Motixafortide and Pembrolizumab Combined to Nanoliposomal Irinotecan, Fluorouracil, and Folinic Acid in Metastatic Pancreatic Cancer: The COMBAT/KEYNOTE-202 Trial

Bruno Bockorny[1,2], Teresa Macarulla[3], Valerya Semenisty[4], Erkut Borazanci[5], Jaime Feliu[6], Mariano Ponz-Sarvise[7], David Gutierrez Abad[8], Paul Oberstein[9], Angela Alistar[10], Andres Muñoz[11], Ravit Geva[12], Carmen Guillén-Ponce[13], Mercedes Salgado Fernandez[14], Amnon Peled[15,16], Marya Chaney[17], Irit Gliko-Kabir[18], Liron Shemesh-Darvish[18], Debby Ickowicz[18], Ella Sorani[18], Shaul Kadosh[19], Abi Vainstein-Haras[18], and Manuel Hidalgo[20]

ABSTRACT

Purpose: Pancreatic ductal adenocarcinoma (PDAC) is largely unresponsive to checkpoint inhibitors. Blockade of the CXCR4/CXCL12 axis increases intratumoral trafficking of activated T cells while restraining immunosuppressive elements. This study evaluates dual blockade of CXCR4 and PD1 with chemotherapy in PDAC.

Patients and Methods: Multicenter, single-arm, phase II study to evaluate the safety and efficacy of motixafortide and pembrolizumab combined with chemotherapy in patients with *de novo* metastatic PDAC and disease progression on front-line gemcitabine-based therapy (NCT02826486). Subjects received a priming phase of motixafortide daily on days 1–5, followed by repeated cycles of motixafortide twice a week; pembrolizumab every 3 weeks; and nanoliposomal irinotecan, fluorouracil, and leucovorin every 2 weeks (NAPOLI-1 regimen). The primary objective was objective response rate (ORR). Secondary objectives included overall survival (OS), progression-free survival (PFS), disease control rate (DCR), safety, and tolerability.

Results: A total of 43 patients were enrolled. The ORR according to RECISTv1.1 was 21.1% with confirmed ORR of 13.2%. The DCR was 63.2% with median duration of clinical benefit of 5.7 months. In the intention-to-treat population, median PFS was 3.8 months and median OS was 6.6 months. The triple combination was safe and well tolerated, with toxicity comparable with the NAPOLI-1 regimen. Notably, the incidence of grade 3 or higher neutropenia and infection was 7%, lower than expected for this chemotherapy regimen.

Conclusions: Triple combination of motixafortide, pembrolizumab, and chemotherapy was safe and well tolerated, and showed signs of efficacy in a population with poor prognosis and aggressive disease.

Introduction

With a rising incidence and mortality, pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States (1). In the past decade, two large phase III trials have established gemcitabine in combination with nab-paclitaxel and FOLFIRINOX (fluorouracil, folinic acid, irinotecan, and oxaliplatin) as accepted front-line regimens for metastatic disease (2, 3). However, resistance to chemotherapy invariably occurs and is a main reason for the dismal 5-year overall survival (OS) of 3% in the metastatic setting (1, 4). The only regimen currently approved in the second line is a combination of nanoliposomal irinotecan, fluorouracil, and folinic acid, leaving an urgent need for patients with advanced disease (5, 6).

Although checkpoint inhibitors have shown efficacy in many tumor types, pancreatic ductal adenocarcinoma (PDAC) is one of the few cancers with essentially no responses (7–9). Even in patients with microsatellite instability-high tumors, the response rate is less than 20% (10). Similarly, dual blockade of CTLA-4 and PD-L1 has shown limited efficacy in PDAC (7). Recent early data with an agonistic CD40 monoclonal antibody in combination with PD1 therapy showed encouraging responses, suggesting that the complex immunosuppressive milieu of pancreatic cancer can be overcome with novel immunotherapy strategies (11).

Prior preclinical studies have shown that inhibition of the CXCR4/CXCL12 pathway increases T-cell infiltration in the PDAC tumor microenvironment (TME) sensitizing tumors to immunotherapy (12, 13).

[1]Beth Israel Deaconess Medical Center, Boston, Massachusetts. [2]Harvard Medical School, Boston, Massachusetts. [3]Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, Barcelona, Spain. [4]Rambam Health Care Campus, Haifa, Israel. [5]HonorHealth/TGen, Scottsdale, Arizona. [6]Hospital Universitario La Paz. IdIPAZ. Cátedra UAM-AMGEN, CIBERONC, Madrid, Spain. [7]Clinica Universidad de Navarra and Program in Solid Tumors (CIMA), Universidad de Navarra, IDISNA, Pamplona, Spain. [8]Grupo Oncologia Fuenlabrada, Madrid, Spain. [9]New York University Langone Health, New York, New York. [10]Atlantic Health System, Morristown, New Jersey. [11]Hospital General Universitario Gregorio Marañón, Universidad Complutense, Madrid, Spain. [12]Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. [13]Servicio de Oncología Médica. Hospital Universitario Ramón y Cajal. IRYCIS. Madrid, Spain. [14]Complejo Hospitalario Universitario de Ourense, Ourense, Spain. [15]Biokine Therapeutics Ltd., Ness Ziona, Israel. [16]Goldyne Savad Institute of Gene Therapy, Hebrew University Hospital, Jerusalem, Israel. [17]Early Development Oncology, Merck & Co., Inc., Kenilworth, New Jersey. [18]BioLineRx, Modi'in, Israel. [19]StatExcellence Ltd., Nesher, Israel. [20]Weill Cornell Medical College, New York, New York.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Manuel Hidalgo, New York-Presbyterian Hospital/Weill Cornell Medical Center, 1305 York Avenue, New York, NY 10021. Phone: 646-962-2084; E-mail: mah4006@med.cornell.edu

Clin Cancer Res 2021;27:5020–7

doi: 10.1158/1078-0432.CCR-21-0929

Translational Relevance

Motixafortide is a small synthetic peptide that binds to CXCR4 with high affinity, inhibiting the CXCR4–CXCL12 pathway, enhancing T-cell access to the tumor microenvironment, and increasing tumor sensitivity to anti–PD-1 therapy in preclinical models and in patients. In this multicenter phase II study of patients with refractory *de novo* metastatic pancreatic cancer, the combination of motixafortide and pembrolizumab with standard chemotherapy was found to be safe and tolerable, with lower than expected incidence of neutropenia and infections, and showed signs of efficacy in a population with poor prognosis and aggressive disease. A randomized study is needed to confirm whether CXCR4 inhibition with motixafortide adds benefits to chemotherapy in the treatment of pancreatic cancer.

We recently reported the first clinical results of this novel strategy of combining the CXCR4 antagonist motixafortide (BL-8040) with pembrolizumab in patients with previously treated metastatic PDAC (14). In the cohort 1 of the COMBAT trial, motixafortide and pembrolizumab combination promoted an increase in $CD8^+$, $CD4^+$, and $CD3^+CD8^+granzymeB^+$ T cells within the TME while decreasing the immunosuppressive myeloid-derived suppressor cells (MDSC) cells (14). Here, we report the clinical results of the cohort 2 of the COMBAT trial with the triple combination of motixafortide, pembrolizumab, and chemotherapy in patients with metastatic PDAC.

Patients and Methods

Study design

This was a multicenter, open-label, two-cohort, phase IIa trial to assess the safety and efficacy of the combination of motixafortide and pembrolizumab in subjects with metastatic PDAC. The immunobiological effects and clinical results of the cohort 1 with motixafortide combined with pembrolizumab were reported previously (14). Here, we present the results of cohort 2, in which subjects with *de novo* metastatic PDAC with disease progression following first-line treatment with gemcitabine-based chemotherapy were enrolled to receive a priming phase of motixafortide monotherapy for five days followed by a combination treatment of motixafortide, pembrolizumab, and chemotherapy. During the monotherapy period, eligible subjects receive daily SC injections of motixafortide (1.25 mg/kg) on days 1–5. From day 8, subjects begin a combination period consisting of liposomal irinotecan (70 mg/m^2) over 90 minutes followed by intravenous leucovorin 400 (mg/m^2) over 30 minutes, followed by intravenous fluorouracil 2,400 (mg/m^2) over 46 hours, every 2 weeks; pembrolizumab 200 mg once every three weeks; beginning on day 10, motixafortide twice a week and at least 24 hours after chemotherapy dosing. The combination therapy continued for up to 35 cycles (approximately two years), or until progression, clinical deterioration or early termination, whichever came first.

The study was approved by institutional review boards or independent ethics committees of all participating institutions and was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice, applicable local regulations and the principles of the Declaration of Helsinki. All patients provided written informed consent. An independent Data Monitoring Committee reviewed the accumulated study data to ensure subject welfare. The study was registered with https://clinicaltrials.gov, number NCT02826486.

Patients

Eligible patients had histologically confirmed *de novo* metastatic pancreatic adenocarcinoma with documented objective radiographic progression after treatment with first-line gemcitabine-based chemotherapy. Only primary metastatic patients were enrolled. Patients with non-metastatic locally advanced disease were ineligible. Subjects must have measurable disease according to Response Evaluation Criteria in Solid Tumors v.1.1 (RECISTv.1.1), Eastern Cooperative Oncology Group performance status of 0–1, and adequate hematologic and end-organ function. Detailed description of inclusion and exclusion criteria is provided in Supplementary Table S1.

Assessments

The objectives of the study were to assess the efficacy and safety of motixafortide in combination with pembrolizumab and chemotherapy in subjects with metastatic PDAC. The primary objective was the assessment of objective response rate (ORR) defined as the proportion of patients with an investigator-assessed confirmed or unconfirmed partial response or complete response as best response per RECIST version 1.1.

Secondary objectives included: OS defined as time from the first dose of study treatment to death from any cause; progression-free survival (PFS); duration of response, defined as time from the first tumor assessment that documented response to the first documented disease progression; disease control rate (DCR), defined as the sum of partial responses (PR), complete responses (CR), and stable disease (SD) according to RECISTv1.1; safety and tolerability. Adverse events were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events, v.4.03. Exploratory objectives were the assessment of changes in tumor marker as potential biomarkers and predictors of response, including the analysis of changes in circulating immune cells subtypes in the peripheral blood, before and after treatment, using flow cytometry.

Flow cytometry

Blood samples for flow cytometry analysis were collected into TransFix/EDTA Vacuum Blood Collection Tubes during monotherapy on days 1 and 5, pre and 4 hours post (±2 hours) BL-8040 administration, and during the combination period at the end of cycle 1, day 15 and at the end of cycle 2, day 21. Cells were stained with antibody panels for 30 minutes at room temperature in the dark followed by red cell lysis using lysing solution (BD Biosciences; 15 minutes) and washing. Antibody panels included CD45 (2D1, BD Biosciences), CD3 (APC/Fire-750, BioLegend), CD4 (RPA-T4, BioLegend), CD8 (SK1, BioLegend), CD19 (HIB19, BioLegend), CD56 (NKH-1, Beckman Coulter), and CD38 (HB7, BioLegend) were used for the assessment of T helper cells (CD3 CD4), cytotoxic T cells (CD3 CD8), activated T cells (expressing upregulated levels of CD38), NK cells ($CD3^-$ $CD56^+$), NKT cells ($CD3^+CD56^+$), and B cells (CD3-$CD19^+$). Stained cells were acquired on FACSLyric flow cytometer (BD Biosciences) and analyzed using FlowJo version 10.

MSI status assessment

MSI status was collected either as baseline information by electronic case report forms ($N = 18$), IHC of mismatch repair proteins ($N = 2$; Neogenomics) or molecular analysis ($N = 19$). In the latter, the MSI Analysis System consists of five nearly monomorphic mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) for MSI

determination and two polymorphic pentanucleotide markers (Penta C and Penta D) for sample identification. MSI analysis was performed according to the manufacturer's directions (Promega MSI Analysis System Version 1.2). The PCR products were analyzed by capillary electrophoresis using an ABI 3500xL Genetic Analyzer (Applied Biosystems). Raw data from the genetic analyzer are transferred to gene mapper software (Applied Biosystems GeneMapper) to determine MSI status based on size (basepair) and height (RFU signal) data. Shifts of ≥2.5 bp difference between normal and tumor fragment size in two or more (>40%) unstable markers (i.e.,) is classified MSI-high.

PD-L1 expression assessment

PD-L1 expression was assessed in formalin-fixed tumor samples at a central laboratory with the use of the commercially available PD-L1 IHC 22C3 pharmDx assay (Dako North America; ref. 15) on the Dako Autostainer system (Agilent Technologies). Biopsies were obtained by core-needle. The scoring system used for PD-L1 expression was the Combined Positive Score 1 (CPS1), measuring PD-L1 expression on both tumor cells and tumor-associated stroma cells. PD-L1 was defined as "positive" if CPS was ≥ 1 and "negative" or not expressed if CPS <1.

Statistical analysis

This was an open label, phase IIa trial to evaluate the safety and tolerability and to estimate the efficacy of the study combination. The planned sample size of 40 subjects was deemed sufficient to characterize the feasibility, safety, tolerability, and to estimate the efficacy of the triple combination on the basis of practical considerations and not statistical power calculations.

Time-to-event outcomes were estimated using the Kaplan–Meier method. Safety was evaluated in all patients who received at least one dose of motixafortide and was summarized in terms of number of events and proportions using descriptive statistics. Intention to treat (ITT) analysis was performed on all subjects who met the eligibility criteria and was allocated to intervention (ITT). Modified ITT (mITT) analysis was conducted on subjects who received at least one dose of combination therapy and had a post baseline imaging. Statistical analyses were done using R version 3.4.4.

Flow cytometry statistical analyses were performed on the log scale using a linear mixed model for repeated measures, incorporating planned *a priori* comparisons using unadjusted two-sided *t* tests. Data were presented as means ± 95% confidence interval (CI).

Results

Patient disposition and baseline characteristics

Patients with histologically confirmed *de novo* metastatic pancreatic cancer with documented disease progression after front-line gemcitabine-based chemotherapy were recruited from 18 sites in 3 countries (Israel, Spain, and United States) to receive a priming phase of motixafortide monotherapy for 5 days followed by a combination of motixafortide, pembrolizumab and nanoliposomal irinotecan, fluorouracil and leucovorin. The study design is shown in Supplementary Fig. S1. Further details of the study population are shown in Supplementary Table S1 and Supplementary Methods. The primary objective was the assessment of ORR per RECIST version 1.1. Secondary objectives included OS, PFS, DCR, duration of response, safety and tolerability. Additional details on the study procedures are provided in Patients and Methods.

A total of 43 patients met eligibility criteria, were enrolled in the study and were included the in the ITT analysis for safety and survival endpoints. Thirty-nine subjects received combination therapy whereas 4 discontinued on monotherapy (one due to worsened disease and three due adverse events) and were withdrawn from the study before starting combination treatment. One patient discontinued due to adverse event and lost follow-up (**Fig. 1**). Thirty-eight subjects were evaluable for efficacy and are referred to as modified ITT (i.e., received at least one dose of combination and had a post baseline CT scan). Median follow-up was 7.7 months (range, 0.75–19.7 months). Among the 43 patients, 24 (56%) were men and 19 (44%) were women, and they had a median age of 68 years (range, 40–85 years). All subjects had metastatic disease at presentation and were refractory to gemcitabine-based chemotherapy at the time of enrollment. Thirty-two (74.4%) patients had metastatic disease to the liver. Baseline patient characteristics and demographics are summarized in Supplementary Table S2.

Safety

The most common adverse events were mild to moderate nausea and vomiting (74.4%) and asthenia (67.4%). Grade 3 or higher treatment-related adverse events were observed in 52.5% of patients, with the most common being nausea and vomiting (18.6%), asthenia (16.3%), and diarrhea (14%). Notably, serious neutropenia (grade ≥ 3) was observed in only 7% of the patients with only one subject (2.3%) developing febrile neutropenia. Reactions at the injection site related to motixafortide was observed in 55.8%, with most being mild to moderate, and 4.7% being grade 3 or higher. A summary of treatment adverse events is provided in **Table 1**. Overall, 5 of 43 patients (11.6%) discontinued treatment because of treatment-related adverse events and there were no treatment-related deaths.

Efficacy

The ORR by RECISTv1.1 in the evaluable population was 21.1% (95% CI, 8.1%–34%) with confirmed ORR of 13.2% (95% CI, 2.4%–23.9%). Stable disease was achieved in 42.1% (95% CI, 26.4%–57.8%), yielding a DCR of 63.2% (95% CI, 47.8%–78.5%). For patients who achieved partial response or stable disease, the median duration of clinical benefit was 5.7 months (95% CI, 4.9–7.3 months). The percentage of change in target lesion size is summarized in **Fig. 2A**. In the IIT population ($N = 43$), median PFS was 3.8 months (95% CI, 1.6–5.1 months) and median OS (mOS) was 6.6 months (95% CI, 4.5–8.7 months; ref. **Fig. 2B**). The efficacy of triple combination was analyzed according to the presence of liver metastasis. In the subgroup with liver metastasis ($N = 30$), the ORR was 16.7% (95% CI, 3.3–30%) and DCR was 56.7% (95% CI, 38.9–74.4%), whereas in the subgroup without liver metastasis ($N = 8$), ORR and DCR were 37.5% (95% CI, 4.0–71%) and 87.5% (95% CI, 64.4–100%), respectively. Patients with liver metastasis had median PFS of 1.9 months (95% CI, 1.5–5.7 months) and mOS of 5.9 months (95% CI, 4.4–9.6 months), whereas subjects without hepatic metastasis had median PFS 5.4 months (95% CI, 1.5–8.0) and mOS of 8.4 months (95% CI, 3.5–10.8; Supplementary Table S3).

PD-L1 expression was evaluated on baseline biopsies through IHC staining and classified as positive (CPS ≥ 1) or negative (CPS<1). The number of respondents was insufficient to establish any association between clinical outcomes and PD-L1 expression. Of 17 samples available for testing, 13 (76%) were from patients with positive PD-L1 expression. Of those, 2 patients experienced partial response and 7 had stable disease. Four patients (24%) had negative PD-L1, including one with partial response and 2 with stable disease (Supplementary Table S4). Microsatellite instability (MSI) status was assessed in 39 patients, of which none was considered MSI-high (Supplementary Table S2).

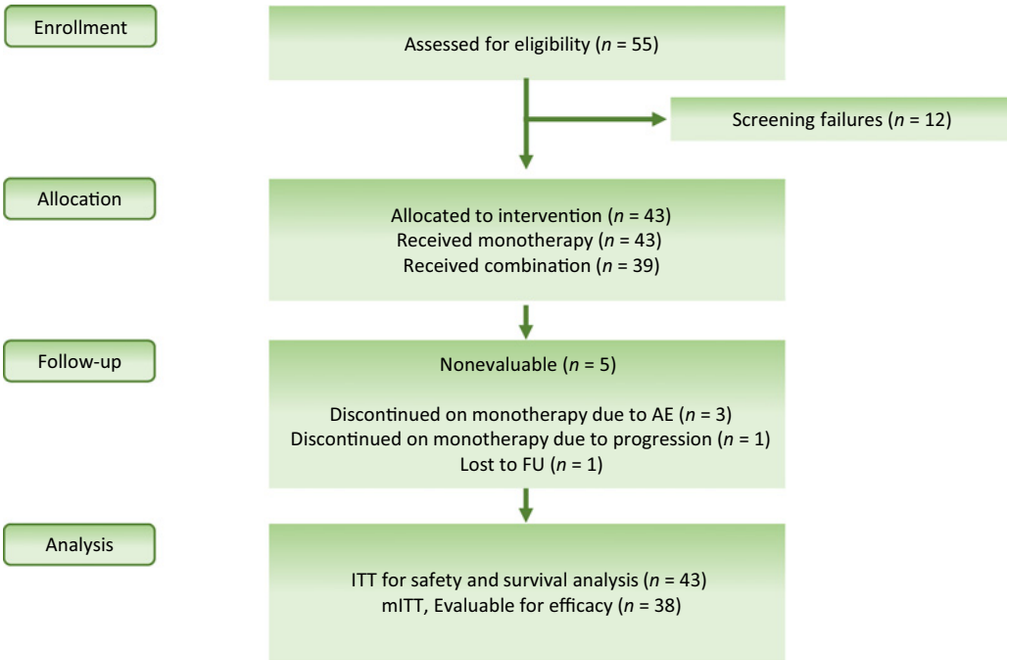

Figure 1.
CONSORT diagram of the Cohort 2 of the COMBAT trial. AE, adverse event; FU, follow up; ITT, intention to treat. mITT, modified intention to treat.

Circulating immune cells

To understand the effects of motixafortide and pembrolizumab with chemotherapy on circulating immune cells, blood samples were obtained before and after treatment and analyzed by flow cytometry and complete blood count. Motixafortide caused rapid and sustained increase of lymphocyte and neutrophil counts starting after the first dose during the monotherapy phase (**Fig. 3A** and **B**). Motixafortide monotherapy also promoted a significant increase in the relative frequency of B cells starting after the first dose, whereas triple combination of motixafortide, pembrolizumab, and chemotherapy led to an increase in activated $CD4^+$ T cells ($CD38^+$; **Fig. 3C**). No substantial effects were observed in the relative frequency of $CD4^+$, $CD8^+$ or NKT^+ cells during monotherapy whereas a small reduction was noted for activated $CD8^+$ cells during monotherapy that normalized on triple therapy (Supplementary Fig. S2). We also did not observe substantial differences in the changes in circulating immune cells between patients who experienced clinical benefit from treatment as compared with those who had progression (Supplementary Fig. S3).

Table 1. Treatment-related adverse events of triple combination of motixafortide, pembrolizumab, and chemotherapy.

Treatment-related adverse events	All grades	Grade≥ 3
Nausea and vomiting	74.4%	18.60%
Asthenia	67.4%	16.30%
Injection site reactions	55.8%	4.70%
Diarrhea	53.5%	14%
Appetite disorders	41.9%	9.30%
Pruritus	39.5%	—
Anemia	37.2%	11.60%
Neutropenia	14.0%	7%
Febrile neutropenia	2.3%	2.3%
Rashes, eruptions, and exanthems	30.2%	—
Gastrointestinal and abdominal pain	30.2%	—
Musculoskeletal and connective tissue pain and discomfort	30.2%	4.60%
Dermal and epidermal conditions	25.6%	—
Edema	23.3%	4.70%
Weight decrease	20.9%	2.30%
Hyperpigmentation disorders	20.9%	—
Gastrointestinal atonic and hypomotility disorders	20.9%	—

Note: Adverse events reported in >20% of patients. Adverse events were assessed by the investigator as probably or possibly related to any of the study drugs, including motixafortide, pembrolizumab, and chemotherapy.

Discussion

The COMBAT/KEYNOTE-202 is a multicenter open-label phase II study to evaluate the efficacy and safety of the CXCR4 antagonist motixafortide with pembrolizumab added to nanoliposomal irinotecan, fluorouracil and folinic acid, the current second-line standard of care for patients with metastatic PDAC. The study results demonstrated that the triple combination achieved clinical benefit, including durable responses, with a safe and tolerable profile in a population with poor prognosis disease.

The observed ORR was 21.1%, confirmed ORR was 13.2%, with DCR of 63.2%, and mOS of 6.6 months in patients with primary metastatic PDAC. It should be noted that this study enrolled a particularly challenging population with all but one patient having *de novo* stage IV disease at initial diagnosis and 74.4% having metastasis to the liver, underscoring the poor prognosis of those patients. Historical data from the NAPOLI-1 trial showed an ORR of 16%, confirmed ORR of 7.7%, DCR of 52% and mOS of 6.1 months for nanoliposomal irinotecan plus fluorouracil and folinic acid in the second-line setting after progression on gemcitabine-based therapy (5).

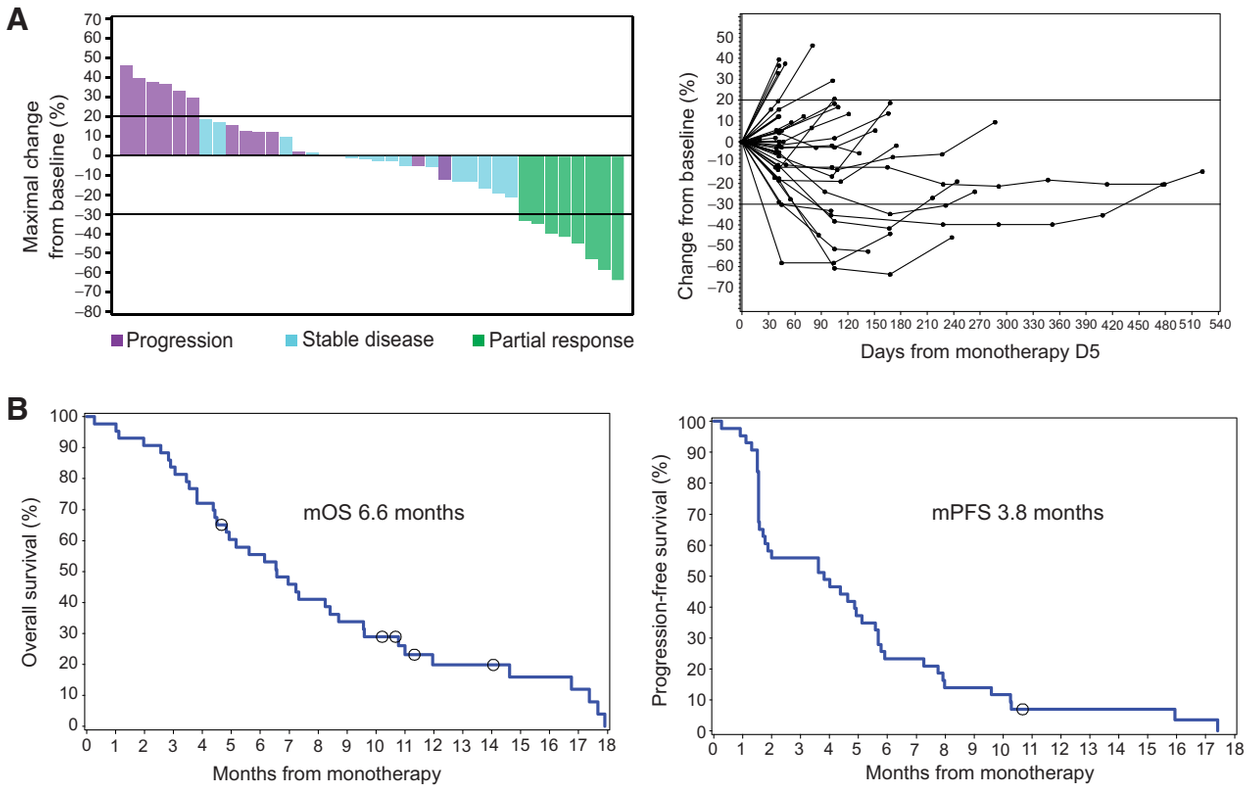

Figure 2.

Response in patients receiving motixafortide and pembrolizumab in combination with chemotherapy. **A,** Left, Waterfall plot analysis for evaluable population (mITT; $N = 38$) showing maximal percentage of change in the sum of longest diameters measured of target lesions as compared with baseline for patients treated with motixafortide and pembrolizumab combined with liposomal irinotecan, fluorouracil, and leucovorin. Right, Spider plot analysis of evaluable population (mITT; $N = 38$) receiving motixafortide, pembrolizumab, and chemotherapy showing the sum of longest diameters (mm) of target lesions by best response, according to RECISTv1.1. **B,** Left, Kaplan–Meier estimates of overall survival in ITT ($N = 43$) population receiving combination treatment. Survival measured in months from monotherapy day 1 to death. Circles displayed identify censoring pattern. Right, Kaplan–Meier estimates of progression-free survival in ITT ($N = 43$) population. Progression-free survival measured in months from monotherapy day 1 to death.

Notably, almost half (47%) of the patients allocated to the NAPOLI-1 regimen arm had early or locally advanced disease at initial diagnosis, which is known to positively impact outcomes (16). Within the subgroup of stage IV disease at diagnosis ($N = 61$), which mirrors our population, the mOS was only 4.7 months with NAPOLI-1 regimen, whereas patients with stage III disease at diagnosis had mOS of 9.0 months (16). Similarly, in a large metanalysis of irinotecan-based therapy in the second line for patients with stage III–IV disease showed ORR of 8.7% and DCR of 29.4%, with mOS of 5.5 months. Notwithstanding the efficacy shown here with triple therapy in patients with aggressive disease, the lack of a control arm with chemotherapy alone prevents definitive conclusions about the impact of CXCR4/PD1 blockade in metastatic pancreatic cancer.

Emerging evidence indicate that the presence of liver metastasis diminishes systemic immunotherapy effect in preclinical models and in patients through a mechanism that is, at least in part, related to depletion of circulating antigen-specific T cells (17). Here, we also observed that patients without liver metastasis appeared to have more benefit from triple combination with ORR of 37.5% and DCR of 87.5%, whereas subjects with hepatic metastasis had ORR of 16.7% and DCR of 56.7%.

The study provides important safety data related to dual CXCR4/PD1 checkpoint inhibition in combination with chemotherapy in the metastatic PDAC setting. Patients showed acceptable tolerability and all adverse events were manageable. The observed safety profile of triple combination therapy was consistent with the profile observed in the NAPOLI-1 regimen with key distinctions (5). In both studies the rate of grade 3–4 nausea and vomiting, fatigue and diarrhea were comparable (5). Remarkably, grade 3–4 neutropenia was observed in 27% of patients who received nanoliposomal irinotecan plus fluorouracil and folinic acid in the NAPOLI-1 study but only in 7% of the COMBAT trial receiving triple therapy, with only one patient experiencing febrile neutropenia. This much lower proportion of severe neutropenia is likely related to motixafortide-mediated CXCR4 inhibition leading to neutrophil mobilization effect via blockade of neutrophil homing to the bone marrow (18, 19). This observation supports the concept that this immunotherapy regimen could be safely combined with more myelosuppressive regimens such as FOLFIRINOX.

The study also demonstrated the effect of motixafortide and pembrolizumab combined to chemotherapy on peripheral immune cells. Consistent with previous pharmacodynamic studies, motixafortide caused a rapid and sustained increase in lymphocytes and neutrophils in the circulation starting after the first dose (14, 19). We observed a substantial relative increase in circulating B cells starting in the priming phase of motixafortide and a relative

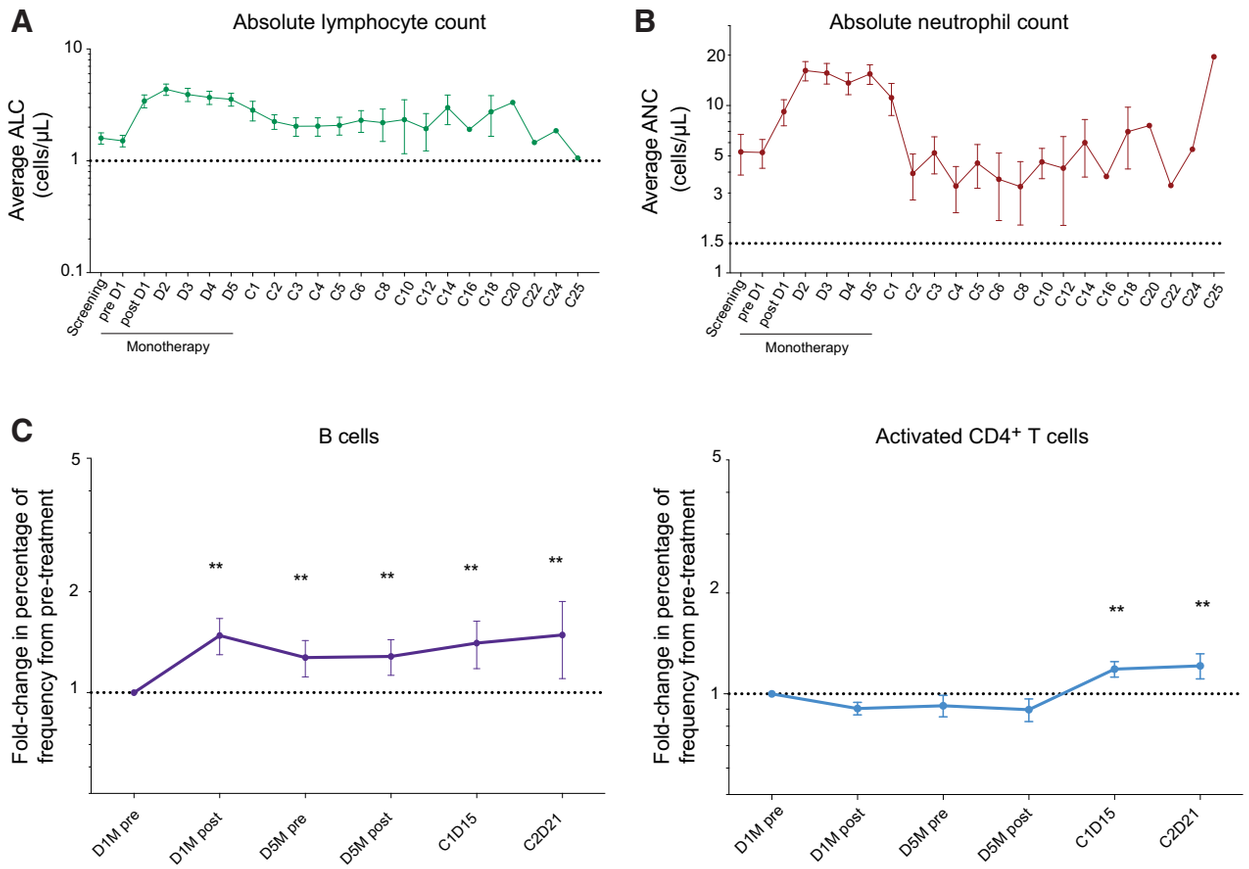

Figure 3.

Effects of triple combination on circulating immune cells. **A,** Absolute lymphocyte count (ALC) as determined by complete blood count. Peripheral blood was drawn at the indicated pre- and post-treatment timepoints. All subjects were sampled during monotherapy and after treatment, but the number of subjects reduced steadily thereafter as patients came off study. Data were available for: Screening $n = 46$, Mono $n = 42$, C1 $n = 38$, C2 $n = 34$, C3 $n = 25$, C4 $n = 20$, C5 $n = 15$, C6 $n = 13$, C8 $n = 9$, C10 $n = 5$, C12 $n = 6$, C14 $n = 3$, C16 $n = 1$, C18 $n = 2$, C20-C25 $n = 1$. **B,** As in (**A**) except for absolute neutrophil count (ANC). **C,** Motixafortide treatment increased the frequency of B cells in the circulation, whereas triple combination increased the relative frequency of activated $CD4^+CD38^+$ T helper cells in the periphery. Data are presented as means ± 95% CI. Statistical analysis was performed on the log scale, incorporating planned *a priori* comparisons using unadjusted two-sided *t* tests (*, $P < 0.05$; **, $P < 0.0005$). Data were available for D1M pre $n = 35$, D1M post $n = 34$, D5M pre $n = 26$, D5M post $n = 25$, C1D15 $n = 26$, and C2D21 $n = 16$. M, monotherapy; C, cycle; D, day. Dotted lines indicate levels at pre-treatment.

increase in the level of activated $CD4^+$ T cells ($CD38^+$) in the periphery starting with triple combination. Given limited number of patients in the study, we did not observe significant differences for the changes in circulating immune cells between responders and non-responders.

Pancreatic cancer has proven largely unresponsive to T-cell checkpoint therapy owing to the complex interplay between its immunosuppressive desmoplastic reaction and regulatory components, restraining antitumor T effector cells and ultimately attenuating antitumor activity (12, 20). Previously, we showed that blockade of the CXCR4/CXCL12 axis with motixafortide increases trafficking of activated T cells in the TME and abrogates immunosuppressive elements such as MDSCs. In the cohort 1 of the COMBAT trial, motixafortide in combination with pembrolizumab (without chemotherapy backbone) led to disease control in nearly a third of the patients with heavily pretreated pancreatic cancer (14). In the cohort 2 presented here, by combining motixafortide and pembrolizumab with the NAPOLI-1 regimen, we observed a potential for higher responses without added toxicity.

The rationale for anti-CXCR4 therapy in pancreatic cancer is further strengthened by a recent study demonstrating that the chemokine CXCL12 derived from cancer-associated fibroblasts forms a coat around pancreatic cancer cells, impairing the trafficking of multiple immune cell types within the TME (21). The study showed that the CXCR4 inhibitor AMD3100 (plerixafor) given through continuous infusion for one week induced antitumor responses and significantly decreased the levels of circulating tumor DNA in patients with PDAC (21), raising the hypothesis that more prolonged exposure to CXCR4 inhibitor might be warranted to avert the immunosuppressive milieu of pancreatic cancer. A new study has been recently initiated to investigate the activity of motixafortide and the anti-PD1 cemiplimab in combination with gemcitabine and nab-paclitaxel for the front-line treatment of metastatic PDAC (NCT04543071). Although pancreatic cancer remains one of the most immune-resistant tumors, such rationale strategies that target non-redundant immune-modulatory pathways in combination with chemotherapy to induce immunogenic cell death may render pancreatic cancer sensitive to immunotherapy.

This study's limitations included the lack of a control arm with NAPOLI-1 regimen, which prevented direct comparison of triple therapy used here. Another study limitation was the limited number of patients, which precluded meaningful appraisal of biomarkers of response. In recent years, several targeted and immune-based therapies have been evaluated in PDAC studies, and almost all have failed to demonstrate efficacy in phase II/III trials, underscoring the challenges in achieving objective responses in the second line in metastatic PDAC (7, 22–25).

In conclusion, motixafortide and pembrolizumab in combination with nanoliposomal irinotecan, fluorouracil and folinic acid showed efficacy in a population with poor prognoses and aggressive disease. Treatment was well tolerated, and the rates of severe neutropenia and infections were substantially lower than expected. A randomized study is warranted to confirm whether CXCR4 inhibition with motixafortide adds benefits to chemotherapy in the treatment of pancreatic cancer.

Authors' Disclosures

B. Bockorny reports other from Bioline Rx during the conduct of the study, as well as other from Erytech Pharma and grants from Nanoview Biosciences outside the submitted work; in addition, B. Bockorny has a patent for PCT/US20/17005 pending and supported by BioLineRx and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ. T. Macarulla reports personal fees from consultancy/advisory role with Amgen, Baxter, Celgene, Incyte, Q&D Therapeutics, Serviere, and Shire during the conduct of the study, as well as grants from AstraZeneca, BeiGene, and Celgene outside the submitted work. E. Borazanci reports other from Ipsen and Merck during the conduct of the study, as well as other from BMS, Minneamrita, BioNTech, Samumed, Northern Biologics, Bioline, and Helix outside the submitted work. J. Feliu reports personal fees from Roche, Amgen, Ipsen, Eisai, Sirtex, MSD, Merck, and Novartis during the conduct of the study. M. Ponz-Sarvise reports grants and non-financial support from Roche, personal fees and non-financial support from Incyte and Astra Zeneca, and grants and non-financial support from BMS outside the submitted work. P. Oberstein reports consulting or advisory role for Merck, BTG Therapeutics, Tyme Inc., and Ipsen and research funding from Merck (institution) and Roche/Genentech (institution). A. Alistar reports other from Atlantic Health System during the conduct of the study, as well as personal fees and other from Merck outside the submitted work. R. Geva reports personal fees from BMS, Lilly, Medison, Roche, Novartis, Janssen, Takeda, MSD, Pfizer, Merck, Eisai, AstraZeneca, Bayer, MSD, Novartis, BI, BOL Pharma, and Roche; grants from Novartis; and other from Merck, Bayer, BMS, Medison, BOL Pharma, and Pyxis outside the submitted work. C. Guillén-Ponce reports grants from BioLineRX during the conduct of the study, as well as non-financial support from Sanofi Aventis, Bayer, Merck Serono, Lilly, and Bristol Myers Squibb and grants from Erytech, BeiGene, AstraZeneca, and Incyte outside the submitted work. M.S. Fernandez reports other from Leo Pharma, Rovi, MSD, Eisai, Sanofi, and Merck and other from Amgen, Servier, and Merck outside the submitted work. A. Peled reports personal fees from Biokine Therapeutics during the conduct of the study, as well as a patent for Pancreatic patent pending. M. Chaney reports other from Merck & Co., Inc. outside the submitted work. E. Sorani reports personal fees from BioLineRX during the conduct of the study, as well as a patent for WO 2020/079692 pending. A. Vainstein-Haras reports personal fees from BioLineRX outside the submitted work, as well as a patent for WO 2020/079692 pending. M. Hidalgo reports grants from Bioline Ltd to Weill Cornell Medicine during the conduct of the study, as well as personal fees from Agenus, BMS, Oncomatrix, Khar, Inxmed, Genchem, Cantargia, and Fibrogen outside the submitted work; in addition, M. Hidalgo has a patent for Method for ACT in PDAC licensed to Peaches SL and Director of BMS. No disclosures were reported by the other authors.

Authors' Contributions

B. Bockorny: Data curation, formal analysis, supervision, investigation, writing–original draft, writing–review and editing. **T. Macarulla:** Data curation, supervision, investigation, writing–review and editing. **V. Semenisty:** Investigation, writing–review and editing. **E. Borazanci:** Supervision, investigation, writing–review and editing. **J. Feliu:** Supervision, investigation, writing–review and editing. **M. Ponz-Sarvise:** Supervision, investigation, writing–review and editing. **D.G. Abad:** Supervision, investigation, writing–review and editing. **P. Oberstein:** Supervision, investigation, writing–review and editing. **A. Alistar:** Supervision, investigation, writing–review and editing. **A. Muñoz:** Supervision, investigation, writing-review and editing. **R. Geva:** Supervision, investigation, writing–review and editing. **C. Guillén-Ponce:** Supervision, investigation, writing–review and editing. **M.S. Fernandez:** Supervision, investigation, writing–review and editing. **A. Peled:** Supervision, investigation, writing–review and editing. **M. Chaney:** Data curation, supervision, project administration, writing–review and editing. **I. Gliko-Kabir:** Resources, data curation, writing–review and editing. **L. Shemesh-Darvish:** Resources, data curation, formal analysis, supervision, writing–review and editing. **D. Ickowicz:** Resources, data curation, methodology. **E. Sorani:** Resources, data curation, methodology, writing–review and editing. **S. Kadosh:** Resources, data curation, formal analysis. **A. Vainstein-Haras:** Conceptualization, resources, data curation, supervision, funding acquisition, writing–review and editing. **M. Hidalgo:** Conceptualization, data curation, supervision, funding acquisition, investigation, project administration, writing–review and editing.

Acknowledgments

We thank the patients and their families for their participation in this study. This work was supported by BioLineRx and Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 12, 2021; revised May 20, 2021; accepted July 2, 2021; published first July 12, 2021.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. CA Cancer J Clin 2021;71:7–33.
2. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med 2013;369:1691–703.
3. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817–25.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7–30.
5. Wang-Gillam A, Li CP, Bodoky G, Dean A, Shan YS, Jameson G, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. Lancet 2016;387:545–57.
6. Wang-Gillam A, Hubner RA, Siveke JT, Von Hoff DD, Belanger B, de Jong FA, et al. NAPOLI-1 phase 3 study of liposomal irinotecan in metastatic pancreatic cancer: final overall survival analysis and characteristics of long-term survivors. Eur J Cancer 2019;108:78–87.
7. O'Reilly EM, Oh DY, Dhani N, Renouf DJ, Lee MA, Sun W, et al. Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: a phase 2 randomized clinical trial. JAMA Oncol 2019;5:1431–8.
8. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti–PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455–65.
9. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 trial of single agent ipilimumab (anti–CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother 2010;33:828–33.
10. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the Phase II KEYNOTE-158 Study. J Clin Oncol 2020;38:1–10.
11. O'Hara MH, O'Reilly EM, Varadhachary G, Wolff RA, Wainberg ZA, Ko AH, et al. CD40 agonistic monoclonal antibody APX005M (sotigalimab) and chemotherapy, with or without nivolumab, for the treatment of metastatic pancreatic adenocarcinoma: an open-label, multicentre, phase 1b study. Lancet Oncol 2021;22:118–31.

12. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti–PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci U S A 2013;110:20212–7.
13. Seo YD, Jiang X, Sullivan KM, Jalikis FG, Smythe KS, Abbasi A, et al. Mobilization of CD8(+) T cells via CXCR4 blockade facilitates PD-1 checkpoint therapy in human pancreatic cancer. Clin Cancer Res 2019;25:3934–45.
14. Bockorny B, Semenisty V, Macarulla T, Borazanci E, Wolpin BM, Stemmer SM, et al. BL-8040, a CXCR4 antagonist, in combination with pembrolizumab and chemotherapy for pancreatic cancer: the COMBAT trial. Nat Med 2020; 26:878–85.
15. Roach C, Zhang N, Corigliano E, Jansson M, Toland G, Ponto G, et al. Development of a companion diagnostic PD-L1 immunohistochemistry assay for pembrolizumab therapy in non–small cell lung cancer. Appl Immunohistochem Mol Morphol 2016;24:392–7.
16. Macarulla Mercade T, Chen LT, Li CP, Siveke JT, Cunningham D, Bodoky G, et al. Liposomal irinotecan + 5-FU/LV in metastatic pancreatic cancer: subgroup analyses of patient, tumor, and previous treatment characteristics in the pivotal NAPOLI-1 trial. Pancreas 2020;49:62–75.
17. Yu J, Green MD, Li S, Sun Y, Journey SN, Choi JE, et al. Liver metastasis restrains immunotherapy efficacy via macrophage-mediated T-cell elimination. Nat Med 2021;27:152–64.
18. Peled A, Abraham M, Avivi I, Rowe JM, Beider K, Wald H, et al. The high-affinity CXCR4 antagonist BKT140 is safe and induces a robust mobilization of human $CD34^+$ cells in patients with multiple myeloma. Clin Cancer Res 2014;20:469–79.
19. Abraham M, Pereg Y, Bulvik B, Klein S, Mishalian I, Wald H, et al. Single dose of the CXCR4 antagonist BL-8040 induces rapid mobilization for the collection of human CD34(+) cells in healthy volunteers. Clin Cancer Res 2017;23:6790–801.
20. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science 2010;330:827–30.
21. Biasci D, Smoragiewicz M, Connell CM, Wang Z, Gao Y, Thaventhiran JED, et al. CXCR4 inhibition in human pancreatic and colorectal cancers induces an integrated immune response. Proc Natl Acad Sci U S A 2020;117:28960–70.
22. Van Cutsem E, Tempero MA, Sigal D, Oh DY, Fazio N, Macarulla T, et al. Randomized phase III trial of pegvorhyaluronidase alfa with Nab-paclitaxel plus gemcitabine for patients with hyaluronan-high metastatic pancreatic adenocarcinoma. J Clin Oncol 2020;38:3185–94.
23. Ramanathan RK, McDonough SL, Philip PA, Hingorani SR, Lacy J, Kortmansky JS, et al. Phase IB/II randomized study of FOLFIRINOX plus pegylated recombinant human hyaluronidase versus FOLFIRINOX alone in patients with metastatic pancreatic adenocarcinoma: SWOG S1313. J Clin Oncol 2019;37: 1062–9.
24. Hecht JR, Lonardi S, Bendell JC, Sim H-W, Macarulla T, Lopez CD, et al. Randomized phase III study of FOLFOX alone and with pegilodecakin as second-line therapy in patients with metastatic pancreatic cancer (SEQUOIA). J Clin Oncol 2020;38:637.
25. Tempero M, Oh D, Macarulla T, Reni M, Van Cutsem E, Hendifar A, et al. Ibrutinib in combination with nab-paclitaxel and gemcitabine as first-line treatment for patients with metastatic pancreatic adenocarcinoma: results from the phase 3 RESOLVE study. Ann Oncol 2019;30:iv126.