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Review

The promises and pitfalls of epigenetic therapies in solid tumours

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ABSTRACT

Epigenetic inactivation of tumour suppressor genes, in contrast to gene mutations, can be modulated or reversed by small molecules. This has lead to several recent studies of drugs targeting epigenetic mechanisms as novel cancer therapies. So far, epigenetic therapies, including HDAC inhibitors and demethylating agents, show considerable activity in haematological malignancies, but their value in the treatment of solid tumours remains much more uncertain. This review will discuss some of the challenges that are expected in the treatment of solid tumours with epigenetic therapies and discuss approaches to overcome these obstacles. There is an increasing need for trials driven by pharmacodynamic biomarkers for these agents, which are aimed at finding the optimum biological dose rather than the maximal-tolerated dose, and also investigating their use in combination with cytotoxics – for example as chemosensitisers. Such trials already suggest that improved tumour delivery and specificity, with decreased normal tissue toxicity, will be required to take full advantage of this class of agents in solid tumours.

1. Background

Epigenetics describe a hereditable change in gene expression without a change in the DNA sequence. Abnormal gene deregulation as a result of genetic and/or epigenetic mechanisms is central to the initiation and maintenance of cancer. Since more key genes are epigenetically silenced in tumours than are genetically silenced, it has been argued that epigenetic mechanisms are the most prevalent driver of tumourigenesis.[1] Furthermore, epigenetic silencing, in contrast to gene mutations, can be modulated or reversed by small molecules.[2] This has led to an ever-increasing number of preclinical and clinical studies of epigenetic therapies. Such therapies have shown a considerable activity in haematological malignancies, but their value in the treatment of solid tumours remains much more uncertain.

One of the most widely studied epigenetic changes is DNA methylation, which occurs in mammalian DNA at CpG dinucleotides, where the hydrogen bond at the fifth position of cytosine becomes methylated.[3] DNA methylation is catalysed by a group of enzymes called the DNA methyl transferases (DNMT's) (Fig. 1). CpG dinucleotides are under represented in the genome; however, there are CpG rich regions (CpG islands) that generally remain unmethylated, and are located in the promoter or first exon regions of approximately 60% of genes. Aberrant methylation of CpG islands occurs in all tumour types and is strongly correlated to transcriptional gene silencing and epigenetic maintenance of the silenced

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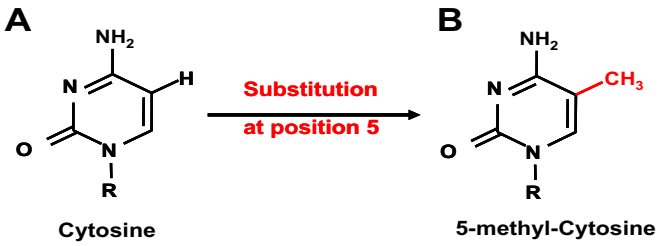

Fig. 1 – Chemical modification of cytosine methylation: (A) the chemical structure of the base cytosine and (B) the chemical structure of 5-methylcytosine following enzymatic transfer of a methyl (CH_3) group.

state.[4] Two examples of physiological roles for methylation are the silencing of the second X chromosome in females (so they do not have twice as much expression of genes), and the silencing of potentially harmful viral sequences.[5] CpG islands at gene transcription start site are usually unmethylated, while other CpG sites in coding sequence are relatively more methylated. In aging and cancer, these contrasting states of methylation tend to reverse,[6–10] with coding CpG sites becoming hypomethylated, while certain CpG islands become hypermethylated (Fig. 2).

DNA methylation at CpG islands is associated with chromatin being in a repressive state for gene transcription. Nucleosomes form the basic repeating unit of chromatin and consist of DNA wrapped around a histone octomer that is formed by four histone partners. In general, condensed chromatin (heterochromatin) mediates transcriptional repression, whereas transcriptionally active genes are in areas of open chromatin (euchromatin). Extending out of the nucleosome are charged amino-terminal histone tails, which are subjected to post-translational modification such as acetylation, phosphorylation and methylation. As an example, the histone position H3-K9 is a site of both acetylation and methylation. Deacetylation of H3-K9 is required for methylation to occur, which is then a repressive epigenetic mark. Trimethylation of H3-K9 results in the recruitment and binding of the transcriptional repressor, heterochromatin protein HP1. HP1 binding to the methylated H3 forms a positive feedback loop, mediating the propagation of heterochromatin over wide chromosomal ranges. Thus, covalent modification of the histone tails directly affects higher-order chromatin structure, and thereby offers a mechanism of epigenetic gene activation or silencing (Fig. 3).

A crucial aspect of both DNA methylation and histone acetylation is that they are reversible. Epigenetic change in gene expression is maintained during cell division and requires active maintenance of the epigenetic signature. Enzymes are required to maintain this epigenetic signature at each cell division and this can, therefore, be manipulated using small molecules that have the potential to be developed into epigenetic drugs. Those most investigated epigenetic therapies to date are the DNMT inhibitors and HDAC inhibitors.

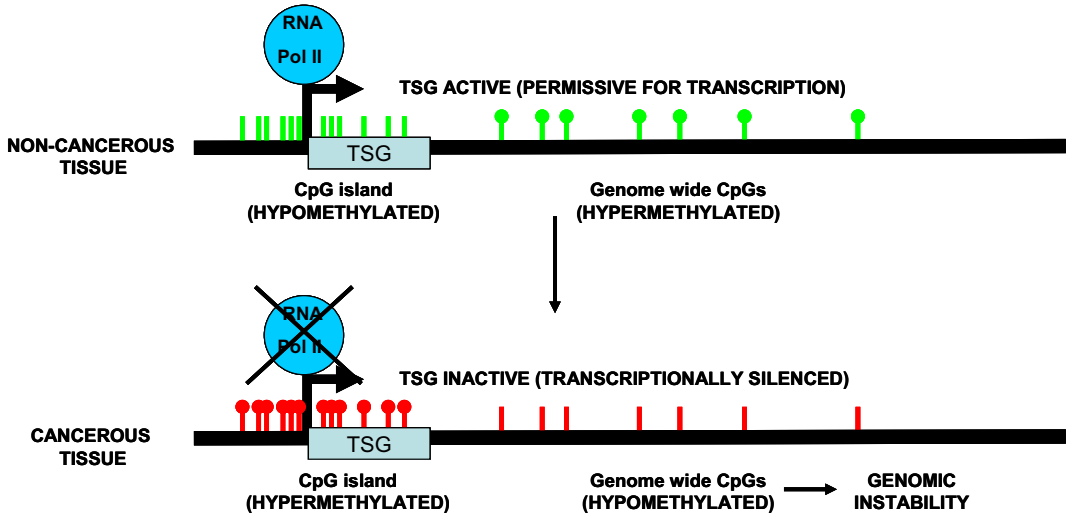

Fig. 2 – DNA methylation and cancer. A representation of a region of DNA in non-cancerous (top; green) and cancerous (bottom; red) tissues showing the differences in DNA methylation in the two phenotypes. In non-cancerous tissue, genome wide hypermethylation of CpGs (closed green circles) and an actively transcribed tumour suppressor gene (TSG) is associated with a hypomethylated CGI (green lines). In cancerous tissue, the opposite is seen with genome wide hypomethylation (red lines) leading to genomic instability, and CGI hypermethylation (closed red circles) contributing to transcriptional silencing of a TSG. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

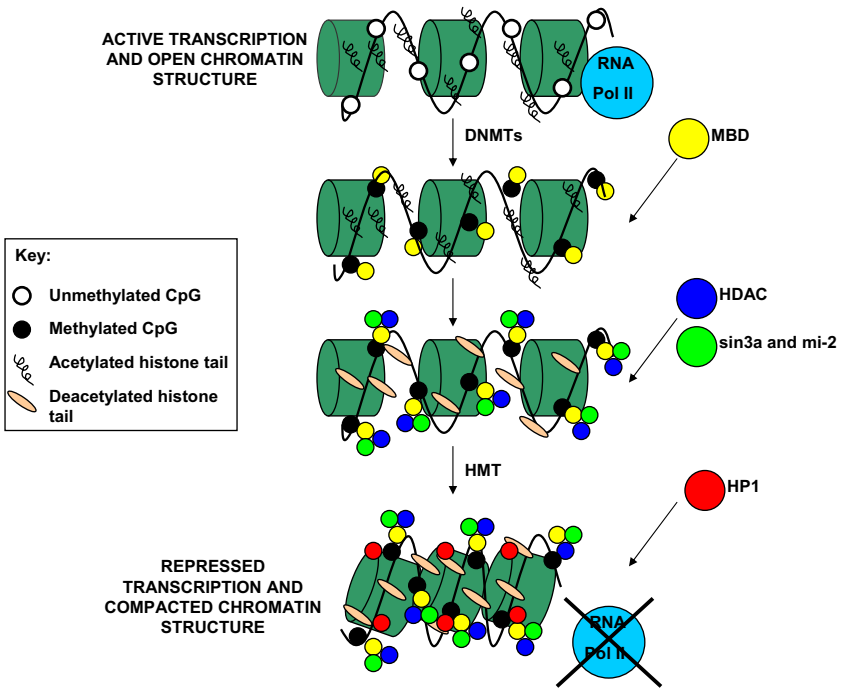

Fig. 3 – Epigenetic mechanism of transcriptional repression and chromatin remodelling. Active transcription is associated with an open chromatin structure, acetylated histones and unmethylated CpGs (white). RNA polymerase (RNA Pol II) and transcription factors can access and transcribe the gene. DNMTs methylate CpGs (black) and bind methyl binding domain, MBD, proteins (yellow). Subsequent recruitment of histone deacetylases, HDAC, (blue) and chromatin remodelling proteins, sin3a/mi-2, (green) leads to remodelling of chromatin and deacetylation of histone tails. Histone methyltransferases, HMTs, methylate lysine residues, allowing binding of heterochromatin protein 1, HP1, (red) to chromatin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. The DNMT inhibitors

2.1. *Nucleoside DNMT inhibitors*

Over 40 years ago, 5-azacytidine, a cytidine ribose nucleoside analogue, was identified as a potential cancer therapy and was subsequently shown to be a DNMT inhibitor.[11] 5-azacytidine is chemically modified to deoxyribonucleoside triphosphate and incorporated into DNA, where it is methylated by DNMT's.[12] However, the enzyme is unable to dissociate from the methylated base, and the DNA methyltransferases become trapped and inactivated as a covalent protein – DNA adduct. As a result, cellular DNA methyltransferase activity is rapidly depleted, and because of continued DNA replication, DNA is progressively demethylated. 5-aza-2′-deoxycytidine (decitabine), the deoxyribose analogue of 5-azacytidine, was subsequently developed, and since it is already in the deoxy form it can be more directly incorporated into DNA, rather than into both RNA and DNA. It was, therefore, suggested as being potentially less toxic than 5-azacytidine. Decitabine does show a greater DNA methylation inhibition and anti-tumour activity at equivalent doses in experimental models[13] but side-effects relating to bone marrow suppression, e.g. febrile neutropaenia, remain problematic. There is, therefore, a need for less toxic DNMT inhibitors. More recently, zebularine,[14] an another derivative of 5-azacytidine, has been developed as a less toxic analogue. It was designed as an oral formulation; however, its oral bioavailability is variable, with mice showing a favourable profile,[15] whilst monkeys have low levels of demethylation relative to dose.[16]

2.2. *Non-nucleoside DNMT inhibitors*

DNA methyltransferase activity can also be inhibited by non-nucleoside inhibitors that, for instance, are catalytic site inhibitors. Controversy surrounds the effect of these drugs.[17–20] Although theoretically they may have less toxicity as they are not incorporated into DNA, they also appear to be much less potent than the DNMT equivalents.[2,21] Antisense oligonucleotides targeting DNMT genes, for example MG98, appear to be effective preclinically. However, in both Phase I and Phase II trials in renal cancer, the drug has shown a lack of efficacy and non-linear pharmacokinetics – resulting in transaminitis and lack of correlation between levels of methylation and toxicity.

2.3. *Clinical trials of demethylating agents in solid tumours*

Several years ago, demethylating agents were tested in a range of solid tumours as single agents and at relatively high, maximally tolerated doses.[22,23] This resulted in few responses and significant neutropaenia. More recently, Issa and colleagues

Table 1 – Table showing examples of some of the clinical trials performed using demethylating agents in solid tumours.

Author Year Phase of study	No. pts	Demethylating agent	Cytotoxic agent	MTD[a]	Cytotoxicity	PD[b] end-points utilised	Surrogate tissues used
Appleton et al.[26] 2007 Phase I	33	6 h, d1, q28 various doses up to 135 mg/m^2	Carboplatin AUC 5 and 6 in 2 separate cohorts d8		Myelosuppression Sepsis Fatigue	5 Methyl-2′-deoxycytidine MAGE1A CGI methylation HbF	PBC Buccal cells Tumour biopsies
Schwartsmann[22] Phase I–II NCSLC	21	Decitabine 45, 67, 90, 120 mg/m^2 for2 h, d1–3, q21	Cisplatin 33 mg/m^2, d1–3, q21	120 mg/m^2	Myelosuppression mucositis		
Thibault[42] Phase II prostate	14	Decitabine 75 mg/m^2 for 1 h, 8 hourly for 3 doses, d1, q5–8 weekly				bFGF	Urine
Pohlmann et al.[27] Phase II cervical	25	Decitabine 50 mg/m^2 for d1–3, q21	Cisplatin 40 mg/m^2, d1–3, q21 initially, reduced to 30 mg/m^2 for toxicity		Febrile neutropaenia resulting in one death		
Momparler[43] Phases I–II Stage IV NSCLC	15	5-Azacytidine 200–660 mg/m^2 for 8 h			Haematological		

a MTD maximal-tolerated dose.
b PD pharmacodynamic.

have argued that these drugs may be more biologically effective in haematological malignancies at lower, less toxic doses.[24] Pharmacodynamic biomarkers can be used to identify maximal biological effect rather than maximum-tolerated dose based on the levels of DNA demethylation in surrogate and tumour tissues. Pharmacodynamic responses measured at various times following treatment can also be used to plan optimal scheduling of these drugs in combination with cytotoxics. For example, it has been suggested from the animal studies[25] that demethylating agents may reverse acquired drug resistance and sensitise tumours to chemotherapeutics such as carboplatin. The mechanistic basis for this sensitisation has been suggested to be re-expression of pro-apoptotic genes silenced during the acquisition of drug resistance. However, demethylation and hence reactivation of gene happen several days after the administration of a demethylating agent and, therefore, correct scheduling to get maximal reversal of epigenetic silencing at the time of treatment with cytotoxic becomes crucial.

Appleton et al. have reported the results of a phase I trial of carboplatin and decitabine in patients with solid tumours, and a randomised phase II study of this combination in relapsed ovarian cancer is currently underway. In the phase I study, 33 patients received escalating doses of decitabine administered as a 6-h infusion on day 1 followed by carboplatin on day 8. This schedule was chosen based on the previous xenograft studies.[25] The major toxicity was myelosuppression. Decitabine induced dose-dependent, reversible demethylation in peripheral-blood cells (PBCs) maximally at day 10. Furthermore, 90 mg/m^2 of decitabine induced demethylation of the MAGE1A CpG island in PBCs, buccal cells, and tumour biopsies, as well as elevation of epigenetically regulated HbF expression. They concluded that decitabine could be combined safely with carboplatin at a dose and schedule that caused epigenetic changes equivalent to or greater than that observed in mice with carboplatin-sensitised xenografts. They recommended a dose/schedule for the phase II trial of decitabine 90 mg/m^2 (day 1) followed by carboplatin AUC 6 (day 8) every 28 days[26] (see Table 1).

Pohlmann et al. described a phase II trial, in which cisplatin and decitabine were used as first-line therapy in patients with recurrent and/or metastatic squamous cancer of the cervix. Decitabine was given at a dose of 50 mg/m^2 for three consecutive days every 21 days. The cisplatin was reduced to 30 mg/m^2 because of toxicity. Twenty one of 25 were patients evaluated for tumour response, 38.1% achieved a partial response, and stable disease was documented in 23.8%. However, 68.0% of patients had CTC Grade III or IV neutropaenia. As this regimen produced very encouraging responses but resulted in a significant haematologic toxicity, the authors felt that they should go forward into a larger trial with growth factor support.[27]

If these agents are to progress within the solid tumour setting, it will be important to address the high incidence of neutropaenia, especially if these drugs are to be used in combination as chemosensitisers with myelosuppressive cytotoxics. If the patient is so profoundly neutropaenic from their drug-sensitising DNMTi that they cannot get a clinically effective dose of cytotoxic in an appropriate time-frame, then

this has clear implications for the feasibility of such combinations.

3. Issues to be considered when designing clinical trials involving demethylating agents

Since nucleoside demethylating agents are S-phase specific, requiring incorporation into DNA and since current agents have short half lives, they may need to be given as repeated daily treatments or as prolonged infusions, rather than as single short duration treatments, in order to ensure that the majority of tumour cells would have passed through S-phase while drug is present. This could have various implications for both patients and staffs. More frequent visits made by patients to hospital have an impact on quality of life and cost implications and a balance needs to be found between dose, schedule and patient convenience. An oral formulation may have advantages in this context. In terms of maintaining demethylation and preventing remethylation of DNA, a more protracted lower dose demethylating regimes may be preferable. However, we do not know what effect prolonged demethylation and epigenetic activation has on other non-cancer-related genes, especially in the normal tissues. Some investigators have raised the concern that the longer term use of demethylating agents could in itself be tumourogenic,[28,29] although both chemopreventive and tumour promoter effects of reduced DNMT expression have been observed in mice.[30]

Variation in drug metabolism in patients could influence the response of patients to DNMT inhibitors and could limit their anti-tumour effectiveness. For instance, cytosine deamination of 5-aza-cytidine and 2-deoxy-5-azacytidine leads to rapid inactivation converting each of them into the respective 5-azauridine compound.[31] There is an inter-patient variability in the level of deamination, and this may influence response to treatment. Recently, Yoo et al. investigated the inhibition of DNMT using short oligonucleotides containing an azapyrimidine. One of these compounds, S110 a 5-AzapG-3 dinucleotide, was protected from deamination by cytidine deaminase, but released 2-deoxy-5-azacytidine in cells.[31] Resistance to cytosine deamination may allow lower doses of DNMT inhibitor to be given with the same effect on demethylation in tumour cells, which could ameliorate some of the myelotoxicity. Further, decitabine has to be triphosphorylated through kinase activity for the nucleotide to be incorporated into DNA; thus there is further potential for inter-patient variability since the levels of kinase expression may affect the response to these agents.

Tumour cells need to be replicating in order for these nucleoside DNMT inhibitors to be incorporated into DNA and for the demethylation to occur. This may be different in different tumour types and it may be that a marker of proliferation such as ki67 would be a useful predictor of benefit. Furthermore, non-replicating subpopulations of cells may be resistant to such agents and stimulating these subpopulations into proliferation may be necessary for these agents to be effective. It has been suggested that subpopulations of cells with stem cell like properties exist, which are generally non-replicating and may be particularly difficult populations of cells to treat with nucleoside DNMT inhibitors. Agents that induce tumour stem cells to replicate or treating tumours at a time when stem cells are maximally replicating may be important for optimal use of DNMT inhibitors.

Table 2 – Types of HDAC inhibitor according to chemical structure.

Chemical structure	Drug name
Hydroxamic acids	SAHA/vorinostat
	PXD101/belinostat
Cyclic tetrapeptides	FK228 (depsipeptide)
Benzamides	MS275 (HDAC Class I selective)
	CI994 (HDAC Class I selective)
	MGCD0103 (HDAC Class I selective)
Electrophilic ketones	2-Trifluoroacetylthiophenes (HDAC Class II selective)
Aliphatic acid group compounds	Phenylbutyrate
	Valproic acid

4. The HDAC inhibitors (HDI's)

HDI's are classified according to their chemical structure, and this is illustrated in Table 2 below. Many of these drugs have been used for years in the treatment of epilepsy and as mood stabilisers but more recently a role for them in the treatment of haematological and solid tumours has been established. There are four classes of HDAC's, based on the homology with yeast and most HDI's target Classes I and II. Each class contains multiple HDAC's, e.g. Class I contains HDAC 1–3, 8, and Class II contains HDAC 4–7, 9 and so an individual HDI is likely to target multiple HDAC's. Some investigators propose that this is the optimal way for these drugs to be efficacious, whereas others hypothesise that more specific drugs targeting individual HDAC's would be more effective and have less unwanted effects.[32]

5. Clinical trials involving HDAC inhibitors

Impressive results in clinical trials of vorinostat have been seen in cutaneous T cell lymphoma with partial responses of around 30% in patients with advanced disease, who often had multiple lines of previous chemotherapy.[33,34] However, the efficacy of these agents in solid tumours has been more mixed with some trials showing encouraging results, whilst others have been more disappointing.

5.1. *Single agent studies*

Steele et al. recently reported the results of phase 1 of PXD101 (Belinostat) in patients with solid tumours. Belinostat was administered from days 1 to 5 in a 21-day cycle. Pharmacodynamic measurements included acetylation of histones extracted from peripheral-blood mononuclear cells, caspase-dependent cleavage of cytokeratin-18 and interleukin-6 levels. Forty-six patients received belinostat at one of the six dose levels (150–1200 mg/m^2/d). The maximum-tolerated dose was 1000 mg/m^2/d intravenous (i.v.). Histone H4 hyperacetylation was observed after each infusion and was sustained for 4–24 h in a dose-dependent manner. Increases in interleukin-6

levels were detected following belinostat treatment. Stable disease was observed in around 40% of patients, and this was associated with caspase-dependent cleavage of cytokeratin-18.

Blumenschein et al. reported a small phase II in recurrent head and neck cancer in 2008.[33] They assessed the efficacy and safety of 400 mg once daily oral vorinostat in patients with recurrent and/or metastatic head and neck cancer. Twelve patients were evaluated for response. The majority had had previous chemotherapy and radiotherapy. One unconfirmed partial response was seen and three patients had stable disease.[33]

5.2. *Studies in combination with cytotoxics*

Many investigators feel that HDAC inhibitors are more likely to be effective in solid tumours if used in combination with cytotoxics. Additive or synergistic activity with conventional anticancer agents has been shown in various models. One explanation for synergistic activity is that an increase in histone acetylation produces a more open DNA conformation which makes chromatin more permissive for gene transcription and hence re-expression of silenced genes. This could lead to increased expression of genes involved in apoptosis, including re-expression of pro-apoptotic genes and hence chemosensitisation.[4] For example, hydroxamate HDAC inhibitors enhance the activity of carboplatin, docetaxel and paclitaxel in ovarian cancer cells[35] and show synergistic activity with paclitaxel and additive activity with carboplatin in serous papillary endometrial cancer cells. However, pre treatment of tumour cells with HDAC inhibitors may also cause increase initial damage and an increased DNA damage response.[36] This could be as a result of a more open chromatin state resulting in greater DNA damage being induced following treatment with DNA damaging therapies, such as ionising radiation and platinum-based chemotherapy.

Ramalingam has investigated vorinostat (SAHA) in the phase 1 setting, combined with standard doses of carboplatin and paclitaxel. Partial responses occurred in 11 of 25 evaluated patients (10 non-small cell lung cancer and 1 head and neck cancer), and stable disease occurred in 7 patients. Febrile neutropaenia was seen. Munster et al. treated patients with increasing doses of valproic acid (VPA) days 1–3 up to 160 mg/m^2/d, followed by epirubicin day 3 in 3-week cycles. Fourty-four patients received at least one cycle of therapy. Partial responses were seen in 22% of patients, and stable disease/minor responses were seen in 39%, despite a median number of three prior regimens. The maximum-tolerated dose and recommended phase II dose were VPA 140 mg/kg/d for 48 h followed by epirubicin 100 mg/m^2. It should be remembered that these patients were heavily pretreated and historically anthracycline resistant.[37]

There are various issues with HDAC inhibitors which are need to be addressed if they are to be used successfully in the solid tumour setting. Firstly, many have a short pharmacokinetic half life – although they do appear to have a reasonably long pharmacodynamic half life as measured for instance by histone acetylation of PBMCs. Secondly, there remains some uncertainty whether histone acetylation really is the chemotherapeutic target and hence, how their effect is mediated. It has been well documented that they have additional acetylated protein targets in the cell, which could affect cell death pathways.[38] Establishing the underlying mechanism for the success of these agents in cutaneous T cell lymphoma, and whether this is relevant to other tumours, may help identify the types of tumours likely to respond to HDAC chemotherapy.

In terms of predicting resistance to these agents, recent work by Richon has shown that persistent activation of STAT1, STAT3 and STAT5 correlates with resistance to vorinostat in lymphoma cell lines.[39] Simultaneous treatment with a pan-Janus-activated kinase inhibitor resulted in synergistic antiproliferative effect and down-regulation of the expression of several antiapoptotic genes. Immunohistochemical analysis of STAT1 and phosphorylated tyrosine STAT3 (pSTAT3) in skin biopsies obtained from CTCL patients enrolled in the vorinostat phase IIb trial showed that nuclear accumulation of STAT1 and high levels of nuclear pSTAT3 in malignant T cells correlate with a lack of clinical response. These results suggest that deregulation of STAT activity plays a role in vorinostat resistance in CTCL, and strategies that block this pathway may improve vorinostat response. It was felt that this could be of prognostic value in predicting the response of CTCL patients to vorinostat.[39]

HDAC inhibitors are generally well tolerated, although dose-dependent toxicities that have been consistently observed include fatigue. Cardiac toxicity is also a consistent feature, with asymptomatic T wave changes being a frequent finding. Significant dysrhythmias, however, are seen only rarely. This T wave flattening has been observed with all the histone deacetylase inhibitors studied at their centre, and despite the different chemical properties of these drugs suggesting a drug class effect.

6. How can epigenetic therapies be optimised for the treatment of solid tumours?

Both demethylating agents and HDACis are now licensed for the treatment of myelodysplastic syndrome and cutaneous T cell lymphoma, respectively. However, they do not yet form part of the treatment of solid tumours. Solid tumours tend to have a lower number of proliferating cells than leukaemias and since epigenetic therapies such as decitabine require DNA replication for their mechanism of action they may be less effective in solid tumours. There are also underlying biological differences in gene regulation between solid and haematological malignancies.

Drug delivery and targeting need to be improved in order to maximise the epigenetic effects whilst decreasing non-specific toxicity. Drugs such as S110 that are less prone to cytosine deamination hold promise; and similarly, drug combinations that have likely synergy without overlapping side-effect profiles. It is also possible that epigenetic therapies may target a different cell population from conventional cytotoxic chemotherapy. Thus, it has been suggested that tumours may relapse due to failure of cytotoxic chemotherapy to eradicate tumour stem or sustaining cell populations.[40] Given the unique epigenetic regulation present in normal stem

Table 3 – Types of clinical approach that could be used include.

Approach	Rationale
Single agent	Switch on a particular TSG fundamental to a cancer
Chemosensitiser	Give prior to a radiotherapy or chemotherapy in order to make treatment more effective, e.g. DNA damaging HDAC inhibitor prior to platinum or XRT.
Maintenance	Give following a course of chemo or XRT to prevent relapse, e.g. a demethylating or HDAC after chemo
Prophylaxis	Give to patients at high risk of developing cancer. Drug would need to be given oral with a favourable toxicity profile and no long-term detrimental effects

cells,[41] it is possible that tumour stem cells may have an increased sensitivity to epigenetic therapies.

As cancer results from a combination of epigenetic and genetic aberrations, it is anticipated that having both 'epigenetic' and 'genetic' therapy/standard cytotoxics available will be helpful. It is not often possible or appropriate to give chemotherapy for a protracted period, but if the dose and schedule of epigenetic treatments can be optimised so as to avoid excessive neutropaenia for example, then there is the possibility of using these agents as maintenance therapies or sensitising agents prior to the standard chemotherapy – or even as prophylactic treatments in patients at high risk of developing malignancy (see Table 3). As these various approaches are being pursued, careful consideration would need to be given to the potential benefit as well as the drawbacks.

7. Conclusion

Epigenetic therapies are finding their place within the haematological arena with high response rates in conditions that were difficult to be treated previously, e.g. Cutaneous T Cell Lymphoma. In order to achieve a similar efficacy in solid tumours due attention is need to be paid to adequate preclinical animal models, which validate biological end-points prior to well-considered clinical trials powered to answer the *right* question in the *right* patient with the *right* drug at the *right* dose and schedule. Such trials (involving both single agent and combination approaches) could include so-called 'window studies' as well as randomised Phase II trials in appropriate situations.

Conflict of interest statement

None declared.

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