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TITLE: Precision Targeting of Castration Resistant Prostate Cancer with a Novel Ferrous Iron-Dependent Therapeutic Delivery and Tumor Imaging Strategy

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14. ABSTRACT

Background Iron is an essential nutrient in biology where, serving in cofactors of diverse enzymes, it enables one-electron oxidation and reduction processes. Access to iron is thus a key driver of proliferative disease and the oncogene MYC has been found to alter iron homeostasis so as to produce an augmented pool of intracellular Fe(II). We recently developed a new drug delivery approach inspired by the Fe(II)-dependent pharmacology of the antimalarial agents artemisinins and arterolane. Our preliminary in vitro and in vivo data suggest that prostate cancer is highly amenable to therapy and imaging using Fe(II)-sensitive reagents. Targeting soluble ferrous iron represents an entirely novel approach to image and treat prostate cancer.

15. SUBJECT TERMS

None Listed

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1. INTRODUCTION:

We propose that mechanistically unrelated anti-cancer therapeutics can be more effectively deployed by administration in a pro-drug form that conditionally releases the therapeutic after chemical reaction with Fe(II), pools of which are augmented in CRPC cells and in the tumor microenvironment. To test and validate our hypothesis we will synthesize and evaluate in multiple prostate cancer models three novel agents, the Fe(II)-activated form of a potent DNA-alkylator (TRX-CBI), the Fe(II)-activated form of enzalutamide (TRX-ENZ), and a novel Fe(II)-targeted therapeutic radionuclide, ^{117}Lu-TRX. We will also image prostate cancer in diverse animal models using an Fe(II)-activated PET probe (^{18}F-TRX). Our objective is to show that castration resistant prostate cancer can be addressed effectively with these novel Fe(II)-targeted approach and that response to therapy can be predicted with ^{18}F-TRX. Successful realization of these objectives via the IDA mechanism will greatly enable our long-term goal of identifying a "theranostic" development candidate that can be progressed toward first-in-human studies.

2. KEYWORDS:

Molecular imaging, cancer theranostics, pro-drug, iron metabolism, fluorine-18, lutetium-177, pharmacology, castration resistant prostate cancer, antiandrogen

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goal for project period two is listed below with the subtasks stipulated in the approved statement of work. In parenthesis, we have indicated the status of each subtask as "completed", "ongoing", or no annotation if the subtask has not yet begun.

Major Task 2: Therapy studies with TRX-CBI				
Subtask 1: 28 day treatment study with TRX-CBI in mice bearing LNCaP-AR xenografts **(ONGOING)**	10-14	Dr. Renslo	Dr. Evans (96 mice)	
Subtask 2: 28 day treatment study with TRX-CBI in mice bearing orthotopic PC3 tumors **(ONGOING)**	12-16	Dr. Renslo	Dr. Evans (48 mice)	
Subtask 3: 28 day treatment study with TRX-CBI in genetically engineered mice **(ONGOING)**	14-18	Dr. Renslo	Dr. Evans	Dr. Ruggero (12 mice)
Milestone(s) Achieved: Determining the anti-tumor activity of TRX-CBI in prostate cancer models	18	Dr. Renslo	Dr. Evans	Dr. Ruggero
Specific Aim 2				
Major Task 1: Synthesis and evaluation of ^{177}Lu-TRX				
Subtask 1: Synthesis of ^{177}Lu-TRX	18	Dr. Renslo	Dr. Evans	
Subtask 2: Determination of the maximum tolerated dose of ^{177}Lu-	19-		Dr.	

TRX	21		Evans (15 mice)	
Subtask 3: Dosimetry calculation for ^{177}Lu-TRX	21-22		Dr. Evans (30 mice)	
Subtask 4: 60 day therapy study with ^{177}Lu-TRX in mice bearing PC3 xenografts	22-28	Dr. Renslo	Dr. Evans (50 mice)	
Milestone(s) Achieved: Determination of the antitumor activity of ^{177}Lu-TRX	28	Dr. Renslo	Dr. Evans	
Major Task 2: Synthesis and evaluation of TRX-ENZ				
Subtask 1: Synthesis of TRX-enzalutamide **(COMPLETED)**	28	Dr. Renslo		
Subtask 2: Antiproliferation studies in vitro with TRX-ENZ in prostate cancer cell lines: LNCaP-AR, LAPC4, VCaP, PC3 VCaP: ATCC **(ONGOING)**	29	Dr. Renslo		
Subtask 3: Pharmacokinetic studies in mice with TRX-ENZ **(COMPLETED)**	30-32	Dr. Renslo	Dr. Evans (72 mice)	
Subtask 4: 28 day treatment study with TRX-ENZ in mice bearing LNCaP-AR xenografts **(ONGOING)**	32-36	Dr. Renslo	Dr. Evans (91 mice)	
Milestone(s) Achieved: Determination of the antitumor activity of TRX-ENZ	36	Dr. Renslo	Dr. Evans	

What was accomplished under these goals?

1) Major activities:

The primary focus of the second project period was to perform antitumor assessment studies with TRX-CBI and synthesize and evaluate the in vivo pharmacology of ^{177}Lu-TRX. Due to COVID-19 and restrictions on type of research we could perform as well as the number of staff allowed to work onsite, we found it challenging to perform large scale animal studies as well as radioactive research. Thus, we opted to focus our efforts on the research in project period three, which was easier for us to achieve with COVID restrictions in place. The goal of project period 3 was to synthesize and evaluate the pharmacology of an Fe(II) activated antiandrogen with a structure like enzalutamide.

2) Specific Objectives:

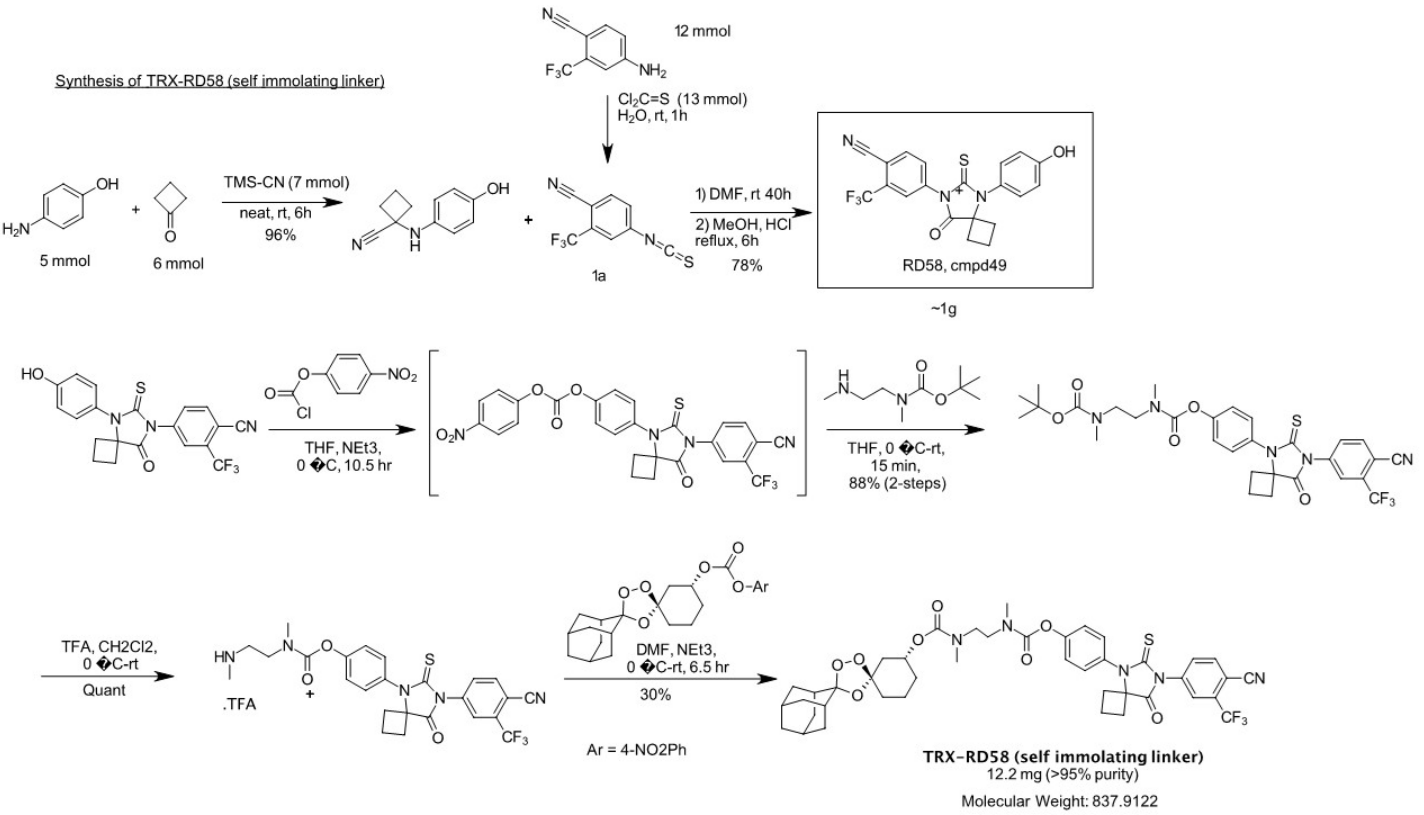
There were several objectives for this project period. The first objective was to synthesize TRX-ENZ, a Fe(II) activated antiandrogen. The second objective was to assess its antitumor effects in vitro. The third objective was to study the pharmacokinetics of the compound in mice. The final objective was to perform antitumor assessment studies with TRX-ENZ in mice.

3) Significant results or key outcomes:

Synthesis of TRX-ENZ:

Because of COVID 19 personnel restrictions, our chemists collaborated with the Renslo laboratory to complete the synthesis of the TRX-ENZ molecule (**Figure 1**). The antiandrogen payload was inspired by the medicinal chemistry published by Jung and Sawyers, as well as the chemical necessity to have a phenolic moiety on the antiandrogen payload to enable coupling to the TRX "caging" motif. The synthesis of the pro-drug is outlined in the figure below and a full description of the rationale for its design and synthetic details is provided in Dr. Renslo's progress report.

Figure 1. A schematic of the synthesis of the Fe(II) activated, prodrug form of an antiandrogen (RD58) sharing structural similarity to apalutamide and enzalutamide.



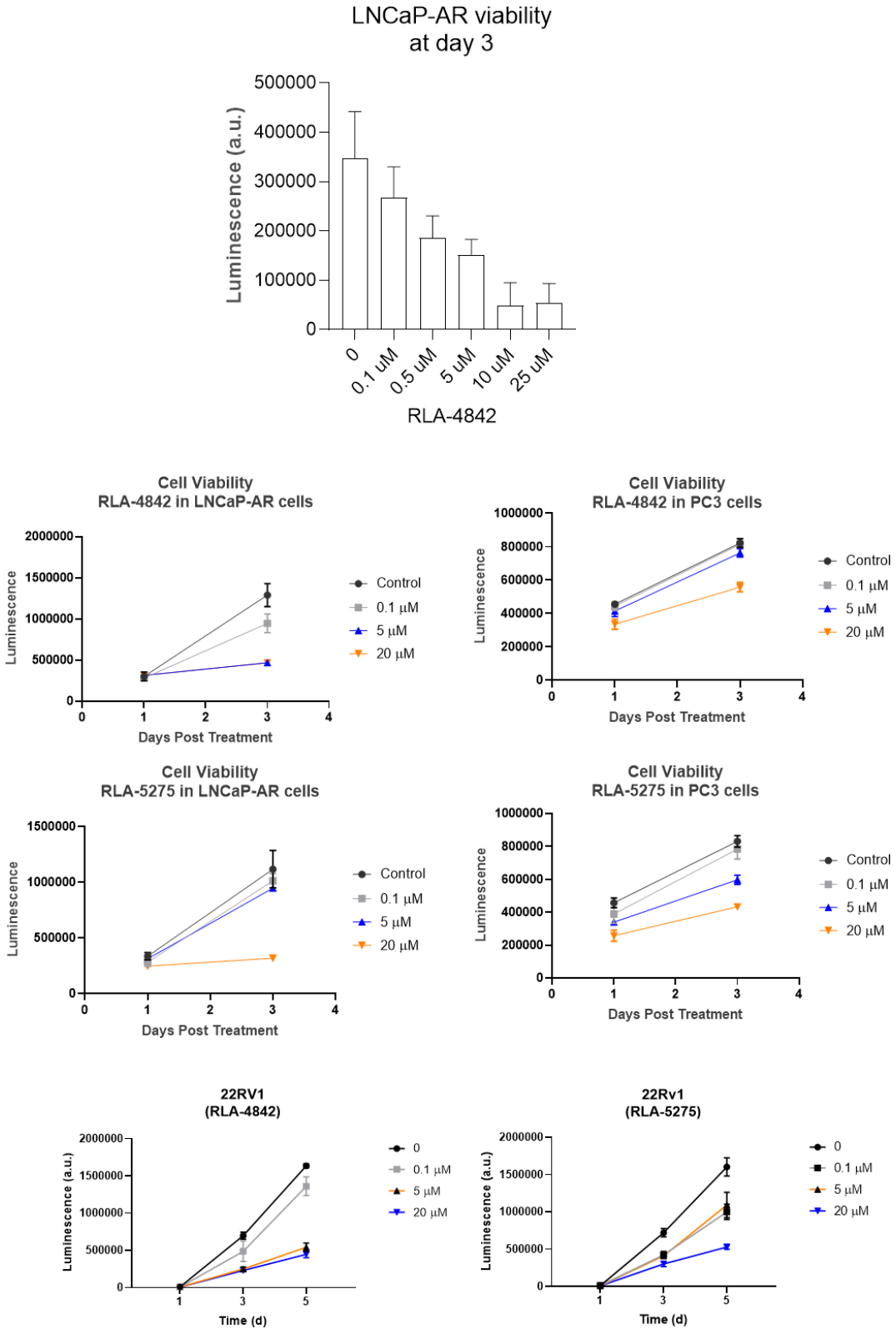
In vitro antiproliferations studies with TRX-ENZ:

We first performed a dose response study in LNCaP-AR cells to determine the concentrations that should be used to profile antiproliferative effects in a larger panel of cell lines (**Figure 2**). We found that TRX-ENZ was active in the low μM range over 3-5 days. Using concentrations of 0.1, 5, and 20 μM, we subsequently profiled the antiproliferative activity of TRX-ENZ in numerous androgen receptor positive human and mouse prostate cancer cell line models. As controls, we treated cells with the uncaged antiandrogen or vehicle. In both LNCaP-AR, 22Rv1, CWR22Pc, and TRAMPC2 cells, we found that TRX-ENZ potently inhibited proliferation at 5 μM compared to vehicle. Remarkably, we also observed that equimolar doses of TRX-ENZ were more

potent inhibitors of proliferation compared to the uncaged antiandrogen. We do not yet understand the mechanistic basis for this effect, but we hypothesize that inhibition of the androgen receptor may actually create a “feed forward” loop by augmenting the labile iron pool within the prostate cancer cell due to transactivation of mTORC1 (transactivation of mTORC1 by androgen receptor inhibition is a phenomenon reported by several labs including Charles Sawyers at MSKCC). We are currently exploring this hypothesis by applying chemical sensors like Ferrofarred to measure treatment induced changes in labile iron pool levels in prostate cancer cells. Moreover, we are continuing to perform mechanism studies like rt-PCR to establish that the pro-drug effectively inhibits androgen receptor transcriptional activity. We will also attempt to parse the role for mTORC1 signaling in cancer cell responsiveness to the pro-drug by co-administering drugs meant to suppress or stimulate mTORC1 signaling.

Another important finding from this project period was that TRX “caging” of the antiandrogen payload attenuates nonspecific toxicity associated with the free antiandrogen. Indeed, this is a well known shortcoming associated with diarylthiohydantoins like enzalutamide and apalutamide, leading to undesirable side effects like neurotoxicity in humans. By treating androgen receptor null PC3 or DU145 cells, we showed that the prodrug had significantly less toxicity than the uncaged antiandrogen. These data foreshadow that higher doses of the prodrug antiandrogen may be tolerated in mice compared to the free antiandrogen, a hypothesis that we will test systematically during year 3 of the project period as COVID restrictions lessen.

Figure 2. In vitro antiproliferative data showing that the TRX-ENZ adduct (termed RLA-4842) as antiproliferative activity against LNCaP-AR and 22Rv1. Moreover, the prodrug appears to be more potent than equimolar doses of the free antiandrogen (termed RLA-5275). Lastly, the TRX "caging" appears to shield AR negative PC3 tumor cells from non-specific toxicity.



4. Other achievements:

We have begun the synthesis of a TRX analogue functionalized with a chelator for radiolabeling with lutetium-177. We plan to conduct labeling and biodistribution studies in preparation for antitumor assessment studies. We have disclosed our findings as one peer reviewed publication and two conference abstracts (see products).

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

We presented out findings at the virtual Society of Nuclear Medicine and Molecular Imaging meeting, and we currently have a manuscript under second revision at the Journal of Nuclear Medicine.

What do you plan to do during the next reporting period to accomplish the goals?

We will revisit the synthesis of ^{177}Lu-TRX and the animal experiments proposed in aims 1 and 2 that we were unable to complete because of COVID 19 restrictions. Specifically, we will continue to establish the mouse prostate cancer models and conduct imaging and biodistribution experiments with ^{18}F-TRX. We will aim to report these studies in a follow-up manuscript to the first publication, which was primarily meant to establish proof-of-concept that Fe(II) can be imaged with PET in normal and a few select cancer models.

We will also begin testing the antitumor efficacy of TRX-CBI and the TRX-ENZ adduct in prostate cancer animal models. We also have all of the cell line models required to establish the animal models.

Lastly, we have begun the synthesis of a TRX conjugate for radiolabeling with Lu-177. We anticipate beginning pilot labeling studies during this project period, and preliminary mouse biodistribution studies prior to antitumor assessment studies.

4. IMPACT:

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

We have shown for the first time that prostate cancer can be effectively treated using a Fe(II) activated prodrug strategy. Achieving this goal was enabled by new chemistry developed by the Renslo laboratory to more efficiently release payloads after reaction with intracellular Fe(II). Moreover, we have shown that TRX caging shields androgen receptor negative cells from nonspecific toxicity, which may increase the tolerated dose of drug to delivery more durable antitumor responses. We are now completing the pharmacokinetic assessment of the TRX-ENZ adduct and will commence animal studies imminently.

What was the impact on other disciplines?

Our interest in developing new anti-cancer diagnostics and therapeutics by targeting the labile iron pool parallels other imaging and medicinal chemistry efforts targeting LIP in non-malignant disorders. Other animal imaging approaches have relied on low resolution imaging modalities like bioluminescence, and some strategies require exotic genetic engineering of mice to express reporter proteins like luciferase. Our nuclear imaging strategy is much higher resolution, absolutely rather than semi-quantitative, and does not require any special genetic manipulation of mice. In this respect, our technology is poised to sensibly complement the ongoing imaging efforts in preclinical animal models of infectious disease by groups like Dr. Chris Chang's laboratory at UC Berkeley. The therapies that we are developing could potentially be applied to non-malignant neoplastic disorders, particularly those driven by mTORC1 or RAS. These include maladies like tuberous sclerosis complex and lymphangioleiomyomatosis, and RASopathies like neurofibromatosis.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

As noted above, we changed the order in which we performed the experiments outlined in the statement of work, as large scale animal studies and studies involving radiochemistry with short lived radioisotopes are very challenging to execute with only one person onsite due to COVID restrictions. Thus, we chose to prioritize the cold chemistry and in vitro studies during this project period and will revisit the remaining imaging, radioligand therapy and antitumor assessment studies during project period 3. As of October 1, 2020, UCSF is currently at 50% capacity, and mice and radioisotopes can be ordered without restrictions or special justification.

Actual or anticipated problems or delays and actions or plans to resolve them

The progress was of course impacted by the COVID 19 pandemic, although we feel that we adapted well and accomplished as much as could be reasonably achieved under suboptimal circumstances.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

Nothing to report. This project is still approved by UCSF IACUC. We can now order animals without restrictions.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Measuring Dynamic Changes in the Labile Iron Pool in Vivo with a Reactivity-Based Probe for Positron Emission Tomography. ACS Cent Sci. 2019 Apr 24; 5(4):727-736.Muir RK, Zhao N, Wei J, Wang YH,

Moroz A, Huang Y, Chen YC, Sriram R, Kurhanewicz J, Ruggero D, Renslo AR, Evans MJ. PMID: 31041393.

Ning Zhao, Yangjie Huang, Yung-hua Wang, Ryan K. Muir, Ying-Chu Chen, Junnian Wei, Pavithra Viswanath, Youngho Seo, Davide Ruggero, Adam R. Renslo, Michael J. Evans "Ferronostics: Measuring Tumoral Ferrous Iron with PET to Predict Sensitivity to Iron-Targeted Cancer Therapies" Journal of Nuclear Medicine, under revision.

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers and presentations.

Measuring dynamic changes in the labile iron pool with a reactivity-based probe for positron emission tomography. Poster abstract at the 2020 Society of Nuclear Medicine and Molecular Imaging Annual Meeting

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or licenses**

  Talukdar, P., Renslo, A.R., Blank, B.R., Muir, R.K., Evans, M.J. Trioxolane agents PCT/US2018/039768, published 03/2019

- **Other Products**

  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Michael Evans, PI – 2.04 calendar months

- Contributions – Dr. Evans supervised the radiochemistry and the animal imaging and biodistribution studies. He also worked with Dr. Chen to analyze and summarize the data for the manuscript submission.

Zhuo Chen, Post-doc – 11.15 calendar months

- Contributions – Dr. Chen performed cell studies to characterize new therapies.

Yangjie Huang, Post-doc – 5.17 calendar months

- Contributions – Dr. Huang performed synthetic organic chemistry to make TRX-ENZ with the Renslo lab.