

drug combinations that can simultaneously target the undifferentiated leukemic cell populations as well as the more mature myeloid lineages (especially monocytes) may represent a powerful way to prioritize the most promising drug combinations for pre-clinical study and for clinical development. Clearly, this report demonstrates just the beginning of utility of this exciting drug screening platform.

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To target the untargetable: elucidation of synergy of APR-246 and azacitidine in TP53 mutant myelodysplastic syndromes and acute myeloid leukemia

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Mutations of the tumor suppressor gene *TP53* represent a common mutation in myeloid malignancies, occurring in 10-20% of patients with *de novo* myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) with profound negative impact on outcomes and a median overall survival (OS) of 6-12 months.¹⁻³ Critically, the clonal burden of *TP53*, that is the variant allele frequency (VAF) and/or allelic state of *TP53*, is intimately tied with the clinical trajectory of these patients and is a robust, independent predictor of survival.^{4,7} Given the poor OS and lack of therapeutic options for *TP53* mutant MDS/AML patients, a number of novel agents are being investigated in this patient group.⁸ Of these, APR-246 has evoked considerable excitement based on its robust clinical efficacy in combination with azacitidine in *TP53* mutant MDS/AML patients.^{9,10} In this issue of *Haematologica*,¹¹ Maslah *et al.* describe compelling preclinical synergy of APR-246 in combination with azacitidine in *TP53* mutated MDS and AML and, more importantly, identify a novel molecular mechanism underlying the observed synergy.

Recent elegant work has definitively identified that *TP53* missense mutations in myeloid malignancies result in a dominant-negative effect without evidence of neomorphic gain-of-function activities, ultimately leading to a selection advantage when exposed to DNA damage.¹² Thus, restoring wild-type function in *TP53* mutant clones would be of profound beneficial impact. APR-246, a methylated PRIMA-1 analog, is a novel, first-in-class, small molecule that selectively induces apoptosis in *TP53* mutant cancer cells. Mechanistically, APR-246 is spontaneously converted into the active species methylene quinclidinone (MQ), which is able to covalently bind to cysteine residues in mutant p53 thereby producing thermo-

dynamic stabilization of the protein and shifting equilibrium toward a functional conformation.^{13,14} APR-246 monotherapy was originally investigated in a phase I trial including AML patients with clinical activity and correlative data identifying activation of p53-dependent pathways.^{15,16}

Maslah *et al.* identified in *TP53* mutant cell lines, *in vivo* models, and primary patient samples that the combination of APR-246 and azacitidine results in a synergistic pro-apoptotic effect as well as a dramatic reduction in cell proliferation *via* cell cycle arrest (Figure 1). As the majority of *TP53* mutations are missense and located in the DNA binding domain, synergy experiments were performed with the SKM1 cell line, which harbors a homozygous hotspot mutation of *TP53* (p. R248Q), and thus is an appropriate representation of clinical disease.¹⁷ Combination therapy of APR-246 and azacitidine resulted in a doubling of apoptotic cells *versus* azacitidine alone as well as 83% of cells undergoing cell cycle arrest in G0/G1. This synergistic effect was confirmed in a xenotransplantation model where combination therapy resulted in a pronounced inhibition of disease progression which occurred early and was durable. Subsequently, the authors interrogated differential gene expression profiles of SKM1 cells treated with either drug alone *versus* the combination of APR-246 and azacitidine. As expected, Gene Set Enrichment Analysis (GSEA) and DAVID analyses of APR-246 treated cells showed robust induction of p53-target genes including *CDKN1A*, *CASP1*, *BAX* and *FAS*, which was confirmed by reverse transcription real-time quantitative polymerase chain reaction (RT-qPCR), resulting in activation of an early apoptotic program. Furthermore, GSEA analysis of “synergistic only” genes (i.e. genes differentially expressed only with combination treatment)

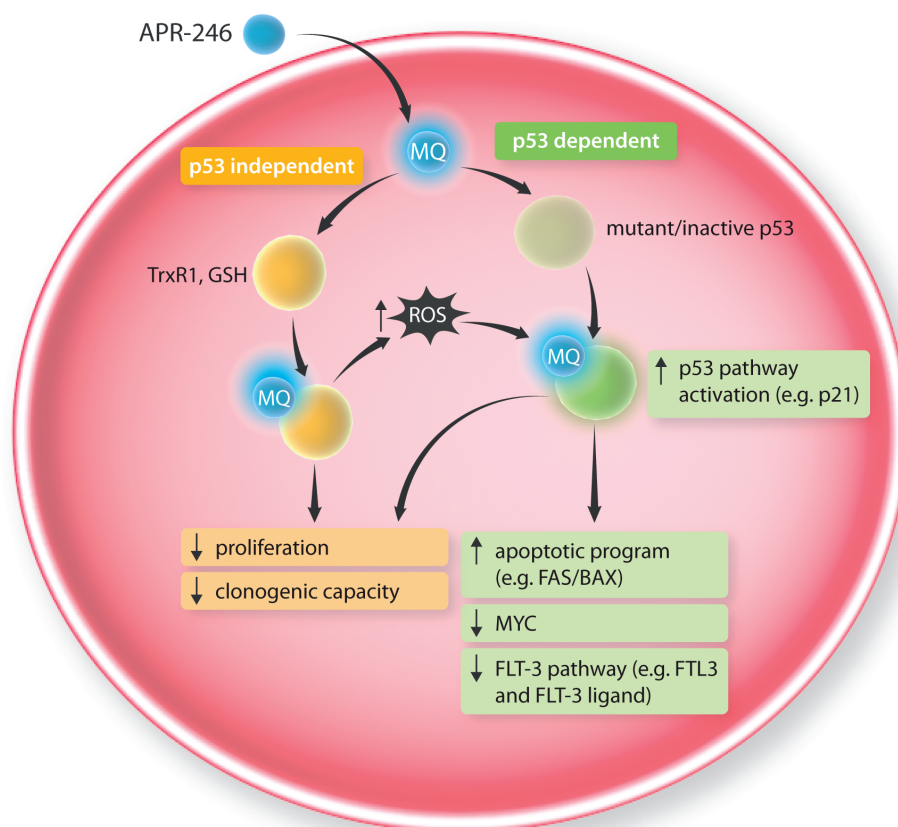


Figure 1. Mechanisms of synergy with APR-246 and azacitidine in *TP53* mutant myelodysplastic syndromes (MDS) / acute myeloid leukemia (AML). GSH: glutathione; MQ: methylene quinuclidinone; ROS: reactive oxygen species; wt: wild-type; TrxR1: thioredoxin reductase 1; FLT-3: fms like tyrosine kinase 3.

identified activation of p53 pathway, induction of apoptosis, and downregulation of *MYC* expression, thus functionally demonstrating restoration of wild-type p53 function. Notably, transcriptome analysis with confirmation by RT-qPCR also identified a novel synergistic mechanism of FLT3 pathway downregulation. Importantly, the inhibition of cell proliferation with combination therapy could be overcome in a dose-dependent fashion in the presence of FLT3 ligand, highlighting a novel therapeutic mechanism of APR-246 that could potentially be exploited in combination with FLT3 inhibitors in future clinical study.

Of importance, synergy was most robust in the presence of *TP53* missense mutations where there is accumulation of misfolded p53 protein, strongly supporting the primary mechanism of APR-246. However, APR-246 also has p53-independent function *via* MQ binding to thioredoxin reductase and glutathione, leading to depletion of glutathione and accumulation of reactive oxygen species (ROS), which can feed forward p53 activation (Figure 1).^{18,19} Indeed, the authors also show synergy in *TP53* knockout mutant cell lines where there is absence of p53, albeit with less synergy than in the missense mutant model. Accordingly, there was significant enrichment of ROS-induced genes with APR-246 treatment. The authors also show data whereby both cell proliferation and clonogenic capacity were strongly inhibited, both in the presence and absence of mutant p53 protein.

Perhaps the most compelling data regarding the synergy of APR-246 and azacitidine originates from the clinical activity in *TP53* mutant MDS/AML patients, where recent

data report an overall and complete remission rate of 87% and 53%, respectively (*clinicaltrials.gov* identifier: NCT03072043).⁹ Similarly, preliminary results from a phase II study of APR-246 and azacitidine by the Groupe Francophone des Myélodysplasies (*clinicaltrials.gov* identifier: NCT03588078) showed comparable response rates.¹⁰ Accordingly, the US Food and Drug Administration has recently granted breakthrough therapy designation for the treatment of patients with *TP53* mutant MDS with the combination of APR-246 and azacitidine and the randomized phase III study of APR-246 and azacitidine *versus* azacitidine is ongoing in MDS patients (*clinicaltrials.gov* identifier: NCT03745716). As *TP53* mutations are strong drivers of negative outcomes in multiple hematologic malignancies, as exemplified by relapsed pediatric acute lymphoblastic leukemia, APR-246 may likely have more broad clinical implications including synergy with traditional cytotoxic agents, as has been recently described.²⁰ Together, shedding light on the synergistic mechanisms underlying APR-246 and azacitidine therapy as presented in this study are critical to continue to advance this novel therapeutic option for patients with the poorest outcomes to traditional treatments.

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Peripheral T-cell lymphoma diagnosis: building a molecular tool

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T-cell lymphoma (TCL) has quite a poor probability of survival (around 25-30% of patients after 5 years), which contrasts with the progress that has recently been made in Hodgkin lymphoma and B-cell lymphoma.¹⁻³ Some recently defined TCL types, such as anaplastic large-cell lymphoma (ALCL), have a better clinical outcome. However, the majority of cases diagnosed with peripheral T-cell lymphoma (PTCL) will eventually die of the disease, and in some specific tumor types, such as intestinal TCL, the prognosis is even more miserable.

Poor survival probability in this context is associated with serious difficulties in lymphoma diagnosis when using routine morphological and immunohistochemistry tools. PTCL classification involves division into multiple subtypes, typically of low frequency and with hazy distinctions (Figure 1). As a consequence, different studies coincide in achieving a very low rate of reproducibility in TCL diagnosis, especially in recognizing ALK-negative ALCL, and distinguishing between PTCL-not otherwise specified (NOS) and PTCL with TFH phenotype or angioimmunoblastic TCL.⁴

An important feature of this situation is that the relative frequencies of the tumor types are quite low, which makes it difficult to design and develop clinical trials, and this hampers the introduction of new drugs for PTCL therapy.

Nevertheless, these difficulties have inspired some research groups to provide essential information about the molecular basis of TCL pathogenesis, and to identify some attractive and challenging therapeutic targets.⁵⁻⁹

Drieux and co-workers,¹⁰ in a joint project involving French, Belgian and Swiss hospitals, are now addressing the radical proposal that molecular diagnosis may give a more precise and reproducible way of classifying TCL cases. Using a technique applicable to paraffin-embedded tissue, they measure the expression of 20 genes, including 17 markers relevant to T-cell classification, one Epstein-Barr virus-related transcript, and frequently mutated variants of RHOA (G17V) and IDH2 (R172K/T). Selected genes allow the identification of several entities: TFH cells, the normal counterparts of angioimmunoblastic TCL; TH1 and TH2 phenotypes, which reflect the diversity of PTCL-NOS; T-regulatory cells, for distinguishing ATLL; the cytotoxic markers, CD30 and ALK, for identifying ALCL; and CD56 and EBER1, to discriminate T/natural killer (NK)-cell lymphomas.

The results validate the solid basis of the currently used PTCL classification scheme, and highlight the similarity between angioimmunoblastic TCL and PTCL-TFH. The findings show a group of cases with simultaneous expression of TFH markers and TH2 (GATA3), and indicate that ALK-negative ALCL is a heterogeneous condition. Cases of PTCL-NOS appear to be extremely heterogeneous,