Functional and mechanistic advantage of the use of a bifunctional anti-PD-L1/IL-15 superagonist

SUPPLEMENTARY METHODS

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Supplementary Methods

PD-L1 binding

The ability of N809 to block PD-L1:PD-1 interactions was measured using the mouse PD-1[biotinylated]:PD-L1 Inhibitor Screening Assay Kit (BPS Bioscience) following the manufacturer's protocol.

IL-15 activity

NK cells were purified from spleens of naïve Balb/C mice (NK Cell Isolation Kit, Miltenyi Biotec). The IL-15 activity of N-809 versus N-803 was examined by culturing NK cells with various concentrations of N-809 or N-803. To measure the PD-L1-bound IL-15 activity of N-809, 96-well plates were coated with mouse PD-L1-Fc fusion protein (BPS Bioscience) overnight at 4^{o}C. Plates were washed, blocked with complete media for 1 hour, and incubated with 200nM of N-809, αPD-L1, or N-803 for 1 hour at 37^{o}C. 200nM N-809 or N-803 was added to the wells that were blocked with αPD-L1, after which NK cells were added. For both studies, the increase in NK cell viability/proliferation was determined after 24-48 hours by Cell Titer Glo (Promega) following the manufacturer's instructions.

Depletion studies

CD8 depletion antibodies (100μg, i.p.) or NK depletion antibody (25μl in 100μl phosphate buffered saline (PBS), i.p.) were administered on days 8, 10, 12, 16 and 19 post-tumor implant. Weekly depletion efficiency was determined in the blood (~50μl) by flow cytometry. Percent reduction of CD8^{+} T cells or NK cells was determined versus undepleted N-809-treated mice (set to 0%).

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Detection and quantification of serum cytokines and tumor chemokines

Serum cytokines were quantified using the V-PLEX Proinflammatory Panel I Mouse Kit and MESO QuickPlex SQ 120 (Meso Scale Diagnostics, LLC). Limits of detection were: IFNγ: 0.04pg/ml, IL-6: 0.61pg/ml, IL-10: 0.95pg/ml, TNFα: 0.13pg/ml. For tumor chemokines, tumors were homogenized in PBS using the gentleMACS Dissociator according to the manufacturer's instructions (Miltenyi Biotec). The supernatant was removed and stored at -80^{o}C until use. Chemokines were detected using the LEGENDplex Mouse 13-Plex Proinflammatory Chemokine Panel and a BD LSRFortessa flow cytometer (Beckton Dickinson) and analyzed with FlowJo FACS Analysis Software v9.9.6 (Treestar).

Discrimination of immune cells in lung parenchyma versus vasculature

To discriminate the presence of lung parenchymal versus intravascular immune cells in 4T1 tumor-bearing mice, mice were injected with anti-CD45.2 (3mg, i.v.) as previously described (1, 2).

TCR sequencing

$CD8^{+}$ T cells were isolated from primary tumor using CD8 TIL MicroBeads (Miltenyi Biotec) and stored at -80^{o}C until use. Genomic DNA was extracted using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's protocol. DNA purity was assessed on the Nanodrop One Spectrophotometer (Thermo Fisher Scientific). TCRβ chains were sequenced using immunoSEQ and analyzed via immunoSEQ Analyzer (Adaptive Technologies).

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Histopathology

Brain, heart, lung, liver, kidney and small intestine were removed and stored in 10% formalin. Paraffin embedded sections were stained for hematoxylin and eosin (H&E) and pathology was determined by an independent certified pathologist (Histoserv, Inc.).

References

1. Knudson KM, Hicks KC, Alter S, Schlom J, Gameiro SR. Mechanisms involved in IL-15 superagonist enhancement of anti-PD-L1 therapy. J ImmunoTher Cancer. 2019;7(1):82.

2. Anderson KG, Mayer-Barber K, Sung H, Beura L, James BR, Taylor JJ, et al. Intravascular staining for discrimination of vascular and tissue leukocytes. Nat Protoc. 2014;9:209-22.