

Do We Need Small Molecule Inhibitors for the Immune Checkpoints?

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T lymphocytes preserve the immunological balance between defending against cancer and preventing continual activated immune responses. The balance between the two extremes requires an intricate network of protein-protein interactions (PPIs) that can either inhibit T cell-mediated immune responses targeted against self-antigens or stimulate them to defend against cancer [1]. While T cells' specificity against cancer is determined by the interaction between the T-cell receptor complex (TCR) and antigenic peptides bound in surface major histocompatibility complex (MHC) molecules, the full activation of T cells requires a second signal obtained by the binding of the co-receptor CD28 on T cells to CD80/86 molecules on activated antigen presenting cells (APCs). Once mobilized, T cells also express other receptors that inhibit their proliferation and cytokine production. Among these receptors are three immune inhibitory checkpoints: Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), programmed death-1 (PD-1) and T cell immunoglobulin mucin-3 (TIM-3). Tumors can evade the immune system via inhibitory checkpoints to attenuate T cells' signaling, leading to a state of immune tolerance [2]. Blocking the immune inhibitory checkpoints pathways recently emerged as a 'game changer' approach in cancer immunotherapy [3-5], with antibodies directed toward PD-1, for example, being selected as 'drug of the year' for 2013 by Science6. These antibodies proved the concept of restoring exhausted T cells' functions and reactivating the immune system to recognize and kill tumor cells [6,7]. More importantly, combination blockage of multiple co-inhibitory pathways has a greater efficacy by preventing accumulation of the unblocked negative co-receptor, allowing T cells to continue to survive, proliferate, and carry out effector functions within the tumor [8-16]. However, despite their outstanding success, they still have numerous disadvantages. These agents are monoclonal antibodies and are very expensive to manufacture and administer, making them financially inaccessible to many. For example, under current market conditions, the treatment cost per quality-adjusted life year (QALY) for a patient with metastatic melanoma exceeds \$500 000 (USD). These antibodies require bolus intravenous injections every 3 weeks, are administered in high dose and have a long half-life. They cannot be quickly withdrawn from the body if adverse events occur (such as autoimmunity) [17,18]. For example, treatment with antibodies targeting CTLA-4 results in life-threatening autoimmune colitis in 5-10% of patients and treatment-related fatalities, while rare, continue to be reported. These limitations suggest that the full potential of the immune checkpoint blockade has yet to be fulfilled and raise an urgent need to explore new modalities that can target the same pathways, but be safer and more effective.

Small molecules can provide this safe therapeutic alternative. They can avoid the problems associated with antibodies while retaining their functionality. Small molecules can provide increased oral bioavailability, increased bio-efficiency and shortened half-life activity for a more controllable treatment, particularly, in the case of autoimmune or other adverse events [17,18]. Small molecules can also offer greater distribution within the tumor tissue, can cross the blood-brain-barrier to treat brain tumors, have higher stability at ambient temperature facilitating purification during production.

Despite all these advantages over current immune checkpoint's antibodies, very limited efforts have been directed to development of

small molecules toward these targets. This is mainly due to the limited structural information on these proteins. For example, current PD-1 crystal structures describe PPIs of mouse PD-1 and human PD-L1 [19] or with mouse PD-L2 [20]. Similarly, there is only one structure for the mouse TIM-3 [21]. Lacking the detailed human protein-protein interactions is a barrier to rationally design small molecule inhibitors for these targets. With the current shortage in understanding these interactions experimentally, Molecular modelling [22-29] can come into play an important role to develop these models and use them to develop small molecules for the immune checkpoints. The last decade has witnessed many successful examples of designing small-molecule modulators for many protein-protein interactions, including orthosteric inhibitors, allosteric regulators, and interfacial binders. Recent examples include discovering small molecule inhibitors for menin and mixed lineage leukemia (MLL) interactions [30]. Some of these molecules can inhibit the menin-MLL interaction at a nanomolar concentration [31]. A more challenging example involves small molecule blockers for the shallow interaction between Interleukin-2 (IL-2) and the IL-2 receptor (IL-2R α) [32,33]. The lead compound, SP4206, traps IL-2 in an unusual conformation and binds with 60nM concentration [34]. Other successful stories include, the discovery of small molecule inhibitors for the EWS-FLI1/RNA helicase [35], the Ras/SOS1 interaction, the xIAP/caspase interaction [36,37], BRaf/CRaf heterodimerization and activation [38,39], urokinase-type plasminogen activator (uPA), urokinase receptor (uPAR) [40], interaction of Interferon- α with Interferon- α receptors (IFNAR2) [41], interaction of ERCC1 with XPF [42], interaction of ERCC1 with XPA [43,44] interaction of p53 with MDM245 and interaction of p53 with MDM4 [45]. All these successful stories call for a similar application of the same methods toward designing small molecules abrogators for the immune checkpoints' protein-protein interactions to provide a safer and more effective alternative for current antibodies (work in progress) [46].

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