SPECIFIC AIMS

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is a highly accessible protein located on the surface of cancer cells that acts as a control switch for immune suppression and metastasis. It allows cancer cells to thrive in an unfavorable inflammatory environment by intercepting the warning signals of tumor formation before they can reach nearby immune cells. Essentially, when chromosomally unstable tumors release DNA fragments into the cytosol, they are bound by the enzyme cGAS, that in turn, catalyzes the formation of 2'3'-cyclic GMP-AMP (2'3'-cGAMP), which triggers an immune response through activation of a downstream pathway called STING (**ST**imulator of **IN**terferon **G**enes). ENPP1 functions as a negative regulator of the STING pathway by cleaving extracellular 2'3'-cGAMP, preventing it from activating STING in neighboring immune cells in the tumor microenvironment (TME). The cleavage of 2'3'-cGAMP also releases the molecule adenosine, known to promote immune suppression and cancer cell migration. Analysis of ENPP1 gene expression in tumors from The Cancer Genome Atlas found that it is expressed in many tumor types, and those with high ENNP1 expression are associated with immune suppression, cancer metastasis, and poor patient outcomes. Moreover, cancers such as breast, and in particular triple-negative breast cancer, show limited efficacy to front-line treatments, including immunotherapies, when tumors express high ENPP1 levels. Altogether, ENPP1 is an attractive target to pursue for several reasons: (1) it is readily accessible, (2) its expression is relatively specific to cancer cells, (3) it is highly expressed in a variety of cancers, and (4) it can be leveraged to sensitize "cold" tumors to immunotherapies. Moreover, while STING is also an interesting target, it, unlike ENPP1, is broadly expressed; hence STING agonists indiscriminately activate STING in multiple cells and tissues, resulting in "off- target" side effects. The effects of an ENPP1 inhibitor would be localized to the TME not only because of its limited expression but also due to the high levels and short half-life of 2'3'-cGAMP.

If a safe and efficacious ENPP1 inhibitor were available, it would have widespread utility for multiple cancer types and, if used in combination with other cancer therapies, may enhance their performance. Towards this end, we have developed an orally bioavailable potent small-molecule inhibitor of ENPP1 called SR-8541A. It inhibits hENPP1 activity with an IC_{50} of 3.6 nM (K_i=1.9 nM) and demonstrates robust selectivity. We have established that it activates the STING pathway, promotes immune cell infiltration, and inhibits cancer spheroid growth. Furthermore, in syngeneic tumor mouse models, SR-8541A demonstrates a synergistic effect with radiation, and a preliminary study also shows synergy with checkpoint inhibitors. To date, we have completed preclinical development activities on SR-8541A that include API development and manufacturing, stability, pharmacokinetics, tolerability, and preliminary toxicology (mouse, rat, dog). Ongoing efforts to be completed before the proposed studies commence include *in vitro* safety pharmacology and PK/PD modeling.

The current objective for our ENPP1 program is to complete non-GLP and GLP preclinical studies necessary to seek IND acceptance for a first-in-human phase I clinical trial. Our initial indication of focus will be in TNBC. To position us to meet these goals, we propose in this Direct to Phase II SBIR application the following aims for our lead molecule SR-8541A:

- **AIM 1: Preclinical evaluation of SR-8541A in combination with FDA-approved drug regimens.** We will work with Charles River Laboratories to evaluate the efficacy of SR-8541A in combination with the chemotherapy drug cisplatin, the checkpoint inhibitors CTLA-4 and PD-1, and the PARP inhibitor olaparib, using syngeneic tumor mouse models of breast cancer.
- **AIM 2: Perform IND enabling dog GLP toxicology study on SR-8541A.** We will work with Charles River Laboratories to conduct a GLP toxicology study in dogs as the rat GLP toxicology is complete.
- **AIM3: cGMP tablet development, manufacture, and initial stability.** We will work with Catalent to determine the human dosage based on the PK/PD modeling data and manufacture clinical-grade tablets necessary to conduct a Phase I clinical trial.

At the conclusion of this work, we will have completed the necessary preclinical research and development of SR-8541A, developed a clinical strategy to test as a single agent or in combination, and submitted the IND application. Next, we will assemble a clinical team to identify the sites where SR-8541A can be clinically tested.