SUPPLEMENTARY INFORMATION

Title: Nicotinamide combined with gemcitabine is an immunomodulatory therapy that restrains pancreatic cancer in mice

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Running title: Nicotinamide and Gemcitabine reduce pancreatic cancer

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Figure S1: Schematic view of generation of tumors and metastases and treatment protocol of NAM+GEM

Various mouse tumor models have been used. Orthotopic Panc-02 model **(A):** 10^6 Panc-02 tumor cells were orthotopically injected into the pancreas of immune competent C57Bl/6 mice as we described earlier[14]. Treatments with NAM and GEM were started 10 days after tumor cell injection, when the tumor was palpable. Briefly, NAM (20 mg/dose) and GEM (1.2 mg/dose) were administered alternately over the period of two weeks. Orthotopic KPC model **(B):** 10^5 tumor cells were orthotopically injected into the pancreas of immune competent C57Bl/6 mice as described above. Treatments with NAM and GEM were started 10 days after tumor cell injection, when a tumor was palpable. Briefly, NAM and NAM were administered alternately over the period of two weeks as described above. Orthotopic Panc-02 model in nude mice **(C):** In this model Panc-02 tumor cells were injected into the pancreas of immune incompetent nude mice with a C57Bl/6 background (Fox n1nu/J (cat #000819) Jackson Laboratories). Treatments with NAM and GEM were started 10 days after tumor cell injection, when a tumor was palpable. Briefly, NAM and NAM were administered alternately over the period of two weeks as described above. Orthotopic Panc-02 model depleted of CD4 and CD8 T cells **(D):** 10^6 Panc-02 tumor cells were injected into the pancreas of immune competent C57Bl/6 mice as we described above. Treatments with NAM and GEM were started 10 days after tumor cell injection, when a tumor was palpable. Briefly, NAM and NAM were

administered alternately over the period of two weeks as described above, and antibodies to CD4 or CD8 T cells were administered between each NAM+GEM treatment, every 3rd day over the period of two weeks.

Figure S2: Apple Juice Plus Pepper (vehicle control) shows no effect on pancreatic cancer in orthotopic Panc-02 mice. Tumors and metastases were generated and treatments with NAM and GEM as described in **Fig S1A.** NAM was delivered in Apple juice plus pepper to prevent glucuronidation of NAM in the liver. The effect of Apple juice plus pepper (vehicle control) was tested on tumors and metastases in the orthotopic Panc-02 model. Tumor weight and number of metastases was determined and subjected statistical analysis (Mann-Whitney). Ns=not significant.

Figure S3: NAM+GEM reduces pancreatic tumors and metastases in orthotopic Panc-02 model. Tumors and metastases were generated and treatments with NAM and GEM as described in **Fig S1A.** Tumors in the pancreas **(A)** and metastases in the liver **(B)** of mice treated with NAM+GEM and control groups are depicted.

Figure S4: NAM+GEM reduces pancreatic tumors and metastases in orthotopic KPC model. Tumors and metastases were generated and treatments with NAM and GEM as described in **Fig S1B.** Tumors in the pancreas **(A)** and metastases in the liver **(B)** of mice treated with NAM+GEM and control groups are depicted.

Figure S5: CD4 and CD8 staining of pancreatic tumors of orthotopic panc-02 model in all treatment groups. Overview of CD4 **(A)** and CD8 **(B)** staining by IHC. Stained sections are shown of each treatment group. Detail of granzyme B- **(C)** and perforin-producing cells **(D)** in pancreatic tumors analyzed by IHC.

Figure S6: CD4 T cells significantly reduces pancreatic cancer by NAM+GEM in orthotopic Panc-02 model. Tumors and metastases were generated and treatments with NAM and GEM as described in **Fig S1D.** Tumors in the pancreas of mice treated with NAM+GEM plus anti-CD4 or anti-CD8 antibodies and control groups are depicted.

Figure S7: NAM+GEM treatment shows little effect on pancreatic cancer in orthotopic immune incompetent panc-02 model (nude mice). Tumors and metastases were generated in nude mice (Fox n1nu/J (000819) and treatments with NAM and GEM as described in **Fig S1C.** These mice lack T cells. Tumors in the pancreas of Panc-02 mice treated with NAM+GEM and control groups are depicted.

Figure S8: Trichrome, αSMA and CD31 staining of pancreatic tumors of orthotopic panc-02 model in all treatment groups. (A) Overview of tumor sections of all treatment groups stained with trichrome. The dark blue areas represent the normal tissues (predominantly acinar cells), and the light blue areas the tumor tissues and tumor stroma. **(B)** Overview of tumor sections of all treatment groups stained with antibodies to α-smooth muscle actin (αSMA) protein. Detail of CD31 expression in pancreatic tumors by IHC **(CD).**

Figure S9: Detail of peritumoral and intratumoral LNS in orthotopic Panc-02 model. NAM **(A),** GEM **(B)** and NAM+GEM **(C)** treatment groups exhibit formation of LNS, with peritumoral LNS most prominently developed in the NAM+GEM group, and intratumoral LNS in the NAM or GEM group. Both intratumoral and peritumoral LNS exhibit formation of vessels, as highlighted by

CD31+ immunostaining, which may allow for migration of T cells to the tumors. A peritumoral LNS surrounded by a fibroblast layer **(D).** The fibroblast layer stained positively with αSMA antibodies, consistent with fibroblast/myofibroblasts **(D).**

Figure S10: Potential immune mechanisms of NAM+GEM in pancreatic cancer. NAM+GEM increased the expression of Ccl21a and Ccl9 in the pancreatic tumors, which are involved in T cell migration and the formation of LNS through chemokines. We also found the production of Granzyme B in the tumors of NAM+GEM-treated mice, which may be responsible for decrease in the expression of HABP (fibrils linking the collagen I fibers), and the subsequent influx of T cells into the pancreatic tumors. NAM+GEM reduced the MDSC and TAM population in the pancreatic tumors, which resulted in improved T cell activation. NAM+GEM also increased the expression of Fcer which is involved in epitope spreading. Indeed, we found CD4 and CD8 T cells (producing IFNγ) activated by survivin (expressed by Panc-02 tumors) through ELISPOT. Moreover, we found that CD4 T cells were involved in the eradication of tumors and metastases in T cell depletions studies *in vivo*, while this was less robust for CD8 T cells.