PS3-12-08

Rac/Cdc42 inhibitor MBQ-167 modulates the breast tumor immune microenvironment.

Anamaris Torres-Sánchez, Ph.D.[1], Ailed M. Cruz-Collazo, Ph.D[1], Nilmary Grafals, Ph.D.[1], Stephanie Dorta-Estremera Ph.D.[2-3], Suranganie Dharmawardhane Ph.D[2,4]

[1]Department of Biochemistry, [2]Department of Microbiology, University of Puerto Rico (UPR) Medical Sciences Campus, San Juan, PR, [3]Division of Clinical and Translational Cancer Research, UPR Comprehensive Cancer Center; [4]MBQ Pharma, Inc., San Juan, PR.

Abstract

A uniform therapeutic approach for metastatic breast cancer has been challenging, especially for triple negative breast cancer (TNBC), due to the inherent heterogeneity and lack of targetable receptors. Therefore, we developed MBQ-167, as a specific inhibitor of the homologous Rho GTPases Rac/Cdc42 and demonstrated its efficacy in pre-clinical mouse models of HER2 (3+) and TNBC. MBQ-167 is currently in Phase 1 clinical trials in advanced BC patients, and so far, have not shown any adverse events, and is bioavailable at acceptable levels. Emerging data show the relevance of the tumor microenvironment (TME) to malignant cancer progression, where immunosuppressive immune cells such as macrophages, neutrophils, and Myeloid-Derived Suppressor Cells (MDSCs) are recruited to the TME to promote tumor cell invasion. Moreover, in the TME, cancer cells may hijack the infiltrating cytotoxic T cells by expressing programmed death ligand (PD-L1), which binds the PD-1 receptor on T cells to induce T cell death. The crosstalk signaling between the immune cells in the TME to instigate metastatic cancer cell migration into the circulatory system is regulated by Rac and Cdc42. Therefore, we investigated the effect of MBQ-167 on the TME and inflammatory responses to further demonstrate its potential as a TNBC therapeutic. By immunophenotyping cells from spleens and tumors following administration of vehicle or MBQ-167 to Balb/C mice bearing 4T-1 TNBC tumors, we found that MBQ-167 treatment significantly reduced macrophages (particularly M2 macrophages), MDSCs, and increased CD8+ T cells. Parallel to a decrease in lung metastases, MBQ-167 increased the CD8+ Granzyme B+ regulatory T cell ratio. Of the cytokines released into the TME to activate inflammatory and immunosuppressive immune cells, we found Interleukin-6 (IL-6) to be significantly decreased in mouse plasma following MBQ-167 treatment. As published by us, gene set enrichment analysis of the transcriptomic data from 4T-1 tumors in Balb/c mice treated with vehicle or MBQ167 demonstrated a statistically significant ~2-fold downregulation in the inflammatory mediators chitinase-3-like protein 1 (CHI3L1) and S100A9 that have also been associated with breast cancer metastasis. Therefore, ELISAs were performed following vehicle, or MBQ-167 using conditioned media from TNBC cells and macrophages in tissue culture, as well as serum from Balb/C mice with 4T-1 TNBC tumors. Results show that MBQ-167 treatment significantly reduced CHI3L1 and S100A8/A9 levels from HER2+ breast cancer cells, macrophages, and serum from Balb/C mice bearing 4T-1 TNBC. In addition to creating a favorable immune environment for inhibition of tumor growth and metastasis, MBQ-167 treatment also increased PDL1 levels in MDSCs and macrophages from spleens and tumors of mice with TNBC tumors following MBQ-167 treatment. Therefore, we tested the effect of MBQ-167 individually (50mg/kg 5X a week for 4 weeks) or in combination with anti-PD1 or anti-PDL1 (10mg/kg every 3 days for 2 weeks) in the 4T-1/Balb/C mouse model. Data show that MBQ-167 administration resulted in 80% reduction in tumor growth with no toxic effects. MBQ-167 was more effective than either anti-PD1 or anti-PDL1 in single or combined therapy in this TNBC mouse model. In conclusion, the Rac/Cdc42 inhibitor MBQ-167 targets metastatic BC cells and immunosuppressive inflammatory cells in the BC TME and is a viable TNBC therapeutic.
Funded by DoD/BCRP HT94252310166, BC220526 and HT94252410094, BC230070.

Introduction

Cancer metastasis is the number one reason for death from epithelial cancers such as breast. Metastasis is driven by complex mechanisms involving cancer cell motility and invasion directed by the Rho family GTPases, Rac, and Cdc42. Rac and Cdc42 do not have to be mutated or overexpressed themselves to regulate cancer progression because they relay signals from multiple oncogenic receptors such as G-protein coupled receptors (GPCR), receptor tyrosine kinases (RTK), toll-like receptors (TLR), and the receptor for advanced glycation end products (RAGE)[1]. These Rho GTPases are ideal molecular targets due to their regulation of proliferation, transformation and invasion, leading to metastasis. Additionally, Rac and Cdc42 have been implanted in the signaling that contributes to recruitment and infiltration of immunosuppressive cells to the tumor microenvironment (TME) (**Fig.1**).

Therefore, we developed a potent dual Rac and Cdc42 inhibitor, called MBQ-167. MBQ-167 is highly effective at targeting cancer and immunosuppressive cells [1-2]. It reduces both tumor burden and prevents liver and lung metastasis in a triple negative breast cancer (TNBC) mouse model (**Fig.2**) [1-3]. Currently, MBQ-167 is undergoing a Phase I clinical trial in advanced breast cancer patients [1-2]. We have demonstrated that MBQ-167 reduces immunosuppressive behavior of macrophages, increases cytotoxic T-cells and decreases inflammatory mediators such as IL-6, CHI3L1 and S100A8/A9, thus promoting an anti-tumor TME[2]. Moreover, MBQ-167 increases PD-L1 expression in immunosuppressive immune cells (**Fig.3-4**). There has been success in immunotherapy for breast cancer, however few TNBCs respond to these therapies. Therefore, the objective of this study is to determine if MBQ-167 can augment the efficacy of current immunotherapy.

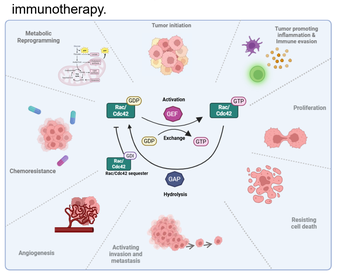


Figure 1. Activation of Rac and Cdc42 drives multiple hallmarks of cancer progression.

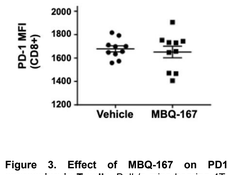


Figure 3. Effect of MBQ-167 on PD1 expression in T cells. Balb/c mice bearing 4T-1 mammary tumors were treated with vehicle or 10mg/kg BW MBQ-167 5x a week for 4 weeks. Mice were sacrificed and the tumors were processed for analysis of PD-1 expression on CD8+ T cells by flow cytometry (n=10).

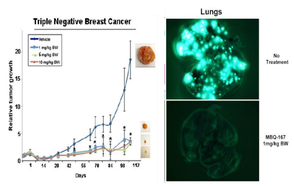


Figure 2. MBQ-167 is a clinical-stage Rac inhibitor that reduces tumor burden and lung metastasis in TNBC mouse models.

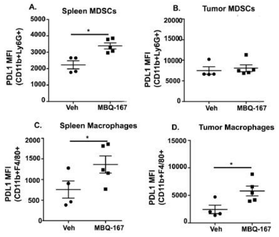


Figure 4. Effect of MBQ-167 on PDL-1 expression in MDSCs and macrophages. SCID mice bearing MDA-MB-231 mammary tumors were treated with vehicle or 5mg/kg BW MBQ-167 (IP) 5x a week for 2 months. The spleens and tumors were extracted at necropsy and subjected to flow cytometry to detect PDL1 in MDSCs and macrophages. N-5. *=p<0.05.

MBQ-167 Is Effective As A Single Agent

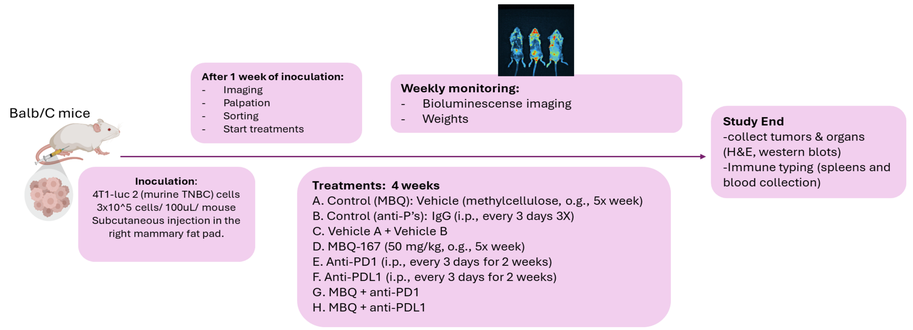
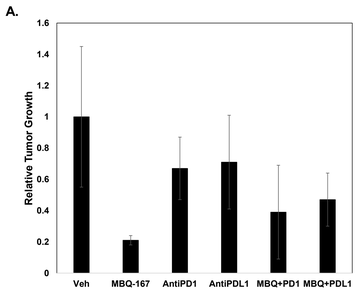
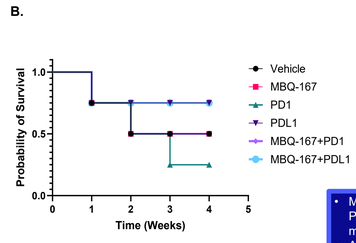


Figure 5. Experimental Strategy for the assessment of MBQ-167 in combination with immunotherapy in a TNBC mouse model. Immunocompetent Balb/C mice were inoculated with luciferase-tagged 4T1 cells at the mammary fat pad. After one week post injection, mice were treated by oral gavage with either 50mg/kg of MBQ-167 as a single agent, or in combination with anti-PD1 or anti-PDL1 antibodies. Tumor growth was imaged by chemiluminescence. After a month, mice were sacrificed. After necropsy, tumors, organs and blood were collected for further studies.





- MBQ-167 is more effective than anti-PD1, anti-PDL1 or combined tumor growth in TNBC mouse model.
- Anti PDL1 therapy was toxic and resulted in 4 deaths 2 weeks after treatment initiation.

Figure 6. MBQ-167 is effective as a single agent in comparison to immunotherapy and in combination, A) Relative tumor growth on day 30 of TNBC mouse models. **B**) Kaplan Meier curve for mouse survival after a month.

MBQ-167 Reduces Lung Metastasis

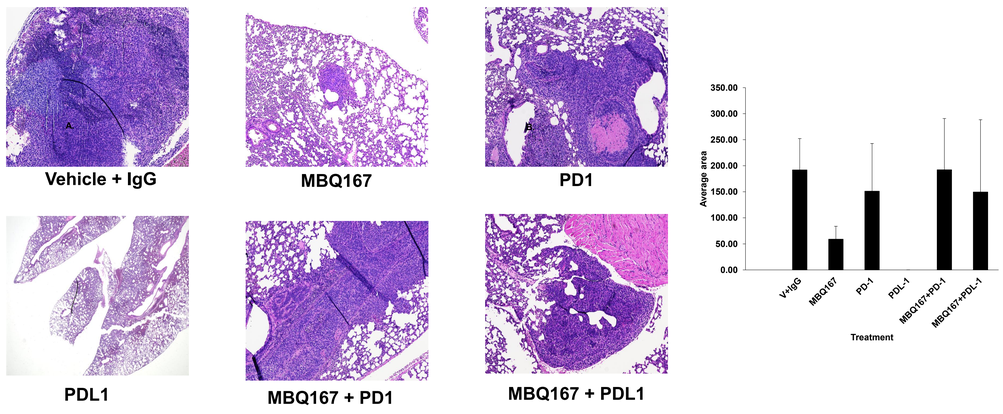


Figure 7. MBQ-167 reduces lung metastasis more effectively as a single agent. Once the study was completed, lungs were collected and fixed in 10% formaldehyde. Subsequently, these fixed lungs were paraffin embedded and cut into sections to stain them with hematoxylin and eosin (H&E) staining. Representative images at 10x are shown.

MBQ-167 Decreases M2 Macrophages In Tumor

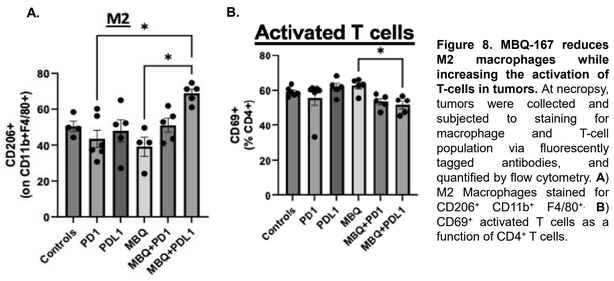


Figure 8. MBQ-167 reduces M2 macrophages while increasing the activation of T-cells in tumors. At necropsy, tumors were collected and subjected to staining for macrophage and T-cell population via fluorescently tagged antibodies, and quantified by flow cytometry. **A**) M2 Macrophages stained for CD206$^+$ CD11b$^+$ F4/80$^+$. **B**) CD69$^+$ activated T cells as a function of CD4$^+$ T cells.

MBQ-167 Decreases Inflammation In TME

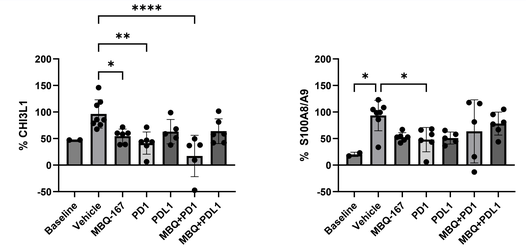


Figure 9. MBQ-167 and anti-PD1 significantly decreases proinflammatory molecules CHI3L1 and S100A8/A9. At the end of the study, blood was collected from mice and centrifuged to obtain the serum. This serum was used for assessing CHI3L1 (Left) and S100A8/A9 (Right). Both ELISA's were conducted per manufacturer's instructions.

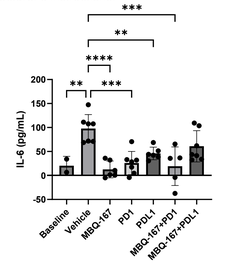


Figure 10. MBQ-167 reduces IL-6 levels both as a single agent and in combination with PD1 therapy. As previously described, serum was obtained from blood samples collected at necropsy. Commercially available ELISA for IL-6 was used and conducted per manufacturers instructions.

Conclusions

- In the mouse TNBC 4T-1 orthotopic tumors in Balb/c immunocompetent model, anti-PD1 and anti-PDL1 immune checkpoint therapies are not as effective as single MBQ-167 therapy.
- Anti PDL-1 treatment was toxic resulting in 50% death by two weeks.
- All treatments resulted in decreased IL-6 in the serum, with MBQ-167 demonstrating a markedly significant decrease, thus signifying reduced inflammation.
- Combination of MBQ and immune checkpoint blockade did not enhance efficacy, with no major changes in tumor immunity.

References

1. Humphries-Bickley, T. et al. 2017. Characterization of a dual Rac/Cdc42 inhibitor MBQ-167 in metastatic cancer. *Mol Cancer Ther.* 16, 805-818.
2. Torres-Sanchez et al. 2023. Rac and Cdc42 inhibitors reduce macrophage function in breast cancer preclinical models. *Front. Oncol.* 13:1152458.
3. Cruz-Collazo et al. 2021. Efficacy of Rac and Cdc42 Inhibitor MBQ-167 in Triple-negative Breast Cancer. *Mol Cancer Ther.* 20(12): 2420–2432. Torres-Sanchez et al. 2023. Rac and Cdc42 inhibitors reduce macrophage function in breast cancer preclinical models. *Front oncol.* 13:1152458.
4. Medina et al. 2022. Characterization of novel derivatives of MBQ-167, an inhibitor of the GTP-binding proteins Rac/Cdc42. *Cancer Res.* 2:1711-1726.

Acknowledgements

We acknowledge that this study was supported by US Army Breast Cancer Research Program W81XWH2010041, HT94252310166, and HT9425-24-1-0094 grants (to SD). NIH/NIGMS 5P20GM103475 is acknowledged for the use of the Keyence microscope system.

Contact: anamaris.torres@upr.edu; ailed.cruzcollazo@upr.edu; su.d@upr.edu