# Impact of Hypomethylating Agents on hTERT Expression and Synergistic Effect in Combination With Imetelstat, a Telomerase Inhibitor, in Acute Myeloid Leukemia Cell Lines

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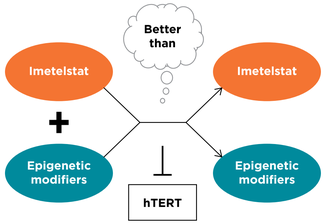
## BACKGROUND

- Acute myeloid leukemia (AML) cells express high levels of the catalytic unit of human telomerase reverse transcriptase (hTERT).
- Telomerase is not expressed in normal tissues, and is only transiently activated in hematopoietic progenitor cells.
- hTERT expression is highly regulated (eg, by epigenetic modification), and reports have suggested correlative links between overexpression and hypermethylation of the hTERT promoter[1]; conversely, normal tissues are largely hypomethylated in this region.[2]
- Imetelstat is a 13-mer oligonucleotide that specifically targets the RNA template of human telomerase and is a potent first-in-class competitive inhibitor of telomerase activity.
- Imetelstat is currently being investigated in clinical trials as a single agent, and recently reported clinical results show activity in patients with essential thrombocythemia or primary, post-essential thrombocythemia, and post-polycythemia vera myelofibrosis.[3,4]
- Imetelstat has limited single-agent activity in the AML cell lines tested up to 4 weeks on treatment (internal data not shown).
- Decitabine (DAC) and 5-azacitidine (AZA) are both DNA methyltransferase inhibitors (DNMTis) that are currently used for the treatment of AML.
- As it has been reported that hTERT expression is modulated by DAC,[5] the combination of imetelstat with DAC or AZA is hypothesized to improve treatment benefit in AML by modulating hTERT expression.

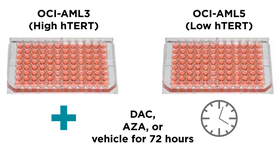
## EXPERIMENTAL OBJECTIVE

- **To determine whether the combination of a DNMTi and imetelstat enhances inhibition of cell viability *in vitro* compared with either agent alone.**

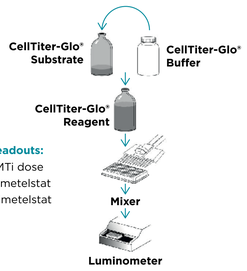



## MATERIALS AND METHODS

- Levels of hTERT RNA expression were investigated across a panel of AML cell lines using a reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) method (**Figure 1**) in order to identify those with high and low hTERT expression levels.
- AML cell lines expressing high hTERT (OCI-AML3) or low hTERT (OCI-AML5) were treated with a single dose of DAC or AZA or dimethylsulfoxide/phosphate-buffered saline (DMSO/PBS) vehicle for 72 hours and assessed for cell viability and hTERT expression (**Figure 2**).
- The effects of DAC or AZA treatment for 72 hours (daily dosing) followed by media alone (**Figure 3**) or imetelstat (**Figure 4**) for 2 or 4 weeks were then examined as follows:




- Cells were passaged weekly and dosed twice weekly with imetelstat (50 µM, 25 µM, 5 µM) or fresh media.
- Cells were monitored for viability with CellTiter-Glo® (Promega) assay immediately after DNMTi (DAC or AZA) dosing for 72 hours, and again after treatment with imetelstat for 2 weeks and 4 weeks.




**Viability readouts:**
- Post DNMTi dose
- 2 weeks imetelstat
- 4 weeks imetelstat

- Flow cytometry performed with BioLegend AnnexinV/ Propidium Iodide Apoptosis Kit.

## RESULTS

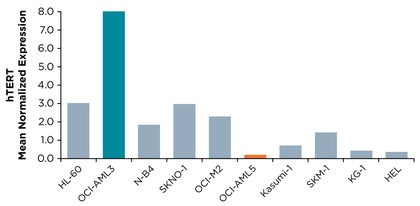



**Figure 1. Determination of hTERT RNA expression in AML cell lines.**
A panel of AML cell lines was measured for hTERT RNA expression using an RT-qPCR method. Levels of RNA expression varied across the lines investigated. Cell lines OCI-AML3 and OCI-AML5 were investigated in single-agent and combination experiments as they represented the bounds of the observed expression range.

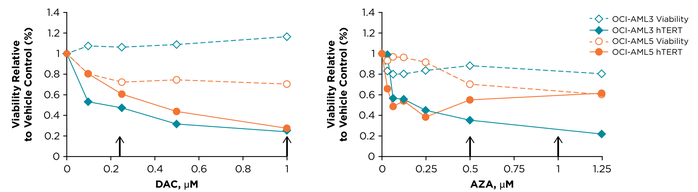



**Figure 2. Effect of single-agent DNMTi on viability and hTERT expression of AML cell lines *in vitro* 72 hours following a single dose.**
AML cell lines OCI-AML3 (high hTERT, teal) and OCI-AML5 (low hTERT, orange) were treated with a single dose of DAC or AZA for 72 hours with no washout period and assessed for cell viability (dashed) and hTERT expression (solid) compared with vehicle (DMSO in PBS). Both lines appear susceptible to AZA at concentrations > 1 µM; however, only OCI-AML5 appears to be sensitive to DAC. High doses of AZA seem to induce expression of hTERT in OCI-AML5. Black arrows highlight concentration points utilized in combinations for experiments demonstrated in Figures 3 and 4. However, it should be noted that daily treatment with DNMTi for 72 hours in experiments in Figures 3 and 4 differed from the single dose in this experiment.

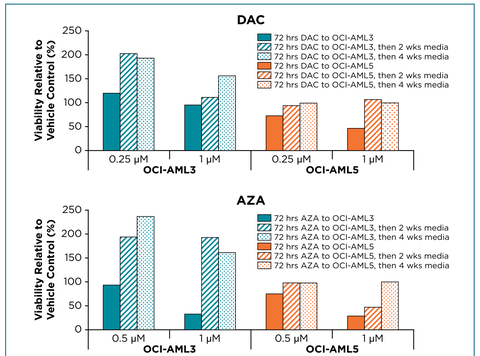



**Figure 3. Recovery time of AML cell lines following treatment with DNMTi.**
Cells were treated with DAC (top) or AZA (bottom) every 24 hours for 72 hours (solid bars), followed by removal of drug, or washout. Cells were then continually cultured in the absence of imetelstat for 2 weeks (diagonally striped bars) or 4 weeks (dotted bars) in parallel with the formal combinations with imetelstat detailed in Figure 4. Both cell lines had reduced viability at 72 hours post dose (solid bars) with the 1 µM concentrations. OCI-AML5 (orange) cells had greater viability reductions in response to either DAC or AZA, as expected based on results in Figure 2. Viability of cells under all conditions had completely recovered by 4 weeks, and as early as 2 weeks in OCI-AML3 (teal).

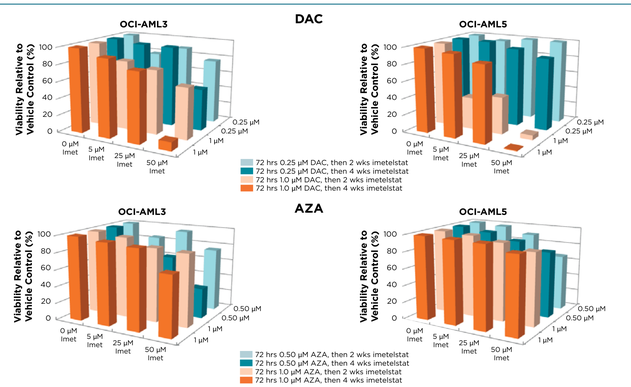



**Figure 4. Viability of AML cell lines after 72 hours treatment with DNMTi (DAC or AZA) followed by long-term treatment with imetelstat for 2 or 4 weeks.**
Cells were pretreated once every 24 hours for 72 hours with 1 of 2 doses of DAC (top) or AZA (bottom). Following washout removal of DNMTi at 72 hours, the cells were then cultured in the presence of biweekly imetelstat treatment as a single agent for up to 4 weeks. Cells generally recovered by 2 weeks, though the highest doses of imetelstat (50 µM) suppressed recovery for both lines in conjunction with DAC. With AZA in combination with imetelstat, reduced viability was noted in OCI-AML3, particularly with the lower (0.5 µM) dose of AZA, but not in OCI-AML5.

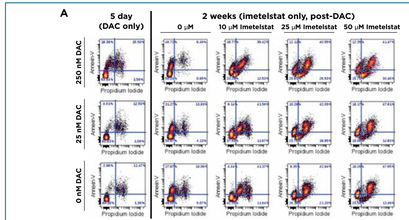



**Figure 5. Flow cytometric analysis of apoptosis in OCI-AML3 *in vitro* post DAC treatment (5 days) followed by imetelstat (in the absence of DAC) for 2 weeks.**
OCI-AML3 cells were pretreated once every 24 hours for 5 days (Panel A) with either dose of DAC or DMSO/PBS (0 nM DAC). Following washout after the fifth day of exposure, cells were cultured in the presence of biweekly imetelstat treatment as a single agent for 2 weeks (Panel B). Populations of dying and dead cells increased in a dose-dependent manner for both DAC and imetelstat, with greatest effect observed at the combination of highest DAC with highest imetelstat doses. Similar experiments were performed with AZA as well as in OCI-AML5 (not shown).

## CONCLUSIONS

- Both DAC and AZA caused dose-dependent decreases in hTERT expression and differential growth inhibition on OCI-AML3 and OCI-AML5 cell lines.
- Upon removal of drugs, growth inhibition by both DAC and AZA was not sustained, and cell proliferation had recovered by 2 weeks in cells with high hTERT expression (OCI-AML3) and by 4 weeks in cells with lower hTERT expression (OCI-AML5).
- Pretreatment with DAC (noting that DAC is more potent than AZA, and a lower initial concentration of DAC was used in all experiments) followed by imetelstat treatment reduced cell viability more than either agent administered alone, and administration of imetelstat after AZA pretreatment prevented or slowed recovery.
- Since treatment with AZA or DAC for 3 days in this study may not generate optimal hypomethylation, a follow-up study with an optimal dosing schedule was conducted.
  - Apoptosis increased in a dose-dependent manner with imetelstat treatment as well as DAC (**Figure 5**) and AZA (data not shown).

## REFERENCES

1. Sui X, et al. *Oncol Lett.* 2013;6:317-322.
2. Renaud S, et al. *Nucleic Acids Res.* 2007;35:1245-1256.
3. Baerlocher GM, et al. *N Engl J Med.* 2015;373:920-928.
4. Tefferi A, et al. *N Engl J Med.* 2015;373:908-919.
5. Zhang X, et al. *Oncotarget.* 2015;6:4888-4900.

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