Review

**TP53 in Myelodysplastic Syndromes: Recent Biological and Clinical Findings**

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Abstract: TP53 dysregulation plays a pivotal role in the molecular pathogenesis of myelodysplastic syndromes (MDS), identifying a subgroup of patients with peculiar features. In this review we report the recent biological and clinical findings of TP53-mutated MDS, focusing on the molecular pathways activation and on its impact on the cellular physiology. In MDS, TP53 mutational status is deeply associated with del(5q) syndrome and its dysregulation impacts on cell cycle, DNA repair and apoptosis inducing chromosomal instability and the clonal evolution of disease. TP53 defects influence adversely the MDS clinical outcome and the treatment response rate, thus new therapeutic approaches are being developed for these patients. TP53 allelic state characterization and the mutational burden evaluation can therefore predict prognosis and identify the subgroup of patients eligible for targeted therapy. For these reasons, in the era of precision medicine, the MDS diagnostic workup cannot do without the complete assessment of TP53 mutational profile.

Keywords: TP53 mutation; p53 expression; myelodysplastic syndrome; del(5q); prognosis; target therapy

1. Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell (HSC) malignancies characterized by bone marrow dysplasia, ineffective hematopoiesis leading to peripheral blood cytopenia, and by the risk of acute myeloid leukemia (AML) transformation [1]. MDS are a group of diseases with a high degree of variability in terms of prognosis, clinical phenotype and response to treatment. This heterogeneity can often be associated to a high genotypic variability among affected individuals, highlighted in the past decade owing to the application of new high throughput technologies, including microarray analysis and next-generation sequencing (NGS) [2,3]. Large-scale analysis of the molecular mechanisms of the disease has enabled the identification of a set of genes that are recurrently mutated in MDS. They are involved in different cellular processes, such as histone modification (e.g., ASXL1, EZH2) and DNA methylation (e.g., TET2, DNMT3A, IDH1, IDH2), signal transduction (e.g., NRAS, JAK2), transcriptional regulation (e.g., RUNX1, TP53), and RNA splicing (e.g., SF3B1, SRSF2, U2AF1, ZRSR2) [4,5]. In this variety of genes, the Homo sapiens tumor protein p53 (TP53) dysregulation plays a crucial role in MDS phenotype, treatment response, and risk of AML transformation [6,7].

TP53 is a tumor suppressor gene that spans 19,144 bp on chromosome 17p13.1 and contains 11 exons. The protein has five functional domains: The transactivation domain and a proline-rich domain in the N-terminal region; the oligomerization domain and a regulatory domain in the C-terminal...
region; the DNA-binding domain (DBD) in the central core \[8,9\]. The protein is an essential transcription factor for cell cycle arrest, DNA repair mechanisms, apoptosis induction, and cellular differentiation regulation \[10,11\]. \TP53\ plays a pivotal role in the cellular apoptotic response to DNA damaging agents, such as cytotoxic chemotherapy and its dysregulation is generally associated with a negative prognostic impact in oncologic diseases \[12,13\]. \TP53\ is the gene most closely studied in cancer, and its role is widely documented in different hematological malignancies: in lymphoid neoplasms such as chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL) and in myeloid diseases such as AML \[14\].

Herein we address \TP53\-mutated MDS, summarizing the recent biological and clinical findings in this patients subgroup. A first section is reserved to the molecular aspects of \TP53\ dysregulation: acquired or constitutive mutations and protein expression, with a special focus on cellular pathways activation and on \TP53\ correlations with karyotype aberrations. The prognostic value of \TP53\ and its influence on treatment decision-making is also discussed, considering the emerging therapeutic strategies that are currently being developed.

2. Biological and Molecular Aspects

2.1. Molecular Pathways Activation

\TP53\ is the most commonly mutated gene in human cancer. Its mutational state in MDS is strongly associated with solitary del(5q) (~20%), or complex karyotypes (CK) with -5/5q- (~70%) \[15,16\]. For this reason, the majority of studies has explored the association of p53 to del(5q) MDS. Deletion of the long arm of chromosome 5 causes the loss of 1.5 megabases, the “commonly deleted region” (CDR), comprising 41 genes situated close to or within 5q32-33 \[17–19\]. Among all the 41 genes in the CDR, those that may play pivotal roles in tumorigenesis include: \RPS14\, which is important in ribosomal function and RNA synthesis, \miR145\ and \miR146\, that intervene in innate immunity and signaling, \CDC25c/PP2A\, a phosphatase that regulates cell division, \SPARC\, that mediates adhesion and \EGR1\ and \DIAPH\, which act as tumor suppressor and cytoskeleton organizer, respectively \[18–20\]. Only with \RPS14\ gene suppression were the maturation and proliferation of erythroid precursors halted, reproducing the del(5q) syndrome phenotype \[18\]. Moreover, \RPS14\ haploinsufficiency was correlated to an enhanced p53 expression in an in vivo model, together with age-dependent progressive anemia, dysmegakaryopoiesis, modification of the stem cell niche, and loss of hematopoietic stem cell quiescence \[21\]. Additional studies demonstrated that after blocking Murine Double Minute-2 (MDM2) using the small molecule Nutlin, p53 was stabilized and activated, a condition that compromised erythropoiesis in a similar way to del(5q) MDS \[22,23\]. In normal conditions, MDM2 is free to bind p53 and MDM2-p53 binding determines p53 ubiquitination and consequent degradation, in a normal cell cycle (Figure 1A). \RPS14\ haploinsufficiency in del(5q) MDS triggers ribosomopathies typified by nucleolar stress, in which ribosome assembly is impeded and small ribosomal proteins (RPs) do not bind to 40S and 60S ribosomal subunits, but are free to bind to MDM2. MDM2-RPs binding prevents MDM2-p53 interaction, resulting in p53 stabilization. This abnormal accumulation of p53 leads to cell cycle arrest, impaired DNA repair, senescence, and apoptosis (Figure 1B). Apoptosis in maturing erythroblasts occurs at the step converting polychromatic to orthochromatic erythroblasts, provoking erythroid hypoplasia, a typical feature of del(5q) MDS \[21\]. Moreover, cytotoxic stresses activate the phosphorylation of both MDM2 and p53 by ATM-Chk1 or ATM-Chk2, thus activating other post-translational modifications, including acetylation, methylation, or sumoylation of MDM2, which reinforce p53 activity \[24,25\] (Figure 1B). In this altered pathway, lenalidomide treatment can intervene, although \TP53\ mutations in del(5q) MDS alter treatment responses and increase the risk of leukemia transformation \[26\].
In del(5q) MDS, a haploinsufficient expression of CK1a plays an important role in the disease pathogenesis and treatment response rate. In fact, lenalidomide induces the degradation of p53 and p53-mediated apoptosis of del(5q) cells because of their haploinsufficient expression of CK1a [27]. Among its various mechanisms, lenalidomide induces proteasome degradation of CK1a, allowing a normal cell cycle. (C) A non del(5q) MDS cell with a low p53 SNP activity in which the MDM2 SNP309 “G” allele enhances MDM2 expression and p53 “C” allele has an improved apoptosis-promoting potential, in part for its major mitochondrial positioning, activating cytosolic liberation of cytochrome C. This condition determines in patients a significantly lower overall survival (OS) and progression-free survival (PFS). (D) Somatic TP53 mutations are related, in MDS patients, with p53 gain of function and dominant negative effect, chromosomal instability, chromotripsis, and clonal evolution.

Among its various mechanisms, lenalidomide induces proteasome degradation of CK1a, the protein product of CSNK1A1, one of the CDR genes [27,28]. Through its interactions with MDM2 and with the b-catenin destruction complex, CK1a negatively regulates both p53 and b-catenin protein levels. In del(5q) MDS, a haploinsufficient expression of CK1a plays an important role in the disease pathogenesis and treatment response rate. In fact, lenalidomide induces the degradation of CK1a, which can be tolerated by normal cells with two copies of CSNK1A1, but results in p53-mediated apoptosis of del(5q) cells because of their haploinsufficient expression of CK1a [27]. Furthermore, the overexpression of CSNK1A1 reduces the lenalidomide sensitivity of BM cells in patients with del(5q) MDS [27].

Few studies have been focused on the role of p53 in non del(5q) MDS. In 2015, two studies explored the influence of single-nucleotide polymorphisms (SNP), in particular on TP53 R72P and MDM2 SNP309. The TP53 R72P mutation features an amino acid variant on the tertiary structure of the p53 binding domain, causing functional variations. The R/R homozygous genotype, because of having a “C” allele, has a better apoptosis-promoting potential than the P/P genotype, in part because of its major mitochondrial positioning, activating cytosolic release of cytochrome C. Instead, the homozygous P/P genotype shows a higher transcriptional efficacy than the R/R genotype, inducing the cell to halt the cell cycle at the G1 level [29]. Moreover, the MDM2 SNP309 “G” allele enhances...
MDM2 expression and consequently decreases p53 expression. When predicting p53 activity (based on TP53 R72P and MDM2 SNP309), it was demonstrated that non-del(5q) MDS patients with a high p53 SNP activity had a significantly longer overall survival (OS) and progression-free survival (PFS) compared to patients with a low p53 SNP activity [6,29,30] (Figure 1C). It is noteworthy that in chronic myeloid leukemia, the same mechanism could be the basis of the therapeutic resistance shown by patients treated with different lines of tyrosine kinase inhibitors treatment [31,32].

Although it is difficult to identify how p53 deregulations in MDS modify pathways within cancer cells, other studies have attempted to correlate the role of p53 in MDS to chromosomal instability and chromothripsis. Chromosomal instability is linked to MDS and it has been hypothesized that MDS arise primarily from DNA repair defects. The incidence of MDS, in fact, is considerably higher in older people and in patients with genetic defects in DNA repair, like Fanconi anemia and Bloom, Werner and Rothmund-Thomson syndromes [33]. Furthermore, almost 50% of patients with MDS exhibit genetic rearrangements and there is evidence that chemo- and radiation therapies dramatically augment the risk for MDS [33]. At first, a xenograft model was used to try to understand the MDS generation of chromosomal instability [34]. Unfortunately, in vitro and xenotransplanted models have a limited ability to reproduce the natural cancer microenvironment [35], thus, this work did not clarify how inactivation of TP53 directly increases chromosomal instability, inducing CK in MDS, nor how it allows chromosomally unstable HSC to bypass senescence or apoptosis and to survive [34].

However, genetic instability modifies the DNA damage responses (DDR) that are presumably implicated in the pathogenesis of MDS [36]. An increase of double-strand breaks (DSB) was observed in MDS, together with an impaired DDR, due to an altered pattern of phosphorylated DDR key proteins, including TP53. A reduced expression of TP53 after phosphorylation presumably indicated impaired downstream signaling of DNA damage and/or defects in downstream components of the DDR [36].

Chromothripsis, instead, is a genetic aberration in which tens to hundreds of clustered genomic rearrangements occur in a one-step catastrophic event [37]. The molecular foundation of this genomic chaos has long been studied, and several possible mechanisms suggested [37,38]. One of these is somatically acquired TP53 mutations. In fact, approximately 50% of AML patients carrying TP53 mutations displayed chromothripsis, and almost all medulloblastomas showing chromothripsis had TP53 mutations [38]. Most of these mutations were mapped on exon 5 of TP53, and all of them were localized in the DBD, which plays a pivotal role in transcriptional transactivation [39]. Chromothripsis was analyzed in 301 MDS samples, carrying out genome-wide analysis of DNA copy number abnormalities and mutational analysis by NGS [39]. Cryptic genomic abnormalities were found in 23.6% of cases, detected mainly in patients with a normal (45%) or non-informative (15%) karyotype by conventional cytogenetics. TP53 deletion and mutation (15%) was an exception, being identified in patients with a complex karyotype. Three (1.2%) high-risk MDS cases (two RAEB-1, with 6% and 8% of BM blasts, respectively, and one RAEB-2, with 12% of BM blasts) displayed chromothripsis and all carried TP53 mutations, underlining the association between TP53 gene alteration and chromosomal abnormalities, and chromothripsis [39] (Figure 1D).

Moreover, TP53 was also investigated for its role during MDS disease progression to AML, that occurs in about 30% of patients [40]. Both linear and branching patterns of evolution have been described: In linear evolution, serial dominant clones appear after the acquirement of supplementary mutations, overgrowing their ancestral clone; branching evolution is, instead, characterized by the appearance of various subclones from one common ancestral clone, and by the coexistence of kindred (sub)clones that carry a partially overlapping set of mutations [40,41]. Furthermore, a “clone sweeping” pattern was proposed, in which a new or a pre-existing subclone sweeps out the other clones and predominates throughout the progression. In this pathway, different kinds of mutations decide the progression fate: “type 1 mutations” cause the transformation from MDS to AML, and “type 2 mutations,” that include TP53, favor the progression from lower-risk to higher-risk MDS [42]. Starting with the initial mutations, subsequent hits are not random, but occur in a specific order and some mutation conjunctions occur more frequently [43]. For example, TP53 dominant mutations were more
likely to precede secondary TP53 mutations, but less likely to precede secondary ASXL1 mutations [43]. Indeed, several TP53 independent clones may coexist, highlighting a broad genetic intratumoral heterogeneity of human tumors. It was observed that all oncogenic TP53 variants were localized in alternative alleles, and each TP53 variant was presumed to belong to an autonomous subclone arising from a wild-type (WT) TP53 founder clone, that requires TP53 inactivation for further progression. All of these subclones present a great dynamic evolution, but it is not known whether this evolution is triggered by treatment, by an intrinsic property of the tumor cells or both [44] (Figure 1D).

More recently, it has been observed that the loss of heterozygosity (LOH) is a frequent but not imperative step during clonal evolution of tumors with TP53 missense mutations [45]. Normally, a tumor suppressor gene requires a biallelic inactivation, and this unusual TP53 behavior has led to the formulation of two hypotheses: the first contemplates an oncogenic gain of function (GOF) of TP53 missense mutants [46], the second envisages a dominant negative effect (DNE), that leads to a selection for TP53 missense mutations, with a non-mutational impairment of the remaining WT allele [47]. To verify the two hypotheses, isogenic human leukemia cell lines of the most common TP53 missense mutations were generated, employing CRISPR-Cas9 [48]. Functional, DNA-binding, and transcriptional analyses highlighted loss of function but no GOF effects. Instead, missense variants in the DBD exert a dominant negative effect, demonstrated through comprehensive mutational scanning of p53 single-amino acid variants and also in mice, where the DNE of p53 missense variants bestows a selective DNA damage benefit to hematopoietic cells [48]. It has also been suggested that p53 GOF is not an autonomous cell phenomenon [35]. The p53 mutant protein establishes a new relationship with the microenvironment, which is in turn conditioned by other extrinsic factors. Thus, a new regulatory circuit is constituted between cancer cells and the microenvironment, in which p53 mutants lie at the molecular heart, and are crucial for the outcome. Solid in vivo evidence revealed the existence of p53 GOF but no in vitro evidence exists because of the impossibility of reproducing the tumor microenvironment. Furthermore, p53 mutant proteins respond to extrinsic signals, becoming stabilized. Thus, a convergence of mechanisms retained from the WT protein, and newly gained by the mutant protein, activate the p53 GOF mutant, and the GOF effects might depend on the degree of activation of the p53 mutant protein [35] (Figure 1D).

A p53 mutant GOF was revealed in clonal hematopoiesis of indeterminate potential (CHIP) [49]. CHIP appears when a single mutant hematopoietic stem and progenitor cell (HSPC) contributes to a considerable clonal amount of mature blood lineages. This condition is common in aged healthy individuals but it is linked to an increased risk of hematological neoplasms, such as MDS and AML [49,50]. A diagnosis of CHIP requires the presence of a somatic mutation with a mutant allele fraction of at least 2% in the peripheral blood and no other evidence of a hematological malignancy [51,52]. TP53 gene ranks in the top five among genes mutated in CHIP and its mutational state in CHIP is similar to that of hematological malignancies. Indeed, approximately 90% of TP53 somatic mutations in CHIP are missense variants in the DBD of the p53 protein. It was shown that mutant p53, but not WT protein, engages EZH2, a key component of Polycomb repressive complex 2 (PRC2), conferring a competitive advantage to HSPCs. In fact, the p53-EZH2 bond reinforces the EZH2 linkage with the chromatin, catalyzing the trimethylation of lysine 27 of histone H3 (H3K27me3) in genes regulating HSPC self-renewal and differentiation [49].

Lastly, in MDS, a TP53 dysregulation resulting from a position effect, as described for other genes in several myeloid neoplasms [33], has never been proved. In such a scenario, TP53 activity, and its pathological dysregulation, play a pivotal role in the molecular pathogenesis of MDS. As already mentioned, TP53 pathway alterations have an impact not only on the biology of the cell but also on the clinical onset and the evolution of the disease, identifying a subgroup of patients with similar features. These issues will be developed below.
2.2. TP53 Allelic State and Mutational Burden

The majority of TP53 mutations are missense variants clustering within the DBD. Consistent with its role as a tumor suppressor, bi-allelic targeting is very frequent. In fact, more than 91% of TP53-mutant cancers exhibit second allele loss due to mutation, chromosomal deletion (involving 17p13 locus), or copy-neutral LOH (cn-LOH) [54]. The TP53 allelic state was recently studied in a cohort of 3324 MDS patients, at diagnosis and treatment naïve; 486 mutations were identified across 378 individuals [55]. Four main TP53 mutational profiles were identified: 1. Mono-allelic mutation \( (n = 125, 33\% \text{ of TP53-mutated patients}) \); 2. multiple mutations without deletion or cnLOH affecting the TP53 locus \( (n = 90, 24\%) \); 3. mutation(s) and concomitant deletion \( (n = 85, 22\%) \); 4. mutation(s) and concomitant cnLOH \( (n = 78, 21\%) \). Additionally, in 24 (0.7%) patients, the TP53 locus was affected by deletion \( (n = 12) \), cnLOH \( (n = 2) \) or isochromosome 17q rearrangement \( (n = 10) \), with no evidence of TP53 mutations. The study showed that the majority (67%) of TP53-mutated MDS patients presents a multiple hit consistent with bi-allelic targeting. The authors demonstrated that, even if TP53 is universally considered as an adverse prognostic biomarker, only the multi-hit TP53 state in MDS is associated with genome instability and the worst clinical outcome, not the bare presence of any TP53 mutation [55]. For these reasons, the MDS diagnostic workup needs to be implemented with a more accurate characterization of the TP53 allelic state. For this purpose, karyotype analysis, still performed by conventional and molecular cytogenetics, must be coupled with NGS approaches, finalized to study the copy-number status of the TP53 gene, its mutational profile, and the variant allele frequency (VAF) of the mutations identified. Moreover, it has been widely demonstrated that the TP53 mutational burden can affect prognosis in MDS [56–58]. A recent study shows the impact of TP53 VAF on the MDS phenotype and outcomes in 219 patients with MDS [56]. MDS patients with a VAF > 40% had a median OS of 124 days; the same OS was not reached in patients with VAF < 20% \( (p < 0.01) \), as validated in an independent cohort \( (p = 0.01) \). TP53 VAF further stratified distinct prognostic groups independently of clinical prognostic scoring systems [56]. The TP53 mutational burden was later studied in a cohort of 154 lower-risk MDS patients, again demonstrating its role in clinical outcome [57]. In fact, evaluation of the OS determined a 6% TP53 VAF threshold as an optimal cut-off for patient stratification. At diagnosis, the median OS was 43.5 months in MDS patients with a VAF > 6% compared to 138 months in WT patients \( (p = 0.003) \); similarly, the median PFS was 20.2 months versus 116.6 months \( (p < 0.0001) \). In contrast, no significant impact on PFS or OS was observed in MDS patients with a VAF < 6%, who remained stable for long periods without progression [57]. The importance of TP53 VAF was recently demonstrated in a study of 80 MDS patients (and 112 AML patients) who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) [58]. In fact, TP53 and EZH2 mutations with a VAF > 33% were associated with poor relapse-free survival (RFS) [58]. In view of these considerations, assessment of the mutational status of the TP53 gene cannot be limited to the simple presence/absence of any mutation, but requires a more complex evaluation. TP53 allelic state characterization and the mutational burden evaluation must therefore be considered as part of the MDS diagnostic workflow. In view of these considerations, assessment of the mutational status of the TP53 gene cannot be limited to the simple presence/absence of any mutation, but requires a more complex evaluation. TP53 allelic state characterization and the mutational burden evaluation must therefore be considered as part of the MDS diagnostic workflow. To this end, great benefit will be offered by the implementation of NGS technologies. Long-read third generation sequencing, already tested in the hematological field [59] and applied for TP53 mutational analysis in other diseases [60–62], could allow a more rapid and better phasing of TP53 mutations, discriminating between mutations occurring in the same allele (in cis) or in different alleles (in trans), as already demonstrated for other targeted genes [62–64]. However, the accuracy improvement of these emerging technologies will be needed before they will be of clinical utility.
2.3. TP53 Germline Mutations and Familial MDS Predisposition

MDS has conventionally been considered a disease related to aging, with a median age of onset in the 7th decade, consistent with the frequency of age-related clonal hematopoiesis [65,66]. On the contrary, these malignancies in children and young adults are very uncommon; in fact, the annual rate of MDS is 0.2 per 100,000 for patients under 40 years of age but a ~300-fold higher incidence of 58 per 100,000 has been registered in patients over 80 years [67]. Several differences between the phenotype of MDS occurring in young patients and the elderly suggest distinct leukemogenic genetic drivers [68]. Indeed, as long ago as 1990, when Li-Fraumeni syndrome (LFS) was linked to TP53 germline mutations, the first evidence about the genetic basis of familial leukemia began to emerge [69]. Since this discovery, many other genes (ANKRD26, CEBPA, DDX41, ETv6, GATA2, RUNX1, SRP72) associated with a hereditary predisposition to MDS/AML have been described [70]. LFS (OMIM #151623) is an autosomal dominant familial cancer predisposition syndrome caused by TP53 germline mutations [71,72], with a risk of malignant transformation to MDS and AML estimated at 8% [70]. In LFS individuals, the constitutive TP53 defect can impact critically on blood cell development and contribute to the emergence of abnormal hematopoietic clones [73]. Genetic screening of 110 MDS samples collected between 1990–2012 was recently performed to investigate the presence of germlines mutations in bone marrow failure genes (FANCA, GATA2, MPL, RTEL1, RUNX1, SBDS, TERT, TINF2, and TP53). Pathological mutations were identified in 15 of 110 (13.6%) pediatric and young adult patients with MDS, three of which were TP53 constitutional mutations associated to LFS individuals [74]. In 2017, pediatric hematologists-oncologists, geneticists, and genetic counselors, following the Childhood Cancer Predisposition Workshop of the American Association for Cancer Research, released recommendations for the surveillance of individuals with a hereditary predisposition to leukemia. In these patients, tumor surveillance is finalized to early disease detection to allow a prompt initiation of treatment, with the aim of minimizing morbidity and mortality. This is particularly useful for more indolent diseases, such as MDS, that evolve over months to years. In these cases it is often possible to detect progressive cytopenia, bone marrow dysplasia, and the emergence of somatic genetic and cytogenetic abnormalities [73]. In this perspective, centers already performing tumor-only target sequencing for the diagnostic workup of MDS/AML patients are optimizing these tests, to detect germline variants of genes associated with familial forms of MDS/AML (e.g., RUNX1, CEBPA, GATA2, TP53), to maximize detection and reorganize patients management [75].

2.4. p53 Protein Expression

As widely discussed, TP53 mutational analysis by NGS is a fundamental step in the MDS diagnostic workflow, but the approach remains expensive, time-consuming, and limited to few referral centers. On the other hand, immunohistochemistry (IHC) is a fast, reproducible, and cost-effective technology that can be used in every routine laboratory to estimate p53 protein expression in bone marrow core biopsy [76]. In fact, IHC cannot normally detect the WT p53 protein, because of its short half-life. On the contrary, mutated proteins can usually be easily detected in formalin-fixed, paraffin-embedded tissues, because they can accumulate in the nucleus due to their prolonged half-life [77]. Therefore, the IHC detection of p53 protein suggests an underlying mutation in the gene [78]. Furthermore, aberrant expression of the p53 protein was correlated with hemizygous TP53 deletion in lymphoproliferative diseases such as CLL (p < 0.001) and multiple myeloma (MM) (p < 0.001) [79–81]. In MDS, a strong correlation between p53 overexpression and gene mutations (p < 0.05) [15] has been widely documented [15,76,82–84]. Importantly, a recent study also showed the association between p53 expression and the TP53 mutations VAF (r = 0.867, p < 0.001), bone marrow (BM) blast percentage (r = 0.362, p = 0.007), cytogenetic characteristics: 17p abnormalities (p = 0.012), 17p deletion (p = 0.014), 5q deletion (p < 0.001), CK (p < 0.001), and a worse outcome [76]. The prognostic impact of p53 expression in MDS was recently studied [84–86]; its overexpression is associated with a more aggressive clinical outcome and adverse histological prognostic factors, such as BM fibrosis [86,87]. In fact, the degree of BM fibrosis was related to parameters of erythropoietic failure, marrow cellularity,
p53 protein accumulation, WTI gene expression, and serum levels of CXCL9 and CXCL10 [87]. The correlation between BM fibrosis and p53 overexpression supports the hypothesis that patients with BM fibrosis at diagnosis can have a worse clinical outcome [88,89]. In view of these considerations, because sequencing technologies are not always available for TP53 mutational status characterization, p53 ICH should be considered a feasible alternative to TP53 sequencing [76].

2.5. TP53 and Karyotype Aberrations

Karyotype is one of the main components of the International Prognostic Scoring System (IPSS) and revised IPSS (IPSS-R), that dictate the basis for MDS prognostication. Del(5q), −7/del(7q), del(20q), +8, and −Y are the cytogenetic anomalies most abundantly explored in MDS [90]. Deletion of the long arm of chromosome 5 is the unique cytogenetic abnormality that identifies a MDS subset, along with morphological features [91]. Del(5q) MDS is observed in 5–10% of cases, showing severe anemia, neutropenia, and a normal or increased platelet count. These patients display a good prognosis and generally respond to lenalidomide treatment [91]. To establish the pathogenic molecular features associated with del(5q), a set of studies was carried out, sequencing genes related to MDS, such as SF3B1, DNMT3A, TP53, TET2, CSNK1A1, ASXL1, JAK2 [15,91,92]. Many patients showed no mutation, or only one in these genes, and in general, the pattern of mutations was similar to that of other MDS subtypes, except for TP53, which was markedly more often mutated in MDS with isolated del(5q) [15,91,92]. Indeed, TP53 exhibits mutations in about 20% of del(5q) and this condition is generally associated with an unfavorable outcome, an aggressive disease course and a higher risk of transformation to AML [30,93]. Various studies have also recognized a correlation between TP53 mutations and resistance to lenalidomide in del(5q) MDS [93,94], highlighting the appearance and/or increase of TP53-mutant clones. Monitoring TP53 clonal evolution could thus predict disease progression better in these patients than just the detection of TP53 mutations at diagnosis, as also recommended in the WHO 2016 classification [1,95,96].

Mutations in the TP53 gene are also identified in over 70% of MDS patients with CK, defined as three or more somatic chromosomal abnormalities present in a single clone [97]. Approximately 10% of MDS have a CK and these patients represent a heterogeneous group whose OS and disease course are influenced by a wide range of chromosomal abnormalities and somatic mutations [97]. Despite a greater structural genomic instability and a high frequency of TP53 mutations, patients with CK-MDS had less somatic mutations in other MDS-associated genes, and these discrepancies were even more evident in the TP53-mutant subgroup, CK-MDS [97]. TP53-mutant CK-MDS patients also had a notably higher BM blast proportion and lower platelet counts, two factors strongly associated with an elevated prognostic risk according to the IPSS-R [97]. Indeed, TP53-mutant CK-MDS patients had an OS of less than half that of non-mutant CK-MDS, relapsed quickly after different kinds of treatment, and hematopoietic clones with TP53 mutations were enriched after chemotherapy [82,96,98].

A monosomal karyotype (MK), instead, is defined as the existence of two autosomal monosomies or one monosomy with at least one additional structural aberration [99]. Its prognostic effect on MDS patients is still open to question: while several studies suggested that MK was linked to a very low OS [100,101], other studies considered that MK should not be regarded as an independent prognostic factor, because the adverse effects of MK on prognosis may be related to CK [102,103]. However, it was demonstrated that mutations of TP53 cluster with MK and this association has a negative impact on the prognosis of MDS patients [104]. TP53 mutations are not associated with a specific chromosomal deletion, nor significantly associated with chromosome 17 abnormality. Further analysis suggested that the number of mutation sites, co-mutation, clonal architecture, and the VAF of the TP53 mutation in MK-MDS all had no effect on OS [99]. Lastly, although uncommon, TP53 mutations also occur in low-grade MDS with a non-complex karyotype. Although poorly characterized in these cases, TP53 mutations showed a lower VAF and generally, patients with a lower VAF had a better survival [105].
2.6. Therapy-Related MDS

The role of TP53 in the MDS pathogenesis is particularly important in the context of therapy-related MDS (t-MDS). The 2016 WHO classification defines “therapy-related myeloid neoplasms” (t-MNs) as MDS and AML exposed to cytotoxic or radiation therapy for an unrelated malignancy or autoimmune disease [1]. t-MDS and therapy-related AML (t-AML) are classified as one entity because of their similar pathogenesis, rapid progression from t-MDS to t-AML, and their equally poor prognosis [106]. The two main classes of chemotherapy implicated in leukemogenesis are alkylating agents and topoisomerase II (TPII) inhibitors [107]. The majority of t-MNs cases are due to the alkylating agents used: mainly melphalan, cyclophosphamide, and chlorambucil [107,108]. These molecules cause direct DNA damage; in fact, alkylation of the DNA bases leads to inter- and intra-strand cross linking, abnormal base pairing, and DNA double-strand breakage [108]. TPII inhibitors, on the contrary, not only induce DNA double-strand breakage, but also interfere with DNA replication, leading to the stabilization of double-stranded breaks, more frequent DNA repair errors and crossover recombination between chromosomes [109]. Currently, t-MNs are considered the result of selection and expansion of pre-existing clonal HSC populations, which have stochastically acquired mutations increasing their fitness and survival capability [110]. The most frequent molecular aberration in t-MNs affects TP53, and the gene is, in fact, mutated in about one-third of these patients [50,111–113]. However, it was shown that the same TP53 mutant clones found at diagnosis had been detected at low frequencies (<1%), 3–6 years before the development of t-MN and even prior to any chemotherapy treatment, supporting the conclusion that t-MN is related to the cytotoxic selection of pre-existing chemo-resistant clones preferentially expanded after treatment [114]. The same mechanism was recently demonstrated in a cohort of MM patients, years before the evolution to t-MN [115]. In these cases, the presence of pre-existing mutant HSC clones was revealed, mainly harboring TP53 mutations, that became the dominant population at the time of t-MN evolution [115]. This mechanism could explain the high frequency of TP53 mutations in these patients. Another genetic event linked to the high prevalence of TP53 mutations in t-MNs is the incidence in elderly patients (over 70 years old) with cancer of CHIP. In the general population, CHIP occurs in about 10% of individuals aged 70 years or older [116]; this incidence grows to 33% in cancer patients of the same age [117]. In a recent study it was shown that TP53 and TET2 are the two most commonly mutated CHIP-associated genes (38%) in these patients before t-MN evolution, and the mean VAF of CHIP mutations had expanded by the time of the t-MN diagnosis [117]. Elderly patients with mutated CHIP-associated genes (such as TP53) therefore have an increased risk of developing t-MNs compared with those without CHIP [117]. t-MDS generally has an aggressive clinical course, often due to TP53 mutations. Patients usually have a poor performance status and commonly show suboptimal responses to conventional chemotherapy; allo-HSCT is therefore the only curative option [113]. The outcome of transplanted t-MDS is, in fact, similar to that of transplanted de novo MDS. Importantly, despite the high prevalence of TP53 mutations in t-MDS, they did not affect transplant outcome, even if more studies are needed, focused on the heterogeneity of TP53-mutated t-MDS to identify which cases could require novel emerging treatments [113].

3. Clinical Implications

3.1. Prognosis and Clinical Outcome

In order to perform the best MDS risk stratification, several prognostic models including the IPSS, WHO based Prognostic Scoring System (WPSS), and IPSS-R have been developed [118,119]. Recently, numerous groups demonstrated the importance of integrating mutational profiling into the IPSS-R for a better MDS patients risk prognostication [5,120–122]. The adverse effects of TP53 alterations on MDS clinical phenotypes and outcome (more aggressive disease and poorer response to treatment) have been widely documented; the role of TP53 mutations as predictors of poor OS in MDS patients has been uniformly described, independently of established risk factors [97,121–125]. On the contrary, SF3B1 mutations identify a subgroup of MDS patients with a relatively good prognosis.
The International Working Group for the Prognosis of Myelodysplastic Syndromes (IWG-PM) has recently recognized SF3B1-mutated MDS as a distinct nosologic entity, characterized by ring sideroblasts, ineffective erythropoiesis, and indolent clinical course [126]. Interestingly, SF3B1-negative MDS with ring sideroblasts have a significantly shorter survival compared to the SF3B1-mutated group and a significantly higher prevalence of TP53 mutations was reported in these patients [126].

In the past years, the correlation between TP53 and MDS outcome has been studied in more detail, paying special attention to the type of TP53 alterations (mutations vs deletions), to characterize the impact of the TP53 mutations burden and the more complex TP53 allelic state. The prognostication of TP53 mutations (mut) and deletions (del) was studied in a large cohort of 3307 patients with hematological malignancies: AML (n = 858), MDS (n = 943), ALL (n = 358), CLL (n = 1148) [14]. MDS was the only entity in which a significant negative impact on OS was shown for all the possible TP53 alterations vs WT patients: TP53mut only (19 vs 65 months, \( p < 0.001 \)), TP53del only (24 vs 65 months, \( p = 0.011 \)), and TP53mut+del (4 vs 65 months, \( p < 0.001 \)) [14]. Interestingly, the impact on OS of mono and bi-allelic TP53 alterations results very different. The same circumstance was highlighted in the last study of the TP53 allelic state implications on MDS prognostication [55]. In a cohort of 3324 patients, it was shown that the two TP53 allelic states were associated with distinct clinical presentations and outcomes. Mono-allelic TP53 altered patients had less cytopenia and lower percentages of BM blasts compared to multi-hit patients. About 50% of patients in the mono-allelic group were classified in IPSS-R as good/very-good risk, whereas 89% of the multi-hit group were stratified as poor/very-poor risk. Moreover, the two allelic states had very different effects on the incidence of AML transformation and on OS [55]. As already discussed, the TP53 mutations burden has also been reported to be of prognostic significance in MDS patients [56–58]. In patients with mono-allelic TP53 mutations, cases with a VAF > 23% had an increased risk of death compared to WT patients (\( p < 0.001 \)), while cases with a VAF ≤ 23% had a similar OS to WT patients. On the contrary, multi-hit patients had poor outcomes across all ranges of VAF [55]. Other authors, analyzing 261 TP53-mutated MDS, developed a multivariable model for OS that included the IPSS-R categories (blast score, cytogenetic score, hemoglobin score, platelet score) and TP53 VAF [98]. In view of these data, the generic adverse role of TP53 alterations in MDS needs to be reconsidered. The simple presence/absence of TP53 mutation/s is not enough to define the patient’s prognosis, and more aspects must be considered. The outcomes of TP53 altered MDS are heterogeneous and their response and prognosis may differ on the basis of the mutation burden and genomic context, even when correcting for clinical biological aspects [98].

### 3.2. Conventional Therapeutic Approaches

The wide range of therapeutic strategies available for TP53-mutated MDS cases was recently excellently described [7]; herein we aim just to summarize the main standard and emerging approaches in order to show how the study of TP53 alterations in MDS patients can influence treatment decision-making and predict the response rate. Conventional therapeutic approaches include hypomethylating agents (HMAs), lenalidomide, and allo-HSCT [7]. The approved HMAs, azacytidine (AZA) and decitabine (DAC), are the standard frontline treatment option in patients with higher-risk MDS [7]. AZA and DAC are two nucleoside analogs able to incorporate into DNA (AZA also in RNA) [127]. Their predominant effect is to inhibit DNA methyltransferase, and revert hypermethylation-induced silencing of tumor suppressor and other cancer-related genes [128,129]. It is not clear whether the TP53 mutational status can influence the response rate to HMAs in MDS patients. Some preclinical studies have demonstrated that TP53 mutations increase the cells sensitivity to HMAs [130,131], and an improved response rate in TP53-mutated MDS was reported in some clinical studies [132,133]. On the contrary, no significant differences in response rate were observed between TP53-mutated and WT patients in other studies [15,134–136]. Interestingly, HMAs induce a TP53 mutational burden reduction (to VAF < 5%), rarely seen with other genes recurrently mutated in MDS [133,134]. This suggests the importance of TP53 analysis not only at the disease onset but also...
during the follow-up, in order to perform a molecular evaluation of the treatment response. The efficacy of HMAs in combination with other therapeutic agents is also under investigation. An ongoing trial (NCT03377725) is evaluating the use of DAC in combination with arsenic trioxide (ATO). ATO is widely reported to be able to degrade and thus inhibit the oncogenic function of mutated p53 [137]; ATO, in fact, suppresses cancer cell growth by targeting mutated p53, for degradation by Pirh2-pathway [138]. Furthermore, it was shown that the combination of ATO and DAC synergistically induces the apoptosis of MDS cells, increasing the levels of reactive oxygen species and inducing the endoplasmic reticulum stress [139]. In TP53-mutated MDS, the potential of DAC plus ATO combination will be evaluated considering the RFS improving and the ability to thoroughly eliminate the TP53-mutated subclone.

In patients with an isolated 5q deletion, characterized by severe, often refractory anemia, the standard treatment option is lenalidomide, an immunomodulatory agent that can reduce transfusion requirements and reverse cytologic and cytogenetic abnormalities [140]. Lenalidomide was shown to be able to stabilize MDM2, accelerating the degradation of p53, that is overexpressed in erythroid precursors in these patients [26]. Different clinical studies have uniformly reported a correlation between TP53 mutations and resistance to lenalidomide in del(5q) MDS [93,94], in terms of a reduced response rate, poorer OS, the appearance and/or increase of TP53-mutant clones, and a higher risk of AML transformation compared to WT patients [7]. For these reasons, the conventional use of lenalidomide in del(5q) MDS should be reassessed in the presence of TP53 mutations, in favor of other therapeutic strategies such as HMAs [7].

The only curative treatment approach for MDS patients is allo-HSCT, that should be considered in all eligible patients [141]. In TP53-mutated MDS, its use is still debated in view of the adverse impact on the outcome of these patients [142–144]. TP53 mutations were shown to be an independent risk factor for a lower OS, higher cumulative incidence of relapse, and lower event-free survival [145]. TP53 mutations were then significantly associated with poor outcomes after transplantation for patients with de novo MDS, primarily owing to a higher prevalence of relapse [145]. Remarkably, it was recently shown that HMAs used as a bridge to allo-HSCT could reduce the adverse outcome observed in these patients [133]. Furthermore, as previously discussed, allo-HSCT is the only curative option for t-MDS cases, regardless of the TP53 mutational status; the outcome of transplanted t-MDS is, in fact, similar to those of transplanted de novo MDS [113].

3.3. Emerging Therapeutic Strategies

In the era of precision medicine, several new approaches are being developed with the purpose of supplanting or supplementing the older drugs mentioned above. The variety of therapies currently under investigation can be classified in two groups, as targeted therapies and immunotherapies. Targeted therapy aims to inhibit or enhance one of the numerous molecular pathways in which p53 is involved. The most promising agent tested to date is APR-246, a methylated derivative of PRIMA-1, which induces apoptosis in human tumor cells through restoring the transcriptional transactivation function of mutant p53 [146]. APR-246 is spontaneously converted to the reactive electrophile methylene quinuclidinone (MQ) that, in the cellular environment, form adducts with thiols (cysteine residues) in mutant p53 [147]. Covalent modification of mutant p53 per se is enough to produce thermodynamic stabilization of the protein toward the WT conformation, inducing apoptosis of the tumor cell. The interaction between APR-246 and p53 was recently better described and cysteine 277 was identified as the primary binding target for MQ in p53 [148]. Cysteine 277 is essential for MQ-mediated thermostabilization of WT, R175H and R273H mutant p53. Moreover, together with cysteine 124, it is required for the functional restoration of R175H mutant p53 in living tumor cells [148]. MQ not only reactivates mutant p53, but also targets antioxidant molecules influencing the cellular redox balance [149]. In fact, it was shown that the MQ has the ability to inhibit the selenocysteine-containing enzyme TrxR1 and to bind and deplete glutathione, inducing a cellular oxidative stress. Dual targeting of mutant p53 and the redox balance induces the elimination of cancer cells [149]. Low doses of APR-246 alone, or in combination with AZA, reactivate the p53 pathway and induce an apoptosis
program. The clinical effect of APR-246, as single agent, was investigated in refractory hematological malignancies, showing its ability to target p53 in vivo [150,151]. Furthermore, the synergistic effect of APR-246 and AZA was demonstrated in TP53-mutated MDS and AML [146]. Several ongoing trials (NCT03745716, NCT03931291, NCT03588078, NCT03072043) show the high efficacy and tolerability of this combination regimen in terms of overall response rate (ORR) and complete remission (CR) for these patients [152]. Notably, a 74% ORR and 59% CR was shown in 27 evaluable MDS patients from the phase Ib/II trial NCT03588078. Furthermore, 88% ORR and 61% CR were reported in 33 evaluable MDS patients from the phase Ib/II trial NCT03072043. Seventeen (52%) evaluable MDS patients from this clinical study discontinued treatment to pursue allo-HSCT [152].

The other agent tested for the purpose of inducing apoptosis in tumor cells is venetoclax, an inhibitor of the antiapoptotic protein Bcl-2. p53 binds to Bcl-2 via the DBD and induces mitochondrial permeabilization, with the release of apoptotic activator proteins [153,154]. In contrast to the WT protein, missense mutants of p53 are unable to form complexes with Bcl-2 in human cancer cells. Clinical studies of venetoclax in combination with HMAs in higher-risk patients with MDS and in AML are currently ongoing. However, it is not clear if this combination is also improving outcomes in TP53-mutated patients [155]. The latest mechanism exploited to solve the deleterious effects of mutant p53 is its inhibition/degradation. The agents tested for this purpose are HDAC inhibitors [156], statins [157], and NEDD8 inhibitors [158]. Ongoing trials are evaluating their efficacy and synergism with the older approved drugs.

The use of immunotherapeutic agents is the other frontline approach in oncology, developed as from the 1990s and constantly evolving. One of the most widely studied immunologic mechanisms to date is the “immune checkpoints,” and the ability of molecules such as PD-1 and CTLA-4 to suppress T-cell-mediated killing of cancer cells [159]. An aberrant upregulation of these genes was observed in CD34+ cells and in peripheral blood mononuclear cells of MDS patients. Furthermore, in a cohort of patients treated with HMAs, cases resistant to therapy had higher relative increments in these genes expression compared with patients who achieved response [159]. In the light of these data, the use of PD-1 and CTLA-4 inhibitors, monoclonal antibodies anti-PD-1 (nivolumab) [160] and anti-CTLA-4 (ipilimumab) [161], was considered in MDS. Moreover, a PD-1 upregulation was shown in TP53-mutated cases in comparison with WT patients [162]. For these reasons, several clinical studies are ongoing to evaluate the efficacy of checkpoint inhibitors, their synergism, and the effect of their combination with conventional therapies.

4. Conclusions

The high phenotypic and clinical heterogeneity of MDS patients mainly has a genetic basis. The introduction of NGS in clinical practice has considerably increased our genomic knowledge of these disorders. In the set of genes recurrently mutated in MDS, alterations of TP53 identify a subgroup of patients with peculiar biological and clinical aspects. In fact, TP53 plays a pivotal role in several molecular pathways implicated in cellular differentiation and the induction of apoptosis. The latest findings show multiple implications of the TP53 allelic state on genome stability, prognosis, and clinical presentation in MDS patients; therefore, TP53 characterization must be viewed as a part of the MDS diagnostic workup. Furthermore, TP53 dysregulation affects the response rate to treatment, and several new approaches are being developed with the aim of supplanting or supplementing the conventional therapeutic strategies. In the era of personalized medicine, the MDS diagnostic process cannot do without a complete assessment of the TP53 mutational profile, to provide physicians with key molecular data for patient management and to identify the patients subgroup that could benefit from targeted therapy.

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Abbreviations

ALL acute lymphoblastic leukemia
allo-HSCT allogeneic hematopoietic stem cell transplantation
AML acute myeloid leukemia
ATO arsenic trioxide
AZA azacytidine
BM bone marrow
CDR commonly deleted region
CHIP clonal hemopoiesis of indeterminate potential
CK complex karyotypes
CLL chronic lymphocytic leukemia
cn-LOH copy-neutral loss of heterozygosity
CR complete remission
DAC decitabine
DBD DNA-binding domain
DDR DNA damage responses
DNE dominant negative effect
DSB double-strand breaks
GOF gain of function
H3K27me3 trimethylation of lysine 27 of histone H3
HMAs hypomethylating agents
HSC hematopoietic stem cell
HSPC hematopoietic stem and progenitor cell
IHC immunohistochemistry
IPSS International Prognostic Scoring System
IPSS-R revised International Prognostic Scoring System
LFS Li-Fraumeni syndrome
LOH loss of heterozygosity
MDM2 Murine Double Minute-2
MDS myelodysplastic syndromes
MK Monosomal karyotype
MM multiple myeloma
MQ methylene quinuclidinone
NGS next generation sequencing
ORR overall response rate
OS overall survival
PFS progression-free survival
PRC2 Polycomb repressive complex 2
RFS relapse-free survival
RP s ribosomal proteins
SNP single-nucleotide polymorphism
t-AML therapy-related acute myeloid leukemia
t-MDS therapy-related myelodysplastic syndromes
t-MNs therapy-related myeloid neoplasms
TP53 tumor protein p53
TPII topoisomerase II
VAF variant allele frequency
WPSS WHO-based Prognostic Scoring System
WT wild-type
References

1. Arber, D.A.; Orazi, A.; Hasseri, 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] [PubMed]

2. Graubert, T.; Walter, M.J. Genetics of myelodysplastic syndromes: New insights. Hematol. Am. Soc. Hematol. Educ. Program 2011, 2011, 543–549. [CrossRef]

3. Raza, A.; Galili, N. The genetic basis of phenotypic heterogeneity in myelodysplastic syndromes. Nat. Rev. Cancer 2012, 12, 849–859. [CrossRef] [PubMed]

4. Papaemmanuil, E.; Gerstung, M.; Malcovati, L.; Tauro, S.; Gundem, G.; Van Loo, P.; Yoon, C.J.; Ellis, P.; Wedge, D.C.; Pellagatti, A.; et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013, 122, 3616–3627. [CrossRef]

5. Haferlach, T.; Nagata, Y.; Grossmann, V.; Okuno, Y.; Bacher, U.; Nagae, G.; Schnittger, S.; Sanada, M.; Kon, A.; Alpermann, T.; et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014, 28, 241–247. [CrossRef] [PubMed]

6. Zhang, L.; McGraw, K.L.; Sallman, D.A.; List, A.F. The role of p53 in myelodysplastic syndromes and acute myeloid leukemia: Molecular aspects and clinical implications. Leukemia Lymphoma 2017, 58, 1777–1790. [CrossRef] [PubMed]

7. Hunter, A.M.; Sallman, D.A. Targeting TP53 Mutations in Myelodysplastic Syndromes. Hematol. Oncol. Clin. North Am. 2020, 34, 421–440. [CrossRef] [PubMed]

8. Bode, A.M.; Dong, Z. Post-translational modification of p53 in tumorigenesis. Nat. Rev. Cancer 2004, 4, 793–805. [CrossRef]

9. Harms, K.L.; Chen, X. The functional domains in p53 family proteins exhibit both common and distinct properties. Cell Death Differ. 2006, 13, 890–897. [CrossRef]

10. Vousden, K.H.; Lu, X. Live or let die: The cell’s response to p53. Nat. Rev. Cancer 2002, 2, 594–604. [CrossRef]

11. Levine, A.J. p53, the cellular gatekeeper for growth and division. Cell 1997, 88, 323–331. [CrossRef]

12. Hamada, M.; Fujiwara, T.; Hizuta, A.; Gochi, A.; Naomoto, Y.; Takakura, N.; Takahashi, K.; Roth, J.A.; Tanaka, N.; Orita, K. The p53 gene is a potent determinant of chemosensitivity and radiosensitivity in gastric and colorectal cancers. J. Cancer Res. Clin. Oncol. 1996, 122, 360–365. [CrossRef] [PubMed]

13. Fujiwara, T.; Grimm, E.A.; Mukhopadhyay, T.; Zhang, W.W.; Owen-Schaub, L.B.; Roth, J.A. Induction of chemosensitivity in human lung cancer cells in vivo by adenovirus-mediated transfer of the wild-type p53 gene. Cancer Res. 1994, 54, 2287–2291. [PubMed]

14. Stengel, A.; Kern, W.; Haferlach, T.; Meggendorfer, M.; Fasan, A.; Haferlach, C. The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL, MDS and CLL: An analysis of 3307 cases. Leukemia 2017, 31, 705–711. [CrossRef]

15. Kulasekararaj, A.G.; Smith, A.E.; Mian, S.A.; Mohamedali, A.M.; Krishnamurthy, P.; Lea, N.C.; Gáken, J.; Pennaneach, C.; Ireland, R.; Czepulkowski, B.; et al. TP53 mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis. Br. J. Haematol. 2013, 160, 660–672. [CrossRef]

16. Kaneko, H.; Misawa, S.; Horiike, S.; Nakai, H.; Kashima, K. TP53 mutations emerge at early phase of myelodysplastic syndrome and are associated with complex chromosomal abnormalities. Blood 1995, 85, 2189–2193. [CrossRef]

17. Solé, F.; Espinet, B.; Sanz, G.F.; Cervera, J.; Calasanz, M.J.; Luño, E.; Prieto, F.; Granada, I.; Hernández, J.M.; Cigudosa, J.C.; et al. Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. Grupo Cooperativo Español de Citogenética Hematológica. Br. J. Haematol. 2000, 108, 346–356. [CrossRef]

18. Ebert, B.L. Deletion 5q in myelodysplastic syndrome: A paradigm for the study of hemizygous deletions in cancer. Leukemia 2009, 23, 1252–1256. [CrossRef]

19. Ebert, B.L.; Pretz, J.; Bosco, J.; Chang, C.Y.; Tamayo, P.; Galili, N.; Raza, A.; Root, D.E.; Attar, E.; Ellis, S.R.; et al. Identification of RPS14 as a 5q- syndrome gene by RNA interference screen. Nature 2008, 451, 335–339. [CrossRef]

20. Ebert, B.L. Molecular Dissection of the 5q Deletion in Myelodysplastic Syndrome. Semin. Oncol. 2011, 38, 621–626. [CrossRef]
21. Schneider, R.K.; Schenone, M.; Ferreira, M.V.; Kramann, R.; Joyce, C.E.; Hartigan, C.; Beier, F.; Brümmendorf, T.H.; Germing, U.; Platzbecker, U.; et al. Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. Nat. Med. 2016, 22, 288–297. [CrossRef]

22. Shangary, S.; Wang, S. Small-Molecule Inhibitors of the MDM2-p53 Protein-Protein Interaction to Reactivate p53 Function: A Novel Approach for Cancer Therapy. Annu. Rev. Pharmacol. Toxicol. 2009, 49, 223–241. [CrossRef]

23. Dutt, S.; Narla, A.; Lin, K.; Mullally, A.; Abayasekara, N.; Meegerdichian, C.; Wilson, F.H.; Currie, T.; Khanna-Gupta, A.; Berliner, N.; et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood 2011, 117, 2567–2576. [CrossRef] [PubMed]

24. Maya, R.; Balass, M.; Kim, S.T.; Shkedy, D.; Leal, J.F.; Shifman, O.; Moas, M.; Buschmann, T.; Ronai, Z.; Shiloh, Y.; et al. ATM-dependent phosphorylation of Mdm2 on serine 395: Role in p53 activation by DNA damage. Genes Dev. 2001, 15, 1067–1077. [PubMed]

25. Brooks, C.L.; Gu, W. Ubiquitination, phosphorylation and acetylation: The molecular basis for p53 regulation. Curr. Opin. Cell Biol. 2003, 15, 164–171. [CrossRef]

26. Wei, S.; Chen, X.; McGraw, K.; Zhang, L.; Komrokji, R.; Clark, J.; Caceres, G.; Billingsley, D.; Sokol, L.; Lancet, J.; et al. Lenalidomide promotes p53 degradation by inhibiting MDM2 auto-ubiquitination in myelodysplastic syndrome with chromosome 5q deletion. Oncogene 2013, 32, 1110–1120. [CrossRef] [PubMed]

27. Fink, E.C.; Ebert, B.L. The novel mechanism of lenalidomide activity. Blood 2015, 126, 2366–2369. [CrossRef] [PubMed]

28. Lee, J.h.; List, A.; Sallman, D.A. Molecular pathogenesis of myelodysplastic syndromes with deletion 5q. Eur. J. Haematol. 2019, 102, 203–209. [CrossRef]

29. McGraw, K.L.; Zhang, L.M.; Rollison, D.E.; Basiorka, A.A.; Fulp, W.; Rawal, B.; Jerez, A.; Billingsley, D.; Sokol, L.; et al. The relationship of TP53 R72P polymorphism to disease outcome and TP53 mutation in myelodysplastic syndromes. Blood Cancer J. 2015, 5, e291. [CrossRef]

30. McGraw, K.L.; Cluzeau, T.; Sallman, D.A.; Basiorka, A.A.; Irvine, B.A.; Zhang, L.; Epling-Burnette, P.K.; Rollison, D.E.; Mallo, M.; Sokol, L.; et al. TP53 and MDM2 single nucleotide polymorphisms influence survival in non-del(5q) myelodysplastic syndromes. Oncotarget 2015, 6, 34437–34445. [CrossRef]

31. Liu, Y.C.; Hsiao, H.H.; Yang, W.C.; Liu, T.C.; Chang, C.S.; Yang, M.Y.; Lin, P.M.; Hsu, J.F.; Lee, C.P.; Lin, S.F. MDM2 promoter polymorphism and p53 codon 72 polymorphism in chronic myeloid leukemia: The association between MDM2 promoter genotype and disease susceptibility, age of onset, and blast-free survival in chronic phase patients receiving imatinib. Mol. Carcinog. 2014, 53, 951–959. [CrossRef] [PubMed]

32. Rossi, A.R.; Breccia, M.; Abruzzese, E.; Castagnetti, F.; Luciano, L.; Gozzini, A.; Annunziata, M.; Martino, B.; Stagno, F.; Cavazzini, F.; et al. Outcome of 82 chronic myeloid leukemia patients treated with nilotinib or dasatinib after failure of two prior tyrosine kinase inhibitors. Haematologica 2013, 98, 399–403. [CrossRef] [PubMed]

33. Lutzmann, M.; Bernex, F.; da Costa de Jesus, C.; Hodroj, D.; Marty, C.; Plo, I.; Vainchenker, W.; Tosolini, M.; Forichon, L.; Bret, C.; et al. MCM8- and MCM9 Deficiencies Cause Lifelong Increased Hematopoietic DNA Damage Driving p53-Dependent Myeloid Tumors. Cell Rep. 2019, 28, 2851–2865.e4. [CrossRef] [PubMed]

34. Salari, A.; Thomay, K.; Himmler, K.; Vajen, B.; Schienke, A.; Hagedorn, M.; Ebersold, J.; Kreipe, H.-H.; Krüger, A.; Schambach, A.; et al. Establishing a murine xenograft-model for long-term analysis of factors inducing chromosomal instability in myelodysplastic syndrome: Pitfalls and successes. Cancer Genet. 2016, 209, 258–266. [CrossRef]

35. Amelio, I.; Melino, G. Context is everything: Extrinsic signalling and gain-of-function p53 mutants. Cell Death Discov. 2020, 6, 16. [CrossRef]

36. Popp, H.D.; Naumann, N.; Kendel, S.; Henzler, T.; Weiss, C.; Hofmann, W.-K.; Fabarius, A. Increase of DNA damage and alteration of the DNA damage response in myelodysplastic syndromes and acute myeloid leukemias. Leuk. Res. 2017, 57, 112–118. [CrossRef]

37. Forment, J.V.; Kaidi, A.; Jackson, S.P. Chromothripsis and cancer: Causes and consequences of chromosome shattering. Nat. Rev. Cancer 2012, 12, 663–670. [CrossRef]

38. Rausch, T.; Jones, D.T.W.; Zapatka, M.; Stütz, A.M.; Zichner, T.; Weissenfeldt, J.; Jäger, N.; Remke, M.; Shih, D.; Northcott, P.A.; et al. Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell 2012, 148, 59–71. [CrossRef]
39. Ábaígar, M.; Robledo, C.; Benito, R.; Ramos, F.; Diez-Campelo, M.; Hermosín, L.; Sánchez-Del-Real, J.; Alonso, J.M.; Cuello, R.; Megido, M.; et al. Chromothripsis is a Recurrent Genomic Abnormality in High-Risk Myelodysplastic Syndromes. *PLoS ONE* **2016**, *11*, e0164370. [CrossRef]

40. Grove, C.S.; Vassiliou, G.S. Acute myeloid leukaemia: A paradigm for the clonal evolution of cancer? *DMM Dis. Model. Mech.* **2014**, *7*, 941–951. [CrossRef]

41. Da Silva-Coelho, P.; Kroeeze, L.I.; Yoshida, K.; Koorenhof-Scheele, T.N.; Knops, R.; Van De Locht, L.T.; De Graaf, A.O.; Massop, M.; Sandmann, S.; Dugas, M.; et al. Clonal evolution in myelodysplastic syndromes. *Nat. Commun.* **2017**, *8*, 1–11. [CrossRef]

42. Makishima, H.; Yoshizato, T.; Yoshida, K.; Sekeres, M.A.; Radivojewitch, T.; Suzuki, H.; Przychodzen, B.J.; Nagata, Y.; Meggendorfer, M.; Sanada, M.; et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat. Genet.* **2017**, *49*, 204–212. [CrossRef] [PubMed]

43. Liu, Y.; Makishima, H.; Kerr, C.M.; Przychodzen, B.P.; Aly, M.; Goyal, A.; Awada, H.; Asad, M.F.; Kuzmanovic, T.; Suzuki, H.; et al. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. *Nat. Commun.* **2019**, *10*, 5386. [CrossRef] [PubMed]

44. Lodé, L.; Ameur, A.; Coste, T.; Ménard, A.; Richebourg, S.; Gaillard, J.-B.; Le Bris, Y.; Béné, M.-C.; Lavabre-Bertrand, T.; Soussi, T. Single-molecule DNA sequencing of acute myeloid leukaemia and myelodysplastic syndromes with multiple TP53 alterations. *Haematologica* **2018**, *103*, e13–e16. [CrossRef] [PubMed]

45. Liu, Y.; Chen, C.; Xu, Z.; Scuoppo, C.; Rillahan, C.D.; Gao, R.; Yao, C.; Kobayashi, M.; Geng, Z.; et al. Deletions linked to TP53 loss drive cancer through p53-independent mechanisms. *Nature* **2016**, *531*, 471–475. [CrossRef]

46. Baugh, E.H.; Ke, H.; Levine, A.J.; Bonneau, R.A.; Chan, C.S. Why are there hotspot mutations in the TP53 gene in human cancers? *Cell Death Differ.* **2018**, *25*, 154–160. [CrossRef]

47. Lee, M.K.; Teoh, W.W.; Tong, W.M.; Wang, Z.Q.; Sabapathy, K. Cell-type, Dose, and Mutation-type Specificity Dictate Mutant p53 Functions In Vivo. *Cancer Cell* **2012**, *22*, 751–764. [CrossRef]

48. Boettcher, S.; Miller, P.G.; Sharma, R.; McConkey, M.; Leventhal, M.; Krivtsov, A.V.; Giacomelli, A.O.; Wang, W.; Kim, J.; Chao, S.; et al. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. *Science* **2019**, *365*, 599–604. [CrossRef]

49. Chen, S.; Wang, Q.; Yu, H.; Capitanio, M.L.; Vemula, S.; Nabinger, S.C.; Gao, R.; Yao, C.; Kobayashi, M.; Geng, Z.; et al. Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway. *Nat. Commun.* **2019**, *10*, 1–14. [CrossRef]

50. Genovese, G.; Kähler, A.K.; Handsaker, R.E.; Lindberg, J.; Rose, S.A.; Bakhoun, S.F.; Chambert, K.; Mick, E.; Neale, B.M.; Fromer, M.; et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *N. Engl. J. Med.* **2014**, *371*, 2477–2487. [CrossRef]

51. Sperling, A.S.; Gibson, C.J.; Ebert, B.L. The genetics of myelodysplastic syndrome: From clonal haematopoiesis to secondary leukaemia. *Nat. Rev. Cancer* **2017**, *17*, 5–19. [CrossRef] [PubMed]

52. Steensma, D.P.; Bejar, R.; Jaiswal, S.; Lindsley, R.C.; Sekeres, M.A.; Hasserjian, R.P.; Ebert, B.L. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **2015**, *126*, 9–16. [CrossRef] [PubMed]

53. Storlazzi, C.T.; Albano, F.; Locunso, C.; Lonoce, A.; Funes, S.; Guastadisegni, M.C.; Cimarosto, L.; Impera, L.; D’Addabbo, P.; Panagopoulos, I.; et al. t(3;12)(q26;q14) in polycythemia vera is associated with upregulation of the HMGA2 gene. *Leukemia* **2006**, *20*, 2190–2192. [CrossRef] [PubMed]

54. Donehower, L.A.; Soussi, T.; Korkut, A.; Liu, Y.; Schultz, A.; Cardenas, M.; Li, X.; Babur, O.; Hsu, T.K.; Lichtarge, O.; et al. Integrated Analysis of TP53 Gene and Pathway Alterations in The Cancer Genome Atlas. *Cell Rep.* **2019**, *28*, 1370–1384.e5. [CrossRef]

55. Bernard, E.; Nannya, Y.; Hasserjian, R.P.; Devlin, S.M.; Tuechler, H.; Medina-Martinez, J.S.; Yoshizato, T.; Shiozawa, Y.; Saihi, R.; Malcovati, L.; et al. Implications of TP53 Allelic State for Genome Stability, Clinical Presentation and Outcomes in Myelodysplastic Syndromes. *bioRxiv:2019*. bioRxiv:2019.12.19.686844.

56. Sallman, D.A.; Komrokji, R.; Vaupe1, C.; Cluzeau, T.; Geyer, S.M.; McGraw, K.L.; Al Ai, N.H.; Lancet, J.; McGinnis, M.J.; Nahas, S.; et al. Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. *Leukemia* **2016**, *30*, 666–673. [CrossRef]
91. Meggendorfer, M.; Haferlach, C.; Kern, W.; Haferlach, T. Molecular analysis of myelodysplastic syndrome.

92. Hosono, N.; Makishima, H.; Mahfouz, R.; Przychodzen, B.; Yoshida, K.; Jerez, A.; LaFramboise, T.; Polprasert, C.; Clemente, M.J.; Shiraishi, Y.; et al. Recurrent genetic defects on chromosome 5q in myeloid neoplasms. Oncotarget 2017, 8, 6483–6495. [CrossRef] [PubMed]
93. Mossner, M.; Jann, J.C.; Nowak, D.; Platzbecker, U.; Giagounidis, A.; Götze, K.;Letsch, A.; Haase, D.; Shirneshan, K.; Braulke, F.; et al. Prevalence, clonal dynamics and clinical impact of TP53 mutations in patients with myelodysplastic syndrome with isolated deletion (5q) treated with lenalidomide: Results from a prospective multicenter study of the German MDS study group (GMDS). *Leukemia* 2016, 30, 1956–1959. [CrossRef] [PubMed]

94. Martinez-Høyer, S.; Docking, R.; Chan, S.; Jadersten, M.; Parker, J.; Karsan, A. Mechanisms of Resistance to Lenalidomide in Del(5q) Myelodysplastic Syndrome Patients. *Blood* 2015, 126, 5228. [CrossRef]

95. Lodé, L.; Ménard, A.; Flet, L.; Richebourg, S.; Loirat, M.; Eveillard, M.; Le Bris, Y.; Godon, C.; Theisen, O.; Gagez, A.L.; et al. Emergence and evolution of TP53 mutations are key features of disease progression in myelodysplastic patients with lower-risk del(5q) treated with lenalidomide. *Haematologica* 2018, 103, e143–e146. [CrossRef]

96. Scharenberg, C.; Giai, V.; Pellagatti, A.; Saft, L.; Dimitriou, M.; Jansson, M.; Jädersten, M.; Grandien, A.; Douagi, I.; Neuberg, D.S.; et al. Progression in patients with low- and intermediate-1-risk del(5q) myelodysplastic syndromes is predicted by a limited subset of mutations. *Haematologica* 2017, 102, 498–508. [CrossRef]

97. Haase, D.; Stevenson, K.E.; Neuberg, D.; Maciejewski, J.P.; Nazha, A.; Sekeres, M.A.; Ebert, B.L.; Garcia-Manero, G.; Haferlach, C.; Haferlach, T.; et al. TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups. *Leukemia* 2019, 33, 1747–1758. [CrossRef]

98. Montalban-Bravo, G.; Kanagal-Shamanna, R.; Benton, C.B.; Class, C.A.; Chien, K.S.; Sasaki, K.; Naqvi, K.; Alvarado, Y.; Kadia, T.M.; Ravandi, F.; et al. Genomic context and TP53 allele frequency define clinical outcomes in TP53-mutated myelodysplastic syndromes. *Blood Adv.* 2020, 4, 482–495. [CrossRef]

99. Ren, Y.; Wang, J.; Zhang, H.; Mei, C.; Ye, L.; Luo, Y.; Zhou, X.; Zhu, S.; Jiang, L.; Wang, L.; et al. TP53 mutations are associated with very complex karyotype and suggest poor prognosis in newly diagnosed myelodysplastic syndrome patients with monosomal karyotype. *Asia. Pac. J. Clin. Oncol.* 2020. [CrossRef]

100. Xing, R.; Li, C.; Gale, R.P.; Zhang, Y.; Xu, Z.; Qin, T.; Li, B.; Fang, L.; Zhang, H.; Pan, L.; et al. Monosomal karyotype is an independent predictor of survival in patients with higher-risk myelodysplastic syndrome. *Am. J. Hematol.* 2014, 89, E163–E168. [CrossRef]

101. McQuilten, Z.K.; Sundararajan, V.; Andrianopoulos, N.; Curtis, D.J.; Wood, E.M.; Campbell, L.J.; Wall, M. Monosomal karyotype predicts inferior survival independently of a complex karyotype in patients with myelodysplastic syndromes. *Cancer* 2015, 121, 2892–2899. [CrossRef]

102. Schanz, J.; Tüchler, H.; Solé, F.; Mallo, M.; Luño, E.; Cervera, J.; Grau, J.; Hildebrandt, B.; Slovak, M.L.; Ohyashiki, K.; et al. Monosomal karyotype in MDS: Explaining the poor prognosis. *Leukemia* 2013, 27, 1988–1995. [CrossRef] [PubMed]

103. Valcárcel, D.; Ademá, V.; Solé, F.; Ortega, M.; Nomdedeu, B.; Sanz, G.; Luño, E.; Cañizo, C.; de la Serna, J.; Ardanaz, M.; et al. Complex, not monosomal, karyotype is the cytogenetic marker of poorest prognosis in patients with primary myelodysplastic syndromes. *J. Clin. Oncol.* 2013, 31, 916–922. [CrossRef] [PubMed]

104. Tefferi, A.; Idossa, D.; Lasho, T.L.; Mudireddy, M.; Finke, C.; Shah, S.; Nicolosi, M.; Patnaik, M.M.; Pardanani, A.; Gangat, N.; et al. Mutations and karyotype in myelodysplastic syndromes: TP53 clusters with monosomal karyotype, RUNX1 with trisomy 21, and SF3B1 with inv(3)(q21q26.2) and del(11q). *Blood Cancer J.* 2017, 7, 658. [CrossRef] [PubMed]

105. Wang, W.; Routhbert, M.J.; Tang, Z.; Ok, C.Y.; Patel, K.P.; Daver, N.; Garcia-Manero, G.; Medeiros, L.J.; Wang, S.A. Characterization of TP53 mutations in low-grade myelodysplastic syndromes and myelodysplastic syndromes with a non-complex karyotype. *Eur. J. Haematol.* 2017, 99, 536–543. [CrossRef] [PubMed]

106. Ganser, A.; Heuser, M. Therapy-related myeloid neoplasms. *Curr. Opin. Hematol.* 2017, 24, 152–158. [CrossRef] [PubMed]

107. Curtis, R.E.; Boice, J.D.; Stovall, M.; Bernstein, L.; Greenberg, R.S.; Flannery, J.T.; Schwartz, A.G.; Weyer, P.; Moloney, W.C.; Hoover, R.N. Risk of Leukemia after Chemotherapy and Radiation Treatment for Breast Cancer. *N. Engl. J. Med.* 1992, 326, 1745–1751. [CrossRef] [PubMed]

108. Fu, D.; Calvo, J.A.; Samson, L.D. Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat. Rev. Cancer* 2012, 12, 104–120. [CrossRef]

109. Cowell, I.G.; Austin, C.A. Mechanism of generation of therapy related leukemia in response to anti-topoisomerase II agents. *Int. J. Environ. Res. Public Health* 2012, 9, 2075–2091. [CrossRef]
110. Chua, C.C.; Fleming, S.; Wei, A.H. Clinicopathological aspects of therapy-related acute myeloid leukemia and myelodysplastic syndrome. *Best Pract. Res. Clin. Haematol.* 2019, 32, 3–12. [CrossRef]

111. Xie, M.; Lu, C.; Wang, J.; McLellan, M.D.; Johnson, K.J.; Wendt, M.C.; McMichael, J.F.; Schmidt, H.K.; Yellapantula, V.; Miller, C.A.; et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* 2014, 20, 1472–1478. [CrossRef]

112. Ok, C.Y.; Patel, K.P.; Garcia-Manero, G.; Routbort, M.J.; Fu, B.; Tang, G.; Goswami, M.; Singh, R.; Kanagal-Shamanna, R.; Pierce, S.A.; et al. Mutational profiling of therapy-related myelodysplastic syndromes and acute myeloid leukemia by next generation sequencing, a comparison with de novo diseases. *Leuk. Res.* 2015, 39, 348–354. [CrossRef] [PubMed]

113. Aldoss, I.; Pham, A.; Li, S.M.; Gendzkhazde, K.; Akkami, M.; Telatar, M.; Hong, H.; Padgeanah, A.; Bedell, V.; Cao, T.; et al. Favorable impact of allogeneic stem cell transplantation in patients with therapy-related myelodysplasia regardless of TP53 mutational status. *Haematologica* 2017, 102, 2030–2038. [CrossRef] [PubMed]

114. Wong, T.N.; Ramsingh, G.; Young, A.L.; Miller, C.A.; Touma, W.; Welch, J.S.; Lampecht, T.L.; Shen, D.; Hundle, J.; Fulton, R.S.; et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature* 2015, 518, 552–555. [CrossRef] [PubMed]

115. Sridharan, A.; Schinke, C.D.; Georgiev, G.; da Silva Ferreira, M.; Thiruthuvananthan, V.; MacArthur, I.; Bhagat, T.D.; Choudhary, G.S.; Aluri, S.; Chen, J.; et al. Stem cell mutations can be detected in myeloma patients years before onset of secondary leukemias. *Blood Adv.* 2019, 3, 3962–3967. [CrossRef] [PubMed]

116. Jaiswal, S.; Fontanillas, P.; Flannick, J.; Manning, A.; Grauman, P.V.; Mar, B.G.; Lindsey, R.C.; Mermel, C.H.; Burtt, N.; Chavez, A.; et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. *N. Engl. J. Med.* 2014, 371, 2488–2498. [CrossRef] [PubMed]

117. Gillis, N.K.; Ball, M.; Zhang, Q.; Ma, Z.; Zhao, Y.L.; Yoder, S.J.; Balasis, M.E.; Mesa, T.E.; Sallman, D.A.; Lancet, J.E.; et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: A proof-of-concept, case-control study. *Lancet Oncol.* 2017, 18, 112–121. [CrossRef]

118. Malcovati, L.; Gерning, U.; Kuendgen, A.; Della Porta, M.G.; Pascutto, C.; Invernizzi, R.; Giagounidis, A.; Hildebrandt, B.; Bernasconi, P.; Knipp, S.; et al. Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. *J. Clin. Oncol.* 2007, 25, 3503–3510. [CrossRef]

119. Greenberg, P.L.; Tuechler, H.; Schanz, J.; Sanz, G.; Garcia-Manero, G.; Solé, F.; Bennett, J.M.; Bowen, D.; Fenaux, P.; Dreyfus, F.; et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood* 2012, 120, 2454–2465. [CrossRef]

120. Nazha, A.; Narkhede, M.; Radivojevitch, T.; Seastone, D.J.; Patel, B.J.; Gerds, A.T.; Mukherjee, S.; Kalaycio, M.; Advani, A.; Przychodzen, B.; et al. Incorporation of molecular data into the Revised International Prognostic Scoring System can improve risk stratification in the patients with myelodysplastic syndrome. *Leukemia* 2016, 30, 2214–2220. [CrossRef] [PubMed]

121. Hou, H.A.; Tsai, C.H.; Lin, C.C.; Chou, W.C.; Kuo, Y.Y.; Liu, C.Y.; Tseng, M.H.; Peng, Y.L.; Liu, M.C.; Liu, C.W.; et al. Incorporation of mutations in five genes in the revised International Prognostic Scoring System can improve risk stratification in patients with myelodysplastic syndrome. *Blood Cancer J.* 2018, 8, 1–13. [CrossRef]

122. Tefferi, A.; Lasho, T.L.; Patnaik, M.M.; Saeed, L.; Mudireddy, M.; Idossa, D.; Finke, C.; Ketterling, R.P.; Pardanani, A.; Gangat, N. Targeted next-generation sequencing in myelodysplastic syndromes and prognostic interaction between mutations and IPSS-R. *Am. J. Hematol.* 2017, 92, 1311–1317. [CrossRef]

123. Bejar, R.; Stevenson, K.; Abdel-Wahab, O.; Galili, N.; Nilsson, B.; Garcia-Manero, G.; Kantarjian, H.; Raza, A.; Levine, R.L.; Neuberg, D.; et al. Clinical Effect of Point Mutations in Myelodysplastic Syndromes. *N. Engl. J. Med.* 2011, 364, 2496–2506. [CrossRef] [PubMed]

124. Gangat, N.; Mudireddy, M.; Lasho, T.L.; Finke, C.M.; Nicolosi, M.; Szuber, N.; Patnaik, M.M.; Pardanani, A.; Hanson, C.A.; Ketterling, R.P.; et al. Mutations and prognosis in myelodysplastic syndromes: Karyotype-adjusted analysis of targeted sequencing in 300 consecutive cases and development of a genetic risk model. *Am. J. Hematol.* 2018, 93, 691–697. [CrossRef] [PubMed]

125. Jiang, L.; Luo, Y.; Zhu, S.; Wang, L.; Ma, L.; Zhang, H.; Shen, C.; Yang, W.; Ren, Y.; Zhou, X.; et al. Mutation status and burden can improve prognostic prediction of patients with lower-risk myelodysplastic syndromes. *Cancer Sci.* 2020, 111, 580–591. [CrossRef] [PubMed]
126. Malcovati, L.; Stevenson, K.; Papaemmanuil, E.; Neuberg, D.; Bejar, R.; Boulwood, J.; Bowen, D.T.; Campbell, P.J.; Ebert, B.L.; Fenaux, P.; et al. SF3B1-mutant myelodysplastic syndrome as a distinct disease subtype—A Proposal of the International Working Group for the Prognosis of Myelodysplastic Syndromes (IWG-PM). *Blood* 2020. [CrossRef]

127. Hollenbach, P.W.; Nguyen, A.N.; Brady, H.; Williams, M.; Ning, Y.; Richard, N.; Krushel, L.; Aukerman, S.L.; Heise, C.; MacBeth, K.J. A comparison of azacitidine and decitabine activities in acute myeloid leukemia cell lines. *PloS One* 2010, 5, e9001. [CrossRef]

128. Mund, C.; Brueckner, B.; Lyko, F. Reactivation of epigenetically silenced genes by DNA methyltransferase inhibitors: Basic concepts and clinical applications. *Epigenetics* 2006, 1, 8–14. [CrossRef]

129. Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat. Rev. Genet.* 2007, 8, 286–298. [CrossRef]

130. Yi, L.; Sun, Y.; Levine, A. Selected drugs that inhibit DNA methylation can preferentially kill p53 deficient cells. *Oncotarget* 2014, 5, 8924–8936. [CrossRef]

131. Nieto, M.; Samper, E.; Fraga, M.F.; González De Buitrago, G.; Esteller, M.; Serrano, M. The absence of p53 is critical for the induction of apoptosis by 5-aza-2′-deoxycytidine. *Oncogene* 2004, 23, 735–743. [CrossRef]

132. Chang, C.K.; Zhao, Y.S.; Xu, F.; Guo, J.; Zhang, Z.; He, Q.; Wu, D.; Wu, L.Y.; Su, J.Y.; Song, L.X.; et al. TP53 mutations predict decitabine-induced complete responses in patients with myelodysplastic syndromes. *Br. J. Haematol.* 2017, 176, 600–608. [CrossRef] [PubMed]

133. Welch, J.S.; Petti, A.A.; Miller, C.A.; Fronick, C.C.; O’Laughlin, M.; Fulton, R.S.; Wilson, R.K.; Baty, J.D.; Duncavage, E.J.; Tandon, B.; et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N. Engl. J. Med.* 2016, 375, 2023–2036. [CrossRef] [PubMed]

134. Falconi, G.; Fabiani, E.; Piciocchi, A.; Criscuolo, M.; Fianchi, L.; Lindfors Rossi, E.L.; Finelli, C.; Cerqui, E.; Ottone, T.; Molteni, A.; et al. Somatic mutations as markers of outcome after azacitidine and allogeneic stem cell transplantation in higher-risk myelodysplastic syndromes. *Leukemia* 2019, 33, 785–790. [CrossRef] [PubMed]

135. Bally, C.; Adès, L.; Renneville, A.; Sebert, M.; Eclache, V.; Preudhomme, C.; Mozzićonacci, M.J.; de The, H.; Lehmann-Che, J.; Fenaux, P. Prognostic value of TP53 gene mutations in myelodysplastic syndromes and acute myeloid leukemia treated with azacitidine. *Leuk. Res.* 2014, 38, 751–755. [CrossRef] [PubMed]

136. Takahashi, K.; Patel, K.; Bueso-Ramos, C.; Zhang, J.; Gumbs, C.; Jabbour, E.; Kadia, T.; Andreff, M.; Konopleva, M.; Di Nardo, C.; et al. Clinical implications of TP53 mutations in myelodysplastic syndromes treated with hypomethylating agents. *Oncotarget* 2016, 7, 14172–14187. [CrossRef] [PubMed]

137. Liu, Q.; Hilsenbeck, S.; Gazitt, Y. Arsenic trioxide-induced apoptosis in myeloma cells: p53-dependent g1 or g2/M cell cycle arrest, activation of caspase-8 or caspase-9, and synergy with APO2/TRAIL. *Blood* 2003, 101, 4078–4087. [CrossRef]

138. Yan, W.; Jung, Y.S.; Zhang, Y.; Chen, X. Arsenic trioxide reactivates proteasome-dependent degradation of mutant p53 protein in cancer cells in part via enhanced expression of Pirh2 E3 ligase. *PLoS ONE* 2014, 9, e103497. [CrossRef]

139. Huang, L.; Liu, Z.; Jiang, H.; Li, L.; Fu, R. Decitabine shows synergistic effects with arsenic trioxide against myelodysplastic syndrome cells via endoplasmic reticulum stress-related apoptosis. *J. Investig. Med.* 2019, 67, 1067–1075. [CrossRef]

140. List, A.; Dewald, G.; Bennett, J.; Giagounidis, A.; Raza, A.; Feldman, E.; Powell, B.; Greenberg, P.; Thomas, D.; Stone, R.; et al. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. *N. Engl. J. Med.* 2006, 355, 1456–1465. [CrossRef]

141. Koreth, J.; Pidala, J.; Perez, W.S.; Deeg, H.J.; Garcia-Manero, G.; Malcovati, L.; Cazzola, M.; Park, S.; Itzykson, R.; Ades, L.; et al. Role of reduced-intensity conditioning allogeneic hematopoietic stem-cell transplantation in older patients with de novo myelodysplastic syndromes: An international collaborative decision analysis. *J. Clin. Oncol.* 2013, 31, 2662–2670. [CrossRef]

142. Della Porta, M.G.; Galli, A.; Bacigalupo, A.; Zibellini, S.; Bernardi, M.; Rizzo, E.; Allione, B.; Van Lint, M.T.; Piolletti, P.; Mareno, P.; et al. Clinical effects of driver somatic mutations on the outcomes of patients with myelodysplastic syndromes treated with allogeneic hematopoietic stem-cell transplantation. *J. Clin. Oncol.* 2016, 34, 3627–3637. [CrossRef] [PubMed]
161. Davids, M.S.; Kim, H.T.; Bachireddy, P.; Costello, C.; Liguori, R.; Savell, A.; Lukez, A.P.; Avigan, D.; Chen, Y.B.; McSweeney, P.; et al. Ipilimumab for patients with relapse after allogeneic transplantation. *N. Engl. J. Med.* 2016, 375, 143–153. [CrossRef]

162. Sallman, D.A.; Amy, M.; Komrokji, R.S.; McGraw, K.; Geyer, S.M.; Eksioglu, E.; Al Ali, N.; Lancet, J.E.; Wei, S.; Padron, E.; et al. Immune Checkpoint Profiling of TP53 Mutant and Wild-Type Myeloid Malignancies: TP53 Mutations Direct Immune Tolerance Via an Immunosuppressive Phenotype. *Blood* 2017, 130, 423.

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