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Mini-review

Biomarkers for predicting clinical responses to HDAC inhibitors

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ABSTRACT

Post-translational modifications of histone and non-histone proteins by acetylation are known to play a key role in tumourigenesis. Pharmacological manipulation of acetylation has been possible with the identification of small molecule inhibitors of histone deacetylases (HDAC), the enzymes responsible for deacetylating lysine residues. An explosion of drug discovery efforts in recent years has led to the development of an extensive group of HDAC inhibitors, many of which have been shown pre-clinically to have potent anti-tumour activity. Clinical trials using these agents are now underway, with Vorinostat (suberoylanilide hydroxamic acid) having been approved by the FDA for treating cutaneous T-cell lymphoma (CTCL) in patients with progressive, persistent or recurrent disease. This review discusses how biomarkers are being identified and used to expand our knowledge of the mechanisms by which HDAC inhibitors exhibit their anti-cancer effects. In the longer term, biomarkers will provide a means towards achieving patient stratification in tumour types that will respond favourably to HDAC inhibitors.

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1. Introduction

Drug discovery and development in cancer research has re-invented itself in recent years with the goal of developing mechanism-based agents that allow targeted cancer therapy. The requirement for these novel agents is to possess increased potency, improved tumour selectivity and reduced toxicities compared to classic cytotoxics, with the promise to prolong survival and improve the quality of life of patients. Targeting specific molecules and cellular pathways has been possible due to the greatly improved understanding of biological processes. It is now clear that therapeutic intervention no longer has to target cellular proliferation alone, although this remains vital, to exert an anti-tumour effect. Cancer is characterised by six hallmarks; self-sufficiency in proliferative growth signals, insensitivity to growth inhibitory and differentiation signals, evasion of apoptosis, acquisition of limitless replicative potential, induction of angiogenesis and induction of invasion and metastasis [1]. Many cellular targets are

involved in each of these processes, which may provide candidates for developing anti-cancer drugs against. Examples of clinically successful mechanism-based anti-cancer agents that target these pathways include Gleevec (imatinib mesylate) which regulates the tyrosine kinase activity of the BCR/ABL fusion protein in chronic myeloid leukaemia and the proteasome inhibitor, Velcade (bortezomib) for treatment of multiple myeloma [2]. Further breakthroughs have also been made with monoclonal antibodies such as Herceptin (trastuzumab) that targets Her2/ErbB2 expressing cancers and the angiogenesis inhibitor Avastin (bevacizumab), an antibody against vascular endothelial growth factor (VEGF) [3]. Despite their successes however, these drugs are suitable for treating a small subset of patients, highlighting the importance for developing additional anti-cancer agents to treat a wider population of cancer sufferers.

Drug discovery remains a complex process consisting of many phases from target validation to clinical development. The FDA has estimated that the attrition rate for investigational new drugs entering clinical trials is 80% [4]. The key question is therefore how could cancer drug discovery be improved so that drugs are less likely to fail

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in clinical trials? One potential solution is to develop predictive biomarkers that enable responsive tumours to be identified. The FDA definition of a biomarker is a measurable molecular, cellular or genetic parameter that can indicate biological or pathological processes or a pharmacological response to a therapeutic agent [5]. Biomarkers can be used for many purposes such as, identifying the most appropriate patients, confirming modulation of drug targets and linking pharmacokinetic and pharmacodynamic end points. Importantly, biomarkers can also assist with decisions regarding the viability of a drug development programme by predicting clinical outcome (Fig. 1). This review will focus on HDAC inhibitors and address the development of biomarkers, together with their potential clinical utility.

1.1. HDAC, acetylation and cancer

One novel area for developing mechanism-based drugs has emerged through a greater understanding of the alterations in cancer cells of epigenetic regulation namely,

remodelling the way in which DNA is accessed through modifications of the chromatin-associated proteins involved in its packaging. Acetylation is a highly dynamic yet reversible process involving the transfer of an acetyl-group (from acetyl-co-enzyme A) onto the ϵ -amino groups of specific lysine residues located within histone tails and a number of non-histone proteins [6]. Two classes of enzyme, histone acetyltransferase (HAT) and histone deacetylase (HDAC), are responsible for the addition and removal of acetyl-groups, respectively, to control the cellular equilibrium of acetylation [6].

HDAC by controlling levels of gene transcription has been widely implicated in regulating a number of physiological processes including cell proliferation, differentiation and apoptosis. This occurs in the cell by HDAC enzymes mediating their effects as components of large protein complexes, and frequently in association with co-repressor proteins like Sin3A, NCoR and SMRT. HDAC interacts with and represses diverse transcription factors [7]. It is somewhat surprising therefore that genome-wide transcript profiling by microarray has indicated that a

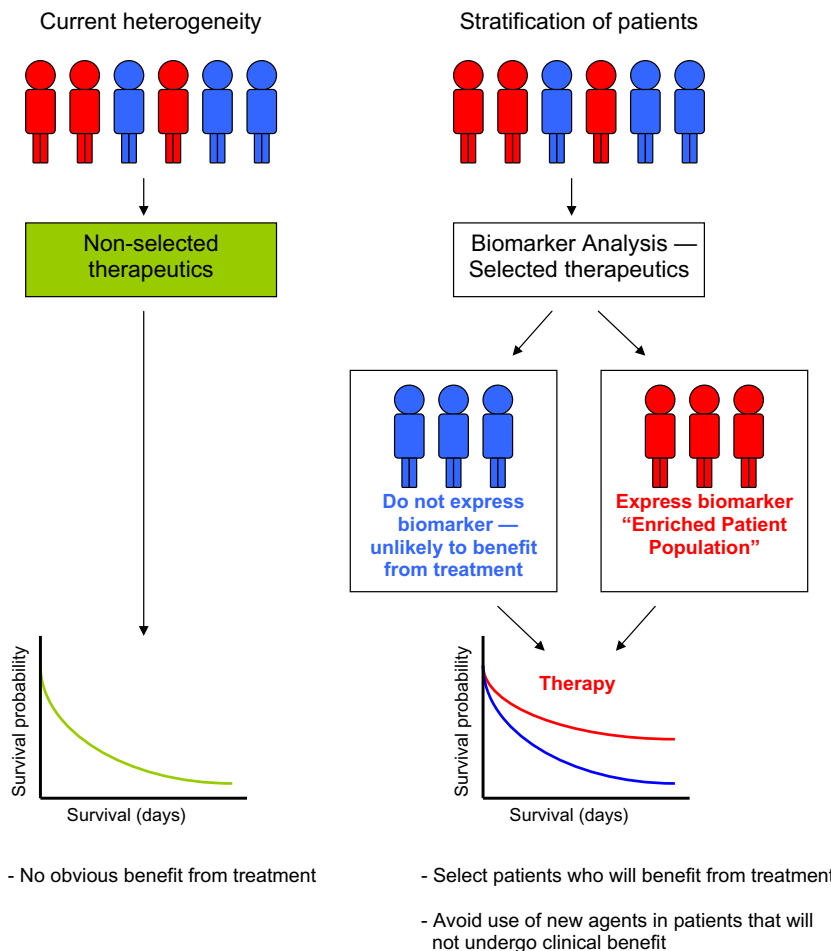


Fig. 1. Biomarker-driven clinical development. Cancer biomarkers will allow patients to be stratified into groups that either respond favourably to a specific drug treatment (red) or identify patients who should avoid a treatment where clinical benefit is unlikely (blue). (For interpretation of color in Fig.1, the reader is referred to the web version of this article.)

relatively small percentage of genes (between 2% and 5%) is influenced by HDAC inhibition [8–10]. To support the idea that HDAC activity is not restricted to the control of chromatin, it is now clear that HDACs regulate an array of non-histone proteins through controlling the level of acetylation, some with importance in tumour cell growth and proliferation (Fig. 2).

HDAC isoforms have sub-family and subunit-specific roles. Class I HDACs are ubiquitously expressed in all cells and may be more significant in regulating proliferation [11], and HDAC2 has been shown to suppress apoptosis in tumour cells [12]. Other HDAC subunits perform specific roles that frequently involve different cellular targets. HDAC6, located in the cytoplasm where it acts as an α -tubulin deacetylase, may participate in regulating cell viability in response to mis-folded proteins [13]. HDAC6 also has the capacity to bind directly to ubiquitinated proteins through a ubiquitin-binding domain, and target cargo proteins for subsequent processing [14]. Class II HDACs meanwhile have a key role in tissue specific events, in particular differentiation. HDAC9 has been implicated in cardiomyocyte differentiation as shown in HDAC9^{-/-} knockout mice, which exhibit increased cardiac growth [15]. HDAC4 acts as a repressor of chondrocyte hypertrophy through interacting with the myocyte-specific enhancer factor 2C transcription factor [16,17], and HDAC7 functions in the negative regulation and apoptosis of T-cells reflecting its interaction with the orphan nuclear receptor Nur77 [18].

1.2. HDAC inhibitors

The involvement of acetylation and HDAC function in cancer has made these enzymes appealing targets for the development of small molecule anti-cancer agents. An explosion of drug discovery programmes using a range of

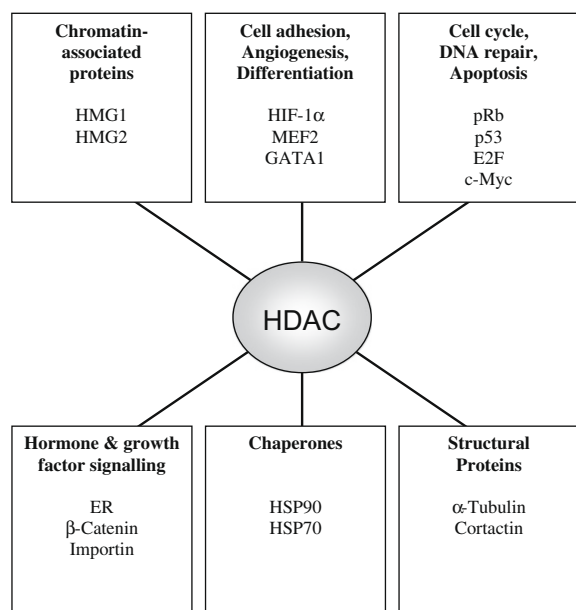


Fig. 2. Examples of non-histone proteins that are direct substrates for HDAC enzymes.

approaches including rational drug design and high-throughput screening, has led to a number of structurally distinct classes of HDAC inhibitors being identified. These include hydroxamic acids, cyclic tetrapeptides, benzamides and short chain fatty acids (Table 1). Hydroxamic acids such as SAHA, PXD101, LBH589 and ITF2537 are the largest class of HDAC inhibitors and represent some of the most potent (nanomolar) HDAC inhibitors known. They have a well-defined mechanism of action, inhibiting HDAC enzymes by mimicking the lysine side chain to enter the HDAC active site where they chelate and disable the zinc moiety [19]. All classes of HDAC inhibitor identified however have been shown to possess anti-tumour activity both *in vitro* and *in vivo* [20].

A number of clinical trials have been completed and many others are ongoing using HDAC inhibitors as single agents and in combination for the treatment of various haematological and solid malignancies with some promising early results. SAHA (Vorinostat), is the most established HDAC inhibitor, and was approved in October 2006 by the FDA for the treatment of advanced forms of cutaneous T-cell lymphoma (CTCL) that have failed multiple other systemic treatment options [21]. Significant single agent activity for FK2228 (romidepsin) has also been demonstrated in peripheral cutaneous T-cell lymphoma (PTCL) [22–24] and encouraging results have also been seen in Hodgkin lymphoma with MGCD0103 [25,26]. From the trials conducted, it is also clear that a major clinical advantage is that HDAC inhibitors are well tolerated in the majority of patients. The future of HDAC inhibitor therapies in haematological and solid malignancy is likely to lie in designing rational combination therapies with other agents that can be predicted to have synergistic or additive effects.

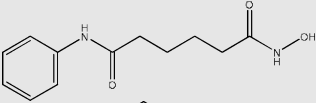
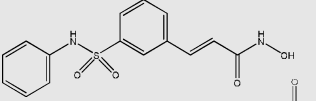
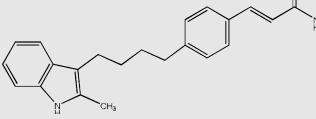
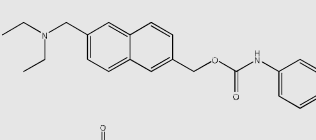
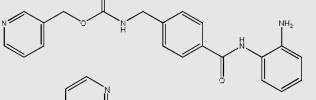
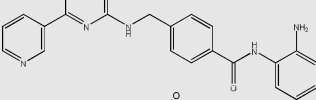
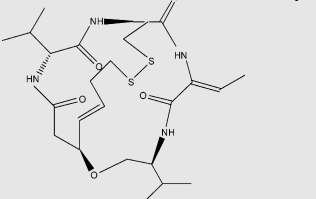
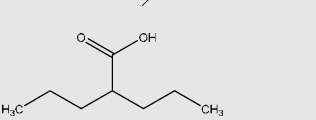
To date, HDAC inhibitors have been shown to synergise with many anti-cancer agents, including cytotoxic agents such as, gemcitabine [27], paclitaxel [28], cisplatin [29], etoposide and doxorubicin [30], as well as mechanism-based agents, including the HSP90 inhibitor 17-AAG [31], the proteasome inhibitor bortezomib [32], and the DNA methylation inhibitor 5'-azacytidine [33]. Since these drugs act via different mechanisms, HDAC inhibitors are likely to act synergistically through a multitude of pathways, such as shifting the balance of pro- and anti-apoptotic genes, inducing reactive oxygen species and inhibiting angiogenesis.

In many of the clinical trials underway, biomarkers are being assessed to elucidate how HDAC inhibitors exert their effect by increasing the understanding of their cellular mechanism of action and in turn identify tumours and stratify patients into groups that may undergo an improved clinical response to HDAC inhibitor-based therapy.

1.3. HDAC enzymes as biomarkers

HDAC enzymes themselves are intricately linked to tumorigenesis, therefore it is plausible that by measuring levels of HDAC enzyme, responsive tumour types can be identified. The easiest way to compare and contrast HDAC levels has been by immunohistochemistry (IHC), which has the benefit of allowing cell localisation and tissue distribu-

Table 1
HDAC inhibitors in clinical trials.

Chemistry	Compound	Structure	Potency	Clinical status
Hydroxamate	SAHA (Vorinostat)		nM	Approved (CTCL) Phases I, II, III
	PXD101 (Belinostat)		nM	Phases I, II
	LBH589 (Panobinostat)		nM	Phases II, III
	ITF2357		nM	Phase I
Benzamide	MS-275		μM	Phase II
	MGCD0103		μM	Phase II
Cyclic tetrapeptide	Depsipeptide (Romidepsin)		nM	Phases I, II
Short chain fatty acids	Valproic acid		mM	Phase I, II

Abbreviation: CTCL, cutaneous T-cell lymphoma.

tion to be observed. A study to examine class I HDAC isoform expression patterns was undertaken using a cohort of 140 colorectal carcinomas. Class I HDACs were found to be highly expressed in the nuclei of a considerable number of the colorectal carcinomas studied; HDAC1 (36.4%), HDAC2 (57.9%) and HDAC3 (72.9%). HDAC expression was found to be highest in proliferating, dedifferentiated tumours, which correlated with patients that had reduced survival times [34].

A similar conclusion was also made when class I HDAC expression was measured in 192 prostate carcinomas by IHC [35]. Following staining of all biopsies, the class I HDACs were strongly expressed in the majority of cases; HDAC1 (69.8%), HDAC2 (74%), HDAC3 (94.8%). High levels of HDAC1 and HDAC2 were again significantly associated with tumour dedifferentiation [35]. These observations suggest that assessing HDAC levels in patients may help identify patient sub-groups who will benefit from HDAC inhibitor treatment.

CTCL has been shown to be the malignancy most responsive to HDAC inhibitors to date [36]. In a panel of 73 CTCL biopsies HDAC1, HDAC2 and also the class II enzyme, HDAC6 were analysed along with histone H4 acetylation to show that they were of prognostic value. This was achieved by relating IHC staining to histological subtypes of CTCL and overall survival [37]. It was concluded that in CTCL, high expression of HDAC2 and histone H4 acetylation were more common in aggressive CTCL compared to indolent forms of the disease. HDAC6 expression was the only HDAC whose high expression was correlated to a favourable outcome independent of CTCL subtype.

These IHC investigations indicates that assessing HDAC levels in patients may help identify patient sub-groups who will benefit from HDAC inhibitor treatment. However, for that to be clinically possible, a more comprehensive analysis of measuring HDAC enzyme levels across a variety of tumours will be necessary as different tumours are likely to be dependent on specific HDAC enzymes.

1.4. Identifying a gene signature of tumour response to HDAC inhibitors

As HDAC inhibitors are involved in the transcriptional regulation of only a small number of genes, there is the possibility that a gene set could be identified as a marker signature of HDAC inhibitor response. One study was carried out on a group of six patients with CTCL, treated with orally administered LBH589. At 0, 4, 8 and 24 h post administration of drug, biopsies were taken from the patients and these analysed by DNA microarray. This study indicated that distinct gene expression profiles over time can be observed. In total, 23 genes showed statistical significance, including those involved in angiogenesis, apoptosis and immune regulation [38]. Of these, four genes were validated by quantitative real-time PCR, two angiogenesis related genes; guanylate cyclase 1A3 (GUCY1A3) and endothelial Tie2/Tek ligands angiopoietin-1 (ANGPT1) and two cell cycle progression genes; the transcription factor COUP-TFII (NR2F2) and cyclin D1 (CCND1).

A similar study was also carried out in a panel of 16 non-small cell lung cancer (NSCLC) cell lines. Following exposure of cell lines to the hydroxamic acids TSA or SAHA, nine genes were identified when correlated in level to drug sensitivity, in particular, three of these genes (NQO1, SEC23A and PSME2) associated with drug activity [39]. Further investigation is continuing to identify whether this nine gene signature will be able to contribute to individualised therapy in NSCLC patients and predict drug sensitivity. In another DNA microarray study, gene expression levels were also assessed with regards to drug sensitivity. Putative HDAC inhibitor biomarkers were identified and four genes, notably v-ski sarcoma viral oncogene homologue (SKI), signal transducer and activator of transcription (STAT1), thymidylate synthetase (TS) and ornithine decarboxylase (ODC1), correlated to PXD101 sensitivity [40].

The DNA microarray studies described here are a small example of the vast literature on HDAC inhibitors and DNA microarrays. In general, it may be possible to identify a gene signature for HDAC inhibitors. However, due to the multi-factorial role of these enzymes, the signature is likely to vary according to tumour type as well as duration of exposure and inhibitor concentration. The additional challenge will be to identify gene changes that can be used as a prognostic signature rather than a response signature of drug effects for future clinical trials. Nevertheless, the transcription changes that have been validated to date will prove invaluable in the quest to understand more about the mechanism of HDAC inhibitor function and could provide a basis for future biomarker studies.

1.5. Surrogate markers

The most extensively used biomarker in HDAC inhibitor trials to date has been histone acetylation, in particular H3 and H4. Preclinical and clinical studies have shown that there are several advantages of measuring histone acetylation. Firstly, histone acetylation is a direct downstream modification regulated by HDAC, which can be monitored within the tumour. Secondly, histone acetylation can be measured in peripheral blood mononuclear cells

(PBMNCs), which are often taken as a surrogate tissue for tumours where biopsies are unobtainable without invasive procedures. The induction of histone acetylation after dosing with HDAC can also be rapidly observed, in a time and concentration-dependent manner [41]. Studies performed using SAHA, also measured histone hyperacetylation in PBMNCs which was found to be rapidly induced by 2–3-fold in patients regardless of dose level or response, and returned to basal levels between drug cycles [42].

It has also been shown in mice that using PXD101 histone H4 acetylation was clearly visible at concentrations of drug known to actively cause tumour regression [41]. However, histone acetylation as a biomarker for predictive treatment outcome has been questioned and while useful as a surrogate for HDAC inhibition, does not appear to reflect tumour response. For example, in a phase I trial using PXD101, drug effects were found to be reversible and at the lowest dose levels, histone acetylation returned to basal levels within 2 h of drug infusion. At the higher doses, histone H4 acetylation was found to plateau such that at the maximum tolerated dose (MTD), further increases in histone acetylation were not observed [43].

Induction of p21 is also commonly used as a marker of HDAC inhibition. As shown by Arts et al. [44], numerous HDAC inhibitors, including hydroxamic acid compounds, R306465, SAHA and PXD101 and also the benzamide HDAC inhibitor MS-275 increased levels of p21, in a concentration-dependent manner. It has also been possible to measure proteins related to HSP90 such as HSP72, which is induced upon inhibition of HSP90 (a result of inhibiting HDAC6) and also c-Raf, an HSP90 client protein, the levels of which decrease when HSP90 is unable to function in response to cell stress [45].

1.6. Predictive biomarkers

The ultimate biomarker is one that informs on the tumour response to the cancer drug. In an attempt to identify predictive biomarkers, a genome-wide loss-of-function screen using shRNA of 8000 genes identified a group of genes that when silenced in the tumour cell prevented HDAC inhibitor-induced apoptosis [46]. One of the genes, HR23B, which shuttles ubiquitinated cargo proteins to the proteasome, has been validated as a sensitivity determinant for HDAC inhibitor-induced apoptosis. HR23B also governs tumour cell sensitivity to drugs that act directly on the proteasome and the level of HR23B was found to influence the response of tumour cells to HDAC inhibitors. In particular, HR23B was identified at high levels in CTCL *in situ*, a malignancy that responds favourably to HDAC inhibitor-based therapy. This result suggests that identifying patients with high HR23B levels would stratify them into a group that would be predicted to benefit from HDAC inhibitor therapy.

2. Conclusions

The future for mechanism-based chemotherapy is exciting, however many challenges remain. HDAC inhibitors are potent anti-proliferative agents with relatively little effect on normal tissues, which clinically are gaining increased

attention. The mechanism by which this anti-tumour activity is mediated remains unclear, although numerous mechanisms have been proposed [9]. A large number of clinical trials are continuing in both haematological and solid malignancies using a wide variety of HDAC inhibitors. While the clinical evaluation of HDAC inhibitors is ongoing, the role of biomarkers will be vital to both stratify patient and tumours into sub-groups that are responsive and likely to undergo clinical benefit, as well as enabling target modulation to be monitored. Ultimately, the use of biomarkers presents the opportunity to maximise the therapeutic advantages to the cancer patient.

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