Entinostat for the treatment of breast cancer

Dario Trapani[1], Angela Esposito[1], Carmen Criscitiello[1], Giulia Viale[1], Luca Mazzarella[1] , Marzia Locatelli[1], Ida Minchella[1], Saverio Minucci[1,2] and Giuseppe Curigliano[1].

[1]Division of Early Drug Development, European Institute of Oncology, Via Ripamonti 435, Milan, Italy.

[2]University of Milan, Department of Oncology and Hematology, Via Festa del Perdono 7, Milan, Italy.

Corresponding author:

Giuseppe Curigliano, MD, PhD, Division of Early Drug Development,

European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy.

e-mail: giuseppe.curigliano@ieo.it.

Abstract

Introduction: Breast cancer accounts for 29% of malignant tumors. It is an heterogenous disease covering a spectrum of different molecular subtypes. Epigenetic aberrations may affect gene expression through DNA and histone proteins modifications thus promoting tumor progression and resistance to anti- tumor treatment.

Area covered: This article explores the potential role of entinostat in the treatment of breast cancer. The clinical trials evaluating entinostat are discussed, highlighting preclinical data and early-phase clinical studies results. The emerging activity of entinostat in several clinical settings is evaluated by focusing on endocrine-resistant, HER2 positive and triple-negative breast cancer with a promising activity as an immune-system boosting.

Expert opinion: Entinostat, a synthetic benzamide derivative class I histone deacetylases (HDACs) inhibitor, inhibits cell proliferation and promotes apoptosis in breast cancer. Several results from clinical trials demonstrate that the addition of an epigenetic therapy to antiestrogen therapy may be an effective approach to targeting resistance pathways in breast cancer, particularly in hormone-positive disease. Agents as entinostat may have a role in immunogenic modulation. Genetic and pharmacological inhibition studies identified HDAC as a key determinant in the reversal of carcinoma immune escape. This offers the rationale for combining HDAC inhibitors with immunotherapy, including therapeutic cancer vaccines.

Keywords: HDAC, endocrine- resistance, entinostat, epigenetic.

1. Introduction

Breast cancer is the mostly diagnosed cancer in women with 249,260 new cases expected for 2016: it accounts for 29% malignant tumors in women with a lifelong risk of 12.5% (1). Breast cancer is an heterogeneous disease covering a spectrum of subtypes with several histological and molecular features affecting prognosis (2). The comprehensive molecular "portrait" of breast cancer reveals four tumor subtypes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-overexpressing and basal-like tumors (2), each one characterized by specific genetic and epigenetic abnormalities. Interestingly, these molecular defined subgroups may recapitulate the four main breast cancer intrinsic subtypes as defined in clinical practice through immunohistochemistry assays based on estrogen (ER) and progesterone receptor (PgR), together with HER2- testing and ki-67 as index of proliferation (3, 4).

Overview of the epigenetic landscapes of cancer

The mechanisms of resistance in breast cancer is the result of a clonal evolution. Furthermore, genome instability and intratumor heterogeneity contributes to drug resistance and may promote the selection of resistant subclones, each one with a specific enabling mutant genotype (5). Genomic instability affects the mutational rate of the tumor through several defects: DNA base and nucleotide excision and mismatch repair, telomere maintenance, double-strand break repair, DNA replication, chromosome segregation (6). The differential gene expression can be related to both genetic and epigenetic mechanisms. As a result tumor heterogeneity can be influenced by non-mutational changes affecting gene expression or, properly, by epigenetic mechanisms. These modifications can alter the DNA primary sequence and chromatin compacting regulation through nucleotide or nucleosome-protein modifications. Indeed, each tumor is characterized by a particular epigenetic profile. Neoplastic cells can display different profiles of nucleotidic methylation on cyosine- guanosine (CpG) rich regions (CpG islands): a hypo-methylated profile is associated with chromosomal instability thus impacting on tumor mutagenic rate and outcome (7). Conversely, hyper- methylation of CpG islands on gene promoter regions leads mostly to gene silencing (8). In addition, expression of tumor suppressor or activating genes can be regulated by post- translational modification of histone proteins such as methylation, acetylation, phosphorylation, ubiquitylation, sumoylation. Histone modifications have been observed in arginine, lysine and serine residues of

histone proteins (9). Histone-tails modifying enzymes govern these post-translational modifications: histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone deacetylases (HDACs) and histone demethylase (HDMTs). As a general rule, histone acetylation leads to transcriptional activation. Specifically, HDACs are collected in 3 class according to the homology with yeast homologous genes. Class I, in particular, comprises HDAC 1, 2, 3 and 8, representing the homologous genes to yeast protein RPD3 (10). The acetylation of lysine residues on histone tails neutralizes the positive charge of the ε-amino groups, which is the determinant of the interaction of histone proteins to DNA by binding to the negatively charged phosphodiester backbone, thus influencing the condensation state of DNA. Therefore, a different state of chromatin compaction can allow the access to transcription factors thus enhancing gene expression (Figure 1). Moreover, acetylated lysine can bind reader proteins through acetylated- lysine recognition domain (i.e. bromodomain) thus recruiting diverse nuclear proteins including chromatin remodeling complex proteins and co-activators of transcription. (11). HDACs remove the acetyl group from the histone tail thus enabling the chromatin to condense and restrict access of bromodomain- containing proteins to the DNA, resulting in gene expression negative regulation. Inhibition of HDAC enzymes affect cellular proliferation through the repression of key genes involved in cell-cycle progression, functioning as a cell growth inhibitor. One of the most extensively studied is the cyclin-dependent kinase (CDK) inhibitor CDKN1A / $p21^{WAF1}$ (CIP1) that has been showed to be increased in cancer cell lines treated with different HDAC- inhibitors (12). Taken together, the possibility to reverse the epigenetic aberrations that cause chemoresistance and impaired response to targeted therapy of tumor cells is called “episensitization”: this possibility of resistance-reversal is attractive as it is amenable to pharmacologic control (13). Interestingly, in-vitro experience with breast cell lines showed an “epigenetic profile” of HDACs expression and mutation. Particularly, an overexpression of HDAC1 has been described in breast and gastric malignant neoplasms (10). This may offer the rationale to target differentially HDAC isoforms as an attempt to target more precisely the different histologically- and molecularly- defined tumors.

2. Chemistry and pharmacology of entinostat

2.1 *Pharmacodynamics*. Entinostat (MS-275) is an oral synthetic benzamide-derivative capable to inhibit HDAC1 and 3 enzymes. Preliminary data showed the potential antiproliferative activity of entinostat in several cell line, including breast cancer *in-vitro* and *in-vivo* xenograft models (14). Translational experiences showed that tumor growth suppression requires 3-4 weeks of exposure to entinostat; this is in accordance with the unique mechanism of action of this drug that targets a general thus finely regulated mechanism of gene expression in order to rescue an anti-proliferative expression pattern (15).

2.2 *Pharmacokinetics*. Pharmacokinetic studies revealed a maximum plasma levels of entinostat reached within 1 h after a single dose administration and a terminal half-life value estimated between 60 and 150 h with a t-max equal to 1h (16).

2.3 *Metabolism*. A renal and biliary clearance of entinostat using a radiolabeled model in baboons and cancer patients model has been studied; a possible entero-hepatic recirculation has been supposed (17). However, liver metabolism seems to be a minor pathway of drug elimination in humans, as showed by Ryan et al. (16).

3. Safety and tolerability

The safety profile of entinostat is favorable; most frequent grade 3 and 4 adverse events are hematological such as thrombocytopenia (63%), anemia (47%), neutropenia (41%), leukopenia (10%) and non- hematological like hypokalemia (8%), and hypophosphatemia (6%) unrelated to nephrotoxicity (18).

4. Preclinical and clinical experiences for breast cancer treatment

4.1 Hormone receptor positive breast cancer

Up to 70% of breast cancer are ER positive at diagnosis; ER-positive breast cancers at initial diagnosis, however, can lose ER- expression, showing different grades of endocrine therapy resistance (19). ER-α repression is often related to an epigenetic aberration. When treated with entinostat, ER-negative breast cancer cells show an increasing response to endocrine treatment with aromatase inhibitors in a dose dependent manner, so re-sensitizing tumor cells to endocrine therapy (20). Histone modifying enzymes can mediate endocrine-resistance of breast cancer

through co-repressor proteins. Tamoxifen is an ER modulator capable of inducing a conformational change of ER that favors the recruitment of co-repressors (21). These proteins can interact with HDAC and mediate a trans-repression of ER- related genes. Accordingly, loss of ER- corepressors, due mainly to mutational events or deletion, may affect the response of tumor to endocrine-treatment, altering the compaction of chromatin; again, this same mechanism can predispose cells to the antitumor effect of HDAC- inhibitors (22). Yardley et al. (23) published a phase 2 study (ENCORE 301) of exemestane and entinostat for the treatment of ER-positive breast cancer progressing on treatment with a nonsteroidal aromatase inhibitor. The schedule adopted was entinostat 5 mg PO weekly continued until progressive disease (PD) or unacceptable toxicity. They showed a drug activity for the combination arm in term of progression free survival -PFS (HR=0.73, p=0.055) and OS (median increase of +8.29 months). Interestingly, ENCORE 301 pharmacodynamics analysis found a predictive role for the hyper-acetylation DNA profile as showed on circulating lymphocytes on liquid biopsy samples: "high acetylators" patients had a better PFS, as found in peripheral blood monocytes, B- and T- lymphocytes with a multiparameter flow cytometry assay (HR=0.32-0.50). The threshold to define the "acetylator" profile was defined as the percent change in protein lysine acetylation at cycle 1 day 15 versus the study derived median value. Thus, a role in endocrine-resistance is conceivable with a potential incorporation of entinostat in endocrine- resistant breast cancer treatment in a similar position as CDK4/6 inhibitors, PI3K/mTOR and AKT/PTEN blockers (24). An ongoing phase3 trial (25), sponsored by the national cancer institute (NCI), will be decisional for the inclusion of entinostat in the armamentarium of this clinical scenario (Table 1).

4.2 HER2- overexpressing breast cancer

About 25% of breast cancer overexpress HER2 oncoprotein and are associated with a worse prognosis than HER2- negative/ER-positive tumors not treated with a targeted therapy (26). At now, anti-HER2 treatment with monoclonal antibodies (i.e. trastuzumab, pertuzumab, TDM-1) and small molecules (lapatinib) are approved in different clinical settings with a significant improvement of the outcome compared to chemotherapy alone. However, resistances to HER2-inhibitors frequently occur, and resistance- crossing strategies are needed (27). The majority of patients under treatment with trastuzumab, despite an initial response, may develop a drug resistance within one year (28). Anti-HER2 treatment resistance can be mediated by the activation of counter-regulatory pathways such as the formation of heterotrimeric complex of HER2 with both HER3 and the insulin-like growth factor-1 (IGF1) or the activation of the PI3K-Akt-mTOR pathway (29). Huang et al reported an

enhancing activity of entinostat when combined with trastuzumab in HER2-overexpressing breast cancer. Particularly, entinostat exhibited a downregulating effect on HER2 and HER3 with a dramatic inactivation of phosphoinositide 3-kinase (PI3K)/Akt signaling, thus disrupting the two main mechanisms of cancer resistance to trastuzumab treatment (30). Moreover, this activity was confirmed in a trastuzumab- resistant cancer model in which the association of trastuzumab and entinostat exhibited a repressive activity on tumor growth with a substantial enhancement of apoptosis (31). Similarly, Lee J et al (32) reported a synergistic activity of entinostat when combined to lapatinib with a significant *in vivo* tumor shrinkage. This antitumor activity was showed to be mediated by a downregulation of phosphorylated Akt and activation of the pro-apoptotic protein Bim1. Moreover, when incorporated in a double- blockade regiment with lapatinib and trastuzumab, the antiproliferative effect of entinostat resulted to be improved in comparison with the single anti-HER2 regimen. Based on these results, a phase 1 study is ongoing for trastuzumab-resistant HER2-positive breast cancer (33): preliminary results of entinostat and lapatinib with or without trastuzumab showed that the triplet is feasible, safe and effective (34).

4.3 Triple negative breast cancer

Triple negative breast cancer subtype (TNBC) encompasses a heterogeneous group of breast malignancies defined by the lack of expression of ER, PgR and HER2. TNBC accounts for almost 15% of breast malignancies and it is associated with an intrinsic biological aggressiveness and generally with a worse prognosis. At least, six distinct molecular subtypes have been described by Lehmann et al (35), differently associated with outcome and response to neoadjuvant chemotherapy (36). However, no target therapy has been so far approved for TNBC and the treatment of choice remains the cytotoxic chemotherapy. Basal- like subtype of TNBC is characterized by a loss of the expression of E-cadherin and a transition into a mesenchymal- type cell with increasing expression of N-cadherin and vimentin; this process can properly be named “epithelial to mesenchymal transition” (EMT). Specific cell features of EMT are loss of intracellular adhesion molecules, acquisition of spindle fibroblast-like morphology and motility thus acquiring a phenotype suitable for metastatizing (37). However, mutations in CDH1 gene, encoding for E-cadherin, are rare and the main mechanism of E-cadherin gene regulation seems to be an epigenetic silencing (38). Cells that activate EMT, seems to acquire the features of tumor initiating cells (TICs), characterized by their ability to reseed a tumor even when few cells are inoculated (39). Shah et al (40) showed a reversal of EMT phenotype in a cell model of basal like TNBC exposed to entinostat with a rescue of E-cadherin expression: migration inhibition and cell morphology change was reported together with

a reduced potential to metastatize. Additionally, entinostat was able to reprogram the TIC phenotype in a basal- like TNBC cellular model: when exposed to entinostat, TNBC cells showed a normalization of CD44/CD24 surface expression together with a growth control of the primary tumor and a reduction of the ability of TNBC cancer cells to colonize distant sites (41). Merino et al reported an *ex-vivo* animal model of combination therapy of entinostat with a differentiating agent (all-trans retinoic acid, ATRA) and doxorubicin (42). In particular, entinostat was able to reverse retinoid acid receptor (RAR-β) epigenetic silencing thus enhancing cell drug sensitivity to ATRA. Additionally, entinostat appeared to reduce topoisomerase II-α and II-β expression, defining a synergistic mechanism of action with doxorubicin. According to these preclinical evidences, a phase 1 trial for solid tumors was published by Pili et al (43) with entinostat plus 13-cis retinoic acid; a preliminary evidence of prolonged stable disease was reported in heavenly pretreated patients with prostate, pancreatic and kidney cancer (43).

4.4 Immune- regulating activity for breast cancer immunotherapy

The key assumption founding the rational basis for cancer immunotherapy is the concept of immune-surveillance. Immune system, indeed, can suppress the development of cancer clones thus offering an endogenous anti-cancer activity (44, 45). The growing success of the "stimulus and co-stimulus axis" immune-checkpoint regulators have led to clinically validated treatments for a wide spectrum of neoplastic disease; truly, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD1) ligand 1 (PDL-1) blocking monoclonal antibodies have defined a new era of immunotherapy for nearly all types of cancer. When treated with a HDAC inhibitor, breast cancer cells show to be more sensitive to the killing activity of cytotoxic T-lymphocytes (CTL) (46). In particular, HDAC 1 inhibition enhances the antigen- mediated cancer cell killing through a HLA- restricted mechanism. Also, HDAC1 is involved in the epigenetic regulation of genes that participates to the antigen recognition process and co-repressors regulation (47). Moreover, HDAC influences chemokine tumor expression and is capable of interfering with anti-cancer immune response to immune checkpoint inhibitors like PD-1 blockers, resulting in a brisker T-cell intratumor infiltration and T-cell–dependent tumor regression (48). Furthermore, HDAC inhibition results in an up-regulation of PDL-1, showing that HDAC-inhibitors may synergistically boost anti- PD1/PD-L1 drugs activity (48). In an immune subset analysis from the previously cited ENCORE 301 trial (25), entinostat was confirmed to produce immunomodulatory effects in a cohort of ER- positive breast cancer patients (50). Particularly, an increase of HLA- DR-positive monocytes was observed together with a significant reduction in granulocytic and monocytic myeloid- derived

suppressor cells. Taken together, these data show an immune-stimulating switch of the association therapy with exemestane and entinostat, hence providing a rationale for immune- checkpoint inhibitor association to endocrine therapy and HDAC- modulators (Table2).

5. Competitor drugs in development

Clinical drug development of entinostat is focusing on breast cancer resistance mechanisms to endocrine therapy and to HER2-targeting agents. Therefore, clinical competitors are being developing contemporary in diverse clinical settings. Endocrine resistance in breast cancer involves different pathways of interest: ER-alpha expression levels, up-regulation of growth factors signaling pathways (HER2, IGFR1, and FGFR1), downstream activation of the mitogen-activated protein kinase (MAPK) or PI3K cascade, CDK4/6, and let-7 miRNA family dysregulation (51). Similarly, trastuzumab resistance can be determined by several aberrations that interest PI3K/ mTOR pathway, cell- cycle control, IGFR1, MAPK (52). For at least each of these anomalies, a drug is under clinical evaluation and some of them are reaching a definite position in clinical field within few months.

6. Expert opinion.

Entinostat is a promising drug under development for the treatment of breast cancer in different clinical settings. Its unique mechanism of action interferes with a general mechanism of tumorigenesis, involving several and different oncogenic pathways that promote and sustain tumor progression. Conversely, entinostat targets class I HDACs (HDAC 1 and 3) so defining a tailored mechanism of action with a specific antitumor activity, too. In particular, entinostat can be combined with targeted- therapy as a booster of clinical activity as well as in association with immune-targeting treatments in support of a stronger antitumor immunity response and incorporated in chemotherapy regimens with potential synergistic activity. In addition, entinostat has a long half- life and can be administered once weekly PO, making this medication unique compared with other HDAC “broad spectrum” modulators such as azacitidine. Even though long term side effects are not completely known, Entinostat may be used for long term and beyond progression of disease –even if contrasting data exists (58)- expecting a persistent antitumor effect: this class of epigenetic- modulator drugs may create a favorable genomic profile prone to a pro-apoptotic and immune- activating context that may act in a promiscuous manner with several combined and sequential partner-drugs. Furthermore, patients may be monitoring before, during and at progression with liquid biopsy to detect a peculiar epigenetic profile predictive of response

to entinostat on circulating cancer cells and immune- competent cells or identify emerging mutation or epigenetic aberration of resistance to HDAC modifiers. Moreover, some of this epigenetic changes (i.e. cycle 1 day 15 lysine acetylation change) may be an early predictor of response to entinostat. However, further prospective data on endocrine resistance reversal, long term toxicity and immune- related adverse events enhancing are still needed. In an optimistic scenario, entinostat will be a promiscuous and transversal drug to be prescribed for almost all breast cancer subtypes as well as in several clinical setting as a general booster of antitumor activity.

Table 1. Synoptic table of entinostat

Drug name (company name)	Entinostat (Syndax Pharmaceuticals, Inc., Waltham, MA) Other names: SNDX-275, MS-275

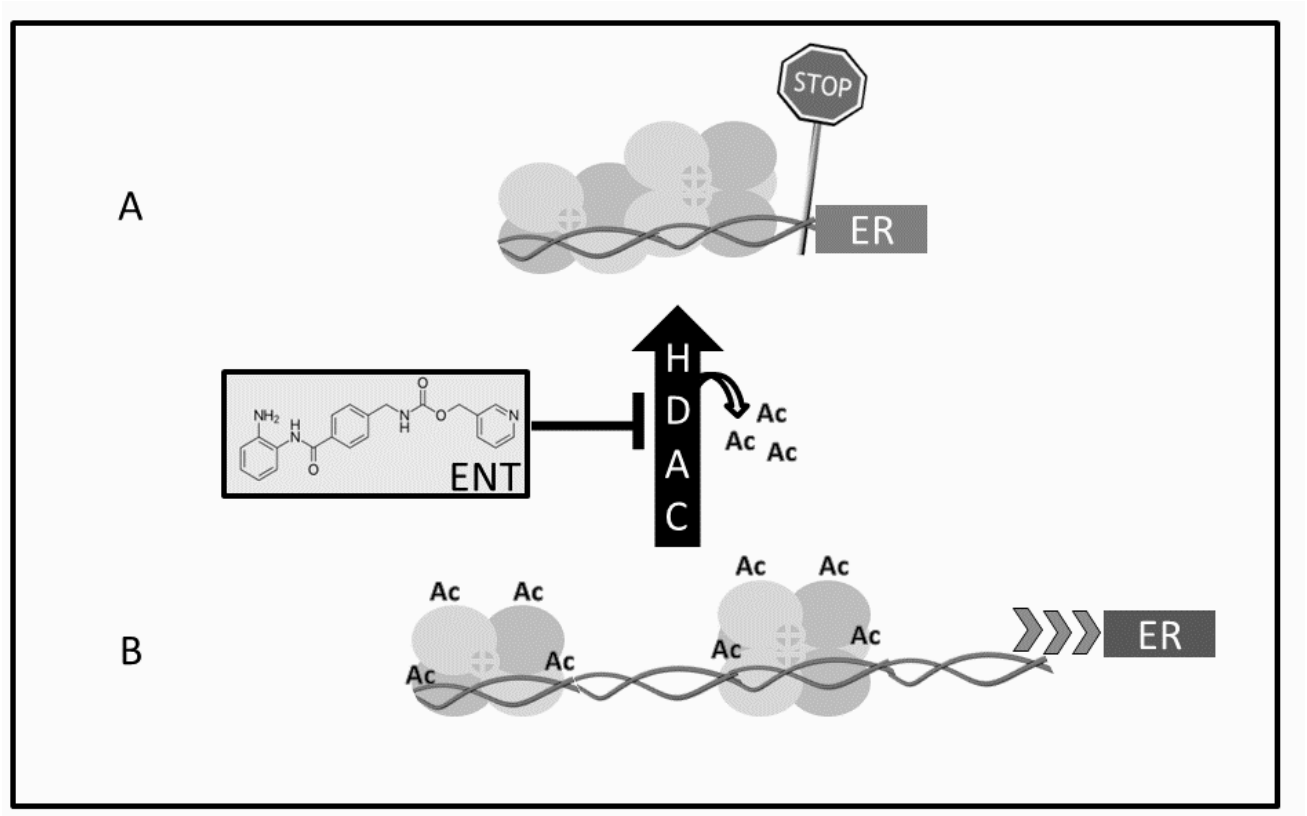
Phase	1, 2 and 3, ongoing
Indication	Hodgkin's lymphoma, leukemia, lung cancer, breast cancer, melanoma, ovarian cancer
Route of administration	Oral
Chemical structure	$C_{21}H_{20}N_4O_3$ Pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl}-carbamate
Pivotal trials ongoing: Ref	22, 24, 32, 33, 52, 53, 54, 55, 56

Table 2. Ongoing trials with entinostat for the treatment of breast cancer

Partner- Drug	**Phase**	**Disease**	**Setting**	**ClinicalTrials.gov identifier**
Exemestane Goserelin	III	HRBC	LABC/M	NCT02115282
Anastrozole	II	TNBC	Neoadjuvant	NCT01234532
Nivolumab Ipilimumab	I	HER2-negative Breast Cancer	LABC/M	NCT02453620
Azacitidine	II	HRBC, TNBC, HER2-positive	LABC/M	NCT01349959
Exemestane	I	HRBC	LABC/M*	NCT02833155
Atezolizumab	Ib	TNBC	LABC/M	NCT02708680
Pembrolizumab	I	HRBC, TNBC, HER2-positive	LABC/M	NCT02909452
Trastuzumab Lapatinib	I	HER2- positive	LABC/M	NCT01434303

*BC, breast cancer. HRBC, estrogen and/or progesterone receptor- positive BC. TNBC, triple negative BC. HER2, human epidermal growth factor receptor 2. LABC/M, locally advanced or metastatic breast cancer (palliative setting). *Only Chinese postmenopausal patients* (Last update October 2016).

Figure 1. Epigenetics modifications are involved in endocrine-resistance of breast cancer



The acetylation of lysine residues on histone tails determines a different state of chromatin condensation thus allowing access to transcription factors and possibly, but not only, enhancing gene expression. Aberrations in the acetylation pattern of cancer cells DNA can repress the expression of estrogen receptor (A) as one of mechanism leading to antitumor activity. When treated with entinostat (B), endocrine- resistant breast cancer cells show an increasing response to the treatment with exemestane, displaying a re-sensitization to aromatase inhibitors. Similar regulatory mechanisms of gene expression are involved in the re- sensitization to trastuzumab in breast cancer progressing to anti-HER2 treatment as well as in the immune- modulatory effects of entinostat (see the text for more details). *HDAC, Histone deacetylase 1 and 3. ENT, Entinostat. ER, oestrogen receptor.*

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