Review

Molecular Classification and Overcoming Therapy Resistance for Acute Myeloid Leukemia with Adverse Genetic Factors

Daisuke Ikeda 1,2*, SungGi Chi 1, Satoshi Uchiyama 1, Hirotaka Nakamura 1, Yong-Mei Guo 1, Nobuhiko Yamauchi 1, Junichiro Yuda 1 and Yosuke Minami 1,*

1 Department of Hematology, National Cancer Center Hospital East, Kashiwa, Chiba 277-8577, Japan; ikeda.daisuke@kameda.jp (D.I.); schi@east.ncc.go.jp (S.C.); suchiyama@east.ncc.go.jp (S.U.); hirotak@east.ncc.go.jp (H.N.); ekaku@east.ncc.go.jp (Y.-M.G.); noyamauc@east.ncc.go.jp (N.Y.);
yuda@east.ncc.go.jp (J.Y.)
2 Department of Hematology, Kameda Medical Center, Kamogawa 296-8602, Japan
* Correspondence: yominami@east.ncc.go.jp; Tel.: +81-4-7133-1111; Fax: +81-7133-6502

Abstract: The European LeukemiaNet (ELN) criteria define the adverse genetic factors of acute myeloid leukemia (AML). AML with adverse genetic factors uniformly shows resistance to standard chemotherapy and is associated with poor prognosis. Here, we focus on the biological background and real-world etiology of these adverse genetic factors and then describe a strategy to overcome the clinical disadvantages in terms of targeting pivotal molecular mechanisms. Different adverse genetic factors often rely on common pathways. KMT2A rearrangement, DEK-NUP214 fusion, and NPM1 mutation are associated with the upregulation of HOX genes. The dominant tyrosine kinase activity of the mutant FLT3 or BCR-ABL1 fusion proteins is transduced by the AKT-mTOR, MAPK-ERK, and STAT5 pathways. Concurrent mutations of ASXL1 and RUNXI are associated with activated AKT. Both TP53 mutation and mis-expressed MECOM are related to impaired apoptosis. Clinical data suggest that adverse genetic factors can be found in at least one in eight AML patients and appear to accumulate in relapsed/refractory cases. TP53 mutation is associated with particularly poor prognosis. Molecular-targeted therapies focusing on specific genomic abnormalities, such as FLT3, KMT2A, and TP53, have been developed and have demonstrated promising results.

Keywords: ELN classification; AML; FLT3-ITD with wild-type NPM1; DEK-NUP214 fusion; KMT2A rearrangement; BCR-ABL1 fusion; haploinsufficiency of GATA2 and mis-expression of MECOM; RUNXI mutation; ASXL1 mutation; TP53 mutation; complex karyotype; menin; anti-CD47 antibody

1. Introduction

Patient-related factors (e.g., age, general condition, and comorbidities) and treatment modality are unarguably essential in predicting the prognosis of acute myeloid leukemia (AML). Nevertheless, tumor-related genetic factors still have a major impact on prognosis [1,2]. The risk classification of AML based on 2017 European LeukemiaNet (ELN) recommendations (Table 1) has been widely adapted in general practice [3]. The original intention of the risk classification was to refine prognostication and improve decision-making for AML patients at diagnosis. The ELN guideline provides a three-group classification (favorable, intermediate, adverse) according to expected overall survival (OS) and relapse rates. Notably, internal tandem duplication (ITD) of the FMS-like tyrosine kinase 3 (FLT3) gene is described in association with nucelophosmin 1 (NPM1) mutation status, with only a high allelic ratio (>0.5) of FLT3-ITD considered a poor prognostic factor. Mutations in the runt-related transcription factor 1 (RUNXI) and additional sex combs such as the 1 (ASXL1) and tumor antigen p53 (TP53) genes were recently added as adverse genetic abnormalities in the 2017 edition. Many of the genetic factors described in this recommendation are closely related to one another. For example, the NPM1 mutation (NPM1mt) is considered to be a favorable factor only if FLT3-ITD is absent or has a low
allelic ratio (<0.5) [4]. Concurrent mutations of ASXL1 and RUNX1 exert poor prognosis, particularly in younger adults [3]. In core-binding factor (CBF) AML, the co-existing KIT proto-oncogene receptor tyrosine kinase (KIT) gene mutation is associated with worse outcome [6]; although, the ELN classification does not include KIT mutation because the negative effect can be negated if a three-log or deeper reduction in minimal residual disease is achieved after induction therapy [7]. Although genetic relationships in AML can be overwhelmingly complex and multifactorial, the ELN classification is a pivotal clinical tool in predicting the outcomes of patients with AML.

Table 1. 2017 ELN risk classification.

| Risk Category | Genetic Abnormality |
|---------------|---------------------|
| Favorable     | t(8;21)(q22;q22.1); RUNX1-RUNXIT1 |
|               | inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 |
|               | Mutated NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> |
|               | Biallelic-mutated CEBPA |
| Intermediate  | Mutated NPM1 and FLT3-ITD<sub>high</sub> |
|               | Wild-type NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> (without adverse-risk genetic lesions) |
|               | t(9;11)(p21.3;q23.3); MLLT3-KMT2A |
|               | Cyogenetic abnormalities not classified as favorable or adverse |
| Adverse       | t(6;9)(p23;q34.1); DEK-NUP214 |
|               | t(8;21)(q22;q22.1); RUNX1-RUNXIT1 |
|               | inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 |
|               | Mutated NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> |
|               | Biallelic-mutated CEBPA |
|               | Mutated NPM1 and FLT3-ITD<sub>high</sub> |
|               | Wild-type NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> (without adverse-risk genetic lesions) |
|               | t(9;11)(p21.3;q23.3); MLLT3-KMT2A |
|               | Cyogenetic abnormalities not classified as favorable or adverse |
|               | t(6;9)(p23;q34.1); DEK-NUP214 |
|               | t(8;21)(q22;q22.1); RUNX1-RUNXIT1 |
|               | inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 |
|               | Mutated NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> |
|               | Biallelic-mutated CEBPA |
|               | Mutated NPM1 and FLT3-ITD<sub>high</sub> |
|               | Wild-type NPM1 without FLT3-ITD or with FLT3-ITD<sub>low</sub> (without adverse-risk genetic lesions) |
|               | t(9;11)(p21.3;q23.3); MLLT3-KMT2A |
|               | Cyogenetic abnormalities not classified as favorable or adverse |

AML with adverse genetic factors, as defined in the ELN classification, uniformly shows resistance to standard chemotherapy and is associated with poor prognosis. Here, we focus on the biological background and real-world etiology of the adverse genetic factors and then describe a strategy to overcome the clinical disadvantages in terms of targeting pivotal molecular mechanisms. In a comprehensive survey of the available preclinical literature, we searched the literature using the key words “ELN”, “FLT3”, “NPM1”, “DEK-NUP214”, “KMT2A”, “BCR-ABL1”, “GATA2”, “MECOM”, “EVI1”, “RUNX1”, “ASXL1”, “TP53”, “complex karyotype”, “menin”, and “anti-cd47 antibody” with the necessary key word “AML”. A systematic literature search was performed using PubMed and the Tip Medical Database, identifying a total of 1839 records including 92 controlled trials, 90 systematic reviews, and 1228 ongoing clinical trials. We chose 169 records according to the theme of this review article.

2. Biology of Adverse Genetic Abnormalities
2.1. DEK-NUP214 Fusion

Normal AML with the fusion of the DEK proto-oncogene (DEK) with the nucleoporin 214 (NUP214) gene or t(6;9)(p23;q34) is a distinct WHO classification category of AML [8] and is associated with poor prognosis [9]. t(6;9)(p23;q34) is a rare chromosomal
abnormality (0.5–4% of AML) and is primarily found in AML with maturation (M2) or acute myelomonocytic leukemia (M4) [10]. DEK protein is a ubiquitous chromatin protein that serves as an architectural protein at promoter or enhancer sites of a subset of human genes [11]. The upregulation of the DEK gene has been clinically noted in many human cancers [12]. The NUP214 protein, also known as CAN, is a component of the nuclear pore complex and plays an important role in the nucleocytoplasmic transport of macromolecules [13,14]. Xenografted mice with human hematopoietic progenitors with t(6;9)(p22;q34) developed AML with a myelomonocytic immunophenotype [15]. In the leukemic cell, the gene expression of the homeobox (HOX) family genes, such as homeobox protein A9 (HOXA9), HOXA10, HOXB3, HOXB4, and PBX homeobox 3 (PBX3), is highly upregulated. Other studies have suggested that DEK-NUP214 is associated with the upregulation of mechanistic targets of rapamycin (mTOR) [16] and the activation of the signal transducer and activator of transcription 5 (STAT5) [17].

2.2. KMT2A (MLL) Rearrangement

The lysine methyltransferase 2A (KMT2A) gene, previously known as mixed lineage leukemia 1 (MLL), is a proto-oncogene that encodes a histone methyltransferase essential in hematopoiesis [18]. A multiprotein complex containing KMT2A mediates the methylation of the fourth lysine residue in histone H3 (H3K4), as well as the acetylation of the sixteenth lysine residue in histone H4 (H4K16) [19], which is required for the upregulation of HOXA9 gene transcription [18]. The KMT2A gene forms oncogenic fusions with a variety of partners, including mixed-lineage leukemia translocated to chromosome 3 (MLLT3), AF4/FMR2 family member 1 (AFF1), and the afadin adherens junction formation factor (AFDN) [20]. Rearrangements of KMT2A are commonly found in AML (approximately 5–10%) and are associated with poor prognosis [21,22]. Recent studies have suggested that oncogenic KMT2A fusion proteins interact with menin and the disruptor of telomeric silencing 1-like (DOT1L), which is required for initiating leukemogenesis [23,24] through the upregulation of specific genes such as HOXA9 and myeloid ecotropic viral insertion site 1 (MEIS1) [25,26]. Menin, a product of the multiple endocrine neoplasia type 1 (MEN1) tumor suppressor gene, is critically involved in leukemogenic mechanisms by linking KMT2A protein with the lens epithelium-derived growth factor (LEDGF) on target genes [27].

2.3. BCR-ABL1 Fusion

ABL proto-oncogene 1 (ABL1) is a non-receptor tyrosine kinase involved in cell growth and survival. Normal ABL is principally located in the nucleus and regulates DNA damage-induced apoptosis [28,29], as well as cytoskeletal remodeling, receptor endocytosis, and autophagy. Several breakpoints have been recognized in pathogenic translocations between chromosome 9q34, the ABL gene location, and chromosome 22q11, the breakpoint cluster region protein (BCR) gene location [30]. The large molecular weight BCR-ABL1-encoded fusion protein (210 kDa; major BCR-ABL1) is associated with chronic myeloid leukemia (CML), while the small molecular weight fusion protein (185–190 kDa; minor BCR-ABL1) is associated with acute lymphoblastic leukemia (ALL). The oncogenic BCR-ABL1 fusion protein is located in the cytoplasm and phosphorylates a variety of proteins [31]. Major BCR-ABL1 is particularly associated with phosphatidylinositol 3-kinase (PI3K), which is required for the BCR-ABL1-dependent proliferation of CML cells [32]. Major BCR-ABL1, but not normal ABL, binds to the adapter molecule CRK-like protein (CRKL) [33], which transduces signals to the oncogene product E3 ubiquitin-protein ligase CBL (CBL) [34]. Major BCR-ABL1 is also related to the constitutive activation of the Janus kinase (JAK)/STAT5 pathway [35] and the indirect activation of RAS proto-oncogene (RAS) proteins [36]. Recent studies have revealed that the BCR-ABL1 fusion protein is a client of the molecular chaperone heat shock protein 90 (HSP90) and the inhibition of HSP90 protein induces the degradation of the pathogenic fusion protein in BCR-ABL1-positive cell lines [37,38].

Normal AML with the BCR-ABL1 fusion gene is a rare entity, accounting for approximately 1% of all AML subtypes [39], and is often challenging to distinguish from the
first presentation of CML in blastic crisis [40,41]. While the majority of CMLs uniformly rely on major BCR-ABL1, both major and minor BCL-ABL1 can be equivalently found in BCR-ABL1-positive AML. A summary of published BCR-ABL1-positive AML cases showed that slightly less than 40% of cases belonged to AML not otherwise specified (AML-NOS) and approximately a quarter of the cases were found to be AML with recurrent genetic aberrations such as CBF-AML and AML with an NPM1 mutation [41]. Notably, when the ELN criteria were applied independently of BCR-ABL1 status, more than three quarters of the BCR-ABL1-positive AML cases were classified as belonging to the non-favorable risk group [41]. Given such unfavorable genetic backgrounds, the prognostic impact of BCR-ABL1 itself has not been confirmed.

2.4. Haploinsufficiency of GATA2 and Mis-Expression of MECOM

The chromosomal translocation and inversion between 3q21 and 3q26 (3q-rearrangement) are well-known recurrent genetic abnormalities in myelodysplastic syndrome (MDS) and AML [42]. The MDS1 and EVII complex locus (MECOM) gene, formerly known as ecotropic virus integration site 1 protein homolog (EVI1), is located in 3q26 and encodes the transcription regulator protein EVII which binds to the promoter region of target genes. EVII protein regulates apoptosis through Jun N-terminal kinase (JNK) and tumor growth factor (TGF)-beta signaling [43,44]. Increased expression of the MECOM gene has been observed in AML with 3q-rearrangement [42]. Katayama and colleagues demonstrated that the inappropriate expression of the MECOM gene in AML with 3q-rearrangement was induced by the recruitment of a GATA2-distal hematopoietic enhancer (G2DHE) to the proximity of the MECOM gene, which subsequently disturbed the function of an allele of the endothelial transcription factor GATA-2 (GATA2) gene [45]. The GATA2 protein was originally identified as a transcription activator that regulates endothelin-1 gene expression in endothelial cells and is also highly expressed in normal hematopoietic progenitor cells [46]. The germ line heterozygous mutation of GATA2 is known to be associated with bone marrow failure, immunodeficiency, lymphedema, and organ dysfunction as well as MDS and AML [47–49]. Considering the report from Dr. Katayama described above, the haploinsufficiency of the GATA2 gene, which is caused by the mis-expression of EVI1 protein, is expected to play a key role in the leukemogenesis of AML with 3q-rearrangement.

3q-rearrangements, such as t(3;3)(q21;q26) or inv(3;3)(q21;q26), are rare cytogenetic abnormalities that are found in approximately 4% of AML [50] and are associated with poor prognosis [50–52]. An analysis of a database of 288 patients with 3q-rearranged AML indicated that the overexpression of EVI1 was observed in 95% of patients with t(3;3)/inv(3;3) and the hazard ratio of death increased by 40% [50]. Interestingly, 3q-rearrangement was frequently associated with monosomy 7 and NRAS mutation.

2.5. Complex Karyotype

In the ELN classification, a complex karyotype is defined as three or more chromosomal abnormalities in the absence of the WHO-designated recurring translocations or inversions, such as t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3), or t(3;3), whereas the UK National Cancer Research Institute Adult Leukaemia Working Group requires four or more chromosomal abnormalities as an adverse risk factor [9]. Complex karyotypes are found in approximately 10% of all AML and are more prevalent (up to 20%) in the elderly [53]. An analysis of a database of 417 AML patients with complex karyotypes showed that three or more chromosomal abnormalities in the absence of strong factors, such as a hyperdiploid karyotype, CBF-AML, and unique adverse-risk aberrations, were associated with reduced OS compared with patients with a normal karyotype [54].

2.6. FLT3-ITD with Wild-Type NPM1

FLT3 is a receptor tyrosine kinase that is expressed in normal hematopoietic progenitor cells and regulates proliferation and survival. FLT3 dimerizes upon binding with FLT3 ligands that are produced by bone marrow stromal cells, which results in
the phosphorylation of the tyrosine residues in the activation loop [55]. Downstream FLT3 signaling involves several pathways. Activated FLT3 promotes the phosphorylation of tyrosine-protein phosphatase non-receptor type 11 (PTPN11), also known as src homology region 2 domain-containing phosphatase 2 (SHP2) and AKT serine/threonine kinase 1 (AKT1) to activate the downstream effector protein mTOR [56,57]. mTOR is a serine/threonine kinase that functions as a part of two distinct signaling complexes—mTOR complex 1 (mTORC1) and 2 (mTORC2)—and regulates the phosphorylation of a wide range of proteins associated with cellular metabolism, growth, and survival [58–60]. FLT3 also phosphorylates the major RAS signaling downstream kinases, namely, mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK) [61]. MAPK and ERK, especially the pairs of MAPK1/ERK2 and MAPK3/ERK1, are involved in an essential component of MAP kinase signaling, regulating a variety of transcription factors (e.g., B-cell lymphoma 6 protein (BCL6) [62]), cytoskeletal elements, and regulators of apoptosis (e.g., myeloid cell leukemia 1 (MCL1) [63]). Although wild-type FLT3 does not significantly affect STAT5 signaling, mutant FLT3 constitutively activates these effector proteins, resulting in the promotion of proliferation and survival [61].

Nucleophosmin 1 (NPM1) protein is involved in a variety of cellular mechanisms including ribosome nuclear export [64], the chaperoning of histones [65], centrosome duplication in concert with breast cancer susceptibility gene 2 (BRCA2) [66], promoting proliferation by antagonizing activating transcription factor 5 (ATF5)-induced G2/M cell cycle blockade [67], the regulation of tumor protein p53 (TP53) and the ADP-ribosylation factor (ARF) [68,69], and the enhancement of myc proto-oncogene (MYC) transcription [70]. Overall, NPM1 functions in supporting cell proliferation and survival. Wild-type NPM1 protein is predominantly located in the nucleus and shuttles between the nucleus and the cytoplasm. However, because NPM1 mutations are concentrated in the nuclear export signal (NES) domain, which enables the export of the protein from the nucleus to the cytoplasm, mutant NPM1 protein inappropriately accumulates in the cytoplasm [71]. Indeed, NPM1 mutation leads to the cytoplasmic localization of several nuclear proteins associated with DNA repair and apoptosis [72], as well as transcription factors such as CCCTC-binding factor (CTCF) and PU.1 [73,74]. The upregulation of HOX genes has been reported in AML with NPM1 mutation [75]. Recent studies have suggested that the interaction between menin and wild-type KMT2A plays a pivotal role in NPM1-mutated AML through upregulating HOXA, HOXB, and MEIS1 [76–78].

FLT3-ITD—a common pattern of activating mutation that leads to the extension of the juxta-membrane domain of FLT3 tyrosine kinase—and NPM1 mutation have opposite prognostic impacts in AML: NPM1 mutation can be a favorable factor only in the absence of FLT3-ITD, and the presence of NPM1 mutation attenuates the adverse prognostic impact of FLT3-ITD [79]. Although the current ELN risk classification does not distinguish wild-type FLT3 from FLT3-ITD with a low allelic ratio (FLT3-ITDlow), some reports have suggested that concurrent NPM1 mutation and FLT3-ITDlow is not related to favorable outcomes [80].

In terms of influence on treatment, a retrospective analysis of the RATIFY trial—a phase 3 trial that demonstrated the efficacy of adding midostaurin to standard chemotherapy in patients with FLT3-mutated AML—indicated that the first-generation FLT3 inhibitor was equally effective for all ELN risk groups regardless of NPM1 mutation status [81].

2.7. RUNX1 Loss-of-Function Mutation

The runt-related transcription factor 1 (RUNX1), previously known as acute myeloid leukemia 1 (AML1) or core-binding factor subunit alpha-2 (CBFA2), protein forms a heterodimeric complex, called CBF, with the core-binding factor B (CBFB) protein. The RUNX1 protein recognizes certain DNA sequence patterns, such as 5′-TGTTGGT-3′, in the target domain and CBFB allosterically enhances RUNX1 function. The CBF complex interacts with promoters and enhancers of a variety of genes and plays an essential role in normal hematopoiesis [82]. A recent study has suggested that RUNX1 gene silencing results in the
upregulation of TP53, which stabilizes the RUNX1 protein via the enhanced expression of the CBFB gene [83].

The pathogenic fusion of RUNX1 with its partner transcriptional co-repressor 1 (RUNXIT1), previously known as eight twenty one (ETO), plays a critical role in the leukemogenesis of CBF-AML via the aberrant transcription factors that contain oncogenic RUNX1-RUNXIT1 or AML1-ETO, fusion proteins [84]. The presence of the RUNX1-RUNXIT1 fusion gene, or t(8;21)(q22;q22.1) translocation, is generally considered a favorable prognostic factor in AML [85]; however, a database analysis revealed that RUNX1 mutation, accounting for 10% of newly diagnosed AML, was independently associated with poor prognosis and co-mutations with epigenetic modifiers (e.g., additional sex combs-like 1 (ASXL1)) and/or spliceosome-related genes (e.g., serine/arginine-rich splicing factor 2 (SRSF2) and plant homeodomain-like finger (PHF6)) predicted notably worse outcomes [86]. A patient-derived xenograft (PDX) mouse model experiment using a human chronic myelomonocytic leukemia (CMML) cell line demonstrated that concurrent mutations of RUNX1 and ASXL1 promoted cell renewal in bone marrow and leukemic transformation, which was induced by the augmented production of hypoxia-inducible factor 1 subunit alpha (HIF1-α) and the consequent upregulation of the inhibitor of DNA binding 1 (ID1) and AKT1 signaling [87]. ID1 is a negative transcriptional regulator and is associated with the immortalization of hematopoietic progenitor cells [88]. On the other hand, a mouse model experiment suggested that Runx1 protein cooperates with Pu.1, and the loss of Runx1 results in the decreased ability of Pu.1 to promote the differentiation of the macrophage lineage [89].

2.8. ASXL1 Loss-of-Function Mutation

ASXL1 protein recognizes adenine N6 methylation in DNA (6 mA) and is a component of the Polycomb repressive deubiquitinase (PR-DUB) complex. PR-DUB removes monoubiquitylation of the 119th lysine residue in histone H2A (H2A-K119Ub) and is degraded following 6 mA formation [90]. H2A-K119Ub is a well-known histone marker related to repressed transcription [91], whereas Polycomb group (PcG) proteins play a critical role in maintaining appropriate gene repression by forming Polycomb repressive complexes (PRC). Contrary to PR-DUB, PRC1 is responsible for the formation of H2A-K119Ub. However, recent studies have suggested that PR-DUB does not simply counteract PRC1, but rather functions in concert with PRC1 [92–94]. A mouse model experiment demonstrated that Asxl1 mutation is accompanied by aberrant PRC1-mediated histone modification, resulting in impaired hematopoiesis similar to low-risk MDS [92]. Overall, the ASXL1 gene acts as a tumor suppressor, at least in terms of hematopoietic regulation.

Nonsense mutation or the frame-shift of the ASXL1 gene, mainly in exon 11 or 12, is found in approximately 10% of AML and is more prevalent in MDS (10–25%) and CMML (40–50%). ASXL1 mutation is known to be mutually exclusive with DNA-methyltransferase 3 alpha (DNMT3A), NPM1, and splicing factor 3b subunit 1 (SF3B1) mutations [95]. In mouse model studies, Asxl1 along with Tet methylcytosine dioxygenase 2 (Tet2) mutation initiated MDS-like hematopoietic impairment [92,96], and concurrent oncogenic mutations such as Kras and Nf1 accelerated the development of myeloid leukemia [97]. ASXL1 mutation is known as an independent adverse prognostic factor of AML [98] and concurrent mutation with RUNX1 is particularly associated with worse prognosis [86].

2.9. TP53 Loss-of-Function Mutation

TP53 protein acts as a major tumor suppressor in many tumor types. TP53 regulates cell division by activating inhibitors of cyclin-dependent kinases (e.g., p21) [99] and induces apoptosis via the stimulation of BCL-2-associated X (BAX) [100] as well as the repression of B-cell/CLL lymphoma 2 (BCL-2) [101]. The pro-apoptotic BAX protein, as well as the BCL-2 homologous antagonist/killer (BAK) protein, is expressed in the outer mitochondrial membrane and is usually deactivated by binding with BCL-2, which also captures cytoplasmic pro-apoptotic BCL-2 homology 3 (BH3)-only proteins. Under apoptotic signaling, free cytoplasmic BH3-only proteins activate the BAX protein which results in mitochondrial...
outer membrane permeabilization (MOMP) and the release of cytochrome c into the cytoplasm, which consequently leads to apoptosis [102,103]. TP53 protein is physiologically neutralized by mouse double minute 2 homolog (MDM2). The tumor suppressor protein p14ARF, an isofrom product of the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene that is upregulated in response to cell proliferation signals, inhibits MDM2 and thereby activates TP53 [104].

TP53 mutation is found in less than 10% of normal AML and 30–40% in secondary AML [105]. TP53 mutation in AML is generally associated with complex karyotypes and universally poor prognosis [106]. TP53-mutated leukemic cells gain enhanced self-renewal capacity and a competitive growth advantage, subsequently accumulating additional mutations, including DNMT3A, TET2, and ASXL1 [107]. Interestingly, TP53-mutated secondary AML and MDS are associated with the increased expression of programmed death ligand 1 (PD-L1), a major immune checkpoint molecule that leads to the anergy of effector T cells on hematopoietic stem cells and decreased numbers of cytotoxic/helper T cells in the bone marrow [108].

The pro-leukemic mechanisms of adverse genetic factors are schematically summarized in Figure 1. The upregulation of HOXA9 is a common phenomenon in AML with KMT2A rearrangement, NPM1 mutation, and DEX-NUP214 fusion. The aberrant tyrosine kinase activity of FLT3-ITD and BCR-ABL1 fusion affects three major signaling pathways: the AKT-mTOR, MAPK-ERK, and STAT5 pathways. Suppressed apoptosis is caused by TP53 mutation and MECOM mis-expression.

![Figure 1. Schematic of pro-leukemic mechanisms of adverse genetic factors. The upregulation of HOX genes, especially HOXA9, plays a key role in AML with DEK-NUP219 fusion, KMT2A rearrangement, or NPM1 mutation. FLT3-ITD and BCR-ABL1 fusion proteins are major tyrosine kinases that promote cell proliferation through the activation of the AKT-mTOR, MAPK-ERK, and STAT5 pathways. The concurrent mutation of ASXL1 and RUNX1 is associated with the upregulation of AKT and HIF1-α. Decreased p53 function and the overexpression of EVI1 lead to impaired apoptotic mechanisms.](image-url)

3. Real-World Etiology of Adverse Genetic Abnormalities

3.1. Ad Hoc Analysis of the Japan Adult Leukemia Study Group (JALSG) AML201 Study

JALSG AML201 was a Japanese multi-center phase 3 randomized study evaluating the equality of standard-dose idarubicin and high-dose daunorubicin as induction therapies, as well as high-dose cytarabine and standard-dose chemotherapies as consolidation therapies in previously untreated AML [109,110]. The ad hoc analysis of the AML201 study focused
on 197 patients in which comprehensive genetic data were available [85]. Favorable, intermediate, and adverse risk groups accounted for 28%, 60%, and 12% of the patient population, respectively. Among adverse genetic factors found in this study, complex karyotype was the most prevalent (8.1%), followed by RUNX1 mutation (5.1%) and the rearrangement of the KMT2A gene (3.0%). Five-year OS was 59.1% in the favorable risk group, 32.6% in the intermediate risk group, and 22.6% in the adverse risk group.

3.2. Hematologic Malignancy (HM)-SCREEN JAPAN 01 Study

This section is composed of a detailed analysis of published data from the Japanese genomic sequencing study HM-SCREEN-Japan 01 [111,112]. Although this study included only a small number of AML patients, it can serve to highlight the characteristics of patients in which conventional treatment was not successful or recommended.

3.2.1. Study Design

HM-SCREEN-JAPAN 01 was a multicenter genomic profiling study in which next-generation sequencing by FoundationOne Heme® was performed for patients with AML. The inclusion criteria were patients with refractory/relapsed AML (R/R) or with previously untreated AML who were ineligible for standard therapy (ND unfit). The available specimens were paraffin-embedded bone marrow clots. The submission of archival specimens was allowed. After submission, annotated genomic reports were returned to the participants. Patients with the FLT3 mutation were allowed to submit specimens multiple times at different opportunities. Clinical information was gathered, including age, sex, treatment modality, response, stem cell transplantation status, and survival information.

3.2.2. ELN Classification

Using conventional cytogenetic tests and next-generation sequencing (NGS), the patients were classified as belonging to non-adverse (favorable or intermediate) or adverse risk groups according to the 2017 ELN recommendation. In Japan, commercially available cytogenetic tests include G-band karyotyping, polymerase chain reaction (PCR) for recurrent rearrangements, FLT3-ITD, and NPM1 mutation. However, these PCR data were not available in the HM-SCREEN-JAPAN 01 study. To supplement the lack of data on FLT3-ITD and NPM1, “classification by conventional cytogenetic tests” was defined as that with G-band karyotyping plus FLT3-ITD and NPM1 information obtained from NGS analysis. Due to the lack of FLT3-ITD allelic ratio assessment, we considered all patients with FLT3-ITD and NPM1 wild type as belonging to the adverse risk group. NGS was performed on bone marrow samples either at the time of diagnosis or relapse.

3.2.3. Conventional Versus NGS-Based Classification

The clinical and cytogenetic characteristics are summarized in Supplemental Table S1. One hundred eighty-two patients were enrolled and fourteen patients were excluded due to unavailable data on G-band karyotyping. Thus, 168 patients were subsequently analyzed, of which 66 (39.3%) and 102 (60.7%) had newly diagnosed and relapsed/refractory AML, respectively. Regarding disease type, 117 (69.6%) patients were diagnosed with normal AML while 41 (24.4%) had AML with myelodysplasia-related changes and 10 (6.0%) had therapy-related myeloid neoplasms. The median age was 63 years (range: 20–91). First, the conventional cytogenetic tests classified 105 (62.5%) patients into the non-adverse risk group and 63 (37.5%) into the adverse risk group. Among the patients in the adverse risk group, the most frequently observed cytogenetic aberration was complex karyotype (25 patients, 39.6%), followed by FLT3-ITD in the absence of mutated NPM1 (15 patients, 23.8%), and KMT2A gene rearrangement (7 patients, 11.1%). Next, we performed NGS-based classification. The timing of NGS analysis was at diagnosis in approximately half of the patients (48.8%). Contrary to the conventional cytogenetic tests, NGS detected an additional 88 adverse gene aberrations, resulting in the re-classification of 39 non-adverse risk patients as adverse risk. Consequently, 66 (39.3%) patients were grouped as non-
adverse risk and 102 (60.7%) as adverse risk (Figure 2). Of the re-classified 39 patients, 19 (48.7%), 14 (35.9%), 6 (15.4%), 4 (10.3%), and 4 (10.3%) harbored mutations of ASXL1, RUNX1, GATA2, TP53 and KMT2A gene rearrangement, respectively. Approximately half of these gene aberrations (51.3%) were detected in the samples at diagnosis. Notably, one re-classified patient harbored the sole TP53 mutation in the specimen at diagnosis without adverse chromosomal abnormalities. Co-mutation was most frequently observed with ASXL1 and RUNX1 in 5/19 (26.3%) patients. Among the 63 patients in the adverse risk group who were not re-classified, NGS detected 49 adverse gene aberrations, of which TP53 mutation was the most frequently found in 32 (50.8%) patients.

Contrary to the re-classified patients, ASXL1 (4, 6.3%) and RUNX1 (7, 11.1%) mutations were found less frequently in the 63 patients in the adverse risk group based on the conventional cytogenetic tests. Moreover, the majority of TP53 mutations (72%) co-occurred with a complex karyotype or a specific aneuploidy involving chromosomes 5 and 7.

3.2.4. Clinical Outcome

After enrollment, many of the patients were treated with non-intensive treatment (64.9%) or best supportive care (9.5%), while 24.4% were treated with intensive chemotherapy. Fifty-nine patients (35.1%) underwent subsequent allogeneic hematopoietic stem cell transplantation (HSCT). When classified based only on the conventional cytogenetic tests, 2-year OS and 2-year progression free survival (PFS) in the non-adverse group was 61.6% (95% confidence interval (CI); 49.3–71.8%) and 46.1% (95% CI; 34.6–56.9%), respectively, while significantly worse outcomes were observed in the adverse group, with a 2-year OS of 39.7% (95% CI; 25.4–53.6%, p = 0.01) and a 2-year PFS of 32.3% (95% CI; 19.9–45.4%, p = 0.02). With NGS analysis, prognosis was similarly discriminated between the non-adverse and adverse groups; a 2-year OS of 68.1% (95% CI; 52.4–79.6%) and a 2-year PFS of 47.9% (95% CI; 33.1–61.3%) in the non-adverse group versus significantly worse outcome in the adverse group, with a 2-year OS of 43.5% (95% CI; 31.7–54.7%, p = 0.01) and
a 2-year PFS of 36.5% (95% CI; 26.1–47.0%, \( p = 0.02 \)). Of note, patients with mutated TP53 had particularly unfavorable outcomes with a 2-year OS of 24.1% (95% CI; 10.4–40.9%) and a 2-year PFS of 17.1% (95% CI; 6.2–32.6%). Patients with mutated TP53 who received HSCT exhibited significantly improved OS compared to those who did not (median OS; 24.5 months, 95% CI; 7.4–38.8, versus 6.8 months, 95% CI; 4.2–8.8, \( p < 0.001 \)).

In summary, the HM-SCREEN-Japan01 study suggested that adverse genetic factors are accumulated in patients with relapsed/refractory AML or those who are ineligible for intensive chemotherapy. Among these genetic abnormalities, TP53 mutation was associated with especially poor outcome. Long-term survival would be expected in patients who underwent HSCT, accounting for only a third of participants of this study (Supplemental Table S1). Molecular-targeting agents specific for adverse genetic factors may be the key to improving the clinical outcomes of patients who are not eligible to HSCT and/or intensive treatment.

4. How to Deal with Specific Adverse Genetic Factors

4.1. FLT3 Inhibitors

Potent FLT3-specific inhibitors such as midostaurin, gilteritinib, and quizartinib are currently available in practice. In the randomized phase 3 RATIFY trial, standard induction therapy (daunorubicin and cytarabine (DNR/AraC), followed by consolidation with high-dose cytarabine) in combination with midostaurin showed longer OS and event-free intervals than chemotherapy alone in patients newly diagnosed with FLT3-mutated AML [113]. The combination of midostaurin and standard therapy followed by midostaurin maintenance also showed better outcomes compared with historical controls (hazard ratio 0.58 in event-free survival) [114]. Gilteritinib monotherapy prolonged survival (9.3 months vs. 5.6 months) compared with conventional salvage therapy (e.g., mitoxantrone, etoposide, and cytarabine (MEC); fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin (FLAG-IDA); low-dose cytarabine (LDAC); and azacitidine) in patients with relapsed/refractory FLT3-mutated AML in the randomized phase 3 ADMIRAL trial [115]. Similarly, quizartinib monotherapy improved OS (hazard ratio 0.76) compared with conventional salvage therapy (e.g., LDAC, MEC, and FLAG-IDA) in the phase 3 QuANTUM-R trial [116]. The novel second-generation FLT3 inhibitor crenolanib has shown possible benefits in combination with conventional chemotherapy in both first-line (anthracyclines/AraC with high-dose AraC) and salvage (high-dose AraC/mitoxantrone) treatment [117–119]. In addition to salvage therapy, FLT3 inhibitors have also been evaluated in upfront combination therapy. In a phase 1 study, gilteritinib in combination with a standard 3 + 7 regimen (idarubicin and cytarabine (IDA/AraC)) showed high response rates (89%) with acceptable toxicity in patients with FLT3-mutated AML [120]. A phase 2 randomized trial comparing gilteritinib versus midostaurin in combination with standard chemotherapy (DNR/AraC) is now ongoing [121]. Front-line combination with quizartinib has also been evaluated in the double-blind phase 3 QuANTUM-First trial [122], and its press release announced superior overall survival compared with standard therapy (DNR/AraC or IDA/AraC) alone in patients with newly diagnosed FLT3-mutated AML [123].

4.2. Inhibition of the AKT, MAPK, and STAT Pathways

The PI3K-AKT-mTOR, RAS-MAPK-ERK, and JAK2-STAT5 pathways are major downstream signals of a variety of tyrosine kinases, including FLT3 and ABL1. The amplification of the PI3K-AKT-mTOR pathway is seen in at least 60% of patients with AML [124]. In addition to the FLT3-ITD and BCR-ABL1 fusion described above, the amplification of AKT signaling can be caused by KIT activation [125], RAS mutations [126], and the overexpression of PI3K [127]. Although mTOR inhibitors as monotherapy showed only a limited efficacy on AML [128,129], dual PI3K/mTOR inhibitors (e.g., NVP-BEZ235, NVPBGT226, and PI-103) induced the cell cycle arrest and apoptosis of leukemic cell lines in preclinical studies [130–132].
Increased levels of phosphorylated JAK2 and STAT5 were noted in AML patient’s bone marrow samples [133,134]. Activating the mutation of FLT3 and JAK2 (e.g., V617F), as well as the functional loss of the suppressor of cytokine signaling-1 (SOCS1) leads to the constitutive activation of JAK2-STAT5 signaling in AML [135]. To date, three JAK inhibitors—ruxolitinib, lestaurtinib, and pacritinib—have been evaluated in clinical studies for AML and/or high-risk myeloproliferative neoplasms (MPNs). Although ruxolitinib failed to demonstrate an appropriate clinical benefit as monotherapy in relapsed/refractory AML [136], the combination therapy of ruxolitinib and decitabine showed a response rate of 61% with tolerable toxicity in patients with high-risk MPN [137]. The sequential administration of lestaurtinib after chemotherapy failed to show a clinical benefit in patients with FLT3-mutated AML in their first relapse in a phase 3 trial [138]. Pacritinib was administered in a small number of patients with relapsed/refractory AML and showed a clinical benefit rate of 43% [139].

Mutations in genes that regulate the RAS-MAPK-ERK pathway, such as NRAS, KRAS, PTPN11, NF1, and KIT are commonly found in patients with AML, and RAS-MAPK-ERK signaling is often highly activated [140]. However, inhibitors of mitogen-activated protein kinase kinase (MEK), a key protein of the RAS-MAPK-ERK pathway, have failed to show meaningful anti-leukemic activity in several clinical studies of either RAS-mutant and RAS wild-type AML [141–143]. RAF proto-oncogene (RAF) kinases also play a key role in this pathway and three isoforms (A-RAF, B-RAF, and C-RAF) have been identified to date. Recently, a novel pan-RAF inhibitor (LY3009120) has demonstrated promising anti-tumor activity in solid tumors and multiple myeloma [144,145]. Tambe and colleagues demonstrated that pan-RAF inhibition induced leukemic cell death in 29% of samples from AML patients [146]. Interestingly, pan-RAF inhibition accompanied the downregulation of MCL1 protein, a negative regulator of apoptosis, and showed synergistic anti-leukemic activity in combination with a BCL-2 inhibitor.

4.3. Menin-KMT2A Inhibitors

As mentioned in the previous section, AML with KMT2A rearrangement (KMT2Ar) is pathologically characterized by upregulated HOXA9 and MEIS1 genes, which are dependent on the interaction of oncogenic KMT2A fusion proteins with other complex-forming proteins such as LEDGF, DOT1L, and menin. Strategies for disrupting the connection between KMT2A protein and menin have recently been investigated. A preclinical study in which PDX mice with KMT2Ar were treated with the orally bioavailable menin-KMT2A inhibitor VTP50469 demonstrated that KMT2A-target genes such as MEIS1 were uniformly suppressed in the bone marrow [147]. Another menin-KMT2A inhibitor, MI-3454, also showed significant anti-leukemic effects along with the downregulation of the MEIS1 gene in a PDX model experiment [148]. Fiskus and colleagues also demonstrated that the menin-KMT2A inhibitor SNDX-50469 induced differentiation and reduced the viability of the leukemic cell lines with KMT2Ar or NPMmt, which was accompanied by the attenuation of BCL-2 and cyclin-dependent kinase 6 (CDK6) levels. Interestingly, the concurrent administration of SNDX-50469 with a BCL-2 inhibitor or a CDK6 inhibitor led to synergistic lethality [149]. Combination therapy with menin-KMT2A inhibitors and FLT3 inhibitors represents a promising strategy for AML with KMT2Ar or NPM1mt and concurrent FLT3 mutation (KMT2Ar/NPM1mt-FLT3mt). Dzama and colleagues showed the synergistic inhibition of the proliferation and enhancement of apoptosis in leukemic cell lines with KMT2Ar/NPM1mt-FLT3mt when treated with FLT3 inhibitors and menin-KMT2A inhibitors [150]. Notably, the first-in-human phase 1 AUGMENT101 study of the menin-KMT2A inhibitor SNDX-5613 as monotherapy showed promising results in patients with relapsed/refractory AML carrying KMT2A rearrangements or NPM1 mutations. Among the 59 patients recruited, clinical responses were observed in 28 patients (47%), including 8 patients (14%) with complete remission (CR) for more than 6 months [151].
4.4. TP53 Stabilizers

A number of small molecules that reactivate mutant TP53 have been identified. Bykov and colleagues demonstrated that one such molecule (PRIMA-1) and its methylated form induced apoptosis in human tumor cells with TP53 mutation [152,153]. APR-246 (eprenetapopt), an alternative name for methylated PRIMA-1, is a first-in-class agent that thermodynamically stabilizes TP53 protein and shifts the equilibrium towards a functional conformation [154]. In a phase 1b/2 clinical study, combination therapy with APR-246 and azacitidine produced a 71% overall response rate in patients with TP53-mutated high-risk MDS or AML [155]. However, the combination strategy did not meet the primary endpoint in a phase 3 clinical trial for patients with TP53-mutated MDS, although the CR rate tended to be superior in the combination group compared to the azacitidine monotherapy group (33.3 vs. 22.4%) [156]. The next-generation TP53 stabilizer APR-548 is now being evaluated in an early-phase clinical trial [157].

Mouse double minute 2 (MDM2) protein is a TP53-specific E3 ubiquitin ligase and functions as a principal cellular antagonist of TP53 [158]. Idasanutlin, a selective MDM2 antagonist, showed a synergistic anti-leukemic effect in combination with a BCL-2 inhibitor in TP53 wild-type AML cell lines [159]. Another MDM2 inhibitor, RG7112, was evaluated in a phase 1 study for patients with acute and chronic leukemia regardless of TP53 mutation status [160]. Thirty-three patients with AML received this agent in its maximal tolerated dose and five patients (15.2%), all TP53 wild-type, achieved complete or partial remission. Thus, MDM2 inhibitors seem appropriate for TP53 wild-type cases. Novel MDM2 inhibitors (e.g., DS-3032b, AMG-232) in combination with hypomethylating agents (HMAs) are now under evaluation in clinical trials (NCT03634228, NCT03041688) for patients with newly diagnosed and relapsed/refractory AML.

Grob and colleagues described the genetic background of 230 patients with TP53-mutated AML and high-risk MDS, among 2200 participants in the Haemato-Oncology Foundation for Adults in the Netherlands and Swiss Group for Clinical Cancer Research (HOVON-SAKK) clinical trials [161]. The most frequent co-mutations were DNMT3A, TET2, and ASXL1 (so-called DTA mutations), accounting for 24.3% of all TP53-mutated patients. These DTA mutations are known to result in disturbed epigenetic modulation within the tumor cells and are associated with better survival when treated with HMAs such as azacitidine [162]. In a meta-analysis of genome sequencing studies in MDS treated with HMAs, TP53 mutation was associated with improved response to HMAs but also linked to worse prognosis [163]. These data suggest that HMA monotherapy may not be sufficient to overcome the adverse impact of TP53 mutation, but represents a promising component of combination therapy with mutant TP53-specific agents.

4.5. Anti-CD47 Antibody

The transmembrane protein CD47, also known as the “don’t-eat-me signal”, is the ligand for signal regulatory protein alpha (SIRP-alpha) on macrophages and dendritic cells, and confers the inhibition of phagocytosis [164]. The binding of CD47 to SIRP-alpha leads to the recruitment of SH2 domain-containing protein tyrosine phosphatase (SHP)1 and SHP2 in the cytoplasm of macrophages, which are negative regulators of cell signaling [165,166]. The expression of CD47 on AML stem cells is associated with poor prognosis [167]. The anti-CD47 antibody magrolimab in combination with azacitidine showed a 57% response rates in patients with previously untreated AML ineligible for intensive therapy in a phase 1b study. Notably, the response rates were slightly better (67%) in TP53-mutated AML in this study [168]. A phase 2 study evaluating the combination therapy of magrolimab with various cytotoxic agents for myeloid malignancies is now in progress [169].

Molecular-targeted agents for AML with adverse genetic factors described to date are summarized in Figure 3.
Treating AML with adverse genetic factors remains challenging, especially in patients who are ineligible for and/or refractory to intensive chemotherapy. In our real-world study, adverse genetic factors were apparently accumulated in patients with relapsed/refractory AML regardless of TP53 mutation status. (C) AML with KMT2A rearrangement, NPM1 mutation, or DEK-NUP214 fusion genes depends on the upregulation of HOXA9, which is initiated by the interaction of menin and KMT2A protein. A menin-KMT2A inhibitor, SNDX-5613, has shown promising results as a monotherapy in patients with relapsed/refractory AML with KMT2A rearrangement or NPM1 mutation. PI3K: phosphatidylinositol 3-kinase, AKT: AKT serine/threonine kinase 1, mTOR: mechanistic targets of rapamycin, MEK: mitogen-activated protein kinase kinase, ERK: extracellular signal-regulated kinases, JAK2: Janus kinase 2, STATs: signal transducer and activator of transcription 5, MDM2: mouse double minute 2, TP53: tumor protein p53, KMT2A: lysine methyltransferase 2A, HOXA9: homeobox protein A9.

5. Conclusions

Figure 3. A summary of molecular-targeted agents for AML with adverse genetic factors. (A) FLT3-ITD and BCR-ABL1 activate the PI3K-AKT-mTOR, RAS-MAPK-ERK, and JAK2-STAT5 pathways to promote leukemic proliferation and leukemogenesis. FLT3 inhibitors (e.g., midostaurin, gilteritinib, and quizartinib) have demonstrated clinical benefit for front-line use in combination with standard induction chemotherapy. Dual PI3K-mTOR inhibitors (e.g., NVP-BEZ235, NVPBG226, and PI-103) induced cell cycle arrest and apoptosis of leukemic cells in preclinical studies. A pan-RAF inhibitor (LY3009120) led to the downregulation of MCL-1 and showed synergistic anti-leukemic activity in combination with a BCL-2 inhibitor. The combination of ruxolitinib, a JAK2 inhibitor, and decitabine, an HMA, showed high response rates with good tolerability in patients with high-risk MPNs. (B) TP53 normally regulates the cell cycle, DNA repair, and apoptosis, and MDM2 counteracts these functions. A first-generation TP53 stabilizer, APR-246, in combination with azacitidine failed to show statistically significant superiority in patients with TP53-mutated MDS in a phase 3 study. However, a next-generation TP53 stabilizer, APR-548, is now under evaluation in an early-phase trial. CD47 is an immune-regulatory tumor antigen that inhibits phagocytosis in macrophages. Although the mechanism of CD47 inhibition is dependent on TP53 function, an anti-CD47 antibody, magrolimab, in combination with azacitidine has demonstrated equal effectiveness in patients with AML regardless of TP53 mutation status. (C) AML with KMT2A rearrangement, NPM1 mutation, or DEK-NUP214 fusion genes depends on the upregulation of HOXA9, which is initiated by the interaction of menin and KMT2A protein. A menin-KMT2A inhibitor, SNDX-5613, has shown promising results as a monotherapy in patients with relapsed/refractory AML with KMT2A rearrangement or NPM1 mutation. PI3K: phosphatidylinositol 3-kinase, AKT: AKT serine/threonine kinase 1, mTOR: mechanistic targets of rapamycin, MEK: mitogen-activated protein kinase kinase, ERK: extracellular signal-regulated kinases, JAK2: Janus kinase 2, STATs: signal transducer and activator of transcription 5, MDM2: mouse double minute 2, TP53: tumor protein p53, KMT2A: lysine methyltransferase 2A, HOXA9: homeobox protein A9.
AML and who were ineligible to intensive therapy, suggesting a relevant need for a new strategy specific for AML with adverse genetic factors. FLT3 inhibitors have been an archetypal example of a successful molecular-targeting strategy for AML with FLT3 mutations, with the clinical benefit of their front-line use in combination with standard chemotherapy being demonstrated in large-scale clinical trials. However, resistance to FLT3 inhibition is a relevant issue to overcome, and pathologic FLT3 mutations (e.g., FLT3-ITD) are not detected in the majority of AML patients. Targeting common pathways essential to adverse genetic factor function, such as HOXA9-related effectors, tyrosine kinase downstream pathways (e.g., AKT-mTOR, MAPK-ERK, and STAT5 pathways), and the loss of TP53 function could be the key to overcoming this issue. Of note, HOXA9 plays a pivotal role in leukemogenesis in AML with KMT2A rearrangement, NPM1 mutation, and DEK-NUP214 fusion, as well as AML with NUP98-involving fusion genes. Given that the interaction of menin and KMT2A protein is essential in initiating the oncogenesis of HOXA9-driven leukemia, menin-KMT2A inhibitors are promising molecular-targeting agents applicable to a considerable proportion of AML patients. Apart from FLT3 inhibitors, the direct inhibition of the PI3K-AKT-mTOR, RAS-MAPK-ERK, and JAK2-STAT5 pathways has been evaluated in preclinical and clinical studies. JAK2 inhibitors may be beneficial in leukemic states arising from MPNs. Although MEK inhibitors did not demonstrate apparent effectiveness for AML, pan-RAF inhibitors, especially in combination with BCL-2 inhibitors, represent a promising strategy in AML therapy. Specific approaches to AML with TP53 mutation remain largely investigational. However, a next-generation TP53 stabilizer is under evaluation in an early-phase trial. An anti-CD47 antibody in combination with HMAs/chemotherapy is another potential strategy for dealing with this category of AML, since this agent seemed equally effective for TP53-mutant and wild-type AML. Although these targeted agents exhibited only a limited anti-leukemic effect in the form of monotherapy, combination therapy with other agents such as conventional chemotherapy, HMAs, and BCL-2 inhibitors represents a promising new strategy.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23115950/s1.

**Author Contributions:** D.I., S.C. and Y.M. were responsible for the preparation and writing of the manuscript; S.U., H.N., Y.-M.G., N.Y. and J.Y. gave academic advises related to the theme of the paper. All authors contributed to and approved the final manuscript; D.I. and S.C. equally contributed to this article. All authors have read and agreed to the published version of the manuscript.

**Funding:** This paper was supported by a National Cancer Research and Development expenses grant (2021-A-11), funded by the National Cancer Center, Japan.

**Institutional Review Board Statement:** The study (HM-SCREEN-Japan) was approved by Institutional Review Board (IRB) of the National Cancer Center Hospital East, and by the IRBs of the individual participating institutions.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study (HM-SCREEN-Japan). Written, informed consent to publish this paper was obtained from each patient.

**Data Availability Statement:** Data sharing not applicable.

**Conflicts of Interest:** Y.M. received research funding from Ono and received honoraria from Bristol-Myers Squibb, Novartis, and Pfizer. The other authors declare no conflict of interest.
References

1. Patel, J.P.; Gönen, M.; Figueroa, M.E.; Fernandez, H.; Sun, Z.; Racevskis, J.; Van Vlierberghe, P.; Dolgalev, I.; Thomas, S.; Aminova, O.; et al. Prognostic Relevance of Integrated Genet Profiling in Acute Myeloid Leukemia. *N. Engl. J. Med.* 2012, 366, 1079–1089. [CrossRef] [PubMed]

2. Papaemmanuil, E.; Gerstung, M.; Bullinger, L.; Gaidzik, VI.; Paschka, P.; Roberts, N.D.; Potter, N.E.; Heuser, M.; Thol, F.; Bolli, N.; et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N. Engl. J. Med.* 2016, 374, 2209–2221. [CrossRef]

3. Döhner, H.; Estey, E.; Grimwade, D.; Amadori, S.; Appelbaum, F.R.; Büchner, H.; Ebert, B.L.; Fenaux, P.; Larson, R.A.; et al. Diagnosis and Management of AML in Adults: 2017 ELN Recommendations from an International Expert Panel. *Blood* 2017, 129, 424–447. [CrossRef] [PubMed]

4. Paschka, P.; Schlenk, R.F.; Gaidzik, VI.; Herzig, J.K.; Aulitzky, T.; Bullinger, L.; Späth, D.; Teleanu, V.; Kündgen, A.; Köhne, C.-H.; et al. ASXL1 Mutations in Younger Adult Patients with Acute Myeloid Leukemia: A Study by the German-Austrian Acute Myeloid Leukemia Study Group. *Haematologica* 2015, 100, 324–330. [CrossRef]

5. Allen, C.; Hills, R.K.; Lamb, K.; Evans, C.; Tinsley, S.; Sellar, R.; O’Brien, M.; Yin, J.L.; Burnett, A.K.; Linch, D.C.; et al. The Importance of Relative Mutant Level for Evaluating Impact on Outcome of KIT, FLT3 and CBL Mutations in Core-Binding Factor Acute Myeloid Leukemia. *Leukemia* 2013, 27, 1891–1901. [CrossRef]

6. Jourdan, E.; Boissel, N.; Chevret, S.; Delabesse, E.; Renneville, A.; Cornillet, P.; Blanchet, O.; Cayuela, J.-M.; Recher, C.; Raffoux, E.; et al. Prospective Evaluation of Gene Mutations and Minimal Residual Disease in Patients with Core Binding Factor Acute Myeloid Leukemia. *Blood* 2013, 121, 2213–2223. [CrossRef]

7. Arber, D.A.; Orazi, A.; Hasserjian, R.; Thiele, J.; Borowitz, M.J.; Le Beau, M.M.; Bloomfield, C.D.; Cazzola, M.; Vardiman, J.W. The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood* 2016, 127, 2391–2405. [CrossRef]

8. Grimwade, D.; Hills, R.K.; Moorman, A.V.; Walker, H.; Chatters, S.; Goldstone, A.H.; Wheatley, K.; Harrison, C.J.; Burnett, A.K.; et al. on behalf of the National Cancer Research Institute Adult Leukaemia Working Group. Refinement of Cytogenetic Classification in Acute Myeloid Leukemia: Determination of Prognostic Significance of Rare Recurring Chromosomal Abnormalities among 5876 Younger Adult Patients Treated in the United Kingdom Medical Research Council Trials. *Blood* 2010, 116, 354–365. [CrossRef]

9. Slovak, M.L.; Gundacker, H.; Bloomfield, C.D.; Dewald, G.; Appelbaum, F.R.; Larson, R.A.; Tallman, M.S.; Bennett, J.M.; Stirewalt, D.L.; Meshinchi, S.; et al. A Retrospective Study of 69 Patients with t(6;9)(P23;Q34) AML Emphasizes the Need for a Prospective, Multicenter Initiative for Rare “poor Prognosis” Myeloid Malignancies. *Leukemia* 2006, 20, 1295–1297. [CrossRef]

10. Hu, H.; Scholten, I.; Gruss, C.; Knippers, R. The Distribution of the DEK Protein in Mammalian Chromatin. *Biochem. Biophys. Res. Commun.* 2007, 358, 1008–1014. [CrossRef]

11. Riveiro-Falkenbach, E.; Soengas, M.S. Control of Tumorigenesis and Chemoresistance by the DEK Oncogene. *Leukemia* 2010, 24, 2932–2938. [CrossRef] [PubMed]

12. Fornerod, M.; van Deursen, J.; van Baal, S.; Reynolds, A.; Davis, D.; Murti, K.G.; Fransen, J.; Grosveld, G. The Human Homologue of Yeast CRM1 Is in a Dynamic Subcomplex with CAN/Nup214 and a Novel Nuclear Pore Component Nup88. *EMBO J.* 1997, 16, 807–816. [CrossRef] [PubMed]

13. Fichtman, B.; Harel, T.; Biran, N.; Zagairy, F.; Applegate, C.D.; Salzberg, Y.; Gilboa, T.; Salah, S.; Shaag, A.; Simonovsky, N.; et al. Pathogenic Variants in NUP214 Cause “Plugged” Nuclear Pore Channels and Acute Febrile Encephalopathy. *Am. J. Hum. Genet.* 2019, 105, 48–64. [CrossRef] [PubMed]

14. Qin, H.; Malek, S.; Cowell, J.K.; Ren, M. Transformation of Human CD34+ Hematopoietic Progenitor Cells with DEK-NUP214 Induces AML in an Immune-compromised Mouse Model. *Oncogene* 2016, 35, 5686–5691. [CrossRef] [PubMed]

15. Sandén, C.; Ageberg, M.; Petersson, J.; Lennartsson, A.; Gullberg, U. Forced Expression of the DEK-NUP214 Fusion Protein Promotes Proliferation Dependent on Upregulation of MTOR. *Biochemistry* 2010, 49, 6576–6586. [CrossRef]

16. Oancea, C.; Rüster, B.; Henschler, R.; Pucetti, E.; Rutherd, M. The t(6;9) Associated DEK/CAN Fusion Protein Targets a Population of Long-Term Repopulating Hematopoietic Stem Cells for Leukemogenic Transformation. *Leukemia* 2010, 24, 1910–1919. [CrossRef]

17. Park, S.; Osmers, U.; Raman, G.; Schwantes, R.H.; Diaz, M.O.; Bushweller, J.H. The PHD3 Domain of MLL Acts as a CYP33-Regulated Switch between MLL-Mediated Activation and Repression. *Biochemistry* 2010, 49, 6576–6586. [CrossRef]

18. Dut, Y.; Milne, T.A.; Tackett, A.J.; Smith, E.R.; Fukuda, A.; Wysocka, J.; Allis, C.D.; Chait, B.T.; Hess, J.L.; Roeder, R.G. Physical Association and Coordinate Function of the H3 K4 Methyltransferase MLL1 and the H4 K16 Acetyltransferase MOF. *Cell* 2005, 121, 873–885. [CrossRef]

19. Slany, R.K. When Epigenetics Kills: MLL Fusion Proteins in Leukemia. *Hematol. Oncol.* 2005, 23, 1–9. [CrossRef]

20. von Neuhoff, C.; Reinhardt, D.; Sander, A.; Zimmermann, M.; Bradtke, J.; Betts, D.R.; Zemanova, Z.; Stary, J.; Bourquin, J.-P.; Haas, O.A.; et al. Prognostic Impact of Specific Chromosomal Aberrations in a Large Group of Pediatric Patients with Acute Myeloid Leukemia Treated Uniformly According to Trial AML-BFM 98. *J. Clin. Oncol.* 2010, 28, 2682–2689. [CrossRef] [PubMed]
22. On Behalf of the Acute Leukemia Working Party EBMT; Pignieux, A.; Labopin, M.; Maertens, J.; Cordonnier, C.; Volin, L.; Socié, G.; Blaise, D.; Craddock, C.; Milpied, N.; et al. Outcome of Allogeneic Hematopoietic Stem-Cell Transplantation for Adult Patients with AML and 11q23/MLL Rearrangement (MLL-r AML). Leukemia 2015, 29, 2375–2381. [CrossRef] [PubMed]

23. Daffon, C.; Craig, V.J.; Mérue, H.; Gräsel, J.; Schacher Engelstier, B.; Hofman, G.; Nijsch, F.; Gaulis, S.; Barys, L.; Ito, M.; et al. Complementary Activities of DOT1L and Menin Inhibitors in MLL-Rearranged Leukemia. Leukemia 2017, 31, 1269–1277. [CrossRef] [PubMed]

24. Yokoyama, A.; Somervaille, T.C.P.; Smith, K.S.; Rozenblatt-Rosen, O.; Meyerson, M.; Cleary, M.L. The Menin Tumor Suppressor Protein Is an Essential Oncogenic Cofactor for MLL-Associated Leukemogenesis. Cell 2005, 123, 207–218. [CrossRef] [PubMed]

25. Armstrong, S.A.; Staunton, J.E.; Silverman, L.B.; Pieters, R.; den Boer, M.L.; Minden, M.D.; Sallan, S.E.; Lander, E.S.; Golub, T.R.; Korsmeyer, S.J. MLL Translocations Specify a Distinct Gene Expression Profile That Distinguishes a Unique Leukemia. Nat. Genet. 2002, 30, 41–47. [CrossRef]

26. Milne, T.A.; Martin, M.E.; Brock, H.W.; Slany, R.K.; Hess, J.L. Leukemic MLL Fusion Proteins Bind across a Broad Region of the Hox A9 Locus, Promoting Transcription and Multiple Histone Modifications. Cancer Res. 2005, 65, 11367–11374. [CrossRef]

27. Yokoyama, A.; Cleary, M.L. Menin Critically Links MLL Proteins with LEDGF on Cancer-Associated Target Genes. Cancer Cell 2008, 14, 36–46. [CrossRef]

28. Yuan, Z.M.; Huang, Y.; Ishiko, T.; Kharbanda, S.; Weichselbaum, R.; Kufe, D. Regulation of DNA Damage-Induced Apoptosis by the c-abl Tyrosine Kinase. Proc. Natl. Acad. Sci. USA 1997, 94, 1437–1440. [CrossRef]

29. Yuan, Z.M.; Huang, Y.; Ishiko, T.; Nakada, S.; Utsugisawa, T.; Kharbanda, S.; Wang, R.; Sung, P.; Shinohara, A.; Weichselbaum, R.; et al. Regulation of Rad51 Function by C-abl in Response to DNA Damage. J. Biol. Chem. 1998, 273, 3799–3802. [CrossRef]

30. Verschraegen, C.F.; Kantarjian, H.M.; Hirsch-Ginsberg, C.; Lee, M.S.; O’Brien, S.; Rios, M.B.; Stass, S.A.; Keating, M.; Talpaz, M. The Breakpoint Cluster Region Site in Patients with Philadelphia Chromosome-Positive Chronic Myelogenous Leukemia. Clinical, Laboratory, and Prognostic Correlations. Cancer 1995, 76, 992–997. [CrossRef]

31. Sattler, M.; Salgia, R. Activation of Hematopoietic Growth Factor Signal Transduction Pathways by the Human Oncogene BCR/ABL. Cytokine Growth Factor Rev. 1997, 8, 63–79. [CrossRef]

32. Skorski, T.; Kanakaraj, P.; Nieborowska-Skorska, M.; Ratajczak, M.Z.; Wen, S.-C.; Zon, G.; Gewirtz, A.M.; Perussia, B.; Calabretta, B. Phosphatidylinositol-3 Kinase Activity Is Regulated by BCR/ABL and Is Required for the Growth of Philadelphia Chromosome-Positive Cells. Blood 1995, 86, 726–736. [CrossRef] [PubMed]

33. Salgia, R.; Uemura, N.; Okuda, K.; Li, J.L.; Picick, E.; Sattler, M.; de Jong, R.; Drucker, B.; Heisterkamp, N.; Chen, L.B. CRKL Links P210BCR/ABL with Paxillin in Chronic Myelogenous Leukemia Cells. J. Biol. Chem. 1995, 270, 29145–29150. [CrossRef] [PubMed]

34. de Jong, R.; ten Hoeve, J.; Heisterkamp, N.; Groffen, J. CRKL Is Complexed with Tyrosine-Phosphorylated Cbl in Ph-Positive Leukemia. J. Biol. Chem. 1995, 270, 21468–21471. [CrossRef] [PubMed]

35. Shuai, K.; Halpern, J.; ten Hoeve, J.; Rao, X.; Sawyer, C.L. Constitutive Activation of STAT5 by the BCR-ABL Oncogene in Chronic Myelogenous Leukemia. Oncogene 1996, 13, 247–254. [PubMed]

36. Gishizky, M.L.; Cortez, D.; Pendergast, A.M. Mutant Forms of Growth Factor-Binding Protein-2 Reverse BCR-ABL-Induced Transformation. Proc. Natl. Acad. Sci. USA 1995, 92, 10889–10893. [CrossRef]

37. Gorre, M.E.; Ellwood-Yen, K.; Chiosis, G.; Rosen, N.; Sawyers, C.L. BCR-ABL Point Mutants Isolated from Patients with Imatinib Mesylate–Resistant Chronic Myeloid Leukemia Remain Sensitive to Inhibitors of the BCR-ABL Chaperone Heat Shock Protein 90. Blood 2002, 100, 3041–3044. [CrossRef]

38. Bagatell, R.; Whitesell, L. Altered Hsp90 Function in Cancer: A Unique Therapeutic Opportunity. Mol. Cancer 2004, 3, 1021–1030. [PubMed]

39. Keung, Y.-K.; Beaty, M.; Powell, B.L.; Molnar, I.; Buss, D.; Pettenati, M. Philadelphia Chromosome Positive Myelodysplastic Syndrome and Acute Myeloid Leukemia-Retrospective Study and Review of Literature. Leuk. Res. 2004, 28, 579–586. [CrossRef]

40. Berger, R. Differences between Blastic Chronic Myeloid Leukemia and Ph-Positive Acute Leukemia. Leuk. Lymphoma 1993, 11, Suppl. (S1), 235–237. [CrossRef]

41. Neudorf, N.R.; Burmeister, T.; Dörken, B.; Westermann, J. BCR-ABL-Positive Acute Myeloid Leukemia: A New Entity? Analysis of Clinical and Molecular Features. Ann. Hematol. 2016, 95, 1211–1221. [CrossRef] [PubMed]

42. Morishita, K.; Parganas, E.; William, C.L.; Whittaker, M.H.; Drabkin, D.; Oul, J.; Taelte, R.; Valentine, M.B.; Ihle, J.N. Activation of EVI1 Gene Expression in Human Acute Myelogenous Leukemias by Translocations Spanning 300–400 Kilobases on Chromosome Band 3q26. Proc. Natl. Acad. Sci. USA 1992, 89, 3937–3941. [CrossRef] [PubMed]

43. Kurokawa, M.; Mitani, K.; Irie, K.; Matsuyma, T.; Takahashi, T.; Chiba, S.; Yazaki, Y.; Matsumoto, K.; Hirai, H. The Oncoprotein Evi-1 Represses TGF-Beta Signalling by Inhibiting Smad3. Nature 1998, 394, 92–96. [CrossRef] [PubMed]

44. Kurokawa, M.; Mitani, K.; Yamagata, T.; Takahashi, T.; Izutsu, K.; Ogawa, S.; Moriguchi, T.; Nishida, E.; Yazaki, Y.; Hirai, H. The Evi-1 Oncoprotein Inhibits c-Jun N-Terminal Kinase and Prevents Stress-Induced Cell Death. EMBO J. 2000, 19, 2958–2968. [CrossRef]

45. Katayama, S.; Suzuki, M.; Yamaoka, A.; Kelekuk-Lukwete, N.; Katsuoka, F.; Otsuki, A.; Kure, S.; Engel, J.D.; Yamamoto, M. GATA2 Haploinsufficiency Accelerates EVI1-Driven Leukemogenesis. Blood 2017, 130, 908–919. [CrossRef]

46. Minegishi, N.; Suzuki, N.; Yokomizo, T.; Pan, X.; Fujimoto, T.; Takahashi, S.; Hara, T.; Miyajima, A.; Nishikawa, S.; Yamamoto, M. Expression and Domain-Specific Function of GATA2 during Differentiation of the Hematopoietic Precursor Cells in Midgestation Mouse Embryos. Blood 2003, 102, 896–905. [CrossRef]
Tamburri, S.; Lavarone, E.; Fernández-Pérez, D.; Conway, E.; Zanotti, M.; Manganaro, D.; Pasini, D. Histone H2AK119 Mono-Ubiquitination Is Essential for Polycomb-Mediator Transcriptional Repression. *Mol. Cell* 2020, 77, 840–856.e5. [CrossRef] [PubMed]

Unl, M.; Masamoto, Y.; Sato, T.; Kamikubo, Y.; Arai, S.; Hara, E.; Kurokawa, M. Modeling ASXL1 Mutation Revealed Impaired Hematopoiesis Caused by Derepression of P16Ink4a through Aberrant PRC1-Mediated Histone Modification. *Leukemia* 2019, 33, 191–204. [CrossRef] [PubMed]

Fang, X.; Xu, S.; Zhang, Y.; Xu, J.; Huang, Z.; Liu, W.; Wang, S.; Yen, K.; Zhang, W. Asxl1 C-Terminal Mutation Perturbs Neutrophil Differentiation in Zebrafish. *Leukemia* 2021, 35, 2299–2310. [CrossRef] [PubMed]

Cao, L.; Li, R.; Wu, X. The Functions and Mechanisms of PR-DUB in Malignancy. *Front. Mol. Biosci.* 2021, 8, 657150. [CrossRef] [PubMed]

Asada, S.; Fujino, T.; Goyama, S.; Kitamura, T. The Role of ASXL1 in Hematopoiesis and Myeloid Malignancies. *Cell Mol. Life Sci.* 2019, 76, 2511–2523. [CrossRef]

Abdel-Wahab, O.; Gao, J.; Adli, M.; Dey, A.; Trimarchi, T.; Chung, Y.R.; Kuscu, C.; Hricik, T.; Ndiaye-Lobry, D.; Lafave, L.M.; et al. Deletion of Asxl1 Results in Myelodysplasia and Severe Developmental Defects in Vivo. *J. Exp. Med.* 2013, 210, 2641–2659. [CrossRef]

Zhang, F.; He, F.; Bai, J.; Yamamoto, S.; Chen, S.; Zhang, L.; Sheng, M.; Zhang, L.; Guo, Y.; Man, N.; et al. Chromatin Regulator Asxl1 Loss and Nf1 Haploinsufficiency Cooperate to Accelerate Myeloid Malignancy. *J. Clin. Investig.* 2018, 128, 5383–5398. [CrossRef] [PubMed]

Schnittger, S.; Eder, C.; Jeromin, S.; Alpermann, T.; Fasan, A.; Grossmann, V.; Kohlmann, A.; Illig, T.; Klopp, N.; Wichmann, H.-E.; et al. ASXL1 Exon 12 Mutations Are Frequent in AML with Intermediate Risk Karyotype and Are Independently Associated with an Adverse Outcome. *Leukemia* 2013, 27, 82–91. [CrossRef] [PubMed]

Wu, M.; Bellas, R.E.; Shen, J.; Sonenshein, G.E. Roles of the Tumor Suppressor P53 and the Cyclin-Dependent Kinase Inhibitor P21WAF1/CIP1 in Receptor-Mediated Apoptosis of WEHI 231 B Lymphoma Cells. *J. Exp. Med.* 1995, 187, 1671–1679. [CrossRef]

Chipuk, J.E.; Kuwana, T.; Boucher-Hayes, L.; Droin, N.M.; Newmeyer, D.D.; Schuler, M.; Green, D.R. Direct Activation of Bax by P53 Mediates Mitochondrial Membrane Permeabilization and Apoptosis. *Science* 2004, 303, 1010–1014. [CrossRef]

Thomas, A.; Giesler, T.; White, E. P53 Mediates Bcl-2 Phosphorylation and Apoptosis via Activation of the Cdc42/JNK1 Pathway. *Oncogene* 2000, 19, 5259–5269. [CrossRef]

Kuwana, T.; Newmeyer, D.D. Bcl-2-Family Proteins and the Role of Mitochondria in Apoptosis. *Biochim. Biophys. Acta* 2000, 1511, 691–699. [CrossRef] [PubMed]

Letai, A.; Bassik, M.C.; Walensky, L.D.; Soricelli, M.D.; Weiler, S.; Korsmeyer, S.J. Distinct BH3 Domains Either Sensitize or Activate Mitochondrial Apoptosis, Serving as Prototype Cancer Therapeutics. *Cancer Cell* 2002, 2, 183–192. [CrossRef]

Quintás-Cardama, A.; Hu, C.; Qutub, A.; Qiu, Y.H.; Zhang, X.; Post, S.M.; Zhang, N.; Coombs, K.; Kornblau, S.M. P53 Pathway Dysfunction Is Highly Prevalent in Acute Myeloid Leukemia Independent of TP53 Mutational Status. *Leukemia* 2017, 31, 1296–1305. [CrossRef]

Nahi, H.; Lehmann, S.; Bengtzen, S.; Jansson, M.; Möllgård, L.; Paul, C.; Merup, M. Chromosomal Aberrations in 17p Predict in Vitro Drug Resistance and Short Overall Survival in Acute Myeloid Leukemia. *Leuk. Lymphoma* 2008, 49, 508–516. [CrossRef] [PubMed]

Christiansen, D.H.; Andersen, M.K.; Pedersen-Bjergaard, J. Mutations With Loss of Heterozygosity of P53 Are Common in Therapy-Related Myelodysplasia and Acute Myeloid Leukemia After Exposure to Alkylating Agents and Significantly Associated With Deletion or Loss of 5q, a Complex Karyotype, and a Poor Prognosis. *JCO* 2001, 19, 1405–1413. [CrossRef]

Barbosa, K.; Li, S.; Adams, P.D.; Deshpande, A.J. The Role of TP53 in Acute Myeloid Leukemia: Challenges and Opportunities. *Genes Chromosomes Cancer* 2019, 58, 875–888. [CrossRef]

Sallman, D.A.; McLemore, A.F.; Aldrich, A.L.; Komrokji, R.S.; McGraw, K.L.; Dhawan, A.; Geyer, S.; Hou, H.-A.; Eksioglu, E.A.; Sullivan, A.; et al. TP53 Mutations in Myelodysplastic Syndromes and Secondary AML Confer an Immunosuppressive Phenotype. *Blood* 2020, 136, 2812–2823. [CrossRef]

Ohtake, S.; Miyawaki, S.; Fujita, H.; Kiyoi, H.; Shinagawa, K.; Usui, N.; Okumura, H.; Miyamura, K.; Nakaseko, C.; Miyazaki, Y.; et al. Randomized Study of Induction Therapy Comparing Standard-Dose Daunorubicin with High-Dose Daunorubicin in Adult Patients with Previously Untreated Acute Myeloid Leukemia: The JALSG AML201 Study. *Blood* 2011, 117, 2358–2365. [CrossRef] [PubMed]

Miyawaki, S.; Ohtake, S.; Fujisawa, S.; Kiyoi, H.; Shinagawa, K.; Usui, N.; Sakura, T.; Miyamura, K.; Nakaseko, C.; Miyazaki, Y.; et al. A Randomized Comparison of 4 Courses of Standard-Dose Multagent Chemotherapy versus 3 Courses of High-Dose Cytarabine Alone in Postremission Therapy for Acute Myeloid Leukemia in Adults: The JALSG AML201 Study. *Blood* 2011, 117, 2366–2372. [CrossRef]

Miyamoto, K.; Fukushima, K.; Chi, S.; Shibayama, H.; Hosono, N.; Yamauchi, T.; Katagiri, S.; Gotoh, A.; Morishita, T.; Yanada, M.; et al. Interim Analysis of Hematologic Malignancies (HM)-Screen-Japan 01: A Mutation Profiling Multicenter Study of Patients with AML. *Blood* 2020, 136, 2–3. [CrossRef]

Hosono, N.; Yamauchi, T.; Chi, S.; Fukushima, K.; Shibayama, H.; Katagiri, S.; Gotoh, A.; Eguchi, M.; Morishita, T.; Ogasawara, R.; et al. Hematologic Malignancies (HM)-Screen-Japan 01: A Mutation Profiling Multicenter Study on Patients with Acute Myeloid Leukemia. *Blood* 2021, 138, 4457. [CrossRef]
Venugopal, S.; Bar-Natan, M.; Mascarenhas, J.O. JAKs to STATs: A Tantalizing Therapeutic Target in Acute Myeloid Leukemia. *Blood Rev.* 2020, 40, 100634. [CrossRef] [PubMed]

Chen, C.-Y.; Tsay, W.; Tang, J.-L.; Shen, H.-L.; Lin, S.-W.; Huang, S.-Y.; Yao, M.; Chen, Y.-C.; Shen, M.-C.; Wang, C.-H.; et al. SOCS1 Methylation in Patients with Newly Diagnosed Acute Myeloid Leukemia. *Genes Chromosomes Cancer* 2003, 37, 300–305. [CrossRef] [PubMed]

Pemmaraju, N.; Kantarjian, H.; Kadia, T.; Cortes, J.; Borthakur, G.; Newberry, K.; Garcia-Manero, G.; Ravandi, F.; Jabbour, E.; Dellasala, S.; et al. A Phase I/II Study of the Janus Kinase (JAK)1 and 2 Inhibitor Ruxolitinib in Patients with Relapsed or Refractory Acute Myeloid Leukemia. *Clin. Lymphoma Myeloma Leuk.* 2015, 15, 171–176. [CrossRef]

Rampal, R.K.; Mascarenhas, J.; Kosiopek, H.E.; Bhave, R.; Henex, E.O.; Wang, E.S.; Gerds, A.T.; Heaney, M.L.; Abboud, C.N.; Kremyrskaya, M.; et al. Efficacy of Combined Ruxolitinib and Decitabine in Patients with Accelerated and Blast-Phase Myeloproliferative Neoplasms: Results of a Phase II Study (MPN-RC 109 Trial). *Blood* 2018, 132, 3027. [CrossRef]

Levis, M.; Ravandi, F.; Wang, E.S.; Baer, M.R.; Perl, A.; Coutre, S.; Erba, H.; Stuart, R.K.; Baccarani, M.; Cripe, L.D.; et al. Results from a Randomized Trial of Salvage Chemotherapy Followed by Lestaurnitib in Patients with FLT3 Mutant AML in First Relapse. *Blood* 2011, 117, 3294–3301. [CrossRef]

Verstovsek, S.; Odenike, O.; Singer, J.W.; Grantson, T.; Al-Fayoumi, S.; Deeg, H.J. Phase 1/2 Study of Pacritinib, a next Generation JAK2/FLT3 Inhibitor, in Myelofibrosis or Other Myeloid Malignancies. *J. Hematol. Oncol.* 2016, 9, 137. [CrossRef]

Ricciardi, M.R.; McQueen, T.; Chism, D.; Milella, M.; Estey, E.; Kaldjian, E.; Sebold-Leopold, J.; Konopleva, M.; Andreeff, M. Quantitative Single Cell Determination of ERK Phosphorylation and Regulation in Relapsed and Refractory Primary Acute Myeloid Leukemia. *Leukemia* 2005, 19, 1543–1549. [CrossRef]

Jain, N.; Curran, E.; Iyengar, N.M.; Diaz-Flores, E.; Kunnavakkam, R.; Karrison, T.; Erba, H.P.; Green, M.; et al. Phase II Study of the Oral MEK Inhibitor Selumetinib in Advanced Acute Myelogenous Leukemia: A University of Chicago Phase II Consortium Trial. *Clin. Cancer Res.* 2014, 20, 490–498. [CrossRef]

Borthakur, G.; Popplewell, L.; Boyiadzis, M.; Foran, J.; Platzbecker, U.; Vey, N.; Walter, R.B.; Olin, R.; Raza, A.; Giagounidis, A.; et al. Activity of the Oral Mitogen-Activated Protein Kinase Kinase Inhibitor Trametinib in RAS-Mutant Relapsed or Refractory Myeloid Malignancies. *Cancer* 2016, 122, 1871–1879. [CrossRef]

Maiti, A.; Naqvi, K.; Kadia, T.M.; Borthakur, G.; Takahashi, K.; Bose, P.; Daver, N.G.; Patel, A.; Alvarado, Y.; Ohanian, M.; et al. Phase II Trial of MEK Inhibitor Binimetinib (MEK162) in Acute Myeloid Leukemia (AML). *Clin. Lymphoma Myeloma Leuk.* 2019, 19, 142–148.e1. [CrossRef] [PubMed]

Peng, S.-B.; Henry, J.R.; Kaufman, M.D.; Lu, W.-P.; Smith, B.D.; Vogeti, S.; Rutkoski, T.J.; Wise, S.; Chun, L.; Zhang, Y.; et al. Inhibition of RAF Isoforms and Active Dimers by LY3009120 Leads to Anti-Tumor Activities in RAS or BRAF Mutant Cancers. *Cancer Cell* 2015, 28, 384–398. [CrossRef] [PubMed]

Müller, E.; Bauer, S.; Stühmer, T.; Mottok, A.; Solch, C.-J.; Steinbrunn, T.; Brüntert, D.; Brandl, A.; Schraud, H.; Kreßmann, S.; et al. Pan-Raf Co-Operates with PDK-Dependent Signalling and Critically Contributes to Melomela Cell Survival Independently from Mutated RAS. *Leukemia* 2017, 31, 922–933. [CrossRef] [PubMed]

Tambe, M.; Karjalainen, E.; Vähä-Koskela, M.; Bulanova, D.; Gjertsen, B.T.; Kontro, M.; Porrka, K.; Heckman, C.A.; Wennenberg, K. Pan-RAF Inhibition Induces Apoptosis in Acute Myeloid Leukemia Cells and Synergizes with BCL2 Inhibition. *Leukemia* 2020, 34, 3186–3196. [CrossRef] [PubMed]

Krivtsov, A.V.; Evans, K.; Gadrey, J.Y.; Eschle, B.K.; Hatton, C.; Uckelmann, H.J.; Ross, K.N.; Perner, F.; Olsen, S.N.; Pritchard, T.; et al. A Menin-MLL Inhibitor Induces Chromatin Changes and Eradicates Disease in Models of MLL-Rearranged Leukemia. *Cancer Cell* 2019, 36, 660–673.e11. [CrossRef]

Kloosowski, S.; Miao, H.; Kemsinska, P.; Wu, T.; Purohit, T.; Kim, E.; Linhares, B.M.; Chen, D.; Jih, G.; Perkey, E.; et al. Menin Inhibitor MI-3454 Induces Remission in MLL1-Rearranged and NPM1-Mutated Models of Leukemia. *J. Clin. Investig.* 2020, 130, 981–997. [CrossRef]

Fiskus, W.; Boettcher, S.; Daver, N.; Mill, C.P.; Sasaki, K.; Birdwell, C.E.; Davis, J.A.; Takahashi, K.; Kadia, T.M.; DiNardo, C.D.; et al. Effective Menin Inhibitor-Based Combinations against AML with MLL Rearrangement or NPM1 Mutation (NPM1c). *Blood Cancer J.* 2022, 12, 5. [CrossRef]

Dzama, M.M.; Steiner, M.; Rausch, J.; Sasda, D.; Schönfeld, J.; Kunz, T.; Taubert, M.C.; McGeehan, G.M.; Chen, C.-W.; Mupo, A.; et al. Synergistic Targeting of FLT3 Mutations in AML via Combined Menin-MLL and FLT3 Inhibition. *Blood* 2020, 136, 2442–2456. [CrossRef]

Stein, E. Safety and Efficacy of Menin Inhibition in Patients (Pts) with MLL-Rearranged and NPM1 Mutant Acute Leukemia: A Phase (Ph) 1, First-in-Human Study of SNDX-5613 (AUGMENT 101). *Blood* 2021, 138, 699. [CrossRef]

Bykov, V.J.N.; Issaeva, N.; Zache, N.; Shilov, A.; Hultcrantz, M.; Bergman, J.; Selivanova, G.; Wiman, K.G. Reactivation of Mutant P53 and Induction of Apoptosis in Human Tumor Cells by Maleimide Analogs *. J. Biol. Chem.* 2005, 280, 30384–30391. [CrossRef]

Bykov, V.J.N.; Zache, N.; Stridh, H.; Westman, J.; Bergman, J.; Selivanova, G.; Wiman, K.G. PRIMA-1MET Synergizes with Cisplatin to Induce Tumor Cell Apoptosis. *Oncogene* 2005, 24, 3484–3491. [CrossRef]

Lambert, J.M.R.; Gorzov, P.; Veprintsev, D.B.; Söderqvist, M.; Segerbäck, D.; Bergman, J.; Fersht, A.R.; Hainaut, P.; Wiman, K.G.; Bykov, V.J.N. PRIMA-1 Reactivates Mutant P53 by Covalent Binding to the Core Domain. *Cancer Cell* 2009, 15, 376–388. [CrossRef]
155. Sallman, D.A.; DeZern, A.E.; Garcia-Manero, G.; Steensma, D.P.; Roboz, G.J.; Sekeres, M.A.; Cluzeau, T.; Sweet, K.L.; McLemore, A.; McGraw, K.L.; et al. Eprenetapopt (APR-246) and Azacitidine in TP53-Mutant Myelodysplastic Syndromes. *J. Clin. Oncol.* 2021, 39, 1584–1594. [CrossRef] [PubMed]

156. Aprea Therapeutics. A Phase III Multicenter, Randomized, Open Label Study of APR-246 in Combination with Azacitidine Versus Azacitidine Alone for the Treatment of (Tumor Protein) TP53 Mutant Myelodysplastic Syndromes. Available online: https://clinicaltrials.gov/ct2/show/NCT03745716 (accessed on 7 October 2021).

157. Aprea Therapeutics. Phase 1 Study to Evaluate Safety and Efficacy of APR-548 in Combination with Azacitidine for the Treatment of TP53-Mutant Myelodysplastic Syndromes. Available online: https://clinicaltrials.gov/ct2/show/NCT04638309 (accessed on 4 November 2021).

158. Moll, U.M.; Petrenko, O. The MDM2-P53 Interaction. *Mol. Cancer Res.* 2003, 1, 1001–1008.

159. Lehmann, C.; Friess, T.; Birzelle, F.; Kiialainen, A.; Dangl, M. Superior Anti-Tumor Activity of the MDM2 Antagonist Idasanutlin and the Bcl-2 Inhibitor Venetoclax in P53 Wild-Type Acute Myeloid Leukemia Models. *J. Hematol. Oncol.* 2016, 9, 50. [CrossRef] [PubMed]

160. Andreeff, M.; Kelly, K.R.; Yee, K.; Assouline, S.; Strair, R.; Popplewell, L.; Bowen, D.; Martinelli, G.; Drummond, M.W.; Vyas, P.; et al. Results of the Phase 1 Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. *Clin. Cancer Res.* 2016, 22, 868–876. [CrossRef] [PubMed]

161. Grob, T.; Al Hinai, A.S.A.; Sanders, M.A.; Kavelaars, F.G.; Rijken, M.; Gradowska, P.L.; Biemond, B.J.; Breems, D.A.; Maertens, J.; van Marwijk Kooy, M.; et al. Molecular Characterization of Mutant TP53 Acute Myeloid Leukemia and High-Risk Myelodysplastic Syndrome. *Blood* 2022, 139, 2347–2354. [CrossRef] [PubMed]

162. Traina, F.; Visconte, V.; Elson, P.; Tabarroki, A.; Jankowska, A.M.; Hasrouni, E.; Sugimoto, Y.; Szpurka, H.; Makishima, H.; O’Keefe, C.L.; et al. Impact of Molecular Mutations on Treatment Response to DNMT Inhibitors in Myelodysplasia and Related Neoplasms. *Leukemia* 2014, 28, 78–87. [CrossRef] [PubMed]

163. Majeti, R.; Chao, M.P.; Alizadeh, A.A.; Pang, W.W.; Jaiswal, S.; Gibbs, K.D.; van Rooijen, N.; Weissman, I.L. CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells. *Cell* 2009, 138, 286–299. [CrossRef] [PubMed]

164. Sallman, D. The First-in-Class Anti-CD47 Antibody Magrolimab Combined with Azacitidine Is Well-Tolerated and Effective in AML Patients: Phase 1b Results. *Blood* 2020, 21, S213.

165. Barclay, A.N.; Brown, M.H. The SIRP Family of Receptors and Immune Regulation. *Nat. Rev. Immunol.* 2006, 6, 457–464. [CrossRef]

166. Latour, S.; Tanaka, H.; Demeure, C.; Mateo, V.; Rubio, M.; Brown, E.J.; Maliszewski, C.; Lindberg, F.P.; Oldenborg, A.; Ullrich, A.; et al. Bidirectional Negative Regulation of Human T and Dendritic Cells by CD47 and Its Cognate Receptor Signal-Regulator Protein-α: Down-Regulation of IL-12 Responsiveness and Inhibition of Dendritic Cell Activation. *J. Immunol.* 2001, 167, 2547–2554. [CrossRef] [PubMed]

167. Oldenborg, P.-A.; Gresham, H.D.; Lindberg, F.P. Cd47-Signal Regulatory Protein α (Sirpα) Regulates Fcy and Complement Receptor–Mediated Phagocytosis. *J. Exp. Med.* 2001, 193, 855–862. [CrossRef] [PubMed]

168. Majeti, R.; Chao, M.P.; Alizadeh, A.A.; Pang, W.W.; Jaiswal, S.; Gibbs, K.D.; van Rooijen, N.; Weissman, I.L. CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells. *Cell* 2009, 138, 286–299. [CrossRef] [PubMed]

169. Gilead Sciences. A Phase 2 Multi-Arm Study of Magrolimab Combinations in Patients with Myeloid Malignancies. Available online: https://clinicaltrials.gov/ct2/show/NCT04778410 (accessed on 23 March 2022).