



Immunotherapy With Human Gamma Delta T Cells—Synergistic Potential of Epigenetic Drugs?

Jaydeep Bhat^{1†}, Léonce Kouakanou¹, Christian Peters¹, Zhinan Yin² and Dieter Kabelitz^{1*}

¹Institute of Immunology, University of Kiel, Kiel, Germany, ²The First Affiliated Hospital, Biomedical Translational Research Institute, Guangdong Province Key Laboratory of Molecular Immunology and Antibody Engineering, Jinan University, Guangzhou, China

Keywords: Bromodomain and ExtraTerminal domain, checkpoint inhibitors, DNA methylation, gamma delta T cells, histone acetylation, immunotherapy, natural-killer group 2 member D, programmed death 1

OPEN ACCESS

Edited by:

Francesco Dieli,
Università degli Studi
di Palermo, Italy

Reviewed by:

Sarina Ravens,
Hannover Medical
School, Germany
Volker Kunzmann,
Universitätsklinikum
Würzburg, Germany

*Correspondence:

Dieter Kabelitz
dietrich.kabelitz@uksh.de

†Present address:

Jaydeep Bhat,
Systems Biology Center,
Division of Intramural
Research, NHLBI, NIH,
Bethesda, MD, United States

Specialty section:

This article was submitted
to T Cell Biology,
a section of the journal
Frontiers in Immunology

Received: 25 January 2018

Accepted: 27 February 2018

Published: 13 March 2018

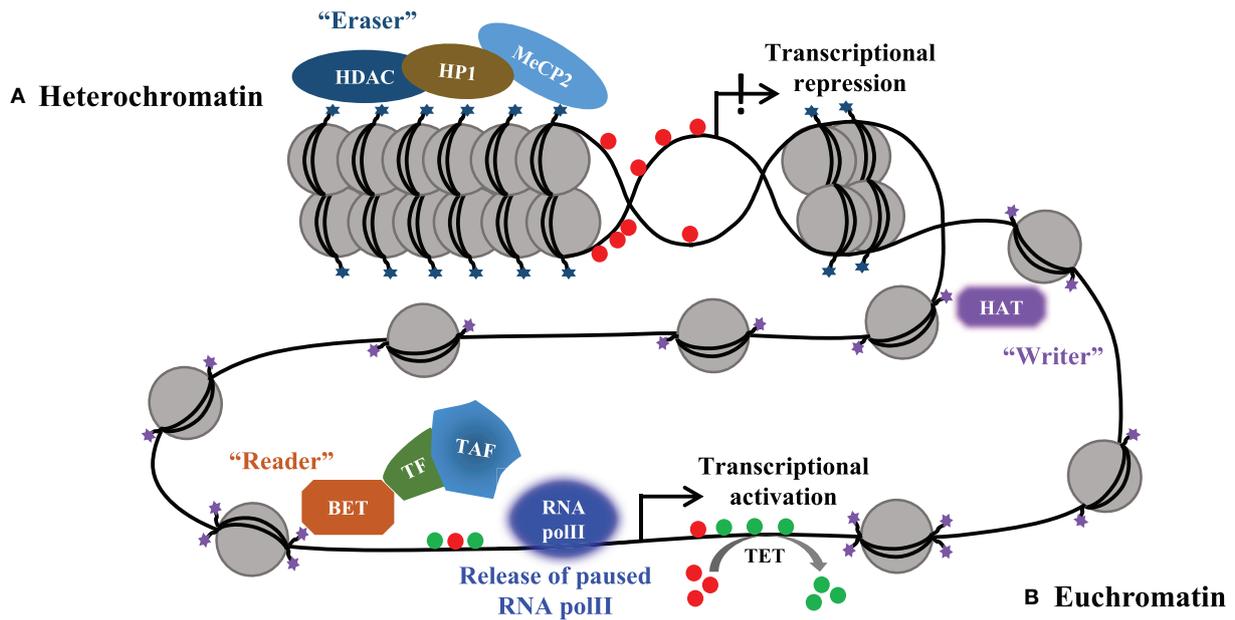
Citation:

Bhat J, Kouakanou L, Peters C,
Yin Z and Kabelitz D (2018)
Immunotherapy With Human
Gamma Delta T Cells—Synergistic
Potential of Epigenetic Drugs?
Front. Immunol. 9:512.
doi: 10.3389/fimmu.2018.00512

INTRODUCTION

Epigenetics has emerged as one of the fastest growing concepts, adding more than 45 new publications every day, spreading through various fields (1). Conrad Waddington coined the term “epigenetics” in 1942; however, a multitude of definitions has been endorsed by different researchers. In essence, Waddington’s definition of “epigenetics” and its redefinition by Holiday is at the heart of cellular function. Hence, it is obvious that epigenetic regulation plays a central role also in the specification, differentiation, and functional plasticity of T lymphocytes (2). T-cell fate decision in progenitor cells, functional CD4 T-cell plasticity, CD8 T-cell differentiation, but also T-cell memory, are all substantially governed by epigenetic mechanisms (3–7). Here, we focus on the current development of drugs targeting major pathways of epigenetic regulation and their possible impact on $\gamma\delta$ T-cell multifunctionality. We aim to develop concepts of how some of these approaches might help to improve the efficacy of $\gamma\delta$ T-cell-based immunotherapies.

The dynamic construction of chromatin organization exists in two principal states, i.e., transcriptionally repressive “heterochromatin” and active “euchromatin.” The heterochromatin formation (**Figure 1A**) is mediated by SET domain, the chromodomain, and plant homeodomain finger, found in the heterochromatin protein 1 (HP1)/chromobox, and the chromodomain helicase-DNA-binding subfamilies, recognizing histone methylation (e.g., H3K9 di- and tri- methylation) (8). Histone deacetylase (HDAC) associates with HP1, then recognize histone methyltransferases and methylated DNA *via* methyl-binding proteins such as MeCP2. HDACs also interact with DNA methyltransferases (DNMTs; the enzymes catalyze DNA methylation), thus forming the regulatory axis of a multiprotein complex responsible for transcriptional repression (9–11). DNA demethylation can be achieved “actively” by the hydroxylation of 5-methylcytosine to 5-hydroxymethyl cytosine mediated by the ten-eleven translocation (TET) enzymes (12, 13). In contrast, the “euchromatin” formation is a complex, multistep process involving post-translational modifications (PTM) of histones and also chromatin-remodeling complex (**Figure 1B**). In addition to other PTM, the acetylation of histone leading to the “euchromatin” formation has already been reported during the 1990s (14). This process of histone lysine acetylation is mediated by HAT and is recognized by the bromodomain (Brd) proteins, additionally recruiting proteins. The Brd proteins are thus categorized as components of HAT complexes, components of chromatin-remodeling complexes, and Brd and ExtraTerminal domain (BET) proteins. BET proteins, particularly Brd2 and Brd3, play a multifaceted role by maintaining euchromatin status and simultaneously “reading” both acetylated histones and transcription factors. By recruiting and coupling the transcriptional machinery to the target gene promoter and/or enhancer sites,



Legends:

- ★ Repressive histone marks (e.g. H3K9me2/3) ● 5-methylcytosine
- ☆ Activation histone marks (e.g. H3K9ac) ● 5-hydroxymethylcytosine

C Epigenetic drugs

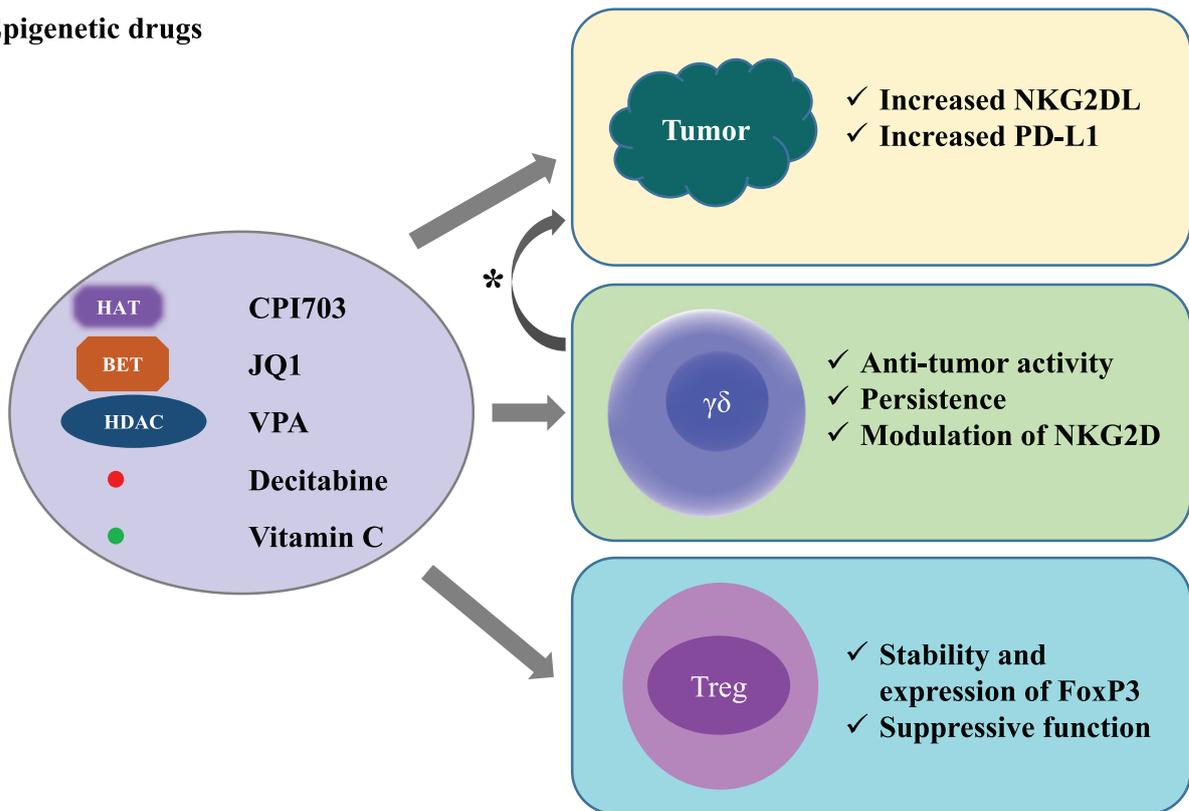


FIGURE 1 | Continued

FIGURE 1 | An overview of epigenetic mechanisms governing cellular processes and the drugs targeting respective epigenetic processes. There are two possible states of chromatin organization: **(A)** the “closed” chromatin associated with heterochromatin formation and transcriptional repression drives gene silencing. **(B)** The mechanistic organization of euchromatin maintains the “open” chromatin confirmation and allows active gene expression. **(C)** Examples of how epigenetic drugs modulate the $\gamma\delta$ T-cell/Treg/tumor interaction. In the circle (left part), the epigenetic drugs (on the right-hand side) that are either in pre-clinical development or clinically approved are listed along with the respective target proteins (on the left-hand side). These are the key proteins for diverse epigenetic processes. The effect of the listed epigenetic drugs on immune cells ($\gamma\delta$ T cells and Treg) and tumor cells are shown in the rectangles (right part). As marked by asterisk (*), the epigenetic drugs are proposed to synergize, leading to increased efficacy of $\gamma\delta$ T cell-based immunotherapy. HDAC, histone deacetylase; HP1, heterochromatin protein 1; MeCP2, methyl-CpG binding protein 2; HAT, histone acetyltransferase; BET, Bromodomain and ExtraTerminal; TF, transcription factor; TAF, transcription-associated factors; RNA polII, RNA polymerase II; TET, ten-eleven translocation; VPA, valproic acid; NKG2D, natural-killer group 2, member D receptor protein; NKG2DL, ligands for NKG2D receptor protein; PD-L1, programmed death ligand 1; FoxP3, forkhead Box P3; Treg, regulatory T cells.

BET proteins further release paused RNA polymerase II for the respective gene activation (15–19). Additionally, the proteins involved in the principal states of chromatin organization have multiple functions including enzymatic activity. Such an important complexity in protein/enzyme function provides a leverage for the epigenetic drugs.

Nonetheless, it is important to realize (but currently not yet a major focus of epigenetic research) that any enzymatic activity (and thus epigenetic regulation) depends on the appropriate cellular metabolism. While the central role of the cellular metabolism for the maintenance of stem cell pluripotency (which is drastically influenced by epigenetics) is well known, the respective roles of metabolic pathways and nutrients availability versus epigenetics for the differentiation and plasticity of immune cells have only recently been appreciated (20, 21).

EPIGENETIC DRUGS

In view of the central role of epigenetic regulation for developmental biology and cellular activation, proliferation, and differentiation, it comes as no surprise that many drugs targeting specific steps of epigenetic regulation have been developed (**Figure 1C**). If suitable for clinical application, such drugs might have broad applications for the treatment of (certain types of) cancer but also autoimmune and chronic inflammatory diseases.

Currently, two hypomethylating agents targeting epigenetic “erasers,” decitabine (5-aza-2'-deoxycytidine) and azacitidine (5-azacitidine) are approved by the US Food and Drug Administration (FDA) for the treatment of myelodysplastic syndromes, but are also used in other clinical conditions (22). The major effect of such agents is to induce hypomethylation of CpG islands thereby allowing re-expression of suppressed genes including tumor suppressor genes. Not unexpectedly, hypomethylating drugs have major effects on immune cells including the stabilization of FoxP3 expression and Treg activity (23). In addition, numerous studies have investigated effects of hypomethylating agents on NK cells, dendritic cells, and T cells [see Ref. (22)]. It is difficult to draw general conclusions as the reported effects may be linked to specific experimental conditions or treatment regimens, but immunomodulatory effects are quite obvious (22). Immunogenicity of tumors might increase due to re-expression of tumor-associated antigens. However, hypomethylating agents might also promote tumor resistance through upregulation of inhibitory molecules like PD-1 and/or PD-L1 (24, 25). Obviously, the complexity of the effects of epigenetic drugs needs to be

carefully evaluated. A major breakthrough in cancer immunotherapy has been the introduction of checkpoint inhibitors into clinical practice. Currently, several trials have been initiated where azacitidine is combined with PD-1/PD-L1 or CTLA-4 checkpoint inhibitors in hematological malignancies and colorectal cancer (26). Another regulator of DNA methylation is Vitamin C (VC). In addition to its antioxidant activity, VC also activates TET enzyme activity and thereby promotes 5-hydroxymethylation of DNA (27, 28).

Like hypomethylating agents, HDAC inhibitors (HDACi) have multiple effects on tumor cells but also on immune cells. In fact, their therapeutic efficacy against cancer is likely to depend on the simultaneous modulation of the immune system (29). Several structural classes of HDACi have been developed. While some HDACi inhibit all HDACs, others are specific for class I and class IIa HDACs (e.g., valproic acid, VPA) or only class I HDAC (e.g., entinostat). Some HDACi including VPA upregulate the expression of NKG2D ligands on tumor cells and thereby augment the susceptibility to recognition and lysis by NK cells and $\gamma\delta$ T cells (30, 31). As of today, several HDACi have been approved by the FDA either as monotherapy or in combination with other drugs, such as with PD-1 or CTLA-4 checkpoint inhibitors (26), for the treatment of hematological malignancies and some solid tumors [see Ref. (26)].

Epigenetic drugs which target epigenetic “readers” are BET inhibitors. The inhibition of BET proteins has a broad impact on gene regulation and may have a therapeutic effect in cancer (18). JQ-1, a pan-BET inhibitor blocks Th17 differentiation and thereby suppresses Th17-related inflammatory diseases in mouse models (19, 32). Importantly, recent studies point to a selective effect of JQ-1 on PD-L1 expression. PD-L1 is a direct target gene of the BET family member Brd4, and BET inhibition by JQ-1 has been found to enhance anti-tumor immunity by suppressing the PD-L1 expression on tumor cells and antigen-presenting cells but also through upregulation of NKG2D ligand MICA on tumor cells (33–35). BET inhibition also affects T-cell differentiation. A recent study reported superior *in vivo* persistence and anti-tumor activity of tumor antigen-specific murine T cells upon adoptive transfer (36). Moreover, BET proteins appear to be interesting targets for synergistic anti-tumor effects in combination with other inhibitors targeting, e.g., PI3-kinase (37), Bcl-2 (38), PARP (39), or HDAC (40). Last but not least, BET inhibitors like JQ-1 might also synergize with checkpoint inhibitors to facilitate efficient anti-tumor immune responses (41). Based on promising pre-clinical results, BET inhibitors have entered clinical trials. However, many details of how BET inhibitors work at

the molecular level and which cells and tissues are differentially affected, are not yet precisely known; therefore, the adverse side effect profile of various BET inhibitors needs to be studied in detail (42).

PLASTICITY OF $\gamma\delta$ T CELLS

$\gamma\delta$ T cells are considered to link innate and adaptive immunity because they can be rapidly activated *via* their T-cell receptor (TCR) in an MHC-independent manner (e.g., recognition of pyrophosphates in the case of human V γ 9V δ 2 T cells) but also express functional innate receptors such as toll-like receptors (43). Importantly, human V γ 9V δ 2 T cells cannot only differentiate into different cytokine-producing subsets, but also may acquire regulatory activity and “professional” antigen-presenting capacity (44). Moreover, $\gamma\delta$ T cells are usually potent cytotoxic effector cells which kill various tumor target cells independent of HLA restriction. Human V γ 9V δ 2 T cells recognize pyrophosphates accumulating in tumor cells exhibiting a dysregulated mevalonate metabolic pathway in a butyrophilin 3A-dependent manner (45). However, most $\gamma\delta$ T cells also express the activating NKG2D receptor, which endows them with a TCR-independent second activation pathway *via* recognition of NKG2D ligands (e.g., MICA/B) on tumor cells. Based on their HLA-independent mode of target cell recognition, $\gamma\delta$ T cells have recently attracted substantial interest as potential effector cells in cell-based cancer immunotherapy (46). This includes the perspective of using allogeneic $\gamma\delta$ T cells from healthy donors since $\gamma\delta$ T cells from the blood of tumor patients are sometimes difficult to expand *in vitro*. The experience of one of us (Zhinan Yin) with over 140 adoptive $\gamma\delta$ T-cell transfers in more than 45 patients with different malignancies indicates that such $\gamma\delta$ T-cell transfers are safe and are well tolerated.

In the murine system, genome-wide histone (H3) acetylation and methylation profiling have identified distinct molecular programs in interferon- γ versus IL-17 producing $\gamma\delta$ T cells (47). It is also well established that epigenetic mechanisms regulate the chromatin accessibility of the TCR γ locus during intrathymic T cell development (48, 49). Currently, however, there is only limited information available as to how epigenetics contributes to the multifunctionality of human $\gamma\delta$ T cells. We have performed a comprehensive analysis of peripheral blood $\alpha\beta$ T cell subsets (CD4⁺, Treg, CD8⁺) and $\gamma\delta$ T cells. In this ongoing work, we expect to obtain information on how $\gamma\delta$ T cells differ from (subsets of) $\alpha\beta$ T cells at the transcriptome and epigenetic level (Bhat et al., unpublished). Moreover, we have investigated the effects of the HDACi VPA on the V γ 9V δ 2 subset of human $\gamma\delta$ T cells upon *in vitro* culture. VPA differentially modulated the expression of certain surface markers (notably CD86, CD54, and NKG2D) on $\gamma\delta$ T cells compared with $\alpha\beta$ T cells (50). For instance, NKG2D receptors on $\gamma\delta$ T cells and their respective ligands on tumor cells were even more affected after VPA treatment (Bhat et al., under revision). We also observed that VPA induced the expression of a non-secreted isoform of IL-4 (IL-4 δ 13) which is known to have regulatory properties (51). Ongoing studies in our laboratories analyze the effects of VC

on the *in vitro* differentiation of human $\gamma\delta$ T cells. VC increases and stabilizes the expression of FoxP3 in transforming growth factor- β (TGF- β)-treated V γ 9V δ 2 T cells and augments the proliferative capacity of V γ 9V δ 2 T cells upon pyrophosphate-induced growth arrest (Kouakanou et al., to be published). RNA-seq and reduced representation bisulfite sequencing analyses of VC-treated human $\gamma\delta$ T cells will provide insights how VC globally affects human $\gamma\delta$ T-cell plasticity at the transcriptional and DNA methylation level. Though our study has been focused on the V γ 9V δ 2 subset, the effect of epigenetic drugs needs to be addressed in the context of distinct subsets of $\gamma\delta$ T cells. Hence, the implication of epigenetic modulation needs to be investigated using different settings. Interestingly, we also found that TGF- β , usually considered as an immunosuppressive cytokine (52), can actually increase the cytotoxic activity of purified $\gamma\delta$ T cells activated by pyrophosphate antigens in the presence of TGF- β (Peters et al., submitted). Thus, a variety of strategies are available to modulate the plasticity of human $\gamma\delta$ T cells.

HOW TO MODULATE THE ANTI-TUMOR POTENTIAL OF MULTIFUNCTIONAL $\gamma\delta$ T CELLS?

Based on the outlined principles, we can envisage a multitude of approaches to enhance the cytotoxic anti-tumor activity of human $\gamma\delta$ T cells, or to modulate their subset phenotype (Bhat et al., under revision), or to revert their detrimental activity (e.g., regulatory activity and/or high-PD-L1 expression of tumor-infiltrating $\gamma\delta$ T cells) (53). DNMT inhibitors and HDACi already in clinical use modulate antigens relevant for $\gamma\delta$ T-cell activation including NKG2D receptor and ligands (22, 26) and thus may increase the efficacy of adoptive $\gamma\delta$ T-cell immunotherapy. Of special interest, however, are established and emerging new BET inhibitors. It will be important to find out whether BET inhibitors like JQ-1 can increase the functionality of *in vitro* expanded $\gamma\delta$ T cells and eventually their persistence and anti-tumor activity similar to what has been described for tumor-reactive CD8⁺ T cells (36). BET inhibitors might also augment $\gamma\delta$ T-cell immunotherapy *via* increasing the expression of NKG2D ligands on tumor cells (35). Furthermore, the recently reported BET inhibitor-mediated inhibition of PD-L1 expression on tumor cells [associated with improved anti-tumor immunity; Ref. (33)] might also extend to the inhibition of PD-L1 expression on tumor-infiltrating $\gamma\delta$ T cells, which has been shown to restrain effective $\alpha\beta$ T-cell responses in pancreatic oncogenesis (54). Last but not least, novel inhibitors have been developed selectively inhibiting the Brd interaction of CBP/EP300, which plays a crucial role in Treg biology (55). By dampening Treg activity, such small molecule inhibitors might also increase the efficacy of $\gamma\delta$ T-cell immunotherapy in cancer patients. Overall, we have a plethora of strategies at hand to potentially increase the efficacy of $\gamma\delta$ T-cell immunotherapy. The challenge is to design the best possible (pre-clinical and clinical) studies to identify efficacious synergistic strategies with acceptable adverse risk profile.

AUTHOR CONTRIBUTIONS

DK and JB wrote the manuscript. LK, CP, ZY, JB, and DK contributed to the discussion of the draft and made final corrections.

REFERENCES

- Deans C, Maggert KA. What do you mean, “epigenetic”? *Genetics* (2015) 199(4):887–96. doi:10.1534/genetics.114.173492
- Bhat J, Helmuth J, Chitadze G, Kouakanou L, Peters C, Vingron M, et al. Stochastics of cellular differentiation explained by epigenetics: the case of T-cell differentiation and functional plasticity. *Scand J Immunol* (2017) 86(4):184–95. doi:10.1111/sji.12589
- Isoda T, Moore AJ, He Z, Chandra V, Aida M, Denholtz M, et al. Non-coding transcription instructs chromatin folding and compartmentalization to dictate enhancer-promoter communication and T cell fate. *Cell* (2017) 171(1):103.e–19.e. doi:10.1016/j.cell.2017.09.001
- Delacher M, Imbusch CD, Weichenhan D, Breiling A, Hotz-Wagenblatt A, Trager U, et al. Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues. *Nat Immunol* (2017) 18(10):1160–72. doi:10.1038/ni.3799
- Luo A, Leach ST, Barres R, Hesson LB, Grimm MC, Simar D. The microbiota and epigenetic regulation of T helper 17/regulatory T cells: in search of a balanced immune system. *Front Immunol* (2017) 8:417. doi:10.3389/fimmu.2017.00417
- Moskowitz DM, Zhang DW, Hu B, Le Saux S, Yanes RE, Ye Z, et al. Epigenomics of human CD8 T cell differentiation and aging. *Sci Immunol* (2017) 2(8):eaag0192. doi:10.1126/sciimmunol.aag0192
- Rodriguez RM, Suarez-Alvarez B, Lavin JL, Mosen-Ansorena D, Baragano Raneros A, Marquez-Kisinousky L, et al. Epigenetic networks regulate the transcriptional program in memory and terminally differentiated CD8+ T cells. *J Immunol* (2017) 198(2):937–49. doi:10.4049/jimmunol.1601102
- Yap KL, Zhou MM. Structure and mechanisms of lysine methylation recognition by the chromodomain in gene transcription. *Biochemistry* (2011) 50(12):1966–80. doi:10.1021/bi101885m
- Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* (2007) 1(1):19–25. doi:10.1016/j.molonc.2007.01.001
- Tampe B, Zeisberg M. Evidence for the involvement of epigenetics in the progression of renal fibrogenesis. *Nephrol Dial Transplant* (2014) 29(Suppl 1):i1–8. doi:10.1093/ndt/gft361
- Honda S, Bicocca VT, Gessaman JD, Rountree MR, Yokoyama A, Yu EY, et al. Dual chromatin recognition by the histone deacetylase complex HCHC is required for proper DNA methylation in *Neurospora crassa*. *Proc Natl Acad Sci U S A* (2016) 113(41):E6135–44. doi:10.1073/pnas.1614279113
- Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. *Nat Rev Genet* (2018) 19:81–92. doi:10.1038/nrg.2017.80
- Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. *Nat Rev Genet* (2017) 18(9):517–34. doi:10.1038/nrg.2017.33
- Hebbes TR, Thorne AW, Crane-Robinson C. A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO J* (1988) 7(5):1395–402.
- Bottomley MJ. Structures of protein domains that create or recognize histone modifications. *EMBO Rep* (2004) 5(5):464–9. doi:10.1038/sj.embor.7400146
- LeRoy G, Rickards B, Flint SJ. The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. *Mol Cell* (2008) 30(1):51–60. doi:10.1016/j.molcel.2008.01.018
- Josling GA, Selvarajah SA, Petter M, Duffy MF. The role of bromodomain proteins in regulating gene expression. *Genes (Basel)* (2012) 3(2):320–43. doi:10.3390/genes3020320
- Shi J, Vakoc CR. The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell* (2014) 54(5):728–36. doi:10.1016/j.molcel.2014.05.016
- Cheung K, Lu G, Sharma R, Vincek A, Zhang R, Plotnikov AN, et al. BET N-terminal bromodomain inhibition selectively blocks Th17 cell differentiation and ameliorates colitis in mice. *Proc Natl Acad Sci U S A* (2017) 114(11):2952–7. doi:10.1073/pnas.1615601114

FUNDING

DK was supported by Deutsche Forschungsgemeinschaft grant Ka 502/19-1. LK is the recipient of a Ph.D. fellowship from the German Academic Exchange Service (DAAD).

- Reid MA, Dai Z, Locasale JW. The impact of cellular metabolism on chromatin dynamics and epigenetics. *Nat Cell Biol* (2017) 19(11):1298–306. doi:10.1038/ncb3629
- Phan AT, Goldrath AW, Glass CK. Metabolic and epigenetic coordination of T cell and macrophage immunity. *Immunity* (2017) 46(5):714–29. doi:10.1016/j.immuni.2017.04.016
- Lindblad KE, Goswami M, Hourigan CS, Oetjen KA. Immunological effects of hypomethylating agents. *Expert Rev Hematol* (2017) 10(8):745–52. doi:10.1080/17474086.2017.1346470
- Lal G, Zhang N, van der Touw W, Ding Y, Ju W, Bottinger EP, et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol* (2009) 182(1):259–73. doi:10.4049/jimmunol.182.1.259
- Heninger E, Krueger TE, Lang JM. Augmenting antitumor immune responses with epigenetic modifying agents. *Front Immunol* (2015) 6:29. doi:10.3389/fimmu.2015.00029
- Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia* (2014) 28(6):1280–8. doi:10.1038/leu.2013.355
- Terranova-Barberio M, Thomas S, Munster PN. Epigenetic modifiers in immunotherapy: a focus on checkpoint inhibitors. *Immunotherapy* (2016) 8(6):705–19. doi:10.2217/imt-2016-0014
- Gillberg L, Orskov AD, Liu M, Harslof LBS, Jones PA, Gronbaek K. Vitamin C—a new player in regulation of the cancer epigenome. *Semin Cancer Biol* (2017). doi:10.1016/j.semcancer.2017.11.001
- Sasidharan Nair V, Song MH, Oh KI. Vitamin C facilitates demethylation of the Foxp3 enhancer in a TET-dependent manner. *J Immunol* (2016) 196(5):2119–31. doi:10.4049/jimmunol.1502352
- West AC, Smyth MJ, Johnstone RW. The anticancer effects of HDAC inhibitors require the immune system. *Oncotarget* (2014) 3(1):e27414. doi:10.4161/onci.27414
- Shi P, Yin T, Zhou F, Cui P, Gou S, Wang C. Valproic acid sensitizes pancreatic cancer cells to natural killer cell-mediated lysis by upregulating MICA and MICB via the PI3K/Akt signaling pathway. *BMC Cancer* (2014) 14:370. doi:10.1186/1471-2407-14-370
- Du XZ, Li QY, Du FW, He ZG, Wang J. Sodium valproate sensitizes non-small lung cancer A549 cells to gamma delta T-cell-mediated killing through upregulating the expression of MICA. *J Biochem Mol Toxicol* (2013) 27(11):492–8. doi:10.1002/jbt.21513
- Mele DA, Salmeron A, Ghosh S, Huang HR, Bryant BM, Lora JM. BET bromodomain inhibition suppresses TH17-mediated pathology. *J Exp Med* (2013) 210(11):2181–90. doi:10.1084/jem.20130376
- Zhu H, Bengsch F, Svoronos N, Rutkowski MR, Bitler BG, Allegranza MJ, et al. BET bromodomain inhibition promotes anti-tumor immunity by suppressing PD-L1 expression. *Cell Rep* (2016) 16(11):2829–37. doi:10.1016/j.celrep.2016.08.032
- Wang H, Cheng F, Xing L, Zhao X, Villagra A, Pinilla-Ibarz J, et al. JQ 1, a selective bromodomain inhibitor, decreased the expression of the tolerogenic molecule PDL1 in antigen-presenting cells (APCs) and restores the responsiveness of anergic CD4+ T cells. *Blood* (2014) 124:2479.
- Abruzzese MR, Bilotta MT, Fionda C, Zingoni A, Soriani A, Vulpis E, et al. Inhibition of bromodomain and extra-terminal (BET) proteins increases NKG2D ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in multiple myeloma cells: role of cMYC-IRF4-miR-125b interplay. *J Hematol Oncol* (2016) 9(1):134. doi:10.1186/s13045-016-0362-2
- Kagoya Y, Nakatsugawa M, Yamashita Y, Ochi T, Guo T, Anczurowski M, et al. BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. *J Clin Invest* (2016) 126(9):3479–94. doi:10.1172/JCI86437
- Andrews FH, Singh AR, Joshi S, Smith CA, Morales GA, Garlich JR, et al. Dual-activity PI3K-BRD4 inhibitor for the orthogonal inhibition of MYC

- to block tumor growth and metastasis. *Proc Natl Acad Sci U S A* (2017) 114(7):E1072–80. doi:10.1073/pnas.1613091114
38. Wang H, Hong B, Li X, Deng K, Li H, Yan Lui VW, et al. JQ1 synergizes with the Bcl-2 inhibitor ABT-263 against MYCN-amplified small cell lung cancer. *Oncotarget* (2017) 8(49):86312–24. doi:10.18632/oncotarget.21146
 39. Karakashev S, Zhu H, Yokoyama Y, Zhao B, Fatkhutdinov N, Kossenkov AV, et al. BET bromodomain inhibition synergizes with PARP inhibitor in epithelial ovarian cancer. *Cell Rep* (2017) 21(12):3398–405. doi:10.1016/j.celrep.2017.11.095
 40. Adeegbe DO, Liu Y, Lizotte PH, Kamihara Y, Aref AR, Almonte C, et al. Synergistic immunostimulatory effects and therapeutic benefit of combined histone deacetylase and bromodomain inhibition in non-small cell lung cancer. *Cancer Discov* (2017) 7(8):852–67. doi:10.1158/2159-8290.CD-16-1020
 41. Adeegbe DO, Freeman GJ, Wong K-K. BET bromodomain inhibition synergizes with immune checkpoint blockade to facilitate anti-tumor response in a murine model of non-small cell lung cancer harboring activating KRAS mutation. *J Immunol* (2016) 196(Suppl 74):10.
 42. Andrieu G, Belkina AC, Denis GV. Clinical trials for BET inhibitors run ahead of the science. *Drug Discov Today Technol* (2016) 19:45–50. doi:10.1016/j.ddtec.2016.06.004
 43. Wesch D, Peters C, Oberg HH, Pietschmann K, Kabelitz D. Modulation of gammadelta T cell responses by TLR ligands. *Cell Mol Life Sci* (2011) 68(14):2357–70. doi:10.1007/s00018-011-0699-1
 44. Vantourout P, Hayday A. Six-of-the-best: unique contributions of gammadelta T cells to immunology. *Nat Rev Immunol* (2013) 13(2):88–100. doi:10.1038/nri3384
 45. Gu S, Nawrocka W, Adams EJ. Sensing of pyrophosphate metabolites by Vgamma9Vdelta2 T cells. *Front Immunol* (2014) 5:688. doi:10.3389/fimmu.2014.00688
 46. Fowler DW, Bodman-Smith MD. Harnessing the power of Vdelta2 cells in cancer immunotherapy. *Clin Exp Immunol* (2015) 180(1):1–10. doi:10.1111/cei.12564
 47. Schmolka N, Serre K, Grosso AR, Rei M, Pennington DJ, Gomes AQ, et al. Epigenetic and transcriptional signatures of stable versus plastic differentiation of proinflammatory gammadelta T cell subsets. *Nat Immunol* (2013) 14(10):1093–100. doi:10.1038/ni.2702
 48. Agata Y, Katakai T, Ye SK, Sugai M, Gonda H, Honjo T, et al. Histone acetylation determines the developmentally regulated accessibility for T cell receptor gamma gene recombination. *J Exp Med* (2001) 193(7):873–80. doi:10.1084/jem.193.7.873
 49. Wagatsuma K, Tani-ichi S, Liang B, Shitara S, Ishihara K, Abe M, et al. STAT5 orchestrates local epigenetic changes for chromatin accessibility and rearrangements by direct binding to the TCRgamma locus. *J Immunol* (2015) 195(4):1804–14. doi:10.4049/jimmunol.1302456
 50. Bhat J, Oberg HH, Kabelitz D. Modulation of human gamma/delta T-cell activation and phenotype by histone deacetylase inhibitors. *Cell Immunol* (2015) 296(1):50–6. doi:10.1016/j.cellimm.2015.01.019
 51. Bhat J, Sosna J, Fritsch J, Quabius ES, Schutze S, Zeissig S, et al. Expression of non-secreted IL-4 is associated with HDAC inhibitor-induced cell death, histone acetylation and c-Jun regulation in human gamma/delta T-cells. *Oncotarget* (2016) 7(40):64743–56. doi:10.18632/oncotarget.11462
 52. Kelly A, Houston SA, Sherwood E, Casulli J, Travis MA. Regulation of innate and adaptive immunity by TGFbeta. *Adv Immunol* (2017) 134:137–233. doi:10.1016/bs.ai.2017.01.001
 53. Chitadze G, Oberg HH, Wesch D, Kabelitz D. The ambiguous role of gammadelta T lymphocytes in antitumor immunity. *Trends Immunol* (2017) 38(9):668–78. doi:10.1016/j.it.2017.06.004
 54. Daley D, Zambirinis CP, Seifert L, Akkad N, Mohan N, Werba G, et al. Gammadelta T cells support pancreatic oncogenesis by restraining alphabeta T cell activation. *Cell* (2016) 166(6):1485.e–99.e. doi:10.1016/j.cell.2016.07.046
 55. Ghosh S, Taylor A, Chin M, Huang HR, Conery AR, Mertz JA, et al. Regulatory T cell modulation by CBP/EP300 bromodomain inhibition. *J Biol Chem* (2016) 291(25):13014–27. doi:10.1074/jbc.M115.708560

Conflict of Interest Statement: DK is a member of the Scientific Advisory Board of Incysus, Ltd. The remaining co-authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Bhat, Kouakanou, Peters, Yin and Kabelitz. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.