PROGRESS REPORT
PI Name: Davide Ruggero
Co-applicant name (if applicable): N/A
Grant title: Looking through a new lens: Posttranscriptional regulation in metastatic breast cancer.
Date of Award: 05/22/2025
RFP name of award: METAvivor Translational Research Award
Date of submitting the report: 04/22/2026

Summary of the project goal (1 paragraph)

This project focuses on understanding the molecular mechanisms underlying drug resistance in estrogen receptor alpha (ERα)-positive breast cancer, particularly the emergence and persistence of drug-tolerant persister (DTP) cells during hormone therapy. While ERα is traditionally recognized as a transcription factor, our work uncovers a novel function of ERα as a cytoplasmic RNA-binding protein that interacts with translation factors such as eIF4A to reprogram post-transcriptional gene expression in DTP cells. Together, these findings establish a paradigm shift in our understanding of ERα function, demonstrating that, beyond its canonical role as a transcription factor, cytoplasmic ERα acts as a potent RNA-binding protein that reprograms the translational landscape under therapeutic stress. Building on this, our proposal will define how ERα, through its interaction with eIF4A1, governs selective translation to sustain survival of DTP cells during hormone therapy. Furthermore, we will delineate how calcium signaling, particularly through the activation of calmodulin-dependent kinases such as CAMK2D and CAMK2B, is co-opted to promote DTP cell persistence and therapeutic resistance. By leveraging HITS-CLIP, quantitative mass spectrometry, translational profiling, and pharmacological interventions using novel clinical translation inhibitors such as zotatifin, this project aims to uncover new vulnerabilities in DTP cell biology. These mechanistic insights will inform innovative therapeutic strategies that concurrently target post-transcriptional regulation and calcium signaling to overcome drug resistance in ERα-positive breast cancer.

Keywords: Rα, drug resistance, translation regulation, breast cancer, DTP cells, eIF4A1, calcium signaling, CAMK2D.

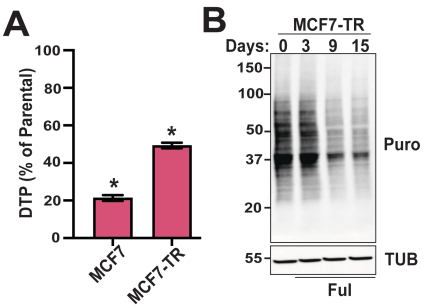

Figure 1: ER+ breast cancer cells form DTPs with reduced protein synthesis upon ERα inhibition. (A) CellTiter-Glo assay after 15 days of fulvestrant (10 μM) treatment quantifies DTP cells in MCF7 and MCF7-TR cell lines. Shown is the mean percent survival and p values of DTP cells compared to the vehicle-treated parental control. Data represent mean ± SD. Significance is shown as *p < 0.0001. (B) Puromycin incorporation assay showing time-dependent reduction in global protein synthesis in MCF7-TR cells during fulvestrant treatment. Tubulin serves as a loading control.

Accomplishments

What were the major goals of the project?

Aim 1: Elucidating the role of calmodulins in DTP cells**.**

Aim 2: Targeting ERα and eIF4A as a combination therapeutic approach for breast cancer metastasis.

What was accomplished under these goals?

The overall goal of this project is to uncover how ERα functions as an RNA-binding protein to regulate selective mRNA translation programs that drive the formation and survival of drug-tolerant persister (DTP) cells in ERα-positive breast cancer. During this reporting period, our studies established a novel post-transcriptional role for ERα in DTP cells, particularly in the regulation of stress response and calcium signaling pathways and identified CAMK2D-centered translational dependencies and ERα-eIF4A–associated mechanisms as candidate therapeutic vulnerabilities relevant to endocrine resistance and metastatic progression in ERα-positive breast cancer.

SOW Task Completion Summary (Year 1):

Major Task 1: Elucidating the role of calmodulins in DTP cells - **40% completed**

Major Task 2: ERα RNA-binding activity shapes the DTP proteome - **25% completed**

Major Task 2.2: Targeting ERα and eIF4A with a combinatorial approach *in vivo*. - not initiated

Specific Aims

Aim 1: Elucidating the role of calmodulins in DTP cells**.**

Rationale

We have made significant progress this year, highlighted by the successful procurement of key reagents and the development of essential molecular and cellular tools to study drug-tolerant persister (DTP) cells in ERα-positive breast cancer. While previous studies of DTP cells have predominantly focused on transcriptional regulation, the role of translational control in supporting DTP survival remains poorly understood. To address this gap, we employed an unbiased proteomic analysis in MCF7-TR (TamR1) cells and found that, despite a global reduction in protein synthesis following endocrine therapy, DTP cells selectively maintain translation of a specific protein. Notably, these include calcium regulators and calmodulin kinases, suggesting a specialized translational program that supports survival under therapeutic stress and reveal potential vulnerabilities. To investigate this mechanism, we have developed key molecular tools, including stable, GFP-tagged overexpression models of CAMK2D and CAMK2B across multiple ER+ breast cancer cell lines, as well as shRNA-mediated knockdown systems. These platforms enable targeted interrogation of calmodulin kinase signaling in DTP cells and provide a functional framework to evaluate their role in endocrine resistance and persistence.

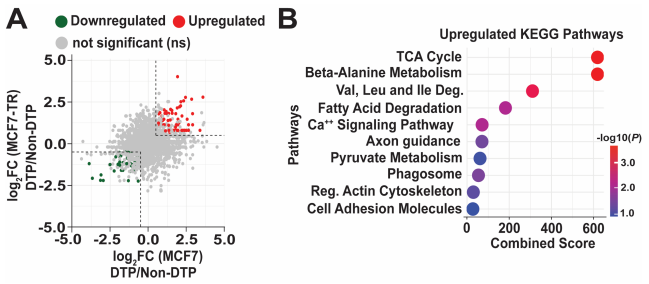

Figure 2: Proteomic analysis of ER+ fulvestrant DTP cells. (A) Mass spectrometry on MCF7 and MCF7-TR cells treated with fulvestrant for 15 days. Scatter plot of log_2 fold change (log_2fc) of proteins abundance, highlighting upregulated (red) and downregulated (green) proteins. (B) KEGG pathway enrichment analysis of upregulated pathways in DTP cells following 15 days of fulvestrant (10 μM) treatment, based on mass spectrometry data from MCF7 and MCF7-TR DTP cells.

Results

To investigate how ERα+ drug-tolerant persister (DTP) cells contribute to endocrine resistance, we utilized MCF7 and MCF7-TR breast cancer cell lines treated with high-dose fulvestrant (10 μM) for 15 days. A small population of cells consistently survived, indicative of DTP cell emergence and providing a robust model to study early, non-genomic resistance mechanisms (Fig. 1A). Under stress conditions, cells typically suppress protein synthesis to reduce energetic demands. We reasoned that DTP cells, under drug-induced stress, would exhibit reduced global protein synthesis. Consistent with this, fulvestrant treatment significantly decreased overall protein

synthesis rates in DTP cells derived from ER$^+$ tamoxifen-resistant and sensitive cells (Fig. 1B). Unbiased proteomic profiling of DTP cells revealed selective upregulation of translationally regulated proteins, with a prominent enrichment in calcium signaling pathways (Fig. 2A and 2B). Calcium ions, through calmodulin and calmodulin-dependent kinases, regulate diverse oncogenic processes—including NFATc3, NF-κB, Src, and AKT—that promote tumor survival and therapy resistance. Yet their role in DTP cells has remained uncharacterized. To assess the involvement of calcium signaling, we measured intracellular calcium using Indo-1 AM dye and flow cytometry. DTP cells derived from MCF7, T47D, and MCF7-TR lines displayed elevated calcium levels following fulvestrant treatment (Fig. 3A). Moreover, chelation of intracellular calcium using BAPTA-AM significantly reduced DTP viability, suggesting calcium is functionally important for their survival (Fig. 3B). To further dissect calcium localization, we developed a lentiviral system encoding GFP-tagged reporters targeted to the cytoplasm, nucleus, ER, and mitochondria, enabling compartment-specific monitoring of calcium distribution in DTP cells. This system is currently being optimized and analyzed to enable investigation of compartment-specific calcium distribution in DTP cells. Interestingly, among the calcium-regulated proteins, CAMK2D—a calmodulin-dependent kinase—was significantly upregulated at the protein level in DTP cells (Fig. 4A, *Upper panel*). While CAMK2D is well characterized in neuronal and developmental contexts, its role in cancer progression and therapy resistance remains largely unexplored. Notably, CAMK2D transcript levels were reduced following 15 days of fulvestrant treatment, suggesting post-transcriptional regulation (Fig. 4A, *Lower panel*). To determine whether CAMK2D is translationally regulated, we performed sucrose gradient polysome profiling. CAMK2D mRNA in untreated cells was mainly found in low translation fractions. In contrast, in DTP cells, CAMK2D mRNA shifted into heavy polysome fractions (Fig. 4B), indicating enhanced translation efficiency under therapeutic stress. To define the functional role of CAMK2D, we initially pursued CRISPR-based knockout approaches but encountered challenges due to multiple transcript variants. We have since implemented an shRNA-based knockdown strategy and are evaluating its effects on DTP survival. In parallel, we have successfully generated stable CAMK2D overexpression models in ERα+ breast cancer lines (Fig. 4C). Together, these findings highlight calcium signaling—particularly CAMK2D—as a novel and functionally important pathway supporting DTP cell survival, and they establish a foundation for targeting translationally regulated vulnerabilities in ERα+ breast cancer.

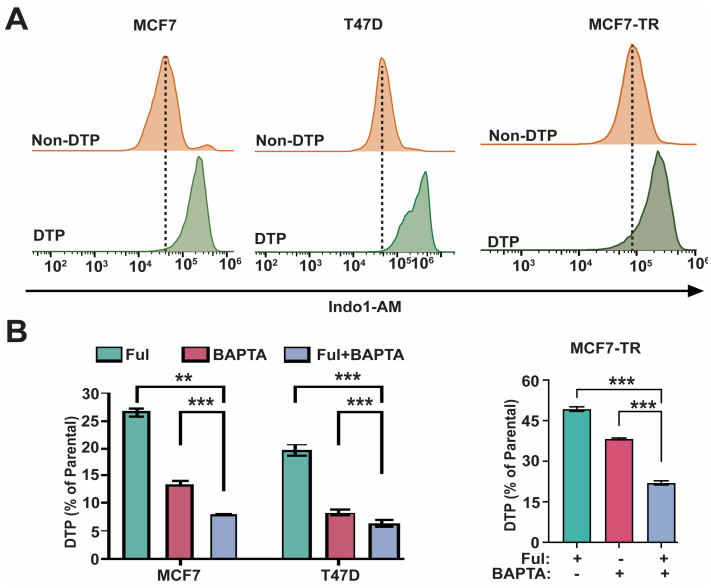

Figure 3: Intracellular calcium expression regulates DTP cell survival. (A) Flow cytometry analysis of intracellular calcium levels in MCF7, T47D and MCF7-TR DTP versus non-DTP cells after 15 days of fulvestrant treatment, using Indo1-AM dye. (B) Survival of DTP cells following 72-hour treatment with BAPTA-AM (100 nM), alone or in combination with fulvestrant, as assessed by CellTiter-Glo assay in MCF7, T47D and MCF7-TR lines. Shown is the mean percent survival of DTP cells compared to the vehicle-treated parental control. Data represent mean ± SD.

Changes to the proposed plan, justification, and alternative approaches for Aim 1

During this reporting period, one important modification to the proposed plan involved the strategy for genetic loss-of-function studies of CAMK2D. Although the original approach relied on CRISPR-based knockout, this strategy proved technically challenging due to the complexity of CAMK2D transcript variants. As an alternative, we established shRNA-based knockdown systems that preserve the mechanistic direction of the project while providing a tractable platform to assess CAMK2D dependence in DTP survival. In parallel, a compartment-specific calcium reporter system has been generated and is currently being optimized. This represents a refinement in experimental timing rather than a change in scientific direction. Together, these adjustments were necessary to ensure experimental rigor and feasibility while preserving the central objective of the aim to define how calcium signaling contributes to DTP cell persistence and metastasis potential.

Aim 2: Targeting ERα and eIF4A as a combination therapeutic approach for breast cancer metastasis.

Major Task 2: Understanding how ERα RNA binding activity shapes the hormone therapy- induced DTP cells proteome- 25 % completed

Major Task 2.1: Understanding the importance of interaction between ERα and eIF4A- 20% completed

Major Task 2.2: Targeting ERα and eIF4A with a combinatorial approach *in vivo*. - not initiated

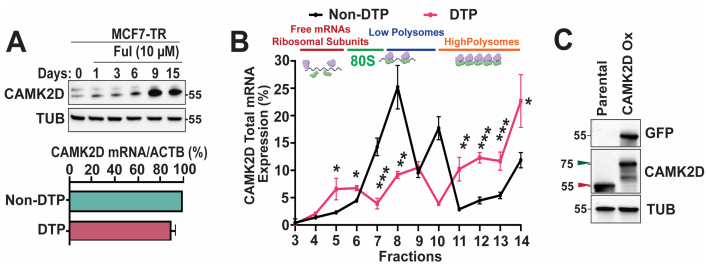

Figure 4: Intracellular calcium expression regulates DTP cell survival. (A) Flow cytometry analysis of intracellular calcium levels in MCF7, T47D and MCF7-TR DTP versus non-DTP cells after 15 days of fulvestrant treatment, using Indo1-AM dye. (B) Survival of DTP cells following 72-hour treatment with BAPTA-AM (100 nM), alone or in combination with fulvestrant, as assessed by CellTiter-Glo assay in MCF7, T47D and MCF7-TR lines. Shown is the mean percent survival of DTP cells compared to the vehicle-treated parental control. Data represent mean ± SD. (C) Western Blot showing stable CAMK2D overexpression models in ERα+ breast cancer lines.

Rationale

Building on prior discoveries from our lab that ERα functions not only as a transcription factor but also as a novel RNA-binding protein (RBP) that interacts with mRNA networks critical for tumor progression, our work extends this concept to the DTP context. Specifically, we show that the post-transcriptional regulatory activity of ERα contributes to the formation and maintenance of DTP cells during endocrine therapy. We further demonstrate an interaction between ERα and eIF4A; however, their functional significance of this interaction in regulating DTP cell survival remains to be elucidated. This interaction may contribute to a specialized translational network that supports the persistence of DTP cells. Collectively, these findings provide critical new insights into the biology of breast cancer persistence and uncover actionable therapeutic targets for eliminating DTP cells and preventing drug-resistant disease progression.

Results

We previously reported that ERα functions beyond its classical nuclear role as a transcription factor by acting as an RNA-binding protein (RBP). This novel RNA-binding activity plays a critical role in promoting breast cancer cell survival, revealing a previously unrecognized mechanism underlying disease progression. Given the RNA helicase activity of eIF4A1, we hypothesized that an interaction between ERα and eIF4A1 may regulate the translation of CAMK2D, contributing to therapy resistance. To test this, we performed co-immunoprecipitation (co-IP) experiments using cytoplasmic lysates from MCF7 and MCF7-TR cells—a tamoxifen-resistant, more tumorigenic derivative of MCF7. These studies revealed a stronger ERα-eIF4A1 interaction in MCF7-TR cells compared to MCF7 cells (Fig. 5A). To dissect the molecular interface of this interaction, we generated a series of ERα deletion constructs (targeting the N-terminal domain, DNA-binding domain, ligand-binding domain [LBD], and activation function domain) and co-transfected them with wild-type eIF4A1 into HEK293T cells. Immunoprecipitation assays demonstrated that the LBD of ERα is required for binding to eIF4A1(Fig. 5B).

While these results define the ERα region involved in this interaction, complementary mapping of the binding interface on eIF4A1 is currently ongoing. To assess the functional relevance of the ERα-eIF4A1 interaction in DTP cells, we attempted to generate MCF7-TR cells stably expressing either wild-type ERα or RNA-binding domain (RBD) mutants. However, due to the limited capacity of MCF7-TR cells to survive single-cell sorting, we have adopted an alternative strategy employing transient knockout of endogenous ERα followed by re-expression of either wild-type or RBD mutant constructs. These experiments are ongoing. We are currently performing polysome profiling and mass spectrometry analyses in these models to define how ERα RNA-binding activity influences the translational landscape of DTP cells during hormone therapy and contributes to mechanisms of persistence and resistance. Collectively, the results obtained to date support the existence of a novel ERα-regulated network of post-transcriptional gene expression that contributes to the formation of drug-tolerant persister (DTP) cells, reveals new vulnerabilities in breast cancer, and informs potential therapeutic interventions.

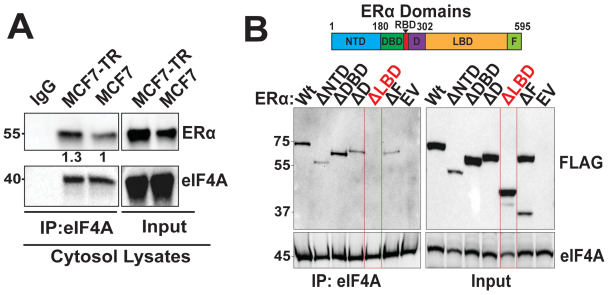

Figure 5: eIF4A and ERα regulation in DTP cells. (A) Co-immunoprecipitation (Co-IP) of ERα with eIF4A from cytosolic lysates of MCF7 and MCF7-TR cells. Lysates were immunoprecipitated with an anti-eIF4A antibody or IgG control, followed by immunoblotting for ERα (ESR1) and eIF4A. Input lysates show total protein levels. Densitometric values are shown. (B) Schematic representation of ERα domain architecture, including the N-terminal domain (NTD), DNA-binding domain (DBD), hinge region (D), ligand-binding domain (LBD), and F domain (Upper panel). HEK293T cells were transiently transfected with FLAG-tagged wild-type ERα or domain-deletion constructs (ΔNTD, ΔDBD, ΔLBD), along with an empty vector (EV) control. Lysates were subjected to immunoprecipitation with anti-eIF4A antibody and immunoblotted for FLAG and eIF4A. Input panels show total protein levels (lower panel).

Changes to the proposed plan, justification, and alternative approaches for Aim 2

The main adjustment under Aim 2 involved the strategy used to model RNA-binding-deficient ERα in MCF7-TR cells. The original experimental design relied on stable cell line generation following single-cell sorting;

however, the MCF7-TR background did not tolerate that workflow. To preserve the conceptual intent of the proposal, we adopted an alternative approach involving with transient knockout of endogenous ERα followed by re-expression of wild-type or RNA-binding-deficient ERα constructs. This modification retains the mechanistic focus on ERα RNA-binding activity while improving feasibility. Sub-aim 2.2, which addresses the in vivo combinatorial studies with zotatifin and fulvestrant, has not yet been initiated as an experimental arm during the present reporting period, but the mechanistic and model-development groundwork established under Aim 2.1 provides the necessary basis for that work.

Achievements and Challenges during the reporting period

Across both aims, the project achieved several major milestones during the reporting period. We established robust and reproducible DTP models in ER-positive breast cancer systems and identified calcium signaling as a prominently enriched, translationally regulated pathway associated with DTP survival. In addition, we strengthened CAMK2D as a candidate effector linking stress adaptation to metastatic competence. We also extended the non-canonical role of ERα into the DTP setting by demonstrating enhanced ERα-eIF4A1 interaction and defining the ligand-binding domain of ERα as a critical region for this interaction. We also encountered technical challenges, including transcript complexity during CRISPR-based CAMK2D targeting and limited viability of MCF7-TR cells during single-cell sorting. Alternative approaches, including shRNA-mediated depletion and transient knockout/re-expression systems, were implemented to address these challenges while maintaining the central objectives of the proposal.

Goals for the upcoming funding period

In the upcoming reporting period, we will continue to investigate the role of ERα RNA-binding activity in translational regulation and DTP cell persistence. To this end, we will perform polysome sucrose gradient assays to assess the translation efficiency of CAMK2D in MCF7 and MCF7-TR cells, as well as in MCF7-TR cells expressing RNA-binding-deficient ERα. RNA isolated from polysome fractions will be analyzed by qPCR to quantify transcript distribution across translational states. To further characterize ERα-dependent translational regulation, we will perform mass spectrometry to compare the global proteomic profiles of MCF7-TR wild-type and RNA-binding-deficient DTP cells. In parallel, we will develop RNA proximity ligation assays to directly examine ERα-RNA interactions in wild-type and RNA-binding-deficient MCF7-TR cells, thereby providing mechanistic insight into ERα-mediated post-transcriptional regulation.

We will also investigate the functional importance of CAMK2D phosphorylation in DTP cells and its role in downstream signaling and metastatic progression. Because phosphorylation of CAMK2D is critical for its kinase activity and activation of downstream targets, we will generate a catalytically inactive phospho-mutant, CAMK2D T287A, and perform phospho-proteomic profiling in DTP cells. To directly test the role of CAMK2D phosphorylation in metastasis, we will implant cells expressing the CAMK2D T287A mutant into mice by intracardiac injection and assess metastatic burden. To evaluate the clinical relevance of this pathway, we will examine protein expression of CAMK2D and additional calcium signaling proteins that were not bound by ERα

but were upregulated in our mass spectrometry analyses, including CAMK2B, CAMK2G, PLCB3, and CAMK1D, using tumor microarrays and matched metastatic samples collected before and after treatment with ERα inhibitors. In parallel, we will use recently procured patient-derived xenograft models obtained from Jackson Laboratories to test the effects of calcium channel blockers and calmodulin inhibitors, including verapamil, KN62, and KN93, on metastatic progression.

In addition, we will evaluate the therapeutic efficacy of zotatifin and fulvestrant, both as single agents and in combination in metastatic mouse models. These models will be generated via intracardiac injection of DTP cells and treatment effects will be assessed across multiple endpoints, including metastasis formation, tumor burden, cell proliferation, cell death, and both overall and tumor-free survival. To further define the translational mechanisms in metastatic disease, tumors will be isolated form key metastatic sites, including liver and lung, and subjected to ribosome profiling to determine translation efficiency in vivo. Finally, we are well positioned to extend these studies to additional ER-positive breast cancer models that exhibit resistance to hormone therapy and display strong metastatic potential, including T47D/T1 and ESR1 mutant models harboring Y537S, Y537C, and D538G alterations.

What was the impact on the development of the principal discipline(s) of the project?

This project has significantly deepened our understanding of how certain breast cancer cells evade hormone therapy and develop resistance. We discovered that the estrogen receptor—traditionally known for controlling gene activity in the nucleus—also binds to RNA, directly influencing protein production in these therapy-resistant cells. Importantly, we identified that calcium signaling, a vital pathway that controls many cell survival and growth processes, is upregulated in these resistant cells. Proteins like CAMK2D, involved in calcium signaling, help the cells survive under treatment stress. This new insight reveals a previously hidden layer of control in breast cancer progression and opens promising avenues for therapies that target both hormone signaling and calcium-dependent survival pathways to prevent resistance.

Publications / Presentations / Patent application section

The data from this project was presented at AACR annual meeting 2026, San Diego

Lay Description of Important Outcomes

This project is focused on defining the molecular mechanisms by which estrogen receptor alpha (ERα)-positive breast cancer cells survive hormone therapy and progress toward metastatic disease. In particular, we are studying a small population of cells known as drug-tolerant persister (DTP) cells, which emerge under treatment stress and are thought to provide the foundation for later relapse and metastatic progression. Our work has uncovered a previously unrecognized function of ERα outside its canonical role as a transcription factor. In

addition to controlling gene expression in the nucleus, ERα appears to function in the cytoplasm as an RNA-binding protein that helps reprogram protein production in therapy-stressed cells. This finding suggests that resistance is driven not only by changes in gene expression, but also by selective control of translation that enables cancer cell survival under endocrine therapy.

A major outcome of this work is the identification of calcium signaling as an important pathway associated with the survival of DTP cells. We found that CAMK2D, a calcium-regulated kinase, is selectively increased at the protein level in these resistant cells and may play a critical role in promoting persistence under hormone therapy. Together, these findings support a model in which ERα-dependent post-transcriptional regulation and calcium signaling cooperate to sustain therapy-resistant cells and promote metastatic competence. By defining these mechanisms, this project is uncovering new therapeutic vulnerabilities and providing the rationale for future strategies that combine endocrine therapy with agents that target translation control, including the clinical eIF4A inhibitor zotatifin, with the goal of preventing resistant cells from driving metastatic breast cancer progression.

2026 METAvivor Research Award Budget With Justification Template

The METAvivor Research Award is for up to $450,000 over two years ($150,000 per year). This amount includes direct costs (salary, materials and supplies, and travel up to $4,500), and indirect costs of up to 5% of the total award. METAvivor funds cannot be used for the purchase of equipment or tuition remission. A detailed explanation justifying the budget for these categories is required. Please complete this template and upload it as a PDF file labeled as **applicantlastname_ MRABudgetandJustification_2026.pdf**.

BUDGET

Applicant Name:	Dr. Davide Ruggero, PhD.		
Project Title:	Looking through a new lens: posttranscriptional regulation in metastatic breast cancer		
Budget Category	**Amount Requested - Initial Budget Period (Year 1)**	**Amount Requested - Additional Budget Period (Year 2)**	**Amount Requested - Additional Budget Period (Year 3)**
Salary (applicant, research specialist and other personnel):	108,704	109,493	111,057
Materials and supplies:	12,576	10,237	8,626
Travel (up to $ 4,500):	0	1,500	1,500
Indirect Costs (F&A) (Cannot exceed 5% of total grant fund i.e. $15,000)	7,143	7,143	7,143
(Please enter any additional budget items)			
Data Network Charges	523	542	561
IT Field Services (ITFS) Desktop Support	256	264	272
GAEL Charges	798	821	841
Mouse Purchase and Housing	20,000	20,000	20,000
Total cost for each period:	**142,857**	**142,857**	**142,857**
Total cost for entire proposed period not to exceed $450,000 total, or $150,000 per year:	150,000	150,000	150,000

Following changes were made to Other Personnel budget for Initial Year –

BUDGET JUSTIFICATION

Other Personnel:

Macey Slota, BS – Graduate Student – (EFFORT:6.0 calendar months (25%) in all years – salary and fringe benefits requested in all years) – Ms. Slota earned her B.S. in Biochemistry and Molecular Biology, Magna Cum Laude from Belmont University, Nashville, TN in 2021. She has extensive experience in cancer biology and RNA biology and has years of research in gene expression in the context of cancer. She is a co-author of two major publications on the role of Myc and tumorigenesis. As a Ph.D. student in my lab, Ms. Slota has been very productive and has been leading the intellectual aspects of the project. She has received wide training in the lab in many technical and conceptual features of post-transcriptional regulation in cancer biology.

Kenya Bonitto, BS – Graduate Student – (EFFORT:6.0 calendar months (25%) in all years – salary and fringe benefits requested in all years) – Ms. Bonitto earned her B.S. in Molecular, Cell, and Developmental Biology from UCLA. She has extensive experience in RNA biology and has years of research in gene expression at the post-transcriptional level and regulation in the context of cellular homeostasis, such as cellular quiescence, one of the key hallmarks of cancer cells. As a Ph.D. student in my lab, Ms. Bonitto has been very productive, leading the intellectual aspects of many projects. She has been receiving wide training in the lab in many technical, and conceptual features of cancer biology.

Adrianna Dabrowska - **Postdoctoral Fellow (EFFORT:6.0 calendar months (25%) in year 01 - salary and fringe benefits requested for initial years) –** Dr. Dabrowska joined Dr. Ruggero's laboratory in January 2022 and she has vast experience in deciphering the role of ribosomal RNA in human biology. Dr. Dabrowska completed her PhD at the Cancer Research UK Beatson Institute, where she worked in mRNA translation control. Dr. Dabrowska has extensive laboratory experience in RNA biology, and she is proficient in many biochemical and biophysical (including low-resolution structure) methods. She is also experienced in investigating how possible changes in rRNA modifications (guided by the snoRNAs) can affect ribosome function. She will help Dr. Noronha study the role of ERα RNA binding activity in DTP cell formation and its impact on the proteome when exposed to hormone therapy inhibitors. She will also explore the involvement of calcium signaling pathways in DTP cells and ERα's regulation of critical pathways linked to cancer cell growth. Additionally, she will investigate the interaction between ERα and eIF4A, a key translation factor, and assess the therapeutic implications of targeting this interaction with a combinatorial therapy in breast cancer models. The goal is to disrupt DTP cell survival and hinder tumor progression.

Following changes Requested for Year 02 (Additional Term) –

BUDGET JUSTIFICATION

Key Personnel:

Dr. D. Ruggero, PhD. - Principal Investigator - (Effort: 0.6 calendar months (5%) – No salary and fringe benefits request. Salary and fringe benefits covered by UCSF funds to allow additional funds for the budget in year 02-

Dr. Ruggero will be responsible for the overall administration and guidance of the proposed research. He has more than 20 years of experience in the fields of ribosome biogenesis and regulation of protein synthesis and has been a pioneer at the forefront of translational control and cancer. His lab has published seminal studies that have significantly advanced our understanding of how defects in translational control lead to cancer development. These include the genetic dissection of translation control downstream the oncogenic mTOR pathway in cancer and its therapeutic implications, which was the cover story of the March 2010 Cancer Cell (Hsieh et al, Cancer Cell 2010), the characterization of the translational landscape of mTOR signaling in steering prostate cancer initiation and metastasis (Hsieh et al, Nature 2012), and the identification of a novel sequence-specific cis-regulatory translation element controlled by mTOR, contained within the 5'UTRs of mRNAs encoding pro-tumorigenic factors (Hsieh et al, Nature 2012). His lab has defined the translational program of the mammalian cell cycle (Stumpf et al, Molecular Cell 2013) and the role of dysregulated eIF4E-dependent translational control in autism (Gkogkas et al, Nature 2013). His lab was the first to genetically prove that an increase in Myc protein synthesis is a key determinant of Myc oncogenic activity (Barna et al, Nature 2008). Dr. Ruggero's recent groundbreaking work has opened an entirely new avenue of study, which has exploited Myc's addiction to enhanced protein synthesis to elucidate an entire network of synthetic lethal interactions. This study is founded upon his identification of a feed-forward metabolic circuit linking protein synthesis with nucleotide metabolism (Cunningham et al, Cell 2014) as well as with the Unfolded Protein Response (Hart et al, Journal of Clinical Investigations 2012), and the discovery that Myc activity converges on translational control via mTOR-directed eIF4E hyperactivation (Pourdehnad et al, Proceedings of the National Academy of Sciences 2013). Additionally, he was the first to demonstrate that the major cap-binding protein eIF4E acts as an oncogene in vivo (Ruggero et al, Nature Medicine 2004) and that eIF4E loss of function is dispensable for normal development (Truitt et al, Cell 2015). Furthermore, his lab has made trendemous progress in unraveling the role of the ribosomal RNA modifications in cancer initiation (Ruggero et al, Science 2003; Bellodi et al, Cancer Research 2010) and how mutations in the enzyme responsible for pseudouridine modifications within the ribosome affect translational control leading to cancer and human disease (Bellodi et al, Cell Reports 2013; Jack et al, Molecular Cell 2011; Bellodi et al, EMBO J 2010; Yoon et al, Science 2006; McMahon et al, eLife 2019). He will be directly responsible for the training and management of personnel engaged in achieving all of the specific aims outlined in the research proposal. He will review the primary data, guide the overall direction of the research, assist with trouble-shooting and alternative approaches, and coordinate with the collaborators. He will coordinate the preparation, submission, and publication of abstracts and manuscripts reporting the results of the project.

Other Personnel:

Macey Slota, BS – Graduate Student – (EFFORT:6.0 calendar months (25%) in all years – salary and fringe benefits requested in all years) – Ms. Slota earned her B.S. in Biochemistry and Molecular Biology, Magna Cum Laude from Belmont University, Nashville, TN in 2021. She has extensive experience in cancer biology and RNA biology and has years of research in gene expression in the context of cancer. She is a co-author of two major publications on the role of Myc and tumorigenesis. As a Ph.D. student in my lab, Ms. Slota has been very productive and has been leading the intellectual aspects of the project. She has received wide training in the lab in many technical and conceptual features of post-transcriptional regulation in cancer biology. She will collaborate with Kenya and Saishma.

Kenya Bonitto, BS – Graduate Student – (EFFORT:6.0 calendar months (25 %) in all years – salary and fringe benefits requested in all years) – Ms. Bonitto earned her B.S. in Molecular, Cell, and Developmental Biology from UCLA. She has extensive experience in RNA biology and has years of research in gene expression at the post-transcriptional level and regulation in the context of cellular homeostasis, such as cellular quiescence, one of the key hallmarks of cancer cells. As a Ph.D. student in my lab, Ms. Bonitto has been very productive, leading the intellectual aspects of many projects. She has been receiving wide training in the lab in many technical, and conceptual features of cancer biology. She will collaborate with Macey and Saishma.

Saishma Hoigebazar - Junior Specialist - (Effort: 4.56 calendar months (38%) - Salary and fringe benefits requested in all years) – Saishma Hoigebazar is replacing Emma Figueredo in this budget. Saishma completed her Bachelor of Science in Biochemistry, Chemistry, and Zoology from St. Aloysius College in 2021. She received her Master of Science in Biochemistry and Molecular Biology in 2023 from Pondicherry University in India. As a junior specialist within the Ruggero lab, she is assisting Dr. Noronha in Aims 1 and 2. She has learned and implemented many techniques in the process of working in the lab, including western blots, RNA extraction and qPCR, recombinant protein expression in bacterial systems, and general tissue culture maintenance.

CONSULTANT: N/A.

SUPPLIES & MATERIALS: Funds in the total amount of $31,439 ($12,576/first year, $10,237/second year, and $8,626/third year). This amount includes molecular biology reagents (including Mass Spec analyses, mass spectrometry, cloning experiments, reporter assays, western blotting, qPT-PCR analysis, and validation of RNA structure analysis) and histology reagents for mouse studies (including the purchase of fixative, embedding materials for frozen sections, slides, slide boxes, primary antibodies for immunofluorescence staining, secondary antibodies, and mounting medium, among others), disposable lab supplies and chemical reagents.

ANIMAL COST AND HOUSING: Funds in the amount of $20,000/year are requested for cage numbers, breed maintaining as explained in the proposal and the purchase of mice.

TRAVEL: Funds in the amount of $1,500 in the second and third year for a total of $3,000 are requested for costs associated with travel for PIs to attend meetings and conferences. This includes airfare, boarding, lodging and registration fees, if any.

OTHER DIRECT COSTS: Data Network Recharge The data network services recharge or data network recharge is a vital component of the University's Enterprise Network Services (ENS), which provides funding for critical equipment in support of UCSF's electronic information flow. Per agreement signed January 24, 2008 with The Department of Health & Human Services (DHHS), Division of Cost Allocation, (cognizant agency to the University), the University does not disclose ENS costs as F&A pool costs and can charge for ENS costs as direct costs.

This cost must adhere to the UCSF Costing Guidelines for the Allowability of Computing Device Support Recharges on Sponsored Project Awards and are permissible under our approved Cost Accounting Standards Board Disclosure Statement (CASB Form DS-2) ensuring that there is no duplicate reimbursement from Federal and non-Federal sponsors. The data network recharge is based on the current rate per FTE, consistent with the current billing rates for the data network recharge. Recharge rates are computed in accordance with the requirements of 2 CFR Part 200 and will be reviewed and adjusted annually.

Calculations are based on the percent effort to be charged to the project for each person named in the grant. The data network recharge rate is implemented and effective as of **(February 2, 2024 of current Budget and Planning Assumption),** and will be approximately **($40 from current Budget and Planning Assumption)** per month, pro-rated per FTE with yearly fluctuation based on actual costs of this service.

Future year rates:

7/1/24 - 6/30/25: $41/month/FTE
7/1/25 - 6/30/26: $42/month/FTE
7/1/26 - 6/30/27: $44/month/FTE
7/1/27 - 6/30/28: $45/month/FTE
7/1/28 - 6/30/29: $46/month/FTE

Total amount of $1,626 has been requested during the course of the study.

IT Field Services (ITFS) Desktop Support, formerly Computing and Communication Device

Support Services (CCDSS) On July 1, 2013, the University initiated direct charging for ITFS as an integral component of its Enterprise Network Services (ENS) to provide support to campus voice and data technology functions. ITFS includes software installation/updates, internet security, hardware setup/ configuration, and centrally managed patching, storage and backup. Direct charging of these support services in conjunction with the Data Network Recharge are covered under Part 3.2.0 – Service Centers, Communication Services, of the University's Cost Accounting Standards Board Disclosure Statement (CASB Form DS-2) ensuring that there is no duplicate reimbursement from Federal and non-Federal sponsors. The ITFS charge is based on the current rate per FTE, consistent with the current billing rates for ITFS. Recharge rates are computed in accordance with the requirements of 2 CFR Part 200 and will be reviewed and adjusted annually.

Calculations are based on the percent effort to be charged to the project for each person named in the grant. The ITFS recharge rate is implemented and effective as of February '24), and will be approximately
$61/- and #102/- (current rate from current Budget and Planning Assumption referred to as ITFS) per month, pro-rated per FTE with yearly fluctuation based on actual costs of this service.

Future year rates:

Basic	Premium
7/1/24 - 6/30/25: $63/month/FTE	7/1/24 - 6/30/25: $105/month/FTE
7/1/25 - 6/30/26: $65/month/FTE	7/1/25 - 6/30/26: $108/month/FTE
7/1/26 - 6/30/27: $67/month/FTE	7/1/26 - 6/30/27: $111/month/FTE
7/1/27 - 6/30/28: $69/month/FTE	7/1/27 - 6/30/28: $115/month/FTE
7/1/28 - 6/30/29: $71/month/FTE	7/1/28 - 6/30/29: $118/month/FTE

A total amount of $792 has been requested during the course of the study.

General, Auto, and Employment Liability (GAEL) insurance assessments:
We are requesting $2,460 for General, Automobile, & Employee Liability (GAEL) expenses. GAEL funds the University's self-insurance program which provides broad coverage for the University's legal or "tort" liability which arises from University activities. Divided into three areas: General Liability, Auto Liability, and Employment Practices Liability, GAEL provides protection of mistakes (negligence) by UCSF employees resulting in personal or bodily injury or damage to third parties. Coverage is applicable worldwide. The GAEL insurance assessment is calculated by applying the current GAEL rate x $100 of salaries budgeted.

GAEL rates:

7/1/24 - 6/30/25:	$0.91
7/1/25 - 6/30/26:	$0.93
7/1/26 - 6/30/27:	$0.95
7/1/27 - 6/30/28:	$0.95
7/1/27 - 6/30/28:	$0.95