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Review

# Reassessing the Potential of Myb-targeted Anti-cancer Therapy

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## Abstract

Transcription factor MYB is essential for the tumorigenesis of multiple cancers, especially leukemia, breast cancer, colon cancer, adenoid cystic carcinoma and brain cancer. Thus, MYB has been regarded as an attractive target for tumor therapy. However, pioneer studies of antisense oligodeoxynucleotides against *MYB*, which were launched three decades ago in leukemia therapy, were discontinued because of their unsatisfactory clinical outcomes. In recent years, the roles of MYB in tumor transformation have become increasingly clear. Moreover, the regulatory mechanisms of MYB, such as the vital effects of MYB co-regulators on MYB activity and of transcriptional elongation on MYB expression, have been unveiled. These observations have underpinned novel approaches in inhibiting MYB. This review discusses the structure, function and regulation of MYB, focusing on recent insights into MYB-associated oncogenesis and how MYB-targeted therapeutics can be explored. Additionally, the main MYB-targeted therapies, including novel genetic therapy, RNA interference, microRNAs and low-molecular-weight compounds, which are especially promising inhibitors that target MYB co-regulators and transcriptional elongation, are described, and their prospects are assessed.

Key words: MYB, MYB inhibitor, therapeutic strategy, targeted therapy, anti-cancer therapy

## Introduction

Cellular MYB (c-Myb) is the homologue of the viral MYB (v-Myb), which is expressed by the avian myeloblastosis virus (AMV) and avian leukemia virus E26, respectively. Aberrant expression of MYB was observed in myeloid and erythroid leukemias [1]. Subsequently, it was identified as a transcription factor that was critical for cell proliferation and differentiation [2]. MYB belongs to a family of transcription factors that comprises two highly homologous proteins, MYBL1/A-Myb and MYBL2/B-Myb that play critical roles in various mammalian tissues [3]. This review will focus on MYB/c-Myb.

Apart from hematopoietic malignancies, aberrant expression of MYB is also associated with many malignant solid tumors [4]. MYB is required at relatively high levels for tumor survival. Therefore, MYB seems to be a target for anti-cancer therapeutics with a strategy to differentiate the excessively proliferating tumor cells [5].

MYB-targeted therapeutics for the treatment of leukemia has been intensely investigated for three decades [6]. However, the use of MYB-targeted therapeutics has been hindered by problems associated with the delivery, efficacy and toxicity of RNA interference approach [7]. Moreover, transcription factors have been considered traditionally almost impossible for targeted therapy. Recent years, new emerging technological advances on targeting of transcription factors with small molecules and new insights regarding the MYB itself have led to exploring novel MYB-targeted

therapeutics [7]. It is hopeful that MYB-targeted therapy shed new light on anti-cancer therapeutics.

## Structure and Function of Myb

The *MYB* gene (Figure 1A) is located on chromosome 6q23.3 and encodes a 75-kDa transcription factor with an N-terminal DNA-binding domain (DBD), a central transactivation domain (TAD) and a C-terminal negative regulatory domain (NRD) [8] (Figure 1B). The TAD is required for the activation of its target genes [5, 7]. The v-Myb protein is truncated at both N- and C- termini. It induces leukemia by transforming immature hematopoietic cells [9]. Truncations at either N- or C-termini of the MYB gene are sufficient to induce transformation [10].

Highly conserved DBD includes three tandem repeats (R1, R2, and R3), the first of which is deleted in the AMV and E26 oncoproteins [4] (Figure 1B and Figure 1C). R1 stabilizes the MYB-DNA complex. R2 and R3 form the core DNA binding domain [11]. Apart from DNA binding, DBD is involved in protein-protein interactions and activation of the target genes, which was identified by studying the activation of the first known MYB-regulated gene, *mim-1*. *Mim-1* was activated by MYB, AMV and E26 proteins by binding to its promoter in reporter gene assays. However, AMV failed to activate the endogenous *mim-1* gene embedded in cellular chromatin; while MYB and E26 proteins activated the endogenous gene. The critical reason is that three amino acid changes only in DBD of AMV (Figure 1C) disrupt its interaction with C/EBPbeta protein [12].

The C-terminal NRD suppresses the transforming activity of MYB [13]. NRD contains EVES motif, which mediates inter- and intra-molecular regulation of MYB [14] (Figure 1B). The N- and C- termini of the MYB protein interact with each other through EVES motif, resulting in transcriptional inactivation. Interestingly, a competing EVES motif is present in p100, which is a ubiquitously expressed transcriptional co-activator in diverse species. MYB adopts an open configuration by interacting with p100, which enables its interaction with additional co-activators [6]. In case of v-Myb, the auto-regulation is absent because of the C-terminal truncation resulting in absence of the EVES motif (Figure 1C).

### Interactions between Myb and its Co-regulators

As shown in Figure 1B, MYB transcription factor, interacts with various co-regulators including C/EBPbeta and p100 [7, 13]. Protein-protein interactions determine the specificity of MYB as well as its ability to induce proliferation or differentiation [9]. For example, as mentioned above, MYB and C/EBPbeta interaction is necessary to activate MYB target genes [12], and the interaction between MYB and p100 regulates MYB activity [6].

CREB-binding protein (CBP) and p300 are also co-activators of MYB, which bind to the MYB TAD and acetylate MYB (Figure 1B), subsequently altering the protein-protein interactions and specificity of MYB [13]. In mice harboring the *MYB* M303V mutation, the disruption of MYB and p300 interaction leads to increased number of hematopoietic stem cells and blocks the development of blood cells [15]. This suggests that MYB and p300 interaction is critical for the normal proliferation and differentiation of hematopoietic stem and progenitor cells.

C-Ski, N-CoR, and mSin3A are 3 co-repressors that bind to the MYB DBD and form a complex with TIF1beta (Figure 1B). TIF1beta binds to the C-terminus and recruits the histone deacetylase complex to MYB, thereby negatively regulating MYB activity. Mutations or deletions in the NRD or DBD decrease the interaction of MYB with its co-repressors and increase its oncogenic transactivation [2]. The c-Ski co-repressor protein competes with CBP for binding to MYB [9]. These studies indicate that interaction with co-activators or co-repressors is critical for MYB-dependent transcription.

Moreover, NRD contains numerous sites for post-translational modifications like acetylation, phosphorylation, sumoylation and ubiquitinylation [13] (Figure 1B). For example, it contains the binding site of Pin1 (Figure 1B), an isomerase that binds to phosphorylated Ser/Thr-Pro motif and upregulates MYB activity [16]. Wnt-1 also induces phosphorylation of MYB at multiple NRD sites, which is followed by ubiquitination and proteasome-dependent degradation of MYB [9]. Therefore, co-regulators and post-translational modifications regulate the activity or stability of MYB. The oncogenic v-Myb protein lacks phosphorylation and ubiquitinylation sites resulting in resistance to Wnt-1 induced degradation. This partially explains the transformational capacity of v-Myb.

A substantial number of MYB target genes are involved in diverse cellular functions like proliferation, differentiation, cell cycle, apoptosis, cell signaling, angiogenesis and cell adhesion [2]. These include cyclin genes (CCNA1, CCNE1, CCNB1), proto-oncogenes (MYC and KIT) and survival gene (BCL-2), which are also critical for tumorigenesis [9]. Other MYB target genes linked to tumorigenesis include COX-2, BCL-$X_L$, MIM1, CD4, CCNB1, HSPA5, HSP70 and GATA3 [2].

### Regulation of Myb Expression by MicroRNAs

The 3'untranslated region (3'UTR) of *MYB* contains binding sites for several miRNAs that regulate the turnover and translation of MYB mRNA [17]. For example, miR-150 binds to the MYB 3'-UTR and down-regulates MYB expression in a stage-specific manner in B-cell development as well as differentiation of other hematopoietic lineages [18]. Granulocytes from patients with primary myelofibrosis (PMF) demonstrate low miR-150 levels associated with MYB overexpression [19]. Other tumor suppressor miRNAs associated with MYB include miRNA-193b-3p and miRNA-103a [20, 21].

## Role of Myb in Normal Cells

*MYB* expression is high in hematopoietic progenitor cells and decreases during differentiation [2]. Antisense knockdown of *MYB* expression in human bone marrow cells decreases the size of colonies and the number of the mononuclear cells [22]. Homozygous *MYB* mutant mice die by embryonic day 15 due to hematopoietic failure [23]. Studies with conditional MYB knockout mice show that precise expression of MYB is necessary for T- and B- cell development, myelopoiesis, erythropoiesis and HSC self-renewal [7]. Therefore, MYB is essential for maintaining the proliferative state of hematopoietic progenitor cells as well as distinct differentiation steps in most hematopoietic cell lineages.

*MYB* expression has also been identified to be critical for the normal differentiation and progenitor cell homeostasis of colonic crypts and neurogenic region of the adult brain [2, 24, 25]. Furthermore, recent conditional knockout studies showed evidence that MYB is globally expressed in many tissues and cells and is required for their normal development [4].

## Role of Myb in Leukemias and Solid Tumors

Elevated MYB expression has been reported in many cases of leukemias, colon and breast cancers [7, 26-29]. Recurrent translocations, duplications and C-terminal deletions have also been reported in leukemia, colon cancer, breast cancer, adenoid cystic carcinoma and brain cancer [2, 9, 30-34] (Figure 1C and Table 1).

Moreover, high MYB expression has been proved to block differentiation and promote proliferation in most human leukemias and brain cancer [7, 35], and to be required for the maintenance of proliferation in primary human leukemia cells [36]. Furthermore, recently, Liu *et al* demonstrated the tumorigenic potential of MYB in zebrafish model [37]. In their study, transgenic zebrafish with hyperactive MYB ($MYB^{hyper}$) exhibited abnormal granulocyte expansion resembling human myelodysplastic syndrome (MDS). And few $MYB^{hyper}$ adult fish developed acute myeloid or lymphoid- and leukemia-like disorders with age. Moreover, the MYB target drug, Flavopiridol, relieved MDS-like symptoms in both $MYB^{hyper}$ embryos and adult fish.

It has been suggested that tissues with essential roles for MYB in development and/or homeostasis like bone marrow, colon, brain and mammary gland are also susceptible to MYB-dependent oncogenesis [2]. Additionally, alternative splicing of human *MYB* transcripts produces multiple forms of the MYB protein that have the same DBD, but different C-termini (Figure 1C). This results in differential MYB activities that correlate with cancer prognosis [38]. For example, MYB9A expression correlates with poor survival for leukemia patients [35].

**Table 1**. Human malignancies associated with MYB

| Study | Tumor entity | Abnormality |
|---|---|---|
| Ferrari *et al* 1985 | AML and ALL | MYB overexpression |
| Barletta *et al* 1987 | Leukemia and lymphoma carrying 6q- deletions | MYB overexpression |
| Tomita *et al* 1998 | TK-6 cell line (CML in T cell blast crisis) | MYB truncation |
| Clappier *et al* 2007 | T-cell acute leukemia | Recurrent chromosomal translocation and genomic duplication of *MYB* locus |
| Murati *et al* 2009 | Acute myelomonocytic leukemia | Genomic gain of the *MYB* locus |
| Quelen *et al* 2011 | Acute basophilic leukemia | Recurrent translocation involving *MYB* |
| Nakano *et al* 2016 | Adult T-cell Leukemia | MYB (unbalanced MYB-9A) overexpression |
| Alitalo *et al* 1984 | Colon cancer | MYB overexpression; amplified *MYB* oncogene |
| Thompson *et al* 1997 | Colon cancer | MYB overexpression; microsatellite deletions in MYB transcriptional attenuator region |
| Hugo *et al* 2006 | Colon cancer | MYB overexpression; mutations in *MYB* intron 1 regulatory sequence |
| Guérin *et al* 1990 | Breast cancer | MYB expression associated with oestrogen-receptor expression |
| Kauraniemi *et al* 2000 | Hereditary breast cancer | MYB overexpression; amplified *MYB* oncogene |
| Persson *et al* 2009 | Adenoid cystic carcinoma | Recurrent translocation involving *MYB* |
| Drier *et al* 2016 | Adenoid cystic carcinoma | Recurrent translocation involving *MYB* |
| Zhang *et al* 2013 | Brain tumor | Recurrent translocation involving *MYB* |
| Ramkissoon *et al* 2017 | Brain tumor | Recurrent translocation involving *MYB* |

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia

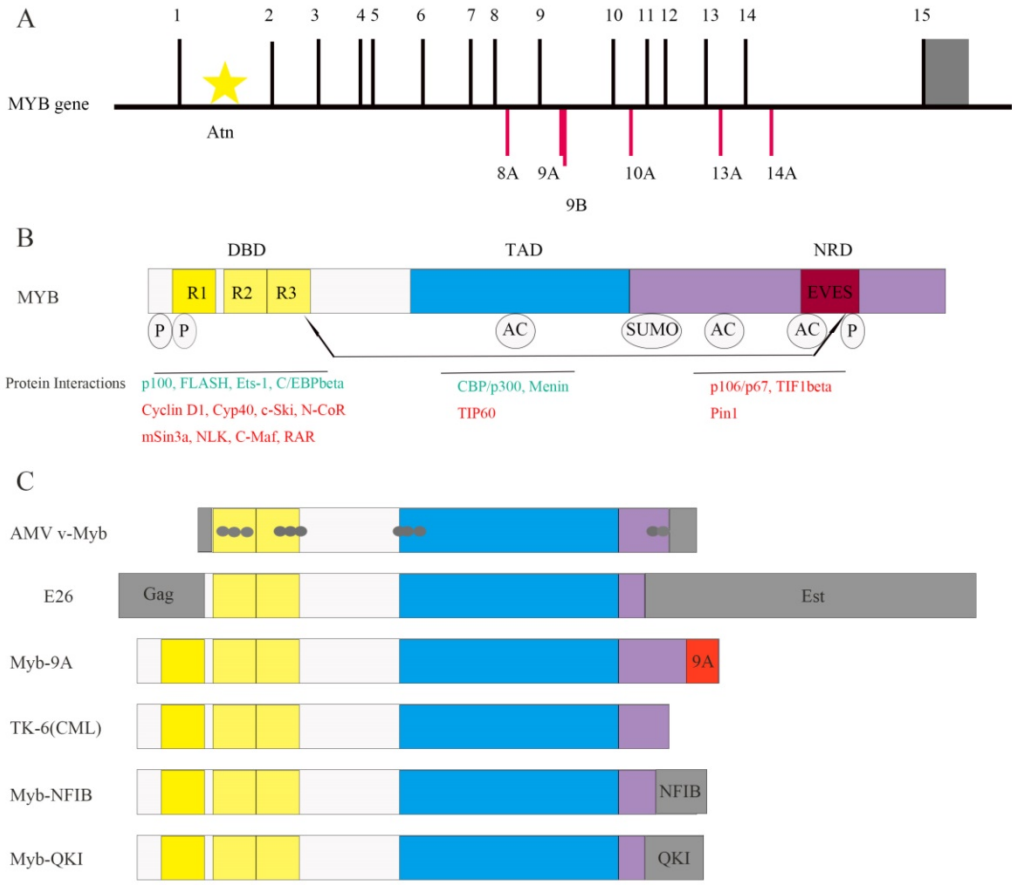


**Figure 1**. Structure of MYB and its co-regulators. (A) The *MYB* proto-oncogene consists of 15 normal exons and many alternative spliced exons such as 8A, 9A, 9B, 10A, 13A and 14A. The 3' untranslated region is shown in gray. Transcription is initiated from multiple start sites and is regulated by the attenuator sequence (Atn) within intron 1.(B) The normal MYB protein contains the DNA-binding domain (DBD), transactivation domain (TAD) and negative regulatory domain (NRD). The post-translational modifications such as phosphorylation (P), acetylation (AC) and sumoylation (SUMO) as well as the EVES peptide sequence that is involved in intra- and intermolecular protein–protein interactions are also shown. The MYB co-activators are listed in green and the co-repressors are listed in red. Arrows indicate potential intramolecular interactions. (C) Schematic representation of MYB variants expressed in leukemia and solid tumors. Both AMV v-Myb and E26 proteins are truncated at both the N- and C-termini of c-MYB. The AMV v-Myb protein contains 6 amino acids derived from the retroviral Gag protein fused to amino acids 72-442 of MYB followed by 13 novel amino acids at the C-terminus (shaded gray). It also has 11 point mutations that cause amino acid changes (gray dots). The E26 protein is a Gag-MYB-Ets fusion protein with 272 amino acids of the retroviral Gag protein fused to MYB, which in turn is fused to 491 amino acids from Ets-1transcription factor at the C-terminus. Aberrant expression of MYB-9A in adult T-cell leukemia correlates with poor outcomes. C-terminal truncated protein has also been identified inTK-6, a chronic myeloid leukemia (CML) cell line. The fusion of *MYB* and *NFIB* genes (translocation 6; 9) generates a MYB-NFIB fusion protein in adenoid cystic carcinoma (ACC) that resembles truncated MYB protein. In brain tumors, *MYB-QKI* fusion gene generates similar MYB fusion protein lacking C-terminal domains.

## Co-operation between Myb and Other Oncogenes

MYB is an essential downstream effector for MLL-ENL and homeobox-mediated transformation in MLL-associated leukemia [39, 40]. In a mouse model of human MLL-ENL leukemia, short-term siRNA suppression of *MYB* effectively inhibited leukemiagenesis by MLL-ENL [39]. Moreover, in mouse model of MLL-AF9 related acute myeloid leukemia (AML), *MYB* knockdown decreased the number of leukemia stem cells (LSCs) [41].

MYB is also a pivotal regulator of oncogenic super-enhancers, which are large regulatory elements that drive the expression of critical oncogenes. In T-cell acute lymphoblastic leukemias, MYB recruits histone H3 lysine 27 (H3K27) acetylase-binding partners like CBP, RUNX1 and GATA-3, which act as a super-enhancer driving the expression of the *TAL1* oncogene [42]. In MLL-associated leukemogenesis, MYB was also identified to bind upstream of MLL through menin and contribute to MLL-mediated methylation of histone H3 at lysine 4 (H3K4) [43].

MYB is also involved in leukemogenesis triggered by other oncogenes like *E2A-HLF*, *BCR-ABL*, *AML1-ETO* and *Setbp1* missense mutants [7, 40]. These oncogenes aberrantly modulate the MYB-related transcriptional program resulting in leukemiogenesis. Therefore, MYB is indispensable for the initiation and/or maintenance of leukemia [7] and is an attractive target for leukemic therapy.

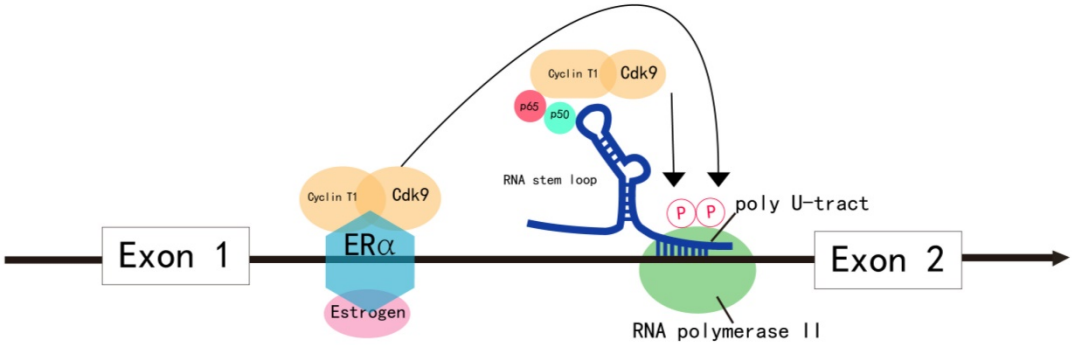


**Figure 2**. Model for the regulation of *MYB* transcriptional elongation. *MYB* transcriptional elongation is regulated by sequences within intron 1, which encode a RNA stem loop and a poly U tract of 19 uridines that stall RNA polymerase II (RNA Pol II). RNA Pol II overcomes transcription blockade through the attenuation region to express full-length *MYB* transcripts in normal cells. In colon cancer, the sequences encoding the stem loop or poly U tract are mutated, which disrupts transcriptional attenuation. In ER+ breast cancer, estrogen receptor α (ERα) and the PTEFB complex are recruited to the transcriptional attenuation region upon estrogen stimulation. Transcriptional block is relieved by the phosphorylation of the Ser-2 residue of RNA Pol II by the CDK9 subunit of PTEFB. In human leukemia cells, *MYB* transcriptional elongation is facilitated by the binding of the NFκB p50-p65 heterodimer to the stem loop, which mediates the recruitment of PTEFB and subsequent Ser-2 phosphorylation of RNA Pol II by CDK9.

### Elongation Control Regulates *Myb* Transcription

In most malignancies, MYB overexpression is due to increased transcriptional rate. *MYB* transcription is predominantly regulated by elongation control sequences within intron 1 that generate a RNA stem loop and a poly U tract, which stalls RNA polymerase II (RNA Pol II). RNA Pol II overcomes this transcription blockade by recruiting positive elongation transcription factor B (PTEFB) to the transcriptional attenuation region or due to mutations in the elongation control sequences of intron 1 (Figure 2). This results in increased transcription of MYB in tumors [2].

In colon cancer, *MYB* expression is regulated by controlling transcriptional elongation by the attenuator sequences within intron 1, which are mutated in many instances [27] (Figure 2). The mutations facilitate transcriptional elongation and increase read-through, thereby increasing *MYB* expression in cancer cells [2].

In estrogen receptor positive (ER+) breast cancer, B lymphoma and erythroleukemia cells, the activity of PTEFB is essential within the transcription attenuation region of the *MYB* gene to resume transcriptional elongation [44-47]. In ER+ breast cancer cells, ER recruits PTEFB when bound to estrogen [44] (Figure 2). The ER/PTEFB complex interacts with the *MYB* gene, phosphorylates RNA Pol II and relieves the attenuation of elongation. In leukemia cells, PTEFB is recruited when the NFκB heterodimer binds to the stem loop resulting in subsequent phosphorylation of RNA Pol II, which facilitates *MYB* transcription [45] (Figure 2). Therefore, suppressing PTEFB activity is a potential strategy to inhibit *MYB* expression in malignancies [48].

## Myb as a Target for Anti-cancer Therapy

Several approaches have been attempted to inhibit aberrant MYB expression in cancer cells. These strategies include disrupting co-regulator interactions, promoting *MYB* transcription attenuation, targeting MYB upstream or downstream effectors, cancer vaccines, antisense oligodeoxynucleotides (short-hairpin RNA), overexpressing miRNAs that target *MYB* directly and low-molecular-weight compounds.

### Antisense ODNs and Short Hairpin RNAs

Three decades ago, Gewirtz *et al* used antisense ODNs or shRNAs against *MYB* to effectively suppress growth and proliferation of primary patient derived leukemia cell lines in vitro [6]. And leukemia cells were found to be more sensitive to *MYB* inhibition than normal hematopoietic cells [49]. The MYB-specific antisense ODNs were also identified to be effective in a K562 leukemia model in SCID mice [50]. However, MYB-specific antisense ODNs showed limited efficacy in the pilot study of chronic myelogenous leukemia patients [51]. On the other hand, the antisense RNA and *MYB* ODNs have been proved to have the capacity to strongly inhibit proliferation of human colorectal cancer cells and transformed neuroectodermal cell lines in vitro. Moreover, the antisense *MYB* ODNs was shown to potentiate the anti-proliferative effects of conventional chemotherapeutic drugs such as taxol, 5-fluorouracil, vinblastine and doxorubicin [52, 53].

Over the last decade, many studies have attempted RNA interference (RNAi) to suppress *MYB*. In a mouse model of MLL-AF9 leukemia, the *MYB* specific shRNAs silenced *MYB* effectively and achieved complete remission and eradication of the aggressive leukemia without preventing normal

myelopoiesis [41]. Furthermore, in mouse model of pre-B-cell acute lymphoblastic leukemia, the *MYB* shRNA decreased proliferation of leukemia cells and increased their sensitivity to cytotoxic agents, thereby significantly delaying disease onset [54].

Many studies explored combining *MYB* antisense ODNs with other targeted therapies. For example, *MYB* antisense ODNs synergized with GD2-specific antibodies achieved long term survival in murine xenograft models of neuroblastoma [55]. The liposomes were coated with the anti-GD2 antibodies (targeted liposomes) and *MYB* antisense ODNs. The GD2-targeted liposomes successfully delivered the ODNs to the target cells and enhanced their toxicity without affecting the normal cells. Additionally, the CpG motifs stimulated the immune cells. Therefore, *MYB* RNAi can effectively inhibit the proliferation of leukemia and other tumor cells and cooperate with other cytotoxic agents or targeted therapies. But, *MYB* RNAi still requires improvements in effective delivery, stability and toxicity for implementation in clinical applications [7].

## *Myb* DNA Vaccine

Overexpression of *MYB* occurs in 80% of colorectal cancer (CRC) and is associated with aggressive disease and poor prognosis [56]. Ramsay *et al* tested *MYB* DNA vaccine in a mouse model of colorectal cancer. The *MYB* DNA vaccine suppressed colorectal tumor growth by inducing the T cell-mediated immunity and increasing the infiltration of immune effector cells at the tumor site [56]. Moreover, in the CRC mice model, the *MYB* DNA vaccine in combination with anti-PD-1 antibody or low dose cyclophosphamide effectively killed tumor cells [57]. Pre-clinical and clinical studies are needed to explore the utility of *MYB* DNA vaccine in clinical applications.

## Inhibition of Myb-coregulator Interactions

P300 has been investigated as a therapeutic target because it is an important co-activator of MYB [7]. In the study on Booreana mice carry a mutant allele of *MYB,* which disrupts its interaction with p300, irradiated recipient mice transplanted with Booreana hematopoietic cells transduced with *AML1-ETO9a* or *MLL-AF9* retroviruses did not develop leukemia [58]. This indicates that disruption of the MYB and p300 interaction is a potential therapeutic strategy in malignancies relying on MYB [59].

The therapeutic efficacy of many small-molecule inhibitors of the MYB/p300 interaction have been investigated [60]. Among these studies, Celastrol, a potent low molecular weight inhibitor, was shown to suppress the proliferative potential of AML cells without affecting normal hematopoietic progenitor cells. Moreover, Celastrol enhanced survival of the mice model of HoxA9/Meis1-driven AML [61].

Also, MYB is recruited to the MLL histone methyl transferase complex through its interaction with menin, and contributes to MLL-associated leukemogenesis [43]. Therefore, the interaction of MYB with menin has been explored as an anti-cancer strategy. Grembecka *et al* demonstrated that several small-molecule inhibitors of the menin-MLL complex blocked transformation by MLL fusion proteins in bone marrow cells (BMC) and relieved the differentiation block in MLL-associated human leukemia cells [62].

## Therapeutic Targeting of *Myb* Elongation

Since PTEFB activity correlates with elevated *MYB* expression, suppressing PTEFB activity is an attractive strategy to inhibit aberrant *MYB* expression [45, 48]. Flavopiridol is an inhibitor of the Cdk9 subunit of PTEFB, which suppresses the expression of *MYB* and its target genes like *BCL-2* and *CCNB1* in ER$^+$ breast cancer cells. Moreover, Flavopiridol is at least 10 times more effective in eliminating ER$^+$ breast cancer cell lines than ER$^-$MYB$^-$ cells. The sensitivity of ER$^+$ breast cancer cells could be reversed by ectopic *MYB* expression. This demonstrated that Flavopiridol is a potential therapeutic agent for ER$^+$ breast cancers and other MYB-dependent cancers [63]. In MYB$^{hyper}$ zebrafish studies, Flavopiridol relieved MDS-like symptoms in both MYB$^{hyper}$ embryos and adult fish [37]. However, clinical trials of Flavopiridol in solid tumors were discontinued because of adverse events and low efficacy [64]. Meanwhile, Flavopiridol shows clinical activity in several hematological malignancies such as chronic lymphocytic leukemia, but, the relevance between MYB suppression and clinical activity is ambiguous [65]. Therefore, new, specific and more effective inhibitors of MYB elongation need to be developed.

## Myb Specific MicroRNAs

Several MYB-targeting miRNAs demonstrate potential as anti-cancer therapeutics. Restoring miR-150 expression in EBV-positive Burkitt lymphoma cell lines decreased MYB levels, which reduced proliferation and induced differentiation in the lymphoma cells. Moreover, MYB knockdown showed similar characteristics to overexpressing miR-150 in the lymphoma cell lines [66]. This showed therapeutic potential of miR-150 and other MYB targeting miRNAs in future anti-cancer therapy.

## Other Approaches for Targeting Myb

Drugs with multiple targets are more effective

than specific target drugs in the treatment of complex diseases such as cancer. The bromodomain and extra-terminal (BET) protein BRD4 has been identified to regulate the expression and activity of MYB in several modes. BRD4 regulates MYB expression through recruiting PTEFB, which promotes *MYB* elongation [67]. It also regulates downstream effectors of MYB such as MYC, and MYB co-regulators including p300/CBP [68, 69]. Hence, BRD4 inhibition is potentially an effective strategy for suppressing MYB. In fact, BRD4 inhibition is being tested as a therapy for acute leukemia and breast cancer with high-level *MYB* expression [70-72].

Additionally, since the positive feedback loop of MYB and Hoxa/Meis is critical for regulating leukemogenesis, Hox is a potential therapeutic target [43]. Also, regulators of MYB degradation like the Wnt-1 signaling pathway have shown therapeutic potential.

Klempnauer *et al* reported several sesquiterpene lactones that suppressed MYB-dependent gene expression specifically [73]. In their studies, mexicanin-I suppressed the proliferation of all *MYB*-expressing tumor cell lines and inhibited the colony growth of blasts from multiple AML patients; while tumor cell lines without *MYB* expression are less sensitive to mexicanin-I [74]. Further investigations are in progress to evaluate the clinical efficacy of these novel low-molecular-weight MYB inhibitors.

More recently, the widely used mebendazole was identified to induce c-MYB degradation via the proteasome by interfering with the heat shock protein 70 (HSP70) chaperone system, which is sufficient to inhibit colony formation by AML cells, but not normal cord blood-derived cells. Moreover, mebendazole is effective at impairing AML progression in mouse experiments [75]. It seems that mebendazole is also a safe and novel therapeutic approach for AML. Further research is ongoing.

## Conclusions

The MYB transcription factor is a suitable target for tumor therapy because its aberrant expression is pivotal for the growth and progression of multiple solid tumors and leukemias. While MYB-targeted antisense ODNs have not been satisfactory in clinical studies, RNAi and miRNAs targeting MYB have shown great promise in preliminary studies. Moreover, better understanding of the regulation of the activity and expression of MYB and efficient inhibitor screens have opened up novel therapeutic avenues for low-molecular-weight inhibitors that target MYB co-regulators or its transcriptional elongation. Multiple inhibitors are effective in inhibiting tumors with elevated MYB expression in laboratory studies [59]. Further investigations are in progress to evaluate their efficacy in pre-clinical and clinical studies. The focus of future studies include studying the efficacy of these inhibitors, their toxicity in animal models or preclinical studies, and screening promising inhibitors individually or in combination with conventional chemotherapeutics or other targeted drugs in clinical trials. Since MYB plays a central role in tumorigenesis, new and effective MYB inhibitors will contribute to improving the survival of patients with MYB-related tumors.

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## Competing Interests

The authors have declared that no competing interest exists.

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