Background Recent clinical trials highlight the potential therapeutic benefit from combining checkpoint immunotherapies (IO) with antibody drug conjugates (ADC) incorporating different cytotoxic warheads. We hypothesize that diverse ADC warheads modulate different aspects of tumor immune biology.

Methods Antibodies specific for tumor antigens B7-H4 and other model antigens were conjugated to either topoisomerase inhibitor linker-payload AZ0133 (AZ'0133) or microtubule inhibitor warhead monomethyl auristatin E (MMAE), and their biologic effects on immune-related endpoints in vitro and anti-tumor activity in vivo in mouse models were determined.

Results In vitro studies demonstrated that AZ'0133-ADCs increases the immunogenicity of tumor cells and enhances the activation and maturation of dendritic cells (DC) to an equal or greater extent compared to MMAE-ADCs. Treatment of human tumor cell lines with B7-H4-AZ'0133 or another model antigen targeting-AZ'0133 ADC increased cell surface expression of MHC class I to a greater extent than the same ADCs conjugated to MMAE. Surface expression of TIGIT ligand PVR/CD155 was exclusively increased by the model antigen targeting-AZ'0133 ADC but not with the same antibody conjugated to MMAE. PD-L1, Nectin-2, MHC class II, and immunogenic cell death markers calreticulin and phosphatidylserine were increased by both AZ'0133- and MMAE-ADCs. Co-culture of immature DCs with tumor cell lines in the presence of model antigen targeting AZ'0133-ADCs induced expression of DC maturation markers and CTLA-4 ligands CD80 and CD86 as well as Nectin-2. The same ADC conjugated to MMAE upregulated only CD80 and decreased TIM-3 expression on DC.

In immunocompetent mouse models, the combination of anti-PD-L1 with either AZ'0133- or MMAE-ADCs drove enhanced anti-tumor activity versus monotherapy controls. In a CT26 syngeneic model expressing mouse B7-H4, the combination of anti-PD-L1 with either a mouse cross-reactive anti-B7-H4 MMAE or anti-B7-H4-AZ'0133 ADC reduced tumor growth rates and increased overall survival versus monotherapy controls. In a CT26 model overexpressing a different human model tumor antigen, the combination of anti-PD-L1 with either AZ'0133- or MMAE-ADCs.

Conclusions AZ'0133 and MMAE ADCs both combine with anti-PD-L1 to enhance anti-tumor activity in vivo. In vitro, whilst both classes of ADC induce immunogenicity and immune activation, they have differing impacts on T cell checkpoint ligand expression.

Ethics Approval All studies involving animals were approved by the Institutional Animal Care and Use Committee at AstraZeneca under protocol number AUP-21-05.