

Is there a role for epigenetic enhancement of immunomodulatory approaches to cancer treatment?

Kirsty J Flower¹, Sadaf Ghaem-Maghani^{1,2}, Robert Brown^{1,3}

¹ Department of Surgery and Cancer, Imperial College London, UK

² West London Gynaecology Cancer Centre, Imperial College NHS Trust, London, UK

³ Section of Molecular Pathology, Institute for Cancer Research, Sutton, UK

Abstract

The efficacy of cancer immunotherapy relies on the ability of the host immune system to recognise the cancer as non-self and eliminate it from the body. Whilst this is an extremely fertile area of medical research, with positive clinical trials showing durable responses, attention must be paid to the subset of patients that do not respond to these treatments. Immune surveillance and immunoediting by the host could itself select for immune-evasive tumour cells during tumour development leading to immunotherapy resistance. One such mechanism of non-efficacy or resistance is the epigenetic silencing of a specific gene required in the immunotherapy response pathway. Epigenetics is the study of the control of expression patterns in a cell via mechanisms not involving a change in DNA sequence. All tumour types show aberrant epigenetic regulation of genes involved in all the hallmarks of cancer, including immunomodulation. Inhibition of key enzymes involved in maintenance of epigenetic states is another important area of research for new treatment strategies for cancer. Could epigenetic therapies be used to successfully enhance the action of immunomodulatory agents in cancer, and are they acting in the way we imagine? An understanding of the effects of epigenetic therapies on immunological pathways in both the tumour and host cells, especially the tumour microenvironment, will be essential to further develop such combination approaches.

Cancer immunotherapy

Attitudes towards cancer immunotherapy have fluctuated dramatically over time. The history of attempting to harness the body's own arsenal to reverse the uncontrollable cell proliferation inherent in cancer dates back to the 1800s, when Coley's toxin [1, 2] was first used in an attempt to provoke an immune response that simultaneously targeted the neoplastic growth, with varying degrees of success. However, the ascent of radiotherapy and chemotherapy, as well as the advent of genomics, leading to the popular view of cancer as a disease of the genome, has led to reduced priority of immunotherapeutic cancer treatments. In the last two decades however, there has been an explosion of immunotherapy successfully modulating the progress of cancer, from the FDA approval of multiple monoclonal antibodies used in a plethora of malignancies [3] to the development of adoptive T cell strategies, an example of the "personalised medicine" approach [4]. This was underscored by the addition of evasion of immune response to the hallmarks of cancer [5].

To understand how tumours evade the immune system, we must first understand how the immune system targets and kills precancerous cells in the process of immune surveillance. Both the innate and adaptive immune responses play important roles in the recognition of malignant cells [6].

Engineered mouse models lacking various components of the immune system have been shown to readily develop tumours when compared to their immunocompetent counterparts (reviewed in [7]), emphasising the importance of each part of the immune system.

The presence of Tumour Associated Macrophages (TAMs) within the tumour microenvironment has largely been seen as a pro-tumourigenic mechanism. They appear to activate and maintain the chronic inflammation process, enhancing invasion, angiogenesis and cancer cell proliferation [8]. However, as part of the innate immune system, macrophages have been shown to exist in two polarised states, with the M1 state acting to attract and activate CD4⁺ T helper cells, and therefore activate adaptive immune pathways [9]. The observed TAMs appear to be more like the M2 polarisation, linked with immune suppressive mechanisms, such as recruitment of CD4⁺ CD25⁺ regulator T cells (Tregs) which function to deactivate cytotoxic T cells [10].

Neutrophils are the most abundant white blood cell and part of the innate immune system. Their identification as Tumour Associated Neutrophils (TANs) highlights their pro-tumourigenic abilities, for example the production of reactive oxygen species (ROS), which can lead to DNA damage and mutation [11, 12]. However, they have also been shown to mirror the plasticity of macrophages and form an anti-tumourigenic state, with immunostimulatory characteristics and cancer cell specific cytotoxic action [13].

The main mechanism of tumour cell killing is via CD8⁺ cytotoxic T-cells. These cells recognise antigens presented by the major histocompatibility complex (MHC) I from affected cells. The T cell receptor specifically recognises and binds to the presented antigen. For the T cell to be activated, a second signal is required, for example the antigenic independent co-stimulatory interaction of CD28 on the T cell with B7 on an antigen presenting cell (APC) resulting in increased T cell expansion, production of cytokines and downstream effects thereof. In the absence of a second signal, T cells are not efficiently activated in response to that antigen [14].

There are two key T cell receptors that negatively regulate T cell activation, CTLA4 (cytotoxic T-lymphocyte associated antigen -4) and PD-1 (Programmed cell Death molecule 1). CTLA4 is upregulated on T cells after activation and competes for the ligand of the stimulatory T cell surface receptor CD28, B7, and causes T cell arrest [15]. PD-1 interacts with ligands PD-L1 and PD-L2 and inhibits T cell activation by blocking kinase activity [16]. Their natural function is as a checkpoint, to control the activation of T cells to ensure that the immune response is appropriate and avoids overactivation against healthy cells [17].

Tumours can evade these immune mechanisms in a number of ways. The secretion of cytokines such as interleukins (IL) -6, -8, -10, and TGF- β from tumours has been shown to affect the activation of T cells and macrophages, and reduce successful immunosurveillance by suppressing inflammation [18]. Upregulation of the checkpoint receptor ligands has been observed, allowing the deactivation of the cytotoxic T cell response [19]. Downregulation of key proteins in the process of antigen presentation, as well as the expression of tumour associated antigens (TAAs) or cancer germline antigens (CGAs) themselves has been shown to assist immune evasion [20]. Overall the immune system has become more tolerant to tumour self-antigens, and the main challenge in effective immunology is overcoming this tolerance.

Multiple approaches have been developed in an attempt to overcome this acquired tolerance. Systemic administration of cytokines such as IFN α and IL2 have had some success, activating the immune system in a non-specific manner [21]. However this non-specificity can lead to toxicity associated side-effects and or simultaneous activation of the repressive Tregs, making it hard to predict the outcome of this therapy [22]. Another approach is to collect T cells from a patient, stimulate them *ex vivo* with a specific antigen and the appropriate cytokines to stimulate them, before transferring them back into the patient, in a process known as adoptive T cell therapy [23]. These activated T cells should be primed for activity against the specific tumour antigens of that particular patient, and mediate a successful immune response on the cancer. However it has been found that only 30-40% of biopsies contain enough T cells within their population for successful expansion, and the process is labour intensive and can take up to 6 weeks [24].

A recent breakthrough in cancer immunotherapy is the use of monoclonal antibodies against the immune checkpoints CTLA4 and PD-1. As described earlier, the activation of these receptors by their ligands leads to inactivation or death of the associated T cell, reducing the immune response. Some cancers have been shown to have an overexpression of ligands, enhancing their immune evasion. In the case of ovarian cancer, it was discovered that PD-L1, the ligand for PD-1, was overexpressed on the cell surface, and this overexpression was associated with poor prognosis [25]. Blocking these receptors with monoclonal antibodies inhibits the inactivation, maintaining an activated cytotoxic T cell state against the tumour [3].

A number of these checkpoint inhibitors have been FDA approved for use in advanced and metastasised non-small cell lung cancer and melanoma [26], and since 2012, a number of these have been awarded FDA Breakthrough Therapy designation, a status which is designed to expedite the development and review of medicines with impressive preclinical data indicating that the drug may demonstrate substantial improvement over existing therapies [27]. In the first phase III study of Ipilimumab, a CTLA4 blocking antibody, in metastatic melanoma, the survival rate at 2 years was 18%, compared with 5% in the control group [28]. A recent pooled analysis of 1861 patients from phase II and III trials confirmed the durability of response to Ipilimumab, finding 20% of patients achieving 3 years survival after treatment [29]. In non-small-cell lung cancer, an improved progression-free survival was observed in a phase II trial for Ipilimumab treatment in combination with carboplatin and paclitaxel [30] and a phase Ib trial in pancreatic cancer showed some promise in combination with a cancer vaccine approach (GVAX, allogeneic pancreatic tumour cells transfected with granulocyte macrophage colony stimulating factor (GM-CSF) gene) [31]. Tremelimumab, another CTLA4 blocking antibody, had some success in a single arm phase II trial of mesothelioma, with a proportion of patients exhibiting disease control and durable partial response [32]. Antibodies targeting PD-1 and PD-L1 have also shown highly durable response rates in large phase I studies [33-35] and continue to impress in phase III randomised trials; a trial testing nivolumab (anti-PD-1) against dacarbazine chemotherapy for advanced melanoma was stopped early due to the improved overall survival observed in the patients receiving nivolumab [36]. Another anti-PD-1 therapy, pembrolizumab has also shown impressive tumour response rates for patients with melanoma previously treated with ipilimumab [37].

Observations of initial disease progression followed by disease response in early CTLA4 trials [38] has led to the proposal of alternative response criteria, known as immune-related response criteria (irRC) [39]. As opposed to standard radiographic response criteria such as RECIST, irRC includes new

lesions to be included in total disease burden estimation and requires confirmation on a subsequent scan to define it as progressive disease. This measure was found to correlate better with overall survival [40].

Epigenetics of cancer

There are many definitions for epigenetics, but it can be broadly defined as the study of changes in organisms and tissues caused by modification of gene expression rather than alteration of the genetic code. It can be used to describe the transmission of heritable gene expression patterns through cell division [41]. The study of epigenetics encompasses several different mechanisms. DNA methylation is the addition of a methyl group to the 5th carbon of a cytosine residue in a CpG context, and is a stable mark with heritable mechanisms during cell division. The genome is mostly CpG-poor, depleted due to spontaneous deamination of methylated cytosines, and most of those cytosines that remain in these sparse regions are methylated [42]. However, regions exist that appear to have conserved a normal density of CpGs, and these regions tend to remain unmethylated, and found in promoter regions. These regions are known as CpG islands [43]. Methylation within a CpG island promoter can be associated with gene silencing, whereas methylation within the body of the gene (known as intragenic methylation, reviewed in [44]) is associated with higher expression of that gene. DNA methylation is thought to facilitate repression of transcription by preventing binding of transcription factors and therefore inhibiting the recruitment of DNA polymerases, or by recruiting histone modifiers with methyl binding domains and creating a repressive environment through another mechanism of epigenetic control, postranslational histone modifications [45, 46]. Such histone modifications, such as methylation, acetylation, and phosphorylation, are found on specific residues of the N terminal tails of histones, which are thought to change chromatin states. In combination, DNA methylation and histone modifications are thought to control accessibility to DNA and govern gene expression. A less well studied mechanism but by no means an unimportant one is the emerging role of miRNAs, which fine-tune gene expression post-transcriptionally. A host of proteins are required for the control of these mechanisms. DNA methyltransferases exist for both *de novo* generation (DNMT3a and 3b) and maintenance (DNMT1) of DNA methylation patterns across the genome, and the discovery of ten-eleven translocation (TET) proteins and their role in iterative oxidation of 5-methyl-cytosine to hydroxyl-, formyl-, and carboxyl- forms of methyl-cytosine have pointed towards a mechanism for active demethylation [47, 48]. Histone modifications are controlled by the reciprocal action of enzyme pairs for each modification, with multiple examples of each type; histone methyltransferases (HMTs) and histone demethylases (HDMs) control methylation on lysine and arginine, histone acetyl transferases (HATs) and histone deacetylases (HDACs) control acetylation of lysine residues, whilst kinases and phosphorylases control the phosphorylation at serine residues.

In the context of cancer, epigenetic regulation has been known to be disrupted for over two decades. Widespread hypomethylation was first reported in human cancer samples compared to normal samples in 1983 [49-51], and localised hypermethylation has been established to be associated with transcriptional repression in several tumour suppressor genes; VHL in renal cancer [52], MLH1 in colorectal, endometrial and gastric cancer [53], and RASSF1A in a multitude of cancers [54] are but a few examples of this phenomenon. The implication of localised hyper methylation and the associated decrease in expression of tumour suppressor genes is clear; loss of expression leads to loss of control of the cell and the acquisition of cancer characteristics. However genome-wide

hypomethylation has very different consequences that are not yet fully understood. DNA methylation has been proposed to contribute to the stability and integrity of the genome, and has a role in protecting against erroneous expression of transposable elements (TEs) and endogenous retroviruses (ERVs) [55]. Loss of this methylation contributes to erroneous expression of these silenced regions, potentially modify the expression of the genes within which these elements are found, as well as global changes in transcription factor levels [56]. It can also lead to aberrant expression of oncogenes, loss of imprinting and chromosomal instability [57]. Alongside this dysregulation of DNA methylation, the enzymes that control this process are also shown to be dysregulated, not only at the level of mRNA expression, but also with changes in protein half-life and stability [58] or mutation [59]. 5-hydroxymethylated cytosine is decreased in cancer [60] which is thought to occur through mutation of the proteins themselves, most often in haematological malignancies [61] or downregulation of the TET genes expression [60, 62].

Variation of epigenetic modulators is a common alteration in malignancies. For example, the overexpression of EZH2, the core catalytic protein the histone methyltransferase PRC2 responsible for the silencing tri-methylation mark on lysine 27 of histone3, has been observed. This is caused by increased copy number or downregulation of associated regulatory microRNAs, as well as a mutation which enhances the ability of EZH2 to perform di- and tri-methylation of H3K27, and is found in a wide range of cancers [63, 64]. Another example is over expression of HDACs, the enzymes catalysing the removal of acetyl groups and creating a repressive environment to silence genes. The overexpression of HDACs has been observed in many cancers, [65-72], and the success of drugs such as vorinostat and romidepsin, (HDAC inhibitors used in the treatment of cutaneous T cell lymphoma [73]) emphasises the role of HDACs in cancer progression. Although the anticancer effects of these HDAC inhibitors are thought to be at least in part due to the accumulation of histone acetylation, the full mechanism is complex and may also include effects to transcription factor acetylation and other non-histone proteins [74-76].

The use of DNA hypomethylating agents azacytadine and decitabine are approved in several forms of myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML) and acute myeloid leukemia (AML). Zebularine is another DNA methylation inhibitor which is not approved for use, and appears to have limited bioavailability [77]. These compounds are cytosine analogues, which require metabolic activation into the tri-phosphorylated version before they can be incorporated into the DNA. These cytosine analogues are recognised by DNMTs as substrates, and initiated by the formation of a covalent bond to the carbon 6 position. Usually this bond is then resolved upon methylation of the carbon 5 position, however in decitabine and azacitadine, this carbon is replaced by a nitrogen, preventing this reaction from taking place, trapping the enzyme in place and inhibiting the methylation action, as well as depleting soluble levels of DNMTs [78, 79]. This leads to degradation of the enzyme over time. However, the formation of these DNA-protein crosslinks has also been shown to initiate DNA damage response [80], indicating the anticancer mechanism of this type of drug is not yet fully elucidated and could encompass multiple modes. SGI-110 is a recent addition to the cytosine analogue family, and has been shown to have less toxicity than azacitadine when used in an *in vivo* xenograft model of bladder cancer [81]. Non-nucleoside inhibitors are also being explored, such as S-adenosylhomocysteine (SAM) hydrolase inhibitor 3-Deazaneplanocin A (DZNep), found initially to reduce the activity of EZH2 [82]. As it acts by inhibiting the synthesis of SAM, the main substrate required for any methylation, it will also inhibit the methylation of DNA [83].

Over-simplification of epigenetics is rife because it is tempting to make broad generalisations about the action of these marks. Methylation within a CpG promoter is usually associated with silencing of that gene; however not all gene promoters contain CpG islands, and not all those CpG islands are modified in this way. Recent work that assessed TCGA cancer DNA methylation and matched expression found that for only a subset of the CpG island associated genes was there a significant correlation between a change in DNA methylation and a change in expression; indeed, cancer specific methylation did not appear to repress gene expression and was instead found at genes with existing low expression in normal tissues [84]. The direction of action is also difficult to ascertain; do we cause DNA methylation changes which change gene expression, or does a change in gene expression, potentially occurring via a different mechanism, modulate the DNA methylation patterns?

Epigenetic enhancement of immunotherapy

The theory behind using epigenetic therapeutics to enhance immunotherapy relies on the observation that modulating epigenetics could create a more immunogenic phenotype in a previously immune-evasive tumour cell, by upregulating the expression of tumour associated antigens or other activating ligands on the surface of the cell, or reversing silencing of the processing and presentation machinery required for immune activation [85, 86]. Multiple trials are underway assessing the combination of epigenetic and immunomodulatory therapies (Table 1). It has been demonstrated that treatment with the DNA demethylating agent azacitadine resulted in upregulation of immunomodulatory pathways in breast, colorectal and ovarian cancer cell lines [87]. Furthermore, it has been observed that PD-L1 expression was upregulated upon treatment with HDAC inhibitors in mouse and human melanoma cell lines. This resulted in a better response to PD-1 blocking antibody therapy in mice [88]. A similar effect was also observed in non-small-cell lung cancer cell lines treated with azacytadine [89]. An important factor to consider in such studies is to ensure that the improved response to PD-1 blocking antibody is observed in an immunocompetent environment, as the initial PD-L1 upregulation could lead to further immune evasion by inactivating T cells. This indicates that the balance between the two treatments will need to be considered carefully. Additionally, the observation that increasing expression of PD-L1 results in a better response to the blocking antibody is perplexing; if the role of the blocking antibody is to inhibit this interaction, then the outcome should be the same if the interaction cannot exist due to low expression of ligand. An alternative mechanism could be responsible through the upregulation of other immune-modulating components.

The re-expression of silenced cytokines in a tumour cell is another possible mechanism for the augmentation of immune response. A recent study found that by inhibiting both EZH2 and DNA methylation using DZNep and decitabine, previously silenced T helper 1-type cytokines CXCL9 and CXCL10 could be re-expressed, which increased T cell tumour infiltration, and slowed tumour growth in a mouse model of ovarian cancer. Additionally use of DZnep and decitabine in conjunction with other immunotherapeutic treatments in mice, such as PD-L1 blocking therapy and adoptive T cell treatment, resulted in improved therapeutic efficacy [90]. Re-activating an immune-suppressive environment appears to have wide-reaching consequences on the system as a whole.

The use of these epigenetic modulators is not gene specific; the effect of demethylation will happen across the genome. The prospect of this is perhaps counterintuitive, when we consider the

observation that widespread hypomethylation in cancer demonstrates that huge regions of the genome in these tumours have already been demethylated, yet still the tumour is immune repressive, begging the question as to how further demethylation can be effective. Localised hypermethylation in cancer has always focussed on tumour suppressor gene promoters, therefore the mechanism of action of epigenetic modulators is, perhaps erroneously, almost completely attributed to action in those regions. As previously mentioned however, DNA methylation has a role to play in protecting the healthy cell against unwanted expression of transposable elements and endogenous retroviruses [55]. It has recently been shown that the re-expression endogenous retroviral elements can be elicited by demethylating agents to stimulate an immune response in colorectal [91] and ovarian [92] cancer, turning this particular epigenetic survival mechanism against the tumour cell, exposing it to immune attack, and revealing that a reliance on existing immune pathways may be implicit in the epigenetic drug response. Other existing epigenetic treatments have been shown to at least partially rely on the immune system for their anticancer mechanism; it has been shown that vorinostat and panobinostat require an intact immune system to effectively kill cancer cells in mouse models of colon cancer and leukemia/lymphoma, and this effect was enhanced by the addition of an IFN- γ inducing agent [93].

An additional possible explanation for observed increased immunogenicity is a concept known as immunogenic cell death, a process by which the drug induced cell death causes the release of danger associated signals that “label” the tumour cells as pathogenic, leading to increased presentation of tumour antigens by the antigen processing cells (APCs), leading to induction of cytotoxic T cell response [94]. The cause of the cell death could be due to re-expression of previously epigenetically silenced genes, but also could be due to general cell toxicity associated with the drug, or cell death due to a different mechanism of action, for example DNA damage. HDAC inhibitors such as vorinostat have been observed to have this effect by improving dendritic phagocytosis of brain tumour cell lines *in vitro* via the release of immunogenic cell death mediators [95].

The consequence of epigenetic modulation can not only be viewed from the perspective of the cancer cell; these therapies act systemically and will have activity in all cells of the body, including components of the immune system itself. Due to the highly adaptive and complex nature of immune response, the effects could be unexpected. Epigenetics plays a role in the differentiation of a naïve T helper cell into either a mature type 1 or type 2 T helper through the modulation of a restrictive chromatin environment of the effector cytokine genes (for example, IL4) in the naïve helper T cell, to a poised state in newly activated progenitor T cells, before final permissive patterns of transcription are fixed in the mature T helper cells [96]. There is also evidence that during V(D)J recombination in both T and B cell receptor generation the modulation of accessibility by epigenetic mechanisms is crucial, with specific chromatin structures determining the targeting of the recombinase activity [97]. Treatment with epigenetic modulators could therefore disrupt these important immune regulatory processes, and affect the immune system in ways that are not conducive to tumour cell killing.

One potential aspect that the full consequences for are not yet known pertains to regulatory T cells (Tregs). Tregs are CD4⁺ CD25⁺ and express the transcription factor Foxp3. Tregs tend to be involved in reducing the immune response or T cell anergy, and are essential for the prevention of autoimmunity. This is achieved by deactivation of cytotoxic T cells. Despite their pivotal role in the inhibition of autoimmunity, it has been shown that the presence of a higher proportion of regulatory

T cells is associated with poorer survival in cancer [98, 99]. The transcription factor Foxp3 is one of the defining features of Tregs, and directs a specific gene expression pattern for this cell fate [100]. It has been postulated that a DNA region specifically demethylated only in Tregs is linked with expression of this gene, and it has been shown that demethylation of this region in non-regulatory T cells induces expression of Foxp3, activating the regulatory T cell gene program [101]. There is therefore potential for DNMT inhibitors to generate stable and specific Tregs; desirable in autoimmune disease, but this outcome in cancer may not be quite so advantageous, generating Tregs which will further repress the immune response in the tumour microenvironment.

Work so far has focussed on examples where epigenetic modulation has enhanced immune response, however some studies have shown a mixed response to epigenetic treatments. For example, zebularine, via the upregulation of IDO, has also been shown to decrease immunogenicity, but only at high doses; at low doses, the immunogenicity is increased [102]. The balance between activated and repressing immunological response appears to be both cell and agent specific, as HDAC inhibition has been shown to both increase expression of tumour associated antigens, for example gp100 in murine melanoma cells [103], and in other systems to downregulate tumour antigens, for example Muc1 in mesothelioma cells [104]. This highlights the complex nature of epigenetic modulation, and the likelihood that it will not work as expected in all cancers or all individuals.

Dysregulation of the epigenetics in immune cells may have occurred concurrently with those affecting cancer cells, especially if a more systemic modulation is at play, or a change in immune surveillance has allowed the cancer to proliferate. Methylation changes in blood have been found associated with cancer development in the peripheral lymphocytes of patients, for example p53 promoter methylation in lung cancer [105, 106] or associated with other cancer risk factors, such as smoking [107]. Primarily used as biomarkers for diagnosis or prognosis, do these changes in peripheral lymphocyte DNA methylation indicate a change of function or expression profile in immune cells, or perhaps a change in cell type proportion? Perhaps the generation of a less competent immune response is required for a carcinogenic microenvironment, allowing neoplastic growth. Interrogation of prospective biomarkers may reveal immune modulatory programmes of change. If these epigenetic changes are functional, then the reversal of their states will be beneficial to cancer treatment. For example, expression of key activating receptors on the surface of immune cells, such as Fas, has been shown to be downregulated and exhibited corresponding epigenetic marks in a mouse model, which could be reversed by inhibiting DNA methylation [108]. In these and other ways, treatment with epigenetic modulating therapies may induce more competent host immune responses.

Concluding remarks

Epigenetic modulating therapies is a promising avenue with which we may successfully enhance immunotherapies, but the use of these drugs will not always be as straightforward as one might hope. Combining the complexities of immune system development and activation, with the equally complex actions of epigenetic mechanisms in both normal and cancerous cells, creates an intricate environment within which a therapeutic balance must be found (Figure 1). As the importance of the tumour microenvironment becomes clearer, continued research to understand this balancing act will be required. *In vitro* studies provide the background knowledge to pursue the clinical relevance

of these potential treatments, and care must be taken in the observation of patients for adverse side effects or unexpected outcomes.

1. Coley, W.B., *The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases.* 1893. *Am. J. med. Sci.*, 1893(105): p. 487-511.
2. Wiemann, B. and C.O. Starnes, *Coley's toxins, tumor necrosis factor and cancer research: a historical perspective.* *Pharmacol Ther*, 1994. **64**(3): p. 529-64.
3. Scott, A.M., J.D. Wolchok, and L.J. Old, *Antibody therapy of cancer.* *Nat Rev Cancer*, 2012. **12**(4): p. 278-87.
4. Rosenberg, S.A. and N.P. Restifo, *Adoptive cell transfer as personalized immunotherapy for human cancer.* *Science*, 2015. **348**(6230): p. 62-8.
5. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* *Cell*, 2011. **144**(5): p. 646-74.
6. Corthay, A., *Does the immune system naturally protect against cancer?* *Front Immunol*, 2014. **5**: p. 197.
7. Dunn, G.P., L.J. Old, and R.D. Schreiber, *The immunobiology of cancer immunosurveillance and immunoediting.* *Immunity*, 2004. **21**(2): p. 137-48.
8. Galdiero, M.R., E. Bonavita, I. Barajon, C. Garlanda, A. Mantovani, and S. Jaillon, *Tumor associated macrophages and neutrophils in cancer.* *Immunobiology*, 2013. **218**(11): p. 1402-10.
9. Sica, A. and A. Mantovani, *Macrophage plasticity and polarization: in vivo veritas.* *J Clin Invest*, 2012. **122**(3): p. 787-95.
10. Biswas, S.K. and A. Mantovani, *Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm.* *Nat Immunol*, 2010. **11**(10): p. 889-96.
11. Gungor, N., A.M. Knaapen, A. Munnia, M. Peluso, G.R. Haenen, R.K. Chiu, R.W. Godschalk, and F.J. van Schooten, *Genotoxic effects of neutrophils and hypochlorous acid.* *Mutagenesis*, 2010. **25**(2): p. 149-54.
12. Sandhu, J.K., H.F. Privora, G. Wenckebach, and H.C. Birnboim, *Neutrophils, nitric oxide synthase, and mutations in the mutatest murine tumor model.* *Am J Pathol*, 2000. **156**(2): p. 509-18.
13. Fridlender, Z.G., J. Sun, S. Kim, V. Kapoor, G. Cheng, L. Ling, G.S. Worthen, and S.M. Albelda, *Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN.* *Cancer Cell*, 2009. **16**(3): p. 183-94.
14. Schwartz, R.H., *A cell culture model for T lymphocyte clonal anergy.* *Science*, 1990. **248**(4961): p. 1349-56.
15. Walker, L.S. and D.M. Sansom, *The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses.* *Nat Rev Immunol*, 2011. **11**(12): p. 852-63.
16. Freeman, G.J., A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, L. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, and T. Honjo, *Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation.* *J Exp Med*, 2000. **192**(7): p. 1027-34.
17. Keir, M.E., M.J. Butte, G.J. Freeman, and A.H. Sharpe, *PD-1 and its ligands in tolerance and immunity.* *Annu Rev Immunol*, 2008. **26**: p. 677-704.
18. Lippitz, B.E., *Cytokine patterns in patients with cancer: a systematic review.* *Lancet Oncol*, 2013. **14**(6): p. e218-28.
19. Zitvogel, L. and G. Kroemer, *Targeting PD-1/PD-L1 interactions for cancer immunotherapy.* *Oncoimmunology*, 2012. **1**(8): p. 1223-1225.
20. Akers, S.N., K. Odunsi, and A.R. Karpf, *Regulation of cancer germline antigen gene expression: implications for cancer immunotherapy.* *Future Oncol*, 2010. **6**(5): p. 717-32.

21. Dranoff, G., *Cytokines in cancer pathogenesis and cancer therapy*. Nat Rev Cancer, 2004. **4**(1): p. 11-22.
22. Su, E.W., C.J. Moore, S. Suriano, C.B. Johnson, N. Songalia, A. Patterson, D.J. Neitzke, K. Andrijauskaite, E. Garrett-Mayer, S. Mehrotra, C.M. Paulos, A.L. Doedens, A.W. Goldrath, Z. Li, D.J. Cole, and M.P. Rubinstein, *IL-2/Ralpha mediates temporal regulation of IL-2 signaling and enhances immunotherapy*. Sci Transl Med, 2015. **7**(311): p. 311ra170.
23. Hinrichs, C.S. and S.A. Rosenberg, *Exploiting the curative potential of adoptive T-cell therapy for cancer*. Immunol Rev, 2014. **257**(1): p. 56-71.
24. Dudley, M.E., J.R. Wunderlich, T.E. Shelton, J. Even, and S.A. Rosenberg, *Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients*. J Immunother, 2003. **26**(4): p. 332-42.
25. Hamanishi, J., M. Mandai, M. Iwasaki, T. Okazaki, Y. Tanaka, K. Yamaguchi, T. Higuchi, H. Yagi, K. Takakura, N. Minato, T. Honjo, and S. Fujii, *Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer*. Proc Natl Acad Sci U S A, 2007. **104**(9): p. 3360-5.
26. Berman, D., A. Korman, R. Peck, D. Feltquate, N. Lonberg, and R. Canetta, *The development of immunomodulatory monoclonal antibodies as a new therapeutic modality for cancer: the Bristol-Myers Squibb experience*. Pharmacol Ther, 2015. **148**: p. 132-53.
27. FDA. *Fact Sheet: Breakthrough Therapies*. 2014 12/10/2014 [cited 2015 25/11/2015]; Available from: <http://www.fda.gov/RegulatoryInformation/Legislation/SignificantAmendmentstotheFDCA/t/FDASIA/ucm329491.htm>.
28. Hodi, F.S., S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbe, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, and W.J. Urba, *Improved survival with ipilimumab in patients with metastatic melanoma*. N Engl J Med, 2010. **363**(8): p. 711-23.
29. Schadendorf, D., F.S. Hodi, C. Robert, J.S. Weber, K. Margolin, O. Hamid, D. Patt, T.T. Chen, D.M. Berman, and J.D. Wolchok, *Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma*. J Clin Oncol, 2015. **33**(17): p. 1889-94.
30. Lynch, T.J., I. Bondarenko, A. Luft, P. Serwatowski, F. Barlesi, R. Chacko, M. Sebastian, J. Neal, H. Lu, J.M. Cuillerot, and M. Reck, *Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study*. J Clin Oncol, 2012. **30**(17): p. 2046-54.
31. Le, D.T., E. Lutz, J.N. Uram, E.A. Sugar, B. Onners, S. Solt, L. Zheng, L.A. Diaz, Jr., R.C. Donehower, E.M. Jaffee, and D.A. Laheru, *Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer*. J Immunother, 2013. **36**(7): p. 382-9.
32. Calabro, L., A. Morra, E. Fonsatti, O. Cutaia, G. Amato, D. Giannarelli, A.M. Di Giacomo, R. Danielli, M. Altomonte, L. Mutti, and M. Maio, *Tremelimumab for patients with chemotherapy-resistant advanced malignant mesothelioma: an open-label, single-arm, phase 2 trial*. Lancet Oncol, 2013. **14**(11): p. 1104-11.
33. Topalian, S.L., F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P.D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L. McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kollia, A. Gupta, J.M. Wigginton, and M. Sznol, *Safety, activity, and immune correlates of anti-PD-1 antibody in cancer*. N Engl J Med, 2012. **366**(26): p. 2443-54.

34. Hamid, O., C. Robert, A. Daud, F.S. Hodi, W.J. Hwu, R. Kefford, J.D. Wolchok, P. Hersey, R.W. Joseph, J.S. Weber, R. Dronca, T.C. Gangadhar, A. Patnaik, H. Zarour, A.M. Joshua, K. Gergich, J. Ellassaiss-Schaap, A. Algazi, C. Mateus, P. Boasberg, P.C. Tume, B. Chmielowski, S.W. Ebbinghaus, X.N. Li, S.P. Kang, and A. Ribas, *Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma*. N Engl J Med, 2013. **369**(2): p. 134-44.
35. Topalian, S.L., M. Sznol, D.F. McDermott, H.M. Kluger, R.D. Carvajal, W.H. Sharfman, J.R. Brahmer, D.P. Lawrence, M.B. Atkins, J.D. Powderly, P.D. Leming, E.J. Lipson, I. Puzanov, D.C. Smith, J.M. Taube, J.M. Wigginton, G.D. Kollia, A. Gupta, D.M. Pardoll, J.A. Sosman, and F.S. Hodi, *Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab*. J Clin Oncol, 2014. **32**(10): p. 1020-30.
36. Postow, M.A., M.K. Callahan, and J.D. Wolchok, *Immune Checkpoint Blockade in Cancer Therapy*. J Clin Oncol, 2015. **33**(17): p. 1974-82.
37. Robert, C., A. Ribas, J.D. Wolchok, F.S. Hodi, O. Hamid, R. Kefford, J.S. Weber, A.M. Joshua, W.J. Hwu, T.C. Gangadhar, A. Patnaik, R. Dronca, H. Zarour, R.W. Joseph, P. Boasberg, B. Chmielowski, C. Mateus, M.A. Postow, K. Gergich, J. Ellassaiss-Schaap, X.N. Li, R. Iannone, S.W. Ebbinghaus, S.P. Kang, and A. Daud, *Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial*. Lancet, 2014. **384**(9948): p. 1109-17.
38. Saenger, Y.M. and J.D. Wolchok, *The heterogeneity of the kinetics of response to ipilimumab in metastatic melanoma: patient cases*. Cancer Immun, 2008. **8**: p. 1.
39. Wolchok, J.D., A. Hoos, S. O'Day, J.S. Weber, O. Hamid, C. Lebbe, M. Maio, M. Binder, O. Bohnsack, G. Nichol, R. Humphrey, and F.S. Hodi, *Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria*. Clin Cancer Res, 2009. **15**(23): p. 7412-20.
40. Hoos, A., A.M. Eggermont, S. Janetzki, F.S. Hodi, R. Ibrahim, A. Anderson, R. Humphrey, B. Blumenstein, L. Old, and J. Wolchok, *Improved endpoints for cancer immunotherapy trials*. J Natl Cancer Inst, 2010. **102**(18): p. 1388-97.
41. Holliday, R., *The inheritance of epigenetic defects*. Science, 1987. **238**(4824): p. 163-70.
42. Bird, A.P., *DNA methylation and the frequency of CpG in animal DNA*. Nucleic Acids Res, 1980. **8**(7): p. 1499-504.
43. Deaton, A.M. and A. Bird, *CpG islands and the regulation of transcription*. Genes Dev, 2011. **25**(10): p. 1010-22.
44. Shenker, N. and J.M. Flanagan, *Intragenic DNA methylation: implications of this epigenetic mechanism for cancer research*. Br J Cancer, 2012. **106**(2): p. 248-53.
45. Bird, A.P., *CpG-rich islands and the function of DNA methylation*. Nature, 1986. **321**(6067): p. 209-13.
46. Pujadas, E. and A.P. Feinberg, *Regulated noise in the epigenetic landscape of development and disease*. Cell, 2012. **148**(6): p. 1123-31.
47. He, Y.F., B.Z. Li, Z. Li, P. Liu, Y. Wang, Q. Tang, J. Ding, Y. Jia, Z. Chen, L. Li, Y. Sun, X. Li, Q. Dai, C.X. Song, K. Zhang, C. He, and G.L. Xu, *Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA*. Science, 2011. **333**(6047): p. 1303-7.
48. Ito, S., L. Shen, Q. Dai, S.C. Wu, L.B. Collins, J.A. Swenberg, C. He, and Y. Zhang, *Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine*. Science, 2011. **333**(6047): p. 1300-3.
49. Feinberg, A.P. and B. Vogelstein, *Hypomethylation distinguishes genes of some human cancers from their normal counterparts*. Nature, 1983. **301**(5895): p. 89-92.
50. Feinberg, A.P. and B. Vogelstein, *Hypomethylation of ras oncogenes in primary human cancers*. Biochem Biophys Res Commun, 1983. **111**(1): p. 47-54.
51. Gama-Sosa, M.A., V.A. Slagel, R.W. Trewyn, R. Oxenhandler, K.C. Kuo, C.W. Gehrke, and M. Ehrlich, *The 5-methylcytosine content of DNA from human tumors*. Nucleic Acids Res, 1983. **11**(19): p. 6883-94.

52. Herman, J.G., F. Latif, Y. Weng, M.I. Lerman, B. Zbar, S. Liu, D. Samid, D.S. Duan, J.R. Gnarr, W.M. Linehan, and et al., *Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma*. Proc Natl Acad Sci U S A, 1994. **91**(21): p. 9700-4.
53. Herman, J.G., A. Umar, K. Polyak, J.R. Graff, N. Ahuja, J.P. Issa, S. Markowitz, J.K. Willson, S.R. Hamilton, K.W. Kinzler, M.F. Kane, R.D. Kolodner, B. Vogelstein, T.A. Kunkel, and S.B. Baylin, *Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma*. Proc Natl Acad Sci U S A, 1998. **95**(12): p. 6870-5.
54. Dammann, R., U. Schagdarsurengin, C. Seidel, M. Strunnikova, M. Rastetter, K. Baier, and G.P. Pfeifer, *The tumor suppressor RASSF1A in human carcinogenesis: an update*. Histol Histopathol, 2005. **20**(2): p. 645-63.
55. Wilson, A.S., B.E. Power, and P.L. Molloy, *DNA hypomethylation and human diseases*. Biochim Biophys Acta, 2007. **1775**(1): p. 138-62.
56. Whitelaw, E. and D.I. Martin, *Retrotransposons as epigenetic mediators of phenotypic variation in mammals*. Nat Genet, 2001. **27**(4): p. 361-5.
57. Ehrlich, M., *DNA methylation in cancer: too much, but also too little*. Oncogene, 2002. **21**(35): p. 5400-13.
58. Agoston, A.T., P. Argani, S. Yegnasubramanian, A.M. De Marzo, M.A. Ansari-Lari, J.L. Hicks, N.E. Davidson, and W.G. Nelson, *Increased protein stability causes DNA methyltransferase 1 dysregulation in breast cancer*. J Biol Chem, 2005. **280**(18): p. 18302-10.
59. You, J.S. and P.A. Jones, *Cancer genetics and epigenetics: two sides of the same coin?* Cancer Cell, 2012. **22**(1): p. 9-20.
60. Yang, H., Y. Liu, F. Bai, J.Y. Zhang, S.H. Ma, J. Liu, Z.D. Xu, H.G. Zhu, Z.Q. Ling, D. Ye, K.L. Guan, and Y. Xiong, *Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation*. Oncogene, 2013. **32**(5): p. 663-9.
61. Scourzic, L., E. Mouly, and O.A. Bernard, *TET proteins and the control of cytosine demethylation in cancer*. Genome Med, 2015. **7**(1): p. 9.
62. Neri, F., D. Dettori, D. Incarnato, A. Krepelova, S. Rapelli, M. Maldotti, C. Parlato, P. Paliogiannis, and S. Oliviero, *TET1 is a tumour suppressor that inhibits colon cancer growth by derepressing inhibitors of the WNT pathway*. Oncogene, 2015. **34**(32): p. 4168-76.
63. Deb, G., A.K. Singh, and S. Gupta, *EZH2: not EZHY (easy) to deal*. Mol Cancer Res, 2014. **12**(5): p. 639-53.
64. Chang, C.J. and M.C. Hung, *The role of EZH2 in tumour progression*. Br J Cancer, 2012. **106**(2): p. 243-7.
65. Choi, J.H., H.J. Kwon, B.I. Yoon, J.H. Kim, S.U. Han, H.J. Joo, and D.Y. Kim, *Expression profile of histone deacetylase 1 in gastric cancer tissues*. Jpn J Cancer Res, 2001. **92**(12): p. 1300-4.
66. Song, J., J.H. Noh, J.H. Lee, J.W. Eun, Y.M. Ahn, S.Y. Kim, S.H. Lee, W.S. Park, N.J. Yoo, J.Y. Lee, and S.W. Nam, *Increased expression of histone deacetylase 2 is found in human gastric cancer*. APMIS, 2005. **113**(4): p. 264-8.
67. Halkidou, K., L. Gaughan, S. Cook, H.Y. Leung, D.E. Neal, and C.N. Robson, *Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer*. Prostate, 2004. **59**(2): p. 177-89.
68. Zhang, Z., H. Yamashita, T. Toyama, H. Sugiura, Y. Ando, K. Mita, M. Hamaguchi, Y. Hara, S. Kobayashi, and H. Iwase, *Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast**. Breast Cancer Res Treat, 2005. **94**(1): p. 11-6.
69. Zhang, Z., H. Yamashita, T. Toyama, H. Sugiura, Y. Omoto, Y. Ando, K. Mita, M. Hamaguchi, S. Hayashi, and H. Iwase, *HDAC6 expression is correlated with better survival in breast cancer*. Clin Cancer Res, 2004. **10**(20): p. 6962-8.
70. Huang, B.H., M. Laban, C.H. Leung, L. Lee, C.K. Lee, M. Salto-Tellez, G.C. Raju, and S.C. Hooi, *Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/WAF1 expression, independent of histone deacetylase 1*. Cell Death Differ, 2005. **12**(4): p. 395-404.

71. Wilson, A.J., D.S. Byun, N. Popova, L.B. Murray, K. L'Italien, Y. Sowa, D. Arango, A. Velcich, L.H. Augenlicht, and J.M. Mariadason, *Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer*. J Biol Chem, 2006. **281**(19): p. 13548-58.
72. Zhu, P., E. Martin, J. Mengwasser, P. Schlag, K.P. Janssen, and M. Gottlicher, *Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis*. Cancer Cell, 2004. **5**(5): p. 455-63.
73. Falkenberg, K.J. and R.W. Johnstone, *Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders*. Nat Rev Drug Discov, 2014. **13**(9): p. 673-91.
74. Ropero, S. and M. Esteller, *The role of histone deacetylases (HDACs) in human cancer*. Mol Oncol, 2007. **1**(1): p. 19-25.
75. Grant, S., C. Easley, and P. Kirkpatrick, *Vorinostat*. Nat Rev Drug Discov, 2007. **6**(1): p. 21-2.
76. Bertino, E.M. and G.A. Otterson, *Romidepsin: a novel histone deacetylase inhibitor for cancer*. Expert Opin Investig Drugs, 2011. **20**(8): p. 1151-8.
77. Holleran, J.L., R.A. Parise, E. Joseph, J.L. Eiseman, J.M. Covey, E.R. Glaze, A.V. Lyubimov, Y.F. Chen, D.Z. D'Argenio, and M.J. Egorin, *Plasma pharmacokinetics, oral bioavailability, and interspecies scaling of the DNA methyltransferase inhibitor, zebularine*. Clin Cancer Res, 2005. **11**(10): p. 3862-8.
78. Stresemann, C. and F. Lyko, *Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine*. Int J Cancer, 2008. **123**(1): p. 8-13.
79. Derissen, E.J., J.H. Beijnen, and J.H. Schellens, *Concise drug review: azacitidine and decitabine*. Oncologist, 2013. **18**(5): p. 619-24.
80. Palii, S.S., B.O. Van Emburgh, U.T. Sankpal, K.D. Brown, and K.D. Robertson, *DNA methylation inhibitor 5-Aza-2'-deoxycytidine induces reversible genome-wide DNA damage that is distinctly influenced by DNA methyltransferases 1 and 3B*. Mol Cell Biol, 2008. **28**(2): p. 752-71.
81. Chuang, J.C., S.L. Warner, D. Vollmer, H. Vankayalapati, S. Redkar, D.J. Bearss, X. Qiu, C.B. Yoo, and P.A. Jones, *S110, a 5-Aza-2'-deoxycytidine-containing dinucleotide, is an effective DNA methylation inhibitor in vivo and can reduce tumor growth*. Mol Cancer Ther, 2010. **9**(5): p. 1443-50.
82. Miranda, T.B., C.C. Cortez, C.B. Yoo, G. Liang, M. Abe, T.K. Kelly, V.E. Marquez, and P.A. Jones, *DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation*. Mol Cancer Ther, 2009. **8**(6): p. 1579-88.
83. Tan, J., X. Yang, L. Zhuang, X. Jiang, W. Chen, P.L. Lee, R.K. Karuturi, P.B. Tan, E.T. Liu, and Q. Yu, *Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells*. Genes Dev, 2007. **21**(9): p. 1050-63.
84. Moarii, M., V. Boeva, J.P. Vert, and F. Reyat, *Changes in correlation between promoter methylation and gene expression in cancer*. BMC Genomics, 2015. **16**(1): p. 873.
85. Sigalotti, L., E. Fratta, S. Coral, and M. Maio, *Epigenetic drugs as immunomodulators for combination therapies in solid tumors*. Pharmacol Ther, 2014. **142**(3): p. 339-50.
86. Maio, M., A. Covre, E. Fratta, A.M. Di Giacomo, P. Taverna, P.G. Natali, S. Coral, and L. Sigalotti, *Molecular Pathways: At the Crossroads of Cancer Epigenetics and Immunotherapy*. Clin Cancer Res, 2015. **21**(18): p. 4040-7.
87. Li, H., K.B. Chiappinelli, A.A. Guzzetta, H. Easwaran, R.W. Yen, R. Vatapalli, M.J. Topper, J. Luo, R.M. Connolly, N.S. Azad, V. Stearns, D.M. Pardoll, N. Davidson, P.A. Jones, D.J. Slamon, S.B. Baylin, C.A. Zahnow, and N. Ahuja, *Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers*. Oncotarget, 2014. **5**(3): p. 587-98.
88. Woods, D.M., A.L. Sodre, A. Villagra, A. Sarnaik, E.M. Sotomayor, and J. Weber, *HDAC Inhibition Upregulates PD-1 Ligands in Melanoma and Augments Immunotherapy with PD-1 Blockade*. Cancer Immunol Res, 2015.

89. Wrangle, J., W. Wang, A. Koch, H. Easwaran, H.P. Mohammad, F. Vendetti, W. Vancrackinge, T. Demeyer, Z. Du, P. Parsana, K. Rodgers, R.W. Yen, C.A. Zahnow, J.M. Taube, J.R. Brahmer, S.S. Tykodi, K. Easton, R.D. Carvajal, P.A. Jones, P.W. Laird, D.J. Weisenberger, S. Tsai, R.A. Juergens, S.L. Topalian, C.M. Rudin, M.V. Brock, D. Pardoll, and S.B. Baylin, *Alterations of immune response of Non-Small Cell Lung Cancer with Azacytidine*. *Oncotarget*, 2013. **4**(11): p. 2067-79.
90. Peng, D., I. Kryczek, N. Nagarsheth, L. Zhao, S. Wei, W. Wang, Y. Sun, E. Zhao, L. Vatan, W. Szeliga, J. Kotarski, R. Tarkowski, Y. Dou, K. Cho, S. Hensley-Alford, A. Munkarah, R. Liu, and W. Zou, *Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy*. *Nature*, 2015. **527**(7577): p. 249-53.
91. Roulois, D., H. Loo Yau, R. Singhanian, Y. Wang, A. Danesh, S.Y. Shen, H. Han, G. Liang, P.A. Jones, T.J. Pugh, C. O'Brien, and D.D. De Carvalho, *DNA-Demethylating Agents Target Colorectal Cancer Cells by Inducing Viral Mimicry by Endogenous Transcripts*. *Cell*, 2015. **162**(5): p. 961-73.
92. Chiappinelli, K.B., P.L. Strissel, A. Desrichard, H. Li, C. Henke, B. Akman, A. Hein, N.S. Rote, L.M. Cope, A. Snyder, V. Makarov, S. Buhu, D.J. Slamon, J.D. Wolchok, D.M. Pardoll, M.W. Beckmann, C.A. Zahnow, T. Mergoub, T.A. Chan, S.B. Baylin, and R. Strick, *Inhibiting DNA Methylation Causes an Interferon Response in Cancer via dsRNA Including Endogenous Retroviruses*. *Cell*, 2015. **162**(5): p. 974-86.
93. West, A.C., S.R. Mattarollo, J. Shortt, L.A. Cluse, A.J. Christiansen, M.J. Smyth, and R.W. Johnstone, *An intact immune system is required for the anticancer activities of histone deacetylase inhibitors*. *Cancer Res*, 2013. **73**(24): p. 7265-76.
94. Kroemer, G., L. Galluzzi, O. Kepp, and L. Zitvogel, *Immunogenic cell death in cancer therapy*. *Annu Rev Immunol*, 2013. **31**: p. 51-72.
95. Sonnemann, J., S. Gressmann, S. Becker, S. Wittig, M. Schmutde, and J.F. Beck, *The histone deacetylase inhibitor vorinostat induces calreticulin exposure in childhood brain tumour cells in vitro*. *Cancer Chemother Pharmacol*, 2010. **66**(3): p. 611-6.
96. Reiner, S.L., *Epigenetic control in the immune response*. *Hum Mol Genet*, 2005. **14 Spec No 1**: p. R41-6.
97. Jaeger, S., B. Fernandez, and P. Ferrier, *Epigenetic aspects of lymphocyte antigen receptor gene rearrangement or 'when stochasticity completes randomness'*. *Immunology*, 2013. **139**(2): p. 141-50.
98. Curiel, T.J., G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J.R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M.L. Disis, K.L. Knutson, L. Chen, and W. Zou, *Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival*. *Nat Med*, 2004. **10**(9): p. 942-9.
99. Bates, G.J., S.B. Fox, C. Han, R.D. Leek, J.F. Garcia, A.L. Harris, and A.H. Banham, *Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse*. *J Clin Oncol*, 2006. **24**(34): p. 5373-80.
100. Marson, A., K. Kretschmer, G.M. Frampton, E.S. Jacobsen, J.K. Polansky, K.D. MacIsaac, S.S. Levine, E. Fraenkel, H. von Boehmer, and R.A. Young, *Foxp3 occupancy and regulation of key target genes during T-cell stimulation*. *Nature*, 2007. **445**(7130): p. 931-5.
101. Lal, G., N. Zhang, W. van der Touw, Y. Ding, W. Ju, E.P. Bottinger, S.P. Reid, D.E. Levy, and J.S. Bromberg, *Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation*. *J Immunol*, 2009. **182**(1): p. 259-73.
102. Liu, H., Z.T. Xue, H.O. Sjogren, L.G. Salford, and B. Widegren, *Low dose Zebularine treatment enhances immunogenicity of tumor cells*. *Cancer Lett*, 2007. **257**(1): p. 107-15.
103. Murakami, T., A. Sato, N.A. Chun, M. Hara, Y. Naito, Y. Kobayashi, Y. Kano, M. Ohtsuki, Y. Furukawa, and E. Kobayashi, *Transcriptional modulation using HDACi depsipeptide promotes*

- immune cell-mediated tumor destruction of murine B16 melanoma*. *J Invest Dermatol*, 2008. **128**(6): p. 1506-16.
104. Roulois, D., C. Blanquart, C. Panterne, F. Gueugnon, M. Gregoire, and J.F. Fonteneau, *Downregulation of MUC1 expression and its recognition by CD8(+) T cells on the surface of malignant pleural mesothelioma cells treated with HDACi*. *Eur J Immunol*, 2012. **42**(3): p. 783-9.
105. Woodson, K., J. Mason, S.W. Choi, T. Hartman, J. Tangrea, J. Virtamo, P.R. Taylor, and D. Albanes, *Hypomethylation of p53 in peripheral blood DNA is associated with the development of lung cancer*. *Cancer Epidemiol Biomarkers Prev*, 2001. **10**(1): p. 69-74.
106. Russo, A.L., A. Thiagalingam, H. Pan, J. Califano, K.H. Cheng, J.F. Ponte, D. Chinnappan, P. Nemani, D. Sidransky, and S. Thiagalingam, *Differential DNA hypermethylation of critical genes mediates the stage-specific tobacco smoke-induced neoplastic progression of lung cancer*. *Clin Cancer Res*, 2005. **11**(7): p. 2466-70.
107. Shenker, N.S., P.M. Ueland, S. Polidoro, K. van Veldhoven, F. Ricceri, R. Brown, J.M. Flanagan, and P. Vineis, *DNA methylation as a long-term biomarker of exposure to tobacco smoke*. *Epidemiology*, 2013. **24**(5): p. 712-6.
108. Walker, P.R., T. Calzascia, V. Schnuriger, D. Chalmers, P. Saas, and P.Y. Dietrich, *Loss of Fas (CD95/APO-1) expression by antigen-specific cytotoxic T cells is reversed by inhibiting DNA methylation*. *Cell Immunol*, 2000. **206**(1): p. 51-8.

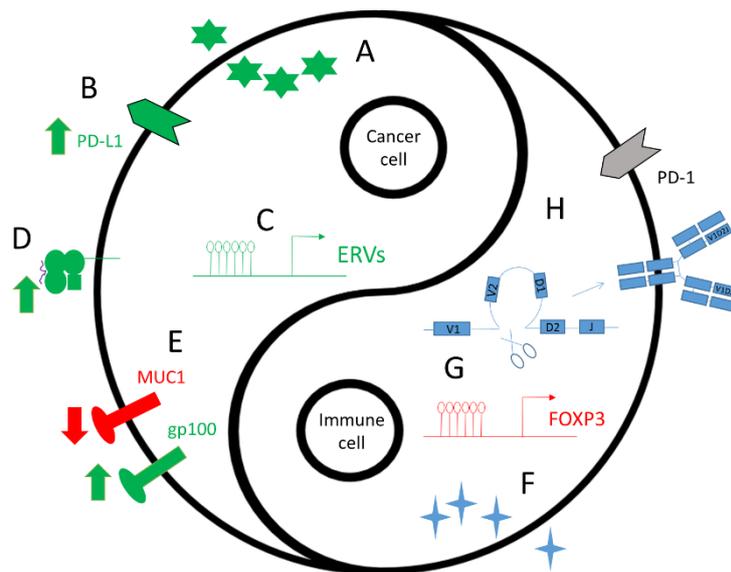


Figure 1: Modulating epigenetics in an interconnected, dynamic system. Epigenetic modifying drugs impact not only tumour cells but also immune response mechanisms. The effect could be positive or negative. These mechanisms include A) increased secretion of chemokines, B) increased expression of cell surface ligands, for example PD-L1, C) expression of endogenous retroviral elements (ERVs), D) increased expression of processing and presentation machinery such as MHC class I, E) modulation of tumour associated antigens (TAAs) expression, F) change in expression of cytokines affecting T cell differentiation, G) induction of Treg production due to increased expression of Foxp3, H) changes in epigenetic mechanisms involved in V(D)J recombination during production of antibodies and cell surface receptors. Green components indicate where epigenetic treatment has a positive, anti-tumour effect in combination with immunomodulatory therapies, components in red

show potential negative outcomes of combination therapies, and blue components indicate systems where epigenetic modulation could affect function of immune pathways, but the outcome is unknown.

ClinicalTrials.gov Identifier	Date	Title	Recruitment status	Epigenetic Drug	Immunotherapy	Other Drugs	Cancer
NCT02546986	First received: August 20, 2015 Last updated: December 23, 2015 Last verified: December 2015	A Phase 2 Multicenter, Randomized, Placebo Controlled, Double Blind Study to Assess the Safety and Efficacy of CC-486 (Oral Azacitidine) in Combination With Pembrolizumab (MK-3475) Versus Pembrolizumab Plus Placebo in Subjects With Previously Treated Locally Advanced or Metastatic Non-small Cell Lung Cancer	Recruiting	CC-486 (Oral Azacitidine)	Pembrolizumab (MK-3475)		Carcinoma, Non-Small-Cell Lung
NCT02395627	First received: March 17, 2015 Last updated: September 29, 2015 Last verified: September 2015	Reversing Hormone Therapy Resistance With Epigenetic-immune Modification: Phase II Trial of Vorinostat, Tamoxifen and Pembrolizumab in Hormone Receptor Expressing Advanced Breast Cancer	Recruiting	Vorinostat	Pembrolizumab	Tamoxifen	Breast Neoplasms
NCT01834248	First received: April 12, 2013 Last updated: June 30, 2015 Last verified: June 2015	A Phase I Study of DEC205mAb-NY-ESO-1 Fusion Protein (CDX-1401) Given With Adjuvant PolyICLC in Conjunction With 5-Aza-2'Deoxycytidine (Decitabine) in Patients With MDS or Low Blast Count AML	Active, not recruiting	Decitabine	DEC-205/NY-ESO-1 Fusion Protein CDX-1401 Poly ICLC		Acute Myeloid Leukemia Alkylating Agent-Related Acute Myeloid Leukemia Chronic Myelomonocytic Leukemia Myelodysplastic Syndrome

							Refractory Anemia With Excess Blasts in Transformation
NCT02518958	First received: August 4, 2015 Last updated: October 29, 2015 Last verified: August 2015	A Phase I, Open-Label, Multiple Ascending Dose Study to Assess the Safety and Tolerability of RRx-001 and Nivolumab in Subjects With Advanced Solid Tumors or Lymphomas For Which There Are No Currently Accepted Life-Prolonging Therapies (PRIMETIME)	Recruiting	Azacitidine	Nivolumab		Malignant Solid Tumor Lymphoma
NCT01120834	First received: April 19, 2010 Last updated: October 27, 2010 Last verified: October 2010	Phase I/II Study of 5-azacitidine in Combination With Vorinostat in Patients With Relapsed or Refractory DLBCL	unknown	Azacytidine	vorinostat		Lymphoma
NCT01928576	First received: August 21, 2013 Last updated: October 13, 2015 Last verified: October 2015	A Randomized Phase II Study of Epigenetic Priming With Azacitidine and Entinostat or Oral Azacitidine Alone Prior to Nivolumab in Subjects With Recurrent Metastatic Non-Small Cell Lung Cancer.	Recruiting	Azacitidine Entinostat	Nivolumab		Non-Small Lung Cancer
NCT01038778	First received: December 18, 2009 Last updated: January 15, 2016 Last verified: January 2016	Phase I/II Study of High Dose Interleukin 2, Aldesleukin, in Combination With the Histone Deacetylase Inhibitor Entinostat in Patients With Metastatic	Recruiting	Entinostat	Aldesleukin		Clear Cell Renal Cell Carcinoma Metastatic Renal Cell Cancer Stage III Renal Cell Cancer Stage IV Renal

		Renal Cell Carcinoma					Cell Cancer
NCT02332889	First received: October 20, 2014 Last updated: November 30, 2015 Last verified: November 2015	A Phase I/Pilot II Trial Combining Decitabine and Vaccine Therapy for Patients With Relapsed or Refractory Pediatric High Grade Gliomas, Medulloblastomas, and CNS PNETs	Recruiting	Decitabine	Vaccine (autologous dendritic cells) Hiltonol		
NCT01241162	First received: November 15, 2010 Last updated: December 2, 2015 Last verified: November 2015	A Phase I Trial Combining Decitabine and Vaccine Therapy for Patients With Relapsed Neuroblastoma and Sarcoma.	Recruiting	Decitabine	Autologous dendritic cell vaccine with adjuvant		Neuroblastoma Ewings Sarcoma Osteogenic Sarcoma Rhabdomyosarcoma Synovial Sarcoma
NCT01966289	First received: October 10, 2013 Last updated: December 8, 2015 Last verified: December 2015	A Pilot Study of SGI-110 in Combination With an Allogeneic Colon Cancer Cell Vaccine (GVAX) and Cyclophosphamide (CY) in Metastatic Colorectal Cancer (mCRC) as Maintenance Therapy	Recruiting	SGI-110	GVAX	CY	Metastatic Colorectal Cancer
NCT02512172	First received: July 2, 2015 Last updated: July 27, 2015 Last verified: July 2015	A Pilot Study of Using Epigenetic Modulators to Enhance Response to MK-3475 in Microsatellite Stable Advanced Colorectal Cancer	Recruiting	Oral CC - 486	Romidepsin MK - 3475		Colorectal Cancer

Table 1: Ongoing clinical trials assessing combinations of epigenetic and immunomodulatory therapies, found at <https://clinicaltrials.gov/> as of January 2016.