Constitutive NF-κB activation in AML: Causes and treatment strategies

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Contents

ARTICLE INFO

ABSTRACT

For more than a decade, it has been known that NF-κB is constitutively activated in a majority of acute myeloid leukemia (AML) patients which contributes to the resistance to apoptosis. Inhibition of NF-κB has been shown to induce apoptosis in AML cells, but the clinical effectiveness of NF-κB inhibitors has been inadequate. In recent years, possible causes underlying this continuous NF-κB activity have been elucidated. It has been shown that chromosomal translocations or mutations leading to development of leukemia drive the increase in NF-κB activity. Furthermore, autocrine/paracrine cytokine signaling and increased expression of NF-κB signaling components play an important role in the continuous NF-κB activation. Moreover, high proteasome activity, which positively regulates NF-κB activity, is often observed in AML patients. In the present study, we described these underlying molecular mechanisms leading to constitutive NF-κB activity and discussed the novel treatment strategies based on the inhibition of NF-κB activation.

1. NF-κB signaling

1.1. Activation of the NF-κB complex

The transcription factor nuclear factor kappaB (NF-κB) is a protein complex which controls the gene expression of genes involved in inflammation and the immune response. Whereas NF-κB induces the expression of various genes involved in proliferation and anti-apoptosis, NF-κB activity has also been recognized as playing a major role in leukemia (Cilloni et al., 2007). NF-κB can be activated by diverse stimuli including cytokines, such as TNFα, IL-1β and Toll-like receptor 4 (TLR-4) (Verstrepen et al., 2008). Consequently, a variety of genes are regulated by NF-κB, such as the anti-apoptotic proteins (cFLIP, BCL-2 and BCL-xL), growth factors, cytokines (IL-1 and IL6), cell adhesion molecules and chemokines (Pahl, 1999). Importantly, only a subset of genes are activated by NF-κB in a stimulus and cell-specific manner. NF-κB complexes are homo- or heterodimers formed by members of the Rel family, which consists of five proteins: p65 (RelA), RelB, c-Rel, p50 and p52. Two major signaling pathways can activate NF-κB, the canonical NF-κB pathway and the non-canonical pathway (Fig. 1). The canonical pathway

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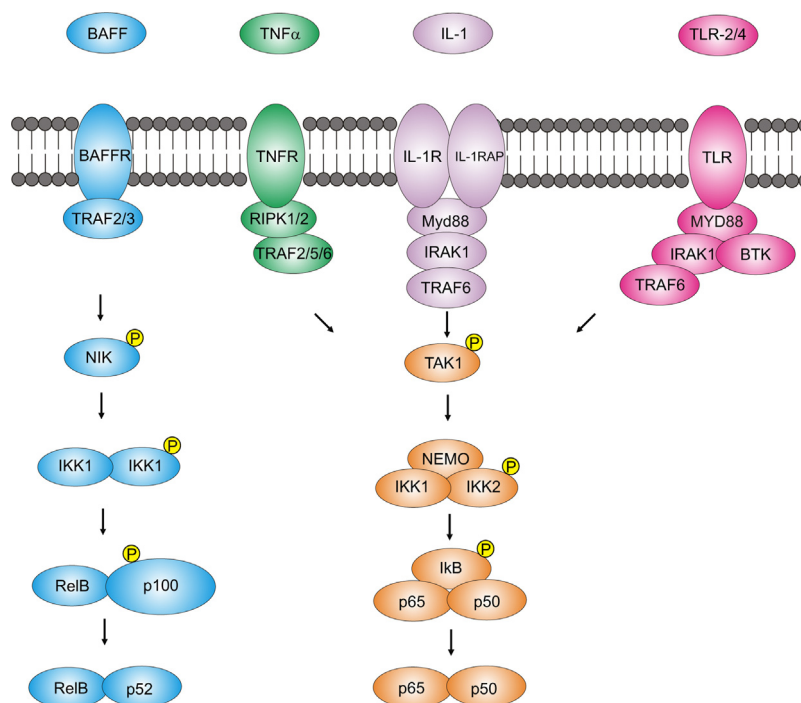   *E-mail address:* e.vellenga@umcg.nl (E. Vellenga).

Fig. 1. Canonical and non-canonical NF-κB signaling.

can be induced by a variety of cytokines including IL-1, TNFα, and TLR-2/4.

Upon binding of IL-1 to the IL-1 receptor (IL-1R), the IL-1R accessory protein (IL-1RAP) and MYD88 form a complex with the IL-1 receptor. This results in the recruitment of the IL-1 receptor associated kinase (IRAK) and TNF-receptor associated factor (TRAF) proteins to the complex. Similarly, binding of TLR-2/4 to the Toll-like receptor (TLR) also induces the association with IRAK and TRAF. Association of TNFα to the TNF receptor leads to the recruitment of receptor-interacting serine/threonine protein kinase (RIPK) and TRAF to the receptor.

Activation of these complexes induces a kinase cascade leading to the activation of NF-κB (Spaargaren et al., 2014; Xiao and Fu, 2011). Firstly, TGF-β activated kinase 1 (TAK1) is phosphorylated at multiple sites including threonine 178, 184 and 187 and serine 192. Phosphorylation of threonine 184 and 187 is mainly necessary for optimal NF-κB activation (Yu et al., 2008). Binding of TAK1 to the TAK1-binding protein 1 (TAB1) and TAK1-binding protein 2 or 3 (TAB2/TAB3) is also required for full activation of TAK1 which then activates the IκB kinase (IKK) complex (Cheung et al., 2004; Sakurai et al., 2000; Takaesu et al., 2000). This complex consists of IKK1/IKKα, IKK2/IKKβ and NEMO/IKKγ (Wang et al., 2001). TAK1-mediated phosphorylation of IKK2 results in activation of the NF-κB complex by phosphorylation of the inhibitory NF-κB protein IκB. IκB proteins, such as IκBα and IκBβ, bind to the nuclear localization domain of the NF-κB complex and thereby prevent nuclear translocation and NF-κB mediated gene expression (Alkalay et al., 1995). Upon phosphorylation of specific serines of IκB (e.g. in the case of IκBα serine 32 and 36), the IκB protein will be ubiquitinated and subsequently degraded by the proteasome (Brown et al., 1995; DiDonato et al., 1997). This directly results in the activation of the canonical NF-κB complex p65/p50.

The non-canonical pathway mainly plays a role in the development of B and T lymphocytes and can be activated by a small number of stimuli, including B-cell activating factor (BAFF), CD40L, receptor activator for NF-κB ligand (RANKL) and lymphotoxinβ. Subsequent NIK activation results in the phosphorylation and activation of the IKK1 dimer. Thereupon, p100 is phosphorylated on serines 886 and 870 by IKK1, which leads to the ubiquitination and partial degradation by the proteasome to p52 (Hoesel and Schmid, 2013). This results in the activation of non-canonical NF-κB complex p52/RelB, ultimately leading to the expression of the target genes.

1.2. NF-κB-mediated gene transcription

Upon activation and translocation to the nucleus of the various NF-κB complexes, these dimers undergo multiple modifications, which subsequently lead to gene transcription (Fig. 2). Phosphorylation on serine 276 and serine 536 of p65 is mediated by protein kinase A (PKA) and IKK, and is required for the binding of p65 to the CBP/p300 complex (Chen et al., 2005; Zhong et al., 1997). The CBP/p300 complex consists of CBP and p300, which associate with various transcription factors and connect them together on sequence specific loci (Chan and La Thangue, 2001). Furthermore,
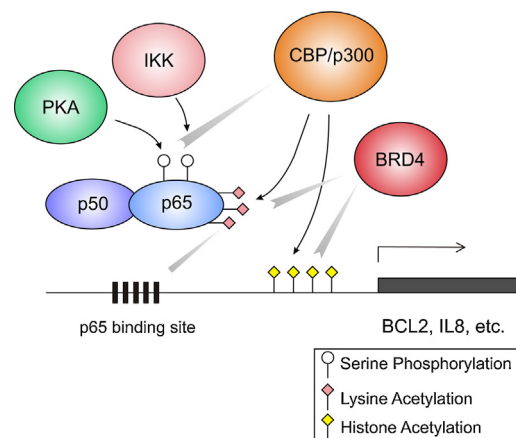

Fig. 2. Model of NF-κB-mediated gene transcription.

the CBP/p300 complex has intrinsic histone acetyltransferase (HAT) activity, leading to acetylation of histones such as H3K27, but it is also able to acetylate proteins like p65 (Chan and La Thangue, 2001; Chen et al., 2001). Upon phosphorylation of p65, CBP/p300 mediates acetylation of p65 on various lysines (e.g. K218, K221 and K310), thereby increasing the DNA binding affinity, decreasing the association with the negative regulator IκBα and promoting the association of the NF-κB complex with bromodomain 4 (BRD4). This results in NF-κB transactivation and gene transcription (Chen et al., 2005; Zou et al., 2014).

BRD4 belongs to the bromodomain and extraterminal domain (BET) family which are involved in transcriptional coactivation and elongation (Zhang et al., 2012a). Acetylated histones and proteins such as p65 are recognized by the BET domain of BRD4 (Mujtaba et al., 2007) leading to the recruitment of pTEFb (Jang et al., 2005; Yang et al., 2005) and chromatin remodeling complexes like the SWI/SNF complex (Shi et al., 2013), subsequently leading to activation of gene transcription. Thus, acetylation of p65 by CBP/p300, acetylation of histones by acetyltransferases like CBP/p300 and the recognition of the acetylation marks by BRD4 may be crucial steps in the transcription of genes regulated by NF-κB.

Selectivity of expression of NF-κB target genes is at least partially mediated by the affinity and specificity of each dimer towards the so-called κB-DNA binding site (GGGRnnYY, $R = A/G$, $Y = T/C$). Hereby, each stimulus can activate a group of genes in a cell-specific manner. For example, it was recently shown that IL-1 induced the recruitment of p65 to more than 400 enhancers in a TAK1-IKK2 dependent manner, which resulted in the recruitment of PolII, Pol(S5)II, AP-1, CBP and p50 at those sites (Jurida et al., 2015).

2. NF-κB activity in normal hematopoiesis

Basal NF-κB activity is required for HSCs self-renewal and differentiation into myeloid and lymphoid lineages. The quality of HSCs was decreased upon deletion of the canonical and non-canonical NF-κB complexes, p65 and RelB/p52 (Stein and Baldwin, 2013; Zhao et al., 2012). Knockout of p65 or double knockout of RelB/p52 resulted in impaired engraftment and self-renewal of HSPCs. In addition, differentiation efficiency of HSCs was affected in p65 knockout mice, whereas deletion of IκBα increased the differentiation of hematopoietic cells, suggesting that NF-κB contributes to the differentiation process. In particular, NF-κB plays a key role in the differentiation and activation of monocytes and granulocytes (Bottero et al., 2006; Gerondakis et al., 1999). Deletion of NF-κB regulators upstream of these NF-κB complexes appeared to be even more severe, as these components also regulate other survival-related pathways.

Besides the phosphorylation of IκBα and subsequent NF-κB activation upon TNFα stimulation, IKK is also able to phosphorylate and inactivate the pro-apoptotic protein BAD on serine 32 (Yan et al., 2013). In addition, deletion of TAK1 resulted in a rapid induction of cell death of normal HSCs, likely due to simultaneous inhibition of the NF-κB, JNK, p38 and ERK pathways (Tang et al., 2008). This effect is largely mediated by TNFα, as the phenotype of TAK1 knockout mice is partially rescued by knockout of both TNF receptors (Takaesu et al., 2012; Xiao et al., 2011). These findings indicate that a basal NF-κB activity is required for proper functioning of the hematopoietic stem and progenitor cells.

3. NF-κB activity in AML

Resistance to cell death and sustained proliferative signaling are hallmarks of cancer development (Hanahan and Weinberg, 2011). NF-κB signaling contributes to these biological process, so constitutive NF-κB activity could result in increased proliferation and survival of AML cells. For 15 years it has been known that NF-κB is constitutively activated in the AML stem cell-enriched CD34+ population cells in a large percentage of patients (Baumgartner et al., 2002; Birkenkamp et al., 2004; Guzman et al., 2001). Constitutive activation of NF-κB has also been detected in various murine AML models, including murine GMPs cells transformed by the retroviral transduction of AML1-ETO, BCR-ABL, MLL-ENL and MOZ-TIF2 (Kagoya et al., 2014). In particular, leukemia-initiating cells are marked by high NF-κB activity. The highest IKK activity and NF-κB activation is found in M4 and M5 AML subtypes compared to M1 and M2 subtypes, suggesting that NF-κB is mainly increased at the monocyte differentiation stage (Baumgartner et al., 2002). This finding is in line with the role of NF-κB in normal hematopoiesis, in which monocyte differentiation and activation coincide with high NF-κB activity.

Even though NF-κB activity is increased in many AML patients, increased NF-κB signaling does not alter steady-state hematopoiesis. Overexpression of constitutive active IKK or p65 in cord blood CD34+ cells did not affect the proliferation, differentiation or self-renewal of these cells (Schepers et al., 2006). These findings suggest that increased NF-κB activity is required mainly for the maintenance of AML cells, but it does not play a major role in myeloid transformation.

More importantly however, initial results with the proteasome inhibitor MG132 and IKK2 inhibitor AS602868 showed that targeting NF-κB provoked cell death in AML cells in vitro and in vivo, whereas normal bone marrow CD34+CD38− cells were less sensitive to NF-κB inhibition (Guzman et al., 2001; Frelin et al., 2005; Guzman et al., 2002). This indicates that AML cells are likely more dependent on NF-κB signaling for their survival than normal bone marrow cells. Therefore, NF-κB was proposed as an attractive candidate to be targeted in AML. Since then, many studies have shown the important function of NF-κB in AML cell survival and the potential role of NF-κB as a target in AML.

In the following subsections we discuss the various routes that have been proposed as being involved in the constitutive activation of NF-κB. Furthermore, we highlight how the interference in these pathways could potentially affect AML cell survival.

3.1. NF-κB activation as direct consequence of the chromosomal translocation/mutation

Amplifications or rearrangements of RelA, RelB, c-rel, NF-κB1 and NF-κB2 or mutations in IκBα are often found in lymphomas, lymphoid leukemias and Hodgkin's disease (Xiao and Fu, 2011; Rayet and Gelinas, 1999). However, genetic alterations of these NF-κB genes have not frequently been found in myeloid leukemias, suggesting that sustained activation of NF-κB is caused differently in AML cells than in lymphoid malignancies (Hoesel and Schmid, 2013; Munzert et al., 2000). One of the causes of constitutive activation of NF-κB is directly related to the initial chromosomal translocation or mutation in the myeloid leukemias (Table 1).

In normal hematopoietic cells it has been shown that the c-terminal region with intact RUNT-domain of AML1 binds to IKK, thereby inhibiting the kinase activity of IKK (Nakagawa et al., 2011). However, the AML1-ETO fusion product, which lacks the c-terminal region of AML1, was no longer able to bind to IKK, which subsequently resulted in NF-κB activation. Inhibition of NF-κB by the proteasome inhibitor bortezomib induced cell death of transformed AML1-ETO cells, showing that maintenance of these AML1-ETO cells is dependent on NF-κB signaling. Moreover, the transcription factor AML-1 induced the expression of the microRNA mir-223 (Fazi et al., 2007; Fukao et al., 2007), which repressed the expression of IKK1 (Li et al., 2010). In contrast, the fusion product AML1-ETO also binds to the mir-223 promoter, upon which histone proteins are aberrantly deacetylated and CpGs are methylated,

Table 1
Genetic aberrations leading to increased NF-κB activation.

Genetic abnormalities	Proposed mechanisms resulting in constitutive NF-κB activation
AML1-ETO translocation	(1) Increased IKK activation by inhibition of AML1 association to IKK
	(2) Increased IKK expression by repression of microRNA-223
C/EBPα mutation	(1) Binding to p50 promotor
	(2) Increased IKK expression by repression of microRNA-223
5q deletion	Increased TRAF6 and p62 expression by loss of mir-146
BCR-ABL translocation	Increased IKK2 activation by activation of PRKD2
FLT3-ITD mutation	(1) Increased TAK1 activation
	(2) Increased IKK2 activation
MOZ-TIF2 translocation	Unknown
MLL translocations	Unknown

resulting in altered chromatin packaging (Fazi et al., 2007). In this context, AML1 is no longer able to bind to the mir-223 promotor, leading to decreased expression of mir-223 and likely to elevated levels of IKK1. The transcription factor C/EBPα also induced the expression of mir-223, whereas C/EBPα mutant proteins failed to express mir-223 (Pulikkan et al., 2010), possibly also resulting in increased expression of IKK1. Nevertheless, it remains unknown whether loss of mir-223 in AMLs carrying the AML1-ETO fusion products or C/EBPα mutants is directly related to decreased expression of IKK. On the other hand, C/EBPα and C/EBPα mutants could further increase NF-κB activity by binding to the p50 promotor, thereby elevating the expression of p50. Moreover, p50 regulates the expression of C/EBPα, thus inducing a positive feedback loop between C/EBPα and p50. It has also been shown that C/EBPα or C/EBPα mutants physically interact with p50 at the promoter site of the anti-apoptotic genes BCL-2 and C-FLIP (Dooher et al., 2011; Paz-Priel et al., 2005, 2009, 2011). Targeting this interaction might therefore abrogate the expression of these genes and induce apoptosis in C/EBPα mutant AML cells.

Deletion of chromosome 5q also resulted in increased NF-κB activity. MicroRNA mir-146, which resides on band q34, repressed the expression of TRAF6 and IRAK1 (Boldin et al., 2011; Starczynowski et al., 2010; Taganov et al., 2006). Recently, it was shown that loss of mir-146 in del(5q) high-risk MDS/AML cells resulted in increased expression of TRAF6 in AML cells (Fang et al., 2014). Increased NF-κB signaling led to overexpression of p62, a scaffold protein of TRAF6. The positive feedback loop then mediated the constitutive activation of NF-κB in these del(5q) AML cells. However, whether loss of mir-146 also depresses the expression of IRAK1 has not yet been investigated.

Constitutive activation of NF-κB also occurs in a BCR-ABL dependent manner. High NF-κB activity has been observed in Ph+ CML blasts (Kirchner et al., 2003). Moreover, transduction of DA1 cells (Hamdane et al., 1997), Ba/F3 cells (Kirchner et al., 2003; Reuther et al., 1998) or murine derived bone marrow cells (Kagoya et al., 2014; Hsieh and Van Etten, 2014) with BCR-ABL resulted in elevated NF-κB activity. It has been proposed that BCR-ABL interacts with protein kinase D2 (PRKD2) and activates PRKD2 by tyrosine phosphorylation (Mihailovic et al., 2004). PRKD2 is then able to activate NF-κB by phosphorylation of IKK2 (Mihailovic et al., 2004), but not IKK1 (Kirchner et al., 2003; Mihailovic et al., 2004), resulting in the phosphorylation and degradation of IκBα. The role of NF-κB in this type of leukemia is also supported by the finding that genetic targeting of NF-κB or pharmacologic inhibition by anti-TNFα antibody both induced cell death in BCR-ABL transformed cells (Hsieh and Van Etten, 2014; Gallipoli et al., 2013).

In addition, it has been observed that overexpression of FLT3 or activating mutations of FLT3 (e.g. FLT3-ITD), which are both found in AML, trigger the NF-κB pathway (Birkenkamp et al., 2004; Takahashi et al., 2005).

FLT3-ITD enhanced the phosphorylation and activation of TAK1, thereby activating the non-canonical NF-κB pathway (Shanmugam et al., 2012). Besides this mechanism, direct interaction between FLT3 and IKK2 has also been observed. Binding of FLT3 to IKK2 resulted in phosphorylation and activation of IKK2, which induced the canonical NF-κB pathway (Grosjean-Raillard et al., 2008).

Contradictory results have been reported regarding the suppression of constitutive NF-κB upon FLT3 inhibition. One study reported that addition of a FLT3 inhibitor induced apoptosis in a subset of high-risk MDS and AML cells, which coincided with a diminished NF-κB activity (Grosjean-Raillard et al., 2008). In contrast, a different study showed that constitutive activation of NF-κB was not changed upon treatment with a FLT3 inhibitor (Birkenkamp et al., 2004). However, in that study no information was given regarding the cytotoxic effects in these AML cells.

As mentioned previously, MOZ-TIF2 and MLL-fusion products might also regulate the activity of NF-κB (Kagoya et al., 2014). However, the mechanism underlying the constitutive activation of NF-κB by the fusion products is not known. Nonetheless, NF-κB plays an important role in leukemic transformation mediated by MLL-fusion proteins. It has been proposed that NF-κB activity is required for the recruitment of MLL oncoproteins to the HOXA9/MEIS1 loci in murine transformed cells (Kuo et al., 2013). Knockdown of p65 induced cytotoxic effects in MLL-rearranged leukemias in vitro and significantly extended the survival of mice. However, whether the epigenetic effect of MLL is also dependent on NF-κB in primary rearranged leukemias has not yet been investigated.

3.2. Autocrine/paracrine signaling induces constitutive NF-κB activity

For several decades, it has been known that a proportional subset of AMLs express high levels of various cytokines, including TNFα, IL-1 and IL-6 (Oster et al., 1989). In line with these findings, murine BM cells transduced with the MLL-AF10 oncogene also expressed high levels of TNFα and IL-1 (Fu et al., 2010). Secretion of TNFα also induces AML growth in an autocrine-dependent manner (Oster et al., 1989). Moreover, it synergizes with IL-3 and GM-CSF to stimulate cell growth by upregulation of IL-3 and GM-CSF receptors (Elbaz et al., 1991; Hoang et al., 1989). Likewise, IL-1 secretion by AML cells has been observed, which stimulated AML cell growth in an autocrine-feedback loop (Oster et al., 1989; Cozzolino et al., 1989; Estrov et al., 1992; Griffin et al., 1987; Rambaldi et al., 1991). IL-1 did this not only by stimulating the expression of GM-CSF by AML cells, but also by stimulating the production of GM-CSF and G-CSF by endothelial cells (Griffin et al., 1987; Delwel et al., 1989). Whereas these cytokines can stimulate their own expression via NF-κB activation, it is plausible that these cytokines contribute to the constitutive activation of NF-κB observed in a subset of AML patients. Indeed, IL-1 and IL-6 secretion was associated with NF-κB activity in primary AML cells (Dokter et al., 1995). Similarly, it has been shown that constitutive NF-κB activation in the murine MLL-ENL, MOZ-TIF2 or BCR-ABL/NUP98-HOXA9 AML models and
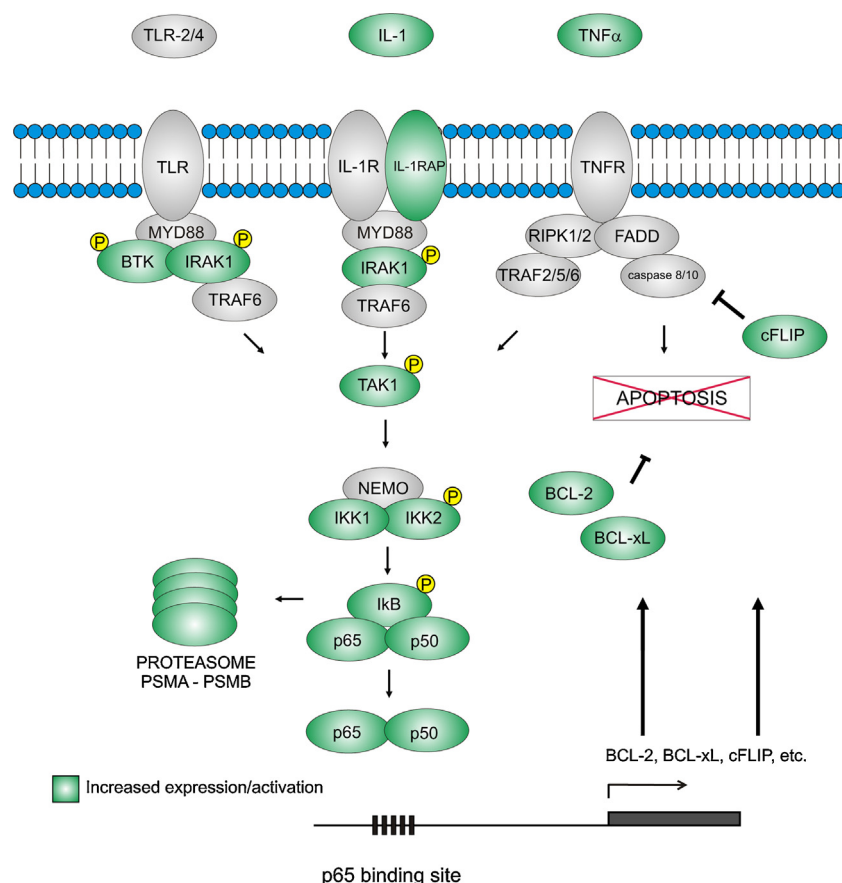
Fig. 3. Increased expression and activation of cytokines and components of the NF-κB pathway drive constitutive NF-κB activation and inhibit apoptosis of AML cells.

human CML cells is dependent on autocrine TNFα signaling (Kagoya et al., 2014; Gallipoli et al., 2013). Furthermore, it has been shown that TNFα expression and secretion was mediated via activation of NF-κB, thus illustrating the positive feedback loop between TNFα and NF-κB. Nevertheless, constitutive NF-κB activation in primary AML cells is probably regulated at the same time by various cytokines and chemokines, considering that the addition of neutralizing antibodies of TNFα, IL-1 or IL-6 to AML cells did not influence the NF-κB activity (Birkenkamp et al., 2004; Dokter et al., 1995).

The crosstalk between AML and the bone microenvironment contributes to the disease progression, and it is plausible that paracrine-related cytokines also enhance the NF-κB activity in AML cells (Zhang et al., 2012b). Recently, it has been shown that the interaction between bone marrow stromal cells and leukemic cells via binding of vascular cell adhesion molecule 1 (VCAM-1) to very late antigen 4 (VLA-4) induced NF-κB activation in the leukemic cells (Jacamo et al., 2014). Besides this interaction, increased SPARC expression has also been observed in AML cells (Alachkar et al., 2014). SPARC is a matricellular protein involved in the communication with the microenvironment (Podhajcer et al., 2008). It was shown that the expression is controlled by the SP1/NF-κB transactivation complex, and that secreted SPARC activated the integrin-linked kinase/AKT (ILK/AKT) pathway in an autocrine-dependent manner. This resulted in increased β-catenin activity, which regulated the self-renewal of AML cells. However, it has not been investigated whether SPARC contributes to the increased NF-κB activity in a positive feedback loop. Nevertheless, a combination of both autocrine and paracrine-related cytokines could most likely be required to induce and maintain constitutive NF-κB activation in AML cells.

3.3. Increased expression of NF-κB signaling components induces constitutive NF-κB signaling

Besides this increased autocrine and paracrine signaling, increased expression and activation of multiple components of the NF-κB signaling pathway could potentially result in constitutive activation of NF-κB (Fig. 3). Indeed, high activity of key components of the NF-κB pathway has been demonstrated in a wide variety of malignancies, hematological and otherwise (Xiao and Fu, 2011). In recent years, increased expression and activation of NF-κB signaling components has also been observed in AML cells.

Firstly, overexpression of the IL-1RAP has been found in stem and progenitors cells from AML and CML patients and has been correlated with the prognosis of AML and MDS patients (Barreyro et al., 2012; Bonardi et al., 2013; de Jonge et al., 2011; Jaras et al., 2010). Furthermore, in cord blood CD34+ cells it has been shown that IL-1RAP expression increased upon retroviral transduction of BCR-ABL (Jaras et al., 2010). Targeting the IL-1RAP protein in AML and CML cells by anti-IL-1RAP antibodies induced antibody-dependent cell-mediated cytotoxicity, indicating that IL-1RAP is a promising target for the treatment of AML (Jaras et al., 2010; Askmyr et al., 2013).

Secondly, increased expression and activation of IRAK1, which can be activated by IL-1 and TLR-signaling, has been detected in high-risk MDS and AML cells (Rhyasen et al., 2013). Inhibition of IRAK1 by an IRAK inhibitor repressed NF-κB activation and suppressed MDS growth in vitro and in vivo. Moreover, simultaneous inhibition of IRAK and BCL-2 induced a synergistic cytotoxic effect, showing that IRAK1 could also be a potential target.

Thirdly, it has been suggested that inhibition of TAK1 could be a therapeutic option in the treatment of AML (Bosman et al., 2014). TAK1 is frequently overexpressed in primary AML cells, and genetic

or chemical suppression of TAK1 induced apoptosis of AML cells in vitro and vivo. TAK1 inhibition suppressed multiple downstream pathways, including NF-κB, ERK, JNK and p38. Cell death mediated by TAK1 inhibition could at least partially be rescued by NF-κB overexpression, suggesting an important role of NF-κB downstream of TAK1 activity. However, upon TAK1-depletion multiple signaling pathways, including JNK, are suppressed, which might contribute to the cytotoxic effect. In line with this hypothesis, it has been found that simultaneous inhibition of NF-κB and JNK has a synergistic effect in TNFα-expressing AML cells (Volk et al., 2014).

In addition, BTK which activates NF-κB downstream of TLR signaling is highly expressed and constitutively phosphorylated in AML (Rushworth et al., 2014).

Together, these findings suggest that components within the NF-κB signaling pathway contribute to the ongoing activation of NF-κB in AML cells. However, the molecular mechanism underlying the increased expression of most of these NF-κB signaling components in AML is still largely unknown. miRNAs might play an important role in the decreased expression of many of the NF-κB signaling components. Indeed, deletion or downregulation of NF-κB-related miRNAs has been found in various hematological malignancies (Xiao and Fu, 2011; Rushworth et al., 2012). As mentioned previously, decreased expression of miRNAs could result in increased expression of TRAF6 and IKK. However, further studies are warranted to unravel the role of miRNAs on the expression of the different components within the NF-κB signaling pathway.

Regulators cooperating with the NF-κB complex in gene activation could potentially also play a role in the constitutive activation of NF-κB. BRD4 has been implicated as playing an important role in leukemia maintenance. Inhibition of BRD4 by using short hairpins significantly delayed MLL-AF9/NRASG12D leukemic outgrowth in mice (Zuber et al., 2011). Secondly, targeting of BRD4 by the chemical compounds JQ1 and IBET-151 induced apoptosis in primary leukemic cells and prolonged the survival of mice in MLL-AF4 and MLL-AF9 leukemia models (Zuber et al., 2011; Dawson et al., 2011). The effect of BRD4 inhibition on the survival of AML cells might be due partly to its association with NF-κB. Recently, it has been shown that constitutive activation of NF-κB in cancer cells is maintained by the binding of p65 to BRD4, thereby suppressing the ubiquitination of p65 and its degradation by the proteasome (Zou et al., 2014). In addition, the effect of the BRD4 inhibitor IBET-151 is partially mediated via inhibition of BCL-2, a key target gene of NF-κB (Dawson et al., 2011). Moreover, TNFα addition and subsequent NF-κB activation resulted in the formation of new super enhancers. These super enhancers are hyperacetylated and have high levels of BRD4 at the expense of the basal super enhancers (Brown et al., 2014). These data could indicate that leukemic cells that have gained constitutively activated NF-κB mediated by TNFa are more dependent on specific super enhancers. However, future studies are required to further illuminate the importance of NF-κB as an effector in BRD4-mediated gene transcription.

Another regulator that cooperates with the NF-κB complex in gene activation is MDM2. Besides its role as co-transcription factor for NF-κB target genes, it also induces the expression of p65 in various cells (Gu et al., 2002; Thomasova et al., 2012). Enhanced expression of MDM2 has been observed in various hematological malignancies, including AML, but it has not been investigated whether this increased expression results in enhanced expression and/or activity of NF-κB in AML cells (Konikova and Kusenda, 2003).

3.4. Increased proteasomal activity is related to constitutive NF-κB activity

Protein homeostasis is essential for eukaryotic cells and is mediated by a tightly regulated synthesis and breakdown of proteins. It was recently reported that an increased or decreased protein synthesis rate largely impaired the function of HSCs, indicating that the synthesis rate of proteins is a highly controlled process in hematopoietic stem cells (Signer et al., 2014). The degradation of proteins is on the other hand mediated via lysosomes and the ubiquitin-proteasome system. Whereas the lysosomal pathway is not selective, the ubiquitin-proteasomal pathway selectively degrades ubiquitinated proteins. The 26S proteasome complex has a barrel-like structure and is composed of two outer α-rings and two central β-rings (20S), each formed by seven subunits and two regulatory caps (19S) that bind to ubiquitinated proteins. Proteolysis is mediated via the β1/PSMB6, β2/PSMB7 and the β5/PSMB5 subunits, which have respectively a caspase-like, trypsin-like and chymotrypsin-like activity. Due to its important role in the degradation of proteins, the proteasome is critically involved in many cellular processes, such as proliferation, apoptosis, DNA repair and possibly carcinogenesis (Crawford and Irvine, 2013). Proteasomal activity is directly related to NF-κB activity via the degradation of ubiquitinated phosphorylated IκBα, as described above. Besides this constitutive proteasome, hematopoietic cells also contain an immunoproteasome variant, which has three catalytic subunits, β5$_i$/PSMB8, β1$_i$/PSMB9 and β2$_i$/PSMB10 (Kuhn and Orlowski, 2012; Niewerth et al., 2013). The immunoproteasome has been recognized as playing an important role in antigen presentation. Increased expression and activity of the proteasome has been observed in leukemic cells compared to normal hematopoietic cells (Kumatori et al., 1990; Ma et al., 2009). In addition, we have observed increased expression of 9 out of the 17 proteasome subunits in AML cells by comparing 66 AML CD34^{+} patient cells versus 22 NBM CD34^{+} cells by microarray (Bonardi et al., 2013; de Jonge et al., 2011). Furthermore, we also detected increased proteasome activity in AML CD34^{+} cells compared to NBM CD34^{+} cells. Increased proteasome activity has also been observed in leukemia-initiating cells (LICs) from murine MLL-ENL, MOZ-TIF2 or BCR-ABL/NUP98-HOXA9 models (Kagoya et al., 2014). Importantly, it has been shown that these elevated levels of proteasome activity are related to increased NF-κB activity in a TNFα-dependent manner.

4. Targeted therapy of NF-κB signaling pathways

Unraveling the molecular mechanism of constitutive NF-κB activation is required to elucidate novel approaches to inhibit NF-κB activity in AML. As described above, various regulators that induce NF-κB activity, such as IRAK1, TAK1 and IKK, have been found to be potential targets for the treatment of AML (Table 2). However, proper validation in in vivo models using primary AML cells is needed to determine the functionality of these inhibitors on AML stem cells. In addition, further insight into the cooperation of NF-κB and BRD4 in gene transcription will be important to determine the extent to which the cytotoxic effects of the BRD4 inhibitors JQ1 and IBET-151 in AML are mediated via repression of NF-κB. Similarly, the relation of MDM2 and NF-κB in gene transcription in AML cells has not been investigated. Inhibition of MDM2 by addition of Nutlin-3 induced apoptosis in AML cells (Kojima et al., 2005). Interestingly, this has been attributed to the reactivation of p53 upon blockade of MDM2. p53 is frequently inactivated in AML cells which is correlated with the expression of MDM2 (Seliger et al., 1996). Thus, targeting of MDM2 might not only induce apoptosis via repression of NF-κB activity, but also via the reactivation of p53. The possible combination of these two effects warrants further investigation into the exact molecular mechanism of the MDM2 inhibitor.

Besides these novel strategies, proteasomal inhibition has already been studied in more detail. Interestingly, the first compound that has been shown to inhibit NF-κB activity in AML cells is

Table 2
Novel targets and agents that inhibit NF-κB activity.

Targets	Compounds
TAK1	5z-7-oxozeaenol, AZ-TAK1, NG25 (Bosman et al., 2014; Tan et al., 2015)
IRAK1	Pacritinib (Komrokji et al., 2015)
BRD4	JQ-1, IBET-151 (Zuber et al., 2011; Dawson et al., 2011)
MDM2	Nutlin-3 (Gu et al., 2002; Kojima et al., 2005)
FLT3	Second-generation inhibitors quizartinib (AC-220),crenolanib (CP-868596) and PLX3397 (Wander et al., 2014)
BTK	Ibrutinib (Rushworth et al., 2014)
Proteasome	Second generation inhibitors carfilzomib and oprozomib (Dick and Fleming, 2010)

the proteasome inhibitor MG-132, which induced apoptosis in AML CD34+ cells while sparing normal CD34+ cells (Guzman et al., 2001). The first proteasome inhibitor that showed clinical effectiveness in AML patients was bortezomib, which primarily binds reversibly to the β5-subunit and blocks the chymotrypsin-like activity of the proteasome (Attar et al., 2008; Blum et al., 2012; Cortes et al., 2004). However, AML stem cells are largely insensitive to bortezomib, which might be due to improper reduction of NF-κB activity and upregulation of the anti-apoptotic protein MCL-1 in AML CD34+ cells (Bosman et al., 2013). Furthermore, neurotoxicity has been observed frequently, warranting the need for other proteasome inhibitors (Attar et al., 2013).

Recently, the second-generation proteasome inhibitors carfilzomib and its orally bio-available derivate oprozomib have been developed. These epoxyketone derivates bind irreversibly to the β5-subunit of the immuno (proteasome), which might increase the efficacy of these compounds by inhibiting the proteasome activity for a longer period (Dick and Fleming, 2010). Clinical activity of carfilzomib has been observed in bortezomib-refractory and bortezomib-relapsed multiple myeloma patients, and less severe side effects compared to bortezomib have been seen up to now (Arastu-Kapur et al., 2011; Siegel et al., 2012; Vij et al., 2012). In vitro experiments have shown that carfilzomib also induces apoptosis in AML blasts (Kraus et al., 2014; Stapnes et al., 2007). Sensitivity of AML blasts is related to a high ratio of immunoproteasome levels compared to constitutive proteasome levels, which is likely due to the increased affinity of the second generation inhibitors to the immunoproteasome (Niewerth et al., 2013; Huber et al., 2012). Furthermore, we have recently shown that carfilzomib effectively induces apoptosis in primitive leukemic cells. In our study, the in vitro stem cell frequency was decreased upon carfilzomib treatment. This coincided with a reduction in quiescent CD34+CD38− cells. However, the clinical effectiveness of the second-generation proteasome inhibitors in AML patients, and accordingly in AML stem cells, has yet to be determined. It will also be important to evaluate the combination of proteasome inhibitors with MCL-1 inhibitors such as obatoclax, since the degradation of the anti-apoptotic protein MCL-1 is also dependent on the proteasome activity.

Inhibition of BTK could be another potential strategy for the treatment of AML. The irreversible BTK inhibitor ibrutinib has already been evaluated in phase 1 and 2 clinical studies, in which it showed cytotoxic effects in a variety of hematological malignancies, including CLL (Herman et al., 2014; Rushworth et al., 2013). In accordance with these results, ibrutinib induced cell death in primary AML cells, which coincided with decreased NF-κB, AKT and ERK activity (Rushworth et al., 2014). BTK is also an important regulatory component of B cell receptor (BCR) signaling. However, it is currently unknown whether inhibition of BCR signaling in AML cells by ibrutinib plays a role in its cytotoxic effects.

As described above, overexpression of FLT3 or activating mutations of FLT3 triggered the NF-κB pathway (Birkenkamp et al., 2004; Takahashi et al., 2005). To further elucidate the mechanism-of-action of FLT3-inhibitors such as AC-220 (Zarrinkar et al., 2009),

it would be interesting to study whether FLT3 inhibitors induce cytotoxicity in AML cells via repression of NF-κB.

Regarding the clinical application of these various inhibitors, it will be necessary to investigate the relationship between the cause of NF-κB activation and the clinical responsiveness of the inhibitors. For example, it has been observed that TNFα-dependent growth of AML cells is not repressed by BTK inhibition. A possible explanation is that the repression of NF-κB signaling by BTK inhibition is compensated by constitutive activation of NF-κB via TNFα. This suggests that ibrutinib most likely has higher efficacy in AML cells expressing low levels of TNFα. In contrast, GM-CSF-, G-CSF- or IL-3-dependent growth was inhibited upon ibrutinib addition, as these cytokines do not activate NF-κB, or activate it to a lesser extent than TNFα (Rushworth et al., 2014; Ebner et al., 2003).

At present it is also unknown whether increased levels of BTK or IRAK1 directly correlate with the sensitivity of AML cells to ibrutinib or to IRAK1 inhibitors. Elucidating these correlations will be essential for the use of these inhibitors in the clinic.

In conclusion, understanding the various molecular mechanisms resulting in constitutive NF-κB activation has provided novel targets to inhibit NF-κB activity. Future research will be necessary to validate the importance of these targets. In addition, unraveling the exact role of the binding partners, such as BRD4 and CBP/p300 in NF-κB mediated transcription, might lead to the identification of novel strategies to inhibit NF-κB.

Conflicts of interest

No conflicts of interest.

Authorship and disclosures

MCJB, JJS and EV wrote the manuscript and all authors approved the final article.

References

Alachkar, H., Santhanam, R., Maharry, K., Metzeler, K.H., Huang, X., Kohlschmidt, J., Mendler, J.H., Benito, J.M., Hickey, C., Neviani, P., Dorrance, A.M., Anghelina, M., Khalife, J., Tarighat, S.S., Volinia, S., Whitman, S.P., Paschka, P., Hoellerbauer, P., Wu, Y.Z., Han, L., Bolon, B.N., Blum, W., Mrozek, K., Carroll, A.J., Perrotti, D., Andreeff, M., Caligiuri, M.A., Konopleva, M., Garzon, R., Bloomfield, C.D., Marcucci, G., 2014. SPARC promotes leukemic cell growth and predicts acute myeloid leukemia outcome. J. Clin. Invest. 124, 1512–1524.

Alkalay, I., Yaron, A., Hatzubai, A., Orian, A., Ciechanover, A., Ben-Neriah, Y., 1995. Stimulation-dependent I kappa B alpha phosphorylation marks the NF-kappa B inhibitor for degradation via the ubiquitin-proteasome pathway. Proc. Natl. Acad. Sci. U. S. A. 92, 10599–10603.

Arastu-Kapur, S., Anderl, J.L., Kraus, M., Parlati, F., Shenk, K.D., Lee, S.J., Muchamuel, T., Bennett, M.K., Driessen, C., Ball, A.J., Kirk, C.J., 2011. Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin. Cancer Res. 17, 2734–2743.

Askmyr, M., Agerstam, H., Hansen, N., Gordon, S., Arvanitakis, A., Rissler, M., Juliusson, G., Richter, J., Jaras, M., Fioretos, T., 2013. Selective killing of candidate AML stem cells by antibody targeting of IL1RAP. Blood 121, 3709–3713.

Attar, E.C., De Angelo, D.J., Supko, J.G., D'Amato, F., Zahrieh, D., Sirulnik, A., Wadleigh, M., Ballen, K.K., McAfee, S., Miller, K.B., Levine, J., Galinsky, I., Trehu, E.G., Schenkein, D., Neuberg, D., Stone, R.M., Amrein, P.C., 2008. Phase I and

pharmacokinetic study of bortezomib in combination with idarubicin and cytarabine in patients with acute myelogenous leukemia. Clin. Cancer Res. 14, 1446–1454.

Attar, E.C., Johnson, J.L., Amrein, P.C., Lozanski, G., Wadleigh, M., DeAngelo, D.J., Kolitz, J.E., Powell, B.L., Voorhees, P., Wang, E.S., Blum, W., Stone, R.M., Marcucci, G., Bloomfield, C.D., Moser, B., Larson, R.A., 2013. Bortezomib added to daunorubicin and cytarabine during induction therapy and to intermediate-dose cytarabine for consolidation in patients with previously untreated acute myeloid leukemia age 60–75 years: CALGB (Alliance) study 10502. J. Clin. Oncol. 31, 923–929.

Barreyro, L., Will, B., Bartholdy, B., Zhou, L., Todorova, T.I., Stanley, R.F., Ben-Neriah, S., Montagna, C., Parekh, S., Pellagatti, A., Boultwood, J., Paietta, E., Ketterling, R.P., Cripe, L., Fernandez, H.F., Greenberg, P.L., Tallman, M.S., Steidl, C., Mitsiades, C.S., Verma, A., Steidl, U., 2012. Overexpression of IL-1 receptor accessory protein in stem and progenitor cells and outcome correlation in AML and MDS. Blood 120, 1290–1298.

Baumgartner, B., Weber, M., Quirling, M., Fischer, C., Page, S., Adam, M., Von, S.C., Waterhouse, C., Schmid, C., Neumeier, D., Brand, K., 2002. Increased IkappaB kinase activity is associated with activated NF-kappaB in acute myeloid blasts. Leukemia 16, 2062–2071.

Birkenkamp, K.U., Geugien, M., Schepers, H., Westra, J., Lemmink, H.H., Vellenga, E., 2004. Constitutive NF-kappaB DNA-binding activity in AML is frequently mediated by a Ras/PI3-K/PKB-dependent pathway. Leukemia 18, 103–112.

Blum, W., Schwind, S., Tarighat, S.S., Geyer, S., Eisfeld, A.K., Whitman, S., Walker, A., Klisovic, R., Byrd, J.C., Santhanam, R., Wang, H., Curfman, J.P., Devine, S.M., Jacob, S., Garr, C., Kefauver, C., Perrotti, D., Chan, K.K., Bloomfield, C.D., Caligiuri, M.A., Grever, M.R., Garzon, R., Marcucci, G., 2012. Clinical and pharmacodynamic activity of bortezomib and decitabine in acute myeloid leukemia. Blood 119, 6025–6031.

Boldin, M.P., Taganov, K.D., Rao, D.S., Yang, L., Zhao, J.L., Kalwani, M., Garcia-Flores, Y., Luong, M., Devrekanli, A., Xu, J., Sun, G., Tay, J., Linsley, P.S., Baltimore, D., 2011. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J. Exp. Med. 208, 1189–1201.

Bonardi, F., Fusetti, F., Deelen, P., van, G.D., Vellenga, E., Schuringa, J.J., 2013. A proteomics and transcriptomics approach to identify leukemic stem cell (LSC) markers. Mol. Cell Proteomics 12, 626–637.

Bosman, M.C., Schuringa, J.J., Quax, W.J., Vellenga, E., 2013. Bortezomib sensitivity of acute myeloid leukemia CD34(+) cells can be enhanced by targeting the persisting activity of NF-kappaB and the accumulation of MCL-1. Exp. Hematol. 41, 530–538.

Bosman, M.C., Schepers, H., Jaques, J., Brouwers-Vos, A.Z., Quax, W.J., Schuringa, J.J., Vellenga, E., 2014. The TAK1-NF-kappaB axis as therapeutic target for AML. Blood 124, 3130–3140.

Bottero, V., Withoff, S., Verma, I.M., 2006. NF-kappaB and the regulation of hematopoiesis. Cell Death Differ 13, 785–797.

Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., Siebenlist, U., 1995. Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. Science 267, 1485–1488.

Brown, J.D., Lin, C.Y., Duan, Q., Griffin, G., Federation, A.J., Paranal, R.M., Bair, S., Newton, G., Lichtman, A.H., Kung, A.L., Yang, T., Wang, H., Luscinskas, F.W., Croce, K.J., Bradner, J.E., Plutzky, J., 2014. NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. Mol. Cell 56, 219–231.

Chan, H.M., La Thangue, N.B., 2001. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J. Cell Sci. 114, 2363–2373.

Chen, L., Fischle, W., Verdin, E., Greene, W.C., 2001. Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science 293, 1653–1657.

Chen, L.F., Williams, S.A., Mu, Y., Nakano, H., Duerr, J.M., Buckbinder, L., Greene, W.C., 2005. NF-kappaB RelA phosphorylation regulates RelA acetylation. Mol. Cell Biol. 25, 7966–7975.

Cheung, P.C., Nebreda, A.R., Cohen, P., 2004. TAB3, a new binding partner of the protein kinase TAK1. Biochem. J. 378, 27–34.

Cilloni, D., Martinelli, G., Messa, F., Baccarani, M., Saglio, G., 2007. Nuclear factor kB as a target for new drug development in myeloid malignancies. Haematologica 92, 1224–1229.

Cortes, J., Thomas, D., Koller, C., Giles, F., Estey, E., Faderl, S., Garcia-Manero, G., McConkey, D., Ruiz, S.L., Guerciolini, R., Wright, J., Kantarjian, H., 2004. Phase I study of bortezomib in refractory or relapsed acute leukemias. Clin. Cancer Res. 10, 3371–3376.

Cozzolino, F., Rubartelli, A., Aldinucci, D., Sitia, R., Torcia, M., Shaw, A., Di, G.R., 1989. Interleukin 1 as an autocrine growth factor for acute myeloid leukemia cells. Proc. Natl. Acad. Sci. U. S. A. 86, 2369–2373.

Crawford, L.J., Irvine, A.E., 2013. Targeting the ubiquitin proteasome system in haematological malignancies. Blood Rev. 27, 297–304.

Dawson, M.A., Prinjha, R.K., Dittmann, A., Giotopoulos, G., Bantscheff, M., Chan, W.I., Robson, S.C., Chung, C.W., Hopf, C., Savitski, M.M., Huthmacher, C., Gudgin, E., Lugo, D., Beinke, S., Chapman, T.D., Roberts, E.J., Soden, P.E., Auger, K.R., Mirguet, O., Doehner, K., Delwel, R., Burnett, A.K., Jeffrey, P., Drewes, G., Lee, K., Huntly, B.J., Kouzarides, T., 2011. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 478, 529–533.

Delwel, R., van, B.C., Salem, M., Bot, F., Gillis, S., Kaushansky, K., Altrock, B., Lowenberg, B., 1989. Interleukin-1 stimulates proliferation of acute myeloblastic leukemia cells by induction of granulocyte-macrophage colony-stimulating factor release. Blood 74, 586–593.

DiDonato, J.A., Hayakawa, M., Rothwarf, D.M., Zandi, E., Karin, M., 1997. A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. Nature 388, 548–554.

Dick, L.R., Fleming, P.E., 2010. Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy. Drug Discovery Today 15, 243–249.

Dokter, W.H., Tuyt, L., Sierdsema, S.J., Esselink, M.T., Vellenga, E., 1995. The spontaneous expression of interleukin-1 beta and interleukin-6 is associated with spontaneous expression of AP-1 and NF-kappa B transcription factor in acute myeloblastic leukemia cells. Leukemia 9, 425–432.

Dooher, J.E., Paz-Priel, I., Houng, S., Baldwin Jr., A.S., Friedman, A.D., 2011. C/EBPalpha, C/EBPalpha oncoproteins, or C/EBPbeta preferentially bind NF-kappaB p50 compared with p65, focusing therapeutic targeting on the C/EBP:p50 interaction. Mol. Cancer Res. 9, 1395–1405.

Ebner, K., Bandion, A., Binder, B.R., de, M.R., Schmid, J.A., 2003. GMCSF activates NF-kappaB via direct interaction of the GMCSF receptor with IkappaB kinase beta. Blood 102, 192–199.

Elbaz, O., Budel, L.M., Hoogerbrugge, H., Touw, I.P., Delwel, R., Mahmoud, L.A., Lowenberg, B., 1991. Tumor necrosis factor regulates the expression of granulocyte-macrophage colony-stimulating factor and interleukin-3 receptors on human acute myeloid leukemia cells. Blood 77, 989–995.

Estrov, Z., Kurzrock, R., Estey, E., Wetzler, M., Ferrajoli, A., Harris, D., Blake, M., Gutterman, J.U., Talpaz, M., 1992. Inhibition of acute myelogenous leukemia blast proliferation by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors. Blood 79, 1938–1945.

Fang, J., Barker, B., Bolanos, L., Liu, X., Jerez, A., Makishima, H., Christie, S., Chen, X., Rao, D.S., Grimes, H.L., Komurov, K., Weirauch, M.T., Cancelas, J.A., Maciejewski, J.P., Starczynowski, D.T., 2014. Myeloid malignancies with chromosome 5q deletions acquire a dependency on an intrachromosomal NF-kappaB gene network. Cell Rep. 8, 1328–1338.

Fazi, F., Racanicchi, S., Zardo, G., Starnes, L.M., Mancini, M., Travaglini, L., Diverio, D., Ammatuna, E., Cimino, G., Lo-Coco, F., Grignani, F., Nervi, C., 2007. Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. Cancer Cell 12, 457–466.

Frelin, C., Imbert, V., Griessinger, E., Peyron, A.C., Rochet, N., Philip, P., Dageville, C., Sirvent, A., Hummelsberger, M., Berard, E., Dreano, M., Sirvent, N., Peyron, J.F., 2005. Targeting NF-kappaB activation via pharmacologic inhibition of IKK2-induced apoptosis of human acute myeloid leukemia cells. Blood 105, 804–811.

Fu, J.F., Hsu, C.L., Shih, L.Y., 2010. MLL/AF10(OM-LZ)-immortalized cells expressed cytokines and induced host cell proliferation in a mouse bone marrow transplantation model. Int. J. Cancer 126, 1621–1629.

Fukao, T., Fukuda, Y., Kiga, K., Sharif, J., Hino, K., Enomoto, Y., Kawamura, A., Nakamura, K., Takeuchi, T., Tanabe, M., 2007. An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. Cell 129, 617–631.

Gallipoli, P., Pellicano, F., Morrison, H., Laidlaw, K., Allan, E.K., Bhatia, R., Copland, M., Jorgensen, H.G., Holyoake, T.L., 2013. Autocrine TNF-alpha production supports CML stem and progenitor cell survival and enhances their proliferation. Blood 122, 3335–3339.

Gerondakis, S., Grossmann, M., Nakamura, Y., Pohl, T., Grumont, R., 1999. Genetic approaches in mice to understand Rel/NF-kappaB and IkappaB function: transgenics and knockouts. Oncogene 18, 6888–6895.

Griffin, J.D., Rambaldi, A., Vellenga, E., Young, D.C., Ostapovicz, D., Cannistra, S.A., 1987. Secretion of interleukin-1 by acute myeloblastic leukemia cells in vitro induces endothelial cells to secrete colony stimulating factors. Blood 70, 1218–1221.

Grosjean-Raillard, J., Ades, L., Boehrer, S., Tailler, M., Fabre, C., Braun, T., de, B.S., Israel, A., Fenaux, P., Kroemer, G., 2008. Flt3 receptor inhibition reduces constitutive NFkappaB activation in high-risk myelodysplastic syndrome and acute myeloid leukemia. Apoptosis 13, 1148–1161.

Gu, L., Findley, H.W., Zhou, M., 2002. MDM2 induces NF-kappaB/p65 expression transcriptionally through Sp1-binding sites: a novel, p53-independent role of MDM2 in doxorubicin resistance in acute lymphoblastic leukemia. Blood 99, 3367–3375.

Guzman, M.L., Neering, S.J., Upchurch, D., Grimes, B., Howard, D.S., Rizzieri, D.A., Luger, S.M., Jordan, C.T., 2001. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood 98, 2301–2307.

Guzman, M.L., Swiderski, C.F., Howard, D.S., Grimes, B.A., Rossi, R.M., Szilvassy, S.J., Jordan, C.T., 2002. Preferential induction of apoptosis for primary human leukemic stem cells. Proc. Natl. Acad. Sci. U. S. A. 99, 16220–16225.

Hamdane, M., David-Cordonnier, M.H., D'Halluin, J.C., 1997. Activation of p65 NF-kappaB protein by p210BCR-ABL in a myeloid cell line (P210BCR-ABL activates p65 NF-kappaB). Oncogene 15, 2267–2275.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Cell 144, 646–674.

Herman, S.E., Mustafa, R.Z., Gyamfi, J.A., Pittaluga, S., Chang, S., Chang, B., Farooqui, M., Wiestner, A., 2014. Ibrutinib inhibits BCR and NF-kappaB signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL. Blood 123, 3286–3295.

Hoang, T., Levy, B., Onetto, N., Haman, A., Rodriguez-Cimadevilla, J.C., 1989. Tumor necrosis factor alpha stimulates the growth of the clonogenic cells of acute myeloblastic leukemia in synergy with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 170, 15–26.

Hoesel, B., Schmid, J.A., 2013. The complexity of NF-kappaB signaling in inflammation and cancer. Mol. Cancer 12, 86.

Hsieh, M.Y., Van Etten, R.A., 2014. IKK-dependent activation of NF-kappaB contributes to myeloid and lymphoid leukemogenesis by BCR-ABL1. Blood.

Huber, E.M., Basler, M., Schwab, R., Heinemeyer, W., Kirk, C.J., Groettrup, M., Groll, M., 2012. Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity. Cell 148, 727–738.

Jacamo, R., Chen, Y., Wang, Z., Ma, W., Zhang, M., Spaeth, E.L., Wang, Y., Battula, V.L., Mak, P.Y., Schallmoser, K., Ruvolo, P., Schober, W.D., Shpall, E.J., Nguyen, M.H., Strunk, D., Bueso-Ramos, C.E., Konoplev, S., Davis, R.E., Konopleva, M., Andreeff, M., 2014. Reciprocal leukemia-stroma VCAM-1/VLA-4-dependent activation of NF-kappaB mediates chemoresistance. Blood 123, 2691–2702.

Jang, M.K., Mochizuki, K., Zhou, M., Jeong, H.S., Brady, J.N., Ozato, K., 2005. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol. Cell 19, 523–534.

Jaras, M., Johnels, P., Hansen, N., Agerstam, H., Tsapogas, P., Rissler, M., Lassen, C., Olofsson, T., Bjerrum, O.W., Richter, J., Fioretos, T., 2010. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. Proc. Natl. Acad. Sci. U. S. A. 107, 16280–16285.

Jurida, L., Soelch, J., Bartkuhn, M., Handschick, K., Muller, H., Newel, D., Weber, A., Dittrich-Breiholz, O., Schneider, H., Bhuju, S., Saul, V.V., Schmitz, M.L., Kracht, M., 2015. The Activation of IL-1-Induced Enhancers Depends on TAK1 Kinase Activity and NF-kappaB p65. Cell Rep.

Kagoya, Y., Yoshimi, A., Kataoka, K., Nakagawa, M., Kumano, K., Arai, S., Kobayashi, H., Saito, T., Iwakura, Y., Kurokawa, M., 2014. Positive feedback between NF-kappaB and TNF-alpha promotes leukemia-initiating cell capacity. J. Clin. Invest. 124, 528–542.

Kirchner, D., Duyster, J., Ottmann, O., Schmid, R.M., Bergmann, L., Munzert, G., 2003. Mechanisms of Bcr-Abl-mediated NF-kappaB/Rel activation. Exp. Hematol. 31, 504–511.

Kojima, K., Konopleva, M., Samudio, I.J., Shikami, M., Cabreira-Hansen, M., McQueen, T., Ruvolo, V., Tsao, T., Zeng, Z., Vassilev, L.T., Andreeff, M., 2005. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukemia therapy. Blood 106, 3150–3159.

Komrokji, R.S., Seymour, J.F., Roberts, A.W., Wadleigh, M., To, L.B., Scherber, R., Turba, E., Dorr, A., Zhu, J., Wang, L., Granston, T., Campbell, M.S., Mesa, R.A., 2015. Results of a phase 2 study of pacritinib (SB1518), a JAK2/JAK2(V617F) inhibitor, in patients with myelofibrosis. Blood.

Konikova, E., Kusenda, J., 2003. Altered expression of p53 and MDM2 proteins in hematological malignancies. Neoplasma 50, 31–40.

Kraus, M., Muller-Ide, H., Ruckrich, T., Bader, J., Overkleeft, H., Driessen, C., 2014. Ritonavir, nelfinavir, saquinavir and lopinavir induce proteotoxic stress in acute myeloid leukemia cells and sensitize them for proteasome inhibitor treatment at low micromolar drug concentrations. Leuk. Res. 38, 383–392.

Kuhn, D.J., Orlowski, R.Z., 2012. The immunoproteasome as a target in hematologic malignancies. Semin. Hematol. 49, 258–262.

Kumatori, A., Tanaka, K., Inamura, N., Sone, S., Ogura, T., Matsumoto, T., Tachikawa, T., Shin, S., Ichihara, A., 1990. Abnormally high expression of proteasomes in human leukemic cells. Proc. Natl. Acad. Sci. U. S. A. 87, 7071–7075.

Kuo, H.P., Wang, Z., Lee, D.F., Iwasaki, M., Duque-Afonso, J., Wong, S.H., Lin, C.H., Figueroa, M.E., Su, J., Lemischka, I.R., Cleary, M.L., 2013. Epigenetic roles of MLL oncoproteins are dependent on NF-kappaB. Cancer Cell 24, 423–437.

Li, T., Morgan, M.J., Choksi, S., Zhang, Y., Kim, Y.S., Liu, Z.G., 2010. MicroRNAs modulate the noncanonical transcription factor NF-kappaB pathway by regulating expression of the kinase IKKalpha during macrophage differentiation. Nat. Immunol. 11, 799–805.

Ma, W., Kantarjian, H., Bekele, B., Donahue, A.C., Zhang, X., Zhang, Z.J., O'Brien, S., Estey, E., Estrov, Z., Cortes, J., Keating, M., Giles, F., Albitar, M., 2009. Proteasome enzymatic activities in plasma as risk stratification of patients with acute myeloid leukemia and advanced-stage myelodysplastic syndrome. Clin. Cancer Res. 15, 3820–3826.

Mihailovic, T., Marx, M., Auer, A., Van, L.J., Schmid, M., Weber, C., Seufferlein, T., 2004. Protein kinase D2 mediates activation of nuclear factor kappaB by Bcr-Abl in Bcr-Abl+ human myeloid leukemia cells. Cancer Res. 64, 8939–8944.

Mujtaba, S., Zeng, L., Zhou, M.M., 2007. Structure and acetyl-lysine recognition of the bromodomain. Oncogene 26, 5521–5527.

Munzert, G., Kreitmeier, S., Bergmann, L., 2000. Normal structure of NFKB2, C-REL and BCL-3 gene loci in lymphoproliferative and myeloproliferative disorders. Leuk. Lymphoma 38, 395–400.

Nakagawa, M., Shimabe, M., Watanabe-Okochi, N., Arai, S., Yoshimi, A., Shinohara, A., Nishimoto, N., Kataoka, K., Sato, T., Kumano, K., Nannya, Y., Ichikawa, M., Imai, Y., Kurokawa, M., 2011. AML1/RUNX1 functions as a cytoplasmic attenuator of NF-kappaB signaling in the repression of myeloid tumors. Blood 118, 6626–6637.

Niewerth, D., Franke, N.E., Jansen, G., Assaraf, Y.G., van, M.J., Kirk, C.J., Degenhardt, J., Anderl, J., Schimmer, A.D., Zweegman, S., de, H.V., Horton, T.M., Kaspers, G.J., Cloos, J., 2013. Higher ratio immune versus constitutive proteasome level as novel indicator of sensitivity of pediatric acute leukemia cells to proteasome inhibitors. Haematologica 98, 1896–1904.

Oster, W., Cicco, N.A., Klein, H., Hirano, T., Kishimoto, T., Lindemann, A., Mertelsmann, R.H., Herrmann, F., 1989. Participation of the cytokines interleukin 6, tumor necrosis factor-alpha, and interleukin 1-beta secreted by acute myelogenous leukemia blasts in autocrine and paracrine leukemia growth control. J. Clin. Invest. 84, 451–457.

Pahl, H.L., 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853–6866.

Paz-Priel, I., Cai, D.H., Wang, D., Kowalski, J., Blackford, A., Liu, H., Heckman, C.A., Gombart, A.F., Koeffler, H.P., Boxer, L.M., Friedman, A.D., 2005. CCAAT/enhancer binding protein alpha (C/EBPalpha) and C/EBPalpha myeloid oncoproteins induce bcl-2 via interaction of their basic regions with nuclear factor-kappaB p50. Mol. Cancer Res. 3, 585–596.

Paz-Priel, I., Ghosal, A.K., Kowalski, J., Friedman, A.D., 2009. C/EBPalpha or C/EBPalpha oncoproteins regulate the intrinsic and extrinsic apoptotic pathways by direct interaction with NF-kappaB p50 bound to the bcl-2 and FLIP gene promoters. Leukemia 23, 365–374.

Paz-Priel, I., Houng, S., Dooher, J., Friedman, A.D., 2011. C/EBPalpha and C/EBPalpha oncoproteins regulate nfkb1 and displace histone deacetylases from NF-kappaB p50 homodimers to induce NF-kappaB target genes. Blood 117, 4085–4094.

Podhajcer, O.L., Benedetti, L.G., Girotti, M.R., Prada, F., Salvatierra, E., Llera, A.S., 2008. The role of the matricellular protein SPARC in the dynamic interaction between the tumor and the host. Cancer Metastasis Rev. 27, 691–705.

Pulikkan, J.A., Dengler, V., Peramangalam, P.S., Peer Zada, A.A., Muller-Tidow, C., Bohlander, S.K., Tenen, D.G., Behre, G., 2010. Cell-cycle regulator E2F1 and microRNA-223 comprise an autoregulatory negative feedback loop in acute myeloid leukemia. Blood 115, 1768–1778.

Rambaldi, A., Torcia, M., Bettoni, S., Vannier, E., Barbui, T., Shaw, A.R., Dinarello, C.A., Cozzolino, F., 1991. Modulation of cell proliferation and cytokine production in acute myeloblastic leukemia by interleukin-1 receptor antagonist and lack of its expression by leukemic cells. Blood 78, 3248–3253.

Rayet, B., Gelinas, C., 1999. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 18, 6938–6947.

Reuther, J.Y., Reuther, G.W., Cortez, D., Pendergast, A.M., Baldwin, A.S., 1998. Jr.: A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. Genes Dev. 12, 968–981.

Rhyasen, G.W., Bolanos, L., Fang, J., Jerez, A., Wunderlich, M., Rigolino, C., Mathews, L., Ferrer, M., Southall, N., Guha, R., Keller, J., Thomas, C., Beverly, L.J., Cortelezzi, A., Oliva, E.N., Cuzzola, M., Maciejewski, J.P., Mulloy, J.C., Starczynowski, D.T., 2013. Targeting IRAK1 as a therapeutic approach for myelodysplastic syndrome. Cancer Cell 24, 90–104.

Rushworth, S.A., Murray, M.Y., Barrera, L.N., Heasman, S.A., Zaitseva, L., MacEwan, D.J., 2012. Understanding the role of miRNA in regulating NF-kappaB in blood cancer. Am. J. Cancer Res. 2, 65–74.

Rushworth, S.A., MacEwan, D.J., Bowles, K.M., 2013. Ibrutinib in relapsed chronic lymphocytic leukemia. N. Engl. J. Med. 369.

Rushworth, S.A., Murray, M.Y., Zaitseva, L., Bowles, K.M., MacEwan, D.J., 2014. Identification of Bruton's tyrosine kinase as a therapeutic target in acute myeloid leukemia. Blood 123, 1229–1238.

Sakurai, H., Miyoshi, H., Mizukami, J., Sugita, T., 2000. Phosphorylation-dependent activation of TAK1 mitogen-activated protein kinase kinase kinase by TAB1. FEBS Lett. 474, 141–145.

Schepers, H., Eggen, B.J., Schuringa, J.J., Vellenga, E., 2006. Constitutive activation of NF-kappa B is not sufficient to disturb normal steady-state hematopoiesis. Haematologica 91, 1710–1711.

Seliger, B., Papdileris, S., Vogel, D., Hess, G., Brendel, C., Storkel, S., Ortel, J., Kolbe, K., Huber, C., Huhn, D., Neubauer, A., 1996. Analysis of the p53 and MDM-2 gene in acute myeloid leukemia. Eur. J. Haematol. 57, 230–240.

Shanmugam, R., Gade, P., Wilson-Weekes, A., Sayar, H., Suvannasankha, A., Goswami, C., Li, L., Gupta, S., Cardoso, A.A., Al, B.T., Sargent, K.J., Cripe, L.D., Kalvakolanu, D.V., Boswell, H.S., 2012. A noncanonical Flt3ITD/NF-kappaB signaling pathway represses DAPK1 in acute myeloid leukemia. Clin. Cancer Res. 18, 360–369.

Shi, J., Whyte, W.A., Zepeda-Mendoza, C.J., Milazzo, J.P., Shen, C., Roe, J.S., Minder, J.L., Mercan, F., Wang, E., Eckersley-Maslin, M.A., Campbell, A.E., Kawaoka, S., Shareef, S., Zhu, Z., Kendall, J., Muhar, M., Haslinger, C., Yu, M., Roeder, R.G., Wigler, M.H., Blobel, G.A., Zuber, J., Spector, D.L., Young, R.A., Vakoc, C.R., 2013. Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. Genes Dev. 27, 2648–2662.

Siegel, D.S., Martin, T., Wang, M., Vij, R., Jakubowiak, A.J., Lonial, S., Trudel, S., Kukreti, V., Bahlis, N., Alsina, M., Chanan-Khan, A., Buadi, F., Reu, F.J., Somlo, G., Zonder, J., Song, K., Stewart, A.K., Stadtmauer, E., Kunkel, L., Wear, S., Wong, A.F., Orlowski, R.Z., Jagannath, S., 2012. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood 120, 2817–2825.

Signer, R.A., Magee, J.A., Salic, A., Morrison, S.J., 2014. Haematopoietic stem cells require a highly regulated protein synthesis rate. Nature 509, 49–54.

Spaargaren, M., de Rooij, M.F., Kater, A.P., Eldering, E., 2014. BTK inhibitors in chronic lymphocytic leukemia: a glimpse to the future. Oncogene.

Stapnes, C., Doskeland, A.P., Hatfield, K., Ersvaer, E., Ryningen, A., Lorens, J.B., Gjertsen, B.T., Bruserud, O., 2007. The proteasome inhibitors bortezomib and PR-171 have antiproliferative and proapoptotic effects on primary human acute myeloid leukaemia cells. Br. J. Haematol. 136, 814–828.

Starczynowski, D.T., Kuchenbauer, F., Argiropoulos, B., Sung, S., Morin, R., Muranyi, A., Hirst, M., Hogge, D., Marra, M., Wells, R.A., Buckstein, R., Lam, W., Humphries, R.K., Karsan, A., 2010. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. Nat. Med. 16, 49–58.

Stein, S.J., Baldwin, A.S., 2013. Deletion of the NF-kappaB subunit p65/RelA in the hematopoietic compartment leads to defects in hematopoietic stem cell function. Blood 121, 5015–5024.

Taganov, K.D., Boldin, M.P., Chang, K.J., Baltimore, D., 2006. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. U. S. A. 103, 12481–12486.

Takaesu, G., Kishida, S., Hiyama, A., Yamaguchi, K., Shibuya, H., Irie, K., Ninomiya-Tsuji, J., Matsumoto, K., 2000. TAB2, a novel adaptor protein,

mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. Mol. Cell 5, 649–658.

Takaesu, G., Inagaki, M., Takubo, K., Mishina, Y., Hess, P.R., Dean, G.A., Yoshimura, A., Matsumoto, K., Suda, T., Ninomiya-Tsuji, J., 2012. TAK1 (MAP3K7) signaling regulates hematopoietic stem cells through TNF-dependent and -independent mechanisms. PLoS One 7, e51073.

Takahashi, S., Harigae, H., Ishii, K.K., Inomata, M., Fujiwara, T., Yokoyama, H., Ishizawa, K., Kameoka, J., Licht, J.D., Sasaki, T., Kaku, M., 2005. Over-expression of Flt3 induces NF-kappaB pathway and increases the expression of IL-6. Leuk. Res. 29, 893–899.

Tan, L., Nomanbhoy, T., Gurbani, D., Patricelli, M., Hunter, J., Geng, J., Herhaus, L., Zhang, J., Pauls, E., Ham, Y., Choi, H.G., Xie, T., Deng, X., Buhrlage, S.J., Sim, T., Cohen, P., Sapkota, G., Westover, K.D., Gray, N.S., 2015. Discovery of type II inhibitors of TGFbeta-activated kinase 1 (TAK1) and mitogen-activated protein kinase kinase kinase 2 (MAP4K2). J. Med. Chem. 58, 183–196.

Tang, M., Wei, X., Guo, Y., Breslin, P., Zhang, S., Zhang, S., Wei, W., Xia, Z., Diaz, M., Akira, S., Zhang, J., 2008. TAK1 is required for the survival of hematopoietic cells and hepatocytes in mice. J. Exp. Med. 205, 1611–1619.

Thomasova, D., Mulay, S.R., Bruns, H., Anders, H.J., 2012. p53-independent roles of MDM2 in NF-kappaB signaling: implications for cancer therapy, wound healing, and autoimmune diseases. Neoplasia 14, 1097–1101.

Verstrepen, L., Bekaert, T., Chau, T.L., Tavernier, J., Chariot, A., Beyaert, R., 2008. TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. Cell Mol. Life Sci. 65, 2964–2978.

Vij, R., Siegel, D.S., Jagannath, S., Jakubowiak, A.J., Stewart, A.K., McDonagh, K., Bahlis, N., Belch, A., Kunkel, L.A., Wear, S., Wong, A.F., Wang, M., 2012. An open-label, single-arm, phase 2 study of single-agent carfilzomib in patients with relapsed and/or refractory multiple myeloma who have been previously treated with bortezomib. Br. J. Haematol. 158, 739–748.

Volk, A., Li, J., Xin, J., You, D., Zhang, J., Liu, X., Xiao, Y., Breslin, P., Li, Z., Wei, W., Schmidt, R., Li, X., Zhang, Z., Kuo, P.C., Nand, S., Zhang, J., Chen, J., Zhang, J., 2014. Co-inhibition of NF-kappaB and JNK is synergistic in TNF-expressing human AML. J. Exp. Med. 211, 1093–1108.

Wander, S.A., Levis, M.J., Fathi, A.T., 2014. The evolving role of FLT3 inhibitors in acute myeloid leukemia: quizartinib and beyond. Ther. Adv. Hematol. 5, 65–77.

Wang, C., Deng, L., Hong, M., Akkaraju, G.R., Inoue, J., Chen, Z.J., 2001. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 412, 346–351.

Xiao, G., Fu, J., 2011. NF-kappaB and cancer: a paradigm of Yin-Yang. Am. J. Cancer Res. 1, 192–221.

Xiao, Y., Li, H., Zhang, J., Volk, A., Zhang, S., Wei, W., Zhang, S., Breslin, P., Zhang, J., 2011. TNF-alpha/Fas-RIP-1-induced cell death signaling separates murine hematopoietic stem cells/progenitors into 2 distinct populations. Blood 118, 6057–6067.

Yan, J., Xiang, J., Lin, Y., Ma, J., Zhang, J., Zhang, H., Sun, J., Danial, N.N., Liu, J., Lin, A., 2013. Inactivation of BAD by IKK inhibits TNFalpha-induced apoptosis independently of NF-kappaB activation. Cell 152, 304–315.

Yang, Z., Yik, J.H., Chen, R., He, N., Jang, M.K., Ozato, K., Zhou, Q., 2005. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol. Cell 19, 535–545.

Yu, Y., Ge, N., Xie, M., Sun, W., Burlingame, S., Pass, A.K., Nuchtern, J.G., Zhang, D., Fu, S., Schneider, M.D., Fan, J., Yang, J., 2008. Phosphorylation of Thr-178 and Thr-184 in the TAK1 T-loop is required for interleukin (IL)-1-mediated optimal NFkappaB and AP-1 activation as well as IL-6 gene expression. J. Biol. Chem. 283, 24497–24505.

Zarrinkar, P.P., Gunawardane, R.N., Cramer, M.D., Gardner, M.F., Brigham, D., Belli, B., Karaman, M.W., Pratz, K.W., Pallares, G., Chao, Q., Sprankle, K.G., Patel, H.K., Levis, M., Armstrong, R.C., James, J., Bhagwat, S.S., 2009. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood 114, 2984–2992.

Zhang, Y., Chen, A., Yan, X.M., Huang, G., 2012a. Disordered epigenetic regulation in MLL-related leukemia. Int. J. Hematol. 96, 428–437.

Zhang, B., Ho, Y.W., Huang, Q., Maeda, T., Lin, A., Lee, S.U., Hair, A., Holyoake, T.L., Huettner, C., Bhatia, R., 2012b. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. Cancer Cell 21, 577–592.

Zhao, C., Xiu, Y., Ashton, J., Xing, L., Morita, Y., Jordan, C.T., Boyce, B.F., 2012. Noncanonical NF-kappaB signaling regulates hematopoietic stem cell self-renewal and microenvironment interactions. Stem Cells 30, 709–718.

Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., Ghosh, S., 1997. The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. Cell 89, 413–424.

Zou, Z., Huang, B., Wu, X., Zhang, H., Qi, J., Bradner, J., Nair, S., Chen, L.F., 2014. Brd4 maintains constitutively active NF-kappaB in cancer cells by binding to acetylated RelA. Oncogene 33, 2395–2404.

Zuber, J., Shi, J., Wang, E., Rappaport, A.R., Herrmann, H., Sison, E.A., Magoon, D., Qi, J., Blatt, K., Wunderlich, M., Taylor, M.J., Johns, C., Chicas, A., Mulloy, J.C., Kogan, S.C., Brown, P., Valent, P., Bradner, J.E., Lowe, S.W., Vakoc, C.R., 2011. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 478, 524–528.

de Jonge, H.J., Woolthuis, C.M., Vos, A.Z., Mulder, A., van den Berg, E., Kluin, P.M., van der Weide, K., de Bont, E.S., Huls, G., Vellenga, E., Schuringa, J.J., 2011. Gene expression profiling in the leukemic stem cell-enriched CD34+ fraction identifies target genes that predict prognosis in normal karyotype AML. Leukemia 25, 1825–1833.