Combination of a novel MELK inhibitor with standard of care treatment results in tumor regression and improved survival in a triple-negative inflammatory breast cancer xenograft model

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Introduction

- Patients with highly aggressive breast cancers, such as triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC)—face clinical challenges, due to advanced stage at diagnosis, high risk for development of metastasis, and limited treatment options.
- We previously demonstrated that MELK is highly expressed in TNBC and IBC, and plays a key role in promoting stemness, epithelial-mesenchymal transition (EMT), and metastasis in a xenograft model[1,2].
- The inhibition of MELK reduced fibronectin (FN1) expression in vitro and in a xenograft mouse model, impairing the formation of extracellular fibronectin fibrils and ultimately decreasing cancer cell motility in TNBC/IBC cells.

Hypothesis

- We hypothesized that MELK expression is higher in TNBC patients and combining MELK inhibitor with standard of care reduce the tumor growth, metastasis and increased survival in novel mouse models.

Objective

- To assess MELK expression, EMT markers, and macrophage infiltration in the tumor microenvironment in patient samples. Additionally, to support clinical translation, to evaluate the efficacy of MELK inhibitors in combination with standard-of-care agents—paclitaxel (PT) and eribulin (ERB) in TNBC/IBC xenograft models.

Results

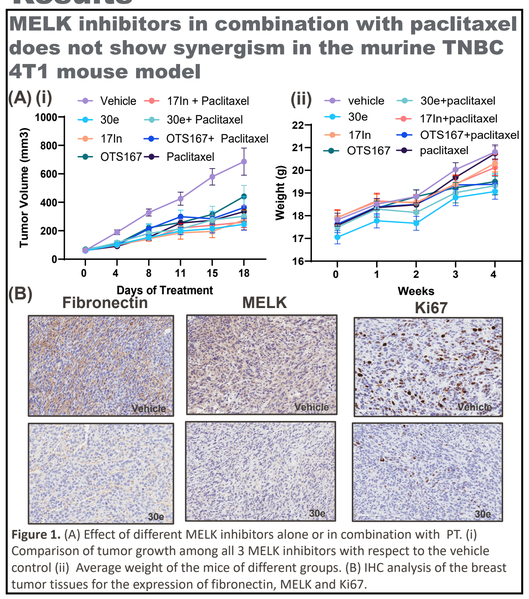


Figure 1. (A) Effect of different MELK inhibitors alone or in combination with PT. (i) Comparison of tumor growth among all 3 MELK inhibitors with respect to the vehicle control (ii) Average weight of the mice of different groups. (B) IHC analysis of the breast tumor tissues for the expression of fibronectin, MELK and Ki67.

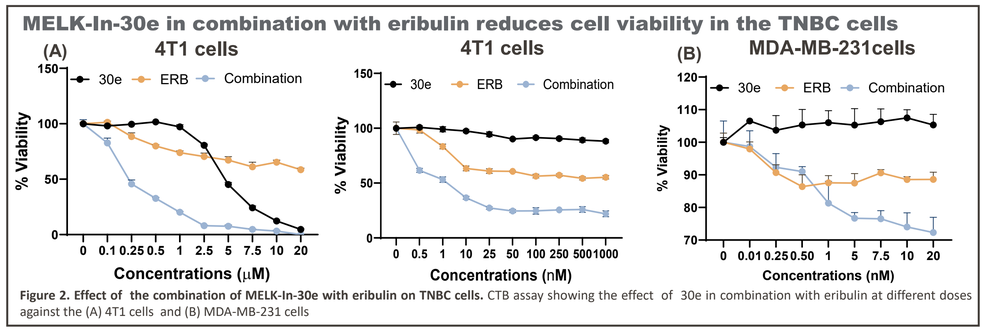


Figure 2. Effect of the combination of MELK-In-30e with eribulin on TNBC cells. CTB assay showing the effect of 30e in combination with eribulin at different doses against the (A) 4T1 cells and (B) MDA-MB-231 cells

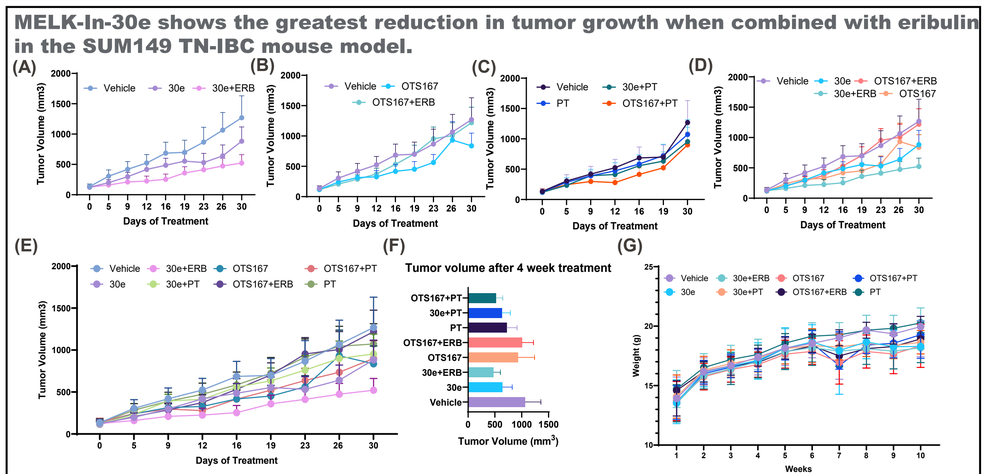


Figure 3. Effect of different MELK inhibitors alone or in combination with the standard of care in TN-IBC orthotopic xenograft model. Comparison of the tumor growth among the (A) MELK-In-30e alone or, (B) OTS167 alone or in combination with eribulin, (C) PT alone or in combination with the 30e and OTS167 as compared to the vehicle control, (D) MELK inhibitors alone or in combination with eribulin. (E) Line graph showing the comparative tumor growth of the different treatment groups with respect to the vehicle control. (F) Bar graph showing the tumor volume of different treatment groups after 4 weeks of treatment. (G) Average weight of the mice of different groups.

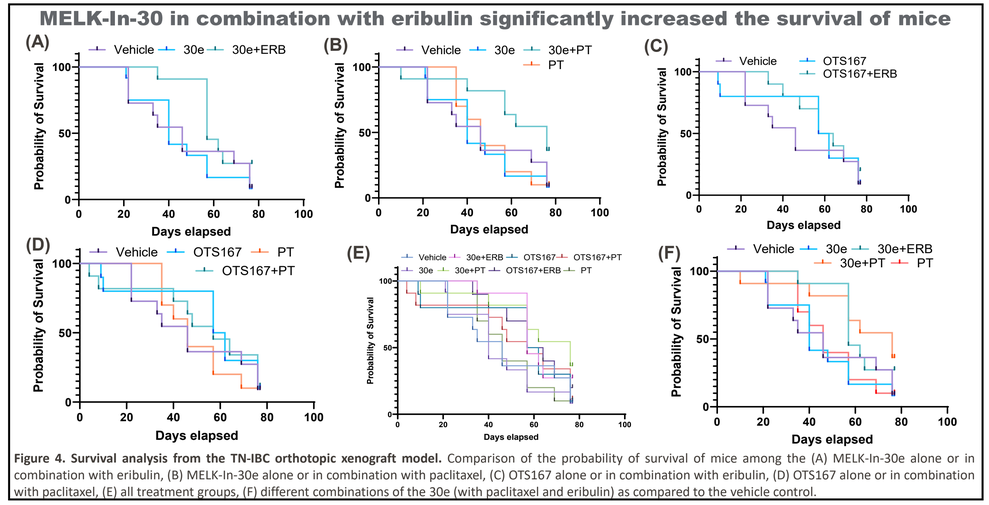


Figure 4. Survival analysis from the TN-IBC orthotopic xenograft model. Comparison of the probability of survival of mice among the (A) MELK-In-30e alone or in combination with eribulin, (B) MELK-In-30e alone or in combination with paclitaxel, (C) OTS167 alone or in combination with eribulin, (D) OTS167 alone or in combination with paclitaxel, (E) all treatment groups, (F) different combinations of the 30e (with paclitaxel and eribulin) as compared to the vehicle control.

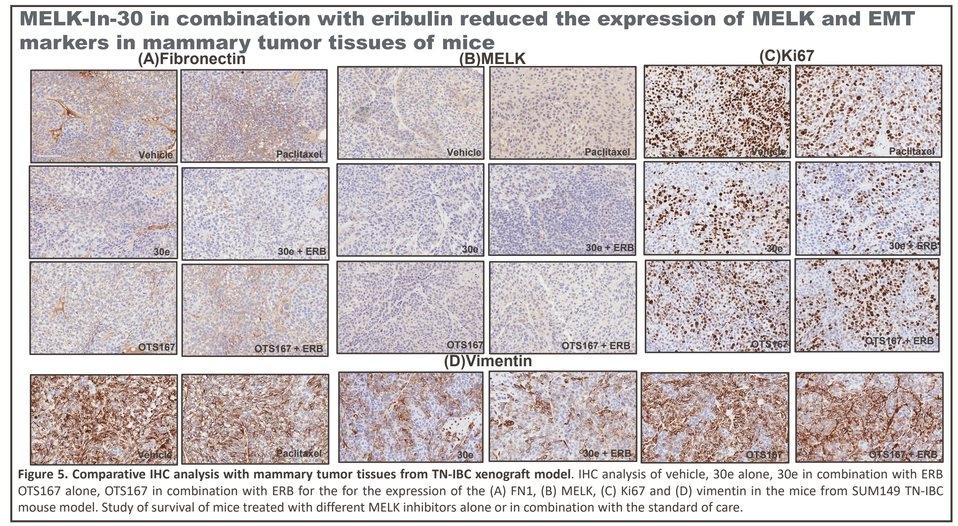
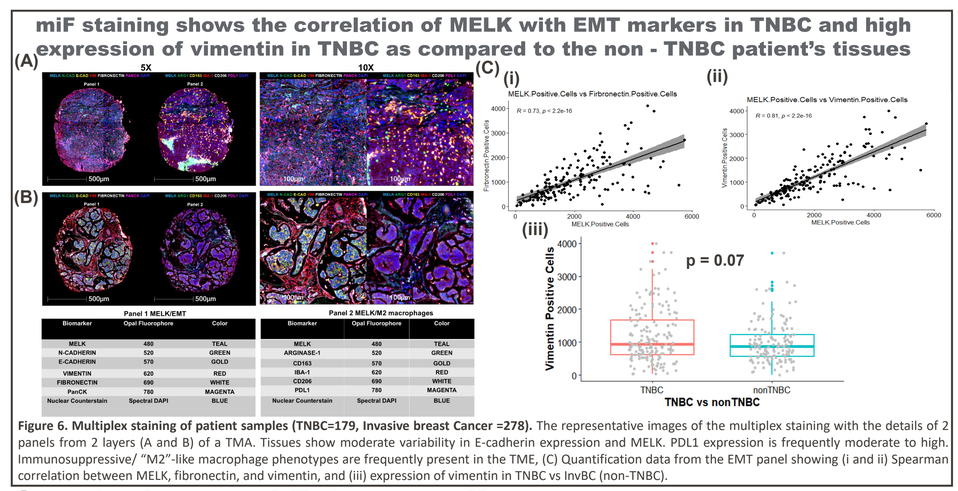


Figure 5. Comparative IHC analysis with mammary tumor tissues from TN-IBC xenograft model. IHC analysis of vehicle, 30e alone, 30e in combination with ERB OTS167 alone, OTS167 in combination with ERB for the for the expression of the (A) FN1, (B) MELK, (C) Ki67 and (D) vimentin in the mice from SUM149 TN-IBC mouse model. Study of survival of mice treated with different MELK inhibitors alone or in combination with the standard of care.



Biomarker	Opal Fluorophore	Color
MELK	480	TEAL
N-CADHERIN	520	GREEN
E-CADHERIN	570	GOLD
VIMENTIN	620	RED
FIBRONECTIN	690	WHITE
PanCK	780	MAGENTA
Nuclear Counterstain	Spectral DAPI	BLUE

Biomarker	Opal Fluorophore	Color
MELK	480	TEAL
ARGINASE-1	520	GREEN
CD163	570	GOLD
IBA-1	620	RED
CD206	690	WHITE
PDL1	780	MAGENTA
Nuclear Counterstain	Spectral DAPI	BLUE

Figure 6. Multiplex staining of patient samples (TNBC=179, Invasive breast Cancer =278). The representative images of the multiplex staining with the details of 2 panels from 2 layers (A and B) of a TMA. Tissues show moderate variability in E-cadherin expression and MELK. PDL1 expression is frequently moderate to high. Immunosuppressive/ "M2"-like macrophage phenotypes are frequently present in the TME, (C) Quantification data from the EMT panel showing (i and ii) Spearman correlation between MELK, fibronectin, and vimentin, and (iii) expression of vimentin in TNBC vs InvBC (non-TNBC).

Conclusions and Future studies

- MELK-In-30e combined with eribulin significantly inhibited tumor growth and suppressed EMT and proliferative markers.
- mIF analysis of patient samples revealed correlation between MELK, FN1 and vimentin. In addition, tumor microenvironment enriched in PD-L1 expression and M2 macrophages, suggesting immune evasion mechanisms.
- These findings provide a rationale for further investigation into MELK-targeted combination therapies to enhance efficacy of existing therapies and immune activation in aggressive cancers.
- We plan to assess the prognostic value of MELK, EMT, and immune markers based on mIF analysis of patient samples.

Acknowledgements

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