Treatment with Nivolumab and All-Trans Retinoic Acid for Patients with Refractory Pancreatic Cancer

Protocol

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1. Abstract

Pancreatic cancer is an aggressive and lethal disease. Even two combination regimens, FOLFIRINOX and albumin-bound paclitaxel in combination with gemcitabine, are superior to gemcitabine alone as the first-line treatments, the prognosis of patients with locally advanced or metastatic pancreatic cancer remains poor, with a median overall survival of 8 to 11 months and an estimated 2-year survival of only 2%. Even more, there is currently no available treatment proven beneficial for patients who fail the second-line therapy with liposomal irinotecan in combination with 5-FU.

Immune checkpoint inhibitors targeting PD-1 or PD-L1 represent a popular treatment option for patients with some kinds of cancers, but previous studies in pancreatic cancer have failed. One critical challenge for immunotherapy in pancreatic cancer is to find efficient therapeutic approaches to reverse this “cold” tumor into a “hot” tumor. Our previous basic studies revealed that all-trans retinoic acid repressed pancreatic adenocarcinoma cell growth and colony formation. Set-to-set genetic analysis revealed that genes regulated by all-trans retinoic acid were predominantly involved in immune response including interleukin-6, tumor necrotic factor-α, IFNs signaling, and PD-L1. Furthermore, mice bearing pancreatic cancer treated with combination of anti-PD-1 and all-trans retinoic acid had a significantly longer survival compared to mice treated with anti-PD-1 or all-trans retinoic acid alone.

Based on the previous findings, we propose this pilot, compassionate use of combination therapy with all-trans retinoic acid and nivolumab for patients with locally advanced or metastatic pancreatic adenocarcinoma who are refractory to current available treatment. This study will also examine ADAR1 as a novel prognostic and predictive biomarker for pancreatic adenocarcinoma patients. This study will help to delineate the role of ADAR1 as a predictive biomarker for immunotherapy, all-trans retinoic acid as a suppressor of ADAR1, and most importantly, the combination of all-trans retinoic acid and nivolumab as an effective anticancer therapy for patients with pancreatic adenocarcinoma.

2. Background

2.1. Pancreatic adenocarcinoma

The incidence of pancreatic cancer is rising, and it is predicted to become the second leading cause of cancer-related death by 2030 in the world (Kenner et al. 2016). The prognosis of patients with advanced or metastatic pancreatic cancer is poor, especially for those who have refractory pancreatic adenocarcinoma. Pancreatic adenocarcinoma is characterized by early metastasis, extensive desmoplasia (Armstrong et al. 2004) and non-immunogenic tumor microenvironment (Riquelme et al. 2018), which are the reasons leading to poor response to treatments.

Currently, potential therapeutic options for pancreatic adenocarcinoma are surgery, radiation, chemotherapy, targeted therapy, and immunotherapy. A chemotherapy drug, gemcitabine, is the standard therapy for pancreatic adenocarcinoma, although the resistance to gemcitabine has been a major challenge (Amrutkar et al. 2017). Gemcitabine resistance comes from several causes including abnormalities in drugs metabolism and desmoplastic reaction caused impairment of drug delivery (Ghaneh et al. 2008). Furthermore, the fact that immunosuppressive tumor microenvironment consistently impedes the functional activation of immune cells (Principe et al. 2016; Beatty et al. 2015), contributes to the low response of pancreatic adenocarcinoma cells for treatments and represents an additional problem in the design of novel therapies for this disease. Thus, one critical challenge of pancreatic adenocarcinoma treatment is to find efficient therapeutic approaches to reverse a “cold” into a “hot” tumor, priming immune response during pancreatic adenocarcinoma progression. The current proposal aims to identify a promising therapeutic approach, with a focus on boosting tumor-specific immunity for pancreatic adenocarcinoma patients.

2.2. Immunotherapy in pancreatic adenocarcinoma

Interferons (IFNs) are cytokines that play a critical role in limiting infectious and suppressing tumor progression due to interferon-stimulated genes expression such as TNF-α, IL-6 and IFNs per se (Schoggins et al. 2019). However a subset of interferon-stimulated genes act as negative pathway regulators and immunosuppressive factors to extinguish signaling and limit therapeutic efficiency of cancers, for example, PD-L1 (Benci et al. 2016). To

date, immune checkpoint inhibitors targeting PD-1 or PD-L1 represent a popular treatment option for patients with certain cancers (Wu et al. 2019). However, a part of cancer patients including pancreatic adenocarcinoma endure resistance to anti-PD1/PD-L1 immunotherapies because of loss of IFNs signaling (de Erauso et al. 2020).

2.3. Adenosine deaminase acting on RNA 1 (ADAR1)

Recently, emerging data suggest that a member of interferon-stimulated genes, double-stranded RNA-specific adenosine deaminase (ADAR1) which is an RNA editing enzyme and a negative regulator of IFNs signaling (Figure 2), promotes immune silencing and tumor viability (Herbert et al. 2019). Several reports indicated reducing ADAR1 expression in tumors increases sensitivity to checkpoint inhibitors (Liu, et al. 2019; Gannon et al. 2018), suggesting that suppression of ADAR1 may be a promising strategy for tumor immunotherapy for cancer patients. However, there is limited understanding of how to inhibit ADAR1 for application of cancer therapy.

2.4. Retinoic acid and pancreatic adenocarcinoma

Retinoic acid plays important roles in cell development, differentiation (Gudas et al. 2011), immune regulation (Larange et al. 2016) as well as tumor progression (Tang et al. 2011). Except for its cytotoxic effect on tumor cells (Shilkaitis et al. 2015), it has been reported that retinoic acid enhances protective antitumor immunity through mechanisms such as induction of cell differentiation and inhibition of immunosuppressive cells (Malkovský et al. 1983; Walkley et al. 2002). In 2004, all-trans retinoic acid (Vesanoid) was approved by FDA to induce remission in patients with acute promyelocytic leukemia (Küley-Bagheri et al. 2018). Regarding the present clinical trials of all-trans retinoic acid in solid tumors, for example, breast cancers, all-trans retinoic acid does not have significant activity in patients with hormone-refractory, metastatic breast cancer (Sutton et al. 1997). These unexpected results of clinical trials emerging may be due to the ignored immune checkpoints up-regulated by all-trans retinoic acid. This proposal is raised for development of a potential therapeutic approach for pancreatic adenocarcinoma patients by regulating tumor immune response. Recently, our laboratory has found several important evidence to support the use of all-trans retinoic acid in pancreatic cancer.

2.5. Key evidence available to support the use of all-trans retinoic acid in pancreatic adenocarcinoma

2.5.1. All-trans retinoic acid represses pancreatic adenocarcinoma cells growth and triggers IFNs signaling

In our previous studies, all-trans retinoic acid represses pancreatic adenocarcinoma cell growth and colony formation (Figure 1A and 1B). Interestingly, set-to-set analysis of the significant gene sets regulated by all-trans retinoic acid revealed a strong overrepresentation of gene sets involved in immune response including interleukin-6, tumor necrotic factor (TNF)-α and IFNs signaling (Figure 1C).

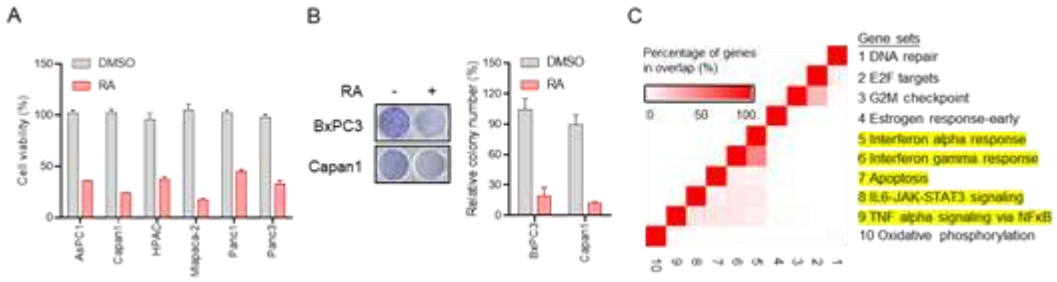


Figure 1. Retinoic acid represses pancreatic adenocarcinoma cells growth and triggers interferons signaling. (A) The effects of retinoic acid on pancreatic adenocarcinoma viability. (B) Colony formation assay. (C) GSEA leading-edge analysis of identified significant gene sets regulated by retinoic acid in MDA-MB231 cells. RA, retinoic acid.

2.4.2. All-trans retinoic acid and PD-L1 in pancreatic adenocarcinoma: all-trans retinoic acid enhances PD-L1 but represses ADAR1 expression

Due to IFNs signaling stimulated by all-trans retinoic acid, we further investigate the effect of all-trans retinoic acid on the downstream of IFNs-stimulated genes. Intriguingly, all-trans retinoic acid specifically induces the expression of PD-L1 instead of other immune checkpoints regulated by IFNs (Figure 2A and 2B). More importantly, ADAR1, a member of interferon-stimulated genes and a negative regulator of IFNs signaling, is decreased by all-trans retinoic acid (Figure 2B). The set-to-set analysis of the

significant gene sets indicates that knockdown of ADAR1 enriches immune gene profiles which are similar to those regulated by all-trans retinoic acid (Figure 2C), implying that all-trans retinoic acid triggers an intense IFNs signaling due to blockage of a negative regulator, ADAR1.

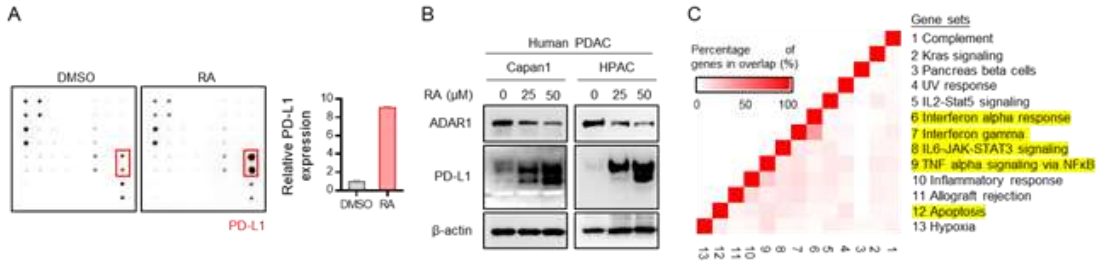


Figure 2. Retinoic acid enhances PD-L1 but represses ADAR1 expression. (A) Representative immune checkpoint array comparing vehicle control (DMSO) versus retinoic acid in HPAC cells. Quantitative data for the relative PD-L1 expression are determined with ImageJ software and represented at the right. (B) Western blot analysis of ADAR1, PD-L1 and β-actin. Cells were treated with retinoic acid with the dose as indicated for 24 h. (C) GSEA leading-edge analysis of identified significant gene sets in ADAR1-knockout H196 cells (GSE122168). RA, retinoic acid.

2.4.3. Combination treatment of anti-PD1 and all-trans retinoic acid improves survival rate in murine pancreatic cancer model

Since all-trans retinoic acid induced PD-L1 and inhibited ADAR1 expression, we further tested for any synergistic effect between anti-PD-1 blockade and all-trans retinoic acid. To this end, we analyzed the in vivo efficacy of all-trans retinoic acid and anti-PD-1 combination treatment in the murine syngeneic Pan02 pancreatic cancer model (Figure 3A). Mice treated with anti-PD-1 antibody and all-trans retinoic acid had significantly smaller tumors compared to mice receiving either monotherapy (Figure 3B). Furthermore, combination treatment was led to a significant improvement in survival compared to both anti-PD-1 and all-trans retinoic acid monotherapy groups (Figure 3C). The average mouse body weight did not differ significantly among all four groups (Figure 3D), suggesting that these regimens did not cause significant toxicity effects. The phenomenon that all-trans retinoic acid inhibit ADAR1 expression can be confirmed in syngeneic Pan02 pancreatic cancer model (Figure 3E).

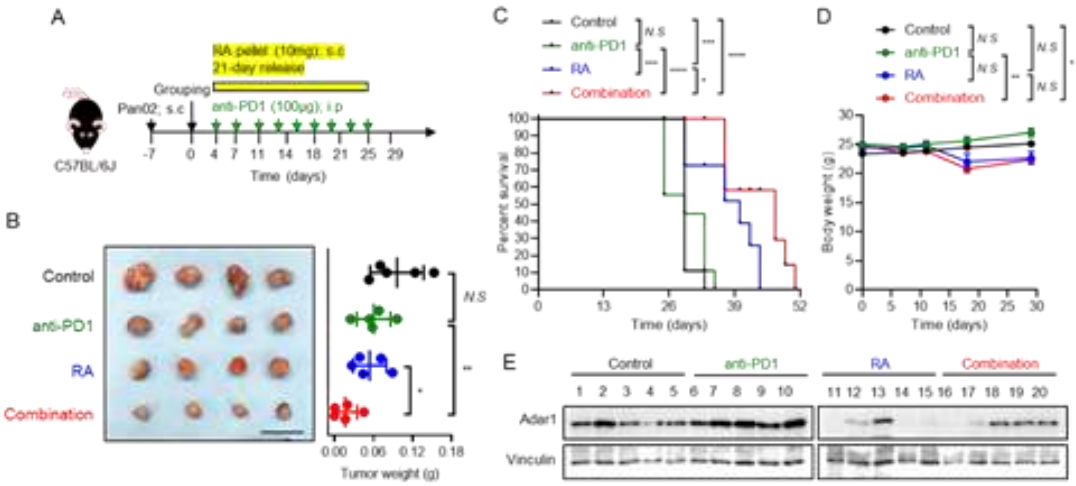


Figure 3. Anti-PD1 antibody and retinoic acid combination treatment improves antitumor effect in murine PDAC model. (A) The outline of schemed treatment strategy and tumor challenge. (B) Left, representative images showing tumors harvested from mice bearing Pan02 tumor cells received indicated treatments. Scale bar, 1.2 cm. Right, tumor weight. (C) Survival rate of mice after systemic therapy. (D) Body weight from all groups. (E) Western blot analysis of adar1 and vinculin protein expression levels in Pan02 cells from subcutaneous murine PDAC model treated with indicated regimens.

2.4.4. ADAR1 positively correlates with poor survival in cancer patients

The data from The Cancer Genome Atlas (TCGA) database shows that ADAR1 mRNA is upregulated in various cancers including pancreatic adenocarcinoma (Figure 4A). ADAR1 proteins positively correlates with poor survival among either breast cancer (p=0.0162 and p=0.0373 in two cohorts, respectively; Figure 4B; Liu et al. 2014) or pancreatic adenocarcinoma patients (p=0.0045; Figure 4C and 4D). Addition of gemcitabine, a cornerstone of pancreatic adenocarcinoma treatment, induces the expression of both ADAR1 and PD-L1 in vitro; however, all-trans retinoic acid can inhibit ADAR1 in gemcitabine treated pancreatic adenocarcinoma cells (Figure 4E). Next, we conducted GSEA to investigate which gene sets are associated with gemcitabine-resistant pancreatic adenocarcinoma. The results indicated gemcitabine-resistant pancreatic adenocarcinoma have negatively enriched hallmark gene sets including interferon-α response (NES = -1.195, $p < 0.0001$; Figure 4F). Since all-trans retinoic acid triggers interferon-α signaling and suppresses ADAR1 as well as induces PD-L1, combination treatment of all-trans retinoic acid and anti-PD1 might be a promising therapeutic strategy

for pancreatic adenocarcinoma patients who have failed gemcitabine chemotherapy.

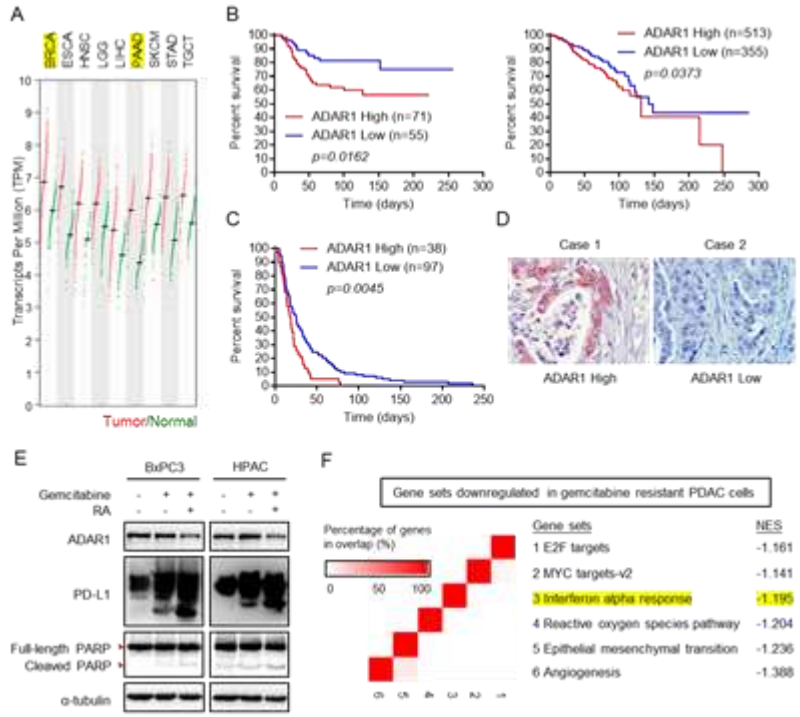


Figure 4. ADAR1 positively correlates with poor survival in cancer patients. (A) ADAR1 gene expression profiled by GEPIA. Tumor (T), red dots; normal tissues (N), green dots. BRCA, breast invasive carcinoma; ESCA, esophageal carcinoma; HNSC, head and Neck squamous cell carcinoma; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors. (B) The correlation between ADAR1 protein expression and breast cancer patients' survival in two cohorts (left, Liu et al., 2014; right, TCGA-RPPA). (C) The correlation between ADAR1 protein expression and PDAC patients' survival in MDA cohort. (D) Representative images of the immunohistochemical staining of ADAR1 in PDAC patients. (E) Western blot analysis showing the effects of combination treatment with gemcitabine and RA. BxPC3

In summary, all-trans retinoic acid represses ADAR1, a member of interferon-stimulated genes and a negative regulator of IFNs signaling. On the other hand, although all-trans retinoic acid simultaneously increases PD-L1 expression for cancer cells to evade immune surveillance, it can be overcome by blockade of PD1/PD-L1 immune checkpoints (Figure 5). Since combination of anti-PD1 antibody and all-trans retinoic acid may thoroughly block self-regulating negative feedback loops of IFNs response, this therapeutic strategy may improve outcomes of pancreatic adenocarcinoma patients.

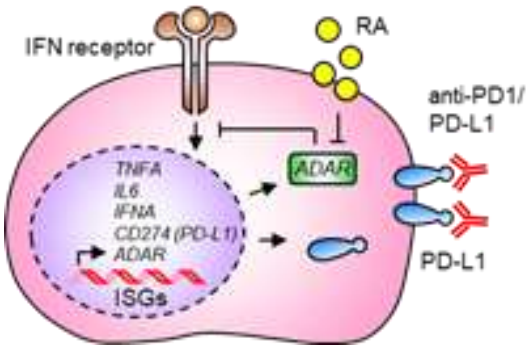


Figure 5. The schema of hypothetic model.

2.5. Proposal of all-trans retinoic acid in patients with refractory pancreatic adenocarcinoma

Based on the previous findings, we propose this pilot, compassionate use of combination therapy with all-trans retinoic acid and nivolumab for patients with locally advanced or metastatic pancreatic adenocarcinoma who are refractory to current available treatment. This study will also examine ADAR1 as a novel prognostic and predictive biomarker for pancreatic adenocarcinoma patients. The major goals of the study is to examine:

1. The anticancer activity of the combination therapy with all-trans retinoic acid and nivolumab in patients with advanced or metastatic pancreatic adenocarcinoma
2. The expression and change of ADAR1 as a prognostic and predictive biomarkers of immunotherapy for pancreatic adenocarcinoma.

This study will help to delineate the role of ADAR1 as a predictive biomarker for immunotherapy, all-trans retinoic acid as a suppressor of ADAR1, and most importantly, the combination of all-trans retinoic acid and nivolumab as an effective anticancer therapy for patients with pancreatic adenocarcinoma. In addition to be a proof of concept study, we hope this combination can be beneficial for patients with refractory pancreatic adenocarcinoma.

3. Objective

3.1. To evaluate the therapeutic efficacy of all-trans retinoic acid in combination with nivolumab in patients with advanced or metastatic pancreatic cancer.

3.2. To evaluate the role of ADAR1 as the predictive biomarker of immunotherapy for pancreatic cancer.

4. Drug information

4.1. Nivolumab

ONO-4538 is a human monoclonal antibody that targets the human PD-1 receptor and is produced in CHO cells by recombinant deoxyribonucleic acid (DNA) technology. The drug substance is an aqueous solution of ONO-4538 that is clear or opalescent and colorless or pale yellow; fine particles may be slightly observed in the solution. The drug product is stored away from light at 2℃–8℃. ONO-4538 specifically binds to the PD-1 receptor and has shown high affinity for human PD-1 and simian PD-1 with dissociation constant (KD) values of 3.06 nmol/L and 3.92 nmol/L, respectively. On the basis of the above, ONO-4538 is expected to exert its antitumor effect by inhibiting the binding between PD-1 and PD-1 ligand and thereby augmenting the activity of antigen-specific T cells, which results in an enhanced tumor-specific immune response. The safety profile of ONO-4538 monotherapy has shown no substantial difference across the tumor types without dose dependency in the incidence, severity, or causal relationship of adverse events and adverse drug reactions. Ongoing clinical studies are evaluating the safety of the combination use of ONO-4538 with other agents, including ipilimumab, cytotoxic chemotherapy agents, angiogenesis inhibitors, and molecular-targeted agents.

Currently, nivolumab has been proved by US FDA for the treatment of: melanoma, non-small cell lung cancer, small cell lung cancer, renal cell carcinoma, classical Hodgkin lymphoma, squamous cell carcinoma of the head and neck, urothelial carcinoma, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer, and hepatocellular carcinoma.

4.2. All-trans retinoic acid (Vesanoid)

Chemically, vesanoid is all-trans retinoic acid (tretinoin) and is related to retinol (Vitamin A). Vesanoid induces maturation of acute promyelocytic leukemia cells. It is available in a 10 mg soft gelatin capsule for oral administration. Each capsule also contains beeswax, butylated hydroxyanisole, edetate disodium, hydrogenated soybean oil flakes, hydrogenated vegetable oils and soybean oil. The gelatin capsule shell contains glycerin, yellow iron

oxide, red iron oxide, titanium dioxide, methylparaben and propylparaben. It is a yellow to light orange crystalline powder with a molecular weight of 300.44.

Vesanoid is not a cytolytic agent but instead induces cytodifferentiation and decreased proliferation of acute promyelocytic leukemia cells in culture and in vivo. In acute promyelocytic leukemia patients, vesanoid treatment produces an initial maturation of the primitive promyelocytes derived from the leukemic clone, followed by a repopulation of the bone marrow and peripheral blood by normal, polyclonal hematopoietic cells in patients achieving complete remission. The exact mechanism of action of vesanoid in acute promyelocytic leukemia is unknown.

The reported adverse effects of vesanoids include (US-FDA package inserts):

(1) Incidence≥10%：headache, fever, malaise, dry skin, bone soreness, hemorrhage, infection, edema, chest tightness, nausea, vomiting, skin rash, mucositis, itching, sweating, visual acuity abnormality, hair loss, abdominal pain, diarrhea, fullness, arrhythmia, flushing, hypotension, hypertension, dizziness, insomnia, depression

(2) Incidence ≥1-10%：visual field abnormality, flank soreness, cellulitis, skin palor, hypothermia, ascites, acidosis, dyspnea, pleural effusion, asthma, pulmonary edema, laryngeal edema, hepatosplenomegaly, hepatitis, oral ulcer, heart failure, anxiety, hallucination, unsteady gait, dysuria, renal dysfunction, enlarged prostate,

(3) Incidence≥0.1-1%：shearing impairment, hypercalcemia, pancreatitis, myositis, organomegaly, hyperbasophilemia

5. Selection of patients

5.1. Inclusion criteria:

Patients will be included in the study if they meet all of the following criteria:
(1) Patients with age ≥ 20 years old
(2) Histologically confirmed pancreatic adenocarcinoma
(3) Unresectable locally advanced, recurrent or metastatic diseases ineligible or unsuitable for further surgical or radiation interventions
(4) Documented disease progression within 6 months after standard chemotherapies or no available standard chemotherapy. The standard chemotherapies include gemcitabine, nab-paclitaxel, S-1, and FOLFIRINOX. Patient who has prior anti-PD1/anti-PD-L1 treatment will not be eligible.
(5) ECOG Performance Status 0-2
(6) Documented measurable disease as defined by RECIST v1.1
(7) Adequate hematologic parameters, and hepatic and renal functions defined as
a.absolute neutrophil count ≥ 1,000/μL
b.platelets ≥ 75,000/μL
c.total bilirubin ≤ 2.5X ULN (≤ 5X ULN if attributable to liver metastases)
d.AST/ALT ≤ 2.5X ULN (≤ 5X ULN if attributable to liver metastases)
e.serum creatinine ≤ 2 mg/dL or creatinine clearance ≥ 30 mL/min (by calculated or 24-hour urine collection)
(8) Normal ECG or ECG without any clinical significant findings
(9) Able to understand and sign an informed consent (or have a legal representative who is able to do so)

5.2. Exclusion criteria:

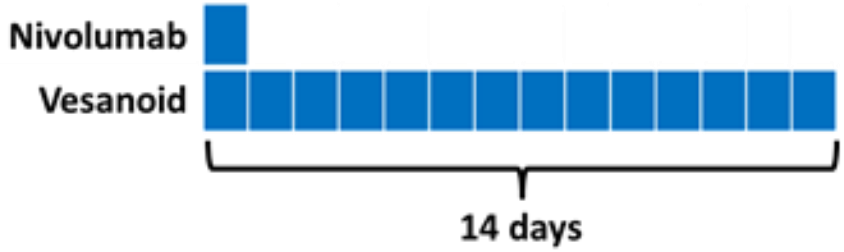
Patients will be excluded from the study if they meet any of the following criteria:
(1) History of allergic reaction to all-trans retinoic acid or nivolumab
(2) Patient with liver cirrhosis with Child-Pugh score ≥ 8 (Late Child-Pugh B and Child-Pugh C)
(3) Active CNS metastasis defined by clinical symptoms, cerebral edema, steroid or anti-convulsant requirement, or progressive growth. Patients with a history of CNS metastasis or cord compression are allowed in the study if they have been treated and are clinically stable

(4) With clinically significant gastrointestinal disorder including bleeding, inflammation, occlusion or diarrhea > grade 1
(5) With uncontrolled intercurrent illness that could limit study compliance or judged to be ineligible for the study by the investigators including, but not limited to, any of the following:
a. ongoing or active infection requiring antibiotic treatment
b. symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia
c. psychiatric illness or social situation that would preclude study compliance
(6) Pregnant or breast feeding women (a urine pregnancy test must be performed on all patients who are of childbearing potential before entering the study, and the result must be negative)
(7) Patients taking the following medications: immunosuppressants, corticosteroids with the exception of administration topically (e.g., external, intra-articular, intranasal, ophthalmic, or inhalational use) or temporarily (e.g., for treatment or prophylaxis of contrast medium allergy or adverse events), antitumor therapies (e.g., chemotherapies, molecular-targeted therapies, immunotherapies), radiopharmaceuticals with the exception of diagnostic purposes, transplant therapies, vitamin A, antifibrinolytic agents (tranexamic acid, aminocaproic acid, aprotinin), inducers (rifampicin, glucocorticoids, phenobarbital and pentobarbital) or inhibitors (ketoconazole, cimetidine, erythromycin, verapamil, diltiazem and cyclosporine) of the hepatic P450 system, and other unapproved drugs (e.g., investigational use of drugs, unapproved combined formulations, unapproved dosage forms).

6. Study treatment

6.1. Overall study design

6.1.1. Number of subject: 10
6.1.2. Study period: 2021.2.1-2022.12.31
6.1.3. Treatment plan
6.1.3.1. Patient will receive oral all-trans retinoic acid (Vesanoid) 45 mg/m^2 on days 1-14, and nivolumab 3mg/kg intravenously on day 1. The treatment cycle will repeat every 2 weeks.



6.1.3.2. The treatment will continue till disease progression, unacceptable toxicity, patients refusal, withdraw of consent, death, or not beneficial for patient by treating physician’s opinion.

6.2. Dosage modification and treatment delay

6.2.1. The dose of nivolumab will be kept without modification. The management of immune-related adverse effects and further use of nivolumab is according to the treatment guideline by The National Comprehensive Cancer Network (NCCN).
6.2.2. The treatment of all-trans retinoic acid will be hold if patient encounter grade 3 or higher hematological or non-hematological toxicities except alopecia or diarrhea which can be controlled by further medication. After resume, the dose of all-trans retinoic acid can be reduced to a lower level if patient has grade 3 non-hematological toxicity or recurrent grade 2 hematological toxicity.
6.2.3. All-trans retinoic acid dose modifications are permanent; no dose escalations are allowed after dose reduction. Any patient who has 2 dose reductions and experiences a toxicity that would cause a third dose reduction must be discontinued from the study drug which is causing the toxicity.

6.2.4 Dose of any drug omitted for toxicity is not replaced or restored; instead, the patient should resume the planned treatment cycles.
6.2.5. Dose modification for non-serious and no-life-threatening toxicities such as alopecia, altered taste, or nail changes may not be required. The final decision is left to the discretion of the treating investigator.
6.2.6. In situations where concomitant toxicities of varying severity exist, dose modification will be tailored by the toxicity with highest National Cancer Institute (NCI) Common Terminology Criteria for Adverse Effect (CTCAE) grade.
6.2.7. If there is delay or modification in administration of study drug due to toxicity, treatment with the other study agent should be discontinued.
6.2.8. If a toxicity related to any component of chemotherapy does not resolve in the same treatment cycle, the administration of that component can be delayed up to 28 days from the next planned dose of the component. If the toxicity does not resolve within 28 days, patient will be withdraw from the study.

6.3. Concomitant therapy

6.3.1. Prohibited therapies through study period
The following treatments are prohibited throughout the study period (i.e., from informed consent through completion of the protocol-specified final examination), unless absolutely necessary for medical reasons.
(1) Immunosuppressants
(2) Corticosteroids with the exception of administration topically (e.g., external, intra-articular, intranasal, ophthalmic, or inhalational use) or temporarily (e.g., for treatment or prophylaxis of contrast medium allergy or adverse events).
(3) Antitumor therapies (e.g., chemotherapies, molecular-targeted therapies, immunotherapies), including antibody products, with the exception of protocol-specified standard therapies
(4) Surgical therapies for malignant tumors
(5) Radiotherapies to the targeted lesions
(6) Radiopharmaceuticals with the exception of diagnostic purposes
(7) Transplant therapies
(8) Inducers (rifampicin, glucocorticoids, phenobarbital and pentobarbital) or inhibitors (ketoconazole, cimetidine, erythromycin, verapamil, diltiazem and cyclosporine) of the hepatic P450 system are suggested to be avoid. Although vesanoid is metabolized by the hepatic P450 system, to date there are no data

to suggest that co-use with these medications increases or decreases either efficacy or toxicity of Vesanoid.
(9) Vitamin A
(10) Antifibrinolytic agents (tranexamic acid, aminocaproic acid, aprotinin)
(11) Other unapproved drugs (e.g., investigational use of drugs, unapproved combined formulations, unapproved dosage forms)

6.3.2. Permitted therapies through study period
(1) For subjects who have experienced infusion-related reactions to the investigational product and may experience recurrences of infusion related reactions, prophylactic medication with acetaminophen or diphenhydramine is recommended before administration of the investigational product.
(2) Corticosteroids may be used in the usual dosage and mode of administration in the study site for subjects in whom chemotherapy-induced nausea/vomiting is concerned.

6.3.3. No data on the effect of food on the absorption of VESANOID are available. The absorption of retinoids as a class has been shown to be enhanced when taken together with food.

6.4. Off study criteria

6.4.1. Radiological evidence of disease progression,
6.4.2. Delayed recovery of treatment-related toxicities, which prohibits the administration of drug in next cycle of treatment on day 29,
6.4.3. Presence of NCI grade 4 non-hematological toxicity,
6.4.4. Presence of NCI grade 3 or higher non-hematologic toxicity after 2 dose reductions,
6.4.5. Presence of severe allergic reaction or anaphylaxis,
6.4.6.Treatment-related event that is deemed life-threatening if the event is considered possibly related to any components of study therapy,
6.4.7.Death of the patient,
6.4.8.The patient requests to be withdrawn from study treatment,
6.4.9. Available potential better alternative treatment at the discretion of in-charged physicians.

7. Study calendar

7.1. The following tests should be performed within one week before the initiation of therapy (as the baseline data) and then be repeated as clinical needed following the initiation of therapy till the end of therapy visit. Baseline image studies (scan and x-film) must be done within 4 weeks prior to the start of therapy.

7.2. Physical examination, comprehensive history, performance status (ECOG score), complete blood cell count, differential blood cell count (CBC/DC), albumin, liver function test (total bilirubin, AST, ALT, alkaline phosphatase, r-GT), serum creatinine, glomerular filtration rate (GFR), fasting blood sugar, electrolyte (Na, K), lipid profile (cholesterol, triglyceride), and coagulation profile (prothrombin time, activated partial thromboplastin time). Other laboratory tests are on the physician's decision clinically.

7.3. GFR is calculated according to Cockcroft-Gault equation [(140-age) x body weight (kg) x (1 if male or 0.85 if female) / (72 x serum creatinine level, mg/dl)] every treatment cycle.

7.4. Serum level of CEA and CA 19-9 will be checked every 2 cycles of treatment.

7.5. Image study (chest X-film + abdominal CT/MRI, or chest + abdominal CT/MRI) will be examined every 8 weeks. For patients with radiographic objective response, scan should be repeated to document tumor response 4 weeks later.

7.6. Chest X-film can be omitted if chest computed tomography is done.

7.7. Bone scan will be done in patients with clinically suspected or evident bony metastases at screening, and be repeated every 4 cycles of treatment afterward.

7.8. Exploratory biomarker

7.8.1. To evaluate the change of cytokine (IFNs, IL-6, IL-2, etc.), 10 ml of blood will be collected during the screening period and every 2 cycles of treatment till

disease progression, end of treatment due to other reasons, or 12 months with whatever comes first.

7.8.2. Tumor tissue will be assessed for the expression of PD-L1 and immune-related genes (PD-L1, ADAR1, etc.) immunohistochemically. Two to 5 slides of tissue from archived tumor are needed.

7.8.3. The tumor tissue and blood for cytokine and immune-related genes determination will be managed at Center for Molecular Medicine at China Medical University Hospital, and be stored for 15 years.

7.9. Flow chart of evaluation

	Baseline	Day 8	q 1 cycle	q 2 cycles	q 4 cycles	q 8 week	EOT[f]	Follow-up
History / physical examination	X	X	X				X	
Performance Status, ECOG	X	X	X				X	
Body weight	X		X				X	
CBC and differential count	X	X	X				X	
Albumin	X			X			X	
Liver function test[a]	X	X	X				X	
Serum Creatinine / Fasting blood sugar / Electrolytes / Lipid profile / Coagulation	X		X				X	

profile[b]								
GFR (glomerular filtration rate)	X		X				X	
CEA, CA19-9	X			X			X	
Chest X-film[c,e]	X					X		
Abdominal (± chest) CT / MRI[e]	X					X	X[g]	X[h]
Bone scan[d,e]	X					X		
Tissue sampling	X							
Serum sampling	X			X			X	

[a]liver function test include total bilirubin, AST, ALT, alkaline phosphatase, γ-GT

[b]electrolytes include Na and K, lipid profile include cholesterol and triglyceride, coagulation profile include prothrombin time and activated partial thromboplastin time

[c]chest X-film can be omitted if chest tomography is done

[d]for patients with clinically suspected or evident bony metastases

[e]chest X-film, abdominal (± chest) CT / MRI and bone scan (if needed) are permitted to have the examination on schedule ± 5 days

[f]end of treatment

[g]for patients without disease progression by last CT/MRI which is examined more than 2 weeks before off-study

[h]every 2 months till disease progression or death

8. Efficacy assessment

8.1. The objective tumor response and duration of response are evaluated according to RECIST v1.1. Response rate and disease control rate will be calculated. Intervals of duration of response, progression-free survival, time to tumor progression and overall survival will also be collected. Tumor marker response will be evaluated.

8.2. The overall survival will be calculated from the date of registration to the date of patient‘s death.

8.3. Time to tumor progression will be calculated from the date of registration to the first date of disease progression.

8.4. Progression-free survival will be calculated from the date of registration to the date of disease progression or patient‘s death of whatever cause.

9. Serious adverse effect and reporting

9.1. Definition of Adverse Events

9.1.1. An adverse event is defined as any unfavorable or unintended sign (including an abnormal laboratory value), symptom, or disease temporally associated with the use of the investigational product, regardless of whether the event is related to the investigational product.
9.1.2. Worsening of the underlying disease, an accompanying symptom of the underlying disease, or a concurrent disease is handled as an adverse event if the worsening is beyond its natural course as medically confirmed.
9.1.3. Tumor enlargement or appearance of a new lesion (except for malignant tumor histologically different from the primary lesion) after the start of the study is thought as an adverse event if the enlargement/appearance is beyond its natural course as medically confirmed.

9.2. Definition of Serious Adverse Events

A serious adverse events is defined as an event that meets the definition of adverse events and:
(1) results in death
(2) is life-threatening
(3) requires inpatient hospitalization for treatment or prolongation of existing hospitalization
(4) results in significant or permanent disability/incapacity
(5) is a congenital anomaly
(6) is otherwise a medically important event

9.3. Criteria for Grading Adverse Events

Adverse events will be graded using the definition by CTCAE 5.0

9.4. Reporting of Serious Adverse Events

Following the subject's written consent to participate in the study, all serious adverse events (SAEs) that occur between the start of the study treatment and 100 days after the end of the study treatment, whether related or not to the investigational product, must be collected, including those thought to

be associated with protocol-specified procedures. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).

The investigator should also report any SAE occurring after these time periods that is believed to be related to any investigational product or protocol-specified procedures.

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

If the investigator believes that an SAE is not related to any investigational product, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

SAEs including overdose, whether related or not to the investigational product, and pregnancies must be reported to the sponsor (or designee) within 24 hours. SAEs must be recorded on the SAE Report Form; pregnancies on a Pregnancy Surveillance Form.

The report forms are to be transmitted via email or confirmed fax transmission to the following, and the original report forms are to remain on site.

9.5. Reporting of Pregnancies

Following initiation of the study treatment, if it is subsequently discovered that a study subject has become pregnant within 28 days after the end of the study treatment, or may have been pregnant during investigational product exposure, the investigational products should be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). The investigator must immediately notify the sponsor (or designee) of this event, and complete and forward a Pregnancy Surveillance Form to the sponsor (or designee) within 24 hours.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcomes and, if possible, newborn information must be reported on the Pregnancy Surveillance Form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to the sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

9.6. Reporting of Overdose

An overdose is defined as the accidental or intentional administration of any dose of a drug product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE (see Section 9.8 for reporting details).

10. Statistical consideration and patient accrual

10.1. This is a compassionate use of combination therapy with all-trans retinoic acid and nivolumab for patients with locally advanced or metastatic pancreatic adenocarcinoma who are refractory to current standard of therapies. As a pilot and prove-of-concept study, the target number of patients is ten.

10.2. Considering the current evidence, the study is worthy to expand to next step of trial if there is 2 or more patients who have response to the combination therapy.

11. Ethical, legal, and administrative aspects

11.1. Data collection

11.1.1. All clinical patient data collected according to the protocol will be stored by PI at Division of Hematology and Oncology, Department of Internal Medicine, China Medical University Hospital.
11.1.2. Patient clinical source documents would include documents such as (although not be limited to) the following: patient hospital/clinic records, physician's and nurse's notes, original laboratory reports, ECG, electroencephalogram, x-ray,pathology and special assessment reports, consultant letters, and screening and enrollment log, etc.

11.2. Study records retention

11.2.1. Following closure of the study, the investigator must maintain all study records in a safe and secure location. The document will be stored in a locked cabinet and the electric data will be stored in a computer with secured password.
11.2.2. The records must be maintained to allow easy and timely retrieval, when needed (eg, audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and personnel.
11.2.3. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy (eg, microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible, are a true and accurate copy of the original, and meet accessibility and retrieval standards, including regenerating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable backup of these reproductions and that an acceptable quality control process exists for making these reproductions.
11.2.4. The minimum retention time will meet the strictest standard applicable to that study center for the study, as dictated by any institutional requirements or local laws or regulations, or the sponsor's standards/procedures; otherwise, the retention period will default to 15 years.

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