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Guadecitabine, in combination with Cyclophosphamide, promotes anti-cancer immunity in BALB/c mice bearing 4T1 mouse mammary carcinoma

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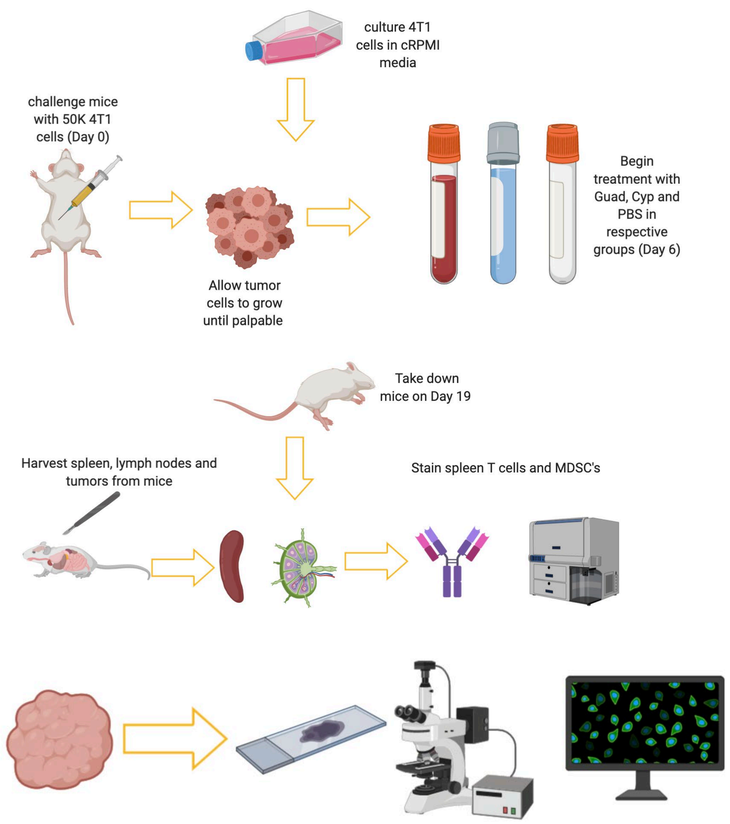
Abstract

Since 1990, death rates in females from breast cancer have been in decline (from ~33 deaths per 100,000 population in 1990 to ~20 deaths per 100,000 population in 2016). Many breast cancers are treated with therapies that target herceptin, estrogen, or progesterone receptors; however, for triple-negative breast cancers (TNBC) that lack these receptors, treatment options are limited and prognosis is often unfavorable. The goal of this study is to design a therapeutic intervention that is able to elicit an effective immune response against the tumor and instill immunological memory to eradicate primary and metastatic lesions. Guadecitabine (Guad) is a second-generation DNA methyltransferase inhibitor (DMNTi) that has been reported to have several antitumor properties such as increased antigenicity and depletion of myeloid-derived suppressor cells (MDSC's). Cyclophosphamide is a FDA approved chemotherapy that has been shown, when given as a low-dose treatment, to selectively deplete regulatory T-cells (T-regs). Both MDSCs and T-regs suppress antitumor immunity. **We hypothesize that the combination of Guad and Cyp will synergize and promote anticancer immunity through increased expression of de novo tumor antigens and depletion of MDSCs and T-regs in a low-dose setting.** To test this hypothesis, BALB/c mice were challenged with murine TNBC 4T1 tumor cells and the 4T1-bearing mice were administered low-dose Guad and Cyp daily for ten consecutive days. This experiment showed a degree of synergy between Guad and Cyp with the dual therapy reducing tumor burden to a greater extent than either monotherapy.

Methods



Flow Panel for **T-cells**:

Zombie Aqua
FoxP3-GFP
TCRbeta-PE
CD4-APC
CD8-Pe/Dazzle
CD44-BV711
CD62L-PeCy7

Flow Panel for **MDSCs**:

Zombie Aqua
FoxP3-GFP
CD11b-BV711
CD11c-BUV395
Ly6C-APC
Ly6G-PE
CD86-PeCy7

Results

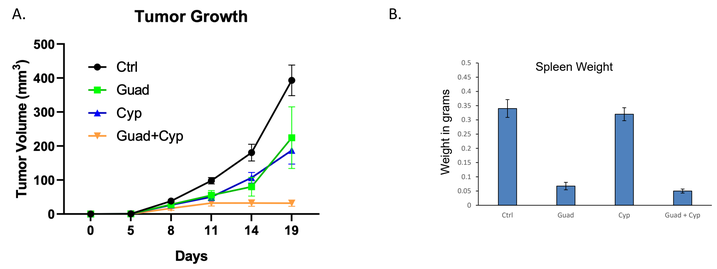


Figure 1. Dual treatment of low-dose guadecitabine with low-dose cyclophosphamide results in decreased tumor burden. Mice were challenged with 4T1 cells and received either vehicle control, low-dose guadecitabine, low-dose cyclophosphamide, or a combination of both therapies. A) Tumor volumes were measured using digital calipers every 3-4 days following tumor challenge. B) On D19, mice were euthanized and spleen weight was taken. Error bars depict SEM.

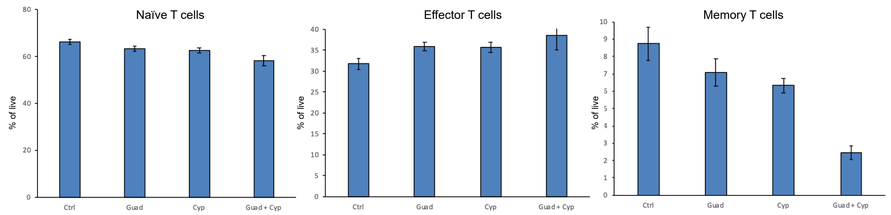


Figure 2. Reduction in the percent of splenic T-regulatory cells in mice receiving both low-dose guadecitabine and cyclophosphamide. Flow cytometry was performed on splenocytes from mice harvested in Fig. 1b to analyze the T-cell compartment. T-cells were defined as live cells that were also TCRbeta+. CD4, CD8, and T-regulatory cells were defined as T cells that expressed CD4, CD8, or FoxP3 respectively. The graphs represent the percent of each type of T-cell of total live T-cells. Error bars depict SEM.

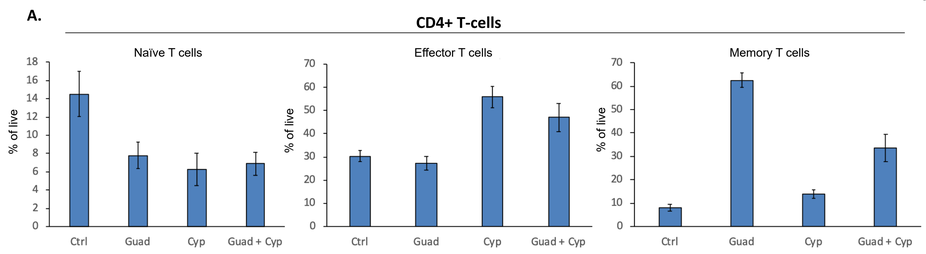
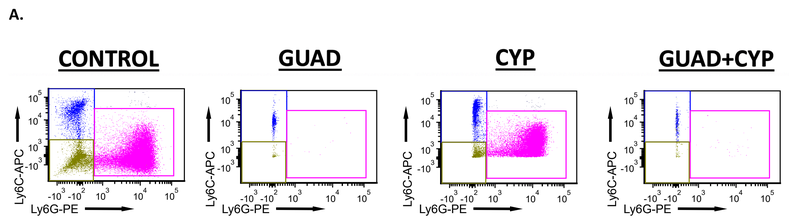
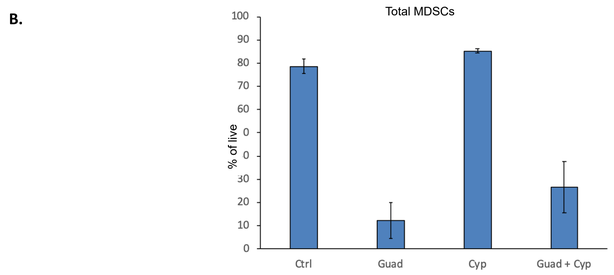


Figure 3. Treatment with low-dose guadecitabine and cyclophosphamide skews splenic T-cells towards an effector/effector-memory phenotype. Flow cytometry was performed on splenocytes from mice harvested in Fig. 1b to analyze the T-cell compartment. T-cells were defined as live cells that were also TCRbeta+. Naïve cells were CD44-, Effector cells were CD44+, and memory cells were CD44+CD62L+. A) Graphs show the naïve, effector, or memory CD4+ T cell population as a percent of total live CD4+ cells. B) Graphs show the naïve, effector, or memory CD8+ T cell population as a percent of total live CD8+ cells. Error bars depict SEM.





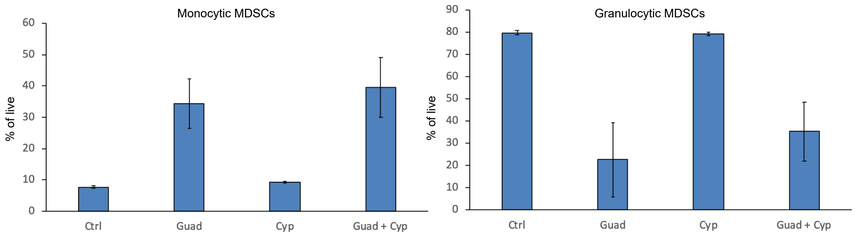


Figure 4. Low-dose guadecitabine depletes splenic MDSCs. Flow cytometry was performed on splenocytes from mice harvested in Fig. 1b to analyze the myeloid compartment. MDSCs were defined as live cells that were also CD11b+ and either Ly6G+ or Ly6C+. A) MDSCs were defined as live cells that were also CD11b+ and either Ly6G+ or Ly6C+. Representative dot plots gated on live CD11b+ splenocytes. B) The graph is representative of the percent of total MDSCs out of live splenocytes. C) Monocytic MDSCs were defined as live CD11b+ Ly6C+ and D) granulocytic MDSCs were defined as live CD11b+Ly6G+. Error bars depict SEM.

Conclusions/Future Direction

Our findings support our hypothesis that combinational therapy with both Guadecitabine and Cyclophosphamide induce an antitumor environment and reduce tumor burden more effectively than either treatment alone. To further solidify our hypothesis, other experimental designs may be beneficial to execute including but not limited to:

- Extending the experiment to produce a survival analysis
- Including counting beads to have cell counts and not only percent
- Conduct the same experiment using C57Bl/6 mice model *in vivo*
- Design and execute an *in vitro* study using same experimental parameters

Acknowledgements

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