Targeted degradation of indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) as a novel anti-cancer strategy

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OVERVIEW/ABSTRACT

Background: The ability to evade the host immune system is a hallmark of cancer and a major contributor to tumor growth and progression. Immunotherapy is a promising strategy for treating cancer that works by stimulating the host immune system. However, many cancers, such as breast cancer, do not respond to current immunotherapies such anti-PD-1/PD-L1 due to the existence of multiple pathways for tumor immunosuppression and resistance, such as enhanced tryptophan (Trp) catabolism. Indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) catalyze the first and rate-limiting step of Trp catabolism to kynurenine (Kyn), and IDO1 expression is correlated with poor response to therapy and poor patient survival in many cancers. In addition, although TDO is constitutively expressed in liver cells, it is also overexpressed in the tumor microenvironment of some cancers, and it is hypothesized that TDO could be used by tumor cells as an escape pathway under IDO1 inhibition. Thus, strategies to target IDO1/TDO activity are expected to dramatically increase the utility of immunotherapy. While IDO1 inhibitors showed great promise in preclinical models and phase I/II trials, these inhibitors did not provide an added benefit in combination with anti-PD-1 therapy in melanoma patients. This clinical failure is thought to be due to the presence of an additional signaling activity of IDO1 that is not affected by the inhibition of the catalytic activity and the existence of other immune escape pathways, such as the TDO pathway.

Objective: We *hypothesize* that the targeted degradation of the Trp catabolic enzymes IDO1 and TDO will overcome immunosuppression, greatly increasing the ability of breast cancers to be treated by immunotherapy. Targeted degradation physically removes a protein from the cell, and the resulting event-driven pharmacology is fundamentally different from the occupancy-driven pharmacology caused by target inhibition. Importantly, the degradation of IDO1 will inhibit Trp catabolism and also prevent IDO1 signaling. Therefore, the *goal* of this proposal is to synthesize proteolysis targeting chimeras (PROTACs) that selectively degrade the Trp catabolic enzyme IDO1 or that selectively degrade both IDO1 and TDO. The dual IDO1/TDO PROTAC strategy is innovative because it represents a mechanism for preventing escape from IDO1 degradation, and no dual degraders that target IDO1/TDO have been reported. Targeted protein degradation is an underexploited, and therefore, innovative approach to IDO1 intervention.

Impact: The advent of immunotherapies has dramatically changed the landscape of cancer treatment planning in the clinic. However, breast cancer has not reaped the benefit of this new class of drugs as much as other cancers due to the presence of multiple immune escape pathways and high IDO1 expression in breast cancer. The novel drug design described in this proposal, which can be used in combination with other clinically approved drugs, has the potential for major impact in breast cancer treatment planning by bringing immunotherapies “back to life”.

Specific Aims: 1) To synthesize and validate novel PROTACs for IDO1 degradation *in vitro*. PROTACs will be synthesized that efficiently degrade IDO1 and IDO1/TDO. Western blot assays will be performed to quantify *in vitro* protein degradation, and mode of action studies will include binding kinetics and an innovative mass spectrometry assay to assess selectivity.

2) To determine the cellular consequences of IDO1 or IDO1/TDO degradation. We will demonstrate that the targeted degradation of IDO1 or IDO1/TDO leads to an expected phenotypic response of decreased Kyn production in cells. Unlike IDO1 inhibition, IDO1 degradation is also expected to reduce levels of phosphorylated IKKα due to the prevention of IDO1 signaling.