# Synergistic Enhancement of Anti-Leukemic Activity by Venetoclax & Radioimmunotherapy in Acute Myeloid Leukemia

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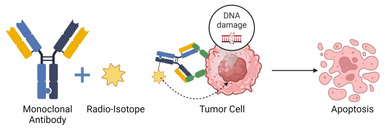
## Background

**Acute Myeloid Leukemia (AML):**

- One of the most common & aggressive blood cancers in adults.
- Intensive chemotherapy can be the best chance for a cure, but is insufficient to eradicate AML and is associated with increased toxicity rates.

**Radioimmunotherapy:**

- Therapy that links radioactive substances to antibodies to deliver radiation to the target and destroy cancer cells.
- Increased selectivity of radiation delivery and decreased off-target toxicity.

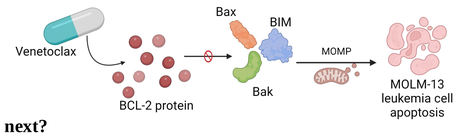



**Astatine-211 ($^{211}$At):**

- α emitter, with high linear energy transfer & around 1 to 2 cells range.
- Causes an overwhelming and irreparable DNA damage that triggers tumor cell death via apoptosis along the BCL-2 pathway.

**Venetoclax:**

- Inhibits BCL-2 anti-apoptotic proteins, and facilitates apoptosis in tumor




**What next?**

- To overcome the inability in eradicating AML, novel therapies are needed such as radioimmunotherapy, and targeted therapy to enhance anti-leukemic effectiveness, and reduce off-target toxicity.

## Hypothesis & Objectives

We hypothesize that the combination of venetoclax and radioimmunotherapy with $^{211}$At will exhibit synergistic effects, leading to an improved survival in disseminated AML mouse models compared to single agents only.

This project aims to assess the synergy between venetoclax and radioimmunotherapy with $^{211}$At in treating AML using the MOLM-13 cell line model.

- ***In vitro* studies:**

**Single Agent Plates:** Asses the effects of venetoclax and $^{137}$Cs irradiation on MOLM-13 cells to identify dosages yielding ~ 50-80% viability to move forward in combination plates.

**Combination Plates:** Asses for a potential synergy between venetoclax and $^{137}$Cs irradiation.

- ***In vivo* studies:**

To observe if venetoclax & $^{211}$At combine synergistically in disseminated AML mouse models.

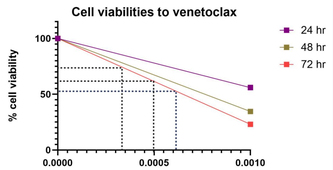
## Methods & Results

### In-Vitro

**Figure 1: Single Agent Plates** – Leukemia cells (MOLM-13) are sensitive to either Venetoclax or $^{137}$Cs irradiation

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | media | media | media | media | media | media | media | media | media | media | media | media |
| B | media | cells | 2 | 1 | 0.55 | 0.5 | 0.1 | 0.075 | 0.058 | 0.041 | cells | media |
| C | media | cells | 2 | 1 | 0.55 | 0.5 | 0.1 | 0.075 | 0.058 | 0.041 | cells | media |
| D | media | cells | 2 | 1 | 0.55 | 0.5 | 0.1 | 0.075 | 0.058 | 0.041 | cells | media |
| E | media | cells | 0.024 | 0.019 | 0.013 | 0.008 | 0.006 | 0.004 | 0.003 | 0.001 | cells | media |
| F | media | cells | 0.024 | 0.019 | 0.013 | 0.008 | 0.006 | 0.004 | 0.003 | 0.001 | cells | media |
| G | media | cells | 0.024 | 0.019 | 0.013 | 0.008 | 0.006 | 0.004 | 0.003 | 0.001 | cells | media |
| H | media | media | media | media | media | media | media | media | media | media | media | media |

Venetoclax (μM)

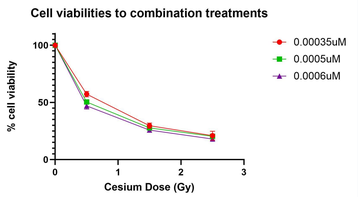



Method: 10,000 MOLM-13 cells/well were treated with venetoclax (2 μM to 0.001 μM) and $^{137}$Cs irradiation (0.5Gy to 15Gy). Cell viability with CellTiter-Glo was assessed after 24, 48, and 72 hours of incubation. Dosages resulting in 50-80% viability were selected for subsequent combination experiments.

**Figure 2: Combination Plates** – Venetoclax at lower doses shows potential synergy with $^{137}$Cs irradiation

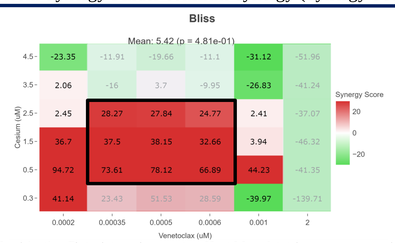
| | Cesium dose (Gy) | Cesium 1x Rep 1 | Cesium 1x Rep 2 | Cesium 1x Rep 3 | 0.50 | 0.50 | 1.5 | 1.5 | 2.5 | 2.5 | Venetoclax 1x Rep 1 | Venetoclax 1x Rep 2 | Venetoclax 1x Rep 3 | Venetoclax dose (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | | | | | | | | | | | | | 0.0 |
| B | 0 | | | | 0.00035 | 0.00035 | 0.00035 | 0.00035 | 0.00035 | 0.00035 | | | | 0.0 |
| C | 0.30 | | | | 0.00035 | 0.00035 | 0.00035 | 0.00035 | 0.00035 | 0.00035 | | | | 0.00020 |
| D | 0.50 | | | | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | | | | 0.00035 |
| E | 1.5 | | | | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | 0.0005 | | | | 0.00050 |
| F | 2.5 | | | | 0.0006 | 0.0006 | 0.0006 | 0.0006 | 0.0006 | 0.0006 | | | | 0.00060 |
| G | 3.5 | | | | 0.0006 | 0.0006 | 0.0006 | 0.0006 | 0.0006 | 0.0006 | | | | 0.001 |
| H | 4.5 | | | | | | | | | | | | | 2.00 |

Venetoclax Combo Doses | Cesium Combo Doses | Media Only | Venetoclax Only | Cesium Only | Control Cells




Methods: 10,000 MOLM-13 cells/well were treated with venetoclax (0.0006 μM to 0.00035 μM), $^{137}$Cs irradiation (0.5Gy to 2.5Gy) or both. Cell viability with CellTiter-Glo was assessed after 48, and 72 hours of incubation after 48 and 72 hours.

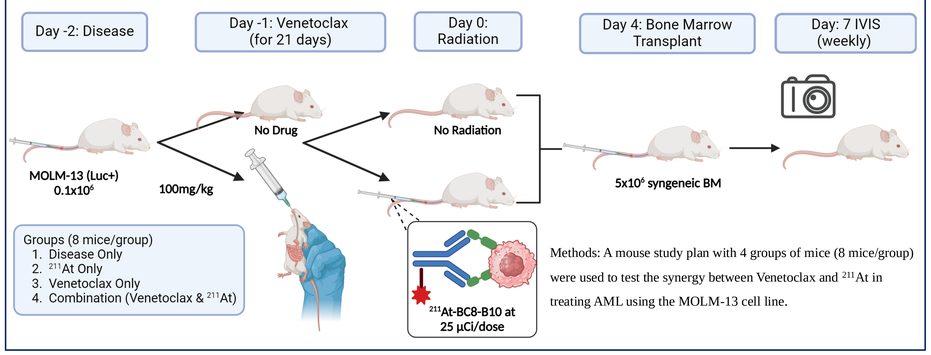
**Figure 3: Heat Map** – Heat Maps from Synergy Finder confirms synergy (Synergy Score: 45.31)




Methods: Synergy Finder was used to analyze Combination Plate data and generate a Heat Map. A total average scores above 10 suggest potential synergy, scores between 10 and -10 indicate an additive effect, and scores below -10 suggest antagonistic effects.

### In-Vivo

**Figure 4A : Mouse Study Schema**

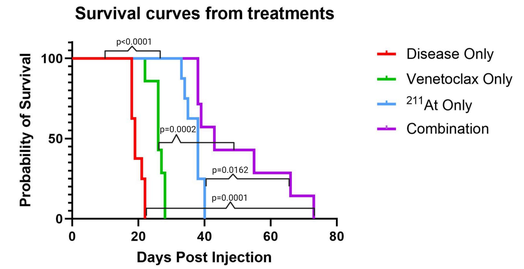



Methods: A mouse study plan with 4 groups of mice (8 mice/group) were used to test the synergy between Venetoclax and $^{211}$At in treating AML using the MOLM-13 cell line.

## Methods & Results

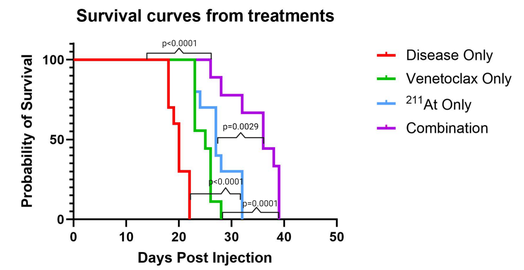
### In-Vivo

**Figure 4B: Survival Curves**– Mice treated with combinations, show improved survival but minimal synergy




Method: NRG mice (n=8/group) were given 100,000 MOLM-13 cells IV (tail vein injections), 1 day later dosed with venetoclax (100 mg/kg), and 2 days later with $^{211}$At radioimmunotherapy (25 μCi), or combination. Mice were followed for survival, survival were calculated by Log-rank (Mantel-Cox) test and plotted on Kaplan-Meier curves.

**Figure 4C: Survival Curves-** Analysis at lower doses of $^{211}$At and venetoclax hints at synergy




Method: NRG mice (n=8/group) were given 100,000 MOLM-13 cells IV (tail vein injections), 1 day later dosed with venetoclax (100 mg/kg), and 2 days later with $^{211}$At radioimmunotherapy (15 μCi), or combination. Mice were followed for survival, survival were calculated by Log-rank (Mantel-Cox) test and plotted on Kaplan-Meier curves.

## Conclusions

- In vitro studies demonstrated a synergistic effect between venetoclax and $^{137}$Cs irradiation.
- Initial in vivo experiments with venetoclax and higher doses of $^{211}$At showed a slightly improved survival. However, subsequent experiments using lower doses of $^{211}$At hinted at synergy when venetoclax was added.

## Future Directions

- Investigate combinations of additional targeted drugs with radiation to improve treatment efficacy.
- Develop an optimal combination based on synergy studies for translation into clinical trials to assess efficacy in humans.
- Confirm the mechanism of synergy between venetoclax and $^{211}$At is through the destabilization of the mitochondrial outer membrane permeabilization with apoptosis.
- Develop a humanized antibody for repeated infusions in the clinic to overcome immune response limitations.

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