

| Cell Line | LD ₂₅ of Cisplatin (µg/ml) | LD ₅₀ of Cisplatin (µg/ml) |
|------------|---------------------------------------|---------------------------------------|
| UMSCC-1 | 0.67 | 1.77 |
| UMSCC-46 | 0.12 | 0.33 |
| UMSCC-74A | 1.53 | 3.07 |
| UPCI SCC90 | 0.28 | 1.11 |

Supplemental Table 1: HNSCC cell lines were treated with a range of cisplatin doses for 72 hours, and cell death was assessed using an annexin/7AAD flow cytometry assay in at least two independent experiments performed in triplicate for each cell line. Cell death curves were generated to estimate the doses required to kill 25% (LD₂₅) or 50% (LD₅₀) for each cell line.

Supplementary Figure S1: Ovalbumin-expressing MOC1 tumor cells (T = target) were allowed to grow in 96-well plates in the presence or absence of cisplatin for 24 hours prior to adding antigen-specific OT-1 T cells (E = effector). Impedance measurements were taken over time to determine tumor cell viability. In B and C, Impedance lines are graphed as averages of 3 replicates that have been normalized to a cell index of 1.0 at 24 hours when CTLs were added. CTLs were pretreated separately with the indicated doses of cisplatin starting at time point 0 hours, then added to the wells with MOC1 ova cells and co-treated with cisplatin throughout the rest of the experiment. Graphs are representative of two independent experiments done in triplicate.

Supplementary Figure S2: Low-dose cisplatin and anti-PD-L1 blockade in combination modestly delayed tumor growth but did not prolonged survival in MOC1 tumor-bearing mice. Mice were injected with 5 x 10⁶ MOC1 tumor cells in the right flank. After 11 days, mice with palpable tumors were randomized and treated concurrently with cisplatin (3 mg/kg/week x 4 weeks) and/or anti-PD-L1 antibody (200 mcg twice/week x 3 doses). Black line: control; Blue line: CDDP only; Gray line: anti-PD-L1; Green line: combination. Data are from the same experiment shown in Fig. 3. **A**, Tumor volume over time. Data are

mean \pm SEM, n = 7-8 mice per group. ****** $p < 0.01$ vs. control by linear regression curve comparison. **B**, Kaplan-Meier survival plot.

Supplemental Figure S3: Cisplatin and anti-PD-L1 blockade induced a slight but short-lived delay of tumor growth in MOC2 tumor-bearing mice that did not improve survival. Mice were injected with 1×10^5 MOC2 tumor cells in the right flank. After 14 days, mice with palpable tumors were randomized and treated concurrently with cisplatin (5 mg/kg/week) and/or anti-PD-L1 antibody (200 mcg twice/week x 3 doses). Black line: control; Blue line: CDDP only; Gray line: anti-PD-L1; Green line: combination. **A**, Tumor volume over time. Data are mean \pm SEM, n = 8 mice per group. ***** $p < 0.05$ vs. control by linear regression curve comparison. **B**, Kaplan-Meier survival plot.

Supplemental Figure S4: Moderate doses of cisplatin and anti-PD-1 do not cause significant kidney toxicity or hearing loss. **A**, kidneys were harvested from MOC1 tumor-bearing mice upon euthanasia, sectioned, and stained with H&E. Examples shown are representative of at least three different animals per treatment group. **B** and **C**, hearing was assessed in control or cisplatin-treated, MOC1 tumor-bearing mice after 6 weeks of treatment by measuring auditory brainstem response thresholds (**B**) and distortion product otoacoustic emission amplitudes (**C**). Data are mean (**B**) \pm SEM (**C**) from two mice per group.