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Near-infrared photoimmunotherapy as a complementary modality to in situ vaccine in a preclinical pancreatic cancer model

Hiroaki Yaku^a, Ken Takahashi^{a,b,*}, Hirokazu Okada^{a,g}, Kouji Kobiyama^{c,d,e}, Masahiro Shiokawa^a, Norimitsu Uza^{a,f}, Yuzo Kodama^f, Ken J. Ishii^{c,d,e}, Hiroshi Seno^a

^a Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

^b Division of Cancer Immunotherapy, Center for Cancer Immunotherapy and Immunobiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

^c Division of Vaccine Science, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

^d International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

^e The University of Tokyo Pandemic Preparedness, Infection and Advanced Research Center, The University of Tokyo, Tokyo, Japan

^f Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kobe University, Kobe, Japan

^g Department of Gastroenterology and Hepatology, National Hospital Organization Kyoto Medical Center, Kyoto, Japan

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ABSTRACT

Pancreatic cancer is one of the most refractory malignancies. In situ vaccines (ISV), in which intratumorally injected immunostimulatory adjuvants activate innate immunity at the tumor site, utilize tumor-derived patient-specific antigens, thereby allowing for the development of vaccines in patients themselves. Near-infrared photoimmunotherapy (NIR-PIT) is a novel therapy that selectively kills cancer cells exclusively in the NIR-irradiated region. Extending our previous research showing that ISV using the unique nanoparticulate Toll-like receptor 9 (TLR9) ligand K3-SPG induced effective antitumor immunity, here we incorporated NIR-PIT into K3-SPG-ISV so that local tumor destruction by NIR-PIT augments the antitumor effect of ISV. In the mouse model of pancreatic cancer, the combination of K3-SPG-ISV and CD44-targeting NIR-PIT showed synergistic systemic antitumor effects and enhanced anti-programmed cell death-1 (PD-1) blockade. Mechanistically, strong intratumoral upregulation of interferon-related genes and dependency on CD8⁺ T cells were observed, suggesting the possible role of interferon and cytotoxic T cell responses in the induction of antitumor immunity. Importantly, this combination induced immunological memory in therapeutic and neoadjuvant settings. This study represents the first attempt to integrate NIR-PIT with ISV, offering a promising new direction for cancer immunotherapy, particularly for pancreatic cancer.

1. Introduction

Pancreatic cancer is one of the leading causes of cancer mortality worldwide [1] and is refractory to standard chemoradiotherapy and current immunotherapies [1,2]. Nonetheless, given that higher levels of tumor-infiltrating T cells are associated with better prognosis in pancreatic cancer [3], T cell-mediated immunity might still operate and immunotherapy enhancing T cell priming/activation would be promising for pancreatic cancer as demonstrated by recent clinical cancer vaccine trials [4,5].

In contrast to conventional vaccination, in situ vaccines (ISV) which stimulates innate immunity at the tumor site can achieve personalized

vaccine by utilizing tumor as a source of vaccine antigens [6]. ISV is clinically feasible for pancreatic cancer because endoscopic ultrasound (EUS)-guided tumor puncture is a routine approach. However, to date, ISV has not yet been clinically implemented for pancreatic cancer.

Toll-like receptor 9 (TLR9) ligand CpG ODNs might be promising adjuvants for ISV because they potently stimulate dendritic cells to produce type I interferon (IFN), and generally have acceptable safety profiles, as proven in past clinical trials [7]. K3-SPG is a nanoparticulate second-generation TLR9 ligand generated by wrapping conventional K-type CpG ODN in schizophyllan [8]. The unique properties of K3-SPG that it is a robust type I IFN inducer with water solubility make it an ideal clinically translatable adjuvant for cancer immunotherapy.

* Corresponding author. Division of Cancer Immunotherapy, Center for Cancer Immunotherapy and Immunobiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

E-mail address: takaken@kuhp.kyoto-u.ac.jp (K. Takahashi).

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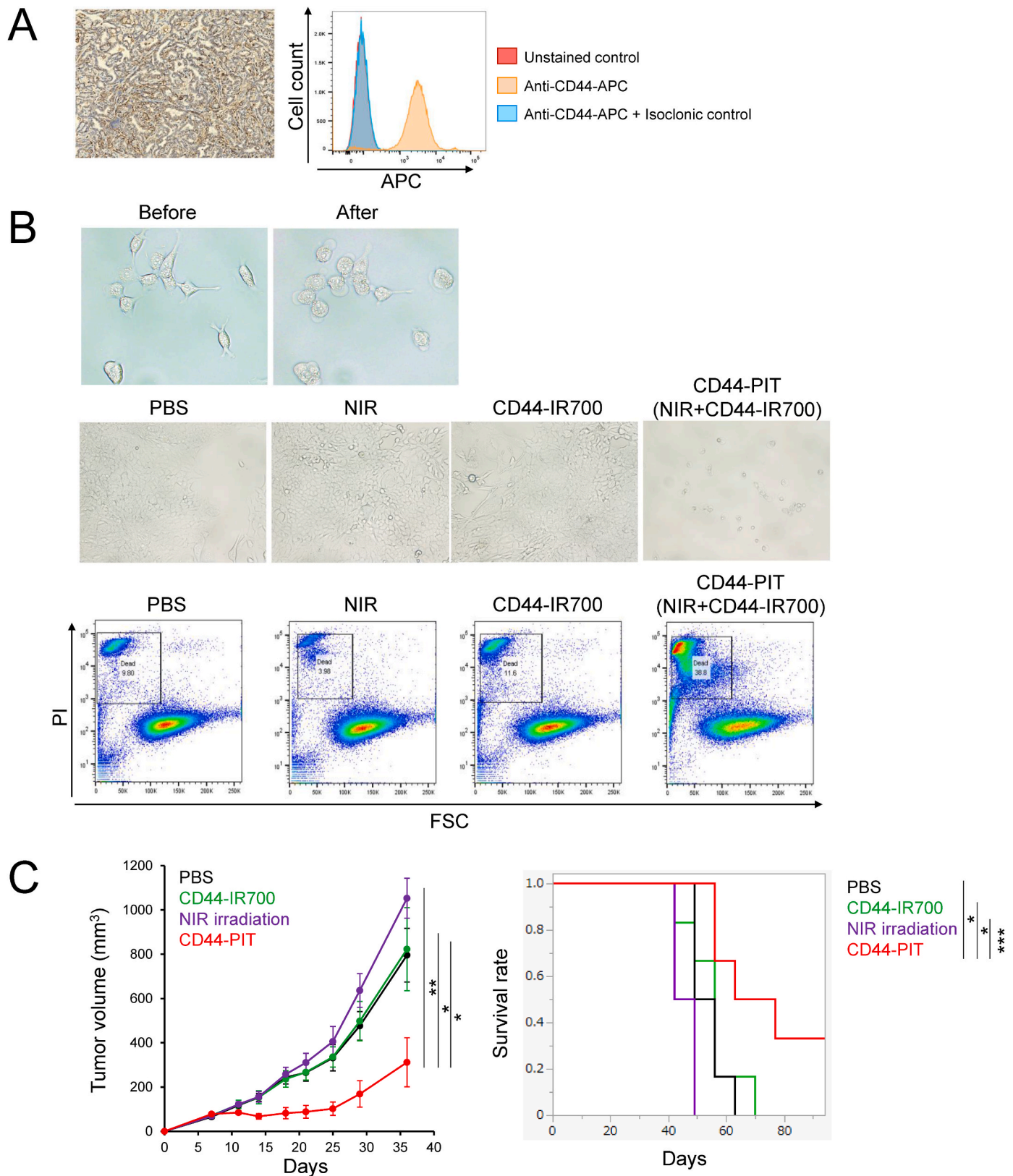


Fig. 1. Establishment of CD44-PIT. A. (Left panel) IHC staining of CD44 on KPC-N. (Right panel) FACS of KPC-N stained with anti-CD44-APC, using unstained and anti-CD44-APC plus isoclonic controls as negative controls. B. In vitro cell destruction of CD44-PIT in KPC-N. (Upper panels) the morphological changes of KPC-N before and 30 min after CD44-PIT. (Middle panels) the morphological changes of KPC-N 3 days after the treatment of either PBS, NIR irradiation, CD44-IR700, or CD44-PIT. (Lower panels) PI staining of KPC-N 30 min after CD44-PIT with the same control groups. C. Tumor volume (left panel) and survival (right panel) were monitored for KPC-N-bearing mice treated with PBS, CD44-IR700 i.t. (10 μ g on day7), NIR irradiation (days 8 and 9), or CD44-PIT (CD44-IR700 i.t.+NIR irradiation, the same dose, light intensity, and schedule as respective monotherapy) (n = 6). Error bars represent the mean $\pm$ SEM. Statistically significant differences were measured by one-way ANOVA with Tukey–Kramer Tests. *: p < 0.05, **: p < 0.01, ***: p < 0.001. i.t., intratumoral injection.

Accordingly, we have previously reported the potent antitumor activity of K3-SPG-ISV in a preclinical pancreatic cancer model [9].

Conventionally, ISV is performed only by adjuvant injection; however, we hypothesize that enforced antigen release by local tumor destructive therapy enhances the effect of ISV. Near-infrared photodynamic therapy (NIR-PIT) is a novel cancer therapy using a conjugate of near-infrared photosensitizer IR700 and an antibody targeting surface tumor antigen, which enables highly selective ablation of cancer cells exclusively within the NIR irradiated region [10]. The original concept of NIR-PIT is a method of local tumor destruction that does not aim to induce systemic antitumor immunity. However, we reason that NIR-PIT might be an optimal complementary modality to ISV because it might have advantages over other local therapies in that it could achieve tumor antigen release by highly selective tumor killing without damaging the surrounding immune environment.

Here, we demonstrate the potent antitumor effect of combination of K3-SPG-ISV and CD44-targeting-NIR-PIT (CD44-PIT). Our results shed light on the potential indication of this combination approach for pancreatic cancer that is clinically accessible via endoscopy.

2. Materials and methods

2.1. Mice

C57BL/6 mice were purchased from Shimizu Laboratory Supplies Japan and maintained under specific pathogen-free conditions. No specific sex-selection method was used in this study. The protocols for mouse experiments were approved by the Institutional Animal Care and Use Committee and Ethics Committee of the Kyoto University Graduate School of Medicine (Med Kyo 22207). All animal experiments were performed in accordance with ARRIVE guidelines.

2.2. Cell lines and reagents

KPC-N and MC38 were documented and cultured as previously described [9]. K3-SPG was prepared as previously described [8].

2.3. Synthesis of IR700-conjugated anti-CD44 antibody

IRDye700DX NHS ester (IR700, hereafter) was obtained from LI-COR Biosciences. The conjugation of IR700 with a monoclonal antibody was previously described [10]. Briefly, anti-CD44 antibody (1 mg, 6.7 nmol/L) was incubated with IR700 (65.1 μ g, 33.3 nmol/L) in 0.1 mol/L Na₂HPO₄ (pH 8.5) at room temperature for 1h, followed by the purification with a Sephadex G50 column (PD-10; Cytiva). Protein concentration was determined using a Coomassie Plus Protein Assay Kit (Thermo Fisher Scientific Inc.). IR700 concentration was measured by absorption at 689 nm using spectroscopy to confirm the number of fluorophore molecules conjugated to each monoclonal antibody. An average of three IR700 molecules was controlled to bound to a single antibody. We have abbreviated IR700-binding anti-CD44 as CD44-IR700.

2.4. In vivo mouse studies

KPC-N- or MC38-bearing mice were established as previously described [9]. At the indicated time points and doses described in the figure legends, mice were injected intratumorally with K3-SPG and/or intratumorally with CD44-IR700, with or without intraperitoneal administration of anti-PD-1 antibody. For T cell depletion, anti-CD8 α antibody (200 μ g) was intraperitoneally injected twice weekly, starting 3 days before K3-SPG injection. For isotype controls, rat IgG2b (200 μ g) was used according to the same protocol. The antibodies used for in vivo studies are listed in Supplementary Table. The tumor length (L) and width (W) were measured at least twice per week. Tumor volume (V) was calculated using formula $V = L \times W \times W/2$. The endpoint criteria

for survival studies included tumor volume >2000 mm³.

2.5. NIR-PIT

In vitro, KPC-N or MC38 cultured overnight in a 6-well plate were incubated with CD44-IR700 (10 μ g/ml) for 6h at 37 °C, irradiated with red-light-emitting diode (670–710 nm, MLL-III-690-1300mW, CNI Laser) at 10J/cm² (50 mW/cm²). In vivo, one and two days after CD44-IR700 injection, NIR light was irradiated at 100J/cm² (400 mW/cm²). The output density was measured using an optical power meter (TS2, CNI Laser).

2.6. Immunohistochemistry

Immunohistochemical analysis was performed as previously described [9]. The antibody and immunoperoxidase labeling reagents are listed in Supplementary Table.

2.7. FACS

FACS was performed as previously described [9]. Antibodies and the reagents used for detection of dead cells are listed in Supplementary Table. Splenocytes for CD8 T cell staining were prepared as previously described [9].

2.8. RNA-seq

Details of RNA extraction, sequencing, data processing, quantification of gene expression, and gene set enrichment analysis (GSEA) are described in Supplementary Method.

2.9. Statistics

Differences between groups were analyzed using one-way analysis of variance (ANOVA) with Tukey–Kramer test for pairwise comparisons or Dunnett's test for comparisons with control. Survival curves were assessed using log-rank tests. Statistical analyses were performed using JMP Pro version 17.0.0 (SAS Institute Inc.). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Establishment of CD44-PIT

CD44 was selected as the target surface antigen for NIR-PIT. CD44 expression on KPC-N (pancreatic cancer cell line) and MC38 (colorectal cancer cell line) was observed by immunohistochemistry and FACS (Fig. 1A and Supplementary Fig. 1A). Microscopic analysis of KPC-N treated with CD44-PIT revealed that NIR-PIT caused rapid swelling and bursting of treated cells after 30 min (Fig. 1B upper panels), and that most of them died after 3 days of culture (Fig. 1B middle panels). PI staining also revealed CD44-PIT-induced cell death (Fig. 1B lower panels). Similar results were observed for CD44-PIT-treated MC38 (Supplementary Fig. 1B). In KPC-N-bearing mice, CD44-NIR-PIT suppressed tumor growth and prolonged survival compared to the control group (Fig. 1C). Tumor suppression and prolonged survival were also observed in CD44-PIT-treated MC38-bearing mice (Supplementary Fig. 1C). Together, CD44-PIT effectively destroyed target cells in vitro and in vivo in multiple cancer models, validating CD44-PIT for further use in ISV experiments.

3.2. K3-SPG-ISV/CD44-PIT combination exerts synergistic and systemic antitumor effect and enhances anti-PD-1 blockade

We assessed the antitumor effects of K3-SPG-ISV in combination with CD44-PIT in KPC-N-bearing mice. For K3-SPG-ISV, our previous study

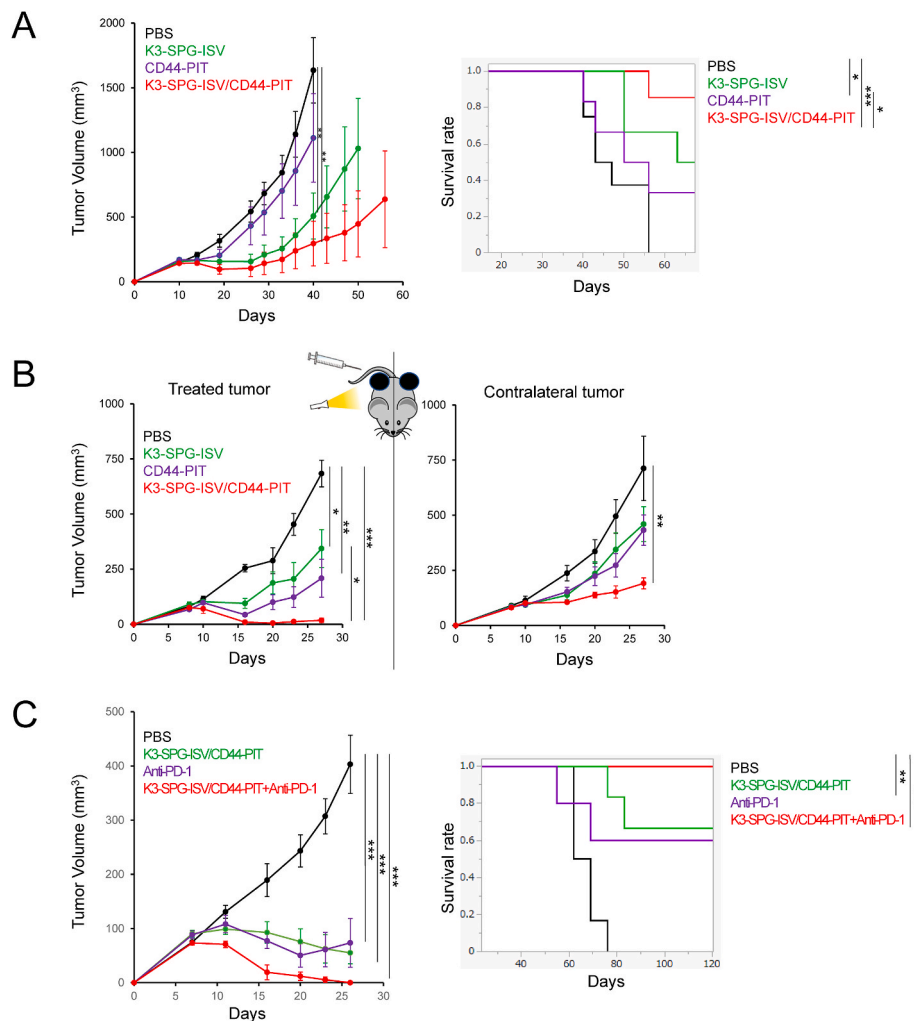


Fig. 2. K3-SPG-ISV/CD44-PIT synergistically and systemically suppresses tumor growth and enhances anti-PD-1 blockade. A. Tumor volume (left panel) and survival (right panel) were monitored in KPC-N-bearing mice treated with PBS, K3-SPG-ISV (10 μ g i.t. on day10), CD44-PIT (CD44-IR700 10 μ g i.t. on day10, followed by NIR irradiation on days 11 and 12), or K3-SPG-ISV/CD44-PIT (the same dose, light intensity, and schedule as respective monotherapy) (n = 6–8). B. Bilaterally KPC-N-bearing mice were treated with PBS, K3-SPG-ISV (10 μ g i.t. on day8), CD44-PIT (CD44-IR700 5 μ g i.t. on day8, followed by NIR irradiation on days 9 and 10), or K3-SPG-ISV/CD44-PIT (the same dose, light intensity, and schedule as respective monotherapy) on right-sided tumor (n = 6). Tumor volumes on the treated (left panel) and contralateral (right panel) sides were monitored. C. Tumor volume (left panel) and survival (right panel) were monitored in KPC-N-bearing mice treated with PBS, K3-SPG-ISV/CD44-PIT (K3-SPG 10 μ g i.t. on day7, CD44-IR700 5 μ g i.t. on day7, followed by NIR irradiation on days 8 and 9), anti-PD-1 antibody (200 μ g i.p. on days 7, 14, 21), or K3-SPG-ISV/CD44-PIT plus anti-PD-1 antibody (same dose, light intensity, schedule as respective therapy) (n = 5–6). Error bars represent mean $\pm$ SEM. Statistically significant differences were measured using one-way ANOVA with Tukey–Kramer Tests. * : p < 0.05, ** : p < 0.01, *** : p < 0.001. i.t., intratumoral injection. i.p., intraperitoneal injection.

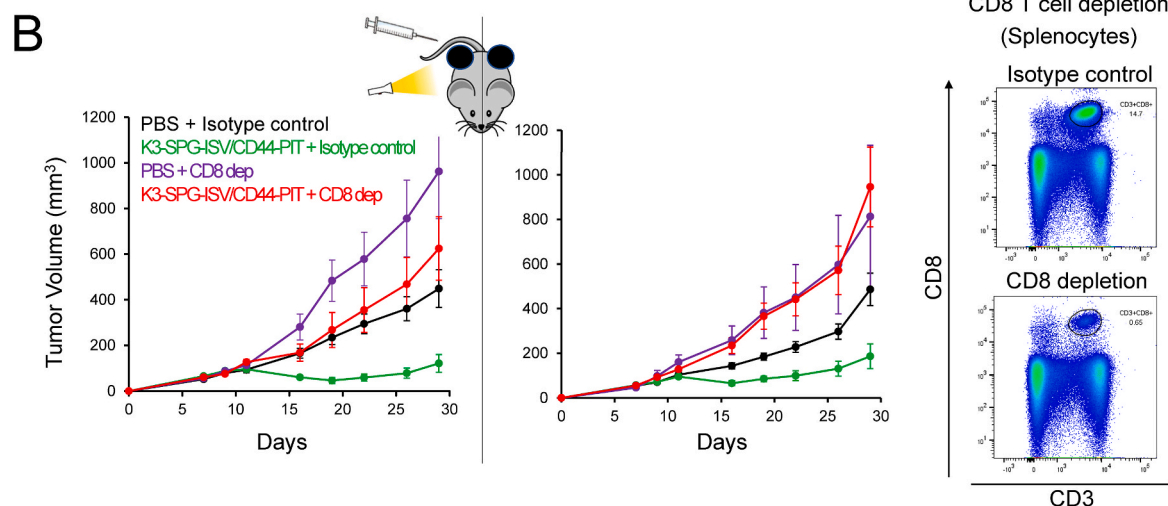
adopted multiple shots of K3-SPG [9]; however, it is preferable to reduce the number of punctures as much as possible for future clinical application. Therefore, in this study, we adopted a single-shot protocol for K3-SPG-ISV. K3-SPG-ISV reduced tumor growth to approximately half that of the control group, while CD44-PIT showed weaker tumor suppression compared to that shown in Fig. 1C (Fig. 2A left panel), presumably reflecting the delay in CD44-PIT initiation in this experimental setting. K3-SPG-ISV/CD44-PIT combination showed a synergistic anti-tumor effect (Fig. 2A left panel) and prolonged survival (Fig. 2A right panel). The synergism of this combination was reproduced in the experiment using MC38-bearing mice, in which the intensity of CD44-PIT was reduced by low-dose administration of CD44-IR700 and initiation of CD44-PIT at later time points (Supplementary Fig. 2).

In bilaterally KPC-N-bearing mice, either K3-SPG-ISV or CD44-PIT alone showed systemic antitumor effects, as revealed by tumor growth inhibition on the contralateral side as well as the treated side (Fig. 2B). K3-SPG-ISV/CD44-PIT synergistically enhanced the systemic antitumor effects of both monotherapies (Fig. 2B).

We evaluated the effect of K3-SPG-ISV/CD44-PIT on PD-1 blockade. In KPC-N-bearing mice, weekly administration of anti-PD-1 antibody suppressed tumor growth at a level comparable to that of K3-SPG-ISV/CD44-PIT, whereas the combination of K3-SPG-ISV/CD44-PIT and PD-1 blockade showed synergistic effects of each respective therapy (Fig. 2C, left panel) and prolonged survival compared to each individual treatment group or no-treatment control (Fig. 2C, right panel).

3.3. K3-SPG-ISV/CD44-PIT induces a broad array of IFN-related genes in the tumor microenvironment and its antitumor activity is CD8 T cell dependent

We conducted RNA-seq to comprehensively analyze the gene expression profiles in the K3-SPG-ISV/CD44-PIT-treated and contralateral tumors, along with untreated controls. Enrichment plots from GSEA and corresponding heat maps of upregulated genes clearly demonstrated the activation of IFN α (type I IFN)- and IFN γ (type II IFN)-mediated pathways in these bilateral tumors, with strong positive enrichment



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Fig. 3. K3-SPG-ISV/CD44-PIT induces a broad array of IFN-related genes in the tumor microenvironment and its antitumoral activity is CD8 T cell dependent. A. Bilaterally KPC-N-bearing mice were treated with PBS or K3-SPG-ISV/CD44-PIT (K3-SPG 10 μ g i.t. on day8, CD44-IR700 5 μ g i.t. on day8, followed by NIR irradiation on day 9) for right-sided tumors (n = 3). Bilateral tumors excised on day 13 after tumor implantation were analyzed by RNA-seq. (Left two panels) The results of treated (sample) and untreated tumors (control). (Right two panels) The results of contralateral (sample) and untreated tumors (control). Both sets display the enrichment plots of IFN α - and IFN γ -mediated pathways and corresponding heat maps of top 50 upregulated genes. NES, normalized enrichment score. FDR, false discovery rate. B. Bilaterally KPC-N-bearing mice were treated with PBS or K3-SPG-ISV/CD44-PIT (the same protocol described in Fig. 2B) at right-sided tumor, together with either an isotype control or anti-CD8 α antibody for CD8 T cell depletion (n = 5). Either antibody was administered intraperitoneally twice weekly for the duration of the experiment, starting on day5. Tumor volumes on the treated (left panel) and contralateral (middle panel) sides are shown. (Right panel) FACS on day29 demonstrated the depletion of CD8 T cells in the splenocytes of anti-CD8 α -treated mice. Error bars represent mean $\pm$ SEM. i.t., intra-tumoral injection.

scores (Fig. 3A). These results presumably reflect direct type I IFN induction by K3-SPG and type II IFN produced by infiltrating antitumor T cells in the treated tumors, while ISG expression in contralateral tumors may be primarily attributed to type II IFN from infiltrating T cells. The overlap in gene expression between type I and II IFNs [11] may explain the similar ISG profiles observed in both tumor locations.

The consequence of innate immune activation is the induction of antitumor CD8 T cells. Under the condition that >95 % depletion of CD8 T cells was achieved (Fig. 3B, right panel), CD8 T cell depletion canceled the antitumor activity of K3-SPG-ISV/NIR-PIT on the treated and contralateral sides, indicating that this combination therapy systemically suppressed tumor growth in a CD8 T cell-dependent manner (Fig. 3B, left panel). CD8 T cell depletion resulted in worsening of tumor growth in the non-treated cohort, suggesting that spontaneously induced antitumor CD8 T cells suppressed tumor progression to some degree.

3.4. K3-SPG-ISV/CD44-PIT induces durable immunological memory in therapeutic and neoadjuvant settings

We assessed the potential of K3-SPG-ISV/CD44-PIT to induce immunological memory in a therapeutic setting (Fig. 4A). As shown in Fig. 4B, KPC-N-bearing mice treated with K3-SPG-ISV/CD44-PIT were rechallenged with KPC-N on the opposite side to mimic recurrence (upper middle and right panels). All cured mice completely rejected the rechallenged tumor cells (lower left panel). In clear contrast, all non-cured mice and age-matched tumor-naïve mice failed to reject the implanted KPC-N (lower middle and right panels). These results indicated that immunological memory was induced in mice that had a complete response to K3-SPG-ISV/CD44-PIT.

Neoadjuvant immunotherapy is an innovative approach applied before curative treatments such as surgical resection with the aim of suppressing recurrence [12]. Since the ISV strategy is harmonious with neoadjuvant immunotherapy, we determined whether K3-SPG-ISV/CD44-PIT is effective in a neoadjuvant setting. The implanted tumors of KPC-N-bearing mice treated with or without K3-SPG-ISV/CD44-PIT were excised one week after the first day of treatment, followed by KPC-N rechallenge 99 days after tumor excision, together with the age-matched tumor-naïve control mice (Fig. 4C). As shown in Fig. 4D, in the neoadjuvant immunotherapy-treated mice (middle panel), tumor recurrence was completely inhibited. In contrast, in both the no-neoadjuvant cohort (left panel) and the age-matched tumor-naïve cohort (right panel), tumor cells engrafted and proliferated, indicating the efficacy of K3-SPG-ISV/CD44-PIT as a neoadjuvant immunotherapy.

4. Discussion

There have been ISV strategies that incorporate local tumor destruction methods [13,14], the general concept of which is to leverage tumor-associated antigen release during tumor cell death, thereby maximizing the antitumor effect of ISV. The present study is the first report that incorporate NIR-PIT into ISV.

The highly selective killing of cancer cells while leaving host tissues undamaged is a notable advantage of NIR-PIT over radiotherapy,

thermal ablation, and photodynamic therapy (PDT), which can harm nearby normal cells, including immune cells. One of the limitations of NIR-PIT is that the penetration depth of NIR light is only approximately 20 mm [15], restricting its use in superficial tumors, although it is still deeper than that of PDT. The development of cylindrical diffusing fiber optics that can be inserted via needles may overcome this limitation [16]. Since tumor puncture by EUS is a routine clinical practice for pancreatic cancer, NIR irradiation using such a cylindrical diffuser system under EUS guidance is feasible. Importantly, both NIR irradiation and intratumoral adjuvant injection can be achieved in a single puncture, which is critical from a clinical safety point of view.

We selected CD44 as a target antigen for NIR-PIT because CD44 is highly expressed on various cancer types including pancreatic cancer, and importantly, it is a well characterized cancer stem cell marker [17, 18]. Therefore, CD44-targeting NIR-PIT offers the advantage of eliminating cancer stem cells, thereby reducing the risk of drug resistance and recurrence. However, targeting CD44 has limitations. One issue is the expression of CD44 in immune cells, which could lead to inadvertent damage and interfere with the immune response. Another limitation is the heterogeneous expression of CD44 within the tumor, which may result in incomplete treatment and recurrence—an issue that is less problematic in radiotherapy and thermal ablation. To overcome these limitations, strategies to protect immune cells, such as using selective delivery systems or optimizing the timing and dosage of the therapy, should be considered. It might also be reasonable to apply a combination approach targeting multiple antigens, taking advantage of NIR-PIT that administration of antibody cocktail is feasible.

For a successful ISV, the choice of adjuvant is critical. We focused on K3-SPG, one of CpG ODNs, because CpG ODNs have been most extensively studied and have a well-documented safety profile in various clinical settings of cancer immunotherapy [7,19]. Although clinical trials of K3-SPG have not yet been conducted to date, given that K3-SPG has advantages over any other class of TLR9 ligands, especially regarding the capability of type I IFN induction [8], utilization of K3-SPG could be one of the best options for NIR-PIT-incorporating ISV. On the other hand, our strategy can also be applied to adjuvants other than TLR9 ligands. Since a number of clinical studies of ISV testing adjuvants targeting innate sensors other than TLR9 have been conducted [20], experiments to verify the usefulness of these adjuvants in the context of the present study are warranted in the future.

The immunosuppressive tumor microenvironment (TME), with the dense stroma characteristic of pancreatic cancer, hinders the infiltration and effectiveness of immune cells [21]. Despite these limitations, recent early phase clinical trials of KRAS mutation-targeting vaccines [4] and personalized mRNA vaccines [5] were reported to be promising. Therefore, our vaccine strategy is expected to be effective in humans, either in combination with PD-1 inhibition or alone, as demonstrated in this preclinical study (Fig. 2). Co-administration of antibodies targeting CD44 and fibroblast activation protein followed by NIR-PIT might not only kill cancer cells but also reduce stromal components such as cancer-associated fibroblasts, thereby overcoming the immunosuppressive TME. Alternatively, breaking the immunosuppressive shield by administration of hyaluronidase might enhance the effect of ISV, although clinical trials of human hyaluronidase PEGPH20 in combination with PD-L1 blockade were negative for pancreatic cancer [22].

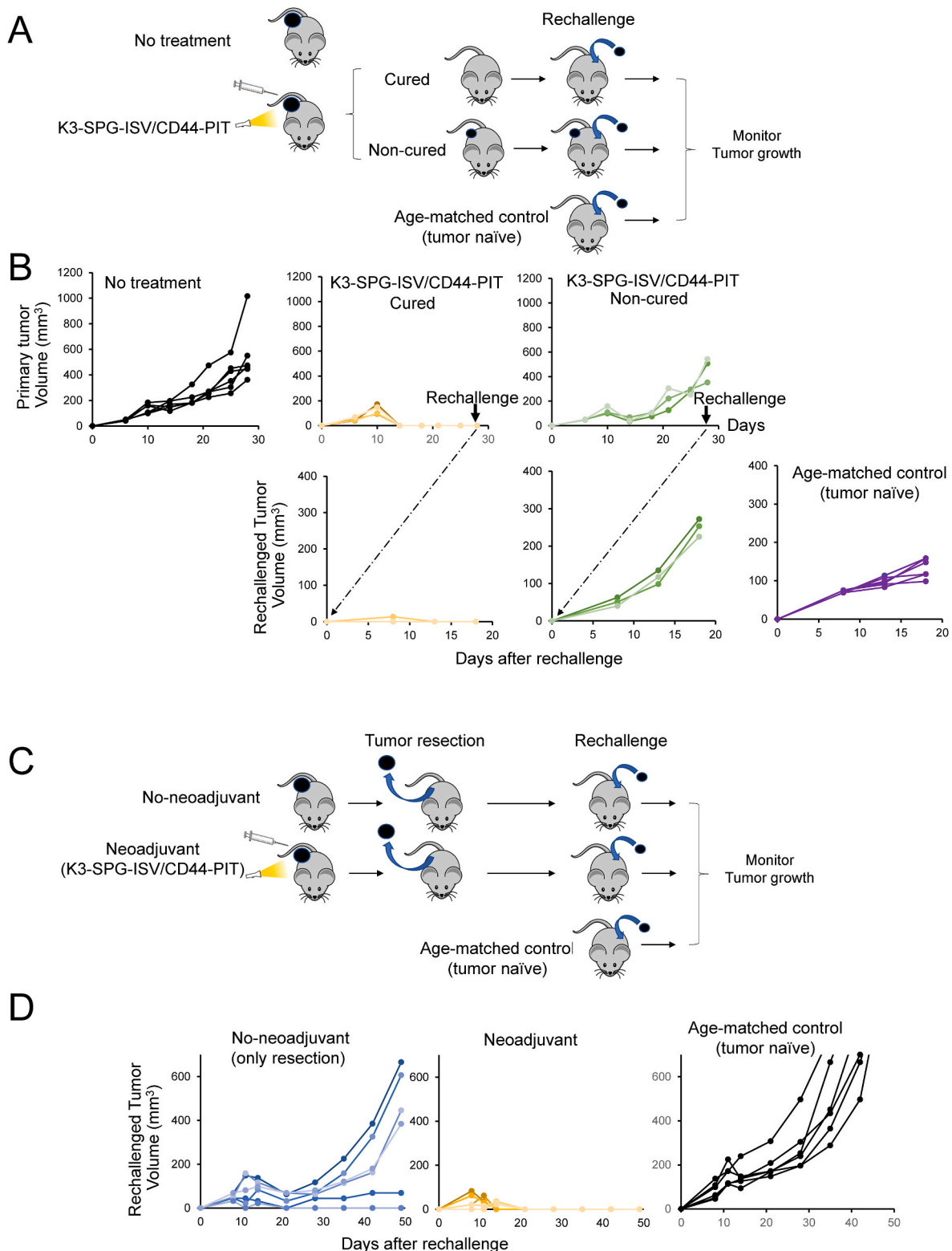


Fig. 4. K3-SPG-ISV/CD44-PIT induces immunological memory and inhibits tumor recurrence in therapeutic and neoadjuvant settings. **A.** Schematic of the experiment in the therapeutic setting. **B.** KPC-N-bearing mice were treated with PBS (no treatment) or K3-SPG-ISV/CD44 -PIT (K3-SPG 10 μ g i.t. on day8, CD44-IR700 10 μ g i.t. on day8, followed by NIR irradiation on days 9 and 10) (PBS: $n = 6$, K3-SPG-ISV/CD44-PIT: $n = 7$). Four K3-SPG-ISV/CD44-PIT treated mice were cured (upper middle panel), and the remaining three had residual tumors (upper right panel). On day27, these seven mice were rechallenged with 1.5×10^6 KPC-N on the opposite side of treatment. As a control cohort, age-matched naïve mice were implanted with the same number of KPC-N ($n = 6$). (Lower panels) Individual tumor growth curves after KPC-N implantation are presented. **C.** Schematic of the experiment in the neoadjuvant setting. **D.** KPC-N-bearing tumors were treated with PBS (no-neoadjuvant) or K3-SPG-ISV/CD44-PIT (neoadjuvant: K3-SPG 10 μ g i.t. on day7, CD44-IR700 10 μ g i.t. on day7, followed by NIR irradiation on days 8 and 9) ($n = 6$). The tumors were excised 14 days after tumor implantation, and 99 days after tumor excision, 1.5×10^6 KPC-N were rechallenged. As another control cohort, age-matched tumor-naïve mice were implanted with the same number of KPC-Ns on the same day ($n = 6$). Individual tumor growth curves after KPC-N implantation are presented. i.t., intratumoral injection.

Elucidation of the interplay between TME and ISV would improve treatment efficacy.

We demonstrated the antitumor activity of K3-SPG-ISV/CD44-PIT in both therapeutic and neoadjuvant settings (Fig. 4). Perioperative (*i.e.* adjuvant and neoadjuvant) immunotherapies are advantageous over conventional therapeutic immunotherapies because early stage cancers are expected to be less immunosuppressed [23]. Since neoadjuvant immunotherapy is administered while the primary tumor remains intact, the ISV approach in neoadjuvant settings would fully leverage the tumor itself as a source of antigens to generate stronger and more diverse immune response. Future clinical studies are warranted to validate the concept of K3-SPG-ISV/CD44-PIT in neoadjuvant setting.

In summary, we first reported the incorporation of NIR-PIT into ISV as a method for local tumor destruction to enhance the antitumor effect of ISV. In a preclinical pancreatic cancer model, K3-SPG-ISV/CD44-PIT induced strong systemic antitumor effects and immunological memory, and enhanced anti-PD-1 therapy, suggesting the potential of NIR-PIT as a complementary modality to ISV. This combination can be applied in both therapeutic and neoadjuvant settings. For clinical translation to pancreatic cancer, it is particularly important to develop optimal devices for NIR-PIT and effective methods to reverse the immunosuppressive TME.

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CRediT authorship contribution statement

Hiroaki Yaku: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ken Takahashi:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Hirokazu Okada:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing – review & editing. **Kouji Kobiyama:** Funding acquisition, Investigation, Methodology, Writing – review & editing. **Masahiro Shiokawa:** Methodology, Writing – review & editing. **Norimitsu Uza:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing. **Yuzo Kodama:** Conceptualization, Writing – review & editing. **Ken J. Ishii:** Funding acquisition, Investigation, Methodology, Writing – review & editing. **Hiroshi Seno:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2024.150534>.

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