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The emphasis of this first year of the award, as planned, has been on synthetic chemistry to obtain materials to test in histology, PET (positron emission tomography) and PDT (photodynamic therapy) studies. We have been successful in preparing samples for the testing studies that begin in year 2. However, as anticipated, the synthetic chemistry work was not without problems and must continue and adapt to overcome challenges that now become evident. For instance, one of the molecules first prioritized, compound 1, was prepared, but only after a great deal of effort; in retrospect it is now clear that this compound has stability issues that make it hard to make, and inappropriate for further studies. Another target compound (2) was then prepared, much more efficiently than the first because it does not have stability issues, and because of the experience we gained from making the first target. This compound has poor solubility characteristics despite the fact that it contains two sulfonic acid groups and may required delivery in micelles; this is something that could not have been predicted until the compound was made. Both structures 1 and 2 are based on the aza-BODIPY dye fragment; as a back-up we have also initiated work on a compound based on a different-dye type, eg compound 3. The original proposal outlined plans to add cytotoxic entities other than PDT agents; for this we entered into a collaboration with a biotechnology company who have provided us a small sample of the previous, highly cytotoxic, compound maytensin A. We have also prepared an agent intended solely for PET, ie compound 4; this takes advantage of very recent advances in the field that enable more efficient capture of 18F- than was possible before, via so-called "Perrin capture agents.

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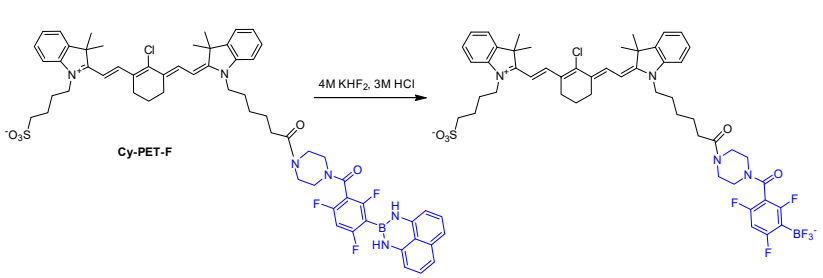
# Theranostics Targeting Metastatic Breast Cancer

The years 3 report thoroughly summarized all results in year 2 and year 3 with all sections such as “Introduction”, “Accomplishments”, “training and professional development” and “impact”. All information included in years 3 report will not be included here. This report summarizes the studies in the period of no cost extension that our team worked on the imaging agent design/synthesis/radiolabeling and in vivo imaging study using primary and metastatic breast cancer models.

## Molecular imaging agent design based on the tumor seeking dye

Our hypothesis is that the tumor seeking near-IR dyes complement targeted approaches using mAbs or other small molecule ligands for cell surface receptors, and could surpass them for some applications. Following the year 3 study, we designed and radiolabeled tumor seeking dye-based radiotracers for PET imaging of breast cancer and to test their capacity for active drug deliver. Compound for $^{18}$F tumor targeting cyanine dye-based tracer (Cy-PET-F) was designed and synthesized in our lab. In vivo optical imaging was performed to study the PK of the compound using orthotopic 4T1 breast cancer model. From the optical imaging, the tumor seeking dye had fairly quick clearance at 24h post injection but high liver and kidney uptake was observed at early time point (Fig. 1). This result suggested $^{18}$F PET will not be suitable for PET imaging of Cy compound because F-18 has short half-life (F-18; $T_{1/2}$ =108 mins). $^{64}$Cu is selected for the following PET study.



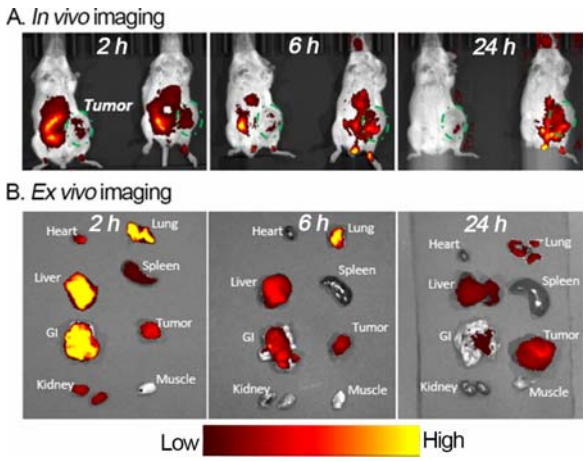
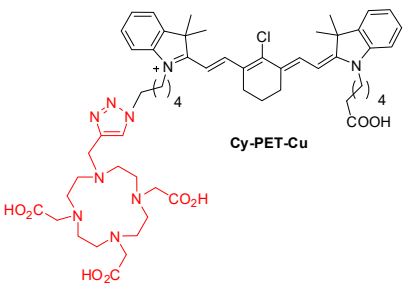




Fig 1. Optical imaging of Cy-PET-F compound on 4T1 tumor bearing Balb/c mice. (A) In vivo imaging was captured at 2h, 6h and 24h post injection of Cy-PET-F via tail vein. (B) Correspondingly, biodistribution study was performed by ex vivo imaging of major organs after each time point of in vivo imaging.

Optical imaging showed good tumor to background ratio for tumor seeking dye at 24h post injection. PET Isotope $^{64}$Cu ($T_{1/2}$ =12.7 h) was selected for the following PET study because of the matched PK of the tumor seeking dye. Chelator DOTA was conjugated to tumor seeking dye (Cy) to get compound Cy-PET-Cu for the Cu-64 radiolabeling. PET imaging of Cy-PET-$^{64}$Cu tracer showed good tumor uptake at 24h post injection in the 4T1 breast tumors (Fig 2), which demonstrated Cy analog can be used as active targeting agent for breast cancer and also can use for active targeted drug delivery.



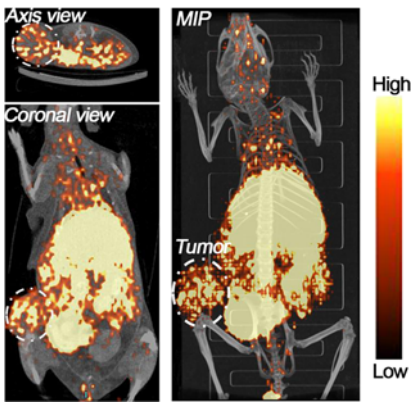




Fig 2. Small animal PET/CT in vivo imaging of Cy-PET-$^{64}$Cu compound in 4T1 tumor bearing mice with axis, coronal view and its maximal intensity projection (MIP) view at 24 h post injection.

## Kinase Inhibitor Cyanine Conjugates, Cy-KIs

Cyclin dependent kinases (CDKs) are critical for cell cycle regulation. Cell cycle arrest in cancer could suppress tumor growth and metastasis, so inhibition of the CDKs is an active research area. The first inhibitors had little or no selectivity between the various members of the series, and they were relatively unsuccessful in pharmaceutical development. Emergence of the first *selective* CDK4/6 inhibitors, notably palbociclib, however, has completely changed the research landscape.[57-59] Thus a second selective CDK inhibitor, ribociclib (**rib**, KISQALI®), was approved in 2017 for treatment of the same cancer sub-type. As of today, palbociclib and ribociclib feature in at least 20 clinical trials for treatment of cancers of breast metastases, non-small cell lung, prostate, pancreas and brain (glioblastoma).We conjugated palbociclib and ribociclib to **1**-Cl and showed their good anti-tumor effects on MDA-MB-231, "triple negative" breast cancer cells. In the no cost extension period, we continued to evaluate the **1-rib** in vivo using a breast cancer brain metastasis model. We showed 1-rib displayed high tumor accumulation and prolonged retention with low uptake in normal organs. These data confirm 1-rib accumulates selectively in breast cancer brain mats and favorable PK of 1-rib compound (Fig 3).

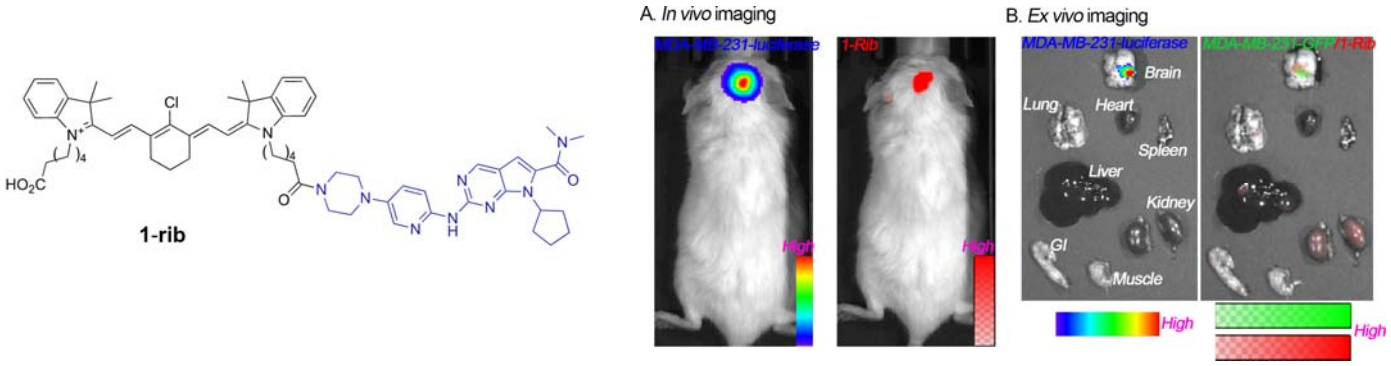



Fig. 3. Optical imaging of 1-Rib compound on MDA-MB-231 brain metastasis bearing NSG mice. The MDA-MB-231 was genetically engineered with luciferase and green fluorescence protein (GFP), and then intracranially implanted into the brain of the mouse to impart a breast tumor metastasis in brain model. (A) In vivo imaging of the 1-Rib was performed by sequential bioluminescence and 1-Rib (@ its emission and excitation) imaging of the tumor bearing mice after 24h injection of the 1-Rib compound. (B) Afterwards, the mice were sacrificed, and the major organs were collected to perform sequential bioluminescence, GFP (@ its emission and excitation) and 1-Rib (@ its emission and excitation) imaging.