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Determining Effective Treatment Regimens for Breast Cancer Using Combined Immunotherapy and Chemotherapy in Vivo

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Abstract

Breast cancer has the highest incidence rate of all cancers globally in women, and those of African descent, especially West African females, face higher rates of triple-negative breast cancer (TNBC), a more aggressive form of breast cancer. Immunotherapy for breast cancer is a relatively new treatment option, and research is ongoing to identify the best combination of treatments for increasing survival of those diagnosed with TNBC. **Eganelisib (IPI-549: a PI3K-gamma inhibitor) shifts immunosuppressive M2 macrophages to stimulatory M1 type and has been shown to augment T cell function; combined with other treatments, it has shown promising results in reducing tumor growth and increasing survival in tumor-bearing mice.** We have been conducting experiments to determine the most effective treatment regimen that will reduce growth of 4T1 mammary cancers, a murine TNBC model in syngeneic Balb/C female mice. Combinations of eganelisib, cyclophosphamide, and anti-PD-1 have been tested to determine how immunotherapy and chemotherapy can induce a strong immune response, resulting in better responses to the treatment. **Our current data indicates that a treatment regimen combining eganelisib, cyclophosphamide, and anti-PD-1 was most effective at suppressing tumor growth, compared to other regimens that only included one or two of these treatments.** Mean tumor sizes of mice treated with a combination of eganelisib and other treatments were 187±70 mm^3, 232±71 mm^3, and 227±59 mm^3 at the end of the experiments, compared to untreated control tumors of 576±137 mm^3, 414±31 mm^3, and 591±200 mm^3, respectively. These results could lead to further research on effective immunotherapy treatment combinations for TNBC.

Introduction

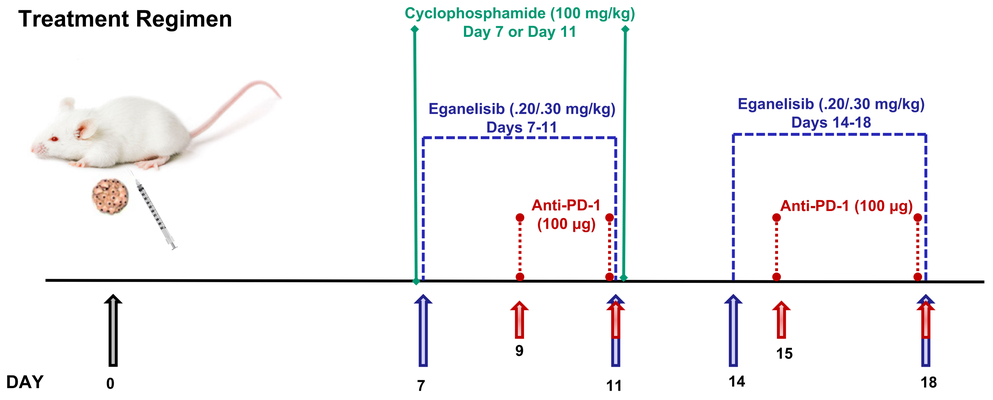
Immune checkpoint inhibitors (ICI), such as anti-PD-1 or anti-PD-L1, have been in the process of development as possible treatment options for TNBC and HER2+ breast cancers. Some have suggested that tumors with high levels of tumor-infiltrating lymphocytes may be most likely to benefit from ICI treatment. PI3Kγ inhibitors, such as eganelisib (IPI-549), are also used in our studies to test its efficacy for reducing tumor growth when given in conjunction with chemotherapy and ICI. In studies with myeloid cells, a correlation between immune checkpoint blockade resistance and increase of immune-suppressive myeloid cells/macrophages warranted a need for an agent that would enhance the tumor immune microenvironment and overcome resistance to immune checkpoint blockade. As PI3Kγ signaling plays a role in suppressing immune response and promoting tumor growth, a PI3Kγ inhibitor, such as eganelisib, could improve immune responses by decreasing M2 macrophages and increasing the activity of $CD8^+$ T cells and increasing cytotoxicity in the immune microenvironment.

Method

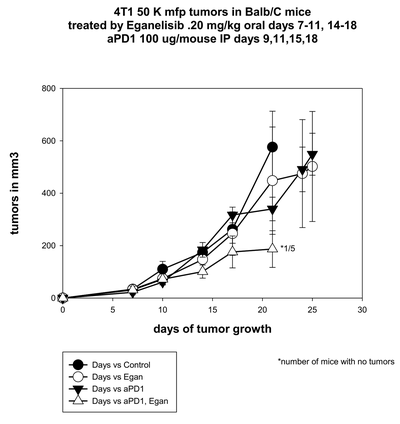
Aim: To determine the sequence and dosages of a treatment regimen with both immunotherapy and chemotherapy to inhibit tumor growth of mice with 4T1 breast cancers

Syngeneic Balb/C female mice are used for the studies, and on day 0, 50,000 cells from the 4T1 breast cancer cell line are injected into the mammary fat pads of each mouse. The current treatments being tested include eganelisib, cyclophosphamide, and anti-PD-1. Eganelisib is administered through oral gavage at a dosage of 0.2 mg/kg or 0.3 mg/kg on days 7-11 and days 14-18 of the experiment. Cyclophosphamide and anti-PD-1 are administered through intraperitoneal injections in the quadrant opposite that of the one where the cancer cells were injected. Cyclophosphamide was given at 100 mg/kg, and different timings of cyclophosphamide administration were tested (either day 7 or day 11) to determine the most effective sequence. Anti-PD-1 was administered to the mice at 100 µg/mouse every three to four days until the end of the treatment regimen.

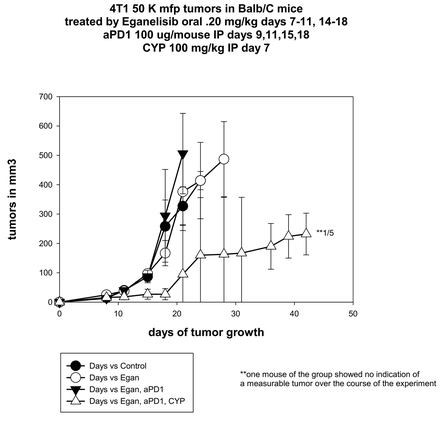
Results



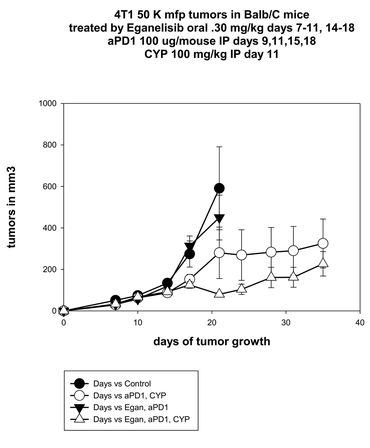
50,000 cells from the 4T1 breast cancer cell line are injected into female Balb/C mice at the mammary fat pads.

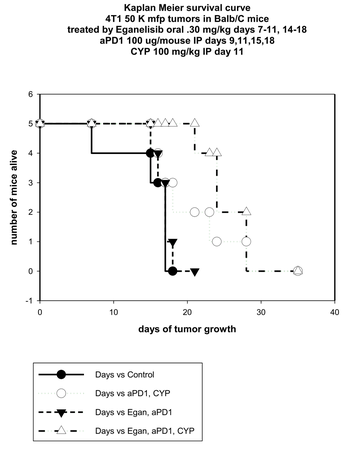


*number of mice with no tumors



**one mouse of the group showed no indication of a measurable tumor over the course of the experiment





All the experiments conducted showed that a combined approach of eganelisib with anti-PD-1 and/or cyclophosphamide was effective in decreasing tumor growth. When eganelisib was given at a dose of 0.2 mg/kg, along with anti-PD-1, the final average tumor volume was 187±70 mm^3, compared to 576±137 mm^3, the final average tumor volume of the control group. Similarly, when the same dosage of eganelisib was combined with anti-PD-1 and cyclophosphamide in a different experiment, the final average tumor volume of the experimental group that received all three treatments was 232±71 mm^3, compared to the control group's tumor volume of 414±31 mm^3. Increasing the dosage of eganelisib from 0.2 mg/kg to 0.3 mg/kg and administering cyclophosphamide at day 11 instead of day 7 seemed to have an effect on tumor growth - the tumor volume of the experimental group that received this increased dosage of eganelisib and different treatment sequencing of cyclophosphamide with the addition of anti-PD-1 had an average tumor volume of 227±59 mm^3, while the untreated control group had a mean tumor volume of 591±200 mm^3. The survival in the experimental group showed more promise compared to the control group mice.

These results show that the sequence of the treatment regimen may play a role on the impact on tumor volume, as well as overall survival. Studying when cyclophosphamide is administered can improve results for decreasing tumor growth by strengthening the immune microenvironment of the tumor. It may be that increasing the immune response prior to chemotherapy may increase the efficacy of the latter. Alternatively, cyclophosphamide, which has been found by some to selectively deplete immunosuppressive Treg cells may increase the efficacy of the immune therapies. Future experiments with different sequences of the same treatments can further reveal the impact of timing for establishing effective treatment regimens that will decrease or even stop tumor growth and increase survival of mice with breast cancer.

References

1. De Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH, Budhu S, Ghosh A, Pink M, Tchaicha J, Douglas M, Tibbitts T, Sharma S, Proctor J, Kosmider N, White K, Stern H, Soglia J, Adams J, Palombella VJ, McGovern K, Kutok JL, Wolchok JD, Merghoub T. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. Nature. 2016 Nov 17;539(7629):443-447. doi: 10.1038/nature20554. Epub 2016 Nov 9. PMID: 27828943; PMCID: PMC5634331.

2. Falvo P, Orecchioni S, Hillje R, Raveane A, Mancuso P, Camisaschi C, Luzi L, Pelicci P, Bertolini F. Cyclophosphamide and Vinorelbine Activate Stem-Like $CD8^+$ T Cells and Improve Anti-PD-1 Efficacy in Triple-Negative Breast Cancer. Cancer Res. 2021 Feb 1;81(3):685-697. doi: 10.1158/0008-5472.CAN-20-1818. Epub 2020 Dec 2. Erratum in: Cancer Res. 2021 Jun 15;81(12):3426. PMID: 33268528.

3. Gonda TA, Fang J, Salas M, Do C, Hsu E, Zhukovskaya A, Siegel A, Takahashi R, Lopez-Bujanda ZA, Drake CG, Manji GA, Wang TC, Olive KP, Tycko B. A DNA Hypomethylating Drug Alters the Tumor Microenvironment and Improves the Effectiveness of Immune Checkpoint Inhibitors in a Mouse Model of Pancreatic Cancer. Cancer Res. 2020 Nov 1;80(21):4754-4767. doi: 10.1158/0008-5472.CAN-20-0285. Epub 2020 Aug 14. PMID: 32816859.

4. Kaneda, M., Messer, K., Ralainirina, N. *et al.* PI3Kγ is a molecular switch that controls immune suppression. *Nature* **539,** 437–442 (2016). https://doi.org/10.1038/nature19834

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