Molecular Immunology 87 (2017) 227–239

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Review article

Epigenetics and immunotherapy: The current state of play

Jennifer Dunn, Sudha Rao*

Health Research Institute, Faculty of Education, Science, Technology and Mathematics, University of Canberra, Bruce, ACT, 2601, Australia

ARTICLE INFO

Keywords:
Epigenetics
Immunotherapy
Immune checkpoint blockade
Combination therapy
DNA methyltransferase inhibitors
Histone deacetylase inhibitors

ABSTRACT

Cancer cells employ a number of mechanisms to escape immunosurveillance and facilitate tumour progression. The recent explosion of interest in immunotherapy, especially immune checkpoint blockade, is a result of discoveries about the fundamental ligand-receptor interactions that occur between immune and cancer cells within the tumour microenvironment. Distinct ligands expressed by cancer cells engage with cell surface receptors on immune cells, triggering inhibitory pathways (such as PD-1/PD-L1) that render immune cells immunologically tolerant. Importantly, recent studies on the role of epigenetics in immune evasion have exposed a key role for epigenetic modulators in augmenting the tumour microenvironment and restoring immune recognition and immunogenicity. Epigenetic drugs such as DNA methyltransferase and histone deacetylase inhibitors can reverse immune suppression via several mechanisms such as enhancing expression of tumour-associated antigens, components of the antigen processing and presenting machinery pathways, immune checkpoint inhibitors, chemokines, and other immune-related genes. These discoveries have established a highly promising basis for studies using combined epigenetic and immunotherapeutic agents as anti-cancer therapies. In this review, we discuss the exciting role of epigenetic immunomodulation in tumour immune escape, emphasising its significance in priming and sensitising the host immune system to immunotherapies through mechanisms such as the activation of the viral defence pathway. With this background in mind, we highlight the promise of combined epigenetic therapy and immunotherapy, focusing on immune checkpoint blockade, to improve outcomes for patients with many different cancer types.

1. Introduction

The recent clinical success of immunotherapy in cancer patients, particularly immune checkpoint blockade, is at least in part due to elegant studies that have led to fundamental discoveries about ligand-receptor interactions between immune and cancer cells within the tumour microenvironment (TME). Distinct ligands expressed by cancer cells engage with cell surface receptors on immune cells, triggering inhibitory pathways that render immune cells immunologically inert or "tolerant". For example, binding of the key T cell surface receptor programmed cell death 1 (PD-1) to the co-inhibitory receptors programmed death ligand 1 (PD-L1) or programmed death ligand 2 (PD-L2) on cancer cells inhibits T cell proliferation, cytokine production, and ultimately results in T cell dysfunction or apoptosis (Dong et al., 2002; Sheppard et al., 2004; Parry et al., 2005). Under normal conditions, these immune checkpoints temper or fine-tune the host immune response to pathogens. However, in the context of cancer, immune checkpoints can be dysregulated or hijacked as a mechanism of immune resistance.

An improved understanding of these molecular mechanisms underlying immune regulation has resurrected the concept of targeting cancer immunologically (Pardoll, 2012; Dolan and Gupta, 2014). Consequently, immunotherapeutic strategies designed to re-activate anti-tumour immune responses and reverse the immunologically tolerant state are now at the forefront of anti-cancer therapy. Similarly, recent elucidation of the role of epigenetics in immune evasion has

Abbreviations: TME, tumour microenvironment; PD-1, programmed cell death 1; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; PTMs, post-translational histone modifications; DNMTi, DNA methyltransferase inhibitor; HDACi, histone deacetylase inhibitor; TAA, tumour-associated antigens; APM, antigen processing and presentation machinery; NK, natural killer; NKG2D, NK group 2D; MICA/B, MHC class I-related chain A/B; ULBPs, ULB16-binding proteins; TRAIL, TNF-related-apoptosis inducing ligand; FASL, FAS ligand; DC, dendritic cell; APC, antigen presenting cells; TFH, T follicular helper; Treg, regulatory T cell; CTL, cytotoxic T lymphocyte; TCR, T cell receptor; HLA, human leukocyte antigen; TAP, transporter associated with antigen presenting; ICAM-1, intercellular adhesion molecule 1; TILs, tumour-infiltrating lymphocytes; CTLA-4, cytotoxic T lymphocyte antigen 4; TNBC, :triple-negative breast cancer; NSCLC, non-small cell lung cancer; AML, acute myeloid leukaemia; CCL, chronic lymphatic leukaemia; mAb, monoclonal antibody; FDA, Food and Drug administration; Ig, immunoglobin; PI3K, phosphoinositide 3-kinase; MDSC, myeloid-derived suppressor cell; CAF, cancer-associated fibroblast; CSC, cancer stem cell; HGF, hepatocyte grown factor; CTAs, cancer testis antigens; HMW-MAA, high molecular weight melanoma-associated protein; Th1, T helper 1; EZH2, enhancer of zeste homologue 2; H3K27, me3: histone 3 lysing 27 trimethylation; DNMT1, DNA methyltransferase 1; ERVs, endogenous retroviral sequences; 5-AZA-dC, 5-aza-2′-deoxycytidine

* Corresponding author.
E-mail addresses: Jenny.Dunn@canberra.edu.au (J. Dunn), Sudha.Rao@canberra.edu.au (S. Rao).

http://dx.doi.org/10.1016/j.molimm.2017.04.012
Received 26 December 2016; Received in revised form 14 April 2017; Accepted 22 April 2017
Available online 14 May 2017

uncovered a role for epigenetic drugs in modulating immune pathways to restore and/or improve immune recognition and immunogenicity. In this way, epigenetic targeting may 'prime' the host immune response for subsequent immunotherapy (Sigalotti et al., 2014; Heninger et al., 2015; Terranova-Barberio et al., 2016). Several studies have demonstrated the efficacy this combined strategy in both clinical studies (Bao et al., 2011; Ishibashi et al., 2016; Krishnadas et al., 2015; Xu et al., 2016) and animal models (Mikyskova et al., 2014; Terracina et al., 2016; Lucarini et al., 2017; Covre et al., 2015; Tellez et al., 2014). Furthermore, immune priming using different epigenetics agents has been observed in combinations with several immunotherapy types such as adoptive cellular immunotherapy (Ishibashi et al., 2016; Terracina et al., 2016), cytokine-based therapy (Lucarini et al., 2017; Gollob and Sciambi, 2007), vaccines (Krishnadas et al., 2015), and immune checkpoint inhibitors (Jazirehi et al., 2014; Yao et al., 2013). Together, these discoveries establish a highly promising basis for combination studies using epigenetic and immunotherapeutic agents in cancer patients.

Even though the concept of partnering epigenetic therapy with immune re-activating strategies such as immune checkpoint therapy is recent, a wave of translational research highlights the potential for this approach in many different cancer types (Terranova-Barberio et al., 2016; Maio et al., 2015; Weintraub, 2016; Chiappinelli et al., 2016a). Furthermore, a number of on-going clinical trials are currently exploring the efficacy of this combined approach (Table 1). This review summarises our current understanding of the key mechanisms of immune evasion in cancer and emphasises the significance of epigenetic immunomodulation of these components in priming the host immune system to immunotherapies. In addition, we highlight the promise of combination epigenetic and immunotherapy regimens, particularly immune checkpoint blockade, for improving outcomes in patients with cancer.

2. Epigenetic therapy

Epigenetic dysregulation is a central mechanism in cancer development and progression (Jones and Baylin, 2002; Esteller, 2008). Epigenetic regulation is defined as heritable modifications to DNA that alter gene expression and chromatin structure without changes to the underlying nucleotide sequence (Esteller, 2008; Jones and Takai, 2001). These epigenetic changes (or marks) include DNA methylation and post-translational histone modifications (PTMs) (Jones and Takai, 2001; Kouzarides, 2007). Epigenetic marks are interdependent, switching genes 'on' and 'off' in response to extracellular signals. With regard to transcriptional regulation, chromatin predominantly exists in two interchangeable states: closed (heterchromatin) or open (euchromatin), which are regulated by a balance between distinct active and repressive epigenetic marks (Fig. 1). Establishing a repressive chromatin structure can preclude access and/or function of transcriptional activators such as RNA polymerases and DNA-binding transcription factors to target genes, and this state is generally associated with transcriptional silencing. In contrast, an open chromatin state is accessible to transcriptional machinery and facilitates active transcription (Li et al., 2007).

Chromatin remodelling regulates a gene's transcriptional state via a number of mechanisms: (1) post-translational modifications of histone proteins; (2) DNA methylation; (3) ATP-dependent chromatin remodelling complexes; (4) histone variant exchange; and (5) the action of non-coding RNAs (such as miRNAs). The most abundant histone modifications are acetylation, methylation, phosphorylation, and ubiquitylation; however, many other modifications have been reported (Kouzarides, 2007). In this way, epigenetic modifications to DNA and histone proteins dynamically shape the chromatin landscape to regulate gene transcription.

Several epigenetic marks have been identified in association with specific chromatin states and transcription levels. DNA methylation predominately occurs at cytosine residues in CpG dinucleotides that are enriched in regions known as CpG islands and is associated with the closed heterochromatin state and transcriptional repression/silencing. Epigenetic modifications to the amino-terminal tails of histone proteins have also been shown to regulate chromatin state and transcription. Histone acetylation of lysine residues (e.g., acetylation of H3K9, H3K14, H4K5, and H4K16) is predominately associated with open chromatin states and active gene transcription. In contrast, histone methylation is more complex and results in different chromatin and transcription states depending on the extent of methylation (e.g., mono-, di-, or tri-methylation). For example, monomethylation of H3K9, H3K27, and H3K79 histone proteins is associated with euchromatin (active transcription), whereas trimethylation of these histones results in a heterochromatin conformation and transcriptional repression.

In addition to the local chromatin state, the 3D nuclear architecture also contributes to transcriptional regulation (Espada and Esteller, 2007; Fedorova and Zink, 2008; Bartova et al., 2008; Schneider and Grosschedl, 2007). Chromatin is spatially organised into higher-order structures that ultimately exhibit a non-random 3D organisation within cell nuclei. The nucleus is an extremely dynamic structure in which many components rapidly and transiently interact, and these dynamic interactions have functional consequences for regulation of gene expression. For example, chromatin domains containing transcriptionally active genes can form chromatin loops that extend away from compact chromosome territories to reposition near transcriptional factories at the center of the nucleus. However, perinuclear repositioning has also been shown to establish transcriptionally silent chromatin. The organisation of the nuclear architecture is thought to mediate gene transcription by controlling accessibility of regulatory DNA elements to transcription factors and RNA polymerases through subnuclear gene positioning and intra-/inter-chromosomal interactions. The impact of nuclear architecture and gene activity is closely related to epigenetic modifications (such as DNA methylation and histone modifications) of individual chromatin domains. For example, it is well established that changes in nuclear organisation are associated with DNA methylation patterns during mammalian pre-implantation development (Bartova et al., 2008; Schneider and Grosschedl, 2007). Furthermore, several inhibitors of histone deacetylase activity have been shown to induce reorganisation of chromatin and histone modifications (Taddei et al., 2001; Bartova et al., 2005). Moreover, chromosome instability and disrupted nuclear morphology is commonly associated with DNA hypomethylation of discrete nuclear regions in cancer cells (Bartova et al., 2008). However, the precise interplay between epigenetic modifications and nuclear architecture remains unclear. In this way, the nuclear architecture is able to contribute, in part, to regulation of gene expression.

Due to the dynamic and reversible nature of epigenetic marks, these alterations represent attractive and therapeutically relevant targets in many diseases including cancer. Current epigenetic therapies are primarily directed towards two functional categories of epigenetic regulators: those that target the "writers", enzymes that establish epigenetic marks, and those that target the "erasers", enzymes that remove epigenetic marks. Specifically, DNA methyltransferase inhibitors (DNMTi; writers) and histone deacetylase inhibitors (HDACis; erasers) are the main epigenetic therapy drug classes. DNMT and HDAC inhibitors exhibit anti-tumour functions by inducing differentiation, apoptosis, growth inhibition, cell cycle arrest, and cell death. DNMTis reactivate gene transcription by inhibiting the action of DNA methyltransferases (which add methyl groups to DNA) by directly incorporating into the DNA and trapping DNMTs for proteosomal degradation. The loss of DNMT is DNA replication dependent, and results in passive hypomethylation of DNA in daughter cells after cell division. Similarly, HDACis block the action of HDACs, which remove acetyl marks from tagged histones to increase global histone acetylation. These inhibitors might also work, at least in part, to re-activate

Table 1
Current clinical trials combining checkpoint inhibitors and epigenetic drugs in various cancer types.

ClinicalTrials.gov identifier	Recruitment status	Phase	Cancer type	Immune checkpoint inhibitor/s	Epigenetic drug/s	Other drugs
NCT02437136	Recruiting	Ib/II	NSCLC and melanoma	Pembrolizumab	Entinostat	
NCT02936752	Not yet recruiting	Ib	MDS following DNMTi-failed therapy	Pembrolizumab	Entionstat	
NCT02546986	Active, not recruiting	II	Advanced/metastatic NSCLC	Pembrolizumab	Oral azacytidine	
NCT02909452	Recruiting	I	Advanced solid tumours	Pembrolizumab	Entinostat	
NCT02697630	Not yet recruiting	II	Metastatic uveal melanoma	Pembrolizumab	Eninostat	
NCT02538510	Recruiting	I/II	Recurrent unresectable/ metastatic HNSCC and SGC	Pembrolizumab	Vorinostat	
NCT02638090	Recruiting	I/II	Stage IV NSCLC	Pembrolizumab	Vorinostat	
NCT02619253	Recruiting	I/Ib	Advanced renal or urothelial cell carcinoma	Pembrolizumab	Vorinostat	
NCT02395627	Recruiting	II	Hormone resistant BC	Pembrolizumab	Vorinostat	Tamoxifen
NCT02901899	Not yet recruiting	II	PR recurrent OC	Pembrolizumab	Guadecitabine	
NCT02900560	Not yet recruiting	II	PR epithelial OC	Pembrolizumab	Oral azacytidine	
NCT02512172	Recruiting	I	MSS advanced CRC	Pembrolizumab	Romidepsin with/without oral azacytidine	
NCT02260440	Active, not recruiting	II	Chemo-refractory metastatic CRC	Pembrolizumab	Azacytidine	
NCT02845297	Recruiting	II	Relapsed/refractory AML	Pembrolizumab	Azacytidine	
NCT02816021	Not yet recruiting	II	MM	Pembrolizumab	Azacytidine	
NCT01928576	Recruiting	II	NSCLC	Nivolumab	Azacytidine with/without entinostat	
NCT02518958	Recruiting	I	Advanced solid tumours or lymphomas	Nivolumab	RRx-001	
NCT02397720	Recruiting	II	AML	Nivolumab	Azacytidine	
NCT02599649	Recruiting	II	MSS	Lirilumab and nivolumab	Azacytidine	
NCT02530463	Recruiting	II	MDS	Nivolumab and/or lirilumab	Azacytidine	
NCT02664181	Not yet recruiting	II	Advanced NSCLC	Nivolumab	Decitabine	Oral THU
NCT02795923	Not yet recruiting	II	NSCLC	Nivolumab	Oral decitabine	Tetrahydrouridine
NCT02543620	Recruiting	I	Metastatic unresectable HER2-negative BC	Nivolumab with/without ipilimumab	Entinostat	
NCT02635061	Not yet recruiting	Ib	Unresectable NSCLC	Nivolumab and ipilimumab	ACY-241	
NCT02890329	Not yet recruiting	I	Relapsed/refractory MDS or AML	Ipilimumab	Decitabine	
NCT02608437	Recruiting	Ib	MM	Ipilimumab	SGI-110	
NCT02032810	Recruiting	I	Unresectable stage III/IV melanoma	Ipilimumab	Panobinostat	
NCT02508870	Recruiting	I	MDS	Atezolizumab	Azacytidine	
NCT02708680	Not yet recruiting	Ib/II	TNBC	Atezolizumab	Entinostat	
NCT0281197	Recruiting	II	MSS-CRC, PR-OC, ER-positive and HER2-negative BC	Durvalumab	Azacytidine	
NCT02281084	Recruiting	II	MDS	Durvalumab	Oral azacytidine	
NCT02117219	Recruiting	I	MDS	Durvalumab with or without tremelimumab	Azacytidine	
NCT02775903	Recruiting	II	MDS, AML	Durvalumab	Azacytidine	
NCT02805660	Recruiting	I/II	Advanced solid tumours and NSCLC	Durvalumab	Mocetinostat	
NCT02915523	No yet recruiting	Ib/II	Refractory/recurrent epithelial OC	Avelumab	Entinostat	

Note: All information on current clinical trials was obtained from ClinicalTrials.gov. Abbreviations: NSCLC: non-small cell lung cancer; MDS: myelodysplastic; DNMTi: DNA methyltransferase inhibitor; HNSCC: head and neck squamous cell carcinoma; SGC: salivary gland cancer; BC: breast cancer; PR: platinum resistant; OC: ovarian cancer; MSS: microsatellite stable; CRC: colorectal cancer; AML: acute myeloid leukaemia; MM: metastatic melanoma; TNBC: triple-negative breast cancer.

gene expression by altering the global nuclear architecture. Loss of DNA methylation and/or increase in histone acetylation can result in a relaxed chromatin configuration, enabling access to transcriptional activators to restore gene transcription. Epigenetic drugs targeting these enzymes can restore, and in some cases overexpress, genes that have been epigenetically silenced in both immune and cancer cells (Sigalotti et al., 2014; Maio et al., 2015; Chiappinelli et al., 2016a). Combining DNMT and HDAC inhibitors generally results in greater re-expression of epigenetically silenced tumour suppressor genes and cell cycle regulators (Tellez et al., 2014).

3. Mechanisms of immune escape in cancer

3.1. Loss of tumour-associated antigens (TAAs), antigen processing and presentation machinery (APM), and co-stimulatory molecules

Both the adaptive and innate arms of the immune system contribute to the immune recognition of malignant cells. The innate immune response relies primarily on natural killer (NK) cells to eliminate malignant cells. NK cells possess activating surface receptors such as NK group 2D (NKGD2), which identify abnormal cells expressing stress-induced ligands (e.g., MHC class I-related chain A and B [MICA, MICB], ULB16-binding proteins [ULBPs]) (Lanier, 2005; Raulet et al., 2013; Waldhauer and Steinle, 2008). NK cell killing of targeted cells is subsequently achieved through engagement of death receptors (e.g., FAS, TNF-related-apoptosis inducing ligand [TRAIL] receptor) on target

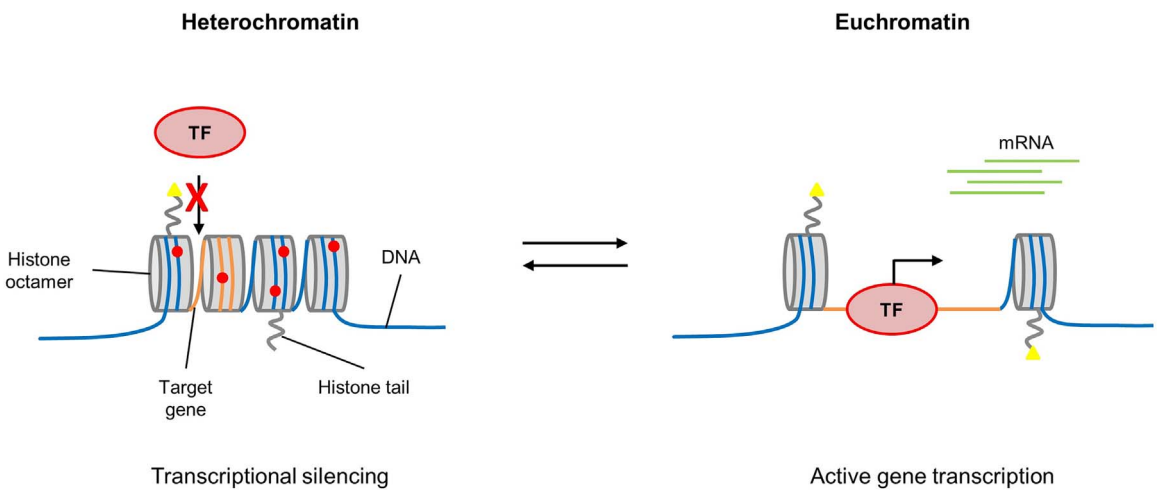

Fig. 1. Epigenetic marks alter DNA accessibility to transcriptional machinery to regulate gene transcription. Epigenetic marks, such as DNA methylation and histone modifications, together determine chromatin accessibility and transcriptional activity. Establishment of repressive epigenetic marks (such as DNA methylation, red dots) are associated with a closed heterochromatin (nucleosomal dense) structure can preclude access and function of transcriptional activators such as RNA polymerases and DNA binding transcription factors (TFs) to target genes, and is generally associated with transcriptional silencing. In contrast, active epigenetic marks (e.g., histone acetylation, yellow triangles) are associated with an open euchromatin state (nucleosomal loss) that is accessible to transcriptional machinery and facilitates active transcription. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cells by NK cell-expressed ligands (e.g., FAS ligand [FASL], TRAIL) and/or the release of cytotoxic granules such as granzymes and perforin (Raulet et al., 2013). Together, these mechanisms contribute to apoptosis of the target tumour cell.

Other innate immune system cells include dendritic cells (DCs) and macrophages, their primary role being to act as professional antigen presenting cells (APCs) and activate the antigen-specific adaptive immune system. Tumour and stromal-derived factors (e.g., growth factors and immunosuppressive cytokines) present in the TME can disrupt the anti-tumour function of macrophages and re-educate them to become tumour-associated macrophages (TAMs) with an M2 immunosuppressive phenotype (predominant in healing tissue) (Bingle et al., 2002; Sica et al., 2006). M2 or repair-type cells promote tumour initiation, progression, and metastasis by producing growth factors (e.g., EGF, FGF-2), angiogenic factors (e.g., VEGF, MMP-9), and inflammatory cytokines (e.g., TNF-α, IL-1) in much the same way that they function in would healing (Bingle et al., 2002).

On APC-mediated activation, naive $CD4^+$ T cells and $CD8^+$ T cells differentiate into different antigen-specific T cell subsets. For example, activation of $CD4^+$ T cells can give rise to T helper cells (such as Th1, Th2, Th17 cells), T follicular helper (TFH) cells, and regulatory T (Treg) cells, whereas activation of naive $CD8^+$ T cells gives rise to effector cytotoxic T lymphocytes (CTLs).

In contrast to the innate arm, cells of the adaptive immune system possess the ability to recognise specific targets and differentiate "self" from "non-self" antigens (Gajewski et al., 2013). Furthermore, activation of adaptive immune cells often results in immunological memory, a feature that enables rapid re-activation of the immune response on future encounters with specific antigens (Dunn et al., 2015). In the TME, adaptive immune responses are primarily mediated by CTLs, the adaptive counterpart to innate NK cells. Similar to NK cells, CTLs induce cancer cell death through death receptor interactions and the secretion of cytotoxic granules (Barry and Bleackley, 2002).

CTL activation is triggered by engagement of the T cell receptor (TCR) to a peptide-human leukocyte antigen (HLA) class I molecule complex expressed on APCs along with a co-stimulatory/accessory signal (for example, the interaction of B7 molecules [CD80 or CD86] on APCs with CD28 on T cells) (Barry and Bleackley, 2002). Assembly of the peptide-HLA complex is a multi-step process involving many different molecules that collectively comprise the antigen processing and presentation machinery (APM; summarised in (Maio et al., 2015; Chiappinelli et al., 2016a)). In turn, peptide-mediated CTL activation induces an intracellular signalling cascade that results in cytokine production and cellular proliferation.

While T cells are a critical cell in anti-tumour immunity, TFH cells, which function to induce B cell differentiation into antibody-secreting cells, have recently been shown to display a key role in the pathogenesis of several cancers in addition to anti-tumour functions (Jia et al., 2015; Ahearne et al., 2014; Gu-Trantien et al., 2013; Shi et al., 2014). For example, patients with hepatocellular carcinoma had fewer and impaired TFH cells compared to control patients, and the TFH cells were associated with disease progression (Jia et al., 2015).

Tregs are also an abundant T cell subpopulation within the TME that are generally immunosuppressive and can suppress or downregulate CTL induction and proliferation (Nishikawa and Sakaguchi, 2014). In the TME, the tumour cells preferentially recruit Tregs, which proliferate in response to tumour-secreting cytokines such as TGFβ. Due to their immunosuppressive role, greater numbers of Tregs are typically associated with poorer prognosis in several cancer types (Wolf et al., 2005; Fu et al., 2007; Curiel et al., 2004; Petersen et al., 2006; Bates et al., 2006; Tang et al., 2014). Furthermore, Treg depletion has been shown to improve responses to immunotherapies (Sutmuller et al., 2001; Comes et al., 2006; Smyth et al., 2006). However, the presence of Tregs has also been associated with positive outcomes, suggesting that the role of Tregs is context dependent (Badoual et al., 2006; Salama et al., 2009; Leffers et al., 2009; Correale et al., 2010).

Malignant cells escape immune-mediated cell death by deploying epigenetic mechanisms to evade host immune recognition and immunogenicity. This acquired immune evasive phenotype is achieved by epigenetic downregulation of many critical molecules required for efficient cancer and immune cell interactions; for example, suppression of TAAs, reduced expression of many APM components, and low cell surface levels of accessory/co-stimulatory molecules, death receptors, and stress-induced ligands.

Reduced expression of TAAs and APM components is correlated with metastasis in several studies (Liu et al., 2012). Negative expression of the co-stimulatory molecule intercellular adhesion molecule 1 (ICAM-1) is associated with cancer progression in oral squamous cell carcinoma and colorectal cancer (Maeda et al., 2000; Usami et al., 2013). For instance, immunohistochemical analysis of metastatic melanoma lesions revealed significant downregulation of the APM chaperones calnexin and calreticulin compared to primary melanoma lesions (Dissemond et al., 2004). Similarly, loss of the TAP-1 and TAP-2 transport proteins and HLA class I molecules have been reported in

Table 2
Characteristics of current FDA-approved checkpoint inhibitors.

Checkpoint target	CTLA-4	PD-1		PD-L1
Drug	Ipilimumab	Nivolumab	Pembrolizumab	Atezolizumab
Brand name	Yervoy	Opdivo	Keytruda	Tecentriq
Developing company	Bristol-Myers Squibb	Bristol-Myers Squibb	Merck & Co*	Genetech/ Roche
FDA-approved indications	Unresectable or metastatic melanoma, adjuvant therapy for stage 3 melanoma	Unresectable or metastatic melanoma, metastatic NSCLC, advanced RCC, Hodgkin lymphoma	Unresectable or metastatic melanoma, metastatic NSCLC, recurrent of metastatic HNSCC	Urothelial carcinoma

Abbreviations: CTLA-4: cytotoxic T lymphocyte antigen 4, PD-1: programmed cell death 1; PD-L1: programmed death ligand 1; NSCLC: non-small cell lung cancer; RCC: renal cell carcinoma; HNSCC: head and neck squamous cell carcinoma. *Known as MSD outside the United States and Canada.

metastatic and high-grade primary versus low-grade primary breast cancer (Kaklamanis et al., 1995; Vitale et al., 1998).

Downregulated expression of any APM molecule can reduce or eliminate the presentation of cell surface peptide-HLA complexes, effectively rendering cancer cells invisible to CTLs and preventing recognition and elimination of cancer cells by the adaptive immune response. In addition, suppression of co-stimulatory molecules can result in CTLs only receiving a partial activating signal which can lead to a state of cell exhaustion, a key feature of which is the increased expression of inhibitory receptors on the T cell surface (e.g., PD-1) (Pardoll, 2012). Together, repression of these APM and co-stimulatory components contributes to the poor immunogenicity of some cancers and facilitates immune evasion and tumour progression.

3.2. Immune checkpoints

The commandeering of immune checkpoints expressed on the surface of CTLs further impedes the immune-mediated cell death of malignant cells. A balance between co-stimulatory and co-inhibitory molecules – known as immune checkpoint receptors – that collectively regulate the duration, quality, and amplitude of the physiological immune response finely regulate the TCR response. In response to pathological infection, these inhibitory pathways normally maintain self-tolerance (the prevention of autoimmunity) and minimise collateral tissue damage from hyperactive T cells.

In the cancer setting, malignant cells are able to switch on or hijack these immune checkpoint pathways as a major mechanism of immune resistance, particularly against CTLs. Cancer cells take advantage of this regulatory mechanism by increasing cell surface expression of co-inhibitory ligands such as PD-L1. Therefore, whilst tumour-infiltrating lymphocytes (TILs) may be present in the TME, they cannot mount an immune response to destroy target cancer cells. Moreover, it has recently been recognised that some tumours exhibit redundancy between different inhibitory pathways, enabling them to resist immune checkpoint therapy and facilitating tumour progression (Koyama et al., 2016).

Two key checkpoint receptors have been extensively studied for immunotherapy: cytotoxic T lymphocyte antigen 4 (CTLA-4) and PD-1. While CTLA-4 is essential during early activation of T cells in secondary lymphoid organs, PD-1 is primarily involved in modulating T cell activation in peripheral tissues including the TME (Pardoll, 2012). CTLA-4, expressed on T cells, competes with the stimulatory receptor CD28 for binding to CD80/CD86 ligands on APCs. The PD-1 receptor is expressed on T cells, B cells, NK cells, monocytes, macrophages, and DCs (Pardoll, 2012). PD-1 binds to two ligands, PD-L1 and PD-L2. PD-L1 is expressed on a variety of cell types including epithelium, muscle, mesenchymal stem cells, T and B cells, DCs, macrophages, and cancer cells, while PD-L2 expression is restricted to immune-related cells such as DCs, macrophages, and mast cells (Pardoll, 2012). Therefore, interfering with the PD-1/PD-L1 pathway may potentially prevent inhibitory signalling and block T cell suppression throughout the TME.

PD-1/L1 is overexpressed in several tumour types including melanoma, ovarian cancer, triple-negative (TNBC) and HER2+ breast cancers, non-small cell lung cancer (NSCLC), and haematological malignancies such as acute myeloid leukaemia (AML) and chronic lymphatic leukaemia (CCL) (Terranova-Barberio et al., 2016).

3.2.1. Immune checkpoint blockade

Monoclonal antibodies (mAbs) that target checkpoint inhibitors such as CTLA-4 (e.g., ipilimumab) and PD-1 (e.g., nivolumab, pembrolizumab) have emerged as powerful weapons against cancer and show significant promise in the treatment of an expanding list of tumour types (Hodi et al., 2010; Ansell et al., 2015; Brahmer et al., 2015).

Currently, there are only four US Food and Drug Administration (FDA)-approved immune checkpoint inhibitors: ipiliumab (Yervoy), a fully human immunoglobulin (Ig) G1 antibody targeting CTLA-4; nivolumab (Opdivo), a human monoclonal IgG4 anti-PD-1 antibody; pembrolizumab (Keytruda), a mAb targeting PD-L1; and atezolizumab (Tecentriq), an IgG1 mAb against PD-L1, which was recently approved to treat locally advanced or metastatic urothelial carcinoma in patients that had failed chemo- or radiotherapy (Table 2) (Markham, 2016; FDA, 2016). Several other checkpoint inhibitors are also being investigated in current clinical trials in readiness for approval (such as lirilumab) (Table 1).

Upon ligation to PD-L1/2, PD-1 suppresses downstream phosphoinositide 3-kinase (PI3K) and Akt signalling via an immunoreceptor tyrosine-based inhibitory motif. In contrast, engagement of the CTLA-4 receptor inhibits PI3K-independent Akt signalling. PD-1/PD-L1 interaction dephosphorylates proteins immediately downstream of the TCR via recruitment of SHP-2 enzymes. Both of these pathways block the TCR response.

These mAbs work by binding to their specific target molecule and blocking receptor-ligand interactions to prevent inhibitory signalling (from the cancer cell). This enables the CTL to elicit an immune response against the target cell. Ipilimumab, a monoclonal antibody that binds to CTLA-4, blocks CTLA-4-CD80/CD86 interactions, while nivolumab or pembrolizumab bind to PD-1 and block engagement to PD-L1 and PD-L2. Atezolizumab blocks the interaction of PD-L1 with PD-1 and CD80. CTLA-4 and PD-1/L1 pathway blockade have been shown to augment T cell activation by inhibiting CTL proliferation and cytokine production, leading to T cell dysfunction or apoptosis (Dong et al., 2002; Sheppard et al., 2004; Parry et al., 2005). In particular, CTLA-4 inhibition plays a major role in suppressing the Treg function. An improved understanding of the mechanisms of action of immune checkpoint inhibitors will help to further broaden the therapeutic impact of these novel anticancer agents.

In particular, PD-1/PD-L1 pathway-targeting immunotherapies have shown highly promising efficacy in a broad spectrum of cancers (Ansell et al., 2015; Brahmer et al., 2015; Topalian et al., 2014). For example, in a phase 3 study comparing nivolumab alone with docetaxel monotherapy in patients with advanced squamous cell NSCLC, nivolumab treatment was associated with a 41% lower risk of death, a 3.2-month longer median survival (9.2 versus 6.0 months), and nearly twice the 1-year survival rate compared to docetaxel monotherapy (Brahmer et al., 2015).

Melanoma has been the most responsive solid tumour to checkpoint blockade, with 20% of patients responding to therapy (Hodi et al., 2010). Patients with advanced melanoma (that have received prior standard therapies) receiving nivolumab have shown long-term responses (1- and 2-year survival rates of 62% and 43%, respectively),

with a median overall survival of 16.8 months (Topalian et al., 2014). Furthermore, dual immune checkpoint therapy has been shown to increase response rates in patients with advanced melanoma. Previously untreated advanced melanoma patients demonstrated significantly longer progression-free survival and objective responses following combined nivolumab and ipilimumab therapy (11.5 months, 57.6%) than patients treated with ipilimumab alone (2.9 months, 43.7%) and in the nivolumab monotherapy (6.9 months, 19%) arm compared to ipilimumab alone (Larkin et al., 2015). Moreover, subgroups of patients with tumours harbouring *BRAF* mutations or positive PD-L1 tumour status experienced longer progression-free survival compared to patients with *BRAF* mutation- or PD-L1-negative tumours.

3.3. Other immunosuppressive cells in the TME

Anti-tumour immunity within the TME can be suppressed by a variety of tumour-infiltrating leukocytes including Tregs, M2 macrophages, myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts (CAFs), and cancer stem cells (CSCs) (Lindau et al., 2013). These different cell types employ multiple mechanisms to suppress the immune response including the secretion of inhibitory cytokines (e.g., IL-10, TGFβ), cell surface expression of co-inhibitory receptors (immune checkpoints), and release of amino acid-depleting enzymes (e.g., arginase and IDO) (Lindau et al., 2013).

Cancer cells have been shown to induce epigenetic changes in normal fibroblasts to transform them into CAFs (Martinez-Outschoorn et al., 2010; Tyan et al., 2011). For example, Tyan et al. showed that breast cancer cells induce hepatocyte growth factor (HGF) secretion by CAFs to enhance tumorigenesis; when normal fibroblasts were cultured with the breast cancer cell line MDA-MB-231, they secreted HGF and adopted a CAF phenotype (Tyan et al., 2011). In turn, CAFs have been shown to promote tumour growth and progression through epigenetic mechanisms such as inducing epithelial-to-mesenchymal transition and cancer stem cell phenotypes in breast cancer cells (Soon et al., 2013; Yu et al., 2014).

4. Epigenetic immunomodulation of the TME primes the immune system for immunotherapy

4.1. Epithelial cancer cells

The capacity of epigenetic drugs such as DMNTis and HDACis to upregulate expression of immune signalling components in cancer cells is well established (Sigalotti et al., 2014; Chiappinelli et al., 2016a; Larkin et al., 2015). Epigenetic drugs upregulate the expression of TAAs, essentially all APM components, surface expression of co-stimulatory molecules, stress-induced ligands and death-inducing receptors, and the expression of checkpoint ligands on tumour cells.

One mechanism by which epigenetic drugs act to restore or improve cancer cell recognition is through increased expression of TAAs. Cancer testis antigens (CTAs) are the best characterised class of epigenetically-regulated TAAs, and epigenetic treatment augments their expression (Fratta et al., 2011). CTAs are expressed in embryonic and germ cells but silenced in mature somatic cells by DNA methylation at the gene promoter (Fratta et al., 2011; James et al., 2006). DNMTis can cause demethylation, resulting in re-expression of CTAs in cancer cells in many different solid tumours (Fratta et al., 2011; James et al., 2006; Weber et al., 1994; Coral et al., 2002; Li et al., 2014). Although HDACis have also been shown to upregulate CTAs, induction is much lower than with DNMTis (Wischnewski et al., 2006). Moreover, combined DNMTi/HDACi treatment upregulates CTAs in some, but not all, cell lines; however, unlike DNMTis alone, the increased expression is not durable. Furthermore, dual epigenetic therapy does not necessarily result in increased recognition and lysis of malignant cells (Weiser et al., 2001).

In addition to CTAs, other TAAs such as high molecular weight melanoma-associated antigens (HMW-MAAs) have been shown to undergo demethylation at the gene promoter with the DNMTi 5-AZA-CdR (decitabine) in melanoma cells, resulting in re-expression of HMW-MAA at both the mRNA and protein levels (Luo et al., 2006). However, not all TAAs are upregulated by HDACis (Roulois et al., 2012).

Epigenetic dysregulation of APM components is thought to be responsible for their reduced expression in cancer. Furthermore, epigenetic regulation can be direct or indirect (Bukur et al., 2012). Both DNMTis and HDACis induce or enhance expression of many APM pathway components including MHC molecules, TAP-1, TAP-2, LMP2, LMP7, and tapasin in a broad range of tumour types (Khan et al., 2008; Setiadi et al., 2008; Magner et al., 2000).

Besides APM components, it is also well established that exposure to epigenetic agents can upregulate surface expression of several co-stimulatory molecules (e.g., CD40, CD80, CD86, and ICAM-1) on tumour cells in addition to enhancing expression of death receptors and stress-induced ligands (Maeda et al., 2000; Magner et al., 2000; Wang et al., 2013; Armeanu et al., 2005; Lopez-Soto et al., 2009; Nakata et al., 2004; Insinga et al., 2005). In particular, these immunomodulatory events increase their sensitivity to immune-mediated cells lysis.

Moreover, epigenetic drugs have been shown to sensitise cancer cells to immune checkpoint therapy by upregulating the immune checkpoints CTLA-4, PD-1, PD-L1, and PD-L2 on tumour cells and TILs, providing a putative mechanism of immune escape (Li et al., 2014; Wrangle et al., 2013; Yang et al., 2014). Furthermore, high tumour cell and TIL expression of PD-L1 has been correlated with good clinical responses to anti-PD-1/PD-L1 therapy (Taube et al., 2014; Herbst et al., 2014).

4.2. Adaptive and innate host immune cells

Epigenetic drugs can also modulate host immune cells as well as epithelial cancer cells. A recently identified mechanism of tumour immune escape is through the epigenetic repression of chemokines important for immune cell infiltration of the TME. Chemokine repression abrogates T cell trafficking, protecting tumour cells from immune responses. In ovarian cancer, tumour production of the T helper 1 (Th1)-type chemokines CXCL9 and CXCL10 is epigenetically repressed by enhancer of zeste homologue 2 (EZH2)-mediated histone H3 lysine 27 trimethylation (H3K27me3) and DNA methyltransferase 1 (DNMT1)-mediated DNA methylation (Peng et al., 2015). Epigenetic modulation using a DNMTi was able to induce chemokine expression and Th1 tumour infiltration. Moreover, HDACis have also been shown to enhance T cell chemokine expression and TME infiltration in lung cancer (Zheng et al., 2016).

The innate immune system can also exploit the action of epigenetic drugs to increase tumour cell recognition and immune-mediated cell lysis. For instance, HDACi treatment can increase expression of the activating receptor NKG2D on the surface of NK cells by increased binding of H3 acetylation across the gene promoter (Zhu et al., 2015). Several different HDACis have also been shown to enhance NK-mediated tumour cell targeting by upregulating the stress-inducing ligands MICA, MICB, and/or ULBP1-3 in tumour cells from many different solid malignancies to increase NK cell killing of tumour cells (Armeanu et al., 2005; Lopez-Soto et al., 2009; Skov et al., 2005; Schmudde et al., 2008; Yamanegi et al., 2012; Berghuis et al., 2012). Furthermore, HDACis with or without DNMTis have been shown to enhance NK cell killing by increasing the expression of death-inducing receptors FAS and TRAIL-R2 on cancer cells (Nakata et al., 2004; Insinga et al., 2005; Yang et al., 2012; Lundqvist et al., 2006). Interestingly, contrary to the results of these studies, Fiegler et al. (Fiegler et al., 2013) recently reported downregulation of B7-H6, a ligand for the activating receptor NKp30, on NK cells following treatment with HDACis in multiple human cancer cell lines. Furthermore, there were reduced NKp30-dependent effector functions in NK

cells (Fiegler et al., 2013). These differences may be due to previous studies using effector populations that primarily exert NKG2D-dependent functions (and decreased NKp30-dependent effector functions), which contributed to an overall increase in NK cytotoxicity in response to HDACi treatment.

Recent studies investigating the function of epigenetic drugs are revealing further mechanisms of action such as MDSC suppression (Kim et al., 2014). For example, a decreased percentage of MDSC and reduced expression of arginine-1 (which impairs T cell proliferation and cytokine production) were found in the TME and spleens of DNMTi-treated mice bearing transgenic prostate adenocarcinoma or MHC class I-deficient TC-1 tumours (Mikyskova et al., 2014). This reduction was associated with an increased percentage of $CD11c^+$ and $CD86^+$/$MHCII^+$ cells, suggesting that the mechanism of action for DNMTi in this case was a result of partial differentiation of MDSCs towards DCs in addition to induction of apoptosis. Moreover, tumour-bearing HDAC11-knockout mice demonstrated an increased suppressive MDSC population compared to wild-type tumour-bearing controls, suggesting that HDAC11 is a negative regulator of MDSC expansion and function and may be targeted using HDACis (Sahakian et al., 2015). In addition to DNA methylation and histone acetylation, several miRNAs and siRNAs have also been shown to target MDSCs by remodelling their characteristics to eliminate cancer cells (Zhang et al., 2016). Epigenetic reprogramming of MDSC differentiation toward the M-type may also be beneficial in tumour elimination as they more specifically inhibit T cell responses. Together, these studies provide further evidence of the broad role that epigenetic drugs play in immunologically priming multiple layers of the immune landscape in the TME. They also highlight the potential of epigenetic drugs to enhance immunotherapeutic outcomes in patients, especially when used as part of combination regimens (Krishnadas et al., 2015; Terracina et al., 2016; Covre et al., 2015; Gollob and Sciambi, 2007). However, it is important to stress that the resulting epigenetic changes at both the chromatin and mRNA levels in host immune cells, particularly T cells, have been significantly neglected and require future study to determine the mechanisms of action of epigenetic drugs in these cells.

4.3. 'Viral mimicry' enhances tumour cell visibility to the immune system

It is primarily thought that increased immunogenicity of tumour cells by DNMTis is mediated by reactivation of epigenetically silenced tumour suppressor programs in cancer cells. Recently, however, two groups (Roulois et al. (Roulois et al., 2015) and Chiappinelli et al. (Chiappinelli et al., 2016b)) have demonstrated that DNA demethylating agents can induce immune signalling in cancer cells by activating dsRNAs derived from endogenous retroviral sequences (ERVs) (Roulois et al., 2015; Chiappinelli et al., 2016b). ERVs, also known as long terminal repeat (LTR) retrotransposons, are an abundant class of retrotransposable elements that comprise approximately 8% of the human genome (Groh and Schotta, 2017; Mager and Stoye, 2015). ERVs are generally transcriptionally silenced by promoter DNA methylation but can be activated in several cancer types (Gimenez et al., 2010; Stengel et al., 2010). ERVs closely resemble the pro-viral integrated form of endogenous retroviruses and are therefore capable of eliciting host innate and adaptive immune responses. It was recently reported that ERV RNAs can trigger signalling via cytosolic pattern recognition receptors (PRRs) and activation of downstream mitochondrial antiviral signalling (MAVS) adaptor molecules in mammals (Zeng et al., 2014).

Roulois et al. (Roulois et al., 2015) recently demonstrated that low dose 5-AZA-CdR (decitabine) treatment of colon cancer cells induces expression of interferon-stimulated genes (ISGs) through activation of viral defence pathways responsive to dsRNAs (Roulois et al., 2015). Following 5-AZA-CdR treatment, there was a robust increase in levels of cytoplasmic dsRNA derived from de-repressed ERV transcripts. This in turn triggered the activation of cytosolic PRR pathway MAD5/MAVS and downstream activation of transcription factor IRF7 and type III interferons, ultimately upregulating the expression of late-response ISGs. Importantly, 5-AZA-CdR was shown to target colorectal cancer-initiating cells (CICs) by activation of the MDA5/MAVS/IRF7 pathway, resulting in reduced CIC frequency. In this way, DNMTis are able to "trick" or reprogram cancer cells to behave as virus-infected cells and induce an anti-viral immune response directed towards cancer cells. Similarly, Chiappinelli et al. (Chiappinelli et al., 2016b) showed that 5-azacytidine (azacytidine/AZA) and 5-AZA-CdR triggered cytosolic sensing of dsRNA in ovarian cancer cells and upregulation of type I interferon-response genes (Chiappinelli et al., 2016b). Together, these studies suggest a novel mechanism for DNMTis in tumour cells by inducing a state of 'viral mimicry' that enhances immune signalling.

However, whilst DNA demethylating agents can increase cancer cell immunogenicity, T cells may still be susceptible to repression by immune checkpoint molecules such as CTLA-4, PD-L1, and PD-L2, allowing tumour immune escape and progression. Interestingly, high viral defence signatures in tumours were significantly associated with durable clinical response in patients receiving anti-CLTA-4 immune checkpoint therapy for advanced melanoma (Chiappinelli et al., 2016b). Furthermore, Chiappinelli et al. (Chiappinelli et al., 2016b) demonstrated that 5-azacytidine sensitised tumours to anti-CTLA-4 immune checkpoint therapy compared to 5-azacytidine or anti-CTLA-4 alone in a mouse model of melanoma (Chiappinelli et al., 2016b). Overall, these studies not only highlight the potential for epigenetic and immunotherapy combination strategies in cancer, but also identify a novel set of IFN-response genes whose activation can track clinical response to immune checkpoint therapy in cancer patients.

DNA methylation was proposed to have primarily evolved in mammals as a nuclear host defence system to shield genomes from parasitic sequence elements such as transcriptionally active retrotransposable elements (Yoder et al., 1997). Active transposable elements can disrupt host genome stability in several different ways including insertion mutations, rearrangements, and chimeric mRNA, which can disrupt host gene expression. It will be important to identify other classes of novel epigenetic enzymes in addition to DNA demethylating agents that can re-educate tumour cells to activate IFN immune signalling. While DNA and histone methyltransferases are essential in heterochromatin formation, additional repressive epigenetic players are also involved in establishing EVR silencing such as the histone demethylase LSD1. A subset of ZGA genes and murine endogenous retroviruses MuERV-L/MERVL are de-repressed in mutant KDM1-deficient murine ES cells, suggesting that LSD1 is required to maintain a repressive chromatin state and silence target ERV activation during early embryogenesis (Macfarlan et al., 2011). It will be interesting to see if inhibition of other epigenetic enzymes such as histone demethylases can induce IFN signalling through activation of ERV transcripts in the cancer setting.

5. Combining epigenetic and immunotherapy to strategically fight cancer

There is, therefore, robust data to support the use of epigenetic drugs in sensitising immunotherapeutic responses via their ability to modulate immune-cancer cell interactions and reverse crucial elements of immune evasion. In an important study that paved the way for combined epigenetic and immunotherapy, dual epigenetic therapy with azacytidine and entinostat (a HDACi selective to class I HDACs) failed to demonstrate significant anti-tumour responses in patients with advanced lung cancer. However, in this study, a small number of patients with advanced NSCLC who progressed after receiving low-dose epigenetic therapy entered a trial for immune checkpoint therapy with nivolumab, an anti-PD-1 checkpoint inhibitor (Wrangle et al., 2013; Juergens et al., 2011). Remarkably, five of the six patients survived six months post-treatment without cancer progression, an unexpected outcome for immunotherapy in NSCLC. These results sparked signifi-

cant interest in the potential of combining epigenetic and immunotherapy in not only NSCLC but also in other tumour types such as melanoma and prostate and colon cancer in human and animal models (Topalian et al., 2014; Peng et al., 2015; Chiappinelli et al., 2016b).

Due to the explosion of interest in cancer immunotherapy, there is a plethora of new research on epigenetic drugs used in combination with different immunotherapies (e.g., adoptive cell therapy, immunostimulatory mAbs, cytokine-based therapy, and vaccination strategies); therefore, this review focuses specifically on combinations with immune checkpoint inhibitors.

Epigenetic modulators can enhance responses to immune checkpoint blockade through several mechanisms such as increased expression of checkpoint inhibitors on tumour cells, induction of chemokine expression on T cells, and reduction of suppressive cell populations in the TME.

As previously discussed, epigenetic modulators enhance cell surface expression of immune checkpoints. Several studies have shown that increased expression of checkpoint inhibitors on tumour cells following epigenetic treatment increases responses to blockade therapy (Wrangle et al., 2013; Woods et al., 2015). For example, Woods et al. (Woods et al., 2015) showed that treatment with HDACis in melanoma-bearing mice upregulated PD-L1 and PD-L2 in melanomas as a result of increased histone acetylation, and combined HDACi-PD-1 inhibition slowed tumour progression and increased survival compared to single agent therapy (Woods et al., 2015).

Recently, Goswami et al. (Goswami et al., 2016) showed that combined inhibition of H3K27me3 (an important epigenetic mark required to maintain Treg function) and anti-CTLA-4 reduced tumour size and the number of Tregs in B16-F10 melanoma-bearing mice compared to anti-CTLA-4 treatment alone, suggesting that H3K27me3 inhibition may increase the efficacy of immune checkpoint therapy (Goswami et al., 2016).

In addition, epigenetic modulators have been shown to increase T cell infiltration into the TME and augment responses to immune checkpoint blockade through the removal of epigenetic marks suppressing chemokine expression in ovarian and lung cancer (Peng et al., 2015; Zheng et al., 2016; Wang et al., 2015). Treatment of ovarian cancer cells with the DNMTi 5aza2′deoxycytidine (5AZAdC) upregulated tumour production of Th-1 chemokines CXCL9 and CXCL10, resulting in increased tumour infiltration with T cells and an improved therapeutic response to PD-L1 checkpoint blockade compared to single therapy alone (Peng et al., 2015). Furthermore, HDACis have been shown to upregulate T cell chemokine expression and enhance responses to PD-1 therapy in lung cancer. (Zheng et al., 2016). However the underlying mechanisms responsible for the increase in chemokine expression require further epigenetic investigation. Similar results have also been demonstrated with checkpoint inhibitors targeting CTLA-4 (Wang et al., 2015). Chemokines play an important role in trafficking immune T cells to the TME; therefore, reduced expression of these molecules can shield tumours from immune responses.

In addition to these mechanisms, HDACis can reduce suppressive cell populations such as MDSCs to augment checkpoint blockade therapies. A recent study examined the effects of two key checkpoint inhibitors, anti-CTLA-4 and anti-PD-1, in conjunction with two epigenetic modulating drugs (5-azacytidine and entinostat) in mice bearing colorectal or metastatic breast cancers (Kim et al., 2014). This combination resulted in primary tumour eradication in 10/11 mice with colorectal cancers and all mice with metastatic breast cancer. Furthermore, metastasis did not develop in the metastatic breast cancer model following combination treatment versus single therapies alone. Further mechanistic studies showed that these epigenetic drugs acted by blocking the suppressive activity of tumour-infiltrating G-MDSCs against T cell killing. However, while entinostat was shown to reduce G-MDSC viability, the specific mechanisms underlying the targeted suppression of G-MDSCs by epigenetic drugs were not examined. This study also highlighted the potential for using combined epigenetic and immunotherapy against poorly immunogenic cancers.

Immunotherapy offers many distinct advantages over standard cancer treatments (e.g., chemotherapy, radiotherapy) including the potential to be applied globally to different cancer subtypes and to elicit specific and durable responses by immunological memory. The ability of epigenetic drugs to specifically prime epithelial cancer cells for host immune responses holds significant promise for future immunotherapy in patients with cancer. Indeed, a number of epigenetic and immunotherapeutic drug regimens have already been used or are under intense investigation in different tumour mouse models (e.g., colon, breast, melanoma) (Terracina et al., 2016; Lucarini et al., 2017; Covre et al., 2015; Gollob and Sciambi, 2007). The results from current clinical trials partnering these two therapies are eagerly anticipated (Table 1).

While the immunological effects of epigenetic agents are well documented, epigenetic drugs such as 5-AZA-CdR can have pharmacological limitations such as a short half-life, sensitivity to inactivation by cytidine deaminase *in vivo*, and pronounced hematopoietic toxicity, all of which may impede its use in combination regimens (Covre et al., 2015). To address these concerns, second-generation epigenetic drugs such as the DNA hypomethylating agent SGI-110 (guadecitabine) are currently in development. Preclinical experience of SG110, a dinucleoitide of 5-AZA-CdR, has shown that it is more convenient and tolerated, achieving biologically relevant hypomethylating effects at lower and less myelosuppressive doses than decitabine while displaying immunomodulatory activity. Importantly, a significant anti-tumour effect was achieved in syngeneic grafts of murine mammary carcinoma TS/A treated with SGI-110 and subsequent murine mAb 9H10 (directed to CTLA-4). This study suggests that it will also be important to explore combinations of second-generation epigenetic inhibitors (particularly DNMTis) and immunotherapeutic strategies across different cancer models. Several second-generation HDACis including vorinostat, panobinostat, and entinostat, have been under investigation in combination with immunotherapy (Table 1).

6. Novel classes of epigenetic drugs for immunotherapy

Whilst the function of HDAC and DNMT inhibitors in immune priming of cancer cells has been explored reasonably thoroughly, the role of many other novel epigenetic drugs has yet to be established. There are several other classes of epigenetic drugs including histone methyltransferases inhibitors, bromodomain inhibitors, and histone demethylase inhibitors which have recently been reported to increase immune signalling in tumour cells. In addition, several epigenetic drugs are in clinical trials or under investigation in cancer and other human diseases that may useful in combination with immunotherapies (Fig. 2).

For example, GSK126, a selective inhibitor of EZH2 methyltransfer-

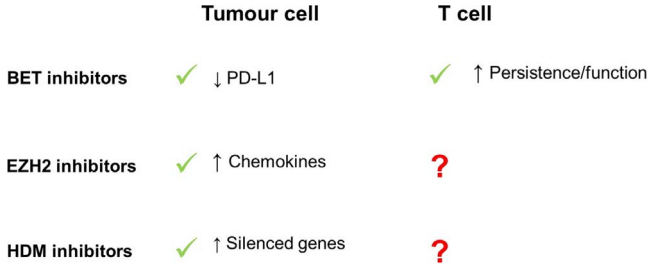

Fig. 2. Novel classes of epigenetic drugs in immunotherapy.Many novel classes of epigenetic enzymes, such as histone methyltransferase inhibitors (GSK126), bromodomain inhibitiors (JQ1), and histone demethylase (HDM) inhibitors (INCB059872), have recently been identified as potential agents for immunotherapeutic treatments. While these drugs have been shown to play a role in tumour cells, their role in the re-education of other cell types within the TME, such as T cells, has yet to be identified. In addition, the role of these drugs in upregulating other tumour cell components involved in immune cell activation like antigen presenting molecules or co-stimulatory molecules remains to be determined.

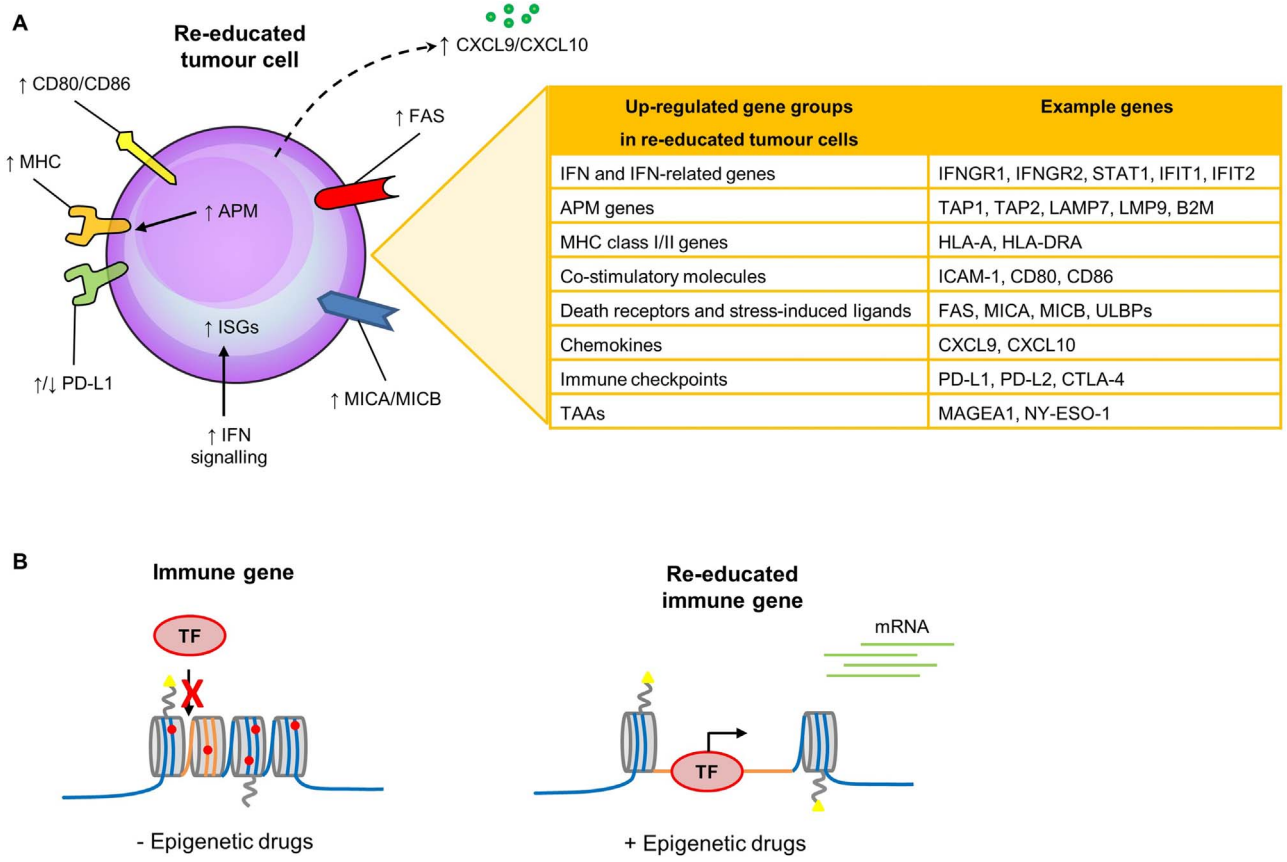

Fig. 3. Re-education of cancers cell towards visibility for immune attack.(**A**) Several key papers have recently identified IFN signalling genes that are (1) activated in response to DNA demethylation (Roulois et al. (Roulois et al., 2015) and Chiappinelli et al. (Chiappinelli et al., 2016b)) or (2) that have genomic defects in immune checkpoint resistant tumours (Gao et al. (Gao et al., 2016)). We hypothesise that these genes, in addition to others key genes that are re-activated in response to different epigenetic treatments (including TAAs, APM components, MHC class I/II molecules, co-stimulatory/accessory molecules, chemokines, and immune checkpoints), can be used to determine the impact of epigenetic therapies on re-education of tumour cells to become more visible for immune attack. (**B**) At the chromatin level, we suggest that immune genes are silenced in a closed heterochromatin state in tumour cells and that the addition of epigenetic drugs re-educates immune genes to become open and transcriptionally active.

ase activity, was shown to synergistically improve the therapeutic efficacy of T cell therapy and increase tumour expression of CXCL9 and CXCL10 and $CD8^+$ T cell infiltration in ovarian cancer (Peng et al., 2015). Similarly, JQ1, a selective bromodomain/BET inhibitor, enhanced T cell persistence and function (Kagoya et al., 2016) and suppressed PD-L1 expression in ovarian cancer to restore cytotoxic T cell responses (Zhu et al., 2016). Furthermore, JQ1 in combination with anti-PD-1 immune checkpoint blockade enhanced anti-tumour responses in lung cancer (Adeegbe et al., 2016).

Recently, histone demethylase inhibitors have been shown to synergise with other classes of epigenetic drugs. Dual inhibition of DNMT and LSD1 was shown to synergistically re-activate epigenetically silenced genes in cancer cells (Han et al., 2013). Similarly, the LSD1 inhibitor INCB059872 was combined with a BET inhibitor to reduce myeloid differentiation and enhance anti-tumour efficacy in a human AML model *in vitro* and *in vivo* (Liu et al., 2016). These studies suggest potential for cancer immunotherapy.

In addition to the immunomodulatory effects discussed in this review, there are other additional expected changes that may occur in response to epigenetic treatment. For example, DNA hypomethylation may result in the activation of silenced retroviral sequences in NK or T cells to induce IFN signalling, as they do in tumour cells. IFN signalling in immune cells may enhance anti-tumour activity and cytokine expression. These effects may also be extended to inhibitors that target other classes of epigenetic enzymes such as LSD1, but this remains to be determined. It has been proposed that immunosuppression by silenced retroviral sequences could contribute to immune evasion by cancer cells. There is evidence that some endogenous retroviruses from the HERV Env family of proteins (e.g., HERV-FRD, HERV-H, HERV-K) display immunosuppressive activity that can impair immune responses to exogenous pathogens and tumours (Kassiotis and Stoye, 2016). This suggests that activation of silenced retroviral transcripts will be dependent on the specific ERVs that are activated. Overall, tumour responses to epigenetic therapy will be determined by the distinct subset of cellular components that make up the TME in individual patients; therefore, it is important to understand the targets of these epigenetic therapies.

7. Can type I IFN and IFN-related genes determine the impact of epigenetic therapies on re-education of tumour cells for immune attack?

Type I IFN possesses the potent ability to activate several immune cell types such as DC cells, NK cells, and CTLs, in addition to dampening the immunosuppressive activities of Tregs and MDSCs (Zitvogel et al., 2015; Minn, 2015; Minn and Wherry, 2016). Furthermore, successful chemotherapy, radiotherapy, and immunotherapy in cancer patients often relies on intact type I IFN signalling and correlates with favourable prognosis in many human cancers (Zitvogel et al., 2015). As previously described, DNA hypomethylating drugs have also been shown act through mechanisms that induce IFN-I and IFN-II signalling to increase tumour recognition and immunogenicity (Roulois et al., 2015; Chiappinelli et al., 2016b).

Given that IFN signalling is so important across several cancer types, we propose that type I IFN and IFN-related genes can be used to determine the impact of various epigenetic therapies on re-education of

tumour cells to immune attack. Recently, Gao et al. (Gao et al., 2016) identified a unique IFN-γ pathway gene signature in anti-CTLA-4-resistant tumour cells that may serve as a biomarker of patient response to immune checkpoint therapy (Gao et al., 2016). This novel gene set includes IFN-γ signalling-related genes (*IFNGR1*, *IFNGR2*, *JAK2*, *IRF1*, *IFIT1*, *IFIT1*, *IFIT3*, *MTAP*, and miR31) and IFN-γ signalling pathway suppressor genes (*SOCS1* and *PIAS4*). In addition, key studies from Roulois et al. (Roulois et al., 2015) and Chiappinelli et al. (Chiappinelli et al., 2016b) that link DMNTi-mediated IFN signalling to ERV activation (as discussed in Section 4) also identify a common set of ISGs whose activation may be used to determine the extent of tumour re-education after epigenetic therapy (Roulois et al., 2015; Chiappinelli et al., 2016b). We hypothesise that these genes, in addition to other key genes re-activated in response to epigenetic therapies (such as those encoding APM components, MHC class I/II molecules, chemokines, immune checkpoints, etc.), can be used to develop an immune gene signature to assess the impact of epigenetic therapies on re-education of tumour cells to become more immunogenic (Fig. 3).

This hypothesis raises many interesting questions that will be important to explore. When is a T cell considered visible to T cells? Do different epigenetic drugs induce common or distinct IFN activation signatures? Does the combination of different epigenetic drugs together with immunotherapeutic strategies, chemotherapy, or other classes of epigenetic drugs further enhance tumour visibility towards immune cells? Which epigenetic drugs alone or in combination with other therapies have the greatest impact on tumour re-education for immune attack? Also, which cell subsets (e.g., T cells) are these epigenetic drugs most suitable for?

It is also important to note that recent papers have shown that type I IFN can also result in immune suppression. For example, upregulation of IFN-γ by T cells (and other immune cells in the TME) can increase PD-L1 expression on tumour cells resulting in T cell exhaustion and adaptive immune resistance (Herbst et al., 2014; Twyman-Saint Victor et al., 2015). In addition, IFN-related signalling can also drive non-immune-mediated resistance to chemotherapy and radiation (Minn, 2015). It has been suggested that activation of IFN signalling through unphosphorylated STAT1, a key IFN-regulated transcription factor pathway, in addition to other negative regulatory proteins, activates a distinct set of ISGs called the interferon-related DNA damage resistance signature (IRDS), which drives immune suppression (Minn, 2015).

8. Conclusions

The recent arrival of immune checkpoint inhibitors is poised to significantly change the management of patients with many different tumour types and is prompting the development of numerous combination strategies as a robust new approach to personalised cancer therapeutics. The interplay between the tumour and immune cells during tumour development is complex, and epigenetic modifications are an important source of many pathological changes leading to immune escape. As highlighted in this review, epigenetic modulators play an important role in shifting the balance of immune inhibition towards immune activation. Epigenetic reprogramming of the immune evasive phenotype synergistically primes the immune system for more effective immunotherapy responses.

However, whilst immunotherapy is showing remarkable promise, mixed tumour regression still represents a clinical challenge as not all cancer types respond to treatment and not all patients in 'responsive' groups experience clinical improvement. Thus, identifying combination strategies for appropriate patient populations is crucial. Furthermore, strategies to treat patients that present with acquired immune resistance are also needed. Finally, the identification of other epigenetic regulators that can prime the immune response to immunotherapy warrants attention. Overall, this review highlights the promise of combining epigenetic therapy and immunotherapy to achieve more effective therapeutic responses in the future, even in poorly immunogenic tumours.

Author Agreement/Declaration

All authors have seen and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Funding

This research was supported by the Australian Postgraduate Award (APA) awarded to JD.

References

Adeegbe, D.O.F., Freeman, G.J., Wong, K.K., 2016. BET bromodomain inhibition synergizes with immune checkpoint blockade to facilitate anti-tumor response in a murine model of non-small cell lung cancer harboring KRAS mutation. J. Immunol. 196 (Supplement 1) 74.10.

Ahearne, M.J., Allchin, R.L., Fox, C.P., Wagner, S.D., 2014. Follicular helper T-cells: expanding roles in T-cell lymphoma and targets for treatment. Br. J. Haematol. 166, 326–335.

Ansell, S.M., Lesokhin, A.M., Borrello, I., Halwani, A., Scott, E.C., Gutierrez, M., Schuster, S.J., Millenson, M.M., Cattry, D., Freeman, G.J., Rodig, S.J., Chapuy, B., Ligon, A.H., Zhu, L., Grosso, J.F., Kim, S.Y., Timmerman, J.M., Shipp, M.A., Armand, P., 2015. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N. Engl. J. Med. 372, 311–319.

Armeanu, S., Bitzer, M., Lauer, U.M., Venturelli, S., Pathil, A., Krusch, M., Kaiser, S., Jobst, J., Smirnow, I., Wagner, A., Steinle, A., Salih, H.R., 2005. Natural killer cell-mediated lysis of hepatoma cells via specific induction of NKG2D ligands by the histone deacetylase inhibitor sodium valproate. Cancer Res. 65, 6321–6329.

Badoual, C., Hans, S., Rodriguez, J., Peyrard, S., Klein, C., Agueznay Nel, H., Mosseri, V., Laccourreye, O., Bruneval, P., Fridman, W.H., Brasnu, D.F., Tartour, E., 2006. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. Clin. Cancer Res. 12, 465–472.

Bao, L., Dunham, K., Lucas, K., 2011. MAGE-A1, MAGE-A3, and NY-ESO-1 can be upregulated on neuroblastoma cells to facilitate cytotoxic T lymphocyte-mediated tumor cell killing. Cancer Immunol. Immunother. 60, 1299–1307.

Barry, M., Bleackley, R.C., 2002. Cytotoxic T lymphocytes: all roads lead to death. Nat. Rev. Immunol. 2, 401–409.

Bartova, E., Pachernik, J., Harnicarova, A., Kovarik, A., Kovarikova, M., Hofmanova, J., Skalnikova, M., Kozubek, M., Kozubek, S., 2005. Nuclear levels and patterns of histone H3 modification and HP1 proteins after inhibition of histone deacetylases. J. Cell Sci. 118, 5035–5046.

Bartova, E., Krejci, J., Harnicarova, A., Galiova, G., Kozubek, S., 2008. Histone modifications and nuclear architecture: a review. J. Histochem. Cytochem. 56, 711–721.

Bates, G.J., Fox, S.B., Han, C., Leek, R.D., Garcia, J.F., Harris, A.L., Banham, A.H., 2006. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J. Clin. Oncol. 24, 5373–5380.

Berghuis, D., Schilham, M.W., Vos, H.I., Santos, S.J., Kloess, S., Buddingh, E.P., Egeler, R.M., Hogendoorn, P.C., Lankester, A.C., 2012. Histone deacetylase inhibitors enhance expression of NKG2D ligands in Ewing sarcoma and sensitize for natural killer cell-mediated cytolysis. Clin. Sarcoma Res. 2, 8.

Bingle, L., Brown, N.J., Lewis, C.E., 2002. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J. Pathol. 196, 254–265.

Brahmer, J., Reckamp, K.L., Baas, P., Crino, L., Eberhardt, W.E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E.E., Holgado, E., Waterhouse, D., Ready, N., Gainor, J., Aren Frontera, O., Havel, L., Steins, M., Garassino, M.C., Aerts, J.G., Domine, M., Paz-Ares, L., Reck, M., Baudelet, C., Harbison, C.T., Lestini, B., Spigel, D.R., 2015. Nivolumab versus docetaxel in advanced squamous-Cell non-Small-Cell lung cancer. N. Engl. J. Med. 373, 123–135.

Bukur, J., Jasinski, S., Seliger, B., 2012. The role of classical and non-classical HLA class I antigens in human tumors. Semin. Cancer Biol. 22, 350–358.

Chiappinelli, K.B., Zahnow, C.A., Ahuja, N., Baylin, S.B., 2016a. Combining epigenetic and immunotherapy to combat cancer. Cancer Res. 76, 1683–1689.

Chiappinelli, K.B., Strissel, P.L., Desrichard, A., Li, H., Henke, C., Akman, B., Hein, A., Rote, N.S., Cope, L.M., Snyder, A., Makarov, V., Budhu, S., Slamon, D.J., Wolchok, J.D., Pardoll, D.M., Beckmann, M.W., Zahnow, C.A., Merghoub, T., Chan, T.A., Baylin, S.B., Strick, R., 2016b. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell 164, 1073.

Comes, A., Rosso, O., Orengo, A.M., Di Carlo, E., Sorrentino, C., Meazza, R., Piazza, T., Valzasina, B., Nanni, P., Colombo, M.P., Ferrini, S., 2006. CD25+ regulatory T cell depletion augments immunotherapy of micrometastases by an IL-21-secreting cellular vaccine. J. Immunol. 176, 1750–1758.

Coral, S., Sigalotti, L., Altomonte, M., Engelsberg, A., Colizzi, F., Cattarossi, I.,

Maraskovsky, E., Jager, E., Seliger, B., Maio, M., 2002. 5-aza-2'-deoxycytidine-induced expression of functional cancer testis antigens in human renal cell carcinoma: immunotherapeutic implications. Clin. Cancer Res. 8, 2690–2695.
Correale, P., Rotundo, M.S., Del Vecchio, M.T., Remondo, C., Migali, C., Ginanneschi, C., Tsang, K.Y., Licchetta, A., Mannucci, S., Loiacono, L., Tassone, P., Francini, G., Tagliaferri, P., 2010. Regulatory (FoxP3+) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. J. Immunother. 33, 435–441.
Covre, A., Coral, S., Di Giacomo, A.M., Taverna, P., Azab, M., Maio, M., 2015. Epigenetics meets immune checkpoints. Semin. Oncol. 42, 506–513.
Curiel, T.J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J.R., Zhang, L., Burow, M., Zhu, Y., Wei, S., Kryczek, I., Daniel, B., Gordon, A., Myers, L., Lackner, A., Disis, M.L., Knutson, K.L., Chen, L., Zou, W., 2004. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat. Med. 10, 942–949.
Dissemond, J., Busch, M., Kothen, T., Mors, J., Weimann, T.K., Lindeke, A., Goos, M., Wagner, S.N., 2004. Differential downregulation of endoplasmic reticulum-residing chaperones calnexin and calreticulin in human metastatic melanoma. Cancer Lett. 203, 225–231.
Dolan, D.E., Gupta, S., 2014. PD-1 pathway inhibitors: changing the landscape of cancer immunotherapy. Cancer Control 21, 231–237.
Dong, H., Strome, S.E., Salomao, D.R., Tamura, H., Hirano, F., Flies, D.B., Roche, P.C., Lu, J., Zhu, G., Tamada, K., Lennon, V.A., Celis, E., Chen, L., 2002. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat. Med. 8, 793–800.
Dunn, J., McCuaig, R., Tu, W.J., Hardy, K., Rao, S., 2015. Multi-layered epigenetic mechanisms contribute to transcriptional memory in T lymphocytes. BMC Immunol. 16, 27.
Espada, J., Esteller, M., 2007. Epigenetic control of nuclear architecture. Cell. Mol. Life Sci. 64, 449–457.
Esteller, M., 2008. Epigenetics in cancer. N. Engl. J. Med. 358, 1148–1159.
FDA, 2016. FDA Approves New, Targeted Therapy for Bladder Cancer. (Accessed 10 October 2016). http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm501762.htm.
Fedorova, E., Zink, D., 2008. Nuclear architecture and gene regulation. Biochim. Biophys. Acta 1783, 2174–2184.
Fiegler, N., Textor, S., Arnold, A., Rolle, A., Oehme, I., Breuhahn, K., Moldenhauer, G., Witzens-Harig, M., Cerwenka, A., 2013. Downregulation of the activating NKp30 ligand B7-H6 by HDAC inhibitors impairs tumor cell recognition by NK cells. Blood 122, 684–693.
Fratta, E., Coral, S., Covre, A., Parisi, G., Colizzi, F., Danielli, R., Nicolay, H.J., Sigalotti, L., Maio, M., 2011. The biology of cancer testis antigens: putative function, regulation and therapeutic potential. Mol. Oncol. 5, 164–182.
Fu, J., Xu, D., Liu, Z., Shi, M., Zhao, P., Fu, B., Zhang, Z., Yang, H., Zhang, H., Zhou, C., Yao, J., Jin, L., Wang, H., Yang, Y., Fu, Y.X., Wang, F.S., 2007. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. Gastroenterology 132, 2328–2339.
Gajewski, T.F., Schreiber, H., Fu, Y.X., 2013. Innate and adaptive immune cells in the tumor microenvironment. Nat. Immunol. 14, 1014–1022.
Gao, J., Shi, L.Z., Zhao, H., Chen, J., Xiong, L., He, Q., Chen, T., Roszik, J., Bernatchez, C., Woodman, S.E., Chen, P.L., Hwu, P., Allison, J.P., Futreal, A., Wargo, J.A., Sharma, P., 2016. Loss of IFN-gamma pathway genes in tumor cells as a mechanism of resistance to anti-CTLA-4 therapy. Cell 167, 397–404 (e399).
Gimenez, J., Montgiraud, C., Pichon, J.P., Bonnaud, B., Arsac, M., Ruel, K., Bouton, O., Mallet, F., 2010. Custom human endogenous retroviruses dedicated microarray identifies self-induced HERV-W family elements reactivated in testicular cancer upon methylation control. Nucleic Acids Res. 38, 2229–2246.
Gollob, J.A., Sciambi, C.J., 2007. Decitabine up-regulates S100A2 expression and synergizes with IFN-gamma to kill uveal melanoma cells. Clin. Cancer Res. 13, 5219–5225.
Goswami, C.J.S., Zhao, H., Zhang, X., Sharma, P., 2016. Epigenetic changes in T cells in response to immune checkpoint blockade. [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research. 2016 Apr 16–20; New Orleans, LA Philadelphia (PA): AACR;, Cancer Res. pp. 76 (Abstract nr 4026).
Groh, S., Schotta, G., 2017. Silencing of endogenous retroviruses by heterochromatin. Cell Mol Life Sci 1–11. http://dx.doi.org/10.1007/s00018-017-2454-8.
Gu-Trantien, C., Loi, S., Garaud, S., Equeter, C., Libin, M., de Wind, A., Ravoet, M., Le Buanec, H., Sibille, C., Manfouo-Foutsop, G., Veys, I., Haibe-Kains, B., Singhal, S.K., Michiels, S., Rothe, F., Salgado, R., Duvillier, H., Ignatiadis, M., Desmedt, C., Bron, D., Larsimont, D., Piccart, M., Sotiriou, C., Willard-Gallo, K., 2013. CD4(+) follicular helper T cell infiltration predicts breast cancer survival. J. Clin. Invest. 123, 2873–2892.
Han, H., Yang, X., Pandiyan, K., Liang, G., 2013. Synergistic re-activation of epigenetically silenced genes by combinatorial inhibition of DNMTs and LSD1 in cancer cells. PLoS One 8, e75136.
Heninger, E., Krueger, T.E., Lang, J.M., 2015. Augmenting antitumor immune responses with epigenetic modifying agents. Front. Immunol. 6, 29.
Herbst, R.S., Soria, J.C., Kowanetz, M., Fine, G.D., Hamid, O., Gordon, M.S., Sosman, J.A., McDermott, D.F., Powderly, J.D., Gettinger, S.N., Kohrt, H.E., Horn, L., Lawrence, D.P., Rost, S., Leabman, M., Xiao, Y., Mokatrin, A., Koeppen, H., Hegde, P.S., Mellman, I., Chen, D.S., Hodi, F.S., 2014. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 515, 563–567.
Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J.C., Akerley, W., van den Eertwegh, A.J., Lutzky, J., Lorigan, P., Vaubel, J.M., Linette, G.P., Hogg, D., Ottensmeier, C.H., Lebbe, C., Peschel, C., Quirt, I., Clark, J.I., Wolchok, J.D., Weber, J.S., Tian, J., Yellin, M.J., Nichol, G.M., Hoos, A., Urba, W.J., 2010. Improved survival with ipilimumab in patients with metastatic melanoma. N. Engl. J. Med. 363, 711–723.
Insinga, A., Monestiroli, S., Ronzoni, S., Gelmetti, V., Marchesi, F., Viale, A., Altucci, L., Nervi, C., Minucci, S., Pelicci, P.G., 2005. Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. Nat. Med. 11, 71–76.
Ishibashi, K., Kumai, T., Ohkuri, T., Kosaka, A., Nagato, T., Hirata, Y., Ohara, K., Oikawa, K., Aoki, N., Akiyama, N., Sado, M., Kitada, M., Harabuchi, Y., Celis, E., Kobayashi, H., 2016. Epigenetic modification augments the immunogenicity of human leukocyte antigen G serving as a tumor antigen for T cell-based immunotherapy. Oncoimmunology 5, e1169356.
James, S.R., Link, P.A., Karpf, A.R., 2006. Epigenetic regulation of X-linked cancer/germline antigen genes by DNMT1 and DNMT3b. Oncogene 25, 6975–6985.
Jazirehi, A.R., Nazarian, R., Torres-Collado, A.X., Economou, J.S., 2014. Aberrant apoptotic machinery confers melanoma dual resistance to BRAF(V600E) inhibitor and immune effector cells: immunosensitization by a histone deacetylase inhibitor. Am. J. Clin. Exp. Immunol. 3, 43–56.
Jia, Y., Zeng, Z., Li, Y., Li, Z., Jin, L., Zhang, Z., Wang, L., Wang, F.S., 2015. Impaired function of CD4+ T follicular helper (Tfh) cells associated with hepatocellular carcinoma progression. PLoS One 10, e0117458.
Jones, P.A., Baylin, S.B., 2002. The fundamental role of epigenetic events in cancer. Nat. Rev. Genet. 3, 415–428.
Jones, P.A., Takai, D., 2001. The role of DNA methylation in mammalian epigenetics. Science 293, 1068–1070.
Juergens, R.A., Wrangle, J., Vendetti, F.P., Murphy, S.C., Zhao, M., Coleman, B., Sebree, R., Rodgers, K., Hooker, C.M., Franco, N., Lee, B., Tsai, S., Delgado, I.E., Rudek, M.A., Belinsky, S.A., Herman, J.G., Baylin, S.B., Brock, M.V., Rudin, C.M., 2011. Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. Cancer Discov. 1, 598–607.
Kagoya, Y., Nakatsugawa, M., Yamashita, Y., Ochi, T., Guo, T., Anczurowski, M., Saso, K., Butler, M.O., Arrowsmith, C.H., Hirano, N., 2016. BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. J. Clin. Invest. 126, 3479–3494.
Kaklamanis, L., Leek, R., Koukourakis, M., Gatter, K.C., Harris, A.L., 1995. Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. Cancer Res. 55, 5191–5194.
Kassiotis, G., Stoye, J.P., 2016. Immune responses to endogenous retroelements: taking the bad with the good. Nat. Rev. Immunol. 16, 207–219.
Khan, A.N., Gregorie, C.J., Tomasi, T.B., 2008. Histone deacetylase inhibitors induce TAP, LMP Tapasin genes and MHC class I antigen presentation by melanoma cells. Cancer Immunol. Immunother. 57, 647–654.
Kim, K., Skora, A.D., Li, Z., Liu, Q., Tam, R.L., Diaz Jr., L.A., Papadopoulos, N., Kinzler, K.W., Vogelstein, B., Zhou, S., 2014. Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc. Natl. Acad. Sci. U. S. A. 111, 11774–11779.
Kouzarides, T., 2007. Chromatin modifications and their function. Cell 128, 693–705.
Koyama, S., Akbay, E.A., Li, Y.Y., Herter-Sprie, G.S., Buczkowski, K.A., Richards, W.G., Gandhi, L., Redig, A.J., Rodig, S.J., Asahina, H., Jones, R.E., Kulkarni, M.M., Kuraguchi, M., Palakurthi, S., Fecci, P.E., Johnson, B.E., Janne, P.A., Engelman, J.A., Gangadharan, S.P., Costa, D.B., Freeman, G.J., Bueno, R., Hodi, F.S., Dranoff, G., Wong, K.K., Hammerman, P.S., 2016. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat. Commun. 7, 10501.
Krishnadas, D.K., Shusterman, S., Bai, F., Diller, L., Sullivan, J.E., Cheerva, A.C., George, R.E., Lucas, K.G., 2015. A phase I trial combining decitabine/dendritic cell vaccine targeting MAGE-A1, MAGE-A3 and NY-ESO-1 for children with relapsed or therapy-refractory neuroblastoma and sarcoma. Cancer Immunol. Immunother. 64, 1251–1260.
Lanier, L.L., 2005. NK cell recognition. Annu. Rev. Immunol. 23, 225–274.
Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J.J., Cowey, C.L., Lao, C.D., Schadendorf, D., Dummer, R., Smylie, M., Rutkowski, P., Ferrucci, P.F., Hill, A., Wagstaff, J., Carlino, M.S., Haanen, J.B., Maio, M., Marquez-Rodas, I., McArthur, G.A., Ascierto, P.A., Long, G.V., Callahan, M.K., Postow, M.A., Grossmann, K., Sznol, M., Dreno, B., Bastholt, L., Yang, A., Rollin, L.M., Horak, C., Hodi, F.S., Wolchok, J.D., 2015. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. N. Engl. J. Med. 373, 23–34.
Leffers, N., Gooden, M.J., de Jong, R.A., Hoogeboom, B.N., ten Hoor, K.A., Hollema, H., Boezen, H.M., van der Zee, A.G., Daemen, T., Nijman, H.W., 2009. Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer. Cancer Immunol. Immunother. 58, 449–459.
Li, B., Carey, M., Workman, J.L., 2007. The role of chromatin during transcription. Cell 128, 707–719.
Li, H., Chiappinelli, K.B., Guzzetta, A.A., Easwaran, H., Yen, R.W., Vatapalli, R., Topper, M.J., Luo, J., Connolly, R.M., Azad, N.S., Stearns, V., Pardoll, D.M., Davidson, N., Jones, P.A., Slamon, D.J., Baylin, S.B., Zahnow, C.A., Ahuja, N., 2014. Immune regulation by low doses of the DNA methyltransferase inhibitor 5-azacitidine in common human epithelial cancers. Oncotarget 5, 587–598.
Lindau, D., Gielen, P., Kroesen, M., Wesseling, P., Adema, G.J., 2013. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology 138, 105–115.
Liu, Y., Komohara, Y., Domenick, N., Ohno, M., Ikeura, M., Hamilton, R.L., Horbinski, C., Wang, X., Ferrone, S., Okada, H., 2012. Expression of antigen processing and presenting molecules in brain metastasis of breast cancer. Cancer Immunol. Immunother. 61, 789–801.

Liu, X.S., Ye, M., Collins, M., Favata, R., Yang, M., Diamond, G., Dostalik, M., Lo, V., He, Y., Wu, C., Combs, L., Yao, A., Hollis, W., Huber, G., Scherle, R., Ruggeri, P., Liu, B.P., Lee, S.H., 2016. Combination of BET inhibitor INC and LSD1 inhibitor INCB059872 is synergistic for the treatment of AML in vitro and in vivo. Cancer Res. AM2016-4702, B054329. http://dx.doi.org/10.1158/1538-7445.

Lopez-Soto, A., Folgueras, A.R., Seto, E., Gonzalez, S., 2009. HDAC3 represses the expression of NKG2D ligands ULBPs in epithelial tumour cells: potential implications for the immunosurveillance of cancer. Oncogene 28, 2370–2382.

Lucarini, V., Buccione, C., Zicchedda, G., Peschiaroli, F., Sestili, P., Puglisi, R., Mattia, G., Zanetti, C., Parolini, I., Bracci, L., Macchia, I., Rossi, A., D'Urso, M.T., Macchia, D., Spada, M., De Ninno, A., Gerardino, A., Mozetic, P., Trombetta, M., Rainer, A., Businaro, L., Schiavoni, G., Mattei, F., 2017. Combining type I interferons and 5-Aza-2'-Deoxycitidine to improve anti-tumor response against melanoma. J. Invest. Dermatol. 137, 159–169.

Lundqvist, A., Abrams, S.I., Schrump, D.S., Alvarez, G., Suffredini, D., Berg, M., Childs, R., 2006. Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. Cancer Res. 66, 7317–7325.

Luo, W., Wang, X., Kageshita, T., Wakasugi, S., Karpf, A.R., Ferrone, S., 2006. Regulation of high molecular weight-melanoma associated antigen (HMW-MAA) gene expression by promoter DNA methylation in human melanoma cells. Oncogene 25, 2873–2884.

Macfarlan, T.S., Gifford, W.D., Agarwal, S., Driscoll, S., Lettieri, K., Wang, J., Andrews, S.E., Franco, L., Rosenfeld, M.G., Ren, B., Pfaff, S.L., 2011. Endogenous retroviruses and neighboring genes are coordinately repressed by LSD1/KDM1A. Genes Dev. 25, 594–607.

Maeda, T., Towatari, M., Kosugi, H., Saito, H., 2000. Up-regulation of costimulatory/adhesion molecules by histone deacetylase inhibitors in acute myeloid leukemia cells. Blood 96, 3847–3856.

Mager, D.L., Stoye, J.P., 2015. Mammalian endogenous retroviruses. Microbiol. Spectr. 3 (MDNA3-0009-2014).

Magner, W.J., Kazim, A.L., Stewart, C., Romano, M.A., Catalano, G., Grande, C., Keiser, N., Santaniello, F., Tomasi, T.B., 2000. Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. J. Immunol. 165, 7017–7024.

Maio, M., Covre, A., Fratta, E., Di Giacomo, A.M., Taverna, P., Natali, P.G., Coral, S., Sigalotti, L., 2015. Molecular pathways At the crossroads of cancer epigenetics and immunotherapy. Clin. Cancer Res. 21, 4040–4047.

Markham, A., 2016. Atezolizumab first global approval. Drugs 76, 1227–1232.

Martinez-Outschoorn, U.E., Pavlides, S., Whitaker-Menezes, D., Daumer, K.M., Milliman, J.N., Chiavarina, B., Migneco, G., Witkiewicz, A.K., Martinez-Cantarin, M.P., Flomenberg, N., Howell, A., Pestell, R.G., Lisanti, M.P., Sotgia, F., 2010. Tumor cells induce the cancer associated fibroblast phenotype via caveolin-1 degradation: implications for breast cancer and DCIS therapy with autophagy inhibitors. Cell Cycle 9, 2423–2433.

Mikyskova, R., Indrova, M., Vlkova, V., Bieblova, J., Simova, J., Parackova, Z., Pajtasz-Piasecka, E., Rossowska, J., Reinis, M., 2014. DNA demethylating agent 5-azacytidine inhibits myeloid-derived suppressor cells induced by tumor growth and cyclophosphamide treatment. J. Leukoc. Biol. 95, 743–753.

Minn, A.J., Wherry, E.J., 2016. Combination cancer therapies with immune checkpoint blockade: convergence on interferon signaling. Cell 165, 272–275.

Minn, A.J., 2015. Interferons and the immunogenic effects of cancer therapy. Trends Immunol. 36, 725–737.

Nakata, S., Yoshida, T., Horinaka, M., Shiraishi, T., Wakada, M., Sakai, T., 2004. Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells. Oncogene 23, 6261–6271.

Nishikawa, H., Sakaguchi, S., 2014. Regulatory T cells in cancer immunotherapy. Curr. Opin. Immunol. 27, 1–7.

Pardoll, D.M., 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 12, 252–264.

Parry, R.V., Chemnitz, J.M., Frauwirth, K.A., Lanfranco, A.R., Braunstein, I., Kobayashi, S.V., Linsley, P.S., Thompson, C.B., Riley, J.L., 2005. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol. Cell. Biol. 25, 9543–9553.

Peng, D., Kryczek, I., Nagarsheth, N., Zhao, L., Wei, S., Wang, W., Sun, Y., Zhao, E., Vatan, L., Szeliga, W., Kotarski, J., Tarkowski, R., Dou, Y., Cho, K., Hensley-Alford, S., Munkarah, A., Liu, R., Zou, W., 2015. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 527, 249–253.

Petersen, R.P., Campa, M.J., Sperlazza, J., Conlon, D., Joshi, M.B., Harpole Jr., D.H., Patz Jr., E.F., 2006. Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients. Cancer 107, 2866–2872.

Raulet, D.H., Gasser, S., Gowen, B.G., Deng, W., Jung, H., 2013. Regulation of ligands for the NKG2D activating receptor. Annu. Rev. Immunol. 31, 413–441.

Roulois, D., Blanquart, C., Panterne, C., Gueugnon, F., Gregoire, M., Fonteneau, J.F., 2012. 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. 42, 783–789.

Roulois, D., Loo Yau, H., Singhania, R., Wang, Y., Danesh, A., Shen, S.Y., Han, H., Liang, G., Jones, P.A., Pugh, T.J., O'Brien, C., De Carvalho, D.D., 2015. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. Cell 162, 961–973.

Sahakian, E., Powers, J.J., Chen, J., Deng, S.L., Cheng, F., Distler, A., Woods, D.M., Rock-Klotz, J., Sodre, A.L., Youn, J.I., Woan, K.V., Villagra, A., Gabrilovich, D., Sotomayor, E.M., Pinilla-Ibarz, J., 2015. Histone deacetylase 11: A novel epigenetic regulator of myeloid derived suppressor cell expansion and function. Mol. Immunol. 63, 579–585.

Salama, P., Phillips, M., Grieu, F., Morris, M., Zeps, N., Joseph, D., Platell, C., Iacopetta, B., 2009. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J. Clin. Oncol. 27, 186–192.

Schmudde, M., Braun, A., Pende, D., Sonnemann, J., Klier, U., Beck, J.F., Moretta, L., Broker, B.M., 2008. Histone deacetylase inhibitors sensitize tumour cells for cytotoxic effects of natural killer cells. Cancer Lett. 272, 110–121.

Schneider, R., Grosschedl, R., 2007. Dynamics and interplay of nuclear architecture, genome organization, and gene expression. Genes Dev. 21, 3027–3043.

Setiadi, A.F., Omilusik, K., David, M.D., Seipp, R.P., Hartikainen, J., Gopaul, R., Choi, K.B., Jefferies, W.A., 2008. Epigenetic enhancement of antigen processing and presentation promotes immune recognition of tumors. Cancer Res. 68, 9601–9607.

Sheppard, K.A., Fitz, L.J., Lee, J.M., Benander, C., George, J.A., Wooters, J., Qiu, Y., Jussif, J.M., Carter, L.L., Wood, C.R., Chaudhary, D., 2004. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. FEBS Lett. 574, 37–41.

Shi, W., Li, X., Cha, Z., Sun, S., Wang, L., Jiao, S., Yang, B., Shi, Y., Wang, Z., Wu, Z., Dai, G., 2014. Dysregulation of circulating follicular helper T cells in nonsmall cell lung cancer. DNA Cell Biol. 33, 355–360.

Sica, A., Schioppa, T., Mantovani, A., Allavena, P., 2006. Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. Eur. J. Cancer 42, 717–727.

Sigalotti, L., Fratta, E., Coral, S., Maio, M., 2014. Epigenetic drugs as immunomodulators for combination therapies in solid tumors. Pharmacol. Ther. 142, 339–350.

Skov, S., Pedersen, M.T., Andresen, L., Straten, P.T., Woetmann, A., Odum, N., 2005. Cancer cells become susceptible to natural killer cell killing after exposure to histone deacetylase inhibitors due to glycogen synthase kinase-3-dependent expression of MHC class I-related chain A and B. Cancer Res. 65, 11136–11145.

Smyth, M.J., Teng, M.W., Swann, J., Kyparissoudis, K., Godfrey, D.I., Hayakawa, Y., 2006. CD4+CD25+ T regulatory cells suppress NK cell-mediated immunotherapy of cancer. J. Immunol. 176, 1582–1587.

Soon, P.S., Kim, E., Pon, C.K., Gill, A.J., Moore, K., Spillane, A.J., Benn, D.E., Baxter, R.C., 2013. Breast cancer-associated fibroblasts induce epithelial-to-mesenchymal transition in breast cancer cells. Endocr. Relat. Cancer 20, 1–12.

Stengel, S., Fiebig, U., Kurth, R., Denner, J., 2010. Regulation of human endogenous retrovirus-K expression in melanomas by CpG methylation. Genes. Chromosomes Cancer 49, 401–411.

Sutmuller, R.P., van Duivenvoorde, L.M., van Elsas, A., Schumacher, T.N., Wildenberg, M.E., Allison, J.P., Toes, R.E., Offringa, R., Melief, C.J., 2001. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. J. Exp. Med. 194, 823–832.

Taddei, A., Maison, C., Roche, D., Almouzni, G., 2001. Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. Nat. Cell Biol. 3, 114–120.

Tang, Y., Xu, X., Guo, S., Zhang, C., Tang, Y., Tian, Y., Ni, B., Lu, B., Wang, H., 2014. An increased abundance of tumor-infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma. PLoS One 9, e91551.

Taube, J.M., Klein, A., Brahmer, J.R., Xu, H., Pan, X., Kim, J.H., Chen, L., Pardoll, D.M., Topalian, S.L., Anders, R.A., 2014. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin. Cancer Res. 20, 5064–5074.

Tellez, C.S., Grimes, M.J., Picchi, M.A., Liu, Y., March, T.H., Reed, M.D., Oganesian, A., Taverna, P., Belinsky, S.A., 2014. SGI-110 and entinostat therapy reduces lung tumor burden and reprograms the epigenome. Int. J. Cancer 135, 2223–2231.

Terracina, K.P., Graham, L.J., Payne, K.K., Manjili, M.H., Baek, A., Damle, S.R., Bear, H.D., 2016. DNA methyltransferase inhibition increases efficacy of adoptive cellular immunotherapy of murine breast cancer. Cancer Immunol. Immunother. 65, 1061–1073.

Terranova-Barberio, M., Thomas, S., Munster, P.N., 2016. Epigenetic modifiers in immunotherapy: a focus on checkpoint inhibitors. Immunotherapy 8, 705–719.

Topalian, S.L., Sznol, M., McDermott, D.F., Kluger, H.M., Carvajal, R.D., Sharfman, W.H., Brahmer, J.R., Lawrence, D.P., Atkins, M.B., Powderly, J.D., Leming, P.D., Lipson, E.J., Puzanov, I., Smith, D.C., Taube, J.M., Wigginton, J.M., Kollia, G.D., Gupta, A., Pardoll, D.M., Sosman, J.A., Hodi, F.S., 2014. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J. Clin. Oncol. 32, 1020–1030.

Twyman-Saint Victor, C., Rech, A.J., Maity, A., Rengan, R., Pauken, K.E., Stelekati, E., Benci, J.L., Xu, B., Dada, H., Odorizzi, P.M., Herati, R.S., Mansfield, K.D., Patsch, D., Amaravadi, R.K., Schuchter, L.M., Ishwaran, H., Mick, R., Pryma, D.A., Xu, X., Feldman, M.D., Gangadhar, T.C., Hahn, S.M., Wherry, E.J., Vonderheide, R.H., Minn, A.J., 2015. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 520, 373–377.

Tyan, S.W., Kuo, W.H., Huang, C.K., Pan, C.C., Shew, J.Y., Chang, K.J., Lee, E.Y., Lee, W.H., 2011. Breast cancer cells induce cancer-associated fibroblasts to secrete hepatocyte growth factor to enhance breast tumorigenesis. PLoS One 6, e15313.

Usami, Y., Ishida, K., Sato, S., Kishino, M., Kiryu, M., Ogawa, Y., Okura, M., Fukuda, Y., Toyosawa, S., 2013. Intercellular adhesion molecule-1 (ICAM-1) expression correlates with oral cancer progression and induces macrophage/cancer cell adhesion. Int. J. Cancer 133, 568–578.

Vitale, M., Rezzani, R., Rodella, L., Zauli, G., Grigolato, P., Cadei, M., Hicklin, D.J., Ferrone, S., 1998. HLA class I antigen and transporter associated with antigen processing (TAP1 and TAP2) down-regulation in high-grade primary breast carcinoma lesions. Cancer Res. 58, 737–742.

Waldhauer, I., Steinle, A., 2008. NK cells and cancer immunosurveillance. Oncogene 27, 5932–5943.

Wang, L.X., Mei, Z.Y., Zhou, J.H., Yao, Y.S., Li, Y.H., Xu, Y.H., Li, J.X., Gao, X.N., Zhou, M.H., Jiang, M.M., Gao, L., Ding, Y., Lu, X.C., Shi, J.L., Luo, X.F., Wang, J., Wang, L.L., Qu, C., Bai, X.F., Yu, L., 2013. Low dose decitabine treatment induces CD80 expression in cancer cells and stimulates tumor specific cytotoxic T lymphocyte

responses. PLoS One 8, e62924.
Wang, L., Amoozgar, Z., Huang, J., Saleh, M.H., Xing, D., Orsulic, S., Goldberg, M.S., 2015. Decitabine enhances lymphocyte migration and function and synergizes with CTLA-4 blockade in a murine ovarian cancer model. Cancer Immunol Res 3, 1030–1041.
Weber, J., Salgaller, M., Samid, D., Johnson, B., Herlyn, M., Lassam, N., Treisman, J., Rosenberg, S.A., 1994. Expression of the MAGE-1 tumor antigen is up-regulated by the demethylating agent 5-aza-2'-deoxycytidine. Cancer Res. 54, 1766–1771.
Weintraub, K., 2016. Take two: combining immunotherapy with epigenetic drugs to tackle cancer. Nat. Med. 22, 8–10.
Weiser, T.S., Ohnmacht, G.A., Guo, Z.S., Fischette, M.R., Chen, G.A., Hong, J.A., Nguyen, D.M., Schrump, D.S., 2001. Induction of MAGE-3 expression in lung and esophageal cancer cells. Ann. Thorac. Surg. 71, 295–301 discussion 301-292.
Wischnewski, F., Pantel, K., Schwarzenbach, H., 2006. Promoter demethylation and histone acetylation mediate gene expression of MAGE −A1, −A2, −A3, and −A12 in human cancer cells. Mol. Cancer Res. 4, 339–349.
Wolf, D., Wolf, A.M., Rumpold, H., Fiegl, H., Zeimet, A.G., Muller-Holzner, E., Deibl, M., Gastl, G., Gunsilius, E., Marth, C., 2005. The expression of the regulatory T cell-specific forkhead box transcription factor FoxP3 is associated with poor prognosis in ovarian cancer. Clin. Cancer Res. 11, 8326–8331.
Woods, D.M., Sodre, A.L., Villagra, A., Sarnaik, A., Sotomayor, E.M., Weber, J., 2015. HDAC inhibition upregulates PD-1 ligands in melanoma and augments immunotherapy with PD-1 blockade. Cancer Immunol. Res. 3, 1375–1385.
Wrangle, J., Wang, W., Koch, A., Easwaran, H., Mohammad, H.P., Vendetti, F., Vancriekinge, W., Demeyer, T., Du, Z., Parsana, P., Rodgers, K., Yen, R.W., Zahnow, C.A., Taube, J.M., Brahmer, J.R., Tykodi, S.S., Easton, K., Carvajal, R.D., Jones, P.A., Laird, P.W., Weisenberger, D.J., Tsai, S., Juergens, R.A., Topalian, S.L., Rudin, C.M., Brock, M.V., Pardoll, D., Baylin, S.B., 2013. Alterations of immune response of non-Small cell lung cancer with azacytidine. Oncotarget 4, 2067–2079.
Xu, P., Hu, G., Luo, C., Liang, Z., 2016. DNA methyltransferase inhibitors: an updated patent review (2012–2015). Expert Opin. Ther. Pat. 26, 1017–1030.
Yamanegi, K., Yamane, J., Kobayashi, K., Kato-Kogoe, N., Ohyama, H., Nakasho, K., Yamada, N., Hata, M., Fukunaga, S., Futani, H., Okamura, H., Terada, N., 2012. Valproic acid cooperates with hydralazine to augment the susceptibility of human osteosarcoma cells to Fas- and NK cell-mediated cell death. Int. J. Oncol. 41, 83–91.
Yang, D., Torres, C.M., Bardhan, K., Zimmerman, M., McGaha, T.L., Liu, K., 2012. Decitabine and vorinostat cooperate to sensitize colon carcinoma cells to Fas ligand-induced apoptosis in vitro and tumor suppression in vivo. J. Immunol. 188, 4441–4449.
Yang, H., Bueso-Ramos, C., DiNardo, C., Estecio, M.R., Davanlou, M., Geng, Q.R., Fang, Z., Nguyen, M., Pierce, S., Wei, Y., Parmar, S., Cortes, J., Kantarjian, H., Garcia-Manero, G., 2014. Expression of PD-L1, PD-L2 PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia 28, 1280–1288.
Yao, Y., Zhou, J., Wang, L., Gao, X., Ning, Q., Jiang, M., Wang, J., Wang, L., Yu, L., 2013. Increased PRAME-specific CTL killing of acute myeloid leukemia cells by either a novel histone deacetylase inhibitor chidamide alone or combined treatment with decitabine. PLoS One 8, e70522.
Yoder, J.A., Walsh, C.P., Bestor, T.H., 1997. Cytosine methylation and the ecology of intragenomic parasites. Trends Genet. 13, 335–340.
Yu, Y., Xiao, C.H., Tan, L.D., Wang, Q.S., Li, X.Q., Feng, Y.M., 2014. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-beta signalling. Br. J. Cancer 110, 724–732.
Zeng, M., Hu, Z., Shi, X., Li, X., Zhan, X., Li, X.D., Wang, J., Choi, J.H., Wang, K.W., Purrington, T., Tang, M., Fina, M., DeBerardinis, R.J., Moresco, E.M., Pedersen, G., McInerney, G.M., Karlsson Hedestam, G.B., Chen, Z.J., Beutler, B., 2014. MAVS, cGAS, and endogenous retroviruses in T-independent B cell responses. Science 346, 1486–1492.
Zhang, C., Wang, S., Liu, Y., Yang, C., 2016. Epigenetics in myeloid derived suppressor cells: a sheathed sword towards cancer. Oncotarget 7, 57452–57463.
Zheng, H., Zhao, W., Yan, C., Watson, C.C., Massengill, M., Xie, M., Massengill, C., Noyes, D.R., Martinez, G.V., Afzal, R., Chen, Z., Ren, X., Antonia, S.J., Haura, E.B., Ruffell, B., Beg, A.A., 2016. HDAC inhibitors enhance T-Cell chemokine expression and augment response to PD-1 immunotherapy in lung adenocarcinoma. Clin. Cancer Res. 22, 4119–4132.
Zhu, S., Denman, C.J., Cobanoglu, Z.S., Kiany, S., Lau, C.C., Gottschalk, S.M., Hughes, D.P., Kleinerman, E.S., Lee, D.A., 2015. The narrow-spectrum HDAC inhibitor entinostat enhances NKG2D expression without NK cell toxicity, leading to enhanced recognition of cancer cells. Pharm. Res. 32, 779–792.
Zhu, H., Bengsch, F., Svoronos, N., Rutkowski, M.R., Bitler, B.G., Allegrezza, M.J., Yokoyama, Y., Kossenkov, A.V., Bradner, J.E., Conejo-Garcia, J.R., Zhang, R., 2016. BET bromodomain inhibition promotes anti-tumor immunity by suppressing PD-L1 expression. Cell Rep. 16, 2829–2837.
Zitvogel, L., Galluzzi, L., Kepp, O., Smyth, M.J., Kroemer, G., 2015. Type I interferons in anticancer immunity. Nat. Rev. Immunol. 15, 405–414.

Glossary

Immunotherapy: Immunotherapy treatments, such as immune checkpoint inhibitors and adoptive t cell therapy, are designed to stimulate the host immune response to combat infection and diseases such as cancer.

Immunosurveillance: Immune surveillance is theories that the immune system patrols the body not only to recognize and destroy invading pathogens but also host cells that become cancerous.

Immune checkpoint blockade: Immune checkpoints refer to inhibitory pathways of the immune system for maintaining self-tolerance and modulating the duration and amplitude of physiological immune responses in peripheral tissues in order to minimize collateral tissue damage. however these checkpoints can become dysregulated in disease to protect pathological cells from the host immune response. blockade of these checkpoints using checkpoint inhibitors to re-activate the immune system is called immune checkpoint blockade.