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ORIGINAL ARTICLE

Combined treatment with crizotinib and temsirolimus is an effective strategy in mantle cell lymphoma and can overcome acquired resistance to temsirolimus

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Abstract

Constitutive activation of the PI3K/AKT/mTOR-pathway plays an important role in the pathogenesis of mantle cell lymphoma (MCL), leading to approval of the mTOR inhibitor temsirolimus for relapsed or refractory MCL. Yet, despite favorable initial response rates, early relapses under treatment have been observed. Therefore, understanding the underlying mechanisms of temsirolimus resistance and developing strategies to overcome it is highly warranted. Here, we established a new temsirolimus-resistant MCL cell line to evaluate the molecular background of resistance to this drug. Transcriptome profiling and gene set enrichment analysis comparing temsirolimus-sensitive and -resistant cell lines showed significant upregulation of PI3K/AKT/mTor-, RAS signaling- and the RTK-dependent PDGFR-, FGFR-, Met- and ALK-signaling-pathways in the resistant cells. Furthermore, *MET*, known as important proto-oncogene and mediator of drug resistance, was among the most upregulated genes in the resistant cells. Importantly, Met protein was overexpressed in both, MCL cells with acquired as well as intrinsic temsirolimus resistance, but could not be detected in any of the temsirolimus sensitive ones. Combined pharmacological inhibition of mTOR and Met signaling with temsirolimus and the RTK inhibitor crizotinib significantly restored sensitivity to temsirolimus. Furthermore, this combined treatment proved to be synergistic in all MCL cell lines investigated and was also active in primary MCL cells. In summary, we showed for the first time that overexpression of *MET* plays an important role for mediating temsirolimus resistance in MCL and combined treatment with temsirolimus and crizotinib is a very promising therapeutic approach for MCL and an effective strategy to overcome temsirolimus resistance.

KEYWORDS
crizotinib, drug resistance, mantle cell lymphoma, met, mTOR, temsirolimus

1 | INTRODUCTION

Mantle cell lymphoma (MCL) is a mature B-cell malignancy representing about 5%–7% of all Non-Hodgkin lymphomas in Western Europe.[1] Although with current standard therapy high initial response rates can be achieved, early relapses and rapid disease progression determine the clinical course of most MCL patients[1] and prognosis is still poor with an overall survival of only 3–5 years.[2] Further understanding of the biology of MCL and the development of numerous novel therapeutic strategies have substantially improved the treatment of MCL over the last years leading to prolonged overall survival. Yet, management of relapsed MCL remains difficult.[3] MCL is characterized by its hallmark chromosomal translocation t(11; 14) (q13; q32) leading to dysregulation of cell cycle due to an aberrant overexpression of Cyclin D1.[2,4] Furthermore, constitutive activation of the B-cell receptor and its downstream pathways, such as the Phosphoinositid-3-Kinase (Pi3K)/Akt/mammalian target of rapamycin(mTOR) pathway seems to play an important role in the molecular pathogenesis of MCL.[5,6] Pi3K induces Akt signaling which subsequently leads to activation of mTOR, a serin/threonine kinase, which mediates cell growth through its most important downstream proteins S6 kinase (S6K) and eukaryotic translation initiation factor 4E (eIF4E).[7–9] The Pi3K/Akt/mTOR pathway can also be activated by different hormone-, growth factor- or cytokine-dependent receptor-tyrosine kinases (RTK) and has various crosslinks to other important intracellular pathways, that mediate cell growth and proliferation, representing a complex signaling network.[10] In summary, the Pi3K/Akt/mTOR pathway plays a central role in mediating cellular survival, proliferation and metabolism and is one of the most frequently dysregulated pathways in cancer, making it an important target for targeted cancer therapies in various neoplasia.[11,12] Based on these findings, temsirolimus, a rapamycin derivate targeting mTOR, was approved in 2009 for the treatment of relapsed and refractory (r/r) MCL after failure of first line therapy.[13] Despite favorable initial response rates, early relapses and disease progression limit the clinical value of temsirolimus as a single agent.[14] Resistance to targeted therapies occurs often and remains a general problem in targeted cancer treatment. Acquired resistance is based on therapy-induced clonal evolution of pre-existing resistant variants in the original cancer cells or by acquisition of new mutations or adaptations.[15,16] Underlying mechanisms can involve pathway reactivation due to additional target mutations or amplification, gain-of-function mutations in upstream or downstream signaling or activation of bypass pathways. Development of mechanism-based combined approaches overcoming selective resistance mechanisms are a potential solution for this common problem. Therefore, understanding of the underlying molecular mechanisms mediating drug resistance is of great importance.[15,17–19] In this study, we investigated the molecular mechanisms leading to temsirolimus resistance in MCL and potential pharmacological strategies to overcome resistance.

2 | RESULTS

2.1 | MCL cell lines show differential response to treatment with the mTor inhibitor temsirolimus

We first evaluated sensitivity to temsirolimus in six established MCL cell lines (Mino, Maver-1, JeKo-1, Granta-519, Z138 and Rec-1). Temsirolimus was shown to be most effective in JeKo-1, Mino and Maver-1, while Rec-1 cells appeared to be intrinsically resistant to temsirolimus (Figure 1A). To investigate the biological background of resistance to temsirolimus, we next established a new temsirolimus resistant cell line (Z138r) by exposing Z138 MCL cells to increasing doses of temsirolimus. As depicted in Figure 1B, temsirolimus was significantly less effective in Z138r compared to Z138s after at least 48 h of treatment. A significant, time- and dose-dependent decrease of ph-mTOR(Ser2448) and its pathway-related targets ph-AKT(Ser 473), target of mTorC2[20] and ph-S6(Ser234/235) ribosomal protein, target of mTorC1, could be observed in the sensitive phenotype (Figure 1C, Figure 1D). In contrast, treatment with temsirolimus did not induce a relevant inhibition of ph-mTOR and ph-AKT in the resistant phenotype. Downstream-target ph-S6 ribosomal protein was only inhibited short-term (Figure 1C, Figure 1D).

2.2 | Temsirolimus resistant MCL cells exhibit a differential gene expression profile compared to sensitive cells

To compare the changes in gene expression profiles induced by acquired resistance to temsirolimus, we next performed transcriptome analysis in Z138s and Z138r. Gene set enrichment analysis (GSEA) of the most frequently dysregulated pathways in cancer showed a significant upregulation of Ras signaling, the Pi3K pathway as well as different RTK pathways (Anaplastic lymphoma kinase (ALK) signaling, Fibroblast growth factor receptor (FGFR) signaling, Hepatocyte growth factor receptor (HGF-Receptor) signaling, Platelet derived growth factor (PDGF) signaling) in the resistant phenotype (Figure 2A; Figure S1). The most significantly upregulated genes of the enriched gene sets are displayed in heatmaps grouped by pathway signaling (Figure 2A). *MET* and *FGFR1* can be found in the core gene sets of Ras and Pi3K signaling indicating their importance as upstream regulators of these intracellular pathways (Figure 2A). Furthermore, both were among the most upregulated genes in the temsirolimus resistant compared to the sensitive phenotype (Figure 2B). Upregulation of *MET* was validated with RTqPCR showing a 11.9-fold increase in mRNA expression in the Z138r cell line compared to Z138s (Figure 2C).

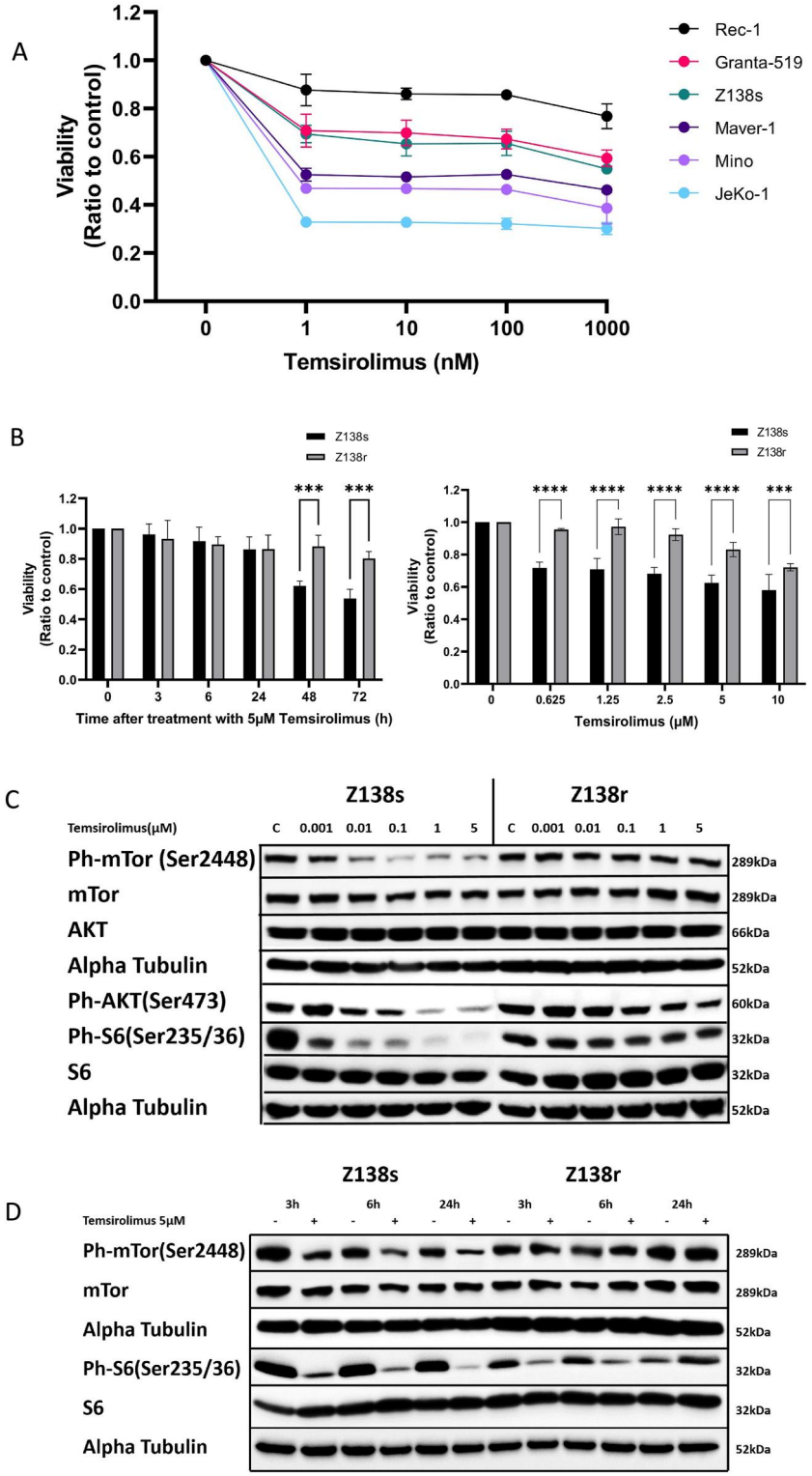

FIGURE 1 A, Six different MCL cell lines were exposed to the indicated concentrations of temsirolimus. After 48 h, viability was assessed using a CellTiterGlo Assay. B, Z138 sensitive and resistant cells were exposed to 5 μM temsirolimus and incubated for the indicated times (left panel) or exposed to the indicated concentrations of temsirolimus for 48 h (right panel). Viability was assessed using Trypan blue staining and digital cell counting. C, Z138 sensitive and resistant cells were treated for 24 h with the indicated concentrations of temsirolimus. Protein expression of ph-mTOR (Ser2448), mTOR, ph-AKT(Ser473), AKT, ph-S6 (Ser235/236) ribosomal protein and S6 ribosomal protein was assessed by Western Blot analysis. Alpha Tubulin was used as an internal control for the antibodies above (D) Z138 sensitive and resistant cells were treated with 5 μM temsirolimus for the indicated times. Protein expression of ph-mTOR (Ser2448), mTOR, ph-S6 (Ser235/236) ribosomal protein and S6 ribosomal protein was assessed by Western Blot analysis. Alpha Tubulin was used as an internal control for the antibodies above. A–D, ($n = 3$, bars represent the mean ± SD, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, one representative Western Blot experiment is shown).

2.3 | Met plays an important role for mediating temsirolimus resistance and combination with crizotinib can completely overcome acquired resistance to temsirolimus

To evaluate the role of Met in mediating temsirolimus resistance we performed an siRNA-mediated Met knockdown in Z138r and Rec-1 cells. Western blot analysis confirmed effective knockdown (Figure S3), yet only a marginal increase of sensitivity to temsirolimus after Met knockdown could be observed (Figure 3A). We next sought to analyze if pharmacological inhibition of Met signaling could restore sensitivity to mTOR inhibition in the resistant cells. We observed an increase in temsirolimus sensitivity upon treatment with the selective Met inhibitor capmatinib leading to similar proliferation inhibition in

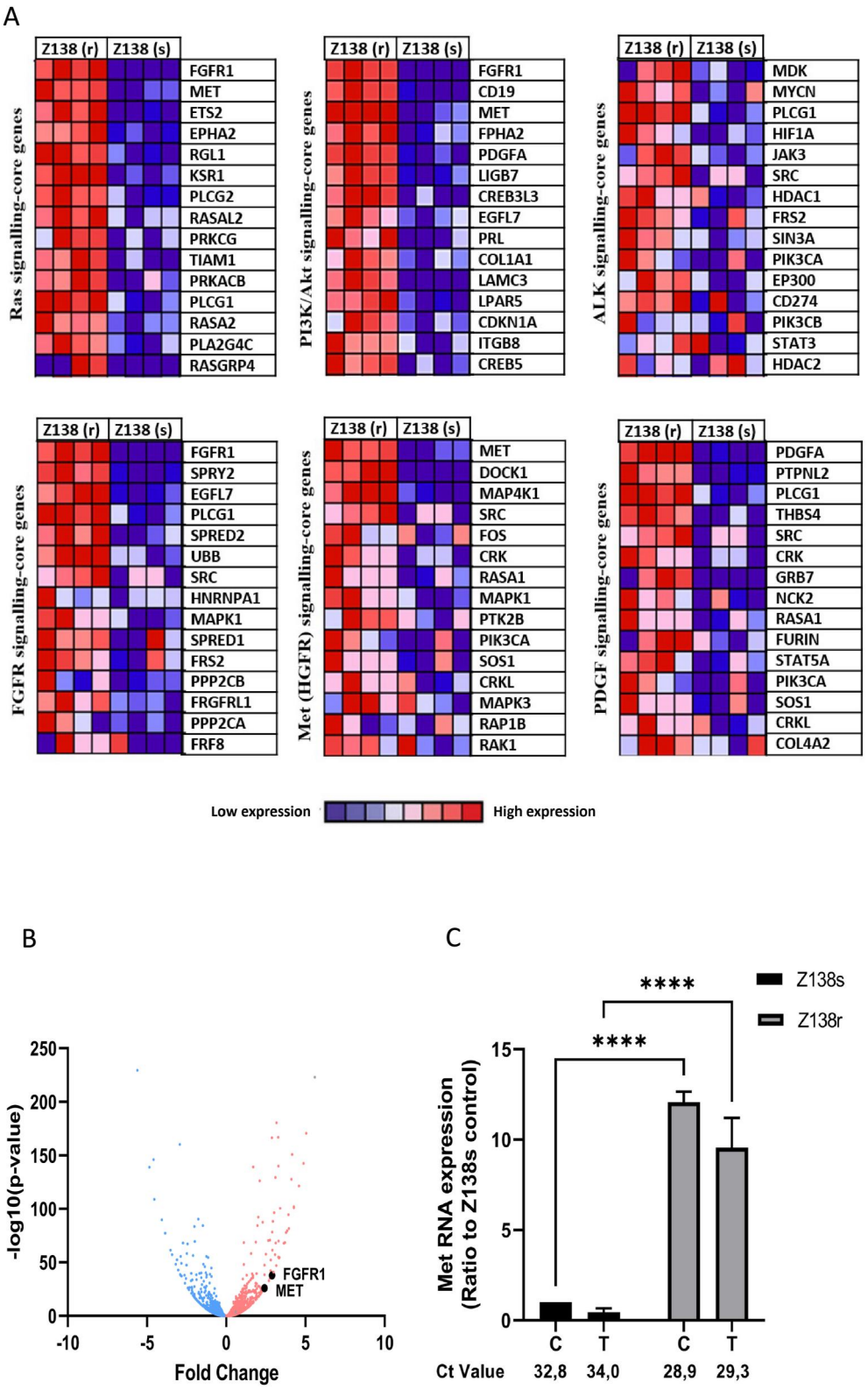

FIGURE 2 A, Heatmaps of the 15 most upregulated core-enriched genes of the indicated gene sets analyzed with the GSEA software, representing significantly upregulated pathways in Z138 resistant cells compared to the sensitive phenotype. B, Volcano plot of RNASeq data showing differentially expressed genes in Z138 resistant compared to Z138 sensitive cells. Fold change (FC) values are mapped to the corresponding -log10 of adjusted *p*-values. Positive FC values (red) indicate a higher gene expression in Z138 resistant compared to Z138 sensitive, whereby negative FC values (blue) indicate a lower gene expression in Z138 resistant. C, RNA expression of *MET* in Z138s and Z138r with (T) and without (C) exposition to 1 μM temsirolimus was assessed by RT-qPCR analysis after 4 h and is depicted as a ratio to the untreated Z138s. Corresponding Ct cycles of the qRT-PCR analysis are indicated ($n = 3$, bars represent the mean ± SD, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

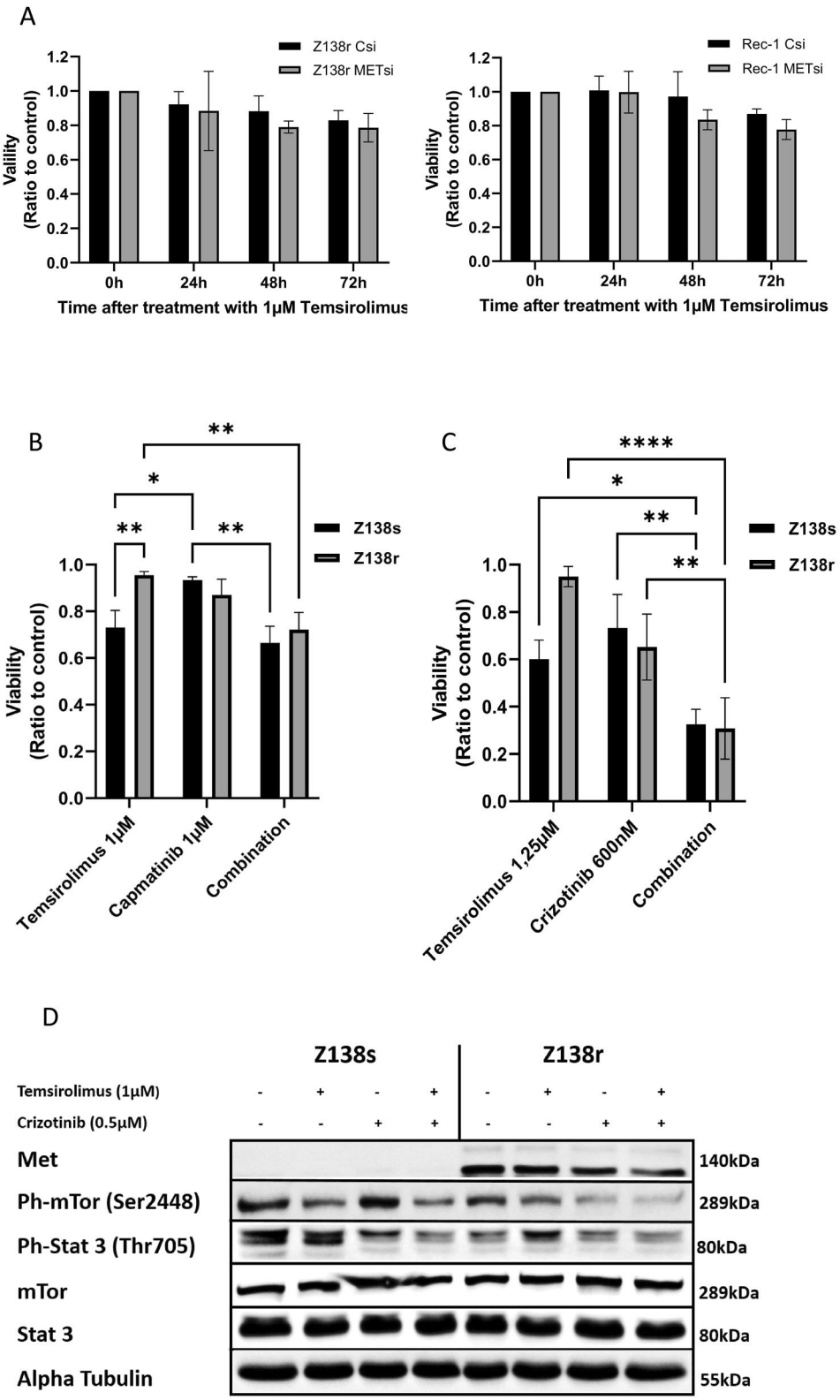

FIGURE 3 A, Knockdown of *MET* was performed in Z138 resistant (C) and Rec-1 (D) cells using *MET* targeting si RNA (METsi). Non-targeting si RNA was used as a control (Csi). 12 h after transfection, cells were treated with 1 μM temsirolimus. Cell viability was assessed after the indicated times using Trypan blue assay (B) Z138 sensitive and resistant cells were exposed to the indicated concentrations of temsirolimus, capmatinib and the combination of both inhibitors. After 48 h, viability was assessed using a CellTiterGlo Assay (C) Z138 sensitive and resistant cells were exposed to the indicated concentrations of temsirolimus, crizotinib and the combination of both inhibitors. After 48 h, viability was assessed using a CellTiterGlo Assay. D, Z138 sensitive and resistant cells were treated with the indicated concentrations of temsirolimus, crizotinib or the combination of the two inhibitors and incubated for 24 h. Protein expression of Met, ph-mTOR (Ser2448) and mTOR, ph-Stat3 (Thr705) and Stat3 was assessed by Western Blot analysis. Alpha Tubulin was used as an internal control. A–D, ($n = 3$, bars represent the mean ± SD, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$, one representative Western Blot experiment is shown).

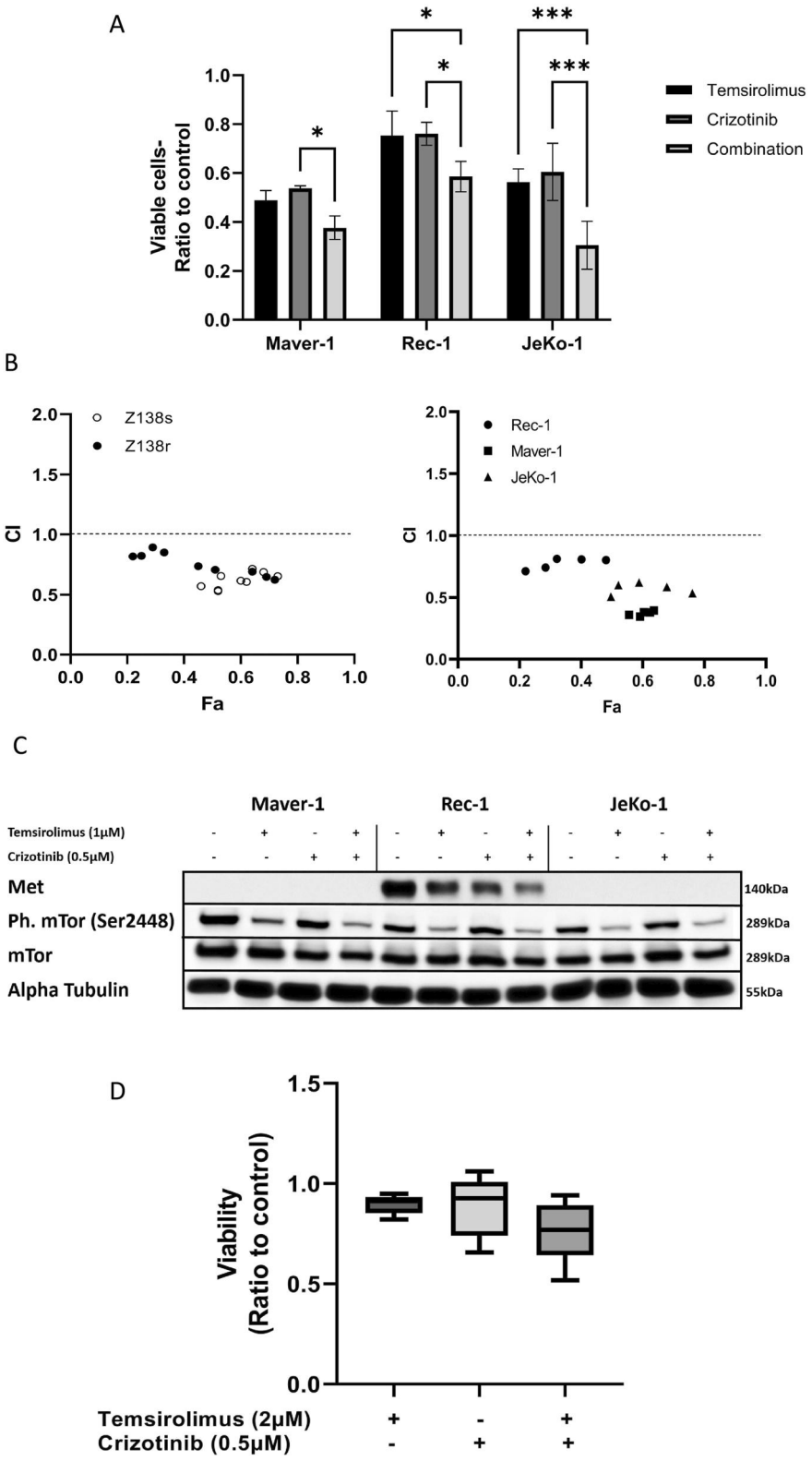

FIGURE 4 A, MCL cell lines Maver-1, Rec-1 and JeKo-1 were treated with temsirolimus (500 nM), crizotinib (700 nM for Maver-1 and JeKo-1, 900 nM for Rec-1) or the combination of both. Viability was assessed using a CellTiterGlo Assay (left panel; $n = 3$, bars represent the mean ± SD). B, left panel) Resistant and sensitive Z138 cells were treated with three different doses of temsirolimus (1.25, 2.5, 5 μM) combined with 3 different doses of crizotinib (400, 500, 600 nM). B, right panel) MCL cell lines Maver-1, Rec-1 and JeKo-1 were treated with five different doses of temsirolimus (1/10/100/500/1000 nM) combined with 5 different doses of crizotinib (400/500/600/700/800 nM for Maver-1 and JeKo-1, 600/700/800/900/1000 nM for Rec) After 48h, number of viable cells was assessed using a CellTiterGlo Assay. Synergy of the combination treatment was assessed using the CompuSyn software by calculation of Combination indices (CI). CI values < 1 indicate

both phenotypes (Figure 3B). Combined treatment of temsirolimus with crizotinib, a small molecule tyrosine kinase receptor inhibitor targeting ALK, MET, RON and ROS1 kinases, proved to be even more effective and could completely overcome acquired drug resistance in Z138r (Figure 3C). This was accompanied by a significant decrease in ph-mTOR protein expression. Interestingly, in Z138s, temsirolimus, but not crizotinib lead to decrease of ph-mTOR expression, whereas in contrast, in the resistant phenotype, crizotinib, but not temsirolimus was able to inhibit mTOR activity. According to the elevated *MET* mRNA levels in the resistant phenotype as shown above in Figure 2C, Westernblot analysis revealed measurable Met protein expression only in Z138r and treatment with combination of temsirolimus and crizotinib induced a marginal decrease of Met expression. Phosphorylation of Met downstream target Stat3 was inhibited upon crizotinib in Z138r and in combination with temsirolimus in both phenotypes, whereby temsirolimus alone led to upregulation of protein activation in Z138r (Figure 3D).

2.4 | Combination of temsirolimus and crizotinib is highly effective and acts synergistically in MCL

The inhibitor combination proved to be effective in the temsirolimus-sensitive JeKo-1 and Maver-1 cell lines and in the temsirolimus-resistant Rec-1 cell line (Figure 4A). Synergistic effects could be observed for all dose combinations in Z138s and Z138r (Figure 4B, left panel; Table S1) as well as in Rec-1, JeKo-1 and Maver-1, however, Rec-1 cells were significantly less sensitive to treatment with crizotinib, wherefore higher doses were used for this cell line (Figure 4B, right panel; Table S2). Western blot analysis revealed extremely elevated Met protein levels in Rec-1, whereas Met could not be detected in Maver-1 and JeKo-1. In line with the results observed in Z138 sensitive, temsirolimus lead to a significant decrease of ph-mTOR in the two temsirolimus sensitive cell lines Maver-1 and JeKo-1, whereas crizotinib did not affect ph-mTOR levels. Similar results could be observed for Rec-1: Crizotinib did not, unlike observed in Z138r, affect ph-mTOR expression. Interestingly, temsirolimus lead to a relevant inhibition of ph-mTOR expression, although the cell line carries an intrinsic temsirolimus resistance. Temsirolimus and crizotinib both lead to a decrease of Met expression in Rec-1 cells (Figure 4C). Finally, we confirmed the activity of combined treatment with temsirolimus and crizotinib in primary MCL cells. Although the observed drug effects were less pronounced compared to our MCL cell lines, a decrease of viability upon combination treatment could be shown compared to the single agents (Figure 4D).

3 | DISCUSSION

The B cell receptor and its downstream pathways have been shown to play a key role in the pathogenesis of MCL, leading to the approval of several targeted inhibitors, including the mTOR inhibitor temsirolimus.[21] Although temsirolimus proved to be effective in the treatment of r/r MCL patients[22] and significantly improved progression free survival (PFS) and objective response rates in these patients,[23] treatment failure due to intrinsic or acquired drug resistance currently limits the clinical success and applicability of this drug.[24] Development of resistance to temsirolimus and other mTOR inhibitors has been described as a major problem in several types of cancer, whereby different underlying cellular escape mechanisms have been postulated. MTOR inhibition-induced activation of upstream kinases like PI3K leading to reactivation of the mTOR pathway[25,26] or compensatory upregulation of other pathways mediating cell survival and proliferation, especially Ras/ERK/MAPK pathway, were among the described resistance mechanisms.[27–29]

In MCL, molecular mechanisms of temsirolimus resistance remain still unknown. Therefore, in this study, we investigated the molecular background of acquired resistance to temsirolimus and explored a strategy to overcome it.

We initially performed transcriptome analysis to compare the changes in gene expression profiles induced by acquired resistance to temsirolimus in the MCL cell line Z138. GSEA analysis revealed, among others, a significant upregulation of the PI3K/Akt/mTOR pathway itself as well as Ras signaling in the resistant phenotype. The Ras signaling pathway mediates intracellular signal transduction in response to different extracellular stimuli and plays a key role in maintaining normal cellular homeostasis, cell survival, growth and proliferation as well as differentiation.[30,31] Pathway dysregulation can be found in approximately one third of all cancers making it an attractive target for cancer therapies.[31] The Ras/ERK/MAPK and the PI3K/AKT/mTOR pathway are known to regulate each other due to different cross-talks and feedback loops. As they partially converge in the same target proteins, they also co-regulate important downstream functions.[29] Furthermore, we found the signaling pathways of several RTK to be among the most significantly modulated ones: FGFR, ALK, HGFR (Met) and PDGF. Fusion, amplification and mutations of *FGFR* can be found in different cancer types.[32,33] *FGFR1*, which is highly

synergistic drug effects. C, Maver-1, Rec-1 and JeKo-1 cells were treated with the indicated concentrations of temsirolimus, crizotinib or the inhibitor combination and incubated for 24 h. Protein expression of Met, ph-mTOR (Ser2448) and mTOR was assessed by Western Blot analysis. Alpha Tubulin was used as an internal control ($n = 3$, one representative Western Blot is shown). D, Primary cells obtained by six different patients were treated with five different doses of temsirolimus (125/250/500/1000/2000 nM) combined with 5 different doses of crizotinib (31.25/62.5/125/250/500). After 48 h, viability was assessed by Trypan blue staining. Obtained results after combining 2 μM temsirolimus with 500 nM crizotinib are displayed in a Box-and-Whisker Plot, showing the medium, minimum and maximum as well as the interquartile range.

overexpressed in Z138r, has also been described as a potential mediator of drug resistance to palbociclib and tyrosine kinase inhibitors targeting EGFR.[34,35] Known downstream pathways involve PI3K/Akt, MAPK/ERK, and JAK/STAT signaling.[36] Overexpression of ALK stimulates downstream signaling through the PI3K/Akt, MAPK/ERK, and STAT3 pathway and can be found in several malignancies leading to increased cancer cell growth and proliferation, survival, angiogenesis and metastasis.[37] Regarding PDGF signaling, there is increasing evidence indicating its involvement in cancer development and progression. PI3K/AKT/mTOR and Ras/ERK signaling may be important downstream pathways.[38–40] HGFR (Met) signaling mediates proliferation and cell survival through different intracellular pathways like Ras/ERK/MAPK, PI3K/AKT/mTOR, STAT and NF-κB signaling.[41] Met signaling is involved in different physiological processes but is also known to play an important role in the pathogenesis of different human cancers, making it an attractive target for cancer therapy.[42] Interestingly, we found *MET* to be among the most upregulated genes in the temsirolimus resistant compared to the sensitive Z138 phenotype. Mutation or amplification of *MET* can promote tumor growth, invasion and dissemination and was shown to correlate with poor clinical outcomes.[43–46] It has already been described as a potential mediator of resistance to chemotherapy, radiotherapy and different targeted therapies, whereby Met overexpression occurred more often than *MET* mutations.[41,42,47–50] Due to its crosslinks to various other RTK, Met is known to mediate drug resistance to RTK inhibitors[41,44,51] and Raf inhibitors.[52] In epithelioid sarcoma, high Met expression was suspected to reduce efficiency of mTOR inhibition by reactivating Akt[48] and expression of c-Met was shown to mediate drug resistance to the mTOR inhibitor everolimus in breast cancer.[53,54] In both studies, combined targeting of mTOR and Met could overcome resistance to mTOR inhibition.[48,54]

Taken together, the different RTK pathways we observed to be upregulated in our temsirolimus resistant Z138 phenotype are all part of a complex and partially overlapping signaling network mainly targeting the same intracellular pathways involved in the mediation of cell growth, proliferation and survival. Especially Met overexpression is considered a common cellular escape mechanism mediating resistance toward several drugs, including mTOR inhibitors.[48,51,53,54] Furthermore, the AKT/mTor-pathway was shown to control Met expression due to a positive feedback loop, indicating an important crosslink between these two components.[55] We therefore sought to further elucidate the role of Met signaling for mediating resistance to mTOR inhibition in MCL. We confirmed a significant upregulation of *MET* RNA and Met protein levels in the resistant phenotype. As evaluated by copy number variation analysis, the *MET* gene was not amplified (Figure S2). Instead, we suspect transcriptional regulation to be responsible for higher Met levels in Z138r. Met protein could only be detected in the resistant cells, yet not in the sensitive ones, which might be due to the extremely low Met levels in Z138s as observed in RT-qPCR experiments, being not detectable by Westernblot analysis. Interestingly, we observed a decrease of *MET RNA* expression upon treatment with temsirolimus. Crizotinib, targeting RTK upstream of the mTor pathway, led to similar results. In line with our observations, different authors have already described the downregulation of Met RNA and protein levels after treatment with temsirolimus and other inhibitors targeting the PI3K/Akt/mTOR pathway.[55–58] Therefore, compensatory upregulation of Met, which is known to be a potent mediator of proliferation and cell survival via different intracellular pathways,[41] could be a rational cellular escape mechanism to mediate temsirolimus resistance. In line with the results in Z138r, we observed extremely elevated Met protein levels in the temsirolimus resistant cell line Rec-1, whereby Met expression could not be observed in the temsirolimus sensitive MCL cell lines. Based on this observation, we postulate that high baseline levels of Met decrease sensitivity to mTOR inhibition and upregulation of Met by the initially sensitive cells could have mediated the acquired resistance in Z138r. We next sought to analyze if sensitivity to mTOR inhibition could be restored by pharmacological inhibition of Met signaling. To evaluate if Met was exclusively responsible for mediating temsirolimus resistance, we combined temsirolimus with the selective Met-inhibitor capmatinib.[59] We observed a relevant increase in temsirolimus sensitivity, but the drug combination could not completely overcome acquired drug resistance. Furthermore, si-RNA mediated knockdown of Met induced only a marginal increase of sensitivity to temsirolimus. In contrast, combination of temsirolimus with the already clinically approved RTK inhibitor crizotinib, targeting Met, RON, ALK and Ros-1[60–62] could completely overcome temsirolimus resistance in the Z138r. As Z138s does nearly not express Met, crizotinib alone was significantly less active active in this phenotype. In contrast, crizotinib led to a decrease of ph-mTor and ph-Stat3, an important downstream target of Met, in Z138r, which was even more pronounced upon combination with temsirolimus. On the other hand, temsirolimus induced compensatory activation of ph-Stat3 in Z138r but not in Z138s. These results are in line with our postulation that Z138r cells compensate for mTor inhibition by upregulation of Met-signaling and combined treatment with temsirolimus and crizotinib can overcome this mechanism, restoring sensitivity to mTor inhibition. Strengthening this hypothesis, in Z138r we observed reactivation of mTor signaling expressed by increased phosphorylation of ribosomal protein S6 after initial inhibition upon temsirolimus. These results suggest that Met may not be the only mediator of temsirolimus resistance. Regarding that our GSEA also revealed an enriched expression of genes involved in the ALK-signaling pathway in Z138r, the better effect of crizotinib on restoring sensitivity to temsirolimus compared to selective Met-inhibitors or *MET* siRNA might be explained by additional ALK inhibition mediated by crizotinib. Nevertheless, we think that the observed results indicate strong involvement of Met in mediating resistance and are in line with the results obtained by different other authors describing upregulation of Met as an important mechanism mediating resistance to mTor inhibitors.[48,53,54] Taken together, treatment with temsirolimus and crizotinib proved to be an effective combined treatment approach in MCL and a promising strategy to overcome resistance to mTor inhibition. Furthermore, combined inhibition of Met and mTor has already been proved to be effective in different preclinical studies.[63–65] In line with this, we observed the combination of temsirolimus and crizotinib to be highly effective for

the treatment of MCL with additive to synergistic effects in all cell lines investigated. Interestingly, the temsirolimus resistant cell line Rec-1 turned out to be less sensitive to crizotinib compared to the other cell lines. Possibly, the extremely elevated Met levels in Rec-1 compensate for the drug effects of the Met-inhibitor crizotinib. On the other hand, treatment with temsirolimus lead to a relevant inhibition of ph-mTOR expression, although the cell line carries an intrinsic temsirolimus resistance. Accordingly, ph-mTor inhibition after treatment with everolimus in cell lines proven to be rapalog resistant, was recently described. In this study, compensatory activation of RAS-signaling mediated drug resistance.[27] Thus, resistance mechanisms in Rec-1 cells might differ from the ones observed in Z138, but still seem to be at least partially linked to Met, as Met knockdown increased sensitivity to Temsirolimus. Elevated Met levels in Rec-1 might compensate for mTor inhibition trough activation of other Met downstream pathways such as the previous mentioned RAS-pathway. Combination of temsirolimus and crizotinib was also shown to be active in primary MCL cell lines, confirming the results obtained with MCL cell lines, although the observed drug effects were less pronounced, presumably due to their limited potential of proliferation *in vitro*. As temsirolimus and crizotinib act mainly cytostatic, observed drug effects in primary cells might be limited.

4 | CONCLUSION

In summary, we showed for the first time that overexpression of Met plays an important role for mediating temsirolimus resistance in MCL and that pharmacological inhibition of Met signaling could effectively increase sensitivity to mTor inhibition. Combination of crizotinib and temsirolimus could completely overcome acquired temsirolimus resistance. Moreover, this drug combination proved to be highly effective and a promising combined treatment approach for MCL, which warrants further investigation in the clinical setting.

AUTHOR CONTRIBUTIONS

Marie Moosburner, Lamija Alibegovic, Johannes Hildebrand, Korbinian Hasselmann and Anton Gaiderov performed experiments. Marie Moosburner, Lamija Alibegovic, Anton Gaiderov, Johannes Hildebrand, Korbinian Hasselmann, Julia Philippou-Massier, Helmut Blum, Luca Fischer and Elisabeth Silkenstedt analyzed and interpreted results. Marie Moosburner, Elisabeth Silkenstedt and Martin Dreyling wrote the manuscript. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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PEER REVIEW

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