**Mice and *in vivo* experiments**

Mice treated with rapamycin were administered a loading dose of 4.5 mg/kg via intraperitoneal (IP) injection, followed by 1.5 mg/kg via IP injection every other day for 21 days. Mice treated with PD0325901 (PD901) were administered 1.5 mg/kg via oral gavage daily for 21 days. These *in vivo* doses were determined from rodent pharmacokinetics and our previous experience with toxicity attributable to these drugs in xenograft models[1, 2]. Mice treated with anti-PD-L1 antibody (clone 10F.9G2, BioXCell) were administered 200 μg IP q3 days for three doses. All treatments were initiated concurrently. Control mice were treated with drug carriers alone (2% ethanol, 5.2% tween 80 and 5.2% PEG 400 in water for rapamycin and 0.5% HPMC and 0.2% tween 80 in water for PD901) and 200 μg rat IgG2b isotype control antibody. Immediately following 21 days of concurrent treatment, some treated and control tumor-bearing mice were euthanized and tissues were processed for analysis as indicated. Remaining mice were followed for primary tumor growth and survival analysis following withdrawal of treatment. All tissues used for flow cytometry were used fresh. Where indicated, systemic CD8 or NK cell depletion was achieved via IP injection of 200 μg of anti-CD8 antibody (clone YTS169.4, BioXCell) or anti-NK1.1 antibody (clone PK136, BioXCell) twice weekly. Depletion of CD8 and NK cells peripherally and from within the tumor microenvironment using this schedule was validated by flow cytometry of splenic or tumor single cell suspensions.

**RT-PCR**

RNA extraction, cDNA synthesis, and RT-PCR was performed as previously described (4) using the following forward and reverse primers:

P15E-5: ATG GAA CCC GTC TCA CTA ACT CTG G

P15E-3: TCA CAG GGC CTG CAC TAC CGA AAT C

Amplified sequences were qualitatively assessed with 2% agarose gel electrophoresis.

**Tissue flow cytometry**

Cell surface staining was performed using flourophore conjugated anti-mouse CD45.2 clone 104, CD3 clone 145-2C11, CD4 clone GK1.5, CD8 clone 53-6.7, NK1.1 clone PK136, CD44 clone IM7, programmed cell death 1 (PD1) clone RMP1-30, CD107a clone 1D4B[3], CD11b clone M1/70, Gr1 clone RB6-8C5, H2-Kb clone AF6-88.5 and H2-Db clone KH95 antibodies (Biolegend) as indicated. H2Kb:KSPWFTTL (p15E604-611) tetramer was purchased from MBL (Woburn, MA). Dead cells were excluded via 7AAD negativity for all cell surface flow experiments. Use of anti-CD8 antibody clone KT15 (MBL) was required with tetramer staining due to epitope compatibility, and specific staining was validated as described previously[4]. Regulatory T-lymphocytes (Tregs) were assayed using the Treg Kit #1 (eBioscience) per protocol along with a LIVE/DEAD fixable viability dye (Life Technologies). Isotype control antibodies and a “fluorescence minus one” method of antibody combination were used for specific staining validation. Data was acquired on a FACSCanto using FACSDiva software (BD Biosciences) and analyzed on FlowJo software vX10.0.7r2.

References

1. Guba, M., Koehl, G.E., Neppl, E., Doenecke, A., Stainbauer, M., Schlitt, H.J., et al., Dosing of rapamycin is critical to achieve an optimal antiangiogenic effect against cancer. Transpl Int, 2005. **18**(1): p. 89-94.

2. Jessen, W.J., Miller, S.J., Jousma, E., Wu, J., Rizvi, T.A., Brundage, M.E., et al., MEK inhibition exhibits efficacy in human and mouse neurofibromatosis tumors. J Clin Invest, 2013. **123**(1): p. 340-7.

3. Betts, M.R., Brenchley, J.M., Price, D.A., De Rosa, S.C., Douek, D.C., Roderer, M., et al., Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J Immunol Methods, 2003. **281**(1-2): p. 65-78.

4. Cash, H., Shay, S., Moree, E., Cariso, A., Uppaluri, R., Van Waes, C., et al., mTOR and MEK1/2 inhibition differentially modulate tumor growth and the immune microenvironment in syngeneic models of oral cavity cancer. Oncotarget, 2015. **6**(34): p. 36400-17.