

Title: Overcoming Apoptosis Resistance in Advanced Prostate Cancer with ADC Combinations and Dual-Payload ADCs

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Background: Antibody-drug conjugates (ADCs) are a class of targeted therapeutics that combine the specificity of monoclonal antibodies with the potency of chemotherapeutic drugs. A typical ADC is comprised of three parts: (1) an antibody that binds to an antigen on the target cell surface, (2) a cytotoxic small molecule (payload), (3) and a linker connecting the two. Despite transformative successes in several solid tumors, clinical activity of ADCs in metastatic castration-resistant prostate cancer (mCRPC) has remained limited, reflecting the molecular and phenotypic heterogeneity of advanced disease and resistance to individual payloads. Dual-payload ADCs and rational ADC combinations offer a strategy to address these limitations by co-targeting complementary mechanisms of tumor-cell survival.

Methods: A screen of established ADC payloads and payload combinations was conducted in mCRPC cell lines to identify synergistic interactors. Custom ADCs targeting clinically relevant mCRPC antigens and incorporating selected payloads were developed. These ADCs were evaluated as single agents and in combination in mCRPC cells and cell line-derived xenografts (CDXs).

Results: Combinatorial drug screens identified drug synergism between DNA-damaging payloads and the Bcl-xL inhibitor (Bcl-xLi). Co-administration of genotoxic payloads with Bcl-xLi led to coordinated activation of the intrinsic apoptosis pathway. Treatment with B7-H3 - seco-DUBA, PSMA - SG3249, or STEAP1 - DXd ADCs induced Bcl-xL upregulation in prostate cancer cells, suggesting a compensatory survival response that may limit ADC-induced apoptosis. Combining genotoxic ADCs with Bcl-xLi enhanced cytotoxicity in prostate cancer models *in vitro* and *in vivo*. Single- and dual-payload ADCs incorporating both a Bcl-xLi and a topoisomerase I inhibitor were developed and evaluated in mCRPC models.

Conclusions: These findings identify Bcl-xL upregulation as a compensatory survival response to ADC-induced genotoxic stress and a mechanism of apoptosis resistance in mCRPC. Co-targeting genotoxic stress and the mitochondrial apoptosis pathway provides a rationale for further development of ADC combinations and dual-payload ADCs bearing complementary, synergistic payloads.

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