Supplementary data

Optimal dosing regimen of CD73 blockade improves tumor response to radiotherapy through iCOS downregulation

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Running title: CD73 blockade sequencing controls MC38 tumor response to IR

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Supplementary methods

Tissue sample preparation

Tumors were digested using the Tumor Dissociation Kit (Miltenyi Biotec) for 40 minutes at 37°C and 1,500 rpm. The cells from the tumors were filtered using cell strainers (70 µm, Miltenyi Biotech) and centrifugated at 1400rpm. Dry pellets were supplemented with 500 µl of methanol, vortexed 5 min, centrifugated (10 min at 15000 g, 4°C), and transferred into injection vials. 300µL of supernatants in vials were then evaporated at 40°C in a Speed-Vacuum Savant (Thermo). UHPLC-Mass Spectrometry (MS) dried extracts were solubilized with 100 µL of MilliQ water and injected into UHPLC-MS.

UHPLC/MS

Targeted UHPLC/MS analyses were performed on a UHPLC 1290 system (Agilent Technologies, Waldbronn, Germany), with an autosampler kept at 4°C, and a peltetier oven for rigorous control of the column temperature. The UHPLC was coupled to a QQQ/MS 6470 (Agilent Technologies) equipped with an electrospray source, using nitrogen as collision gas. Samples were injected into a Zorbax Eclipse plus C18 (100 mm × 2.1 mm, particle size 1.8 µm, Agilent) column protected by a guard column C18 (5 mm × 2.1 mm, particle size 1.8 µm). Column oven maintained at 40°C during analysis. The gradient mobile phase consisted of 0.5 mM DBAA (Sigma-Aldrich) (A) and ACN (B). The flow rate was set to 0.4 ml/min, and gradient as follow: 10% B (initial conditions) maintained for 3 minutes, then increased to 95% B in 1 min and maintained 2 min, to finally equilibrate to initial conditions, 10% B, for 1 min. The QQQ/MS was operated in both positive and negative mode. The gas temperature was set to 350°C with a gas flow of 12 l/min. The capillary voltage was set to 5.0 kV in positive mode. MRM scan mode was used for targeted analysis. Peak detection and integration were performed using the Agilent Mass Hunter quantitative software (B.10.1).