Impact of the CDK4/6-targeted degradation by PROTACs on immune checkpoint signaling in cancer

Modern oncology is founded on five treatment pillars: surgery, radiotherapy, chemotherapy, targeted treatments, and immunotherapy. The last two methods only recently joined the repertoire of personalized therapies, with the immunotherapy being the newest addition. Immunotherapy represents one of the major breakthroughs in the treatment of cancer. Current therapies focus on harnessing the immune system, by interfering with immune checkpoints to unleash antitumor T cell responses. Immune checkpoint blockade (ICB) with antibodies specific for cytotoxic T lymphocyte–associated protein (CTLA)-4 or programmed cell death 1 (PD-1) result in durable and long lasting clinical benefits leading to tumor regression also in metastatic cancer. These dramatic responses are however confined to a "substantial" minority of patients. A "substantial" minority of patients means that the ICB response rates for monotherapies range from 19% for anti-CTLA-4 to 43.7% for anti-PD-1 antibodies. Combination therapy with anti-CTLA-4 and anti-PD-1 has achieved a response rate of 57.6% (Larkin, J. *et al*. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N. Engl. J. Med.* 373, 23–34 (2015)). There is thus an urgent need to develop combination immunotherapy strategies to benefit even more patients. We have undertaken to study one of these combinations, namely that between the anti-PD-L1 ICB and CDK4/6. Deregulated cell cycle progression is a hallmark of human cancer, and targeting cyclin-dependent kinases (CDK4/6 proteins) to block cell proliferation has been validated as an effective anti-cancer therapy. Currently, several compounds are available that selectively inhibit CDK4 and CDK6. These compounds are potent, however, they exhibit elevated toxicity. CDK4/6 targeting is now under evaluation in a large number of clinical trials, both as single agents as well as in combination with other modalities. Therefore, it is of paramount importance to obtain a better understanding of the mechanisms behind their anti-tumor activity. Our research proposal addresses these timely and important issues of the mechanism of the involvement of the CDK4/6 kinases and PD-1/PD-L1 in tumorigenesis.

The research objective of our project is to characterize the mechanism of degradation of CDK4 and CDK6 cell cycle kinases by PROTACs - small-molecule degrades capable of inducing selective degradation of target proteins. In particular, we are interested in studying how these CDK4/6 PROTACS modulate the tumor immune microenvironment and synergize with the Immune Checkpoint Blockade (ICB)-induced responses.

PROteolysis-TArgeting Chimeras (PROTACs) are hetero-bifunctional molecules that recruit an E3 ubiquitin ligase to a given substrate protein resulting in its targeted degradation. Our PROTACs consist of a small-molecule inhibitor of CDK4/6 linked together with a spacer to a small-molecule that binds a specific E3 ubiquitin ligase.

Eliminating the target protein via protein degradation using PROTAC molecules has many advantages. Compared to direct traditional inhibitors, PROTACs demonstrate sub-stoichiometric activity where one PROTAC molecule is capable of inducing multiple rounds of degradation. Deactivation of an oncoprotein occurs at lower inhibitor concentration compared to traditional small-molecule inhibitors. Thus PROTACs in general exhibit lower toxicities in comparison with direct conventional inhibitors.