Imprime (β-1,3/1,6 glucan) synergizes with a CD40 agonist to stimulate T cell dependent anti-tumor activity in a poorly immunogenic model ofpancreatic carcinoma

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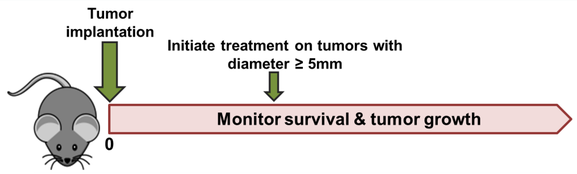
ABSTRACT

While some solid tumors show remarkable sensitivity to immunotherapy, pancreatic ductal adenocarcinoma (PDAC) has demonstrated impressive resistance. This poor responsiveness to immunotherapy may reflect multiple mechanisms necessary for generating productive T cell immunosurveillance including ineffective tumor antigen presentation by myeloid cells. Here, we hypothesized that combining two immune agonists capable of "licensing" myeloid cells with enhanced T cell stimulatory properties would synergize to reverse the poorly immunogenic state of pancreatic cancer and in doing so, drive productive T cell dependent anti-tumor immunity. **METHODS**: C57BL/6 mice were challenged subcutaneously with a syngeneic PDAC cell line (7940B.PDA) derived from a tumor arising spontaneously in the KrasG12D/+; Trp53R172H/+; Pdx-1 Cre (KPC) mouse model. Mice with tumors measuring approximately 5 mm in diameter were treated with defined combinations of gemcitabine chemotherapy, a CD40 agonist (FGK45), and Imprime (a yeast β-1,3/1,6 glucan that coordinately activates innate and adaptive immune responses through pattern recognition receptors). CD4 and CD8 T cells were depleted in vivo using GK1.5 and 2.43 antibodies, respectively. Tumor growth curves and overall survival were determined. **RESULTS:** Monotherapy with Imprime or a CD40 agonist only modestly delayed tumor outgrowth compared to chemotherapy alone. Delivering chemotherapy 48 hours prior to Imprime administration produced a two-fold increase in the median overall survival but responses were not durable and all mice relapsed. In contrast, concurrent single administration of Imprime and a CD40 agonist induced complete and durable regressions in 60% of mice. Tumor-free mice resisted tumor re-challenge and therapeutic efficacy was completely abrogated with CD4/CD8 cell depletion. **CONCLUSIONS:** The combination of two myeloid-directed immune agonists (Imprime and a CD40 agonist) shows promising activity for stimulating T cell dependent anti-tumor immunity against PDAC, a poorly immunogenic cancer.

BACKGROUND

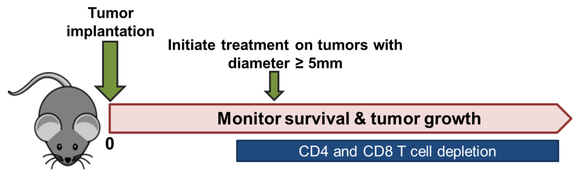
Immunotherapies, using immune checkpoint antibodies (e.g. anti-PD1/PDL1 and anti-CTLA-4) designed to sustain and enhance the productivity of an ongoing immune response, have produced impressive results in several solid cancers. However, in PDAC, the immunosuppressive microenvironment precludes T cells from mounting anti-tumor responses, even with antibody blockade of checkpoint molecules. Thus, we investigated the role for a novel combination of immune agonists, FGK45 and Imprime, in a preclinical model of murine PDAC. FGK45 is a CD40 agonist (rat IgG2a antibody) that "licenses" antigen-presenting cells, which in turn stimulate antigen-specific T cell responses. Imprime is a yeast β-1,3/1,6 glucan that stimulates pathogen recognition receptors found in innate immune cells. Previous work has demonstrated that FGK45 improves responses to cytotoxic chemotherapy (gemcitabine), but anti-tumor responses remain transient without effective T cell activation. Overcoming inhibition of T cell activation can be achieved by stimulation with Imprime, which redirects innate immune cells away from an immunosuppressive phenotype and drives a pro-inflammatory milieu that supports T cell activation.

STUDY #1 DESIGN



Group	D-2 Treatment	D+0 Treatment
1	N/A	N/A
2	N/A	Imprime
3	N/A	FGK45
4	N/A	Imprime and FGK45
5	Gemcitabine	N/A
6	Gemcitabine	Imprime
7	Gemcitabine	FGK45
8	Gemcitabine	Imprime and FGK45

STUDY #2 DESIGN



Group	D+0 Treatment	Depletion
1	N/A	N/A
2	Gemcitabine	N/A
3	FGK45	N/A
4	Imprime	N/A
5	Imprime and Gemcitabine	N/A
6	Imprime and FGK45	N/A
7	Imprime and Gemcitabine	CD4 & CD8 T cells
8	Imprime and FGK45	CD4 & CD8 T cells

RESULTS

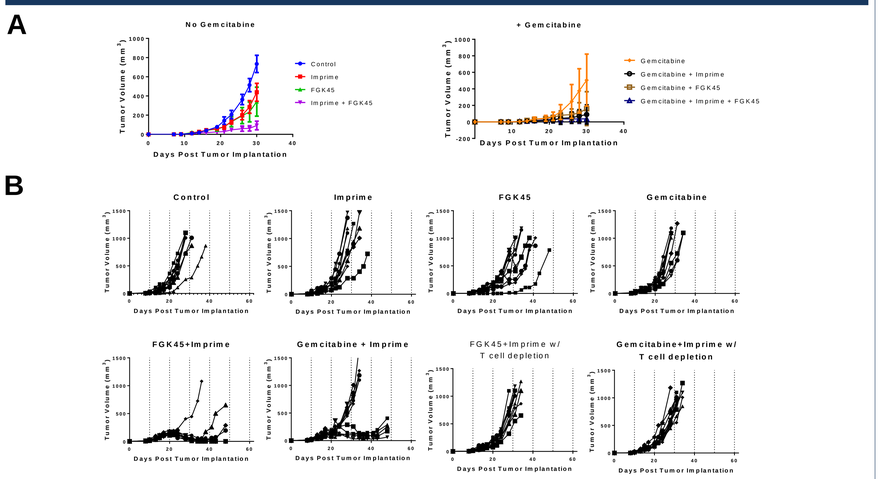


Figure 1. Combination treatment with FGK45 and Imprime synergizes to enhance the efficacy of gemcitabine chemotherapy in an immunocompetent model of murine PDAC. (A) Tumor growth curves of mice receiving control, FGK45, and Imprime combination treatments (n=10), with and without gemcitabine. (B) Tumor growth of treatment groups with or without T cell depletion (n=10).

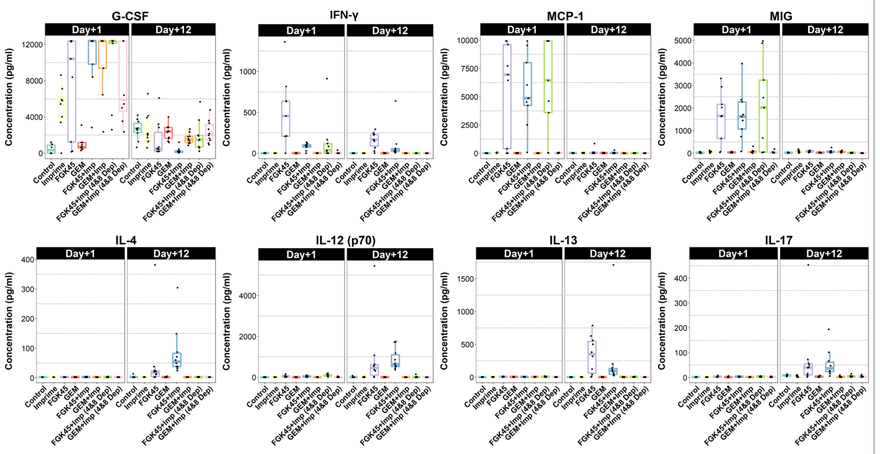


Figure 2. Systemic release of pro-inflammatory cytokines in the peripheral blood following combination treatment with FGK45 and Imprime.

RESULTS

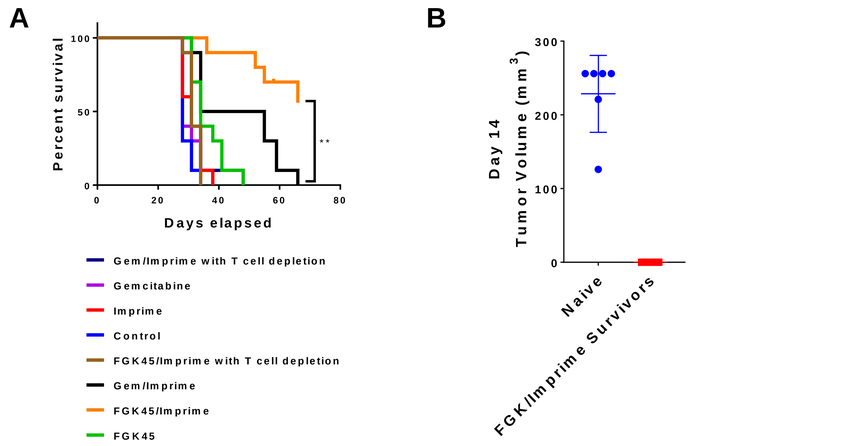


Figure 3. Dual agonist combination of Imprime and FGK45 produces long-term anti-tumor responses in a T cell dependent manner. (A) Overall survival of mice bearing subcutaneous PDAC tumors treated with combination regimens, with and without depletion of T cells. (B) Tumor outgrowth following tumor re-challenge of naïve mice (n=6) and FGK45/Imprime-treated survivors (n=6) from (A).

CONCLUSIONS

- Combination of two myeloid-directed agonists (Imprime and a CD40 agonist) produced durable T cell dependent anti-tumor immunity in a poorly immunogenic model of PDAC.
- Chemotherapy combined with Imprime and a CD40 agonist to produced enhanced anti-tumor activity.
- CD40 agonist + Imprime treated mice experiencing complete regressions resisted tumor re-challenge, implicating treatment-induced immune memory.
- Potent myeloid activation using multiple agonists can restore productive T cell immunosurveillance in a poorly immunogenic tumor in the absence of a T cell agonist of checkpoint inhibitor.

MATERIALS & METHODS

Tumor growth: Tumor cells (7940b.PDA) were cultured in DMEM supplemented with 10% bovine serum and L-glutamine. Tumor cells were harvested by trypsinization, and $5x10^5$ tumor cells suspended in phosphate buffered saline (PBS) were injected into the subcutis of C57BL/6 mice. Mice were monitored 2-3 times per week for tumor growth by calipers. In Study 1, a cohort of mice received gemcitabine (120 mg/kg) on day -2. On day 0, mice were treated with or without FGK45 (0.1 mg), Imprime (1.2 mg), or both.

T cell depletion: Depleting antibodies (clones GK1.5 and 2.43) were prepared at 0.2 mg in 200 µl PBS. Mice received 0.2 mg of GK1.5 and 0.2 mg of 2.43 by intraperitoneal injection on days 0,4,7, and 11.

Survival analysis. Mice were monitored 2-3x per week for tumor growth until tumors reached >1000 mm^3 or animals demonstrated tumor-related discomfort/distress).

Luminex bead array: Serum was collected by retro-orbital bleed on day +1. Serum was then evaluated for cytokines/chemokines using Luminex platform to analyze 26 serum cytokines and chemokines: G-CSF, IFN-γ, MCP-1, MIG, IL-2, IL-4, IL-12(p70), IL-13, IL-17

Tumor re-challenge. Mice previously challenged with 7940b.PDA that showed complete regression with FGK45+Imprime treatment (n=6) and control, naïve Pdx mice (n=6). Mice in both groups were re-challenged in the left flank with $5x10^5$ 7940b.PDA cells and monitored 2-3 times per week for tumor engraftment and outgrowth.

REFERENCES

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2. Chan AS et al. Imprime PGG-Mediated Anti-Cancer Immune Activation Requires Immune Complex Formation. PLoS One. 2016 Nov 3;11(11):e0165909.

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