

## REVIEW

# Bromodomain protein inhibition: a novel therapeutic strategy in rheumatic diseases

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**To cite:** Klein K. Bromodomain protein inhibition: a novel therapeutic strategy in rheumatic diseases. *RMD Open* 2018;4:e000744. doi:10.1136/rmdopen-2018-000744

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/rmdopen-2018-000744>)

Received 25 July 2018

Revised 28 September 2018

Accepted 17 October 2018

## ABSTRACT

The reading of acetylation marks on histones by bromodomain (BRD) proteins is a key event in transcriptional activation. Small molecule inhibitors targeting bromodomain and extra-terminal (BET) proteins compete for binding to acetylated histones. They have strong anti-inflammatory properties and exhibit encouraging effects in different cell types *in vitro* and in animal models resembling rheumatic diseases *in vivo*. Furthermore, recent studies that focus on BRD proteins beyond BET family members are discussed.

## INTRODUCTION

Acetylation of histones is associated with the establishment of euchromatin, which is a prerequisite for the activation of gene transcription and expression. The opposing functions of histone acetyl transferases (HAT) and histone deacetylases (HDAC) control the acetylation of lysine residues on histones but both protein families have also been shown to act on non-histone proteins.<sup>1</sup> HDAC inhibitors have been widely studied in rheumatic diseases (RMD) *in vitro* and *in vivo*, underscoring the importance of protein lysine acetylation in this context.<sup>2,3</sup>

The recognition of  $\epsilon$ -N-acetylation modifications on histones is a key event in the reading of epigenetic marks. Bromodomains (BRD) exclusively recognise acetylation motifs. BRDs form a bundle of four alpha helices, linked by interhelical loop regions of variable length and sequence that form the hydrophobic lysine acetylation binding site and contribute to substrate specificity. The structural features of BRDs are in sharp contrast to other epigenetic reader domains such as chromodomains which hold a  $\beta$ -barrel structure.<sup>4</sup> Many BRDs have good druggability, based on prediction from available structures and computational analysis.<sup>5</sup> Frequently, BRDs are flanked by several other epigenetic reader domains, in multidomain BRD proteins, or they occur in tandem arrangement in bromodomain

## Key messages

- Inhibition of bromodomain and extra-terminal (BET) proteins (BRD2, BRD3, BRD4) has anti-inflammatory effects.
- BET protein inhibition alleviates arthritis and fibrosis.
- Bromodomain proteins of distinct families are important in Th17 cell differentiation.
- There is a need for studies beyond the use of pan-inhibitors to unravel the role of individual bromodomain proteins.

and extra-terminal domain (BET) proteins (BRD2, BRD3, BRD4, BRDT).<sup>6</sup>

Reading of acetylation marks by BRD enables the regulation of gene expression through a wide range of activities. Either BRD proteins act as scaffolds that enable the recruitment and binding of large protein complexes or they function as transcription factors and coregulators themselves. Additionally, BRD proteins can contain several catalytic domains enabling them to act as methyltransferases, ATP-dependending chromatin remodelling complexes or HATs and helicases.<sup>7</sup>

## Involvement of BRD proteins in diseases

The human genome encodes 61 BRDs that are present in 46 diverse proteins. These proteins cluster into eight major BRD families (I–VIII),<sup>6</sup> with BET proteins being the foremost studied group. Dysfunction of BRD proteins has been linked to the development of several diseases. Mutations in BRD proteins, copy number alterations, misexpression and BRD-containing fusion proteins, resulting from chromosomal translocation, were observed in several cancers.<sup>7</sup> Targeting BRD proteins has transitioned to the centre of epigenetic drug development in the last decade. Even some of the beneficial effects of HDAC inhibitors might be attributed to BRD,

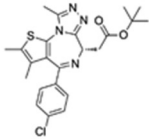
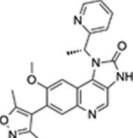
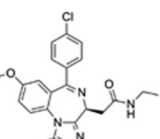
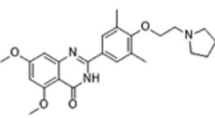


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molecule name	structure	targets
JQ-1		BRD2, BRD3, BRD4, BRDT
I-BET151		BRD2, BRD3, BRD4, BRDT
I-BET762		BRD2, BRD3, BRD4, BRDT
RVX-297		BRD2, BRD3, BRD4, BRDT (BD2 selective)

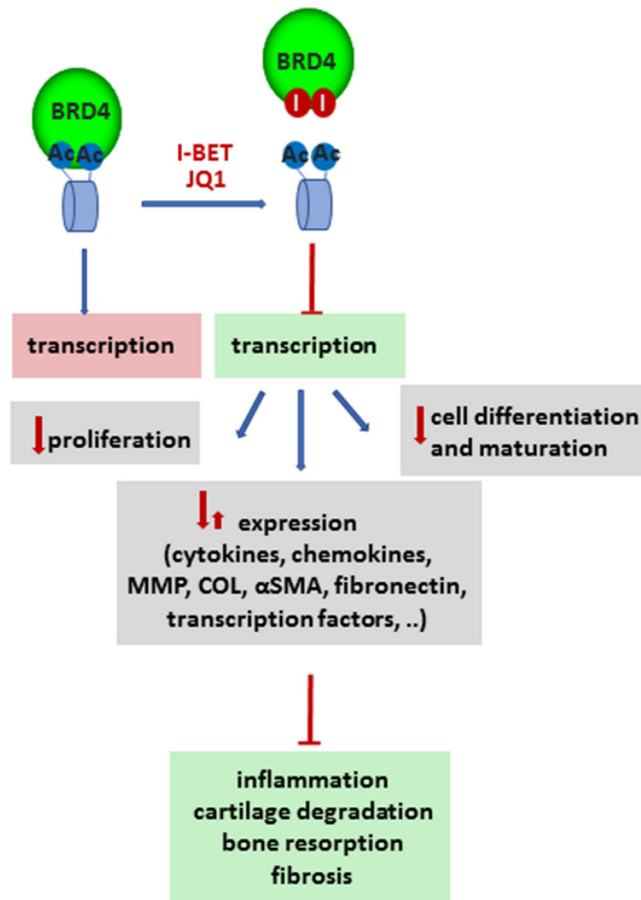
**Figure 1** Inhibitors and their chemical structures targeting BET proteins. BD2, bromodomain 2; BET, bromodomain and extra-terminal.

since HDAC inhibitors can induce a selective depletion of BRD proteins from the cells' nuclei.<sup>8</sup>

### BET protein inhibitors

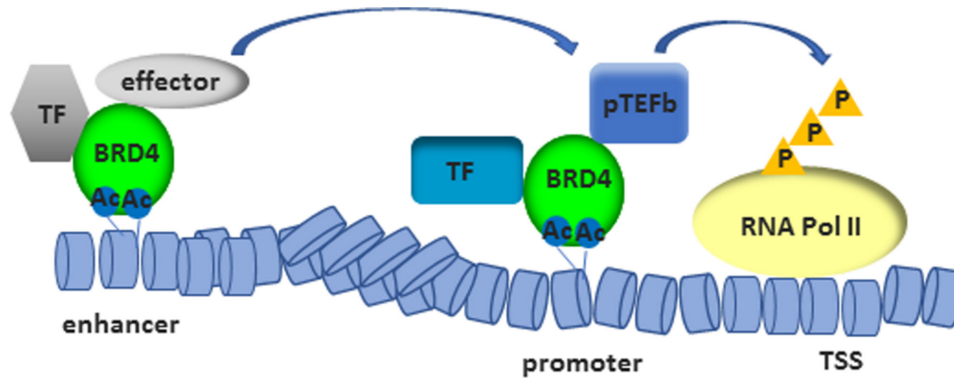
In 2010, the first small molecule inhibitors targeting BET proteins, JQ1 and I-BET, have been described by Filipakopoulos *et al* and Nicodeme *et al*, which exhibited anticancer and anti-inflammatory activities, respectively.<sup>9,10</sup> The availability of inhibitors has led to a significant increase in the BRD protein research. BET inhibitors (figure 1), targeting BRD2, BRD3, BRD4, and the testis-specific BRDT have been tested in many cell types, diseases and disease models suggesting a role of BET proteins far beyond cancer, such as inflammation,<sup>10–18</sup> fibrosis,<sup>19–23</sup> asthma<sup>24,25</sup>, coronary artery<sup>26</sup> and cardiovascular disease,<sup>27,28</sup> Alzheimer's disease,<sup>29,30</sup> autism-like syndrome,<sup>31</sup> graft-versus-host disease<sup>32</sup> and a variety of autoimmune conditions.<sup>33–37</sup> BET inhibitors bind to the proteins' BRD, compete for binding to acetylated histones and displace BET proteins from nuclear chromatin. This prevents the transcription of BRD protein target genes (figure 2). By now, at least 14 different small molecule inhibitors targeting BRD proteins have entered clinical trials for different forms of cancer, but also for the treatment of type 2 diabetes mellitus, cardiovascular disease and coronary artery disease.<sup>7,10</sup>

The most-studied member among BET proteins is BRD4. BRD4 confers the ability to direct the assembly of HAT-dependent chromatin complexes (figure 3), leading to the recruitment of positive transcription elongation factor b



**Figure 2** Inhibitors, such as JQ1 and I-BET, targeting BET proteins compete with the BRD for binding to acetylated histones. Thereby, they prevent the transcription of their target genes depending on the cell type. This leads to decreased proliferation, suppressed cell differentiation and maturation and altered gene expression. BET inhibitors lead to decreased inflammation, cartilage destruction, bone resorption and fibrosis. AC, acetylated; BET, bromodomain and extra-terminal; BRD, bromodomain; col, collagen; MMP, matrix metalloproteinase; αSMA, alpha smooth muscle cell actin.

(p-TEFb)<sup>38,39</sup> and subsequent phosphorylation of RNA polymerase II (RNA Pol II).<sup>40</sup> Additionally, p-TEFb-independent transcriptional activation via the extra-terminal domain of BRD4 has been shown.<sup>41</sup> BRD4 has also kinase activity and binds and phosphorylates RNA Pol II<sup>42</sup> and acetylates H3K122, a residual critical for nucleosome stability, whose acetylation leads to histone eviction.<sup>43</sup> Furthermore, by binding BRD4 to the acetylated lysine-310 of p65, BRD4 regulates the transcriptional activity of nuclear factor kappa light chain enhancer of activated B cells (NF-κB). This interaction was shown to be sensitive to treatment with JQ1, which induced the ubiquitination and subsequent degradation of the constitutively active nuclear form of p65.<sup>44</sup> This data suggests an active role of BRD4 in transcription. Recent work has demonstrated the recruitment of BRD4 to enhancers<sup>45</sup> and its involvement in assisting transcriptional elongation at enhancers (enhancer RNA (eRNA)) and gene bodies.<sup>46</sup> Therefore,



**Figure 3** By binding to acetylated histones, BRD4 contributes to the assembly of histone acetyltransferase-dependent chromatin complexes at gene promoters and to the recruitment of TF and p-TEFb. This leads to the phosphorylation of RNA Pol II. BRD4 binds also acetylated histones in enhancers and assists transcriptional elongation at enhancers and gene bodies. AC, acetylated; BRD4, bromodomain protein 4; P, phosphorylation; p-TEFb, positive transcription elongation factor B; RNA Pol II, RNA polymerase II; TF, transcription factors; TSS, transcriptional start site.

BET inhibitors have the potential for a dual targeting of gene expression in RMD, by affecting the inflammatory environment and by altering cell-type specific enhancer activity.<sup>9 47</sup> Treatment with JQ1 was shown to preferentially affect the expression of super enhancer-associated gene transcripts compared with genes associated with bound BRD4 to normal enhancers.<sup>47</sup> Super enhancers are cell type specific and consist of clusters of enhancers that span a long range of DNA (>10 kb). They are densely occupied by master transcription factors and histone marks and often guide eRNA expression.<sup>48 49</sup> Super enhancers were demonstrated to specifically regulate genes associated with cell identity and disease.<sup>47</sup> Tumour necrosis factor alpha (TNF $\alpha$ ) was shown to be sufficient to alter the enhancer landscape of vascular endothelial cells, with a BRD4 eviction from basal super enhancers and a redistribution to de novo enhancers as well as super enhancers in a p65-dependent manner.<sup>50</sup> Also, I-BET was reported to primarily affect the expression of genes from de novo enhancers, whereas promoters enriched for histone 3-acetylation and histone 4-acetylation already at basal levels (primed loci) were less affected.<sup>10</sup> Likewise, a disease-specific, inflammation-associated super enhancer signature was identified in synovial fluid-derived CD4<sup>+</sup> memory/effector T cells from patients with juvenile idiopathic arthritis (JIA). JQ1 treatment of these cells inhibited immune-related super enhancer activity and preferentially suppressed disease-associated gene expression. In contrast, the enhancer signature of peripheral blood-derived CD4<sup>+</sup> memory/effector T cells from patients with JIA was relatively similar to those from healthy controls and less affected by JQ1 treatment.<sup>51</sup> It remains to be elucidated whether the altered enhancer profile is causally related to disease pathogenesis or the consequence of the exposure to a chronic inflammatory milieu.

BET inhibitors can target a variety of cell types in RMD and have been tested in different mouse models resembling different aspects of RMD (table 1). Several different compounds are used in RMD research. Inhibitors in this

review are named based on their description in the respective original paper. BET inhibitors block the maturation and differentiation of several cell types, including T cell subsets,<sup>33 34 52 53</sup> dendritic cells<sup>54</sup> and osteoclasts.<sup>55 56</sup> BRD protein members of three distinct families have been shown to interfere with the differentiation and function of Th17 cells,<sup>34 37 52 57 58</sup> suggesting a broad role of BRD proteins in autoimmune disorders. Furthermore, BET inhibitors affect the inflammatory responses of B cells,<sup>59</sup> macrophages,<sup>10 11 13</sup> synovial fibroblasts (SF)<sup>15 60 61</sup> and chondrocytes.<sup>62 63</sup>

### BET protein inhibition in macrophages

Treatment of murine bone marrow-derived macrophages (BMDM) with I-BET or JQ1 suppressed the expression of key lipopolysaccharide (LPS)-inducible cytokines, chemokines and transcription factors.<sup>10 11 13</sup> In line with the in vitro data, injection of I-BET or JQ1 before the initiation of LPS-induced or *Salmonella typhimurium*-induced endotoxic shock was able to prevent or attenuate death of mice.<sup>10 11</sup> Anti-inflammatory effects of BET inhibitors were later also confirmed in human monocytes,<sup>36 64</sup> where I-BET151 diminished both autocrine interferon (IFN)- $\beta$  production and transcriptional responses to IFN- $\beta$ .<sup>64</sup> Some of these effects might be attributed to the function of BRD3, since the knockout of BRD3 in murine RAW246.7 macrophages inhibited the virus-triggered IFN- $\beta$  production.<sup>65</sup>

Whereas whole transcriptome data is available of LPS-stimulated BMDM treated with I-BET<sup>10</sup> or JQ1,<sup>13</sup> respectively, very little is known on the individual roles of BRD2, BRD3 and BRD4 on regulating the inflammatory response of BMDM and only few direct target genes are known. Individual silencing of BRD2, BRD3 and BRD4 in BMDM was sufficient to decrease the LPS-induced expression of interleukin (IL)6, IL1 $\beta$ , TNF $\alpha$  and monocyte chemoattractant protein-1.<sup>10 11</sup> The expression of these genes was also significantly decreased in LPS-stimulated BMDM derived from *brd2* lo mice, a genetically modified

**Table 1** Inhibitors targeting BET proteins have been tested in animal models resembling diverse characteristic of rheumatic diseases

Animal model	Inhibitor	Administration	References
<b>Arthritis</b>			
CIA (mouse)	JQ1	intraperitoneal	Mele <i>et al</i> <sup>34</sup>
CIA (mouse)	JQ1	n.d.	Zhang <i>et al</i> <sup>67</sup>
CIA (mouse)	JQ1	intraperitoneal	Xiao <i>et al</i> <sup>60</sup>
CAIA (mouse)	RVX-297	oral	Jahagirdar <i>et al</i> <sup>37</sup>
CIA (mouse, rat)	RVX-297	oral	Jahagirdar <i>et al</i> <sup>37</sup>
K/BxN serum transfer (mouse)	I-BET151	intraperitoneal	Park-Min <i>et al</i> <sup>55</sup>
<b>Osteoporosis</b>			
OVX (mouse)	I-BET151	intraperitoneal	Park-Min <i>et al</i> <sup>55</sup>
<b>Osteoarthritis</b>			
DMM (mouse)	I-BET151	intraperitoneal	Dai <i>et al</i> <sup>62</sup>
<b>Systemic lupus erythematosus</b>			
MRL-lpr (mouse)	JQ1	oral	Wei <i>et al</i> <sup>36</sup>
<b>Psoriasis</b>			
IMQ (mouse)	JQ1	topical	Nadeem <i>et al</i> <sup>77</sup>
<b>Ankylosing spondylitis</b>			
HLA-B27/β2m (rat)	I-BET151	intraperitoneal	Fan <i>et al</i> <sup>82</sup>
<b>Fibrosis</b>			
Bleomycin (mouse)	JQ1	oral (chow)	Tang <i>et al</i> <sup>85</sup>
Bleomycin (mouse)	JQ1	oral (chow)	Tang <i>et al</i> <sup>22</sup>
UUO (mouse)	I-BET151	intraperitoneal	Xiong <i>et al</i> <sup>23</sup>
TAD (mouse)	JQ1	intraperitoneal	Duan <i>et al</i> <sup>20</sup>
LAD (mouse)	JQ1	intraperitoneal	Duan <i>et al</i> <sup>20</sup>
CCI4 (mouse)	JQ1	intraperitoneal	Ding <i>et al</i> <sup>19</sup>

BET, bromodomain and extra-terminal; CAIA, collagen antibody induced arthritis; CCI4, carbon tetrachloride; CIA, collagen-induced arthritis; DMM, destabilisation of the medial meniscus; HLA, human leucocyte antigen; IMQ, imiquimod; LAD, left anterior descending; n.d., not defined; OVX, ovariectomy; TAD, transverse aortic constriction; UUO, unilateral ureteral obstruction.

mouse model with a hypomorph phenotype and a 50% reduction of BRD2 expression in all tested tissues.<sup>11</sup>

Chromatin immunoprecipitations (ChIP) of *TNFα* and *IL6* promoter regions in BMDM identified first gene-specific and distinct mechanisms behind BRD2 and BRD4 functions. Whereas BRD2 and BRD4 bound to the *IL6* promoter, the *TNFα* promoter was bound only by BRD2 but not by BRD4. Since silencing of BRD4 was sufficient to decrease the *TNFα* secretion in LPS-stimulated BMDM, a chromatin-independent effect of BRD4 was suggested by the authors.<sup>11</sup> Some of the anti-inflammatory effects of BET inhibitors might also be mediated by a direct effect of BET inhibitors on the activation of NF-κB pathways by decreasing the *TNFα*-induced phosphorylation of NF-κB inhibitor α (IκBα).<sup>12 66</sup> However, these effects were not consistently observed and might be cell-type dependent or related to the treatment protocol and inhibitor used.<sup>10 15 60</sup>

### BET protein inhibition in synovial fibroblasts

Besides the anti-inflammatory role of BET inhibitors in macrophages, I-BET151 and JQ1 were also sufficient to

decrease the *TNFα*-induced, *IL1β*-induced, as well as the Toll-like receptor ligand-induced inflammatory response of SF and to decrease SF proliferation.<sup>15 60 61</sup> The changes in gene expression of cytokines and chemokines translated into a reduced chemoattractance towards peripheral blood mononuclear cell.<sup>15</sup> Silencing of BRD2 or BRD4 was equally efficient in downregulating the *TNFα*-induced expression of matrix metalloproteinases (MMP) and cytokines in SF.<sup>60</sup> In addition, BRD4 was shown to control migration and invasion of SF.<sup>67</sup> Similar to macrophages, only few target genes of individual BET proteins are known in SF. Recently, a study by Nagpal *et al* provided new insights into the mechanisms behind the activity of JQ1 in SF. JQ1 was shown to reduce the occupancy of both BRD2 and BRD4 in approximately 2000 regions and RNA Pol II in approximately 600 regions genome-wide in *IL1β*-treated SF. The authors showed that JQ1 reduced the BRD2/BRD4 occupancy in the *IL6* and *IL8* super enhancer regions in SF,<sup>61</sup> consistent with a general function of BET proteins on super enhancers in different cell types.<sup>50 51 68 69</sup>

Besides the regulatory function of BET proteins in SF under inflammatory conditions, BET reading regulated the expression of *HOX* genes in a joint-specific manner under non-inflammatory conditions. I-BET151 decreased the basal expression of genes encoded in the 5 *HOXA* cluster, but not of genes encoded in the 5 *HOXC* cluster, which distinguish SF derived from upper versus lower extremity joints.<sup>70</sup> This might be relevant to arthritis, since the expression of specific *HOX* genes widely overlaps with the occurrence of certain forms of arthritis in specific joints.<sup>71</sup> As an exception among BRD proteins, BRD4 was shown to stick to the chromatin during mitosis in murine P19 embryonal carcinoma cells.<sup>38</sup> This feature makes BET proteins to the key candidates that maintain the transcriptional memory in SF and other cells.

### BET protein inhibition in endothelial cells

Endothelial cell activation and dysfunction are hallmarks of systemic autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and systemic sclerosis (SSC).<sup>72–74</sup> JQ1 treatment or silencing of BRD2 or BRD4 in human umbilical vein endothelial cells (HUVEC) suppressed the vascular endothelial growth factor (VEGF)-induced tube formation, vascular hyperpermeability and migration. The effects were accompanied with a reduced VEGF-induced phosphorylation of VEGF receptor 2 (VEGFR2), p21-activated kinase 1 and endothelial nitric oxide synthase, factors which are important for angiogenesis and permeability.<sup>75</sup> In addition, BET BRD inhibition was shown to efficiently block the endothelial activation under inflammatory conditions. JQ1 or silencing of BRD2 and BRD4 in HUVEC cells suppressed the TNF $\alpha$ -induced or LPS-induced expression of adhesion molecules, translating into a reduced adhesion of leukocytes to activated HUVEC. Pretreatment of HUVEC with JQ1 suppressed the TNF $\alpha$ -induced phosphorylation and degradation of I $\kappa$ B $\alpha$  and reduced the nuclear levels of p65, indicating that the observed effects were at least partially due to a reduced activation of the NF- $\kappa$ B pathway.<sup>16</sup> In line with this data, JQ1 decreased the p65 recruitment to IL6 and IL8 promoters in serum-stimulated human pulmonary microvascular endothelial cells (HPMEC), reducing the expression and secretion of IL6 and IL8. Furthermore, JQ1 reduced HPMEC proliferation, with increased expression of cyclin-dependent kinase (CDK) inhibitors CDKN1A and CDKN2D and decreased expression of CDK2, CDK4 and CDK6.<sup>76</sup>

### BET inhibitors in mouse models of arthritis

As a proof of concept for the use of BRD inhibitors in RMD in vivo, in 2013, Mele *et al* showed that intraperitoneal injections of JQ1, over a period of 2 weeks, protected mice from collagen-induced arthritis (CIA), evident by reduced arthritis scores and histological assessment.<sup>34</sup> These findings were later confirmed by two other groups.<sup>60,67</sup> Recently, the use of a BRD2 specific and orally active BET inhibitor, RVX-297, was shown to be efficient

in decreasing the arthritis scores in CIA mice and rats, as well as in mice with collagen antibody-induced arthritis.<sup>37</sup> The beneficial effects of BET inhibitors in arthritis models in vivo were mainly attributed to the suppressive effects on Th17 cell differentiation and the reduced expression of Th17-associated cytokines, including IL17, IL21 and granulocyte macrophage-colony stimulating factor.<sup>34,37</sup> The aberrant generation and activation of Th17 cells, a subset of T helper cells, is one of the key mechanisms leading to autoimmune conditions.<sup>53</sup> JQ1 or a combined silencing of BRD2 and BRD4 also interfered with the differentiation of human Th17 cells and suppressed the expression of steroid receptor-type nuclear receptors (ROR)A and RORC, encoding the transcription factors ROR $\alpha$  and ROR $\gamma$ t that drive Th17 differentiation, as well as IL17A and IL21.<sup>34</sup> These findings were later confirmed by differentiating Th17 from CD4<sup>+</sup> T cells derived from healthy controls, patients with ankylosing spondylitis (AS) and psoriatic arthritis in the presence and absence of JQ1. In the same study, CBP30, a selective CBP/p300 inhibitor was described as a suppressant of human Th17 responses. In comparison to JQ1, CBP30 affected the expression of fewer genes in CD4<sup>+</sup> T cells, but similarly downregulated the expression of RORC, IL17A and IL21.<sup>52</sup> JQ1 was also shown to interfere with the RORC/IL17 pathway in imiquimod (IMQ)-induced skin inflammation, a mouse model for psoriasis-like skin disease. Topical application of JQ1 before and after IMQ-induced skin inflammation exhibited protective effects as indicated by reduced ear thickness, ear myeloperoxidase activity and decreased skin expression of RORC and IL17A.<sup>77</sup>

Mele *et al* showed that JQ1 had no impact on the differentiation of T cell subsets other than Th17 from human naive T cells, such as Th1, Th2 or regulatory T cells.<sup>34</sup> In contrast, the administration of I-BET762, in early phases of T cell differentiation from naive murine T cells had long-lasting effects on the proinflammatory function of murine Th1 cells accompanied by an increase of anti-inflammatory genes such as IL10. Furthermore, I-BET762 did not affect the expression of RORC in this study, but was sufficient to reduce the expression of IL17A.<sup>33</sup> In a doxycyclin-inducible mouse model for sustained and reversible silencing of BRD4 in vivo, the absence of BRD4 interfered with a normal haematopoiesis, leading to a depletion of CD4<sup>+</sup> and CD8<sup>+</sup> single-positive T cell subsets, as well as a significant reduction in Lineage<sup>-</sup>Sca1<sup>+</sup>cKit<sup>+</sup> haematopoietic stem cells in reconstituted bone marrow.<sup>78</sup> Differences in the results of the differentiation of different T cell subsets on BET inhibition might be due to different experimental setups or different inhibitors and inhibitor concentrations used. Bolden *et al*, for example, showed that the levels of BRD4 silencing (moderate vs strong) were essential in obtaining phenotypic changes in the intestine of mice.<sup>78</sup>

### BET inhibitors in mouse models of SLE

Oral administration of JQ1 decreased the glomerular damage, nephritis, delayed the onset and decreased the

severity of proteinuria in MRL-lpr mice, a murine model for SLE. The effects were accompanied by decreased serum concentrations of B-cell activating factor, IL1 $\beta$ , IL6, IL17 and IFN $\gamma$  and augmented IL10. In addition, JQ1 treatment decreased serum concentrations of anti-dsDNA antibodies, as well as immune complex deposition in kidneys,<sup>36</sup> a hallmark of human SLE. This is in line with the finding that BRD4 interacts with octamer-binding transcription factor 2 (Oct2), which is crucial for B-cell specific gene expression. JQ1 reduced the binding of Oct2 to the immunoglobulin kappa locus (*IGK*) promoter, leading to a BET inhibitor-mediated decrease in immunoglobulin production in B cells.<sup>59</sup> In contrast to the study by Wei *et al*,<sup>36</sup> the expression of LPS-induced IL10 was decreased in regulatory B cells (B<sub>reg</sub>) by JQ1 treatment.<sup>79</sup> This might represent a detrimental effect of JQ1, since B<sub>reg</sub> cells are critically involved in suppression of inflammation and known to inhibit inflammatory immune diseases including CIA.<sup>80</sup>

### BET proteins in bone homeostasis and cartilage metabolism

BET proteins have been suggested to play a role in bone homeostasis, which is regulated by bone-resorbing osteoclasts and bone-synthesising osteoblasts.<sup>81</sup> I-BET151 and JQ1 suppressed the human and mouse nuclear factor  $\kappa$ B ligand (RANKL)-induced osteoclastogenesis in vitro and in vivo.<sup>12 55</sup> Therapeutic dosing of I-BET151 was sufficient to increase bone mass in postovariectomy osteoporosis. In K/BxN serum-induced arthritis, a mouse model resembling the inflammatory effector phase of arthritis, I-BET151 suppressed bone resorption, possibly by targeting osteoclast differentiation along with suppressing the ongoing inflammation. I-BET151 was shown to target nuclear factor of activated T cells C1, the master regulator of osteoclastogenesis by suppressing MYC.<sup>55</sup> In the same study, the authors showed that I-BET151 could inhibit the differentiation of osteoblasts in vitro by using fivefold to 10-fold higher concentrations than those required for the suppression of osteoclastogenesis.<sup>55</sup> In another study, I-BET151 inhibited the expression of MMP3, MMP9, RANKL and osteoprotegerin in human MG63 osteoblast cells treated with serum derived from patients with AS. The expression of these genes was also suppressed in HLA-B27/ $\beta$ 2m transgenic AS Lewis rats, a rodent model for AS, on treatment with I-BET151.<sup>82</sup> Unfortunately, no other outcomes of I-BET151 were studied in this animal model. Both, JQ1 and I-BET151 were shown to exhibit protective effects on cartilage, by downregulating the expression of major factors involved in extracellular matrix degradation in human, murine and rat chondrocytes.<sup>62 63</sup> On surgical destabilisation of the medial meniscus (DMM), postsurgical treatment with I-BET151 from day 2 on prevented articular cartilage damage, but only at earlier and not at later time points after treatment.<sup>62</sup> Whether this timely limited effect of I-BET151 in the DMM model was due to a general suppression of inflammation or other mechanisms was not further studied.

### BET proteins in fibrosis

Fibrosis of the skin and internal organs, as well as vasculopathy are hallmarks of SSc.<sup>83</sup> Transforming growth factor  $\beta$  (TGF $\beta$ ) and downstream mothers against decapentaplegic homolog (SMAD) signalling molecules are key factors mediating the disease.<sup>84</sup> Inhibition of BET proteins was efficient to inhibit lung,<sup>22 85</sup> liver,<sup>19</sup> renal<sup>23</sup> and heart fibrosis<sup>20</sup> in vivo.

Treatment of human dermal fibroblasts (DF) with JQ1 decreased the expression of alpha smooth muscle cell actin ( $\alpha$ SMA), fibronectin, collagen (COL) 1A1, nicotinamide adenine dinucleotide phosphate oxidase (NOX)4<sup>21</sup> and TGF $\beta$ -induced cytokine secretion.<sup>85</sup> Silencing of BRD4 in DF reduced stress fibre formation and resembled the effects on gene expression that were observed by JQ1 treatment at least partially.<sup>21</sup> Therefore, some other BET proteins might additionally be involved in the beneficial effects mediated by JQ1 in DF. In pancreatic stellate cells, which are key regulators of the collagen I production and fibrosis in pancreatic ductal adenocarcinoma, silencing of BRD4 suppressed the expression of COL1A1 and COL1A2, whereas silencing of BRD2 or BRD3 led to opposite effects in this cell type. However, JQ1 was still sufficient to suppress collagen production in vitro and in vivo.<sup>86</sup>

In lung fibroblasts, both JQ1 and I-BET or silencing of either BRD2 or BRD4, decreased the TGF $\beta$ -induced expression of  $\alpha$ SMA.<sup>22</sup> Administration of JQ1 with the chow to mice with bleomycin-induced fibrosis prevented lung fibrosis.<sup>22 85</sup> The expression of BRD4 was shown to be elevated in tubular and interstitial cells in humans with kidney disease, as well as in kidneys of mice with renal fibrosis induced by unilateral ureteral obstruction (UUO). Treatment of renal interstitial fibroblasts with I-BET151 or silencing of BRD4 reduced the TGF $\beta$ -induced expression of  $\alpha$ SMA, fibronectin and COL1. In line with this, I-BET151 alleviated renal fibrosis in the UUO model.<sup>23</sup> BET protein inhibition with JQ1 was also sufficient to suppress innate inflammatory and profibrotic transcriptional networks in mice subjected to transverse aortic constriction and in mice with permanent surgical ligation of the proximal left anterior descending coronary artery, resembling severe heart failure from prolonged pressure overload and massive anterior myocardial infarction, respectively. Together these data suggest beneficial effects of BET protein inhibition in multiorgan fibrosis and BRD4 as the major target in this context.

### BRPF proteins in RMD

Studies on other BRD proteins than BET proteins in RMD are very rare. Recently, two studies on the role of BRD and plant homeodomain finger-containing (BRPF) family proteins were published. The BRPF family consists of three members: BRPF1, BRPF2 (BRD1) and BRPF3. BRPF proteins comprise several functional domains in addition to a BRD, including two plant homeodomains connected by a zinc finger and a PWWP domain.<sup>6</sup> Pharmacological inhibition of BRPF proteins using the

three pan-BRD-inhibitors PFI-4, OF-1 and NI-57 strongly impaired RANKL-induced differentiation of murine and human primary monocytes into bone resorbing osteoclasts accompanied by a decreased expression of osteoclast markers.<sup>56</sup> In synovial tissues, BRPF2 expression was verified in SF and macrophages. BRPF2 exerts cell-type and stimulus-specific effects in SF and monocyte-derived macrophages (MDM). Silencing of BRPF2 in SF decreased proliferation and led to rather proinflammatory and prodestructive effects. In contrast, silencing of BRPF2 in human MDM exhibited mild anti-inflammatory effects, indicated by a decrease of the LPS-induced expression of TNF $\alpha$ .<sup>87</sup> This data underscore the need for careful testing of BRD protein function and their inhibition in more than one cell type.

### TRIM proteins in RMD

Another interesting group of BRD proteins is a subgroup of tripartite motif (TRIM) family proteins. TRIM proteins are multidomain proteins and are implicated in innate signalling pathways.<sup>88</sup> Among TRIM proteins, TRIM28, TRIM33, TRIM24 and TRIM66 contain a BRD and are known as positive and negative transcriptional regulators.<sup>6</sup> So far, no specific inhibitors targeting these TRIM proteins are available but an inhibitor with a dual activity targeting the BRD of BRPF1B and TRIM24 has been described.<sup>89</sup> Knockdown of TRIM28 in human macrophages potentiated the IFN-regulatory factor 5-mediated expression of TNF $\alpha$ .<sup>90</sup> Another study showed that TRIM33 is overexpressed in muscle and skin tissues of patients with dermatomyositis and skin tissues from patients with other autoimmune diseases including psoriasis.<sup>91</sup> Studies in knockout mice revealed that TRIM33 acts as a transcriptional repressor in macrophages in vitro and in vivo, leading to a high sensitivity to endotoxin challenge. At early stages of haematopoiesis, TRIM33 deficiency was associated with an impaired production of monocytes/macrophages. Deficiency of TRIM33 in mature myeloid cells increased the expression of a subset of known LPS-repressible genes, whereas the expression of only few LPS-inducible genes was decreased.<sup>92</sup> In addition, studies in mice showed that TRIM33 and TRIM28 play a crucial role in the differentiation of Th17 cells. The knockout of TRIM33 or TRIM28 in CD4<sup>+</sup> T cells reduced the expression of IL17 and IL17F but had, in contrast to the BET protein-regulated inhibition of Th17 differentiation, no effect on Th17-related transcription factors such as ROR $\gamma$ t.<sup>57 58</sup> These data point to different mechanisms underlying the BET protein-mediated and TRIM-mediated Th17 differentiation. In the absence of TRIM33 or TRIM28, the *IL17* locus was enriched for suppressive histone marks, or reduced in active epigenetic marks, respectively, whereas the *IL10* locus was enriched for activating H3K4me3 in TRIM33 knockout cells, leading to decreased and increased expression of the respective genes.<sup>57 58</sup> TRIM33 orchestrated its effects by forming a transcriptional complex with ROR $\gamma$ t and Smad2.<sup>57</sup> On the other hand, a STAT3-dependent recruitment of TRIM28

was required for the ROR $\gamma$ t recruitment and function at the *IL17-IL17F* locus.<sup>58</sup>

### CONCLUSION AND OUTLOOK

The development of small molecule inhibitors targeting BET proteins has raised new possibilities in the future treatment of RMD. Their effects among different mouse models are encouraging and raise the perspective that BET inhibitors might be used in the future for the treatment of different RMD including their comorbidities, such as cardiovascular events or chronic obstructive pulmonary disease. Preclinical and basic research studies provide indication of potential complications of BET inhibitor therapies, such as sterility, retroviral activation and impact on the haematopoietic development. The latter concern was confirmed by results of first phase 1 clinical studies in patient cohorts with lymphoma or multiple myeloma and patients with acute leukaemia (ClinicalTrials.gov, number NCT01713582). These studies reported reversible haematological toxicities, in particular thrombocytopenia, anaemia and neutropenia as the main related adverse events that were frequent among all dose levels. Few non-haematological events were reported, including gastrointestinal events and fatigue with increasing frequency and severity at the highest doses.<sup>93 94</sup> Given that common BET inhibitors might be considered as non-hormonal approach to male contraception and also target the testis-specific BRD protein, a BET protein essential for spermatogenesis, one of the adverse effects of BET inhibitor treatment could be male sterility.<sup>95–97</sup> In addition, BET inhibitors are capable of reactivating latent retroviral gene expression.<sup>98 99</sup> Although the exact role of retroviruses in RMD is incompletely understood, there is consensus about their role as triggers of autoimmunity<sup>100 101</sup>. Therefore, the use of BET inhibitors in patients with RMD might bear an unpredictable risk.

Ten years after the first publication of BET inhibitors, there is still remarkably little known on individual functions of single BET proteins. The majority of research has focused on the use of pan-BET inhibitors in different cell types in vitro and in mouse models in vivo and only few studies do a more in depth analysis on the underlying mechanisms of BET protein inhibition. Some studies have identified small numbers of target genes of selected BET proteins, mainly of BRD4, but full transcriptome analysis of BET proteins in different cell types and conditions are missing. Recent studies focused on the oral administration of BET inhibitors<sup>36 37 85</sup> or the use of BRD2-selective inhibitors such as RVX-297.<sup>37</sup> The good druggability of BRD will likely lead to the continuing development of inhibitors that will further boost the research in this field.

Studies on BRD proteins outside of the BET protein family are only appearing in the last few years and are still rare. Inhibitors targeting BRPF family proteins have only recently been developed<sup>56 102 103</sup> and will likely boost the research on BRPF proteins also in the field of RMD.

So far, no TRIM protein-specific inhibitors are available for research, likely due to their dual function as activators and repressors of transcription. However, the interference of TRIM proteins with the differentiation of Th17 cells suggests that also TRIM protein inhibition might be a successful treatment in autoimmune conditions.

**Contributors** KK planned and wrote the manuscript.

**Funding** The author has not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent** Not required.

**Provenance and peer review** Commissioned; externally peer reviewed.

**Data sharing statement** No additional data are available.

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## REFERENCES

- Choudhary C, Weinert BT, Nishida Y, *et al.* The growing landscape of lysine acetylation links metabolism and cell signalling. *Nat Rev Mol Cell Biol* 2014;15:536–50.
- Klein K, Gay S. Epigenetics in rheumatoid arthritis. *Curr Opin Rheumatol* 2015;27:76–82.
- Tough DF, Tak PP, Tarakhovskiy A, *et al.* Epigenetic drug discovery: breaking through the immune barrier. *Nat Rev Drug Discov* 2016;15:835–53.
- Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene* 2007;26:5521–7.
- Vidler LR, Brown N, Knapp S, *et al.* Druggability analysis and structural classification of bromodomain acetyl-lysine binding sites. *J Med Chem* 2012;55:7346–59.
- Filippakopoulos P, Picaud S, Mangos M, *et al.* Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* 2012;149:214–31.
- Fujisawa T, Filippakopoulos P. Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. *Nat Rev Mol Cell Biol* 2017;18:246–62.
- Mackmull MT, Iskar M, Parca L, *et al.* Histone Deacetylase Inhibitors (HDACi) Cause the Selective Depletion of Bromodomain Containing Proteins (BCPs). *Mol Cell Proteomics* 2015;14:1350–60.
- Filippakopoulos P, Qi J, Picaud S, *et al.* Selective inhibition of BET bromodomains. *Nature* 2010;468:1067–73.
- Nicodeme E, Jeffrey KL, Schaefer U, *et al.* Suppression of inflammation by a synthetic histone mimic. *Nature* 2010;468:1119–23.
- Belkina AC, Nikolajczyk BS, Denis GV. BET protein function is required for inflammation: Brd2 genetic disruption and BET inhibitor JQ1 impair mouse macrophage inflammatory responses. *J Immunol* 2013;190:3670–8.
- Meng S, Zhang L, Tang Y, *et al.* BET inhibitor JQ1 blocks inflammation and bone destruction. *J Dent Res* 2014;93:657–62.
- Das A, Chai JC, Yang CS, *et al.* Dual transcriptome sequencing reveals resistance of TLR4 ligand-activated bone marrow-derived macrophages to inflammation mediated by the BET inhibitor JQ1. *Sci Rep* 2015;5:16932.
- Jung KH, Das A, Chai JC, *et al.* RNA sequencing reveals distinct mechanisms underlying BET inhibitor JQ1-mediated modulation of the LPS-induced activation of BV-2 microglial cells. *J Neuroinflammation* 2015;12:36.
- Klein K, Kabala PA, Grabiec AM, *et al.* The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann Rheum Dis* 2016;75:422–9.
- Huang M, Zeng S, Zou Y, *et al.* The suppression of bromodomain and extra-terminal domain inhibits vascular inflammation by blocking NF- $\kappa$ B and MAPK activation. *Br J Pharmacol* 2017;174:101–15.
- Huang W, Haynes AC, Mukherjee R, *et al.* Selective inhibition of BET proteins reduces pancreatic damage and systemic inflammation in bile acid- and fatty acid ethyl ester- but not caerulein-induced acute pancreatitis. *Pancreatology* 2017;17:689–97.
- Suarez-Alvarez B, Morgado-Pascual JL, Rayego-Mateos S, *et al.* Inhibition of bromodomain and extraterminal domain family proteins ameliorates experimental renal damage. *J Am Soc Nephrol* 2017;28:504–19.
- Ding N, Hah N, Yu RT, *et al.* BRD4 is a novel therapeutic target for liver fibrosis. *Proc Natl Acad Sci U S A* 2015;112:15713–8.
- Duan Q, McMahon S, Anand P, *et al.* BET bromodomain inhibition suppresses innate inflammatory and profibrotic transcriptional networks in heart failure. *Sci Transl Med* 2017;9:eaah5084.
- Ijaz T, Jamaluddin M, Zhao Y, *et al.* Coordinate activities of BRD4 and CDK9 in the transcriptional elongation complex are required for TGF $\beta$ -induced Nox4 expression and myofibroblast transdifferentiation. *Cell Death Dis* 2017;8:e2606.
- Tang X, Peng R, Ren Y, *et al.* BET bromodomain proteins mediate downstream signaling events following growth factor stimulation in human lung fibroblasts and are involved in bleomycin-induced pulmonary fibrosis. *Mol Pharmacol* 2013;83:283–93.
- Xiong C, Masucci MV, Zhou X, *et al.* Pharmacological targeting of BET proteins inhibits renal fibroblast activation and alleviates renal fibrosis. *Oncotarget* 2016;7:69291–308.
- Clifford RL, Patel JK, John AE, *et al.* CXCL8 histone H3 acetylation is dysfunctional in airway smooth muscle in asthma: regulation by BET. *Am J Physiol Lung Cell Mol Physiol* 2015;308:L962–L972.
- Nadeem A, Ahmad SF, Al-Harbi NO, *et al.* Inhibition of BET bromodomains restores corticosteroid responsiveness in a mixed granulocytic mouse model of asthma. *Biochem Pharmacol* 2018;154:222–33.
- Nicholls SJ, Ray KK, Johansson JO, *et al.* Selective BET protein inhibition with apabetalone and cardiovascular events: a pooled analysis of trials in patients with coronary artery disease. *Am J Cardiovasc Drugs* 2018;18:109–15.
- Gilham D, Wasiak S, Tsujikawa LM, *et al.* RVX-208, a BET-inhibitor for treating atherosclerotic cardiovascular disease, raises ApoA-1/HDL and represses pathways that contribute to cardiovascular disease. *Atherosclerosis* 2016;247:48–57.
- Cully M. Cardiovascular disease: BET inhibitor attenuates heart failure. *Nat Rev Drug Discov* 2017;16:453.
- Magistri M, Velmeshev D, Makhmutova M, *et al.* The BET-bromodomain inhibitor JQ1 reduces inflammation and tau phosphorylation at Ser396 in the brain of the 3xTg model of alzheimer's disease. *Curr Alzheimer Res* 2016;13:985–95.
- Benito E, Ramachandran B, Schroeder H, *et al.* The BET/BRD inhibitor JQ1 improves brain plasticity in WT and APP mice. *Transl Psychiatry* 2017;7:e1239.
- Sullivan JM, Badimon A, Schaefer U, *et al.* Autism-like syndrome is induced by pharmacological suppression of BET proteins in young mice. *J Exp Med* 2015;212:1771–81.
- Sun Y, Wang Y, Toubai T, *et al.* BET bromodomain inhibition suppresses graft-versus-host disease after allogeneic bone marrow transplantation in mice. *Blood* 2015;125:2724–8.
- Bandukwala HS, Gagnon J, Togher S, *et al.* Selective inhibition of CD4+ T-cell cytokine production and autoimmunity by BET protein and c-Myc inhibitors. *Proc Natl Acad Sci U S A* 2012;109:14532–7.
- Mele DA, Salmeron A, Ghosh S, *et al.* BET bromodomain inhibition suppresses TH17-mediated pathology. *J Exp Med* 2013;210:2181–90.
- Fu W, Farache J, Clardy SM, *et al.* Epigenetic modulation of type-1 diabetes via a dual effect on pancreatic macrophages and  $\beta$  cells. *Elife* 2014;3:e04631.
- Wei S, Sun Y, Sha H. Therapeutic targeting of BET protein BRD4 delays murine lupus. *Int Immunopharmacol* 2015;29:314–9.
- Jahagirdar R, Attwell S, Marusic S, *et al.* RVX-297, a BET bromodomain inhibitor, has therapeutic effects in preclinical models of acute inflammation and autoimmune disease. *Mol Pharmacol* 2017;92:694–706.
- Dey A, Chitsaz F, Abbasi A, *et al.* The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. *Proc Natl Acad Sci U S A* 2003;100:8758–63.
- Yang Z, Yik JH, Chen R, *et al.* Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. *Mol Cell* 2005;19:535–45.
- Brès V, Yoh SM, Jones KA. The multi-tasking P-TEFb complex. *Curr Opin Cell Biol* 2008;20:334–40.
- Rahman S, Sowa ME, Ottinger M, *et al.* The Brd4 extraterminal domain confers transcription activation independent of pTEFb by recruiting multiple proteins, including NSD3. *Mol Cell Biol* 2011;31:2641–52.



42. Devaiah BN, Lewis BA, Cherman N, *et al.* BRD4 is an atypical kinase that phosphorylates serine2 of the RNA polymerase II carboxy-terminal domain. *Proc Natl Acad Sci U S A* 2012;109:6927–32.
43. Devaiah BN, Case-Borden C, Geggone A, *et al.* BRD4 is a histone acetyltransferase that evicts nucleosomes from chromatin. *Nat Struct Mol Biol* 2016;23:540–8.
44. Zou Z, Huang B, Wu X, *et al.* Brd4 maintains constitutively active NF- $\kappa$ B in cancer cells by binding to acetylated RelA. *Oncogene* 2014;33:2395–404.
45. Liu W, Ma Q, Wong K, *et al.* Brd4 and JMJD6-associated anti-pause enhancers in regulation of transcriptional pause release. *Cell* 2013;155:1581–95.
46. Kanno T, Kanno Y, LeRoy G, *et al.* BRD4 assists elongation of both coding and enhancer RNAs by interacting with acetylated histones. *Nat Struct Mol Biol* 2014;21:1047–57.
47. Lovén J, Hoke HA, Lin CY, *et al.* Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 2013;153:320–34.
48. Pefanis E, Wang J, Rothschild G, *et al.* RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. *Cell* 2015;161:774–89.
49. Ko JY, Oh S, Yoo KH. Functional enhancers as master regulators of tissue-specific gene regulation and cancer development. *Mol Cells* 2017;40:169–77.
50. Brown JD, Lin CY, Duan Q, *et al.* NF- $\kappa$ B directs dynamic super enhancer formation in inflammation and atherogenesis. *Mol Cell* 2014;56:219–31.
51. Peeters JG, Vervoort SJ, Tan SC, *et al.* Inhibition of super-enhancer activity in autoinflammatory site-derived T cells reduces disease-associated gene expression. *Cell Rep* 2015;12:1986–96.
52. Hammitzsch A, Tallant C, Fedorov O, *et al.* CBP30, a selective CBP/p300 bromodomain inhibitor, suppresses human Th17 responses. *Proc Natl Acad Sci U S A* 2015;112:10768–73.
53. Stadhouders R, Lubberts E, Hendriks RW. A cellular and molecular view of T helper 17 cell plasticity in autoimmunity. *J Autoimmun* 2018;87:1–15.
54. Toniolo PA, Liu S, Yeh JE, *et al.* Inhibiting STAT5 by the BET bromodomain inhibitor JQ1 disrupts human dendritic cell maturation. *The Journal of Immunology* 2015;194:3180–90.
55. Park-Min KH, Lim E, Lee MJ, *et al.* Inhibition of osteoclastogenesis and inflammatory bone resorption by targeting BET proteins and epigenetic regulation. *Nat Commun* 2014;5:5418.
56. Meier JC, Tallant C, Fedorov O, *et al.* Selective targeting of bromodomains of the bromodomain-PHD fingers family impairs osteoclast differentiation. *ACS Chem Biol* 2017;12:2619–30.
57. Tanaka S, Jiang Y, Martinez GJ, *et al.* Trim33 mediates the proinflammatory function of Th17 cells. *J Exp Med* 2018;215:jem.20170779–68.
58. Jiang Y, Liu Y, Lu H, *et al.* Epigenetic activation during T helper 17 cell differentiation is mediated by Tripartite motif containing 28. *Nat Commun* 2018;9:1424.
59. Shim JM, Lee JS, Russell KE, *et al.* BET proteins are a key component of immunoglobulin gene expression. *Epigenomics* 2017;9:393–406.
60. Xiao Y, Liang L, Huang M, *et al.* Bromodomain and extra-terminal domain bromodomain inhibition prevents synovial inflammation via blocking I $\kappa$ B kinase-dependent NF- $\kappa$ B activation in rheumatoid fibroblast-like synoviocytes. *Rheumatology* 2016;55:173–84.
61. Nagpal S, Krishna V, Yin X. Integration of chromatin conformation, transcriptome and genome-wide landscape of brd2 and brd4 binding motifs identifies mechanisms of bet inhibitor action in rheumatoid arthritis synovial fibroblasts. *Ann Rheum Dis* 2018;77(Suppl 2):A192.
62. Dai J, Zhou S, Ge Q, *et al.* Bi-directional regulation of cartilage metabolism by inhibiting BET proteins-analysis of the effect of I-BET151 on human chondrocytes and murine joints. *J Orthop Surg Res* 2018;13:118.
63. An QD, Li YY, Zhang HX, *et al.* Inhibition of bromodomain-containing protein 4 ameliorates oxidative stress-mediated apoptosis and cartilage matrix degeneration through activation of NF-E2-related factor 2-heme oxygenase-1 signaling in rat chondrocytes. *J Cell Biochem* 2018;119:7719–28.
64. Chan CH, Fang C, Yafilina A, *et al.* BET bromodomain inhibition suppresses transcriptional responses to cytokine-Jak-STAT signaling in a gene-specific manner in human monocytes. *Eur J Immunol* 2015;45:287–97.
65. Ren W, Wang C, Wang Q, *et al.* Bromodomain protein Brd3 promotes I $\kappa$ B transcription via enhancing IRF3/p300 complex formation and recruitment to I $\kappa$ B promoter in macrophages. *Sci Rep* 2017;7:39986.
66. Huang B, Yang XD, Zhou MM, *et al.* Brd4 coactivates transcriptional activation of NF- $\kappa$ B via specific binding to acetylated RelA. *Mol Cell Biol* 2009;29:1375–87.
67. Zhang QG, Qian J, Zhu YC. Targeting bromodomain-containing protein 4 (BRD4) benefits rheumatoid arthritis. *Immunol Lett* 2015;166:103–8.
68. Di Micco R, Fontanals-Cirera B, Low V, *et al.* Control of embryonic stem cell identity by BRD4-dependent transcriptional elongation of super-enhancer-associated pluripotency genes. *Cell Rep* 2014;9:234–47.
69. Zuber V, Bettella F, Witoelar A, *et al.* Bromodomain protein 4 discriminates tissue-specific super-enhancers containing disease-specific susceptibility loci in prostate and breast cancer. *BMC Genomics* 2017;18:270.
70. Frank-Bertoncelj M, Trenkmann M, Klein K, *et al.* Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat Commun* 2017;8:14852.
71. Ospelt C, Frank-Bertoncelj M. Why location matters - site-specific factors in rheumatic diseases. *Nat Rev Rheumatol* 2017;13:433–42.
72. Atehortúa L, Rojas M, Vásquez GM, *et al.* Endothelial alterations in systemic lupus erythematosus and rheumatoid arthritis: potential effect of monocyte interaction. *Mediators Inflamm* 2017;2017:1–12.
73. Mostmans Y, Cutolo M, Giddelo C, *et al.* The role of endothelial cells in the vasculopathy of systemic sclerosis: A systematic review. *Autoimmun Rev* 2017;16:774–86.
74. Bordy R, Totoston P, Prati C, *et al.* Microvascular endothelial dysfunction in rheumatoid arthritis. *Nat Rev Rheumatol* 2018;14:404–20.
75. Huang M, Qiu Q, Xiao Y, *et al.* BET bromodomain suppression inhibits VEGF-induced angiogenesis and vascular permeability by blocking VEGFR2-mediated Activation of PAK1 and eNOS. *Sci Rep* 2016;6:23770.
76. Mumby S, Gambaryan N, Meng C, *et al.* Bromodomain and extra-terminal protein mimic JQ1 decreases inflammation in human vascular endothelial cells: Implications for pulmonary arterial hypertension. *Respirology* 2017;22:157–64.
77. Nadeem A, Al-Harbi NO, Al-Harbi MM, *et al.* Imiquimod-induced psoriasis-like skin inflammation is suppressed by BET bromodomain inhibitor in mice through RORC/IL-17A pathway modulation. *Pharmacol Res* 2015;99:248–57.
78. Bolden JE, Tasdemir N, Dow LE, *et al.* Inducible in vivo silencing of Brd4 identifies potential toxicities of sustained BET protein inhibition. *Cell Rep* 2014;8:1919–29.
79. Lee MB, Lee JH, Hong SH, *et al.* JQ1, a BET inhibitor, controls TLR4-induced IL-10 production in regulatory B cells by BRD4-NF- $\kappa$ B axis. *BMB Rep* 2017;50:640–6.
80. Mauri C, Gray D, Mushtaq N, *et al.* Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med* 2003;197:489–501.
81. Rodan GA. Bone homeostasis. *Proc Natl Acad Sci U S A* 1998;95:13361–2.
82. Fan J, Zhao J, Shao J, *et al.* I-BET151 inhibits expression of RANKL, OPG, MMP3 and MMP9 in ankylosing spondylitis *in vivo* and *in vitro*. *Exp Ther Med* 2017;14:4602–6.
83. Denton CP, Khanna D. Systemic sclerosis. *The Lancet* 2017;390:1685–99.
84. Lafyatis R. Transforming growth factor  $\beta$ --at the centre of systemic sclerosis. *Nat Rev Rheumatol* 2014;10:706–19.
85. Tang X, Peng R, Phillips JE, *et al.* Assessment of Brd4 inhibition in idiopathic pulmonary fibrosis lung fibroblasts and in vivo models of lung fibrosis. *Am J Pathol* 2013;183:470–9.
86. Kumar K, DeCant BT, Grippo PJ, *et al.* BET inhibitors block pancreatic stellate cell collagen I production and attenuate fibrosis in vivo. *JCI Insight* 2017;2:e88032.
87. Klein K, Kato M, Frank-Bertoncelj M, *et al.* Evaluating the bromodomain protein BRD1 as a therapeutic target in rheumatoid arthritis. *Sci Rep* 2018;8:11125.
88. Hatakeyama S. TRIM family proteins: roles in autophagy, immunity, and carcinogenesis. *Trends Biochem Sci* 2017;42:297–311.
89. Bennett J, Fedorov O, Tallant C, *et al.* Discovery of a chemical tool inhibitor targeting the bromodomains of TRIM24 and BRPF. *J Med Chem* 2016;59:1642–7.
90. Eames HL, Saliba DG, Krausgruber T, *et al.* KAP1/TRIM28: an inhibitor of IRF5 function in inflammatory macrophages. *Immunobiology* 2012;217:1315–24.
91. Scholtissek B, Ferring-Schmitt S, Maier J, *et al.* Expression of the autoantigen TRIM33/TIF1 $\gamma$  in skin and muscle of patients with dermatomyositis is upregulated, together with markers of cellular stress. *Clin Exp Dermatol* 2017;42:659–62.
92. Gallouet AS, Ferri F, Petit V, *et al.* Macrophage production and activation are dependent on TRIM33. *Oncotarget* 2017;8:5111–22.

93. Amorim S, Stathis A, Gleeson M, *et al.* Bromodomain inhibitor OTX015 in patients with lymphoma or multiple myeloma: a dose-escalation, open-label, pharmacokinetic, phase 1 study. *Lancet Haematol* 2016;3:e196–e204.
94. Berthon C, Raffoux E, Thomas X, *et al.* Bromodomain inhibitor OTX015 in patients with acute leukaemia: a dose-escalation, phase 1 study. *Lancet Haematol* 2016;3:e186–e195.
95. Shang E, Nickerson HD, Wen D, *et al.* The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing proteins, is essential for male germ cell differentiation. *Development* 2007;134:3507–15.
96. Gaucher J, Boussouar F, Montellier E, *et al.* Bromodomain-dependent stage-specific male genome programming by Brdt. *Embo J* 2012;31:3809–20.
97. Matzuk MM, McKeown MR, Filippakopoulos P, *et al.* Small-molecule inhibition of BRDT for male contraception. *Cell* 2012;150:673–84.
98. Banerjee C, Archin N, Michaels D, *et al.* BET bromodomain inhibition as a novel strategy for reactivation of HIV-1. *J Leukoc Biol* 2012;92:1147–54.
99. Bartholomeeusen K, Xiang Y, Fujinaga K, *et al.* Bromodomain and extra-terminal (BET) bromodomain inhibition activate transcription via transient release of positive transcription elongation factor b (P-TEFb) from 7SK small nuclear ribonucleoprotein. *J Biol Chem* 2012;287:36609–16.
100. Nelson PN. Retroviruses in rheumatic diseases. *Ann Rheum Dis* 1995;54:441–2.
101. Tugnet N, Rylance P, Roden D, *et al.* Human Endogenous Retroviruses (HERVs) and autoimmune rheumatic disease: is there a link? *Open Rheumatol J* 2013;7:13–21.
102. Bouché L, Christ CD, Siegel S, *et al.* Benzoisoquinolinediones as potent and selective inhibitors of BRPF2 and TAF1/TAF1L bromodomains. *J Med Chem* 2017;60:4002–22.
103. Igoe N, Bayle ED, Tallant C, *et al.* Design of a chemical probe for the Bromodomain and Plant Homeodomain Finger-Containing (BRPF) family of proteins. *J Med Chem* 2017;60:6998–7011.