metastasierten Stadium bereits vorbehandelt. Trastuzumab wurde vor Beginn der MCT bei 12,5 % der Patientinnen im Rahmen der (neo-)adjuvanten Therapie und bei 10 % der Patientinnen im metastasierten Stadium verabreicht. Das Regime führte zu einer ORR von 20,0 % und einer CBR ≥ 24 Wochen von 75,0 % (PR: 20,0 % und SD: 55,0 %). Das mediane PFS betrug 7,4 Monate (95 % CI 3,2-11,5) und das mediane OS war 5,8 Monate (95 % CI nicht erreicht) [94].

In einer randomisierten Phase-II-Studie wurde die Wirksamkeit einer dualen Anti-HER2-Therapie mit oder ohne metronomisches CTX bei älteren HER2-positiven MBC-Patient:innen untersucht. 80 Patient:innen, von denen 70 % ein potenzielles Gebrechlichkeitsprofil gemäß dem geriatrischen Screening-G8-Score (≤ 14) aufwiesen, wurden 1:1 randomisiert und haben entweder intravenöse Standard-therapie mit Trastuzumab und Pertuzumab oder Trastuzumab und Pertuzumab plus metronomisches CTX 50 mg einmal täglich erhalten. Die CBR betrug 86 % in der Gruppe mit MCT und 77 % in der Gruppe ohne MCT. Die zusätzliche Gabe von CTX bei älteren und gebrechlichen Patient:innen mit HER2-positivem MBC verlängerte das mediane PFS um 7 Monate im Vergleich zur alleinigen dualen HER2-Blockade. Das 1-Jahres-OS war 83,8 % (95 % CI 67,3-92,4) in der Gruppe mit CTX und 67,3 % (95 %

CI 49,4-80,0) in der Gruppe ohne CTX [96]. Zusammenfassend kann man sagen, dass die Kombinationen von MCT mit Anti-HER2-Therapien möglich sind und nach Nutzen-Risiko-Abwägung und individueller Beratung der Patient:innen eingesetzt werden können [2, 95, 98].

Nebenwirkungsprofil und Lebensqualität

Einer der wichtigsten Gründe für den Einsatz der MCT ist deren günstiges Nebenwirkungsprofil. Es wird angenommen, dass die MCT im Vergleich zur CCT seltener unerwünschte Ereignisse wie Polyneuropathie, Myelosuppression, Mukositis und Alopezie hervorruft [12, 23, 53, 99]. Dies kann wesentlich zur Erhaltung der HRQoL beitragen, die eines der Hauptziele der Behandlung im metastasierten Stadium darstellt [6]. Außerdem ist es von großer Bedeutung, Medikamente zu finden, die über einen langen Zeitraum verabreicht werden können, ohne dass es zu einer Dosisakkumulation und einer Häufung inakzeptabler Nebenwirkungen kommt. Bislang fehlen jedoch genaue Daten, die einen direkten Vergleich zwischen MCT und CCT in Bezug auf Verträglichkeit und Lebensqualität ermöglichen.

In der ersten Studie zur metronomischen CTX/MTX-Kombination von Colleoni et al., die insgesamt 63 Patient:innen mit einem Eastern Cooperative Oncology Group (ECOG)-Score von ≤ 3 ausgewertet hat, traten nur wenige unerwünschte Ereignisse auf. Nur 10 % der Therapiezyklen wurden verschoben und 7 % der Zyklen wurden in einer reduzierten Dosierung verabreicht. Die häufigste Toxizität war Leukopenie Grad 1 (35 % der Patient:innen) und Übelkeit/Erbrechen Grad 1 (20 % der Patient:innen). Bei neun (15 %) Patient:innen kam es zum Anstieg der Transaminasen Grad 3, wobei bei sechs (9 %) Patient:innen gleichzeitig eine hepatische Metastasierung vorlag und nach Unterbrechung bzw. Reduktion von MTX eine komplette Normalisierung der Leberwerte beobachtet wurde [23]. In der retrospektiven Analyse mit insgesamt 186 Patientinnen, die die CTX/MTX-Kombination erhalten haben, wurde bei 44 (23,6 %) Patientinnen mindestens ein unerwünschtes Ereignis dokumentiert. Es gab keine Toxizität ≥ Grad 3. Die häufigsten Nebenwirkungen waren Übelkeit Grad 1 (7,1 %), Fatigue Grad 1 (4,2 %) und Erhöhung der Transaminasen Grad 1 (1,8 %) und Grad 2 (1,2 %) [76].

Ähnliche Ergebnisse wurden auch in unserer CTX/MTX-Kohorte **(II)** beobachtet, wenn MTX nicht in der üblichen Dosierung von 2,5 mg am Tag 1 und 4 zweimal täglich, sondern einmal täglich 2,5 mg alle zwei Tage eingenommen wurde. Insgesamt brachen drei (8,6 %) Patientinnen die Therapie aufgrund von Thrombozytopenie, gastrointestinalen Beschwerden oder Fatigue (jeweils eine Patientin) ab. Vier (11 %) Patientinnen beendeten nur die MTX-Therapie, hauptsächlich wegen Übelkeit/Erbrechen (zwei Patientinnen), Fatigue (zwei Patientinnen) und Sehstörungen (eine Patientin). Eine (3 %) Patientin entwickelte eine hämorrhagische Zystitis, ein Therapieabbruch war jedoch nicht erforderlich. Leukopenie und Anämie wurden nicht als Gründe für einen Therapieabbruch dokumentiert [58].

In der Real-World-Analyse **(I)** waren Übelkeit/Erbrechen, Diarrhoe und Thrombozytopenie die häufigsten Gründe für einen vorzeitigen Therapieabbruch. Insgesamt wurde die MCT bei drei (4,2 %) Patientinnen aufgrund der Toxizität und bei sieben (9,7 %) Patientinnen aufgrund des schlechten Allgemeinzustandes vorzeitig abgebrochen, was im Vergleich zur vorherigen und nachfolgenden Behandlung ähnlich war (7,5 % bzw. 10,9 % aufgrund der Toxizität und 0,0 % bzw. 10,9 % aufgrund des schlechten Allgemeinzustandes) [54]. Die metronomische Gabe von CAPE in einer Dosierung von 500 mg dreimal täglich [77] bzw. 1500 mg einmal täglich [78] war ebenfalls gut verträglich ohne hämatologische Toxizitäten ≥ Grad 3. Die einzigen hochgradigen unerwünschen Ereignisse waren Hand-Fuß-Syndrom Grad 3 bei vier (9,1 %) Patientinnen in der Studie von Li et al. [77] bzw. bei drei (5 %) Patientinnen in der Studie von Fedele et al. [78].

In unserer Phase-II-Studie VinoMetro **(IV)** mit einer täglichen oralen VRL-Gabe von 30 mg und einer möglichen Reduktion aufgrund Toxizität auf 20 mg täglich betrug die mediane Tagesdosis von VRL 27,3 (14,7-30,0) mg. Insgesamt wurden 73 unerwünschte Ereignisse gemeldet (8,1 pro Patientin). 37 (50,7 %) unerwünschte Ereignisse standen im Zusammenhang mit der Studienbehandlung (4,1 pro Patientin). Die häufigsten Nebenwirkungen waren Übelkeit (55,6 %), Fatigue (44,4 %) und Diarrhoe (33,3 %). Nebenwirkungen ≥ Grad 3 wurden bei zwei (22,2 %) Patientinnen dokumentiert. Eine (11,1 %) Patientin starb in Folge von Pneumonie und febriler Neutropenie nach 12 Tagen Behandlung [75]. Das Nebenwirkungsprofil vom oralen VRL mit einer fraktionierten Gabe (3x/Woche) scheint mit einer Inzidenz von

unerwünschten Ereignissen ≥ Grad 3 von 2,2 % bis 9,0 % günstiger zu sein [17, 70, 72]. In den Studien waren die häufigsten Nebenwirkungen ≥ Grad 3 Anämie (1,1-9,0 %), Neutropenie (0,0-9,0 %) und Diarrhoe (1,1-3,0 %) [17, 70, 72]. Die metronomischen Kombinationsbehandlungen (CTX/CAPE, VRL/CAPE und CTX/CAPE/VRL) zeigten ebenfalls keine erhöhte Rate an unerwünschten Ereignissen. Die häufigsten Nebenwirkungen ≥ Grad 3 waren Hand-Fuß-Syndrom (1,0-7,0 %), hämatologische Toxizitäten (0,8-5,8 %), Übelkeit/Erbrechen (1,0-2,1 %) und Diarrhoe (0,4-2,0 %) [25, 26, 67, 69].

Studien, in denen die MCT mit best supportive care verglichen wird und Daten zur HRQoL und patient reported outcome ausgewertet werden, sind ebenfalls unerlässlich, um die Rolle der MCT beim MBC zu definieren, da dies in diesem Zusammenhang von besonderem Interesse ist. Bisher existieren kaum Daten zu diesem Thema. In der Phase-II-Studie von Perroud et al. wurde bei 20 MBC-Patient:innen, die mit CTX 50 mg einmal täglich und Celecoxib, einem Cyclooxygenase-2-Hemmer, 200 mg zweimal täglich behandelt wurden, die HRQoL mittels Fuctional Assessment of Cancer Therapy Breast (FACT-B)-Fragebogen, Brief Pain Inventory-Fragebogen und ECOG-Score ausgewertet. In der kleinen, stark vorbehandelten Patientenkohorte konnte keine signifikante Verschlechterung der HRQoL und des Schmerzempfindens während der MCT festgestellt werden. Bei 26,7 % der Patient:innen kam es zu einer Verbesserung und bei 33,3 % der Patient:innen zu einer Verschlechterung des ECOG-Scores während der MCT [99]. In der randomisierten Phase-II-Studie von Dal Lago et al. führte die Hinzunahme von metronomischem CTX zur Trastuzumab/Pertuzumab-Kombination bei Patient:innen mit einem HER2-positiven MBC zur Verlängerung des medianen PFS um 7 Monate ohne Einfluss auf die HRQoL, die mittels European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30-Fragebogen gemessen wurde [98].

Um weitere Daten zur HRQoL unter der MCT zu ermitteln, wird aktuell durch unsere Arbeitsgruppe eine nicht-interventionelle, unizentrische, prospektive Studie “PROmetronomic-Patient Reported Outcome unter metronomischer Chemotherapie mit Cyclophosphamid ± Methotrexat bei metastasiertem Mammakarzinom und platinresistentem Ovarialkarzinomrezidiv” in der Klinik und Poliklinik für Geburtshilfe und Frauengesundheit der Universitätsmedizin Mainz durchgeführt. In der Studie

werden gesundheitsbezogene Patientendaten bei Patientinnen mit MBC und platinresistentem Ovarialkarzinomrezidiv ausgewertet, die mit MCT (CTX 50 mg einmal täglich ± MTX 2,5 mg jeden zweiten Tag) behandelt werden. Patientinnen mit mindestens zwei endokrinen Therapielinien (bei HR-positivem Tumor) und mindestens zwei Chemotherapielinien (MBC) bzw. mit mindestens einer platinbasierten und einer platinfreien Chemotherapie (platinresistentes Ovarialkarzinomrezidiv) in der Vorbehandlung werden eingeschlossen. Primär werden Daten zur HRQoL und zu unerwünschten Ereignissen mit den Fragebögen EORTC QLQ-C30 Version 3.0, EORTC QLQ-BR23 Version 1.0 (MBC)/EORTC QLQ-OV28 Version 1.0 (platinresistentes Ovarialkarzinomrezidiv) und Hospital Anxiety and Depression Scale, deutsche Version (HADS-D) elektronisch im CANKADO-Programm erhoben. Sekundäre Endpunkte sind die DCR ≥ 12 und 24 Wochen, das PFS und OS. Im Rahmen des translationalen Forschungsansatzes werden während der MCT Blutproben gesammelt und für die Krankheitskontrolle potenziell relevante Biomarker in Bezug auf Antiangiogenese und Immunmodulation analysiert. Die Rekrutierung wurde nach 24 Monaten im August 2022 abgeschlossen. Die Auswertung ist nach Abschluss der Follow-up-Phase im August 2023 geplant und die HRQoL-Ergebnisse werden Ende 2023 erwartet.

Die meisten Patient:innen mit einer unheilbaren Malignomerkrankung ziehen eine orale gegenüber einer intravenösen Therapie vor [100-102]. Ein Fragebogen zur Bewertung der Wahrnehmung der oralen antitumoralen Therapien zeigte eine hohe Akzeptanz bei den meisten MBC-Patient:innen. In der Studie trug die orale Verabreichung dazu bei, dass sich die Patient:innen weniger krank fühlten und die Krankheit besser bewältigten [103]. In der VinoMetro-Studie **(IV)** konnten wir zeigen, dass im Median 91 % (49-100 %) der Patientinnen die Therapie mit VRL 30 mg täglich genau nach Anordnung des Prüfarztes eingenommen haben [75]. Darüber hinaus ermöglicht die MCT eine einfache tägliche oder fraktionierte Verabreichung mit der Möglichkeit einer individuellen Dosisanpassung im Falle von Toxizitäten und erfordert im Vergleich zur intravenösen CCT seltener Klinikaufenthalte und Vorstellungen in den onkologischen Ambulanzen [104]. Das letztere wurde spätestens während der Covid-19-Pandemie relevant, als die Möglichkeit einer Vorstellung und Behandlung in Kliniken und onkologischen Ambulanzen über eine gewisse Zeit eingeschränkt war. Einen weiteren Vorteil der MCT im Vergleich zur intravenösen Verabreichung der

Chemotherapie stellt die verlängerte Plasmakonzentration und damit ein größeres therapeutisches Fenster dar [13, 80]. Schließlich bewerteten Bocci et al. in einer pharmakoökonomischen Analyse die metronomische Kombination von CTX/MTX als kostengünstig [105]. Der Einsatz der MCT könnte dabei helfen, die Kosten im Gesundheitswesen zu senken und auch Patient:innen in ressourcenarmen Ländern eine antitumorale Therapie anzubieten [28, 106, 107].

Kombination der metronomischen Chemotherapie mit zielgerichteten Substanzen

Der multimodale Wirkmechanismus und die gute Verträglichkeit der MCT bieten die Möglichkeit einer Kombination mit neuen zielgerichteten Substanzen [53, 108].

Einer der möglichen therapeutischen Ansätze besteht darin, die antiangiogenen Eigenschaften und die Effektivität der MCT durch die Kombination mit VEGF-Inhibitoren zu verstärken. Die Kombination von metronomischem CTX 50 mg einmal täglich, CAPE 500 mg dreimal täglich und Bevacizumab 10 mg/kg intravenös alle zwei Wochen zeigte bei 46 MBC-Patient:innen eine vielversprechende Wirksamkeit und minimale Toxizität. 68 % (95 % CI 51-81) der Patient:innen erreichten eine CBR ≥ 24 Wochen und die mediane TTP betrug 10,5 Monate (95 % CI 6,5-18,0). Neutropenie ≥ Grad 3 trat bei 4,3 % der Patient:innen auf. Zu den nicht-hämatologischen unerwünschten Ereignissen ≥ Grad 3 gehörten arterielle Hypertonie (17,4 %), Erhöhung der Transaminasen (4,3 %) und Übelkeit/Erbrechen (4,3%) [109].

In einer multizentrischen, randomisierten Phase-III-Studie wurde Bevacizumab 10 mg/kg intravenös alle zwei Wochen entweder mit Paclitaxel 90 mg/m^2 intravenös am Tag 1, 8 und 15 alle vier Wochen (Arm A, n=73) oder mit der metronomischen Kombination aus CTX 50 mg einmal täglich und CAPE 500 mg dreimal täglich (Arm B, n=74) als Erstlinienbehandlung bei Patient:innen mit HER2-negativem MBC verglichen. Die meisten Patient:innen (85,9 % im Arm A und 76,5 % im Arm B) hatten eine HR-positive Erkrankung; Lebermetastasen traten bei 57,7 % bzw. 54,4 % der Patient:innen auf. In Bezug auf den primären Endpunkt, das Auftreten von unerwünschten Ereignissen ≥ Grad 3, gab es keinen signifikanten Unterschied zwischen den Behandlungsarmen (25 % versus 24 %, p=0,96). Die DCR lag bei 79 % (95 % CI 70-89) im Arm A und 64 % (95 % CI 53-74) im Arm B; das mediane PFS

betrug 10,3 Monate (95 % CI 8,7-11,4) versus 8,5 Monate (95 % CI 6,5-11,9) (p=0,83). Die einzigen statistisch signifikanten Unterschiede hinsichtlich der HRQoL waren niedrigere Raten an Alopezie und Taubheitsgefühl im metronomischen Arm. Die Behandlungskosten waren zwischen den beiden Gruppen gleich hoch [79]. In einer Dosisfindungsstudie zeigte die Kombination von metronomischem CTX 50 mg einmal täglich, VRL 20-40 mg 3x/Woche und Bevacizumab 15 mg/kg intravenös alle drei Wochen eine gute Wirksamkeit und Verträglichkeit bei 15 vorbehandelten MBC-Patient:innen. 80 % der Patient:innen hatten eine HR-positive Erkrankung und 33 % der Patient:innen eine HER2-positive Erkrankung, die zusätzlich Trastuzumab in der Standarddosierung erhielten. Die CBR lag bei 66,6 % und die mediane TTP bei 6,9 Monaten. 20 % der Patient:innen entwickelten eine arterielle Hypertonie Grad 3, andere Toxizitäten ≥ Grad 3 wurden nicht beobachtet [89].

Montagna et al. untersuchten die Wirksamkeit und Sicherheit von metronomischem CTX 50 mg einmal täglich und CAPE 500 mg dreimal täglich in Kombination mit Bevacizumab 15 mg/kg alle drei Wochen und Erlotinib, einem Tyrosinkinase-Inhibitor des epidermalen Wachstumsfaktorrezeptors (EGFR), 100 mg einmal täglich bei 26 unbehandelten Patient:innen mit HER2-negativem MBC. Die Autoren dokumentierten eine CBR von 75 % (95 % CI 53-90) und eine mediane TTP von 10,8 Monaten (95 % CI 5,3-17,3). Die Verträglichkeit war gut mit wenigen Nebenwirkungen ≥ Grad 3 (arterielle Hypertonie bei 8 %, Diarrhoe und Thrombose bei je 4 % der Patient:innen) [110]. Im Vergleich dazu zeigte eine Phase-I-Studie mit Kombination aus metronomischem CTX 50 mg einmal täglich und MTX 2,5 mg zweimal täglich am Tag 1 und 2/Woche und Vandetanib, einem Tyrosinkinase-Inhibitor des VEGFR sowie des EGFR, 100-300 mg einmal täglich bei 23 vorbehandelten MBC-Patient:innen nur eine eingeschränkte klinische Aktivität (CBR ≥ 24 Wochen von 25 %) und ein relativ häufiges Auftreten von unerwünschten Ereignissen Grad ≥ 3 (Erhöhung der Transaminasen bei 17,4 %, Hautausschlag bei 13,0 % sowie Fatigue und Diarrhoe bei jeweils 8,7 % der Patient:innen) [111].

Poly-ADP-Ribose-Polymerasen (PARP) sind Enzyme, die an der Reparatur von Einzelstrang-DNA-Brüchen beteiligt sind [112]. Wenn PARP gehemmt werden, bleiben Einzelstrangbrüche bestehen und führen zu blockierten Replikationsgabeln und Doppelstrangbrüchen [113]. Neben der PARP-Inhibitor-Monotherapie bei Karzinomen

mit gestörten DNA-Reparaturmechanismen wäre die Kombination der PARP-Inhibitoren mit Zytostatika, die DNA-Schäden verursachen, wie zum Beispiel CTX, denkbar [114, 115]. Die Hemmung von PARP scheint die Tumorzellen für zytotoxische Wirkstoffe, die DNA-Schäden verursachen, zu sensibilisieren und damit ihren Effekt zu verstärken [116]. Die Multimodalität von metronomischem CTX in Kombination mit einem PARP-Inhibitor könnte daher einen vielversprechenden therapeutischen Ansatz darstellen [116, 117]. Anampa et al. untersuchten die Wirksamkeit und Sicherheit von metronomischem CTX in Kombination mit Veliparib, einem PARP-Inhibitor, bei Patient:innen mit HER2-negativem MBC. Die Kombination aus einer kontinuierlichen oralen Gabe von Veliparib 200 mg zweimal täglich und CTX 50-125 mg einmal täglich wurde gut vertragen und führte bei 43 % der Patient:innen mit einer Keimbahn-BRCA-Mutation und bei 11 % der Patient:innen mit einem unauffälligen oder unbekannten BRCA-Mutationsstatus zum objektiven Ansprechen oder SD ≥ 24 Wochen [118].

Der Phosphatidylinositol-3-Kinase (PI3K)/AKT/Mammalian Target of Rapamycin (mTOR)-Signalweg spielt eine der Schlüsselrollen bei der Regulierung von Wachstum, Stoffwechsel, Migration und Überleben der Zellen [119-121]. Genomische Veränderungen in diesem Signalweg sind bei vielen Malignomen, darunter auch bei BC, nachweisbar [122, 123]. Es wird geschätzt, dass 60-70 % der BC-Patient:innen mindestens eine Mutation im PI3K/AKT/mTOR-Signalweg aufweisen, wobei das PIK3CA-Gen, das für die p110α-Untereinheit von PI3K kodiert, das am häufigsten mutierte Gen ist [122, 124, 125]. Die Häufigkeit von PIK3CA-Mutationen ist bei den verschiedenen BC-Subtypen unterschiedlich: 34,5-48,3 % bei HR-positivem, 22,7-42,2 % bei HER2-positivem und 8,3-25,0 % bei triple-negativem BC [125-127]. Die daraus resultierende Überaktivierung des PI3K-Signalwegs fördert das Tumorwachstum, die Resistenz gegen verschiedene systemische Therapien und führt zu einer Verschlechterung der Prognose [128-132]. Es kann postuliert werden, dass die PI3K-Hemmung, insbesondere in Kombination mit anderen Substanzen, einen neuen Therapieansatz in der Onkologie darstellt [133]. Die SOLAR-1-Studie zeigte eine signifikante Verlängerung des medianen PFS und eine Verbesserung des medianen OS um 7,9 Monate, wenn Alpelisib, ein oral verfügbarer, α-selektiver PI3K-Inhibitor und Degrader, zu Fulvestrant bei der Behandlung von Patient:innen mit einem PIK3CA-mutierten, HR-positiven, HER2-negativen MBC hinzugefügt wurde [134, 135]. Präklinische Modelle haben gezeigt, dass die Hemmung des PI3K-Signalweges zu

einer Sensibilisierung der Karzinomzellen gegenüber der Zytostatika führen und die Resistenzentwicklung verhindern kann [136, 137]. Das Ziel unserer Studie **(V)** war es, die antitumoralen Effekte der Kombination von niedrig dosiertem metronomischem VRL und Alpelisib in verschiedenen BC-Zelllinien zu untersuchen.

Die HR-positiven, HER2-negativen, PIK3CA-mutierten, humanen BC-Zelllinien MCF-7 und T-47D sowie die triple-negativen, humanen BC-Zelllinien ohne PIK3CA-Mutation MDA-MB-231 und BT-549 wurden mit VRL (Navirel®; Medac, Wedel, Deutschland) und Alpelisib (Piqray®, Novartis Pharma AG, Basel, Schweiz) alleine sowie in Kombination kontinuierlich über 3 und 7 Tage behandelt, um das metronomische Dosierungsschema zu simulieren. Die eingesetzten Konzentrationen von VRL entsprachen den Serumkonzentrationen von metronomisch behandelten Patient:innen (0,63-5 ng/ml), d. h. viel niedrigeren Konzentrationen im Vergleich zur MTD von CCT [138, 139]. Die eingesetzten Konzentrationen von Alpelisib entsprachen den Serumkonzentrationen von Patient:innen mit der zugelassenen Dosis von 300 mg/Tag (500-1000 ng/ml) [140]. Darüber hinaus wurde Alpelisib im Hinblick auf eine mögliche Verringerung der Nebenwirkungen *in vivo* auch in niedrigeren Konzentrationen (10 ng/ml und 100 ng/ml) getestet. Wir konnten bei der Kombination von niedrig dosiertem metronomischem VRL und Alpelisib eine signifikante Verringerung der Zellviabilität und der Zellproliferation mit synergistischen antitumoralen Effekten bei HR-positiven, HER2-negativen, PIK3CA-mutierten BC-Zelllinien zeigen, nachgewiesen im Isobologramm. Das Wachstum der triple-negativen PIK3CA-Wildtyp-Zelllinien wurde durch VRL signifikant gehemmt, nicht aber durch Alpelisib alleine (**V**). Diese Ergebnisse bestätigen die Hypothese, dass PI3K-Inhibitoren die zytotoxische Aktivität von Mikrotubuli hemmenden Substanzen in PIK3CA-mutierten BC-Zelllinien verstärken können [136, 137, 141].

Taselisib, ein selektiver Inhibitor der PI3Kα-, δ- und γ-Isoformen der Klasse I, und Ipatasertib, ein AKT-Inhibitor, zeigten zusammen mit Mikrotubuli hemmenden Zytostatika einen signifikanten Synergismus in Bezug auf die antiproliferativen, proapoptotischen und antimetastatischen Effekte bei PIK3CA-mutierten BC-Zellen [141]. Zum Vergleich wollten wir in unserer Arbeit das metronomische Dosierungsschema simulieren, indem wir die Zellen über einen längeren Zeitraum kontinuierlich mit einem niedrig dosierten Chemotherapeutikum behandelten. Die

synergistischen zytotoxischen Effekte bei den beiden PIK3CA-mutierten BC-Zelllinien waren nach einer 7-tägigen Behandlung stärker als nach einer 3-tägigen Behandlung, was auf eine günstige Wirkung der kontinuierlichen Langzeitverabreichung von VRL und Alpelisib hindeutet.

In den Western-Blot-Analysen war die p110α-Expression in den PIK3CA-mutierten Zelllinien MCF-7 und T-47D nicht beeinträchtigt bzw. herunterreguliert und in den PIK3CA-Wildtyp-Zelllinien MDA-MB-231 und BT-549 nicht signifikant hochreguliert. Es kann davon ausgegangen werden, dass die p110α-Expression bei den getesteten Konzentrationen in den HR-positiven, HER2-negativen Zelllinien mit einer Überaktivierung von p110α aufgrund der PIK3CA-Mutation keine klare Abhängigkeit von Alpelisib zeigte. Im Gegensatz dazu wiesen die triple-negativen PIK3CA-Wildtyp-Zelllinien Regulationsmechanismen auf, die mit einer Hochregulierung des nicht überaktivierten Proteins als Reaktion auf die Behandlung mit Alpelisib verbunden waren. Zudem konnten wir zeigen, dass auch niedrigere Alpelisib-Konzentrationen (10 ng/ml und 100 ng/ml) in Kombination mit niedrig dosiertem metronomischem VRL zu einer signifikanten Verringerung der Zellviabilität von PIK3CA-mutierten Zellen führten, und die antitumorale Aktivität war mit den Effekten von 1000 ng/ml Alpelisib vergleichbar. Dies deutet darauf hin, dass oberhalb einer bestimmten Konzentration von VRL und Alpelisib keine signifikante Potenzierung der antitumoralen Wirkung beobachtet werden konnte. Die Kombination der Wirkstoffe in niedrigeren Dosen könnte jedoch die Nebenwirkungen verringern, weshalb die Wirksamkeit und Verträglichkeit *in vivo* weiter untersucht werden soll.

Da es Hinweise gibt, dass die Hemmung des PI3K-Signalwegs die Resistenz gegen Zytostatika verhindern und deren Wirksamkeit verstärken kann, könnte die Kombination von PI3K-Inhibitoren mit MCT, die ihre Wirkung multimodal über die Hemmung der Angiogenese, die Immunmodulation, Auswirkungen auf das Tumorstroma und direkte Zytotoxizität entfaltet, einen neuen vielversprechenden Ansatz für die Behandlung von MBC darstellen [20, 136, 137]. Das Ziel wäre es, die synergistischen Effekte auch *in vivo* zu erzielen, Arzneimittelresistenzen zu überwinden und die einzelnen Arzneimitteldosen und die Toxizitäten zu verringern.

In dieser Studie wurden HR-positive, HER2-negative Zelllinien mit einer PIK3CA-Mutation und triple-negative Zelllinien ohne PIK3CA-Mutation analysiert, sodass wir nicht eindeutig sagen können, ob die gezeigte signifikante Verringerung der Zellviabilität und der Zellproliferation allein von der PIK3CA-Mutation abhängt, unabhängig vom HR- und HER2-Status. Obwohl wir die Hypothese aufstellen, dass die PIK3CA-Mutation in erster Linie für die beobachteten synergistischen Effekte und die Wirkung von Alpelisib verantwortlich ist, halten wir die aktuell durch unsere Arbeitsgruppe initiierte Untersuchung von HR-positiven, HER2-negativen BC-Zelllinien ohne PIK3CA-Mutation sowie von triple-negativen Zelllinien mit einer PIK3CA-Mutation für besonders wichtig.

Die vorliegende Arbeit **(V)** demonstrierte eine signifikante Verringerung der Zellviabilität und der Zellproliferation mit synergistischen Effekten durch die Kombinationsbehandlung mit niedrig dosiertem metronomischem VRL und Alpelisib bei HR-positiven, HER2-negativen, PIK3CA-mutierten BC-Zelllinien. Des Weiteren zeigten wir, dass auch niedrigere Konzentrationen von Alpelisib in Kombination mit niedrig dosiertem VRL zu signifikanten antitumoralen Effekten führten [142]. Dies liefert eine Rationale für weitere Bemühungen zur Evaluation dieser Kombination *in vivo* mit dem Ziel der Verbesserung des Toxizitätsprofils.

Weitere Entwicklungen der metronomischen Chemotherapie

Bei den meisten der bisherigen Studien zu MCT beim MBC handelt es sich um Phase-II-Studien, in denen häufig kleine, heterogene Patientengruppen mit unterschiedlichen Wirkstoffen und unterschiedlichen Dosierungen behandelt werden. Aufgrund dieser Tatsachen wurden größere Vergleichsstudien initiiert, um die positiven Auswirkungen der MCT zu verifizieren. Derzeit werden Ergebnisse zu metronomischem VRL 30 mg einmal täglich (EudraCT 2016-002165-63) und 50 mg dreimal wöchentlich (EudraCT 2014-003860-19 und NCT03854617) im Vergleich zum oralen VRL in konventioneller Dosierung als Erst- oder Zweitlinienbehandlung von HER2-negativem MBC erwartet.

Darüber hinaus wurden vielversprechende Ergebnisse mit der Kombination von CAPE 500 mg dreimal täglich und einem Aromatasehemmer mit einer CBR ≥ 24 Wochen von 77,3 % und einem medianen PFS von 16,2 Monaten (95 % CI 6,24-26,17) bei MBC-Patient:innen nach Progress unter einem Aromatasehemmer in der ersten

Therapielinie beobachtet [77]. Die Kombination von MCT und endokriner Therapie könnte dank ihrer nachgewiesenen Wirksamkeit bei gleichzeitig beherrschbarem Toxizitätsprofil im Gegensatz zur Kombination von CCT und endokriner Therapie eine potenzielle Therapiestrategie für Patient:innen mit einem HR-positiven MBC darstellen, bei denen CDK 4/6-Inhibitoren kontraindiziert oder nicht verfügbar sind [70, 143-145]. Weitere Erkenntnisse über dieses interessante Therapieschema werden von den laufenden Studien (NCT02767661, NCT04571437, NCT02583828, NCT05411380) erwartet.

Auch bei HER2-positivem MBC werden neue Therapiestrategien mit MCT entwickelt [53, 146]. Die oral verfügbare Anti-HER2-Substanz Pyrotinib wird aktuell in Kombination mit metronomischem CAPE 500 mg dreimal täglich untersucht (NCT03923166). Eine weitere Phase-II-Studie (NCT04941885) testet Inetetamab, einen neuen monoklonalen Anti-HER2-Antikörper, in Kombination mit metronomischem CTX 50 mg einmal täglich und einem Aromatasehemmer bei Patient:innen mit einem HR-positiven, HER2-positiven MBC.

Aufgrund der immunmodulatorischen Wirkung der MCT sind Studien zur Kombination von MCT und Immuncheckpoint-Inhibitoren wie Anti-PD-1- und Anti-PD-L1-Wirkstoffen von besonderem Interesse [147]. Präklinisch gibt es bereits Hinweise, dass MCT die Effektivität von Anti-PD-L1-Substanzen verstärken kann [148-151]. In der Phase-I-Studie Movie wurde die Kombination von Durvalumab, einem PD-L1-Inhibitor, Tremelimumab, einem CTLA-Inhibitor, und metronomischem VRL bei 14 Patient:innen mit einem fortgeschrittenen Karzinom, davon neun Patient:innen mit MBC untersucht. Insgesamt waren die Toxizitäten wie Colitis, Diarrhoe und Hyperthyreose gut beherrschbar und die Krankheitskontrolle wurde bei der Hälfte der Patient:innen dokumentiert, sodass diese Kombination weiter in einer Phase-II-Studie (NCT03518606) untersucht wird [152]. Außerdem werden in Phase-II-Studien weitere Kombinationen wie zum Beispiel CTX/Pembrolizumab (NCT03139851, NCT03971045), VRL/Toripalimab (NCT04389073) und CTX/Avelumab/Pexa-Vec (NCT02630368) bei Patient:innen mit MBC untersucht.

Neben dem Fokus auf die Behandlung des MBC gibt es zunehmend Daten zum Einsatz von MCT in früheren Stadien der Erkrankung [153-155]. Die meisten klinischen

Studien untersuchen MCT als Erhaltungstherapie nach abgeschlossener adjuvanter Chemotherapie oder als (neo-)adjuvante Therapie bei Patient:innen, bei denen eine CCT nicht in Frage kommt [156]. Das Ziel der Behandlung ist es, das krankheitsfreie Überleben zu verlängern bzw. die Rate an pathologischer Remission zu erhöhen. Bianco et al. untersuchten 223 Patient:innen mit TNBC im Stadium II oder III bei Erstdiagnose, die eine Anthrazyklin/Taxan-haltige neoadjuvante Chemotherapie erhielten und darunter keine pathologische Remission erfuhren. Die Patient:innen wurden postneoadjuvant mit einem CMF (CTX, MTX, 5-Fluorouracil)-Standardschema oder einer metronomischen Kombination CTX/CAPE oder CTX/MTX behandelt. 16,6 % der Patient:innen erhielten keine adjuvante Chemotherapie. Das mediane Follow-up betrug 9,9 Jahre. In dieser retrospektiven Studie konnte die MCT im Gegensatz zum CMF-Standardschema im Vergleich zum Chemotherapie-freien Vorgehen einen Benefit in Bezug auf das krankheitsfreie Überleben mit einer Hazard Ratio von 0,46 und 95% CI 0,26-0,81, p=0,008 zeigen [157].

In der randomisierten Phase-III-Studie IBCSG wurden insgesamt 1086 Patient:innen mit einer HR-negativen BC-Erkrankung nach Abschluss der adjuvanten Standard-chemotherapie mit oder ohne metronomische Kombination CTX/MTX im Rahmen einer Erhaltungstherapie für ein Jahr behandelt. 18,9 % der Patient:innen wiesen eine HER2-positive Erkrankung auf und erhielten zusätzlich Trastuzumab. In der Gesamtpopulation führte die erweiterte adjuvante Therapie mit CTX/MTX zu keiner Verbesserung des krankheitsfreien Überlebens. In der TNBC-Kohorte mit einem positiven Nodalstatus kam es jedoch zu einer relativen und absoluten Reduktion der Rezidivrate um 24 % bzw. 7,9 % [153]. Auch weitere Studien beim frühen BC unterstützen die Annahme, dass MCT möglicherweise durch die immun-modulatorischen Wirkmechanismen insbesondere bei TNBC klinisch bedeutsam sein könnte [158-161].

Zusammenfassung

Trotz der Vielzahl potenzieller Behandlungsoptionen für MBC besteht nach wie vor ein hoher medizinischer Bedarf an neuen Behandlungskonzepten, die eine ausreichende und langfristige antitumorale Wirkung bei einem überschaubaren Toxizitätsprofil bieten. Die MCT ist definiert als eine kontinuierliche Verabreichung niedrig dosierter Zytostatika und wirkt im Vergleich zur CCT nicht nur zytotoxisch sondern multimodal

[19, 20, 53]. Die MCT wird daher zunehmend als eine mögliche Behandlungsoption für MBC angesehen [2, 8]. Dennoch sind die Daten unzureichend, um unter anderem zu definieren, welche Patient:innen am meisten profitieren und welcher Wirkstoff beziehungsweise welche Wirkstoffkombination am besten geeignet ist.

In unserer Real-World-Analyse **(I)** lag die DCR ≥ 24 Wochen 31,9 % und das mediane PFS und OS betrugen 17,0 Wochen und 58,0 Wochen. CTX/MTX war mit dem Anteil von 86,1 % aller Patientinnen das am häufigsten verwendete metronomische Therapieschema, gefolgt von VRL bei 6,9 %, CTX/CAPE bei 4,2 % und CTX bei 2,8 % der Patientinnen. Es gab keine signifikanten Unterschiede hinsichtlich der DCR und Überlebensraten bei den verschiedenen metronomischen Schemata [54]. In der Kohortenanalyse mit CTX/MTX **(II)** wurde die DCR ≥ 24 Wochen bei 31,4 % der Patientinnen erreicht. 43 % der Patientinnen zeigten ein Therapieansprechen und die mediane DoR lag bei 22 Wochen [58]. Diese Ergebnisse bestätigen die Erkenntnisse aus früheren Studien zu MCT, wobei der Vergleich dieser Daten aufgrund der heterogenen Patientenpopulationen, die mit unterschiedlichen metronomischen Therapieschemata behandelt worden waren, nur bedingt möglich ist.

Die Studienlage zum Vergleich der MCT mit den derzeitigen Standardtherapien ist limitiert, sodass die genaue Positionierung der MCT im Behandlungsalgorithmus für MBC erschwert ist. In unserer Fall-Kontroll-Studie **(III)** mit insgesamt 120 MBC-Patientinnen wurde die Wirksamkeit der MCT mit der CCT in Bezug auf Alter, Ausmaß der Metastasierung, Chemotherapielinie und HR-/HER2-Status verglichen. Der primäre Endpunkt DCR ≥ 24 Wochen unterschied sich nicht signifikant zwischen der MCT- und der CCT-Population (30,0 % versus 22,5 %, p=0,380). Das mediane PFS betrug sowohl in der MCT- als auch in der CCT-Gruppe 12,0 Wochen (p=0,218). Auch bei der DoR (31,0 versus 20,5 Wochen, p=0,383) und beim Therapieansprechen (37,5 % versus 30,0 %, p=0,417) zeigten sich keine signifikanten Unterschiede zwischen den beiden Therapiekonzepten [59].

In allen drei retrospektiven Studien **(I-III)** wurden Subgruppenanalysen durchgeführt, um eine Patientenpopulation zu identifizieren, die möglicherweise besonders von der MCT profitieren könnte. Wir zeigten, dass MCT auch eine Behandlungsoption für jüngere Patient:innen darstellen kann. Das mediane PFS (17,0 Wochen sowohl bei

jüngeren als auch bei älteren Patientinnen) und das mediane OS (58,0 Wochen bei jüngeren versus 52,0 Wochen bei älteren Patientinnen) waren hinsichtlich des Alters ohne signifikante Unterschiede (p=0,389 bzw. p=0,237). Außerdem beobachteten wir ein signifikant längeres medianes PFS (34,0 Wochen versus 13,0 Wochen, p<0,001) und OS (91,0 Wochen versus 39,0 Wochen, p=0,003) in der Subgruppe ohne multiple Metastasen im Vergleich zu der Subgruppe mit multiplen Metastasen **(I)** [54]. Die DCR ≥ 24 Wochen betrug bei Patientinnen ohne multiple Metastasen 36,0 % in der MCT-Gruppe und 18,0 % in der CCT-Gruppe (p=0,096) und das mediane PFS betrug 16,0 Wochen versus 12,0 Wochen (p=0,064) mit einem Trend zur Signifikanz **(III)** [59]. Die Wirksamkeit der MCT war in unseren Studien unabhängig von der Therapielinie, sodass sowohl wenig als auch stark vorbehandelte Patient:innen von diesem Therapieschema profitieren können. Die prospektive Phase-II-Studie VinoMetro **(IV)** untersuchte zum ersten Mal ein metronomisches Schema mit einer täglichen oralen VRL-Gabe von 30 mg als Erstlinienchemotherapie bei HR-positiven, HER2-negativen MBC-Patientinnen nach endokriner Resistenz. Der primäre Endpunkt CBR ≥ 24 Wochen lag bei 22,2 % und damit deutlich unter der erwarteten CBR, wobei die Studie nach Einschluss von neun Patientinnen aufgrund einer Toxizität Grad 5 frühzeitig beendet worden war [75]. Neben der Wirksamkeit der MCT bei Patientinnen mit einer HR-positiven Erkrankung konnten wir zeigen, dass auch Patientinnen mit TNBC von der MCT profitieren können. Das mediane PFS betrug sowohl in der HR-positiven Subgruppe als auch in der TNBC-Subgruppe 17,0 Wochen (p=0,144). Das mediane OS betrug 58,0 Wochen bzw. 52,0 Wochen (p=0,379) **(I)** [54]. Bei den Patientinnen mit TNBC blieb die DCR ≥ 24 Wochen, ähnlich wie in der HR-positiven Subgruppe, in der Gruppe der metronomisch behandelten Patientinnen bei 30,0 %, in der Gruppe der CCT sank die DCR auf 5,0 % (p=0,095) **(III)** [59].

Unsere Ergebnisse deuten darauf hin, dass das Alter, Ausmaß der Metastasierung, Therapielinie und HR-Status nicht die entscheidende Rolle bei der MCT spielen. Grundsätzlich gilt jedoch, dass aufgrund der effektiven und relativ gut verträglichen Substanzen MCT erst in späteren Therapielinien empfohlen wird. Ein früher Einsatz der MCT kann unter Umständen bei älteren Patient:innen und Patient:innen mit Komorbiditäten, für die eine CCT nicht geeignet ist, sinnvoll sein. Die MCT kann aber auch aufgrund des relativ geringen Toxizitätsprofils eine Therapiealternative für Patient:innen mit Wunsch nach einer nebenwirkungsarmen Therapie darstellen. Die

Rate an Therapieabbrüchen aufgrund von unerwünschten Ereignissen lag in unseren Kohorten bei 4,2 % bzw. 8,6 % **(I, II)** [54, 58].

Angesichts des zunehmenden Einsatzes von zielgerichteten immunmodulatorischen und antiangiogenen Therapien bei MBC und der multimodalen Eigenschaften der MCT könnte die Kombination dieser Wirkstoffe einen neuen vielversprechenden Ansatz bieten. In unserer präklinischen Arbeit **(V)** konnten wir nachweisen, dass die Kombination von niedrig dosiertem VRL und Alpelisib, einem oralen α-selektiver PI3K-Inhibitor und Degrader, zu einer signifikanten Verringerung der Zellviabilität und der Zellproliferation mit synergistischen Effekten bei HR-positiven, HER2-negativen, PIK3CA-mutierten BC-Zelllinien führte. Darüber hinaus wurden auch bei niedrigeren Dosen von Alpelisib in Kombination mit VRL antitumorale Effekte beobachtet, was eine Grundlage für weitere Untersuchungen dieser Kombination *in vivo* liefert [142].

Zusammenfassend kann angenommen werden, dass die MCT aufgrund der nachgewiesenen Wirksamkeit und der niedrigen Nebenwirkungsrate als eine wertvolle Behandlungsoption bei ausgewählten Patient:innen angesehen werden kann. Um jedoch die angemessene Rolle der MCT im Behandlungsalgorithmus für MBC festzulegen und die am besten geeignete Patientenpopulation zu ermitteln, sollte die MCT in randomisierten Studien im Vergleich zu aktuellen Standardtherapien untersucht werden.

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Real-World Experience of Metronomic Chemotherapy in Metastatic Breast Cancer: Results of a Retrospective Unicenter Study

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Metronomic chemotherapy · Metastatic breast cancer · Disease control rate · Survival · Toxicity

Abstract

Introduction: Metronomic chemotherapy (MCT) is increasingly used in oncology due to its favorable therapeutic index. There is still a lack of evidence for MCT in metastatic breast cancer (MBC). In this retrospective unicenter study, we demonstrated real-word data on MCT in MBC. ***Methods:*** MBC patients who received metronomic oral cyclophosphamide (CTX) (50 mg daily) and methotrexate (MTX) (2.5 mg every other day), CTX and capecitabine (CAPE) (500 mg thrice daily), CTX, or vinorelbine (VRL) (30 mg daily) alone for at least 4 weeks between 2009 and 2021 were included. The primary endpoint was disease control rate (DCR) ≥24 weeks. Secondary endpoints were progression-free survival (PFS) and overall survival (OS). Patient characteristics and therapy response were analyzed using χ^2 test. For survival analyses, Kaplan-Meier estimator and log-rank test were used. ***Results:*** Seventy-two patients were identified. Sixty-two patients received CTX/MTX, three CTX/CAPE, two CTX, and five VRL. Median age at diagnosis MBC and at start of MCT was 59.0 years and 64.5 years, respectively. 72.2% tumors were hormone receptor positive and 27.8% were triple-negative. 54.2% patients had more than two different metastases. 80.6% patients showed visceral involvement. 31.9% patients achieved DCR ≥24 weeks. Median PFS was 17.0 weeks (95% CI 14.5–19.5) and median OS was 58.0 weeks (95% CI 29.0–87.0). MCT showed similar DCR ≥24 weeks and clinically meaningful but not statistically significant shorter median PFS compared to prior therapy (31.9% versus 32.8% [p = 0.570] and 17.0 weeks versus 20.0 weeks [p = 0.093], respectively) and statistically significant higher DCR ≥24 weeks and longer median PFS compared to subsequent therapy (31.9% versus 17.4% [p = 0.038] and 17.0 weeks versus 12.0 weeks [p = 0.006], respectively). Three (4.2%) patients terminated MCT because of toxicity. ***Conclusion:*** In this real-world retrospective study, MCT was effective and well tolerated and may thus represent a valuable treatment option in selected MBC patients.

Introduction

Metastatic breast cancer (MBC) represents one of the leading cause of cancer mortality worldwide [1]. In general, MBC is an incurable disease with a median overall survival (OS) of about 3 years and a 5-year survival rate of about 25% [2, 3]. Hormone receptor (HR), human epidermal growth factor receptor 2 (HER2), breast cancer 1/2 gene (BRCA 1/2), phosphatidylinositol-4,5-bisphos-

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phate 3-kinase catalytic subunit alpha, programmed death-ligand 1 status, as well as biological age, tumor burden, and prior therapies critically influence prognosis [2–4]. Considering these prognostic factors, the therapeutic goal is to achieve chronification of the disease with a prolongation of survival and preservation of quality of life [5].

Metronomic chemotherapy (MCT), defined as a chronic administration of conventional chemotherapeutic agents at low doses without prolonged drug-free breaks, was first mentioned by Hanahan and Kerbel in early 2000 [6, 7]. Since then, numerous studies with MCT have shown promising results. Most of the data originate from phase 2 and retrospective trials, with breast cancer being the most studied tumor entity. As there is growing evidence of efficacy and good tolerability, MCT is increasingly appreciated as a possible treatment option for MBC [2, 3]. Oral administration of MCT is safe and popular among patients because of its ease of use and flexibility of drug dosing in case of toxicities [8–10]. Dose accumulations associated with intolerable side effects are rare, so the medication can be administered for longer periods of time [11]. The mechanisms of action are not truly cytotoxic but rather multimodal, particularly via inhibition of angiogenesis, immunomodulation, and effects on tumor stroma [6, 7, 12, 13]. It is assumed that MCT is not simply a different way of administering chemotherapy (CT) but a truly new treatment option [14, 15]. Nevertheless, the data are still insufficient to identify which patients will benefit most and which agent or combination is most appropriate. This retrospective study aimed to investigate the efficacy and toxicity of MCT in real-world settings, taking into account the treatment prior and subsequent to MCT as well as clinicopathological parameters.

Materials and Methods

Study Population and Treatment

MBC patients who received MCT in the form of oral cyclophosphamide (CTX) (50 mg daily) and methotrexate (MTX) (2.5 mg every other day), CTX (50 mg daily) and capecitabine (CAPE) (500 mg thrice daily), CTX (50 mg daily) alone, or vinorelbine (VRL) (30 mg daily) for at least 4 weeks between February 2009 and December 2021 at the University Medical Center Mainz were selected for this retrospective analysis. Patients with HER2-positive tumors and patients with presence of additional cancer were excluded. No antiemetic treatment was routinely given to patients during MCT. Clinicopathological and follow-up data until March 2022 were collected as previously reported by our group [16]. Patients who had not progressed or died by the cutoff date of March 2022 were censored. The manuscript was written in accordance with the STROBE Statement checklist for cohort studies of the EQUATOR network reporting guidelines [17].

Clinical Outcomes

The primary endpoint was disease control rate (DCR) ≥24 weeks. DCR included stable disease, partial response, and complete response. Secondary endpoints were progression-free survival (PFS) and OS. Disease-free interval was defined as the time from completion of primary therapy for early breast cancer to the evidence of recurrence or metastatic disease. The therapy efficacy was assessed using the standard clinical and imaging methods. Assessment of safety and tolerability of MCT was conducted according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03. For subgroup analyses, we stratified the patients by age at start of MCT (younger: ≤ median age vs. older: > median age), HR status (HR positive: estrogen/progesterone receptor positive and HER2 negative vs. triple negative: estrogen/progesterone receptor negative and HER2 negative), number of different metastatic sites (without multiple metastases: ≤2 different metastatic sites vs. multiple metastases: >2 different metastatic sites), and number of prior CT lines (nonheavily pretreated: <2 CT lines vs. heavily pretreated: ≥2 CT lines).

Statistical Analyses

Statistical analyses were performed using the SPSS statistical software system, version 27.0 (SPSS Inc., Chicago, IL, USA). Patient characteristics were analyzed descriptively using median and range for continuous data and relative and absolute frequencies for categorical data. The associations between clinicopathological characteristics and therapy response were analyzed by applying a Pearson's χ^2 test. For PFS and OS analyses, Kaplan-Meier estimator and log-rank test were used. All tests were two sided and a $p < 0.05$ was considered statistically significant.

Results

Patient Characteristics

A total of 72 patients were included into the study (Table 1). Conventional CT was administered in 40 (59.7%) patients as the main therapy immediately prior to MCT and in 36 (78.3%) patients subsequent to MCT. Five (6.9%) patients received MCT as first-line therapy and 62 (86.1%) patients showed progressive disease from prior treatment at the start of MCT. No concomitant treatment such as radiotherapy, endocrine or targeted therapy was observed in this cohort.

Therapy Response

DCR ≥24 weeks was achieved in 23 (31.9%) patients (Table 2). Median PFS and OS were 17.0 weeks (95% confidence interval [CI] 14.5–19.5) and 58.0 weeks (95% CI 29.0–87.0), respectively. CTX/MTX was the most common metronomic regime with DCR ≥24 weeks of 30.6%. There were no significant differences in DCR and survival between different metronomic regimens, with 62, 3, 2, and 5 patients receiving CTX/MTX, CTX/CAPE, CTX, and VRL, respectively. Compared to MCT, therapy prior to MCT showed a similar DCR ≥24 weeks of 32.8% ($p = 0.570$) and therapy subsequent to MCT showed a significantly lower DCR ≥24 weeks of 17.4% ($p = 0.038$) (Table 2).

Table 1. Patient characteristics

Characteristics	Patients ($n = 72$), n (%)
Age at diagnosis of BC, years	
Median	50.5
Range	29.0–80.0
Age at diagnosis of MBC, years	
Median	59.0
Range	33.0–86.0
Age at start of MCT, years	
Median	64.5
Range	35.0–87.0
Time between diagnosis of MBC and start of MCT	
≤2 years	31 (43.1)
≤5 years	24 (33.3)
>5 years	17 (23.6)
HR status	
HR positive	52 (72.2)
HR negative (TNBC)	20 (27.8)
Disease status	
De novo MBC	10 (13.9)
DFI ≤5 years	20 (27.8)
DFI ≤10 years	21 (29.2)
DFI >10 years	21 (29.2)
Metastatic sites	
Median	3.0
Range	1–7
Number of metastatic sites	
≤2	33 (45.8)
>2	39 (54.2)
Metastatic sites	
Bone	49 (68.1)
Liver	35 (48.6)
Lung	34 (47.2)
Pleura	20 (27.8)
Peritoneum	13 (18.1)
Distant lymph nodes	36 (50.0)
Cerebrum	7 (9.7)
Soft Tissue	16 (22.2)
Prior therapy for metastatic disease	
CT	
Median	1.5
Range	0–6
Lines of CT	
<2	36 (50.0)
≥2	36 (50.0)
Endocrine therapy	
Median	2.0
Range	0–7
Lines of endocrine therapy	
<2	28 (38.9)
≥2	44 (61.1)
Agents for endocrine therapy	
SERM	11 (15.3)
SERD	41 (56.9)
AI nonsteroidal	42 (58.3)
AI steroidal	31 (43.1)
GnRH agonist	6 (8.3)
Targeted therapy	
None	40 (55.6)
Anti-HER2 therapy	3 (4.2)
VEGF inhibitor	14 (19.2)
PD-L1 inhibitor	1 (1.4)
mTOR inhibitor	20 (27.8)
CDK 4/6 inhibitor	10 (13.9)
PARP inhibitor	1 (1.4)
Anti-resorptive therapy	
None	25 (34.7)
Bisphosphonates	31 (43.1)
Denosumab	20 (27.8)
Radiotherapy	
None	26 (36.1)
Bone	39 (54.2)
Cerebrum	7 (9.7)

MCT, metronomic chemotherapy; BC, breast cancer; MBC, metastatic breast cancer; HR, hormone receptor; TNBC, triple-negative breast cancer; DFI, disease-free interval; SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor degrader; AI, aromatase inhibitor; GnRH, gonadotropin-releasing hormone; HER2, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor; PD-L1, programmed death-ligand 1; mTOR, mammalian target of rapamycin; CDK, cyclin-dependent kinase; PARP, poly-ADP-ribose polymerase.

Regarding age, median PFS (17.0 weeks in both younger and older patients) and OS (58.0 weeks in younger vs. 52.0 weeks in older patients) did not show any significant differences ($p = 0.389$ and $p = 0.237$, respectively) (Fig. 1a). Median PFS in both HR-positive and triple-negative subgroup was 17.0 weeks ($p = 0.144$) (Fig. 1b). Median OS was 58.0 weeks (95% CI 17.2–98.8) in patients with HR-positive disease and 52.0 weeks (95% CI 11.0–93.0) in patients with triple-negative breast cancer (TNBC) ($p = 0.379$). Patients without multiple metastases had significantly longer median PFS and OS than those with multiple metastases (34.0 weeks [95% CI 20.6–47.4] vs. 13.0 weeks [95% CI 11.5–14.5], $p < 0.001$, and 91.0 weeks [95% CI 53.7–128.3] vs. 39.0 weeks [95% CI 26.1–51.9], $p = 0.003$, respectively) (Fig. 1c). Median PFS and OS did not differ between nonheavily pretreated and heavily pretreated patients (17.0 weeks [95% CI 15.8–18.2] vs. 13.0 weeks [95% CI 9.3–16.7], $p = 0.764$, and 52.0 weeks [95% CI 2.0–102.0] vs. 58.0 weeks [95% CI 31.4–84.6], $p = 0.457$, respectively) (Fig. 1d). In terms of prior and subsequent therapy, MCT showed a clinically relevant but not statistically significant shorter median PFS compared to prior therapy (17.0 weeks vs. 20.0 weeks, $p = 0.093$) and a significantly longer median PFS compared to subsequent therapy (17.0 weeks vs. 12.0 weeks, $p = 0.006$) (Table 2).

Safety Results

The most common adverse events leading to discontinuation of MCT (≥ grade 3) were nausea/vomiting and diarrhea (2 patients) and thrombocytopenia (1 patient) (Table 3). In the CTX/MTX group, 5 (8.1%) patients discontinued MTX without termination of CTX. Two (3.2%) patients terminated MTX due to nausea/vomiting and each (1.6%) patient due to fatigue, mucositis, and vision impairment, respectively. Neutropenia or anemia ≥ grade 3 did not occur in our cohort. Overall, MCT was terminated early due to toxicity in 3 (4.2%) patients and low performance status in 7 (9.7%) patients, which was similar compared with prior and subsequent treatment (7.5% and 10.9% due to toxicity and 0.0% and 10.9% due to low performance status in prior and subsequent therapy, respectively).

Discussion

In this retrospective unicenter study analyzing MCT in real-world settings, CTX/MTX was the most common metronomic regimen, accounting for 86.1% of all patients, followed by VRL in 6.9%, CTX/CAPE in 4.2%, and CTX in 2.8% patients. No concomitant treatment such as radiotherapy, endocrine or targeted therapy was given in our cohort. In the largest multicenter retrospective study VICTOR-6, which collected data of 597 MBC patients who received MCT between January 2011 and December 2016 in 43 Italian Oncology sites, most patients (79.3%) received MCT as single agent (VRL 34.6%, CAPE 22.3%, and CTX 20.7%). The use of VRL- and CTX-based regimens increased during the observation period (2011: 16.8% and 17.1%, 2016: 29.8% and 25.6%, respectively) [11]. In a meta-analysis conducted by Liu et al. [18], subgroup analysis did not show any significant difference in the clinical benefit rate (CBR) among different metronomic agents as well as between MCT alone and the combination regimens. In the present study, there were also no significant differences regarding DCR and survival among the different metronomic regimens.

The primary endpoint DCR ≥24 weeks was 31.9%, which was in line with the results of previous studies [11, 19–21]. In the VICTOR-6 study, the relatively high DCR of 74.4% could be attributed to the fact that patients were included into the study regardless of their response to prior treatment. In addition, the definition of the timing of DCR was not clearly specified. Finally, the patient populations studied were very heterogeneous and can only be compared with caution [11]. In our analysis, which included only patients with disease progression or intolerable toxicity from prior treatment, CTX/MTX was the most common metronomic regimen with a DCR ≥24 weeks of 30.6%. The results were consistent with a DCR

Table 2. Therapy response to MCT and its prior and subsequent therapy

Therapy	MCT overall	CTX/MTX	CTX/CAPE	CTX	VRL	Therapy prior to MCT	Therapy subsequent to MCT
Patients, *n* (%)	72	62 (86.1)	3 (4.2)	2 (2.8)	5 (6.9)	67	46
DCR ≥24 weeks, *n* (%)	23 (31.9)	19 (30.6)	1 (33.3)	1 (50.0)	2 (40.0)	22 (32.8)	8 (17.4)
p value	0.919					0.570	0.038
SD/PR/CR (≥24 weeks), *n* (%)	15 (20.8)/7 (9.7)/1 (1.4)	11 (17.7)/7 (11.3)/1 (1.6)	1 (33.3)/0 (0.0)/0 (0.0)	1 (50.0)/0 (0.0)/0 (0.0)	2 (40.0)/0 (0.0)/0 (0.0)	14 (20.9)/8 (11.9)/0 (0.0)	8 (17.4)/0 (0.0)/0 (0.0)
p value	0.932					0.763	0.001
PFS (weeks) (median, 95% CI)	17.0 (14.5–19.5)	17.0 (13.9–20.1)	17.0 (9.0–25.0)	4.0 (N/A)	17.0 (15.9–18.1)	20.0 (16.6–23.4)	12.0 (9.1–14.9)
p value	0.410					0.093	0.006
OS (weeks) (median, 95% CI)	58.0 (29.0–87.0)	47.0 (25.7–68.3)	138.0 (N/A)	4.0 (N/A)	160.0 (112.8–207.2)	N/A	N/A
p value	0.127					N/A	N/A

CTX, cyclophosphamide; MTX, methotrexate; CAPE, capecitabine; VRL, vinorelbine; DCR, disease control rate; SD, stable disease; PR, partial response; CR, complete response; CI, confidence interval; PFS, progression-free survival; OS, overall survival.

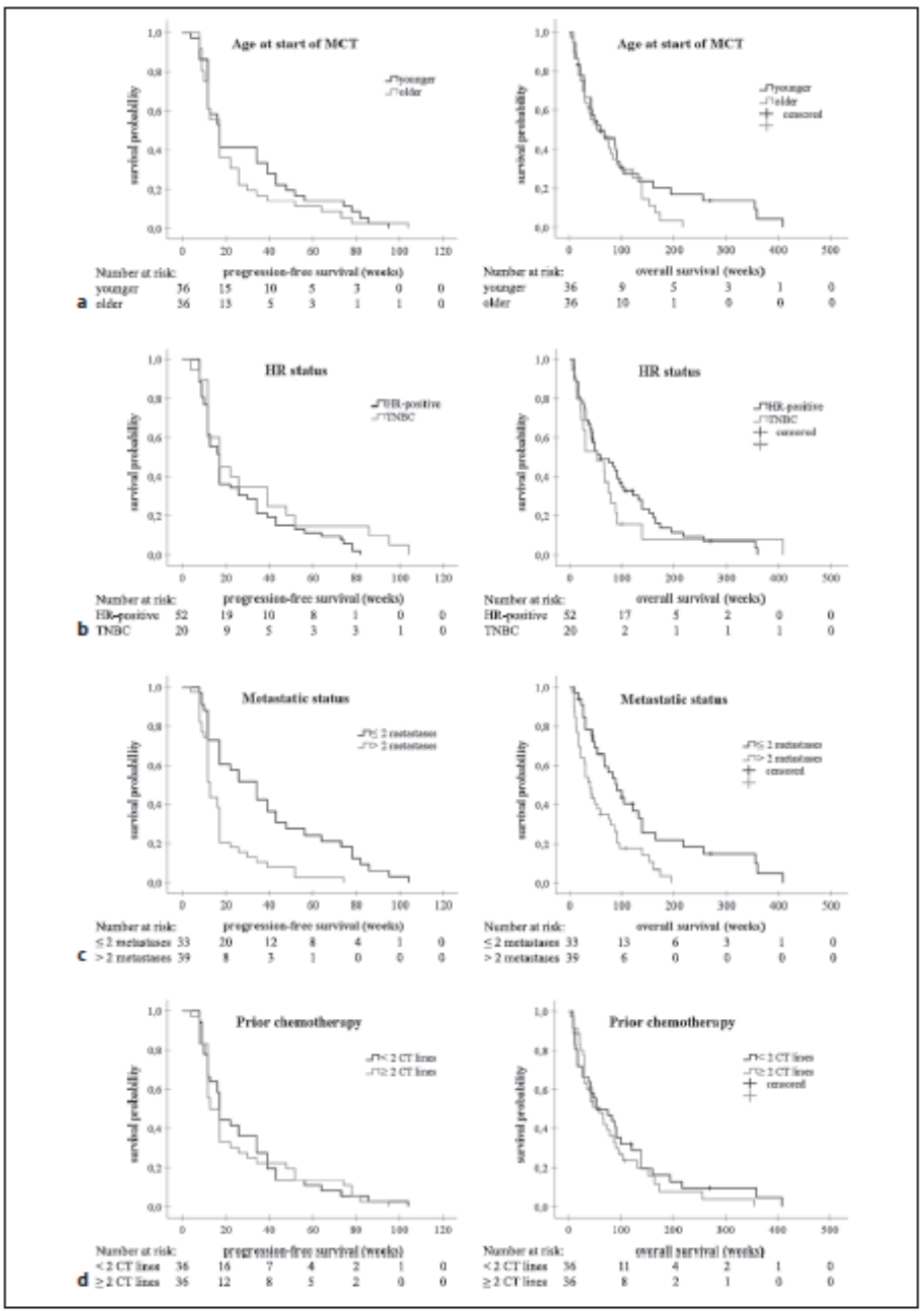

Fig. 1. Kaplan-Meier analyses of PFS and OS regarding age at start of MCT (**a**), HR status (**b**), number of different metastatic sites (**c**), and number of prior CT lines (**d**). **a** Younger versus older: median PFS: both 17.0 weeks, log rank: $p = 0.389$, median OS: 58.0 weeks versus 52.0 weeks, log rank: $p = 0.237$. **b** HR positive versus TNBC: median PFS: both 17.0 weeks, log rank: $p = 0.144$, median OS: 58.0 weeks versus 52.0 weeks, log rank: $p = 0.379$. **c** ≤2 metastases versus >2 metastases: median PFS: 34.0 weeks versus 13.0 weeks, log rank: $p < 0.001$, median OS: 91.0 weeks versus 39.0 weeks, log rank: $p = 0.003$. **d** <2 prior CT lines versus ≥2 prior CT lines: median PFS: 17.0 weeks versus 13.0 weeks, log rank: $p = 0.764$, median OS: 52.0 weeks versus 58.0 weeks, log rank: $p = 0.457$. MCT, metronomic chemotherapy; HR, hormone receptor; TNBC, triple-negative breast cancer; PFS, progression-free survival; OS, overall survival; CT, chemotherapy.

Table 3. Therapy termination

a Adverse events leading to discontinuation of MCT

MCT agent		Patients ($n = 72$), n (%)
CTX/MTX		62
	PD	46 (74.2)
	Death	6 (9.7)
	Low performance status	7 (11.3)
	Nausea/vomiting and diarrhea	2 (3.2)
	Thrombocytopenia	1 (1.6)
MTX alone (CTX not terminated)	Fatigue	1 (1.6)
	Nausea/vomiting	2 (3.2)
	Mucositis	1 (1.6)
	Vision impairment	1 (1.6)
CTX/CAPE		3
	PD	3 (100.0)
CTX		2
	PD	1 (50.0)
	Death	1 (50.0)
VRL		5
	PD	5 (100.0)

b Termination of MCT compared to prior and subsequent therapy

Therapy		Therapy prior to MCT	MCT	p value	Therapy subsequent to MCT	p value
Patients, n		67	72		46	
Therapy termination	PD	62 (92.5)	55 (76.4)	0.255	28 (60.9)	0.486
	Death	0 (0.0)	7 (9.7)		7 (15.2)	
	Toxicity	5 (7.5)	3 (4.2)		5 (10.9)	
	Low performance status	0 (0.0)	7 (9.7)		5 (10.9)	
	Without termination	0 (0.0)	0 (0.0)		1 (2.2)	

MCT, metronomic chemotherapy; CTX, cyclophosphamide; MTX, methotrexate; PD, progressive disease; CAPE, capecitabine; VRL, vinorelbine.

≥24 weeks of 31.2% in the Chinese population, 40.3% of whom received CTX/MTX as maintenance therapy without disease progression from previous treatment [20]. Survival analyses revealed median PFS and OS of 17.0 weeks and 58.0 weeks, respectively, which was consistent with the results of previous studies [11, 20, 22, 23].

In the subgroup analyses, we observed a significantly longer median PFS (34.0 weeks vs. 13.0 weeks, $p < 0.001$) and OS (91.0 weeks vs. 39.0 weeks, $p = 0.003$) in the subgroup without multiple metastases compared to the subgroup with multiple metastases. It can be assumed that patients with few metastases are more likely to have less aggressive disease with a lower probability of extensive visceral involvement. We did not find any significant differences regarding age at start of MCT, HR status, and CT line in survival analyses. In the VICTOR-1 study, metronomic combination of VRL and CAPE had an acceptable efficacy profile (overall response rate 33%, CBR 67%) and was well tolerated in MBC patients aged ≥70 years [24]. Also in older and frail HER2-positive MBC patients, addition of metronomic CTX to trastuzumab plus pertuzumab increased median PFS by 7 months compared to dual HER2 blockade alone and was relatively well tolerated [25]. Patients with HR-positive, HER2-negative metastatic disease resistant to endocrine-based therapy and who do not require rapid tumor response are generally suitable for MCT [2, 14]. However, MCT may also be promising in patients with TNBC, as demonstrated in the current analysis, which did not show any significant differences in survival between the HR-positive and the TNBC cohort. In the subgroup analysis of the VICTOR-6 study, patients with triple-negative MBC showed a median PFS, OS, and DCR of 6.0 months (95% CI 4.9–7.2), 12.1 months (95% CI 9.6–16.7), and 64.9%, respectively [26]. In the present study, similar to most trials, patients with HER2-positive tumors were excluded. In recent years, there has been an increasing use of the combination of MCT with anti-HER2 therapies. In the HEX trial, the

combination of standard trastuzumab and metronomic CTX (50 mg once daily) plus CAPE (500 mg thrice daily) in 60 patients with untreated HER2-positive MBC demonstrated favorable efficacy with a median PFS of 11.0 months (95% CI 6.3–15.6) and a CBR of 78.2% [27]. Also, the combination of metronomic VRL (40 mg thrice weekly) and trastuzumab showed activity with a median PFS of 7.4 months (95% CI 3.2–11.5) and a CBR of 75% [28].

MCT showed similar DCR ≥24 weeks and clinically meaningful but not statistically significant shorter median PFS compared to prior therapy (31.9% vs. 32.8% [p = 0.570] and 17.0 weeks vs. 20.0 weeks [p = 0.093], respectively) and statistically significant higher DCR ≥24 weeks and longer median PFS compared to subsequent therapy (31.9% vs. 17.4% [p = 0.038] and 17.0 weeks vs. 12.0 weeks [p = 0.006], respectively). Based on these results, it can be assumed that MCT was as effective as standard prior therapy and that patients experienced significant disease progression during subsequent therapy. It is important to note that MCT was administered in 5 (6.9%) patients in first-line setting and 24 (33.3%) patients received no further therapy after MCT because of low performance status or death. Nearly 20% of patients terminated MCT due to low performance status or death. This suggests that there were many frail and pretreated patients in our cohort, so this should be taken into consideration when interpreting and comparing the results with other studies. The extent to which the efficacy of MCT, as illustrated by comparable DCR ≥24 weeks and median PFS with the prior treatment line, might improve OS remains speculative because of several limitations. This single-arm study was designed to describe the experience of a single institution. A cross-study comparison with historical cohorts of MBC is difficult due to the heterogeneity of the cohort, which included patients in first-line setting as well as heavily pretreated patients who were treated with different agents.

MCT was well tolerated. Only 3 (4.2%) patients terminated MCT due to toxicity, which was similar compared with prior and subsequent treatment. The favorable toxicity profile of MCT with similar results has also been demonstrated in previous studies in which 2.2–14.9% of patients discontinued MCT due to toxicity [11, 20, 23, 29]. Compared with conventional CT, MCT is associated with fewer adverse events such as polyneuropathy, myelosuppression, mucositis, and hair loss and may thus contribute substantially to the maintenance of quality of life, which is one of the main goals of treatment at this stage [19, 30]. However, to date, data allowing a direct comparison between MCT and conventional CT in terms of tolerability and quality of life are lacking.

There were limitations in the present study. The retrospective design can lead to selection bias, so the interpretation of the presented results is limited. The vast majority of patients received MCT before or at the time of introduction of cyclin-dependent kinase 4/6 inhibitors for HR-positive, HER2-negative MBC and pembrolizumab for triple-negative MBC. Available studies on MCT often show small, heterogeneous patient populations with different agents and different doses. In addition, the metronomic agents are often combined with each other as well as with other substances. It is therefore difficult to interpret the presented data and the exact role of MCT in terms of current recommendations for MBC treatment. Parts of the clinical data from our MCT cohort were already published elsewhere [16, 31, 32]. In this study, MCT patients were included regardless of the active agent, and OS data were presented for the first time.

Conclusion

This real-world retrospective unicenter study on MCT showed favorable efficacy with minimal toxicity in MBC patients. The data presented support the premise that MCT is appreciated as a valuable treatment option in selected patients. However, to establish the appropriate role of MCT in the treatment algorithm for MBC and to identify the most appropriate patient population, no effort should be spared to test the efficacy and safety of MCT in randomized controlled trials compared with modern treatment regimens.

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Statement of Ethics

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the World Medical Association Declaration of Helsinki. Data collected in this study were obtained in the context of routine medical care. Ethical approval for the use of data for research purposes as well as consent for participation and publication were not required for this study in accordance with the Ethics Committee of the Landesaerztekammer Rheinland-Pfalz, Germany. Written informed consent from participants was not required in accordance with local/national guidelines.

Conflict of Interest Statement

Slavomir Krajnak: lecture: Novartis and Roche. Research funding: Novartis. Travel reimbursement: Novartis and PharmaMar. Katharina Anic: lecture: AstraZeneca, Clovis Oncology, and MSD. Katrin Almstedt: lecture: AstraZeneca, Pfizer, and Roche. Anne-Sophie Heimes: lecture: Medupdate GmbH, Pfizer, and Roche. Annette Hasenburg: advisory board: AstraZeneca, GSK, LEO

Pharma, MSD, PharmaMar, Roche, and Tesaro. Lecture: AstraZeneca, Celgene, Clovis Oncology, LEO Pharma, MedConcept GmbH, Med update GmbH, Medicultus, Pfizer, PharmaMar, Roche, Streamedup! GmbH, and Tesaro. Marcus Schmidt: lecture: AstraZeneca, BioNTech, Daiichi Sankyo, Eisai, Lilly, MSD, Novartis, Pantarhei Bioscience, Pfizer, Pierre Fabre, Roche, and SeaGen. Research funding: AstraZeneca, BioNTech, Eisai, Genentech, Novartis, Pantarhei Bioscience, Pfizer, Pierre-Fabre, Roche, and SeaGen. Travel reimbursement: Pfizer and Roche. Marco Johannes Battista: advisory board: Eisai, GSK, MSD, PharmaMar, Roche, and Tesaro. Lectures: Astra Zeneca, Clovis Oncology, GSK, MSD, PharmaMar, Roche, and Tesaro. Research funding: AstraZeneca, Clovis Oncology, MSD, and Novartis. All other authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization and methodology: Slavomir Krajnak, Marcus Schmidt, and Marco Johannes Battista; formal analysis and investigation: Slavomir Krajnak and Jana Krajnakova; writing – original draft: Slavomir Krajnak; writing – review and editing: Slavomir Krajnak, Jana Krajnakova, Katharina Anic, Katrin Almstedt, Anne-Sophie Heimes, Valerie Catherine Linz, Amelie Loewe, Mona Wanda Schmidt, Annette Hasenburg, Marcus Schmidt, and Marco Johannes Battista. All authors read and approved the final manuscript.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Explorative Analysis of Low-Dose Metronomic Chemotherapy with Cyclophosphamide and Methotrexate in a Cohort of Metastatic Breast Cancer Patients

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Keywords

Adverse events · Breast cancer · Metastasized · Chemotherapy · Metronomic chemotherapy · Cyclophosphamide · Methotrexate

Summary

Background: Low-dose metronomic chemotherapy (LDMC) is increasingly used in metastatic breast cancer (MBC). In this retrospective analysis, we examined the therapeutic effects and side effects of LDMC in a cohort of MBC patients. **Methods**: Patients with MBC were included when LDMC with oral cyclophosphamide (CTX) and methotrexate (MTX) was administered between 2009 and 2015. The primary endpoint was disease control rate (DCR) ≥ 24 weeks after the start of LDMC. Secondary endpoints were duration of progression-free survival (PFS), rates of discontinuation due to side effects, and DCR with regard to subgroups. **Results**: Retrospective data of 35 patients were available for this analysis. 31% patients achieved DCR. The median PFS was 12 weeks. 9% of patients discontinued LDMC due to adverse events. DCR was 37% in the first 2 lines and 25% in further lines of therapy. 22% of patients with multiple metastases and 35% with ≤2 different metastatic sites achieved DCR. DCR was achieved in 33% of hormone receptor(HR)-positive patients and 27% of HR-negative patients. **Conclusion**: The DCR of 31% is in line with the results of previous phase II studies. LDMC was well tolerated. Subgroup analysis was not able to identify a group in which LDMC was more efficient.

Introduction

The concept of conventional chemotherapy is based on the maximum tolerated dose (MTD) given at 2- to 4-week intervals. The aim of this therapy schedule is to affect rapidly dividing cells and to kill as many malignant cells as possible [1]. In addition, non-malignant highly proliferative cells are also affected by these agents. Thus, adverse events like mucositis, myelosuppression, and hair loss are common and represent clinically relevant sequelae [2]. In order to preserve quality of life for patients with advanced cancer, other strategies with fewer side effects have been evaluated [3, 4]. In hormone receptor(HR)-positive metastatic breast cancer (MBC) patients without severe symptoms of disease, endocrine therapy should be applied as an initial therapy. In the case of disease progression or in patients with HR-negative MBC, cytostatic agents with or without targeted therapies are recommended [5].

The daily administration of cytostatic drugs in low doses is called low-dose metronomic chemotherapy (LDMC) [1, 6]. Many studies showed that LDMC used for palliation can achieve disease control and prolonged overall survival without severe side effects [7]. Over the last decade, clinicians have begun to consider introducing LDMC much earlier into the treatment plan [8, 9]. In comparison with conventional chemotherapy, the lower doses of LDMC may induce fewer side effects [10–13], which may lead to a lower rate of discontinuation due to side effects such as myelosuppression [10]. In this explorative retrospective analysis, the therapeutic effects and the side effects of LDMC with cyclophosphamide (CTX) and methotrexate (MTX) in patients with MBC were examined. Furthermore, we tried to define a subgroup of patients in which LDMC is more favorable.

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Table 1. Clinical patient characteristics

Median age at start of treatment (range), years	64 (35–83)
Number of current chemotherapy lines, n	
≤2	19
1	8
2	11
>2 (heavily pretreated)	16
3	10
4	3
5	1
6	1
7	0
8	1
Number of metastatic sites at start of treatment, n	
≤2	26
1	13
2	13
>2 (multiple metastases)	9
3	5
4	4
Metastatic sites, n	
Bone	20
Liver	17
Lung	13
Lymph	9
Pleura	6
Other (thoracic wall, cutaneous, cerebral, peritoneum)	6
Hormone receptor status, n	
Positive	24
Negative	11
HER2 status, n	
Positive	1
Negative	34

Patients and Methods

We screened patients with MBC who received CTX 50 mg daily and MTX 2.5 mg every second day at the Department of Gynecology and Obstetrics of the University Medical Center Mainz between 2009 and 2015. Patient characteristics such as number of chemotherapy lines and different metastatic sites, metastasis location, HR status, and HER2 status were extracted from the patient files. The therapeutic efficacy was determined as disease control rate (DCR) for ≥24 weeks after the start of LDMC medication. DCR included complete remission (CR), partial remission (PR), and stable disease (SD). Progression-free survival (PFS) was defined as the time from the start of therapy to the detection of progressive disease (PD) or death. Duration of response (DoR) was defined as the time from documentation of tumor response to PD. Toxicity was assessed by common terminology criteria for adverse events (CTCAE) version 4.0. For subgroup analysis, we stratified the patients by number of chemotherapy lines (heavily pretreated ≥2 chemotherapy lines), number of different metastatic sites (multiple metastases ≥2 different metastatic sites), and HR and HER2 status. The SPSS statistical software system, version 19.0. was used for statistical analyses (IBM Corp., Armonk, NY, USA).

Results

Retrospective data of 35 patients were available for this analysis. Patient characteristics are displayed in table 1. Briefly, the median age was 64 years (range 35–83 years). All patients were pretreated with an endocrine or targeted therapy and/or other cytostatic drugs. The treated cohort received a median of 2 (range 1–8) lines of chemotherapy (including LDMC). A median of 2 (range 1–4) different metastatic sites of MBC were present; the most frequent location was bone (20 patients), liver (17 patients), and lung (13 patients). 24 patients presented with HR positivity, and 1 patient showed HER2 positivity at diagnosis.

Table 2. Treatment response (disease control rate ≥ 24 weeks)

Treatment response after ≥24 weeks (n = 35)	n (%)
Progressive disease	24 (68.6)
Disease control rate	11 (31.4)
Stable disease	4 (11.4)
Partial remission	6 (17.1)
Complete remission	1 (2.9)

Table 3. Therapy duration, progression-free survival (PFS), and duration of response (DoR)

	Mean	Median	Minimum	Maximum
Therapy duration, weeks	26.6	12	6	86
PFS, weeks	27	12	6	86
DoR, weeks	36.2	22	8	74

Table 4. Adverse events

Adverse events (n = 35)	n (%)
Cyclophosphamide/methotrexate (MTX) dropouts	3 (8.6)
Thrombocytopenia	1 (2.9)
Fatigue	1 (2.9)
Nausea/vomiting	1 (2.9)
MTX dropouts	4 (11.4)
Fatigue	2 (5.7)
Nausea/vomiting	2 (5.7)
Visual impairment	1 (2.9)
Hemorrhagic cystitis	1 (2.9)

DCR was achieved in 11 (31%) patients (tables 2, 3). 1 (3%), 6 (17%), and 4 (11%) patients achieved CR, PR, and SD, respectively. The remaining 24 (69%) patients showed PD. The median PFS was 12 weeks (range 6–86 weeks). Therapy response was documented in 15 (43%) patients, and the median DoR was 22 weeks (range 8–74 weeks).

During LDMC, discontinuation of therapy due to adverse events occurred (table 4): 3 (9%) patients receiving CTX/MTX stopped therapy due to intolerable side effects. Thrombocytopenia, gastrointestinal complaints, and fatigue were the most common reasons for early termination of therapy. 4 (11%) patients stopped only MTX, mainly because of gastrointestinal symptoms and visual impairment. 1 (3%) patient developed hemorrhagic cystitis but no therapy termination was necessary.

Subgroup analysis did not reveal a specific group of patients who showed benefit from LDMC (table 5, fig. 1). 4/16 (25%) heav-

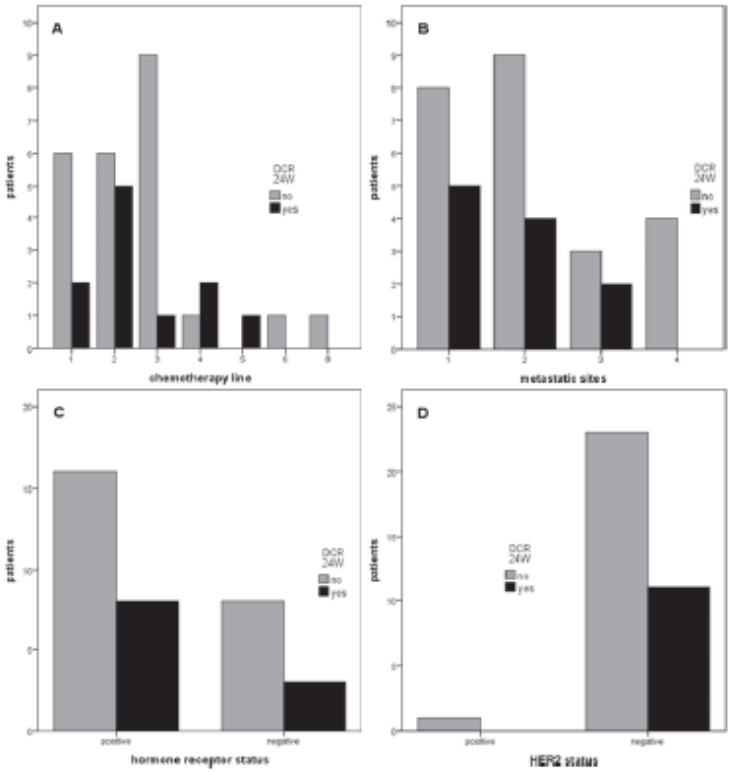

Fig. 1. Treatment response (disease control rate ≥ 24 weeks, DCR 24W) with regard to subgroups: **A** number of chemotherapy lines; **B** number of different metastatic sites; **C** hormone receptor (HR) status; **D** HER2 status.

Table 5. Treatment response (disease control rate ≥ 24 weeks) with regard to subgroups

Subgroup	Rate, n (%)	p value
DCR depending on chemotherapy line		0.493
≤2	7/19 (36.8)	
>2	4/16 (25)	
DCR depending on number of metastatic sites		0.685
≤2	9/26 (34.6)	
>2	2/9 (22.2)	
DCR depending on hormone receptor status		1.00
Positive	8/24 (33.3)	
Negative	3/11 (27.3)	
DCR depending on HER2 status		–
Positive	0/1 (0)	
Negative	11/34 (32.4)	

ily pretreated and 7/19 (37%) non-heavily pretreated patients achieved DCR (p = 0.493). 2/9 (22%) patients with multiple metastases and 9/26 (35%) with ≤2 different metastatic sites achieved DCR (p = 0.685). 8/24 (33%) HR-positive and 3/11 (27%) HR-negative patients achieved DCR (p = 1.00). We did not conduct a comparison regarding HER2 status and therapy response due to the lack of HER2-positive patients (HER2-positive: n = 1; HER2-negative: n = 34).

Discussion

In this explorative analysis of 35 heavily pretreated MBC patients, LDMC was efficient and well tolerated. The treated cohort varied in age, number of pretreatments, and number of metastatic sites before the start of LDMC. The DCR of 31% was in line with the results of previous phase II studies in which the DCR/clinical benefit rate (CBR) was achieved in 24–51% of patients [10, 11, 14]. CR, PR, and SD were observed in 1, 6, and 4 patients, respectively. The median PFS was 12 weeks (range 6–86 weeks), and the median DoR was 22 weeks (range 8–74 weeks). Orlando et al. [15] showed in a long-term follow-up study that metronomic CTX/MTX was feasible and provided a prolonged clinical benefit (CB) in 16% of patients without cumulative toxicity despite prolonged use. In a prospective, non-randomized, phase II clinical trial, Perroud et al. [12] showed in patients with advanced breast cancer a CB of 55% (11/20) at 24 weeks after the beginning of treatment with metronomic CTX and celecoxib, a selective cyclooxygenase-2 inhibitor. Moreover, serum concentration of vascular endothelial growth factor (VEGF) decreased and soluble vascular endothelial growth factor receptor 2 (sVEGFR-2) increased during treatment. Circulating endothelial cells (CECs) increased in patients with CB at the time of progression. Perroud et al. [12] postulated baseline VEGF and VEGF/sVEGFR-2 to be potential predictive biomarkers of re-

sponse, and CECs of follow-up, in metronomic chemotherapy. Because of the well-known anti-angiogenic effects of LDMC, combinations with other agents targeting VEGF were evaluated [16, 17]. A phase II trial with metronomic CTX and capecitabine in combination with bevacizumab showed a CBR of 68% ≥ 24 weeks and a mild toxicity profile in heavily pretreated MBC patients [18]. The orally available VEGFR inhibitor, vandetanib, given metronomically with metronomic CTX/MTX, obtained a favorable therapy response (DCR of 25% ≥ 24 weeks) with a good toxicity profile in MBC [19].

LDMC with CTX/MTX was well tolerated. 7 (20%) patients discontinued therapy due to adverse events. 3 (9%) patients stopped both CTX and MTX therapy due to intolerable side effects. Thrombocytopenia, gastrointestinal complaints, and fatigue were the most common reasons for early termination of therapy. 4 (11%) patients stopped MTX but continued CTX, mainly because of gastrointestinal symptoms and vision impairment. 1 (3%) patient developed hemorrhagic cystitis but no therapy interruption was necessary. Leukopenia and anemia were not documented as reasons for therapy termination. In the study by Colleoni et al. [10], MBC patients with Eastern Cooperative Oncology Group (ECOG) scores ≤ 3 treated with metronomic CTX/MTX presented with few adverse events. The most frequent toxicity was grade 1 leukopenia, which was observed in 35% of cases. Only 10% of the cycles were delayed and 7% of the courses were administered at reduced dosages, mainly due to leukopenia and an increase in transaminases [10].

In our subgroup analysis, no significant differences were found regarding chemotherapy lines, number of different metastatic sites, and HR status. Not surprisingly, we observed that therapy response was worse with an increasing number of metastatic sites and chemotherapy lines. It can be assumed that the LDMC should be investigated not only in heavily pretreated patients but also in MBC patients without symptoms and need for rapid response. Recently, LDMC was also examined as a component of the adjuvant and neoadjuvant treatment of breast cancer [9, 20, 21].

LDMC should be discussed as a feasible low-dose variation of dose-dense therapy. Compared to MTD chemotherapy, LDMC is associated with significantly lower cumulative doses and less toxicity [1, 2, 6, 21]. Despite this finding, the antitumor effects of LDMC might be comparable or even superior to conventional MTD regimens [22–24]. The main efficacy of LDMC is induced by anti-angiogenesis and immune system modulation [25–29]. Furthermore, LDMC exerts inhibiting effects on the tumor and, in contrast to MTD regimens, also on tumor-initiating cells [30, 31].

The main experience with LDMC in MBC arises from phase II studies. The most frequently administered therapy consists of CTX and MTX [10, 11, 13, 14, 32, 33]; however, prospective randomized studies are lacking [7]. Other orally available drugs with proven efficacy in MBC, like vinorelbine or capecitabine, also showed high potential in metronomic schedules [34, 35]. Moreover, there is still a lack of randomized studies of LDMC in comparison to approved chemotherapies to determine the exact role of LDMC in the palliative treatment of MBC patients. To evaluate the efficacy and advantages of LDMC in comparison with conventional chemotherapy in the palliative treatment of MBC, we have initiated a case-control study. The retrospective design of the current analysis limited the examination of further important aspects like quality of life. In the next step, we will prepare a prospective case-control analysis of LDMC and conventional chemotherapy to examine subgroups and determine the patient collective which will obtain the best benefit from LDMC in the treatment of MBC.

Conclusion

In conclusion, our retrospective analysis of metronomic CTX/MTX indicates that LDMC is a feasible and well tolerated therapy option in MBC patients without the need for rapid response. However, effectiveness and tolerability should be examined in prospective randomized trials.

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Disclosure Statement

The authors state that there are no conflicts of interest to declare.

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CLINICAL TRIAL

Low-dose metronomic chemotherapy as an efficient treatment option in metastatic breast cancer—results of an exploratory case–control study

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Abstract

Purpose There is growing interest in low-dose metronomic chemotherapy (LDMC) in metastatic breast cancer (MBC). In this retrospective case–control analysis, we compared the efficacy of LDMC and conventional chemotherapy (CCT) in MBC.

Methods Each LDMC patient receiving oral cyclophosphamide (CTX) (50 mg daily) and methotrexate (MTX) (2.5 mg every other day) was matched with two controls who received CCT. Age, number of chemotherapy lines and metastatic sites as well as hormone receptor (HR) status were considered as matching criteria. Primary endpoint was disease control rate longer than 24 weeks (DCR). Secondary endpoints were progression-free survival (PFS), duration of response (DoR) and subgroup analyses using the matching criteria.

Results 40 cases and 80 controls entered the study. 30.0% patients with LDMC and 22.5% patients with CCT showed DCR ($p=0.380$). The median PFS was 12.0 weeks in both groups ($p=0.218$) and the median DoR was 31.0 vs. 20.5 weeks ($p=0.383$), respectively. Among younger patients, DCR was 40.0% in LDMC vs. 25.0% in the CCT group ($p=0.249$). DCR was achieved in 33.3% vs. 26.2% non-heavily pretreated patients ($p=0.568$) and in 36.0% vs. 18.0% patients without multiple metastases ($p=0.096$), respectively. In the HR-positive group, 30.0% LDMC vs. 28.3% CCT patients showed DCR ($p=1.000$). Among triple-negative patients, DCR was achieved in 30.0% LDMC and 5.0% CCT patients ($p=0.095$).

Conclusions We demonstrated a similar efficacy of LDMC compared to CCT in the treatment of MBC. Thus, LDMC may be a valuable treatment option in selected MBC patients.

Introduction

Metastatic breast cancer (MBC) is an incurable but treatable disease. Thus, it is crucial to achieve disease control with preservation of quality of life (QoL) [1]. In the last decades low-dose metronomic chemotherapy (LDMC) gained increasing popularity [2, 3]. LDMC is defined as a continuous administration of cytotoxic drugs at low doses, distinctly lower than the maximum tolerable dose (MTD) of conventional chemotherapy (CCT) [4]. Consequently, compared to MTD the lower doses of chemotherapeutic drugs may induce less adverse events like myelosuppression, mucositis or hair loss [5–7]. It is assumed that LDMC is not simply a different way of administering chemotherapy but a truly new treatment option [3, 8, 9]. This alternative strategy has been used especially in elderly patients, not eligible for a CCT [8]. The orally available and well-established cytostatic agents like cyclophosphamide (CTX), methotrexate (MTX), vinorelbine (VRL) and capecitabine (CAPE) are suited for metronomic chemotherapy. The best experience about LDMC arises from phase II studies, however phase III studies are still lacking. Furthermore, to the best of our knowledge, there is insufficient experience regarding the efficacy of metronomic chemotherapy, compared to CCT in MBC.

In this retrospective case–control study, the efficacy of metronomic administered CTX/MTX and CCT was compared in matched pairs and subgroup analyses were performed to define patients in which LDMC might be a more effective treatment option.

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Methods

MBC patients receiving LDMC with oral CTX (50 mg daily) and MTX (2.5 mg every other day) at the Department of Gynecology and Obstetrics of the University Medical Center Mainz, Germany between 2009 and 2018 were selected for this retrospective analysis as previously described [10]. Each LDMC patient was matched with two patients, who received CCT, if matching criteria (measurable metastatic disease, age at start of therapy, number of chemotherapy lines and different metastatic sites as well as hormone receptor (HR) status) were met. No antiemetic treatment was routinely given to patients in the LDMC group. In the CCT group only patients without therapy termination due to toxicity were included. No concomitant treatment like radiotherapy, endocrine or targeted therapy was allowed. HER2-positive patients and patients with presence of additional cancer were excluded.

Primary endpoint was disease control rate longer than 24 weeks (DCR). DCR included stable disease (SD), partial response (PR) and complete response (CR). Secondary endpoints were progression-free survival (PFS), duration of response (DoR), as well as DCR and PFS in subgroups. The DoR was defined as the time from documentation of tumor response to progression disease (PD) or death. The therapy efficacy was assessed using the standard clinical and imaging methods. For subgroup analyses we used the a priori determined matching criteria to obtain comparable populations of same size. Thereby, we stratified the patients by age at start of LDMC/CCT (younger: ≤ median age vs. elderly: > median age), the number of chemotherapy lines (non-heavily pretreated: ≤ 2 chemotherapy lines vs. heavily pretreated: > 2 chemotherapy lines), number of different metastatic sites (no multiple metastases: ≤ 2 different metastatic sites vs. multiple metastases: > 2 different metastatic sites) and by HR status (HR-positive: oestrogen/progesterone positive and HER2-negative vs. triple-negative). SPSS (statistical software system, version 23.0. IBM Corp., Armonk, NY, U.S.) was used for statistical analyses. Patient characteristics and therapy response (DCR and therapy response in subgroups) were analysed by applying a Fisher's Exact test. For PFS and DoR analysis Kaplan–Meier estimator was used. The Log-rank test was used for the comparisons of survival curves between LDMC and CCT group. A Cox regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) in the analysis of PFS and DoR. All tests were two-sided and $p < 0.05$ was considered as statistically significant. Written informed consent was obtained from all patients included in the study.

Results

Patient characteristics

In total, 120 patients (40 cases and 80 controls) entered the study. Patient characteristics are shown in Table 1. The median age at first diagnosis (FD) of MBC was 59 (33–82) years in the LDMC group and 59 (28–81) years in the CCT group ($p = 0.544$). The median age at start of therapy was 63 (range 35–83) years and 61 (range 30–81) years ($p = 0.230$), respectively. In the HR-positive cohort, 93.3% LDMC patients and 71.7% CCT patients had at least one endocrine therapy in MBC prior to LDMC or CCT treatment ($p = 0.026$). 47.5% vs. 51.3% patients received adjuvant or neoadjuvant chemotherapy at FD of breast cancer (BC) ($p = 0.847$). After FD of MBC, 52.5% patients received less than 2 chemotherapy lines before LDMC/CCT ($p = 1.000$) (Table 1). 62.5% patients had up to 2 different metastatic lesions ($p = 1.000$). In the LDMC group as well as in the CCT group 25.0% patients showed triple-negative disease ($p = 1.000$). As demonstrated in Table 1, all matching criteria were met. The most frequent site of metastases in the LDMC group as well as in the CCT group were bone (62.5% vs. 70.0%, $p = 0.417$), liver (52.5% vs. 52.5%, $p = 1.000$) and lung (40% vs. 40%, $p = 1.000$), respectively. There were no significant differences regarding number and localization of metastatic lesions between the two groups (Table 2). In the CCT group, the most frequently used chemotherapy regimen was capecitabine (27.5%) and pegylated liposomal doxorubicin (26.3%). Taxanes were administered in 17.5% patients and eribulin in 12.5% patients (Table 3).

Therapy response

DCR was achieved in 30.0% LDMC patients and in 22.5% CCT patients ($p = 0.380$) (Table 2). 12.5%, 15.0%, 2.5% LDMC patients vs. 18.8%, 3.8%, 0.0% CCT patients showed SD, PR, CR, respectively (Table 4). The median PFS was 12.0 weeks (95% CI 9.9–14.1) in the LDMC group, as compared with 12.0 weeks (95% CI 10.5–13.5) in the CCT group, HR for progression or death was 0.796; 95% CI 0.541–1.170; $p = 0.245$ (Fig. 1). The median DoR was 31.0 weeks in the LDMC group and 20.5 weeks in the CCT group ($p = 0.383$) (Table 4), HR for progression or death was 0.749; 95% CI 0.385–1.459; $p = 0.396$. Therapy response was detected in 37.5% LDMC patients and in 30.0% CCT patients ($p = 0.417$) (Table 4).

Table 1 Patient characteristics

	LDMC	CCT	*p*
	n = 40	*n* = 80	
Median age at start of therapy (range) (years)	63 (35–83)	61 (30–81)	0.230
Median age at FD MBC (range) (years)	59 (33–82)	59 (28–81)	0.544
Median age at FD BC (range) (years)	51 (29–80)	52 (26–79)	0.506
Age at start of therapy			
Younger	20 (50.0%)	40 (50.0%)	1.000
Elderly	20 (50.0%)	40 (50.0%)	
Chemotherapy line			
Non-heavily pretreated	21 (52.5%)	42 (52.5%)	1.000
Heavily pretreated	19 (47.5%)	38 (47.5%)	
Metastatic sites			
No multiple metastases	25 (62.5%)	50 (62.5%)	1.000
Multiple metastases	15 (37.5%)	30 (37.5%)	
HR status			
HR-positive	30 (75.0%)	60 (75.0%)	1.000
Triple-negative	10 (25.0%)	20 (25.0%)	

LDMC low-dose metronomic chemotherapy, *CCT* conventional chemotherapy, *FD* first diagnosis, *MBC* metastatic breast cancer, *BC* breast cancer, *HR* hormone receptor

Table 2 Localization of metastatic lesions

	LDMC	CCT	p
Metastatic sites	No. of patients (%)		
Bone	25 (62.5%)	56 (70.0%)	0.417
Liver	21 (52.5%)	42 (52.5%)	1.000
Lung	16 (40.0%)	32 (40.0%)	1.000
Pleura	7 (17.5%)	10 (12.5%)	0.579
Peritoneum	2 (5.0%)	9 (11.3%)	0.333
Lymph	12 (30.0%)	23 (28.8%)	1.000
Cerebrum	1 (2.5%)	8 (10.0%)	0.269
Soft tissue (thoracic wall, cutis)	5 (12.5%)	10 (12.5%)	1.000

Table 3 Chemotherapeutic substances in the conventional chemotherapy group

Chemotherapy	No. of patients (%)
Capecitabine	22 (27.5%)
Pegylated liposomal doxorubicin	21 (26.3%)
Taxane (paclitaxel/nab-paclitaxel/docetaxel)	14 (17.5%) (4 (5.0%)/ 8 (10.0%)/ 2 (2.5%))
Eribulin	10 (12.5%)
Carboplatin + gemcitabine	5 (6.3%)
Vinorelbine	4 (5.0%)
Other (doxorubicin/carboplatin/fluorouracil/capecitabine + vinorelbine)	4 (5.0%) (each 1 (1.3%))

Therapy response in subgroups

In the subgroup analyses, 40.0% younger LDMC patients and 25.0% younger CCT patients showed DCR ($p=0.249$) (Fig. 2a). 20.0% elderly patients achieved DCR in both treatment groups ($p=1.000$). Among non-heavily pretreated patients, DCR was 33.3% in the LDMC and 26.2% in the CCT group ($p=0.568$). In the heavily pretreated group, 26.3% vs. 18.4% patients showed DCR ($p=0.509$). DCR was achieved in 36.0% LDMC patients and in 18.0% CCT patients ($p=0.096$) without multiple metastases and in 20.0% vs. 30.0% with multiple metastases ($p=0.722$). 30.0% vs. 28.3% HR-positive patients ($p=1.000$) and 30.0% vs. 5.0% triple-negative patients achieved DCR ($p=0.095$), respectively.

The median PFS in younger patients was 15.0 weeks in the LDMC group and 14.0 weeks in the CCT group ($p=0.212$) (Fig. 2b), HR for progression or death was 0.719; 95% CI 0.415–1.243; $p=0.237$ (Fig. 2c). Elderly patients showed a median PFS of 12.0 weeks in both groups ($p=0.627$) (Fig. 2b). The median PFS in non-heavily pretreated patients was 17.0 weeks vs. 15.0 weeks ($p=0.531$) (Fig. 2b), HR for progression or death was 0.849; 95% CI 0.500–1.442; $p=0.544$ (Fig. 2c). In the heavily pretreated subgroup, the median PFS was 12.0 weeks for both treatment groups ($p=0.235$) (Fig. 2b). The median PFS in patients without multiple metastases was 16.0 weeks vs. 12.0 weeks ($p=0.064$) (Fig. 2b), HR for progression or death was 0.642; 95% CI 0.392–1.053; $p=0.079$ (Fig. 2c). In the cohort with multiple metastases, the median PFS was 12.0 weeks in both groups ($p=0.684$) (Fig. 2b). Regarding receptor status, the median PFS was 12.0 weeks vs. 14.0 weeks in the HR-positive group ($p=0.570$) and 12.0 weeks in both triple-negative groups ($p=0.081$) (Fig. 2b).

Table 4 Therapy response

		LDMC $n=40$	CCT $n=80$	p
DCR (n (%))		12 (30.0%)	18 (22.5%)	0.380
Therapy response after 24 weeks	PD	28 (70.0%)	62 (77.5%)	
	SD	5 (12.5%)	15 (18.8%)	
	PR	6 (15.0%)	3 (3.8%)	
	CR	1 (2.5%)	0 (0.0%)	
Median PFS (range) (weeks)		12.0 (6–86)	12.0 (4–100)	0.218
Median duration of response (range) (weeks)		31.0 (12–74)	20.5 (12–88)	0.383
Therapy response (n (%))		15 (37.5%)	24 (30.0%)	0.417

DCR Disease Control Rate, *PFS* progression-free survival, *PD* progression disease, *SD* stable disease, *PR* Partial response, *CR* Complete response

Discussion

In this retrospective case–control study 120 MBC patients were evaluated regarding the efficacy of the chemotherapy treatment. The primary endpoint DCR did not differ significantly between LDMC and CCT group (30.0% vs. 22.5%, $p=0.380$). The impact of metronomic CTX/MTX in our cohort of HR-positive and HER2-negative MBC patients as measured by DCR after 24 weeks of treatment was in line with previous studies [11–13]. Gebbia et al. [6] observed a higher PR rate in the cohort of patients with the combination CTX/MTX as compared to that treated with CTX alone (20% vs. 14%, $p=0.45$). The median PFS was 12.0 weeks in the LDMC as well as in the CCT group ($p=0.218$). Furthermore, DoR (31.0 vs. 20.5 weeks, $p=0.383$) and therapy response (37.5% vs. 30.0%, $p=0.417$) failed to show any significant differences between LDMC and CCT group. Moreover, the rate of treatment response may also depend on patient characteristics like age, metastatic spread, HR status as well as previous treatment. In the subgroup of younger patients, DCR was documented in 40.0% patients in the LDMC group and in 25.0% patients in the CCT group ($p=0.249$). According to current recommendations for treatment of MBC, LDMC is primarily intended for elderly and frail patients, who are not suitable for conventional dosis of chemotherapy [14–16]. However, we have shown that LDMC can also be a treatment option for younger patients. Based on previous data from phase II studies, LDMC regimens provide promising results in the first-line setting with a clinical benefit rate (CBR) of up to 78% and a median time to progression (TTP) of up to 22 months [17–19]. Among the non-heavily pretreated subgroup, 33.3% LDMC patients and 26.2% CCT patients showed DCR ($p=0.568$). More importantly, it is well established that the duration of disease control decreases with the increasing number of chemotherapy lines [20]. In the subgroup without multiple metastases, LDMC patients showed DCR twice as often as in the control group (36.0% vs. 18.0%, $p=0.096$) and the median PFS was 16.0 weeks vs. 12.0 weeks ($p=0.064$) with a trend towards significance. In the HR-positive group, we found no differences in DCR between the two groups. However, among triple-negative patients, 30.0% patients with LDMC compared to 5.0% patients with CCT showed DCR ($p=0.095$) resulting in a borderline significance in favor of LDMC. A beneficial effect of the metronomic combination of VRL and CAPE was also shown in the triple-negative subgroup (28 patients) in the VICTOR-2 study [21]. The DCR was 53.7% and the median PFS was 4.7 months. Furthermore, LDMC with CTX/MTX was well-tolerable with almost only grade 1–2 toxicities. The most frequent adverse events were leukopenia (1–49%), nausea/vomiting (3–39%) and gastric pain (6–7%). Elevated values of transaminases, observed in up to 60% patients (10% grade 3–4), were mostly attributable to concomitant hepatic metastases or recovered with reduction or transient interruption of MTX [10, 12, 22]. In order to reduce hepatic toxicity and simplify the drug administration we modified the MTX schedule (2.5 mg every other day instead of 2.5 mg twice a day on days 1 and 4 every week) and found no grade 3–4 hepatic toxicities [10].

By reference to current experience, endocrine-based therapy should be provided as the first choice for MBC with positive HR status except in the case of life-threating disease [23]. In the last decade, new options as cyclin-dependent-kinase (CDK) inhibitors and immune checkpoint inhibitors for the treatment of MBC were established [24–26]. Moreover, LDMC has gained increasing interest through its multi-targeted nature. In addition to direct cytotoxic effect, LDMC induces indirect effects on tumor cells by modulation of tumor microenvironment via inhibition of angiogenesis and stimulation of immune response [27–29]. Thus, while the anti-tumor response to LDMC may be delayed, the effect is more likely to be sustained, owing to the decreased selection of resistant tumor cell clones and the suppression of anti-tumour immunity with a decreased likelihood of disease relapse [30]. Oral administration of well-tolerable LDMC including improvement

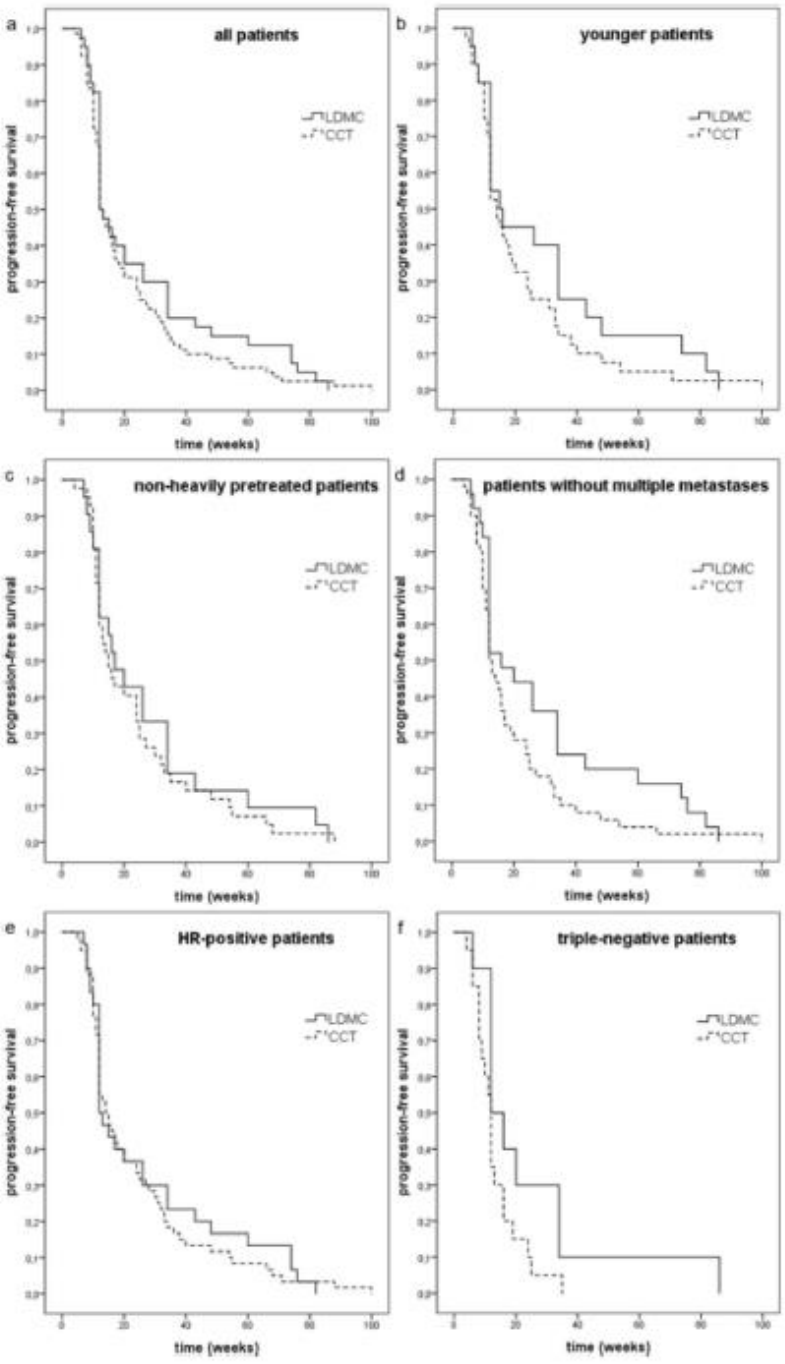
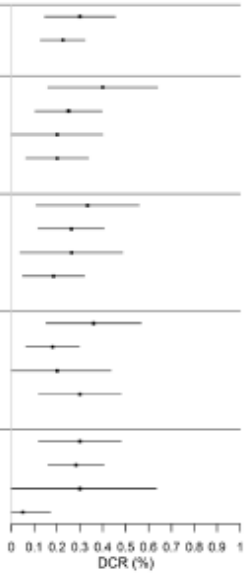


Fig. 1 Kaplan–Meier analysis of progression-free survival. **a** all patients: median PFS in LDMC and CCT: 12.0 weeks vs. 12.0 weeks, Log-rank: $p = 0.218$. **b** younger patients: median PFS in LDMC and CCT: 15.0 weeks vs. 14.0 weeks, Log-rank: $p = 0.212$. **c** non-heavily pretreated patients: median PFS in LDMC and CCT: 17.0 weeks vs. 15.0 weeks, Log-rank: $p = 0.531$. **d** patients without multiple metastases: median PFS in LDMC and CCT: 16.0 weeks vs. 12.0 weeks, Log-rank: $p = 0.064$. **e** HR-positive patients: median PFS in LDMC and CCT: 12.0 weeks vs. 14.0 weeks, Log-rank: $p = 0.570$. **f** triple-negative patients: median PFS in LDMC and CCT: 12.0 weeks vs. 12.0 weeks, Log-rank: $p = 0.081$. *LDMC* low-dose metronomic chemotherapy), *CCT* conventional chemotherapy

a

Subgroup	No. of patients with DCR	DCR (%) (95% CI)
all LDMC	12/40	0.30 (0.15 - 0.45)
all CCT	18/80	0.22 (0.13 - 0.32)
age		
younger LDMC	8/20	0.40 (0.16 - 0.64)
younger CCT	10/40	0.25 (0.10 - 0.40)
elderly LDMC	4/20	0.20 (0.00 - 0.40)
elderly CCT	8/40	0.20 (0.06 - 0.34)
chemotherapy line		
non-heavily pretreated LDMC	7/21	0.33 (0.11 - 0.56)
non-heavily pretreated CCT	11/42	0.26 (0.12 - 0.41)
heavily pretreated LDMC	5/19	0.26 (0.04 - 0.49)
heavily pretreated CCT	7/38	0.18 (0.05 - 0.32)
metastatic sites		
no mult. metastases LDMC	9/25	0.36 (0.15 - 0.57)
no mult. metastases CCT	9/50	0.18 (0.06 - 0.30)
mult. metastases LDMC	3/15	0.20 (0.00 - 0.44)
mult. metastases CCT	9/30	0.30 (0.12 - 0.48)
HR status		
HR-positive LDMC	9/30	0.30 (0.12 - 0.48)
HR-positive CCT	17/60	0.28 (0.16 - 0.41)
triple-negative LDMC	3/10	0.30 (0.00 - 0.63)
triple-negative CCT	1/20	0.05 (0.00 - 0.17)



b

Subgroup	No. of patients	median progression-free survival (weeks) (95% CI)
all LDMC	40	12.0 (9.9 - 14.1)
all CCT	80	12.0 (10.5 - 13.5)
age		
younger LDMC	20	15.0 (6.2 - 23.8)
younger CCT	40	14.0 (11.5 - 16.5)
elderly LDMC	20	12.0 (10.8 - 13.2)
elderly CCT	40	12.0 (10.6 - 13.4)
chemotherapy line		
non-heavily pretreated LDMC	21	17.0 (9.5 - 24.5)
non-heavily pretreated CCT	42	15.0 (10.5 - 19.5)
heavily pretreated LDMC	19	12.0 (10.6 - 13.4)
heavily pretreated CCT	38	12.0 (10.7 - 13.3)
metastatic sites		
no mult. metastases LDMC	25	16.0 (11.1 - 20.9)
no mult. metastases CCT	50	12.0 (9.9 - 14.1)
mult. metastases LDMC	15	12.0 (9.5 - 14.5)
mult. metastases CCT	30	12.0 (9.6 - 14.4)
HR status		
HR-positive LDMC	30	12.0 (9.6 - 14.4)
HR-positive CCT	60	14.0 (8.9 - 19.1)
triple-negative LDMC	10	12.0 (5.8 - 18.2)
triple-negative CCT	20	12.0 (11.0 - 13.0)

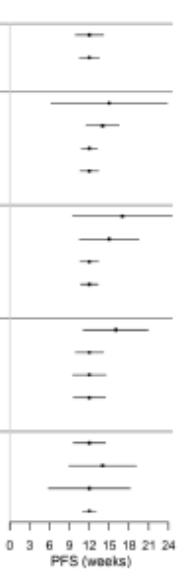


Fig. 2 **a** Disease control rate in subgroups. **b** Median progression-free survival in subgroups. **c** Hazard ratio for progression or death in subgroups

c

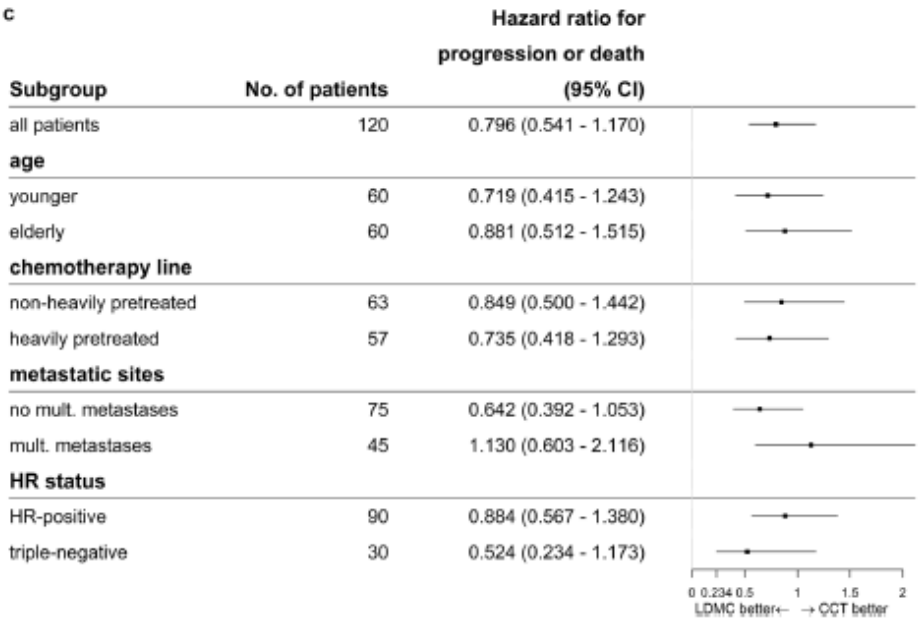


Fig. 2 (continued)

of QoL of patients and reduced healthcare costs as well as having benefits over intravenous administration such as prolonged plasma drug concentration or increased therapeutic window makes LDMC attractive in clinical practice [31, 32]. Apart from that there are still several aspects that need to be clarified, such as patient selection, the choice of cytotoxic drug used for treatment, its optimal dose and decision between single versus doublet agent administration [8, 33]. Nevertheless, based on previous studies, LDMC represents a therapy option for MBC patients without need for rapid response and can be recommended according to the Breast Committee of the German Gynecological Oncology Working Group in HR-positive, HER2-negative MBC patients after anthracycline and taxane pretreatment [34, 35]. In addition to HR-positive patients, we demonstrated a favorable effect also in the triple-negative subgroup. Considering that, the therapeutic goal in advanced disease is on the one hand to maintain the QoL and on the other hand to control the disease, LDMC is a valuable option. It may be administered particularly in asymptomatic patients with endocrine resistance and triple-negative disease to prolong PFS and delay the onset of the often more toxic CCT with MTD regimen. Furthermore, combination of LDMC with antiangiogenic and immunomodulatory substances seems to be promising [36–40]. Further analyses are needed to gain detailed experience about the role of LDMC in the management of MBC, including QoL issues and combination therapies with e.g. immunomodulatory drugs.

To the best of our knowledge, the presented analyses are the first to compare and show a similar efficacy of LDMC and CCT in terms of age, previous chemotherapy and severity of metastatic lesions. However, the retrospective character limits the validity of the presented data. In particular, since the median age at first diagnosis MBC was 59 for both groups and the median age at start of therapy was 63 in LDMC and 61 in CCT group, it can be assumed that LDMC patients had a less aggressive disease and/or better response to prior therapies compared to CCT patients. Reliable information on the toxicity of the administered therapies is not presented. Moreover, the validity of our conclusions is impaired by the study design and should be regarded as hypothesis generating. Therefore, we try to overcome this limitation and prepare a prospective non-interventional study to gather further insights about LDMC in MBC regarding patient-reported outcome, QoL, safety and efficacy, named PROmetronomic.

In conclusion, in our retrospective case–control study we could demonstrate a similar efficacy of LDMC compared to CCT in the treatment of MBC. Our analyses support further efforts to investigate the LDMC in selected MBC patients.

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Compliance with ethical standards

Conflict of interest All other authors declare that they have no conflict of interest. S. Krajnak received speaker honoraria from Roche Pharma AG. He received research funding from Novartis and travel reimbursement from PharmaMar. K. Almstedt received speaker honoraria from Roche Pharma AG, Pfizer Pharma GmbH and AstraZeneca. R.Schwab received speaker honoraria from Roche Pharma AG and AstraZeneca. A. Hasenburg received honoraria from AstraZeneca, Celegen, MedConcept Gm, Med update GmbH, Medicultus, Pfizer, Promedicis GmbH, Pierre Fabre, Softconsult, Roche Pharma AG, Streamedup!GmbH and Tesaro Bio Germany GmbH. She is a member of the advisory board of PharmaMar, Promedicis GmbH, Pierre Fabre Pharma GmbH, Roche Pharma AG and Tesaro Bio Germany GmbH. She received research funding from Celgene. M. Schmidt received honoraria for speaker or consultancy role from AMGEN, AstraZeneca, Eisai, Lilly, Myelo Therapeutics, Novartis, Pantarhei Bioscience, Pfizer, and Roche Pharma AG. He received research funding from AstraZeneca, BioNTech, Eisai, Genentech, Myelo Therapeutics, Novartis, Pantarhei Bioscience, Pfizer, Pierre-Fabre, and Roche. He received travel reimbursement from Pfizer and Roche Pharma AG. M.J. Battista received honoraria for speaker or consultancy role from AstraZenca, MSD, PharmaMar, Roche Pharma AG, TEVA and Tesaro. He received travel reimbursement from Celgene, PharmaMar and Pierre Fabre.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Phase II study of metronomic treatment with daily oral vinorelbine as first-line chemotherapy in patients with advanced/metastatic HR+/HER2– breast cancer resistant to endocrine therapy: VinoMetro—AGO-B-046

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Abstract

Purpose Metronomic chemotherapy (MCT) is an increasingly used treatment option in hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2–) advanced/metastatic breast cancer (MBC) after failure of endocrine-based therapies.

Methods VinoMetro was a multicentre, open-label, single-arm, phase II study of metronomic oral vinorelbine (VRL; 30 mg/day) as a first-line chemotherapy (CT) in patients with HR+/HER2– MBC after endocrine failure. The primary endpoint was the clinical benefit rate (CBR) at 24 weeks.

Results Between January 2017 and April 2019, nine patients were enrolled. The CBR was 22.2% (90% confidence interval [CI] 4.1–55.0), $p = 0.211$. The median progression-free survival (PFS) was 12.0 weeks (95% CI 11.3–12.7). Grade 3–4 adverse events (AEs) occurred in 22.2% of patients. One patient died of febrile neutropenia.

Conclusion VinoMetro (AGO-B-046) was closed early after nine patients and occurrence of one grade 5 toxicity in agreement with the lead institutional review board (IRB). Metronomic dosing of oral VRL in HR+/HER2– MBC as first-line CT after failure of endocrine therapies showed only limited benefit in this population.

Trial registration number and date of registration ClinicalTrials.gov Identifier: NCT03007992; December 15, 2016.

Keywords Metronomic chemotherapy · Daily oral vinorelbine · Metastatic breast cancer · Clinical benefit rate

Introduction

With an incidence of 2.1 million and a mortality of 0.6 million cases per year, breast cancer (BC) represents a major disease burden worldwide (Bray et al. 2018). Hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2–) disease represents the largest group of cancer subtypes (68%), with the majority of patients in this group showing a lower proliferation index (luminal A 44%; luminal B 24%) (Voduc et al. 2010). Advanced/metastatic breast cancer (MBC) is a treatable but still generally incurable disease (Cardoso et al. 2020; Thomssen et al. 2020). The goal of care in this situation is to reach a prolonged overall survival (OS) with adequate quality of life (QoL) and thus to establish a disease chronification using treatment options that provide an optimal therapeutic index (Harbeck and Gnant 2017). Single-agent chemotherapy

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(CT) is recommended as a preferred choice for patients with HR+/HER2−, non-life-threatening MBC after failure of endocrine treatment (Ditsch et al. 2020). Among available agents, vinorelbine (VRL) represents a standard treatment option in this situation, as it provides proven efficacy and a good safety profile (Fumoleau et al. 1993; Terenziani et al. 1996). Oral administration of VRL shows a level of efficacy comparable to the intravenous treatment, while providing a favourable safety profile and the additional advantages of an oral treatment (Aapro et al. 2019; Blancas et al. 2019; Freyer et al. 2003; Steger et al. 2018). Metronomic chemotherapy (MCT), describing the administration of low doses on a continuous and high administration frequency basis, has been shown to mediate good tumour control and maintain an excellent safety profile (Cazzaniga et al. 2019a; Krajnak et al. 2020; Liu et al. 2017; Orlando et al. 2020). This therapy option generally represents an approach of high interest in the treatment of solid tumours, as it offers the advantage of exposing the tumour to a significant amount of drug while improving safety for the patients (Cazzaniga et al. 2019b). It provides for fractionated, frequent, long-term administration of single doses of medication without pauses until disease progression or unacceptable toxicity (Gennari et al. 2011; Liu et al. 2017). MCT may have a complementary mechanism of action as compared to conventional CT, with an additional anti-angiogenic effect, thus counteracting tumour regrowth that may occur between conventional CT cycles (Kerbel and Shaked 2017; Natale and Bocci 2018). Moreover, MCT suppresses regulatory T cells (Tregs) and induces the maturation of dendritic cells, thereby leading to an anti-tumour immune response (Andre et al. 2017; Chen et al. 2017). The ease of oral VRL administration allows for flexible treatment schedules including a more frequent and metronomic dose application. Various schedules have been evaluated including a fractionated regimen (day 1, 3, 5) and daily intake (Adamo et al. 2019; Addeo et al. 2010; Guetz et al. 2017). In patients with non-small-cell lung cancer, the daily administration of VRL up to 40 mg per day was well-tolerated (Banna et al. 2018; Guetz et al. 2017). For these reasons, VinoMetro aimed to investigate a truly metronomic schedule with daily oral VRL in HR+/HER2− MBC patients following endocrine resistance, by assessing efficacy and safety of doses well below the maximum tolerated dose in advanced breast cancer patients with visceral metastases.

Methods

Study design

VinoMetro (ClinicalTrials.gov Identifier NCT03007992) was an investigator-initiated national, multicentre, open-label, single-arm phase II study sponsored by the University Medical Centre of the Johannes Gutenberg-University Mainz, Germany. It was initially planned to conduct the trial in 8 AGO-B (Arbeitsgemeinschaft Gynäkologische Onkologie–Breast) centres in Germany. As the study was stopped early upon request of the institutional review board (IRB) due to occurrence of one grade 5 toxicity, only two of these centres actually enrolled and treated patients between January 2017 and April 2019. The study was conducted in accordance with the 1987 Declaration of Helsinki and the Good Clinical Practice (ICH-GCP) guidelines. Approval of the protocol was obtained from the local ethics committee for each participating centre. Written informed consent was obtained from all patients prior to the performance of any trial specific procedure.

Patients and treatment

Eligible patients were female, ≥ 18 years, with ECOG performance status ≤ 1 and estimated life expectancy ≥ 16 weeks. Further inclusion criteria were histologically confirmed BC and locally advanced or metastatic disease, previously untreated by palliative CT and not amenable to any curative treatment. Moreover, the included patients had to present with HR+ disease determined by ≥ 1% positive-stained cells for oestrogen receptor (ER) and/or progesterone receptor (PR) by immunohistochemistry (IHC) as well as HER2− disease (IHC 0–1 + or IHC 2+, confirmed as FISH or CISH negative) in the primary tumour or a metastatic site. Only patients with relapse ≤ 12 months from end of adjuvant endocrine therapy or progression during/after the first line of endocrine therapy in the metastatic setting and/or being no longer a candidate for further endocrine therapy were included. Prior (neo-) adjuvant CT was allowed if the interval between end of CT and date of registration was > 12 months. Prior treatment with everolimus and/or cyclin-dependent kinase (CDK) 4/6 inhibitors as part of endocrine-based therapy was allowed. Presence of ≥ 1 measurable lesion as per RECIST 1.1 (Schwartz et al. 2016), which had not been previously irradiated as well as adequate bone marrow, hepatic and renal functions was required. The main exclusion criteria were prior vinca-alkaloids, aggressive disease-requiring combination CT and cerebral involvement. Patients with no recovery to ≤ grade 1 side effects (exception: alopecia) of any prior antineoplastic treatment, current peripheral neuropathy ≥ grade 2 and dysphagia or inability to swallow oral medication were also excluded.

Oral VRL (Navelbine® soft capsules) was administered at a daily dose of 30 mg (flat dose without any adaptation to body weight or body surface area) without breaks. One treatment cycle was defined as 28 days of therapy. Treatment was continued until disease progression, occurrence of unacceptable toxicity, patient's refusal, or investigator's decision to stop the treatment. Dose adjustments to 20 mg per day and

dose delays were permitted in those patients who were unable to tolerate the dosing. Supportive care during the study was provided in accordance with established clinical standards and protocols. Blood tests including hematocrit, hemoglobin, red blood cell count (RBC), platelets, white blood count (WBC), differential (basophils, eosinophils, lymphocytes, monocytes, neutrophils) as well as clinical chemistry, including SGPT, SGOT, gamma-GT, alkaline phosphatase, total bilirubin, and creatinine, were performed weekly in the first two cycles and every two weeks afterwards.

Study evaluation

The primary endpoint was the clinical benefit rate (CBR; complete response [CR] + partial response [PR] + stable disease [SD]) at 24 weeks after start of metronomic treatment with daily oral VRL. Secondary objectives were to further assess the efficacy of metronomic VRL in terms of overall response rate (ORR; CR + PR), duration of disease control (DoDC), duration of stable disease (DoSD), progression-free survival (PFS), time-to-treatment-failure (TTF) and OS. Tumour measurements by computed tomography scan or magnetic resonance imaging were performed at screening (chest and abdomen/pelvis; within 28 days prior to the first intake of study medication) and repeated every 12 weeks (±7 days) until end of study treatment. Whole-body bone scintigraphy and further potential imaging were performed according to clinical indication. Clinical response was determined using the revised RECIST guidelines version 1.1 (Schwartz et al. 2016). Assessment of safety and tolerability of metronomic VRL was conducted according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

Statistical analysis

The minimum required level of efficacy (p0) was set to a CBR of 35% based on a prior trial investigating the standard-monotherapy with oral VRL in the first-line setting of patients with HR+/HER2− MBC (Freyer et al. 2003). An increase in CBR by 20% when using the metronomic treatment rated as being clinically relevant and accordingly the target CBR (p1) was set to 55%. Simon's two-stage minimax design was used. The null hypothesis that the true response rate is 0.35 (p0) was tested against a one-sided alternative. In the first stage, 21 patients were to be accrued. If there had been ≤ 8 patients with clinical benefit at 24 weeks in these 21 patients, the study would have been stopped. Otherwise, 18 additional patients would have been accrued for a total of 39. This design would yield a type I error rate of 0.05 and power of 0.80 when the true response rate was 0.55 (p1). Considering an anticipated dropout rate of approximately 15%, a total of 45 patients had to be accrued for this trial.

ORR and DCR were summarized as percentage rate and 95% confidence interval (CI). DoDC and DoSD were also analysed descriptively. PFS, TTF and OS were assessed using the Kaplan–Meier method.

Results

Patient characteristics

Between January 2017 and April 2019, 9 patients were recruited from two active cancer centres in Germany. Patient characteristics are listed in Table 1. The median age was 63.0 years (52.0–77.0). At the time of the first diagnosis (FD) of BC 2 (22.2%) and 7 (77.8%) patients had a T1 and T2 tumour, respectively. 3 (33.3%) patients were node-negative and 6 (66.7%) patients were node-positive (N1). None of the patients presented distant metastases at the time of FD. 5 (55.6%) and 4 (44.4%) tumours showed histological grade 2 and grade 3 cancer, respectively. 6 (66.7%) tumours were ER/PR-positive and 3 (33.3%) only ER-positive. 8 (88.9%) patients were postmenopausal. The median number of measurable metastatic lesions was 2.0 (1.0–3.0) with the liver being predominantly affected (9/17). The median size of these lesions was 50 mm and ranged from 15 to 71 mm. All patients presented with visceral metastases at the time of start of treatment. Local R0 surgical therapy as well as radiotherapy was performed in all patients within initial treatment (Table 2). 8 (88.9%) patients received adjuvant CT. Median number of prior lines of endocrine-based therapy was 3.0 (2.0–4.0) and the most common agents were tamoxifen (23.3%), letrozole (23.3%) and fulvestrant (20.0%).

Therapy response

The primary endpoint CBR at 24 weeks after start of metronomic treatment with daily oral VRL was 22.2% (90% CI 4.1–55.0), $p = 0.211$ (Table 3). There was no objective response achieved after 12 weeks of treatment. The results of DoDC and DoSD are identical because only SD could be achieved. Median DoDC/DoSD was 45.8 weeks (31.4–60.1). Median PFS was 12.0 weeks (95% CI 11.3–12.7) (Fig. 1), with a median TTF of 13.4 weeks (95% CI 9.0–17.8) (Fig. 2). Two patients who terminated the study early without opportunity for follow-up were censored in the calculation of PFS. Altogether three patients died, hence median OS could not be estimated.

Safety results

The median number of completed treatment cycles was 4.0 (0–18). One patient reached treatment cycle 18 and another one treatment cycle 10. All other patients discontinued the

Table 1 Patient characteristics

Characteristics		Patients ($n=9$) (%)
Age (years)	Median	63.0
	Range	52.0–77.0
T_TNM	T1	2 (22.2%)
	T2	7 (77.8%)
	T3	0 (0.0%)
	T4	0 (0.0%)
N_TNM	N0	3 (33.3%)
	N1	2 (22.2%)
	N2	2 (22.2%)
	N3	2 (22.2%)
M_TNM	M0	9 (100.0%)
	M1	0 (0.0%)
Histological grade	G1	0 (0.0%)
	G2	5 (55.6%)
	G3	4 (44.4%)
HR status	ER-positive	9 (100.0%)
	ER-negative	0 (0.0%)
	PR-positive	6 (66.7%)
	PR-negative	3 (33.3%)
Menopausal status	Perimenopausal	1 (11.1%)
	Postmenopausal	8 (88.9%)
Measurable metastatic lesions	Median	2.0
	Range	1.0–3.0
Measurable metastatic lesions n (events) ($n=17$)	Liver	9 (52.9%)
	Pleura	2 (11.8%)
	Cranium	1 (5.9%)
	Lymph nodes distant	3 (17.6%)
	Lymph nodes locoregional	1 (5.9%)
	Soft tissue	1 (5.9%)
Metastatic lesions n (patients)	Liver	9 (100%)
	Lung	1 (11.1%)
	Pleura	2 (22.2%)
	Peritoneum	1 (11.1%)
	Cranium	1 (11.1%)
	Bone	7 (77.8%)
	Lymph	3 (33.3%)
	Soft tissue	1 (11.1%)

T tumour size, *N* nodal status, *M* distant metastasis, *G* grade, *HR* hormone receptor, *ER* oestrogen receptor, *PR* progesterone receptor

treatment at cycle 5 or earlier. The median adherence to therapy was 91% (49–100) (Table 3). The median average daily dose of VRL was 27.3 mg (14.7–30.0).

In total, 73 adverse events (AEs) were reported (8.1 per patient). 37 (50.7%) AEs were assessed as related to study treatment (4.1 per patient). The most frequent clinical AEs were nausea (55.6%), fatigue (44.4%) and diarrhoea (33.3%) (Table 4). Grade 3–4 AEs, including elevated liver enzymes, were documented in 2 (22.2%) patients. 1 (11.1%) grade 5 AE with a fatal outcome occurred as a consequence of neutropenic fever and pneumonia. The patient was 77 years old and had received tamoxifen and letrozole in combination with a CDK 4/6 inhibitor without (neo-) adjuvant CT prior to start of study treatment. The study medication was begun 4 weeks after prior therapy with normal blood values and was administered for only 12 days before the onset of pneumonia and febrile neutropenia. 4 days later, the patient died as a result of septic shock. After the occurrence of this grade 5 AE and taken into account the limited efficacy observed so far, it was decided in agreement with the lead

Table 2 Prior therapy before study treatment

Therapy		Patients ($n=9$) (%)
Surgical therapy	Yes	9 (100.0%)
	R0 surgery	9 (100.0%)
Adjuvant chemotherapy	Yes	8 (88.9%)
	No	1 (11.1%)
Adjuvant chemotherapy n (events) ($n=10$)	EC	3 (30.0%)
	EC-paclitaxel	3 (30.0%)
	FEC-docetaxel	2 (20.0%)
	Other	2 (20.0%)
Endocrine-based therapy	Yes	9 (100%)
	No	0 (0.0%)
Lines of endocrine-based therapy	Median	3.0
	Range	2.0–4.0
Endocrine-based therapy n (events) ($n=30$)	Tamoxifen	7 (23.3%)
	Letrozole	5 (16.7%)
	Letrozole/ CDK 4/6 inhibitor	2 (6.6%)
	Fulvestrant	5 (16.7%)
	Fulvestrant/CDK 4/6 inhibitor	1 (3.3%)
	Anastrozole	2 (6.7%)
	Exemestane	5 (16.7%)
	Leuprorelin/goserelin	3 (10.0%)
Radiotherapy	Yes	9 (100%)
	No	0 (0.0%)
Radiotherapy n (events) ($n=23$)	Breast	6 (26.1%)
	Thorax wall	3 (13.0%)
	Lymphatic region	2 (8.7%)
	Axilla	2 (8.7%)
	Bone metastasis	10 (43.5%)

EC epirubicin/cyclophosphamide, *FEC* 5-fluorouracil/epirubicin/cyclophosphamide, *CDK* cyclin-dependent kinase

Table 3 Therapy response

Clinical benefit rate at 24 weeks after start of vinorelbine		Patients ($n=9$) (%)
	Yes	2 (22.2%)
	No	7 (78.8%)
	90% confidence interval	4.1–55.0
	p value ($p0=35\%$)	0.211
Duration of DoDC/DoSD (weeks)		Patients ($n=2$)
	Mean (SD)	45.8 (20.3)
	Median	45.8
	Range	31.4–60.1
	Missing	7 (78.8%)
Adherence to therapy (%)	Median	91.0
	Range	49.0–100.0
Average daily dose (mg)	Median	27.3
	Range	14.7–30.0
Total amount of study medication taken (mg)	Median	2430.0
	Range	360.0–15, 120.0

Adherence to therapy = Percentage of patients who took the study medication according to the study protocol

DoDC duration of disease control, *DoSD* duration of stable disease, *SD* standard deviation

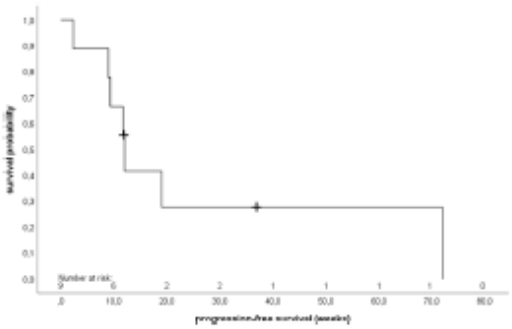

Fig. 1 Kaplan–Meier analysis of progression-free survival (PFS) ($n=9$). The median PFS was 12.0 weeks (95% confidence interval 11.3–12.7). + (censored)

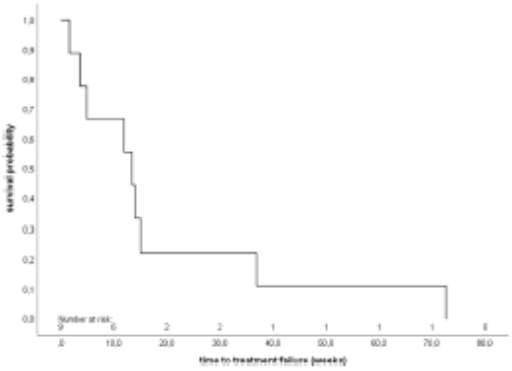

Fig. 2 Kaplan–Meier analysis of time to treatment failure (TTF) ($n=9$). The median TTF was 13.4 weeks (95% confidence interval 9.0–17.8)

Table 4 Adverse events

Adverse event	Patients ($n=9$) (%)			
	Overall	Grade 3	Grade 4	Grade 5
Neutropenia	1 (11.1%)	0	0	0
Anaemia	1 (11.1%)	0	0	0
Thrombocytopenia	0	0	0	0
Febrile infection	1 (11.1%)	0	0	1 (11.1%)
Fatigue	4 (44.4%)	0	0	0
Stomatitis	0	0	0	0
Nausea	5 (55.6%)	0	0	0
Vomiting	1 (11.1%)	0	0	0
Diarrhoea	3 (33.3%)	0	0	0
Constipation	0	0	0	0
Elevated liver enzymes	3 (33.3%)	2 (22.2%)	0	0

IRB to terminate the study early without activating the other centres.

Discussion

In VinoMetro, the observed CBR of metronomic VRL as a first-line CT in nine patients was 22.2%, well below the expected CBR. Moreover, after death of a patient in septic shock, the study was closed early in agreement with the IRB after careful risk / benefit analysis. To reduce potential publication bias, we decided to publish our results accordingly.

In the last years, therapy options for MBC increased steadily. Treatment choice should take several important factors into account. HR, HER2 status, germline BRCA status as well as PIK3CA mutation status in HR-positive and PD-L1 in triple-negative breast cancer (TNBC) should be assessed to allow for targeted therapies. Biological age, menopausal status, tumour burden, comorbidities and previous therapies with their toxicities are also crucial for decision-making (Cardoso et al. 2020; Thomssen et al. 2020). Nowadays, endocrine therapy combined with a CDK 4/6 inhibitor should be considered as a first choice in patients with HR+/HER2– MBC without life-threatening disease. In case of endocrine resistance, the guidelines recommend sequential single-agent CT as the preferred choice for MBC. Combination CT should be reserved for patients with rapid clinical progression, life-threatening visceral metastases, and/or the need for rapid symptom/disease control (Cardoso et al. 2020; Ditsch et al. 2020; Thomssen et al. 2020). Nevertheless, there still remains a high medical need for new therapy options in MBC that prolong the time between endocrine failure and CT, the latter being potentially associated with impaired QoL and more severe side effects. In this respect, the present phase II study evaluated the efficacy and safety of metronomic daily VRL. The primary endpoint CBR at 24 weeks after start of study treatment was 22.2%. There was no objective response achieved after 12 weeks of treatment. The median DoDC/DoSD was 45.8 weeks (31.4–60.1). The median PFS was 12.0 weeks (95% CI 11.3–12.7) and the median TTF was 13.4 weeks (95% CI 9.0–17.8).

These results in a small patient number showed only a limited efficacy of metronomic VRL compared to previous studies (Table 5). Addeo et al. treated 34 MBC patients with oral VRL 70 mg/m^2 as first-line treatment, fractioned on days 1, 3 and 5, for 3 weeks on and 1 week off, every 4 weeks, for a maximum of 12 cycles. The median age was 75 years (70–84). The primary endpoint ORR was 38% (95% CI 28–48) and CBR at 12 weeks after start of treatment was 68% (95% CI 61–82). However, only 32% of patients suffered from visceral metastases compared to the present study where all patients showed visceral involvement (Addeo et al. 2010). In a study by De Iuliis et al., 32 MBC patients treated

Table 5 Metronomic vinorelbine in patients with metastatic breast cancer

Study	Phase/clinical setting	Treatment	Number/age of patients (years)	Tumour	Results	
					Efficacy	Toxicity
Addeo et al. (2010)	II; first line	VRL (70 mg/m^2) days 1, 3 and 5 (3 weeks on, 1 week off), q4w	34; median age (range) 74 (70–84)	ER+ or unknown status 62%	ORR 38% (95% CI, 28–48) mPFS 7.7 months (95% CI, 6.9–9.05) mOS 15.9 months (95% CI, 13.1–15.9)	Haematologic toxicity (grade 3/4) 24%; non-Haematologic toxicity (grade 3/4) 18%
De Iuliis et al. (2015)	II; several lines	VRL 30 mg one day on and one day off without interruptions	32; median age (range) 76 (69–83)	–	CBR 50%	No grade 3/4 toxicities
Cazzaniga et al. 2016, Victor-2 study	II; first line / ≥ second line	VRL 40 mg days 1,3 and 5, CAPE 500 mg thrice daily without interruptions	80 (35/45); median age (range) 66.3 (38.0–85.6) / 64.9 (44.0–82.7)	HR+ 65% Triple-negative 35%	CBR 45.7% (95% CI, 28.8–63.4) / 51.1% (95% CI, 35.8–66.3) ORR 35.5% (95% CI, 19.2–54.6) / 25.6% (95%CI, 13.5–41.2) mPFS 6.7 months (IQR, 4.7–11.3)/7.2 months (95% CI 2.8–11.5)	Haematologic toxicity (grade 3/4) 18%; non-haematologic toxicity (grade 3/4) 31%
Brems-Eskildsen et al. (2020), XeNa trial	II; first and second line	Arm A: VRL (60–80 mg/m^2) day 1 and 8 + CAPE 2000 mg/m^2 day 1–14 / Arm B: VRL 50 mg 3 times a week + CAPE 2000 mg/m^2 day 1–14	118 (60 / 58); median age 60.8 / 60.9	ER+ 82% / 78%; all HER2−	CBR 46.8% / 51.7% mPFS 7.1 (95% CI, 3.9–10.3) / 6.3 (95% CI, 4.1–8.5) mOS 23.3 months (95% CI 20.2–26.4) / 22.3 months (95% CI, 14.3–30.3)	Haematologic toxicity (grade 3/4) 16% / 5%; non-haematologic toxicity (grade 3/4) 11% / 17%
Montagna et al. (2017), VEX trial	II; first line / ≥ second line	VRL 30–40 mg 3 times a week, CTX 50 mg daily, CAPE 500 mg thrice daily	108 (43 / 65); mean age (SD) 52.6 (10.1) / 55.2 (10.0)	HR+	CBR 81% / 74% mTTP 25.1 months (95% CI, 14.2–39.1) / 11.2 months (95% CI, 9.2–17.0)	Grade 3/4: 21% / 15%
Sanna et al. (2020)	Phase I; ≥ 1 prior line of therapy	VRL 20–40 mg 3 times per week, CTX 50 mg daily, bevacizumab 15 mg/kg q 3w (HER2 + patients: + trastuzumab q 3w)	15; median age (range) 61 (29–72)	ER + 80% HER2+ 33%	CBR 66.6% mPFS 6.9 months	Grade 3/4 toxicity 20%

VRL vinorelbine, *ER+* oestrogen receptor-positive, *ORR* overall response rate, *CI* confidence interval, *mPFS* median progression-free survival, *mOS* median overall survival, *CBR* clinical benefit rate, *CAPE* capecitabine, *HR +* hormone receptor-positive, *IQR* interquartile range, *HER2−* human epidermal growth factor receptor 2-negative, *CTX* cyclophosphamide, *SD* standard deviation, *mTTP* median time to treatment failure, *HER2+* human epidermal growth factor receptor 2-positive

with oral VRL 30 mg every other day showed CBR of 50%. The median age was 76 years (69–83) and the patients were pre-treated with several CT lines (De Iuliis et al. 2015). In the VEX trial, Montagna et al. showed a significant activity and good tolerability of MCT in HR+MBC patients when VRL was administered in combination with cyclophosphamide (CTX) and capecitabine (CAPE). VRL 30 or 40 mg three times a week, CTX 50 mg once daily and CAPE 500 mg thrice daily received 43 patients as first-line CT and 65 patients as ≥ second-line CT. Visceral disease at the time of study inclusion was reported in 71% of patients. CBR for more than 6 months was 81% in the naive and 74% in the pre-treated group, the median time to progression (TTP) was 25.1 months (95% CI 14.2–39.1) and 11.2 months (95% CI 9.2–17.0), respectively (Montagna et al. 2017).

On the basis of favourable efficacy results with ORR of 4–31%, CBR of 49–56% and median PFS of 3.7–8.2 months, oral weekly VRL is considered as an active oral alternative to intravenous CT in the first-line CT treatment of MBC (Blancas et al. 2019; Freyer et al. 2003; Steger et al. 2018). It is noteworthy that these results are similar to the results of metronomic VRL regimens. In the VICTOR-2-study, MBC patients were treated with metronomic VRL 40 mg three times a week and CAPE 500 mg three times a day. The CBR was 48.8% (95% CI 37.4–60.2) and the median PFS was 6.7 months (95% CI 4.7–11.3) in the first-line treatment group and 7.2 months (95% CI 2.8–11.5) in the ≥ second-line treatment group (Cazzaniga et al. 2016). The efficacy of metronomic VRL 50 mg three times a week combined with standard CAPE treatment in HER2− MBC could be confirmed in the randomized phase II study XeNa. In the metronomic group with visceral involvement in 86% of patients, the CBR was 51.7% (95% CI 39.1–64.9) and the median PFS was 6.3 months (95% CI 4.1–8.5) without significant difference compared to the standard treatment (Brems-Eskildsen et al. 2020). Another retrospective study by Cazzaniga et al. which collected data from 584 h+/HER2− MBC patients treated with MCT showed an increased use of VRL-based regimens during the last years (2011: 16.8%—2016: 29.8%). 79.3% of patients received MCT as single agent. In the first-line setting, the highest ORR and DCR were observed for VRL-based regimens (single agent: 44% and 88%; combination: 36.7% and 82.4%, respectively). The median PFS was 7.2 months (95% CI 5.3–10.3) for VRL single agent and 9.5 months (95% CI 8.8–11.3) for VRL combinations. The median OS was 22.7 months (95% CI 13.0–43.5) for VRL single agent and 30.9 months (95% CI 26.2–34.7) for VRL in combination regimens (Cazzaniga et al. 2019b).

A meta-analysis of randomized trials including 2,269 MBC patients has shown that longer first-line CT duration is associated with marginally longer OS and a substantially longer PFS (Gennari et al. 2011). Thus, it is of high importance to find anticancer agents that could be administered for a long period without dose accumulation and accumulation of unacceptable side effects. In the present study 22.2% of patients presented grade 3–4 AE. More favourable results were reported in previous studies on MCT. The incidence of grade 3–4 events was 6–24%. The most frequent AEs grade 3–4 were anaemia and neutropenia (≤9%), elevated liver enzymes (5%) and gastrointestinal disorders (<5%). Discontinuations due to AEs were observed up to 9% (Addeo et al. 2010, Cazzaniga et al. 2016, Cazzaniga et al. 2019b, De Iuliis et al. 2015, Montagna et al. 2017).

Most patients with incurable cancer prefer oral to intravenous therapy (Liu et al. 1997). A questionnaire assessing the perception of oral anticancer treatment could demonstrate a high acceptance of oral CT by most MBC patients. Oral administration helped the patients to feel less ill and to reduce the effort in coping with the disease (Catania et al. 2005). Moreover, metronomic VRL allows for easy daily or fractioned administration with the possibility of individual dose adjustment in case of toxicities and require less frequent hospital visits compared to standard intravenous CT (Gebbia and Puozzo 2005).

Taken together, there is evidence of increasing use of MCT, especially metronomic VRL, in MBC (Cazzaniga et al. 2019b; Sanna et al. 2020; Xu et al. 2020). However, randomized trials and phase III studies are lacking and there is currently not enough evidence about which regimen and which administration should be preferred (Cardoso et al. 2020; Cazzaniga et al. 2019a). The VinoMetro study did not confirm the results of previous studies of oral metronomic VRL, although daily oral administration has not been previously studied in MBC patients. One possible reason to explain these discrepancies could be the fact that all patients in VinoMetro had HR+ disease and visceral metastases. Another possible reason could be the daily administration of VRL as a single agent. Finally, given the small sample size, chance could be an additional explanation. Further insights with regard to metronomic VRL (fractionated regimen) are currently being generated in two randomized studies (TempoBreast: NCT03007992; TempoLung: EudraCT 2014-003859-61) comparing the metronomic with the conventional regimen in MBC and advanced non-small-cell lung cancer.

A weakness of our study is the limited sample size due to early study termination, thus the interpretation of the presented results is limited. A strength, however, is the prospective design evaluating for the first time the effectiveness and safety of daily administered low-dose metronomic VRL as first-line therapy in endocrine-resistant MBC with visceral metastases.

Conclusions

This phase II study had to be closed early. The results in a small patient cohort showed only limited benefit of this treatment regimen in ER +/HER2− MBC patients with visceral metastases and progressive disease after endocrine-based therapy. The clinical relevance of the presented VRL administration in MBC should be evaluated in further trials.

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Author contributions MS: Conceptualization; MS, CR, LS, CR: Methodology; LS, CR, SK, MS: Formal analysis and investigation; SK: Writing–original draft preparation; TD, LS, CRO, CRU, TF, CT, NH, MS: Writing–review and editing; MS: Funding acquisition; MS: Resources; MS: Supervision; All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest Slavomir Krajnak received speaker honoraria from Roche Pharma AG, research funding from Novartis and travel reimbursement from PharmaMar outside the submitted work. Thomas Decker reports personal fees from Lilly and Novartis outside the submitted work. Christian Rosé is an employee of Pierre-Fabre. Tanja Fehm received honoraria from Onkowissen, Pfizer, Novartis, Roche, MSD, Daichii Sankyo, AstraZeneca outside the submitted work. Christoph Thomssen received honoraria for advisory boards and lectures from Amgen, Astra-Zeneca, Celgene, Daiichi Sankyo, Eisai, Lilly, MSD, Nanostring, Novartis, Pfizer, Pierre Fabre, Puma, Roche, Vifor outside the submitted work; he received research support (by discount prizes) from American Diagnostica, Affymetrix, Nanostring. Nadia Harbeck received honoraria for consulting and/or lectures from Astra Zeneca, Daiichi-Sankyo, Lilly, MSD, Novartis, Pierre Fabre, Pfizer, Roche, Sandoz/Hexal, Seattle Genetics outside the submitted work. Marcus Schmidt reports grants from Pierre-Fable during the conduct of the study; he received honoraria for consulting and/or lectures from Amgen, AstraZeneca, Eisai, Lilly, Myelo Therapeutics, Novartis, Pantarhei Bioscience, Pfizer, Pierre-Fabre, Roche and Seattle Genetics outside the submitted work. He received research funding from AstraZeneca, BioNTech, Eisai, Genentech, German Breast Group, Myelo Therapeutics, Novartis, Palleos, Pantarhei Bioscience, Pierre-Fabre, and Roche. He received travel reimbursement from BioNTech, Pantarhei Bioscience, Pfizer and Roche; in addition, he has a patent for EP 2951317 B1 and a patent for EP 2390370 B1 issued. All other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study were in accordance with GCP, with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz and local ethics committee for each participating centre. Informed consent was obtained from all individual participants participating in the study.

Informed consent to participate Informed consent to participate in the study was obtained from all participants.

Informed consent for publication All participants consented to the publication of the study.

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ANTI-TUMOR EFFECTS OF LOW-DOSE METRONOMIC VINORELBINE IN COMBINATION WITH ALPELISIB IN BREAST CANCER CELLS

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ABSTRACT

In metastatic breast cancer (MBC), *PIK3CA* mutations, activating the phosphatidylinositol 3-kinase (PI3K) signaling pathway seem to be associated with chemotherapy resistance and poor outcome. Inhibition of the PI3K signaling pathway may lead to sensitization and prevention of the development of resistance to cytotoxic drugs. The present study aimed to investigate the anti-tumor activity of low-dose vinorelbine (VRL) combined with alpelisib, an α-selective PI3K inhibitor and degrader, in breast cancer (BC) cells. Human BC cell lines MCF-7, T-47D [both hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, *PIK3CA*-mutated], MDA-MB-231 and BT-549 (both triple-negative, wild-type *PIK3CA*) were exposed to a combination of low-dose VRL and alpelisib for 3 and 7 days. Cell viability was detected by the Alamar blue assay, and cell proliferation was determined by the BrdU incorporation. The effect of the substances on the p110α protein expression that is encoded by *PIK3CA* gene was investigated by Western blot. Low-dose VRL plus alpelisib showed synergistic anti-tumor effects and significantly inhibited cell viability and proliferation of MCF-7 and T-47D cells. Even lower alpelisib concentrations (10 ng/ml and 100 ng/ml) combined with low-dose metronomic VRL led to a significant reduction of cell viability of *PIK3CA*-mutated cells, and the anti-tumor activity was comparable with the effects at 1000 ng/ml alpelisib. Cell viability and proliferation of MDA-MB-231 and BT-549 cells were inhibited by VRL but not by alpelisib alone. This indicates that alpelisib did not significantly affect the cell growth of triple-negative, *PIK3CA* wild-type BC cells. The p110α expression was downregulated or not affected in *PIK3CA*-mutated cell lines, and not significantly upregulated in *PIK3CA* wild-type cell lines. In conclusion, combination of low-dose metronomic VRL and alpelisib showed synergistic anti-tumor effects and significantly inhibited the growth of HR-positive, HER2-negative, *PIK3CA*-mutated BC cells, providing a rationale for further efforts to evaluate this combination *in vivo*.

Keywords: Low-dose metronomic chemotherapy, *PIK3CA* mutation, vinorelbine, alpelisib, breast cancer

INTRODUCTION

With an incidence of 2.3 million and a mortality of 0.7 million cases per year, female breast cancer (BC) represents the most commonly diagnosed cancer and the fifth leading cause of cancer mortality worldwide (Sung et

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al., 2021). In the advanced/metastatic breast cancer (MBC), the goal of care is to achieve a disease chronification and preservation of the health-related quality of life (Harbeck and Gnant, 2017). Despite the plethora of possible treatment options for MBC, there is still a high medical need for new treatment options that provide sufficient and long-term anti-tumor effect with a manageable toxicity profile.

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway plays one of the key roles in the regulation of cellular growth, metabolism, migration, and survival (Bilanges et al., 2019; Khezri et al., 2022; Li et al., 2021). Genomic alterations of this pathway are detectable in many cancers, including BC (Millis et al., 2019; Naeem et al., 2022). It is estimated that 60-70 % of BC patients have at least one mutation in the PI3K/AKT/mTOR pathway, whereas *PIK3CA* gene, which encodes the p110α subunit of PI3K, is the most frequently mutated gene (Lee et al., 2015; Millis et al., 2019; Xiao et al., 2021). *PIK3CA* mutation frequencies are different among BC subtypes: 34.5-48.3 %, 22.7-42.2 % and 8.3-25.0 % in HR-positive, HER2-positive, and in triple-negative BC, respectively (Martinez-Saez et al., 2020; Stemke-Hale et al., 2008; Xiao et al., 2021). The resulting overactivation of the PI3K pathway promotes tumor growth, resistance to various systemic therapies and poor outcome (Dong et al., 2021; Mosele et al., 2020; Rasti et al., 2022; Sobhani et al., 2018; Yang et al., 2019). Consequently, PI3K inhibition, especially in combination with other substances, is expected to be a new approach in the treatment of BC (Fuso et al., 2022). SOLAR-1 study demonstrated a significant prolongation of progression-free survival (PFS) and 7.9-month improvement in median overall survival (OS) when alpelisib, an orally bioavailable, α-selective PI3K inhibitor and degrader, was added to fulvestrant treatment of patients with *PIK3CA*-mutated, hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced BC (Andre et al., 2019, 2021). Preclinical models have shown that inhibition of the PI3K signaling pathway can lead to a sensitization as well as a prevention of the development of resistance to cytotoxic drugs (Badinloo and Esmaeili-Mahani, 2014; Rajput et al., 2019). Metronomic chemotherapy (MCT), defined as continuous daily administration of chemotherapeutic agents at low doses, has shown promising results in MBC (Cazzaniga et al., 2019b; Krajnak et al., 2020; Liu et al., 2017; Montagna et al., 2022). Based on its proven efficacy and good tolerability, MCT is increasingly perceived as a possible treatment option for MBC (Cardoso et al., 2020). MCT is considered a multimodal therapy that exerts its effects via immunomodulation, inhibition of angiogenesis, and direct cytotoxic effects (Andre et al., 2017; Cazzaniga et al., 2021). Due to the multimodal mechanisms of action, the combination with PI3K inhibitors may represent a new promising approach in the BC treatment, preventing resistance to chemotherapy and improving prognosis. Thus, this study aimed to investigate the anti-tumor activity of low-dose metronomic vinorelbine (VRL) combined with alpelisib in various BC cell lines.

MATERIALS AND METHODS

Cell culture and treatment

Human BC cell lines MCF-7 (HR-positive, HER2-negative with a p.E545K missense mutation in the *PIK3CA* gene), T-47D (HR-positive, HER2-negative with a p.H1047R missense mutation in the *PIK3CA* gene), MDA-MB-231 and BT-549 (both triple-negative, wild-type *PIK3CA*), were all obtained from American Type Culture Collection (Rockville, MD, USA). BC cells were cultured in RPMI 1640 culture medium (Thermo Fisher Scientific, Waltham, MA, USA), containing 10 % fetal calf serum and 1 % penicillin-streptomycin. Cells were incubated in a moistened atmosphere at 37 °C and 5 % CO_2. For all functional assays, cells were detached by trypsin-ethylenediaminetetraacetic acid (Sigma Aldrich, St. Louis, MO, USA) and seeded in 96-well plates (Greiner

Bio-One, Frickenhausen, Germany) at 3,000 cells/well (passage 10/semiconfluence). VRL (Navirel®; Medac, Wedel, Germany), (stock solution 10 mg/ml) diluted in Aqua dest., was added at levels corresponding to the concentration in serum of metronomically treated patients (0.63-5 ng/ml VRL), *i.e.* at much lower concentrations compared to maximum tolerated dose of conventional chemotherapy (Bocci and Kerbel, 2016; Briasoulis et al., 2013). Alpelisib (Piqray®, Novartis Pharma AG, Basel, Switzerland) (stock solution 2 mg/ml), diluted in 100 % dimethyl sulfoxide (DMSO) (Carl Roth GmbH, Karlsruhe, Germany), was added at concentrations equivalent to serum concentration in patients receiving an approved dose of 300 mg/day (500-1000 ng/ml) (Juric et al., 2018). In addition, in view of a possible reduction of side effects *in vivo*, alpelisib was tested also at lower concentrations (10 and 100 ng/ml). The treatment with both substances was performed continuously for 3 and 7 days to simulate the metronomic dosing schedule.

Cell viability assay

Cell viability was measured using the Alamar blue assay kit (Thermo Fisher Scientific). After 3 and 7 days of treatment, 100 µl/well Alamar blue Cell Viability Reagent solution [(1:10 in Dulbecco's Phosphate-Buffered Solution (DPBS)] was added to the cell cultures and incubated for 4 hours at 37 °C and 5 % CO_2 in aluminum foil due to the photosensitivity of the redox dye for fluorescence measurements. The absorbance was measured at 590 nm (650 nm reference wavelength) using a GloMax® multi detection system microplate reader (Anthos Labtec Instruments, Cambridge, UK).

Cell proliferation assay

Cell proliferation was detected by the Bromdeoxyuridine (BrdU) incorporation kit (Roche, Basel, Switzerland). 10 µl/well BrdU labeling solution (1:100 in DPBS) was added to the cells and incubated for 3 hours at 37 °C and 5 % CO_2. Afterwards supernatants were discarded and 200 ml/well Fix-Denat solution and 100 µl/well anti-BrdU antibody solution (1:100 in antibody dilution solution) were added. After incubation for 60 min at room temperature the absorbance was measured at 450 nm (690 nm reference wavelength) using a GloMax® multi detection system microplate reader (Anthos Labtec Instruments).

Western blot

To prepare protein extracts from cell culture, tumor cells were seeded on 143 mm^2 cell culture plates. The cell number was chosen according to the proliferation rate so that the culture dishes were semiconfluent at the time of protein extraction. One day after seeding, the cells were treated for 7 days. For protein extraction, cells were washed with DPBS and mechanically removed with a cell scraper. The solution was centrifuged at 1200 rpm for 5 minutes. The supernatant was discarded and the pellet was dissolved with up to 1 ml of lysis buffer. The solution was transferred into a 2 ml reaction tube and placed on ice. After incubation for 30 min on ice the samples were centrifuged for 10 min at 14000 g. The supernatant was transferred to a new tube and stored at -20 °C. The BCA Protein assay kit (Thermo Fisher Scientific) was used to determine the protein concentration of the extracts. Equal amounts of protein (12.5 µg per lane) were separated by size using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 10 % polyacrylamide gels. Gels were transferred on polyvinylidene difluoride (PVDF) membrane by semi-dry blotting. Membranes were blocked for 1 hour according to the antibody manufacturer's instructions and then incubated with a primary antibody in blocking solution overnight at 4 °C on a roll mixer. The monoclonal antibody against PI3K p110α (Cell Signaling Technology, Danvers, MA, USA) was used at a dilution of 1:500. β-actin antibody (Sigma Aldrich) was employed at a dilution of 1:2000. After washing, the membranes were incubated with HRP-linked secondary antibody (Agilent, Santa Clara, CA, USA) at a dilution of 1:4000 (PI3K p110α Western blot) or 1:2000 (β-actin Western blot) for 1 hour at

room temperature, and after washing the bound antibodies were visualized by adding of an enhanced chemiluminescent solution (PerkinElmer Inc, Waltham, MA, USA) and detected in a chemiluminescence detector (ProteinSimple, San Jose, CA, USA). For quantification bands were quantified by densitometry evaluation using a computer-based pixel counting system (AlphaView, ProteinSimple). These values were referenced to the corresponding β-actin values of the same membrane as a loading control.

Statistical analysis

All experiments were performed in triplicates and repeated three times. The absorbance in Alamar blue and BrdU assay for the untreated control group was regarded as 100 % cell viability and 100 % cell proliferation, respectively. The results of absorbance for the treated groups were indicated as the mean ± standard deviation of three separate experiments and displayed as percentages relative to that of the control group. Student's *t*-test (Microsoft Excel 2013 v15.0; Microsoft Corporation, Redmond, WA, USA) was used to evaluate the statistical significance of the results. All p-values represented two-sided tests and statistical significance was assumed at a value of $p<0.05$. To quantify the effects of combination treatment, the combination index (CI)-isobologram equation based on the Chou-Talalay method was used (Chou, 2006). Based on the experimentally determined dose-response curves, the inhibitory concentrations $(IC)_{50}$ and IC_{80} were calculated by interpolation and used as a reference for further investigation of any synergistic effects according to the CI method. The CI was calculated according to the formula shown in formula 1 and was interpreted as follows: CI<1, synergism; CI=1, additive effect, and CI>1, antagonism (range ± 5 %). The graphical presentation was performed as a classical isobologram with isoboles of the IC_{50} or IC_{80} and presentation of the corresponding CI values in relation to the corresponding isoboles within the diagram.

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} \quad [1]$$

where CI = combination index (CI). $(D)_1$ and $(D)_2$: required concentrations of the active compounds in combination to achieve the defined effect. $(D_x)_1$ and $(D_x)_2$: required concentrations of each active compound considered individually to achieve defined effect.

RESULTS

Effects of low-dose vinorelbine plus alpelisib on cell viability

In both HR-positive, *PIK3CA*-mutated cell lines, the combination of low-dose VRL and alpelisib decreased cell viability with increasing concentrations after both 3 and 7 days of treatment (Figure 1a-1d). Even the combination of 0.63 ng/ml VRL and 10 ng/ml alpelisib significantly reduced cell viability of MCF-7 cells by 33.5 % ($p=0.005$) (Figure 1a) and T-47D cells by 22.8 % ($p=0.016$) (Figure 1c) after 3 days. At 2.5 ng/ml VRL plus 10 ng/ml alpelisib, cell viability of MCF-7 was 33.4 % after 3 days ($p<0.001$) and 19.1 % after 7 days ($p=0.002$), and decreased slightly to 26.8 % ($p=0.002$) and 13.5 % ($p<0.001$) at the highest concentrations (Figure 1a, 1b). Similar response of VRL plus alpelisib on cell viability was observed in T-47D cells. At 2.5 ng/ml VRL plus 100 ng/ml alpelisib, cell viability of T-47D was 48.0 % after 3 days ($p<0.001$) and 18.1 % after 7 days ($p<0.001$), and decreased slightly to 36.8 % ($p<0.001$) and 10.6 % ($p<0.001$) at the highest concentrations (Figure 1c, 1d).

In the triple-negative, *PIK3CA* wild-type cell lines, the combination of low-dose VRL and alpelisib reduced cell viability with increasing concentrations after both 3 and 7 days of treatment (Figure 1e-1h). Concentrations of 2.5 ng/ml VRL plus 10 ng/ml alpelisib significantly decreased cell viability of MDA-MB-231 cells by 56.9 % ($p=0.026$) (Figure 1f) and BT-549 cells by 71.7 % ($p=0.023$) (Figure 1h) after 7 days. The strongest effects with the lowest cell viability of MDA-MB-231 cells (19.7 %, $p<0.001$) and

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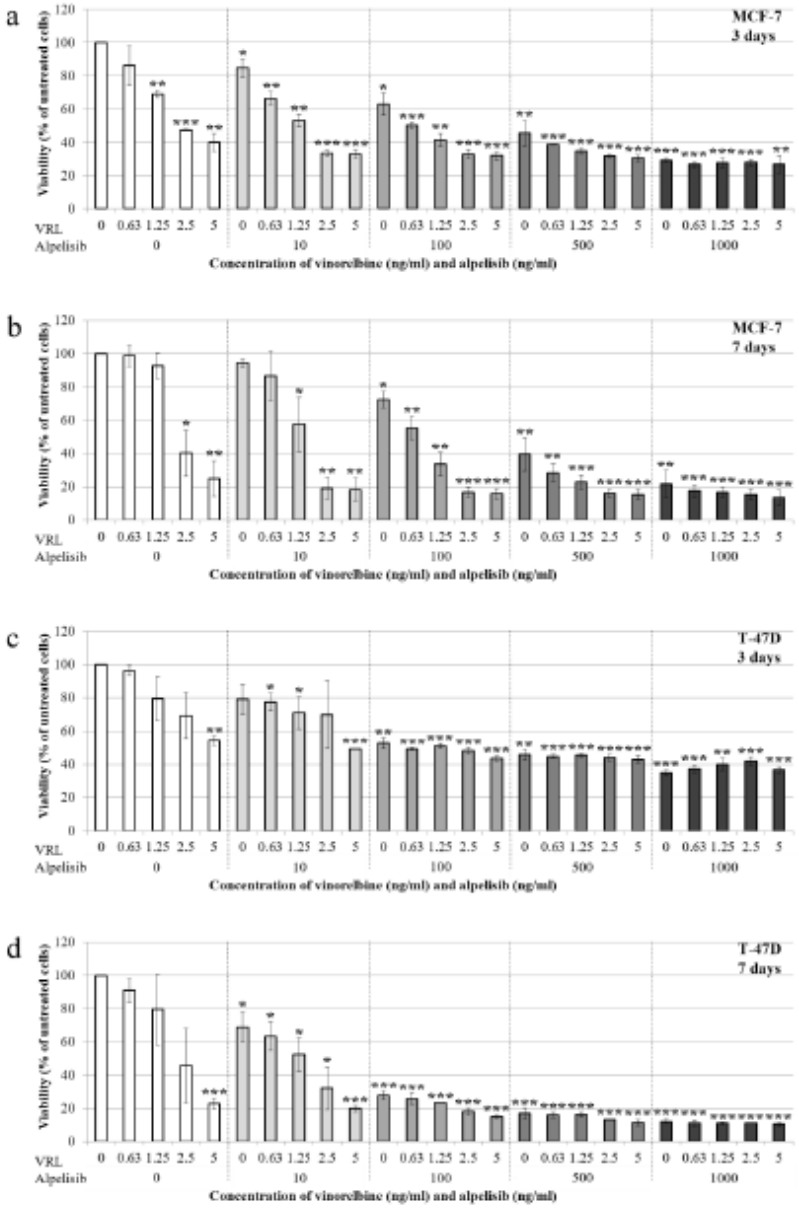
BT-549 cells (14.4 %, p=0.002) were observed at 5 ng/ml VRL and 1000 ng/ml alpelisib after 7 days of treatment (Figure 1f, 1h). In contrast to HR-positive cells with a *PIK3CA* mutation, the reduction of cell viability was not or only marginally affected by alpelisib alone. Concretely, alpelisib in concentrations below 1000 ng/ml did not significantly affect cell viability of the triple-negative *PIK3CA* wild-type cells tested.



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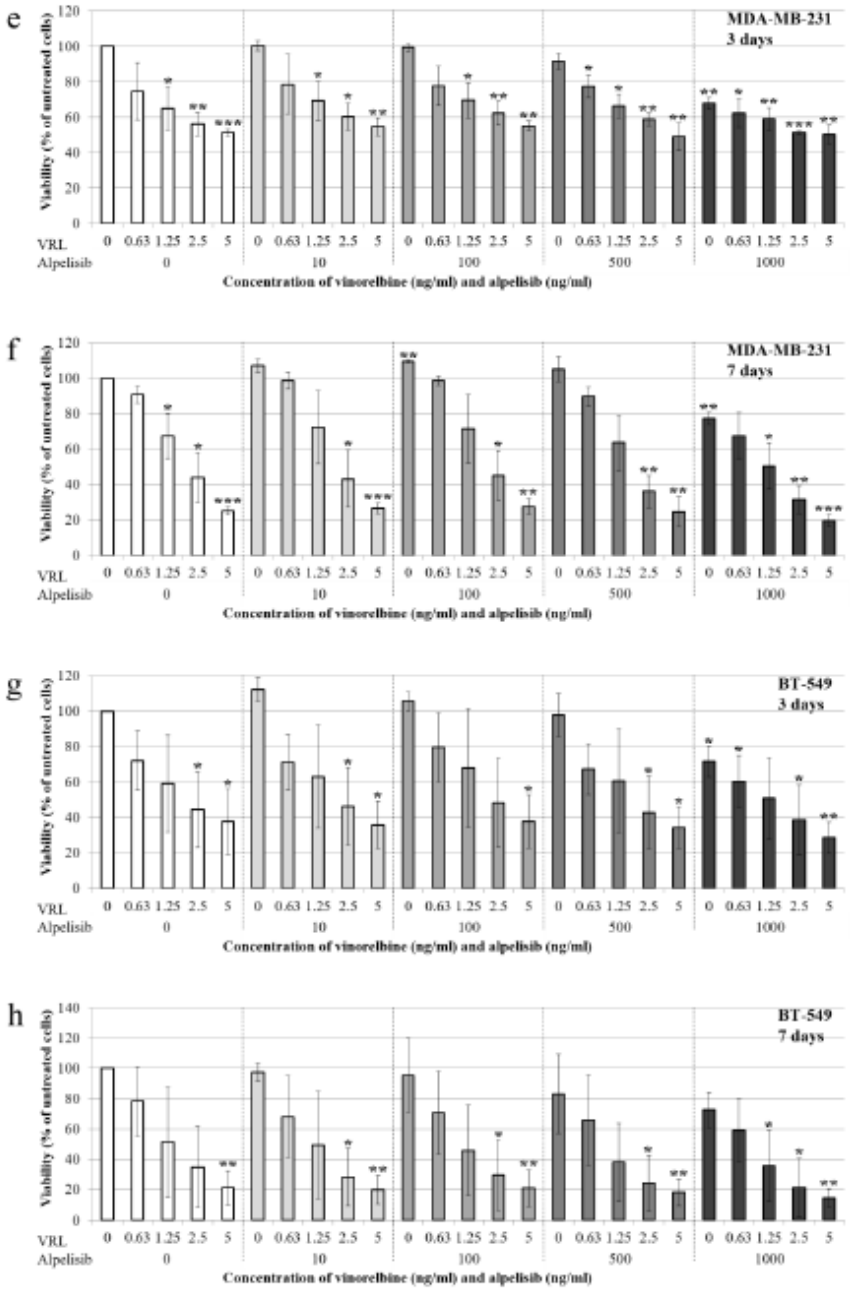

Figure 1: Effects of low-dose vinorelbine plus alpelisib on cell viability of the MCF-7 (a, b), T-47D (c, d), MDA-MB-231 (e, f) and BT-549 (g, h) cell line. Alamar blue assay was used to measure cell viability after 3 and 7 days of treatment with low-dose vinorelbine plus alpelisib. The results are shown as the mean ± standard deviation of three separate experiments. Statistical significance was assumed at *p<0.05, **p<0.01 and ***p<0.001.

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Effects of dimethyl sulfoxide on cell viability

The effects of DMSO on cell viability after 7 days of treatment were assessed at a concentration corresponding to the highest alpelisib concentration (0.5 % DMSO≙1000 ng/ml alpelisib) and in a concentration corresponding to a lower alpelisib concentration (0.1 % DMSO≙100-500 ng/ml alpelisib). In all cell lines tested DMSO at concentrations 0.1-0.5 % did not significantly affect the cell viability (Figure 2). However, the viability of BT-549 cells decreased by 24.7 % (p=0.319) when treated with 0.5 % DMSO, and thus this cell line was not used for further cell culture experiments due to potential confounding of the results.

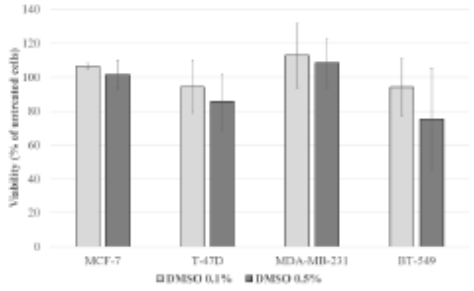

Figure 2: Effects of DMSO on cell viability of MCF-7, T-47D, MDA-MB-231 and BT-549 cells after 7 days of treatment at a concentration corresponding to the highest alpelisib concentration (0.5 % DMSO≙1000 ng/ml alpelisib) and in a concentration corresponding to a lower alpelisib concentration (0.1 % DMSO≙100-500 ng/ml alpelisib). The results are shown as the mean ± standard deviation of three separate experiments. Statistical significance was assumed at *p<0.05, **p<0.01 and ***p<0.001.

Effects of low-dose vinorelbine plus alpelisib on cell proliferation

In both HR-positive, *PIK3CA*-mutated cell lines, the combination of low-dose VRL and alpelisib decreased cell proliferation with increasing concentrations after 7 days of treatment (Figure 3a, 3b). Similar to the cell viability results, the reduction of cell proliferation was detectable also with single agents. Combination of 2.5 ng/ml VRL and 10 ng/ml alpelisib significantly reduced cell proliferation of MCF-7 by 32.9 % (p=0.004) and the highest concentrations reduced cell proliferation by 65.1 % (p=0.005) after 7 days of treatment (Figure 3a). In T-47D cells, even the lowest concentration of 0.63 ng/ml VRL plus 10 ng/ml alpelisib significantly decreased cell proliferation by 68.1 % (p=0.004) after 7 days of treatment (Figure 3b). At 2.5 ng/ml VRL plus 10 ng/ml alpelisib, cell proliferation was 13.5 % (p<0.001) and varied only marginally with the minimal value of 10.3 % (p<0.001) at higher concentrations.

In the triple-negative, *PIK3CA* wild-type MDA-MB-231 cells, the combination of low-dose VRL and alpelisib reduced cell proliferation with increasing concentrations. Combination of 2.5 ng/ml VRL and 10 ng/ml alpelisib significantly decreased cell proliferation of MDA-MB-231 cells by 57.8 % (p=0.021) after 7 days of treatment (Figure 3c), whereby increasing concentrations of alpelisib did not show further anti-tumor activity. The lowest cell proliferation of around 30.0 % was achieved at 5 ng/ml VRL and at alpelisib concentrations of above 100 ng/ml (p=0.010). This indicates that alpelisib did not significantly affect the cell proliferation of triple-negative, *PIK3CA* wild-type MDA-MB-231 cells.

Synergistic effects of low-dose vinorelbine plus alpelisib on cell viability and proliferation in HR-positive, PIK3CA-mutated cell lines

To determine the synergistic effects of low-dose VRL plus alpelisib on cell viability according to the isobole method and to calculate the CI in MCF-7 cells, concentration of 100 ng/ml alpelisib was used. For the application of the isobole method the IC_{50} and IC_{80} were calculated and dose-response curves for single compounds were generated (Figure 4). After 3 days of treatment, 0.59 ng/ml and 7.81 ng/ml VRL was required to achieve the IC_{50} and IC_{80}, respectively (Figure 5a). The CI was 0.372 for the IC_{50} and 1.018 for the IC_{80} value, so conceptually synergistic and additive effects could be assumed, respectively. The syn-

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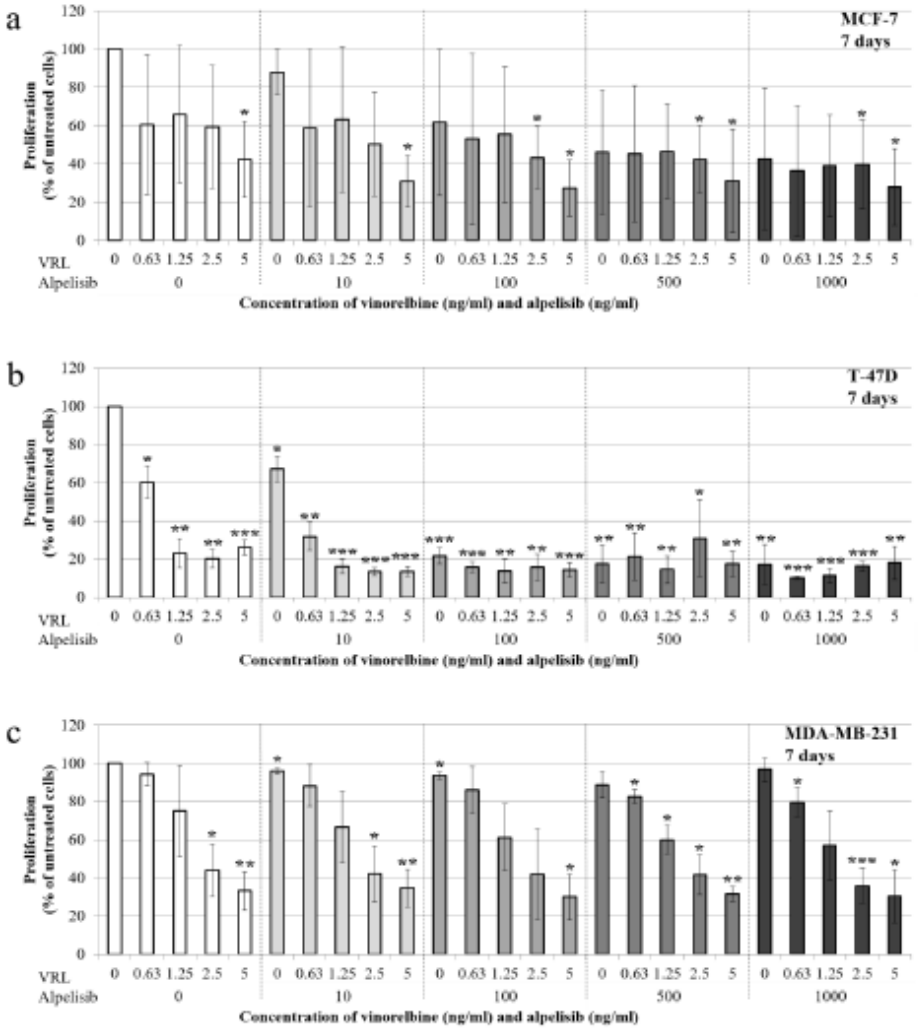

Figure 3: Effects of low-dose vinorelbine plus alpelisib on cell proliferation of the MCF-7 (a), T-47D (b) and MDA-MB-231 (c) cell line. BrdU incorporation was used to measure cell proliferation after 7 days of treatment with low-dose vinorelbine plus alpelisib. The results are shown as the mean ± standard deviation of three separate experiments. Statistical significance was assumed at *p<0.05, **p<0.01 and ***p<0.001.

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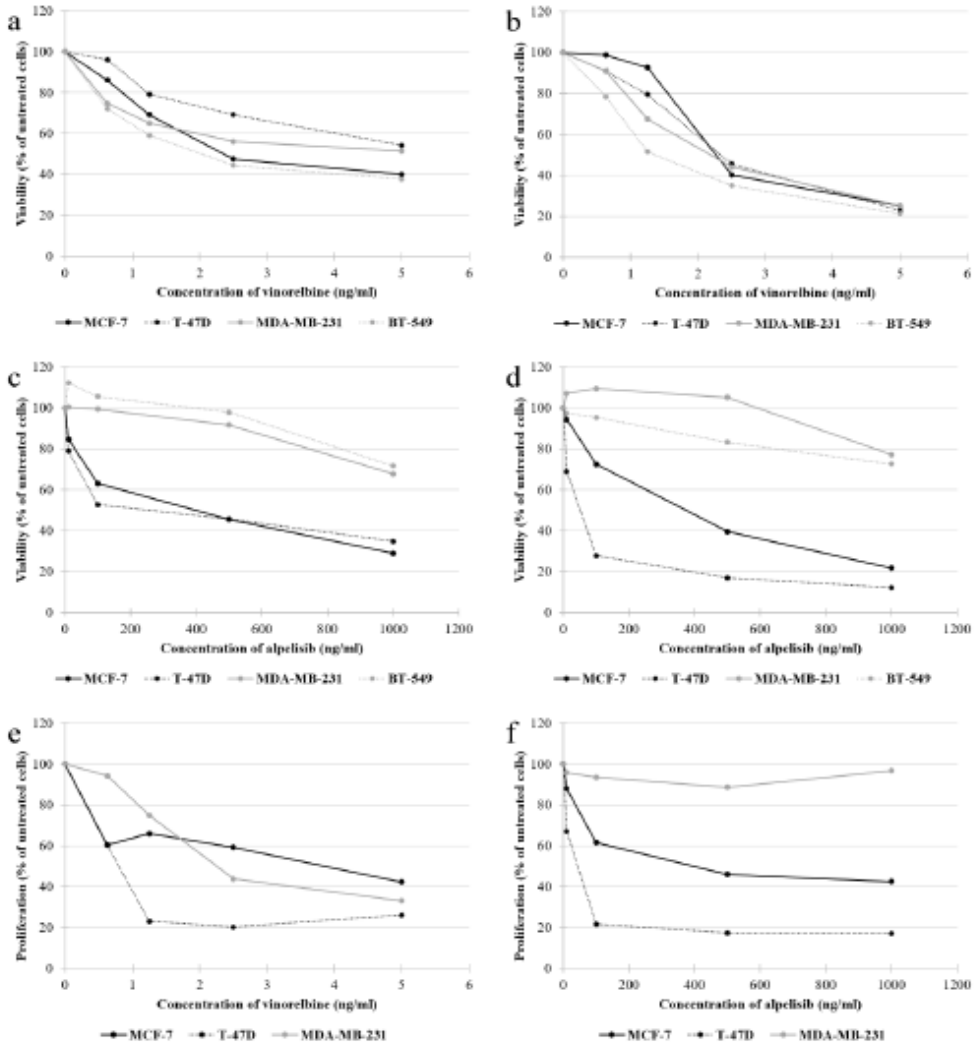

Figure 4: Dose-response curves of vinorelbine and alpelisib regarding cell viability: vinorelbine (a, b), alpelisib (c, d) after 3 and 7 days of treatment and regarding cell proliferation: vinorelbine (e), alpelisib (f) after 7 days of treatment. The results are shown as the mean of three separate experiments. The standard deviation results are shown in Figure 1 and Figure 3, respectively, and have been omitted from this figure for clarity.

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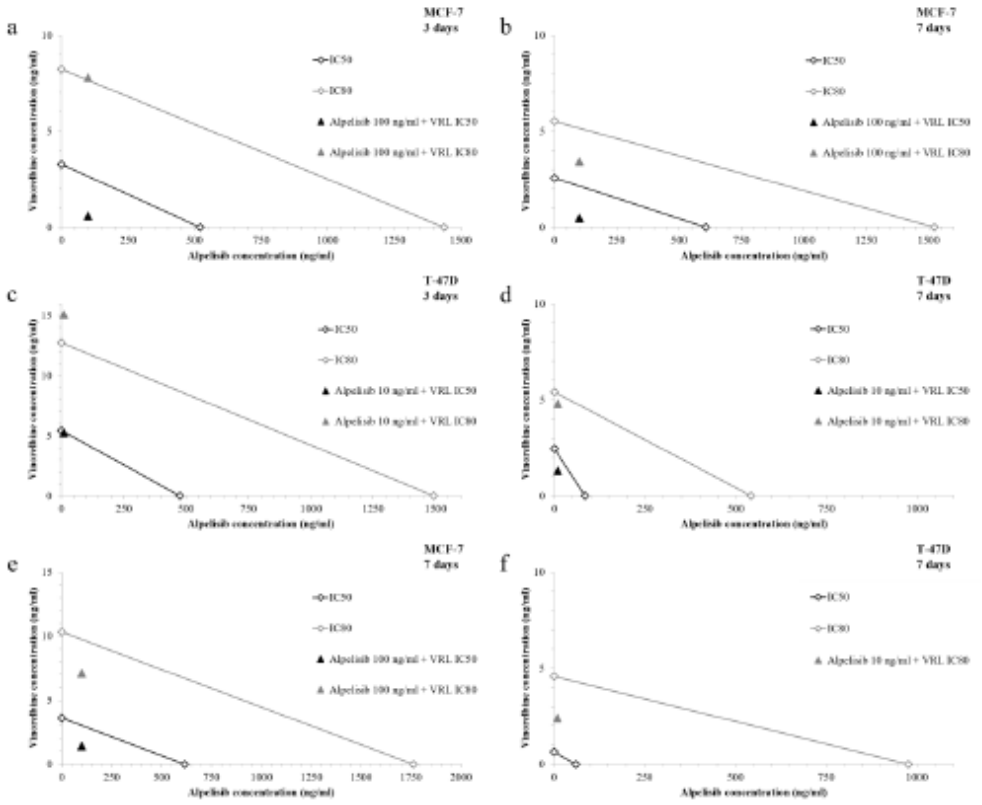

Figure 5: Combination index-isobologram for cell viability of the MCF-7 (**a**, **b**) and T-47D (**c**, **d**) cell line after 3 and 7 days of treatment and for cell proliferation of the MCF-7 (**e**) and T-47D (**f**) cell line after 7 days of treatment with low-dose vinorelbine plus alpelisib. The inhibitory concentrations $(IC)_{50}$ and IC_{80} were calculated by interpolation and used as a reference. The combination index (CI) was calculated according to the formula shown in formula [1] and was interpreted as follows: CI<1, synergism; CI=1, additive effect, and CI>1, antagonism (range ± 5 %). The graphical presentation was performed as classical isobologram with isoboles of the IC_{50} or IC_{80} and presentation of the corresponding CI values in relation to the corresponding isoboles within the diagram.

ergistic effects were stronger after 7 days of treatment. The IC_{50} value was reached at 0.48 ng/ml VRL (CI=0.351) and the IC_{80} value at 3.43 ng/ml VRL (CI=0.685) (Figure 5b). For the calculation of the CI in T-47D cells, concentration of 10 ng/ml alpelisib was used to achieve interpretable data. After 3 days of treatment, 5.23 ng/ml and 15.08 ng/ml VRL was required to achieve the IC_{50} (CI=0.987) and IC_{80} (CI=1.190) value (Figure 5c). The synergistic effects were evident after 7 days of treatment. The IC_{50} was reached at 1.29 ng/ml VRL (CI=0.648) and the IC_{80} at 4.79 ng/ml VRL (CI=0.906) (Figure 5d).

To determine the synergistic effects of low-dose VRL plus alpelisib on cell proliferation according to the isobole method and to calculate CI in MCF-7 cells, concentration of 100 ng/ml alpelisib was used. After 7 days of treatment, 1.41 ng/ml and 7.10 ng/ml VRL was required to achieve the IC_{50} and IC_{80} value, and the CI of 0.554 and 0.744 revealed synergistic effects of the agents, respectively (Figure 5e). In T-47D cells, the isobole

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method of calculating CI with 10 ng/ml alpelisib showed that 2.39 ng/ml VRL was required to reach IC_{80} value after 7 days of treatment (Figure 5f). The CI was 0.533, suggesting synergistic effects. The calculation of IC_{50} seemed implausible and was not performed due to the strong reduction of cell proliferation already at low concentrations.

Effects of low-dose vinorelbine plus alpelisib on p110α expression

To further examine the effects of low-dose VRL and alpelisib in the *PIK3CA*-mutated and *PIK3CA* wild-type cell lines, we analyzed the expression of the p110α protein that is encoded by the *PIK3CA* gene. In the MCF-7 cell line, the p110α expression was not significantly affected at most concentrations tested by VRL, alpelisib or combination of both substances (Figure 6a). In T-47D cells, the p110α expression was downregulated by alpelisib (Figure 6a). In both triple-negative, *PIK3CA* wild-type cell lines MDA-MB-231 and BT-549, an increase of the p110α expression induced by alpelisib was detected (Figure 6b). VRL at a concentration of 5 ng/ml and alpelisib at concentrations of 500 ng/ml and 1000 ng/ml led to a significant reduction in the number of cells tested. This resulted in a substantial reduced protein concentration in the cell lysate during protein extraction, making it impossible to obtain the amount of protein required for a Western blot. Therefore, these concentrations of the agents are missing in the Western blot analyses.

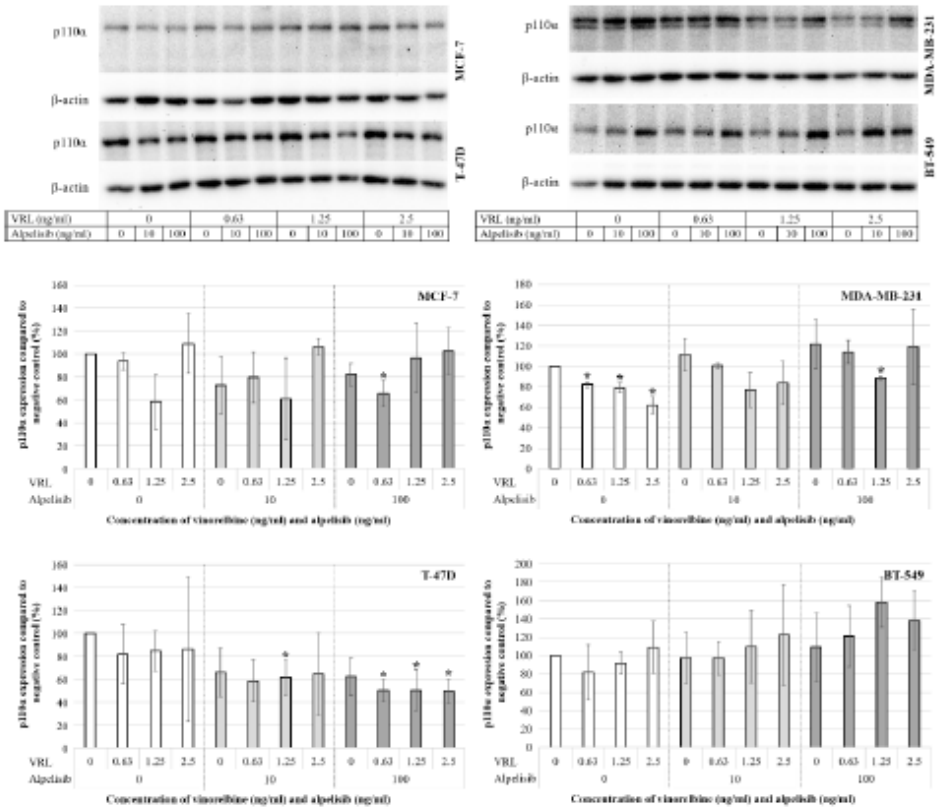

Figure 6: Western blot analyses of the p110α expression in the *PIK3CA*-mutated cell lines MCF-7 and T-47D (a) and the *PIK3CA* wild-type cell lines MDA-MB-231 and BT-549 (b) after 7 days of treatment with low-dose vinorelbine plus alpelisib. 12.5 µg protein was separated in a 10 % polyacrylamide gel. The order of the concentrations of substances differs between the bars and bands.

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DISCUSSION

In this study, the combination of low-dose metronomic VRL and alpelisib revealed a significant reduction of cell viability and proliferation with synergistic anti-tumor effects in HR-positive, HER2-negative, *PIK3CA*-mutated BC cell lines. The growth of triple-negative, *PIK3CA* wild-type cell lines was significantly inhibited by VRL but not by alpelisib alone. These results confirm the hypothesis that PI3K inhibitors may potentiate cytotoxic activity of anti-microtubule agents in *PIK3CA*-mutated BC cell lines, as previously described (Badinloo and Esmaeili-Mahani, 2014; Morgillo et al., 2017; Rajput et al., 2019). Taselisib, a selective inhibitor of class I PI3Kα, δ-, and γ-isoforms, and ipatasertib, an AKT inhibitor, plus anti-microtubule chemotherapy showed significant synergism in terms of antiproliferative, pro-apoptotic, and anti-metastatic effects in *PIK3CA*-mutated BC cells (Morgillo et al., 2017). In particular, MCF-7 (HR-positive, HER2-negative), BT474 (HR-positive, HER2-positive), KPL-4 (HR-negative, HER2-positive), and SUM159 (triple-negative) BC cell lines with *PIK3CA* mutation were treated for 3 days with taselisib or ipatasertib and VRL, eribulin, and paclitaxel in concentrations of IC_{50}. Combined treatment with taselisib and anti-microtubule agents exerted a strong reduction of cell viability with synergistic effects and a significant increase of apoptotic cells in all cell lines tested compared to single treatment. In addition, the combination of taselisib or ipatasertib and eribulin significantly inhibited motility and migration of SUM159 mesenchymal cells compared to single-agent treatment. Here, by way of comparison, we intended to simulate the metronomic dosing regimen by continuously treating the cells with low-dose chemotherapeutic agent over an extended period of time and observed similar results with combination of alpelisib and VRL. In both *PIK3CA*-mutated BC cell lines, we observed a CI<1, assuming synergistic effects of low-dose VRL and alpelisib. The synergistic cytotoxic effects were after 7 days of treatment even stronger compared to 3 days of treatment, suggesting a favorable impact of continuous long-term administration of VRL and alpelisib. In the Western blot analyses, the p110α expression was not affected or downregulated in the *PIK3CA*-mutated cell lines MCF-7 and T-47D, respectively, and not significantly upregulated in the *PIK3CA* wild-type cell lines MDA-MB-231 and BT-549. It may be assumed that the p110α expression did not show any clear dependance on alpelisib at concentrations tested in HR-positive, HER2-negative cell lines with an overactivation of p110α due to the *PIK3CA* mutation. In contrast, the triple-negative *PIK3CA* wild-type cell lines showed regulatory mechanisms associated with upregulation of the non-overactivated protein as a response to treatment with alpelisib.

Serum concentration achieved in patients taking 300 mg alpelisib once daily on a regular basis averages 1000 ng/ml (Juric et al., 2018). Here, we showed that even lower alpelisib concentrations (10 ng/ml and 100 ng/ml) combined with low-dose metronomic VRL led to a significant reduction of cell viability of *PIK3CA*-mutated cells, and the anti-tumor activity was comparable with the effects at 1000 ng/ml alpelisib. This indicates that above a certain concentration of VRL and alpelisib, no significant potentiation of the anti-tumor effect could be observed. The combination of the agents in lower doses may reduce side effects, so the efficacy and tolerability will be further evaluated *in vivo*.

Hyperactivation of PI3K/AKT/mTOR pathway has also been associated with triple-negative breast cancer (TNBC) via loss of phosphatase and tensin homolog (PTEN), a direct counterpart of PI3K (Lopez-Knowles et al., 2010). Therefore, Rajput and colleagues (2019) assessed the combinatory effect of eribulin and buparlisib, a pan-class I PI3K inhibitor, in TNBC cell lines and patient-derived xenograft (PDX) models. In the BT-549, HCC1806, and MBA-MB-231 TNBC cell lines as well as WHIM3 and WHIM12 PDX derived cell lines with loss of PTEN, reduction of cancer stem cell population and synergistic cytotoxic effects between eribulin

and buparlisib were observed. In the randomized phase III trials BELLE-2 and BELLE-3, treatment with buparlisib and fulvestrant demonstrated efficacy with significant prolongation of PFS compared to fulvestrant alone in pretreated HR-positive, HER2-negative MBC patients. However, the unfavorable toxicity profile of buparlisib including elevation of liver enzymes, hyperglycemia, depression, and rash led to terminate further development of the agent (Baselga et al., 2017; Di Leo et al., 2018). In the randomized phase III study SANDPIPER, taselisib in combination with fulvestrant also met its primary endpoint and underlined the efficacy in HR-positive, HER2-negative, *PIK3CA*-mutated MBC patients. However, the combination did not demonstrate clinical utility given its safety profile and modest clinical benefit (Dent et al., 2021). Combination of fulvestrant and alpelisib (300 mg once daily) versus placebo was evaluated in the randomized phase III study SOLAR-1. The addition of alpelisib demonstrated a significant 5.3-month improvement in median PFS and a 7.9-month improvement in median OS in HR-positive, HER2-negative, *PIK3CA*-mutated MBC patients. In the cohort without *PIK3CA*-mutated BC, there was no significant difference in the median PFS (7.4 months versus 5.6 months), suggesting that the *PIK3CA* gene may represent a predictive biomarker for alpelisib activity in this population (Andre et al., 2019). In the overall population, the most frequent adverse events of grade 3 or 4 were hyperglycemia (36.6 % versus 0.7 %) and rash (9.9 % versus 0.3 %). In the SOLAR-1 *PIK3CA*-mutated cohort, only 5.9 % of patients had previously received cyclin-dependent kinase 4/6 inhibitor (CDK4/6i). Since endocrine therapy combined with CDK4/6i has become the standard first-line treatment for HR-positive, HER2-negative MBC, a phase II trial, BYLieve (NCT03056755), was conducted to evaluate alpelisib plus fulvestrant in MBC patients who had progressed on immediate prior CDK4/6i plus aromatase inhibitor. In a cohort A, alpelisib plus fulvestrant met its primary endpoint, with 50.4 % of patients alive without progression after 6 months of treatment (Rugo et al., 2021).

Considering that clinical trials evaluating the combination of alpelisib plus endocrine therapy have shown favorable efficacy and manageable toxicity profile in HR-positive, HER2-negative, *PIK3CA*-mutated MBC patients, there is a promising approach to investigate alpelisib with chemotherapeutic agents as well. VRL, an orally available anti-microtubule agent, represents a standard treatment option in MBC (Aapro et al., 2019; Huang et al., 2020). In addition, VRL is one of the most commonly used agents for MCT with proven efficacy and excellent safety profile (Cazzaniga et al., 2019b; Liu et al., 2021). Based on the growing body of evidence, MCT can be considered as a suitable treatment option in selected MBC patients. Especially patients with HR-positive, HER2-negative metastatic disease resistant to endocrine-based therapy and not requiring rapid tumor response are generally suitable for MCT (Cazzaniga et al., 2019a; Krajnak et al., 2022). Alpelisib is an approved, orally bioavailable, α-selective inhibitor of PI3K for use in combination with fulvestrant to treat HR-positive, HER2-negative, *PIK3CA*-mutated MBC patients after endocrine-based therapy (Andre et al., 2019). Alpelisib targets the two most common *PIK3CA* mutations and is 50 times more potent against PI3Kα than other isoforms (Fritsch et al., 2014). As there is evidence that inhibition of the PI3K pathway may prevent resistance to chemotherapy and potentiate its efficacy, the combination of PI3K inhibitors with MCT affecting angiogenesis, immune response, and tumor cells via direct cytotoxicity may represent a new promising approach for the treatment of MBC, aiming to achieve synergistic effects, overcome drug resistance, or decrease the drug dose and toxicities. The fact that both agents are specifically indicated for the treatment of HR-positive, HER2-negative MBC after CDK4/6i pretreatment also supported the investigation of the substances in combination.

In this study, HR-positive, HER2-negative cell lines with a *PIK3CA* mutation and triple-negative cell lines without a *PIK3CA* mutation were analyzed, so we cannot clearly state whether the significant reduction in the cell viability and proliferation shown depended on the *PIK3CA* mutation alone regardless of HR and HER2 status. Although we hypothesize that the *PIK3CA* mutation was primarily responsible for the synergistic effects and the effect of alpelisib shown, we consider the evaluation of HR-positive, HER2-negative cell lines without *PIK3CA* mutation as well as the evaluation of triple-negative cell lines with a *PIK3CA* mutation to be of particular importance.

In conclusion, the present work revealed a significant reduction of cell viability and proliferation with synergistic effects of low-dose metronomic VRL and alpelisib in HR-positive, HER2-negative, *PIK3CA*-mutated BC cell lines. In addition, we showed that even lower doses of alpelisib in combination with low-dose VRL result in significant anti-tumor effects, providing a rationale for further efforts to evaluate this combination *in vivo* with the intent of improving the toxicity profile.

Conflict of interest

SK received speaker honoraria from Roche Pharma AG and Novartis Pharma GmbH Germany, research funding from Novartis Pharma GmbH Germany and travel reimbursement from PharmaMar and Novartis Pharma GmbH Germany.

KA received speaker honoraria from Clovis Oncology, MSD und AstraZeneca.

ASH received speaker honoraria from Pfizer Pharma GmbH and honoraria from Medupdate GmbH.

MS received honoraria for speaker or consultancy role from AMGEN, AstraZeneca, Eisai, Lilly, Myelo Therapeutics, Novartis, Pantarhei Bioscience, Pfizer, and Roche. He received research funding from AstraZeneca, BioNTech, Eisai, Genentech, Myelo Therapeutics, Novartis, Pantarhei Bioscience, Pfizer, Pierre-Fabre, and Roche Pharma AG. He received travel reimbursement from Pfizer and Roche. In addition, MS has a patent for EP 2390370 B1 issued and a patent for EP 2951317 B1issued.

MJB received honoraria and expenses from Astra Zeneca, Clovis Oncology, GSK, MSD, Pharma Mar, Roche Pharma AG and Tesaro Bio Germany GmbH. He is consultant to Eisai, GSK, MSD, Pharma Mar, Roche Pharma AG and Tesaro Bio Germany GmbH. He received funded research from AstraZeneca, Clovis Oncology, MSD and Novartis.

AH received honoraria from AstraZeneca, Celegen, MedConcept Gm, Med update GmbH, Medicultus, Pfizer, Promedicis GmbH, Pierre Fabre, Softconsult, Roche Pharma AG, Streamedup!GmbH and Tesaro Bio Germany GmbH. She is a member of the advisory board of PharmaMar, Promedicis GmbH, Pierre Fabre Pharma GmbH, Roche Pharma AG and Tesaro Bio Germany GmbH. She received research funding from Celgene.

All remaining authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Authors' contributions

Conceptualization: SK, MJB and WB; investigation and formal analysis: SK, JPT, PFH and WB; writing – original draft: SK and WB; writing – review and editing: SK, KA, ASH, AL, MS, AH and MJB.

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