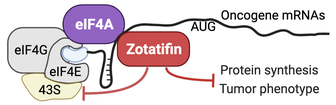
Targeting EIF4A triggers an interferon response to suppress triple-negative breast cancer

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Background

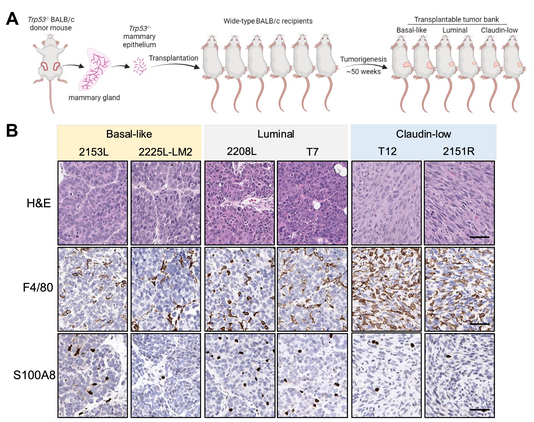
Protein synthesis, frequently dysregulated in cancers, represents an understudied cancer vulnerability. The eukaryotic translation initiation factor 4A (eIF4A) is an RNA helicase that unwinds secondary structures in the 5′-UTR of mRNAs to facilitate translation initiation. Zotatifin, the first-in-class eIF4A inhibitor, is currently undergoing a Phase I/II clinical trial for heavily pretreated ER+ breast cancers (NCT04092673), where partial responses were observed (5 of 19) when zotatifin was combined with fulvestrant and abemaciclib. However, the effects of Zotatifin on triple-negative breast cancer (TNBC) and its interactions with chemotherapy or immunotherapy have not been reported in immuno-competent models.



Objectives

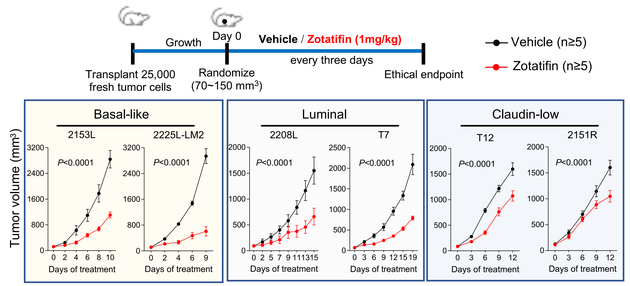
Our study aimed to investigate the effects and mechanisms of Zotatifin on tumor progression and metastasis, both as a monotherapy and in combination with chemo- or immuno-therapy, using diverse syngeneic TNBC mouse models.

Syngeneic TNBC preclinical models

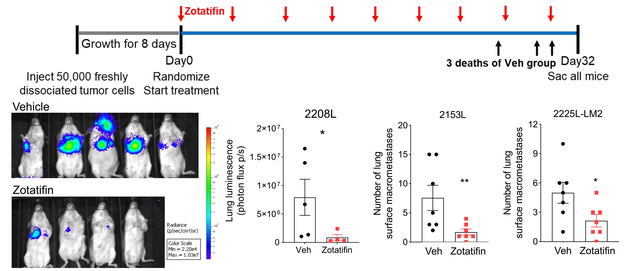


A, Scheme depicting the generation of syngeneic *Trp53*-null preclinical models. Donor mammary epithelium from *Trp53*-null BALB/c mice was transplanted *in situ* into cleared mammary fat pad of wild-type recipient hosts, resulting in the derivation of heterogeneous *Trp53*-null transplantable mammary tumors representative of the different intrinsic molecular subtypes of human breast cancer. **B,** Top, representative images of H&E staining and IHC staining of F4/80 and S100A8 in *Trp53*-null models used in this study.

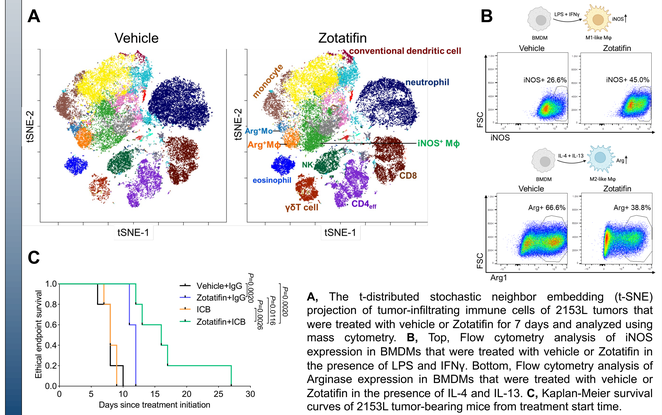
Zotatifin monotherapy inhibits tumor growth



Zotatifin monotherapy inhibits lung metastasis

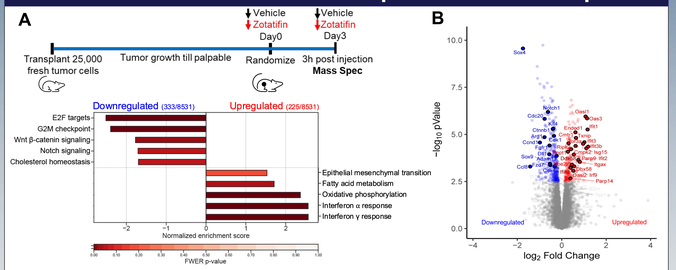


Zotatifin remodels the tumor immune microenvironment and sensitizes tumors to immune checkpoint blockade.



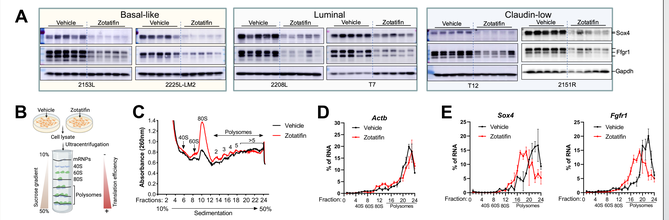
A, The t-distributed stochastic neighbor embedding (t-SNE) projection of tumor-infiltrating immune cells of 2153L tumors that were treated with vehicle or Zotatifin for 7 days and analyzed using mass cytometry. **B,** Top, Flow cytometry analysis of iNOS expression in BMDMs that were treated with vehicle or Zotatifin in the presence of LPS and IFNγ. Bottom, Flow cytometry analysis of Arginase expression in BMDMs that were treated with vehicle or Zotatifin in the presence of IL-4 and IL-13. **C,** Kaplan-Meier survival curves of 2153L tumor-bearing mice from treatment start time.

Zotatifin remodels the tumor proteomic landscape



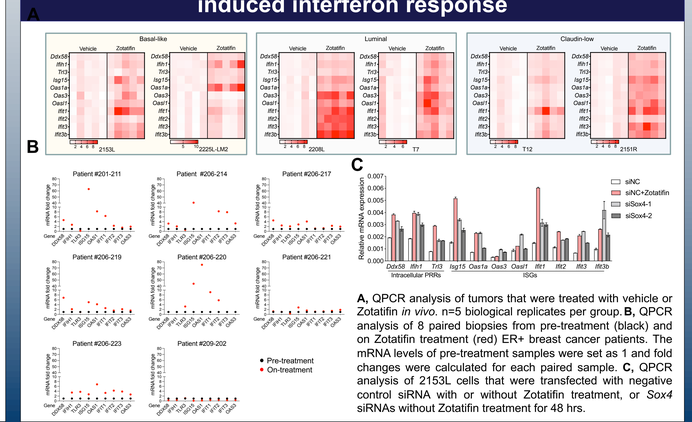
A, Mice with 2153L tumors were treated with either vehicle or Zotatifin for two doses spanning 3 days before mass spectrometry analysis. GSEA of mass spectrometry results is summarized as the normalized enrichment score in vehicle or Zotatifin treated 2153L tumor tissues. **B,** Volcano plot showing relative fold change (log2) in protein abundance versus $-\log_{10}(P$ values) from 2153L tumors treated with Zotatifin compared with vehicle. Proteins that demonstrate a significant change in expression (FDR $q<0.05$) are colored, with decreased expression on the left in blue and increased expression on the right in red. Genes that are critically involved in cell proliferation, stem cell signaling, and interferon response pathways are labeled.

Zotatifin inhibits the translation of Sox4 and Fgfr1 mRNAs



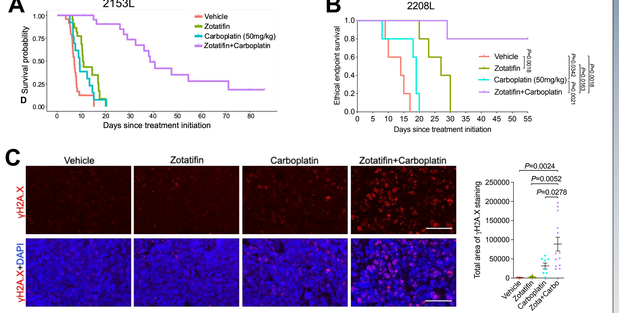
A, Immunoblotting analysis of tumors that were treated with vehicle or Zotatifin *in vivo*. n = 5 biological replicates per group. **B,** Illustration for polysome profiling analysis. **C-E,** Polysome profiling of 2153L cells that were treated with vehicle or 40 nM Zotatifin for 2 hrs. **C,** Representative polysome profiles from three biological replicates. **D,** Distribution of *Actb* mRNA across the different fractions. **E**, Distribution of *Sox4* and *Fgfr1* mRNAs across the different fractions.

Inhibition of Sox4 translation contributes to Zotatifin-induced interferon response



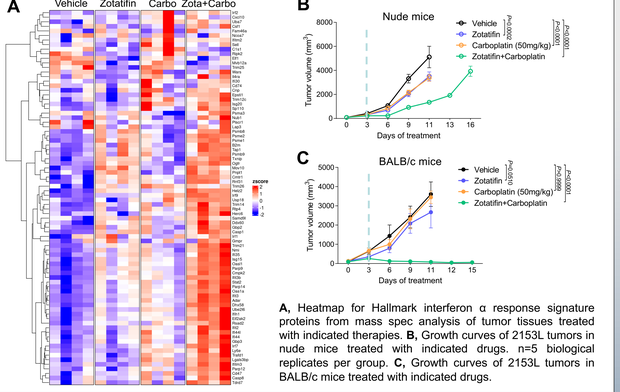
A, QPCR analysis of tumors that were treated with vehicle or Zotatifin *in vivo*. n=5 biological replicates per group. **B,** QPCR analysis of 8 paired biopsies from pre-treatment (black) and on Zotatifin treatment (red) ER+ breast cancer patients. The mRNA levels of pre-treatment samples were set as 1 and fold changes were calculated for each paired sample. **C,** QPCR analysis of 2153L cells that were transfected with negative control siRNA with or without Zotatifin treatment, or *Sox4* siRNAs without Zotatifin treatment for 48 hrs.

Zotatifin synergizes with carboplatin to inhibit tumor growth



A, Kaplan-Meier survival curves of 2153L tumor-bearing mice from treatment start time. **B,** Kaplan-Meier survival curves of 2208L tumor-bearing mice from treatment start time. Mice were randomized and treatment was initiated when tumors reach ~210 mm³ volume. **C,** Left, representative images of IF staining of γH2A.X (in red) in 2153L tumors that were treated with indicated drugs for 3 days. Scale bar, 50 μm. Right, quantification of IF staining.

Zotatifin synergizes with carboplatin to induce an interferon response and promote T cell-dependent tumor inhibition.



A, Heatmap for Hallmark interferon α response signature proteins from mass spec analysis of tumor tissues treated with indicated therapies. **B,** Growth curves of 2153L tumors in nude mice treated with indicated drugs. n=5 biological replicates per group. **C,** Growth curves of 2153L tumors in BALB/c mice treated with indicated drugs.

Impact and Significance

These studies revealed a vulnerability of eIF4A in TNBC, identified potential pharmacodynamic biomarkers for Zotatifin, and provided a rationale for new combination regimens comprising Zotatifin and chemotherapy or immunotherapy as treatments for TNBC.

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