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Multiple modes of action of eribulin mesylate: Emerging data and clinical implications

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ABSTRACT

Eribulin mesylate (eribulin) is a synthetic analogue of the marine-sponge natural product halichondrin B. Eribulin exhibits potent antiproliferative activities against a variety of human cancer cell types in vitro and in vivo, and is used for the treatment of certain patients with advanced breast cancer or liposarcoma who are refractory to other treatments. The antiproliferative effects of eribulin have long been attributed to its antimitotic activities. Unlike other microtubule-targeting agents, eribulin inhibits microtubule polymerization through specific plus end binding, thus interfering with microtubule dynamic instability. Non-mitotic effects of eribulin on tumor biology have also been established in laboratory settings including: tumor vasculature remodeling, increased vascular perfusion, reduced hypoxia, and phenotypic changes involving reversal of epithelial-to-mesenchymal transition (EMT), resulting in reduced capacities for migration, invasion, and seeding lung metastases in experimental models. Preclinical data suggest that increased perfusion following eribulin treatment improves delivery of subsequent drugs. Supporting evidence for eribulin's non-mitotic effects in the clinical setting include increased tumor oxygen saturation, reduced hypoxia, phenotype changes consistent with EMT reversal, and genotype changes consistent with shifts from nonendocrine-responsive, luminal B, to endocrine-responsive, luminal A, breast cancer subtypes. Finally, potential biomarkers for eribulin response have been established based on tumor-phenotype and gene-expression profiles. Overall, preclinical and clinical data support both antimitotic and non-mitotic mechanisms of eribulin that may underlie the survival benefit observed in various clinical trials.

Introduction

Eribulin mesylate (eribulin) is an anticancer agent used in patients with advanced or metastatic breast cancer previously treated with an anthracycline and a taxane, where it has demonstrated a significant overall survival (OS) advantage [1]. Similarly, in a phase 3 trial, eribulin improved OS in patients with advanced liposarcoma (dedifferentiated, myxoid/round cell, or pleomorphic) previously treated with an anthracycline [2]. Although eribulin's current clinical approvals in the United States and Europe are in advanced breast cancer and liposarcoma [3,4], it has shown antitumor activity against a wide range of other tumor types in preclinical cancer models. For instance, in human tumor xenograft models, eribulin has shown antitumor activity against colon cancer, Ewing's sarcoma, fibrosarcoma, glioblastoma, head and neck cancer, leiomyosarcoma, melanoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, and small cell lung cancer [5–7]. Eribulin has also shown activity in a variety of preclinical pediatric tumor models, including several B- and T-cell leukemias and lymphomas, ependymoma, malignant rhabdoid and Wilms' tumors, medulloblastoma, neuroblastoma, osteosarcoma, and rhabdomyosarcoma [8].

Chemically, eribulin is a pharmaceutically optimized, fully synthetic macrocyclic ketone analogue of the macrocyclic lactone portion of halichondrin B, a marine sponge natural product [7,9]. Biochemical investigations into the mechanism of action of halichondrin B by the US National Cancer Institute and other investigators in the early 1990s revealed that its tubulin-based antimitotic activities were mechanistically unique compared with other microtubule-targeting agents (MTAs), including taxanes and vinca alkaloids [10,11]. Following

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eribulin's first synthesis in the late 1990's, initial studies showed that it retained mechanistic similarity with halichondrin B [7], with subsequent work extending these observations to include mechanistically unique aspects of microtubule dynamics inhibition via binding to exposed β-tubulin subunits at microtubule plus ends [12,13].

In addition to tubulin-based mechanistic differences between eribulin and other MTAs, eribulin exhibits different clinical profiles compared with these drugs, likely due to more recently defined non-antimitotic effects of eribulin on tumor biology and the tumor microenvironment. For instance, preclinical work has shown that eribulin induces tumor vasculature remodeling leading to mitigation of hypoxia, phenotypic shifts toward epithelial phenotypes, diminished migration and invasiveness, and decreased capacity to seed metastases in preclinical models [14,15]. Eribulin has been shown to significantly improve OS in certain patients with breast cancer or liposarcoma who had previously progressed on other therapies [1,2], highlighting the potential utility of a compound that has effects on tumor biology in addition to its antimitotic mechanisms. The purpose of this review is to summarize eribulin's antimitotic and non-antimitotic mechanisms of action, first established in the laboratory and more recently supported by clinical observations. The review will further evaluate the implications of these mechanisms for clinical practice, including the possibility of identifying biomarkers predictive of eribulin response.

Eribulin: Modes of action

Tubulin-based antimitotic mechanism of action

Eribulin was initially established as having tubulin-based antimitotic activities that potently suppressed proliferation. In an early preclinical study performed by Towle and colleagues [7], eribulin (known at the time as ER-086526) exhibited antiproliferative activity against 8 different human cancer cell lines but, importantly, showed little or no activity against quiescent human fibroblasts. Like other MTAs, eribulin diminished both the rate and extent of tubulin polymerization in vitro. Due to disruption of the mitotic spindle by eribulin, cells were blocked in the prometaphase stage of mitosis and accumulated in the G_2–M cell cycle phase together with progressive depletion of G_1 and S phases. Eribulin-induced accumulation of cells in G_2–M was confirmed in another study with lymphoma and pancreatic cancer cells, which also established that sustained mitotic blockade led to initiation of apoptosis within 8–10 h after eribulin exposure [16].

Other studies have investigated the binding sites and mechanisms of tubulin polymerization inhibition. Unlike other MTAs, which inhibit both the growth and shortening phases of microtubule dynamics, eribulin inhibits only the growth phase, which prevents normal mitotic spindle assembly during prometaphase [12]. Treatment of the human osteosarcoma cell line U-2OS with eribulin at concentrations that inhibited mitosis, decreased the centromere relaxation rate by 21% and increased the time microtubules were in stasis by 67%, which reduced microtubule-dependent spindle tension at the kinetochores [17]. These data demonstrated that the inhibitory effects of eribulin on microtubule dynamics, first defined in interphase cells [12], also occurred during mitosis and were thus the driving force behind prevention of normal mitotic spindle formation [17].

Eribulin displays complex binding characteristics, with a high affinity for microtubule plus ends and a lower affinity for soluble tubulin [18]. Recent X-ray crystallographic studies and other biophysical experiments demonstrated that eribulin binds to the β-tubulin subunit, which functions in protofilament plus-end elongation [13]. Furthermore, single eribulin molecules are sufficient to induce erratic microtubule growth and sudden catastrophic depolymerization [13,18]. Overall, the complex binding characteristics of eribulin appear to upset the normal equilibrium between dynamic microtubules and soluble tubulin, resulting in a net depolymerization of microtubules despite the evidence that these effects are rooted in eribulin's inhibition of microtubule growth [13,18].

Microtubule plus ends become extensively splayed when bound to vinblastine, indicating its preference for binding to β-tubulin at interfaces between α/β-tubulin heterodimers, thus deforming individual protofilaments from linearity [12,18]. In contrast, eribulin prefers binding to open β-tubulin at microtubule plus ends, thus blocking microtubule polymerization with little or no effect on shortening, protofilament linearity, or end splaying [13,18]. Finally, the antimitotic effects of eribulin are functionally irreversible at the cellular level, unlike those of paclitaxel, vinblastine, colcemid, and nocodazole [19]. Even with short-term or intermittent exposure to eribulin, long-lasting effects on cell viability are seen, which have been associated with long-term cellular retention of the compound. Such irreversibility is thought to contribute to eribulin's effectiveness under intermittent dosing conditions [19].

Non-mitotic mechanism of action

In more recent studies, eribulin has been shown to exert non-mitotic mechanisms that fall into 3 categories: effects on tumor vascular remodeling and perfusion, reversal of epithelial-to-mesenchymal transition (EMT), and decreased capacity for migration and invasion. In terms of activity, these non-mitotic effects occur in the surviving residual tumor, including its component tumor cells, following eribulin's antimitotic, cytotoxic activity. Preclinical and clinical evidence of these non-mitotic mechanisms are described below.

Effects on tumor vascular remodeling and perfusion

Effects of eribulin on vascular remodeling and tumor perfusion were reported by Funahashi and colleagues in 2014 [14]. Using human triple-negative breast cancer xenograft models, they showed that a single dose of eribulin improved perfusion of tumor cores within 5 days. This effect was independent of suppressed tumor growth, as inhibition of tumor growth by capecitabine in the same study did not increase perfusion. The authors then showed that the increased perfusion observed following eribulin treatment was related to alterations in tumor vasculature: both microvessel density and the proportion of small vessels were increased [14]. More recent studies have similarly shown that eribulin increases tumor perfusion in xenograft models of soft tissue sarcoma (STS), including both leiomyosarcoma and liposarcoma [6,20].

Eribulin-induced changes in tumor vasculature in both breast cancer and STS are likely beneficial in several ways. These changes are associated with reduced hypoxia, a known driver of both drug resistance and metastasis [6,14]. In tumor xenograft models, eribulin treatment led to suppression of genes that are known to be involved in hypoxic signaling cascades, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Notch, Eph, and Wnt pathways [14]. Importantly, expression of VEGF was not significantly decreased in an in vitro model of angiogenesis. In contrast, the reduced in vivo expression of VEGF, which is upregulated by the hypoxic tumor microenvironment, supports the concept that eribulin-induced reversal of hypoxia via vascular remodeling drives, at least partially, the observed changes in gene regulation. In support of this concept, the hypoxia marker carbonic anhydrase 9 (CA9) is also decreased in eribulin-treated xenograft models [14]. Another potential benefit of eribulin-induced vascular remodeling and increased perfusion may be improved tumor delivery, and thus efficacy, of subsequently administered drugs. This could be particularly beneficial in the case of residual tumor remaining after treatment with other therapies. Indeed, preclinical studies in human breast cancer xenograft models support eribulin's ability to improve delivery of subsequently administered drugs. For example, prior treatment with eribulin improved the antitumor activity of capecitabine (Fig. 1) [14]. Additionally, in a preclinical sequencing study, giving a single dose of eribulin followed one week later by a single dose of paclitaxel was more effective than the reverse sequence of paclitaxel before eribulin [21]. Taken together, these data support the concept

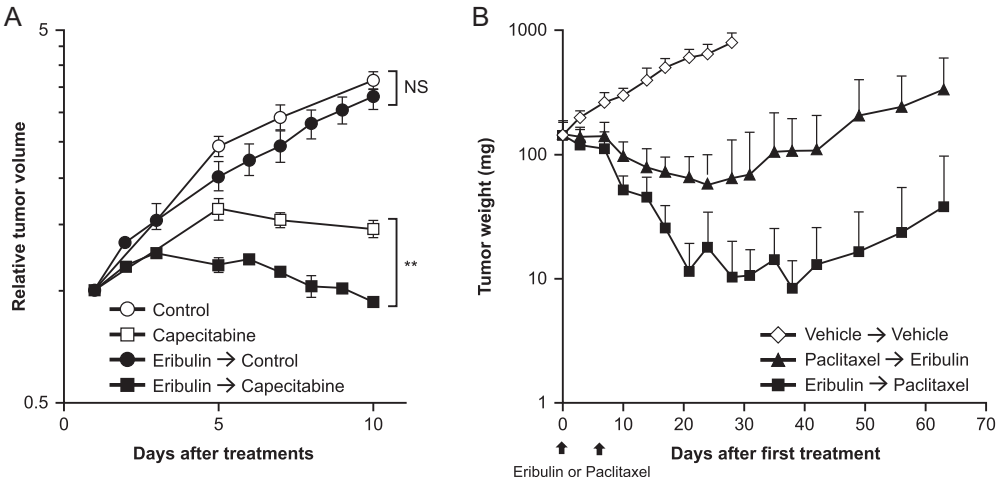

Fig. 1. Effects of prior treatment with eribulin on antitumor activity of capecitabine (A) and paclitaxel (B) in the MDA-MB-231 human breast cancer xenograft model. **A.** Effects of prior treatment with eribulin on antitumor activity of capecitabine in the MDA-MB-231 human breast cancer xenograft model in nude mice. When the mean MDA-MB-231 xenograft tumor volumes in 24 mice reached approximately 200 mg, nude mice were randomly divided into control (non-treatment, 6 mice), capecitabine (540 mg/kg, QD8, p.o., 6 mice) and eribulin (1.0 mg/kg, QD1, i.v., 12 mice) groups. Following treatment in the eribulin group, when tumor volumes had recovered to approximately the initial tumor size 12 days later (i.e. approximately 200 mg), eribulin-treated mice were then randomly divided into new eribulin-pretreated control (no further treatment, 6 mice) and eribulin-pretreated capecitabine (540 mg/kg, QD8, p.o., 6 mice) groups. The days of randomization of both the initial treatment-naive group (24 mice) and eribulin-pretreated group (12 mice) were both considered to be day 1 for purposes of the visual comparison shown here. Data are means +/− SEM. $^{**}P < 0.05$ versus after eribulin treatment by Student's *t*-test. i.v., intravenously; NS, not significant; p.o., per os; QD1, single dose; QD8, daily for 8 days; SEM, standard error of the mean. **B.** Antitumor effect of paclitaxel following eribulin was significantly superior to that of eribulin following paclitaxel. Eribulin was given once at 1 mg/kg on either day 0 or 7, with paclitaxel given once at 40 mg/kg on either day 7 or 0, respectively. Fig. 1A adapted with permission from Funahashi et al. [14]; Fig. 1B adapted with permission from Ozawa et al. [21].

that eribulin's effects on tumor vascular remodeling result in improved perfusion, mitigation of hypoxia via modulation of gene expression, and improved drug delivery.

The effects of eribulin on tumor vasculature stand in direct contrast with the established effects of other MTAs, which are typically categorized as either antiangiogenic or vascular-disrupting [22–24]. These differences are likely due to differences between the molecular mechanisms of eribulin and other MTAs. For example, although paclitaxel and eribulin have similar effects on gene expression in endothelial cells, the two drugs have markedly different effects on vascular pericytes, with only a 12% overlap in affected genes in this cell type [25]. Such differential effects on pericyte gene expression may explain the observation that eribulin impaired the interactions between pericytes and endothelial cells more than paclitaxel, resulting in greater anti-vascular effects [25].

Effects on epithelial-to-mesenchymal transition

EMT is the process by which cells of epithelial origin acquire mesenchymal phenotypes through epigenetically driven changes in gene and protein expression, transforming largely stationary cells with epithelial characteristics to more migratory and invasive cells with mesenchymal characteristics [26]. EMT underlies the metastatic spread of tumor cells and can also contribute to chemo- and radio-resistance, stem cell self-renewal [27], and immunosuppression [28]. Many signals from the tumor microenvironment induce EMT, including hypoxia, certain proinflammatory cytokines, some extracellular matrix components, and mechanical properties of the local tumor environment [29]. EMT is a dynamic state and is reversible through a process termed the mesenchymal-to-epithelial transition (Fig. 2) [26]. Preclinical studies have shown that eribulin reverses EMT in cultured human triple-negative breast cancer cells [15]. In a gene expression profiling study, eribulin downregulated 13 genes related to EMT in breast cancer cell lines [30]. Furthermore, regulation of EMT pathway genes may serve as a biomarker for response to eribulin because breast cancer models that overexpress these genes were sensitive to eribulin, but not paclitaxel [30]. Indeed, paclitaxel resistance has been associated with EMT [31] in breast cancer cells, the opposite of the reported effects of eribulin. Finally, Dezsö and colleagues suggest that these findings potentially point to an EMT/mesenchymal-to-epithelial transition gene expression profile that might identify sub-populations of patients who may derive greater benefit from eribulin versus paclitaxel treatment [30].

Substantial preclinical evidence exists supporting the idea that eribulin can reverse EMT. In breast cancer cell lines in vitro, treatment with eribulin increased gene expression of epithelial markers (i.e., *CDH1* and *KRT18*), while downregulating levels of mesenchymal markers (i.e., *CDH2*, *VIM*, *TWIST1*, *SNAI2*, *ZEB1*, and *ZEB2*), leading to reversal of EMT after 7 days of eribulin exposure [15]. Corresponding eribulin-induced shifts in EMT/mesenchymal-to-epithelial transition protein expression-level profiles were observed in a breast cancer tumor xenograft model in vivo [15]. Using protein analysis in a non-cancer EMT model, eribulin reversed EMT at least partially through downregulation of the transforming growth factor (TGF)-β/Smad signaling pathway [15], processes that may be related to altered interactions between Smad proteins and microtubules following eribulin binding [15,32]. Recent work by Dybdal-Hargreaves and colleagues additionally suggests that eribulin's effects on E-cadherin expression during EMT reversal are driven by disrupted interactions between microtubules and signaling scaffold complexes consisting of p130Cas and phosphorylated Src, leading to release of tonic inhibition of cell surface E-cadherin expression [33].

Eribulin-induced reversal of EMT has also been established in other cancer models. When assessed against three oral squamous cell carcinoma (OSCC) cell lines, eribulin showed approximately 100-fold more activity against the cell line with mesenchymal phenotype, compared with two other cell lines with epithelial phenotypes [34]. Along with growth inhibition, eribulin also reversed EMT in the mesenchymal OSCC line as assessed by characteristic changes in expression of epithelial and mesenchymal markers. Remarkably, epidermal growth factor receptor (EGFR) was upregulated in these same cells after eribulin treatment, leading to de novo sensitivity to growth inhibitory effects of the anti-EGFR monoclonal antibody cetuximab. Eribulin also blocked TGF-β-induced EMT in one of the OSCC lines with epithelial phenotype [34]. Taken together, these observations point to eribulin's ability to induce cellular differentiation responses in OSCC cells involving reversal of EMT (or blocking its induction by TGF-β) and inducing EGFR-signaling pathways that can be newly accessed by anti-EGFR drugs. This suggests the intriguing possibility that combinations of eribulin and cetuximab might be particularly effective in anti-EGFR–-refractory cancers due to synergistic interactions between the two drugs [34]. A recent preclinical study by Asano et al. showed combinatorial antitumor effects of eribulin plus the EGFR kinase inhibitor erlotinib in

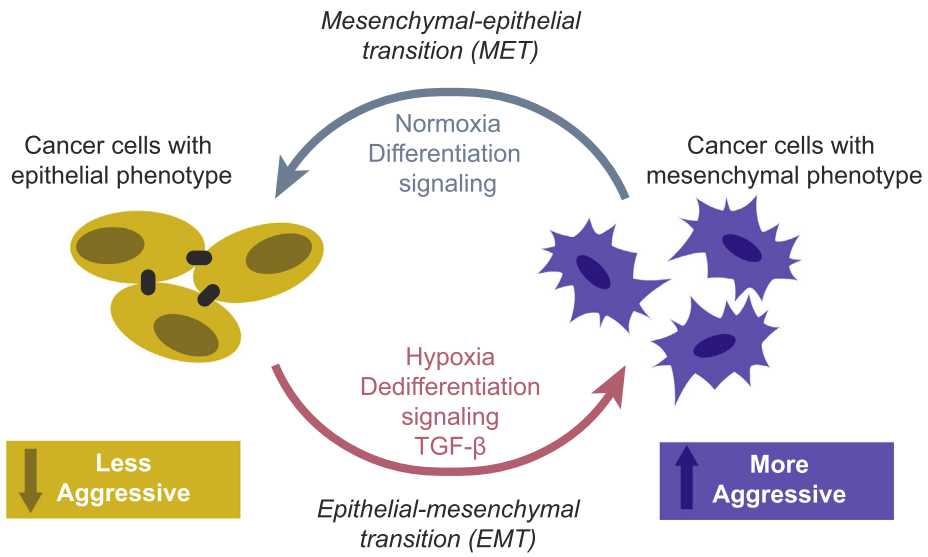

Fig. 2. Cancer cells can reversibly change phenotype via epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition processes. Black ovals connecting the epithelial cells on the left represent adhesion molecules such as E-cadherin.

a non-small cell lung cancer xenograft model [35], which further supports the potential clinical use of eribulin in combination with anti-EGFR therapies.

In an STS preclinical setting, eribulin also induced differentiation responses, albeit in ways distinct from those seen in breast cancer models [6]. Based only on the breast cancer results, one might have expected to see induction of epithelial markers and reductions of mesenchymal markers in liposarcoma and leiomyosarcoma cell lines, yet this was not observed. Instead, eribulin treatment of these cell types led to differentiation responses associated with increased expression of adipocyte and smooth muscle differentiation markers in the liposarcoma and leiomyosarcoma cell lines, respectively [6]. Thus, a picture emerges of eribulin as an inducer of cellular differentiation, with the phenotypic 'direction' of such effects being dependent on the cell type of origin: eribulin induces a more differentiated epithelial phenotype in breast cancer (cell type of origin: epithelium) yet a more differentiated mesenchymal phenotype in liposarcoma and leiomyosarcoma (cell type of origin: mesenchyme).

Effects on migration, invasion, and metastasis

The concept that epithelial cancers with mesenchymal phenotypes are more prone to invade and metastasize is well established [36]. Correspondingly, preclinical studies have shown that in breast cancer cell lines in which eribulin reverses EMT, the drug also reduces the capacity of tumor cells to migrate and invade in vitro [15]. Another study showed that eribulin pretreatment of cells dramatically reduced their capacity to seed lung metastases in an in vivo lung experimental metastasis model, an observation that was also associated with a marked increase in survival [15]. In both studies, the effects of eribulin were greater than those observed with 5-fluorouracil (5-FU), an active metabolite of capecitabine [15]. Notably, in the in vivo experimental metastasis model, 100% mortality was seen by days 15 and 21 in the control and 5-FU groups, respectively; in contrast, 60% of the animals in the eribulin group were still alive on day 80 at the conclusion of the study [15]. Although further studies are needed to fully understand the relationships between eribulin's effects on migration and invasion and its reversal of EMT, the crucial role of EMT as a central mechanism that induces invasion and metastasis [37,38] suggests a causative relationship between these biological processes [15].

Additional corroborating evidence for potential anti-invasive effects of eribulin arises from studies in preclinical models of small-bowel adenocarcinoma (SBA) [39]. At relevant in vitro concentrations, eribulin significantly inhibited the growth of SIAC1 SBA cells and depressed tumor growth in an in vivo SIAC1 SBA xenograft model. Furthermore, eribulin decreased Wnt/β-catenin signaling, leading to reduced β-catenin levels. The downregulation of both wild-type and mutant β–catenin suggests that eribulin may have antitumor activity in SBA and may act through mechanisms affecting the stability of β-catenin [39]. Because Wnt/β-catenin signaling is related to invasive tumor formation [40], these studies suggest a route by which eribulin may modify tumor behavior and microenvironment, resulting in a less aggressive, less invasive tumor phenotype.

Other non-mitotic effects observed with eribulin

In addition to the non-mitotic effects of eribulin on tumor vasculature, EMT, and migration and invasion, eribulin has also been reported to exhibit other activities in preclinical cancer models. For example, in a study of breast cancer cell lines, eribulin significantly reduced the proportion of cancer stem cells (CSCs) in both estrogen-receptor (ER)-positive and ER-negative cell lines [41], suggesting the possibility that anti-CSC activities of eribulin might contribute to the clinically observed increase in OS observed in patients with metastatic breast cancer.

Intriguingly, the microtubule-binding properties of eribulin may actually contribute to the non-mitotic mechanisms of action of the compound. In addition to their function in mitotic spindle assembly, microtubules play crucial roles in interphase (non-mitotic) cells to help regulate signal transduction and intracellular trafficking. For example, eribulin's inhibition of TGF-β/Smad signaling results in decreased nuclear localization of Smad2/3 [32], an effect likely mediated by eribulin's inhibition of microtubule dynamics, based on the known binding of Smad proteins to microtubules [42,43]. The inhibition of TGF-β/Smad signaling by eribulin ultimately suppresses the transcription and protein expression of Snail [32], a key transcription factor that drives EMT [44]. In addition, studies of the mechanisms by which eribulin increases cell surface E-cadherin expression showed that eribulin-induced microtubule depolymerization inhibits interactions between Src and the signaling scaffold p130Cas, leading to reduced phospho-Src at the cell cortex, thus facilitating rapid deployment of intracellular E-cadherin to the cell surface [33].

Importantly, microtubules are also essential for intracellular transport in neuronal cells, which depends on microtubules for trafficking along the elongated axons and dendrites [45]. In this regard, the nature

of interactions between eribulin and microtubules may underlie some of the clinical benefits seen with eribulin relative to other MTAs. In mouse models of peripheral neuropathy, a 2-week regimen of eribulin at its maximum tolerated dose (MTD) did not affect caudal or digital nerve conduction velocity or amplitude, and caused milder, less-frequent, morphological effects on dorsal root ganglia and sciatic nerve compared with maxiumum tolerated dosing of paclitaxel and ixabepilone [46]. In follow-up studies by the same group, pre-existing neuropathy induced by paclitaxel in the mouse models was exacerbated less severely by subsequent treatment with eribulin compared with paclitaxel [47]. This could be explained, at least in part, by the observation that microtubule-dependent axonal transport in an in vitro vesicle motility assay was less inhibited by eribulin than by paclitaxel, ixabepilone, or vincristine [48]. In aggregate, these preclinical studies of neuropathy are consistent with clinical observations suggesting that eribulin provokes or exacerbates lower levels of neurotoxicity, myalgia/arthralgia or fatigue in patients compared with other microtubule-targeting chemotherapeutic agents [49,50].

Overall, given the potential therapeutic relevance of the multifarious non-mitotic processes that eribulin affects, it seems likely that such effects contribute to eribulin's unique patterns of clinical activity. In randomized, phase 3 trials of eribulin, patients with metastatic breast cancer and advanced STS showed statistically significant improvements in OS, with negligible or no effect on progression-free survival [1,2]. Conceptually, this implies that residual tumors after progression have been altered in ways that ultimately lead to prolonged survival. For example, eribulin-induced tumor vascular remodeling with increased perfusion may increase the delivery and, thus, the effectiveness of subsequently administered drugs [14]. Likewise, eribulin-induced reversal of EMT may decrease CSC self-renewal as well as the tendency of cancer cells to invade and metastasize, both effects supporting prolonged survival even after progression [15].

Clinical support for eribulin's non-mitotic mechanisms of action

Emerging clinical evidence corroborates eribulin's non-mitotic mechanisms of action that were originally defined in the preclinical setting. Indeed, such mechanisms may help to explain the unique therapeutic activities reported with eribulin in large-scale, randomized, clinical trials. A summary of preclinical and clinical evidence supporting eribulin's various mechanisms of action is presented in Table 1.

Clinical evidence for eribulin's tumor vascular remodeling effects is supported by studies employing a variety of techniques. For example, Ueda and colleagues [51] used diffuse optical spectroscopic imaging (DOSI), a noninvasive infrared imaging technique, to assess the oxygenation status of breast tumors in patients before and 7 days after a single dose of eribulin. Guided by ultrasound mapping to define tumor boundaries, the authors measure tumor concentrations of oxygenated and deoxygenated hemoglobin, which then allowed derivation of overall oxygen saturation within the tumor. Their results showed statistically significant decreases in deoxygenated hemoglobin, with corresponding increases in oxygen saturation, in patients' breast tumors 7 days after a single administration of eribulin. In another arm of the same study, the only statistically significant effect of a single dose of the anti-VEGF monoclonal antibody bevacizumab was a decrease in oxygenated hemoglobin, conceptually opposite to that of eribulin and thus highlighting differences between the vascular effects of the two agents [51].

To conclusively demonstrate that increased oxygenation of the tumor as a whole was associated with decreased intracellular hypoxia, Ueda and colleagues [52] used ^{18}F-fluoromisonidazole-positron emission tomography/computed tomography (FMISO-PET/CT) as well as DOSI to measure both intracellular hypoxia and overall tumor oxygen saturation, respectively, after three cycles of eribulin treatment of a patient with breast cancer. The DOSI results confirmed that, similar to their earlier findings after a single eribulin dose [51], eribulin treatment for three cycles (6 doses) also resulted in increased overall tumor oxygen saturation. Importantly, the FMISO-PET/CT results confirmed corresponding reductions in intracellular hypoxia. Together, these two reports [51,52] showed that, in breast cancer patients, eribulin treatment is associated with increased tumor oxygenation and decreased intracellular hypoxia. That such effects in breast cancer patients are driven by the increased tumor microvessel density seen in preclinical models [14] is suggested by the results of Yardley and colleagues [53], who showed statistically significant increases in anti-CD31 staining for endothelial cells in patient tumor biopsies taken after eribulin-cyclophosphamide neoadjuvant treatment relative to pretreatment biopsies. Although numerical increases in anti-CD31 staining were also seen in the docetaxel-cyclophosphamide neoadjuvant comparator arm, such increases were not statistically significant, arguing against any role of cyclophosphamide in the eribulin-cyclophosphamide arm, and supporting the concept that eribulin's effects on vascular remodeling are not simply driven by general class effects shared by all MTAs.

Emerging clinical evidence also supports eribulin's non-mitotic mechanisms that involve phenotypic changes associated with EMT reversal in breast cancer. In a study of patients with locally advanced or metastatic breast cancer, Goto and colleagues [54], obtained paired tumor biopsies from 10 patients receiving eribulin treatment, 5 of whom showed clinical responses (partial response). Among the responders, 5 of 5 patients showed statistically significant increases in tumor expression of the epithelial marker E-cadherin, with 4 of the 5 responders also showing significant decreases in expression of the hypoxia marker CA9 [54,55]. These results are consistent not only with the preclinically defined mechanisms of eribulin-induced reversal of EMT and hypoxia mitigation, but also with the notion that such non-mitotic effects of eribulin may contribute to its therapeutic benefits. In the same study, the authors also examined expression of various immune markers before and after eribulin treatment, including CD8, programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), PD-L2, and the regulatory T cell (Treg) marker FOXP3 [54]. Remarkably, in the same 5 of 5 responding patients, eribulin treatment was associated with statistically significant reductions in expression of the immunosuppressive drivers PD-L1 and FOXP3 [54]. Given the well-established link between EMT and immunosuppression [56,57], it is tempting to speculate that eribulin's apparent reversal of EMT and mitigation of hypoxia in these patients' tumors might have also led to a reduced immunosuppressive tumor microenvironment.

The induced morphological changes and upregulation of differentiation markers observed in preclinical studies may be particularly relevant to certain types of STS, for example dedifferentiated liposarcoma. In the phase 3 study in patients with advanced leiomyosarcoma and liposarcoma, eribulin had impressive activity in patients with dedifferentiated liposarcoma, showing approximately a 10-month gain in median OS compared with dacarbazine (18.0 vs. 8.1 months; HR = 0.43; 95% CI, 0.23–0.79) [58], which may be indicative of eribulin-induced differentiation of cancer cells. Eribulin also exhibited unprecedented activity in pleomorphic liposarcoma, an aggressive and highly resistant malignancy (median OS, 22.2 vs. 6.7 months; HR = 0.18; 95% CI, 0.04–0.85), albeit in a small number of patients (n = 23) [58]. Evidence indicates that other types of STS, including leiomyosarcoma, may also benefit from the non-mitotic mechanisms of eribulin. In a study of patients with leiomyosarcoma, proteomics and genomics analyses revealed elevated levels of the epithelial marker E-cadherin in a subset of leiomyosarcoma, which was associated with better survival [59]. This led the study authors to conclude that mesenchymal-to-epithelial transition mediated by the E-cadherin repressor Slug is a clinically beneficial process [59], consistent with the observed antiproliferative and differentiation-inducing activities of eribulin in leiomyosarcoma preclinical models [6]. In the phase 3 trial of eribulin in sarcoma, an OS benefit was not observed in the subgroup of patients with LMS [2]. Nevertheless, objective responses in patients with leiomyosarcoma were observed [2]. For vascular sarcomas (e.g., angiosarcomas or solitary fibrous tumors), there is currently also

Table 1
Summary of preclinical and clinical evidence supporting eribulin's non-mitotic mechanisms of action.

Non-mitotic mechanism	Preclinical evidence	Clinical evidence
Vascular remodeling	• Increased perfusion and microvessel density in breast cancer xenografts [14] • Increased perfusion in STS xenograft models [6,20] • Reduced expression of genes regulating hypoxia [14] • Improved antitumor activity for subsequent treatments [14,21]	• Significant decreases in deoxygenated hemoglobin with corresponding increases in oxygen saturation in breast tumors after eribulin treatment [51] • Decreased intracellular hypoxia in a breast tumor after eribulin treatment (case study) [52] • Significant increases in endothelial cell staining in tumor biopsies after eribulin/cyclophosphamide neoadjuvant treatment [53]
Reversal of EMT (breast cancer, OSCC)	• Increased expression of epithelial genes and protein markers and decreased expression of mesenchymal genes and protein markers in breast cancer cell lines and tumor xenograft models [15] • Rapid deployment of epithelial marker E-cadherin to cell surface following eribulin treatment of TN breast cancer cells [33] • Upregulation of EMT/mesenchymal-to-epithelial transition-related gene pathways was predictive of eribulin sensitivity in breast cancer model [30] • Upregulation of *E-cadherin* and downregulation of *N-cadherin*, *vimentin* and *Snail* in an OSCC cell line with initial mesenchymal phenotype [34]	• Statistically significant increases in tumor expression of E-cadherin and significant decreases in expression of the hypoxia marker CA9 [54,55]
Increased differentiation (STS, OSCC)	• Increased expression of adipocyte and smooth muscle differentiation markers in LPS and LMS cell lines, respectively [6] • Upregulation of EGFR with consequent acquisition of sensitivity to anti-EGFR monoclonal antibody cetuximab in an OSCC cell line with initial mesenchymal phenotype [34]	• Not yet available
Decreased capacity for migration, invasion, and metastasis	• Reduced the capacity of TN breast cancer cells to migrate and invade in vitro [15] • Reduced capacity of TN breast cancer cells to seed lung metastases in an in vivo lung experimental metastasis model [15] • Inhibited the growth of SIAC1 cells and depressed tumor growth in a xenograft mouse model of SBA [39] • Decreased the expression of molecules involved in the Wnt/β-catenin pathway, leading to a reduction in β-catenin levels [39]	• Not yet available
Decreased immunosuppressive environment	• Not yet available	• Statistically significant reductions in expression of the immunosuppressive markers PD-L1 and FOXP3 [54]

CA9, carbonic anhydrase 9; EMT, epithelial-to-mesenchymal transition; FOXP3, forkhead box P3; LMS, leiomyosarcoma; LPS, liposarcoma; OSCC, oral squamous cell carcinoma; PD-L1, programmed death-receptor ligand 1; SBA, small bowel adenocarcinoma; SIAC1, small intestinal adenocarcinoma 1; STS, soft tissue sarcoma; TN, triple-negative.

interest in the use of therapies with novel anti-angiogenic and vascular remodeling properties [60].

Another clinical example of eribulin inducing changes toward less aggressive tumor phenotypes comes from analysis of the SOLTI1007 phase 2 neoadjuvant study [61]. In this study, Prediction Analysis of Microarray 50-gene classifier (PAM50) gene-expression profiling [62] of breast cancers was performed to categorize tumors into 1 of 5 intrinsic subtypes at baseline and after 1 and 4 cycles of neoadjuvant eribulin treatment [61]. Of 83 patients at baseline, 38.6% and 39.8% were categorized as having luminal A and luminal B tumor subtypes, respectively. After one cycle of eribulin treatment (82 patients), the proportion of luminal B tumors decreased to 31.7%, with luminal A tumors increasing to 41.5%. Remarkably, after 4 cycles of eribulin treatment (73 patients), the proportion of luminal B tumors had decreased by half relative to baseline: 19.2%; while the proportion of luminal A tumors had increased to 57.5%. Percentages of tumors represented by the non-luminal subtypes (HER2 enriched, normal-like, and basal-like) remained relatively constant at 21.6%, 26.8%, and 23.3% for baseline, 1 cycle, and 4 cycles, respectively, indicating that most of the eribulin-induced phenotypic shifts could be accounted for by shifts from luminal B to luminal A subtypes. While both luminal A and luminal B tumors are frequently ER positive, luminal A tumors are typically less aggressive and more hormonally responsive, which translates into a better long-term prognosis [63]. In contrast, the luminal B subtype is typically more aggressive and associated with hormonal insensitivity. The trial found that a pathological complete response with eribulin treatment was more likely in patients with a luminal B phenotype compared with a luminal A phenotype. Thus, an intriguing implication of the SOLTI1007 results is that eribulin treatment may trigger increased hormonal sensitivity in luminal B patients, providing a rationale for combining eribulin with hormone therapies in patients who present with the luminal B tumor subtype at baseline [61].

In a recent report, Kobayashi and colleagues [64] investigated the time-to-treatment-failure (TTF) of endocrine therapies among 25 postmenopausal patients with luminal metastatic breast cancers who had received at least two endocrine therapies prior to eribulin and at least one endocrine therapy after eribulin. Not surprisingly, in 76% of patients, TTF was shorter for the second endocrine therapy compared with the first *(mean, −8.6 months)*, consistent with an expected reduction in efficacy of sequential endocrine-based therapies. However, in 64% of patients, TTF was longer for the endocrine therapy immediately following eribulin compared with the endocrine therapy immediately preceding eribulin *(mean, +1.4 months)*, indicating improved efficacy of endocrine-based therapies after eribulin treatment. Among patients who experienced an increase in the length of TTF for the second endocrine therapy, the length of the increase was similar between patients regardless of whether the endocrine therapy was pre- or post-eribulin (mean, 2.8 months and 3.4 months, respectively). However, the percentage of patients who experienced a longer TTF was significantly larger (64% vs 24%; $p = 0.018$) for patients who received eribulin preceding their second endocrine therapy compared with after. Overall, these data are consistent with predictions from the SOLTI1007 results, and support the concept that eribulin treatment of luminal breast cancers may increase hormonal responsiveness as a result of eribulin-

induced phenotypic shifts from luminal B to luminal A subtypes. Moreover, these results support the notion that eribulin improves response to subsequent lines of therapy, including endocrine-based.

Implications for clinical practice

In the pivotal phase 3 study of eribulin in patients with recurrent or metastatic breast cancer, patients receiving eribulin had significantly improved OS compared with those receiving treatment of physician's choice (13.1 vs. 10.6 months; hazard ratio [HR] = 0.81; 95% confidence interval [CI], 0.66–0.99) [1]. Furthermore, patients receiving eribulin had a significantly higher objective response rate by independent review (12% vs. 5%; $P = 0.002$). The discovery of eribulin's non-mitotic modes of action raises interesting questions about its use in the clinic. Many cancers are known to be strongly dependent on the biological processes now known to be affected by eribulin. These cancer types include not only the current indications for eribulin, advanced breast cancer and advanced liposarcoma in patients who are refractory to other treatments, but also other cancers thus highlighting the need for further clinical evaluation of eribulin in cancer types that depend on such processes [29]. Overall, eribulin's non-mitotic mechanisms appear to play a role in the potential efficacy of the drug, by increasing tumor perfusion, reducing metastatic potential, and changing cancer morphologies and phenotypes. For example, the reversal of EMT observed in preclinical and clinical studies may be particularly relevant to the survival advantages conferred by eribulin in metastatic breast cancer, in which phenotypic conversion is tightly linked to cell migration and invasion of primary tumors [65].

The possibility for utilization of combination therapies, including pairing of eribulin with immunotherapies, is of emerging clinical interest. For example, in a recently published phase 1 trial of patients with HER2-negative metastatic breast cancer, the combination of eribulin plus balixafortide (a CXCR4 antagonist) resulted in a 30% objective response rate (n = 54) [66]. The most common TEAEs were fatigue, neutropenia, infusion-related reactions, alopecia, constipation and nausea. Of note, based on these results, in April 2018, the FDA granted a fast-track designation to the combination of balixafortide and eribulin for the treatment of patients with HER2-negative MBC who regressed after treatment with ≥2 chemotherapies in the metastatic setting [67]. Looking toward the future, as of June 2018 there were over 20 active studies listed on ClinicalTrials.gov exploring the use of eribulin in combination with other agents [68].

A more in-depth understanding of the full spectrum of the anti-tumor activity of eribulin may help improve the selection of patients who are most likely to benefit from this therapy. Recently, a microRNA biomarker signature for eribulin response was identified in a group of patients with advanced STS [69]. In this exploratory substudy of patients from a phase 2 sarcoma trial with eribulin (EORTC trial 62052 [70]), the pattern of microRNA expression was significantly different between patients who responded to eribulin (defined as absence of progression at week 12) and nonresponders. Preliminary analysis of the microRNA targets supports the multiple modes of action of eribulin summarized in this review. Among responding patients, the microRNA signature reflected downregulation of genes involved in the cell cycle, cell survival and apoptosis, as well as upregulation of genes involved in growth arrest, suppression of cancer stemness and metastasis, and modulation of chemoresistance [69]. Confirmation of these findings using tissue samples from the phase 3 trial in sarcoma is ongoing (NCT01327885).

Because eribulin is now believed to directly impact the tumor microenvironment, research into prognostic markers related to these mechanisms of action has been initiated. Tumor-infiltrating lymphocytes (TILs) are markers of immune response, and have been shown to predict therapeutic efficacy in some cancer models [71]. In a recent study, the prevalence of TILs was analyzed in patients with metastatic breast cancer who received eribulin [72]. Among the patients with triple-negative breast cancer, patients with high levels of TILs had significantly improved disease-free survival and OS compared with patients with low levels of TILs ($P = 0.033$ and $P = 0.042$, respectively). However, in non-triple-negative breast cancer patients, no significant difference in disease-free survival or OS was observed between patients with high or low levels of TILs ($P = 0.878$ and $P = 0.535$, respectively) [72]. These data indicate that the presence of TILs may be a method of predicting the potential efficacy of eribulin therapy in patients with triple-negative breast cancer.

Overall, the emerging evidence surrounding the mechanisms of action of eribulin paints a picture of a potent molecule with a unique spectrum of mechanisms and pleiotropic antitumor effects. By affecting both mitotic and non-mitotic cancer-relevant mechanisms through novel pathways, eribulin may be a promising drug in a variety of cancers, particularly advanced, refractory breast cancer and STS. Further elucidation of the non-mitotic activities of eribulin may improve our understanding of the best ways to use this compound—whether alone or in combination—to ultimately improve patient outcomes in diseases that have remained refractory to current treatments.

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Conflicts of Interest

BAL is a full-time employee of Eisai Inc., which manufactures and markets the clinically formulated mesylate salt form of eribulin as Halaven®.

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Contributions

All authors were involved in the writing and revision of this manuscript and have provided final approval to submit.

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