A Phase III Randomized Trial of Integrated Genomics and Avatar Models for Personalized Treatment of Pancreatic Cancer: The AVATAR Trial

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ABSTRACT

Purpose: Pancreatic ductal adenocarcinoma (PDAC) has limited treatment options. We compared the efficacy of comprehensive precision medicine against that of the conventional treatment in PDAC.

Patients and Methods: We report a phase III trial of advanced PDAC in which patients were randomized (1:2) to a conventional treatment treated at physician's discretion (arm A) or to precision medicine (arm B). Subjects randomized to arm B underwent a tumor biopsy for whole-exome sequencing and to generate avatar mouse models and patient-derived organoids for phenotypic drug screening, with final treatment recommended by the molecular tumor board. The primary objective was median overall survival (OS).

Results: A total of 137 patients were enrolled with 125 randomized, 44 to arm A and 81 to arm B. Whole-exome sequencing was performed in 80.3% (65/81) patients of arm B, with potentially actionable mutations detected in 21.5% (14/65). Experimental models were generated in 16/81 patients (19.8%). Second-line treatment was administered to 39 patients in the experimental arm, but only four (10.2%) received personalized treatment, whereas 35 could not receive matched therapy because of rapid clinical deterioration, delays in obtaining study results, or the absence of actionable targets. The median OS was 8.7 and 8.6 months ($P = 0.849$) and the median progression-free survival was 3.8 and 4.3 months ($P = 0.563$) for the conventional and experimental arms, respectively. Notably, the four patients who received personalized treatment had a median OS of 19.3 months.

Conclusions: Personalized medicine was challenging to implement in most patients with PDAC, limiting the interpretation of intention-to-treat analysis. Survival was improved in the subset of patients who did receive matched therapy.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal neoplasms, and it is expected to become the second leading cause of cancer-related deaths in the United States by 2030 (1). Despite the significant progress that has been made in understanding the complex genetic and molecular landscape of PDAC, conventional treatment recommendations for advanced disease are empirical and largely based on patient's age and performance status (2, 3). Germline mutations of *BRCA1/2* and microsatellite instability status alter recommendations only for a minority of patients (4, 5). Personalized medicine is an approach that considers an individual's unique genetic, molecular, and clinical characteristics to tailor treatment strategies, holding promise for improving treatment efficacy for recalcitrant cancers such as PDAC (6, 7).

Recent genomic and bioinformatic efforts have defined new pancreatic cancer subtyping classification, but their ability to implement clinical decision-making has been limited (8–10). Patient-derived organoid (PDO) technologies allow culturing and expansion of pancreatic tumor tissue *ex vivo* (11, 12), enabling DNA and RNA sequencing, biomarker discovery, and high-throughput drug screening testing, in some cases mirroring clinical responses of patients (13–16).

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Translational Relevance

The AVATAR trial is the first multicenter randomized phase III study to assess the feasibility and efficacy of an integrative precision medicine approach in pancreatic cancer, including genomic and bioinformatic analyses of tumor biopsies combined with tumor modeling in patient-derived xenograft and patient-derived organoid models to guide treatment decisions. The study showed that personalized medicine did not improve survival as compared with standard of care in an intention-to-treat population, with the median overall survival of 8.6 and 8.7 months for each arm, respectively ($P = 0.849$). Most patients could not receive a matched therapy because of premature clinical deterioration, delays in obtaining study results, or absence of actionable targets. Notably, for the subjects who received a matched therapy, the median OS was 19.3 months, serving as a proof of concept that precision medicine may benefit a subset of patients. Future efforts are needed to better select patients to realize the full benefit of precision oncology.

Similarly, patient-derived xenografts (PDX) represent an important system for understanding tumor biology, therapeutic responsiveness, and resistance mechanisms, with some studies showing its potential to complement clinical trials in informing therapeutic decisions (17, 18). In a large retrospective analysis of the Know Your Tumor registry trial (19), 26% of patients with pancreatic cancer were found to have actionable molecular alterations, and individuals who received molecularly matched therapy seemed to derive survival benefit (19). Despite these encouraging results, the feasibility of a comprehensive personalized medicine approach for the treatment of PDAC has not been formally investigated in a randomized clinical trial.

Here, we present the results of the AVATAR trial, the first multicenter prospective randomized phase III study designed to compare the efficacy of conventional treatment with that of an integrative precision medicine approach, including genomic and bioinformatic analyses of tumor biopsies combined with tumor modeling in PDX and PDO models to guide treatment decision in pancreatic cancer.

Patients and Methods

Study design and participants

The Integrated Genomics and Avatar Mouse Models for Personalized Treatment of Pancreatic Cancer (AVATAR trial) is a phase III multicenter, open-label, prospective, randomized study conducted at five cancer centers in Spain. Patients with newly diagnosed, histologically confirmed metastatic pancreatic adenocarcinoma were enrolled. Subjects were required to have measurable disease based on RECIST v1.1, Eastern Cooperative Oncology Group (ECOG) performance status ≤1, and adequate organ function at baseline. Eligible subjects were randomized at 1:2 to a conventional treatment strategy (arm A) or to a personalized treatment strategy (arm B). Randomization was stratified according to the presence or absence of liver metastasis and ECOG performance status 0 or 1 (**Fig. 1**). Patients from both arms were followed up at the investigator's discretion, depending on the assigned treatment regimen as well as tolerance to it. Response was evaluated every 8 to 12 weeks from the start of treatment throughout the study according to the investigator's criteria.

The study was approved by the ethical review board of each institution (EudraCT: 2015-004860-12) and was conducted in accordance with ethical principles derived from the Declaration of Helsinki for medical research involving human subjects and good clinical practice and all applicable laws, regulations, and scientific guidelines. All the patients provided written informed consent before enrollment. The authors vouch for the adherence to the trial protocol and the completeness and accuracy of the data.

Treatment

Patients randomized to the conventional treatment (arm A) received standard chemotherapy regimens or investigational drugs in the context of a clinical trial, at the discretion of the treating oncologist. Standard drugs were administered according to the recommendations of regulatory authorities summarized in the corresponding summary of product characteristics. Acceptable chemotherapy regimens for the treatment of metastatic pancreatic adenocarcinoma according to National Comprehensive Cancer Network (NCCN) v1. 2013 guidelines included FOLFIRINOX (combination of fluorouracil, leucovorin, irinotecan, and oxaliplatin), gemcitabine plus nab-paclitaxel, gemcitabine plus cisplatin, gemcitabine plus erlotinib, gemcitabine plus capecitabine, fluoropyrimidines plus oxaliplatin, or clinical trial participation. Second-line and subsequent treatments were decided at the investigator's discretion. Participation in clinical trials with investigational drugs was allowed.

Subjects randomized to personalized treatment (arm B) underwent a tumor biopsy, preferably from a liver implant and before initiating first-line treatment. However, if the patient's treatment needed to be initiated, biopsy could be performed during the first 2 months of initial treatment administration. Patients then received first-line treatment at the discretion of the investigator according to the NCCN v1. 2013, as above. The freshly collected tumor tissues were submitted to next-generation sequencing (NGS) and to generate PDO and mouse avatar models (PDX). Genomic data were analyzed by bioinformatics to select the most promising targeted drugs from a database of more than 2,000 compounds. The results were reviewed by the molecular committee consisting of medical oncologists with expertise in pancreatic cancer, computational biologists, and cancer biologists using a cloud-based virtual molecular tumor board to select the most promising single or combination agents to undergo efficacy testing in the PDO models. A high-throughput screening was developed on the PDOs, using a panel of 24 drugs. The drugs that showed the most efficacies were also tested in efficacy assays on the avatar mouse models. Based on these efficacy results and known safety data for the agent/regimen, a consensus treatment recommendation was finally made for participants at the time of progression. Whenever the preclinical models were not already available, the tumor committee suggested a potential treatment only based on the potential targets suggested by the NGS results. If the physician considered standard chemotherapy treatment to be preferable at the time of first progression, then personalized treatment was used at later lines.

Outcomes and assessment

The primary objective of this study was to compare the median overall survival (OS) from the time of randomization until the time of death, between the conventional treatment arm and the personalized treatment strategy. Secondary objectives included

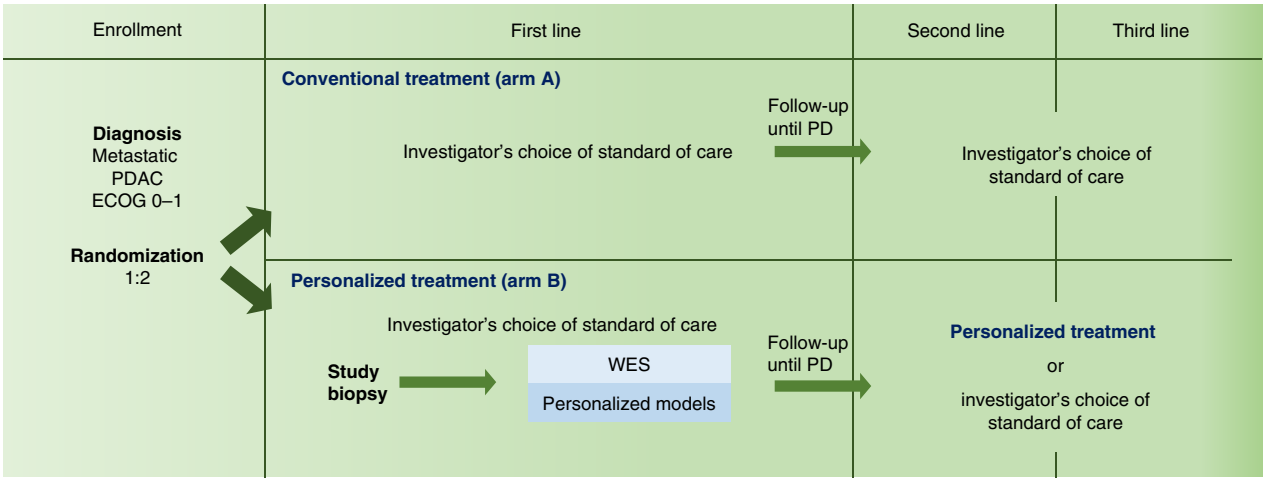

Figure 1.
Study design. PD, progressive disease.

comparison of the objective response rates (ORR) according to RECIST v1.1. and progression-free survival (PFS) between the two arms. Exploratory objectives included analysis of the genomic landscape of metastatic PDAC and the feasibility to generate a library of avatar mouse models of metastatic PDAC.

Statistical analysis

The study was designed to test the main hypothesis that the OS is improved with a personalized treatment approach compared with conventional treatment in metastatic PDAC. The 1-year OS in this patient population is estimated to be 20%. With an α of 0.05 and a power of 80%, the sample size needed to detect a 20% to 40% improvement in survival is 146, with 49 in the control arm and 97 in the experimental arm. Time-to-event outcomes were estimated using the Kaplan–Meier method. Intention-to-treat (ITT) analysis was performed on all subjects who met the eligibility criteria and was allocated to intervention. Modified ITT analysis was conducted on subjects who received at least one dose of treatment and had a postbaseline imaging for RECIST response and PFS assessment. Statistical analyses were done using the software package IBM SPSS Statistics release version 26.

Patient material processing

Hepatic metastatic samples from core needle biopsies were freshly collected into RPMI medium added with penicillin–streptomycin 1:100 v/v. Tumor samples, free from fat and necrotic tissue, were cut into small pieces of 2 to 3 mm^3 and embedded in Matrigel (Corning Matrigel Basement Membrane Matrix, 354234). Five- to six-week-old female NOD/SCID gamma mice (strain NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wji}/SzJ) provided by Charles River Laboratories were anesthetized using isoflurane gas, anesthesia administered with buprenorphine dosed at 0.2 mg/kg. Subsequently, each piece of the tumor sample was implanted subcutaneously, using an 18-gauge trocar, in one flank of the lower back of the mice. Due to the scarcity of the tumor samples derived from liver biopsies, in this first phase (F1 or engraftment phase), only two or three NOD/SCID gamma mice, instead of the usual five, were used to be implanted.

Establishment of PDX models

Five- to six-week-old female athymic nude-Foxn1 (nude/nude) mice [strain Crl:UN(NCr)-Foxn1^{nu}], provided by Charles River Laboratories, were anesthetized as mentioned above. Xenografts obtained from F1 were excised, and a part was cut into small 3 × 3 × 3-mm fragments and then implanted subcutaneously in both mice flanks (as described in F1), in a group of five to eight mice (F2 or expansion phase). The remaining part of the xenograft was cryopreserved and/or processed for future biological studies. When the tumors from F2 reached a size of about 1,500 mm^3, they were explanted, cut into 3 × 3 × 3-mm fragments, and finally implanted into the experimental cohorts of mice that were treated with the drugs (F3 and successive).

PDX treatments

Xenografts from the treatment phases (F3 and beyond) were randomized into the treatment groups when their sizes reached about 200 mm^3. Experimental groups were formed by five to eight mice, and the cohort number depended on the number of treatments preset for each model.

Gemcitabine and nab-paclitaxel were administered, both as a single agent and combination, at concentrations of 150 mg/kg i.p. twice a week and 50 mg/kg i.v. once a week, respectively. Both treatments lasted 28 days. Palbociclib in lactate buffer was administered in combination with nab-paclitaxel at a dose of 75 mg/kg orally once daily during a 28-day period. Nab-paclitaxel was also administered as a single agent at a concentration of 100 mg/kg i.v. once daily for 3 days followed by a 2-day rest period, during a 28-day period. Five doses of irinotecan 50 mg/kg i.v. were administered every 4 days. Sunitinib 80 mg/kg p.o. was prepared in saline added with methylcellulose and administered once daily for 21 days. Doxorubicin at 10 mg/kg i.v. was administered once weekly for three doses. Mitomycin C 4 mg/kg i.v. was administered once every 4 days for three doses. Trametinib 2 mg/kg orally dissolved in Tween 80 with methylcellulose was administered once daily for 21 days. Dabrafenib at 100 mg/kg orally was administered daily for 2 weeks. Olaparib at 20 mg/kg i.p. was administered daily in corn oil, for 4 weeks. If not otherwise specified, drugs were prepared in saline buffer.

Tumor size was evaluated three times a week by caliper measurement, using the formula tumor volume = (length × width^2)/2, as previously reported (20). Relative tumor growth inhibition (TGI) was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice (T/C). In all cases, experiments terminated on day 28. We consider having a significant response when the TGI% was higher than 50% after 28 days of treatment. Animals were checked daily for any symptom of toxicity or discomfort. Whenever the symptoms of humane endpoint emerge, including 15% loss of weight after three consecutive days, labored respiration, and/or persistent hypothermia, or when the tumor reaches 1,500 mm^3, whichever comes first, the mice were euthanized by CO_2 inhalation.

Establishment of PDO models

The xenograft proceeding sample was minced into 0.5- to 1.0-mm fragments and added with resuspension media (DMEM plus 1% BSA and 10% penicillin–streptomycin), pelleted at 1,500 rpm and incubated for 30 minutes in digestion media [DMEM plus 1% penicillin–streptomycin and 1:100 collagenase/dispase (Sigma)] at 37°C. The latter was added with a little bit more of resuspension media and pelleted at 1,500 rpm at 4°C for 5 minutes. Pellet was incubated into a water bath for 30 minutes, after being added with 2 mL of Accutase (Sigma). Resuspension media (1 mL) were added and moved to a tissue strainer. Eventually, 1 mL more of resuspension media was needed to let the digested cells flow. After centrifugation at 1,500 rpm for 5 minutes at 4°C, pellet was resuspended in 2 mL of culture media [DMEM plus 1% penicillin–streptomycin, 1:2,000 hydrocortisone (1 mg/mL), and PaTOM growth factor cocktail (obtained from Muthuswamy laboratory; ref. 11) plus 5% GFR-Matrigel (Corning) and 10 μmol/L Y267632 Rock Inhibitor (Tocris Bioscience) prepared in sterile PBS]. The resuspended cells were finally transferred to a Matrigel-coated 12-well dish. Cell cultures were monitored, and media were changed every 3 to 4 days.

PDO susceptibility testing

The PDO model used for these studies was established and stored as cryopreserved stocks. Early passage (<15) cultures were used for the proposed studies. Organoids were dissociated with collagenase/dispase and then by TrypLE to generate singles. Cells were diluted in organoid culture medium at a density of 50,000 cells/mL. Cells were seeded into 96 wells at 100 μL/well. Cells were allowed to grow for 4 days to form organoids. The medium was replaced and incubated with drugs dispensed into wells using a Tecan D300e digital dispenser (Tecan) for 72 hours at 5 μmol/L concentrations: capecitabine, carboplatin, dabrafenib, dasatinib, decitabine, doxorubicin, erlotinib, everolimus, fluorouracil, gemcitabine, lapatinib, paclitaxel, palbociclib, mitomycin C, olaparib, oxaliplatin, pemetrexed, SN-38, sorafenib, sunitinib, trametinib, venetoclax, vinorelbine, and vorinostat. Each experiment included three control arms: untreated, carrier, and general toxin. The impact of drug treatment was analyzed using CytoTox-Glo assay (Promega) after 72 hours to determine both changes in total cell number for calculating growth inhibition and changes in cell death with reference to the untreated control as outlined in the manufacturer's instructions. Each treatment was analyzed by three technical replicates over two different passages. The results were used for calculating the dose of drug needed for 50% growth inhibition.

Cryopreservation of PDO

After aspiration of the old media, 0.5 mL/well digestion media was added, and the 12-well plate was incubated at 37°C for 2.5 to 3 hours. About 1 mL of resuspension medium was added and centrifuged at 1,500 rpm for 5 minutes at 4°C. The pellet was resuspended in 500 μL cold Accutase and incubated at 37°C for 30 to 40 minutes. After the addition of 500 μL of resuspension medium and gentle stirring, the sample was centrifuged at 1,500 rpm for 5 minutes at 4°C, and the pellet was resuspended in CryoStor freezing media (Sigma-Aldrich) plus 10 μmol/L Y267632 Rock Inhibitor (Tocris Bioscience).

Genetic analysis

DNA was extracted from both formalin-fixed, paraffin-embedded tissue and peripheral blood according to the manufacturer's protocol. For whole-exome sequencing (WES), library preparation was performed using Agilent SureSelect v6 (Agilent), and sequencing was performed using Illumina NovaSeq 6000 (Illumina). Paired tumor blood samples were analyzed together following a custom algorithm developed in-house in a subset of genes according to a virtual panel of 53 genes (Supplementary Table S1), which included several filtering tiers as follows: quality control, population frequency, impact of the variants, Gene Ontology, cancer databases for somatic variants, pharmacogenetic impact of the variants, and pathogenicity prediction according to several pathogenic predictors (dbNSFP, CADD, and REVEL). Differentiation between mosaic and germline samples was also analyzed by reviewing the allele frequency in the tumor tissue and peripheral blood. Five percent threshold was imputed as the cutoff for somatic variant allele frequency, according to previous reports on the sensibility of the WES in somatic variant detection. Variant classification was performed according to American College of Medical Genetics and Genomics (ACMG) guidelines for germline variants (21) and Association for Molecular Pathology (AMP) guidelines for somatic variants (22). Actionable genomic events were defined as those for which there is available evidence supporting effective therapeutic targeting either by a labeled or off-label drug [European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets; ref. 23].

Data availability

The data generated in this study are available in a de-identified manner to qualified researchers upon reasonable request to the corresponding author. The DNA sequencing data have been uploaded at the European Variation Archive repository under the accession number PRJEB81716 and can be accessed via https://www.ebi.ac.uk/ena/browser/view/PRJEB81716.

Results

Patient characteristics

Between June 2016 and June 2020, 137 patients from five Spanish centers were assessed for eligibility in the study. Twelve patients did not meet the eligibility criteria. A total of 125 subjects were randomized, with 44 assigned to the conventional arm and 81 to the experimental arm (**Figs. 1** and **2**). Baseline characteristics are presented in **Table 1**. See Supplementary Table S2 for key PDAC demographics to compare with the population enrolled in this study. The median age at diagnosis was 62.4 years and 45.6% were female, and those with ECOG status of 0 or 1 were 42.4% and 57.6%, respectively. First-line gemcitabine plus nab-paclitaxel was the first-line regimen used in 59.1% of patients of the conventional arm and 53.8% of the experimental group. First-line FOLFIRINOX was used

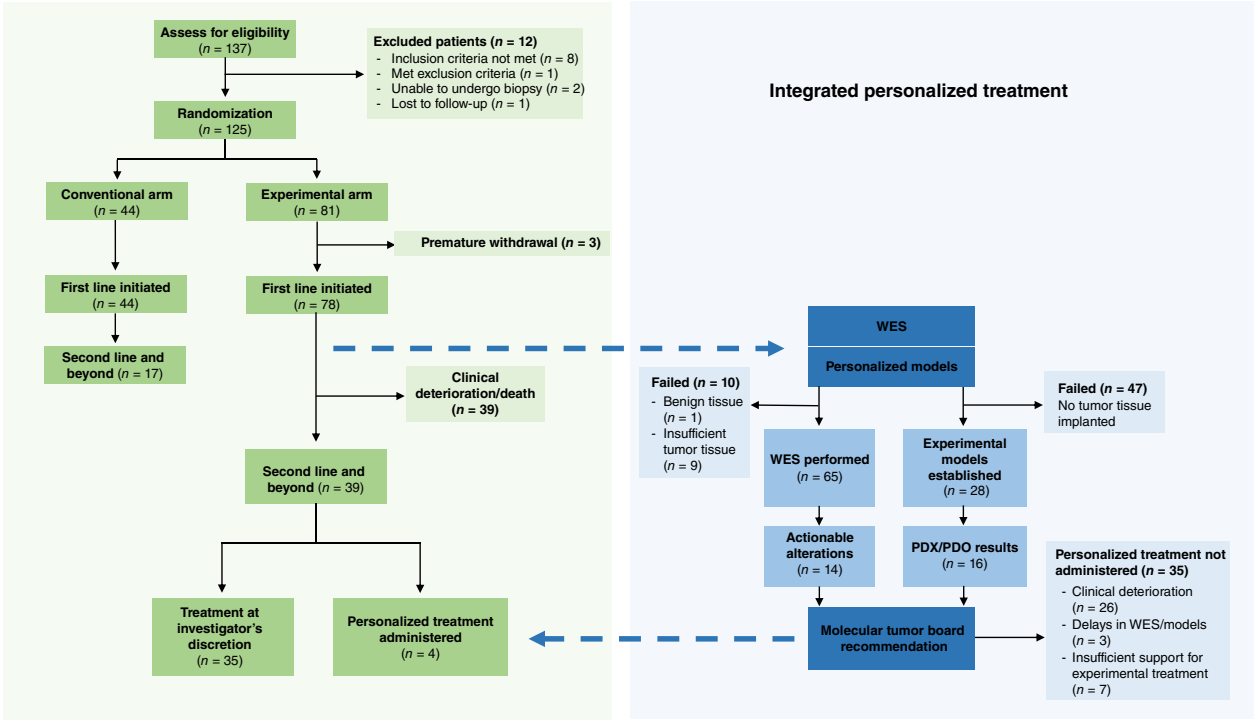

Figure 2.
CONSORT diagram. Subjects in whom personalized treatment was not administered ($n = 35$) may have more than one overlapping reason.

in 9.1% of subjects of the conventional arm and 5.1% of the experimental group. Other regimens included clinical trial treatments used in 31.8% of conventional arm and 41.1% of experimental arm subjects.

Genomic analysis

Among the 81 patients randomized to the experimental arm, 75 underwent biopsy for genomic analysis and avatar model development. WES was performed on 65 samples of the 75 obtained biopsies. Ten subjects had failed NGS because of insufficient tumoral tissue. We detected potential actionable genomic alterations suitable to therapeutic targeting in 14 (21.5%) of the 65 individuals who underwent WES (**Fig. 3**).

WES of both tumor tissue and blood revealed a total of 71 variants detected in the 39 genes tested from 72 samples from 65 individuals (**Fig. 3**; Supplementary Table S3). Fifty-six variants (78.8%) were detected at the somatic level (only in the tumor tissue) and 15 variants (21.1%) at the germline level (both in the DNA from tumor tissue and peripheral blood). *KRAS* and *TP53* were the genes with the highest rate of pathogenic variants detected at the somatic level, with $KRAS^{G12D}$ being the most prevalent variant, detected in 18 samples. At the germline level, three pathogenic variants were detected in *BRCA2* and one in *ATM* (**Fig. 3**; Supplementary Table S3). We also detected a likely pathogenic variant in *TCF3*, which has been associated with type 8A agammaglobulinemia both in autosomal dominant and recessive patterns of inheritance (24). Main clinical features in addition to agammaglobulinemia included recurrent infections, decreased circulating B cells, and severely decreased levels of serum immunoglobulins. The majority of the variants detected have already been detected in other patients with cancer, although there are some variants not previously reported. Notably, more than one pathogenic variant was identified in several patients. For instance, in sample PAN122, four different variants were identified in *KRAS*, *TP53*, *ARID1A*, *KDM6A*, *KMT2C*, and *AURKA* (**Fig. 3**; Supplementary Table S3). The combination of two variants was identified in *KRAS* and *TP53* in 20 individuals. The majority of the variants detected were missense, although nonsense, frameshift, splice site, and synonymous were also observed (**Fig. 3**). In 25 cases, neither somatic nor germline variants were identified. Additionally, 10 variants of unknown significance were detected in several genes without a clear association with the development of any disease and in which further analyses are required (i.e., family segregation study or functional *in vitro* or *in vivo* assays) to understand the role of these variants.

Experimental model generation and drug screening

Twenty-eight experimental PDX avatar models were generated from samples obtained of the 75 biopsies performed. Twenty-three PDOs of the previous 28 were also established. At least one experimental model was generated in 35% of patients included in the experimental arm (**Fig. 2**).

A panel of 24 drugs was used for high-throughput screenings in 8 of the 23 PDOs established. Thirteen of the 28 avatar models were treated in efficacy assays (Supplementary Table S4). The drugs used to treat the animal models were selected according to the results from WES analysis integrated with the high-throughput screenings conducted on the PDO models.

Integrated personalized treatment recommendation

Following the analyses of the 14 patients in whom WES detected a potential actionable genomic alteration suitable to therapeutic

Table 1. Baseline characteristics.

Characteristic	Conventional arm *N* = 44 *n* (%)	Experimental arm *N* = 81 *n* (%)	Total *N* = 125 *n* (%)
Median age, years (range)	62.7, range (46–84)	62.2, range (38–82)	62.4, range (38–84)
Gender			
Female	19 (43.2)	38 (46.9)	57 (45.6)
Male	25 (56.8)	43 (53.1)	68 (54.4)
ECOG performance status			
0	17 (38.6)	36 (44.4)	53 (42.4)
1	27 (61.4)	45 (55.6)	72 (57.6)
Liver metastasis			
Yes	39 (88.6)	73 (90.1)	112 (89.6)
No	5 (11.4)	8 (9.9)	13 (10.4)
Tumor stage			
I	1 (2.3)	1 (1.23)	2 (1.6)
II	1 (2.3)	2 (2.47)	3 (2.4)
III	5 (11.4)	10 (12.3)	15 (12)
IV	36 (81.8)	67 (82.8)	103 (82.4)
Not available	1 (2.3)	1 (1.23)	2 (1.6)
Prior surgery			
Yes	8 (18.2)	17 (21)	25 (20)
No	36 (81.8)	64 (79)	100 (80)
Prior radiotherapy			
Yes	3 (6.8)	5 (6.2)	8 (6.4)
No	41 (93.2)	76 (93.8)	117 (93.6)
First-line chemotherapy			
Gemcitabine/nab-paclitaxel	27 (61.4)	43 (53.1)	70 (56)
FOLFIRINOX	4 (9.1)	4 (4.9)	8 (6.4)
Clinical trial regimen	13 (16)	31 (38.3)	44 (35.2)
No treatment	0 (0)	3 (3.7)	3 (2.4)

targeting, combined with the drug testing of 16 subjects' models (8 PDOs and 13 PDXs), a total of 4 patients received a molecularly matched therapy in second or subsequent lines. Thirty-five of the 39 patients (90%) who started a second-line therapy did not receive molecularly matched therapy. The main underlying reasons for these individuals not to receive a matched therapy were swift clinical deterioration in 26 subjects, preventing second-line treatment from commencing. Other overlapping reasons included delayed genomic analysis due to technical issues in three patients, whereas in seven individuals, the suggested matched therapy was not authorized by the molecular committee. The description of patients treated with preclinical model recommendation is shown in Supplementary Table S4.

Clinical outcomes

A total of 125 subjects were randomized, with 44 assigned to the conventional arm and 81 to the experimental arm, with three having premature withdrawal (**Fig. 2**). All 44 patients in the conventional arm received first-line treatment, whereas 17 (38.6%) received second-line or subsequent lines of treatment. In the experimental arm, 78 individuals received first-line treatment, whereas 39 (50%) received a second or subsequent lines of therapy. Of these 39 patients receiving a second or subsequent lines, 4 (10%) received a molecularly matched therapy. The most commonly used second-line regimen included irinotecan, which is present in about 50% of regimens.

At the time of the final analysis in October 2021, 88% of patients had died. With a median follow-up of 9.8 months, the median OS was 8.7 months [95% confidence interval (CI), 4.5–12.9] for the conventional group and 8.6 months (95% CI, 6.4–10.8) for the experimental group ($P = 0.849$; **Fig. 4**). The 1-year OS was 31.8% (95% CI, 18.0%–45.6%) for the conventional group and 33.5% (95% CI, 23.2%–43.8%) for the personalized group. The median PFS for the first line of treatment was 3.9 months (95% CI, 3.3–4.5) for the conventional group and 4.4 months (95% CI, 3.1–5.7) for the experimental group ($P = 0.651$). In the second-line setting, the PFS was 2.4 months (95% CI, 1.7–3.1) and 2.0 months (95% CI, 1.5–2.2) for the conventional and experimental groups, respectively ($P = 0.649$). Similarly, no differences in PFS were observed in the third- and fourth-line settings (**Table 2**; Supplementary Table S5). Notably, for the four patients who received personalized treatment recommended by the molecular committee, the median OS was 19.32 months.

The confirmed ORR according to investigators for the first-line treatment was 34.4% in the conventional arm versus 25.4% in the experimental arm ($P = 0.611$). In the second line, the ORR was 12.5% and 7.1% for the conventional and experimental arms, respectively ($P = 0.886$). No patients achieved partial or complete responses in the third- or fourth-line settings (**Table 2**; Supplementary Table S5).

Clinical vignette

The benefits of personalized medicine in PDAC are demonstrated in the following case narratives, in which nonstandard drugs such as sunitinib, liposomal doxorubicin, and mitomycin C demonstrated clinical benefit in patients with advanced disease.

Patient Panc-136 was a 73-year-old male with PDAC (*KRAS*^{G12D}, *TP53*, and *FANCD2*) treated per the physician's choice with gemcitabine/nab-paclitaxel/BBI-608 in the first line on a clinical trial, with treatment lasting for 4.5 months. The patient received fluorouracil, leucovorin, and oxaliplatin (FOLFOX) in the second line also per the physician's choice, having progression within 6 weeks. He then received fluorouracil, leucovorin, and irinotecan (FOLFIRI) combined with mitomycin C in the third line according

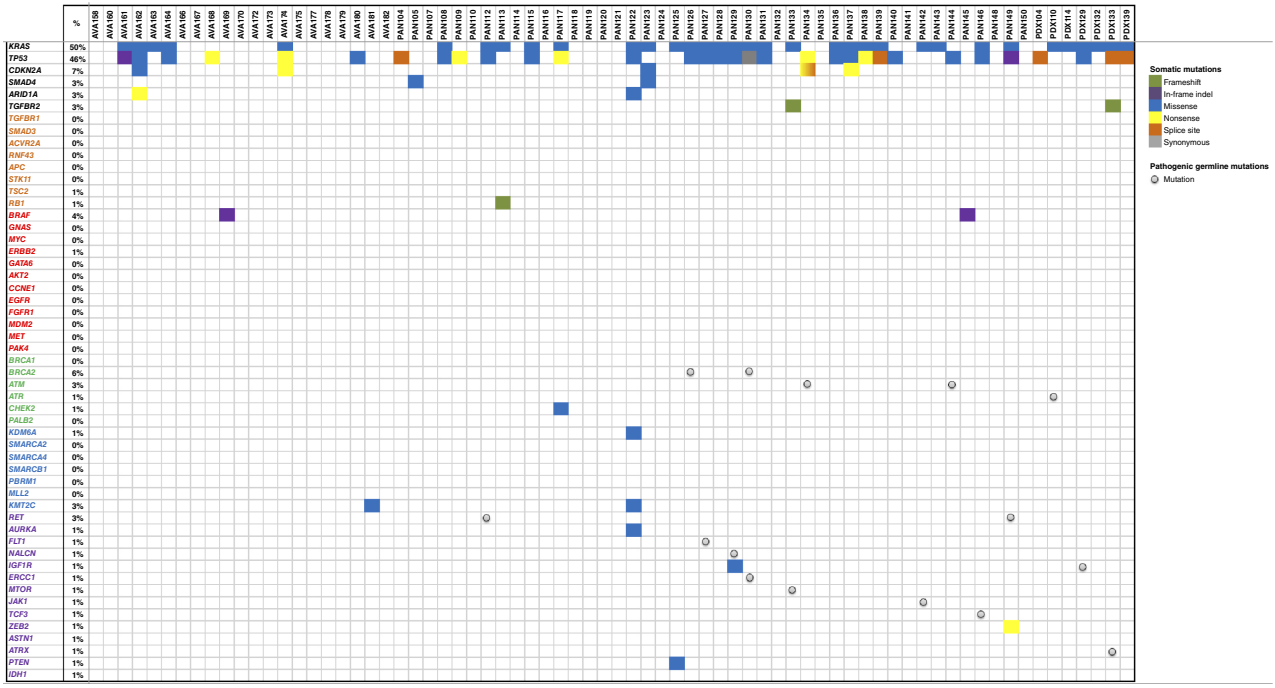

Figure 3.

Summary of WES data. Columns represent each study sample analyzed for WES ($n = 76$). Rows represent genes in which relevant variants were detected with their respective prevalence. Genetic variants were classified as somatic and germline, as detailed in the color-coded legend. In 26 samples, no mosaic or neither germline variants were identified.

to high-throughput drug screening on PDO, with disease control lasting for 14 months.

Patient Panc-137 was a 71-year-old female with PDAC (*KRAS*^{G12D}, *TP53*, *SMAD4*, and *CDKN2A* loss) treated with gemcitabine/nab-paclitaxel in the first line per the physician's choice with treatment lasting

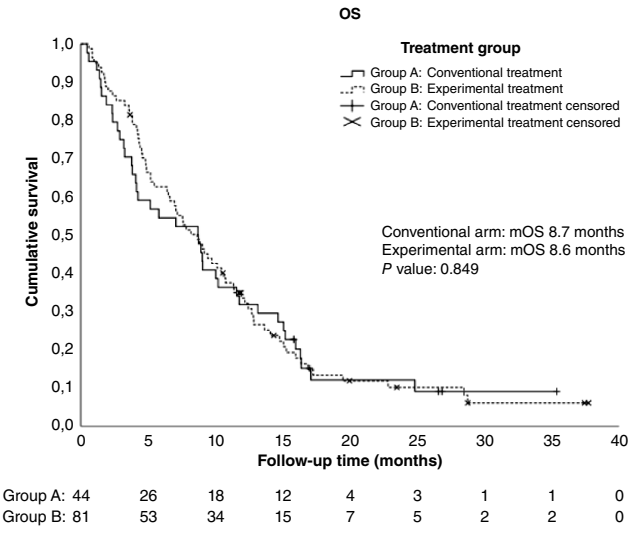

Figure 4.

OS. Kaplan–Meier estimate of OS in the ITT population (arm A, $n = 44$; arm B, $n = 81$). Survival measured in months from the time of randomization. + and × displayed in the curves identify censoring pattern.

for 9 months, followed by FOLFIRI in the second line for 5 months. Upon progression, she received sunitinib in the third line guided by high-throughput drug screening on PDO, with disease control lasting for 5 months. Accordingly, PDX results also confirmed increased sensitivity to sunitinib (TGI 96.5%).

Patient Panc-146 was a 66-year-old female with PDAC (*KRAS*^{G12D}, *TP53*, and *PIK3R6*) treated per the physician's choice in the first line with gemcitabine/nab-paclitaxel for 5 months and in the second line on a clinical trial with FOLFOX/nivolumab/cabiralizumab having progression within 2 months. She then received fluorouracil/liposomal irinotecan guided by high-throughput drug screening on PDO, with disease control lasting for 13.8 months. The patient subsequently received fourth-line therapy with liposomal doxorubicin also guided by PDO results, with treatment lasting for 11 weeks.

Patient Panc-105 was a 70-year-old female with PDAC (*KRAS*^{G12R}, *TP53*, and *SMAD4*) treated per the physician's choice in the first line with gemcitabine/nab-paclitaxel ± ibrutinib in a double-blinded clinical trial, having progression within 2 months. She received the second line with FOLFIRINOX achieving tumor control for 13 months. This recommendation was guided by WES results and supported by PDX data, which showed that the second best tumor growth inhibition after gemcitabine/nab-paclitaxel (TGI 92%) was achieved with irinotecan (TGI 59%). Other options included trabectedin (TGI 54%), pemetrexed (TGI 35.3%), and palbociclib (TGI 21.5%).

Discussion

The AVATAR trial is the first prospective randomized study to formally assess the feasibility and efficacy of a comprehensive personalized medicine approach in the care of patients with metastatic

Table 2. Efficacy results.

	Conventional arm (n = 44)	Experimental arm (n = 81)	P value
OS (months)	8.7	8.6	0.849
Evaluable in the first line	$n = 32$	$n = 63$	
ORR (n, %)	11 (34.4%)	16 (25.4%)	0.661
DOR (months)	3.5	4.4	0.508
PFS (months)	3.8	4.3	0.563
Evaluable in the second line	$n = 8$	$n = 28$	
ORR (n, %)	1 (12.5%)	2 (7.1%)	0.886
DOR (months)	2.8	5.2	0.707
PFS (months)	2.4	1.9	0.417

In the conventional arm, 44 subjects initiated first-line therapy. Of those, 32 were evaluable for response in the first line and 8 were evaluable in the second line. In the experimental arm, 81 subjects initiated first-line treatment. Of those, 63 were evaluable for response after first-line therapy and 28 were evaluable for the second line.
Abbreviation: DOR, duration of response.

PDAC. The study showed that this integrated personalized strategy did not improve OS as compared with standard of care in an ITT population, with the median OS of 8.6 and 8.7 months for each arm, respectively ($P = 0.849$).

This large multicenter effort exposed many real-world challenges of implementing an integrated personalized medicine in a highly aggressive disease such as PDAC. The integration of PDX and PDO models to inform treatment in the second line and beyond can be lengthy, and this turnaround time is often incompatible with the pace of clinical deterioration that most patients with PDAC experience. In our study, only 45.9% of patients were able to start a second-line treatment. This low proportion of candidates entering the second line underscores the suboptimal efficacy of the standard-of-care first-line chemotherapy in the metastatic setting (25, 26). In fact, a swift clinical deterioration was the main reason for these individuals not to receive a matched therapy, occurring in 65% of the cases. Notably, of the 39 individuals in the experimental group who were able to start a second or subsequent lines of therapy, 4 (10%) received a molecularly matched therapy achieving a median OS of 19.5 months, much higher than the 8.7 months observed in the conventional arm.

Similar barriers were reported in the Know Your Tumor registry trial (19). In this large retrospective study, 1,856 patients with pancreatic cancer received molecularly tailored therapy recommendations based on tumor multiomics profiling. However, of those, only 2% (46 subjects) ultimately received a matched therapy. These 46 subjects also showed an improvement in OS compared with those who did not receive a matched therapy.

Among the obstacles to the implementation of personalized medicine included challenges to obtain high-quality molecular testing, lack of an actionable alteration, and accessibility to a matched therapy. Still, the authors reported that for the small subset of patients (2%) who actually received a matched therapy, the median OS was significantly longer than the OS of those who only received unmatched therapies [2.6 vs. 1.5 years; HR, 0.42 (95% CI, 0.26–0.68); $P = 0.0004$; ref. 19]. Together with our data, these results suggest that a personalized medicine approach might not be attainable for "all comers" patients with metastatic PDAC at present.

Our study highlighted the paucity of therapeutic targets in metastatic PDAC. WES failed to identify any potentially actionable somatic and germline alterations in 51 of the 65 patients tested (78.5%). In addition, following analyses of genomic and experimental model drug testing results, the molecular committee assessed that the available published data to support a particular therapeutic intervention were inadequate in seven cases. The lack of actionable genetic alterations is undoubtedly a major contributor to poor outcomes in PDAC. The vast majority of mutations involve *KRAS* and *TP53*, genes felt to be "undruggable" until recently.

The recent exciting development of sotorasib and adagrasib for the 1% to 2% of patients with PDAC with $KRAS^{G12C}$ showed promising response rates of 21% to 50% (27, 28). Another promising advance for patients with pancreatic cancer treatment is the development of $KRAS^{G12D}$ inhibitors, as these variants are present in 35% to 45% of patients (29) according to both the literature and our results presented herein. Recent preclinical studies targeting the G12D with a small-molecule inhibitor MRTX1133 showed deep and durable tumor regressions in autochthonous PDAC models (30). The phase I trial with this agent is currently enrolling patients with solid tumors including PDAC (NCT05737706), whereas other agents such as RMC-6236 and LUNA18 are also targeting specific RAS variants (NCT05379985 and NCT05012618).

In summary, the limitations of this study include the inability of use of matched therapy for a broader population in the experimental arm, which was directly related to the paucity of targetable genomic alterations in PDAC as well as the fast pace for clinical deterioration, incompatible with the lengthy time to generate genomic and PDO/PDX data. Due to only a limited number of patients in the personalized therapy arm actually receiving a matched drug, caution should be taken to derive definitive conclusions about the real effect of personalized medicine in PDAC. With the knowledge and experience acquired by this pioneer AVATAR trial, we anticipate that the further development of novel *KRAS* inhibitors will have a significant potential to change the landscape of precision medicine for a broader population of patients with metastatic PDAC.

Future studies should also consider an alternative design in which all patients receive the same first-line therapy, and upon randomization to the experimental arm, a research biopsy is done for NGS and PDO/PDX generation to guide matched therapy for the second line, whereas the control arm would receive a standard-of-care second-line regimen.

Despite these challenges, the AVATAR trial highlights a promising outcome in the small subset of patients who received personalized treatment as recommended by the molecular committee. The findings of this study contribute to a better understanding of the role of precision medicine in the clinical management of patients with PDAC and provide insights into the potential benefits

and limitations of a comprehensive precision medicine approach in the treatment of this aggressive malignancy. Future studies are needed to understand whether personalized medicine is suitable for the subset of patients with a less aggressive course in the first-line setting, which may allow additional time for experimental models to be established and inform individualized therapeutic decisions in the second line and beyond.

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Authors' Contributions

F. Sarno: Data curation, formal analysis, supervision, validation, project administration, writing–review and editing. **J. Tenorio:** Investigation, writing–review and editing. **S. Perea:** Investigation, writing–review and editing. **L. Medina:** Supervision, investigation. **R. Pazo-Cid:** Supervision, investigation. **I. Juez:** Investigation, writing–review and editing. **R. Garcia-Carbonero:** Investigation, writing–review and editing. **J. Feliu:** Investigation, writing–review and editing. **C. Guillen-Ponce:** Supervision, investigation. **P.P. Lopez-Casas:** Writing–review and editing. **C. Guerra:** Investigation, writing–review and editing. **Y. Duran:** Investigation, writing–review and editing. **J.F. López-Acosta:** Investigation, writing–review and editing. **C. Alonso:** Investigation, writing–review and editing. **E. Esquivel:** Investigation, writing–review and editing. **A. Dopazo:** Investigation, writing–review and editing. **D. Akshinthala:** Data curation, writing–review and editing. **S.K. Muthuswamy:** Investigation, writing–original draft. **P. Lapunzina:** Investigation, writing–review and editing. **B. Bockorny:** Data curation, formal analysis, writing–original draft, writing–review and editing. **M. Hidalgo:** Conceptualization, formal analysis, investigation, writing–review and editing.

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Note

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin 2023;73:17–48.
2. Sohal DPS, Kennedy EB, Cinar P, Conroy T, Copur MS, Crane CH, et al. Metastatic pancreatic cancer: ASCO guideline update. J Clin Oncol 2020;38:3217–30.
3. Taieb J, Prager GW, Melisi D, Westphalen CB, D'Esquermes N, Ferreras A, et al. First-line and second-line treatment of patients with metastatic pancreatic adenocarcinoma in routine clinical practice across Europe: a retrospective, observational chart review study. ESMO Open 2020;5:e000587.
4. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. N Engl J Med 2019;381:317–27.
5. 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.
6. Ramanathan RK, Weiss GJ, Posner RG, Rajeshkumar NV, Jameson G, Aziz M, et al. A phase 2 trial of personalized cytotoxic therapy based on tumor immunohistochemistry in previously treated metastatic pancreatic cancer patients. J Gastrointest Oncol 2017;8:925–35.
7. Dorman K, Zhang D, Heinrich K, Reeh L, Weiss L, Haas M, et al. Precision oncology in pancreatic cancer: experiences and challenges of the CCCMunich^{LMU} molecular tumor board. Target Oncol 2023;18:257–67.
8. Puleo F, Nicolle R, Blum Y, Cros J, Marisa L, Demetter P, et al. Stratification of pancreatic ductal adenocarcinomas based on tumor and microenvironment features. Gastroenterology 2018;155:1999–2013.e3.
9. Waddell N, Pajic M, Patch A-M, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015;518:495–501.
10. Bailey P, Chang DK, Nones K, Johns AL, Patch A-M, Gingras M-C, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531:47–52.
11. Huang L, Holtzinger A, Jagan I, BeGora M, Lohse I, Ngai N, et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nat Med 2015;21:1364–71.
12. Boj SF, Hwang C-I, Baker LA, Chio II, Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015;160:324–38.
13. Grossman JE, Muthuswamy L, Huang L, Akshinthala D, Perea S, Gonzalez RS, et al. Organoid sensitivity correlates with therapeutic response in patients with pancreatic cancer. Clin Cancer Res 2022;28:708–18.
14. Tiriac H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. Cancer Discov 2018;8:1112–29.
15. Aguirre AJ, Nowak JA, Camarda ND, Moffitt RA, Ghazani AA, Hazar-Rethinam M, et al. Real-time genomic characterization of advanced pancreatic cancer to enable precision medicine. Cancer Discov 2018;8:1096–111.
16. Huang L, Bockorny B, Paul I, Akshinthala D, Frappart P-O, Gandarilla O, et al. PDX-derived organoids model in vivo drug response and secrete biomarkers. JCI Insight 2020;5:e135544.
17. Nardella C, Lunardi A, Patnaik A, Cantley LC, Pandolfi PP. The APL paradigm and the "co-clinical trial" project. Cancer Discov 2011;1:108–16.
18. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther 2011;10:1311–6.

19. Pishvaian MJ, Blais EM, Brody JR, Lyons E, DeArbeloa P, Hendifar A, et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the know your tumor registry trial. Lancet Oncol 2020;21:508–18.
20. Euhus DM, Hudd C, LaRegina MC, Johnson FE. Tumor measurement in the nude mouse. J Surg Oncol 1986;31:229–34.
21. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.
22. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 2017;19:4–23.
23. Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). Ann Oncol 2018;29:1895–902.
24. Al Sheikh E, Arkwright PD, Herwadkar A, Hussell T, Briggs TA. TCF3 dominant negative variant causes an early block in B-lymphopoiesis and agammaglobulinemia. J Clin Immunol 2021;41:1391–4.
25. Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med 2011;364:1817–25.
26. 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.
27. Strickler JH, Satake H, George TJ, Yaeger R, Hollebecque A, Garrido-Laguna I, et al. Sotorasib in KRAS p.G12C-mutated advanced pancreatic cancer. N Engl J Med 2023;388:33–43.
28. Bekaii-Saab TS, Yaeger R, Spira AI, Pelster MS, Sabari JK, Hafez N, et al. Adagrasib in advanced solid tumors harboring a $KRAS^{G12C}$ mutation. J Clin Oncol 2023;41:4097–106.
29. Moore AR, Rosenberg SC, McCormick F, Malek S. RAS-targeted therapies: is the undruggable drugged? Nat Rev Drug Discov 2020;19:533–52.
30. Kemp SB, Cheng N, Markosyan N, Sor R, Kim I-K, Hallin J, et al. Efficacy of a small-molecule inhibitor of KrasG12D in immunocompetent models of pancreatic cancer. Cancer Discov 2023;13:298–311.