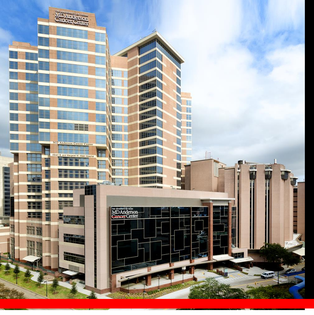

# A Novel Second-generation MELK specific inhibitor targets triple negative inflammatory breast cancer (TN-IBC)

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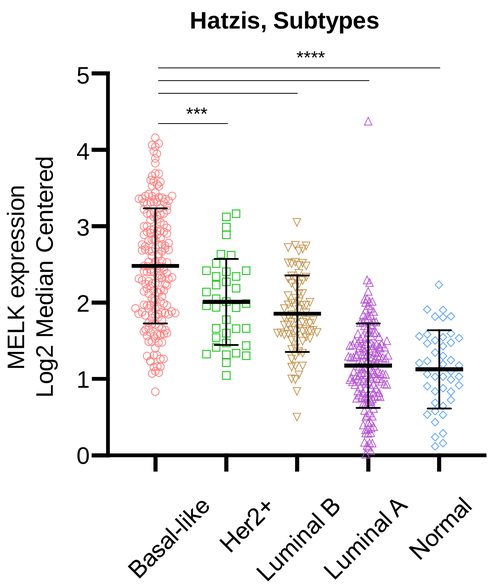
## Introduction

- At least 20% of breast cancers are “triple-negative” (TNBC). TNBC is often associated with poor prognosis and high metastasis.
- Inflammatory breast cancer (IBC), another highly aggressive breast cancer, accounts for 1-5% of all breast cancers, but causes about 8-10% of U.S. breast cancer deaths
- Approximately one-third of cases of IBC are also TNBC.
- Patients with TN-IBC are treated with the standard-of-care multimodality therapy, but thus far resulted in poor outcomes.
- MELK has been shown to be overexpressed in various cancer types, including TNBC.




- Our previous studies have shown that Knockdown of MELK in TNBC cells reduced the CSC phenotype, reversed epithelial-mesenchymal transition (EMT), and blocked invasion and metastasis.

## Hypothesis

- A novel specific MELK inhibitor reduces cancer stemness, tumorigenicity and invasiveness of TN-IBC cells.

## Methods and Materials

- The use of standard assays included cell proliferation, colony formation, soft agar, migration, flow cytometry, and western blotting.

## Results

### 1. Inhibition of MELK decreases cell proliferation

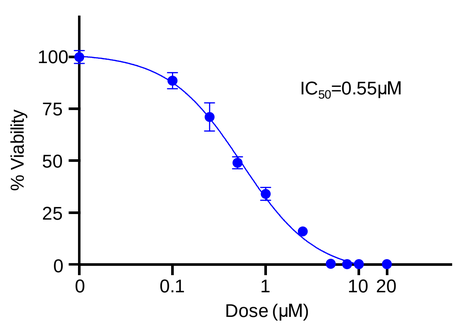



**Fig. 1 Effect of MELK inhibitor 30e on viability.** Estimating the $IC_{50}$ of MELKi 30e, a second-generation MELK inhibitor, using CellTiter-Blue viability assay.

### 2. Inhibition of MELK decreases colony formation capabilities

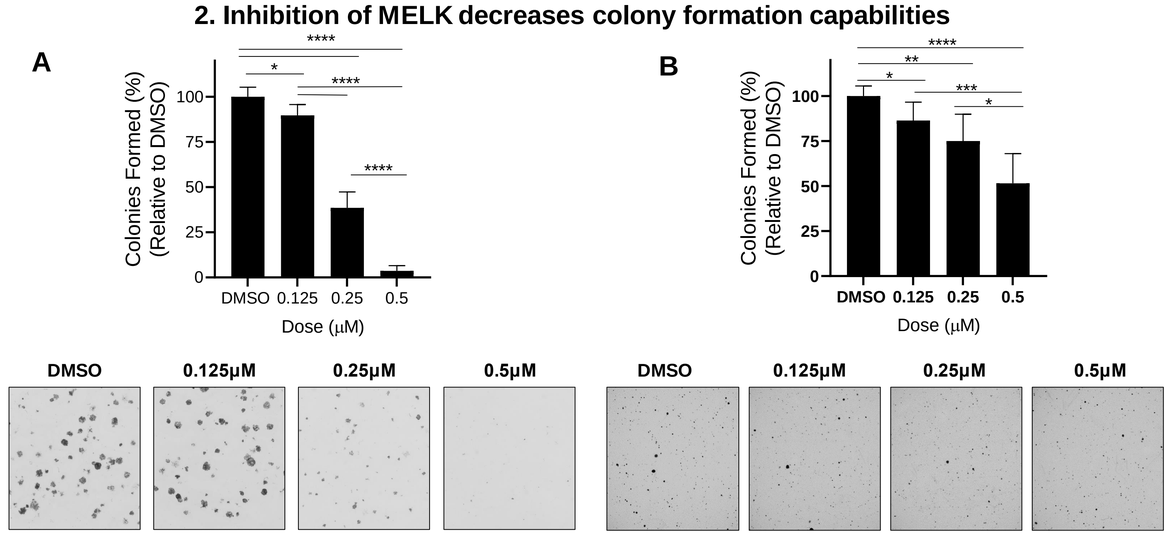



**Fig. 2 Effect of MELK inhibitor 30e on triple-negative and inflammatory breast cancer cell line SUM149 on colony formation. (A)** Colony formation and **(B)** anchorage-independent growth in soft agar. *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001, and ****$P$ < 0.0001 by Welch-ANOVA with Games-Howell’s multiple comparisons test.

### 3. Inhibition of MELK reduces cancer stemness in CD24⁻/CD44⁺ population

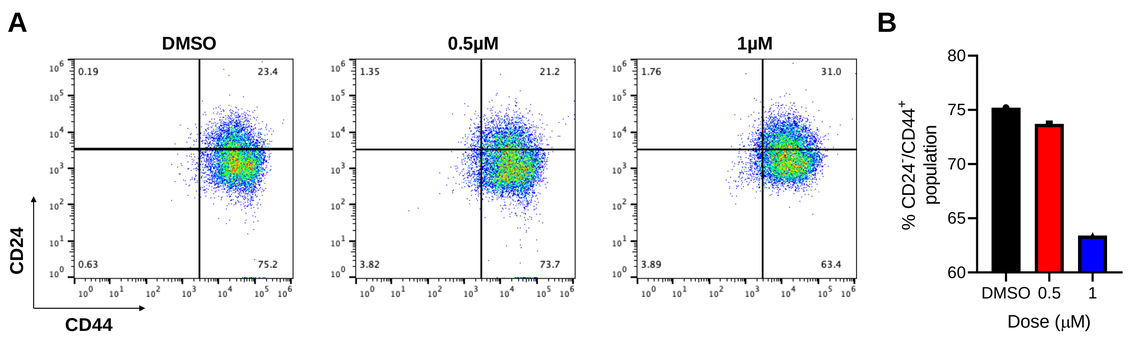



**Fig. 3 Effect of MELK inhibitor 30e on triple-negative and inflammatory breast cancer cell line SUM149 on CD24 and CD44. (A)** Expression level of CD24 and CD44 and **(B)** percentage of cells with CD24⁻/CD44⁺ surface marker expression in SUM149 cells after 48-hour treatment.

### 4. MELK inhibition reduces cancer stemness in ALDH1⁺ population

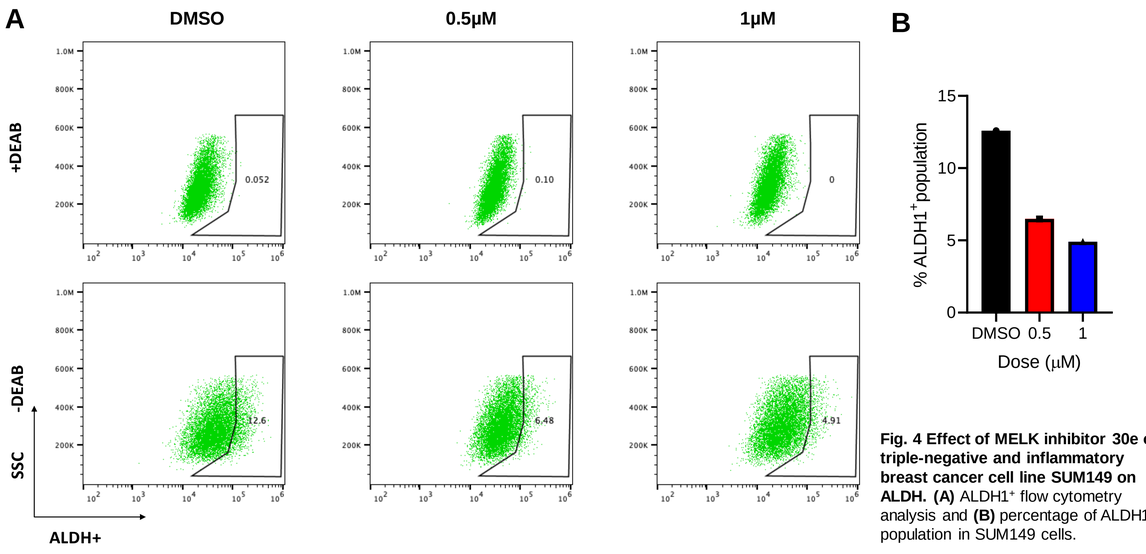



**Fig. 4 Effect of MELK inhibitor 30e on triple-negative and inflammatory breast cancer cell line SUM149 on ALDH. (A)** ALDH1⁺ flow cytometry analysis and **(B)** percentage of ALDH1⁺ population in SUM149 cells.

## Conclusions and Future Directions

- We show that treating cells with a novel selective MELK inhibitor resulted in significant reduction in colony forming ability, migratory capacity and stemness.
- Our findings highlight the therapeutic potential for MELK-In-30e, a second-generation MELK-specific inhibitor as an approach for TN-IBC targeted therapy.
- Future studies will determine the molecular mechanisms of MELK-In-30e and its therapeutic efficacy in a TN-IBC xenograft mouse model to pave the way for this promising target to be translated for clinical use.

## Funding Sources

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- Morgan Welch IBC Bootwalk Seed Funding
- MDACC-Bridge-Seed Funding

## References

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- Pitner M, Taliaferro J, Dalby K, Bartholomeusz C. *Expert Opinion on Therapeutic Targets.* 2017; 21: 849-859.

### 5. MELK inhibition decreases migration

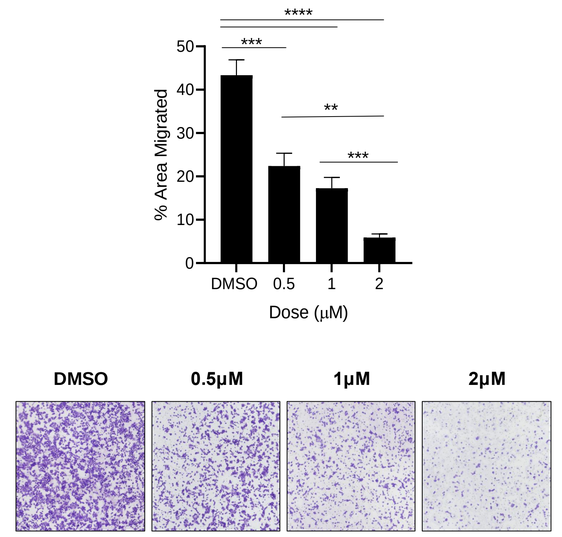


**Fig. 5 Effect of MELK inhibitor 30e on triple-negative and inflammatory breast cancer cell line SUM149 on migration.** *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001, and ****$P$ < 0.0001 by Welch-ANOVA with Games-Howell’s multiple comparisons test.

### 6. MELK inhibition reduces EMT-markers related mRNA expression levels

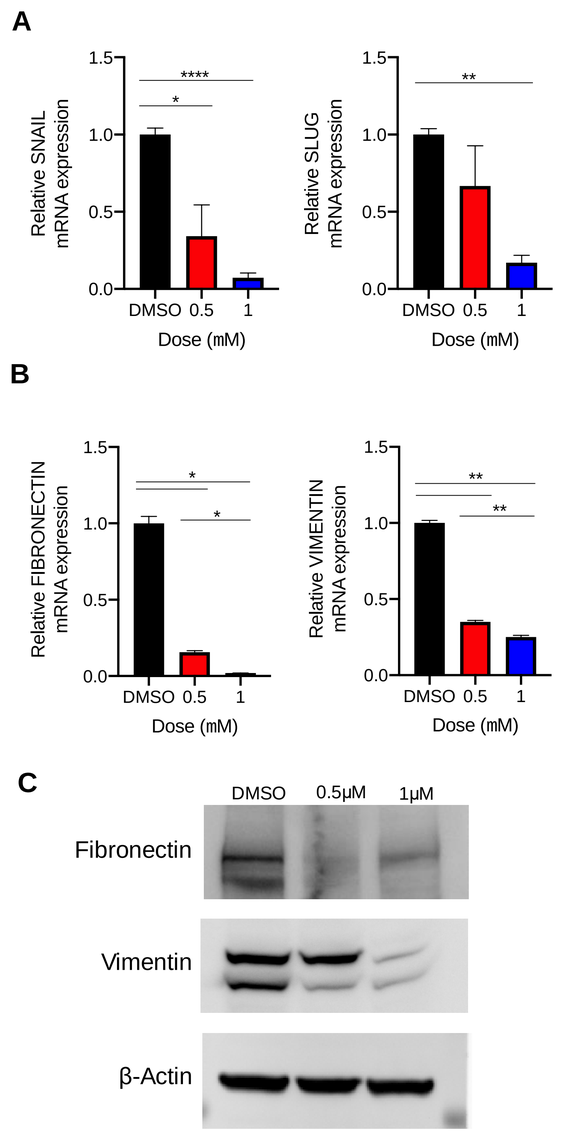


**Fig. 6 Effect of MELK inhibitor 30e on triple-negative and inflammatory breast cancer cell line SUM149 on mRNA expression levels.** RT-qPCR was performed for **(A)** EMT transcriptional factors (Snail and Slug) and **(B)** EMT mesenchymal factors (fibronectin and vimentin) using GAPDH as loading control after 48-hour treatment. *$P$ < 0.05, **$P$ < 0.01, and ****$P$ < 0.0001 by unpaired t-test with Welch’s correction. **(C)** Western blotting of SUM149 cells for EMT markers (fibronectin and vimentin) and loading control β-actin.