Highly lipophilic prodrugs of PD-1-PD-L1 inhibitor BMS-1 for targeted delivery to mesenteric lymph nodes

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Background: Although a substantial number of immune checkpoint inhibitors (ICIs) have been reported, there has been a relatively low number of low molecular weight immunotherapeutics. Large molecules such as monoclonal antibodies, in many cases, cannot be accurately targeted to the sites of action, such as directly draining lymph nodes, limiting their efficacy and leading to systemic adverse effects. Therefore, small molecules in many cases are a preferable choice. The hypothesis of this project is that lipophilic prodrugs of BMS-1 (programmed cell death protein 1 (PD-1)/ programmed death-ligand 1 (PD-L1)) inhibitor could be delivered to mesenteric lymphatics via chylomicrons (CMs) pathway, obtaining a high concentration of the released drug in the lymph nodes. Mesenteric lymph nodes are the directly draining nodes for many gastrointestinal cancers, and targeting immunotherapeutics to these sites of action can improve treatment outcomes of these types of cancers.

Methods: Five prodrugs of BMS-1 were designed and their association with CM was predicted using an *in-silico* model as previously reported (Gershkovich et al, 2009). They were esterified by linking to a desired alcohol at room temperature using 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride as coupling agent, 4 dimethylaminopyridine as catalysts and dichloromethane as the solvent. For experimental artificial CM association, tested drugs were added into artificial CM emulsion (1.75 μM final concentration) and incubated at 37 °C for 1 h with stirring. Samples were ultracentrifuged at 268,350 g, 15 °C for 45 min, and 1 mL of the upper layer was collected for further preparation. An ideal situation is that the prodrug is stable in intestinal lumen but releases the drug efficiently in lymph nodes. For conversion tests, the tested drugs were added to rat plasma (as a substitute for lymph fluid) or simulated intestinal fluid with added esterase enzyme. Samples were incubated at 37 °C for 2 h with stirring at 200 rpm, with 100 μL collected at intervals and reactions were terminated with 300 μL cold ACN. For triglyceride (TG) solubility, over 5 mg prodrug was dissolved in 100 μL olive oil and incubated at 37 °C for 72 h with stirring. The mixture was filtered, and the filtrate diluted 10× with acetone, ethanol, and methanol. All samples were analysed using an LC-MS method.

Results: Five prodrugs of BMS-1 were designed using an *in silico* model for prediction of association with CMs and synthesized. Prodrug1,2 and 3 were synthesized using cetyl alcohol, hexyl alcohol and ethanol. Prodrug 4 and 5 contain one or two double bonds and synthesized using Oct-2-en-1-ol or geraniol. A bioanalytical method of simultaneous determination of BMS-1 and its prodrugs in biological samples was developed and optimized. *In vitro* association percentage with CMs of prodrug 2 (86.4 ± 12.5%) increased dramatically (86 fold) compared to BMS-1 (1.0 ± 0.5%). Prodrug 2 has a greater solubility (47.55 ± 1.01) in TG compared to BMS-1 (0.17 ± 0.02). However, the half-lives of active drug release from prodrug 2 in fasted state simulated intestinal fluid and rat plasma (as a substitute for lymph fluid) were 13.17 and 13.23 h, respectively. Similar results were obtained for the other three prodrugs.

Conclusions: Five synthesized prodrugs do not appear to be suitable lipophilic prodrug candidates, because BMS-1 was slowly or not at all released from the prodrugs in the rat plasma. However, three prodrugs have high affinity to artificial CMs and relatively high solubility in olive oil. Further design and evaluation of more promising compounds is needed to assess their suitability for targeted delivery of BMS-1 to mesenteric lymph nodes.