DRUG EVALUATION

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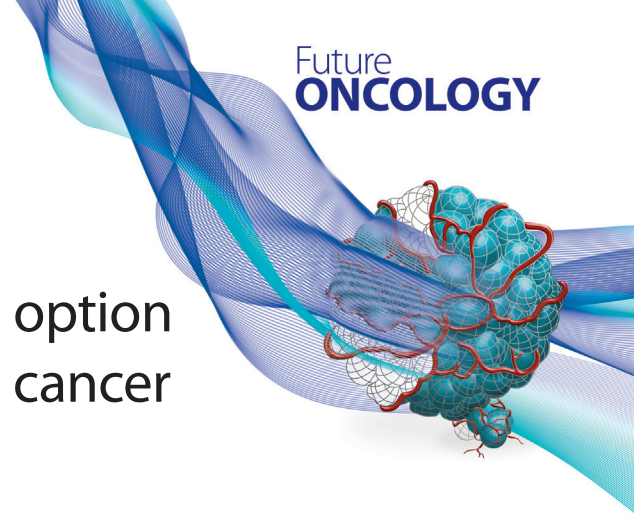

Entinostat: a promising treatment option for patients with advanced breast cancer

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Entinostat is a synthetic benzamide derivative histone deacetylase (HDAC) inhibitor, which potently and selectively inhibits class I and IV HDAC enzymes. This action promotes histone hyperacetylation and transcriptional activation of specific genes, with subsequent inhibition of cell proliferation, terminal differentiation and apoptosis. This oral HDAC inhibitor has been evaluated in Phase I and II trials in patients with advanced malignancies, and is in general well tolerated. Entinostat does not currently have regulatory approval for clinical use; however promising preclinical and clinical data exist in hormone-resistant breast cancer. An ECOG-ACRIN Phase III registration study is ongoing in advanced breast cancer (E2112, NCT02115282) and aims to confirm the overall survival advantage observed with the combination of exemestane and entinostat/placebo in the Phase II setting (ENCORE301 trial). This article provides an overview of the chemistry, pharmacokinetics/pharmacodynamics and available clinical data for entinostat with a focus on advanced breast cancer.

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KEYWORDS
• breast cancer • clinical trials • entinostat • epigenetics • histone deacetylase inhibitor

Breast cancer is the most common malignancy in women in western societies, and is a significant global health problem. It is estimated that more than 240,000 women will be diagnosed with breast cancer in the USA in 2016 [1]. Despite the many advances that have been made in the early detection and adjuvant treatment of breast cancer in recent decades, a significant proportion of patients with breast cancer continue to develop recurrent or metastatic disease. Consequently, breast cancer remains the second leading cause of cancer deaths in women [1]. New treatment strategies which reduce further the recurrence risk of this disease, and improve survival and quality of life for those with advanced or metastatic breast cancer are thus urgently required.

A number of factors guide the most appropriate treatment strategy for patients with advanced or metastatic breast cancer including the breast cancer subtype; defined by expression of the estrogen (ER), progesterone (PR) and HER2 receptors. For patients with hormone receptor-positive (ER- and/or PR-positive) advanced breast cancer, the largest subgroup of breast cancer, endocrine therapy that blocks estrogen signaling remains the primary approach. The recent addition of novel agents including CDK and mTOR inhibitors to an endocrine therapy backbone has resulted in improved progression-free survival (PFS) in this patient population [2–4]. Despite the wide range of treatment options available, treatment resistance is inevitable and approximately 40,000 women per year continue to die from this disease in the USA [1].

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A promising potential mechanism of overcoming treatment resistance may lie in the use of epigenetic modifiers [5]. Epigenetics as a field refers to alterations in gene expression that are not, as with genetic mutations, a result of changes in the DNA sequence of a gene. Epigenetic alterations instead may result in changes in chromatin structure leading to a repressive chromatin state and silencing of both gene expression and transcription of DNA into RNA. Epigenetic alterations include both histone hypoacetylation and abnormal methylation of DNA in the promoter region of important genes. The dynamic equilibrium between histone acetylation and deacetylation is regulated by the enzymes histone acetyltransferase and histone deacetylase (HDAC).

Epigenetic alterations including aberrant HDAC activity have been documented in breast cancer. In breast cancer core biopsy specimens, for example, HDAC1 expression was associated with ER and PR expression, earlier stage of disease at diagnosis and improved disease-free survival [6]. A study in a large cohort of breast cancer patients (n = 238) reported that protein expression of HDAC1 was increased in hormone receptor-positive tumors, HDAC2 expression was increased in higher grade tumors and HDAC3 expression was correlated with hormone receptor-negative status and high grade [7]. These and other studies have prompted the development and clinical testing of epigenetic modifiers including HDAC inhibitors as anticancer therapeutics [8].

Entinostat is a HDAC inhibitor which has not yet been approved for clinical use by any regulatory agency, but has received 'breakthrough designation' status from the US FDA in combination with exemestane for the management of advanced breast cancer. This review summarizes the chemistry, pharmacokinetics, pharmacodynamics and available clinical data for entinostat with a focus on its potential role in the management of patients with advanced breast cancer.

Overview of the market

Several therapeutic options exist for blocking estrogen signaling in the management of hormone receptor-positive breast cancer. Tamoxifen is a selective estrogen receptor modulator which binds to the ER and selectively inhibits transcriptional activity in breast cancer cells (estrogen antagonist). It also functions to activate the receptor in other tissues including bone and endometrium (estrogen agonist). The aromatase inhibitors (AIs) function by inhibiting the aromatase (CYP19A1) enzyme, which catalyzes conversion of androgens to aromatic estrogens in the adrenal gland and adipose tissue resulting in profound estrogen deprivation in postmenopausal women. Nonsteroidal AIs that bind reversibly to aromatase include anastrozole and letrozole, while exemestane is a steroidal AI that functions through irreversible binding and inactivation of the enzyme [9]. Fulvestrant is an ER antagonist or selective estrogen receptor downregulator administered as an intramuscular injection which is increasingly used as a treatment option [10].

De novo and acquired resistance ultimately develops to endocrine therapies through various proposed mechanisms, for example aberrant signaling via the PI3K/Akt/mTOR intracellular signaling pathway [11]. The combination of exemestane and everolimus (mTOR inhibitor) has been approved for use by the US FDA and the EMA for patients with metastatic breast cancer whose disease has progressed on a nonsteroidal AI. This was based on the observed PFS benefit of this regimen versus exemestane plus placebo (6.9 vs 2.8 months, respectively, hazard ratio [HR] for progression or death: 0.43; 95% CI: 0.35–0.54; $p < 0.001$) with no overall survival (OS) advantage observed [2]. This was the first approval of a novel agent combined with endocrine therapy for patients with advanced breast cancer.

More recently, the FDA has given approval to the combination of palbociclib, a CDK 4/6 inhibitor, and letrozole in the first-line setting for patients with hormone receptor-positive advanced breast cancer [12]. This was based on a significant improvement in PFS observed for the combination arm compared with letrozole alone (median PFS: 20.2 and 10.2 months, respectively; HR: 0.488; 95% CI: 0.319–0.748; $p = 0.0004$) in the PALOMA-1 trial [12]. A Phase III trial (PALOMA-2) has confirmed these results [13]. Palbociclib has also been approved in combination with fulvestrant for those who have already experienced disease progression after an AI (PALOMA-3) [4].

Another potential mechanism of overcoming resistance to endocrine therapy lies in the use of epigenetic modifiers such as the HDAC inhibitor entinostat. Although no HDAC inhibitors have regulatory approval for clinical use to date in solid tumors, significant clinical benefit has previously been observed with the combination

of exemestane and entinostat in patients with advanced breast cancer who have already been treated with a nonsteroidal AI [14]. The results from this study have led to the development of a Phase III ECOG-ACRIN registration trial E2112 (NCT02115282). Results from this study are eagerly awaited.

Entinostat

• The HDAC enzymes

HDACs cause post-translational acetylation of core nucleosomal histones, which affects chromatin structure and thus regulates gene expression including those important for cell survival, proliferation, differentiation and apoptosis [15]. HDACs also act as members of a protein complex which recruits transcription factors to the promoter region of genes and regulate cell cycle regulatory protein acetylation status [16]. As high HDAC expression and histone hypoacetylation have been noted in cancer in the setting of transcriptional repression of genes, the HDAC inhibitors have been investigated as therapeutic agents in cancer [17].

• Chemistry & mechanism of action

Currently available HDAC inhibitors target a variety of HDAC isoenzymes with class I (HDAC 1, 2, 3 and 8), class II (HDAC 4–7 and 9–10) and class IV (HDAC 11) activity. HDAC inhibitors may be class-specific inhibitors such as entinostat and romidepsin, or pan or nonspecific HDAC inhibitors such as vorinostat, belinostat and panobinostat. Entinostat is a synthetic benzamide derivative and member of the substituted pyridylcarbamate class of HDAC-inhibiting compounds with oral bioavailability **(Figure 1)** [18,19]. It potently and selectively inhibits class I and IV HDACs, promoting histone hyperacetylation and transcriptional activation of specific genes [20,21]. These actions can result in inhibition of cell proliferation, terminal differentiation and apoptosis. Its isoform selectivity may result in better safety and efficacy profiles when compared with nonselective pan-HDAC inhibitors.

• Pharmacokinetics & metabolism

Entinostat is an oral bioavailable drug with a moderate variability in exposure [22]. The pharmacokinetics of this agent were linear over dosages ranging from 2 to 12 mg/m^2 in prior studies **(Table 1)** [23–25]. However, large variability in the time to maximal concentration (T_{max}) exists and ranges from 0.5 to 60 h, but is reduced

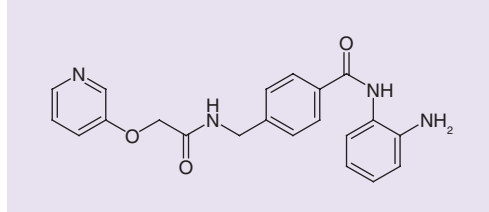

Figure 1. Entinostat structure.

in a fasting state to 0.25–2 h [26]. While the initial development involved a body surface area-based dosing approach, analysis of covariates (e.g., body surface area, BMI, lean body mass) in 64 patients supports the utility of a flat-fixed dose which has been utilized in subsequent clinical trials [22]. Entinostat's half-life ranged from an average of 33–150 h, which is much longer than what was predicted from preclinical studies. In assessment of factors altering the pharmacokinetics of entinostat, it was noted that plasma protein binding is lower in humans by approximately twofold and is presumed to be due to the near equal binding to both albumin and α-1 acid glycoprotein [27]. It has been proposed that the long half-life may be due to enterohepatic recirculation. Yet, *in vitro* experiments point to Phase I and II metabolism as a minor route of elimination with no drug-mediated transport by OATP1B1 and OATP1B3 [28]. Entinostat does inhibit cytochrome P450 enzymes albeit at higher than clinically achievable concentrations and is therefore likely to cause drug–drug interactions via this mechanism. Currently, the factors contributing to the unique pharmacokinetic profile remain unknown. Lastly, entinostat has been noted to penetrate into the CNS utilizing indirect measures of acetylation and pharmacodynamics but direct assessments utilizing imaging have demonstrated poor penetration [29,30]. Based on the current clinical pharmacology knowledge, it is recommended that entinostat be administered as a flat-fixed dose once per week or every 2 weeks on an empty stomach, at least 1 h before or 2 h after a meal.

• Preclinical antitumor activity

Entinostat has demonstrated promising antitumor activity in both *in vitro* and *in vivo* models of human malignancy. It has the ability to inhibit HDACs *in vitro* and has growth-inhibiting activity in tumor cell culture and murine human xenograft models [18,19]. *In vitro*, entinostat demonstrated antiproliferative activity against various human tumor cell lines derived from

Table 1. Pharmacokinetic parameters from select Phase I single-agent studies of entinostat.

Study	Entinostat dose and schedule	C_{max} (ng/ml)	$t_{½}$ (h)	CL/F (l/h/m²)	AUC (ng h/ml)	Ref.
Ryan *et al.*† (2005)	10 mg/m² every 2 weeks	45.1 ± 59.3	51.6 ± 10.5	20.5 ± 6.0	529 ± 171	[23]
Kummar *et al.*† (2007)	6 mg/m²/week × 4 weeks of 6-week cycle	30.9 ± 30.1	33.4 ± 21.8	47.5 ± 44.9	174.9 ± 97.4	[24]
Gojo *et al.*† (2007)	8 mg/m²/week × 4 weeks of 6-week cycle	53.1 ± 92.4	33.4 ± 12.7	66.7 ± 54.5	328 ± 168	[25]
Gore *et al.*‡ (2008)	6 mg/m² every 2 weeks or	19 (285)	105 (20.1)	14.7 (62.8)	409 (62.8)	[31]
	2 mg/m²/week × 3 weeks of 4-week cycles or	4 (67.3)	87.9 (54.6)	19	102	
	4 mg/m²/week × 3 weeks of 4-week cycles	7.15 (120)	150 (47)	11 (27.9)	365 (27.9)	

†Mean ± SD.
‡Geometric mean (% coefficient of variation).
Data shown reflects the maximum tolerated dose.
AUC: Area under the plasma concentration-time curve; CL/F: Oral clearance; C_{max}: Maximum plasma concentration; $t_{½}$: Half-life.

both solid tumor and hematologic malignancies [18]. *In vivo*, entinostat inhibited the growth of a range of human tumor xenografts, including models of lung, prostate, breast, pancreas and renal cell carcinoma among others [32].

Preclinical studies have also demonstrated promising findings when entinostat was combined with various targeted therapies and chemotherapeutic agents, resulting in enhanced antiproliferative activity, synergy and the ability to overcome treatment resistance [33,34]. In addition, entinostat has demonstrated immunomodulatory activity that may translate to direct effects on the tumor microenvironment and enhanced antitumor immune responses [20,35]. HDAC inhibitors have been shown to affect immune responses by regulating cytokine production [36], altering activation and function of macrophage and dendritic cells [37], regulating the transcription of major histocompatibility class I and II [38], or the activation of costimulatory molecules [39] and increasing the function of Tregs and enhancing their immunosuppressive effect *in vivo* [40]. Recent preclinical investigations suggest that entinostat has the ability to target Tregs thus enhancing the immune response when combined with either IL-2 in a renal cell carcinoma model or a survivin based vaccine therapy in a castration resistant prostate cancer model [41].

• Preclinical activity in breast cancer

Preclinical research to date specifically supports the investigation of HDAC inhibitors in breast cancer. HDAC inhibitors are thought to be able to target breast cancer through several different approaches including relief of transcriptional repression, and an impact on epithelial–mesenchymal transition (EMT). Reactivation of silenced *ER* has been observed in preclinical models in tumors which are considered hormone receptor-negative [42]. Restoration of sensitivity to endocrine therapy has also been observed in both ER-negative [43] and ER-positive tumors [34,44]. Entinostat has been shown to induce not only re-expression of ER-α, but also the androgen receptor and the aromatase enzyme both *in vitro* and in triple-negative breast cancer (ER/PR/HER2-negative, TNBC) xenografts [44].

The combination of entinostat and letrozole has also been shown to result in a significant and durable reduction in letrozole-resistant xenograft tumor volume when compared with treatment with either agent alone [34]. Entinostat was found to increase ER expression and aromatase activity but downregulate HER2, and also phosphorylated HER2/MAPK and AKT. Rather than overcoming endocrine resistance by epigenetic silencing, it appeared that entinostat impacted posttranslational and transcriptional modulation of HER2 [34]. These experiments have provided the strong rationale for combining epigenetic modifiers with endocrine therapy in breast cancer clinical trials. Interestingly, many studies also indicate that a strategy which combines HDAC and DNA methyltransferase (DNMT) inhibitors is more efficacious than either agent alone with respect to both re-expression of silenced genes and

restoration of response to endocrine therapies such as AIs [45–47]. Finally, preclinical studies also support the combination of entinostat with HER2-targeted therapy. The combination of entinostat and lapatinib resulted in significant *in vivo* tumor shrinkage or growth inhibition in HER2-positive xenograft mouse models when compared with either agent alone. Mechanistic studies elucidated that these effects were as a result of downregulation of phosphorylated Akt, activation of FOXO3 transcriptional activity and subsequent induction of the pro-apoptotic protein Bim1. Entinostat was also found to sensitize treatment-resistant HER2-overexpressing cells to the combination of trastuzumab and lapatinib [48]. These results have prompted the development of a clinical trial in patients with HER2-positive breast cancer **(Table 2)**.

Immune checkpoint blockade has also been shown to lead to expansion of cytotoxic T effector cells in solid tumor models including breast cancer, but these are not fully functional unless immune suppressor cells are reduced by treatment with epigenetic modulators including entinostat [50]. This preclinical data revealed that the combination of epigenetic therapy (azacitidine, entinostat) with combined immune-checkpoint blockade (nivolumab, ipilimumab) led to eradication of tumors and long-term cure in mouse models of breast and colon cancer. Complete regression of all primary tumors three weeks after treatment initiation and 80% survival 100 days after tumor implantation [50].

Additional approaches in breast cancer may include the effect of HDAC inhibitors on EMT. Additional studies by the same investigators examined TNBC models with a basal phenotype. E-cadherin transcription was found to be increased after treatment of cell lines with entinostat, along with reduced expression of mesenchymal markers. Entinostat increased E-cadherin promoter activation by reducing Twist and Snail association with the promoter, and also inhibited cell migration. These findings suggest that entinostat can reverse EMT and reduce the development of metastases [51]. This effect has been observed in other tumor types with various HDAC inhibitors [52,53]. Entinostat has also been shown in TNBC cells to reduce the percentage of tumor-initiating cells, the CD44high/CD24low cell population, ALDH-1 activity and expression of known tumor-initiating cell markers [54].

Clinical development

• Entinostat monotherapy trials

Entinostat has been evaluated in a number of Phase I and II trials in patients with both solid tumors and hematologic malignancies, including breast cancer **(Table 3)**. In a Phase I study of single agent entinostat in patients with advanced and refractory solid tumors or lymphoma, dosing of entinostat was initially daily but was changed to every 14 days since dose-limiting toxicities were observed at the first dose level [23]. In a subsequent study, weekly dosing of entinostat was investigated and was found to be well tolerated in 22 patients with refractory malignancies [24]. Two additional Phase I trials have been reported which have investigated alternative dosing schedules [25,31].

Table 2. Select ongoing clinical trials with entinostat in breast cancer.

Clinical trial	Phase	Eligibility	Regimen	Estimated completion date	Clinical end points
NCT02453620 (target n = 45)	I/II	Advanced solid tumors (escalation) Advanced HER2-negative breast cancer (expansion)	Entinostat + nivolumab ± ipilimumab	January 2017	Phase I: safety RP2D Phase II: ORR
NCT01434303 (target n = 37)	I	Advanced HER2-positive breast cancer	Entinostat and lapatinib and trastuzumab	June 2016	Safety RP2D
NCT02708680 (target n = 88)	I/II	Advanced TNBC	Entinostat + atezolizumab	June 2017	Phase I: Safety RP2D Phase II: PFS
NCT02115282 (target n = 300)	III	Advanced HER2-negative breast cancer	Entinostat or placebo + exemestane	July 2017	PFS and/or OS

NSCLC: Non-small-cell lung cancer, ORR: Overall response rate, OS: Overall survival; PFS: Progression-free survival; RP2D: Recommended Phase II dose; TNBC: Triple-negative breast cancer; TTP: Time to progression.

Table 3. Select completed clinical trials with entinostat in breast cancer.

Clinical trial	Agents	Study design/dosing/primary end point	Study population	Clinical results	Ref.
NCT00676663 (n = 130) ENCORE301	Entinostat + exemestane	Randomized Phase II design Entinostat 5 mg/placebo weekly, plus exemestane 25 mg daily PFS	Advanced ER-positive breast cancer, progression on prior nonsteroidal AI	Improved median PFS: 4.28 vs 2.27 months, improved median OS: 28.1 vs 19.8 months (entinostat vs placebo)	[14]
NCT01234532 (target n = 45)	Entinostat + anastrozole or tamoxifen	Single-arm pilot and Phase II study Entinostat oral weekly, anastrozole/tamoxifen daily oral Determine RP2D, safety and tolerabilty (pilot)/change in Ki67, ER pre- and post-therapy (Phase II)	Primary operable TNBC	Terminated early due to low accrual	
NCT01349959 (n = 40)	Entinostat + AZA	Phase II single arm study AZA 40 mg/m^2 days 1–5 and 8–10 every 28 days, entinostat 7 mg days 3 and 10 Response rate by RECIST criteria	Advanced breast cancer: TNBC and hormone-resistant	Primary end point not met	[49]

AI: Aromatase inhibitor; AZA: Azacitidine, ER: Estrogen receptor; OS: Overall survival; PFS: Progression-free survival; RP2D: Recommended Phase II dose; RECIST: Response evaluation criteria in solid tumors; TNBC: Triple-negative breast cancer.

• Combination with demethylating agents

A number of Phase I and II combination epigenetic strategies have also been investigated in advanced solid tumors and hematologic malignancies. Preclinical evidence across multiple tumor types has suggested that the combination of an HDAC inhibitor with a DNMT inhibitor is more efficacious than either agent alone at re-expression of silenced genes [47,55,56]. The combination of the DNMT inhibitor 5-azacitidine (AZA) with entinostat, for example, was demonstrated to be tolerable and effective in clinical testing in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia. Clinical responses were observed in 14/30 (46%) of patients [57]. The recommended Phase II dose was determined to be AZA 50 mg/m^2/day subcutaneously for 10 days and entinostat 4 mg/m^2/day orally on day 3 and day 10 of a 28 day cycle. However, a randomized Phase II clinical trial in patients with MDS and acute myeloid leukemia did not demonstrate a benefit of this combination over single agent AZA. One possible explanation for this lack of benefit is the limited demethylation observed with the combination, suggesting a pharmacodynamic antagonism when the agents are combined on this schedule [58].

Other trials initiated at the same time sought to test this combination of epigenetic modifying agents in patients with solid tumors. The combination of AZA and entinostat was explored in patients with advanced non-small-cell lung cancer [59]. Low-dose AZA (40 mg/m^2) was administered on days 1–5 and 8–10, and entinostat 7 mg on days 3 and 10 of a 28-day cycle. The regimen was well tolerated and associated with objective responses including one complete response and a partial response in a patient without progression of disease for 2 years after study completion. Another surprising finding, noted with long-term follow-up was the observation of unexpected major objective responses to subsequent anticancer strategies in a significant number of patients, despite the patients being heavily pretreated. This included major objective responses in 21% receiving chemotherapy (4/19) [59], and 5 of 5 treated subsequently with immune-checkpoint agents had either durable response (3/5) or prolonged stable disease (>6 months) [J Brahmer, Pers. Comm. November 2016]. This has provided additional evidence for the ability of epigenetic agents to prime tumor cells to respond to subsequent therapies [60].

A multicenter Phase II clinical trial of the same schedule of AZA and entinostat was designed in patients with advanced TNBC and hormone-resistant breast cancer. This was based on preclinical evidence suggesting that a combination of epigenetic modifiers may be more successful in re-expression of silenced genes and restoration of hormonal therapy responsiveness [45,47]. Patients were offered transition to an optional continuation phase at the time of disease progression in which the same epigenetic therapy was continued with the addition of endocrine therapy if deemed clinically acceptable [49]. No disease responses were observed in 13 patients with advanced TNBC. One partial response was observed with

epigenetic therapy alone in the hormone-resistant cohort such that the cohort was expanded to 27 patients. Unfortunately, no further responses were observed in this phase of the study and the study did not therefore meet its primary end point. However, an additional response was observed in the optional continuation phase in a patient who experienced disease progression with epigenetic therapy alone (one of 15 patients enrolled in this phase). Combination epigenetic therapy was well tolerated and the results from the optional continuation phase suggested that some women may benefit from epigenetic therapy and/or reintroduction of endocrine therapy beyond progression.

• Combination with endocrine therapy

Whether the addition of an HDAC inhibitor to endocrine strategies for breast cancer can reverse treatment resistance and therefore improve breast cancer outcomes has also been investigated in the clinic. The ENCORE301 randomized Phase II study evaluated the role of entinostat in combination with exemestane (steroidal AI) in the advanced breast cancer setting. The trial was supported by the preclinical experiments described above in letrozole-resistant mouse models, which indicated that the combination of entinostat and exemestane was more effective than either agent alone in reducing tumor volumes [34]. Postmenopausal women who had received ≤1 prior chemotherapy and were progressing on a nonsteroidal AI were randomized to exemestane 25 mg daily plus entinostat 5 mg weekly versus exemestane plus placebo [14]. A significant improvement in PFS was noted in the entinostat arm versus placebo (median 4.3 vs 2.3 months, respectively, HR: 0.73; 95% CI: 0.50–1.07; p = 0.055). OS, an exploratory end point, was also significantly longer in the entinostat arm versus the placebo arm (28.1 vs 19.8 months, respectively, HR: 0.59; 95% CI: 0.36–0.97; p = 0.036). A Phase III confirmatory study is ongoing (E2112, NCT02115282).

• Combination with immune-therapy

Additional areas of active investigation include the evaluation of the role of epigenetics in priming the immune system to respond to immune checkpoint blockade. A number of studies are ongoing which combine entinostat with immune-checkpoint blockade, in an effort to convert traditionally 'nonimmunogenic' tumors into tumors which can respond to immune therapy. These trials are based on the preclinical data and clinical observations noted above. For example, a Phase I clinical trial is actively recruiting patients to evaluate the combination of entinostat, nivolumab with or without ipilimumab in advanced solid tumors, and incorporates a dose expansion phase in patients with HER2-negative breast cancer (NCT02453620, **Table 2**). A Phase Ib/II trial of entinostat in combination with atezolizumab in TNBC is planned (NCT02708680, **Table 2**).

Safety & tolerability

Adverse events (AEs) with entinostat appeared to be dependent upon dose, schedule and patient characteristics [61]. Commonly occurring AEs in monotherapy studies included fatigue, gastrointestinal effects (nausea, vomiting, anorexia and diarrhea), hematologic abnormalities (predominantly anemia, thrombocytopenia, neutropenia and leukopenia) and metabolic abnormalities (hypoalbuminemia, hypophosphatemia, hyperglycemia and hyponatremia). Grade 3 and 4 AEs occurring in >5% of patients with solid tumors receiving entinostat monotherapy have included hypophosphatemia (21%), anemia (13%), fatigue (10%), neutropenia (9%) and hyponatremia (9%). Although hematologic abnormalities are common, they are seldom dose-limiting. At or below the maximum tolerated dose, most side effects in earlier studies were mild to moderate and manageable with supportive care including use of anti-emetics. Based on completed Phase I and II studies, a cumulative dose of approximately 20 mg entinostat given in a 28-day cycle has been established as a well-tolerated regimen delivered at either 5 mg every week, 10 mg every 2 weeks, or 7–15 mg weekly for 3 out of 4 weeks.

The Phase II study of AZA and entinostat in patients with advanced HER2-negative breast cancer noted that grade 3 and 4 drug-related toxicities were infrequent. Neutropenia (17.5%) and leukopenia (17.5%) were the most common hematologic AEs observed. The most frequent nonhematologic AEs included urinary tract infection (10%), fatigue (5%) and hypophosphatemia (5%) [49]. In the Phase II ENCORE301 study the combination of exemestane and entinostat/placebo was overall well tolerated with the side effect profile consistent with prior experience. Entinostat/placebo was administered in this study as a 5 mg weekly dose. Fatigue, GI toxicity

(nausea, vomiting, diarrhea), hematologic toxicity (anemia, neutropenia, thrombocytopenia), dyspnea, peripheral edema and decreased weight were the most frequent AEs, occurring in approximately 15% of patients treated with entinostat. The overall rate of AEs was higher in the entinostat arm (vs placebo) with grade 3 AEs occurring in 44 versus 23%, respectively. Grade 4 AEs occurred in 6 versus 3%, and AEs led to study discontinuation in 11 versus 2%, respectively [14].

The safety of entinostat in combination with immunotherapy is currently unknown and will be elucidated pending on-going clinical trials (NCT02453620, NCT02437136 and NCT02708680).

Pharmacodynamics

HDAC inhibitors appear to have multiple actions on cancer cells. These may include their impact on gene expression or regulation of function of other proteins. Unfortunately, it is not clear which effect contributes to the observed clinical activity [26,62].

HDAC inhibitors target the HDAC enzymes, resulting in hyperacetylation of histone tails. This appears to directly related to their impact on changes in gene expression. Preclinical investigations have suggested that this histone acetylation may occur approximately thirty minutes after exposure to HDAC inhibitors, with the effect on chromatin remodeling occurring after more prolonged exposure to these agents (24–48 h minimum) [63]. The hyperacetylation of target histones observed with administration of the HDAC inhibitors is comparable in tumor samples and peripheral blood mononuclear cells (PBMCs) when assessed by standard western blot analysis and other techniques thus allowing PBMCs to be a surrogate biomarker [63,64].

Histone hyperacetylation has been observed in clinical trials to date with entinostat at all doses, confirming the intended pharmacologic effect. However, until recently, studies have failed to show a clear correlation between the level of hyperacetylation and response to therapy [65]. This may relate to the small sample sizes in some studies or variation in the HDAC inhibitor being investigated. In a subset analysis examining protein acetylation in PBMCs at 2 weeks after commencement of entinostat (n = 27) and endocrine therapy in the ENCORE301 study, the median PFS was significantly longer in those exhibiting protein lysine hyperacetylation versus not (8.5 vs 2.7 months, HR: 0.32, 95% CI: 0.13–0.79) [28]. This potential predictive biomarker of response is also being evaluated in a Phase III E2112 study with the hope of confirming initially the prognostic value of this biomarker (NCT02115282).

A significant reduction in granulocytic myeloid-derived suppressor cells (MDSCs; -14.67 vs +20.56%; p = 0.03) and monocytic MDSCs (-62.3 vs +1.97%; p = 0.002) has also been observed in PBMCs in a *post hoc* analysis of samples from entinostat treated patients in ENCORE301 [66]. CD40, a costimulatory receptor required for MDSC-mediated immune suppression, was also significantly downregulated in the majority of MDSC subsets. A significant increase in the number of HLA-DR+ monocytes (34.1 vs -11.38%; p = 0.0004) and level of HLA-DR expression on monocytes (16.3 vs -4.7%; p = 0.015) was also observed. These findings may in part explain the improved OS seen in the entinostat-treated patients. These interesting results in addition to preclinical observations have also led to the development of studies in patients with advanced cancer including HER2-negative breast cancer, which combine epigenetic modifiers with immune checkpoint agents (e.g., NCT02453620). These efforts hope to improve on results seen to date with the immune checkpoint agents in tumors not traditionally felt to be 'immunogenic'.

Additional potential pharmacodynamics markers of HDAC inhibitor activity have been noted in other clinical trials. Histone hyperacetylation and higher baseline HDAC2 levels correlated with response in a clinical trial which enrolled 43 patients with hormone receptor-positive metastatic breast cancer treated with tamoxifen and the HDAC inhibitor vorinostat [65]. HR23b expression has also been shown to be a potential biomarker of response to HDAC inhibitors in various malignancies [67,68]. HR23b plays an important role in proteasome activity and was identified in a genome-wide loss of function screen to be involved with tumor sensitivity to HDAC inhibitors [69]. Future studies should aim to validate these potential biomarkers further in carefully designed studies.

Regulatory affairs

Based on the promising results of the ENCORE301 study, the FDA granted 'breakthrough designation' to the combination of exemestane and entinostat in 2013. A Phase III

multicenter international registration trial led by the ECOG-ACRIN co-operative group (E2112) is ongoing in advanced breast cancer. E2112 is investigating entinostat in combination with endocrine therapy in patients with locally advanced or metastatic breast cancer who have experienced disease progression after a nonsteroidal AI (NCT02115282, Table 2). If the primary end point of the study (PFS and/or OS) is met, this may lead to FDA approval for this regimen in patients with advanced breast cancer.

Conclusion

Entinostat is an oral HDAC inhibitor which has been evaluated in Phase I and II trials in patients with advanced malignancies, with a favorable risk–benefit profile.

This synthetic benzamide derivative and member of the substituted pyridylcarbamate class of HDAC-inhibiting compounds potently and selectively inhibits class I and IV HDACs. Entinostat induced histone hyperacetylation facilitates transcriptional activation

EXECUTIVE SUMMARY

Mechanisms of action

- Entinostat is a synthetic benzamide derivative and member of the substituted pyridylcarbamate class of histone deacetylase (HDAC) inhibiting compounds which potently and selectively inhibits class I and IV HDACs.
- Entinostat induced histone hyperacetylation allows transcriptional activation of a distinct set of genes resulting in inhibition of cell proliferation, terminal differentiation and apoptosis.

Pharmacokinetic properties

- Entinostat is a moderately lipophilic orally administered agent with a variable absorption rate and moderate plasma protein binding.
- Entinostat has a long half-life (~100 h) and the predominant route of metabolism remains unclear.

Clinical efficacy

- Entinostat does not currently have regulatory approval for clinical use.
- Entinostat has been evaluated in a number of Phase I and II trials in cancer patients with both solid tumors and hematologic malignancies.
- Promising data have been reported from the ENCORE 301 randomized Phase II study evaluated the role of entinostat in combination with endocrine therapy in the advanced breast cancer setting. A significant improvement in progression-free survival was noted in the entinostat arm versus placebo (median 4.3 vs 2.3 months, respectively). Overall survival was also significantly longer in the entinostat arm versus the placebo arm (28.1 vs 19.8 months, respectively) in an exploratory analysis.
- A confirmatory ECOG-ACRIN Phase III registration study (E2112) is ongoing in advanced breast cancer (NCT 02115282).

Safety & tolerability

- Entinostat has been found to be generally well tolerated, with a favorable benefit–risk profile, both as a single agent and in combination regimens.
- The most common grade ≥3 adverse events associated with entinostat include hypophosphatemia, anemia and fatigue in patients with solid tumors. In hematologic malignancies the most common were hematologic.

Drug interactions

- No significant drug–drug interactions have been reported.

Dosage & administration

- Entinostat is available in 1 and 5 mg tablets. The drug exhibits a food effect and should be administered on an empty stomach, at least 1 h before or 2 h after a meal.
- Clinical studies have explored a variety of dosing schedules. Due to the long half-life (approximately 100 h), it is recommended that entinostat be administered orally once per week or every 2 weeks.
- A 5 mg once weekly dose is being investigated in combination with exemestane in the Phase III E2112 study (NCT02115282).

of cancer-associated genes which leads to inhibition of cell proliferation, terminal differentiation and apoptosis. Entinostat has a long half-life and thus is administered weekly in clinical trials.

The potential for the HDAC inhibitors to overcome resistance to endocrine therapy in advanced breast cancer patients is of great interest to the breast cancer community, but requires confirmatory trials before any transition to the clinic. That entinostat has been designated as a 'breakthrough therapy' by the FDA is extremely encouraging. The ECOG-ACRIN Phase III registration study (E2112) is ongoing in advanced breast cancer (NCT02115282) and aims to confirm the OS advantage observed with the combination of exemestane and entinostat/placebo in the Phase II setting.

Combination strategies incorporating immune checkpoint agents are also ongoing and hope to improve the efficacy of immune therapy in solid tumors and breast cancer, without increasing toxicity profiles. It is key, however, that robust biomarkers of response to novel agent are developed which can predict which patients may benefit from these treatments. In this era of personalized medicine, investigators should continue to collaborate together to design preclinical experiments and clinical trials such that the right patients may benefit from these agents in the not too distant future.

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