Targeting TP53 mutation in AML

David A. Sallman

H. Lee Moffitt Cancer Center and Research Institute, USA

Myelodysplastic syndromes (MDS) represent a heterogeneous group of malignant stem cell neoplasms hallmarked by ineffective hematopoiesis and risk of leukemic transformation. Of all somatic mutations identified in MDS, *TP53* mutations are associated with the most inferior outcomes across independent studies with a median OS of 6-12 months despite standard of care therapies.[1,2] Importantly, there is little, if any, significant difference between TP53 mutant MDS and AML as most often patients are oligoblastic and outcomes to standard and novel therapy have been similar to date. Notably, the variant allele frequency (VAF) and/or allelic status of *TP53* to be strongly concordant with disease phenotype and further stratifies survival over binary mutation analysis alone.[1,3-5] Importantly, clearance of *TP53* (i.e. VAF <5%) predicted for improved OS whereas clonal expansion significantly predicted for inferior OS which remained predictive of OS in multivariate analysis. Importantly, clearance of *TP53* in the setting of therapy, i.e. HMA therapy or allogeneic stem cell transplantation, has also been identified to be a positive predictor of outcomes[6]. APR-246 (eprenetapopt) is a novel small molecule anti-cancer compound that reactivates mutated and non-functional p53 and targets the cellular redox balance, two Achilles heels of cancer cells. APR-246 is a pro-drug that spontaneously releases the active drug species, methylene quinuclidinone (MQ), at physiologic pH. MQ forms a covalent bond with cysteine residues in p53 and the binding event thermodynamically stabilizes p53 protein, shifting the dynamic equilibrium away from the unfolded/misfolded state and toward the wild-type p53 conformation. Additionally, APR-246 has p53 independent activity via an increase in reactive oxygen species as well as more recent data identifying early cell death by APR-246 is mediated via ferroptosis[7,8]. We have conducted a Phase 1b/2 combination study of sequential APR-246 and azacitidine in HMA-naïve, *TP53* mutant MDS and oligoblastic AML (≤30% blasts; ClinicalTrials.gov identifier NCT03072043). The phase 1b/2 results showed the combination to be well tolerated with no dose-limiting toxicities and a recommended phase 2 dose of APR-246 as a fixed dose of 4500mg days 1-4 in combination with AZA[9]. Treatment related APR-246 side effects include nausea, vomiting, dizziness and transient neuropathy; the majority of which were G1/G2. The overall response rate was 71% with 44% achieving CR. Overall, 19/55 (35%) patients underwent allogeneic stem cell transplant, with a median overall survival of 14.7 months. Similar data have also recently been reported by the GFM showing comparable response rates and long-term data will be presented at the upcoming ASH 2021 meeting which highlight particularly improved outcomes in patients who achieve CR and/or TP53 VAF clearance and are bridged to transplant with a median OS that was not reached[10]. These data support the ongoing phase 3 study of APR-246 in combination with azacitidine versus azacitidine alone (NCT03745716). Unfortunately, the trial has failed to meet its primary endpoint of increase CR (33.3% in combination vs 22.4% in control arm; P=0.13) although survival follow-up is ongoing and these data are yet to be formally presented to help evaluate for differential responses from the earlier phase 2 studies. Additionally, novel triplet therapy with venetoclax as well as azacitidine in combination with APR-246 as post-transplant maintenance are being investigated and will be presented at the ASH 2021 annual meeting (NCT04214860/NCT03931291). Lastly, a 2nd generation oral agent (APR-548) has entered the clinic in late 2021. In addition, there are several additional azacitidine combinations which are ongoing in MDS and AML patients where molecular subset data are available. In *TP53* mutant AML, azacitidine in combination with venetoclax did achieve an increased CR/CRi rate of 47%, although these patients had a short median duration of CR/CRi of 5.6 months and a median OS of 7.2 months, similar to survival outcome data with single agent HMA, with recent data showing *TP53* as a major driver of resistance to venetoclax.[11,12] This combination is ongoing in MDS patients

(NCT02942290). More recently, azacitidine in combination with magrolimab, an inhibitor of the macrophage immune checkpoint CD47, was presented at the 2020 EHA congress showing very high response rates in MDS patients (91% ORR, 42%, CR), including high responses in *TP53* mutant MDS patients (NCT03248479). More recently, data was presented on the *TP53* mutant AML cohort at ASH of 2020. Importantly, in the *TP53* mutant AML cohort of evaluable patients (n=29), the CR/CRi rate was 59% with a median OS of 12.9 months to date although follow up was short (median follow up of 4.7 months). Patients have achieved high depth of response with a 44% complete cytogenetic response and 29% MRD negativity. These data support the ongoing phase 3 open-label study of azacitidine + magrolimab vs azacitidine + venetoclax in unfit AML patients and versus induction chemotherapy in fit patients with a primary endpoint of OS in the non-intensive group (NCT04778397). Importantly, additional novel triplet strategies are underway for both all-comer elderly AML patients and TP53 specific patient populations (e.g. azacitidine + magrolimab + venetoclax). *TP53* mutant MDS/AML patients represent a molecular cohort with very poor outcomes and lack of disease modifying therapy. The clonal burden of *TP53* is intimately associated with outcomes in this patient group and novel therapies targeting this mutation are urgently needed. The treatment landscape of these patients is encouraging as the combination of azacitidine with APR-246 or magrolimab have been well tolerated and produced significantly improved response rates. Ideally, future translational data will further elucidate the underpinnings driving the poor outcomes for this molecular subgroup to lead to additional novel therapeutic strategies.

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