3 Insights into the Pathobiology of Secondary AML

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Abbreviations

AHD	Antecedent hematological disorder
AML	Acute myeloid leukemia
CBF	Core binding factor
CIBMTR	Center for International Bone Marrow Transplant Research
EBMT	European Society for Blood and Bone Marrow Transplantation
HCT	Hematopoietic cell transplantation
JAK	Janus kinase
MDS	Myelodysplastic syndrome
MPN	Myeloproliferative neoplasm
SNP	Single-nucleotide polymorphism
TP	Tumor protein
WHO	World Health Organization

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3.1 Introduction: What Is Secondary AML?

Acute myeloid leukemia (AML) is the most common type of acute leukemia, the incidence of which increases with advancing age. The etiology remains elusive for the most part, but development following a prior cytotoxic agent or as a consequence of an antecedent myeloid disorder has been widely recognized. In the 1997 World Health Organization (WHO) classification of neoplastic disease of the hematopoietic and lymphoid tissues, AML with multilineage dysplasia, defined as dysplastic changes in two or more cell lines, and therapy-related AML were recognized as distinct entities due to morphological differences from de novo AML, characteristic cytogenetic abnormalities, and worse prognosis [1].

Secondary acute myeloid leukemia (s-AML) in the current era informally refers to the AML that evolves from an antecedent hematological disease (AML-AHD), usually a myeloid malignancy such as myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), or aplastic anemia; has myelodysplasia-related changes (AML-MRC); or develops after exposure to a cytotoxic chemotherapy or radiation treatment for a prior neoplasm (t-AML) (Table 3.1). Per the 2016 WHO classification, AML with MRC is defined as AML meeting at least one of the following criteria: (a) presence of 50% of more dysplastic cells in at least two cell lines and in the

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Table 3.1 What is included in secondary AML

	Details
AML-AHD	• Preceding MDS • Preceding MPN • Preceding MDS/MPN overlap syndromes • Bone marrow failure syndrome
AML-MRC	• Dysplasia in >50% cells in at least two cell lines and absence of favorable-risk mutations of *NPM1*, biallelic *CEBPA* or del(9q) • MDS-related cytogenetic abnormality • AML-AHD with preceding MDS or MDS/MPN
t-AML	• Type I – Alkylating agents—cyclophosphamide, bendamustine, melphalan, busulfan, carmustine, chlorambucil, thiotepa Platinum agents—cisplatin, carboplatin Antimetabolites—azathioprine, fludarabine – 5–7 years after exposure – Preceding MDS phase or MDS-related changes at diagnosis – Del(5q), del(7q), monosomy 7, complex karyotype, *TP53* mutations • Type II – Topoisomerase II inhibitors—anthracyclines (daunorubicin, doxorubicin, epirubicin), etoposide, mitoxantrone – 2–3 years after exposure – Balanced chromosomal translocations involving 11q23 (*KMT2A/MLL*) or 21q22 (*RUNX1/AML1*) • Radiation exposure

AML-AHD acute myeloid leukemia with an antecedent hematological disorder, *AML-MRC* acute myeloid leukemia with myelodysplasia related changes, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *t-AML* therapy-related acute myeloid leukemia

absence of favorable-risk mutations of NPM1 or biallelic CEBPA or del(9q) [2]; (b) an antecedent hematologic disorder (MDS or MDS/MPN); or (c) presence of an MDS-related cytogenetic abnormality.

t-AML has been associated with prior chemotherapy, typically alkylating agents and DNA topoisomerase II inhibitors, as well as prior radiation therapy. Alkylating agents such as melphalan, cyclophosphamide, and nitrogen mustard may lead to dysplastic changes similar to MDS, bi- or tri-lineage cytopenias, abnormalities of chromosomes 5 and 7 or both, and complex karyotype, typically with a latency of over 5 years from exposure [3–6]. These karyotypes have been reported to comprise 76% of all abnormal karyotype in one series of patients with t-AML or therapy-related MDS [7]. t-AML associated with topoisomerase II inhibitors, for example, doxorubicin, etoposide, and mitoxantrone, is characterized by shorter interval (around 2–3 years) between exposure and diagnosis, absence of preceding MDS, and a genetic abnormality involving translocation of *MLL* gene on chromosome 11, band q23, and *RUNX1/AML1* gene on chromosome 21, band q22 [7–10]. t-AML has also been described following the use of other chemotherapy agents such as azathioprine, 5-flourouracil, methotrexate, 6-mercaptopurine, and fludarabine [7, 11, 12]).

3.2 Why Does It Matter?

Two large population studies reported that s-AML constituted approximately one in every four cases of AML diagnosed between 1997–2006 and 2000–2013 in these respective studies [13, 14]). More specifically, AML-AHD has been reported in approximately 16–19% and t-AML in approximately 7% of all AML diagnoses in several studies [13–16]. This number may reasonably be expected to rise due to improved survival following chemotherapy for prior malignancies.

More importantly, numerous studies over the years have shown inferior survival in patients with s-AML compared to de novo AML, which makes it imperative to recognize this as a prognostic factor, and indeed an unmet need for treatment options [13–16]. Response to standard chemotherapy in patients with s-AML has also traditionally been reported to be lower than response rates seen with de novo AML. This is likely a result of a higher incidence of high-risk genetic and molecular features, genetic alterations, or selection of chemotherapy-resistant clones in cases of prior treatment of the AHD. The high-risk cytogenetic and mutational profile in

s-AML, such as mutations in tumor protein (*TP53*) and complex karyotype, may render leukemia cells less responsive to chemotherapy and a poorer overall survival [17]. Mechanism of resistance and resulting lower response to chemotherapy in s-AML have been attributed to an over-expression of multidrug resistance gene 1 (MDR1) which results in increase in efflux pumps such as p-glycoprotein leading to a decreased intracellular concentrations and overall exposure to anthracycline chemotherapy, hence rendering the resistance to drugs [18, 19]. Other plausible mechanisms of resistance include expression of proteins conferring multidrug resistance such as multidrug resistance-associated protein 1 (MRP1) and lung resistance protein (LRP) [20, 21]. Of these, MDR1 expression and the resultant drug efflux has been reported to increase with increasing age from 17% in age <35 years while being 39% in age >50 years, which at least partly contributes to decreased responses to chemotherapy in s-AML which is commonly seen in older patients [19]. Additionally, patients with s-AML are older and may have been treated previously with cytotoxic agents, both of which can possibly limit the ability to utilize high dose or cytotoxic chemotherapy to treat s-AML [16, 22].

To summarize, understanding the biology as well as management strategies for s-AML is important in treatment planning and risk-stratification as the incidence of this diagnosis is anticipated to rise, response as well as survival in these patients is inferior compared to de novo AML, and treatment options can be limited by age or other patient factors.

3.3 Biology and Genomics of Secondary AML: Are They Different and How?

Clonal hematopoiesis plays an important role in the development of s-AML from both an AHD and in t-AML [23, 24]. It is now being realized that clonal hematopoiesis exists early, likely even prior to exposure to cytotoxic therapy in t-AML. Subsequent exposure to chemotherapy results in selection of a drug resistant preleukemic clone. Mutant *TP53* clones have been found years prior to chemotherapy exposure or diagnosis of t-AML [25, 26]. Recent studies have also shown that both hematopoietic and stromal compartments of the bone marrow are involved in the malignant transformation of hematopoiesis and that mesenchymal stem cells undergo remodeling upon exposure to MDS cells [27].

3.3.1 AML-AHD from Preceding MDS

Transformation of MDS to AML (AML-MDS) is mediated by clonal evolution or increase in the number of mutations as well as expansion of existing mutant clones. A variety of cytogenetic abnormalities and mutations in genes affecting splicing machinery and chromatin modifiers have been reported more commonly in AML-MDS than in de novo AML, including *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, and *STAG2* [28]. Paired sampling of MDS and AML-MDS bone marrow samples suggested that at least one new driver mutation occurs in most (59% in the study) patients in the process of transformation, except in patients with *TP53* mutation [28]. Most of these mutations were in genes encoding myeloid transcription factors (*RUNX1*, *CEBPA*, *GATA2*) and signal transduction proteins (*FLT3* or *RAS*). Another study comparing samples of patients with s-AML versus high-risk MDS showed enrichment of *NRAS*, *FLT3*, *WT1*, *NPM1*, *IDH1/2*, *PTPN11* genes in s-AML [29]. Whole exome sequencing studies have demonstrated that mutations in signaling pathways (such as *NRAS* and *PTPN11*) occur or expand significantly during transformation of disease [30]. This was, however, not observed for mutations associated with DNA methylation or splicing machinery, which were noted to expand at the initial stages of MDS but not at the time of progression to s-AML. Another study using a comprehensive transcriptome sequencing in CD34+ bone marrow cells identified two subgroups of MDS by gene expression profiling: one with increased expression of

genes in the erythroid/megakaryocytic lineage and the second with upregulation of genes related to immature progenitor cells. The later demonstrated upregulation of various signaling pathways and downregulation of pathways related to metabolism and DNA repair and was exclusively associated with leukemic transformation and shorter survival [31].

Identification of the above-mentioned mutations in patients with MDS may herald emerging AML subclones and potentially identify patients at risk for transformation to s-AML.

3.3.2 AML-AHD from MPN

MPN are another set of myeloid disorders that can potentially transform into AML (AML-MPN) with a rather dismal prognosis [32]. In a single-center study of 91 patients, a clonal abnormality was identified in 91% patients including complex karyotype (54%), core binding factor (CBF) gene mutations (3%), and chromosome 5 or 7 abnormalities (32%) [32]. The three known driver mutations in myelofibrosis, *JAK2V617F*, *CALR* or *MPL*, also impact time to leukemia transformation. Mutations in *CALR* were associated with lesser risk of transformation when compared with "triple-negative" (absence of all three mutations) and *JAK2V617F*, but with no difference compared to mutations in *MPL* [33]. Subsequently, genomic profiling of samples from patients with AML-MPN has delineated differences from genetics patterns of de novo AML. Recurrent point mutations in *TET2*, *ASXL1*, *SRSF2*, *TP53*, and *IDH1/2* have been reported to be more common in AML-MPN than in de novo AML [34–37]. Single-nucleotide polymorphism (SNP) array analysis of 88 chronic phase MPN and 71 MPN-blast phase samples showed three-times higher genomic changes in the latter [38]). Aberrations in chromosomes 3p (*FOXP1*), 4q (*TET2*), 7p (*IKZF1*), 7q (*CUX1*), 8q (*MYC*), 12p (*ETV6*), 17p (*TP53*), 21q (*RUNX1*) were seen more commonly in AML-MPN samples (MPN-blast phase) than in chronic phase [38, 39]. Genomic profiling of AML-MPN samples demonstrated a higher frequency of somatic *TP53* mutations accompanying *JAK2V617F* mutations, in contrast to chronic phase MPN samples where *TP53* mutations were less frequent [40]. *TP53* loss in combination with *JAK2V617F* mutation led to expansion of blasts, in both the bone marrow and the peripheral blood, and fully penetrant AML in murine models, thus biologically validating these genomic observations.

Collectively, these data demonstrate that recurrent mutations in epigenetic regulators, transcription factors, spliceosome complex members as well as *TP53* have been associated with the leukemia transformation of MPNs. This mutational spectrum appears to differ from that observed in de novo AML, thus potentially explaining biological differences and clinical behavior of AML-MPN.

3.3.3 t-AML

t-AML has a high representation of high-risk or unfavorable cytogenetic abnormalities and somatic mutations. In a series of 306 patients with t-MDS/t-AML from a single center, over 70% patients had del(5q), del(7q), or monosomy 7 [7]. Of these, chromosome 7 abnormalities [del(7q) and monosomy 7] occur in almost half of these patients and is associated with mutations that activate the RAS pathway [41, 42]. *TP53* mutations occur in up to 37% of patients with t-AML and can be associated with del(5q) as well as complex karyotype [28, 43]. Around one-third patients with t-AML can harbor mutations in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, or *STAG2* [28]. Additionally, association of *MLL* rearrangements and exposure to topoisomerase II has been described above.

Overall, the genetic composition of t-AML is notable for a higher frequency of unfavorable mutations that are associated with poor response to therapy and inferior outcomes.

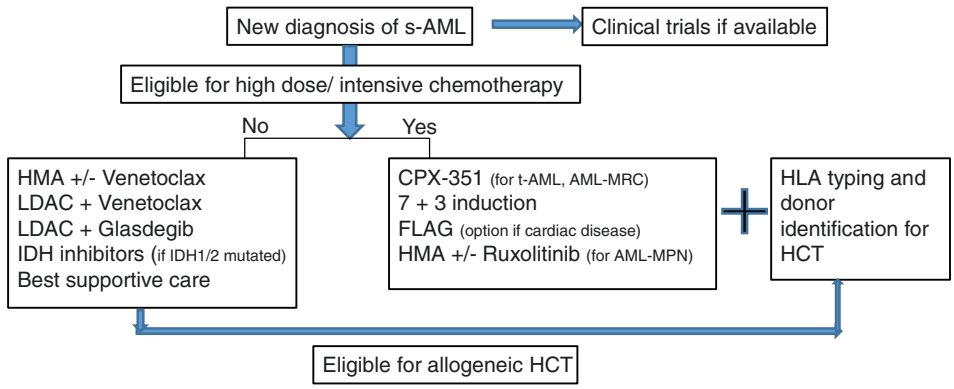
3.4 Management of s-AML: How to Think of Treatment Options?

The overall prognosis of patients with s-AML remains poor whether they arise from an antecedent MDS, MPN, or t-AML; and treatment options have not changed significantly in over four decades. A proposed treatment schema with contemporary options is depicted in Fig. 3.1.

3.4.1 Induction and Chemotherapy: Past, Present, and Future

Anthracycline-based therapy, in combination with cytarabine, in the historic "7+3" regimen has remained the traditional therapy for s-AML, although response rates have been lower than that with de novo AML [13, 14, 44]. While both AML-AHD and t-AML show inferior outcomes compared to de novo AML, s-AML arising from AHD other than MDS was associated with an even lower survival in patients over 60 years of age with adverse karyotype [13].

CPX-351 is a novel agent, recently approved by Food and Drug Administration (FDA) and European Medicine Agency (EMA), for treatment of newly diagnosed t-AML or AML-MRC. It is the dual-drug liposomal encapsulation of cytarabine and daunorubicin at a fixed 5:1 molar ratio at which maximal synergistic activity had been demonstrated in preclinical models [45–47]. The encapsulated liposomal formulation improves pharmacokinetic characteristics and allows for intracellular delivery which is enhanced in leukemia cells compared to normal cells. The particular benefit of CPX-351 in patients with s-AML was seen in a randomized phase 2 study conducted in patients with newly diagnosed AML of ages 60–75 years comparing CPX-351 with the standard 7+3 regimen [48]. While a statistical significant advantage in overall survival or event-free survival was not seen for the overall study population, a planned subgroup analysis in high-risk patients including those with s-AML showed a significantly superior overall survival in this subgroup (HR 0.46, $p = 0.01$). These findings were confirmed in a phase 3 trial of patients with ages 60–75 years with newly diagnosed t-AML or AML-MRC. The study showed significantly superior overall survival compared to 7+3 induction (9.56 vs 5.95 months, HR 0.69, $p = 0.003$) along with



FLAG, fludarabine, cytarabine, plus G-CSF; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; HMA, hypomethylating agents; IDH, isocitrate dehydrogenase; LDAC, low dose cytosine arabinoside (cytarabine); s-AML, secondary acute myeloid leukemia; t-AML, therapy related AML; AML-MRC, AML with myelodysplasia related changes

Fig. 3.1 Treatment schema for secondary AML

higher remission rates (47.7% vs 33.3%, $p = 0.016$) and improved event-free survival (2.53 vs 1.31 months, HR 0.74, $p = 0.021$) [49]. An exploratory landmark analysis in patients who underwent allogeneic stem cell transplantation (HCT) after induction was notable for a significantly favorable overall survival with CPX-351 (median not reached vs 10.25 months, HR 0.46, $p = 0.009$). Although this analysis was influenced by confounding factors such as that the decision to undergo HCT was not randomized, these results do establish CPX-351 as a suitable treatment option as a bridge to HCT.

Azanucleosides are pyrimidine analogs which at lower doses have established themselves as potent inhibitors of DNA methylation and are commonly referred to as hypomethylating agents. Due to the noted significance of DNA methylation in transformation of MDS to AML, hypomethylating agents have an established role in treatment of patients with s-AML especially those who are not candidates for high dose chemotherapy [50, 51]. The two commonly used hypomethylating agents are 5-azacytadine and 5-aza-2′-deoxycytidine (or decitabine). Azacitidine was FDA approved for use in MDS based on data from the AZA-001 trial which included patients with up to 30% blasts, which would now be classified as AML for >20% blasts, and showed improved survival compared to best supportive care [52]. Subsequently, a phase 3 study (AZA-AML-001) was conducted in patients with AML, including AML-AHD and AML-MRC, who were ≥65 years old and continued to show improved overall survival with azacitidine versus best supportive therapy (median, 8.9 vs 4.9 months, HR 0.74) [53]. Similarly, decitabine was compared to conventional therapy in older patients with AML, resulting improved response rate (17.8% vs 7.8%, OR 2.5, $p = 0.001$) although no statistical improvement in survival was reported in the primary analysis (7.7 vs 5 months, $p = 0.108$) until an unplanned analysis a year later (HR 0.82, $p = 0.037$) [54]. Both azacitidine and decitabine have been reported to have responses in AML-MPN although most reports are relatively small series. Decitabine showed responses in 6/21 (29%) patients with AML-MPN, including one partial response, with the response lasting a median of 7 months [55]. Azacitidine therapy resulted in an overall response in 10 (38%) with complete response in 2 (8%) of the 26 patients with AML-MPN, with median duration of response of 9 months [56]. Ruxolitinib, a selective *JAK1* and *JAK2* inhibitor, as a single agent also has moderate activity with responses seen in 3/18 (17%) patients with AML-MPN, of which 2 were complete responses [57]. Given the individual activity with these agents and the synergistic activity demonstrated in murine models of *JAK2V617*-driven AML, a phase 1 trial was conducted using a combination of decitabine and ruxolitinib in patients with accelerated phase (10–19% blasts in peripheral blood or bone marrow) and blast phase (>20% blasts in peripheral blood and bone marrow) [58]. No dose-limiting toxicity was observed, and overall responses were seen in 5/13 (39%) patients with AML-MPN.

The more recent success story in the treatment paradigm of AML is venetoclax, an orally administered B-cell leukemia/lymphoma-2 (bcl-2) inhibitor, that has been studied in various combinations with hypomethylating agents as well as low-dose cytarabine in older patients with AML who are not candidates for intensive therapy. Approximately one quarter to half of the patients in these studies have s-AML and have shown responses ranging from 8% to 35% in these patients when utilized as upfront therapy [59–61]. It should be noted though that in these studies with venetoclax/hypomethylating agent combinations, no patients with prior hypomethylating agent exposure were included (Table 3.2).

3.4.2 Transplantation or No Transplantation? The Perpetual Enigma

Prospective or randomized studies to compare outcomes with versus without HCT do not exist and are unlikely to be conducted. Studies to date show variable outcomes regarding this, which is likely the result of patient selection in these sin-

Table 3.2 Broad therapeutic categories in secondary AML

	Study	Number of patients	CR/CRi	Median OS	Clinical pearls
CPX-351 (cytarabine:daunorubicin 5:1)	Phase 2 study (compared to 7+3) [48]	Total *N* in CPX-351 arm = 85 s-AML = 33 (39%)	58% in s-AML	12.1 months	Prolonged cytopenias, neutropenic fever but without infection-related deaths
	Phase 3 study (compared to 7+3) [49]	Total *N* in CPX-351 arm = 153 t-AML = 30 (20%) AML-MDS = 71 (46%) AML with preceding CMML = 11 (7%) AML with MDS related cytogenetic abnormalities = 41 (27%)	48% in s-AML	9.5 months	
Hypomethylating agents (±V)	Aza, randomized [53]	Total *N* in Aza arm = 129 AML-MDS = 44 (34%) AML-MRC = 72 (56%) (overall s-AML = 100%)	24.8% in s-AML	8.9 months in s-AML	Cytopenias/myelosuppression
	Aza, retrospective [56]	*N* with AML-MPN in Aza arm = 26	12% in AML-MPN	8 months in AML-MPN	
	Dec, open label phase 3 [54]	Total *N* in Dec arm = 242 s-AML = 87 (36%)	26% overall	7.7 months	Myelosuppression/febrile neutropenia
	Dec, retrospective [55]	Total *N* = 45: AML-MPN = 21	24% in AML-MPN	6.9 month in AML-MPN (10.5 months in responders, 4 months in non-responders)	
	V + Aza/ Dec, phase 1b [59]	Total *N* = 145 s-AML = 36 (25%)	67% in s-AML	Not reached (95% CI, 14.5 months—not reached)	Cytopenias; no tumor lysis observed; patients with prior HMA exposure excluded
	V + Dec10, phase 2 [60]	Total *N* = 48: s-AML = 7 (15%)	71% in s-AML	Not reached (95% CI, 1.8 months—not reached)	Neutropenia, febrile neutropenia, 4% patients had tumor lysis syndrome

(continued)

Table 3.2 (continued)

	Study	Number of patients	CR/CRi	Median OS	Clinical pearls
Rux ± HMA	Rux, phase 2 [57]	Total *N* = 38 AML-MPN = 18 (47%)	17% in AML-MPN	–	Thrombocytopenia
	Dec + Rux, phase 1 [58]	Total *N* = 21 AML-MPN = 13 (62%)	19% overall (in AML-MPN and MPN-AP)	7.2 months (95% CI, 2.2—not reached)	Myelosuppression, pneumonia
Low dose cytarabine + V	V + LDAC, phase 1b/2 [61]	Total *N* = 82 s-AML = 40 (49%)	35% in s-AML	10.1 months for overall cohort	Venetoclax dose interruptions between subsequent cycles in 55% patients, due to delayed neutrophil (10%) and platelet (12%) recovery

AML acute myeloid leukemia, *AML-MDS* acute myeloid leukemia transformed from underlying myelodysplastic syndrome, *AML-MPN* acute myeloid leukemia transformed from underlying myeloproliferative neoplasm, *Aza* azacitidine, *CI* confidence interval, *CMML* chronic myelomonocytic leukemia, *Dec* decitabine, *Dec10* decitabine 10 days, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *MPN-AP* MPN-accelerated phase, *N* number of patients, *Rux* ruxolitinib, *s-AML* secondary AML, *t-AML* therapy related AML, *V* venetoclax

gle institution or retrospective studies, but do favor improved outcomes in patients who undergo HCT after achieving a complete response [62–67]. While HCT remains the only potential curative option, s-AML has been identified as an independent risk factor for poorer outcomes after HCT [68]. Hence, careful consideration to the various clinical and genetic factors that impact HCT outcomes is warranted to identify who would benefit most from an HCT. Data from the Center for International Bone Marrow Transplant Research (CIBMTR) using HCT data between 1990 and 2004 for t-AML or t-MDS and from European Society for Blood and Bone Marrow Transplantation (EBMT) of HCTs performed between 2000 and 2016 for s-AML have demonstrated inferior survival with advancing age at HCT, poor risk cytogenetics, active disease at HCT, and alternative donors (other than HLA-identical sibling or partially or well-matched unrelated donor) [69, 70]. Among genetic markers, a study from Japan Marrow Donor Program, that included 24% patients with s-AML showed that patients with mutations in *NRAS* (HR 1.64, $p = 0.0075$), *TP53* (HR 1.49, $p = 0.0096$), *CBL* (HR 1.55, $p = 0.024$) as well as complex karyotype (HR 1.45, $p = 0.046$) had particularly inferior outcomes post-HCT [68].

Emergence of therapeutic options prior to HCT increases the optimism for ability to achieve deeper remissions and possibly improving the outcomes from HCT. The possibility of incorporation of the novel therapies as maintenance strategies after HCT and availability of these drugs for treatment of relapse post-HCT further pave the way to utilize the graft-versus-leukemia in combination with the targeted agents.

3.5 Future Directions

Conventional therapeutic options have demonstrated limited activity in s-AML. Identification of novel recurrent somatic mutations in MDS, MPN, as well as post-MPN AML have helped to improve our understanding of disease biology, as well as to elucidate novel therapeutic possibilities. An example is the presence of *IDH1/2* mutations that is seen in over 20% patients with AML-MPN (versus around 4% in chronic phase MPN) [71, 72]. Ivosidenib and enasidenib have shown promising activity in relapsed/ refractory AML with *IDH1* and *IDH2* mutations, respectively [73, 74]. Whether these agents can be used in combination with other active agents in patients with s-AML as front-line therapy is being actively studied with encouraging early results. As mentioned above, mutations in *TP53* are also enriched in patients with progression to AML following MDS or MPN, which presents a particularly challenging problem. The role of investigational drugs such as APR-246, with the potential ability to restore the function of point mutant *TP53* in tumor cells, is currently being explored in *TP53* mutated myeloid malignancies in combination with azacitidine (NCT 03072043). Additionally, novel venetoclax-based or CPX-351-based combination studies with targeted therapies are in progress. Collectively, these recent biological and clinical insights have the potential to alter the historically poor clinical course of patients with s-AML, and hopefully yield better options and outcomes.

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