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Dear Application Review Subcommittee members,

We thank the CIRM team for recommending our grant for funding based on the strengths of the proposal and programmatic considerations. The goal of this proposal is to advance a small molecule inhibitor that targets breast cancer stem cells in Triple Negative Breast Cancer (TNBC). Chemotherapy and newer targeted therapies have been useful for treating patients with metastatic TNBC, they are limited by the emergence of treatment-resistant cancer cells. Thus, most patients with metastatic disease either relapse or fail to respond to standard treatment of TNBC, including chemotherapy or immunotherapy, despite millions of dollars spent on clinical trials for TNBC. **It is clear there is no cure to permanently eradicate metastasis once it has occurred**. There is a large body of evidence that breast cancer cells expressing stem cell pathway genes, often called cancer stem cells, are resistant to present therapies including chemotherapy, radiation, targeted therapies and even immunotherapies. However, it has been difficult to develop therapeutic agents that effectively target the cancer stem cells. Our studies have identified CDK19 as a potential cancer stem cell therapeutic target that spares normal stem cells. These cells are present in metastatic breast tumors. Hence, we hypothesize that if CDK19, a cancer stem cell target, can be inhibited, breast cancer metastasis can be eliminated or slowed.

There was consensus among the reviewers that the project holds the necessary significance and potential impact, has sound rationale, is well planned, feasible, and upholds the principles of diversity, equity and inclusion. Some of the referees raised some concerns. However, most of these concerns were actually addressed in the proposal. A couple were not included because of space limitations. In general, there were 4 issues raised by some referees. 1) Our CDK19-selective inhibitors should be compared to published dual inhibitors for efficacy. 2) The candidate drug should be assayed for the ability to kill breast cancer stem cells. 3) A subset of reviewers wished us to test whether the inhibitors activate an immune response against TNBC stem cells. 4) A subset of referees requested more detailed preclinical pharmacology.

As described below, none of these concerns are valid.

1) Our CDK19-selective inhibitors should be compared to published dual inhibitors for efficacy.

Due to limitation of space, these comparisons were not included in our proposal. We have compared our current STF-2410 (CDK19-selective inhibitor) against multiple published dual CDK8/19 inhibitors. Our selective inhibitor is significantly more effective than published dual inhibitors. Although our CDK19-specific inhibitors appear to have the best efficacy, as we generate more compounds, we will test both CDK19-specific and CDK19/CDK8 dual inhibitors for efficacy, and any that have excellent anti-breast cancer stem cell activity will be tested for toxicity.

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2) The candidate drug should be assayed for the ability to kill breast cancer stem cells.

As stated in our proposal, such assays will be done. As described in **Assay 7** of our proposal, "*For each drug, 10 different mice will be treated. Tumor growth and survival will be measured and in case of residual tumor cells in any treatment groups, the number of remaining cancer stem cells capable of proliferation in vitro and in vivo will be determined*." Our lab routinely profiles treated breast PDX tumors by flow cytometry, and perform *in vitro* and *in vivo* limiting dilution assays, in organoid assays and in tumorigenicity assays in mice, and scRNA-seq, respectively, to determine frequency of cancer stem cells. In fact, as noted by the reviewers our lab pioneered all of those assays. Due to the limitation of space, we were unable to give a detailed description of how we would determine and verify the elimination of breast cancer stem cells. However, our description of how CDK19 was discovered to be a breast cancer stem cell target utilized all of the assays that the referee(s) want us to do. Indeed, the reviewers agreed that we had proven CDK19 to be a breast cancer stem cell target. Our statement for **Assay 7** was meant to say that we would do the requested assays, we apologize that because of space limitations we did not make this clearer.

3) A subset of reviewers wished us to test whether the inhibitors activate an immune response against TNBC stem cells.

We agree that such studies are important. But they are not critical for advancing a CDK19 inhibitor to the clinic. Our data shows that CDK19 inhibition kills cells through a cell-intrinsic mechanism independent of the microenvironment or immune system. Other people's data shows that CDK19/CDK8 inhibition activates anti-tumor T-cells, including CAR T-cell therapies. We will test our final candidate drugs for immune modification activities. Again, even if they do not have such activity our data still supports advancing them to the clinic.

However, the published data regarding immune modulation by CDK19/CDK8 inhibition gives another potential avenue for use of our inhibitor to enter the clinic for the treatment other cancers that respond to immunologic therapies, or for improving CAR T-cell therapeutics. We think that this should make this project more attractive to CIRM, since it increases the chances for the drug being approved by the FDA as a human therapeutic.

4) A subset of referees requested more detailed preclinical pharmacology "***Addressing vital factors such as administration route, dosage, and potential toxicities will contribute to a more comprehensive plan***."

We agree that administration route, dosage, and toxicity are all important, as well as a myriad of other factors such as formulation, blood-brain penetration, drug metabolism, unfavorable off target interactions such as hERG inhibition, Cytochrome p450 metabolism, interactions with renal and hepatic transporters, etc. are all critical for identifying a clinical candidate to submit to the FDA for a IND. Both PI's, Drs. Clarke and Gray, have experience in advancing drugs from IND-enabling preclinical studies, including efficacy and toxicity studies, into the clinic. Space limitations preclude anything except outlining the steps needed for an IND application.

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Thus, all the concerns of a subset of the reviewers are easily addressed. Bringing a drug targeting TNBC stem cells to the clinic is a major, unmet clinical need. Delaying funding of this project for a year is unwarranted and could significantly delay advancing such a treatment into the clinic. We hope that the ARS supports the recommendation of the CIRM team and approves this grant.

Sincerely yours,

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