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PHARMACOLOGIC TUMOR PD-L1 DEPLETION REDUCES CHK2 CONTENT AND SENSITIZES TUMORS TO SMALL MOLECULE CHK1 DNA DAMAGE REPAIR INHIBITORS

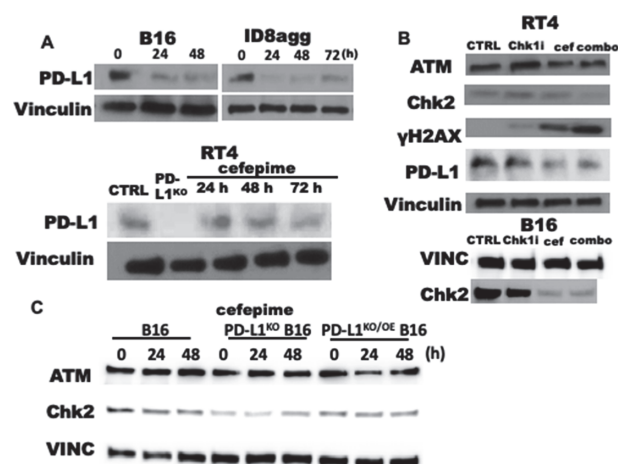
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Background Tumor PD-L1 canonically signals to PD-1 on immune cells to evade immune destruction.¹ We reported that tumor PD-L1 also mediates diverse pathologic intracellular signals,²⁻³ including promoting the ATM/Chk2 DNA damage response, and that genetically PD-L1 deficient tumors are sensitized to Chk1 inhibitor therapy.⁴ DNA damage increases cytosolic DNA, which induces immunogenic STING signals through inflammatory type I interferon and cytokine production.⁵

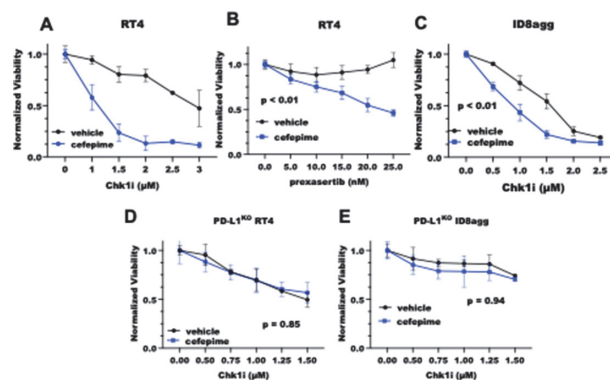
Methods We conducted a high-throughput drug screen that identified the β -lactam cephalosporin antibiotic cefepime as a pharmacologic tumor PD-L1 depleting agent. In vitro tests of β -lactam antibiotics used 80 μ M and the Chk1 inhibitors rabusertib and prexasertib were used at indicated concentrations. Cell lines were RT4 human bladder cancer, ID8agg murine ovarian cancer, and B16 mouse melanoma. Viability was by MTT and proteins by immunoblot. We challenged NSG mice (n = 5 per group) with RT4 (SQ) and treated with cefepime (200 mg/kg), rabusertib (2.5 mg/kg), vehicle, or combination daily.

Results Cefepime at pharmacologically relevant concentrations depletes tumor PD-L1 and phenocopies genetic tumor PD-L1 depletion by decreasing Chk2 protein and increasing DNA damage (γ H2AX) (figure 1). Chk2 is depleted by cefepime in CTRL cells, but not in PD-L1KO or PD-L1 overexpressing cells, and sensitizes PD-L1+ tumor cells to Chk1 inhibitors in vitro in a PD-L1-dependent manner (figures 1 and 2). Combining cefepime with rabusertib in vivo significantly prolonged severely immunodeficient NSG mice survival in RT4 challenge versus cefepime alone while rabusertib alone was not effective (figure 3). Antimicrobial mechanisms, reported to influence tumor treatment responses,⁶ are unlikely in NSG mice. To test β -lactam contributions to cefepime efficacy, we found that ceftazidime, a structurally related cephalosporin, also depletes tumor PD-L1 and Chk2 protein and sensitizes tumors to rabusertib in a PD-L1 dependent manner (figures 4 and 5). Structurally-unrelated β -lactam antibiotics did not sensitize tumors to rabusertib. Both cefepime and ceftazidime activate tumor STING, suggesting they could augment immunotherapies by increasing tumor immunogenicity (figure 6).

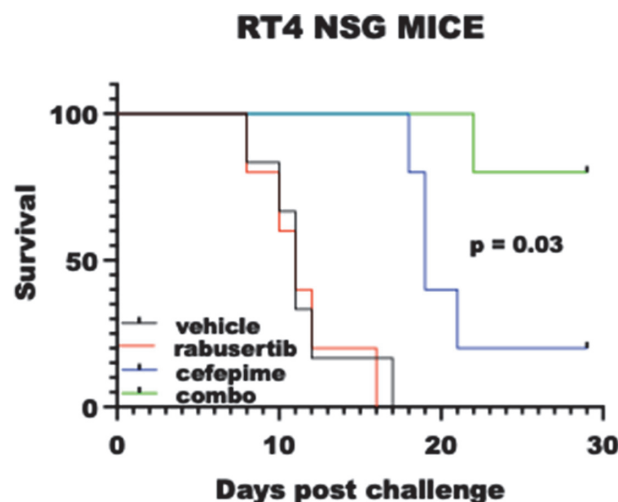
Conclusions As genetic PD-L1 depletion is not yet clinically feasible, we provide pharmacologic means to deplete tumor PD-L1 to improve clinical treatment efficacy as a rapidly translated approach. Cefepime is the prototype agent, but ceftazidime is structurally similar with significant activity, suggesting important structure activity relationships to explore. Pharmacologic tumor PD-L1 depletion could augment other standard of care approaches, including immunotherapy, and deserves further investigation.



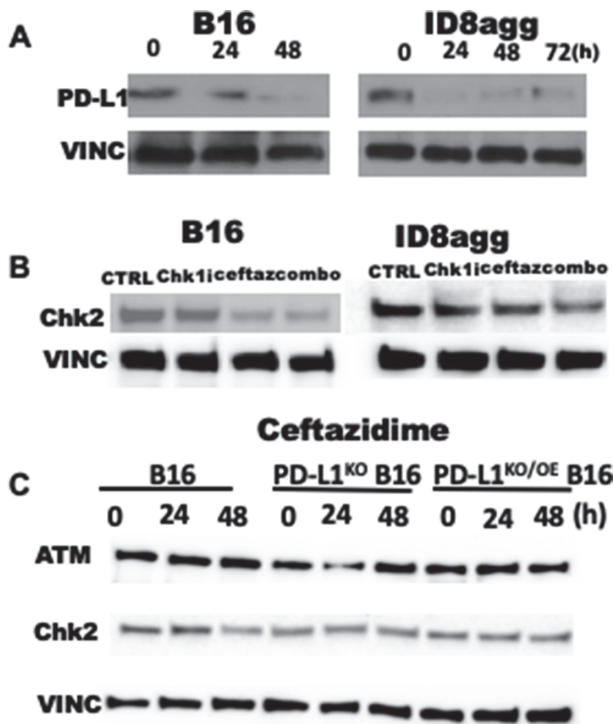
Abstract 755 Figure 1 Cefepime depletes tumor PD-L1 and Chk2 and induces γ H2AX



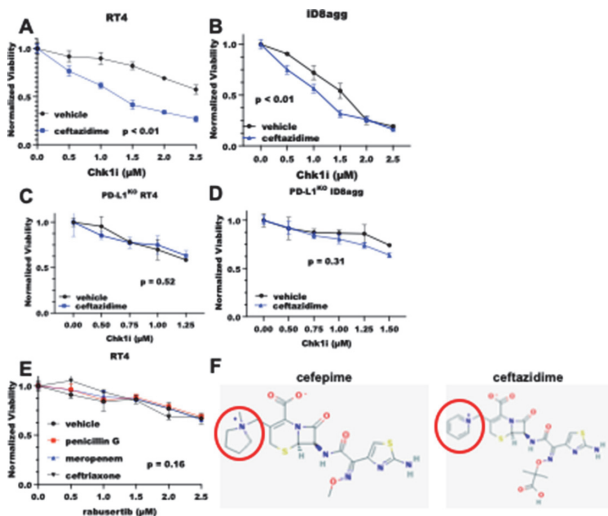
Abstract 755 Figure 2 Cefepime sensitizes tumors to Chk1 inhibitors in a PD-L1 dependent manner



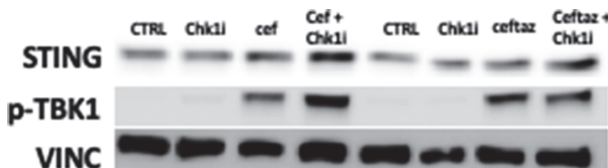
Abstract 755 Figure 3 Cefepime combined with Chk1 inhibitors in vivo prolongs NSG mouse survival



Abstract 755 Figure 4 Ceftazidime depletes tumor PD-L1 and Chk2



Abstract 755 Figure 5 Ceftazidime, but not other B-lactam antibiotics, sensitizes RT4 to Chk1 inhibitors in a PD-L1 dependent manner



Abstract 755 Figure 6 Cefepime and ceftazidime induce STING and p-TBK1

REFERENCES

1. Dong H, Strome SE, Salomao DR, Tamura H, Fumiya H, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;**8**:793–800.
2. Clark CA, Gupta HB, Gangadhara S, Pandeswara S, Lao S, Yuan B, et al. Tumor-Intrinsic PD-L1 signals regulate cell growth, pathogenesis, and autophagy in Ovarian Cancer and Melanoma. *Cancer Res* 2016;**76**(23):6964–6974.
3. Gupta HB, Clark CA, Yuan B, Sareddy G, Pandeswara S, Padron AS, et al. Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. *Signal Transduct Target Ther* 2016;1.
4. Kornepati AV, Zhang D, Hambright HG, Kari SC, Deng Y, Clark CA, et al. Cell-intrinsic programmed death ligand-1 (PD-L1) inhibits cytotoxic chemo, promotes DNA damage repair, and enhances ATM/ATR signaling following exposure to DNA damaging agents in bladder, melanoma, and ovarian cancer cells. *J Immunol* 2019;**202**(1):195.17.
5. Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, et al. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. *Immunity* 2013;**39**(3):482–495.
6. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpnits TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018;**359**(6371):97–103.

Ethics Approval This protocol was approved by the UTHSCSA IACUC.

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