PHARMACOLOGIC TUMOR PDL1 DEPLETION AS A TRANSLATIONAL APPROACH TO INHIBIT TUMOR-INTRINSIC PDL1 SIGNALS AND CREATE NOVEL TREATMENT VULNERABILITIES

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Background Canine oral surface PDL1 signals to PD1 on immune cells to evade antitumor immunity are well-defined. However, tumor-intrinsic PDL1 mediates additional pathologic signals. Our lab recently showed that tumor PDL1 promotes homologous recombination DNA damage repair and ATM/ Chk2 DNA damage sensing. Genetic tumor PDL1 depletion renders tumors sensitive to Chk1 and PARP inhibitors. However, anti-PDL1 antibodies were unable to replicate treatment sensitivity, and genetic PDL1 depletion is not yet clinically feasible. We hypothesized that pharmacologic tumor PDL1 depletion could replicate genetic PDL1 depletion to sensitize tumors to therapy as a translational application of tumor PDL1 depletion.

Methods We conducted a high-throughput drug screen enriched for FDA-approved molecules in RFP-PDL1 B16 cells to identify PDL1 depleting drugs (PDDs). B16 cells were treated with 2.5 or 10 mM of screen drug for 48 hours, and RFP intensity was measured by fluorescence. We use various biochemical, cell biology, and genetic techniques to interrogate in vitro PDD mechanisms and in vivo studies of treatment and immune outcomes using human and transplantable murine cell lines of distinct cancers.

Results We identified the FDA-approved, structurally similar cephalosporin antibiotics cefepime and ceftazidime and the alkylating agent chlorambucil as PDDs with distinct signaling and treatment consequences. Cefepime and ceftazidime potently depleted PDL1 in several tumor lines and phenocopied genetic PDL1 depletion by inducing DNA damage and significantly depleting Chk2 protein. Cefepime and ceftazidime PDL1-dependently generated synthetic lethality to small molecule Chk1 inhibitors in vitro, and cefepime generated Chk1 inhibitor efficacy in vivo. Other beta-lactams failed to deplete PDL1, suggesting the beta-lactam ring is dispensable for PDL1 depletion. Cefepime and ceftazidime replicated additional genetic PDL1 depletion outcomes, including STING and autophagy induction and tumor stemness reduction in vitro. Cefepime induced an immune Th1 signature in vivo in tumor-bearing mice, consistent with STING induction and suggesting PDDs could improve immunotherapy efficacy. Strikingly, the PDD chlorambucil potently depleted ovarian cancer PDL1 and induced anti-PDL1 efficacy in PDL1-replete, anti-PDL1-resistant tumors through an NK-dependent mechanism. These data suggest tumor immunogenicity consequences of chlorambucil-mediated tumor PDL1 depletion.

Conclusions PDDs induce novel cancer treatment vulnerabilities with high clinical translational potential. We identify several FDA-approved drugs that deplete tumor PDL1, disrupt its pathogenic tumor-intrinsic signals, and induce small molecule synthetic lethality and anti-PDL1 efficacy improvement. We identified other PDDs offering opportunities as translational targets, which we are now progressing to phase I clinical trials.

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Ethics Approval All animal studies were approved by the UT Health San Antonio Institutional Animal Care and Use Committee (Number 09128).