

## Eicosanoid Regulation of Bladder Cancer

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Unresolved inflammation plays a critical role in bladder cancer initiation and progression. Controlling the local and systemic inflammatory response is critical to preventing cancer progression. Chemotherapy, the standard of care for advanced bladder cancer, disrupts the resolution of inflammation and is only partially effective in preventing tumor recurrence. Although immunotherapy induces a durable response in a subset of patients with advanced bladder cancer, a majority of patients do not respond. Thus, there is a critical unmet need to improve chemotherapy and immunotherapy in bladder cancer. We have developed dual COX-2/sEH inhibitors (e.g., PTUPB), which target two key enzymes in the arachidonic acid cascade: cyclooxygenase-2 (COX-2) and soluble epoxide hydrolase (sEH). Since chemotherapy and immunotherapy both induce tumor-promoting inflammation via an eicosanoid and cytokine storm, we hypothesized that dual COX-2/sEH inhibition using PTUPB would enhance immunotherapy in experimental bladder cancer via anti-inflammatory and pro-resolution mechanisms. When syngeneic (MB49) bladder tumors reached ~200 mm<sup>3</sup> in immunocompetent mice, treatment was initiated with PTUPB, anti-CTLA-4, anti-PD1, gemcitabine, cisplatin, or combinations thereof. Dual COX-2/sEH inhibition in combination with chemotherapy (gemcitabine and cisplatin) and immune checkpoint blockade (anti-CTLA-4 or anti-PD1) induced tumor regression via synergistic anti-tumor activities. Chemotherapy and immunotherapy induced the expression of ER stress response genes (e.g., *BiP* and PD1) and the angiogenic factors (e.g. EGF and VEGF-C) in bladder cancer tissue, which was counter-regulated by PTUPB. PTUPB also prevented chemotherapy-induced toxicity. Our results demonstrate for the first time that dual COX-2/sEH inhibition is a novel therapeutic approach to enhance immunotherapy in bladder cancer without overt toxicity.