COMBINED IRREVERSIBLE ELECTROPORATION AND LOCAL CD40 AGONISM STIMULATE NEOANTIGEN SPECIFIC SYSTEMIC IMMUNE RESPONSES THAT INHIBIT LIVER METASTASIS IN AN ORTHOTOPIC PANCREATIC CANCER MODEL

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Background Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, and most patients present with either locally advanced or metastatic disease. Irreversible Electroporation (IRE) is a non-thermal method of ablation, used clinically in locally advanced PDAC, but most patients eventually develop distant recurrence. We have previously shown that IRE alone is capable of generating protective, neoantigen-specific immunity. Here we aim to generate meaningful therapeutic immune effects by combining IRE with local (intratumoral) delivery of CD40 agonistic antibody (CD40Ab).

Methods KPC46 organoids were generated from a tumor-bearing male KrasLSL-G12D-p53LSL-R172H-Pdx-1-Cre (KPC) mouse. Orthotopic tumors were established in the pancreatic tail of B6/129 F1J mice via laparotomy (KPC46O). Candidate neoantigens were identified by mutanome profiling of tumor. Tumors were monitored by ultrasound, and when they reached 4-5 mm, mice were randomized to either sham laparotomy, IRE alone, CD40Ab alone, or IRE followed immediately by CD40Ab injection. Metastatic disease and immune infiltration in the liver were analyzed 14 days post-procedure using flow cytometry and multiplex immunofluorescence assay with spatial analysis.

Results Sham-treated KPC46O mice showed a median survival of 14 days post-procedure due to rapid development of metastasis and increasing tumor burden. IRE or CD40Ab alone improved the median survival to 21 and 24 days, respectively, but significantly (p<0.01) lower than the median survival of >35 days achieved by the combination of IRE+CD40Ab. CD40Ab had a significant effect on metastatic disease with average liver weights significantly lower in the IRE+CD40Ab group than the Sham group (p<0.01) or IRE alone (p<0.05). Immunohistochemistry of metastatic nodules in the liver revealed a significantly (p<0.01) higher infiltration of CD8+ T-cells in the IRE+CD40Ab group than the other groups. Multiplex immunofluorescence imaging also revealed a 4-6-fold increase in the density of CD80+CD11c+ activated dendritic cells (p<0.05), which were spatially distributed throughout the tumor unlike the sham group, where they were restricted to the periphery. In contrast, CD4+FoxP3+ T-regulatory cells (p<0.05) and Ly6G+ MDSCs (P<0.01) were reduced and restricted to the tumor periphery in the IRE+CD40Ab group. T-cells from the IRE+CD40Ab group recognized more peptides (65 ± 9.3%) representing candidate neoantigens than did T-cells from IRE or Sham groups suggesting the dendritic cell activation and improved antigen presentation caused by IRE+CD40Ab treatment leads to wider tumor neoantigen recognition.

Conclusions IRE can induce local tumor regression and generate neoantigen-specific immune responses. Addition of CD40Ab to IRE improved neoantigen recognition, thereby generating a strong systemic anti-tumor T-cell response that inhibited metastatic disease progression.