2017-11-30

Synergy of anti-leukemic effects between anti-thymocyte globulin, busulfan, and fludarabine

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Determining synergy of anti-leukemic effects between ATG with Busulfan and Fludarabine

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BACKGROUND

- Acute myeloid leukemia (AML): immature myeloblasts proliferate and build up in the bone marrow and overflow into the bloodstream
- Hematopoietic stem cell transplantation (HCT) as treatment for AML
  - Healthy HSCs and other immune cells are infused into recipient
  - Risk: graft versus host disease (GvHD)
  - Anti-GvHD drug: anti-thymocyte globulin (ATG)
    - Unlike other drugs, ATG does not increase chances for relapse
- ATG: polyclonal antibody consisting of a collection of immunoglobulin molecules each identifying a different epitope
  - Used for immunosuppression of patient's cells
  - Targets multiple antigens expressed on surface of various immune cells
- Busulfan: one of the drugs used in killing leukemia cells
  - Alkylating agent that inhibits the transcription of DNA into mRNA, preventing replication
  - Proposed that it adds alkyl groups to DNA and links guanine with adenine to prevent helicase from separating DNA for replication
- Fludarabine: another drug used for its anti-leukemic properties
  - F-ara-ATP (active metabolite) competes as alternative substrate with DNA to directly inhibit DNA polymerases, primases, and DNA ligases
- **This study determines whether synergy exists when ATG is combined with either busulfan or fludarabine**
  - **Synergy may lead to higher treatment efficacy**
  - **Individual drug dosages can be decreased so that the desired effect could remain with less risk for toxicity and side effects**

HYPOTHESIS

ATG synergizes with Busulfan and Fludarabine to present heightened anti-leukemic effects.

METHODS

- Different concentrations of ATG were combined with different concentrations of Busulfan and Fludarabine separately
- Pre-conditioned leukemic samples of acute myeloid leukemia patients were used for testing
- Cell death was quantified using flow cytometry after staining with 7AAD
- Wilcoxon Matched Pairs Test to test whether the difference in cell death between combined drugs and separate drugs is significant

RESULTS

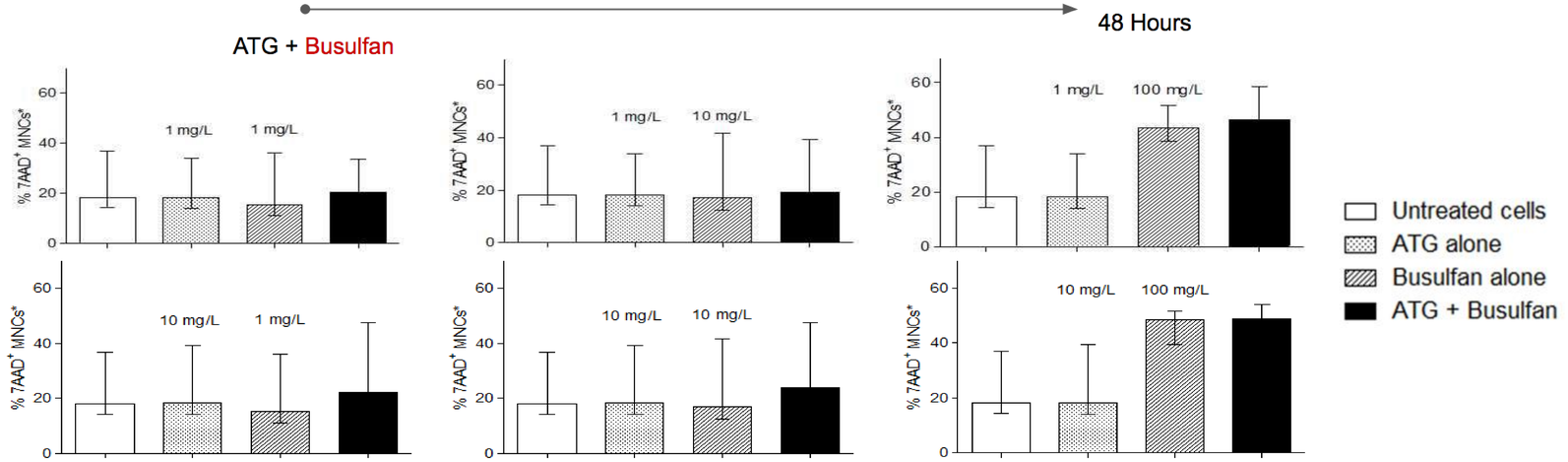

Figure 1. Cell death quantified by %7AAD+ from an average of 4 different patients when incubated with ATG and Busulfan separately and together in different concentrations for 48 hours. Increased cell death in some trials when ATG is combined with busulfan. Wilcoxon Matched Pairs Test deemed the difference not significant.

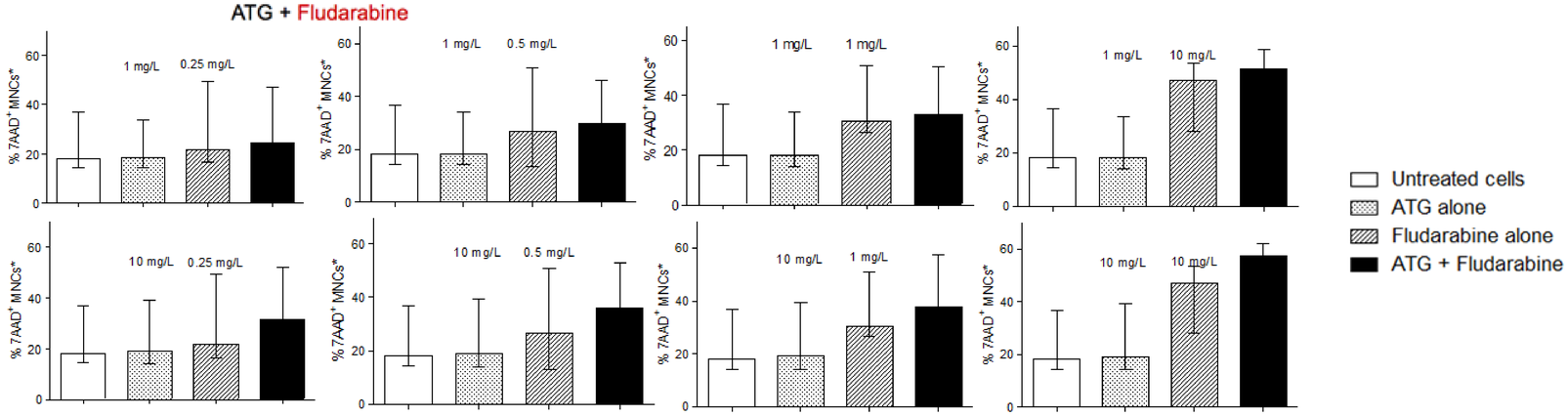

Figure 2. Cell death quantified by %7AAD+ from an average of 4 different patients when incubated with ATG and Fludarabine separately and together in different concentrations for 48 hours. Increased cell death in most trials when ATG is combined with fludarabine. Wilcoxon Matched Pairs Test deemed the difference not significant.

CONCLUSION

Although there were no significant differences, it could be due to the fact that only the cells from 4 different replicates were used to calculate an averaged result. Furthermore, other concentrations not tested in this experiment could yield better results. If synergy exists between ATG and Busulfan or Fludarabine, Busulfan or Fludarabine should be given to patients together with ATG in the conditioning schedule to allow synergism to occur, leading to more cancer cell death with less risk for side effects.

ACKNOWLEDGEMENTS

We thank the AML patients who donated blood for the research that will help future AML patients in their treatment. I would also like to thank Poonam Dharmani-Khan and Cindy Miskolczi-McCallum for teaching me how to use the flow cytometer and other equipment in the lab for my project. Lastly, I would like to thank the O'Brien Institute for Public Health for the funding that made this project possible.

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