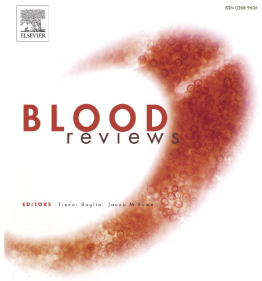

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A p53-JAK-STAT Connection Involved in Myeloproliferative Neoplasm Pathogenesis and Progression to Secondary Acute Myeloid Leukemia

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Abstract

Since the discovery of *JAK2* V617F as a highly prevalent somatic acquired mutation in the majority of myeloproliferative neoplasms (MPNs), it has become clear that these diseases are driven by pathologic activation of JAK2 and eventually of STAT5 and other members of the STAT family. The concept was strengthened by the discovery of the other activating driver mutations in *MPL* (thrombopoietin receptor, TpoR) and in calreticulin gene, which all lead to persistent activation of wild type JAK2. Although with a rare frequency, MPNs can evolve to secondary acute myeloid leukemia (sAML), a condition that is resistant to treatment. Here we focus on the role of p53 in this transition. In sAML mutations in *TP53* or amplification in genes coding for negative regulators of p53 are much more frequent than in de novo AML. We review studies that explore a signaling and biochemical interaction between activated STATs and p53 in MPNs and other cancers. With the development of advanced sequencing efforts, strong evidence has been presented for dominant negative effects of mutated p53 in leukemia. In other studies, gain of function effects have been described that might be cell type specific. A more profound understanding of the potential interaction between p53 and activated STATs is necessary in order to take full advantage of novel p53–targeted therapies.

Keywords:
Myeloproliferative Neoplasms, p53 mutant, Secondary Acute Myeloid Leukemia, STATs, Transcription.

Introduction

BCR-ABL1-negative myeloproliferative neoplasms include Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) (1). They are clonal malignant diseases resulting from acquisition of JAK-STAT activating driver mutations, of which JAK2 V617F is the most prevalent (2). The worst outcome of MPN is their transition to secondary AML (sAML), which is often marked by poor survival. 2-5% cases of ET and PV and around 15-30% cases of PMF transform to sAML (3-5). There are a number of mutations experimentally shown to contribute to this transition. Here, a special focus is laid on p53 and its role in the chronic MPN diseases and secondary AML.

Leukemias are a group of life-threatening clonal malignant disorders of blood-forming tissues, including the bone marrow and the lymphatic system. They are generally classified into two main groups: acute leukemia including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and chronic leukemia (chronic lymphoblastic leukemia (CLL), and chronic myeloid leukemia (CML)). The term acute and chronic refer to the evolution of the disease without treatment. The term leukemia arises from an old classification meaning that malignant cells are present in the blood. In fact, there is a continuum between leukemia and other malignant hematological diseases: certain myelodysplastic syndromes (MDS) are close to AML (3) and there are many homologies between CML and the other myeloproliferative BCR-ABL negative disorders. In addition, in lymphoid leukemia there are also many similarities between CLL and ALL and different types of lymphoma.

Acute leukemias are characterized by a block in differentiation and excessive blast accumulation. Among them, AML is the most common acute leukemia in adults. In 2019 alone, an estimated 21,450 new cases were diagnosed in United States and over 10,900 patients died from this disease (6). AML can itself be sub-classified as *de novo* AML, and secondary AML, which occur as a progression of CML, MPNs and MDS, or after chemotherapy or radiation treatment or age related.

p53: an overview

p53, also called "the guardian of the genome", is a transcription factor, a sequence specific DNA binding protein, that maintains genomic integrity. Being discovered in 1979, as a 53 kDa protein, it was found as a protein bound to the large T antigen of Simian Virus 40 (7, 8). Ten years later in 1989, strong experimental evidence established that p53 (or *TP53* for the gene) was the paragon of tumor suppressor able to inhibit cell growth and oncogenic transformation (9). Simultaneous work suggested that *TP53* mutations are common in a variety of human tumors (10, 11). p53 protein is composed of three domains: i) N-terminus, which contains two transactivation domains followed by a proline rich domain that mediates protein-protein interactions; ii) the core domain or DNA-binding domain that allows binding to the promoters of target genes; iii) the C-terminus, contains the nuclear localization signal, the oligomerization domain, and the regulatory domain that contains two MDM2-binding sites (Fig. 1). In unstressed cells, p53 is normally maintained at a very low level, hardly detectable by standard immunological staining. These low levels of p53 are mainly maintained by a post-transcriptional mechanism implicating regulators like MDM2 (an E3 ubiquitin ligase,) which ubiquitinates p53 targeting it for 26S-proteosomal degradation (12). In addition to its key roles in cell cycle arrest, apoptosis, and DNA repair, p53 was also

shown to be involved in many other biological processes, such as glucose and lipid metabolism, and oxidative stress (13).

Mutations in *TP53*

TP53 mutations occur in about half of all human cancers. The rate of mutation and the mutational spectra differ among various cancers (14). Deep sequencing analysis revealed that the majority of mutations are missense in nature and clustered within the DNA binding domain. A recent integrative study performed on 32 different cancer types from 10,225 patients revealed that over 91% of cancers with p53 mutations display loss of both *TP53* alleles (15). A number of compelling lines of experimental evidence suggests that specific allelic variants exert loss of function for p53 physiologic anti-proliferative and pro-apoptotic functions, potential gain of function (GOF) activities and dominant negative effects (Fig. 2). Kato et al. created 2,314 mutants of *TP53* by single nucleotide substitution throughout the entire length of *TP53* ORF except for the initiation codon. By functional assays in yeast, they showed loss of function and transcriptional inactivity associated with *TP53* mutants in cancers (16). Giacomelli et al. using mutagenesis by integrated TilEs created and characterized a library of around 8,000 *TP53* mutants representing all possible single amino acid variants of p53. They found commonly observed TP53 hotspot mutants that do not exhibit gain-of-function activities or dominant negative effects (17). Based on mutational signatures and using computational modeling approaches, the enrichment of various TP53 mutants was hypothesized to occur as a function of the particular tissue of origin.

These studies showed that the mutational landscape of *TP53* is quite complex with vast number of mutations in different tissues exhibiting different effects. Intriguingly, engineered p53 mutants were described that exert tumor suppressor effects *in vivo* in the absence of p53-mediated cell cycle arrest, apoptosis and senescence (18). Such a p53 mutant where three lysine (K117, K161, K162) that are acetylated are substituted to arginine retain the ability to regulate target metabolic target genes, but cannot induce transcriptional effects leading to the classical functions of p53, yet this mutant remains tumor suppressive in a mouse model (18). Therefore, other non-canonical gene targets are being uncovered that might be at the basis of the tumor suppressor effects of p53 during oncogenesis; these have recently been reviewed in (19).

The classical functions of p53 are to respond to DNA-damaging stress by cell cycle arrest and apoptosis. However, p53 can also promote cell survival during metabolic stress, as for example serine starvation; this function leads to metabolic reprograming of cancer cells (20). Certain p53 mutants retain the ability to protect against glutamine and serine starvation, while they cannot execute elimination of cells after DNA-damaging stress (21, 22). Increased p53 protein stability for certain missense mutants along with weak remnant transcriptional activity can explain why certain mutants can exert weak p21 transcriptional activity which can mediate resistance to treatment.

p53 and hematopoiesis

p53 plays a crucial role in hematopoiesis by regulating the proliferative capacity and self-renewal of hematopoietic stem cells (HSCs) (23). p53 exerts a cytoprotective role by maintaining HSCs quiescence and negatively regulating HSC self-renewal. Mice lacking wild type p53 exhibit an increase in the HSC pool with an excess of proliferation (24, 25). There are certain differences between p53 deficient HSC in comparison to normal HSC. In a

competitive repopulation assay *tp53*$^{-/-}$ bone marrow cells (BMCs) engrafted two to four times more efficiently than *tp53*$^{+/+}$ BMCs in a lethally irradiated mouse. Recipients of *Trp53*$^{-/-}$ donors had 2 to 3 fold more Lin(-)Sca-1(+)c-kit(+)CD34(-) BMCs than the recipients of *Trp 53*$^{+/+}$ donors at 5 months after transplantation. However only 44% of recipient of *Trp 53*$^{-/-}$ donors survived 5 months after transplantation compared with 92% of recipients of *Trp53*$^{+/+}$ donors (25). Aside from the hematopoietic system, p53 negatively regulates stem cells in other tissue systems as well. Furthermore, suppression of p53 increased the efficiency of reprogramming for induced pluripotent stem cells (26). The effect of p53 to restrain pluripotency is manifest also in tissue stem cells and its loss may allow cells within a tumor to acquire stem cell-like transcriptional signatures. This has been shown for breast cancer, where p53 loss was correlated with stem-cell like gene expression and depletion of differentiation genes regulated by polycomb repressive complex 2 (27).

p53, myeloproliferative neoplasms and other myeloid malignancies

In the context of the roles of the p53 pathway in hematopoiesis (28), the prevalence of *TP53* mutations in Myeloproliferative Neoplasms (MPNs) is low, but it carries high significance (Fig. 3) (29-33). In one study, only 5 patients in a cohort of 197 patients harbored a mutated *TP53*, but 4 of these 5 patients progressed to AML (31). *TP53* mutations in heterozygous state existed for an extended period of time during chronic MPN phase without clonal expansion (31). Loss of another WT allele by either chromosomal deletion or uniparental disomy was associated with rapid expansion of the homozygous clone, ultimately leading to leukemic transformation (31). Thus, patients with *TP53* mutations represent a high-risk group. Another study found *TP53* mutations in both *JAK2* V617F mutated (1 of 7) as well as *JAK2* wild-type secondary AML (3 of 9) (34). Half of these patients did not harbor *TP53* mutations in the MPN chronic phase. This suggests that acquisition of *TP53* mutation might be a late, but crucial event in MPN progression to sAML or *TP53* mutations present at very low allele frequencies not detectable by standard NGS, but that are selected during time, eventually by the treatment. Indeed, in therapy-induced AML it has been shown that the *TP53* mutations were already present at extremely low level before treatment, but are selected by the treatment as they confer a resistance to therapy (35). The situation in sAML of MPN might represent an exception from the notion that loss of heterozygosity of *TP53* mutation is frequent, but not mandatory in cancer, with genetic loss around the *TP53* gene being also a potential contributor to the oncogenesis (36). Although the number of sAML cases of MPNs that has been studied in great detail is low, data so far argue that for this particular subtype of sAML, the appearance of sAML coincides with mainly homozygous or compound heterozygous missense mutations of *TP53* (30, 31) rather than the classical missense – gene loss pair or heterozygous missense mutation or frameshift/gene loss (36).

In another study, deep sequencing analysis of peripheral blood samples collected from 212 women years before diagnosis of AML revealed the presence of *TP53* mutants (21 of 189 samples analyzed) (37). Patients carrying *TP53* mutations exhibited shortened latency of disease in comparison to patients with non-mutated *TP53*.

p53 dysfunction is frequently occurring event in AML, not just as a consequence of mutations in *TP53,* but also in the context of overexpression of MDM2 or MDM4 (negative regulators of p53) via chromosomal duplication or gene amplification). Since *TP53* mutations represent a low incidence event in hematological malignancies in contrast to solid tumors, a study showed that MDM2 and MDM4 duplication are present with higher frequencies in

patients expressing WT *TP53* alleles, thus leading to a phenotype close to a loss of function of p53 (38).
In a study of chromosomal aberrations associated with post-MPN sAML, authors found that more than 25% patients transformed to leukemia carried *TP53* mutations (30).
The incidence of *TP53* mutations in complex karyotype-AML (CK-AML) is exceptionally high. Around 70% cases of CK-AML were found as having *TP53* mutations and its alteration remains the most important prognostic factor (39).

Development of leukemia in the mice deficient for *Trp53*, overexpressing JAK2 V617F after retroviral transduction highlights the role of WT p53 in preventing leukemic transformation (40, 41). When bone marrow of WT p53 was transduced in parallel with JAK2 V617F, a PV like disease was detected (41), as expected from the previous mouse models of retrovirally transduced or knock-in/transgenic models. It has been shown previously that codon 248 of p53 protein is the most frequently mutated in MDS and AML (39, 42). To better understand the effect of this mutant, Chen et al. generated humanized *p53R248W* knock-in mice expressing human p53 mutant protein from the endogenous murine *Trp53* promoter, and observed a clonal expansion of HSCs expressing mutant p53 (43).

Overall, 25% of sAML post-MPN carry *TP53* mutations, while another 20%-25% carry amplification of negative regulators of p53, such as MDM4 (Fig. 2) (31) While 50% of sAML on MPNs carry defects in p53, it will be of great interest to study in depth the patients with sAML evolving from MPNs that do not carry *TP53* mutations or *MDM2* amplifications. Remarkably, four genetically related West Indian families with predisposition to acquisition of MPN driver mutations and ET development were found to share a duplication of a 700 kb region coding on chromosome 14q32 (44). This duplication results in enhanced thrombopoietin (TPO) sensitivity of megakaryocyte progenitors and potentiation of driver *JAK2*, *MPL* and *CALR* mutations during MPN development. In these West Indian families that essentially develop ET, there is a frequent progression to sAML. No *TP53* mutations or MDM4 amplification were detected in these sAML patients, while IDH1/2 or TET2 mutations were detected. Of interest the enhanced TPO-sensitivity in the West Indian families required two of the several genes amplified, *ATG2B* and *GSKIP*. However, other families with different duplications in 14q32 also exhibited predisposition to hematological malignancies, essentially MDS, and in those families ATG2B and GSKIP were not amplified, but may have been deregulated by long-range chromatin interactions (45). Although it cannot be excluded that another gene such as TCL1A or a lncRNA present in these duplications could be responsible of the predisposition. Future studies will be required to determine the link between MPN predisposition, enhanced ligand-dependent versus persistent JAK-STAT activation and events leading to sAML progression.
In contrast, the frequency of *TP53* mutations in de novo AML is low (around 8 – 10%) (39, 46, 47).

Functional consequence of *TP53* mutations

Mutations in p53 can have three different effects: i) Loss of function (LOF), where p53 loses the capacity to induce its target genes; ii) Gain of function (GOF), this concept was first introduced in 1993, after a study showing that expression of p53 mutant can transform p53-null cells in vitro and p53 deficient mice in vivo, which suggests that this p53 mutant confers additional phenotypes over p53-null cells (34, 48). Functional differences between LOF and

GOF can be exemplified by the different phenotypes observed in Li-Fraumeni Syndrome (LFS), where patients harboring germline LOF mutations tend to develop cancer later in life, while patients harboring GOF mutations tend to develop cancer in early stages of their lives; iii) Dominant negative mutants, which exert dominant negative effect on remaining WT allele of p53, by forming heterotetramers that result in the inactivation of the wild type form (14, 49). The higher frequency of mutated *TP53* over the simple loss of WT *TP53* in human cancer suggests a selective advantage of such mutants in tumor initiation or progression, as reviewed in (50). Mutations in *TP53* exert such an impact on structure of the p53 protein by inducing possible novel interactions with other proteins such as other transcription factors, and interfere with their physiological functions.

Higher mutant p53 protein stability leads to its accumulation, which is a crucial for the oncogenic GOF activity. This stability can be achieved by different mechanisms. Mutant p53 aggregates by virtue of the hydrophobic core of the DNA binding domain. Upon mutation this core that contains an aggregation-nucleating sequence gets exposed on the mutation site (51). Also, mutant p53 loses its ability to induce expression of MDM2, a degrader of p53, thus maintaining mutant p53 at higher levels. Once stabilized mutant p53 may exert GOF activities. The biological relevance of these functions is now debated and can be cell-type specific.

Mutant p53 R248Q was reported to physically interact with HSP90 and once stabilized, it leads to constitutive activation of JAK2-STAT3 signaling pathway and enhances size and invasiveness of colonic tumors (52). Removal of mutant p53 abrogates such effects. In human erythroleukemia cell line (HEL), harboring 8 copies of *JAK2* V617F and homozygous *TP53* M133K mutation, mutant p53 M133K was found to interact with persistently activated STAT5 and induce gene expression of non-canonical genes (53).
Mutant p53 promotes genomic instability as a consequence of acquisition of GOF. Cyclin A1, which is overexpressed in de novo AML, along with cdk2 phosphorylates mutant p53 at S315, this in turn leads to stable complex formation with topoisomerase I, triggering hyper-recombination in mutant p53 cells (54). Mutant p53 is also known to regulate the expression of *TDP2* (tyrosyl-DNA phosphodiesterase 2) gene, which encodes a protein required to repair etoposide induced DNA double strand breaks (55). GOF p53 mutants were shown to bind and upregulate a set of chromatin regulatory genes including acetyltransferases and methyltransferases, which alter the histone acetylation and methylation pattern genome wide (56).

Two recent studies highlight the puzzling complexity of the role of *TP53* mutations in AML. One study in CK-AML (Complex Karyotype-AML) demonstrated the role of mutant p53 R172H (mouse homolog of human R175H) in maintaining leukemia cell stemness by inducing FoxH1 (57). FoxH1 is a transcription factor, which is generally expressed during embryonic development. Mutant p53 elevated the expression of FoxH1, leading to aberrant self-renewal of HSPCs. Therefore, a strong association between mutant p53 GOF and stemness was suggested for CK-AML. In contrast, another study assessing the role of the most frequently occurring *TP53* mutations in high risk MDS (58) found that misesense mutations in *TP53* led to decreased p53 binding to canonical sites and only dominant negative effects, but not GOF both in engineered isogenic AML cell line models (K562 and MOLM13) and in mouse bone marrow transplant experiments (59). While this study used AML cell lines that are not perfect models for secondary AML of MPNs, as K562 is a blast crisis on BCR-ABL-positive chronic myeloid leukemia and MOLT13 is a relapse of acute monocytic leukemia secondary to MDS, these data on a similar set of TP53 mutations shed

light on the mechanism of action of p53 mutants. The high risk MDS *TP53* mutations represent an interesting set as they are present in the oncogenic pre-leukemia phase (58). No evidence was found for an additional GOF transcriptional program of missense *TP53* mutations when Chip-seq and RNA-seq data were compared (59). Furthermore, when RNA-seq data were interpreted independently of DNA binding data, no alternative general program was detected (59). Remarkably, for one p53 mutant (R282W) increased DNA binding to both canonical and non-canonical sites was observed, but without stronger induction from the former and induction from latter promoters (59). The study did not report any gain of function features and provided strong evidence for a dominant negative effect of mutant p53 over wild type p53 in MDS and AML (59). Finally, examination of the clinical evolution of 154 patients with *TP53*-mutated AML from the German-Austrian AML Study Group did not reveal a more severe evolution or an increased set of additional mutations in missense *TP53* mutated patients versus stop-codon p53-null patients (59).

More experimental evidence is needed in the future to validate the dominant negative-only versus gain-of-function only role of *TP53* mutations in AML. One possibility is that GOF effects are manifest in a tissue-specific manner where levels of expression of certain partners of p53 mutants are sufficiently high.

p53 and STATs

The somatic activating mutation JAK2 V617F is acquired in >70% of BCR-ABL-negative MPNs (PV, ET and PMF) (60-63). This mutation leads to activation of downstream signaling pathways linked to cytokine receptors in cytokine independent fashion. The members of signal transducer and activator of transcription (STAT) transcription factor family STAT5, STAT3 and STAT1 are constitutively activated. When activated by cytokines, STATs regulate transcription of genes involved in cell survival, growth and differentiation in a transient manner. Persistently hyperactivated STATs are oncogenic both in mouse models and patients (64). Signaling and biochemical studies pointed to differential STAT5 signaling by ligand-dependent versus constitutively active cytokine receptors. In hematopoietic cells transformed by oncoproteins that activate JAK2-STAT5 persistently, or by BCR-ABL it was mainly STAT5B that was constitutively activated (65, 66). Sequencing of native sequences after ChIP with anti-STAT5 antibodies in cytokine-dependent and - transformed cells indicated that in transformed cells, STAT5B bound in the chromatin not only to high affinity, but also to low affinity GAS (gamma interferon activated sequence) sites (65).

Several studies indicated that cross-talk exists between the JAK-STAT pathway and p53 function (Fig. 4). STAT1 decreases the expression of MDM2, thus stabilizing p53 (67). STAT1 binds p53 and accentuates transcriptional effects of p53 on certain p53-responsive apoptotic genes like *Noxa* and *Bax* (67). Another study showed that persistently activated STAT3 may disable p53 without the requirement for TP53 mutations. Activated STAT3 interacts with the promoter of the *TP53* gene, inhibiting p53 expression (68). Consequently, oncogenic transformation with constitutively activated STAT3 may be achieved without acquisition of *TP53* mutation (68).

Fritsche et al. reported that WT p53 and p53 mutants retaining intact DNA-binding domain inhibited the transactivation potential of prolactin induced STAT5, as measured by STAT5 luciferase reporter assays (69). The authors could not detect any interaction between p53 and

STAT5. They have shown that p53 did not prevent STAT5-DNA binding as assessed by gel shift assays, suggesting an interference with STAT5 transcriptional activity, but not DNA-binding capacity (69).
In contrast, Girardot et al. have shown, by co-immunoprecipitation, that p53 interacts (directly or indirectly) with both tyrosine phosphorylated and un-phosphorylated (U) STAT5 (53). This interaction is mediated by the DNA binding domain of STAT5, as truncations of the N-terminus (1-136) and C-terminus (at 749, thus lacking 749-794) did not disrupt the interaction. Furthermore, p53 WT was able to inhibit STAT5 transcriptional activity (53). Given that the U-STAT5 was shown to bind to chromatin and prevent binding of lineage specific factors responsible of megakaryocyte differentiation (70), the interaction between p53 and U-STAT5 may be relevant in chromatin regulation over the differentiation pathway. In contrast to its effects on STAT5, p53 WT exerted no transactivation inhibition on STAT1 reporter gene (69). Ren et al. showed that STAT5 down-regulates expression of nucleophosmin 1 (NPM1), which is a known stabilizer of p53 (71). Thus, decreased NPM1 reduces p53 levels (72). Along the same lines, Falini et al. reported that mutated NPM1 is aberrantly localized in the cytoplasm of myeloid blasts of AML patients (73) and is able to dampen p53 pathway (74).

Of interest, persistently activated STAT5 was shown to synergistically bind along with p53 mutants or p53 WT to promoters of non-canonical STAT5/p53 target genes (53). This leads, for example, to pathologic expression of *LPP* gene that hosts *miR-28* (in intron 6). *miR-28* is pathologically overexpressed in 30% of MPNs and downregulates TpoR mRNA along with that of several genes involved in megakaryocyte differentiation (75). Genome-Wide chromatin immunoprecipitation studies showed chromatin co-localization of STAT5 and p53 at around 460 genomic positions in proximal promoter (53). Chromatin immunoprecipitation assay showed that STAT5 inhibition, with Ruxolitinib, or *TP53* knockdown was sufficient to decrease the expression of genes binding both STAT5 and p53 (53). Besides *LPP* and *miR-28*, several other genes are induced by STAT5/p53 complex. These are *ATP5J*, *CRABP1*, *GTF2A2*, *VEGFC*, *NPY5R* and *LEF1*. Those have been found to be overexpressed in MPN patient platelets, especially in ET and myelofibrosis (53, 75). Further studies are required to determine the potential role of such pathologic gene expression and whether this may contribute to progression to sAML.
One interesting finding is that Suppressor or Cytokine Signaling 1 (SOCS1) that is induced by persistent JAK-STAT activation was found to activate/stabilize p53 and to be required in senescent fibroblasts for induction of a subset of p53-target genes (76). This finding might provide an interesting link between inflammation and induction/stabilization of p53 in disorders such as MPNs.
p53 was shown to interact with protein inhibitor of activated STATs y (PIASy) coded by *PIAS4*, which leads to inhibition of p53 trans-activating functions (77). Furthermore, WT p53 exerts an inhibitory role on constitutively active STAT3 at different levels: DNA binding, phosphorylation and transcriptional activities (71).

Taken together, these studies suggest an inverse correlation between levels of persistently activated STATs and expression and physiologic function of WT p53. While the majority of MPNs retain WT TP53, the very high prevalence of TP53 mutations or events leading to functional inactivation of p53 in sAML remains puzzling and requires further exploration. A different path to AML phenotype appears to be taken in *de novo* AML (39, 46, 47). A better understanding of the differences between the roles of p53 between sAML and *de novo* AML might prove beneficial with respect to novel therapeutic approaches in sAML. The cross-talk between persistently activated JAK-STAT pathway in MPNs and sAML would need to be

compared to the cross-talk between p53 and other oncogenic pathways invoked in sAML evolving after on MDS, anti-cancer therapy or age related sAML, as they are likely to involve some common structural mechanisms at the level of chromatin spatial organization.

Regulation of p53 by other pathologically activated signaling pathways

Acquisition of JAK2 V617F leads to accumulation of the La protein via activation of PI-3'-kinase/mTOR pathway, an RNA- binding phosphoprotein which interacts with the mRNA of MDM2 and stabilizes it. The stabilized mRNA results in enhanced translation of MDM2, this in turn degrades p53. Unaltered MDM2 mRNA and MDM2 protein levels in *JAK2* non-mutated cells point out the negative regulation of p53 by JAK2 V617F (78). The combination of MDM2 antagonist, Nutlin-3, with low dose of IFN-α significantly reduces the proliferation of PV CD34+ cells. This indicated that the attenuating effect exerted by JAK2V617F on p53 is reversed by MDM2 antagonist (55).

WT p53 function is also suppressed by abnormal signaling pathways and interrelated mechanisms. Ras/Raf/MEK/ERK and Ras/PI3K/PTEN/Akt/mTOR regulate the activity of WT p53 (79). Gilkes et. al showed that Ras overexpression and ERK hyper-phosphorylation lead to upregulated expression of MDM2 and MDM4, thus impairing p53 activity (80). Another study reported the presence of a Ras responsive element in the promoter of MDM2 and showed modulation of MDM2 expression by Ras/Raf/MEK/MAP kinase (81). Aberrant activation of Ras/Raf/MEK/ERK pathway, present in most hematopoietic malignancies, leads to elevated levels of MDM2 and enhanced degradation of WT p53 (82-85). Finally, NF-κB is frequently constitutively active in AML, especially *de novo* AML (86). NF-κB responsive sites were identified in the promoter of MDM2 (87). Increased expression of MDM2 led to deregulation of p19ARF (negative regulator of MDM2 and p53 stabilizer) further accentuating WT p53 dysfunction.

CEBPA not only functions as a transcription factor, targeting lineage specific genes, but also is a potent cell cycle inhibitor. KLF4 is a lineage specific transcription factor and promotes monocyte differentiation. It has been reported that CEBPA transcription is activated by p53 and KLF4 defining a p53-KLF4-CEBPA axis; this axis is deregulated in *de novo* AML, with impaired function of p53 and levels of KLF4 and CEBPA (88). In the same study, the authors reported that in PBMCs isolated from AML patients, the level of functional nuclear p53 protein was found significantly reduced in comparison to healthy PBMCs.

Future considerations

Several studies have reported biochemical and functional interactions between p53 and STATs. An inverse correlation appears to exist between levels of persistently activated STATs and expression and physiologic function of WT p53. Loss of both p53 alleles, especially by homozygous missense or compound missense mutations in *TP53* appears to be characteristic to establishment of secondary acute leukemia on MPNs, which is very hard to treat. *TP53* mutations are also key for progression of MDS and establishment of age-related and therapy-related AML. What is specific for sAML of MPNs is the co-existence of strong persistent STAT5 activation throughout the chronic phase and during oncogenesis and loss of physiologic function of p53 especially by missense mutations. Further studies are necessary to define the relevance of all reported biochemical interactions for oncogenesis under conditions where certain p53 domains being naturally unstructured may exert a tendency to

interact with other proteins, especially when mutations induce stability, and this in addition to the core hydrophobic interactions.

Research agenda

Several small molecules are emerging that could re-activate certain p53 mutants. It will be important to obtain a clear picture on whether such small molecules can prevent dominant negative effects or gain of function effects in various cancers, including sAML and in which cellular and signaling context they may be effective. Furthermore, associations between homozygous or compound heterozygous TP53 mutations with other epigenetic or splicing clonal mutations should be studied. A major future point is to understand whether a mechanistic link exists between *TP53* and *RUNX1* mutations in the establishement of sAML.

Practice points

TP53 mutations, as well as amplifications of MDM2/4 genes should be carefully assessed in MPN molecular diagnosis, as homozygous or compound heterozygous *TP53* mutations are associated with sAML.

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Potential conflicts of interest

Stefan N. Constantinescu is co-founder of MyeloPro Diagnostics and Research GmbH, Vienna, a biotechnology company aiming to seek treatments for mutant calreticulin positive myeloproiferative neoplasms. There is no conflict of interests.

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Figure legends

Figure 1: Domain structure of p53. TAD, trans-activating domain; NES, nuclear export signal; DBD, DNA Binding Domain; NLS, nuclear localization signal, TET, tetramerization domain; REG, regulatory domain.

Figure 2: Putative effects of p53 mutated proteins in acute myeloid leukemia.
Upon mutation, p53 can either exert dominant negative effect on remaining WT p53 or can acquire neomorphic functions. Mutant p53 interacts with other protein partners (transcription factors, molecular chaperones) or can directly function as a transcription factor, which may lead to diverse outcomes, such as induction of non-canonical genes, stabilization of oncogenic protein, aberrant signaling pathways, self-renewal of leukemic cells.

Figure 3: *TP53* mutations in Acute Myeloid Leukemia.
TP53 mutations in hematological malignancies are rare events as opposed to solid tumors (≤50%). However, *TP53* mutations are frequent during transformation of Myeloproliferative Neoplasms (MPN) to secondary Acute Myeloid Leukemia (sAML). MDM4 (negative regulator of *TP53*) amplification occurs during leukemic transformation of MPNs in a mutual exclusive manner with *TP53* mutations.

Figure 4: Regulation of p53 protein function in context of activated STATs.
Activated STAT1 exerts an inhibitory effect on MDM2, thereby stabilizing p53. The highly prevalent JAK2 V617F mutated protein in MPNs induces expression of La protein via the PI3K/Akt/mTOR pathway leading to up-regulation of MDM2 that promotes the degradation of p53. While WT p53 inhibits cytokine induced STAT5 signaling, constitutively activated STAT5 deregulates p53 via by preventing the stabilization effect of Nucleophosmin (NPM1) on p53. p53 and constitutively active STAT3 negatively regulate each other. Protein Inhibitor of Activated STAT Protein (PIAS*y*) negatively regulates p53.

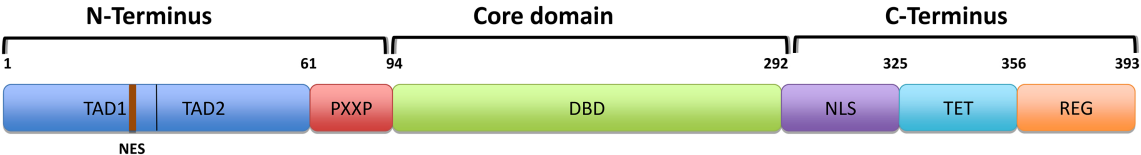
N-Terminus
Core domain
C-Terminus
1
61
94
292
325
356
393
TAD1
TAD2
PXXP
DBD
NLS
TET
REG
NES

Figure 1

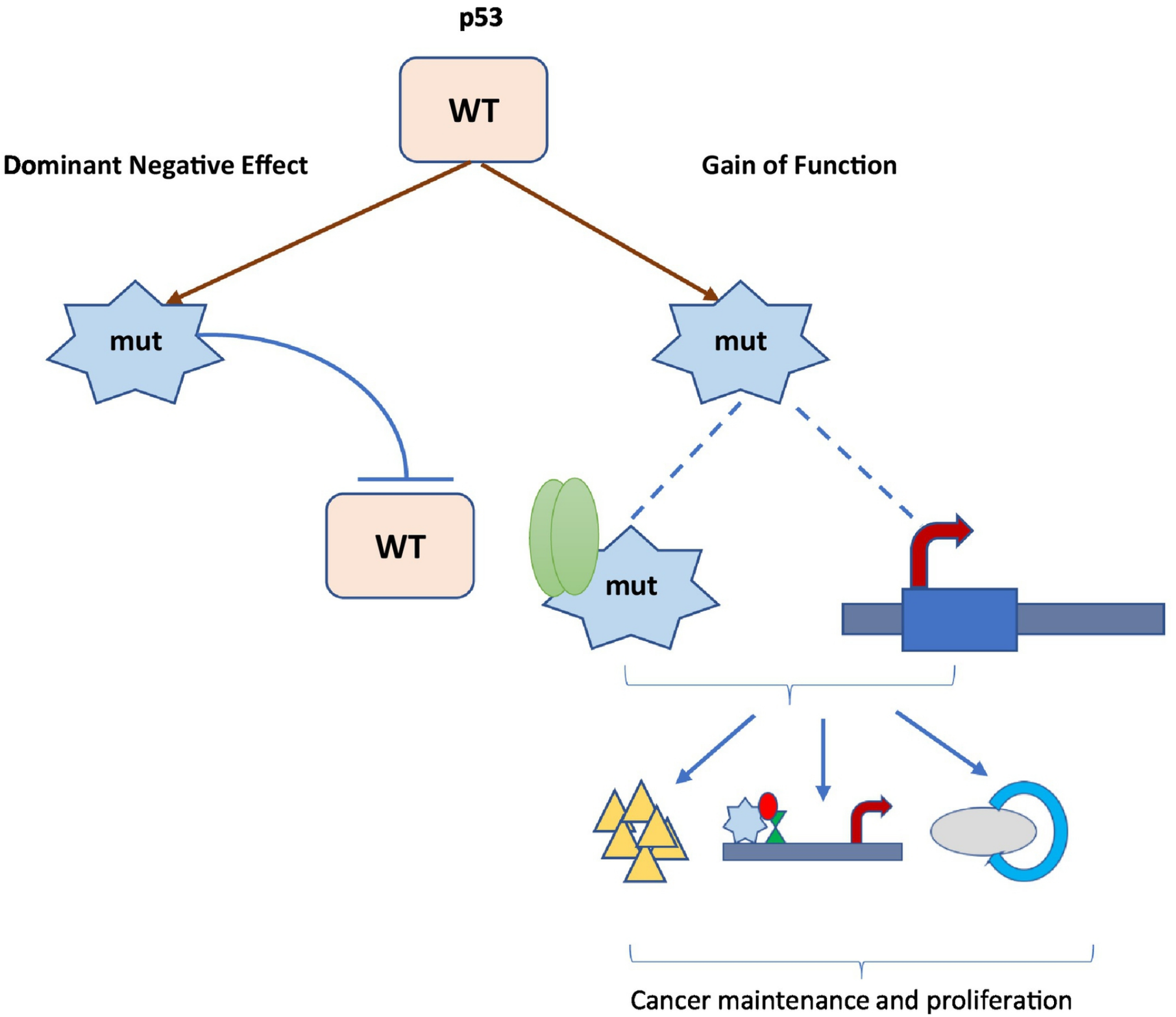
p53
WT
Dominant Negative Effect
Gain of Function
mut
mut
WT
mut
Cancer maintenance and proliferation

Figure 2

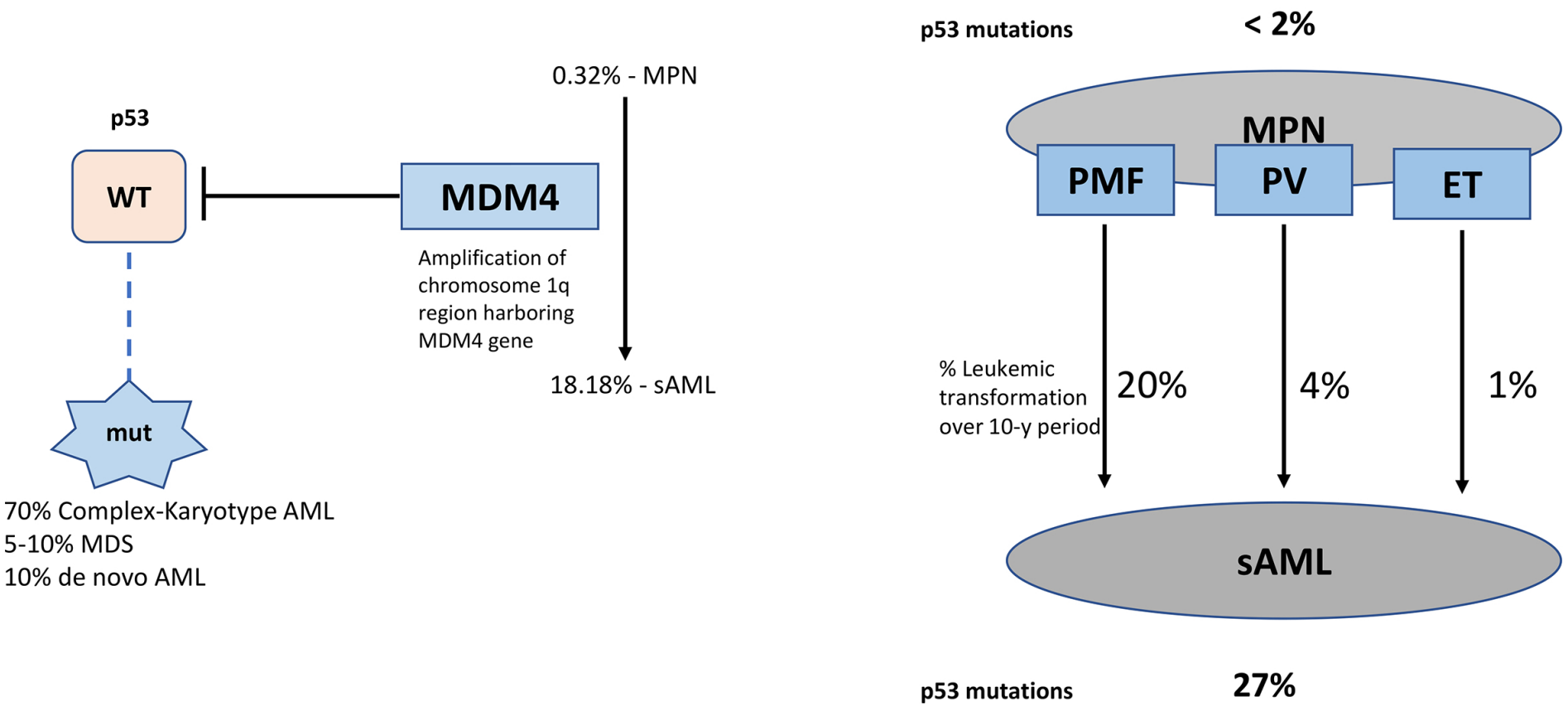
p53
WT
MDM4
mut
0.32% - MPN
Amplification of chromosome 1q region harboring MDM4 gene
18.18% - sAML
70% Complex-Karyotype AML
5-10% MDS
10% de novo AML
p53 mutations
< 2%
MPN
PMF
PV
ET
% Leukemic transformation over 10-y period
20%
4%
1%
sAML
p53 mutations
27%

Figure 3

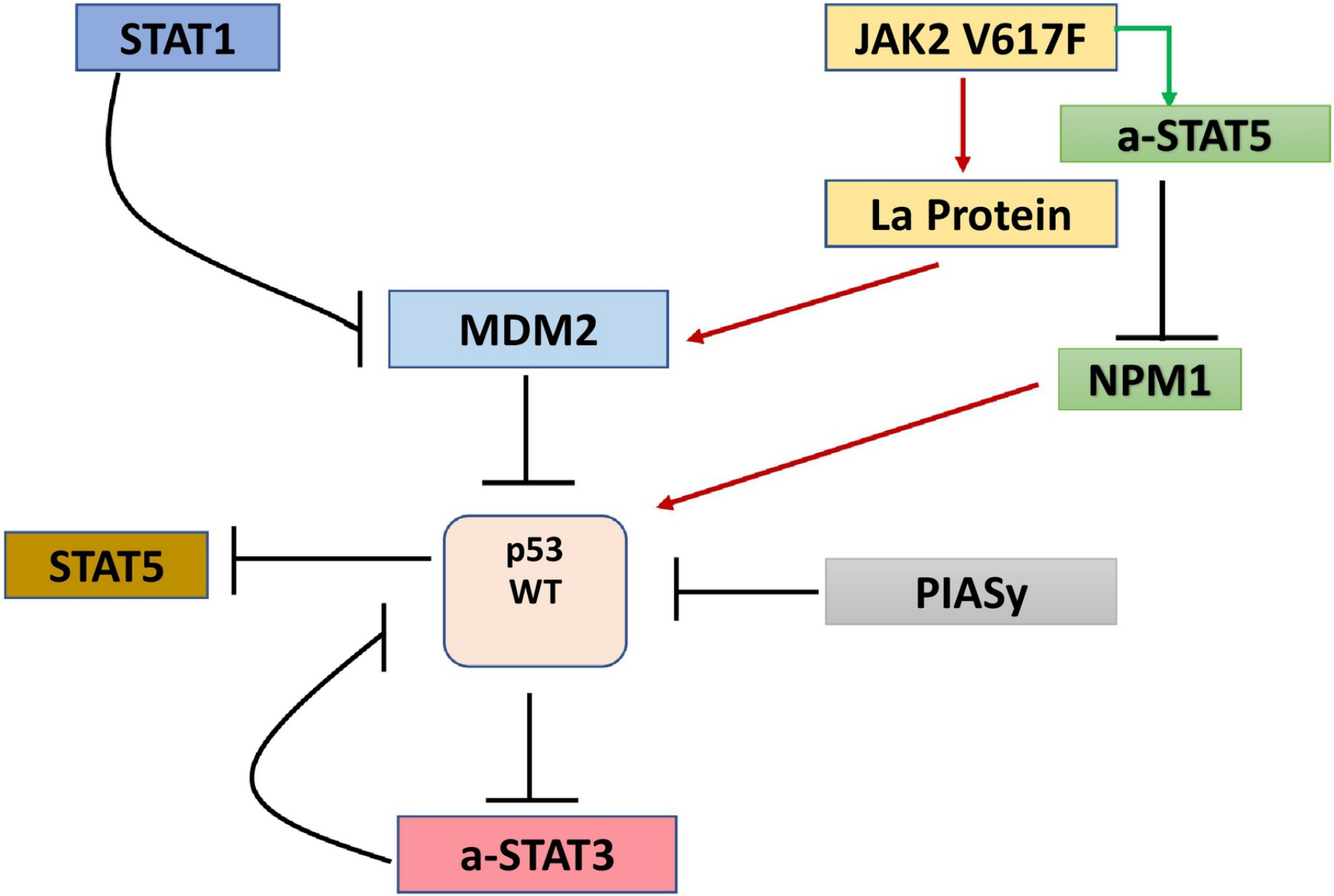
STAT1
JAK2 V617F
a-STAT5
La Protein
MDM2
NPM1
STAT5
p53
WT
PIASy
a-STAT3

Figure 4