Acute myeloid leukemia (AML) has limited treatment options apart from chemotherapy which ultimately provides only temporary reprieve from tumor burden. As such, improved therapies and novel mechanisms of targeting the disease are needed.

Hypothesis

Preclinical data suggest hTERT downregulation induces apoptosis via disruptive interactions between hTERT and BCL-2 in tumor cells.

Inhibition of telomerase and BCL-2 in conjunction will yield greater anti-tumor effect in AML as compared to either treatment alone.

Methodology

In vitro investigations in AML cell lines
- Five AML lines were treated with imetelstat and/or ABT-199 for 48 or 96 hours
- Cell viability was then assessed by cytometry using an Annexin V/Propidium iodide (PI) assay, with viable cells defined as Annexin V-PI
- Additional samples were collected for RNA and protein extracts to evaluate hTERT expression by RT-qPCR and telomerase activity by qPCR-TRAP

In vivo study in the disseminated MOLM-13 AML model
- Female SCID-beige mice at 6-8 weeks were used as approved by the Institutional Animal Care and Use Committee of Janssen R&D, Spring House, PA
- Mice were injected with 1 million MOLM-13 AML cells intravenously via lateral tail vein on Day 0 and randomly assigned to treatment groups of ten mice per group
- Starting on Day 1, imetelstat treatment was administered intraperitoneally every 3 to 4 days for 4 weeks
- Starting on Day 5, ABT-199 treatment was started and administered daily for 28 days
- Mice were monitored 2x weekly for body weight and adverse effects
- Median survival was determined and percent increased life span (±% KS) were calculated compared to vehicle controls

Ex vivo efficacy in purified PBMCs from AML patient blood
- PBMCs were FcγR-blocked from whole blood of four AML patients
- Cells were treated for 16 or 40 hours and assessed for viability by flow cytometry alone

Results

In vitro investigations in AML cell lines
- Imetelstat reduced viability of AML leukemic cells
- Imetelstat reduces the viability of AML leukemic cells in vitro
- Imetelstat showed both dose and time dependent activity in enhancing cell killing of AML leukemia cells

Ex vivo efficacy in mouse xenograft model
- Combining drugs yields enhanced survival in MOLM-13 mouse model of AML
- Imetelstat plus ABT-199 was well tolerated by SCID-beige mice – morbidity was observed for single agent and controls only due to tumor growth

Survival curve and increased life span analysis show that the combination yields the greatest outcome, with 4 mice remaining alive 80 days after drug treatment ended. NM = mismatch oligonucleotide control

Conclusions

- Three of five investigated AML lines exhibited both dose-response to ABT-199 alone, and enhanced blast death was observed when combining with imetelstat. Mean ± standard deviation of four patient samples.
- Imetelstat enhanced apoptosis induced by ABT-199 in AML cell lines in vitro and AML patient samples ex vivo; with greater synergistic activity observed at longer treatment times
- Both hTERT expression and telomerase activity were greatly decreased by imetelstat treatment and further reduced by combination with ABT-199
- Prolonged survival was achieved in vivo when combining imetelstat with ABT-199; four mice remained alive ~80 days post end of treatment suggesting a potential cure in these animals

References

- Tefferi A et al. NEJM 2015; 373: 908
- Neutheicher G et al. NEJM 2015; 373: 933
- Tefferi A et al. Blood; 6: 4405
- Tefferi A et al. NEJM 2015; 373: 906
- Beilhack G and Wang X. “Apoptosis,” 2013
- Tan and Teragaki Y. Canc Res 2014; 74: 1639
- http://cancersoncology.com/analysis.jsp

Figure 1. Single-agent response of AML cell lines in vitro
- Three of five lines are sensitive to ABT-199 at 48 hour exposure, consistent with the literature. ABT-199 insensitive lines show moderate sensitivity to imetelstat (right).

Figure 2. In vitro combination synergy assessment
- In vitro combination synergy assessment
- In vitro investigations in AML cell lines
- Five AML lines were treated with imetelstat and/or ABT-199 for 48 or 96 hours
- Cell viability was then assessed by cytometry using an Annexin V/Propidium iodide (PI) assay, with viable cells defined as Annexin V-PI
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Figure 3. Synergistic induction of apoptosis by combining ABT-199 with imetelstat
- Imetelstat pushes early apoptotic cells (Annexin V+/PI-) into late apoptosis and cell death (Annexin V+/PI+). Dot plots show differences in cells treated with Smrt-199 in combination with 10 μM and 25 μM imetelstat versus untreated controls.

Figure 4. hTERT expression and telomerase activity are modulated by the combination
- In vitro investigations in AML cell lines
- Five AML lines were treated with imetelstat and/or ABT-199 for 48 or 96 hours
- Cell viability was then assessed by cytometry using an Annexin V/Propidium iodide (PI) assay, with viable cells defined as Annexin V-PI
- Additional samples were collected for RNA and protein extracts to evaluate hTERT expression by RT-qPCR and telomerase activity by qPCR-TRAP

Figure 5. Combining drugs yields enhanced survival in MOLM-13 mouse model of AML
- Imetelstat plus ABT-199 was well tolerated by SCID-beige mice – morbidity was observed for single agent and controls only due to tumor growth

Survival curve and increased life span analysis show that the combination yields the greatest outcome, with 4 mice remaining alive 80 days after drug treatment ended. NM = mismatch oligonucleotide control

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Ex vivo proof of concept

Figure 6. Imetelstat in combination with ABT-199 reduces viability of AML leukemic cells in vivo
- Leukemic blasts from AML patients’ PBMCs showed dose response to ABT-199 alone, and enhanced blast death was observed when combined with imetelstat. Mean ± standard deviation of four patient samples.