Background Adenosine deaminase acting on RNA (ADAR1) catalyses deamination of adenosine (A) to inosine (I) in double-stranded (ds) RNAs. There are 2 isoforms of ADAR1 (p110 in the nucleus; p150 in cytoplasm) and both modify self dsRNA in coding and non-coding regions. The ADAR1 p150 isoform features a Z-DNA/Z-RNA binding domain at the N-terminus and is produced from an interferon (IFN)-inducible promoter. ADAR1 p150 edits 3'-untranslated region dsRNAs comprising of inverted Alu repeats and thereby suppresses MDA5-MAVS-IFN signaling. ADAR1 is frequently overexpressed and aids in the development of various cancers. As a monotherapy and in combination with checkpoint inhibitors, radiation, and chemotherapeutic modalities, inhibition of ADAR1 has promising anti-tumor effects. Herein, we outline the discovery of a potential first-in-class ADAR1 inhibitor for cancer immunotherapy.

Methods AVA-ADR-001, a novel first-in-class ADAR1 p150 inhibitor was identified through in silico and high throughput cell and non-cell based assays. Direct binding studies of AVA-ADR-001 with Za domain of ADAR1 p150 was confirmed by fluorescence spectroscopy and surface plasmon resonance. Interferon dependent cytotoxicity and reversal by ISRIB was evaluated. Finally, the anti-tumor efficacy of AVA-ADR-001 was evaluated in B16F10 syngeneic melanoma mice model as monotherapy and in combination with anti-PD1. SAR optimization was focused on developing analogues of AVA-ADR-001 with binding affinity for the Za domain superior to AVA-ADR-001.

Results We have discovered a potential first-in-class small molecule inhibitor of the ADAR1 enzyme, which exhibits a strong in-vitro IFN response in an MDA5 dependent manner. In vitro binding studies have confirmed direct binding of AVA-ADR-001 with Za domain of the ADAR1 p150 isoform. Direct binding study by SPR demonstrates a family of AVA-ADR-001 analogues with superior affinity (sub-uM) for Za domain compared to AVA-ADR-001. Our ADAR1 inhibitors have micromolar EC50 and demonstrated anti-tumor efficacy against B16F10 syngeneic melanoma solid model. 100 μg of AVA-ADR-001 treatment resulted in 45% tumor growth inhibition (TGI) compared 33% TGI in Anti-PD1 group. Interestingly, upon combination with AVA-ADR-001 and anti-PD1 TGI was significantly increased to 56%. Additionally, several interferon stimulated genes (ISGs) along with T-cell activation markers were significantly upregulated in tumor samples of the combination group.

Conclusions To our knowledge, AVA-ADR-001 is the first disclosure of a selective small molecule inhibitor of ADAR1 p150. AVA-ADR-001 is a potent and selective first-in-class ADAR1 inhibitor which has shown significant IFN induction in vitro and in vivo in the tumor microenvironment resulting in substantial tumor growth inhibition as monotherapy and synergistically in combination with Anti-PD1.